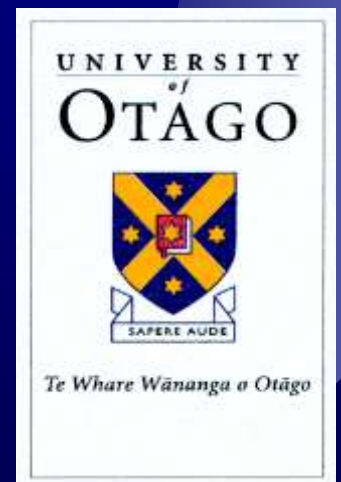


# Early Craniofacial Development

By Murray C Meikle  
Biological Foundations of Orthodontics  
and Dentofacial Orthopaedics

Seminar 1

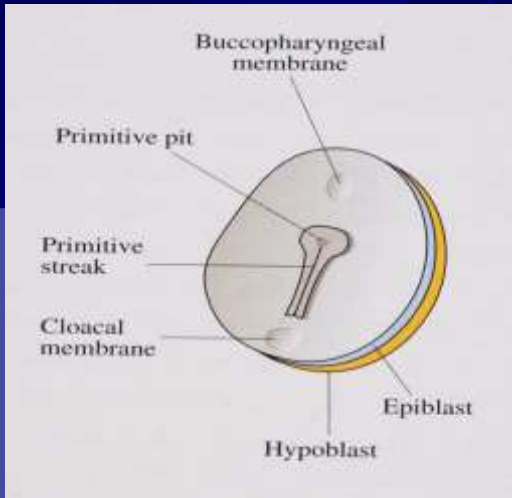
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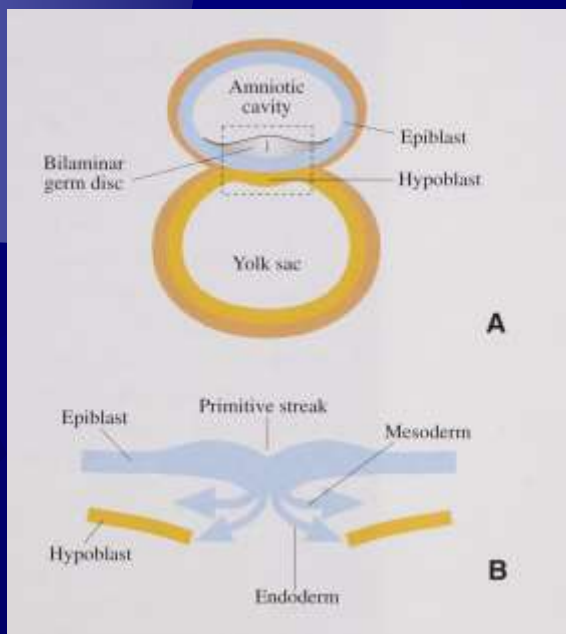
# Origin of the vertebrate head

- The most striking change coincident with the evolution of vertebrates more than 500 million years ago was the co-option of ectoderm from the neural plate to provide a second source of mesenchyme or ectomesenchyme to form cartilage, bone and dentine.
- It has been proposed that the craniofacial skeleton thus represents a new structure or neomorph added to the front end of a primitive amphioxus-like protochordate; the so-called 'new head' hypothesis of Gans and Northcutt (1983).
- The neural crest, the population of cells derived from the neural plate, is the key to understanding the development of the head which begins with the formation of the neural tube.

# Gastrulation and neurulation



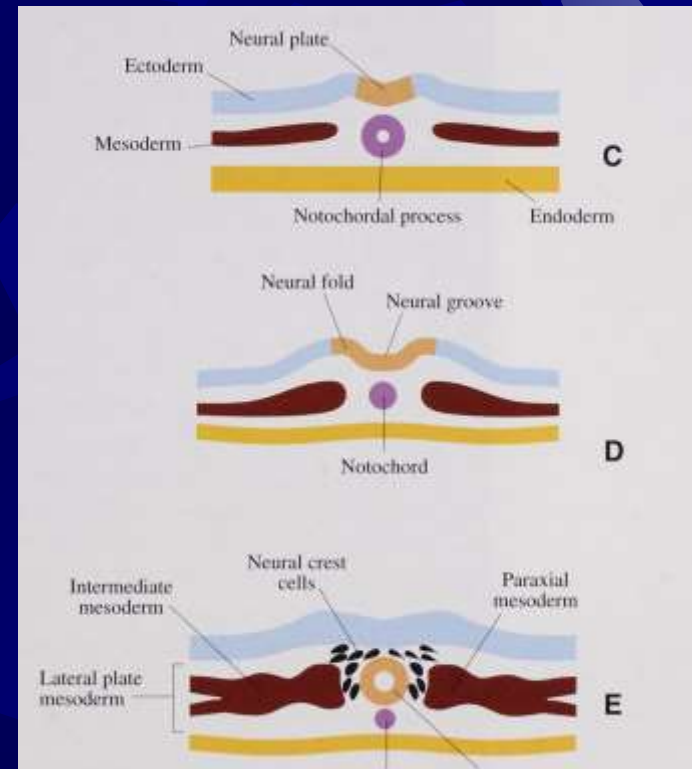
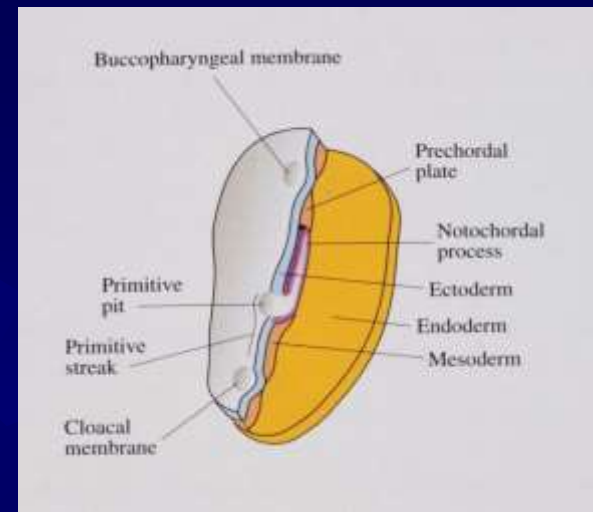
- Top. Dorsal view of the bilaminar embryonic disc. Neural tube formation or neurulation is preceded by gastrulation, the process that converts the bilaminar germ disc into the 3 germ layers of the embryo.

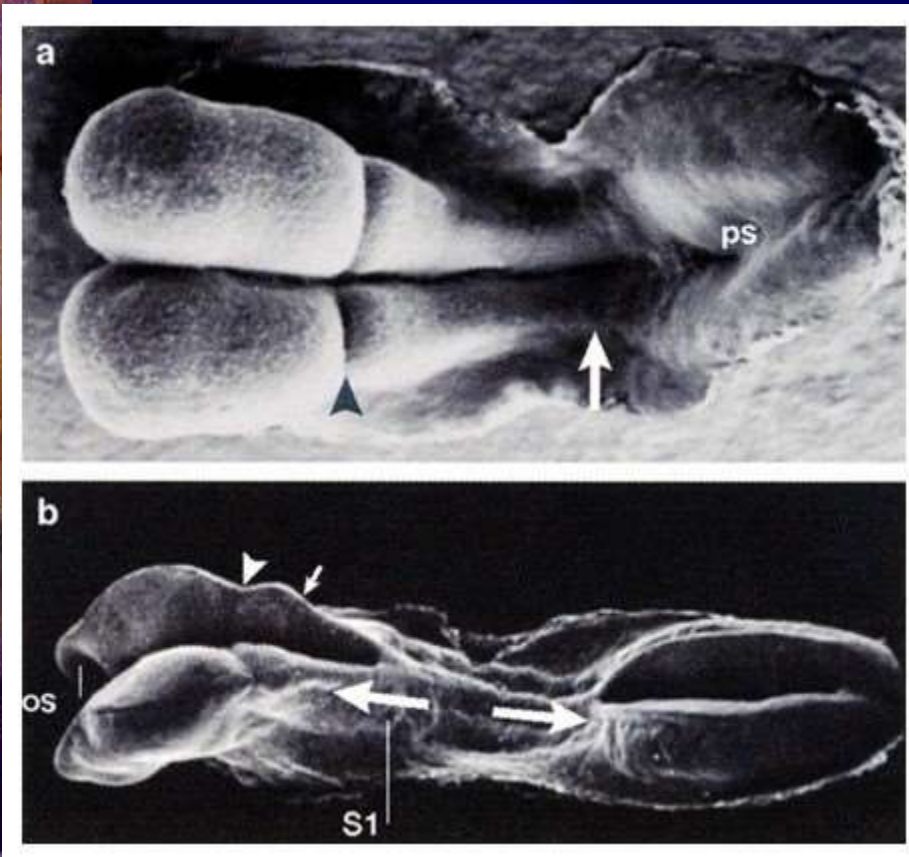


- A. Cross-section through the bilaminar embryonic disc, amnion and yolk sac. Gastrulation begins with the ingress of epiblastic cells through the primitive streak (day 15–16 in humans).
- B. Some invade and replace the hypoblast to form the endoderm; others migrate between the epiblast (which becomes the ectoderm) and the endoderm to form the mesoderm.

# Neurulation

- ✦ C. Dorsal view (top) and cross-section through the trilaminar germ disc. The appearance of the neural plate, a thickening of the ectoderm in the midline marks the beginning of neurulation (day 18 in humans).
- ✦ D. The edges of the neural plate become elevated to form the neural folds flanking a central depression, the neural groove.
- ✦ E. The neural folds fuse at the level of the first 7 somites to form the neural tube resulting in the separation of the neurectoderm from the surface ectoderm.
- ✦ From Meikle (2002). *Craniofacial Development, Growth and Evolution*.

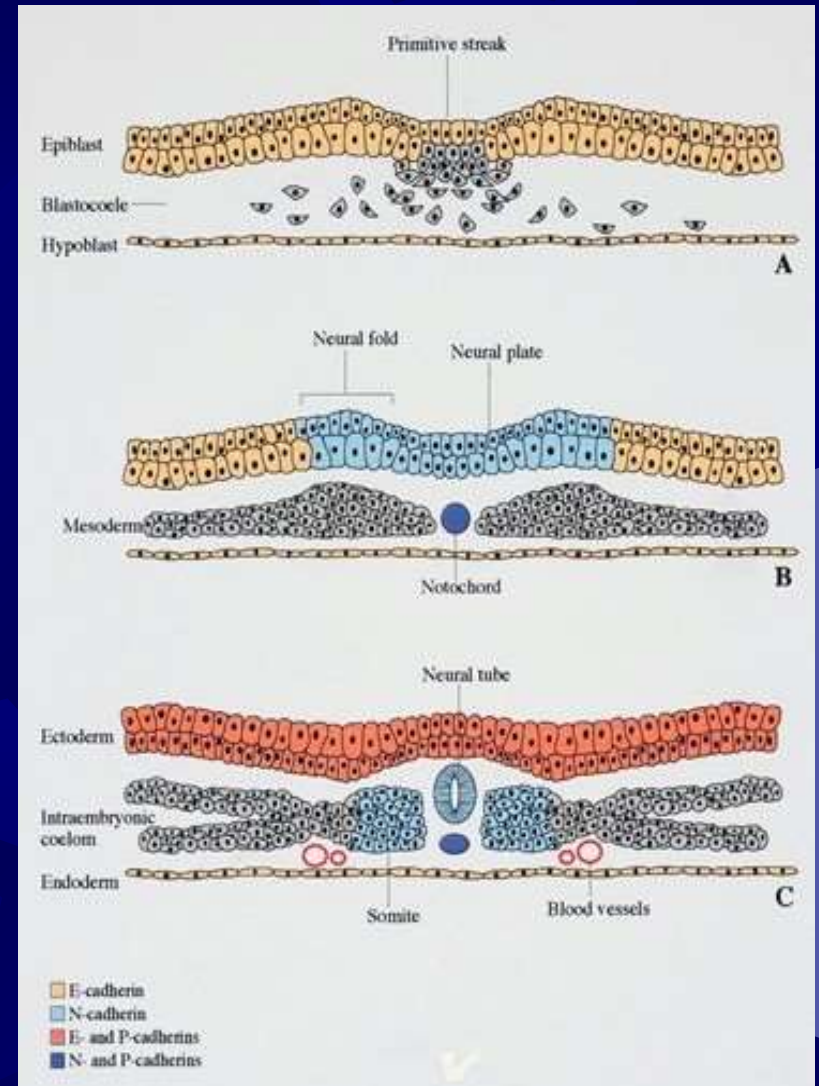




- (a) SEM of neural-fold-stage rat embryo, embryonic day, E 8.5, equivalent to 20 days post-ovulation human. The preotic sulcus (arrowhead) divides the future brain into forebrain plus midbrain plus rostral hindbrain - future rhombomeres (1 and 2) and the more caudal hindbrain (r 3–8). The arrow indicates the brain–spinal cord boundary; ps, primitive streak.
- (b) 8-somite rat embryo (human 22d). Neural tube closure is initiated at the 7-somite stage in rodents and humans at the brain–spinal cord junction and proceeds cranially and caudally (arrows); arrowhead, preotic sulcus; os, optic sulcus; S1, first somite.
- From Wilkie and Morriss-Kay (2001). *Nature Reviews Genetics* 2,458–468.

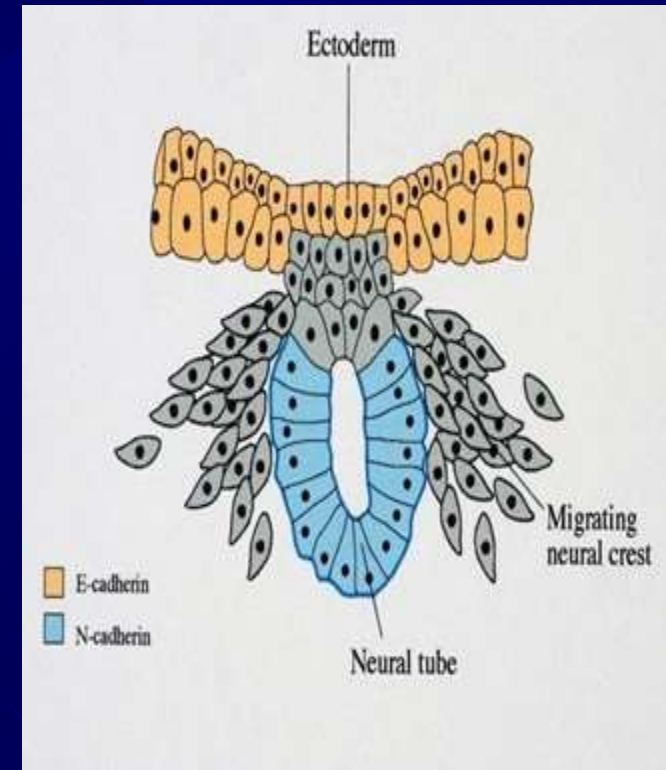
# Role of cell adhesion

- A. During gastrulation the cells of the epiblast down-regulate E-cadherin expression coincident with epithelial–mesenchymal transformation (EMT), and migrate to form the mesoderm.
- B. Neural plate cells increase N-cadherin expression.
- C. With the completion of neural tube formation, ectodermal cells express E- and P-cadherin and the notochord N- and P-; as mesodermal cells condense into somites they express N-cadherin.
- Redrawn from Lodish *et al.* (1995). *Molecular Cell Biology*.

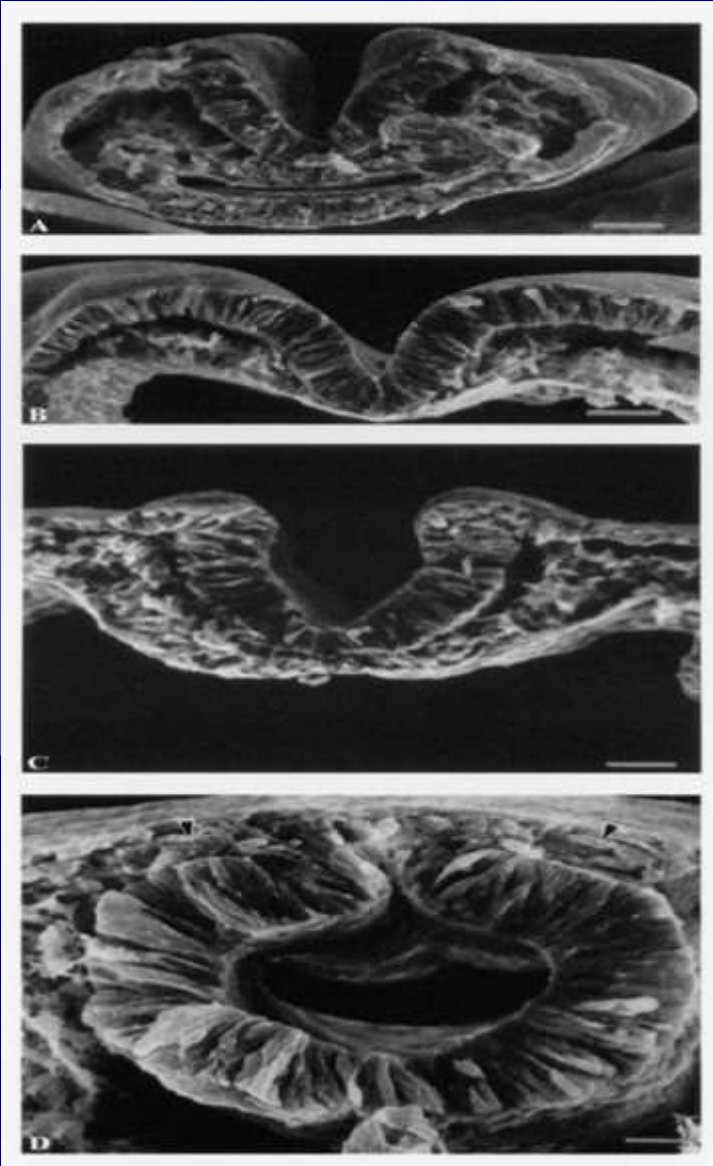


# Neural crest cell migration

- With the onset of epithelial–mesenchymal transformation, N-cadherin and other cell adhesion molecules are down-regulated in pre-migratory and migratory neural crest cells (grey).
- This alters the attachment of the cells to each other and their extracellular matrices, permitting migration. Synthesis of membrane-associated proteinases also facilitates cell migration.
- From Meikle (2002). *Craniofacial Development, Growth and Evolution*.

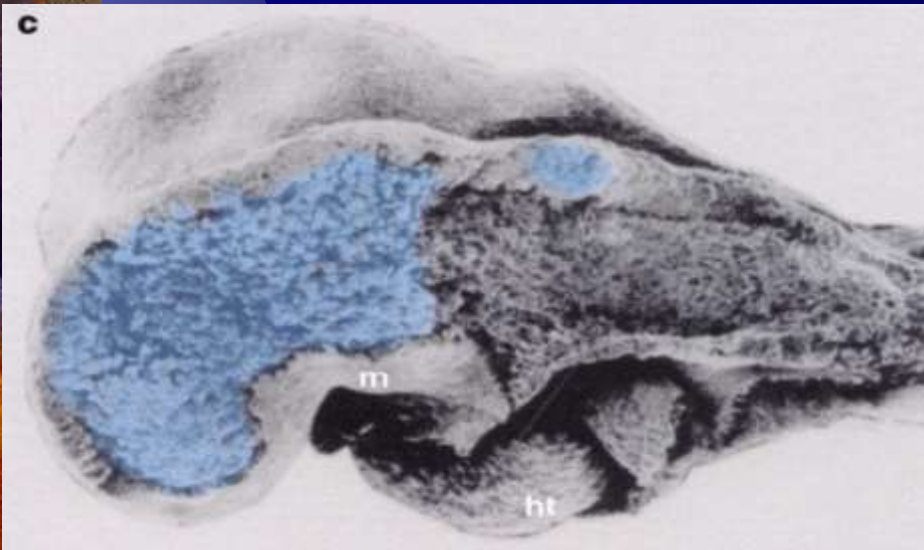
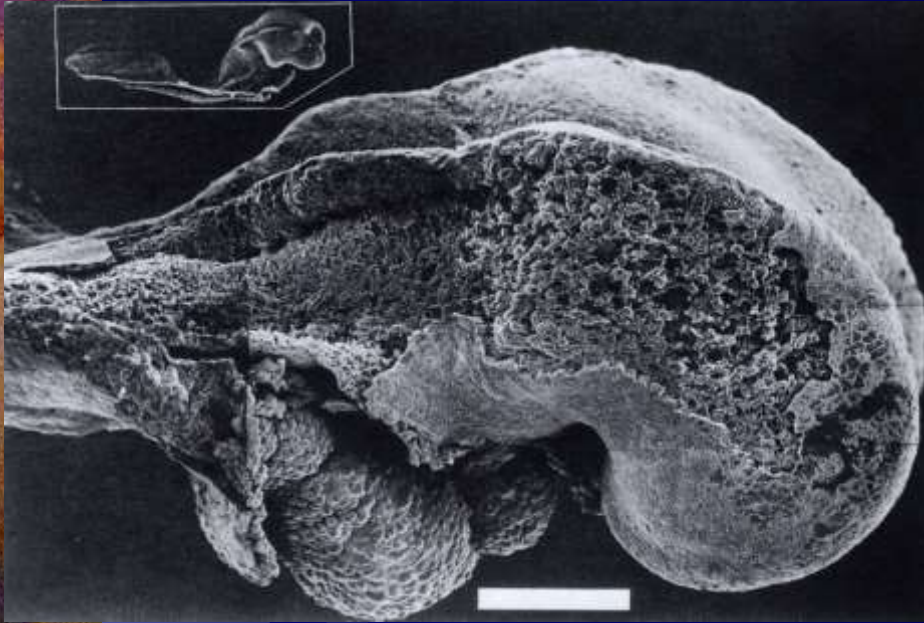


# Neural tube formation



- SEMs showing neural tube formation in cross-section in the chick.
- A–C are sections through the neural groove. In C convergence has begun and the neural folds are well formed (Hamburger and Hamilton (HH) stage 9 embryo).
- D. Section through the neural tube (future midbrain) of a stage 10 embryo; neural crest cells (arrowheads) are migrating from the neural folds.
- Following fusion, neural crest cells start to migrate (day 22 in humans) to diverse locations and functions throughout the body.
- From Schoenwolf (1982). *Scanning Electron Microscopy* 1, 289–308.

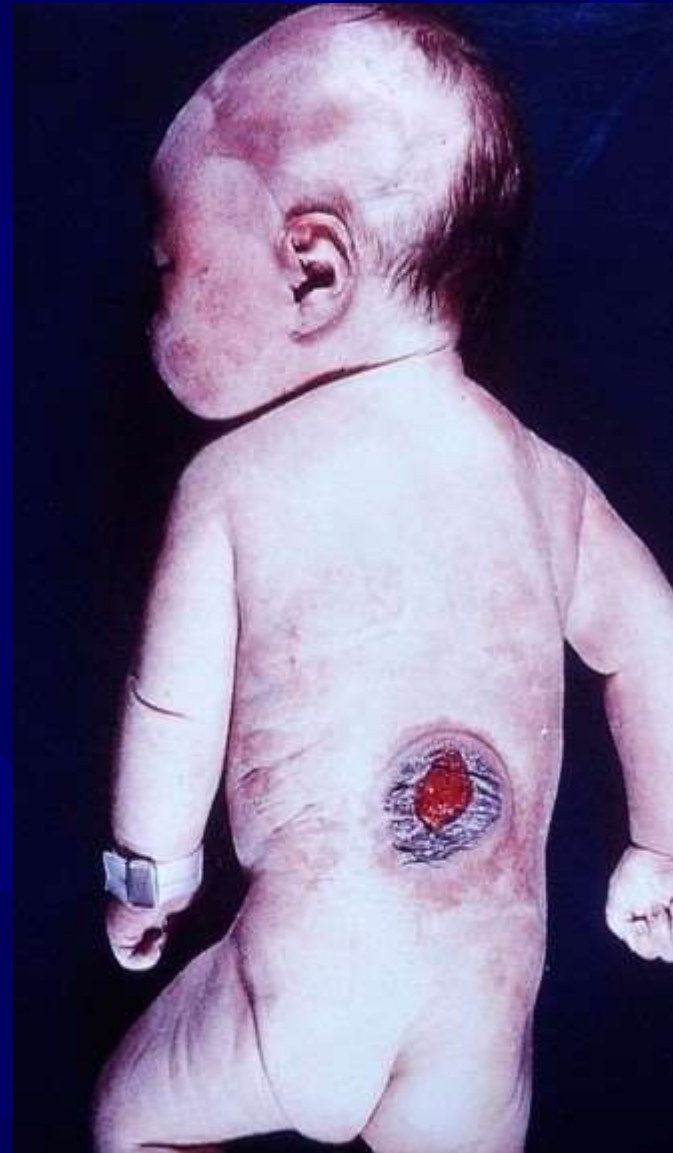
# Mammalian cranial crest cells



- Mammalian cranial crest cells migrate from widely open neural folds. In this late 7-somite rat embryo with the surface ectoderm removed, neural tube closure has begun in the trunk. Bar measures 100  $\mu\text{m}$ . Courtesy of G Morriss-Kay.
- Midbrain and hindbrain neural crest cells (blue) are migrating from wide open cranial neural folds towards the forebrain and first branchial arch in this 8-somite stage rat embryo. Mandibular part of first branchial arch, m; heart, ht.
- From Wilkie and Morriss-Kay (2001). *Nature Reviews Genetics* 2,458–468.

# Spina bifida

- At its anterior end the neural tube expands to form the brain, while the narrow caudal part overlying the notochord becomes the spinal cord.
- Incomplete closure of the caudal or posterior neuropore results in the clinical manifestation of spina bifida, the severity of which depends on how much of the tube remains open.
- From Larsen (1998). *Essentials of Human Embryology*.

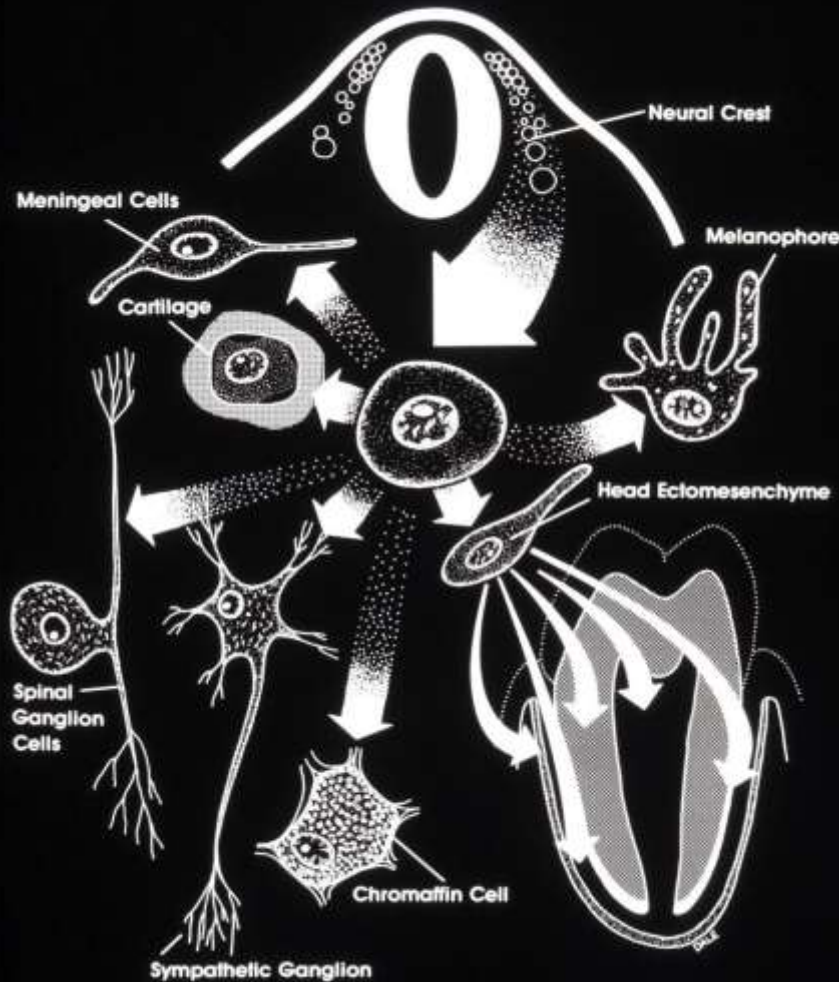


# Anencephaly

- Non-closure of the rostral or anterior neuropore at 28 days leads to the malformation of anencephaly, a fatal condition in which the forebrain degenerates and the cranial vault fails to form.
- The incidence of neural tube defects is 1:500 live births.
- It has been estimated that neural tube defects can be prevented by approximately 50% with folic acid (vitamin B12) supplements during pregnancy.
- From Larsen (1998). *Essentials of Human Embryology*.

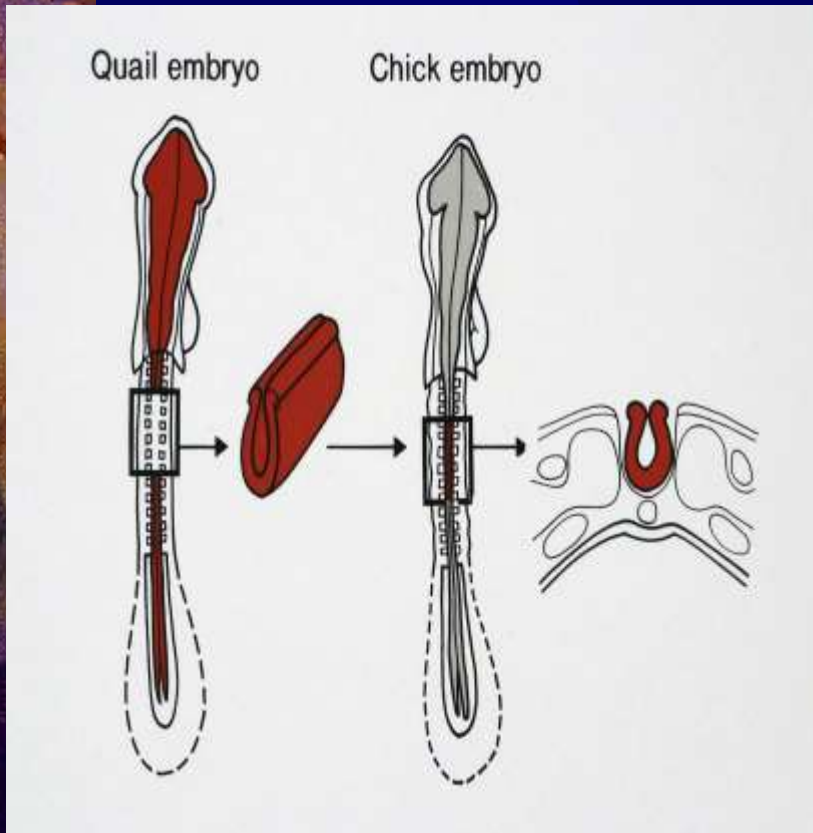


# Derivatives of neural crest cells



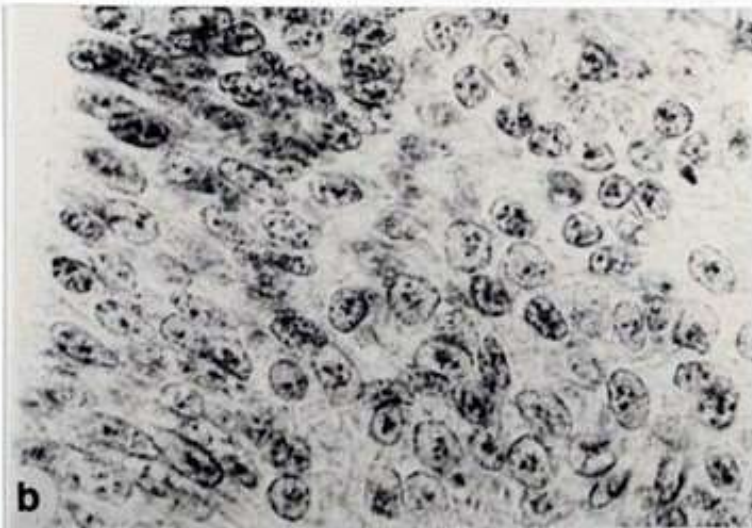
- Derivatives of the cranial neural crest. Bone and cartilage of the prechordal skull; dermal bones of the craniofacial skeleton; odontoblasts; dermis of face and neck; meninges of the brain; neurones of the cranial nerve ganglia; calcitonin (C cells) of thyroid; melanocytes.
- Trunk neural crest derivatives. Meningeal coverings of the spinal cord; peripheral nervous system; Schwann cells; adrenaline producing cells of adrenal medulla; melanocytes.
- From Ten Cate (1980). *Oral Histology*.

# Fate mapping of neural crest



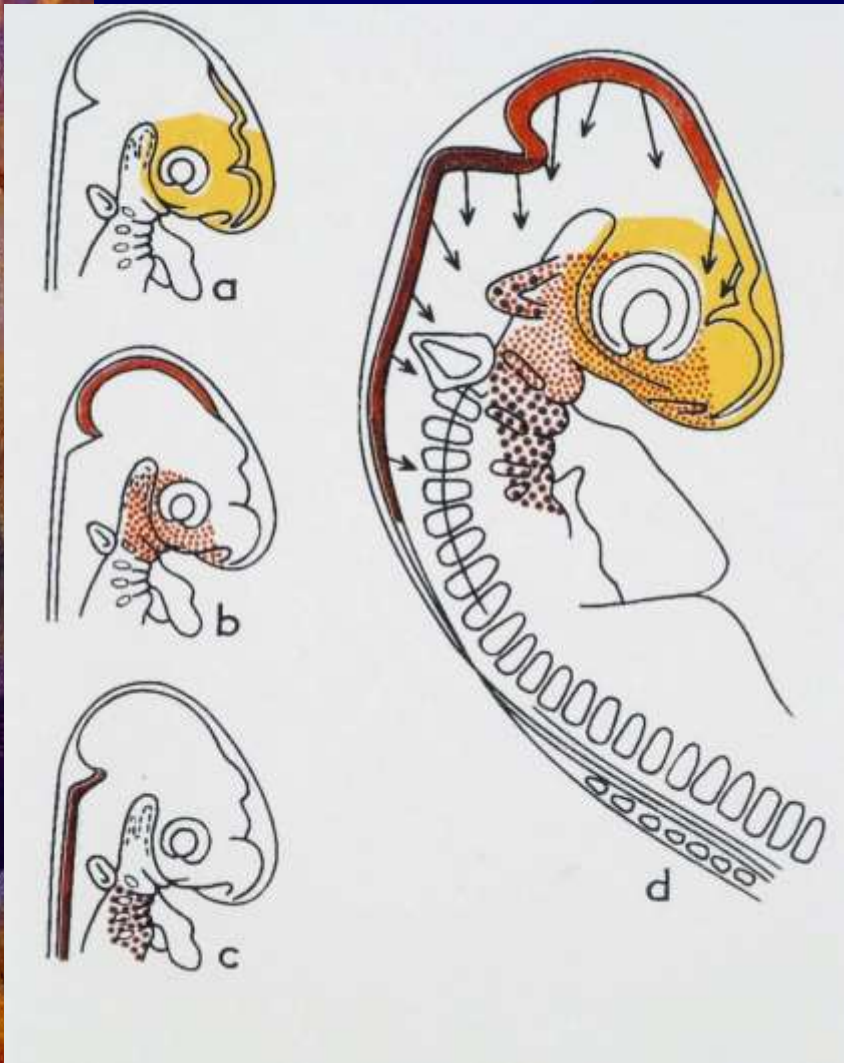
- A number of methods have been used in the past to map the fate of neural crest cells including vital dyes and  $^3\text{H}$ -thymidine autoradiography. Vital dyes are too toxic for long term use, and  $^3\text{H}$ -thymidine rapidly becomes diluted with cell division.
- Crest cell fate has been most successfully mapped by Nicole Le Douarin and her colleagues in Paris using the quail–chick chimera model.
- In this technique part of the neural tube from the Japanese quail is removed and grafted into the corresponding position in the chick host and the fate of the cells followed by histology.
- Modified from Le Douarin (1982). *The Neural Crest*.

# Quail and chick neuroblasts



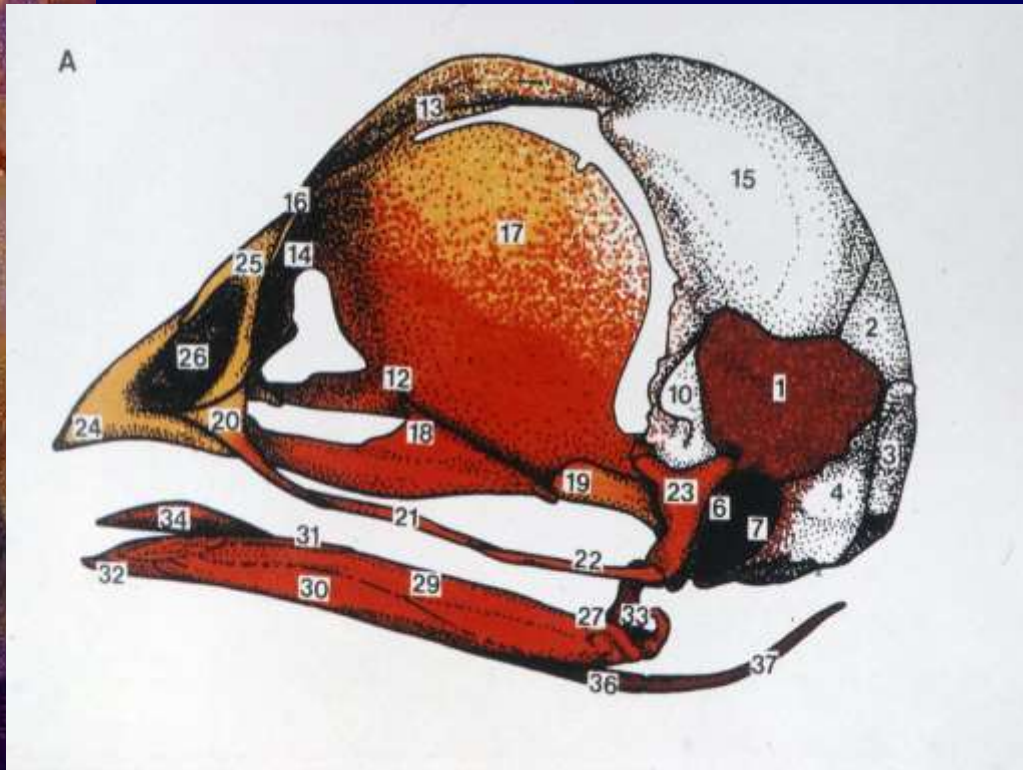
- Neuroblasts (a) from an 8-day Japanese quail (*Coturnix coturnix japonicum*) and (b) chick (*Gallus domesticus*) stained with the Fielgen–Rossenbeck reaction for DNA. X600.
- Quail cell DNA is concentrated into a single mass of heterochromatin, whereas in chick nuclei the DNA is dispersed in small chromocentres. It is therefore relatively easy with experience to distinguish quail cells by their large DNA-rich nucleoli
- From Le Douarin (1982). *The Neural Crest*.

# Destination of crest cells in the head



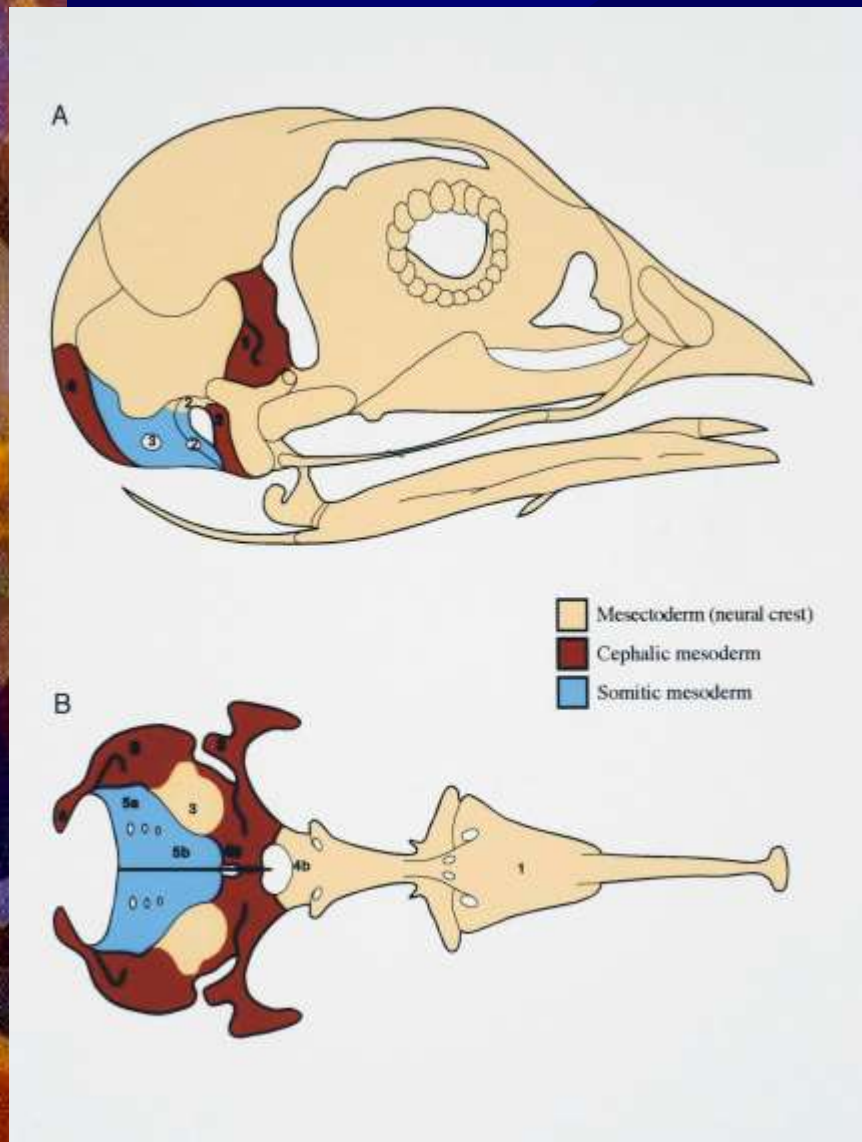
- Data from quail–chick transplantation have shown the extensive contribution of neural crest cells to the facial skeleton. These figures show the localization of neural crest-derived cells in the head and branchial region of an HH stage 20 chick embryo.
- Cells migrating from (a) the forebrain (prosencephalon) have been mapped to the frontonasal, orbital and maxillary regions, and (b) cells from the mesencephalon (midbrain) to the maxilla, palatine complex and lower jaw. (c) cells from the rhombencephalon (hindbrain) migrate into the branchial arches.
- From Le Douarin (1982). *The Neural Crest*.

# The avian skull



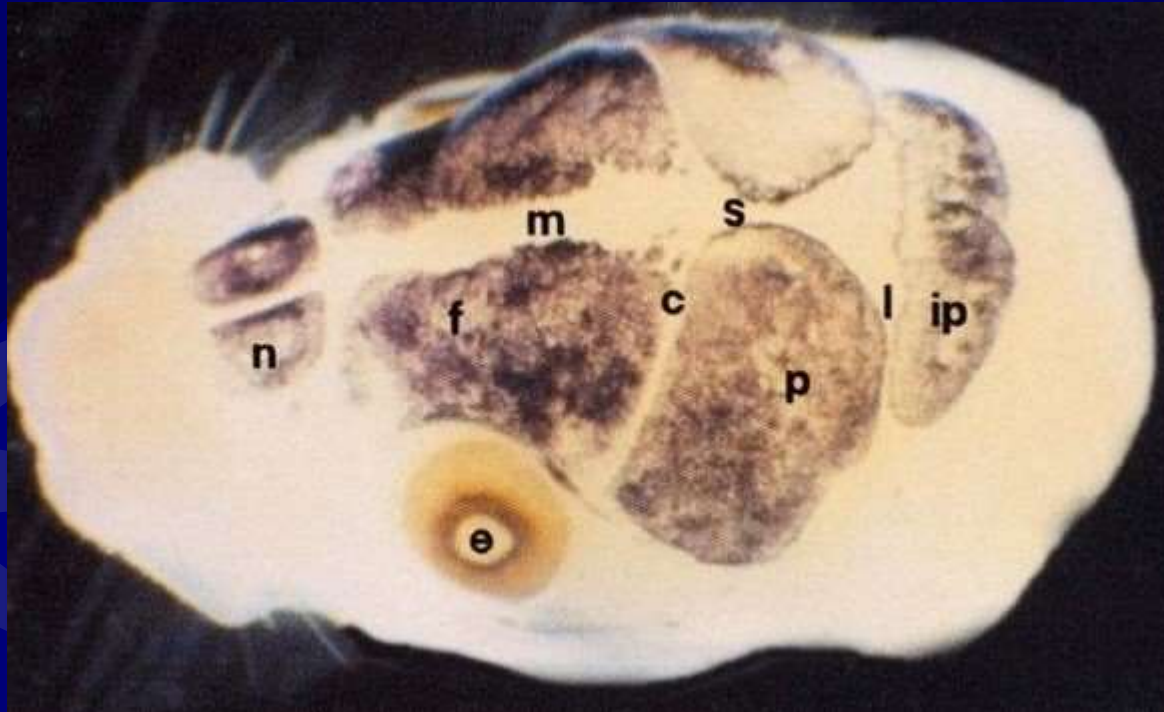
- This figure summarizes the distribution of cephalic neural crest cells in the avian skull following quail–chick transplantation experiments.
- Yellow indicates bones derived from forebrain neural crest, red midbrain neural crest and brown the hindbrain.
- Originally it was thought that in birds, the frontal and parietal bones of the cranial vault were derived from the cephalic mesoderm (white).
- From Le Douarin (1982). *The Neural Crest*.

# The avian skull



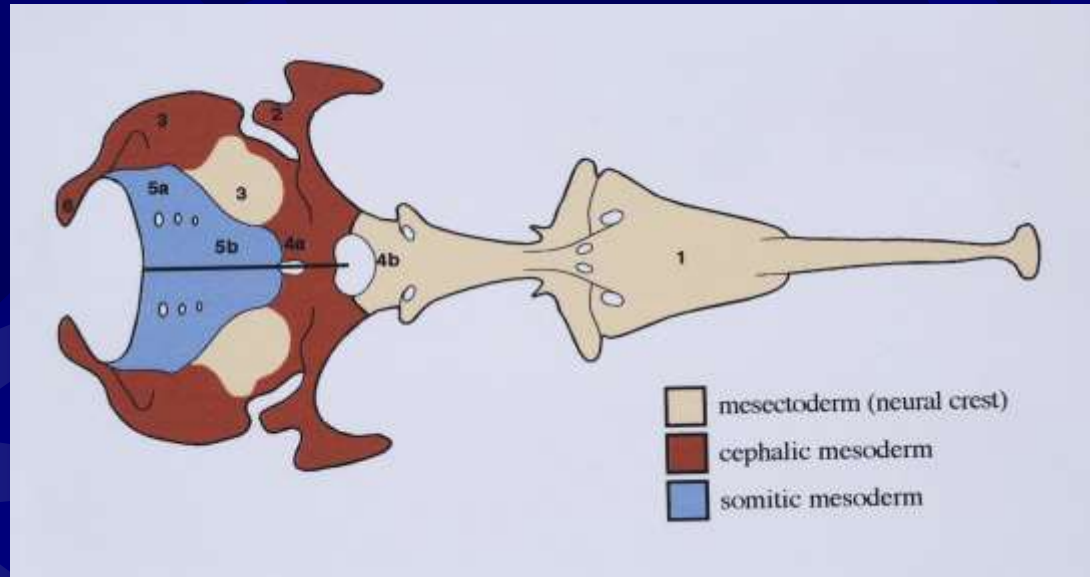
- Subsequent research using the quail–chick chimera model by the Le Douarin group, showed that the frontal, parietal and squamosal bones were also derived from neural crest.
- A. Cephalic skeleton of a bird. The bones of the lower jaw and most of the bones of the skull are derived from ectomesenchyme.
- B. Dorsal view of the chondrocranium (E9). The black line represents the notochord. The prechordal skull is derived from neural crest cells.
- Redrawn from Couly *et al.* (1993). *Development* **119**, 409–429.

# Ossification of the cranial vault



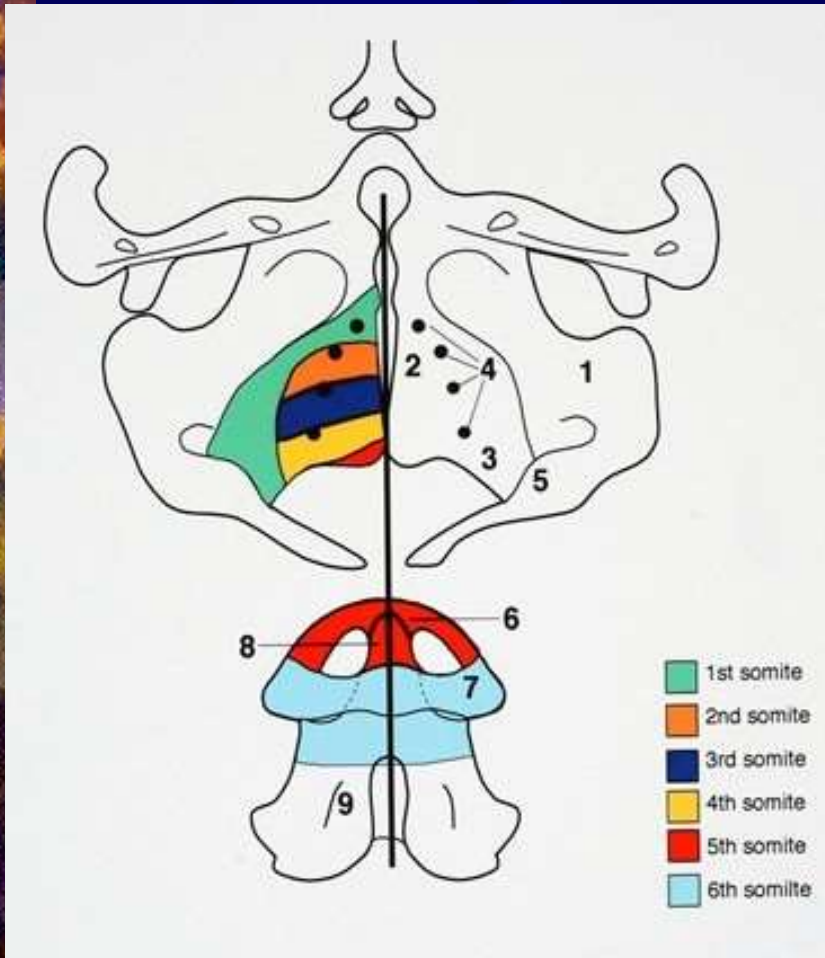
- Ossification of the cranial vault and formation of the cranial sutures. E16 mouse head (equivalent to 4 months in humans). Whole mount *in situ* hybridization showing the expression of the bone differentiation marker Spp-1 (secreted phosphoprotein-1).
- Bones: n, nasal; f, frontal; p, parietal; ip, interparietal. Sutures: m, metopic, c, coronal; s, sagittal; l, lambdoid; e, eye.
- From Wilkie and Morriss-Kay (2001). *Nature Reviews Genetics* 2,458–468.

# Origin of the chondrocranium



- ✦ The prechordal chondrocranium is derived from cells of the neural crest. Posterior to the anterior tip of the notochord, the chordal chondrocranium is derived from cephalic or somitic mesoderm.
- ✦ The sella turcica represents the boundary between the ectomesodermal and mesodermal parts of the cranial base and the speno-occipital synchondrosis the boundary between the cephalic and somitic mesoderm.
- ✦ The otic capsule is formed by cells from three sources: neural crest, paraxial mesoderm and the first somite.

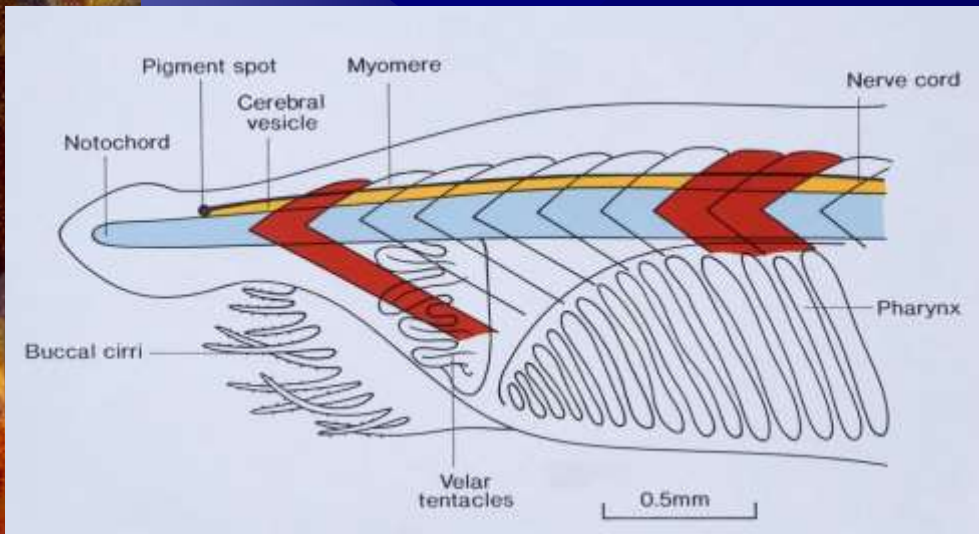
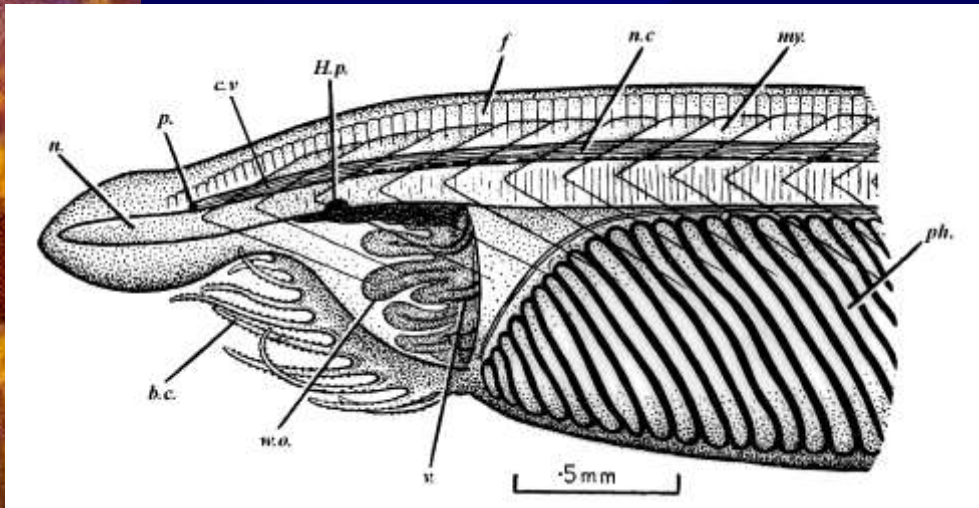
# Occipitalization of the vertebrae



- During evolution there has been a progressive incorporation of the first five somites into the occipital region of the skull.
- In jawless vertebrates (agnatha) no somitic mesoderm participates in the formation of the skull. In mammals the five occipital somites fuse to form the basiocciput.
- In addition to contributing to the basiocciput, the 5th somite (red) forms the anterior arch of the atlas and the dens (odontoid process) of the axis.

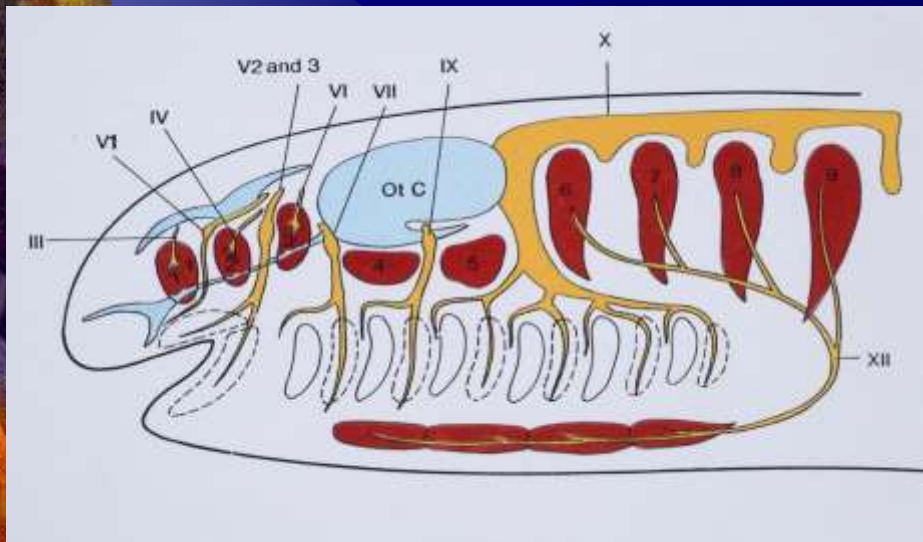
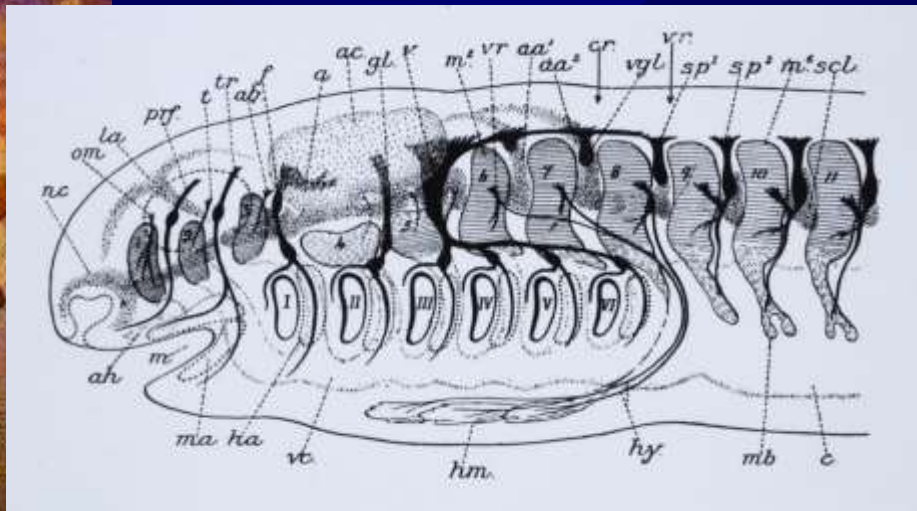
Redrawn from Couly *et al.* (1993). *Development* 119, 409–429.

# Segmentation of the head



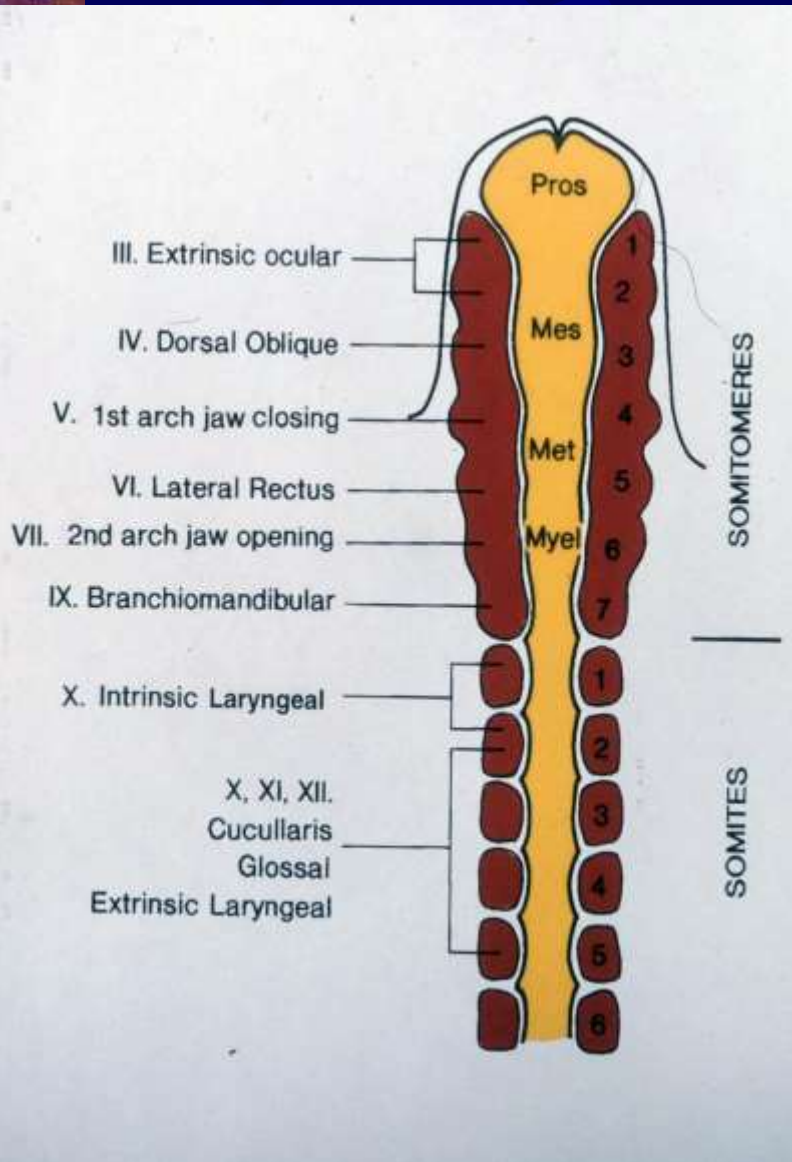
- ☀ Segmentation or metamerism is the process that divides a body into duplicated sections. To what extent the vertebrate head is segmented has a long and controversial history.
- ☀ The protochordate amphioxus (*Branchiostoma lanceolatum*) shows many of the morphological features thought to characterize the chordate body plan and is segmented from end to end. Muscle is segmented into myomeres with corresponding segmentation of the peripheral nervous system. The pharynx shows a similar segmental pattern.
- ☀ From Young (1962). *The Life of Vertebrates*.

# The classical scheme



- In the dogfish *Scyllium canicula*, the paraxial mesoderm is segmented into myotomes up to its anterior end (Balfour, 1876–1878).
- This provided the basis for what became called the ‘classical scheme’ of segmentation and nervous innervation of the head by Goodrich (1930).
- He proposed that the paraxial mesoderm was segmented into 3 preotic somites (innervated by cranial nerves III, IV, VI), 2 metotic somites that are lost, and 3–4 postotic somites (XII) which form the tongue muscles.
- Redrawn from Goodrich (1930). *Studies on the Structure and Development of Vertebrates*.

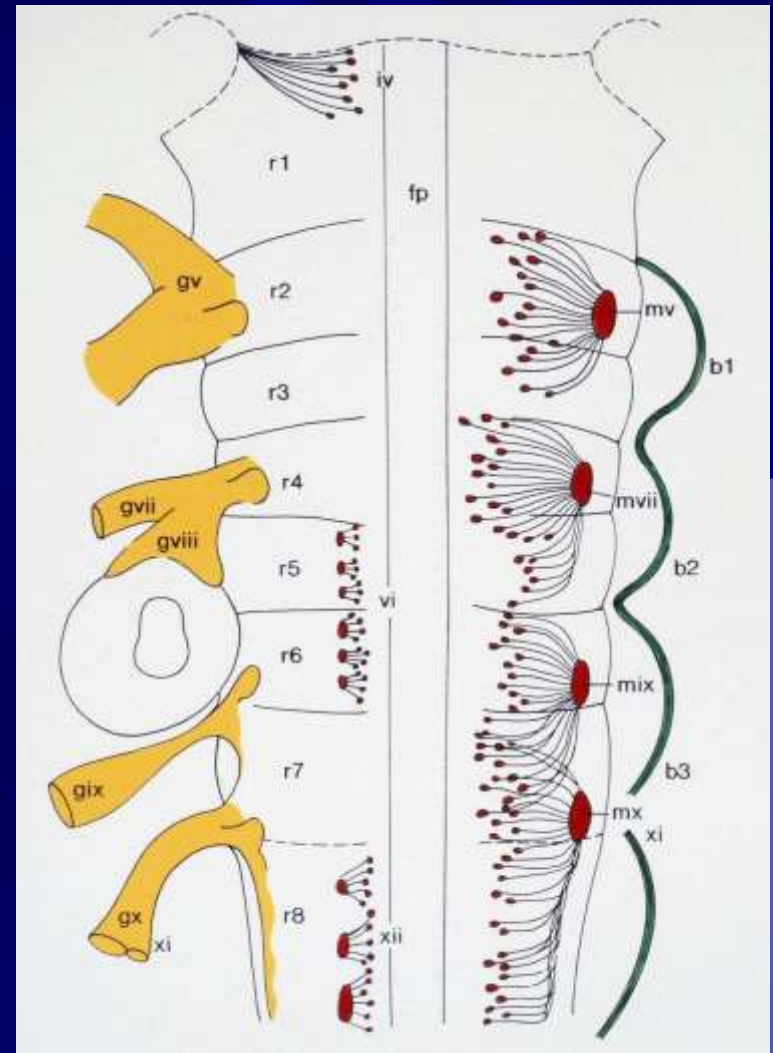
# Somitomes



- Based on SEM studies Meier (1979,1984) proposed that the cephalic mesoderm is segmented and loosely arranged into somitomes.
- Somitomes S1 and S2 give rise to extrinsic eye muscles innervated by cranial nerve III and S3 by nerve IV; S4 forms the mandibular muscles (nerve V) and S5 the lateral rectus muscles (nerve VI); the classical view that the metotic segments (4 and 5) were transient was incorrect. S6 forms the second arch muscles, and S7 the third arch (branchiomandibular) muscles.
- Redrawn from Noden (1991). *Journal of Craniofacial Genetics and Craniofacial Biology* 11,192–2123.

# Segmentation of the hindbrain: rhombomeres

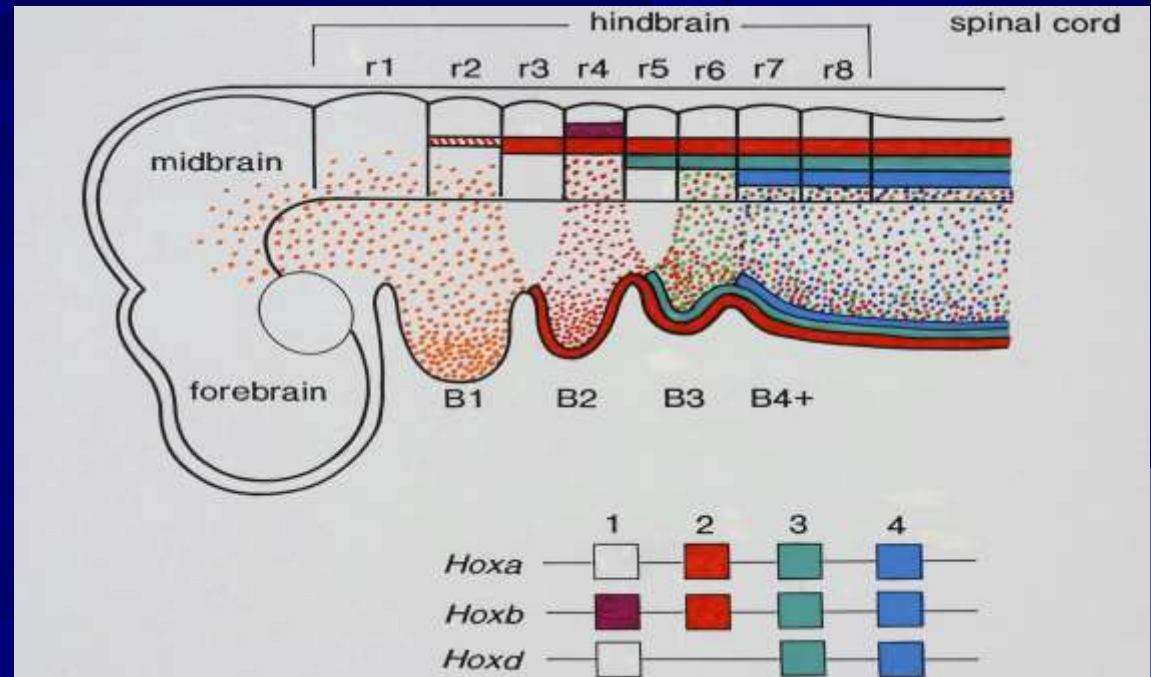
- The neural tube is also segmented and divided into a series of transient swellings (neuromeres), known in the hindbrain as rhombomeres.
- Rhombomeres are spatially related to the pharyngeal arches and their cranial nerves, and are true lineage-restricted cellular compartments.
- This figure shows the relationship of sensory ganglia (yellow, gv–gx), branchial motor nuclei and exit points (red, mv–mxi) and somatic motor nuclei and exit points (iv, vi, xii) to rhombomeres (r1–r8) and branchial arches (b1–b3).



Redrawn from Lumsden and Keynes (1989). *Nature* **337**, 424–428.

# Hox gene expression

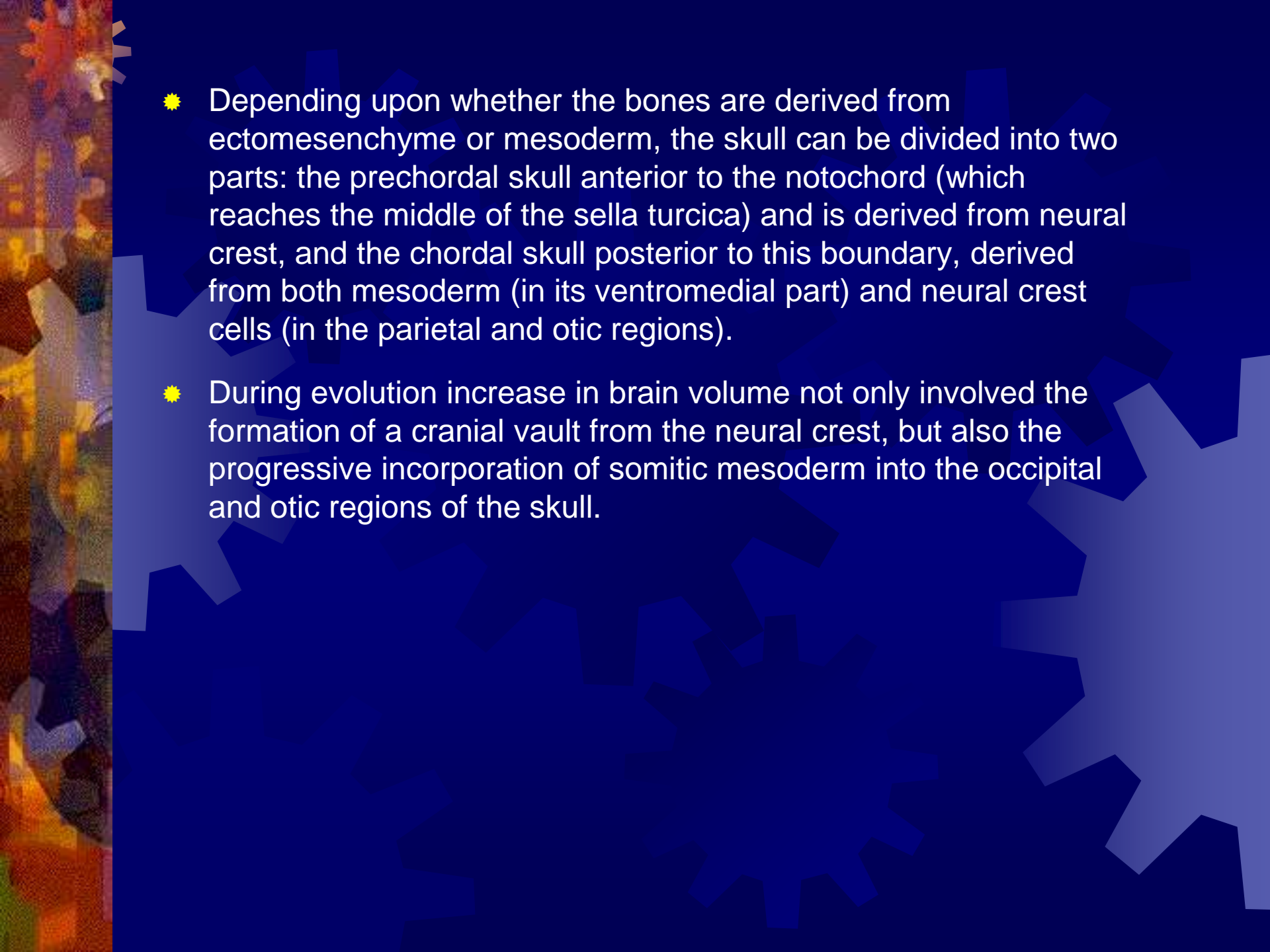
Modified from Krumlauf (1993). *Trends in Genetics* 9, 106–112.



- This figure shows the relation between domains of *Hox* gene expression in rhombomeres (r1–8) and neural crest migration into the branchial arches (B1–B4).
- Neural crest cells from r1 and r2 migrate into the mandibular arch, r4 cells into the hyoid arch, and r6–r8 cells into the other arches. Crest cells carry this *Hox* code as they migrate to the periphery.

# Summary

- The vertebrate head owes its origin to the co-option of ectoderm from the neural plate to provide a second source of mesenchyme (ectomesenchyme) to form cartilage, bone and dentine. The remainder of the mesenchyme in vertebrate embryos is derived from the mesoderm.
- The key to understanding the development of the head is the neural crest, a population of migratory cells derived from the neural plate. With neural tube formation (day 22 in humans) neural crest cells migrate from the neural folds to diverse sites throughout the embryo. Only in the head, however, do crest cells give rise to cartilage and bone.
- Quail–chick transplantation experiments have shown the extensive contribution of the neural crest to craniofacial development and the facial skeleton.

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- Depending upon whether the bones are derived from ectomesenchyme or mesoderm, the skull can be divided into two parts: the prechordal skull anterior to the notochord (which reaches the middle of the sella turcica) and is derived from neural crest, and the chordal skull posterior to this boundary, derived from both mesoderm (in its ventromedial part) and neural crest cells (in the parietal and otic regions).
  - During evolution increase in brain volume not only involved the formation of a cranial vault from the neural crest, but also the progressive incorporation of somitic mesoderm into the occipital and otic regions of the skull.