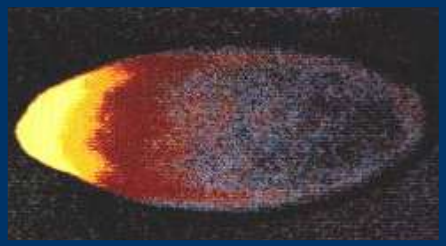
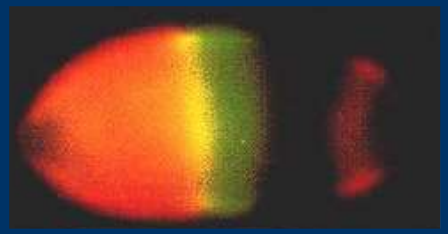
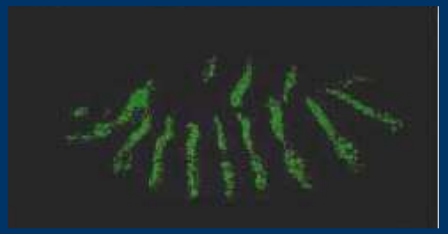





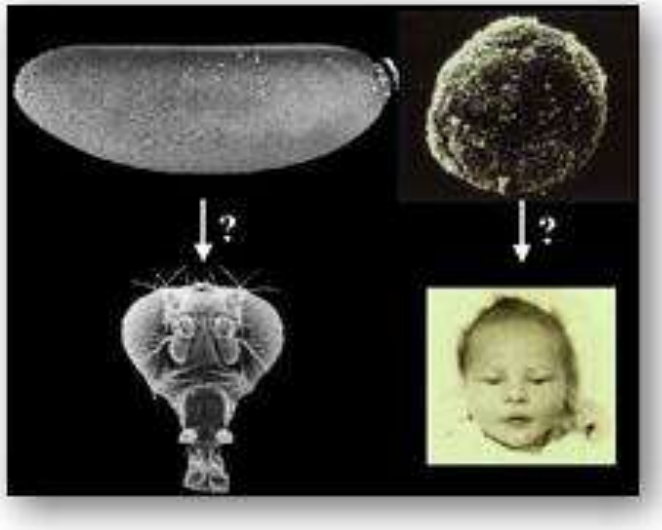
Regulatory mechanism of ontogenesis III.

by Krisztina H.-Minkó
Semmelweis University,
Dept. of Anatomy, Histology and Embryology
2018

How regulating genes work together in Drosophila?

„Drosophila and human development are homologous processes. They utilize closely related genes working in highly conserved regulatory networks. Unlike humans, Drosophila is subject to easy genetic manipulation. As a result, most of what we know about the molecular basis of animal development has come from studies of model systems such as Drosophila."

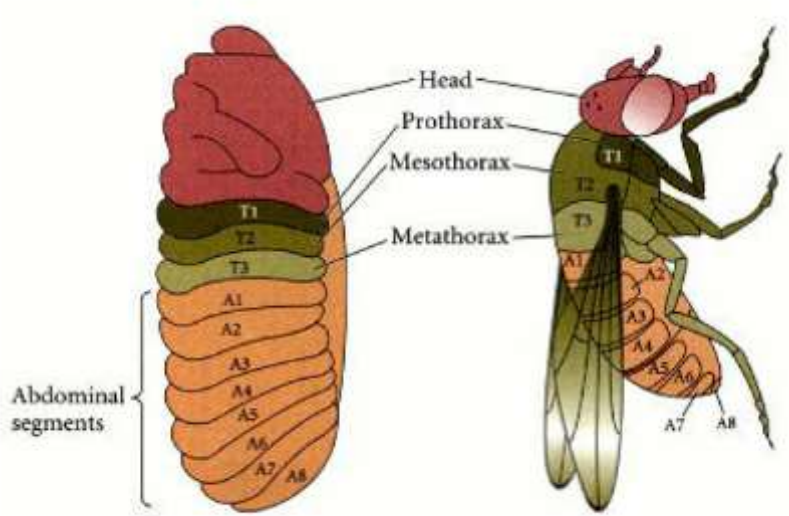
Albert J. Courey, professor of biochemistry at UCLA



One of the most important realizations has been the conservatism of the genes that guide development. Sequencing studies have shown remarkably few changes in the nucleotide bases of many developmentally regulated genes that are represented in species ranging from worms to *Drosophila* to humans. Because of this phylogenetic conservatism, it has been possible to identify mammalian counterparts of genes that are known from genetic studies to have important developmental functions in other species.* It is also clear that the same gene may function at different periods of development and in different organs. Such reuse greatly reduces the total number of molecules that are needed to „control" development. Before and after birth, specific genes may be expressed in normal and abnormal processes.

One of the principal themes in contemporary cancer research is the role of mutant forms of developmentally important genes (e.g., proto-oncogenes) in converting normal cells to tumor cells.

Similarities



Early mitotic divisions in a Drosophila embryo (close-up)
http://www.youtube.com/watch?v=r7SGcmgcqRc&feature=related

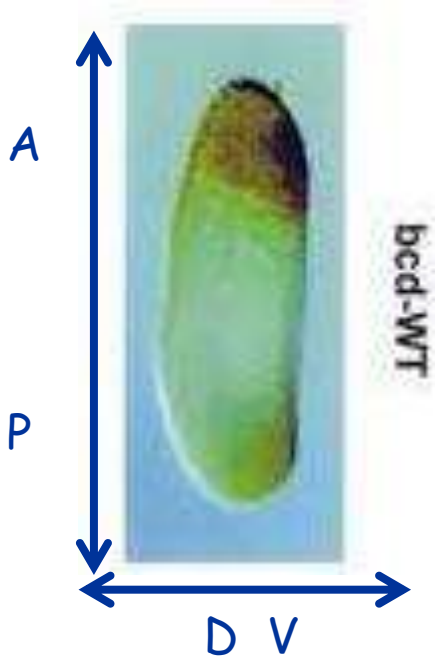
Early mitotic divisions and gastrulation of a Drosophila embryo
http://www.youtube.com/watch?NR=1&v=YMrlSRts5Ts&feature=endscreen

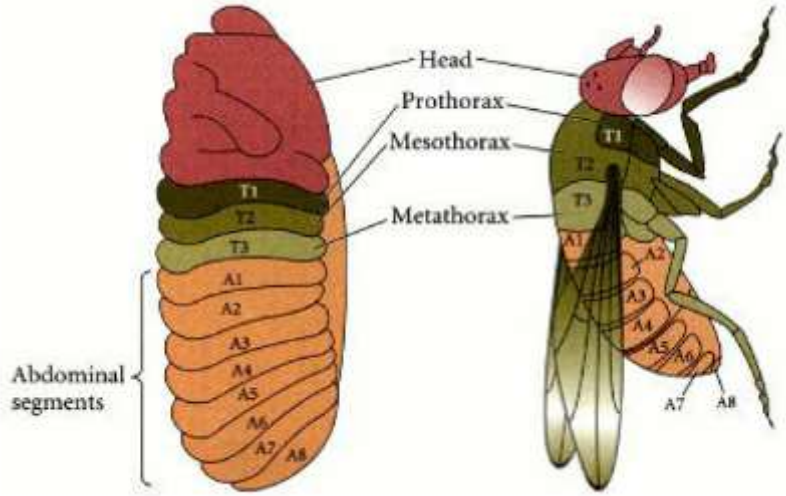
http://www.youtube.com/watch?v=RQtHE99Flv4

Syncytial divisions in the Drosophila embryo periphery
http://www.youtube.com/watch?v=YO-n5ZP5Lsk

Early Developmental Genetics in *Drosophila*

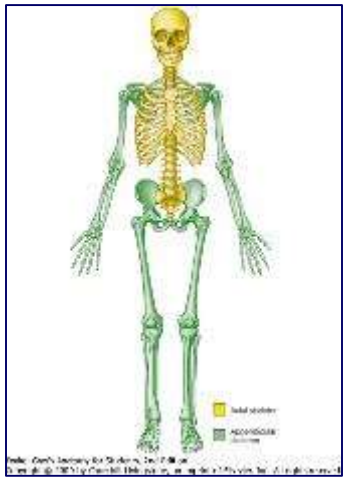
Despite the discovery and characterization of many developmentally important genes in mammals, the basic framework for understanding the molecular basis of embryonic development still rests largely on studies of developmental genetics in *Drosophila*. Although the earliest stages of human development occur under less rigid genetic control than those of *Drosophila* an exposure to the fundamental aspects of early *Drosophila* development nevertheless sets the stage for a deeper understanding of molecular embryogenesis in mammals.





Mechanisms and constraints shaping the evolution of body plan segmentation*

K.H.W.J. ten Tusscher*



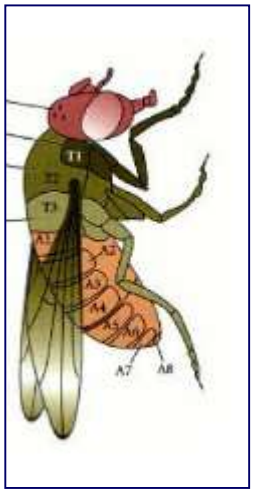
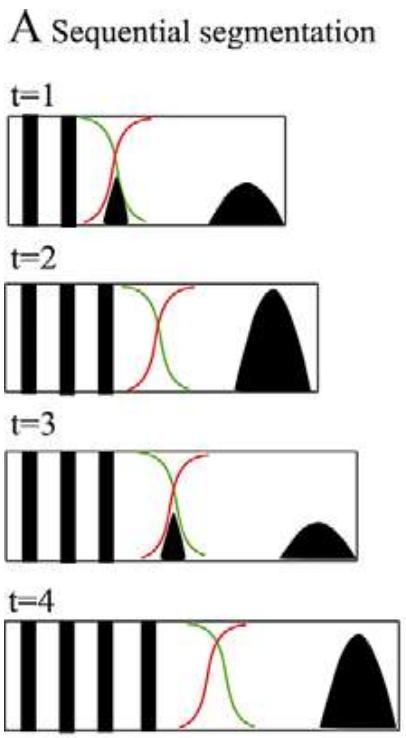
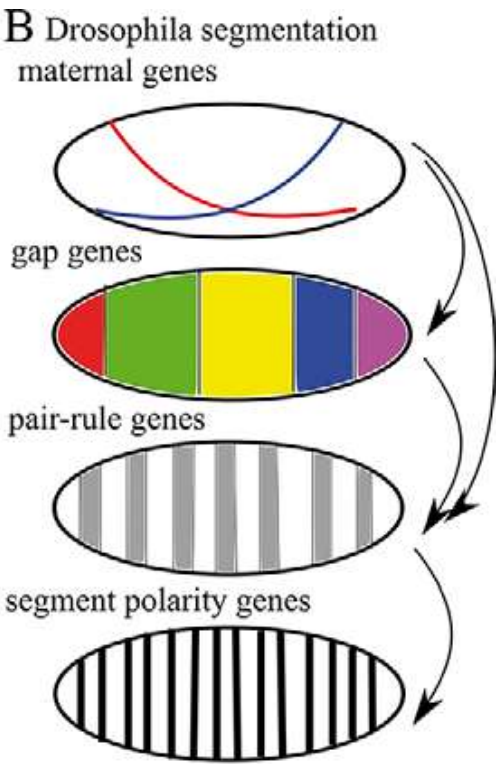


Fig. 2. Schematic depiction of sequential, clock driven (A) and simultaneous Drosophila-type (B) segmentation.

In A) aschematised overview of **clock-and-wavefront type segmentation** is shown. The increasing and decreasing black curve in the back represents the posterior growth zone oscillator, the intersection between the red and green curve represents the wavefront that determines where the transition from temporal oscillations into spatial stripes occurs. Over time, sequentially more segments are laid down, resulting in growth of the segmented domain.

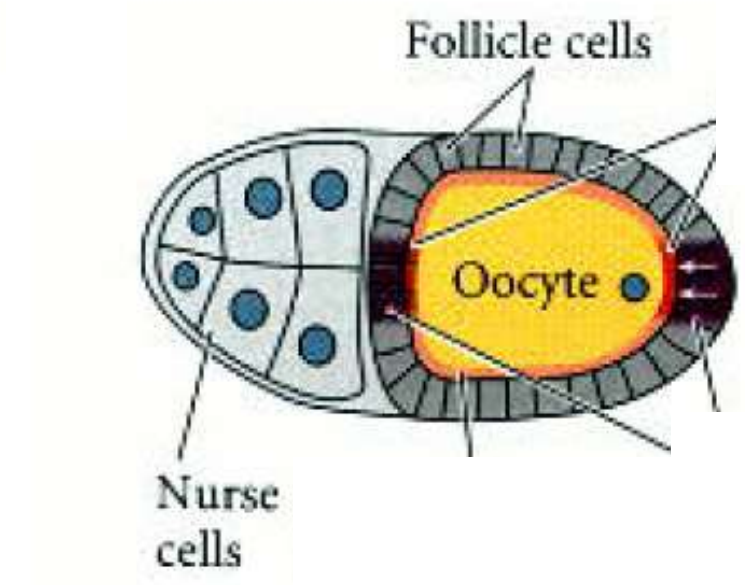
In B) a schematised overview of the Drosophila segmentation hierarchy is shown, depicting the information transfer from maternal, to gap, to pair rule to segment polarity genes that results in a subdivision of the axis into smaller and smaller domains.

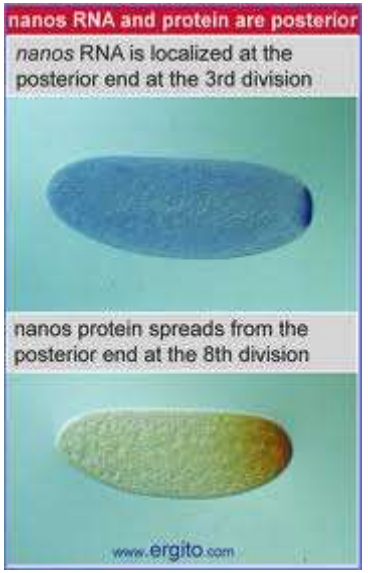


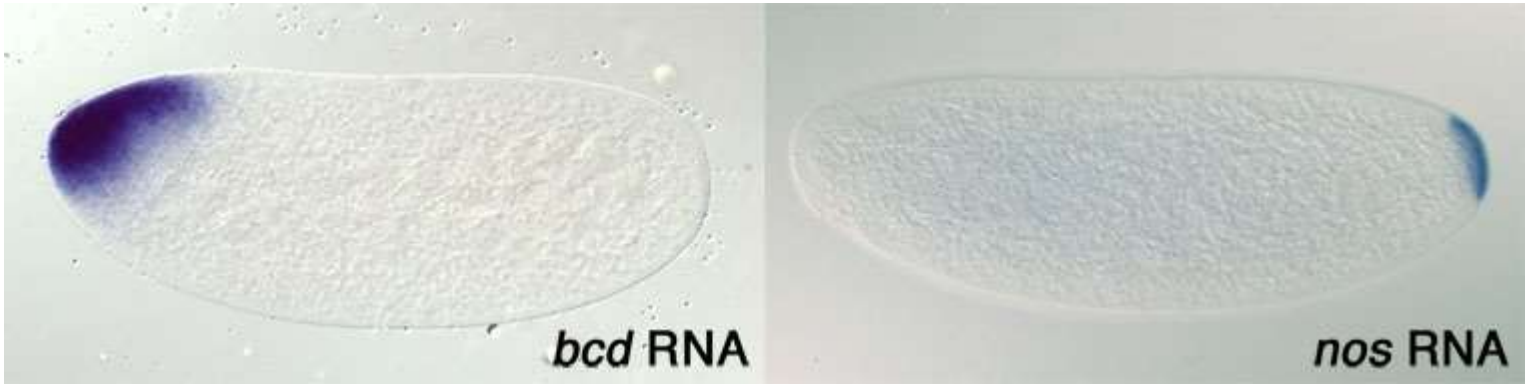


Eur. Phys. J. E (2013) **36**: 54
DOI 10.1140/epje/i2013-13054-7

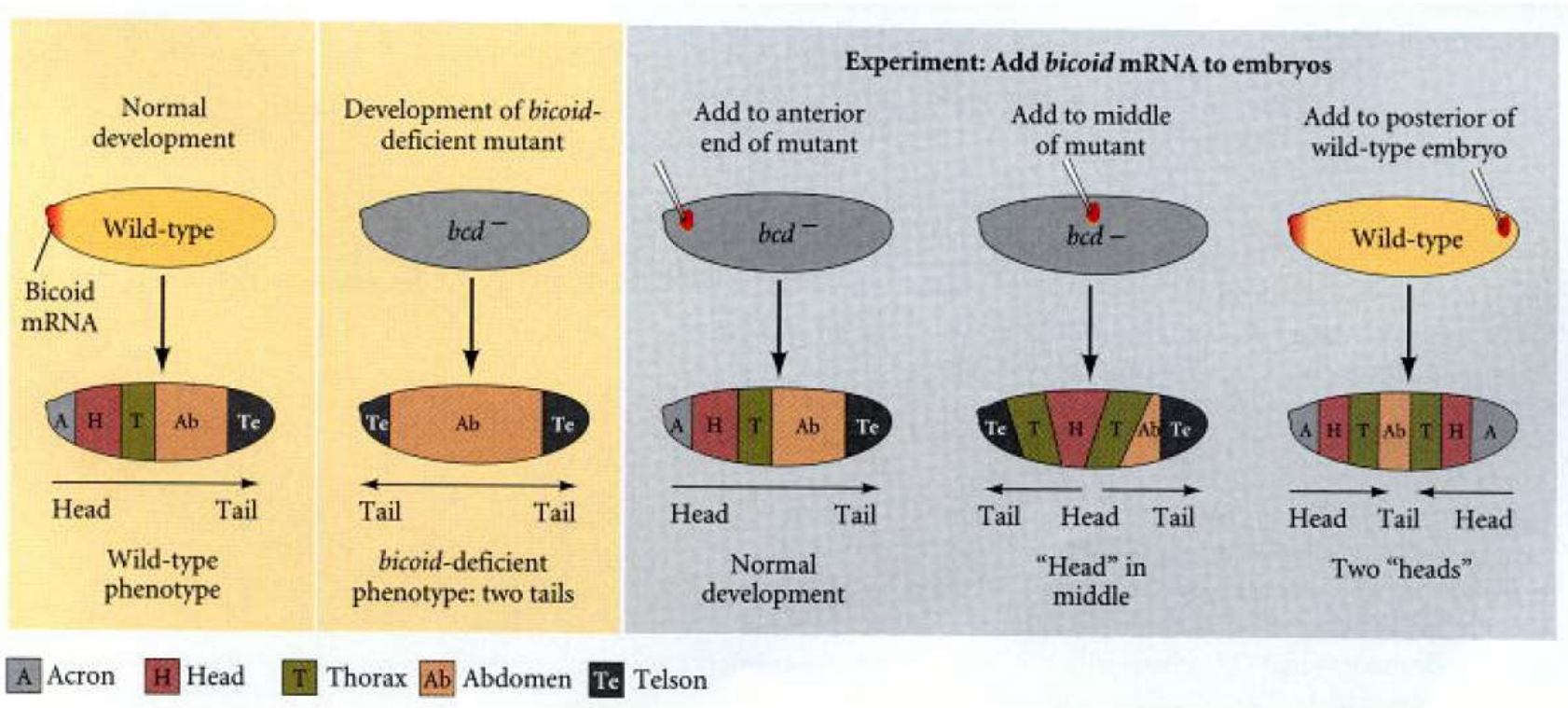
Establishment of morphogenetic gradients in the oocyte





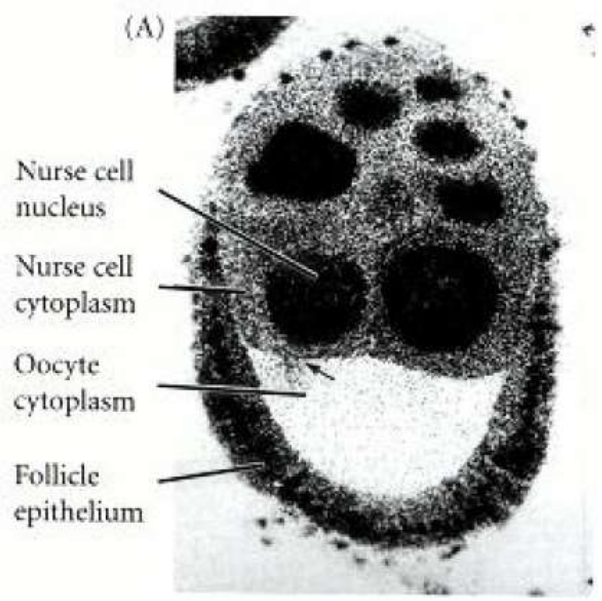


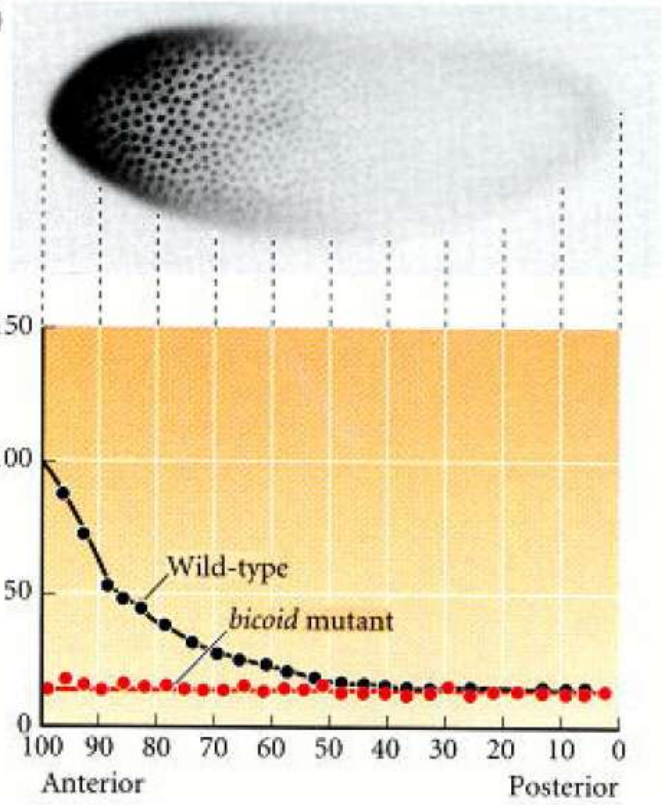
Experiments showing the importance of bicoid gene in the development of the head structures



Search for bicoid homolouges on PubMed Nucleotids:

1. member of the RIEG/PITX homeobox family, which is in the bicoid class of homeodomain proteins. Members of this family are involved in **organ development and left-right asymmetry**. This protein acts as a transcriptional regulator involved in **basal and hormone-regulated activity of prolactin.** [provided by RefSeq, Jul 2008].
2. member of the bicoid subfamily of the paired homeobox transcription factor family. The encoded protein is critical to the **maintenance and regionalization of the forebrain and midbrain** during development. It may also have important functions in **sense organ development, pituitary function, and in the regulation of blood cell production.** [provided by RefSeq, Jul 2008].





Further studies have strengthened the view that the product of the wild-type *bicoid* gene is the morphogen that controls anterior development. First, *bicoid* is a maternal effect gene.

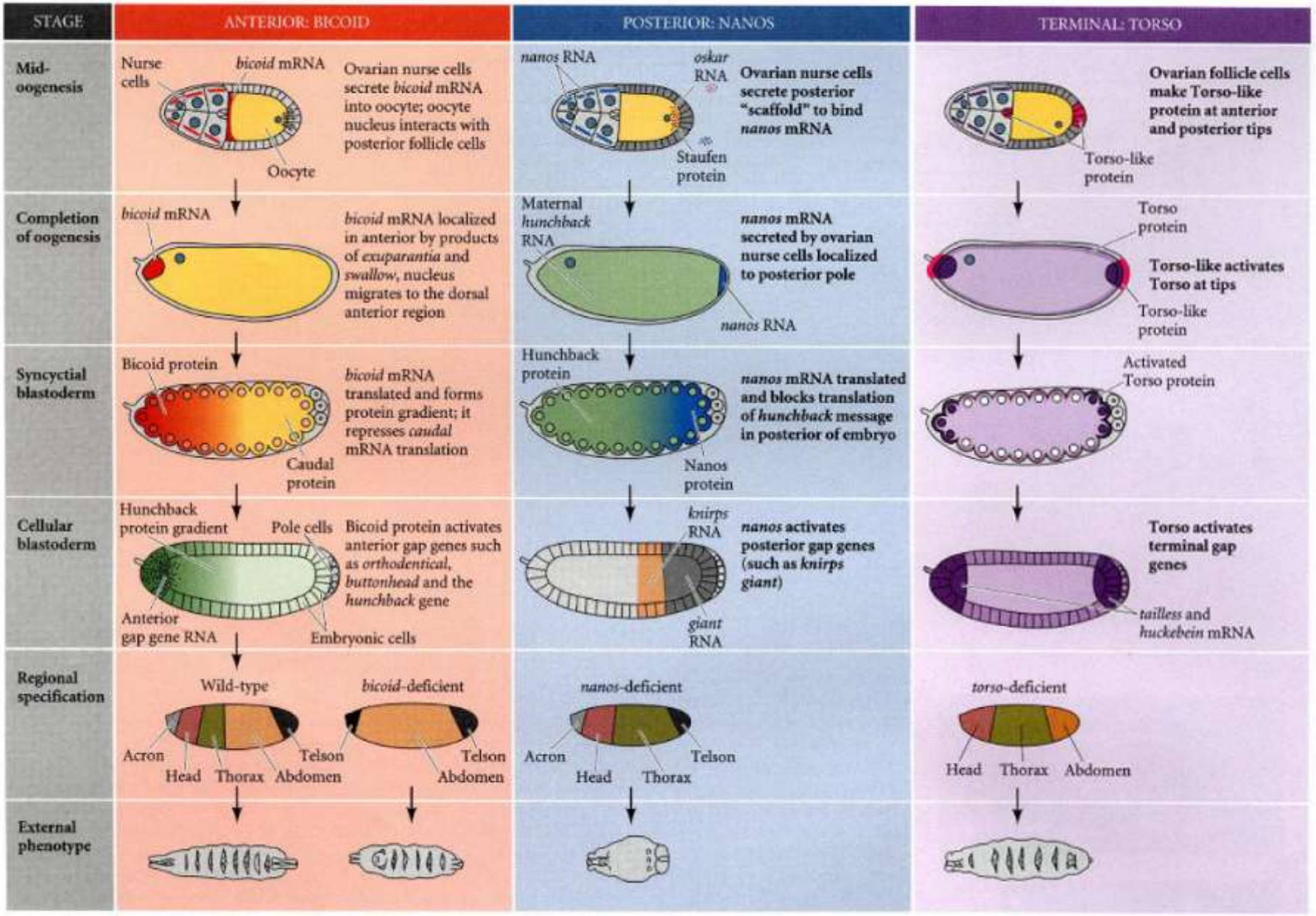
Messenger RNA from the mother's *bicoid* genes is placed in the embryo by the mother's ovarian cells (Figure 9.13; Frigerio et al. 1986; ABerleth et al. 1988).

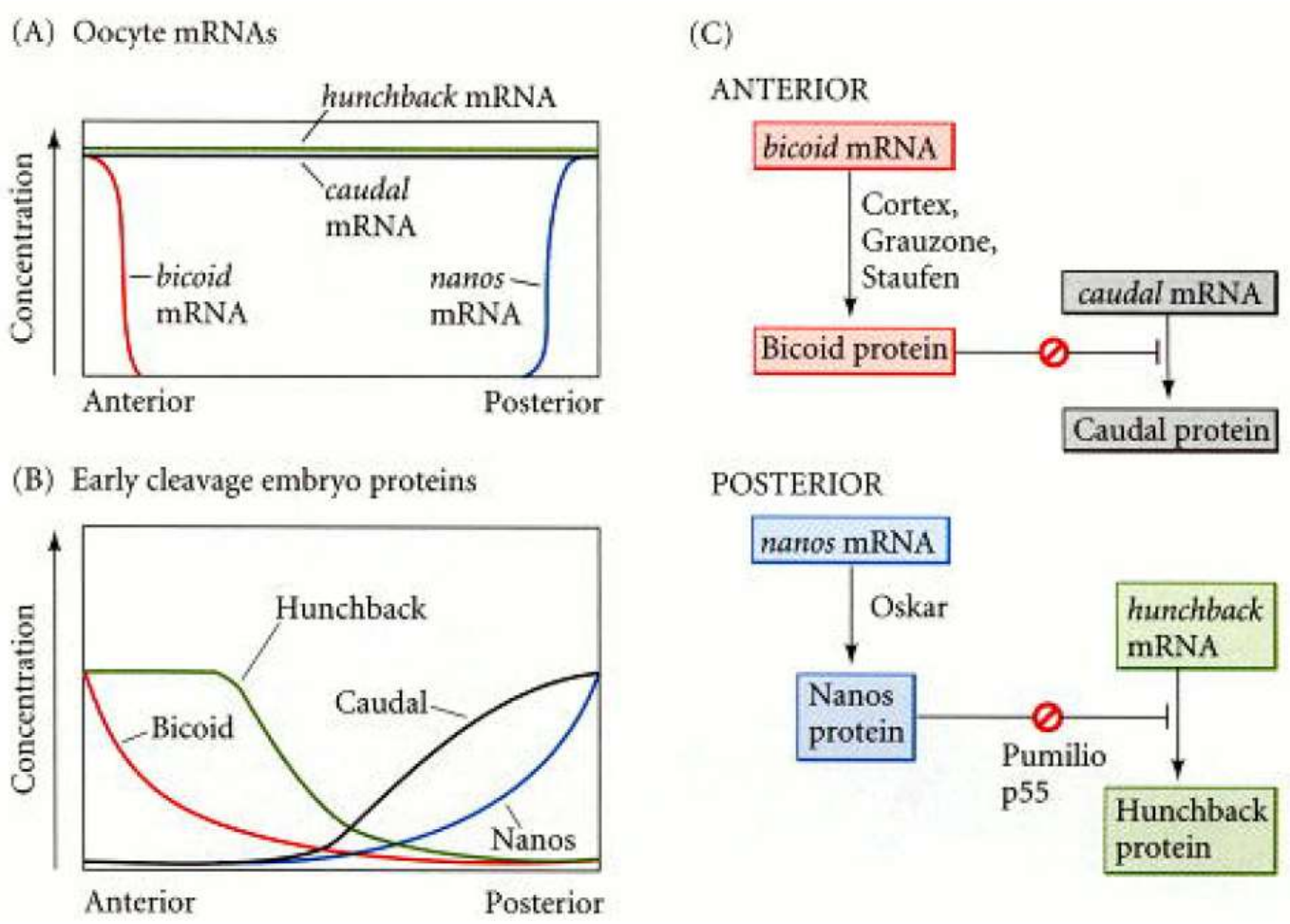
Table 9.1. Maternal effect genes that effect the anterior-posterior polarity of the *Drosophila* embryo

Gene	Mutant phenotype	Proposed function and structure
AnteriroGroup		
bicoid (*bcd*)	Head and thorax deleted, replaced by inverted telson	Graded anterior morphogen; contains homeodomain; represses caudal
exuperantia (*exu*)	Anterior head structures deleted	Anchors bicoid mRNA
swallow (*swa*)	Anterior head structures deleted	Anchors bicoid mRNP
PosteriroGroup		
nanos (*nos*)	No abdomen	Posterior morphogen; represses hunchback
tudor (*tud*)	No abdomen, no pole cells	Localization of Nanos
oskar (*osk*)	No abdomen, no pole cells	Localization of Nanos
vasa (*vas*)	No abdomen, no pole cells; oogenesis defective	Localization of Nanos
valois (*val*)	No abdomen, no pole cells; cellularization defective	Stabilization of the Nanos localization complex
pumilio (*pum*)	No abdomen	Helps Nanos protein bind hunchback message
caudal (*cad*)	No abdomen	Activates posterior terminal genes
TerminalGroup		
torso (*tor*)	No termini	Possible morphogen for termini
trunk (*trk*)	No termini	Transmits Torsolike signal to Torso
fs(1)*Nasrat*[*fs*(1)*N*]	No termini; collapsed eggs	Transmits Torsolike signal to Torso
fs(1)*polehole*[*fs*(1)*ph*]	No termini; collapsed eggs	Transmits *Torsolike* signal to *Torso*

cloning and functional analyses of nanos2 and nanos3; demonstrated an **evolutionarily conserved function of nanos proteins in germ cell development** [nanos2] [nanos3] Bioinfo for nanos sequence

The molecular model: protein gradients in the early embryo




(A) Oocyte mRNAs
hunchback mRNA
caudal mRNA
bicoid mRNA
nanos mRNA
Concentration
Anterior
Posterior
(B) Early cleavage embryo proteins
Hunchback
Caudal
Bicoid
Nanos
Concentration
Anterior
Posterior
(C)
ANTERIOR
bicoid mRNA
Cortex, Grauzone, Staufen
caudal mRNA
Bicoid protein
Caudal protein
POSTERIOR
nanos mRNA
Oskar
hunchback mRNA
Nanos protein
Pumilio p55
Hunchback protein

Embryonic development of *Drosophila* is under tight genetic control. In the earliest stages, the dorsoventral and anteroposterior axes of the embryo are established by the actions of batteries of **maternal-effect genes** (Fig. 4-1). When these broad parameters have been established, the oval-shaped embryo undergoes a series of three sequential steps that result in the segmentation of the entire embryo along its anteroposterior axis. The first step in segmentation, under the control of what are called **gap genes**, subdivides the embryo into broad regional domains. **Loss-of-function gap mutants result in loss of structure, or gaps**, in the body pattern several segments in width. In the second step, a group of **pair-rule genes** is involved in the formation of seven pairs of stripes along the craniocaudal axis of the embryo. The third level in the segmentation process is controlled by the **segment-polarity genes**, which work at the level of individual segments and are involved in their anteroposterior organization. *The segmentation process results in a regular set of subdivisions along the anteroposterior axis of the early *Drosophila* embryo, but none of the previously mentioned developmental controls imparts specific or regional characteristics to the newly formed segments. This function is relegated to two large clusters of **homeotic genes** found in the **antennapedia** complex and the **bithorax** complex. The specific genes in these two complexes determine the morphogenetic character of the body segments, such as segments bearing antennae, wings, or legs. Mutations of homeotic genes have long been known to produce bizarre malformations in insects, such as extra sets of wings or legs instead of antennae (hence the term *antennapedia*).

*In *Drosophila*, each stripe (segment) is subdivided into anterior and posterior halves. The posterior half of one segment and the anterior half of the next are collectively known as a **parasegment**.

Figure 4-1 Sequence of genetic control of early development in Drosophila. Within each level of genetic control are listed representative genes.

Genetic hierarchy	Functions	Representative genes	Effects of mutation
Maternal-effect genes 	Establish gradients from anterior and posterior poles of the egg	Bicoid Swallow Oskar Caudal Torso Trunk	Major disturbances in anteroposterior organization
Segmentation genes  *Gap genes*	Define broad regions in the egg	Empty spiracles Hunchback Kruppel Knirps Orthodenticle Tailless	Adjacent segments missing in a major region of the body
 *Pair-rule genes*	Define 7 segments	Hairy Even skipped Runt Fushi tarazu Odd paired Odd skipped Paired	Part of pattern deleted in every other segment
 *Segment-polarity genes*	Define 14 segments	Engrailed Gooseberry Hedgehog Patched Smoothened Wingless	Segments replaced by their mirror images
Homeotic genes 	Determine regional characteristics	Antennapedia complex Bithorax complex Hox in mammals!	Inappropriate structures form for a given segmental level

Maintenance genes: maintain state of homeotic genes

After fertilisation , maternal gene products laid down in the egg, such as *bicoid* mRNA, are translated. They provide positional information which activates the zygotic genes. The four main classes of zygotic genes acting along the antero-posterior axis are the gap genes, the pair-rule genes, the segmentation genes, and the selector, or homeotic genes.

The gap genes define regional differences that result in the expression of a periodic pattern of gene activity by the pair-rule genes, which define the parasegments and foreshadow segmentation. The segmentation genes elaborate the pattern in the segments, and segment identity is determined by the selector genes.

Table 9.2. Major genes affecting segmentation pattern in *Drosophila*

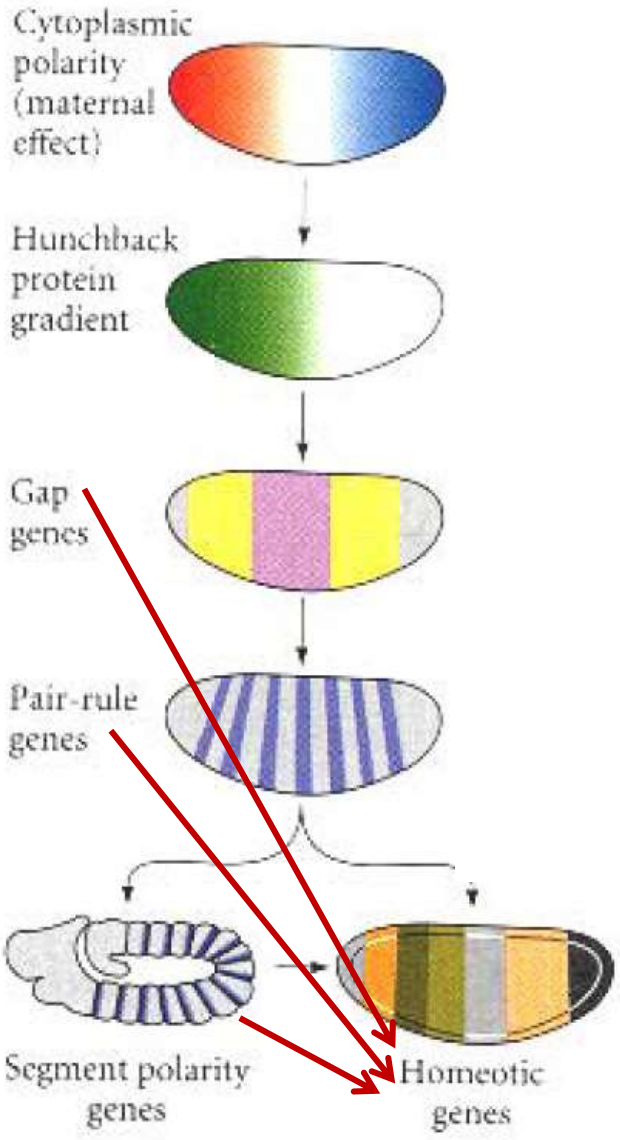
Category		Category	
Gap genes	*Krüppel (Kr)*	Pair-rule genes Secondary	*fushi tarazu (ftz)*
	knirps (kni)		*odd-paired (opa)*
	hunchback (hb)		*odd-skipped (slp)*
	giant (gt)		*sloppy-paired (slp)*
	tailless (tll)		*paired (prd)*
	huckendein (hkb)		
	buttonhead (btd)	Segment	*engrailed (en)*
	empty spiracles (ems)	polarity genes	*wingless (wg)*
	orthodenticle (otd)		*cubitus interruptus*^{D} (*ci*^{D})
			hedgehog (hh)
Pair-rule genes Primary	*hairy (h)*		*fused (fu)*
	even-skipped (eve)		*armadillo (arm)*
	runt (run)		*patched (ptc)*
			gooseberry (gsb)
			pangolin (pan)

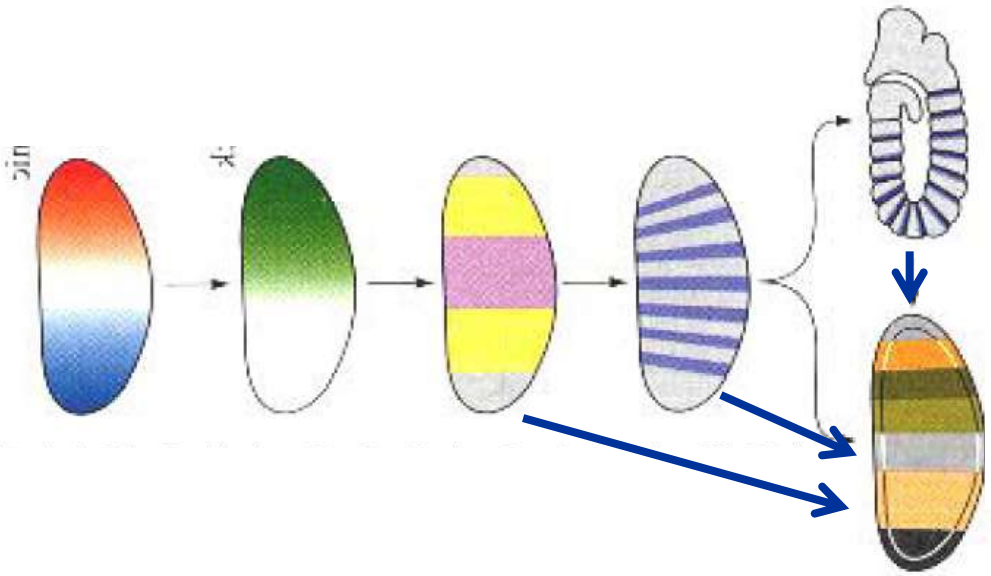
The Origins of Anterior-Posterior Polarity

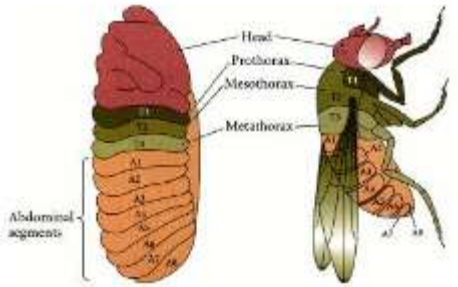
SUMMARY

The anterior-posterior polarity of the embryo, larva, and adult has its origin in the anterior-posterior polarity of the egg (Figure 9.8). The **maternal effect genes** expressed in the mother's ovaries produce messenger RNAs that are placed in different regions of the egg. These messages encode transcriptional and translational regulatory proteins that diffuse through the syncytial blastoderm and activate or repress the expression of certain zygotic genes. Two of these proteins, **Bicoid** and **Hunchback**, regulate the production of anterior structures, while another pair of maternally specified proteins, **Nanos** and **Caudal**, regulates the formation of the posterior parts of the embryo. Next, the zygotic genes regulated by these maternal factors are expressed in certain broad (about three segments wide), partially overlapping domains. These genes are called **gap genes** (because mutations in them cause gaps in the segmentation pattern), and they are among the first genes transcribed in the embryo.

Differing concentrations of the gap gene proteins cause the transcription of **pair-rule genes**, which divide the embryo into periodic units. The transcription of the different pair-rule genes results in a striped pattern of seven vertical bands perpendicular to the anterior-posterior axis. The pair-rule gene proteins activate the transcription of the **segment polarity genes**, whose mRNA and protein products divide the embryo into 14 segment-wide units, establishing the periodicity of the embryo. At the same time, the protein products of the gap, pair-rule, and segment polarity genes interact to regulate another class of genes, the homeotic selector genes, whose transcription determines the developmental fate of each segment.






Head
Prothorax
Mesothorax
Metathorax
Abdominal segments

Hox genes again...

The *Drosophila* **antennapedia-bithorax** complex consists of eight homeobox-containing genes located in two clusters on one chromosome. Mice and humans possess at least 39 homologous homeobox genes (called ***Hox***genes in vertebrates [***HOX*** in humans]), which are found in four clusters on four different chromosomes (Fig. 4-5). The *Hox* genes on the four mammalian chromosomes are arranged in 13 **paralogous groups**.

Vertebrate *Hox* genes play a prominent role in the craniocaudal segmentation of the body, and their spatio temporal expression proceeds according to some remarkably regular rules. The genes are activated and expressed according to a strict sequence in the 3' to 5' direction, corresponding to their positions on the chromosomes. Consequently, in *Drosophila* and mammals, 3' genes are expressed earlier and more anteriorly than 5' genes (Fig. 4-6). Mutations of *Hox* genes result in morphological transformations of the segmental structures in which a specific gene is normally expressed. Generally, **loss-of-function mutations** result in posterior-to-anterior transformations (e.g., cells of a given segment form the structural equivalent of the next most anterior segment), and **gain-of-function mutations** result in anterior-to-posterior structural transformations. Figure 4-7 illustrates an experiment in which injection of an antibody to a homeodomain protein into an early frog embryo resulted in the transformation of the anterior spinal cord into an expanded hindbrain. Although *Hox* genes were originally described to operate along the main body axis, sequential arrays of expression are found in developing organs or regions as diverse as the gut, the limbs, blood cells, and the internal and external genitalia. The expression of isolated *Hox* genes also occurs in locations as diverse as hair follicles, blood cells, and developing sperm cells. The principal function of the *Hox* genes is involved in setting up structures along the main body axis, but ordered groups of *Hox* genes are later reused in guiding the formation of several specific nonaxial structures.

The regulation of *Hox* gene expression is complex. A major regulator along parts of the anteroposterior axis of the developing central nervous system is retinoic acid, but this effect is mediated by other genes. At a different level, *Hox* expression is influenced by modifications of chromatin and the three-dimensional organization of the chromosomes. Even after the transcription has occurred, recently discovered microRNAs may cleave Hox mRNAs and inactivate them.

Figure 4-5 Organization of the human HOX complex. Genes on the 3' ends of each of the complexes are expressed earlier and more anteriorly than those on the 5' end (right). (Based on review by Scott MP: Vertebrate homeobox nomenclature, Cell 71:551-553, 1992.)

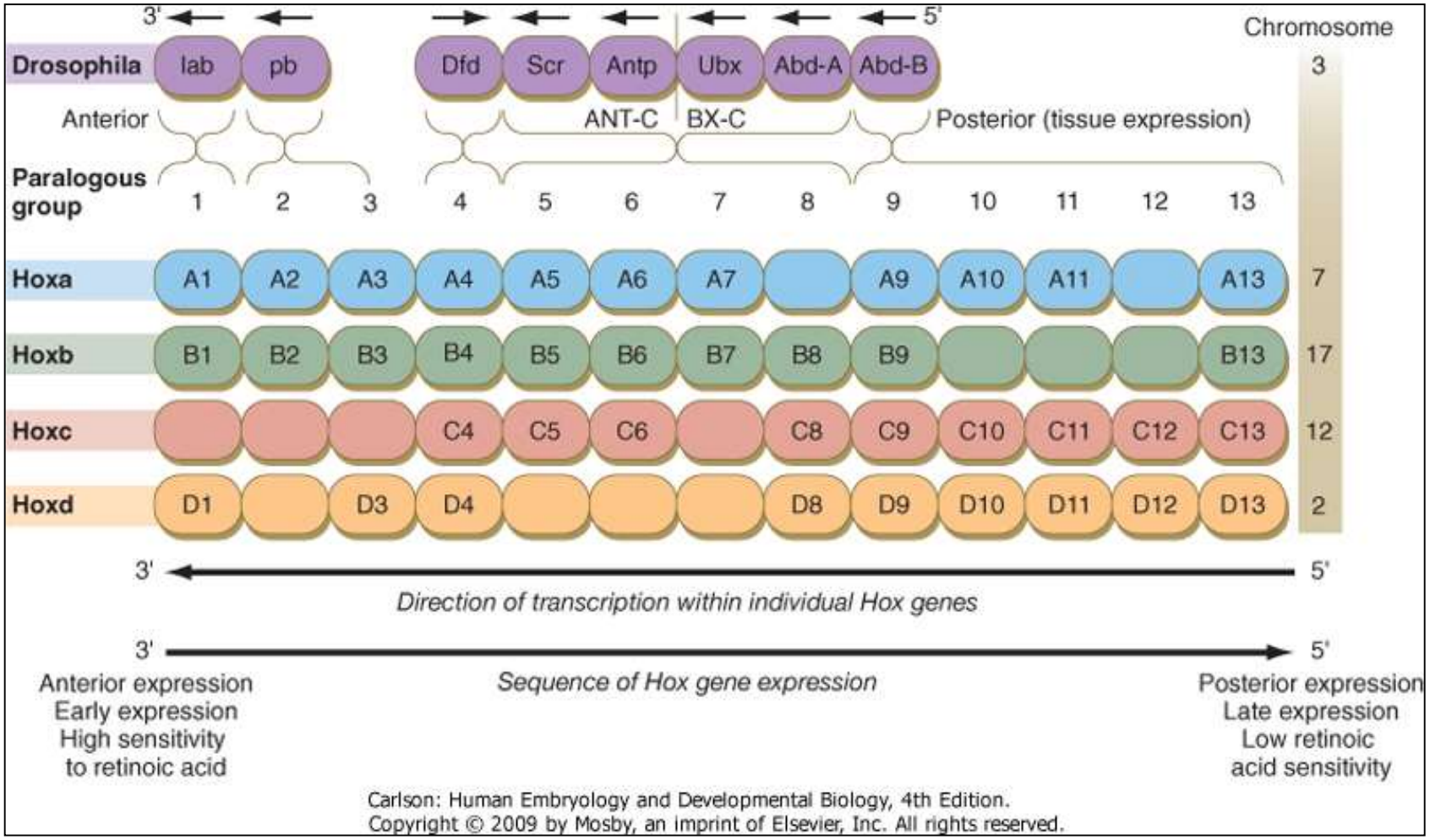
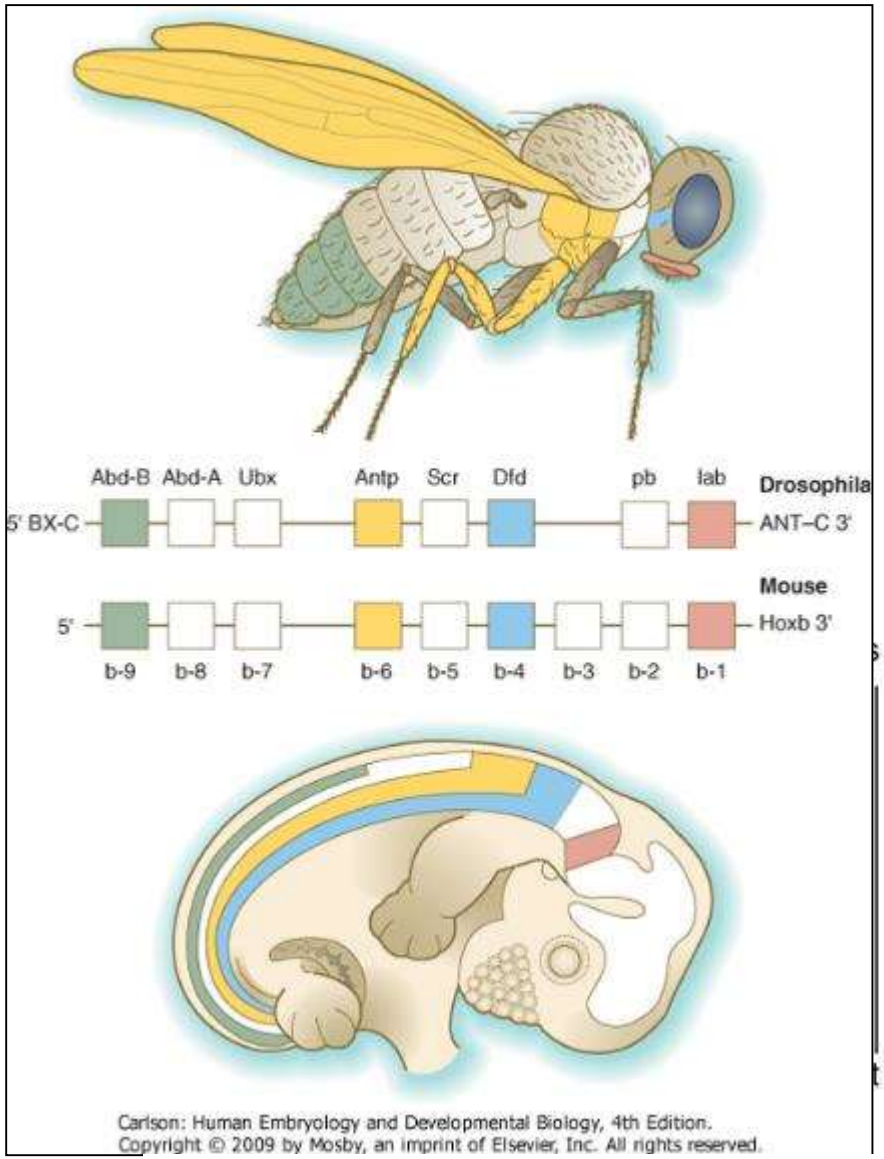
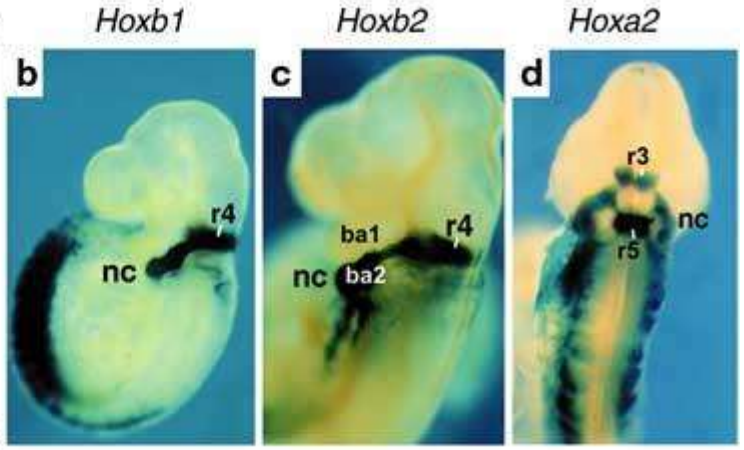


Figure 4-6 Organization of certain homeobox-containing genes of Drosophila and mouse and their segmental expression in the body. (Based on review by DeRobertis EM, Oliver G, Wright CVE: Homeobox genes and the vertebrate body plan, Sci Am 263:46-52, 1990. Copyright Patricia J. Wynne.)



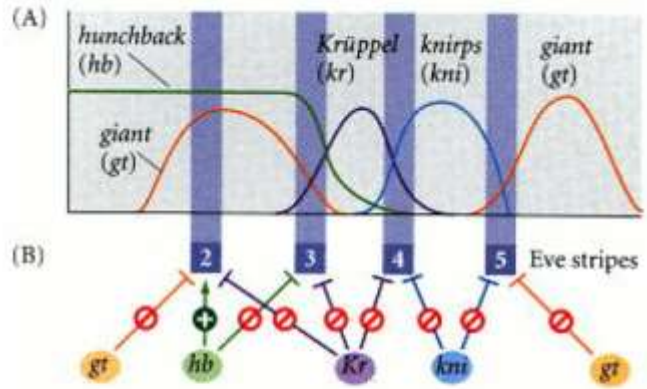
In situ hybridisations showing
Hox expression in the mouse



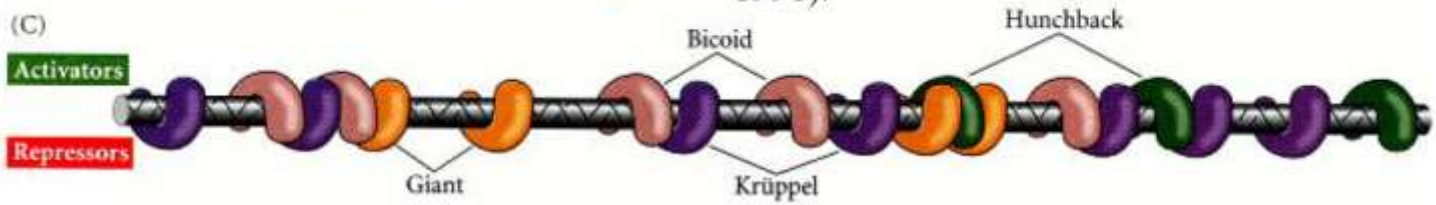
Paul Trainor & Robb Krumlauf
Nature Cell Biology **2**, 96 - 102 (2000)

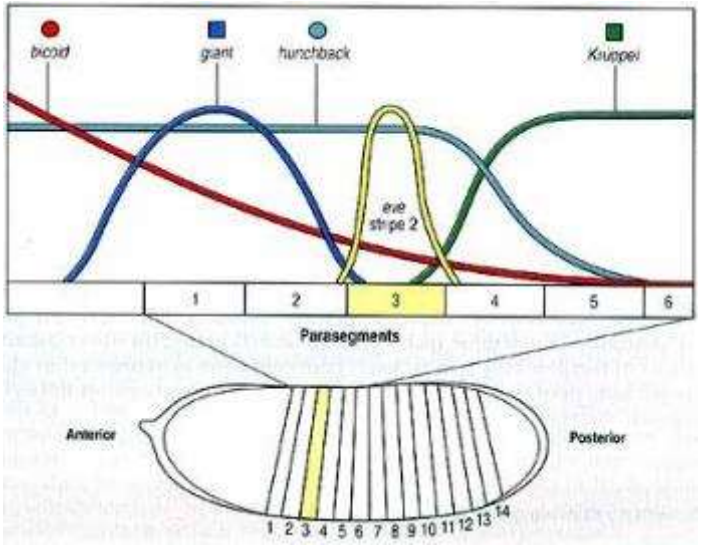
Let's go back to what it means: enhancer?!

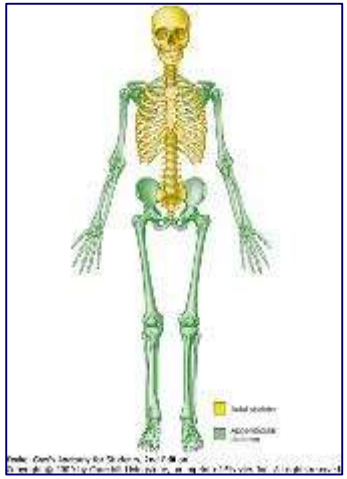
One of the best-studied enhancers is that for the *even-skipped* gene.



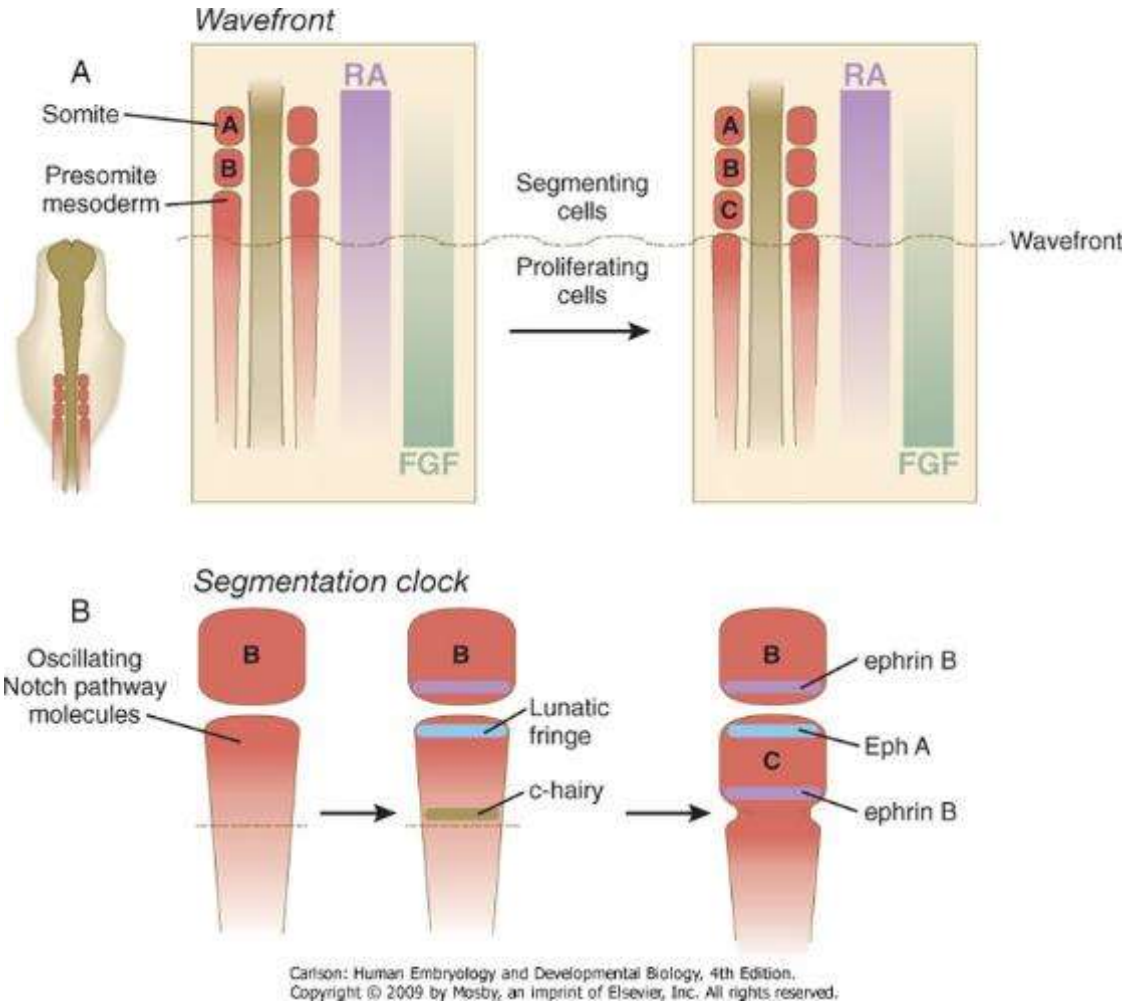
The structure of this enhancer is shown in Figure 9.22A. It is composed of modular units arranged in such a way that each unit regulates a separate stripe. For instance, the second *even-skipped* stripe is repressed by both Giant and Krüppel proteins and is activated by Hunchback protein and low concentrations of Bicoid (Figure 9.23; Small et al. 1991, 1992; Stanojevic et al. 1991).





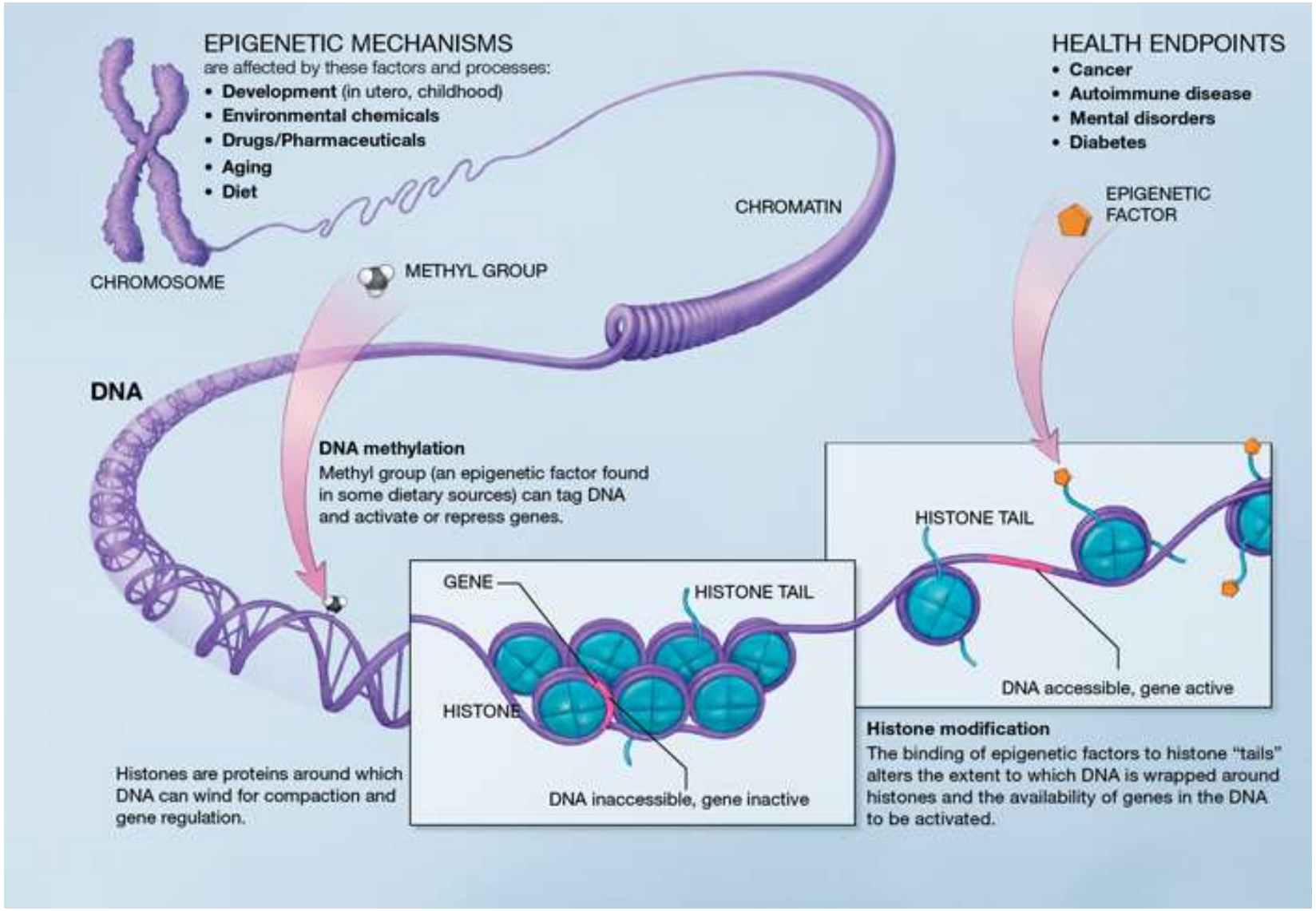


clock-and-wavefront type segmentation in higher animals



Epigenetic regulation

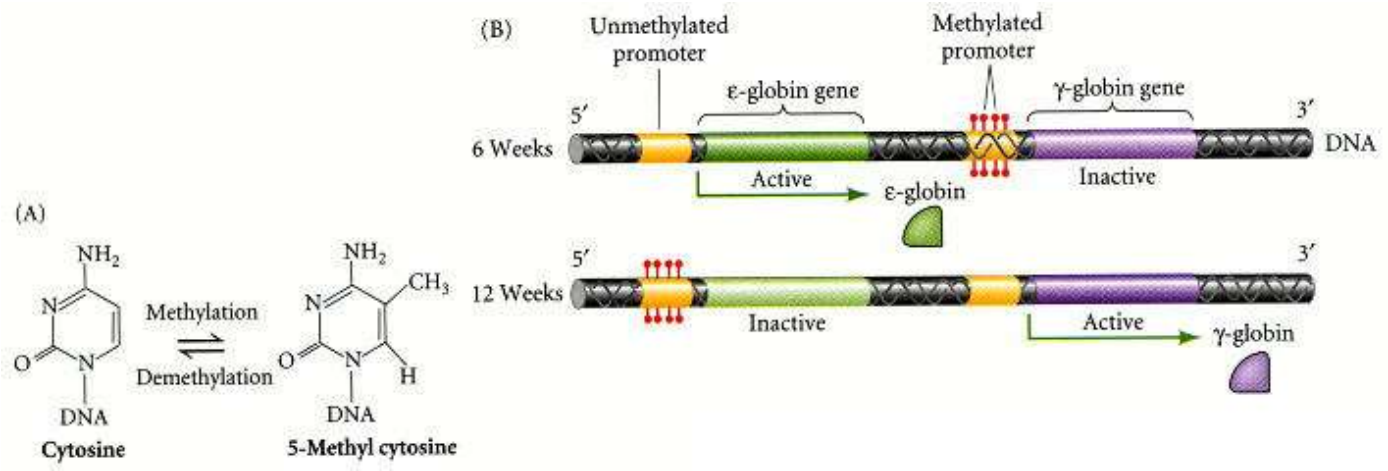
(it does not alter the fundamental DNA sequence)


EPIGENETIC MECHANISMS
are affected by these factors and processes:
• Development (in utero, childhood)
• Environmental chemicals
• Drugs/Pharmaceuticals
• Aging
• Diet
HEALTH ENDPOINTS
• Cancer
• Autoimmune disease
• Mental disorders
• Diabetes
CHROMOSOME
CHROMATIN
METHYL GROUP
EPIGENETIC FACTOR
DNA
DNA methylation
Methyl group (an epigenetic factor found in some dietary sources) can tag DNA and activate or repress genes.
GENE
HISTONE TAIL
HISTONE
DNA inaccessible, gene inactive
HISTONE TAIL
DNA accessible, gene active
Histones are proteins around which DNA can wind for compaction and gene regulation.
Histone modification
The binding of epigenetic factors to histone "tails" alters the extent to which DNA is wrapped around histones and the availability of genes in the DNA to be activated.

Chromatin „memory”

Histon acetylation (loose chromatin, transcription)
deacetylation (rigid chromatin, cell division)

DNA methylation (silenced genes, no transcription on methylated DNA)



Acetylation of the lysine residues at the N terminus of histone proteins removes positive charges, thereby reducing the affinity between histones and DNA. This makes RNA polymerase and transcription factors easier to access the promoter region. Therefore, in most cases, histone acetylation enhances transcription while histone deacetylation represses transcription.

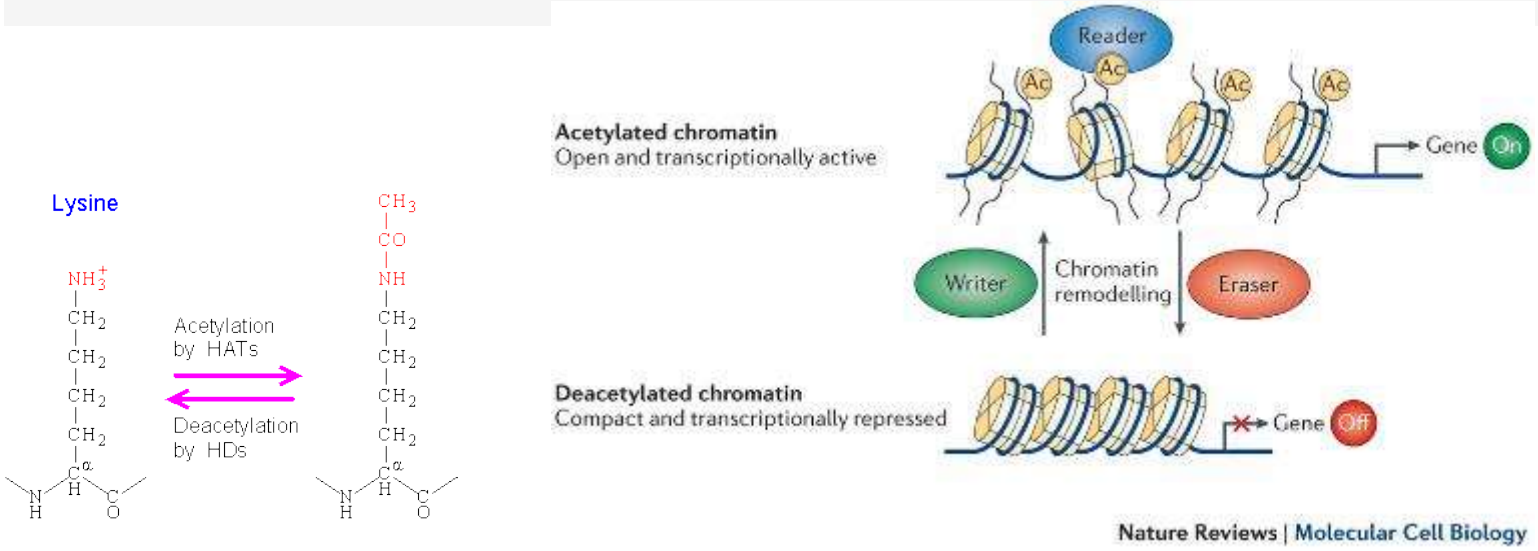
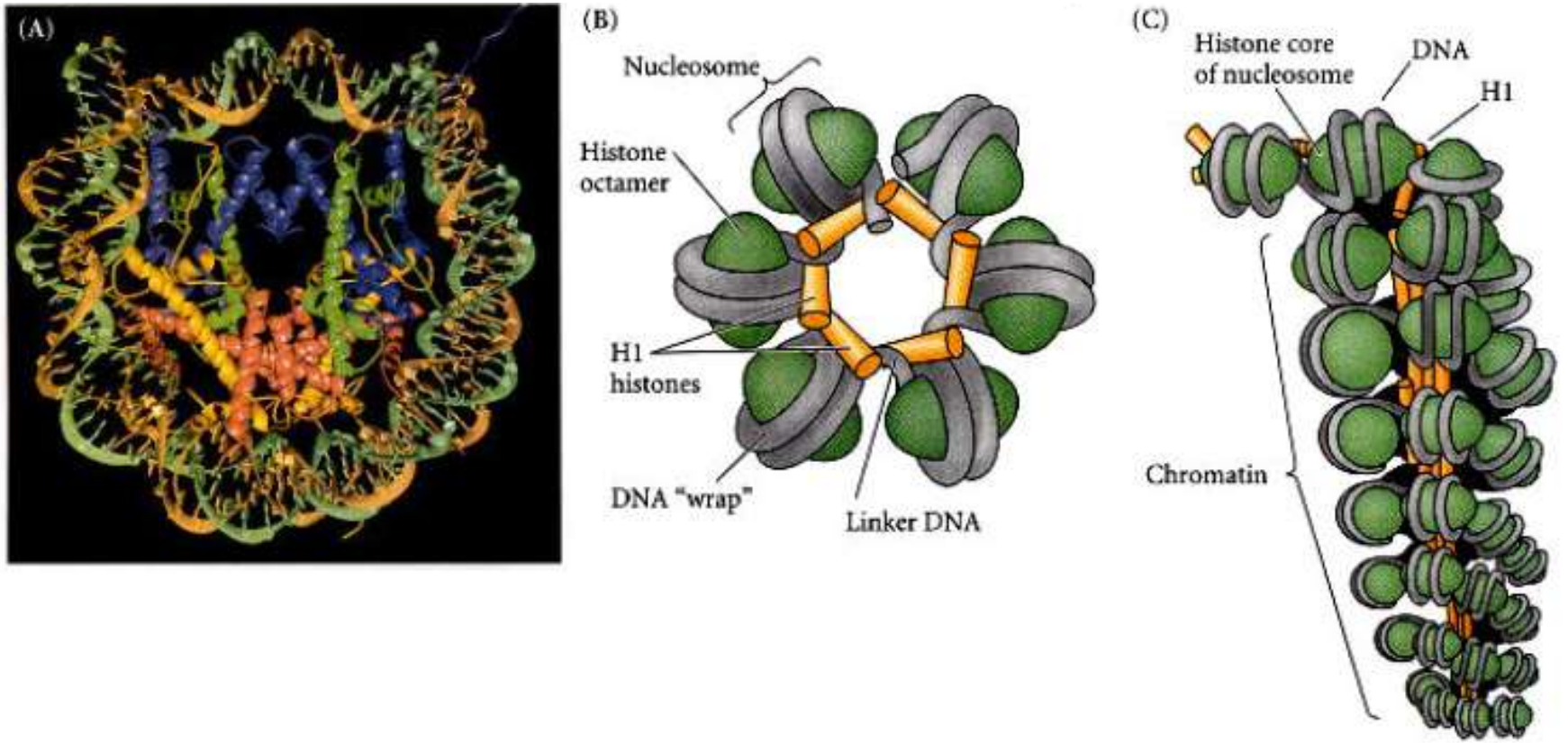


Figure 4-G-1. Acetylation and deacetylation of the lysine residue.

Histone acetylation is catalyzed by **histone acetyltransferases** (**HATs**) and histone deacetylation is catalyzed by **histone deacetylases** (denoted by **HDs** or **HDACs**). Several different forms of HATs and HDs have been identified. Among them, **CBP/p300** is probably the most important, since it can interact with numerous transcription regulators.

The "default" condition of chromatin is a repressed state



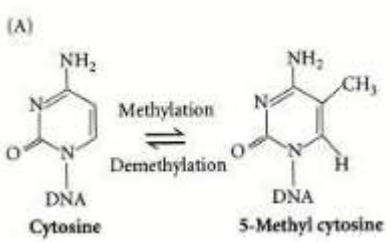
Histone H1 is found in the 60 or so base pairs of "linker" DNA between the nucleosomes (Weintraub 1984). This H1-dependent conformation of nucleosomes inhibits the transcription of genes in somatic cells by packing adjacent nucleosomes together into tight arrays that prohibit the access of transcription factors and RNA polymerases to the genes (Thoma et al. 1979; Schlissel and Brown 1984). It is generally thought, then, that the "default" condition of chromatin is a repressed state, and that tissue-specific genes become activated by local interruption of this repression (Weintraub 1985). BUT HOW? WE ONLY START TO UNDERSTAND IT…

Importance of DNA methylation I.

The consequence of the lack of massive advance storage of ribosomes and RNAs during mammalian oogenesis is that the zygote must rely on embryonic gene products very early during cleavage, typically by the two-cell or four-cell stage (four to eight cells in a human). There does not seem to be, however, a sharp transition between the cessation of reliance on purely maternal gene products and the initiation of transcription from the embryonic genome. Some paternal gene products (e.g., isoforms of β-glucuronidase and β_2-microglobulin) appear in the embryo very early, while maternal actin and histone mRNAs are still being used for the production of corresponding proteins. As an indication of the extent to which the early embryo relies on its own gene products, development past the two-cell stage does not occur in the mouse if mRNA transcription is inhibited. In contrast, similar treatment of amphibian embryos does not disrupt development until late cleavage, at which time the embryos begin to synthesize the mRNAs required to control morphogenetic movements and gastrulation.

Mature eggs and sperms are transcriptionally inactive. A major reason for this is that their DNA is highly methylated. **Methylation**, which occurs on CpG dinucleotides, normally inactivates the associated gene. Such inactivation is often called **epigenetic regulation** because it does not alter the fundamental DNA sequence. Methylation can inactivate informational genes or their regulators (e.g., enhancers or promoters). Pronounced cycles of global methylation and demethylation occur during the life span of an individual (Fig. 3-4). Within 4 hours after fertilization, the paternally derived genome undergoes a rapid massive demethylation. Demethylation of the maternally derived genome occurs more gradually until the early morula, at which stage all of the DNA is maximally demethylated. Remethylation ensues in the inner cell mass, until by the late blastocyst stage it returns to maximal levels. Within the germ cell line, the high methylation levels characteristic of the early embryo fall after the primordial germ cells have entered the genital ridge. During later gametogenesis, remethylation occurs. This remethylation imprints maternal or paternal characteristics on the gametes and for some genes has profound effects on the embryos produced from these gametes. For the first couple of days after fertilization, transcriptional activity in the cleaving embryo is very low. Similarly, fertilized eggs and early mammalian embryos possess a limited capacity for the translation of mRNAs. The factor limiting translational efficiency may be the small number of ribosomes stored in the egg. As cleavage proceeds, products from maternally and paternally derived chromosomes are active in guiding development. Haploid embryos commonly die during cleavage or just after implantation. There is increasing evidence, however, that the control of early development involves more than simply having a diploid set of chromosomes in each cell.

Importance of DNA methylation II.



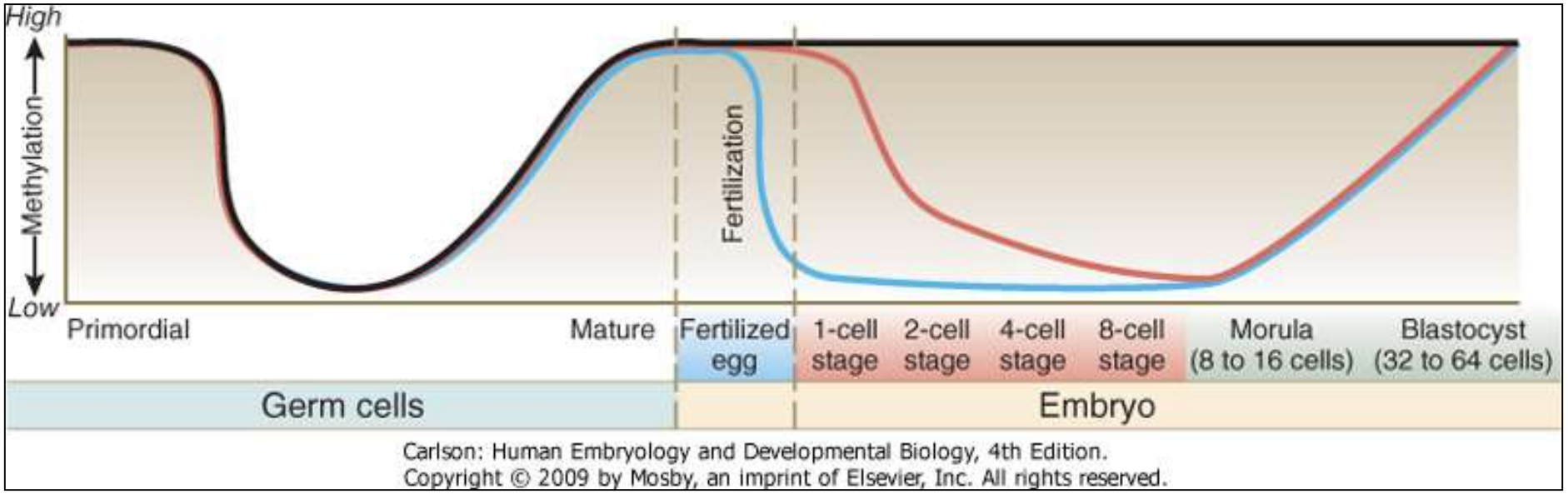
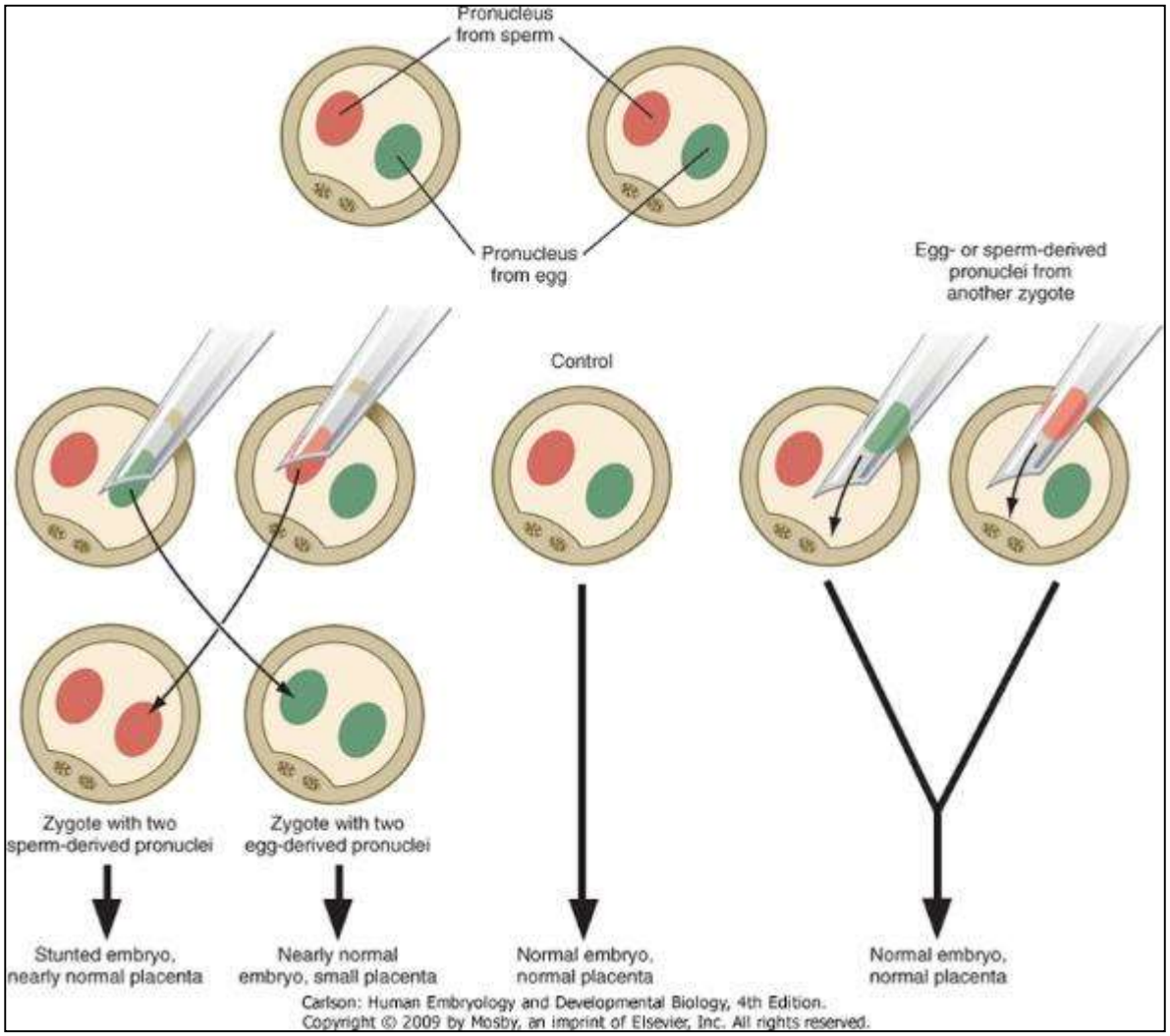


Figure 3-4 Methylation of various classes of genes during gamete maturation and cleavage. Migrating primordial germ cells are highly methylated, but they lose their methylation on entering the primitive gonad. Methylation is then lost and later reacquired during late stages of gamete maturation. After fertilization, methylation remains high in imprinted genes (black line), but falls in nonimprinted paternally (blue line) and maternally (red line) derived genes. By the blastocyst stage, high methylation levels have returned. (Modified from Santos F, Dean W: Epigenetic reprogramming during early development in mammals, Reproduction 127:643-651, 2004.)

The tale of parental imprinting…

Experimentation, coupled with observations on some unusual developmental disturbances in mice and humans, has shown that the expression of certain genes derived from the egg differs from the expression of the same genes derived from the spermatozoon.
Called **parental imprinting**, the effects are manifest in different ways…

Figure 3-5 Experimental demonstrations of parental imprinting by the use of pronuclear transplants.



What is the mechanism? Probably methylation.

Parental Imprinting

It is possible to remove a pronucleus from a newly inseminated mouse egg and replace it with a pronucleus taken from another inseminated egg at a similar stage of development (Fig. 3-5). If a male or female pronucleus is removed and replaced with a corresponding male or female pronucleus, development is normal. If a male pronucleus is removed and replaced with a female pronucleus (resulting in a zygote with two female pronuclei), however, the embryo itself develops fairly normally, but the placenta and yolk sac are poorly developed. Conversely, a zygote with two male pronuclei produces a severely stunted embryo, whereas the placenta and yolk sac are nearly normal.

Parental imprinting occurs during gametogenesis through mechanisms that are still unclear. Methylation of DNA is considered to be one of the major means of imprinting. Methylation of DNA results in the differential expression of paternal and maternal alleles of the imprinted genes.The imprinted genes operate during development and possibly into adulthood, but a given imprint is not passed on to that individual's progeny. Instead, the parental imprints on the genes are erased, and new imprints, corresponding to the sex of that individual, are established in the oocytes and sperm during gametogenesis. Not all genes are parentally imprinted. Present estimates suggest that up to 2% of all mammalian genes are imprinted.

Conditions and Syndromes Associated with Parental Imprinting

A striking example of paternal imprinting in humans is a **hydatidiform mole**, which is characterized by the overdevelopment of trophoblastic tissues and the extreme underdevelopment of the embryo. This condition can result from the fertilization of an egg by two spermatozoa and the consequent failure of the maternal genome of the egg to participate in development or from the duplication of a sperm pronucleus in an "empty" egg. This form of highly abnormal development is consistent with the hypothesis that paternal imprinting favors the development of the trophoblast at the expense of the embryo

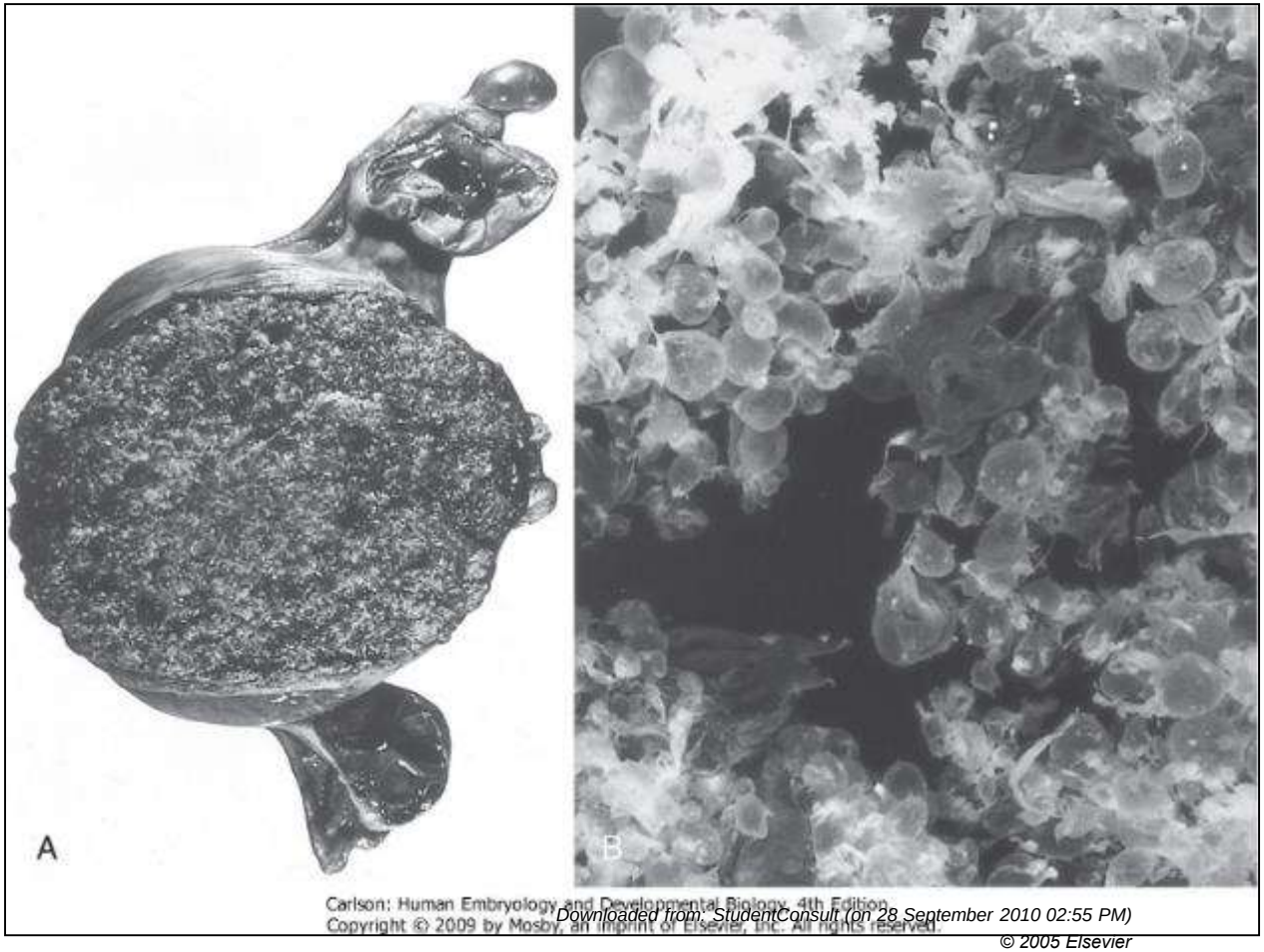


Figure 7-16 A, Distended uterus containing a hydatidiform mole. The ovaries (top and bottom) contain bilateral theca lutein cysts. B, View at greater magnification showing swollen villi. (A from Benirschke K, Kaufmann P: Pathology of the human placenta, ed 2, New York, 1990, Springer-Verlag. B courtesy K. Benirschke, San Diego.)

Conditions and Syndromes Associated with Parental Imprinting

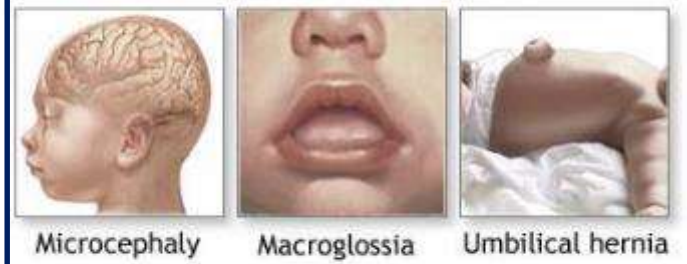
Several other syndromes are also based on parental imprinting.

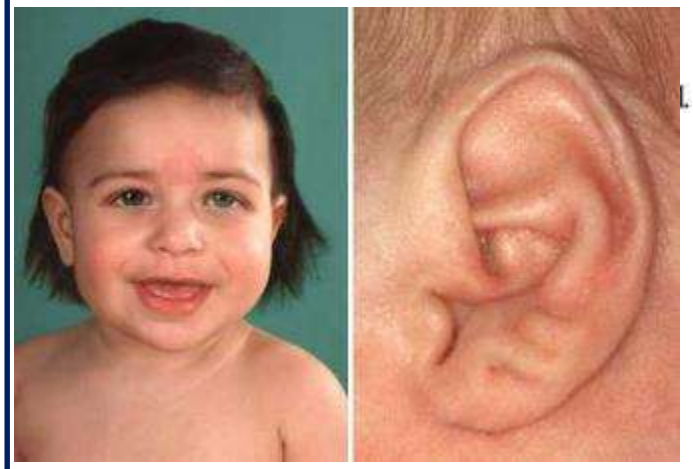
Beckwith-Wiedemann syndrome, characterized by fetal overgrowth and an increased incidence of childhood cancers, has been mapped to the imprinted region on chromosome 11, which contains the genes for insulinlike growth factor-II (IGF-II, which promotes cell proliferation) and H19 (a growth suppressor). It occurs when both alleles of the *IGF-2* gene express a paternal imprinting pattern.

Another instructive example involves deletion of the long arm of chromosome 15. Children of either sex who inherit the maternal deletion develop **Angelman's syndrome**, which includes severe mental retardation, seizures, and ataxia.

A child who inherits a paternal deletion of the same region develops **Prader-Willi syndrome**, characterized by obesity, short stature, hypogonadism, a bowed upper lip, and mild mental retardation.

Beckwith-Wiedemann syndrome





Angelman's syndrome

Prader-Willi syndrome

X-Chromosome Inactivation

Another example of the inequality of genetic expression during early development is the pattern of X-chromosome inactivation in female embryos. It is well known from cytogenetic studies that one of the two X chromosomes in the cells of females is inactivated by extreme condensation. This is the basis for the **sex chromatin**, or **Barr body**, which can be shown in cells of females, but not the cells of normal males.

The purpose of X-chromosome inactivation is dosage compensation, or preserving the cells from an excess of X-chromosomal gene products.

X-chromosome inactivation is initiated at the **X-inactivation center**, a unique locus on the X chromosome. ***XIST*** (**X-i**nactive **s**pecific **t**ranscript), one of the genes in the X-inactivation center, produces a large RNA with no protein coding potential. XIST RNA remains in the nucleus and coats the entire inactive X chromosome, thus not allowing any further transcription from that chromosome. In the inactivated X chromosome, the *XIST* gene is unmethylated and expressed, whereas in the active X chromosome, this gene is methylated and silent. Genetic studies show a complex ontogenetic history of X-chromosome inactivation (Fig. 3-6). In the female zygote, both X chromosomes are transcriptionally inactive, although not through the actions of XIST, because of the global inactivation of transcription in the early cleaving embryo. By the four-cell stage and into the morula stage, the paternally derived X chromosome becomes inactivated as the result of parental imprinting. Then as the embryo forms the blastocyst, the paternally derived X chromosomes in the trophoblast and the hypoblast remain inactivated, but within the cells of the inner cell mass both X chromosomes become active. As the cells of the inner cell mass begin to differentiate, the somatic cells undergo random permanent XIST-based X-chromosome inactivation of either the maternal or the paternal X chromosome. Within the germ cell line, activation of both X chromosomes occurs during the first meiotic division.

Ontogenetic history of X-chromosome inactivation

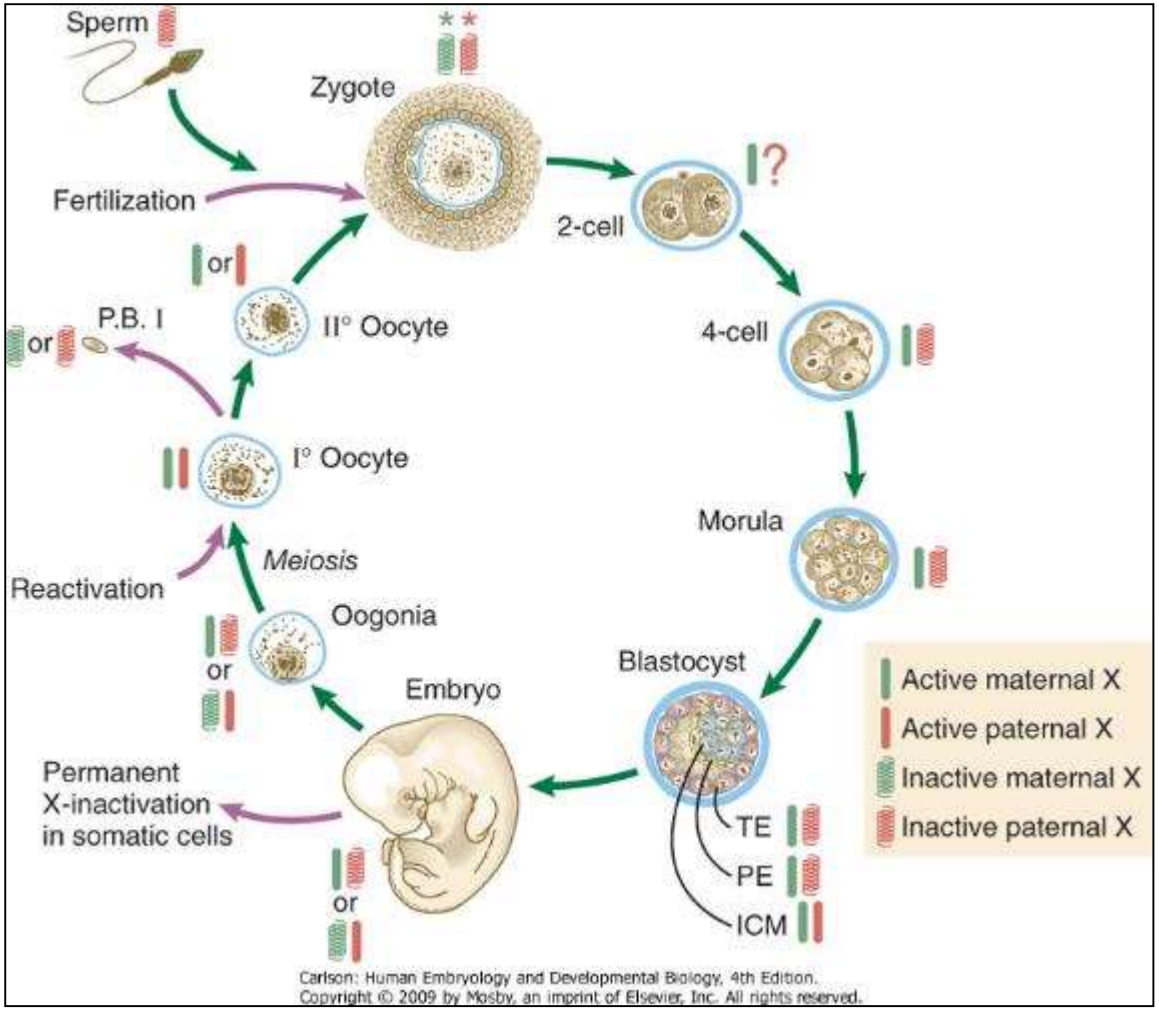
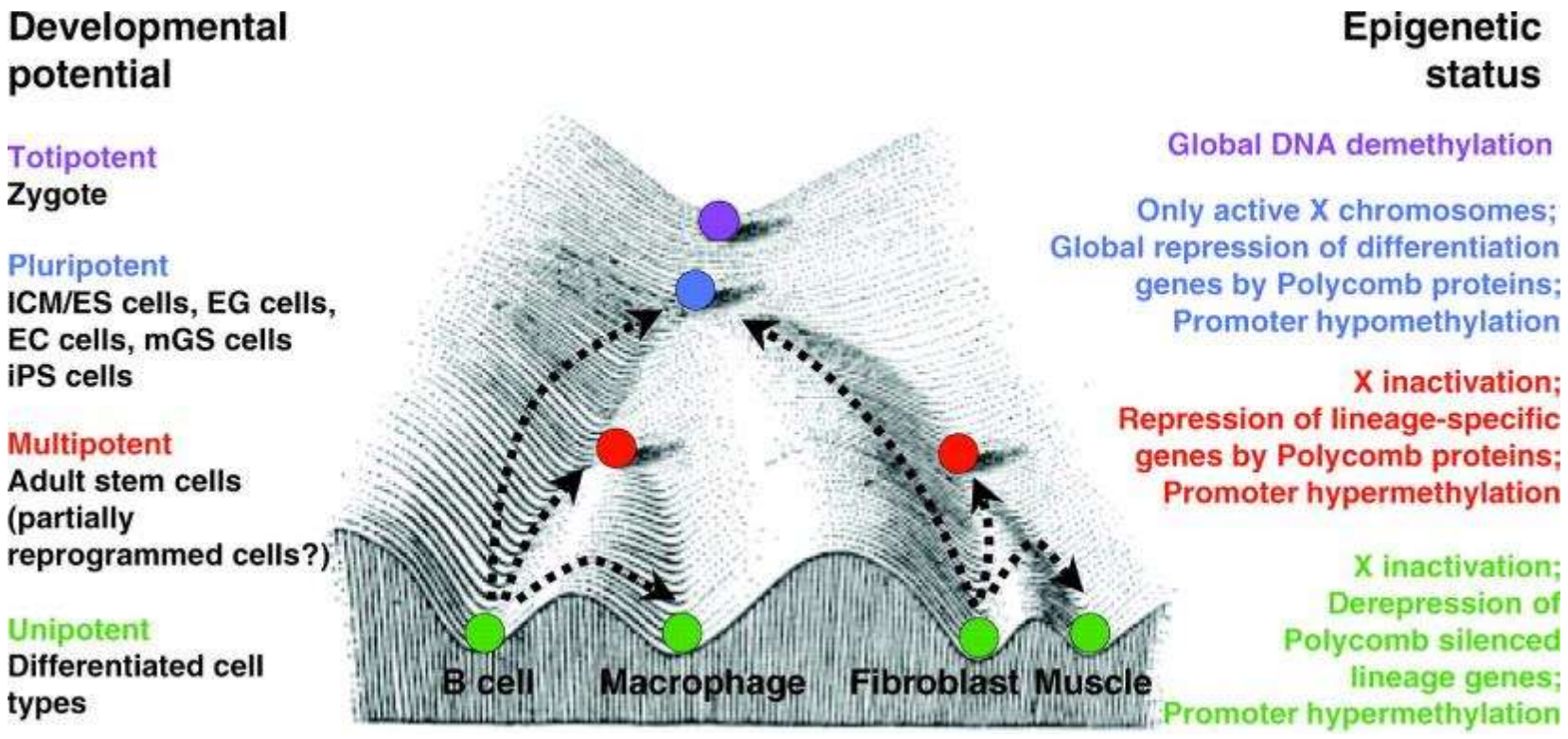


Figure 3-6 X-chromosomal inactivation and reactivation during the mammalian life cycle. The red and green symbols refer to inactivated paternally (red) and maternally (green) derived X chromosomes. ICM, inner cell mass; P.B. I, first polar body; PE, primitive (extraembryonic) endoderm; TE, trophectoderm. (Based on Gartler SM, Riggs AD: Mammalian X-chromosome inactivation, Annu Rev Genet 17:155-190, 1983; and Thorvaldsen JL and others: X-tra! X-tra!! News from the mouse X chromosome, Dev Biol 298:344-353, 2006.)

The developmental potential and epigenetic states of cells at different stages of development.

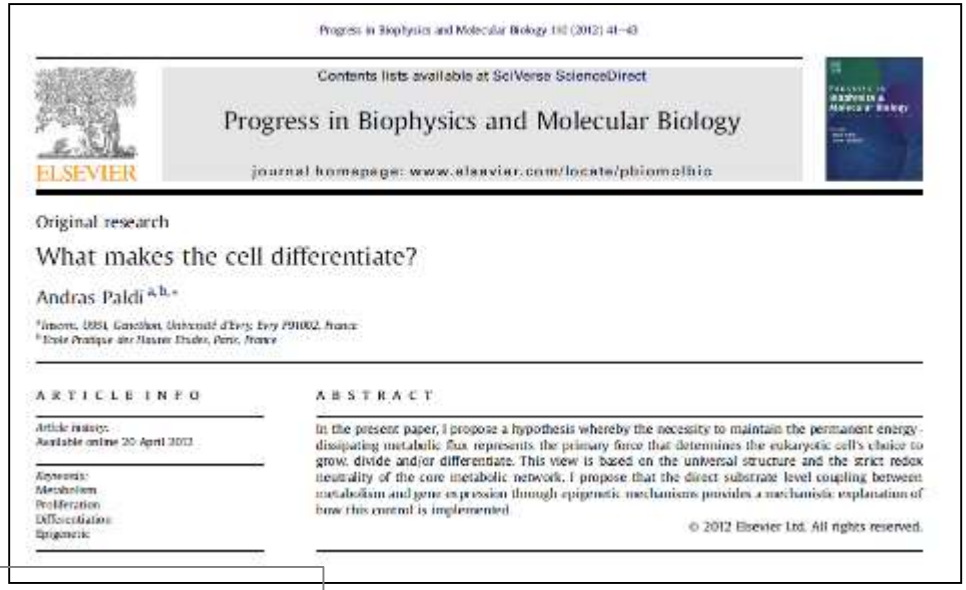


Hochedlinger K , Plath K Development 2009;136:509-523

A system biologist view:

Cell's choice controlled by substrates (nutrients)?

Be careful what you eat! It can have an epigenetic impact on your cells!



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Original research

What makes the cell differentiate?

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ABSTRACT

In the present paper, I propose a hypothesis whereby the necessity to maintain the permanent energy-dissipating metabolic flux represents the primary force that determines the eukaryotic cell's choice to grow, divide and/or differentiate. This view is based on the universal structure and the strict redox neutrality of the core metabolic network. I propose that the direct substrate level coupling between metabolism and gene expression through epigenetic mechanisms provides a mechanistic explanation of how this control is implemented.

The necessity to maintain the permanent energy-dissipating metabolic flux and the universal structure of the metabolic network can explain why the nutrient/oxygen ratio controls the cell's choice to grow, divide and/or differentiate. Our increasing understanding of the direct substrate level coupling between metabolism and gene expression through epigenetic mechanisms provides a mechanistic explanation of how this control is implemented. This explanatory scheme also provides a rational basis to explain the formation of tissues and ordered multicellularity as a result of the metabolic cooperation and complementation of the cells. If true, this view can also explain how the perturbations of the nutrient/oxygen ration disturb the normal metabolic cooperation and can result in pathologies characterized by the disruption of the normal tissular structure, such as degenerative disorders or cancer. Although most of the existing data are fully consistent with the view described in this paper, dedicated empirical investigations will reveal the exact role of the metabolic control on cell proliferation and differentiation.

Thank you for your attention!

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Bicoid expression: http://www.eb.tuebingen.mpg.de/departments/3-genetics/drosophila/uwe-irion/localisation-of-rna

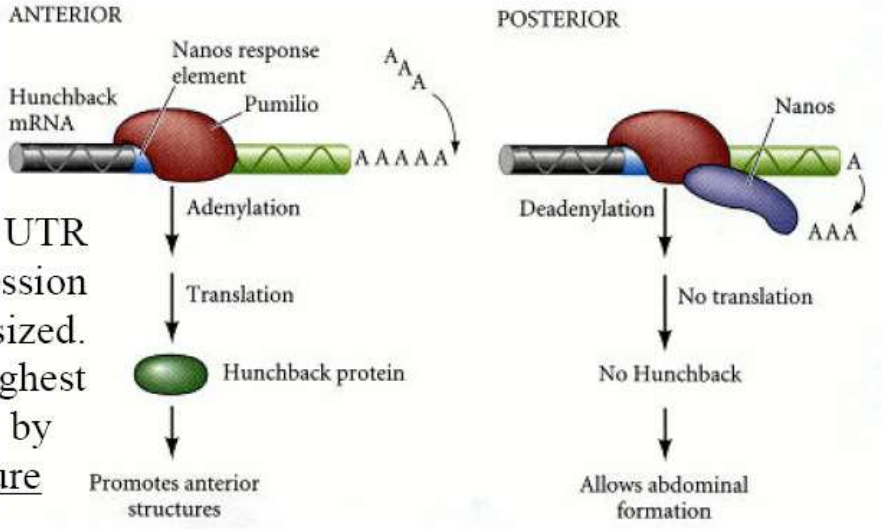
http://courses.bio.indiana.edu/L104-Bonner/F09/imagesF09/Gene%20Regulation/Embryology.html

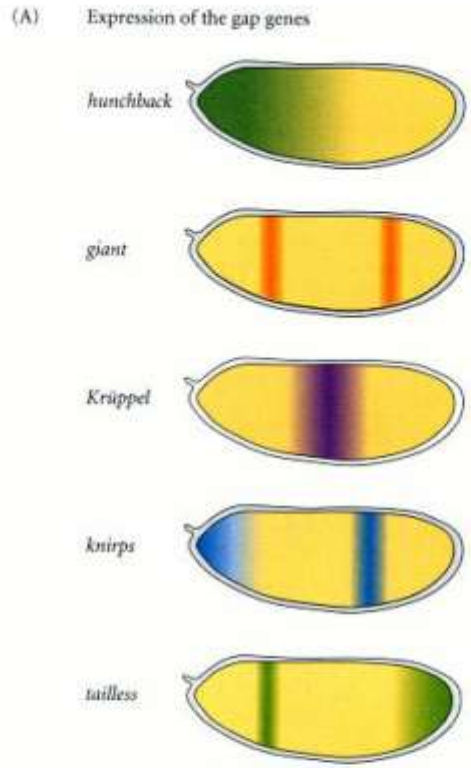
Further reading about Drosophila:

The posterior organizing center: localizing and activating nanos

The posterior organizing center is defined by the activities of the *nanos* gene (Lehmann and Nüsslein-Volhard 1991;Wang and Lehmann 1991; Wharton and Struhl 1991). The *nanos* RNA is produced by the ovarian nurse cells and is transported into the posterior region of the egg (farthest away from the nurse cells). The *nanos* message is bound to the cytoskeleton in the posterior region of the egg through its 3′ UTR and its association with the products of several other genes (*oskar, valois, vasa, staufen,* and *tudor*).* If *nanos* or any other of these maternal effect genes are absent in the mother, no embryonic abdomen forms (Lehmann and Nüsslein-Volhard 1986; Schüpbach and Wieschaus 1986).

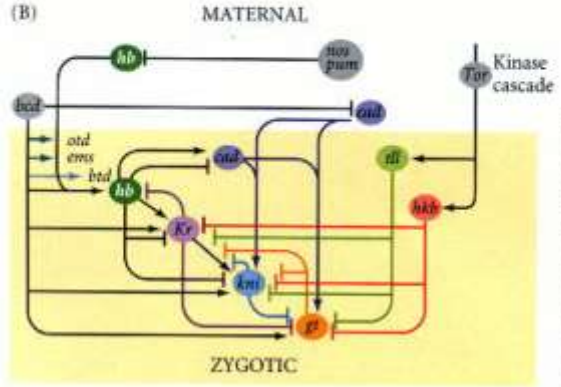
The *nanos* message is dormant in the unfertilized egg, as it is repressed by the binding of the Smaug protein to its 3′ UTR (Smibert et al. 1996). At fertilization, this repression is removed, and Nanos protein can be synthesized. The Nanos protein forms a gradient that is highest at the posterior end. Nanos functions by inactivating *hunchback* mRNA translation (Figure 9.16, see also Figure 9.11; Tautz 1988).





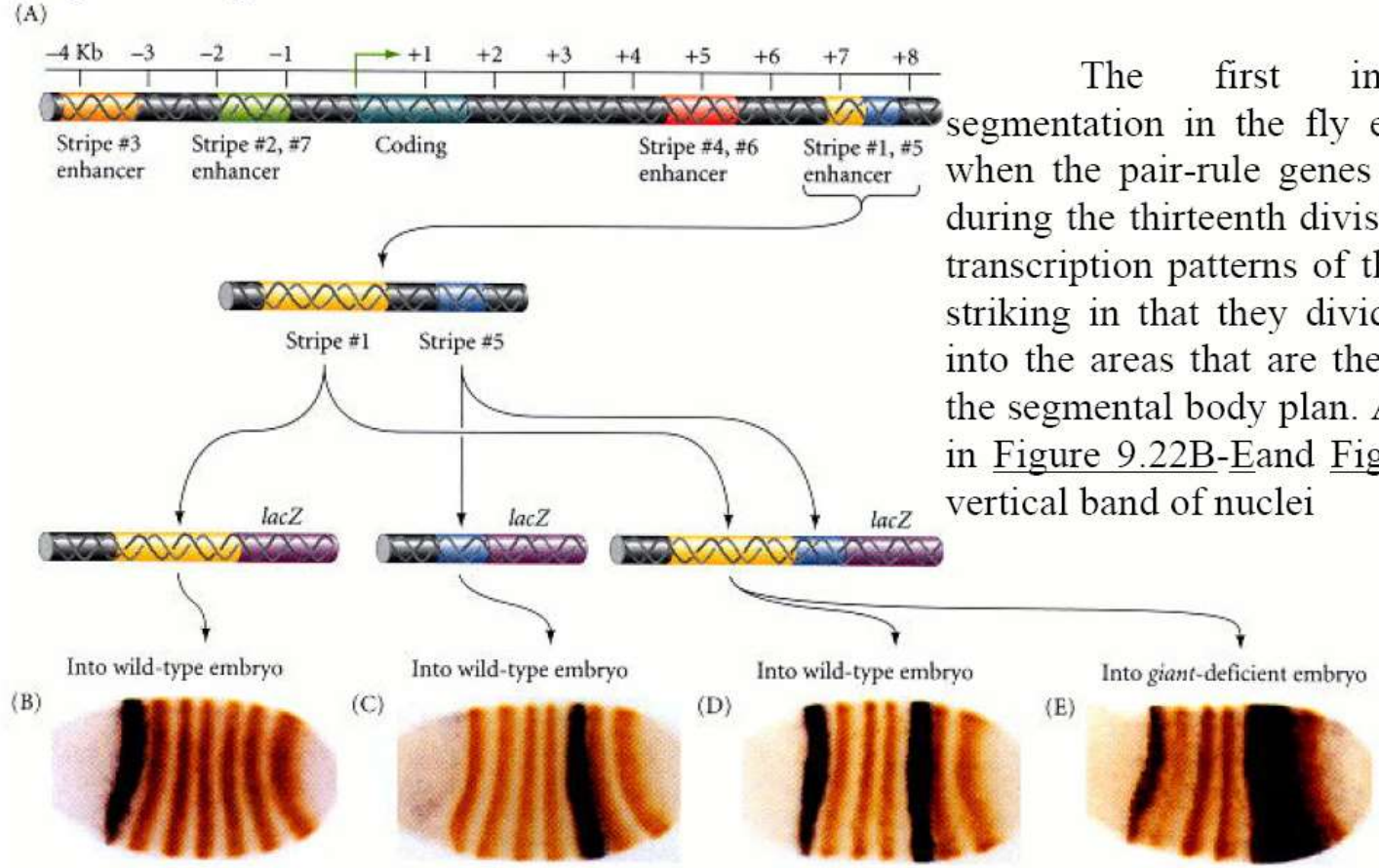
The gap genes were originally discovered through a series of mutant embryos that lacked groups of consecutive segments (Figure 9.21; Nüsslein-Volhard and Wieschaus 1980). Deletions caused by mutations of the *hunchback*,*Krüppel*, and *knirps* genes span the entire segmented region of the *Drosophila* embryo. The *giant* gap gene overlaps with these three, and mutations of the *tailless* and *huckebein* genes delete portions of the unsegmented termini of the embryo.

The expression of the gap genes is dynamic. There is usually a low level of transcriptional activity across the entire embryo that becomes defined into discrete regions of high activity as cleavage continues (Jäckle et al. 1986). The critical element appears to be the expression of the Hunchback protein, which by the end of nuclear division cycle 12 is found at high levels across the anterior part of the embryo, and then forms a steep gradient through about 15 nuclei. The last third of the embryo has undetectable Hunchback levels. The transcription patterns of the anterior gap genes are initiated by the different concentrations of the Hunchback and Bicoid proteins.

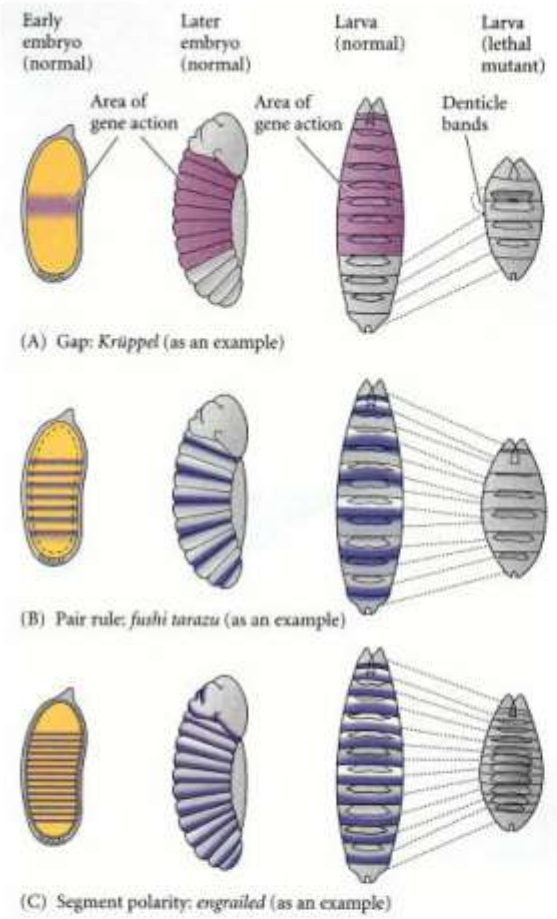


High levels of Hunchback protein induce the expression of *giant*, while the *Krüppel* transcript appears over the region where Hunchback begins to decline. High levels of Hunchback protein also prevent the transcription of the posterior gap genes (such as *knirps*) in the anterior part of the embryo (Struhl et al. 1992). It is thought that a gradient of the Caudal protein, highest at the posterior pole, is responsible for activating the abdominal gap genes *knirps* and *giant*.

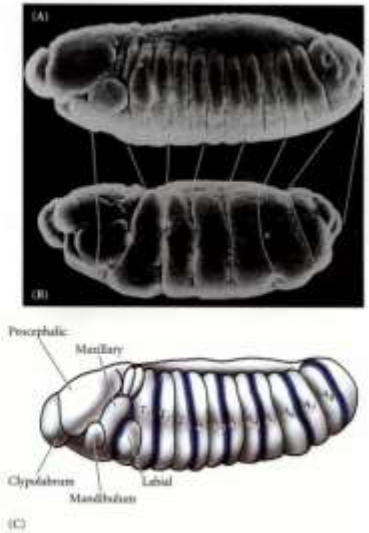
The pair-rule genes



The first indication of segmentation in the fly embryo comes when the pair-rule genes are expressed during the thirteenth division cycle. The transcription patterns of these genes are striking in that they divide the embryo into the areas that are the precursors of the segmental body plan. As can be seen in Figure 9.22B-Eand Figure 9.8C, one vertical band of nuclei (the cells are just beginning to form) expresses a pair-rule gene, then another band of nuclei does not express it, and then another band of nuclei expresses it again. The result is a "zebra stripe" pattern along the anterior-posterior axis, dividing the embryo into 15 subunits (Hafen et al. 1984). Eight genes are currently known to be capable of dividing the early embryo in this fashion; they are listed in Table 9.2. It is important to note that not all nuclei express the same pair-rule genes. In fact, within each parasegment, each row of nuclei has its own constellation of pair-rule gene expression that distinguishes it from any other row.



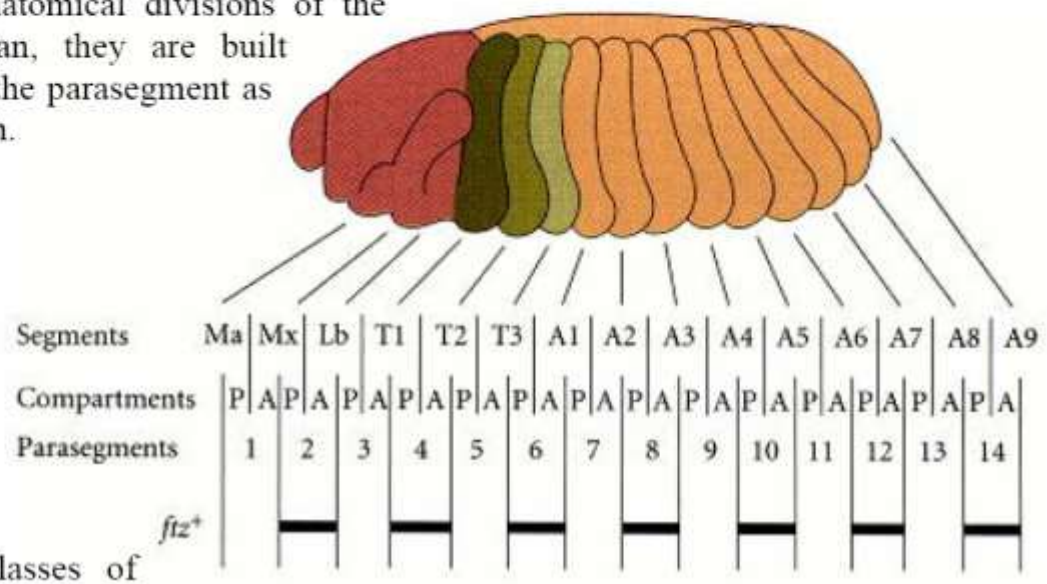
The products of these genes subdivide the broad gap gene regions into parasegments. Mutations of pair-rule genes, such as *fushi tarazu* (Figures 9.8C, 9.19B, 9.20), usually delete portions of alternate segments. Finally, the **segment polarity** genes are responsible for maintaining certain repeated structures within each segment. Mutations in these genes cause a portion of each segment to be deleted and replaced by a mirror-image structure of another portion of the segment. For instance, in *engrailed* mutants, portions of the posterior part of each segment are replaced by duplications of the anterior region of the subsequent segment (Figures 9.19C, 9.8D). Thus, the segmentation genes are transcription factors that use the gradients of the early-cleavage embryo to transform the embryo into a periodic, parasegmental structure.



After the parasegmental boundaries are set, the pair-rule and gap genes interact to regulate the homeotic selector genes, which determine the identity of each segment. By the end of the cellular blastoderm stage, each segment primordium has been given an individual identity by its unique constellation of gap, pair-rule, and homeotic gene products (Levine and Harding 1989).

The Segmentation Genes

The process of cell fate commitment in *Drosophila* appears to have two steps: specification and determination (Slack 1983). Early in development, the fate of a cell depends on environmental cues, such as those provided by the protein gradients mentioned above. This specification of cell fate is flexible and can still be altered in response to signals from other cells. Eventually, the cells undergo a transition from this loose type of commitment to an irreversible determination. At this point, the fate of a cell becomes cell-intrinsic. The transition from specification to determination in *Drosophila* is mediated by the **segmentation genes**. These genes divide the early embryo into a repeating series of segmental primordia along the anterior-posterior axis. Mutations in segmentation genes cause the embryo to lack certain segments or parts of segments. Often these mutations affect **parasegments**, regions of the embryo that are separated by mesodermal thickenings and ectodermal grooves. The segmentation genes divide the embryo into 14 parasegments (Martinez-Arias and Lawrence 1985). The parasegments of the embryo do not become the segments of the larva or adult; rather, they include the posterior part of an anterior segment and the anterior portion of the segment behind it (Figure 9.18). While the segments are the major anatomical divisions of the larval and adult body plan, they are built according to rules that use the parasegment as the basic unit of construction.

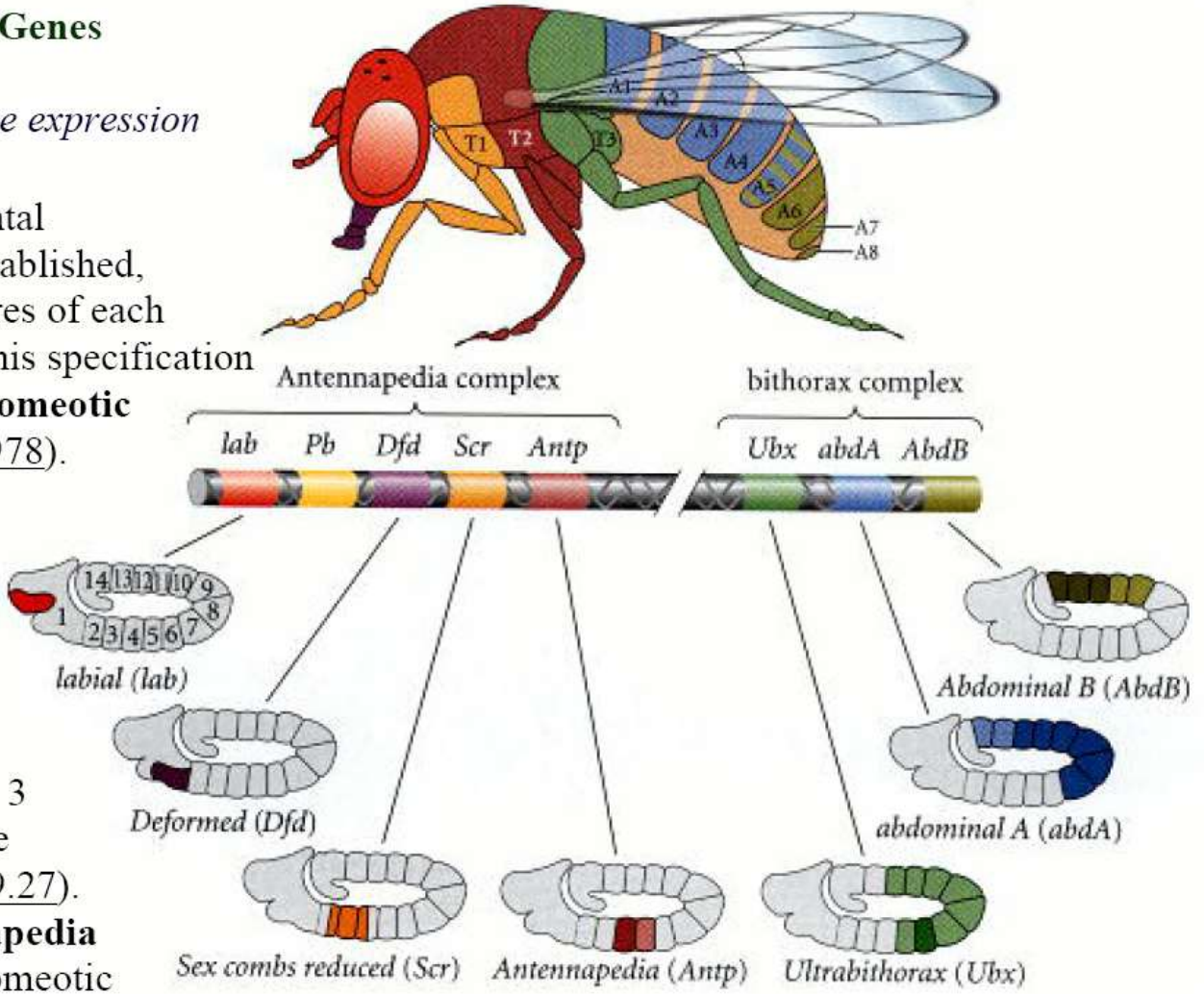


There are three classes of

The Homeotic Selector Genes

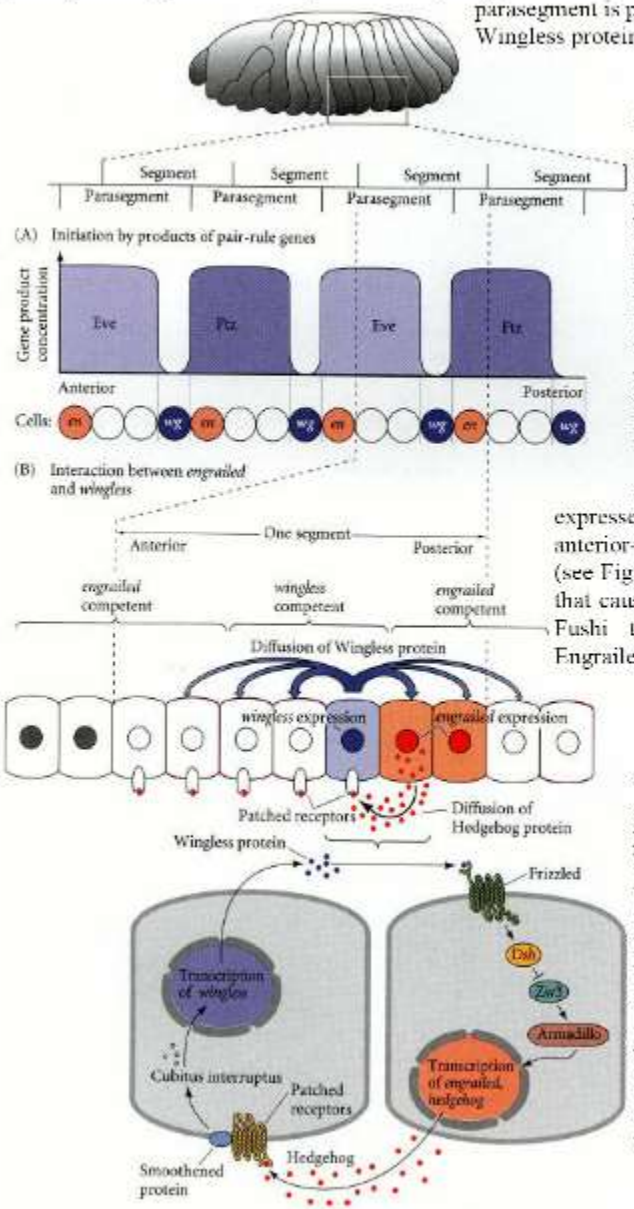
Patterns of homeotic gene expression

After the segmental boundaries have been established, the characteristic structures of each segment are specified. This specification is accomplished by the **homeotic selector genes** (Lewis 1978).



There are two regions of *Drosophila* chromosome 3 that contain most of these homeotic genes (Figure 9.27). One region, the **Antennapedia complex**, contains the homeotic genes *labial* (*lab*), *Antennapedia* (*Antp*), *sex combs reduced* (*scr*), *deformed* (*dfd*), and *proboscipedia* (*pb*). The *labial* and *deformed* genes specify the head segments, while *sex combs reduced* and *Antennapedia* contribute to giving the thoracic segments their identities.

The development of the normal pattern relies on the fact only one row of cells in each parasegment is permitted to express the Hedgehog protein, and only one row of cells in each parasegment is permitted to express the Wingless protein.



The key to this pattern is the activation of the *engrailed* gene in those cells that are going to express the Hedgehog protein. The *engrailed* gene is activated when cells have high levels of the Even-skipped, Fushi tarazu, or Paired transcription factors. Moreover, it is repressed in those cells that receive high levels of Odd-skipped, Runt, or Sloppy-paired proteins.

As a result, Engrailed is expressed in fourteen stripes across the anterior-posterior axis of the embryo (see Figure 9.8D). (Indeed, in mutations that cause the embryo to be deficient in Fushi tarazu, only seven bands of Engrailed are expressed.)

These stripes of *engrailed* transcription mark the anterior boundary of each parasegment (and the posterior border of each segment). The *wingless* gene is activated in those bands of cells that receive little or no Even-skipped or Fushi tarazu proteins, but which do contain the Sloppy-paired protein. This causes *wingless* to be transcribed solely in the row of cells directly anterior to the cells where *engrailed* is transcribed (Figure 9.25).