Poly (A)-containing RNA Synthesis in *Xenopus laevis* Embryos

Noriyuki Sagata, Takahiro Nakahashi, Koichiro Shiokawa and Kiyotaka Yamana

Department of Biology, Faculty of Science, Kyushu University 33, Fukuoka, 812 Japan

**ABSTRACT.** Poly(A)-containing RNA synthesis was studied in isolated cells from *Xenopus laevis* embryos at various stages of early development. Sedimentation analysis revealed little stage-dependent differences in the distribution; the size was heterogeneous and ranged from >40S to 18S, with an average of 25S (ca. 1.4 × 10^6 daltons). The total amount of newly synthesized poly(A)-containing RNA increased sharply as development proceeded. On a per-cell basis, however, the synthesis of poly(A)-containing RNA was much more active in blastula cells than in later embryonic cells.

Gene expression in early embryonic development has been well studied, and a growing body of information on the synthesis of “DNA-like RNA” as putative messenger RNA in embryos of amphibians (3, 8) and sea urchins (7, 11) exists. Recently, most, if not all, messenger RNAs in eukaryotes have been shown to contain poly (A) sequences at their 3' termini. This has provided a powerful tool for messenger isolation. In Amphibia such as *Xenopus, Rana* or *Triturus*, poly(A)-containing RNA synthesis occurs in oocytes (5, 12, 17, 18, 24), kidney cells (18), liver cells (1, 19, 25, 26) and in tadpole tails (10). However, little attention has been paid to the quantitative aspect of poly(A)-containing RNA synthesis during embryogenesis.

Our recent papers have characterized poly(A)-containing RNA synthesized in isolated neurula cells of *Xenopus laevis* (20, 23). Further studies on the characterization and quantification of poly(A)-containing RNA have now been carried out throughout early developmental stages. Results show that there is little stage-dependent difference in size distribution and that synthesis occurs during the blastula stage at a much higher rate than during later stages. A preliminary account of the present work was given at the 8th International Congress of the International Society of Developmental Biologists, Tokyo (21).

**MATERIALS AND METHODS**

Isolation and isotopic labeling of embryonic cells. Embryos of *Xenopus laevis* were obtained and dissociated into isolated cells at the blastula [stages 8–9 (14)], gastrula (stages 10 –11), neurula (stages 15–16) and tailbud (stages 22–24) stages. Cells were labeled at 21°C with either (8, 3-3H) adenosine (30 μCi/ml; 31 Ci/mmole) or (5-3H) uridine (70 μCi/ml; 25 Ci/mmole) in complete Stearns’ solution as described previously by Shiokawa and Yamana (22). Labeled cells were washed once with fresh Stearns’ solution and immediately subjected to RNA extraction.

Extraction and fractionation of RNA. The methods used were essentially the same as those...
described previously by Sagata et al. (20). RNA was extracted from labeled cells with chloroform-phenol (1:1) in 0.1 M sodium acetate, pH 5.0, containing 0.5% SDS (sodium dodecylsulfate) and 10 µg/ml of PVS (polyvinylsulfate). It was purified by repeated precipitation with 0.1 M NaCl-70% ethanol. The RNA was fractionated on a poly(U)-Sepharose column, then the poly(A)-containing RNA obtained underwent sedimentation analysis in 28 ml of a 5–30% sucrose density gradient in 10 mM sodium acetate-10 mM EDTA, pH 5.0, at 22,500 rpm for 18 hr in a Hitachi RSP rotor. The radioactivity in each fraction was counted in Triton-toluene-2,5-diphenyloxazole in a scintillation spectrometer.

The poly(A)-containing RNA obtained was treated with RNase A (20 µg/ml) and T1 (10 units/ml) in 10 mM Tris-HCl, pH 7.5, containing 0.3 M NaCl. RNase-resistant materials were electrophoresed on SDS-10% polyacrylamide gels. More than 97% of the RNase-resistant materials could be rendered acid-soluble after treatment with RNase T2 (10 µg/ml) in the above buffer.

**Determination of the specific radioactivity of UTP and of the cell number.** Isolated cells prepared from 30 embryos at each developmental stage were labeled for 1.5 and 3 hr with ³H-uridine (70 µCi/ml) as described above, then mixed with 270 non-labeled, sibling embryos. The whole was then homogenized in 0.5 N perchloric acid (PCA). PCA-soluble materials were purified, then chromatographed on columns of Dowex-1 (13). The optical density of the fractions was measured and the radioactivity was counted as described above. The content of UTP per embryo was about 450 pmoles and showed no change between the blastula and tailbud stages. This agrees with the results of Woodland and Pestell (29) and of Nakanashi and Yamana (13). The average specific radioactivity of UTP during the 3 hr-labeling was calculated according to Humphreys (9). A ten-fold increase in specific radioactivity occurred between the blastula and tailbud stages, due mainly to the sharp increase in incorporation into the nucleotide.

PCA-insoluble materials were washed successively with 0.5 N PCA and ethanol-ether (1:1), then extracted with 0.5 N PCA for 20 min at 100°C. The hot PCA extract was used to determine the radioactivity in the RNA and the amount of DNA. The cell number per embryo was calculated from the DNA content, using a value of 6 pg DNA per diploid cell (6). The results are in good agreement with those of Dawid (6).

**RESULTS**

**Characterization of poly(A)-containing RNA.** Isolated cells were labeled with ³H-uridine for 3 hr at each developmental stage, after which the poly(A)-containing RNA was purified from the bulk of the radioactive RNA with a poly(U)-Sepharose column. The poly(A)-containing RNA comprised 8, 12, 16 and 16% of the total labeled RNA in the blastula, gastrula, neurula and tailbud embryo cells, respectively. When fractionated in a sucrose density gradient, the poly(A)-containing RNA was distributed heterogeneously from 10S to >40S, with an average S-value of 25 (1.4 × 10⁶ daltons) regardless of the embryonic stage used (Fig. 1). This size value was much smaller than that of the 30 min-labeled poly(A)-containing RNA reported previously (20). Thus the present poly(A)-containing RNA may be mainly cytoplasmic (cf. 23). Cell fractionation experiments showed that more than half of the poly(A)-containing RNA labeled under the present conditions was cytoplasmic and that a quarter was polysomal (unpublished data). The possibility that heterogeneous size distribution might be due to molecular aggregation has previously been excluded in a sedimentation study under denaturing conditions (20).
Poly(A)-RNA in *Xenopus* Embryos

Poly(A) sequences were obtained from poly(A)-containing RNA labeled for 3 hr with $^3$H-adenosine (70 μCi/ml). Poly(A)-containing RNA, equivalent to either 15 embryos (for blastulae and gastrulae) or 7 embryos (for neurulae and tailbuds), was centrifuged on 5-30% sucrose gradients. The positions of the 4S, 18S and 28S RNAs, each designated by an arrow, were determined with rat liver RNA as the reference. (A), blastulae; (B), gastrulae; (C), neurulae and (D), tailbud embryos.

Poly(A) sequences were obtained from poly(A)-containing RNA labeled for 3 hr with $^3$H-uridine at each developmental stage. Poly(A) sequences comprised about 10 per cent of the poly(A)-containing RNA, and appeared on acrylamide gels as a relatively homogeneous peak moving slower than 5S RNA (Fig. 2). These features of the poly(A) sequence persisted throughout the developmental stages. From the relative rate of migration (15), the major component of the poly(A) sequences was estimated as approximately 140 nucleotides long. This size was intermediate between values for 30 min-labeled and 5 hr-labeled poly(A) sequences cited previously (20). Also in sea urchin embryos, the size distribution of poly(A) sequences is relatively unchanged throughout their development (30).

**Quantification of poly(A)-containing RNA synthesis.** Isolated cells were labeled with $^3$H-uridine under the conditions described above. The content of UTP and the radioactivity incorporation into it were measured. Specific radioactivities of UTP at the different developmental stages were obtained as described in the methods section.
The average number of cells per embryo and the amount of radioactivity incorporated into poly(A)-containing RNA were also determined. Radioactivity incorporation into poly(A)-containing RNA was calculated on the proportion in the total labeled RNA. The radioactivity in the poly(A)-containing RNA increased as development proceeded. Absolute amount of newly synthesized poly(A)-containing RNA were calculated on the basis of radioactivity incorporated and on the specific radioactivity of the UTP (Fig. 3). Results show that a blastula and a tailbud embryo synthesized 2 ng and 12 ng of poly(A)-containing RNA per hour, respectively. On a per-cell basis, however, the situation was reversed: the rate was highest at the blastula stage (0.8 pg/hr), decreasing sharply from the gastrula stage on and reached a constant low level (0.11 pg/hr) at the tailbud stage (Fig. 3).

**DISCUSSION**

Our purpose was to characterize and quantify poly(A)-containing RNA synthesis during the early development of *Xenopus laevis*. The advantage of employing isolated cells, rather than intact embryos, and the reliability of the results obtained lie in the markedly higher activity of isolated cells in incorporating exogenously supplied labeled nucleosides and amino acids (22), and the fact that the temporal changes in RNA synthesized during culture are the same as those of intact embryos during their development (22).
Poly(A)-RNA in *Xenopus* Embryos

Sedimentation and electrophoretic analysis showed that the size of poly(A)-containing RNA newly synthesized during early development and that of the poly(A) sequences were, on the average, 1.4 × 10^6 and 50,000 daltons, respectively. A similar size for poly(A)-containing RNA has been observed with oocytes (18), cultured liver cells (19), cultured tadpole tails (10) and cultured kidney cells (18, Misumi *et al.*, to be published) of *Xenopus laevis*. No stage-dependent difference has been found in the size of poly(A)-containing RNA during early development. Probably the size does not change significantly throughout life, since, as described above, a similar size for poly(A)-containing RNA has been obtained in certain somatic cells.

A dramatic change, however, is seen during development when the rate of synthesis

---

**Fig. 3.** The absolute amount of poly(A)-containing RNA synthesized at various developmental stages. Isolated cells from 30 embryos at each developmental stage were labeled for 1.5 and 3 hr with \(^3\)H-uridine (70 μCi/ml) as in the experiment in Fig. 1, then they were mixed with 270 non-labeled, sibling embryos. The specific radioactivity of UTP, the incorporation into the total RNA and DNA contents were determined as described in Methods. The amount of radioactivity in the poly(A)-containing RNA was determined from the proportions in the total labeled RNA as described in the experiment in Fig. 1. The absolute amount of the poly(A)-containing RNA synthesized was calculated by dividing the radioactivity incorporated by the average specific radioactivity of UTP during a 3 hr-labeling (9, 13). It is shown as the amount synthesized per embryo or per cell per hr. The nucleotide composition of the poly(A)-containing RNA obtained was assumed to be equal to that of DNA-like RNA (2).
was expressed on a per-embryo or per-cell basis. The amount of poly(A)-containing RNA synthesized per-embryo increased sharply from the blastula (2 ng/hr) to the tailbud (12 ng/hr) stages. In contrast, on per-cell basis, it had the highest value at the blastula (0.8 pg/hr) stage, then decreased sharply to a very low level (0.1 pg/hr) during gastrulation and later stages.

Similar changes have been observed for “DNA-like RNA” as putative messenger RNA. For example, the rate of DNA-like RNA synthesis calculated from the results of Brown and Littna (3) are 0.25 and 0.05 ng/cell/hr for the early gastrula (stages 9-10) and neurula (stages 15-16) stages, respectively. These values are somewhat lower than ours, but the discrepancies may be due to differences in the methods used. However, exactly the same trend can be observed between the results of Brown and Littna and ours: the rate was several times higher at the blastula stage than at the neurula stage.

From the data given in Fig. 1 and from the estimated average molecular weight ($1.4 \times 10^6$ daltons), the number of poly(A)-containing RNA molecules newly synthesized can be calculated. Thus, about 7,000 molecules were synthesized per min per cell at the blastula stage; a figure ten times higher than that at the tailbud stage.

The biological significance of the higher rate of poly(A)-containing or messenger RNA synthesis in the blastula cells is not yet clear. However, since at least 50% of these newly synthesized poly(A)-containing RNA could be cytoplasmic, they are likely to raise the polysomal level per cell and participate in active protein synthesis, together with maternal messenger RNA. The total polysome content per embryo has been shown to increase sharply during cleavage and blastulation while the amount of persisting messenger RNA decreases gradually during blastulation and gastrulation (4, 28). The higher activity of synthesis may also be related to DNA-dependent RNA polymerase II whose activity is much higher at the blastula stage than it is at the tailbud and later stages (16).

Several points should be clarified to establish the validity of the present results. First, our estimates might have been affected by the degradation of RNA during extraction. However, sedimentation profiles of the total labeled RNA showed no detectable amounts of degradation products. Second, the turnover of poly(A)-containing RNA could be ruled out because poly(A)-containing RNA was accumulated almost linearly for at least 5 hr during labeling (20). Finally, intracellular compartmentalization of the UTP would cause ambiguity in calculating the absolute amount synthesis. However, in Hela cells a single pool has been shown, at least for messenger RNA (27), and this does not favor compartmentalization in embryonic cells of *Xenopus laevis*.

**Acknowledgment.** We thank Dr. K. Hori for his generous gift of RNase T2. This work was supported in part by a grant from the Ministry of Education of Japan.

**REFERENCES**


(Received for publication, January 27, 1978)