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An essay on the similarities and differences between inductive interactions in anuran and urodele embryos

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Abstract. As a first step towards providing a conceptual approach to understanding similarities and differences in the mechanisms which guide inductive interactions among related organisms (e.g. various amphibia), a set of five principles is offered here. These principles were formulated by analyzing literature examples of classical embryological phenomena and by performing experiments with activin, a peptide growth factor which is currently suspected to play for a role in mesoderm induction. Mechanisms which account, at least in part, for the observed differences between anuran and urodele inductive processes can be derived from these principles.
Key words. Amphibian inductive interactions; anuran embryonic induction; urodele inductive interactions; activin action; mesoderm induction.

Arbitrary choices

This essay asks a question frequently posed by contemporary embryologists: to what extent do the inductive interactions which drive organogenesis display similarities and differences when common anuran and urodele laboratory species are compared? By inductive interactions we mean those processes by which one group of cells controls the fate of neighbouring cells. Our essay will consider inductive interactions to represent a broad class of cellular interactions, as have other authors (e.g. ref. 1). We will, however, emphasize mesoderm induction as a prototypic case. It is better understood than most other inductive interactions, and we have first-hand knowledge of it since we ourselves have collected some of the experimental data. Other phenomena, such as those which control the differentiation of migratory cells (e.g. primordial germ cells), will also be mentioned. Two general components are believed to be involved in virtually all of these inductive interactions: the 'go-forward' signalling system (e.g. a peptide growth factor), and the target cell's (or tissue's) response mechanism (e.g. a receptor protein on the cell surface, coupled to intracellular activators of nuclear transcription factors). Our focus will be on the signalling components, since they are better understood than the components which are involved in the response process (i.e. the competence system).

In this modern era of embryology a single species of anuran (*Xenopus*) and a limited number of urodeles (e.g. axolotl = *Ambystoma*; *Pleurodeles*; *Cynops*; etc.) have come to dominate the experimental approaches. Whether those species will eventually prove to have been the most useful ones remains of course to be seen. After all, there exist approximately 3500 species of anurans and 350 species of urodeles, as well as another 150 or so species of caecelians (legless amphibia). Amphibia have been successful in adapting to a wide range of habitats, ranging from tropical forests to temperate deserts. Thus, they represent a rich source of biological diversity (reviewed in ref. 2).

Historical reasons, often somewhat abitrary, account in most instances for the choice of *Xenopus* and a few urodeles as experimental material. *Xenopus*, for example, 'has become a favorite amphibian for laboratory research owing to its ready response to gonadotrophic hormones. The ovulation response was first used for pregnancy diagnosis, then later exploited to provide embryos for experimental work' (quoted from Deuchar ref. 3). The axolotl likewise entered developmental biology through the back door. H. M. Smith's account of its early use in developmental biology [4] is fascinating and begins with an account of Cortes's troops reaching Mexico city in 1519. Shortly thereafter, Spanish friars documented the role of the axolotl in Aztec life, and eventually – in 1863 – a shipment of axolotls arrived at the Natural History Museum in Paris. That group of animals gave rise to the experimental material used by leading embryologists of the era (e.g. Dumeril, Chauvin, and Weismann). Descendants of that stock found their way throughout European and eventually American research laboratories. Being very hardy, relatively fecund, and easy to grow in the laboratory, the axolotl became the most popular amphibian model of its day for experimental embryology.

The few other urodeles which have dominated the embryological scene have less illustrious histories, but nevertheless fit this pattern: history, availability, and

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convenience rather than a highly reasoned choice account for the widespread adoption of these organisms by laboratory experimentalists.

In contemporary developmental biology it has not always been this way. The nematode *Caenorhabditis elegans* began its rise to prominence less than 25 years ago when S. Brenner established a new experimental paradigm [5]. His choice of *C. elegans* was a deliberate one, since the organism contains a total of only 959 cells, and is now almost completely analyzed genetically. Likewise, zebra fish were deliberately chosen for particular reasons as a model system (reviewed in ref. 6). But those few examples represent anomalies. The vast majority of developmental model systems in use today grew to prominence because they offered particular practical advantages to individual laboratory scientists. Once adopted by a pioneering group, an organism often became part of the experimental culture associated with a particular research problem.

General considerations such as biological diversity and phylogenetic history have almost always proven to be low in priority when adopting an organism as a model system. Practical considerations such as ease of collecting eggs, having a short generation time, and hardiness have usually predominated.

Thus, we are left with *Xenopus* and two or three urodeles as standards of reference for comparing anurans and urodeles. Substantial differences, as well as many general similarities, characterize the inductive interactions in these species, as will be reviewed below. These similarities and differences will be examined here using a set of five guiding principles and a series of examples for comparing the key features of anuran and urodele inductive interactions.

First principle – terminal aspects of morphogenesis show the most differences among organisms

As organisms evolve, their ability to generate adaptive changes generally becomes limited. As has been previously argued (e.g. ref. 7), embryos are not highly streamlined space rockets; rather, they are more akin to bloated bureaucracies. They are inclined to resist change, and with evolution tend to get bigger and bulkier. Their cytoplasmic information systems, which represent a geometric expansion of the information contained in the triplet genetic code, impose complex constraints on the possibilities for adaptive change. That is, the interlocking nature of cytoplasmic information systems limits the opportunities for *major* restructuring events to occur during evolution. Hence, change is generally achieved in small increments through the modification of preexisting structures. Genetic change (e.g. mutation) which deletes or substantially alters one portion of an inductive interaction is not easily accommodated because of the interdependence of developmental processes. Evolutionary change is therefore most easily accomplished by modification of the terminal features of a morphogenetic process.

Comparison of axial (somite) structure development between anurans and urodeles illustrates this principle well. Cell interactions and morphogenetic patterning appear to be completely different among various anurans and urodeles, as the diagrams in figure 1 illustrate. Indeed, it has been remarked that among the vertebrate class, it is easier to identify ways in which members differ from each other than to list uniquely 'amphibian' characteristics they share [8]. These differences include adult sizes, egg sizes, and developmental rates, as well as sequences of embryological processes. Such major differences should not be surprising, since various groups which exist today are positioned at the end of long independent evolutionary lineages that dis-

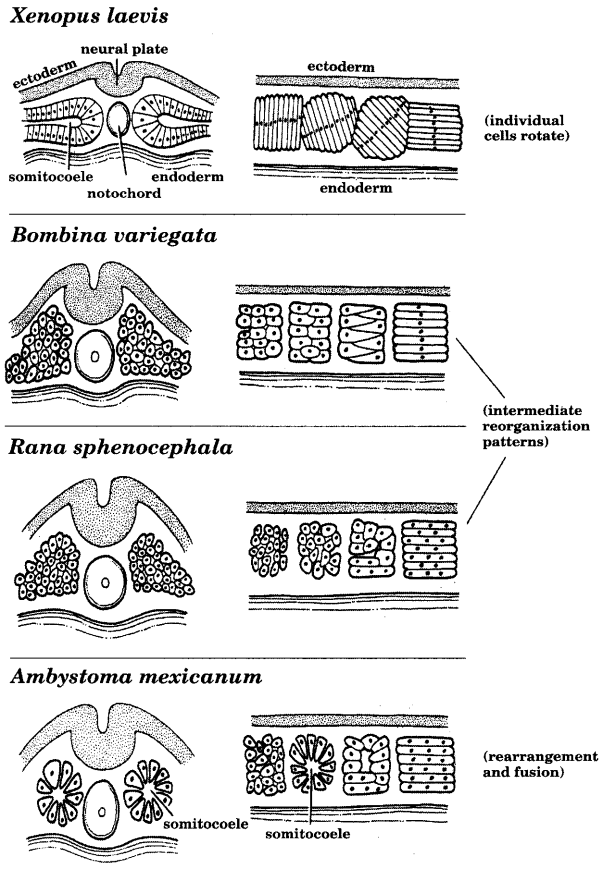

Figure 1. Somite cell arrangements (left) and reorganization patterns (right) displayed by various common laboratory amphibia are very different. *Xenopus* somite precursor cells arrange parallel to one another and later rotate (as individual cells) 90 degrees. Axolotl cells, in contrast, organize as rosettes (around a myocoel) and later rearrange. Unlike axolotl myocytes, *Xenopus* cells elongate and do not fuse. The *Xenopus* myocyte nuclei become transiently polyploid, whereas axolotl myocytes remain diploid. *Xenopus* muscle cells become electrically coupled relatively early. Axolotl muscle cells, by contrast, remain uncoupled during early stages. Other species (e.g. *Bombina* and *Rana*) exhibit intermediate patterns (modified from Malacinski et al. [9]).

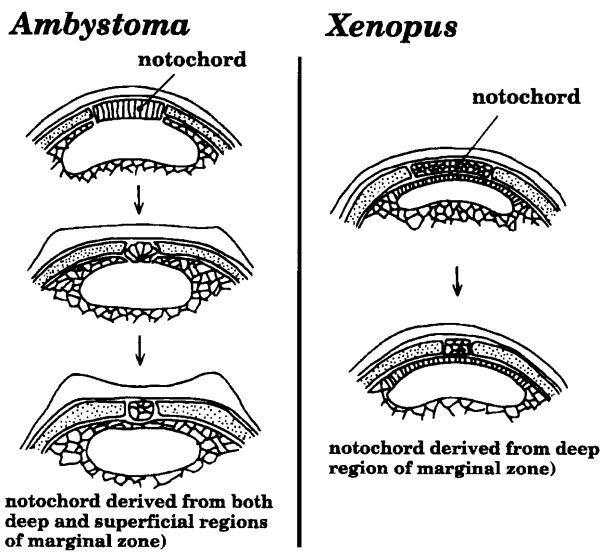

Figure 2. Anuran notochord formation shows sharp contrasts to urodele pattern concerning cell origins and re-arrangement patterns (modified from Hanken [9]).

play remarkable diversity even within closely related groups, as well as between groups. Pugh [2] has even commented that it is 'surprising that enough similarity remains after so long a separation (of independent lineages) to allow us to recognize the three orders as a subclass'. Thus, the diversity in somite patterning shown in figure 1 is perhaps understandable.

A somewhat earlier inductive event – notochord formation – also shows substantial differences between anurans and urodeles (reviewed in ref. 10). The embryological origin of notochord cells differs among anuran species, as well as between anurans and urodeles. Figure 2 contrasts the notochord formation patterns in the axolotl and in *Xenopus*. However, despite these differences in morphogenesis, the fully formed notochord displays strikingly similar features at the fine-structure level [11].

Even earlier events (e.g. gastrulation movements) show substantial differences. *Xenopus* and axolotl gastrulation are illustrated in figure 3. The differences in the origin of the mesoderm and surrounding cells, which eventually grow and differentiate into the axial structure tissues described above, are illustrated.

When the developmental pathway for somites at earlier stages is investigated, similarities rather than the differences highlighted above emerge. First, both anuran and urodele embryos employ an inductive interaction between vegetal hemisphere blastomeres and marginal zone cells for mesoderm specification [12]. Second, the molecular signalling mechanism – peptide growth factors – appears to be similar between the two.

Second principle – molecular components in induction

Recently, substantial insight has been obtained into the role peptide growth factors play as signalling molecules for induction. A variety of peptide growth factors are currently prime candidates for the 'natural inducer' in mesoderm formation (see below). Of these, activin has emerged as an especially useful probe for highlighting the similarities between anuran and urodele mesoderm induction mechanisms. In this essay it will, therefore, be used as a model to compare the molecular features of inductive interactions.

Activin A (also known as erythroid differentiation factor) is a member of the transforming growth factor beta (TGF-β) family, which includes other peptide growth factors such as Vgl and bone morphogenetic protein (BMP) (reviewed in ref. 13). It is a homodimer consisting of two inhibin βA chains, with a native molecular weight of 24,000 daltons. It is well known to be capable of inducing various mesoderm tissues in *Xenopus* blastula ectoderm cultures. The inducing effects of activin are concentration-dependent [14]. In fact, the precise array of tissues induced in *Xenopus* explants depends largely on the concentration of activin added to the culture medium. At relatively low activin concentrations blood cells, mesenchyme and coelomic epithelium are induced. At moderate concentrations muscle and neural tissues are greatly enhanced. At relatively high concentrations (e.g. 50 ng/ml) notochord tissue is prominently displayed by activin-treated explants.

Whether activin is the 'natural' (i.e. endogenous) inducer is at present not certain. Other peptide growth factors are capable of eliciting similar responses in ectoderm explants. Basic and acidic fibroblast growth factor (FGF) and BMP, for example, have also been demonstrated to possess mesoderm-inducing activity (reviewed in ref. 13). Nevertheless activin is a strong candidate, for unlike many of the other peptide growth factors it is present in *Xenopus* oocytes and blastulae [15], it is effective at very low concentrations (e.g. 0.3 ng/ml), different doses yield unique histological features, and overexpression of activin generates outgrowths [16] and partial duplication of the embryonic axis [17]. A cautious interpretation of the activin data is, however, called for, in view of the recent observation that overexpression of mRNA for follistatin (an activin antagonist) in whole *Xenopus* embryos does not interfere with mesoderm formation [17].

It is of course possible that in the whole embryo a combination of peptide growth factors, rather than a single component such as activin, acts to induce mesoderm. FGF is especially potent as an inducer of ventral mesodermal structures, while activin appears to induce (especially at very low concentrations) a broader range of mesodermal tissues, including both dorsal and ventral types. Although in various bioassays any one

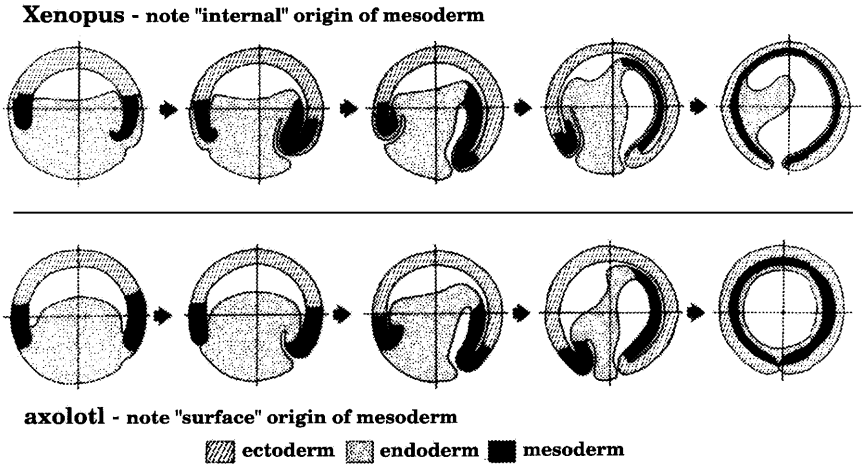

Figure 3. Schematic diagrams of cross sections of gastrulae illustrating the different location of mesoderm precursor cells in *Xenopus* and in the axolotl. *Xenopus* (top) mesoderm cells arise internally, whereas axolotl (bottom) mesoderm cells originate on the embryonic surface (from Malacinski et al. [9]).

growth factor may appear as 'sufficient' for induction, no single growth factor in the whole embryo may be 'necessary' if combinatorial usage and redundancy characterize the regulatory circuits [18]. Despite these potential complications, the activin system is a useful model for gaining insight into the extent to which the molecular basis of mesoderm inductive interactions is similar in anurans and urodeles.

Literally dozens of studies on mesoderm induction by growth factors have been carried out with *Xenopus* (reviewed in ref. 13). Only one report [19] has, however, explored the effects of activin in urodele (newt) explant assays. The overall newt response pattern to activin in those first experiments looked superficially similar to the *Xenopus* induction pattern. In order to obtain for this essay a more rigorous comparison of the response of explants of anuran and urodele tissue to activin, we expanded the urodele observations to include axolotl embryos. Figure 4 illustrates outside views of axolotl explants treated with various concentrations of activin. Control explants (fig. 4A) developed into a mass of atypical epidermis with a highly wrinkled surface. At relatively low activin concentrations smooth surfaces formed, while at higher concentrations multiple differentiations, as shown by elongation and convolution (figs. 4C, D), were observed.

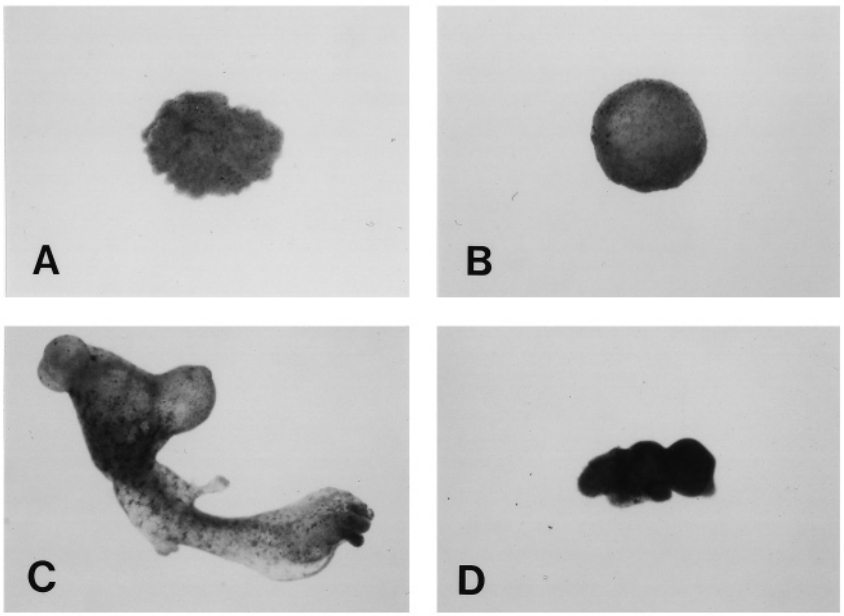

Figure 4. Outside views of 14 day axolotl ectoderm cultures treated with various concentrations of activin A (added to the Holtfreter's solution culture medium): (*A*) control (no activin); (*B*) 0.1 ng/ml; (*C*) 5.0 ng/ml; and (*D*) 100 ng/ml. Details of the culturing methods and other procedures employed for collecting the data included in figures 4–6 are included in Moriya and Asashima [19].

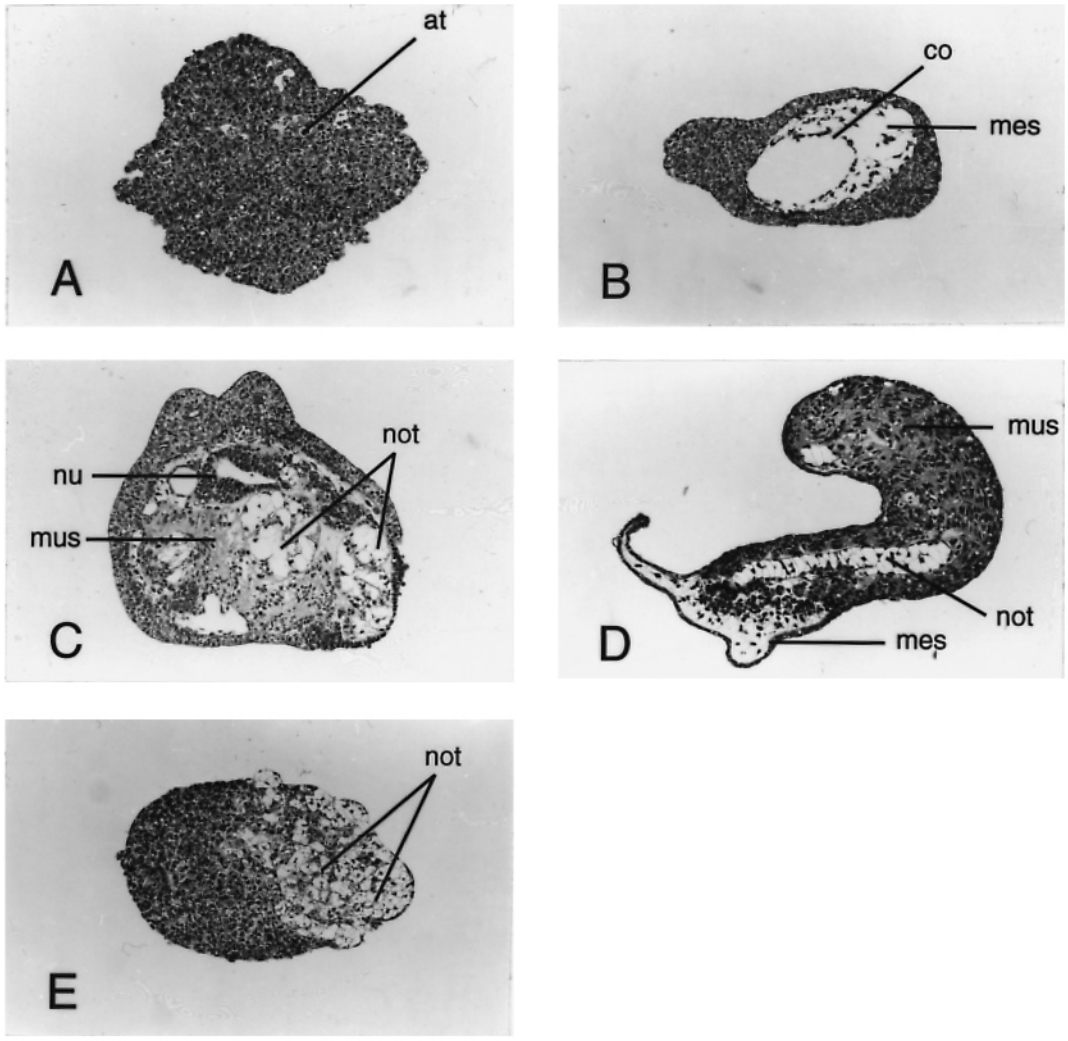

Figure 5. Histological sections of axolotl animal cap explants treated with various concentrations of activin A during a 14 day culture period. (*A*) control (no activin); at = atypical epidermis; (*B*) 0.5 ng/ml (co = coelomic epidermis; mes = mesenchyme cells); (*C*) 1.0 ng/ml (nu = neural tissue; not = notochord; mus = muscle); (*D*) 5.0 ng/ml (mus = muscle; not = notochord; mes = mesenchyme); (*E*) 100 ng/ml (not = notochord).

Histological observations of typical axolotl explants treated with activin are shown in figure 5. As described in the figure legend, lower activin concentrations promoted differentiation of coelomic epidermis and mesenchyme cells, while higher concentrations enhanced muscle and notochord development. A more comprehensive set of data is summarized in figure 6.

When the above data for axolotl embryos are combined with the previously published data for the newt [19], and then compared with the *Xenopus* data, the similarities are striking. Throughout the activin concentration range coelomic epithelium/mesenchyme, then muscle/notochord, and finally neural tissue (perhaps as a 'secondary' induction) differentiate. Those observations on both anurans and urodeles are compatible with the various gradient theories which have been proposed [20, 21] to account for the differentiation of a wide spectrum of tissues in response to a limited number of signalling molecules.

The similar responses of *Xenopus* and newt/axolotl to activin are consistent with earlier observations on hybrid tissue recombinations. Faulhaber reported [22] that a *Xenopus* dorsal lip is capable of inducing neural tissues in axolotl host ectoderm, and axolotl dorsal lip tissue induces *Xenopus* ectoderm to differentiate into neural structures. Thus, although terminal aspects of tissue/organ development have diverged among anurans and urodeles, the initial inductive steps of organogenesis show striking similarities.

Third principle – expression patterns for differentiation products can vary substantially among amphibian species

Despite the apparent similarities in the ability of cultured ectoderm from anurans and urodeles to respond to peptide growth factors, substantial differences in the in vivo differentiation of embryonic muscle are obvious.

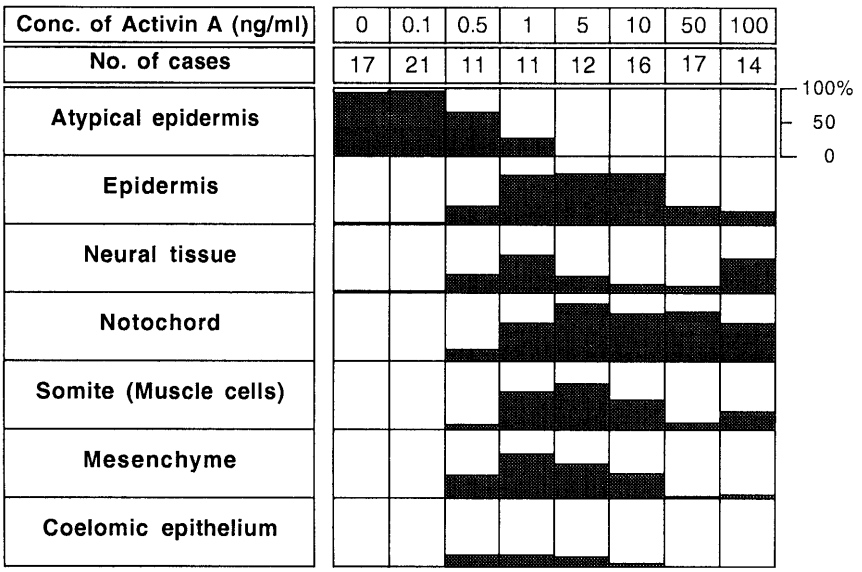

Figure 6. Composite of observations of mesoderm-inducing activity of activin A on axolotl ectoderm. After 14 days cultured explants were examined histologically. The differentiation frequency for various tissue types is indicated by the height of the dark bars.

Dramatic spatial and temporal differences in actin/myosin accumulation have been described. In anurans accumulation of actin/myosin is initiated relatively early (during gastrulation), whereas in urodele embryos those proteins accumulate only after somite segmentation is well underway [23]. For anurans (e.g. *Xenopus* and *Bombina*) actin/myosin accumulate more or less uniformly within an individual somite. Urodeles (e.g. axolotl and *Cynops*) exhibit a much more complex accumulation pattern. An anterior/posterior gradient and medial/lateral polarity of actin/myosin accumulation are observed within individual somites [24].

Something of a paradox thus emerges: although key components of the molecular signalling mechanism which drives mesoderm induction are most likely similar (if not identical) in anurans and urodeles, the above data imply that the timing patterns for the use of those regulatory components are probably very different, since the differentiations they promote are timed so differently. Such timing changes are often referred to as reflecting 'heterochrony' (changes in relative timing of developmental processes (reviewed in ref. 25). This third principle can therefore be considered to include the heterochrony observed in the above example and in several other well-known examples from amphibian embryology (e.g. neoteny, direct development, etc.).

Fourth principle – phylogenetic history has imposed constraints on adaptive change

Tissues and organs which evolved early are no doubt relatively fixed in their developmental pathway. Included among these are the sensory systems and apparatus of muscular movement, which, being so directly related to survival, no doubt became sophisticated relatively early in evolution. Extending the bureaucracy metaphor mentioned earlier one step further, it can be imagined that once an organizational framework is established, change is most easily accomplished by adding layers to the bureaucracy (recall the first principle re. terminal additions), rather than by restructuring its fundamental morphological characteristics.

Two features of regulatory systems impose constraints on adaptive change: (l) the network format which distinguishes most regulatory circuits, and (2) the reciprocal nature of many inductive interactions. Networks of signalling systems are well known. Classical embryological manipulations have revealed that although a single cell type or tissue normally provides the 'go-forward' signal in an inductive interaction, alternative sources of the signal also exist. For example, Jacobson [26] has demonstrated that lens induction can be experimentally modified by surgically removing the retina (the normal inducing tissue). In the absence of the retina, endoderm and heart mesoderm act as inducing tissue.

Although networks might provide flexibility due to their built-in redundancies, it is unlikely that networks can accommodate major changes. The high degree of integration provided by networks preclude major transformations. Thus, the widespread occurrence of vestigial structures among virtually all vertebrates is easily accounted for. The interlocking nature of developmental pathways makes it highly likely that when a major component is superseded (made obsolete) by the evolutionary emergence of a more suitable version, the old component will be retained. Networks and reciprocal interactions act as stabilizing forces in phylogenetic progression.

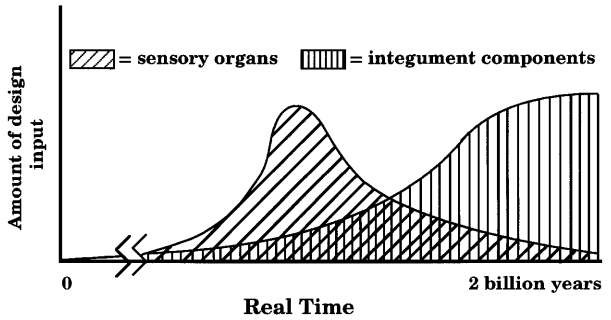

Figure 7. Inductive interactions which evolved early (e.g. sense organs) provide structural components which become fundamental and rigid properties (like the foundation of a building) that are not easily modified by evolutionary change. Components evolving later (e.g. integument features) are more easily modified.

An example of this phenomenon is the now classic tissue recombinant experiments in which bird oral epithelium was co-cultured with mouse oral mesenchyme. Chick tissue was induced to form enamel organs and in a few instances, complete teeth [27]. Why does the bird genome maintain the developmental program for tooth formation? Presumably because some component of the tooth program continues to play a role in another, interlocked developmental program which is necessary for embryogenesis of one or another structure.

In figure 7 the fourth principle is illustrated. Scales, feathers, hair, and other integument components, having evolved relatively recently as terminal modifications of precursor structures, display greater predisposition to morphological transformation than do sensory structures or motility functions.

Fifth principle – phylogenetic divergence has yielded some sharp distinctions among anuran and urodele inductive processes

Primordial germ cell (PGC) development differs dramatically between anurans and urodeles. In anurans (*Xenopus* and *Rana* have been most extensively studied), PGC development is guided by the so-called germ plasm, a mitochondria-rich zone of subcortical cytoplasm containing electron-dense bodies ('germinal granules') localized in the vegetal hemisphere of the uncleaved egg. It is similar in many regards to the polarplasm of *Drosophila* eggs [28]. Germ plasm is believed to act by programming the cells which inherit it during cleavage to differentiate into migratory PGCs (fig. 8). Thus, PGCs are preformistically established as a result of the action of a localized 'cytoplasmic determinant' (reviewed in ref. 29).

Among urodele embryos (e.g. *Pleurodeles*, axolotl, *Triturus*) no similar cytologically distinctive germ plasm can be detected in the early cleavage stage embryo. Instead, PGCs are thought to arise epigenetically from mesoderm cells which are induced by the endoderm (reviewed in ref. 30) (fig. 9). When the PGCs begin to

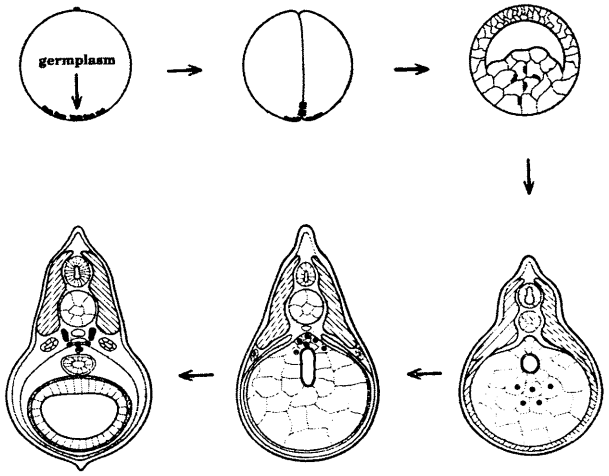

Figure 8. Germ plasm is localized in the uncleaved anuran egg in the subcortical cytoplasm of the vegetal hemisphere, here shown in cross section. During cleavage cells which contain the germ plasm are displaced towards the floor of the blastocoel. Later, PGCs migrate within the endoderm to the genital ridges (modified from Nieuwkoop and Sutasurya [30]).

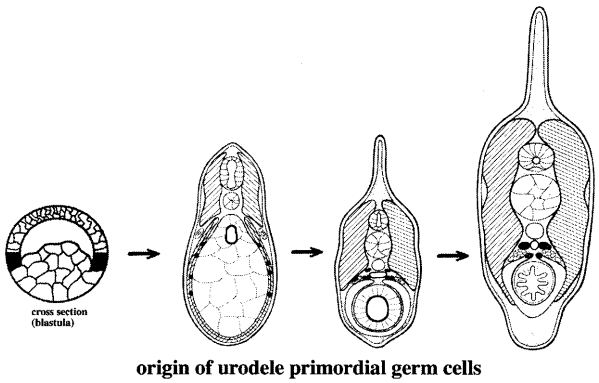

Figure 9. PGCs in urodeles arise in presumptive lateral plate mesoderm (shown in cross section of blastula) as the result of inductive interaction with underlying endodermal cells. Later they migrate along the mesoendodermal interspace into the genital ridges (redrawn, based on Nieuwkoop and Sutasurya [30]).

differentiate, a distinctive germ plasm can be detected for the first time in embryogenesis. The later-appearing urodele germ plasm is indistinguishable from the earlier (unfertilized eggs) recognizable anuran germ plasm.

The point of view expressed by Niewkoop and Sutusyura [30] is that the discrepancy in the mode of origin of PGCs reflects different phylogenetic origins of the two groups of amphibia. Thus, the variations in the ontogeny of PGCs is easily reconciled with the fifth principle. Accepting that rationale does, however, introduce a conflict with the first principle (terminal aspects show most differences). Once PGCs are formed, later aspects of differentiation are virtually identical in anuran and urodele embryos. It is the earlier aspects of

PGC formation which are different among those two amphibian groups.

Two alternative, somewhat iconoclastic, explanations are however possible: (1) that germ plasm is not a determinant at all, and anuran PGC formation indeed mimics that of urodeles; and (2) that germ cells, since they are potentially immortal (unlike somatic cells), exhibit highly idiosyncratic ontogenies which do not necessarily coincide with the general principles associated with somatic tissues and organs.

Concluding remarks

Too few anuran and urodele species have been investigated in sufficient detail to formulate long-lasting generalizations about similarities in inductive phenomena. The species which have been studied represent 'organisms selected for convenience'. It therefore remains to be determined whether key variations in the patterns which have emerged from *Xenopus*/*Rana* and axolotl/*Pleurodeles*/*Cynops* comparisons represent widespread phenomena. Likewise, too few inductive processes are understood with the level of detail required for making insightful generalizations.

These caveats notwithstanding, as a starting point for generating dialogue among research scientists we have offered a conceptual framework, in the form of a set of five principles, to guide the organization of data which will emerge in the future. The formulation of such principles can perhaps be criticized as being premature, since so few amphibian species have been systematically examined for the features of their inductive interactions. In that context the following Chinese proverb is, however, appropriate: *a journey of a thousand miles begins with a single step*.

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