GENE EXPRESSION AND EMBRYOGENESIS IN AMPHIBIANS

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Contents

Summary

A central topic in embryology and molecular developmental biology is the early embryonic gene expression and regulation resulting in the formation of the complex adult organism (Grunz 1997, 1999).

In the last 5 years the knowledge about the molecular process during ontogenesis and evolution has dramatically improved. The scientific community of developmental biologists were strongly encouraged by the Nobel prize for 3 developmental biologists in 1995. This acknowledgment of representatives of this scientific field together with the new molecular techniques resulted in an exponential increase of gold rush-like research activities. It could be shown that many genes and their products responsible for the main pathways and pattern formation in eukaryotic organisms are evolutionary conserved. Such homologeous genes (some of them function as master control genes) are found in all phyla of the animal Kingdom. On the basis of these data new hypothesis about convergent development including new definitions of homology and analogy have been established (Halder et al., 1995a, 1995b; DeRoberts and Sasai, 1996;

DeRobertis, 1997; Grunz, 1999). Comparatives studies on the function of homologeous genes in invertebrates (Drosophila) and vertebrates (Xenopus) suggest that there existed common ancestors of these so different organisms like protostomia and deuterostomia. Nuclear transfer of somatic cells already shown in amphibians over 20 years ago could also be recently performed in mammals. These techniques (therapeutic cloning) will be used to generate replacement tissues and organs circumventing the immunological problems correlated with heterologeous organ transfer. The creation of more complex 3-dimensial vital organs, such as kidney, liver and heart under cell culture condition will be a great challenge. The amphibian ectoderm as pluripotant tissue comparable to the mammalian stem cells will also in the next future be a valuable tool to study the complicated molecular mechanism correlated with tissue and organ engineering.

So far only in amphibians it was possible to create 3-dimensional organs like heart and kidney under in vitro (cell culture) conditions, which could be used as surrogating organs in rescue experiments

1. Introduction

A central topic in Biology and Medicine is the formation of the body plan of the growing organism starting from the relative simple structure of the fertilized egg to the highly complex embryo and adult organism (see also reviews Grunz, 1997, 1999, 2000; Harland and Gerhard, 1997; DeRobertis et al.,1997). In higher organisms like invertebrates and vertebrates including humans the early embryonic development will be triggered by maternal and zygotic factors. The information for all forthcoming steps of development is stored in the huge DNA of the oocyte and every embyonic cell. However, the knowledge about the exact structure of the genome (base sequences) of an organism is not sufficient to understand the ontogenetic (individual embryonic development) and also phylogenetic (evolutionary) processes. Therefore many laboratories focus their studies on gene activation and gene regulation. Sophisticated temporal and spatial gene expression within the developing embryo collaborate with specific cell-to-cell interactions. A lot of genes code for transcription factors and special signalling factors like embryonic inducing factors. Several of them are homologues to growth factors. Since similar signal cascades leading to specific gene activation are found in normal as well as in cancer cells, the studies of cell differentiation including early embryonic development is of interest for both developmental biology and cancer research. Of special interest is the fact that comparative studies of genes and their functions in invertebrates and vertebrates (including humans) gave new insights into the mechanisms of evolution (urbilateralia-hypothesis see below). This holds true also for a specific class of closely related genes (master control genes) responsible for the eye formation of animals of all animal phyla, where eye structures or light senistive organs were found. Furthermore techniques of Molecular Developmental Biology will be essential for the progress in tissue and organ engineering. In the near future production of 3-dimensional organs like heart, liver or pancreas under cell culture conditions will be an outstanding chellange in biology and (transplantation) medicine (therapeutic cloning). Finally gold rush like research activities can be observed in the field of transgenic animals and plants or cloned organisms. The method of nuclear transplantation first used to produce genetically identical frogs (Gurdon and DeRobertis, 1977; Gurdon and Colman,1999). The method was recently modified to produce clones

of sheep, mice, cows and goals. If such methods will be applied to humans this is not only a technical problem for the scientific community, but a basic question for the whole society in the context of the potential benefits and risks for humans.

2. Historical background

The organizer experiment of Hans Spemann and Hilde Mangold in 1924 initiated gold-rush like activities for the search of inducing factors (Spemann and Mangold 1924, Hamburger, 1988). Spemann earned for this organizer concept the nobelprize in 1935. Frequently mentioned as Einsteck-technique it is one of the best known experiments in Biology. The organizer experiment could show that a part of the future dorsal side of a newt embryo is able to "organize" a secondary embryonic axis including the complete central nervous system, when transplanted to the ventral side of a host embryo (Grunz, 1999, 2000). Therefore Spemann named this area organizer (German: Organisator). As will be shown below the formation of the body axis of the developing embryo starting from the uncleaved egg and ending up in a complex organism is still a central topic of molecular genetics and molecular developmental biology. The observation of Spemann stimulated the scientific community after 1924 to look for (chemical) factors, so called inducing factors correlated with the organiser phenomena.

3. The search for inducing factors

Since the organizer is able to stimulate the formation of a secondary embryo with complete brain structures, embryologist began with the search of inducing factors shortly after the discovery of the organizer in the30s and 40s. The aim was to find a neuralizing factor responsible for the brain formation. At this time the nature of such factors were absolutely obscure. Spemann already could show that minced organizer tissue shows week neuralizing activity, indicating that chemical factors are responsible for the inductive processes. Therefore several laboratories tested different molecules like lipids, methylen blue, urea, sterols and even kaolin or silicon (reviewed in detail by Saxén and Toivonen, 1962). It was very confusing and disappointing that many of these chemically different agents, caused neural induction and differentiation either in the einsteck-experiment or in the sandwhich-experiment (Holtfreter, 1933).

In the latter experiment omnipotent competent ectoderm (comparable to mammalian stem cells) serves as the reacting tissue (Holtfreter, 1933). Today a similar test (one animal cap only) is known as animal cap assay and is used in many laboratories to perform gain or loss of function studies or to test growth factor with morphogenetic activity. At that time the responding tissue, the ectoderm, was considered less important. Confusing was the fact that ectoderm under certain conditions (high pH, ammonia, urea, etc.) will form several histotypic tissues (mainly neural structures) without the influence of the organizer.

Holtfreter postulated that some cells of the reacting tissue will be damaged by the treatment with different substances. This so called "sublethal cytolysis" of some cells was considered to cause neuralization of neighbouring, still vital cells (Holtfreter, 1947). He concluded from these data that no specific external inducer is needed and that neuralization takes place by the release of masked inactive factors. Meanwhile this

"autoneuralization" can be explained by the "neural default model" of the ectoderm (see below) formulated on the basis of our data with dissociated ectoderm (Grunz and Tacke, 1989, 1990; Wilson and Hemmati-Brivanlou, 1995; Hemmati-Brivanlou and Melton; 1997). The neural induction of the ectoderm without any influence of the organizer were quite irritating in the 1930s. This confusing situation was the reason that many scientists lost interest in this research field.

Nevertheless, a few groups in Finland, Japan and Germany continued to search for early embryonic inducers. Amphibian embryos themselves were not suitable to isolate inducing factors by biochemical methods. All amphibians cells contain a large amount of yolk and lipids, which severely interfere with traditional biochemical isolation and purification steps.

Furthermore it must be expected that such factors were present in low concentrations. However, a main short coming was the fact that the scientists did not have any idea about the exact chemical nature of such factors (small chemical molecules or polymeric molecules like nucleic acids, proteins, carbohydrates etc.). In the 30s Holtfreter could show that coagulated chick embryo extract was an effective mesoderm inducer (Holtfreter, 1933 a, 1933 b). A further key experiment was performed by Chuang, a PhD candidate of Holtfreter, later director of the Shanghai Institute of Cell Biology. He could show that the mesodermalizing activity of liver of guinea pig is lost after short boiling in hot water. However, the liver still could trigger competent ectoderm to form neural tissue (Chuang, 1939, 1940). These data indicate that the so called heterogeneous inducer liver contains a heat sensitive mesodermalizing and a heat stable neuralizing factor. Similar results about heterogeneous inducers were independently performed by Toivonen (1940). It should be mentioned that tissues of adult animals as source for the isolation of inducers were chosen, since in contrast to amphibian embryos (at this time Rana and Triturus only) liver, kidney or bone marrow from rat, mice or guinea pig were available in large quantities. Both findings, including activity of chicken extract and liver and bone marrow of guinea pig were the basis for extensive search for embryonic inducing factors in 3 laboratories in Japan (Tuneo Yamada), Finland (Sulo Toivonen and Lauri Saxén) and in Germany (Heinz Tiedemann). On the basis of their results with combination of different heterogeneous inducers Saxén and Toivonen formulated later the double-gradient-hypothesis (Toivonen and Saxén, 1955; Saxén and Toivonen, 1962), which was supported by the fractionation experiments with chicken embryo extracts of the group of Tiedemann (Tiedemann and Tiedemann, 1964). Nearly simultaneously the teams in Nagoya (Yamada and coworkers) and in Heiligenberg later Berlin (Tiedemann and coworkers) started extensive studies to isolate inducing factors from kidney, liver, bone marrow of guinea pig and trunks of 11 days old chicken embryos (Yamada and Takata, 1956; Tiedemann and Tiedemann, 1956a). Of central interest were the observations of both groups that apparently inducing factors are proteins in nature. In different approaches they could show that nucleoprotein fractions of liver did not loose inducing activity after treatment with ribonuclease (Yamada, Hayashi and Takata, 1958). In contrast treatment with pepsin abolished the inducing activity (Hayashi, 1958).

Tiedemanns group used the phenolextraction method and found inducing activity in the phenol layer (where the proteins will be dissolved). Nucleic acids and polysaccharides

enriched in the aqueous phase did not show any inducing activity (Tiedemann et al. 1960). These findings were indeed of outstanding importance for all later studies. Nearly all factors with mesodermalizing and neuralizing activities are protein in nature.

3.1 Isolation of the first embryonic inducing factor from chicken embryos – a milestone for our understanding of evolutionary conserved proteins

The group of Tiedemann only continued with enormous technical efforts to isolate the so called vegetalizing factor from large quantities of chicken embryos. The factor was named vegetalizing factor, since it induces mesodermal and endodermal derived tissues in competent ectoderm. Endodermal and mesodermal derivatives emerge from the vegetal (vegetative) hemisphere of the embryo (Figure 1). Finally after a period of 28 years it could be proven that this factor is not a mysterious substance, but is close related to activins, which belong to the TGFß-superprotein family. In the 50ies and even up till the early 90ies biologists could not understand why Tiedemann's group isolated the factor from chicken and used as test system the Einsteck experiment with amphibian embryos. Many considered the inductions in competent ectoderm (formation of notochord, somites, pronephros, blood cells etc.) as unspecific effects.

With our present knowledge about evolutionary conserved genes and their products we know that homologuous factors are found in Drosophila, Xenopus, Zebrafish and mice and also in humans. Activin shows mesoderm inducing activity also in the chicken embryo and probably also in humans. Therefore we now can imagine that the group of Tiedemann was quite ahead of the main stream of this time.

3.1.1 The vegetalizing factor (a homologue of activin) – the first embryonic inducing factor available in highly purified form

After long lasting biochemical isolation techniques the molecular weight of the vegetalizing factor (25-28 KDa) was determined by size exclusion chromatography and zone centrifugation (Tiedemann and Tiedemann, 1957). The molecular weight of the subunit determined by SE (Size exclusion)-HPLC in 50 % formic acid is 13000 KDA (Geithe et al., 1981, Schwarz et al., 1981). After 28 years tremendous effects is could be shown that the factor is activin A or an Activin A homologue (Tiedemann et al., 1992).

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