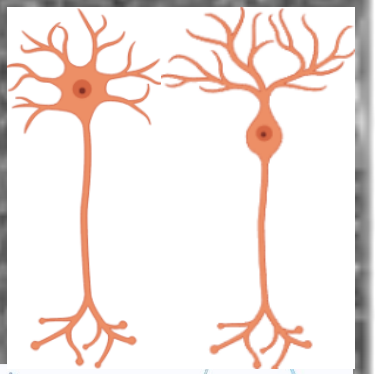
MEDIZINISCHE UNIVERSITÄT
INNSBRUCK

Celulele stem:
de la modelare neuropatologica la terapii specifice



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Institutul de Neuroanatomie
Departamentul de Anatomie, Histologie si Embriologie
Universitatea de Medicina din Innsbruck, Austria

DIVERSITATEA CELULELOR NOASTRE

sau

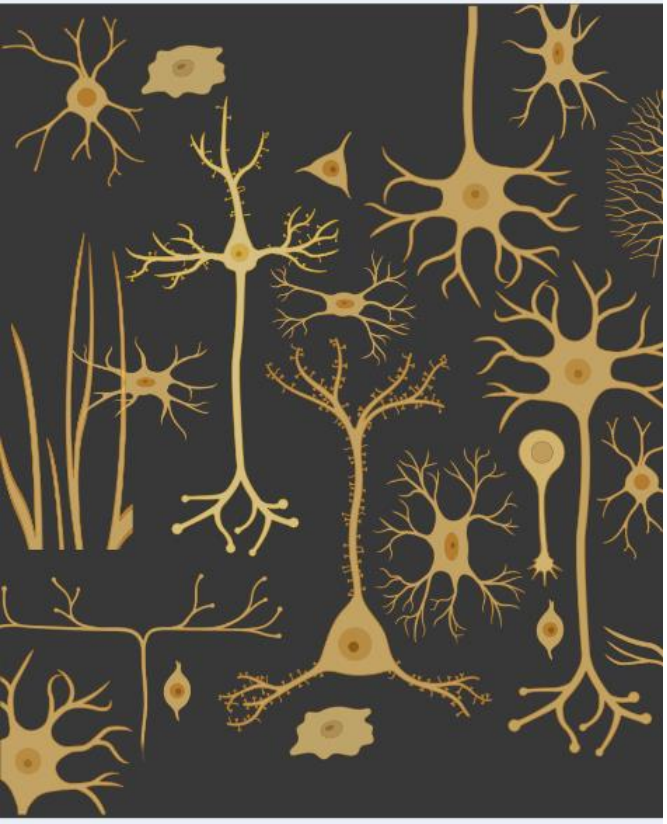
corpul uman la rezolutie celulara

3×10^{13}

30.000 miliarde

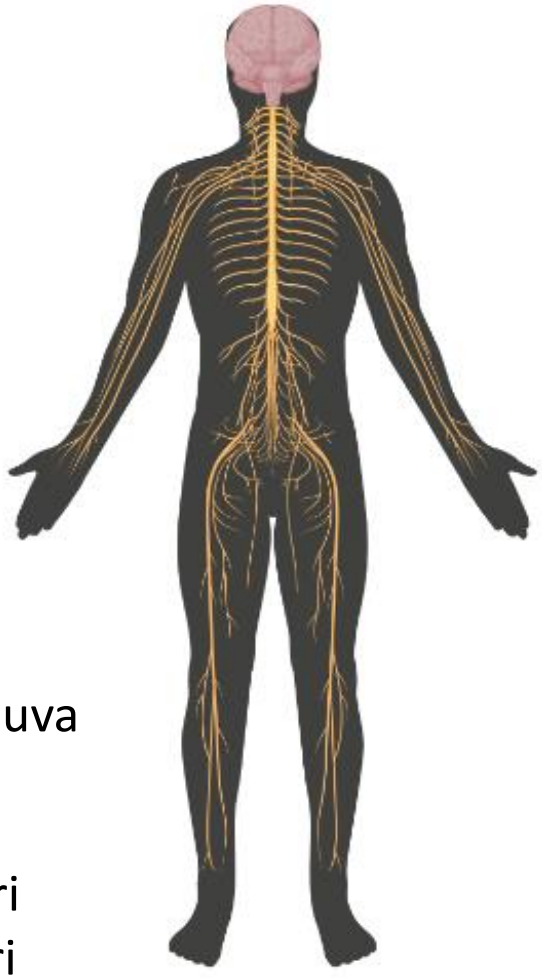
75-80% hematii

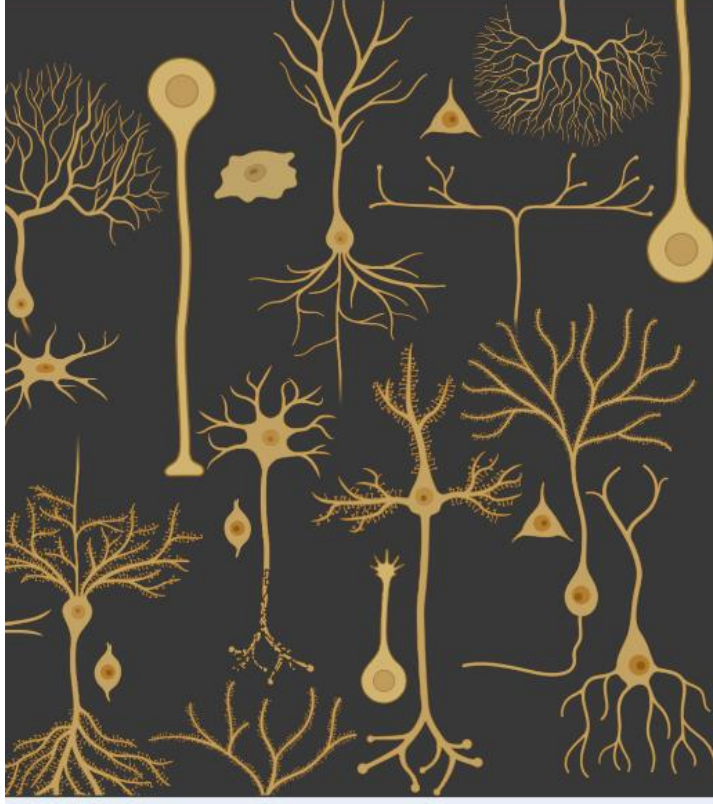
5000 miliarde



Sistemul nervos

200 miliarde (10^9) celule neurale





- Central

telencefal, cerebel,

ax: trunchi cerebral +maduva spinarii

neuroni:

excitatori

inhibitori

celule gliale/progenitoare

- Periferic

neuroni:

senzitivi

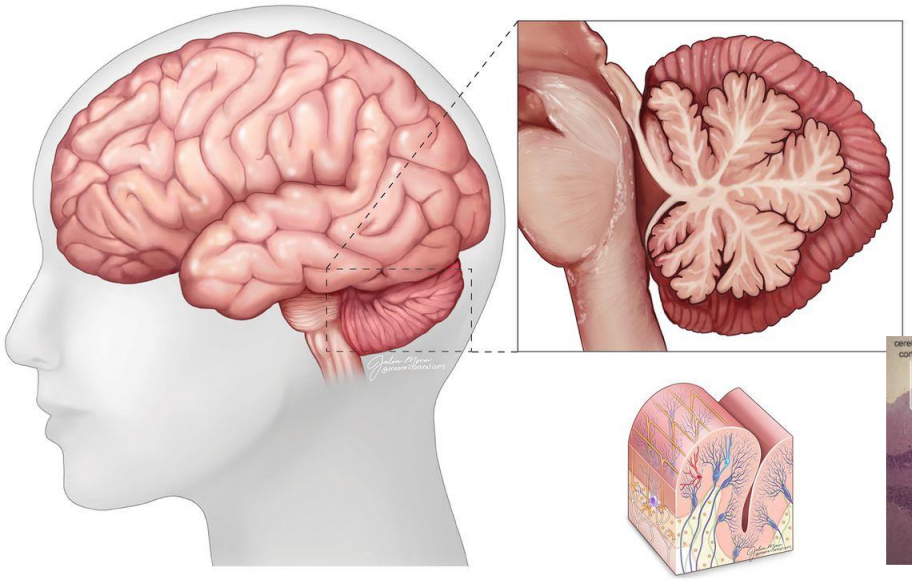
autonomi

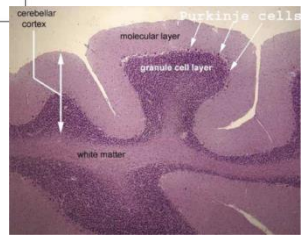
simpatici

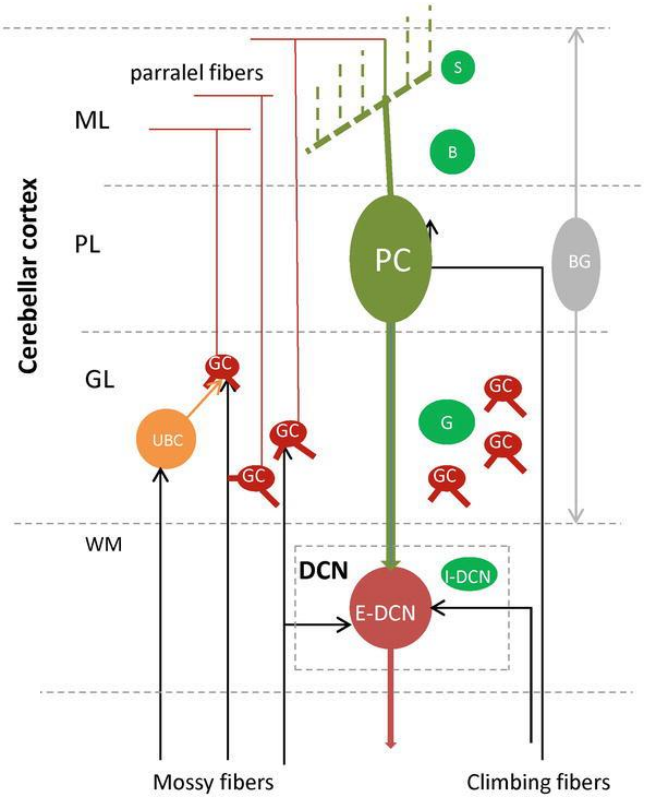
parasimpatici

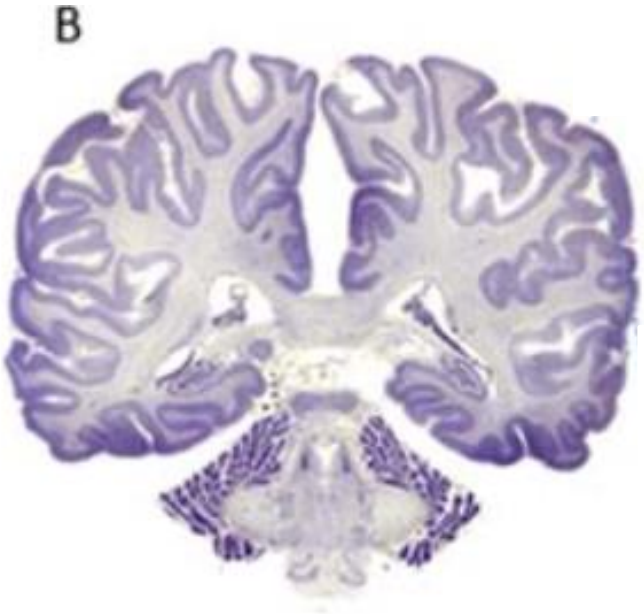
celule gliale/progenitoare

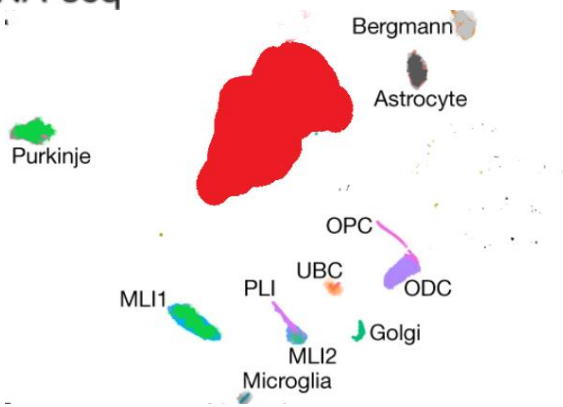
16 + 70 miliarde neuroni



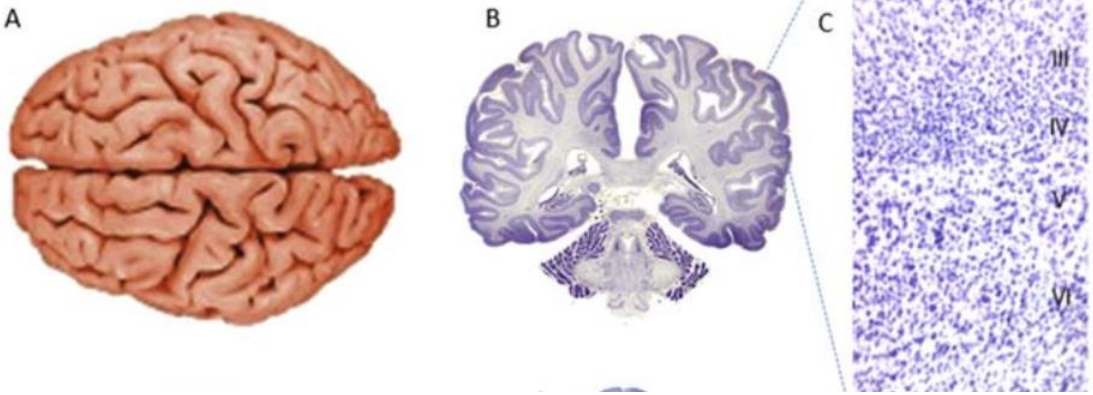


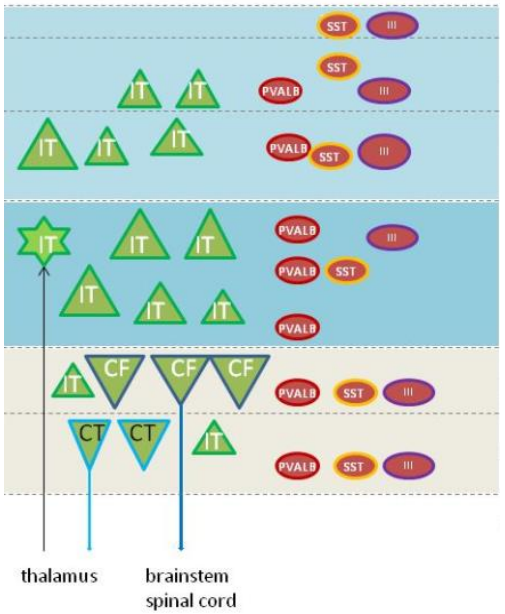


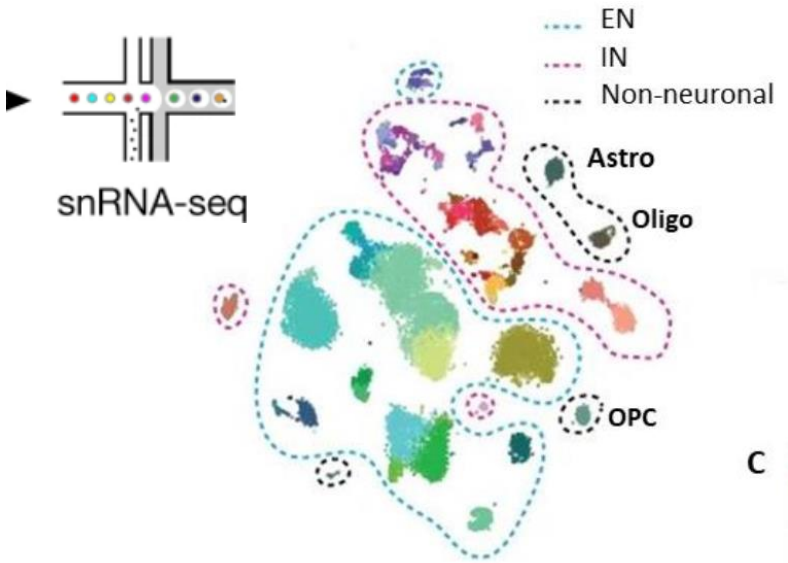


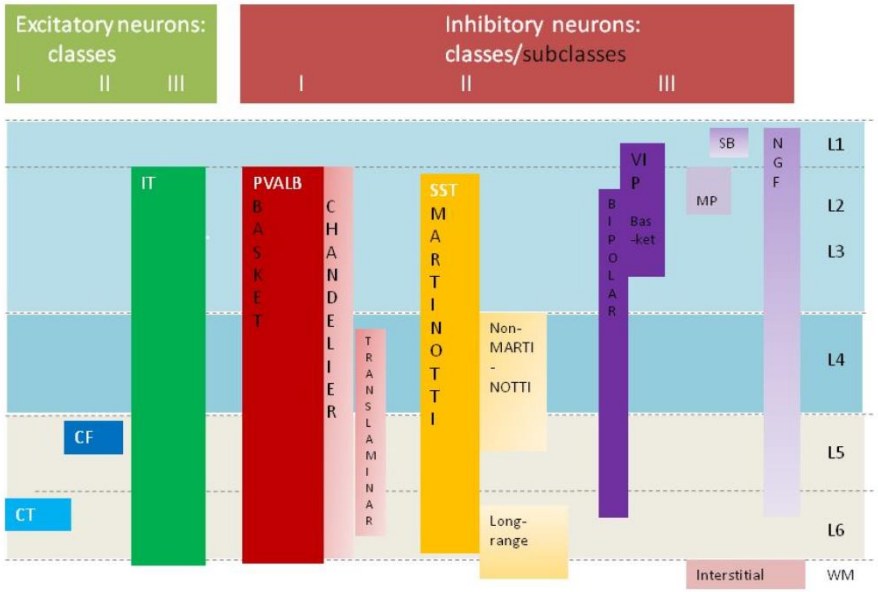


Kozareva et al Nature 2021



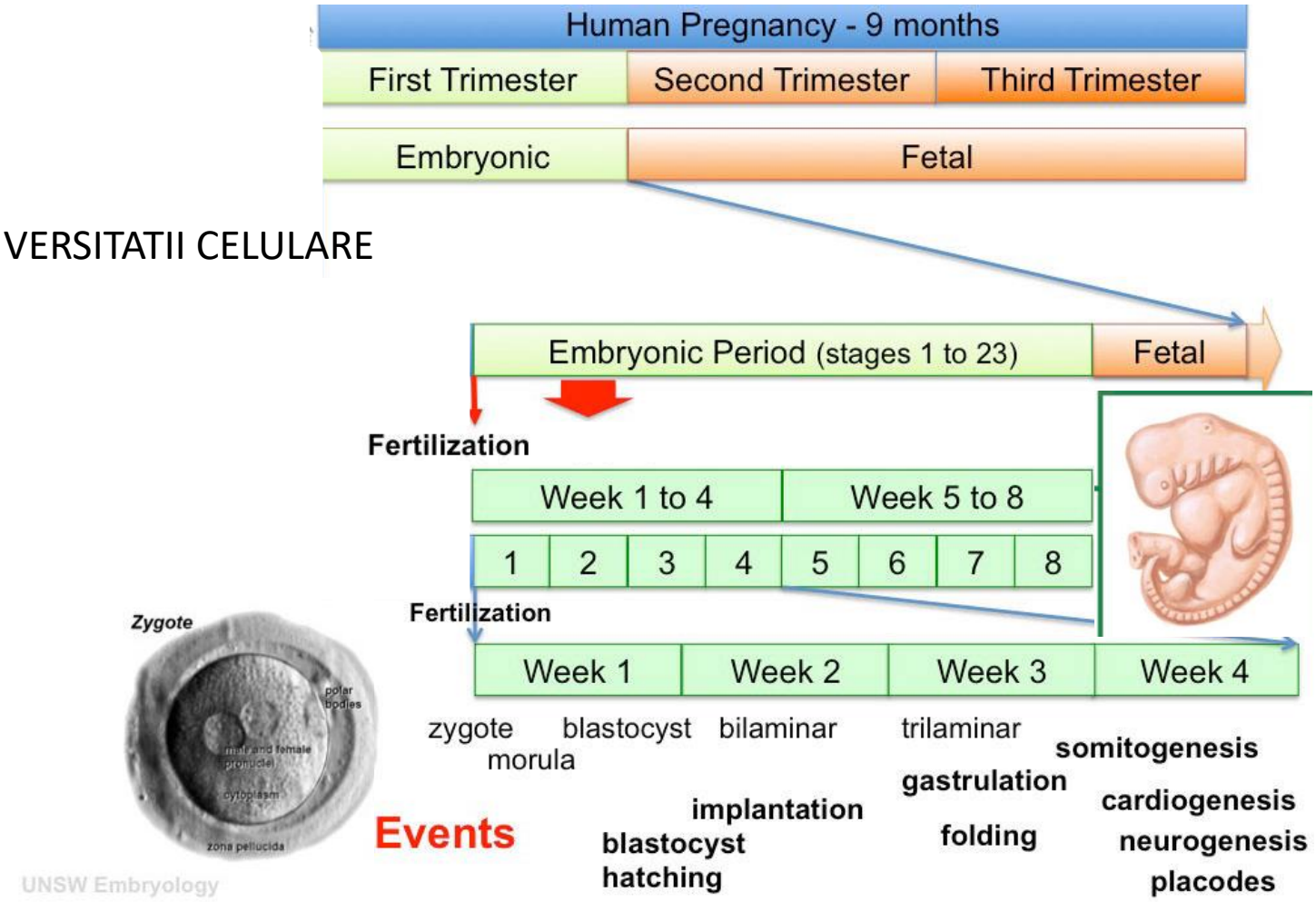




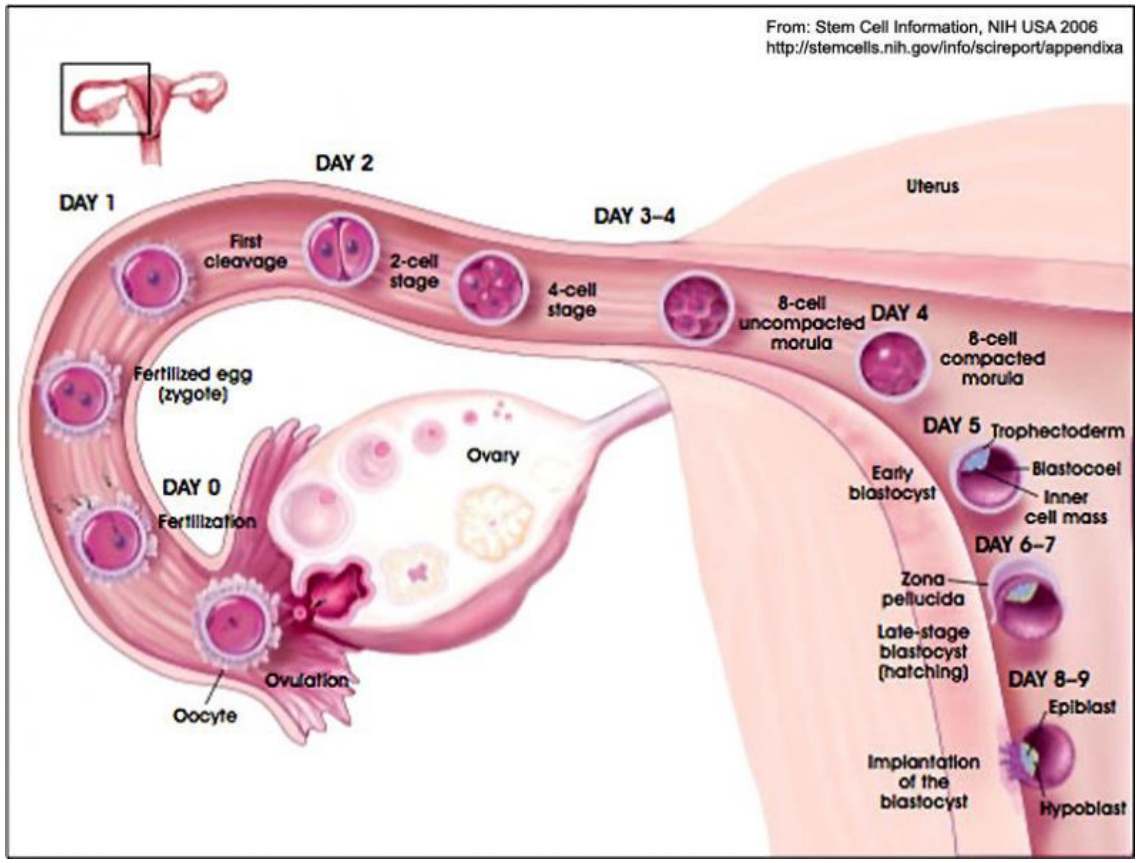


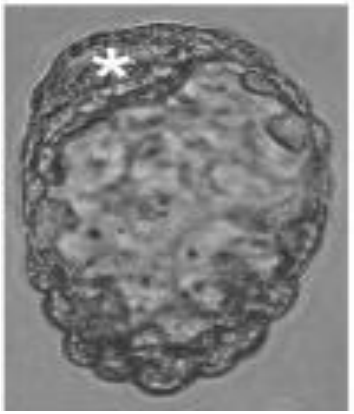
Tasic et al Nature 2018

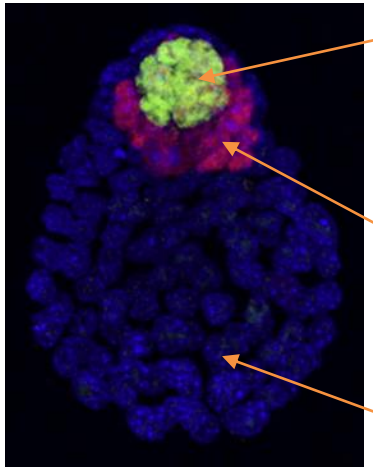
SURSA DIVERSITATII CELULARE



SAPT 1-2







EPIBLAST
OCT4

Hipoblast
GATA4

Trofoblast

1 week

Blastocyst

2 weeks

Embryo

3 weeks

Gastrulation occurs.

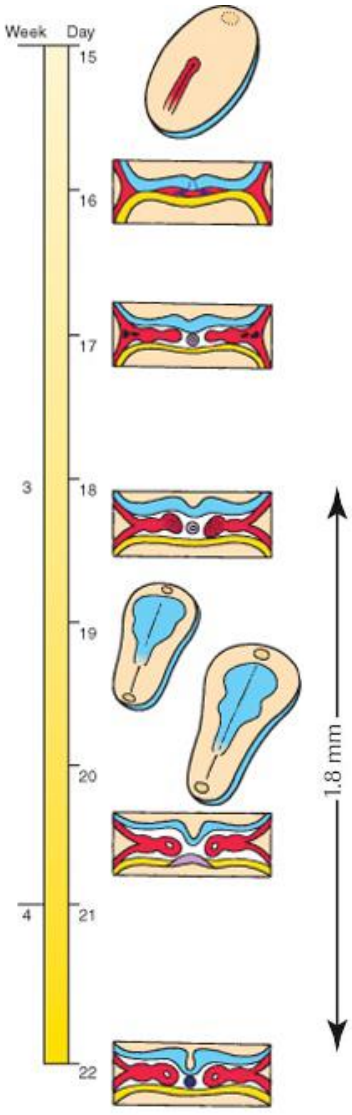
4 weeks

Folding of the embryo occurs, and the vertebrate body plan is established.

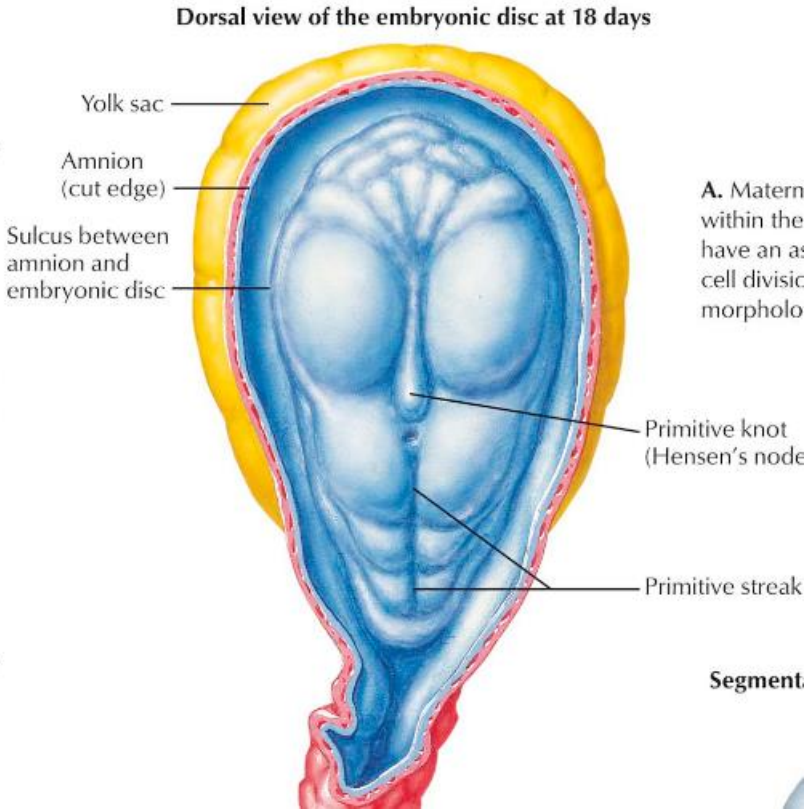
1

Fetus

Saptamana 3



Schoenwolf et al: Larsen's Hu
Copyright © 2008 by Church



A. Matern
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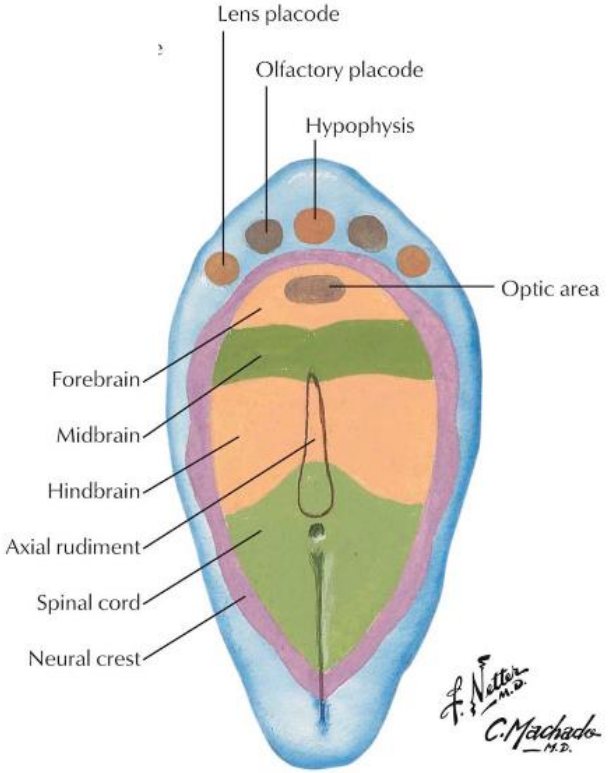
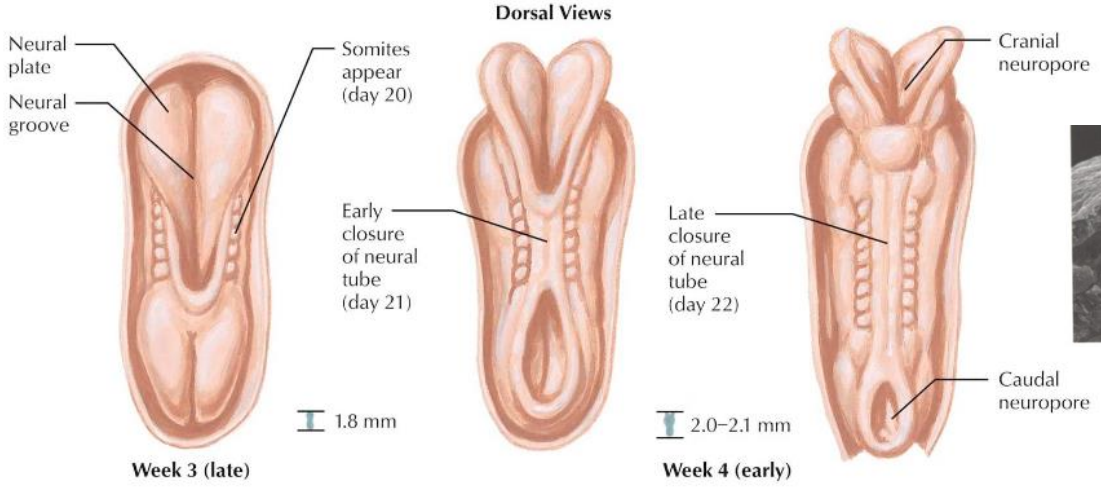
have an a
cell divisic
morpholo

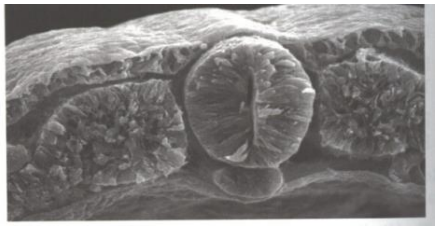
Segment

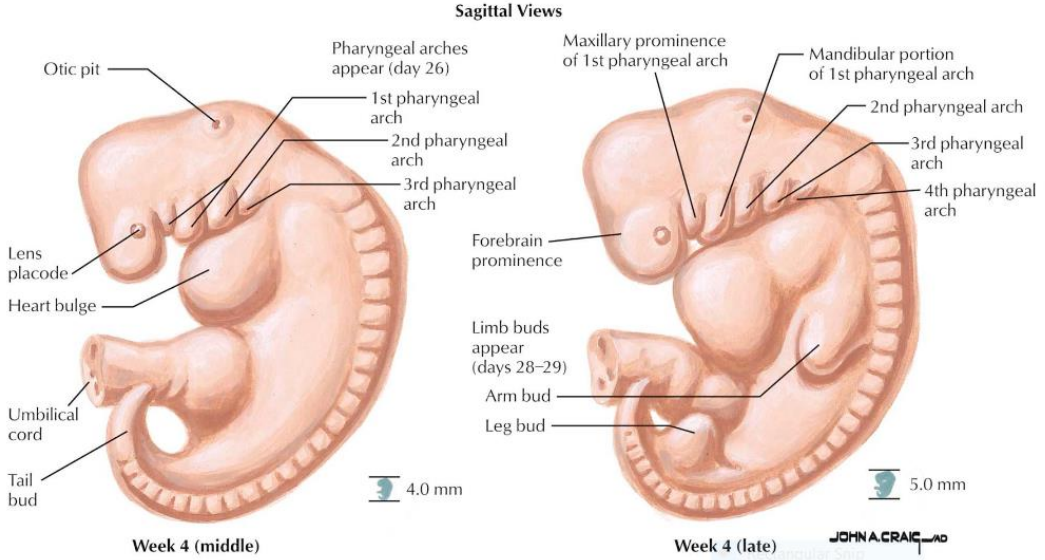


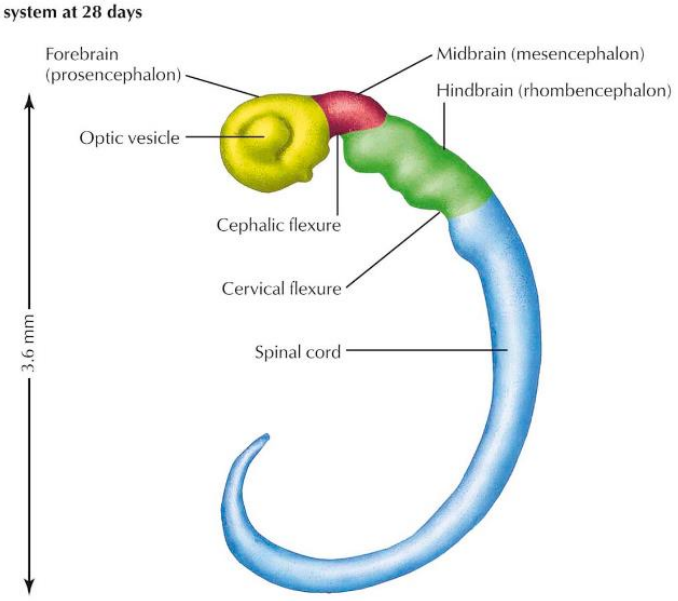
Developmental fates of
local regions of ectoderm
of embryonic disc at 18 days

NEURULATIA

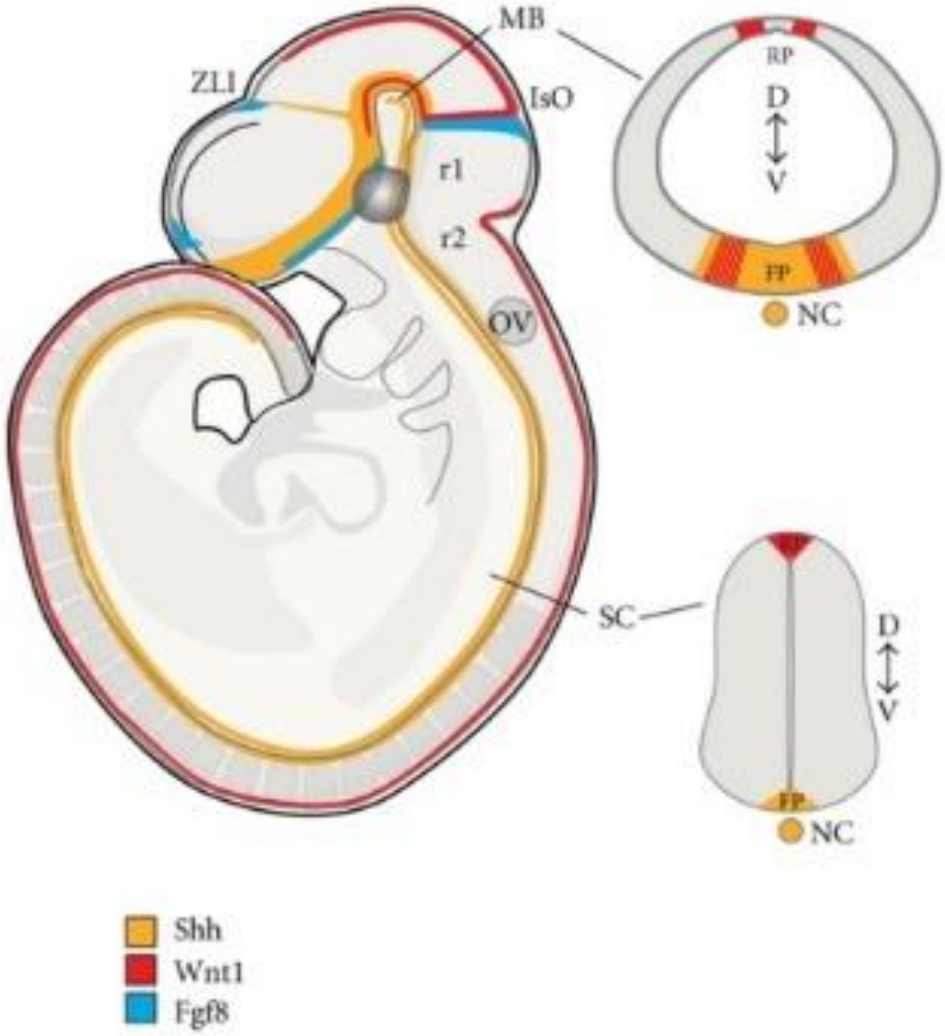

Dorsal Views
Neural plate
Neural groove
Somites appear (day 20)
Early closure of neural tube (day 21)
1.8 mm
Week 3 (late)
Cranial neuropore
Late closure of neural tube (day 22)
Caudal neuropore
2.0–2.1 mm
Week 4 (early)



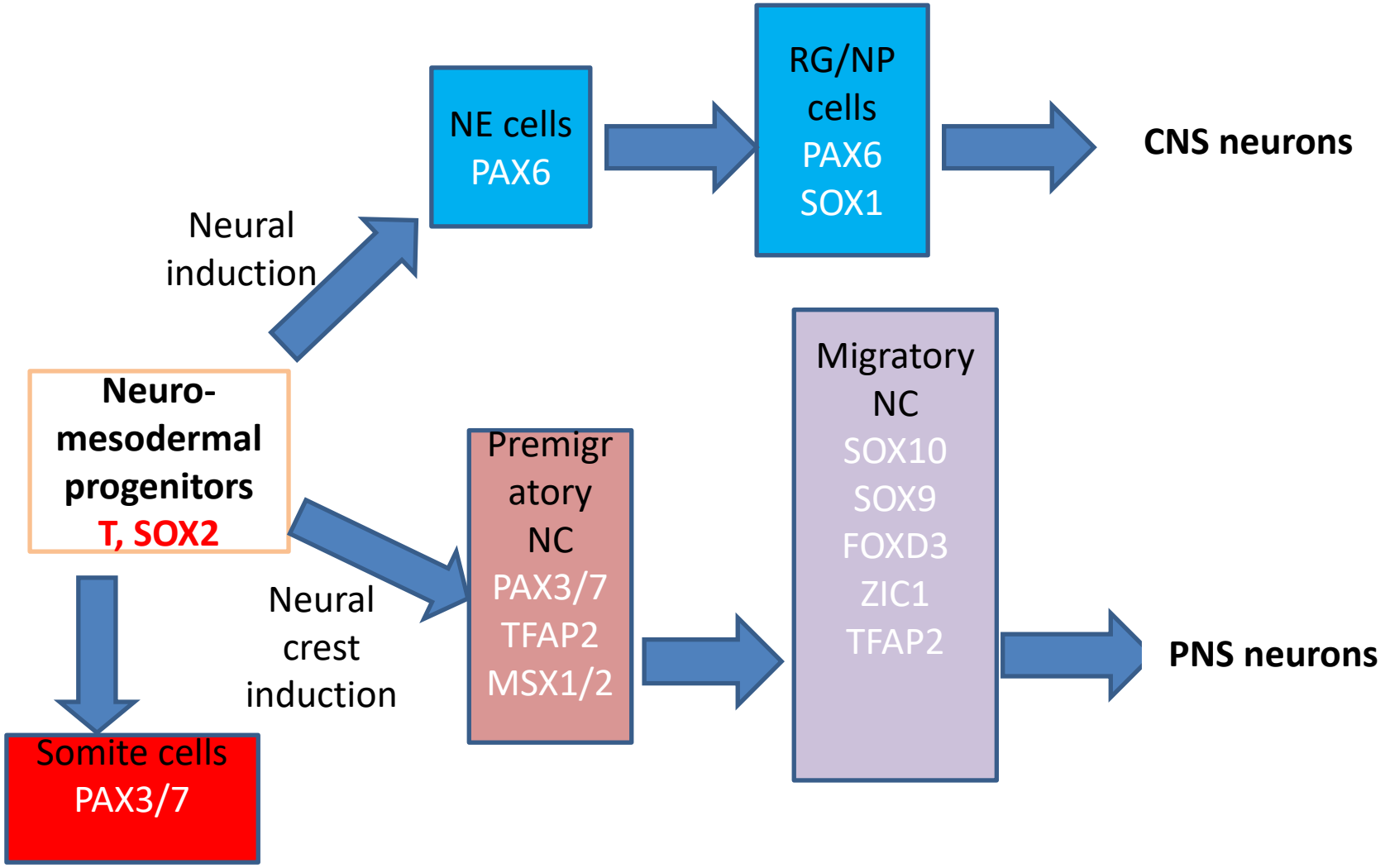

Sagittal Views
Otic pit
Pharyngeal arches appear (day 26)
1st pharyngeal arch
2nd pharyngeal arch
3rd pharyngeal arch
Lens placode
Heart bulge
Umbilical cord
Tail bud
4.0 mm
Week 4 (middle)
Maxillary prominence of 1st pharyngeal arch
Mandibular portion of 1st pharyngeal arch
2nd pharyngeal arch
3rd pharyngeal arch
4th pharyngeal arch
Forebrain prominence
Limb buds appear (days 28–29)
Arm bud
Leg bud
5.0 mm
Week 4 (late)
JOHN A. CRAIG, MD


Central nervous system at 28 days
Forebrain (prosencephalon)
Midbrain (mesencephalon)
Hindbrain (rhombencephalon)
Optic vesicle
Cephalic flexure
Cervical flexure
Spinal cord
3.6 mm

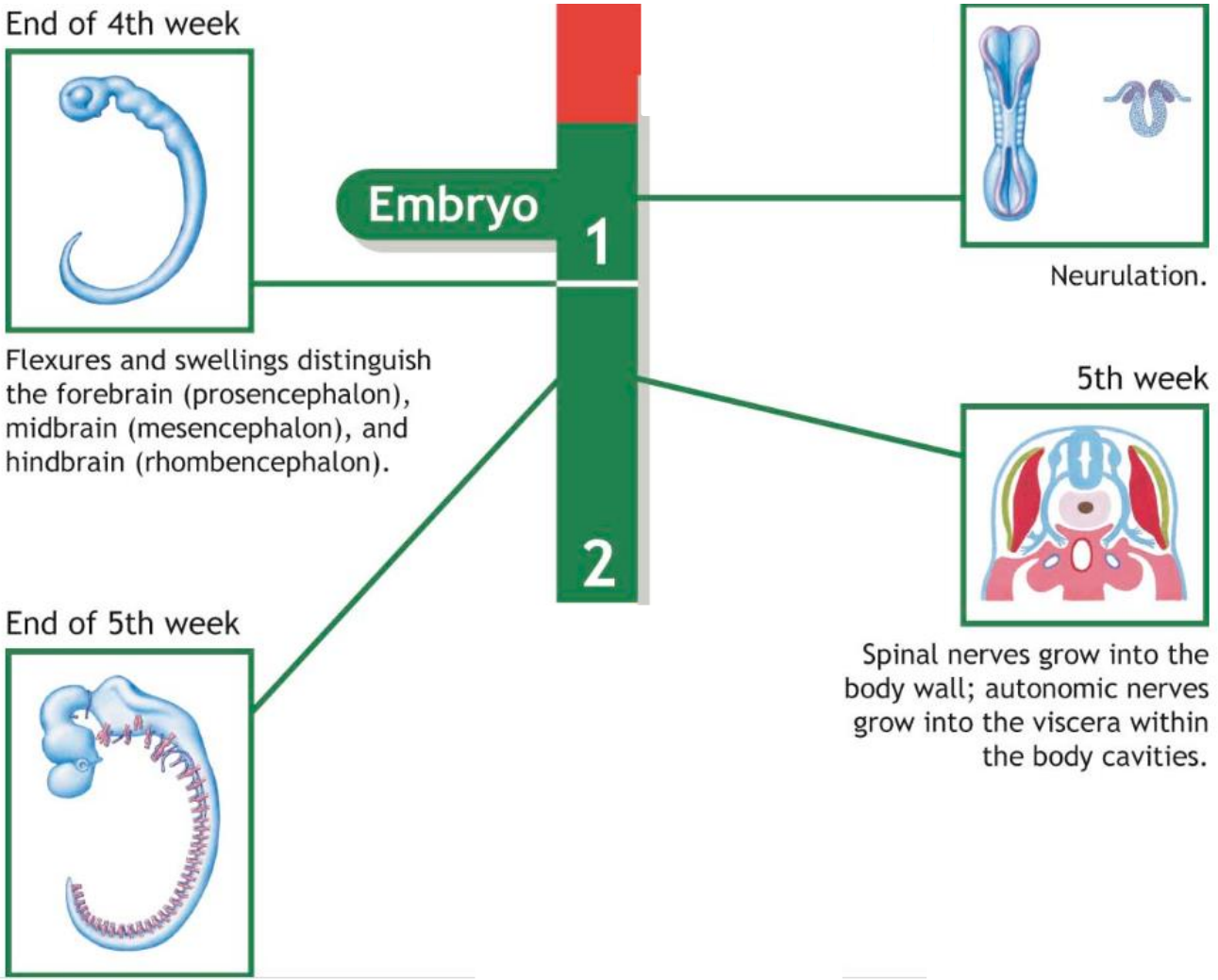
Patterning A-P si D-V

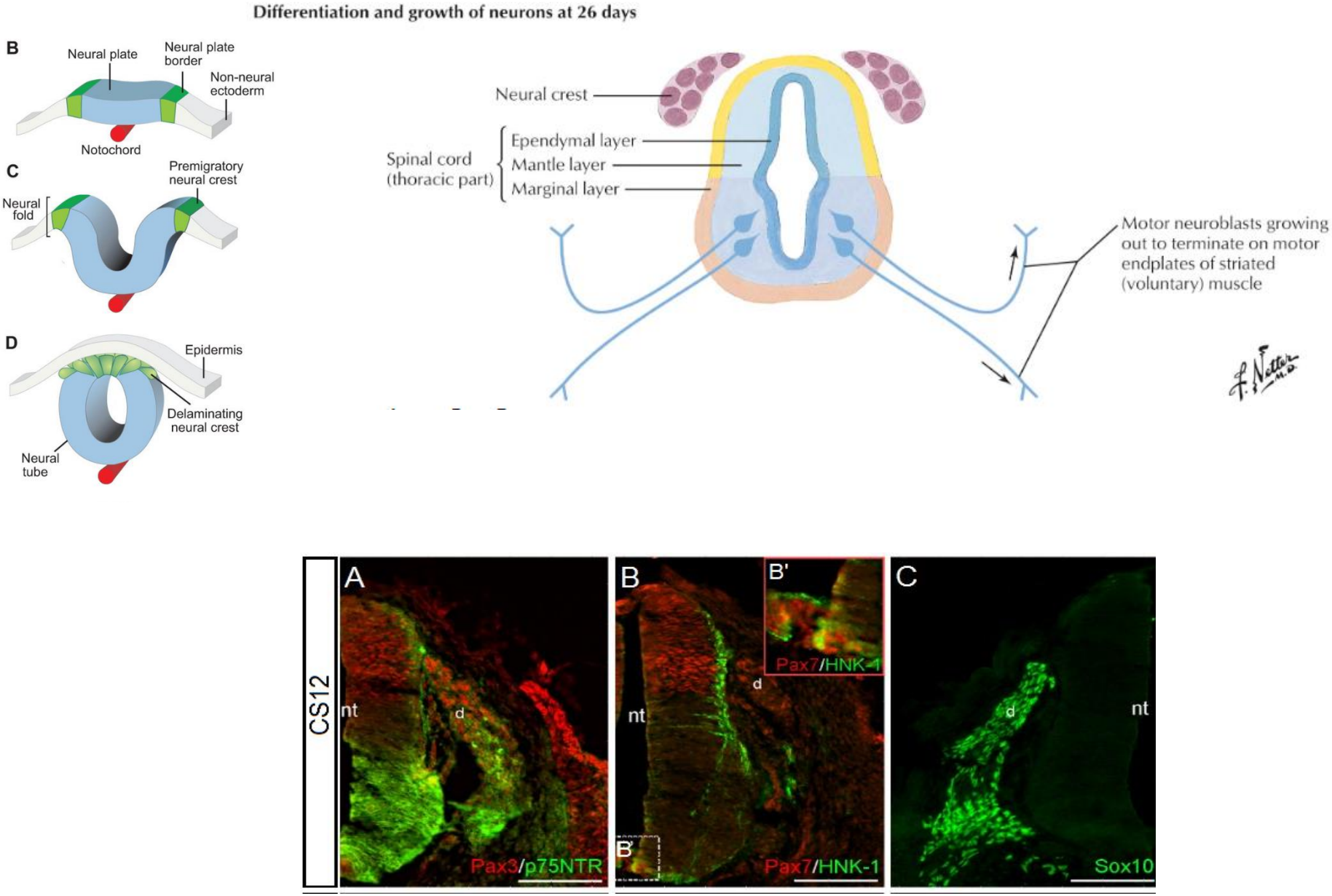


TRUNK Milestones for CNS and PNS neurogenesis

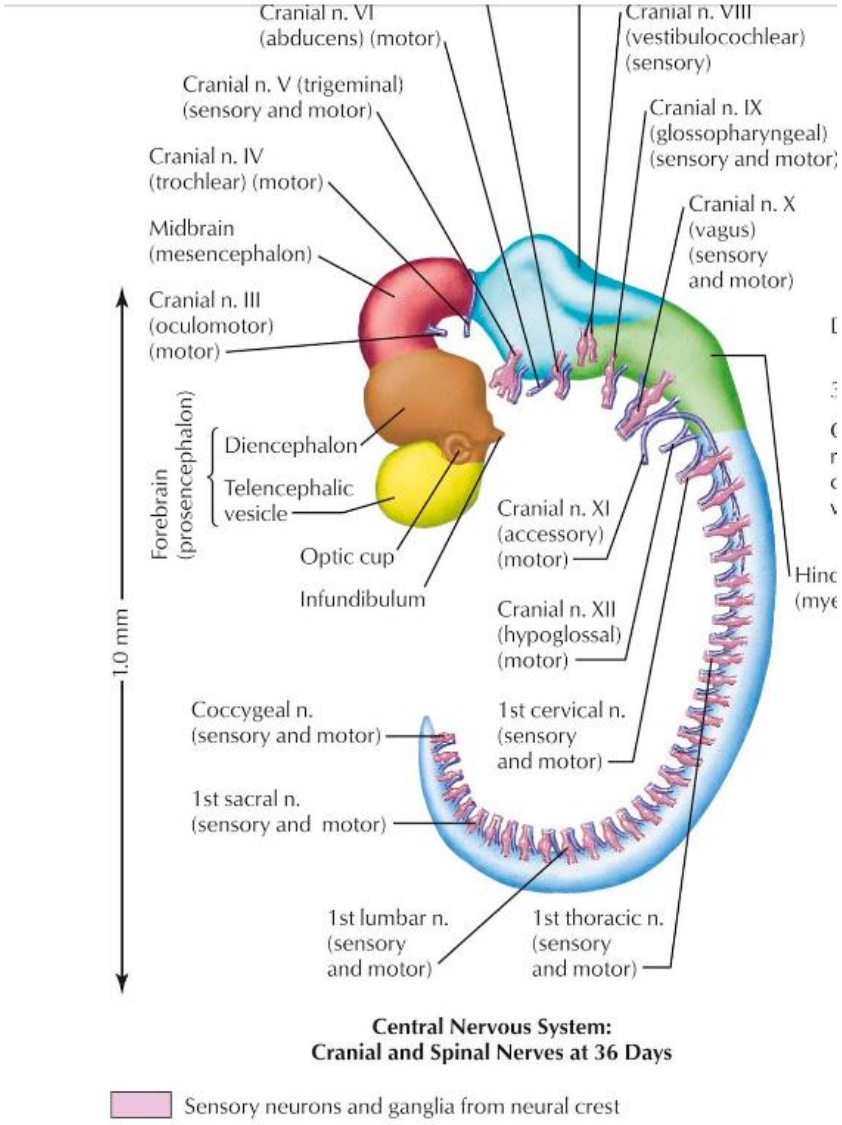


Saptamanile 4 si 5



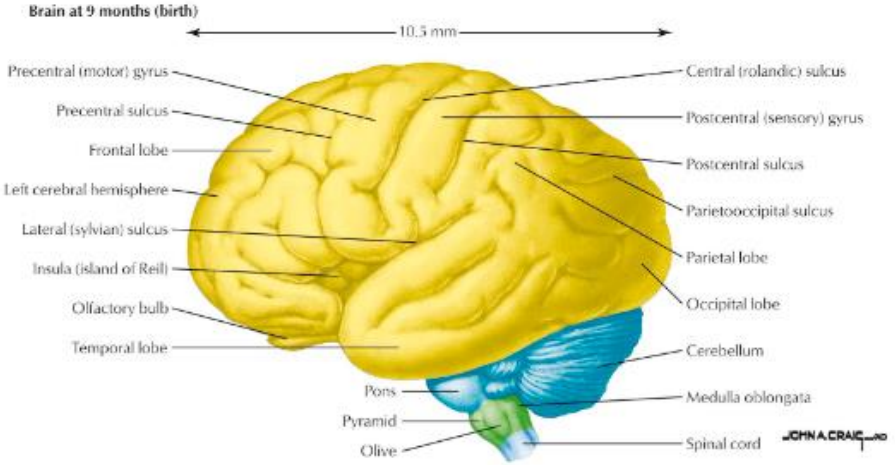


Betters et al 2012

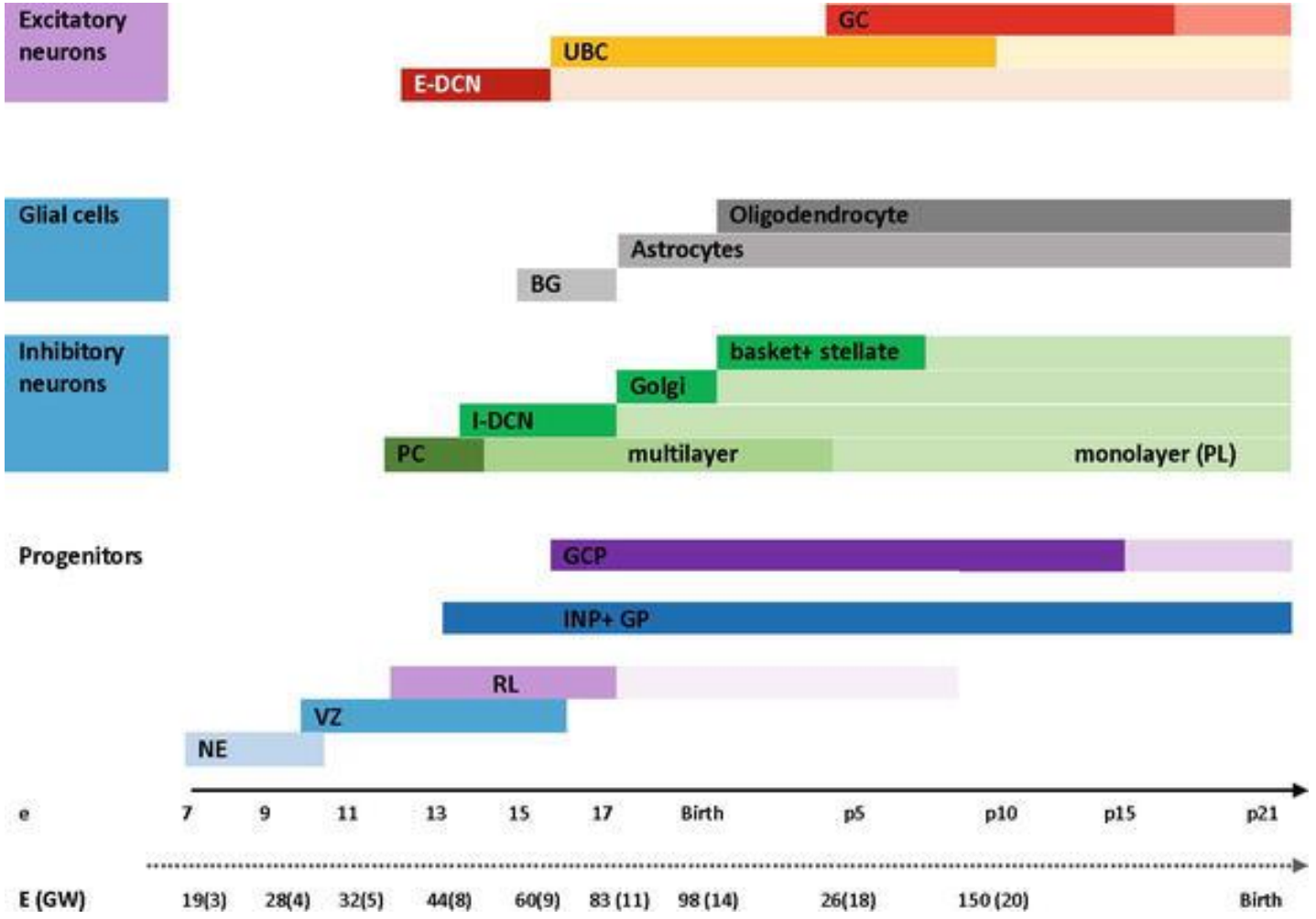


**Central Nervous System:
Cranial and Spinal Nerves at 36 Days**

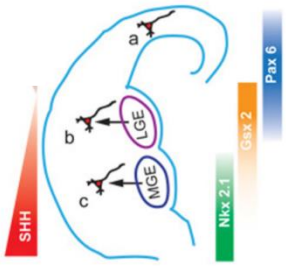
Sensory neurons and ganglia from neural crest

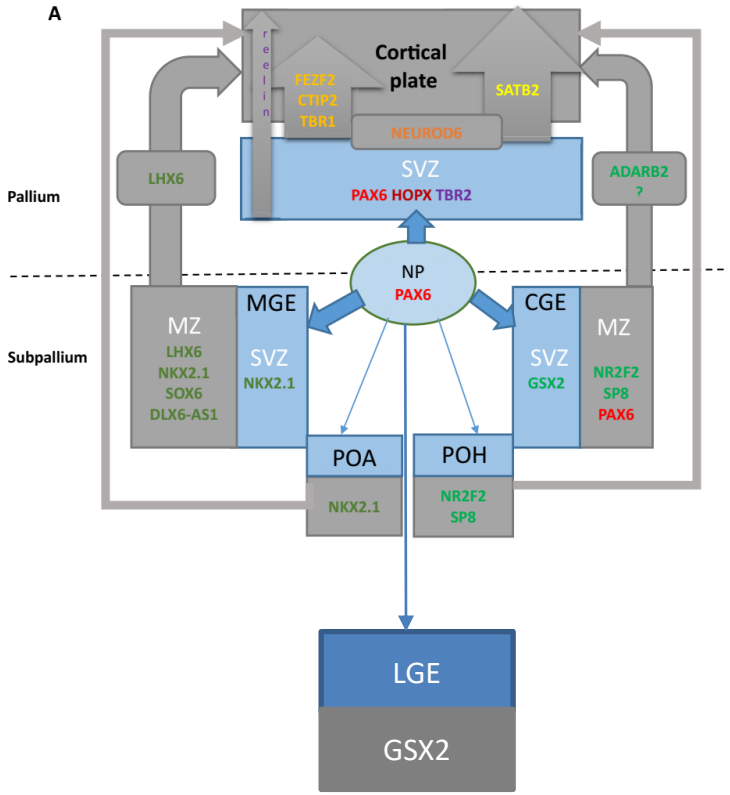


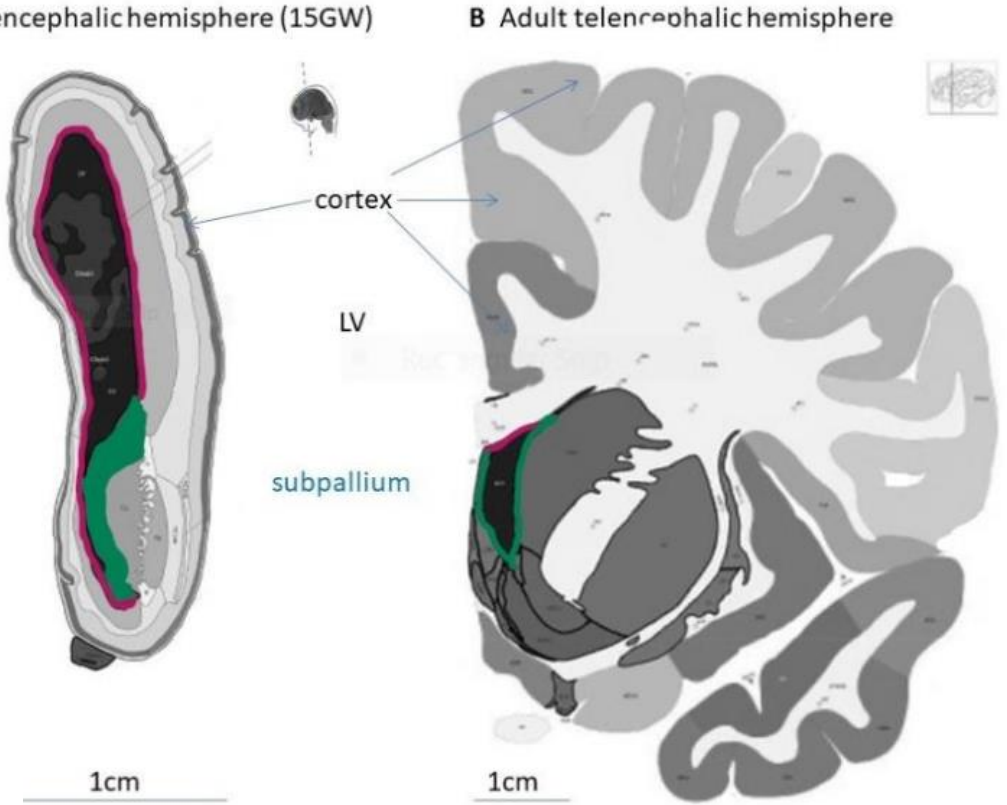
Sfarsitul saptamanii a 5a

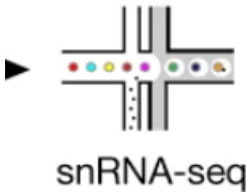


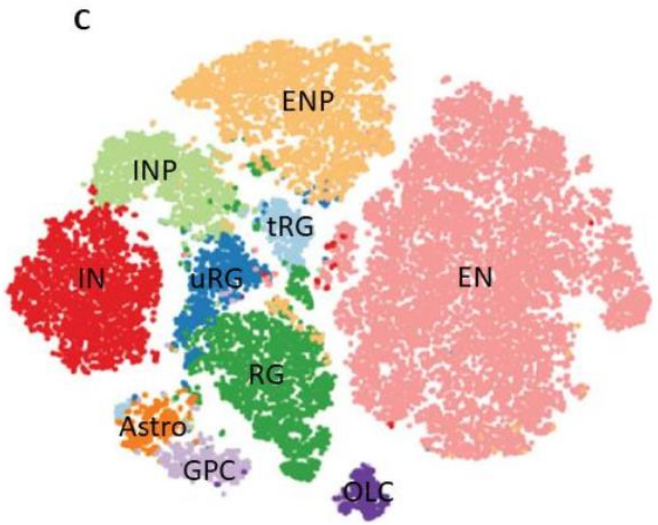
Dezvoltarea cerebelului-Etape



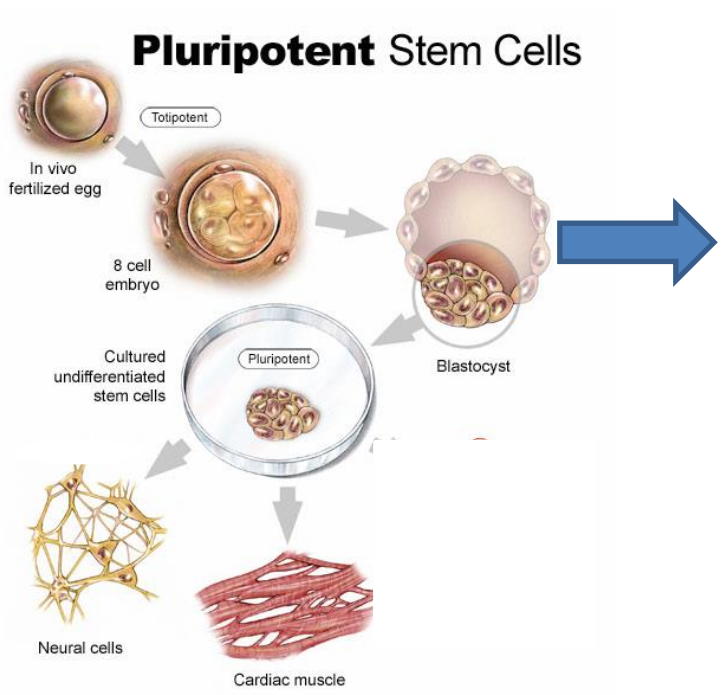


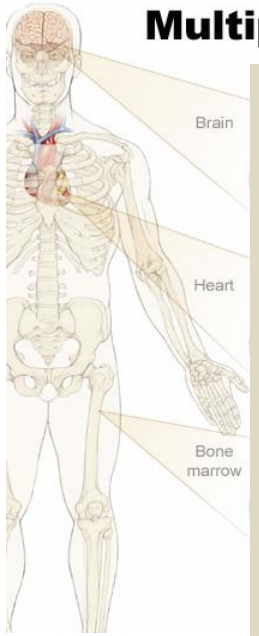






Dezvoltarea TELENCEFALALULUI





Neural stem cells

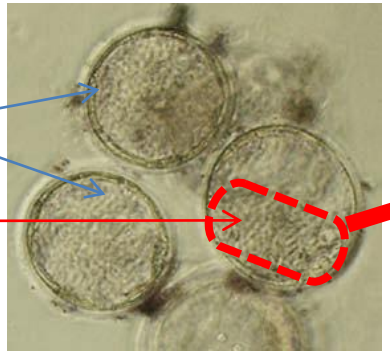
Heart stem cells

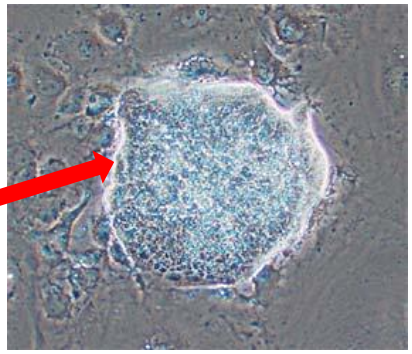
Hematopoietic stem cells

Intestinal stem cells

Epidermal stem cells

PNS stem cells/satellite cells



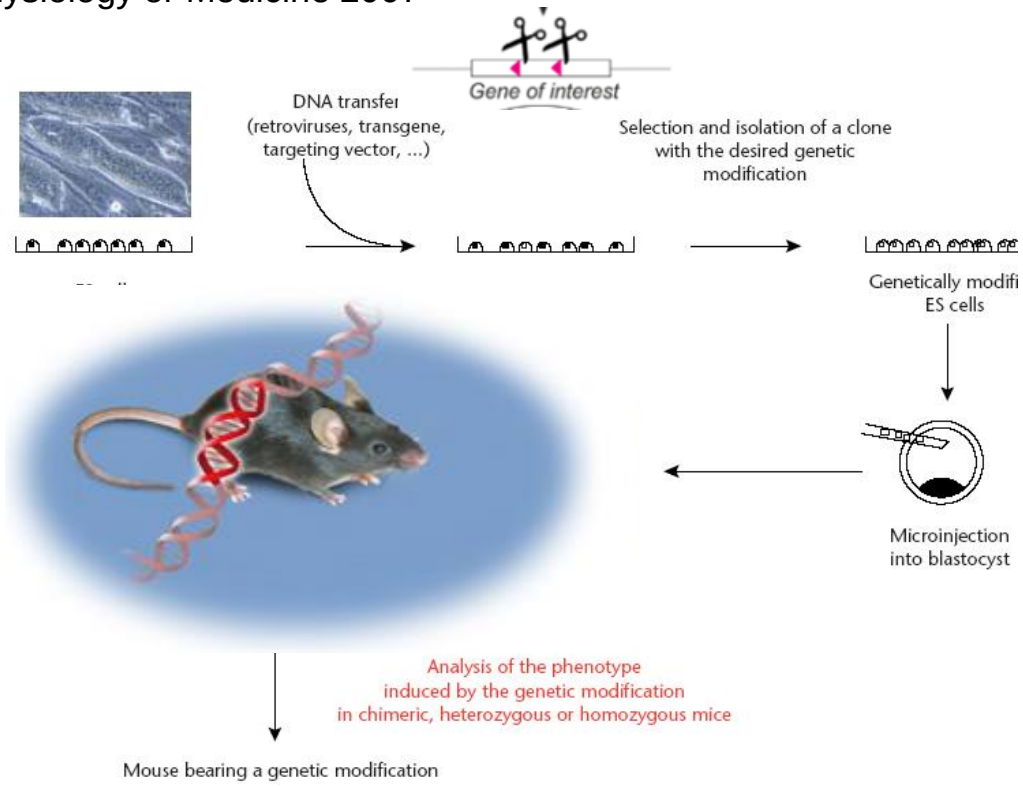


mESC colony

The Nobel Prize in Physiology or Medicine 2007

1981: Evans & Kaufman

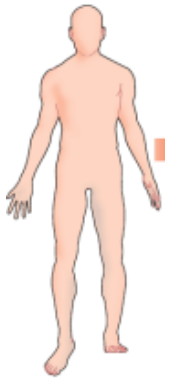
mESCs



Celulele stem embrionare

http://nobelprize.org

Celulele stem embrionare umane

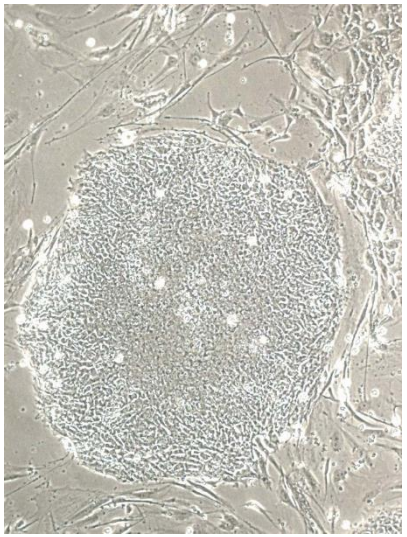


1998: First

Embryonic Stem Cell Lines Derived from Human Blastocysts

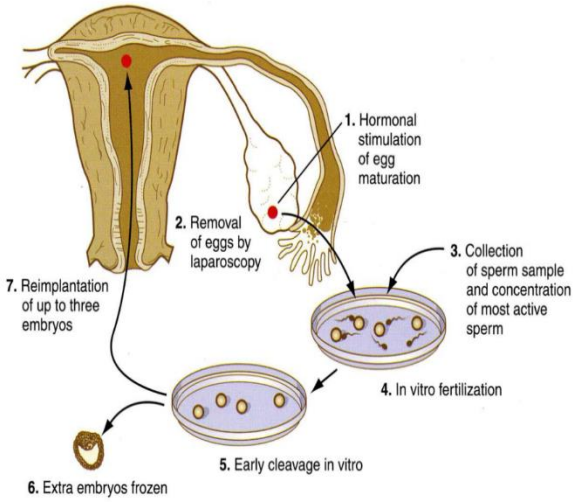
www.sciencemag.org SCIENCE VOL 282 6 NOVEMBER 1998

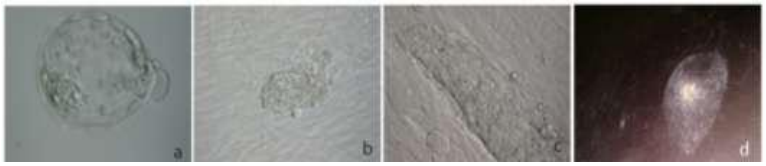
James A. Thomson & co-workers



In vitro fertilization

Steptoe & Edward 1998 (Nobel Prize 2010)



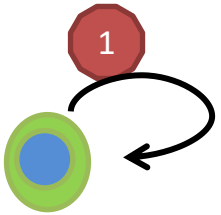


Derivation of hESC lines

>1000 hESC lines

Ström et al. 2007

Proliferare (self-renewal)



Conditii definite:

Substrat (laminin, vitronectin)

time

FGF2 + TGFβ/Activin ✓

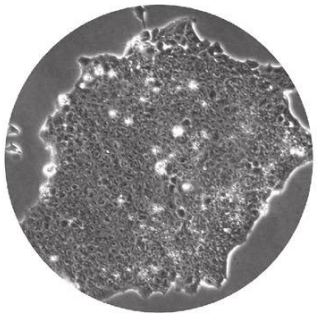
Cell population doubling time:

30-36h

2D

Reactoare ✓

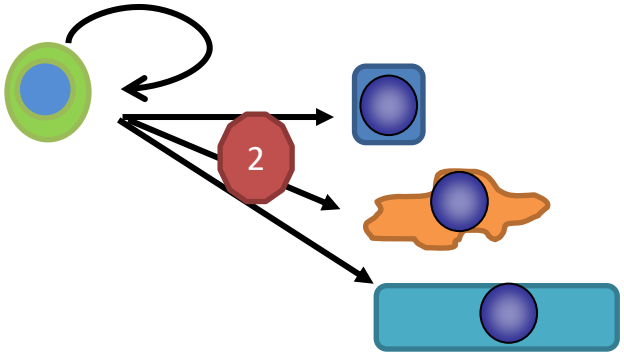
3D



Manipulare genetica

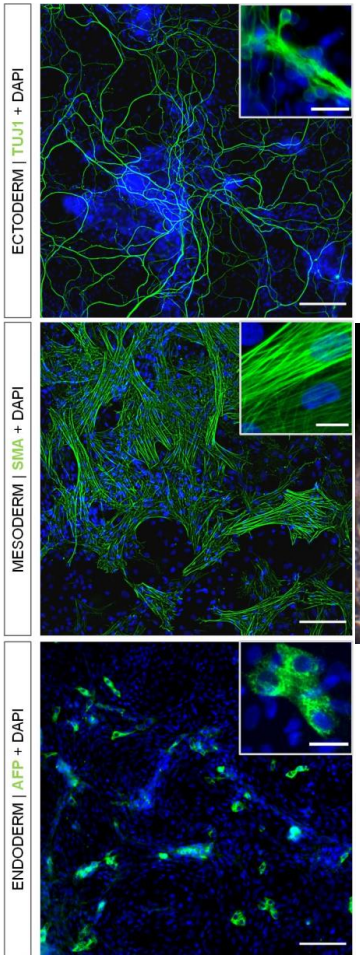
✓

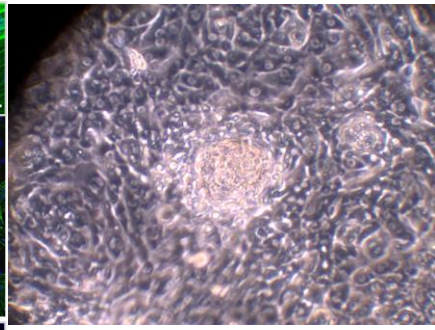
2. Diferentiere



In vitro:

- Spontana
- Directionata





“RETROSINTEZA”

NEURONILOR DIN SNP

time

Neural border induction

Induction, of neural crest cells (NCCs)

Migratory NCCs

Specification Neuronal differentiation

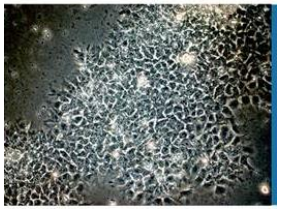
Peripheral NEURONS, Glial cells, Other derivatives

PSC

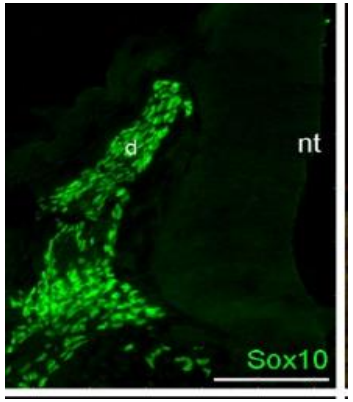
Stage 1

Stage 2

Stage 3



✓ **SOX10**

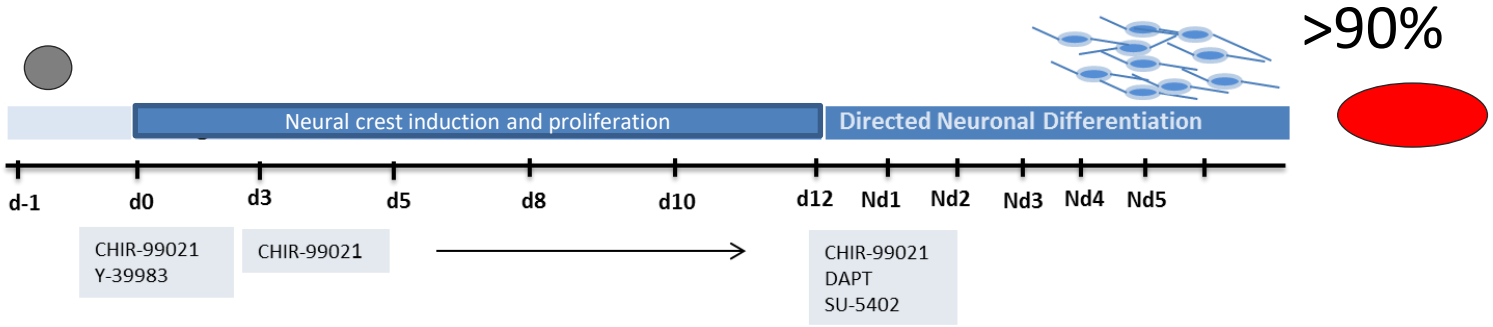


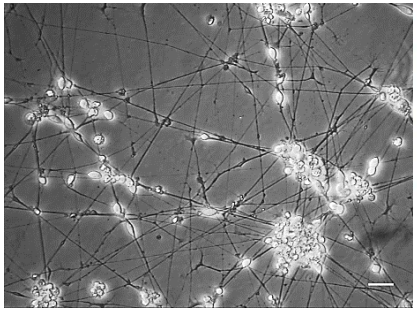
Eigentler et al *Stem Cell Dev* 2013

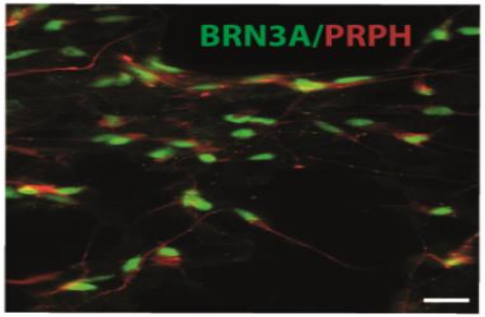
Anita Erharter

Lisa Knaus

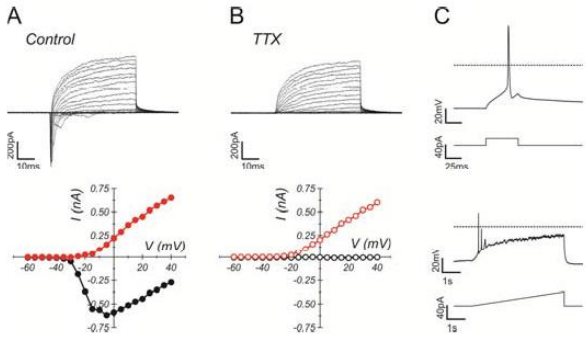
PERIPHERAL SENSORY NEURONS



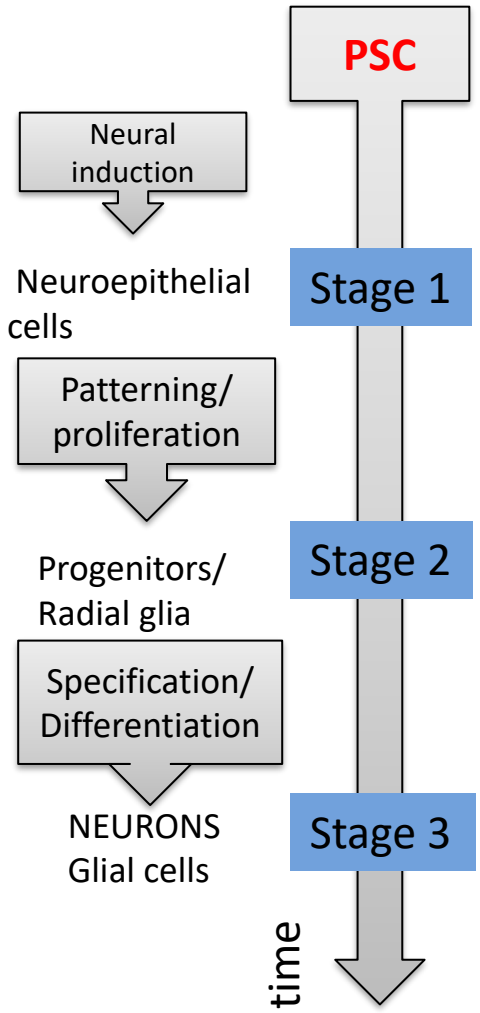


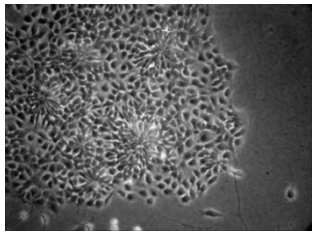


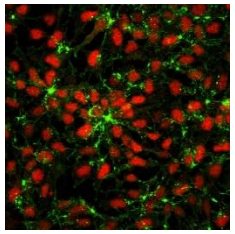
- ✓ express voltage-dependent ion channels
- ✓ generate action potentials
- ✓ TTX-sensitive Na+ channels

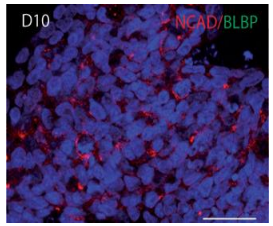


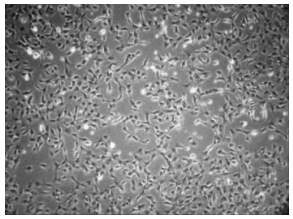
Modeling CNS neurogenesis

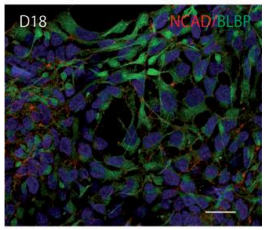






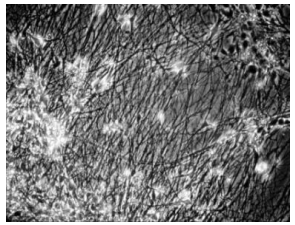


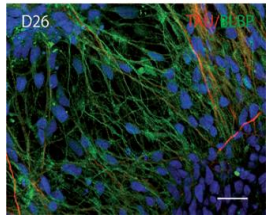




FOXG1,

NKX2.1 or EMX1 or OLIG2....





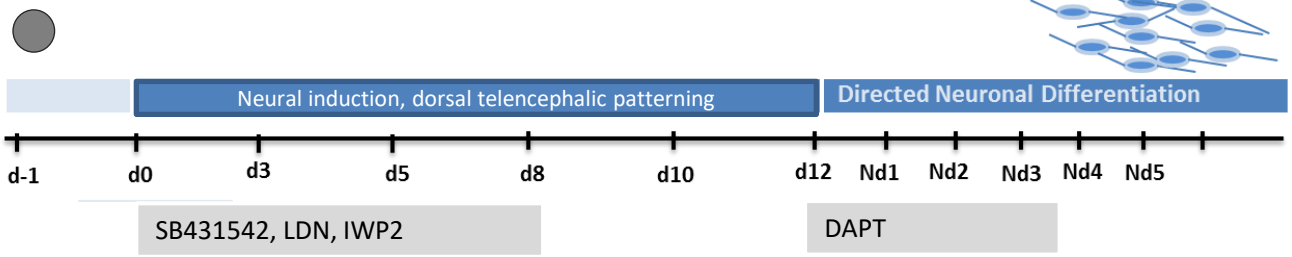
in vivo NEURONS *in vitro*

Nat et al *Glia* 2007

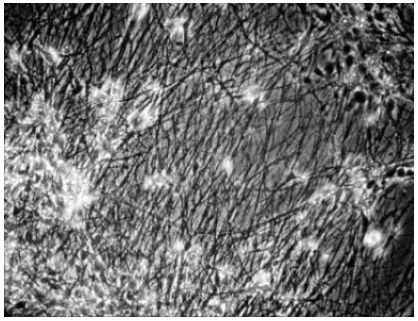
Nat et al *Stem Cell Dev* 2012

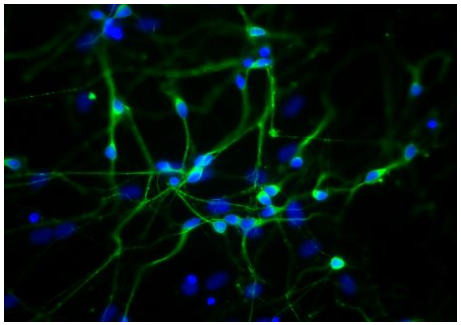
Carlo Bavassano

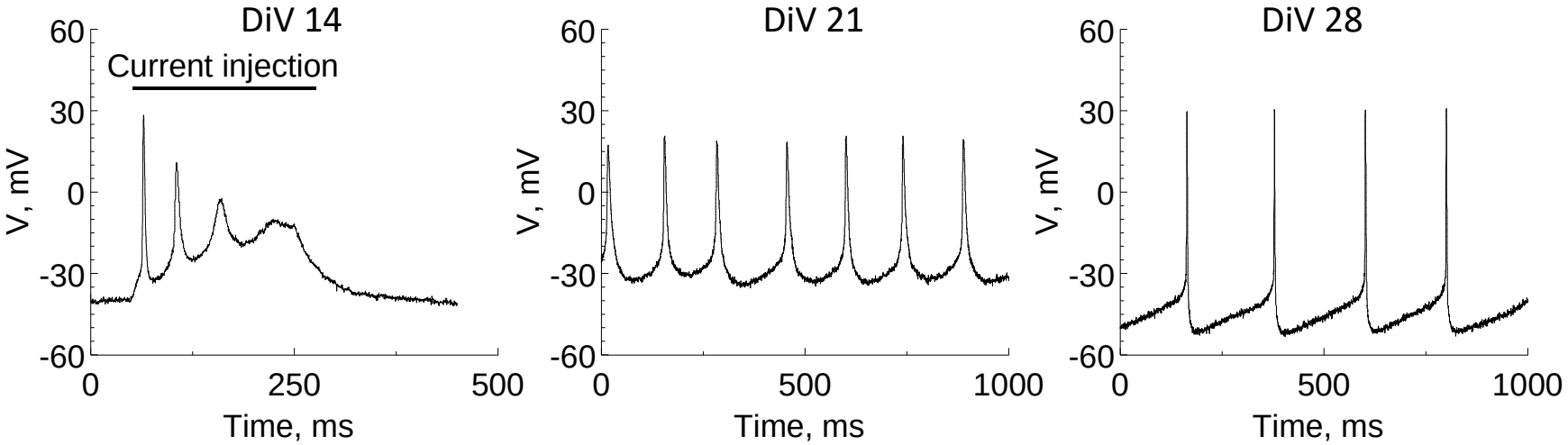
Dorsal telencephalic (cortical) neurons



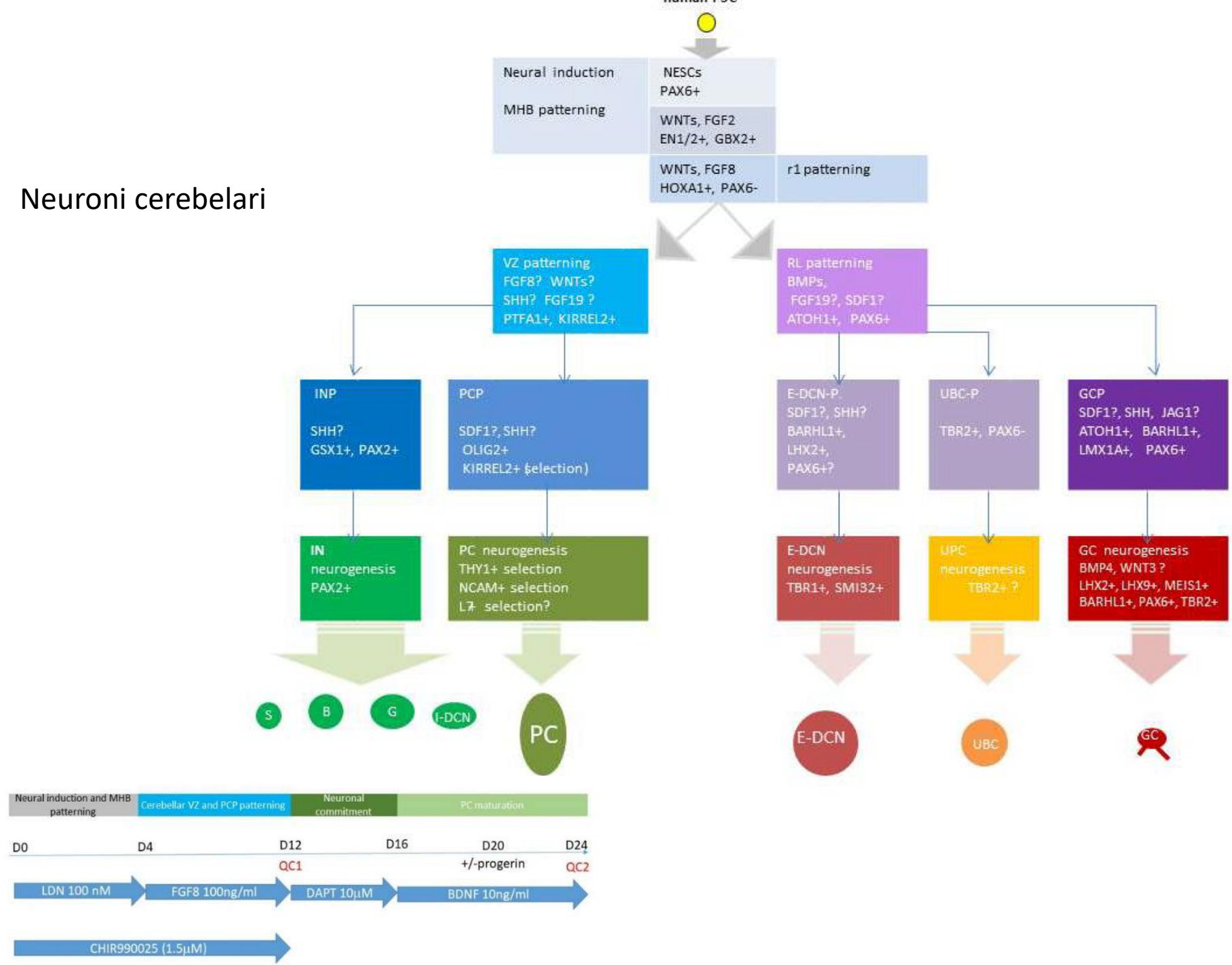
>90%

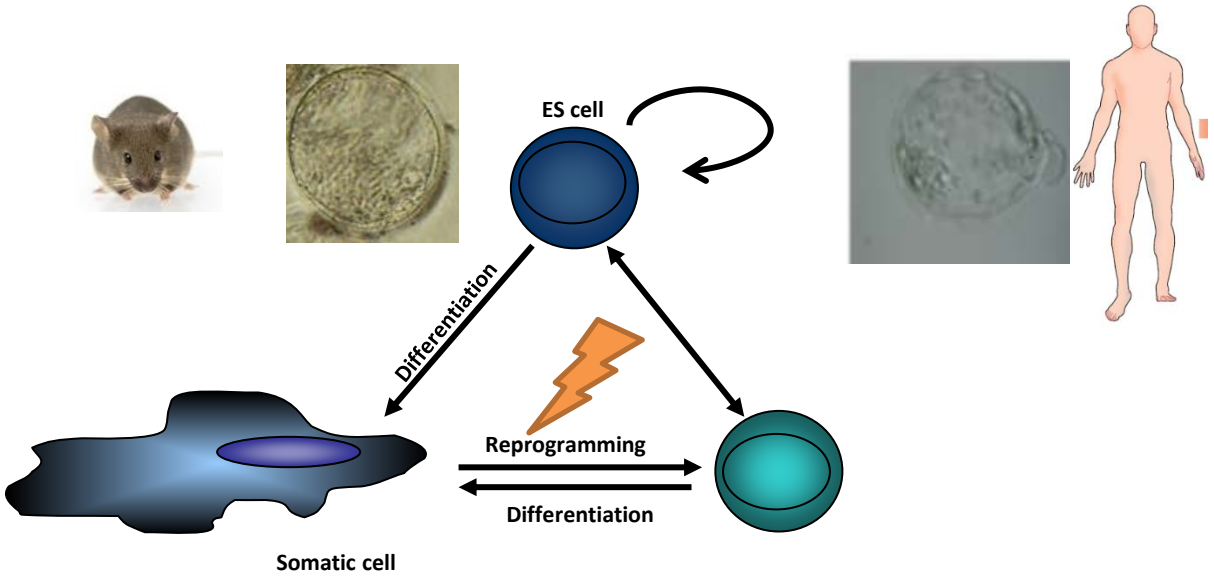






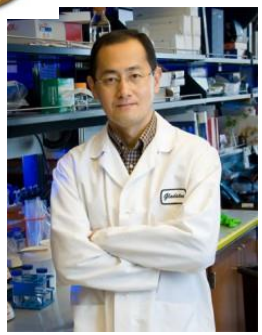
Neuroni cerebelari





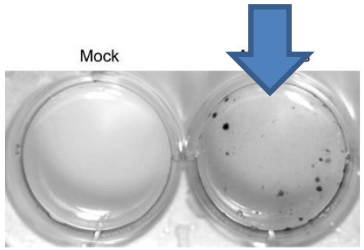
Reprogramare pentru pluripotenta?

Nobel Prize in Physiology or Medicine 2012
"For the discovery
that mature cells can be reprogrammed to become pluripotent"



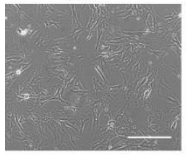
24 factori

Mock

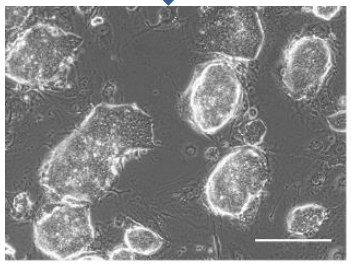


AP staining

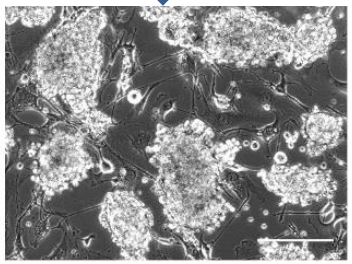
MEF



4 factori



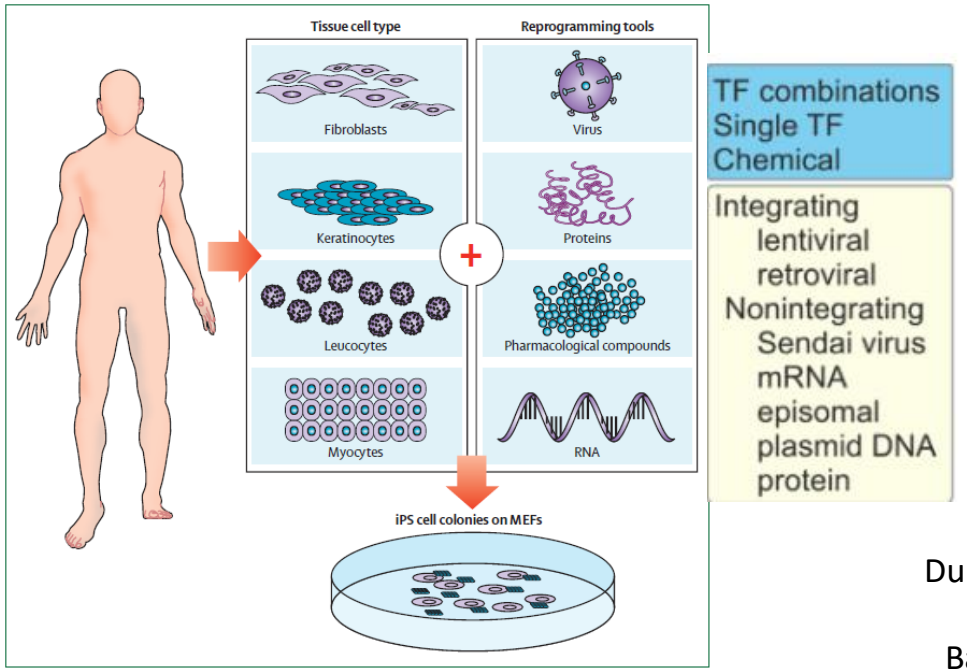
3 factori



Oct4- O
Sox2- S
Klf4- K
c-Myc- M

iPSCs

Takahashi & Yamanaka
Cell 2006

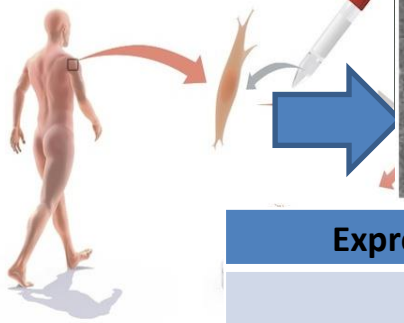


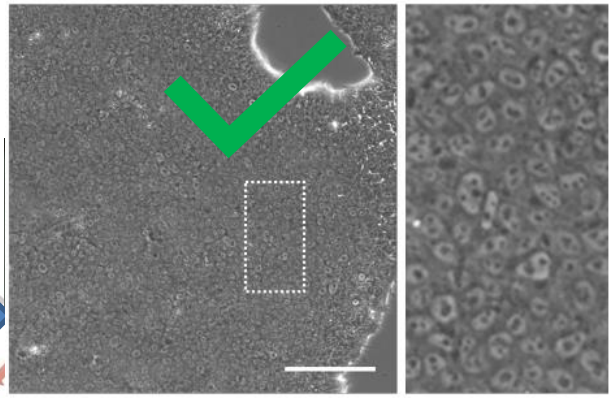
Eficienta ~ 0.01-4%

Mecanism epigenetic

Durata de generare si caracterizare: 3-6 luni

Banci de celule stem pluripotente induse





Gene of interest

linii iPS validate

Expression Profile

Genotype

Epigenetic profile

snRNA-seq

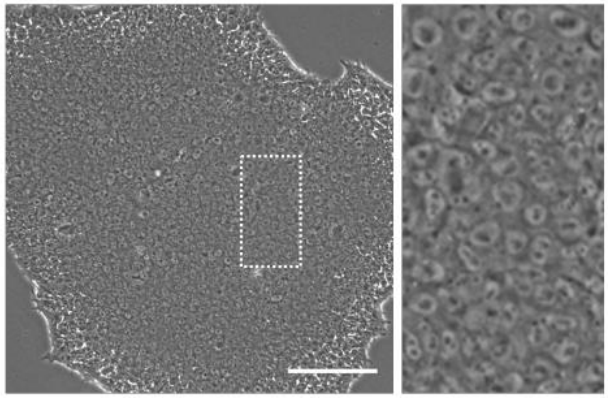
scATAC-seq

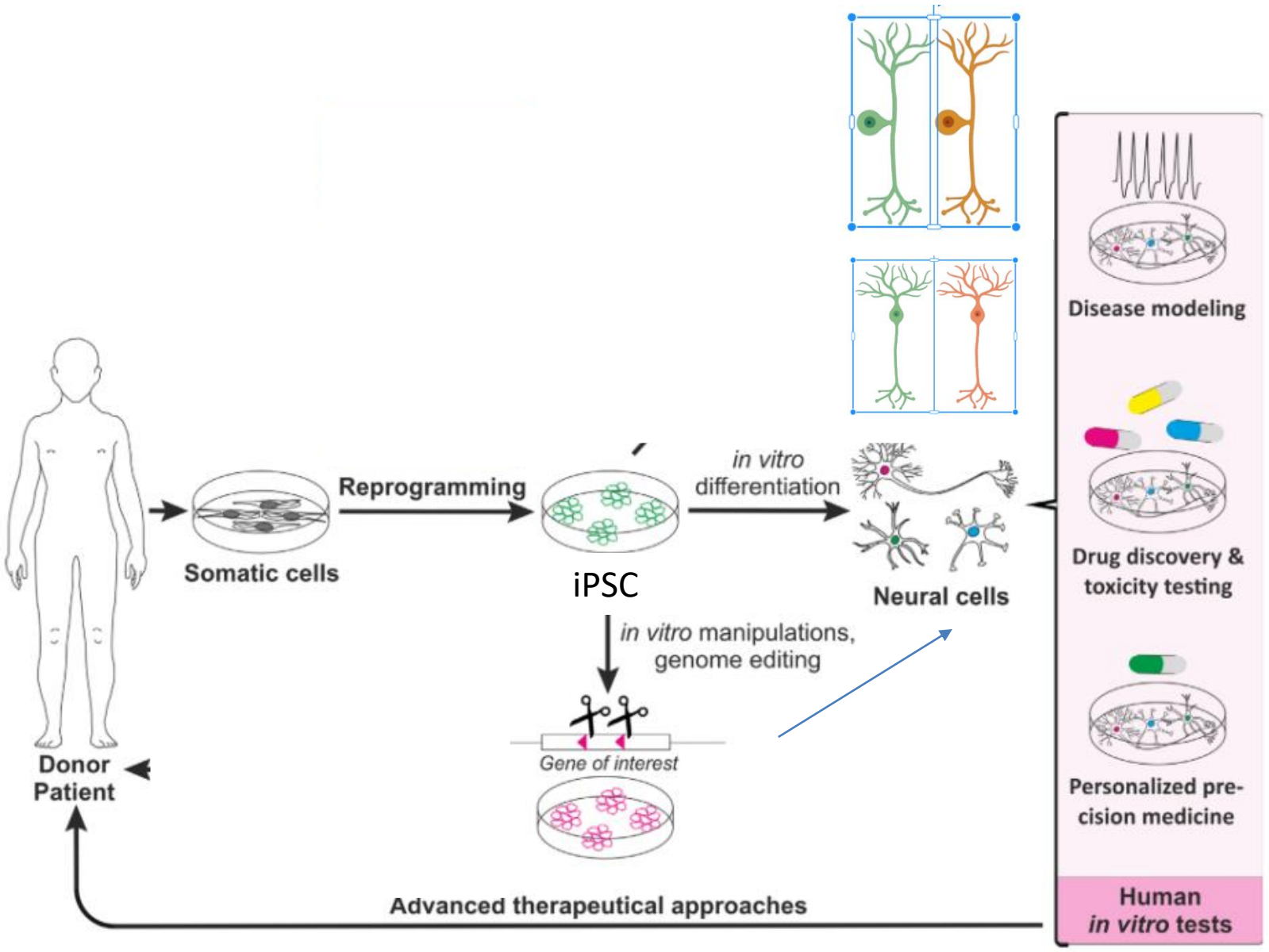
multiomics

Diferentiere directionata

Diferente genetice intre liniile celulare

Linii hESC, „gold standard“




Donor Patient
Somatic cells
Reprogramming
iPSC
in vitro differentiation
Neural cells
in vitro manipulations, genome editing
Gene of interest
Advanced therapeutical approaches
Disease modeling
Drug discovery & toxicity testing
Personalized pre-cision medicine
Human in vitro tests

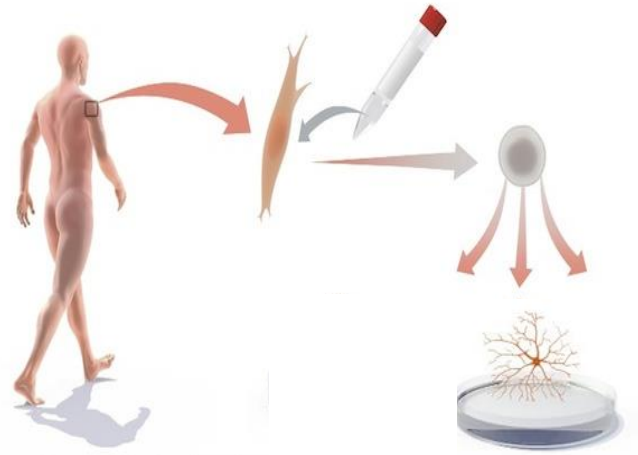
Modelarea bolilor???

In vivo

+multe gene/mecanisme conservate
+/- durata de viata
- background genetic

In vitro

+MUTATII UMANE



+Neuroni, cardiomiocite…..

Investigarea mecanismelor patologice (initiere si progresie) in celule umane relevante.

Boli Neurodegenerative :

Complexitate ridicata!

MECANISM NEELUCIDAT

FARA TRATAMENT SPECIFIC!

Polinucleotide in Tandem!!!

Boala Huntington (HD)

Exonic (CAG)n

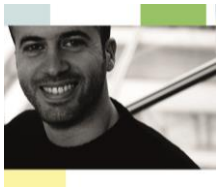
PolyQ

ATAXII MONOGENICE

Ataxia Spinocerebelara tip 6 (SCA6), **ataxia Friedreich (FRDA)**…

(*CACNA1A*) (*FXN*)

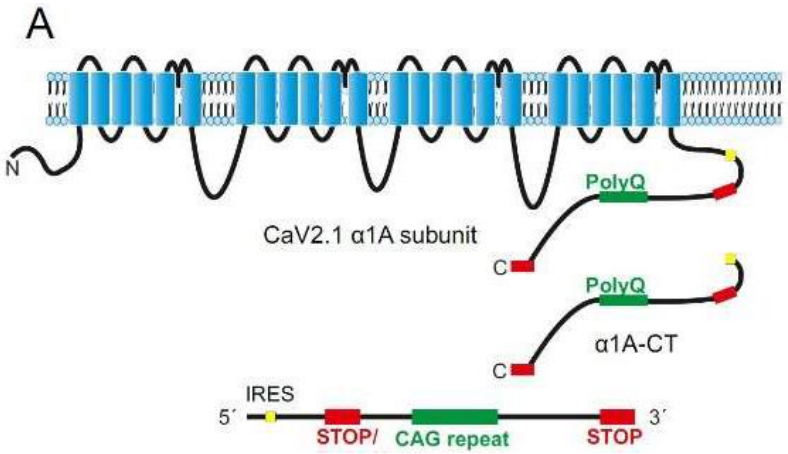
Exonic (CAG)n Intronic (GAA)n



Bavassano et al *Stem Cell Dev* 2017

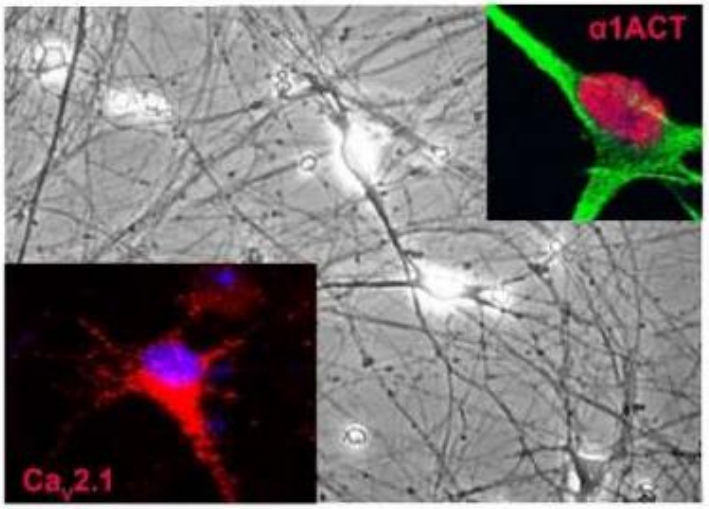
Eigentler et al *Stem Cell Dev* 2013

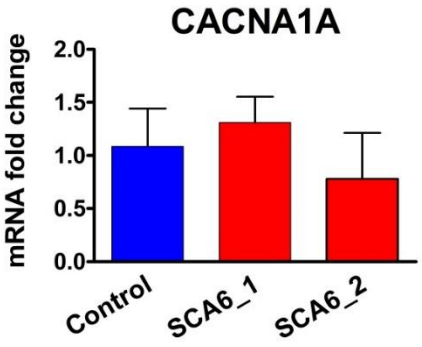
Spinocerebellar Ataxia type 6 (SCA6) model

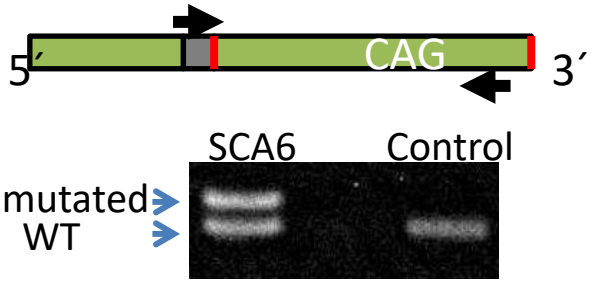


Exon 47

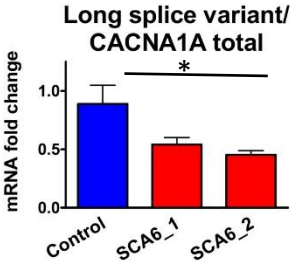
PolyQ sequence range:
8–14 normal (13, 14)
>20 pathological (23, 23)

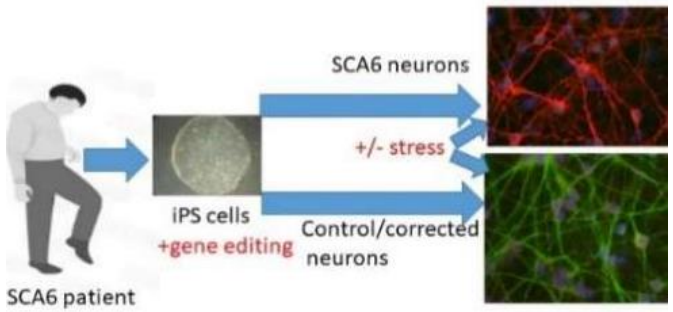


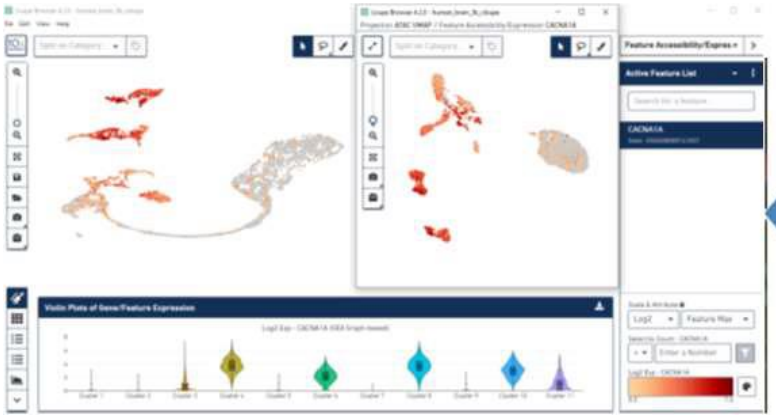




Long splice variant is expressed in neurons





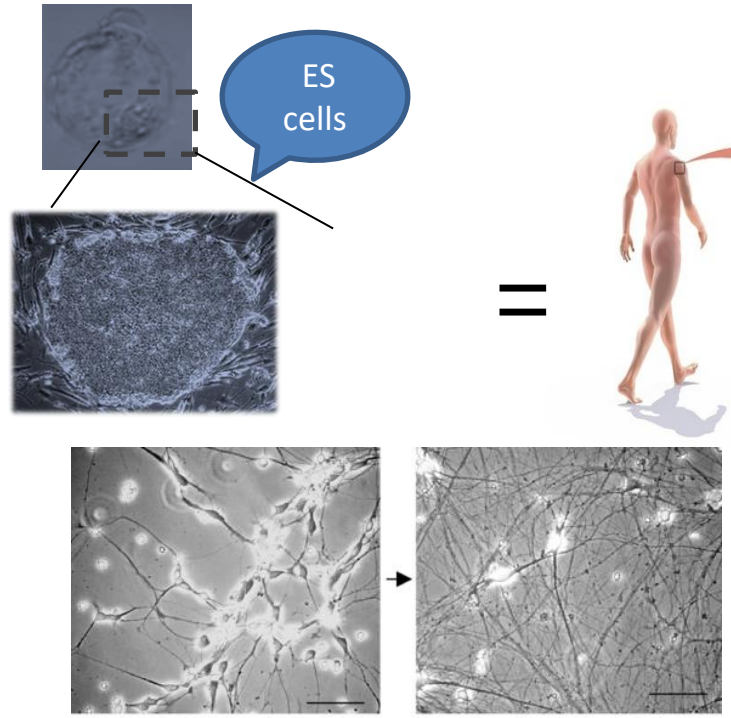


Searching for disease phenotypes/mechanisms

Testing for correction/treatment approaches

snRNA-seq

Concluzii

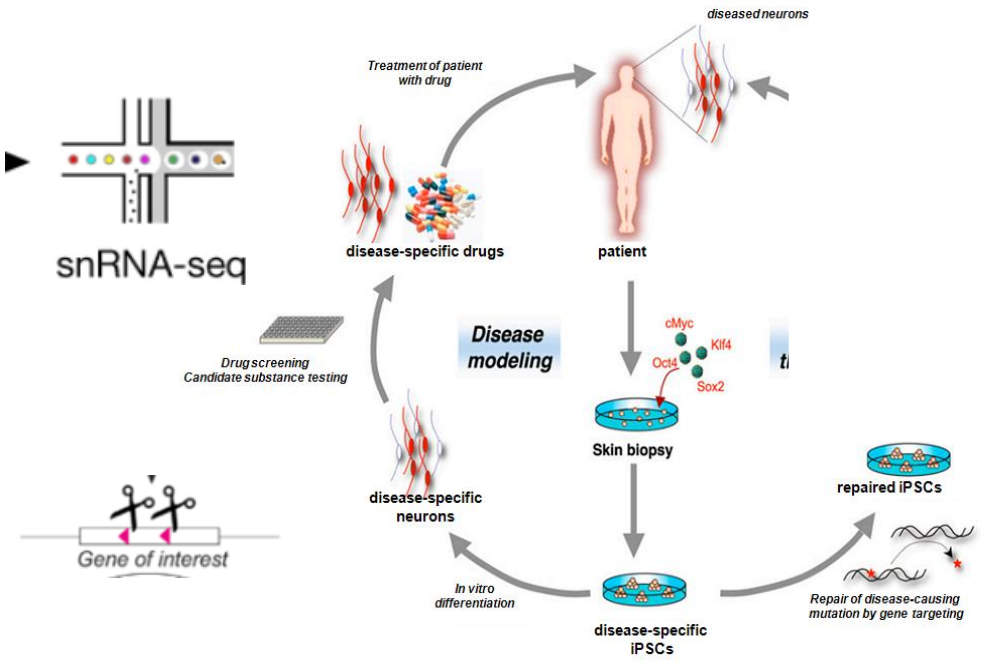


- No ethical concerns
- Patient-specific

Technology: routine, no safetly concern for non-integrative methods

Multiomic approaches for characterization

Progress in directed differentiation, efficiency for some classes of neurons



control,
healthy neurons

control,
healthy iPSCs

- differentiation into the most affected cell types:
- impact of the genetic background reduced by using isogenic lines as control

✓ Forced ageing, induced stress

Search for early phenotype and disease progression!

Disease „in a dish”, especially for monogenic diseases

Isogenic lines and multiomics

Drug/toxicity/treatments/corrections testing on human/patient neurons