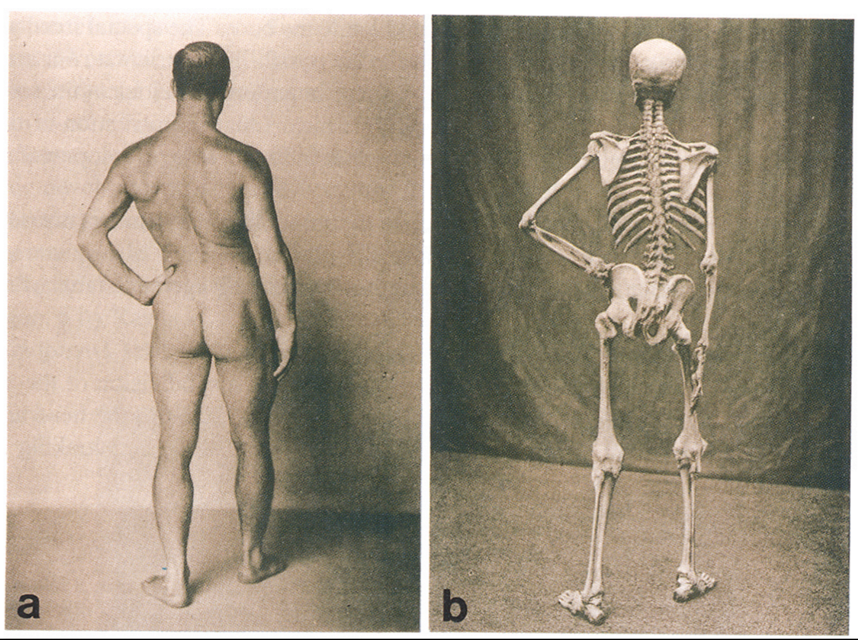
Developmental concepts

•Specification: Squishy and zen-like idea that a cell has an identity. Assay cell-identity in many ways (morphology, molecules, movement, etc.).

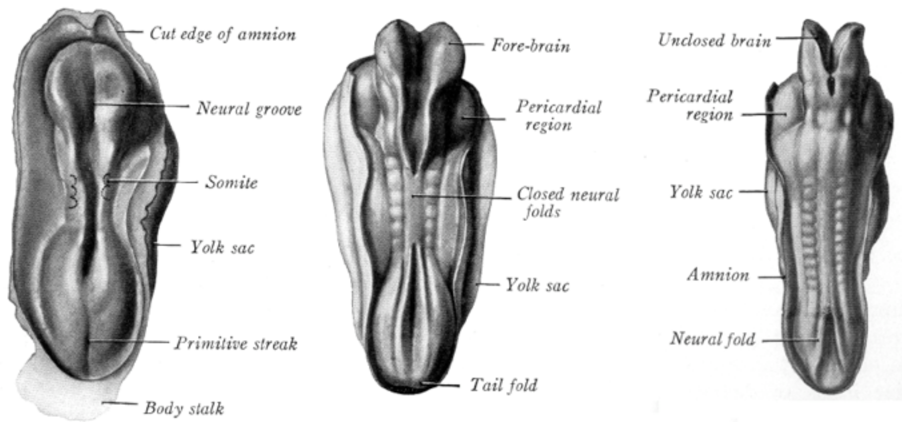
•Commitment (=determination): A cell's identity cannot be changed by a change in the environment. Commitment is an OPERATIONAL definition (when I challenged the cell in THIS way, its fate did not change).

•Differentiation: Expression of structure and functional properties (myosin, AChR, etc)

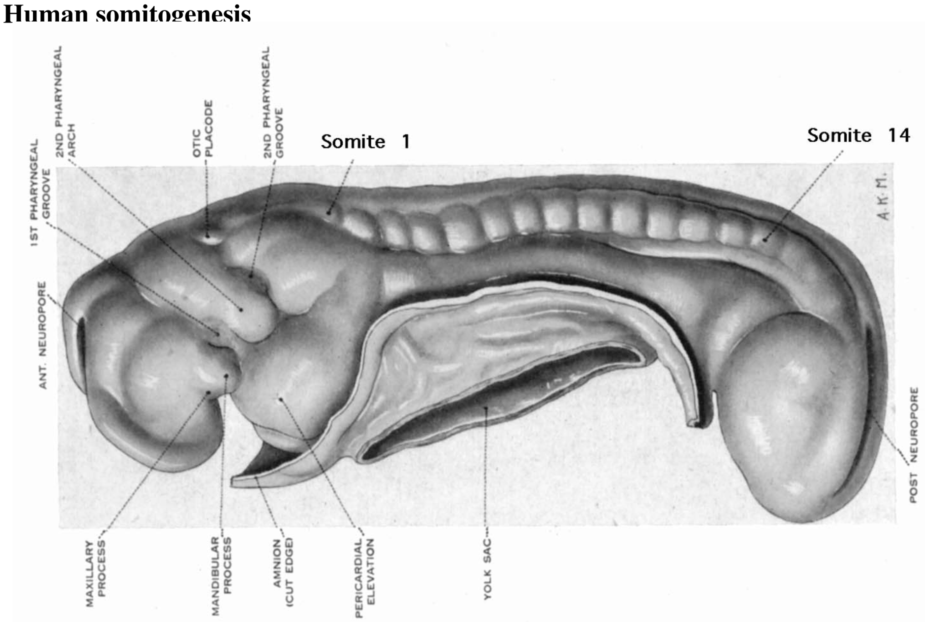
Vertebrate segmentation

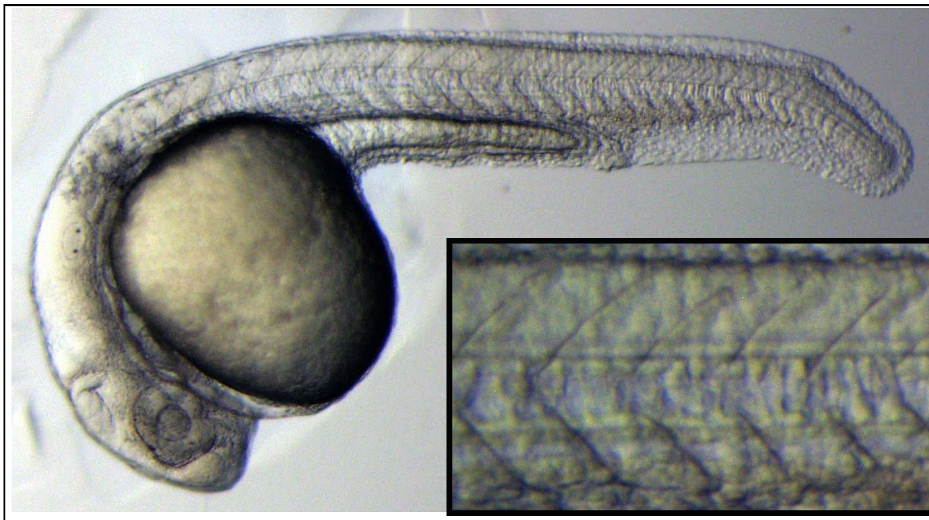


Somitogenesis

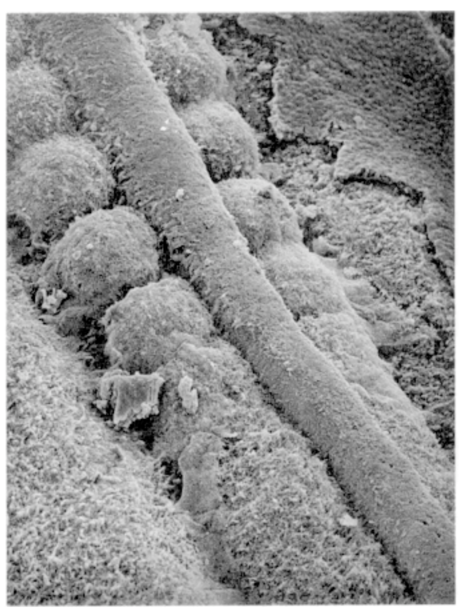


Human somitogenesis

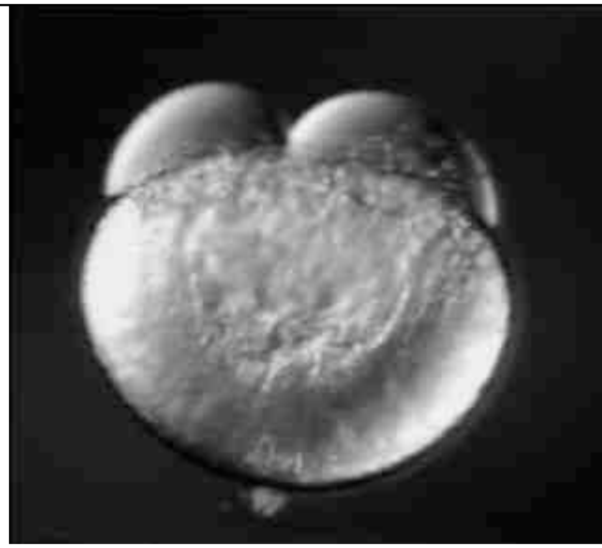


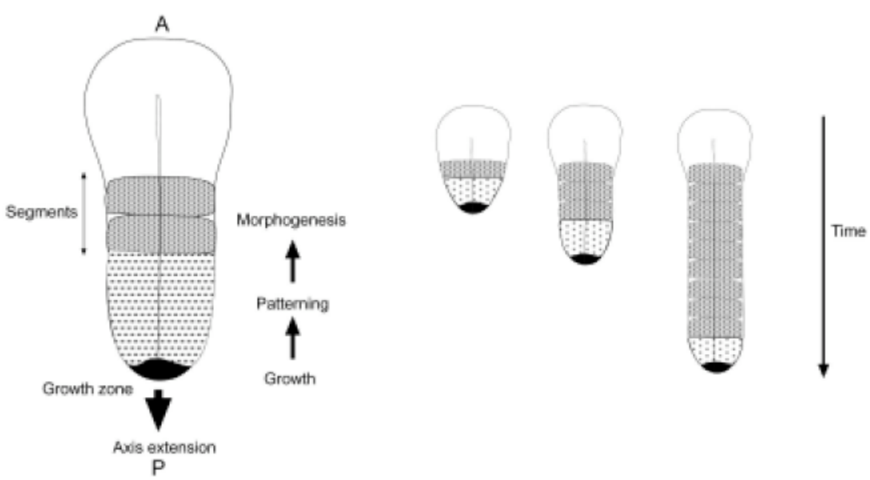


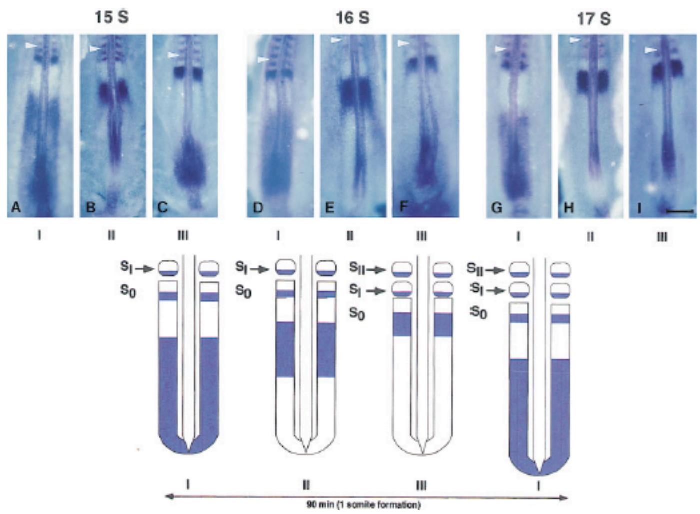
somitogenesis

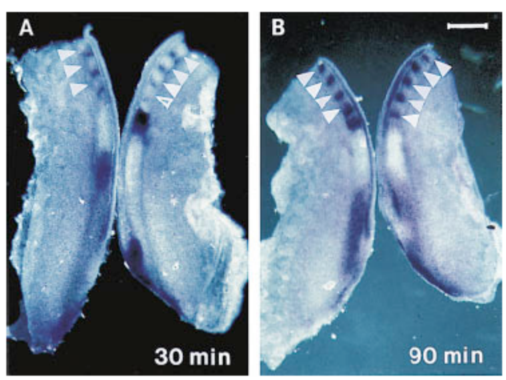


Flipbook



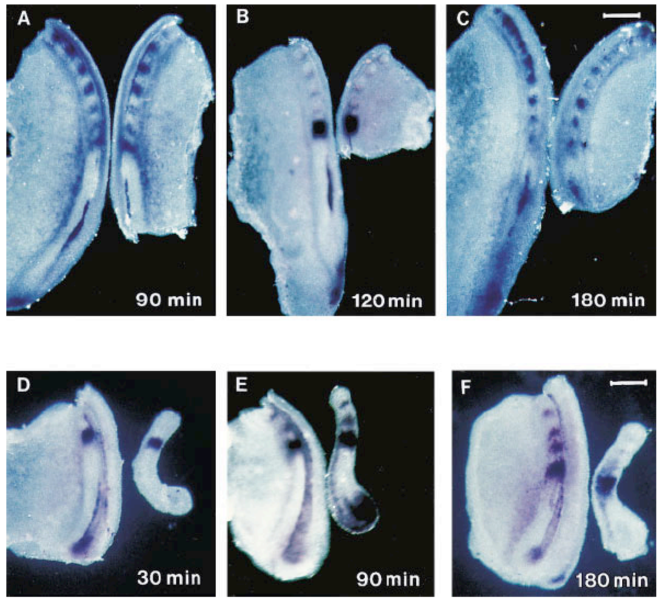

A
Segments
Morphogenesis
Patterning
Growth
Growth zone
Axis extension
P
Time

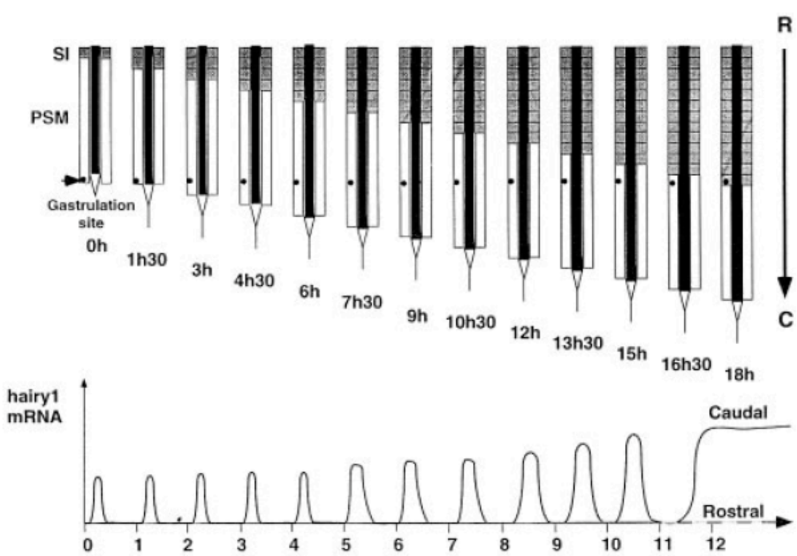




Palmeirim, et al., 1997

Autonomous to pre-somitic mesoderm

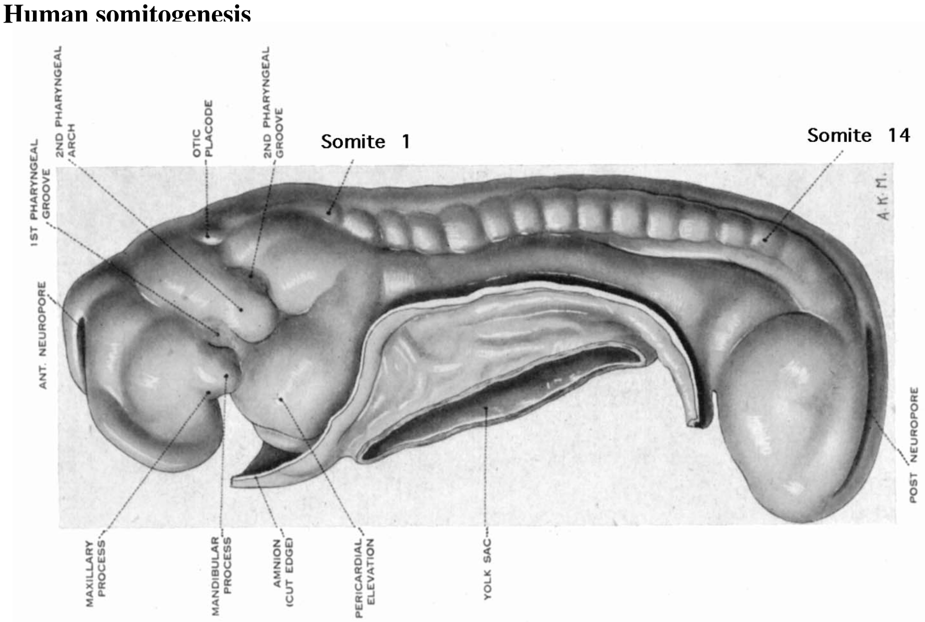




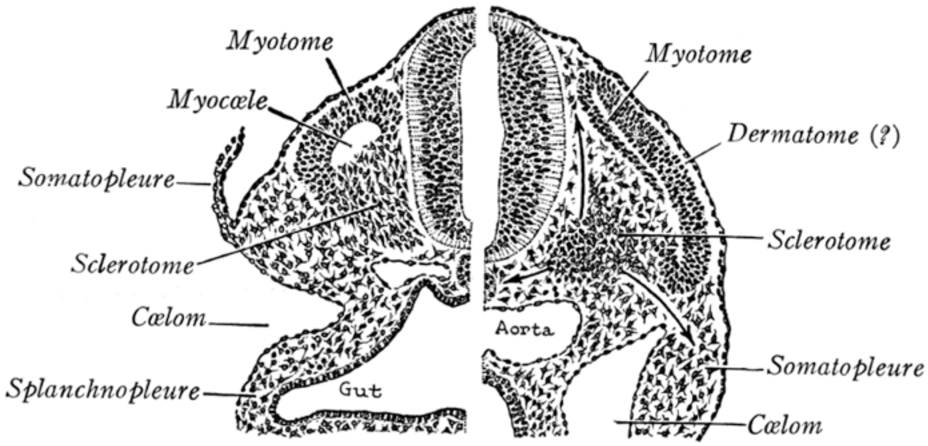
hairy1 in blue, central nervous system in grey; head to the right

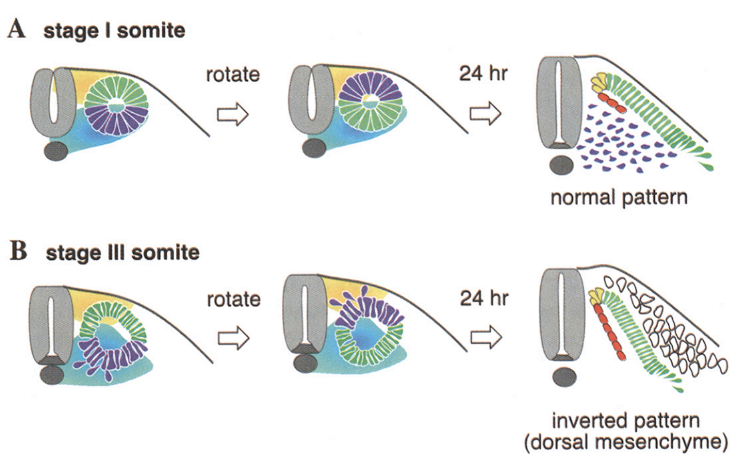
click to run

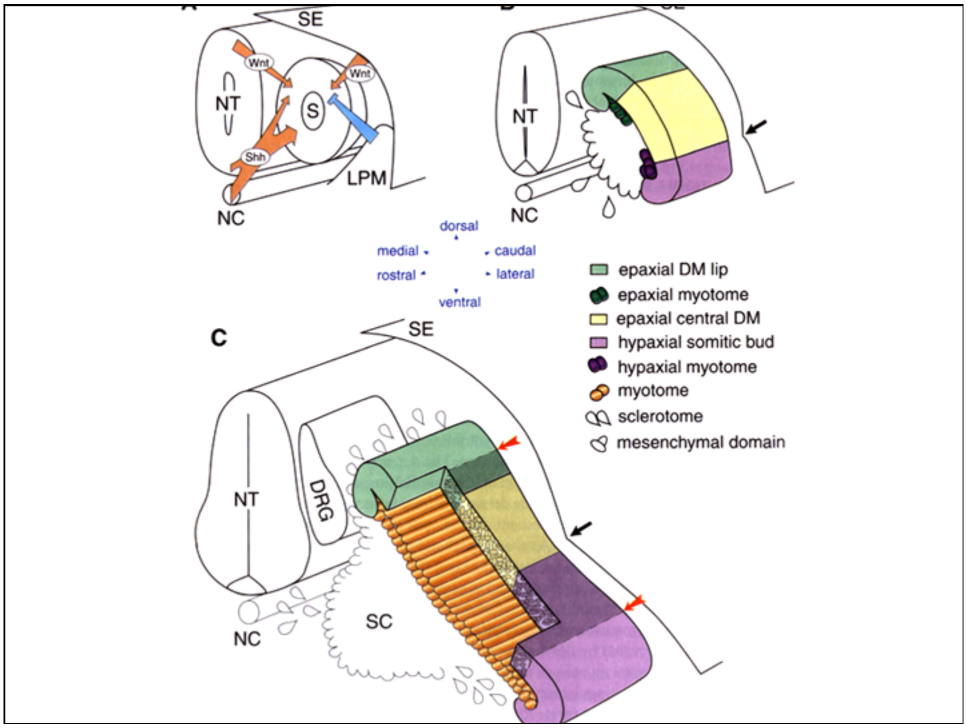
Human somitogenesis

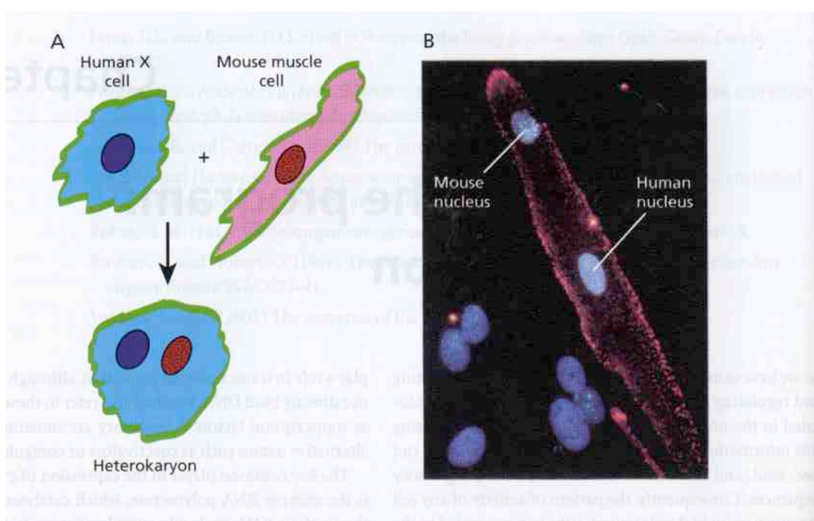


Somitic anatomy, maturation

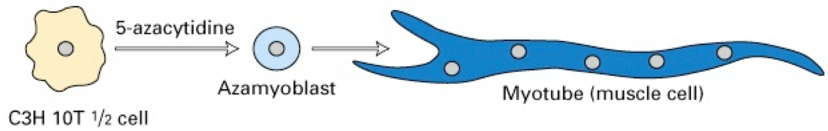





A
B
C
SE
NT
S
Wnt
Wnt
Shh
LPM
NC
DRG
SC
dorsal
ventral
medial
rostral
caudal
lateral
epaxial DM lip
epaxial myotome
epaxial central DM
hypaxial somitic bud
hypaxial myotome
myotome
sclerotome
mesenchymal domain

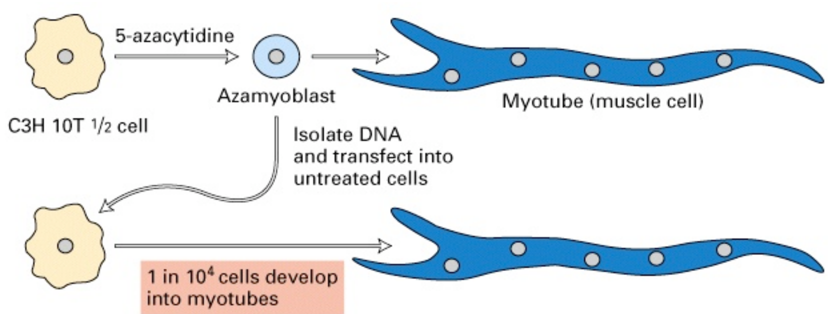

A
Human X cell
Mouse muscle cell
+
Heterokaryon
B
Mouse nucleus
Human nucleus

Experimental system

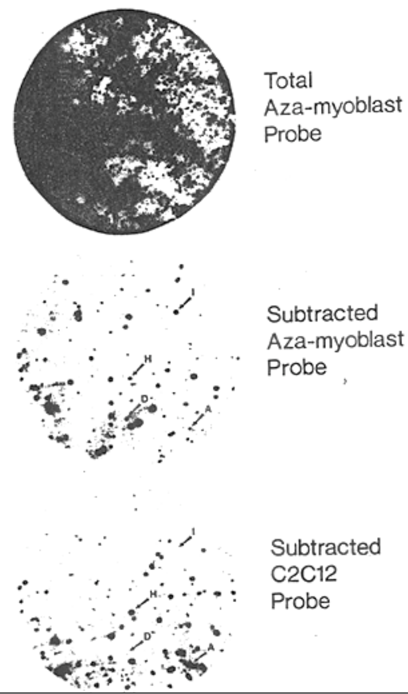


10T 1/2 fibroblasts differentiate into muscle cells when treated with 5-azacytidine

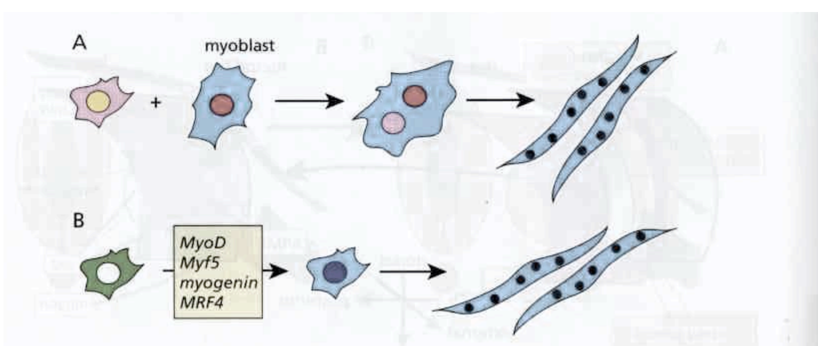
DNA induces differentiation



- DNA from treated cells causes differentiation of non-treated cells
- Only a few genes control development

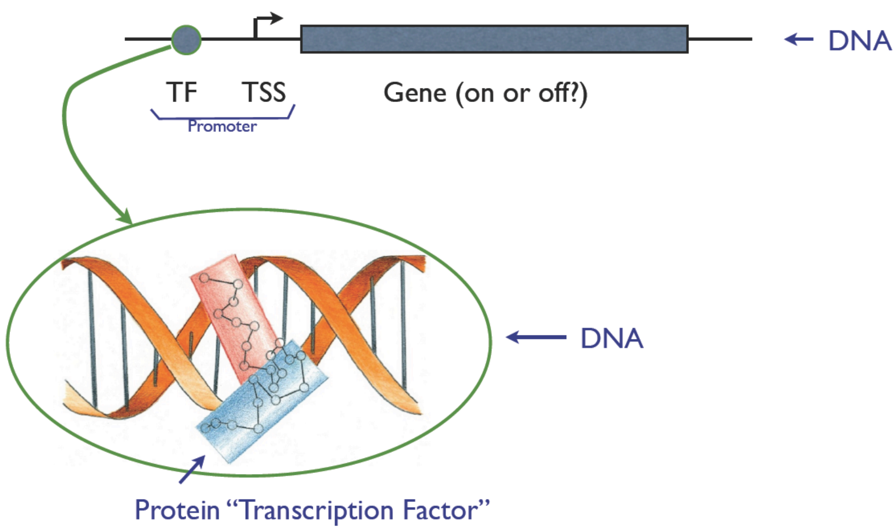


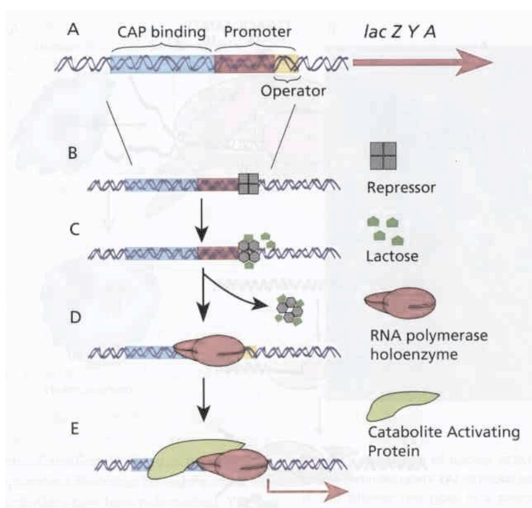
Total
Aza-myoblast
Probe
Subtracted
Aza-myoblast
Probe
Subtracted
C2C12
Probe

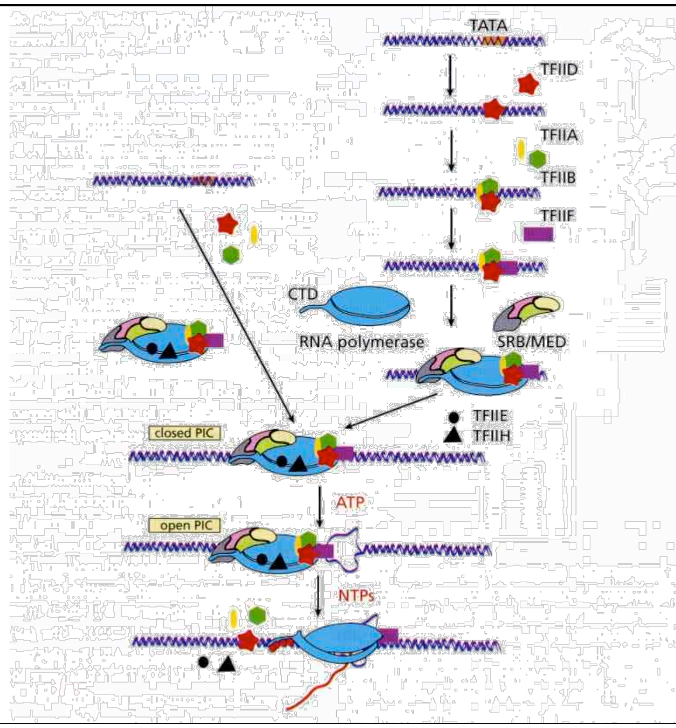


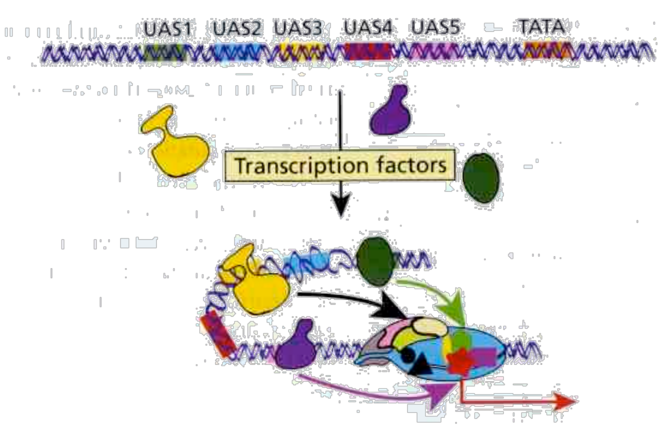
A
myoblast
B
MyoD
Myf5
myogenin
MRF4

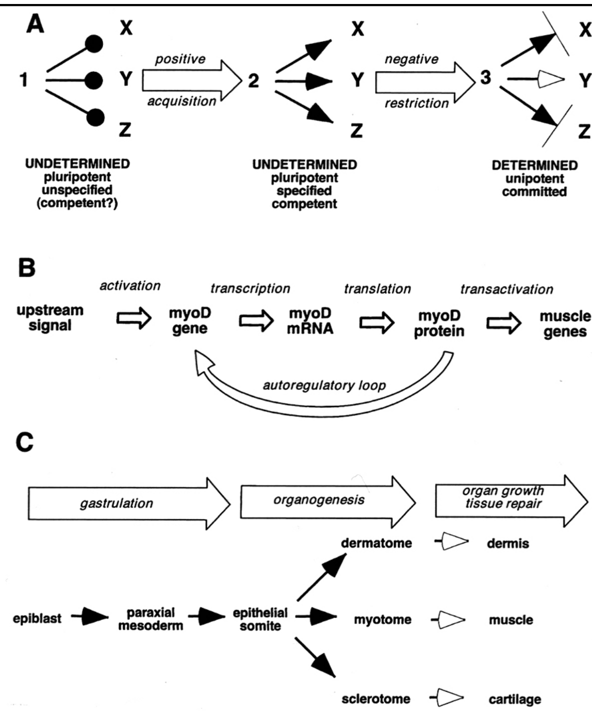
How transcription factors work (simplified)



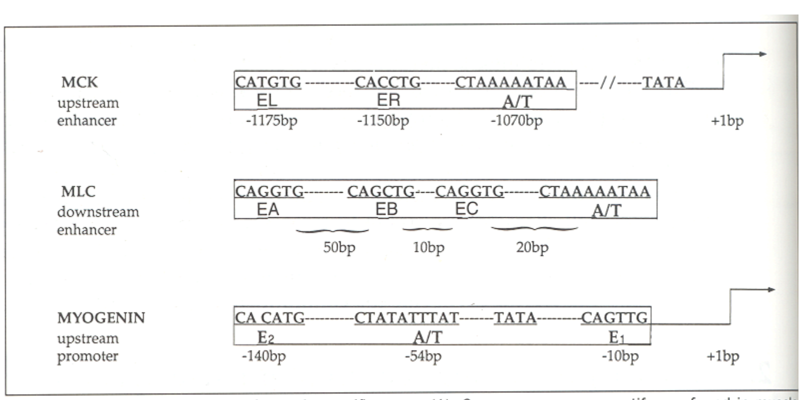


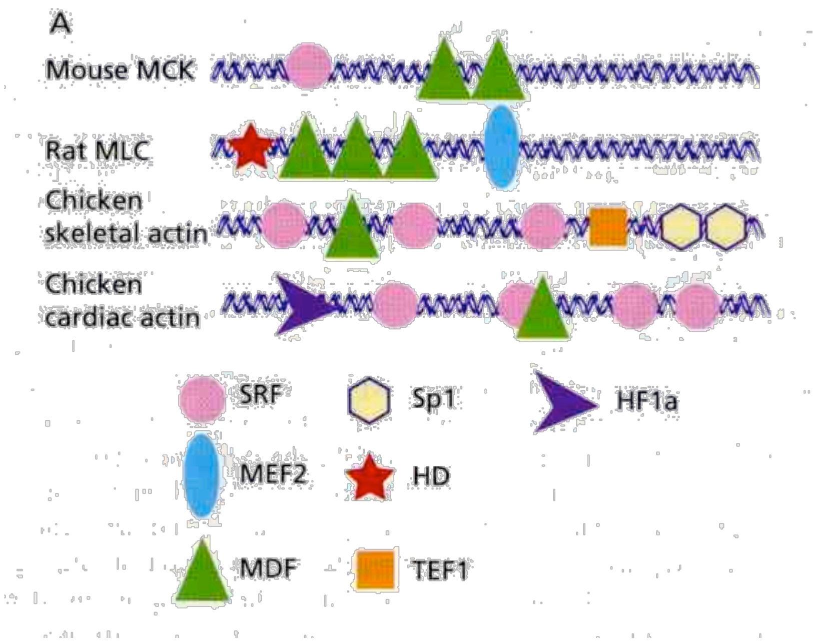

TATA
TFIID
TFIIA
TFIIB
TFIIF
CTD
RNA polymerase
SRB/MED
TFIIE
TFIIH
closed PIC
ATP
open PIC
NTPs


UAS1
UAS2
UAS3
UAS4
UAS5
TATA
Transcription factors



- Regulatory genes turn on structural genes
- Structural genes include myosin, metabolic enzymes, troponin, etc.
- Structural genes have similar sequences in their promoters.





Myogenic genes

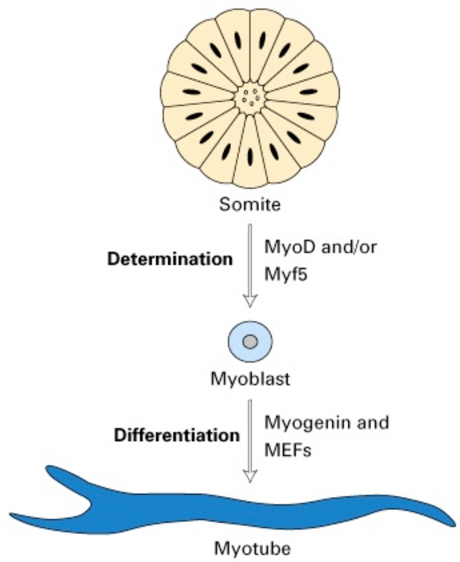
Four genes can convert cells into muscle cells

- *myoD* (first identified)
- *myogenin*
- *myf5*
- *mrf4*

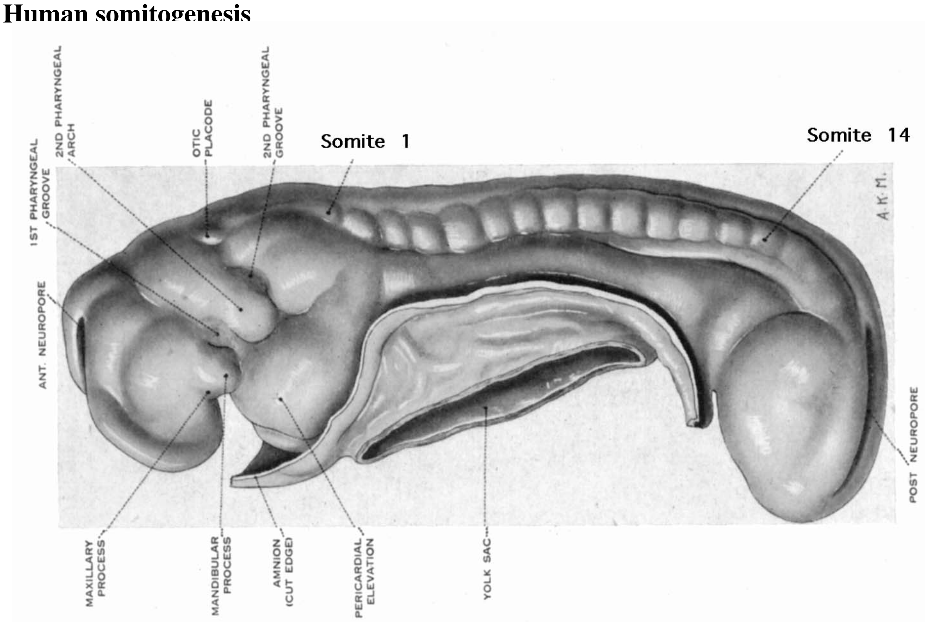
All are homologous transcription factors

Genetic control of myogenesis

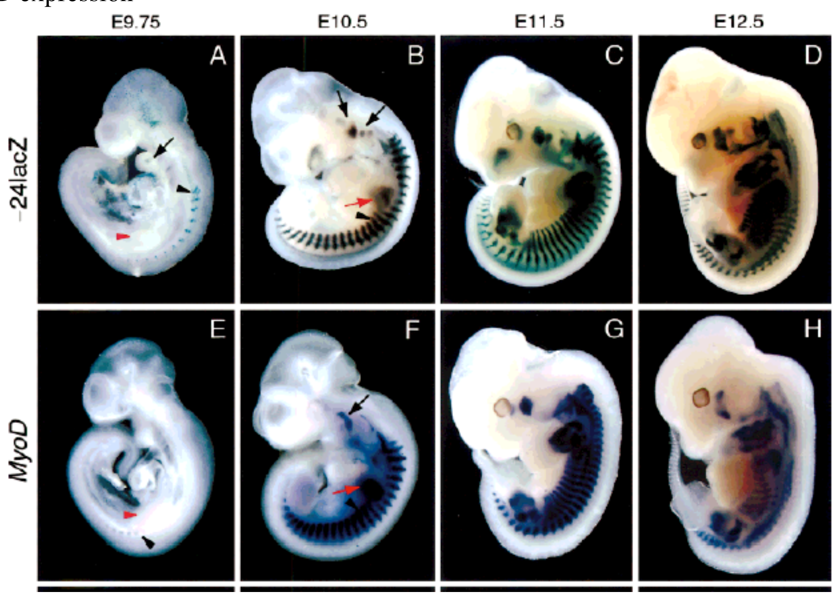
- *myoD* and *myf5* expressed prior to cell fusion
- *myogenin* expressed as cells fuse
- *mrf4* expressed later



Human somitogenesis



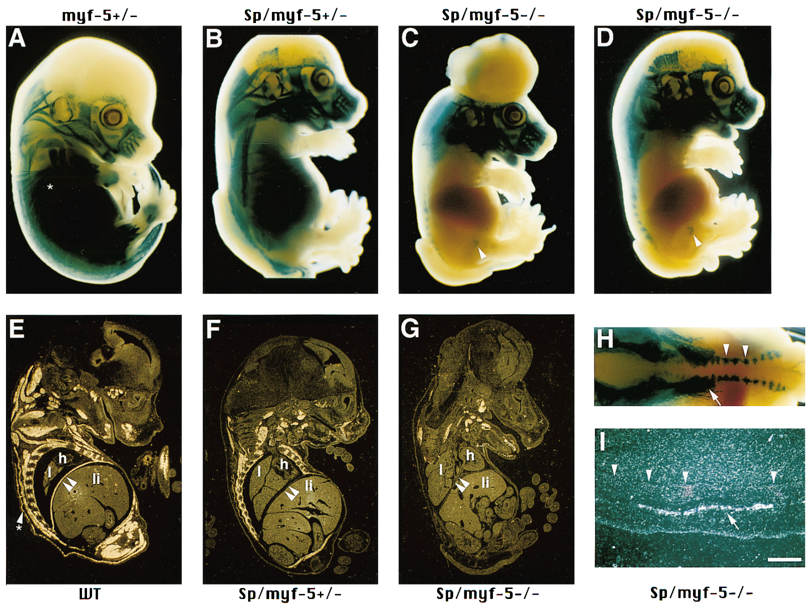
MyoD expression



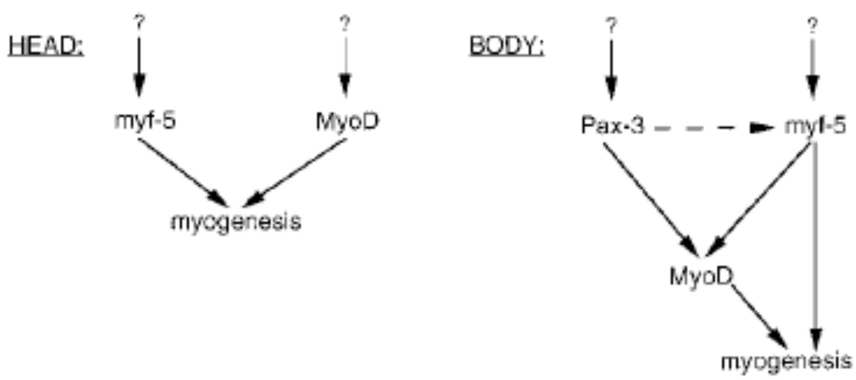
Myogenic gene disruption

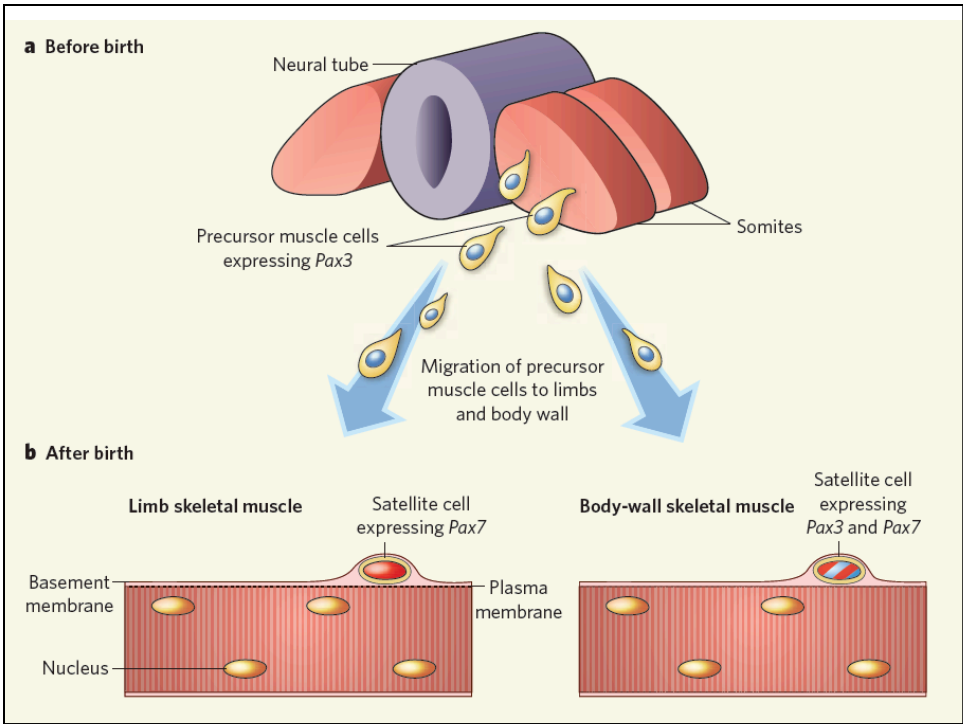
Gene Knocked Out	Phenotype*		
	Viable	Myoblasts	Muscle
myoD	Yes	+	+
myf5	Yes	+	+
myoD; myf5	No	−	−

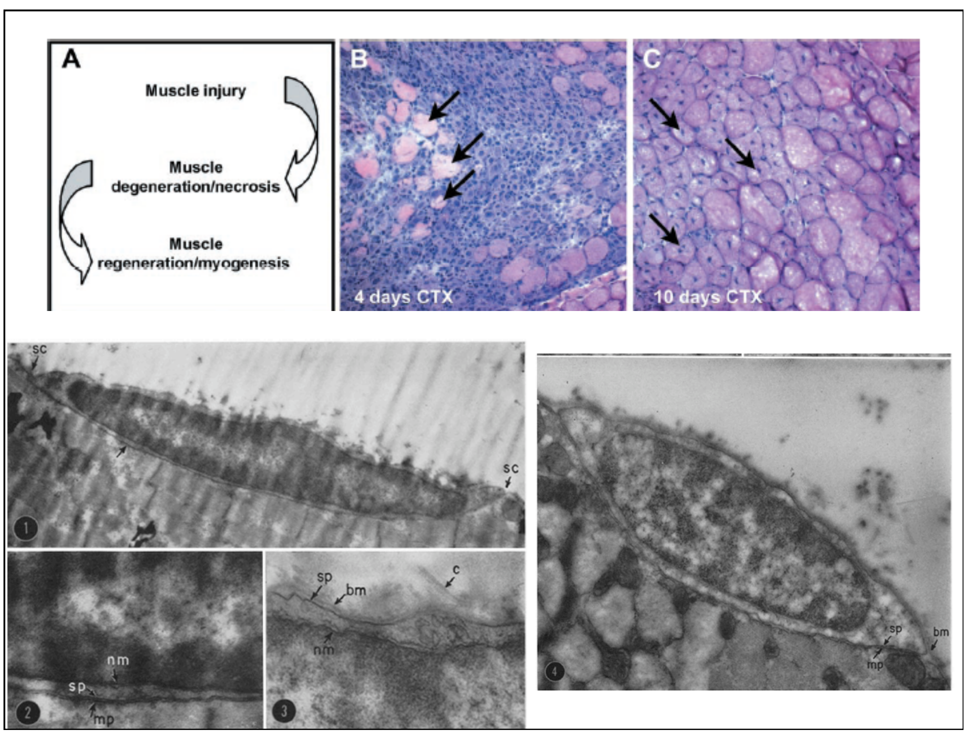
What are possible models (hypotheses) regarding the function of *myoD* and *myf5*?



Pax3 function in myogenesis

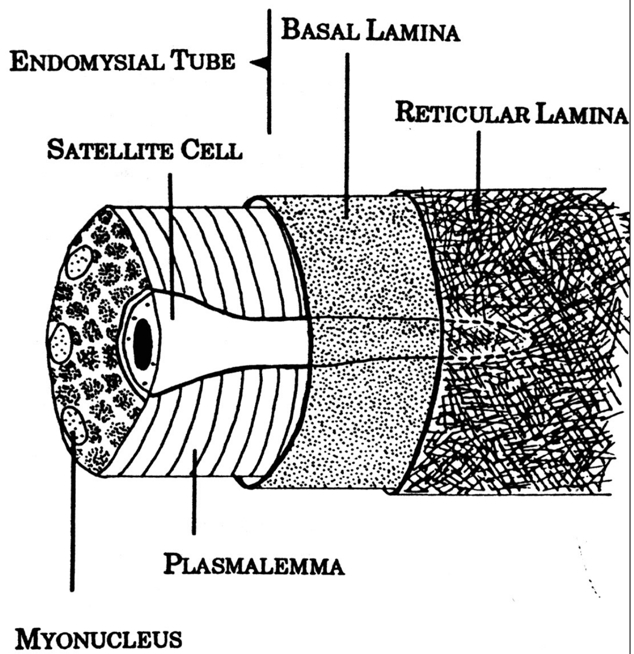



a Before birth
Neural tube
Somites
Precursor muscle cells expressing Pax3
Migration of precursor muscle cells to limbs and body wall
b After birth
Limb skeletal muscle
Satellite cell expressing Pax7
Body-wall skeletal muscle
Satellite cell expressing Pax3 and Pax7
Basement membrane
Plasma membrane
Nucleus


A
Muscle injury
Muscle degeneration/necrosis
Muscle regeneration/myogenesis
B
4 days CTX
C
10 days CTX
sc
sp
bm
nm
mp
c

Satellite cells

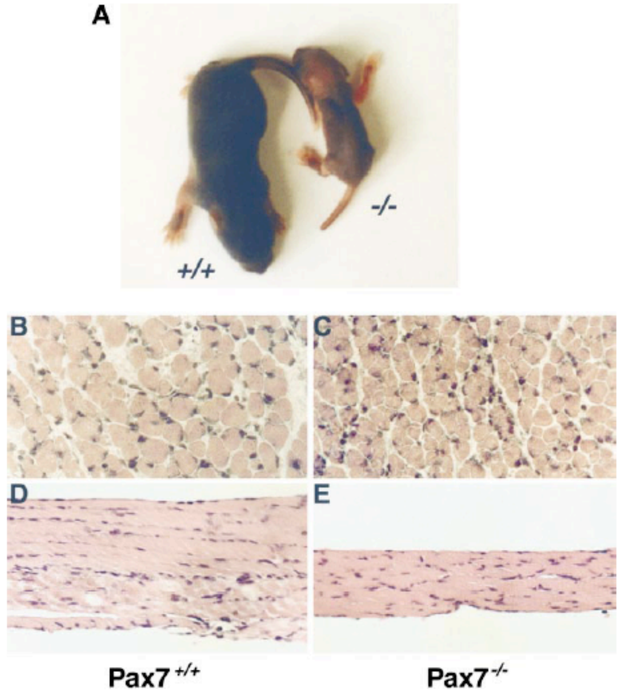
Bischoff



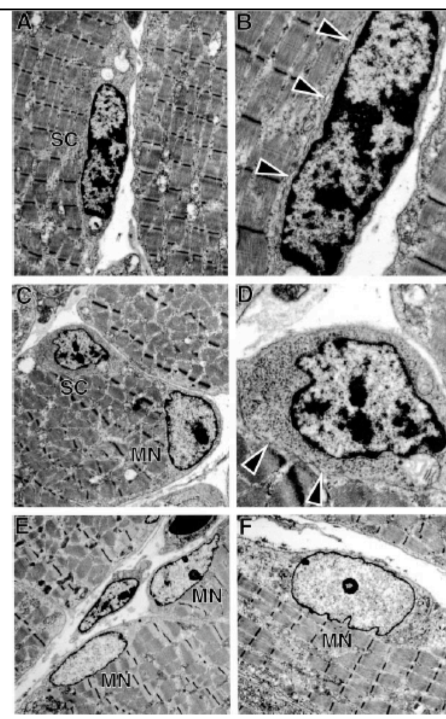
A Pax3/Pax7-dependent population of skeletal muscle progenitor cells

Frédéric Relaix[1], Didier Rocancourt[1], Ahmed Mansouri[2] & Margaret Buckingham[1]

Pax7 mutants are small



No satellite cells in mutants!
A-D are wt

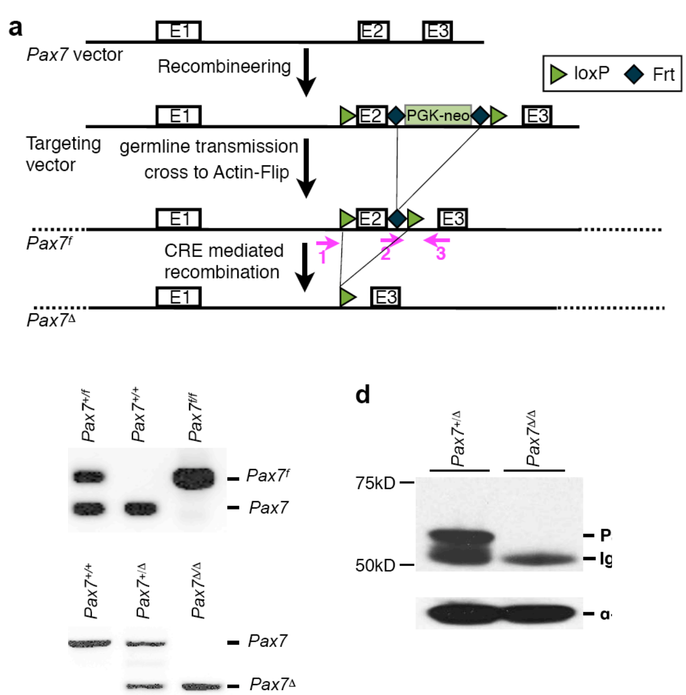


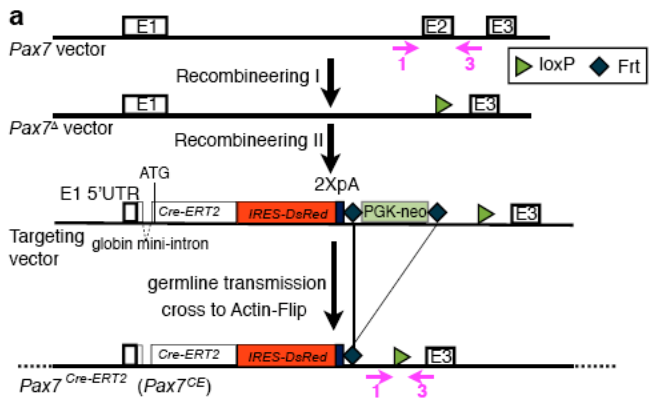
Vol 460|30 July 2009|doi:10.1038/nature08209 nature

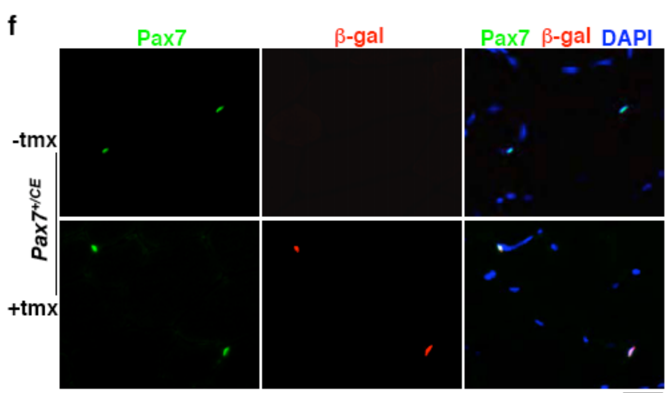
LETTERS

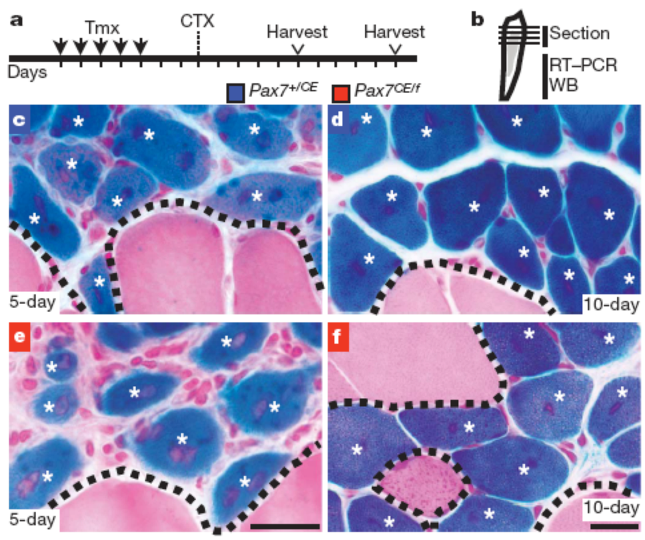
Adult satellite cells and embryonic muscle progenitors have distinct genetic requirements

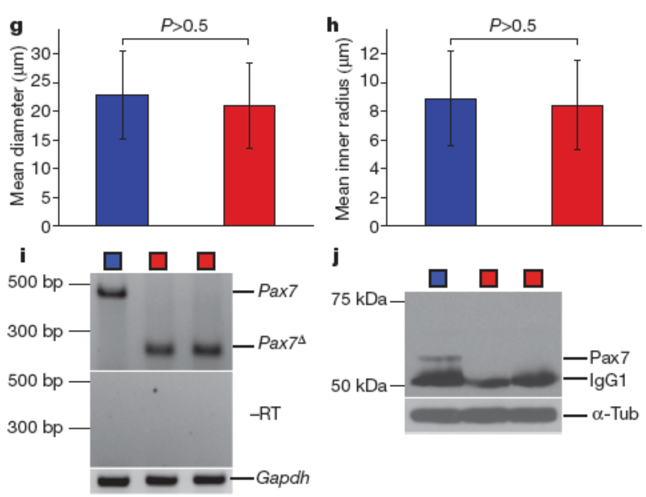
Christoph Lepper[1,2], Simon J. Conway[3] & Chen-Ming Fan[1]

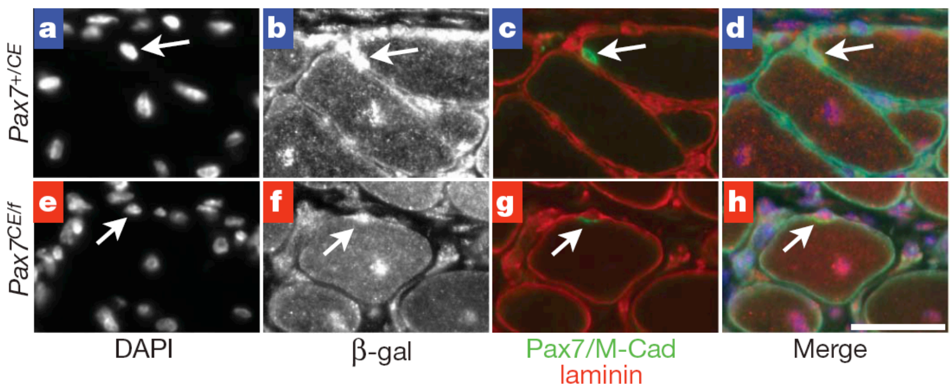



a
E1
E2
E3
Pax7 vector
1
3
loxP
Frt
Recombineering I
E1
E3
Pax7Δ vector
Recombineering II
ATG
2XpA
E1 5'UTR
Cre-ERT2
IRES-DsRed
PGK-neo
E3
Targeting vector
globin mini-intron
germline transmission
cross to Actin-Flip
Cre-ERT2
IRES-DsRed
E3
Pax7 Cre-ERT2 (Pax7CE)
1
3

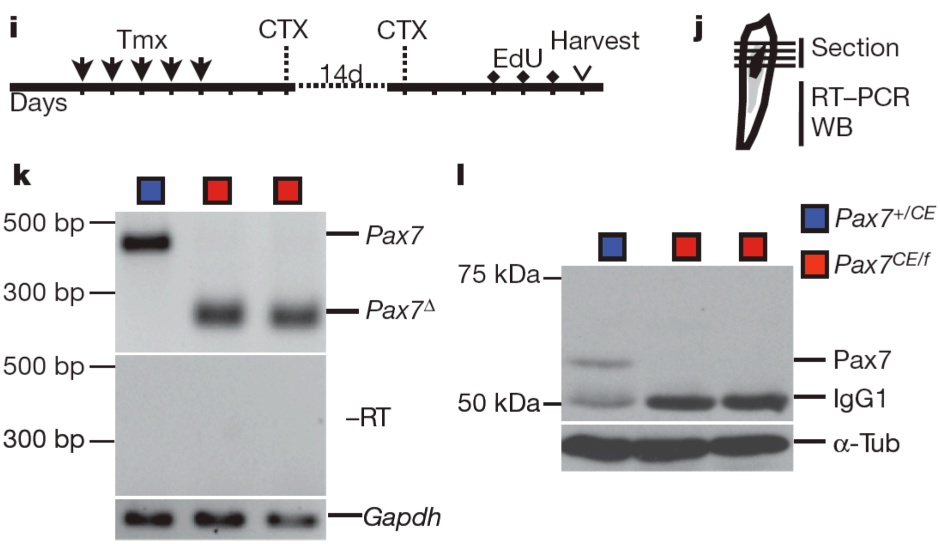

f
Pax7
β-gal
Pax7 β-gal DAPI
-tmx
Pax7+/CE
+tmx


a
Tmx
CTX
Harvest
Harvest
Days
b
Section
RT–PCR
WB
Pax7+/CE
Pax7CE/f
c
d
e
f
5-day
10-day
5-day
10-day

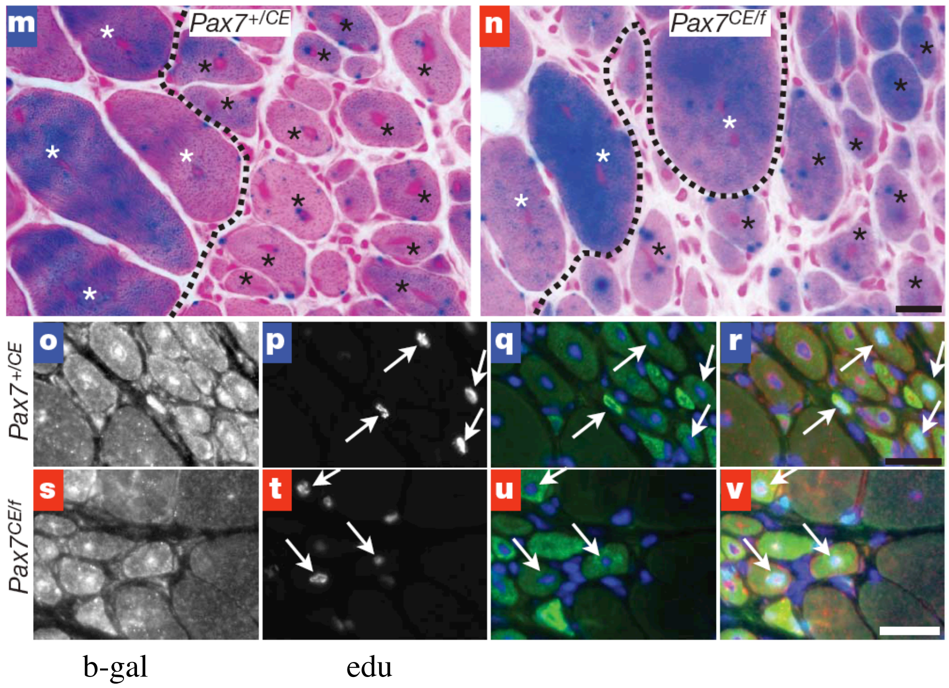

g
P>0.5
Mean diameter (μm)
h
P>0.5
Mean inner radius (μm)
i
500 bp
300 bp
500 bp
300 bp
Pax7
Pax7Δ
–RT
Gapdh
j
75 kDa
50 kDa
Pax7
IgG1
α-Tub



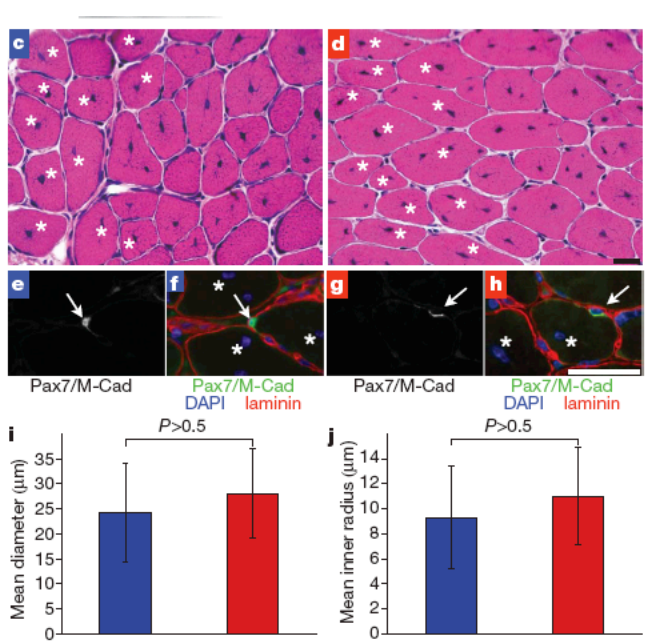
a
b
c
d
e
f
g
h
Pax7+/CE
Pax7CE/f
DAPI
β-gal
Pax7/M-Cad
laminin
Merge



i
Tmx
CTX
CTX
14d
EdU
Harvest
Days
j
Section
RT–PCR
WB
k
500 bp
300 bp
500 bp
300 bp
Pax7
Pax7Δ
–RT
Gapdh
l
Pax7+/CE
Pax7CE/f
75 kDa
50 kDa
Pax7
IgG1
α-Tub



m
Pax7+/CE
n
Pax7CE/f
o
p
q
r
s
t
u
v
Pax7+/CE
Pax7CE/f
b-gal
edu



c
d
e
f
g
h
Pax7/M-Cad
Pax7/M-Cad
DAPI laminin
Pax7/M-Cad
Pax7/M-Cad
DAPI laminin
i
P>0.5
Mean diameter (µm)
0
5
10
15
20
25
30
35
j
P>0.5
Mean inner radius (µm)
0
2
4
6
8
10
12
14