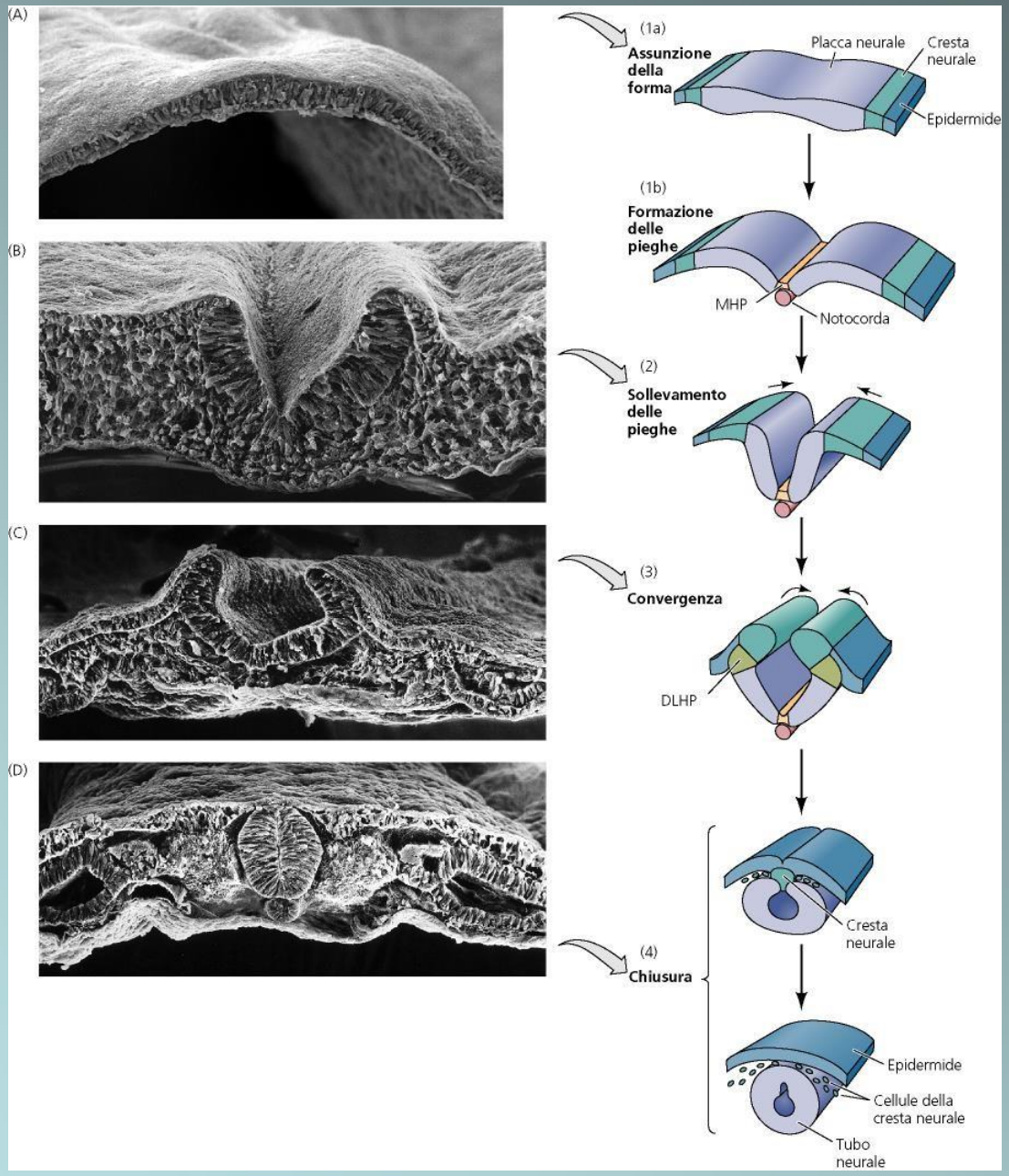
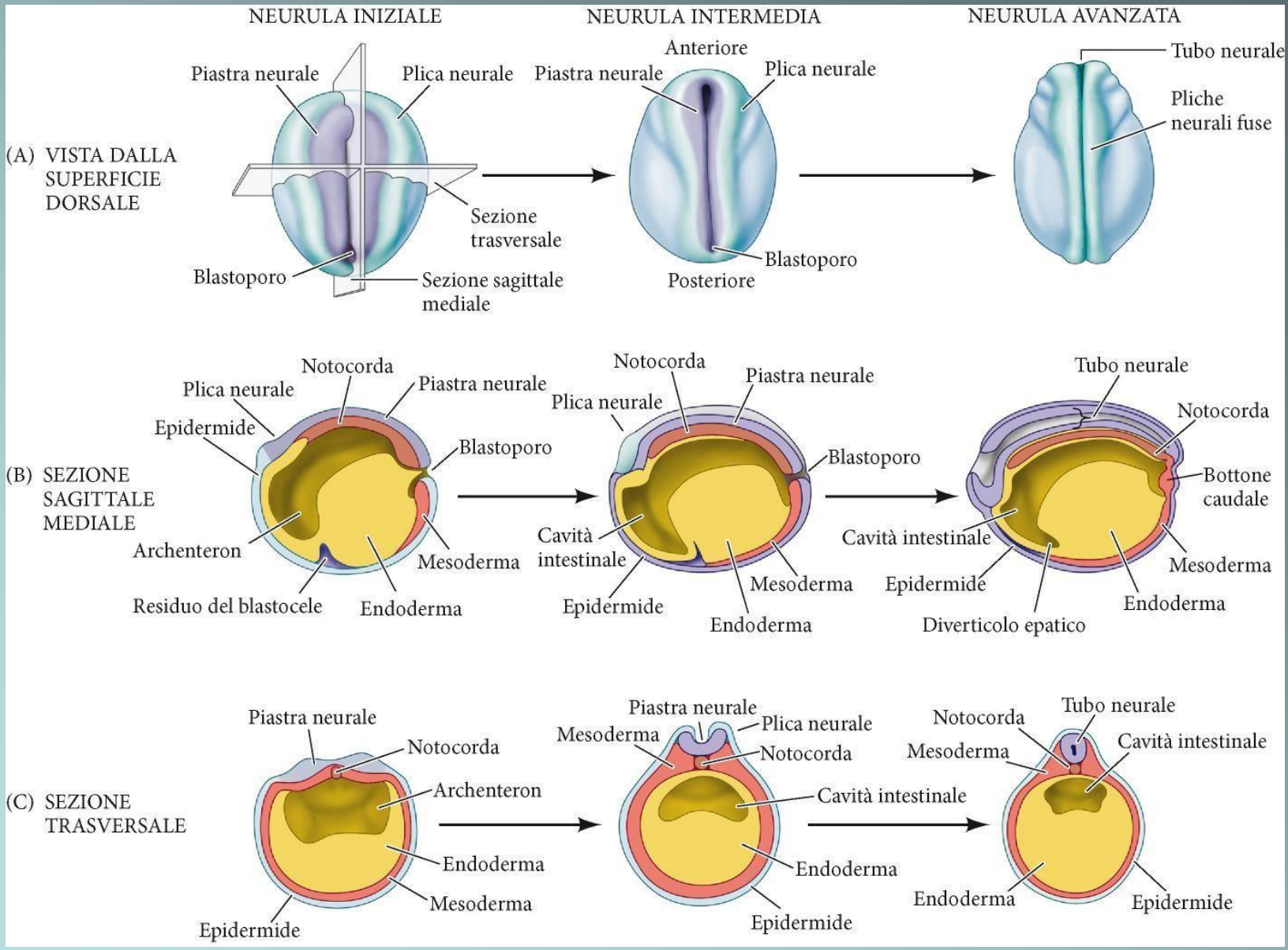
Definizione della polarità antero-posteriore e dorso ventrale nel Sistema nervoso

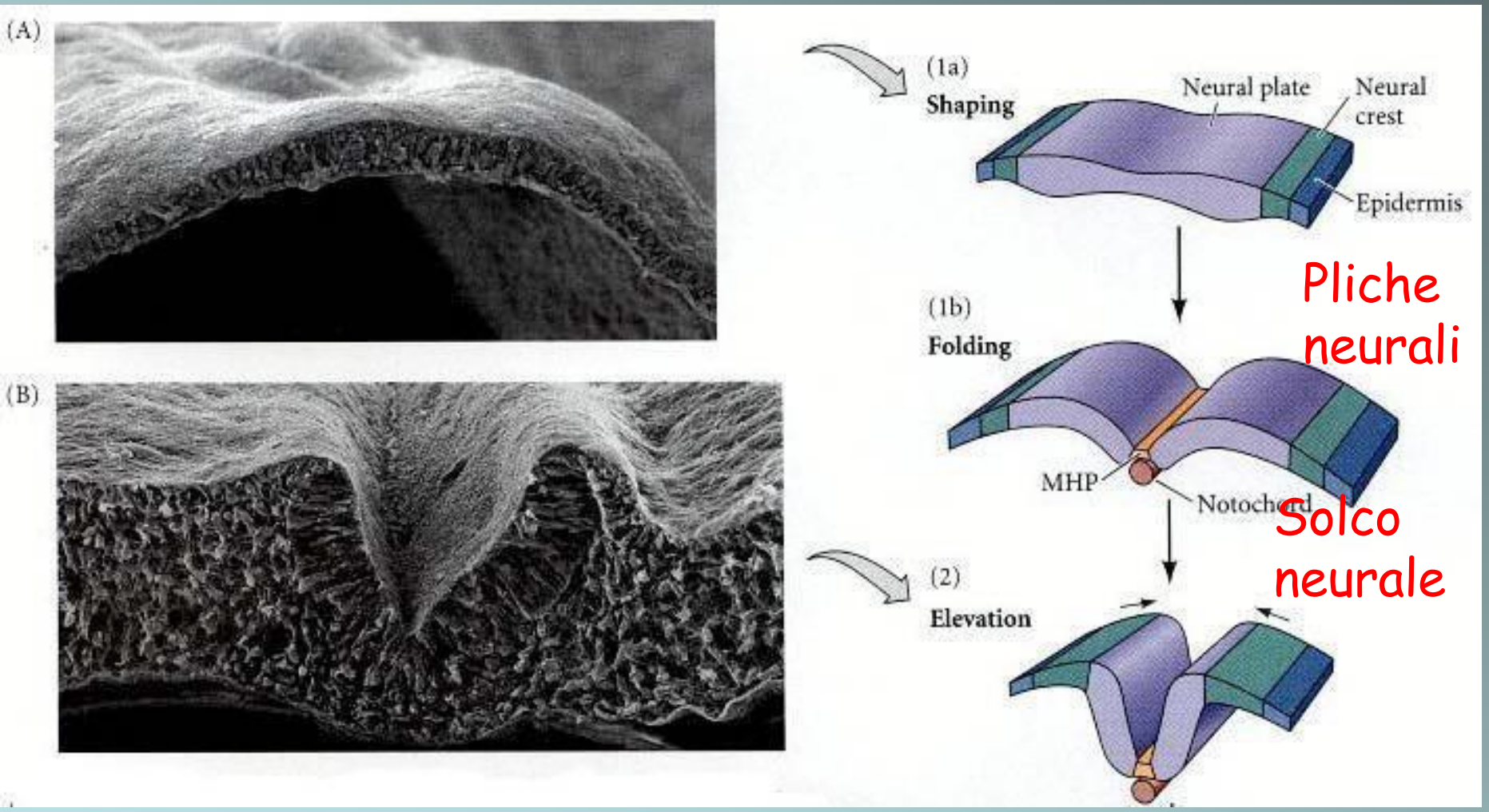

(A)
(B)
(C)
(D)
(1a)
Assunzione della forma
Placca neurale
Cresta neurale
Epidermide
(1b)
Formazione delle pieghe
MHP
Notocorda
(2)
Sollevamento delle pieghe
(3)
Convergenza
DLHP
(4)
Chiusura
Cresta neurale
Epidermide
Cellule della cresta neurale
Tubo neurale


NEURULA INIZIALE
NEURULA INTERMEDIA
NEURULA AVANZATA
(A) VISTA DALLA SUPERFICIE DORSALE
Piastra neurale
Plica neurale
Sezione trasversale
Blastoporo
Sezione sagittale mediale
Anteriore
Piastra neurale
Plica neurale
Blastoporo
Posteriore
Tubo neurale
Pliche neurali fuse
(B) SEZIONE SAGITTALE MEDIALE
Notocorda
Plica neurale
Piastra neurale
Epidermide
Blastoporo
Archenteron
Mesoderma
Residuo del blastocele
Endoderma
Notocorda
Piastra neurale
Plica neurale
Blastoporo
Cavità intestinale
Epidermide
Mesoderma
Endoderma
Tubo neurale
Notocorda
Bottone caudale
Cavità intestinale
Mesoderma
Epidermide
Endoderma
Diverticolo epatico
(C) SEZIONE TRASVERSALE
Piastra neurale
Notocorda
Archenteron
Endoderma
Mesoderma
Epidermide
Piastra neurale
Plica neurale
Mesoderma
Notocorda
Cavità intestinale
Endoderma
Epidermide
Tubo neurale
Notocorda
Cavità intestinale
Mesoderma
Endoderma
Epidermide

La neurulazione primaria

Avviene nelle regioni anteriori dell'embrione

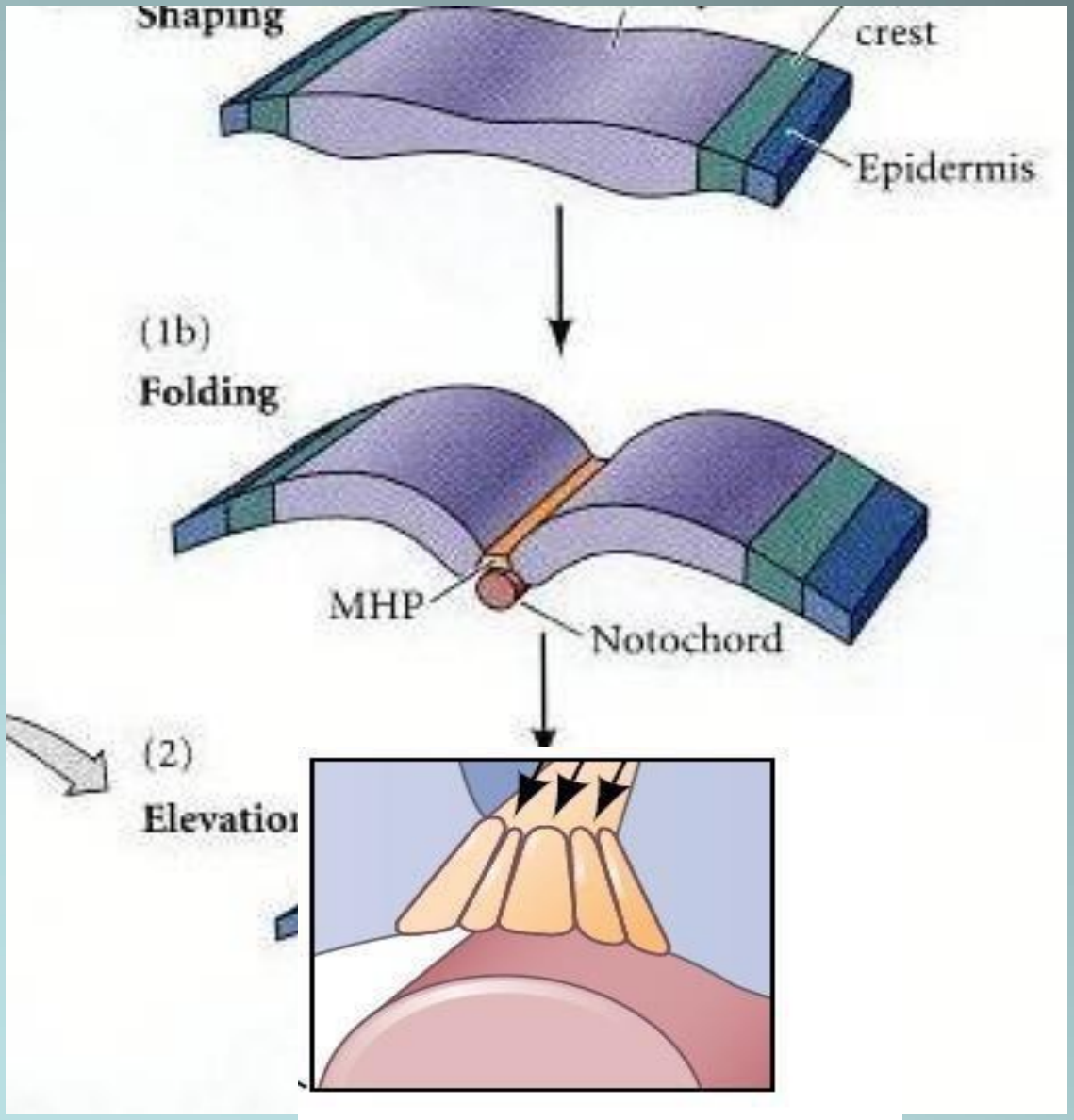
Piastra neurale



Pliche neurali

Solco neurale

Punti cardine per la neurulazione



MHP: cellule del punto cardine mediale.

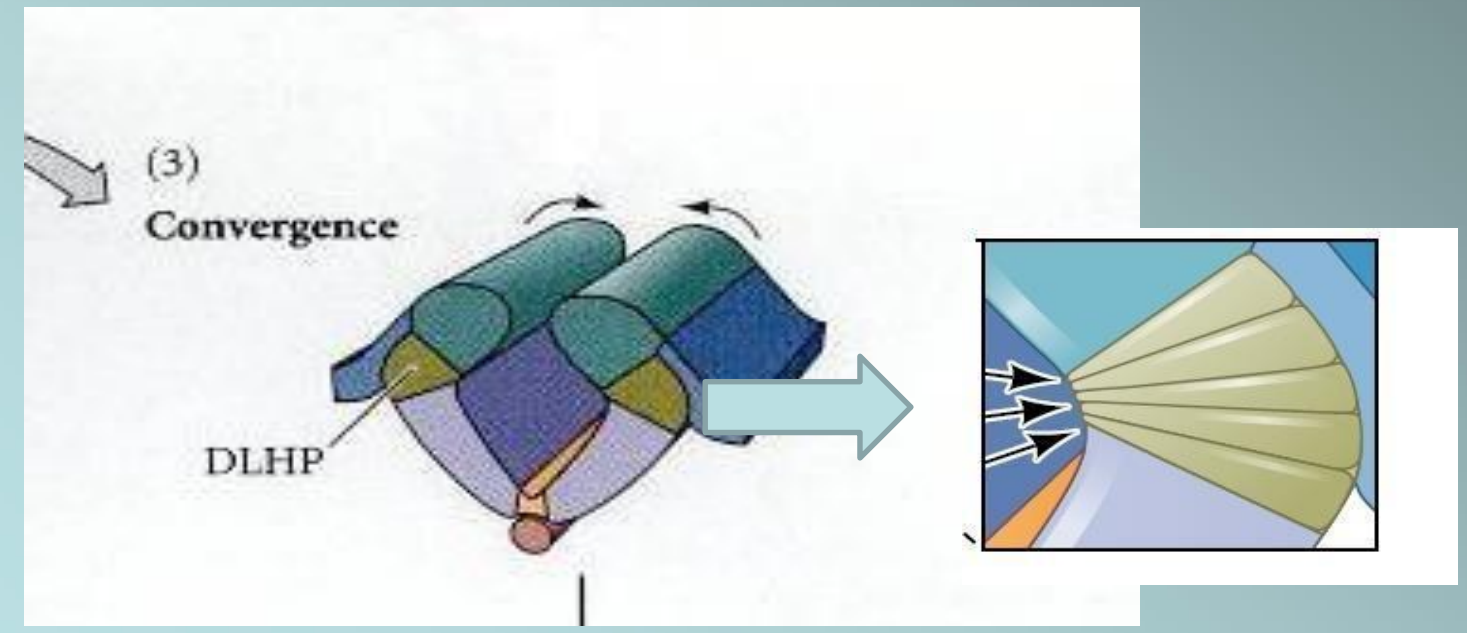
Cellule posizionate sopra la notocorda che acquisiscono una forma a cuneo
(modificazioni del citoscheletro di actina)

Punti cardine per la neurulazione

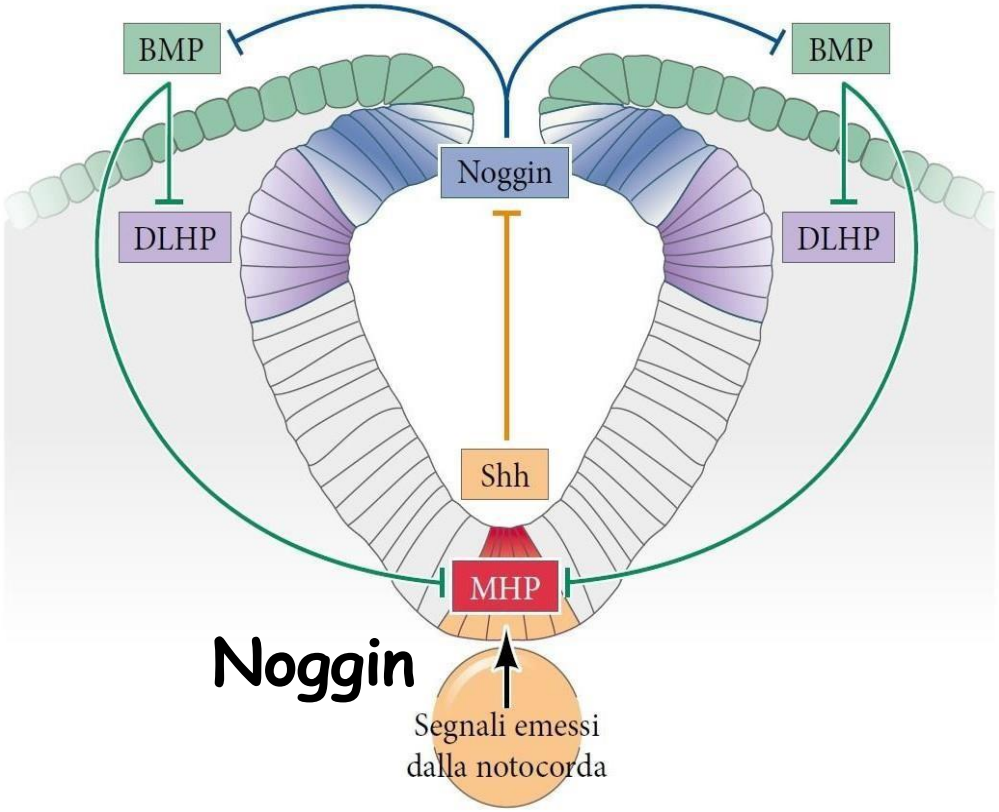
DLHP: cellule del punto cardine dorsolaterale.

Cellule posizionate ai lati della piastra neurale che acquisiscono una forma a cuneo (modificazioni del citoscheletro di actina)

Maggiore proliferazione nella porzione dorsale



La formazione dei punti cardine per la neurulazione sono regolati da gradienti di BMP, Noggin e SHH



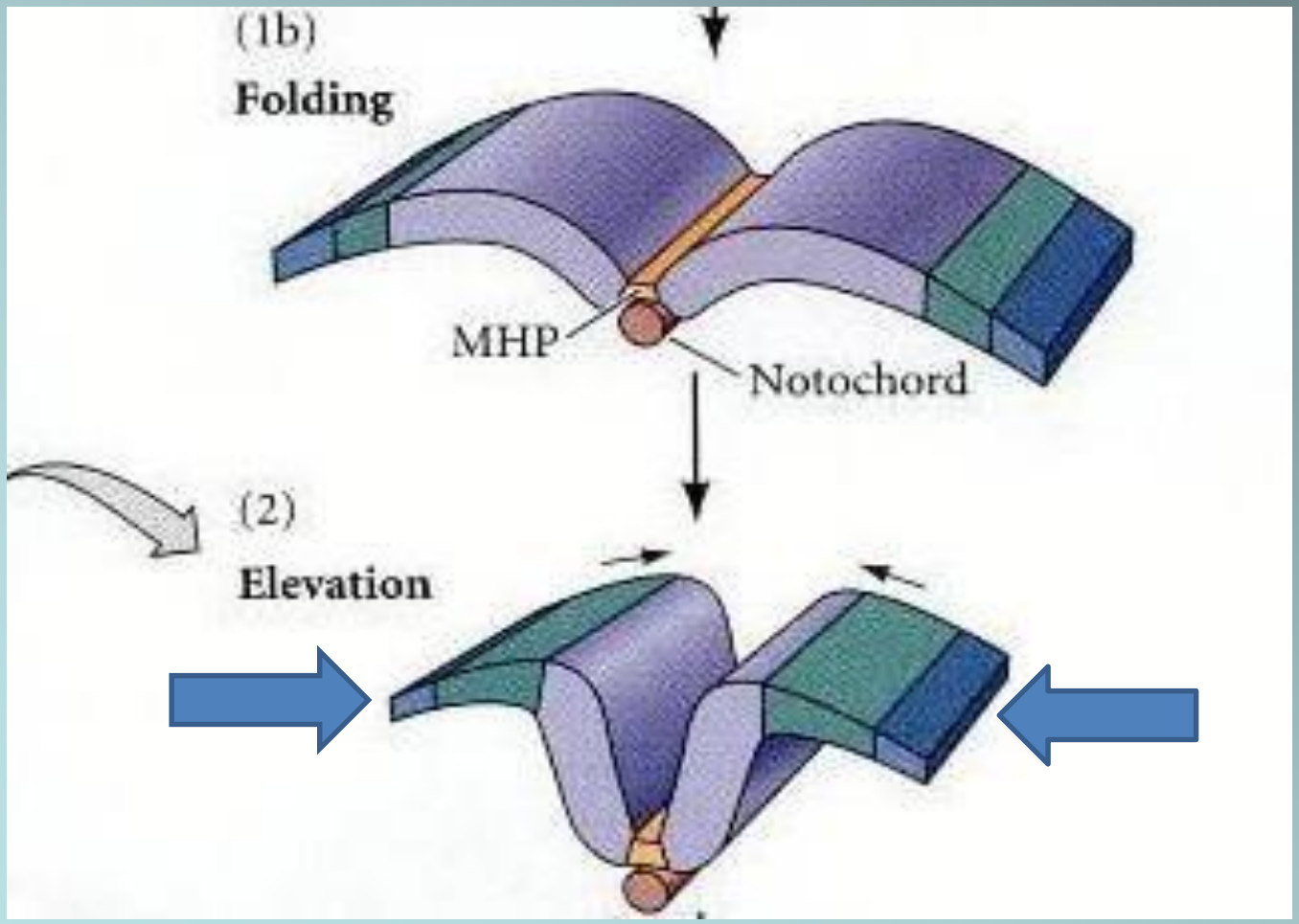
BMP inibisce la formazione di punti cardine

Noggin inibisce BMP e permette la formazione dei punti cardine

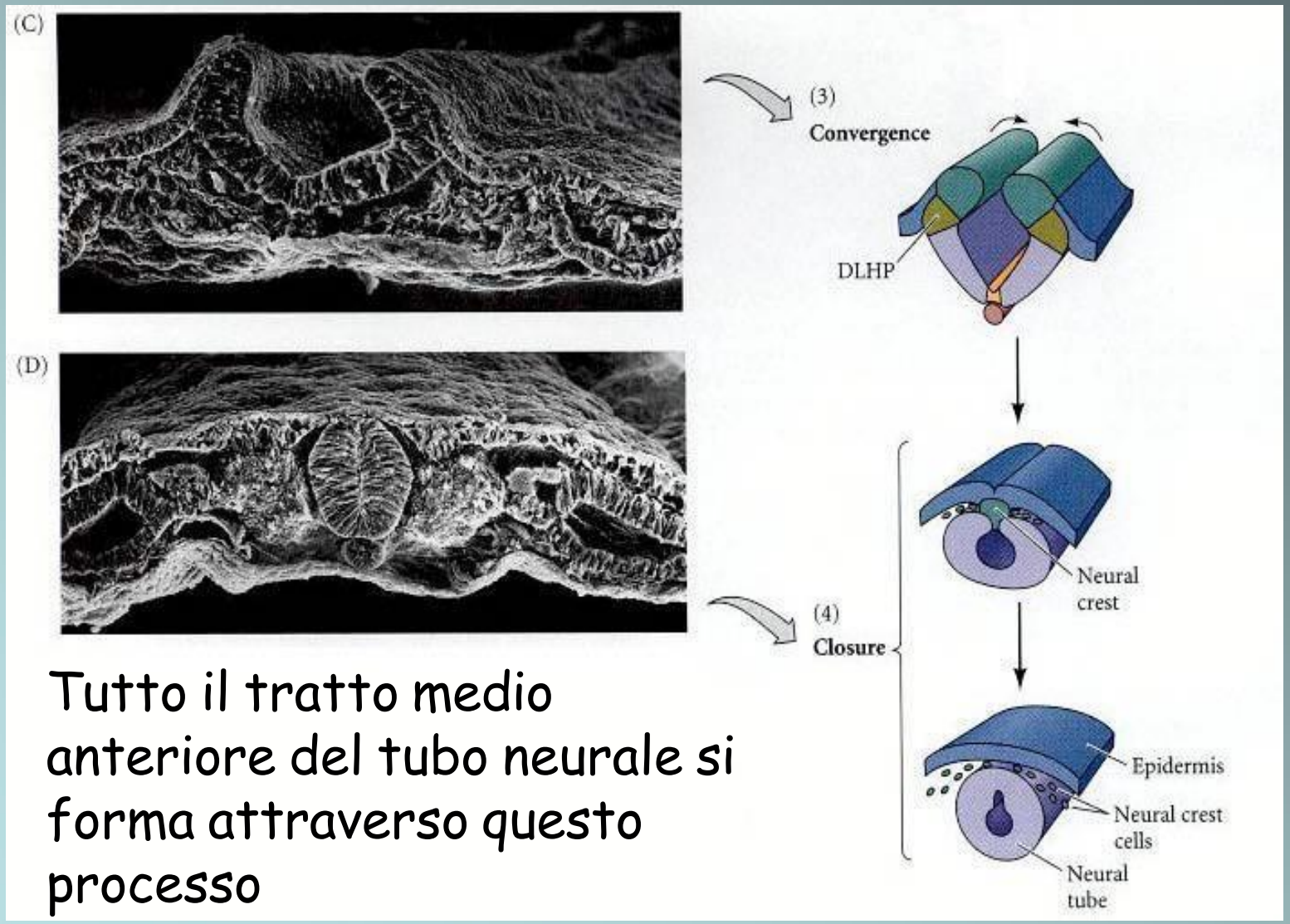
SHH determina la piastra neurale ventrale ed blocca la formazione di punti cardine

Ruolo dell'ectoderma prossimale nella neurulazione

Spinta dell'ectoderma verso la linea mediale aiuta a far piegare la piastra neurale

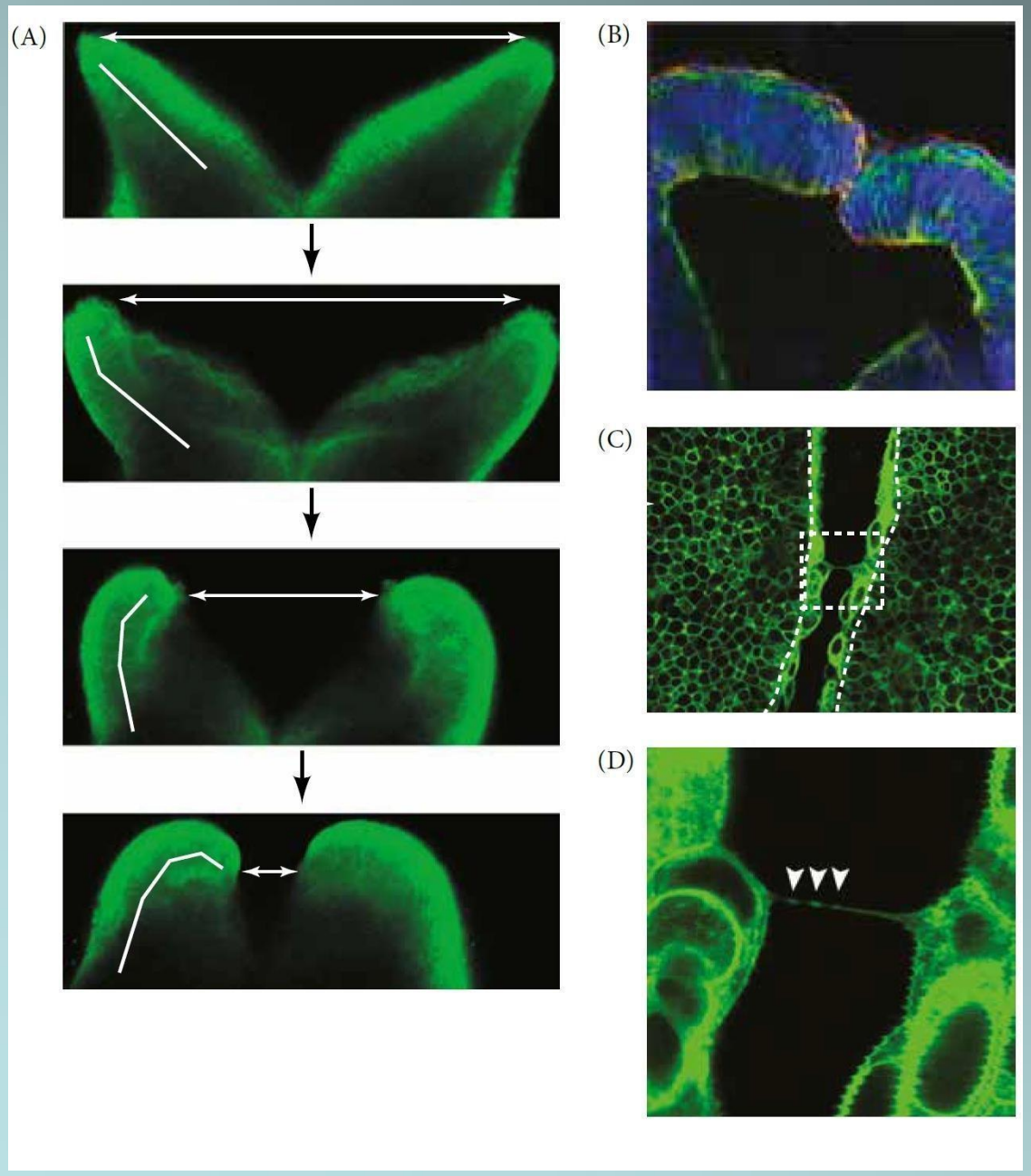


La neurulazione primaria



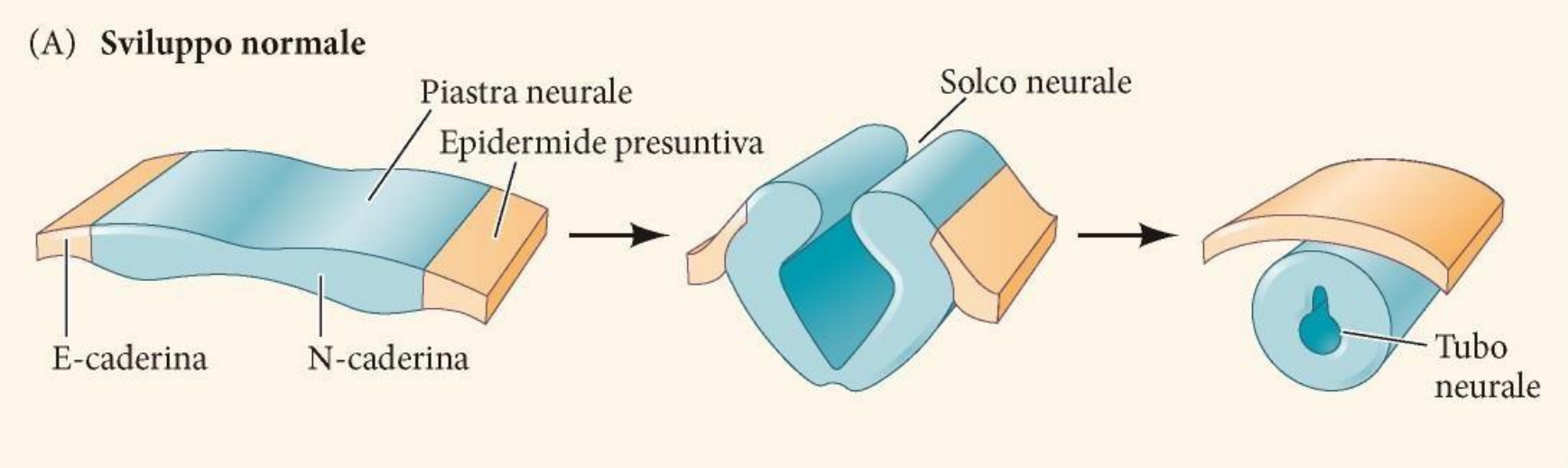
Tutto il tratto medio anteriore del tubo neurale si forma attraverso questo processo

Avvicinamento per la chiusura del tubo neurale

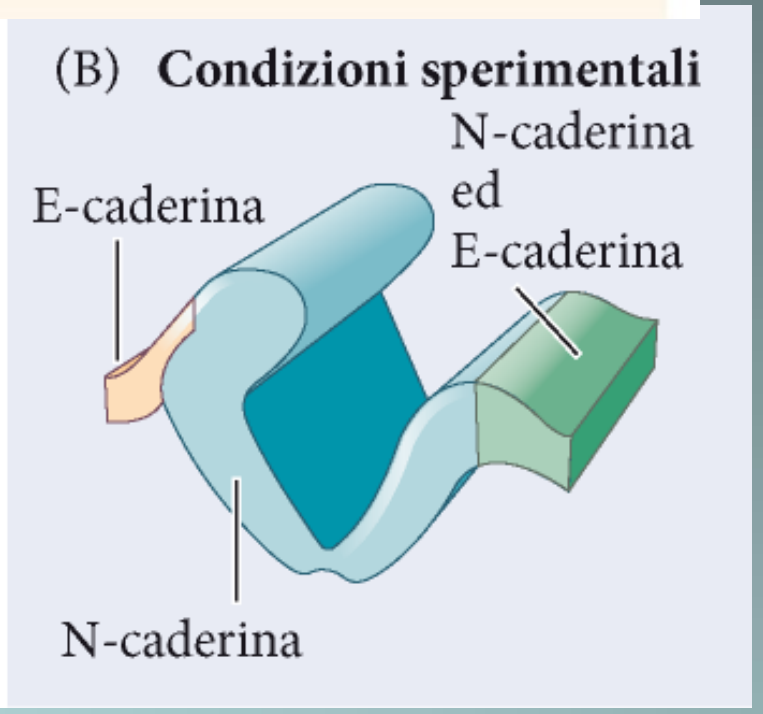


Presenza di filopodi "ponte" tra l'ectoderma non neuronale ai due lati delle pliche

Chiusura del tubo neurale

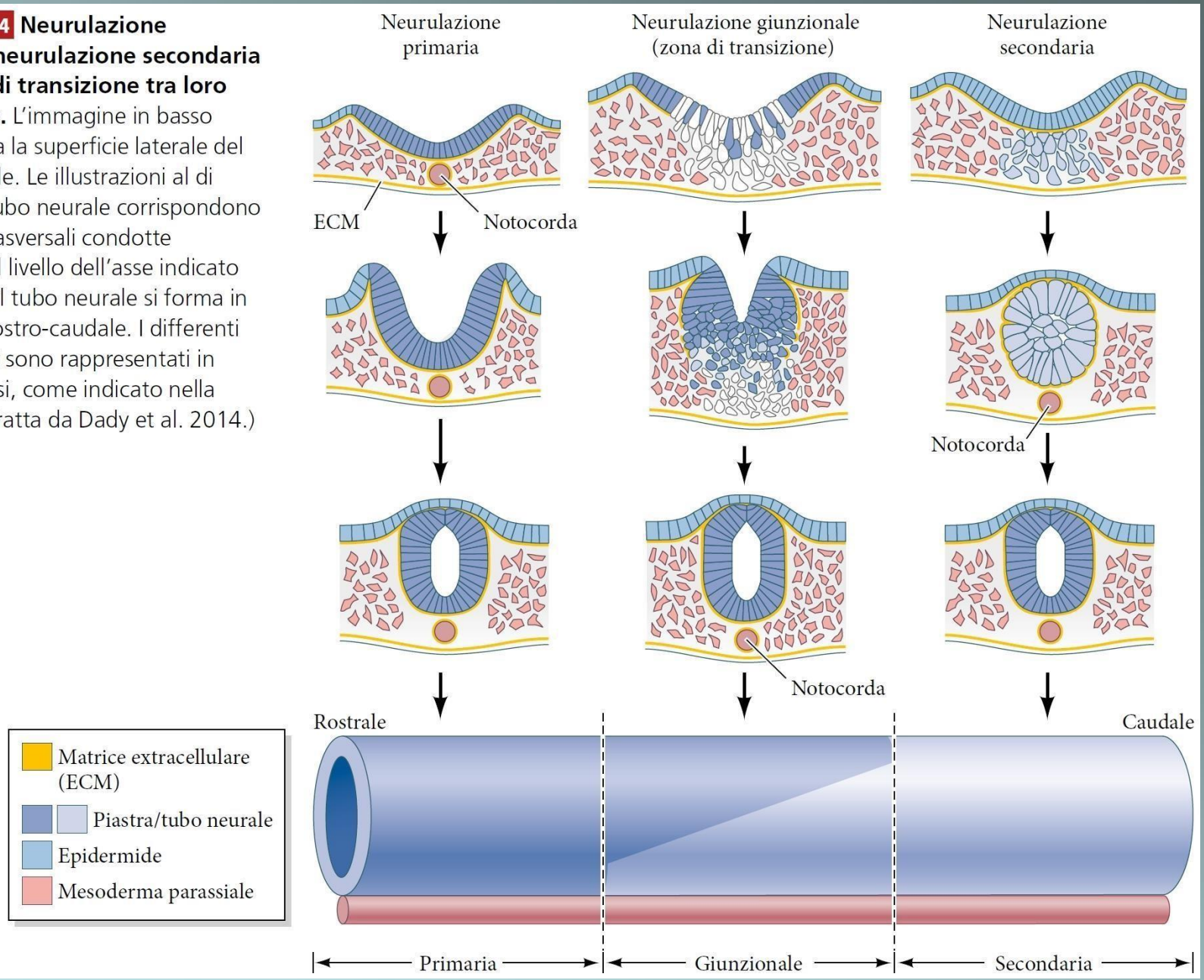


L'espressione di N-caderina è fondamentale per la chiusura del tubo neurale



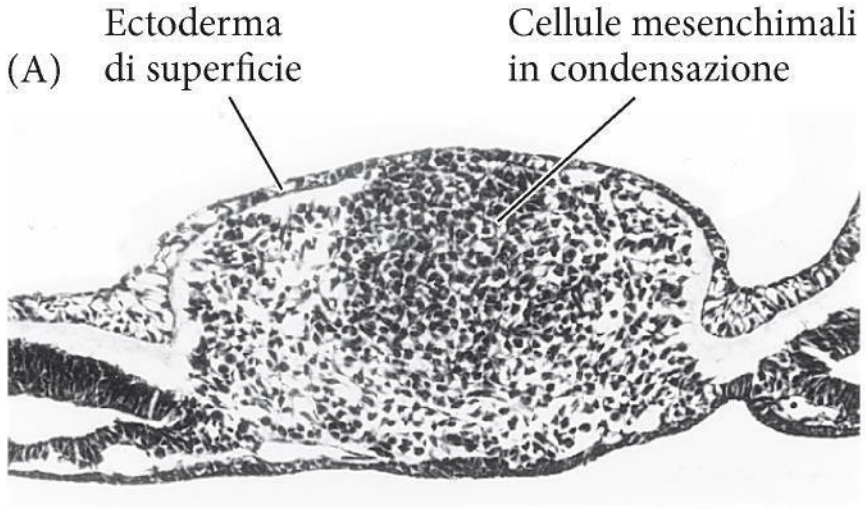
Neurulazione primaria e secondaria

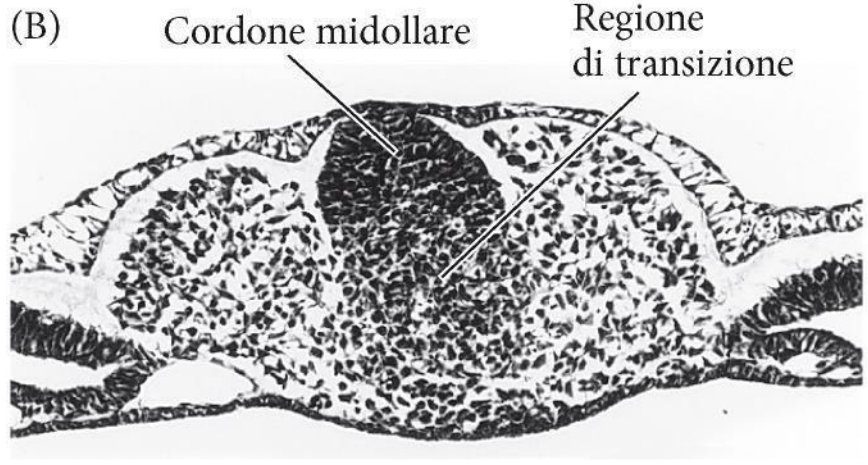
FIGURA 13.4 Neurulazione primaria, neurulazione secondaria e le zone di transizione tra loro interposte. L'immagine in basso rappresenta la superficie laterale del tubo neurale. Le illustrazioni al di sopra del tubo neurale corrispondono a sezioni trasversali condotte attraverso il livello dell'asse indicato via via che il tubo neurale si forma in direzione rostro-caudale. I differenti tipi cellulari sono rappresentati in colori diversi, come indicato nella legenda. (Tratta da Dady et al. 2014.)

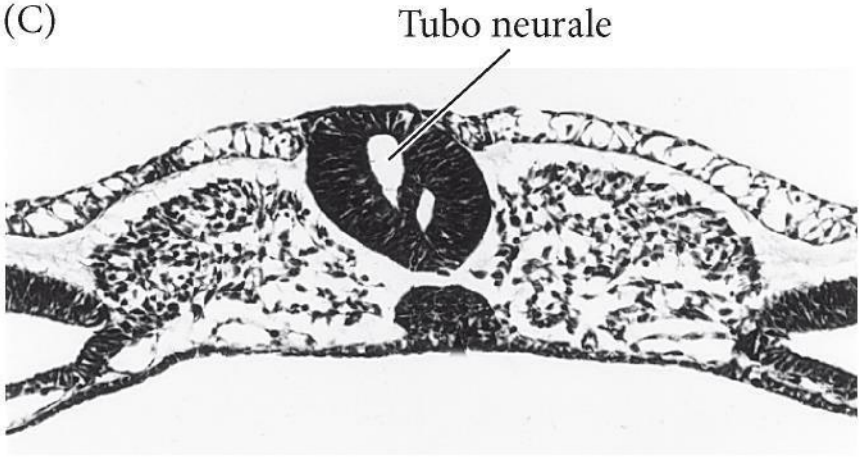


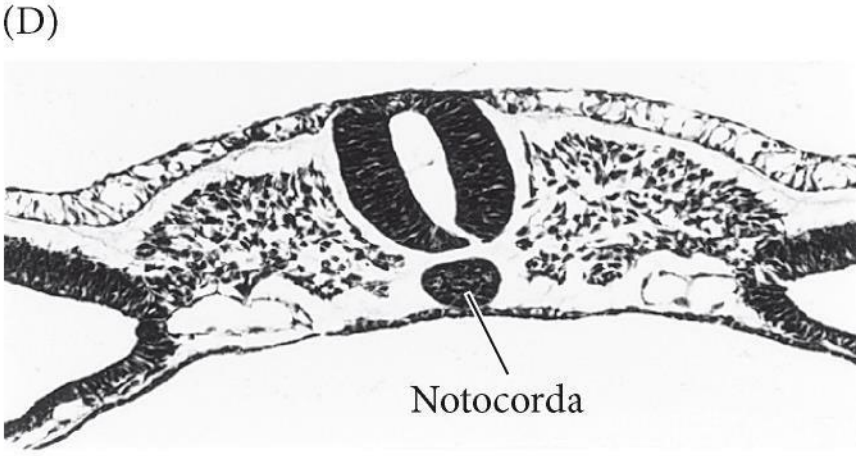
La neurulazione secondaria: condensazione e cavitazione

- Avviene nelle regioni posteriori dell'embrione a partire da cellule di natura mesenchimale
- Processo di transizione mesenchima-epitelio

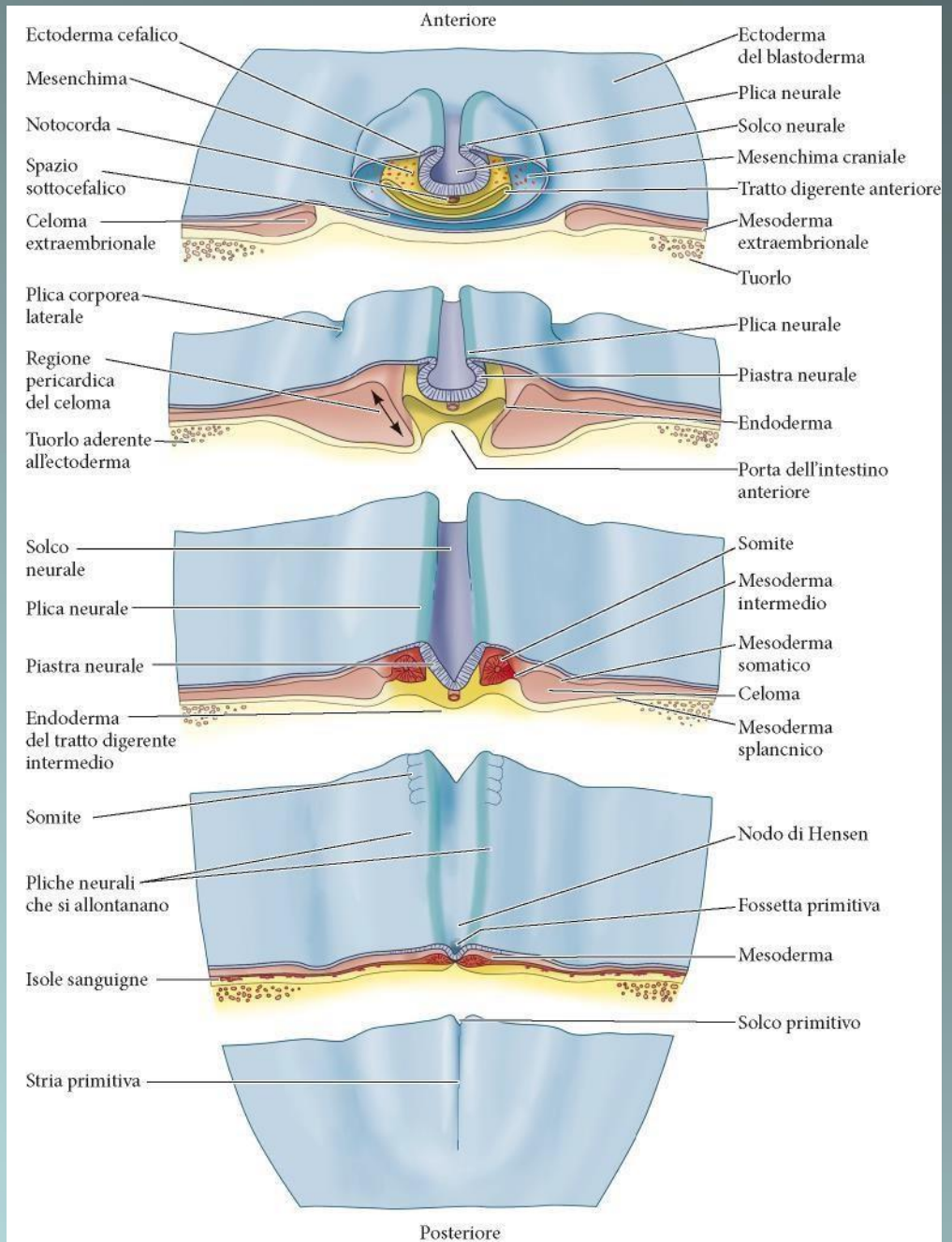




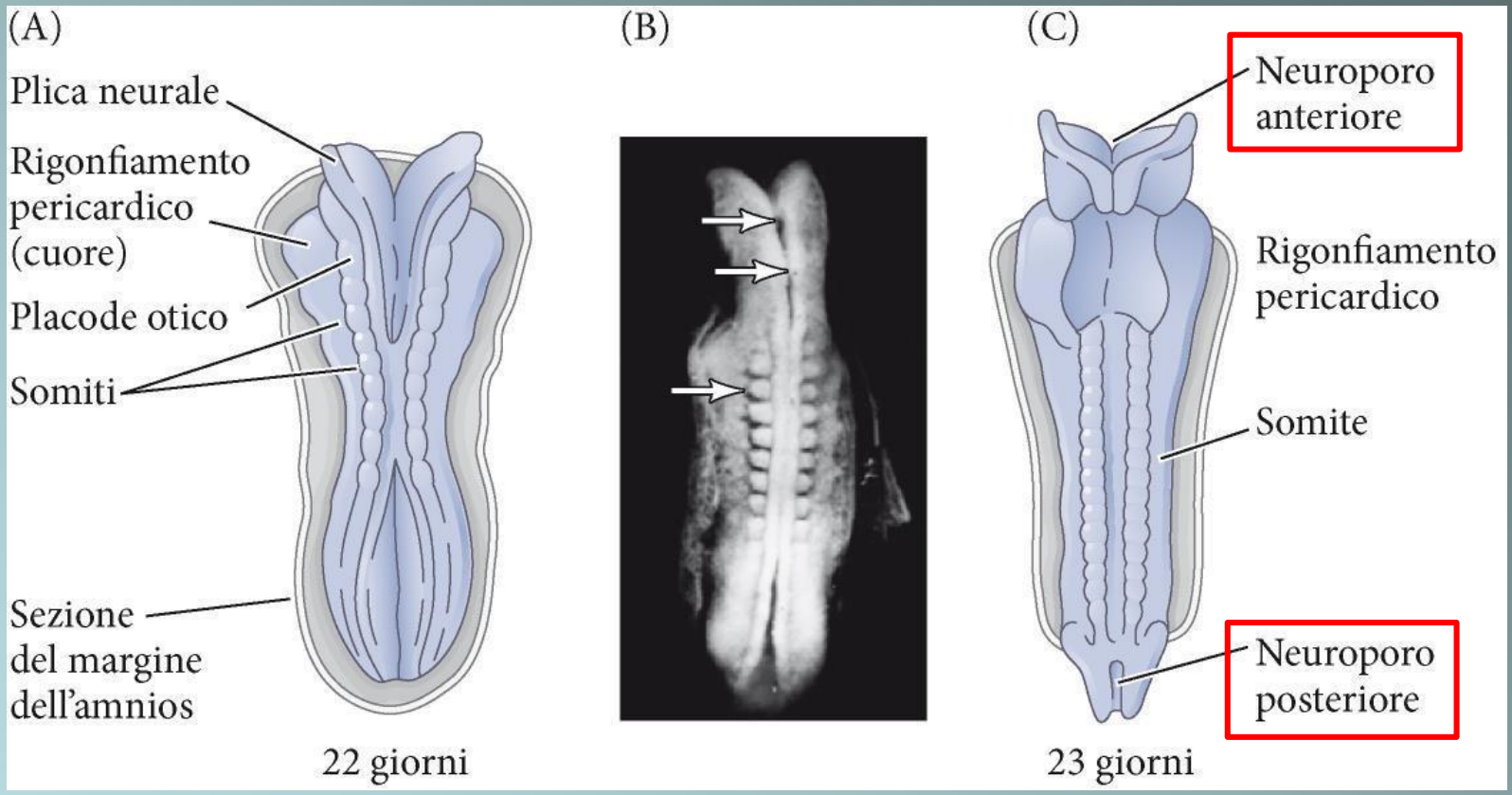




Negli uccelli, la chiusura del tubo neurale avviene prima nella porzione cefalica e dopo nella porzione caudale.



Nei mammiferi la chiusura del tubo neurale avviene in diversi punti contemporaneamente

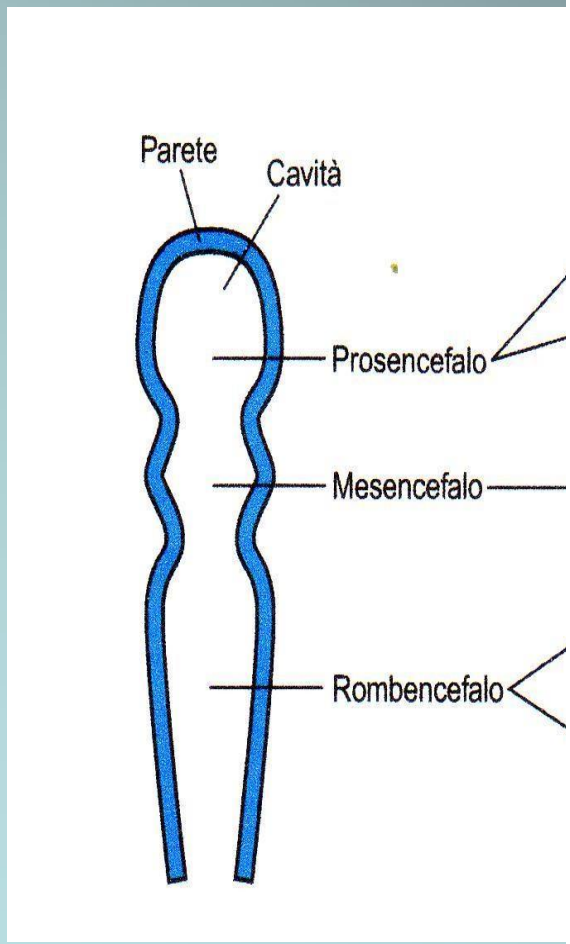


Difetti nella chiusura del tubo neurale. Frequenza: 1:500

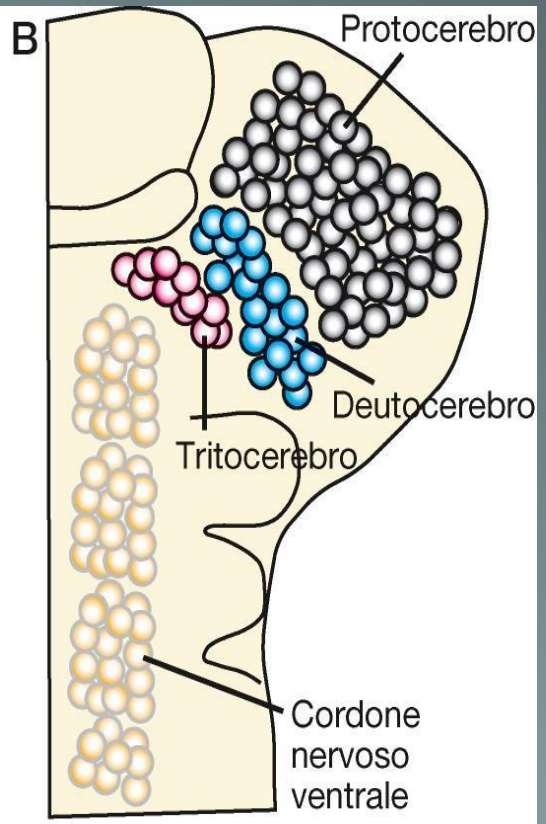
Difetti anteriori: anencefalia

Difetti posteriori: spina bifida

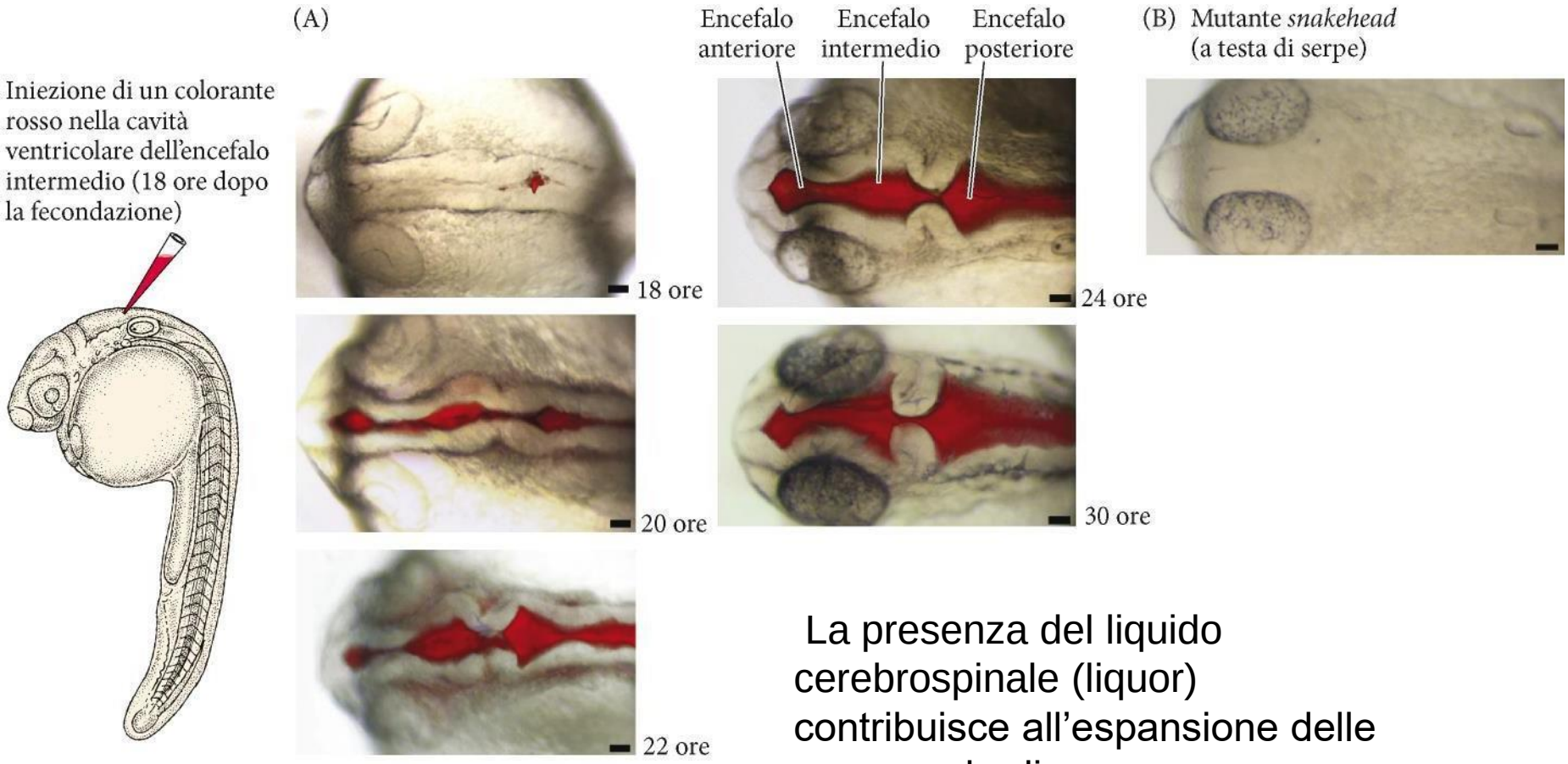
Regionalizzazione SN





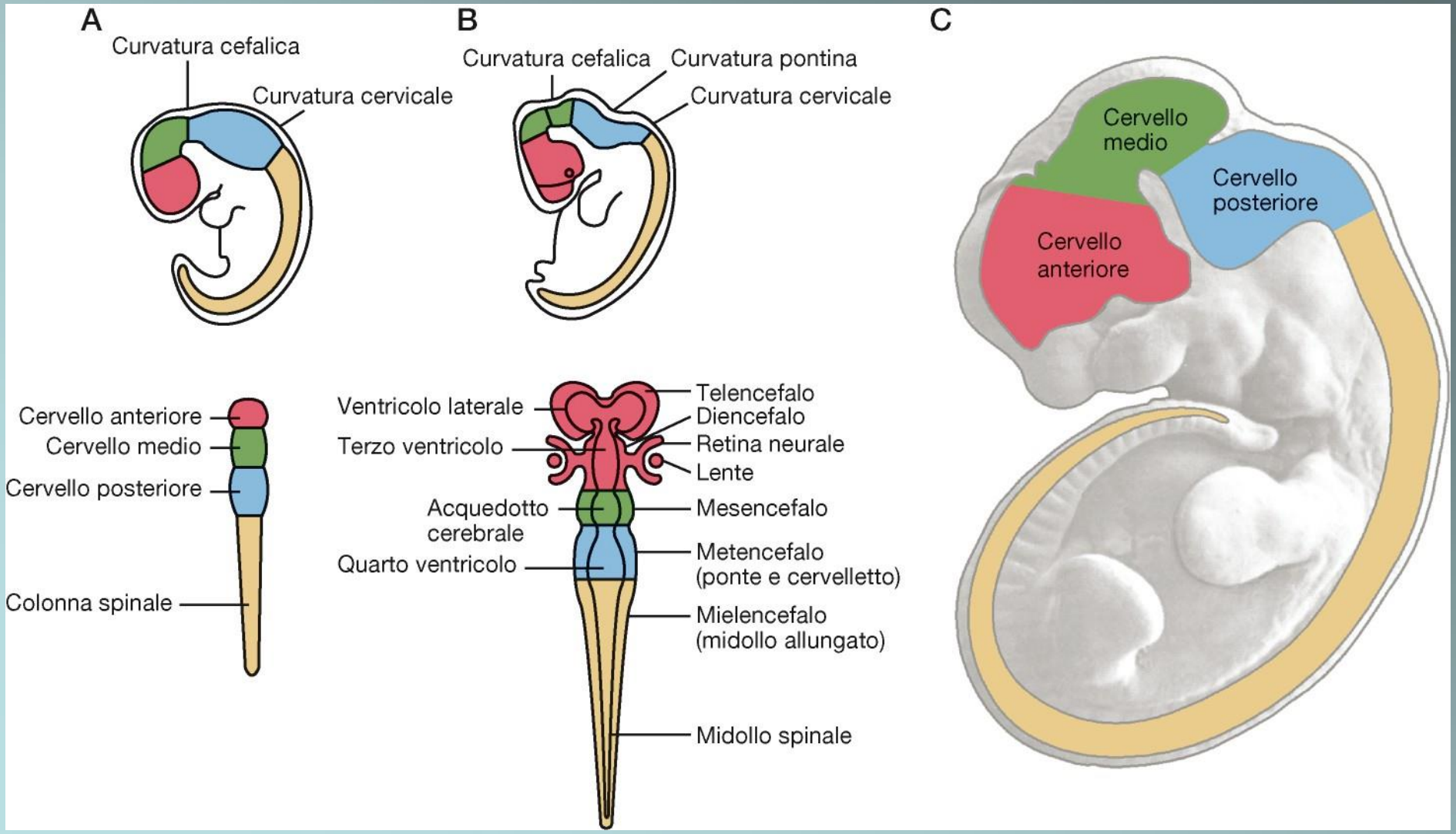


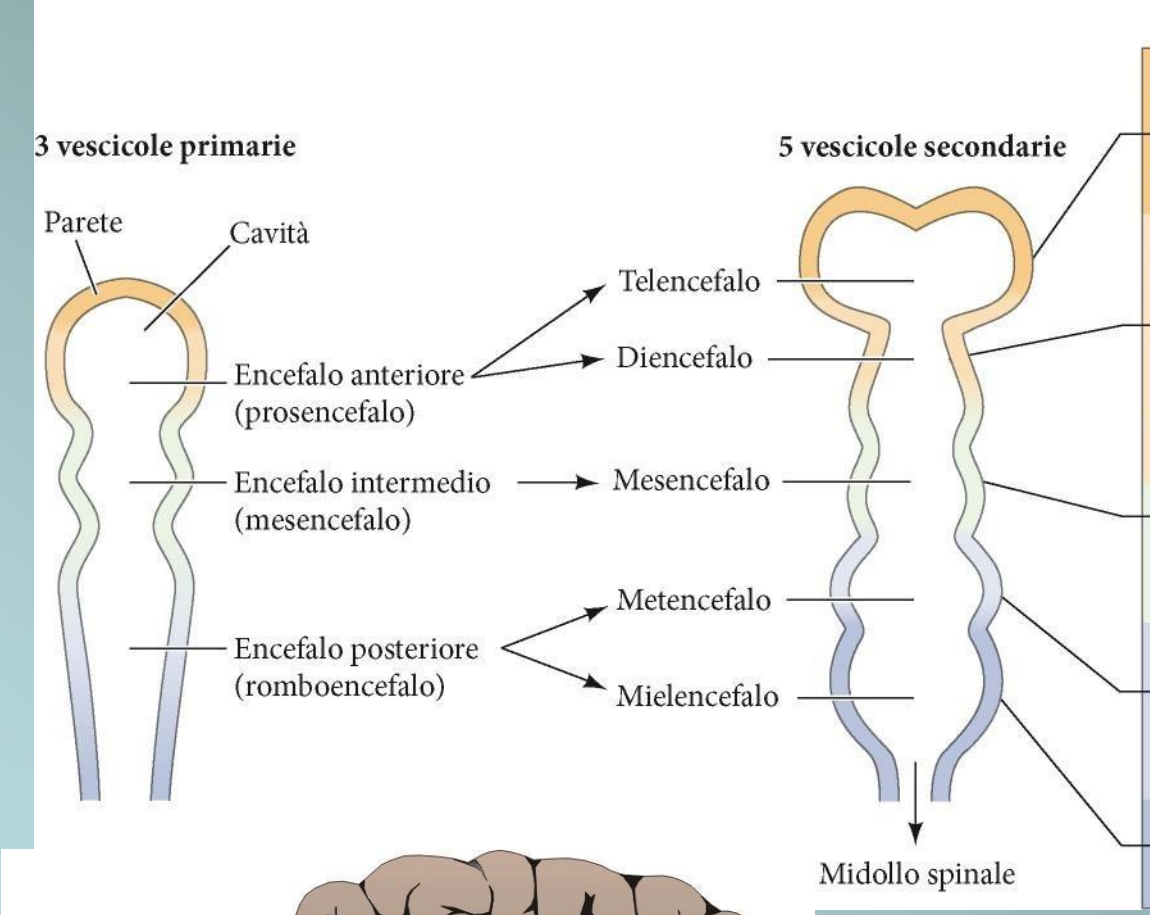
Formazione dell'encefalo: ruolo del liquido contenuto nella cavità del tubo neurale

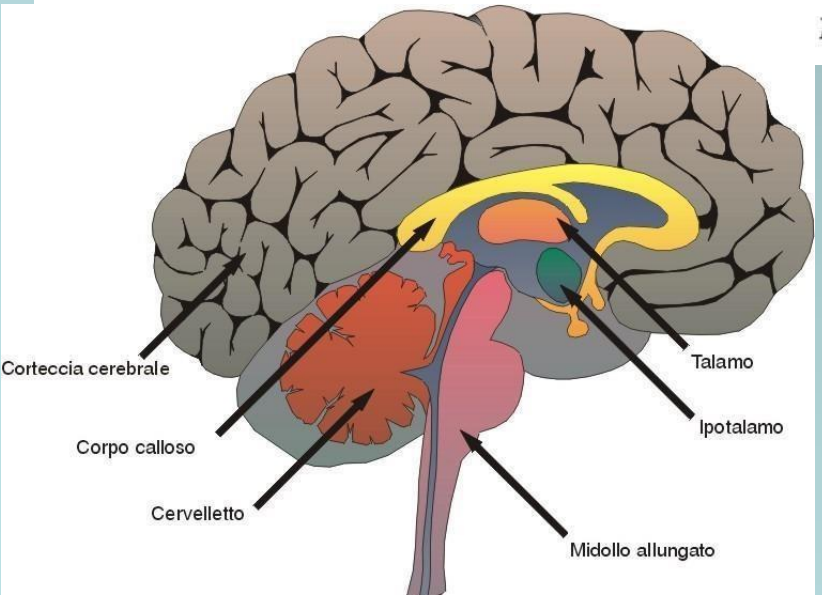


La presenza del liquido cerebrospinale (liquor) contribuisce all'espansione delle aree cerebrali

Regionalizzazione SN nel Vertebrato

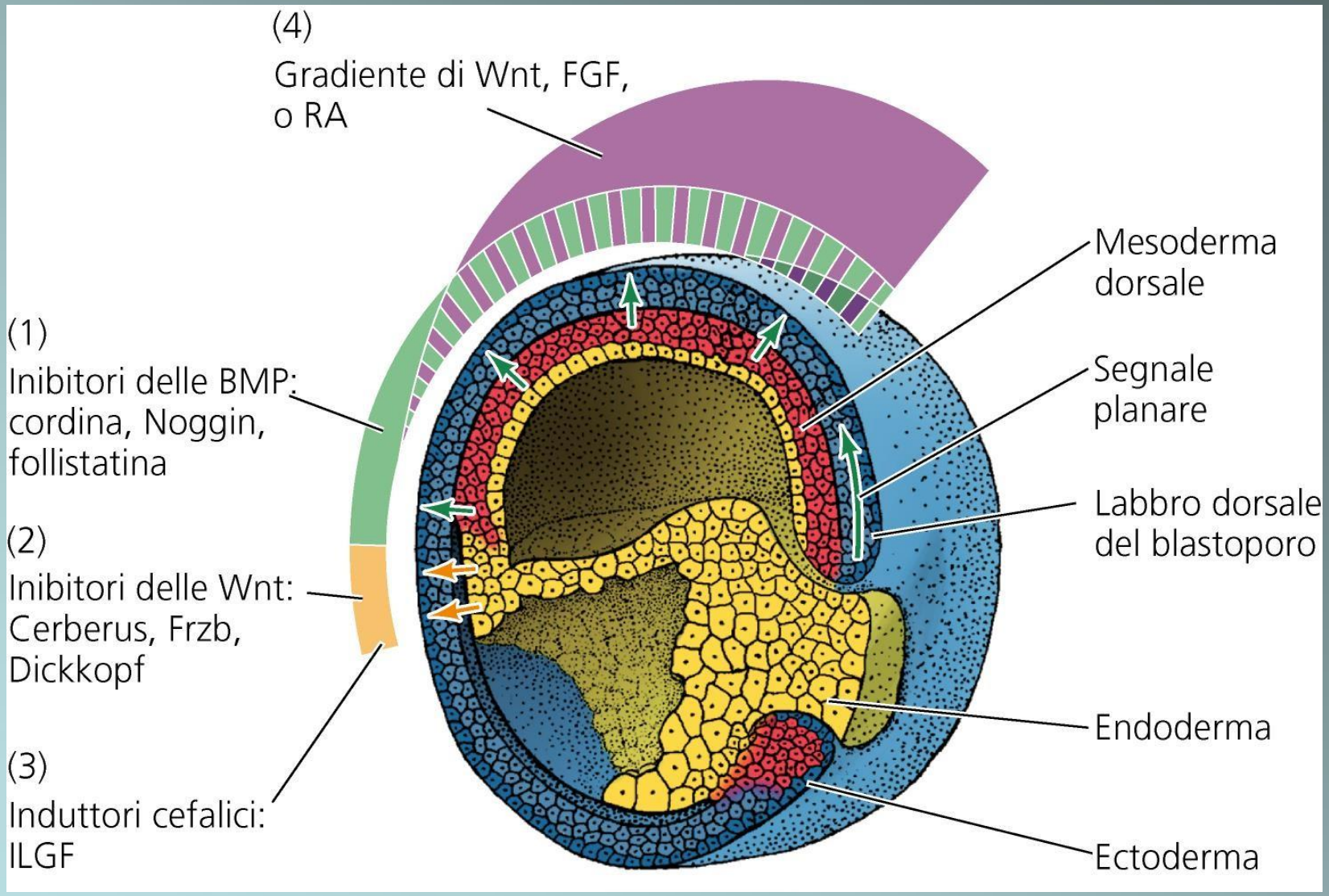






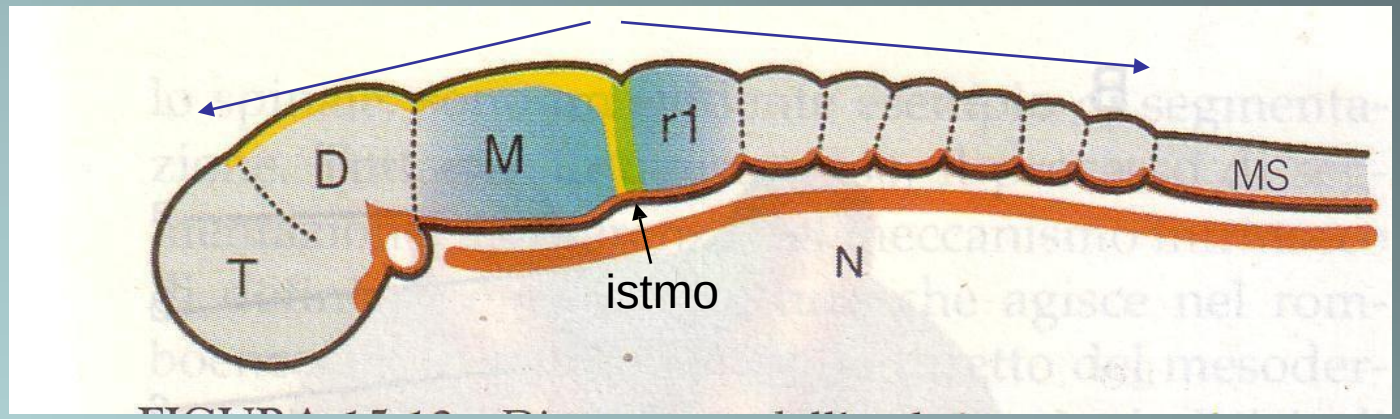
Regionalizzazione del tubo neurale

Asse antero-posteriore



La definizione dell'asse antero posteriore è dipendente da specifiche molecole e dalla loro concentrazione

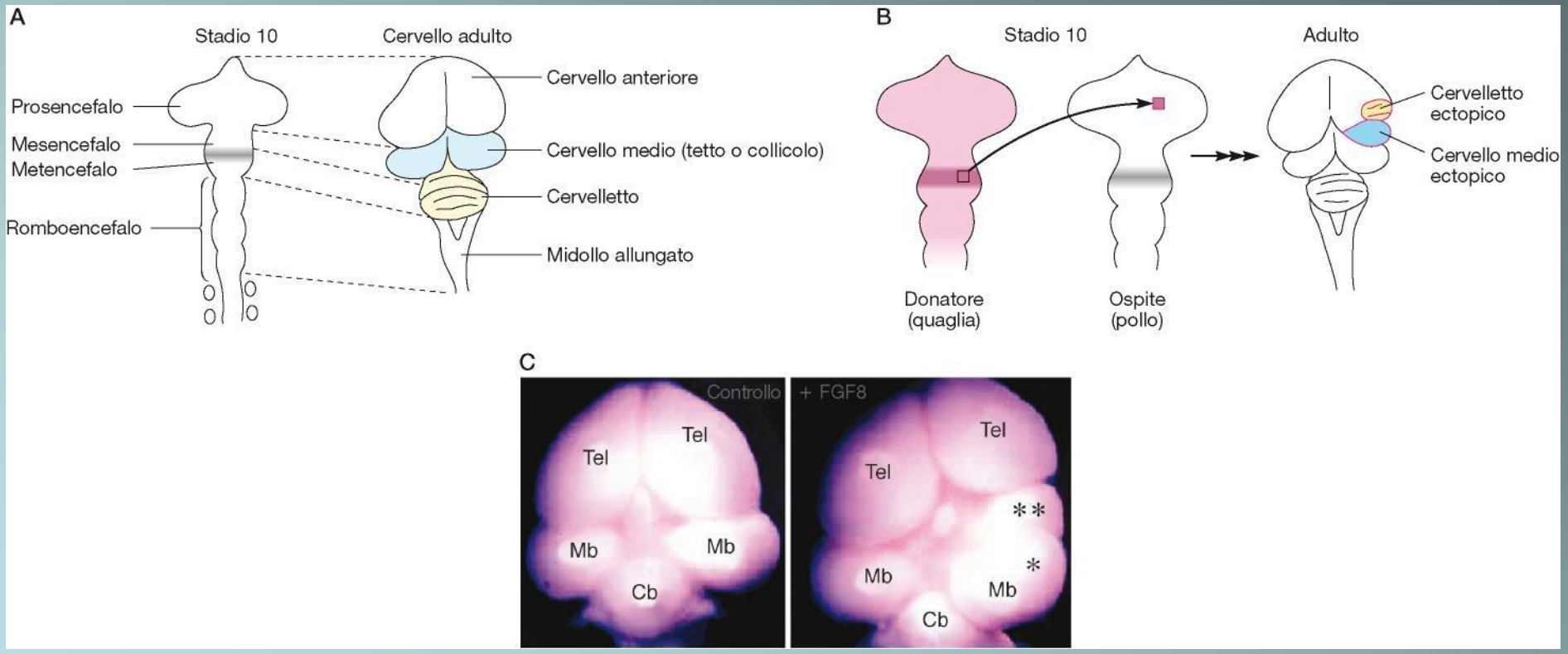
I geni di confine



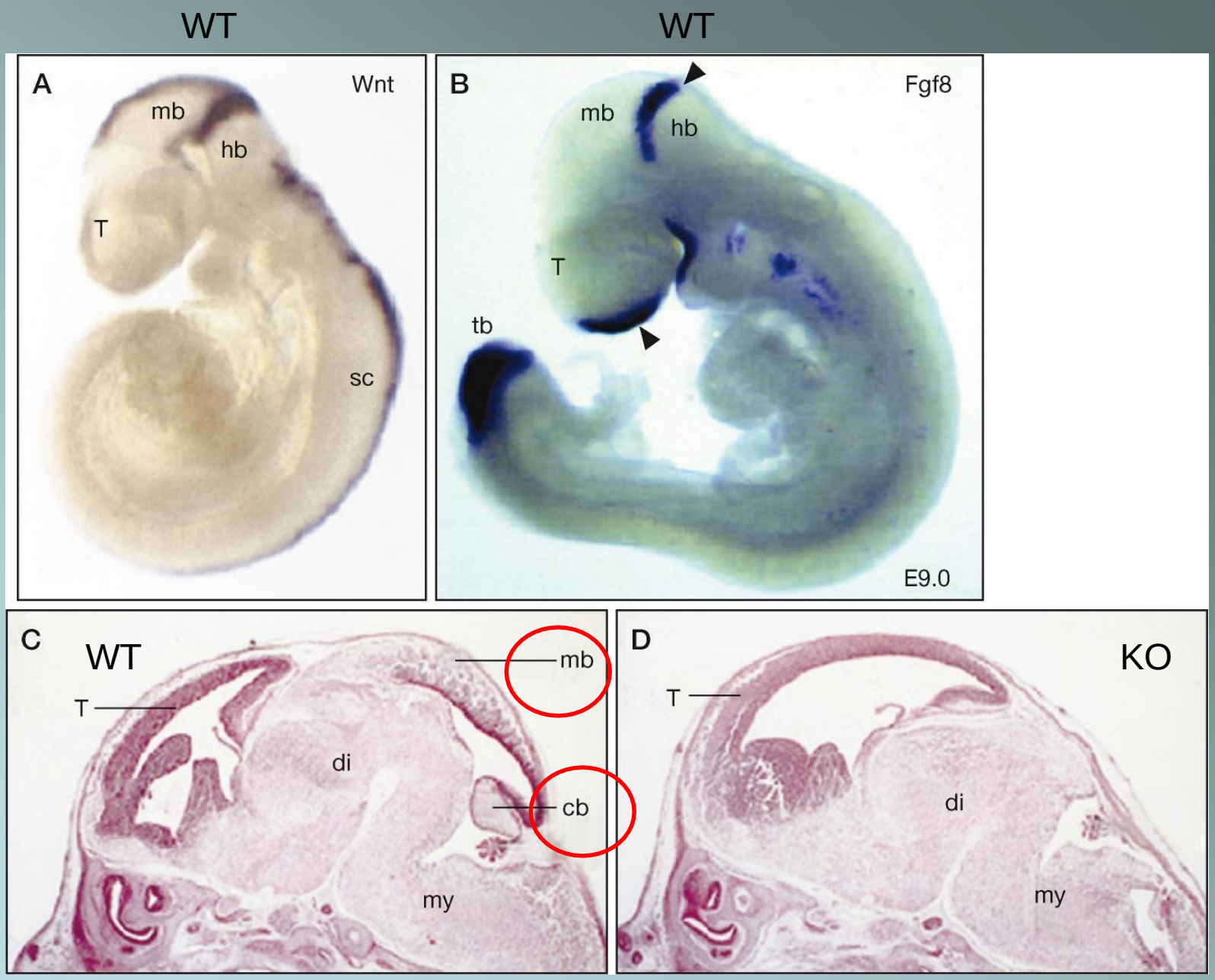
FgF8: Marca il confine nella zona dell'istmo tra mesencefalo e romboencefalo

WNT: Dalla zona dell'istmo alla regione rostrale

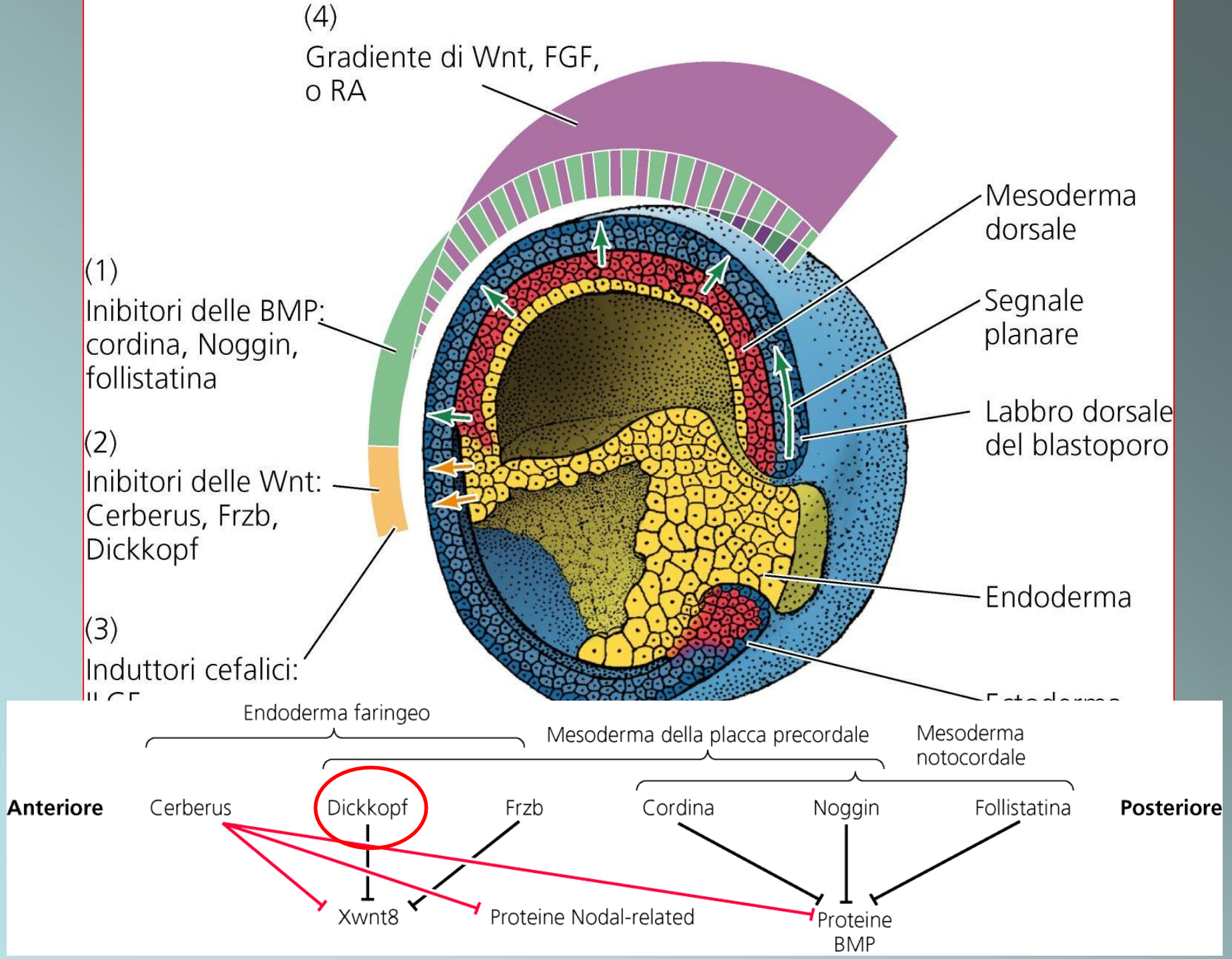
Engrailed: massima concentrazione nell'istmo e segue poi gradiente in direzione rostrale e caudale

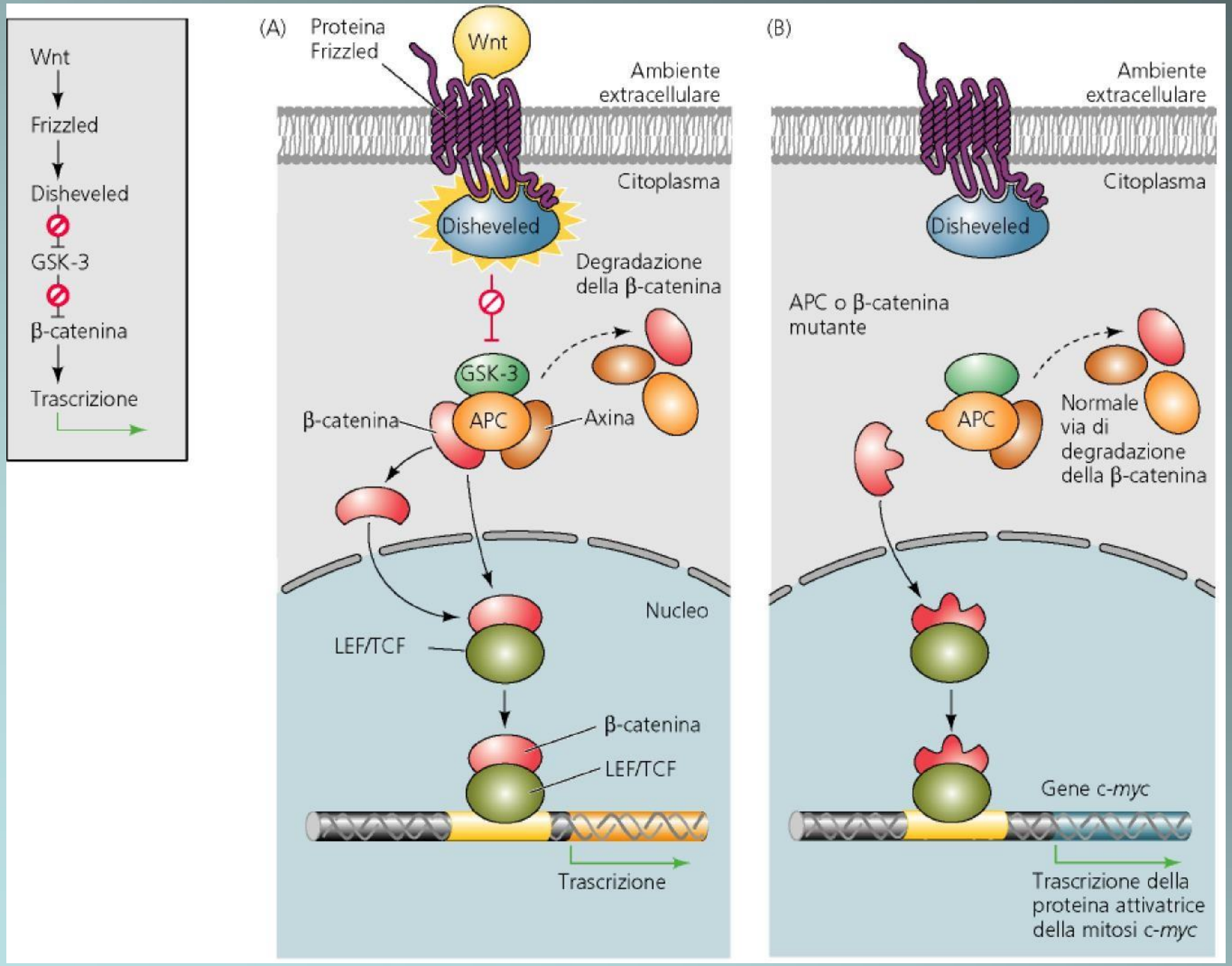


1. Trapianto della regione dell'istmo nel cervello anteriore
2. Trapianto di sfera imbevuta di FGF8

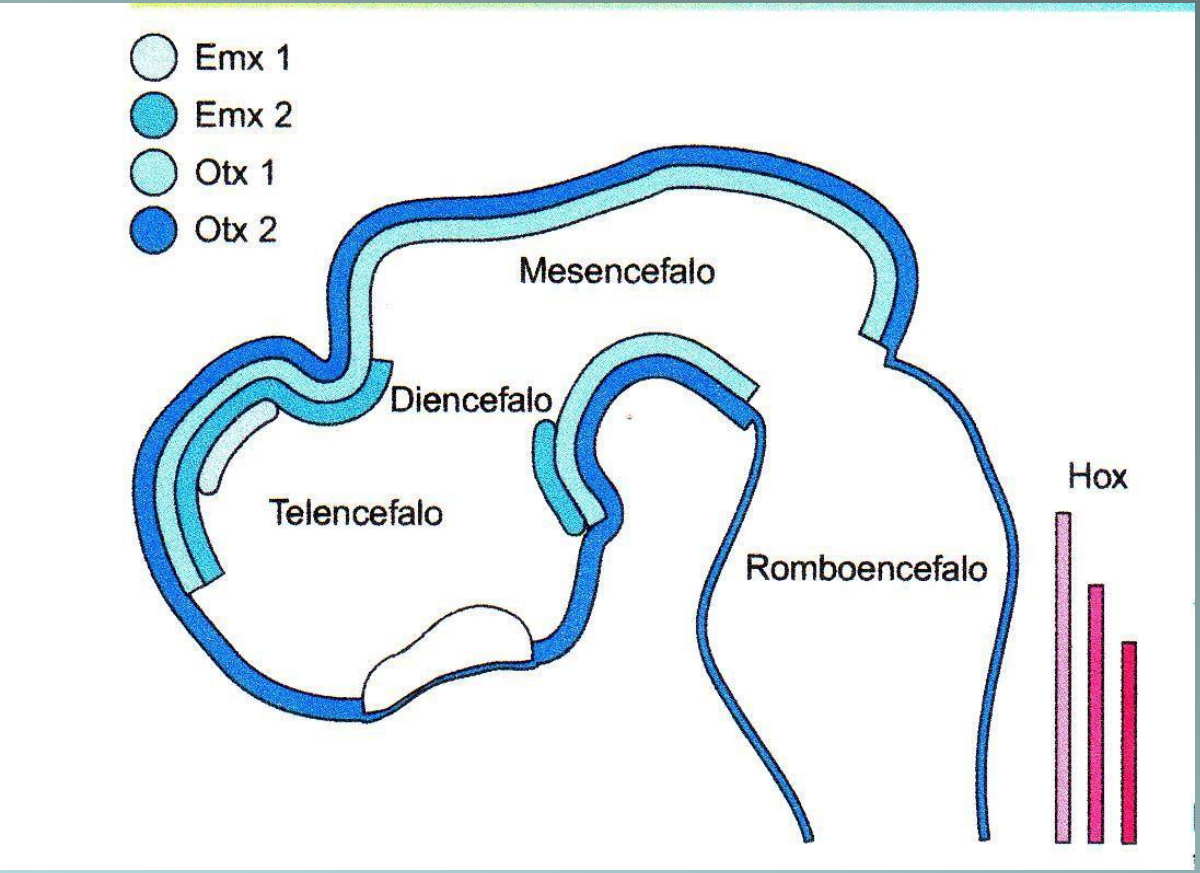


KO WNT1 non presenta regione mesencefalica
Il KO di WNT1 mostra assenza di espressione anche di engrailed 1


(4)
Gradiente di Wnt, FGF, o RA
(1)
Inibitori delle BMP: cordina, Noggin, follistatina
(2)
Inibitori delle Wnt: Cerberus, Frzb, Dickkopf
(3)
Induttori cefalici:
Mesoderma dorsale
Segnale planare
Labbro dorsale del blastoporo
Endoderma
Endoderma faringeo
Mesoderma della placca precordale
Mesoderma notocordale
Anteriore
Cerberus
Dickkopf
Frzb
Cordina
Noggin
Follistatina
Posteriore
Xwnt8
Proteine Nodal-related
Proteine BMP


Wnt
Frizzled
Disheveled
GSK-3
β-catenina
Trascrizione
(A)
Proteina Frizzled
Wnt
Ambiente extracellulare
Citoplasma
Disheveled
Degradazione della β-catenina
GSK-3
β-catenina
APC
Axina
Nucleo
LEF/TCF
β-catenina
LEF/TCF
Trascrizione
(B)
Ambiente extracellulare
Citoplasma
Disheveled
APC o β-catenina mutante
APC
Normale via di degradazione della β-catenina
Gene c-myc
Trascrizione della proteina attivatrice della mitosi c-myc

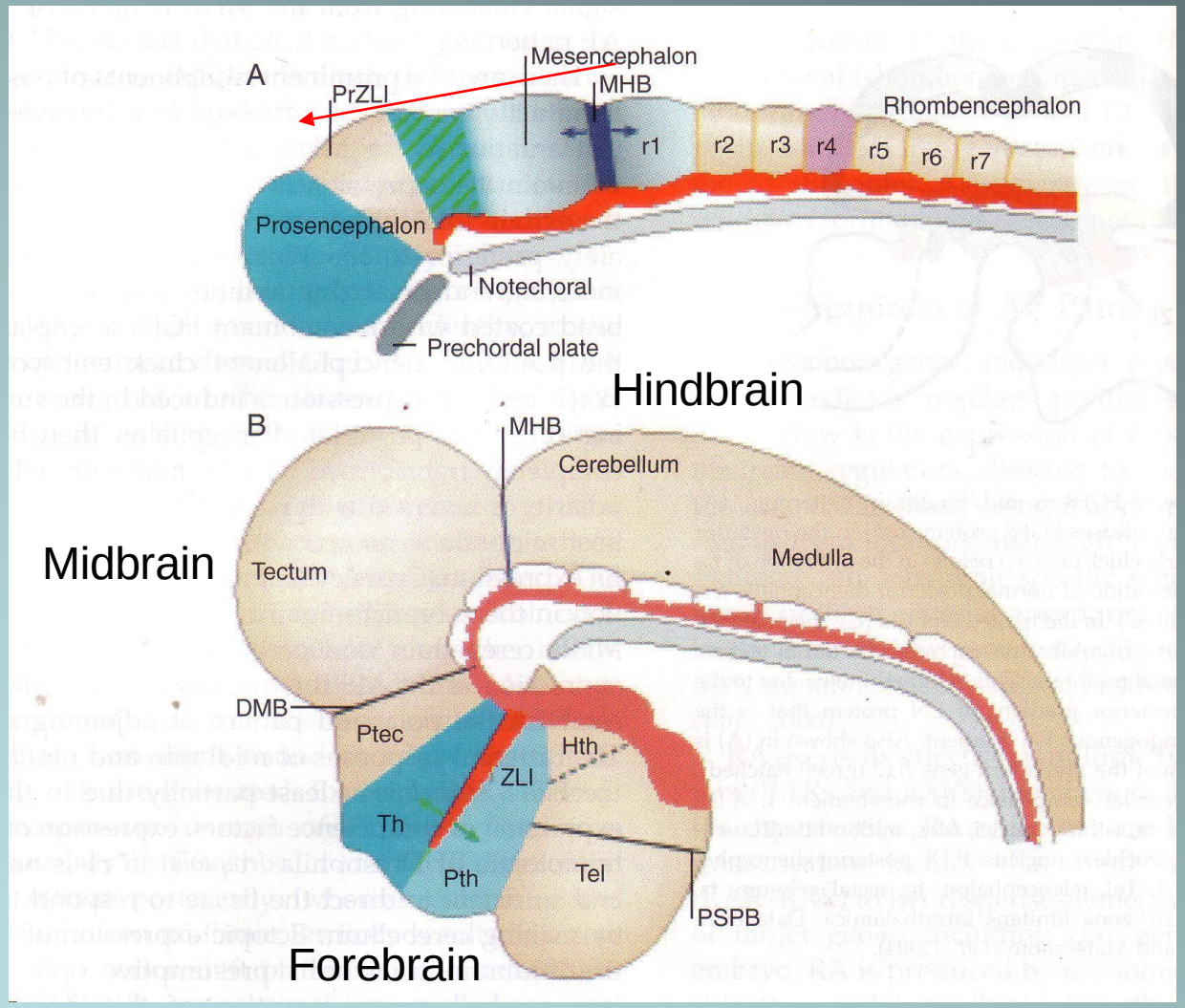
Gli omeotici contribuiscono alla regionalizzazione del Forebrain e del Midbrain



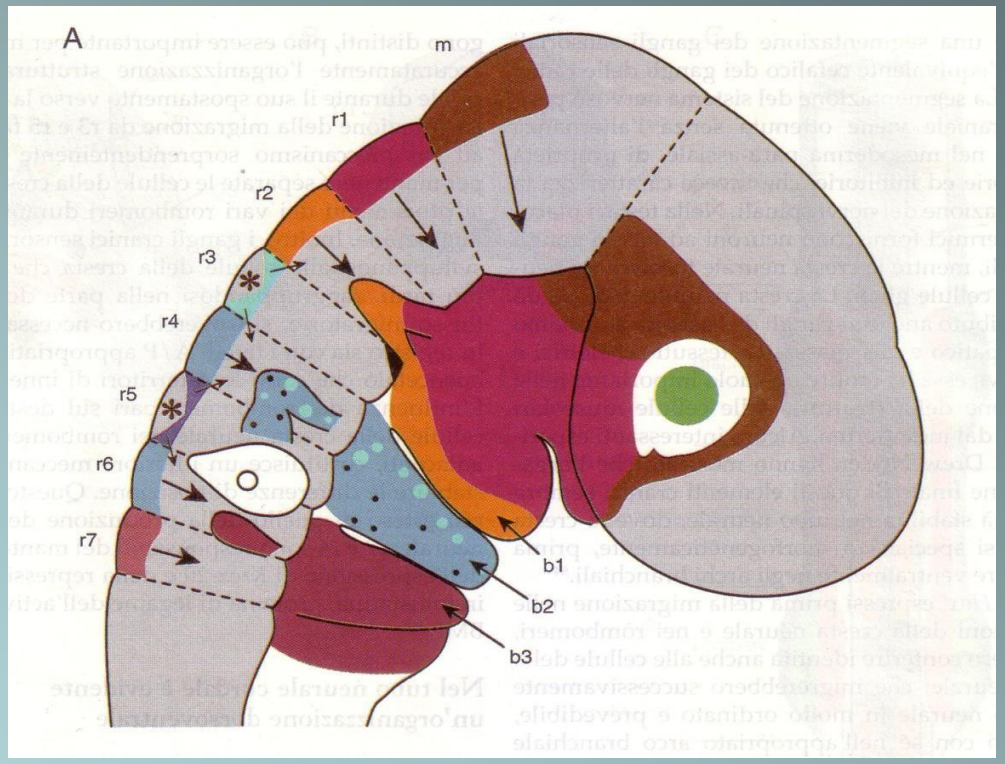
Otx: omologo del gene *orthodenticle (otd)*
Emx: omologo di *empty spiracles (ems)*

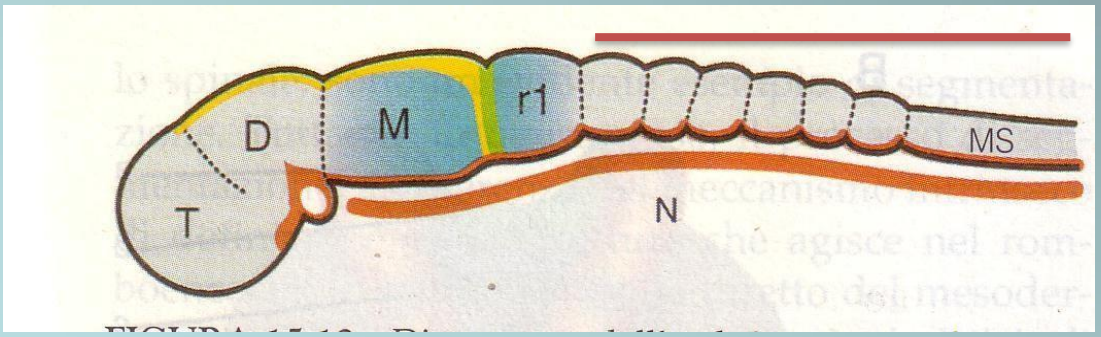
emx1< emx2<otx1<otx2

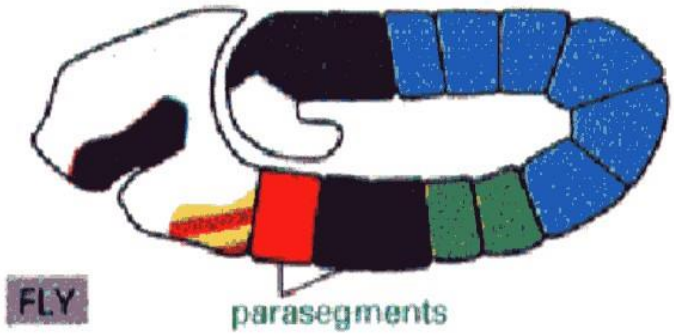
→ La loro parziale sovrapposizione contribuisce a definire le regioni del telencefalo, diencefalo e mesencefalo

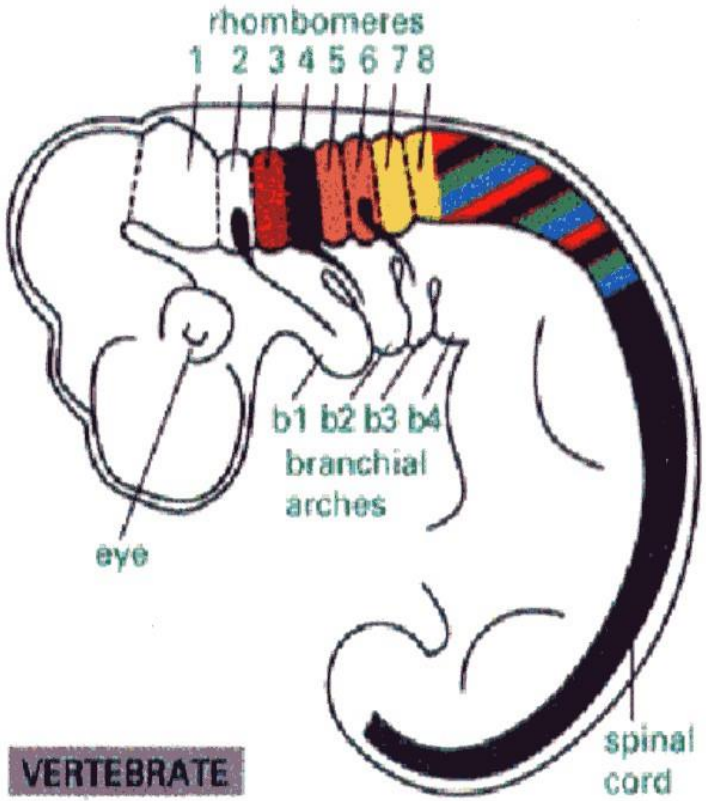

A
Mesencephalon
MHB
PrZLI
Rhombencephalon
r1
r2
r3
r4
r5
r6
r7
Prosencephalon
Notechoral
Prechordal plate
Hindbrain
B
MHB
Cerebellum
Midbrain
Tectum
Medulla
DMB
Ptec
Hth
ZLI
Th
Pth
Tel
PSPB
Forebrain

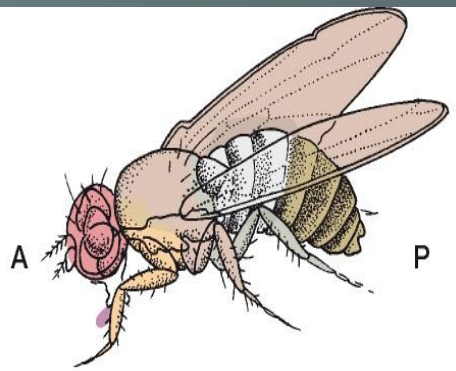
La segmentazione del Romboencefalo

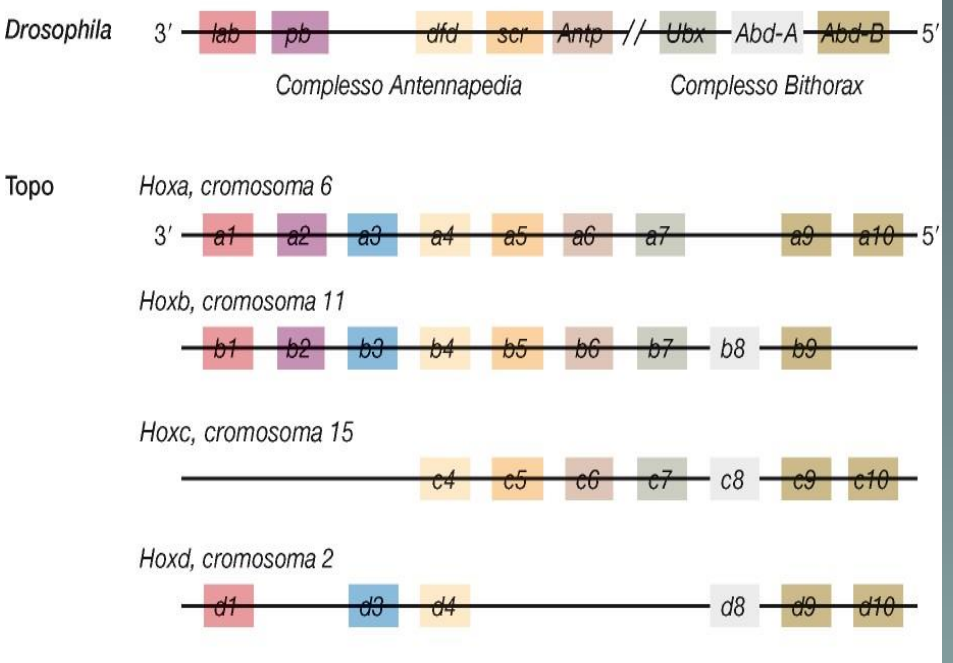


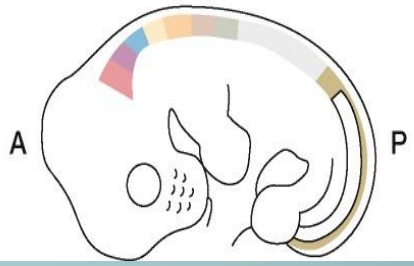



FLY
parasegments

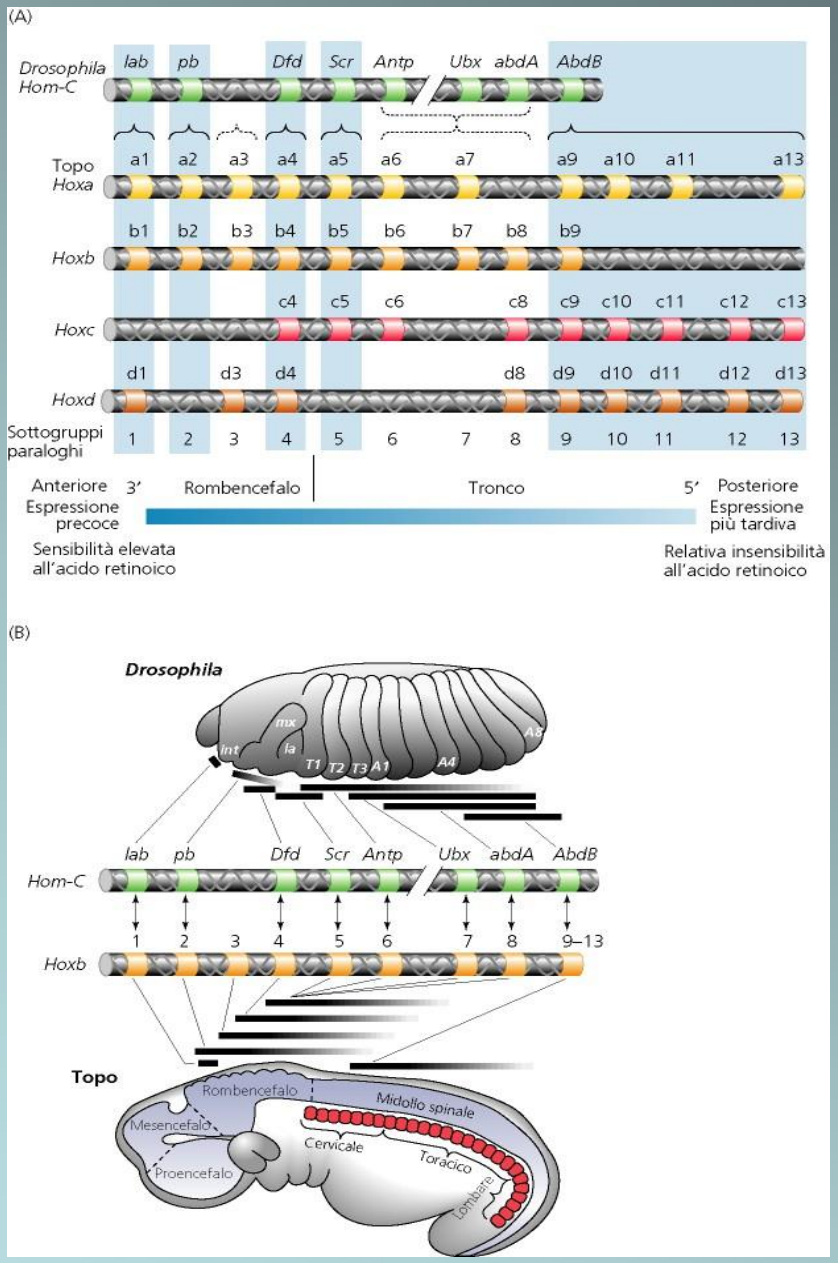

rhombomeres
1 2 3 4 5 6 7 8
b1 b2 b3 b4
branchial
arches
eye
spinal
cord
VERTEBRATE


A
P

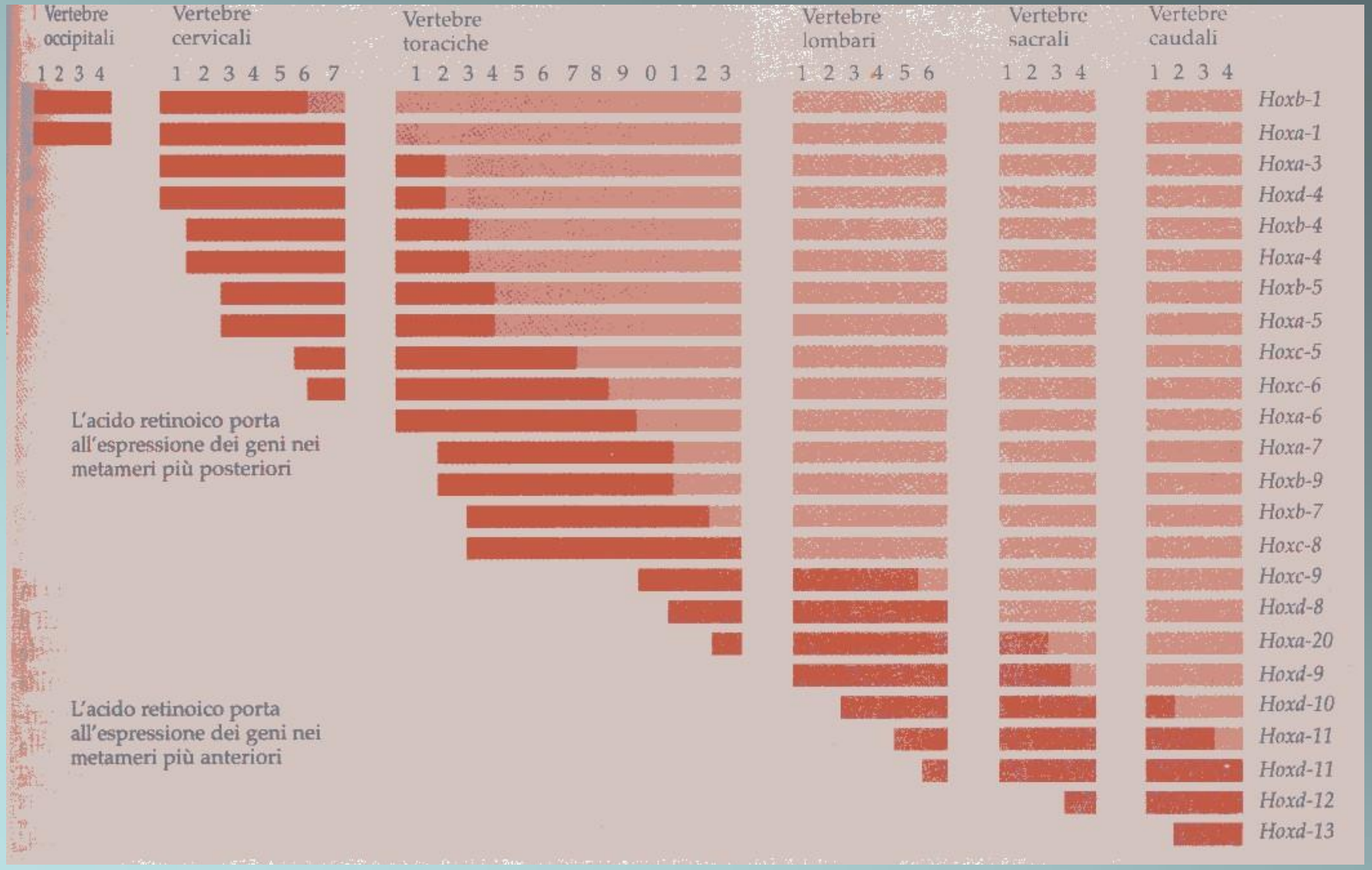

Drosophila
3′ lab pb dfd scr Antp // Ubx Abd-A Abd-B 5′
Complesso Antennapedia
Complesso Bithorax
Topo
Hoxa, cromosoma 6
3′ a1 a2 a3 a4 a5 a6 a7 a9 a10 5′
Hoxb, cromosoma 11
b1 b2 b3 b4 b5 b6 b7 b8 b9
Hoxc, cromosoma 15
c4 c5 c6 c7 c8 c9 c10
Hoxd, cromosoma 2
d1 d3 d4 d8 d9 d10


A
P

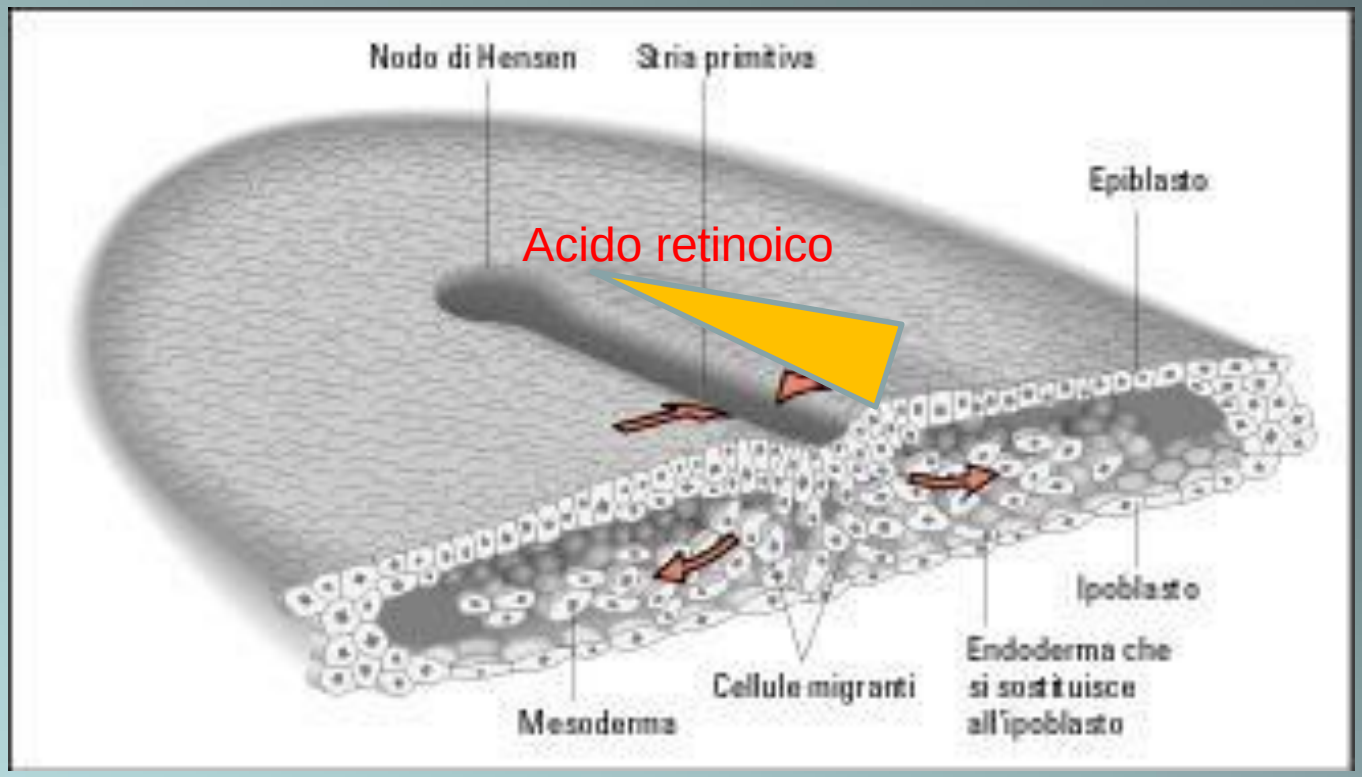
Accensione progressiva dalla regione 3’ al 5’

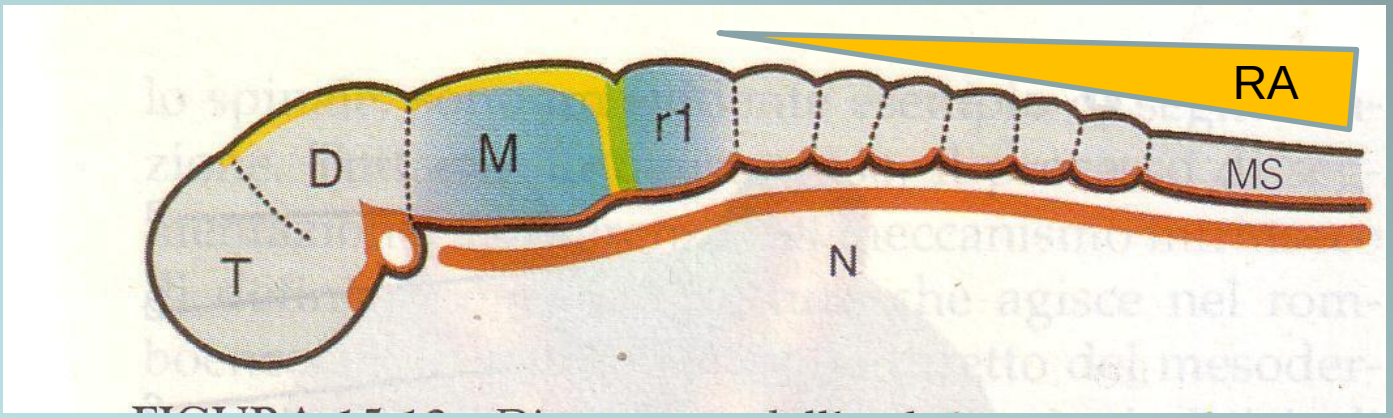


Progressiva accensione dei geni HOX lungo l'asse A-P

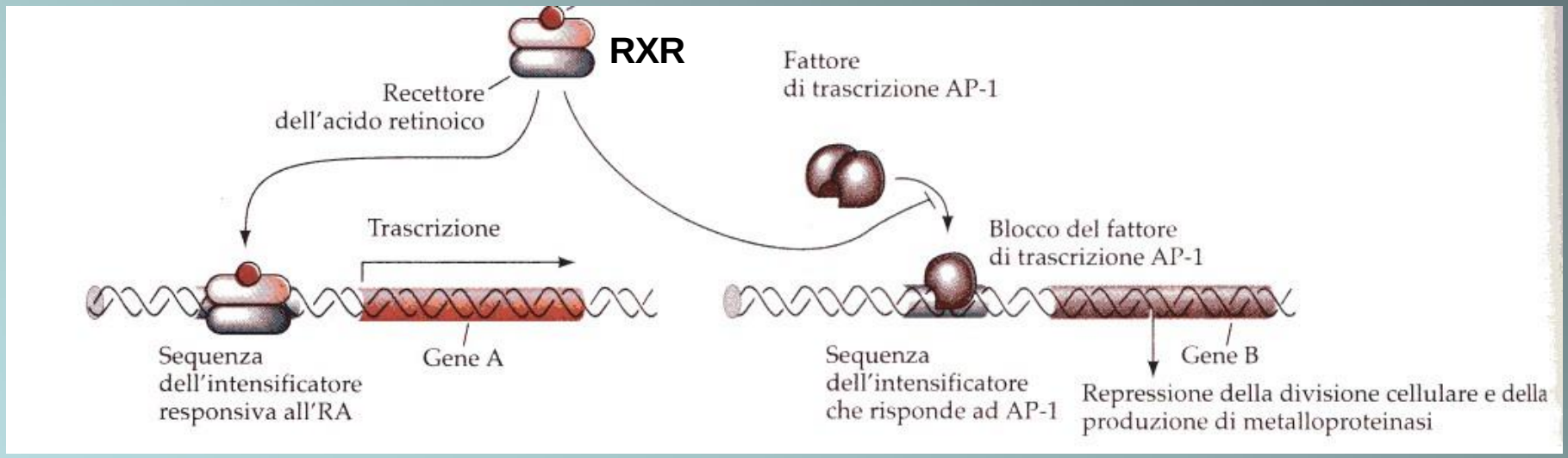


Gradiente di Acido Retinoico





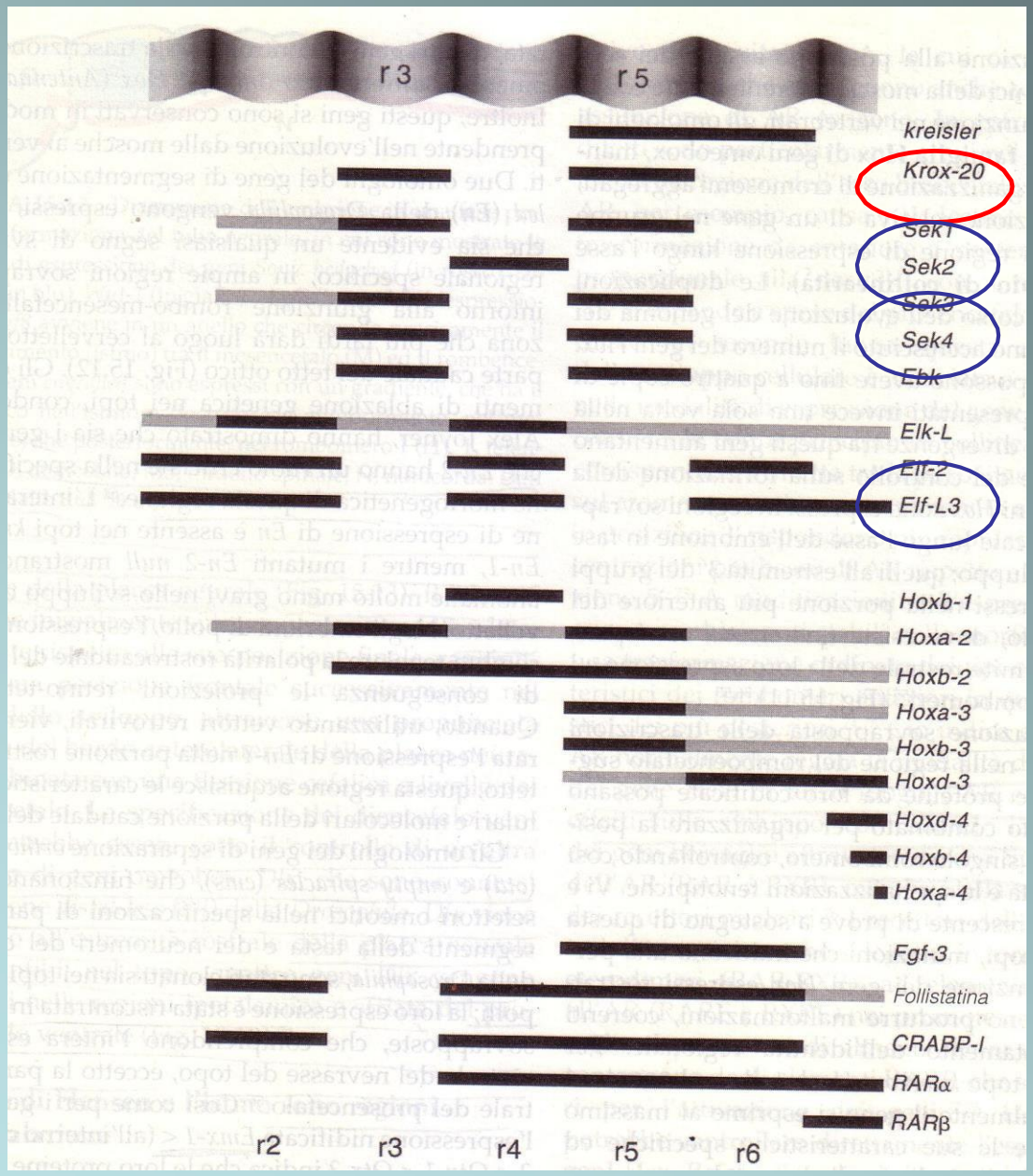
RA controlla accensione dei geni HOX



RAR e RXR = recettori intracitoplasmatici per RA- permettono traslocazione di RA nel nucleo

CRABP= binding protein per acido retinoico – non permette la traslocazione di RA nel nucleo

Il confine tra i rombomeri

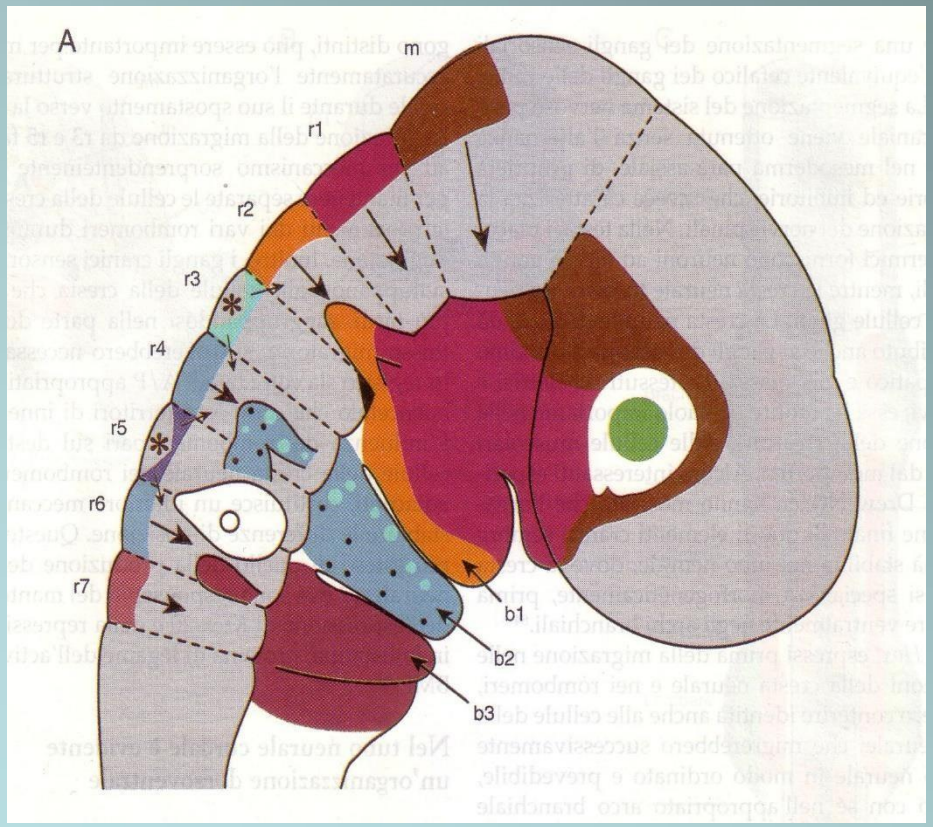


Recettori per Eph (Sek) e i loro ligandi Elf

1. Efrine si esprimono nei rombomeri dispari

2. Recettori per efrine si esprimono nei rombomeri pari

Migrazione delle cellule di cresta neurale



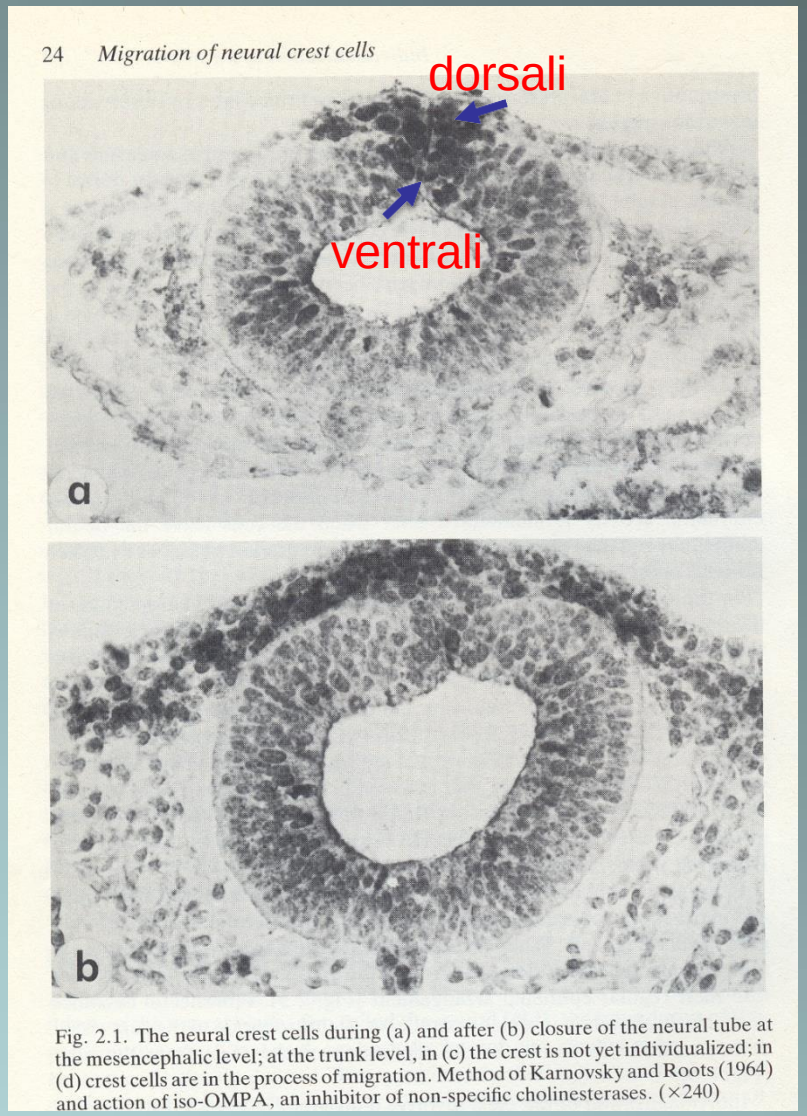
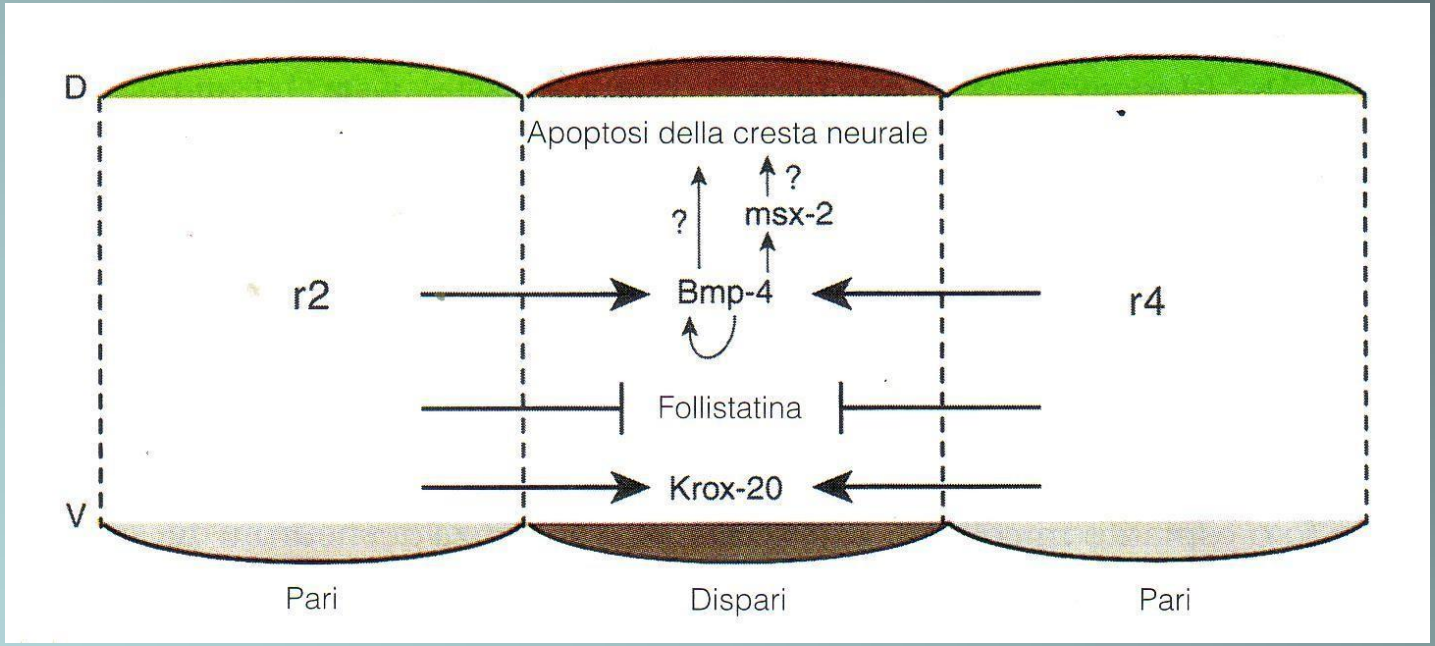


Fig. 2.1. The neural crest cells during (a) and after (b) closure of the neural tube at the mesencephalic level; at the trunk level, in (c) the crest is not yet individualized; in (d) crest cells are in the process of migration. Method of Karnovsky and Roots (1964) and action of iso-OMPA, an inhibitor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

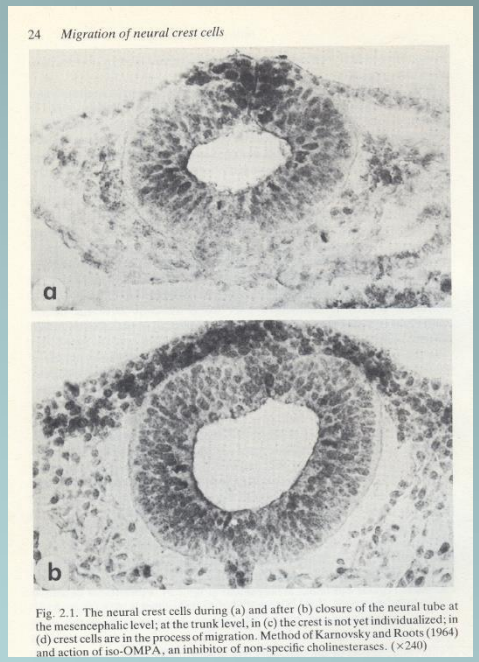
Interazioni tra i rombomeri

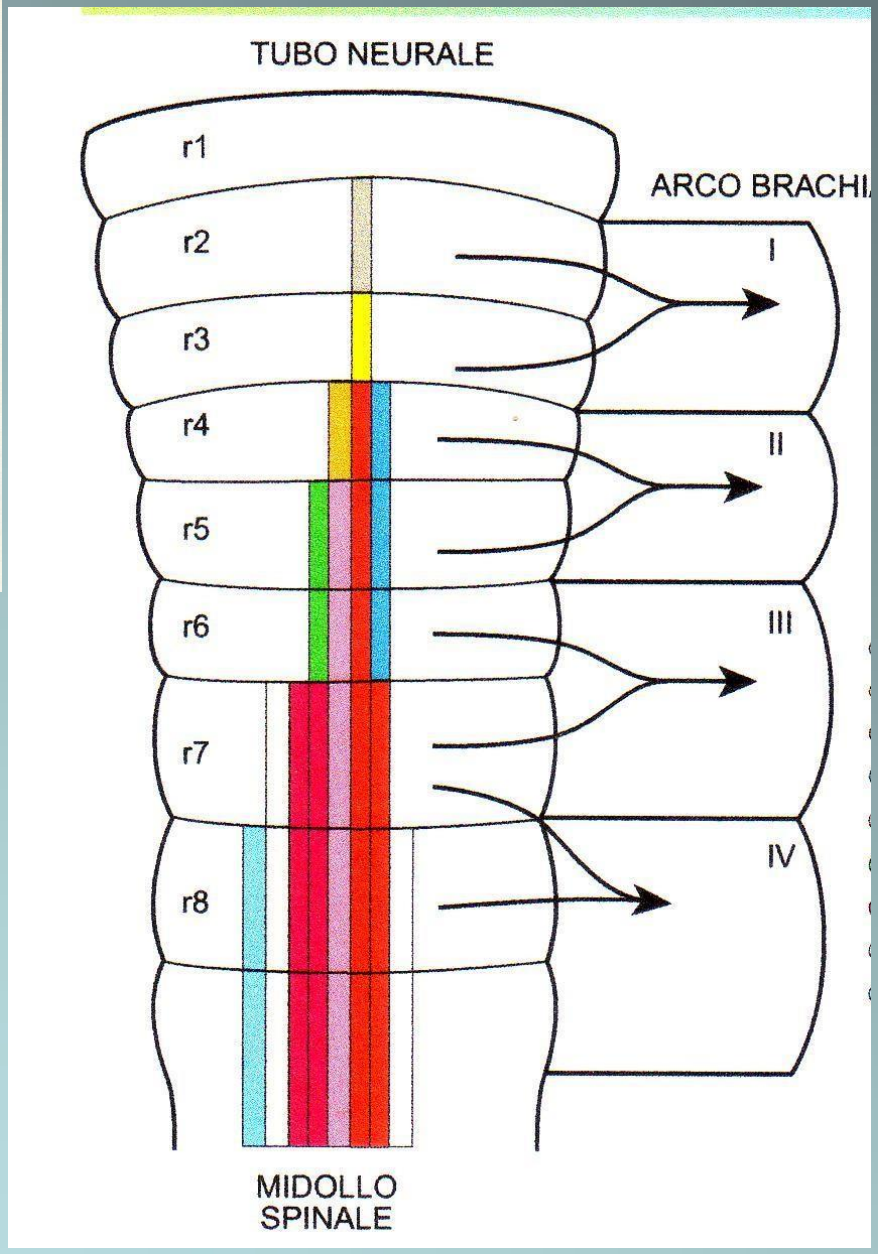


Le cellule della cresta neurale più dorsali dei rombomeri r3 e r5 vengono indotte a morire sotto influenza dei rombomeri pari.

Questo effetto è mediato da BMP4

Prima ondata migratoria: migrano le NC dorsali





Prima ondata migratoria

Coinvolge principalmente le cellule dorsali del r2 e r4

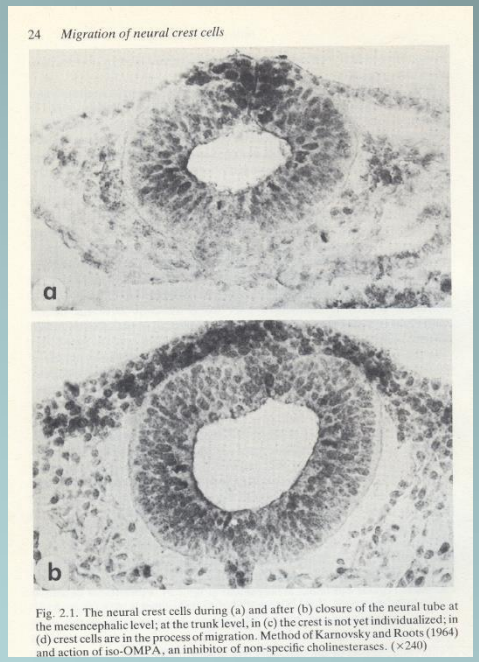
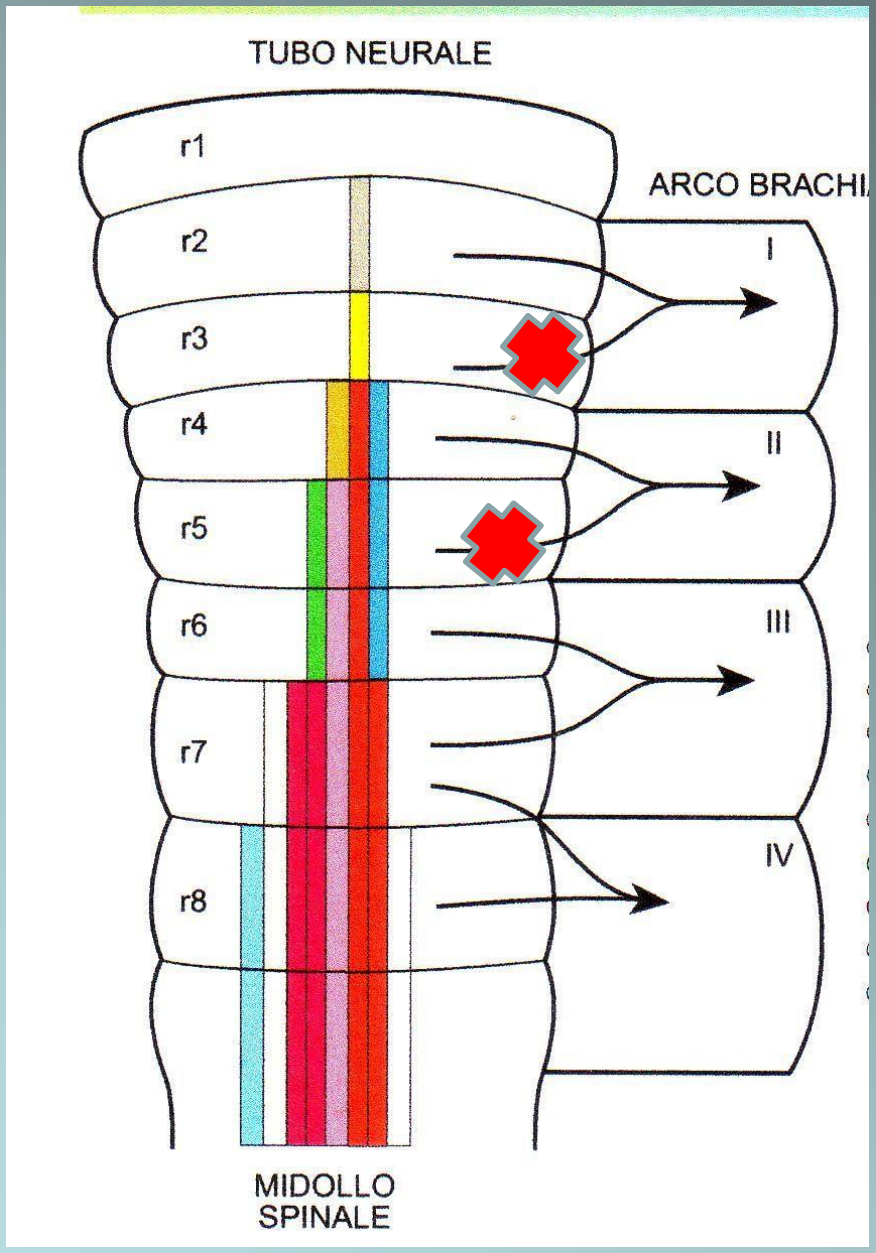


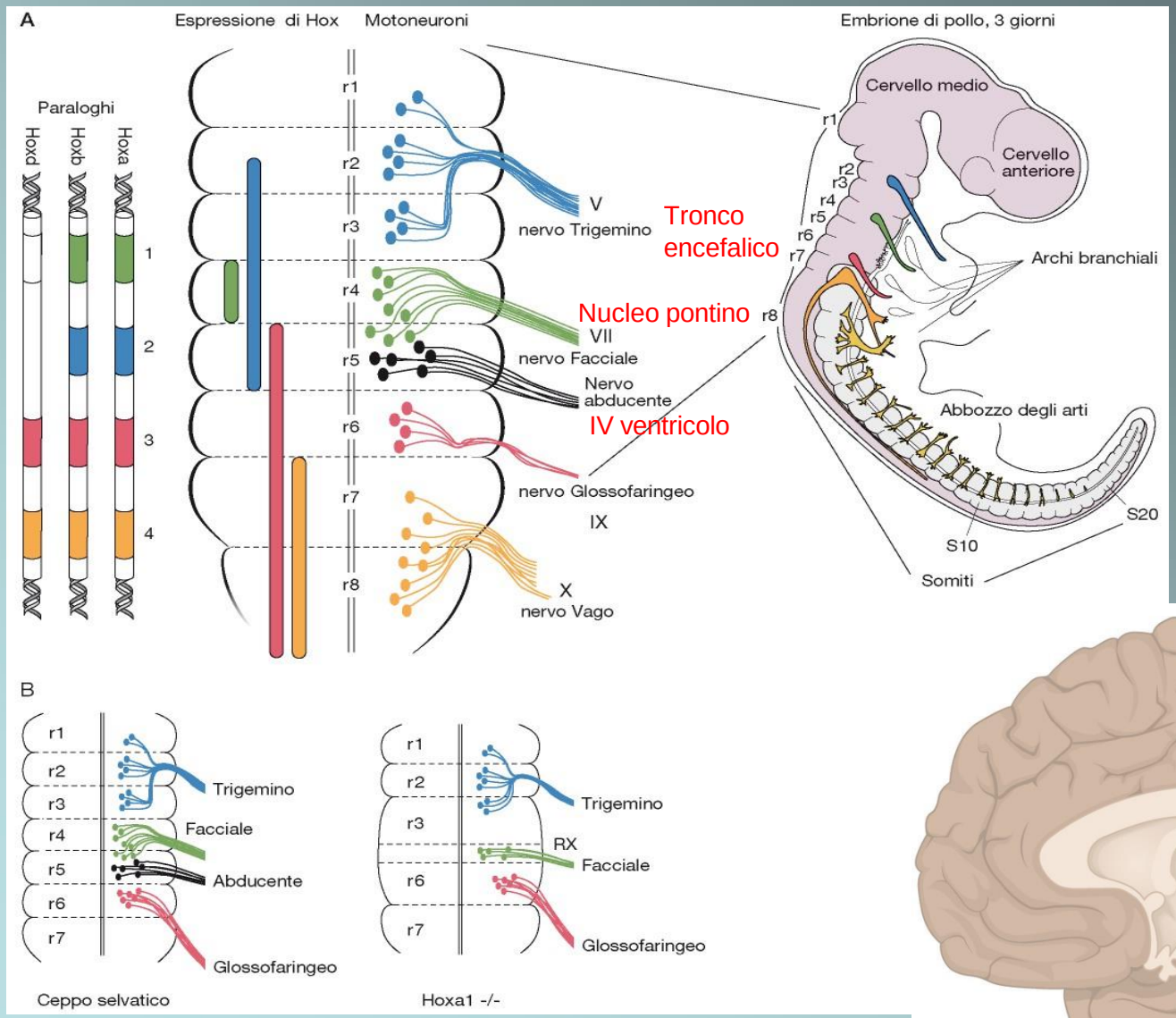
Fig. 2.1. The neural crest cells during (a) and after (b) closure of the neural tube at the mesencephalic level; at the trunk level, in (c) the crest is not yet individualized; in (d) crest cells are in the process of migration. Method of Karnovsky and Roots (1964) and action of iso-OMPA, an inhibitor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

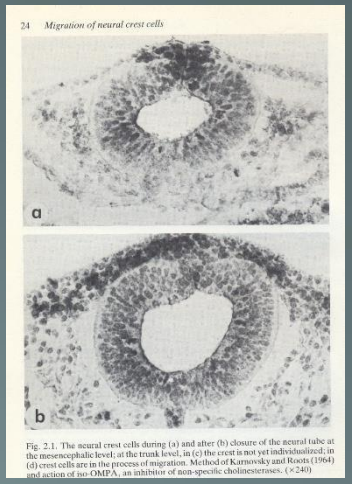


mandibolare

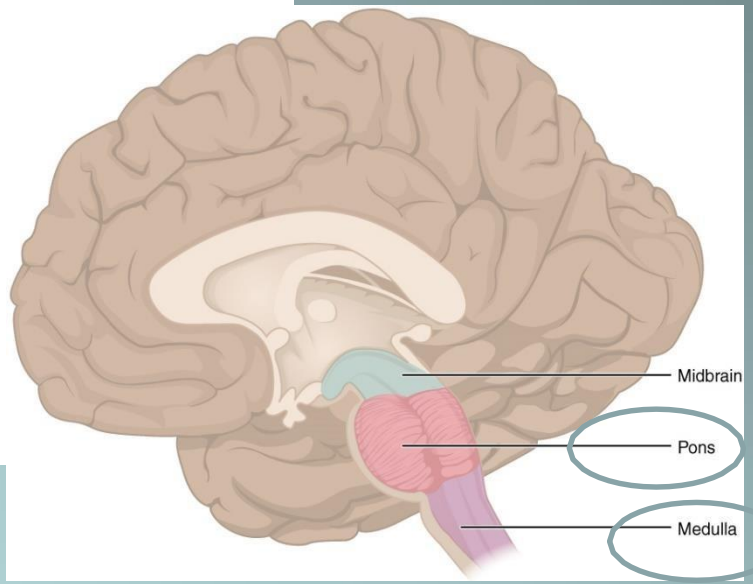
ioide

Seconda ondata migratoria





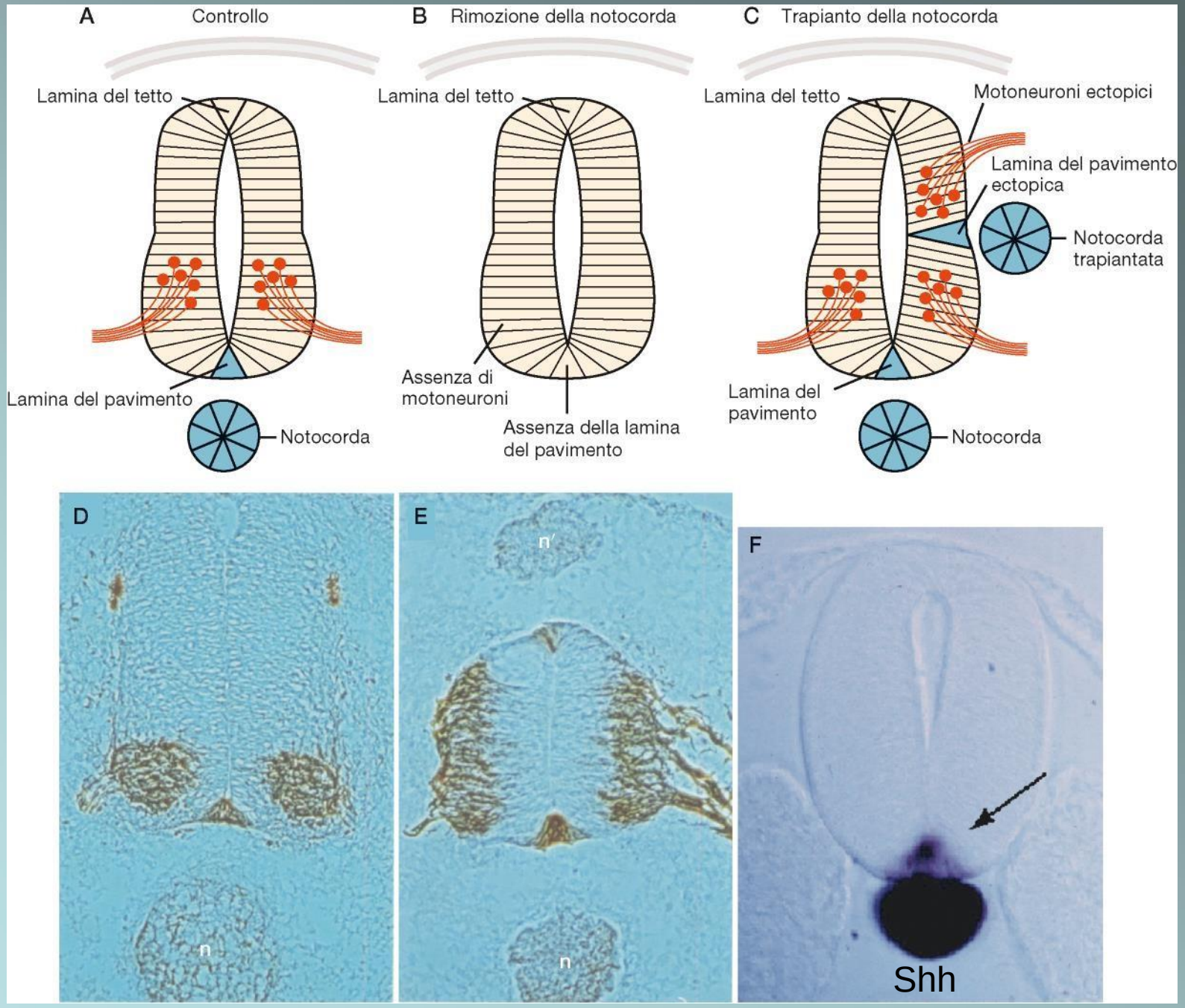
Coinvolge tutte le cellule più ventrali dei vari rombomeri



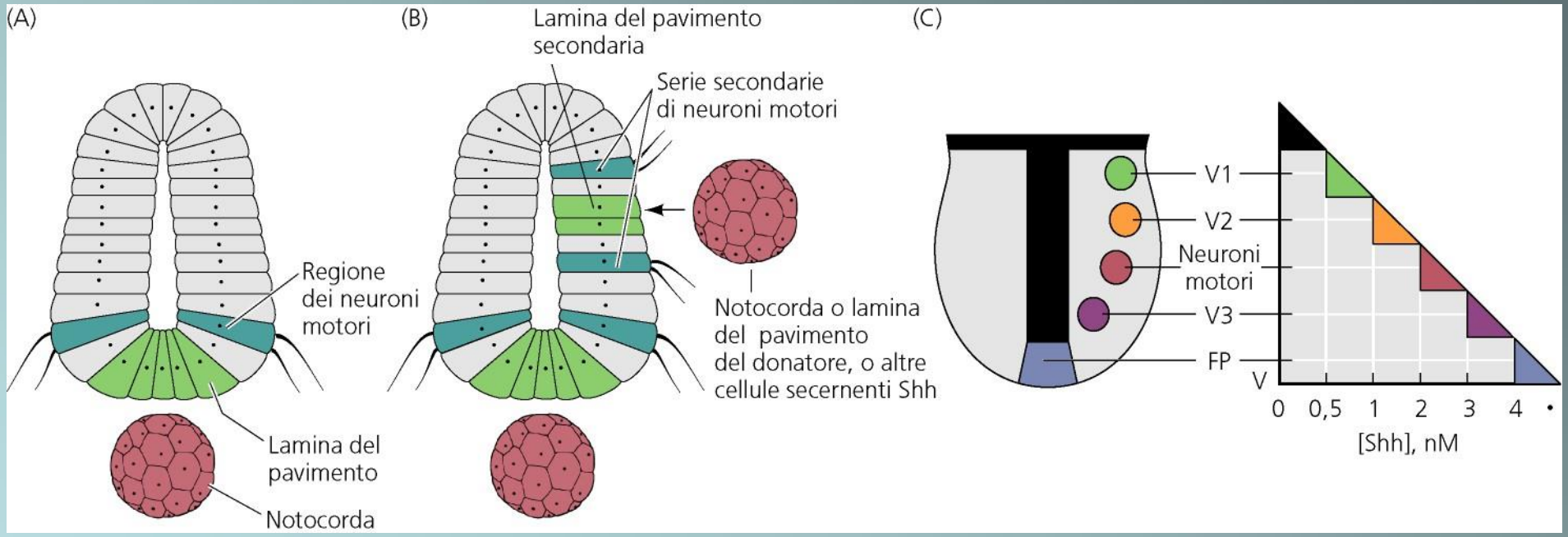
Quindi……

- La segmentazione transiente del romboencefalo è determinata da differenziale accensione dei geni hox e Krox20
- La segmentazione transiente garantisce l'uscita ordinata delle NC dal tubo neurale (prima le dorsali e poi le ventrali)
- Questa migrazione coordinata permette alle NC di raggiungere specifiche tasche faringee nei giusti tempi per avviare la formazione dei differenti derivati cellulari

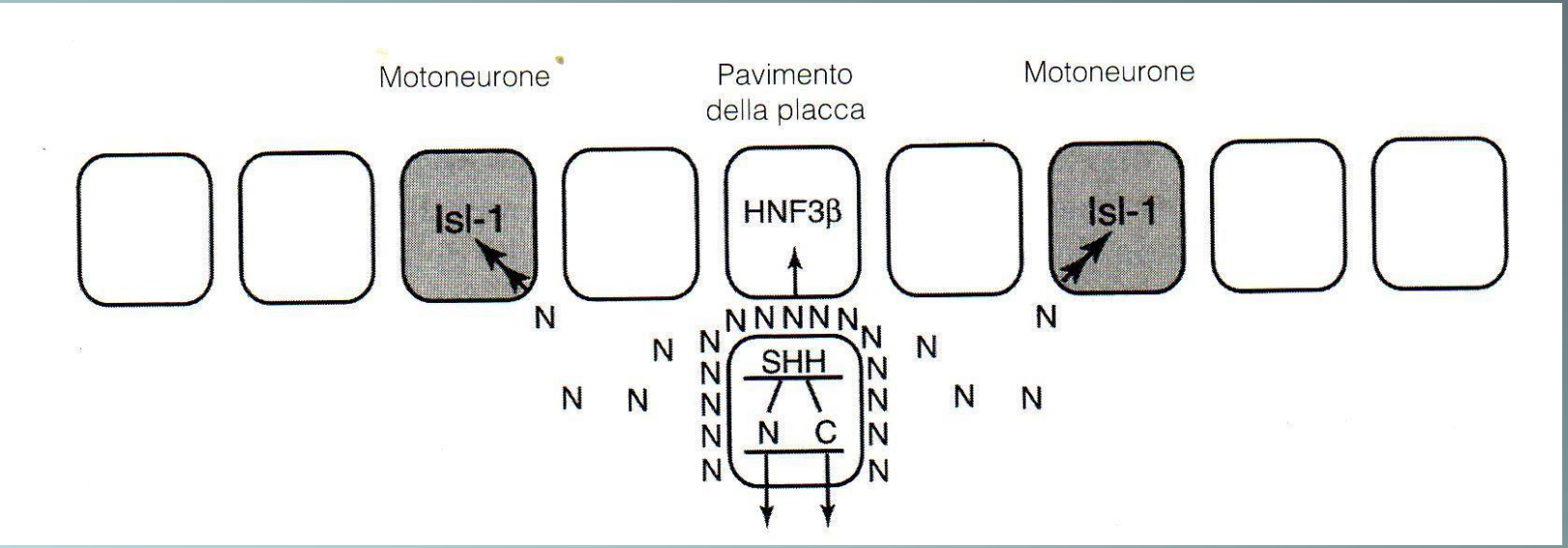
Asse Dorso Ventrale


A
Controllo
Lamina del tetto
Lamina del pavimento
Notocorda
B
Rimozione della notocorda
Lamina del tetto
Assenza di motoneuroni
Assenza della lamina del pavimento
C
Trapianto della notocorda
Lamina del tetto
Motoneuroni ectopici
Lamina del pavimento ectopica
Notocorda trapiantata
Lamina del pavimento
Notocorda
D
n
E
n′
n
F
Shh

Distribuzione del gradiente di Shh



Effetti prodotti dal gradiente di Shh

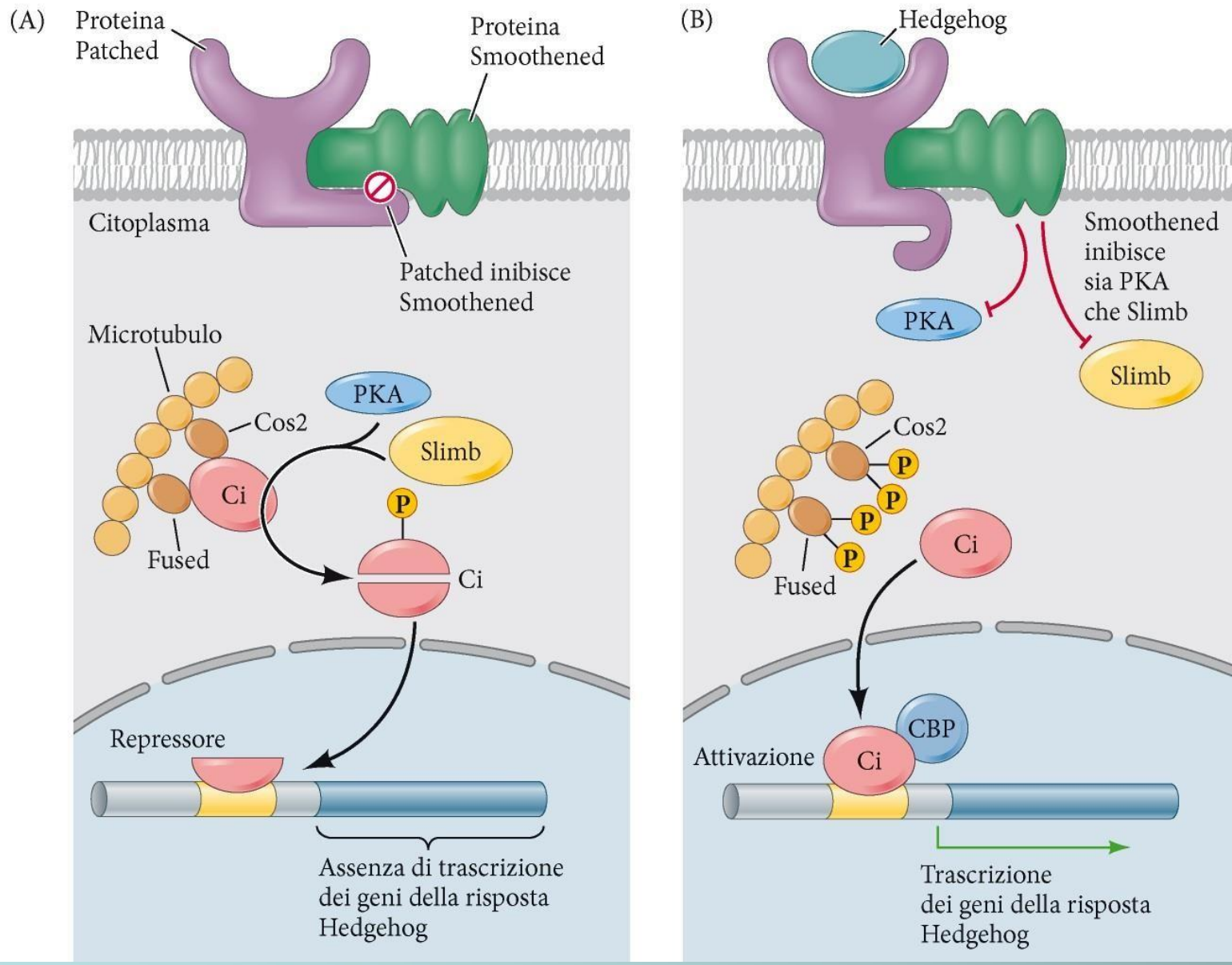


Alte concentrazioni di Shh inducono la lamina del pavimento (HNF3β)

Minori concentrazioni di Shh inducono la zona dei motoneuroni (islet1)

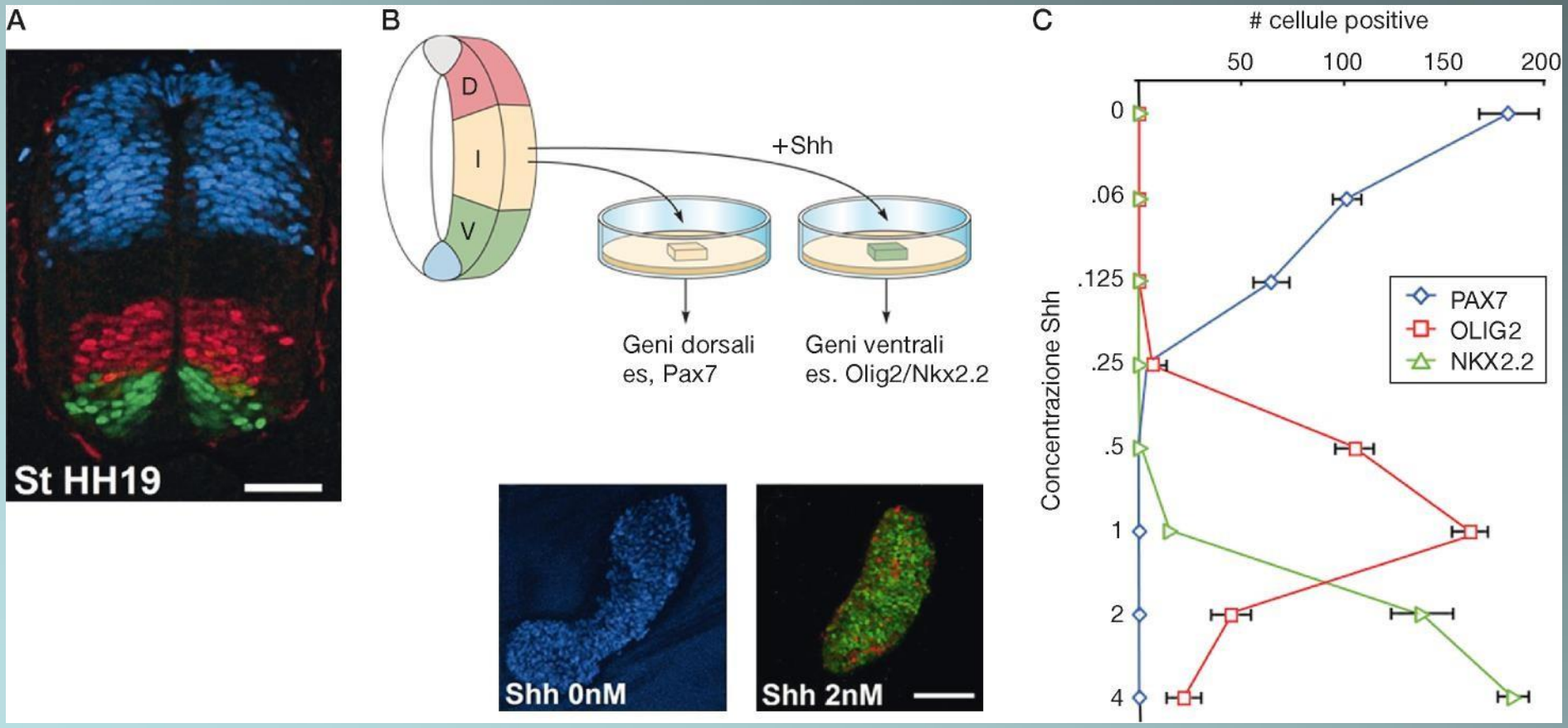
Via di trasduzione del segnale di Sonic HedgeHog

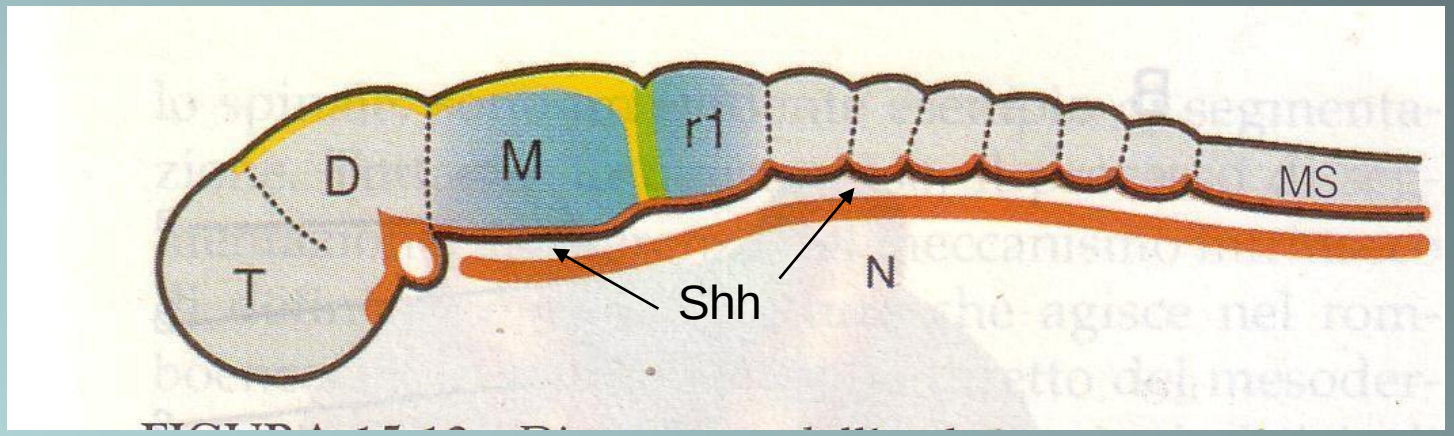
Hedgehog
Patched
Smoothened
Attivazione effettuata dalla proteina Ci
Trascrizione



CI: cubitus interruptus.

GLI nei mammiferi

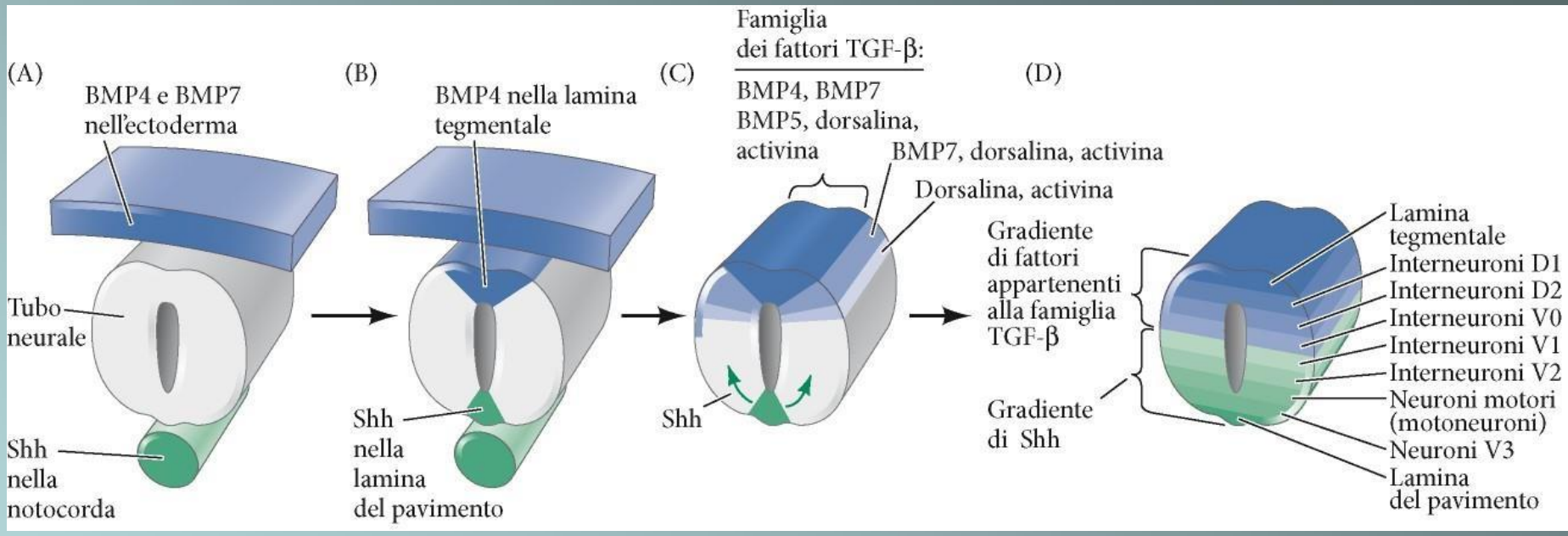

A
St HH19
B
D
I
V
+Shh
Geni dorsali
es, Pax7
Geni ventrali
es. Olig2/Nkx2.2
Shh 0nM
Shh 2nM
C
cellule positive
50
100
150
200
0
.06
.125
.25
.5
1
2
4
Concentrazione Shh
PAX7
OLIG2
NKX2.2



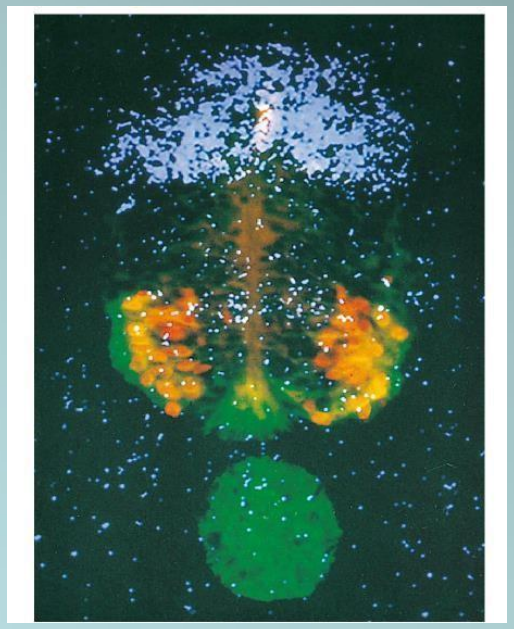
Shh è distribuito lungo tutto l'asse ventrale del tubo neurale

Influenza la formazione oltre che dei motoneuroni spinali anche i neuroni serotoninergici del nucleo del *Rafe (romboencefalica)* e quelli dopaminergici della *substantia nigra (mesencefalica).*

Differenziamento del tubo neurale



Gradienti opposti di sonic-hedgehog e fattori della famiglia TGF-beta



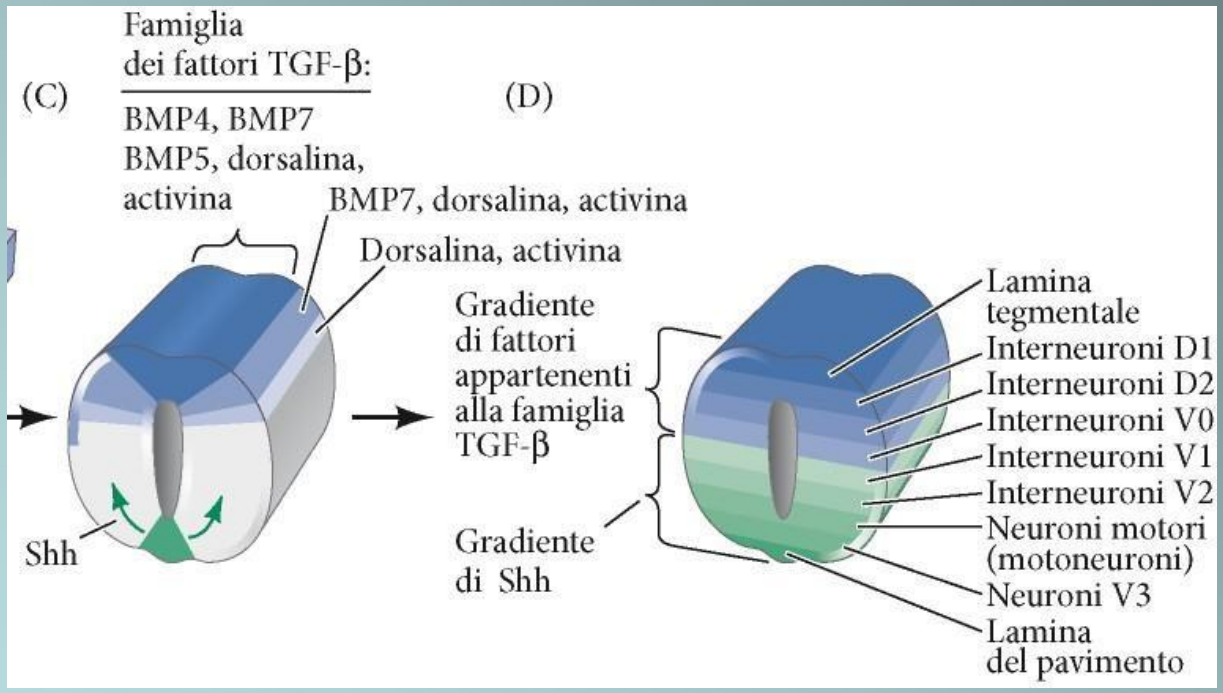
Dorsalin, BMP (blu)

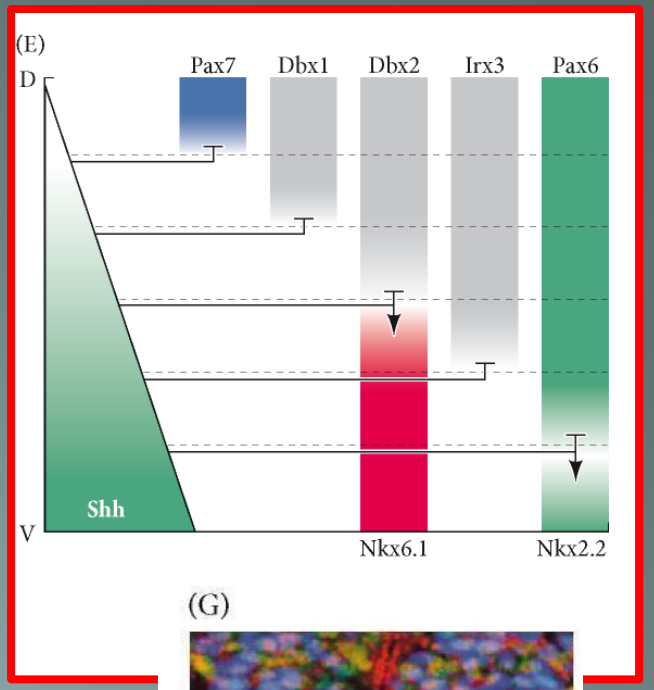
Interneuroni

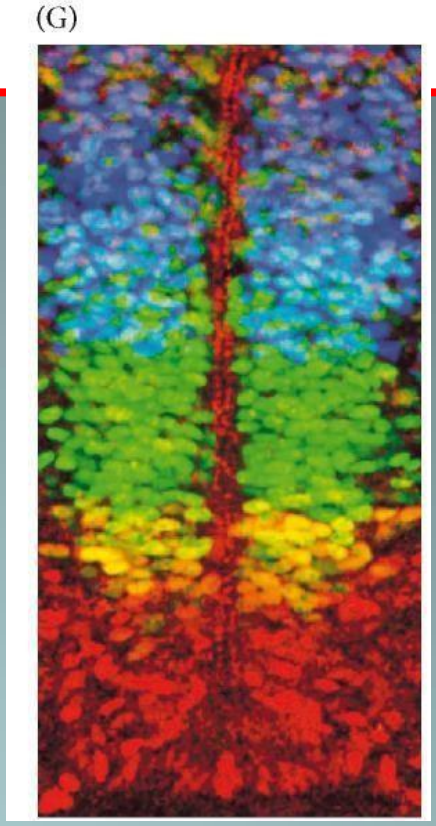
Motoneuroni (arancio)

Sonic-hedgehog (verde)

Differenziamento dorso-ventrale del tubo neurale



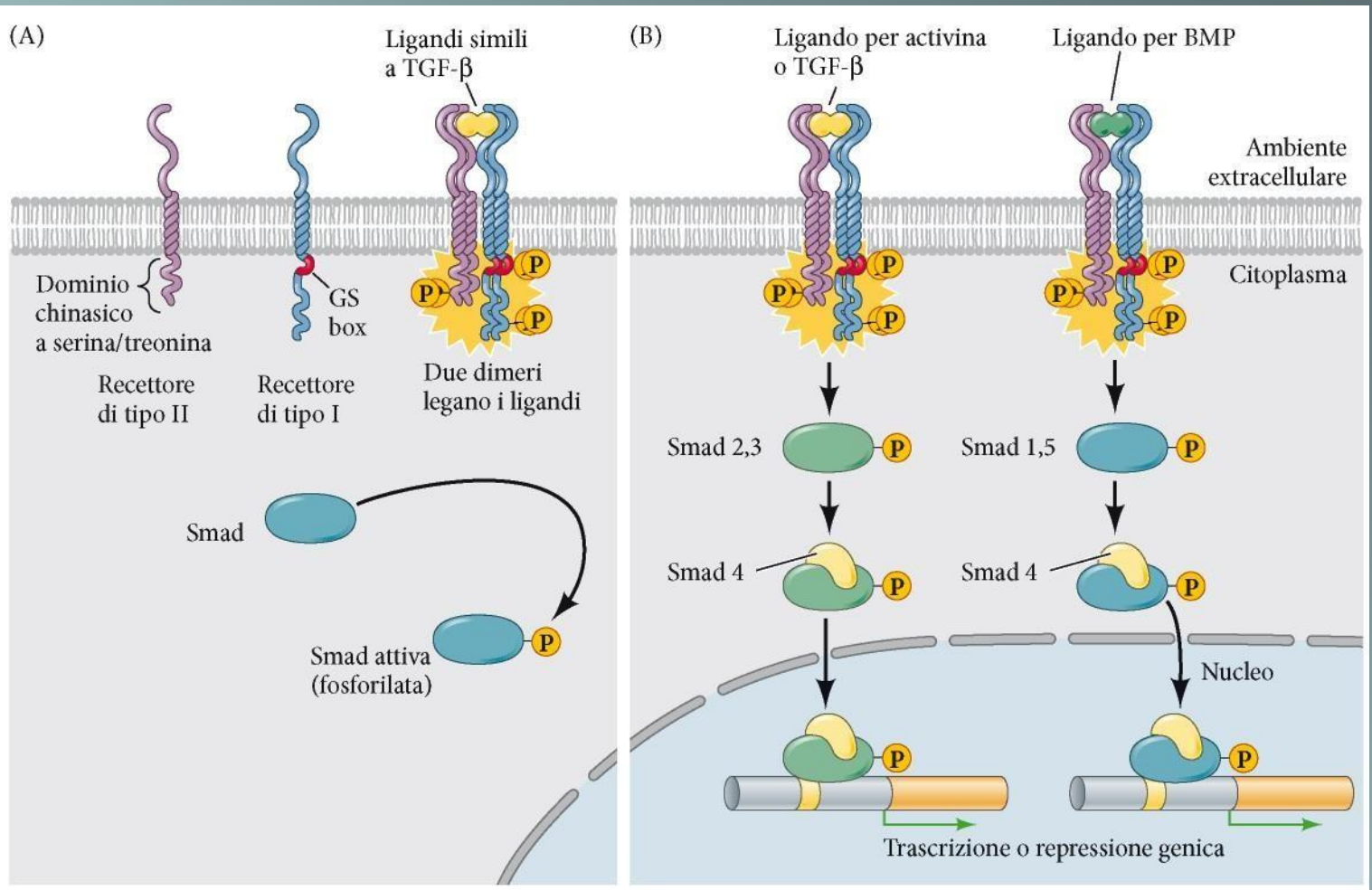


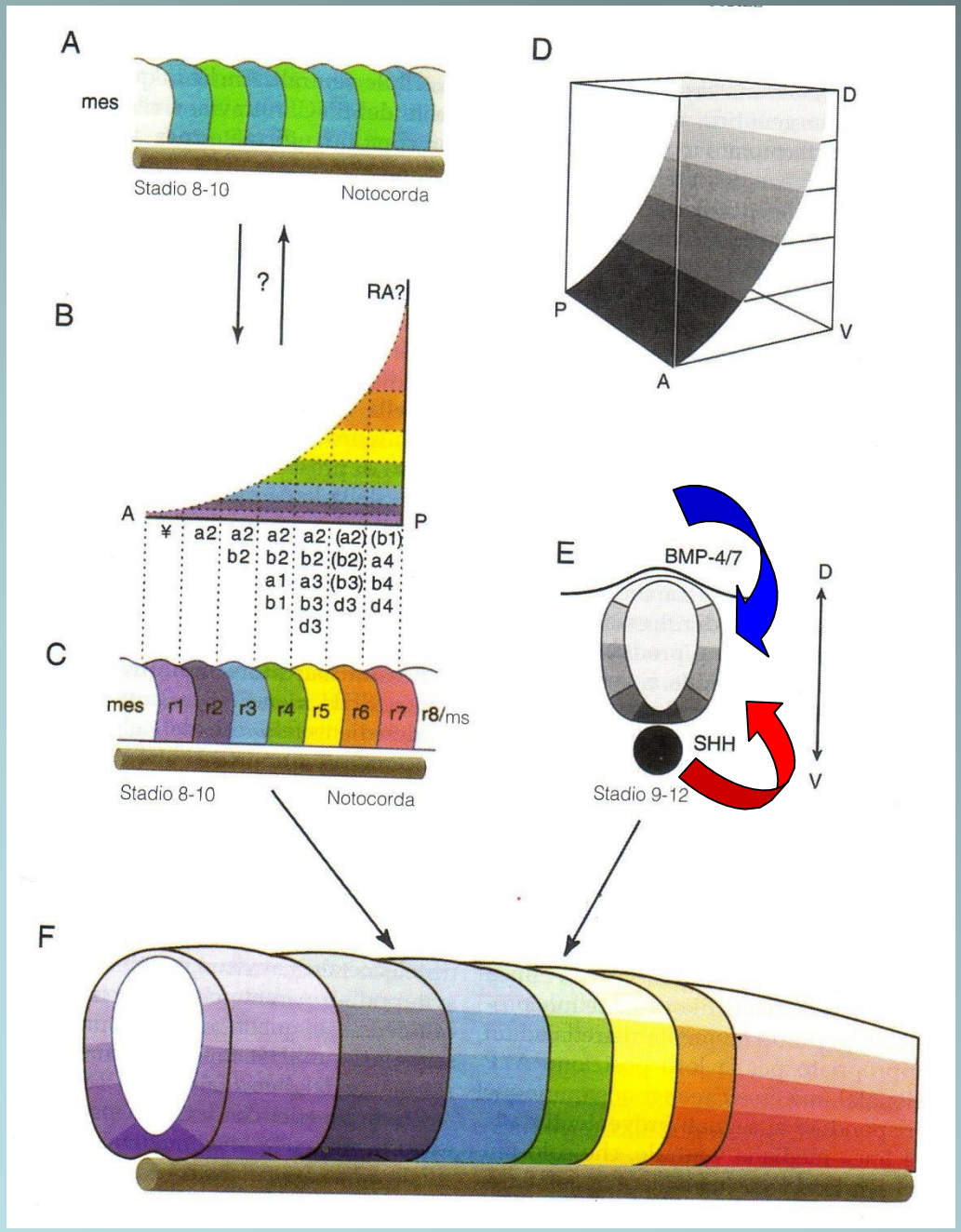


I gradienti di fattori diffusibili stabiliscono dei territori specifici di espressione di fattori trascrizionali

Trasduzione del segnale dei fattori della famiglia TGFβ

Ligando della superfamiglia TGF-β
↓
Recettore II
↓
Recettore I
↓
Attivazione di Smad
↓
Dimerizzazione di Smad
↓
Neotrascrizione

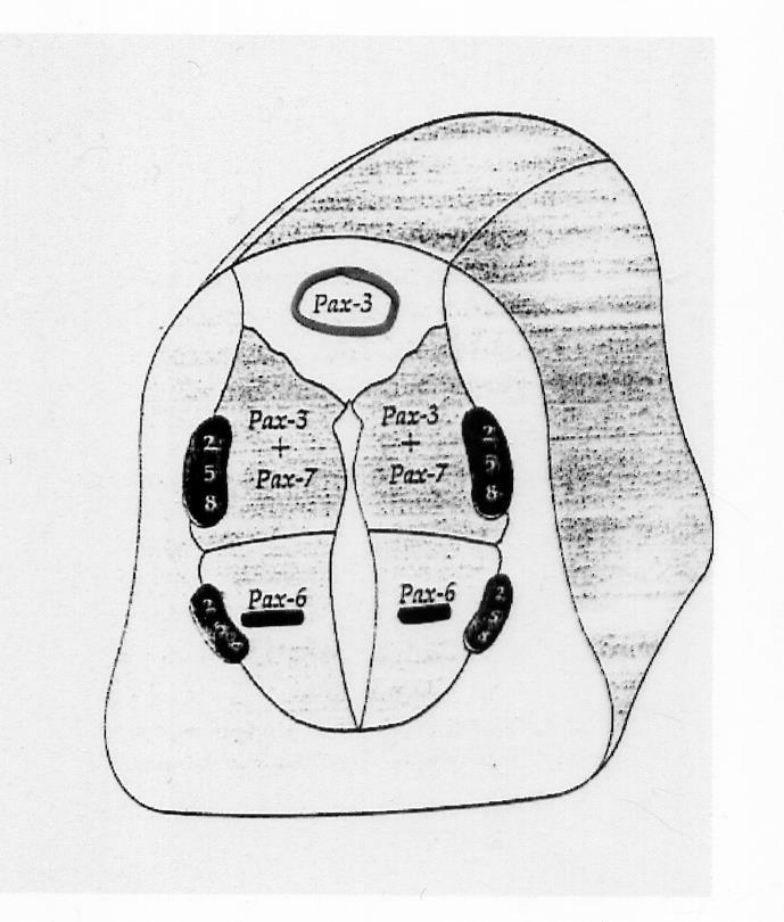




BMP4-7: gradiente dorso-ventrale

Shh: gradiente ventro-dorsale

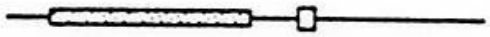
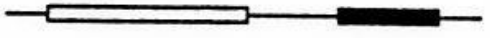
Geni Pax



- **Sono omologhi dei geni *paired* di Drosophila**

- **Regolano l'asse D-V del tubo neurale**
- **Pax 3 è richiesto per la chiusura del tubo neurale**
- **Pax 3 e Pax 7 sono sotto controllo di gradienti di BMP-4/7**
- **Pax 6 è controllato da gradienti di Shh**

Mutazioni dei geni PAX

Gene	Chromo-some	Structural Features PD OP HD	Mouse Mutants	Homologous Human Syndromes
Pax -1	2		*undulated (un)* (sclerotome)	
Pax -2	19			Nervo ottico (coloboma)
Pax -3	1		*splotch (sp)* (SNC)	*Waardenburg Syndrome I* (dif. vdito fat. ment.)
Pax -4	6			
Pax -5	4	 ?		
Pax -6	2		*small eye (sey)* (occhio)	*Aniridia* (Iride)
Pax -7	4			
Pax -8	2		*Danforth´s short tail (Sd)* ?	

Integrazione tra segnali che agiscono nell'asse D/V

