Differential accumulation of U1 and U4 small nuclear RNAs during *Xenopus* development

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We showed previously that those U1 small nuclear RNA (snRNA) genes of *Xenopus laevis* which are transcribed very actively in early embryos are quiescent in mature (stage VI) oocytes (Forbes et al. 1984). Although that study demonstrated that differential control of snRNA genes occurred, it did not describe snRNA accumulation during development. Using high-resolution polyacrylamide gels in combination with Northern blot hybridization and RNA sequence analyses, we show here that *Xenopus* has at least three classes of U1 and U4 snRNAs that are distinguishable by their differential expression of oocytes, embryos, tadpoles, and frogs. Adult snRNAs appear to be synthesized constitutively throughout *Xenopus* development and comprise the major species in tissues from large tadpoles and frogs. Embryonic snRNAs are the principal species accumulating during the two periods of rapid snRNA synthesis, i.e., in previtellogenic oocytes and early embryos. Tadpole RNAs are minor species that are most prominent in young feeding tadpoles. Transcription of both embryonic and adult snRNA genes is activated at the midblastula transition (MBT), but expression of the embryonic genes is switched off selectively within a few days after MBT. Although the precise timing of this inactivation differs significantly for U1 and U4 genes, the overall pattern of differential expression is common to U1 and U2 snRNA genes. Because of sequence differences between the snRNAs accumulating at various stages, the resulting populations of snRNPs could have different splice-site specificities leading to altered patterns of pre-mRNA splicing during development.

[Key Words: U1 snRNA; U4 snRNA; developmental control of snRNAs; transcription of snRNAs; *Xenopus laevis*; small nuclear RNAs]

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At particular periods in *Xenopus* development, the need for rapid accumulation of small nuclear RNAs (snRNAs) becomes very pronounced. For example, after the midblastula transition (MBT) (Newport and Kirschner 1982), when the blastomeres are still dividing rapidly, the synthesis of large amounts of snRNAs is required (Forbes et al. 1983). In the case of U1 snRNA, this requirement is met by transcription of a large family of embryonic U1 RNA genes (Forbes et al. 1984). The resulting U1 RNAs differ slightly in sequence from the majority of the U1 RNAs expressed in cells from differentiated tissues (e.g., kidney), suggesting that the expression of the embryonic genes might serve other functions than simply to furnish sufficient amounts of snRNAs. Since most of the major species of snRNAs, including U1, U2, U4, U5, and U6, participate in the splicing of pre-messenger RNAs (pre-mRNAs) (for review, see Padgett et al. 1986 and references therein), we and others have proposed that differential expression of snRNA genes during development might be responsible, in part, for selection of alternative splice sites during pre-mRNA processing (Forbes et al. 1984; Lund et al. 1985; Laski et al. 1986).

Cultured *Xenopus laevis* cells contain more than seven different electrophoretically separable species of U1 RNA. We previously showed that two of these RNAs, called xU1b1 and xU1b2 (formerly xU1a and xU1b, respectively; Lund et al. 1984; Ciliberto et al. 1985; Krol et al. 1985), account for more than 95% of the U1 RNAs synthesized during early embryogenesis; hence, they are referred to as embryonic U1 RNAs. A different subset of RNAs, called xU1a1 to xU1a5 (formerly called xU1c-g, respectively), is synthesized in tissue culture cells and in mature stage VI oocytes (Forbes et al. 1984); these are called adult U1 RNAs. Although it has been shown that large amounts of U1 (and U2) snRNAs accumulate during early oogenesis (Forbes et al. 1983; Fritz et al. 1984), it is not known whether the U1 RNAs synthesized in immature and mature oocytes are the same.

The two xU1b genes are arranged pairwise in large tandem arrays and are present in over 500 copies per haploid genome. In contrast, the xU1a genes appear to be more dispersed and present in less than 10 copies each (Lund et al. 1984; Zeller et al. 1984).

To delimit precisely the stages at which the different classes of U1 genes are expressed, we have examined the patterns of accumulation of xU1a and xU1b RNAs throughout *Xenopus* development. Our results demonstrate that xU1a RNAs are present in all transcriptionally active tissues investigated, whereas the two xU1b RNAs accumulate almost exclusively in previtellogenic oocytes and in early embryos. We also show that this pattern of developmental control extends to another family of *Xenopus* snRNA genes, encoding U4 RNAs. Furthermore, we present evidence for a third class of xU1 and xU4 RNAs, which accumulate mostly in tadpoles.

Results

To determine which classes of U1 RNAs were present in various tissues during *Xenopus* development we have taken advantage of the striking separation of the different species of *X. laevis* U1 RNAs, which can be obtained in a nondenaturing gel (Forbes et al. 1984; Lund et al. 1984). The separated RNAs, which we have characterized previously by RNA sequence analyses (Forbes et al. 1984), were detected by Northern blot hybridization using a U1 RNA-specific probe derived from a human U1 gene (Lund and Dahlberg 1984).

Embryonic U1 RNAs are stockpiled in oocytes and accumulate in early embryos

Although the expression of the xU1a, or so-called adult, RNAs predominates in mature stage VI oocytes (Forbes

et al. 1984), it was unknown which types of U1 RNAs were synthesized in oocytes at earlier stages of development, when 4–8000 somatic cell equivalents of U1 RNAs accumulated (Forbes et al. 1983; Fritz et al. 1984). As shown in Figure 1 (lanes 2–6) almost all of the stored U1 RNAs were of the embryonic (xU1b) type rather than of the adult (xU1a) type. Since the amounts of embryonic U1 RNAs per oocyte did not increase significantly between stages II and III (compare lanes 3 and 4) and remained constant thereafter, expression of the embryonic genes appeared to be restricted to the previtellogenic stages of oogenesis. In contrast to oocytes, follicle cells (like other somatic cells, see below) contained primarily adult xU1a RNAs (compare lanes 6 and 7; data not shown). Although xU1a RNAs are the major species synthesized in stage VI oocytes, these molecules contribute only a very small amount of the U1 RNAs present in mature oocytes.

We previously demonstrated that the synthesis of two embryonic U1 RNAs predominates in late blastula and gastrula embryos (Forbes et al. 1984). The preferential synthesis of these RNAs, as well as the abrupt onset of transcription at MBT (Newport and Kirschner 1982; Forbes et al. 1983), results in the rapid accumulation of xU1b1 and xU1b2 RNAs immediately after MBT (Fig. 2A, lane 3). During the next few days, the level of accumulated embryonic RNAs (per embryo) increased only slightly, apparently reaching a maximum around the hatching or feeding tadpole stage (see lanes 12 and 13, which were exposed for shorter times than lanes 1–11, to reveal the changes in RNA levels). Throughout early embryogenesis, the level of xU1b1 RNA exceeded that of xU1b2. Presumably, this difference in accumulation resulted from the preferential transcription of xU1b1 versus xU1b2 genes (Forbes et al. 1984) due to competition between the two kinds of xU1b genes (Lund et al., this issue).

Figure 1. The stockpile of U1 RNAs in oocytes consists of embryonic RNAs. Northern blot analysis of the U1 RNAs that accumulate during oogenesis. (Lanes 2–6) RNAs of 2.5 defolliculated oocytes of the stages indicated (Dumont 1972); (lane 7) RNAs from about three stage V–VI oocytes plus (+) follicle cells; (lanes 1 and 8) RNAs of about 10^4 frog tissue culture (TC) cells. Total RNAs were separated by electrophoresis in a 15% (19:1) nondenaturing polyacrylamide gel and transferred electrophoretically to Gene Screen Plus membrane; the blot was hybridized to a 32 P-labeled U1-specific Sp6 RNA probe. The autoradiogram shown represents a 20-hr exposure without intensifying screen.

Adult U1 RNAs accumulate both in embryos and adult tissues

The expression of xU1a genes is readily detectable in late gastrula embryos (Fig. 2A, lane 3) and could also be observed as early as stage 9, in late-blastula embryos (data not shown). This indicates that transcription of both embryonic and adult U1 RNA genes is activated at MBT. However, in contrast to the situation with embryonic RNAs, the adult RNAs continue to accumulate throughout development. If all of the U1 genes were expressed with the same efficiency at MBT, each of the multiple xU1a RNAs would account for only 1–2% of the total U1 RNAs accumulating. Thus, the low levels of the xU1a RNA synthesis early in development probably result from the relatively low abundance of xU1a genes, which together comprise less than 10% of all *X. laevis* U1 genes (Lund et al. 1984; Zeller et al. 1984). Because of the continued synthesis of xU1a RNAs, concomitant with the slow disappearance of xU1b RNAs, the adult RNAs emerge as the major class in tadpoles

Figure 2. The accumulation of embryonic U1 RNAs is developmentally controlled. Northern blot analyses of the U1 RNAs that accumulate during embryogenesis (A), or in tissues from tadpoles (B) or frogs (C). (A) (Lanes 1–13) Total RNAs corresponding to 0.2 embryos of the stages indicated; (lanes 1–11 and lanes 12 and 13) 40- and 8-hr autoradiographic exposures, respectively; (lanes 11 and 12) the same sample. (B) (Lanes 1–4) Total tadpole RNAs (corresponding to less than 0.1 tadpole); (lanes 5 and 6) total RNAs of tadpole liver. The arrows on the right indicate minor species of xU1c RNAs which appear to be tadpole specific. (C) (Lanes 1–5) Total RNAs of brain (B), kidney (K), liver (L), testis (T), or ovary (O), respectively. Polyacrylamide gel electrophoresis and Northern blot hybridizations were performed as in Fig. 1. The developmental stages, indicated above the lanes in C, are according to Nieuwkoop and Faber (1967). In A, the stages are 8 (blastula), 12/13 (gastrula), 18/19 and 21/22 (neurula), 26 and 29/30 (tailbud), 35/36 (hatching tadpole), 39/40, 41/42, and 43/44 (swimming tadpole), and 48 (feeding tadpole).

(stages 50–59) or newly metamorphosed froglets (stage 66) (Fig. 2B, lanes 5 and 6).

Adult frog tissues, like liver, brain, and kidney contain almost exclusively xU1a RNAs (Fig. 2C, lanes 1–3). However, both testis and ovary have significantly elevated levels of embryonic xU1b RNAs (lanes 4, 5). The presence of xU1b RNAs in frog testis resembles the situation in mouse testis (Lund et al. 1985), in which embryonic mU1b RNAs accumulate in large amounts, apparently as a result of expression of embryonic U1 genes in the dividing germ line cells (E. Lund, unpubl.). The predominance of embryonic U1 RNAs in ovaries can be accounted for by a stockpile of xU1b RNAs which is present in stage I and II oocytes (Fig. 1).

Expression of U4 snRNAs is also developmentally controlled

To determine whether the less abundant U4 RNA, which also participates in pre-mRNA processing (Berget and Roberson 1986; Black and Steitz 1986), might be subject to similar developmental control, Northern blot analyses were performed as above except that the RNAs were separated in partially denaturing polyacrylamide gels (Fig. 3). As was the case with xU1 RNAs, the patterns of accumulated xU4 RNAs changed strikingly during development and multiple variants of xU4 RNAs accumulated in a coordinate fashion at several stages.

Since the predominant forms of U4 RNAs in tissues from large tadpoles or adult frogs (Fig. 3C,D) appeared to be the same as the two major species in tissue culture cells (Fig. 3A), these RNAs were further characterized by analyses of 32 P-labeled RNAs isolated from the cultured

Figure 3. Developmental control of *X. laevis* U4 RNA gene expression. Northern blot analyses of xU4 RNAs that accumulate during oogenesis (A) and embryogenesis (B) or in tissues from tadpoles (C) and frogs (D). (A) (Lanes 2–6) Total RNAs of 2.5 defolliculated oocytes of the indicated stages (Dumont 1972); (lane 7) RNAs of intact stage V–VI oocytes plus (+) follicle cells; (lane 1, TC) RNAs of cultured frog kidney cells. (B) (Lanes 1–6) Total RNAs of 0.2 embryos; the stages (Nieuwkoop and Faber 1967) are 12/13 (gastrula), 21/22 (neurula), 31/32 (tailbud), 43/44 (swimming tadpole), and 48 (feeding tadpole). (C) (Lanes 3–9) Total RNAs of individual tadpoles from a single fertilization; (lanes 10 and 11) total RNAs of tadpole liver. The samples in lane 1 (TC) and lane 2 (48) correspond to those of lane 1 of A and lane 6 of B, respectively. (D) (Lanes 1–5) Total RNAs of liver (L), kidney (K), brain (B), testis (T), and ovary (O), respectively. Only testis and ovary contain significant amounts of xU4b embryonic RNAs. The identities of the various bands of U4 RNAs (a1–2, b, and c1–3) are discussed in the text. Total RNAs were separated by electrophoresis in partially denaturing, 12% (30:0.8) polyacrylamide gels, containing 7 M urea gels, and the membrane blots were hybridized with the Sp6-U4 RNA probe derived from a human U4 RNA pseudogene (Bark et al. 1985).

Figure 4. Identification of *X. laevis* embryonic and adult U4 RNAs. Polyacrylamide gel (A) and RNase T1 fingerprint (B) analyses of the U4 RNAs synthesized in *Xenopus* cultured cells and early embryos (cf. Fig. 5A). (A) (Lanes 1 and 2) Total and anti- m_3G antibody-precipitable 4–8S RNAs of cultured frog kidney cells. (Lane 3) Markers of mouse snRNAs run in the same gel. Uniformly ^{32}P -labeled RNAs from *X. laevis* cultured kidney cells or mouse C1300 cells were separated by electrophoresis in a partially denaturing 12% (30:0.8), 7 M urea gel as in Fig. 3. The assignments of U1, U2, U4, and U5 snRNAs were by RNase T1 fingerprint analyses (Fig. 4B and data not shown). Note that cognate frog and mouse snRNAs have different gel mobilities. (B) Uniformly ^{32}P -labeled xU4 RNAs were purified from cultured cells (A) or early embryos (Fig. 5) (see Materials and methods) and subjected to two-dimensional RNase T1 fingerprinting according to Barrell (1971). Panels I, II, and IV show the fingerprints of xU4a1, xU4a2, and xU4b RNAs, respectively. Panel III is a schematic summary indicating the RNase T1 oligonucleotides which are common to all three xU4 RNAs (open ellipses) or specific to xU4a1 RNA (hatched ellipses), xU4a2 RNA (dotted ellipses), or xU4b RNA (closed ellipses), respectively. Redigestion with RNase T2 demonstrated that most, if not all, of the xU4a-specific oligonucleotides differed in sequence rather than in the base modification (data not shown). Many of the common oligonucleotides, including those containing the 5' and 3' termini, are identical in sequences to those of mouse U4 RNAs (Kato and Harada 1981).

cells. As shown in Figure 4A, both forms of xU4 RNAs were precipitable by anti- m_3G antibodies (Bringmann et al. 1983) and, when present in snRNPs (Lerner and Steitz 1979), by anti-Sm antibodies (data not shown). Furthermore, RNase T1 fingerprint analyses (Fig. 4B, panels I and II) confirmed that they were distinct, but closely related, variants of U4 RNA. Because the levels of these RNAs are much greater in adult tissues than in early embryos or oocytes (Fig. 3), we call them the xU4a (adult) RNAs. Like the adult xU1 RNAs, both of the xU4a RNAs were present in follicle cells (compare lanes 6 and 7 of Fig. 3A).

The other major form of xU4 RNA, called xU4b, was stockpiled in oocytes (Fig. 3A) and accumulated in gastrula embryos, but not during later stages of embryogenesis (Fig. 3B). Analyses of newly synthesized RNAs isolated from pulse-labeled embryos (Fig. 5) demonstrated that xU4b RNA was synthesized only during a brief period immediately after MBT but not in early neurula embryos, when other forms of xU4 RNAs could be distinguished (see also Fig. 3B). RNase T1 fingerprinting of the xU4b RNA from gastrula embryos (Fig. 4B, panel IV) unequivocally showed that this RNA was a distinct species, differing in sequence from both of the adult xU4a RNAs, as summarized in panel III.

Despite the overall similarity in the patterns of accumulation of xU4b and xU1b RNAs, the synthesis of these RNAs was not strictly coordinated during early embryogenesis. While synthesis of xU4b RNA had ceased in early neurula embryos, transcription of the xU1b genes continued at that stage (Fig. 5C; cf. Fig. 2 and Forbes et al. 1984).

A third class of U1 and U4 RNAs accumulates in tadpoles

A third class of xU1 and xU4 RNAs (called xU1c and xU4c RNAs, respectively) was revealed by analyses of the RNAs accumulating during tadpole development (Figs. 2B, 3B, and 3C). The xU1c and xU4c RNAs, like most other classes of *X. laevis* snRNAs, appeared to consist of several variants (c1, c2, and c3). Since the patterns of these RNAs differed in sibling tadpoles of similar developmental age (Fig. 3C and data not shown), it is unlikely that these variants correspond to modified forms of the adult or embryonic RNAs. Thus, this novel class of snRNAs, which is most prominent in tadpoles, apparently is encoded by polymorphic multigene families.

Figure 5. Transcriptional control of embryonic snRNA genes during early embryogenesis. (A) Analyses of the newly synthesized snRNAs accumulating during MBT and late blastula (B, lane 1), gastrula (G, lane 2), or early neurula (N, lane 3). Developing embryos were labeled by injection of $^{32}\text{PO}_4^{3-}$ at various stages of development. Total RNAs were isolated from groups of 20 embryos, labeled from stages 7.5–10 (blastula), stage 10–12.5 (gastrula), or stage 12.5–15/16 (neurula), respectively. The total RNAs corresponding to about four embryos were analyzed by gel electrophoresis as in Fig. 3. (B) Comparison of xU2 RNAs synthesized in embryos (B, G, and N, lanes 2–4) and cultured cells (a, c, and TC, lanes 1, 5, and 6). The U2 RNAs from the bands indicated by arrowheads in A and from the U2 band in lane 1 of Fig. 4A, were re-electrophoresed in 15% nondenaturing gels as in Fig. 1. The markers (lanes 1 and 5) were the U2 RNAs in bands a and c of lane 6; these two RNAs (plus b) were further characterized by RNA T1 fingerprinting (see text). (C) Developmental control of embryonic xU1 RNA synthesis. (Lanes 1 and 4 and lanes 2 and 5) U1 RNAs synthesized in gastrula (G) or neurula (N) embryos, respectively. The U1 RNAs from bands b1 and b2 (arrowheads) in A were re-electrophoresed in a nondenaturing gel as in B. (Lane 3) Markers of xU1b1, xU1b2, and 5.8S ribosomal RNAs synthesized in injected oocytes; (lane 6) markers of adult xU1a RNAs from the fastest migrating band of xU1 RNAs in lane 1 of Fig. 4A.

Modification of U2 RNA is controlled during early embryogenesis

When snRNAs of uniformly labeled tissue culture cells (Fig. 4) or pulse-labeled embryos (Fig. 5A) were analyzed in partially denaturing gels, only a single band of xU2 RNA was evident. In contrast, several bands of U2 RNAs could be resolved when the U2 RNAs eluted from these gels were re-electrophoresed in a nondenaturing gel (Fig. 5B). Moreover, the patterns of U2 RNA bands differed between RNAs labeled at various times after MBT (lanes 2–4) and between the RNAs of embryos and cultured cells (lanes 1 and 5). RNase T1 fingerprints of the U2 RNAs from gastrula embryos and tissue culture cells revealed no detectable primary sequence differences, but instead indicated that the RNAs differed at

the level of base modification (data not shown). Thus, it is possible that posttranscriptional modification of *X. laevis* U2 RNAs is developmentally controlled.

Discussion

We have demonstrated that the accumulation of both U1 and U4 snRNAs is developmentally regulated in *Xenopus*. Three classes of snRNAs could be distinguished according to their accumulation at different stages of development. The adult RNAs (xUa), which appear to be constitutively expressed throughout development, the embryonic RNAs (xUb), which accumulate primarily during oogenesis and early embryogenesis, and the tadpole RNAs (xUc), which, although in low amounts, are most prominent in the feeding tadpole stages.

Developmental regulation of base modifications may also occur. Although electrophoretically separable forms of xU2 RNAs are detectable, they all appear to have the same primary sequence and to differ primarily in the extent of ribose methylations. The embryonic U1 RNAs of both mice and frogs also show consistent modification differences from the adult U1 RNAs, i.e., the conserved Ap residue at position 70 contains a 2'-O-methyl group only in the adult RNAs (Kato and Harada 1985; Lund et al. 1985; our unpublished results).

Function of developmental control of snRNA accumulation

As a result of the developmental switches described here, the populations of several snRNAs change substantially during development. Moreover, "embryonic" Sm antigens have been detected in *Xenopus* oocytes, early embryos, and testis (Fritz et al. 1984). Although these proteins have not been shown to be associated specifically with the embryonic snRNAs, which are abundant in these same tissues, that is likely to be the case. It is tempting to speculate that such changes in the complement of snRNPs could alter the patterns of pre-mRNA processing, for example by allowing for alternative splicing to occur.

An additional (or alternative) function for developmental control of snRNA gene transcription appears to be the need for rapid synthesis of large amounts of snRNAs during specific periods in early oogenesis (see below) and embryogenesis (Forbes et al. 1984). In this regard it should be noted that the embryonic (xU1b) genes are at least an order of magnitude more abundant than the adult (xU1a) genes (Lund et al. 1984; Zeller et al. 1984).

Embryonic forms of U1 RNAs also accumulate during early mouse development (Lund et al. 1985). In that case there is no need for a high rate of snRNA synthesis since the rate of cell division is not accelerated at any particular stage. Thus, if developmental control of snRNA transcription has a function in mouse embryos, it appears to be needed for accumulation of particular U1

RNAs, presumably with unique properties, rather than for the production of a large amount of U1 RNAs.

Control during oocyte development

In stage I and II oocytes (as well as in MBT embryos), the preferential accumulation of the embryonic xU1b and xU4b RNAs appears to be a direct result of the relative gene copy numbers (see above). The cessation of xU1b RNA accumulation prior to stage III of oogenesis indicates that the embryonic genes might be switched off during the stage when lampbrush chromosomes are at their maximum (Dumont 1972; Davidson 1976). Since embryonic xU1b genes injected into stage IV, V, or VI oocytes are transcribed very efficiently (Ciliberto et al. 1985; Krol et al. 1985; Lund et al., this issue; C.J. Bostock and E. Lund, unpubl.), presumably only the genes and not the transcription factor(s) are (temporarily) inactivated at this stage.

The stockpiling of $1-2 \times 10^9$ molecules of xU1b RNAs per oocyte (4–8000 somatic cell equivalents; Forbes et al. 1983) requires a rapid rate of synthesis of these molecules. Since each tetraploid oocyte contains a total of about 4×10^3 copies of embryonic U1 genes and since each gene can be transcribed about 10 times per minute (assuming the RNA polymerase II transcription rates summarized by Davidson 1976), it would take a total of $(1-2 \times 10^9)/(4 \times 10^3 \times 10)$ minutes or approximately 20–40 days to generate the stockpile of embryonic snRNAs; this time is commensurate with the length of the previtellogenic stages of oogenesis.

Follicle cells, in contrast to oocytes, contain mainly xU1a RNAs (Fig. 1). Since these cells outnumber the oocyte by several thousandfold (Dumont 1972), we were concerned that contamination with transcripts from follicle cells could have accounted for our earlier observation that xU1a RNA is synthesized in mature oocytes (Forbes et al. 1984). When stage VI oocytes, which had been injected with radioactive GTP and incubated overnight, were defolliculated, much of the newly synthesized xU1a RNA was, in fact, removed with the labeled somatic 5S RNA (which accounted for as much as 50% of the total labeled 5S RNAs; see also Wakefield and Gurdon 1983) (E. Lund, unpubl.). However, the remainder of the labeled xU1 RNAs, which fractionated with oocytes together with all of the labeled oocyte 5S RNA, was xU1a RNA; little, if any xU1b RNA was found in either fraction. Thus, stage VI oocytes, and not just follicle cells, do synthesize xU1a RNAs.

These results and the data presented earlier (Forbes et al. 1984) indicate that xU1b RNA synthesis is minimal in mature oocytes. Since the level of xU1b RNA does not decline in stage VI oocytes (Fig. 1), we conclude that the turnover of the stockpiled xU1b RNA (which are in snRNPs) must be negligible.

Mechanisms of control of snRNA synthesis during embryogenesis

During early embryogenesis, the most striking control of snRNA synthesis is the activation of transcription at

MBT (Newport and Kirschner 1982; Forbes et al. 1983). Since accumulation of both adult and embryonic snRNAs occurs at that time (Fig. 2), we conclude that this control is not specific to the embryonic snRNA genes. Shortly thereafter, however, the expression of embryonic snRNA genes apparently is switched off selectively, while transcription of adult (and tadpole) genes continues.

The mechanism(s) responsible for the inactivation of the embryonic (and later presumably also the tadpole) snRNA genes is unknown, but the control of expression of *Xenopus* oocyte and somatic 5S RNA genes (see, for example, Gottesfeld and Bloomer 1982; Wormington et al. 1982; Brown and Schlissel 1985; Guinta and Korn 1986) serves as an excellent model. By analogy, we propose that in early embryos, immediately after MBT, an excess of transcription factors can support expression of all snRNA genes, but as DNA replication proceeds, competition for these factor(s) becomes established. Under such conditions, the genes that replicate earliest and/or have the highest affinity for the limiting factor(s) would be the ones that continue to be transcribed; for example, we would expect that xU4a genes would replicate before xU4b genes and that affinities for the limiting transcription factor(s) would be in the same order.

We have recently found that cloned xU1b genes are subject to correct transcription controls when injected into embryos (Lund and Dahlberg, in prep.); thus, it is now possible to test directly if the developmental control is mediated as proposed above.

Materials and methods

Preparation of oocytes and embryos

Adult male and female *X. laevis* frogs were purchased from Xenopus I (Ann Arbor, Michigan), kept at 18°C, and fed chopped liver three times a week. Unfertilized eggs were isolated as described elsewhere (Lund et al., this issue) and fertilization of freshly laid eggs was carried out according to Newport and Kirschner (1982). Fertilized eggs were allowed to develop at room temperature (22–24°C) and the developmental stages of embryos and tadpoles were determined according to Nieuwkoop and Faber (1967). Intact stage V and VI oocytes were dissected manually from whole ovaries. Defolliculated oocytes were obtained by incubation of small pieces of ovary in 0.2% collagenase followed by manual collection of the oocytes of different stages (Dumont 1972).

Isolation of unlabeled RNAs for northern blot analyses

Total nucleic acids were prepared from oocytes, eggs, or early embryos by homogenization in proteinase K lysis buffer (Krol et al. 1985; Lund et al., this issue). In the case of larger tadpoles and tissues from frogs, homogenization was in urea lysis buffer as described previously for isolation of RNAs from mouse tissues (Lund et al. 1985).

For Northern blot analyses of U1 RNAs, total RNAs were separated by electrophoresis in nondenaturing gels [containing 15% (19:1) polyacrylamide, $\frac{1}{2} \times$ TEB (1 $\times$ TEB is 85 mM Tris-borate, pH 8.3, 2.8 mM EDTA); Lund et al. 1983] which are capable of resolving the multiple species of *X. laevis* U1 RNAs (Forbes et al. 1984). For analyses of U4 RNAs, we used partially denaturing gels [containing 12% (30:0.8) polyacrylamide, 7 M

urea and $1 \times$ TEB; Lund et al. 1985] which were found to resolve at least six different forms of xU4 RNAs.

Northern blot analyses

Electrophoretic transfer of RNAs to Gene Screen Plus membrane (New England Nuclear), the conditions of hybridization and the Sp6-U1 probe were described earlier (Lund et al. 1985). The Sp6-U4 probe was made by cloning a 64-bp *Pst*I fragment from a human U4 pseudogene (kindly provided by U. Pettersson; Bark et al. 1985) into the pGEM1 cloning vector (Promega Biotec). The Sp6 probes were labeled with [32 P]GTP (400 Ci/mmol, Amersham), according to Melton et al. (1984). Autoradiographic exposures of the blots were for 4–40 hr without intensifying screens.

Isolation of labeled RNAs for RNase T1 fingerprint analyses

For pulse-labeling of embryo RNAs, embryos at various stages of development were injected with 20 nl containing 2 μ Ci of 32 PO $_4^{3-}$. After 4–8 hr of incubation (depending on the stage of development), total nucleic acids were prepared as above.

Uniformly 32 P-labeled RNAs from cultured frog kidney cells (kindly provided by D.D. Brown) or mouse C1300 cells (Lund et al. 1985) were obtained by incubation of one-half to two-thirds confluent cells (one 75-cm 2 flask) with 1–2 mCi of 32 PO $_4^{3-}$ in 10 ml of phosphate-free medium. Total RNAs were prepared as above using urea lysis buffer. Frog snRNAs were identified by precipitation with anti-m $_3$ G antibody (generously provided by R. Lührmann) according to Bringmann et al. (1983). For further analyses by RNase T1 fingerprinting, different species of xU2 and xU4 RNAs were purified by electrophoresis in two consecutive polyacrylamide gels: a partially denaturing 12% (30:0.8), 7 M urea gel followed by a 15% (19:1) nondenaturing gel (see above).

Two-dimensional RNase T1 fingerprinting was done according to Barrell (1971) using homomix C and polyethyleneimine thin-layer plates for the second dimension.

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