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Intercellular signalling in mesoderm formation during amphibian development

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SUMMARY

The mesoderm of amphibian embryos arises through an inductive interaction in which a signal from the vegetal hemisphere of the blastula-stage embryo acts on overlying equatorial cells. Strong candidates for endogenous mesoderm-inducing signals include members of the fibroblast growth factor (FGF) and activin families. In this paper we show that cells form different mesodermal cell types in response to different concentrations of these factors, and that graded distributions of activin and FGF can, in principle, provide sufficient positional information to generate the body plan of the *Xenopus* embryo.

1. INTRODUCTION

The first inductive interaction in amphibian development, and perhaps in the development of all vertebrates, is mesoderm induction (see reviews by Smith (1989); Dawid & Sargent (1990); Whitman & Melton (1989)). In amphibia this occurs when a signal from the vegetal hemisphere of the blastula-stage embryo acts on overlying equatorial cells (figure 1*a*,*b*). Several factors have been identified which may be involved in this interaction, including members of the activin family (Albano *et al.* 1990; Asashima *et al.* 1990; Smith *et al.* 1990; Thomsen *et al.* 1990), fibroblast growth factor (Slack *et al.* 1987; Kimelman & Kirschner 1987) and bone morphogenetic protein-4 (Köster *et al.* 1991; Dale *et al.* 1992; Jones *et al.* 1992). In addition, members of the Wnt family (Smith & Harland 1991; Solol *et al.* 1991; Chakrabarti *et al.* 1992) and the recently discovered noggin (Smith & Harland 1992), may be involved in imposing pattern on the mesoderm.

Members of the activin family are among the strongest candidates for an endogenous mesoderm-including factor. Treatment of presumptive ectodermal cells of the *Xenopus* blastula (the animal cap, which is normally out of range of the natural mesoderm-inducing signal) results in those cells forming mesodermal cell types such as muscle and nonochord, rather than epidermis (figure 1*c*,*d*). Activin-like protein is present in the early *Xenopus* embryo (Asashima *et al.* 1991; G.-D. Geux & J. C. Smith, unpublished observations), and over-expression of a mutated activin receptor during early development causes loss of mesodermal structures (Hemmati-Brivanlou & Melton 1992). In this article we ask to what extent activin might provide cells not only with the information to form mesoderm, but also with information about their position within the embryo (see Wolpert 1969) and what sort of mesoderm they should form. We suggest that this information is transmitted by different concentrations of activin, and go on to discuss mechanisms by which this might occur.

2. CONCENTRATION-DEPENDENT EFFECTS OF ACTIVIN

To study the mesoderm-inducing effects of different concentrations of activin on embryonic cells, it is desirable that all cells are exposed to the same concentration of factor. To achieve this, presumptive ectodermal cells of the animal pole region of the *Xenopus* blastula are dispersed by culture in calcium- and magnesium-free medium. Groups of such cells are exposed to different concentrations of activin for one hour before being reaggregated, cultured overnight to the neurula stage, and then assessed for expression of various region-specific mesodermal markers. The result of one such experiment is shown in figure 2, which shows that increasing concentrations of activin cause the formation of more anterior and dorsal mesoderm (Green & Smith, 1990; Green *et al.* 1992).

At low concentrations, activin does not divert cells from their normal fate of epidermis. At slightly higher doses, expression of *Xhox3*, *XlHbox6* and *Xbra* is induced. These genes are expressed predominantly in the posterior and lateral mesoderm of the neurula (Ruiz i Altaba & Melton 1989; Wright *et al.* 1990; Smith *et al.* 1991), and although *Xbra* is also expressed elsewhere at this stage (see below and figure 3), its combination with *Xhox3* and *XlHbox6* is characteristic of this region. *Xhox3*, *XlHbox6* and *Xbra* are switched off with increasing activin concentrations and muscle-

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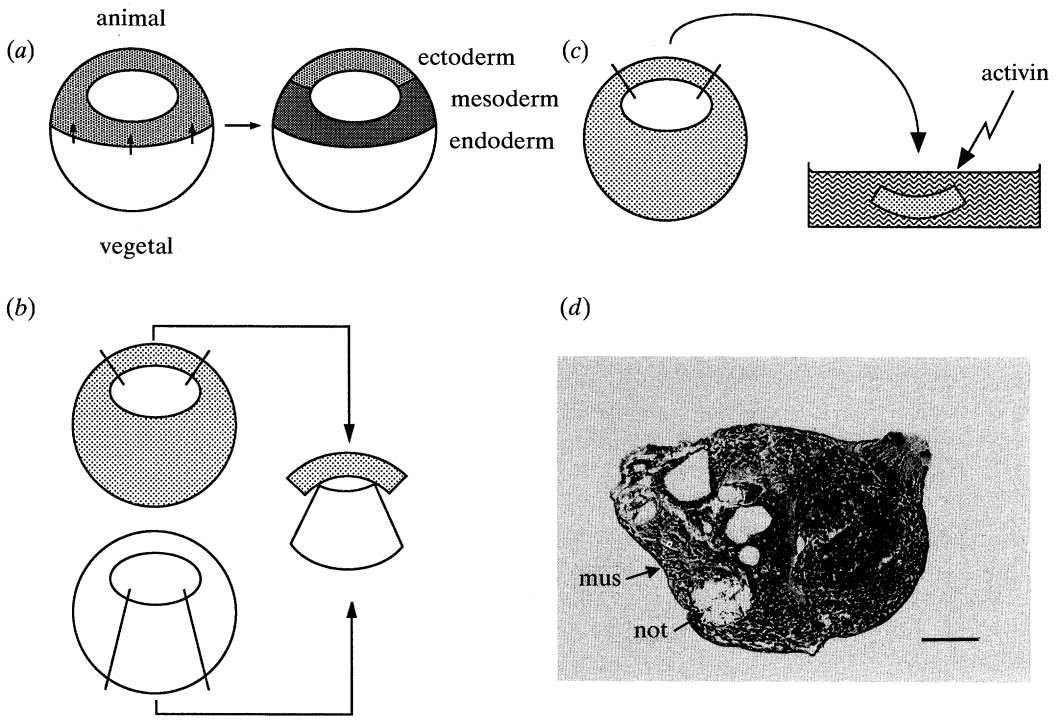

Figure 1. Mesoderm induction. (*a*) Mesoderm is formed from the equatorial region of the blastula-staged embryo as the result of an inductive signal produced by cells of the vegetal hemisphere. (*b*) Demonstration of mesoderm induction. Cells from the animal cap of a lineage-labelled blastula are juxtaposed with cells from the vegetal hemisphere. (*c*) Treatment of animal pole tissue with activin. (*d*) A section through a *Xenopus* animal pole region treated with activin and cultured for three days. Note notochord (not) and muscle (mus). Scale bar is 100 μm.

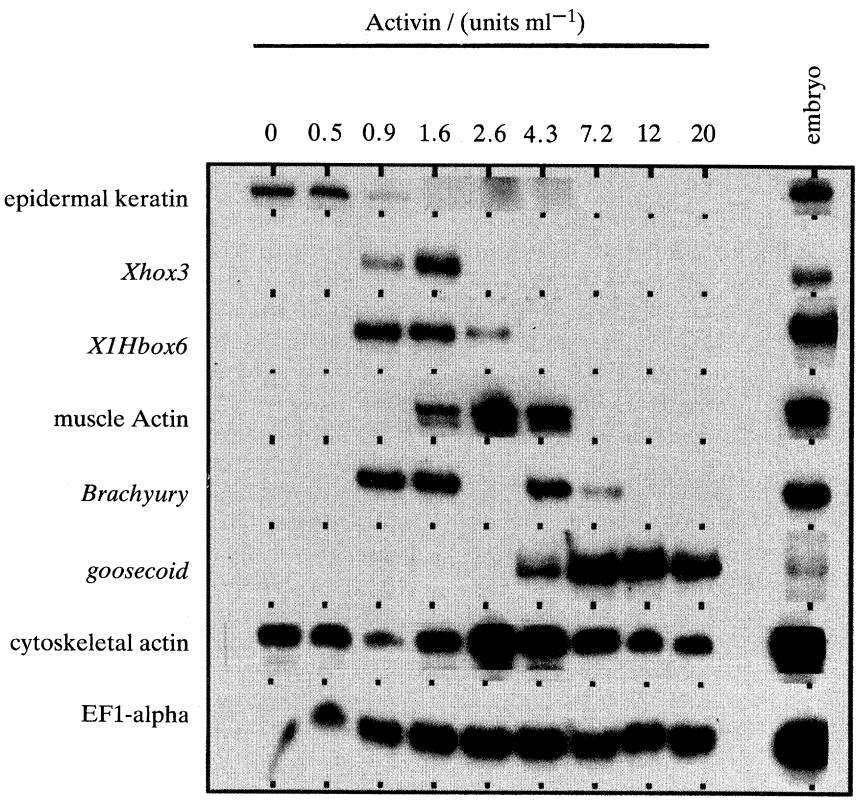

Figure 2. Activin induces different region-specific markers according to its concentration. Dispersed cells derived from blastula animal pole regions were exposed to a 1.7-fold dilution series of activin. After re-aggregation and culture to the neurula stage they were analysed for expression of epidermal keratin, *Xhox3*, *XlHbox6*, muscle-specific actin, *Xenopus Brachyury* and *goosecoid*. EF-1α and cytoskeletal actin serve as loading controls.

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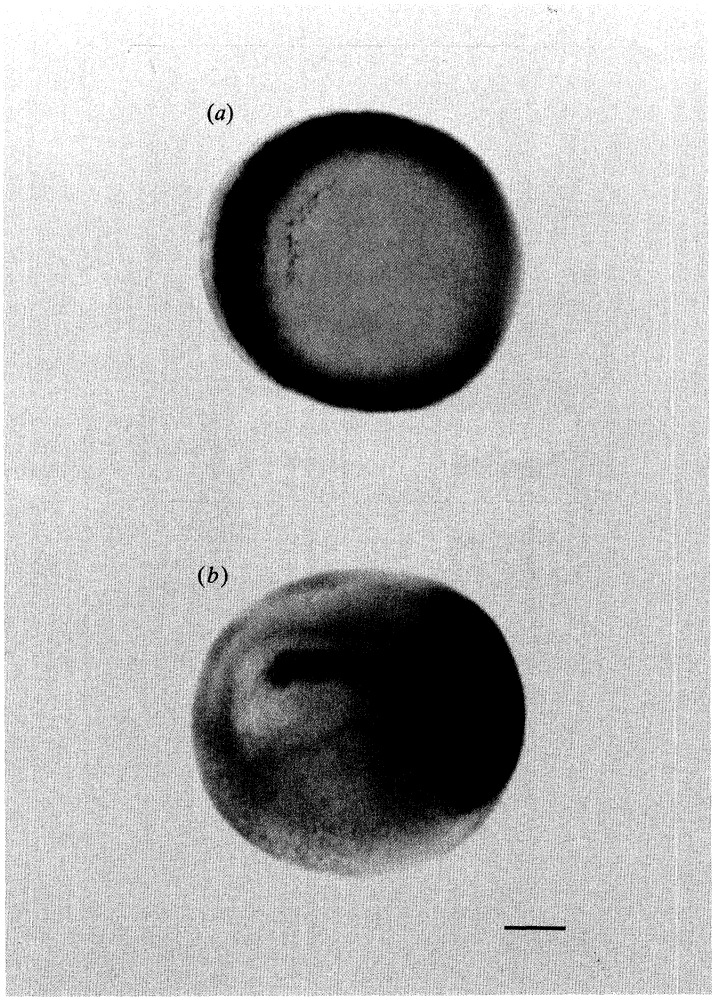

Figure 3. Wholemount *in situ* hybridization showing expression of *Xbra* in the marginal zone of an early gastrula (*a*) and in the notochord and posterior domains of a late gastrula (*b*). In (*a*) the embryo is viewed from the vegetal side and dorsal is to the top. In (*b*) anterior is to the top. Scale bar in (*b*) is 200 μm, and also applies to (*a*).

specific actin is switched on. The muscle window overlaps with a second *Xbra*-expressing window. Thus, as activin concentration is increased *Xbra* expression is first turned on, then off, then on again and finally, at high doses, off. These two windows of *Xbra* induction may relate to the two domains of *Xbra* expression *in vivo*; at neurula stages, *Xbra* is expressed both in the notochord and also posteriorly, around the blastopore (Smith *et al.* 1991; figure 3). High doses of activin induce expression of *goosecoid* (*gsc*), which is expressed in the dorsal lip, or 'organizer', of the *Xenopus* gastrula (Cho *et al.* 1991; Blumberg *et al.* 1991), and which eventually forms notochord and prechordal plate mesoderm.

It is remarkable that an increase in activin concentration of merely 80% is sufficient to divert cells from forming epidermis to expressing maximal levels of *XlHbox6*, and we discuss below mechanisms by which this sharp 'threshold' effect might occur. It is also necessary to consider, however, whether the differential activation of mesoderm-specific genes we observe is due to instruction or selection; that is, whether the target population of cells is homogeneous, and treatment with activin *instructs* cells to activate certain genes, or whether the target population is heterogeneous, with, for example, some cells predisposed to activate *Xhox3*, some muscle-specific actin and some *gsc*. In this case, activin concentration would *select* which cells express their predisposed fate. Although there is evidence suggesting that the intact animal cap is heterogeneous (Ruiz i Altaba & Jessell 1991; Sokol & Melton 1991), there are two pieces of evidence suggesting that induction in the dispersed cell regime is instructive. First, when aggregates are allowed to differentiate for several days and then analysed by immunocytochemistry, it is possible to identify some which consist essentially entirely of notochord cells (Green *et al.* 1992). Such a result cannot be explained by selection. The second experiment uses embryos made radially ventral by ultraviolet light irradiation of their vegetal hemispheres (Scharf & Gerhart 1980). Such embryos form no axial structures such as notochord or muscle, but prospective ectoderm derived from them behaves like that derived from control embryos in the dispersed cell experiments described above; that is, they activate muscle-specific actin and *goosecoid* (J. B. A. Green, H. V. New and J. C. Smith, unpublished observations). This cannot

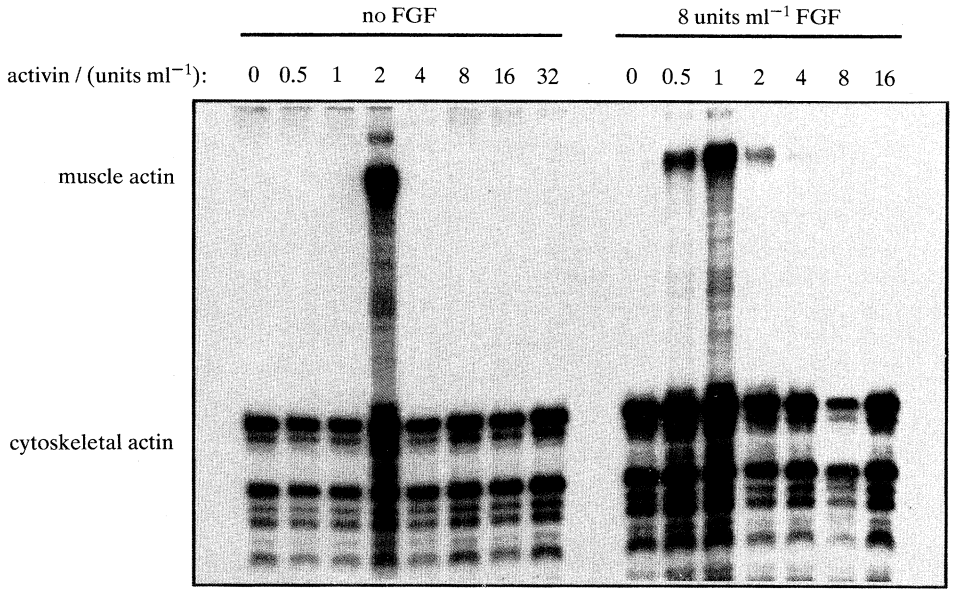

Figure 4. FGF modifies activin thresholds. In the left-hand panel, dispersed cells were exposed to increasing concentrations of activin in the absence of FGF. After re-aggregation and culture to the neurula stage they were analysed for expression of muscle-specific actin. In the right-hand panel cells were exposed to the same concentrations of activin in the absence of FGF. Note that the presence of FGF broadens the range of concentrations over which muscle-specific actin is expressed, and lowers the activin concentration at which maximum muscle gene expression is obtained.

be explained by a selection model, because such embryos should contain no cells predisposed to express these genes. Overall, therefore, the evidence favours an instructive role for activin.

3. ACTIVIN THRESHOLDS ARE MODIFIED BY THE PRESENCE OF OTHER FACTORS

The above results suggest that one way in which pattern might be established in the mesoderm of the amphibian embryo is through a gradient of activin: activin concentration would be high in the prospective anterior and dorsal mesoderm and lower in posterior and ventral regions. Although there is no direct evidence to support this model, it is intriguing that pattern formation in the *Drosophila* embryo is specified by a gradient of *decapentaplegic* activity; *decapentaplegic*, like activin, is a member of the transforming growth factor type β family (see Ferguson & Anderson 1992*a*,*b*). It is unlikely, however, that an activin gradient would be sufficient to specify all the mesodermal cell types in the embryo, despite the fact that over-expression of a mutated activin receptor causes loss of all mesodermal structures (Hemmati-Brivanlou & Melton 1992).

Another candidate for an endogenous mesoderm-inducing factor is fibroblast growth factor (FGF), which induces posterior and ventral mesoderm from *Xenopus* animal pole regions (Slack *et al.* 1987; Green *et al.* 1990). Evidence for a role for FGF in mesoderm formation *in vivo* comes from experiments in which a dominant-negative FGF receptor is over-expressed; this causes loss of posterior mesodermal structures (Amaya *et al.* 1991). These results raise the possibility that activin and FGF might interact to establish the correct pattern of mesodermal cell types in the embryo. To investigate this question, the responses of dispersed animal cap cells to different concentrations of FGF were first examined. This experiment revealed two significant differences between activin and FGF. Firstly, FGF treatment was not observed to activate expression of *goosecoid*. Second, whereas *Xhox3* and *XlHbox6* are activated only at low concentrations of activin (Green *et al.* 1992), expression of these genes persisted even at the highest concentrations of FGF. The significance of these results can best be appreciated through experiments in which the two factors are combined. Figure 4 shows an experiment in which activin concentration is varied in the presence of a fixed concentration of FGF. In this case, the sharp peaks of expression of muscle genes are broadened, and the activin concentration required for maximal expression of muscle-specific actin is lowered. Taken together, these results suggest that an activin gradient which is high on the dorsal side of the embryo and low on the ventral, and an FGF gradient which is high in the marginal zone and lower towards the vegetal hemisphere, may be sufficient to specify the correct spatial pattern of cellular differentiation in the amphibian embryo (see figure 5 and Green *et al.* (1992)).

This suggestion is consistent with what little is known about FGF and activin distribution in the *Xenopus* embryo. For the former, Shiurba *et al.* (1991) show that FGF epitopes are rare in the animal hemisphere and more abundant in the rest of embryo. At morula and blastula stages, staining is stronger in

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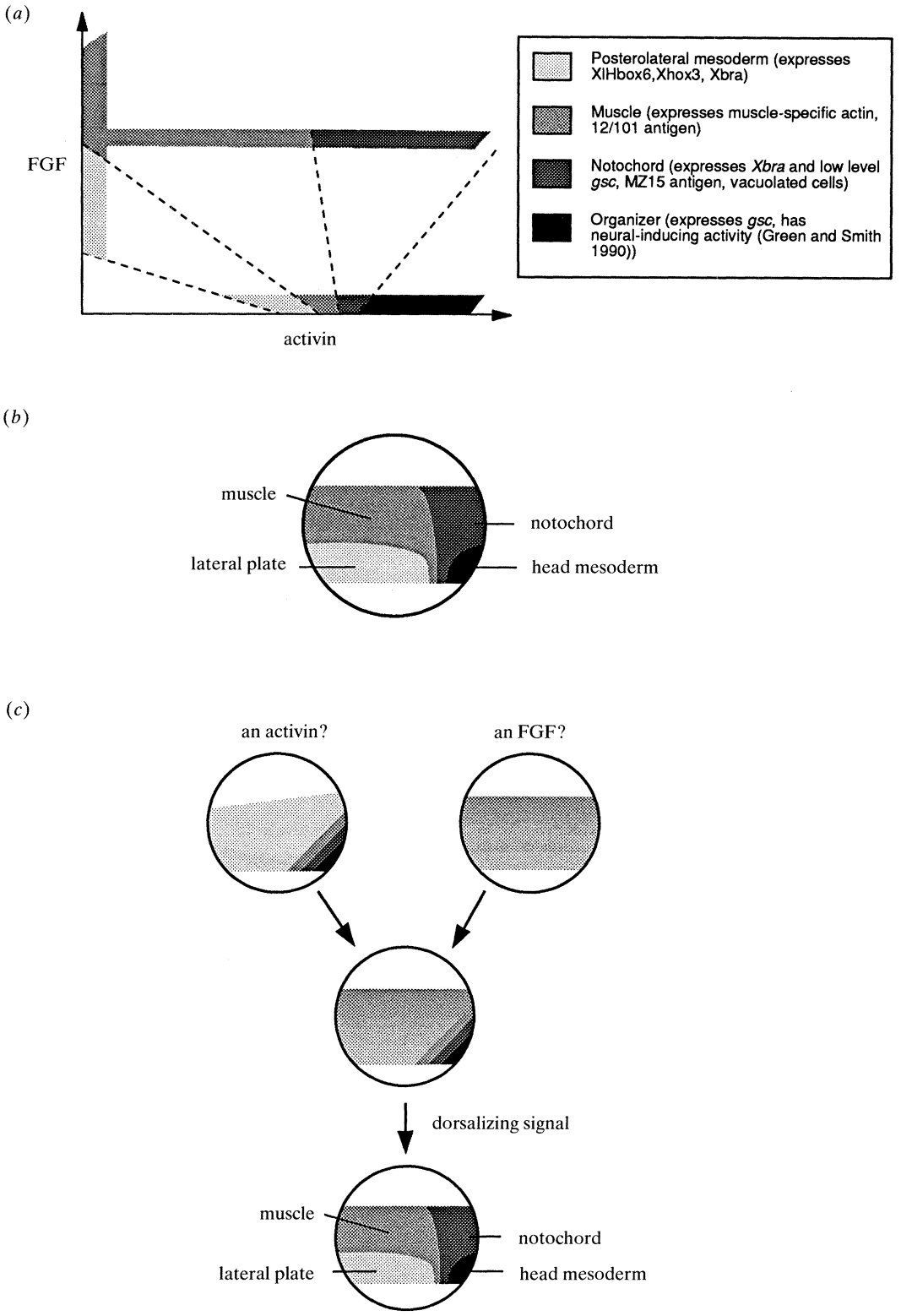

Figure 5. Dose-dependent effects of activin and FGF and their correspondence to two axes of the mesodermal blastula fate map. (*a*) Summary of the data shown in figures 2 and 4 and in Green *et al.* (1992). Dashed lines interpolate thresholds at different FGF concentrations assuming that they vary smoothly. (*b*) Schematic diagram of the *Xenopus* blastula fate map based on the fate map of Keller (1976). (*c*) Two-gradient hypothesis suggesting how gradients of activin- and FGF-like activities might specify the fate map. A putative activin-like gradient has a high point near the prospective dorsal lip, and a putative FGF-like gradient is radially symmetrical about the animal-vegetal axis. It is high in the upper marginal zone and trails off gradually towards the vegetal pole. Both gradients are in the form of maternal proteins. Simple superimposition of the separate effects of the two gradients would give rise to the blastula specification map (Dale & Slack 1987). Interaction between the gradients takes place during gastrula stages and may require the action of an activin-induced third signal ('dorsalization'; Dale & Slack 1987). See Green *et al.* (1992) for further explanation.

the marginal zone than in more vegetal cells but the distribution appears to be uniform in the dorsoventral axis: that is, radially symmetrical.

Nothing is known about the localization of activin protein in the *Xenopus* embryo, but the results from experimental embryology and the spatial distribution of activin-induced 'immediate-early' genes suggest that the highest concentration of activin would be near the dorsal marginal zone of the blastula, the prospective organizer. For example, it is only dorso-vegetal blastomeres that are capable, like activin, of inducing notochord from animal pole tissue (Boterenbrood & Nieuwkoop 1973; Dale *et al.* 1985), and genes such as *goosecoid*, *Xlim-1* and *Xfkh1*, which are induced by activin but not by FGF (Cho *et al.* 1991; Dirksen & Jamrich 1992; Taira *et al.* 1992), are expressed exclusively at the dorsal lip of the blastopore. It may be that activin-like activity is not restricted exclusively to this region because another activin-inducible gene, *Mix 1*, is expressed over the entire vegetal hemisphere as well as in the marginal zone (Rosa 1989).

Although these results are consistent with the notion that activin and FGF gradients specify positional information in the embryo, it is very unlikely that they represent the whole story. As described above, there are other potential mesoderm-inducing factors present in the embryo, as well as factors which can modulate mesodermal patterning, and further experiments are required to elucidate the roles of these molecules. Nevertheless, the dramatic mesoderm-inducing effects of these molecules, together with the results of dominant-negative receptor experiments, indicate that both activin and FGF play an important role in mesoderm formation, and their dose-dependent effects (particularly the very dramatic effects of activin) deserve further analysis.

4. DO DIFFERENT ACTIVIN RECEPTORS MEDIATE DIFFERENT MESODERM-INDUCING SIGNALS?

How do different concentrations of activin induce expression of different genes, and what makes the thresholds so sharp? To answer this question it is necessary to turn to the activin signal transduction pathway. Cross-linking experiments reveal that cell-surface receptors for activin fall into two classes, analogous to those observed for the TGFβ receptor. The type I activin receptor has a relative molecular mass (M_r) of $50–60 \times 10^3$, while the type II has an M_r of $70–80 \times 10^3$ (see Attisano *et al.* 1992). Type I receptors are poorly characterized, but a type II receptor was recently cloned by Mathews & Vale (1991). The cytoplasmic portion of this receptor contains a serine/threonine kinase domain, and it therefore differs from many other growth factor receptors, such as those for FGF, which are tyrosine kinases. Several *Xenopus* activin receptors have since been identified by Kondo *et al.* (1991), Hemmati-Brivanlou and Melton (1992), Mathews *et al.* (1992) and Nishimatsu *et al.* (1992). The functions of these various receptors are unclear, but as discussed below,

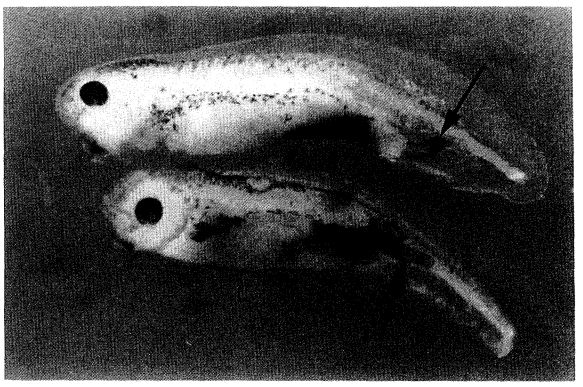

Figure 6. Microinjection of the XSTK9 putative activin receptor into ventral cells of two *Xenopus* embryos causes formation of additional tails (arrows).

it may be significant that different receptors may show different affinities for activin (Attisano *et al.* 1992).

Might different activin receptors transduce different mesoderm-inducing signals? One attractive possibility is that a high affinity receptor might respond to low concentrations of activin to induce expression of *Xhox3*, and that a lower affinity receptor might respond only to higher concentrations and activate *goosecoid*. There is no direct evidence for such an idea, but preliminary experiments indicate that over-expression of the XSTK9 receptor in early embryos induces tail formation (figure 6), while the related XAR7 induced additional trunks (Kondo *et al.* 1991). We are now investigating whether, as the above model predicts, XSTK9 has a higher affinity for activin than XAR7. There is little evidence from other systems that different growth factor receptors might transduce different signals, although in human foreskin fibroblasts, platelet-derived growth factor (PDGF) types AB and BB induce reorganization of actin filaments whereas PDGF AA does not. This suggests that actin reorganization is mediated by the PDGF type B, but not type A, receptor (Hammacher *et al.* 1989). More recently, Eriksson *et al.* (1992) have studied the responses of porcine aortic endothelial cells expressing equal levels of the PDGFα- or β-receptors. Some of the effects of PDGF on these cells, such as the ability to migrate chemotactically up a concentration gradient of ligand, were transduced only by the β-receptor.

The presence of different activin receptors may help explain how different genes are activated at different activin concentrations, but what makes the thresholds so sharp? To understand this we turn to the *Brachyury* gene.

5. BRACHYURY IS AN IMMEDIATE-EARLY RESPONSE TO ACTIVIN

The mouse *Brachyury* (*T*) mutation was first identified because heterozygous mutant animals have short tails (Dobrovolskaia-Zavadskaia 1927). Homozygous embryos die at mid-gestation, and characteristically lack a notochord and posterior mesodermal structures, as well as having a severely reduced allantois. The *T* gene was cloned by Herrmann *et al.* (1990). Its sequence, and the fact that it is a nuclear protein,

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suggest that it is a transcription factor (Schulte-Merker *et al.* 1992), and its expression pattern is consistent with a role in early mesoderm formation: transcripts are present in future mesoderm of the primitive streak at 7 to 9.5 day embryos, and in the notochord of 8.5 day and older embryos (Wilkinson *et al.* 1990). The *Xenopus* homologue of *Brachyury*, *Xbra*, has been studied by Smith *et al.* (1991). Expression of the *Xenopus* gene is similar to that of the mouse, with transcripts present in presumptive mesoderm at the early gastrula stage (figure 3*a*) and in the notochord and blastopore lip region of the late gastrula and early neurula (figure 3*b*). As with other mesoderm-specific genes, transcription of *Xbra* can be induced in animal pole tissue by contact with vegetal pole cells or by treatment with activin or FGF. This response is rapid, and can occur in dispersed cells and in the presence of a protein synthesis inhibitor, indicating that it is an 'immediate-early' response to mesoderm induction. The inducibility of the *Brachyury* gene by mesoderm-inducing factors has also been observed in the zebrafish (Schulte-Merker *et al.* 1992), suggesting that it is a general feature of vertebrate development.

6. WIDESPREAD EXPRESSION OF *BRACHYURY* CAUSES ECTOPIC MESODERM FORMATION; MUSCLE GENE ACTIVATION OCCURS WITH A SHARP THRESHOLD

The phenotype of homozygous *T*/*T* mouse embryos indicates only that *Brachyury* is required for formation of posterior mesoderm, but recent experiments in *Xenopus* indicate that expression of this gene is sufficient to activate mesoderm-specific genes and to induce the formation of mesodermal cell types (Cunliffe & Smith 1992). Injection of *Xbra* mRNA into the animal hemisphere of *Xenopus* embryos causes them to form ectopic mesoderm, and animal caps isolated from such embryos activate mesoderm-specific genes such as *Xsna* (Sargent & Bennett 1990), muscle-specific actin and the posteriorly-expressed *Xhox3* (Ruiz i Altaba & Melton 1989). Epidermal keratin expression is reduced, as might be predicted, but *goosecoid* expression is not induced, suggesting that *Xbra* induces posterior mesoderm.

To study threshold effects of *Xbra*, a two-fold dilution series of *Xbra* mRNA was injected into embryos, animal caps were dissected from them, and muscle-specific actin gene expression was analysed at the late neurula stage. Although a concentration of 0.13 ng nl^{-1} *Xbra* mRNA failed to induce muscle, strong expression was elicited on injection of RNA at 0.25 ng nl^{-1}, and the level of induction remained constant thereafter (figure 7). This suggests that one way in which threshold responses to increasing extracellular concentrations of activin or FGF may occur is by a corresponding gradual increase in *Brachyury* concentration being translated into a sharp activation of downstream genes such as muscle-specific actin. As discussed below, such a sharp activation might occur through a series of events similar to those which have been reported to establish thresholds in *Drosophila*. The suggestion that increasing concentrations of activin cause a gradual increase in *Brachyury* would appear to contrast with the results shown in figure 2, where activation of *Brachyury* occurs with a sharp threshold. This experiment was analysed at the neurula stage, however, and it is possible that an initially graded dose–response profile of *Brachyury* is translated into a sharper pattern by interactions with other immediate-early genes, as occurs in the regulation of gap gene expression in *Drosophila* (Jäckle *et al.* 1986). This is under investigation.

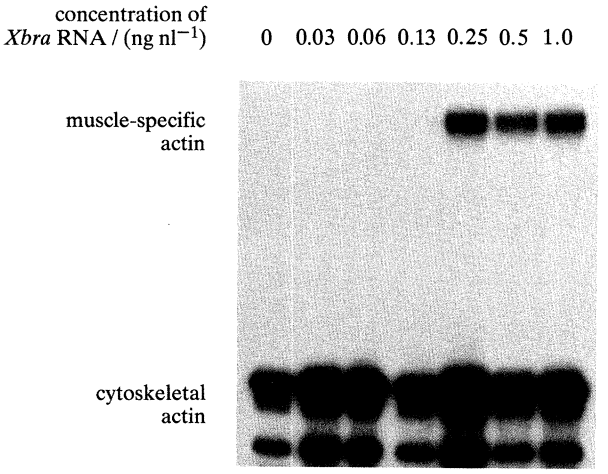

Figure 7. Threshold effects of Brachyury injection. Animal caps were dissected from embryos which had received increasing amounts of *Xbra* mRNA. Concentrations of 0.25 ng nl^{-1} and above induced expression of muscle-specific actin.

7. CONCLUSIONS

This work described in this paper suggests that formation of the body plan in amphibian development occurs through threshold responses to gradients of inducing factors such as activin and FGF. We have shown that embryonic cells have the ability to distinguish between small differences in factor concentration and then to activate different genes at different concentrations, essentially as predicted by Walpert (1969). To understand how this occurs, many questions must be addressed, and as we discuss below, the answers may come from work in *Drosophila*.

The first question concerns the mechanism by which graded distributions of mesoderm-inducing factors might be established. Although the distribution of FGF in the early embryo is consistent with a role in mesodermal patterning (Shiurba *et al.* 1991; see Green *et al.* 1992), there are no clues as to how this radially symmetrical distribution is established. In the case of activin, however, it may be possible to study the formation of a gradient by analogy with *Drosophila*. The functional gradient of the *decapentaplegic* gene product depends on the activities of the *tolloid*, *shrew* and *short gastrulation* genes, and it will be of interest to study the homologues of these genes in *Xenopus*. So far, only the *Xenopus tolloid* has been cloned and, interestingly, it proved to be related to bone morphogenetic protein-1 (Shimell *et al.* 1991), a metalloprotease

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which is likely to be involved in processing members of the BMP-4/activin family to yield the mature, active form.

The next step is to ask how different concentrations of extracellular factors might activate different genes, and how the thresholds are so sharp. One possibility, discussed above, is that different receptors transduce different signals; we suggest, for example, that XAR1 might transduce a 'trunk' signal, and XSTK9 a 'tail' signal. The mechanism by which this occurs will require greater understanding of the activin receptor signal transduction pathway. There remains, however, the question of how the graded signals are translated into sharp responses; that is, where in the signal transduction pathway does the 'sharpening' occur?

Experiments in which *Brachyury* mRNA is injected into *Xenopus* embryos suggest that thresholds might arise through a gradual increase in mesoderm-inducing factor concentration being translated into a gradual increase in intracellular *Brachyury* concentration, and that it is this which causes a sharp activation of, for example, muscle genes. Precedent for the first part of such an idea in *Drosophila* comes from the dorsoventral system where a graded distribution of the *toll* ligand is translated into a graded nuclear distribution of the *dorsal* morphogen (see St Johnstone & Nüsslein-Volhard 1992). Dorsal, a transcription factor related to NFκB, subsequently causes a sharp activation of the *snail* gene in the mesoderm of *Drosophila* (see below).

The mechanism by which a graded distribution of a transcription factor such as Dorsal or Brachyury might produce a sharp activation of responding genes has been analysed by Driever and Nüsslein-Volhard (1989) and Struhl *et al.* (1989), who studied the activation of *hunchback* by the product of the *bicoid* gene. The results suggest that the sharpness of the response depends on cooperative binding of the transcription factor to the promoter of the responding gene, that the affinities of the binding sites set the spatial limits of expression, and that their number determines the overall level of expression. With this in mind, it will be of great interest to identify targets of *Brachyury*. A further sharpening of thresholds may occur through interactions between gradients. The activation of *snail* by *dorsal* is remarkably sharp, and work by Ip *et al.* (1992) has established that this sharpness requires the *twist* gene product, a basic helix-loop-helix protein. *twist* is also activated by *dorsal*, and is expressed in somewhat steeper gradient than the nuclear gradient of Dorsal. An interaction between Dorsal and Twist then establishes the sharp on–off pattern of *snail*. The integration of two gradients in establishing a single sharp threshold has also been demonstrated in the antero-posterior axis of *Drosophila*, where gradients of Hunchback and Bicoid interact to regulate expression of *Krüppel*. (Hoch *et al.* 1992; see review by Ingham & Smith (1992)). It is not yet known whether such interactions happen in vertebrates, but it seems very likely that it does.

Clearly, comparisons between *Xenopus* and *Drosophila* will prove fruitful for the analysis of thresholds in vertebrates, but equally clearly we have a long way to go. We have not considered the roles of other inducing factors present in the early vertebrate embryo, such as BMP-4 and *noggin*, and nor have we discussed putative transcription factors such as *goosecoid*, *Xlim-1* and *Xfkh1* (Cho *et al.* 1991; Dirksen & Jamrich 1992; Taira *et al.* 1992). A complete analysis of the roles of all these proteins will be a formidable task.

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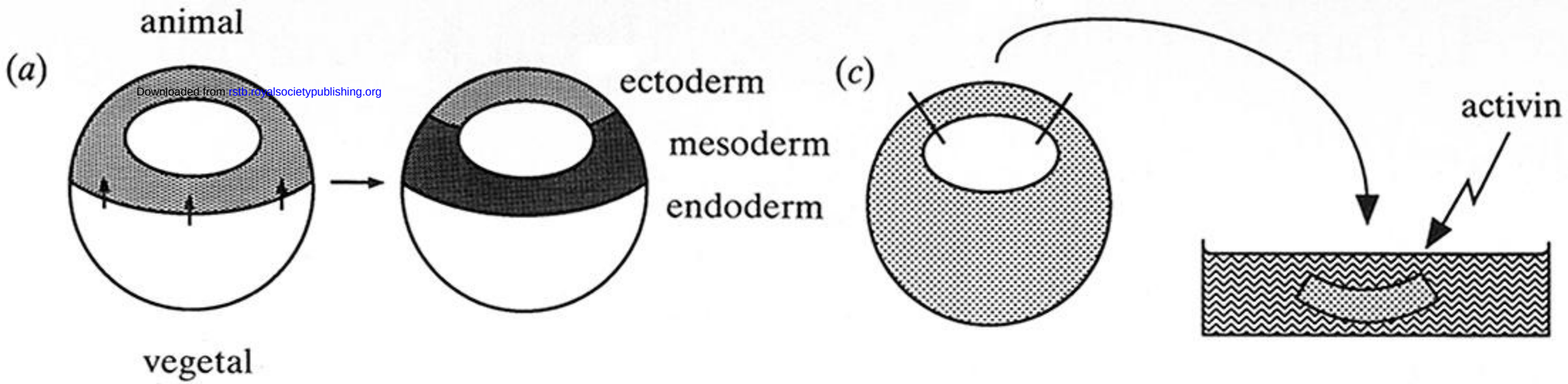
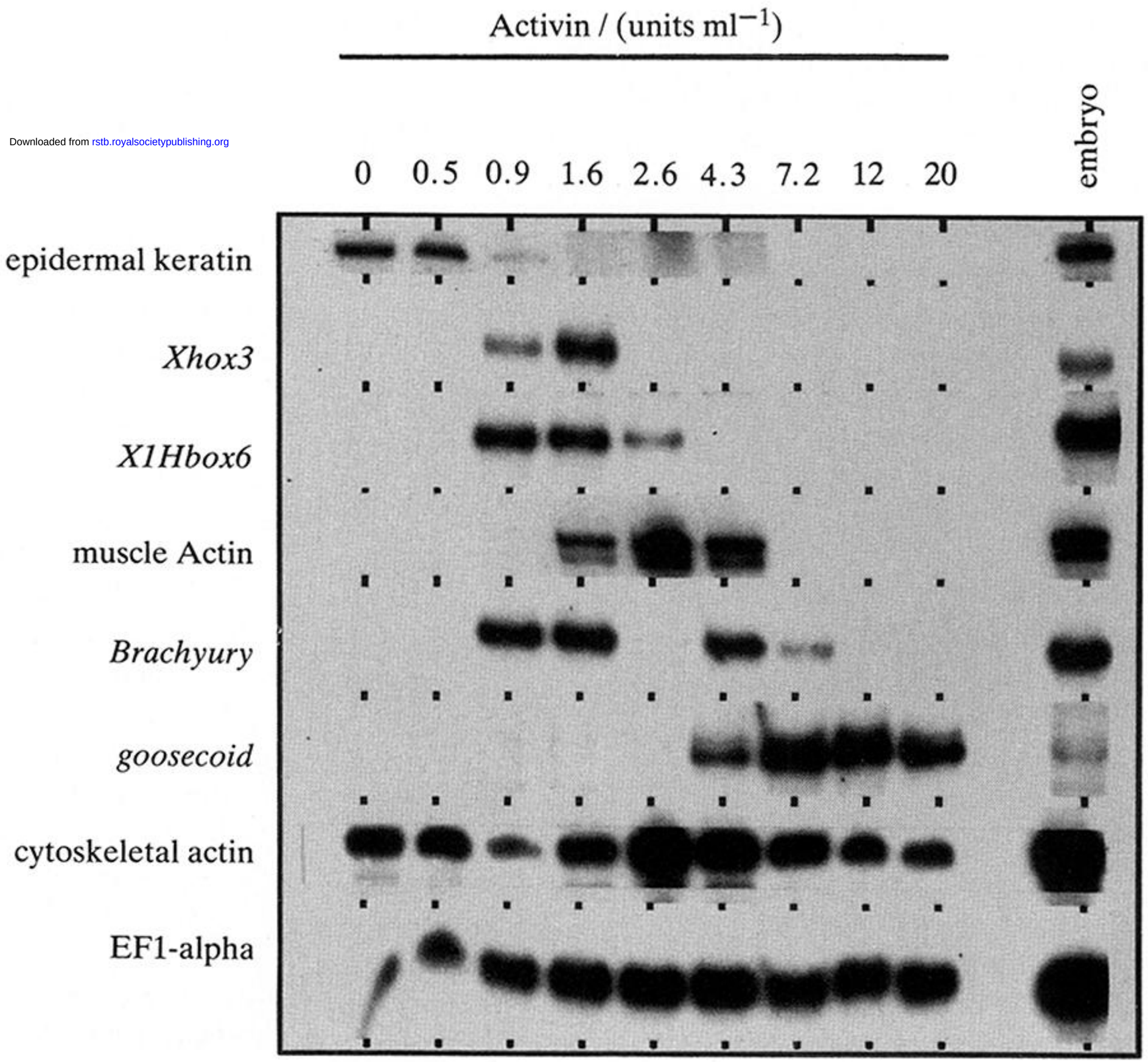
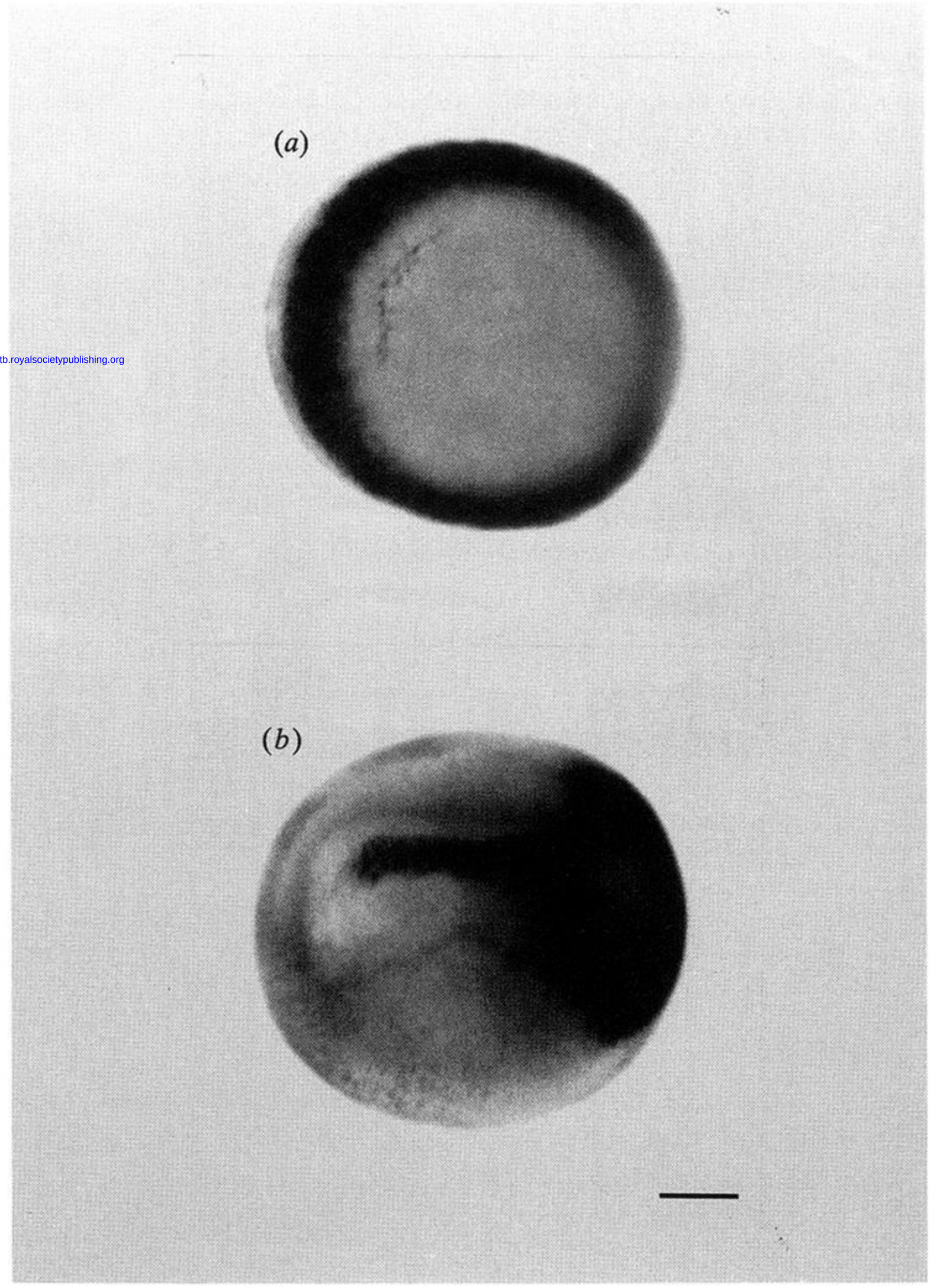


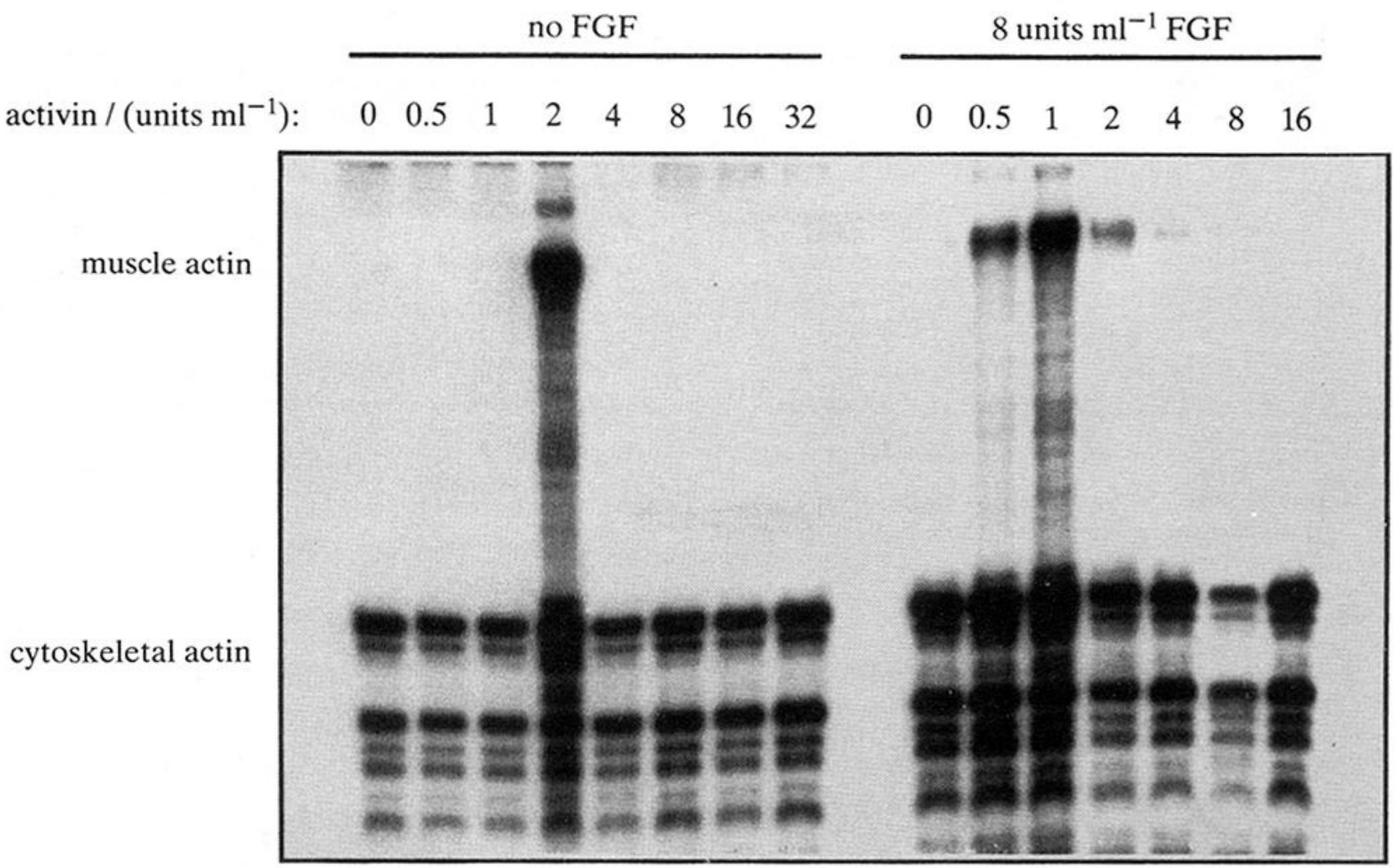
gure 1. Mesoderm induction. (*a*) Mesoderm is formed from the equatorial region of the blastula-staged embryo as e result of an inductive signal produced by cells of the vegetal hemisphere. (*b*) Demonstration of mesoderm duction. Cells from the animal cap of a lineage-labelled blastula are juxtaposed with cells from the vegetal misphere. (*c*) Treated of animal pole tissue with activin. (*d*) A section through a *Xenopus* animal pole region ated with activin and cultured for three days. Note notochord (not) and muscle (mus). Scale bar is 100 µm.



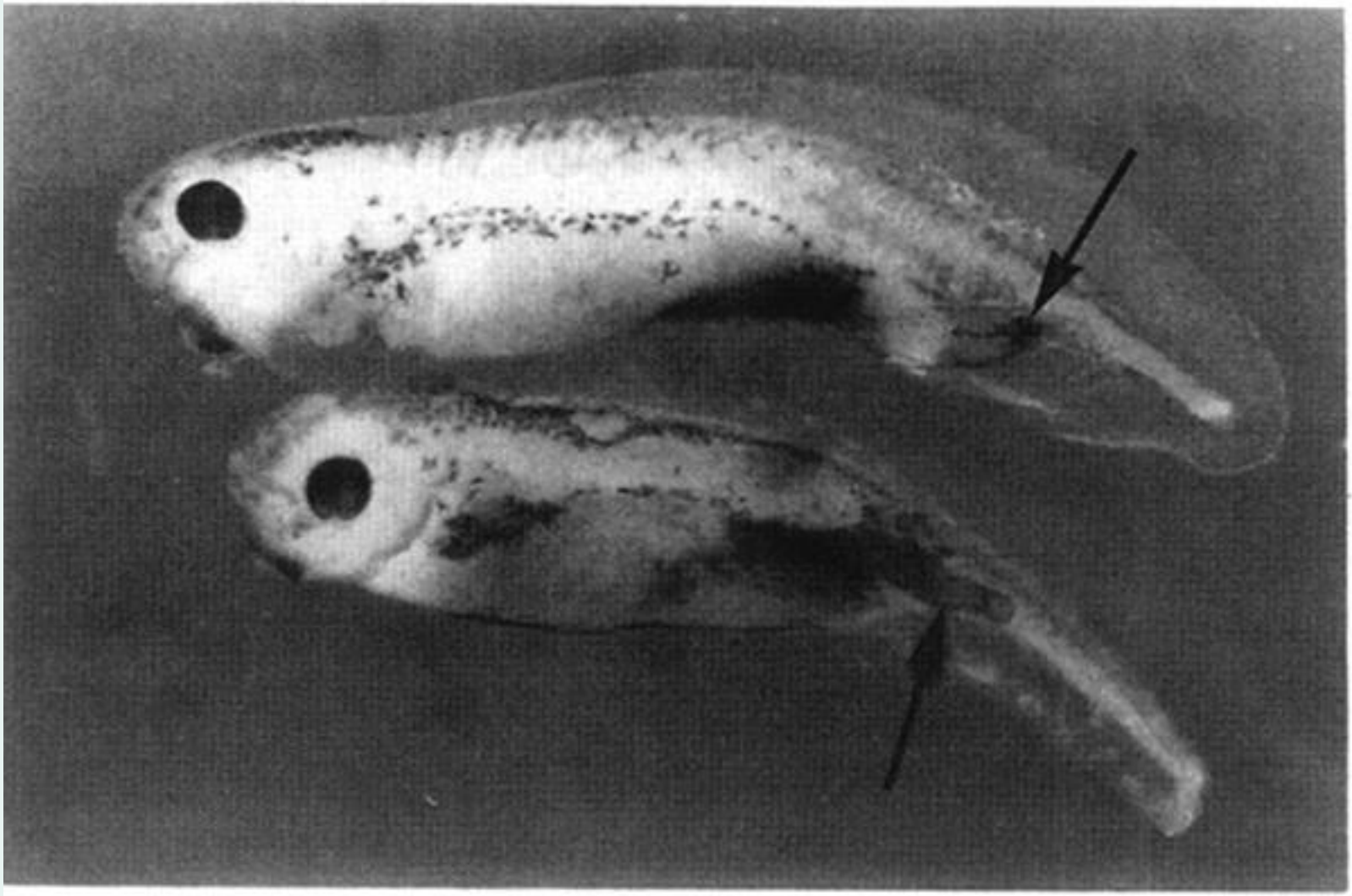
gure 2. Activin induces different region-specific markers according to its concentration. Dispersed cells derived m blastula animal pole regions were exposed to a 1.7-fold dilution series of activin. After re-aggregation and lture to the neurula stage they were analysed for expression of epidermal keratin, *Xhox3*, *XlHbox6*, muscle-specific tin, *Xenopus Brachyury* and *goosecoid*. EF-1α and cytoskeletal actin serve as loading controls.



gure 3. Wholemount *in situ* hybridization showing expression of *Xbra* in the marginal zone of an early gastrula (*a*)
d in the notochord and posterior domains of a late gastrula (*b*). In (*a*) the embryo is viewed from the vegetal side
d dorsal is to the top. In (*b*) anterior is to the top. Scale bar in (*b*) is 200 μm, and also applies to (*a*).



gure 4. FGF modifies activin thresholds. In the left-hand panel, dispersed cells were exposed to increasing ncentrations of activin in the absence of FGF. After re-aggregation and culture to the neurula stage they were alysed for expression of muscle-specific actin. In the right-hand panel cells were exposed to the same ncentrations of activin in the absence of FGF. Note that the presence of FGF broadens the range of concentrations er which muscle-specific actin is expressed, and lowers the activin concentration at which maximum muscle gene pression is obtained.



gure 6. Microinjection of the XSTK9 putative activin ceptor into ventral cells of two *Xenopus* embryos causes rmation of additional tails (arrows).

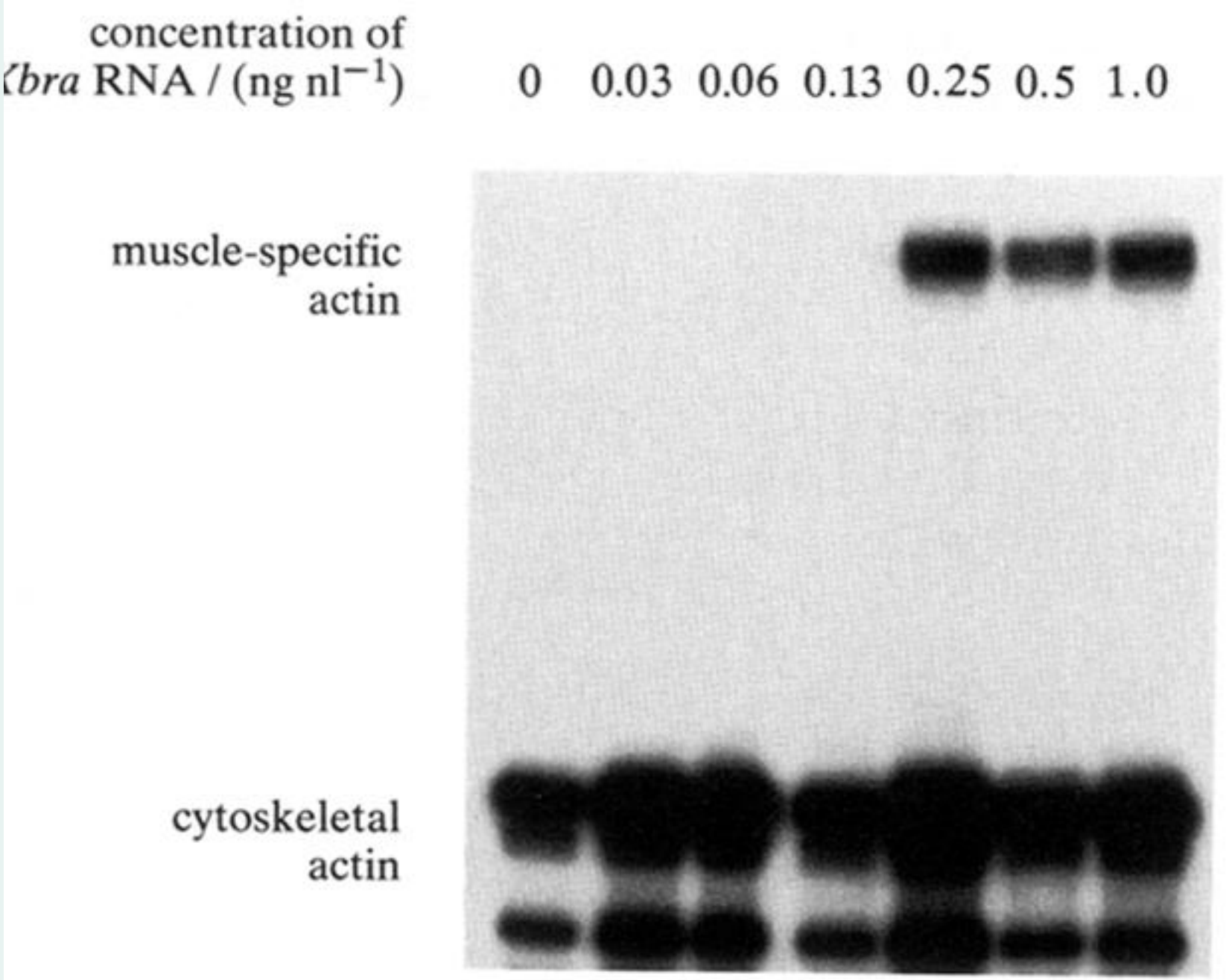


Figure 7. Threshold effects of Brachyury injection. Animal caps were dissected from embryos which had received increasing amounts of *Xbra* mRNA. Concentrations of 0.25 ng nl^{-1} and above induced expression of muscle-specific actin.