The role of meiotic nuclear envelope components in chromosome dynamics and meiotic progression

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zur Erlangung des naturwissenschaftlichen
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der Julius-Maximilians-Universität Würzburg

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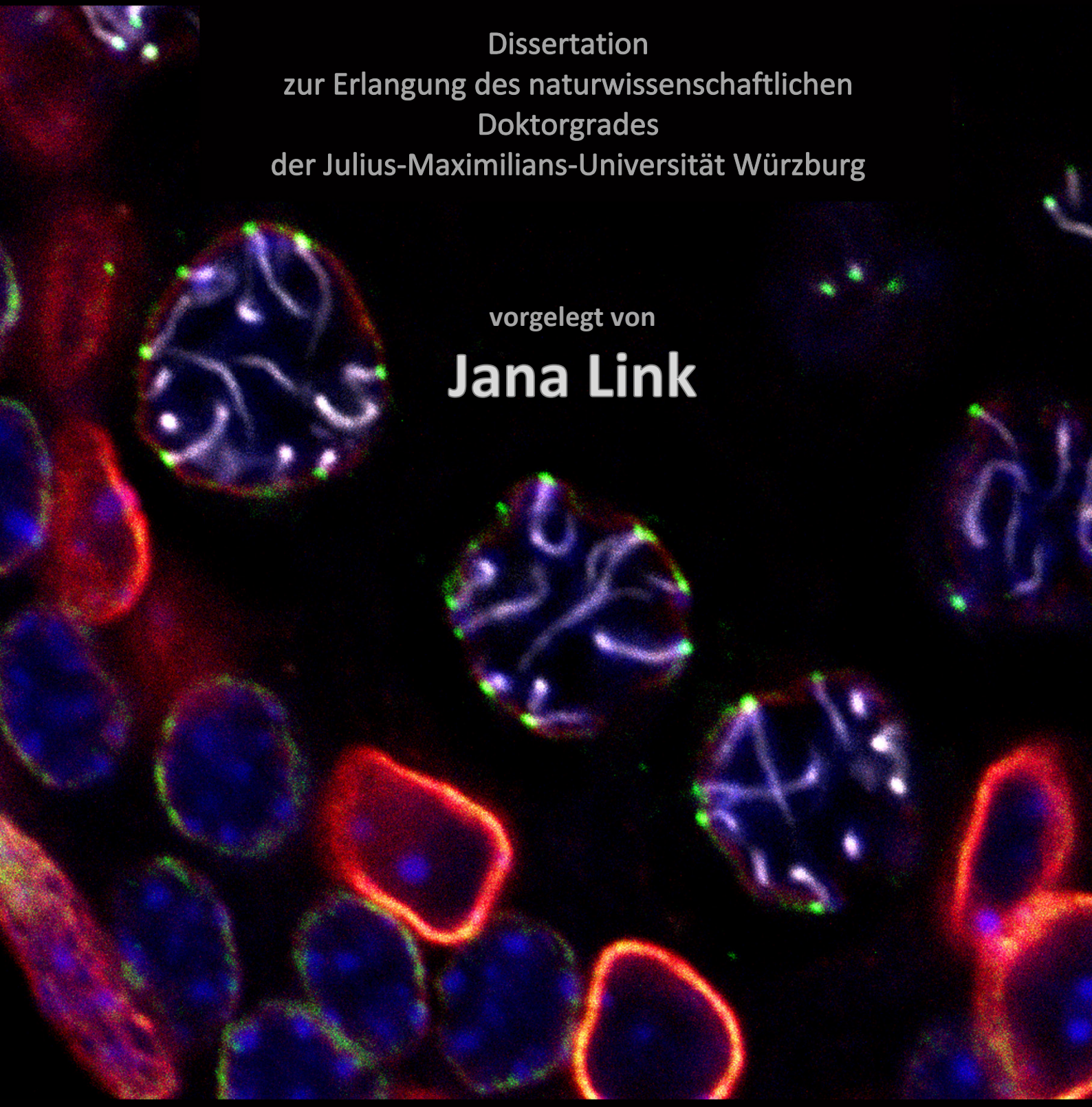

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Illustrated cover page: Immunofluorescence showing Lamin B (red), SYCP3 (white), SUN1 (green) and DNA (blue) in a murine testis cross section including pachytene spermatocytes. By Jana Link.

Summary

Meiosis is the specialised cell division which produces haploid germ cells, capable of developing into fertile gametes, from diploid progenitor cells. During meiosis, chromosomes undergo strictly regulated and strongly conserved dynamic processes, at the beginning of which the telomeres are actively tethered and intimately attached to the nuclear envelope (NE). The attached telomeres are then moved within the NE through cytoskeletal forces to cluster within a restricted region, forming the highly conserved bouquet stage. Subsequently, the bouquet is released simultaneously to the completion of the synaptonemal complex assembly tightly linking homologous chromosome pairs together. In combination these processes are essential for the successful completion of meiosis. Because the meiotic NE serves as a platform for telomere attachment and movement it can be assumed to be critically involved in these events crucial for fertility. However, the precise roles of many meiotic NE proteins in the attachment and movement of telomeres still remain elusive. Therefore, it was the aim of this thesis to investigate the functions of two mammalian meiotic NE components in telomere attachment and dynamics.

The first part of this thesis is concerned with the meiosis-specific lamin C2. Lamin C2 is the only A-type lamin expressed during meiosis and has in previous studies shown to feature altered meiosis-specific properties, clearly distinguishing it from somatic lamins. Because lamin C2 is enriched at sites of telomere attachment, exhibits a high mobility within the nuclear lamina and influences NE integrity, it has been postulated that it may locally increase NE flexibility to allow efficient meiotic telomere movement. Therefore, possible functions of lamin C2 in the movement of attached telomeres were investigated in this thesis by studying the bouquet formation and release of pubertal mice specifically lacking lamin C2. This revealed that lamin C2 deficient mice show a delayed bouquet release, leading to severe defects in the synaptic pairing of homologous chromosomes, which in turn results in infertility of the males. Therefore, the efficient repositioning of attached meiotic telomeres, facilitated by lamin C2, seems essential for completing meiosis.

The second part of this thesis focuses on the protein complex responsible for the attachment of meiotic telomeres to the NE and their coupling to the cytoskeleton. The so-called LINC complex is composed of SUN domain proteins in the inner nuclear membrane interacting with KASH domain proteins of the outer nuclear membrane. In previous studies it had been shown that SUN1, SUN2 and KASH5 localise to the attached meiotic telomeres. Regarding the meiotic role of SUN2, however, contradicting results have recently been discussed, showing the need for further investigations. Using an available SUN1 deficient mouse strain, this thesis was able to show that SUN2 is sufficient for telomere attachment *per se* although telomere attachment is impaired in SUN1 deficient mice leading to infertility. It is also demonstrated that SUN2 forms a functional LINC complex together with KASH5 to mediate this telomere attachment. This LINC complex in the absence of SUN1 is able to move attached telomeres into a bouquet-like cluster formation. Therefore, this demonstrates that SUN2 is involved in the functional attachment and movement of meiotic telomeres.

In summary, this thesis has shown SUN2 and the meiotic nuclear lamina to be directly involved in or essential for the highly conserved attachment and movement of telomeres, making them critical for a successful meiosis. The meiotic NE is therefore in this thesis demonstrated to be a determinant of mammalian fertility.

Zusammenfassung

Die Meiose, eine spezialisierte Zellteilung, produziert aus diploiden Vorläuferzellen haploide Keimzellen, welche sich zu befruchtungsfähigen Gameten entwickeln können. Chromosomen durchlaufen während der Meiose stark regulierte, evolutionär hochkonservierte Bewegungen. Zunächst werden die Telomere aktiv und stabil an der Kernhülle verankert. Angeheftete Telomere werden durch das Cytoskelett entlang der Kernhülle bewegt um sich in einer begrenzten Region anzureichern und das chromosomale Bouquet bilden. Das Bouquet wird durch gerichtete Telomerbewegungen anschließend wieder aufgelöst während die finalen Schritte des Zusammenbaus des Synaptonemalkomplexes stattfinden. Diese Prozesse sind in ihrer Summe essentiell für den erfolgreichen Ablauf der Meiose. Da die meiotische Kernhülle als Plattform für die Anheftung und Bewegung der Telomere dient, kann angenommen werden, dass sie in diese für die Fertilität kritischen Prozesse involviert ist. Die genaue Funktion von Kernhüllenproteinen in der Anheftung und Bewegung meiotischer Telomere ist trotzdem zu großen Teilen noch unverstanden. Deshalb war es Ziel dieser Arbeit zwei Komponenten der meiotische Kernhülle und deren Rolle in Telomeranheftung und –dynamik zu untersuchen.

Der erste Teil dieser Arbeit beschäftigt sich mit dem meiosespezifischen Lamin C2, welches das einzige während der Meiose exprimierte A-typ Lamin ist. Weil Lamin C2 in Bereichen der Telomeranheftung angereichter ist, eine hohe Mobilität in der Kernhülle aufweist und Kernhülleneigenschaften verändern kann, wurde postuliert dass es lokal die Flexibilität der Kernhülle steigern könnte um leichtere Bewegungen der Telomere zu ermöglichen. Demzufolge sollte in dieser Arbeit eine mögliche Rolle von Lamin C2 in der effizienten Bewegung angehefteter Telomere anhand von jungen Lamin C2-defizienten Mäuse untersucht werden. Dies ergab, dass Lamin C2-defiziente Mäuse eine verlangsamte Auflösung des meiotische Bouquets zeigten, was Defekte in der Homologenpaarung verursacht und letztlich zur Infertilität der Männchen führt. Schlussendlich, scheint die effiziente Bewegung angehefteter Telomere, ermöglicht durch Lamin C2, damit essentiell für einen erfolgreichen Ablauf der Meiose.

Der zweite Teil dieser Arbeit ist auf einen Proteinkomplex fokussiert welcher für die Anheftung meiotische Telomere und deren Verbindung zum Cytoskelett verantwortlich ist. Dieser LINC complex besteht aus SUN-domänenproteinen der inneren Kernmembran welche mit KASH-domänenproteinen der äußeren Kernmembran interagieren. Aus früheren Studien ist bekannt, dass SUN1, SUN2 und KASH5 an angehefteten Telomeren lokalisieren. Die meiotische Funktion von SUN2, jedoch, wird aktuell anhand widersprüchlicher Ergebnisse diskutiert. Durch die Verwendung SUN1-defizienter Mäuse konnte diese Arbeit zeigen, dass obwohl die partiell unvollständige Telomeranheftung in der Abwesenheit von SUN1 zu Infertilität führt, SUN2 dennoch für Telomeranheftung an sich ausreichend ist. Um diese Anheftung zu vermitteln, bildet SUN2 einen funktionellen LINC complex mit KASH5 welcher angeheftete Telomere in der Abwesenheit von SUN1 in bouquet-ähnliche Konformationen führt. Demzufolge demonstriert diese Arbeit, dass SUN2 an der funktionellen Telomeranheftung und –bewegung beteiligt ist.

Zusammenfassend hat diese Arbeit gezeigt, dass SUN2 und die meiotische Lamina in die hochkonservierte Anheftung und Bewegung von Telomeren direkt involviert oder für sie essentiell

sind, und somit unabdingbar für eine erfolgreiche Meiose. Damit definiert diese Arbeit die meiotische Kernhülle als eine Determinante für die Fertilität von Säugern.

1. Introduction

The biological significance of any sexually reproducing organism can, in its essence, be reduced to its ability to produce viable and well adapted offspring. Gametes capable of fertilisation, both maternal and paternal, are absolutely indispensable for the formation of a zygote. Therefore, the development of competent gametes could, in a way, be perceived as perhaps the most essential of all developmental processes. A critical, highly complex part of the formation of mature gametes is meiosis. Meiosis, responsible for the reduction of the genome from a diploid to a haploid state prior to the fusion of two gametes, has been observed with fascination and investigated since the late nineteenth and early twentieth century. The historical studies and drawings of scientists like Theodor Boveri and Walter Sutton, studying chromosome reduction and segregation in amphibians, arthropods or lower eukaryotes such as roundworms and flatworms, are still perceived as milestones of science today (Boveri, 1892; Sutton, 1903). However, many of the processes observed over 100 years ago are, until today, not fully understood. Numerous open questions still remain about the mechanisms and molecular players of meiosis but also about the significance and regulation of the prominent chromosomal reorganisation during meiosis including the attachment and movement of telomeres within the NE. The extraordinary conservation of many of these meiotic aspects throughout the animal kingdom, however, today allows the usage of a number of model organisms suited for a broad diversity of scientific aims and problems. The mouse, being a mammal, has shown to be a well suited model organism for a number of scientific questions, especially those related to human health and fertility.

The development of mature and mobile gametes in male mice, as a whole referred to as spermatogenesis, may further be divided into the meiotic phase and the later post-meiotic stage. Meiosis is preceded by subsequent rounds of mitotic division of the spermatogonia, the germ cell progenitor stem cells. The meiotic phase, including both the first and second meiotic division, is then followed by the post-meiotic development of the spermatids into mature sperm, referred to as spermiogenesis. Because an efficient and correct meiotic division is the basis for the development of competent gametes in many sexually reproducing organisms, it has remained an important challenge until today to continuously deepen and broaden our understanding of the basic mechanisms and regulators of meiosis.

1.1 Meiosis – a special type of cell division essential for gametogenesis

Meiosis is a specialised form of cell division, reducing the prior diploid genome to a haploid one. However, because DNA is replicated before meiosis, two subsequent division are needed to reduce the genome from its replicated diploid sate (2n 4c) to the haploid state (1n 1c). In the first of these divisions, meiosis I, homologous chromosomes are separated from each other, creating two haploid daughter cells (1n 2c). This first reductional division is then followed by meiosis II, where sister chromatids are separated from each other, resulting in the final haploid gamete (1n 1c).

Meiotic divisions can be subdivided into several stages. Meiosis I consists of a prophase I, the longest and most complex phase, followed by metaphase I, anaphase I and telophase I, leading into meiosis II. Some of the most important and fascinating processes in the development of gametes take place during prophase I. These involve the pairing and synapsis of homologous chromosomes and meiotic recombination between non-sister chromatids as well as simultaneous large scale chromosome reorganisations including conserved telomere-led chromosome movements. All of these closely interdependent events are together essential prerequisites to achieve the finite goal of meiosis: to produce gametes competent for fertilisation.

Prophase I (see Figure 1.1) is further subdivided into distinguished phases. It starts after the transition of stem cell derived gametogonia into meiocytes with the leptotene stage. At the beginning of leptotene, DNA double strand breaks (DSBs) are introduced into the genome as a preparatory mechanism for the homologous pairing and meiotic recombination later during prophase I (Sun et al., 1989). As leptotene proceeds, chromosomes start to condense and telomeres are tethered to the nuclear envelope (NE) and stably connected to it (Page and Hawley, 2003; Scherthan, 2007). Simultaneous chromosome reorganisation leads to the alignment of homologous chromosomes and the axial elements of the synaptonemal complex (SC) begin to form. The SC is a proteinaceous structure, which, when it is completely assembled, stably connects homologous chromosomes to each other. The axial elements of chromosomes at this stage build the structural backbone for the not yet paired homologs. The attached telomeres are, as part of the chromosome reorganisation, actively moved along the NE, clustering within a restricted area to form the so-called meiotic bouquet at the transition into zygotene (Zickler and Kleckner, 1998). These chromosome movements lead to the complete alignment of homologous chromosomes by the zygotene stage, the second stage of prophase I. As zygotene proceeds, the axial elements assemble completely and first structural connections between homologous chromosomes are made by transverse filaments linking short stretches of axial elements with each other (Page and Hawley, 2003). Axial elements, once they are connected to each other, are from then on called lateral elements. Regarding meiotic recombination, the induced DSBs are now precisely processed by DSB repair mechanisms. When all pairs of homologs have been tightly connected with each other through transverse filaments, the SC has assembled completely, marking the pachytene stage of prophase I. This stage, in which all pairs of homologs are fully synapsed, is the longest of prophase I. The sex chromosomes X and Y of male mammals build an exception during the synapsis formation in pachytene, as these only synapse in a short homologous region, the pseudo-autosomal region, leaving the rest of both X and Y unpaired (Kauppi et al., 2012). The intimate connection between homologs during pachytene allows recombination events to take place in order to exchange maternal and paternal genetic material

between homologous chromosomes. The stable connection between homologous chromosomes is also critical for the correct alignment during metaphase and the attachment of the meiotic spindle. Furthermore, the formerly clustered telomeres are actively dispersed over the NE during pachytene, releasing the meiotic bouquet. Towards the end of pachytene, the synapsis starts to disassemble at the transition into diplotene, the fourth stage of prophase I (Page and Hawley, 2004). During diplotene, telomeres also start to detach from the NE. At the end of diplotene, loci along the chromosomes where recombination has led to crossing over, the so-called chiasmata, are the only regions where homologous chromosomes are still tightly connected to each other. Elsewhere along the chromosomes, the transverse filaments of the SC are dissolved. Metaphase I, where the homologous chromosomes are aligned within the cell, follows diplotene and marks the end of prophase I. The correct and unambiguous pairing of the homologous pairs during pachytene and their stable connection at chiasmata during diplotene are essential for the proper attachment of the spindle to the kinetochores and therefore the correct alignment of the homologous pairs at metaphase I. The bipolar spindle attaches to the kinetochores of the paired homologs such that homologous pairs are pulled apart from each other during anaphase. The faithful separation of homologs is absolutely critical for the equal separation of the chromosomes into two haploid daughter cells.

Telomere tethering & movement – a conserved feature of leptotene & zygotene

Telomeres are actively tethered to the NE at the onset of meiosis. These attached telomeres are laterally moved within the NE in the course of chromosome rearrangements during prophase I. The telomere-led chromosome movements observed during prophase I lead to the active formation and release of the highly conserved bouquet stage (see Figure 1.1). Most meiotic model organism, with only very few exceptions, exhibit this distinguished clustering of telomeres in the earlier stages of prophase I, when telomeres are commonly orientated towards the centrosome or spindle pole body (SPB) (Scherthan, 2001). The tightest clustering of telomeres is usually observed at the leptotene/zygotene transition, after which telomeres are actively dispersed again. Although some clustering may still be observed during early pachytene, by the end of pachytene when homologous chromosomes are fully paired, telomeres are dispersed evenly over the NE again. At the end of diplotene, the telomeres are finally detached from the NE.

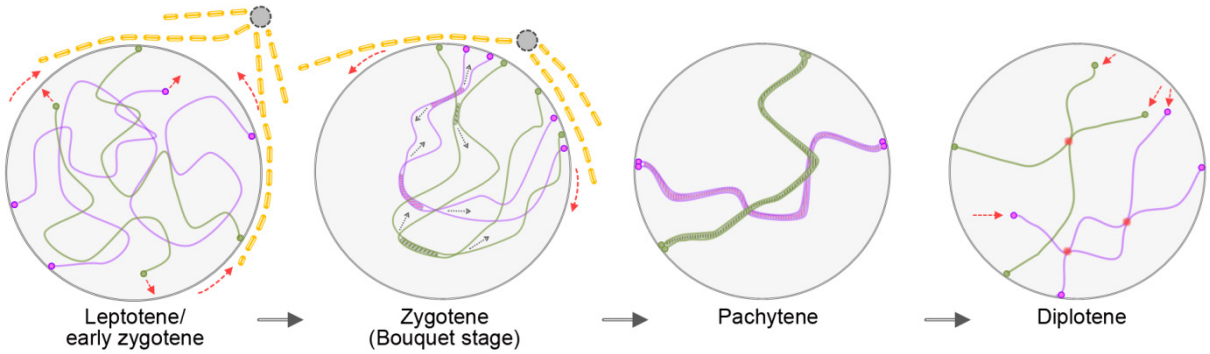

Figure 1.1: Prophase I of meiosis in mice. During **leptotene** and into **early zygotene,** the later axes of homologous chromosomes (green and purple) start to assemble as the chromatin starts to condense. Telomeres are actively tethered to the NE, and once stably attached to it, moved along the NE (red arrows). The movement in mice is most likely mediated by cytoplasmic microtubules (in yellow) organised by the centrosome (in grey). At the transition to **zygotene** and during zygotene the telomeres are clustered in a confined region of the NE, forming the characteristic bouquet. The bouquet is probably oriented towards to centrosome. Simultaneously, the transversal filament assembly is initiated at several sites and the synapsis proceeds along the chromosomes, zipping them together (grey arrows). Towards the transition from zygotene into pachytene, telomeres are actively dispersed within the NE again (red arrows), releasing the meiotic bouquet. At **pachytene**, full synapsis of the homologous chromosomes is reached and telomeres are evenly distributed across the NE. The full synapsis during pachytene allows recombination events to take place. At **diplotene**, the intimate association of homologous chromosomes is released again as the SC disassembles. The homologs are only still connected at the chiasmata, the sites of crossing-over (in red). Telomeres detach from the NE at the end of prophase I (red arrows). Figure adapted from Kracklauer et al., 2012.

Demonstrated by the extraordinary evolutionary conservation of the bouquet stage, meiotic telomere attachment and polarization within the NE must have continuously been selected for throughout evolution. However, the significance and actual role of the bouquet stage still remains somewhat elusive. Historically, the bouquet was commonly compared to the, visually similar, somatic Rabl configuration. This chromosomal arrangement where centrosomes of somatic chromosomes are clustered together, leading to the telomeres passively clustering by “hanging freely” from the centromeres in a U-shaped arrangement, can be observed in numerous cell types of diverse organisms. However in recent years it has become increasingly evident that the bouquet configuration of meiotic chromosomes is a functional, actively formed, highly regulated and *de novo* structure at the onset of meiosis, rather than an evolutionary relict derived from the Rabl configuration (Zickler and Kleckner, 1998; Harper et al., 2004).

The movement of attached telomeres into and out of the bouquet is an active process for which the NE serves as platform. Generally, the telomere movements are directed by forces generated by components of the cytoskeleton. These are mediated to the attached telomeres via an extremely conserved membrane protein complex, the so-called LINC complex (linker of nucleoskeleton and cytoskeleton) (Crisp et al., 2006). In any model organism investigated so far, the components of the cytoskeleton interact with the outer nuclear membrane (ONM) component of the LINC complex, the KASH domain proteins. These interact with SUN domain proteins in the inner nuclear membrane (INM), which attach the telomeres to the NE. This protein complex is therefore essential for the attachment as well as movement of the telomeres within the NE, leading to the meiotic bouquet.

Regarding the function of the meiotic bouquet, and thus the reason for its strong conservation, a number of scenarios are currently discussed. The formation of the bouquet coincides with the

alignment of homologous chromosomes as well as with the induction of the synapsis between them. Also many processes relevant for DSB repair and recombination occur within the same time frame as the meiotic bouquet. Because the attached telomeres are moved into close proximity during the formation of the bouquet, it may serve to enhance the alignment, pairing or synapsis between homologous chromosomes (Scherthan et al., 1996). Furthermore, the restricted space of the clustered chromatin within the nucleus may also increase the efficiency of protein recruitment for synapsis elongation, DSB repair and recombination processes (Moens et al., 1998). Finally, because the resolution of the meiotic bouquet coincides with the completed synapsis of homologous chromosomes during pachytene, it may also be that the bouquet resolution is important for the resolution of non-homologous and therefore weaker connections between heterologous chromosomes. In this way, the meiotic bouquet would be a mechanism to prevent defects in homologous pairing (Zickler and Kleckner, 1998). Such defects in homologous pairing would result in defective separations of homologs at metaphase or even in apoptosis prior to this.

Most of the studies investigating the role of the meiotic bouquet are conducted in yeast, although some studies have also been undertaken in mice. The majority of the studies indicate that the bouquet is indeed relevant for the efficient progression of meiosis and especially for the correct pairing of homologous chromosomes (Niwa et al., 2000; Chikashige et al., 2007; Liebe et al., 2004). Defects in bouquet formation or its absence affect synapsis formation and DSB repair efficiency. Also heterologous associations and other pairing defects seem to increase when bouquet stage formation is impaired. However, interdependencies with DSB repair and recombination events often make clear conclusions regarding the significance of the bouquet from the studies rather difficult, especially in mammals.

Although telomere attachment and bouquet conformation are highly conserved across sexually reproducing organisms, some variations in structure as well as in spatial and timely extent between species are observed. The bouquet conformation is, depending on the species, confined to a smaller or larger region of the NE and may be very transient or more extended throughout prophase I (Bass, 2003). For instance, the fission yeast *S.pombe* exhibits a very tight bouquet with all telomeres attached to the SPB. Additionally, this bouquet stage is maintained throughout prophase I, whilst the entire nucleus performs back-and-forth motion within the cell, the so-called horsetail movements. In mice, the clustering of telomeres is less tight, being only restricted to one hemisphere of the nucleus. This rather transient clustering is most prominent at the leptotene/zygotene transition but may also to some extent last into early pachytene (Liebe et al., 2006). Similarly, the extremely well conserved general mechanism of meiotic telomere attachment via the LINC complex also shows some variation between different species. Through KASH domain proteins, which are able to interact with different components of the cytoskeleton, different species have evolved diverse strategies to move the attached telomeres. In the budding yeast *S.cerevisiae*, for example, it has been demonstrated, that cytoplasmic actin cables tightly surrounding the nucleus are responsible for the active movement of attached meiotic telomeres (Koszul et al., 2008), whereas in mice the attached telomeres are coupled to the microtubule network instead (Morimoto et al., 2012).

Synapsis formation & recombination – interdependent processes of zygotene & pachytene

Simultaneously to bouquet formation in early prophase I, preparations for synapsis formation and recombination are already underway (see Figure 1.2). The very beginning involves the introduction of DSBs into the genome of early prophase I meiocytes by a meiosis-specific endonuclease, Spo11. Homologs of this protein are found in all common meiotic model organisms such as mice, *S.cerevisiea*, *S.pombe* or *C.elegans* (Romanienko and Camerini-Otero, 1999; Romanienko and Camerini-Otero, 2000; Lin and Smith, 1994; Keeney et al., 1997; Dernburg et al., 1998). In most of these, the induction of DSBs into the meiotic genome is an essential prerequisite for the correct formation of a stable synapsis between homologs chromosomes. This is particularly well demonstrated for mice, which are unable to form a complete SC in the absence of Spo11, resulting in infertility of both males and females (Romanienko and Camerini-Otero, 1999; Romanienko and Camerini-Otero, 2000).

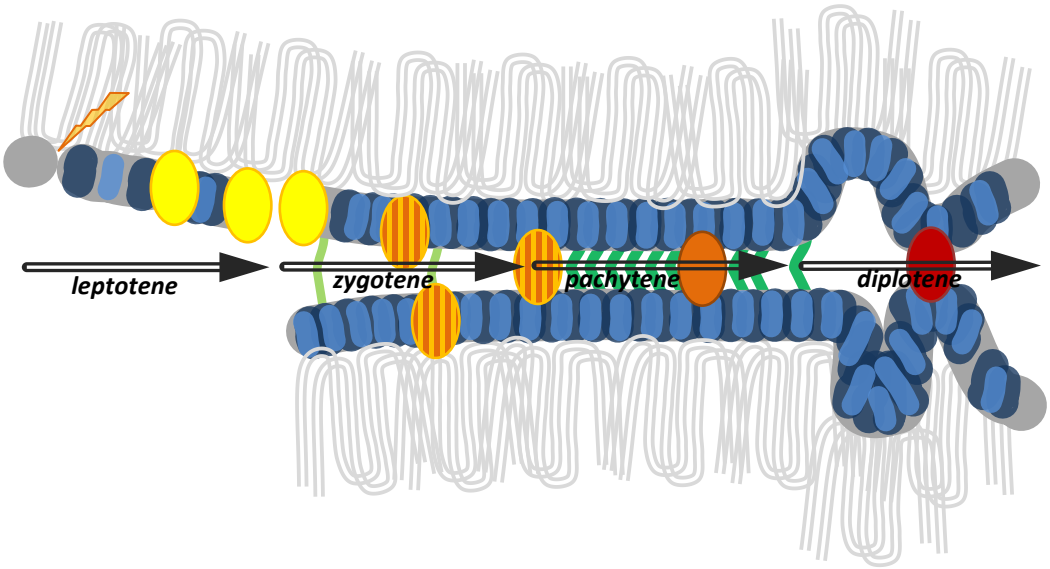

Figure 1.2: Progression of SC assembly and recombination in prophase I. The induction of DSBs into the genome (yellow bolt) at the beginning or even before **leptotene** is the earliest event in SC assembly and recombination. During leptotene, the axial elements, mainly composed of SYCP2 and SYCP3 (dark and light blue ovals), are starting to assemble. Simultaneously, homologous chromosomes are aligning with each other and early recombination nodules (yellow ovals), containing replication and DNA repair proteins, are formed numerously along the axes. During **zygotene**, first associations (light green lines) are established between the aligned axial elements. Early recombination nodules are reduced in number and change in their protein composition, developing into transition nodules (orange, yellow striped ovals). **Pachytene** is reached when the transversal filaments (dark green lines), mainly composed of SYCP1, are established between the now-called lateral elements. The SC is now fully matured. Transition nodules are further reduced in number and changed in proteins composition, becoming late recombination nodules (orange ovals) and marking the future sites of crossing-over. At **diplotene**, the SC starts to disassemble. Homologous chromosomes are not linked to each other anymore with exception of the actual sites of crossing-over, the chiasmata (red ovals).

The successive repair of the induced DSBs is a highly regulated mechanism which runs in parallel to SC assembly and axis formation. The axial elements, building the structural backbone of the SC, are mainly composed of SYCP3 and SYCP2 in mammals and are formed during leptotene and

zygotene. The scaffold of the axial elements is furthermore composed of the so-called cohesin core, constituted of a number of cohesin proteins (Cohen et al., 2006; Fraune et al., 2012). A number of studies using specific knockout mouse models of SC components have shown SYCP3 to be the most prominent component of the axial elements. Mice lacking SYCP3 are unable to form a functional and complete SC, leading to infertility of the male mice (Yuan et al., 2000). Furthermore, SYCP3 is responsible for recruiting SYCP2 to the axial elements, yet SYCP3 localises to the axial elements independently of SYCP2. Additionally, the proper assembly of the axial element, including SYCP3 and SYCP2, is essential for the efficient formation of the cohesin core (Pelttari et al., 2001). Yet it has also been shown that the axial elements assemble efficiently and functionally only if the cohesion core is also formed completely (Revenkova et al., 2004). Therefore, these early stages of SC assembly are strongly interdependent of each other and at the same time prerequisites for the maturation of the synapsis.

Simultaneously to the elongation of the axial elements and the cohesin core, DSB repair mechanisms progress to form so-called early recombination nodules, closely associated with the axial elements. These nodules, in mice over 200 per cell, are characteristically composed of the meiotic RecA-homolog RAD51 and DMC1 and indicate sites of strand invasion of the aligned, although not yet paired, homologs as part of the DNA damage repair pathway (Ashley et al., 1995; Baarends and Grootegoed, 2003; Pawlowski and Cande, 2005). As the DSB repair progresses, a large fraction of these early nodules, around 200 in murine meiocytes, mature into transition nodules. The transition nodules are now located between the axial elements of aligned homologous chromosomes, mediating their first structural contact. This initial contact is further supported by axial associations, which form first filamentous connections between axial elements of the aligned homologs. Transition nodules are characterised by proteins such as RPA, BLM, MSH4 and MSH5 and are observed during zygotene, thus temporally coinciding with the initiation of synapsis (Plug et al., 1997; Plug et al., 1998; Fraune et al., 2012). In mammals the main structural component of the synapsis forming between the axial elements, from now onwards called lateral elements, is SYCP1. SYCP1 initiates the formation of the ladder like central region of the SC by connecting both lateral elements of the homolog pair and interacting with other proteins of the central region such as SYCE1 and SYCE3. Again, these processes of DSB procession and synapsis initiation have shown to be tightly interconnected and interdependent of each other and with other events during prophase I. Regarding mammalian meiosis, it has become evident, that the localisation of RAD51 and DMC1 as well as the formation of transition nodules are important for the efficient formation of synapsis and homolog recognition (Pawlowski and Cande, 2005; Fraune et al., 2012). However, the timely and correct progression of early recombination nodules into transition nodules and later into recombination events is also dependent on the formation of an intact synapsis between the homologous chromosomes. The synapsis initiation itself is strongly dependent on SYCP1, as mice deficient for SYCP1 are unable to initiate and form a stable synapsis (de Vries et al., 2005). This initiation and formation of the synapsis is in turn dependent on the efficient formation of the axial or lateral elements as studies have shown that SYCP1 can only assemble the central element of the SC correctly by loading onto the previously formed axial elements (Yuan et al., 2000). Furthermore, SYCP1 is also responsible for the recruitment of other central element proteins necessary for synapsis initiation, such as SYCE1 and SYCE3 (Costa et al., 2005; Schramm et al., 2011). These

examples illustrate that the early stages of homologous recombination and intermediate stages of synapsis formation are, once again, strongly interconnected and simultaneously dependent on earlier events of leptotene and zygotene.

Once synapsis formation has been initiated between the lateral elements of homologous chromosomes, it spreads rather quickly along the entire axis of the homolog pair, zipping them intimately together. In mice, this elongation of the SC is mediated by proteins of the central element such as SYCE2 and TEX12, which are recruited to the existing complex of SYCP1, SYCE1 and SYCE3 (Fraune et al., 2012). By pachytene, all homologous chromosomes have developed a mature SC, stably connecting the homologous chromosomes to each other. Regarding the progression of meiotic recombination, within the same timeframe transition nodules are severely reduced in number and transformed into recombination nodules by specifically recruiting MLH1 (Baker et al., 1996). The recombination nodules are associated with the central element of the SC and most likely mark the sites of actual crossing over events. The maturation of transition nodules into recombination nodules, and thus the completion of meiotic recombination, is only possible if the SC is assembled completely and fully functionally, at least regarding the male meiosis. Since this is not the case in most knockout mouse models of meiotic recombination or SC proteins, the majority of these lack MLH1 foci on chromosomes axis or elicit reduced foci numbers even if they proceed to a pachytene or pachytene-like stage of prophase I (Schramm et al., 2011; de Vries et al., 2005; Revenkova et al., 2004). Since the formation of the SC is dependent on the assembly of the axial elements as well as on the initiation and elongation of the central element, meiotic recombination is dependent on any of these processes. However, to some extent, the converse is true as well. The induction and progression of meiotic recombination and especially DSB repair is also a prerequisite for the efficient initiation and assembly of the SC (Pawlowski and Cande, 2005). Similar interdependencies are also described in other meiotic model organisms, mainly in yeast, indicating that although single proteins involved in these processes differ considerably between species, once again major mechanisms seem to be conserved well throughout evolution. As has become evident from these examples, it is quite true to say that defects in homologous pairing and synapsis formation or in homologous recombination consequently lead to defects of each other, highlighting the intimate interplay between these processes and the progression of prophase I in general.

In mice, this complex interplay between synapsis formation, homologous recombination and meiotic progression is further complicated by the fact, that there seem to be substantial differences in this regulation between males and females. In general, male meiotic progression is more stringently controlled leading to frequent apoptosis of spermatocytes carrying defects. Apoptosis is commonly induced at different stages of prophase I, usually during pachytene at the latest, depending on the severity of the meiotic defects. In females however, meiosis often progresses much further or is even completed despite the presence of meiotic defects. This sexual dimorphism is observed in a number of meiotic mouse models, ranging from targeted SC proteins to NE components (Yuan et al., 2000; Link et al., 2013a; Liu et al., 1998; Herran et al., 2011).

Defects in meiotic progression – causes for infertility

All of the processes described above, DSB induction, telomere-led chromosome movements, DSB repair, homologous recombination, synapsis initiation and elongation, are together essential for the correct and complete progression of meiosis (Page and Hawley, 2004). Because of the strong interdependency of all of these events on one another, defects in virtually any of these result in further defects downstream in prophase I (Cohen et al., 2006; Liebe et al., 2004). The severest consequence of any meiotic defect is the induction of apoptosis during or after prophase I, which is often observed in both males and females of meiotic knockout mouse models. By inducing apoptosis in all meiocytes, a mechanism to prevent defective sperm and oocytes from maturing, animals result in being infertile. A perhaps less severe, yet no less common, consequence of meiotic defects is the formation of chromosome nondisjunction, usually occurring during metaphase I. Chromosome nondisjunction is mostly a consequence of incorrect or incomplete homologous recombination, more often observed in females than males (Hunt and Hassold, 2002). Chromosomes can only segregate correctly at metaphase I if each pair of homologs has at least one chiasma. Chiasmata, the sites of gene conversion resulted in crossing-over, are the last points at which homologous chromosomes are tightly connected to each other once the SC has disassembled at and after diplotene. If a pair of chromosomes lacks its only chiasma, meiotic spindles cannot attach to the kinetochores properly, resulting in an uneven dispersal of chromosomes causing aneuploidy in the daughter gamete progenitor cells (Hunt and Hassold, 2002).

In human females, an estimated one of four oocytes exhibit an error in meiotic progression. Furthermore, 35% of all spontaneous pregnancy aborts is predicted to be caused by aneuploidy of the oocyte (Page and Hawley, 2003). This highlights the importance to understand meiotic progression in more detail and especially the connections, regulations and mechanisms of recombination, chromosome dynamics and synapsis formation during mammalian meiosis.

1.2 The nuclear lamina – a versatile component of the nuclear envelope

As discussed in the context of meiotic telomere-led chromosome movements, the NE is not only a passive barrier between the cytoplasm and the nucleus. Rather, the NE including all its components is a highly structured, active, regulatory platform. Generally, the NE is structurally composed of two membranes: the inner nuclear membrane (INM) facing the nucleoplasm and the outer nuclear membrane (ONM) facing the cytoplasm. Both membrane systems are structurally and functionally distinct from each other, forming a lumen of approximately 30-50 nm between them, the perinuclear space (PNS) (Liu et al., 2007). The ONM is continuous with the membrane of the endoplasmic reticulum (ER), therefore linking the lumen of the ER with the PNS. The INM and ONM only meet at sites where nuclear pore complexes (NPCs) are inserted into the NE. NPCs are integrated into both membrane systems forming a channel connecting the cytoplasm with the nucleoplasm. These NPCs are essential components of the NE for the active and passive shuttling of proteins, RNA and other molecules between the nucleus and the cytoplasm.

The third structural component of any metazoan NE, besides the INM and ONM, is the nuclear lamina. The nuclear lamina is a dense protein meshwork of 20-50 nm thickness on the nucleoplasmic face of the INM (Gerace et al., 1978; Liu et al., 2007). It is an essential component of the NE which interacts intimately with membrane proteins of the INM as well as with chromatin and proteins inside the nucleus, creating an interactive network within the nucleus. The nuclear lamina is well known for its manifold roles in nuclear organisation, structure and regulation through which it contributes tremendously to the regulatory functions of the NE.

Structure & composition of the lamina – an assembly mediated by functional domains of the lamin proteins

The nuclear lamina is mainly composed of lamin proteins. Lamins are intermediate filament type V proteins, localised exclusively within the nucleus and are conserved throughout metazoans. In vertebrates lamins are differentiated into A- and B-type lamins depending on biochemical, structural and functional features. A-type lamins are characterised by a neutral isoelectric point, their dissociation from the lamina during mitotic divisions and their tissue specific expression patterns. A-type lamin expression is developmentally regulated and in general mostly observed in differentiated cells (Stuurman et al., 1998; Gruenbaum et al., 2005; Benavente et al., 1985; Lehner et al., 1987). A-type lamins are not expressed in undifferentiated stem cells and are detectable in mouse embryonic tissue only from 9 days post fertilisation onwards (Rober et al., 1989; Schatten et al., 1985; Stewart and Burke, 1987; Constantinescu et al., 2006). Besides the expression in somatic cells, A-type lamins are furthermore present in cells undergoing gametogenesis (Schatten et al., 1985). In mammals, four different A-type lamin isoforms are expressed, all encoded by a single gene, the *LMNA* gene (Fisher et al., 1986; McKeon et al., 1986; Stuurman et al., 1998) (see Figure 1.3). Lamin A and C are the main somatic *LMNA* gene products and only differ at the C-terminus from each other. Lamin A is encoded by exons 1 through 12 of the *LMNA* gene locus, whereas lamin C is only encoded by exons 1 through 10, resulting in a shorter C-terminal domain (Nakajima and Abe, 1995). The C-terminus of lamin A, but not of lamin C, includes the conserved CAAX – sequence also found in B-type lamins (Fisher et al.,

1986). A third somatic A-type lamin is laminAΔ10, which lacks part of the coding region of exon 10 of the *LMNA* gene and was found to be expressed in a variety of different tissues and cell types (Machiels et al., 1996). Finally, a meiosis-specific *LMNA* splice variant, lamin C2, is found in male and female meiotic germ cells. It is encoded by an alternative starting exon of the *LMNA* gene (exon 1a), which modulates its N-terminal domain. Through this alternative starting exon, lamin C2 exhibits a unique hexapeptide sequence (GNAEGR) in its N-terminus (Alsheimer et al., 2000). Furthermore, lamin C2 shows the same shortened C-terminus as lamin C, missing the above mentioned CAAX-sequence found in other lamin proteins (Furukawa et al., 1994; Nakajima and Abe, 1995).

B-type lamins, on the contrary, exhibit an acidic isoelectric point, remain attached to NE membrane during mitosis and are more broadly expressed during development and differentiation (Stuurman et al., 1998; Gruenbaum et al., 2005). The expression of at least one B-type lamin is furthermore essential for cell viability (Hutchison, 2002). In mammals three B-type lamin isoforms are expressed by two different genes. Lamin B1 is expressed from the *LMNB1* locus, whereas lamin B2 and the germline-specific variant B3 are both encoded by the *LMNB2* gene (Hoger et al., 1988; Zewe et al., 1991; Furukawa and Hotta, 1993). Lamin B3 is, compared to its somatic counterpart B2, modulated by a unique shortened N-terminal domain.

Generally, lamin proteins all share a conserved protein domain structure typical for intermediate filament proteins. This tripartite structure is characterised by a central α-helical rod domain, flanked by a non-helical N-terminal 'head'-domain as well as a non-helical C-terminal 'tail'-domain (Figure 1.3). Both, the central rod-domain as well as the non-helical domains of lamins, play critical roles for lamina function and structure. The α-helical rod domain, subdivided into four helical segments, is able to form two-stranded α-helical coiled-coils (Stuurman et al., 1998). This makes it essential for the parallel dimerization of two lamin proteins, which is the critical first step of lamina assembly (Herrmann and Aebi, 2004). The highly conserved ends of the central rod domain, both N- and C-terminally, serve further essential functions in the polymerisation of higher ordered lamina structures. The lamin dimers associate longitudinally into long head-to-tail polymers, constructing polar protofilaments. These in turn assemble laterally and in an anti-parallel fashion into lamin filaments which are the basis for the complex lamin network. Both the longitudinal association into protofilaments and the lateral assembly into filaments are mediated by the ends of the central rod domain described above (Heitlinger et al., 1992; Stuurman et al., 1996).

The C-terminal non-helical 'tail'-domain of lamins exhibits several features essential for lamin function. A strongly conserved component of this C-terminal domain is an immunoglobulin-like structural motif (Ig-fold), which has been demonstrated to be involved in interactions with numerous lamin-binding proteins (Krimm et al., 2002). The nuclear localisation signal (NLS), responsible for the exclusively nuclear localisation of lamins, is also located within the C-terminal domain, in between the Ig-fold and the central rod. Finally, a so-called CAAX- sequence, essential for the posttranslational modifications, is located at the very C-terminal end of all lamins except for lamin C and C2 (Dechat et al., 2010).

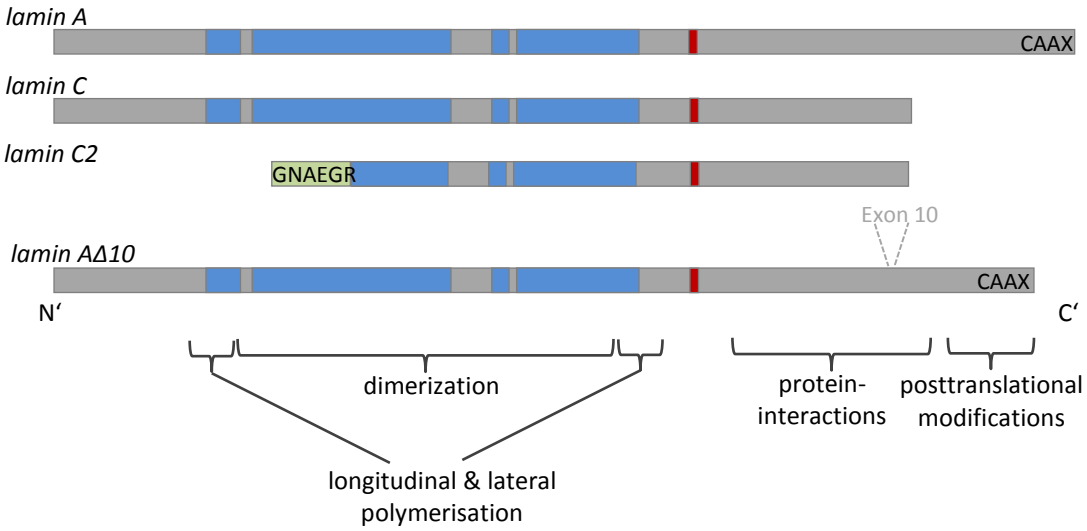

Figure 1.3: Domain structure and function of mammalian A-type lamins. The central α-helical rod domain (coiled-coils depicted in blue) of all A-type lamins consist of four helical segments. Only lamin C2 is an exception to this, as the alternative starting exon encodes for the unique hexapeptide GNAEGR (light green box), substituting the N-terminal end of the central rod domain. The N-terminal and C-terminal ends of the central rod domain are essential for higher order lamin polymerisation, whereas the central region of the rod domain is essential for dimerization of lamin proteins. The C-terminal region of all A-type lamins contains a NLS (red box) followed by the Ig-fold, which is critical for numerous interactions with lamin binding proteins. Lamin A and AΔ10 both have the highly-conserved CAAX-box at their C-terminal end, serving as a target and regulatory element of the posttranslational modifications of A-type lamins. The shortened C-term of lamin C and C2 do not contain a CAAX-box.

The CAAX-sequence is a highly conserved series of amino acids (cysteine-aliphatic residue-aliphatic residue-random residue) at the very C-terminal end of A- and B-type lamins, except for lamin C and C2 (Burke and Stewart, 2006). The first posttranslational modification targeted at the CAAX-sequence is the farnesylation of the cysteine. This is followed by the proteolytic cleavage of –AAX by the metalloproteinase Zmptse24 (Leung et al., 2001; Bergo et al., 2002; Pendas et al., 2002). The cysteine is methylated after this cleavage, resulting in increased hydrophobicity of the lamin proteins, facilitating their association with the INM and their assembly into the lamina (Holtz et al., 1989). Once the farnesylated lamin A is incorporated into the lamina, it is further modified by again being targeted by Zmspte24. This cleavage, cleaving off another 15 amino acids including the farnesylated cysteine, finally results in the mature lamin A (Sinensky et al., 1994; Burke and Stewart, 2006). The posttranslational modifications of lamins encoding the CAAX-box are essential for lamina function as they are required for their integration into the nuclear lamina. Lamin C can only be incorporated into the lamina in the presence of the modified lamin A, because it is missing the CAAX box and its modifications needed for the association into the lamina (Horton et al., 1992).

General functions of the lamina – roles beyond structure & scaffold

The nuclear lamina serves a wide variety of functions in nuclear organisation and maintenance through its huge diversity of protein and chromatin interactions within the nucleus. Initially, the lamina was described to mainly fulfil mechanical functions in maintaining nuclear integrity. It has been demonstrated in vivo and in vitro that the nuclear lamina is responsible for regulating nuclear

shape and size (Goldberg et al., 1995; Sullivan et al., 1999). In mice, A-type lamins especially are important for the maintenance of a regular nuclear shape and mechanical stiffness of the NE (Sullivan et al., 1999). These mechanical functions of the nuclear lamina have recently been extended by the discovery of the interaction of lamins with components of the LINC complex (Haque et al., 2006; Lee et al., 2002). Through the connection to the LINC complex, and thus indirectly to the cytoskeleton, the lamina seems to regulate NE stability on multiple levels and be involved in nuclear positioning (Mattioli et al., 2011; Starr, 2007). However, besides these structural responsibilities, the lamina also serves a multitude of other functions, which are equally as important but seemingly more complex to understand.

Lamins are able to interact direct and indirectly with chromatin through multiple chromatin binding domains and interactions with core histones (Hoger et al., 1991; Luderus et al., 1992; Stierle et al., 2003; Glass et al., 1993; Taniura et al., 1995). Generally, heterochromatin tends to located at the nuclear periphery, probably in intimate contact with the nuclear lamina. Because the lamina has been shown to be essential for the correct localisation of heterochromatin to the nuclear periphery, probably through its chromatin interactions, it is directly involved in chromatin organisation (Filesi et al., 2005; Goldman et al., 2004). If these lamina-chromatin interactions are disturbed, this may lead to the mislocalisation of heterochromatin which is often associated with a misregulation of gene silencing. Gene regions within the heterochromatin territories are usually silent or weakly transcribed genes, which through their relocation into the nuclear interior may escape silencing mechanisms, leading to an overexpression or general deregulation (Capell and Collins, 2006).

Lamins also interact directly with components of signalling pathways involved in gene regulation (Andres and Gonzalez, 2009; Dauer and Worman, 2009). For example, lamins directly bind MAN1, a member of the highly conserved LEM-domain family, which interacts with components of the SMAD-pathway. This pathway is well-known for its regulation of gene expression through members of the TGF-ß family. Through the SMAD-pathway, lamins are likely to influence cell differentiation processes by gene expression regulation (Gruenbaum et al., 2005). Furthermore, lamins A and C also regulates DNA replication by interacting directly with PCNA of the DNA-replication complex (Shumaker et al., 2008). Similarly, lamins have been shown to interact with RNA-polymerase complexes as well as DNA-repair complexes, probably illustrating only a few of the multiple possibilities of the lamina to influence gene regulation.

The understanding of the functions of the nuclear lamina has now evolved from a prior mainly structural role to a complex protein network, which influences many cellular functions either directly or indirectly through a variety of protein-protein interactions. Many of these functions have become evident through pathological consequences of lamina malfunctions. A collection of diseases is associated with defects in lamina function and structure; the laminopathies. Laminopathies describe a collection of syndromes affecting a variety of tissues and organs. Symptoms differ extremely in severity and extent between affected individuals, even of the same laminopathy. Most laminopathies are associated with mutations within the *LMNA* gene, even though a clear correlation of mutations with symptoms is often difficult due to the broad spectrum of phenotypes (Burke and Stewart, 2006; Capell and Collins, 2006; Worman et al., 2010). Although lamins are expressed in any cell type, only a restricted set of tissues is usually affected by these mutations within lamins. Myopathies, affecting

skeletal or heart muscle, are frequently observed in laminopathy patients with a mutation within the central rod domain of lamin A/C. The autosomal-dominant form of Emery-Dreifuss Muscular Distrophy is one example of a laminopathy affecting skeletal and heart muscle tissue, leading to severe muscle wasting and cardiomyopathies (Bonne et al., 1999). Along with muscle tissue, nerves and fat tissue are also often affected by laminopathies. A prominent lipodystrophy is the Dunnigan-type familial partial lipodystrophy, caused by mutations within exon 8 and 11 of the *LMNA* gene (Hegele et al., 2000). The perhaps most severe, and yet least frequent, consequence of mutations within the *LMNA* gene are observed in patients of the Hutchinson-Gilford Progeria Syndrome (HGPS). HGPS is most frequently caused by a point mutation leading to the activation of a splice donor site which results in a shortened lamin A mRNA transcript and protein isoform. The posttranslational modification of this protein is incomplete, leading to changes in the localisation and polymerisation of other "normal" lamin isoforms. This change in lamina composition has severe effects on nuclear integrity and growth (Eriksson et al., 2003; Goldman et al., 2004). The complicated relationship between mutations within *LMNA* and disease phenotypes once again demonstrates the complex functional network the lamina is involved in. Our mechanistic understanding of how these laminopathies are caused on a cellular level are still incomplete despite numerous knock-out mouse strains modelling a number of laminopathy-associated phenotypes.

There is a limited number of, not necessarily mutually exclusive, hypotheses to explain the diverse phenotypes of laminopathies on the one hand and the restriction to a limited set of affected tissues on the other hand (reviewed in (Ho and Lammerding, 2012; Capell and Collins, 2006). The "structural hypothesis" postulates that defects in lamina function mainly result in reduced mechanical stability of the cell, especially regarding shearing forces, and are responsible for the observed phenotypes. In this scenario, the limited capability of cells to cope with mechanical stress is a cause of increased cellular and tissue damage. This hypothesis serves as a well-suited explanation for syndromes in which contractile tissues, such as skeletal or heart muscle, are affected. The contractile tissues are exposed to an increased amount of mechanical stress and reduced nuclear stability, caused by lamina defects, which has been shown to result in increased cellular damage (Broers et al., 2004). This hypothesis is clearly limited in explaining phenotypes in which predominantly fat or neuronal tissues are affected, as these are not exposed to increased amounts of mechanical stress. Another frequently discussed scenario is the "gene regulation hypothesis". In this hypothesis, the defects in lamina function lead to deregulation of gene expression, causing the observed cellular defects (Goldman et al., 2004). The effect of lamin mutations on tissue-specific transcription factors could also explain the selected effect on limited tissues. Finally, a number of other ideas are here combined to a more general "cell-cycle deregulation and DNA-damage-hypothesis". Data indicates, that some mutations within *LMNA* cause defects in renewal of adult mesenchymal stem cells (Pekovic and Hutchison, 2008). On the other hand, defects in lamins have been observed to be associated with delayed or inefficient cell differentiation and maintenance of differentiated states. These defects in addition to the observed reduced DNA damage repair capabilities of cells associated with laminopathy phenotypes all together create the picture that defects within lamina structure or function deregulate all aspects of cell fate, maintenance and lifespan. This combination of inefficient cell proliferation and differentiation and accelerated cellular ageing would explain both reduced cell growth and tissue degeneration observed in several

laminopathies. However, it is most likely that the complex effect of lamina defects and the diverse phenotypes of laminopathies are explained by a combination of any of these hypotheses, rather than any single cause.

The lamina during meiosis – unique proteins & implications for specialised functions

During male germ cell development, including both meiosis and post-meiotic development, the nucleus undergoes severe remodelling. The NE of meiotic cells differs in its molecular features and composition quite substantially from that of somatic cells. This may be a consequence of the different and specialised functional requirement of the NE in meiotic cells, including the ability to attach and move meiotic telomeres. This nuclear reorganisation also includes the nuclear lamina. The nuclear lamina of meiotic and post-meiotic male germ cells differs from that of somatic cells both in composition and structure. During germ cell development, only one A-type lamin, lamin C2, and two B-type lamins, lamins B1 and B3, are expressed. Lamin C2 is specifically only expressed during meiosis. Somatic lamins A and C are not expressed during meiosis and lamin B3 is only expressed during spermiogenesis (Furukawa et al., 1994; Furukawa and Hotta, 1993; Alsheimer and Benavente, 1996). Therefore, the composition of the nuclear lamina in meiotic cells is much more restricted in terms of lamin isoforms than the lamina of somatic cells. The meiotic nuclear lamina is, in male and female meiosis, only composed of Lamin B1 and Lamin C2. Also the distribution of lamin proteins within the nuclear lamina in meiotic cells differs significantly to the distribution of lamins in somatic cells. Lamins in somatic cells usually show an even distribution around the NE, localising to the nuclear lamina. Lamin C2, however, shows an uneven distribution in the NE enriching in patches within the nuclear lamina. These sites of lamin C2 enrichment have been demonstrated to coincide with the sites of meiotic telomere attachment (Alsheimer et al., 1999).

As mentioned earlier, Lamin C2 is encoded by an alternative starting exon of the *LMNA* gene, resulting in a shortened N-terminal domain. Relatively to its somatic counterpart, lamin C, lamin C2 is missing the N-terminal non-helical domain and a substantial part of the N-terminal region of the central rod domain. Instead the N-terminus of lamin C2 is composed of a specific hexapeptide sequence (GNAEGR), which has been shown to be a target for myristoylation. (Alsheimer et al., 2000). In somatic lamins A and C, this N-terminal part of the central rod-domain has been shown to be essential for higher ordered lamin polymerisation (Stuurman et al., 1998; Herrmann et al., 2004). Furthermore, the somatic lamins A and C have been described to be virtually immobile once they are incorporated into the lamina, probably due to the tight integration into the lamina network, which is mediated by the described domains (Broers et al., 1999; Gilchrist et al., 2004). This immobility of somatic lamins within the nuclear lamina, leading to the tight and rigid lamina network, may well be responsible for the mechanical and stabilising function of the somatic lamina, described above. Lamin C2, however, has been demonstrated to be by far more mobile within the nuclear lamina (Jahn et al., 2010). In transfection experiment, lamin C2 forms distinct plaques within the nuclear lamina of the transfected somatic cells. In these cells lamin C2 has been shown to be highly mobile both between distinct plaques of lamin C2 as well as in between distinct plaques (Jahn et al., 2010). It has been demonstrated, that the increased mobility of lamin C2 is due to the shortened N-terminal domain, which is missing the N-terminal end of the central rod essential for higher order

polymerisation. The thereby altered polymerisation properties of lamin C2 seem to cause a less rigid incorporation into to meiotic lamina, than the corporation of lamin A or C into the somatic lamina. The myristoylation site within the unique N-terminal domain of lamin C2 has been demonstrated not to be responsible for the increased mobility, but rather serves as an alternative pathway to incorporate lamin C2 into the lamina (Alsheimer et al., 2000; Jahn et al., 2010). In lamins containing a CAAX-box, the posttranslational modifications of this motif are responsible and essential for the incorporation into the lamina. Somatic lamin C, which does not have a CAAX-box, is recruited to the lamina by lamin A (Horton et al., 1992). Since the C-terminal domain of lamin C2 does also not include a CAAX-box and there are no other A-type lamins expressed during meiosis, the recruitment through the myristoylation seems to be another adaptation of the meiotic lamina.

Furthermore, lamin C2 has been demonstrated to influence NE integrity (Jahn et al., 2010). The transfection and ectopic expression of lamin C2 in somatic cells influences the distribution of other, endogenous, NE proteins, such as SUN1. In these experiments, the expression of lamin C2 additionally induced changes in nuclear shape. The changes in nuclear shape indicate that the expression of lamin C2 substantially changes the structural properties of the NE, probably also in meiotic cells. These unique properties of the meiosis-specific lamina suggest that it plays a central role during the functional reorganisation of the nucleus during meiosis. Altered mechanical properties of the meiotic NE, for example, would have immense implications for its possible functions, especially regarding structural roles, which could then be quite different compared to those of the somatic lamina. However, the distinct role of meiotic lamin C2 within the NE in meiotic cells still remains to be demonstrated.

Because lamin C2 is enriched at the sites of telomere attachment, shows increased mobility compared the somatic lamins and influences NE features and protein distributions, it has been postulated to be either directly involved in the attachment of meiotic telomeres to the NE or locally change NE properties during meiosis. Lamin C2 could locally increase NE flexibility and could thereby facilitate the highly conserved and dynamic telomere movements of prophase I within the NE (Alsheimer et al., 1999; Jahn et al., 2010).

1.3 The LINC complex – the linker between nucleoplasm and cytoskeleton

The NE consists of many more components than the nuclear membranes and the lamina. An integral party of the NE are the membrane proteins embedded within the INM and the ONM. KASH domain proteins in the ONM are able to interact with SUN domain proteins in the INM, forming a multimeric proteins complex which spans the NE. This complex, generally composed of KASH and SUN domain proteins, is the so-called LINC complex (linker of nucleoskeleton and cytoskeleton) (Crisp et al., 2006). The SUN and KASH proteins interact directly with each other within the PNS, thus enabling the LINC complex to traverse through the entire NE (McGee et al., 2006; Starr and Fridolfsson, 2010) (see Figure 1.4). Diverse members of the KASH domain protein family have been demonstrated to interact with different components of the cytoskeleton, generating connections to the actin network, microtubules and intermediate filaments (Wilhelmsen et al., 2006). SUN domain proteins, on the other hand, are able to interact with the nuclear lamina and chromatin on the nucleoplasmatic face of the INM (Razafsky and Hodzic, 2009). Therefore, the LINC complex provides a mechanical bridge between nuclear components and the cytoskeleton, allowing direct communication between the cytoplasm and the nucleus.

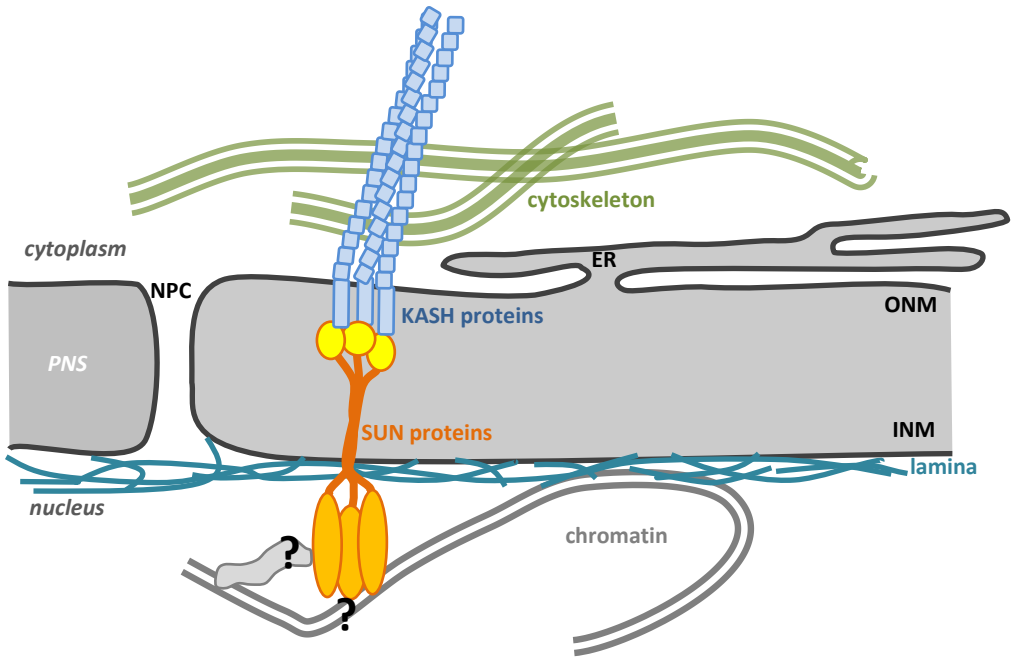

Figure 1.4: The location and structure of a LINC complex within the nuclear envelope. SUN-domain protein trimers are located within the INM, interacting with KASH-domain proteins of the ONM. SUN-domain proteins have been shown to interact with various nuclear proteins, such as lamins. Direct interactions with chromatin are less well described and other SUN protein binding factors are most likely still unknown. KASH proteins interact with different cytoskeletal components within the cytoplasm. Therefore the LINC-complex bridges the NE, connecting the nucleus and its content to the cytoskeleton.

Proteins of the LINC complex – highly conserved components from yeast to man

SUN domain proteins are a family of highly conserved proteins which represent the INM component of the LINC complex. Members of the SUN protein family have been characterised almost within any eukaryotic model organism investigated to date. At least one SUN protein within any organism is usually almost ubiquitously expressed, like UNC-84 in *C.elegans* (Lee et al., 2002) or SUN1 and 2 in mice (Hodzic et al., 2004; Padmakumar et al., 2005). Although SUN domain proteins are found in most known model organisms, the number of SUN domain proteins expressed within one species ranges substantially. In the yeasts *S.pombe* and *S.cerevisiae*, for example, only one SUN domain protein is known in each, Sad1 and Mps3, respectively. In mammals five SUN domain proteins are expressed from different genes (Starr, 2009), of which at least one (SUN1) additionally encodes for multiple splicing isoforms (Gob et al., 2010). Regarding organisms with multiple SUN domain proteins, expression patterns of SUN proteins also vary (Kracklauer et al., 2013). For example, tissue specific expression of some SUN domain proteins is observed like for the murine SUN3, 4 and 5 which are restricted to the testis (Gob et al., 2010; Frohnert et al., 2010; Kracklauer et al., 2013). The strong evolutionary conservation and ubiquitous expression of SUN proteins on the one hand and the specialised tissue restricted expression of other SUN protein isoforms on the other hand indicate that SUN domain proteins must be serving multiple functions ranging from general cell maintenance to cell type specific roles.

All SUN domain proteins in any species are characterised by at least three shared features (Starr, 2009; Starr and Fridolfsson, 2010). Firstly, all SUN proteins exhibit at least one hydrophobic amino acid sequence serving as a transmembrane domain, defining them as integral membrane proteins. Secondly, the localisation of SUN domain proteins is restricted exclusively to the INM, where the C-term of the proteins is located within the PNS and the N-term is facing the nuclear interior. Finally, the name-giving SUN domain is located at the C-terminal end of the protein, within the PNS. The SUN domain is an extremely conserved amino acid sequence of approximately 200 residues in length (Hodzic et al., 2004; Padmakumar et al., 2005; Crisp et al., 2006). Its name is derived from the protein family members first characterised to contain the SUN domain in yeast and *C.elegans* (Sad1 and UNC-84). Furthermore, many of the known SUN proteins also feature at least one C-terminal coiled-coil domain located between the conserved SUN domain and the transmembrane domain (Starr and Fridolfsson, 2010). Although these coiled-coil motifs are not conserved on the amino acid level, their structural role in the polymerisation of homo- or heteropolymers of SUN proteins seems to be conserved quite well (Zhou et al., 2012; Sosa et al., 2012). On the contrary, the N-terminal nucleoplasmic domain of SUN proteins is evolutionarily not well conserved, varying substantially in size and structure between SUN proteins (Rothballer et al., 2013; Starr and Fridolfsson, 2010). This variation of the nucleoplasmic domain is associated with different potential interaction partners of SUN domain proteins within the nucleus. Regarding mammalian SUN domain proteins little is yet known about the interactions and regulation of nuclear SUN-binding components (Haque et al., 2006; Mejat and Misteli, 2010). It is, however, proposed that the nucleoplasmic interaction of SUN proteins with the lamina or chromatin is responsible for the specific retention of SUN domain proteins to the INM (Razafsky and Hodzic, 2009; Haque et al., 2006; Hasan et al., 2006).

The other conserved component of the LINC complex, the KASH domain proteins, are the ONM component of this NE spanning protein complex. Similarly to SUN domain proteins, KASH domain proteins have also been characterised in a wide variety of model organism, ranging from yeast to mouse. In all organisms, at least one KASH domain protein is usually almost ubiquitously expressed, similarly to some SUN domain proteins, like Nepsrin1 and 2 in mammals or ANC-1 in *C.elegans* (Zhang et al., 2001; Starr and Han, 2002). Nonetheless, the number of KASH proteins expressed differs widely between species, ranging from one clearly defined protein in *S.cerevisiae* Csm4, over three in *C.elegans* to at least five in mammals (Starr, 2009; Kracklauer et al., 2013). However, the many splicing isoforms of some of the KASH domain proteins, like the mammalian Nesprin2 for example, and the weak conservation between KASH proteins makes it rather difficult to clearly define the number of actual KASH domain proteins in any one organism (Zhang et al., 2005; Mellad et al., 2011). In accordance with the large number of KASH domain proteins and their many splicing isoforms, the expression patterns of KASH proteins may vary greatly, like the mammalian KASH5 for example, shows an extremely restricted expression pattern (Morimoto et al., 2012).

KASH domain proteins are generally less strictly conserved than SUN domain proteins, but they share a conserved motif in the C-terminal domain. This name-giving KASH domain is a relatively short amino acid sequence localised at the C-terminal end of the proteins. It consists of a transmembrane domain followed by approximately 30 weakly conserved amino acids and a stop codon (Razafsky and Hodzic, 2009). The last four amino acids of this sequence is the only strongly conserved region consisting of a PPPX motif (Rothballer et al., 2013; Starr, 2009). The name of the KASH domain is derived from the homology of conserved members of this protein family in *Drosophila*, *C.elegans* and mammals (Klarsicht, ANC-1 and SYNE homology). The KASH-domain resides within the PNS whereas the N-terminal domain is facing the cytoplasm, locating the KASH proteins within the ONM. The vast majority of KASH domain proteins and their isoforms are located exclusively to the ONM (Roux et al., 2009; Zhang et al., 2010; Wilhelmsen et al., 2005; Starr, 2007) nonetheless, for some KASH protein isoforms a localisation within the INM and interactions with lamins have also been reported. In any case, the KASH domain is always located within the PNS (Zhang et al., 2005; Mislow et al., 2002). KASH domain proteins show enormous variation in their N-terminal cytoplasmic domains. Therefore, direct and specific interactions of KASH proteins with all components of the cytoskeleton have been demonstrated. For example, mammalian Nesprins1 exhibit cytoplasmic actin binding properties, the *C.elegans* protein UNC-83 interacts with the microtubule network and the mammalian Nesprin3 shows interactions with plectin, linking it to the intermediate filament cytoskeleton (Fridolfsson et al., 2010; Wilhelmsen et al., 2005; Starr and Fridolfsson, 2010; Starr and Fischer, 2005). In summary, the variability of the KASH proteins reflects the adaptability and wide spectrum of LINC complex compositions and their cytoplasmic binding partners.

Just over a decade ago, Lee at al. proposed the first “bridging model”, in which an INM protein and an ONM protein were postulated to interact with each other thus bridging the entire NE (Lee et al., 2002). The identification of SUN and KASH domain proteins and their demonstrated direct interaction with each other substantiated this “bridging model”, leading to the development of the LINC complex model (Malone et al., 1999; Starr and Han, 2002; Starr et al., 2001; Crisp et al., 2006).

In the recent years evidence has accumulated from diverse model organisms in which differently composed LINC complexes have been shown to exist and function in a well conserved manner. This led to more detailed insights in LINC complex structure and composition. Several studies suggested that SUN domain proteins are likely to form either homo- or heteropolymers. A favoured model was that SUN domain proteins from hetero- and homodimers mediated by their coiled-coil motifs (Wang et al., 2006; Lu et al., 2008). Surprisingly, very recent crystallography studies unravelling the LINC complex were able to show, that LINC complexes are composed of SUN domain protein trimers interacting with three KASH domain peptides (Zhou et al., 2012; Sosa et al., 2012). The SUN domain proteins were indeed shown to interact through their coiled-coil motifs, creating a stem-like structure, on which the globular SUN domains are place, much like a clover-leave (Sosa et al., 2012; Rothballer et al., 2013) (compare to Figure 1.4). Because mammalian SUN1 and 2 have additionally been shown to interact with each other, it is plausible that heterotrimers of SUN domain proteins exist within LINC complexes, increasing the variability of possible LINC complex compositions further.

Functions of LINC complexes – a broad variety of roles mediated by specialised protein combinations

As indicated by the broad spectrum of SUN and KASH proteins found in the common model organism, LINC complexes have been demonstrated to be involved in a large number of different cellular processes. This diversity of LINC complex function is further increased by the ability of some SUN proteins to interact with different KASH partners, performing a cell-type specific “partner switching” (Hiraoka and Dernburg, 2009). This is for instance seen in *C.elegans* where the SUN domain protein UNC-84 is able to interact with two different KASH domain proteins, UNC-83 and ANC-1. Because UNC-83 is connected to the microtubule network and ANC-1 to actin filaments within the cytoplasm, UNC-84 is therefore selectively able to interact with different components of the cytoskeleton (Razafsky and Hodzic, 2009).

One of the first described functions of a complete LINC complex, including its SUN and KASH partners, was associated with nuclear migration in *C.elegans* (Starr and Fischer, 2005; Starr et al., 2001). Mutations within UNC-83 and UNC-84 lead to phenotypes in which uncoordinated nuclear migration in the early embryogenesis of *C.elegans* was observed (Starr et al., 2001; Malone et al., 1999). The demonstrated interaction of UNC-84 and UNC-83 and the ability of UNC-83 to bind to centrosome associated microtubules elucidated the role of the LINC complex in the directed nuclear migration observed in *C.elegans* embryos (Meyerzon et al., 2009). Similarly, in *Drosophila* a LINC complex was shown to be essential for nuclear migrations in the developing eye. Here the SUN protein Klaroid interacts with the KASH domain partner Klarsicht, which in turn is again connected to the microtubule network (Mosley-Bishop et al., 1999; Kracklauer et al., 2007). Therefore, it has been demonstrated in different organisms that the LINC complex is responsible for microtubule dependent directed nuclear migrations, which are relevant during development and embryogenesis.

Once directed nuclear migration is successfully completed, nuclei need to be anchored at the correct position within the cell. Differently composed LINC complexes have been demonstrated to be essential for this nuclear anchorage. In *C.elegans*, the SUN domain protein UNC-83 is able to selectively interact with the KASH protein ANC-1, to specifically anchor nuclei in an actin dependant

manner (Starr and Han, 2002). Comparably, in vertebrates, Nesprin1 and 2 interact with SUN1 and 2 to anchor nuclei at distinct positions within the cell (Zhang et al., 2007; Lei et al., 2009).

In *Drosophila* and mammals, specialised LINC complexes are furthermore suggested to be involved in the directed nuclear shaping of developing sperm. In these cases, the mechanical forces of the cytoskeleton are proposed to be transferred to the NE by the LINC complex to achieve the non-spherical nuclear shape (Kracklauer et al., 2013). In this context, mammalian SUN3 has been shown to interact with Nesprin1, which colocalises with the associated microtubule manchette and in turn is suggested to be involved in shaping spermatid nuclei (Gob et al., 2010).

As demonstrated by the functions in nuclear positioning and nuclear shaping the LINC complex transfers mechanical forces of the cytoskeleton onto the nucleus and into the nuclear interior. However, recently it has become evident that the roles of the LINC complex go beyond the mechanical linking of the cytoskeleton and the nucleus. LINC complexes in several species seem to also be involved in signal cascades, cell regulation pathways and NE organisation and structure (Fridkin et al., 2009; Mejat and Misteli, 2010; Rothballer et al., 2013).

Another highly specialised LINC complex function is the strongly conserved attachment and repositioning of meiotic telomeres within the NE (Chikashige et al., 2007). The involvement of the LINC complex in these processes essential for fertility has been demonstrated to function in a highly conserved manner from yeast to mice. This particular function of the LINC complex is discussed in more detail below.

Meiotic LINC complexes – the players that tether & move meiotic chromosomes

A widely conserved attribute of meiosis is the transient yet stable attachment of telomeres to and their directed repositioning within the NE. In any species investigated so far, this robust connection of telomeres to the NE is mediated by the LINC complex, transferring forces of the cytoskeleton onto the attached telomeres. Although the general mechanism of meiotic telomere attachment is exceptionally well conserved, the proteins involved in this attachment differ from species to species (Hiraoka and Dernburg, 2009) (see Table 1.1). In some organisms, components of meiotic LINC complexes are meiosis-specific SUN or KASH proteins. In other cases, ubiquitously expressed SUN or KASH proteins are redistributed or specifically regulated to become part of the meiotic LINC complex upon entry into meiosis. However, in many species, neither all components of the meiotic LINC complexes nor their regulation are completely unravelled yet. Additionally, how chromosome ends attach stably and specifically during meiosis to the LINC complexes is only beginning to be understood. Seemingly, adaptor proteins connecting chromosome ends to the LINC complex have evolved, at least in some species.

Amongst the first meiotic LINC complex of which KASH and SUN partners were described was that of the fission yeast *S.pombe* (Chikashige et al., 2007). In *S.pombe*, the ubiquitously expressed SUN protein Sad1 is normally associated with the SPB. Upon entry into meiosis, it transiently relocalises into smaller foci distributed within the NE. These Sad1 foci have been shown to colocalise with the attached telomeres which are moved towards to the SPB where they cluster to form the

bouquet conformation (Hagan and Yanagida, 1995; Jin et al., 2002). Sad1 has been shown to interact with the KASH protein Kms1, thus forming a functional LINC complex which tethers meiotic telomeres to the NE (Niwa et al., 2000). This LINC complex is connected to the microtubule cytoskeleton through the interaction of Kms1 with dynein, transferring the cytoskeletal forces onto the meiotic telomeres (Miki et al., 2004).

Although meiotic telomeres in *S.pombe* are attached through a conserved LINC complex, adaptor proteins connecting the telomeres to Sad1 seem to be *S.pombe* specific, as homologs have to date not been found in other species. In *S.pombe* the conserved telomeric proteins Taz1 and Rap1 bind to the meiosis-specific proteins Bqt1 and 2, which together with Bqt3 and 4, build a protein complex to connect the telomeres to the LINC complex (Chikashige et al., 2006; Chikashige et al., 2009). Additionally, the clustered attachment of meiotic telomeres to the SPB in *S.pombe* lasting throughout prophase I represents a rather peculiar case compared to other model organisms. Especially, the prominent horse-tail movements observed in *S.pombe,* in which the entire nucleus is moved back and forth whilst the telomeres are attached to the SPB, is a specialised meiotic mechanism. Therefore, perhaps, *S.pombe* may have also evolved special connector proteins to meet these peculiar meiotic requirements.

Table 1.1: Known LINC complexes involved in meiotic telomere tethering in common model organism.

Organism	nuclear interaction partner	SUN-domain protein	KASH-domain protein	cytoskeletal partner	reference
S.pombe	Bqt1-4	Sad1	Kms1	Microtubules	(Miki et al., 2004; Chikashige et al., 2009; Chikashige et al., 2006)
S.cerevisiea	Ndj1	Mps3	Csm4(?)	Aktin	(Conrad et al., 2008; Wanat et al., 2008)
C.elegans	HIM-8, ZIM1-3	SUN-1	ZYG-12	Microtubules	(Malone et al., 2003; Penkner et al., 2009)
M.musculs	?	SUN1/SUN2	KASH5	Microtubules	(Schmitt et al., 2007; Ding et al., 2007; Morimoto et al., 2012)

In the budding yeast *S.cerevisiae*, meiotic telomere attachment is also mediated by a meiotic LINC complex, but here not as many components have been clearly defined to date. Certainly, the SUN domain protein Mps3 is responsible for telomere attachment. Mps3 binds to the Csm4 protein, which is implicated to be the KASH binding partner (Conrad et al., 2008; Wanat et al., 2008). Mps3 is,

similar to Sad1, also a ubiquitously expressed SUN proteins which performs specialised functions during meiosis. The KASH protein Csm4 probably connects the attached telomeres to the actin cytoskeleton, in contrast to *S.pombe* Kms1, which interacts with the microtubule cytoskeleton. So regarding the cytoskeletal forces which drive telomere dynamics during prophase I, clear species-specific differences are observed. In *S.cerevisiae* too, specific nuclear proteins essential for the attachment of the telomeres to the SUN protein have been identified. In *S.cerevisiae* this adaptor protein is Ndj1. In the absence of Ndj1, telomeres fail to attach to the NE (Wu and Burgess, 2006). This, in some ways, is similar to the situation in *S.pombe* regarding the Bqt-proteins.

In *C.elegans*, the meiotic attachment of chromosome ends is mediated by a LINC complex composed of the ubiquitously expressed SUN-1 and ZYG-12 (Malone et al., 2003; Penkner et al., 2007). ZYG-12 links the attached telomeres to the microtubule cytoskeleton, similarly to the situation of Kms1 in *S.pombe* meiosis. In *C.elegans* as well, meiotic sub-telomeric regions are linked to SUN-1 through specific connector proteins. In the case of *C.elegans* these are the zinc finger proteins ZIM1-3 and HIM-8 (Phillips and Dernburg, 2006; Phillips et al., 2005). However, the meiotic attachment and subsequent movement of chromosome ends in *C.elegans* is somewhat unique. *C.elegans* chromosomes have short repetitive sub-telomeric regions, which are responsible for the initiation of homolog recognition and pairing. The ZIM proteins, and HIM-8 for the X-chromosome, bind to these pairing centres at one end of each chromosome. This leads to the attachment of only one end per chromosome to the NE, instead of both ends like in yeast or mammals. Despite this chromosomal peculiarity, *C.elegans* is the only model organism in which the regulation of the SUN and KASH proteins constituting the meiotic LINC complex, and their switch from somatic to meiotic functions, is beginning to be understood. Kinases, associated with the pairing centres, phosphorylate meiosis-specific phosphorylation sites of SUN-1 at leptotene/zygotene (Penkner et al., 2009). This leads to the meiosis-specific localisation of SUN-1 and the increased mobility of the SUN-1/ZYG-12 LINC complex in the NE (Labella et al., 2011). This meiosis-specific phosphorylation of SUN-1 has shown to be essential for the efficient movement of meiotic telomeres. Furthermore, very recently it has also been elucidated that the subsequent dephosphorylation of SUN-1 at and after pachytene is crucial for the further meiotic progression (Woglar et al., 2013). Although *C.elegans* exhibits unique chromosome behaviour during meiosis, the molecular mechanisms to regulate meiotic LINC complex function and meiotic progression may still be conserved in other organisms.

Although many components of the meiotic LINC complex in mammals have been identified recently, its regulation and composition is less well understood than in yeast or especially *C.elegans*. After the induction of meiosis, the ubiquitously expressed SUN proteins SUN1 and 2 relocalise into distinct foci which colocalise with the attached telomeres (Schmitt et al., 2007; Ding et al., 2007). The essential role of SUN1 during mammalian meiosis is demonstrated in SUN1 deficient mice, which are infertile due to impaired meiotic telomere attachment (Ding et al., 2007). However, the particular role of SUN2 within the meiotic LINC complex is not as well defined yet. The meiosis-specific, novel KASH protein KASH5 has been identified recently. KASH5 is able to interact with SUN1 and 2, and is part of the mammalian meiosis-specific LINC complex responsible for the attachment of telomeres to the NE (Morimoto et al., 2012). KASH5 also shows interactions with the microtubule cytoskeleton.

Most likely the forces responsible for the directed repositioning of the meiotic telomeres within the NE are generated by microtubules and transferred through KASH5. This would be similar to the situation in fission yeast and *C.elegans*, where cytoskeletal forces moving attached telomeres are generated by microtubules as well. The telomere dynamics of mammals, however, are somewhat different to those observed in yeast or *C.elegans.* All telomere are attached to the NE in mammals, clustering rather transiently, especially in mice. The most prominent clustering is observed at the leptotene/zygotene transition but is already resolved by pachytene.

How telomeres are connected to the SUN domain proteins during mammalian meiosis is at the moment entirely unclear. Meiosis-specific adaptor proteins, comparable to yeast and *C.elegans*, have not been identified so far. Also non-meiosis specific telomere proteins or other chromatin associated binding partners have not shown to interact with SUN1 or 2 in a meiosis-dependent manner. Furthermore, the redistribution of SUN1 and 2 from their homogeneous somatic distribution within the entire NE to the distinct meiotic foci and the regulation of the meiosis-specific telomere interaction are also not understood yet. Phosphorylation-dependent regulation of SUN1 or 2, as observed in *C.elegans,* could be possible in mammals as well but have not explicitly been demonstrated to date. Also the significance of two, somewhat similar, meiotic SUN domain proteins expressed in mammals is still unclear. Whether SUN1 and SUN2 fulfil partially redundant or separate functions during meiosis is still unknown. Therefore, especially in mammalian meiosis, many questions regarding meiotic telomere attachment and movement still remain to be answered.

1.4 Aims of this thesis – investigating meiotic telomere attachment and dynamics from different perspectives

Meiotic telomere attachment and chromosome movement are extremely conserved phenomena throughout most sexually reproducing organisms. It has been demonstrated in various organisms from yeast to mice that the attachment of telomeres to the NE and their efficient repositioning into and out of the bouquet conformation are essential for the efficient completion of meiosis and thus for fertility. However, many aspects of telomere attachment and movement are not well understood yet. Therefore, it was the aim of this thesis to investigate the contribution of different NE components to the processes of efficient telomere attachment and repositioning during murine meiosis. Furthermore, this thesis aimed to get deeper insight into the role of mammalian meiotic telomere movements in general, including the formation and release of the meiotic bouquet.

Understanding the role of the meiotic lamina – the lamin C2 knockout mouse model

As described above, the composition and molecular features of the meiotic lamina are considerably different than those of the lamina of somatic cells (Alsheimer and Benavente, 1996; Furukawa and Hotta, 1993; Furukawa et al., 1994). Whereas the somatic lamina is renowned for its multiple roles in maintaining nuclear shape and integrity as well as chromosome organisation, the role of the meiotic lamina is less clear. Male mice deficient for all A-type lamins were observed to be infertile, exhibiting meiotic defects which lead to apoptosis of spermatocytes (Alsheimer et al., 2004). However, which precise role the nuclear lamina plays during meiosis remained elusive in this study as a differentiation between the laminopathy-associated secondary effects on fertility and direct effects caused by the absence of meiosis-specific lamin C2 was not possible. To further investigate the specific role of the meiotic lamina, without possible somatic secondary effects, a novel, lamin C2-specific knockout mouse model was constructed (Schmitt, 2008).

Due to the molecular features of lamin C2, it has been postulated, that lamin C2 may be able to alter NE properties to locally increase its flexibility. This would then allow the attached meiotic telomeres to move more efficiently within the NE to facilitate the formation and release of the bouquet conformation. Therefore an aim of this thesis was to investigate the dynamic chromosome behaviour in mice of the lamin C2 knockout strain and hence to gain insight into the possible functions of the nuclear lamina, and of lamin C2 in particular, in the conserved formation and release of the meiotic bouquet.

Investigating the meiotic functions of SUN2 – the SUN1 knockout mouse model

Both SUN1 and SUN2 have been described to be part of the murine meiotic LINC complex responsible for tethering telomeres to the NE (Ding et al., 2007; Schmitt et al., 2007). In a previous study it was shown that the absence of SUN1 causes severe defects in meiotic telomere attachment, leading to infertility in SUN1 deficient mice (Ding et al., 2007; Chi et al., 2009). The precise role of SUN2, however, is currently controversially discussed due to contradicting published results. Whether SUN1 and SUN2 fulfil separate or partially redundant functions during meiosis, as they do in

somatic cells, is still unknown (Lei et al., 2009). Therefore, the contribution of SUN2 to the mammalian meiotic LINC complex is unclear. Using mice of an available SUN1 knockout strain allows studying the functions of SUN2 in the absence of SUN1. Hence, a further aim of this thesis was to investigate the actual meiotic role of SUN2 in telomere attachment and movement, which in particular would become evident in the mice lacking SUN1. This would consequently allow gaining deeper insights into the mechanism of mammalian meiotic telomere attachment and its role as a requirement for directed chromosome movements.

2. Results

2.1 The role of the meiotic nuclear lamina in chromosome dynamics

As described earlier, the nuclear lamina of meiotic cells is composed of fewer and different lamin proteins than the lamina of somatic cells. Regarding A-type lamins, somatic lamins A and C are not expressed in meiocytes. Instead the shorter meiosis-specific splice variant lamin C2 is the only A-type lamin present in the lamina of spermatocytes. To directly investigate the role of meiosis-specific lamin C2, a novel knockout mouse model was previously constructed, where the lamin C2 specific exon 1a of the *LMNA* gene was targeted (Schmitt, 2008). By eliminating this lamin C2 specific exon, only the expression of lamin C2 was disrupted, but leaving the expression of the somatic lamins A and C intact. Prior to this thesis, the characterisation of the lamin C2 knockout mouse, here referred to as lamin $C2^{-/-}$, showed that mice are of normal size and weight, showing no laminopathy-associated phenotype (Gob, 2011). Male mice of this strain were infertile, suggesting that lamin C2 does indeed play an essential role, at least during male meiosis. Females of this strain, however, were fertile although it could be demonstrated that lamin C2 is also expressed in oocytes (Jahn, 2012). On-going investigation of the male mice of the lamin $C2^{-/-}$ strain furthermore demonstrated dramatically increased rates of apoptosis in the pachytene stage, which could explain for the observed infertility. More detailed analyses of pachytene staged oocytes and spermatocytes revealed that mice lacking lamin C2 exhibit defects in the pairing and synapsis formation of homologous chromosomes (Gob, 2011; Link, 2010; Link et al., 2013a). In the males over 99% of spermatocytes at a pachytene-like stage exhibit defects in homologous pairing in the form of incomplete synapsis, heterologous associations and univalent chromosomes (see Figure 2.1A and (Link, 2010). Similar defects were observed in lamin $C2^{-/-}$ oocytes but less severe and less frequent (Jahn, 2012; Link et al., 2013a). Because pioneering studies investigating lamin C2 observed an enrichment of lamin C2 within the NE at sites of meiotic telomere attachment, it had originally been suggested that it may have a role in the attachment of telomeres to the NE and/or their directed movements within the NE (Alsheimer et al., 1999). However, work of a diploma thesis could previously show that the attachment of meiotic telomeres in lamin C2 deficient spermatocytes was actually not impaired (see Figure 2.1B and (Link, 2010). Due to the molecular features of lamin C2, it had also been suggested that lamin C2 may locally increase NE flexibility to facilitate movements of the attached telomeres within the NE (Jahn et al., 2010). Because lamin C2 is not essential for the attachment of meiotic telomeres *per se*, the first part of this current thesis focuses on the possible role of lamin C2 in telomere movements and comprises most of the work published in Link et al., 2013 (Link et al., 2013a).

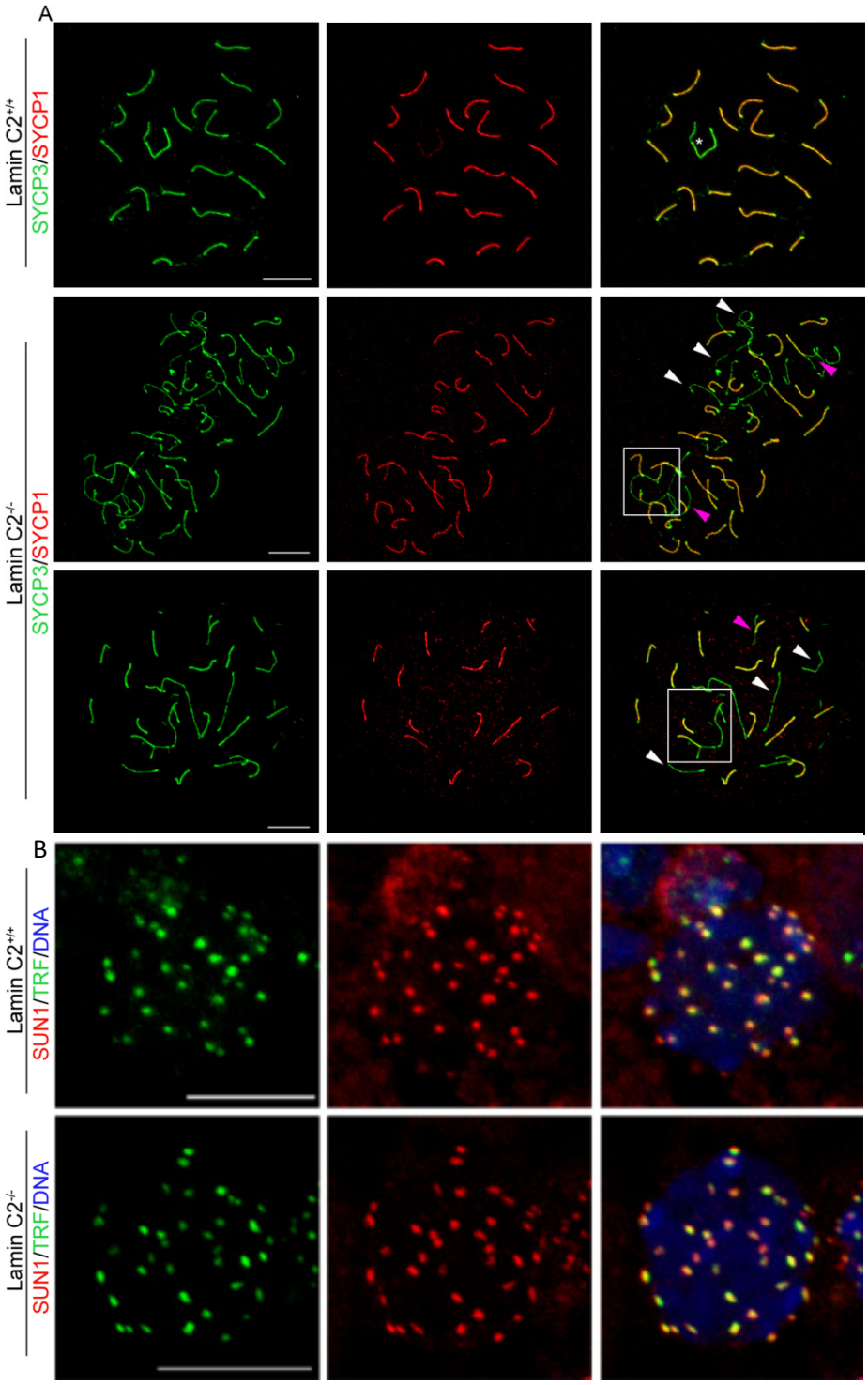

Figure 2.1: Defective homologous pairing and intact telomere attachment in lamin C2 deficient mice. (A) Immunofluorescence on chromosome spreads of wildtype and knockout spermatocytes using rb anti-SYCP3 (in green) and gp anti-SYCP1 (in red) antibodies. In the lamin $C2^{+/+}$ spermatocytes at pachytene all homologs are fully paired shown by the colocalisation of SYCP3 and SYCP1 in the merge. Only the X and Y chromosomes build an exception to this. Here only the small pseudo-autosomal region is labelled with SYCP1 and paired (indicated by the asterisk). The lamin $C2^{-/-}$ spermatocytes shown are also in a pachytene-like state indicated by the presence of some fully paired homologous chromosomes. However, numerous pairing defects are observed. Univalent chromosomes (white arrowheads) are observed in almost any spermatocyte. Partially paired synapses are also observed (magenta arrowheads) as well as heterologous associations (white rectangles). Scale bars 10 µm. (B) Immunofluorescence and Z-projections of swab preparations of spermatocytes labelled by rb anti-TRF (green) and gp anti-SUN1 (red) antibodies. DNA is counterstained using Hoechst-33528 (blue). In both lamin C2 wildtype and knockout spermatocytes all TRF signals (labelling telomeres) and SUN1 signals colocalise. This indicates that all telomeres are attached in the absence and presence of lamin C2. Scale bars 10 µm.

Bouquet stage formation is not altered in lamin C2 deficient spermatocytes

The telomere-led chromosome dynamics that include the formation and release of the bouquet conformation occur in mice from leptotene extending into early pachytene. The first wave of spermatogenesis, starting approximately at 8-9 days postpartum (dpp), offers an ideal time frame for the investigation of telomere movements because the first prophase I of meiosis progresses almost synchronously within the testis of young mice. Leptotene spermatocytes are commonly enriched in mice aged 9-10 dpp, zygotene spermatocytes are mostly found in mice of approximately 11-12 dpp, whereas spermatocytes in mice older than 12 dpp are usually in the transition into pachytene or in early pachytene. Mice aged 14 dpp commonly show enrichment of mid-pachytene spermatocytes, with the progression continuing as the age of the mice increases. By preparing tissue samples for paraffin embedding from littermate wildtype and lamin $C2^{-/-}$ mice ranged between 10 to 14 dpp it was possible to analyse the meiotic progression of these stages and to construct a timeline of the chromosome dynamics. To investigate whether telomeres in lamin C2 mice are able to cluster to form a bouquet, 7µm paraffin embedded testis tissue sections of lamin $C2^{-/-}$ and lamin $C2^{+/+}$ littermate mice of 12 and 14 dpp were subjected to immunofluorescence experiments using an anti-SYCP3 antibody to label SC structures and an anti-SUN1 antibody to indirectly label the attached telomeres. By analysing 3 dimensional reconstructions or Z-projections of confocal images from entire spermatocyte nuclei, the clustering pattern of SUN1 foci in the NE and the directionality of the SCs could be evaluated (Figure 2.2).

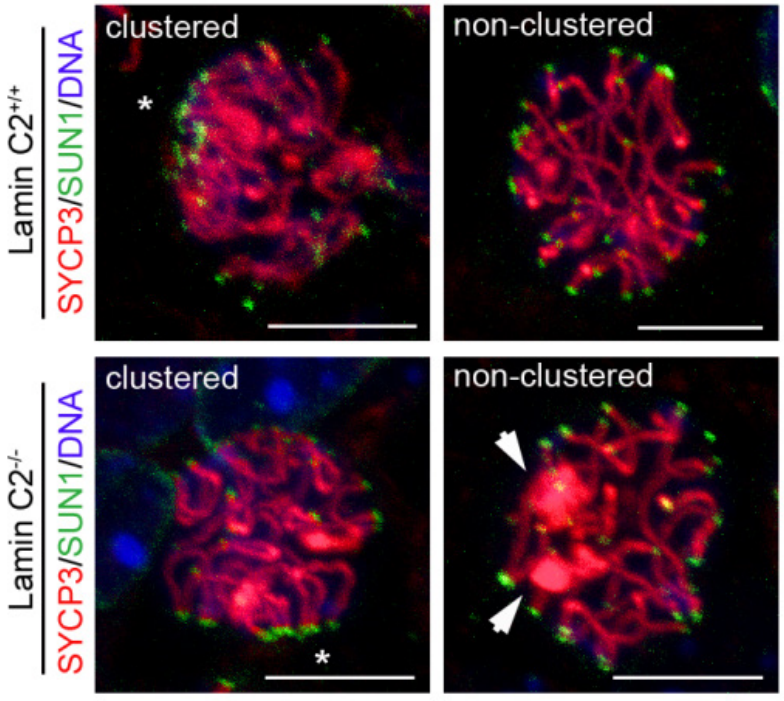

Figure 2.2: Z-Projections of spermatocytes of lamin $C2^{+/+}$ and lamin $C2^{-/-}$ mice showing clustered and non-clustered SUN1 foci patterns. Immunofluorescence on 7µm paraffin sections using rb anti-SYCP3 (in red) and gp anti-SUN1 (in green) antibodies, DNA labelled with Hoechst-33258 (blue). SUN1 foci are located at sites of SC-end attachment, indirectly labelling telomeres. Spermatocytes showing clearly clustered telomere patterns are found in both wildtype and knockout preparations (asterisks indicate direction of bouquet conformation). Spermatocytes with non-clustered telomeres are found in later meiotic stages of both lamin $C2^{+/+}$ and $^{-/-}$ mice. SYCP3 aggregates, indicating defects in homologous pairing, are only found in the lamin $C2^{-/-}$ spermatocytes (arrowheads). Scale bars 5µm.

In the wildtype clustered SUN1 patterns, resembling bouquet conformations, were commonly observed in spermatocytes of mice of 12 dpp. In sibling lamin $C2^{-/-}$ mice comparable bouquet stages were also observed. In bouquet spermatocytes of both genotypes the Z-projections evidently show the polarised orientations of the SC axis, ending at the clustered SUN1 foci (see Figure 2.2). As expected, in older aged mice (14 dpp) spermatocytes with non-clustered SUN1 foci were also detected in both wildtype and knockout males. These spermatocytes

show pachytene or pachytene-like staged cells, as judged by the progression of SC assembly, corresponding well to the on-going first wave of spermatogenesis. Although the lamin $C2^{-/-}$ spermatocytes reach a pachytene-like stage, the earlier mentioned defects in homologous pairing are visible in form of accumulations of the SYCP3 signal within the nucleus. Similar SYCP3 aggregations are not detectable in the wildtype pachytene cells (see arrowheads in Figure 2.2). Generally, these images demonstrate that telomeres in spermatocytes from lamin C2 deficient mice are able to cluster to form a meiotic bouquet.

Bouquet stage release is significantly delayed in lamin C2 deficient spermatocytes

Both, the formation and release of the bouquet stage are a closely regulated active processes occurring in zygotene to pachytene spermatocytes. As the first wave of spermatogenesis progresses, clustered telomere patterns are expected to decline correspondingly to the meiotic progression and age of the mice. Although lamin C2 deficient spermatocytes are able to cluster telomeres into conformations resembling a wildtype bouquet, as shown above, the question remains whether the timely dynamics of bouquet formation and release are comparable to the wildtype siblings. To analyse whether the chromosome dynamics, especially regarding the bouquet release, are temporally altered in lamin $C2^{-/-}$ males, a timeline of telomere movement was constructed using tissues from 10-14 dpp mice. At each age, tissues were prepared from three separate littermate pairs of wildtype and knockout siblings. Paraffin sections of each tissue were subjected to the immunofluorescence experiment described above using anti-SYCP3 and anti-SUN1 antibodies (Figure 2.3A). Confocal images of 100-200 single entire nuclei per mouse were reconstructed 3 dimensionally and analysed for their SUN1/telomere distribution. By this means, spermatocytes showing clustered or non-clustered telomere patterns were quantified (Figure 2.3B).

This quantification revealed, that at 10 dpp similar amounts of spermatocyte nuclei showed clustered telomere patterns in both wildtype (74.2%) and knockout (77.6%) mice. This demonstrates again, that bouquet formation is neither affected in its timely occurrence nor in its frequency in lamin $C2^{-/-}$ males. Further analyses demonstrated that in the wildtype, the percentage of spermatocytes exhibiting clustered telomere patterns decreases with increasing age of the mice, as would be expected, due to the on-going meiotic progression and the associated bouquet stage release (57.8% at 11 dpp, 45.4% at 12 dpp, 28.7% at 13 dpp, 20.5% at 14 dpp). In the lamin C2 knockout littermates the fraction of spermatocytes showing clustered telomere patterns also decreases, but significantly slower than in the wildtype (57.8% at 11 dpp, 51.8% at 12 dpp, 45.4% at 13 dpp, 43.9% at 14 dpp). This leads to a highly significant difference in bouquet staged cells at 13 dpp, where roughly the 1.5 fold amount of cells with clustered telomere patterns are observed in lamin $C2^{-/-}$ mice compared to lamin $C2^{+/+}$ mice (mean p-value of Pearson's Chi-Squared Test: 6.5×10^{-3}). This difference increases with meiotic progression such that by 14 dpp, lamin C2 deficient mice even show the approximately 2.5 fold amount of bouquet-staged spermatocytes compared to their wildtype siblings (mean p-value of Pearson's Chi-Squared Test: 7.6×10^{-7}). The significantly elevated numbers of bouquet-staged spermatocytes in lamin C2 deficient males at 13 and 14 dpp evidently

demonstrate that bouquet stage release in lamin C2$^{-/-}$ mice is significantly delayed compared to their wildtype siblings.

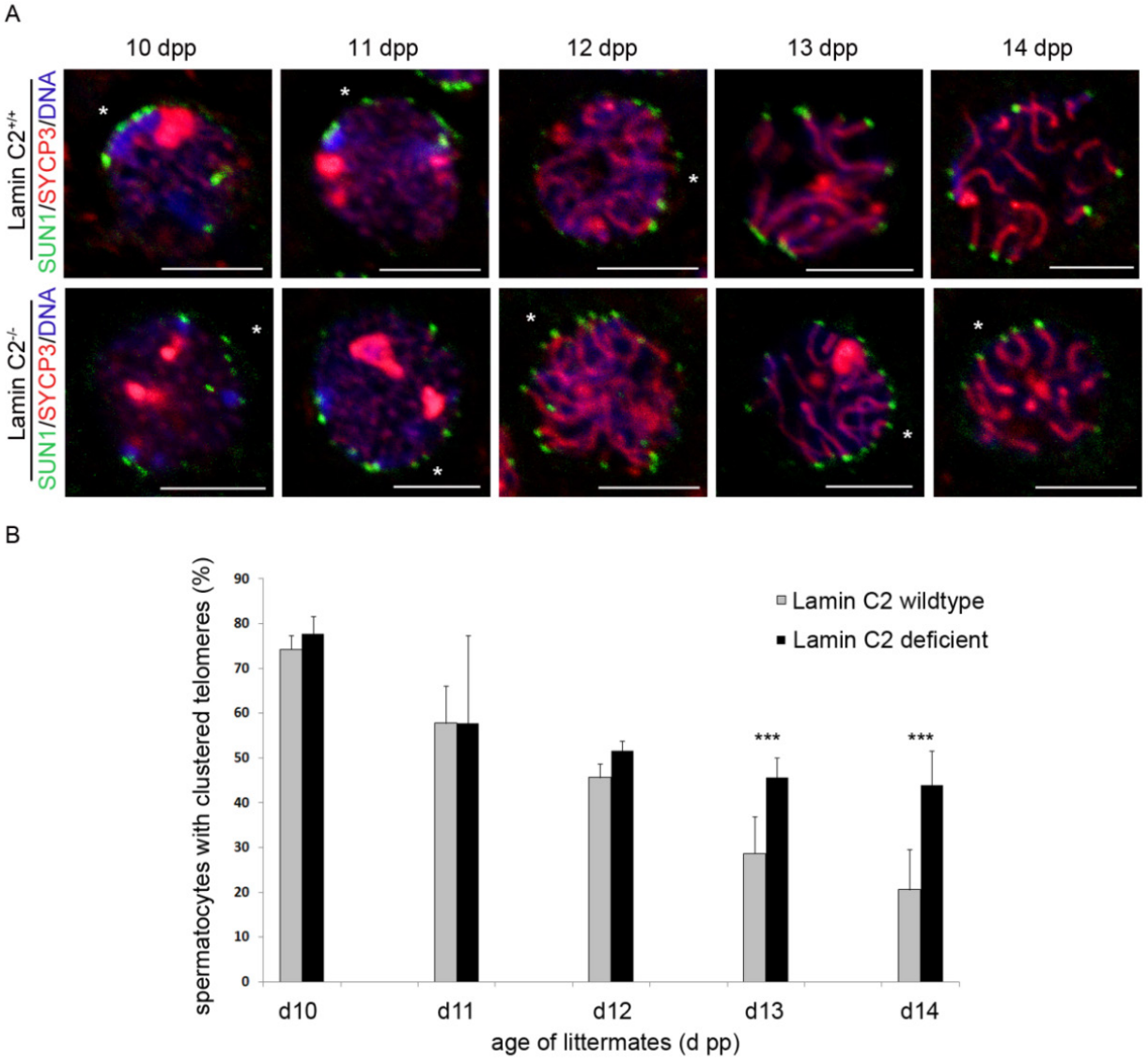

Figure 2.3: Bouquet resolution in spermatocytes of 10 to 14 dpp lamin C2$^{+/+}$ and $^{-/-}$ littermates. (A) Immunofluorescence on paraffin sections of littermate wildtype and knockout males from 10 to 14 dpp using rb anti-SYCP3 (in red) and gp anti-SUN1 (in green) antibodies, DNA labelled with Hoechst-33258 (blue). In the lamin C2$^{+/+}$ mice, spermatocytes with bouquet conformations (indicated by asterisks) are commonly found up to 12 d pp. At 13 and 14 dpp many spermatocytes clearly show dispersed SC-end/SUN1 foci patterns again once the bouquet stage is resolved. In lamin C2$^{-/-}$ spermatocytes, relatively tight chromosome-end clustering is observed from 10 to 14 dpp (clusters indicated by asterisks). Scale bars 5µm. (B) Quantification of mean spermatocyte numbers with clustered and non-clustered telomere patterns at 10 to 14 dpp in 3 pairs of littermate wildtype and knockout mice each, p-value analysed with Pearson's Chi-Squared Test. d10 p= 0.28 (wt n=517, ko n=580 cells), d11 p=0.15 (wt n=667, ko n=703 cells), d12 p=0,29 (wt n=736, ko n=780 cells), d13 p=6.5x10^{-3} (wt n=639, ko n=648 cells), d14 p=7.6x10^{-7} (wt n=700, ko n=682 cells). *** p< 0.01.

Tight telomere clustering is normally a hallmark of leptotene and zygotene. To analyse whether the SC assembly in spermatocytes of 14 dpp lamin C2$^{-/-}$ males exhibiting telomere clusters is also retained at a leptotene/zygotene stage or is processed further, the state of SC assembly was investigated. To compare the state of SC assembly in lamin C2$^{-/-}$ and $^{+/+}$ spermatocytes, paraffin sections of testis tissue of 14 dpp lamin C2$^{-/-}$ and $^{+/+}$ siblings were subjected to immunofluorescence

experiments using anti-SYCP1 and anti-SUN1 antibodies (Figure 2.4). Because SYCP1 is a component of the transversal filaments of the SC, it only labels paired chromosome axis which are a hallmark of a pachytene or pachytene-like spermatocytes.

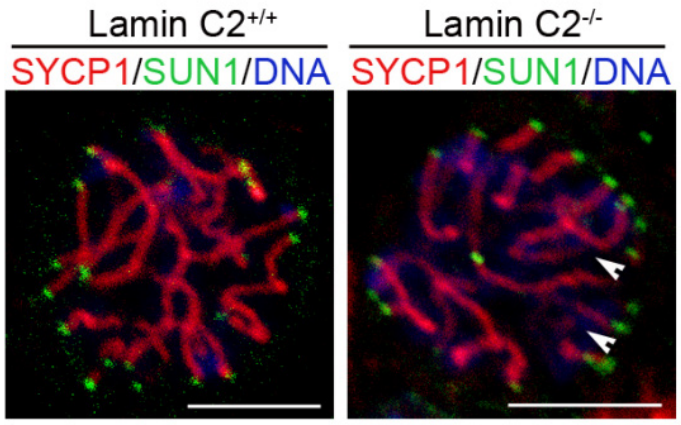

Figure 2.4: SC-assembly progression in 14 dpp lamin C2$^{+/+}$ and $^{-/-}$ spermatocytes. Z-projections of immunofluorescences on 7µm paraffin sections of 14 dpp littermate wildtype and knockout males using rb anti-SYCP1 (in red) and gp anti-SUN1 (in green) antibodies, DNA labelled with Hoechst-33258 (blue). The continuous SYCP1 stained SC axis in both wildtype and knockout spermatocytes show the advanced synapsis formation in both animals. The state of SC assembly is similar in wildtype and knockout. Arrowheads indicate regions of missing SYCP1 staining, probably due to defects in homologous pairing. Scale bar 5µm.

The long SYCP1-labelled chromosome axes in the wildtype spermatocytes clearly show that virtually all homologous chromosomes are paired. Similarly, lamin C2$^{-/-}$ spermatocytes also show long stretches of SYCP1 labelled SC axes. Therefore, as judged by the state of SC assembly, spermatocytes of the lamin C2 deficient mice have reached a comparable stage of meiotic progression, despite their retained telomere clustering. Nonetheless, frequent defects in homologous pairing are also observed in these preparations as demonstrated by the gaps in the SYCP1 stain (indicated by arrowheads in Fig. 2.4). These analyses indicate that SC assembly in the absence of lamin C2 progresses on to the recruitment of SYCP1, connecting homologous chromosomes through a stable synapsis. Despite the defects in homologous pairing and retained clustering of telomeres in lamin C2$^{-/-}$ spermatocytes, SC progression is not halted in the absence of lamin C2. Rather, lamin C2$^{-/-}$ spermatocytes seem to proceed into a pachytene-like stage even though the telomere clustering resembles a leptotene/zygotene hallmark. Conclusively this demonstrates that the progression of prophase I continues on, past the leptotene/zygotene transition, even though telomere clusters are not resolved efficiently.

DSB-repair & recombination are incomplete in lamin C2 deficient males

The progression of SC assembly is only one aspects of meiotic progression. Other important aspects of meiotic progression are the induction and subsequent repair of DSBs during leptotene and zygotene as well as recombination events during and after pachytene. The induction of DSBs into the genome at leptotene and their subsequent repair at zygotene are essential prerequisites for meiotic recombination at later stages during prophase I. Additionally, efficient recombination, SC- assembly and chromosome dynamics are processes that occur interdependently of each other. Because the repair of DSBs and subsequent meiotic recombination are temporally strictly regulated events, they can be used to evaluate the progression of prophase I. To analyse the progression of DSB repair and recombination and to determine the latest stage reached in lamin C2 deficient spermatocytes, prominent recombination markers were used in immunocytochemical approaches (Figure 2.5). The earliest stages of DSB-repair are analysed by using anti-γH2AX antibodies. The histone-variant γH2AX

specifically associates with unrepaired DSBs in the genome, strongly labelling chromatin during leptotene and early zygotene. As the DSBs are repaired, the intensity of the γH2AX signal is successively reduced within the nucleus. As the chromatin surrounding the paired X and Y chromosomes at pachytene develops into the sex-body, γH2AX is specifically recruited here. During pachytene, γH2AX therefore intensively labels the sex-body chromatin whilst it is no longer detected in the chromatin surrounding the autosomes (Figure 2.5A). RAD51, a component of early recombination nodules, specifically marks early stages of meiotic recombination. These early recombination nodules are detected in large numbers on chromosome axes during early zygotene especially. As the early nodules are processed, the RAD51 foci are reduced in number and intensity. By late zygotene and pachytene, no RAD51 foci are detected any more on the paired chromosomes axes (Figure 2.5B). RPA is specifically found in the later transition nodules, therefore the RPA foci are most prominently and frequently found on chromosome axes during zygotene. As spermatocytes progress into pachytene, RPA foci are significantly reduced until by mid-pachytene no RPA foci are detectable any more (Figure 2.5C). MLH1, as a marker of late recombination nodules, finally indicates crossing-over events and later sites of chiasmata. MLH1 is therefore not detectable before pachytene. During pachytene each synapsed homologous pair exhibits at least one MLH1 focus, marking the site of crossing-over (Figure 2.5D).

To compare the progression of DSB repair and recombination in wildtype and lamin C2 deficient spermatocytes, chromosome spreads of pachytene wildtype and pachytene-like lamin C2 deficient spermatocytes were subjected to specific antibodies against the above described recombination markers. Each recombination marker antibody was colocalised with an anti-SYCP3 antibody to simultaneously label the paired and non-paired chromosomes axes. Pachytene-like stages of lamin C2 deficient spermatocytes were judged by the presence of, at least some, fully paired homologous chromosomes within the cell. In the chromosome spreads of wildtype pachytene spermatocytes (Figure 2.5A), no γH2AX-signal is detectable within the chromatin associated with the paired homologous SC axis. The γH2AX-labelling only shows the strong characteristic sex-body chromatin staining. RAD51 foci are, as expected, not present in wildtype pachytene spermatocytes anymore and RPA foci are already reduced to a minimum (Figure 2.5B and C). MLH1 foci however are clearly present on every homolog, marking sites of crossing over events and future chiasmata (Figure 2.5D). In the pachytene-like spermatocytes deficient of lamin C2, a strong persistent γH2AX-signal is found indicating the presence of unrepaired DSBs (Figure 2.5A'). However, the characteristic labelling of the sex-body chromatin is also visible, although X and Y are seemingly unpaired in this chromosome spread. RAD51 and RPA foci persist in the pachytene-like lamin $C2^{-/-}$ spermatocytes, both on unpaired and fully paired chromosome axis (Figure 2.5B' and C'). This shows that components of early and transition nodules are recruited to the chromosome axis, yet are not processed completely. Finally, MLH1 foci are entirely absent in lamin C2 deficient spermatocytes (Figure 2.5D'). In summary, the analyses of recombination markers demonstrate that DSBs are induced in the absence of lamin C2, as shown by the labelling of γH2AX. Furthermore, components of early recombination nodules (i.e. RPA and RAD51) are recruited to the chromosome axes, indicating that at least DSB repair and the early recombination events are initiated. Yet, as shown by the persistence of RAD51 and RPA, early recombination nodules are not processed correctly. Finally, recombination does not occur in lamin

C2$^{-/-}$ spermatocytes, as indicated by the absence of MLH1 foci. Additionally, the recombination analyses conducted here clarify that spermatocytes deficient for lamin C2 reach a pachytene-like state, as demonstrated by the fully paired homologous chromosomes and the sex-body formation labelled specifically by γH2AX.

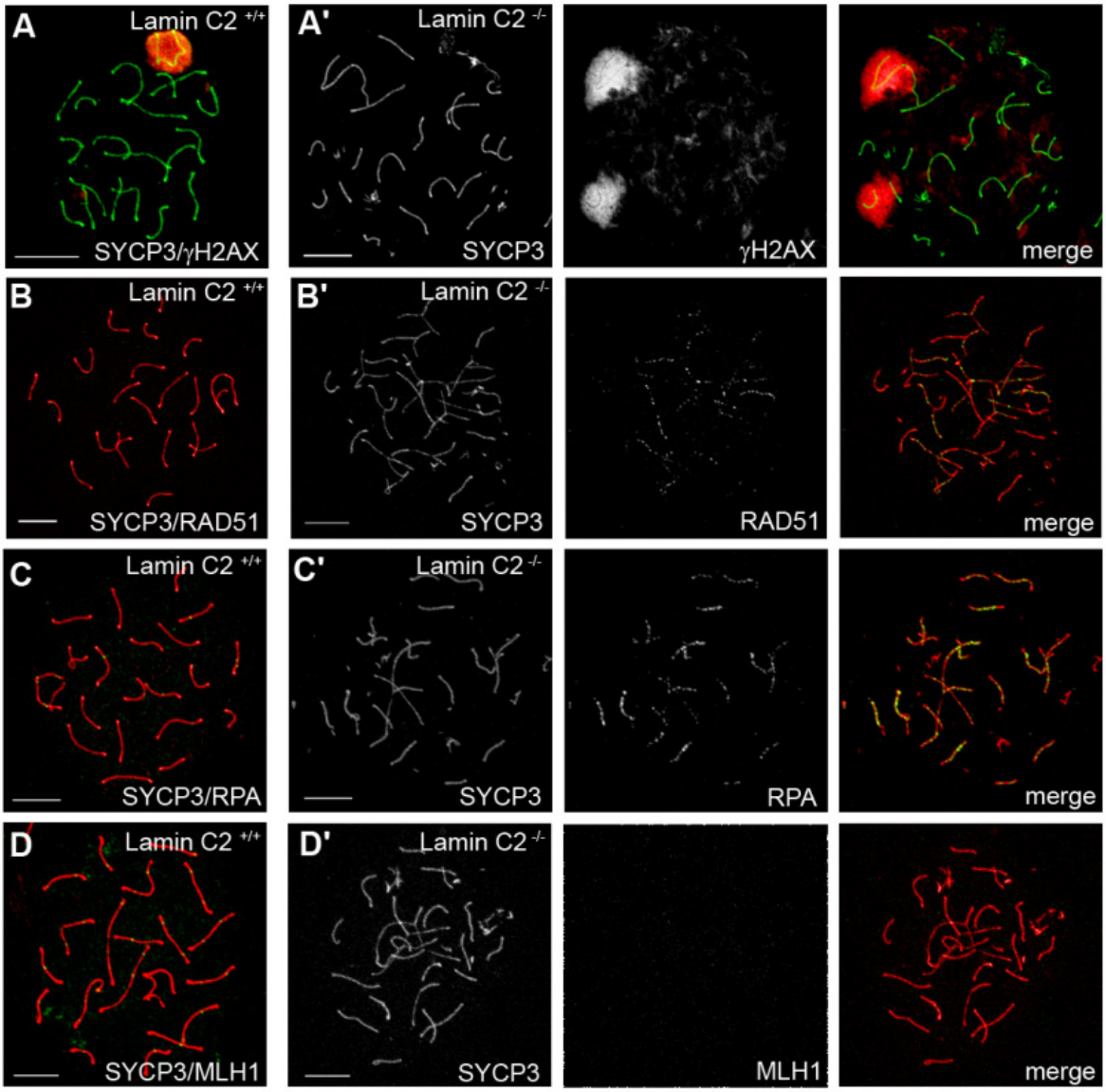

Figure 2.5: Progression of DSB-repair and recombination in lamin C2$^{-/-}$ spermatocytes. Immunofluorescences on chromosome spreads of wildtype pachytene and pachytene-like knockout spermatocytes. (A) Mouse anti-γH2AX (in red) and rb anti-SYCP3 (in green) antibodies on wildtype and knockout (A') spermatocytes. The persistent γH2AX staining in knockout spermatocytes is clearly visible, although the sex-body specific signal is also present, despite unpaired X and Y chromosomes. (B) Rb anti-RAD51 (green) and gp anti-SYCP3 (in red) antibodies on wildtype and (B') knockout spermatocytes. RAD51 foci aberrantly persist on fully paired chromosome axes of knockout spermatocytes. (C) Mouse anti-RPA (in green) and rb anti-SYCP3 (in red) antibodies on wildtype and knockout (C') spermatocytes. Numerous RPA foci are found on paired chromosome axes of lamin C2$^{-/-}$ spermatocytes. (D) Mouse anti-MLH1 (in green) and rb anti-SYCP3 (in red) antibodies on chromosome spreads of wildtype and knockout (D') males. At least one MLH1 focus per paired homolog is present in wildtype spermatocytes. In knockout spermatocytes MLH1 foci are absent. Scale bars 10 µm.

The DSB repair and recombination in lamin C2 deficient males contrasts the situation found in lamin C2 deficient females. Since lamin C2$^{-/-}$ females were found to be fertile, their DSB repair and recombination was also analysed (Jahn, 2012; Link et al., 2013a). The analyses revealed that in the oocytes MLH1 is partially recruited to the paired homologous chromosomes, indicating that some

recombination does take place. This is the case even though the processing of induced DSBs was slightly slower and less efficient than in the wildtype. However, the number of MLH1 foci per oocyte was decreased and the frequency of oocytes missing obligate MLH1 foci was increased compared to the control oocytes. Therefore, even though recombination proceeds to late events in lamin C2 deficient females, recombination frequencies are significantly decreased (Jahn, 2012).

In summary, the data presented in this section clearly shows that lamin C2 is essential for meiotic chromosome movements. More precisely, lamin C2 is required for the efficient release of the meiotic bouquet stage. Furthermore, the observed impaired telomere dynamics seem to lead to severe defects in synapsis formation, DSB repair and homologous recombination, causing infertility in the male mice and reduced recombination frequencies in the female mice.

2.2 The role of SUN2 in meiotic telomere attachment and movement

The faithful and stable attachment of meiotic chromosomes to the NE is required for the highly conserved telomere movements observed in prophase I and is one of many prerequisites for the successful completion of meiosis. In mammals, earlier studies have indicated that the attachment of meiotic telomeres is mediated by the INM proteins SUN1 and SUN2, which in meiosis localise to the attached telomeres (Ding et al., 2007; Schmitt et al., 2007). Furthermore, the ONM KASH-domain protein KASH5 also specifically localises to the attached meiotic telomeres and is able to interact with both SUN1 and 2 (Morimoto et al., 2012). KASH5 links the attached telomeres to the microtubule cytoskeleton through its cytoplasmic interactions with dynactin. Studies in SUN1 deficient mice have demonstrated that meiotic telomere attachment is impaired in the absence of SUN1, leading to meiotic abrogation and, as a consequence, the male and female SUN1$^{-/-}$ mice are infertile. However, the role of SUN2 in meiotic telomere attachment is still not very clear. SUN1 and SUN2 share the same localisation during meiosis, forming distinct foci at the attached telomeres. Therefore, they may also fulfil similar, partially redundant functions in the attachment of the meiotic telomeres. To investigate the precise meiotic function of SUN2 in more detail, this second part of the thesis focuses on the function of SUN2 in meiotic telomere attachment and chromosome dynamics. These investigations were undertaken in an available SUN1 deficient mouse strain (Chi et al., 2009), because this offers the opportunity to analyse the function of SUN2 in meiotic telomere attachment in the absence of SUN1. In this particular SUN1 deficient mouse strain, exons 10-11 of the SUN1 gene are targeted, resulting in a functional knockout of SUN1 (Chi et al., 2009). This section of the thesis comprises most of the work published in Link et al., 2013b ((Link et al., 2013b)*submitted)*.

Telomere attachment is still partially intact in SUN1 deficient spermatocytes

SUN1 has been demonstrated before to be essential for the complete attachment of meiotic telomeres to the NE. Mice deficient for SUN1 show impaired telomere attachment, exhibit severe defects in homologous pairing and synapsis and both males and females are infertile (Ding et al., 2007; Chi et al., 2009). Nonetheless, SUN2 has also been described to localise to attached telomeres in a wildtype situation (Schmitt et al., 2007). Additionally, studies conducted in SUN1 and SUN1/SUN2 deficient mice have shown, that in somatic cells SUN1 and SUN2 fulfil, at least partially, redundant functions (Yu et al., 2011; Lei et al., 2009). It is therefore possible, that SUN1 and SUN2 fulfil partially redundant functions during meiosis, too. To investigate the meiotic role of SUN2 in telomere attachment in detail, the localisation of telomeres in spermatocytes deficient for SUN1 was carefully analysed. For this 7 µm paraffin sections of testis tissue of adult SUN1 deficient mice (Chi et al., 2009) and wildtype littermates were subjected to a double immunofluorescence using anti-lamin B and anti-SYCP3 antibodies together with fluorescently labelled telomere probes (TeloFISH). Using these rather thick paraffin sections ensured that entire nuclei were structurally preserved within the sections. By producing confocal images of these preparations, it was possible to analyse the localisation of the labelled telomeres within the nuclei in respect to the labelled lamina (Figure 2.6). Telomeres which are attached to the NE should colocalise with the nuclear lamina. In the wildtype control spermatocytes all labelled telomeres clearly colocalise with the lamina, resembling telomeres

embedded within the lamina. Therefore, the wildtype pachytene spermatocytes show the complete meiotic telomere attachment, as expected. Analyses of the SUN1 deficient pachytene-like spermatocytes revealed, consistent with the previously published results (Ding et al., 2007), that some labelled telomeres are located within the nuclear interior, away from the NE. These telomeres clearly do not colocalise with the labelled lamina. This demonstrates the impaired telomere attachment described before (yellow arrows in Figure 2.6). Detailed analyses, however, revealed a number of telomeres, surprisingly, which colocalise with the labelled lamina. These telomeres embedded within the lamina resemble telomeres that may still be attached to the NE, even in the absence of SUN1 (white arrowheads in Figure 2.6).

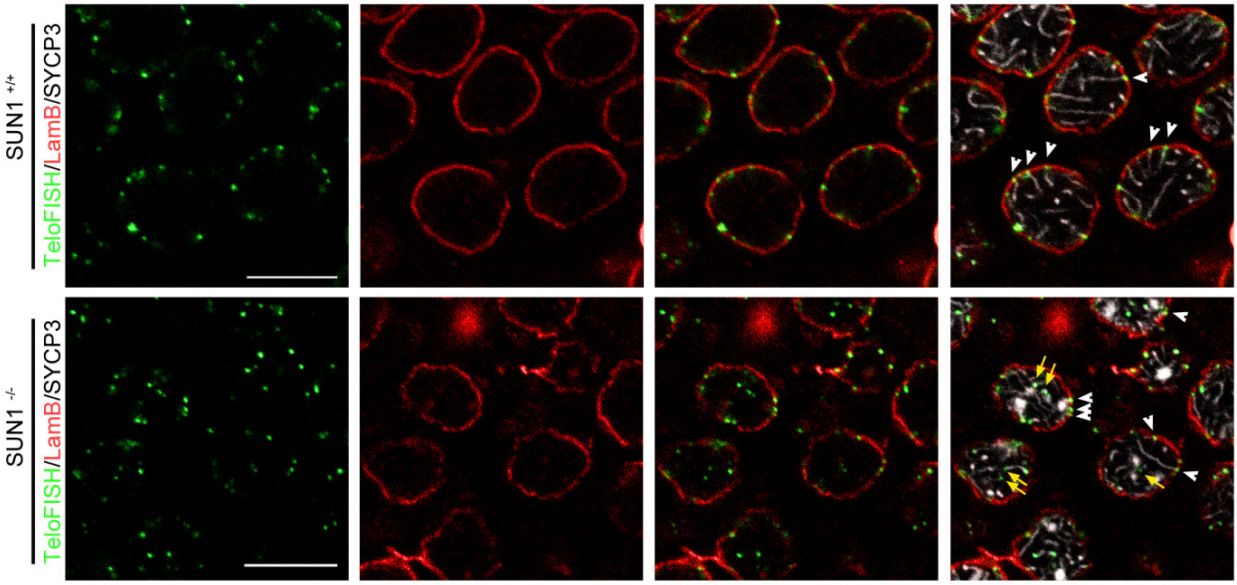

Figure 2.6: Telomere attachment in wildtype and SUN1 knockout spermatocytes. Immunofluorescence using goat anti-lamin B (in red) and rabbit anti-SYCP3 antibodies (in grey) in combination with telomere fluorescent in situ hybridisation (in green; TeloFISH) on 7µm paraffin sections of adult SUN1 wildtype and knockout testis tissue. In the wildtype spermatocytes all telomere signals co-localise with the nuclear lamina (white arrowheads), showing complete telomere attachment. In the SUN1$^{-/-}$ spermatocytes internal telomeres detached from the NE are detected (yellow arrows) but clearly some telomeres also co-localise with the nuclear lamina (white arrowheads). Scale bars 10 µm.

To clarify whether the peripheral telomeres in SUN1 deficient mice are truly attached to the NE or are only in close proximity to the lamina, samples of SUN1 wildtype and knockout testis were prepared for electron microscopy (Figure 2.7). For this particular experiment, tissue from a different SUN1 deficient mouse strain (Ding et al., 2007), in addition to the tissue from the SUN1 deficient mouse strain provided by K.T. Jeang (Chi et al., 2009), was also analysed. The SUN1 deficient mouse strain published by Ding et al., is constructed by a different knockout strategy, targeting exons 10-13 of the SUN1 gene. This also results in a functional knockout of the SUN1 protein (Ding et al., 2007). Testis tissue from both SUN1 deficient strains was investigated regarding possible telomere attachment to achieve a thorough and complete analysis. In control spermatocytes of both strains, attachment sites showing the typical structure of the SC tightly associated with the NE are frequently found, as expected (Figure 2.7A and B). The structure of the SC, including central and lateral elements, can be seen clearly in the electron micrographs. At the attachment sites, the SC typically shows canonical thickenings where it is in intimate contact with the INM. This also can be observed

in some of the electron micrographs shown (black arrowheads in 2.7). Furthermore, filamentous structures traversing the PNS and reaching into the cytoplasm can commonly be seen in well preserved electron microscope samples at sites of telomere attachment. These have been suggested to be part of the LINC complex attaching the telomeres to the NE and connecting them with the cytoplasm (Schmitt et al., 2007; Benavente et al., 2004). Intriguingly, sites where paired chromosome axes are in intimate contact with the NE were found in the samples of both SUN1 deficient mouse strains, as well (Figure 2.7A and B). These SCs, leading to the NE, seem to be structurally intact, containing lateral and central elements. In fact, multiple examples of such sites where SCs are in intimate contact with the NE were found in mice deficient for SUN1 (Figure 2.8). In some cases, even the typical conical thickening of the SC is observed where SCs attach to the NE (black arrowheads in insets of 2.8A and B). Additionally, some unpaired axes were also observed to be in intimate contact with the NE in the absence of SUN1 (Figure 2.8C). In general, the observed sites of telomere attachment in both SUN1 deficient mouse strains in the electron micrographs indeed resemble the wildtype attachment sites. This indicates that in the absence of SUN1 peripheral telomeres can be found which are truly attached to the NE on the electron microscope level. Furthermore, images from both strains indicate that this telomere attachment is independent of the genetic background and targeting strategy of the SUN1 deficient mice. Finally, although the pairing and synapsis of homologous chromosomes has been shown to be defective in SUN1 deficient mice (Ding et al., 2007; Chi et al., 2009), some partially completed synaptonemal complexes seem to be able to attach to the NE achieving wildtype-like attachment sites in the absence of SUN1.

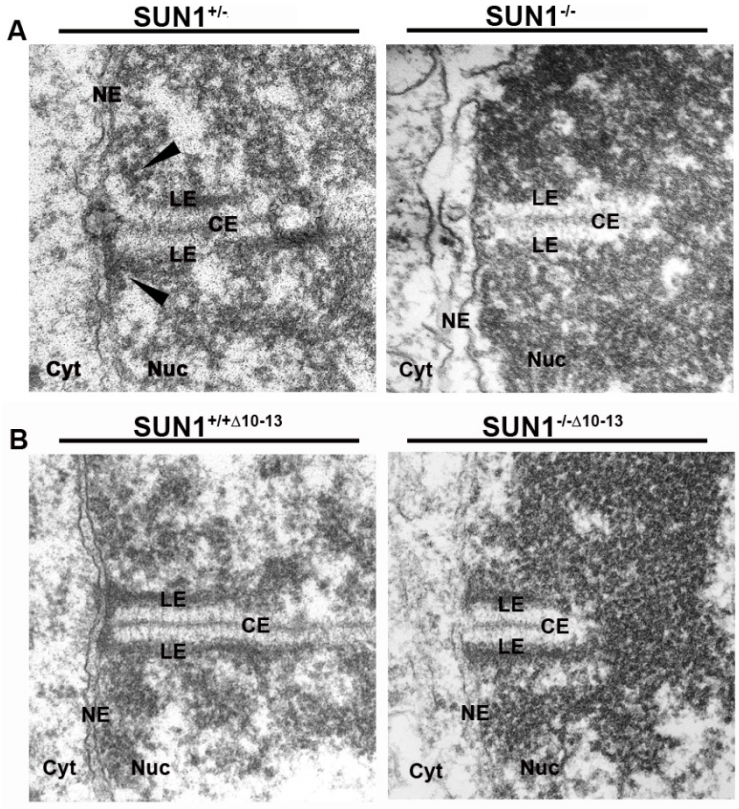

Figure 2.7: Telomere attachment sites in wildtype and SUN1 deficient spermatocytes of two SUN1 deficient mouse strains. (A) Electron micrographs of control and knockout spermatocytes of the SUN1 deficient mouse strain published in Chi et al. (Chi et al., 2009) (B) Electron micrographs of wildtype and SUN1$^{-/-}$ spermatocytes of the additional SUN1 deficient mouse strain used in this experiment, published by Ding et al. (Ding et al., 2007). Telomere attachment sites are observed in control and knockout spermatocytes of both SUN1 deficient mouse strains. Intimate association of paired SC axes are observed in both preparations. Conical thickening at SC ends indicating attachment plates are also visible in some examples (black arrowheads). Cyt cytoplasm, Nuc nucleoplasm, NE nuclear envelope, LE lateral element, CE central element.

Similarly to the situation in male SUN1 deficient mice, females deficient for SUN1 also show partial attachment of meiotic telomeres to the NE. The attachment of the telomeres to the NE is visible in the immunofluorescence approach described above as well as in electron micrographs.

Here too, paired SC stretches were found which are tethered to the NE forming wildtype-like attachment sites (Leubner, 2012).

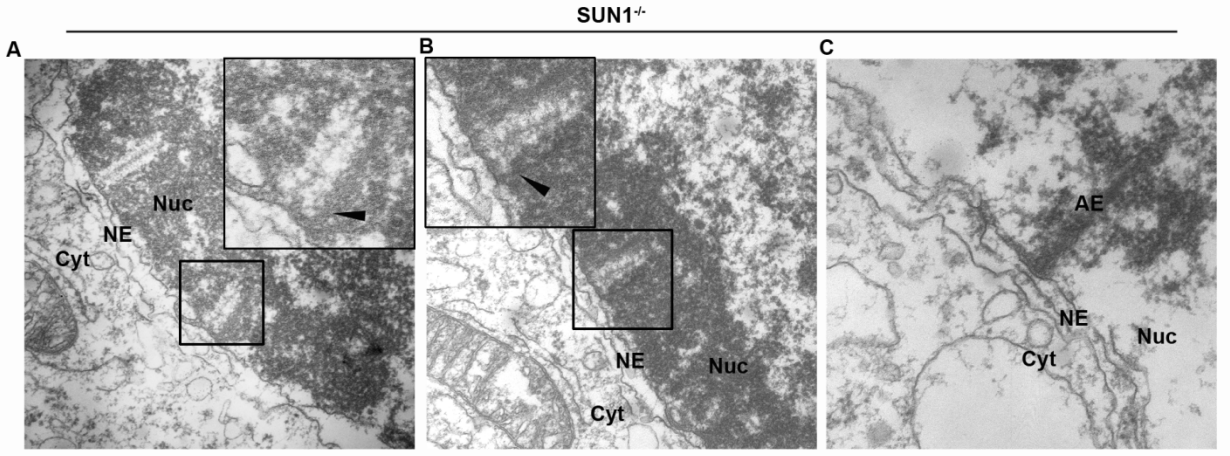

Figure 2.8: Telomere attachment sites in SUN1 deficient spermatocytes. (A-B) Intimate associations of SCs with the NE in SUN1 deficient spermatocytes of the strain published by Chi et al., 2009. Insets show magnifications of the indicated attachment sites. Canonical thickenings are partially observed where the SC interacts with the NE (arrowheads). (C) Attachment of an unpaired chromosome axis (axial element) to the NE. Cyt cytoplasm, Nuc nucleoplasm, NE nuclear envelope, LE lateral element, CE central element, AE axial element.

As shown by the colocalisation of telomeres with the lamina in the immunofluorescences and especially by the attachment sites in the electron micrographs, telomere attachment is not entirely lost in the absence of SUN1. To define the proportion of telomeres which are still attached to the NE in the absence of SUN1, telomere attachment in pachytene spermatocytes was quantified in wildtype and SUN1 knockout littermate males. For this, 7 µm paraffin sections of 14 dpp littermates were subjected to the previously described immunofluorescence approach. In this approach, the nuclear lamina and SC were labelled using respective specific antibodies in colocalisation with fluorescently labelled telomere probes. In these samples, peripheral telomeres colocalising with the nuclear lamina and internal telomeres were quantified in 3 dimensional reconstructions or sequential optical sections of confocal images of entire nuclei. In the pachytene spermatocytes of the 14 dpp wildtype mice all telomeres were found to colocalises with the nuclear lamina (2.10C). This resembled the expected situation of a complete telomere attachment in wildtype pachytene cells. The quantification of telomere attachment in pachytene spermatocytes of the SUN1 deficient littermates surprisingly revealed a high frequency of peripheral telomeres in colocalisation with the nuclear lamina (69.8%, Figure 2.10C). This indicates that a large portion of telomeres is attached to the NE in the absence of SUN1.

In summary, the data of the immunofluorescence and electron microscopy demonstrated that some telomeres are located peripherally and that, unexpectedly, telomeres are still attached to the NE in the absence of SUN1. Quantifications of pachytene spermatocyte revealed that actually approximately two thirds of all telomeres are still attached to the NE in the absence of SUN1. This clearly contradicts the results published before (Ding et al., 2007).

Telomeres remain stably attached throughout prophase I in SUN1 deficient spermatocytes

The quantification of telomere attachment in mid-pachytene spermatocytes described above revealed that roughly two thirds of the telomeres in pachytene-stage cells are able to attach to the NE in the absence of SUN1. However, the question remains whether this telomere attachment is stable throughout the progression of prophase I. It would be possible that in the absence of SUN1 telomeres attach to the NE in even greater numbers at earlier meiotic stages and detach again until pachytene. Also it could be possible that telomere attachment is delayed in the absence of SUN1. To analyse the progression of telomere attachment in comparison to wildtype mice, the attachment of telomeres was quantified, as described above, on paraffin testis sections of 12 dpp aged littermate mice. Because leptotene and zygotene spermatocytes were found within the testis of the 12 dpp aged mice, the quantification of telomere attachment was conducted separately for leptotene and zygotene spermatocytes.

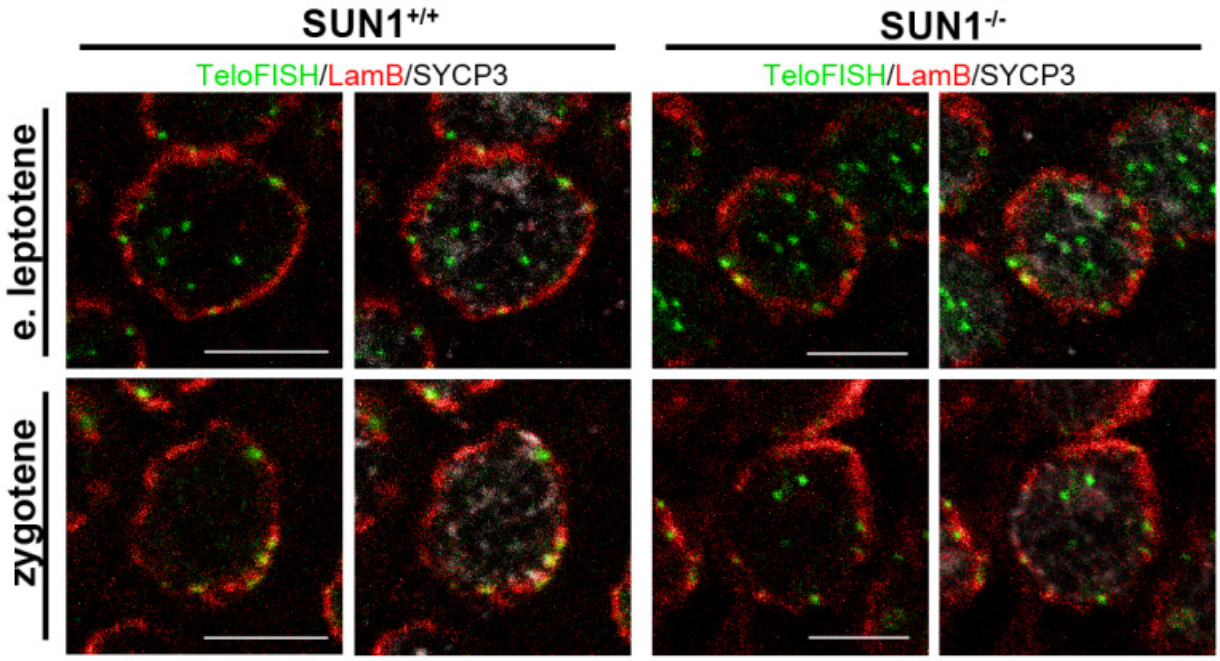

Figure 2.9: Differentiation of leptotene and zygotene spermatocytes in tubules of 12 dpp SUN1 wildtype and SUN1 deficient males. TeloFISH combined with immunofluorescent labelling of goat anti-lamin B and rabbit anti-SYCP3 antibodies on 7µm paraffin testis sections of 12 dpp SUN1 wildtype and knockout littermates. By comparing states of SC assembly and telomere attachment early leptotene (e. leptotene) staged spermatocytes may be distinguished from zygotene spermatocytes. In leptotene wildtype spermatocytes internal non-attached telomeres are detected. In zygotene wildtype spermatocytes only attached telomeres in colocalisation with the lamina are observed. In knockout spermatocytes, both attached and non-attached telomeres are detected in leptotene and zygotene spermatocytes. Scale bars 5 µm.

Leptotene and zygotene spermatocytes were differentiated according to their state of SYCP3 aggregation and assembly (Figure 2.9). Early leptotene spermatocytes do not show thread-like SYCP3 signals because the assembly of the SC is only just commencing. Zygotene spermatocyte, however, clearly show thread-like SC signals, as by then the unpaired axial elements have formed or are starting to form.

The quantification in the wildtype leptotene spermatocytes (Figure 2.10A) revealed a telomere attachment frequency of 77.7%. In these cells, telomere attachment is probably not completed yet due to the very early meiotic stage. According to this, these early leptotene spermatocytes observed are at the moment in the process of telomere attachment. In the knockout spermatocytes of comparable leptotene stages 64.5% of the telomeres are attached. Therefore, the SUN1 deficient leptotene spermatocytes show a slightly reduced frequency of telomere attachment compared to the wildtype control cells. Because nonetheless, a large fraction of telomeres is already attached in the SUN1$^{-/-}$ mice, this indicates that telomere attachment is induced at comparable stages in the absence of SUN1 as in the wildtype. However, here too, the observed attachment frequency of pachytene spermatocytes (68.9%) is not yet reached, indicating that SUN1 deficient spermatocytes during early leptotene are also still in the process of telomere attachment. Quantifying telomere attachment in zygotene spermatocytes (Figure 2.10B) revealed that by this stage the telomere attachment of wildtype spermatocytes is completed, as 100% of telomeres are attached. In contrast, the knockout zygotene spermatocytes of the same stage still only show 71.2% of all telomeres attached to the NE. The frequency of telomere attachment reached in zygotene SUN1 knockout spermatocytes is very similar to the frequency observed in the knockout pachytene spermatocytes (Figure 2.10C). This suggests that no more than roughly two thirds of the telomeres ever become attached in the absence of SUN1. Furthermore, this also indicates that telomeres which become attached to the NE, remain attached. Therefore, the attachment of telomeres found in the absence of SUN1 seems to be stable throughout prophase I.

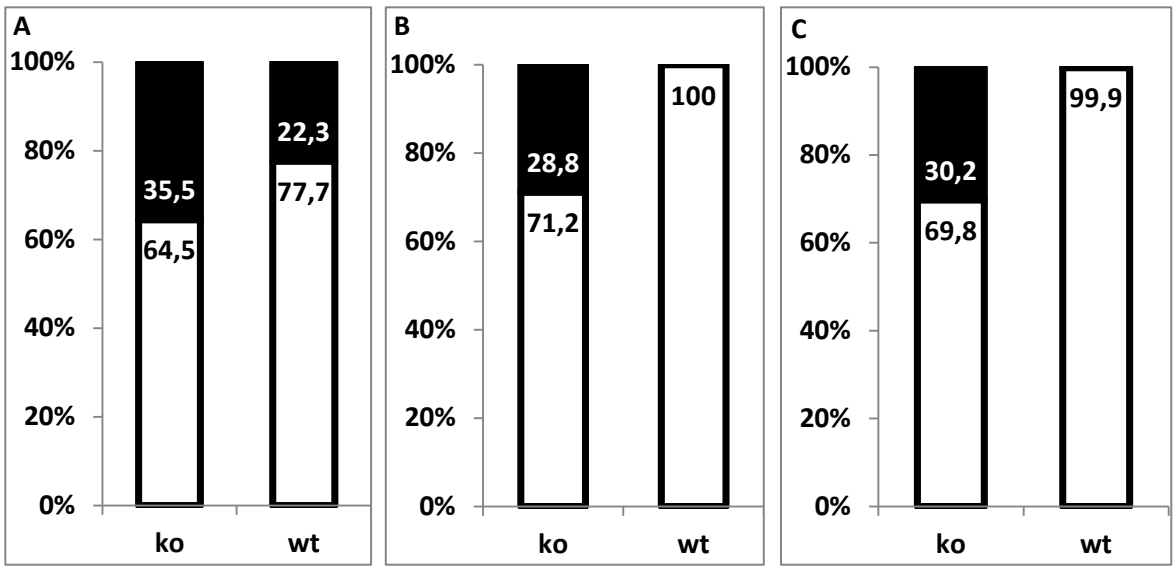

Figure 2.10: Quantification of telomere attachment in leptotene, zygotene and pachytene spermatocytes of SUN1 wildtype and SUN1 deficient mice. Telomere attachment was quantified in spermatocytes in 7µm testis sections of 12 and 14 dpp littermates subjected to TeloFISH in combination with immunofluorescent labelling of SYCP3 and lamin B. (A) Telomere attachment in early-/mid leptotene spermatocytes enriched in tubules of 12 dpp SUN1$^{+/+}$ and SUN1$^{-/-}$ mice (WT n= 16 spermatocytes, 772 telomeres; KO n = 13 spermatocytes, 645 telomeres). (B) Telomere attachment in zygotene spermatocytes enriched in tubules of 12 dpp SUN1$^{+/+}$ and SUN1$^{-/-}$ mice (WT n= 5 spermatocytes, 194 telomeres; KO n= 7 spermatocytes, 337 telomeres), (C) Telomere attachment in pachytene spermatocytes enriched in tubules of 14 dpp SUN1$^{+/+}$ and SUN1$^{-/-}$ mice. (WT n= 54 spermatocytes, 2138 telomeres; KO n= 31 spermatocytes, 1150 telomeres).

In summary, the quantifications revealed that the telomeres which attach to the NE in SUN1$^{-/-}$ mice during leptotene, remain attached throughout prophase I in males.

SUN2 and KASH5 mediate telomere attachment in SUN1 deficient spermatocytes

Meiotic telomeres in the absence of SUN1 are frequently and stably attached to the NE, as demonstrated above. Generally, meiotic telomere attachment is, as described in detail before, mediated by the LINC complex. Previous studies in mice have shown that SUN1 and SUN2 specifically localised to the attached telomeres during meiosis (Ding et al., 2007; Schmitt et al., 2007). KASH5, a novel ONM KASH protein, also localises to the attached meiotic telomeres and connects them to components of the cytoskeleton (Morimoto et al., 2012). Which of these LINC complex components are possibly also involved in the attachment of the telomeres in the absence of SUN1, is not clear. Because SUN2 has been shown to localise to attached meiotic telomeres, it may be possible that SUN2 is also involved in the attachment of meiotic telomeres in the absence of SUN1. To investigate whether SUN2, the only other known SUN domain protein to be expressed in meiotic cells, mediates the telomere attachment in SUN1 deficient mice, a SUN2-specific antibody was raised and affinity purified. To analyse the SUN2 localisation, this anti-SUN2 antibody was used in colocalisation with an anti-SYCP3 antibody in immunofluorescence experiments on SUN1 wildtype and knockout spermatocytes. The immunofluorescence analyses of the wildtype testis sections revealed that SUN2 foci are found to be located within the NE at the end of SCs (Figure 2.11). Therefore, the SUN2 foci are located at the attached telomeres of the wildtype spermatocytes. This is in accordance with earlier publications, which described the NE protein SUN2 to localise at attached telomeres during prophase I (Schmitt et al., 2007). Strikingly, similar SUN2 foci were also detectable in the SUN1 deficient spermatocytes. Here too, the SUN2 foci are exclusively found within the nuclear periphery at ends of SCs and no SUN2 foci are found within the nuclear interior. This demonstrates again that some telomeres area attached in the absence of SUN1 and shows that SUN2 is localised within the NE at the attached telomeres also in the absence of SUN1. Additionally, this indicates that the attachment of telomeres in the absence of SUN1 is indeed mediated by SUN2.

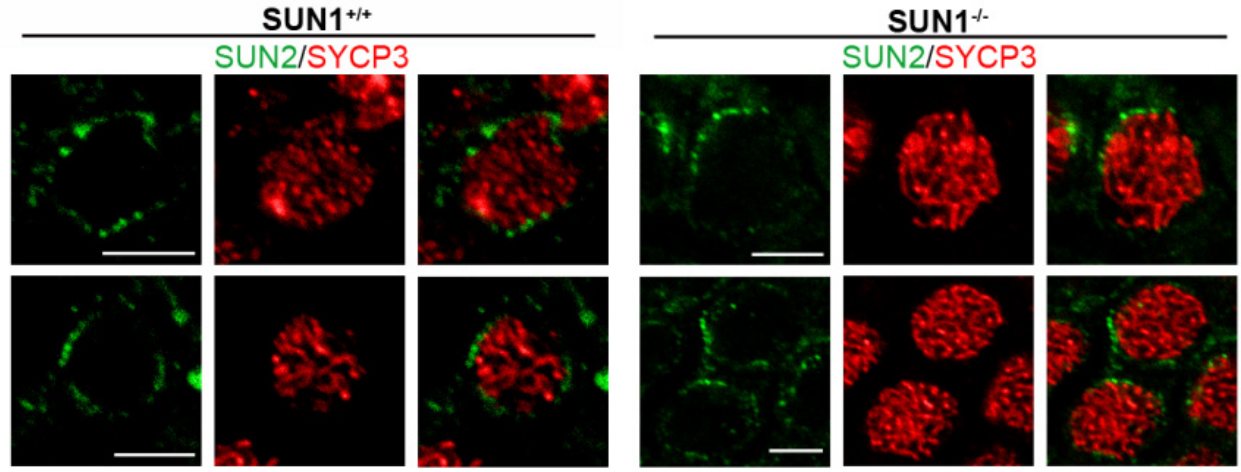

Figure 2.11: SUN2 localisation on SUN1$^{+/+}$ and SUN1$^{-/-}$ spermatocytes. Immunofluorescence using gp anti-SUN2 and rabbit anti-SYCP3 antibodies on 3 µm paraffin sections of 12 dpp aged SUN1 wildtype and SUN1 deficient male mice. Clearly in SUN1$^{+/+}$ spermatocytes of late leptotene/early zygotene (top row) and late zygotene (bottom row) stages SUN2 foci are located in the nuclear periphery at the ends of SC axes. In SUN1$^{-/-}$ spermatocytes similar SUN2 foci are also detected, here too located at the ends of SCs.

The analyses using a SUN2-specific antibody indicated that SUN2 is involved in the attachment of telomeres in the absence of SUN1. However, it still remained to be determined if SUN2 is also part of a complete LINC complex, connecting the attached telomeres to the cytoskeleton. If SUN2 is part of a complete LINC complex in the absence of SUN1, this meiotic LINC complex can be assumed to include KASH5 because it is the only known mammalian meiotic KASH-domain protein. To investigate whether KASH5 is localised to the attached telomeres in the absence of SUN1, immunofluorescence analyses using a KASH5-specific antibody were conducted. Testis sections of SUN1 wildtype and knockout mice were subjected to anti-KASH5 and anti-SYPC3 antibodies (Figure 2.12) to visualize a potential localisation of KASH5 relative to the SC axes. In wildtype pachytene spermatocytes strong KASH5 foci were detected at the distal ends of SC axes. This specific KASH5 localisation was in accordance with published results and demonstrates that the telomeres in the wildtype spermatocytes are attached and connected to the cytoskeleton through a complete LINC complex (Morimoto et al., 2012). Detailed analyses of the SUN1 deficient spermatocytes also showed KASH5 foci that were located at the ends of SCs within the nuclear periphery. However, the KASH5 signals in the knockout spermatocyte had a significantly reduced intensity compared to the signals detected in the wildtype cells. Nonetheless, this shows that the attachment of telomeres in the absence of SUN1 involves both SUN2 and KASH5.

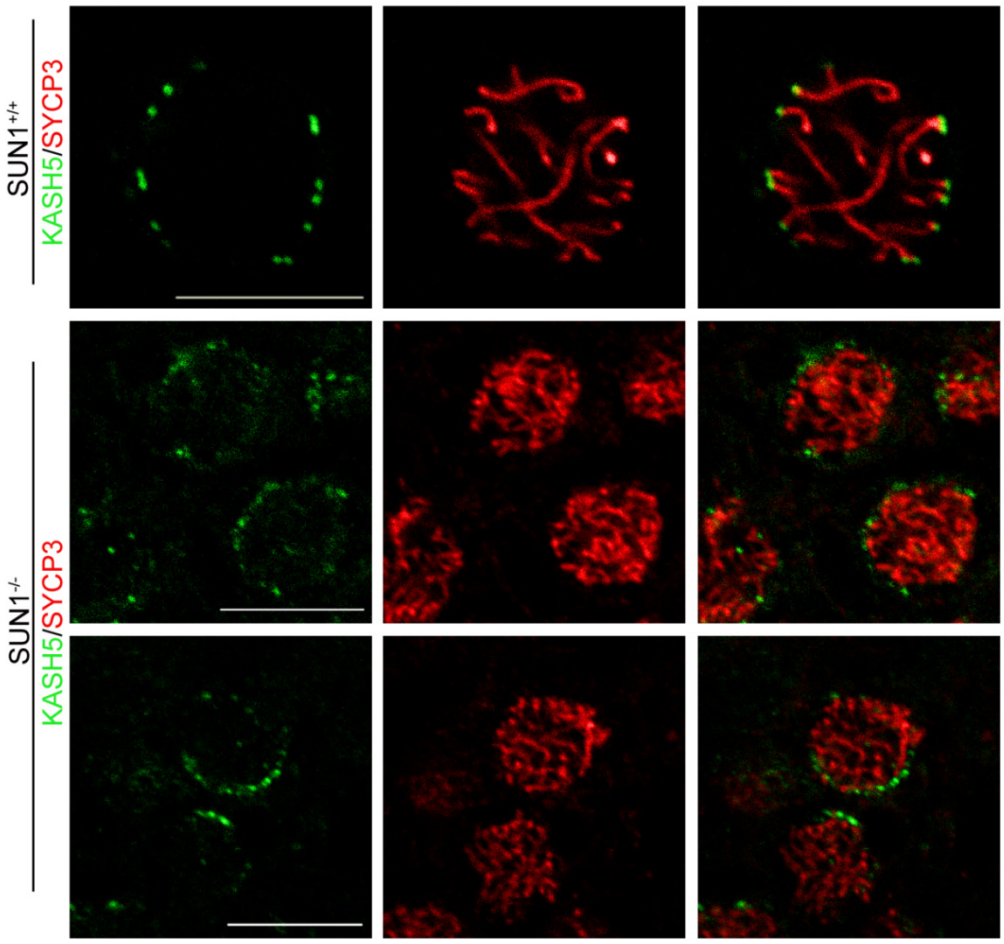

Figure 2.12: KASH5 localisation on SUN1$^{+/+}$ and SUN1$^{-/-}$ spermatocytes. Immunofluorescence using mouse anti-KASH5 and rabbit anti-SYCP3 antibodies on 3µm paraffin sections of 12 dpp aged SUN1 wildtype and SUN1 deficient male mice. The pachytene wildtype spermatocytes show the expected KASH5 foci located at the ends of the paired SCs. In SUN1 deficient spermatocytes KASH5 foci are also found to be located at the ends of SCs although the signal intensity is reduced substantially. Scale bars 5 µm.

The analyses above have shown separately that both SUN2 and KASH5 are localised at the ends of SCs within the NE in the absence of SUN1. If SUN2 and KASH5 are involved in one complete LINC complex to attach meiotic telomeres to the NE and link them to the cytoskeleton, this requires their localisation within the NE to overlap. To complement the separate localisations of SUN2 and KASH5, co-immunofluorescences using anti SUN2- and KASH5-antibodies synchronously were performed on SUN1 wildtype and knockout testis sections (Figure 2.13). The immunofluorescence analyses of wildtype spermatocytes show a clear colocalisation of the KASH5 and SUN2 signals in the periphery of the nucleus. Both LINC complex components show the expected pattern of distinct, overlapping foci in the NE. This indicates that in the wildtype SUN2 and KASH5 may be part of one LINC complex, as they share the same localisation. In the SUN1 deficient spermatocytes SUN2 and KASH5 foci are also observed to co-localise clearly in the nuclear periphery. This indicates that, also in the absence of SUN1, SUN2 and KASH5 are involved in the same LINC complex. This final piece of evidence conclusively reveals that the telomere attachment to the NE in the absence of SUN1 is mediated by SUN2 and KASH5, probably forming a complete LINC-complex. Therefore, the attached telomeres in the SUN1 deficient spermatocytes should also be connected to the cytoskeleton.

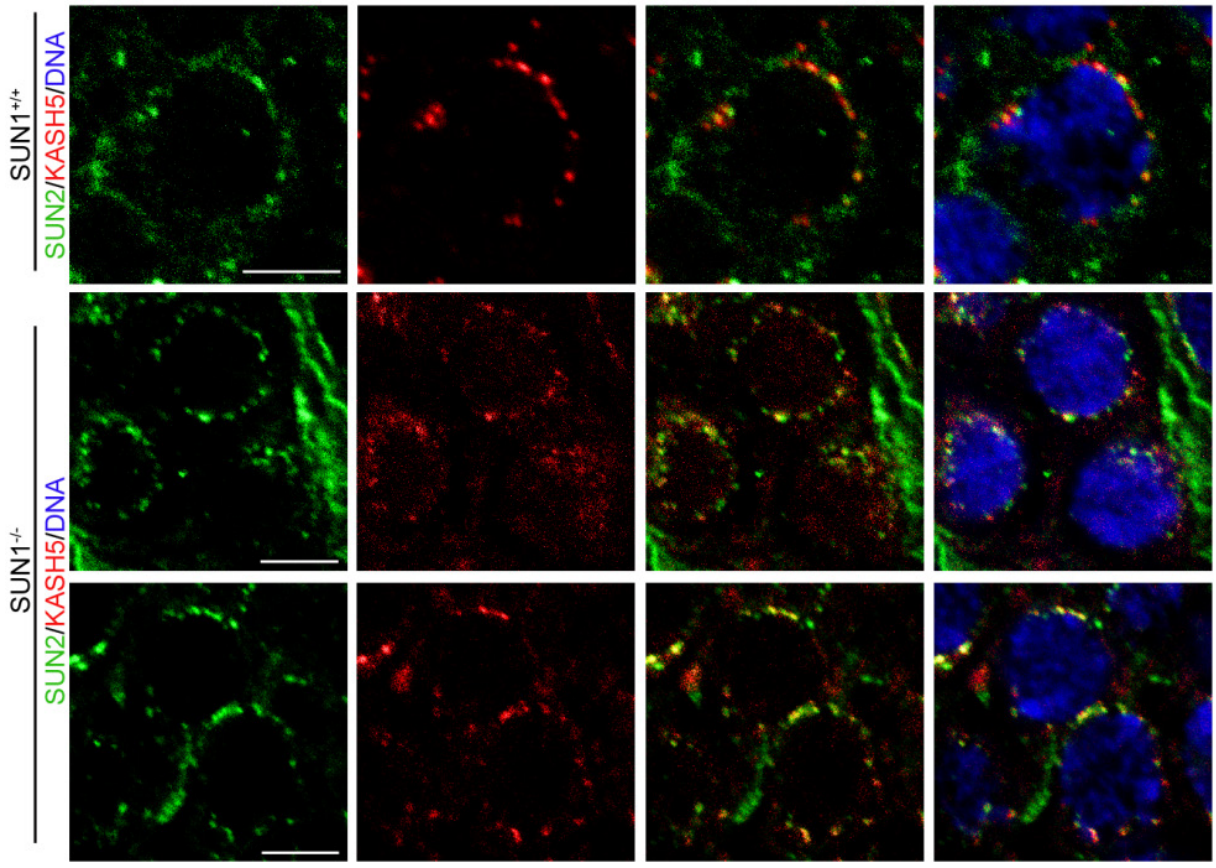

Figure 2.13: KASH5 and SUN2 colocalisation on SUN1$^{+/+}$ and SUN1$^{-/-}$ spermatocytes. Immunofluorescence using gp anti-SUN2 and rabbit anti-KASH5 antibodies on 3µm paraffin sections of 12 dpp aged SUN1 wildtype and SUN1 deficient male mice. DNA is counterstained using Hoechst 33528. In both the wildtype and knockout spermatocytes SUN2 and KASH5 focus patterns colocalise at the periphery of the nuclei. Scale bars 5 µm.

Attached telomeres in SUN1 deficient mice are able to form bouquet-like clusters

The previous analyses have demonstrated clearly that in SUN1 deficient mice a relatively large proportion of telomeres is attached to the NE, despite the absence of SUN1. Through further investigations it has also become evident that the attachment of telomeres in the absence of SUN1 is mediated by a SUN2-KASH5 LINC complex, which may connect the attached telomeres to the cytoskeleton. Generally, the cytoskeleton generates the forces which move the attached telomeres during meiosis to perform the highly conserved telomere-led chromosome dynamics. The movement of telomeres within the NE requires a complete and functional LINC complex. Therefore, it was a further aim to elucidate if the attachment of telomeres to the NE in mice lacking SUN1 is not only physically stable but also functional. A basic functionality of the meiotic LINC complex includes the ability to cluster attached telomeres within a restricted region of the NE to form the meiotic bouquet. Therefore, it was first investigated if the attached telomeres in the SUN1 deficient mice are at all able to cluster within a bouquet or bouquet-like conformation. For this, testis tissue sections of 12 dpp littermate wildtype and knockout mice were used as here leptotene and zygotene staged spermatocytes should be accumulated within the tubules, leading to an enrichment of bouquet stages. By using anti-KASH5 and anti-SYCP3 antibodies, only telomeres which are attached to the NE are labelled indirectly by the KASH5 antibody. Confocal images of these preparations were used to reconstruct entire nuclei three dimensionally, thus enabling to distinguish between spermatocytes with clustered or non-clustered telomere patterns. In the wildtype mice, leptotene and zygotene spermatocytes with both clustered and non-clustered telomere patterns were observed as would be expected for mice of this age (Figure 2.14). Interestingly, in the SUN1 deficient leptotene and zygotene spermatocytes, clustered telomere patterns were also observed commonly. This demonstrates that the LINC complex attaching telomeres in the SUN1 deficient mice is sufficiently functional to allow the formation of bouquet-like clusters at leptotene or zygotene.

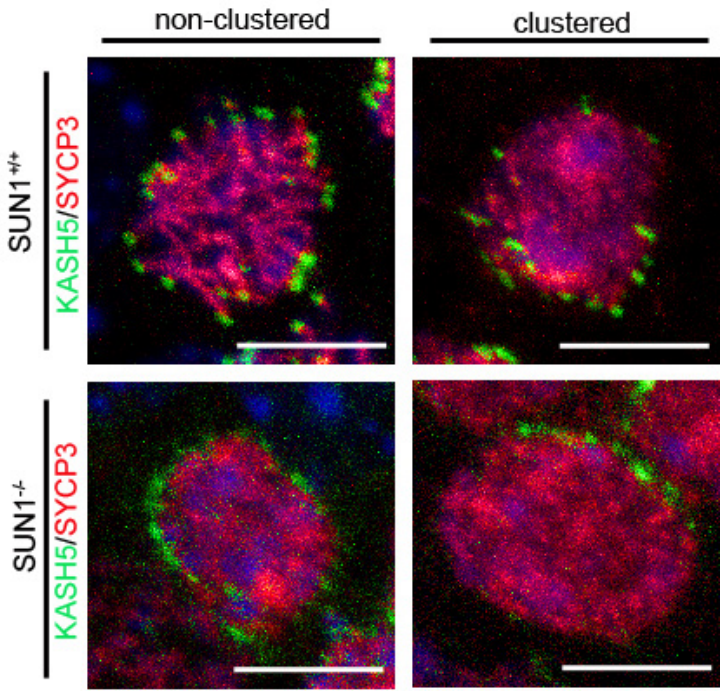

Figure 2.14: Distributions of attached telomeres in spermatocytes of wildtype and SUN1 deficient 12 dpp littermates. Z-projections of entire nuclei with non-clustered and clustered patterns of attached telomeres labelled through the mouse anti-KASH5 antibody (green). SCs are visualised by a rb anti-SYCP3 (red) antibody and DNA is counterstained using Hoechst 33528. In wildtype spermatocytes clustered and non-clustered KASH5 foci are clearly distinguishable. Spermatocytes exhibiting non-clustered KASH5 patterns are commonly of later meiotic stages than those showing clustered patterns. Similarly, tightly clustered KASH5 patterns are also found in earlier stages of SUN1 deficient spermatocytes. Scale bars 10µm

As demonstrated by the bouquet-like clusters in the absence of SUN1, the SUN2-KASH5 LINC complex is *per se* functional to move and attach telomeres during meiosis. Moving on from this conclusion, the next investigations were conducted to define whether bouquet-like clustered telomere patterns are a rather rare event in SUN1 deficient mice or occur at similar frequencies compared to the wildtype. The frequency of bouquet formation indicates whether the timely process of bouquet formation is comparable between wildtype and knockout siblings, thereby investigating the functionality of the SUN2-KASH5 LINC complex further. Bouquet frequencies were quantified in spermatocytes of 12 dpp littermate knockout and wildtype mice. For this, confocal images of single nuclei of wildtype and knockout spermatocytes labelled by anti-KASH5 and anti-SYCP3 antibodies were reconstructed three dimensionally and judged for their distribution of KASH5 foci within the NE. The wildtype spermatocytes showed a relatively high frequency of clustered telomere patterns (70% with clustered telomere patterns; n= 50 spermatocytes). This is in accordance with the progression of the first wave of spermatogenesis in this age and characteristic for leptotene/zygotene spermatocytes. Intriguingly, in the knockout spermatocytes similar frequencies of bouquet-like conformations are also observed (79.6% with clustered telomere patterns; n=64 spermatocytes). These quantifications therefore indicate that the formation of bouquet-like clusters of telomeres attached in the absence of SUN1 occurs at comparable frequencies as in the wildtype. Furthermore, the bouquet formation does not seem to be delayed in the absence of SUN1. Thus, the attachment of meiotic telomeres through SUN2 and KASH5, demonstrated in the SUN1 deficient spermatocytes, is *per se* capable of functionally attaching meiotic telomeres to the NE.

In summary, these data clearly show that telomere attachment is impaired in the absence of SUN1 but not completely absent as published before (Ding et al., 2007). Although this impaired telomere attachment is severe enough to cause male and female infertility, it has become clear that SUN2 is indeed sufficient for the stable and functional attachment of meiotic telomeres *per se*, forming a LINC complex with KASH5.

3. Discussion

Meiosis is an essential process in the formation of gametes competent for fertilisation, as it reduces the former diploid genome to a haploid one. The correct and exact formation of haploid gametes is essential for their successful fusion at fertilisation and the subsequent development of a viable zygote. During meiosis, chromosomes are involved in a highly regulated choreography, leading to the faithful pairing of homologous chromosomes. The stable and unambiguous pairing of homologous chromosomes during prophase I of meiosis is critical for their correct alignment at metaphase I and their separation by the bipolar spindle in anaphase I. Incorrect or instable pairing results in the unequal separation of the genome into non-competent gametes or commonly even in apoptosis of gametes prior to their maturation. Either defects result in the partial or complete infertility of the organism. To achieve the precise pairing of homologous chromosomes and an efficient meiotic progression, telomeres attach to the NE at the beginning of prophase I. The attached telomeres perform highly conserved movements within the NE, leading to the formation of the bouquet stage and its subsequent release. The attachment of telomeres to the NE and their directed movement within the NE are highly conserved and special features of meiosis. The intimate connection between chromatin, chromatin associated proteins and the NE is stable enough to withstand forces generated within the nucleus and yet flexible enough to allow the motion of attached telomeres within the NE. Because the attachment of telomeres only occurs in cells of meiotic prophase I, it can be assumed to be mediated by meiosis-specific and/or highly regulated components of the NE. Furthermore, both the attachment of telomeres to the NE and their efficient movement along it have shown to be critical for the correct pairing of the homologous chromosomes and therefore for fertility. The NE of meiotic cells differs substantially in its composition and structure to that of somatic cells. The meiosis-specific NE and lamina mediate the telomere attachment and are the platform for the telomere movement unique to meiosis. However, the functional or regulatory role of many of the meiotic NE components, especially regarding telomere attachment and repositioning, is still unclear. Also, the precise function and significance of the highly conserved telomere movement is not entirely understood yet.

In this thesis, the functions of meiotic NE components in telomere attachment and movement, and thus their significance for mammalian fertility, were investigated. Specifically, the role of the meiotic nuclear lamina in telomere attachment and repositioning was analysed. Since lamin C2 is the only A-type lamin expressed during meiosis, these investigations were performed using a lamin C2 deficient mouse strain (section 2.1). Separately, the precise meiotic role of SUN2, which to date is controversially discussed, was also analysed. Although SUN2 has been described to localise to attached telomeres, a clear meiotic function in telomere attachment remained to be demonstrated. Due to the shared localisation of SUN2 with SUN1 to attached telomeres, and their potentially redundant functions, the investigations were performed in a SUN1 deficient mouse strain (section 2.2).

Work in this thesis was able to define the meiotic nuclear lamina as a determinant for the correct pairing of homologous chromosomes. Furthermore, the role of meiotic SUN2 in LINC complex formation and functions, and thus in telomere attachment and movement, was also elucidated.

Therefore, this thesis substantiates the critical role of the meiotic NE as a functional and regulatory platform for processes essential for fertility.

3.1 Contributions of nuclear envelope components to meiotic telomere attachment

The NE is substantially reorganised at the onset of meiosis through the expression of meiosis-specific components and the relocalisation and regulation of other non-meiosis-specific components. This extensive reorganisation of the NE is also reflected in the meiotic lamina. The lamina of meiotic cells is only composed of lamin B1 and the meiosis-specific A-type lamin C2 (Alsheimer and Benavente, 1996; Jahn et al., 2010). Lamin C2 exhibits a number of peculiar, unique features which distinguish it clearly from other somatic A-type lamins (Alsheimer et al., 1999; Alsheimer et al., 2000; Jahn et al., 2010; Jahn, 2012).

The specifically meiotic expression of lamin C2 and its unique features have raised the question of the function of lamin C2, and thereby the function of the nuclear lamina, in meiotic cells in vivo. Because the expression and localisation of lamin C2 coincides with the attachment and movement of meiotic telomeres to the NE, it had been postulated that lamin C2 plays essential roles in these meiosis-specific processes. Lamin C2 was suggested to be directly involved in or essential for the attachment of telomeres to the NE and/or their repositioning within the NE (Jahn, 2012; Jahn et al., 2010; Alsheimer et al., 1999). To investigate the role of lamin C2 in detail and in vivo, a lamin C2 deficient mouse strain was constructed (Schmitt, 2008). In this mouse strain, the expression of lamin C2 was specifically disrupted, leaving the expression of somatic lamins A and C intact. This was particularly important, as the disruption of somatic lamins A and C lead to laminopathy-associated phenotypes of the mice, showing severe effects on health and viability (Alsheimer et al., 2004). However, the lamin C2 deficient mice were viable and healthy, yet males were infertile. This indicated that lamin C2 does indeed fulfil an essential meiotic function. Further analyses revealed that the lamin C2 deficient males showed severe defects in homologous pairing, leading to apoptosis of the spermatocytes, which explains the infertility (Schmitt, 2008; Jahn, 2012). Therefore, a component of the NE seems to be the cause for defective homologous pairing of the chromosomes. The most direct way for the NE in meiosis to influence chromosome behaviour is at the attached telomeres. Because lamin C2 additionally enriches at the sites of telomere attachment, a possible function of lamin C2 in the attachment of meiotic telomeres was investigated. Recent work revealed however, that telomere attachment is not affected in the absence of lamin C2. Rather, telomeres are quantitatively attached to the NE both in lamin C2 deficient and control spermatocytes. Therefore, a function of lamin C2 in the attachment of telomere to the NE could be excluded (Link, 2010; Link et al., 2013a). By this, the nuclear lamina of meiotic cells has thus been demonstrated to not be directly involved in or essential for the attachment of telomeres to the NE. Because, however, lamin C2 is essential for male fertility, it is nonetheless assumed to be serving another role critical for meiosis. A different proposed function of lamin C2, especially due to its unique ability to influence NE integrity and its high mobility, was that it may be involved in or essential for the movement of attached telomeres. This aspect was thoroughly investigated in this thesis. The role of the meiotic nuclear lamina in telomere movement is discussed in detail below (section 3.2).

Defects in telomere attachment have in other meiotic models been demonstrated to be the cause for meiotic abrogation and thus infertility (Ding et al., 2007; Viera et al., 2009). The LINC complex, composed of SUN and KASH domain proteins, is in a widely conserved manner essential for the correct and efficient attachment of telomeres to the NE. Studies in *S.pombe,* in *S.cerevisiae* and *C.elegans* have all demonstrated that a SUN domain protein in the INM together with a KASH domain protein of the outer nuclear membrane are essential for the attachment of telomeres to the NE. In *S.pombe* the only expressed SUN domain protein Sad1 is transiently relocalised to tether attached telomeres to the SPB upon induction of meiosis (Jin et al., 2002; Chikashige et al., 2006). Similarly, in *C.elegans* the SUN domain protein SUN-1 is relocalised into distinct foci associated with the attached chromosomes at the induction of meiosis (Penkner et al., 2007; Penkner et al., 2009). In mice, the almost ubiquitously expressed SUN domain proteins SUN1 and SUN2 have been shown to relocalise during meiosis as well, forming distinct foci within the NE at sites of telomere attachment (Ding et al., 2007; Schmitt et al., 2007). SUN1 has been demonstrated to be required for meiotic telomere attachment, as SUN1 deficient mice show defective telomere attachment and are consequently infertile (Ding et al., 2007). The impaired telomere attachment leads to defects in the pairing of the homologous chromosomes, which is then the cause for the apoptosis and observed infertility of the male and female mice deficient for SUN1. Although SUN2 has been demonstrated to also localise to the attached telomeres in mice, its contribution to telomere attachment has previously remained elusive. Additionally, contradicting published results have led to a controversial discussion regarding the meiotic role of SUN2. Therefore it was an aim of this thesis to elucidate the meiotic function of SUN2 in LINC complex formation and function. In somatic cells SUN1 and SUN2 also share their homogenous distribution within the NE. Here, studies in SUN1 deficient and SUN1/2 deficient mice have shown that SUN1 and SUN2 play partially redundant roles in nuclear positioning (Lei et al., 2009). Therefore, if SUN2 is involved in meiotic telomere attachment as well, this would indicate a, at least partially, redundant role of SUN1 and SUN2 in meiosis as well.

To study the meiotic function of SUN2 in this thesis, without influences of the co-localising SUN1, the localisation of telomeres within the nucleus was investigate in detail in SUN1 deficient spermatocytes. Surprisingly, immunofluorescence analyses and investigation on the electron microscopy level of SUN1$^{-/-}$ spermatocytes clearly demonstrate that a fraction of telomeres is still attached to the NE, even in the absence of SUN1. Multiple attachment sites observed in the electron micrographs resembled the wildtype attachment sites of telomeres. To validate this observation, additional testis tissue was obtained from a separately developed SUN1 deficient mouse strain (Ding et al., 2007) and analysed for telomere attachment in the electron microscope. In this SUN deficient mouse strain as well, multiple telomeres were found to be attached to the NE by wildtype-looking attachment sites. Quantifying the attached telomeres in the SUN1$^{-/-}$ mice showed that approximately two thirds of the telomeres are attached in the absence of SUN1. By further quantifying telomere attachment in leptotene and zygotene spermatocytes, it was also demonstrated that telomeres which manage to attach in the absence of SUN1, remain stably attached to the NE throughout prophase I. This indicates that in the absence of SUN1 a majority of telomeres is still stably attached to the NE. Further analyses in this thesis showed a distinct localisation of SUN2 foci at NE-attached SC ends in the SUN1 deficient spermatocytes. This demonstrated that SUN2 is involved in the attachment of the telomeres in the absence of SUN1. The SUN2 foci located at the attached

telomeres in SUN1 deficient mice were furthermore co-localised with KASH5, the meiosis-specific KASH domain protein involved in telomere attachment. Therefore, the analyses demonstrated that SUN2 is involved in a complete LINC complex with KASH5 and that this LINC complex is capable of attaching meiotic telomeres stably to the NE. This indicates in summary that SUN1 and SUN2 play, at least partially, redundant roles during mammalian meiosis because they are both able to stably attach telomeres to the NE.

In somatic cells, partially redundant functions of SUN1 and SUN2 have been described in nuclear positioning and anchorage by analysing SUN1 and SUN1/SUN2 double knockout mice (Zhang et al., 2009; Yu et al., 2011; Lei et al., 2009). The partially redundant role of SUN1 and SUN2 during meiosis demonstrated in this thesis therefore is not a distinctive feature of meiotic SUN1/SUN2 function but rather seems to be in addition to the description of the general partially redundant function of mammalian SUN1 and SUN2. Generally, SUN1 and SUN2 seem to be able to partially compensate each other's functions, both in somatic and meiotic cells. This is also demonstrated by the SUN1-independent localisation of SUN2 in somatic cells as well as meiotic cells ((Yu et al., 2011), this thesis section 2.2). The compensation of SUN1 by SUN2 is additionally demonstrated by the absence of a somatic phenotype in SUN1 deficient mice. In these mice, SUN2 apparently compensates all somatic SUN1 functions sufficiently to allow normal development, cell growth and cellular phenotype. Merely the meiotic function of SUN1 seems to be compensated only partially, resulting in the impaired attachment of meiotic telomeres and infertility. To investigate the single function of SUN2 in comparison to SUN1 directly, a SUN2-deficient mouse strain would have to be evaluated. If SUN1 and SUN2 do fulfil partially redundant functions and are reciprocally able to compensate each other, at least in part, a supposed SUN2 deficient mouse should exhibit a similar phenotype to $SUN1^{-/-}$ mice. Apparently however, SUN2 deficient mice, used for the construction of the SUN1/SUN2 double knockout mice, showed no meiotic or somatic phenotype (Lei et al., 2009). This would suggest that SUN1 alone is sufficient to attach all meiotic telomeres to the NE. This observation contradicts a hypothesis of partially redundant meiotic function of SUN1 and SUN2. However, the constructed SUN2 deficient mouse strain used in this publication has not been clearly demonstrated to be a functional and complete knockout. In this approach, exons 11-16 of the SUN2 gene were targeted, which include the coiled-coil domain and the first half of the SUN-domain (Lei et al., 2009). However, the transmembrane domains and a substantial C-terminal part of the SUN domain are left intact. This could essentially result in an alternatively spliced shortened, yet partially functional, transmembrane protein, which may fulfil partial functions of the SUN2 protein. A similar situation is found in apparent *LMNA*-null mice, where in fact a shortened A-type lamin isoform is still expressed (Jahn et al., 2012). Therefore these results regarding the SUN2 deficient mice have to be handled with caution. A complete functional knockout of the SUN2 protein, especially including the transmembrane domain, would have to be analysed to elucidate if SUN1 fully or partially compensates meiotic SUN2 function.

Mammalian KASH domain proteins Nesprin1 and 2 are also suggested to fulfil, at least in some cell types, partially redundant or similar functions (Yu et al., 2011). However, both Nesprin1 and 2 are found in an enormous variety of splicing isoforms. The demonstrated compensation of similar functions is only restricted to splicing variants found in the developing eye. Multiple other splicing isoforms of Nesprin1 and 2 are suggested to function in a tissue specific manner, connecting the

nucleus to versatile components of the cytoskeleton. A similar situation, showing the compensation of one LINC complex component by another, has not been demonstrated in other species yet. However, the possibility of SUN domain proteins compensating each other is, for example, *per se* excluded in fission and budding yeast as both express only one SUN domain protein. In *C.elegans*, where two SUN domain proteins are known to exist, redundant functions have not been reported (Fridkin et al., 2004; Malone et al., 1999). The almost ubiquitous expression of two similar SUN domain proteins in mammals seems to be a feature of more complex organisms, as neither *C.elegans* nor *D.melanogaster* are known to express two ubiquitous SUN domain proteins. Therefore the partially redundant functions, and possible compensations, are only likely in organisms expressing multiple LINC complex components in similar expression patterns.

The partial redundancy of SUN1 and SUN2 in meiosis, demonstrated clearly in this thesis, leads to an impaired telomere attachment in the SUN1 deficient mice, but not a complete loss of telomere attachment as published earlier (Ding et al., 2007). A complete loss of telomere attachment in mice has, until today, not been observed in any meiotic knockout mouse model. In Smc1β deficient mice, telomere attachment has been observed to be impaired, but not completely lost (Adelfalk et al., 2009). Smc1β is a meiosis-specific cohesin essential for the complete sister chromatid cohesion. In $Smc1\beta^{-/-}$ spermatocytes, one firth of the telomeres fail to attach to the NE, resulting in reduced numbers of SUN1 foci. In this mouse model, the impaired telomere attachment is probably induced by telomeric instability. Nonetheless, in this mouse model, as well, telomere attachment is only disrupted but not entirely lost. In mice deficient for CDK2 (cyclin-dependant kinase 2) disrupted telomere attachment was also observed. Here around 50% of the telomeres were still attached in the absence of CDK2, leading to male and female infertility (Viera et al., 2009). In mice, it seems that the absence of a single protein involved in telomere attachment or the regulation of attachment is not sufficient to prevent telomere attachment completely. This contradicts the situation in fission yeast for example. Here, the absence of a single protein, bqt4, is sufficient for the complete loss of telomere attachment. Bqt4 is a yeast-specific connector protein between the chromosome ends and Sad1 and its absence cannot be compensated by any other protein (Chikashige et al., 2009). The only partial defects in telomere attachment observed in mammalian meiosis suggest extensive redundancy, beyond that of SUN1 and SUN2, in the mechanism involved in telomere attachment in mammals. Mammalian telomere attachment seems to be regulated by a multi-factored network in which a number of components are able to perhaps partially compensate each other.

Although telomere attachment is partially disturbed in the SUN1 deficient mice, SUN2 has clearly demonstrated to be involved in a complete LINC complex interacting with KASH5 to attach meiotic telomeres in vivo, at least in the absence of SUN1. Nonetheless, SUN2 is also localised, together with KASH5, at attached telomeres in wildtype spermatocytes ((Schmitt et al., 2007) and this thesis section 2.2). Furthermore, KASH5 has been demonstrated to interact with both SUN1 and SUN2, indicating that a SUN2/KASH5 LINC complex is also likely in the presence of SUN1 (Morimoto et al., 2012). SUN1 and SUN2 have shown to be able to interact with each other through their coiled-coil domains, forming both hetero- and homopolymers and they are likely to also interact in vivo (Lu et al., 2008; Wang et al., 2006). A very recent crystallography study elucidating the structure of an entire LINC complex could show that SUN proteins within a LINC complex, in this particular case SUN2, actually form trimers rather than dimers (Sosa et al., 2012). Therefore it is conceivable that

SUN1 and SUN2 form heterotrimers or homotrimers during meiosis as well, to attach the telomeres to the NE. Also the parallel presence of separate SUN1 and SUN2 homotrimers to attach a telomere in a cooperative manner is possible. However, in which protein ration SUN1 and SUN2 are involved in the attachment of meiotic telomeres is entirely unknown. Possibly, the single amount of either SUN1 or SUN2 expressed during meiosis is not sufficient for the complete and stable attachment of all telomeres, explaining the incomplete meiotic attachment. In either constellation, a SUN domain protein trimer interacts with three KASH domain peptides to form a hexameric, NE-spanning LINC complex (Sosa et al., 2012; Rothballer et al., 2013). Meiotic LINC complex compositions of a variety of organisms are known to date. Nonetheless, the molecular structure and protein numbers involved is so far only elucidate in mammals. Because however SUN domain proteins are so well conserved, including their coiled-coil domain, it may be quite plausible to argue that SUN domain proteins of other organisms also form trimers in vivo as part of a complete LINC complex. In the organisms in which only one SUN domain protein is present in meiotic cells for example, of course the formation of heterotrimers is not possible. The possibility of heterotrimer formation is then restricted to mammals, at least in the context of meiosis, because this is the only known group where two similar SUN domain proteins are expressed at the same time within the same cell. If however, heterotrimers of SUN1 and SUN2 are at all formed in vivo, is still unclear.

SUN1 and SUN2 have been demonstrated to fulfil functions in nuclear position in somatic cells through their interactions with Nesprin1 and 2. Upon the induction of meiosis, these ubiquitously expressed SUN domain proteins significantly alter a number of features. Their localisation changes from the homogenous dispersion within the NE in somatic cells into distinct foci at attached telomeres in meiotic cells. Here they now no longer interact with Nesprin1 or 2, but instead with the meiosis-specific KASH5. How this switch from the somatic to the meiotic function is achieved is entirely unknown in mammals. In *C.elegans*, the switch of somatic developmental functions of a SUN domain protein to its meiotic function is beginning to be understood. Here, the SUN domain protein SUN-1 is regulated through meiosis-specific phosphorylation sites. Upon the meiosis-specific phosphorylation through PLK-2, which is associated with the pairing centres during leptotene and zygotene, SUN-1 becomes increasingly mobile within the NE and forms the aggregations within the NE characteristic for prophase I. The phosphorylation has been demonstrated to be essential for the meiotic function of SUN-1 (Penkner et al., 2009; Woglar et al., 2013). In mammals, CDK2 is demonstrated to be an active kinase during meiosis and mammalian SUN1 may also exhibit potential phosphorylation sites. However, it remains to be demonstrated if the meiotic functions of mammalian SUN1 or 2 are also regulated by meiosis-specific phosphorylations.

Other important proteins are also essential for telomere attachment during meiosis, although they are not directly part of the LINC complex. The chromosome ends should selectively, and in a meiosis-specific manner, interact with the SUN domain components of the meiotic LINC complex. In mammals it is entirely unclear how this meiosis-specific interaction of telomeres with SUN1 and SUN2 is achieved in meiotic cells but avoided in somatic cells. In fission yeast a complex of meiosis-specific adaptor proteins, bqt1 and bqt2, is responsible for the connection between ubiquitous telomeric proteins and Sad1 (Chikashige et al., 2006). Due to the meiosis-restricted expression of the bqt-protein complex, the stable associating of telomeres with the LINC complex only occurs in meiotic cells. Similarly, in *C.elegans* a family of meiosis-specific zinc-finger proteins (ZIM-1 to ZIM-3

and HIM-8) have shown to be responsible for the connection between sub-telomeric regions of the chromosomes and the SUN domain protein of the meiotic LINC complex (Phillips and Dernburg, 2006; Phillips et al., 2005). The attachment of so-called pairing centres of meiotic chromosomes to the NE in *C.elegans* represents a somewhat special case in comparison to the attachment of telomeres to the NE in yeast or mammals. Therefore, it is not surprising that the Zinc-finger protein family responsible for this attachment is not conserved beyond nematodes (Phillips and Dernburg, 2006). Nonetheless, the ZIM-proteins are functional homologs of the meiosis-specific bqt proteins in yeast, attaching chromosomes specifically during meiosis to the LINC complex. Although the adaptor proteins discovered to date seem to be quite species specific, the general mechanism of expressing meiosis-specific adaptor proteins to mediate the chromosomal attachment to the LINC complex may be conserved.

3.2 Roles of the nuclear envelope in meiotic telomere dynamics

The directed and active repositioning of attached telomeres within the NE is also an extremely conserved feature of meiosis. The telomere dynamics include the formation of the meiotic bouquet at the leptotene/zygotene transition and its subsequent release before pachytene. The telomere-led chromosome dynamics coincide with the synapsis formation between homologous chromosomes as well as with events of DSB repair and recombination. Although the bouquet is found in almost any sexually reproducing species its precise function, and therefore the reason for its strong evolutionary conservation, is still somewhat debated. The telomere-led chromosome movements have been implicated to be involved in the progression of homolog search and synapsis formation as well as recombination events. In any case, defects in efficient chromosome dynamics have been demonstrated, mostly in yeast, to be associated with inefficient meiotic progression and therefore reduced fertility (Davis and Smith, 2006; Zickler and Kleckner, 1998). Nonetheless, regulating factors, driving forces as well as clear functions of the bouquet and consequences of its absence yet remain to be demonstrated, particularly in mammals. Especially how single components of the meiotic NE function in or regulate the telomere-led chromosome dynamics is still unclear.

The NE of meiotic cells substantially differs to that of somatic cells, as discussed in detail above. Part of this reorganisation is reflected in the nuclear lamina. The somatic lamina is well known for its rigidity and stabilizing functions. The nuclear lamina of meiotic cells, however, greatly differs to the somatic lamina in its structure and compositions. This implies different properties of the meiotic lamina which are perhaps associated with different functions. This change of lamina composition is exemplified by the lack of lamins A and C and the unique expression of lamin C2 as the only A-type lamin. Lamin C2 displays a number of unusual features compared to its somatic counterparts, as discussed in detail before. It influences NE shape and integrity and is highly mobile within the nuclear lamina (Jahn et al., 2010). Furthermore, lamin C2 has also been demonstrated to influence the distribution of other NE proteins when expressed ectopically in somatic cells (Jahn et al., 2010). The increased mobility of lamin C2 has been shown to be caused by a molecular peculiarity of lamin C2. Lamin C2 is an N-terminally shortened product of the *LMNA* gene, in comparison to the lamins A and C. The altered N-terminal domain is encoded through a meiosis-specific alternative starting exon

(exon 1a). The N-terminal region not found in lamin C2, the N-terminal globular head domain and the N-terminal end of the central rod, has in other somatic lamins been shown to be essential for the higher order polymerisation of the lamins. This region is substituted by a unique hexapeptide sequence (GNAEGR), which includes a myristoylation site (Alsheimer et al., 2000). Studies have clearly shown that the shortened N-terminal domain, and not the myristoylation site, is responsible for the increased mobility of lamin C2 within the nuclear lamina (Jahn et al., 2010). This altered domain structure seems to influence the polymerisation features of lamin C2, to make it less rigidly incorporated into the nuclear lamina. Nonetheless, a direct function of lamin C2 remained to be demonstrated in vivo.

In vivo, lamin C2 is expressed in oocytes and spermatocytes throughout prophase I, coinciding with the attachment and movement of telomeres as well as with synapsis formation and recombination events (Alsheimer et al., 1999; Jahn, 2012). Lamin C2 is located within the meiotic nuclear lamina inhomogeneously, enriched at sites of telomere attachment (Alsheimer et al., 1999). Lamin C2 has been demonstrated to be essential for male fertility, as shown by the infertility of lamin C2 deficient male mice (Schmitt, 2008). Furthermore, the homologous pairing and synapsis in lamin C2 deficient mice was found to be severely defective, causing meiotic abrogation during pachytene in male mice (Link, 2010; Link et al., 2013a). Males lacking lamin C2 exhibit synapsis defects in almost any spermatocytes, whereas female mice lacking lamin C2 exhibit synapsis defects in roughly one third of the oocytes (Jahn, 2012). Although lamin C2 is enriched at sites of telomere attachment, it was recently demonstrated that lamin C2 is dispensable for the attachment of meiotic telomere to the NE. Spermatocytes deficient for lamin C2 show a quantitative attachment of all telomere to the NE, exhibiting no difference to wildtype control spermatocytes (Link, 2010). Although lamin C2 is dispensable for the attachment of telomeres to the NE, it must be serving essential functions during meiotic progression as demonstrated by the infertility of mice lacking lamin C2 (Schmitt, 2008; Link et al., 2013a). Due to the unique properties of lamin C2, including its expression patter, high mobility and influence on NE integrity, it is likely that it is involved in the movement of attached telomeres, since it is dispensable for their attachment.

To investigate the possible function of lamin C2 in telomere movement, and therefore the function of the meiotic lamina, it was part of this thesis to study telomere dynamics in mice deficient for lamin C2 (section 2.1). The analyses within this thesis regarding the frequency of bouquet staged spermatocytes in pubertal knockout and wildtype littermates clearly demonstrated that spermatocytes lacking lamin C2 are capable of forming a meiotic bouquet. However, the elevated number of lamin $C2^{-/-}$ spermatocytes with clustered telomere patterns compared to the wildtype littermate cells of the ages 13 and 14 dpp indicated a significant delay in the release of the meiotic bouquet induced by the lack of lamin C2. Therefore, lamin C2 is demonstrated to be essential for the efficient release of the bouquet stage. Although telomere clusters are usually a hallmark of leptotene and zygotene, analyses of the progression of SC assembly demonstrated that SC assembly is not halted at a leptotene or zygotene stage in lamin C2 deficient spermatocytes despite the retained clustering. The SC assembly of older lamin C2 deficient mice was comparable to those of wildtype littermates, exhibiting pachytene-like SC structures in spermatocytes with clustered telomeres and the SC progression occurred despite of the frequent pairing defects. Another measure of meiotic progression, besides SC assembly, is the induction and subsequent repair of DSB during prophase I.

The induction and repair of DSBs, combined with subsequent events of recombination, are strictly regulated processes which can be used to monitor meiotic progression. Analyses investigating DSB repair and recombination in this thesis have demonstrated that DSBs are introduced into the genome in the absence of lamin C2, however their repair is inefficient and incomplete. Early stages of DSB repair, marked by RAD51 and RPA, are reached but never successfully completed. This leads to the absence of recombination events in the lamin $C2^{-/-}$ males. Nonetheless, the complete pairing of some homologous chromosomes in each spermatocyte, together with the DSB repair analyses, indicate that spermatocytes deficient for lamin C2 proceed to a pachytene-like stage. Since no later meiotic stages of lamin C2 deficient spermatocytes are detected, apoptosis is likely to occur at a pachytene-like stage preventing meiotic progression beyond this point. This contradicts the situation observed in females, as here recombination does occur, although at reduced rates (Jahn, 2012; Link et al., 2013a). Additionally, oocytes deficient for lamin C2 do proceed beyond pachytene, as lamin C2 deficient females are fertile. In summary, the analyses in this thesis lead to the conclusion, that lamin C2 is essential for the efficient release of the meiotic bouquet. Since the attachment of telomeres is intact in lamin C2 deficient mice, the inefficient telomere movement seems to cause the defects in homologous pairing observed in male and females lamin $C2^{-/-}$ mice. These pairing defects in consequence are likely to cause the inefficient DSB repair in males and the reduced recombination in females. It had been postulated before that lamin C2, through its unique properties, may locally alter NE properties to allow efficient telomere movement (Alsheimer et al., 2000; Jahn et al., 2010). Work in this thesis, together with the work on lamin C2 females, substantiates this model. The delayed bouquet resolution observed in the absence of lamin C2 indicates that lamin C2 is essential for the efficient movement of attached telomeres within the NE. Taking into account that lamin C2 exhibits increased molecular mobility and the ability to alter NE properties, it seems to locally and temporally modulate the NE to increase its flexibility and therefore allow the efficient movements of telomeres. The lack of lamin C2 then could lead to an increasingly rigid NE and thereby interfere with the directed movement of the attached telomeres, causing the observed delay in bouquet resolution (see figure 3.1).

Lamin C2 has been demonstrated to be essential for the efficient release of the meiotic bouquet. The inefficient release of the bouquet stage seems to be the underlying cause for the defects in homologous pairing, leading to inefficient DSB repair and absent recombination in males. Elevated bouquet frequencies have also been observed in mice with defects in double strand break induction ($SPO11^{-/-}$ mice) and with defects in late recombination events ($MLH1^{-/-}$ mice) (Liebe et al., 2004). In these models it seems, that the failure in the very early and very late recombination events leads to a delay in meiotic telomere dynamics thus causing the elevated bouquet frequencies. This contrasts the situation in lamin $C2^{-/-}$ males, where DSB are induced and early recombination proteins are recruited but the latest recombination stages are never reached due to the prior meiotic abrogation. Therefore, in lamin $C2^{-/-}$ males, defects in DSB induction or repair do not seem to cause the elevated bouquet frequencies. Rather the inefficient DSB repair and recombination seems to be the consequence of delayed bouquet release. This illustrates, that not only SC formation, DSB repair and recombination are interdependent processes of each other, but furthermore that these processes together are interdependent of the telomere-led chromosome dynamics as well. Obviously, defects occurring prior to the formation of the bouquet, like the absence of DSB induction, are able induce

prolonged bouquet stages, but also the inefficient release of the bouquet stage is able to induce defects of intermediate recombination events. Furthermore, the inefficient bouquet release is likely to induce pairing defects. This illustrates the complex and highly interdependent nature of mammalian meiotic progression. In lamin C2 deficient females, for that matter, MLH1 foci are present showing that the lack of lamin C2 does not prevent recombination *per se* from occurring (Jahn, 2012). This, in addition, supports that the delay in telomere movement causes the observed meiotic defects and not vice versa. Additionally it shows, that the processes of prophase I may be differently dependant on each other or regulated by varying mechanism in males and females.

The sexual dimorphism observed in the lamin C2 deficient mouse strain, is quite prominent. Males deficient for lamin C2 exhibit severe defects in homologous pairing, absent homologous recombination and apoptosis of spermatocytes at mid-pachytene. This leads to the complete infertility of these male mice. Females of the same strain exhibit less severe defects in homologous pairing and recombination events occur at late pachytene, although at reduced rate. DSB repair was observed to be less efficient, probably causing the reduced recombination frequency (Jahn, 2012). Nonetheless, lamin C2 deficient females are fertile. This sexual dimorphism is also observed in knockout mouse models of other meiotic proteins. A prominent example of this is the SYCP3-deficient mouse strain in which males are infertile due to the defects in synapsis formation, yet females defective for SYCP3 are fertile (Yuan et al., 2000). In both of these mouse models, defective synapsis leads to infertility of the males but not of the females. This contrasts the situation in mouse models which show defects in telomere attachment, like the SUN1 or CDK2 knockout mice, where males and females are equally affected and both infertile (Ding et al., 2007; Viera et al., 2009). Regarding defects in homologous pairing, spermatocytes seem to be more stringently controlled than oocytes. This is exhibited by the induced apoptosis in spermatocytes but not in oocytes. Therefore it seems that the accurate pairing, probably facilitated by telomere movements, is more crucial for the male meiosis than for the female meiosis. This is not true for the complete attachment of telomeres which seems equally as important in both males and females. These differences in meiotic monitoring in males and females indicated that different aspects of meiosis, perhaps including telomere movement, may be of different significance in males and females. Other aspects, like the complete attachment of telomeres, are however generally important for the successful completion of meiosis.

The analyses of the lamin C2 deficient mouse model in this thesis demonstrated that the nuclear lamina during mammalian meiosis is essential to facilitate the high mobility of attached telomeres in the NE during prophase I. It may well be, that the NE and nuclear lamina of somatic cells would not be flexible enough to allow the high mobility of the attached telomeres within the NE. This mobility of attached telomeres within the NE is a general feature of meiosis. In *C.elegans*, it is achieved through meiosis-specific phosphorylation of the SUN protein involved in chromosome attachment (Penkner et al., 2009). Here too, the unmodified, somatic SUN-1 may not be mobile enough within the membrane to facilitate the rapid movements. If further reorganisation of the NE is required in *C.elegans* to facilitate telomere movement, is still unclear. Since *C.elegans* only expresses one lamin protein, which has been demonstrated to be also expressed during meiosis, it is unlikely, that a meiosis-specific nuclear lamina is involved in nuclear reorganisation (Liu et al., 2000). Nonetheless, species-specific meiotic adaptations to either increase the mobility of LINC complex components

within the NE or to increase flexibility of the nuclear lamina and NE seem to be essential to facilitate the efficient repositioning of attached telomeres across taxa.

Besides a flexible NE and lamina, the efficient repositioning of meiotic telomeres also requires a stable connection to the cytoskeleton. Cytoskeletal forces are needed to move the attached telomeres. The attachment is mediated by a meiotic LINC complex, which in mammals has previously been shown to consist of SUN1 and SUN2 in the INM together with KASH5 in the ONM (Schmitt et al., 2007; Ding et al., 2007; Morimoto et al., 2012) (this thesis sections 2.2 and 3.1). The meiotic function of SUN1 in telomere attachment has previously been demonstrated in SUN1 deficient mice, which show impaired telomere attachment (Ding et al., 2007). Detailed analyses in this thesis (section 2.2), however, have demonstrated that approximately two thirds of all telomeres in SUN1 deficient spermatocytes are still attached to the NE. Therefore, telomere attachment in the absence of SUN1 is impaired, but not entirely absent, contradicting earlier results (Ding et al., 2007). Further analyses in this thesis have demonstrated, that SUN2 is responsible for the telomere attachment in the absence of SUN1, indicating that SUN1 and SUN2 fulfil, at least partially, redundant functions during meiosis in telomere attachment. Furthermore, attachment of telomeres in the absence of SUN1 was found to involve KASH5. Indeed, the demonstrated colocalisation of KASH5 and SUN2 in SUN1 deficient spermatocytes within this thesis shows that SUN2 is capable of attaching telomeres to the NE together with KASH5. This indicates the presence of a complete LINC complex comprised of SUN2 and KASH5 in the absence of SUN1 and likely also in wildtype spermatocytes. This complete LINC complex in the absence of SUN1 suggests that telomeres which are attached are also connected to the cytoskeleton.

In order for the complete LINC complex in the absence of SUN1 to be functional, it should be able to transfer the forces of the cytoskeleton to the attached telomeres, to achieve telomere-led chromosomes dynamics. To investigate the functionality of the SUN2/KASH5 LINC complex, analyses investigating the distribution of KASH5 foci in SUN1 deficient spermatocytes were undertaken (sections 2.2). The three dimensional reconstructions clearly showed that attached telomeres in SUN1 deficient mice are able to form bouquet-like clusters. Furthermore, cluster frequencies at 12 dpp were comparable between wildtype and SUN1 knockout siblings. Therefore, the SUN2/KASH5 LINC complex in the absence of SUN1 is not only a complete but appears to be also a functional LINC complex, required and sufficient for moving attached telomeres into bouquet-like clusters. SUN2, in this context, therefore fulfils redundant functions with SUN1, being part of a complete and functional meiotic LINC complex. This implies that in a wildtype situation the meiotic LINC complex responsible for the attachment of telomeres would be composed of the earlier mentioned heterotrimers or homotrimers of SUN1 and SUN2 in the INM interacting with three KASH5 peptides in the ONM.

The demonstrated functionality of the SUN2/KASH5 LINC complex in the absence of SUN1 clearly indicates that SUN1 and SUN2 are functionally redundant during meiosis. The presence of two meiotic, at least partially but functionally, redundant SUN domain proteins seems to be unique to mammals. The KASH domain partner involved, KASH5, has been demonstrated to interact with dynactin, linking the mammalian attached telomeres to the microtubule cytoskeleton. Therefore, it is very likely that the movement of attached telomeres in mammals is generated by microtubules. This is also found in other model organism. In *C.elegans* meiotic telomere attachment is mediated by the

SUN domain protein SUN-1 and the KASH partner ZYG-12 (Minn et al., 2009; Penkner et al., 2009). ZYG-12 has been demonstrated to interact with dynein, linking the attached telomeres to the microtubules (Malone et al., 2003). Similarly, in the fission yeast, the attachment of meiotic telomeres is also mediated by the SUN domain protein Sad1 interacting with the KASH partner Kms1, which also interacts with the microtubule cytoskeleton (Miki et al., 2004). Meiotic attached telomeres in the budding yeast are, contrasting the situation in mammals, fission yeast and *C.elegans*, probably coupled to the actin cytoskeleton instead (Wanat et al., 2008). In summary, it seems that microtubules are commonly responsible for the meiotic telomere-led chromosome movement in a number of species. In some species, however, species-specific adaptations to this seem to have evolved, involving other cytoskeletal forces through the high diversity of KASH domain partners in LINC complexes to move attached telomeres.

In the SUN1 deficient mice investigated in this thesis, it has been demonstrated that telomeres which are attached through a functional LINC complex are able to proceed to cluster formation even when not all telomeres within a cell are connected to the NE. Therefore, the complete attachment of all telomeres to the NE is not a prerequisite for telomere movement or cluster formation. Meiotic progression seems to proceed even in the presence of non-attached telomeres within a cell, as far as it can. This indicates that there may be no meiotic molecular checkpoint monitoring the complete attachment of telomeres to the NE. Other defects in meiotic progression are however detected by checkpoint mechanisms. A recently investigated checkpoint mechanism during meiosis is involved in the detection of unpaired chromosomes. In mice, it has become evident, that meiosis-specific well-conserved proteins (HORMAD1 and 2) preferentially localise to unsynapsed chromosomes (Kogo et al., 2012; Wojtasz et al., 2012). The accumulation of these in the case of unpaired chromosomes during pachytene results in the recruitment of the checkpoint kinase ATR, which can result in apoptosis of the defective gamete. This molecular checkpoint mechanism therefore seems to avoid the maturation of gametes with pairing defects, which would lead to homolog non-disjunction during metaphase and anaphase I. This checkpoint mechanism detects defects in homologous pairing irrespective of whether they are caused by defects in the attachment of telomeres to the NE, defective telomere movements or any other causes of synapsis errors. This perhaps explains why there may be no need for a separate checkpoint mechanism to monitor the complete attachment of telomeres to the NE.

Telomere attachment and movement have been closely investigated in this thesis. It has become evident that telomere attachment and movement are together highly conserved features of meiosis, yet to some extent are also separate events. Telomere attachment *per se* is of course a prerequisite for the movement of the telomeres within the NE. However, telomere attachment is not dependent on telomere movement. This is demonstrated in the lamin C2 deficient mouse model, where telomeres are all attached, but not efficiently moved. Furthermore, the movement of telomeres is not dependent on the attachment of *all* telomeres to the NE. This has been demonstrated by the SUN1 deficient mouse model, where telomeres are moved despite the incomplete attachment of telomeres to the NE. It has furthermore become evident that telomere attachment and movement both have in common that they are dependent on and regulated by components of the meiotic NE.

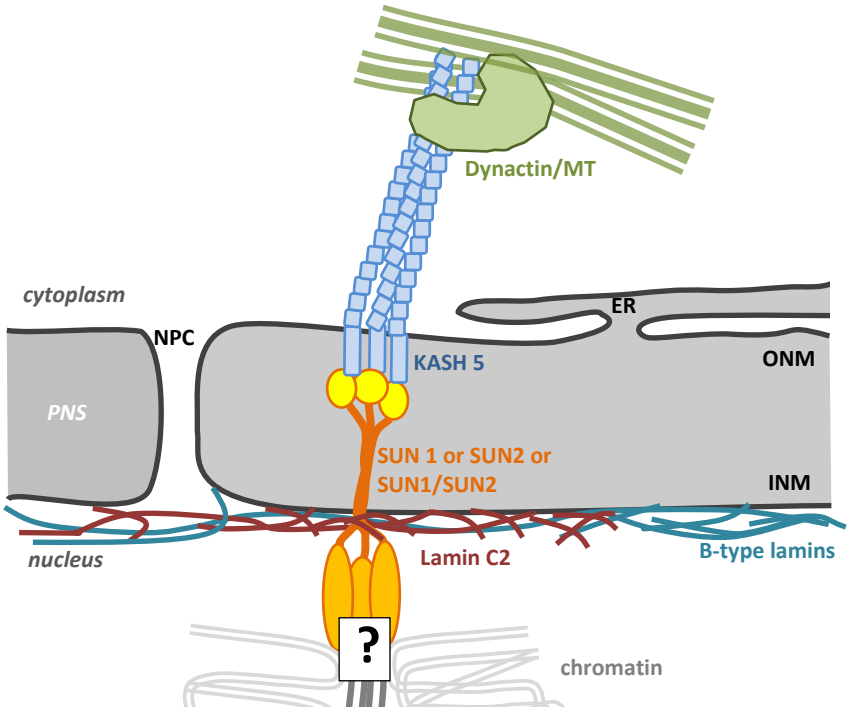

Figure 3.1: Meiosis-specific adaptations of the NE essential for telomere attachment and movement. The paired homologous chromosomes are attached to a protein trimer of SUN domain proteins within the INM. This protein trimer can potentially be a SUN1 or SUN2 homotrimer or a SUN1/SUN2 heterotrimer in variable protein ratios. How the chromatin in mammals is connected to the SUN domain proteins yet unknown. The SUN domain trimer directly interacts with three KASH5 peptides located in ONM. These are connected to the microtubules (MT) through dynactin. In the nuclear lamina, lamin C2 enriches within the lamina at sites of telomere attachment. This most likely alters NE properties locally to increase flexibility and allow the efficient movement of the attached telomeres. The combination of the locally increased NE flexibility and the stable attachment of telomeres to the cytoskeleton seems essential for the efficient movement and attachment of meiotic telomeres and thus for fertility.

The meiosis-specific reorganisation of the meiotic NE is critical for the successful attachment and movement of telomeres to the NE, as demonstrated here by the meiosis-specific LINC complex and nuclear lamina (Figure 3.1). The complete attachment of telomeres is essential for mammalian fertility because it is a prerequisite for the faithful pairing of the homologous chromosomes. This work has demonstrated, that also the efficient movement of the telomeres within the NE, and especially the release of the meiotic bouquet, is critical for the correct pairing of homologous chromosomes and thus for fertility. Furthermore, the efficient movement of attached telomeres within the NE seems to require two essential features: Firstly, an increased flexibility of the meiotic NE, in mammals induced by a unique meiotic lamina. Secondly, the stable attachment of telomeres to the NE and their connection to the cytoskeleton in form of a functional LINC complex. The meiosis-specific features of the NE, the meiotic LINC complex and the increased flexibility of the nuclear lamina in mammals, fulfil these requirements and are therefore meiosis-specific adaptations of the NE critical for fertility.

3.3 Possible functions of the formation and release of meiotic bouquet

The meiotic bouquet is amongst the earliest described strictly conserved hallmarks of meiosis. Due to its distinctive microscopic appearance the bouquet confirmation had first been described in the late nineteenth hundreds and has been found in almost any sexually reproducing organism since (Scherthan, 2001). The bouquet is a feature of the earlier stages of prophase I. At the beginning of prophase I, during leptotene, telomeres are actively tethered to the NE and stably attached to it. This attachment is, in a widely conserved manner, mediated by the LINC complex. Thereby, the attached telomeres are also connected to the cytoskeleton. Cytoskeletal forces during leptotene and zygotene are responsible for the active repositioning of the attached telomeres within the NE. However, it is not entirely clear if telomeres are first polarised within a nucleus and attached in an already somewhat clustered region or if telomeres are attached in a randomly dispersed manner and consecutively moved across relative long distances to cluster within the NE. Either way, the directed telomere movements culminate in the tightly clustered pattern of the meiotic bouquet. Although the extent of clustering ranges between species, generally the clustered telomere patterns are clearly distinguishable from non-clustered telomeres. The tightest clustering of telomeres is usually observed at the leptotene/zygotene transition and during early zygotene. Subsequently, as the pairing of homologous chromosomes and DSB repair progresses, telomere clustering is resolved. This too, is an active process mediated by forces generated by the cytoskeleton. By mid-pachytene, when all homologous chromosomes have paired completely, telomeres are evenly dispersed over the NE again. The efficient movement of attached meiotic telomeres within the NE requires a stable attachment of telomeres to the NE and cytoskeleton as well as an increased mobility and/or flexibility of the NE itself (compare to sections 3.1 and 3.2). Defects in telomere attachment and movement have deleterious effects on fertility in organisms ranging from yeast to mice. Although this indicates highly conserved and important functions of bouquet formation and release, the functions of the meiotic bouquet are still not completely understood.

The lamin C2 and SUN1 mouse models investigated in this thesis indicated that diverse components of the NE influence the ability of efficient bouquet formation and release. Furthermore, defects in chromosome dynamics seem to have severe consequences for the residual meiotic progression. In the lamin C2 mouse model a delayed bouquet resolution was observed. This seems to cause severe pairing defects in both female and male mice lacking lamin C2. This in turn leads to the absence of recombination and infertility of lamin C2 deficient males. Females lacking lamin C2 do progress to late, albeit reduced, recombination events and are fertile (Link et al., 2013a; Jahn, 2012; Link, 2010). Therefore, in this case the delayed bouquet release does not prevent recombination *per se* from occurring, but it greatly reduces the efficiency of homologous synapsis formation and recombination. The SUN1 deficient knockout mouse model, on the other hand, also exhibits defects in pairing and synapsis but not caused by reduced telomere motion but rather by impaired telomere attachment. In this case, the telomeres which were attached were able to move into telomere clusters efficiently. Here too, recombination was observed, although at much reduced rates, in oocytes of SUN1 deficient mice, again showing that impaired telomere attachment *per se* does not completely prevent recombination either (Leubner, 2012; Link et al., 2013b). Reduced rates of

recombination and inefficient synapsis formation are also observed in other model organisms which show defects in bouquet formation or release. Such studies have often been conducted in yeast. Studies in the fission yeast *S.pombe* have shown that mutations in Kms1, which is responsible for transferring cytoskeletal forces to the attached telomeres, lead to the absence of bouquet formation (Niwa et al., 2000). In these mutants, the absence of bouquet formation and release lead to reduced rates of homologous pairing and increased rates of irregular, non-homolgous pairing during prophase I. Furthermore, the absence of telomere movements also lead to increased irregular recombination. The prophase I of *S.pombe* represents a somewhat special case, as telomeres are normally attached to and clustered at the SPB throughout prophase I (Chikashige et al., 2007). Furthermore, *S.pombe* does not exhibit the common SC structure, but rather a functionally similar, SC-related structure connecting homologous chromosomes. This may make extrapolations to other meiotic model organisms somewhat difficult. Nonetheless, also in *S.cerevisiae* similar effects on meiotic progression are observed in the absence of efficient telomere-led movement, although prophase I here differs quite substantially to that of *S.pombe*. Mutations in *S.cerevisiaes* Csm4 also result in the absence of a bouquet formation and release (Wanat et al., 2008). Here too, efficient homologous pairing is significantly reduced in the absence of the directed telomere-led chromosomes dynamics and cross-over frequencies are altered (Conrad et al., 2008). It has therefore been suggested here, as well as in the studies of *S.pombe*, that the bouquet conformation, either its formation or release, increases the efficiency and accuracy of homologous pairing and DBS repair as well as of recombination events. However, all of these mutations result in the absence of the bouquet formation which leads to the reduced homologous pairing and other defects. In the lamin C2 mouse model, bouquet formation is not absent. Rather, the specific release of the bouquet stage is disrupted, still leading to similar defects as observed in the yeast models. This may suggest that the disruption of bouquet release, rather than its general absence, causes the defects in homologous pairing and recombination. Furthermore, this may also suggest separate functions for the formation and release of the meiotic bouquet.

Indeed, it has been postulated that the release of the bouquet may be important for resolving heterologous, and thus weaker, connections during prophase I (Zickler and Kleckner, 1998; Koszul and Kleckner, 2009). In this concept, the directed dispersion of telomeres over the NE after the mature bouquet stage is thought to be an efficient way to resolve heterologous associations between non-homologous chromosomes, thus avoiding defects in synapsis formation (Zickler and Kleckner, 1998). In a way, this would resemble untying the knots within a ball of wool by loosening its ends. This model seems quite appealing for a number of reasons. Firstly, the timing of bouquet resolution correlates well with the completion of SC formation in most species described. In mice for example, bouquet resolution occurs up until early pachytene, which is exactly the time when transverse filaments of the SC assemble between homologous chromosomes (Scherthan et al., 1996). Secondly, avoiding defects in synapsis formation is essential for the faithful segregation of homologous chromosomes into competent gametes. Therefore it seems plausible that this is a feature where selective pressures of evolution could operate, thus perhaps partially explaining the strong conservation of the bouquet formation and release.

Larger chromosome movements during meiosis, not restricted to bouquet formation or release, are also frequently observed. The fission yeast *S.pombe* is a prominent example of this, exhibiting the

so-called horsetail movements throughout long phases of prophase I. During these 2-3 hours (Zickler and Kleckner, 1998) the entire nucleus, led by the SPB, performs rigorous back-and-forth motion (Chikashige et al., 1994). Mammalian pre-meiotic and meiotic nuclei also show large chromosome movements, although not as dramatic as the horsetail movement observed in yeast. Rotational motions of the entire nucleus have been observed in mammals at pre-leptotene as well as during leptotene and zygotene (Parvinen and Soderstrom, 1976). Generally, these more global chromosomal rearrangement before and during pachytene are also thought to provide a stirring force to meiotic nuclei to loosen unwanted, and thus weaker, connections within chromosomes, between non-homologous chromosomes or to other nuclear components like the NE or lamina. The release of the bouquet formation, seen in the context of the more global chromosome movements, may be an additional means to resolve interlocking between chromosomes or any other kind of incorrect association of homologous chromosomes before crossing over occurs and homologs get separated from each other. Therefore, the bouquet could possibly be a central mechanism, amongst others, which have evolved to avoid defects in homologous pairing and thus aneuploidies in later gametes.

There are, however, a number of other concepts suggesting different roles for these dramatic meiotic chromosome movements, which involves both the formation and the resolution of the bouquet stage. The perhaps simplest model suggest that the confinement of telomeres to a limited area of the NE enhances the search of homologous chromosomes essential for pairing and synapsis during meiosis, simply by increasing proximity of homologs to each other (Scherthan et al., 1996). However, in several organisms the formation of the bouquet actually occurs too late during prophase I to be responsible for the alignment and pairing of homologous chromosomes. Mostly, the majority of homologs have already aligned, at least partially, by the time the fully mature bouquet has formed. For instance in mice, pairing and alignment of homologous chromosomes occurs well before leptotene/zygotene transition, which is when the clustering of telomeres is established. It has been shown that a substantial amount of alignment even occurs before axial element formation or DSB induction and thus before the onset of leptotene (Boateng et al., 2013). Sub-telomeric regions of homologous chromosomes establish pre-leptotene pairing, which remain associated throughout leptotene and zygotene. Thus this early homology search and pairing, at least of sub-telomeric regions, must be accomplished without the influence of the bouquet stage. Furthermore, it has also been demonstrated in yeast as well that more global rapid telomere movements before and in the earliest stages of meiosis are responsible for establishing meiotic chromosome pairing (Lee et al., 2012). Therefore, the formation of the bouquet seems to be dispensable for the establishment of homologous pairing. This may indicate, that bouquet formation, or its release, is more important for the release of heterologous associations than for the establishment of homologous associations.

Although bouquet formation seems to be, at least partially, dispensable for the alignment and early pairing of homologous chromosomes, it has also been discussed that the bouquet possibly facilitates pairing or synapsis by increasing the efficiency of SC elongation. Also, the efficiency of the intermediate stages of recombination has been argued to be increased by the bouquet stage. Studies regarding these lines of arguments have again mostly been conducted in strains of yeast mutants, where it has been observed that failure to form a bouquet does not necessarily abrogate meiosis completely, but rather reduces the efficiency of homologous pairing, recombination and SC

formation (Niwa et al., 2000; Chikashige et al., 2007). The main concept in these models have in common that the probability and ability of recombination complexes and SC proteins to load on to and efficiently recruit to homologous chromosome pairs is increased as the proximity of homologous chromosomes increases. Therefore, the clustering of telomeres is argued to efficiently drive mid-prophase processes forward (Moens et al., 1998). Similarly it has also been discussed, that the bouquet possibly serves as a backup mechanism for late stages of homologous pairing. In this case, bringing together yet unpaired homologous chromosomes during late zygotene or pachytene may further support SC formation or elongation (Zickler and Kleckner, 1998).

In conclusion, the clear function of the highly conserved bouquet conformation is still unclear. However, it is becoming increasingly evident that global chromatin movement at the onset of meiosis and during early meiotic stages, the formation of the bouquet stage and the release of the bouquet may fulfil partially separate functions. The formation of the bouquet may facilitate the recruitment of DSB repair proteins and SC components, thereby driving prophase processes ahead. The release of the bouquet stage is implicated to be critical for resolving heterologous, thus incorrect and weaker, associations in this and other work. Therefore, the bouquet stage and its active release seem to be a mechanism to promote efficient prophase progression and subsequently prevent heterologous associations. Therefore it is critically involved in the efficient generation of gametes competent for fertilization, explaining its strong evolutionary conservation.

The meiotic NE is the platform for the attachment and movement of the telomeres. Meiosis-specific adaptations of the NE allow the attachment and movement of telomeres during meiosis, as discussed in earlier sections of this thesis. Amongst these meiotic-adaptations is the meiosis-specific regulation of LINC complex components to stably attach meiotic telomeres and the proposed increase NE flexibility to allow their efficient repositioning within it. These adaptations of the NE are therefore essential prerequisites for the formation and release of the meiotic bouquet. The interdependencies of the meiotic NE and successful meiosis, promoted by the formation and release of the meiotic bouquet, highlight the important roles of meiotic NE component. Therefore, the specialised meiotic NE is demonstrated to be essential for fertility.

4. Future prospects

The NE of meiotic cells has been demonstrated, both in this and other work, to substantially differ to the NE of somatic cells. The meiosis-specific expression and regulation of NE components play critical roles in the successful progression and completion of meiosis. Especially the highly conserved attachment of telomeres to the NE at the beginning of prophase and the subsequent telomere movements within the NE are directly influenced by components of the meiotic NE. The function of the meiotic nuclear lamina in telomere movement and the meiotic function of SUN2 in telomere attachment were elucidated in this thesis. Nonetheless, many open questions about telomere attachment and movement still remain, especially regarding mammalian meiosis.

SUN1 and SUN2 are SUN domain proteins expressed in somatic and meiotic cells, which have been demonstrated to fulfil partially redundant functions. In somatic cells, this has been demonstrated before. Here SUN1 and SUN2 fulfil functions in nuclear positioning together with the KASH proteins Nepsrin1 and 2. The partially redundant function in meiotic telomere attachment was demonstrated in this thesis. SUN1 and SUN2, together with the meiosis-specific KASH5, are responsible for attaching mammalian telomeres during meiosis. How is the switch between somatic SUN1/SUN2 function and the meiosis-specific SUN1/SUN2 function in telomere attachment regulated? It is entirely unknown in mammals, how the homogenous somatic distribution of SUN1/SUN2 is induced to change to the meiosis-specific localisation exclusively to attached telomeres. In *C.elegans* this switch between somatic and meiotic SUN protein function has shown to be regulated by meiosis-specific phosphorylations of SUN-1. If this is also the case for the mammalian SUN1/SUN2 is unclear, could however be possible. For this, phosphorylation-dependant investigations of SUN1/SUN2 functions would have to be conducted. This could be achieved by the in vitro-phosphorylation of SUN1 or SUN2 and possible observed changes in molecular features or protein mobility. Other unanswered questions of mammalian telomere attachment include the connection between telomeres and the SUN domain proteins of the meiotic LINC complexes. It is entirely unknown how telomeres bind to SUN1/SUN2 during meiosis. In *C.elegans* and yeast, species-specific adaptor-proteins have been found, which mediate this connection. However, homologous proteins in mammals remain to date unknown. To investigate this, biochemical approaches including protein interaction assays or pull-down assays would have to be conducted to identify potential meiotic proteins interacting with SUN1 or SUN2. The in vivo function of these potential interaction partners would most likely have to be validated in knockout mouse models afterwards.

Regarding meiotic telomere dynamics, numerous unanswered questions remain as well. Due to the lack of a meiotic cell culture system, the in vivo observation of telomere movement is very challenging. However, fluorescently tagging either SUN-proteins during meiosis or telomeric proteins, providing that their molecular function is not impaired by this, would allow establishing a short-term culture of spermatocytes to investigate telomere movements during prophase I directly. This may well provide novel information about the time frame, extent or regulation of bouquet formation and release. This in turn could provide direct detailed insights into its functions, especially when comparing it to mouse models where bouquet formation or release is impaired. Using this approach could also allow to investigate the influence of other meiotic proteins, like CDK2, SC-

components or cohesins, on meiotic telomere dynamics. This would furthermore deepen our understanding of regulatory players in mammalian meiotic telomere dynamics and therefore critical components of mammalian fertility.

5. Materials and Methods

5.1 Materials used

Chemicals

All chemicals used in this study were obtained from Merck, Roth, Applichem, Serva or Sigma-Aldrich unless stated otherwise.

Mice strains and breeding

All mouse tissue used in this study was taken from mice bred and brought up in the animal facility of the Biocenter, University Wuerzburg. Mice of the lamin $C2^{-/-}$ strain were constructed in the research groups of Ricardo Benavente or Manfred Alsheimer (Schmitt, 2008; Link et al., 2013a). Heterozygous males and females were used to breed litters with homozygous wildtype, heterozygous and homozygous knockout pups for preparation of tissue and sustaining the knockout strain. Heterozygous mice of the $SUN1^{-/-}$ strain were kindly provided by K.T. Jeang (Chi et al., 2009) and used to establish the mouse strain in the animal facility of the Biocenter. Here too, heterozygous males and females were used for breeding and obtaining control and knockout animals. All experiments investigating the meiotic role of SUN2 were conducted in this mouse strain. Only for additional electron microscopy analyses, mouse testis tissue was provided by another SUN1 deficient mouse strain (Ding et al., 2007). The tissue for electron microscopy was kindly provided by R. Xu.

Antibodies

Primary antibodies used in this study were:

Table 4.1 Primary antibodies used in this study. Rb rabbit, Gp guinea pig, M mouse, Gt goat;

Antibody	Host animal	Antibody Epitope	Manufacturer or Reference	Dilution used in IF	
				Paraffin sections	Frozen sections, swabs & chromosome spreads
SYCP3	Rb	N-terminal	Novus Biologicals, Cambridge	1:150	1:300-400
SYCP3	Gp	N-terminal	Seqlab, Göttingen	1:100	-
SYCP1	Rb	C-terminal	Seqlab, Göttingen	1:100	-
SUN1	Gp	AA 427-722	Seqlab, Göttingen	1:300-400	-
SUN2	Gp	AA 248-469	Seqlab, Göttingen	1:200	1:400
KASH5	M	AA 242–648	Morimoto et al., (2012)	1:300	1:500
KASH5	Rb	AA 421-612	Seqlab, Göttingen	1:400	-

Lamin B	Gt	C-terminal	Santa Cruz Biotechnology, Santa Cruz CA	1:100	-
TRF1/2-A	Rb	N-terminal	ADI/ Biotrend, Köln	-	1:100
Digoxigenin Fab Fragment	M	Digoxigenin	Roche Diagnostics, Mannheim	1:50	-
Digoxigenin Fab Fragment conj. Fluorescein	M	Digoxigenin	Roche Diagnostics, Mannheim	1:25	-
Digoxigenin Fab Fragment conj. AP	M	Digoxigenin	Roche Diagnostics, Mannheim	1:10.000 (used in Dot Blot)	
RAD51	Rb	unknown	Calbiochem Merck, Darmstadt	-	1:100
RPA	M	unknown	Calbiochem Merck, Darmstadt ,	-	1:400
ɣH2AX	M	AA 134-142 phospho-Ser139	Millipore Merck, Darmstadt	-	1:500
MLH1	M	unknown	BD Bioscienes, Heidelberg	-	1:30

Secondary antibodies used in this study were:

Table 4.2 Secondary antibodies used in this study. Rb rabbit, Gp guinea pig, M mouse, Gt goat;

Antibody	Host animal	Manufacturer	Dilution used
Texred anti-Rb	Gt	Dianova, Hamburg	1:50
Texred anti-M	Gt	Dianova, Hamburg	1:50
Texred anti-Gt	Bovine	Dianova, Hamburg	1:50
Cy2 anti-Rb	Gt	Dianova, Hamburg	1:50
Alexa 488 anti-Rb	Gt	Life Technologies, Darmstadt	1:400
Cy2 anti-M	Gt	Dianova, Hamburg	1:50
Cy2 anti-Gp	Gt	Dianova, Hamburg	1:50
Alexa 647 anti-Rb	Gt	Dianova, Hamburg	1:100

Oligonucleotides

Primers in this study were designed for PCR reactions used for the genotyping of the mice of the SUN1 and Lamin C2 deficient strains. Only the oligonucleotides "Telo_1" and "Telo_2" were not used for PCR. These were telomere specific probes used for the fluorescent labelling of telomeres in vivo (TeloFISH).

Table 4.3 Oligonucleotides used in this study.

Primer	Sequence	Temperature [°C]	Application
LC2_F1_KOP9_5'	CACCAGAGCCCTACCAGACTCTC	60	Amplification of WT
LC2_del_KOP10_3'	AAAGCTTACACCAGCTCTGGATTC	56	Lamin C2 allele
LC2_neo_KOP11_5'	GAAGTGTATGTGGAACAGAGGCTG	58	Amplification of KO
LC2_neo_KOP12_3'	GCTTCCTCGTGCTTTACGGTATC	57	Lamin C2 allele
SUN1_KOP1_5'	GGCAAGTGGATCTCTTGTGAATTCTTGAC	60	Amplification of WT
SUN1_KOP2_3'	GTAGCACCCACCTTGGTGAGCTGGTAC	64	and KO SUN1 allele
Telo_1	$(TAACCC)_7$	-	TeloFISH labelling of
Telo_2	$(GGGTTA)_7$	-	telomeres

Equipment and Software

Table 4.4 Special equipment used in this study

Camera AxioCamn MRm	Zeiss, Oberkochen
Centrifuge MIKRO 200/ MIKRO 200R	Hettich, Tuttlingen
Confocal laser scanning microscope TCS-SP2 AOBS	Leica, Wetzlar
Cryomicrotome 2800 Frigocut E	Reichert-Jung, Depew NY
Electrophoresis Power Supply	Peqlab, Erlangen
Epifluorescence microscope Axiophot Stereo HB050	Zeiss, Oberkochen
Gradient Thermocycler T100	Bio-Rad, Munich
Heating block	Liebisch (through Hartenstein, Würzburg)
Hybridisation Oven MINI 10	MWG Bioech, Ebersberg
Hybridisation Oven/ Incubator	Memmert, Schwabach
iMIC microscope	Till Photonics, Munich
Infinite M200	Tecan, Männedorf
Paraffin microtome	Leitz, Wetzlar
Peel-a-way® tissue embedding moulds	Plano, Wetzlar
Thermocycler Primus 25 advanced	Peqlab, Erlangen
UV Stratalinker 1800	Stratagene, La Jolla, CA

Table 4.5 Software used in this study.

Software	Application
Leica confocal software	Image acquisition
iMIC live acquisition software package	Image acquisition
CLC	DNA sequence analysis
Adobe Photoshop	Image analysis and processing
ImageJ	Image analysis, 3D reconstructions
R statistical Project	Statistical analysis
Microsoft Excell	Statistical analysis and data management

5.2 Molecular methods

Genomic DNA preparation

- **SDS-Mix(pH 7.5)**

  17 mM Tris/HCl
  17 mM EDTA
  170 mM NaCl
  0.85 % SDS

- **Proteinase K**

  20 mg/ml in 10 mM $CaCl_2$

- **6 M NaCl solution**
- **100% EtOH p.a., ice cold**
- **80% EtOH p.a.**

For the genotyping of wildtype, heterozygous or homozygous knockout mice genomic DNA was extracted from tail tip samples. For this, tail tips were digested with Proteinase K in small reaction tubes (1.5 ml) by adding 500 µl of SDS-Mix and 50 µl of Proteinase to each tail tip. Digestions was conducted at 56°C in rotating hybridisation tubes over night or at least for 4 hours. Subsequently, 250 µl of 6 M NaCl solution was added to each tube and mixed by again rotating the tubes in the hybridisation oven for 10 minutes. Cell debris and proteins were precipitated by centrifugation (8 min, 9000 rpm) and 500 µl of the clear supernatant was transferred to a new reaction tube. Genomic DNA was precipitated by adding the double amount (1000 µl) of ice cold EtOH and inverting quickly, followed by centrifugation (10 min, 14000 rpm, 4°C). The supernatant was discarded and the DNA pellet washed twice with 80% EtOH. Washing was conducted by adding 1000 µl 80% EtOH to the pellet, centrifugating (10 min, 14000 rpm) and discarding the supernatant. Finally, the supernatant was removed and the pellet dried for several hours at room temperature or for 30 min at 60°C. DNA was resolved in 30 µl of water for at least 10 min at 60°C and stored at 4°C. The DNA concentration was measured at 260/280 nm using the Infinite M200 (Tecan).

Mice genotyping by PCR amplification of genomic DNA

Using specific primers for each mouse strain, the wildtype or knockout alleles were amplified by PCR (polymerase chain reaction) from genomic DNA. Phusion DNA-Polymerase was used for this as it is a fast and sensitive DNA polymerase. For this application, the annealing temperatures of the primers (see table 4.3) were adapted to the Phusion polymerase by using an appropriate algorithm (http://www.thermoscientificbio.com/webtools/tmc).

Phusion-PCR reaction:

300-400ng	genomic DNA
1 µl	5’ primer (10pmol/µl)
1 µl	3’ primer (10pmol/µl)
10µl	5x Phusion Puffer (GC)
1 µl	dNTPs (each 10 mM)
1.5µl	DMSO
0.3-0.5µl	Phusion DNA polymerase
Add.	50µl H_2O

Phusion PCR program:

Initial denaturation	2 min	98°C
Denaturation	20 sec	98°C
Annealing	15 sec	depending on primer (table 4.3)
Elongation	15 sec/1 kb	72°C
Finale elongation	7 min	72°C
Storage	∞	8°C

The denaturation, annealing and elongation was repeated 25-30 times

DNA electrophoresis

- **20x SB Buffer (pH8)**

  200 mM NaOH
  pH titrated using Borate

- **Ethidiumbromide (10mg/ml)**
- **6x DNA Loading dye**

  60% Glycerol
  30% 200 mM EDTA
  10% H_2O
  4% Orange G and 2% Xylencyanol

To separate the products of a PCR reaction according to their size, an agarose DNA electrophoresis was conducted. Agarose gels used in this context were 1% (weight/volume) agarose gels in 1x SB Buffer. The SB-buffer containing the agarose was heated until the agarose was fully dissolved. Evaporated water was added back into the hot solution according to the weight lost during the heating and the agarose was cooled to approximately 50°C. Ethidiumbromide was added into the warm agarose to an end concentration of 0.1µg/ml and mixed gently. The solution was poured into the gel cast and hardened at room temperature. The DNA probes were mixed with the loading dye

and brought into the gel pockets. Finally, the gel was run at 150-300 V for 10-30 min in 1x SB buffer and afterwards analysed under UV light and documented.

5.3 Microscopic Methods

Mouse tissue preparation

Mouse tissue used in this study was obtained from 10- 14 day old or adult male mice. For this, the respective mice were sacrificed by CO_2 and cervical dislocation and the required tissues removed immediately. All subsequently described methods for tissue embedding and preparations were conducted using such freshly dissected tissue.

Paraffin tissue embedding and sectioning

- **10x PBS**

  1,4 M NaCl
  26 mM KCl
  64 mM Na_2HPO_4
  14 mM KH_2PO_4

- **37% formaldehyde**
- **EtOH** *(50%-100% diluted in H_20)*
- **Tert-Butanol**
- **Paraffin pellets** *(solidification at 57-60°C)*

For paraffin embedding, testes or pieces thereof were fixed in 1% formaldehyde in PBS for 3 hours. Afterwards, the testis were washed in PBS for 1 hour to remove the formaldehyde and subsequently dehydrated in an increasing ethanol series. For this, testes were incubated in 50,60,70,80,90 and 100% ethanol in water for at least one hour each. Following the incubation in 100% Ethanol, the tissue was furthermore incubated in tert-Butanol for another hour. From the butanol, the tissue was taken directly into melted, pre-heated, liquid paraffin in an incubator at 58-60°C. The tissue was incubated in the warm paraffin for another 24 hrs before transferring it into a fresh plastic mould containing melted paraffin. Here it is important to note, that the tissue has to sink into the liquid paraffin rather than floating on it. Whilst the paraffin is liquid, the position of the tissue within the paraffin was adjusted using a warm needle. Once the tissue was placed at the desired position within the mould, the paraffin remained in the incubator until it had again become entirely liquid. After this, the paraffin containing the tissue was cooled at room temperature and stored at 4°C.

The paraffin embedded testis were cross sectioned to 3-7µm using a sliding microtome (Leitz). Sections were transferred from the blade of the microtome onto a water droplet placed onto Superfrost Plus (Menzel Glas, Braunschweig) slides using a small brush. To stretch the sections and remove folds in the tissue or paraffin, the sections on top of the water droplets were gently heated by transferring the slides to a heating plate of 58°C. The slides were removed from the heating plate

as soon as the sections were observed to be fully stretched. To remove the water underneath the paraffin section, the slide was carefully slanted and the water removed with a paper towel. Residual water between the paraffin section and the slide was removed by rigorous shaking of the slide. Sections were dried over night at room temperature before preparing them for immunofluorescence experiments.

Frozen tissue embedding and sectioning

Tissue embedded for cryosectioning was snap frozen using liquid nitrogen. For this, the freshly prepared testis were placed into a container filled with methylbutane, which was cooled in liquid nitrogen. The tissue was incubated in the cold methylbutane for approximately 3 min before it was removed from the methylbutane using tweezers. Prior to this, a small reaction tube (1.5 ml) for every prepared testis was filled with 1000 µl methylbutane and cooled at -70°C. After transferring the snap frozen tissue to these prepared tubes they were stored at -70°C. Here it is important to note, that once the tissue has been snap frozen, thawing and refreezing should be avoided as this will induce the formation of ice crystals within the tissue.

The snap frozen tissue was sectioned to 5-7µm using a cryomicrotome. For this the frozen tissue was quickly transferred into a cryomicrotome and fixed to the sectioning block using tissue freezing medium. Sections were transferred to Superfrost Plus slides and air dried for at least 30 min at room temperature. If the tissue did not thaw substantially, it was removed from the block and transferred back into the methylbutane for storage at -70°C.

Tissue preparation for electron microscopy

- **Buffered glutaraldehyde solution (pH 7.2)**
  2.5% glutaraldehyde
  50 mM KCl
  2.5 mM MgCl
  50 mM cacodylate

- **Cacodylate buffer (pH 7.2)**
  50 mM cacodylate

- **Osmium tetroxide**
  2% osmium tetroxide in 0.05 mM cacodylate buffer

- **Uranyl acetate**
  0.5% in water

- **EtOH** *(50, 70, 90, 96 and 100% in water)*

To prepare testis for electron microscopy, the tunica surrounding the testis was removed using fine tweezers. Single tubuli were transferred to buffered glutaraldehyde solution for 45 min and subsequently washed 5 times for 3 min each in cacodylate buffer. After this, the tubuli were incubated in osmium tetroxide at 0°C for 1.5 to 2 hours. Excess osmium was removed by washing the tubules 5 times for 5 min each in H_2O on ice. Afterwards, the tissue was contrasted in uranyl acetate at 4°C over night. After the contrasting, the tubules were again washed in H_2O (5 times 3 min each) followed by an increasing ethanol series to dehydrate the tissue. For this, the tissue was incubated in 50,70,90 and 96% EtOH once for 30 min each and in 100% EtOH thrice for 30 min. Following the dehydration, the tissue was incubated in propylene oxide thrice for 30 min each and over night in a 1:1 mixture of propylene oxide and epon. Finally, the epon was changed once before hardening it at 60°C. The embedded tissues were used for ultrathin sectioning for electron microscopy.

Chromosome spreads

- **Hypertonic buffer (pH 8.2)**

  30 mM Tris/HCl
  17 mM Na-Citrate
  5 mM EDTA
  50 mM Sucrose
  5mM DTT (from 1M stock solution)

- **100 mM sucrose (sterile-filtered)**

- **PFA-Solution (pH 9.2)**

  1% Paraform-aldehyde in water
  titrated using NaOH and sterile-filtered
  0.15% Triton-X (1.5 ml of 10% stock solution)

The chromosome spreads used in this study were produced using a dry-down method adapted from de Boer et al. (de Boer, 2009). For this, the tunica surrounding the freshly prepared testes was removed using fine tweezers and single tubuli were transferred into the hypertonic buffer. Tubuli were left to swell in the hypertonic buffer for 30-60 min. After this, a 20 µl droplet of the sterile sucrose solution was transferred onto a slide and a tubule from the hypertonic buffer placed into it. The cells were washed out of the tubule by resuspending thoroughly using a small pipette (10 µl). Larger pieces of debris were removed using tweezers after the resuspension. A new slide was covered with the PFA solution and tilted so that the entire volume of the solution accumulated at one corner of the slide. Into this droplet, the 20 µl sucrose solution containing the cells from the tubule were transferred. The volume of the solutions containing the cells was spread evenly over the entire slide and the slides were placed into a moisture chamber. The slides were incubated in the closed moisture chamber for 2 hours to allow the cells to settle onto the slides. Subsequently, another incubation with the lid of the moisture chamber left ajar for 30 min followed. Finally, the

slides were left to dry in the open moisture chamber for at least 2 hours. The dried slides were carefully wrapped in tinfoil and stored at -70°C until use.

Immunofluorescences on paraffin tissue sections

- **Rotihistol (Xylene-substitute)**
- **EtOH (100-50% in water)**
- **Unmasking solution (1:100 in water)**
- **10x PBS**

  1,4 M NaCl
  26 mM KCl
  64 mM Na_2HPO_4
  14 mM KH_2PO_4

- **0.1% Triton-X in PBS**
- **1x PBT (pH 7.4)**

  PBS
  1.5% BSA
  0.1% Tween-20

- **or Blocking solution**

  PBS
  5% Milk
  5% FCS

- **Hoechst 33258 (end concentration of 15µg/ml in PBS)**
- **Glycerol in PBS (1:1)**

To prepare paraffin sections for immunofluorescence analyses the paraffin was first removed from the tissue, the tissue was rehydrated and subjected to antigen retrieval protocols. The antigen retrieval is essential for allow the antibody to efficiently bind to its epitope in the tissue. A heat induced antigen retrieval at a low pH is chosen here to achieve best results (unmasking). The slides with paraffin sections were incubated in Rotihistol twice for 10 min. From this, the tissue was moved to an decreasing ethanol series (100, 90, 80, 70 and 60%) in which it was incubated for at least 2 min each. Subsequently, slides were placed into 50% ethanol and incubated for 10 min to ensure a complete exchange of water/ethanol within the tissue. Following the 50% ethanol, the sections were collected in water. Whilst the tissue was in the ethanol series, the unmasking solution was placed within the table autoclave and pre-heated to 125°C at 1.5 bar. The sections were taken from the

water into the hot unmasking solution and remained in there until the autoclave had again reached 125°C and 1.5 bar, at which temperature the actual unmasking was conducted. The duration of the unmasking depended on the fixation and thickness of the tissue sections. Sections between 2 and 7 µm were subjected to unmasking of 12-30 min, respectively. Following the unmasking, the pressure and temperature of the autoclave was slowly decreased and the sections moved into PBS at room temperature. It is important to note here, that the boiling of the unmasking solution was avoided. After a short incubation in PBS, slides were moved to 0.1% triton for 10 min and then washed twice for 5 min in PBS again.

Following the unmasking of sections, two different protocols were used in this study. For the analyses concerning lamin C2, unspecific binding sites in the tissue sections were blocked by an incubation with PBT in moisture chambers for 1-2 hours. After blocking, the primary antibodies were incubated together for 1-2 hours on the sections in the moisture chambers. After washing the slides twice for 7 min each in PBS, sections were subjected to the according secondary antibodies in the moisture chambers for 20 min. 2-3 drops of Hoechst were added to the secondary antibodies for another 10 min after which slides were again washed twice in PBS. Finally, sections were covered with glycerol/PBS and a cover slip.

For immunofluorescence analyses of the SUN1 deficient and SUN1 control mice a different protocol was used following the unmasking and triton incubation. For these applications, unspecific binding sites were blocked using the supernatant of the centrifuged milk-containing blocking solution (16,000 g, 30 min, 4°C). Blocking was conducted before any antibody incubation for 30 min in a moisture chamber and all antibodies, both primary and secondary, were incubated separately, pre-diluted in the supernatant of the blocking solution. Additionally, antibodies were all centrifuged (16,000g, 30 min, 4°C) in the blocking solution supernatant prior to incubation. Following the first blocking, the first primary antibody was incubated for 2 hours at room temperature or over nights at 4°C. After this, slides were washed thrice for 5 min in PBS and again blocked for 30 min. For antibody incubations over night, this first washing was conducted at 37°C, all other washing steps at room temperature. This was followed by the incubation of the first secondary antibody for 20 min after which slides were again washed (3 times 5 min in PBS) and blocked. After this, the second primary antibody was incubated for 2 hours and slides again washed and blocked. Finally, the slides were subjected to the second secondary antibody for 20 min to which 2-3 drops of Hoechst were added for an additional 10 min. Before covering the sections with glycerol/PBS and a cover slip, slides were once again washed thrice for 7 min in PBS.

Telomere fluorescent in situ Hybridisation (TeloFISH) on paraffin sections

To directly visualise telomeres, fluorescently labelled telomere probes were used on paraffin sections (telomere fluorescent in situ hybridisation (TeloFISH)).

Labelling probes

Two separate telomere probes (see table 4.3; Telo_1 and Telo_2) were labelled by one digoxigenin molecule each using the terminal transferase (Tdt; ThermoScientific Waltham, MA).

Digoxigenin labelling reaction:

100 pmol	Oligonucleotide (Telo_1 or Telo_2)
4 µl	5 x TdT-Buffer
1 µl	Dig-ddUTPs (Roche Diagnostics)
2 µl	TdT
Add.	20 µl H_20

Labelling was performed at 37°C for 1 hour and probes were stored at -20°C until use.

Dot Blot

- **2x SSC (pH 7.4)**
  0.3 m NaCl
  0.03 M Na-Citrate

- **Dig-Buffer (pH 7.5)**
  100 mM Maleic acid
  150 mM NaCl

- **Blocking solution I**
  Blocking solution (Roche Diagnostics) 1:10 in Dig-Buffer

- **Antibody solution**
  alkaline phosphatase Anti-Dig antibody (Roche Diagnostics) 1:10,000 in Blocking solution I

- **Washing Buffer (pH 7.5)**
  100 mM Maleic acid
  150 mM NaCl
  0.3% Tween 20 (30 ml from 10% stock solution)

- **Detection Buffer (pH 9.5)**
  100 mM Tris-HCl
  100 mM NaCl

The efficiency of the digoxigenin labelling can be monitored by conducting a dot blot in which descending concentrations of each labelled oligonucleotide are blotted onto a membrane and detected on X-ray film through a light reaction caused by a alkaline phosphatase conjugated anti-Dig antibody. For this the following dilutions of both labelled probes were used:

20 pg/µl
10pg/µl
5 pg/µl
2pg/µl
1pg/µl
0.1pg/µl

A drop of 0.5µl of each dilution of each probe was transferred onto the positively charged nylon membrane (Roche Diagnostics) and dried at room temperature. Two Whatman filters (Whatman, Dassel) of the size of the nylon membrane were soaked in 2x SSC and the dry nylon membrane placed in between the two wet Whatman filters onto a glass plate. The nylon membrane between the Whatman filters was autocross linked twice with 120 mJoule. After shortly washing the nylon membrane in water (5 min), the membrane was baked at 80°C for 30 min. Following this, unspecific binding sites on the nylon membrane were blocked by a 30 min incubation in blocking solution prior to the incubation with the antibody solution for another 30 min. Subsequently, the nylon membrane was washed twice for 15 min in washing buffer before subjecting it to the detection buffer for 5 min. Finally, the CSPD- ready-to-use solution (Roche Diagnostics) was used to induce the light reaction catalysed by the alkaline phosphatase. For this, 1ml/100cm^2 of CSPD solution was brought on to membrane and the membrane enclosed into a transparent film. After 5 min of incubation at room temperature, the membrane was incubated at 37°C for another 10 min before placing an X-ray film onto the membrane in a dark room to detect the light signal. The X-ray film was developed according to standard procedures.

TeloFISH with co-immunofluorescence on paraffin sections

- **Rotihistol (Xylene-substitute)**
- **EtOH (100-50% in water)**
- **Unmasking solution (1:100 in water)**
- **10x PBS**

  1,4 M NaCl
  26 mM KCl
  64 mM Na_2HPO_4
  14 mM KH_2PO_4

- **0.1% Triton-X in PBS**
- **1x PBT (pH 7.4)**

  PBS
  1.5% BSA

0.1% Tween-20

- **Glycerol in PBS (1:1)**

- **2x SSC (pH 7.4)**
  0.3 m NaCl
  0.03 M Na-Citrate

- **Hybridisation solution**
  2x SSC
  30% Formamide
  10% Dextrane sulphate
  250µg/ml E.coli DNA

- **Digoxigenin-labelled telomere probes**

- **TBS (pH 7.4)**
  150 mM NaCl
  10 mM Tris/HCl

- **TBS/BR (pH 7.4)**
  0.5% Roche Blocking solution (Roche Diagnostics) in TBS

- **TBST (pH 7.4)**
  150 mM NaCl
  10 mM Tris/HCl
  0.05% Tween 20

- **Hoechst 33258** *(end concentration of 15µg/ml in PBS)*

The preparation and antigen retrieval of the paraffin sections for TeloFISH is identical to that described for immunofluorescences on paraffin sections (see above). After removing the paraffin by Rotihistol, rehydrating the tissue through a decreasing ethanol series, performing unmasking and permeabilisation by triton, the slides were prepared for the hybridisation through an incubation in 2x SSC for 5 min. Simultaneously, the hybridisation solution and both labelled telomere probes were all separately heated to 95°C for 15 min. After this, per each 40 µl of hybridisation solution 4 µl of each labelled probe were mixed into the heated solution and quickly brought onto the slides in a moisture chamber. The closed moisture chamber containing the sections with hybridisation solution and probes were pre-hybridised at 95°C for 20 min. Hybridisation occurred after this over night at 37°C. Washing away unbound telomere probes was done twice for 10 min in 2x SSC at 37°C following the hybridisation. To block any unspecific binding sites, the tissue was then incubated for 30 min with TBS/BR after which the slides were subjected the mouse anti-digoxigenin antibody (1:50 in TBS/BR)

for 60 min. After two washing steps in TBST for 10 min each, a normal immunofluorescence protocol was continued as described above. For this, slides were first blocked for 60 min in PBT and then subjected to a primary antibody, followed by washing in PBS and a secondary antibody. If desired, the tissue was then subjected to another primary antibody followed by washing and another secondary antibody to achieve a double co-immunofluorescence in combination with TeloFISH. In any case, Hoechst was added to the last secondary antibody for the last 10 min to counterstain the DNA before the final washing in PBS and covering the sections with glycerol/PBS and a coverslip.

Immunofluorescence on frozen sections

- **10x PBS**
  1,4 M NaCl
  26 mM KCl
  64 mM Na_2HPO_4
  14 mM KH_2PO_4

- **1% Formaldehyde in PBS**

- **0.1% Triton-X in PBS**

- **1x PBT (pH 7.4)**
  PBS
  1.5% BSA
  0.1% Tween-20

- **Hoechst 33258** *(end concentration of 15µg/ml in PBS)*

- **Glycerol in PBS (1:1)**

To prepare cryosections for immunofluorescence experiments, the dried cryosections were fixed using 1% formaldehyde in PBS for 10 min in a moisture chamber. To achieve a mild permeabilisation of the tissue, the slides were subsequently subjected to 0.1% triton for 5 min only. After thorough washing in PBS (twice for 7 min), unspecific binding sites were blocked by a 1-2 hour incubation with PBT in moisture chamber. After this, slides were subjected to both primary antibodies for 1 hour, washed twice in PBS for 5 min and subjected to the corresponding secondary antibodies for 30 min. All antibodies were diluted in PBS and incubations were conducted in moisture chambers. For the last 10 min of the secondary antibody incubation, 2-3 drops of Hoechst were added to counterstain the DNA. After washing the slides again twice in PBS for 5 min, they were covered with a coverslip using glycerol/PBS.

Immunofluorescence on chromosome spreads

- **10x PBS**

  1,4 M NaCl
  26 mM KCl
  64 mM Na_2HPO_4
  14 mM KH_2PO_4

- **Blocking solution**

  PBS
  5% Milk
  5% FCS

- **Hoechst 33258 (end concentration of 15µg/ml in PBS)**

- **Glycerol in PBS (1:1)**

The slides with the chromosome spreads stored at -70°C were thawed at room temperature and the tinfoil was unwrapped once the slides hat reached room temperature. To prepare the slides for immunofluorescence assays, the slides were washed three times for 5 min each in PBS. The blocking solution was centrifuged (16,000 g, 4°C for 30 min) and the supernatant was used for all blocking steps as well as to dilute and incubate all antibodies in. Slides were blocked for 30 min using approximately 500 µl of the supernatant of the blocking solution in a moisture chamber. After this, the first primary antibody was incubated for 1 hour diluted in blocking solution after which slides were washed twice for 5 min each in PBS. Following this, slides were again subjected to blocking solution for 30 min, followed by the incubation of the first secondary antibody for 20 min. After washing twice in PBS, slides were again blocked followed by the incubation of the second primary antibody for 1 hour. After further washing (twice 5 min in PBS) and blocking for 30 min, slides were subsequently subjected to the second secondary antibody for 30 min. For the last 10 min of this incubation, 2-3 drops of Hoechst were added onto the slides. Finally slides were washed three times for 5 min each in PBS and covered with a coverslip using glycerol/PBS.

5.4 Image acquisition and analysis

Microscopy

Most immunofluorescent analyses were conducted using a Leica TCS-SP2 AOBS confocal laser scanning microscope (Leica Microsystems, Mannheim) using the 63x/1.40 HCX PL APO oil immersion objective. Images were usually acquired using the 4 times optical zoom of the Leica confocal software and scanned at 800 Hz with 1024x1024 pixels. This resolution allowed image analysis, quantification of fluorescent signals and 3 dimensional reconstructions. Maximum projections for image analysis were calculated using the Leica confocal software or manual projections were calculated using the Z-projection tool (maximum projection) in ImageJ.

Images for the quantifications of bouquet staged cells in three littermate pairs of 10-14 dpp lamin C2 mice were additionally acquired using the iMic Microscope (Till Photonics, Munich) equipped with the 100x/1.40 NA oil immersion objective. This epifluorescence microscope system allows acquiring image series composed of epifluorescence images recorded at subsequent focal planes at a distance of 100 nm to each other. By this, similar images series are produced as with the confocal microscope, except for the increased background signal from the focal planes above and below. Image acquisition is much faster than when using the confocal microscope and the image quality of samples of high signal intensity is nonetheless sufficient for three dimensional reconstructions even without further image processing. Therefore, these images were used to quantify the second and third littermate pair of each age to speed up the data acquisition and reach the large number of sample spermatocytes.

3D reconstructions

For the quantifications of bouquet staged spermatocytes in littermate knockout and wildtype pubertal males of the lamin C2 and SUN1 deficient mice, confocal or iMIC image series of single spermatocytes were reconstructed three dimensionally. By this, the distribution of labelled telomeres within the nuclear periphery could be judged and therefore nuclei with clustered telomere patterns distinguished from nuclei with non-clustered telomere patterns. For three dimensional reconstructions of spermatocytes, paraffin sections of 7 µm were usually chosen for immunofluorescence approaches to ensure that entire nuclei were preserved in the tissue. The whole nuclei were scanned with approximately 40-70 optical sections at a distance of 0.122 µm using the confocal laser scanning microscope or 100 nm at the iMIC. The three to four separate colour channels acquired were merged in ImageJ and the merge used for further analysis. If necessary, adjustments to colour brightness were made to each channel also in ImageJ. Image properties (voxel width, height and depth) were adjusted according to the Leica log text file of each confocal image series. Using the brightest point mode of the 3 D project tool in ImageJ, these image series were used

to produce to scale three dimensional reconstructions of single spermatocytes. Other settings of the 3 D project tool were usually kept to the default state.

Quantifications of telomere attachment

Attached and non-attached telomeres in spermatocytes of wildtype and knockout SUN1 mice were quantified in preparations in which the nuclear lamina and SC were labelled using respective specific antibodies in co-localisation with fluorescently labelled telomeres (TeloFISH). Telomere attachment was only quantified in spermatocytes which were scanned entirely using the confocal microscope. The separate colour channels of the acquired image series were merged in ImageJ and if necessary their brightness adjusted. Telomeres which were classified to be attached showed a clear co-localisation with the labelled nuclear lamina. Telomeres located within the nuclear interior, well away from the nuclear lamina, where classified to not be attached. By this classification, the co-localisation or interior localisation of each telomere signal was judged and quantified in the image series analysed in ImageJ.

Quantifications of bouquet formations

The quantification of clustered or non-clustered telomere patterns was performed in spermatocytes of pupertal males of the lamin C2 deficient strains labelled by SUN1 and SYCP3 with respective antibodies. In spermatocytes from males of the SUN1 strain, attached telomeres were labelled with an anti-KASH5 antibody in colocalisation with SYCP3. By scanning entire nuclei and performing three dimensional reconstructions of the image series, the distribution of the attached telomeres could be judged. The murine bouquet stage is, compared to other species, characterised by a relatively weak telomere clustering within one hemisphere of the nucleus only. Therefore, spermatocytes exhibiting the majority of telomere signals within one hemisphere of the nucleus were classified to be within the bouquet stage. Spermatocytes not showing such a clustered telomere pattern in the three dimensional reconstructions where classified to be non-clustered.

Statistical analysis

Statistical analyses performed in this thesis were conducted in R (version 2.10.1; http://www.r-project.org) or Microsoft Excel 2010. The Pearson's Chi-Squared Test was used to identify significant differences between populations and was always performed in R applying default settings. Regarding the quantification of the bouquet frequencies in 10-14 dpp lamin C2 littermates, a separate p-value was determined for each littermate at each age. For data representation and better understanding, the mean average of all three p-values of each age was taken to determine a single mean p-value for each age showing the significant or non-significant difference between the littermate knockout and

wildtype animals. Any standard deviations shown were determined using the standard deviation (STABW.N) function of Microsoft Excel 2010 (Microsoft Office Corporation 2010).

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7. Erklärung

Hiermit erkläre ich ehrenwörtlich,

dass ich die vorliegende Arbeit unter Verwendung der angegebenen Hilfsmittel und Quellen selbständig angefertigt habe,

dass ich diese Dissertation in gleicher oder ähnlicher Form in keinem anderen Prüfungsverfahren vorgelegen habe,

und dass ich noch keine akademischen Doktorgrade erworben habe und früher auch noch nicht versucht habe einen akademischen Doktorgrad zu erwerben.

Würzburg, _____

_____

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8. Curriculum Vitae

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Date of Birth	**08.05.1986**
Nationality	***German***

Scientific education

since May 2010	**PhD candidate** (Dr. rer. nat. cand.) at the University of Wuerzburg and graduate student in the Graduate School of Life Sciences, University of Wuerzburg.
April 2010	**Diploma in Biology**, University of Wuerzburg (achieved final grade "very good")
July 2009- April 2010	**Diploma Thesis** "The functional organisation of the meiotic nucleus; Synapsis, recombination and meiotic telomere attachment in Lamin C2 deficient mice" at the Dept. of Cell and Developmental Biology, University of Wuerzburg, in the laboratory of Ricardo Benavente.
Oct. 2004- April 2010	Study of Biology at the University of Wuerzburg **Main subject** Cell and Developmental Biology **Other Subjects** Genetics; Animal ecology and tropical biology
June 2004	Graduated from the International School of Tanganyika, Dar es Salaam/Tanzania with a bilingual (German/English) International Baccalaureate Diploma (achieved 39 points); equivalent to the German "Allgemeine Hochschulreife" of the grade 1.5.

Scholarship

May 2010- May 2013 **PhD Scholarship** of the Research Training Group "Molecular basis of organ development in vertebrates" ("Graduiertenkolleg" 1048) by the DFG (German Research Foundation) at the University of Wuerzburg

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9. Lebenslauf

Jana Link
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Geburtsdatum	**08.05.1986**
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Wissenschaftlicher Werdegang

seit Mai 2010	**Doktorandin** (Dr. rer. nat. cand.) der Universität Würzburg, Lehrstuhl für Zell- und Entwicklungsbiologie
April 2010	**Diplom der Biologie**, Universität Würzburg (Note: "Sehr gut")
Juli 2009- April 2010	**Diplomarbeit** "Funktionelle Organisation des meiotischen Zellkerns: Synpasis, Rekombination und Telomeranheftung meiotischer Chromosomen bei Lamin C2-defizienten Mäusen" am Lehrstuhl für Zell- und Entwicklungsbiologie, Universität Wuerzburg.
Okt. 2004- April 2010	**Biologiestudium** an der Universität Würzburg **Hauptfach** Zell- und Entwicklungsbiologie **Nebenfächer** Genetik; Tierökologie und Tropenbiologie
Juni 2004	**Erlangung der allgemeinen Hochschulreife** an der International School of Tanganyika, Dar es Salaam/Tanzania mit bilingualem (Deutsch/Englisch) International Baccalaureate Diploma (Note: 39 points); Gleichwertig dem Deutschen Abitur der Note 1.5 (anerkannt durch das Hessische Kultusministerium)

Stipendium

Mai 2010- Mai 2013 **Doktorandenstipendium** des Graduiertenkollges **"**Molecular basis of organ development in vertebrates" (GK 1048) der DFG an der Universität Würzburg

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10. Publications

Peer-reviewed articles

Link J, Jahn D, Schmitt J, Göb E, Baar J, Ortega S, Benavente R, Alsheimer M (2013) The Meiotic Nuclear Lamina Regulates Chromosome Dynamics and Promotes Efficient Homologous Recombination in the Mouse. *PLoS Genetics 9:e1003261.*

Link J, Leubner M, Schmitt J, Göb E, Benavente R, Jeang K T, Xu R, Alsheimer M (2013) SUN2 in mammalian meiotic LINC complex formation and functions *(submitted)*

Kracklauer MP, **Link J**, Alsheimer M (2013) LINCing the Nuclear Envelope to Gametogenesis. *Current Topics in Developmental Biology; 102:127-57.*

Published abstracts

Link J, Jahn D, Schmitt J, Göb E, Baar J, Ortega S, Benavente R, Alsheimer M. The Meiotic Nuclear Lamina Regulates Chromosome Dynamics and Promotes Efficient Homologous Recombination in the Mouse. *(International Joint Meeting of the German Society for Cell Biology and Society for Developmental Biology;* ***Heidelberg, March 2013****)*

Link J, Jahn D, Schmitt J, Göb E, Baar J, Ortega S, Benavente R, Alsheimer M. The Meiotic Nuclear Lamina Regulates Chromosome Dynamics and Promotes Efficient Homologous Recombination in the Mouse. *(Dynamic Organization of nuclear Function, CSHL Meeting;* ***Cold Spring Harbor, NY, September 2012)***

Link J, Jahn D, Schmitt J, Göb E, Baar J, Ortega S, Benavente R, Alsheimer M. Loss of lamin C2 causes defective meiotic chromosome dynamics and male infertility.*(Annual conference of the German Genetics Society;* ***Wuerzburg, September 2011)***

Link J, Jahn D, Göb E, Benavente R, Alsheimer M. Loss of lamin C2 causes defective meiotic chromosome dynamics and male infertility. *(The EMBO Conference on Meiosis;* ***Napels, September 2011)***

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