Inhibitor of Ribosomal RNA Synthesis in *Xenopus laevis* Embryos. VII. Inhibition of 40S Pre-rRNA Synthesis in *Xenopus* Neurula Cells

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ABSTRACT. A fraction containing acid-soluble materials (PCA-extract) from *Xenopus* early blastulae preferentially inhibits the incorporation of [³H]uridine into 18S and 28S rRNA in *Xenopus* neurula cells. Pulse-labeling experiments revealed that whereas tubercidin, a known inhibitor of rRNA processing, produced a marked accumulation of the label in 40S pre-rRNA, *Xenopus* inhibitor suppressed labeling of the pre-rRNA. When tubercidin was added to cells whose activity for rRNA synthesis had been lowered by the *Xenopus* inhibitor, there was still an accumulation of label in the pre-rRNA. These results indicate that *Xenopus* inhibitor suppresses the synthesis of rRNA at transcription rather than at processing.

During the embryogenesis of *Xenopus laevis*, rRNA synthesis starts at about the mid-blastula stage (12, 13). A fraction containing an acid-soluble extract prepared from *Xenopus* early blastulae that had been partially purified with charcoal inhibits the synthesis of 18S and 28S rRNA preferentially when administered to isolated *Xenopus* neurula cells (5, 15, 17). In *Xenopus laevis*, rRNA is transcribed as a 40S pre-rRNA which is processed to form 18S and 28S mature rRNA (2, 4, 7, 16). Therefore, as in the mechanism of inhibition of rRNA synthesis by the *Xenopus* inhibitor, two steps must be considered; transcription and processing. Our reported experiments show that the *Xenopus* inhibitor acts at the transcription level rather than in post-transcriptional processing.

Embryos of *Xenopus laevis* were dejellied and used at the early blastula (stage 7) (9) or neurula (stage 21–22) or tailbud (stage 24–25) stage. An inhibitor of rRNA synthesis was prepared from early blastulae by homogenizing the embryos with 0.3 N PCA (5, 15). As the control, acid-soluble material was obtained from tailbud embryos. These materials were adsorbed on charcoal columns then eluted with ammonia-alcohol and used after repeated lyophilization (5, 15). They are here called the blastula and tailbud PCA-extracts.

To test the activity of the inhibitors, we isolated cells (14) obtained from 15 neurulae and labeled them with [³H] uridine (25 Ci/mole) for 1 h in 1 ml of medium in the presence or absence of PCA-extract equivalent to 20–60 embryos. As a reference substance, tubercidin, a known inhibitor of rRNA processing in *Xenopus* kidney cells (2), was administered at 0.5–1.0 µg/ml to normal or inhibited cultures.

Abbreviations used: PCA, perchloric acid; SDS, sodium dodecylsulfate.

Labeled RNA was extracted from the frozen cells by phenol treatment in 0.1 M sodium acetate, pH 5.0, and 1 mg/ml bentonite-0.5% SDS. The final aqueous phase was made 0.2 M in NaCl, then RNA was precipitated with 2.5 volumes of ethanol. The RNA was electrophoresed on 0.5% agarose-2.0% polyacrylamide gels. Identification of the 40S rRNA precursor was made as described previously by Misumi *et al.* (7). Tubercidin was a gift from Dr. M. Saneyoshi, Hokkaido University.

When neurula cells were labeled for 1 h, the 40S rRNA precursor and 28S and 18S mature rRNA appeared as distinct peaks on the electrophoretic profiles in addition to 4S RNA and RNA of heterogeneous size distribution (Fig. 1A). This profile is very similar to that of primary cultured *Xenopus* kidney cells pulse-labeled in the logarithmic phase of growth (7). When neurula cells were treated with the blastula PCA-extract, the accumulations of 40S pre-rRNA, 28S and 18S mature rRNA were

Fig. 1. Inhibition of the labeling of 40S pre-rRNA by PCA-extracts. Cells from 15 neurulae were cultured in 1 ml of medium. The PCA-extract obtained from 20 embryos was added to the medium. Cells then were cultured for 2 h, after which they were pulse-labeled in the same medium with 30 μ Ci of [3 H] uridine for 1 h. Shaded areas represent the 40S pre-rRNA, and dotted areas the 28S and 18S rRNA. A: Control, B: blastula PCA-extract and C: tailbud PCA-extract.

greatly reduced, the respective inhibitions being about 90, 81 and 87% (Fig. 1B). The labeling of heterogeneous non-ribosomal RNA also was inhibited; but, to a much less extent, about 60% for heterogeneous RNA and 50% for 4S RNA. When tailbud PCA-extract was tested, there was no preferential inhibition of rRNA labeling (Fig. 1C).

The effect of tubercidin on the pattern of RNA labeling is shown in Fig. 2. Clearly the labeling of 18S and 28S rRNA was greatly inhibited, whereas that of 40S pre-rRNA was concomitantly increased. A comparison of the results shown in Figs. 1 and 2 reveals that the effect of blastula PCA-extract differed markedly from that of tubercidin; the former inhibited the labeling of 40S pre-rRNA, whereas the latter enhanced the accumulation of 40S pre-rRNA.

Fig. 2. Effects of tubercidin on the processing of 40S pre-rRNA. Cells from 15 neurulae were treated with $0.5 \mu\text{g/ml}$ of tubercidin for 2.5 h, then labeled as in Fig. 1 in the same medium for 1 h with $30 \mu\text{Ci}$ of $[^3\text{H}]\text{uridine}$. The 4S RNA region is not included. A: control and B: treated with tubercidin.

For *Xenopus*, 40S pre-rRNA is accepted to be the primary transcript (2, 4, 7, 8, 16) although there have been some reports suggesting the existence of a larger precursor (1, 11). Thus, there is the implication that the PCA-extract suppresses formation of the primary transcript.

The processing of pre-rRNA clearly was not inhibited by the blastula PCA-extract because under the inhibition conditions used small amounts of 28S and 18S rRNA were formed (Fig. 1). The possibility must, however, still be considered that rRNA transcription was not inhibited although rapid degradation, or "wastage", took place. If 40S pre-rRNA was formed normally but was degraded rapidly in the PCA-extract-treated neurula cells, there would be little accumulation of 40S pre-rRNA in those

Fig. 3. Effects of tubercidin on the pattern of RNA labeling in cells treated with the blastula PCA-extract. Cells from 15 neurulae were cultured for 2 h in the presence of blastula PCA-extract obtained from 60 embryos. Tubercidin then was added to the medium at $1 \mu\text{g/ml}$, and after 10 min of culture, the cells were labeled for 1 h in the same medium with $100 \mu\text{Ci}$ of $[^3\text{H}]$ uridine. A: control, B: treated with the blastula PCA-extract and C: first treated with the PCA-extract then with tubercidin.

cells when rRNA processing was inhibited by tubercidin. We therefore first treated neurula cells with the blastula PCA-extract for 2 h (as in Fig. 1B) then treated them with tubercidin (as in Fig. 2B).

As Fig. 3AB shows, the blastula PCA-extract again inhibited the labeling of 40S pre-rRNA, 18S and 28S rRNA. When these inhibited cells were subsequently treated with tubercidin, formation of 18S and 28S rRNA was suppressed almost completely, the degree of suppression roughly corresponding to the amount of the label accumulated in the 40S pre-rRNA (Fig. 3C). Thus, in cells treated with the blastula PCA-extract, 40S pre-rRNA was accumulated without being degraded.

Because the accumulation of 40S pre-rRNA could be induced by tubercidin in cells treated with the blastula PCA-extract as it was in normal cells (Fig. 2B), the possibility of very rapid degradation of pre-rRNA in the blastula PCA-extract-treated cells is unlikely. We concluded that the blastula PCA-extract neither causes extensive degradation of the pre-rRNA nor inhibits its processing. Instead, the blastula PCA-extract appears to inhibit rRNA synthesis at the transcription stage.

Reeder (10) reported that a PCA-extract prepared from unfertilized *Xenopus* eggs inhibited the transcription of rRNA but not that of 5S RNA in *in vitro* systems constructed with the purified 5S DNA templates and soluble fraction from *Xenopus* oocyte nuclei. We (3) also recently obtained results which show that crude *Xenopus* blastula extract inhibits the transcription of mouse rDNA in an *in vitro* system that was constructed by Muramatsu and his group (6). The results obtained from our present *in vivo* experiments are consistent with the results obtained in the *in vitro* experiments described above (3, 10), and are further evidence that *Xenopus* cleavage embryos contain a factor which negatively regulates rRNA synthesis (5, 15).

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