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Embryonic Induction and Cell Differentiation During the Amphibian Development

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Since H. Spemann and H. Mangold (1924) discovered the "organizer", the morphogenetic center in the newt embryos, many attempts have been made to isolate the inducing substance which causes cell differentiation and morphogenesis in the presumptive ectoderm (undifferentiated cell mass) of the embryos. I have approached this embryonic induction problem in a wide variety of ways, from experimental morphology to molecular biology.

First, I proved that the capacity to induce a secondary set of axial structures is not a property of the egg cortex. Rather, as embryogenesis proceeds through cleavage division to blastulation, first, endodermal cells, then later the dorsal side of the embryo progressively acquire primary embryonic induction capacity.

Using the molecular approach, I focused on discovering the nature of the mesodermal inducing substance. Initially I isolated mesodermal inducing or vegetalizing inducing substances from chicken embryo's extract, carp swim bladder and the embryo itself. Using these materials, some properties and features of these factors were elucidated; protein in nature, molecular weight 25,000-30,000, homodimer (monomer 12,000–13,000). But with those factors, the amino acids sequence of the protein was not determined. From those basic experiments I developed the idea that it would be better to isolate the mesodermal inducing factors from various types of mammalian cultured cell lines. Presently there exists a vast number of cell lines which are of different origins, secrete different proteins. Mesoderm induction was assayed by examining the differentiation of mesoderm-type features in cultures of presumptive ectoderm of the newt and *Xenopus* embryos. The assay system also examined erythroid differentiation activity (EDF activity) in order to test the relationship between mesoderm induction and activin A (=EDF). Of 22 mammalian cell lines examined, six strains were positive for both mesoderm-inducing activity and EDF activity. The conditioned medium of one of them, K562 myelomonocyte, has the highest inducing activity. Then I purified the mesodermal inducing substance using by biochemical methods such as ammonium sulfate precipitation, DEAE-Toyopearl column chromatography and reverse phase HPLC. Finally, I succeeded in isolating the pure substance as a single band by SDS-PAGE. I determined its molecular weight to be 25,000 daltons. This mesodermal inducing substance has the same amino acid sequence of activin A (or Erythroid Differentiation Factor=EDF) and consists of a homodimer of 12,500 daltons.

Recently, the mesoderm-inducing effects of the transforming growth factor (TGF-β) family of proteins have been widely examined.

Independent of those efforts I determined that activin A possesses mesodermal inducing activity. I used the purified activin A made by recombinant cells. First I reported that activin A is the most effective of the range of inducing substances, having its activity at the concentration of 1 ng/ml. At the concentration of 10 ng/ml, it has 100% mesodermal inducing activity. In the explants I can see notochord, muscle and mesenchymal tissues in high frequencies.

Though presently several mesodermal inducing factors such as bFGF, TGF-β_2, TGF-β_1, ECDGF, and so on are known, I have shown that activin can

induce the mesodermal tissues at the highest frequency by the lowest concentrations of the factor.

Next, *Xenopus* liver genomic DNA library was screened using a rat activin A cDNA as a probe to identify the *Xenopus* homologue of the mammalian activin gene. Some independent genomic DNA clones were isolated. The reduced amino acid sequence of these genes all showed almost complete conservation of the distribution of cysteine residues. By Northern analysis we could detect some of their mRNAs in the embryo. One of them is expressed from stage 9 to the tailbud stage. Recently, I have also tried to isolate the activin protein itself from the early embryos. This may demonstrate that the activin and its related proteins are the natural inducer of embryonic development.