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Developmental biology for undergraduate students at the University of Palermo, Italy

GIOVANNI GIUDICE* and KAROLY ONORATO

Dipartimento di Biologia cellulare e dello sviluppo, Viale delle Scienze, Palermo, Italy

ABSTRACT This article describes a course for undergraduate students required for graduation in the Biological Sciences given by one of us (G. Giudice). The second author (K. Onorato) is responsible for computer graphics. Included in the description is a review of the various topics which are discussed in the lectures, as well as a collection of the graphics which accompany many of the lecture topics. Axis formation is reviewed in various model systems, and organogenesis, as it is understood at the molecular level in several genetic systems, is presented. Sex determination is reviewed in *Drosophila* and *Caenorhabditis,* followed by a discussion of developmental phenomena which involve clear-cut cellular interactions. Finally, assorted topics such as urodele limb regeneration, cell division control, aging and left-right asymmetry determination are presented.

KEY WORDS: *undergraduate, Italy, developmental biology, computer graphics, axis formation, lecture*

Background Information

Scholarly Interests of the Author

My scientific work started in 1956 and since then has primarily concerned the developmental biology of sea urchin embryos, using molecular and cellular approaches. Current aspects being researched include heat-shock proteins, nitrous oxide producton at fertilization, apoptosis, and macomolecular synthesis in mitochondria.

Representative Publications

My teaching experience began at the University of Palermo back in 1960, when I taught a microbiology course (I have an MD background); then, starting in 1964, I taught comparative anatomy for 26 years at the same university. Afterward, I began teaching developmental biology, which I continue to teach nowadays. I have, however, carried out research in developmental biology all my university life. I also have had experience teaching developmental biology in other countries, as a visiting professor at the universities of Chicago, Nagoya, Moscow, and others. Here are some recent representative citations relevant to my developmental biology teaching:

GIUDICE, G. (1998), Embriologia molecolare. In *Biologia dello sviluppo.* (Eds Raunich, L., Giudice, G and Manelli, H.). Piccin, Padova, Italy, pp. 87-248.

GIUDICE, G. (1999). Genes and their products in sea urchin development. *Curr. Top. Dev. Biol.* 43: 41-116.

GIUDICE, G. (2001). Conserved cellular and molecular mechanisms in development. *Cell Biol. Int.* 23: 1081-1090.

General Teaching Philosophy

The guiding theme of this course is to explain developmental biology phenomena in molecular terms, as studied in selected model organisms. The obvious caveat—beware of the danger of oversimplified generalizations—is of course emphasized. The most common model systems are described, although for lack of time, the zebra fish model is drastically reduced.

I believe first of all that developmental biology is the basis for understanding several aspects of biology, including evolution. I therefore attempt to enhance student interest by talking in the introductory lectures about the fascinating problem of how—from an egg—an organism arises which may be composed of billions of cells, connected with billions of other cells, each occupying its precise place, among the billions of billions of places it might occupy. I then go on to explain that the solutions of embryology problems may be looked for in molecular terms. I do not in fact believe that the reductionist method of inquiry can be easily dismissed. Nevertheless, I believe we should also be aware that we need to search for holistic solutions as well. I expect students to understand the scope of developmental biology and to be able thereafter to use what they have learned to either go on in research and/or pass correct information to other people, both in school and in life in general.

General Features of the Course

This course was presented to students of the University of Palermo during the academic year 2001-2002. These students had

***Address correspondence to:** Dr. Giovanni Giudice. Dipartimento di Biologia cellulare e dello sviluppo, Viale delle Scienze, 90128, Palermo, Italy. Fax: +39-091-6577430. e-mail: giudice@unipa.it

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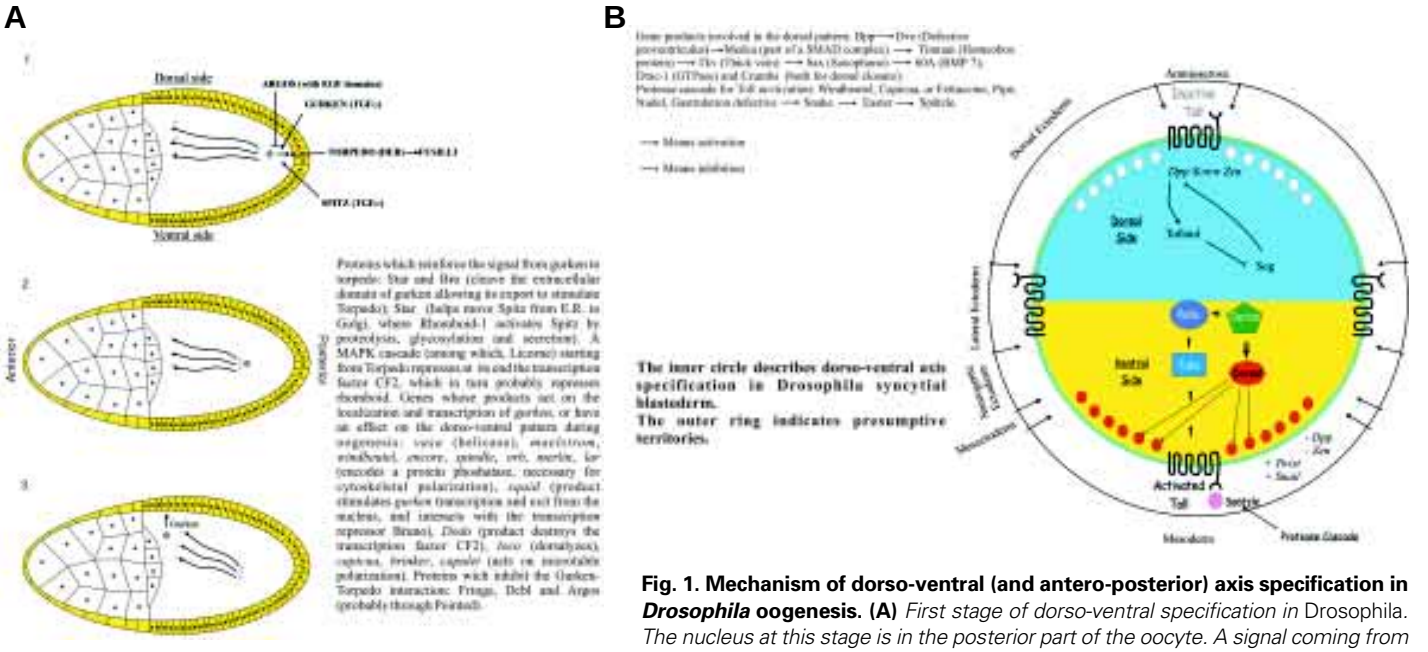


Fig. 1. Mechanism of dorso-ventral (and antero-posterior) axis specification in *Drosophila* oogenesis. (A) *First stage of dorso-ventral specification in* Drosophila. *The nucleus at this stage is in the posterior part of the oocyte. A signal coming from the nucleus, and consisting of the protein Gurken (a member of the TGFα family) goes to the receptor Torpedo (a* Drosophila *epidermal growth factor receptor) of the posterior follicular cells. This in turn stimulates the production of the protein Fusilli. The protein Spitz reinforces this signal, whereas the protein Argos inhibits it, as shown in part 1. This causes the inversion of microtubule polarity, as shown in part 2. The nucleus is therefore transported to the dorsal anterior part of the oocyte, and there it sends to the follicular cells of that zone another Gurken signal, which gives the first indication that this is the dorsal anterior site, as shown in part 3. The inset shows updating and details.* **(B)** *A later stage of dorso-ventral specification in* Drosophila. *The dorsal side of the syncytial blastoderm is in blue, and the ventral side is in yellow. The protease Spätzle activates the ubiquitous receptor Toll only on the ventral side; this stimulates in turn the protein tube, which causes activation of the kinase Pelle. The latter phosphorylates, and therefore inactivates, the ankyrin Cactus, causing it to release, only on the ventral side, the ubiquitous protein Dorsal, which therefore is permitted to leave the cytoplasm for the nuclei only on the ventral side. This causes the activation in the ventral nuclei of the genes* twist *and* snail*, and the inhibition of the genes* dpp *and* zen. *On the dorsal side of the figure, interactions are shown between the genes or proteins Dpp, Screw and Zen, which activate the protein Tolloid; this inhibits the gene* Short gastrulation*, which in turn inhibits* dpp. *The inset shows details and updating.*

already attended the university for two and a half years, and had previously taken several courses, including year-long courses in cell biology, comparative anatomy, genetics, biochemistry, and molecular biology. The course had a value of 8 credits, and was given through 64 lecture presentations (approx. 60 minutes each). In addition to this course, various pertinent laboratory (i.e., "practical") courses were given by other instructors. Graduation in Biological Sciences was obtained after passing 27 exams in 5 years, and after defending an experimental thesis. Typically, 100 students enrolled in the course each year.

Beginning in 2002, Italian law introduced, besides the 5-year degree, a degree to be achieved after 3 years of university study. For that program, my developmental biology course had to be shortened to 4 credits, and consequently two sections (those on fertilization and control of mitosis and meiosis) were deleted. In future years, the short program will continue, supplemented by 2 years of so-called "specialization" (also with an experimental thesis), which will add up to a total of 5 years for a degree in Biology.

The present description applies to the original 8-credit course, and also to the 4-credit course, except that the two sections mentioned above are omitted. It should be noted that students in the "short graduation" program have also enrolled in the same prerequisite courses mentioned above, albeit for proportionally shorter times.

Special Features of the Course

The undergraduate learning experience is accomplished through illustrating and commenting on approximately 160 figures, examples of which are presented here. Each year, these are updated and innovations and additional details added. In fact, beginning next year(2003), I will distribute to each student a CD. This will contain all the figures for the course, which will represent all they need to learn, plus updates and details, only for their further information and for consultation. In the present paper, a simplified example of this updating is illustrated in the inset of each figure.

Lecture Topics

Introductory Lecture

An introductory lecture is dedicated to the totipotency of nuclear DNA in somatic embryonic cells, starting with the classic experiments of nuclear transplantation in amphibians (Briggs and King, 1952) and going on to the experiments of Wilmut *et al.* (1997) and other recent experiments which involve cloning from adult cells.

Establishment of Embryonic Axes in Drosophila

Next, I state that one of the main problems of developmental biology is that of the establishment of embryonic axes. In fact,

as I used to say to my students, "if when I was an embryo, I was not able to distinguish between my oral and aboral poles, I would feel quite embarrassed in talking today in front of you." More seriously, the problem is an old one and was first addressed in sea urchins. Hörstadius (1939) and Runnström (1928) formulated the first rules about the animal and vegetal poles. Those ideas have been recently revived by groups such as those of Davidson (Ransick and Davidson, 1995), Angerer and Angerer (2000), McClay (Logan *et al.*, 1999), Gache (Emily-Fenouil *et al.*, 1998), Giudice and Di Carlo (DiCarlo *et al.*, 1996), and those experiments are thus described in detail in this course.

What are described there and reported here in the illustrations are the experiments which provide examples of the various means by which the goal of establishing embryonic axes is achieved in *Drosophila*. For example, Fig. 1 illustrates the establishment of the dorso-ventral axis. The story starts with oogenesis, during which (through the Gurken signal) the nucleus is moved to an anterior and dorsal position by an inversion of microtubule polarity, and consequently signals an anterior and

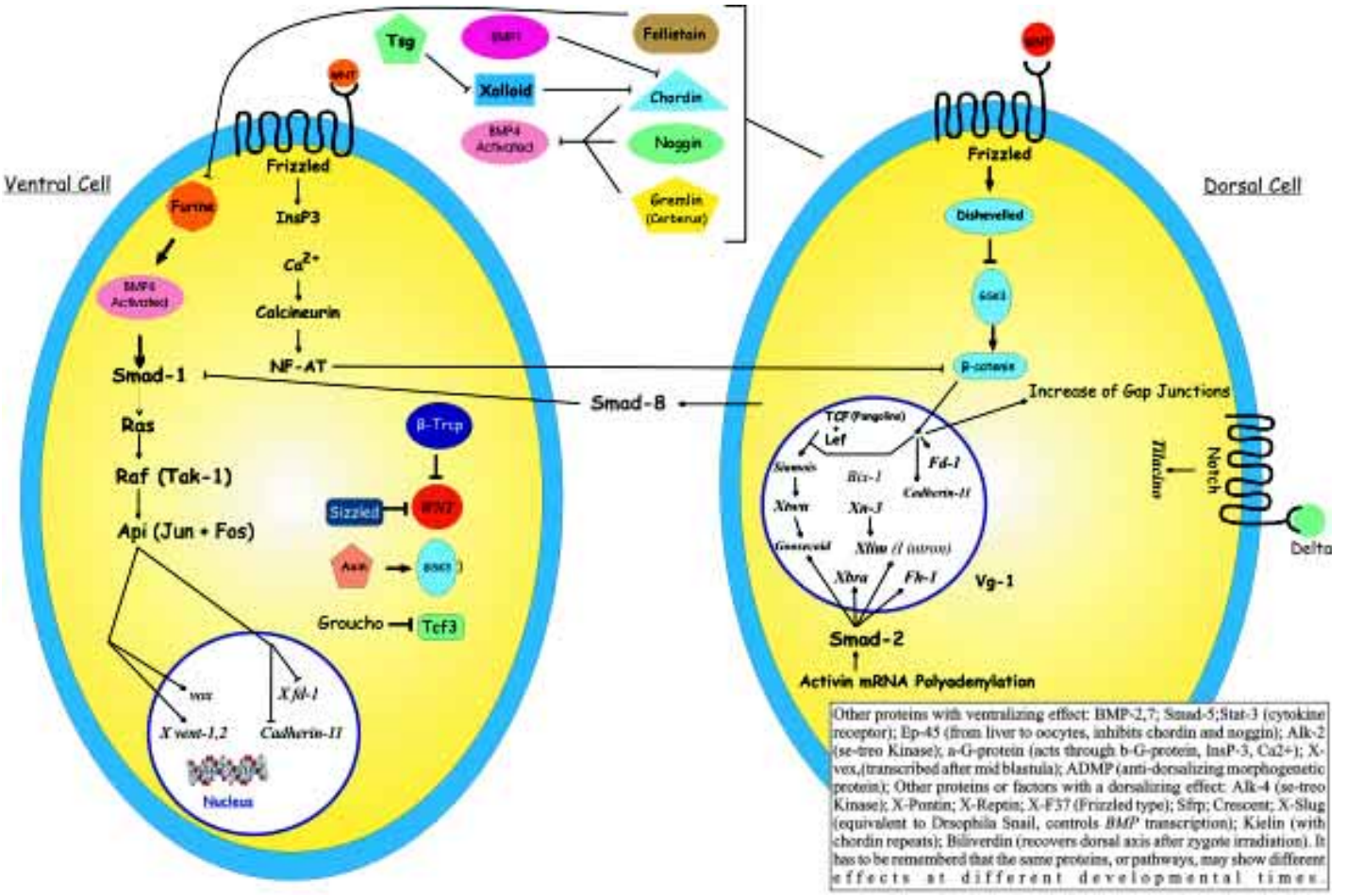
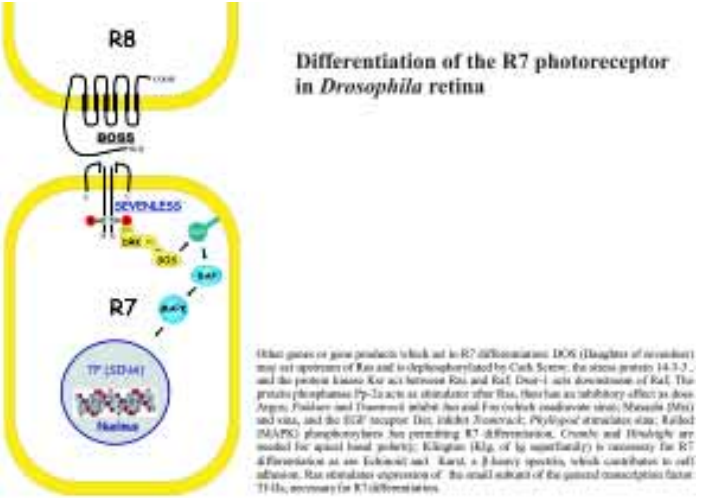
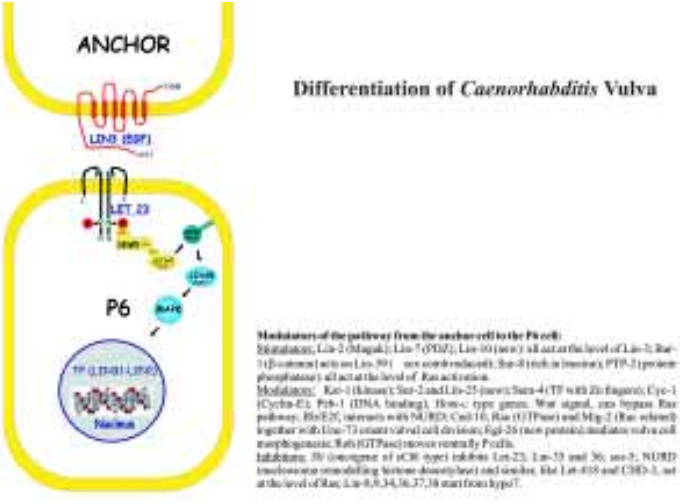


Fig. 2. Two mesodermal cells of *Xenopus laevis* mid-blastula. *A typical dorsal cell and a typical ventral cell are shown side by side, with the genes which are activated in each. In the dorsal cell, a Wnt protein activates the receptor Frizzled, which activates the protein Disheveled; this in turn inhibits the glycogen synthetase kinase-3, which therefore does not phosphorylate β-catenin, thus permitting it to enter the nucleus. There, together with the transcription factors Tcf and Lef, it activates a series of genes such as* siamois, Xtwn *and* goosecoid. *At the same time* Activin *mRNA is polyadenylated and its protein stimulates the gene* smad-2, *which activates* Xbra, *again* goosecoid, Fh-1, *and binds to the first intron of* Xlim, *to which Xn-3 also binds. The gene* bix-1 *is also activated in the dorsal cell, in which an increased number of gap junctions is also observed. In the same cell a Notch type receptor is stimulated by a Delta type ligand, and as a consequence the gene* tilacino *is activated. The protein Smad-8 is produced by the dorsal cell, which inhibits the gene* smad-1 *active in the ventral cell. The dorsal cell produces and exports the proteins Gremlin, Noggin and Chordin, which directly inhibit Bmp-4 produced by the ventral cell, while Follistatin, produced also by the dorsal cell, indirectly inhibits Bmp-4 by inhibiting the protease Furin, which is responsible for Bmp-4 maturation. Conversely, the ventral cell produces Bmp-1 and Xolloid which proteolyze Chordin. The inhibitor of Xolloid, Tsg is also shown. Mature Bmp-4 activates* smad-1 *in the ventral cell, starting the chain Ras, Raf, and Api, which results in the activation of the genes* vox *and* vent-1 *and -2, and in the inhibition of the genes* Xfd-1 *and* cadherin-11. *Always in the ventral cell, another Wnt protein activates a different Wnt pathway by stimulating a Frizzled receptor, which causes IP3 production and therefore* $Ca2^{+}$ *release, calcineurin activation and production of the protein NFAT. The latter might in turn inhibit at the level of β-catenin the Wnt pathway active in the dorsal cell. The canonical Wnt pathway is, conversely, inhibited in the ventral cell, because the proteins Sizzled and β-trcp inhibit Wnt, and the protein Groucho inhibits Tcf3, while the protein Axin stimulates Gsk3. The inset shows updates and additions.*

Other genes or products which are involved in ascidian development: *Ci hox, Hr hox* (both active in the head); *Ci-vnolo-1, 2, 3* and *Ci-vtf-1* (all active in the gut); *Ci-Bra-1* with LiCl homeobox) expressed downstream of β-catenin, for gut; Ci brachyury, active in the chord and suppressed outside of it by Ci sal I and by Ci snx, activates at least 40 chord-specific genes; FGF-4,6,9 and MEK signaling act in chord induction by presumptive endoderm; Ci VegTr, macho-1and As-T2 act probably in muscle differentiation, as do proteins P-58 and Myoplasmin; Ci mrlf (MyoD family); Hr BMP (ventralizing); Hr eal, Pox-6; Hr pspk-1, Hr Wnt-5, five genes (posterior end markers) are all most active posteriorly; HrWnt-5 helps chord cell movement; Synaptotagmin is necessary for synaptic transmission; about 1,000 maternal genes have been recently identified through expressed sequence tag (EST) analysis.





dorsal fate (Fig. 1A). The same story continues with the strategy, which is also used for signaling the Acron and Telson positions (not explained here), of having a receptor evenly distributed on the egg periphery but a ligand asymmetrically distributed. Here the receptor is Toll, which by a protease

Fig. 3. (Top Left) The ascidian morula. *Genes which are active in the species* Molgula oculata, M. occulta *and in the hybrid. Note that in* M. occulta *(whose embryo has no tail)* Manx *is lacking,* MoccMa-1 *(equivalent of* Mocu Ma-1*) is mutated both in the open reading frame and in the promotor. The gene* Ca-1 *is at first active in* M. occulta, *then becomes inactivated, while* Ca-2 *is inactive. In the hybrid,* Manx *is active, as is* FH-1. *The inset shows updating and additions.*

Fig. 4. (Middle Left) The ligand Boss present on the surface of the photoreceptor R8 stimulates the receptor Sevenless on the surface of the photoreceptor R7. *Sevenless gets autophosphorylated and, through the general adaptor Drk, stimulates the protein Sos (son of sevenless); which activates the GTPase Ras, moving it to the membrane; which in turn activates the MAP kinase chain starting with Raf. This leads eventually to the activation of the gene encoding the transcription factor Sina (seven in absentia). The inset shows updating and additions.*

Fig. 5. (Bottom Left) Lin 3, a ligand on the surface of the anchor cell, stimulates Let 23, a receptor on the surface of the P6 cell, which gets autophosphorylated and, through a general adaptor, here called Sem5, stimulates the protein Let 341, equivalent to Sos. *This activates the GTPase Ras, here called Let60, moving it to the membrane, and this in turn activates the MAP kinase chain starting with a Raf, here called Lin 45. At the end, genes for two transcription factors, Lin 31 and Lin1, are modulated. The inset shows updating and additions.*

cascade is activated by the ligand Spätzle only ventrally. Therefore, Toll induces phosphorylation only ventrally and inactivates the protein Cactus. This inactivation releases the protein Dorsal from the cytoplasm, thus allowing its transfer to the nuclei of the syncytial blastoderm only on the ventral side. Here, Dorsal acts as a transcription factor and activates the synthesis of the RNAs for the proteins Twist and Snail, while inhibiting the syntheis of RNAs for Decapentaplegic (Dpp) and Zerknult (Zen) (see Fig. 1B). As shown in the same figure, while this happens on the ventral side, opposite phenomena take place on the dorsal side.

On the dorsal side, there is maximum activation of Zerknult synthesis and, through a not yet fully understood mechanism, activation of *dpp*. Thus, a dorso-ventral gradient opposite to that of Short gastrulation (Sog) is formed, owing also to the fact that Dpp activates the protease Tolloid, which inactivates the protein Sog (which in turn inhibits *dpp*). The fate map of different embryonic territories is also indicated in the same illustration. Other genes involved are indicated in the inset of the illustration. It is made clear to students that the main mechanism is that of a gradient of nuclear concentrations of dorsal components, with different thresholds of sensitivity to various target genes, as recently reviewed by Stathopoulos and Levine (2002).

Amphibian Embryonic Axis Formation

Another paradigmatic pattern of embryonic axis establishment—amphibia—is also described in this course. I begin with a brief analysis of animal and vegetal pole positions. Then I present a description of the different mRNA locations along this axis, and of their mechanisms of transport and location, cortical rotation at fertilization, and consequent fixation of the dorso-ventral axis. Primary induction is then introduced in detail. Finally, attention is focused on the dorso-ventral potentialities of the embryonic mesoderm, as described in Fig. 2, where the genomic activities of a typical mesodermal dorsal cell and that of a ventral one are schematically indicated. The activity of the canonical Wnt pattern

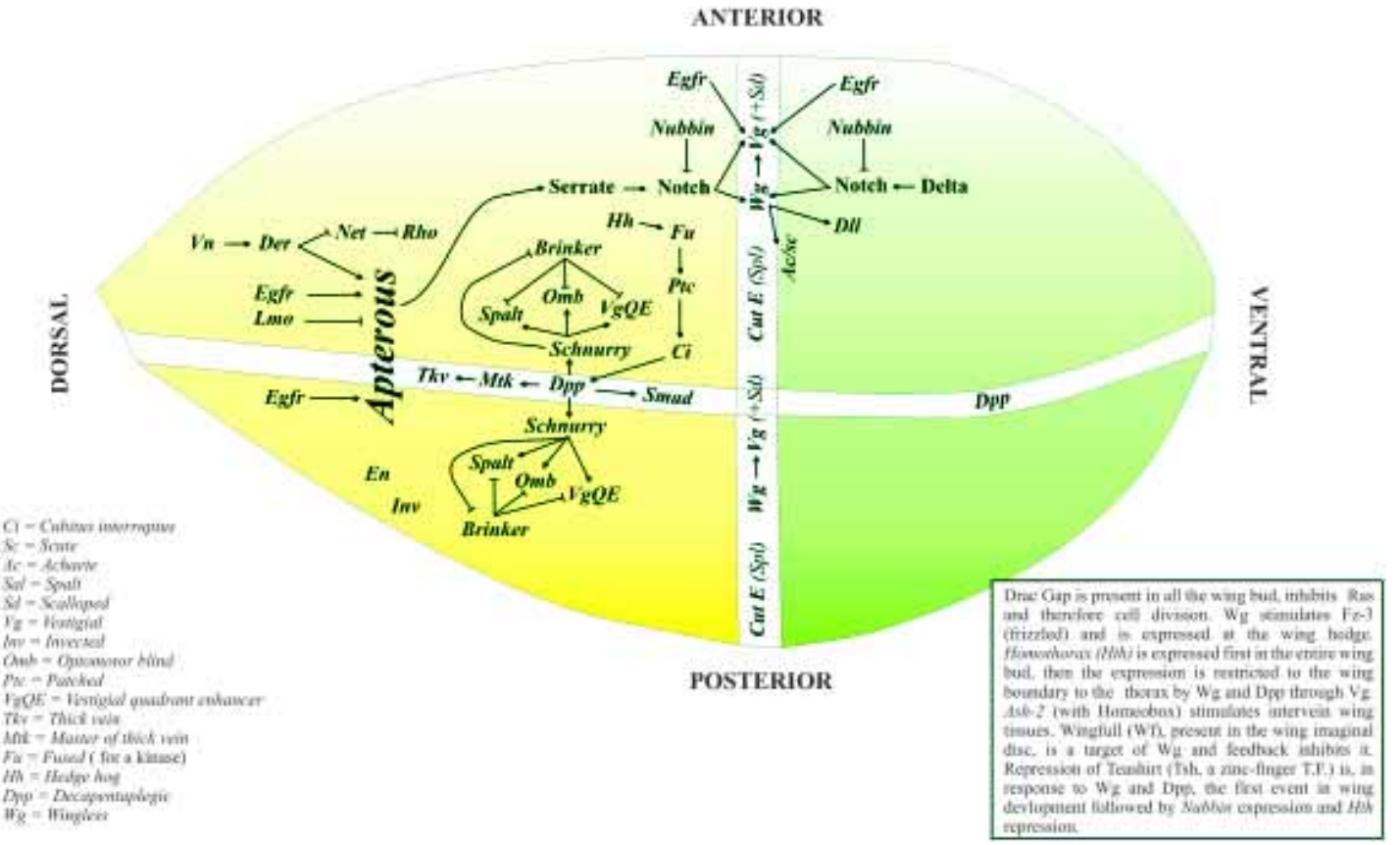


Fig. 6. Genes expressed in the *Drosophila* wing presumptive territories. *The different presumptive territories of the wing imaginal disc are shown on the same plane in different colors and color gradations, i.e., yellow, dorsal; green, ventral; darker, posterior and paler, anterior. A double white line indicates the boundary between these regions, and bears the names of the genes expressed there. Note that* apterous *is expressed only dorsally, both anteriorly and posteriorly, and is stimulated by EGF-r and Der and inhibited by Lmo. Der acts downstream of* Vn *and inhibits* Net*, which inhibits* Rho. Apterous *stimulates the production of the ligand Serrate which is the dorsal stimulator of the receptor Notch, which is stimulated by the ligand Delta on the ventral side. Also the genes* en *(*engrailed*) and* inv *are expressed only dorsally.* Dpp *is expressed at the antero-posterior boundary and there stimulates the genes* mtk *and then* tkv*, and also* smad. dpp *also stimulates* schnurry*, which both anteriorly and posteriorly stimulates the genes* spalt, omb *and* vgqe*, which are all inhibited by* brinker. Dpp *is stimulated by* hh *which acts through* fu, ptc *and ci.* Wg *acts at the dorso-ventral boundary, where it stimulates* vg *(plus* sd*).* Vg *on the ventral side stimulates* ac-sc *and* dll *(distal-less). The genes* cu *and* e(split) *(enhancer of Split) act at the same boundary. Notch is stimulated both ventrally and dorsally by EGF-r (epidermal growth factor) and inhibited by Nubbin. The inset shows updating and additions.*

is present in dorsal cells, at least at certain stages, whereas this is repressed in a variety of ways in the ventral cells. I stress how dorsalizing activity is exerted through the production of evolutionarily conserved proteins such as Noggin, Gremlin and Chordin, which directly inhibit the ventralizing protein Bmp4, and through Follistatin, which inhibits Bmp4 maturation.

The recent paper of Saneyoshi *et al.* (2002) is also discussed. It describes an alternative pattern of Wnt, which is active in ventral cells, in an InsP3-dependent manner. It may interfere with the canonical Wnt pattern of the dorsal cells by inhibiting it at the level of Gsk3-β-catenin. That may provide a unifying explanation between the reported vegetalizing effects of Li in sea urchins and the ventralizing one in amphibians by activation of β-catenin movement to the nuclei from one side (Emily-Fenouil *et al.*, 1998), and InsP3 regeneration from the other side (Giudice *et al.*, 1992).

At this time the attention of students is directed towards the concept that members of the same metabolic pathway can produce different effects. For example, the T-cell factor, which activates s*iamois* expression dorsally while inhibiting it ventrally (Brannon *et al.*, 1997), or to the different effects of activin, according to the number of receptors occupied (activation of *Xbra* for 100 receptors occupied, and activation of g*oosecoid*, and consequent inhibition of *Xbra,* for 300 receptors occupied). The theory of Geoffrey Saint-Hilaire, revived by De Robertis and Sasai (1996) and the concept of Urbilateria are also discussed in this set of lectures.

Axis Formation in Ascidians

The establishment of embryonic axes is also discussed in ascidians, as an example of mosaic eggs. I describe the genes which are expressed in different body parts, starting with the

oocyte and the movements of its various plasms during maturation and fertilization. Emphasis is placed on the experiments of Swalla and Jeffery (1996), which are aimed at understanding the molecular mechanisms that bring about formation of the notochord (see Fig. 3). Those experiments demonstrate some of the genes which come in to play in one species with an embryonic tail and another species which does not have a tail, during embryonic life, and in the hybrid. The genes *manx1*, *MocuMa 1*, and *ca 1* and *2* (for cytoskeletal actins) are present in the genus *Molgula* species *oculata*, which has an embryonic tail, together with the genes *caudal* and *FH1*. Whereas in the species *occulta* of the same genus, *manx* is absent and *MocuMa 1* is mutated. *Ca 1*, although with a slightly different sequence, appears also in *M. occulta* for a short period, then disappears. *Ca 2* is absent in *M. occulta*. *Manx 1* and *FH1* are present in the hybrid.

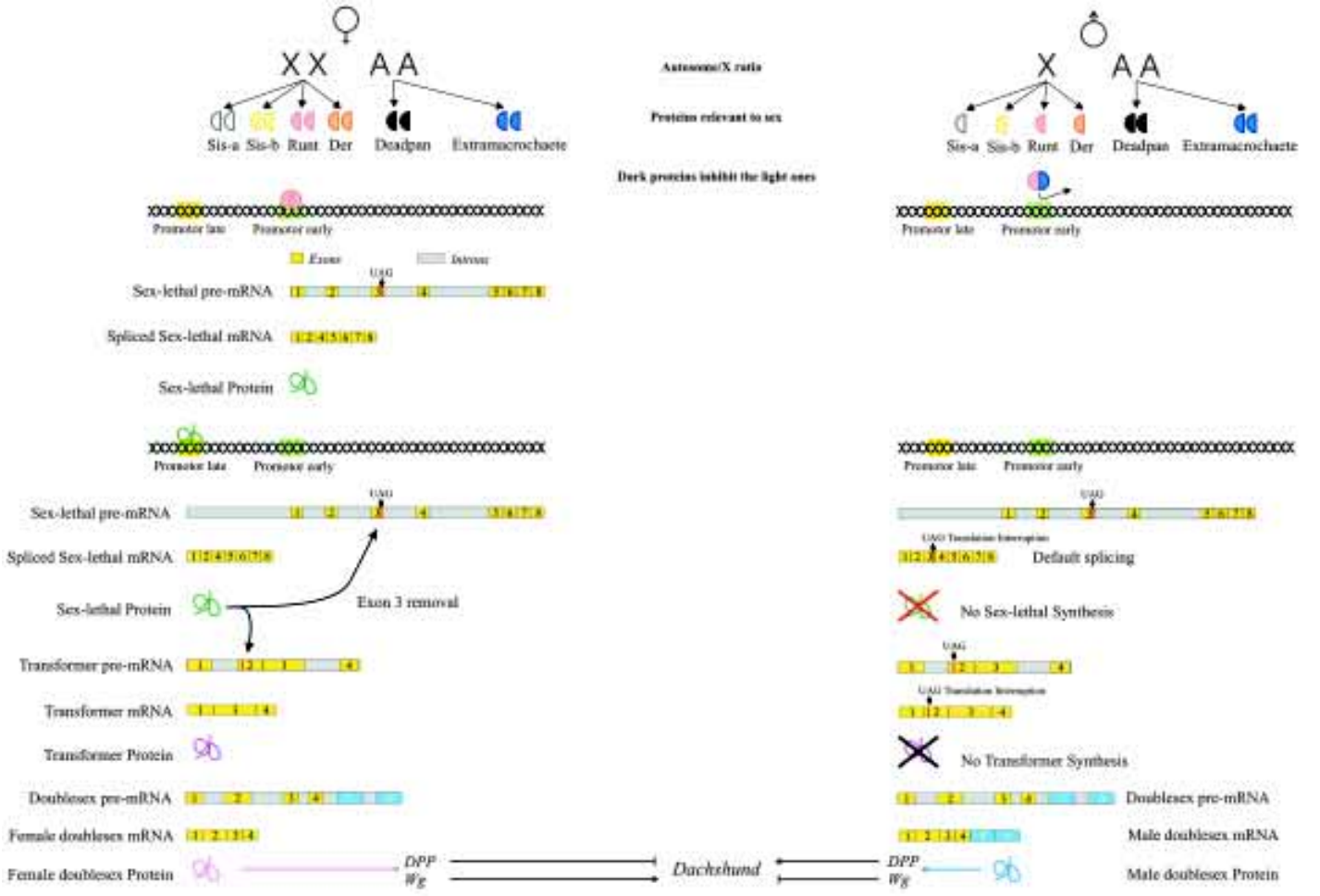


Fig. 7. Sex determination in *Drosophila*. *The chromosomes of each sex are shown in the first line: female to the left and male to the right. The second line represents transcription factors synthesized by each sex. The dark-colored factors inhibit the light ones. This is relevant to the activation of the early promotor of the gene* sex lethal, *which, as schematically indicated in the third line, is activated only in females. The fourth line represents* sex-lethal *pre-mRNA with introns and exons in different colors. In the fifth line the spliced* sex lethal *mRNA is shown, and in the next line the sex-lethal protein, synthesized only in females. The seventh line shows again* sex-lethal *DNA, and the fact that the sex-lethal protein binds the late promotor and activates transcription starting from that. On the right, it is shown, however, that the late promoter is activated also in the male, through a less-well-understood mechanism. The eighth line shows the* sex-lethal *pre-mRNA in both sexes. The ninth line shows the crucial difference: in the female, due to the action of the sex-lethal protein, exon 3 is removed, whereas in the male it is not, and because it contains a stop codon, again the sex-lethal protein is not synthesized in the male, as shown in the following line. The presence of sex-lethal protein also has an effect on the splicing of another sex relevant gene, i.e.,* Transformer. *Here again, as shown in lines 11, 12 and 13, because of the action of the sex-lethal protein, the pre-mRNA is differently spliced in the two sexes, so in the female the final product does not contain exon 3, whereas in the male it does. Since it contains a stop codon in the male, the transformer protein again is not synthesized. In the following two lines, the fate of the pre-mRNA of a third gene relevant to sex, i.e.,* doublesex, *is shown. Here again, there is different splicing in the two sexes, and the final messengers contain a different number of exons: from 1 to 4 in the female and from 1 to 6 in the male. Two different Doublesex proteins are therefore synthesized. This, as illustrated in the last two lines, seems to have an effect on development, since it has been shown that in the male the gene* dachshund *is inhibited by Wg and stimulated by Dpp, whereas the reverse is true in the female, where* dachshund *is stimulated by Wg and inhibited by Dpp.*

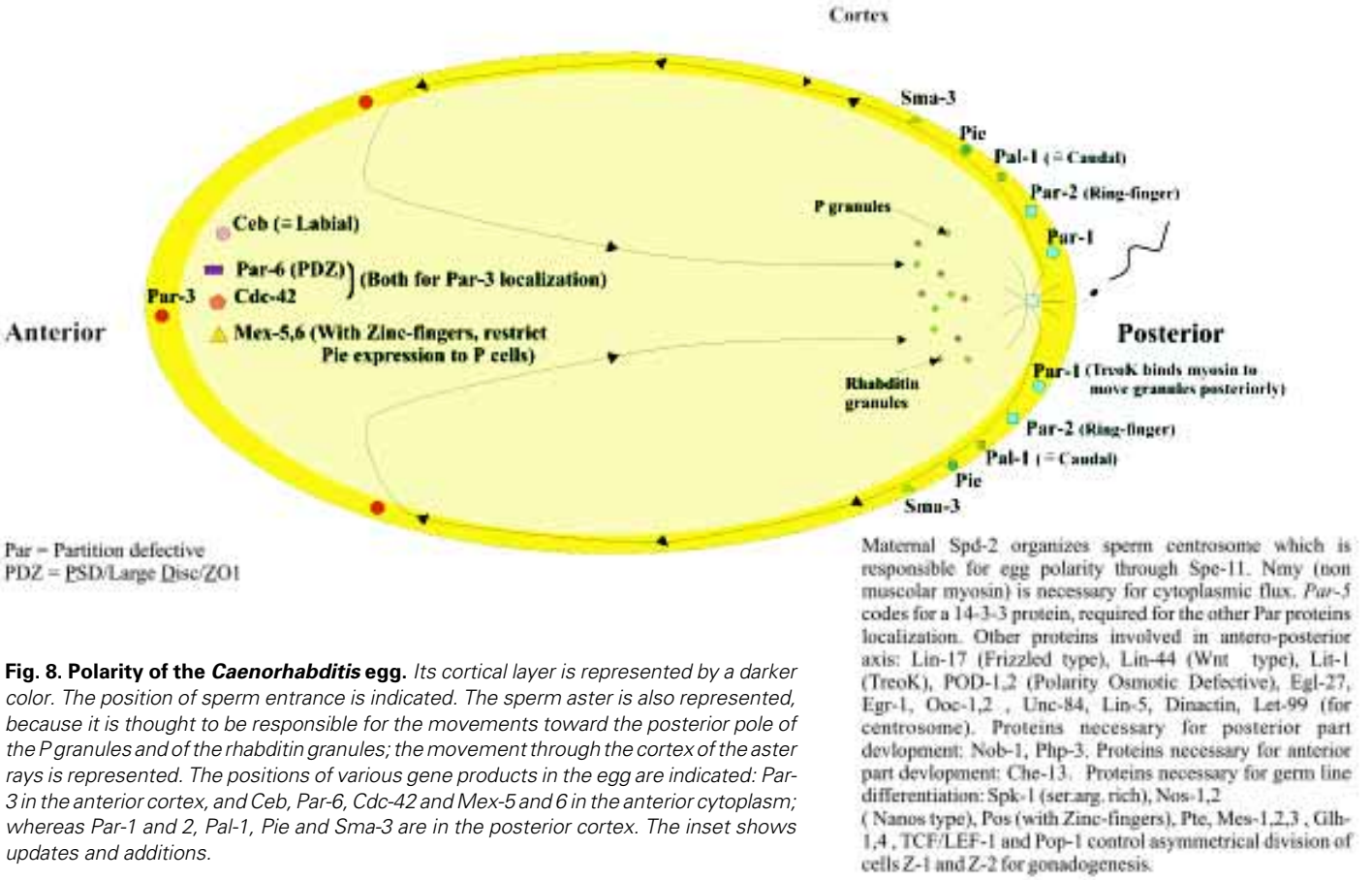


Fig. 8. Polarity of the *Caenorhabditis* egg. *Its cortical layer is represented by a darker color. The position of sperm entrance is indicated. The sperm aster is also represented, because it is thought to be responsible for the movements toward the posterior pole of the P granules and of the rhabditin granules; the movement through the cortex of the aster rays is represented. The positions of various gene products in the egg are indicated: Par-3 in the anterior cortex, and Ceb, Par-6, Cdc-42 and Mex-5 and 6 in the anterior cytoplasm; whereas Par-1 and 2, Pal-1, Pie and Sma-3 are in the posterior cortex. The inset shows updates and additions.*

Organogenesis

The development of two organs which have been studied in detail is then described. One is the *Drosophila* eye and the second is the *Caenorhabditis* vulva. For the first (see reviews by Dominguez and Hafen, 1996, and Frankfort and Mardon, 2002), after a morpholgical description and enumeration of most of the genes involved, the scheme reported in Fig. 4 is discussed. It deals with the differentiation of the R7 photoreceptor. Here, the importance of signal transduction is stressed, showing one of the first complete examples of signal transduction starting with a ligand, Boss on the R8 cell, which activates a tyrosine kinase receptor Sevenless, on the surface of the R7 cell. Then, through the general adaptor Drk the protein Sos (son of sevenless) is activated, which transmits the signal to the monomeric G protein Ras, which in turn activates Raf, thereby initiating the MAP kinase pathway, which at the end activates the gene *sina*. The roles of other genes and gene products involved in this network, for example, Jun, Fos, Pokkury, Tramtrack and others are also mentioned in the addendum (inset of Fig. 4). At this point, general schemes are presented which seek molecular explanations of morphological phenomena such as furrow progression and ommatide orientation. An attempt to provide a gene hierarchy in eye formation is given by discussing the experiments of ectopic eye formation of Gehring (Halder *et al.*, 1995) and the new theories about eye evolution presented by Gehring (2002).

As for vulva differentiation in *Caenorhabitis*, the concept of equivalence of cell groups is introduced. After explanations of the different cell destinies in vulva formation, an example of cellular interaction with signal transduction very much reminiscent of R7 differentiation in the *Drosophila* eye is presented, as shown in Fig. 5. Here the signal starts from the anchor cell by a ligand (called Lin 3) and is tranferred to a tyrosine kinase receptor on the surface of the P6 cell (Let 23), through a general adaptor (here called Sem5) to an equivalent of Sos (Let 341), then to a Ras GTPase (Let 60), and on to a Raf (Lin 45), thereby initiating a MAP kinase cascade terminating with the regulation of the transcription factors Lin 1 and Lin 31. A complex network of activators and inhibitors of this pathway is shown to the students by clicking on the pertinent choice in the CD (Fig. 5 inset). The importance of the so-called lateral inhibition of cell P6 on cells P5 and P7 is discussed, and at this time the concept of Notch signaling is introduced.

Another selected example of organ differentiation is the *Drosophila* wing, where a complex set of genes is at work. Unfortunately, however, a precise regulatory circuit has not yet been worked out (see Fig. 6). Here, genes which are expressed in the anterior and posterior parts and in the dorsal and ventral parts of the wing imaginal disc are shown using different color gradations for the different territories. The importance of the genes expressed at borders between territories is stressed. The influence of such genes as *vestigial* and *wingless* and of the

Notch pathways on different target genes and the effects of different ligands in the dorsal and ventral sides are discussed.

Sex Determination

Another subject treated in detail is sex determination. After a general introduction, the two examples reviewed in detail are those of *Drosophila* and *Caenorhabditis*. The first is shown in Fig. 7. Here, the first row shows the general concept of sex chromosome/autosome ratio for sex determination and which genes account for that. In the following rows, the alternative splicings of the transcripts of the genes *sex lethal*, *transformer* and *doublesex* are indicated, along with their possible terminal consequences.

The concept of germ plasm and germ-cell differentiation is described for *Drosophila*, *Caenhorhabditis* and amphibians. The example of *Caenorhabditis* is described in Fig. 8, where how cell polarity is already present in the egg is shown first.

Molecular mechanisms by which some genes are made active only in the P-cell line, which will give rise to germ cells, are explained in the lectures.

Cellular Interactions

The subject of cellular interactions is treated starting with the classical experiments that I performed on dissociation and reaggregation of sea urchin cells (Giudice, 1962), which represented the first example of reconstitution of entire embryos from dissociated cells (I can't resist a showing a little pride!). Then, I move through some historical experiments on the subject, and then on to more modern questions of cellular interactions in sea urchins, such as those raised by the groups of Davidson (Oliveri *et al.,* 2002), and of McClay (Sherwood and McClay, 2001). Next, cellular interactions in *Caenorhabditis* are reviewed, in order to show some recurring molecular mechanisms for signaling cell differentiation through interaction, such as the already described Wnt pathway for gut differentiation in this embryo (see Maduro and Rothman, 2002, for a recent review). This is found in Fig. 9, where it is shown how one can find an equivalent of the Wnt pathway in the signaling of the *mom* (more mesenchyme) genes. In fact, a Wnt molecule leaving the surface of the P2 cell stimulates a receptor of the Frizzled type (called Mom5) on the surface of the EMS cell, which inhibits a kinase of the Shaggy type, thereby inhibiting phosphorylation of a β-catenin type molecule, here called Wormadillo (the armadillo of the worm), thereby permitting its transfer to the nucleus.

The example of *Dyctyostelium discoideum* is then introduced as a simple model of differentiation and for studies of cellular interactions. Especially interesting are the molecular mechanisms underlying the important decision of whether to become a spore cell and survive or to become a stalk cell and die for the sake of the community.

In connection with cellular interactions, the problem of nerve cone growth and pathfinding is also discussed, by illustrating the roles of key molecules such as Ephrins, Robo, Slit and others (see Patel and Van Vactor, 2002, and Cooper, 2002, for reviews).

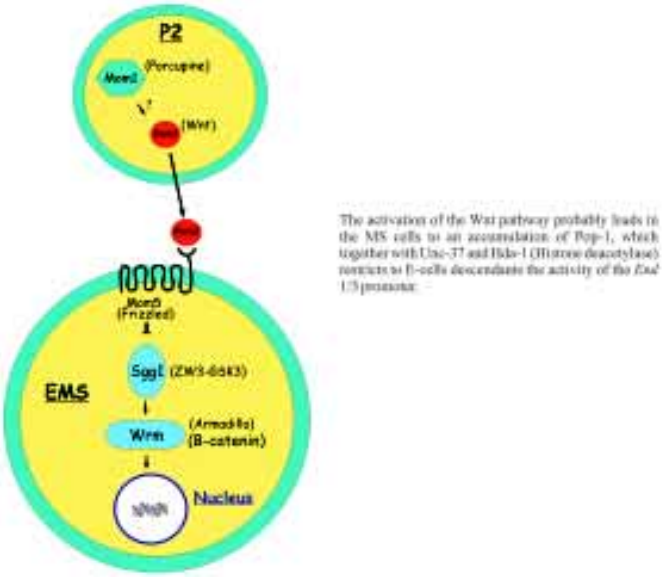


Fig. 9. Interactions between cells P2 and EMS in *Caenorhabditis elegans* and use of the Wnt pathway. *The P2 cell is shown in the upper part. A protein, called Mom1 (equivalent to* Drosophila *Porcupine), probably causes export of a molecule called Mom2 (equivalent to Wnt) which goes to stimulate a receptor called Mom5 (equivalent to frizzled), which inhibits Sgg1 (shaggy-1, equivalent to ZW3 of* Drosophila *and to Gsk3 of vertebrates). This causes the lack of phosphorylation of Wrm (wormadillo, i.e., armadillo of the worm, equivalent to β-catenin of vertebrates) thus permitting its tranfer to the nucleus. The inset reports updating and additions.*

Additional Topics

Another question related to embryonic axes which has been dealt with in my course is that of limb development and growth in vertebrates (see Capdevila and Izpisua Belmonte, 2001, for a review). The subject is begun with a review of somite cell migration and signals starting from the neural tube and notochord (see Fig. 10A). Then, I go on to explain the gene networks operating in antero-posterior and proximo-distal growth and differentiation (see Fig. 10B). Finally, I illustrate the action of genes for dorso-ventral differentiation and interdigital apoptosis (see Fig. 10C). A special scheme, not shown here, is dedicated to the factors involved in muscle differentiation. As for the subject of apoptosis, this is introduced when discussing *Caenorhabitis*, for historical reasons.

What is stressed in Fig. 10A is the fact that again we find an opposition to the effects of proteins such as Noggin, Chordin, Gremlin and Follistatin from the dorsal side of the neural tube, and to those of Bmp4 from its ventral side. Also, here again a Wnt pathway is involved, starting from the neural tube, going through Sonic hedgehog and Noggin from the medial side and inhibiting Bmp4 on the lateral side. Other relevant issues reviewed include oscillations in the activity of the genes *hairy* and *lunatic fringe* (for somitic segmentation). More complex is the situation shown in Fig. 10B, where the ZPA (zone of polarizing activity) is schematically indicated below on the left side and the AER (apical ectodermal ridge) on the right. The possible cascade effects of endogenous retinoic acid on *shh, BMP-2,-4,-7, gli* and *patched* are shown. Stimuli coming from the AER, essentially consisting of FGF-1-2-4,-7,-8 and -10, and going to stimulate proliferation of the underlying mesenchyme progress zone, and genes such as *Msx-1* and *-2* are indicated. Also, the posterior-anterior gradient of the products of genes such as *slug* (proximally), and *Cek8* (distally) is indicated, among other things in Fig. 10B. Fig. 10C shows, on the left side, the genes at work in the dorsal face of the

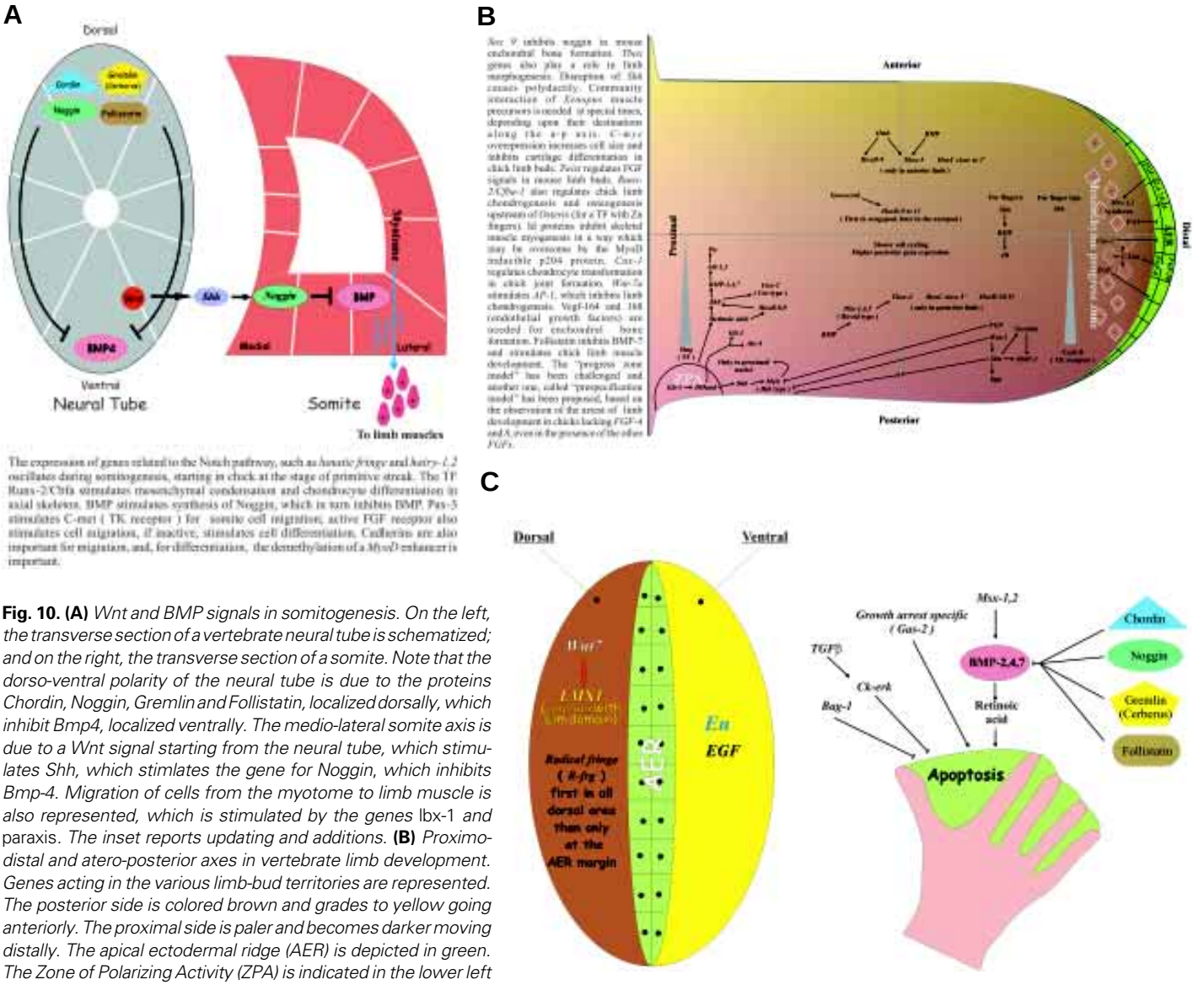


Fig. 10. (A) *Wnt and BMP signals in somitogenesis. On the left, the transverse section of a vertebrate neural tube is schematized; and on the right, the transverse section of a somite. Note that the dorso-ventral polarity of the neural tube is due to the proteins Chordin, Noggin, Gremlin and Follistatin, localized dorsally, which inhibit Bmp4, localized ventrally. The medio-lateral somite axis is due to a Wnt signal starting from the neural tube, which stimulates Shh, which stimlates the gene for Noggin, which inhibits Bmp-4. Migration of cells from the myotome to limb muscle is also represented, which is stimulated by the genes* lbx-1 *and* paraxis. *The inset reports updating and additions.* **(B)** *Proximo-distal and atero-posterior axes in vertebrate limb development. Genes acting in the various limb-bud territories are represented. The posterior side is colored brown and grades to yellow going anteriorly. The proximal side is paler and becomes darker moving distally. The apical ectodermal ridge (AER) is depicted in green. The Zone of Polarizing Activity (ZPA) is indicated in the lower left corner. From this, a retinoic acid stimulus departs, which stimulates in the order* shh, BMP-2, 4, 7, gli-1,3 *and* ptc. Shh *also stimulates* cux-2, *and retinoic acid stimulates* hoxb-8 *and* -9, *which in turn stimulate* shh. *A posterior-anterior gradient of the transcription factor Slug (indicated by a triangle) is present in the proximal side. On the posterior and proximal side,* gli-3 *stimulates* dhand, *which stimulates Shh to move Meis to nuclei, only on the proximal side. Inhibitory stimuli to the* meis *gene leave the* FGF *and* wnt *genes acting on the distal side; there, positive stimuli go from* shh *to* dpp, *Gremlin and BMP-2, while Gremlin inhibits BMP-2.* Meis *acting proximally and* shh *acting distally send inhibitory stimuli to each other. On the extreme right, i.e., distally, a series of stimuli, represented by the* FGF-1,-2,-4,-7,-8 *and* -10 *products, go to the mesenchymal progress zone, and more precisely to* Msx-1,-2*; while stimuli represented by* EGF *products return from the latter to AER. Inhibitory stimuli from* P-63 *and* Cux-1 *go also from the mesenchyme progress zone to AER. Stimuli from AER go from* FGF-10 *to* FGF-8 *and from this to* C-Lim *on the mesenchyme cell membrane. The presence of Syndecan in the mesnchyme progress zone is also reported, together with a posterior-anterior gradient of the Check-8 receptor in the distal part (again indicated by a triangle). In the center of the figure (not to indicate a location there) other information is provided: i.e., that* omb *stimulates* hoxD-9 *only in the anterior limb and, together with BMP, stimulates* Tbox-5, *and that, only in the anterior limb, the* hoxC *genes close to the 3' end of DNA are expressed; whereas a network represented in the order by* BMP *and* pitx *stimulates* Tbox-4 *only in the posterior limb; that only there, the* hoxC *genes close to the 5' end of DNA are expressed, as are the* hoxD *genes 10 and 11.* HoxD *from 9 to 13 are expressed at first in the zeugopod, and later in the autopod. Genes necessary for finger development are in the order* shh, BMP *and* IB*; while* ihh *(indian hedgeheog) is expressed only at the fingertips. Inset reports updating and additions.* **(C) Left:** *Vertebrate limb bud: dorso-ventral polarity.* **(C) Right:** *Programmed cell death of the interdigital cell membrane in terrestrial vertebrates. The left side indicates the genes expressed on the dorsal side and and on the ventral side of a vertebrate limb; the right side indicates the genes expressed for apoptosis of the interdigital membrane in terrestrial vertebrates. The* Wnt *gene is expressed on the dorsal side, where it stimulates* LMX-1. *The gene* Radical Fringe *is at first expressed in all the dorsal side, and then becomes restricted to the zone adjacent to the AER. The genes* Engrailed *and for the epidermal growth factor are expressed on the ventral side. A network consisting of the genes* Msx-1,-2, BMP-2,-4 *and* -7, *in that order, stimulates apoptosis through retinoic acid, while the proteins chordin, gremlin, noggin and follistatin inhibit the BMPs. Another stimulator of apoptosis is* gas-2*; while inhibitory stimuli are sent by TGFβ through* Ck-erk *and by* Bag-1.

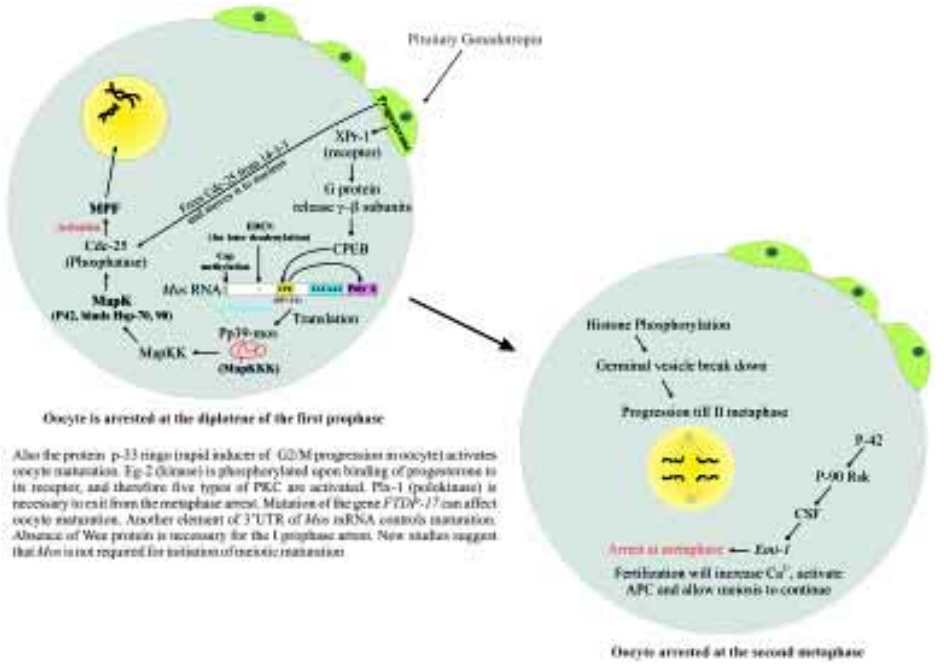


Fig. 11. *Xenopus* oocyte maturation. *The upper left drawing represents a* Xenopus *oocyte, surrounded by some follicular cells. The nucleus is depicted with two of its chromosomes, indicating that the oocyte is arrested at the diplotene of prophase I. At the moment of maturation, pituitary gonadotropins are secreted, which stimulate progesterone secretion by the follicular cells. This binds to a progestrone receptor (Xpr-1), which causes a G protein to release its βγ subunits. This stimulates binding of a Cpeb (cytoplasmic polyadenylation element binding protein) to the CPE (made by 6 U and 1 A) of the mRNA of the* Mos *RNA, till that moment inactive in the oocyte cytoplasm. This causes* Mos *mRNA polyadenylation and activation, with consequent synthesis of the pp39 protein. Another mechanism of activation of the* Mos *mRNA is methylation of its cap. Also the sequence necessary for polyadenylation (AAUAAA) is depicted on the* Mos *mRNA, and the sequence called EDEN, necessary for future de-adenylation is indicated. The pp39 synthesis starts a MAP kinase cascade (pp39 is itself a MAP kinase) culminating with activation of Cdc-25 phosphatase and consequent MPF (maturation promoting factor) dephosphorylation and activation. Progesterone is shown to free Cdc-25 from the protein 14-3-3, thus allowing Cdc-25 to enter the nucleus; while MPF stabilizes pp39. The described activation of MPF causes the events depicted in the oocyte at the lower right part of the figure, i.e, histone phosphorylation, germinal vesicle breakdown and progression until metaphase II, where the oocyte again arrests, because of the chain of activations illustrated there. That is, p-42 activates p-90 rsk, which activates a cytostatic factor (CSF), which through the action of the* Emi-1 *gene blocks the oocyte at that stage. Fertilization will then cause an increase of* Ca^{2+}*, activation of the anaphase promoting complex (APC) and completion of meiosis.*

limb and those at work in the ventral face. The same figure on the right side indicates the role of genes for Noggin, Chordin,Gremlin and Follistatin in contrasting the effect of Bmp-4, which stimulates apoptosis in the interdigital spaces of terrestrial vertebrates.

Regeneration is another subject treated in the course, starting with *Hydra* as an example. All the genes and factors which are known to be involved are reviewed. Then, the case of the urodele limb, where the classical experiments of Singer (Singer and Caston, 1972) on the role of the nervous system are recalled. Next, liver regeneration in rats, planarian regeneration, and finally the latest on central nervous system (CNS) regeneration in adult mammals is reviewed. For mammalian CNS regeneration, the negative role of myelin and the identification of relevant amino acids in the Nogo protein (GrandPrè *et al.*, 2002) are discussed.

As already stated, fertilization and cell division are dealt with only in the 5-year course. As for fertilization, the invertebrate example is treated first, starting with sea urchins. Sperm activation, the acrosome reaction, identification of molecules such as bindin, sperm cell receptor, jelly receptor, and egg receptor for sperm, and the cortical reaction are reviewed. Theories about Ca^{2+} liberation are discussed next. Then, I pass on to examples of vertebrates and return to sperm activation and capacitation, to Ca^{2+} movement, to the role of ZP3 and to identification of receptors. I finally discuss the physiological changes that accompany fertilization in some species.

As for cell division, special attention is given to the checkpoints and to meiosis and oocyte maturation. Two cases are described in particular, that of the *Xenopus* oocyte and that of *Asterias*. Fig. 11 describes the general scheme of the former, where the oocyte is arrested at the diplotene of the first meiotic prophase (shown on the upper left) and progesterone, which stimulates polyadenylation and consequent translation of the *mos* RNA. This starts the MAP kinase cascade and, consequently, dephosphorylation and activation of MPF. This allows a meiotic progression until the second metaphase (shown on the lower right), where a second arrest occurs owing to the cytostatic factor, until fertilization increases free Ca^{++} and allows the anaphase-promoting complex to permit completion of meiosis.

Finally, two other problems are briefly treated: aging, especially in *Caenorhabditis* and *Drosophila*, and left-right asymmetry in vertebrates. The relevant molecules for both phenomena are described and the possible role of nodal ciliary rotation in asymmetry (see Mercola and Levin, 2001, for a review of the latter) is mentioned.

Plant developmental biology is not treated in this course but in another course, called Developmental Biology II, taught in Palermo by Prof. Ida Albanese.

Textbooks

The following textbooks are recommended for consultation: Gilbert (2000), Slack (2001), Wolpert (2002), and Raunich *et al.* (1998).

Examination Format

One final oral examination for the 5- and 3-year courses, plus a written test during the first half of the 3-year course are given.

Sample Examination Questions

How is dorsoventral polarity generated in the *Drosophila* embryo?

How is maturation of the *Xenopus* oocyte achieved?

Acknowledgments

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