

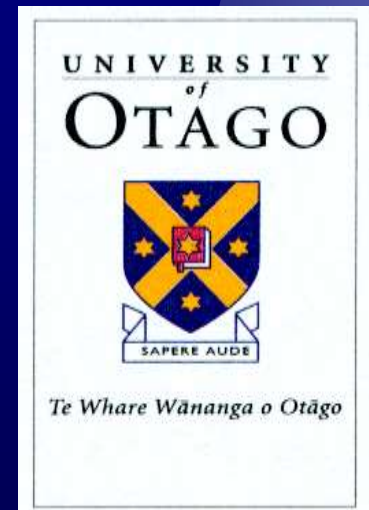
Genes and Development

By Murray C Meikle

Biological Foundations of Orthodontics
and Dentofacial Orthopaedics

Seminar 2

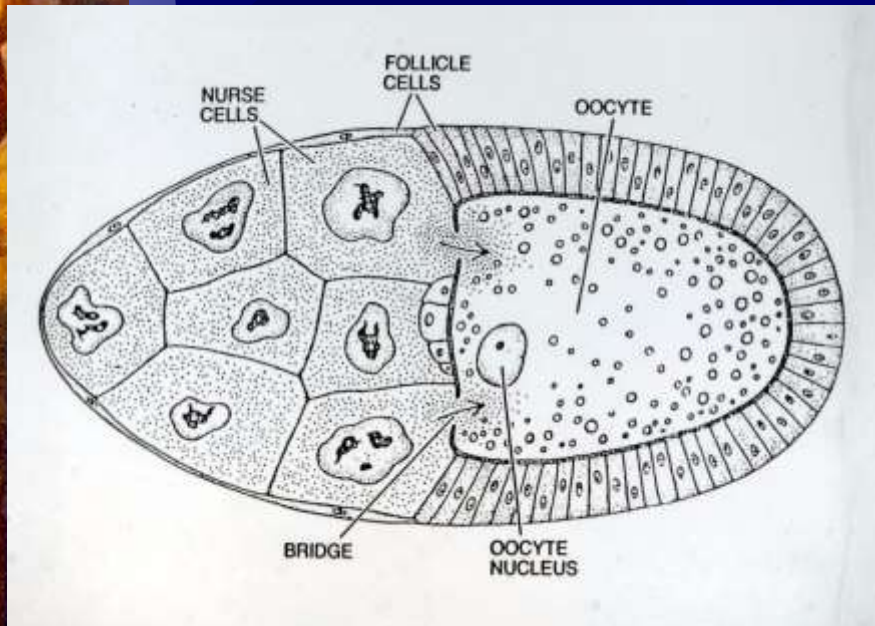
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Drosophila genetics

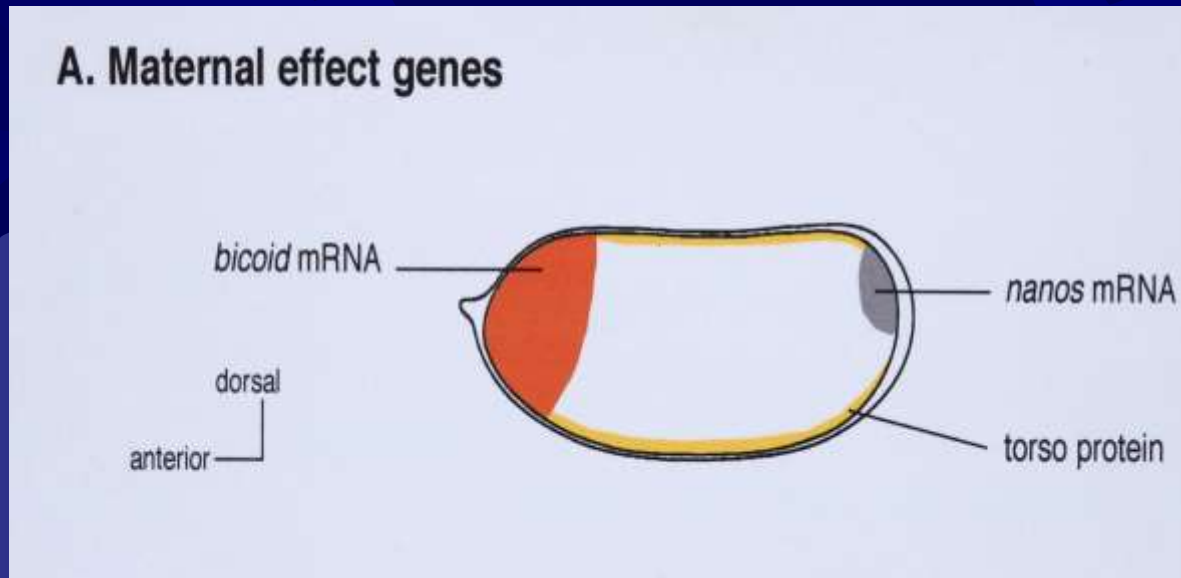
- ✿ To understand the role of genes in the control of vertebrate morphogenesis (the process by which embryonic form and structure is achieved) it is necessary to look at the development of the fruit fly *Drosophila melanogaster* which has become the reference model for developmental genetics.
- ✿ *Drosophila* genetics is important because the body axes of developing mammals are specified by the same or homologous genes that specify the *Drosophila* body axes.

Maternal effect genes



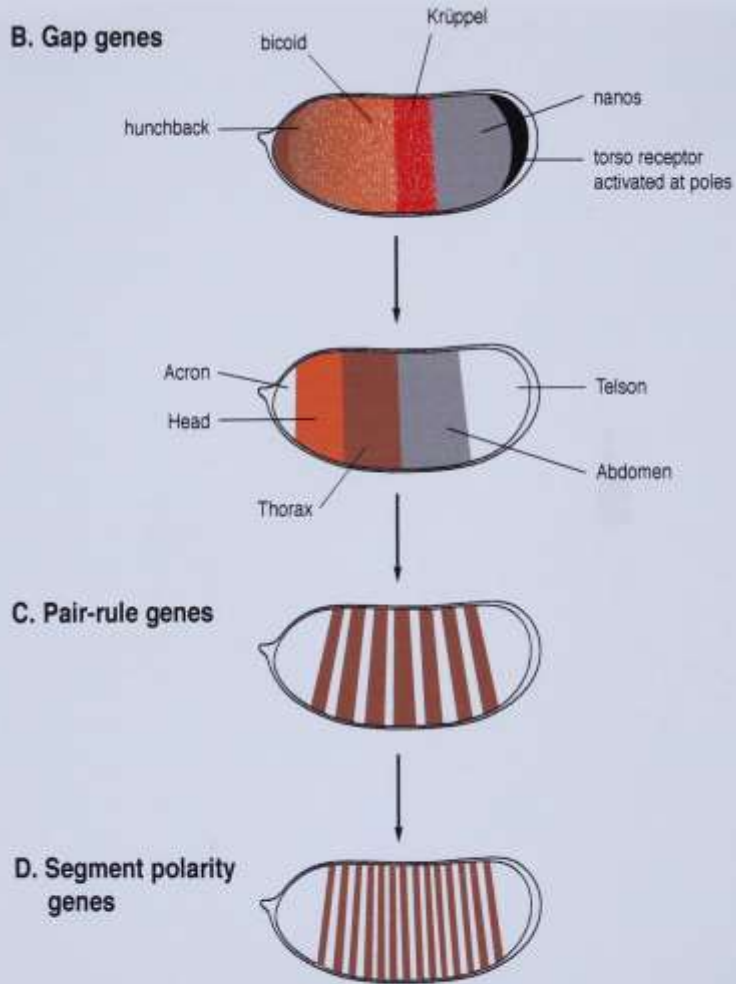
- The polarity and pattern of the *Drosophila* embryo are determined by a small number of maternally-derived genes termed maternal effect genes.
- These produce messenger RNAs that are deposited in different regions of the egg during oogenesis and encode diffusible 'form generating molecules' or morphogens which act as local determinants of cell differentiation.
- This means that the primary axes of the embryo are already determined when the egg is fertilized, in marked contrast to vertebrates.
- From Gehring (1985), *Scientific American* 235, 137-146.

Specification of anteroposterior axis



- The anteroposterior axis is specified by the *bicoid* and *nanos* genes. Their mRNAs are synthesized in the nurse cells of the follicle and deposited at the anterior and posterior poles. Their proteins bicoid and nanos diffuse away from the poles forming gradients and acting as morphogens.
- The terminal gene *torso* which encodes a tyrosine kinase receptor is expressed ubiquitously, but only activated at the poles.
- From Meikle (2002), *Craniofacial Development, Growth and Evolution*.

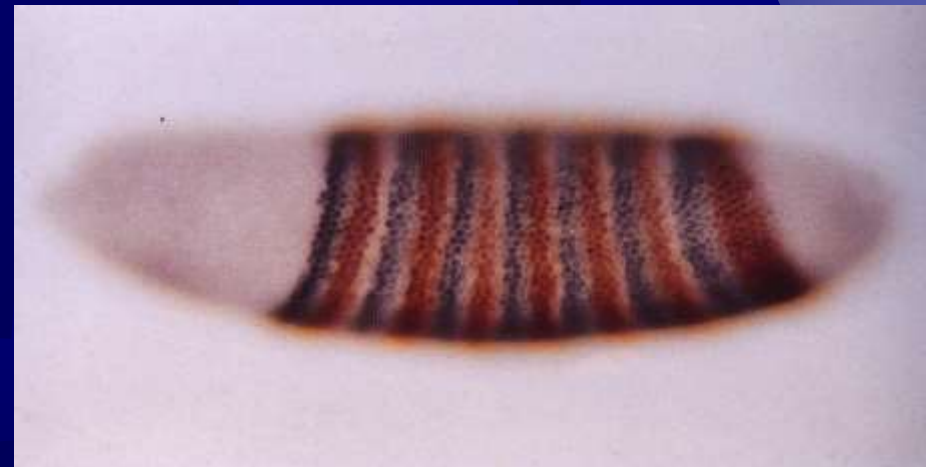
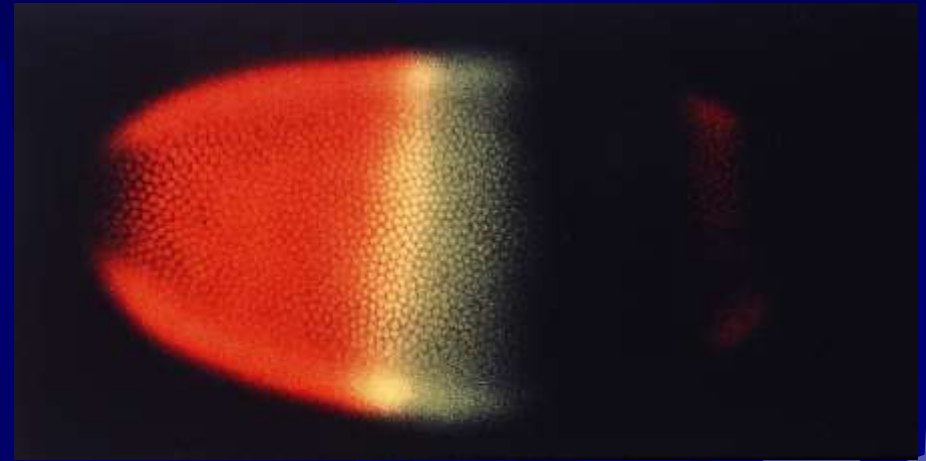
Segmentation genes divide *Drosophila*



- ☀ Maternal effect genes act as transcriptional regulators of zygotic segmentation genes to generate complexity. These include:
- ☀ Gap genes such as *hunchback* and *Krüppel* (B) that define broad territories in the embryo.
- ☀ Pair-rule genes (C) which are the first zygotic genes to be expressed in a periodic pattern of 7 stripes in alternating segments.
- ☀ Segment polarity genes (D) further subdivide the seven segments into 14 distinct bands.
- ☀ From Meikle (2002), *Craniofacial Development, Growth and Evolution*.

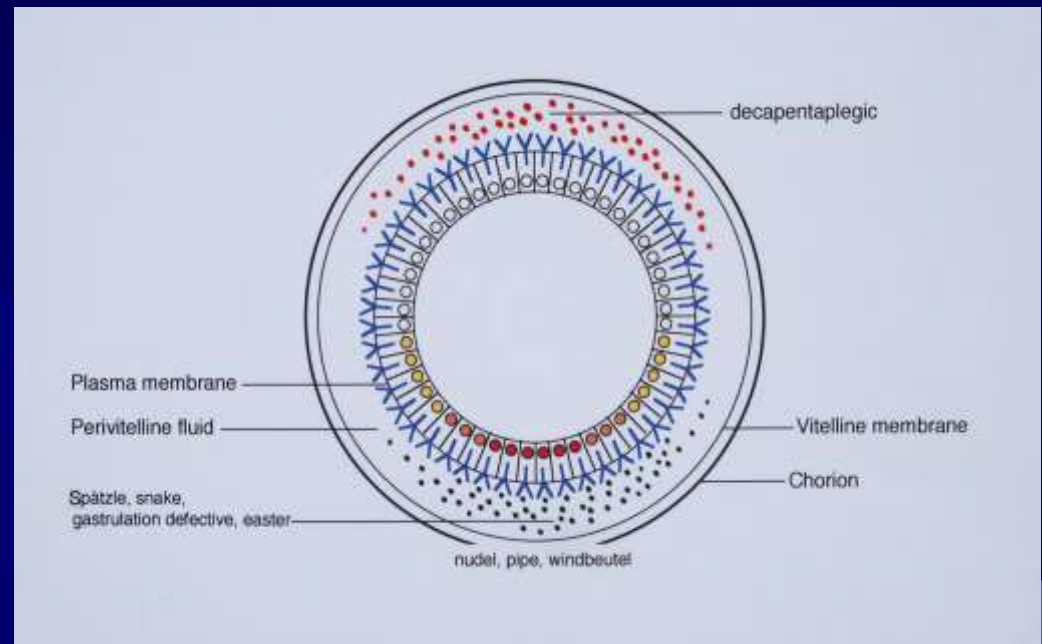
Segmentation genes

- ✦ Expression of the gap genes *hunchback* (red) and *Krüppel* (green). Mutations in these genes cause multiple adjacent segments to be missing.
- ✦ Expression of the pair-rule genes *even-skipped* (grey) and *fushi tarazu* (brown) in alternating parasegments
- ✦ From Lawrence (1992), *The Making of a Fly: The Genetics of Animal Design*.



Dorsoventral specification

Modified from Steward and Govind (1993), *Current Opinion in Genetics and Development* **3**, 556–561.



- ✿ The dorsoventral axis is patterned by different maternal genes that depend on activation of toll *receptors* (blue Y) in the plasma membrane along the ventral midline by Spätzle protein (black).
- ✿ Activation of toll receptors establishes a gradient of nuclear dorsal protein (red circles) with maximum concentration ventrally.
- ✿ Cells that lack dorsal protein liberate decapentaplegic (red dots) into the dorsal vitelline space. The vertebrate homologue of dpp is the bone morphogenetic protein BMP-4.

Are vertebrates upside-down insects?

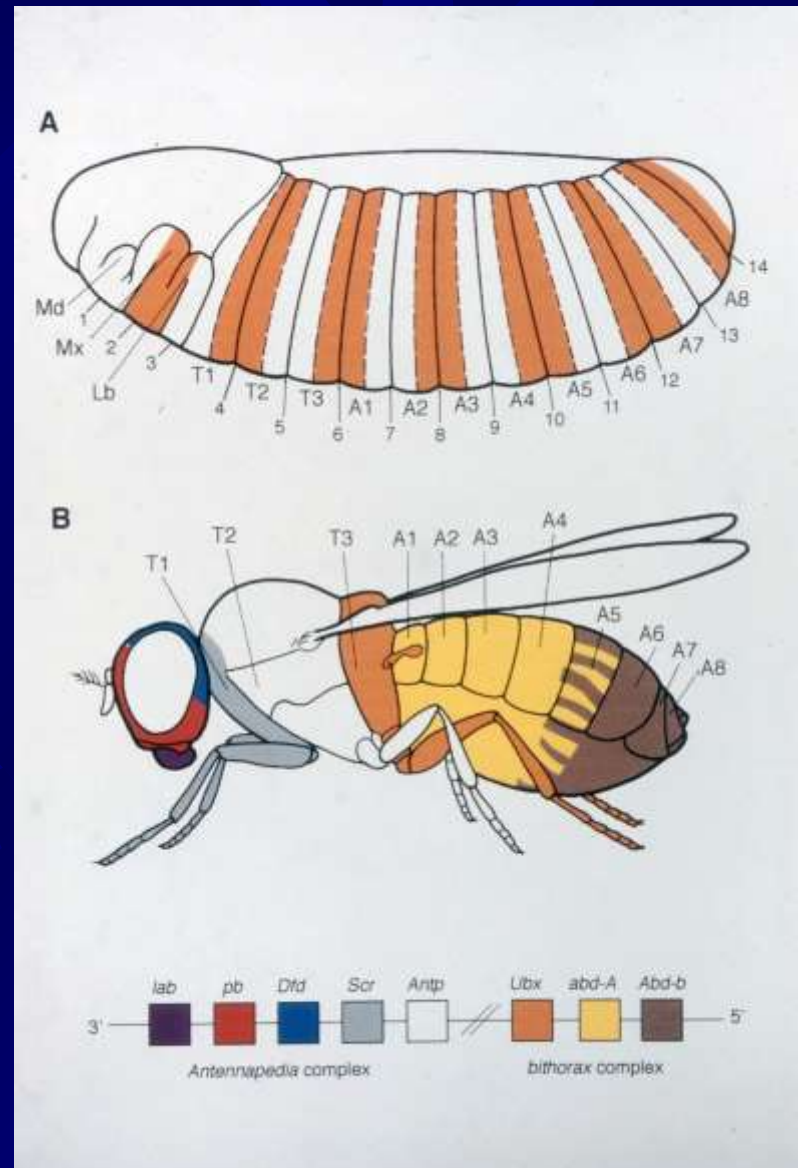
- ✱ Unlike vertebrates in which the neural tube lies along the dorsal midline, in *Drosophila* the nerve cord is ventral. In 1822 the French zoologist Étienne Geoffroy Saint-Hilaire proposed that vertebrates and arthropods have a common body plan or Bauplan, but with an inverted dorsoventral axis.
- ✱ This provoked great controversy at the time, but at the molecular level it seems he was right; the answer lies in the expression of dpp and Bmp-4 which are both secreted proteins of the TGF- β superfamily of growth factors.
- ✱ In *Drosophila*, as described in the previous slide, dpp is produced in the dorsal region of the blastoderm embryo, where it is blocked by a protein called sog (short gastrulation); in the frog *Xenopus*, BMP-4 is synthesized ventrally and opposed by chordin (the vertebrate homologue of sog). Sog/chordin induce neurulation by blocking the effects of dpp/BMP-4.

Homeotic selector genes specify segment identity

- ✱ Segmentation genes may be sufficient to specify the body pattern of annelids in which most segments are alike; the segments in insects, however, differ from one another.
- ✱ In *Drosophila* once the segments are formed they receive a specific identity according to their position along the AP axis, *i.e.* legs or antennae, thorax or abdomen.
- ✱ Segment identity is specified by homeotic selector genes which are expressed in a segment-specific manner. Defects in these genes which result in the appearance of recognizable appendages in inappropriate segments is termed homeosis [Gr. *homoiosis*, likeness, resemblance].

The homeotic complex (HOM-C)

- Homeotic selector genes are clustered in two complexes, *Antennapedia* and *bithorax* collectively known as the homeotic complex (HOM-C).
- The *Antennapedia* complex (ANT-C) contains 5 homeotic genes and specifies the morphological characteristics of anterior parasegments up to number 5.
- The *bithorax* complex (BX-C) contains 3 homeotic genes which specify parasegments 5-14.
- From Meikle (2002), *Craniofacial Development, Growth and Evolution*.



Homeotic transformation



- Homeotic mutants in *Drosophila* produce deviations in the head and body segments.
- A typical mutant phenotype called *Ultrabithorax* develops four wings by substituting a second pair of wings for the halteres in the third thoracic segment. Other mutants, for example *Antennapedia*, substitute legs in place of antennae, and in *Ophthalmoptera*, wings in place of eyes.
- Many of these mutants are essentially manifestations of earlier evolutionary characteristics; it would appear that each segment is capable of producing a variety of appendages with the appropriate signal.

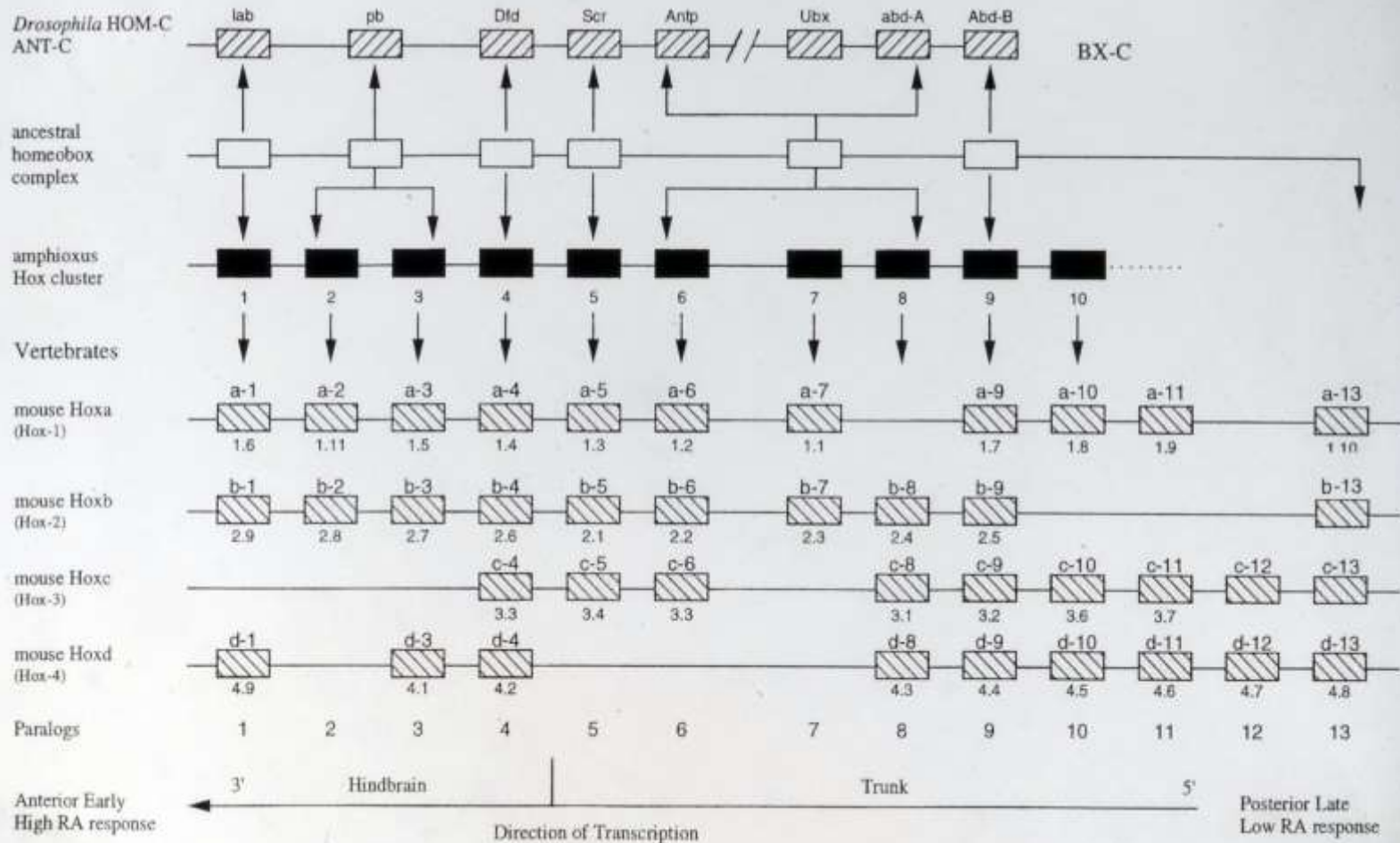
The homeobox and homeodomain

- Homeotic transformations result from mutations in a highly conserved DNA sequence termed the homeobox (so-called because it was discovered independently in two homeotic genes *Ultrabithorax* and *Antennapedia*).
- The homeobox is a nucleotide sequence of 180 base pairs encoding a 60–61 amino acid DNA-binding domain within the protein product termed the homeodomain.
- Homeodomain-containing proteins in all known cases act as transcription factors regulating the activity of other genes (referred to as downstream genes) within the genome. They occur in all animal phyla.

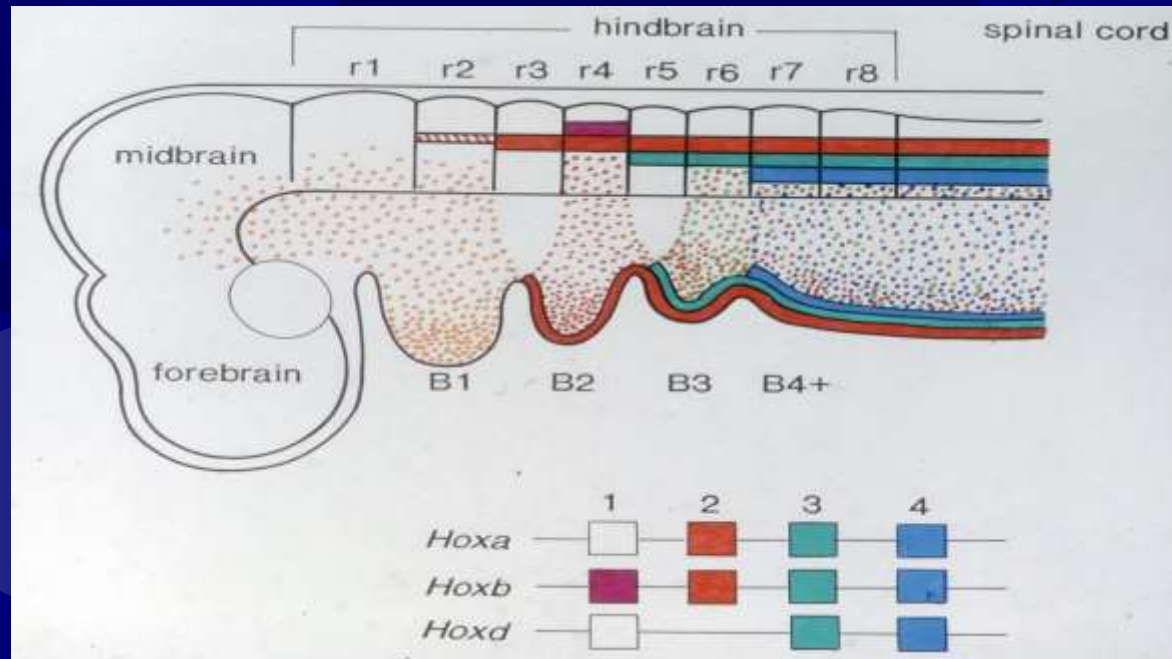
The *Hox* gene family

- Vertebrate homeotic genes that are the evolutionary homologues of *Drosophila* HOM-C are known as *Hox* genes.
- The mouse and human genomes consist of 4 *Hox* complexes (A–D) totalling 39 genes which probably arose from a common ancestral gene (see next slide for the relationship between vertebrate *Hox* genes and the *Drosophila* HOM-C complex).
- This primordial homeobox gene via a series of duplication and divergent steps, generated an ancestral homeobox complex which predated the evolutionary separation of vertebrates and arthropods more than 500 million years ago.
- The homeotic genes that regulate development in *Drosophila* have been conserved during evolution and adapted to generate the complexity of form and function that characterizes more advanced organisms including mice and men.

Drosophila HOM-C, amphioxus, and vertebrate Hox gene clusters



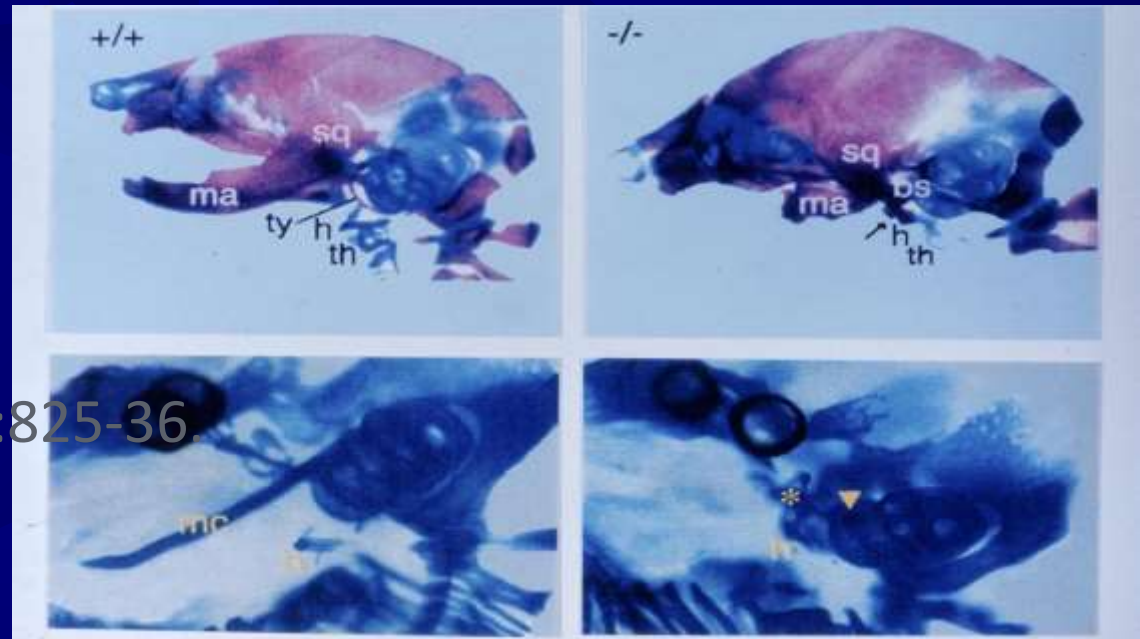
The Branchial *Hox* code



- ✦ In the pharyngeal arches neural crest cells are positionally specified by classical *Hox* genes along the anteroposterior axis in a manner analogous to their counterparts in *Drosophila*.
- ✦ Populations of neurogenic and mesenchymal crest cells carry this code as they migrate to the periphery, which accounts for the co-ordination of rhombomeric and pharyngeal segmentation.
- ✦ Modified from Krumlauf (1993). *Trends in Genetics* 9, 106–112.

Epithelial–mesenchymal interactions are required for differentiation

From Yanagisawa *et al.* (1998), *Development* 125, 825–36.



- ✿ Interactions with embryonic epithelia are required for neural crest cell differentiation into cartilage, bone and dentine.
- ✿ The importance of interactions with epithelia can be seen in this figure in which the targeted disruption of two members of the endothelin gene family has severely affected mandibular development; ma, mandible; mc, Meckel's cartilage; h, hyoid; sq, squamosal bone.

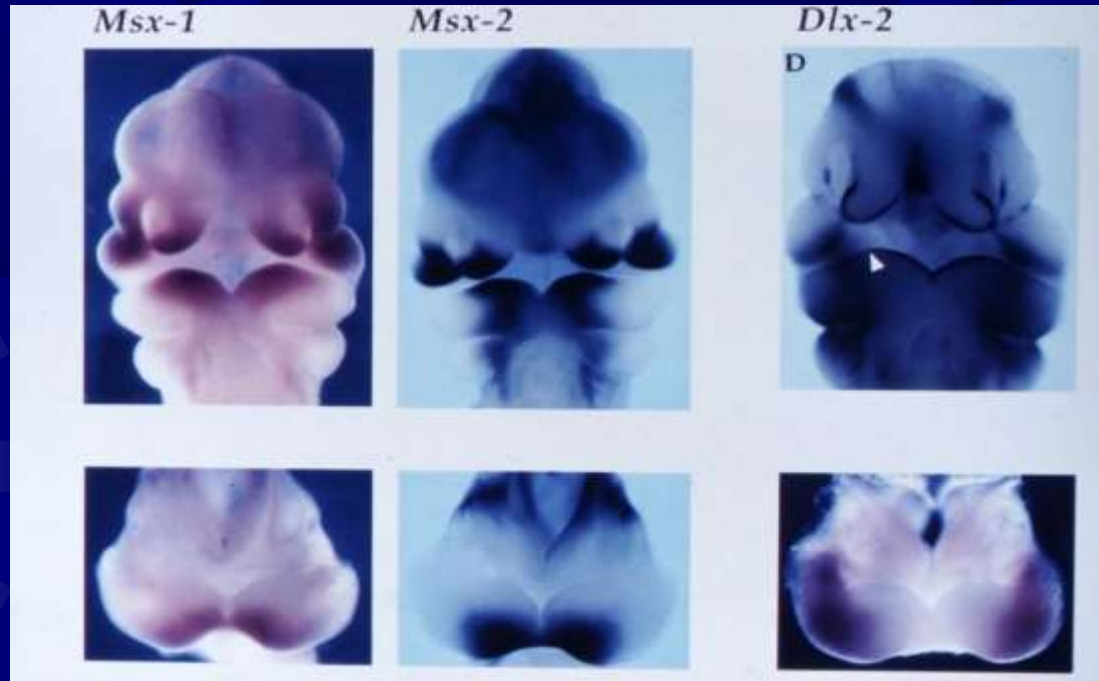
Hox gene mutations

- Loss-of-function mutations in mice have been shown to produce abnormalities resembling certain human syndromes.
- Neural crest cells from r6 migrate into the 3rd and 4th branchial arches. Targeted disruption of the *Hoxa-3* gene produces mutants with a severely deficient or absent thymus, thyroid and parathyroid glands, shortened neck vertebrae and heart malformations, a phenotype resembling the DiGeorge syndrome in *Homo sapiens*.
- Mutations of *Hoxb-1* expressed in r4 in which the motor neurones of the facial (VII) nerve are located, result in a phenotype that resembles Möbius syndrome (congenital facial paralysis).

Positional specification of the first branchial arch

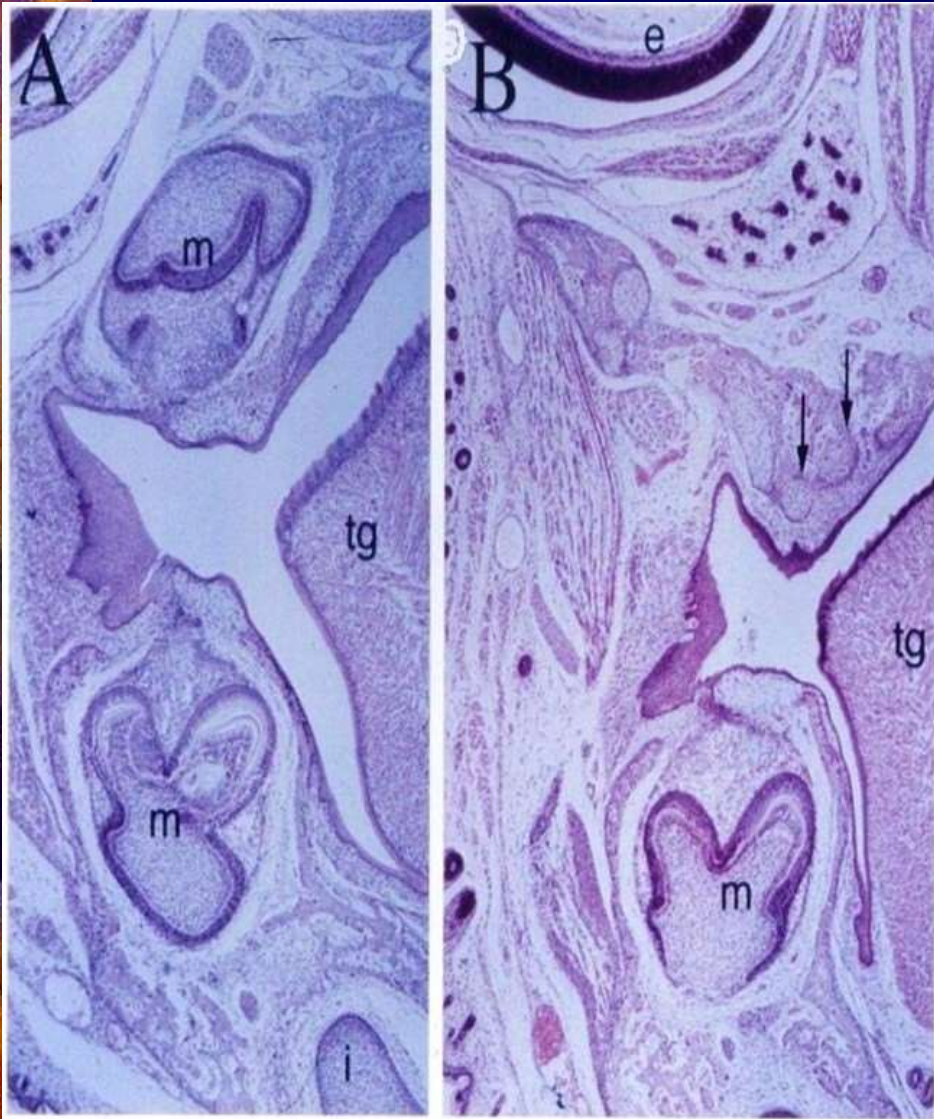
- ✱ Since *Hox* genes (with the exception of *Hoxa-2* which is weakly expressed in r2) are not expressed in crest cells migrating from rhombomeres r1 and r2 into the first branchial arch, neural crest cells from r1 and r2 are positionally specified by homeobox-containing genes that are not related to *Drosophila* HOM-C.
- ✱ Such genes are responsible for the dorsoventral specification of structures within each arch and include homeobox transcription factors such as *Msx-1* and *Msx-2*, the *Dlx* family, and *Prx-1* (formerly *Mhox*).

Msx and *Dlx* genes in craniofacial patterning



- ✿ Expression patterns of *Msx-1* and *Msx-2* in the distal (ventral) regions of the maxillary and mandibular processes, and *Dlx-2* in the proximal (dorsal). Their role in tooth development will be discussed in Seminar 7.
- ✿ Homozygous *Msx-1* (-/-) mouse mutants have a cleft secondary palate and a failure of tooth and alveolar bone development in the premaxilla and anterior part of the mandible.
- ✿ From Thomas and Sharpe (1998). *European Journal Oral Sciences* **106**, 48–54.

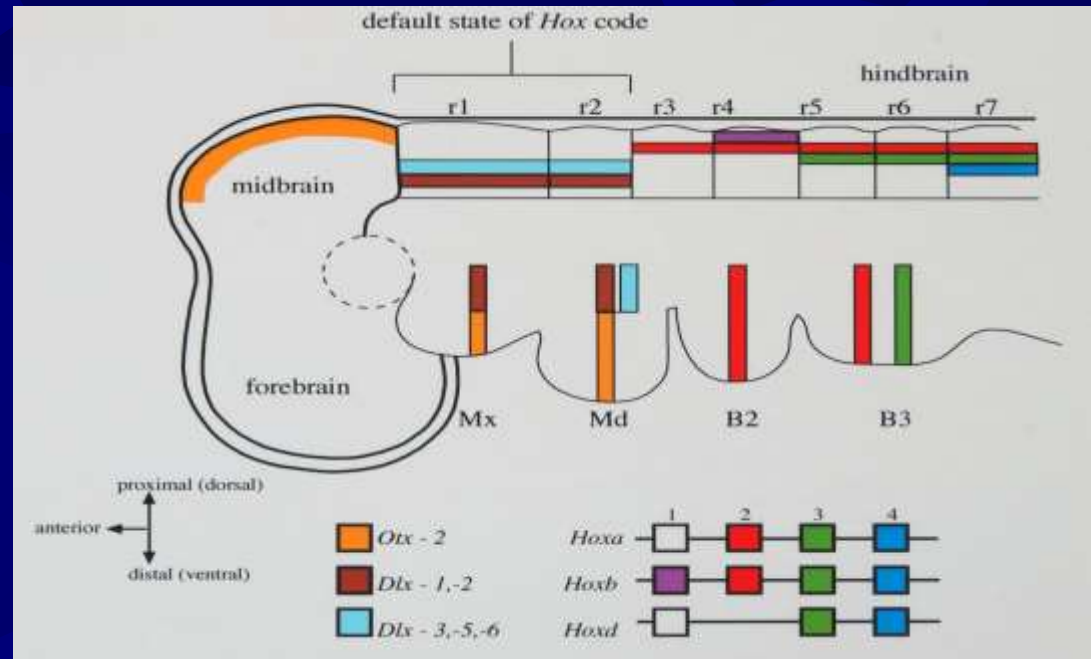
Dlx genes in craniofacial patterning



- *Dlx* genes are the vertebrate homologues of the *Drosophila* *distal-less* (*dll*) gene.
- In vertebrates there are six known *Dlx* genes. In the branchial arches *Dlx-1*, *-2*, *-3*, *-5*, *-6* are expressed in the proximal mesenchyme of the mandible and 2nd (hyoid) arch. In the proximal region of the maxilla only *Dlx-1*, and *Dlx-2* are expressed.
- Null mutants for *Dlx-2* alone and *Dlx-1* and *-2* together as shown in figure B, result in defects of the maxilla (including absence of the molar teeth), squamosal bone and stapes.
- Courtesy of L Christensen.

Dlx and Otx gene expression

From Meikle (2002),
*Craniofacial Development,
Growth and Evolution.*



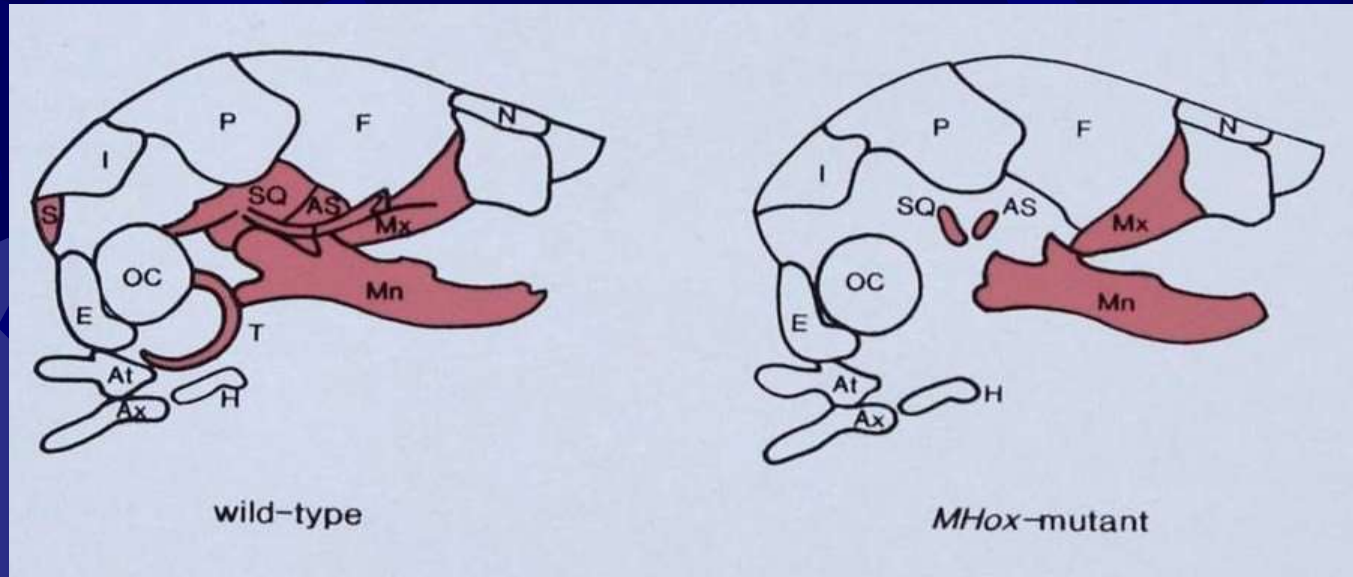
- The maxillary (Mx) and mandibular (Md) processes of the first branchial arch are populated by neural crest cells from the distal midbrain and rhombomeres r1 and r2, i.e. a different axial origin.
- Mandibular arch ectomesenchyme is therefore divided into 2 domains: (1) a proximal domain patterned by a so-called default state of *Hoxa-2* from r1 and r2 (*Dlx* genes in the above) and (2) a distal domain patterned by *Otx-2* and derived from the midbrain.

Sequence homology of *dll* and related genes

<i>Drosophila dll</i>	MRKPRTIYSSLQLQQLNRRFQRTQYLALPERAELAASLGLTQTQVKIWFQNRRSKYKMMK
	◆ ◆ ◆ ◆ ◆
<i>AmphiDll</i>	MRKPRTIYTSFQLQQLNRRFQRYQYLALPERAELAAQLGLTQTQTVKIWFQNRRSKYKMLMK
	◆ ◆ ◆ ◆ ◆ ◆ ◆ ◆ ◆ ◆
<i>Dlx-1</i>	IRKPRTIYSSLQLQALNRRFQQTQYLALPERAELAASLGLTQTQVKIWFQNKRSKFKKLMK
	◆ ◆ ◆ ◆ ◆ ◆ ◆ ◆ ◆ ◆
<i>Dlx-2</i>	VRKPRTIYSSFQLAALQRRFQKTQYLALPERAELAASLGLTQTQVKIWFQNRRSKFKKMWK

- ★ Comparison of the homeodomain amino acids of *Drosophila dll*, amphioxus *AmphiDll*, mammalian *Dlx-1* (identical in mouse and human) and *Dlx-2*.
- ★ The homeodomain is highly conserved with five substitutions (out of 61) between *dll* and *AmphiDll*, five between *dll* and *Dlx-1*, and nine between *AmphiDll* and *Dlx-1*.
- ★ From Meikle (2002), *Craniofacial Development, Growth and Evolution*.

Prx-1 (formerly *Mhox*) mutant mice

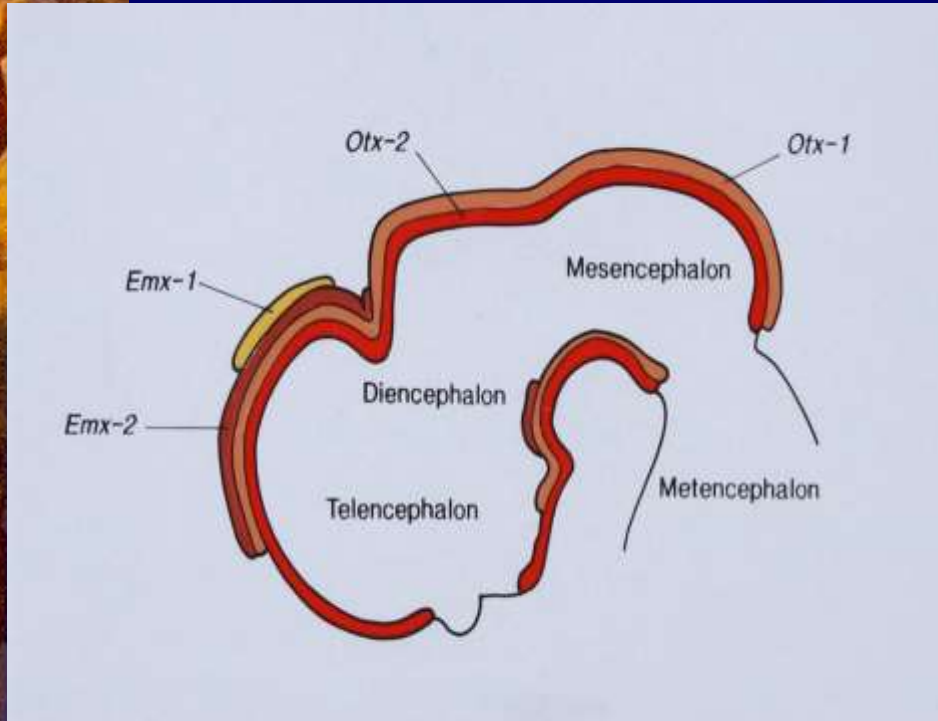


- The role of individual genes in patterning the craniofacial region has been widely studied in mice using both gain-of-function and loss-of-function mutations.
- This figure shows the effects of a *Prx-1*(-/-) loss-of-function mutation on the skull. Only remnants of the squamosal and alisphenoid bones remain in the mutant, resulting in the absence of a CMJ. The coronoid, condylar and angular processes are hypoplastic, and the tympanic, gonial and zygomatic bones are absent.
- Redrawn from Martin *et al.* (1995), *Genes and Development*. **9**, 1237–1249.

Positional specification of forebrain and midbrain neural crest

- ✱ Although HOM-C genes such as *lab* (labial), *Dfd* (deformed) and *Scr* (sex combs reduced) contribute to fly head patterning, none of these genes cover the most anterior parts of the embryo.
- ✱ The most anterior cells employ other genes including at least three types of homeobox genes (*distal-less*, *orthodenticle* and *empty spiracles*) not present in the *Hox* cluster.
- ✱ The vertebrate homeobox genes related to these fly head genes are expressed in patterns similar to those of the fly genes, suggesting evolutionary conservation of function despite the markedly different final morphologies.

Otx and Emx genes in patterning the forebrain/midbrain



- As discussed earlier, the critical anterior gene in patterning *Drosophila* is the maternal effect gene *bicoid*.
- Two of the known downstream targets of bicoid protein are *orthodenticle (otd)* and *empty spiracles (ems)*.
- In the mouse, two *otd*-related genes (*Otx-1* and *Otx-2*), and two *ems*-related genes (*Emx-1* and *Emx-2*) have been isolated.
- The overlapping domains of expression of the genes span the developing forebrain and midbrain.
- Redrawn from Finkelstein and Boncinelli (1994), *Trends in Genetics* **10**, 312–315.

Otx-2 and *Lim-1* mutants

From Shawlot and Behringer (1995),
Nature **374**, 425–430.

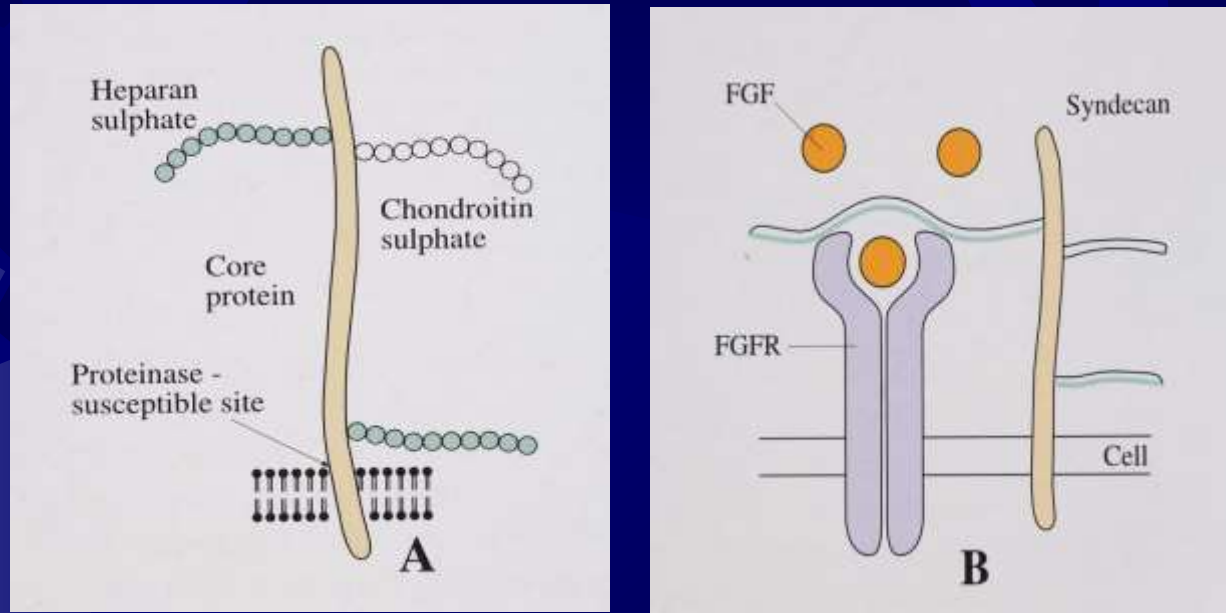


- ✦ The importance of these genes can be seen in *Otx-2* (-/-) mutant mice which lack an anterior head with no structures anterior to the alisphenoid; they are anencephalic leaving the hindbrain and trunk relatively unaffected.
- ✦ Heterozygotes (*Otx-2* +/-) are otocephalic and micrognathic. The mutant phenotype is similar to the *Lim-1* mutants shown in this figure.

Signalling molecules in craniofacial patterning

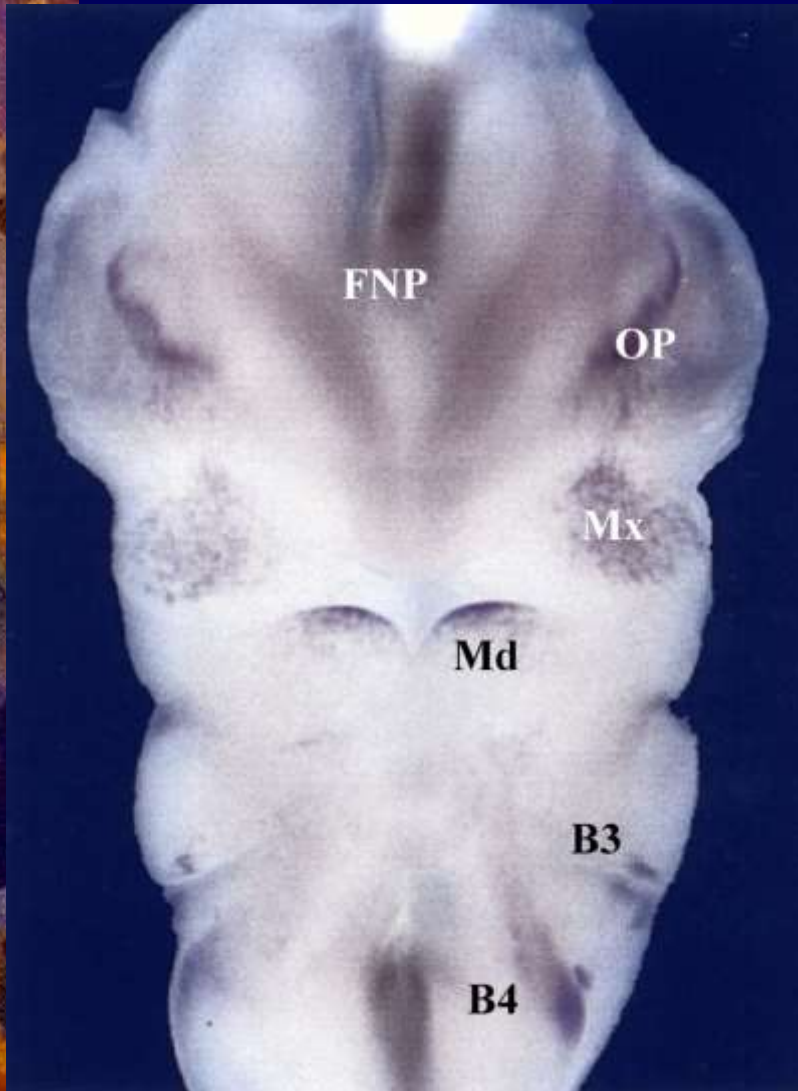
- Secreted molecules are part of a signalling cascade that controls the outgrowth and patterning of the developing facial primordia.
- Members of the fibroblast growth factor (FGF), bone morphogenetic protein (BMP) and hedgehog (Hh) families have all been shown to be expressed in signalling centres through the embryo.
- These include the limb bud, notochord and developing teeth, where they play important roles in mediating epithelial–mesenchymal interactions. Their role in craniofacial development will be discussed in more detail later.

FGF signalling



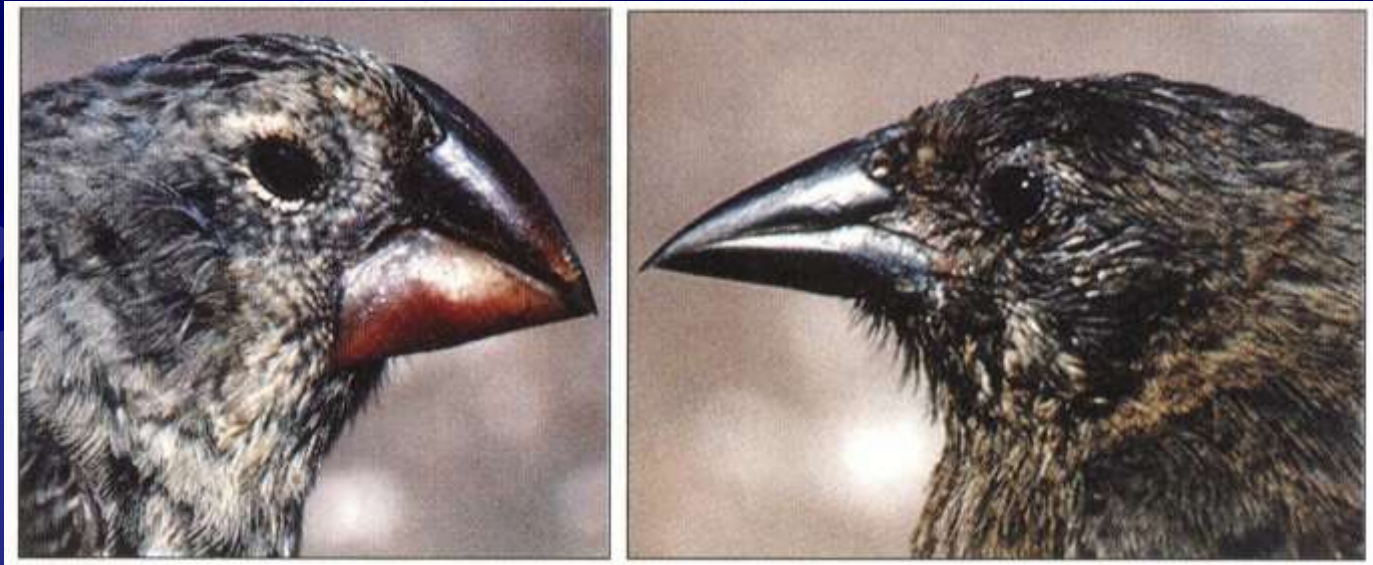
- FGFs are important regulators of cell proliferation, differentiation and migration. FGF signals are mediated by the FGF receptor (FGFR) family of tyrosine kinase receptors. In Seminar 5 the effect of mutations in *FGFR* genes on the aetiology of craniosynostosis will be discussed.
- (B) is a theoretical model of the binding of FGFs to the receptor in the presence of syndecan (A), an obligatory co-factor, resulting in receptor dimerization and signal transduction.
- Redrawn from Green *et al.* (1996), *BioEssays* **18**, 639–646.

BMP-4 signalling



- ✦ In addition to its roles in dorso-ventral specification of the embryo and in bone induction, BMP-4 also plays an important role in facial patterning.
- ✦ This figure shows the epithelial expression of *Bmp-4* in a HH stage (3.5 days) chick embryonic face.
- ✦ *Bmp-4* is expressed in the epithelia of the olfactory placodes (OP), maxillary process (Mx), mandibular process (Md) and the third and fourth branchial arches (B3, B4) and the frontonasal process (FNP) which gives rise to the beak prominence. See next slide.
- ✦ Whole mount *in situ* hybridization using digoxigenin-labelled RNA probes. Courtesy of J P Bogardi and P Francis-West.

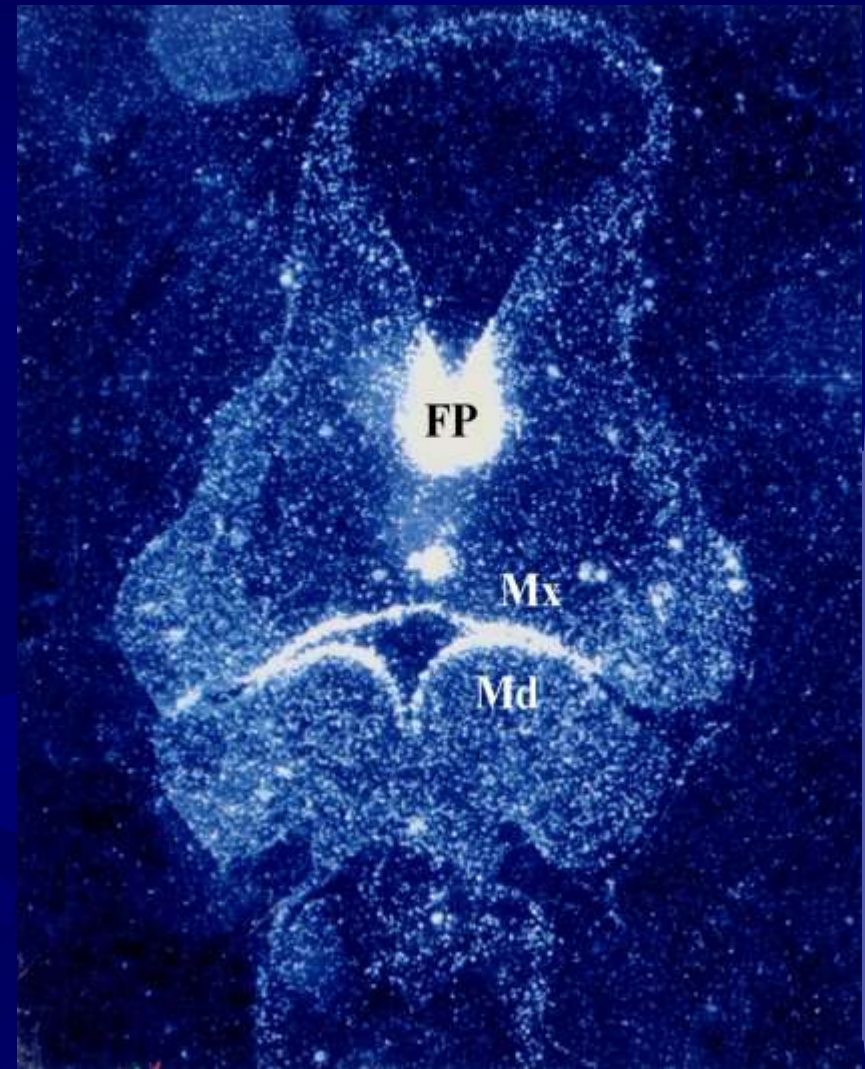
BMP-4 and Darwin's finches



- The variation in beak morphology of the 14 closely related species of Darwin's finches on the Galápagos and Cocos Islands, collected by Charles Darwin and others on the *Beagle* in 1835, is a classic example of species diversification by natural selection.
- Two research teams have recently shown that variation in the expression of the *Bmp-4* gene in beak prominences, plays a key role in determining the different shapes and sizes of beaks. Ground finches with larger beaks than cactus finches, make more BMP-4 protein and at an earlier stage.
- From *Science* (2004). Left, ground finch; right, cactus finch.

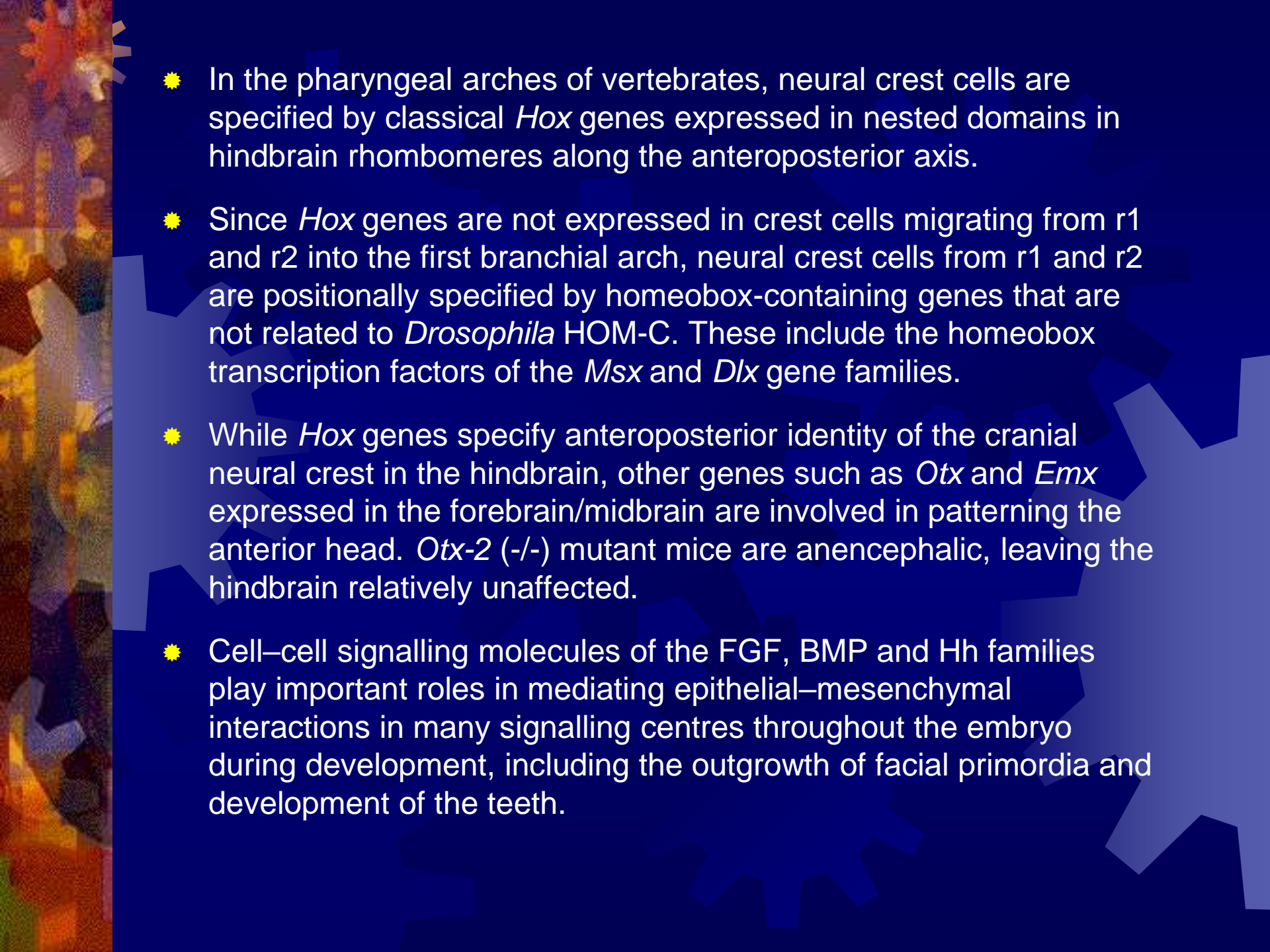
Sonic hedgehog signalling

- SHH acts as a cell–cell signalling factor involved in patterning the limbs, notochord, floor plate of the neural tube and somites.
- In the limb bud SHH initiates expression of secondary signalling molecules including BMP-2 in the mesoderm and FGF-4 in the ectoderm.
- SHH is also involved in midline signalling; loss-of-function mutations in SHH result in holoprosencephaly and a deficit in midline structures.
- Courtesy of M Cobourne; ³⁵S radiolabelled *in situ* hybridization, E10.5 mouse.



Summary

- Anteroposterior segmentation in vertebrates is patterned by the same homeotic genes that specify the *Drosophila* body axis.
- In other words, the homeotic genes that regulate development of *Drosophila* have been conserved during 500 million years of evolution, and adapted to generate the complexity of form and function that characterizes more advanced organisms including man.
- The homeotic genes of *Drosophila* are clustered in two complexes, the *Antennapedia* complex (ANT-C) and the *bithorax* complex (BX-C) collectively known as the homeotic complex (HOM-C).
- Vertebrate homeotic genes that are the evolutionary homologues of *Drosophila* HOM-C are termed *Hox* genes.

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- ✱ In the pharyngeal arches of vertebrates, neural crest cells are specified by classical *Hox* genes expressed in nested domains in hindbrain rhombomeres along the anteroposterior axis.
 - ✱ Since *Hox* genes are not expressed in crest cells migrating from r1 and r2 into the first branchial arch, neural crest cells from r1 and r2 are positionally specified by homeobox-containing genes that are not related to *Drosophila* HOM-C. These include the homeobox transcription factors of the *Msx* and *Dlx* gene families.
 - ✱ While *Hox* genes specify anteroposterior identity of the cranial neural crest in the hindbrain, other genes such as *Otx* and *Emx* expressed in the forebrain/midbrain are involved in patterning the anterior head. *Otx-2* (-/-) mutant mice are anencephalic, leaving the hindbrain relatively unaffected.
 - ✱ Cell–cell signalling molecules of the FGF, BMP and Hh families play important roles in mediating epithelial–mesenchymal interactions in many signalling centres throughout the embryo during development, including the outgrowth of facial primordia and development of the teeth.