Cell, Vol. 38, 681–689, October 1984, Copyright © 1984 by MIT 0092-8674/84/100681-09 $02.00/0

Differential Expression of Multiple U1 Small Nuclear RNAs in Oocytes and Embryos of Xenopus laevis

Douglass J. Forbes,*† Marc W. Kirschner,* Daniel Caput,‡ James E. Dahlberg,§ and Elsebet Lund§
*Department of Biochemistry and Biophysics
University of California
San Francisco, California 94143
‡Chiron Corporation
Emeryville, California 94608
§Department of Physiological Chemistry
University of Wisconsin
Madison, Wisconsin 53706

Summary

The small nuclear RNA, U1, is a highly conserved, 165 nucleotide long RNA which has been implicated in the processing of mRNA precursors. We present evidence that in the amphibian X. laevis there exist at least seven species of U1 RNA, which differ in sequence but not in length. Strikingly, these RNAs are not coordinately expressed. Two of the U1 RNAs are the predominant U1 species transcribed in the late blastula–early gastrula stages of Xenopus embryogenesis. These two RNAs, designated xU1a and xU1b, are not synthesized in significant amounts in stage 6 oocytes; a different set of U1 RNAs are expressed during late oogenesis. In a Xenopus cultured cell line, all of the U1 RNA species are expressed. Possible functions and developmental significance of these multiple U1 RNA species are discussed.

Introduction

The small nuclear RNA, U1 snRNA, ubiquitous to eucaryotes from insects to mammals, is highly conserved in sequence (for review see Busch et al., 1982). In most organisms, U1 RNA appears to be homogeneous in sequence, although mouse has two slighly different U1 variants (Lerner and Steitz, 1979). Because of the complementarity between the highly conserved 5′ terminal sequence of U1 RNAs and the 5′ intron–exon junctions of pre-mRNAs, it was proposed that U1 RNA was involved in the splicing of introns from messenger RNA precursors (Lerner et al., 1980; Rogers and Wall, 1980; Mount and Steitz, 1981). Recent experimental evidence supporting this hypothesis includes the finding that isolated particles containing U1 RNA and its associated proteins (U1 snRNPs), when mixed with a synthetic messenger RNA precursor, specifically bind to and protect the 5′ intron–exon junction from RNAase digestion (Lerner and Steitz, 1979; Mount et al., 1983). Furthermore, monoclonal and polyclonal antibodies against U1 RNP particles block RNA processing in vitro (Yang et al., 1981; Padgett et al., 1983).

The uniformity of the U1 RNA 5′ terminal sequence between species would imply an essential role in RNA processing. It is possible that structures other than the 5′ end of the U1 molecule, such as the remainder of the molecule or the specific U1 snRNP proteins, might also influence mRNA precursor splicing (Ohshima et al., 1981). Specifically, differences in these structures might allow differential RNA splicing. The use of alternative 3′ splice sites to control gene expression has been observed in several instances in the production of both viral and cellular gene products (see review by Ziff, 1980; Tyler et al., 1981, and references therein; Schwarzbauer et al., 1983).

The expression of U1 RNA is turned on at a high rate at the onset of Xenopus embryonic transcription (the 4000 cell embryo; Forbes et al., 1983). We reasoned that the resulting increase in U1 RNA might allow for altered patterns of Xenopus gene expression during development. We have, therefore, analyzed the U1 RNAs that are present before and after this transition as a further step in determining possible roles for U1 RNA.

Using a nondenaturing polyacrylamide gel, we can distinguish at least seven different species of U1 RNA in a Xenopus cultured cell line, both on the basis of electrophoretic mobility in the nondenaturing gel and RNAase T1 fingerprint analysis. In addition, we have obtained three U1 cDNA clones which differ in sequence, using late gastrula U1 RNA as a cDNA template. This heterogeneity of Xenopus laevis U1 RNAs has been used to determine whether the U1 RNA species are expressed coordinately. We report here that differential expression is observed; two of the U1 RNA variants are the primary U1 species expressed when transcription is turned on in early embryogenesis, whereas a different set of at least four U1 RNAs are synthesized during late oogenesis. All species appear to be expressed in cultured cells. In related work, we have found that the two embryonic U1 RNAs are encoded by tandemly repeated U1 RNA genes which represent the majority of U1 genes in Xenopus (Lund et al., submitted).

Results

U1 RNA from Xenopus Cultured Cells Consists of at Least Seven RNA Species

When the U1 RNA from a cultured Xenopus laevis kidney-derived cell line was prepared by hybrid selection and examined by electrophoresis in a nondenaturing gel, a heterogeneous group of bands was observed. Six bands of U1 RNA were resolved in this gel (Figure 1A, lane 1). These are designated I, II, III, IV, V, and VI. Hybrid-selected U1 RNA isolated from cultured human fibroblasts was included for reference and displayed no heterogeneity (lane 2).

The multiple Xenopus laevis U1 RNA bands in Figure 1A could represent different lengths of U1 RNA, either generated naturally by cleavage of a single U1 RNA species or by degradation during RNA isolation. Differences in the lengths of the Xenopus U1 RNAs were ruled out by electrophoresis of the same samples in a fully denaturing (sequencing) polyacrylamide gel. All of the U1 RNAs, from

† Present address: Department of Biology, University of California, San Diego, California 92093.

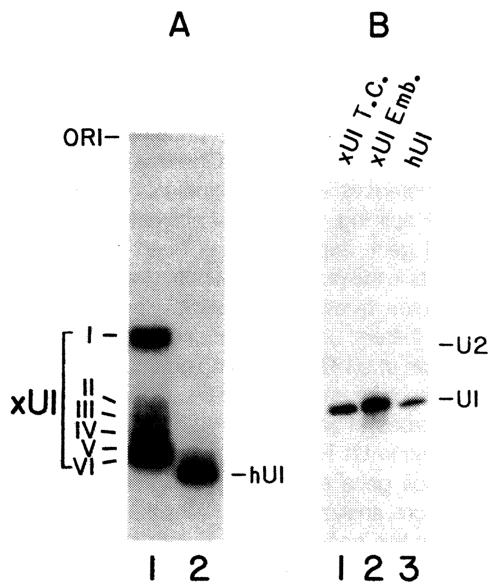

Figure 1. Xenopus U1 RNAs Are Heterogeneous in Sequence but Not in Length

Purified Xenopus and human U1 RNAs were compared by polyacrylamide gel electrophoresis under nondenaturing (A) and denaturing (B) conditions. ^{32}P-labeled U1 RNAs were isolated from the total RNAs of a Xenopus kidney-derived cultured cell line or cultured human fibroblasts by hybrid selection using filter-bound human U1 DNA. ^{32}P-labeled Xenopus embryonic U1 RNAs were obtained as described in the legend to Figure 5A. (A) Autoradiographic analyses of U1 RNAs from Xenopus (lane 1) or human (lane 2) cultured cells in a 15% (19:1) nondenaturing polyacrylamide gel. (B) Analysis in an 8% (30:0.8) 7 M urea-containing (sequencing) gel of U1 RNAs from Xenopus (lane 1) and human (lane 3) cultured cells and from Xenopus gastrula stage embryos (lane 2). The position of Xenopus U2 RNA is indicated as a size marker. Autoradiograms are shown.

Gel-purified U1 RNAs of bands I II, V, and VI showed the same relative gel mobilities upon reelectrophoresis in similar 15% gels, whereas the U1 RNAs of bands III and IV displayed more variation in electrophoretic mobility; i.e., the separation between bands II, III, IV, and V were frequently less distinct (see for example Figure 5A, lane 1 and Figure 6, lanes 2 and 7).

both the Xenopus laevis and the human cultured cells, were identical in size, as shown in Figure 1B (lanes 1 and 3). Xenopus laevis embryonic U1 RNA from gastrula stage embryos migrated as a slightly broader band on this denaturing gel (Figure 1B, lane 2), reflecting slight length heterogeneity at the 3′ ends (see fingerprint analysis below).

In order to determine whether the individual Xenopus U1 RNA bands seen in nondenaturing gels indicated the existence of different U1 RNA species rather than conformational isomers of a single U1 RNA species, we eluted and fingerprinted the U1 RNA in each band of Figure 1A, lane 1. Representative fingerprints of the U1 RNAs from four bands (I, II, III, and VI) are shown in Figures 2A–D. The identification of oligonucleotides was done both by comparison to the fingerprint of human U1 (Branlant et al., 1981; Nohga et al., 1981) and by using the sequence data obtained for Xenopus U1 cDNA clones (see below). Differences were observed between each of the U1 RNA fingerprints. A clear example of these heterogeneities is the presence of an oligonucleotide 11 residues long in the fingerprint of RNAs of bands III and VI (h; open arrow in Figures 2C and 2D), and the replacement of that 11-mer with a 5-mer and 6-mer in the RNAs of bands I and II (d and e; solid arrows in Figures 2A and 2B). The 5-mer and 6-mer presumably derive from the substitution of a G residue in the middle of the II-mer sequence, a substitution also supported by sequence data for U1 cDNA clones (see below). Similarly, a 7-mer oligonucleotide (#13) was found in the RNAs of bands I and VI (Figures 2A and 2D), distinguishing them from the RNAs of bands II and III, which contained a tetramer and trimer (c and #8, respectively). These and other characteristic changes are presented in the schematic figure (Figure 2F) and are summarized in Table 1.

The differences between RNAs, based on the presence or absence of particular oligonucleotides, allowed us to conclude that Xenopus cultured cells synthesize at least seven different U1 RNAs. In several instances, an individual band appeared to contain a single unique species of RNA (i.e., bands III and VI contained single RNA species which we designated xU1e and xU1g, respectively; see Table 1). In the RNAs of the other bands, submolar yields of oligonucleotides derived from the same regions of U1 RNA indicated that the band contained two (or more) U1 RNA species. The RNAs could be distinguished by characteristic oligonucleotides. For example, the RNA of band I from cultured cells gave rise to a fingerprint containing the two oligonucleotides, AUCACG (#12; Figure 2A) and AUCAUG (f; Figure 2A). However, embryonic U1 RNA of band I contained only the oligonucleotide AUCACG (#12; Figure 2E). Using such data, we have designated the U1 variant present in embryonic band I as xU1a, and those present in cultured cell band I as xU1a and xU1c. Similarly, band II contained two variants (Figure 2B; see Table 1), the minor species, designated xU1b, which is expressed in embryos (see below) and the major one, designated xU1d, which is expressed in oocytes and cultured cells. Finally, bands IV and V appeared to be mixtures of the RNA species from bands III and VI, as well as a distinct additional U1 variant designated xU1f (fingerprint not shown; see Table 1).

The possibility that the differences in the RNAase T1 fingerprints might be a reflection of the presence of modified bases (e.g., methylated G residues) in some of the U1 RNA species was ruled out by RNAase T2 digestion of RNAs from each of the six bands. No differences in modification were observed (data not shown). Thus we conclude that Xenopus laevis cultured cells express at least seven variants of U1 RNA.

U1 RNAs Present in Embryonic Cells Are Heterogeneous

In order to determine the sequence complexity of embryonic U1 RNAs, cDNA clones were made using total U1 RNA isolated from late gastrula embryos as a template. A total of 17 cDNA clones were isolated in pBR322 and the cDNA inserts of four clones, designated U1cD1 through D4, were transferred into M13 vectors and sequenced (Sanger et al., 1980). As shown in Figure 3, the longest

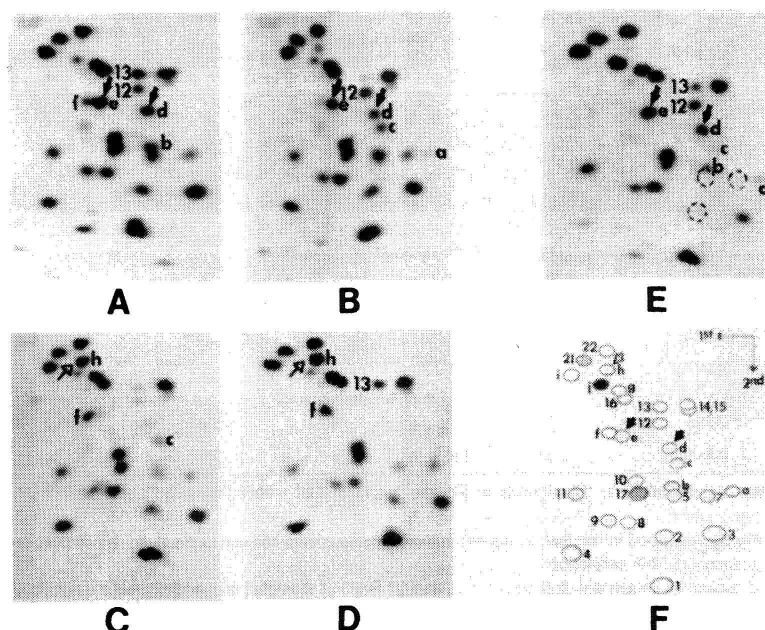

Figure 2. RNAase T_1 Fingerprint Analysis of Somatic and Embryonic U1 RNA Species of Xenopus

^{32}P-labeled, gel-purified U1 RNAs from cultured cells (Figure 1A) or early gastrula stage embryos (Figure 5A) were characterized by two-dimensional RNAase T_1 fingerprint analysis. The fingerprints shown in Figure 2 are of cultured cell U1 RNAs isolated from bands I (A), II (B), III (C), and VI (D) in lane 1 Figure 1A and of total embryonic U1 RNAs (E), corresponding to the sample in lane 3 of Figure 5A. The somatic and embryonic U1 RNAs were labeled with $^{32}PO_4^{3-}$ and α-^{32}P-UTP, respectively. In (E), the positions of oligonucleotides which would not be labeled by α-^{32}P-UTP are indicated by dashed circles. A schematic summary of the Xenopus laevis U1 RNA fingerprints is presented in (F): oligonucleotides that are common to human and Xenopus U1 RNA (see Figure 3) are numbered according to Nohga et al. (1982). Oligonucleotides that are specific to Xenopus U1 RNAs are indicated by letters (a–j) (see Table 1). The hallmark oligonucleotides discussed in the text are the 11-mer labeled h (in [C] and [D]) and the pentamer and hexamer, labeled e and d, respectively (in [A], [B], and [E]); they replace oligonucleotide #19 of human U1 RNA. In Xenopus somatic U1 RNAs the 5′ and 3′ terminal oligonucleotides (#21 [dotted circle] and #17 [cross-hatched circle], respectively, in [F]) are identical to those of human U1 RNA. In embryonic U1 RNAs the majority of the molecules contain extra nucleotide(s) at their 3′ ends as evidenced in (E) by the presence of large amounts of the 3′ overlap oligonucleotide (filled circle labeled j in [F]). Oligonucleotides g ($UUCUCCCAG_{(51)}$) and i ($CAUAAUUUCUG_{(133)}$) replace oligonucleotides #20 and #18 of human U1 RNA, respectively. Oligonucleotide b is a breakdown product which is particularly abundant in U1 RNAs of band I. The first dimension was high-voltage electrophoresis at pH 3.5; the second dimension was homochromatography (Barrell, 1971).

insert extends 146 nucleotides from the 3′ end of the U1 RNA. The sequences (Figure 3) are aligned to display the sequence of the encoded RNA with respect to the human U1 RNA sequence (Branlant et al., 1980). The Xenopus laevis U1 RNA sequences closely resemble that of human U1 RNA, differing at most in eight positions within the regions that could be compared. The differences are evenly distributed between base-paired stems and the open loops of the putative U1 RNA secondary structure (Mount and Steitz, 1981; Branlant et al., 1981); the differences in the cDNA clones are also found in the RNA fingerprints of various U1 RNA species (see legend to Figure 4). Three of the four clones studied differed in sequence from each other; the differences were seen at five sites, positions 63, 79, 108, 115, and 131. These are shown in Figure 4, where the RNA has been folded into a putative secondary structure (Mount and Steitz, 1981).

Clones U1cD1, U1cD2, and U1cD4 all contain a G at position 63. This G residue would, if the RNAs corresponding to these cDNA clones were digested by RNAase T1, give rise to the hallmark pentamer and hexamer oligonucleotides present in the U1 RNAs of bands I and II (oligonucleotides d and e; Figures 2A and 2B). Furthermore, the sequence of clone U1cD1 (and probably U1cD4) is entirely consistent with its having been copied from an xlU1b RNA molecule (Table 1 and data not shown). The nucleotide sequences of clones U1cD2 and U1cD3 suggest that they were copied from minor U1 RNAs present in bands I and IV, respectively. Clearly, at least three different U1 genes exist and are transcribed prior to or during the late gastrula period of embryogenesis.

The U1 RNAs Expressed in Early Embryogenesis Are a Subset of Those in Cultured Cells

We have previously demonstrated (Forbes et al., 1983) that U1 RNA synthesis is turned on at a very high rate at the midblastula stage of development, which is the onset of embryonic transcription (Bachvarova and Davidson, 1966; Newport and Kirschner, 1982). To determine whether all the species of U1 RNA are transcribed in these early stages, or whether there is a differential expression of various U1 RNA genes at this time, we isolated ^{32}P-

Table 1. Characteristic Oligonucleotides of Individual X. laevis U1 RNAs

Tissue[a]	Gel Mobility[a]	U1 RNA Species[b]	RNAase T_1 Oligonucleotides[c] 12 or f		13 or c (+8)		h or d + e		a
Embryonic	Band I	xU1a	+		+			+	
	Band II	xU1b	+			+		+	+
Somatic	Band I	xU1a	+		+			+	
		xU1c		+	+			+	
	Band II	xU1b	+			+		+	(+)
		xU1d	+			+		+	
	Band III	xU1e		+		+	+		
	Band IV	xU1f	+		+		+		
		(xU1g)							
	Band V	(xU1e)							
		(xU1g)							
	Band VI	xU1g		+	+		+		
Oocyte	Bands III–VI	(Mixture)	(+)	+	+	+	(+)	+	

[a] The sources of somatic, embryonic, and oocyte U1 RNAs are described in the legends to Figures 1 and 5. Gel mobility refers to analyses in 15% nondenaturing polyacrylamide gels (see Figure 1A).
[b] The different species of U1 RNAs were designated arbitrarily as described in the text. In agreement with previous studies (see Lund et al., 1983) prefix x stands for Xenopus and lower case letters indicate different primary U1 RNA sequences.
[c] RNAase T_1 oligonucleotides refer to labeling in Figure 2. The nucleotide sequences and positions (within U1 RNA) of individual oligonucleotides are deduced to be the following (see Figure 3): a, CCG_{80}; c, $AAAG_{115}$; 8, UCG_{118}; 13, $AAACUCG_{118}$; d, $CUCAG_{63}$; e, $CCAUUG_{69}$; h, $CUCAUCCAUUG_{69}$; f, $AUCAUG_{35}$; 12, $AUCACG_{35}$. The assignments of sequences to these oligonucleotides are discussed in the text and were confirmed by redigestion with pancreatic or T_2 RNAases. + indicates molar or half molar amounts, whereas (+) indicates a lesser amount of the oligonucleotide.

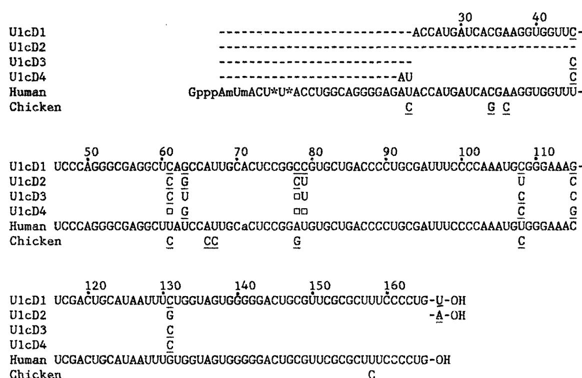

Figure 3. Nucleotide Sequences of Four Xenopus laevis U1 cDNA Clones

cDNA clones to U1 RNAs from gastrula stage embryos were isolated and sequenced as described in Experimental Procedures. The nucleotide sequences of four U1cDNAs (as RNAs), are shown in the upper four lines, aligned with respect to the sequence of human U1 RNA (Branlant et al., 1980). U1 RNA sequences not included in the cDNA clones are designated by dashes (– – –). Certain residues in clones U1cD3 and D4 could not be precisely identified because of difficulties caused by long runs of poly (C) and poly (A) added in the cloning protocol; these are indicated by open squares (□). Two cDNA clones (D1 and D2) were derived from a U1 RNA molecule containing an additional residue at the 3′ end. Nucleotide sequences different from the human U1 sequence are underlined. The A_m residue at position 71 of human U1 RNA is indicated by (a). For comparison the sequence of chicken U1 RNA is presented in the bottom line, showing only the residues in which this sequence differs from that of human U1 RNA (Branlant et al., 1980).

labeled U1 RNAs synthesized during the first 10–11 hr after fertilization and analyzed them by electrophoresis in nondenaturing polyacrylamide gels, as above.

As shown in Figure 5A, the embryonic U1 RNAs constituted a subset of the cultured cell RNA and were composed primarily of RNA species that comigrated with bands I and II of cultured cell U1 RNA. An RNAase T1 fingerprint of the mixture of the two embryonic RNA species is presented in Figure 2E. The major component corresponded to xU1a RNA seen in cultured cells, whereas the minor component corresponded to the less abundant xU1b RNA (see Table 1). These assignments were confirmed by RNAase T1 fingerprinting of the individual embryonic U1 RNAs, xU1a and xU1b (data not shown; Lund et al., submitted). Thus the early Xenopus embryo expresses primarily two of the seven U1 RNAs expressed in cultured cells.

A Different Subset of U1 RNAs Is Expressed during Late Oogenesis

The U1 RNAs synthesized in fully grown oocytes differed from the embryonic U1 RNAs and constituted another subset of those expressed in cultured cells (compare Figure 5B, lanes 1 and 2 with Figure 5A, lanes 1–3). The major U1 RNAs labeled in stage 6 oocytes (Figure 5B) had electrophoretic mobilities corresponding to the cultured cell RNAs of bands III through VI, clearly differing from embryonic xU1a RNA (Figure 5B, lane 2). Because of the difficulty involved in obtaining large quantities of labeled oocyte-specific RNAs, it was necessary to do fingerprint analysis on these RNAs as a mixture of types rather than as individual gel-purified RNAs. Nevertheless, this analysis confirmed that the majority of the U1 RNAs synthesized in stage 6 oocytes corresponded to xU1e, xU1f, and xU1g (fingerprint not shown). We conclude that the embryonic and the newly synthesized late oocyte U1 RNAs constitute two separate classes of U1 RNAs, both of which are expressed in cultured cells.

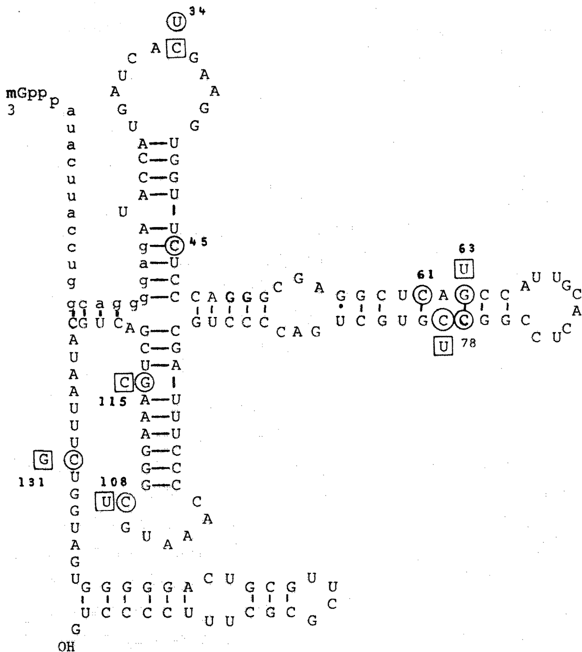

Figure 4. Proposed Secondary Structure of Xenopus U1 RNA Showing Sequence Differences

The Xenopus U1 RNA sequence derived from analysis of clone U1cD1 is shown. The RNA is arranged in the secondary structure proposed for human U1 RNA by Mount and Steitz (1981). Those nucleotides at the 5′ end that were not included in clone U1cD1 are shown in small letters and were taken from the known human sequence and the sequence of Xenopus genomic U1 RNA clones (Krol, Lund, and Dahlberg, unpublished results). The circled residues are nucleotides that differ from the human U1 RNA sequence. The boxed residues indicate nucleotide changes found in other Xenopus U1 cDNA clones. The sequence heterogeneities at positions 34, 61, 63, 79, and 115 are also seen in the RNA fingerprints of different U1 RNA species and those at positions 63 and 115 are also apparent in restriction site heterogeneity in genomic U1 clones (Lund et al., submitted; unpublished results).

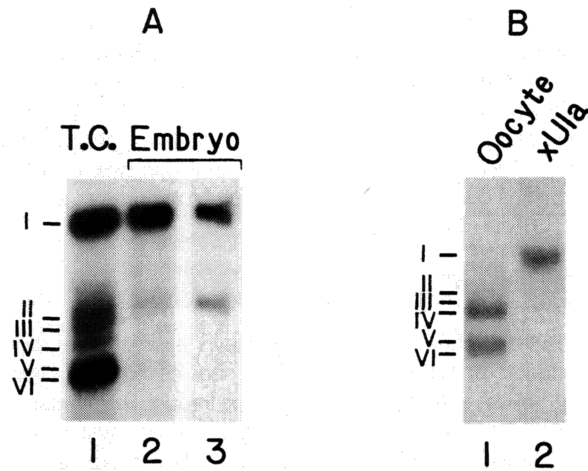

Figure 5. Differential Expression of Multiple U1 RNA Species in Early Embryos and Stage 6 Oocytes

Xenopus U1 RNAs isolated from gastrula stage embryos (A) or stage 6 oocytes (B) were compared to cultured cell U1 RNAs by electrophoresis in 15% polyacrylamide gels as in Figure 1A. ^{32}P-labeled U1 RNAs were purified from total RNAs either by hybrid selection (oocytes and cultured cells) or by gel electrophoresis (embryos). (A) Analysis of somatic U1 RNAs (lane 1) and embryonic U1 RNAs from two independent experiments isolated 11 hr (lane 2) or 10 hr (lane 3) after fertilization. The embryonic U1 RNAs, designated xU1a and xU1b as described in the text, were further characterized by RNAase T_1 fingerprinting (Figure 2E). (B) Analysis of U1 RNAs from stage V–VI oocytes (lane 1). Purified embryonic xU1a RNA (lane 2) was included as a marker. Autoradiograms are shown. The RNAs of the major bands in lane 1 were designated xU1e, xU1f, and xU1g on the basis of RNAase T_1 fingerprint analysis.

Patterns of U1 RNA Synthesis during Early Embryogenesis

In order to determine when the U1 RNA species that are present in oocytes and cultured cells (xU1c-f) are first observable in Xenopus embryogenesis, we examined newly synthesized U1 RNA from several embryonic stages. Embryos were allowed to develop for 6½ hr until the onset of transcription; at that time ^{32}P-orthophosphate was injected into the blastocoel cavity. At varying times after this injection, embryos were lysed and the RNA analyzed as in Figure 5. Figure 6 shows the U1-sized RNA from embryos at 9 hr (lane 3), 11 hr (lane 4), 13½ hr (lane 5), and 23½ hr (lane 6) after fertilization. Samples containing U1 RNA from Xenopus cultured cells (lanes 2 and 7), as well as other purified RNAs, were included as markers. The synthesis of additional U1 RNAs (bands III–VI) was first observed at 13½ hr (late gastrula; lane 5). Although these additional bands were more prominent in the 23½ hr embryonic RNA sample (lane 6), it appeared that the two embryonic U1 RNAs, xU1a and xU1b, continued to be the predominant species synthesized, even 12–14 hr after the onset of embryonic transcription. Expression of the 5.8S

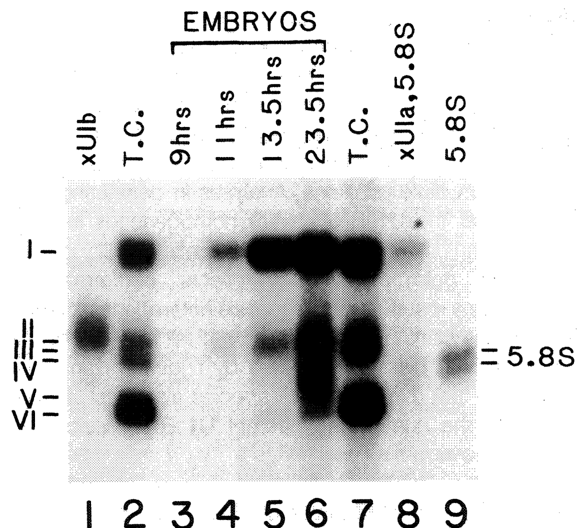

Figure 6. Developmental Regulation of U1 RNA Expression

Polyacrylamide gel analysis of newly synthesized U1 RNAs isolated from different stages of Xenopus development. The synthesis of U1 RNAs during early embryogenesis was monitored by injection of $^{32}PO_4^{3-}$ into the blastocoel cavity of 6½ hr old embryos and subsequent harvesting of embryos at 9 hr (lane 3), 11 hr (lane 4), 13½ hr (lane 5), and 23½ hr (lane 6) after fertilization. Gel-purified U1 RNAs were displayed on a 15% gel along with markers of ^{32}P-labeled xU1b RNA (lane 1), cultured cell U1 RNAs (lanes 2 and 7), xU1a RNA + 5.8S RNA (lane 8), and 5.8S RNA (lane 9). The xU1a and xU1b RNAs were obtained from Xenopus oocytes injected with cloned copies of the embryonic U1 RNA genes (Lund et al., submitted).

ribosomal RNA genes is also observed in the 23½ hr old embryos, indicating that despite the injection, the developmental program proceeded correctly (5.8S genes normally turn on around 13 hr of Xenopus development; Brown and Littna, 1964; Busby and Reeder, 1983). Northern blot analysis of total RNA from oocytes, embryos, and adult liver also gave results consistent with new U1 RNA species being synthesized at the gastrula stage of embryogenesis (data not shown).

Discussion

We have shown that Xenopus laevis expresses multiple species of U1 RNA. Seven U1 species can be distinguished in a Xenopus kidney cell line through the combined use of a nondenaturing polyacrylamide gel system and fingerprint analysis. Early Xenopus embryos express two of these species, xU1a and xU1b, whereas fully grown oocytes express a second subset of the U1 RNA species.

It is unlikely that preferential degradation explains the apparent lack of synthesis of xU1a and xU1b RNAs in the late oocyte; these embryonic U1 RNAs accumulate in large amounts when genes encoding them are injected into the oocyte (Lund et al., submitted). Moreover, Drosophila and mouse U1 RNAs are stable when injected into fertilized eggs (Forbes et al., 1983), as is human U1 RNA when injected into oocytes (De Robertis et al., 1982). Therefore, it is probable that the differential expression of the U1 gene occurs at the transcriptional level.

Xenopus laevis U1 RNAs Are Heterogeneous in Sequence

The existence of sequence variants of Xenopus U1 RNAs was documented in two ways: First, RNAase T1 fingerprints of U1 RNAs isolated from individual bands in the nondenaturing gel were clearly different. Second, the sequences of cDNA clones made to embryonic U1 RNAs differed in multiple positions. Analyses in denaturing gels showed that the U1 RNAs were homogeneous in size; therefore, the differences in electrophoretic mobility in nondenaturing gels presumably reflects conformational changes as a result of this sequence heterogeneity. Since some of the cDNA sequences do not fall into any of the major U1 RNA classes predicted by fingerprint analysis, we believe that minor U1 RNA species also exist, further increasing the number of different U1 RNA species in Xenopus laevis.

The Expression of Multiple U1 RNA Genes Is Developmentally Controlled

The expression of U1 RNA in oocytes and embryos has been studied previously (Forbes et al., 1983). U1 RNAs are synthesized throughout oogenesis (Zeller et al., 1983; R. Gelfand and L. D. Smith, personal communication) and the mature Xenopus laevis oocyte contains stored U1 RNA molecules in amounts sufficient for 4000–8000 nuclei (Forbes et al., 1983). During the first 12 cleavages of early Xenopus embryogenesis, no detectable U1 RNA transcription occurs (Forbes et al., 1983), consistent with the finding that all embryonic transcription is turned off during these cleavages (Brown and Littna, 1964; Bachvarova and Davidson, 1966; Newport and Kirschner, 1982). At the onset of embryonic transcription (the midblastula transition), the major RNA polymerase II transcripts are the small nuclear RNAs, U1, U2, U4, U5, and U6. Approximately 2×10^5 U1 RNA molecules are transcribed per cell per hour and the rate of transcription of these RNAs at this stage appears to be about 10- to 20-fold higher than in Xenopus cultured cells (Forbes et al., 1983). Thus U1 RNA genes are expressed in oocytes, shut off in early cleavage stage embryos, and very actively transcribed at the onset of embryonic transcription in the 4000 cell embryo.

As described here, different sets of U1 RNA genes are expressed in stage 6 oocytes and early embryos. Specifically, we have shown that at the onset of embryonic transcription, the vast majority of U1 RNAs synthesized consists of two species, xU1a and xU1b, which we have called the embryonic U1 RNAs. We have found that these embryonic U1 RNAs are encoded by genes that are tandemly arranged and reiterated approximately 500-fold per haploid genome (Lund et al., submitted). In contrast, the U1 RNAs synthesized in late oocytes consist of multiple species which appear to be encoded by the minor families of dispersed U1 RNA genes (Lund et al., submitted). (We note that the U1 RNAs synthesized in stage 6 oocytes may not be representative of the accumulated RNAs in these cells.)

Possible Models for the Control of Embryonic U1 RNA Gene Expression

The abundant U1 RNA synthesis that is observed in embryogenesis could be explained in two ways. First, it is possible that there is a preferential activation of particular RNA polymerase II transcription units at the onset of embryonic transcription. This would allow for the expression of specific small nuclear RNA genes such as the embryonic xU1a and xU1b genes and the major U2 RNA gene family (Mattaj and Zeller, 1983). Alternatively, all snRNA genes transcribed by RNA polymerase II might in fact be turned on. In this case, the abundance of the different U1 RNAs synthesized in the embryo (primarily xU1a and xU1b) would reflect gene dosage: i.e., the 50-fold excess of embryonic genes over each of the other U1 RNA genes. Since the two embryonic U1 RNAs are encoded by equal numbers of genes (Lund et al., submitted), a gene dosage model does not explain the 5- to 7-fold difference between the levels of newly synthesized xU1a and xU1b RNAs, implying other mechanisms of control exist. In any case it is clear that the embryonic U1 RNA genes are not expressed during late oogenesis, but are activated at the 4000 cell stage of embryogenesis.

There may also be a preferential inactivation of the embryonic U1 RNA genes later in development, in order to account for the relatively low levels of xU1a and xU1b RNAs observed in cultured cells and the later embryonic stages. It must be noted, however, that the tissue culture

cells may aberrantly express the various U1 genes. We are at present determining the pattern of expression of the U1 genes in adult tissues. If the tissue culture cell U1 pattern is representative of somatic tissues, it would be necessary to invoke stage-specific regulation of the expression of the highly repeated U1 RNA genes at more than one stage of development.

Differential Control of Highly Reiterated RNA Genes in Xenopus laevis

The pattern of expression of Xenopus U1 RNA is reminiscent of the differential expression of the somatic and oocyte-specific 5S ribosomal RNA genes of Xenopus (Wegnez et al., 1972; Ford and Southern, 1973; Ford and Brown, 1976; Harper et al., 1983). In the case of 5S rRNA, 20,000 tandemly repeated 5S genes are expressed primarily in oogenesis and not in adult tissues, whereas a smaller class of 400 reiterated 5S rRNA genes are expressed at all times (Brownlee et al., 1974; Peterson et al., 1980). At the molecular level, this differential expression of Xenopus 5S RNA genes has recently been related to the abundance of a 5S-specific positive transcription factor and to the maintenance of stably active or inactive transcription complexes in the chromatin (Wakefield and Gurdon, 1983; Wormington et al., 1983).

In the case of U1 RNA, it appears that the abundant class of genes is expressed in early embryogenesis but not late in oogenesis. It is conceivable that a factor which is required for transcription of embryonic genes is produced or activated for the first time at the onset of transcription in embryos. This factor would either be absent or inactive in stage 6 oocytes. Alternatively, the late oocyte may contain an inhibitor of transcription of the xlU1a and xlU1b genes, which is later destoryed or diluted out in the 12 cell divisions preceding the onset of transcription in the embryo.

What Is the Functional Significance of the Developmental Control of U1 RNA Gene Expression?

The differential expression of the highly reiterated U1 RNA genes may reflect the requirement for high levels of a particular variant of U1 RNA, or simply large amounts of U1 RNA in general, at a particular developmental stage. For example, the transcription of a large number of embryonic U1 RNA genes may be required simply to supply a full complement of U1 RNAs to all cells. The clustering of many identical transcription units could facilitate simultaneous activation of a large number of genes, as is thought to be the case with the 5S genes (Peterson et al., 1980). If this explanation is correct, then the nucleotide sequence differences between stage 6 oocyte and embryonic U1 RNAs may not be significant, but rather represent neutral positions within the U1 RNA structure.

More interestingly, the stage-specific RNA products may be functionally distinct. If this were the case for U1 RNA, the nucleotide sequence differences between variants could be important for the developmental control of messenger RNA splicing. In the Xenopus U1 RNAs, the nucleotide changes appear to be confined to a few positions in the U1 RNA sequence. No changes appear to be located within the 5′ proximal sequences that are proposed to basepair with the 5′ exon–intron border in pre-messenger RNAs. Thus it is unlikely that the observed changes could directly affect the processing at the 5′ donor splice site. It is, however, possible that the variable nucleotides are important for selection of the 3′ acceptor splice site. This could be accomplished by the incorporation of different species of U1 RNAs into functionally distinct U1 snRNP particles (Kinlaw et al., 1982; Hinterberger et al., 1983). By changing the complement of snRNP proteins, the U1 snRNPs might bind only to specific types of mRNA precursors, or the variant U1 snRNPs might interact differently with other snRNPs, thereby promoting the use of alternative splice sites.

This is the first example of differential control of U1 RNA synthesis. Furthermore, the Xenopus laevis U1 RNA gene system offers a unique opportunity to identify and analyze mechanisms of developmental regulation of RNA polymerase II transcription units. Lastly, the study of differential expression of multiple U1 RNA genes may be quite useful in determining the precise function(s) of U1 RNA.

Experimental Procedures

U1 cDNA Cloning and Sequencing

U1 RNA from late gastrula stage Xenopus embryos was isolated by fractionation of total gastrula RNA on a denaturing 5% polyacrylamide gel containing 7M urea (Forbes et al., 1983). The U1 RNAs were eluted from the gel and used as a template for synthesis of double-stranded cDNA which was then cloned into the plasmid, pBR322, according to Goddard et al. (1983). The U1 cDNA inserts were subcloned into the M13 vectors, Mp8 and Mp9 (Messing and Vieira, 1982), and sequenced by the methods of Sanger et al. (1980).

Isolation of ^{32}P-Labeled Embryonic U1 RNAs

Labeled embryonic RNAs were obtained in two ways: (1) Fertilized dejellied Xenopus eggs were injected with 50 nl (1–2 μCi) of α-^{32}P-rUTP or α-^{32}P-rGTP (in 10 mM potassium phosphate, 0.1 mM EDTA) in the animal pole of the egg (Forbes et al., 1983) and allowed to develop for 10–11 hr. (2) Fertilized, dejellied Xenopus eggs were allowed to develop for 6½ hr (until the onset of embryonic transcription) prior to injection of 4 μCi (50 nl) of $^{32}PO_4^{3}$ into the blastocoel cavity. At varying times after injection (9 to 23½ hr) the embryos were lysed in 100 mM NaCl, 10 mM Tris-HCl (pH 7.5), 1 mM MgCl. For isolation of total nucleic acids an equal volume of 5 M guanidine thiocyanate, 50 mM Tris-HCl (pH 7.5), 10 mM EDTA, 5% 2-mercaptoethanol was added prior to phenol-chloroform extraction. After ethanol precipitation the nucleic acids were fractionated on a denaturing gel (Forbes et al., 1983). The U1 RNA was eluted from the gel and ethanol precipitated before further analyses in a nondenaturing gel or by RNAase T_1 fingerprinting (see below).

Preparation of ^{32}P-Labeled U1 RNAs from Cultured Cells, Ovarian Tissue, and Oocytes

Kidney-derived X. laevis cultured cells (kindly provided by D. D. Brown) were maintained at 20°–22°C in DME medium supplemented with 10% fetal calf serum. For labeling, 3–5 × 10^6 cells were incubated for 24–40 hr with 0.2 μCi of ^{32}-PO_4^{3} in phosphate-depleted medium (Peters et al., 1977). The preparation of total nucleic acids (by cell lysis in urea-containing buffer) as well as the purification of U1 RNAs by hybrid selection to filter-bound human U1 DNA have been described in detail elsewhere (Lund et al., 1983). For labeling of oocytes, stage V–VI oocytes (Dumont, 1972) were manually dissected from a single ovary, injected with 20 nl (1–2 μCi) of α-

^{32}P GTP per oocyte, and incubated for 20-24 hr at 19°C as previously described (Murphy et al., 1982). Total ^{32}P-labeled nucleic acids were prepared from oocytes by proteinase K digestion followed by phenol-chloroform extractions according to Murphy et al. (1982). The U1 RNAs were purified by hybrid selection (as above) prior to polyacrylamide gel analyses (see below).

Analyses of U1 RNAs

Hybrid-selected or gel-purified U1 RNAs were analyzed by electrophoresis in nondenaturing 15% (19:1) polyacrylamide gels run in 45 mM Tris-base, 45 mM boric acid, 1.4 mM EDTA (pH 8.3) for 20–24 hr at 20V/cm (Lund et al., 1983). Maximal separation of X. laevis U1 RNAs was obtained using a 1.5 mm thick, 20 cm long gel which was run at 400V in the 4°C cold room (see Figure 1A). When a thinner (0.4 mm) gel of identical length and polyacrylamide composition was run at a slightly higher voltage (500V) in the cold room, X. laevis U1 RNAs produced quite different patterns of bands (data not shown). Similar changes in the relative mobilities of these U1 RNAs were observed when a 15% nondenaturing gel of a slighly different composition or crosslinking was used (D. Forbes, unpublished data). Thus the exact structure and/or running temperature of the 15% gels appeared to be crucial for separation of the different species of X. laevis U1 RNAs.

For analysis under denaturing conditions, U1 RNAs were electrophoresed in 8% (19:1) polyacrylamide gels, containing 7 M urea and 90 mM Tris-base, 90 mM boric acid, 2.8 mM EDTA (pH 8.3). The thin (0.4 mm) gels (Sanger et al., 1980) were run at 50V/cm at room temperature.

Gel-purified U1 RNAs were eluted and subjected to RNAase T_1 fingerprinting (Sanger et al., 1965) as described previously (Lund et al., 1983).

Acknowledgments

E. L. and J. E. D. thank J. L. Mitchen for excellent technical assistance and D. D. Brown for supplying the X. laevis cultured cell line. The authors would like to thank E. Schenborn, J. Skuzeski, J. Newport, R. Miake-Lye, and D. Drubin for critical reading of the manuscript and C. Hernandez for preparation of the manuscript. D. F. and M. K. thank Dr. T. Kornberg for generous support. This work was supported in part by an American Cancer Society Senior Postdoctoral Fellowship-California Division (#S-14-82) to D. F. and by grants to M. K. from the American Cancer Society and the National Institutes of Health and to J. E. D. and E. L. from the National Institutes of Health and the National Science Foundation.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 21, 1984; revised July 19, 1984

References

Bachvarova, R., and Davidson, E. H. (1966). Nuclear activation at the onset of amphibian gastrulation. J. Exp. Zool. *163*, 285–295.

Barrell, B. G. (1971). Fractionation and sequence analysis of radioactive nucleotides. In Procedures in Nucleic Acids, Volume 2, G. L. Cantoni and D. R. Davies, eds. (New York: Harper), pp. 751–779.

Branlant, C., Krol, A. Ebel, J.-P., Lazar, E., Gallinaro, B., Jacob, M., Sri-Wadada, J., and Jeanteur, P. (1980). Nucleotide sequences of nuclear U1A RNAs from chicken, rat, and man. Nucl. Acids Res. *8*, 4143–4154.

Branlant, C., Krol, A., Ebel, J.-P., Gallinaro, B., Lazar, E., and Jacob, M. (1981). The conformation of chicken, rat and human R1 RNAs in solution. Nucl. Acids Res. *9*, 841–858.

Brown, D. D., and Littna, E. (1964). Variations in the synthesis of stable RNAs during oogenesis and development of Xenopus laevis. J. Mol. Biol. *8*, 688–695.

Brownlee, G. G., Cartwright, E. M., and Brown, D. D. (1974). Sequence studies of the 5S DNA of Xenopus laevis. J. Mol. Biol. *89*, 703–718.

Busby, S. J., and Reeder, R. H. (1983). Spacer sequences regulate transcription of ribosomal gene plasmids injected into Xenopus embryos. Cell *34*, 989–996.

Busch, H., Reddy, R., Rothblum, L., and Choi, Y. C. (1982). SnRNAs, snRNPs, and RNA processing. Ann. Rev. Biochem. *51*, 617–654.

De Robertis, E. M., Lienhard, S., and Parisot, R. F. (1982). Intracellular transport of microinjected 5S and small nuclear RNAs. Nature *295*, 572–577.

Dumont, J. N. (1972). Oogenesis in Xenopus laevis I. Stages of oocyte development in laboratory maintained animals. J. Morphol. *136*, 153–179.

Forbes, D. F., Kornberg, T. B., and Kirschner, M. W. (1983). Small nuclear RNA transcription and ribonucleoprotein assembly in early Xenopus development. J. Cell Biol. *97*, 62–72.

Ford, J. P., and Brown, R. D. (1976). Sequences of 5S ribosomal RNA from Xenopus mulleri and the evolution of 5S gene-coding sequences. Cell *8*, 485–493.

Ford, J. P., and Southern, E. M. (1973). Different sequences for 5S RNA in kidney cells and ovaries of Xenopus laevis. Nature New Biol. *241*, 7–12.

Goddard, J. M., Caput, D., Williams, S. R., and Martin, D. W., Jr. (1983). Cloning of human purine-nucleoside phosphorylase cDNA sequences by complementation in E. coli. Proc. Nat. Acad. Sci. USA *80*, 4281–4285.

Harper, J. E., Price, J., and Korn, L. J. (1983). Chromosomal mapping of Xenopus 5S genes: somatic-type versus oocyte type. Nucl. Acids Res. *11*, 2313–2323.

Hinterberger, M., Petersson, I., and Steitz, J. A. (1983). Isolation of small nuclear ribonucleoproteins containing U1, U2, U4, U5 and U6 RNAs. J. Biol. Chem. *258*, 2604–2613.

Kinlaw, C. S., Dusing-Swartz, S. K., and Berget, S. M. (1982). Human U1 and U2 small nuclear ribonucleoproteins contain common and unique polypeptides. Mol. Cell. Biol. *2*, 1159–1166.

Lerner, M. R., and Steitz, J. A. (1979). Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus. Proc. Nat. Acad. Sci. USA *76*, 5495–5499.

Lerner, M. R., Boyle, J. A., Mount, S. M., Wolin, S. L., and Steitz, J. A. (1980). Are snRNPs involved in splicing? Nature *283*, 220–224.

Lund, E., Bostock, C., Robertson, M., Christie, S., Mitchen, J. L., and Dahlberg, J. E. (1983). U1 small nuclear RNA genes are located on human chromosome 1 and are expressed in mouse–human hybrid cells. Mol. Cell. Biol. *3*, 2211–2220.

Mattaj I. W., and Zeller, R. (1983). Xenopus laevis U2 snRNA genes: tandemly repeated transcription units sharing 5′ and 3′ flanking homology with other RNA polymerase II transcribed genes. EMBO J. *2*, 1883–1891.

Messing, J., and Vieira, J. (1982). A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. Gene *19*, 269–276.

Mount, S. M., and Steitz, J. A. (1981). Sequence of U1 RNA from Drosophila melanogaster: implications for U1 secondary structure and possible involvement in splicing. Nucl. Acids Res. *9*, 6351–6368.

Mount, S. M., Hinterberger, M., Pettersson, I., and Steitz, J. A. (1983). The U1 small nuclear RNA–protein complex selectively binds a 5′ splice site in vitro. Cell *33*, 509–518.

Murphy, J. T., Burgess, R. R., Dahlberg, J. E., and Lund, E. (1982). Transcription of a gene for human U1 small nuclear RNA. Cell *29*, 265–274.

Newport, J. W., and Kirschner, M. W. (1982). A major developmental transition in early Xenopus embryos: I. Characterization and timing of cellular changes at the midblastula stage. Cell *30*, 675–686.

Nohga, K., Reddy, R., and Busch, H. (1981) Comparison of RNase T_1 fingerprints of U1, U2 and U3 small nuclear RNAs of HeLa cells, human normal fibroblasts and Novikoff hepatoma cells. Cancer Res. *41*, 2215–2220.

Ohshima, Y., Itoh, M., Okada, N. and Miyata, T. (1981) Novel models for RNA splicing that involve a small nuclear RNA. Proc. Nat. Acad. Sci. USA *78*, 4471–4474.

Padgett, R. A., Mount, S. M., Steitz, J. A., and Sharp, P. A. (1983). Splicing of messenger RNA precursors is inhibited by antisera to small nuclear ribonucleoprotein. Cell *35*, 101–107.

Peters, G., Harada, F., Dahlberg, J. E., Panet, A., Haseltine, W. A., and Baltimore, D. (1977). Low-molecular-weight RNAs of Moloney murine leu-

kemia virus: identification of the primer for RNA-directed DNA synthesis. J. Virol. *21,* 1031–1071.

Peterson, R. C., Doering, J. S., and Brown, D. D. (1980). Characterization of two Xenopus somatic 5S DNAs and one minor oocyte-specific 5S DNA. Cell *20,* 131–141.

Rogers, J., and Wall, R. (1980). A mechanism for RNA splicing. Proc. Nat. Acad. Sci. USA *77,* 1877–1879.

Sanger, F., Brownlee, G. G., and Barrell, B. G. (1965). Two-dimensional fractionation procedure for radioactive nucleotides. J. Mol. Biol. *13,* 373–398.

Sanger, F., Coulson, A. R., Barrel, B. G., Smith, A. J. H., and Roe, B. A. (1980). Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. *143,* 161–178.

Schwarzbauer, J. E., Tamkun, J. W., Lemischka, I. R., and Hynes R. O. (1983). Three different fibronectin mRNAs arise by alternative splicing within the coding region. Cell *35,* 421–431.

Tyler, B. M., Cowman, A. F., Adams, J. M., and Harris, A. W. (1981). Generation of long mRNA for membrane immunoglobulin α2a chains by differential splicing. Nature *293,* 406–408.

Wakefield, L., and Gurdon, J. B. (1983). Cytoplasmic regulation of 5S RNA genes in nuclear transplant embryos. EMBO J. *2,* 1613–1619.

Wegnez, M., Monier, R., and Denis, H. (1972). Sequence heterogeneity of 5S RNA in Xenopus laevis. FEBS Lett. *25,* 13–20.

Wormington, W. M., Schlissel, M., and Brown, D. D. (1983). Developmental regulation of Xenopus 5S RNA genes. Cold Spring Harbor Symp. Quant. Biol. *47,* 879–884.

Yang, V. W., Lerner, M. R., Steitz, J. A., and Flint, S. J. (1981). A small nuclear ribonucleoprotein is required for splicing of adenoviral early RNA sequences. Proc. Nat. Acad. Sci. USA *78,* 1371–1375.

Zeller, R., Nyffenegger, T., and De Robertis, E. M. (1983). Nucleocytoplasmic distribution of snRNPs and stockpiled snRNA-binding proteins during oogenesis and early development in Xenopus laevis. Cell *32,* 425–434.

Ziff, E. B. (1980). Transcription and RNA processing by the DNA tumor viruses. Nature *287,* 491–499.

Note Added in Proof

The work referred to throughout as Lund et al., submitted, is in press: Lund, E., Dahlberg, J. E., and Forbes, D. J. (1984). The two embryonic U1 snRNAs of Xenopus laevis are encoded by a major family of tandemly repeated genes. Mol. Cell. Biol.