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Localization of c-myc expression during oogenesis and embryonic development in *Xenopus laevis*

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Summary

The expression of the proto-oncogene c-myc during oogenesis and embryonic development was followed by *in situ* hybridization using a cytological protocol adapted to amphibian embryos. The c-myc RNA was highly expressed in the cytoplasm of young oocytes and was further diluted during oocyte growth without specific localization. From the neurula stage on, new myc transcripts were detected and the whole embryo appeared positive with antisense myc RNA probes relative to control sense RNA probes. In addition, a spatial localization of high levels of the transcript was also observed in specific areas of the developing embryo, including the epidermis, gill buds, optic vesicles and lens placodes. These observations might indicate a specific role of the c-myc gene during the differentiation of these tissues. Alternatively, this high level of myc expression might prevent such tissues from entering into terminal differentiation during the growth of the embryo.

Key words: c-myc, *Xenopus laevis*, proto-oncogene, oogenesis, RNA, differentiation.

Introduction

Expression of c-myc proto-oncogene has been correlated with cell proliferation in a number of experimental systems (for reviews, see Heldin & Westermark, 1984; Bishop, 1985). We previously reported the characterization of the *Xenopus laevis* c-myc cDNA sequence and showed that this proto-oncogene was closely homologous to its mammalian counterpart (Taylor *et al.* 1986). Northern blot analysis showed that this gene was expressed as a stable maternal mRNA during oogenesis. After fertilization, the myc mRNA level decreased until the gastrula stage, when it reached a minimum value per embryo. At the neurula stage, the myc mRNA content increased again and we assumed that it was a consequence of new transcription in the embryo (Taylor *et al.* 1986; King *et al.* 1986). This biochemical analysis of the steady state myc mRNA level was done on whole embryos and did not provide information about spatial localization of the transcript. Localization of specific transcripts during embryogenesis can provide information on early cell determination before any morphological change occurs. Thus specific localization of maternal mRNA during oogenesis has been recently demonstrated (Rebagliati *et al.* 1985; King & Barklis, 1985; Melton, 1987) and might, at least partly, account for mesoderm induction in the developing *Xenopus* embryo (Weeks & Melton, 1987; Kimelman & Kirschner, 1987). We used *in situ* hybridization to assay for localization of myc transcripts during oogenesis and embryonic development. For that purpose, we adapted to amphibian embryos a protocol designed for cytological studies in yolk-laden insect eggs and embryos (Pétavy, 1985). We confirm that the myc proto-oncogene is highly expressed in early oocytes and show that its transcript is uniformly distributed in the cytoplasm. From stage-III to stage-VI oocytes (Dumont, 1972), the myc mRNA content is diluted in the expanding cytoplasm and does not exhibit a detectable preferential localization. From the neurula stage on, when new myc transcripts are detected, we show that their distribution is not uniform throughout

different tissues. The correlation between the localized c-myc expression in a developing embryo and the putative role of c-myc in cell proliferation and differentiation is discussed.

Materials and methods

Collection of oocytes and embryos

Xenopus laevis adults imported from South Africa (South Africa Farms, Fish Hoek) were accommodated and fed as described by Gurdon (1967). Oocytes, eggs and embryos were obtained as previously described (Taylor *et al.* 1986). Unless specified, *in situ* hybridizations were performed with albino *Xenopus* embryos to avoid confusion between pigment granules and silver grains.

Preparation of cut sections

Preliminary experiments using standard paraffin embedding for oocytes and early embryos were not successful in our hands. The technique, originally described by Pétavy (1985) for yolk-laden insect eggs and embryos, was adapted to *Xenopus* embryos. It gave satisfying results for both cytological studies and *in situ* hybridizations. The oocytes and embryos were fixed with freshly prepared 4% paraformaldehyde in 0·5× PBS, pH 7·4 for 1 h at 4°C, washed three times in 1× PBS for 30 min each. Oocytes and embryos were then closely coated with 1·4% (w/v) agarose at 60°C according to Pétavy (1985). Specimens were cast in molds filled with the agarose solution. After chilling, the agarose gel blocks were trimmed as little cubes containing specimens. They were then treated by sequential immersion in 50% ethanol (2×20 min); 70% ethanol (2×20 min); 50% ethanol, 50% ethoxy-ethanol (2×20 min); 66% ethanol, 33% 2-ethoxy-ethanol (2×20 min), pure 2-ethoxy-ethanol (4×30 min), and butanol-1 (several changes of 24 h each). The specimens were placed 20 min at 50°C in a 1:1 mixture of butanol-1 and ester wax (Gurr). This final embedding medium was then processed through five baths of pure ester wax at 50°C, under increasing vacuum in order to evaporate butanol-1 and facilitate infiltration with ester wax. Blocks were then cast in molds containing melted ester wax and stored at 4°C. 7·5 μm sections were mounted on poly-L-lysine-coated slides. After removal of ester wax by toluene and rehydration in decreasing ethanol concentrations, they were stained with 0·1% toluidine blue.

RNA probes

A 680 bp *Hin*cII–*Pvu*II fragment from myc, including part of Exon II and Exon III (Taylor *et al.* 1986) was subcloned into the transcription vector Bluescribe (Stratagene) in both orientations, linearized with *Bam*HI and transcribed with T3 RNA polymerase (Stratagene). Thus, both sense and antisense probes were synthesized. The radioactive nucleotide was ^{35}S-UTP, 400 Ci mM^{-1} (Amersham). The probes had a specific activity of 4–6×10^8 cts min^{-1} μg^{-1} and were purified as described by Angerer *et al.* (1987*a*,*b*). Template DNA was removed by digestion with RNase-free DNase (Promega). Reduction of probe fragment length was performed as described by Angerer *et al.* (1987*a*,*b*), to an average length of 100 nucleotides as determined by electrophoresis through a 6% denaturing polyacrylamide gel.

DNA probes

The 680 bp *Hin*cII–*Pvu*II myc insert separated by acrylamide gel electrophoresis was used for random-priming labelling according to Feinberg & Vogelstein (1983) using ^{35}S-dATP, 400 Ci mmole^{-1} (Amersham). Reduction of probe length was carried out using digestion with *Hin*fI and *Pst*I. Control probes were derived from pUC18 digested by *Hae*II and *Bgl*I and labelled by random-priming. Probe length was further reduced by digestion with *Pvu*I. Fragments were purified by filtration on Biogel P100. The DNA probes had a specific activity of 3–5×10^8 cts min^{-1} μg^{-1} and the length of fragments ranged from 70 to 200 bp.

In situ *hybridization*

In situ hybridization was carried out on 7·5 μm sections following Cox's *et al.* (1984) and Angerer's *et al.* (1987) protocols, with minor modifications.

Prehybridization

Prehybridization treatment included removal of ester wax, treatment with 0·2 N-HCl for 20 min at room temperature, digestion with 1 μg ml^{-1} proteinase K (Merck) in 20 mM-Tris–HCl, pH 7·4, 2 mM-$CaCl_2$ for 15 min at 37°C, treatment with 0·25% acetic anhydride in triethanolamine buffer, followed by two rinses in 2×SSC and dehydration in ethanol.

Hybridization

For DNA probes, hybridization was performed at 30°C in 50% formamide, 4×STE buffer pH 7·4 (0·6 M-NaCl, 10 mM-Tris–HCl, pH 7·4, 1 mM-EDTA), 1×Denhardt's, 8% dextran sulphate, 10 mM-DTT, 100 μg ml^{-1} *E. coli* sonicated DNA, 100 μg ml^{-1} tRNA, 0·06 μg ml^{-1} ^{35}S-labelled DNA probe (5–7×10^5 cts min^{-1} per slide). Slides were kept overnight at 30°C in a moist chamber (Gall & Pardue, 1969). For RNA probes, hybridization was performed at 48°C in 50% deionized formamide, modified STE buffer (0·3 M-NaCl, 10 mM-Tris–HCl, pH 7·5, 5 mM-EDTA), 1×Denhardt's, 8% dextran sulphate, 10 mM-DTT, 100 μg ml^{-1} yeast tRNA, 0·06 μg ml^{-1} ^{35}S-labelled RNA probe (5–7×10^5 cts min^{-1} per slide), for 16 h.

Post-hybridization washes

For DNA probes, slides were washed twice in 50% formamide, 4×STE, 10 mM-DTT at 30°C, twice in 50% formamide, 2×STE, 10 mM-DTT at 30°C, once in 50% formamide 1×STE, 10 mM-DTT at room temperature. For RNA probes, slides were washed twice in 2×SSPE at room temperature for 1 h, once in 2×SSPE, 50% formamide, 10 mM-DTT at 60°C for 30 min, treated with 20 μg ml^{-1} RNAse A and 1 i.u. ml^{-1} RNAse T1 in 4×SSPE at 37°C for 30 min, washed in 4×SSPE at 37°C for 15 min, 2×SSPE for 1 h at room temperature, 1×SSPE, 0·5×SSPE and 0·1×SSPE for 30 min each at room temperature.

Autoradiography

Slides were dipped in Kodak NTB2 emulsion and exposed for 5 to 19 days at 4°C. After development, they were stained for 20–30 s with 0·04 % Giemsa in 10 mM-potassium phosphate buffer pH 7·3.

Quantification of autoradiographic silver grains

Images of the sections formed by a Leitz Orthoplan microscope were scanned by a Plumbicon camera and analysed by a Quantimet 720 (Cambridge Instruments) connected to a PDP 11/34 computer (Digital equipment). The number of silver grains was counted over an area of the sections. The diameter of the oocytes was determined by encircling the sections with a lightpen on the screen.

Results

C-myc RNA is uniformly distributed in the cytoplasm of young oocytes and is diluted during oocyte growth

Precise localization of transcripts by *in situ* hybridization requires that good preservation of cell and tissue structure be compatible with mRNA accessibility and stability. The use of paraffin embedding has been found suitable for most tissue sections (Angerer *et al.* 1987; and our unpublished observations). Although used with success with oocytes and *Xenopus* embryos (Dworkin-Rastl *et al.* 1986; Melton, 1987; Kintner & Melton, 1987; Jamrich *et al.* 1987) this technique often gave poor oocyte sections in our hands. As yolk platelets appeared responsible for these drawbacks, we adapted for amphibians (Materials and methods) a technique originally described for insect yolk-laden eggs and embryos (Pétavy, 1985). This gave satisfactory results for all stages of oogenesis and embryogenesis (Figs 1, 4 and 5), preserving the integrity of both nuclear and cytoplasmic substructures such as nucleoli, yolk platelets and membranes (Fig. 1 and its legend).

Fig. 2A,B shows oocytes after hybridization with a ^{35}S-DNA probe and autoradiography with a 7-day exposure. Stage-I oocytes show no signal from the nucleus but a strong signal over the cytoplasm, indicating a high concentration of myc mRNA. In all experiments, hybridizations with a control pUC18 DNA probe were performed in parallel and no signal above background was observed (Fig. 2G). Fig. 2C and D show stage-I oocytes after hybridization with a ^{35}S-labelled antisense RNA probe. Although the specific activity and the exposure time were the same as for the experiments with the DNA probe, the signal is much higher, due to the higher sensitivity of RNA probes as compared to DNA probes. In all cases, hybridizations were performed in parallel with a control sense RNA probe; Fig. 2H shows the background signal with that control. With both DNA and RNA probes, the labelling was intense in previtellogenic stage-I oocytes (Fig. 2A–D) and decreased in later stages (Fig. 2E,F). From stage II on, oocytes accumulate yolk while their diameter increases from 300 μm to 1200 μm. The concentration of myc mRNA decreased during this period of oocyte growth. However, as quantified and discussed below, the total number of myc transcripts per cell remained relatively unchanged. In all cases, myc RNA appeared uniformly distributed in the oocyte cytoplasm and no specific localization was detected whatever the stage of oocyte growth.

Quantification of c-myc-RNA autoradiographic signals during oogenesis

The signal observed on a series of sections hybridized with c-myc was quantified during oocyte growth, as described in Materials and methods. RNA probes reproducibly gave higher signals as compared to DNA probes (Fig. 3A) as observed by Cox *et al.* (1984). This difference might reflect the low sensitivity of DNA probes at low transcript density. Thus it is likely that when the silver grains density is close to the background level, the number of transcripts is underestimated. We also noted, in repeated experiments, that the background level is steady when the exposure time is increased. Values of 9×10^{-3} grains per μm^2 and 8×10^{-3} grains per μm^2 were registered after 7- and 19-day exposures, respectively, with hybridizations using RNA probes. This observation might indicate that latent grains in the photographic emulsion are the source of most of total background (Cox *et al.* 1984). A stage-VI oocyte (10^9 μm^3) contains $4{\cdot}8\times10^6$ myc transcripts (Taylor *et al.* 1986), or 0·48 transcripts per 100 μm^3. In cut sections from such oocytes, a mean value of 1 grain per 100 μm^2 was registered with 7-day exposures with probes of 4 to 6×10^8 cts min^{-1} μg^{-1}. For a density of 1 transcript per 100 μm^3 cell and with a probe of $2{\cdot}5\times10^9$ cts min^{-1} μg^{-1}, 1·6 grains per 100 μm^2 per day would have been obtained. In another study using a 500 nucleotide probe (specific activity $2{\cdot}5\times10^6$ cts min^{-1} μg^{-1}) *versus* 680 nucleotides in our case, the same calculation suggested a maximum of 1·8 grains per 100 μm^2 per day (Angerer *et al.* 1987), which is similar to the sensitivity of the technique used here. Fig. 3A shows that the grain density reached a maximum level in small oocytes (100 μm diameter). From that stage until stage-III oocytes (450–600 μm), the grain density decreased about fourfold. During that period the volume of the oocyte increased from 0·523 to 113 nl, and so the actual number of silver grains per oocyte increased (Fig. 3B), and reached a near plateau level in oocytes above 600 μm diameter. In Fig. 3B, the number of grains was compared to the level of myc RNA as determined by slot blot and Northern blot

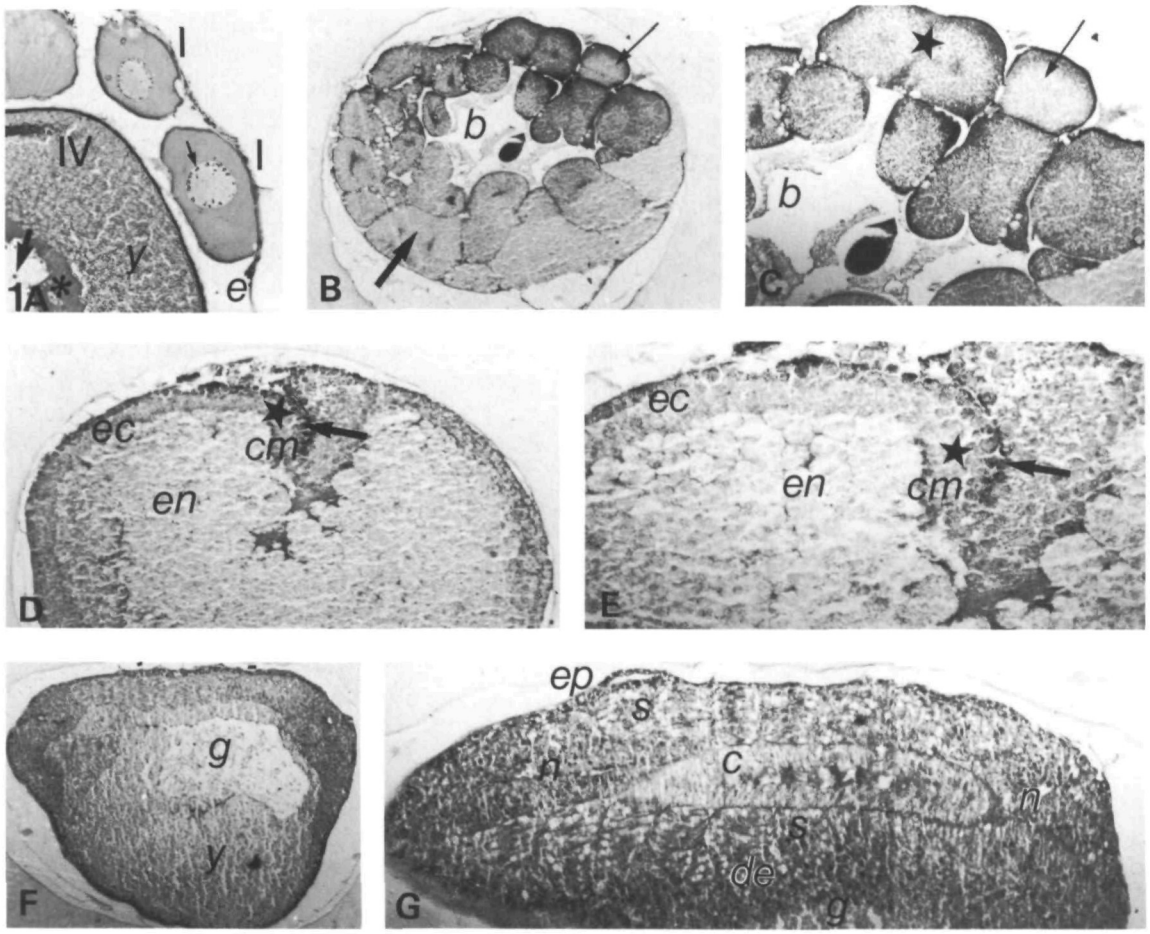

Fig. 1. Histological sections of *Xenopus* oocytes and embryos embedded by the ester-wax procedure. *Xenopus* oocytes and embryos were fixed and embedded as described in Materials and methods and 7·5 μm sections were stained with toluidine blue. (A) Stage-I and -IV oocytes. Stage-I oocytes are previtellogenic, the nucleus is clearly visible, has a regular shape and nucleoli can be seen at its periphery (thin arrow). Stage-IV oocytes have accumulated yolk platelets (*y*), the outline of the nucleus is irregular and the number of peripheral nucleoli decreases (thick arrow); a rim of toluidinic cytoplasm separates the nucleus from the surrounding yolk platelets (asterisk), ×100. *e*, oocyte envelopes (ovarian and follicular epithelia). (B) Late morula stage-5 embryo (Nieuwkoop & Faber, 1967). Large blastomeres containing larger yolk platelets are located at the vegetal hemisphere (thick arrow) and smaller blastomeres at the animal hemisphere (thin arrow); *b*, blastocelic cavity, ×50. (C) is a detail of B; a blastomere is dividing (asterisk), ×100. (D) Stage-10 early gastrula embryo. Gastrulation is indicated by the appearance of a slightly bent furrow (arrow); the fold on the left of this furrow is the dorsal lip of the blastopore (star); at this level, the future chordomesoderm invaginates (*cm*). *ec*, ectoderm; *en*, endoderm, ×70. (E) Detail of D, ×140. (F and G) Stage-22 neurula embryo: parasagittal sections. (F) General view, ×50; (G) detail of the dorsal region, ×110. *c*, notochord; *de*, dorsal endoderm; *ep*, epidermis; *g*, gut; *n*, neural tube; *s*, mesodermal cords beginning to divide into metamerized somites; *y*, yolk endoderm.

quantification (Taylor *et al.* 1986). Accumulation of myc RNA per oocyte was first observed in 100 μm diameter oocytes and reached a maximum level at stage III of oocyte growth (450–600 μm diameter). The *in situ* hybridization data were thus in agreement with the biochemical data previously reported.

C-myc RNA is highly expressed in specific tissues of the embryo

For all experiments described below, RNA probes were used and the results obtained have been reproduced in five independent experiments. The total amount of myc mRNA per embryo progressively decreases after fertilization (Taylor *et al.* 1986) and reaches a minimal value at the gastrula stage. During the early cleavage stages, the grain density of the whole embryo was low but a faint positive signal above background was reproducibly observed only at the morula stage in blastomeres of low yolk content in the animal hemisphere (not shown). We have not been able to detect any positive signal in the gastrula embryo. We previously showed that at neurula stage,

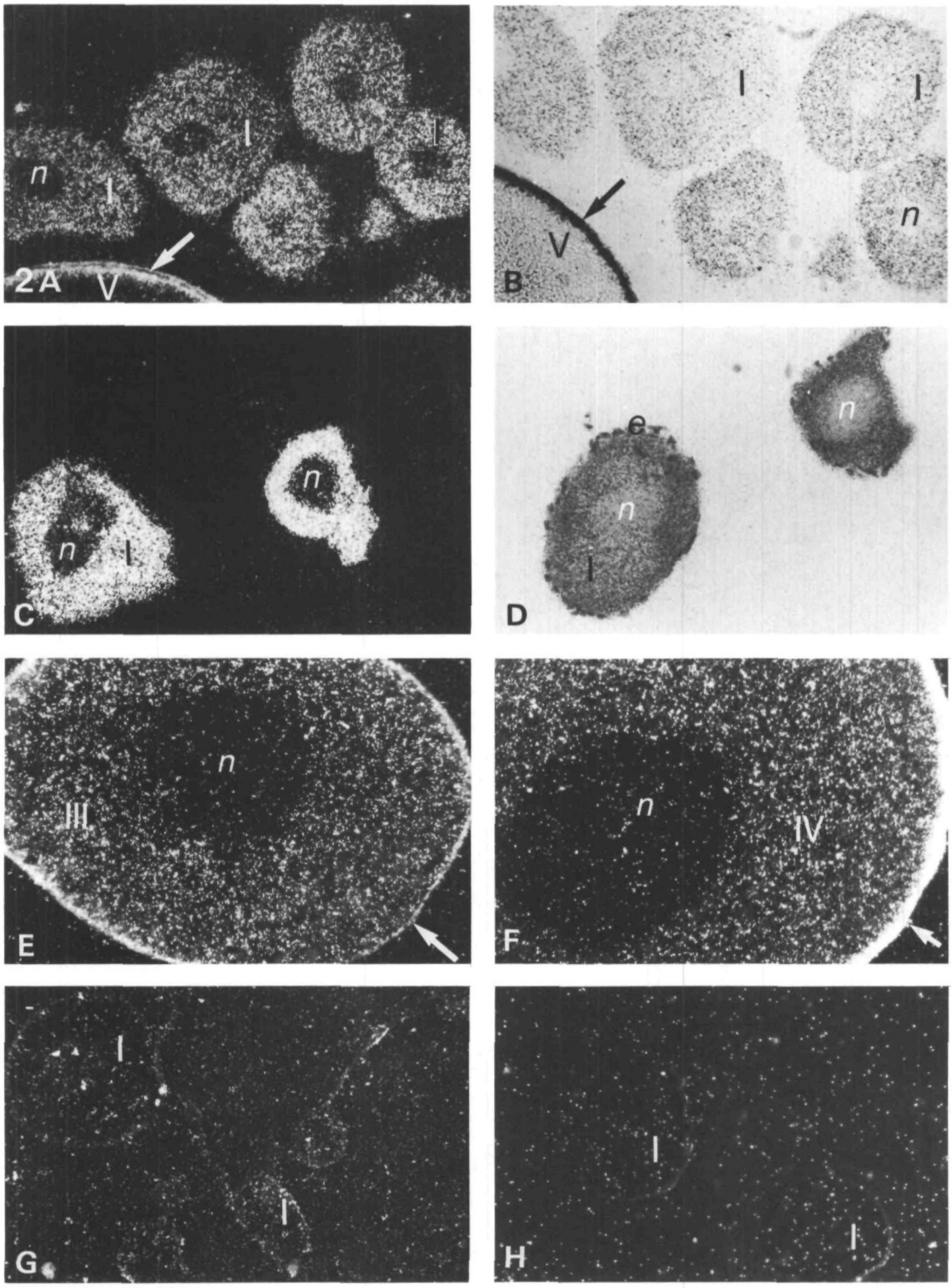

Fig. 2. Localization of c-myc RNA during oogenesis. *In situ* hybridization of myc ^{35}S-DNA probes (A,B) and myc antisense ^{35}S-RNA probes (C–F) to *Xenopus* oocytes sections. A high level of labelling was detected throughout the cytoplasm of young oocytes both with DNA (A,B) or RNA probes (C,D). In later stage-III and -IV oocytes (E,F), the density of silver grains is lower and still uniformly distributed in the cytoplasm. The white ring around the oocyte (arrow) is due to pigment granules in the cortex of wild-type oocyte. (G) Hybridization with a pUC18 DNA probe and (H) with a sense myc RNA probe: no signal above background can be observed. Labels as Fig. 1. (A,C,E–H) Dark-field illumination; (B,D) brightfield illumination. (E) ×120; (A,F,G) ×130; (B,C,D,H) ×180.

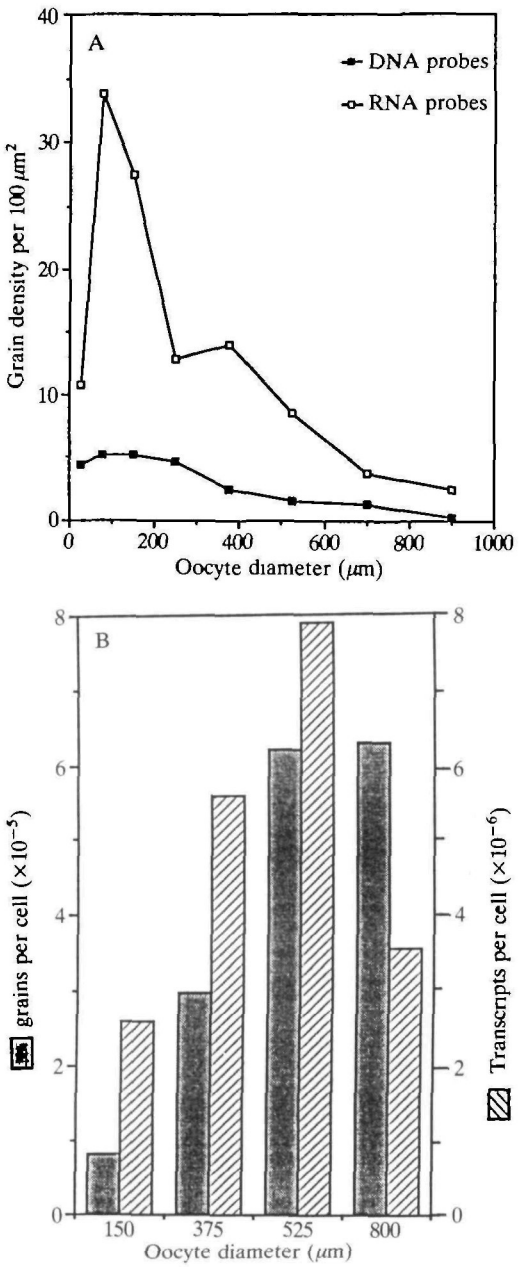

Fig. 3. Quantification of *in situ* hybridization signals during oogenesis. Quantification of silver grain density was done as described in Materials and methods. (A) Values were corrected for the grain density background as a function of oocyte diameter. Values are the average of several measurements in different sections. The average background values were 9×10^{-3} grains per μm^2 for RNA probes and 7×10^{-3} grains per μm^2 for DNA probes. (B) The number of grains per oocyte was calculated from the oocyte diameter and the thickness of the section (7·5 μm) with correction for the background level. It was compared with the number of myc RNA transcripts as determined by both slot blot and Northern blot hybridizations (Taylor *et al.* 1986). Histograms are displayed as a function of oocyte growth.

the average myc RNA content per average embryonic cell started to increase (Taylor *et al.* 1986). Using *in situ* hybridization, a positive signal was also observed for the neurula stage (Fig. 4). The whole embryo appeared faintly positive but the grain density was higher in posterior epidermis, neural tube, notochord and endoblast (see legend to Fig. 4). At neurulation, a number of maternal RNAs that had been degraded during the cleavage stage are again synthesized (Shiokawa *et al.* 1981; Dawid *et al.* 1985). In addition, new specific genes are expressed (Dworkin *et al.* 1984). The average cell cycle is markedly lengthened as compared to the early blastula stage but the rate of cell division is still high in the embryo (Newport & Kirschner, 1982). The background of positive labelling observed was therefore expected, but the heterogeneity of labelling that was reproducibly detected was not foreseen. This heterogeneity was accentuated at later stages of development.

At the tailbud stage, a striking pattern of distribution of silver grains was observed (Fig. 5). Once again the whole embryo was positive as compared to control sense probe hybridization (not shown) but the truncal and caudal epidermis as well as the buds of the external gills displayed a higher concentration of myc RNA (Fig. 5A,D). Strong signals were also observed in the optic cups and lens placodes (Fig. 5A–C,E,F) which result from a thickening of the epidermis induced by the optic cup. No differentiated cell can be seen in these areas. The labelling also extended over the cephalic epidermis (Fig. 5E). The average grain density was about sixfold higher on the optic cups and lens placodes than on endoderm sections. Thus the concentration of myc RNA was sixfold higher in the optic cup cells. It is difficult to correlate these values with a given transcript density. As a tentative approach, however, oocyte sections were always mounted on the same slides as embryo sections so that hybridization conditions would be identical. Since the number of transcripts per oocyte is known quite acccurately (Taylor *et al.* 1986), comparison with the grain density on oocyte sections and tailbud sections enabled a rough estimate of the number of transcripts per cell volume in optic cup cells. This value corresponded to more than 100 transcripts per cell of the optic cup tissue.

Discussion

We previously reported that myc mRNA was uncoupled from cell proliferation during oogenesis and was accumulated as a stable maternal mRNA. The measure of the steady-state level of this transcript during development showed a degradation of 90 % of the stored myc mRNA during the early cleavage

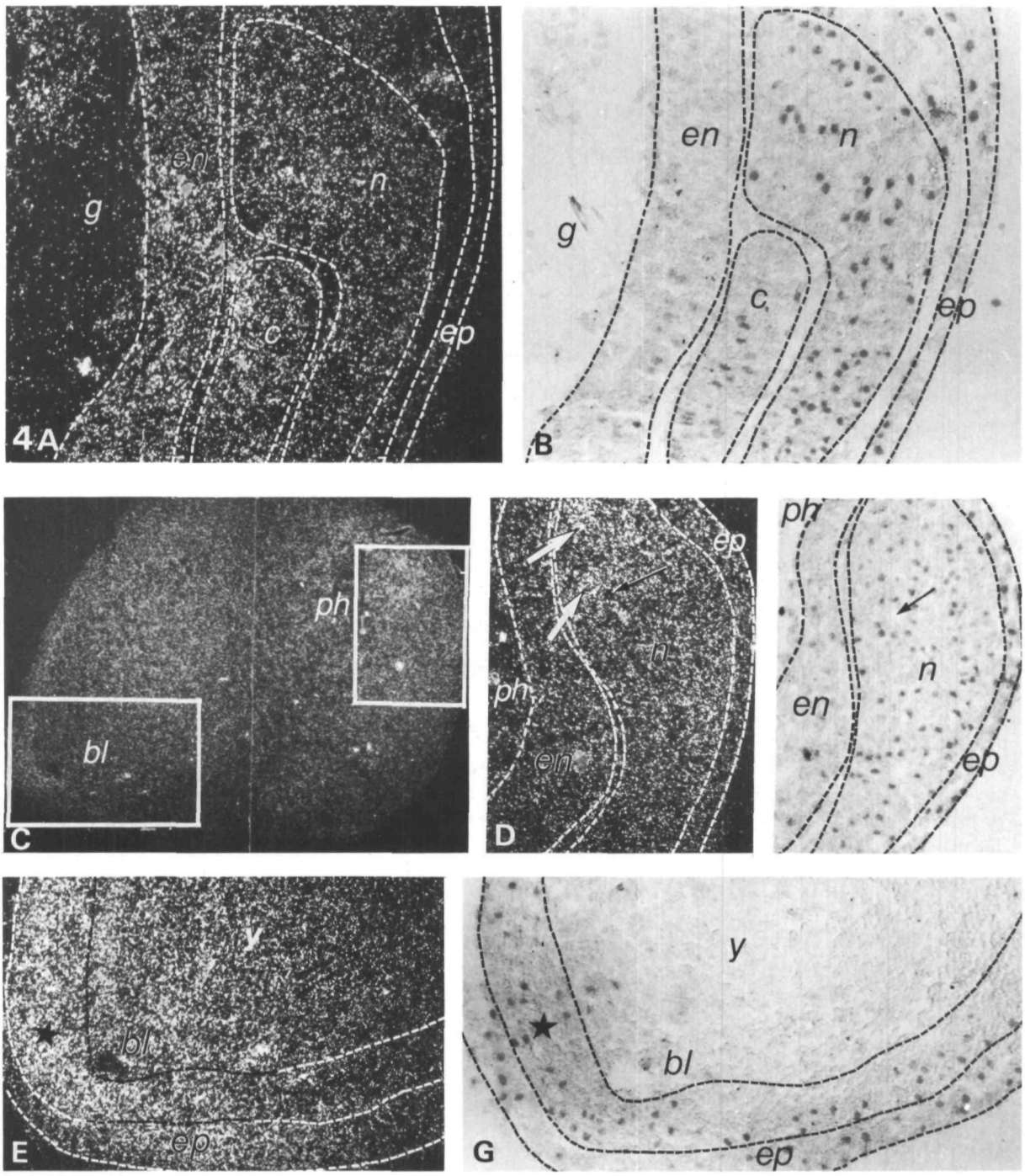

Fig. 4. Localized expression of c-myc in neurula embryos. Stage-22 (neurula) *Xenopus* embryo sections were hybridized with a ^{35}S-labelled antisense c-myc RNA probe. (A,C,D,F) Dark-field illumination; (B,E,G) bright-field illumination. (A,B) Sagittal sections. The neural tube (*n*), notochord (*c*) and dorsal endoderm (*en*), exhibit a signal above the background of the whole embryo. *g*, gut; *ep*, epidermis. (C–G) Frontal sections. In (C) the neurula is oriented so that the anterior region is on the right side. The section becomes partially sagittal on the right side due to the twisting of the embryo by the cytological protocol. The cut plane crosses both the foregut or pharyngeal cavity (*ph*) and hindgut near the blastopore (*bl*) which remains as a posterior opening of the gut (future anus). (D) and (E) are an enlarged detail of C (rectangle on the right side). This detail shows the region located anterodorsally toward the pharyngeal cavity and including the pharyngeal endoderm (*en*), neural tube (*n*) and head epidermis (*ep*). The hybridization signal is generally high in these tissues, with some areas more intensely labelled (thick arrows). Thin arrow, neural lumen. (F) and (G) are another detail of C (rectangle on the left side). Signals are detected over yolk-laden endoderm (*y*). The grain density is higher both over the posterior epidermis (*ep*), the mesoderm, and immediately underlying endoderm (star), all of which are composed of moderately yolk-laden blastomeres. (C) ×80; (D,E) ×170; (A,B) ×190; (F,G) ×200.

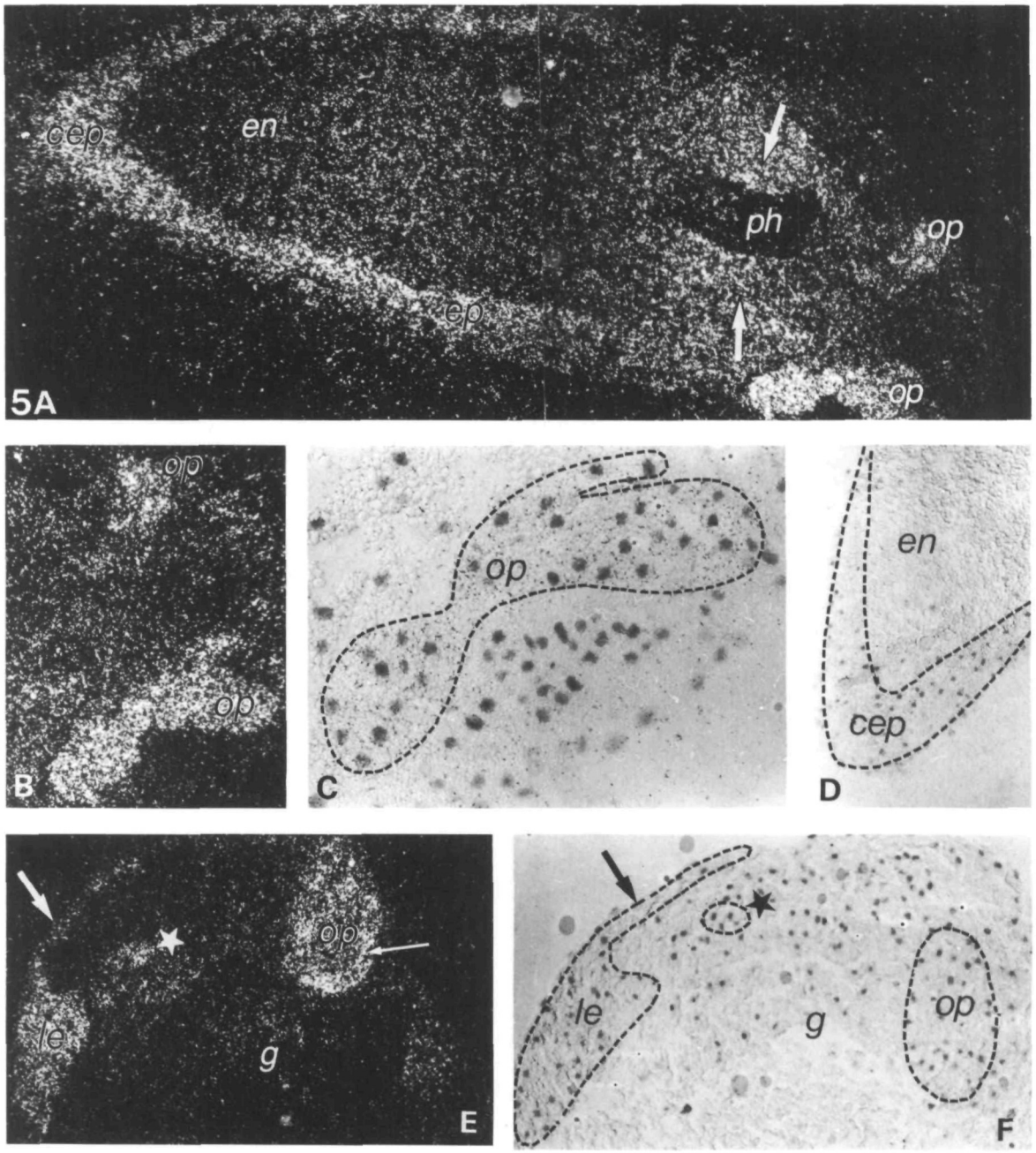

Fig. 5. Localized expression of c-myc in the tailbud embryo. Embryo frontal sections were hybridized with a ^{35}S-labelled antisense c-myc RNA probe. (A,B,E) Dark-field illumination; (C,D,F) bright-field illumination. A is a general view which shows that the probe hybridized intensely to epidermis (*ep*), optic cups (*op*) and lateral pharyngeal walls (arrows). Abbreviations: *cep*, caudal epidermis; *en*, yolk-laden endoderm; *ph*, pharyngeal cavity. B and C are a detail of A in the cephalic region. (D) Caudal region. (E) Frontal section of the anterior part of the embryo showing an intense signal in the right optic cup (*op*), the left lens placode (*le*) and the cephalic epidermis (thick arrow). Periphery of the right optic cup is preferentially labelled (thin arrow) and might include the wall of the cup; the less preferentially labelled inner region might include part of the cup cavity. The left optic cup (asterisk) is more weakly labelled because the cut plane barely crosses the cup. *g*, gut. (A,D) ×50; (B,E,F) ×90; (C) ×140.

stage, followed by new transcription from the neurula stage onward (Taylor *et al.* 1986). These observations did not exclude the possibility of different patterns of variation within cells or between tissues. Therefore, *in situ* hybridization was applied to examine the *in situ* localization of myc transcripts during development.

It has been proposed that the localization of maternal determinants in unfertilized eggs may direct

development of the early embryo (review by Davidson, 1987). We show here that myc RNA is uniformly distributed in the cytoplasm of oocytes, at all stages of their growth (Fig. 2), and thus cannot be considered as a localized determinant. The data obtained by quantification of autoradiographic silver grains are in accordance with biochemical data previously published (Taylor *et al.* 1986): the total number of grains per oocyte, which is related to the total number of myc transcripts, increases from young oocytes (100 μm diameter) through stage-III oocytes (450–600 μm diameter) when it reaches a maximum level (Figs 2, 3). This myc RNA pool does not expand further during late oogenesis and is diluted in the cytoplasm of the still growing oocyte. Morula-stage embryos were weakly positive and we have not been able to detect a positive signal at the gastrula stage. This is in accordance with the decrease in myc RNA content induced by fertilization as previously reported (Taylor *et al.* 1986).

The observation of a high level of steady-state myc RNA in oocytes was interpreted as resulting from a specific regulation of the myc gene (Taylor *et al.* 1986). During the early cell cleavage stage of *Xenopus* development, the embryo divides every 30 min, in absence of detectable expression which first appears at the midblastula stage (Bachvarova & Davidson, 1966; Newport & Kirschner, 1982). The correlation between c-myc mRNA levels and cell proliferation is the most characteristic feature of the regulation of c-myc expression (Kelly *et al.* 1983; Cole, 1986 for a review), at least partly through post-transcriptional control (Dani *et al.* 1984; Dony *et al.* 1985; Knight *et al.* 1985). Thus, the accumulation of c-myc RNA during oogenesis could be interpreted as an uncoupling of c-myc expression and cell proliferation necessary to provide a myc RNA stockpile before the active cell proliferation period induced by fertilization. The absence of localization of this mRNA pool in the oocyte is in agreement with a role of c-myc in proliferation, as well as the observation of steady-state levels of c-myc expression in whole embryos from the neurula stage on since the embryo is growing at that stage.

The detection of high levels of myc RNA above this constitutive expression, spatially restricted to epidermis, optic vesicles and lens placodes, is more difficult to interpret. A spatial localization of c-myc expression was detected as early as the neurula stage and was more pronounced at the tailbud stage. It has been suggested that the transcripts appearing only after the gastrula stage are tissue-specific in tadpoles and code for differentiation-specific proteins (Dworkin *et al.* 1984). On the other hand, since most maternal transcripts are destroyed by the neurula stage, the high level of new transcription seen at that stage merely represents the replacement of homologous maternal transcripts (Shiokawa *et al.* 1980; Dawid *et al.* 1985). Indeed, the quantitative dominance among zygotic genes of maternally active genes is a general feature of most mid-stage embryos (Davidson, 1987 and references therein). If elevated myc RNA level is coordinated with rapid cell proliferation, then clusters of labelled and unlabelled cells might identify tissues with a high division rate at a given stage of embryogenesis. The different *in situ* signals between endodermic and epidermic cells of the tailbud might support such an interpretation (Woodland & Gurdon, 1968). The stronger signal observed in lens placodes is more difficult to reconcile with this supposition. Different studies have shown that constitutive c-myc expression has a major influence on cell differentiation. Thus c-myc expression in murine erythroleukemia cells blocks their terminal differentiation although their commitment is not altered (Coppola & Cole, 1986). Similarly, quail myogenic cells infected with a retrovirus fail to differentiate into myotubes (Falcone *et al.* 1985). A down regulation of c-myc expression has been correlated with cell differentiation both for hematopoietic cells (Westin *et al.* 1982) and for teratocarcinoma cells (Dony *et al.* 1985; Dean *et al.* 1986). Overall, these reports indicate that constitutive myc expression is incompatible with terminal cell differentiation. Microscopic examination of the tailbud shows that in the cephalic areas, optical vesicles grow rapidly to give rise to two large eyes in the young tadpole. However, at this stage, no cytological sign of differentiation is evident. Thus, the high level of myc-mRNA localized in these cells may prevent them from entering terminal differentiation in the still growing embryo.

Alternatively, c-myc expression may be a positive effector for the differentiation of this specific tissue. In a few instances, a transient increase in the c-myc RNA level was observed during cell differentiation (Lachman & Skoultchi, 1984; Greenberg & Ziff, 1984). More recently, a specific and transient elevation of c-myc mRNA was reported in differentiating chick lens cells and this has been correlated with a withdrawal from the cell cycle (Nath *et al.* 1987). Our observations on c-myc expression in the developing *Xenopus* embryo, together with these reports, suggest that, in addition to a specific role in cell proliferation, expression of the c-myc gene might have a distinct role in cell differentiation.

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