Proc. Natl. Acad. Sci. USA
Vol. 76, No. 8, pp. 3957–3961, August 1979
Cell Biology

Transcription of spacer sequences in genes coding for ribosomal RNA in *Xenopus* cells

(restriction endonuclease fragments of ribosomal DNA/hybrid plasmids/RNA·DNA hybridization/nucleolus)

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Communicated by V. Prelog, May 22, 1979

ABSTRACT **Untreated *Xenopus* cells synthesize RNA molecules (5S–23S) containing sequences complementary to the external "nontranscribed" spacer of the genes coding for rRNA. In cells incubated in the cold, maturation cleavages of the rRNA precursors are inhibited. Concomitantly, the relative proportion of spacer transcripts detectable in newly synthesized RNA is increased and some sequences complementary to those of the nontranscribed spacer are found in the heavy shoulder of the 40S precursor rRNA peak. In such events transcription initiation seems to take place within the spacer because its middle and right *Bam*HI endonuclease fragments are preferentially transcribed, whereas only few RNA sequences complementary to the left spacer fragment are found. It is concluded that at least some spacer regions contain promoters for transcription and can be transcribed either into a special class of "spacer transcripts" or into molecules covalently linked to rRNA precursor.**

It is still unclear which sequences of eukaryotic genes coding for pre-rRNA or proteins are actually transcribed and whether transcription initiation starts only from a single defined initiation site. The rRNA genes (rDNA) of *Xenopus*, which are located in the nucleolus, are among the best-studied eukaryotic genes (for reviews see refs. 1–4). Their first stable transcript is a rRNA molecule (pre-rRNA) of M_r 2.6×10^6 and a sedimentation coefficient of 40 S. After prolonged labeling times no molecules larger than 40S have been found hybridizing to rDNA (5). However, no 5′-terminal polyphosphates were detected on the 40S pre-rRNA from cultured *Xenopus* cells (6). On the other hand, it has recently been shown that the 40S pre-rRNA isolated from oocytes may be capped by an enzyme preparation from vaccinia virus, and thus seems to carry a polyphosphate at its 5′ end (7). These results do not, however, exclude other initiation events elsewhere in rDNA, especially if the resulting transcripts are highly unstable.

There are various observations in the literature (for reviews see refs. 4, 8, and 9) indicating that the first stable pre-rRNA is not necessarily the only transcript of rDNA. In the electron microscope, the spacer intercepts between the 40S matrix units of *Xenopus* and *Triturus* are often found associated with isolated lateral fibrils or even with dense transcriptional complexes forming the so-called prelude regions (10, 11). In isolated nuclei of *Rana*, methylated, G+C-rich RNA molecules larger than the 40S pre-rRNA have been observed (12). Moreover, *Xenopus* cells grown in culture treated with fluorouridine synthesize large RNA molecules that seem to contain sequences of both the 40S pre-rRNA and the external "nontranscribed" spacer (ENTS) of rDNA (13). Accordingly, changes in the ultrastructure of rDNA transcription units such as the presence of transcriptional complexes along nearly the entire deoxynucleoprotein axis have been observed after fluorouridine treatment (14).

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In the present study we have used a recombinant plasmid containing only ENTS sequences as a hybridization probe to monitor spacer transcription. The results show that, in untreated *Xenopus* cells, heterogenously sized RNA molecules are transcribed from the "nontranscribed" spacer of rDNA. In cold-treated cells, which show similar changes in RNA metabolism as fluorouridine-treated cells, there are in addition ENTS sequences found in fast-sedimenting RNA molecules.

MATERIALS AND METHODS

Labeling and Fractionation of RNA. Established kidney epithelial cell lines from *Xenopus laevis* (15) were labeled by addition of [^{3}H]uridine to the normal culture medium at 50 μCi/ml (1 Ci = 3.7×10^{10} becquerels). Incubation was for either 3 hr at 27°C (normal temperature) or 1 hr at 27°C followed by 2 hr at 7°C (cold treatment), which allowed for the metabolization of the radioactive precursors. Labeling with $^{32}PO_4$ was carried out by preincubating the cells for 6–10 hr in phosphate-free Eagle's medium without added serum. Then $^{32}PO_4$ was added at 100 μCi/ml, together with cytosine arabinonucleoside at 40 μg/ml to block DNA synthesis (16). Incubation conditions (27°C or 7°C) were as described above.

After incubation the cells were quickly washed with 0.1 M sodium acetate buffer and lysed in the petri dishes by adding 3 ml of extraction buffer (0.1 M sodium acetate pH 5/0.5% sodium dodecyl sulfate/4 μg of polyvinylsulfate per ml). Extraction, fractionation, and isolation of the 40S and "45S" RNA samples on sucrose gradients and on composite agarose/acrylamide gels were performed as described (13). Enrichment of 23S RNA was carried out by electrophoresis on 3% acrylamide gels. For hybridization, the RNA samples were resuspended in either 2× standard saline citrate (standard saline citrate contains 0.15 M NaCl and 0.015 M sodium citrate) or 5× standard saline citrate/50% formamide.

rDNA Recombinant Plasmids. Several rDNA recombinant plasmids were used as hybridization probes. The hybrid plasmid CD18 (17) consists of the vector pSC101 (18) and a M_r 3×10^6 *Eco*RI endonuclease fragment of *Xenopus laevis* rDNA that includes only rRNA structural sequences (19) (see Fig. 1). Plasmid CD42 (17) contains a M_r 4.2×10^6 *Eco*RI fragment of *Xenopus* rDNA with some structural sequences and the entire ENTS (19) (see Fig. 1). This plasmid was also used to create the *Bam*HI restriction fragments L, M, and R (see Fig 1) for hybridization analysis by the Southern technique (20).

The recombinant plasmid HM5 was prepared in our laboratory. It consists of the vector pBR313 (21) carrying the middle (M) fragment of ENTS sequences generated by *Bam*HI restriction (cf. Fig. 1). This DNA was used as a probe for detecting

Abbreviations: rDNA, DNA containing the genes for ribosomal RNA; pre-rRNA, rRNA precursor; ENTS, external nontranscribed spacer of rDNA; ETS, external transcribed spacer.

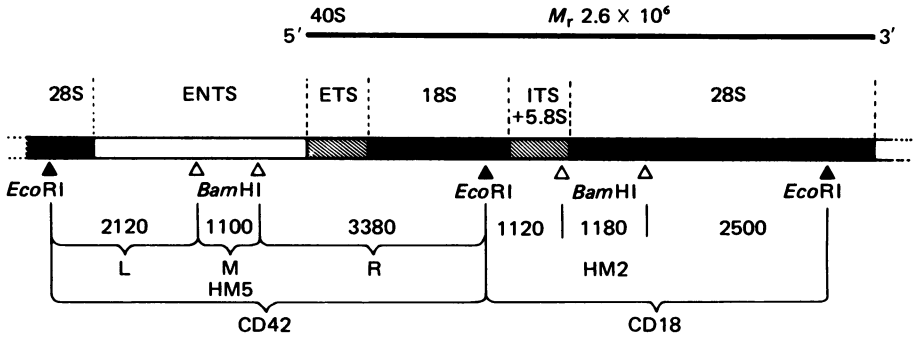

FIG. 1. Map of *Xenopus laevis* rDNA and its cloned restriction fragments. The plasmid strains CD42, CD18, and HM5 were used for hybridization assays of rRNA and of spacer transcripts. The different studies elaborating the rDNA sequence arrangement (external nontranscribed spacer, ENTS; external transcribed spacer, ETS; 18S rRNA; internal transcribed spacer, ITS, with the 5.8S rRNA moiety; 28S rRNA) as well as the 5′-3′ polarity of the 40S pre-rRNA are extensively reviewed elsewhere (4). The numbers above the brackets give the approximate length of the different fragments in base pairs.

spacer transcripts. The vectors of these different plasmids, pSC101 and pBR313, were used as blanks in all the hybridization experiments. All growth of cells and handling of recombinant DNA have been carried out according to U.S. National Institutes of Health guidelines (P2, EK1) as recommended by the Swiss Academy for Medical Sciences.

Hybridization Experiments. Covalently closed circular plasmid DNA was purified from detergent-lysed (Triton X-100) bacterial cells by cesium chloride/ethidium bromide density gradient centrifugation (22) followed by centrifugation on 15–30% sucrose gradients. The circular DNA was denatured and broken by incubation for 1 hr at 37°C in 0.5 M NaOH, neutralized with 2 vol of neutralization mixture (1 vol 1 M Tris·HCl, pH 8/1 vol 1 M HCl/2 vol 3 M NaCl) to give a final pH of 8 (according to M. Birnstiel, personal communication). This solution was then mixed with 20 vol of cold 6× standard saline citrate and quickly loaded onto 12-mm nitrocellulose filters. Hybridization was carried out in 2× standard saline citrate at 66°C for 16 hr.

To monitor the ENTS sequences transcribed into RNA, the DNA of plasmid CD42 was digested by *Eco*RI, precipitated with ethanol, resuspended in buffer, and digested with *Bam*HI restriction enzyme. The fragments were separated on a 0.8% agarose gel and transferred onto Millipore filter strips (20). The RNA samples were resuspended in 5× standard saline citrate/50% formamide and hybridized to the filter-bound DNA fragments at 37°C for 16 hr. The filters were then washed, treated with RNase, prepared for fluorography, and exposed with a sensitized Kodak Royal X O-mat film (23).

RESULTS

The analysis on composite agarose/polyacrylamide gels of RNA labeled for 3 hr at normal temperature (27°C) shows a predominant peak of 40S pre-rRNA as well as some label already processed into mature 18S and 28S rRNA. In cold-treated cells, the overall RNA synthesis is reduced and the processing of the pre-rRNA is inhibited. The 40S pre-rRNA is therefore present as a predominant peak of RNA of M_r 2.6 × 10^6 but, in addition, a heterogeneously sized population of RNA molecules forms a broad shoulder on the heavy side of this peak. Moreover, there is a small radioactive peak appearing in a position corresponding to about M_r 1.2 × 10^6 that is not detectable in RNA samples taken from untreated cells. Fig. 2 shows the pattern of RNA samples from cells labeled at 27°C and 7°C that have been electrophoresed on the same agarose/acrylamide composite gel. The pattern of RNA synthesis in cold-treated cells closely resembles the pattern of RNA synthesis in the presence of fluorouridine (13).

In order to determine whether any RNA sequences are transcribed from the so-called nontranscribed spacer of rDNA, we have used DNA of plasmid HM5 as a hybridization probe. This recombinant plasmid contains the central *Bam*HI fragment of ENTS. The sequence content of this rDNA insert has been analyzed in different experiments, the results of which may be briefly summarized as follows. Hybridization of nick-translated HM5 DNA with Southern blots of *Eco*RI-digested total *Xenopus* DNA from normal and anucleolate mutant embryos (0-nu, cf. ref. 24) shows that hybridization is limited to a single band in the 2-nu DNA and that there is no detectable hybridization to other DNA fragments. The 0-nu DNA, devoid of ribosomal genes, does not react at all with ENTS sequences present in the HM5 recombinant plasmid (data not shown). This indicates that the sequences of the ENTS present in plasmid HM5 are not represented elsewhere in the genome. In another experiment (not shown) the nick-translated HM5 DNA has been hybridized to Southern blots of the three *Eco*RI/*Bam*HI rDNA fragments of plasmid CD42. A crossreaction is observed between the middle (M) and the right (R) (see Fig. 1) spacer fragments, indicating that the two fragments have some com-

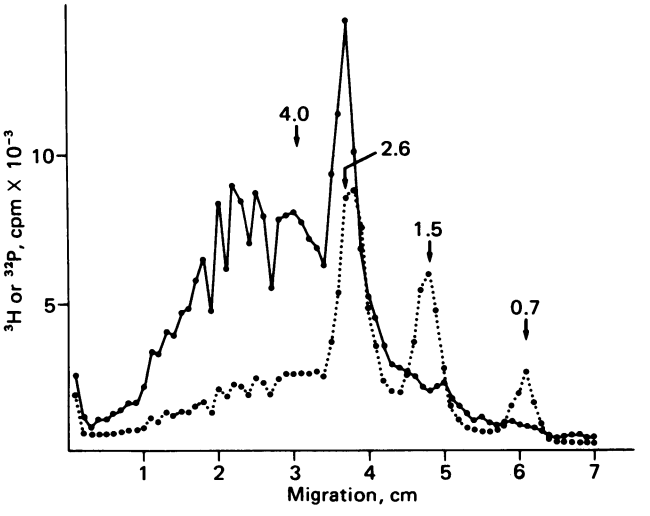

FIG. 2. Effect of cold treatment on overall RNA synthesis in *Xenopus* cells. *Xenopus* cells were labeled with [^{3}H]uridine for 3 hr at 27°C (●·····●) and with $^{32}PO_4$ for 3 hr at 7°C (●—●). Aliquots (0.5 A_{260} unit) of each RNA sample were mixed, treated with DNase, and electrophoresed on a composite agarose/acrylamide gel. Ordinate, ^{3}H or ^{32}P cpm incorporated into RNA. The numbers above the arrows indicate the nominal molecular weights of the 18S (0.7 × 10^6), 28S (1.5 × 10^6), and 40S (2.6 × 10^6) rRNA species.

mon sequences. These common sequences, however, must be limited to the ENTS portion of the right *Bam*HI fragment, because we never have observed hybridization of the HM5 DNA with 40S pre-rRNA. The left spacer fragment does not crossreact with HM5 sequences. These observations are in accordance with rDNA sequencing data (ref 25; see *Discussion*). It thus appears that HM5 DNA represents a specific hybridization probe for RNA molecules complementary to the middle and right ENTS sequences of rDNA.

Total RNA from both untreated and cold-treated cells has been fractionated on sucrose gradients and each fraction has been hybridized to filter-bound DNA of the HM5 plasmid. The results of such experiments (Figs. 3 and 4) show that, in untreated *Xenopus* cells (27°C), intermediate-sized RNA molecules are found with sedimentation coefficients ranging from about 5 S to 23 S that hybridize to the "nontranscribed" spacer sequences in the presence of a large excess of unlabeled 18S and 28S rRNA. A large proportion of these spacer transcripts sediments at 23 S and appears to have a molecular weight of 1.2×10^6 on gel electrophoresis. After cold treatment, these RNA molecules complementary to the ENTS are still present and now represent a larger proportion of the total radioactivity incorporated into RNA. In all experiments using cold-treated cells (see, for instance, Fig. 3) the size distribution of spacer transcripts is altered in that the smallest RNA species hybridizing to ENTS are less frequent and the "23S" peak is shifted by one to two fractions toward the heavy side of the gradient. In addition, some molecules hybridizing to the spacer DNA are now found also in the heavy region of the gradient, sometimes forming a distinct peak at 45S. Control hybridization with vector pBR313 (data not shown) or with pSC101 DNA (Fig. 4) have not yielded any RNase-resistant radioactive material. A series of filters loaded with CD18 DNA has been used as a probe for the distribution along the gradient of rRNA structural sequences (cf. Fig. 1). This probe hybridizes with the 40S pre-rRNA as well as with its heavy shoulder, but not with the smaller labeled RNA species (Fig. 4).

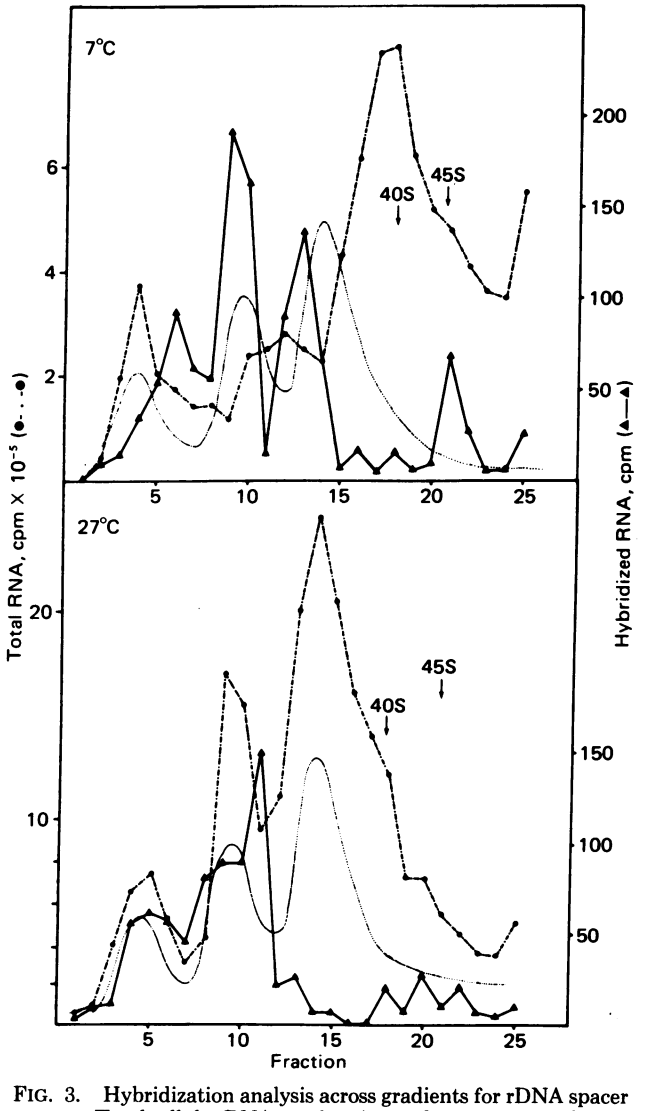

FIG. 3. Hybridization analysis across gradients for rDNA spacer sequences. Total cellular RNA was fractionated on sucrose gradients. ·····, A_{260} profile. The arrows indicate the positions of 40S and 45S RNA. Sedimentation is from left to the right. Total trichloroacetic acid-precipitable cpm of each fraction are given by the left ordinate (●–·–●). The right ordinate gives the RNase-resistant cpm hybridized to filter-bound HM5 DNA (0.5 μg per filter) minus cpm of blank filters loaded with pBR313 DNA (▲—▲). (*Upper*) RNA from cells labeled at 7°C for 3 hr; (*Lower*) labeled at 27°C for 3 hr.

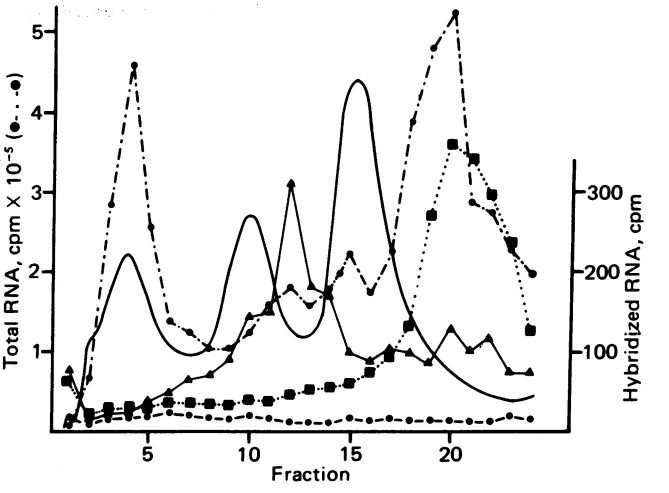

FIG. 4. Localization of 40S spacer sequences in RNA from cold-treated cells. The RNA from cold-treated cells (7°C) was separated on a gradient (—, A_{260} profile) and the cpm incorporated into the different fractions was determined by trichloroacetic acid precipitation of aliquots (●–·–●). Material in each fraction was hybridized on filters loaded with 0.5 μg of CD18 DNA (■·····■), HM5 DNA (▲—▲), or vector pSC101 DNA ●—●. The scale of the RNase-resistant hybridized cpm is given in the right ordinate.

A discrete peak in the 23S region of gradients (M_r 1.2×10^6) was previously observed in RNA preparations from fluorouridine-treated cells (13). Because at that time no plasmid containing exclusively spacer sequences was available, this 23S RNA was not analyzed. The analysis of 23S RNA from fluorouridine-treated cells has since been performed by hybridization on nitrocellulose filters loaded with equal amounts of the different rDNA plasmids and has indicated that 23S RNA hybridizes preferentially to HM5 and CD42 DNA, whereas hybridization to CD18 DNA is relatively low. The 40S RNA reacts strongly with both CD42 and CD18 sequences but not at all with the HM5 ENTS fragment. These results (not shown) indicate that the 23S RNA fraction contains mainly ENTS sequences.

The RNA of cold-treated cells has been analyzed further by hybridizing 23S, 40S, and 45S samples to restriction fragments of rDNA. Such fragments were obtained by double digestion of CD42 DNA (cf. Fig. 1) with *Eco*RI and *Bam*HI restriction enzymes. The fragments were then separated on gels and transferred onto Millipore filter strips. Fig. 5 shows autoradiographs of hybridization experiments carried out with 23S, 40S, and 45S RNA samples purified from cold-treated cells. As

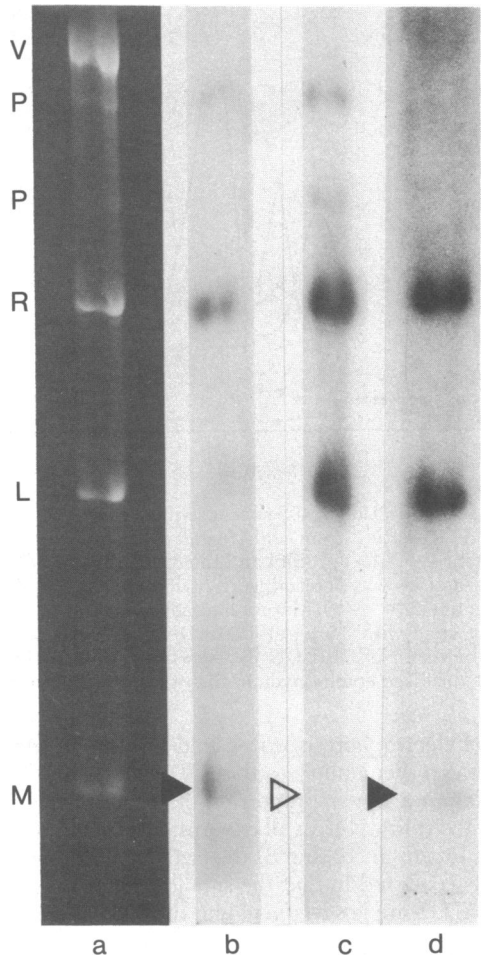

FIG. 5. Hybridization of various RNA fractions to rDNA spacer fragments. The DNA of plasmid strain HM456 was cleaved with *Eco*RI and *Bam*HI enzymes, fractionated on gels (a) and transferred to Millipore strips. The DNA on one strip each was hybridized with 23S RNA (b), with 40S pre-rRNA (c), or with 45S RNA (d) obtained from cold-treated cells. The left margin indicates the localization of the bands in the ribosomal repeating unit. V, vector; P, partial digests; R, right, L, left, and M, middle *Bam*HI spacer fragment (cf. Fig. 1).

expected, the 40S pre-rRNA reacted with two of the rDNA fragments—i.e., the 28S sequences located on the left *Bam*HI fragment (L in Fig. 1) and the ETS and 18S sequences of the right (R) fragment. 23S RNA, on the other hand, reacted with the middle *Bam*HI (M) fragment, which contains only ENTS sequences, and with the R fragment. For the latter we probably measured the reaction to its spacer sequences, because there was a large excess of unlabeled 18S rRNA present in the 23S RNA sample. There was hardly any detectable reaction of 23S RNA with the left (L) ENTS sequences. The 45S RNA, which was heavily contaminated with labeled 40S pre-rRNA, reacted strongly with the two fragments containing 40S structural sequences, but nothing can be said about its reactivity with the ENTS component of these fragments. This RNA did show, however, a faint reaction with the middle (M) *Bam*HI fragment of the ENTS.

These experiments indicate that the 23S RNA contains sequences complementary to the middle and right *Bam*HI fragments of the rDNA spacer and to few, if any, sequences complementary to the left spacer region. Moreover, in cold-treated cells there are also some spacer sequences found in the heavy region of the gradients. It is, however, not yet proven that they are covalently linked to 40S RNA sequences.

DISCUSSION

The use of a cloned rDNA spacer fragment as a hybridization probe allows the unambiguous identification of spacer transcripts. The characterization of the rDNA fragment contained in plasmid HM5 has shown that the sequences of the insert are present only in rDNA and that they are limited to the ENTS. Using this specific probe, we could demonstrate that normal *Xenopus* cells synthesize RNA molecules from the so-called nontranscribed spacer of rDNA.

The spacer transcripts in normal cells appear to be heterogeneous in size and sediment at between 5S and 23S. It is possible that the smallest of these molecules are cleavage products of 23S molecules because their distribution varies from one experiment to another, whereas the 23S peak is always present. The relative amount of spacer transcripts in cold-treated cells is increased as compared to total radioactivity incorporated into RNA. Concomitantly the smallest spacer transcripts disappear and the broad "23S" peak is slightly shifted toward the heavy side of the gradient. Some RNA sequences complementary to the ENTS are also found sedimenting at 45S. These changes in rRNA metabolism closely resemble the alterations observed in fluorouridine-treated cells (13, 14). This effect on the size of spacer transcripts may be explained by the mode of action of reduced temperature and of fluorouridine. Fluorouridine has been shown to be incorporated into RNA and to inhibit maturation cleavages of rRNA (cf. refs. 13, 26 and 27).

Cold treatment has been chosen because it had been shown that reduced temperature modifies the processing of 45S pre-rRNA in HeLa cells (28). We now observe that incubation of *Xenopus* cells in the cold inhibits rRNA maturation and conclude that both treatments, fluorouridine and cold, exert their action through inhibition of rRNA cleavage. Thus, the observed effects on rDNA transcription, such as a relative accumulation of 23S spacer transcripts and the appearance of RNA sequences complementary to ENTS in the heavy region of gradients, might be due to stabilization of a class of normally highly unstable RNA molecules. If this is true, the rapidly sedimenting RNA molecules (45S) would represent covalently linked ENTS and 40S sequences that have escaped cleavages usually taking place during transcription (see also refs. 4, 9, and 14). This implies that no proper termination occurs between transcription of ENTS and 40S sequences. In untreated cells no large molecules (>40S) containing spacer sequences are observed (see also ref. 5). Moreover, the prelude regions are normally separated from the 40S matrix units (10, 11). This separation thus seems to be due to rapid cleavages taking place on the ENTS transcripts.

The analysis of the sequence content of spacer transcripts indicates that only a few, if any, of the sequences of the left spacer fragment are represented and that the transcripts observed contain primarily sequences from the middle and right spacer fragments. When we compare the size of the 23S RNA (up to M_r 1.2×10^6) with the length of these two DNA fragments (1.8 kilobases in CD42), it appears that the RNA molecules from cold-treated cells, besides containing ENTS sequences, must also include ETS or even 18S sequences. This is also indicated by the hybridization of 23S RNA from fluorouridine-treated cells, which weakly reacts with rDNA structural sequences of plasmid CD18. Again this suggests that some spacer transcripts remain linked to 40S sequences. On the other hand, it must be mentioned that in *Xenopus* cells the ENTS are heterogeneous in length and vary from about 3 to 6.3 kilobases (cf. refs. 3 and 4). This heterogeneity seems to be due to a variable number of short repeats in the ENTS (25). Obviously, this heterogeneity of spacer sequences will also contribute to the size heterogeneity of the spacer transcripts. At

least part of the "23S" spacer transcripts may arise from large spacer individuals and need not contain 40S sequences.

It is difficult to say in what proportion of rDNA repeat units transcription in ENTS takes place. An exact quantitation of spacer transcripts was not possible because neither their half-life nor their specific radioactivity could be determined. Even the exact amount of 40S pre-rRNA synthesized is difficult to determine because of the unknown amount of hybridization competition by preformed 40S and contaminating 28S sequences. As an estimate, however, it seems probable that among the genes actively transcribing 40S pre-rRNA only a few also transcribe spacer sequences. This situation is also suggested by electron microscopy observations, in which transcriptional complexes have been found only in a low proportion of the spacers (11). Even in the presence of fluorouridine, where the suggested stabilization of RNA chains leads to a marked increase in spacer-associated transcriptional complexes, most genes still show seemingly untranscribed spacer intercepts (14).

The most crucial question arising from the present observations concerns the initiation of the transcriptional events in the spacer. In this context it is important to remember that about 22% of 40S pre-rRNA molecules are capable of being capped (7). Considering the capping efficiency, it has been calculated that at least 85% of these molecules carry a polyphosphate group on their 5′ end (7). On the basis of this finding, it has been suggested that spacer transcription is due to a failure of proper termination at the 3′ end of the 40S transcriptional unit, coupled with a variable degree of processing of the aberrant transcripts. The present results argue against this interpretation. The left spacer fragment, adjacent to the 3′ end of the preceding transcriptional unit, is in fact the least represented in the ENTS transcripts. Moreover, in spread transcriptional units, the prelude regions are clearly separated from the preceding matrix unit by a stretch of seemingly untranscribed deoxynucleoprotein axis (9–11). It thus seems that spacer transcription rather results from initiation events in the spacer itself.

The proposed existence of transcription initiation site(s) within the ENTS is further corroborated by rDNA sequencing data that have recently become available (25). The spacer sequences around the *Bam*HI sites show high homology with sequences near the starting region of the 40S pre-rRNA transcription. It has therefore been suggested that the DNA sequences near the 5′ end of the pre-rRNA transcription unit have been duplicated and displaced into the spacer by saltation of an intervening short DNA sequence (25). These data add new aspects to the possible significance of spacer transcription (for earlier discussions see refs. 4, 9, and 14). If the transposed regions near the *Bam*HI sites contain the 40S initiation site, as well seems possible in the light of the present observations, it is possible, for instance, that the transcription of spacer sequences is due to "illicit" initiation in such transposed starting regions (25). This situation might then represent an intermediate step in the evolutionary increase in the length of the pre-rRNA, but such transcription need not have a functional physiological role in the present organism. It cannot be excluded, however, that the initiation events in the ENTS represent functional variants of the 40S pre-rRNA transcription unit that may be utilized in some of the pre-rRNA gene units of normal *Xenopus* cells.

We thank Prof. M. L. Birnstiel (Zürich, Switzerland) for communicating and discussing the sequencing data prior to publication, and Prof. W. W. Franke (Heidelberg, West Germany) for his continuous interest in this study. Prof. M. Fischberg (Geneva) kindly provided heterozygous anucleolate mutant frogs. This work was supported by Swiss National Science Foundation Grants 3.716.76 and 3.300.0.78.

1. Birnstiel, M. L., Chipchase, M. & Speirs, J. (1971) *Prog. Nucleic. Acid Res. Mol. Biol.* **11**, 351–389.
2. Maden, B. E. H. (1971) *Prog. Biophys. Mol. Biol.* **22**, 127–177.
3. Reeder, R. H. (1974) in *Ribosomes*, eds. Lengyel, P., Nomura, M. & Tissières, A. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 489–518.
4. Rungger, D. & Crippa, M. (1977) *Prog. Biophys. Mol. Biol.* **31**, 247–269.
5. Bird, A., Rogers, E. & Birnstiel, M. L. (1973) *Nature (London) New Biol.* **242**, 226–230.
6. Slack, J. M. W. & Loening, U. E. (1974) *Eur. J. Biochem.* **43**, 59–67.
7. Reeder, R. H., Sollner-Webb, B. & Wahn, H. L. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5402–5406.
8. Loening, U. E. (1975) in *Biochemistry of the Cell Nucleus: Mechanism and Regulation of Gene Expression*, eds. Hidvegi, E. J., Simegi, J. & Venetianer, P. (North-Holland, Amsterdam), Vol. 33, pp. 151–157.
9. Franke, W. W., Scheer, U., Spring, H., Trendelenburg, M. F. & Krohne, G. (1976) *Exp. Cell. Res.* **100**, 233–244.
10. Scheer, U., Trendelenburg, M. F. & Franke, W. W. (1973) *Exp. Cell Res.* **80**, 175–190.
11. Scheer, U., Trendelenburg, M. F. & Franke, W. W. (1977) *Chromosoma* **60**, 147–167.
12. Caston, J. D. & Jones, P. H. (1972) *J. Mol. Biol.* **69**, 19–38.
13. Rungger, D. & Crippa, M. (1977) *Exp. Cell Res.* **107**, 227–237.
14. Rungger, D., Crippa, M., Trendelenburg, M. F., Scheer, U. & Franke, W. W. (1978) *Exp. Cell Res.* **116**, 481–486.
15. Rafferty, K. A., Jr. (1969) in *Biology of Amphibian Tumors*, ed. Mizell, M. (Springer, Berlin), pp. 52–81.
16. Wilson, M. C., Melli, M. & Birnstiel, M. L. (1974) *Biochem. Biophys. Res. Commun.* **61**, 404–409.
17. Morrow I. F., Cohen, S. N., Chang, A. C. Y., Boyer, H. W., Goodman, H. M. & Helling, R. B. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1743—1747.
18. Cohen, S. N., Chang, A. C. Y., Boyer, H. W. & Helling, R. B. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 3240–3244.
19. Wellauer, P. K. & Reeder, R. H. (1975) *J. Mol. Biol.* **94**, 151–161.
20. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
21. Rodriguez, R. L., Bolivar, F., Goodman, H. M., Boyer, H. W. & Betlach, M. (1976) in *Molecular Mechanisms in the Control of Gene Expression*, ICN-UCLA Symposia on Molecular and Cellular Biology, eds. Nierlich, D. P., Rutter, W. J. & Fox, C. F. (Academic, New York), pp. 471–477.
22. Cohen, S. N., Chang, A. C. Y. & Hso, L. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2110–2114.
23. Laskey, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56**, 335–341.
24. Elsdale, T. R., Fischberg, M. & Smith, S. (1958) *Exp. Cell Res.* **14**, 642–643.
25. Boseley, P., Moss, T., Mächler, M., Portmann, R. & Birnstiel, M. L. (1979) *Cell* **17**, 19–31.
26. Hadjiolov, A. A. & Nicolaev, N. (1976) *Prog. Biophys. Mol. Biol.* **31**, 95–144.
27. Wilkinson, D. S. & Pitot, H. C. (1973) *J. Biol. Chem.* **248**, 63–68.
28. Stevens, R. H. & Amos, H. (1971) *J. Cell. Biol.* **50**, 818–829.