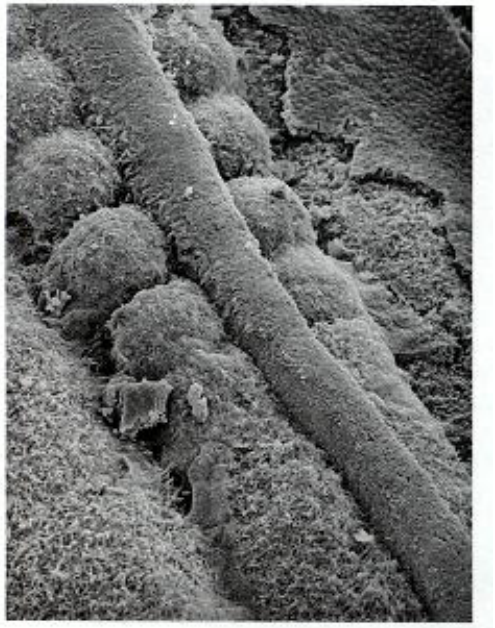
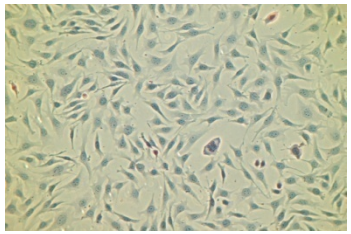
Myogenesis

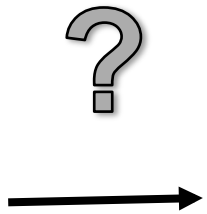
Gilbert (11th edition) Chapter 17 pp566-572 575-579

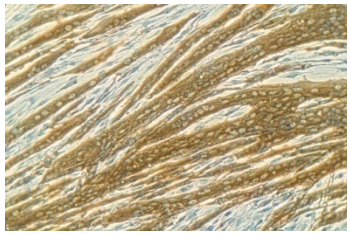
Gilbert (10th edition) Chapter 12 pp 428- 432

9th edition Chapter 11 425- 428

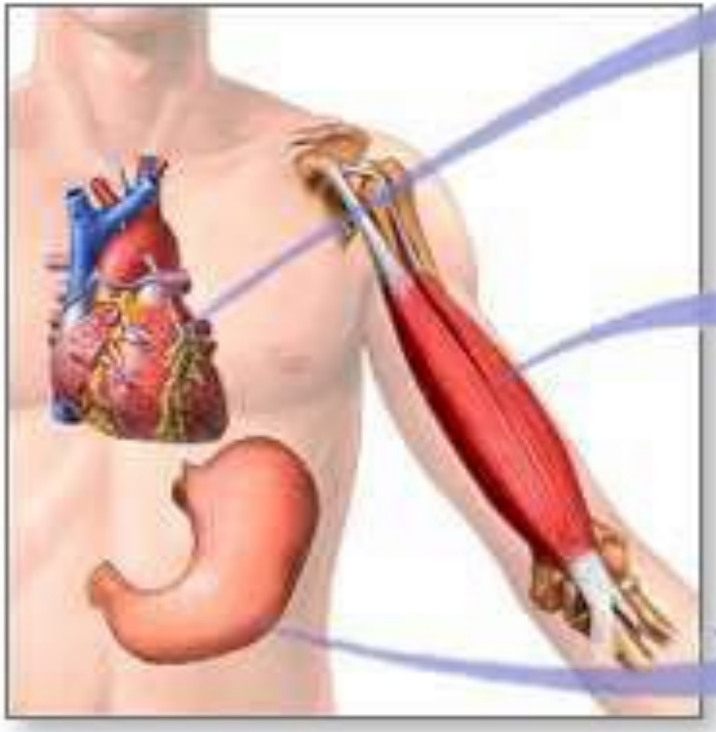








Muscle types

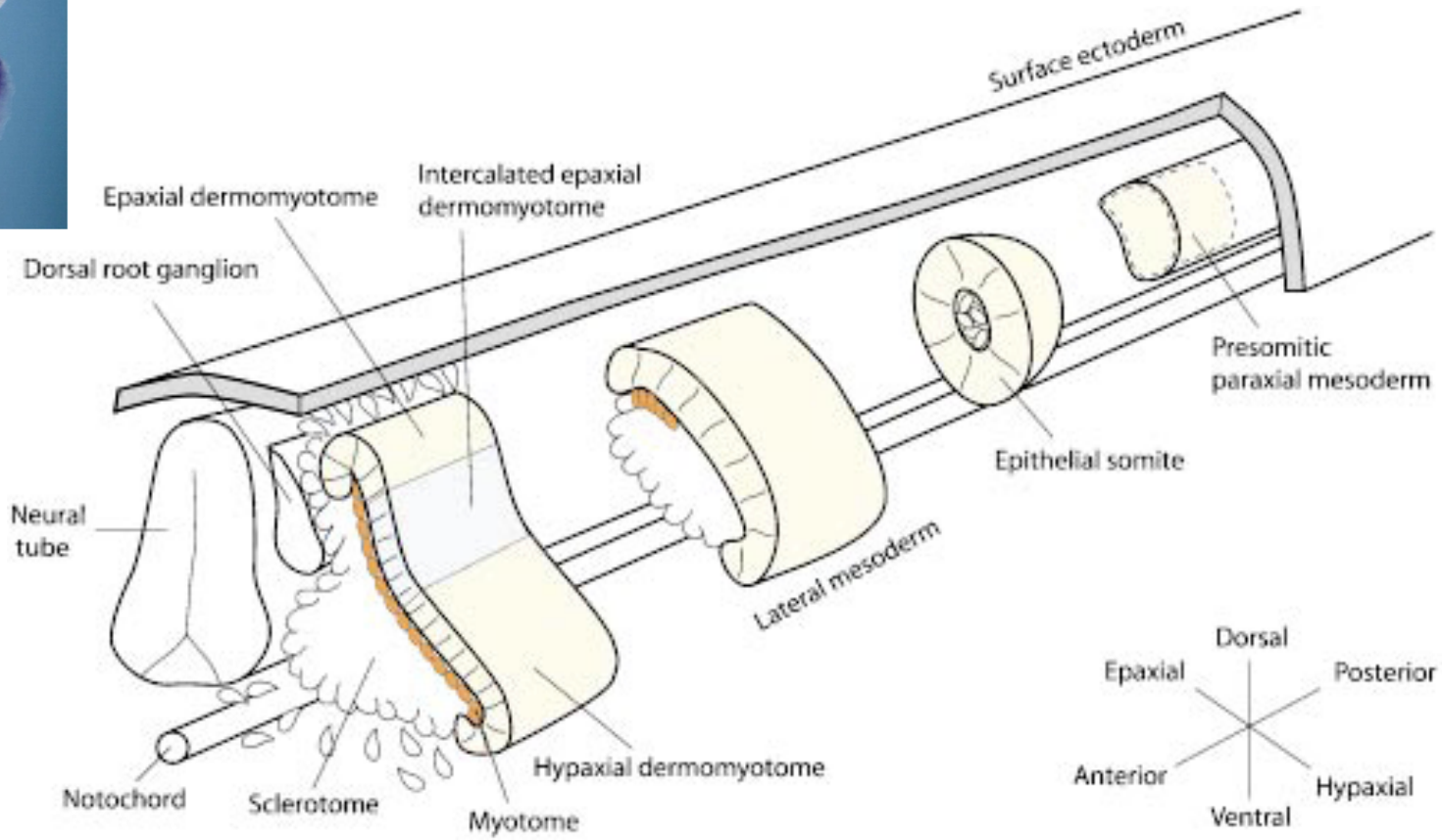


Cardiac muscle

Skeletal muscle cell

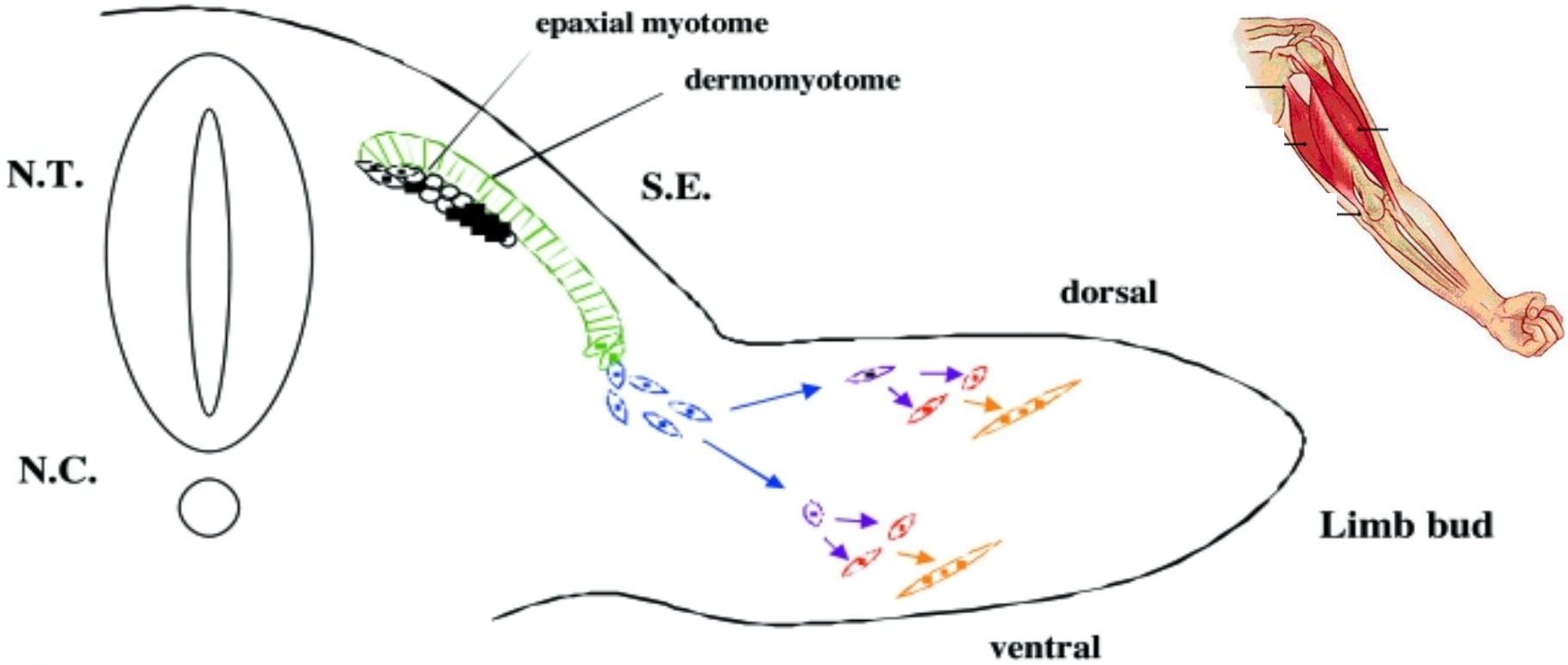
Smooth muscle

Muscle progenitor cell commitment *in vivo*



Buckingham. M, *J. Anat.* (2003)

Skeletal muscle differentiation *in vivo*



Delamination : Pax3 → c-met

Migration : c-met/HGF, Lbx1

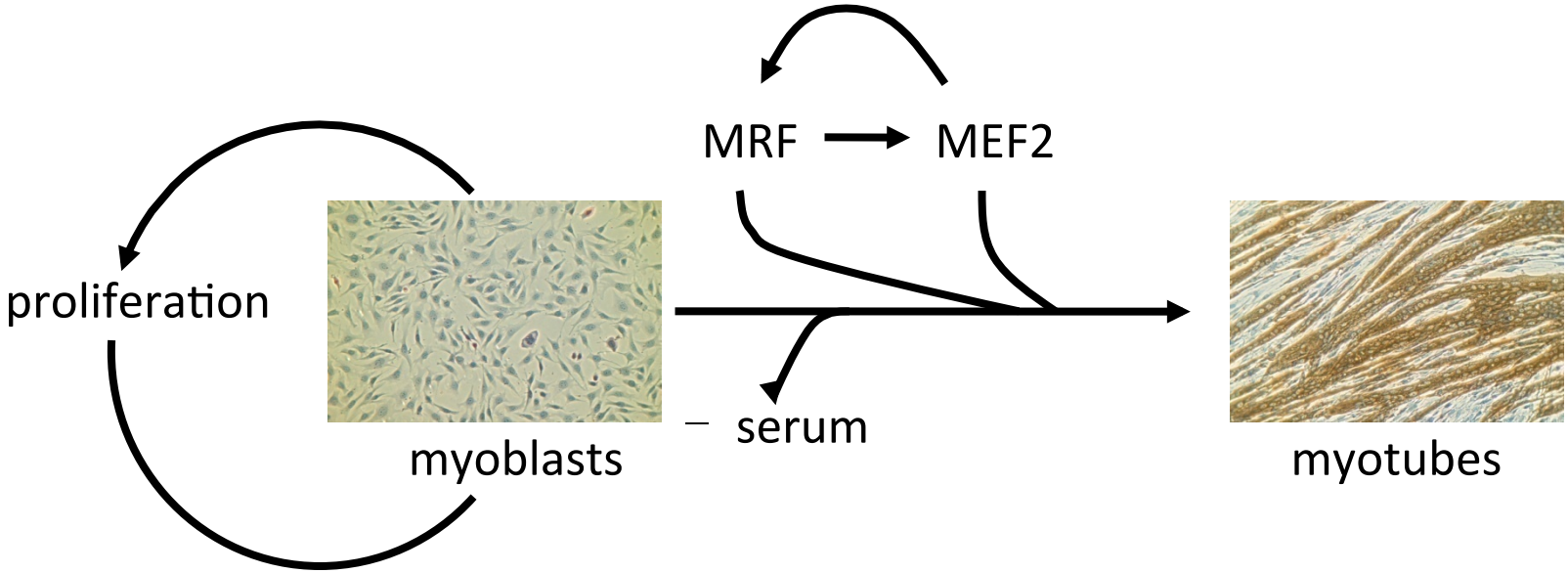
Proliferation : ? Pax3, c-met, Mox2, Msx1, Six, (Myf5, MyoD)

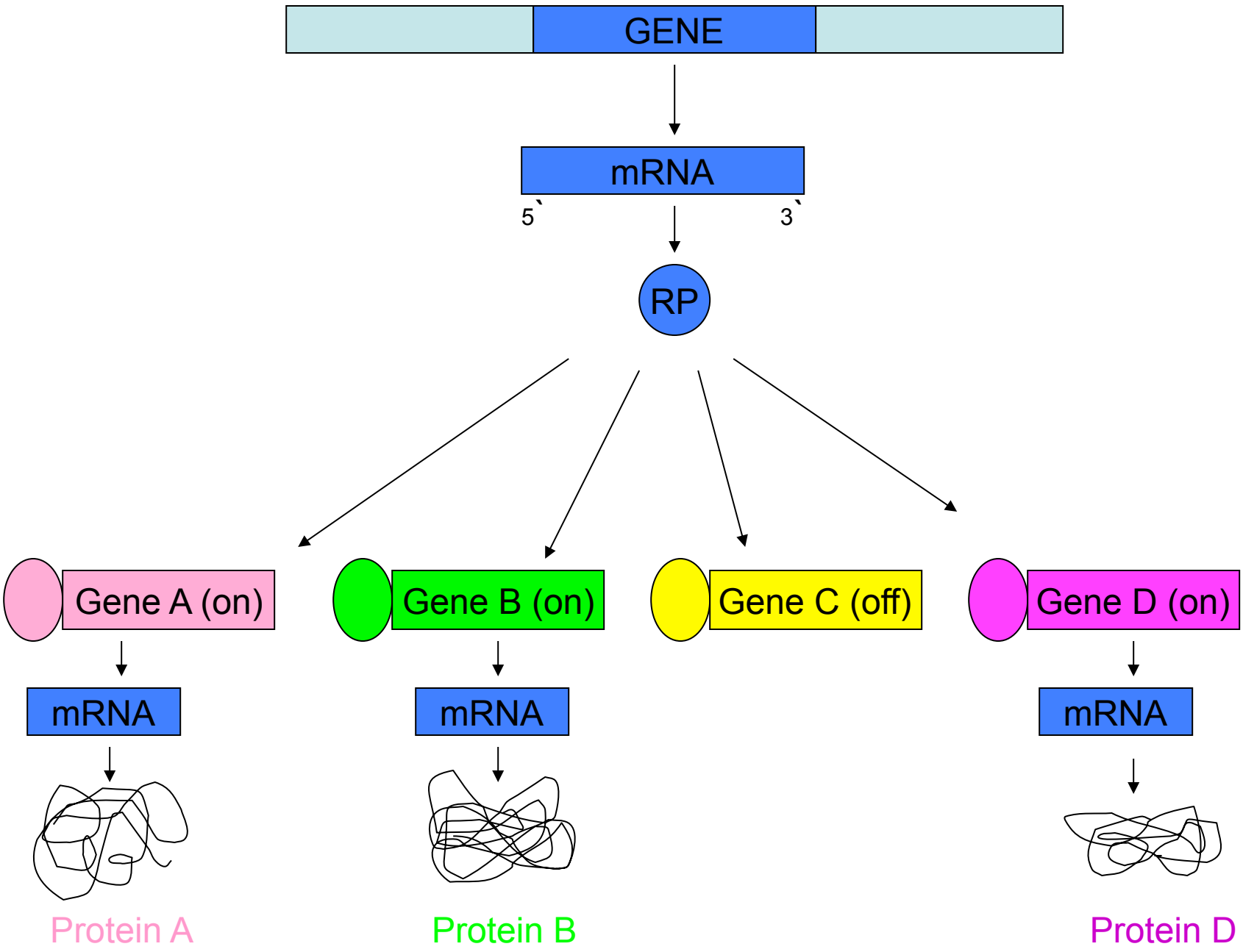
Determination : Myf5, MyoD

Differentiation : Myogenin, (MyoD, Mrf4), Mef2, Six ...

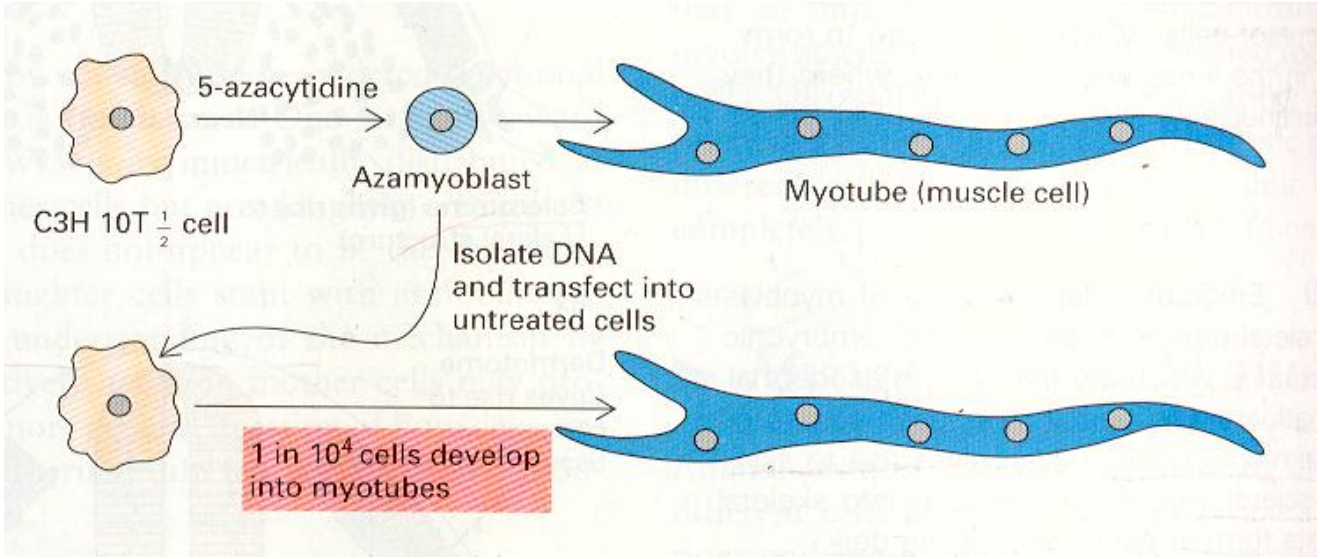
Buckingham. M, *J. Anat.* (2003)

Cell culture model of muscle cell development



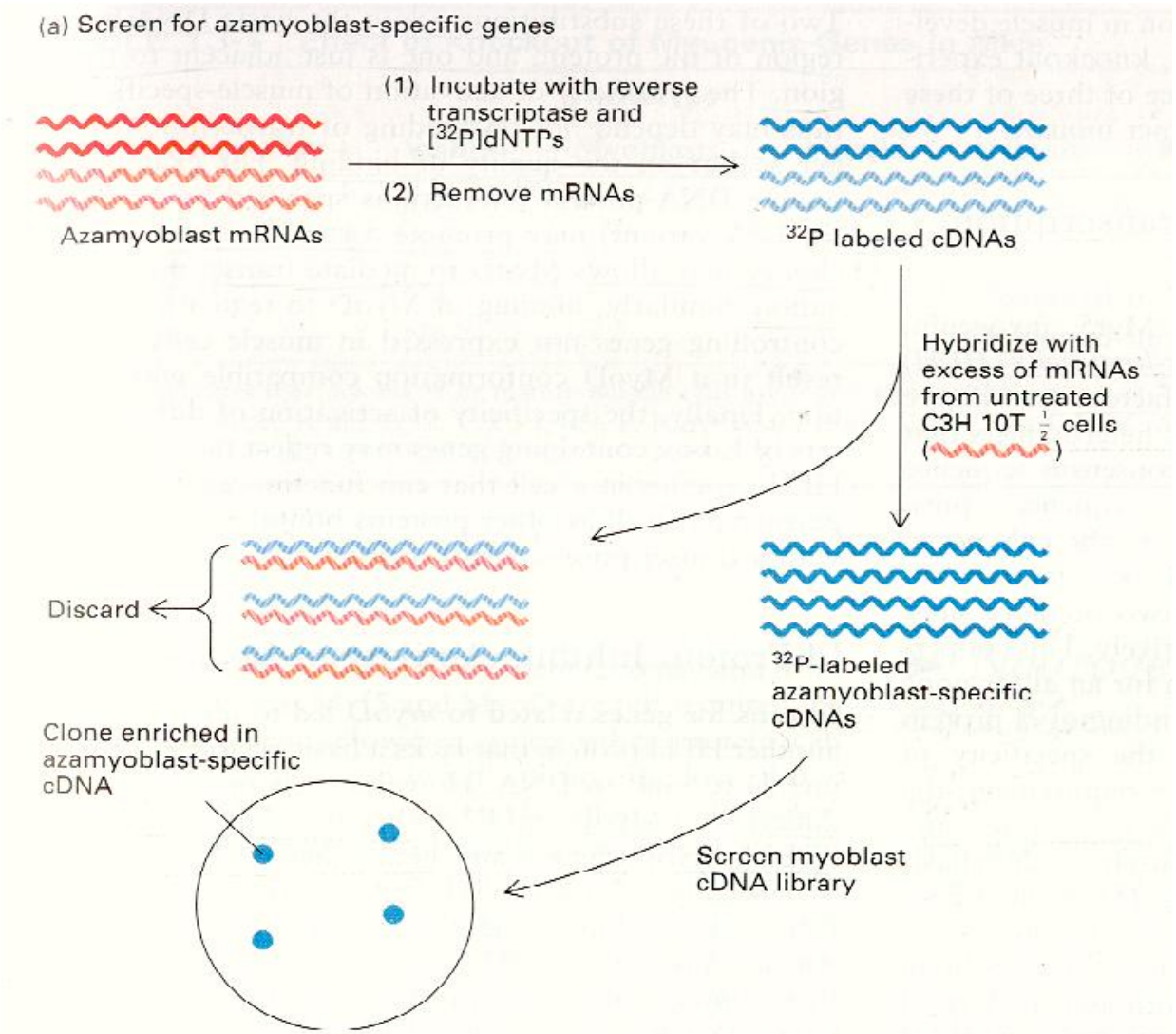

GENE
mRNA
5`
3`
RP
Gene A (on)
Gene B (on)
Gene C (off)
Gene D (on)
mRNA
mRNA
mRNA
Protein A
Protein B
Protein D

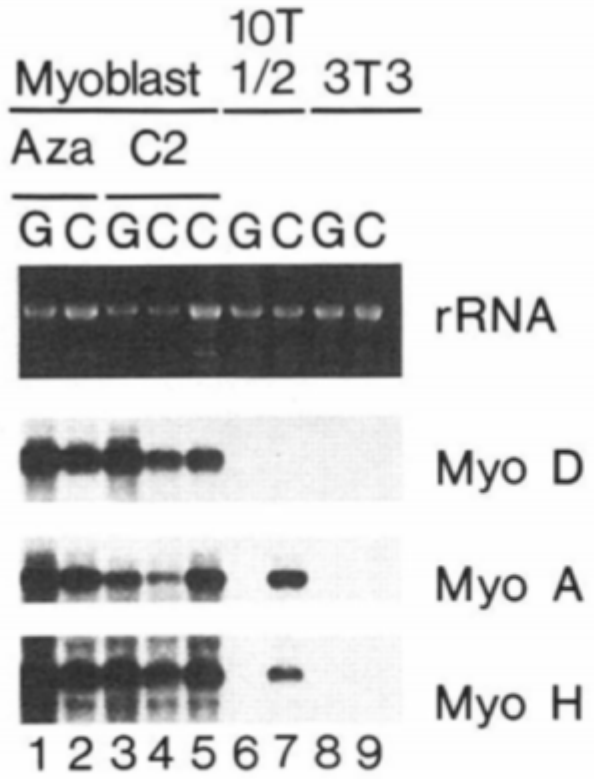
Experimental system for studying mammalian myogenesis



Davis *et* al., 1987 Cell. 51(6): 987–1000

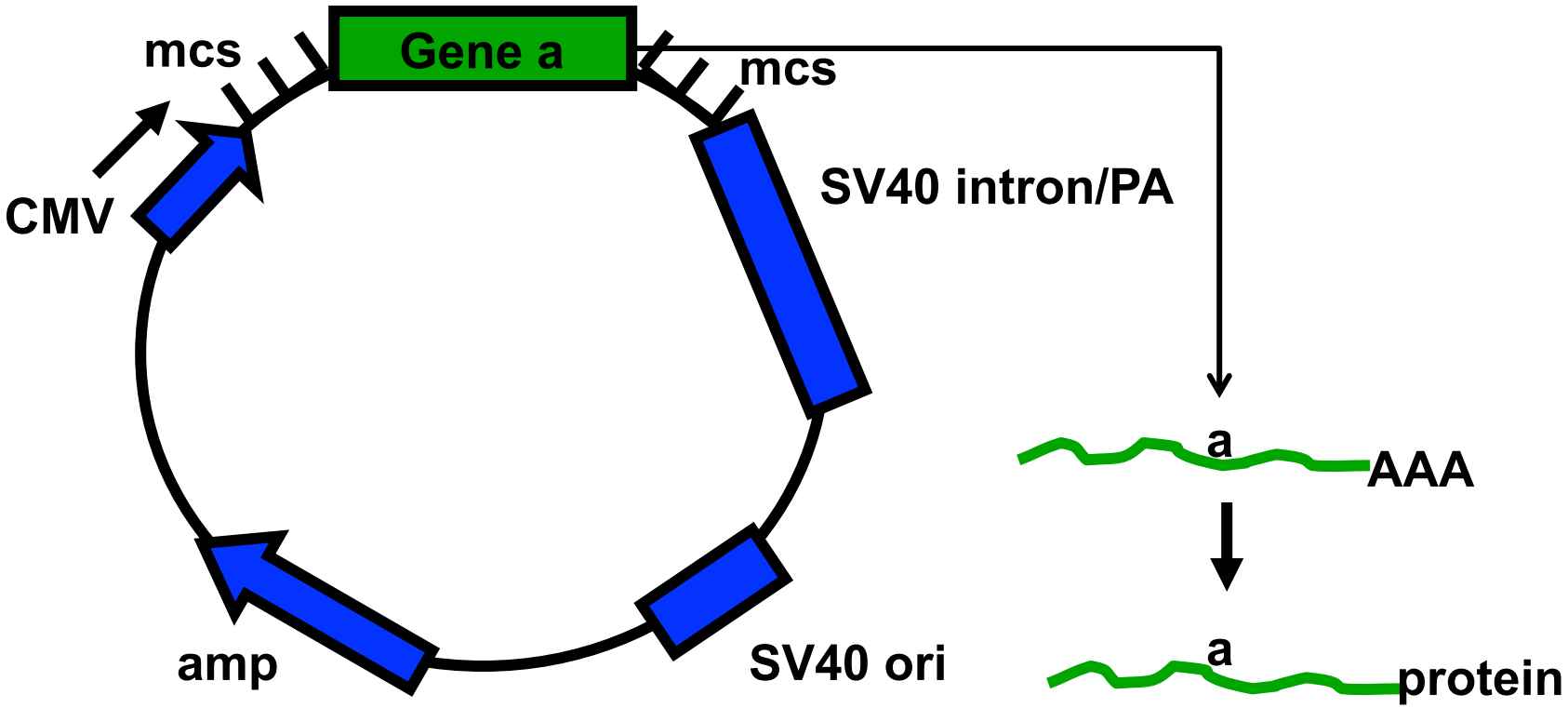
Identification and assay of genes that drive myogenesis



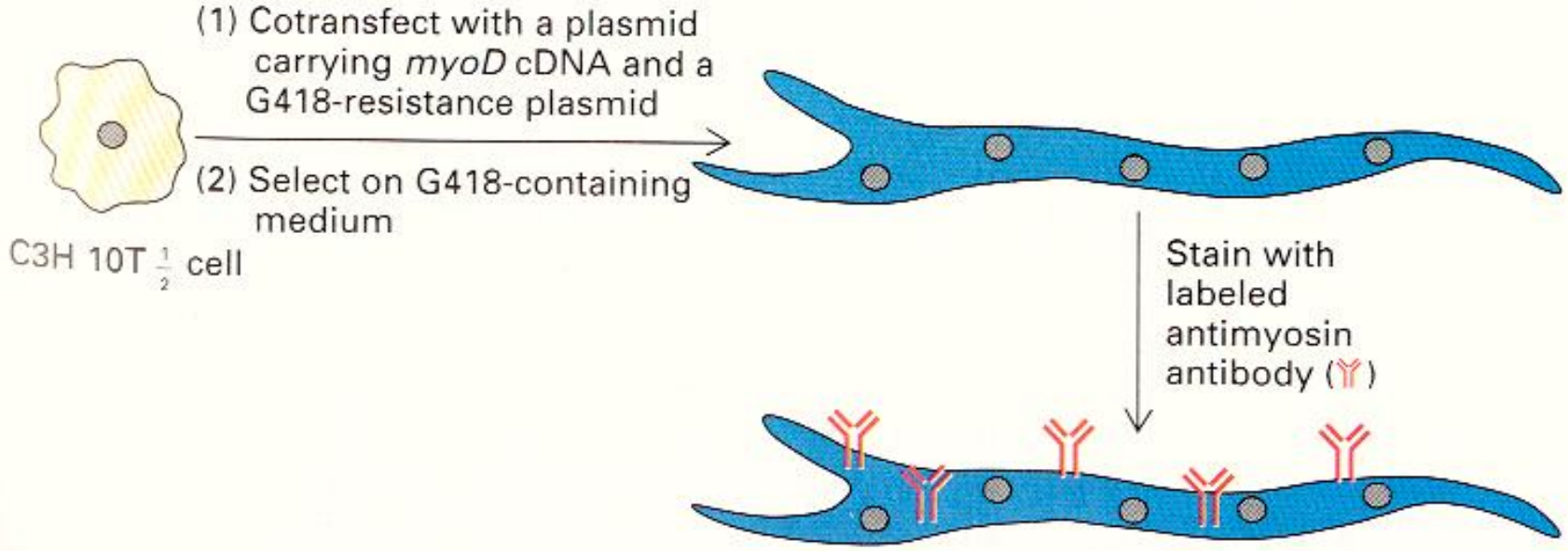


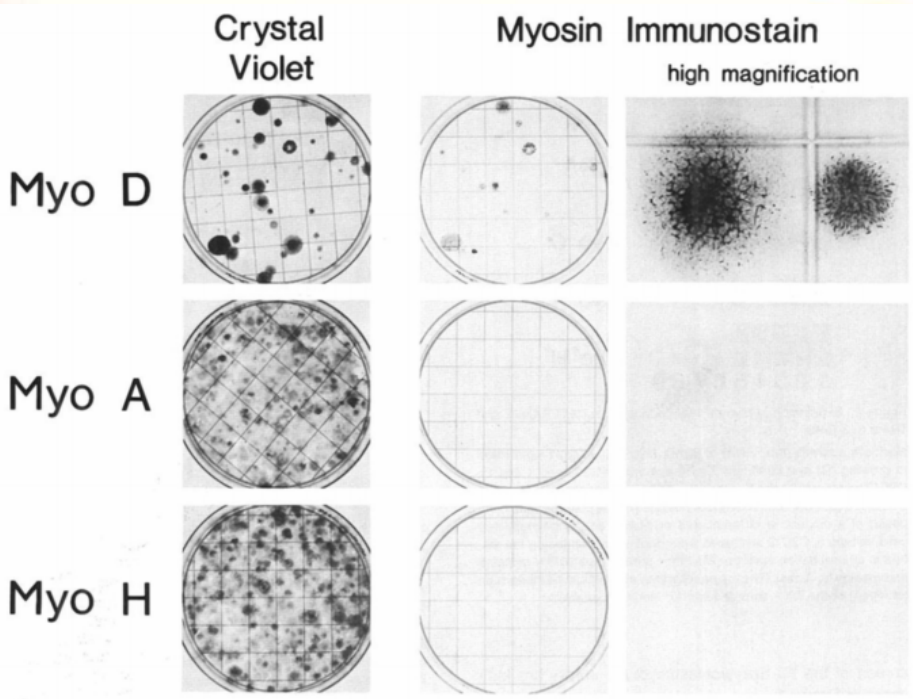
Davis *et* al., 1987 Cell. 51(6): 987–1000

Mammalian Expression Vector



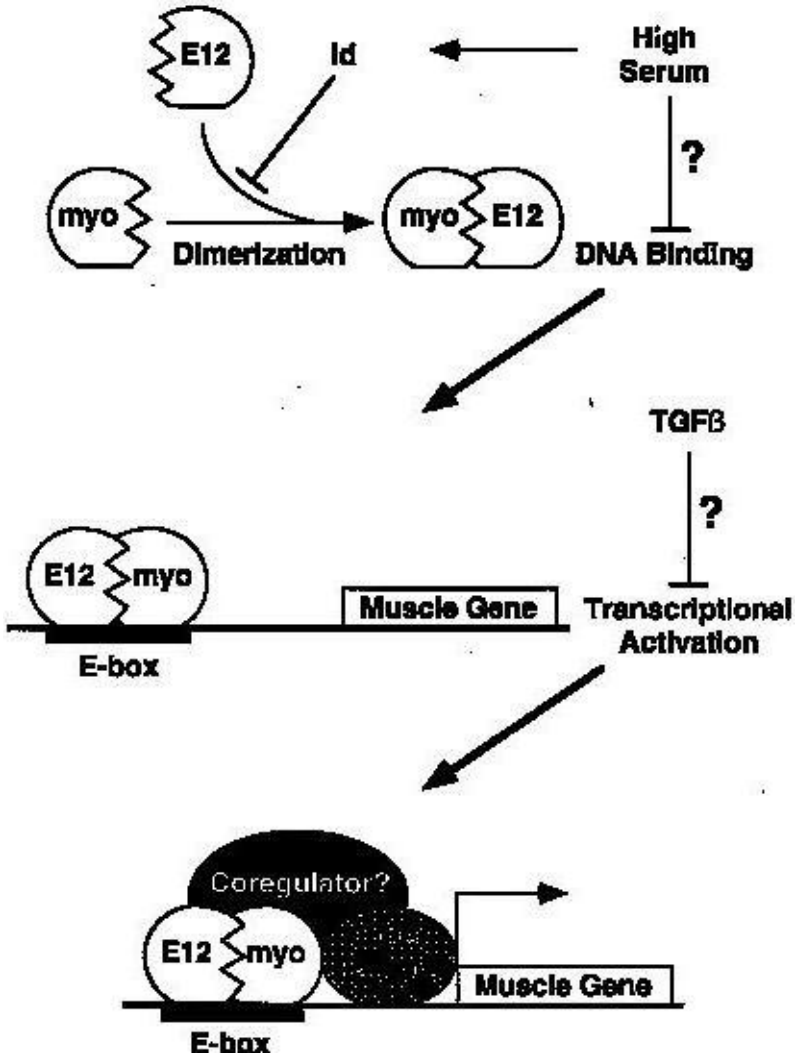
(b) Assay for myogenic activity of *myoD* cDNA



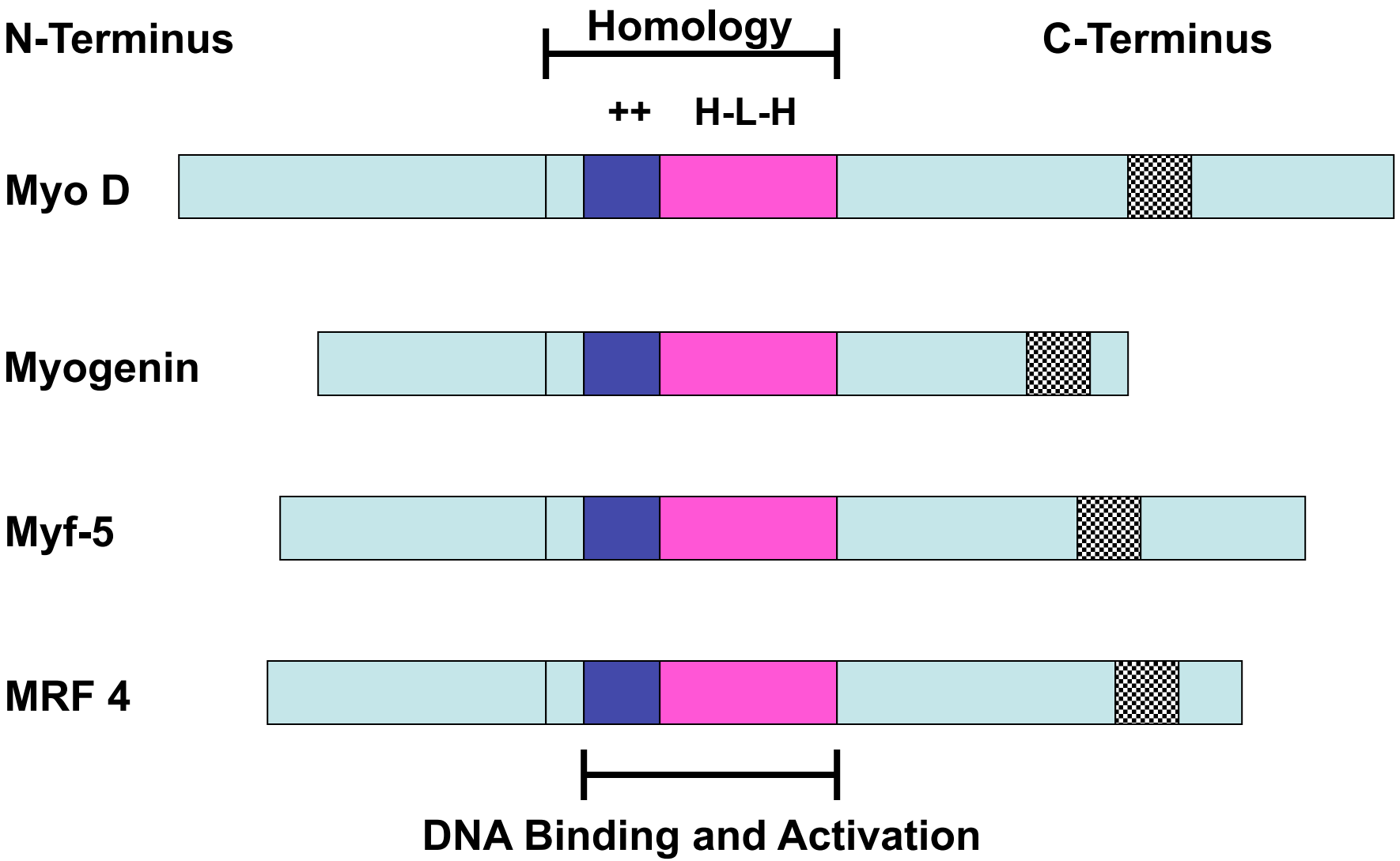


Davis *et* al., 1987 Cell. 51(6): 987–1000

Regulation of Muscle Gene Expression



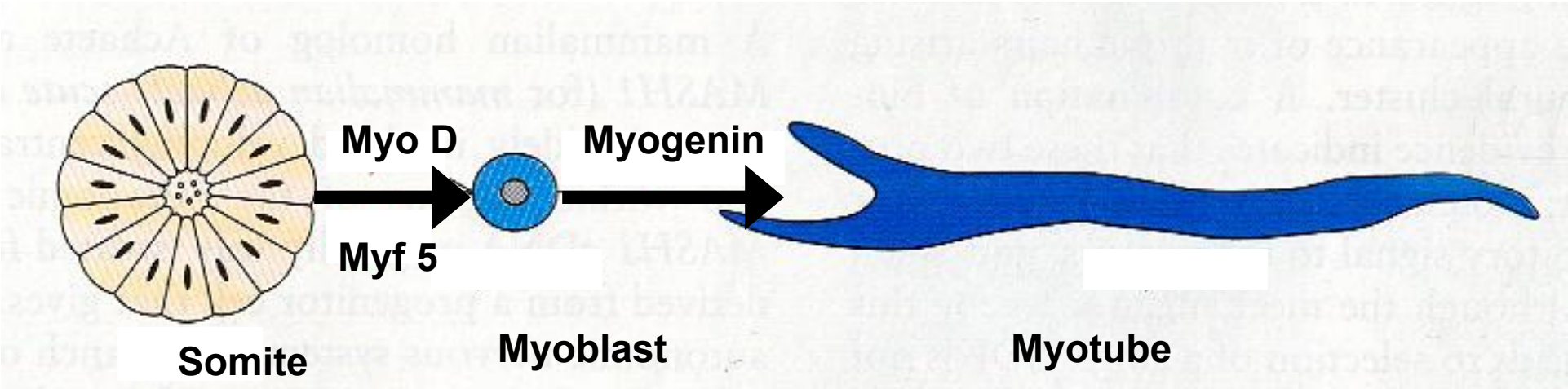
Myogenic Regulatory Factors



Effects of ‘knockout’ of myogenic genes in mice

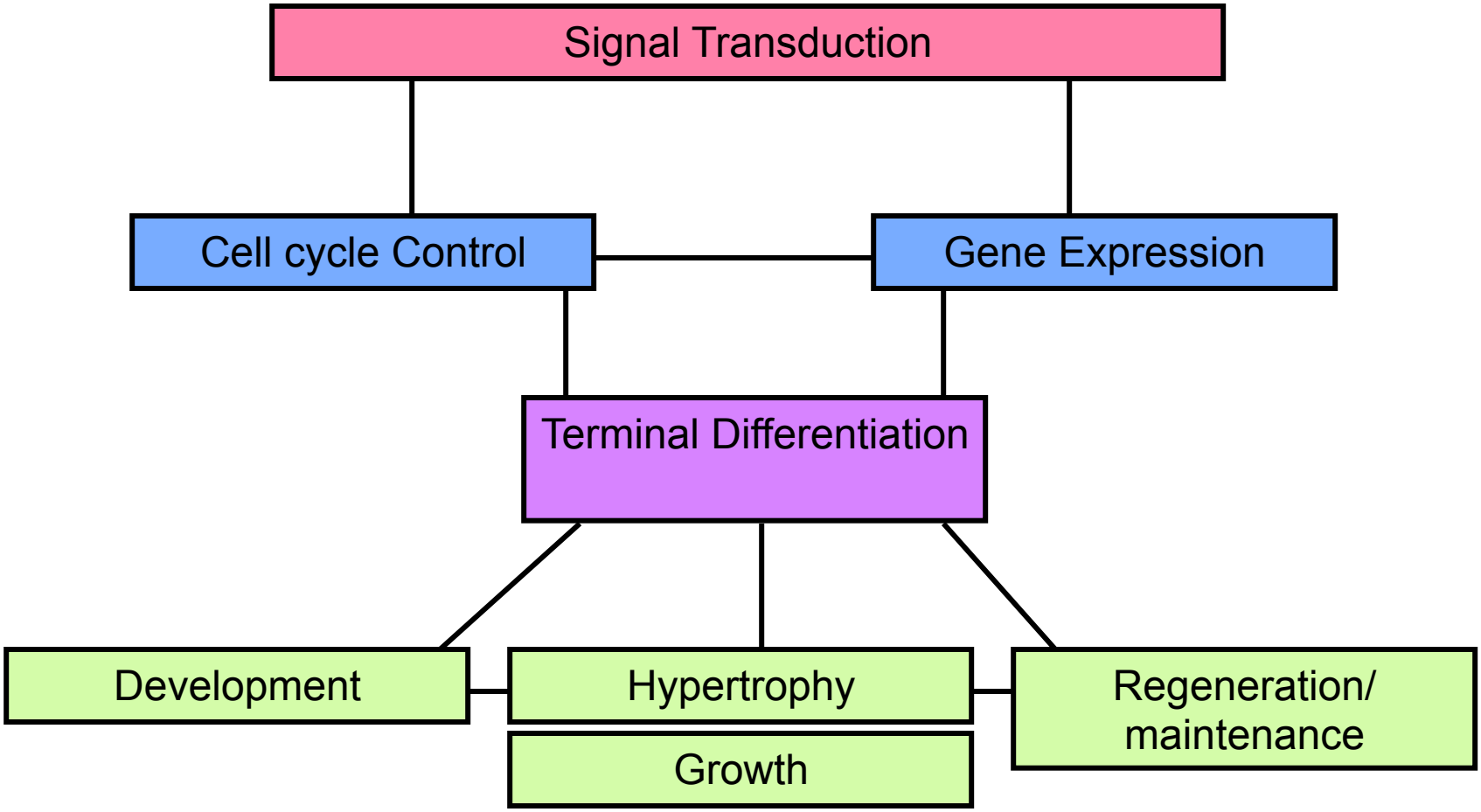
Gene KO	Viable	MB	Muscle	Role of Myogenic Factor
MyoD	Yes	+	+	?
Myf 5	Yes	+	+	?
MyoD; Myf5	No	-	-	MB formation or survival
Myogenin	No	+	-	MB differentiation

Model of genetic control of mammalian skeletal muscle development.



Regulation of cell cycle in cellular differentiation

A "systems biology" approach to understanding development



Cell Cycle Control

Features of cell division control

(1) Regulate passage through the cell cycle

(2) Control the transition of cells into and out of the proliferative state

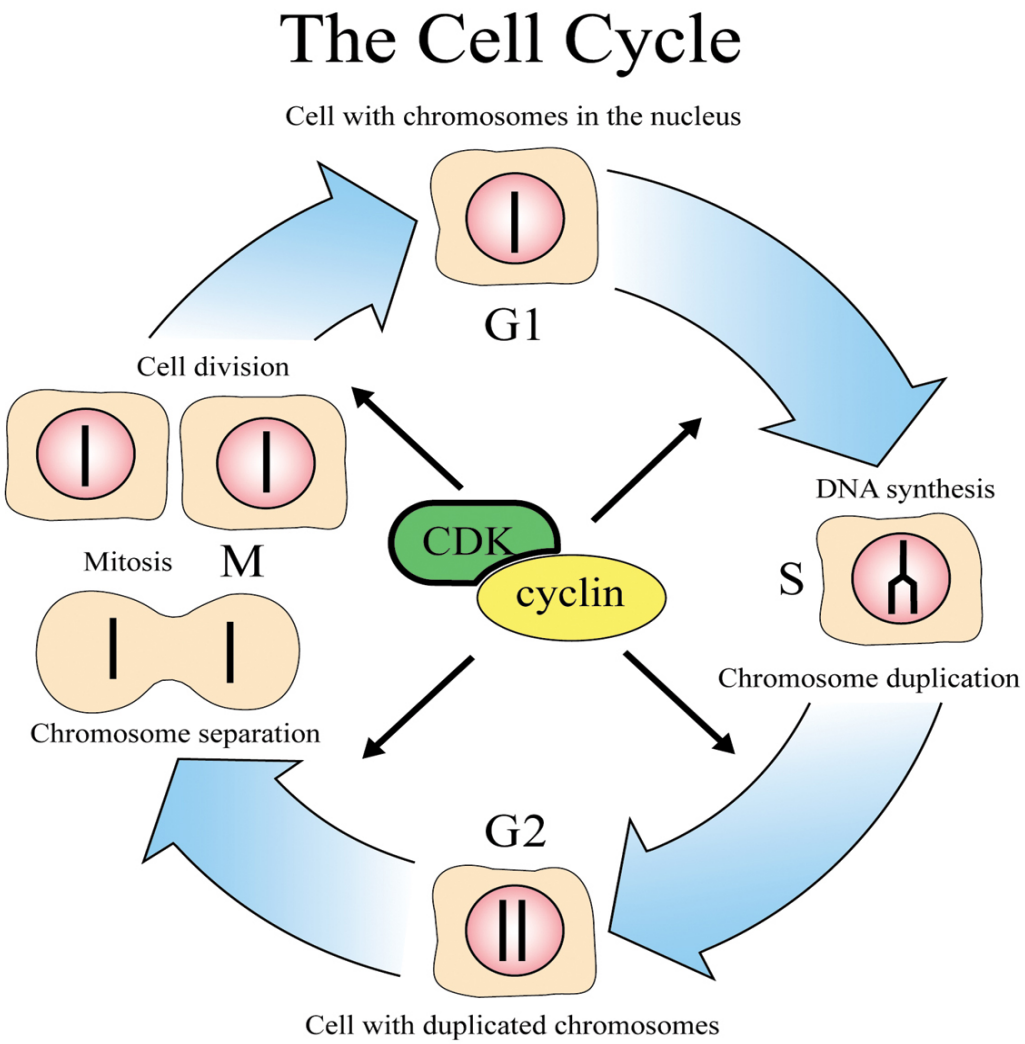
Design features of a cell cycle control system:

(A) Cells must not enter mitosis until the nuclear DNA has been replicated

(B) Cytokinesis should not proceed if spindle malfunction has prevented proper chromosome segregation in mitosis

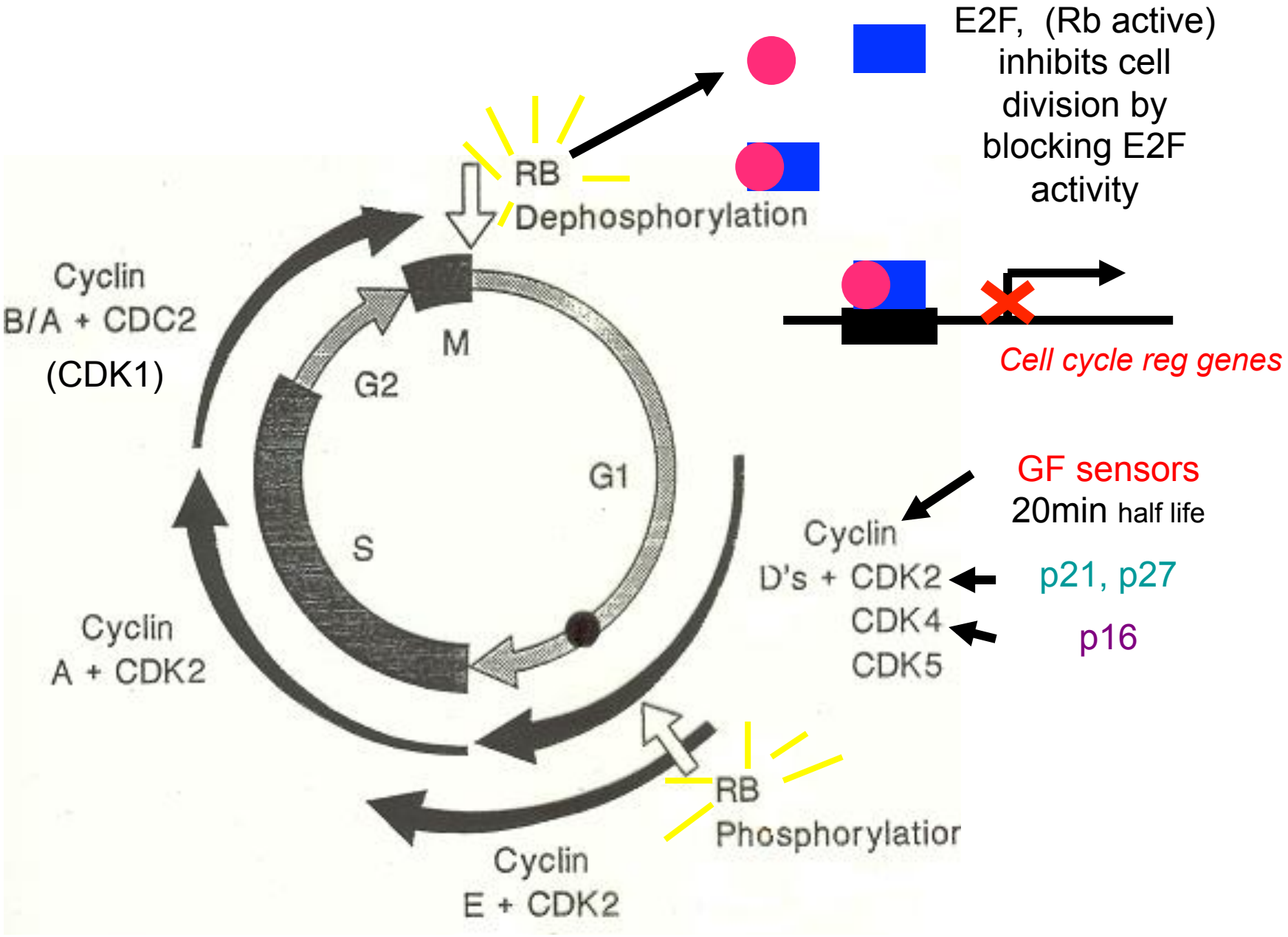
(C) A control system must respond to developmental/ environmental cues to assume that the DIVIDE/ DIFFERENTIATE choice is correctly made

(D) Chromosome replication must NOT begin if mitosis has not been completed since the last round of replication



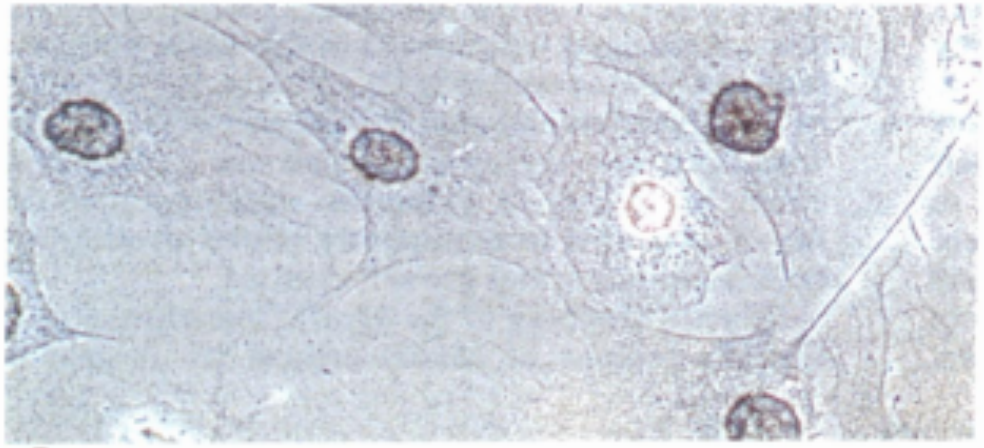
The 2001 Nobel Prize in Physiology or Medicine:
Leland H. Hartwell, R. Timothy (Tim) Hunt and Paul M. Nurse for their discoveries of "key regulators of the cell cycle"

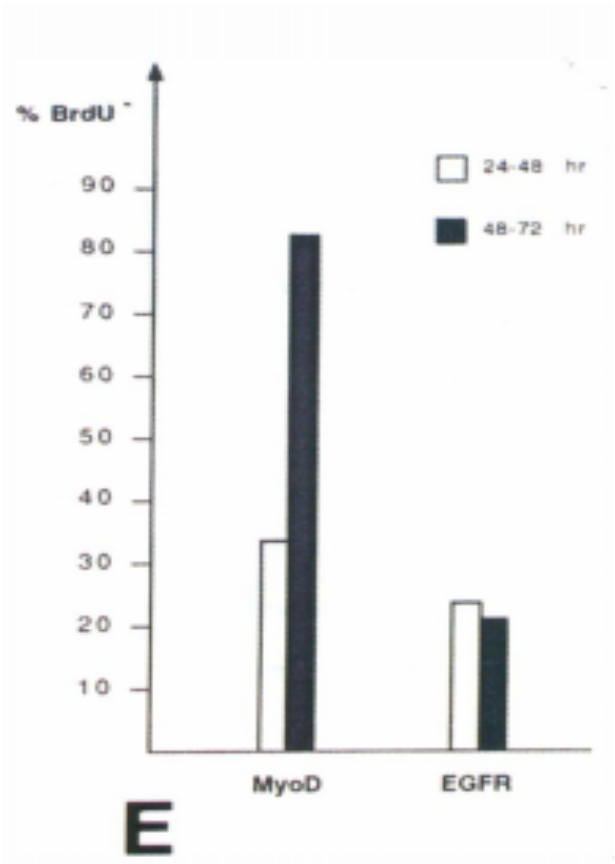
Leland H. Hartwell (cdc mutants in *S. cerevisiae* (“The Hutch”1970),
R. Timothy (Tim) Hunt (Cyclins in sea urchins 1980, ICRF)
and Paul M. Nurse (cdk1 in *S. Pombe* 1987,ICRF)


E2F, (Rb active) inhibits cell division by blocking E2F activity
RB
Dephosphorylation
Cyclin
B/A + CDC2
(CDK1)
M
G2
G1
S
Cell cycle reg genes
GF sensors
20min half life
Cyclin
D's + CDK2
CDK4
CDK5
p21, p27
p16
Cyclin
A + CDK2
RB
Phosphorylatior
Cyclin
E + CDK2

MyoD involved in growth arrest

BrdU incorporation as an index of cell cycle progression

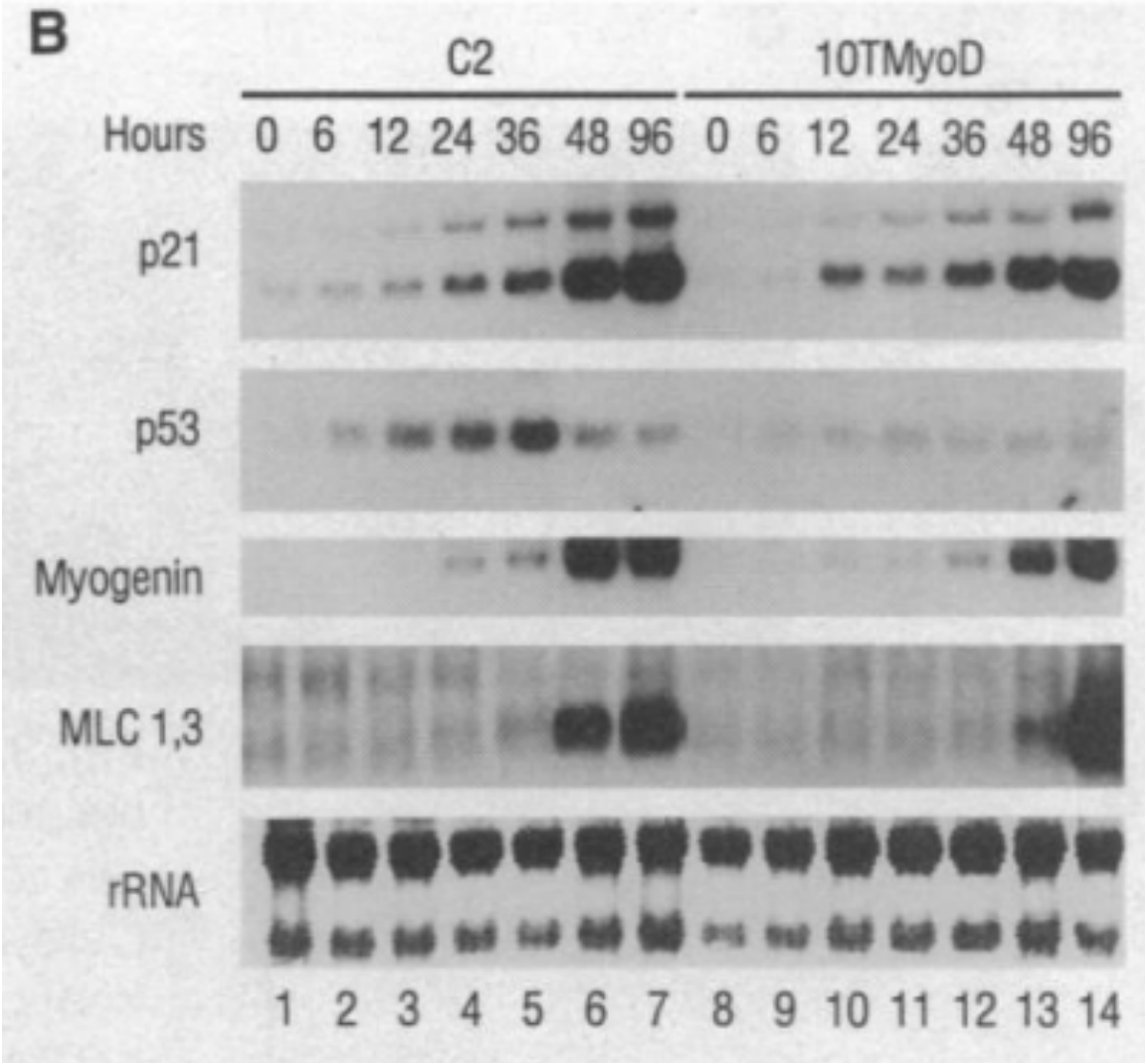




MyoD correlated with BrdU (-)

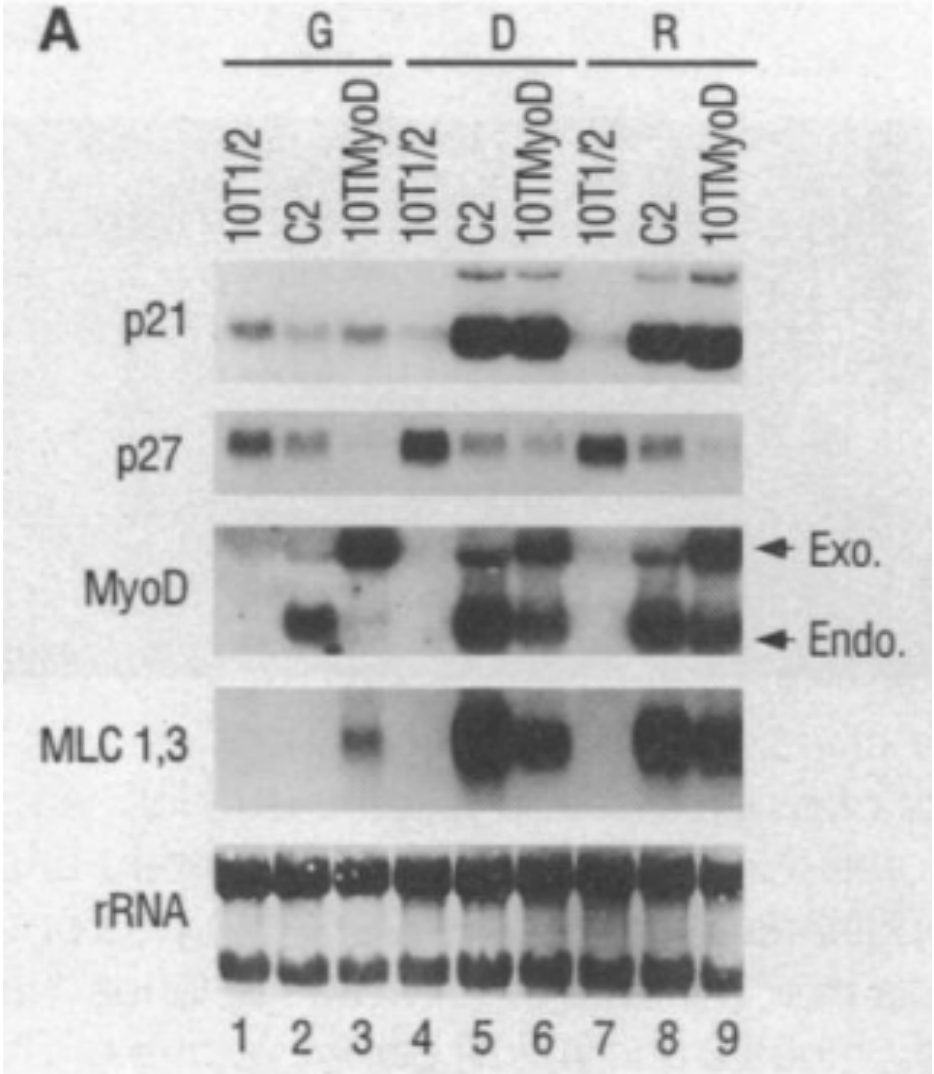
Crescenzi et al. (1990) 87,8442-8446 Proc. Natl. Acad.Sci

Correlation of terminal cell cycle arrest of skeletal muscle with induction of p21 by MyoD



Halevy et al. (1995) Science Vol. 267: 1018-1021

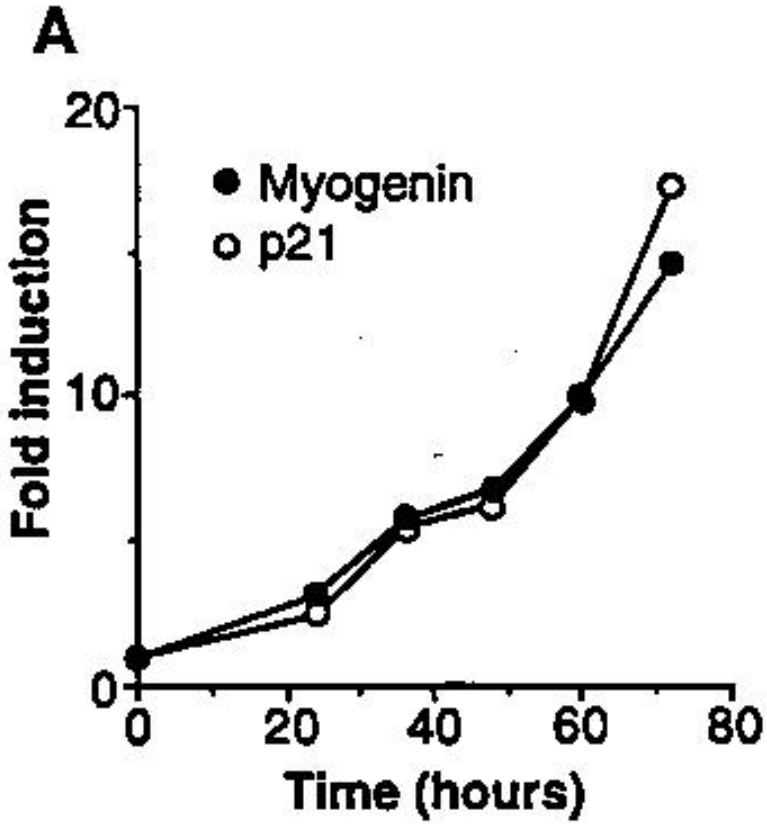
Correlation of terminal cell cycle arrest of skeletal muscle with induction of p21 by MyoD



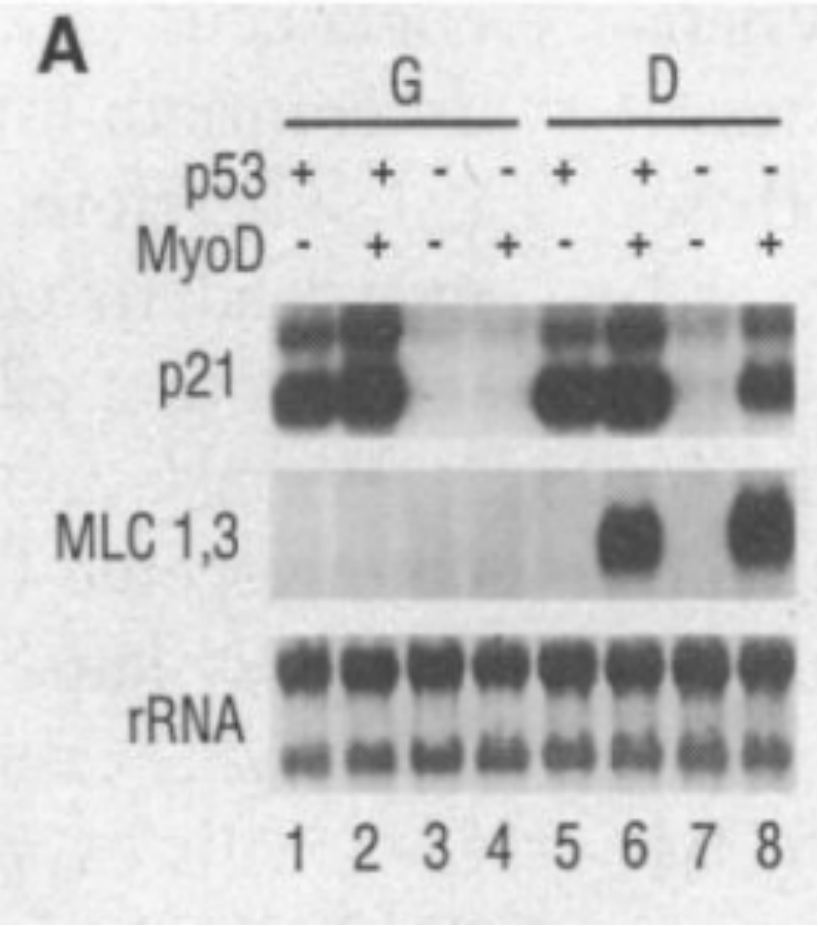
Halevy et al. (1995) Science Vol. 267: 1018-1021

Cell type	MyoD $^{+}$ cells		MyoD $^{-}$ cells	
	p21^{+} %	Brd U^{-} %	p21^{+} %	Brd U^{-} %
CV1	46.7	50.3	1.5	1.9
U205	13.0	14.5	5.2	3.2

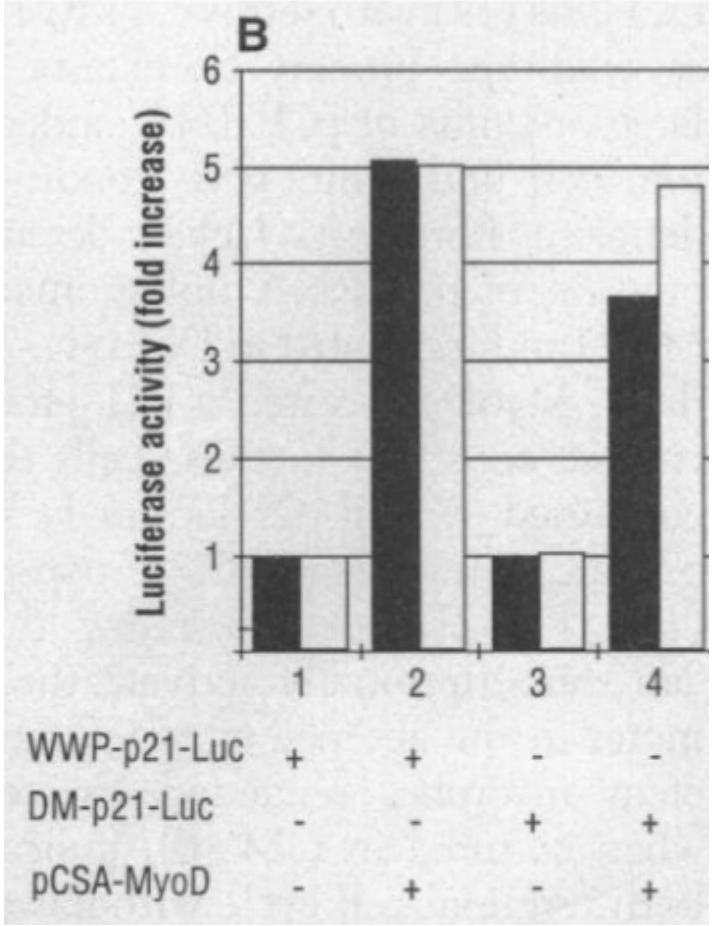
Induction of p21 mRNA during myoblast differentiation *in vitro*



Induction of p21 by MyoD in the absence of p53

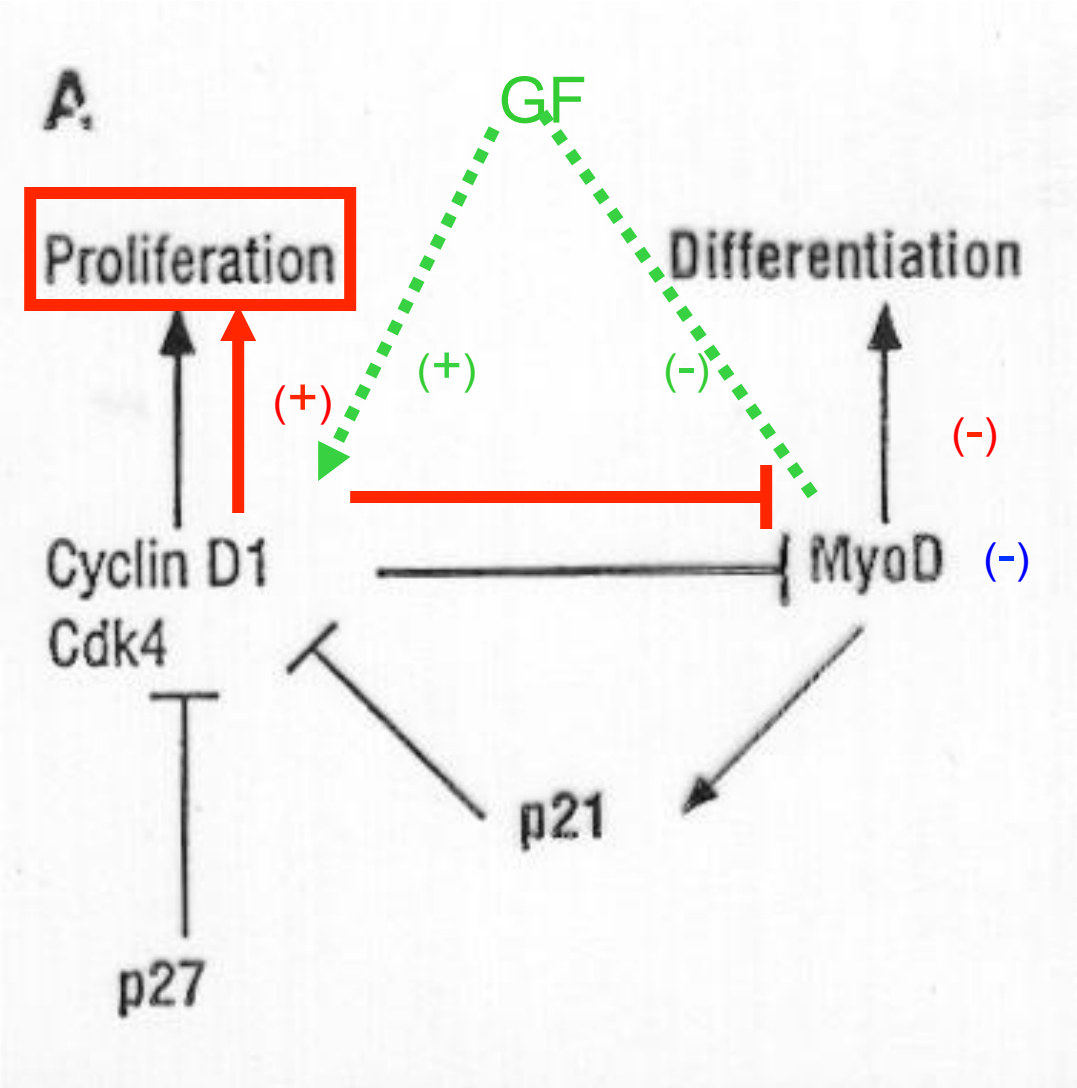


Northern analysis of p21 expression in wild type (lanes 1,2,5, & 6), and p53-deficient (lanes 3, 4, 7, & 8).

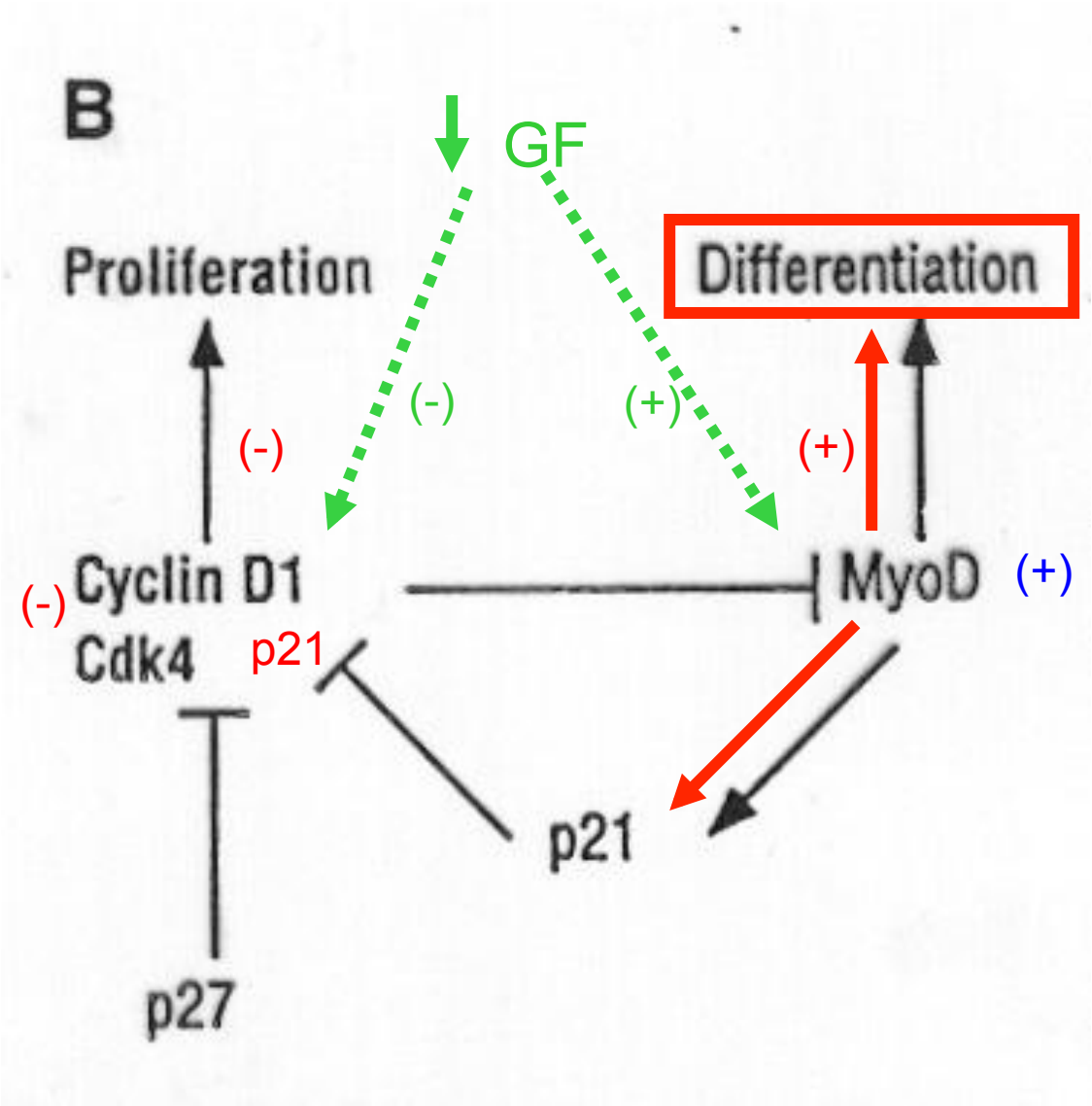


Transcriptional activation of p21 promoter constructs by MyoD.

A positive feedback loop maintains the proliferative state in myoblasts in growth media



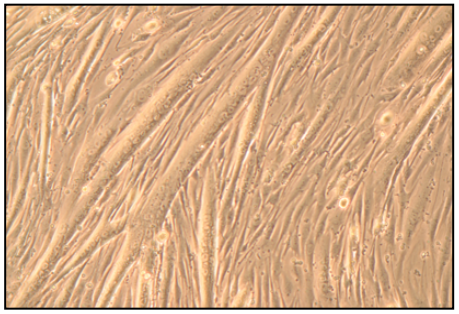
A positive feedback loop maintains permanent cell cycle withdrawal of differentiated myocytes



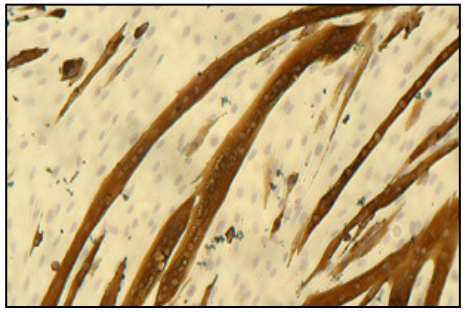
The end

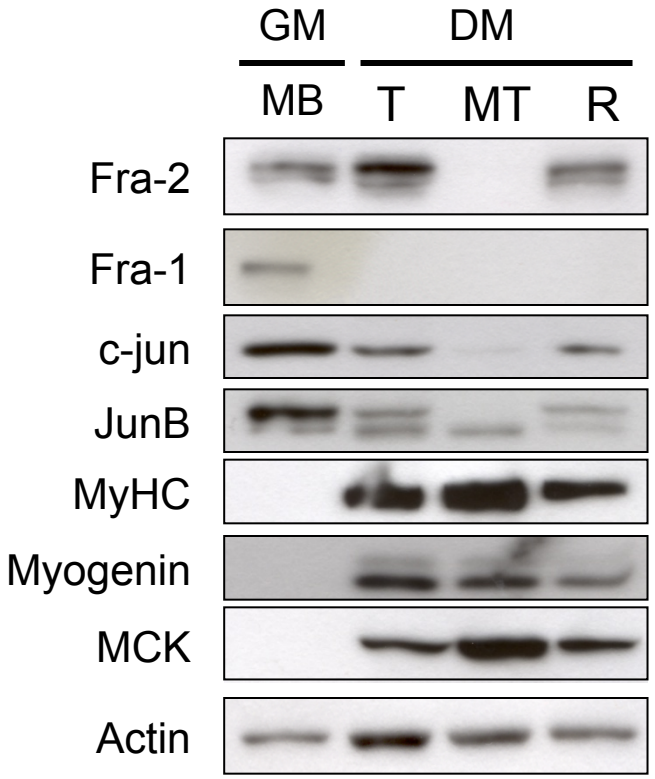
Fra-2 expression is restricted to mononucleated cells in differentiation conditions

Cells in culture



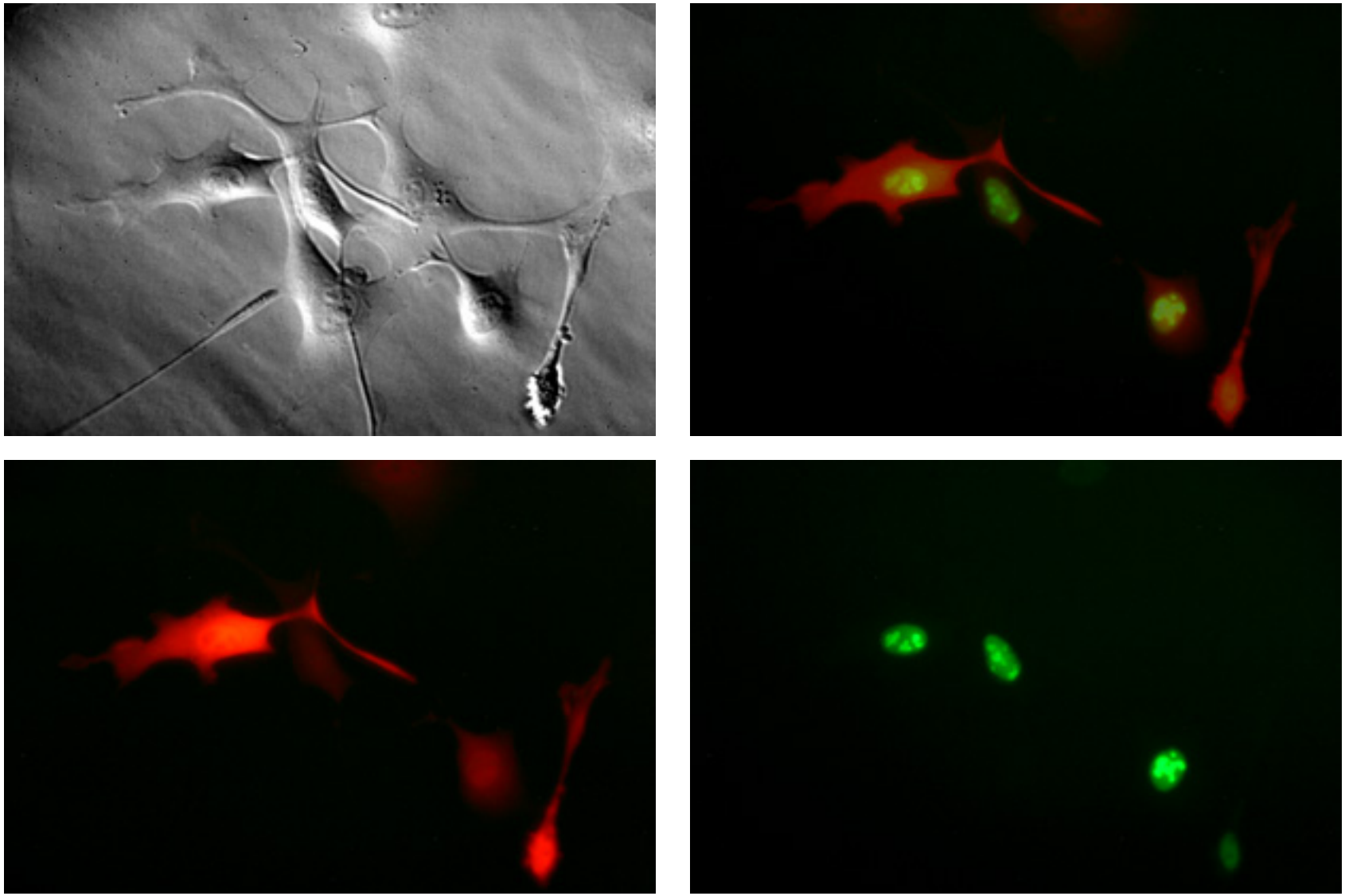
Immunocytochemistry





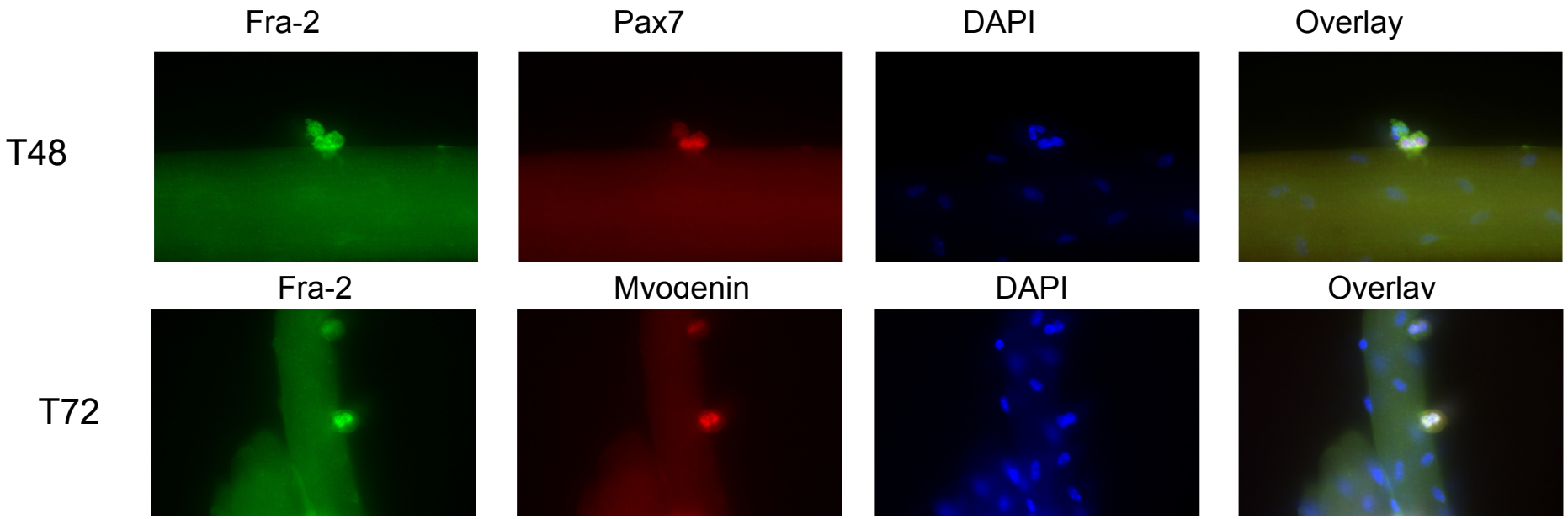
T = total, MT = MT only, R = reserve cells

EYFP-c-Jun

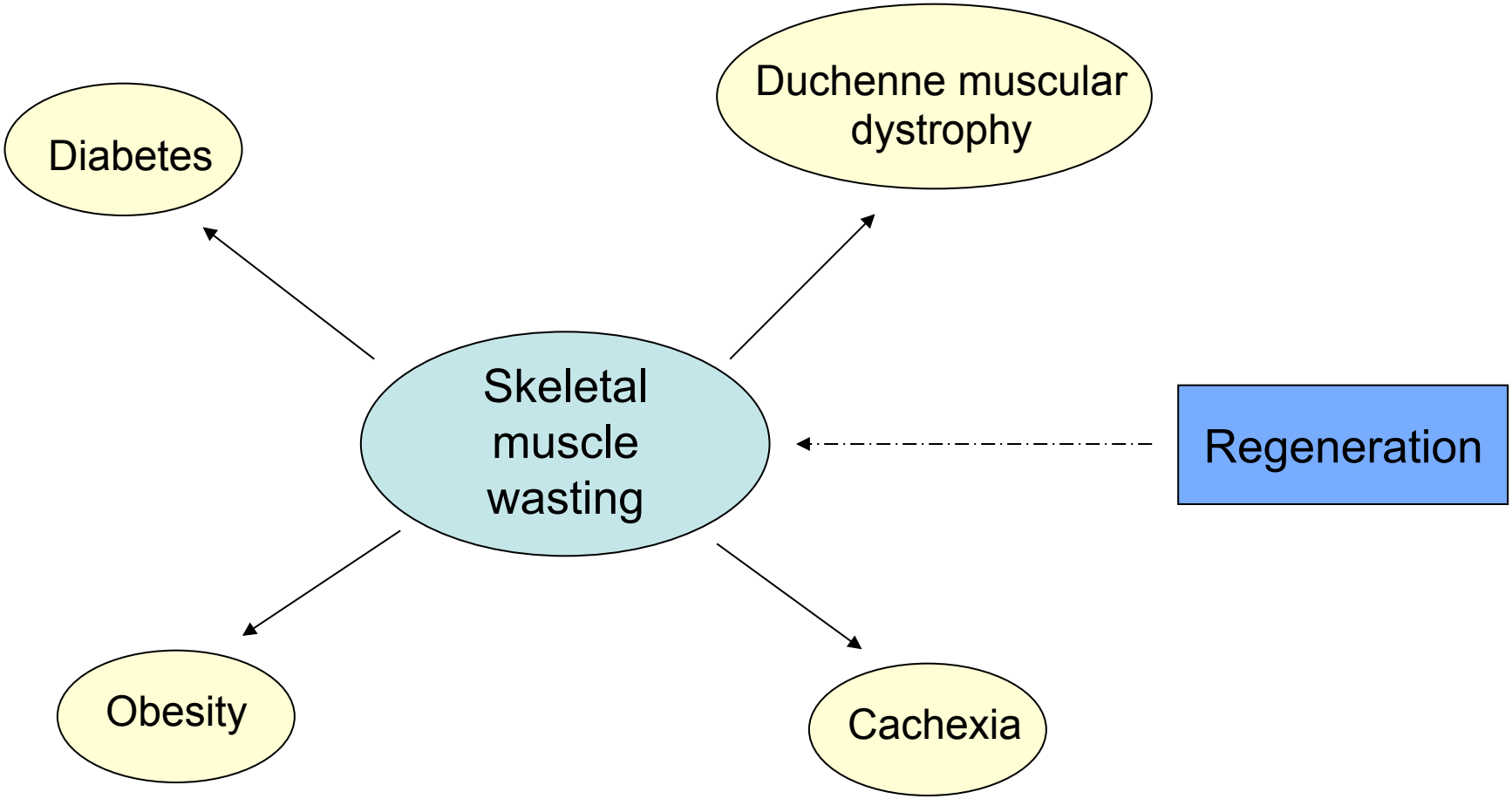


C2C12 in GM T. Miyake

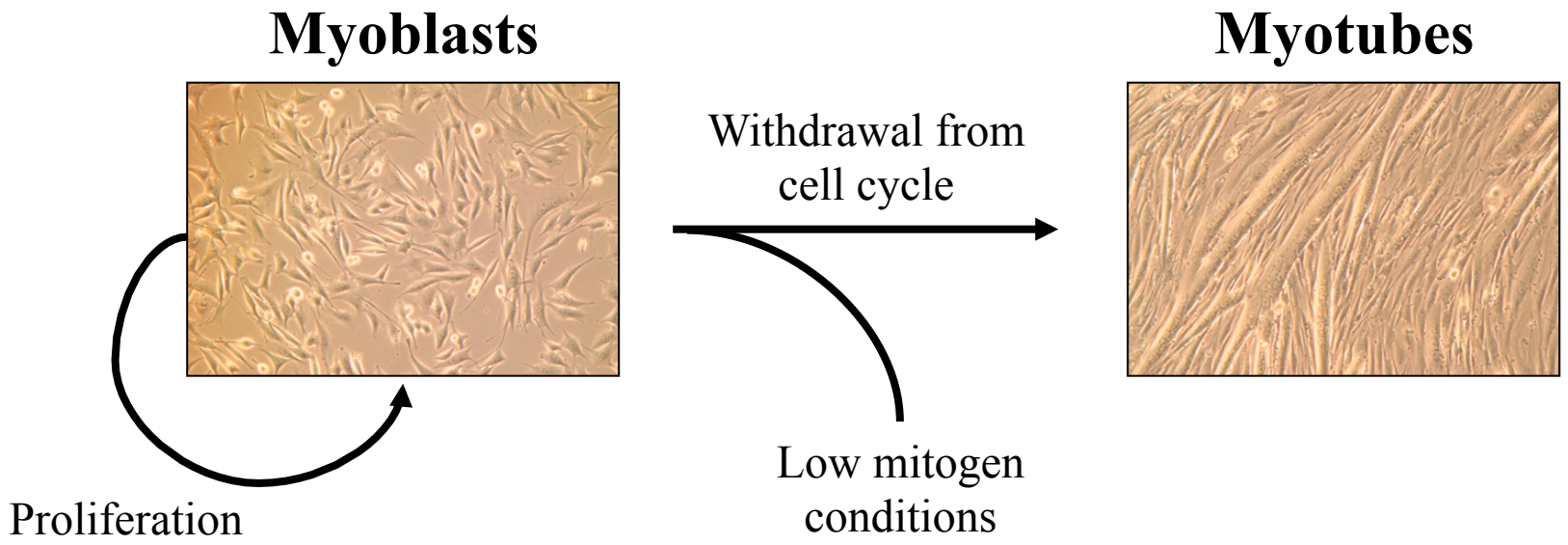
Fra-2 is expressed in Satellite cells



Dr. Henry Collins-Hooper (University of Reading)


Diabetes
Duchenne muscular dystrophy
Skeletal muscle wasting
Regeneration
Obesity
Cachexia

C2C12 cell line: Model for myogenesis



MyoD → MEF2

Myf5

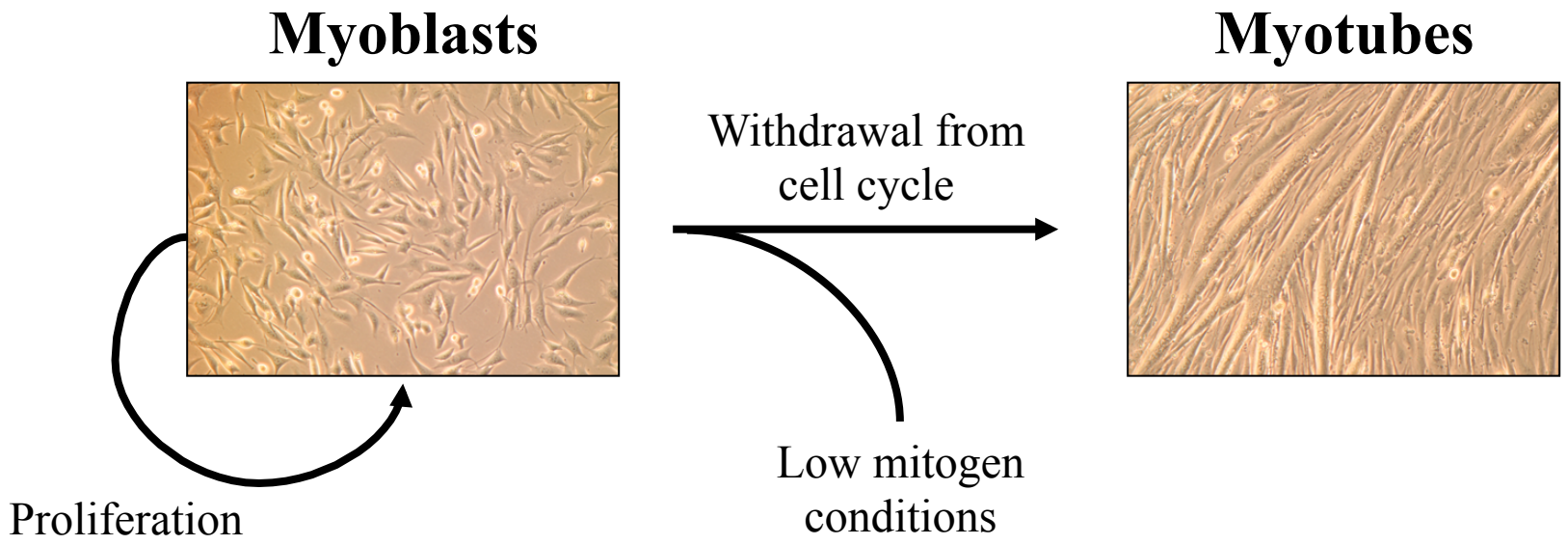
Myogenin

MRF4

Smad

AP-1

C2C12 cell line: Model for myogenesis



MyoD → MEF2

Myf5

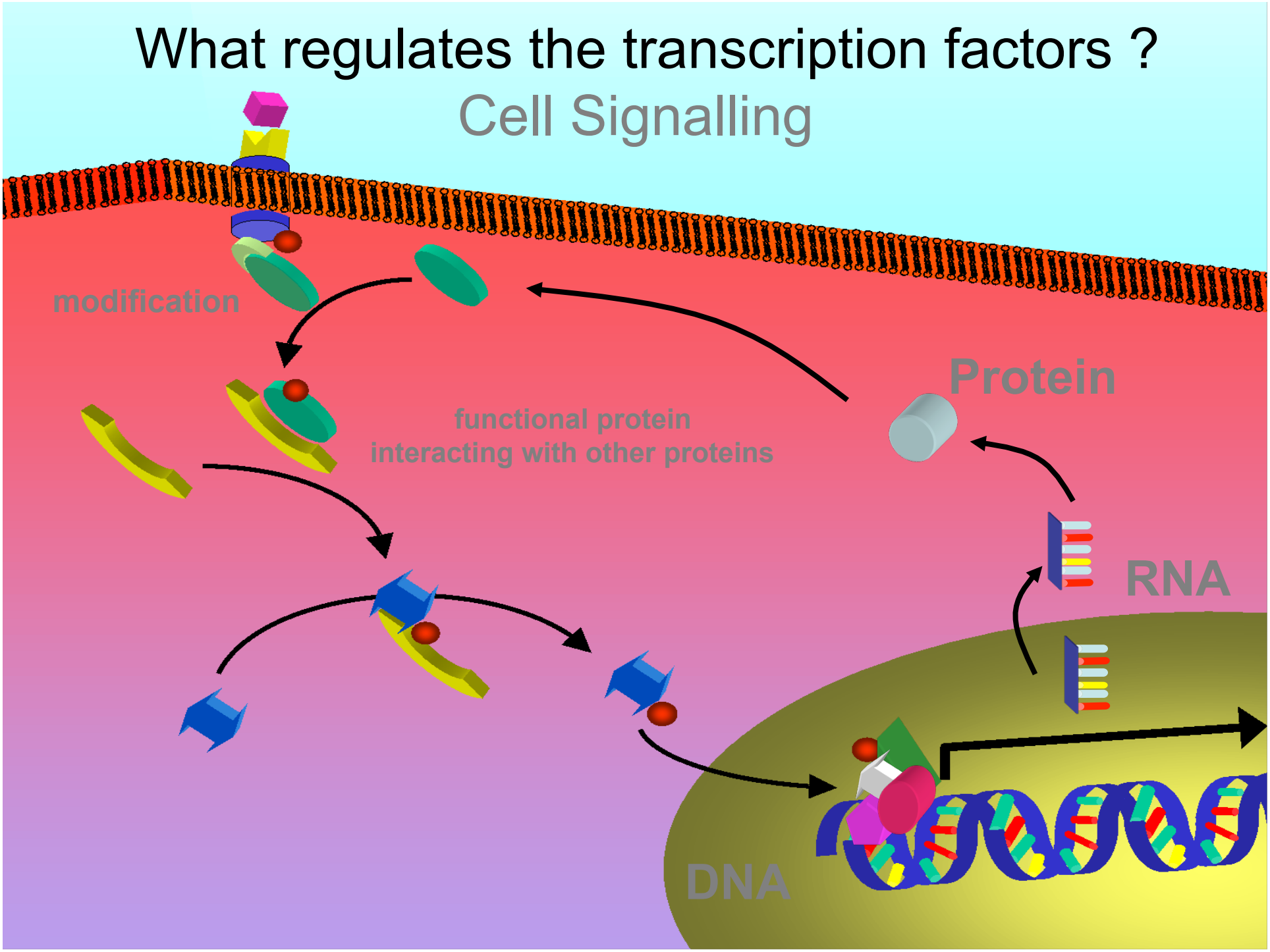
Myogenin

MRF4

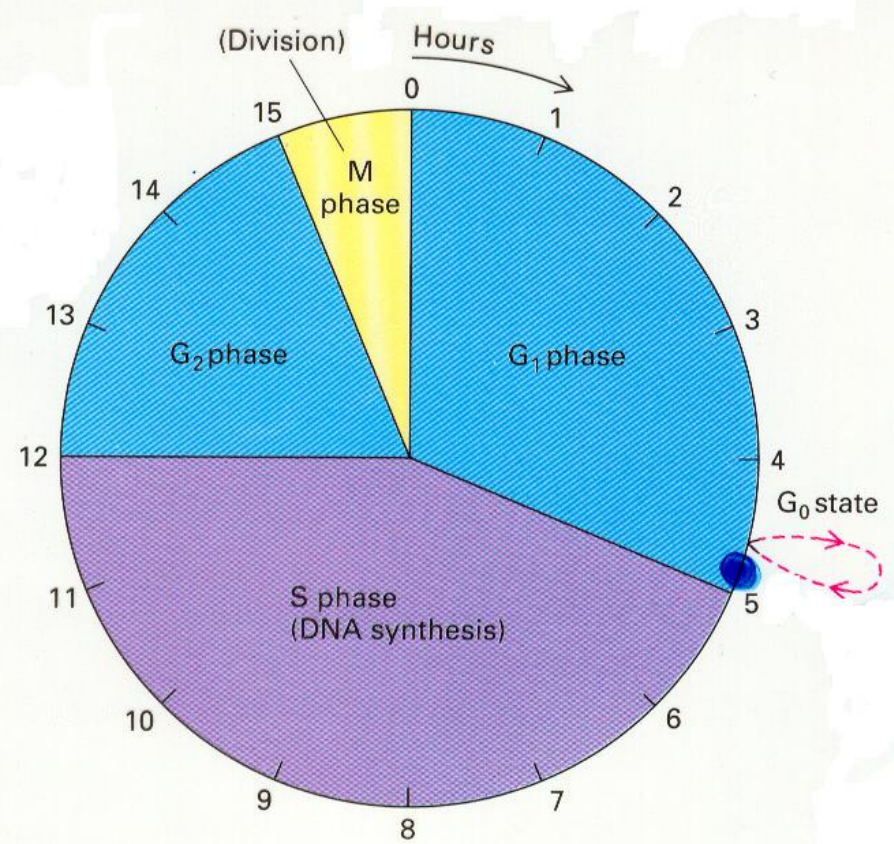
Smad

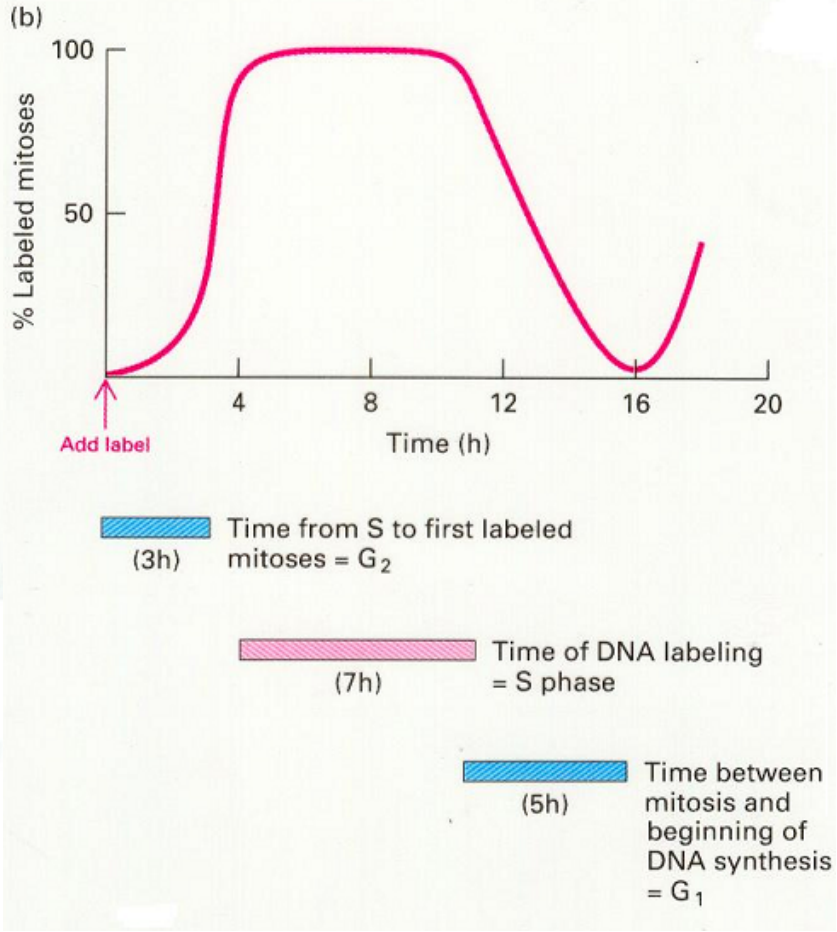
AP-1

What regulates the transcription factors ?

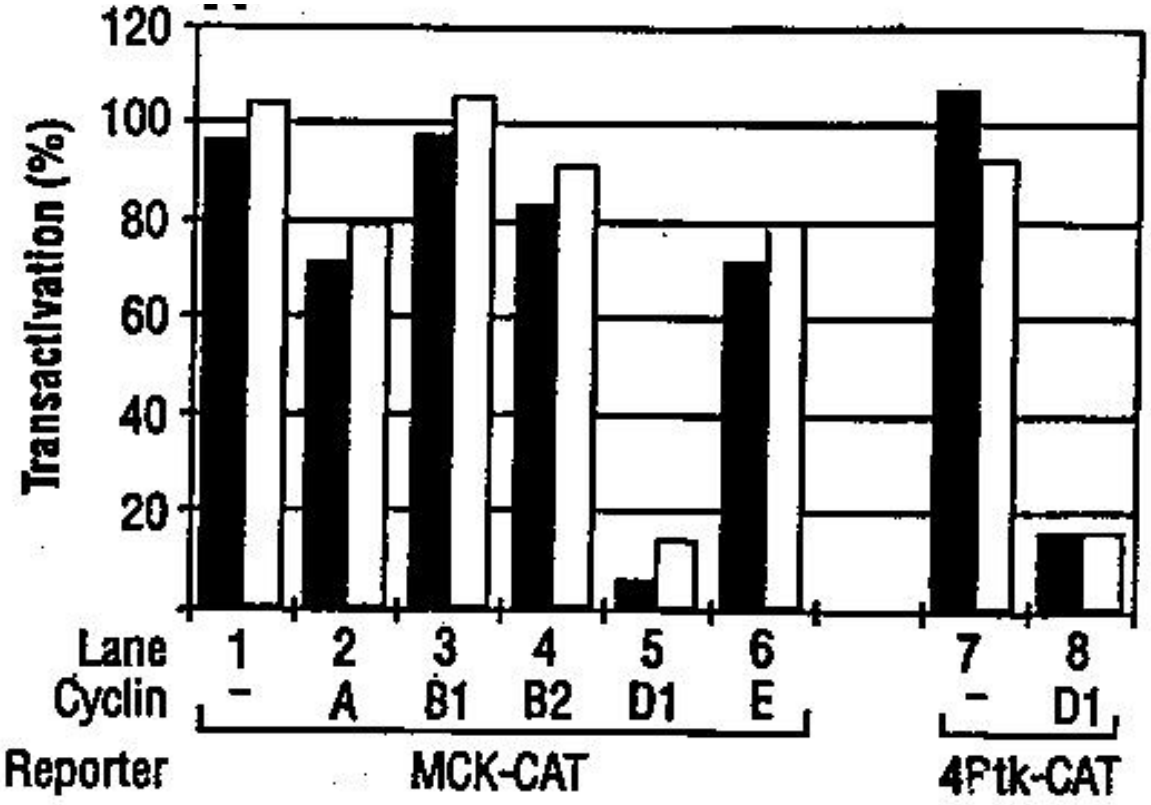


The cycle in a mammalian cell having a generation time of 16 hours

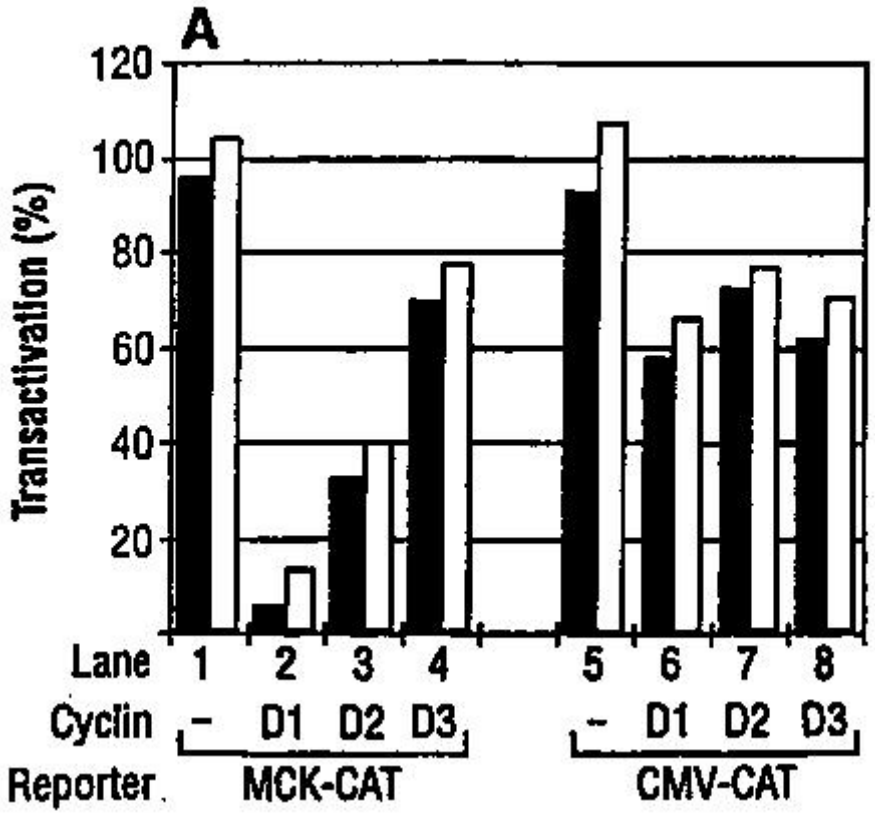




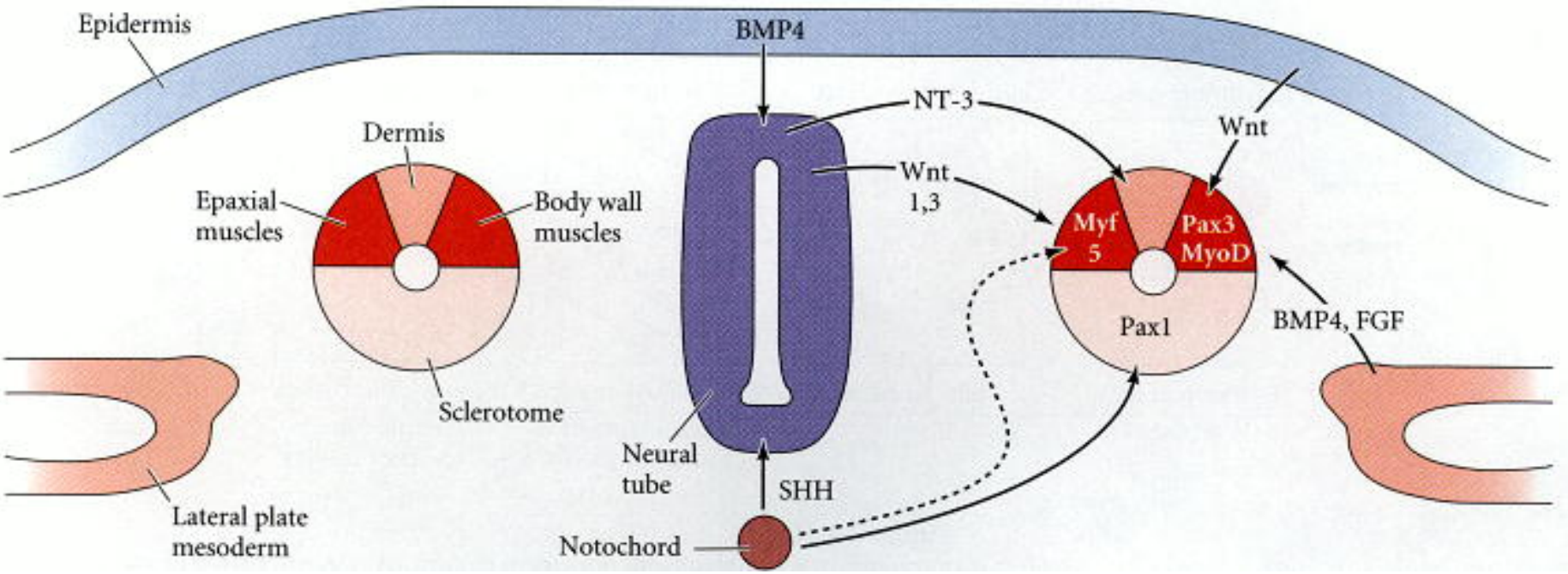
Inhibition of myogenic differentiation in myoblasts by cyclin D1- dependent kinase

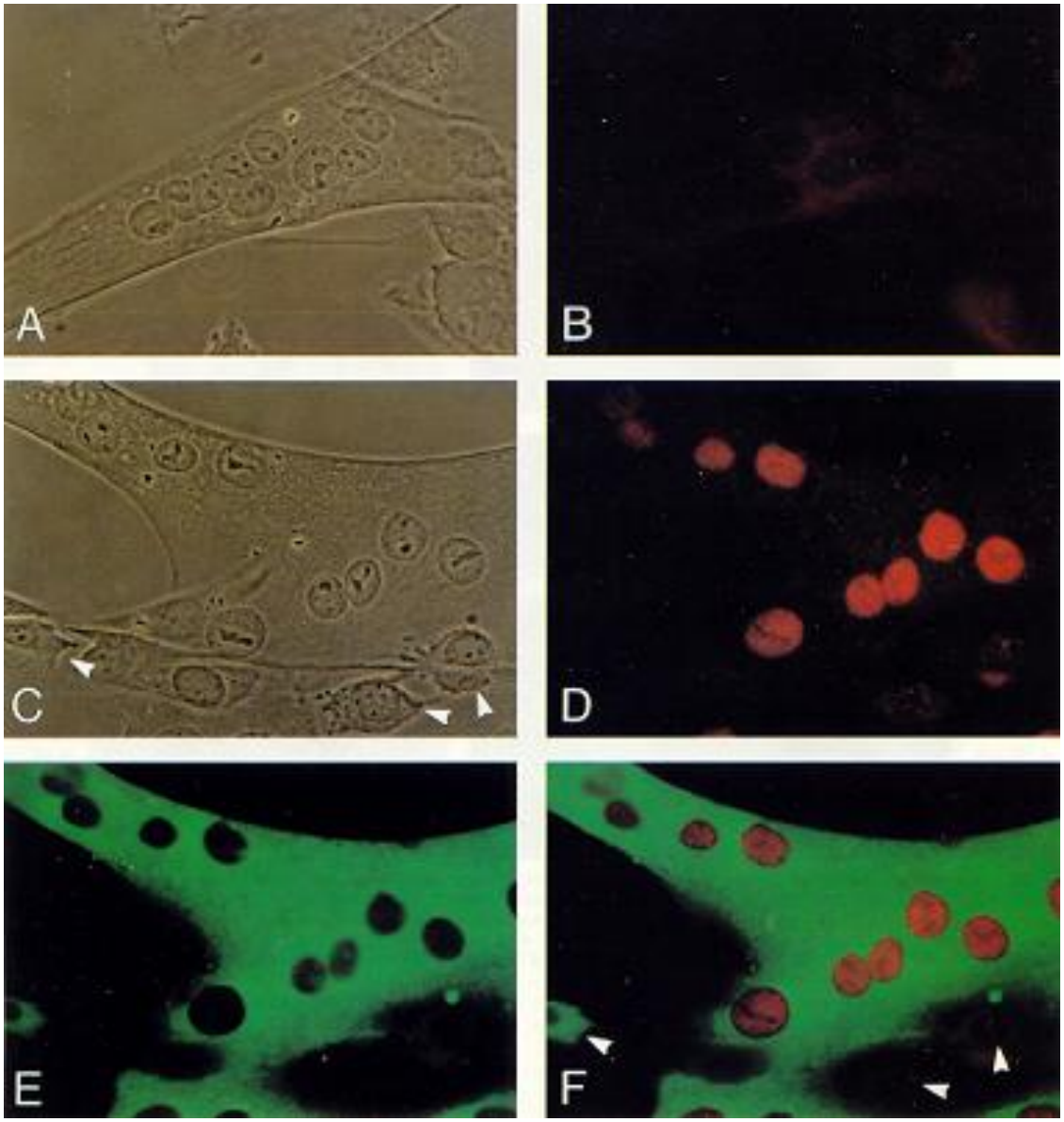


Differential inhibition of MyoD function



Model of major postulated interactions in the patterning of the somite:
Signaling between NT, NC and somite




A
B
C
D
E
F

Transfection: DNA transfer into cultured mammalian cells

- Microinjection
- Electroporation
- Viral Transfer
- Calcium Phosphate
- Lipid
  - lipofectamine
- Cationic polymer
  - Turbofectamine