Temporal and tissue-specific expression of the proto-oncogene c-fos during development in Xenopus laevis

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Summary

We describe the isolation and complete sequence of the Xenopus c-fos proto-oncogene. c-fos expression throughout Xenopus development was analysed using a homologous probe derived from the cloned gene. c-fos RNA is accumulated during oogenesis to reach a plateau of $2 \times 10^5$ transcripts per stage VI oocyte, suggesting an unusual stability of the c-fos message. The amount of RNA per embryo decreases substantially after fertilisation to reach a level corresponding to less than 0.1 molecule per cell at the tailbud stage. Subsequently, at the swimming tadpole stage, the amount of c-fos mRNA increases; an increase that is correlated with the start of skeleton formation. In the newly metamorphosed froglet, c-fos mRNA shows a marked tissue-specific distribution, with the highest level in intestine and lowest in gall bladder, lung and spleen. We also demonstrate that the Xenopus c-fos gene is serum-inducible in Xenopus cultured cells, a property attributable to a promoter sequence known as the Serum Response Element (SRE). A protein activity (indistinguishable from Serum Response Factor) in both whole cell and nuclear Xenopus embryo extracts binds specifically to the SRE and is present at an approximately constant level throughout early development. Our results suggest roles for c-fos in aspects of both the rapid cell proliferation and cell differentiation characteristic of early Xenopus development.

Key words: c-fos, oncogenes, development, gene expression, Xenopus, SRF.

Introduction

Fertilisation of the amphibian egg triggers a period of rapid cell cleavage resulting in the division of egg cytoplasm into a thousand daughter cells within a few hours. Embryological studies have demonstrated that elements of the future body plan are established during this period despite the limited morphological and biochemical differentiation that has been documented. In subsequent hours, the cell division rate slows dramatically and the embryo undergoes the complex morphogenetic changes of gastrulation, during which regional and tissue-specific patterns of gene expression commence. The molecular mechanisms regulating these events are little understood but recent studies have implicated polypeptide growth factors as the earliest signals for regional specialisation (reviewed in Almendral et al. 1988). The earliest of these are transcriptionally activated within minutes of exposure to the growth factors and may comprise regulatory genes that control cell growth (Lau and Nathans, 1987).

The best characterised of such 'growth-related' genes is the proto-oncogene, c-fos (Muller, 1986; Verma, 1986). Its viral homologue (v-fos) is the transforming gene of the FBJ and FBR murine sarcoma viruses and many studies have implicated c-fos in the control of both cell proliferation and differentiation (Curran, 1988). Most recently, the c-fos gene product has been identified as a transcription factor (Chiu et al. 1988), responsible in conjunction with several others for the regulation of genes containing the AP1 binding site within their promoters (reviewed in Curran and Franza, 1988). Expression of the c-fos gene is itself modulated by a wide variety of environmental stimuli (including growth factors) suggesting that the gene acts as a nuclear 'third messenger' linking extracellular stimuli with the genetic responses they elicit, such as cell...
growth and differentiation (Herrlich and Ponta, 1989). For this reason, it is possible that genes such as c-fos play an important role in the cell and environmental interactions that regulate embryonic development. In this study, we report the structure of the amphibian c-fos gene and its expression during early Xenopus development. Our results suggest a role for c-fos in both the rapid cell proliferation and cell differentiation that occurs in early Xenopus development.

Materials and methods

Isolation and characterisation of the c-fos gene

A Xenopus laevis genomic library (Krieg and Melton, 1985) was screened using a 5 kb NotI/BglII fragment from the plasmid p6A711 (Treisman, 1985) as probe. This contains the entire human c-fos gene (VanStraaten et al. 1983). Positive recombinants were identified and purified as described by Maniatis et al. (1982) and hybridising fragments subcloned into plasmid vectors. The location of the Xenopus c-fos promoter was established using subcloned fragments as competitors for the Xenopus cytoskeletal actin SRE in a DNA mobility shift assay with embryo nuclear extracts (see below).

The nucleotide sequence of the entire gene was determined using the dideoxy method (Sanger et al. 1982, 1984). Each nucleotide was sequenced an average of four times on each DNA strand and the consensus analysed using the ANALYSEQ and Diagon programs.

RNA analysis

Stage VI oocytes, unfertilised eggs and embryos were obtained as outlined previously (Dumont, 1972; Gurdon, 1977; Gurdon and Wickens, 1983; Mohun et al. 1986). Embryos were staged as described by Nieuwkoop and Faber (1956). Total nucleic acid was extracted from oocyte, eggs and embryos as described previously (Mohun et al. 1984). Samples were analysed by an RNase protection assay (Zinn et al. 1983) using the equivalent of a single oocyte/egg/embryo in each hybridisation. Nuclease-resistant fragments were fractionated on thin 6% acrylamide sequencing gels and their sizes estimated from DNA markers electrophoresed in parallel. An RNA probe was synthesised from the plasmid pSP71Xfos with T7 polymerase, using the method of Melton et al. (1984). pSP71Xfos contains 210 nucleotides of the first exon of the gene. In addition to full-length protection, a prominent, smaller protected fragment (approximately 110 bp fragment) is observed. These were cloned into the EcoRI site of pUC18. The probe XDYAD1 was excised by cutting with BamHI and BstNI to release a 145 bp fragment. The binding activity of this sequence was compared with the Xenopus cytoskeletal actin SRE sequence (Act; Mohun et al. 1987), three synthetic derivatives (Act-L, Act-R and Act-L*; Treisman, 1987) and the Xenopus cardiac actin promoter CARG box 1 sequence (Mohun et al. 1986, 1989).

Results

Nucleotide sequence of the Xenopus c-fos gene

The entire nucleotide sequence of the Xenopus c-fos gene is shown in Fig. 1. The precise transcription initiation site has not been determined, but has been assigned to an A residue 25 nucleotides downstream from the putative TATA box. The gene encodes a transcript of 2060 nucleotides and contains three introns within its protein coding portion.

(a) Structure of the c-fos promoter

Transcriptional regulation of the mammalian c-fos genes has been extensively studied in the last few years and a number of sequences within the promoter have been identified as regulatory elements. In Fig. 2A, regions of the human, mouse and chicken c-fos promoters are aligned with their frog counterpart to illustrate the conservation of these cis-regulatory sequences. The most proximal of these to the TATA box is a ten nucleotide sequence (...-43 to -52 in the Xenopus gene) calculated from trace label incorporation. Serial dilutions of the sense transcripts were hybridised in parallel with embryo RNA samples, using the anti-sense RNA probe derived from pSP71Xfos. Amounts of c-fos RNA in the embryo samples were estimated by comparison with the signals from the serial dilutions of sense transcript, electrophoresed on the same gel.

For Northern blots, 10 μg of total RNA was fractionated on 1% formaldehide-agarose gels and blotted onto Genescreen. Filters were hybridised with probes containing Xenopus c-fos exon sequence as described elsewhere (Hopwood et al. 1989).

Cell culture

Xenopus A6 kidney fibroblast cells were cultured as described previously (Mohan et al. 1987). For serum stimulation assays, semi-confluent cultures were transferred into medium lacking serum for 36 h. Cells were then stimulated by refeeding with medium containing 20% foetal calf serum. For TPA stimulation, semi-confluent cultures were treated by the addition of TPA to a final concentration of 30, 100 or 500 nm in the culture medium. Similarly, A23187 was added to similar final concentrations to test the effect of the calcium ionophore. Cells were harvested at 0, 5, 30 and 120 min after stimulation and levels of c-fos transcripts compared with control cultures maintained in normal growth medium.

DNA mobility shift assays

DNA mobility shift assays were performed as described elsewhere (Taylor et al. 1989) using a neurula embryo nuclear extract or whole cell extracts from different developmental stages (Mohun et al. 1989). The Xenopus c-fos SRE sequence was synthesised as the 26mer oligonucleotides 5'-AAATTGTGTTCCTTATAGTTATCT-3' and 5'-AATAGATACACCTTATAAGGAAAAAC-3'. These were cloned into the EcoRI site of pUC18. The probe XDYAD1 was excised by cutting with BamHI and BstNI to release a 145 bp fragment. The binding activity of this sequence was compared with the Xenopus cytoskeletal actin SRE sequence (Act; Mohun et al. 1987), three synthetic derivatives (Act-L, Act-R and Act-L*; Treisman, 1987) and the Xenopus cardiac actin promoter CARG box 1 sequence (Mohun et al. 1986, 1989).
Xenopus c-fos expression

Fig. 1. Nucleotide sequence of the Xenopus laevis c-fos gene. The sequence of a 3530 nucleotide fragment containing the c-fos gene is shown along with the polypeptide sequence it encodes. Nucleotides are numbered with respect to an assigned transcription start site (*). Intron/exon boundaries are shown in lower case.

that can mediate transcriptional activation by cAMP and resembles the CRE sequence of other cAMP responsive genes and the ATF sequence of adenovirus early gene promoters (Fisch et al. 1989). The same element is required for basal c-fos promoter activity (Gilman et al. 1986; Fisch et al. 1987).

Further upstream (—284 to —298 in the Xenopus gene) lies a 15 nucleotide sequence known to comprise a protein binding site in vitro (Piette and Yaniv, 1987) that matches the API recognition sequence (Angel et al. 1987; Lee et al. 1987) and resembles the more degenerate AP4 consensus (Mermod et al. 1988). Adjacent to the API sequence lies the serum response element (SRE), a twenty nucleotide imperfect dyad (Treisman, 1985) that provides the recognition sequence for the SRF/P67 transcription factor (Treisman, 1985).
Fig. 2. Comparison of vertebrate c-fos gene sequences. (A) Sequence homologies in the promoter sequences of vertebrate c-fos genes. Sequences from the mouse (Van Beveren et al. 1983), chick (Fujiwara et al. 1987) and frog gene promoters are aligned with the human (Van Straaten et al. 1983) sequence and their relative positions with respect to the transcription start site indicated. Gaps (.) have been introduced to permit optimum alignment of the sequences. Five conserved regions are identified: (1) SCM inducible site; (2) serum response element; (3) API site; (4) cAMP response element; (5) TATA box.

(B) Sequence homologies in the 3' non-coding region of vertebrate c-fos genes. Sequences from the mouse, chick, rat and frog genes are aligned with the human gene sequence. Only differences from the human sequence are shown. Gaps (-) have been introduced to permit optimal alignments. Each is numbered with respect to the gene transcription start site, except for the rat sequence, which was obtained from a cDNA (Curran et al. 1987). Regions of homology across all five species are shown in bold. A 67 base pair sequence implicated in the rapid turnover of c-fos mRNA is underlined.

1986, 1987; Prywes and Roeder, 1987; Schroter et al. 1987) and mediates transient transcriptional activation of the c-fos gene in cultured cells by a wide variety of environmental stimuli (see below). A second factor that also binds the SRE has recently been characterised (Ryan et al. 1989). In the frog gene, the SRE lies from -298 to -371, but it is evident from Fig. 2A that the optimal alignment with the mammalian SREs is achieved by allowing for a three nucleotide insertion in the left half of the chick and frog SREs. The nucleotide adjacent to the 5' G residue of the SRE in the human and mouse genes is another G. This GG pair is necessary for binding of the P62 protein in conjunction with SRF/P67 (Shaw et al. 1989). Both the chick and frog promoters lack this GG pair, possessing instead a T residue in place of the first G. However, a GG pair can be identified three nucleotides further upstream.

Finally, a fifteen nucleotide sequence upstream of the SRE comprises the binding site of an inducible factor distinct from the ubiquitous SRF/P67 and P62 proteins (Hayes et al. 1987). The same sequence is required for basal promoter activity and can act as a cAMP responsive promoter element (Fisch et al. 1989). In the frog gene, this sequence lies from -355 to -369 whilst its counterpart in the mammalian and avian genes is 16-17 nucleotides closer to the SRE (see Fig. 2A).

A number of additional cis-acting sites have been identified in functional studies of mammalian c-fos genes that are not obviously conserved across species. These include a region between -76 and -97 in the human gene containing an eight base pair direct repeat 5'-GCGCCACC-3', which is absent from the mouse, chick and frog genes (Fisch et al. 1987); a region between -214 and -277 in the human promoter (containing direct and indirect repeats), which is required for transcriptional activation by nerve growth factor (Visvader et al. 1988); lastly, a region around -150 in the mouse gene that has not been characterised further (Gilman et al. 1986).

(b) Conservation of the c-fos transcript sequence
No significant homology can be detected between the
118 nucleotide 5' untranslated sequence of the Xenopus c-fos transcript and those encoded by the mammalian or avian genes. In contrast, several conserved regions are evident in the extensive 3' untranslated regions of these mRNAs (Fig. 2B). Functional studies have demonstrated that a conserved 67-nucleotide AU-rich sequence is at least in part responsible for the rapid turnover of c-fos transcripts and confers instability on heterologous transcripts (Treisman, 1985; Fort et al. 1987; Rahmsdorf et al. 1987; Wilson and Treisman, 1988; Shyu et al. 1989). A similar AU-rich region is required for instability of the human granulocyte-monocyte colony stimulating factor (GM-CSF) transcript and has been proposed as a recognition signal that identifies a large number of lymphokine, cytokine and proto-oncogene mRNAs that are rapidly degraded (Shaw and Kamen, 1986).

The precise sequences required to signal c-fos transcript degradation have yet to be defined and sequence comparisons of the human, mouse, rat and chick mRNAs yield little information since they are so similar over the last 250 nucleotides that encompass the AU-rich region. The frog sequence is however much more divergent; regions conserved between mammalian, avian and amphibian sequences are restricted to 21 nucleotides, which include the first ten of the 67 base AU-rich element, a seven nucleotide motif (5'-AAG-CATT-3') approximately 30 nucleotides upstream from the poly A addition signal and the hexanucleotide poly A addition signal itself. Interestingly, although the amphibian transcript contains an AU-rich region, apart from the first ten nucleotides, its sequence diverges from that shared by the other species.

(c) Conservation of the c-fos polypeptide sequence c-fos is one of a number of oncogenes that encode nuclear proteins that have recently been shown to be transcription factors. The c-fos gene product forms a heterodimer with the product of another proto-oncogene, c-jun, and the resulting protein activates transcription by binding to the API enhancer sequence (Chiu et al. 1988). Two distinct regions have been identified in the c-fos protein. A short, central sequence contains a ‘leucine zipper’ motif (Landschultz et al. 1988) and is essential for formation of functional c-fos/c-jun heterodimers (Scheurmann et al. 1989). Adjacent to this on the amino-terminal side is a cluster of basic residues that is required for recognition of the API binding site (Gentz et al. 1989; Turner and Tjian, 1989). Both of these regions are conserved in the frog c-fos protein (see Fig. 3). Similarly, the carboxy terminal end of the protein is highly conserved across species although it is clear from Fig. 3 that the amino terminal half of the c-fos proteins is the more highly conserved.

Expression of c-fos during Xenopus early development Northern blot analysis detected a single 2.2 kb transcript in total and poly(A)+ RNA from ovary tissue and unfertilised eggs, suggesting that the c-fos transcript was accumulated during oogenesis (Fig. 4A). We have consistently noticed that ovary RNA contains significantly more c-fos mRNA than that obtained from an equivalent number of defolliculated oocytes. Either the follicle cells surrounding each oocyte contain a considerable amount of the transcript or the manner by which the ovary fragments are obtained and treated prior to RNA extraction results in the activation of c-fos expression in the tissue (see below). Since little c-fos mRNA was detected in developing embryos, we used a more sensitive RNAase protection assay to quantify levels of transcript through early development (Fig. 4B). The c-fos transcript can be detected in previtellogenic (stage I) oocytes and accumulates to a peak level by stage III of oogenesis. It then declines to approximately half of this level during subsequent oocyte growth (Fig. 4B, lanes 3–7). After fertilisation, levels fall as the embryo develops and by tailbud stage, the steady state level of c-fos mRNA is only 1–2% of that detected in stage III oocytes. This contrasts with the rapid accumulation of cytoskeletal actin transcripts detected in the same RNA samples.

In order to quantify the amounts of c-fos mRNA, we synthesised in vitro a fragment of c-fos mRNA complementary to the probe and used this as a quantitative standard in protection assays (see Materials and methods; data not shown). We estimate that the amount of c-fos mRNA in the full-grown oocyte as approximately 2×10^5 transcripts, falling to less than one-third of this amount in the gastrula embryo. By the tailbud stage, the amount has fallen to 10^4 molecules per embryo. For comparison, we repeated the procedure for the ubiquitous, type 5 cytoskeletal actin mRNA, a prevalent transcript in both oocytes and embryos. Approximately 2×10^6 cytoskeletal actin transcripts are accumulated in the stage VI oocyte and activation of the gene during gastrulation results in the accumulation of 5×10^7 transcripts by the tailbud stage. Interestingly, c-fos transcript levels rise several fold during later development of swimming tadpoles, the increase being detected from stage 41 (Fig. 4B, lanes 12 and 13). By stage 46, the tadpole contains approximately 10^5 molecules of c-fos mRNA.

Tissue specialisation and organogenesis are essentially complete in the swimming tadpole and by stage 46 feeding has commenced. To examine whether the accumulation of c-fos transcripts from stage 41 onwards occurs in a regional or tissue-specific manner, we compared the levels of c-fos mRNA in a variety of organs dissected from newly metamorphosed froglets. The levels of c-fos mRNA varied widely between different tissues and organs (Fig. 5). The highest level was detected in intestine (lane 5); relatively high levels were also detected in kidney, brain, skin and stomach (lanes 6, 10, 11 and 12 respectively); intermediate levels were found in liver and limb skeletal muscle (lanes 3 and 9); very few or no transcripts were detected in gall bladder, lung or spleen (lanes 2, 7 and 8 respectively).

Expression of c-fos in cultured Xenopus cells In mammalian cells, c-fos mRNA and protein are present in very low amounts in cultured cells, but expression of the gene is rapidly and transiently induced...
Fig. 3. Comparison of vertebrate c-fos gene products. The deduced polypeptide sequences of the mouse (Van Beveren et al. 1983), chick (Fujiiwara et al. 1987), rat (Curran et al. 1987), human (Van Straaten et al. 1983) and frog c-fos proteins are aligned and differences from the human sequence indicated. Gaps (-) have been introduced to permit optimum alignment. Asterisks identify the five conserved leucine residues of the 'leucine zipper' motif and the adjacent basic region of the proteins is highlighted (*).

by a variety of environmental stimuli. Transcriptional activation by many of these agents, including growth factors, serum mitogens and phorbol esters is mediated by the SRE protein-binding site upstream of the c-fos promoter (see Norman et al. 1988 and references therein). We have previously found that, in the *Xenopus* kidney fibroblast cell line, the SRE functions as a constitutive promoter element for the cytoskeletal actin genes (Mohun et al. 1987) and that the *Xenopus* c-fos SRE (XDYAD 1) can efficiently replace the actin SRE in this activity (T.M. unpublished data). However, our attempts to elicit serum responsive transcription from these cells were unsuccessful. We now find that after prolonged culture in serum-free medium, kidney fibroblasts will indeed respond to serum mitogens with increased c-fos transcription.

Within five minutes of stimulation by serum, c-fos transcripts were detected in the A6 fibroblast cell line. These accumulated rapidly, reaching a peak within 30 min and declined to barely detectable levels over the following 90 min (Fig. 6, lanes 2–5). Expression of c-fos is also stimulated by treatment of cultures with the phorbol ester, TPA, but is unaffected by the calcium ionophore A23187 (Fig. 6, compare lanes 7–9 with lanes 10–12). No synergistic effect was detected when cells were treated with both TPA and the ionophore (compare lanes 7 and 8 with lanes 13 and 14). Cytoskeletal actin mRNA levels are unchanged by serum, TPA
Fig. 4. Expression of c-fos during Xenopus early development. (A) Northern blot hybridised with a Xenopus c-fos exon probe. Lane 1: 10 µg ovary RNA. Lane 2: 10 µg unfertilised egg RNA. Lane 3: 0.5 µg ovary poly(A)+ RNA. The size of the single c-fos transcript was estimated from Xenopus and bacterial ribosomal RNA markers (not shown). (B) RNASpase protection assay to monitor the level of c-fos transcripts through development. Samples were each hybridised with probes for c-fos, type 5 cytoskeletal actin and SS RNAs (upper, middle and lower panels respectively). In the upper panel, the position of undigested c-fos probe is indicated (probe). Full-length protection of the probe by c-fos transcripts results in a protected fragment of 210 nucleotides (Fosx). Two smaller protected fragments are also produced of approximately 110 nucleotides. The middle and lower panels show only the major protected fragments obtained with the cytoskeletal actin and SS RNA probes. Lane 1: tRNA. Lanes 2–7: oocyte RNA (stages I–VI respectively). Lane 8: unfertilised egg RNA. Lanes 9–12: blastula, gastrula, neurula and tailbud RNA respectively. Lanes 13 and 14: tadpole RNA (stages 41 and 46 respectively). Each lane contained approximately 20 µg of total RNA. Subsequent O.D. measurements demonstrated that significantly more RNA was included in hybridisations shown in lanes 3, 4 and 8. This is consistent with the elevated levels of cytoskeletal actin and SS RNA as estimated by densitometry.

c-fos gene is attributable to the SRE (XDYAD1–T.M. unpublished data).

Protein-binding characteristics of the Xenopus c-fos SRE

As the SRE mediates the action of many environmental stimuli on the expression of c-fos in cultured cells, it is likely that the same sequence is important for the regulation of c-fos during embryogenesis. We have therefore sought to characterise the protein(s) present in embryo extracts that can bind to the c-fos SRE sequence.

In earlier studies, we found a binding activity present in ovary S100 and embryo nuclear extracts that bind specifically to the cytoskeletal actin SRE and synthetic SRE sequences (Mohun et al. 1987; Taylor et al. 1989). Furthermore, an indistinguishable binding activity was detected using a CArG box sequence (Mohun et al. 1989; Taylor et al. 1989), a promoter element necessary for muscle-specific expression of cardiac and skeletal actin genes (Minty and Kedes, 1986; Mohun et al. 1989; Walsh, 1989). The Xenopus c-fos SRE (XDYAD1 – see Methods for abbreviations) forms a similar, specific complex in bandshift experiments using the nuclear extract from neurula (stage 18) embryos (Fig. 7). The cytoskeletal actin SRE, cardiac actin CArG box1 and synthetic SRE-like sequences all compete with varying affinities for the c-fos SRE-binding activity (Fig. 7) and the mobility of the fos SRE/protein complex is identical to that formed with any of the other binding sites (data not shown).

We have previously proposed that all these sequences are bound by the same factor in the neurula extract, the amphibian equivalent of the mammalian transcription factor, SRF (Taylor et al. 1989). The relative efficiency
Fig. 5. Tissue distribution of c-fos transcripts in metamorphosing frogs. The relative levels of c-fos mRNA in various organs dissected from newly metamorphosed frogs was assessed using the RNAse protection assay. The position of the 210 nucleotide fragment obtained from full-length protection of the probe by c-fos mRNA is indicated (Fosx). P: undigested probe. Lane 1: tRNA. Lane 2: gall bladder. Lane 3: liver. Lane 4: heart. Lane 5: intestine. Lane 6: kidney. Lane 7: lung. Lane 8: spleen. Lane 9: limb skeletal muscle. Lane 10: brain. Lane 11: skin. Lane 12: stomach. Each tissue RNA sample contains similar amounts of total RNA as assessed by RNAse protection with the 5S probe using the same samples (data not shown).

Fig. 6. Expression of c-fos in cultured Xenopus cells. A6 kidney cells were assayed by RNAse protection for the presence of c-fos transcripts after treatment with serum, the phorbol ester, TPA, or the calcium ionophore, A23187. 20 µg of total RNA was included in each assay and protected fragments representing c-fos mRNA are indicated (Fosx). Lane P: undigested probe. Lane 1: tRNA control. Lanes 2-5 show the effect of serum stimulation on c-fos levels. RNA was extracted directly from cells 0, 5 30 and 120 min after refeeding of serum-starved cells. Lanes 6-14 show the effect of TPA and A23187 on A6 cultures. These were added to the cell growth medium containing 10% foetal calf serum. After 30 min of treatment, cells were harvested from each plate and RNA isolated. Lane 6: A6 RNA control. Lanes 7-9: 30, 100 and 500 nM final TPA concentration. Lanes 10-12: 30, 100 and 500 nM final A23187 concentration. Lane 13: 30 nM-TPA and 30 nM-A23187. Lane 14: 100 nM-TPA and 100 nM-A23187. Lane M: molecular weight markers (HindIII digested pBR322; 220 and 154 nucleotides). In the lower panels, the same samples were assayed for type 5 cytoskeletal actin transcripts.

Discussion

The Xenopus c-fos transcript, like its mammalian
counterpart which has a half-life of approximately ten minutes (Rahmsdorf et al. 1987), is very unstable in cultured fibroblasts. This is indicated by its rapid induction in response to serum and its subsequent rapid disappearance. In contrast, the message is extremely stable during the earliest stages of frog development, accumulating during oogenesis and being inherited by the fertilised egg.

Using the quantification described here, it is possible to estimate the half-life of the c-fos mRNA in the oocyte. In later oogenesis the amount of c-fos mRNA is maintained at an approximately constant level of $2 \times 10^5$ transcripts. If it is assumed that the c-fos gene is being transcribed at the maximum rate for a PolII gene of 10 transcripts per gene per minute (Perlman and Rosbash, 1978; Ng et al. 1984), then the transcripts must have a half-life of a few days in order for the constant level to be maintained. Of course if the c-fos gene were transcribed at less than the above maximum rate, then the estimated half-life would be longer still. What is clear is that the stability of the Xenopus c-fos transcript is very much greater in the oocyte than in a normal, somatic cell. A similar situation was found previously for transcripts from another Xenopus proto-oncogene, c-myc, which is also very unstable in normal, somatic cells (Taylor et al. 1986). The accumulated, maternal c-fos mRNA is degraded after fertilisation so that less than one third of the message remains in gastrulae. Again c-myc was found to behave similarly (Taylor et al. 1986).

The mechanisms responsible for c-fos transcript stability in oocytes and its rapid degradation in embryos are as yet unknown. We note that both c-fos and c-myc mRNAs contain motifs implicated in message instability (see Results and S.Vriz and M.T. unpublished data) and it may be possible to use the experimental flexibility provided by Xenopus oocytes to identify sequences and factors responsible.

Whilst the stability of c-fos and c-myc mRNAs is unusual, it is maybe not surprising that such mRNAs are accumulated in the oocyte. The period of development following fertilisation is characterised by a series of rapid cell divisions that occur in the absence of transcription and growth (see for example Newport and Kirschner, 1982). It is supported by the maternal stockpile of the oocyte, whose general biochemical composition is equivalent to 10–100,000 somatic cells (Laskey, 1979 for review). The stabilisation of normally highly labile mRNAs may be just one specialisation of the oocyte to produce this maternal stockpile of components to support the rapid cleavage phase of early development. An example of another such specialisation is the uncoupling of histone synthesis from DNA synthesis in the oocyte.

We have no direct information on the role of the accumulated, maternal c-fos mRNA; however, since one function of c-fos protein is as a transcription factor, it is reasonable to suggest that c-fos has a role in oocyte transcription. This might be, at least partly, in the production of other growth-related gene products that are required to support the cleavage phase of Xenopus early development. A role for c-fos as a transcription factor in the earliest period of embryo development is precluded by the absence of transcription, but the c-fos product could also function as a transcriptional activator during post-MBT development. This period is characterised by cell and tissue differentiation.

By the tailbud stage there are only $10^5$ c-fos mRNAs per embryo, which corresponds to a level of $<0.1$ molecules per cell. However, there is a subsequent increase in the c-fos mRNA content during the swimming tadpole stage of development. Nonetheless, the total amount of $5 \times 10^5$ transcripts still corresponds to a very low average number of molecules per cell. If this increase is functionally important, it is probable that there is a localisation of the message. Whilst we do not know whether this is the case, we do know that in the
newly metamorphosed froglet different organs contain very different amounts of c-fos mRNA. It is also noteworthy that at the time of increased c-fos expression from stage 40 onwards, the frog skeleton begins to form through mesenchymatous condensation, chondrification and subsequent perichondrial ossification. In both the mouse and rat, c-fos expression has been implicated in skeletal development (Muller et al. 1982; Ruther et al. 1987; Dony and Gruss, 1987; De Togni et al. 1988).

The first described response of the c-fos and c-myc proto-oncogenes in normal cells was a dramatic increase in their expression in quiescent fibroblasts and lymphocytes responding to mitogens (Kelly et al. 1983; Greenberg and Ziff, 1984; Moore et al. 1986). Many systems have since been described where c-fos and c-myc are expressed together, often when linked to a cell proliferative response. Both genes have also been implicated in aspects of cell differentiation. This is also true of the present study. However, the two Xenopus genes show both quantitative and qualitative differences in expression. By the mid-neurula stage, the mean number of c-myc transcripts is approximately 50 per cell, which is the same as that found in proliferating cultured cells. In contrast, c-fos mRNA levels have fallen to an average of less than one molecule per cell at bud embryos there are localised high levels of c-myc. Taken together these results demonstrate that the expression of the early genetic response to growth factors in mouse fibroblasts. Mol. cell. Biol. 8, 2191–2201.

References


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