Gilmartin, J. L. Hall, N. J. Cowan, *J. Mol. Biol.* **182**, 11 (1985); M. G. S. Lee, S. A. Lewis, C. D. Wilde, N. J. Cowan, *Cell* **33**, 477 (1983).
30. HMGCoA reductase, human: K. L. Luskey and B. Stevens, *J. Biol. Chem.* **260**, 10271 (1985). HMGCoA, yeast: M. E. Basson, M. Thorsness, J. Rine, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5563 (1986).
31. Cytochrome c, yeast: M. Smith *et al.*, *Cell* **16**, 753 (1979); K. Narita and K. Titani, *J. Biochem.* **65**, 259 (1969). Cytochrome c, human: H. Matsubara and E. L. Smith, *J. Biol. Chem.* **238**, 2732 (1963); *ibid.* **237**, PC3575 (1962).
32. Citrate synthase, yeast: M. Suissa, K. Suda, G. Schatz, *EMBO J.* **3**, 1773 (1984); D. P. Bloxham, D. C. Parmelee, S. Kumar, K. A. Walsh, K. Titani, *Biochemistry* **21**, 2028 (1982); S. Remington, G. Wiegand, R. Huber, *J. Mol. Biol.* **158**, 111 (1982). The comparison is with the porcine enzyme.
33. RAS protein, yeast: R. Dhar, A. Nieto, R. Koller, D. Dereo-Jones, E. M. Scolnick, *Nucleic Acids Res.* **12**, 3611 (1984).
34. Transforming protein 1, human: J. P. McGrath *et al.*, *Nature* **304**, 501 (1983); K. Shimizu *et al.*, *ibid.*, p. 497; D. J. Capon *et al.*, *ibid.*, p. 507; E. Taparowsky, K. Shimizu, M. Goldfarb, M. Wigler, *Cell* **34**, 581 (1983).
35. Glucose transporter, human: M. Mueckler *et al.*, *Science* **229**, 941 (1985). Glucose transporter, yeast: J. L. Celenza, L. Marshall-Carlson, M. Carlson, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2130 (1988).
36. We thank J. Mulholland and C. Watanabe for the homology searches and C. Morita for preparation of figures.

Xenopus laevis in Developmental and Molecular Biology

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***Xenopus laevis* is a prime system for the study of embryogenesis in vertebrates. Both prelocalized information in the egg and inductive interactions between cells contribute to the ordered increase in complexity during development. Embryonic induction, discovered in amphibians, is being studied intensely in *Xenopus*; recent work suggests a role for growth factors in this process. Contributions of the *Xenopus* system to the analysis of ribosomal and 5S RNA genes, and the diverse and highly productive applications of the oocyte injection technology, are also summarized.**

PERHAPS THE BEST KNOWN EXPERIMENT IN EMBRYOLOGY IS the Spemann and Mangold experiment on embryonic induction, defining what these researchers called the "organizer" (*1, 2*). Induction is widespread and fundamentally important phenomenon in biology; in its broadest terms it describes any interaction between cells or groups of cells that affects differentiation. As such, induction also occurs in adult organisms, but the term is usually used in the context of embryogenesis, when the processes that generate new tissues and cell types are most active. How different tissues with their great morphological and functional diversity are formed from the comparatively simple egg is the basic question of developmental biology. Inductive interactions constitute one of the two general developmental mechanisms—cytoplasmic localization of information in the egg being the other—that are thought to be instrumental in setting up regional differences in the embryo, which result in a complex organized structure. Although induction events occur in the development of all animals, this phenomenon has been studied most extensively in amphibians, the phylogenetic class in which it was discovered. The original work involved newts, but more recently *Xenopus laevis* has become the animal of choice for studies of induction as well as many other aspects of embryogenesis, in particular at the interface of molecular and developmental biology.

The advantages of *Xenopus* as an experimental animal include its easy husbandry, the fact that it is a vertebrate, the accessibility of embryonic material from the earliest stage onward, and the comparatively large size of the egg and embryo that facilitates physical manipulations. These advantages, in spite of the limitation of the almost total inapplicability of classical genetics, have stimulated much research on *Xenopus* over the past three decades. In this article we discuss three areas in which this system has made important contributions: (i) the role of localized cytoplasmic information and of inductive interactions in the establishment of the polarity and initial tissue differentiation in the embryo, and on the nature and molecular basis of embryonic induction; (ii) the study of genes for RNA components of the ribosome and the control of their expression; and (iii) the productive use of the *Xenopus* oocyte as a "super test tube" in a broad range of studies on translation and transcription.

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The Spemann-Mangold Organizer Concept

Amphibian gastrulation begins with cell migrations at the dorsal side of the embryo. Cells moving up along the blastocoel roof form the presumptive dorsal mesoderm (chordamesoderm), and the ectoderm overlying this tissue develops into the central nervous system (CNS). As the earliest externally visible sign of gastrulation the dorsal lip forms at a subequatorial position in conjunction with these migrations, marking the future dorsal side of the embryo. The Spemann-Mangold experiment involved the transplantation of the dorsal lip from one embryo into the ventral side of another, leading to the development of two dorsal axes in the host (*1–3*). Figure 1 illustrates such an experiment: a cross section of a host embryo is shown at the late neurula stage with two neural tubes, two notochords, and duplicated somites. In external morphology a second head or tail is formed with almost complete duplication of the embryo as a "Siamese twin" in certain cases. Most of the tissue in

the duplicated axis, including mesodermal as well as neural derivatives, originates from the host, that is, it is induced by the implanted dorsal lip, which was therefore named the organizer (*1, 2*).

Fascination with the organizer concept gradually turned to frustration as attempts to isolate a factor with organizing activity met with varied difficulties (*4*). Although we are far from understanding the relevant phenomena and therefore not in a position to explain these difficulties from a historical perspective, it appears that a major reason for a lag in progress for many years, in addition to insufficient molecular techniques, was the way in which the problem had been defined (*5*). Our present view is that organizing a second axis is a complex phenomenon that may be separated into at least two components, mesoderm induction and neural induction, with the mesoderm induction occurring at an earlier stage than the organizer effect. Substantial progress in this area has come, therefore, not only from advances in technology, but also from a dissection of the problem into components that could be handled experimentally.

Determination of Polarity

In *Xenopus*, one embryonic axis is determined by the structure of the egg, the second is determined after fertilization. The egg is radially symmetrical around the animal-vegetal axis, which is defined by the center of the pigmented and unpigmented halves of the egg. This axis, which is set up during oogenesis, defines the future anterior-posterior polarity of the tadpole (Fig. 2); it is thus an example of a developmental outcome predetermined by cytoplasmic localization of information in the egg. The second major axis of the tadpole is fixed only after fertilization. Sperm entry can occur anywhere in the animal hemisphere, and the sperm entry point (SEP) normally defines the future ventral side. The way in which this polarity determination comes about has been explained primarily through the work of Gerhart and colleagues (*6*). A movement of cytoplasm relative to the cortex takes place between fertilization and first cleavage, and it is the direction of this movement that is the most accurate predictor of the future dorsal-ventral polarity of the embryo (Fig. 3) (*7, 8*). This movement, which can be abolished by ultraviolet (UV) irradiation or cold shock, may be driven by a mechanism that is dependent on microtubules (*9*). Eggs treated with UV irradiation that do not undergo cytoplasmic movement develop into defective embryos lacking a dorsal axis and all dorsal structures including CNS, notochord, and skeletal muscle (*9, 10*); yet irradiated eggs can be completely rescued if they are rotated before first cleavage, thereby generating gravity-driven cytoplasmic movement (*9*). This is an important point since no substance is added to the egg to achieve rescue; whatever substance may be affected can be regenerated by the egg as a consequence of cytoplasmic movements.

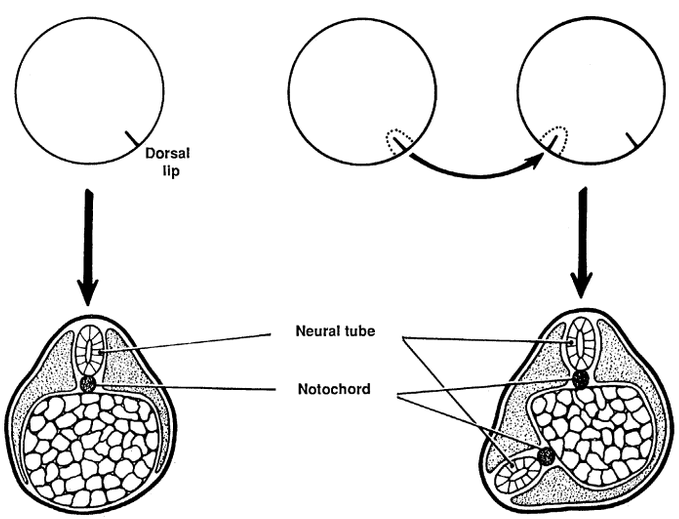
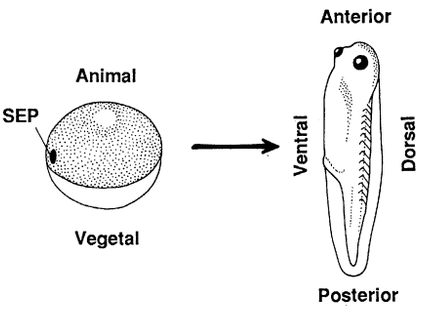


Fig. 1. Induction of a secondary axis in the amphibian embryo. Gastrulation starts with an invagination at one side, forming the dorsal lip, shown at the right of the schematized gastrula at the top. Cells originating from the area of the dorsal lip organize the dorsal axis, as shown in a cross section of a neurula below. Transplantation of a small segment of tissue including the dorsal lip into the ventral side of another embryo, as indicated on the top right, leads to the formation of a double-axis embryo. The second axis, including mesodermal and neural derivatives, is formed mostly from host tissue, that is, it is induced by the transplant. Adapted from (*1*).

Fig. 2. Relation between the polarity of the fertilized egg and that of the tadpole.



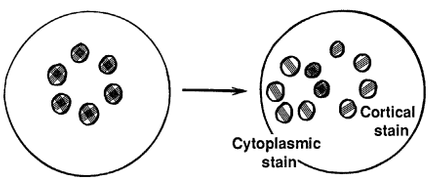


Fig. 3. Cytoplasmic movement before first cleavage as visualized by differential staining. A spot pattern was imprinted on the vegetal surface of a fertilized egg by staining through a grid. Two dyes were used: One stains the cortex (fluoresceinated potato lectin) and a second stains subcortical cytoplasm (Nile blue). The egg was embedded in gelatin, which allows normal development but immobilizes the cortex. About 1 hour after fertilization, the cytoplasm had rotated relative to the cortex by about 30°, as visualized by the staining pattern. Movement did not substantially distort the pattern. [Drawn after Vincent *et al.* (*7*)].

The spatial cue generated by cytoplasmic movement is translated into inductive capacity. Eggs treated with UV irradiation, destined to become axis deficient, can also be rescued after cleavage has been initiated by the transplantation of vegetal dorsal blastomeres from a normal embryo into an irradiated host (Fig. 4). Not only is rescue achieved, but dorsal structures such as CNS and notochord are formed from the host, not the graft (*11, 12*). Thus, the implanted normal cells do not simply replace structures that had been destroyed, but they induce the differentiation of host cells that are therefore capable of responding to the appropriate signal. This result suggests that in normal embryos vegetal dorsal blastomeres are the source of an inductive signal that generates dorsal structures by influencing the fate of other cells. The dorsal blastomeres acquire this inductive capability as a consequence of cytoplasmic movements during the first cleavage cycle. At what time, in what way, and by which molecular mechanism are these inductive signals generated, transmitted, and received?

Mesoderm Induction

The Nieuwkoop experiment. The initial evidence for an inductive interaction responsible for mesoderm differentiation in *Xenopus* was obtained by experiments illustrated in Fig. 5. Animal region explants (animal caps), when cultured in standard buffered salt

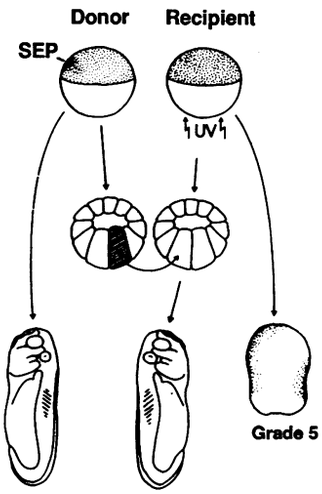


Fig. 4. Ultraviolet irradiation of fertilized eggs results in defective embryos lacking dorsal structures (Grade 5). Such embryos could be rescued by transplantation of two normal vegetal-dorsal blastomeres at the 64-cell stage. A normal dorsal axis is formed on the implanted side entirely from the cells of host embryo. [Reprinted from (*11*), with permission of Academic Press]

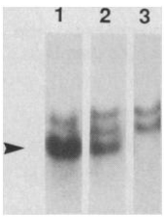


Fig. 6. Muscle induction in animal caps by XTC-CM and TGF-β2. The accumulation of α-actin mRNA has been used as a measure of muscle differentiation; muscle is a major derivative of dorsal mesoderm (*21*). The arrowhead points to α-actin mRNA; the other bands are cross-hybridizing cytoskeletal actin mRNAs. Lane 1, animal cap induced by XTC-CM; lane 2, animal cap induced by TGF-β2 (200 ng/ml); lane 3, uninduced control (*52*).

solution, differentiate only along epidermal lines; yet, when placed in contact with vegetal (future endodermal) tissue, such caps will form a variety of mesodermal tissues including notochord, muscle, kidney, and blood (*13*). These results were interpreted as suggesting that normal mesodermal development, which arises from centrally located cells, the so-called marginal zone, also requires an inductive signal; but this conclusion does not follow directly. The fact that a blastula animal cap can be induced toward mesoderm does not prove that mesoderm in a normal embryo, arising from different cells, requires induction. There are good reasons to believe, however, that it does. One is the rescue of UV-irradiated embryos by blastomere transplant (Fig. 4), in which dorsal mesoderm forms from the same cells as in a normal embryo but would fail to differentiate from these cells without the inductive signal. A second reason is an experiment in which the cells of *Xenopus* embryos were dissociated and widely dispersed, thus preventing interactions. Cells divide for many hours under these conditions. The cells were kept dispersed through the period of cleavage and blastula development, when induction is presumed to occur, later reaggregated for additional culture, and then assayed for gene activation. It was found that muscle-specific genes, such as the α-actin gene, were not expressed, whereas keratins, specific for epidermal differention, were expressed at normal levels (*14*). This result suggests that cell interactions during blastula stages are required for the differentiation of muscle, which is a major derivative of dorsal mesoderm.

Studies with XTC mesoderm-inducing activity. Inducing factors have been obtained from various sources over many years (*15*). A

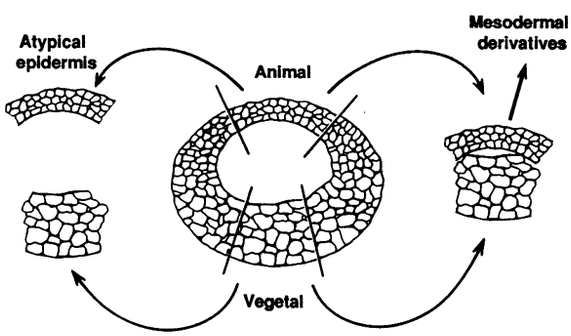


Fig. 5. An animal explant (animal cap) derived from a *Xenopus* blastula (middle), and cultured in salt solution, differentiates along the epidermal pathway, which is a major fate of the cells of this region in normal embryogenesis; vegetal tissue cultured alone yields little differentiation. Culture of an animal cap in contact with vegetal tissue leads to varied mesodermal structures (right) that develop from the animal explant (*13*).

breakthrough in this area came from the recent discovery by Smith of the powerful inducing effect of conditioned medium of XTC cells (XTC-CM) (*16*). This cell line was generated some time ago from a metamorphosing tadpole (*17*); the tissue of origin of the XTC cells is not clear, nor is it understood why cells from such a late stage should secrete inducing factor. These unknowns, and the fact that the active principle has not yet been purified to homogeneity, have not diminished the value of XTC-CM as a reproducible source of a soluble, highly effective inducing activity.

Animal caps induced by endodermal tissue or by XTC-CM differentiate into the whole range of mesodermal derivatives (*13, 16*). How these different tissues are generated is a critical question, especially if one considers that the normal embryo produces this range of tissues in an orderly way along its dorsal-ventral axis. Thus, it is insufficient to discuss mesoderm induction as such without considering the establishment of dorsoventral polarity. One result of studies already completed with XTC-CM is that a concentration-dependent effect occurs in which high levels induce mostly dorsal tissues (notochord and muscle), whereas lower levels lead to the differentiation of ventral mesodermal derivatives such as kidney and blood (*18*). Any conclusion from this result is preliminary since the possible involvement of multiple factors has not been excluded. But because the active principle in XTC-CM appears to be a single component, the result suggests that there is no need to postulate separate dorsal and ventral mesoderm-inducing factors; a graded distribution of a single factor might suffice. Yet one cannot postulate that the nature of the induced tissue depends entirely on a specific concentration of factor along the gradient. Such a mechanism would not be precise enough to assure a properly organized embryo, and interactions between neighboring induced cell groups may have to be invoked.

The nature of mesoderm inducer: Relation to TGF-β2. The relation of mesoderm-inducing activity to growth factors has been implied by several recent experimental results, connecting the field of embryonic induction to a large and highly active area of exploration. Slack *et al.* (*19*) showed that fibroblast growth factor (FGF) can induce ventral mesoderm as well as small amounts of muscle; Kimelman and Kirschner (*20*) observed the muscle-inducing effect of FGF to be potentiated by transforming growth factor β (TGF-β); and Rosa *et al.* (*21*) found that TGF-β2 alone effectively induces muscle (Fig. 6). Although none of these heterologous factors was as effective as the homologous XTC-CM, the activity of XTC-CM in muscle induction was inhibited by antibodies to TGF-β2 but not by antibodies to TGF-β1 (*21*). These results suggest that the active principle in XTC-CM is structurally related but not identical to mammalian TGF-β2.

TGF-β is a large family of factors with a variety of biological functions (*22*). The family includes a gene named *Vg1* (*23*), whose messenger RNA (mRNA) is accumulated in a localized manner in the vegetal region of *Xenopus* oocytes (*24*). The product of this gene is a good candidate for a component of the mesoderm induction system.

A summary of polarity determination and induction. Although the molecules and mechanisms involved in these processes are not fully

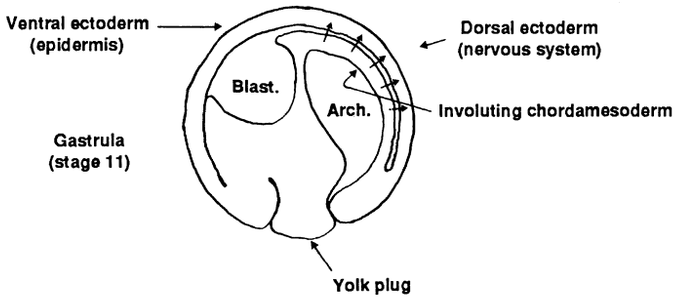


Fig. 7. Neural induction during gastrulation. The drawing represents a cross section through a midgastrula stage. The small arrows pointing from the chordamesoderm to the dorsal ectoderm symbolize signal transfer in neural induction. Abbreviations: Blast., blastocoel; and Arch., archenteron.

understood, the following hypothetical scenario seems reasonable at this time. Factors localized in the egg (as exemplified by, but not limited to, *Vg1*) are displaced through cytoplasmic movements to bestow distinct properties on different vegetal blastomeres, which subsequently induce the mesoderm in the marginal zone. Dorsal vegetal blastomeres induce dorsal mesoderm, including the Spemann-Mangold organizer, which in turn induces the neural plate during gastrulation. Interactions between the different induced tissues are likely to ensure the emergence of an ordered global structure. This scenario is a simplification and is only meant as an aid to visualization of events, rather than as an established series of mechanistically understood processes.

Future Work on Cytoplasmic Localization and Embryonic Induction

The cell and molecular biology of mesoderm induction has entered a phase of rapid exploration. We may expect clarification of questions on the identity of inducers in the near future. The issue of establishment of dorsal-ventral polarity will be an important focus. Furthermore, attention will be focused on receptors for inducer molecules, an issue of interest in terms of transduction of the signal, but especially as an approach to the problem of competence, that is, the ability—highly regulated in development—of certain cells to respond to an inducing signal.

Although we have emphasized induction as an important phenomenon in early development, localization of information in the egg contributes greatly to specification of the embryo [for a general discussion, see (*25*)]. One approach to this question is the search for localized macromolecules in the egg, some of which may be regulatory in nature, for example, *Vg1* (*23, 24*). Another approach starts from the consideration that the cell-autonomous activation of epidermal keratin genes is likely to be controlled by prelocalized factors (*26*). The study of such factors can be approached by defining the sequences of keratin genes that control their activation.

Neural induction and the organizer. While mesoderm is established by interactions during cleavage and blastula, neural induction takes place during gastrulation by the effect of migrating dorsal mesoderm on the overlying ectoderm (Fig. 7) (*27*). The CNS develops from the induced ectoderm. As such, neural induction may be thought of as a separate event that follows mesoderm induction, yet the situation is somewhat confusing because of overlapping ranges of inducing ability and competence. In fact, the classical organizer induction generates both dorsal mesoderm and CNS in the host (*1–3*). The cellular and molecular basis of neural induction will be amenable to detailed investigation when this phenomenon can be isolated and analyzed as a distinct entity.

Xenopus in Molecular Biology: Key Advances in Gene Isolation and Expression

The *Xenopus* system was used in the first isolation of a gene from any eukaryote (*28*), the initial studies on gene amplification (*29*), the earliest example of accurate transcription of a cloned eukaryotic gene (*30*), and the first isolation, cloning, and detailed characterization of a eukaryotic transcription factor (*31*).

Ribosomal RNA genes. Paradoxically, in view of the general lack of information about the genetics of *Xenopus*, it was probably the existence of the anucleolate mutation that caused investigators to focus on the ribosomal DNA (rDNA) of this animal. This mutation allowed the conclusion that the nucleolus is the site of rRNA synthesis (*32*), containing the rRNA genes (*33*). Soon it became clear that the ribosomal genes of *Xenopus* are tandemly repeated and, because of their high copy number and their distinctive base composition, could be physically separated from the bulk chromosomal DNA (*28*). *Xenopus* genes encoding components of the ribosome became the prototype for repeated gene families.

In the analysis of the structure and regulation of expression of rRNA genes, *Xenopus* has been a prime system. A complex arrangement of control regions has been identified (*34*). Each gene (repeat unit) has a basic promoter, a series of enhancer elements that have additive effects, and several duplicated promoter elements that can initiate transcription in vitro, and under some conditions in vivo (Fig. 8). Transcription termination on rDNA has proved even more surprising than initiation. It was believed at first that the 3′ end of mature 28*S* rRNA corresponds to the termination site. This proved inaccurate: transcription extends far into what was originally called the nontranscribed spacer, generating transcripts that are rapidly processed to the mature molecules (Fig. 8).

5S RNA genes. These genes, isolated and extensively characterized by Brown, have yielded many insights into questions of transcriptional mechanisms. Achievement of accurate expression of 5*S* RNA genes (*30*) allowed a detailed analysis of the cis-regulatory regions of the 5*S* DNA. The unexpected result pointed to an intragenic control region (*35*), a feature now generally recognized in genes transcribed by polymerase III (*36*). TFIIIA, one of three factors required for 5*S* RNA gene transcription, was isolated and cloned primarily through the efforts of Roeder and colleagues (*31*) and has become a

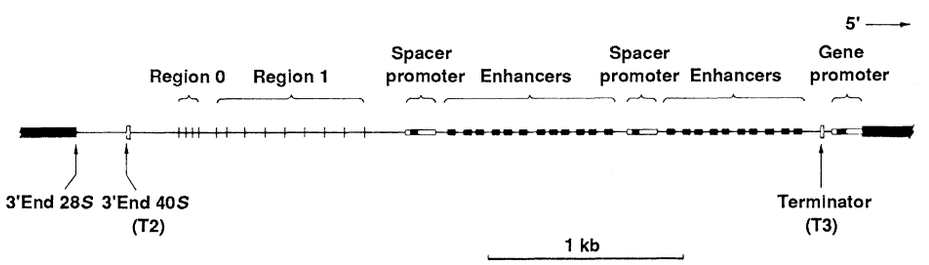


Fig. 8. Summary of regulatory regions in ribosomal RNA genes of *Xenopus*, showing promoters, enhancers, terminators, and major 3′ termini (*31*). [From (*53*), with permission]

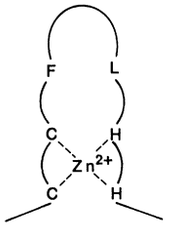


Fig. 9. The zinc finger motif. Protein domain recognized originally in TFIIIA (*37*) and found in many proteins (*38*). Conserved amino acids are shown in one-letter code. Abbreviations for the conserved amino acids follow: C, Cys; F, Phe; H, His; and L, Leu. The number of residues between conserved amino acids are: Cys-Cys, 2 or 4; Cys-Phe, 3, rarely 2; Phe-Leu, 5; Leu-His, 2, rarely 3; His-His, 3, rarely 4. Coordination of a zinc ion is hypothetical in most cases.

prototype for eukaryotic transcription factors. Recognition of a repeated structural feature in TFIIIA, the so-called zinc finger (Fig. 9), led to significant further insights (*37*). The zinc finger is a structural motif in which four residues coordinate a Zn^{2+} ion that is presumed to help fold the protein domain into a configuration suitable for DNA binding. The zinc finger motif has since been recognized in numerous known or suspected DNA-binding regulatory proteins (*38*); it is a second major motif of DNA-binding proteins in addition to the helix-turn-helix motif recognized earlier (*39*). Detailed analysis of the binding of TFIIIA to the internal control region of 5*S* DNA and exploration by mutagenesis of the protein regions involved (*40*) have led to a detailed understanding of the DNA-protein interaction.

The most interesting aspect of the 5*S* RNA system, however, is the existence of oocyte-specific and somatic cell–specific genes and their differential regulation. In a comparatively simple, well-understood system, this property provides a model for the general question of differential gene regulation, one of the basic questions in biology. Although the molecular mechanisms of differential 5*S* RNA gene activity are not understood at present, the relevance of stable transcription complexes, and of changing factor concentrations has been stressed (*41*). Brown (*42*) has suggested that stable transcription complexes may represent a general mechanism in establishment or preservation of the differentiated state, a hypothesis that should provide stimulation for useful experimentation.

Regulation of class II genes in embryogenesis. In the past 2 years progress has been made in this system toward understanding the control of genes transcribed by polymerase II. Two highly regulated genes, GS17, which is expressed only during gastrulation (*43*), and α-actin, a muscle-specific gene (*44*), have been cloned and introduced as purified DNA into fertilized eggs. Both of these genes are correctly regulated by the embryo; cloned GS17 is transcribed at the correct time because of the presence of a gastrulation-specific enhancer element located about 700 bases upstream from the initiation site (*45*). The α-actin genes are also controlled by elements residing in the upstream flanking region (*44*). Similar results have been obtained with a cloned epidermal keratin gene (*46*). The ability of the embryo to incorporate exogenous DNA into its regulatory circuitry is an important advantage in mapping cis-acting elements that regulate the transcriptional responses to temporal, positional, or inductive developmental cues.

The Uses of *Xenopus* Oocytes as an Expression System

The frog oocyte translates injected mRNAs with great efficiency, processes the resulting proteins, and distributes them into the correct compartment. The oocyte also transcribes accurately many, although not all, genes injected into its large nucleus. These powerful techniques were developed through the efforts of Gurdon (*30, 47*) and are now used widely. In particular, translation in the oocyte has broad applicability for characterization of mRNA and protein products and as an aid in cloning of complementary DNA (*48*). Transcription of genes injected into the oocyte nucleus has not been used as widely as mRNA translation, but the *Xenopus* oocyte provided an important early expression system for 5*S* and ribosomal RNA genes (*30, 34*). Recently, the oocyte provided a functional transcription factor assay. Injection of nuclear extracts from sea urchins stimulated the transcription of sea urchin histone genes in the frog oocyte (*49*), and injection of a purified protein factor allowed the expression of an introduced *Drosophila* heat-shock gene without actual heat shock (*50*). A corollary of transcriptional studies is the work on RNA processing and transport that takes advantage of the ability to introduce genes, RNA precursor molecules, and protein or ribonucleoprotein factors into the nucleus and determine both the nature and localization of the products (*51*). Thus, the *Xenopus* oocyte continues to provide investigators with new opportunities in a variety of applications.

REFERENCES AND NOTES

1. H. Spemann and H. Mangold, *Wilhelm Roux Arch. Entwicklungsmech. Org.* **100**, 599 (1924).
2. O. Mangold, *Hans Spemann* (Wissenschaftliche Verlagsgesellschaft, Stuttgart, ed. 2, 1982).
3. R. L. Gimlich and J. Cooke, *Nature* **306**, 471 (1983); M. Jacobson and U. Rutishauser, *Dev. Biol.* **116**, 524 (1986); J. Smith and J. M. W. Slack, *J. Embryol. Exp. Morphol.* **78**, 299 (1983).
4. L. Saxén and L. Toivonen, in *A History of Embryology*, T. J. Horder, J. A. Witkowski, C. C. Wylie, Eds. (Cambridge Univ. Press, Cambridge, 1986), pp. 261–274.
5. I. B. Dawid, *Trends Biochem. Sci.* **12**, 34 (1987).
6. J. Gerhart, M. Danilchik, J. Roberts, B. Rowning, J.-P. Vincent, in *Gametogenesis and the Early Embryo*, J. G. Gall, Ed. (Liss, New York, 1986), pp. 305–319.
7. J.-P. Vincent, G. F. Oster, J. C. Gerhart, *Dev. Biol.* **113**, 484 (1986).
8. J.-P. Vincent and J. C. Gerhart, *ibid.* **123**, 526 (1987).
9. S. R. Scharf and J. C. Gerhart, *ibid.* **79**, 181 (1980); *ibid.* **99**, 75 (1983).
10. P. Grant and J. F. Wacaster, *ibid.* **28**, 454 (1972); G. M. Malacinski, A. J. Brothers, H.-M. Chung, *ibid.* **56**, 24 (1977).
11. R. L. Gimlich and J. C. Gerhart, *ibid.* **104**, 117 (1984).
12. R. L. Gimlich, *ibid.* **115**, 340 (1986).
13. S. Sudarwati and P. D. Nieuwkoop, *Wilhelm Roux Arch. Entwicklungsmech. Org.* **166**, 189 (1971); O. Nakamura and K. Kishiyama, *Proc. Jpn. Acad.* **47**, 407 (1971); P. D. Nieuwkoop, *Adv. Morphog.* **10**, 1 (1973); H. Grunz and L. Tacke, *Wilhelm Roux's Arch. Dev. Biol.* **195**, 467 (1986); J. B. Gurdon, S. Fairman, T. J. Mohun, S. Brennan, *Cell* **41**, 913 (1985).
14. T. D. Sargent, M. Jamrich, I. B. Dawid, *Dev. Biol.* **114**, 238 (1986).
15. H. Tiedemann, in *Biochemistry of Differentiation and Morphogenesis,* Colloquium Mosbach, L. Jaenicke, Ed. (Springer-Verlag, Berlin, 1982), vol. 33, pp. 275–287; J. Born *et al.*, *Biol. Chem. Hoppe-Seyler* **366**, 729 (1985).
16. J. C. Smith, *Development* **99**, 3 (1987).
17. M. Pudney, M. G. R. Varma, C. J. Leake, *Experientia* **29**, 466 (1973).
18. J. Cooke, J. C. Smith, E. J. Smith, M. Yaqoob, *Development* **101**, 893 (1987). A similar idea was proposed by E. C. Boterenbrood and P. D. Nieuwkoop [*Wilhelm Roux Archiv. Entwichlungsmech. Org.* **173**, 319 (1973)].
19. J. M. W. Slack, B. G. Darlington, J. K. Heath, S. F. Godsave, *Nature* **326**, 197 (1987).
20. D. Kimelman and M. Kirschner, *Cell* **51**, 869 (1987).
21. F. Rosa *et al.*, *Science* **239**, 783 (1988).
22. M. B. Sporn, A. B. Roberts, L. M. Wakefield, B. De Crombrugghe, *J. Cell. Biol.* **105**, 1039 (1987).
23. D. L. Weeks and D. A. Melton, *Cell* **51**, 861 (1987).
24. M. R. Rebagliati, D. L. Weeks, R. P. Harvey, D. A. Melton, *ibid.* **42**, 769 (1985); D. A. Melton, *Nature* **328**, 80 (1987).
25. E. H. Davidson, *Gene Activity in Early Development* (Academic Press, New York, ed. 3, 1986).
26. M. Jamrich, T. D. Sargent, I. B. Dawid, *Genes Dev.* **1**, 124 (1987).
27. B. I. Balinsky, *Introduction to Embryology* (Saunders, Philadelphia, ed. 4, 1975).
28. D. D. Brown and C. S. Weber, *J. Mol. Biol.* **34**, 661 (1968); *ibid.*, p. 681; M. L. Birnstiel, J. Speirs, I. Purdom, K. Jones, U. E. Loening, *Nature* **219**, 454 (1968); I. B. Dawid, D. D. Brown, R. H. Reeder, *J. Mol. Biol.* **51**, 341 (1970).
29. D. D. Brown and I. B. Dawid, *Science* **160**, 272 (1968); J. G. Gall, *Proc. Natl. Acad. Sci. U.S.A.* **60**, 553 (1968).
30. D. D. Brown and J. B. Gurdon, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2064 (1977).
31. D. R. Engelke, S.-Y. Ng, B. S. Shastry, R. G. Roeder, *Cell* **19**, 717 (1980); A. M. Ginsberg, B. O. King, R. G. Roeder, *ibid.* **39**, 479 (1984).
32. D. D. Brown and J. B. Gurdon, *Proc. Natl. Acad. Sci. U.S.A.* **51**, 139 (1964).
33. H. Wallace and M. Birnstiel, *Biochim. Biophys. Acta* **114**, 296 (1966).
34. R. H. Reeder, *Cell* **38**, 349 (1984); T. Moss, *ibid.* **30**, 85 (1982); P. Labhart and R. H. Reeder, *ibid.* **45**, 431 (1986); J. Windle and B. Sollner-Webb, *Mol. Cell. Biol.* **6**, 1228 (1986); *ibid.*, p. 4585; R. H. Reeder *et al.*, *Nucleic Acids Res.* **15**, 7429 (1987); K. Mitchelson and T. Moss, p. 9577 (1987); R. F. De Winter and T. Moss, *J. Mol. Biol.* **196**, 813 (1987); for a broad review on rRNA transcription see B. Sollner-Webb and J. Tower [*Annu. Rev. Biochem.* **55**, 801 (1986)].

35. D. F. Bogenhagen *et al.*, *Cell* **19**, 27 (1980); S. Sakonju *et al.*, *ibid.*, p. 13.
36. E. P. Geiduschek and G. P. Tocchini-Valentini, *Annu. Rev. Biochem.*, in press.
37. J. Miller, A. D. McLachlan, A. Klug, *EMBO J.* **4**, 1609 (1985).
38. J. M. Berg, *Science* **232**, 485 (1986); U. B. Rosenberg *et al.*, *Nature* **319**, 336 (1986); A. Ruiz i Altaba, H. Perry-O'Keefe, D. A. Melton, *EMBO J.* **6**, 3065 (1987); H. Blumberg, A. Eisen, A. Sledziewski, D. Bader, E. T. Young, *Nature* **328**, 443 (1987); J. T. Kadonaga *et al.*, *Cell* **51**, 1079 (1987).
39. C. O. Pabo and R. T. Sauer, *Annu. Rev. Biochem.* **53**, 293 (1984).
40. K. E. Vrana, M. E. A. Churchill, T. D. Tullius, D. D. Brown, *Mol. Cell. Biol.*, **8**, 1684 (1988).
41. D. F. Bogenhagen, W. M. Wormington, D. D. Brown, *Cell* **28**, 413 (1982); A. P. Wolffe and D. D. Brown, *ibid.* **51**, 733 (1987).
42. D. D. Brown, *ibid.* **37**, 359 (1984).
43. P. A. Krieg and D. A. Melton, *EMBO J.* **4**, 3463 (1985).
44. T. J. Mohun *et al.*, *ibid.* **5**, 3185 (1986); C. Wilson *et al.*, *Cell* **47**, 589 (1986).
45. P. A. Krieg and D. A. Melton, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2331 (1987).
46. E. Jonas, A. M. Snape, T. D. Sargent, unpublished observations.
47. J. B. Gurdon *et al.*, *Nature* **233**, 177 (1971).
48. D. A. Melton, *Method. Enzymol.* **152**, 288 (1987).
49. R. Maxson *et al.*, *Mol. Cell. Biol.* **8**, 1236 (1988); J. Mous, H. Stunnenberg, O. Georgiev, M. Birnstiel, *ibid.* **5**, 2765 (1985).
50. C. Wu *et al.*, *Science* **238**, 1247 (1987).
51. H. G. Stunnenberg and M. L. Birnstiel, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6201 (1982); G. M. Gilmartin, F. Schaufele, G. Schaffner, M. L. Birnstiel, *Mol. Cell. Biol.* **8**, 1076 (1988); M. Zasloff, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6436 (1983); P. de la Peña and M. Zasloff, *Cell* **50**, 613 (1987).
52. F. Rosa, unpublished data.
53. R. H. Reeder, unpublished data.
54. We thank D. Brown, J. Cooke, J. Smith, and F. Rosa for unpublished material and R. Reeder for Fig. 8.

The Nematode *Caenorhabditis elegans*

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In *Caenorhabditis elegans* patterns of cell division, differentiation, and morphogenesis can be observed with single-cell resolution in intact, living animals. Mechanisms that determine behaviors of individual cells during development are being dissected by means of genetic, cell biological, and molecular approaches.

THE NEMATODE *Caenorhabditis elegans* WAS ORIGINALLY selected for study as the result of a deliberate search for a multicellular organism that could be analyzed with the ease and resolution characteristic of studies of microorganisms (*1*). Features of the *C. elegans* life cycle facilitate genetic analysis, and features of its development and anatomy make it possible to analyze multicellular processes in the living animal at the level of individual cells. Since then a concerted effort has been made by members of the field to develop the system for studies of neurobiology, development, and cell biology. A detailed genetic map and methods for genetic analysis have been compiled (*1–3*), a complete cell-by-cell description of the development and anatomy of *C. elegans* has been achieved (*4–7*), and now a library of ordered cosmid clones representing the genome is nearing completion (*8*).

Caenorhabditis elegans is well suited to genetic studies for several reasons. First, it takes about 3 days to do a genetic cross in *C. elegans*; this is about the time required for genetic crosses in yeast. In addition, unlike many closely related nematode strains, *C. elegans* reproduces by self-fertilization. Self-fertilization allows new mutations to become homozygous automatically, without requiring brother-sister matings. The ability to freeze strains allows mutant stocks to be maintained indefinitely. Finally, genes defined by mutation can be cloned easily by transposon tagging or by injection of cloned DNA sequences from the genetic region of interest.

Each cell in the developing nematode is visible under the light microscope (Fig. 1). Furthermore, the entire cell lineage, from egg to adult, is essentially the same in each animal and is known precisely (*4, 5*) (Fig. 2). This means that any process involving the behaviors of individual cells in a multicellular context can be analyzed at the level of the single cell.

Finally, development of *C. elegans* appears to involve mechanisms that are conserved throughout the animal kingdom. When nematode lineages were found to be invariant, it seemed possible that much of nematode development would be controlled by mechanisms internal to individual lineages, and not by cell-extrinsic signaling, which plays such an important role in the development of higher organisms. Because the majority of *C. elegans* lineages are invariant, showing that cell-cell communication occurs is problematic. In order to show that cellular interactions influence cell fate, one must be able to alter a cell's behavior by changing its environment, for example by ablating other cells with a laser microbeam. By using this and other approaches, many examples of cellular interactions have now been discovered; it is clear that cell-cell communication plays a central role in *C. elegans* development (*9*). Furthermore, molecules that regulate *C. elegans* development have been found to contain homology to familiar vertebrate growth factor precursors and cell surface receptors (*10*), and to evolutionarily conserved homeodomains (*11*). Thus, with *C. elegans* it is possible to use genetics and single-cell analysis to dissect regulatory mechanisms of general significance and also to gain insights into how these mechanisms are modified to produce different kinds of animals.

We will first describe nematode development, then discuss experimental approaches available, and finally mention some current topics in *C. elegans* development, neurobiology, and cell biology. More comprehensive reviews are also available (*12–17*).

Overview of *C. elegans* Development

Caenorhabditis elegans adults are about 1 mm in length and live in the soil, feeding on microorganisms. They are sexually dimorphic, with hermaphrodites having two X chromosomes and males only one. Hermaphrodites have a total of 959 somatic nuclei (*5*), and a

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