The transcription of *Xenopus laevis* embryonic U1 snRNA genes changes when oocytes mature into eggs

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***X. laevis* stage VI oocytes respond differently from unfertilized eggs when injected with the genes for *X. laevis* embryonic U1 RNAs, xU1b1, and xU1b2. Upon maturation of oocytes into eggs, the efficiency of transcription decreases greatly and the ratio of xU1b1 to xU1b2 RNA transcription changes. Moreover, DNA replication is now required for transcription. Because of differences in the 5'-flanking regions of the two xU1b genes, xU1b2 RNA transcription predominates after injection into oocytes; in contrast, xU1b1 RNA transcription predominates after injection into unfertilized eggs. Our results also indicate that in oocytes a factor that interacts with sequences close to the coding region is limiting, whereas in eggs a factor that recognizes far-upstream sequences required for enhancer activity is limiting. Qualitatively, expression of the embryonic xU1b genes injected into eggs closely resembles that of the endogenous genes during early embryogenesis.**

[**Key Words:** Embryonic U1 RNA genes; snRNA transcription factors; *X. laevis* oocytes and unfertilized eggs; microinjection]

Received November 4, 1986; accepted November 26, 1986.

Xenopus laevis cells contain more than seven variant forms of U1 small nuclear RNA (U1 snRNAs) (Forbes et al. 1984) which are encoded by at least two different multigene families (Lund et al. 1984; Zeller et al. 1984). Following activation of transcription in early embryos at the midblastula transition (MBT) (Newport and Kirschner 1982b; Forbes et al. 1983a), the newly synthesized U1 RNAs are primarily the two so-called embryonic U1 RNAs, xU1b1 and xU1b2 (Forbes et al. 1984; Lund and Dahlberg, this issue). These RNAs (formerly called xU1a and xU1b, respectively; Lund et al. 1984; Krol et al. 1985) are also the major species of U1 RNAs stockpiled during early oogenesis (Forbes et al. 1983b; Fritz et al. 1984; Lund and Dahlberg, this issue). As embryonic development proceeds, the synthesis of multiple species of adult U1 RNAs (the xU1a RNAs, formerly called xU1c-g RNAs) predominates (Lund and Dahlberg, this issue).

The genes that code for the embryonic U1 RNAs are both present in at least 500 copies per haploid genome and comprise more than 90% of the *X. laevis* U1 genes. The xU1b1 and xU1b2 genes are paired in 1.8-kb repeat units organized in large tandem arrays (Lund et al. 1984; Zeller et al. 1984). Upon injection into stage VI oocytes,

cloned copies of the full-length repeat unit are transcribed efficiently to give mature xU1b RNAs (Ciliberto et al. 1985; Krol et al. 1985).

Although the xU1b gene repeats contain one copy each of the xU1b1 and xU1b2 genes, the synthesis of xU1b2 RNA predominates under standard conditions of DNA injection (Krol et al. 1985). This is in contrast to the normal in vivo situation in stage I and II oocytes and in embryos at the midblastula transition (MBT) when transcription of the endogenous xU1b1 genes is either the same as, or more than, that of the endogenous xU1b2 genes (Forbes et al. 1984; Lund and Dahlberg, this issue).

We have examined the transcription of U1b genes after injection into stage VI oocytes and unfertilized eggs, which represent developmental stages between the early oocyte and the MBT embryo. Stage VI oocytes are transcriptionally very active, but incapable of replicating DNA whereas activated but unfertilized eggs resemble early cleavage embryos in that they support high amounts of DNA replication, but low levels of RNA transcription (for review, see Gurdon and Melton 1981).

We show here that in both injected oocytes and injected eggs, the differential rate of transcription of one embryonic gene over the other is based on limiting levels of transcription factors. Moreover, different factors appear to be limiting in the two types of cells since the competition for them is mediated by different control regions upstream of the xU1b1 and xU1b2

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coding sequences. In eggs, but not in oocytes, the formation of active transcription complexes requires DNA replication.

Results

Transcription in oocytes

The two embryonic U1 RNA genes of *X. laevis*, xU1b1 and xU1b2, differ by only two base pairs in the coding regions (Ciliberto et al. 1985; Krol et al. 1985), but the encoded RNAs can be readily distinguished by polyacrylamide gel electrophoresis (Forbes et al. 1984; Lund et al. 1984). However, the 5'-flanking regions of these genes differ significantly. To determine how these flanking sequences influence the relative transcription levels of the two genes, we constructed a variety of chimeric xU1b genes in which two sections of the 5'-flanking region were interchanged, as illustrated in Figure 1. The region more proximal to the coding region

includes the snRNA gene "TATA-box," i.e., sequences that are essential for correct transcription initiation of U1 RNA (Skuzeski et al. 1984; Ciliberto et al. 1985; Krol et al. 1985; Murphy et al. 1987); accordingly, we call this the proximal promoter region. The more distal sequences, upstream of about position -220, contain the snRNA transcriptional enhancer; we call this the enhancer region. We note that both regions include considerably more sequence information than the TATA box or the enhancer.

When equal amounts of the genes for the two xU1b RNAs are injected separately into oocytes, using standard conditions (4–8 ng of DNA injected per oocyte), both genes are expressed equally well. However, when the genes are coinjected either on the same or separate plasmids, xU1b2 RNA accumulates at five- to tenfold the rate of xU1b1 RNA (Fig. 2; Krol et al. 1985). Neither the ratio of accumulated xU1b1 to xU1b2 RNAs (see Fig. 2A) nor the intracellular distribution of the two RNAs (i.e., nuclear versus cytoplasmic localization)

Figure 1. Structures of wild-type and chimeric *X. laevis* embryonic U1 genes. (A) The standard wild-type xU1b repeat unit containing one copy each of the xU1b1 (b1) and xU1b2 (b2) genes (1/1/1:2/2/2). The open and closed circles, boxes, and arrows indicate the positions of homologous regions between the two xU1b genes corresponding to the enhancer, the U1 TATA box, and the U1 coding sequences, respectively. The coding regions of the b1 and b2 genes differ at positions 78 and 114, downstream of the *Bcl*I sites at position +27. The use of these symbols as cartoons of xU1b genes (Fig. 1B,C) is shown below the map. The positions of restriction enzyme cleavage sites used in constructions of chimeric templates are indicated. (P) *Pst*I; (S) *Stu*I; (H) *Hind*III; (B) *Bcl*I; (R) *Rsa*I; (M) *Mlu*I. (B) Example of a mutant xU1b repeat DNA. The U1 coding region and 5'-flanking region sequences of the b1 and the b2 genes, respectively, were joined via the *Bcl*I sites and this mutant b1 gene (2/2/1) and the wild-type b2 gene (2/2/2) were religated to generate the chimeric repeat (2/2/1:2/2/2) as described in Materials and methods. (C) xU1b genes with truncated or chimeric 5'-flanking sequences. The truncated (short) b1 and b2 genes, (1_s/1/1) and (2_s/2/2), contain 269 and 289 bp of 5'-flanking sequences, respectively. The genes with the chimeric promoters, (1_s/2/2) and (2_s/1/1), contain the enhancer regions of the b1 gene (SH, positions -269 to -222) and the b2 gene (RM, positions -289 to -219), respectively. (H) and (M) indicate restriction enzyme cleavage sites that were converted to *Xho*I sites.

Figure 2. Transcription of xU1b genes with wild-type and chimeric promoter regions in injected oocytes. (A) Time course of xU1b RNA synthesis in oocytes injected with wild-type (panel 1) or mutant (panels 2–4) xU1b repeat DNAs. As indicated by the cartoons (cf. Fig. 1), the mutant templates contained tandemly arranged xU1b genes with different (panel 2) or identical (panels 3 and 4) promoter regions. About 30–40 oocytes were each coinjected with 6 ng of a template DNA and 0.5 μ Ci of [α - 32 P]GTP and pools of 10 oocytes were harvested for RNA preparation after the indicated times of incubation at 18°C. Total RNAs equivalent to two oocytes were analyzed by electrophoresis in 12% (30:0.8), 7 M urea gels (Krol et al. 1985). For simplicity, only the regions of the gels containing the b1 and b2 U1 RNAs are shown. Autoradiograms were exposed for 20–24 hr without intensifying screens. (B) Competition between xU1b genes with chimeric promoter regions. The mutant xU1b repeat templates contained genes with different (panels 1–3) or identical (panels 4 and 5) 5' proximal promoter regions. Conditions of injections and gel analyses of total RNAs (isolated after 18–20 hr of incubation) were as in A. Preferential transcription of one gene over the other (panels 1–3) was observed independent of whether the 5' distal promoter regions were different (panel 1) or identical (panels 2 and 3).

(data not shown) changes as a function of time of incubation. Therefore, the differential rates of accumulation of the xU1b RNAs presumably reflect different rates of transcription of the two xU1b genes, rather than differences in RNA stability or snRNP formation.

5'-flanking sequences responsible for preferential accumulation of xU1b2 RNA To determine which region of the xU1b genes was responsible for the preferential accumulation of xU1b2 RNA, chimeric genes were injected in which the 5'-flanking sequences had been exchanged (Fig. 1B). When the entire 5'-flanking regions of the two genes were exchanged, the ratio of the levels of xU1b1 to xU1b2 was reversed, so that the amount of xU1b1 RNA was higher than that of xU1b2 RNA (Fig. 2A, panel 2). No preferential accumulation was observed when the 5'-flanking regions of the two genes were identical (Fig. 2A, panels 3 and 4). These patterns of gene expression were independent of the relative positions of the chimeric xU1b genes within the cloned repeats (data not shown), indicating that the 5'-flanking regions of the two embryonic genes compete for one or more transcription factors that are limiting in injected oocytes.

Competition between genes was unaffected by switching the regions containing the enhancer (Fig. 2B). However, preferential transcription of an xU1b gene resulted when it contained the more proximal region of the xU1b2 promoter (Fig. 2B, compare panels 1–3). This

conclusion was confirmed by the observation that xU1b genes with different enhancer regions but identical 5'-proximal promoter regions were equally well transcribed (panels 4 and 5). We conclude that preferential transcription of the normal xU1b2 gene results from the relative efficiency with which sequences close to its coding region bind a limiting transcription factors.

xU1b genes compete only when injected in large amounts To obtain a more quantitative measure of the limiting factor, we examined the dose-response curve for transcription of the wild-type xU1b genes in injected oocytes. As illustrated in Figure 3, the two genes were transcribed equally well when less than 1 ng per oocyte of the wild-type repeat DNA was injected, mimicking the expression of the endogenous xU1b genes during early oogenesis (Lund and Dahlberg, this issue). However, the xU1b2 gene was preferentially expressed after injection of more than 1 ng of DNA per oocyte. Thus, the limiting factor is present in quantities sufficient to accommodate 1 ng of template, a level well in excess of both the 10^{-5} ng of endogenous xU1b repeat DNA in oocytes and the 10^{-2} ng of repeat DNA present in mid-blastula embryos (cf. legend to Fig. 3B).

At nonsaturating concentrations of injected DNA, the levels of xU1b RNA transcripts per molecule of template were five- to tenfold higher than those of the heterologous human U1 RNA gene, hU1-1D (Murphy et al.

Figure 3. Efficiency of transcription of *X. laevis* embryonic U1 genes and a human U1 gene in injected oocytes. (A) About 15–20 oocytes were each coinjected with 0.5 μ Ci of [α -³²P]GTP and the indicated amounts of wild-type xU1b repeat DNA (1/1/1:2/2/2) (lanes 1–5) (or similar amounts of wild-type human U1 DNA, not shown). After 18 hr of incubation, total RNAs were isolated from pooled oocytes and the RNAs equivalent to three oocytes were analyzed by electrophoresis in a non-denaturing 15% (19:1) polyacrylamide gel. Only the region of the gel containing the U1 RNAs is shown. (B) The amounts of xU1b1 (b1), xU1b2 (b2), and human U1 (hU1) RNAs synthesized in injected oocytes as a function of template DNA concentration. The number of U1 RNA molecules produced were estimated from the known specific activity of the injected precursor (see Materials and methods) and the measured radioactivity in the bands of U1 RNAs in the gel shown in A and other comparable gels (not shown). The amounts of injected template DNA that correspond to 10⁶ xU1b genes per oocyte (arrow) and the number of endogenous xU1b genes per embryo at the mid-blastula transition (=MBT) are indicated. One oocyte has about 2×10^3 endogenous copies of each xU1b gene.

1982; Skuzeski et al. 1984) (Fig. 3B). This difference probably reflects more efficient recognition by the frog oocyte transcription factors of the homologous *X. laevis* genes.

Transcription in unfertilized eggs

Transcription in unfertilized eggs resembles embryonic U1 RNA synthesis The relative transcription efficiencies of injected xU1b genes in stage VI oocytes (i.e.,

xU1b2 $\geq$ xU1b1) do not reflect the transcription efficiencies of endogenous genes in early embryos (i.e., xU1b2 < xU1b1; Forbes et al. 1984; Lund and Dahlberg, this issue). To develop a transcription system that more closely resembles that of early embryos, we analyzed the expression of cloned xU1b genes upon injection into unfertilized eggs.

Pricking unfertilized eggs with a microinjection needle mimicks fertilization by activating the eggs to initiate DNA replication (Gurdon et al. 1969) and other events of the cell cycle (Hara et al. 1980). Although this leads to an apparent deterioration of the eggs within 5–10 hr after activation (Gurdon and Melton 1981), we found that expression of injected xU1b genes was detectable only after a lag period of at least 6 hr (Fig. 4, compare lanes 3 and 5; see also Fig. 7, below; Bendig and Williams 1984).

When the wild-type xU1b gene repeat unit (Fig. 1A) was injected into eggs, both xU1b RNAs accumulated

Figure 4. Transcription of embryonic U1 genes in injected unfertilized eggs. Comparison of xU1b RNA synthesis in injected unfertilized eggs and in gastrula embryos. Dejellied eggs were injected with or without 5 ng of wild-type xU1b repeat DNA (1/1/1:2/2/2) DNA plus [α -³²P]GTP and incubated for 6 or 18 hr, as indicated. Total nucleic acids were isolated from pools of 10–12 eggs and analyzed as in Fig. 2, except that the autoradiogram was exposed for 30 hr with intensifying screen. The products from the equivalent of four eggs injected with (lanes 3 and 5) or without (lanes 2 and 4) xU1b repeat DNA are shown. Total nucleic acids from gastrula embryos (lane 1) were obtained by pulse-labeling with ³²PO₄³⁻ between 9–13 hr after fertilization (cf. Lund and Dahlberg, this issue).

(Fig. 4, lane 5) but the absolute levels of xU1b1 RNA transcription was at least 50-fold less than in oocytes (cf. legend to Fig. 4). However, the ratio of xU1b to xU1b2 RNAs resembled that seen normally in early gastrula embryos (lane 1). In the absence of injected DNA, the transcripts from endogenous templates in activated eggs were principally mitochondrial RNAs (Fig. 4, lanes 2 and 4; cf. Gurdon and Melton 1981).

Sequences responsible for the preferential transcription of xU1b1 genes When experiments with chimeric genes were performed in eggs, the differential accumulation again correlated with the type of 5'-flanking region of the gene (Fig. 5); furthermore, the effects were independent of the gene order within the plasmid DNA (data not shown), indicating competition for a limiting transcription factor. In contrast to the situation in oocytes, the xU1b1 5'-flanking region was the more efficient one in eggs (panels 1 and 2). Injection of genes with the same 5'-flanking regions resulted in the production of equal amounts of xU1b1 and xU1b2 RNAs (panel 3). The less efficient xU1b2 promoter functioned well when injected alone (panel 4).

Sequences upstream of the enhancer region of the xU1b1 gene are not responsible for its preferred transcription (panel 5). However, replacement of the enhancer regions of the xU1b1 genes with that of the xU1b2 gene impaired the ability of the chimeric genes to

compete (compare panel 6 with 1, and panel 7 with 3). We conclude that a transcription factor that interacts with sequences in the enhancer region probably is limiting in injected unfertilized eggs; thus, it differs from the factor that is limiting in oocytes.

Transcription control during in vitro maturation of oocytes We tested whether the difference in transcriptional dominance, which distinguishes expression of the injected xU1b genes in oocytes and eggs, could be reproduced by in vitro maturation of preinjected oocytes. Maturation of stage VI oocytes occurs within 10–15 hr of incubation with progesterone, as monitored by germinal vesicle breakdown (Gurdon 1967). In vitro-matured oocytes, like in vivo-matured eggs, are activated by pricking (Harland and Laskey 1980).

Initially (Fig. 6A), the injected xU1b genes were transcribed very efficiently both in progesterone-treated and in control oocytes (i.e., more xU1b2 than xU1b1 in lanes 1 and 2); presumably the RNAs in lane 1 were made before RNA synthesis ceased at germinal vesicle breakdown (LaMarca et al. 1975; Gelfand and Smith 1983). After maturation, (Fig. 6B), when the in vitro-matured oocytes were activated by the injection of the labeled GTP, the xU1b genes were again transcribed; however, at this time more xU1b1 RNA than xU1b2 RNA was made (lane 2), thereby reproducing the transcriptional dominance seen in in vivo-matured eggs and in early embryos (Fig. 4). As with unfertilized eggs, U1 RNA synthesis in in vitro-matured oocytes was detectable only after a lag period of several hours (Fig. 6B, lanes 1 and 2) and the efficiency of transcription was much lower than that of untreated control oocytes (compare lanes 1 and 2 with lanes 3 and 4 and see legend to Fig. 6B). Also, the change in transcription of endogenous DNAs was evident since chromosomal, but not mitochondrial, gene expression was suppressed.

Embryonic U1 RNA transcription in eggs depends on DNA replication Activation of eggs by pricking stimulates the replication of both endogenous and injected DNAs (Gurdon et al. 1969; Harland and Laskey 1980). Since replication increases the amounts of both total DNA and specific templates, we investigated how DNA concentrations affected the transcription of injected xU1b genes in eggs.

The amounts of xU1b RNAs synthesized by 18 hr were dose dependent over a range of injected template from 0.15 to 5 ng DNA, but the ratio between the two RNAs remained unchanged (Fig. 7A). When 25 ng of carrier pBR322 DNA was included to keep the total amount of injected DNA relatively constant, the accumulation of xU1b RNAs still increased in response to higher levels of template (lanes 4–6), indicating that the level of competent template was a limiting feature in determining xU1b transcription in activated eggs.

The presence of carrier pBR322 DNA did significantly increase transcription of injected xU1b genes (Fig. 7A, lanes 1–3); presumably, this was due to titration of non-specific inhibitors of transcription that are present in eggs (Newport and Kirschner 1982b). In some eggs that

Figure 5. Transcriptional dominance of xU1b1 genes with wild-type, truncated, or chimeric 5'-flanking regions in unfertilized eggs. Groups of 10–12 unfertilized eggs were coinjected with [α - 32 P]GTP and the templates indicated (at 5 ng DNA per egg) and incubated for 18–20 hr; total nucleic acids were analyzed as in Fig. 4, but only the regions of the gels containing b1 and b2 U1 RNAs are shown. The templates were wild-type (panel 1) or mutant (panels 2 and 3) xU1b tandem repeats, each of which contained two genes with either different (panels 1 and 2) or identical 5'-flanking regions. The template in panel 4 contained only a single mutant xU1b gene. The other templates were mutant xU1b repeats containing genes with truncated (1_s/1/1) (panel 5) or chimeric (2_s/1/1) promoter regions (panels 6 and 7) in combination with genes wild-type b2 (panels 5 and 6) or wild-type b1 (panel 7) promoter regions.

Figure 6. Expression of injected embryonic genes during progesterone-induced maturation of stage VI oocytes. The synthesis of xU1b RNAs in oocytes injected with the wild-type xU1b repeat DNA (5 ng per oocyte) was examined during (A) and after (B) progesterone-induced maturation. (A) Injected oocytes were labeled for 18 hr in the presence (lane 1) or absence (lane 2) of progesterone (1 μ g/ml of incubation medium) as shown schematically. (B) Preinjected matured oocytes (progesterone treated) (lanes 1 and 2) or control oocytes (lanes 3 and 4) were labeled for 4 hr (lanes 1 and 3) or 20 hr (lanes 2 and 4) as outlined in the diagram below. Total nucleic acids equivalent to three oocytes (A, lanes 1 and 2), or four (B, lanes 1 and 2) or 0.4 (B, lanes 3 and 4) oocytes, respectively, were analyzed as in Fig. 4. Autoradiograms were exposed for 20 hr without screens (A, lanes 1 and 2) or for 48 hr with intensifying screens (B, lanes 1–4).

were very proficient in DNA replication (data not shown), this inhibitory factor apparently could be titrated by injection of as little as 5 ng of DNA (compare Fig. 7B, lanes 3 and 7).

As noted earlier (Fig. 4), xU1b transcripts did not accumulate within the first 6 hr after DNA injection into eggs. Injection of larger amounts of template evidently reduced this lag period (Fig. 7B, compare lanes 2 and 6), indicating that at least part of the lag was needed for synthesis of additional templates. Control experiments (not shown) demonstrated that the lag could also be shortened, but not abolished, by coinjection of nonspecific (pBR322) DNA, indicating that the newly synthesized DNA was titrating transcriptional inhibitors.

Because injection of very large amounts of template DNA did not abolish the lag period (Fig. 7B, lane 5, and data not shown), we tested whether replication of the template was needed for formation of an active transcription complex. Injected eggs were treated either with aphidicolin, which blocks the action of DNA polymerase α (Ikegami et al. 1978), or with cycloheximide, which blocks replication by disrupting the cell cycle (Wasserman and Smith 1978; Harland and Laskey 1980; Miake-Lye et al. 1983). Both compounds inhibited xU1b RNA synthesis completely (Fig. 7C). Moreover, the inhibition occurred even when large amounts of xU1b template were injected (Fig. 7B, lanes 4 and 8; cf. Bendig and Williams 1984). In control experiments (not shown) we found that cycloheximide did not affect xU1b transcription in injected stage VI oocytes. Thus, transcription of injected xU1b genes in activated eggs, but not oocytes, apparently requires ongoing DNA replication.

Discussion

We have demonstrated that the two *X. laevis* embryonic U1 genes (xU1b1 and xU1b2) differ from each other in their abilities to form active transcription complexes after injection into either oocytes or unfertilized eggs. The limiting factor in injected stage VI oocytes interacts with sequences in the proximal promoter region of the xU1b genes, whereas the limiting factor in injected eggs recognizes more distal sequences, probably corresponding to the enhancer. Furthermore, in contrast to oocytes, transcription of injected xU1b genes in activated unfertilized eggs is dependent on DNA replication.

Transcription of exogenous xU1b genes resembles that of endogenous genes The endogenous xU1b genes are transcriptionally quiescent in both stage VI oocytes and unfertilized eggs (Forbes et al. 1984). Nevertheless, exogenous xU1b genes are expressed and, when appropriate amounts of these genes are injected, the ratios of the levels of the two xU1b RNAs resemble those observed in vivo in related cell types. For example, equal amounts of xU1b1 and xU1b2 RNAs accumulate in stage VI oocytes that are injected with nonsaturating amounts of xU1b repeat DNA (i.e., 10–100 pg); correspondingly, the two embryonic U1 RNAs accumulate in a 1:1 ratio in pre-tellogenic oocytes (Lund and Dahlberg, this issue). Likewise the ~5:1 ratio of accumulated xU1b1 and xU1b2 RNAs in injected eggs closely resembles the ratio of these RNAs synthesized normally at MBT (Forbes et al. 1984; Lund and Dahlberg, this issue).

Injection of relatively large amounts of the xU1b repeat DNA (with one copy each of the xU1b1 and xU1b2

Figure 7. Expression of xU1b genes in unfertilized eggs depends on DNA concentration and replication. (A) Analyses of xU1b RNAs from eggs injected with 1.5 μ Ci of [α - 32 P]GTP and 0.15 ng (lanes 1 and 4), 1 ng (lanes 2 and 5) or 5 ng (lanes 3 and 6) of wild-type xU1b repeat DNA (1/1/1:2/2/2) alone (lanes 1–3) or plus 25 ng of pBR322 DNA (lanes 4–6). Incubation was for 18–20 hr and nucleic acids equivalent to three eggs were analyzed as in Figs. 4 and 5, except the autoradiograms were exposed for 10 days without intensifying screens. (B) Analyses of xU1b RNAs from eggs injected with 5 ng (lanes 1–4) or 25 ng (lanes 5–8) of wild-type xU1b repeat DNA (1/1/1:2/2/2) per egg. Injected eggs were incubated in the absence (lanes 1–3 and 5–7) or presence (lanes 4 and 8) of cycloheximide (100 μ g/ml of medium) for the indicated times. (C) Analyses of xU1b RNAs from eggs injected with 5 ng of wild-type xU1b repeat DNA (1/1/1:2/2/2) per egg and incubated for 18–20 hr in the absence (lane 1) or presence of cycloheximide (100 μ g/ml of medium) (lane 2) or aphidicolin (20 μ g/ml of medium) (lane 3).

genes) appears to saturate the transcriptional capacity of oocytes, as evidenced by the competition between xU1b1 and xU1b2 genes (Figs. 2 and 3). Under these conditions, the ratio of xU1b RNAs reflects not gene dosage (1:1), but the ability of the two embryonic genes to compete for a limiting transcription factor (cf. Westin et al. 1984; Murphy et al. 1987). Judging from the amount of injected DNA required for manifestation of this competition, we infer that the store in stage VI oocytes of the limiting transcription factor would be sufficient to accommodate all of the xU1b genes present in the embryo at the onset of endogenous snRNA transcription at MBT (Fig. 3). We recognize that there is no evidence that the concentration of this factor is the same in oocytes and at MBT.

Injected U1 RNA genes are very efficiently transcribed in oocytes The difference in template activities between the *X. laevis* xU1b genes and the human U1 gene in injected oocytes (Fig. 3) indicates that U1 transcription factors are, at least to some extent, species specific. Preliminary studies using human–frog chimeric U1 genes suggest that the proximal region of the 5′-flanking sequences (position –1 to –200) is responsible for some of this difference in promoter strength (M. Schimerlik and E. Lund, unpubl.).

Irrespective of whether the snRNA genes are homologous (i.e., *X. laevis*) or heterologous (i.e., human or mouse), U1 and U2 RNA genes are by far the most active RNA polymerase II transcription units yet observed in injected oocytes or eggs (for a comparison with other genes, see review by Gurdon and Melton 1981; Bendig

and Williams 1984). Under favorable conditions (i.e., in injected oocytes), the *X. laevis* embryonic U1 genes produce an average of 20–40 transcripts per gene per hour (Fig. 3), and are thus transcribed with an efficiency comparable to that of injected *X. laevis* 5S RNA and tRNA genes (Gurdon and Melton 1981); the actual rate of U1 RNA synthesis per gene is probably higher since not all of the injected genes would be expected to form active transcription complexes (Gargiulo et al. 1983). The fidelity of transcription, as measured by the percentage of U1 transcripts with correct 5′ and 3′ ends (Lund et al. 1984; Skuzeski et al. 1984), is also much higher than that of most other RNA polymerase II genes (Wickens et al. 1980; Bendig and Williams 1984).

Inactivation of U1 RNA transcription factors during oocyte maturation The striking differences between U1 gene transcription in oocytes and eggs, both in terms of the overall level of transcription and in the ratio of accumulated products, could result from any of several changes that occur upon maturation of oocytes. For example, some transcription factors may be inactivated or destroyed, as is the case with 5S rRNA-specific transcription factor (TFIIIA) (Shastri et al. 1984); selective inactivation, or loss, of a previously abundant factor that binds to the enhancer region would account for our observations. Alternatively, after nuclear breakdown the transcription factors and template would be in a different environment, and at different concentrations. This change in conditions might alter the efficiencies or specificities of factor–template interactions. We note that the same results were obtained both with templates

that had been injected into the nucleus prior to in vitro maturation and with templates injected directly into eggs; thus, the effects that we are measuring are not simply a result of injecting DNA into a cell that has no nucleus.

It is unclear what is responsible for the lag in transcription of xU1b genes after activation of eggs. Although synthesis of additional amounts of template and of total DNA takes place during this period, other events related to the maintenance of the cell cycle clock are also occurring (for example, see Miake-Lye et al. 1983; Newport and Kirschner 1984). Thus, the lag may reflect the time necessary to activate or reactivate one or more transcription factors. In that regard it may well be analogous to the period prior to MBT, when embryos are transcriptionally inactive (Newport and Kirschner 1982a).

Coupling of transcription and DNA replication in injected eggs The stimulation of injected xU1b genes in unfertilized eggs by nonspecific DNA is consistent with the notion that inhibitory factors (e.g., histones or nucleases) must be titrated out (or inactivated) before transcription from chromatin can occur (Newport and Kirschner 1982b). However, transcription also appears to require the act of DNA replication because regardless of the amount of carrier DNA injected, aphidicolin and cycloheximide block U1 RNA synthesis. This requirement is probably not specific to snRNA genes, since similar results have been reported for transcription of the *X. laevis* α - and β -globin genes and the herpes simplex virus thymidine kinase gene in injected unfertilized eggs (Bendig and Williams 1984).

The reasons for the dependence of RNA polymerase II transcription on DNA replication remains unclear. Replication may mediate necessary structural changes in the template DNA or chromatin, for example, to allow the exchange of the postulated general repressor of transcription (Newport and Kirschner 1982b) with gene-specific transcription factors. Alternatively, the effect of replication might be indirect if expression of injected RNA polymerase II genes were coupled to the formation of nuclear structures (Forbes et al. 1983a).

Materials and methods

Adult female *Xenopus laevis* were purchased from Xenopus I (Ann Arbor, Michigan). [α - 32 P]GTP was from Amersham Corporation. Restriction enzymes were from Promega Biotec or New England Biolabs. Cycloheximide and aphidicolin were from Sigma Corp.

Injection of oocytes and eggs

Preparation and injection of *X. laevis* oocytes were as previously described (Skuzeski et al. 1984; Krol et al. 1985), except that oocytes were centrifuged for 10 min at 750g and 5°C, animal hemisphere up, to facilitate injection into the nuclei. Each oocyte received 0.5 μ Ci of [α - 32 P]GTP and between 0.01 and 10 ng of supercoiled plasmid DNA (as indicated in the figure legends) in a total volume of 20 μ l. Unless otherwise stated, incubation was at 18°C for 18–20 hr in MBS-H [88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO₃, 10 mM HEPES (pH 7.5), 0.8 mM

MgSO₄, 0.3 mM Ca(NO₃)₂, 0.4 mM CaCl₂, 10 μ g/ml penicillin, 10 μ g/ml streptomycin; Gurdon 1976]. Maturation of injected oocytes was induced by incubation with progesterone (1 μ g/ml of medium) for 15–18 hr, and matured oocytes were dissected individually to score for germinal vesicle breakdown (Gurdon 1967).

X. laevis eggs were obtained by injecting mature females with human chorionic gonadotropin (500–700 Units) 15–20 hr prior to egg collection. Eggs were laid in MMR (0.1 M NaCl, 2 mM KCl, 1 mM MgSO₄, 2 mM CaCl₂, 5 mM HEPES, 0.1 mM EDTA (pH 7.8), 100 μ g/ml penicillin, 50 μ g/ml streptomycin; Newport and Kirschner 1982a) and immediately dejected by treatment with 2–3% cysteine (pH 7.8) for about 5 min at room temperature. After rinsing several times with MMR, the eggs were transferred to injection medium, MMR with 5% Ficoll (Newport and Kirschner 1982a). Each egg was injected with 1.0 μ Ci of [α - 32 P]GTP and varying amounts of template DNA (as indicated in the figure legends) in a total volume of 20 μ l. Incubation was for 3–20 hr (as indicated) at 18°C. For inhibition of DNA replication, injected eggs were incubated in medium containing 100 μ g/ml of cycloheximide or 20 μ g/ml of aphidicolin.

Analysis of RNA and DNA synthesis

Total nucleic acids were extracted from individual or pooled oocytes or eggs and the accumulated transcripts were analyzed as described previously by electrophoresis in partially denaturing gels [containing 12% (30:0.8) polyacrylamide, 7 M urea, and 1 $\times$ TEB (85 mM Tris-borate, pH 8.3, 2 mM EDTA) (Krol et al. 1985)] or native gels [15% (19:1), 1/2 $\times$ TEB] (Forbes et al. 1984). Autoradiography of the wet gels was for 20–240 hr without, or for 24–48 hr with, intensifying screens.

The number of U1 RNA molecules synthesized per oocyte (Fig. 3) was determined from the known specific activity of the [α - 32 P]GTP precursor and the amount of radioactivity incorporated into newly synthesized U1 RNAs. The radioactivity of gel bits containing the labeled U1 RNAs was determined by Cerenkov counting and generally ranged from 0.2×10^3 to 2×10^3 dpm of U1 RNA synthesized per oocyte in 20 hr. The specific activity of the incorporated [32 P]GTP was 1.1×10^6 dpm per 250 pmoles (0.5 μ Ci injected at ~ 1.25 pmoles plus ~ 250 pmoles of endogenous GTP) or about 4×10^3 dpm per pmole of GTP. Thus, 1000 dpm of U1 RNA corresponds to 0.25 pmoles of GTP or, assuming 40 residues of GMP per U1 RNA, approximately 6 fmoles ($\sim 3.6 \times 10^9$ molecules) of U1 RNA. Similarly, the number of U1 genes injected was calculated to be 1.5×10^8 copies of each xU1b gene per 1 ng of xU1b repeat DNA, which has a molecular mass of 4×10^6 daltons.

DNA synthesis in injected eggs (data not shown) was monitored by agarose gel electrophoresis of RNase-treated total nucleic acids.

Construction of DNA templates

The full-length *X. laevis* embryonic U1 repeat DNA containing both of the xU1b genes, pX1U1b(1/1/1/2/2/2) [formerly pX1U1-AB; Fig. 1A], and the two *Pst*I subclones containing the separate xU1b genes, pX1U1b(1/1/1) and pX1U1b(2/2/2) [formerly pX1U1-A(P) and pX1U1-B(P), respectively] have been described previously (Krol et al. 1985). Chimeric xU1b genes, having the 5'-flanking region of one gene and the coding region of the other gene, were constructed by cleavage of pX1U1b(1/1/1) and pX1U1b(2/2/2) DNAs with *Bcl*II (at position +27 of the U1 coding region) and *Bam*HI (at position 375 of pBR322) and purification of the resulting two *Bcl*II–*Bam*HI fragments of each DNA by preparative agarose gel electrophoresis. The smaller

fragment from one clone was then religated to the larger fragment from the other clone, and vice versa, to generate pX1U1b(1/1/2) and pX1U1b(2/2/1) (cf. Fig. 1B).

To generate xU1b genes with chimeric promoter regions, the truncated xU1b genes pX1U1b(1_s/1/1) and pX1U1b(2_s/2/2) [formerly pX1U1-A(SP) and pX1U1-B(RP), respectively] were used. In both clones, the restriction enzyme cleavage site located immediately downstream of the enhancer region (i.e., the *Hind*III site at position -222 of the xU1b1 gene and the *Mlu*I site at position -219 of the xU1b2 gene; cf. Fig. 1C) was converted to an *Xho*I site by insertion of an *Xho*I linker (CCTCGAGG) (Maniatis et al. 1982). The enhancer regions between xU1b genes were switched by exchange of *Xho*I-*Bam*HI fragments, as outlined above for the exchange of entire 5'-flanking regions.

Reconstruction of mutant xU1b gene repeat units, containing various combinations of wild-type and chimeric genes, was carried out according to the scheme devised by Krol et al. (1985) for the reconstruction of the full-length wild-type repeat unit from the two xU1b subclones.

Preparation of DNAs for injection

All plasmids to be used as templates for transcription analyses were propagated in strain CAG 1574, whereas plasmids to be used for constructions involving cleavage with *Bcl*I were grown in strain GM 2163 (*dam*⁻, *dcm*⁻). Plasmid DNAs were prepared by the alkaline lysis method (Birnboim and Doly 1979), and supercoiled DNAs were collected after one round of CsCl-ethidium bromide density gradient centrifugation. Ethidium bromide was removed by extraction with isopropyl alcohol, and the DNAs were precipitated directly from the CsCl solutions by addition of 3 volumes of 70% ethanol. The recovered DNA was resuspended in 0.25 M NaOAc and reprecipitated with 2 volumes of 96% ethanol. The final pellet of DNA was dissolved in the appropriate volume of injection buffer containing 10 mM Tris (pH 7.6), 1 mM EDTA.

Acknowledgments

We thank H. Neuman deVegvar for setting up our oocyte injection system, M. Wickens for useful discussions, and P. Tregloan for typing the manuscript. This work was supported by National Institutes of Health grant GM 30220 and National Science Foundation grant PCM 83-09618 to J.E.D. and E.L.

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Genes Dev. 1987, 1:

Access the most recent version at doi:[10.1101/gad.1.1.47](https://doi.org/10.1101/gad.1.1.47)

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