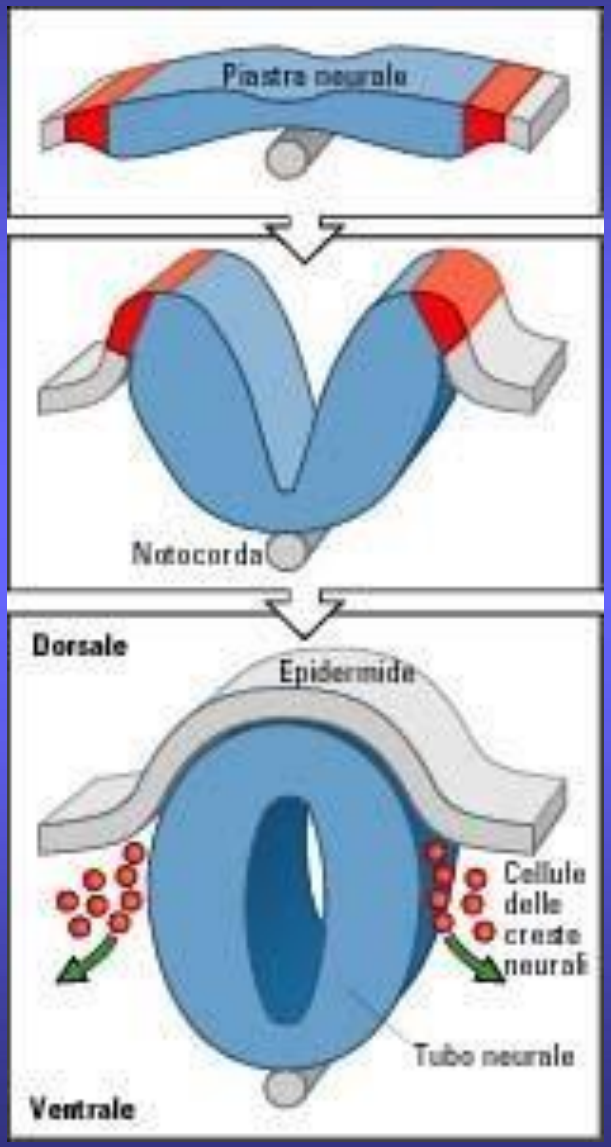
Specificazione
delle Creste Neurali

Origine delle cellule delle creste neurali



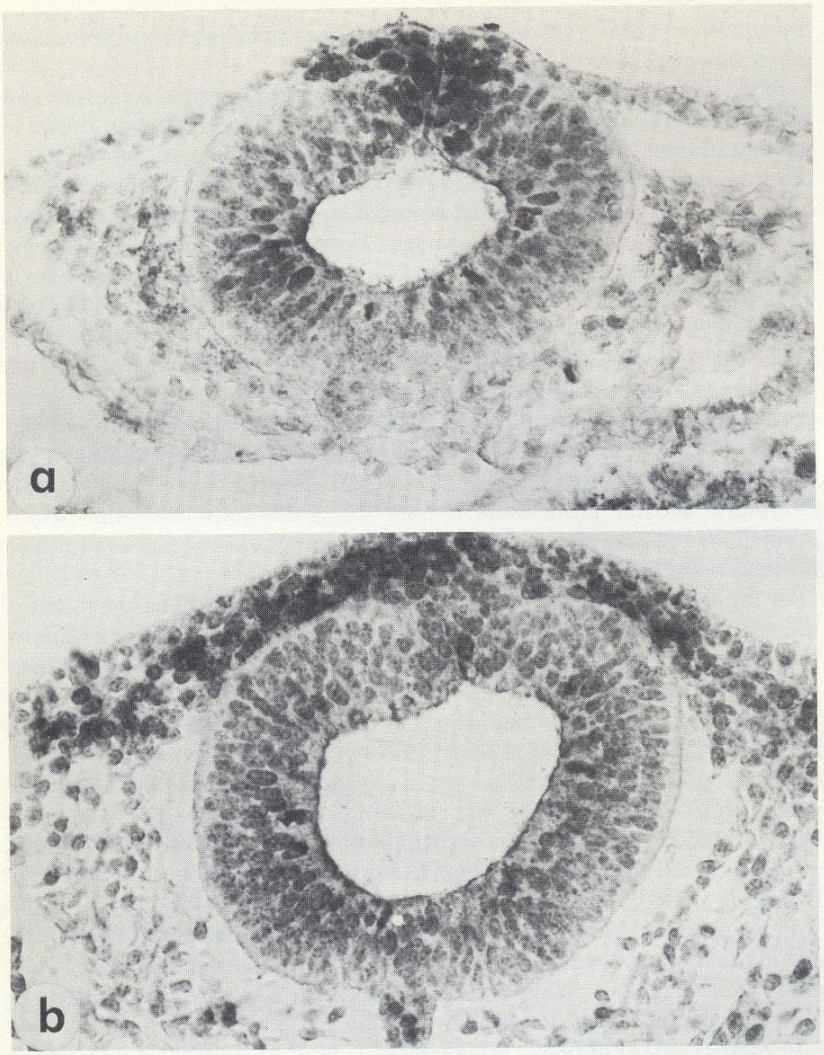
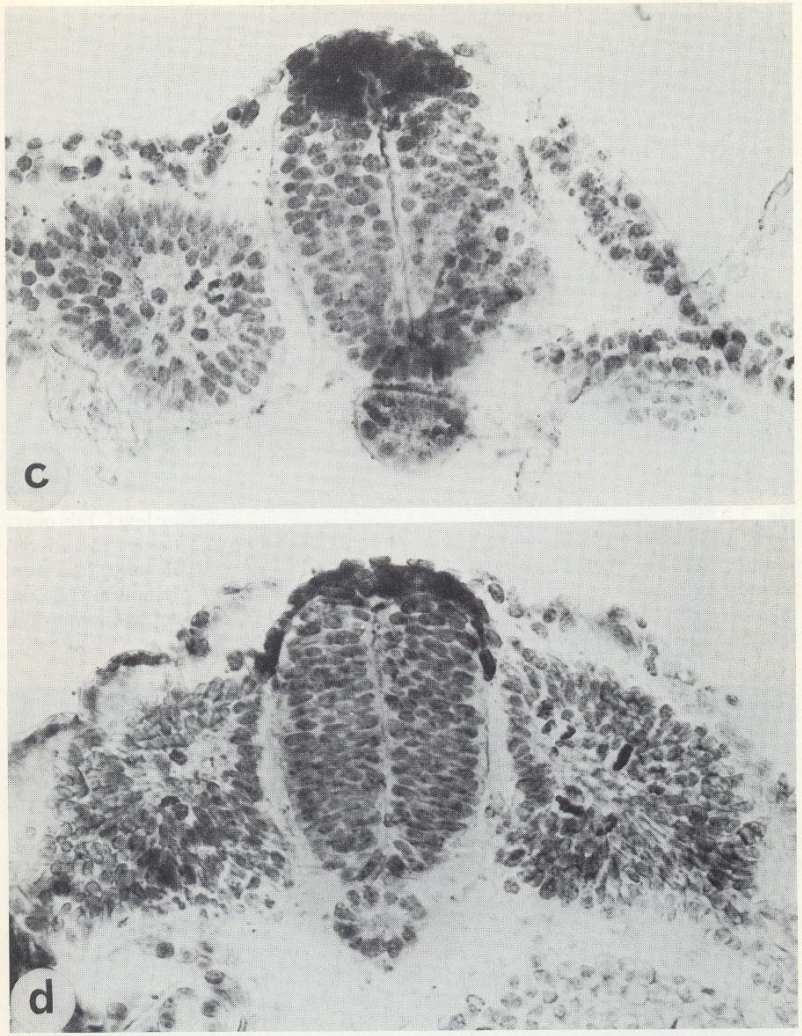


Fig. 2.1. The neural crest cells during (a) and after (b) closure of the neural tube at the mesencephalic level; at the trunk level, in (c) the crest is not yet individualized; in (d) crest cells are in the process of migration. Method of Karnovsky and Roots (1964) and action of iso-OMPA, an inhibitor of non-specific cholinesterases. (×240)



Classificazione sulla base della localizzazione della cresta neurale lungo l'asse antero-posteriore

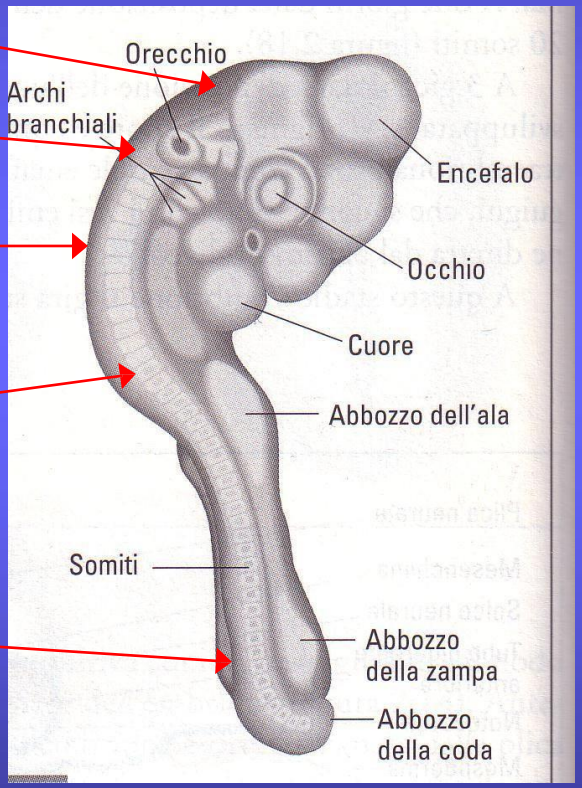
CN cefalica

CN cervicale

CN cardiaca
(I-III somite)

CN del tronco

CN sacrale

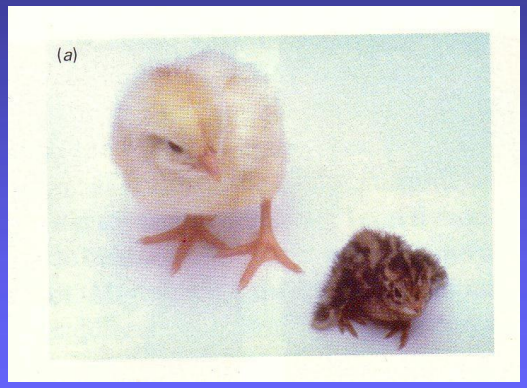


Come studiare le cellule della cresta neurale

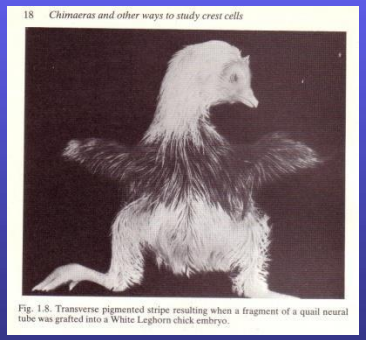
- Trapianti di cresta neurale sottoposte precedentemente a incorporazione di timidina triziata

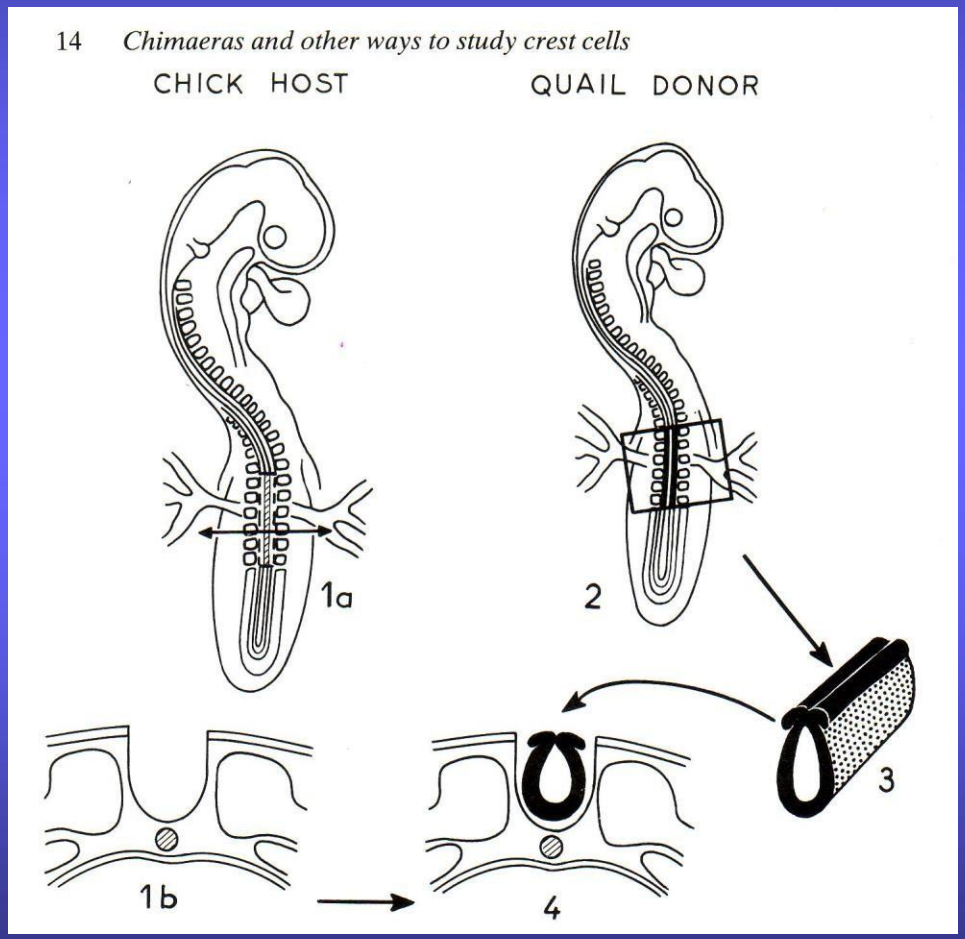
- Asportazioni di regioni della cresta neurale

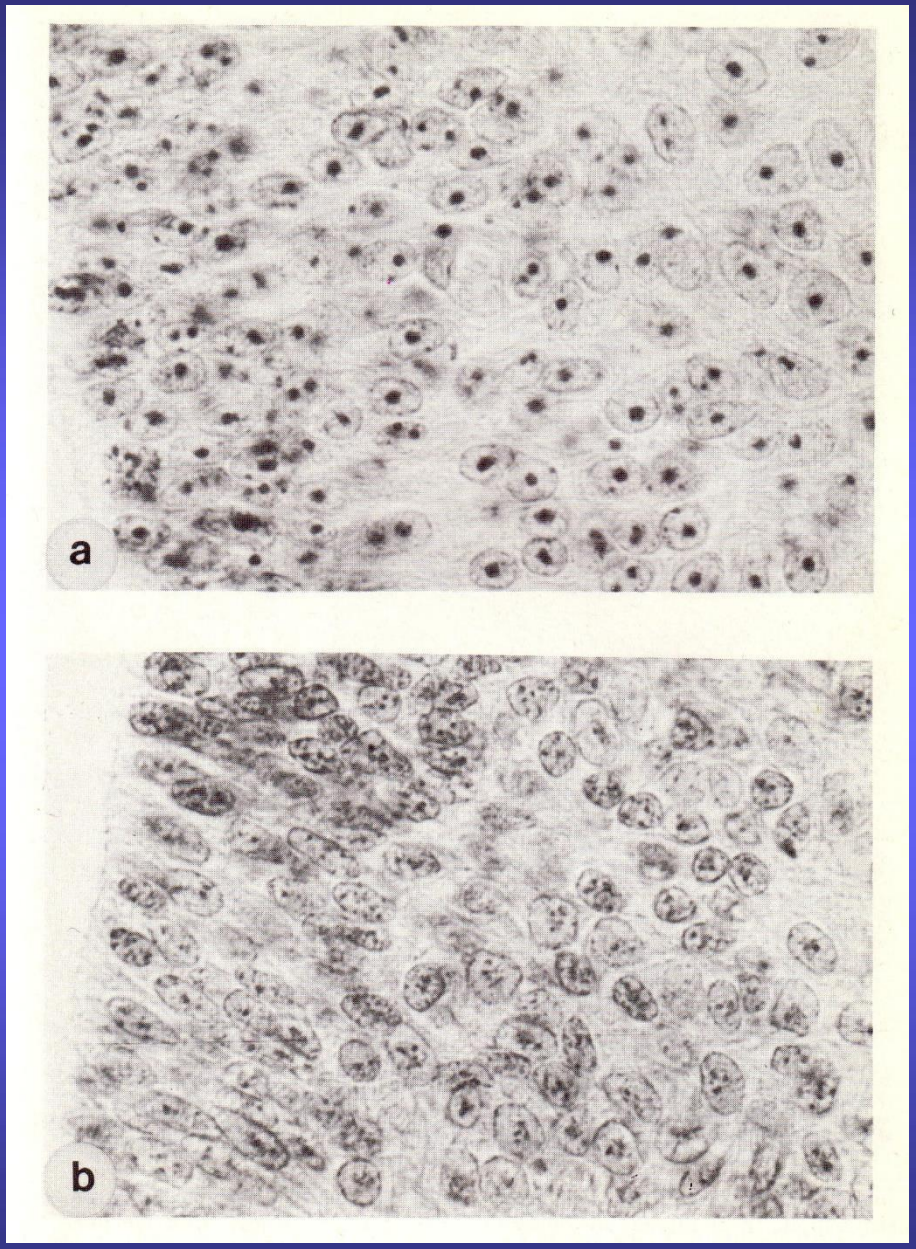
Gli ibridi quaglia-pollo

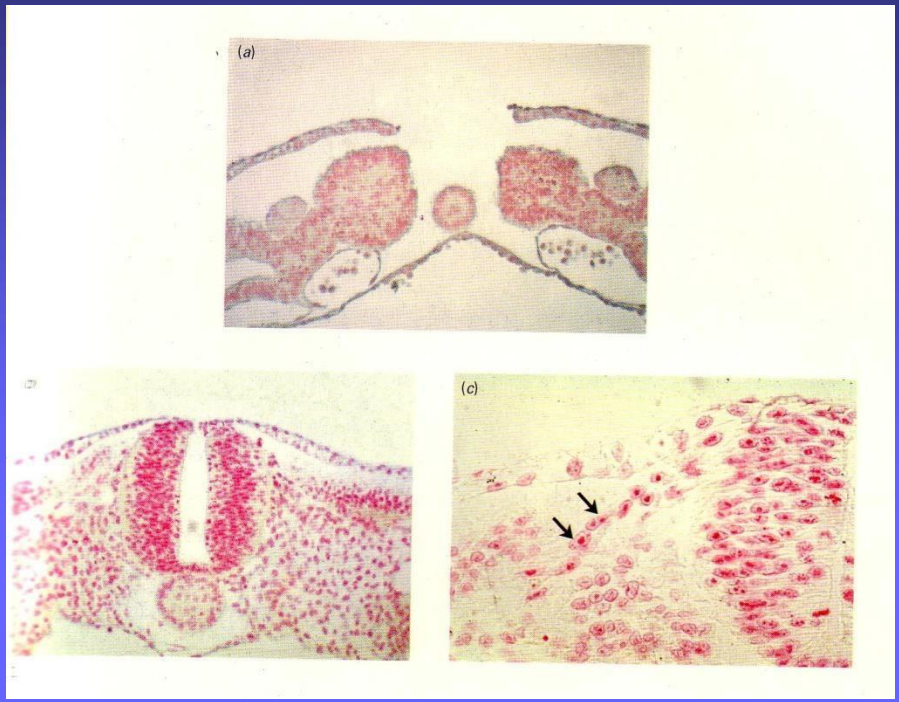


N. Le Douarin (1968)

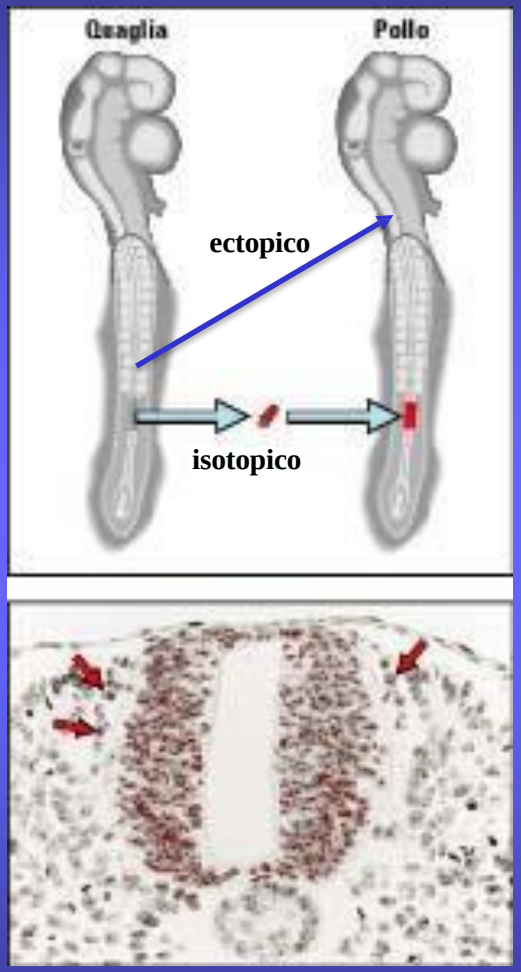








Colorazione Feulgen



1. Trapianti in regioni isotopiche (dimostrano una correlazione tra il sito di inizio della migrazione e il tipo di derivato)

2. Trapianti in regioni ectopiche (es. cresta neurale cervicale al posto della cresta neurale del tronco) (dimostrano che le cellule di cresta neurale non sono già determinate all'inizio della migrazione)

CN cefalica → Mesoderma e cartilagini della testa

CN cervicale → Gangli parasimpatici

CN cardiaca (I-III somite) → Derivati muscolari e connettivali delle grandi arterie

CN del tronco → Gangli simpatici e spinali

CN sacrale → Gangli parasimpatici e gangli enterici

I derivati cellulari delle creste neurali

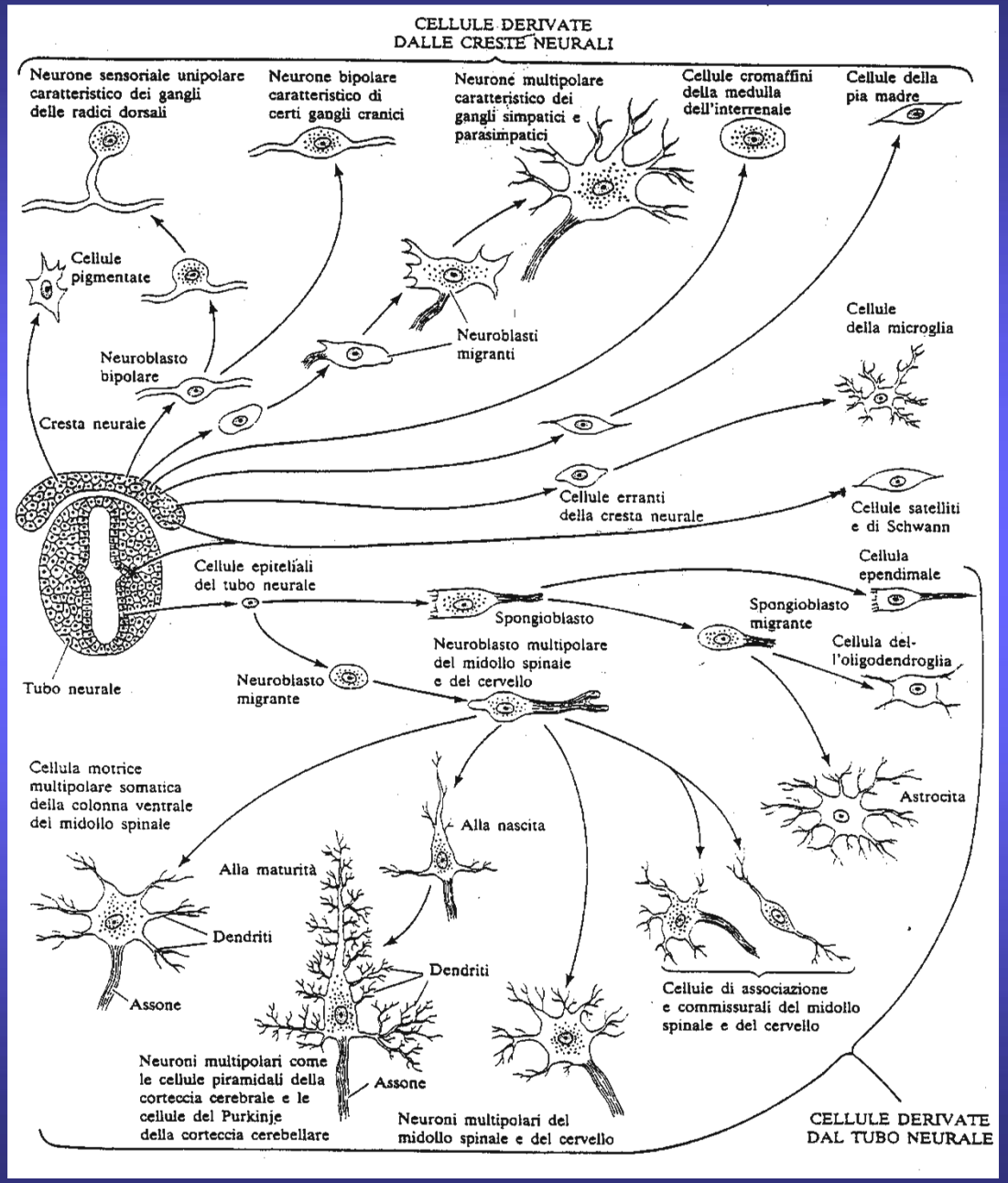
- Sistema nervoso periferico:
  gangli spinali
  gangli simpatici
  gangli parasimpatici
  Cellule di Schwann

- Cellule pigmentate:
  melanociti

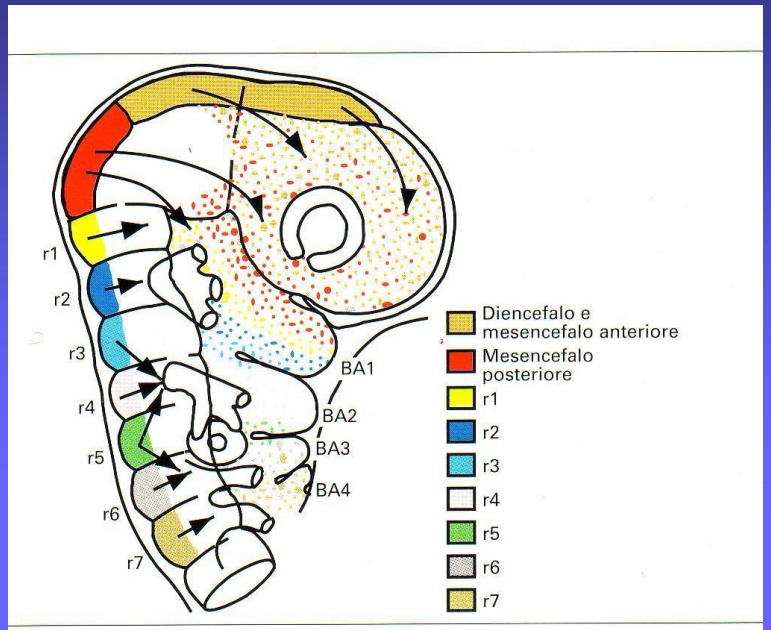
- Derivati endocrini:
  midollare del surrene

- Derivati mesenchimali:
  cartilagine ed ossa della faccia

- Derivati connettivali:
  connettivo e muscolatura delle arterie
  endotelio e stroma corneale

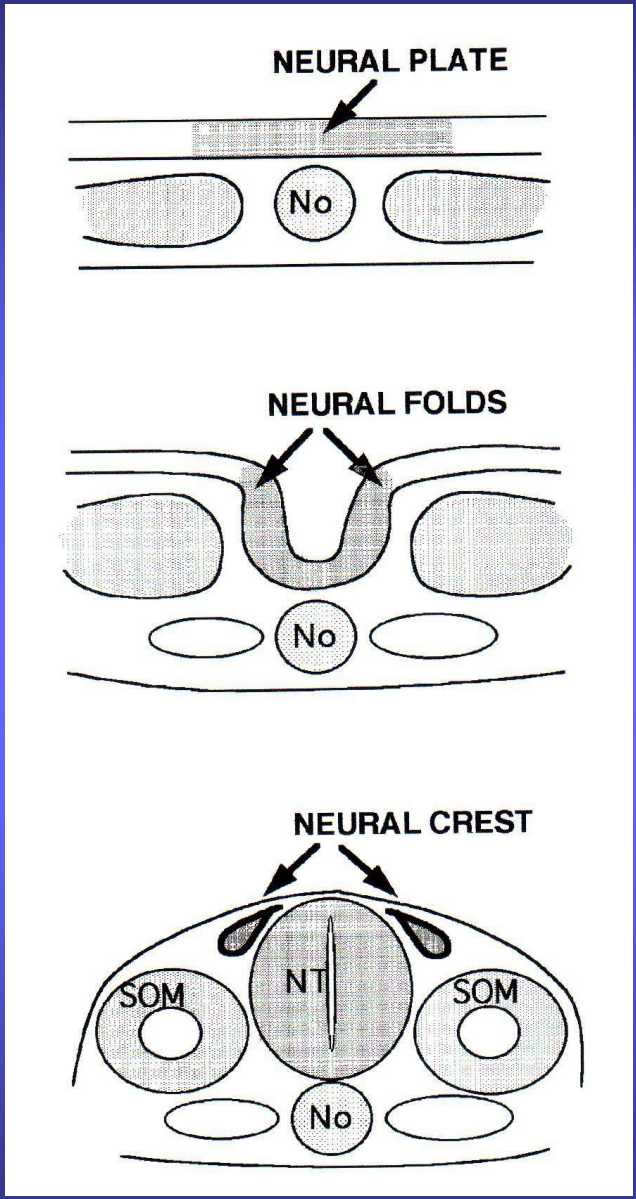


Cresta neurale cefalica



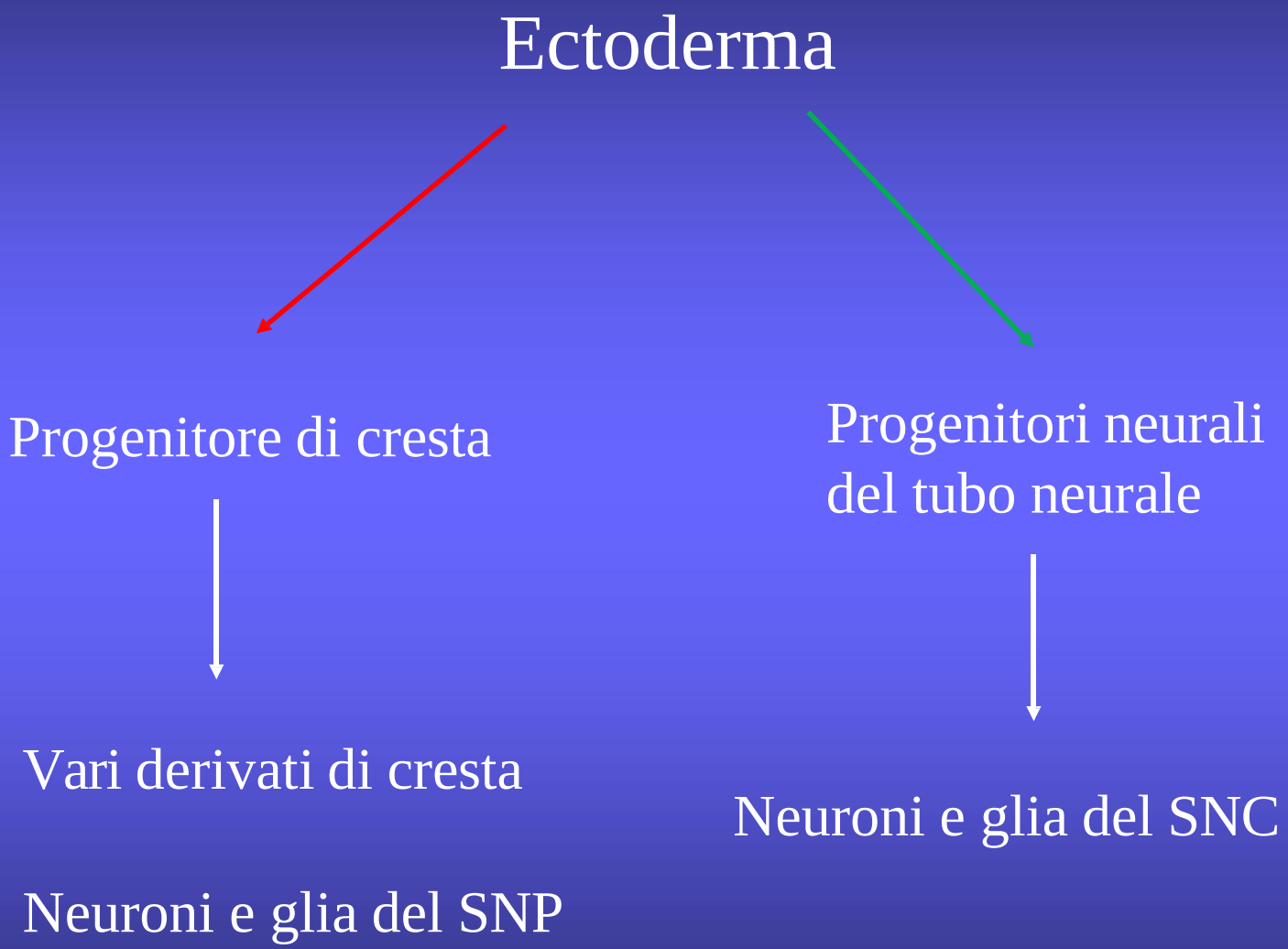
Derivati:

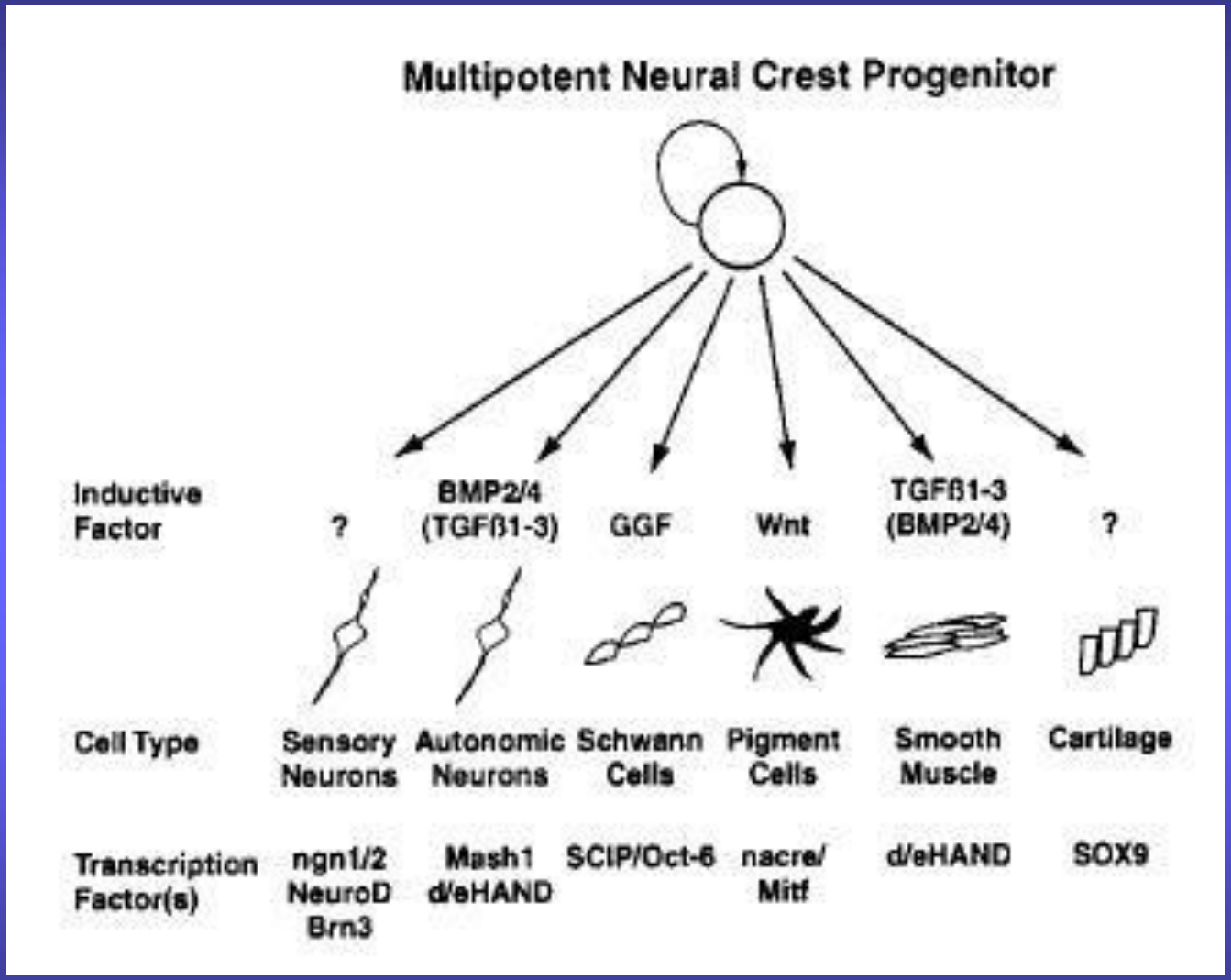
1. mesenchima della testa,
2. neuroni e nervi cranici,
3. connettivo di timo, tiroide e paratiroide
4. cartilagine della mandibola e dell'orecchio interno



Le cellule delle creste neurali vengono ad originarsi dagli stessi precursori del tubo neurale ?

I progenitori della NC vengono determinati e segregati alla chiusura del tubo neurale, benchè il territorio di cresta cominci ad essere definito ancor prima della neurulazione.

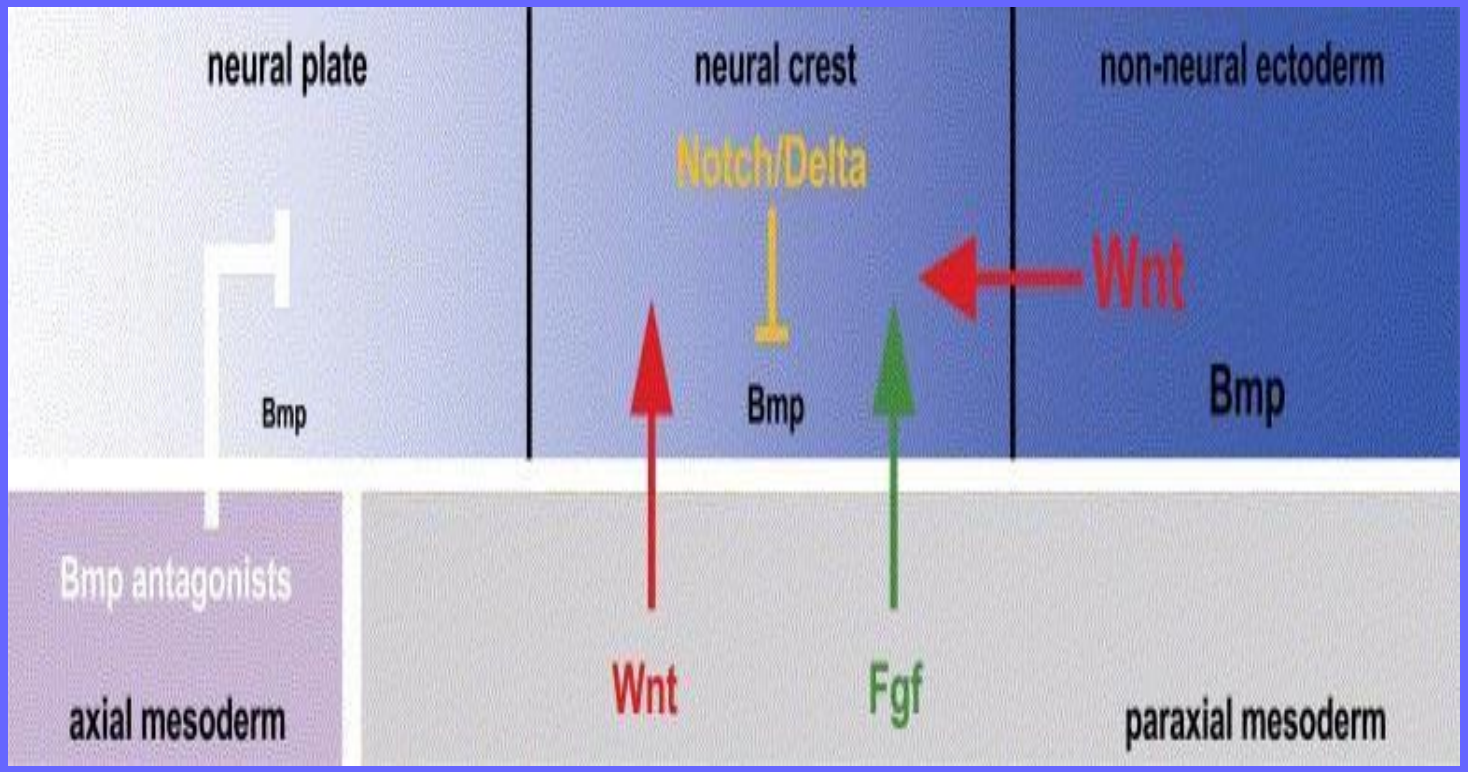

Ectoderma
Progenitore di cresta
Progenitori neurali del tubo neurale
Vari derivati di cresta
Neuroni e glia del SNP
Neuroni e glia del SNC

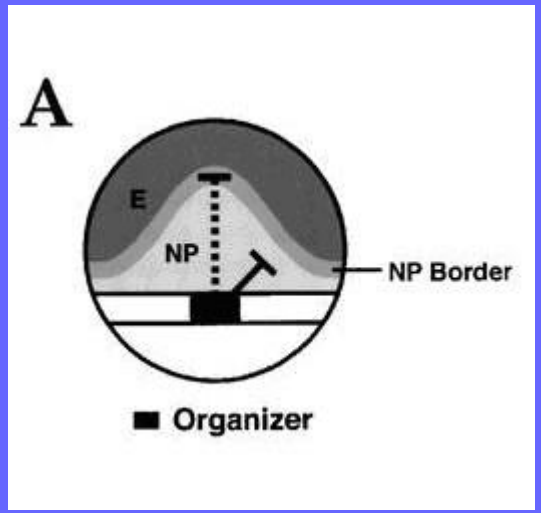

Multipotent Neural Crest Progenitor
Inductive Factor
?
BMP2/4 (TGFß1-3)
GGF
Wnt
TGFß1-3 (BMP2/4)
?
Cell Type
Sensory Neurons
Autonomic Neurons
Schwann Cells
Pigment Cells
Smooth Muscle
Cartilage
Transcription Factor(s)
ngn1/2 NeuroD Brn3
Mash1 d/eHAND
SCIP/Oct-6
nacre/ Mitf
d/eHAND
SOX9

- **Quali sono i fattori che determinano la NC ?**

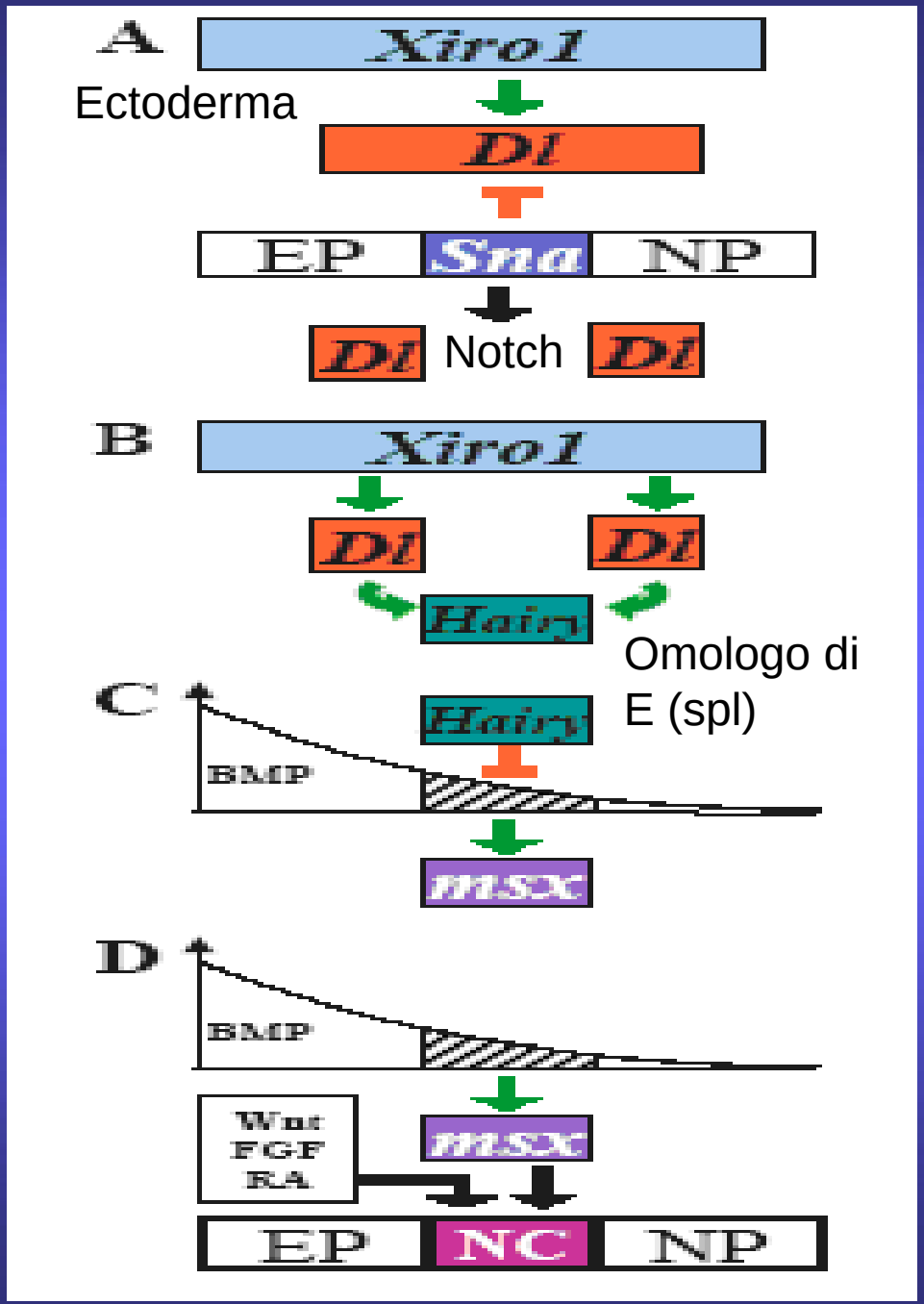
- La placca neurale e l'epidermide sono responsabili della determinazione delle cellule della NC

- I fattori coinvolti sono BMP e WNT





I segnali che agiscono a livello del territorio di cresta.



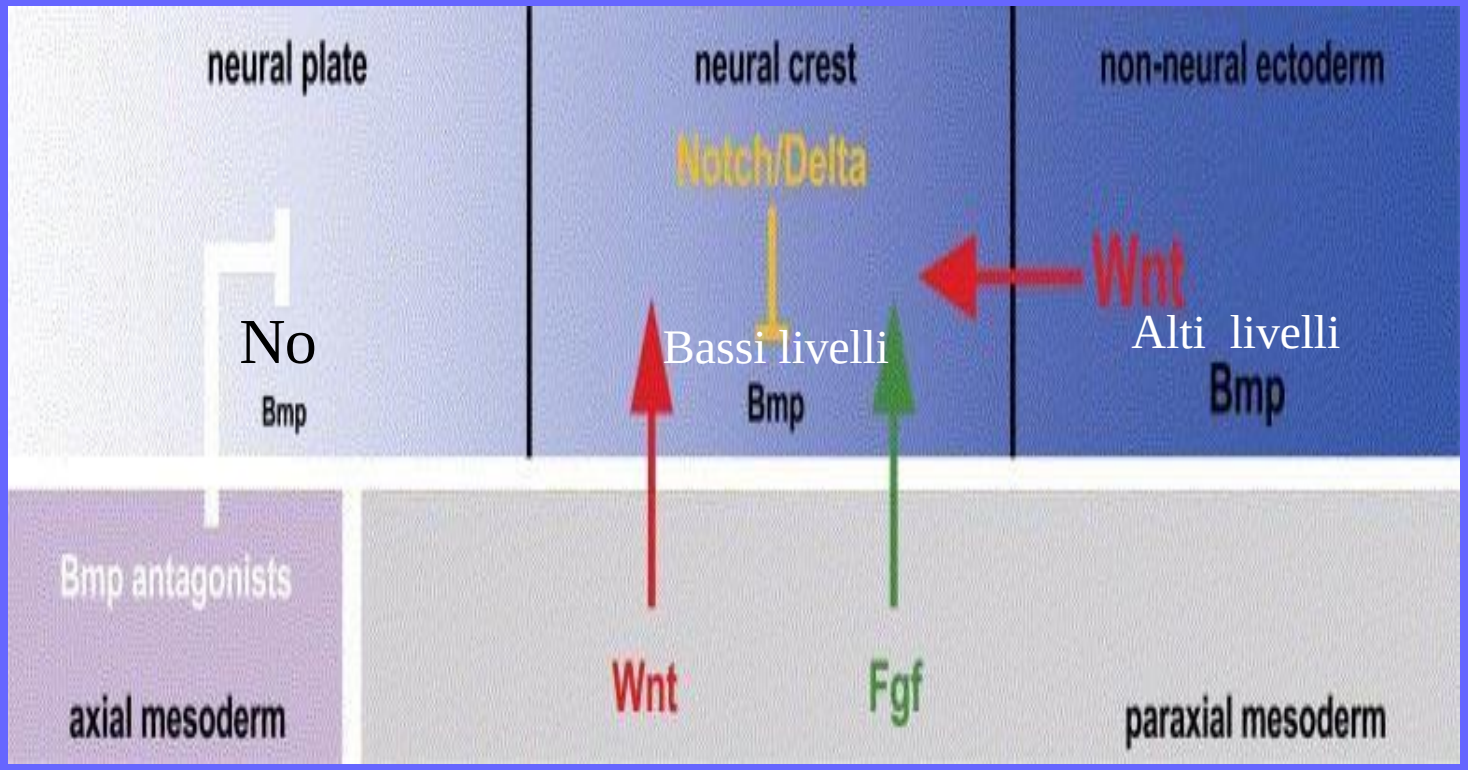
Gastrula iniziale

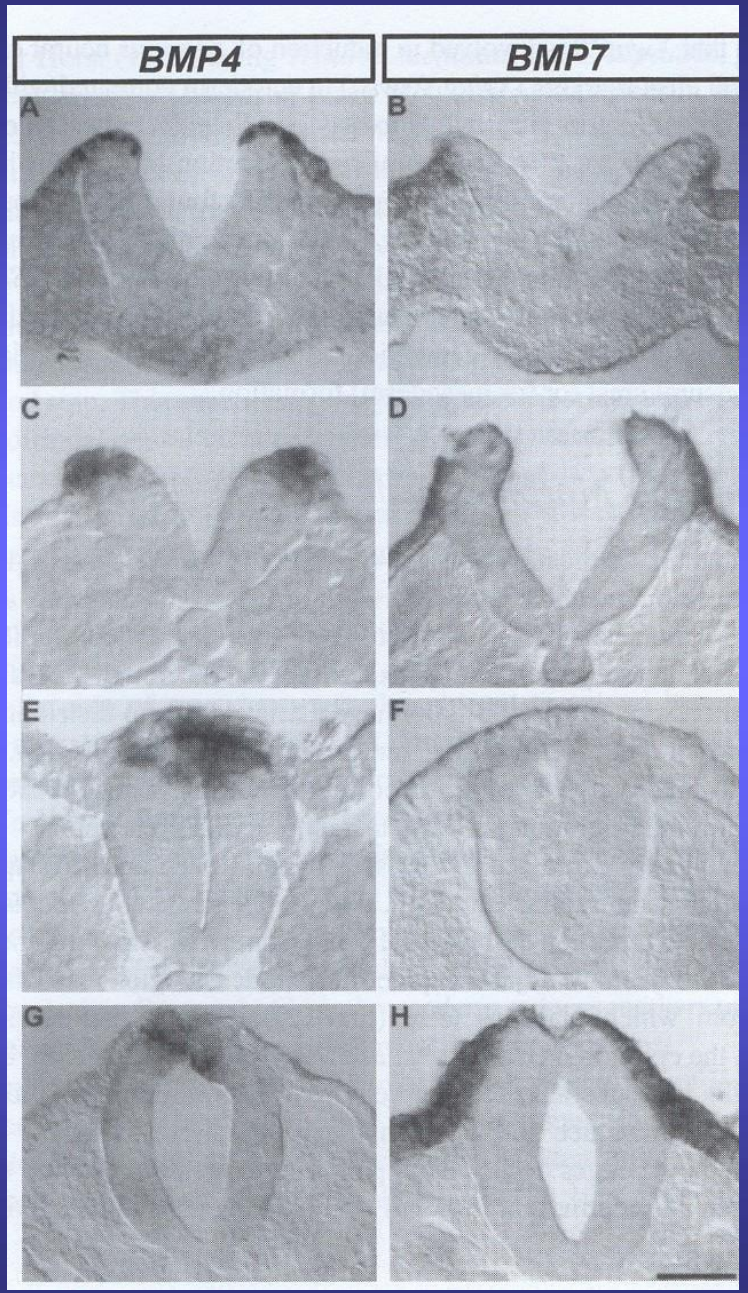
Gastrula tardiva

Hairy controlla a livello trascrizionale i livelli di BMP

La cascata Delta-Notch e la inibizione di BMP

Mayer et al, 2004

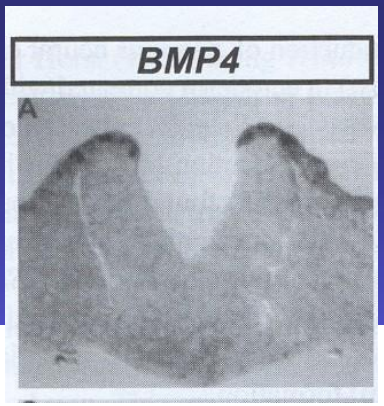

neural plate
neural crest
non-neural ectoderm
Notch/Delta
Wnt
No
Bmp
Bassi livelli
Bmp
Alti livelli
Bmp
Bmp antagonists
axial mesoderm
Wnt
Fgf
paraxial mesoderm

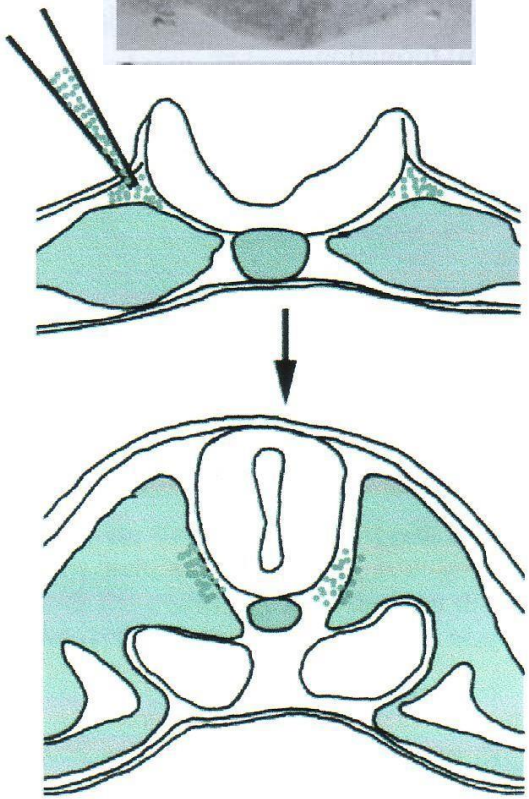


BMP4 è uno dei responsabili della formazione dei progenitori NC

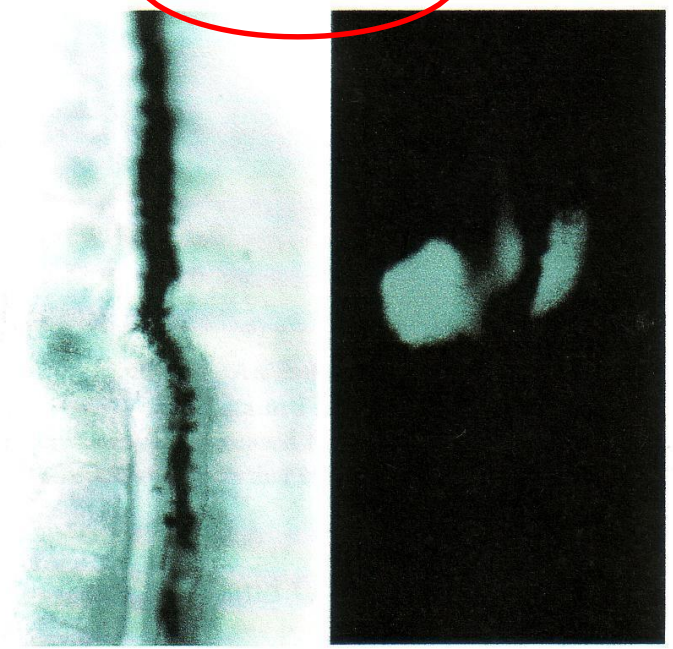
L’azione di BMP è tempo dipendente:

1. Fase precoce: BMP indipendente
2. Fase intermedia: BMP dipendente
3. Fase tardiva: BMP indipendente

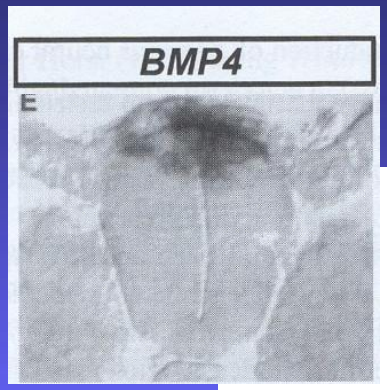


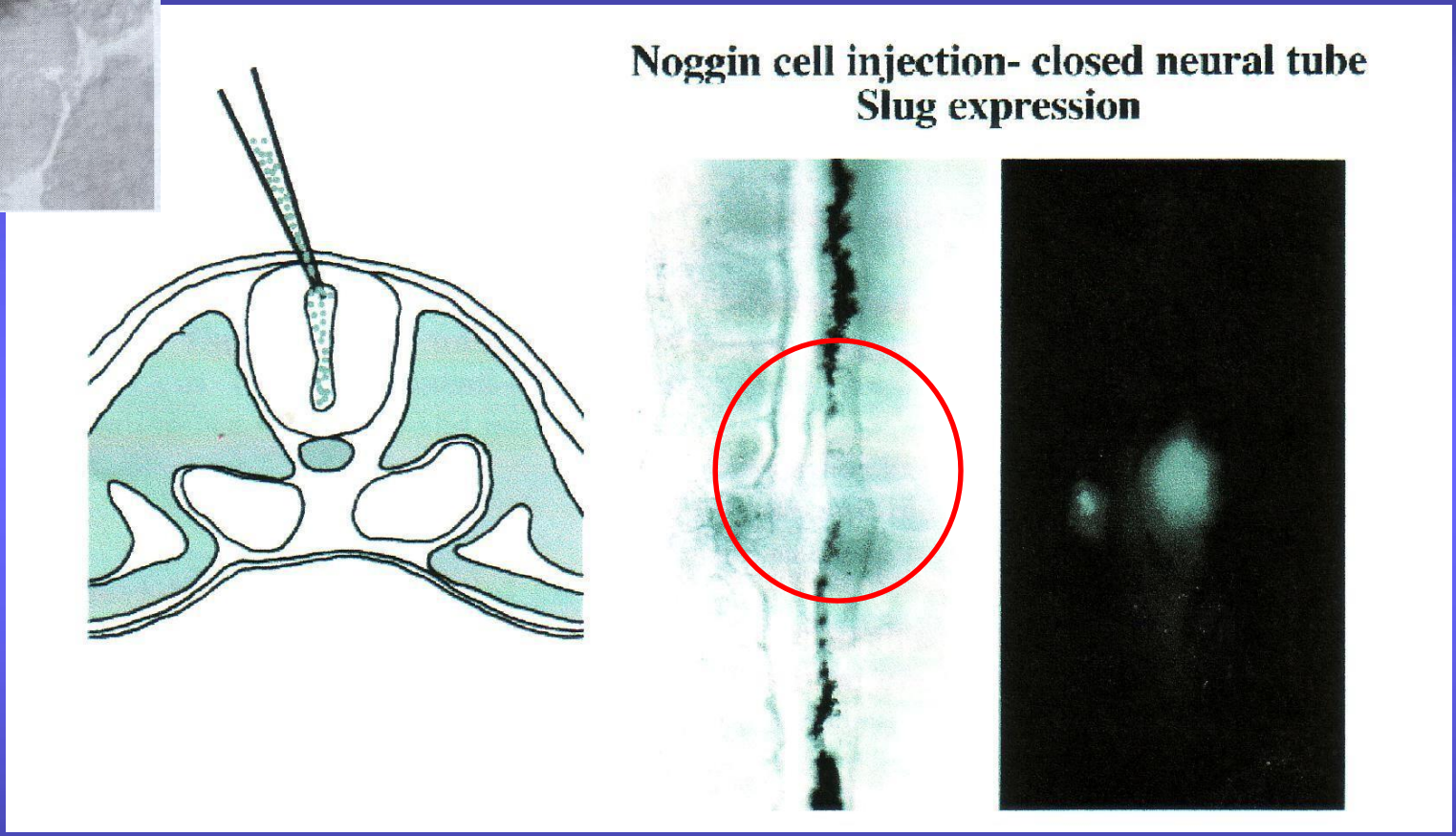


Noggin cell injection- open neural plate
Slug expression

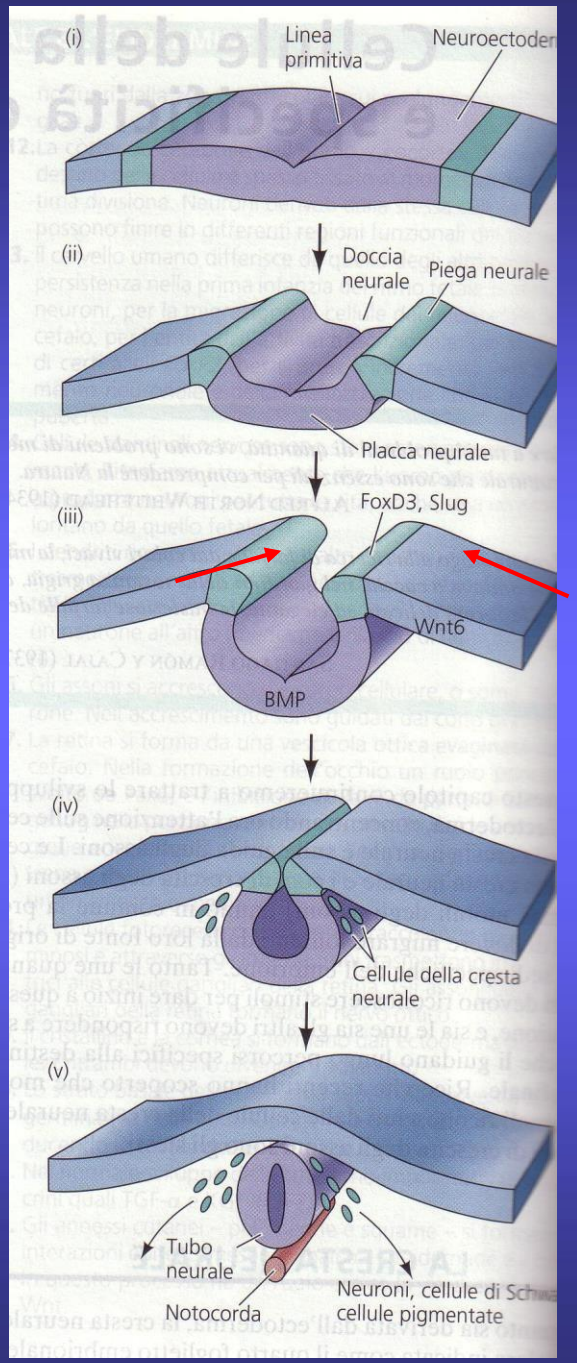


L’iniezione di cellule producenti noggin quando il NT è aperto non impedisce la formazione della NC





Quando le cellule producenti noggin vengono iniettate nel tubo neurale chiuso, si inibisce la formazione della NC



WNT prodotto a livello dell'epidermide collabora alla determinazione delle cellule della NC

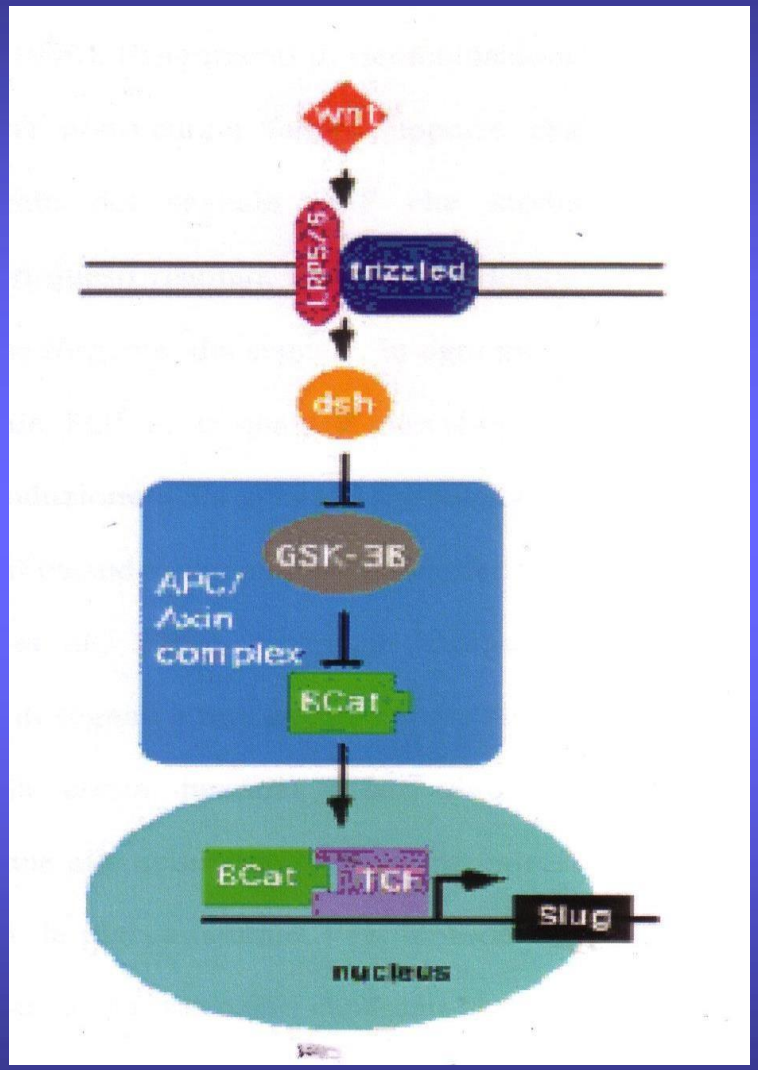
Marcatori di cresta FOXD3 e Slug

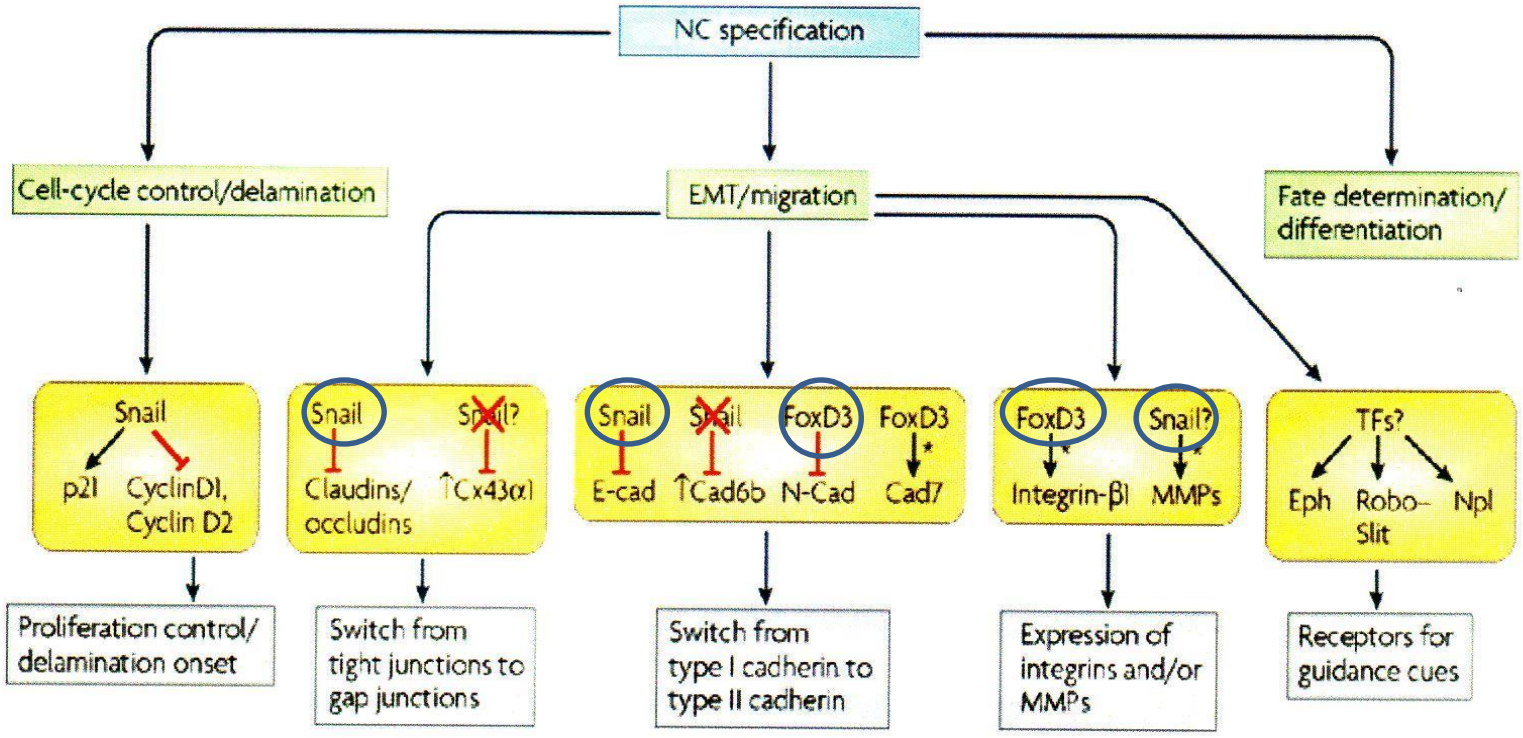
In risposta ai segnali BMPe WNT viene acceso il gene **FoxD3** nella zona della cresta neurale

Segue l'accensione di **Slug** che impartisce le proprietà mesenchimali alle cellule della NC.

Slug è una fattore di trascrizione a dita di zinco che reprime l'espressione di alcuni geni (es. causa la inibizione dell'espressione delle N-caderine).

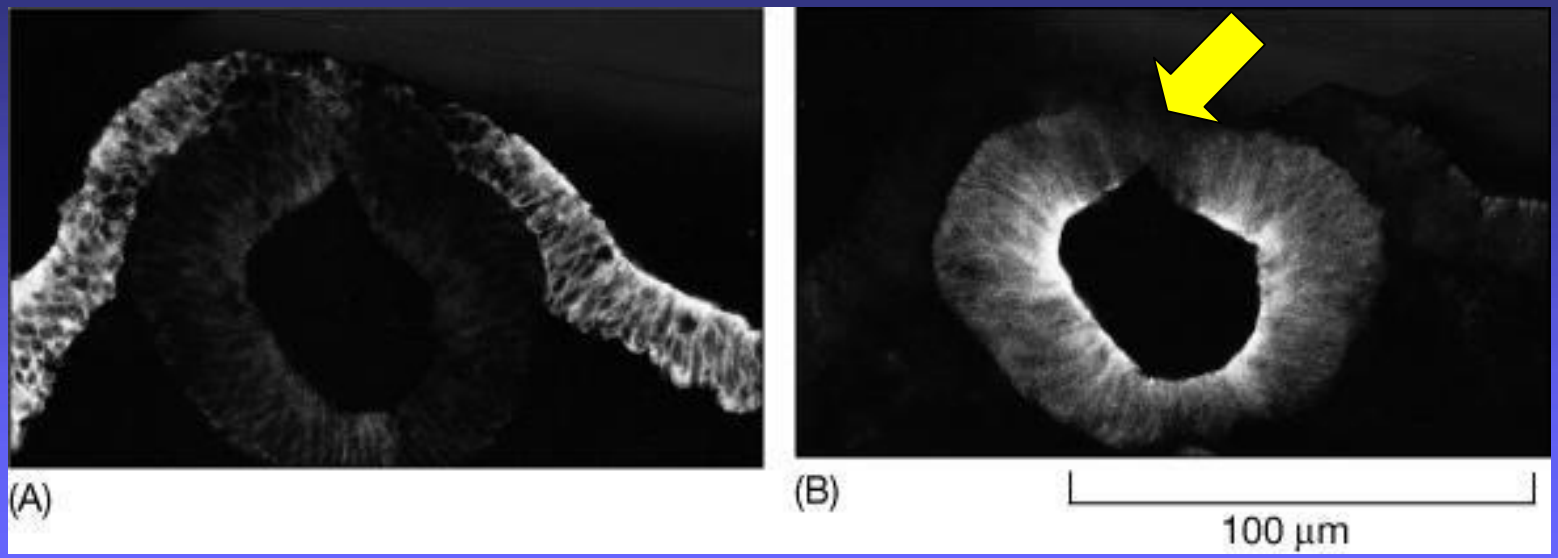
La cascata WNT e Beta catenina regolano l'accensione di Slug

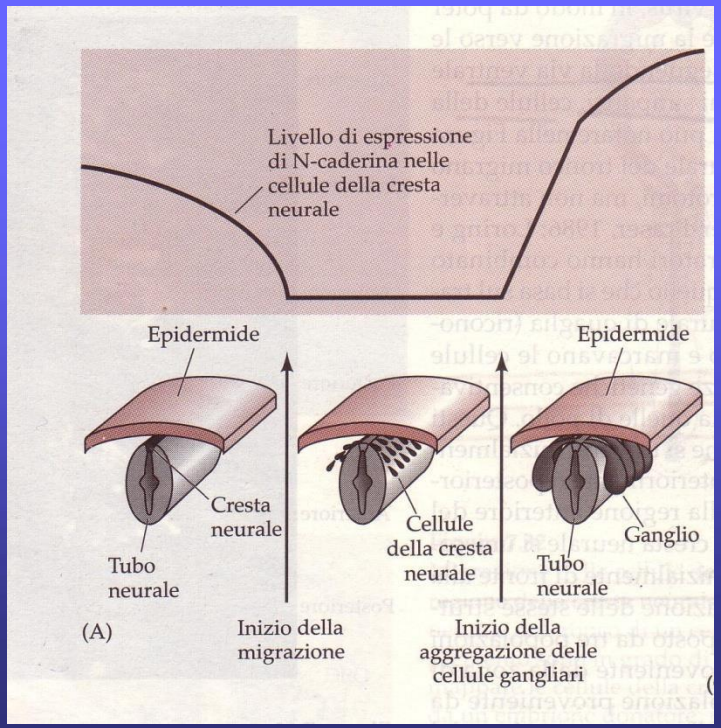



NC specification
Cell-cycle control/delamination
EMT/migration
Fate determination/ differentiation
Snail
p21
CyclinD1, Cyclin D2
Proliferation control/ delamination onset
Snail
Snail?
Claudins/ occludins
↑Cx43α1
Switch from tight junctions to gap junctions
Snail
Snail
FoxD3
FoxD3
E-cad
↑Cad6b
N-Cad
Cad7
Switch from type I cadherin to type II cadherin
FoxD3
Snail?
Integrin-β1
MMPs
Expression of integrins and/or MMPs
TFs?
Eph
Robo–Slit
Npl
Receptors for guidance cues
Nature Reviews | Molecular Cell Biology

E-caderina

N-Caderine





• Ruolo della matrice extracellulare

• Substrati permissivi: fibronectina, laminina, collagene, alcuni proteoglicani

• Espressione di integrine
•specifiche (es. β1)

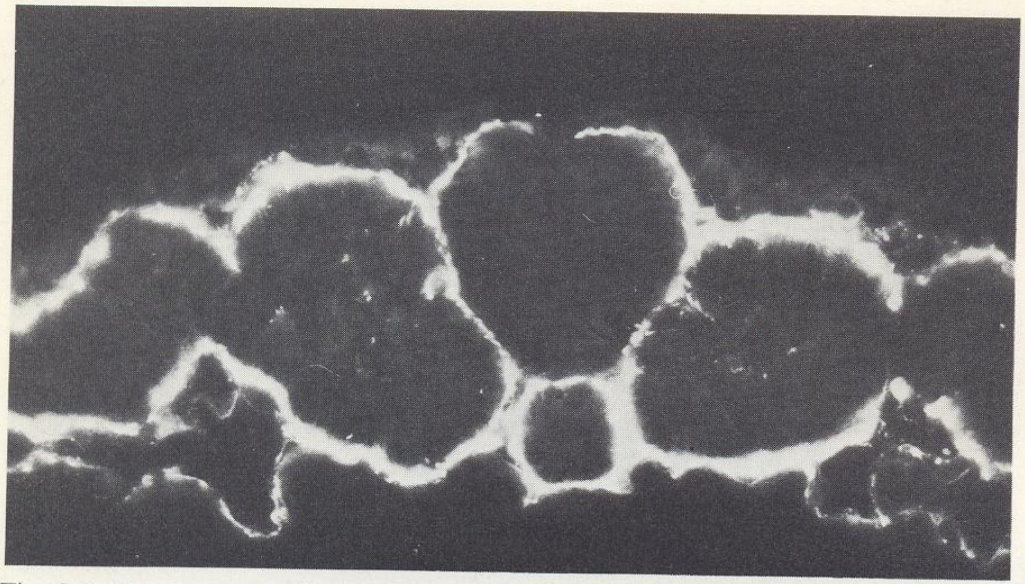
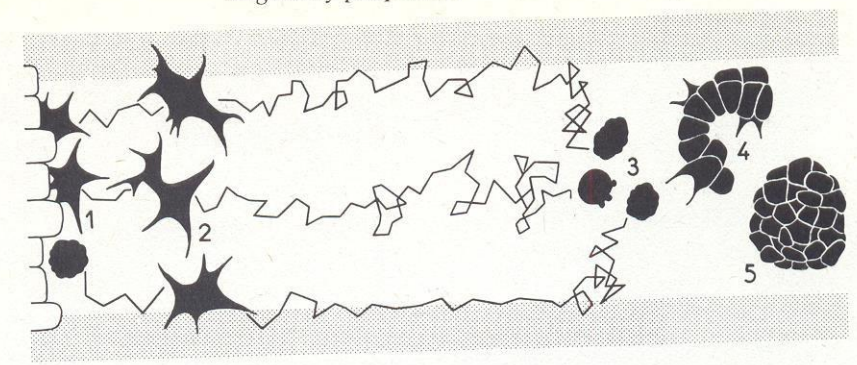


Fig. 2.2. Transverse section of a 15-somite embryo at the last somite level. Fibronectin is present as a basement membrane component of all the tissues (ectoderm, somite, neural tube, notochord and endoderm). The neural tube basement membrane is not completed on its dorsal aspect. *Note*: Just before neural crest cell migration no space is available in the fibronectin-rich presumptive pathways. Immunofluorescence staining of fibronectin. (×260)



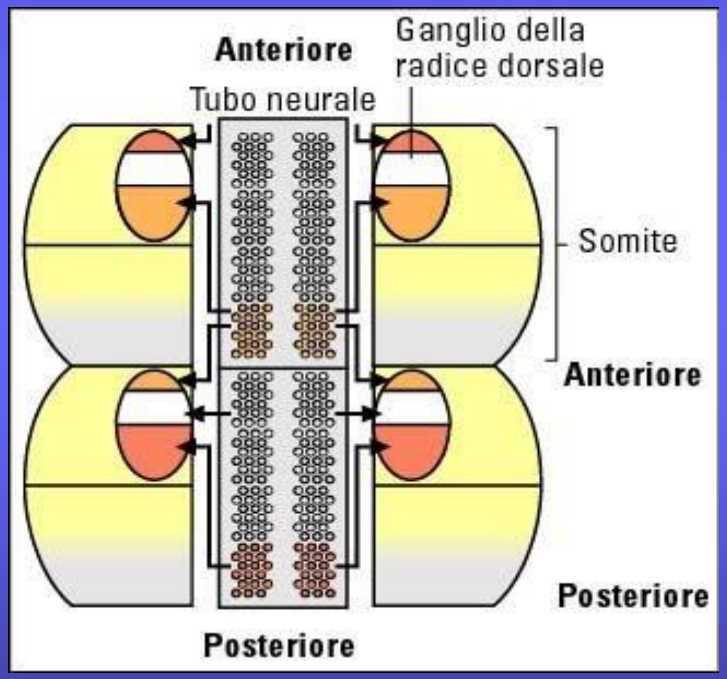
Fig. 2.18. *In vitro* culture of neural crest cells on a fibronectin substratum. Neural crest cells migrate as a quasi-confluent layer following very precisely the pathways containing fibronectin. (From Rovasio *et al.*, 1982.) (×170)

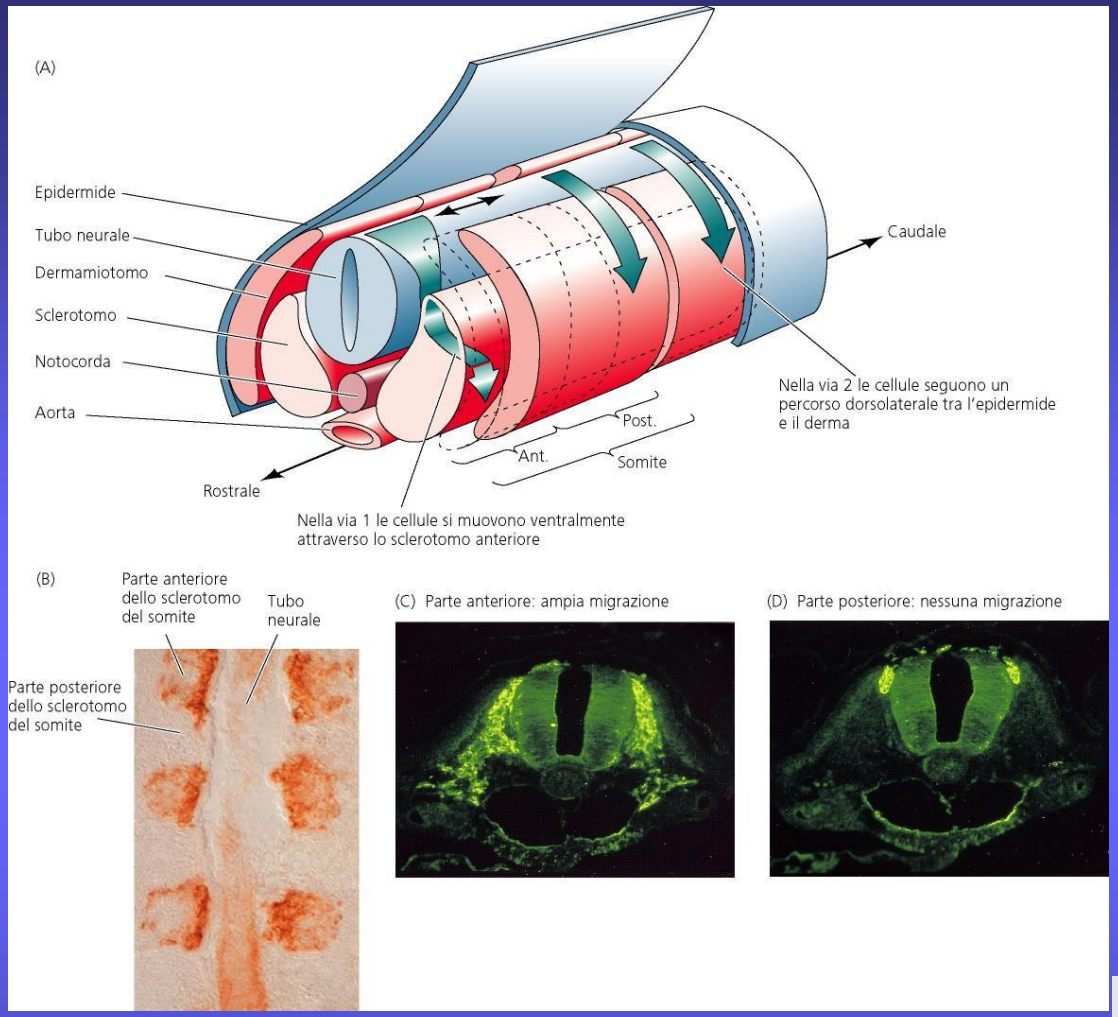
Substrato di fibronectina è di supporto alle cellule di cresta



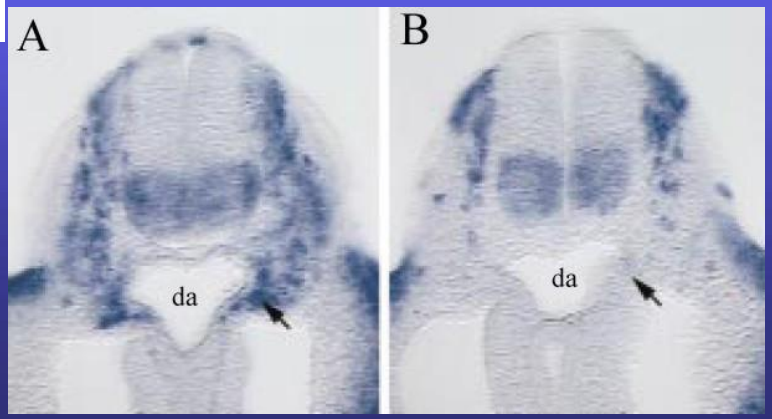
La Migrazione delle NC è regolata da diversi fattori

- Molecole inibitorie: alcuni proteoglicani, efrine, T-caderine





Il segnale di neuregulina media la migrazione delle cellule della NC



Vie di migrazione

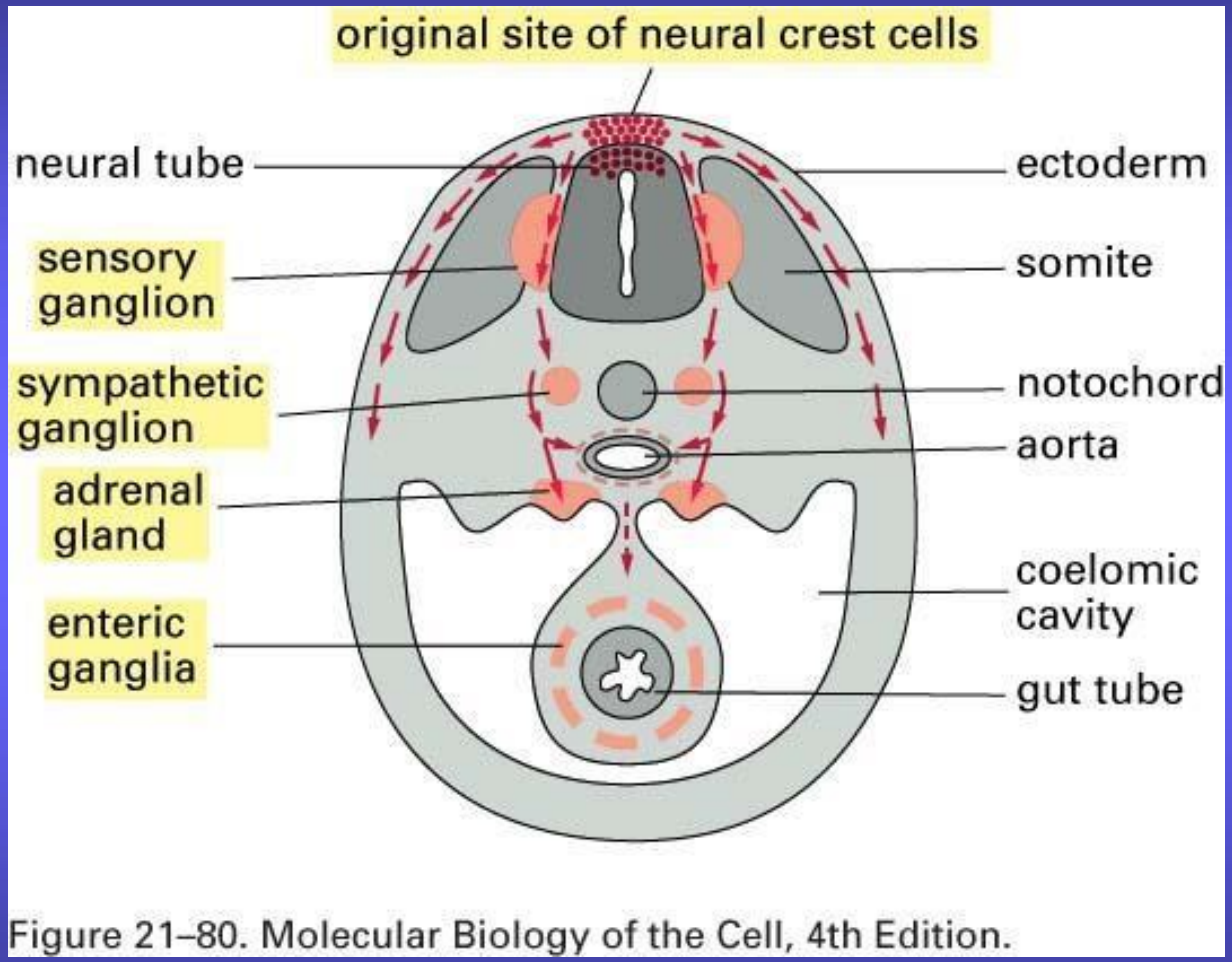


Figure 21–80. Molecular Biology of the Cell, 4th Edition.

Dorso laterale

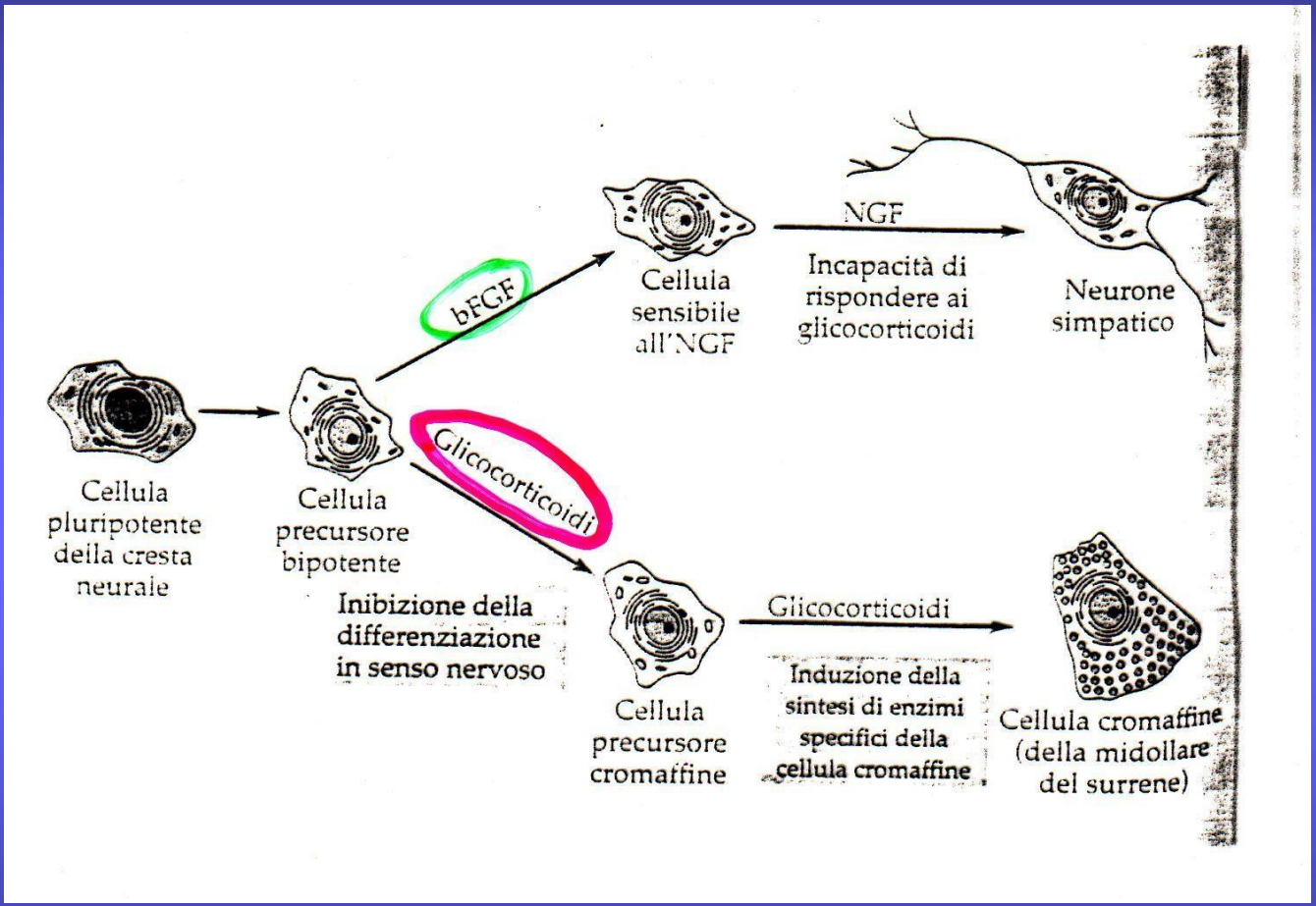
Latero ventrale

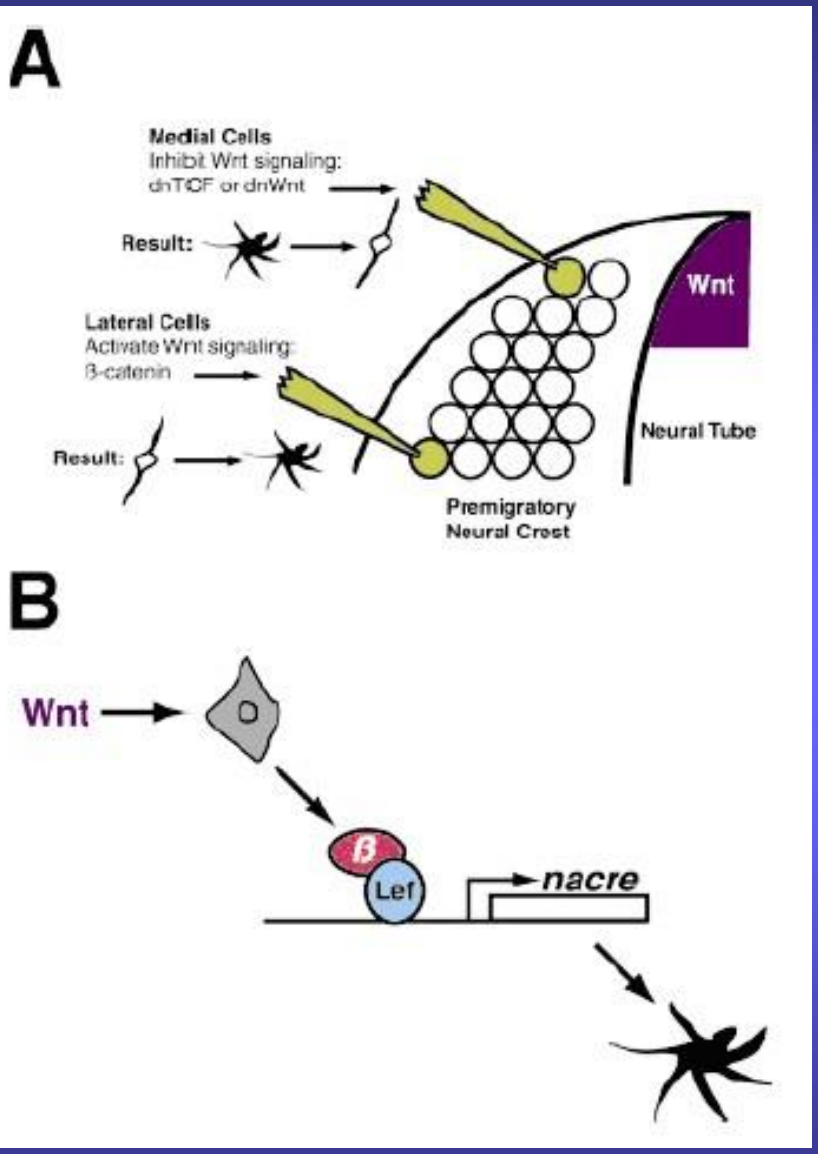
DETERMINAZIONE FINALE

1. Segnali locali provenienti dal tubo neurale e dalla notocorda

2. Fattori di crescita rilasciati e depositati sulla matrice extracellulare

Segnali inducenti per le NC





Il segnale WNT e beta-catenina è importante per la determinazione della via di melanocita

Prima ondata migratoria (NC dorsali): neuroni

Seconda ondata migratoria (NC ventrali): melanocita