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My perpetual cycle: from student to researcher to teacher to student . . .

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ABSTRACT This contribution stems from the personal experience of the author regarding how he became acquainted with embryology and how he finally entered the field of developmental biology. It reports his feelings as a student of the Histology and Embryology course as it was taught in the late 1970s, and his present efforts in teaching developmental biology to university students. In the Developmental Biology course at Pisa University today, students are taught the tissue, molecular and genetic mechanisms that regulate development of several model systems. *Drosophila* is introduced at the beginning, because of the great knowledge that it has brought to the unraveling of the molecular aspects of development and because it allows several basic concepts to be introduced, and vertebrate systems follow. Other topics include the classic experiments on amphibian systems, which are explained in the light of recent molecular advances, as well as the genetically more versatile vertebrate systems such as the mouse.

KEY WORDS: *lecture, tutorial, graduation thesis*

Background Information

Scholarly Interests of the Author

In recent years, the author has developed a scientific interest in the early development of the anterior central nervous system (CNS) and, in particular, the role of homeobox transcription factors in the specification of the anterior CNS and the nature of the relevant signaling involved in induction of these genetic activities. More recently, the role of transcription factors in retinal cell determination has also been a focus of the author's research. The experimental system in use is the frog *Xenopus laevis*, in which the role of specific genetic activities is easily monitored by misexpression techniques and in the context of tissue recombination experiments. Other fields of research or intellectual interest relate to early events in development, such as mesoderm induction, limb development and developmental evolution of structures.

Representative Publications

I have always been interested in development, even when research in our institute was focused on the structure and evolution of the urodele genome. When we decided to shift to development, I was very happy to be part of the new project.

LUPO, G., HARRIS, W.A., BARSACCHI, G. and VIGNALI, R. (2002). Induction and patterning of the telencephalon in *Xenopus laevis*. *Development* 129: 5421-5436.

VIGNALI, R., COLOMBETTI, S., LUPO, G., ZHANG, W., STACHEL, S., HARLAND, R.M. and BARSACCHI, G. (2000). *Xotx5b*, a new member of the *Otx* gene family, may be involved in anterior and eye development in *Xenopus laevis*. *Mech. Dev.* 96: 3-13.

VIGNALI, R., POGGI, L., MADEDDU, F. and BARSACCHI, G. (2000). HNF1β is required for mesoderm induction in the *Xenopus* embryo. *Development* 127: 1455-1465.

General Features of the Course

Developmental biology is a fundamental topic in the Biological Sciences course of study at Pisa University, where it was introduced and promoted as a basic course by Prof. Giuseppina Barsacchi in 1990. Italian university courses of study have, at present, a different organization from university courses in America or in the rest of Europe. The degree is now obtained after 4 or 5 years (depending on the subject; Biology is a 5-year curriculum), following the defense of a graduation thesis; in Biology this is usually an experimental thesis. Developmental biology is taught to undergraduate students in their third year, after they have completed courses in mathematics, physics, general and organic chemistry, introductory cell biology (cytology) and histology, and first courses in genetics and biochemistry during their first two years. Molecular Biology is taught in parallel with Developmental Biology, and we coordinate with the Molecular Biology teacher so

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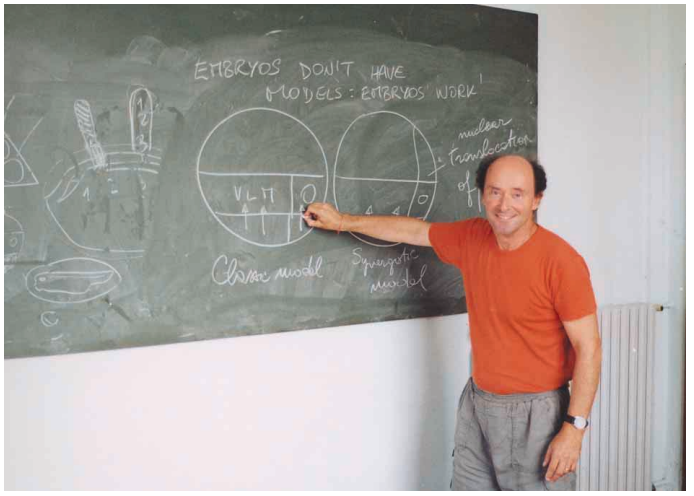

Fig. 1. The author coping with mesoderm induction models in a tutorial session. *(University of Pisa, Italy, 2002).*

that development can be introduced in molecular terms at the appropriate time. Because we like to start with *Drosophila*, we usually introduce some basic concepts and mechanisms (such as promoters, transcription factors, DNA binding and transactivation domains) right at the beginning of the course, before they are covered in the Molecular Biology course. We usually do this in a "soft" way, which is soon refined and deepened by the molecular biology teacher. There are two classes: one is taught by me and a parallel one by Giuseppina Barsacchi. Usually 30-40 students attend the lectures. There are three 45-min lectures per week, over the period from early November to the end of May, with two short interruptions, for a total of 60 lectures. These are given with the aid of slides (PowerPoint has just been installed) and overheads, but I quite like to use the blackboard in many instances (Fig. 1). In addition to lectures, there are two hours per week for the students to freely come and discuss with the teacher any subjects they need to deepen or clarify. These sessions are rather informal and, although sometimes a single student comes, there are usually several, so the discussion is collective; usually only the blackboard is used for these sessions. There are no laboratories connected with this course, which is different from other courses, such as biochemistry, molecular biology, zoology, and botany. However, students who are accepted for their experimental graduation thesis receive full training in our lab in frog experimental embryology, *in situ* hybridization and immunohistochemistry procedures, RNA injections, and DNA transfection, including all the associated techniques of molecular biology (Figs. 2,3).

All this will need to change, because in Italy we are now (year 2002) reforming the whole course of Biology study, making it more similar to the English and American models, with a first-level degree (Laurea) earned after a three-year period of undergraduate studies, followed by two more years to obtain a second-level degree (Laurea Specialistica), and then by a three-year Ph.D. program. A Ph.D. program did not exist some 20 years ago and right now directly follows the present five-year degree program. In this reformed course of studies, Developmental Biology will be a second-semester course in the second year, with a total of 40 lectures. This change is will provide an opportunity to renew the basic courses and make them more essential. This will require, I think, more synthetic abilities from the teachers.

Course Structure

The course is structured as traditional lectures in which the teacher's main goal is to stimulate curiosity about how embryos develop and, in particular, how genes control this process in time and space. For this reason, the course considers a sequence of issues in development. It is in relation to such issues that the different experimental models are progressively presented and their development described in causal terms. For example, we consider how cells at one end of the embryo make a head and those at the other end make a tail, and how this process is progressively regulated during developmental time by the action of genes. This is a big question, and to solve it, *Drosophila* is ideal. I am convinced that the fact itself that a great part of what we know in molecular terms about vertebrate development is a consequence of the study of *Drosophila* is an extremely stimulating paradox for the curiosity of the students. This is why, as I say later, it has proved extremely successful to start the course with the fruitfly, after a brief general introduction.

Course Content

I. Introduction
- A. Concept of development. From descriptive to experimental embryology to developmental biology.
- B. Model systems in developmental biology.

II. Developmental Processes
- X. Differentiation
- Y. Patterning (regional specification)
- Z. Morphogenesis

III. Drosophila *as a model system for the study of three-dimensional patterning*
- A. *Drosophila* oogenesis
- A. Segmentation and gastrulation in *Drosophila*
- B. Body plan in *Drosophila*: parasegments, segments and compartments

IV. Specification of the body axes in Drosophila*: the anteroposterior axis*
- A. Positional information and the "French flag" model
- B. Morphogenetic gradients
- C. Screening of mutants and identification of master regulatory genes
- D. Maternal effect genes: *bicoid, caudal*, maternal *hunchback,* and *nanos*
- E. Zygotic genes: gap, pair rule, and segment polarity genes
- F. Homeotic genes and the homeobox
- G. Vertebrate *Hox* genes and evolutionary conservation of homeotic genes
- H. Head development in fly and vertebrates
- I. The terminal system

V. Specification of the body axes in Drosophila*: dorsoventral axis*
- A. Follicle cells and the oocytes
- B. The protein Dorsal and the regulation of its translocation to the nucleus

VI. Mosaic and regulative development: concepts and experiments

VII. Sea urchin as a regulative model system: development of the sea urchin embryo

A. Cleavage
B. Gastrulation
C. The pluteus
D. Cell interactions in sea urchin development

VIII. Amphibians as a vertebrate model system; the Xenopus *embryo*
A. Amphibian egg and animal-vegetal polarity
B. Cortical rotation and the establishment of dorsoventral polarity
C. Cleavage, gastrulation, neurulation and organogenesis

IX. Inductive interactions in frog development
A. Hans Spemann and the organizer experiment
B. Mesoderm induction and specification of the organizer region
C. The Nieuwkoop center and the two-, three- and four-signal model
D. Molecules involved in mesoderm induction
E. Cortical rotation and nuclear translocation of β-catenin
F. A synergistic model for mesoderm induction and organizer specification
G. Neural induction and dorsoventral patterning of mesoderm
H. BMP and BMP antagonists; wnt and wnt antagonists

X. Neural induction
A. Vertical or planar signals?
B. Activation-transformation model
C. Neural inducing molecules

XI. Dorsoventral inversion: the case of Drosophila *short gastrulation/dpp* vs. Xenopus *chd/BMP4*

XII. Chick development
A. The egg
B. Cleavage and gastrulation
C. Formation of the amnion, chorion and allantois

XIII. Mammalian development
A. The egg
B. Cleavage and gastrulation
C. The placenta
D. Mammalian model systems: the mouse
E. Genetic recombination techniques in the mouse

XIV. Limb development

Textbooks for Assigned Readings

GILBERT, S.R. (2000). *Developmental Biology.* Sinauer, Sunderland, MA

WOLPERT, L.(1998). *Principles of Development.* Current Biology/Oxford University Press, London/Oxford. This text was translated into Italian by Giuseppina Barsacchi.

Examinations

Examinations are discussions of some of the topics of the course. Questions are asked in order to be developed as a "short dissertation" by the student. After the start, we tend to go deeper into the topic to get a better idea of the level of preparation of the student. Primarily, we tend to give a better evaluation to those students that show good thinking skills and that are able to discuss and argue about the subject, even if they do not have all the exact notions and details. The idea is to reward those that have understood the mechanisms and concepts, rather that those who just know the right answer. We can ask them to think of experiments that were not described in detail during the course, and forecast the results and tell why they would expect such a result: for instance, "How would you cause a gene to be expressed in a particular ectopic location, and what phenotype would you expect from this and why?" Or we could ask what kind of approach the student would use to trace back cells within the embryo and if and how this relates to cell specification. One typically asked question is "What is the difference between fate maps and specification maps?" However, we also like the students to have a basic, descriptive three-dimensional idea of what an embryo looks like. So we would not let a student pass who puts the neural tube right in the middle of the embryo with the digestive tube around it!!!

My Introduction to Embryology was Brief, but Stimulating

My introduction to embryology came during my first experience with a university course of studies. The first lecture I attended at the university was in fact part of the Histology and Embryology course at the University of Pisa during the 1976-77 academic year. This classical course was taught to first-year students and was designed to describe the main features of animal tissues and how germ layers and organ primordia eventually form during embryogenesis. Unfortunately, after the initial part of the course devoted to histology, only a small section was dedicated to embryology (at least in my class). It was merely descriptive embryology, reporting on how creatures, ones that seemed "strange" to me, such as amphioxus, arise from a single fertilized egg. There was, of course, time devoted to describing vertebrate embryogenesis. The lectures appeared to me to be very clear. The simple description of how embryos developed opened up for me a completely new world that I had previously ignored. It was for me rather extraordinary to learn how germ layers formed and reached their final positions during gastrulation. No less interesting and surprising was how organ primordia were generated during early embryonic stages.

That brief introduction to embryology, as stimulating as it was, did not excite me nearly as much as the information I would later read and study in textbooks. The textbooks reported that a small piece of tissue from a young amphibian gastrula was able to promote and direct the development of an additional embryo on the ventral side of a host embryo (Spemann and Mangold, 1924). Also mentioned was how animal and vegetal cells of a sea urchin embryo could be variously combined to produce a normal pluteus (reviewed in Hörstadius, 1973). I must say that all this was impressive and gave me a vague idea about how biologists were trying to understand the mechanisms through which different parts of the body are made.

However, what I still do not understand is why, what appeared to me to be the most interesting part of the embryogenesis story, experimental design/data, was not taught in the lectures. Had I been the teacher, I would certainly have liked to teach these experiments. As will be described later, the Developmental Biology course I now participate in begins with *Drosophila* studies, mainly because they have such a firm experimental basis.

The various experiments were neatly described and appeared to me to be properly interpreted. They led to the establishment of key concepts such as gradients, morphogens, induction, etc. Nevertheless, despite the impression generated by the experiments described in the textbooks of the time (Houillon, 1973; Balinsky, 1975), there were very few hints regarding the molecular explanation for those observations. This is not to say that there were no ideas about how to explain them. In fact, most of the observations reported in the textbooks were fortified by careful

Fig. 2. A Ph.D. student in our lab injecting mRNA into early *Xenopus* embryos. *(University of Pisa, Italy, 2002).*

arguments on the various alternative possibilities that would explain the data. They usually ended up with one or more working hypothesis to be further tested. In many cases, however, these hypotheses remained simple assumptions, which were therefore debatable. For instance, in reviewing the formation of the polar lobe in *Ilyanassa*, and the null effects of centrifugation on this process, Balinsky (1975) concludes that polar lobe formation does not depend on yolk, but probably upon some other factors that are not affected by centrifugation. It could depend either on a fixed cytoplasmic network that allows yolk granules to move around without breaking, or on a similarly centrifugation-resistant cortex. While he states that the first hypothesis is not substantiated by ultrastructural or physical observations, the second would be consistent with some experimental data showing that cortical granules do not move upon centrifugation. But I believe that today this evidence would be considered indirect and not conclusive. Another example is the description of egg activation, where he dedicates a whole chapter to the several working hypotheses that had been proposed to explain this phenomenon only to conclude, after a few pages, that although this problem was still unresolved, the predictable hypotheses had become few and more focused, so future research would surely be fruitful.

The Thoughts, Feelings, and Ideas which Filled this Young Student's Head

One of my recurrent thoughts in my early student years was that the cytoplasm of different regions of the egg, such as the gray crescent of amphibian egg, and particular tissues of the embryo that are able to promote special developmental events clearly have special qualities. But the molecular composition of these special "organ-forming substances" or egg plasms was vague and undetermined. The earliest molecular characterizations of developing embryos were reported in some instances to show how changes in general metabolism accompanied developmental processes: there were measures of the rate of oxygen usage; or of glycogen metabolism; or of protein synthesis in different parts of the amphibian or sea urchin embryo during gastrulation, a stage that was identified as a critical step in development (reviewed by Balinsky, 1975).

Those characterizations could not, however, provide a direct and causal link to explain special developmental events. Those studies only recorded metabolic changes during development. Balinsky's textbook also considered gene activities: he devoted a whole chapter to differentiation and clearly stated that "differentiation is the production of specific protein systems," that is, different repertoires of proteins expressed in different cell types. He analyzed a few examples of changing patterns in protein synthesis and gene activities during differentiation. These examples were relative to the terminal products of differentiation, so they could not provide a mechanism through which the differentiated state was actually reached. And yet the notion that genes somehow controlled development was becoming clear to him, since in analyzing gene activities during gastrulation, he stated that it would be interesting to know whether the mRNA produced immediately before gastrulation was the same or different from the RNA present in the unfertilized egg, or in the fertilized or cleaving egg.

The crucial significance of the developmental regulation of gene activities was reported in terms of the changing pattern of RNA synthesis in textbooks such as Ebert's *Interacting Systems during Development* (1965) and Balinsky's *An Introduction to Embryology* (1975). Those textbooks contained examples of developmental regulation of sets of tRNA and rRNA genes. However, in my personal experience as a student, I found that in Browder's *Developmental Biology* (1980), especially, genes received more attention in relation to developmental processes. That textbook contained quite ample descriptions of changes in gene expression. Most of them were related to tRNA, rRNA, or to heterogeneous nuclear mRNA. The changing composition of hnRNA (heterogeneous nuclear RNA) during development was characterized in terms of size in sucrose sedimentation gradients or in terms of its so-called hybridization (sequence) complexity. Although the reviews provided in those textbooks gave the clear idea that the quality of RNA changes during development, the whole picture was still missing two important pieces, inviting the following questions: How are these changes regulated during development? and What are the molecular mechanisms through which changes in gene regulation actually direct development phenomena?

In those years (1970s), little was known about gene regulation, especially in eukaryotes. It was not altogether clear, for example, how proteins (and possibly RNA of specific types) might regulate transcription. Moreover, the identities of the hnRNA molecules were unknown, and no functional data at that time showed that one specific type or class of genes could have a specific effect on development. At least this was the theme we learned from our course at the University of Pisa. All this was somehow frustrating to students like me. We were left with the scent of something that

we could not taste the flavor of! Ironically, I find it curious that today fruitfly development has become a major chapter in all developmental biology textbooks, when I recall that a picture of a *bithorax Drosophila* mutant is shown in the Introduction to Browder's textbook to make the simple point that genes are involved in the control of developmental pathways (without any further details being discussed).

Especially ironic was the fast turn of events. A major breakthrough was the discovery of the homeobox and its extensive conservation (McGinnis *et al.,* 1984a, b; Scott and Wiener, 1984; Carrasco *et al.,* 1984). It came a little later, too late for me, as a student, to appreciate its significance. We now know that those discoveries were crucial for unraveling the developmental events of the fruitfly and of other systems as well (Lawrence, 1992). For that reason, I introduce our Developmental Biology course by explaining to students how fortunate they are today to be able to learn so many details of how organisms develop and to have this explained in sophisticated molecular terms: this is something we were not able to experience as recently as 20 years ago, when I was a student.

Reversing the Usual Order: Teaching Developmental Biology with *Drosophila* Molecular Genetics as the Starting Point

I taught my first Developmental Biology course, devoted to undergraduate students, two years ago. I have about 30 students in a class. In our department, we have a strong tradition of experimental work on amphibians, which was initiated many years ago by Prof. Giorgio Mancino and continued by Profs. Giuseppina Barsacchi and Irma Nardi. Before the topics of our research moved to developmental biology, we focused our research on cytogenetics, and in particular on the study of metaphase and lampbrush chromosomes in newts. Significantly, the topic of our research changed after Giuseppina Barsacchi left off teaching the Comparative Anatomy course to take over the new course in Developmental Biology in 1990. In fact, this research move was in great part due to the interest she found in her new course: a nice case of feedback of teaching upon research. Today, in spite of our familiarity with the amphibian system, we choose to begin the Developmental Biology course with a review of discoveries made in *Drosophila*. The main reason is that *Drosophila* is a wonderful model for experimental studies. It provides the student with the perfect synthesis that takes place between descriptive embryology, genetics and molecular biology, the main disciplines from whose close interaction modern developmental biology originates.

Teaching *Drosophila* meant for me lots of studying, since my familiarity with this model system was not very extensive. Thus, I became a "student" of sorts, and as I taught this subject, I remembered what it was like to be a student. I could empathize with the students in the class, which likely contributed to their favorable responses to the course.

We at the University of Pisa think that teaching *Drosophila* development, and in particular its anteroposterior (AP) patterning, gives us the opportunity to introduce several concepts that will be continuously referred to during the course: First, the importance of mutants and screening procedures, which have allowed many classes of developmentally important genes to be characterized (Lewis, 1978; Nusslein-Volhard and Wieschaus**,** 1980). This allows us to make the point that the isolation of mutants links developmental patterning to genetic activities. Second, the role of maternal components at the beginning of development, which are essential for initiating successive developmental steps. This permits us to introduce genetic cascades or networks as a progressive display from maternal information. Third, the concepts of morphogen and gradient, which are active on the promoters of *Drosophila* genes. These allow us to describe the transformation of continuous gradients into discrete, well-delimited and periodic patterns, to subdivide the fruitfly body into developmental modules (Lawrence, 1992). In this section, we also introduce the main categories of molecules which are active during development (e.g., transcription factors, signaling molecules, receptors, and intracellular transducer molecules). We try to provide some details of the molecular interplay of transcription factors with the promoters of target genes to elucidate how this progressive subdivision and identification of the different parts takes place. We believe that unless some detail is given here, the mechanisms of fruitfly AP patterning will remain obscure to the students.

When we first began teaching using this *Drosophila*-first format, we were a bit fearful that too many details would generate confusion in the minds of our students. To our surprise, however, students asked to know more about *Drosophila*. We therefore believe that students are fully able to understand how fly patterning actually works. Of course, one of the parts students seem to prefer is that about homeotic genes: I must admit that I agree with their sentiment, because I had the same impression when I first learned about them. Thus, to maintain student interest at a high level, we introduce vertebrate *Hox* genes at this point. This serves to highlight the evolutionary and functional conservation of these genes and to show examples of homeotic transformation in vertebrates (Lufkin *et al.,* 1992; Rijli *et al.,* 1993; Ramirez-Solis *et al.,* 1993). In addition, we discuss how changes in regulation of *Hox* genes might have had a crucial role in major morphological changes during vertebrate evolution, a point I will return to later.

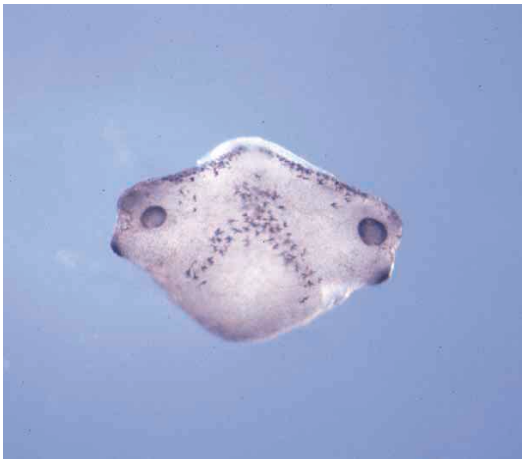

Fig. 3. Dorsalizing effects of *Xwnt8* injection. Xwnt8 *mRNA (25 pg) was injected at the 4 cell stage in one ventral blastomere. This embryo was injected by a student as part of his experimental activity for the preparation of his graduation thesis (see "General Features of the Course" section of this paper). Students (and also seniors) are always impressed to see how efficient and spectacular the effect of some injections can be.*

It should perhaps also be mentioned that this nontraditional strategy of beginning a Developmental Biology course with *Drosophila* may reflect somewhat of an overreaction to the feelings I myself had as a student. Recall, I was rather frustrated with the lack of understanding of the molecular basis of developmental phenomena back in the 1970s.

After the main aspects of *Drosophila* development have been described, we assume that many primary concepts are clear to students. Also, we presume that students are familiar with many of the molecular players and pathways which regulate development. For example, students know what a homeotic gene is and what a homeobox and a DNA-binding domain are. They also have an idea of the basic features of the canonical wingless pathway. They need only to transform this into the Wnt/β-catenin homologs to have the corresponding vertebrate pathway. Thus, we believe we are ready to shift the emphasis of the course to other topics.

Introduction to the Classics follows Reviews of *Drosophila*

The "organizer" experiment (Spemann and Mangold, 1924) is perhaps the best-known experiment in developmental biology. It has always given me a special feeling, both when I learned about it and when I teach about it. I also have the impression that students are always surprised to learn about the special quality of the organizer tissue. What is important here is to underline that only a part of the tissues that participate in the secondary axis are derived from the donor, and that there is a substantial contribution by host tissues that are "induced" to become something different from what they would normally become. It is absolutely vital here to stress the ingenious approach of using two differently pigmented species for this experiment. That seems to me absolutely clever: only this, in fact, allowed them to conclusively demonstrate induction ("primary induction," as it was called) of the host ventral tissues. But here came the surprise (one of the many in developmental biology): the organizer itself is induced, and its induction is part of a more general process that has been called mesoderm induction. At this point of the course, we temporarily freeze the analysis of molecular properties of the organizer to move to the study of how the organizer itself is induced. I will get back to mesoderm induction later in this contribution, because I think the story of research on mesoderm induction is a particular example of "trial and error" in science and of how research has possibly been misled by orthodoxy. Let's go back for the moment to the organizer. In spite of a long-lasting effort to identify and isolate the biochemical principles responsible for primary induction, only in relatively recent times, with the advent of molecular biology, has the molecular basis for the Spemann's organizer activity become clear. We use the story of the organizer and its molecular components as an opportunity to tell students the history of how this knowledge was gained. In doing this, we like to outline the rationale of the strategy used to identify genetic activities involved in the Spemann's organizer phenomenon. We therefore report the idea of looking for homeobox-containing genes in cDNA libraries from dorsal blastopore lips, which led to the identification of *goosecoid (gsc),* the first organizer gene (Cho *et al.,* 1991). We also report on the alternative functional approach of injecting pools of mRNA copied from a library derived from lithium chloride-treated gastrula embryos. That approach allowed initial selection of the *wnt8* and *noggin* genes (Smith and Harland, 1991, 1992). Both approaches show how important it is to choose the right experimental material at the start in order to optimize the chance of success. We also introduce the expression studies and functional assays that can be used to prove that a gene has some activity similar to that of the organizer, that is, the ability to rescue UV-ventralized embryos or to induce secondary axes. Since these properties may also be shared by earlier activities, being expressed at the right time and in the right place is an important aspect to consider before concluding that a gene is a main player in the organizer. Next, we describe how some of these gene products were initially shown to dorsalize both ectodermal and mesodermal explants (Lamb *et al.,* 1993; Smith *et al.,* 1993; Sasai *et al.,* 1994, 1995), thus explaining the neural inducing and dorsalizing activities of the dorsal blastopore lip. I think that these were exceptionally important discoveries, which crowned decades of research on the biochemical and genetic properties of the organizer.

Mesoderm Induction and Orthodoxy in Biology

Many of us were raised with the conviction that the "primary induction" demonstrated by Spemann and Mangold (1924) was the first inductive event in amphibian development. But we now know that mesoderm induction comes first. Strange to say, although the fundamental findings of Nieuwkoop (1969) and Ogi (1969) were at the turning of the seventies, there is no mention of this process in textbooks such as that of Balinsky (1975) or even that of Browder (1980), so, in fact, I learned about mesoderm induction only much later.

As I said previously, it is my impression that the story of mesoderm induction can be instructive in terms of how science proceeds and how *orthodoxy* in science can be misleading when not challenged by science's best instrument, that is, experimentation. I got interested in mesoderm induction while working with my colleagues on the zygotic transcription factor HNF1β which was found to have a permissive role in mesoderm induction (Vignali *et al.,* 2000). When I first got to study mesoderm induction, I learned that this process has already begun as early as the 32-cell stage and hence relies on maternal signaling molecules, released from the vegetal pole before zygotic transcription starts at the midblastula transition (MBT) (Jones and Woodland, 1987; reviewed in Harland and Gerhart, 1997; Kimelman and Griffin, 1998). The best candidates for this induction were found to be FGF- or activin-like molecules, which could promote formation of mesoderm in animal caps (Kimelman and Kirschner, 1987; Slack *et al.,* 1987; Smith *et al.,* 1988; Asashima *et al.,* 1990; Smith *et al.,* 1990; Sokol *et al.,* 1990; Thomsen *et al.,* 1990). Because there were maternal FGF (aFGF and bFGF) proteins and maternal TGF-β (Vg1) mRNA in the early embryo (Weeks and Melton, 1987; Kimelman *et al.,* 1988; Slack and Isaacs, 1989; Shiurba *et al.,* 1991), and early activin-like activities in the early embryo (Asashima *et al.,* 1991), a reasonable conclusion was that these factors initiated mesoderm induction very early, well before the MBT. The crucial role of FGF-like and TGF-β-like factors was further substantiated by results with dominant negative receptors (Amaya *et al.,* 1991; Hemmati-Brivanlou and Melton, 1992). However, the role of these maternal FGFs was questioned because they lacked a signal sequence to allow efficient secretion; on the other hand, although an active form of Vg1 was shown to act as an axial mesoderm inducer (Thomsen and Melton,

1993), the role of Vg1 was also questioned because an active form of Vg1 had never been detected *in vivo*. When other new candidates were found, such as eFGF (Isaacs *et al.,* 1992) and nodal-related proteins (Jones *et al.,* 1995; Joseph and Melton, 1997), they as well did not fulfill the expected requirements. In fact, they were expressed only zygotically, whereas the search was for maternal signaling molecules: they were seen as a "relay factor, maintaining or intensifying the initial mesoderm induction signals to allow continued formation and differentiation of the mesoderm" (Jones *et al.,* 1995) or considered to act "in the relay or maintenance, rather than the initiation, of mesoderm-inducing signals" (Joseph and Melton, 1997). Hence, still around 1998, in spite of circumstantial evidence that FGF-like and TGF-β-like molecules were involved in mesoderm induction, none of the proposed candidates proved completely satisfying and the exact identity of the mesoderm inducers proved elusive. And yet, there were some hints that the story might be different from what the "orthodoxy" had assumed. Students in biology should realize that sometimes in science you might have to question even what seems robust evidence. At this point, I like to mention what I think was a very important piece of evidence that possibly was not given sufficient attention, and which could inoculate the germ of doubt. While studying the role of β-catenin, Wylie and colleagues found that there was little, if any, induction of the ventral mesodermal marker *Xwnt-8* in animal caps by vegetal explants before the MBT, while this same marker was highly activated in animal caps by post-MBT vegetal explants, suggesting that "mesoderm induction signals coming from the vegetal mass may occur after MBT" (Wylie *et al.,* 1996). I think this experiment was the first clear evidence that the bulk of mesoderm induction is zygotic. I like to describe this experiment because it immediately struck me with the possibility that the story was different from the common view. But data were required to prove this, and the orthodox view of mesoderm induction still prevailed until the successive discovery of the maternally encoded transcription factor VegT and the clarification of its role at the onset of zygotic transcription: now it is clear that mesoderm induction is essentially a zygotic event (Zhang *et al.,* 1998; Kofron *et al.,* 1999). Many FGF and TGF-β players that had been found over the years, and which had not been completely satisfying as candidate mesoderm inducers because of their zygotic expression, were back in the field. They were already there, in fact. It has been found that VegT is able to activate many of the genes for these secreted factors (Clements *et al.,* 1999; Yasuo and Lemaire, 1999; Hyde and Old, 2000; see also review by Kimelman and Griffin, 2000), and every piece seems now in place again.

Evolution and Developmental Biology

As a student, one of the most interesting courses I attended was that of Comparative Vertebrate Anatomy. It was a great course where you could touch evolution at work, modifying structures and organs. Many of the differences that were described could be traced back to development, so the study of embryology was tightly connected with that of comparative anatomy. Therefore, we learned that the Gnathostome skull is characterized by a series of visceral arches, the first two, the oral arch (maxilla and mandibula) and the hyoid arch, having a distinct morphology, and the others, the branchial arches, having similar morphology. Moreover, the Gnathostome skull also has an occipital region where a number of vertebral primordia have fused together. But how were these different morphologies generated during evolution? At the time I was a student, this was almost impossible to tell except in rather general and vague terms, such as supposing that genes may mutate and these mutations might sum to change some structures or cause fusion of vertebrae or morphological changes in the branchial arches. But there appeared to be no key to solve this aspect. Today, there are quite a few examples of gene knockout or ectopic expression that lead to atavistic phenotypes, suggesting that major morphological changes in evolution could have occurred after regulative changes of developmental gene expression. The work by Lufkin *et al.* (1992), who showed that ectopic expression of *Hox-d4* in the occipital region leads to ectopic neural arches replacing the occipital region, and other studies, which showed that knocking out *Hox-a2* causes homeotic transformation of the second visceral arch into the second arch (Rijli *et al.,* 1993; Gendron-Maguire *et al.,* 1993), are the standard examples I use to show how developmental control mechanisms are related to evolutionary changes.

Another topic that has always attracted me is limb development, since it can be discussed in relation to several aspects of development, such as growth, patterning, differentiation and morphogenesis. At this point, students have a clear concept of induction and it is therefore easy for them to learn about reciprocal interactions of the apical ectodermal ridge (AER), progress zone (PZ), and zone of polarizing activity (ZPA). What I find particularly interesting here is the relationship among the patterning genes and molecules (wnts, SHH, FGFs, fringe) and the way they are integrated, through mutual interactions among the AER, PZ and ZPA, such that the coordinated three-dimensional development of the limb results (Johnson and Tabin, 1997). The role of cell death in shaping the limb is also discussed at this time. Moreover, I like to consider other aspects of evolution in this section of the course, in particular, the evolution of the tetrapod limb and its relationship to the fin (Sordino *et al.,* 1995; Shubin *et al.,* 1997), and the genetic basis for the absence of limbs in snakes (Cohn and Tickle, 1999). These evolutionary changes may also be explained in terms of regulatory changes in gene expression (Shubin *et al.,* 1997; Tickle, 2002).

Epilogue

I am now continuing with my teaching experience, and I must say that I find it stimulating. There are several reasons that I enjoy it. First, students seem in general genuinely interested in developmental biology, and therefore they provide feedback by asking questions and making suggestions on how the course might be improved. For example, at the end of the course, an anonymous evaluation form is distributed to students to be filled out, concerning the degree of interest and clarity of lectures, interactions with the teacher, etc. Among the more frequent suggestions is that a tighter link be made to human development, and that more about development of the nervous system be included. A second reason I enjoy teaching is that, as I am active in developmental biology research, I can update at least some of the topics by including the latest published data, and it is always nice to bring the students to the leading edge of science. Furthermore, by teaching also aspects of developmental biology other than the ones I am directly involved in with my research, I have the opportunity to widen my own interests and to continue learning. That is part of my perpetual cycle.

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