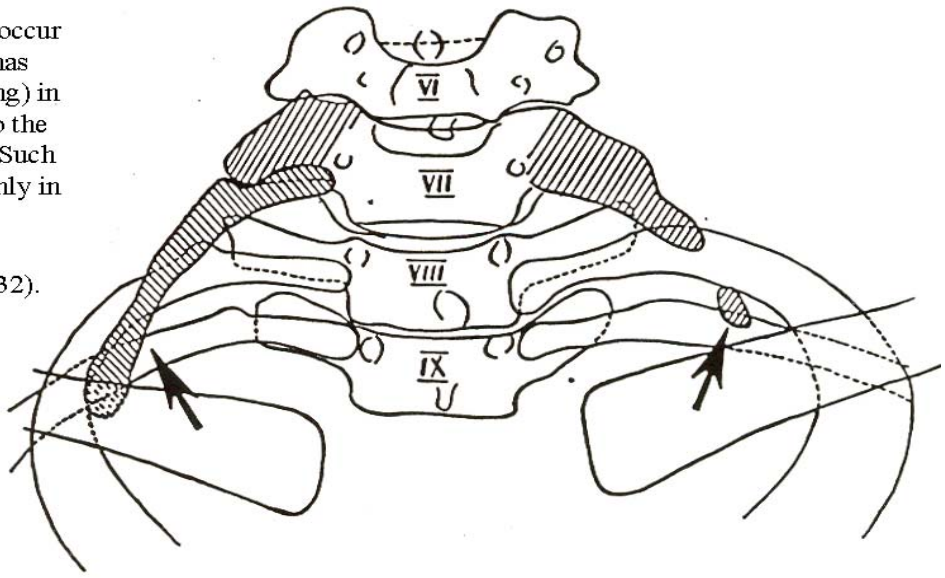
EVO-DEVO: Evolution of animal design – Lecture 4

Hox Genes

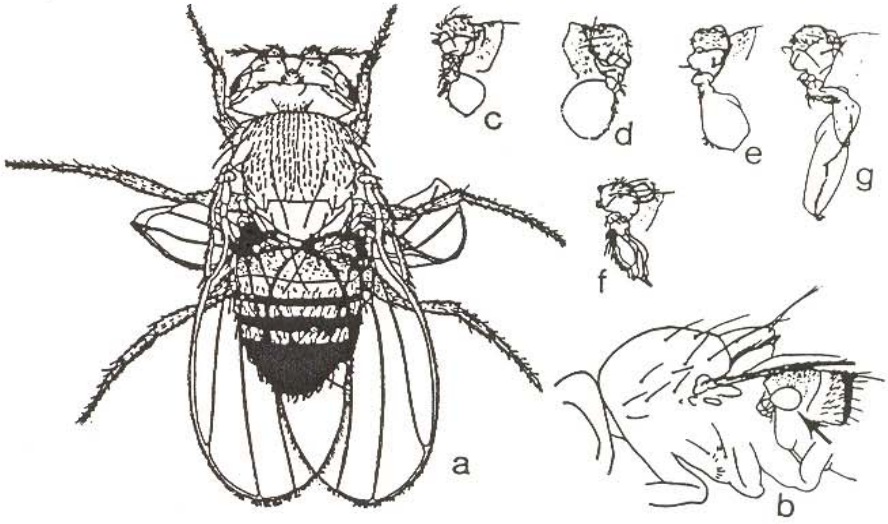
Naturalists were interested in transformations of one region of the body into another, in order to explain the discontinuous jumps in body form found in the evolution of species. Changes of one body part into another were called homeotic transformations. The moth shown below has a leg transformed into a wing. In 1923 Bridges and Morgan isolated bithorax, a four-winged fly, showing that homeotic transformations had a genetic basis.

Drawing of a moth, having an extra wing (indicated by the arrow) that has replaced a leg. Transformations like this, of one region of the body into the likeness of another, attracted the attention of William Bateson who sought to prove that the discontinuity between species is the result of the discontinuity of natural variation.

Homeotic transformations occur in humans. This skeleton has extra ribs (shown by shading) in the neck region, attached to the seventh cervical vertebra. Such cervical ribs occur commonly in human populations and are usually not deleterious. (Modified from Kuhne, 1932).



There are also variations among humans. For example, extra ribs sometimes occur in the neck region (attached to the seventh cervical vertebra). Although most people have 12 pairs of ribs, some have one less and some have one more, especially in the lower back (lumbar) region; when a vertebra in this region acquires a pair of ribs, the pair by definition is considered to be thoracic. In humans, extra ribs occur among members of particular families; the trait appears to be inherited, passed genetically from parents to their children. Thus, an extra rib in humans, and in other mammals as well, constitutes a true homeotic transformation in exactly the sense proposed long ago by Bateson.



The *bithorax* homeotic mutation in the fruit fly, *Drosophila*, as originally depicted by Bridges and Morgan. (a) A mutant fly with an extra pair of wings. (b) A normal fly showing the balancer (haltere) organ (at arrow). (c to g) Various degrees of transformation of the balancer organ into winglike structures. (Modified from Bridges and Morgan, 1923.)

Plate 1
Antennapedia. *The head of a normal (wild-type) fruit fly on the left is compared to the homeotic* Antennapedia *mutant head on the right. In the mutant the antennae are transformed into middle legs.*

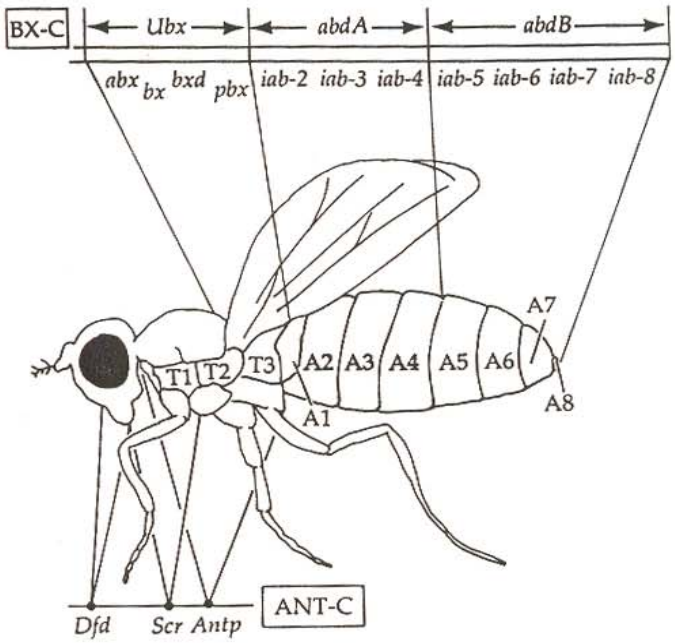
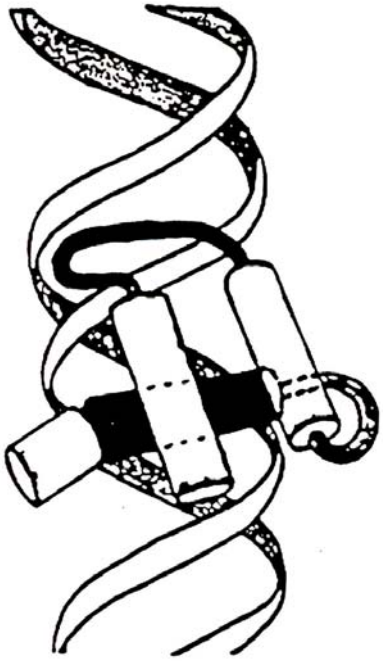
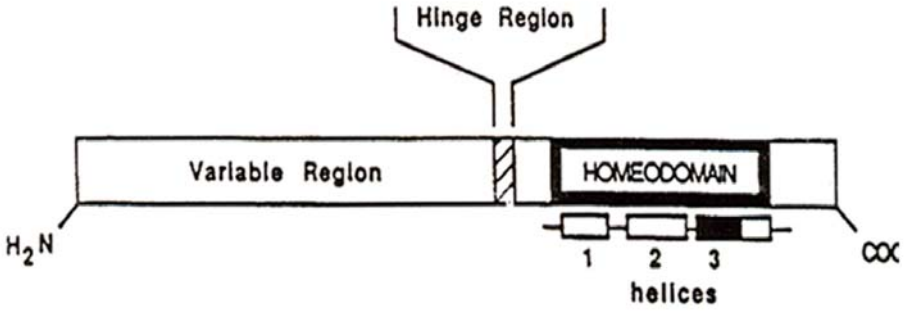


Fig. 9.3 The homeotic genes within the bithorax (BX-C) and *Antennapedia* (ANT-C) complexes in *Drosophila* to illustrate the anterior-posterior regionalization of the specification of body parts. The three genes of ANT-C [*Deformed* (*Dfd*), *Sex Combs reversed* (*Scr*) *and Antennapedia* (*Antp*)] specify head and thoracic segments T1–T3. Within the BX-C complex, *Ultrabithorax* (*Ubx*) specifies T3, while *abdominal A* and *Abdominal-B* (*abd-A, Abd-B*) specify abdominal segments A1–A4 and A5–A8 respectively. Reproduced from Gilbert (1988) *Developmental Biology* 2nd edn, Sinauer Inc., New York.





Homeobox genes code for proteins that bind DNA. The proteins are composed of a variable region, which can differ greatly in its amino acid sequence from one species to another, and a region called the homeodomain (coded for by the homeobox in chromosomes) that chemically binds to specific sequences of DNA and is very similar (evolutionarily conserved) in all multicelled animals. The homeodomains of these proteins consist of 60 amino acids and are composed of three segments (helices) of which helix 3 binds to the major groove of the DNA of chromosomes. Because most homeodomains are very similar in this segment, all bind to similar sites in DNA.

The first vertebrate homeobox gene was isolated by low stringency hybridization with a 600 bp *Drosophila* fragment containing the Antennapedia homeobox. The cloned lamda DNA hybridized not only with Antp, but also with Ultrabithorax and fushi-tarazu. Vertebrates had a homeobox. Hox genes are transcription factors of the helix-turn-helix class.

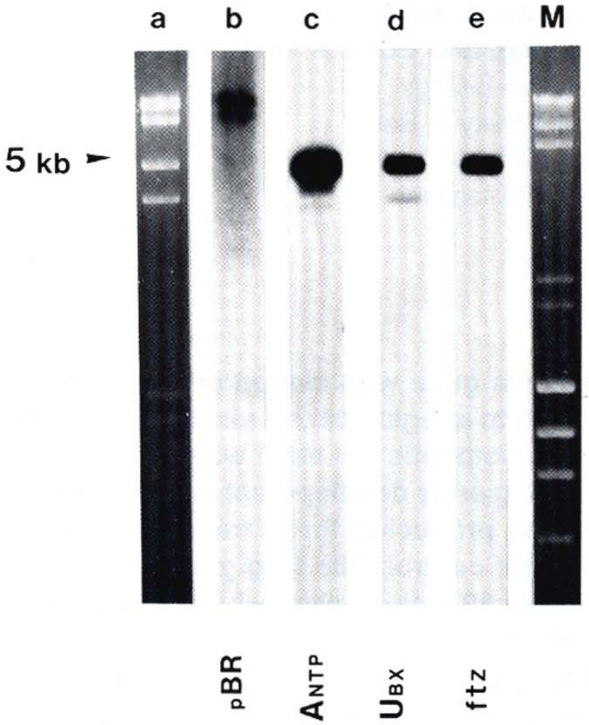
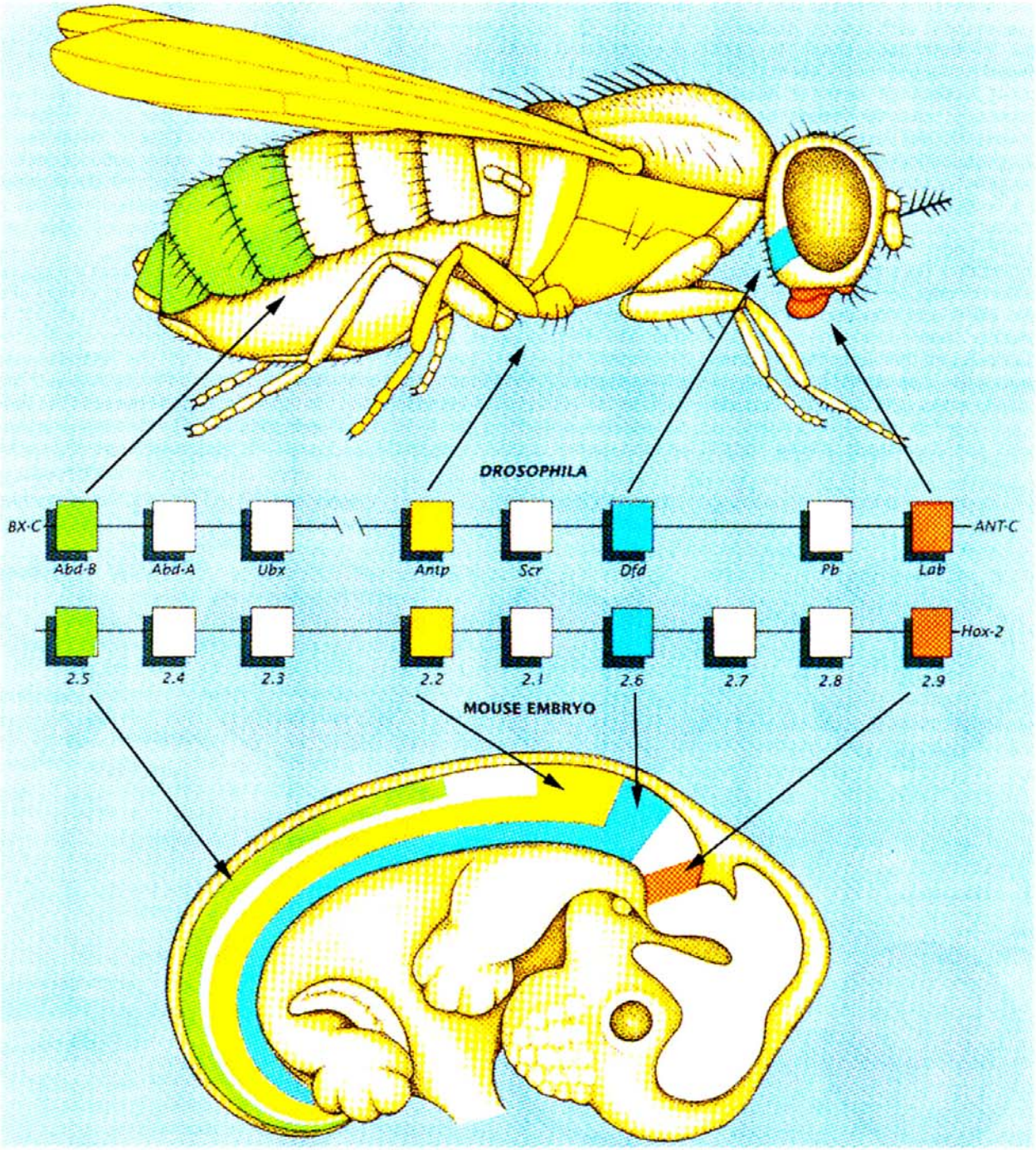


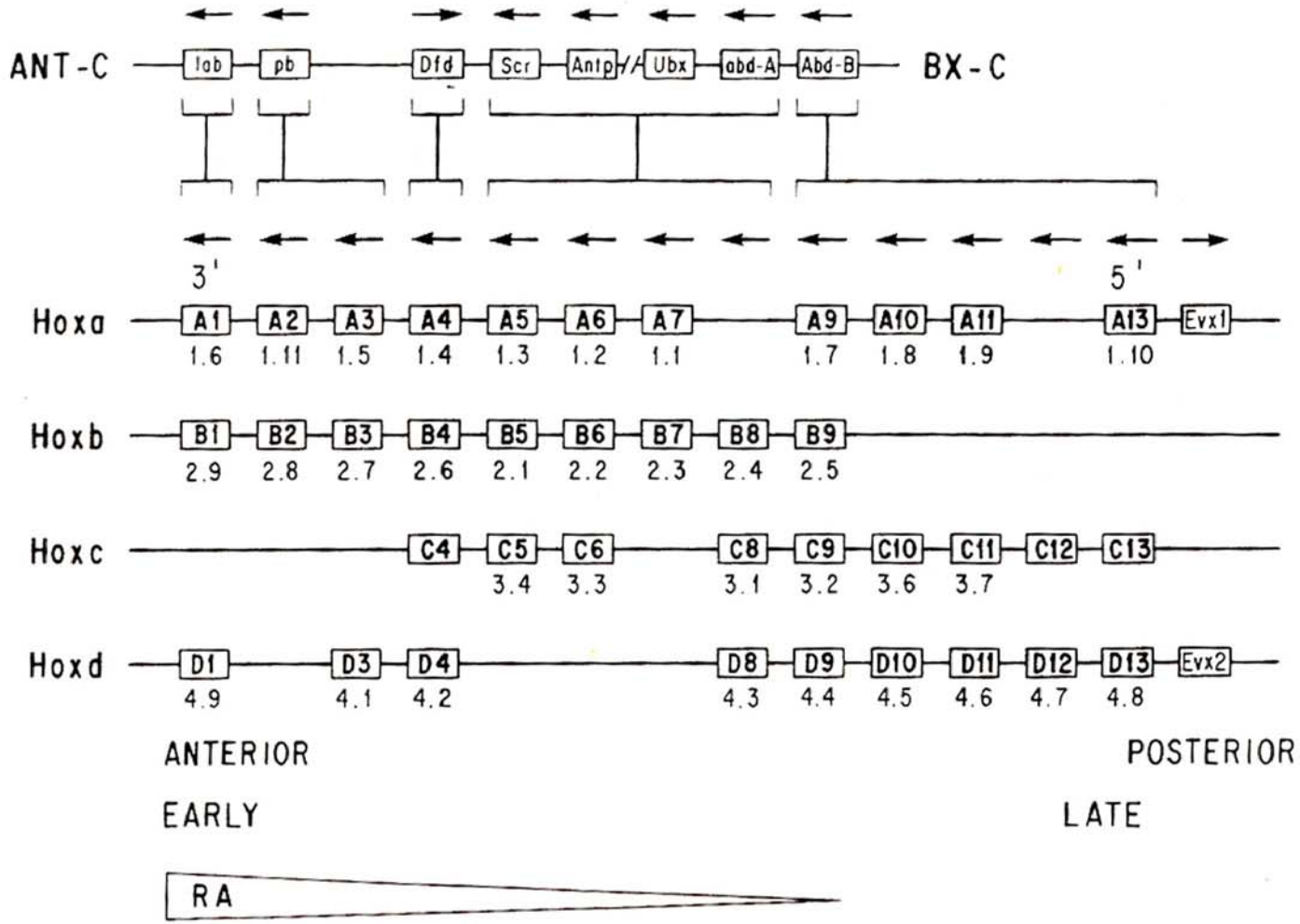
Figure 2. The Xenopus Clone**HoxC6** Cross-Hybridizes with the Drosophila Development-Controlling Genes *Antennapedia, Ultrabithorax,* and *fushi-tarazu*

λ**HoxC6** was digested to completion with Eco RI, run in several lanes of a 1% agarose gel, and transferred to nitrocellulose. Strips containing individual lanes were hybridized to various probes at low-stringency conditions. Lane a, ethidium bromide-stained agarose gel of Eco RI, digested λAC1; lane b, hybridized with pBR 322; lane c, hybridized with *Antp* subcloned in a plasmid; lane d, hybridized to *Ubx;* lane e, hybridized to *ftz;* M, molecular weight markers obtained by digesting bacteriophage λDNA with Hind III and ϕX174 DNA with Hae III. Details of the recombinant plasmids used for nick translation are given by McGinnis et al. (1984b). A band of about 5 kb hybridizes with the three Drosophila genes.

Carrasco, McGinnis, Gehring and De Robertis
Cell 37, 409-414 (1984)

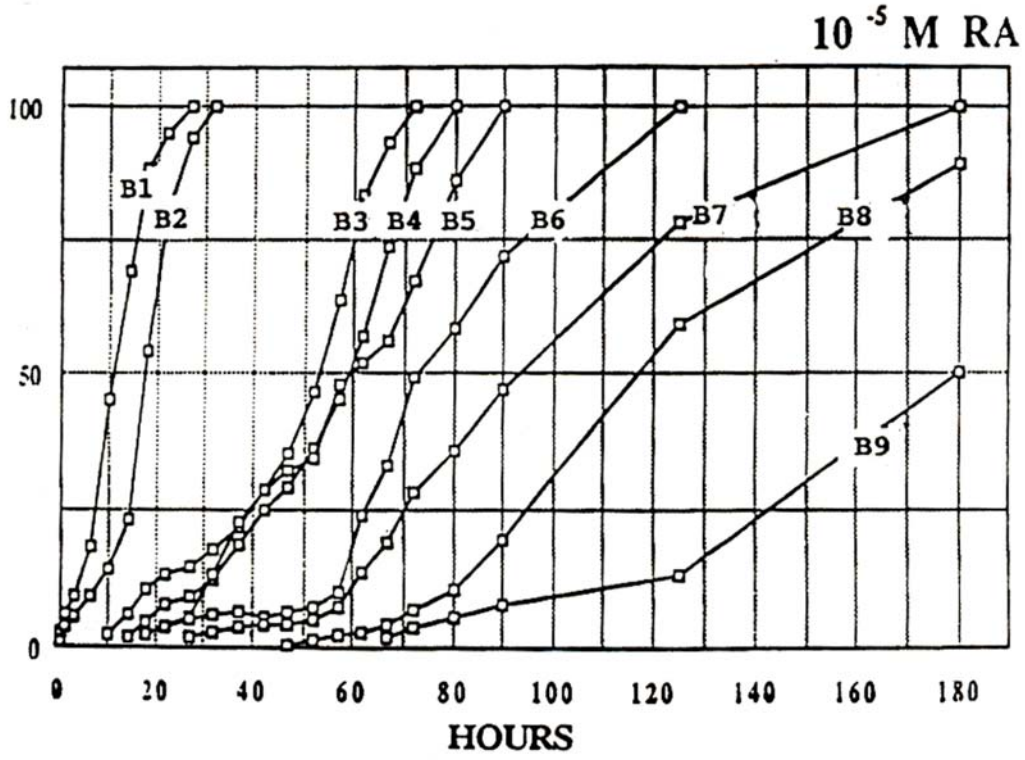


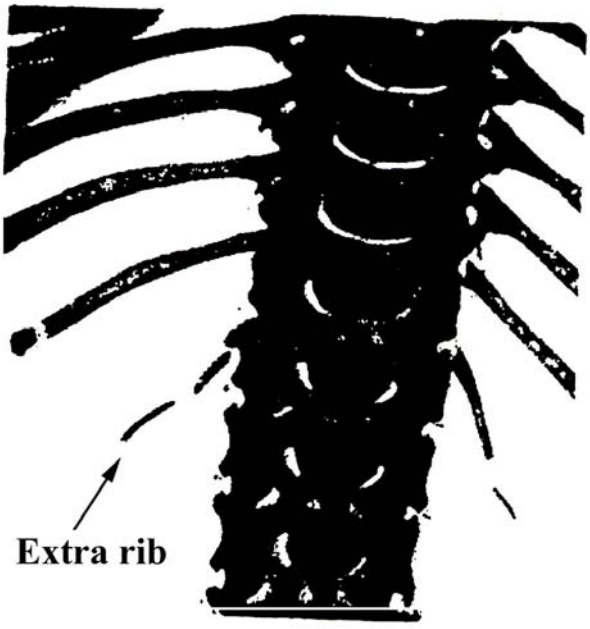
Homeobox genes are clustered in the chromosomes and are expressed in the body in the same order in which they occur in the chromosomal DNA. For example, genes of the *labial* class (*Lab*, upper row, far right) are expressed toward the front end of the fruit fly, whereas genes of the *Abdominal-B* class (*Abd-B*, upper row, far left) are expressed toward the rear. As shown below, the same relations occur in the mouse. The exceptional conservation of these relations suggests that the gene system that controls anteroposterior cell distributions arose very early in the evolutionary history of the Metazoa. (Reproduced from E. De Robertis et al., *Scientific American*, July 1990, pp. 46-52).



Vertebrates have four Hox complexes, with about 10 genes each. They can be aligned in 13 groups. They display colinearity: a) Temporal colinearity: genes on one end of the complex are expressed first, those on the other (posterior) end are turned on last, b) Spatial colinearity: the more anteriorly expressed genes are in one end, the more posterior ones at the other end of the gene complex, c) Anterior Hox genes are activated sequentially by retinoic acid, and d) Hox genes are activated sequentially also in the developing limb bud and in the genitalia.

Retinoic acid activates homeobox genes sequentially in cultured teratocarcinoma cells

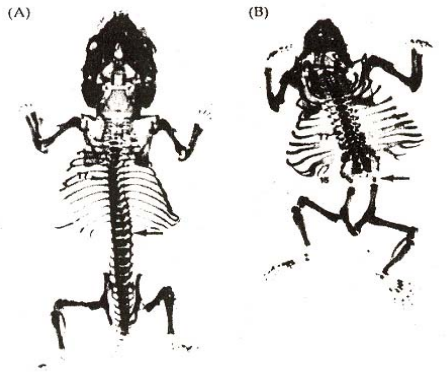




Homeotic transformations in the mouse induced by gene knockouts of homeobox genes expressed in the trunk. (A) Partial transformation of the first lumbar vertebra into a thoracic vertebra by the knockout of the *Hoxc-8* gene. Thoracic vertebrae, but not lumbar vertebrae, have ribs associated with them.

- Extensive studies with mouse knockouts support the view that cells "know" their position along the anterior-posterior axis based on the combination of Hox genes that they express.

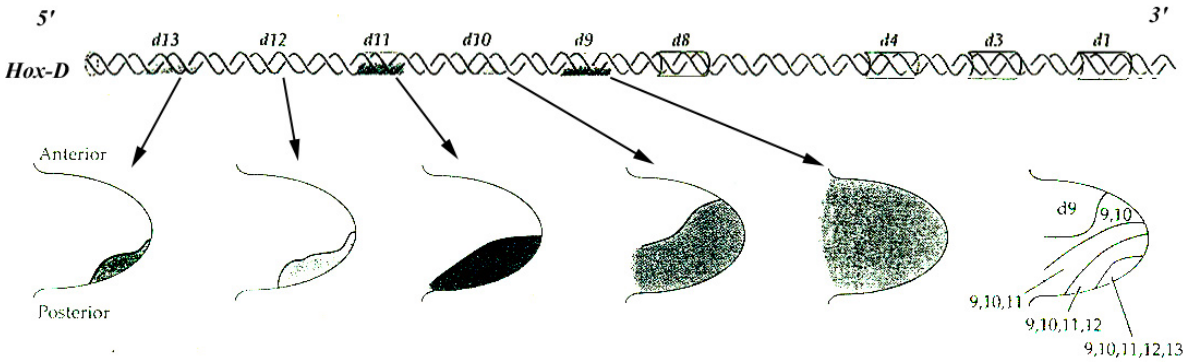
- The activation of Hox genes also explains the teratogenenicity of Retinoic Acid (Isoretinoin, Accutane), a vitamin A derivative used for the treatment of cystic acne. Despite label warnings, Accutane has been taken by at least 160,000 women of childbearing age. The most common abnormality found is cleft palate and other head and neck malformations. Formation of the palate roof through fusion of the palate is a complex process, and the cells in this region of the anterior head do not express genes of the Hox complexes. Exposure to RA causes the border of Hox gene expression to be displaced anteriorly. The expression of Hox genes in the wrong place is the cause of RA-induced congenital malformations. In mouse, it has been documented that the RA is a potent teratogen that acts through the activation of Hox genes.

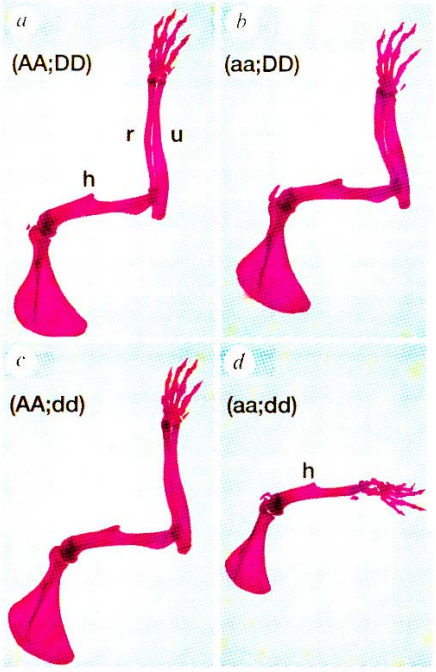


The figure shows changes in the axial skeleton (vertebrae and ribs) caused by RA exposure in utero on day 8. (A) The wild-type mouse has 7 cervical vertebrae, 13 thoracic vertebrae, 6 lumbar vertebrae, 4 sacral vertebrae, and tail vertebrae. (B) RA-treated neonate with 15 thoracic vertebrae (15 ribs) and aplasia of sacral and tail region.

- Hox proteins can form heterodimers with Pbx, a gene homologous to *Drosophila* Extradenticle. When a Hox/Pbx heterodimer is bound to DNA, it functions as an activator of transcription, whereas Hox homodimers tend to act as repressors. Pbx is so called because it is translocated in 30% of childhood Pre-B lymphocytic leukemias (OMIM 176310). The translocation forms a fusion of the homeodomain of Pbx-1 to the activation domain of another transcription factor.

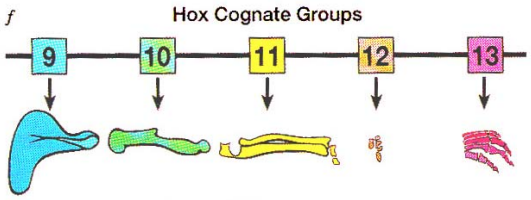
Nested expression (as in Russian dolls) of ABD-B type Hox genes in developing limb buds





Non-allelic complementation: Hox genes in different paralogous groups can compensate for each other. For example, cell proliferation of the radius and ulna is regulated by HoxA-11 and HoxD-11. Only in double mutants the entire region is lost.

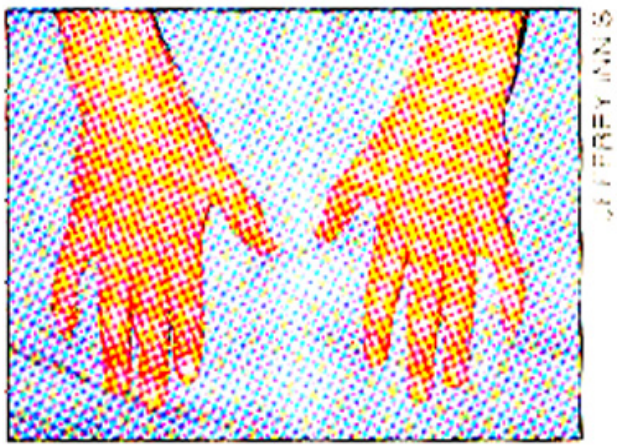
Loss of the radius and ulna in the double-mutant mouse.
Dorsal view of the right forelimbs from adult mouse skeleton preparations.



NATURE · VOL 375 · 29 JUNE 1995

Remember the Hand-Foot-Genital syndrome described earlier? It is caused by mutations in Hoxa 13. Patients have shortened thumbs and large toes and, interestingly, genital malformations as well.

A fabulous online resource for molecular disease is located at htpp://www.ncbi.nlm.nih.gov. In "search", scroll down from PubMed to OMIM. Click on OMIM (Online Mendelian Inheritance of Man). You can search OMIM direclty for the numbers provided for each disease in the lecture notes, or you can use a disease or gene name. Do this for OMIM # 14000. this Hand-Foot-Genital syndrome seems a bizarre disease, but you have a good molecular and embryological understnading of what causes it (read also abstract of reference 9 in this OMIM entry).



Science *275*, 1568 (March 1997)

Hox genes and the evolution of body forms

In *Drosophila*, the *Antp-C* and *Bx-C* are believed to have played a critical role in the evolution of insects. Flies probably evolved from insects with two pairs of wings (most insects have 4, not 2 wings). Insects probably evolved from millipede-like creatures, which had legs on each segment and very few specializations at either the anterior or the posterior. Thus Lewis proposed that Hox genes evolved to cause increasing specialization at the anterior and posterior of the fly, i.e., the specialization of segments away from a middle, or ground state, toward a more posterior or tail-like identity. For his work on homeotic genes, Ed Lewis was one of the recipients of the 1995 Nobel Prize in Physiology and Medicine.

Clinical-Embryological Correlation
Hox transcription factors in development and disease:
Hand-Foot-Genital syndrome

Hand-Foot-Genital syndrome is a dominant hereditary disease caused by nonsense mutations that truncate Hoxa 13 in the DNA-binding helix. In Hand-Foot-Genital syndrome males may have hypospadias, and females defects in uterine septation and urethral malformations. The thumb and great toe are shortened. How does this come about?

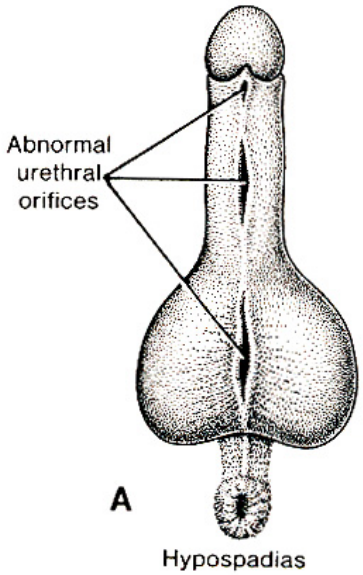


Figure 15-28. (*A*) Hypospadias. 1:125 male births

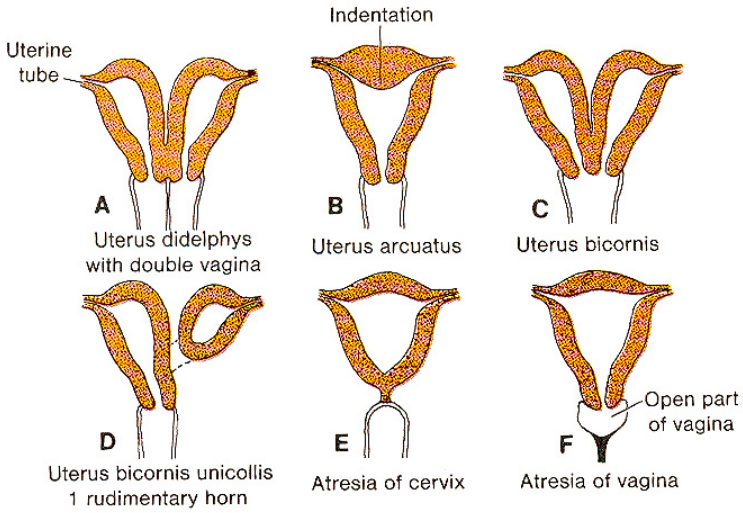


Figure 15-25. Schematic representation of the main abnormalities of the uterus and vagina, caused by persistence of the uterine septum.

(From Langman's Medical Embryology, a great book).

Uterine septation results from incomplete fusion of Müllerian ducts.

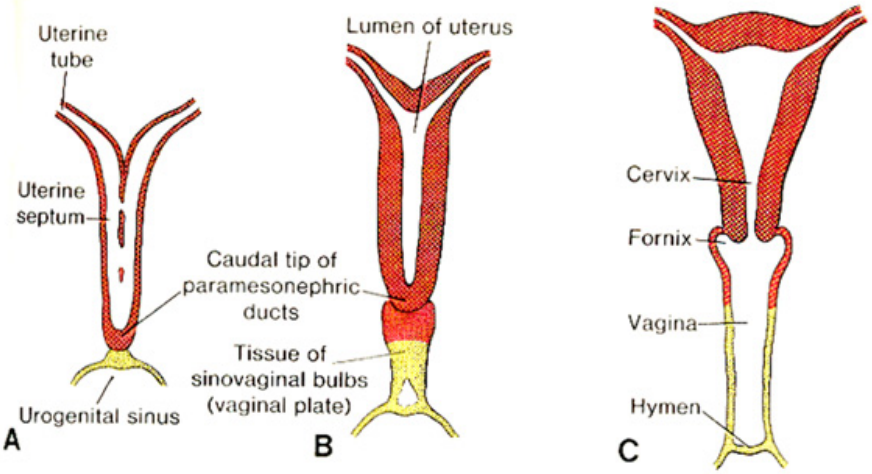


Figure 15-23. Schematic drawing showing the formation of the uterus and vagina. (*A*) At 9 weeks. Note the disappearance of the uterine septum. (*B*) At the end of the 3rd month. Note the tissue of the sinovaginal bulbs. (*C*) Newborn. The upper 3rd of the vagina and the fornices are formed by vacuolization of the paramesonephric tissue and the lower two-thirds by vacuolization of the sinovaginal bulbs.

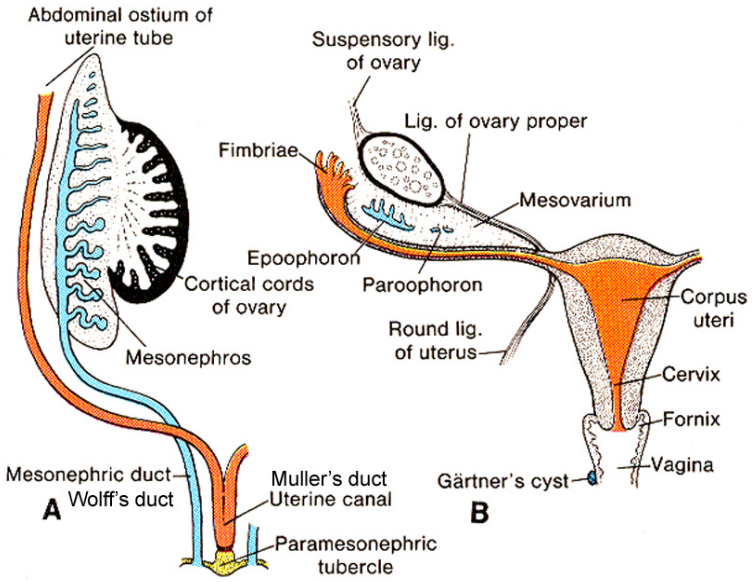


Figure 15-21. (*A*) Schematic drawing of the genital ducts in the female at the end of the 2nd month of development. Note the paramesonephric or Müllerian tubercle and the formation of the uterine canal. (*B*) The genital ducts after descent of the ovary. The only parts remaining of the mesonephric system are the epoophoron, paroophoron, and Gärtner's cyst. Note the suspensory ligament of the ovary, the ligament of the ovary proper, and the round ligament of the uterus.

Hypospadias in males is related to the development of the cloaca and external genitalia. Development of the genitalia is identical in male and females until the seventh week of pregnancy. The hindgut forms an enlargement called the cloaca. The cloaca does not open to the outside initially, and is separated by the cloacal membrane. The anterior part will become the urogenital membrane, the posterior the anal membrane. The genital tubercle will become the glans or clitoris. The urethra is formed by folding, so that the epithelium forms a tube.

This folding process is controlled by an inducing center in the epithelium (which secretes sonic hedgehog and FGF-8) and causes induction of Abd-B type Hox genes in the genital tubercle mesoderm.

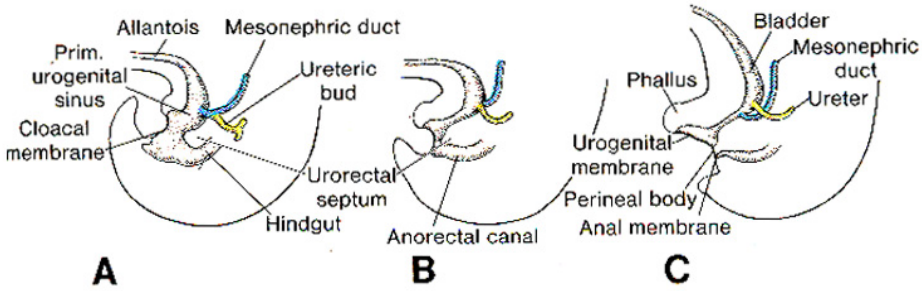


Figure 15-10. Diagrams showing the division of the cloaca into the urogenital sinus and anorectal canal. Note that the mesonephric duct is gradually absorbed into the wall of the urogenital sinus and that the ureters enter separately. (*A*) End of the 5th week, (*B*) 7 weeks, and (*C*) 8 weeks.

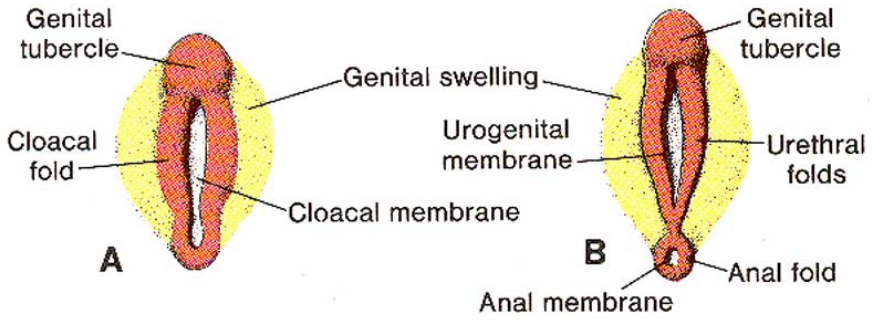


Figure 15-26. The indifferent stage of the external genitalia. (*A*) At approximately 4 weeks, and (*B*) at approximately 6 weeks.

The genital tubercle forms the glans or clitoris; the genital/urethral folds the shaft of the penis or the labia minora; and the genital swelling the scrotum or the labia majora.

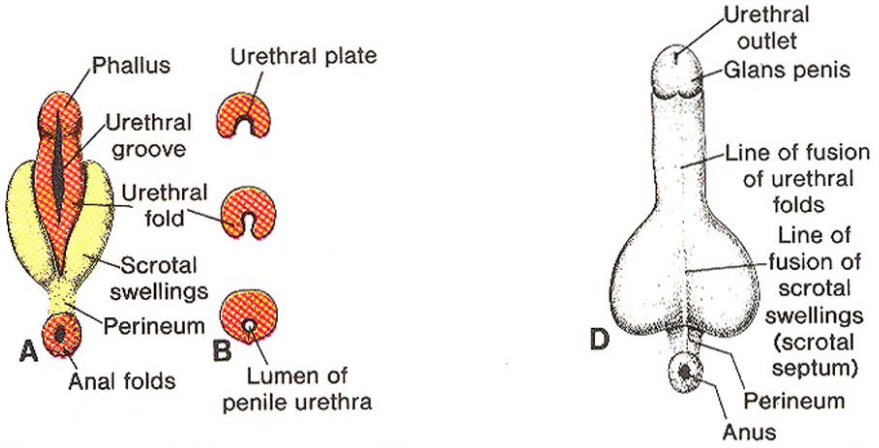


Figure 15-27. (*A*) Development of the external genitalia in the male at 10 weeks. Note the deep urethral groove flanked by the urethral folds. (*B*) Transverse sections through the phallus during the formation of the penile urethra. The urogenital groove is bridged over by the two urethral folds. (*D*) In the newborn.

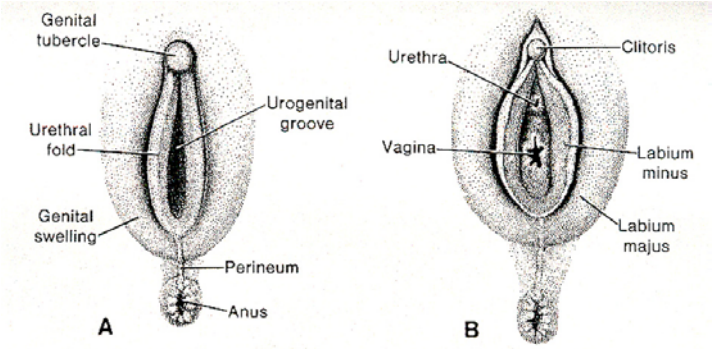
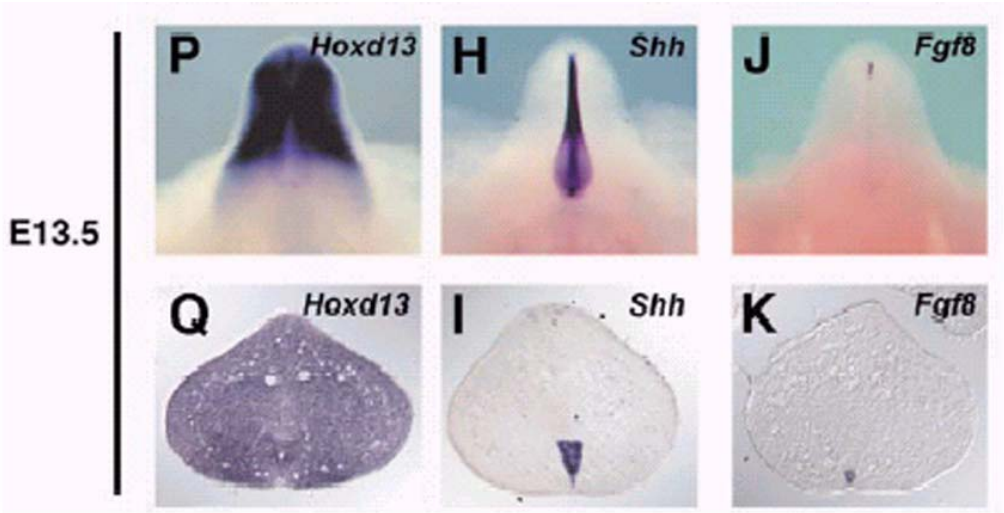


Figure 15-29. Development of the external genitalia in the female at 5 months (*A*), and in the newborn (*B*).

Hox genes are expressed in genital tubercle, and their expression is driven by growth factors secreted by the urethral epithelium.

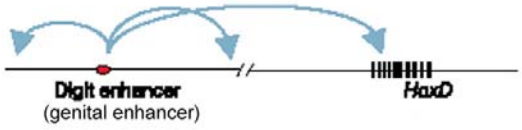


From Perriton et al., Dev. Biol. *247*, 26-46 (2002)

Hoxd 13 mutant mice have small penial bone (os penis). Hoxa13;Hoxd13 double mutants have no genital tubercle, hands or feet. Sonic hedgehog mutant mice no genital tubercle at all. The hypospadias in Hand-Foot-Genital Syndrome is caused by failure of urethral folding. A more frequent cause of hypospadias in humans is hormonal teratogenesis during pregnancy (e.g., diethylstilbestrol).

The Hand and Foot phenotype is caused is caused by the Hox expression in limb development. Hox 13 is expressed in digit 1. The sequence of Hox expression in the digits is regulated by a digit enhancer located 300 kilobases away. The gene closest to the enhancer is expressed at the highest levels ("quantitative colinearity"). Surprisingly, deletion of Hox 13 had less phenotypic effect than an inactivating mutation that left the Hox 13 gene in its place. The same enhancer used for the digits is also used to deploy Hox genes sequentially in the genital tubercle. We will discuss the work of Denis Tubule

The use of Hox genes for the anteroposterior axis, limb development and genitalia has important evolutionary consequences. Regulation by a common digit-genital enhancer helps explain why some Hox genes are conserved as linear arrays of genes in the DNA, and how developmental mechanisms can place constraints on Evolution.



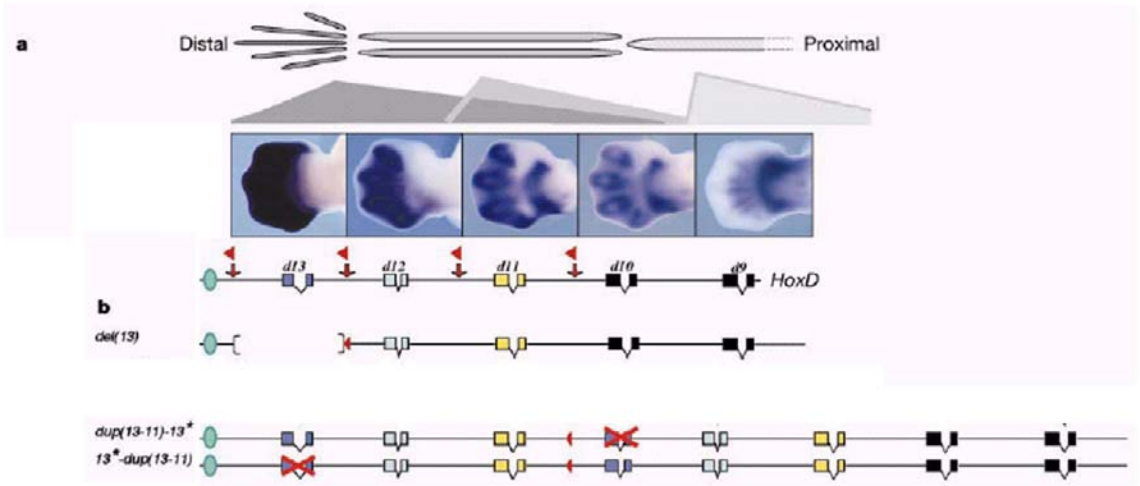


Figure 1 Collinearity in developing limbs. **a**, Scheme of a forelimb skeleton (top) and expression of five of the most 5′ *Hoxd* genes in limb buds at day 12.5 (bottom). Following their genomic order, expression of these genes is progressively restricted to the most distal part of the developing limbs (grey triangles). *Hoxd* genes are also expressed following a gradient of transcriptional efficiency within the distal domain itself, such that *Hoxd13* is most strongly expressed, whereas genes located in a more 3′ direction display progressively less robust expression levels (quantitative collinearity[8]). Whereas only *Hoxd13* is expressed in presumptive digit I, *Hoxd9* is barely detectable in any of the digits. **b**, Allelic series.

Denis Duboule
Nature *420*, 145-150 (2002)

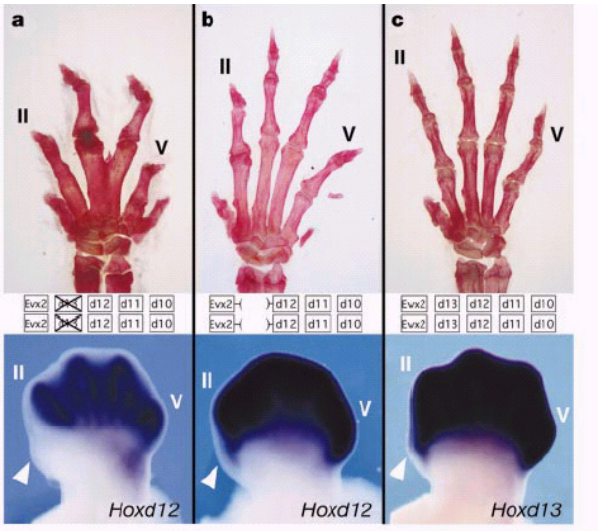


Figure 2 Targeted deletions induce regulatory reallocations. Comparison between digit phenotypes (top) and expression of 5′ *Hoxd* genes (bottom) associated with either the disruption or the deletion of the corresponding loci. Crosses indicate gene inactivation; brackets indicate deletion breakpoints. **a**, Inactivation of *Hoxd13* leads to an overall reduction in the size of digits, partial fusion between digits III and IV, and a supernumerary digit in most cases[23]. **b**, In contrast, deletion of the same locus has little effect (compare with control in **c**).

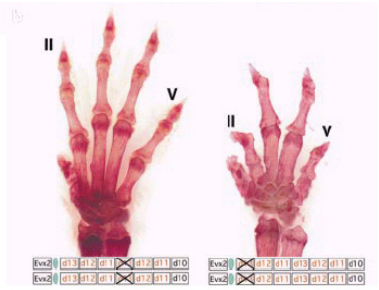


Figure 4 Supernumerary loci titrate the effect of the digit enhancer in a polar fashion.

Conclusion: Molecular Medicine can explain the pathogenesis of complex of diseases such as Hand-Foot-Genital Syndrome.

Pax-6 induces eyes

Up to this moment we have only discussed homeobox genes of the *Antennapedia*-type (*Hox* genes) of which there are about 40 in the mammal. But this is only the tip of the iceberg. The vast majority of homeobox genes correspond to the non-*Ant*-class. *Drosophila*, for example, contains 113 homeobox genes in its genome. These other homeobox genes form families of transcription factors that have names such as *PAX*, *POU*, *Oct*, *Lim*, *Emx*, *Otx*, *Msx* and are all transcription factors.

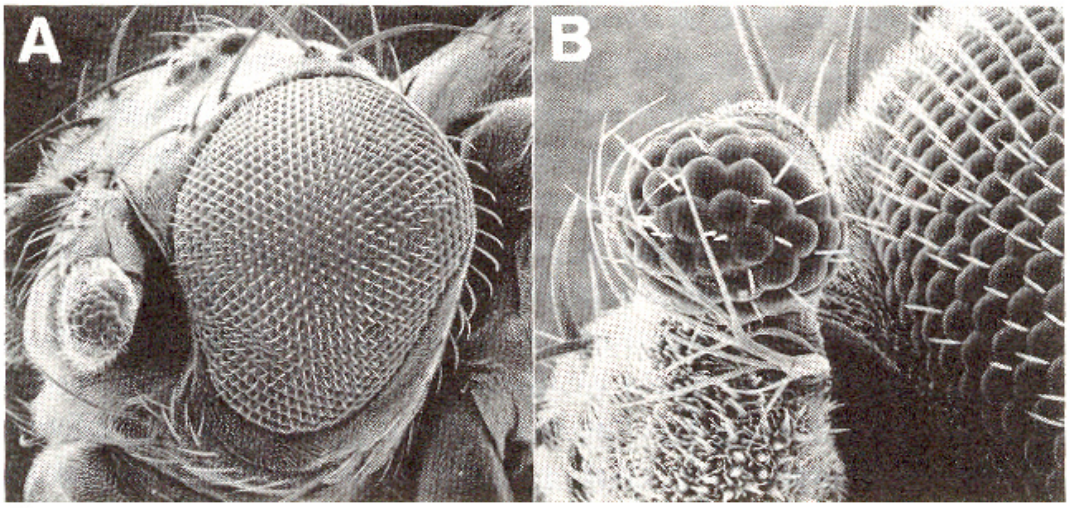


Figure 13.7
Eye induced on the antenna of a fly with the mouse Pax-6 (Small eye) *gene: (A) overview; (B) higher magnification. Scanning electron micrograph by G. Halder and A. Hefti.*

One of the most interesting ones is *Pax-6*, which encodes the human Aniridia gene. *Pax-6* is the sixth member of a family of homeobox genes related to *Drosophila* paired. Aniridia is a human mutation in which the iris is lost and the retina is hypoplastic in heterozygotes (OMIM # 106210). In homozygotes there is fetal lethality and complete loss of eyes and olfactory epithelium. The aniridia gene was cloned by positional cloning and found to be the human homologue of mouse *Pax-6*. Mouse *Pax-6* has spontaneous mutations, called *small-eye*, which as homozygotes also lack eyes and nasal epithelium. In *Drosophila*, a mutation called *eyeless* has been known since 1915. The group of Walter Gehring cloned this gene and found it to be the homologue of *Pax-6*. When expressed artificially in transgenic fruit flies, the eyeless gene gave rise to ectopic eyes in places such as the antenna, haltere or leg. What is more, ectopic expression of the mouse *Pax-6* in *Drosophila* also led to the formation of ectopic fly eyes. This led to the surprising realization that the eyes of flies and humans come from an ancestral photosensitive cell already present in a common ancestor 500 million years ago, that had this regulatory gene in place (Halder et al., 1995). Thus, the eyes of all metazoans arose once in evolution and were then modified by natural selection.

The eye seems to have evolved before the brain (jellyfish) and there are eye structures even in unicellular dinoflagelates:

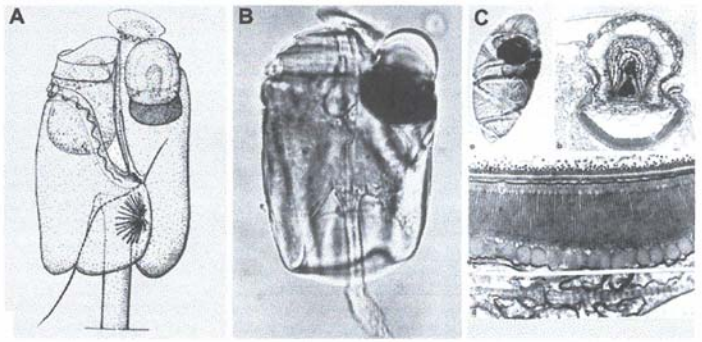
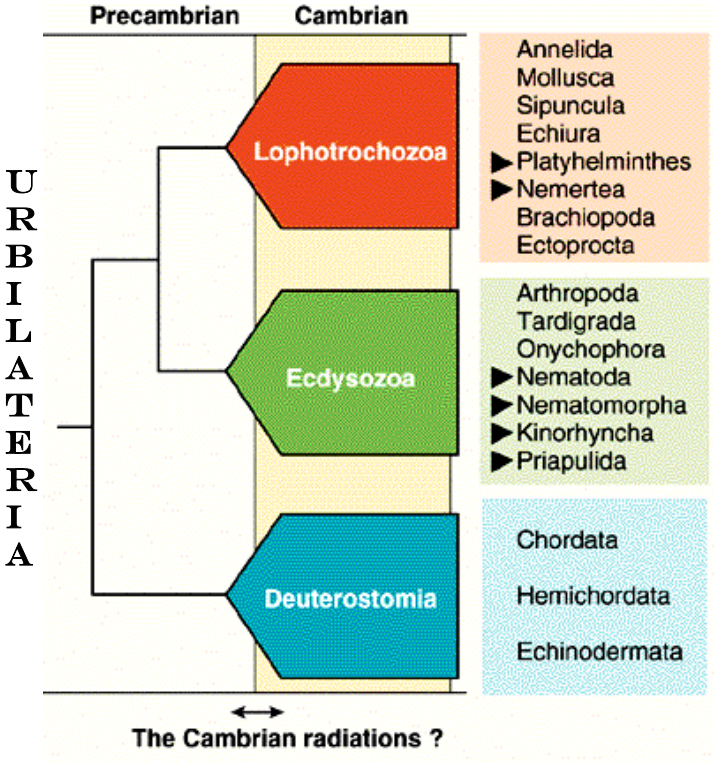


Fig. 11. Eye organelle of the unicellular dinoflagellate *Erythropsis pavillardi*. **(A)** *Schematic drawing.* **(B)** *Light micrograph showing the lens and the shielding pigment.* **(C)** *Stacked membranes resembling the arrangement in photoreceptor cells in the retina of multicellular organisms. After Greuet, 1965. (Courtesy of Marie-Odile Soyer).*

The common ancestor

The common ancestor of protostomes and deuterostomes (called Urbilateria for Ur = primaeval, bilateria = bilateral animal) was a complicated creature indeed.



Jim Lake's new phylogenetic tree (UCLA)

The Urbilateria had:

1) Hox gene complexes
2) A dorso-ventral system provided by *sog/chd* and *dpp/BMP*
3) Genes regulating formation of photoreceptors (*Pax-6*)
4) A contractile blood vessel (with tinman/Nkx2.5 and MEF2 transcription factors).
5) Segmentation genes?

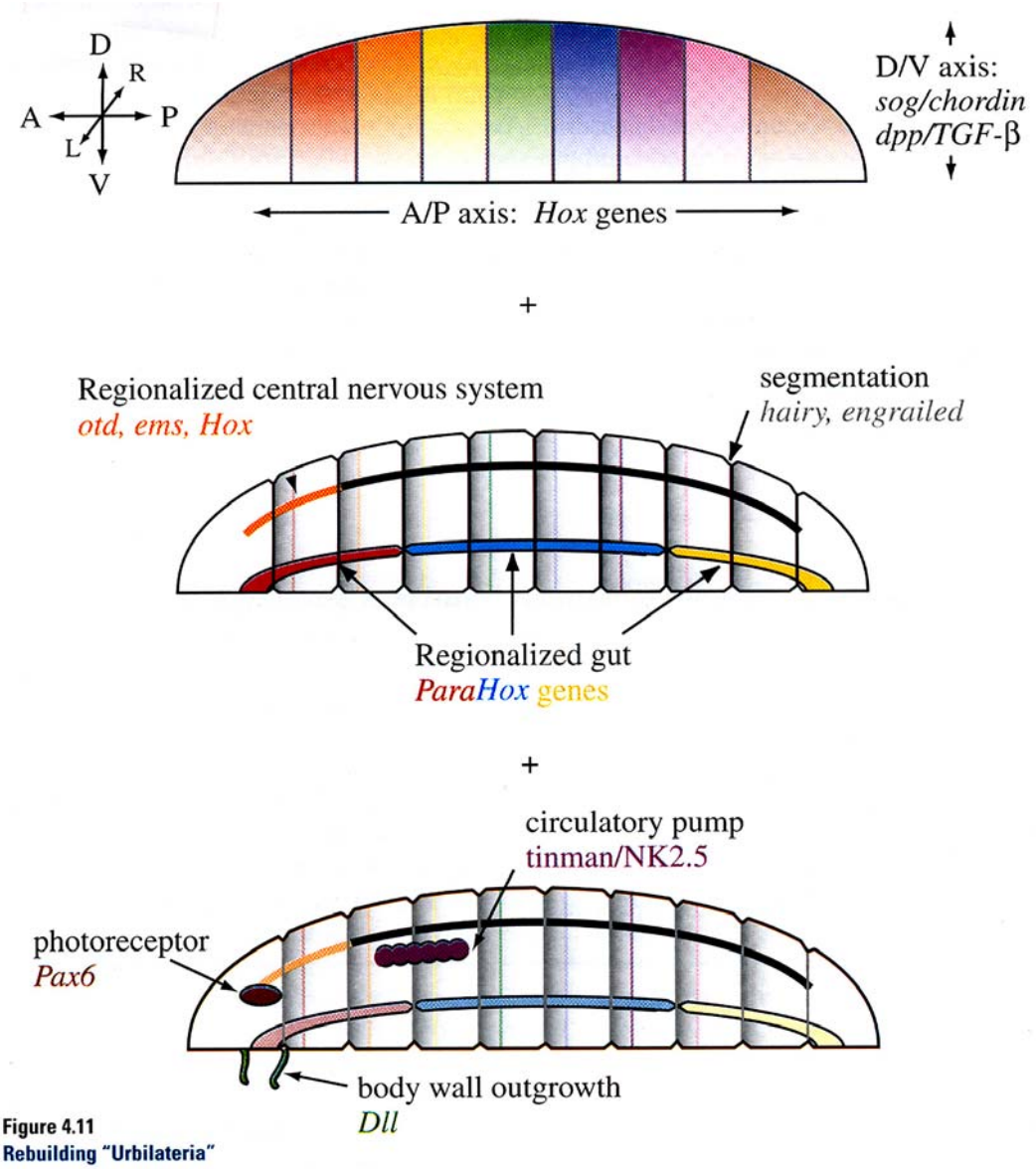


Figure 4.11
Rebuilding "Urbilateria"

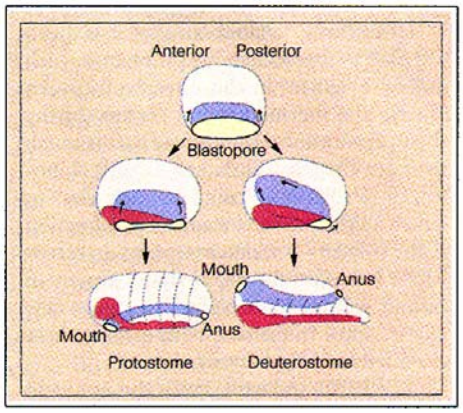
The possible features of the common bilaterian ancestor are deduced from the conservation of genes and their developmental functions between arthropods (*Drosophila*) and vertebrates (mouse). (**top**) Patterning of the D/V axis may have been controlled by ancestral genes of the short gastrulation (*sog*)/chordin and TGF-β families. The A/P axis was probably subdivided by nested, overlapping domains of *Hox* gene expression. (**middle**) Different tissue layers were regionally patterned along the A/P axis, including the gut (*ParaHox* genes) and the nervous system [*orthodenticle* (*otd*), *empty spiracles* (*ems*), *Hox*]. Segmentation may have evolved under the regulation of ancestral *hairy* and *engrailed* genes. (**bottom**) Primitive versions of a photoreceptor organ, a circulatory pump, and an outgrowth/appendage might have been present in the bilaterian ancestor, under the regulatory control of the ancestral *Pax6, tinman,* and *Dll* genes.

These and other common elements suggest that all Bilateria are derived from a complex ancestor (that existed over 550 million years ago). This represents a major change in evolutionary thinking, suggesting that the constraints imposed by the previous history of species played a greater role in the outcome of animal evolution than anyone would have predicted until recently.

Evolving protostomes and deuterostomes

Zoologists classify bilateral animals that possess a coelomic cavity into two fundamental groups: the protostomes – such as arthropods, annelids and molluscs; and the deuterostomes – such as echinoderms and chordates (chordates include ascidians, amphioxus and vertebrates). In protostomes, the mouth is formed at or close to the initial site (proto, first; stomo, mouth) of the blastopore, the site at which the endo-mesoderm, shown here in mauve, involutes into the interior of the embryo. In many protostomes the anus is formed at the blastopore as well. In deutero-stomes, the mouth is formed secondarily (deutero, second) by a perforation of the ectoderm, and the anus is formed at or close to the site of the original blastopore. In addition, most protostomes have a ventral nerve cord which is traversed by the mouth and connects to a supraoesophageal ganglion (brain) in the anterior. In most deuterostomes the central nervous system (CNS, in red) is dorsal and is not traversed by the gut. There are other differences, but these suffice for the present discussion.

Developmental studies suggest that protostomes and deuterostomes had a



common ancestor that was complex and segmented, raising the question of how such different body plans evolved. We of course do not know how the adult Urbilateria looked; it could have had either an open gut/blastopore (not shown) or it could have resembled an adult protostome or deuterostome (bottom diagrams). However, by modifying the blastopore during gastrulation it is possible to envisage how the transition could have happened, provided one assumes that the CNS is formed near the blastopore[13].

As shown in the figure, in protostomes such as annelids the slit-like ventral blastopore gives rise to the mouth and anus by closing along its central portion. The fusion of the lateral blastopore lips results in the dorso-ventral inversion of the CNS, generating posteriorly a nerve cord ventral to the gut, and anteriorly a supraoesophageal ganglion (bottom left). In deuterostomes the anterior part of the blastopore, corresponding to the mouth, does not form at all and endomesodermal involution takes place from the posterior, eventually leading to formation of the anus. The CNS is induced in nearby ectoderm by proteins secreted by the invaginating endomesoderm, and by the end of gastrulation the deuterostome mouth is perforated secondarily, with the gut and CNS remaining in different sides of the animal throughout their length. The deuterostome depicted here is a frog tadpole, which if inverted would adopt its normal dorsoventral position. Thus, although the Urbilateria ancestor was complex in its adult form (presumably having segments, heart, eyes and appendages), the potential to give rise to widely divergent body plans resided in its mechanisms of embryonic development. **E.M.De R.**

NATURE | VOL 387 | 1 MAY 1997

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