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Region-specific regulation of the actin multi-gene family in early amphibian embryos

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The actin multi-gene family shows both spatial and temporal regulation during early embryogenesis in the amphibian *Xenopus laevis*. Both muscle-specific and ubiquitous cytoskeletal actin genes are activated at the end of gastrulation; transcription of the α-cardiac and α-skeletal actin genes is restricted to the somitic mesoderm and its muscle-forming derivatives providing a convenient molecular marker for this early embryonic tissue.

Introduction

During embryogenesis, equipotent cells of the embryo embark upon divergent pathways of differentiation to provide the embryonic tissues and organ primordia from which the larval and adult organism is constructed. A long standing goal of embryologists has been to discover the mechanisms that control embryonic cell differentiation and that confine the cells of different embryo regions to distinct developmental fates. The amphibian embryo is especially suited for these studies since the normal biochemical and morphological events of embryo development have been extensively characterized. Amphibian embryos are also sufficiently large to permit a wide range of experimental manipulations such as dissection and tissue grafting (see, for example, Slack *et al.* Gerhart *et al.*, this symposium).

In a molecular approach to this problem we have investigated when the various regions of a frog embryo establish distinct patterns of gene expression. Using a molecular marker for a particular embryonic tissue, the somite region of the mesoderm, we have begun to study the origins of these cells and the mechanisms by which their gene activity is regulated.

Actin gene expression in amphibians

One of the earliest tissues to differentiate in the amphibian embryo is embryonic muscle which forms as segmented myotome blocks along most of the larval body axis (Hamilton 1969; Muntz 1975). The myotomes are fully contractile in the tailbud embryo long before other organs such as the liver, heart or gut are fully formed or functional, and the larval tadpole can swim several days before it feeds (Nieuwkoop & Faber 1967). As in other vertebrates (reviewed in Buckingham & Minty 1983), muscle cells in amphibians contain a unique set of contractile proteins, the most abundant of which is muscle-specific actin. Genes encoding these proteins are expressed only in contractile tissue and in all vertebrates so far studied, their control is exerted at a transcriptional level.

As well as the muscle-specific actin, vertebrates contain other closely related actins that are ubiquitous among cells and comprise a major component of the cytoskeleton. In total, six different actins have been found in vertebrates; two striated muscle types (α-skeletal and

α-cardiac), two smooth muscle types and two cytoskeletal forms (β- and γ-types). The multigene family that encodes these abundant proteins is therefore subject to tissue-specific control which is established early in embryonic development. We have used molecular cloning techniques to study the expression of both cytoskeletal and muscle-specific actin genes during the earliest stages of amphibian embryogenesis.

Cloning and characterization of actin cDNAs

Actin gene transcripts can be detected by hydridization to radio-labelled, complementary DNA (cDNA) probes, and to obtain these we have isolated several actin cDNAs from cDNA libraries derived from embryonic and adult muscle RNA (Mohun *et al.* 1984). From the nucleotide sequence of each recombinant we have been able to predict their protein-coding potential and design probes that will hybridize only to the transcripts of individual actin genes. Such probes are derived from the untranslated mRNA 3′-trailer sequence of each cDNA, since any protein coding portion will cross hybridize with the same region of all other actin mRNAs. Hybridization of these probes to northern blots of adult tissue RNA has provided the *in vivo* identity of three actin cDNAs. Two are muscle-specific, the α-cardiac and α-skeletal forms, while the third is a cytoskeletal type. Alpha-skeletal actin mRNA is detected solely in adult skeletal muscle tissue, and α-cardiac transcripts are unique to heart tissue. By contrast, the cytoskeletal actin mRNAs are detected to varying degrees in all adult tissues. Nucleotide sequence analysis has established that the cytoskeletal actin cDNA encodes the γ-like type 8 actin protein previously identified by peptide mapping (Vandekerckhove *et al.* 1981). Using an S1 nuclease protection assay, we estimate that at least 99% of the muscle-specific actin gene transcripts in adult frog muscle are of the α-skeletal type. We detect no co-expression of α-cardiac and α-skeletal actin genes in either skeletal muscle or heart tissue.

Developmental expression of actin genes

The same procedures have been used to examine actin gene expression in early embryonic development (figure 1). Three conclusions can be drawn from these experiments. First, the abundance of all three actin mRNAs increases dramatically within the first day after fertilization and before morphological differentiation of myotome blocks is complete. Second, by using S1 protection analysis with RNA from individually staged embryos we first detect transcripts from the muscle-specific genes shortly after gastrulation (embryo stage 14). We conclude that unless the muscle actin gene transcripts show a dramatic developmentally regulated increase in stability, their genes must be activated at the end of gastrulation. Third, a relatively constant low level of cytoskeletal actin mRNA is detected at all stages before gastrulation, including oöcytes and fertilized eggs. We suspect that this results from a maternal actin mRNA pool that is inherited by the early embryo (figure 2).

Regional activation of muscle actin genes

By the onset of muscle actin gene activation the amphibian embryo comprises the three embryonic tissue layers: ectoderm, mesoderm and endoderm (which differ in their cellular morphology). By dissecting apart various regions of each of the three layers from neurulating

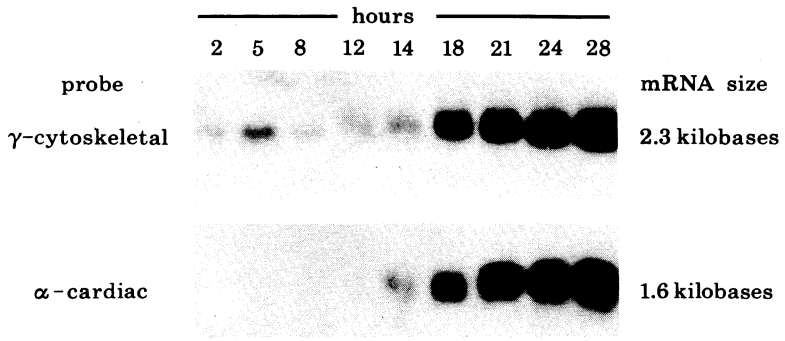

FIGURE 1. The accumulation of actin mRNAs during early embryogenesis in *Xenopus*. Total RNA was extracted from early embryos at successive stages of development from 2 to 28 h after fertilization, and 10 μg of each sample electrophoresed on a formaldehyde agarose gel. After blotting on to nitrocellulose, the RNA was hybridized to a single-stranded DNA probe, radio-labelled with ^{32}P and specific for a particular actin mRNA. The figure shows the results of two such experiments with probes for the γ-cytoskeletal and α-cardiac actin mRNAs. The α-cardiac actin message-specific probe hybridizes to a single size class of short mRNAs (1600 nucleotides). The same size class hybridizes to the α-skeletal actin message-specific probe. Both muscle actin mRNAs show similar developmental profiles. γ-cytoskeletal actin mRNA (2300 nucleotides) can be detected at all stages of development. The levels of all actin mRNAs studied show a dramatic increase after gastrulation.

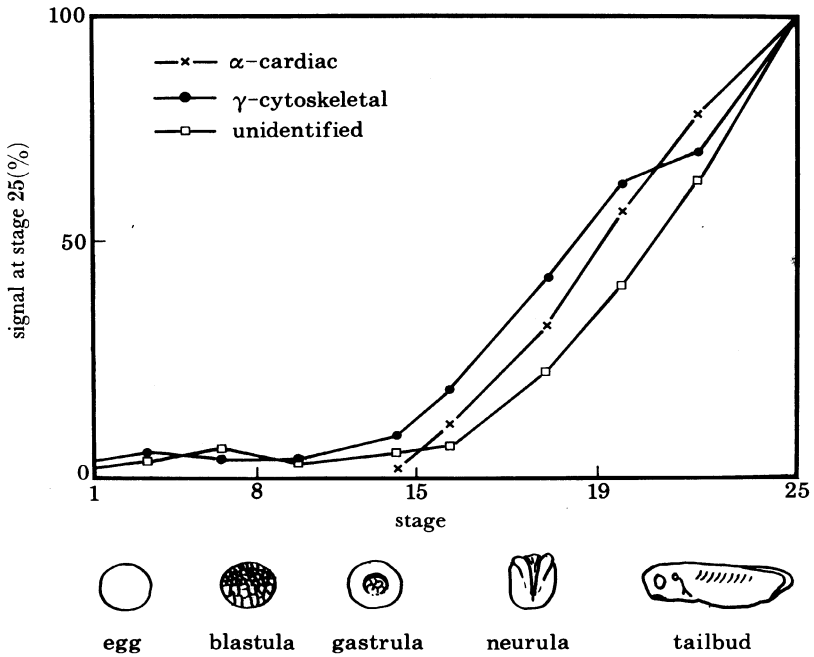

FIGURE 2. Relative levels of actin mRNAs accumulated during early development. RNA blots such as those in figure 1 were scanned with a Joyce–Loebl densitometer and values standarized by using the signals obtained from serial dilutions of tailbud RNA samples included in each blot. The three curves represent the accumulated levels of γ-cytoskeletal, α-cardiac and an unidentified third actin mRNA (probably encoding a cytoskeletal protein). Values given represent the percentage fraction of the signal obtained for each particular probe with RNA from tailbud embryos.

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embryos (figure 3) we have found that transcription of the muscle actin genes is initiated only in the cells of the mesoderm (Mohun *et al.* 1984). Furthermore, no α-cardiac or α-skeletal actin mRNA can be detected by the sensitive S1 protection assay in the most dorsal, axial strip of the mesoderm mantle which by this embryonic stage has separated to form the notochord. The muscle-specific mRNAs are found almost exclusively in the dorsal mesoderm that flanks the notochord. It is this tissue, the somitic mesoderm, from which the segmentally arranged embryo somites (and in later stages the myotomes) are subsequently derived. Thus, from the time of their activation, the muscle actin genes are active in only a fraction of embryonic cells and provide a convenient molecular marker for amphibian somitic mesoderm.

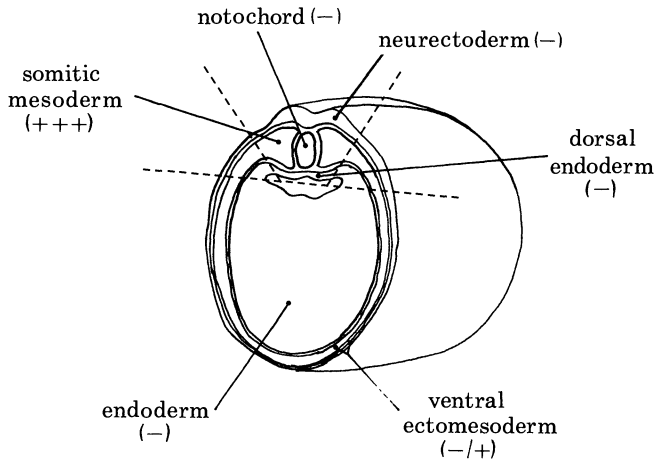

FIGURE 3. Diagram of stage 18 neurula embryo. Dotted lines indicate cuts made in the embryo to allow separation of the six fragments identified. RNA was extracted from each fragment and tested for the presence of actin mRNAs by S1 nuclease protection. The distribution of muscle-specific actin mRNAs is shown in the figure. Both α-skeletal and α-cardiac actin mRNA are found almost exclusively within the somitic mesoderm. A minor, variable signal is found for both with the ventral ectomesoderm and might arise from the heart primordium that has formed from this region of the mesoderm.

From both classical embryological studies and more recent work (see Slack 1983), it is known that the dorsal mesoderm becomes committed to notochord and somite formation during the period of gastrulation (stage 10) several hours before muscle fibres or their constituent proteins can first be detected (Ballantine *et al.* 1979). Our present results now indicate that cell commitment in the presumptive somite region of the mesoderm precedes somite-specific gene expression (i.e. muscle actin gene activation) by about five hours when assayed by the most sensitive methods available. We can conclude that the molecular mechanisms that underlie embryonic cell determination act before genes that encode products of terminal differentiation are activated.

THREE LEVELS OF ACTIN GENE REGULATION

The results described above suggest that the actin multi-gene family is regulated at a number of different levels during amphibian development and these may be summarized as follows.

(1) All actin genes appear to be activated at the end of gastrulation (approximately stage 13), and their transcripts are first detected shortly afterwards. Other genes active in early

embryos, including those transcribed by all three RNA polymerases, are first expressed at various times both before and after actin gene expression commences (see, for example, Shiokawa *et al.* 1979; Woodland *et al.* 1979; Newport & Kirschner 1982; Sargent & Dawid 1983). There must therefore be a specific mechanism that selects the actin genes for activation at this developmental stage.

(2) Individual actin genes are activated in a cell-type specific manner. In particular, both muscle-specific actin genes are co-expressed in approximately equal amounts in somitic mesoderm and its myotome derivatives. The contractile, embryonic muscle of the tadpole contains both α-cardiac and α-skeletal actin mRNA and presumably utilizes both proteins. Muscle actin transcripts are first detected only shortly before synthesis of α-actin proteins commences (Ballantine *et al.* 1979), indicating that the expression of these genes is regulated primarily at the transcriptional level. The cytoskeletal actin genes are activated in all tissues of the embryo to varying degrees.

(3) A third level of regulation is exerted in later stages to ensure that the co-expression of muscle actin genes in muscle tissues is abolished and individual muscle-specific members of the multi-gene family are expressed in the appropriate muscle type. In this respect amphibia differ from other vertebrates which show a significant level of co-expression in adult muscle tissues (Minty *et al.* 1982; Mayer *et al.* 1983; Gunning *et al.* 1983).

Future prospects

Our most recent studies have begun to examine the controls that establish cell-type specific actin gene activation in the early embryo. We already know that the tissue-specific activation of muscle actin genes does not require the normal cell–cell contacts that arise during the complex cell movements of gastrulation. Indeed, before any cell movements, a restricted group of cells in the blastula embryo have gained all the information necessary for effecting an appropriately timed gene switch. For example, an embryo fragment comprising the equatorial third of a mid-blastula embryo (stage 8) will activate the α-cardiac actin gene at the same time as control embryos when cultured in isolation (cf. Sudarwati & Nieuwkoop 1971). In such experiments, the S1 nuclease protection assay is sensitive enough to be used on individual embryo fragments. Equivalent sized fragments comprising the animal and vegetal pole regions of the embryo do not make the same gene switch. On the basis of similar experiments we have found that the ability to activate these genes autonomously is the property of cells located equatorially in the earliest embryo stages and perhaps reflects a localization in the cytoplasm of the new fertilized egg. Our future goal is to identify the macromolecular basis of such a localization. Our results with blastula fragments are described by Gurdon *et al.* (1984).

Finally, we have begun to examine one of the inductive interactions that classical studies have documented in early embryos. In blastula stage embryos, the presumptive mesoderm can be removed, the resulting endodermal and ectodermal fragments recombined and successfully cultured (see Sudarwati & Nieuwkoop 1971). Axial tissues (i.e. mesodermal derivatives) can be found in the cultured recombinants. The results suggest that the endoderm is capable of inducing mesoderm formation from the overlying ectoderm tissue. We have found that the recombinant embryos activate the muscle actin genes at the appropriate time and we are now using this gene switch as a sensitive assay for the inductive process.

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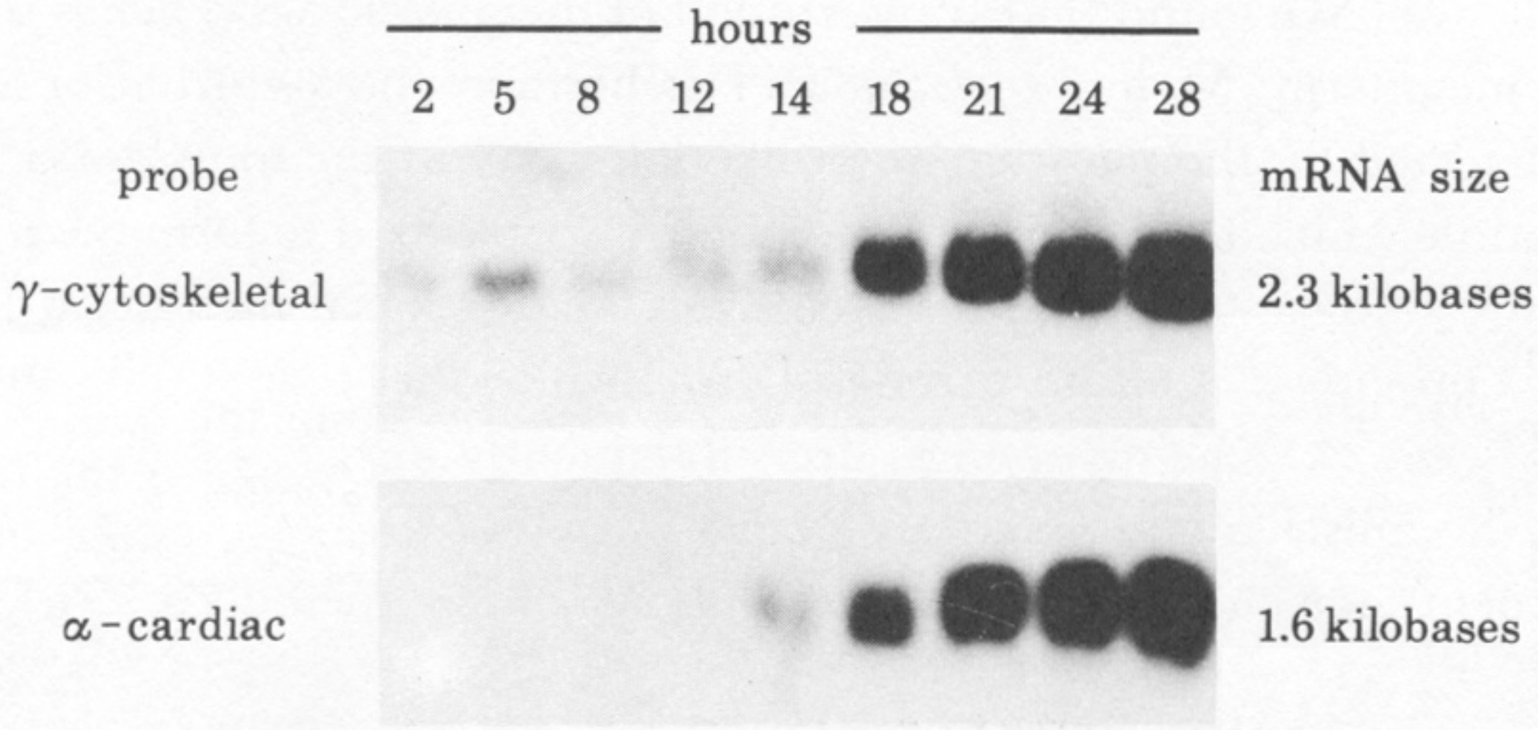

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