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Activation of embryonic genome in chick

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Summary

The earliest stages of development in most animals are under the control of maternally inherited information. The initiation of embryonic gene expression has been reported at the mid-blastula in amphibians and the mid-2-cell stage to the early morula in mammals. In chick embryos, embryonic gene expression was detectable at stage X (morula) and showed marked activation at stage XIII (blastula) with a gradual increase thereafter. Synthesis of rRNA and tRNA was low at stage X and was already the major class of RNA at stage XIII in chick embryos. The observed upregulation of RNA synthesis seems to coincide with a period of extensive fine structural differentiation when the first major cellular migrations start and signal the formation of the primitive streak in the chick embryo.

Keywords: Gene activity, RNA, Chick embryo

Introduction

Early development of the embryo appears to be largely or exclusively under the control of maternal factors stored in the oocyte during oogenesis and does not require transcription of the embryonic genome in many species (review by Davidson, 1986). Organisms differ in the timing and the abruptness of the transition from the cytoplasmic control of development to the regulation of development by nuclear transcription. The transcription from maternal factors to activation of the embryonic genome transcription occurs at the mid-blastula in the frog embryo (Bacharova & Davidson, 1996; Newport & Kirschner, 1982*a*, *b*; Kimelman *et al.*, 1987). *Drosophilia* embryos generally follow the *Xenopus* pattern in that bulk transcription does not commence until after cleavage stage during cycle 14 just prior to the onset of gastrulation (Lamb & Laird, 1976; McKnight & Miller, 1976; Zalokar, 1976; Anderson & Lengyel, 1979; Edgar & Schubiger, 1986). Sea urchin lacks a stark transition from maternal to embryonic control. Although enucleated sea urchin eggs can develop through blastula stages (Harvey, 1936), and although there is a burst of nuclear transcription from mid-blastula nuclei, there seems to be no time in sea urchin development when the embryonic nucleus is not functioning (Wilt, 1964; Nemer & Infante, 1965; Kedes & Gross, 1969; Rinaldi & Monroy, 1969; Levner, 1974; Brandhorst, 1980). Dependence on expression of the embryonic genome cannot be detected until the mid-2-cell stage in the mouse (Braude, 1979*a*, *b*; Flach *et al.*, 1982; Clegg & Piko, 1983; Bolton *et al.*, 1984; Latham *et al.*, 1982), the 4- and 8-stage cell stages in the human (Braude *et al.*, 1988) and the 8-cell stage in the sheep (Crosby *et al.*, 1988). In the chick, embryos at stage X are not sensitive to transcriptional inhibition, but some of the major qualitative changes that occur in embryos at stage XIII are dependent on transcription (Zagris & Matthopoulos, 1987, 1988).

The chick blastoderm at stage X (homologous to the morula in amphibians) is a flat compacted disc about 2 mm in diameter. Development of the chick embryo (Hamburger & Hamilton, 1951; Vakaet, 1962, 1970; Eyal-Giladi & Kochav, 1976) from the blastoderm at stage X involves sequentially: the formation of the epiblast (upper layer) and the hypoblast (lower layer) from the blastoderm disc; the interaction between the epiblast and the newly formed hypoblast (blastula stage, stage XIII) and induction of the primitive streak (initiation of gastrula formation, stage HH2); the migration of cells to form the primitive streak (homologous to the blastopore in amphibians, stage HH3–4),

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the site through which cells migrate to form mesoderm and definitive endoderm; and the interaction between the dorsal mesoderm (chordamesoderm) and its overlying ectoderm to form the neural plate (stage HH5) that forms the nervous system. The objective of the present work was to study the onset of genome activation during development of the chick embryo. The results of a comparison of patterns of RNA synthesis in the early chick embryo are presented.

Materials and methods

Labelling of embryos

Fertilised chicken eggs (Epirus Poultry Co., Ioannina, Greece) were used. The chick embryo in a freshly laid chicken egg is at stage X, which is homologous to the morula in amphibians. Embryos at stages XIII (blastula), HH2 (initial primitive streak), HH3–4 (intermediate to definitive streak) and HH5 (head process) were obtained after incubation of eggs at 37 °C. Roman and arabic numerals indicate the stages of chick embryo development according to Eyal-Giladi & Kochav (1976) and Hamburger & Hamilton (1951), respectively. Embryos were removed from the eggs and the adhering yolk cleaned off with fine dissecting needles in chick Ringer solution. Support 'rafts' were made of the vitelline membrane (New, 1955) and the cleaned embryos were flattened separately, epiblast side against the surface of the raft.

Each raft was placed on 0.3 ml chick Ringer solution containing 180 μCi of ^{32}P-orthophosphate (37 MBq/0.1 ml, 1 mCi/0.1 ml, Amersham) per millimetre for 5 h at 38 °C. Labelling was stopped on ice, embryos were detached from the vitelline membrane rafts, grouped separately and stored at –80 °C overnight until RNA extraction. Sterile procedures were employed throughout this work.

RNA isolation and gel electrophoresis

For the analysis of high-molecular-weight RNA, embryos at stages X, XIII and HH2 (12 embryos per stage) were homogenised separately in 3 volumes of lysis buffer (50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 5 mM ethylenediamine-tetraacetic acid (EDTA), 200 μg proteinase K/ml and 0.5% sodium dodecyl sulphate (SDS), and RNA was extracted with phenol–chloroform–isoamyl alcohol, and ethanol precipitated as described by Sambrook *et al.* (1989).

To determine acid-insoluble radioactivity, 1 μl of a homogenate before the RNA phenol extraction was spotted onto a Whatman GF/C filter. Filters were placed in a series of trichloroaceitc acid and ethanol–ether washes. Radioactivity was measured by scintillation counting in toluene containing 0.5% PPO (2,5-diphenyloxazole) and 0.03% POPOP. (2,2'-*p*-phenylene-bis[5-phenyloxazole]).

The RNA content of embryos at stages X, XIII and HH2 was quantified spectrophotometrically from homogenates of whole unlabelled embryos. Total RNA is pure with an $A_{260/280}$ ratio of 1.7–2.0.

Equal amounts of RNA from embryos at stages X, XIII and HH2 were fractionated on 0.8% agarose/formaldehyde gels for the determination of high-molecular-weight RNA as described by Sambrook *et al.* (1989).

For the analysis of low-molecular-weight RNA, embryos at each of stages X, XIII, HH3–4 and HH5 were homogenised in a buffer containing 50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.5% SDS, 3.5 M urea and 0.2 M NaCl, followed by phenol extraction as described previously (Guialis *et al.*, 1987). Precipitated nucleic acids were resuspended in 20 mM Tris-HCl, pH 7.4, 10 mM MgCl and digested with 40 units DNase (RN-ase free) at 37 °C to remove residual DNA. Following re-precipitation of RNA molecules, lower-molecular-weight RNA species were resolved on 10% polyacrylamide–7 M urea gels and uniformity of RNA loads was verified by visual inspection of ethidium bromide (EtBr, 0.5 μg/ml) stained gels.

Visualisation of ^{32}P-labelled RNAs was possible after exposing dried gels to Kodak X-AR5 X-ray film at –80 °C.

Molecular weight of embryonic RNAs was estimated using *E. coli* rRNA (Mino Tech) and a 50–2000 bp DNA ladder (Promega) as markers.

Results and discussion

The onset of genome activation during development of the chick embryo was studied in the present work by identifying the newly transcribed RNA species. This was achieved by incubating the embryos in the presence of ^{32}P-orthophosphate followed by RNA isolation and subsequent resolution on denaturing RNA gels.

The mean values of total RNA yields were 3.73 μg and 3.53 μg RNA per embryo at stages X and XIII, respectively. Variation of these mean values was 5–10%, which represents different experiments. The radioactivity incorporated into RNA increased significantly at stage XIII. The specific activities of RNA at stages X and XIII were 5.27 and 19.95 (cpm $\times 10^{-3}$/μg RNA), respectively. The specific activity of RNA increased at stage XIII without accumulation of RNA content, suggesting a high rate of turnover.

Electrophoretic patterns of newly synthesised low- and high-molecular-weight RNA from embryos at stages X, XIII, HH2, HH3–4 and HH5 labelled with ^{32}P-orthophosphate are shown in Figs. 1 and 2, respectively. In Fig. 1, initial staining of the gel with EtBr was used

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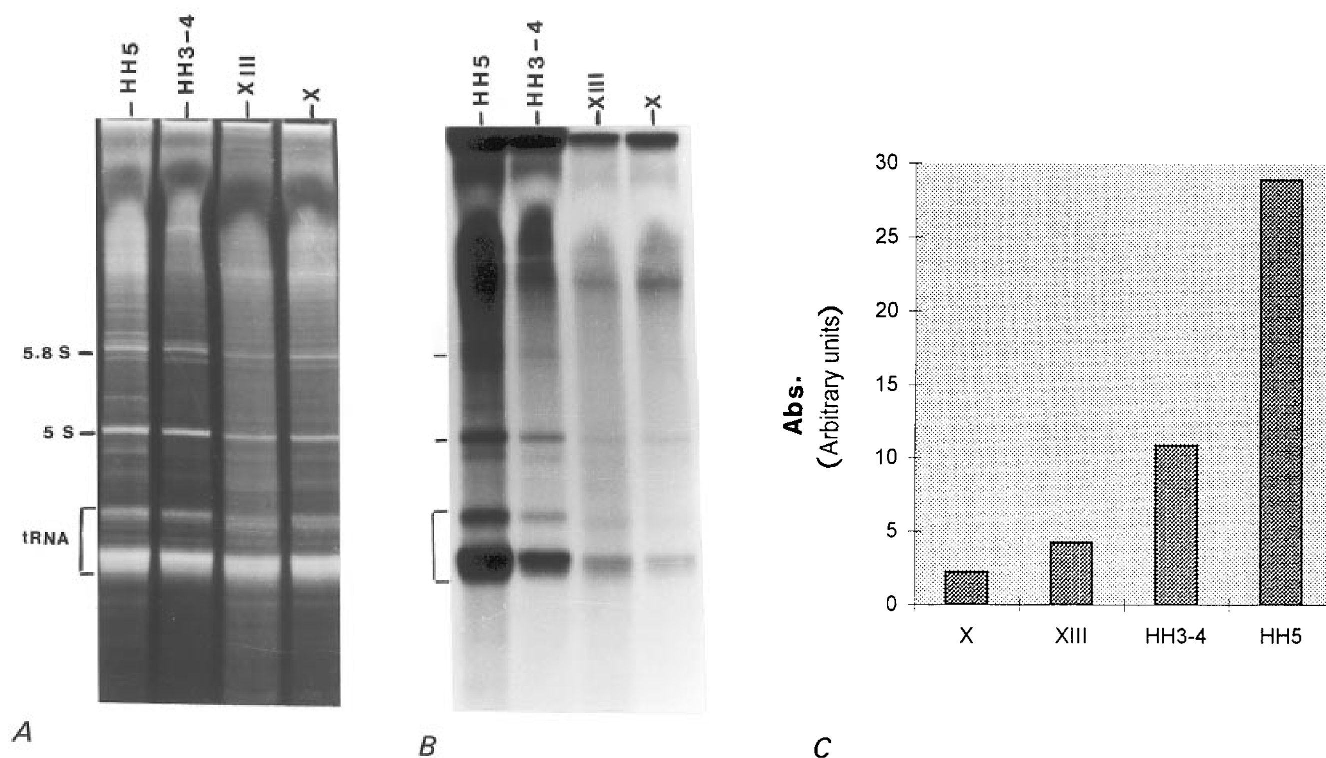

Figure 1 Newly synthesised low-molecular-weight RNA species during early development of chick embryo. Total RNA was extracted from chick embryos at stages X, XIII, HH3–4 and HH5 following a 5 h incubation in the presence of ^{32}P-orthophosphate. Low-molecular-weight RNAs were resolved on a denaturing polyacrylamide/urea gel and visualised by staining with EtBr (*A*). ^{32}P-labelled RNA species were detected following autoradiography of the same gel (*B*). The position of migration of marker 5.8S, 5S and tRNA species is indicated. Relative expression (absorbance in arbitrary units) of newly synthesised RNAs at stages X, XIII, HH3–4 and HH5 was quantified by densitometric scans of the labelled tRNA band on the autoradiogram (*C*).

as a way of normalising for the total RNA applied on the gel in order to allow for expected differences in the RNA content of the different stages. As seen in Fig. 1*A*, roughly equal amounts of low-molecular-weight RNAs, corresponding to embryonic stages X, XIII, HH3–4 and HH5, were resolved on the gel. The bulk of these RNAs belonged to the abundant 5.8S and 5S rRNA, as well as to the tRNA species, as was expected. Some of the additional RNA bands may correspond to spliceosomal snRNAs as judged from their mobility in the gel (Guialis *et al.*, 1987 and references therein). Autoradiography of the gel (Fig. 1*B*) revealed the low-molecular-weight RNA species that were transcribed in embryonic stages X, XIII, HH3–4 and HH5. As seen, newly synthesised tRNAs (5.8S and 5S) and tRNA were detectable as early as stage X, with a gradual increase thereafter. The rate of synthesis of these low-molecular-weight species is shown schematically in the histogram of Fig. 1*C* that was produced following scanning of the major tRNA band of the autoradiograph.

In Fig. 2, equal amounts of RNA (10 μg RNA) from chick embryos at stages X, XIII and HH2 were applied to each separate well on a slab gel. Synthesis of high-molecular-weight RNA was low in embryos at stage X (Fig. 2). Similar results to stage X were obtained with RNA isolated from embryos at stage XI to XII (mid-blastula) (results not shown). In embryos at stage XIII there was an abrupt, marked increase in synthesis of RNA and prominent bands associated with 28S and 18S rRNA were shown in the electrophoretic pattern (Fig. 2). As shown in the histograms of Fig. 1*C* and 2*B*, there was a 2- to 3-fold increase in the synthesis of both low- and high-molecular-weight RNAs between stages X and HH2.

Several studies investigating the relationship between embryonic gene expression and the timing of developmental events make use of the selective action of α-amanitin, which is a specific inhibitor of the nucleoplasmic enzyme RNA polymerase II that catalyses synthesis of heterogeneous nuclear RNA (Lindell *et al.*, 1970; Zybler & Penman, 1971; Levey & Brinster, 1978; Braude *et al.*, 1988). Previous work using α-amanitin raised the possibility that normal development of chick embryo in the early stages, including protein synthesis, does not require transcription of the embryonic genome (Zagris & Matthopoulos, 1987, 1988). Our experiments had shown that the polypeptides synthesised in the

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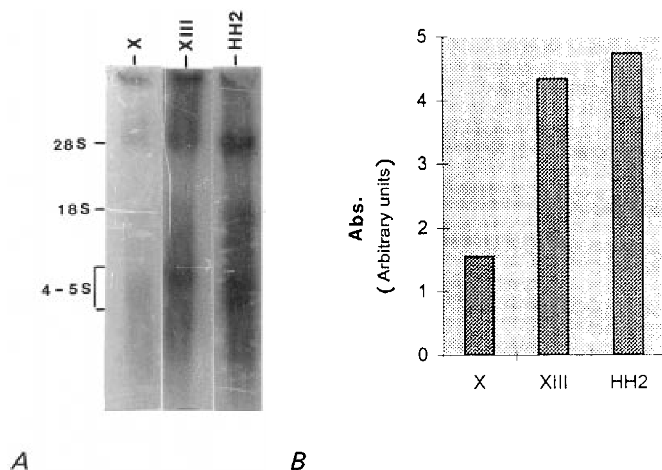

Figure 2 Newly synthesised high-molecular-weight RNA species during early development of chick embryo. Total RNA was extracted from chick embryos at stages X, XIII and HH2 following a 5 h incubation in the presence of ^{32}P-orthophosphate. Equal amounts of RNA were loaded onto a denaturing agarose/formaldehyde gel and ^{32}P-labelled RNA species detected following autoradiography of the gel (*A*). Molecular weight of embryonic RNAs was estimated relative to *E. coli* rRNA marker. Relative expression (absorbance in arbitrary units) of newly synthesised high-molecular-weight RNAs at stage X, XIII and HH2 was quantified by densitometric scans of the labelled 28S rRNA band on the autoradiogram (*B*).

chick embryo during stage X were insensitive to transcriptional inhibition by α-amanitin (Zagris & Matthopoulos, 1988). In the present work, electrophoretic analysis of the newly synthesised RNA presented in Fig. 1 and 2 showed that the embryonic genome was functioning at stage X. Then, protein synthesis would seem to depend predominantly, if not exclusively, on the recruitment of maternal mRNA rather than on embryonic gene expression in chick at stage X (Zagris & Matthopoulos, 1987). At stage XIII, marked activation of high- and lower-molecular-weight RNA synthesis was evident, with rRNA being already the major class of RNA (Figs. 1, 2). In previous work (Zagris & Matthopoulos, 1987), inhibition by α-amanitin was associated with qualitative and quantitative changes in the pattern of protein synthesis in the hypoblast and in the epiblast, respectively, in chick at stage XIII.

The observed upregulation of RNA synthesis described in the present work, and the qualitative and quantitative changes in the pattern of protein synthesis provoked by α-amanitin as described in previous work (Zagris & Matthopoulos, 1987, 1988), seem to coincide with a period of extensive fine structural differentiation when hypoblast interacts with epiblast and induces morphogenetic movements and formation of the primitive streak in the epiblast of the chick embryo. Development of the chick embryo proceeded to initiation of cellular migrations for formation of the primitive streak (early gastrula, stage HH2), but formation of the definitive primitive streak (stage HH4) seemed to depend on expression of the embryonic genome and was sensitive to transcriptional inhibition by treatment with α-amanitin (Zagris & Matthopoulos, 1987, 1988). The abrupt change at stage XIII thus seemed to be the start of synthesis of RNA which might have some relation to the beginning of gene transcription occurring at gastrulation in chick embryos. The initiation of zygotic gene expression has been reported at the mid-blastula in amphibians and the mid-2-cell stage to early morula in mammals. In the chick embryo, the zygotic genome is of low activity at stage X but shows marked activation and proceeds maximally during stage XIII, and this may be of evolutionary relevance.

Acknowledgements

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