Genes and mechanisms involved in early embryonic development in *Xenopus laevis*

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ABSTRACT Our laboratory is studying genes involved in the regulation of the balance between cell growth and differentiation during embryonic development in *Xenopus*. We have analyzed the developmental expression of the proto-oncogenes c-myc, and KiRas 2B, the proliferating cell nuclear antigen (PCNA), and the tumor suppressor gene p53. These genes, usually expressed during cell proliferation, are expressed in the oocyte in large quantities, but the majority of their maternal RNAs are degraded by the gastrula stage. The expression of c-myc and the localization of the protein indicate that c-myc has the characteristics expected for a gene involved in the regulation of the mid-blastula transition, when zygotic expression is turned on in the embryo. Its expression during late development or during regeneration indicates that it enables the cells to remain competent for cycling during organogenesis. *In vitro* systems that reproduce the principal cellular functions during early development are used as model systems to understand the mechanisms involved in early embryogenesis.

KEY WORDS: *proto-oncogenes, early development, oogenesis, cell cycle, chromatin replication*

Introduction

The cytological and histological similarities between an embryonic cell and a cancer cell have been recognized for more than a century. Evidence has accumulated that some RNAs or proteins specifically expressed in a number of tumors are expressed during the genesis of an individual only during its fetal life. With the discovery of proto-oncogenes (Stehelin *et al.*, 1976; Bishop, 1987 for a review), a genetic approach to the relation between cancer and development became rapidly possible. Our laboratory is involved in the study of the gene products responsible for the regulation of cell growth and differentiation during development. Among the genes studied are proto-oncogenes and proteins involved in chromosomal replication events. Our approach includes both *in vivo* and *in vitro* studies of such proteins, and our research is with *Xenopus laevis* as a model system of vertebrate development.

Results

Presence of proto-oncogenes in Xenopus laevis

Using controlled hybridization conditions we have been able to detect proto-oncogenes in *Xenopus* (Moreau *et al.*, 1989). Members of all the oncogene families were detected and oncogenes encoding for the ras family are well conserved (Fig. 1). The sequencing has further confirmed the presence of the proto-oncogenes src (Steele, 1985), c-myc (King *et al.*, 1986; Taylor *et al.*, 1986), mos (Sagata *et al.*, 1987), int1 (Nordermeer *et al.*, 1988), Ki-Ras 2B (Andeol *et al.*, 1990) and the tumor suppresor gene p53 (Soussi *et al.*, 1987).

Expression of proto-oncogenes involved in cell proliferation during oogenesis and embryogenesis

During oogenesis a number of components involved in early development are already stored as maternal RNAs or proteins (Davidson, 1986 for a review). We are interested in genes involved in early embryonic development and the control of the cell cycle during this period. The expression of four genes possibly involved in such regulation was analyzed: c-myc and p53 are nuclear proto-oncogenes involved in the positive or negative control of cell proliferation (Cole, 1986; Soussi *et al.*, 1990 for reviews); KiRas 2B is a cytoplasmic proto-oncogene involved in the G1-S transition (Barbacid, 1987 for review); the proliferative cell nuclear antigen (PCNA) is a protein involved in chromosomal replication during S phase (Prelich and Stillman, 1989). We have cloned these genes from a *Xenopus*

Abbreviations used in this paper: SDS, sodium dodecyl sulfate; DAB, diaminobenzidine; SSC, sodium saline citrate; SSPE, sodium saline phosphate ethylenediamine tetraacetic acid.

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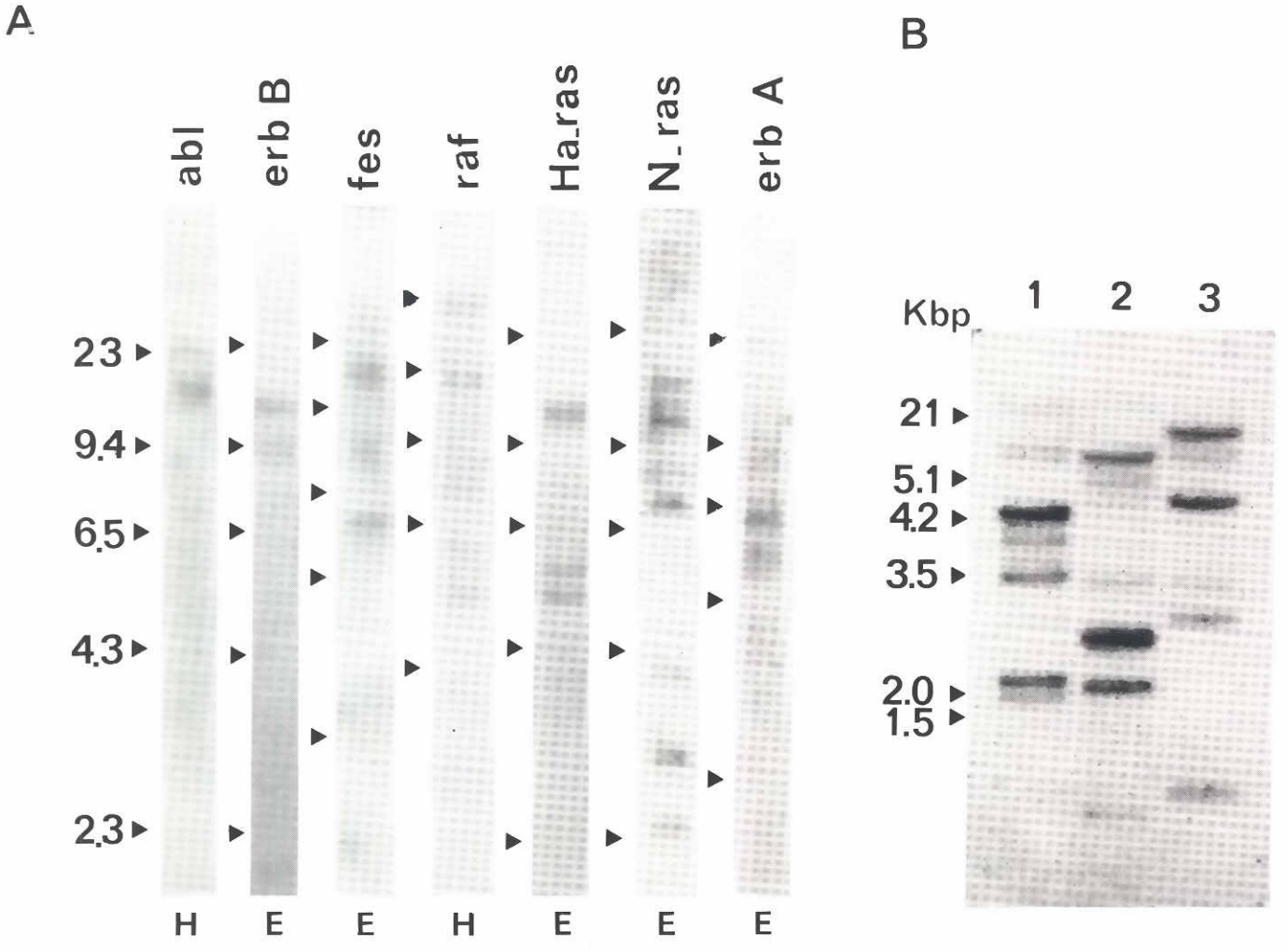

Fig. 1. Detection of proto-oncogenes in the genome of *Xenopus laevis*. **(A)** *Analysis by Southern blotting of high molecular weight* Xenopus *genomic DNA (10μg) digested with Eco RI (E) or Hind III (H), by heterologous probes corresponding to three different families of oncogenes as described in Moreau et al. (1980). a) protein kinase: abl, erbB, fes, and raf; b) ras family: Ha-ras and N-ras; c) nuclear oncogenes: erbA. The filters were hybridized at 42° in 5X SSPE and 35% formamide and washing was carried out at a final stringency of 0.1X SSC 42° C (abl, erbB, fes, erbA) and 55° C (N ras, Ha-ras and raf).* **(B)** *Hybridization was with the homologous* Xenopus *Ki Ras 2B probe (Andéol* et al., *1990). Bam H1 (lane 1), Eco RI (lane 2) and Hind III (lane 3) were used. Molecular weight markers are indicated.*

oocyte cDNA library and have analyzed their expression during development (Taylor *et al.*, 1986; Soussi *et al.*, 1987; Vriz *et al.*, 1989; Andéol *et al.*, 1990; Leibovici *et al.*, 1990). In all cases at least two DNA bands were observed by Southern blot hybridization of *Xenopus* genomic DNA (Fig. 1B), consistent with the tetraploid evolution of *Xenopus laevis* (Bisbee *et al.*, 1977). At the amino acid level, the homology between the *Xenopus* genes and human counterparts was 51% (p53), 70% (c-myc), 89% (PCNA) and 92% (Ki-Ras 2B).

It is interesting to note that two c-myc genes were expressed in *Xenopus* (Vriz *et al.*, 1989). C-myc I encodes for the major c-myc gene expressed, and is expressed both during oogenesis and embryonic development. C-myc II transcripts constitute 5-10% of the level of total c-myc RNA in the oocyte and are present only during oogenesis (Fig. 2). Such different expressions of two c-myc genes during development, with one oocyte specific gene and one somatic type gene, is reminiscent of polymerase III 5S RNA gene expression in *Xenopus*. We do not have a clear explanation of the biological significance of such different expressions for c-myc. The specific maternal expression of c-myc II might be linked to strictly maternal features of the oocyte such as rDNA amplification, although it is not clear if the corresponding protein is expressed during early oogenesis.

Fig. 3 is a summary of the expression of c-myc, KiRas 2B, and PCNA during oogenesis and development. The RNA levels for each gene analyzed were determined (Andéol *et al.*, 1990 for details). A high level of expression was observed in early stages of oogenesis, and by mid-oogenesis the maximum accumulation of RNA was reached. The values are close to the prevalence of stored maternal RNAs in the mature oocyte (Davidson, 1986). During the cleavage stage, the three maternal RNAs analyzed are degraded, although with different kinetics. C-myc RNA is progressively degraded from fertilization, whereas the bulk of PCNA and KiRas 2B become degraded between the midblastula transition and the gastrula

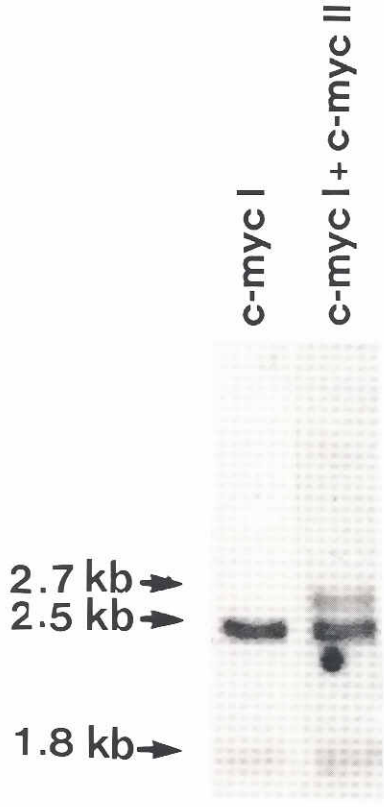

Fig. 2. Transcripts of the two c-myc genes are expressed during oogenesis. *Oocyte poly(A)⁺ RNA was analyzed by Northern blot hybridization. The blot was first probed with an oligonucleotide specific for c-myc I RNA and further hybridized with an oligonucleotide specific for c-myc II RNA, without dehybridization (see Vriz* et al., *1989 for details).*

stages (Taylor *et al.*, 1986; Andéol *et al.*, 1990; Leibovici *et al.*, 1990). As a result of the rapid cleavage stage and degradation of the maternal stores, the number of mRNA copies per embryonic cell for each gene analyzed falls to a range of 10 (c-myc I) and to 500 (KiRas 2B) RNA molecules at gastrula. Zygotic expression of C-myc and PCNA is detected at neurula, and KiRas 2B at the tadpole stage. The p53 RNA level remains stable from the egg to the gastrula-neurula stage, when it becomes undetectable (unpublished observations). Thus, during embryonic development, there is not a specific stage for new zygotic expression of genes encoding maternal transcripts. However, most of the maternal store has been degraded by gastrula and new zygotic expression is a specific property of each gene rather than a developmental stage (see also Davidson, 1986 for a review).

Translation of the maternal transcripts of c-myc, PCNA and KiRas 2B also occurs during oogenesis. It is interesting to note that a ras protein is already present in the oocyte before maturation (Fig. 4), indicating that its presence is not sufficient *per se* to induce maturation events. Thus the possible involvement of ras protein in maturation induced by progesterone (Birchmeier *et al.*, 1985) is not the direct result of the induction of Ras RNA translation. This result raises the possibility that either post-translational events or synthesis of a specific subset of the ras family is the event associating ras with the maturation process.

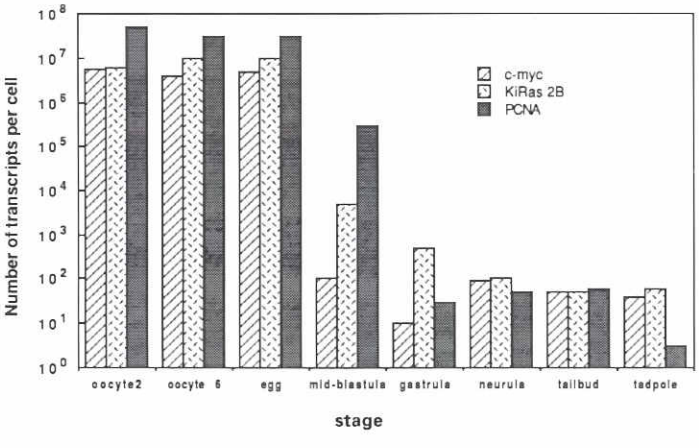

Fig. 3. Expression of c-myc, KiRas 2B and PCNA during embryonic development. *The level of expression of c-myc, KiRas and PCNA was quantitated during oogenesis and development. The values are reported as number of RNA molecules per cell for certain developmental stages. (See text and references cited for details).*

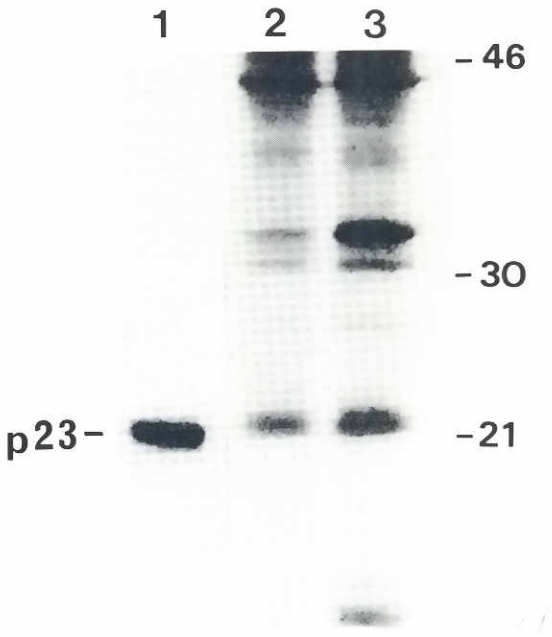

Fig. 4. Detection of the ras protein in oocytes. *Lane 1 is an* in vitro *translation product of KiRas 2B mRNA. Immature oocyte extracts (lane 2), or extracts from oocytes matured by progesterone (lane 3), were immunoprecipitated with a ras antibody and analyzed by SDS gel electrophoresis (Andéol* et al., *1990, for details).*

Involvement of c-myc protein in embryonic development

The three main characteristics of the proto-oncogene c-myc in proliferating cells are localization of the protein in the nucleus, expression correlating with cell proliferation, and a rapid turnover of both the RNA and protein (Cole, 1986, for a review). During embryonic development in *Xenopus*, however, these features are not found. C-myc is expressed at an unusual level in the non-dividing oocyte, which contains 5×10^5 times the amount of c-myc RNA or protein in a somatic cell (King *et al.*, 1986; Taylor *et al.*, 1986; Gusse *et al.*, 1989). During this period, both the mRNA and protein

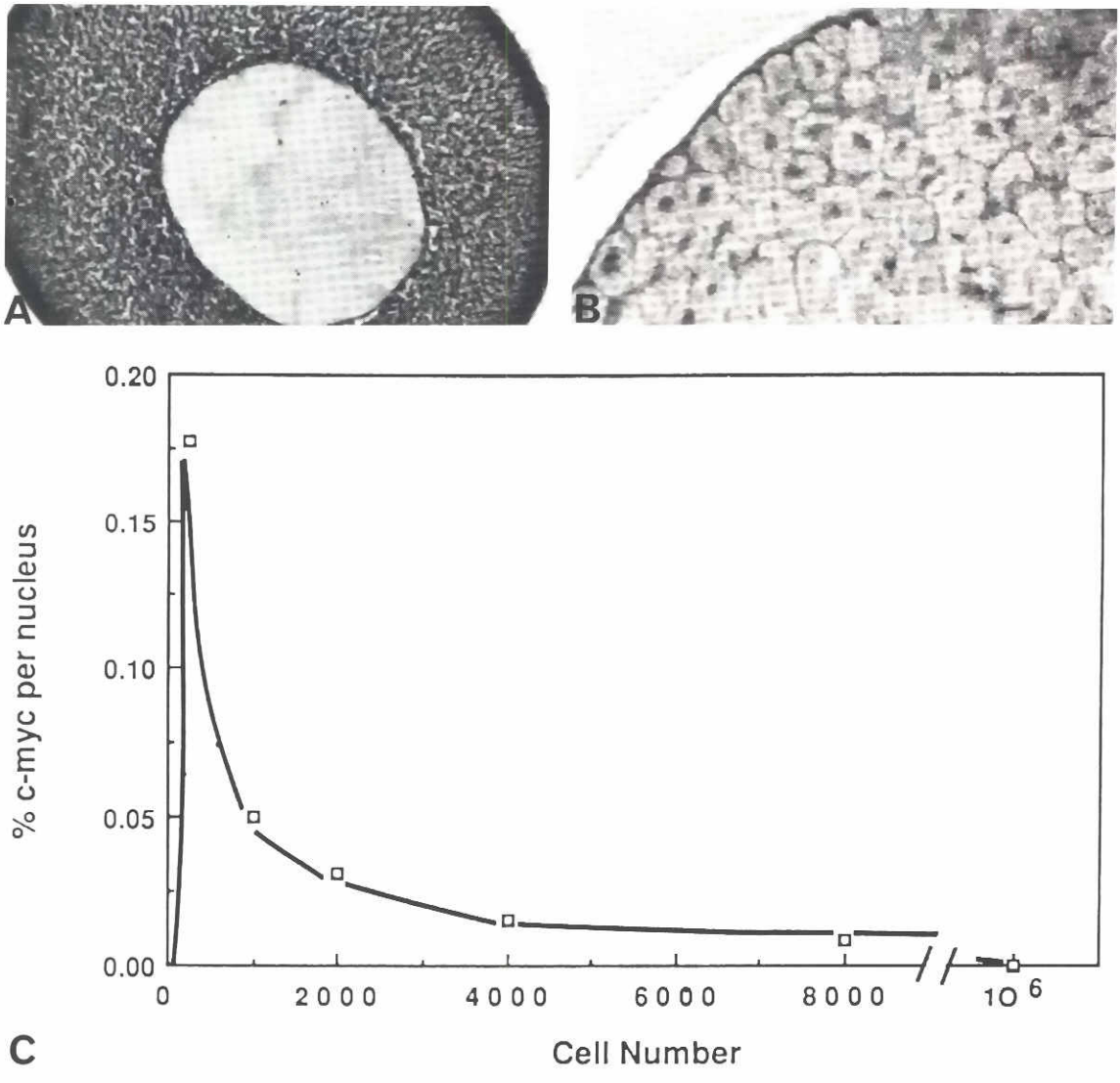

Fig. 5. *In situ* localization of c-myc protein. *Immunolocalizations with a c-myc antibody are as described (Gusse* et al., *1989), using a peroxidase-DAB reaction. c-myc protein is localized in the cytoplasm in the stage V oocyte* **(A)**, *and the nuclei of morula stage* **(B)**. **(C)** *is the fraction of c-myc protein per nucleus during development from the oocyte (O) to the tadpole stage.*

are stable and the protein is localized in the cytoplasm of the oocyte (Taylor *et al.*, 1986; Gusse *et al.*, 1989 and Fig. 5), in contrast with its nuclear location in somatic cells (Cole, 1986). Storage in the cytoplasm of proteins involved in nuclear metabolism is not a general property of the oocyte. For example, PCNA protein, an accessory protein for replication, is localized in the nucleus in oocyte and in somatic cells (Fig. 6). This paradox could be explained if c-myc was a key protein involved in early embryonic development. The stockpiling of a high level of c-myc protein may be understood as the necessity of the oocyte to store components involved in activating the rapid cell division which follows fertilization. During this period, the egg divides every 30 min, without G1 or G2 phases, and no transcription is detectable until the 12th cell cycle (Bachvarova *et al.*, 1966; Newport and Kirschner, 1982). The stockpiling of this protein in the cytoplasm of the oocyte might be a solution to avoid any activation of mitotic nuclear events dependent on its normal nuclear location. This hypothesis was confirmed by the fate of c-myc products after fertilization. Fertilization rapidly triggers a massive translocation of c-myc into the embryonic nuclei, which become very quickly saturated with c-myc protein (Fig. 5). Then an exponential decrease of c-myc protein per nucleus is observed until an equilibrium is reached when the embryo reaches the 12th cell cycle (Gusse *et al.*, 1989). Thus the timing of titration of the cytoplasmic c-myc protein by the nuclei correlates with the onset of zygotic expression in the embryo. Based on a series of elegant experiments, Newport and Kirschner (1982) proposed that the midblastula transition was under the control of a nuclear protein, stored in large excess in the cytoplasm of the oocyte. They proposed that such a protein should translocate to the nucleus after fertilization and that the timing of new zygotic expression was set after the nuclei had entirely titrated this cytoplasmic factor. Our observations of the developmental expression of c-myc show that it has all the characteristics expected

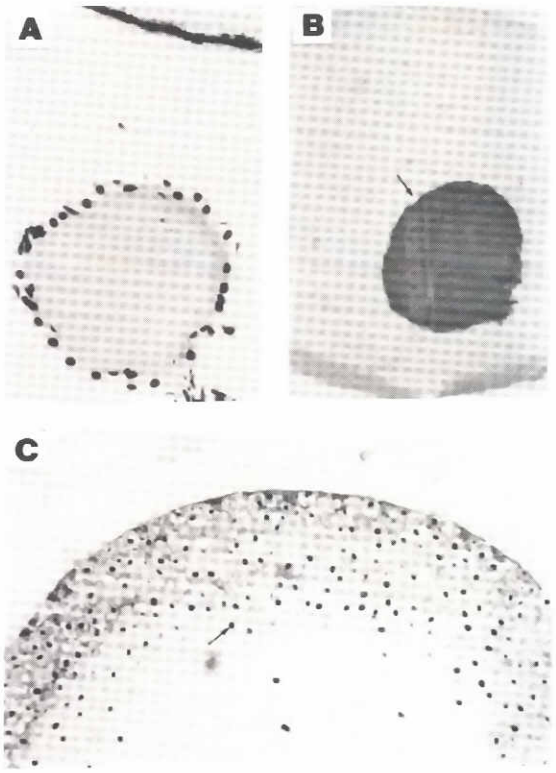

Fig. 6. *In situ* localization of PCNA protein. *Immunolocalization of PCNA protein was with a PCNA antibody and revelation with a peroxidase-DAB reaction (Leibovici* et al., *1990). PCNA is localized in the nucleus of both somatic cells* **(A)**, *oocytes* **(B)**, *or gastrula embryos* **(C)**. *Arrows point to nuclei.*

for a gene involved in the midblastula transition. The maternal c-myc protein pool is further degraded within two hours after the onset of gastrulation, confirming that the requirement for a large amount of this protein is restricted to early development. We do not understand the events that trigger the entry of c-myc into the nuclei after fertilization, but we did note that the protein was phosphorylated at the same time (Gusse *et al.*, 1989).

During organogenesis, beginning at the neurula stage, the zygotic c-myc gene is also expressed. We observed an enhanced expression in the epiderm and during the formation of the eye in the young tailbud embryo (Hourdry *et al.*, 1988 and Fig. 7). Such specific localization was interpreted as a necessity for the embryo to maintain these early differentiating tissues in a state competent for growth. A similar phenomenon was observed during limb regeneration, when a spatial localization of high levels of c-myc in the intermediate cell layers of the wound epithelium might prevent terminal differentiation and compel cells to proliferate during the process of regeneration (Géraudie *et al.*, 1989, and Fig. 7).

In vitro *systems for defining the function of early development genes*

During early development, the *Xenopus* embryo is committed to cell proliferation. (i) The early cell cycles after fertilization are very rapid and the eggs are not rate-limited for commitment to S phase (Newport and Kirschner, 1982). (ii) *Xenopus* eggs contain a stockpile of enzymes and precursors for DNA synthesis and chromatin assembly (reviewed in Laskey, 1979; Davidson, 1986). Our approach has been to develop *in vitro* systems able to reproduce accurately both DNA structure formation and DNA replication, in order to better understand DNA replication and its regulation in relation to chromosomal organization during early development.

Two types of *in vitro* systems can be distinguished (see Fig. 8). A low-speed supernatant extract from activated *Xenopus* eggs, or a high-speed supernatant extract from *Xenopus* eggs. The low-speed system was initially described by Lohka and Masui (1983) and has gained popularity in the last few years for studying cell cycle associated events. In this system multiple rounds of replication can occur on DNA from sperm nuclei (Blow and Laskey, 1986; Almouzni and Méchali, 1988c; Hutchison *et al.*, 1988), and pronuclear formation seems to be a prerequisite for DNA replication (Blow and Laskey, 1988). It is possible in this system to study the key events in the precise regulation of initiation of DNA replication that permits one and only one round of replication per cell cycle. Some possible candidates for such a role are tested in our laboratory. These include proto-oncogenes, especially c-myc, a protein that might be important in replication (Iguchi-Ariga *et al.*, 1987). In addition, the structural organization of chromatin and associated changes after replication are also being studied to determine whether some specific signal could explain why newly replicated DNA does not overreplicate during one cell-cycle.

The high-speed extract was used in our laboratory with a circular single-stranded DNA template to study chromatin assembly. With this substrate, extracts obtained from high-speed centrifugation perform complementary DNA strand synthesis coupled to chromatin assembly (Méchali and Harland, 1982; Almouzni and Méchali, 1988a and Figs. 8 and 9). Nucleosomes are formed on the newly replicated DNA, and the overall reaction mimics the events occuring during chromosomal replication on the lagging strand at the replication fork. In contrast, with double-stranded DNA template, not replicated in these extracts, chromatin is assembled less efficiently and the rate of assembly is dramatically decreased (Figs. 8 and 9). Thus with the two templates two DNA functions can be studied separately: with single stranded DNA the events of elongation at the lagging strand during replication replicated chromatin are reproduced, while with double-stranded DNA unreplicated chromatin is obtained. In both cases, addition of ATP/Mg^{2+} leads to a change in chromatin organization that defines a physiological spacing of nucleosomes (Almouzni and Méchali, 1988b). We have recently used this system, with DNA templates that include cloned genes, to study the possible effect on regulation of transcription of DNA synthesis linked to chromatin assembly (Almouzni *et al.*, 1990). It appears that both DNA synthesis and chromatin organization can affect transcription of these genes.

The use of these two cell free systems enables us to examine either part of the machinery (high-speed extract), or the whole machinery used in the cell cycle (low-speed extract). Such tools should help us to better understand DNA replication and its regulation in eukaryotic cells at the early stages of development and its relationship with gene expression.

Concluding remarks

Our observations show that the expression of proto-oncogenes and other genes possibly involved in cell growth is uncoupled from cell proliferation during oogenesis. This observation is in agreement

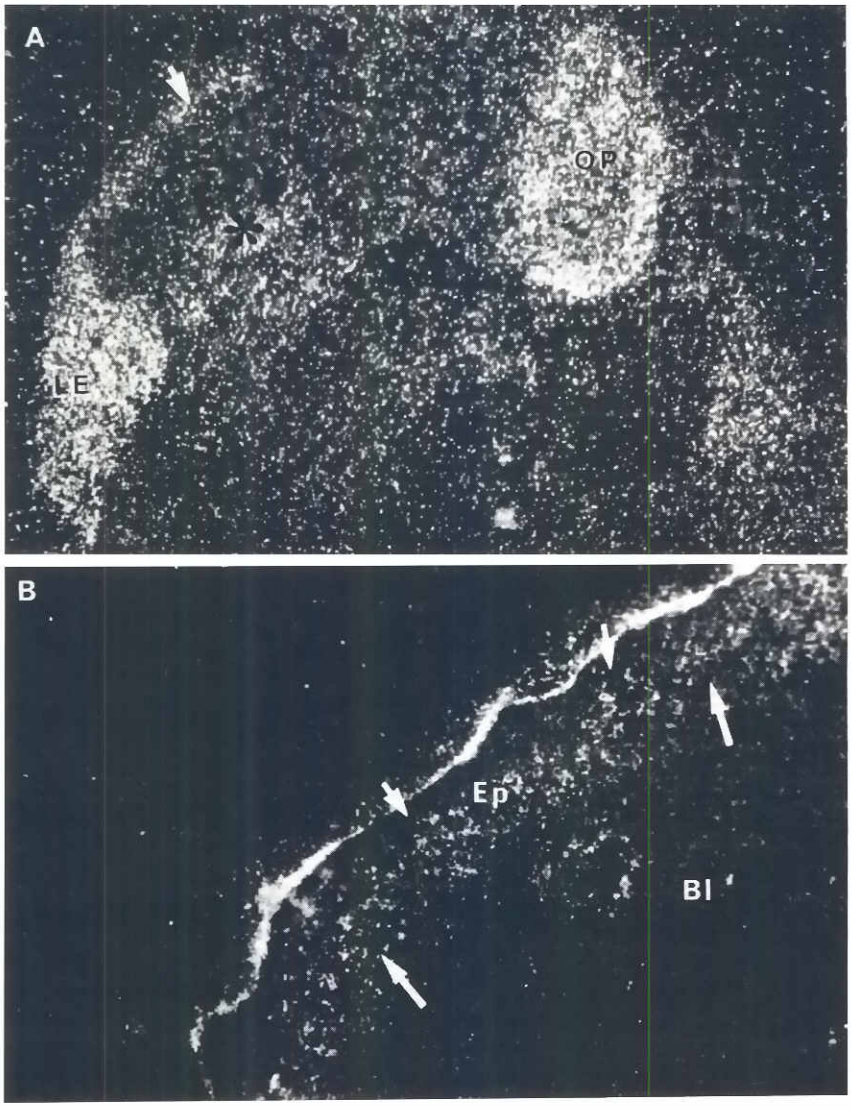

Fig. 7. Expression of c-myc during embryonic induction of the eye and during limb regeneration. In situ *localization was with a ^{35}S-labeled antisense c-myc RNA probe, and observed by darkfield illumination.* **(A)** *Frontal section of the anterior part of a tailbud embryo showing c-myc expression in the right optic cup (op) and the lens placode (le). The cephalis epidermis (thick arrow) is also labeled. The section is through the top of the left optic cup (asterisk).* **(B)** *Regenerates were harvested 1 month after amputation of young postmetamorphic froglets and routinely processed for histology. Note the gradient of transcripts in the wound epidermis (Ep). Transcripts are only present in the deepest layers of the epidermis (long arrows). Well-differentiated keratinocytes located at the outermost layers do not express c-myc transcription (short arrows). Mesenchymal cells of the blastema (Bl) located beneath the wound epidermis are moderately labeled. ^{35}S-labeled sense RNA probes are used as controls.*

with the role of maternal products in the cleavage stage of the embryo (Davidson, 1986, for review). During this stage, there is division without overall growth of the embryo, and a number of genes whose expression is usually coupled to cell proliferation are already expressed before fertilization. The best examples are probably the histone genes. In somatic cells, histone genes are strictly expressed during S phase, and in the absence of DNA synthesis histone mRNA is degraded within a few minutes. In contrast, histone genes are expressed during oogenesis and a large pool of histone molecules is present in the unfertilized egg, ready to be incorporated in the chromatin of newly made nuclei during the cleavage stage (Woodland, 1980). Thus, the regulation of c-myc, Ki-ras 2B, and PCNA during embryonic development can be explained by an important role of these genes during early development. However, important differences are observed with the c-myc proto-oncogene. A high level of c-myc protein is restricted to only one phase of development, the early cleavage stage. Most of the c-myc protein is rapidly degraded by the gastrula stage, and further expression of c-myc in the embryo appears similar to the constitutive expression of c-myc in proliferating somatic cells. In contrast, the PCNA protein, a subunit of DNA polymerase-δ (So and Downey, 1988), is expressed at the same steady state level in the embryo during the entire development period. Another main difference between the regulation of c-myc and the regulation of other genes involved in nuclear events linked to cell proliferation, is the localization of the protein in the cell. Thus, PCNA, histones, and DNA polymerase-a are localized in the nucleus of the oocyte although the oocyte is not dividing. C-myc protein is in the cytoplasm of the oocyte, a unique case of cytoplasmic localization for this protein. A strong correlation between the entry of c-myc into the nucleus and the onset of the cleavage stage is observed at fertilization. This different fate of c-myc protein relative to other proteins like PCNA, histone or DNA polymerase-a, might be explained if the entry of c-myc into the nucleus was a fundamental regulatory event linked to the expression of this gene. Thus the entry of c-myc into the nucleus might be an event sufficient to trigger the cleavage stage,

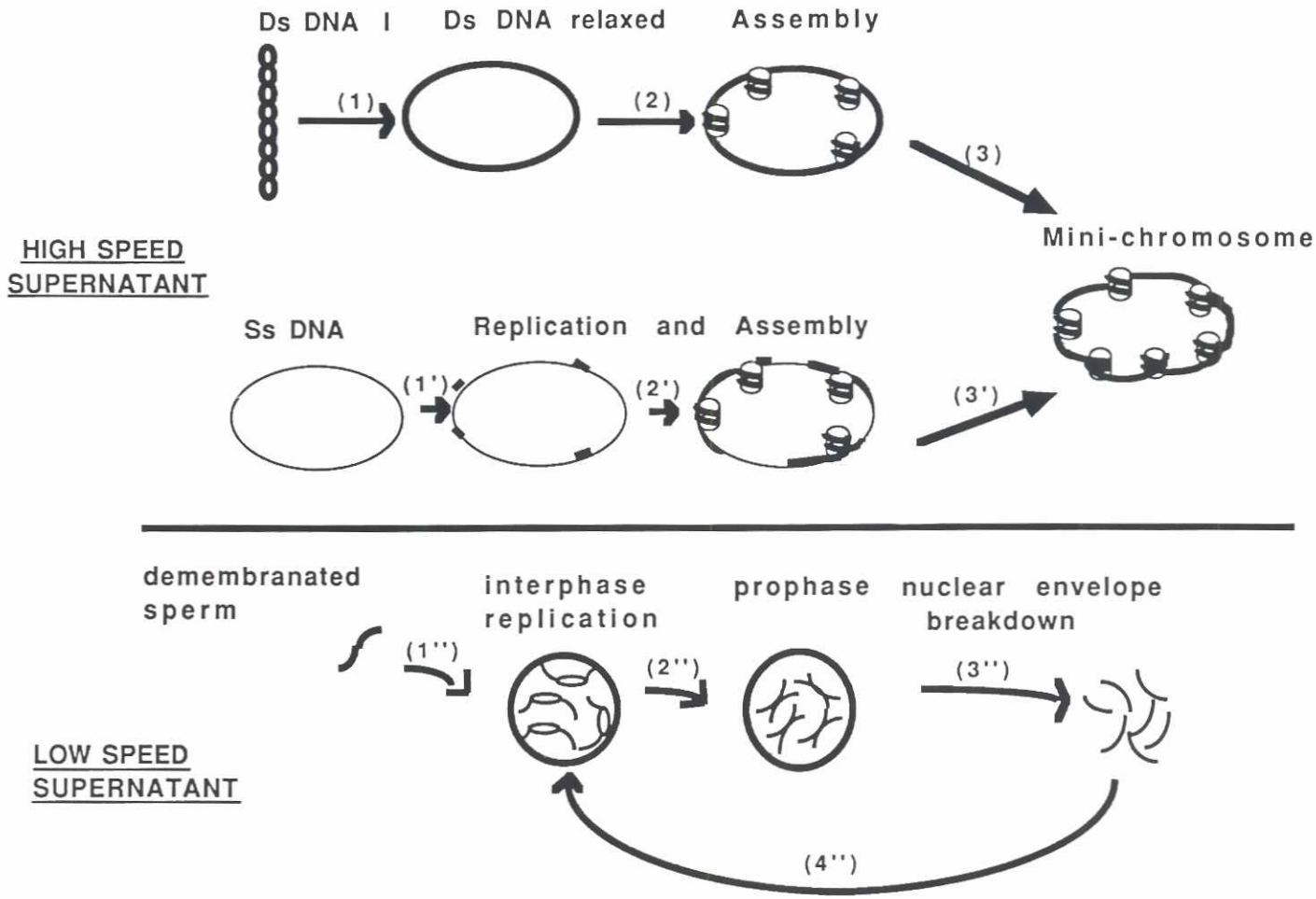

Fig. 8. Two possible cell-free systems from *Xenopus* eggs. The high speed supernatant (HSE): *double-stranded supercoiled DNA (Ds DNAI) is converted to a relaxed form (Ds DNA relaxed) and then assembled into chromatin. Relaxation involves topoisomerase I (1), and nucleosome formation uses the endogenous histone pool while topoisomerase I relaxes the arising constraints (2). ATP/Mg^{2+} is required for final spacing and stability of the minichromosome (3). Single-stranded DNA (Ss DNA) is converted to a double-stranded form assembled as a minichromosome. Primers are synthesized by a primase activity (1'). Elongation involves DNA polymerase-a using dNTPs; ligase assures the joining of the new fragments. Concurrently nucleosome deposition occurs using the histone stockpile (2'). Ligation of the final okazaki-like fragments leads to a minichromosome. ATP/Mg^{2+} is required throughout the process (1', 2', 3').* The low speed supernatant (LSE): *demembranated sperm nuclei acquire a new membrane and then follow a cycle. Decondensation of sperm DNA occurs and formation of a nuclear membrane is obtained using the lamina from the extract. The interphase nuclei undergo DNA synthesis (1''). The entry into mitosis is probably driven by MPF activity (2''), and chromatin is dispersed after the nuclear membrane breaks down (3''). A new interphase nucleus is produced as MPF activity disappears and a new cycle begins (4'').*

and maintaining this protein in the cytoplasm of the oocyte might be crucial to avoid its activation in the oocyte.

Such a regulatory event can be analyzed *in vitro* using *Xenopus* egg extracts. During recent years, these extracts have been found to be unique in their ability to reproduce principal cell functions *in vitro* with good efficiency. DNA replication (Méchali and Harland, 1982; Blow and Laskey, 1986; Almouzni and Méchali, 1988a), chromatin assembly (Laskey *et al.*, 1977; Almouzni and Méchali, 1988b), transcription (Wolffe and Brown, 1987), and translation (Patrick *et al.*, 1989 and our unpublished results) are effective *in vitro*. More complex reactions like the formation of nuclei (Newport, 1987) and nuclear transport are also reproduced *in vitro* (Newmeyer *et al.*, 1986). Our observations relative to chromatin assembly coupled to DNA replication *in vitro* show that it might be possible now to dissect the events regulating initiation of DNA replication and nuclear synthesis during early development. Thus, complementation between *in vivo* and *in vitro* observations is an unusual possibility provided by *Xenopus* embryos to understand one of the most spectacular but poorly understood phenomena in biology: the commitment of the egg to development.

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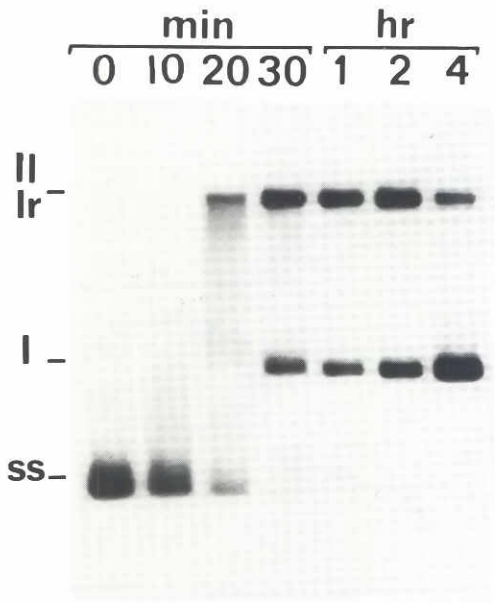

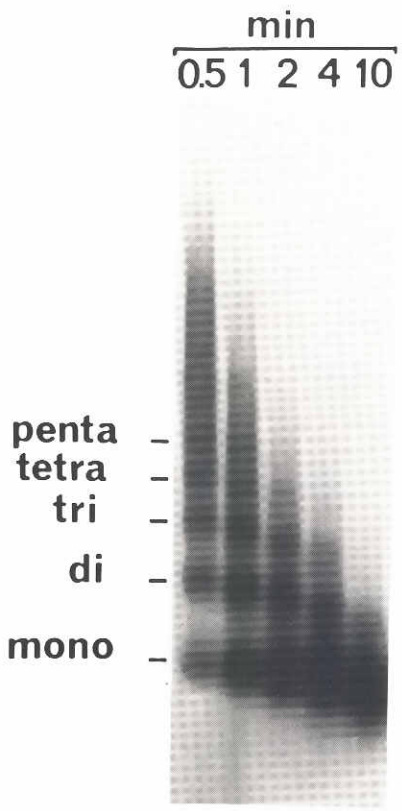

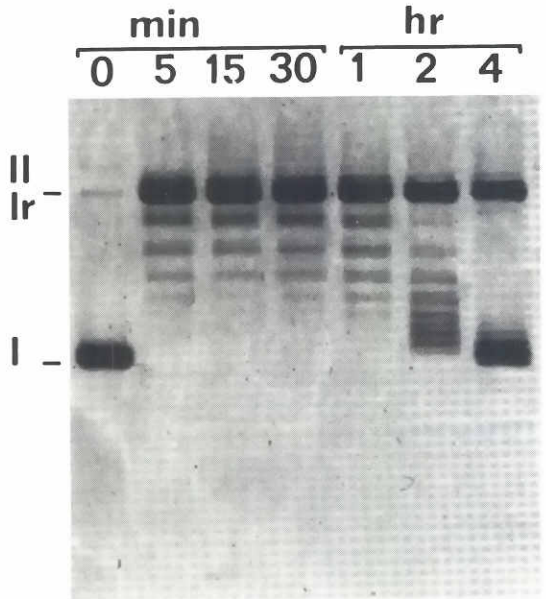

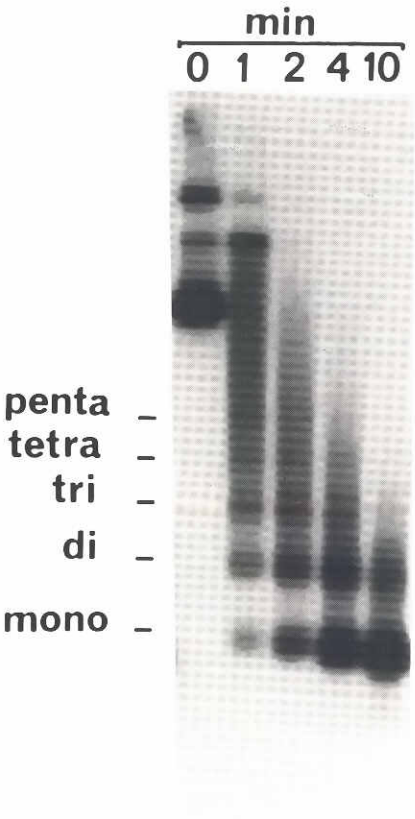

Fig. 9. Chromatin assembly during replication and on non-replicating DNA. (A) *A ^{32}P-labeled M13 single-sranded DNA was added to a Xenopus high speed egg extract and replication was analyzed by separation of the replication products on agarose gels.* **(B)** *Kinetics of micrococcal nuclease digestion of the replication products.* **(C)** *Chromatin assembly of PUC18 double-stranded DNA added to the Xenopus egg extract, and analyzed on agarose gel electrophoresis after phenol-chloroform extraction.* **(D)** *Kinetics of micrococcal nuclease digestion of the chromatin assembled on double stranded DNA. Analysis was by agarose gel electrophoresis. Molecular weight markers (M) were from a ØX174-HaeIII digestion. (See Fig. 8 and Almouzni and Méchali, 1988a and b for details).*

References

ALMOUZNI, G. and MÉCHALI, M. (1988a). Assembly of spaced chromatin promoted by DNA synthesis in extracts from *Xenopus* eggs. *EMBO J. 7:* 665-672.

ALMOUZNI, G. and MÉCHALI, M. (1988b). Assembly of spaced chromatin: Involvement of ATP and DNA topoisomerase activity. *EMBO J. 7:* 4355-4365.

ALMOUZNI, G. and MÉCHALI, M. (1988c). *Xenopus* egg extracts: a model system for chromatin replication. *Biochem. Biophys. Acta, 951:* 443-450.

ALMOUZNI, G., MÉCHALI, M. and WOLFFE, A.P. (1989). A competition exists between transcription complex assembly and chromatin assembly on replicating DNA. *EMBO J.* (In press).

ANDÉOL, Y., GUSSE, M. and MÉCHALI, M. (1990). Characterization and expression of a *Xenopus* RAS during oogenesis and development. *Dev. Biol.* (In press).

BACHVAROVA, R. and DAVIDSON, E.H. (1966). Nuclear activation at the onset of amphibian gastrulation. *J. Exp. Zool.163:* 285-295.

BARBACID, M. (1987). Ras genes. *Annu. Rev. Biochem. 56:* 779-827.

BIRCHMEIER, C., BROEK, D. and WIGLER, M. (1985). Ras proteins can induce meiosis in *Xenopus* oocytes. *Cell 43:* 615-621.

BISBEE, C.A., BAKER, M.A. and WILSON, A.C. (1977). Albumin phylogeny for clawed frogs *(Xenopus). Science 195:* 785-787.

BISHOP, J.M. (1987). The molecular genetics of cancer. *Science 235:* 305-311.

BLOW, J.J. and LASKEY, R.A. (1986). Initiation of DNA replication in nuclei and purified DNA by a cell-free extract of *Xenopus* eggs. *Cell 47:* 577-587.

BLOW, J.J. and LASKEY, R.A. (1988). A role for the nuclear envelope in controlling DNA replication within the cell cycle. *Nature 332:* 546-548.

BROOKS, P., DOHET, C., ALMOUZNI, G., MÉCHALI, M. and RADMAN, M. (1989). Mismatch repair involving localized DNA synthesis in extracts of *Xenopus* eggs. *Proc. Natl. Acad. Sci. USA 86:* 4425-4429.

COLE, M.D. (1986). The myc oncogene: its role in transformation and differentiation. *Annu. Rev. Genet. 20:* 361-384.

DAVIDSON, E.H. (1986). *Gene Activity in Early Development.* Academic Press, New York.

FOX, A.M., BREAUX, C.B. and BENBOW, R.M. (1980). Intracellular localization of DNA polymerase activities within large oocytes of the frog, *Xenopus laevis. Dev. Biol. 80:* 79-95.

GERAUDIE, J., HOURDRY, J., BOEHM, K., SINGER, M. and MÉCHALI, M. (1989). C-myc proto-oncogene expression during newt limb regeneration. In *NATO Advanced Research Workshop: Recent Trends in Regeneration Research* (Eds. V. Kiortsis, S. Koussoulakos and H. Wallace).

GERAUDIE, J., HOURDRY, J., VRIZ, S., SINGER, M. and MÉCHALI, M. (1990). Enforced c-myc expression during blastema formation of forelimb regenerate in the froglet, *Xenopus laevis. Proc. Natl. Acad. Sci. USA* (In press).

GUSSE, M., GHYSDAEL, J., EVAN, G., SOUSSI, T. and MÉCHALI, M. (1989). Translocation of a store of maternal cytoplasmic c-myc protein into nuclei during early development. *Mol. Cell. Biol. 2:* 5395-5403.

HOURDRY, J., BRULFERT, A., GUSSE, M., SCHOVAERT, D., TAYLOR, M.V. and MÉCHALI, M. (1988). Localization of c-myc expression during oogenesis and embryonic development in *Xenopus laevis. Development 104:* 631-641.

HUTCHISON, C.J., COX, R. and FORD, C.C. (1988). The control of DNA replication in a cell-free extract that recapitulates a basis cell cycle *in-vitro. Development 103:* 553-566.

IGUCHI-ARIGA, S.M.M., ITANI, T., KIJI, Y. and ARIGA, H. (1987). Possible function of the c-myc product: promotion of cellular DNA replication. *EMBO J. 6:* 2365-2371.

KING, M.W., ROBERTS, J.M. and EISENMAN, R.N. (1986). Expression of the c-myc proto-oncogene during development of *Xenopus laevis. Mol. Cell. Biol. 6:* 4499-4508.

LASKEY, R.A. (1979). Biochemical processes in early development. In *Companion to Biochemistry* (Eds. A.T. Bull, J.R. Lagnado, J.O. Thomas and K.F. Tipton). Longman, London, pp. 137-160

LEIBOVICI, M., GUSSE, M., BRAVO, R., LACROIX, J.C. and MÉCHALI, M. (1990). Characterization and developmental expression of *Xenopus* proliferating cell nuclear antigen (PCNA). *Dev. Biol.* (In press).

LOHKA, M.J. and MASUI, Y. (1983). Formation *in vitro* of sperm pronuclei and mitotic chromosomes induced by amphibian ooplasmic components. *Science 220:* 719-721.

MÉCHALI, M., GUSSE, M., VRIZ, S., TAYLOR, M., ANDEOL, Y., MOREAU, J., HOURDRY, J., LEIBOVICI, M., BRULFERT, A., ALMOUZNI, G. and MOUSSERON-GRALL, S. (1988). Proto-oncogenes and embryonic development. *Biochimie 70:* 895-899.

MÉCHALI, M. and HARLAND, R. (1982). DNA synthesis in a cell-free system from *Xenopus* eggs. Priming and elongation on single-stranded DNA *in vitro. Cell 30:* 93-101.

MÉCHALI, M., TAYLOR, M.V., GUSSE, M., EVAN, G.I. and HOURDRY, J. (1987). The *Xenopus* myc proto-oncogene: message stabilisation and post transcriptional regulation during early development. *Cell Differ. 20:* 197.

MOREAU, J., LEGUELLEC, R., LEIBOVICI, M., COUTURIER, A., PHILIPPE, M. and MÉCHALI, M. (1988). Detection of proto-oncogenes in the genome of the amphibian *Xenopus laevis. Oncogene 4:* 443-449.

NEWMEYER, D.D., LUCOCQ, J.M., BURGLIN, T.R. and DE ROBERTIS, E.M. (1986). Assembly *in-vitro* of nuclei active in nuclear protein transport: ATP is required for nucleoplasmin accumulation. *EMBO J. 5:* 501-510.

NEWPORT, J.W. (1987). Nuclear reconstitution *in vitro*: stages of assembly around protein-free DNA. *Cell 48:* 205-217.

NEWPORT, J.W. and KIRSCHNER, M. (1982a). A major developmental transition in early *Xenopus* embryos. I. Characterization and timing of cellular changes at the midblastula stage. *Cell 30:* 675-686.

NEWPORT, J.W. and KIRSCHNER, M. (1982b). A major developmental transition in early *Xenopus* embryos. II. Control of the onset of transition. *Cell 30:* 687-696.

NORDERMEER, J., MEIJLINK, F., VERRIJZER, P., RIJWIJK, F. and DESTREE, O. (1989). Isolation of the *Xenopus* homologue of int-1 wingless and expression during neurula stages of early development. *Nucl. Acids Res. 17:* 11-18.

PATRICK, T.D., LEWER, C.E. and PAIN, V.M. (1989). Preparation and characterization of cell free protein synthesis systems from oocytes and eggs of *Xenopus laevis. Development 106:* 1-9.

PRELICH, G. and STILLMAN, B. (1988) Coordinated leading and lagging strand synthesis during SV40 DNA replication *in vitro* requires PCNA. *Cell 53:* 117-126.

SAGATA, N., OSKARSSON, M., COPELAND, T., BRUMBAUGH, J. and VANDE WOUDE, G.F. (1987). Function of c-mos proto-oncogene product in meiotic maturation in *Xenopus* oocytes. *Nature 335:* 519-525.

SOUSSI, T., CARON DE FROMENTEL, C., MÉCHALI, M., MAY, P. and KRESS, M. (1987). Cloning and characterization of a cDNA from *Xenopus laevis* coding for a protein homologous to human and murine p53. *Oncogene 1:* 71-78.

SOUSSI, T., CARON DE FROMENTEL, C. and MAY, P. (1990). Structural aspects of the p53 protein in relation to gene evolution. *Oncogene 5:* 101-108.

STEELE, R.E. (1985). Two divergent cellular src genes are expressed in *Xenopus laevis. Nucl. Acids Res. 13:* 1748-1761.

STEHELIN, D., VARMUS, H.E., BISHOP, J.M. and VOGT, P.K. (1976). DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature 260:* 170-173.

SO, A.G., MILLS, A.P. and MORRIS, N.R. (1977). Assembly of SV40 chromatin in a cell-free system from *Xenopus* eggs. *Cell 10:* 237-243.

TAYLOR, M.V., GUSSE, M., EVAN, G.I., DATHAN, N. and MÉCHALI, M. (1986). *Xenopus* myc proto-oncogene during development: expression as a stable maternal mRNA uncoupled from cell division. *EMBO J. 5:* 3563-3570.

VRIZ, S. and MÉCHALI, M. (1989). Analysis of 3'-untranslated regions of seven c-myc revealed conserved elements prevalent in post-transcriptionally regulated genes. *FEBS Lett. 251:* 201-206.

VRIZ, S., TAYLOR, M. and MÉCHALI, M. (1989). Differential expression of two c-myc genes during embryonic development. *EMBO J. 8:* 4091-4097.

WOLFFE, A.P. and BROWN, D.P. (1987). Differential 5S RNA gene expression *in vitro. Cell 51:* 733-740.

WOODLAND, H.R. (1980) Histone synthesis during the development of *Xenopus. FEBS Lett. 121:* 1.