Structural organization of nascent transcripts and hnRNA molecules in amphibian oocytes

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Abstract

Comparisons of relative lengths of lampbrush loops, nascent RNP transcripts and hnRNA molecules from oocytes of amphibia with different C-values show that there is an increasing trend in loop, and transcriptional unit, length with increase in genome size but no increasing trend with respect to RNA contour length. The formation of duplex regions and circles in RNP fibrils indicates that RNA processing may occur within the nascent fibrils. The hnRNA molecules from oocytes of the various amphibia readily form intermolecular duplex structures. These complementary sequences have a low kinetic complexity and are transcribed from highly repetitive sequences distributed throughout the genome. Their possible function is considered.

Introduction

The immediate products of transcription can be viewed in two ways, either as the nascent RNP fibrils seen in Miller spreads (1, 2) or as the hnRNA molecules extracted from nuclei. The relationship between these forms is important for the integration of microscopical observation with biochemical data and consequently for a clearer understanding of early post-transcriptional events. Amphibian oocytes are particularly useful in that the germinal vesicles are highly active in transcription (3) and can be easily hand-isolated. Therefore, features of both transcribing chromatin and hnRNA molecules can be studied by direct procedures. Furthermore by using previtellogenic oocytes any analysis is uncomplicated by the transcription of rRNA sequences, since nucleolar activity is negligible at this early oogenic stage.

In this study two features of nascent transcripts and hnRNA molecules have been considered: their relative lengths, which relate to early cleavage events; and their ability to form duplex structures, which relates to the transcription of inverted repeat sequences that may have a regulatory function (4, 5). An added complication in examining amphibia, but one that can be turned to advantage for the purpose of studying functional relationships, is that there exists a large range of genome sizes (C-values as expressed in pgDNA/ haploid nucleus) in a series of closely related organisms.

Transcript length and hnRNA length

The dimensions of transcriptional units have been compared in lampbrush chromosome preparations and in Miller spreads from oocytes of amphibians with different C-values. Oocytes of as near the same previtellogenic stage were used in each instance. As shown in Table 1 there is a general increase in lampbrush loop length and in transcription unit length with increase in genome size. In order to examine whether such an increase is reflected in the lengths of RNA molecules which can be isolated from germinal vesicles, total populations of nuclear RNA molecules were sized by electron microscopic contour analysis. Al-

Table 1. Comparison of germinal vesicle transcript length and hnRNA length in amphibia with different C-values.

Organism	C-value (pg)	(ratio)	Average loop length (μm)	Transcriptional unit length (μm)	hnRNA length (μm) maximum	mean
Xenopus laevis	3.1	1	5–10	2–10	4.7	0.56
Triturus cristatus	23	7.4	40–50	3–27	10.1	1.69
Amphiuma means	65	21	nd	nd	6.8	1.03
Necturus maculosus	78	25	>100	nd	6.1	1.04

nd: not determined

though the total nuclear RNA represents both chromatin-bound and unbound forms, previous labeling experiments have demonstrated that as much as half of the nuclear RNA extracted from previtellogenic oocytes is derived from the nascent RNP transcripts of lampbrush chromosomes (6). However, it was found that in *Triturus* oocytes the range in lengths of hnRNA molecules (up to 7 μm) is much less than the range in lengths of transcriptional units (3–27 μm). (It should be borne in mind that the lengths of transcriptional units, as well as loops, are minimum estimates that do not take into account any compaction of DNA within the chromatin axis.) In no instances are RNA molecules detected which approach the length of the longest RNP fibrils of average sized transcriptional units. In fact the size distribution of *Triturus* hnRNA is not very much different from the size distribution of *Triturus* oocyte poly (A)$^+$RNA (data not shown). Since the RNA extraction procedure is gentle and excludes nuclease activity (germinal vesicles are rapidly transferred into ice-cold 70% ethanol, pelleted, digested with proteinase K and the RNA sedimented through 5.7 M' CsCl), it is concluded that the RNA transcripts are rapidly processed *in vivo* and that cleavage may occur within the growing RNP fibrils, the linear continuity of the fibrils being maintained by means of protein components. This view is supported by contour length analysis of hnRNA molecules extracted from oocytes of different C-value amphibia which shows that there is little increasing trend with respect to RNA length. It has been demonstrated previously (7) that in cell lines of *Drosophila* and *Aedes, Aedes* has a 5-6 fold larger genome than does *Drosophila* and produces hnRNA which is on average 2-2.5 fold longer. This is similar to the situation observed here in the comparison of *Xenopus* and *Triturus,* although the results, taken together, favour the view that the hnRNA molecules are already cleavage products.

Formation of stem-loop structures in nascent transcripts

Previous experiments involving *in situ* hybridization of denatured DNA probes to nascent RNA transcripts (8) have indicated that processing occurs in the nascent RNP fibril by scission and loss of 5'-terminal RNA sequences. Evidence for excision of RNA sequences from within nascent transcripts comes from an examination of spreads of *Triturus* oocyte chromatin. Stem-loop and circular RNP structures are readily formed along the nascent RNP fibril axis and apparently involve the pairing of inverted-repeat sequences to form the neck region.

Stem-loop and circular structures can also be seen frequently in hnRNA spread preparations and the relative dimensions of RNP and RNA forms (Fig. 1) are compatible with the view that they are
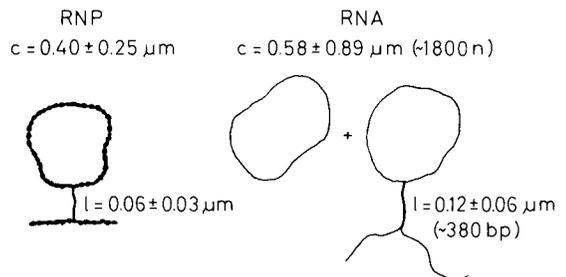

Fig. 1. Dimensions of stem-loop structures observed in nascent RNP fibrils and in hnRNA from germinal vesicles of *Triturus.* (c), circumference of loop; (l), length of stem; (n) equivalent length in nucleotides; (bp), equivalent length in base pairs.

Table 2. Formation of duplex structures in the hnRNA from amphibian oocytes.

Organism	C-value (pg)	Intramolecular duplex % RNA	Intermolecular duplex		
			% RNA	$R_0t\ \frac{1}{2}$	T_m
Xenopus	3.1	5	7.5	4×10^{-4}	82
Triturus	23	5	10	3×10^{-2}	79
Amphiuma	65	1.5	2	2×10^{-1}	75
Necturus	78	4.5	8.5	1.5×10^{-1}	74

derived from the same source. However, the sequences contained within the circles are, as yet, undetermined and further work is required to demonstrate that the electron microscopic observations relate unequivocally to sequence excision *in vivo*.

Formation of intermolecular duplexes in hnRNA

In addition to intramolecular hairpin and stem-loop structures, when denatured hnRNA from amphibian germinal vesicles is incubated under conditions favoring molecular hybridization (in 0.5 M NaCl, 1 mM EDTA, 10 mM Pipes, pH 6.4, 10% formamide at 56 °C) there results extensive intermolecular hybridization, as judged by electron microscopic examination. Intermolecular duplex formation has already been described in detail for HeLa cell hnRNA (9). Here a study was made of the extent of duplex formation, the kinetics of the process and the thermal stability of the duplex regions in hnRNA isolated from the germinal vesicles of different C-value amphibia. In these experiments, total hnRNA, which had been ^{3}H-labeled *in vivo* with all four nucleotide triphosphates, was used and the extent of duplex formation at various times was assayed by counting the ribonuclease-resistant acid-precipitable radio-activity. Thermal stability was assayed by decrease in ribonuclease resistance on increasing the

temperature of a solution of annealed hnRNA molecules in 0.1 × SSC. In addition to the biochemical assays, the reactions were checked by electron microscopic examination of spread RNA preparations. The main findings are as follows:

1. In *Triturus* most of the intermolecular duplex structures fall into a size class of 0.07 ± 0.015 μm, which is equivalent to about 220 base pairs, with a less frequent class of 0.64 ± 0.31 μm, or 2000 base pairs. In *Necturus* the duplex regions are generally longer but less well matched.

2. The proportion of RNA sequences capable of rapidly forming duplex structures is different in different C-value organisms (with no trend) but the sequence complexity of complementary repeats increases with C-value (see Table 2).

3. Culture cells from the same organisms (*Xenopus* and *Triturus*) give complexity values for RNA complementary repeats which are similar to the oocyte values (not shown).

4. Duplex structures formed in the hnRNA from higher C-value organisms have a lower thermal stability (see Table 2).

When the complementary repeat sequences are isolated, after ribonuclease digestion of annealed molecules, and hybridized with a vast excess of amphibian DNA, it is found that these sequences are transcribed from DNA sequences that are repeated in the genome of *Triturus* in the order of 2 × 10³ times and in the genome of *Necturus* in the order of 6 × 10³ times (see Table 3). However, the

Table 3. Hybridization of RNA complementary-repeat sequences with excess DNA.

Source of RNA	Source of DNA	% RNA hybridized*	$C_0t\ \frac{1}{2}$ RNA/DNA	$C_0t\ \frac{1}{2}$ single copy DNA	Gene repeat frequency
Triturus	*Triturus*	62	22	4×10^4	1.8×10^3
Necturus	*Necturus*	58	25	1.4×10^5	5.6×10^3
Triturus	*Necturus*	8	–	–	

*at a C_0t value of 10^3M.sec

extent of cross-species hybridization is low, indicating that the inverted-repeat sequences transcribed in different amphibia are not homologous.

Taking the various results together, the formation of intermolecular duplexes in *Triturus* oocyte hnRNA invoves a total sequence complexity of 15 000 nucleotides (equivalent to the $R_0t \frac{1}{2}$ value of 3×10^{-2} M.sec.). Since most of the duplex regions have a length equivalent to 220 nucleotides and the average repeat frequency for each is 2×10^3, then there are about 70 families of different sequence of about 220 nucleotides in length, each transcribed from 2×10^3 genetic loci. Preliminary experiments in hybridizing complementary repeat sequences to lampbrush chromosomes *in situ* indicate that these sequences have a widespread distribution in the genome.

Transcription during oogenesis: Possible functions

During amphibian oogenesis, 5–10% of the genome is transcribed from lampbrush chromosomes irrespective of the C-value of the organism (3). Therefore the analytical complexity of germinal vesicle hnRNA may be as much as 3×10^8 nucleotides in *Xenopus*, 2×10^9 nucleotides in *Triturus* and 7×10^9 nucleotides in *Necturus*. Nevertheless, the cytoplasmic poly(A)$^+$ RNA of different C-value amphibia is transcribed from single copy genes and summates to a fairly constant sequence complexity of 2×10^7 nucleotides (10). Therefore the vast majority of transcribed sequences, especially in high C-value organisms, has no obvious function. Several possibilities have been suggested for the apparently excessive levels of transcription from lampbrush chromosomes:

1. Materials are stored during oogenesis for use throughout early embryogenesis (11). The stored material may exist as mRNP, as protein or as nuclear restricted sequences.

2. There may be continuous transcription through many sequences with excision of the relatively rare essential sequences. This mechanism appears to be more efficient than precise gene regulation. Evidence is presented in this report that there is extensive processing within the nascent transcripts of high C-value organisms.

3. There may be a physiological function in maintaining a level of transcription proportional to the genomic and nuclear size. For instance, redundant sequences may be used for the simple purpose of swelling the nucleus to increase the number of nuclear pores to assist the exit of essential components such as rRNA sequences (12).

4. There may exist in oocytes an elaborate control mechanism concerned with the preprogramming of activities required for early development. This might occur through nucleic acid interaction (4, 5) or through the production of regulatory proteins.

In the present report we have described complementary repeat sequences which are transcribed into the hnRNA of amphibian oocytes and which may have some such regulatory function.

Acknowledgement

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