PATHWAYS OF NUCLEOCYTOPLASMIC TRANSLOCATION OF RIBONUCLEOPROTEINS

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INTRODUCTION

In the prokaryotic cell there exists only one membranous barrier controlling mobility of molecules and particles, namely the cell surface membrane. By contrast, the eukaryotic cell exhibits a diversity of membrane barriers. Apart from the separation of the cellular interior from the extracellular space by the plasma membrane, these cells have various intracellular vesicles and cisternae bounded by membranes, and also intracellular compartmentalizations constituted by envelopes of two membranes, which can be different in character, such as the inner and outer membranes of the mitochondria and plastids, or can be homologous, as in the nuclear envelope. The nuclear envelope is a continuous perinuclear cisterna which is in luminal continuity with the endoplasmic reticulum (ER), and is unique insofar as it represents an ubiquitous subdivision of the plasmatic phase of the cell by separating the compartment of genome localization and transcription (the nucleus) from that of translation (cytosol). It is also unique in not representing a continuous double membrane sheath; rather it has a variable number of regular and constitutive interruptions ('pores') at which both membranes are fused. These 'pore complexes' (for definition see below) allow passage of molecules or particles through a plasmatic channel of 50–80 nm diameter which is not obstructed by a membrane diaphragm. It is important to note, however, that this characteristic eukaryote structure is transitory and dynamic since (*a*) it can be transitorily disintegrated, without harming the viability of the cell, as for example in some cell cycle stages of those cells which have an 'open' mitosis and meiosis as well as in special cell or nuclear differentiation processes such as in sperm development and in pronuclei formation (for references see Stevens & André, 1969; Longo & Anderson, 1968, 1969; Moses & Wilson,

1970; Bajer & Molè-Bajer, 1972; Kessel, 1973; Franke, 1974*a*; Franke & Scheer, 1974); and (*b*) it can vary its structural parameters including size, shape, cisternal width, frequency of pores, and its associations with other membranes and organelles such as ER, dictyosomes, periplastidal cisternae, centrioles, microtubules and mitochondria, in response to changes in cell differentiation and physiology (for reviews see Stevens & André, 1969; Feldherr, 1972; Wischnitzer, 1973; Kessel, 1973; Franke, 1974*a*; Franke & Scheer, 1974).

ACCESSORY STRUCTURES INVOLVED IN NUCLEOCYTOPLASMIC COMPARTMENTALIZATION

In some cells the nucleoplasm is separated from the cytosol phase not only by the nuclear envelope but also by various perinuclear structures such as annulate lamellae (see, e.g. Plate 14), Golgi apparatus formations, aggregates of mitochondria and/or 'heavy bodies' (references in Kessel, 1971 and Franke & Scheer, 1974). Such juxtanuclear structures do not form a continuous barrier around the whole nuclear surface. There is, however, one nuclear type known, the giant nucleus in the rhizoids of some Bryopsidales (green algae – the most prominent member being *Acetabularia*), in which the entire nuclear envelope is surrounded, at a distance of approximately 70 nm, by another porous cisterna, the 'perinuclear lacuna', which is in continuity with the large vacuolar labyrinth of these cells (Plate 8; Werz, 1964; Van Gansen & Boloukhère-Presburg, 1965; Boloukhère, 1970; Zerban, Wehner & Werz, 1973; Franke *et al.* 1974). Consequently, this 'secondary nuclear envelope' provides another 'zone of exclusion' for cytoplasmic and nuclear particles. The intermediate zone sandwiched between the true and the secondary envelope contains only tangles of fine filaments and occasional membranous vesicles or cisternal pieces. However, the pores in the perinuclear lacuna are larger than the nuclear pores (although they obviously do not allow penetration of cytoplasmic ribosomes) and are not structurally identical or related to the true 'pore complexes' described below.

PASSAGE OF IONS AND SMALL MOLECULES ACROSS THE NUCLEAR ENVELOPE

Earlier studies of Loewenstein and associates, applying electrophysiological micromethods, have suggested that the mobility of ions is hindered at the level of the nuclear envelope in some cell types, specifically in dipteran salivary gland cells, but not in others such as oocytes from various animals

(Kanno & Loewenstein, 1963; Loewenstein & Kanno, 1963*a*, *b*; Loewenstein, 1964; Ito & Loewenstein, 1965; Kanno, Ashman & Loewenstein, 1965; Wiener, Spiro & Loewenstein, 1965). This was taken, together with observations of accumulations of some ions in the nucleus (Abelson & Duryee, 1949; Naora *et al.* 1962), to be an indication of the semipermeable character of the nuclear envelope and the existence of a mechanism for active uptake of ions into the nucleus (for reviews see Goldstein, 1964; Feldherr, 1972). However, recent data support the interpretation that the accumulation of specific ions in nuclei is due either to a higher relative solvent space, as in the hyaline giant nuclei of the amphibian oocytes (Abelson & Duryee, 1949; Riemann, Muir & MacGregor, 1969; Century, Fenichel & Horowitz, 1970), or to the binding of the specific ions to certain nuclear and cytoplasmic structures (Feldherr & Harding, 1964; Horowitz & Fenichel, 1970; Siebert & Langendorff, 1970; Siebert, 1972). Although no special reinvestigations have been made as to the ion permeability resistance of the insect salivary gland nuclei (see above), the majority of workers at the moment strongly favours the concept that ion movements are not significantly hindered at the nuclear envelope of most, if not all, nuclear types. It might well be, however, that the migrating ions are immediately and transitorily bound to the non-membranous 'gel-like' materials associated with the nuclear pore complexes (see below). These ions may exist in some form of steady state equilibrium; they may even be locally adsorbed or bound at these structures.

Charged and uncharged small molecules with molecular weights of up to a few thousand daltons also rapidly penetrate the nuclear envelope. This is true for glycerol, sucrose, sugar phosphates, and larger saccharides (Goldstein & Harding, 1950; Horowitz & Fenichel, 1968; Horowitz, 1972; Kohen, Siebert & Kohen, 1971; Horowitz, Moore & Paine, 1973; for experiments with isolated nuclei see Kodama & Tedeschi, 1968; however, see also the work of Stirling & Kinter, 1967, interpreted as an indication for a delayed cytoplasmic–nuclear equilibration of galactose in the hamster intestinal mucosa), for amino acids (e.g. Mirsky & Osawa, 1961; Kostellow & Morrill, 1968; for corresponding studies with isolated nuclei see Allfrey, Meudt, Hopkins & Mirsky, 1961), nucleosides and nucleotides, including such important metabolites as ATP and nicotinamide dinucleotide (e.g. Allfrey *et al.* 1961; Lee & Holbrook, 1965; Kohen *et al.* 1971; for reviews see: Siebert, 1972; Feldherr, 1972; Kay & Johnston, 1973). There are, to our knowledge, no reports indicating that any diffusible low molecular weight component of the cytosol is strictly excluded from the nucleus. Concentration gradients of such compounds across the nuclear envelope need not be interpreted as indicative of active transport; but rather as due to differences

of the water solvent space (e.g. Horowitz, 1972) or to the binding of the compound in question to specific nuclear or cytoplasmic constituents.

Nothing can be said as to the pathway of the nucleocytoplasmic exchange of such charged or uncharged small molecules. Although the similarity of the permeability characteristics of the nuclear envelope with those of a cytoplasmic volume element of similar dimensions (keep in mind, however, the aforementioned contrasting data of Loewenstein's group) suggests that the bulk flow is through the pores and not across the membranes of the perinuclear cisterna, the existence of a complex translocation system involving both routes cannot be excluded at present.

PASSAGE OF LARGE MOLECULES AND OF PARTICLES THROUGH THE NUCLEAR ENVELOPE

When one studies the nucleocytoplasmic distribution of large solutes (i.e. those with molecular weight higher than approximately 5000 daltons) and particles, one usually finds a limitation by size that is rather independent of the chemical nature of the specific particle. Such a size control 'sieving' mechanism has been described for polysaccharides (Horowitz *et al.* 1973), in which it appears to begin at effective molecular diameters above 6 nm (earlier literature reviewed by Feldherr & Harding, 1964). Proteins which are synthesized in the cytosol can be rapidly translocated into the nucleus, sometimes within fractions of a second. These proteins can be specifically accumulated there, examples being the histones and various other special nuclear proteins, and nucleocytoplasmic exchange of proteins has been experimentally demonstrated (reviews: Feldherr & Harding, 1964; Goldstein, 1964; Gurdon, 1970; Feldherr, 1972; Paine & Feldherr, 1972). There is a marked limitation of protein uptake from the cytoplasm into the nucleus by molecular size, and sieving appears to begin at diameters around 6 nm (Paine & Feldherr, 1972). There might also exist a preference for accumulating positively charged proteins in the nucleus compared to neutral or negatively charged proteins. From studies using ferritin and gold globules of defined sizes (coated with polyvinylpyrrolidone) Feldherr (1964, 1965, 1966) was able to establish that (*a*) the absolute upper size limit for transportation into the nucleus is 13.5 ± 1 nm particle diameter, (*b*) that such molecules and particles apparently exclusively migrate through the nuclear pores, and (*c*) that they are usually observed in the very centre of the pore and are excluded from the pore periphery (see below). No studies have been made as to the inverse situation; i.e. migration from nucleus into cytoplasm. Although these investigations clearly demonstrate the existence of the trans-pore pathway for such particles, they do not rule

out the existence of alternative pathways such as via formation of nuclear envelope pockets followed by detachment and membrane disintegration (e.g. Szollosi, 1965; Kessel, 1973; Jaworska & Lima-de-Faria, 1973*a*, *b*; Jaworska, Avanzi & Lima-de-Faria, 1973; Franke, 1974*a*; Franke & Scheer, 1974), by a sequence of single membrane vesicle formations which include the specific material and translocate it in a membrane flow like mechanism (e.g. Hinsch, 1970; Franke, 1974*a*), or via direct translocation through one of the membranes or through gaps of the envelope cisterna (see the ideas of Tashiro, Matsuura, Morimoto & Nagata, 1968 and Scharrer & Wurzelmann, 1969).

NUCLEOCYTOPLASMIC MIGRATION OF ORGANELLES AND LARGE AGGREGATES

Normally the nuclear envelope establishes the nucleus as a 'zone of exclusion' for large particulate cytoplasmic components including the ribosomes (e.g. Plates 3, 4, 5, 14), and as a reservation for the chromosomal and extrachromosomal deoxyribonucleoproteins (DNP) and the various structures functioning in transcription and processing of the ribonucleoproteins (RNP). However, in a variety of nuclei one can observe, occasionally or regularly, large particles which are components normally exclusive to the cytoplasm. These include microtubules and microfilaments, fat droplets, membrane cisternae and vesicles, glycogen particles, endosymbiotic bacteria and aggregated virions (references in Franke & Scheer, 1974). For most of these intranuclear structures one can suppose that they have originated by being entrapped in the reconstitution of the nuclear envelope in mitotic anaphase–telophase stages or can form *de novo* in the nucleoplasm from monomeric or micellar constituents (e.g. for the microtubules, the membranes, the fat bodies) or even, in the case of glycogen, be synthesized *in situ*. However, one has to assume that intranuclear symbionts, for example, those that have been described in some euglenoid algae (Leedale, 1969) and in the macronuclei of *Paramecium* (Beale, Jurand & Preer, 1969), have found an – as yet unknown – pathway for nuclear penetration, since these cells have a strictly intranuclear ('closed') mitosis.

POSSIBLE PATHWAYS OF NUCLEOCYTOPLASMIC MOVEMENTS OF RNA AND RIBONUCLEOPROTEINS

The eukaryotes have at least three different genetic systems of protein synthesis, namely mitochondrial, plastidal and nucleocytoplasmic, among which the last is by far the predominant, particularly in quantitative aspects. In the current concept of this protein synthetic system it is assumed that

the nuclear envelope separates the compartment of transcription from that of translation and that the newly synthesized tRNAs, rRNAs and mRNAs or their precursors migrate through the nuclear envelope into the cytoplasm (reviews: Goldstein & Plaut, 1955; Prescott, 1964; Goldstein, 1964; Georgiev, 1967; Spirin, 1969; Maden, 1971).* This phenomenon is demonstrable by nucleoside labelling *in vivo* and autoradiography in those cells in which the pool of endogeneous precursors for RNA synthesis is relatively low (e.g. Zalokar, 1960). Plates 1(*a*) and 3 show the distribution of radioactive nucleosides incorporated into RNA after brief pulse labelling in such an organism, the ciliate *Tetrahymena pyriformis*. Here, as was first shown by Prescott (1962*a*, *b*), the precise confinement of the radioactivity to the macronucleus is evident in the pulse-label situation, and it is also clear in this organism that most of this radioactivity is translocated into the cytoplasm in a subsequent period of chase in medium containing only non-radioactive nucleosides (Plate 1(*b*)). Due to the predominance of ribosomal RNA formation in these nuclei, one usually notes an enrichment of radioactivity over the – in certain stages peripherally accumulated – nucleoli (Plates 1(*a*) and 3(*b*); cf. Leick, 1969; Leick & Anderson, 1970; Satir & Dirksen, 1971; Eckert, Franke & Scheer, 1974). Such an almost complete nucleocytoplasmic chase of newly synthesized RNA is not observed in many other cell types, since the rate appears to depend on the specific relative amounts of radioactivity incorporated into nucleus-specific nucleolar and non-nucleolar RNAs (such as the 4–7S RNA category; for reviews see Busch & Smetana, 1970; Sirlin, 1972), the relative turnover rates of the nuclear heterogeneous pre-mRNAs, the efficiency of the nucleocytoplasmic translocation machinery, the sizes of precursor pools, and possibly some flow of RNAs from the cytoplasm into the nucleus as reported by Goldstein and associates in *Amoeba* (e.g. Goldstein & Trescott, 1970; Wise & Goldstein, 1973).

Recent biochemical and structural studies have brought some insight into the processes and the organization of transcription of the cistrons for various categories of RNAs, in particular for those coding for the ribosomal RNAs and transfer and mRNAs (e.g. Darnell, 1968; Miller & Beatty,

* Some alternative concepts such as those including a transfer of informative DNA molecules from the nucleus to the cytoplasm, followed by a transcription of these DNA sequences into (putatively messenger-like) RNA molecules (cf. Bell, 1969, 1971; Koch, 1972, 1973; Koch & v. Pfeil, 1971, 1972; see, however, also the contrasting references as collected in the recent articles by Williamson, McShane, Grunstein & Flavell, 1972; Meinke, Hall & Goldstein, 1973) are not considered in the present article. It is hard to evaluate the positive evidence for such ideas (see also Franke *et al.* 1973), in particular in cell systems in which thymidine labelling is totally restricted to the mitochondria and the nucleus.

1969*a–c*; Busch & Smetana, 1970; Dawid, Brown & Reeder, 1970; Grierson, Rogers, Sartirana & Loening, 1970; Loening, 1970; Miller, Beatty, Hamkalo & Thomas, 1970; Perry *et al.* 1970; Weinberg & Penman, 1970; Birnstiel, Chipchase & Speirs, 1971; Burdon, 1971; Wensink & Brown, 1971; Brown, Wensink & Jordan, 1972; Daneholt, 1972; Georgiev *et al.* 1972; Lambert, 1972; Miller & Bakken, 1972; Miller & Hamkalo, 1972; Sirlin, 1972; Charret & Charlier, 1973; Chen & Siddiqui, 1973; Darnell, Jelinek & Molloy, 1973; Derksen, Trendelenburg, Scheer & Franke, 1973; Hamkalo & Miller, 1973; Jelinek *et al.* 1973; Littauer & Inouye, 1973; Scheer, Trendelenburg & Franke, 1973; Stewart & Letham, 1973; Trendelenburg, Scheer & Franke, 1973; Weinberg, 1973). It seems to be a general principle that DNA regions, which are separated from each other by shorter or longer (for tRNA and 5S rRNA see also: Brown *et al.* 1971; Sirlin, 1972; Clarkson, Birnstiel & Serra, 1973; Clarkson, Birnstiel & Purdom, 1973) 'spacer' intercepts, are transcribed into precursor molecules from which the specific RNAs are produced by a subsequent characteristic cascade of hydrolytic cleavages, the 'processing'. During and after this processing, chemical modifications of both sugar and base moieties can occur in a pattern specific for the RNA category; in particular, various methylation reactions. While the tRNAs (and probably also the 5S rRNA) and their precursors are generally assumed to be 'naked',* that is, not tightly associated with distinct proteins, it is clear for the mRNAs and rRNAs that, while being synthesized at the template, they become immediately covered with proteins and then appear to be released in the form of nuclear 'informosomes' or the nucleolar preribosomal RNP fibrils or granules (for surveys see: Vaughan, Warner & Darnell, 1967; Warner & Soeiro, 1967; Moulé & Chauveau, 1968; Rogers, 1968; Liau & Perry, 1969; Narayan & Birnstiel, 1969; Samarina, Lukanidin, Molnar & Georgiev, 1968; Spirin, 1969; Busch & Smetana, 1970; Faiferman, Hamilton & Pogo, 1971; Maden, 1971; Mirault & Scherrer, 1971; Niessing & Sekeris, 1971; Kumar & Warner, 1972; Sirlin, 1972; Albrecht & Van Zyl, 1973; Simard, Sakr & Bachellerie, 1973; Williamson, 1973). How these growing RNP fibrils are stored or translocated from the point of termination is not clear. Regarding the nucleolus, the prevailing hypothesis proposes that a transfer occurs from the *pars fibrosa* of the nucleolar interior to the *pars granulosa*, which in most nucleolar types is located in the nucleolar periphery. This is indicated from long term pulse and pulse-chase labelling experiments (e.g. Plate 4) as well as from studies using inhibitors of transcription (for references see: e.g., Granboulan & Granboulan, 1965; Busch & Smetana,

* For exceptions during early amphibian oogenesis see, however, Thomas, (1970), Denis & Mairy (1972), Ford (1972).

1970; Das, Micou-Eastwood, Ramamurthy & Alfert, 1970; Fakan & Bernhard, 1971, 1973). Concomitant with this transfer, processing of the precursor molecule takes place. The typical granular substructure of the nucleolus cannot, however, be clearly correlated with the structural nucleolar components described in isolated preparations (Vaughan *et al.* 1967; Warner & Soeiro, 1967; Rogers, 1968; Liau & Perry, 1969; Narayan & Birnstiel, 1969; Busch & Smetana, 1970; Craig & Perry, 1970; Kumar & Warner, 1972; Shepherd & Maden, 1972; Simard *et al.* 1973) or in spread preparations of *pars granulosa*. In the latter, only very long beaded-string fibril structures are seen; there being no distinct or clearly defined granular moieties (Miller & Beatty, 1969*b*, *d*; Miller & Hamkalo, 1972).

As to the precursors of messenger RNAs, it is widely assumed that after transcription and complexing with proteins, most of the large or small precursor molecules become attached at their 3′-ends with a (perhaps somewhat variably long) stretch of polyadenylate. These complexes are then degraded and processed into functional smaller molecules before being transported into the cytoplasm (for references see Adesnik, Salditt, Thomas & Darnell, 1972; Darnell *et al.* 1973; Jelinek *et al.* 1973; Weinberg, 1973). There are, however, at least two known examples of giant RNA molecules entering the cytoplasm, namely the Balbiani ring-derived mRNA coding for the secretory slime protein of chironomid larvae (Daneholt & Hosick, 1973), and an RNA species synthesized during sea urchin embryogenesis (Giudice, Sconzo, Ramirez & Albanese, 1972). It is not known at what nuclear structures the individual poly-A-polymerization and the processing steps take place, although there is some evidence that such activities may be located in the nuclear informosomes (Niessing & Sekeris, 1973).

Little is known about the mechanism by which the RNAs and the RNPs are further translocated from the site of transcription, or of possible transient intranuclear storage (as perhaps in the *pars granulosa* in the case of the rRNA-containing precursor molecules) into the cytoplasm. It is obvious that such transport mechanisms must somehow be selective, since certain specific RNA molecules remain in the nucleus or, even more specifically, remain in association with distinct nuclear components (nucleoli or chromatin). Another statement which may be made is that this translocation, at least for the functionally defined species of the tRNAs, rRNAs and mRNAs, is strictly vectorial: backflow into the nucleus, of either these RNAs or the particles in which they reside, has not been described. (The cytonucleoplasmically shuttling RNAs discussed by Wise & Goldstein (1973) apparently do not belong to these classes.) Neither ribosomes, polyribosomes nor informosomes of the cytoplasmic type can be demonstrated in an intact nucleus (see, e.g., Plate 5).

PLATE 1

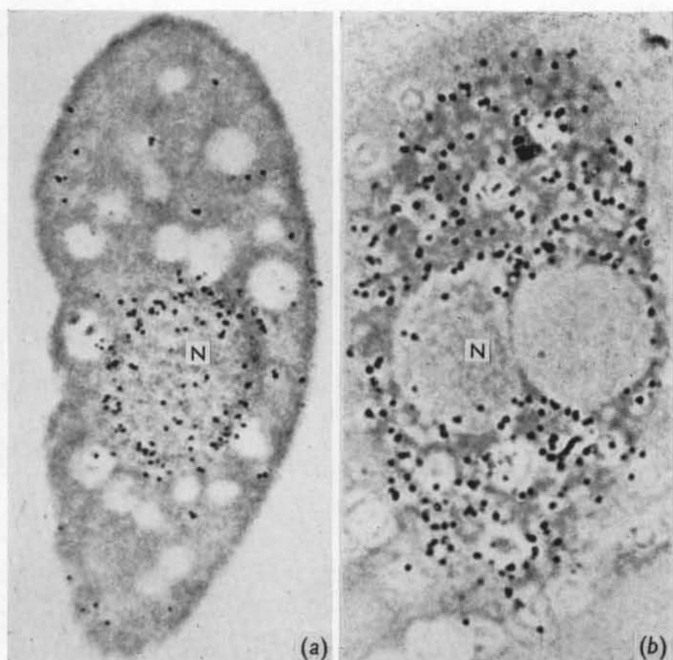
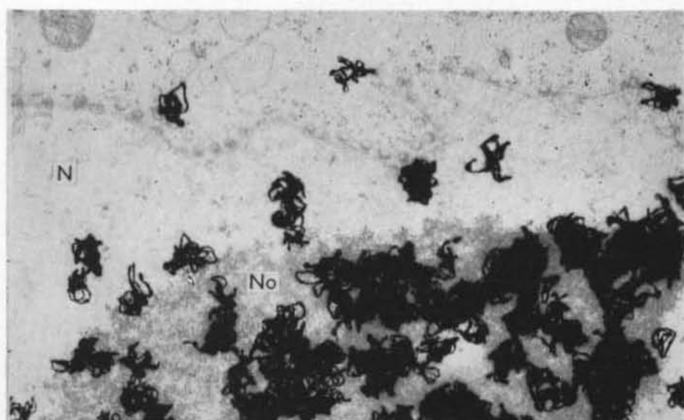
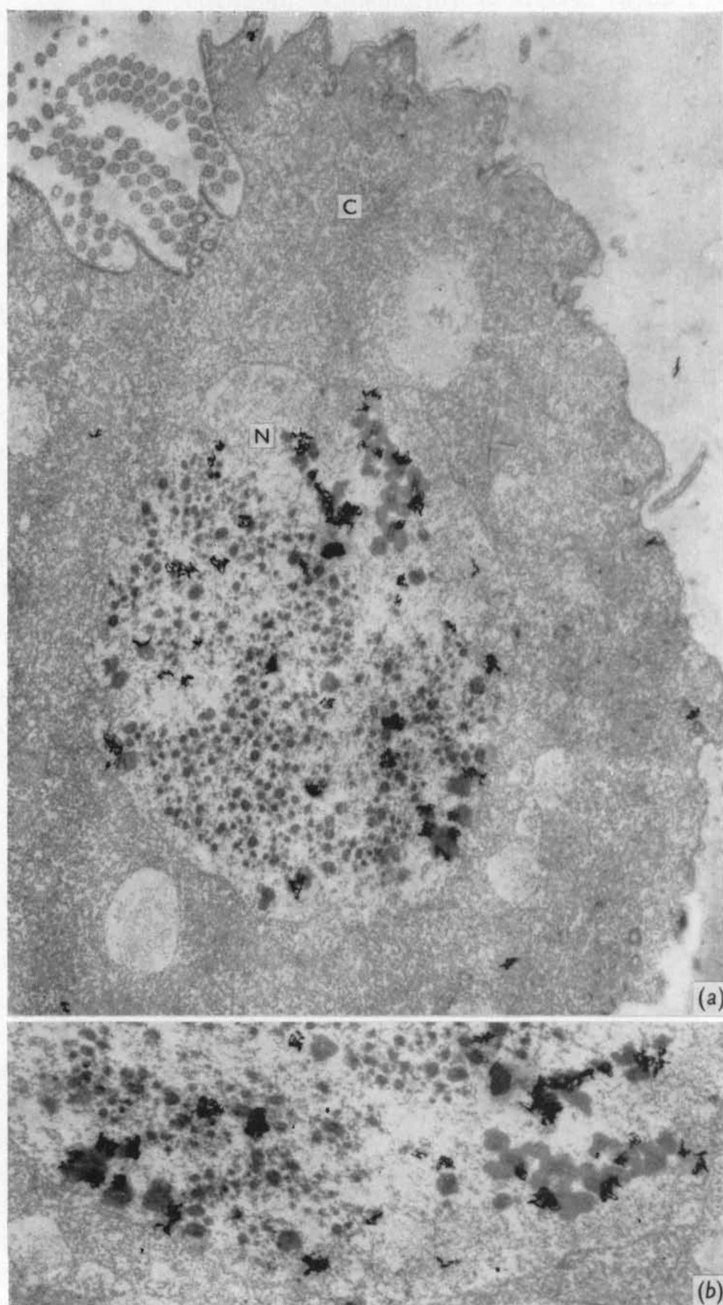


PLATE 2



For explanations see p. 279



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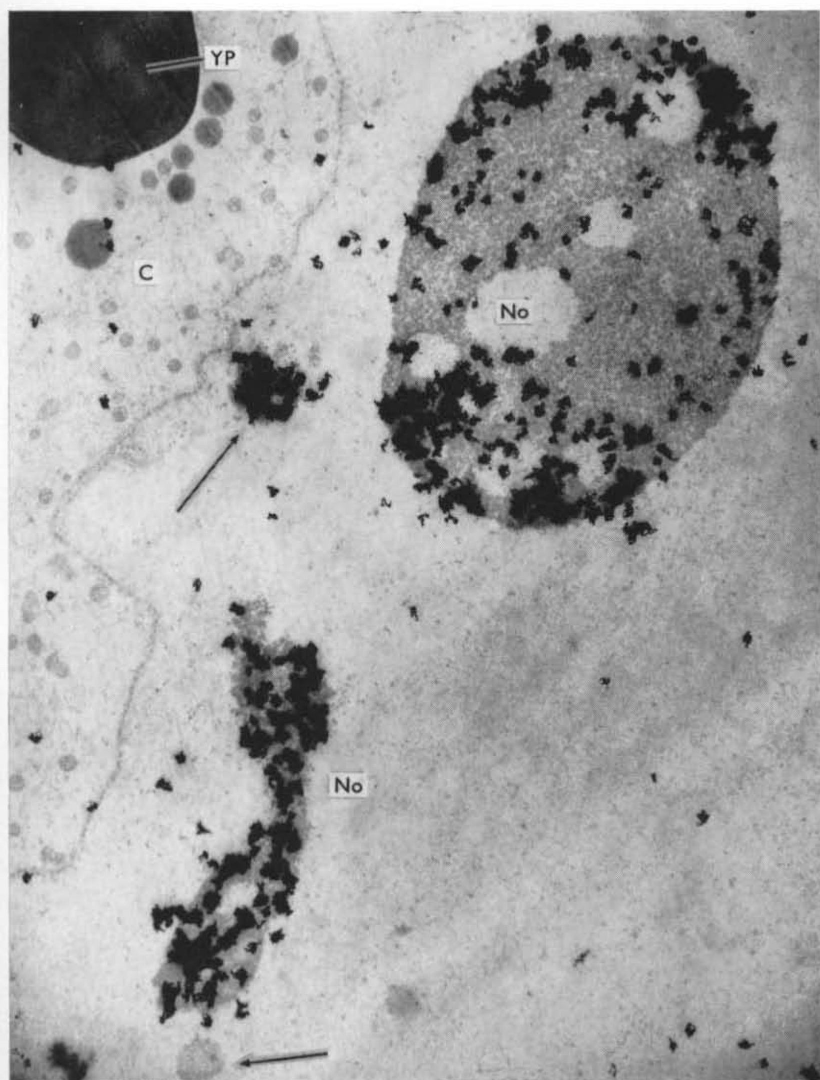

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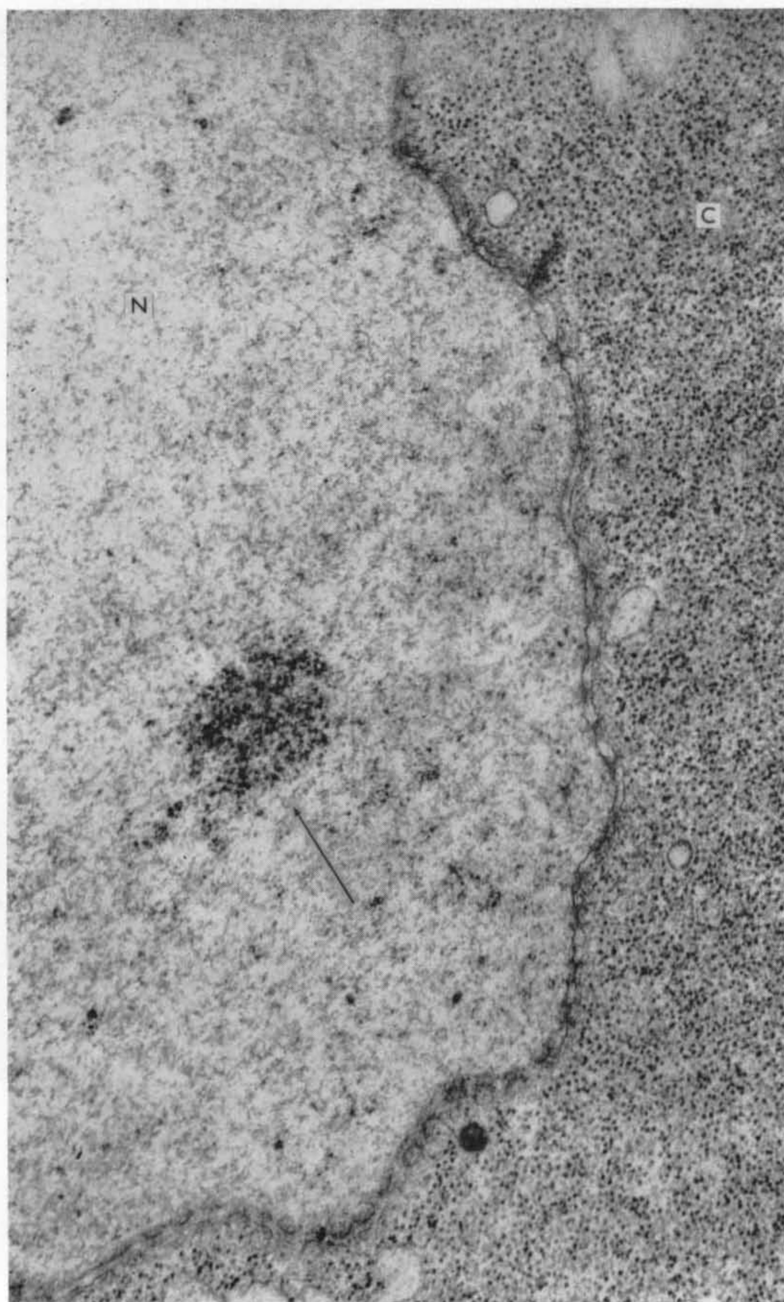
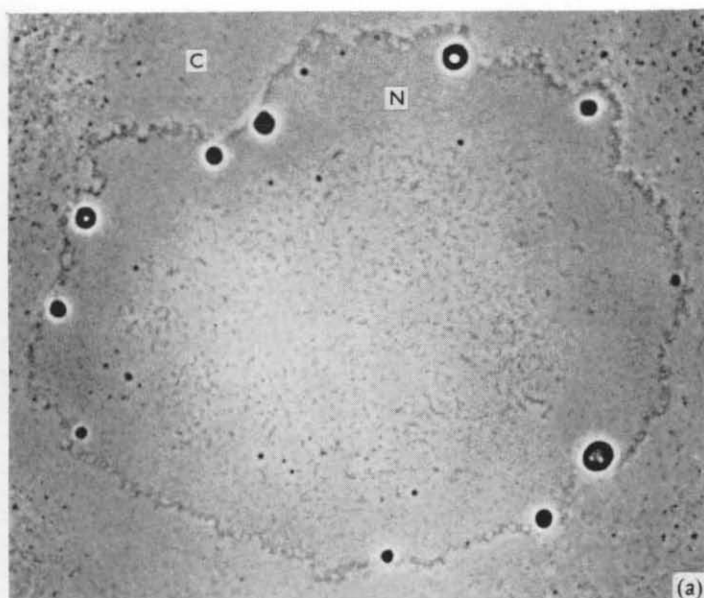


For explanation see p. 280

PLATE 6



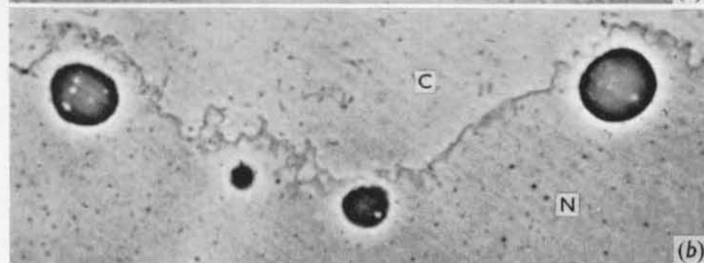
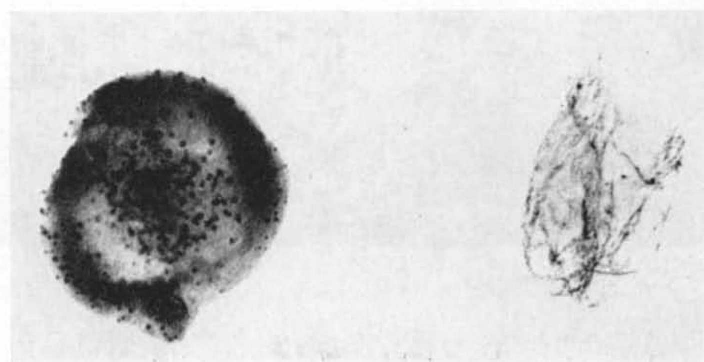
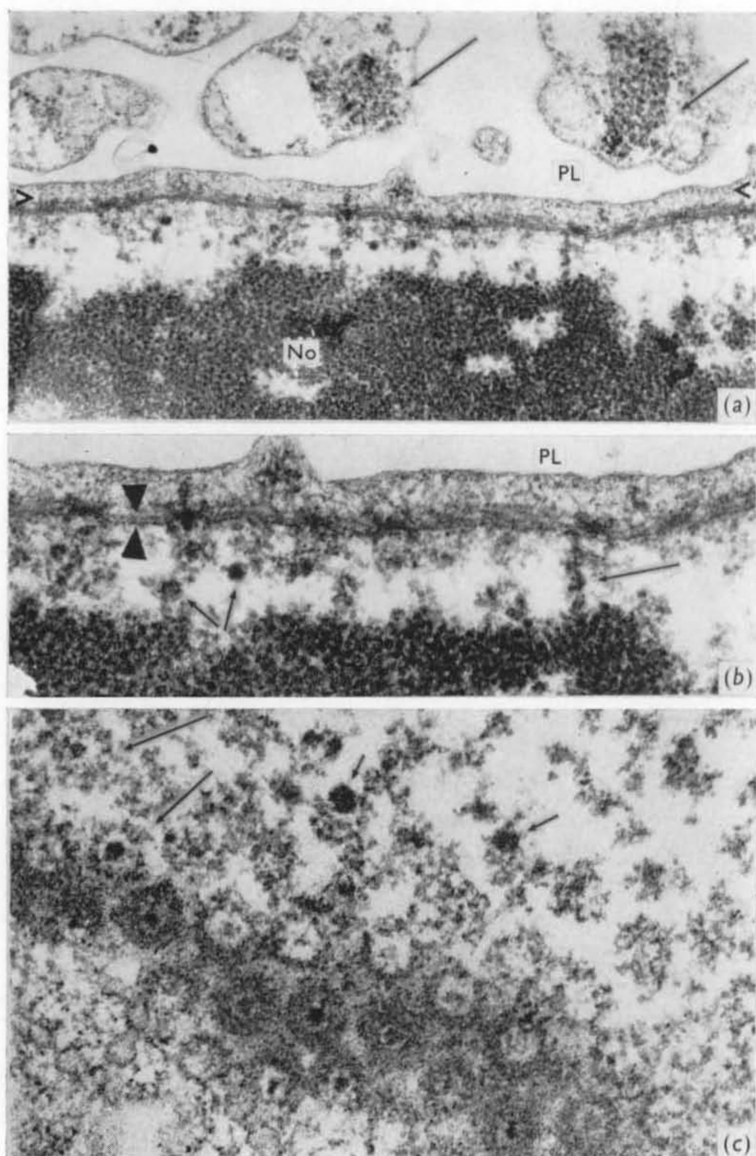


PLATE 7



For explanations see p. 280



For explanation see p. 281

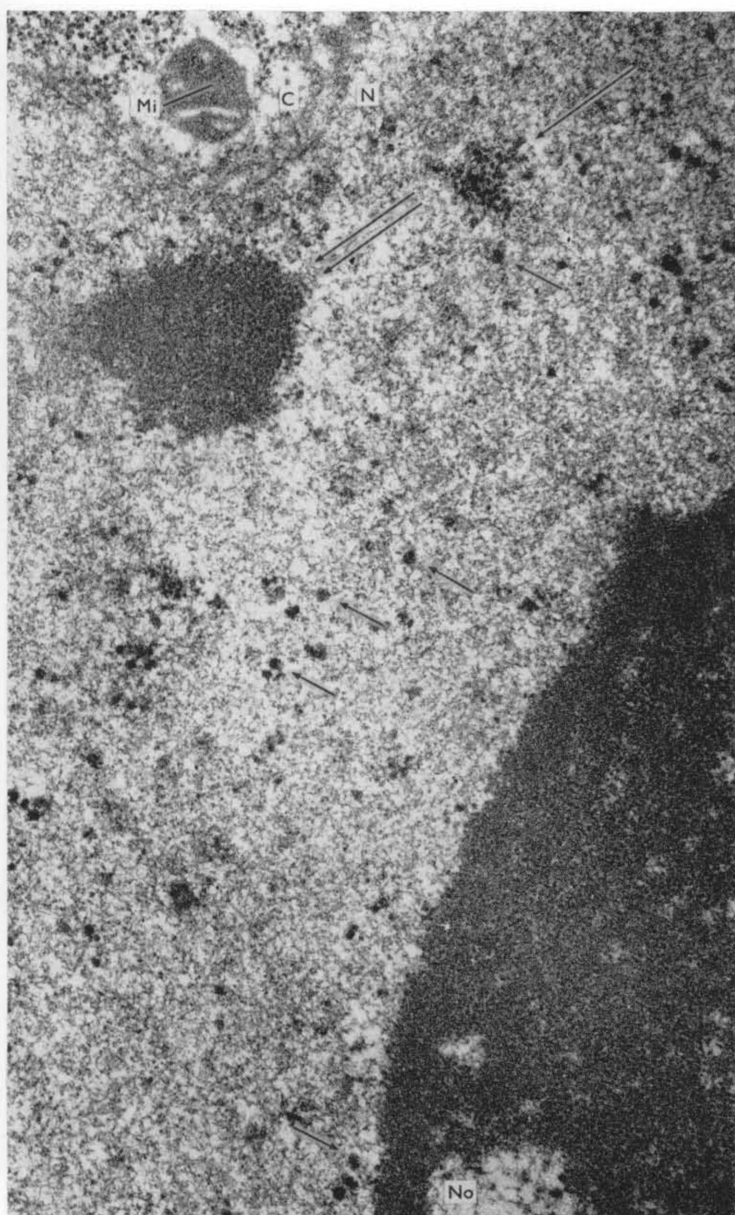


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PLATE 10

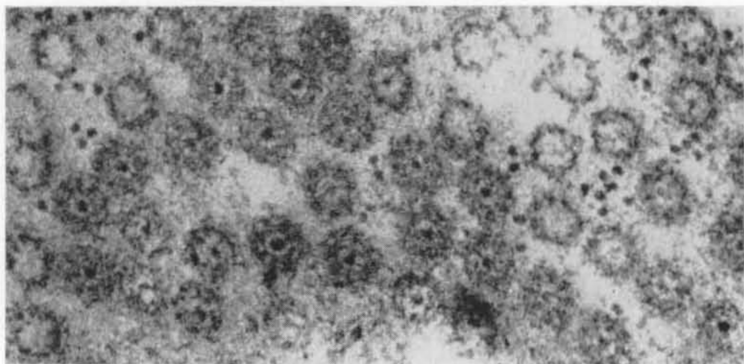
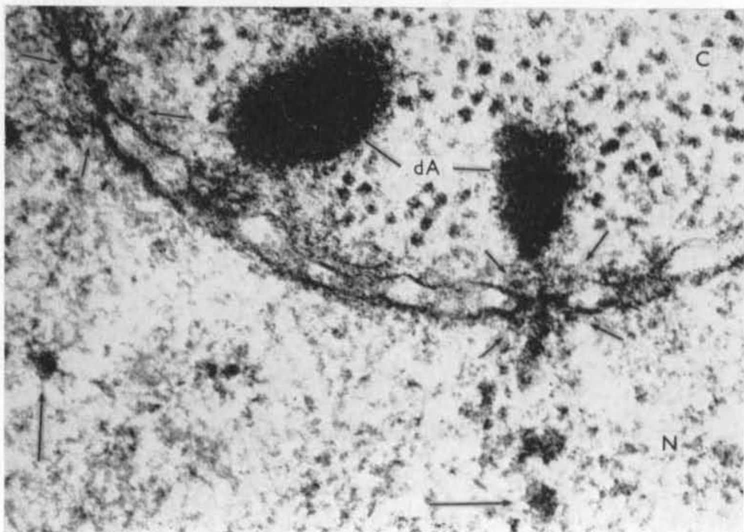


PLATE 11

PLATE 12



For explanations see pp. 281–2

PLATE 13

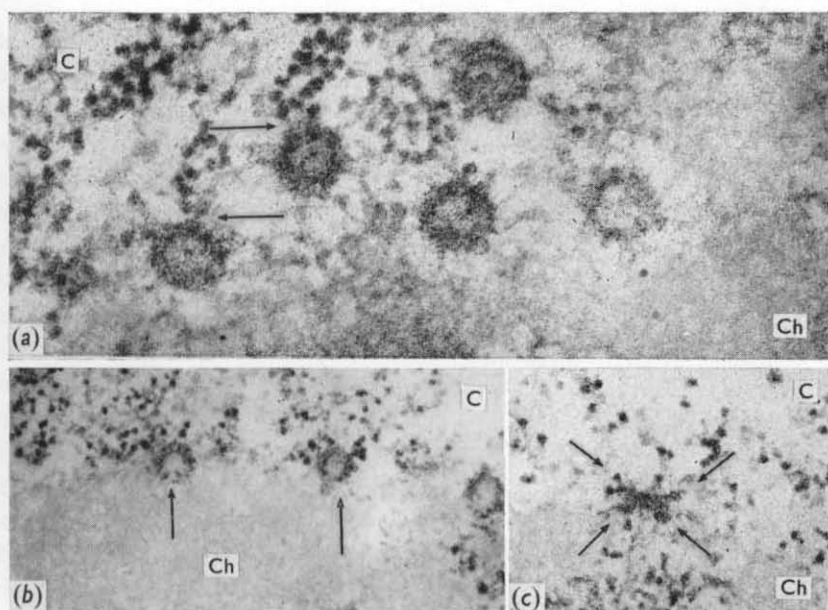
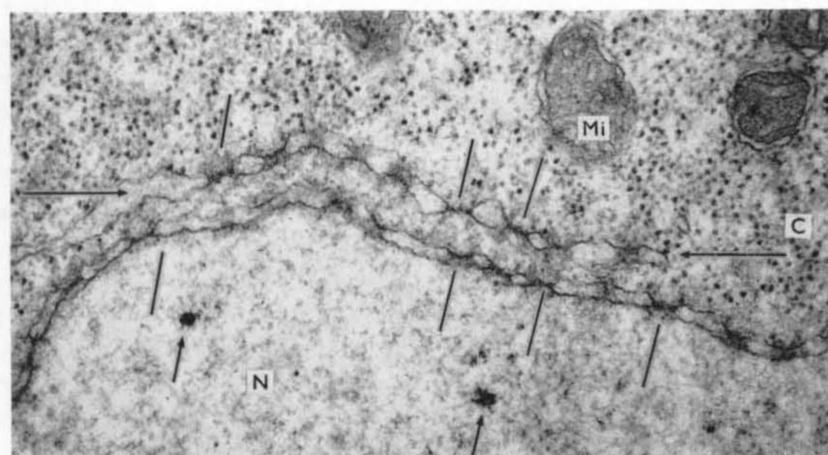
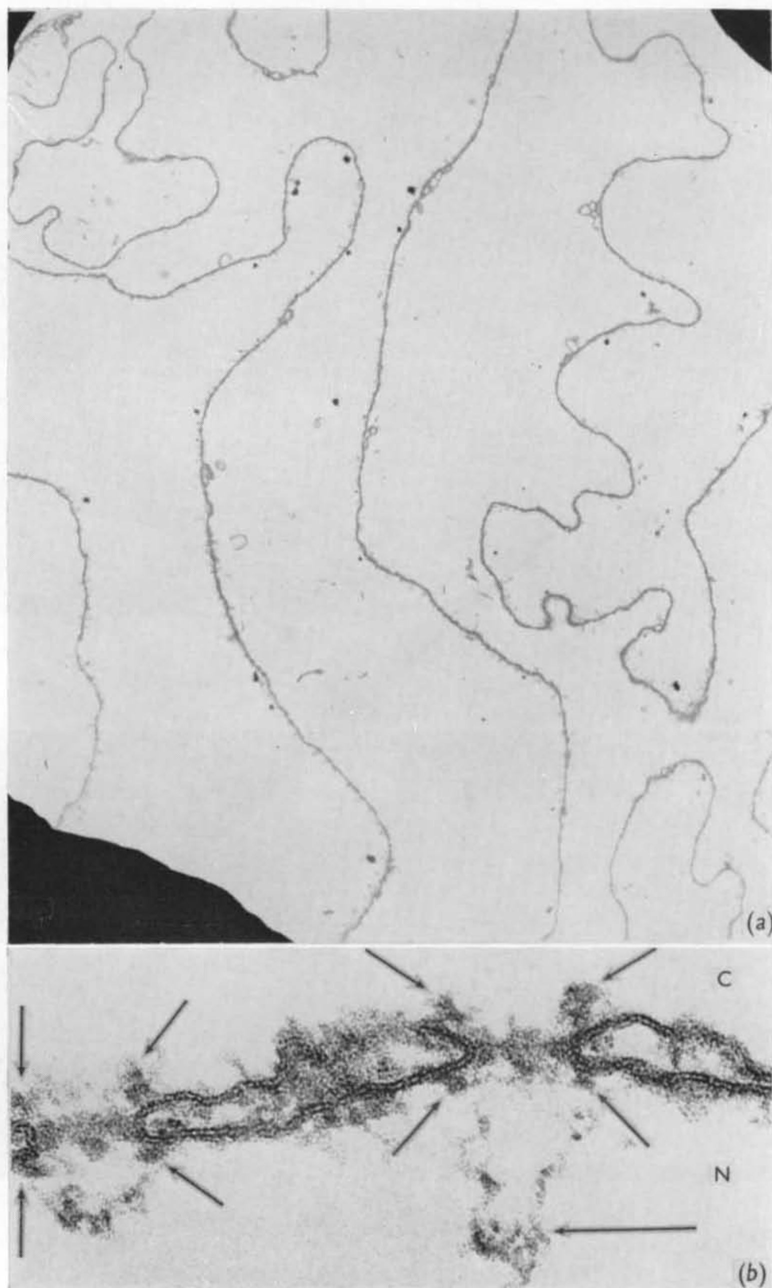


PLATE 14



For explanations see p. 282



For explanation see p. 282

Most concepts of the nucleocytoplasmic translocation of newly synthesized RNAs have included the idea that the structural moieties passing the nuclear envelope contain the fully processed RNA molecules already complexed with the proper proteins and are individualized, that is, present in a free nuclear pool which is exchangeable with similar or identical units in the cytoplasm. Especially detailed models have been developed for the nucleocytoplasmic transfer of ribosomal components. These models have envisaged that mature ribosomal subunits migrate from the nucleus into the cytoplasm, the smaller subunit being exported at a significantly faster rate than the larger subunit (e.g.: Penman, 1966; Penman, Smith & Holtzmann, 1966; Vaughan *et al.* 1967; Maden, 1968; Weinberg & Penman, 1970; Nosal & Radouco-Thomas, 1971). In gel electrophoretic separations we have consistently found, however, that in purified nuclei (isolated, for example, from the amphibian oocyte and the amicronucleate strain GL of the ciliate *Tetrahymena pyriformis*) the large mature rRNA component is not detectable in the nucleus in significant amounts and that the smallest precursor rRNA component has a markedly lower electrophoretic mobility than the mature rRNA prepared from the cytoplasmic ribosomes (Fig. 1; cf. Scheer, 1973; Scheer *et al.* 1973; Franke & Scheer, 1974; Eckert *et al.* 1974). Similar observations have also been made by Gall (1966) and Rogers (1968) in nuclei isolated manually from *Triturus viridescens* and *Amblystoma mexicanum* oocytes, by Ringborg & Rydlander (1971) in the nuclei dissected from chironomid salivary gland cells, by Hogan & Gross (1972) in nuclei isolated from sea urchin embryos, and by Sillevis Smitt *et al.* (1970, 1972) in isolated yeast nuclei. There are also indications that the small (18S) rRNA might likewise not be present in the nucleus, since only a precursor component with a slightly lower mobility is observed in the gels (see, e.g. Fig. 1 (*a*), and, for yeast, Udem & Warner, 1973). Therefore, we conclude, in contrast to the earlier schemes (Penman, 1966; Penman *et al.* 1966; Perry, 1967, 1969; Vaughan *et al.* 1967; Maden, 1968; Weinberg & Penman, 1970; Nosal & Radouco-Thomas, 1971), that in many cells the smallest nuclear precursors of the rRNAs differ either in conformation or in molecular weight from the rRNAs themselves, which leads us to hypothesize that either the processing of the 28 and 18S rRNAs is not finished within the nucleus or that the translocation of the mature rRNAs into the cytoplasm is extremely fast so that the steady state concentrations of these RNAs in the nucleus are very low. It is not clear whether the nucleocytoplasmically migrating rRNA and other RNA moieties are present as free components *in vivo*, as has been concluded from a variety of biochemical studies using disrupted and/or extracted nuclei. From ultrastructural observations of the intimate integration of the nucleolar

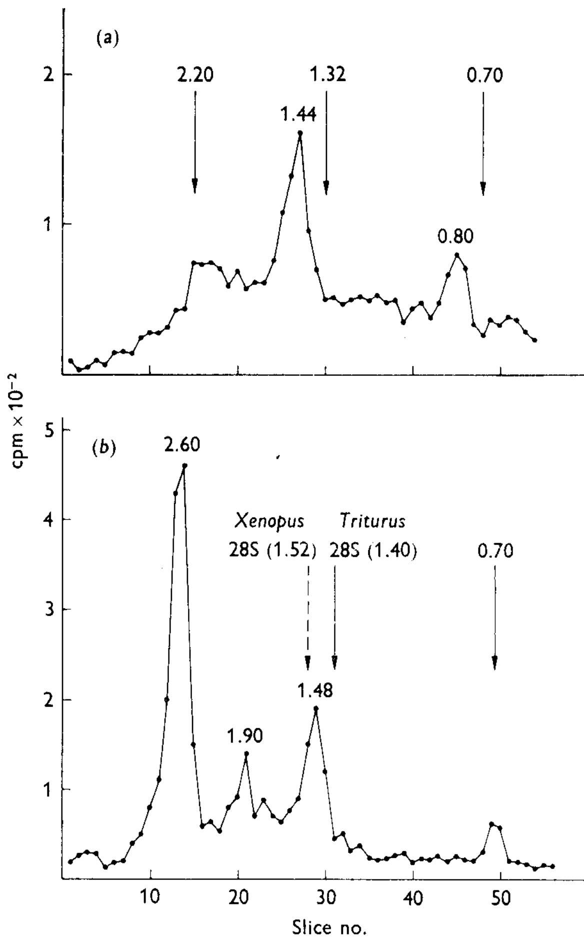


Fig. 1. Gel electrophoresis of [^{3}H]uridine labelled RNA extracted from isolated macronuclei of *Tetrahymena pyriformis* (*a*) and isolated nuclei of *Triturus alpestris* lampbrush stage oocytes (*b*). The *Tetrahymena* cells were pulse-labelled for 8 min and chased in non-radioactive medium for 90 min (for further details see Plate 1 and Eckert *et al.* 1974). Macronuclei were isolated using a modified version of the method described by Franke (1967) and Eckert (1972); the RNA was extracted with pronase–sodium dodecylsulfate (SDS). The lampbrush stage oocytes obtained from *Triturus alpestris* ovaries were incubated in Eagle's medium (diluted 1 to 1) containing a mixture of all four tritiated nucleosides (100 μCi ml^{-1} each) for 12 h at 18 °C. In each experiment 20 nuclei were manually isolated and extracted with pronase–SDS (for details see Scheer *et al.* 1973). Gel electrophoresis was performed on slabs of 2.25 % acrylamide–0.5 % agarose composite gels under conditions described by Ringborg *et al.* (1970). Mature rRNAs extracted from the microsomal fraction of *Tetrahymena* (*a*) and from ooplasmic ribosomes in the case of the amphibians (*b*) were run in parallel on the same gels; their positions are indicated by the arrows (*Tetrahymena*: 1.32 and 0.70 × 10^6 D, *Triturus*: 1.40 and 0.70 × 10^6 D). (*a*) The RNA isolated from *Tetrahymena* macronuclei after a 90 min chase shows

and interchromatinic granules or nodules with the surrounding RNP-containing fibrillar strands (e.g. Monneron & Bernhard, 1969), as well as from the findings that almost all RNA is recovered in a form sedimentable with a few thousand **g** within 10 min after disruption of amphibian germinal vesicles at appropriate ionic strength, we feel that it is an alternative hypothesis worth pursuing to envisage all these RNA moieties as being integrated into very large granulofibrillar network structures.

A challenge for electron microscopists has been the question of which route such macromolecular RNP aggregates follow when leaving the nucleus. Pathways to be considered (compare the reviews of Feldherr, 1972; Kessel, 1973; Franke & Scheer, 1974) include, for example, inclusion of nuclear RNP in vesicles formed at the inner nuclear membrane, followed by detachment into the perinuclear cisterna, fusion with the outer nuclear membrane and finally release of vesicle contents into the cytosol (for similar processes see the mechanisms of extrusion of various nuclear viruses described by Darlington & Moss, 1968, 1969; Nii, Morgan & Rose, 1968; Kitajima, Lauritis & Swift, 1969; Sylvester & Richardson, 1970; see also the discussions of Hinsch, 1970, and Gulyas, 1971) and the production and release of large nuclear envelope evaginations (such processes have been demonstrated, e.g., in the extrusions of nucleolus-like bodies in some mammalian oocytes by Szollosi, 1965; in the amplified rDNA-containing nucleoli of the house cricket by Jaworska & Lima-de-Faria, 1973*a*, *b* and Jaworska *et al.* 1973; and for the developing eggs of some ferns: Bell, 1972). However, most observations indicate that the normal way of translocation of RNP structures is via the nuclear pores:

(*a*) In some nuclei one can observe direct fibrillar connections between the nucleolar cortex RNP and the constituents of the nuclear pore complexes, which exhibit a stainability similar to that of the nucleolar structures. Such connections are especially conspicuous in situations where the nucleoli have accumulated at the nuclear envelope in periods of high ribosome formation rate, such as in the amphibian oocyte lampbrush stage

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two prominent radioactive peaks corresponding to molecular weights of 1.44 and 0.80×10^6 D, which migrate more slowly than the corresponding mature rRNAs. (The pre-rRNA of this organism has an apparent molecular weight of 2.20×10^6 D.) (*b*) In manually isolated nuclei from *Triturus* oocytes no significant amounts of mature 28S rRNA can be found. The peak corresponding to a molecular weight of 1.48×10^6 D presumably represents an intermediate stage in the development of the rRNA. Both the 28S rRNAs from *Xenopus laevis* (1.52×10^6 D) and *Triturus alpestris* (1.40×10^6 D) ovary ribosomes served as markers for molecular weight determinations (see also Rogers & Klein, 1972). The pre-rRNA in *Triturus* has an apparent molecular weight of 2.6×10^6 D. Some radioactivity is also found in the region of the 18S rRNA.

(Plate 6 and Fig. 2(*c*); see also Miller, 1966; Lane, 1967; Franke & Scheer, 1970*b*; Scheer, 1972), in the macronuclei of exponentially growing cultures of *Tetrahymena pyriformis* (references in Satir & Dirksen, 1971), and in protrusions from giant nucleoli in the primary nucleus of *Acetabularia* (Plate 8(*a*), (*b*)). Fibrillar connections are also seen between the pore complex structures and distinct large aggregates of granules which apparently are derived from the nucleolar cortex (e.g. Lane, 1967; Franke & Scheer, 1970*b*).

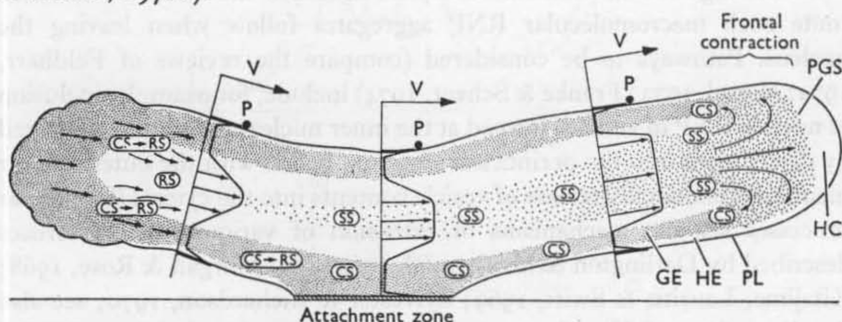

Fig. 2. Associations of various structures that are probably composed of ribonucleoprotein with the nuclear pore complexes. The cytoplasmic side is indicated by the mitochondria. For further details see text.

(*b*) Each pore complex is the terminal attachment site for a bundle of aggregated fibril–coil structures which appear to contain RNP and traverse the nuclear interior. These constitute the so-called 'interchromatinic channels' (cf. Watson, 1959) or the 'ribonucleoprotein network' (cf. Busch & Smetana, 1970). They react, in cytochemical tests and in differential extractions, in the same manner as the constituents of the pore complex (e.g. Plate 13; cf. Franke & Falk, 1970).

(*c*) A special class of 35–55 nm large granules are frequently seen in association with the nucleolar surface as well as in the nucleoplasmic space between the nucleoli and the nuclear envelope. These granules, which exhibit in cytochemical tests a typical RNP reaction, can also frequently be seen in association with the nuclear pore complexes or even within the interior of the nuclear pore (e.g. Plates 8(*c*) and 9; this case is depicted under (*b*) in Fig. 2).

(*d*) Fractions of isolated nuclear membranes contain a significant amount of RNA which, unlike the ribosomal RNA, is resistant to high salt extraction and which is different from the average ribosomal RNA (Table 1 gives determinations in nuclear membrane fractions from various cell types all isolated by a procedure identical to or slightly modified from that described by Franke *et al.* 1970; for details of preparation compare:

Table 1. *Gross compositions (% dry weight of total) of nuclear membranes isolated* from different cells and tissues*

	Hen erythrocytes	Rat liver	Pig liver	Calf thymus	Onion root tip	Macronuclei of *Tetrahymena pyriformis*
Protein	75.4	75.5	74.8	70.0	73.8	75.5
Phospholipids	13.0	16.1	18.2	15.4	15.2	18.0
Non-polar lipids	3.7	2.8	3.0	3.7	2.9	—
RNA	4.0	3.6	2.8	3.8	6.1	2.6
DNA	3.8	2.0	1.2	7.0	1.9	3.9

* Isolation procedures were similar for all objects (for references see text).

Zentgraf, Deumling, Jarasch & Franke, 1971; Eckert, 1972; Jarasch, Reilly, Comes & Kartenbeck, 1973; Eckert *et al.* 1974; Franke, 1974*b*; for discussions of nuclear membrane associated RNA see: Zbarsky, 1972; Monneron, Blobel & Palade, 1972; Kay & Johnston, 1973; Kessel, 1973; Kasper, 1974). The large nuclear envelopes from amphibian oocytes, to which no considerable amount of chromosomal DNA is attached, also contain large amounts of RNA (Scheer, 1972). Since these preparations contain only the nuclear membrane proper and the granules and fibrils associated with the pore complex (Plate 15), it can be concluded that this RNA is located largely in the non-membrane components of the nuclear pore complexes. This is particularly clear in preparations from mature oocytes where the nucleoli have become detached and only very few ribosomes are associated with the outer nuclear membrane (Scheer, 1972). When one treats such manually separated (see Plate 7) nuclear envelopes with non-ionic detergents, such as with 0.8% Triton-X-100 for 10 min in a 'nucleoprotein stabilizing solution' (0.04 M KCl, 0.04 M NaCl, 15 mM $MgCl_2$, 10 mM Tris-HCl pH 7.0), the membrane becomes solubilized and the non-membranous nuclear pore complex constituents are obtained in a form that is still sedimentable at low speed. Almost all of the initial RNA is recovered in such a pellet (Scheer & Franke, unpublished data).

(*e*) The nuclear pore complex has a highly ordered subarchitecture which consists of (i) an inner and outer ring (annulus) on either pore margin containing eight symmetrically distributed granules, (ii) eight symmetrically arranged tips of material projecting from the pore wall, (iii) an occasionally observed central dense element of a somewhat variable size and shape, and (iv) a variety of radially, concentrically and axially oriented fibrils, the most prominent usually being the nucleoplasmic fibrils terminating at the inner (nuclear) annulus and at the central granule (Fig. 3; for detailed descriptions see the reviews: Franke, 1970*a*; Franke & Scheer,

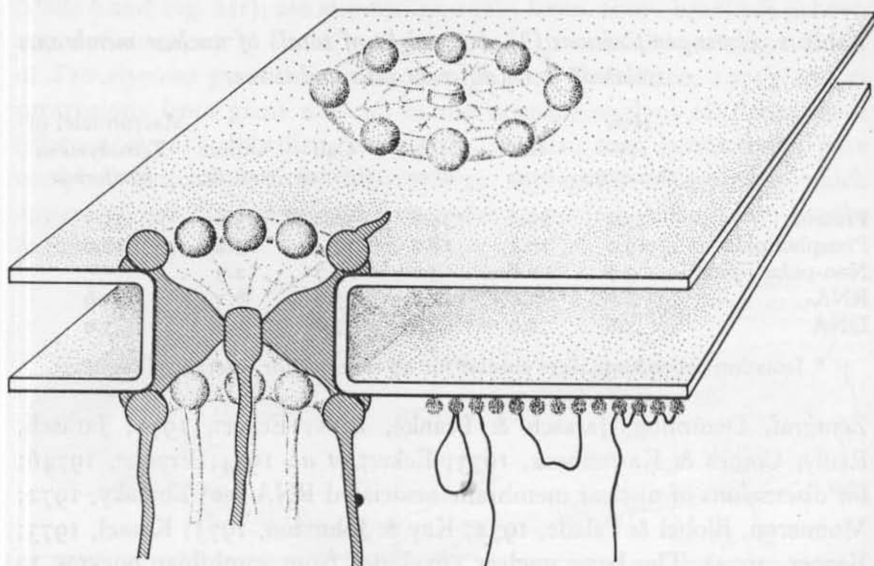

Fig. 3. Pore complex model emphasizing the compact appearance of the non-membranous constituents. Eight annulus granules are arranged symmetrically at the margin of either side of the pore; conical tips of dense material project from the pore wall and the annuli into the pore lumen ('peripheral granules', cf. Roberts & Northcote, 1970, 1971). In the pore centre one frequently notes a central element of variable size and shape. Fibrils terminate at the granules of the inner (nucleoplasmic) annulus and the central element. Short fibrils sometimes extend from the outer (cytoplasmic) annulus granules into the cytoplasm. For reasons of clarity the various arrangements of fibrils within the pore lumen are not included in this model. In the inter-pore regions of somatic cells strands or loops of chromatin are attached at certain sites on the inner nuclear membrane where they often appear to terminate in distinct granules.

1970*a*; Roberts & Northcote, 1970, 1971; Feldherr, 1972; Engelhardt & Pusa, 1972; LaCour & Wells, 1972; Fabergé, 1973; Hanzely & Olah, 1973; Kessel, 1973). All these non-membranous components react in cytochemical tests in a manner analogous to RNP-containing structures and different from DNP structures, which are often associated with the inter-pore areas (Plate 13; e.g. Mentré, 1969; Franke & Falk, 1970). The identical fine structural organization is present in the intranuclear and cytoplasmic annulate lamellae (AL; reviews: Kessel, 1968; Wischnitzer, 1970; Franke & Scheer, 1974).* This fact demonstrates that the nuclear pore complexes,

* The granular substructures of the pore complexes frequently exhibit a fibrillar (unravelled) appearance, perhaps also depending on the specific fixation procedure. This is especially striking within AL stacks where the many pore complexes make up a fibrillar textured zone in between the cisternae (e.g. Plate 14).

although they appear to be the gateways for nucleocytoplasmic exchange, are not structures unique to the nuclear envelope; i.e. they are not functionally exclusive to such translocation phenomena.

(*f*) The nuclear pore complexes show, in various cell types, close relationships to cytoplasmic polyribosomes (e.g. Plates 10, 13; Gall, 1956; Mepham & Lane, 1969; Franke, 1970*a*; Jacob & Danieli, 1972; for further references see Franke & Scheer, 1974). The annulus granules, however, differ from the ribosomes by being somewhat larger and less electron opaque after the usual staining treatments, thus suggesting a lower density of packing (Plates 10, 11, 13; compare Scheer, 1972). This is diagrammed as case (*f*) in Fig. 2.

(*g*) Associations of perichromatin granules, which are also ribonucleoproteinaceous in nature (Monneron & Bernhard, 1969), with the nuclear envelope are likewise not uncommon. As was first noted by Monneron & Bernhard (1969), it is obvious that these granules never appear to enter the pore lumen but rather seem to unravel into fine filaments which are revealed in connection with the pore walls.

(*h*) A very impressive mode of transfer through the nuclear pore complexes has been noted in the salivary glands of chironomid larvae (Beermann, 1964; Stevens & Swift, 1966; Vazquez-Nin & Bernhard, 1971). In these cells aggregates of heavily stainable granules of a relatively uniform diameter of 40–50 nm appear to derive from the Balbiani ring, migrate to the nuclear pores, elongate into approximately 15 nm thick rods and then force through the very centre of the pore, thereby assuming a transitory dumbbell-like shape. After passage through the pores these aggregates then reassume their spherical shape and can be identified in the nuclear vicinity.

A similar mode of transportation has been described for the larger perinuclear aggregates observed during amphibian oogenesis (e.g. Clérot, 1968; Franke & Scheer, 1970*b*; Eddy & Ito, 1971; this type of movement is seen in Plate 12 and diagrammed under (*d*) in the scheme of Fig. 2), the RNA-containing helices of *Amoeba proteus* (Stevens, 1967; Wise, Stevens & Prescott, 1972; for quotations of similar structures in macronuclei of *Euplotes* see also Kessel, 1973), various viruses some of which release only their nucleic acid content through the pore centre during nuclear infection (from the cytoplasm) (DeZoeten & Gaard, 1969; Morgan, Rosenkrantz & Medmis, 1969; Summers, 1969, 1971; Chardonnet & Dales, 1970), and the characteristic granulo-fibrillar aggregates of the 'nuclear bodies' or 'sphaeridia' (Büttner & Horstmann, 1967; Dupuy-Coin, Lazar, Kalifat & Bouteille, 1969; Rupec, 1969; this latter case is sketched as (*e*) in Fig. 2). While for some of these structures a nucleic

acid content has been demonstrated, there are also reports on some of the structures mentioned which indicate a purely proteinaceous character, e.g. for the perinuclear bodies observed during amphibian oogenesis (Clérot, 1968; Eddy & Ito, 1971; Gerin, 1971). A trans-pore passage of related types of dense bodies accumulated in the juxtanuclear cytoplasm, for example, the 'chromatoid bodies' occurring in various spermiogeneses and the 'polar granules' of some insect eggs (reviews: Fawcett, Eddy & Phillips, 1970; Comings & Okada, 1972, Fawcett, 1972; Mahowald, 1972), might also be supposed from some of the published micrographs, but has not yet been directly demonstrated.

This mode of transportation also illustrates that only the central region of the pore complexes (of maximally about one third of the diameter of the pore lumen) is accessible to the migratory particles. It also recalls to mind the above-mentioned studies of Feldherr and others who have noted both a size limitation for particle transfer from the cytoplasm into the nucleus and a restriction of particle transfer to the very centre of the pore. From such findings it is reasonable to conclude that, at least in many situations, the central granules of the pore complexes may represent such particulate material en route (Stevens & Swift, 1966; Franke & Scheer, 1970*b*; Wunderlich, 1969, 1972), but there is also strong evidence that central granules cannot be generally regarded as representing such migrating material, in particular not newly formed RNP (for detailed discussions see, e.g., Eckert, Franke & Scheer, 1972; Franke, 1974*a*; Franke & Scheer, 1974).

(*i*) Although one can see in electron microscopic autoradiographs, after long-pulse as well as after pulse-chase labelling experiments with RNA precursors, more or less heavy accumulations of silver grains over the nuclear envelope (e.g. Plate 2; see also Dhainaut, 1970), it is obvious that the resolution of the emulsions used (half distance radii for 60 % confidence of *c.* 150 nm) does not allow a clear-cut correlation of the label with the nuclear envelope. In particular, such micrographs do not allow a distinction between incorporation of precursors into the RNA of, for example, the nuclear pore complexes and ribosomes associated with the outer nuclear membrane. One can, however, isolate and purify such nuclear envelopes (as described above, Plate 7) from labelled amphibian oocytes in mid-to-late lampbrush stages (unfortunately the incorporation of nucleosides into such RNA is too low in the mature oocytes in which nucleoli do not adhere to the envelope, thus facilitating the preparation of very pure nuclear membrane material) and then separately analyse the RNA associated with the nuclear content (the 'gelified' aggregate ball, illustrated in Plate 7) and that recovered with the nuclear envelope

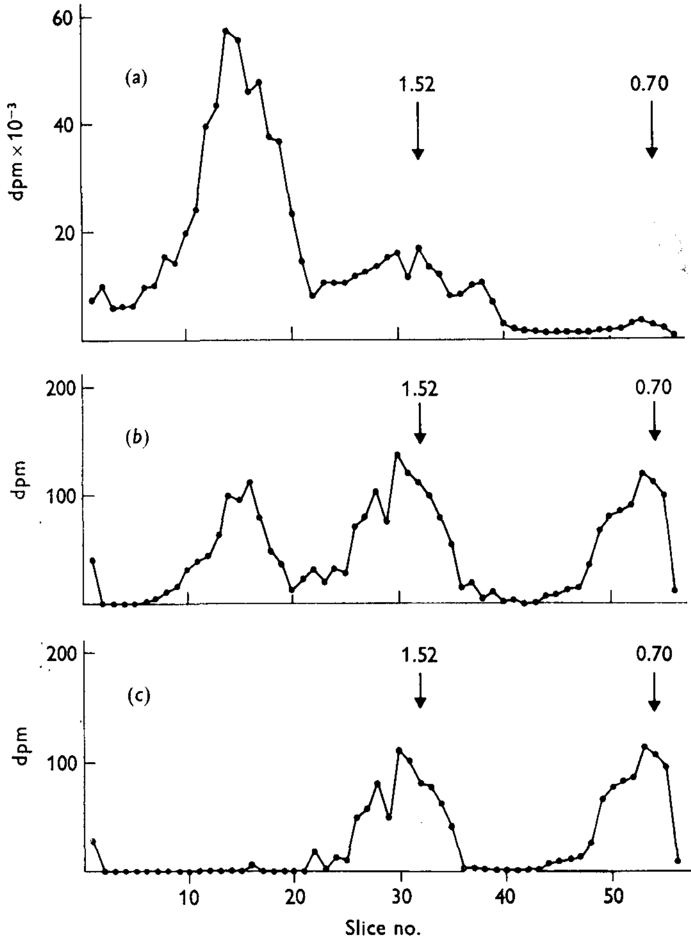

Fig. 4. Electrophoretic analysis of labelled RNA of isolated nuclear envelope (*b*, *c*) and the corresponding nucleoplasmic fractions (*a*). Lampbrush stage oocytes were selected from a *Xenopus laevis* ovary and incubated for one day at 25 °C in Eagle's medium (diluted 1 to 1) containing all four tritiated nucleosides (100 μCi ml^{-1} each). 200 nuclei were isolated and fractionated manually into nuclear envelopes and aggregated nuclear contents. The fractions were collected in ice-cold ethanol. RNA was extracted by incubating the pellets in 0.02 M Tris–HCl buffer (pH 7.4) containing 0.5 % SDS and 1 mg ml^{-1} predigested pronase at 25 °C for 10 min. 20 μg rRNA extracted from *Xenopus* ovary ribosomes was added as a marker. The RNA was precipitated by adding NaCl (to a final concentration of 0.1 M) and two volumes of ethanol and was then kept at −20 °C for several hours. The pelleted RNA was suspended in 20 μl electrophoresis buffer containing 0.2 % SDS and applied to slabs of 2.25 % acrylamide–0.5 % agarose gels (for further details see Ringborg *et al.* 1970, and Scheer *et al.* 1973). The position of the marker rRNAs (with molecular weights of 1.52 and 0.70 × 10^6 D) is indicated by the arrows. (*a*) RNA from 200 nuclear contents. Most of the radioactivity is present in the

(Fig. 4). Data obtained in this manner show that the pattern of the RNA associated with the nuclear envelope is different from that found in the nuclear interior, the latter basically reflecting that of the nucleoli, and shows an enrichment (Fig. 4(*b*), (*c*)) of RNA molecules of slightly lower electrophoretic mobility than the mature cytoplasmic rRNA. These results can serve as a basis for hypothesizing that either a conformational change of rRNA occurs within the nuclear pore complexes or that the final processing of rRNA (and probably the final assembly with ribosomal proteins as well) takes place very late in the process of nucleocytoplasmic translocation, namely in association with the nuclear envelope. From analyses of the RNAs associated with the nuclear membrane it is also evident that only a quantitatively negligible amount could reflect truly membrane-bound RNA of the type found in the endoplasmic reticulum system in various other cell types (for reviews see: Moulé, 1968; Shapot & Davidova, 1971; Pitot *et al.* 1969). From these morphological observations and preliminary analyses of the RNA associated with the nuclear envelope, we propose the working hypothesis summarized in the Scheme 1 which not only envisages the nuclear pore complexes as the major gateways for nucleocytoplasmic translocation of the ribonucleoproteins (see also the reviews: Gall, 1964; Stevens & André, 1969; Gouranton, 1969; Franke & Scheer, 1970*a*, *b*; Radouco-Thomas, Nosal & Radouco-Thomas, 1971; Feldherr, 1972; Kessel, 1973; Kay & Johnston, 1973; Franke & Scheer, 1974), but also includes the idea that the mature ribosomal sub-units do not exist in the nucleus and that their RNAs might be processed and assembled with the ribosomal proteins at the level of the nuclear envelope, probably in association with the nuclear pores. (It is important to consider, however, that the association of the 5S RNA with the RNP containing the pre-rRNA has already occurred within the nucleolus: e.g. Perry, 1969; Maden, 1971; Burdon, 1971; Sirlin, 1972.)

As to the translocation of messenger RNA containing informosome-like particles, only very little is known. It is tempting to suggest a messenger RNA content for the granules derived from the Balbiani ring, mentioned

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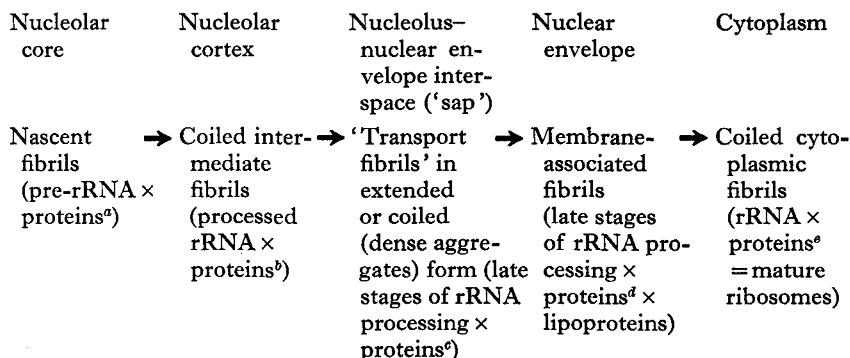
pre-rRNA region corresponding to a molecular weight of about 2.6×10^6 D. (*b*) RNA extracted from 200 isolated nuclear envelopes. (*c*) Here the RNA distribution shown in (*b*) was corrected for nucleoplasmic contamination which is revealed by the presence of some pre-rRNA, by subtracting the percentage of radioactivity of the corresponding fractions of (*a*) and assuming that all the primary precursor-rRNA of about 2.6×10^6 D represents such contamination. The RNA associated with the nuclear envelope showed a significant enrichment of labelled RNA which migrates more slowly than the mature 28S rRNA and (possibly) the 18S rRNA.

Scheme 1. *Nucleocytoplasmic translocation of ribosomal ribonucleoproteins (a hypothetical scheme)*



Nucleolar core		Nucleolar cortex		Nucleolus–nuclear envelope interspace ('sap')		Nuclear envelope		Cytoplasm
Nascent fibrils (pre-rRNA × proteins[a])	→	Coiled intermediate fibrils (processed rRNA × proteins[b])	→	'Transport fibrils' in extended or coiled (dense aggregates) form (late stages of rRNA processing × proteins[c])	→	Membrane-associated fibrils (late stages of rRNA processing × proteins[d] × lipoproteins)	→	Coiled cytoplasmic fibrils (rRNA × proteins[e] = mature ribosomes)

above (Beermann, 1964; Stevens & Swift, 1966), and similarly Takamoto (1966) discussed the presence of mRNA in the aggregates found during amphibian oogenesis (see above). Relevant in this connection are the recent findings of binding of mRNA–protein complexes to the nuclear envelope (Faiferman, 1973) and of a nuclear membrane-associated, high salt resistant, poly-A-polymerase-like activity (Kay, Johnston & Franke, 1974).

Assuming that the newly synthesized RNA leaves the nucleus via the pore complexes one can calculate the translocation (export) rate of total RNA (and in some cell types one can approximate that of rRNA) per average pore complex (NPFR; Franke, 1970*b*). From such calculations, made in various cell types and differentiation stages (e.g. Franke, 1970*b*; Scheer, 1970, 1973; Wunderlich, 1972), it is apparent that great differences in the rates, from zero to 127×10^{-18} g rRNA pore^{-1} min^{-1}, can occur (summarized by Franke & Scheer, 1974). Such calculations, and the findings that neither the frequency nor the total number of nuclear pores plays a regulatory role in directly controlling RNA transport efficiency (references in Feldherr, 1972; Franke & Scheer, 1974), have led to the conclusion that the RNA translocation efficiency is a variable which is characteristic for the specific physiological status of the pore complex.

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REFERENCES

ABELSON, P. H. & DURYEE, W. R. (1949). Radioactive sodium permeability and exchanges in frog eggs. *Biol. Bull. mar. biol. Lab., Woods Hole* **96**, 205–17.

ADESNIK, M., SALDITT, M., THOMAS, W. & DARNELL, J. E. (1972). Evidence that all messenger RNA molecules (except histone messenger RNA) contain poly (A) sequences and that the poly (A) has a nuclear function. *J. molec. Biol.* **71**, 21–30.

ALBRECHT, C. & VAN ZYL, I. M. (1973). A comparative study of the protein components of ribonucleoprotein particles isolated from rat liver and hepatoma nuclei. *Exp. Cell Res.* **76**, 8–14.

ALLFREY, V. G., MEUDT, R., HOPKINS, J. W. & MIRSKY, A. E. (1961). Sodium-dependent 'transport' reactions in the cell nucleus and their role in protein and nucleic acid synthesis. *Proc. natn. Acad. Sci. U.S.A.* **47**, 907–32.

BAJER, A. S. & MOLÈ-BAJER, J. (1972). Spindle dynamics and chromosome movements. *Int. Rev. Cytol., Suppl.* **3**, 1–271.

BEALE, G. H., JURAND, A. & PREER, J. R. (1969). The classes of endosymbiont of *Paramecium aurelia*. *J. Cell Sci.* **5**, 65–91.

BEERMANN, W. (1964). Control of differentiation at the chromosomal level. *J. exp. Zool.* **157**, 49–61.

BELL, E. (1969). I-DNA: its packaging into I-somes and its relation to protein synthesis during differentiation. *Nature, Lond.* **224**, 326–8.

BELL, E. (1971). Information transfer between nucleus and cytoplasm during differentiation. *Symp. Soc. exp. Biol.* **25**, 127–43.

BELL, P. R. (1972). Nucleocytoplasmic interaction in the eggs of *Pteridium aquilinum* maturing in the presence of thiouracil. *J. Cell Sci.* **11**, 739–55.

BERNHARD, W. (1969). A new staining procedure for electron microscopical cytology. *J. Ultrastruct. Res.* **27**, 250–65.

BIRNSTIEL, M. L., CHIPCHASE, M. & SPEIRS, J. (1971). The ribosomal RNA cistron. *Progr. Nucleic Acid Res.* **11**, 351–89.

BOLOUKHÈRE, M. (1970). Ultrastructure of *Acetabularia mediterranea* in the course of formation of the secondary nuclei. In *Biology of Acetabularia*, 145–75, eds Brachet, J. & Bonotto, S. New York: Academic Press.

BROWN, D. D., WENSINK, P. C. & JORDAN, E. (1971). Purification and some characteristics of 5S DNA from *Xenopus laevis*. *Proc. natn. Acad. Sci. U.S.A.* **68**, 3175–9.

BROWN, D. D., WENSINK, P. C. & JORDAN, E. (1972). A comparison of the ribosomal DNAs of *Xenopus laevis* and *Xenopus muelleri*: the evolution of tandem genes. *J. molec. Biol.* **63**, 57–73.

BURDON, R. H. (1971). Ribonucleic acid maturation in animal cells. *Progr. Nucleic Acid Res.* **11**, 33–79.

BUSCH, H. & SMETANA, K. (1970). *The Nucleolus*. New York: Academic Press.

BÜTTNER, D. W. & HORSTMANN, E. (1967). Das Sphaeridion, eine weit verbreitete Differenzierung des Karyoplasma. *Z. Zellforsch.* **77**, 589–605.

CENTURY, T. J., FENICHEL, I. R. & HOROWITZ, S. B. (1970). The concentrations of water, sodium and potassium in the nucleus and cytoplasm of amphibian oocytes. *J. Cell Sci.* **7**, 5–13.

CHARDONNET, Y. & DALES, S. (1970). Early events in the interaction of adenovirus with HeLa cells. II. Comparative observations on the penetration of types 1, 5, 7 and 12. *Virology* **40**, 478–85.

CHARRET, R. & CHARLIER, M. (1973). Visualisation de la transcription et de la traduction dans les mitochondries de *Tetrahymena pyriformis*. *J. Microscopie* **17**, 19–26.

CHEN, G. S. & SIDDIQUI, M. A. Q. (1973). Biosynthesis of transfer RNA: in vitro conversion of transfer RNA precursors from *Bombyx mori* to 4S RNA by *Escherichia coli* enzymes. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2610–13.

CLARKSON, S. G., BIRNSTIEL, M. L. & SERRA, V. (1973). Reiterated transfer RNA genes of *Xenopus laevis*. *J. molec. Biol.* **79**, 391–410.

CLARKSON, S. G., BIRNSTIEL, M. L. & PURDOM, I. F. (1973). Clustering of transfer RNA genes of *Xenopus laevis*. *J. molec. Biol.* **79**, 411–29.

CLÉROT, J.-C. (1968). Mise en évidence par cytochimie ultrastructurale de l'émission de protéines par le noyau d'auxocytes de batraciens. *J. Microscopie* **7**, 973–92.

COMINGS, D. E. & OKADA, T. A. (1972). The chromatoid body in mouse spermatogenesis. Evidence that it may be formed by the extrusion of nucleolar components. *J. Ultrastruct. Res.* **39**, 15–23.

CRAIG, N. C. & PERRY, R. P. (1970). Aberrant intranucleolar maturation of ribosomal precursors in the absence of protein synthesis. *J. Cell Biol.* **45**, 554–64.

DANEHOLT, B. (1972). Giant RNA transcript in a Balbiani ring. *Nature New Biol.* **240**, 229–32.

DANEHOLT, B. & HOSICK, H. (1973). Evidence for transport of 75S RNA from a discrete chromosome region via nuclear sap to cytoplasm in *Chironomus tentans*. *Proc. natn. Acad. Sci. U.S.A.* **70**, 442–6.

DARLINGTON, R. W. & MOSS, L. H. (1968). Herpes virus envelopment. *J. Virol.* **2**, 48–55.

DARLINGTON, R. W. & MOSS, L. H. (1969). The envelope of Herpes virus. *Progr. Med. Virol.* **11**, 16–45.

DARNELL, J. E. (1968). Ribonucleic acid from animal cell. *Bact. Rev.* **32**, 262–90.

DARNELL, J. E., JELINEK, W. R. & MOLLOY, G. R. (1973). Biogenesis of mRNA: genetic regulation in mammalian cells. *Science, Washington* **181**, 1215–21.

DAS, N. K., MICOU-EASTWOOD, J., RAMAMURTHY, G. & ALFERT, M. (1970). Sites of synthesis and processing of ribosomal RNA precursors within the nucleolus of *Urechis caupo* eggs. *Proc. natn. Acad. Sci. U.S.A.* **67**, 968–75.

DAWID, I. B., BROWN, D. D. & REEDER, R. H. (1970). Composition and structure of chromosomal and amplified ribosomal DNAs of *Xenopus laevis*. *J. molec. Biol.* **51**, 341–60.

DENIS, H. & MAIRY, M. (1972). Recherches biochimiques sur l'oogenèse. 1. Distribution intracellulaire du RNA dans les petits oocytes de *Xenopus laevis*. *Eur. J. Biochem.* **25**, 524–34.

DERKSEN, J., TRENDELENBURG, M. F., SCHEER, U. & FRANKE, W. W. (1973). Spread chromosomal nucleoli of *Chironomus* salivary glands. *Exp. Cell Res.* **80**, 476–9.

DHAINAUT, A. (1970). Etude en microscope électronique et par autoradiographie à haute résolution des extrusions nucléaires au cours de l'ovogenèse de *Nereis pelagica*. *J. Microscopie* **9**, 99–118.

DEZOETEN, G. A. & GAARD, G. (1969). Possibilities for inter- and intracellular translocation of some icosahedral plant viruses. *J. Cell Biol.* **40**, 814–23.

DUPUY-COIN, A. M., LAZAR, P., KALIFAT, S. R. & BOUTEILLE, M. (1969). A method of quantitation of nuclear bodies in electron microscopy. *J. Ultrastruct. Res.* **27**, 244–9.

ECKERT, W. A. (1972). Über den Einfluss transkriptions- und translationshemmender Antiobiotika auf den Kern-Cytoplasma-Übertritt von RNA bei *Tetrahymena pyriformis* GL. Ph.D. Thesis, University of Freiburg i.Br., 1–158.

ECKERT, W. A., FRANKE, W. W. & SCHEER, U. (1972). Actinomycin D and the central granules in the nuclear pore complex: thin sectioning versus negative staining. *Z. Zellforsch.* **127**, 230–9.

ECKERT, W. A., FRANKE, W. W. & SCHEER, U. (1974). Nucleocytoplasmic translocation of RNA in *Tetrahymena pyriformis* GL and its interference with inhibitors of transcription and translation. Submitted.

EDDY, E. M. & ITO, S. (1971). Fine structural and radioautographic observations on dense perinuclear cytoplasmic material in tadpole oocytes. *J. Cell Biol.* **49**, 90–108.

ENGELHARDT, P. & PUSA, K. (1972). Nuclear pore complexes: 'Press-stud' elements of chromosomes in pairing and control. *Nature New Biol.* **240**, 163–6.

FABERGÉ, A. C. (1973). Direct demonstration of eight-fold symmetry in nuclear pores. *Z. Zellforsch.* **136**, 183–90.

FAIFERMAN, I. (1973). MRNA-protein complex bound to the nuclear membrane. *J. Cell Biol.* **59**, 96a.

FAIFERMAN, I., HAMILTON, M. G. & POGO, A. O. (1971). Nucleoplasmic ribonucleoprotein particles of rat liver. II. Physical properties and action of dissociating agents. *Biochim. biophys. Acta* **232**, 685–95.

FAKAN, S. & BERNHARD, W. (1971). Localization of rapidly and slowly labelled nuclear RNA as visualized by high resolution autoradiography. *Exp. Cell Res.* **67**, 129–41.

FAKAN, S. & BERNHARD, W. (1973). Nuclear labelling after prolonged ^{3}H-uridine incorporation as visualized by high resolution autoradiography. *Exp. Cell Res.* **79**, 431–44.

FAWCETT, D. W. (1972). Observations on cell differentiation and organelle continuity in spermatogenesis. In *The Genetics of the Spermatozoon*, 37–68, eds Beatty, R. A. & Gluecksohn-Waelsch, S. Edinburgh and New York.

FAWCETT, D. W., EDDY, E. M. & PHILLIPS, D. M. (1970). Observations on the fine structure and relationships of the chromatoid body in mammalian spermatogenesis. *Biol. Reprod.* **2**, 129–53.

FELDHERR, C. M. (1964). Binding within the nuclear annuli and its possible effect on nucleocytoplasmic exchanges. *J. Cell Biol.* **20**, 188–92.

FELDHERR, C. M. (1965). The effect of the electron opaque pore material on exchange through the nuclear annuli. *J. Cell Biol.* **25**, 43–53.

FELDHERR, C. M. (1966). Nucleocytoplasmic exchanges during cell division. *J. Cell Biol.* **31**, 199–203.

FELDHERR, C. M. (1972). Structure and function of the nuclear envelope. In *Advances in Cell and Molecular Biology*, **2**, 273–307, ed. Du Praw, E. J. New York: Academic Press.

FELDHERR, C. M. & HARDING, C. V. (1964). The permeability characteristics of the nuclear envelope at interphase. *Protoplasmatologia, Vienna* **5**, 35–50.

FORD, P. J. (1972). Ribonucleic acid synthesis during oogenesis in *Xenopus laevis*. In *Oogenesis*, 167–91, eds Biggers, J. D. & Schuetz, A. W. Baltimore: University Park Press.

FRANKE, W. W. (1967). Zur Feinstruktur isolierter Kernmembranen aus tierischen Zellen. *Z. Zellforsch.* **80**, 585–93.

FRANKE, W. W. (1970*a*). On the universality of nuclear pore complex structure. *Z. Zellforsch.* **105**, 405–29.

FRANKE, W. W. (1970*b*). Nuclear pore flow rate. *Naturwiss.* **57**, 44–5.

FRANKE, W. W. (1974*a*). Structures, functions and biochemistry of the nuclear envelope. *Int. Rev. Cytol.* (in press).

FRANKE, W. W. (1974*b*). Zellkerne und Kernbestandteile. In *Biochemische Zytologie der Pflanzenzellen*, 15–40, ed. Jacobi, G. Stuttgart: G. Thieme Verlag.

FRANKE, W. W., BERGER, S., FALK, H., SPRING, H., SCHEER, U., HERTH, W., TRENDELENBURG, M. F. & SCHWEIGER, H. G. (1974). Morphology of the nucleocytoplasmic interaction during the development of *Acetabularia* cells. I. The vegetative phase (submitted).

FRANKE, W. W., DEUMLING, B., ERMEN, B., JARASCH, E. & KLEINIG, H. (1970). Nuclear membranes from mammalian liver. I. Isolation procedure and general characterization. *J. Cell Biol.* **46**, 379–95.

FRANKE, W. W., DEUMLING, B., ZENTGRAF, H., FALK, H. & RAE, P. M. M. (1973). Nuclear membranes from mammalian liver. IV. Characterization of membrane-attached DNA. *Exp. Cell Res.* **81**, 365–92.

FRANKE, W. W. & FALK, H. (1970). Appearance of nuclear pore complexes after Bernhard's staining procedure. *Histochemie* **24**, 266–78.

FRANKE, W. W. & SCHEER, U. (1970*a*). The ultrastructure of the nuclear envelope of amphibian oocytes: a reinvestigation. I. The mature oocyte. *J. Ultrastruct. Res.* **30**, 288–316.

FRANKE, W. W. & SCHEER, U. (1970*b*). The ultrastructure of the nuclear envelope of amphibian oocytes: a reinvestigation. II. The immature oocyte and dynamic aspects. *J. Ultrastruct. Res.* **30**, 317–27.

FRANKE, W. W. & SCHEER, U. (1974). Structures and functions of the nuclear envelope. In *The Cell Nucleus*, **1**, ed. Busch, H. New York: Academic Press (in press).

GALL, J. G. (1956). Small granules in amphibian oocyte nucleus and their relationship to RNA. *J. biophys. biochem. Cytol. Suppl.* **2**, 393–6.

GALL, J. G. (1964). Electron microscopy of the nuclear envelope. *Protoplasmatologia, Vienna* **5**, 4–25.

GALL, J. G. (1966). Nuclear RNA of the salamander oocyte. *Nat. Cancer Inst. Monogr.* **23**, 475–88.

GEORGIEV, G. P. (1967). The nature and biosynthesis of nuclear ribonucleic acid. *Progr. Nucleic Acid Res. molec. Biol.* **6**, 259–351.

GEORGIEV, G. P., RYSKOV, A. P., COUTELLE, C., MANTIEVA, V. L. & AVAKYAN, E. R. (1972). On the structure of transcriptional unit in mammalian cells. *Biochim. biophys. Acta* **259**, 259–83.

GERIN, Y. (1971). Etude par cytochimie ultrastructurale des corpuscules périnucleaires présent dans le jeunes oocytes de *Ilyanassa obsoleta* Say (Mollusca gastéropode). *J. Embryol. exp. Morph.* **25**, 423–38.

GIUDICE, G., SCONZO, G., RAMIREZ, F. & ALBANESE, J. (1972). Giant RNA is also found in the cytoplasm in sea urchin embryos. *Biochim. biophys. Acta* **262**, 401–3.

GOLDSTEIN, L. (1964). Combined nuclear transplantation and isotope techniques for the study of nuclear activities. *Protoplasmatologia, Vienna* **5**, 51–71.

GOLDSTEIN, L. & HARDING, C. V. (1950). Osmotic behaviour of isolated nuclei. *Fedn Proc. Fedn Am. Socs exp. Biol.* **9**, 48–9.

GOLDSTEIN, L. & PLAUT, W. (1955). Direct evidence for nuclear synthesis of cytoplasmic ribose nucleic acid. *Proc. natn. Acad. Sci. U.S.A.* **41**, 874–80.

GOLDSTEIN, L. & TRESCOTT, O. H. (1970). Characterization of RNAs that do and do not migrate between cytoplasm and nucleus. *Proc. natn. Acad. Sci. U.S.A.* **67**, 1367–74.

GOURANTON, J. (1969). L'enveloppe nucléaire. *Ann. Biol.* **8**, 385–409.
GRANBOULAN, N. & GRANBOULAN, P. (1965). Cytochimie ultrastructurale du nucléole. II. Etude des sites de synthèse du RNA dans le nucléole et le noyau. *Exp. Cell Res.* **38**, 604–19.
GRIERSON, D., ROGERS, M. E., SARTIRANA, M. L. & LOENING, U. E. (1970). The synthesis of ribosomal RNA in different organisms: Structure and evolution of the rRNA precursor. *Cold Spring Harbor Symp. Quant. Biol.* **35**, 589–98.
GULYAS, B. J. (1971). Nuclear extrusion in rabbit embryos. *Z. Zellforsch.* **120**, 151–9.
GURDON, J. B. (1970). Nuclear transplantation and the control of gene activity in animal development. *Proc. R. Soc. Lond., Ser. B* **176**, 303–14.
HAMKALO, B. A. & MILLER, O. L. (1973). Electron microscopy of genetic activity. *Ann. Rev. Biochem.* **42**, 379–96.
HANZELY, L. & OLAH, L. V. (1973). Fine structure and distribution of nuclear pores in root tip cells of *Allium sativum*. *Trans. Amer. microsc. Soc.* **92**, 35–43.
HINSCH, G. W. (1970). Possible role of intranuclear membranes in nuclear-cytoplasmic exchange in spider crab oocytes. *J. Cell Biol.* **47**, 531–5.
HOGAN, B. & GROSS, P. R. (1972). Nuclear RNA synthesis in sea urchin embryos. *Exp. Cell Res.* **72**, 101–14.
HOROWITZ, S. B. (1972). The permeability of the amphibian oocyte nucleus, in situ. *J. Cell Biol.* **54**, 609–25.
HOROWITZ, S. B. & FENICHEL, I. R. (1968). Analysis of glycerol-^{3}H transport in the frog oocyte by extractive and radioautographic techniques. *J. gen. Physiol.* **51**, 703–30.
HOROWITZ, S. B. & FENICHEL, I. R. (1970). Analysis of sodium transport in the amphibian oocyte by extractive and radioautographic techniques. *J. Cell Biol.* **47**, 120–31.
HOROWITZ, S. B., MOORE, L. C. & PAINE, P. L. (1973). The effective size of nuclear envelope pores. *J. Cell Biol.* **59**, 148a.
ITO, S. & LOEWENSTEIN, W. R. (1965). Permeability of a nuclear membrane: change during normal development and changes induced by growth hormone. *Science, Washington* **150**, 909–10.
JACOB, J. & DANIELI, G. A. (1972). Electron microscope observations on nuclear pore-polysome association. *Cell Diff.* **1**, 119–25.
JARASCH, E. D., REILLY, C. E., COMES, P., KARTENBECK, J. & FRANKE, W. W. (1973). Isolation and characterization of nuclear membranes from calf and rat thymus. *Hoppe-Seyler's Z. physiol. Chem.* **354**, 974–86.
JAWORSKA, H. & LIMA-DE-FARIA, A. (1973*a*). Amplification of ribosomal DNA in *Acheta*. VI. Ultrastructure of two types of nucleolar components associated with ribosomal DNA. *Hereditas* **74**, 169–86.
JAWORSKA, H. & LIMA-DE-FARIA, A. (1973*b*). Amplification of ribosomal DNA in *Acheta*. VII. Transfer of DNA–RNA assemblies from the nucleus to the cytoplasm. *Hereditas* **74**, 187–204.
JAWORSKA, H., AVANZI, S. & LIMA-DE-FARIA, A. (1973). Amplification of ribosomal DNA in *Acheta*. VIII. Binding of H^3-actinomycin to DNA in the nucleus and cytoplasm. *Hereditas* **74**, 205–10.
JELINEK, W., ADESNIK, M., SALDITT, M., SHEINESS, D., WALL, R., MOLLOY, G., PHILIPSON, L. & DARNELL, J. E. (1973). Further evidence on the nuclear origin and transfer to the cytoplasm of polyadenylic acid sequences in mammalian cell RNA. *J. molec. Biol.* **75**, 515–32.
KANNO, Y. & LOWENSTEIN, W. R. (1963). A study of the nucleus and cell membranes of oocytes with an intra-cellular electrode. *Exp. Cell Res.* **31**, 149–66.

KANNO, Y., ASHMAN, R. F. & LOEWENSTEIN, W. R. (1965). Nucleus and cell membrane conductance in marine oocytes. *Exp. Cell Res.* **39**, 184–9.

KASPER, C. B. (1974). Chemical and biochemical properties of the nuclear envelope. In *The Cell Nucleus*, ed. Busch, H. New York: Academic Press (in press).

KAY, R. R. & JOHNSTON, I. R. (1973). The nuclear envelope: current problems of structure and of function. *Sub-Cell Biochem.* **2**, 127–66.

KAY, R. R., JOHNSTON, I. R. & FRANKE, W. W. (1974). Nuclear sub-location of Mg^{2+}-activated poly A polymerase activity (submitted).

KESSEL, R. G. (1968). Annulate lamellae. *J. Ultrastruct. Res. Suppl.* **10**, 1–82.

KESSEL, R. G. (1971). The origin of the Golgi apparatus in embryonic cells of the grasshopper. *J. Ultrastruct. Res.* **34**, 260–75.

KESSEL, R. G. (1973). Structure and function of the nuclear envelope and related cytomembranes. *Prog. surf. membr. Sci.* **6**, 243–329.

KITAJIMA, E. W., LAURITIS, J. A. & SWIFT, H. (1969). Morphology and intracellular localization of a bacilliform latent virus in sweet clover. *J. Ultrastruct. Res.* **29**, 141–50.

KOCH, J. (1972). The cytoplasmic DNAs of cultured human cells. *Eur. J. Biochem.* **26**, 259–66.

KOCH, J. (1973). Cytoplasmic DNAs consisting of unique nuclear sequences in hamster cells. *FEBS Letters* **32**, 22–6.

KOCH, J. & PFEIL, H. v. (1971). Transport of nuclear DNA into the cytoplasm in cultured human liver cells. *FEBS Letters* **17**, 312–14.

KOCH, J. & PFEIL, H. v. (1972). Transport of nuclear DNA into the cytoplasm in cultured animal cells. A survey. *FEBS Letters* **24**, 53–6.

KODAMA, R. M. & TEDESCHI, H. (1968). Studies on the permeability of calf thymus nuclei isolated in sucrose. *J. Cell Biol.* **37**, 747–60.

KOHEN, E., SIEBERT, G. & KOHEN, C. (1971). Transfer of metabolites across the nuclear membrane. A microfluorometric study. *Hoppe-Seyler's Z. physiol. Chem.* **352**, 927–37.

KOSTELLOW, A. B. & MORRILL, G. A. (1968). Intracellular sodium ion concentration changes in the early amphibian embryo and the influence on nuclear metabolism. *Exp. Cell Res.* **50**, 639–94.

KUMAR, A. & WARNER, J. R. (1972). Characterization of ribosomal precursor particles from HeLa cell nucleoli. *J. molec. Biol.* **63**, 233–46.

LACOUR, L. F. & WELLS, B. (1972). The nuclear pores of early meiotic prophase nuclei of plants. *Z. Zellforsch.* **123**, 178–94.

LAMBERT, B. (1972). Repeated DNA sequences in a Balbiani ring. *J. molec. Biol.* **72**, 65–75.

LANE, N. J. (1967). Spheroidal and ring nucleoli in amphibian oocytes. *J. Cell Biol.* **35**, 421–34.

LEE, H.-J. & HOLBROOK, D. J. (1965). Evidence for carrier-mediated transport of adenine by isolated rat liver nuclei. *Arch. Biochem. Biophys.* **112**, 98–103.

LEEDALE, G. F. (1969). Observations on endonuclear bacteria in euglenoid flagellates. *Österr. Bot. Z.* **116**, 279–94.

LEICK, V. (1969). Formation of subribosomal particles in the macronuclei of *Tetrahymena pyriformis*. *Europ. J. Biochem.* **8**, 221–8.

LEICK, V. & ANDERSON, S. B. (1970). Pools and turnover rates of nuclear ribosomal RNA in *Tetrahymena pyriformis*. *Eur. J. Biochem.* **14**, 460–4.

LIAU, M. C. & PERRY, R. P. (1969). Ribosome precursor particles in nucleoli. *J. Cell Biol.* **42**, 272–83.

LITTAUER, U. Z. & INOUYE, H. (1973). Regulation of tRNA. *Ann. Rev. Biochem.* **42**, 439–70.

LOENING, U. E. (1970). The mechanism of synthesis of ribosomal RNA. *Symp. Soc. gen. Microbiol.* **20**, 77–106.

LOEWENSTEIN, W. R. (1964). Permeability of the nuclear membrane as determined with electrical methods. *Protoplasmatologia, Vienna* **5**, 26–34.

LOEWENSTEIN, W. R. & KANNO, Y. (1963*a*). The electrical conductance across the membrane of some cell nuclei. *J. Cell Biol.* **16**, 421–5.

LOEWENSTEIN, W. R. & KANNO, Y. (1963*b*). Some electrical properties of a nuclear membrane examined with a microelectrode. *J. gen. Physiol.* **46**, 1123–9.

LONGO, F. J. & ANDERSON, E. (1968). The fine structure of pro-nuclear development and fusion in the sea urchin, *Arbacia punctulata*. *J. Cell Biol.* **39**, 339–69.

LONGO, F. J. & ANDERSON, E. (1969). Cytological events leading to the formation of the two-cell stage in the rabbit: association of the maternally and paternally derived genomes. *J. Ultrastruct. Res.* **29**, 86–118.

MADEN, B. E. H. (1968). Ribosome formation in animal cells. *Nature, Lond.* **219**, 685–9.

MADEN, B. E. H. (1971). The structure and formation of ribosomes in animal cells. *Progr. Biophys. molec. Biol.* **22**, 129–77.

MAHOWALD, A. P. (1972). Oogenesis. In *Developmental Systems: Insects*, vol. 1, 1–47, eds Counce, S. I. and Waddington, C. H. New York: Academic Press.

MEINKE, W., HALL, M. R. & GOLDSTEIN, D. A. (1973). Physical properties of cytoplasmic membrane-associated DNA. *J. molec. Biol.* **78**, 43–56.

MENTRÉ, P. (1969). Présence d'acide ribonucléique dans l'anneau osmiophile et le granule central des pores nucléaires. *J. Microscopie* **8**, 51–68.

MEPHAM, R. H. & LANE, G. R. (1969). Nucleopores and polyribosome formation. *Nature, Lond.* **221**, 288–9.

MILLER, O. L. (1966). Structure and composition of peripheral nucleoli of Salamander oocytes. *Nat. Cancer Inst. Monogr.* **23**, 53–66.

MILLER, O. L. & BAKKEN, A. H. (1972). Morphological studies of transcription. *Acta endocrinol., Copenh., Suppl.* **168**, 155–77.

MILLER, O. L. & BEATTY, B. R. (1969*a*). Visualization of nucleolar genes. *Science, Washington* **164**, 955–7.

MILLER, O. L. & BEATTY, B. R. (1969*b*). Extrachromosomal nucleolar genes in amphibian oocytes. *Genetics Suppl.* **61**, 134–43.

MILLER, O. L. & BEATTY, B. R. (1969*c*). Portrait of a gene. *J. Cell Physiol.* **74**, *Suppl.* 1, 225–32.

MILLER, O. L. & BEATTY, B. R. (1969*d*). Nucleolar structure and function. In *Handbook of Molecular Cytology*, 605–19, ed. Lima-de-Faria, A. Amsterdam: North-Holland.

MILLER, O. L., BEATTY, B. R., HAMKALO, B. A. & THOMAS, C. A. (1970). Electron microscopic visualization of transcription. *Cold Spring Harbor Symp. Quant. Biol.* **57**, 505–12.

MILLER, O. L. & HAMKALO, B. A. (1972). Visualization of RNA synthesis on chromosomes. *Int. Rev. Cytol.* **33**, 1–25.

MIRAULT, M.-E. & SCHERRER, K. (1971). Isolation of preribosomes from HeLa cells and their characterization by electrophoresis on uniform and exponential-gradient-polyacrylamide gels. *Eur. J. Biochem.* **23**, 372–86.

MIRSKY, A. E. & OSAWA, S. (1961). The interphase nucleus. In *The Cell*, vol. II, 677–770. ed. Brachet, J. & Mirsky, A. E. New York: Academic Press.

MONNERON, A. & BERNHARD, W. (1969). Fine structural organization of the interphase nucleus in some mammalian cells. *J. Ultrastruct. Res.* **27**, 266–88.

MONNERON, A., BLOBEL, G. & PALADE, G. E. (1972). Fractionation of the nucleus by divalent cations. *J. Cell Biol.* **55**, 104–25.

MORGAN, C., ROSENKRANZ, H. S. & MEDMIS, B. (1969). Structure and development

of viruses as observed in the electron microscope. x. Entry and uncoating of adenovirus. *J. Virol.* **4**, 777–96.

MOSES, M. J. & WILSON, M. H. (1970). Spermiogenesis in an iceryine coccid, *Steatococcus tuberculatus* Morrison. *Chromosoma* **30**, 373–403.

MOULÉ, Y. (1968). Biochemical characterization of the components of the endoplasmic reticulum in rat liver cell. In *Structure and Function of the Endoplasmic Reticulum in Animal Cells*, 1–12, ed. Gran, F. C. London and New York: Academic Press.

MOULÉ, Y. & CHAUVEAU, J. (1968). Particules ribonucléo-protéiques 40S des noyaus de foie de rat. *J. molec. Biol.* **33**, 465–81.

NAORA, H., NAORA, H., IZAWA, M., ALLFREY, V. G. & MIRSKY, A. E. (1962). Some observations on differences in composition between the nucleus and cytoplasm of the frog oocyte. *Proc. natn. Acad. Sci. U.S.A.* **48**, 853–9.

NARAYAN, K. S. & BIRNSTIEL, M. L. (1969). Biochemical and ultrastructural characteristics of ribonucleoprotein particles isolated from rat-liver cell nucleoli. *Biochim. biophys. Acta* **190**, 470–85.

NIESSING, H. & SEKERIS, C. E. (1971). Further studies on nuclear ribonucleoprotein particles containing DNA-like RNA from rat liver. *Biochim. biophys. Acta* **247**, 391–403.

NIESSING, H. & SEKERIS, C. E. (1973). Synthesis of polynucleotides in nuclear ribonucleoprotein particles containing heterogeneous RNA. *Nature New Biol.* **243**, 9–12.

NII, S., MORGAN, C. & ROSE, H. M. (1968). Electron microscopy of herpes simplex virus. II. Sequence of development. *J. Virol.* **2**, 517–536.

NOSAL, G. & RADOUCO-THOMAS, C. (1971). Ultrastructural study on the differentiation and development of the nerve cell. The 'nucleus-ribosome' system. In *Advances in Cytopharmacology*, **1**, 433–46, eds Clementi, F. & Ceccarelli, B. New York: Raven Press.

PAINE, P. L. & FELDHERR, C. M. (1972). Nucleocytoplasmic exchange of macromolecules. *Exp. Cell Res.* **74**, 81–98.

PENMAN, S. (1966). RNA metabolism in the HeLa cell nucleus. *J. molec. Biol.* **17**, 117–30.

PENMAN, S., SMITH, I. & HOLTZMAN, E. (1966). Ribosomal RNA synthesis and processing in a particulate site in the HeLa cell nucleus. *Science, Washington* **154**, 786–9.

PERRY, R. P. (1967). The nucleolus and the synthesis of ribosomes. *Progr. Nucleic Acid Res. molec. Biol.* **6**, 219–57.

PERRY, R. P. (1969). Nucleoli: the cellular sites of ribosome production. In *Handbook of Molecular Cytology*, 620–84, ed. Lima-de-Faria, A. Amsterdam: North-Holland.

PERRY, R. P., CHENG, T.-Y., FREED, J. J., GREENBERG, J. R., KELLEY, D. E. & TARTOF, K. D. (1970). Evolution of the transcription unit of ribosomal RNA. *Proc. natn. Acad. Sci. U.S.A.* **65**, 609–16.

PITOT, H. C., SLADEK, N., RAGLAND, W., MURRAY, R. K., MOYER, G., SOLING, H. D. & JOST, J. P. (1969). A possible role of the endoplasmic reticulum in the regulation of genetic expression. The membron concept. In *Microsomes and Drug Oxidations*, 59–79, eds Gillette, J. R., Conney, A. H., Cosmides, G. J., Estabrook, R. W., Fonts, J. R. & Mannering, G. J. New York: Academic Press.

PRESCOTT, D. M. (1962*a*). Synthetic processes in the cell nucleus. II. Nucleic acid and protein metabolism in the macronuclei of two ciliated protozoa. *J. Histochem. Cytochem.* **10**, 145–53.

PRESCOTT, D. M. (1962*b*). Tritiated precursors employed for the study of nuclear

function and nuclear replication. In *Use of Radioisotopes in Animal Biology and the Medical Sciences*, **1**, 71–86. New York: Academic Press.

PRESCOTT, D. M. (1964). Cellular sites of RNA synthesis. *Progr. Nucleic Acid Res.* **3**, 33–57.

RADOUCO-THOMAS, C., NOSAL, G. & RADOUCO-THOMAS, S. (1971). The nuclear-ribosomal system during neuronal differentiation and development. In *Chemistry and Brain Development*, eds Paoletti, R. & Davison, A. N. New York: Plenum Press.

RIEMANN, W., MUIR, C. & MACGREGOR, H. C. (1969). Sodium and potassium in oocytes of *Triturus cristatus*. *J. Cell Sci.* **4**, 299–304.

RINGBORG, U., DANEHOLT, B., EDSTRÖM, J.-E., EGYHÁZI, E. & LAMBERT, B. (1970). Electrophoretic characterization of nucleolar RNA from *Chironomus tentans* salivary gland cells. *J. molec. Biol.* **51**, 327–40.

RINGBORG, U. & RYDLANDER, L. (1971). Nucleolar derived RNA in chromosomes, nuclear sap and cytoplasm of *Chironomus tentans* salivary gland cells. *J. Cell Biol.* **51**, 355–68.

ROBERTS, K. & NORTHCOTE, D. H. (1970). Structure of the nuclear pore in higher plants. *Nature, Lond.* **228**, 385–6.

ROBERTS, K. & NORTHCOTE, D. H. (1971). Ultrastructure of the nuclear envelope; structural aspects of the interphase nucleus of sycamore suspension culture cells. *Microscopica Acta* **71**, 102–20.

ROGERS, M. E. (1968). Ribonucleoprotein particles in the amphibian oocyte nucleus. *J. Cell Biol.* **36**, 421–32.

ROGERS, M. E. & KLEIN, G. (1972). Amphibian ribosomal ribonucleic acids. *Biochem. J.* **130**, 281–8.

RUPEC, M. (1969). Ausschleusung von Sphaeridien aus dem Zellkern. *Naturwiss.* **56**, 223.

SAMARINA, O. P., LUKANIDIN, E. M., MOLNAR, J. & GEORGIEV, G. P. (1968). Structural organization of nuclear complexes containing DNA-like RNA. *J. molec. Biol.* **33**, 251–63.

SATIR, B. & DIRKSEN, E. R. (1971). Nucleolar ageing in *Tetrahymena* during the cultural growth cycle. *J. Cell Biol.* **48**, 143–54.

SCHARRER, B. & WURZELMANN, S. (1969). Ultrastructural studies on nuclear-cytoplasmic relationships in oocytes of the African lung fish, *Protopterus aethiopicus*. I. Nucleolo-cytoplasmic pathways. *Z. Zellforsch.* **96**, 325–43.

SCHEER, U. (1970). Strukturen und Funktionen der Porenkomplexe in der Amphibien-Eizelle. Ph.D. Thesis, Universität Freiburg, 1–174.

SCHEER, U. (1972). The ultrastructure of the nuclear envelope of amphibian oocytes. IV. On the chemical nature of the nuclear pore complex material. *Z. Zellforsch.* **127**, 127–48.

SCHEER, U. (1973). Nuclear pore flow rate of ribosomal RNA and chain growth rate of its precursor during oogenesis of *Xenopus laevis*. *Devl Biol.* **30**, 13–28.

SCHEER, U., TRENDELENBURG, M. F. & FRANKE, W. W. (1973). Transcription of ribosomal RNA cistrons. *Exp. Cell Res.* **80**, 175–90.

SHAPOT, V. S. & DAVIDOVA, S. Y. (1971). Liporibonucleoprotein as an integral part of animal cell membranes. *Progr. Nucleic Acid Res. molec. Biol.* **11**, 81–101.

SHEPHERD, J. & MADEN, B. E. H. (1972). Ribosome assembly in HeLa cells. *Nature, Lond.* **236**, 211–14.

SIEBERT, G. (1972). The biochemical environment of the mammalian nucleus. *Sub-Cell. Biochem.* **1**, 277–92.

SIEBERT, G. & LANGENDORF, H. (1970). Ionenhaushalt im Zellkern. *Naturwiss.* **57**, 119–24.

SILLEVIS SMITT, W. W., NANNI, G., ROZIJN, TH. H. & TONINO, G. J. M. (1970).

Sedimentation characteristics of RNA from isolated yeast nuclei. *Exp. Cell Res.* **59**, 440–46.

SILLEVIS SMITT, W. W., VLAK, J. M., SCHIPHOF, R. & ROZIJN, TH. H. (1972). Precursors of ribosomal RNA in yeast nucleus. *Exp. Cell Res.* **71**, 33–40.

SIMARD, R., SAKR, F. & BACHELLERIE, J.-P. (1973). Ribosomal precursor particles in ascites tumor cell nucleoli. *Exp. Cell Res.* **81**, 1–7.

SIRLIN, J. L. (1972). *Biology of RNA.* New York: Academic Press.

SPIRIN, A. S. (1969). Informosomes. *Europ. J. Biochem.* **10**, 20–35.

STEVENS, A. R. (1967). Machinery for exchange across the nuclear membrane. In *The Control of Nuclear Activity*, 189–211, ed. Goldstein, L. Englewood Cliffs, N.J.: Prentice-Hall, Inc.

STEVENS, B. J. & ANDRÉ, J. (1969). The nuclear envelope. In *Handbook of Molecular Cytology*, 837–71, ed. Lima-de-Faria, A. Amsterdam: North-Holland.

STEVENS, B. J. & SWIFT, H. (1966). RNA transport from nucleus to cytoplasm in *Chironomus* salivary glands. *J. Cell Biol.* **31**, 55–77.

STEWART, P. R. & LETHAM, D. S. (eds) (1973). *The Ribonucleic Acids.* Berlin: Springer-Verlag.

STIRLING, C. E. & KINTER, W. B. (1967). High-resolution radioautography of galactose-^{3}H accumulation in rings of hamster intestine. *J. Cell Biol.* **35**, 585–604.

SUMMERS, M. D. (1969). Apparent *in vivo* pathway of granulosis virus invasion and infection. *J. Virol.* **4**, 188–90.

SUMMERS, M. D. (1971). Electron microscopic observations on granulosis virus entry, uncoating and replication processes during infection of the midgut cells of *Trichoplusia ni. J. ultrastruct. Res.* **35**, 606–25.

SYLVESTER, E. S. & RICHARDSON, J. (1970). Infection of *Hyperomyzus lactucae* by sowthistle yellow vein virus. *Virology* **42**, 1023–42.

SZOLLOSI, D. (1965). Extrusion of nucleoli from pronuclei of the rat. *J. Cell Biol.* **25**, 545–62.

TAKAMOTO, T. (1966). Ultrastructural transport mechanism of messenger ribonucleic acid in the young oocytes of amphibians. *Nature, Lond.* **211**, 772–3.

TASHIRO, Y., MATSUURA, S., MORIMOTO, T. & NAGATA, S. (1968). Extrusion of nuclear materials into cytoplasm in the posterior silk gland cells of silkworm, *Bombyx mori. J. Cell Biol.* **36**, C5–C10.

THOMAS, C. (1970). Ribonucleic acids and ribonucleoproteins from small oöcytes of *Xenopus laevis. Biochim. biophys. Acta* **224**, 99–113.

TRENDELENBURG, M. F., SCHEER, U. & FRANKE, W. W. (1973). Structural organization of the transcription of ribosomal DNA in oocytes of the house cricket. *Nature New Biol.* **245**, 167–70.

TRENDELENBURG, M. F., SCHEER, U. & FRANKE, W. W. (1974). Effect of actinomycin D on the template association of nascent pre-rRNP in amphibian oocyte nucleoli (submitted).

UDEM, S. A. & WARNER, J. R. (1973). The cytoplasmic maturation of a ribosomal precursor RNA in yeast. *J. Biol. Chem.* **248**, 1412–16.

VAN GANSEN, P. & BOLOUKHÈRE-PRESBURG, M. (1965). Ultrastructure de l'algue unicellulaire *Acetabularia mediterranea* LmX. *J. Microscopie* **4**, 347–62.

VAUGHAN, M., WARNER, J. R. & DARNELL, J. E. (1967). Ribosomal precursor particles in the HeLa cell nucleus. *J. molec. Biol.* **25**, 235–51.

VAZQUEZ-NIN, G. & BERNHARD, W. (1971). Comparative ultrastructural study of perichromatin- and Balbiani ring granules. *J. Ultrastruct. Res.* **36**, 842–60.

WARNER, R. & SOEIRO, R. (1967). Nascent ribosomes from HeLa cells. *Proc. natn. Acad. Sci. U.S.A.* **58**, 1984–90.

WATSON, M. L. (1959). Further observations on the nuclear envelope of animal cells. *J. biophys. biochem. Cytol.* **6**, 147–55.

WEINBERG, R. A. (1973). Nuclear RNA metabolism. *Ann. Rev. Biochem.* **42**, 329–54.

WEINBERG, R. A. & PENMAN, S. (1970). Processing of 45S nucleolar RNA. *J. molec. Biol.* **47**, 169–78.

WENSINK, P. C. & BROWN, D. D. (1971). Denaturation map of the ribosomal DNA of *Xenopus laevis*. *J. molec. Biol.* **60**, 235–47.

WERZ, G. (1964). Untersuchungen zur Feinstruktur des Zellkernes und des perinucleären Plasmas von *Acetabularia*. *Planta* **62**, 255–71.

WIENER, J., SPIRO, D. & LOEWENSTEIN, W. R. (1965). Ultrastructure and permeability of nuclear membranes. *J. Cell Biol.* **27**, 107–17.

WILLIAMSON, R. (1973). The protein moieties of animal messenger ribonucleoproteins. *FEBS Letters* **37**, 1–6.

WILLIAMSON, R., MCSHANE, T., GRUNSTEIN, M. & FLAVELL, R. A. (1972). 'Cytoplasmic' DNA from primary embryonic cell cultures is not informational. *FEBS Letters* **20**, 108–10.

WISCHNITZER, S. (1970). The annulate lamellae. *Int. Rev. Cytol.* **27**, 65–100.

WISCHNITZER, S. (1973). The submicroscopic morphology of the interphase nucleus. *Int. Rev. Cytol.* **34**, 1–48.

WISE, G. E. & GOLDSTEIN, L. (1973). Electron microscope localization of nuclear RNAs that shuttle between cytoplasm and nucleus and nuclear RNAs that do not. *J. Cell Biol.* **56**, 129–38.

WISE, G. E., STEVENS, A. R. & PRESCOTT, D. M. (1972). Evidence of RNA in the helices of *Amoeba proteus*. *Exp. Cell Res.* **75**, 347–52.

WUNDERLICH, F. (1969). The macronuclear envelope of *Tetrahymena pyriformis* GL in different physiological states. II. Frequency of central granules in the pores. *Z. Zellforsch.* **101**, 581–7.

WUNDERLICH, F. (1972). The macronuclear envelope of *Tetrahymena pyriformis* GL in different physiological states. V. Nuclear pore complex-A controlling system in protein biosynthesis? *J. Membrane Biol.* **7**, 220–30.

ZALOKAR, M. (1960). Sites of protein and ribonucleic acid synthesis in the cell. *Exp. Cell Res.* **19**, 559–76.

ZBARSKY, I. B. (1972). Nuclear envelope isolation. In *Methods in Cell Physiol.* **5**, 167–98, ed. Prescott, D. M. New York: Academic Press.

ZENTGRAF, H., DEUMLING, B., JARASCH, E.-D. & FRANKE, W. W. (1971). Nuclear membranes and plasma membranes from hen erythrocytes. I. Isolation, characterization and comparison. *J. biol. Chem.* **246**, 2986–95.

ZERBAN, H., WEHNER, M. & WERZ, G. (1973). Über die Feinstruktur des Zellkerns von *Acetabularia* nach Gefrierätzung. *Planta* **114**, 239–50.

EXPLANATION OF PLATES

PLATE 1

Nucleocytoplasmic transfer of RNA as demonstrated in a pulse-chase experiment. Exponentially growing cells of *Tetrahymena pyriformis* GL were labelled with [^{3}H]uridine (20–30 μCi ml^{-1}, sp. radioactivity 24.9 Ci mmol^{-1}) for 8 min at 28 °C. After centrifugation (3000 *g*, 3 min) the pellet was divided into two aliquots. One sample was processed immediately for autoradiography (*a*), the other was resuspended in culture medium containing non-radioactive uridine (same concentration). After a chase period of 120 min at 28 °C the cells were pelleted in ice-cold culture medium and processed for autoradiography (*b*). Pellets were fixed at room temperature in 2 % glutaraldehyde (buffered with Na-cacodylate at pH 7.2) for 30 min, then thoroughly washed in the same buffer and postfixed in 2 % OsO_4 (pH 7.2). After several washes in distilled water the cells were extracted in the cold with 5 % TCA for 5 min and washed again in water. Dehydrated samples were embedded in Epon 812. Semithin sections (1 μm) were coated with Kodak AR 10 stripping film, exposed for 10 days, developed, and photographed under phase contrast. In the pulse-labelled cells silver grains were found almost exclusively over the macronucleus (N; note the accumulation of label in the nuclear periphery). After the chase the macronucleus is almost free of grains but the cytoplasm is heavily labelled. (A large vacuole is located next to the macronucleus.) (*a*) × 2900; (*b*) × 1750. (For details see Eckert *et al.* 1974.)

PLATE 2

Electron microscopic autoradiograph of the nuclear periphery of a *Triturus alpestris* lampbrush stage oocyte. Isolated oocytes were incubated with tritiated RNA precursors as described in the legend of Plate 4. The nucleolar (No) cortex is highly labelled. Some silver grains are found over the fibrillar network which spans between the nucleolus and the nuclear envelope and some grains are located over the nuclear envelope. × 16000.

PLATE 3

Electron microscope autoradiograph of the ciliate *Tetrahymena pyriformis* GL, labelled for 8 min with [^{3}H]uridine. Labelling conditions and fixation were similar to those given in Plate 1. (Ultrathin sections were coated with Ilford L-4 emulsion.) Due to the predominance of rRNA synthesis in these cells, there is a preferential labelling of the numerous partially clustered nucleoli which are located in the nuclear periphery. Very few silver grains were detected in the cytoplasm [C; (*a*)]. (*b*) Shows a peripheral part of the macronucleus at higher magnification: the nucleoli are heavily labelled whereas only few grains are associated with the chromatin bodies. (*a*) × 5200; (*b*) × 7000.

PLATE 4

Labelling of (extrachromosomal) amplified nucleoli (No) with [^{3}H]uridine in a *Triturus alpestris* lampbrush stage oocyte. Isolated oocytes were incubated in tissue culture medium (Eagle's buffer, diluted 1 to 1 with distilled water) containing 200 μCi ml^{-1} [^{3}H]uridine (sp. radioactivity 27 Ci mmol^{-1}) at 18 °C for 4 h. The cells were fixed with glutaraldehyde/OsO_4 and embedded in Epon 812 (for details see Plate 1 and Trendelenburg *et al.* 1974). Ultrathin sections were coated with the Ilford L-4 emulsion and exposed for 10 weeks. The upper nucleolus shows a preferential labelling in the polar cap regions, the nucleolus in the bottom part is cut tangentially to such a polar region. The upper arrow points to a heavily labelled (presumably nucleolus-derived) electron dense aggregate situated near the nuclear envelope; the arrow at the bottom margin points to an unlabelled dense aggregate near a nucleolus (No). C, cytoplasm; YP, yolk platelet. ×4000.

PLATE 5

The nuclear envelope represents the boundary layer between the cytoplasm (C) and the nucleoplasm (N) as demonstrated in a lampbrush stage oocyte of *Xenopus laevis*. The cytoplasm is packed with ribosomes which are stored there for future embryonic growth and development. No ribosomes can be seen within the nucleoli and the nucleoplasm. The only nucleoplasmic structure is fibrillogranular material which is sometimes aggregated into electron-dense masses (arrow). ×33000.

PLATE 6

(*a*) The mid-phase of amphibian oogenesis is characterized by the lampbrush configuration of the chromosomes (located in the central part of the nucleus) and the peripheral location of the amplified nucleoli in the 'germinal vesicle' (N); C, cytoplasm. (*b*) Demonstrates at higher magnification the typical arrangement of these nucleoli immediately adjacent to the nuclear envelope. (*Triturus alpestris* oocytes were fixed sequentially in glutaraldehyde/OsO_4 and embedded in Epon; 1 μm sections were cut and photographed under phase optics.) (*a*) ×430; (*b*) ×1300.

PLATE 7

Products of the manual separation of the nuclear envelope 'ghost' from the aggregated nucleoplasm of a nearly mature *Triturus alpestris* oocyte. The light micrograph shows, at the left, the nucleoplasmic aggregate 'ball' with numerous nucleoli and, at the right, the isolated nuclear envelope (for further details see Scheer, 1972). ×68.

PLATE 8

Electron micrographs of the primary (giant) nucleus in the rhizoid of the green alga, *Acetabularia mediterranea*. The nucleus is ensheathed by a special cisterna (the 'perinuclear lacuna', PL), which is continuous with the intricate vacuolar labyrinth of these rhizoids and is separated by a zone about 70 nm broad (indicated by the arrowheads in (*a*)) from the nuclear envelope. This 'intermediate zone' is clearly different from the cytoplasm since it contains only finely fibrillar material and, occasionally, some small membranous structures. Most of these fibrils traverse the intermediate zone, and seem to link the nuclear envelope (which is marked by the triangles in (*b*)) with the inner membrane of the perinuclear lacuna, which thus constitutes something like a 'secondary nuclear envelope' [(*a*), (*b*).] The plasmatic material of the intermediate zone is in continuity with the cytoplasm through channels, i.e. fenestrations in the perinuclear lacuna. The juxtanuclear cytoplasm is characterized by numerous large and electron-dense aggregates (arrows in (*a*)). The nucleolar cortex (No) appears to be in structural continuity with the nuclear pore complexes via fibrillar strands (indicated, e.g., at the right arrow in (*b*)). Densely staining nuclear granules (25 to 50 nm in diameter) are associated with this fibrillar network (e.g. pair of arrows at left in (*b*)). In slightly oblique grazing sections (*c*) such granules are frequently found at the nucleoplasmic side of the pore complexes (arrows) and in the centre of a great many of the pores ('central granules'). They are usually not seen in the intermediate zone (lower left part of (*c*)). (*a*) ×36000; (*b*) ×67000; (*c*) ×91000.

PLATE 9

Demonstration of possible ribonucleoprotein structures in the nuclear periphery of a *Triturus alpestris* lampbrush stage oocyte. In ultrathin sections selectively stained according to the method of Bernhard (1969) the stain is preserved not only in structures which are known to contain RNA like the nucleolus (No) and the ribosomes, but also in the numerous approximately 50 nm large nuclear globules (some of which are indicated by the small arrows) found either close to the nucleolar cortex, free in the nucleoplasm, or aggregated into larger units (longer arrow). The double arrow points to a fibrillogranular body (presumably nucleolus-derived) near the nuclear envelope. Mi, mitochondrion; C, cytoplasm; N, nucleus. ×36000.

PLATES 10 AND 11

Comparison of the electron microscopic appearance of the annulus granules of the pore complex with ribosomes. In tangential (Plate 10) and transverse (Plate 11) sections the annulus subunits appear less densely stained, larger and more variable in shape than the adjacent ribosomes attached to the outer nuclear membrane. Plate 10, section tangential to the isolated nucleus of a mature *Xenopus laevis* oocyte. Plate 11, cross-section of a nuclear envelope fragment isolated from a rat hepatocyte (arrows denote annulus granules). PC, perinuclear cisterna. Plate 10, ×76000; Plate 11, ×180000.

PLATE 12

Previtellogenic and early lampbrush stages of amphibian oogenesis (here in *Triturus alpestris*) are characterized by the juxtanuclear accumulation of dense aggregates (dA). Electron dense material penetrates the nuclear envelope, in a rod-like configuration, via the central portion of the pore lumen and accumulates on the cytoplasmic side as a more spheroid body (annulus subunits of pore complexes are denoted by the shorter arrows). Fibrillar connections are also observed between the dense cytoplasmic aggregates and the pore complexes (e.g. at the dark body in the centre). The two longer arrows in the lower part point to nuclear globules (cf. Plate 9). N, nucleus; C, cytoplasm. ×85000.

PLATE 13

Electron micrograph of a meristematic onion root tip nucleus as revealed after application of the differential staining method of Bernhard (1969). Peripheral chromatin (Ch) is bleached whereas the pore complex substructures, especially the annulus granules and the central elements, have retained the stain, although at a lower degree than the ribosomes in the cytoplasm (C). In tangential sections (*a*), (*b*) polysomes on the outer nuclear membrane are often seen in close associations with the outer annulus of pore complexes (arrows in (*a*) and (*b*)). In (*c*), a cross-section through the nuclear envelope and a pore complex is presented which illustrates the high electron contrast as compared to the adjacent bleached chromatin (the arrows point to the annular subunits). Some interchromatinic fibrils terminate at the inner annulus. (*a*) ×100000; (*b*) ×60000; (*c*) ×70000.

PLATE 14

Cross-section through the nuclear periphery of a *Xenopus laevis* lampbrush stage oocyte. A profile of a single cytoplasmic annulate lamella (denoted by the two horizontal long arrows) lies in close proximity to and parallel with the nuclear envelope. Note the identical subarchitecture of the pore complexes (bars) in the nuclear envelope and in the annulate lamella. No ribosomes are recognized in the zone between the nuclear envelope and the annulate lamella; here are seen only fibrils which often appear to connect the corresponding pore complexes of both cisternal systems. The short arrows point to electron-dense nuclear globules. N, nucleus; C, cytoplasm; Mi, mitochondrion. ×50000.

PLATE 15

Survey electron micrograph (*a*) of a nuclear envelope manually isolated from a mature *Xenopus laevis* oocyte which demonstrates the purity and structural integrity of this membrane fraction. (*b*) Shows the same preparation at higher magnification. The nuclear membrane reveals a distinct dark-light-dark 'unit membrane' pattern. The small arrows point to the annulus granules lying upon the pore margins. 'Projecting tips' of dense material protrude from the pore wall into the lumen, and the pore centre is occupied by a central element. The long arrow denotes the nuclear fibrils attached to the annulus. N, nucleoplasmic side: C. cvtoplasmic side. (*a*) ×3700; (*b*) ×185000.