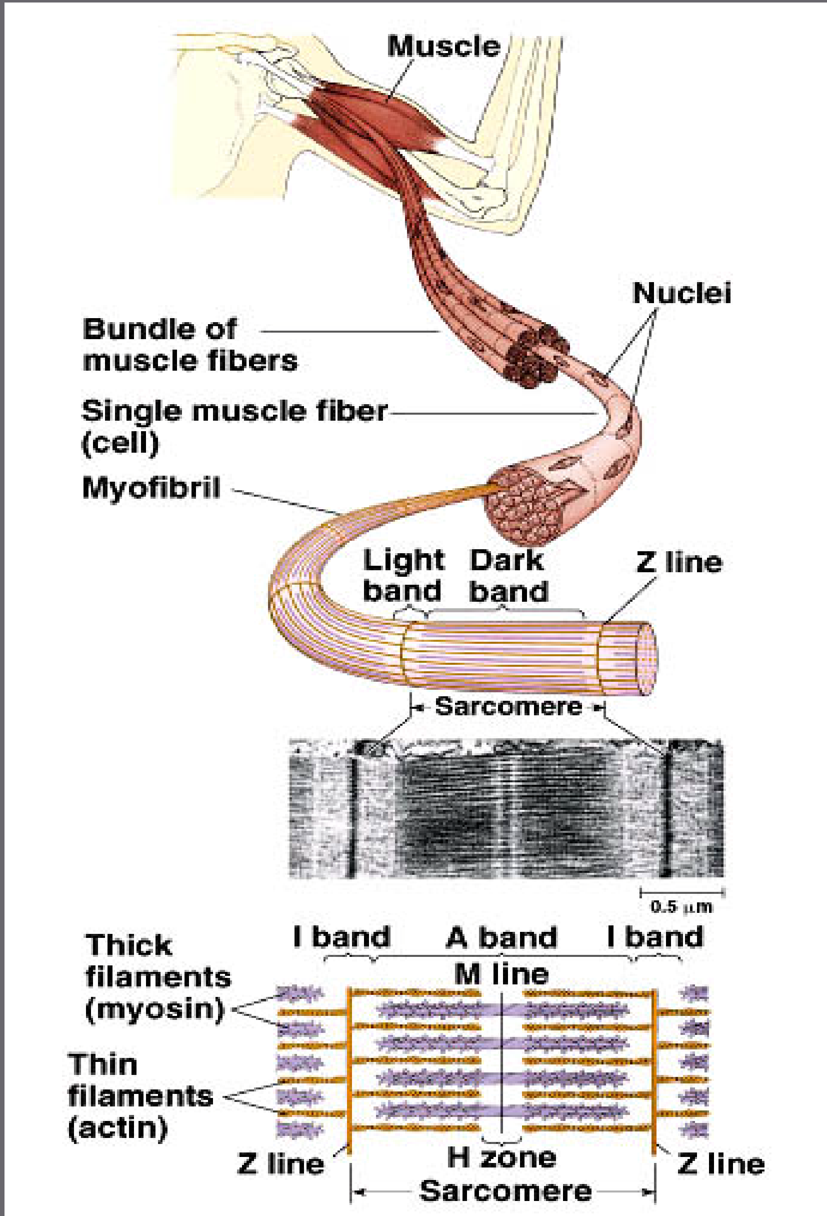
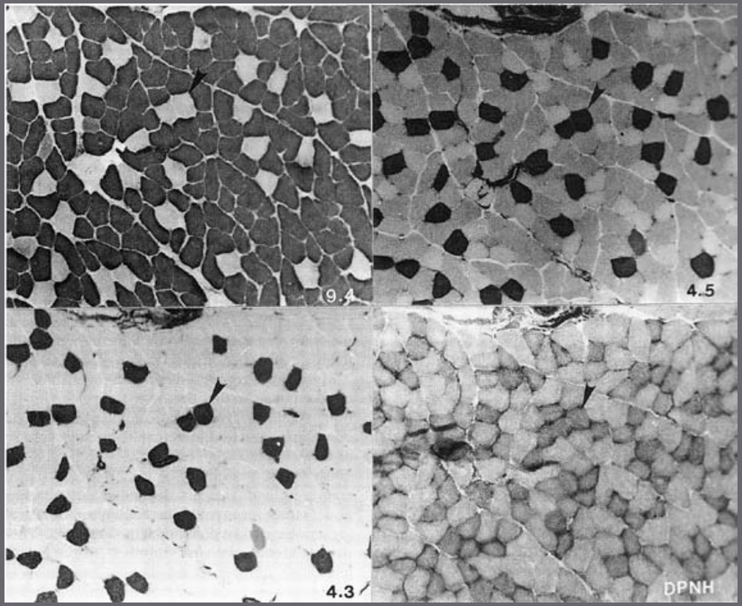


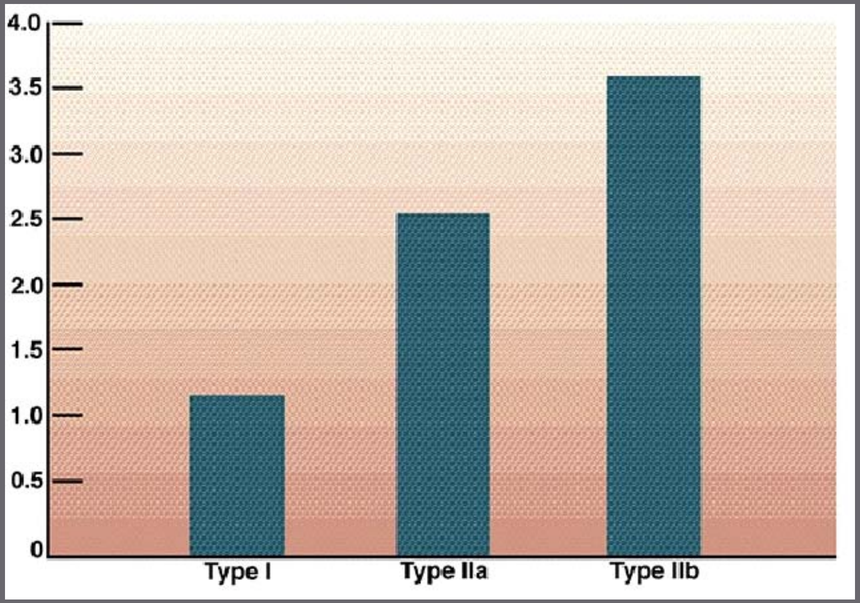
Muscles, muscle fibres and myofibrils

Fast and slow twitch fibres

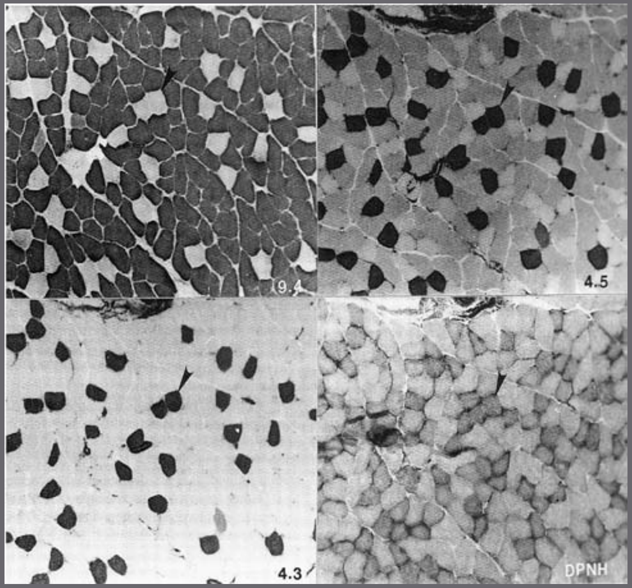
Rat hindlimb muscle – ATPase staining
at different pH and NADH



Muscle fibre shortening velocity
lengths/second



Properties of Muscle Fiber Types



Fast fibers **Slow fibers**

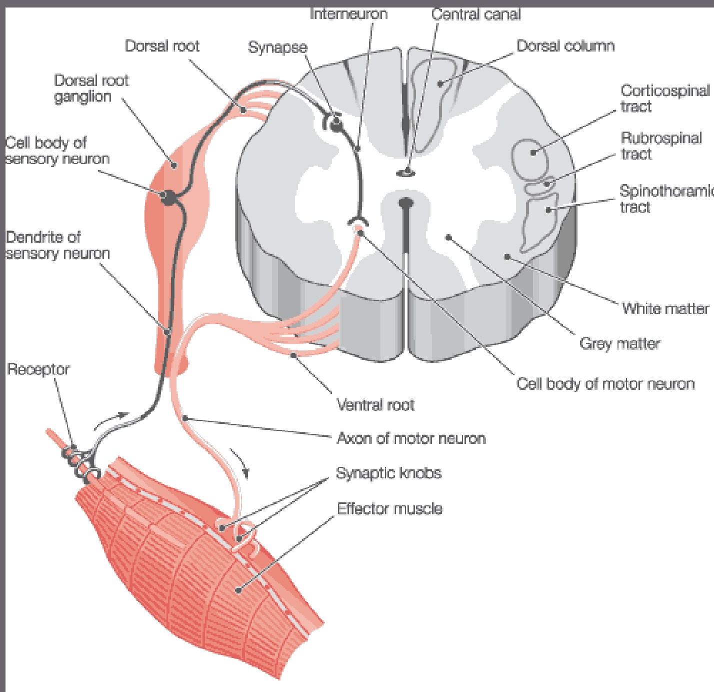
Characteristic	**IIb**	**IIx**	**IIa**	**Type I**
V_{max} (speed of shortening)	Highest	Intermediate		Low
Resistance to fatigue	Low	High/moderate		High
Predominant energy system	Anaerobic	Combination		Aerobic
Myoglobin	Low	Medium		High
Capillary density	Low	Medium		High

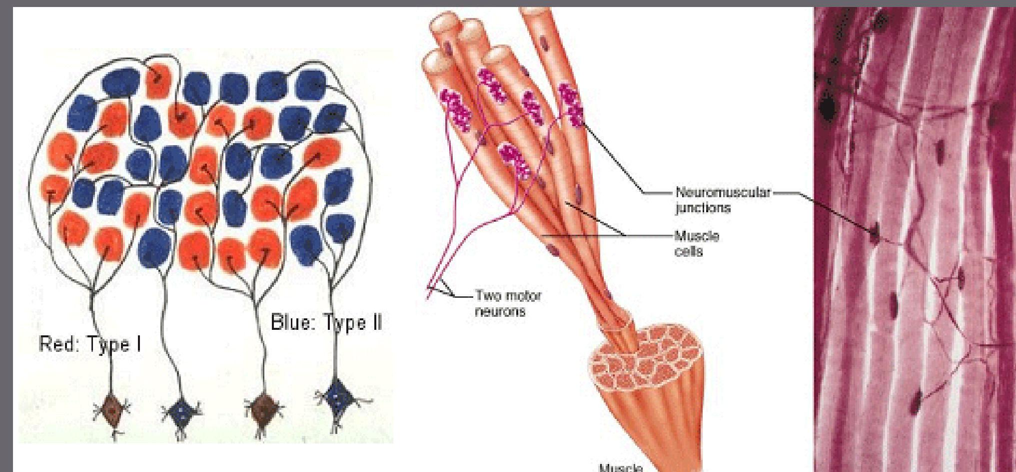
Fibre-specific genes and their expression pattern in adult striated muscle

Gene family	Gene expressed				
	Slow	Fast			Heart
	I	IIA	IIX/D	IIB	
Myosin heavy chain	MyHCI/slow/β	MyHC2A	MyHC2X	MyHC2B	MyHCI/slow/β MyHCα
Myosin light chain 1	MLC1SA MYL3	MYL1			MYL4 MYL3
Myosin light chain 2	MYL2	MYL5			MYL7 MYL2
Troponin C	TNNC1	TNNC2			TNNC1
Troponin T	TNNT1	TNNT3			TNNT2
Troponin I	TNNI1	TNNI2			TNNI3

Motor control of muscle fibres

Motor unit – the α-motor neuron and all the fibres under its control





Motor units
may control <5 muscle fibres in the eye or small hand muscles or >2000 fibres in the gastrocnemius

Importance of muscle fibre types

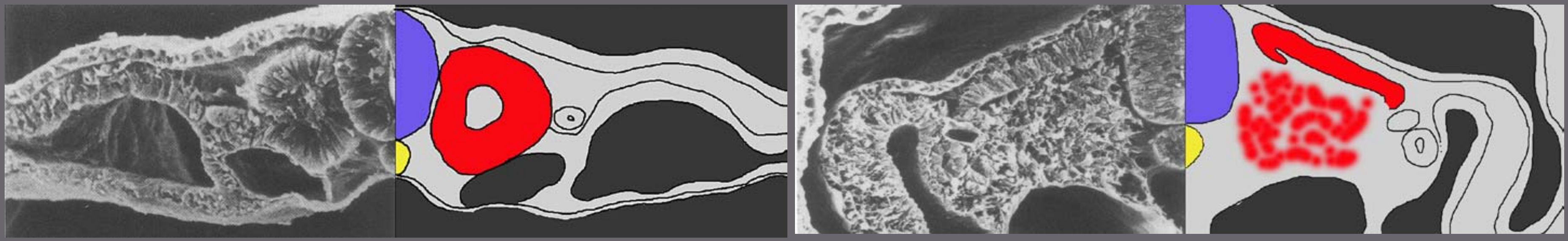
Athletic performance – marathon runners versus sprinters

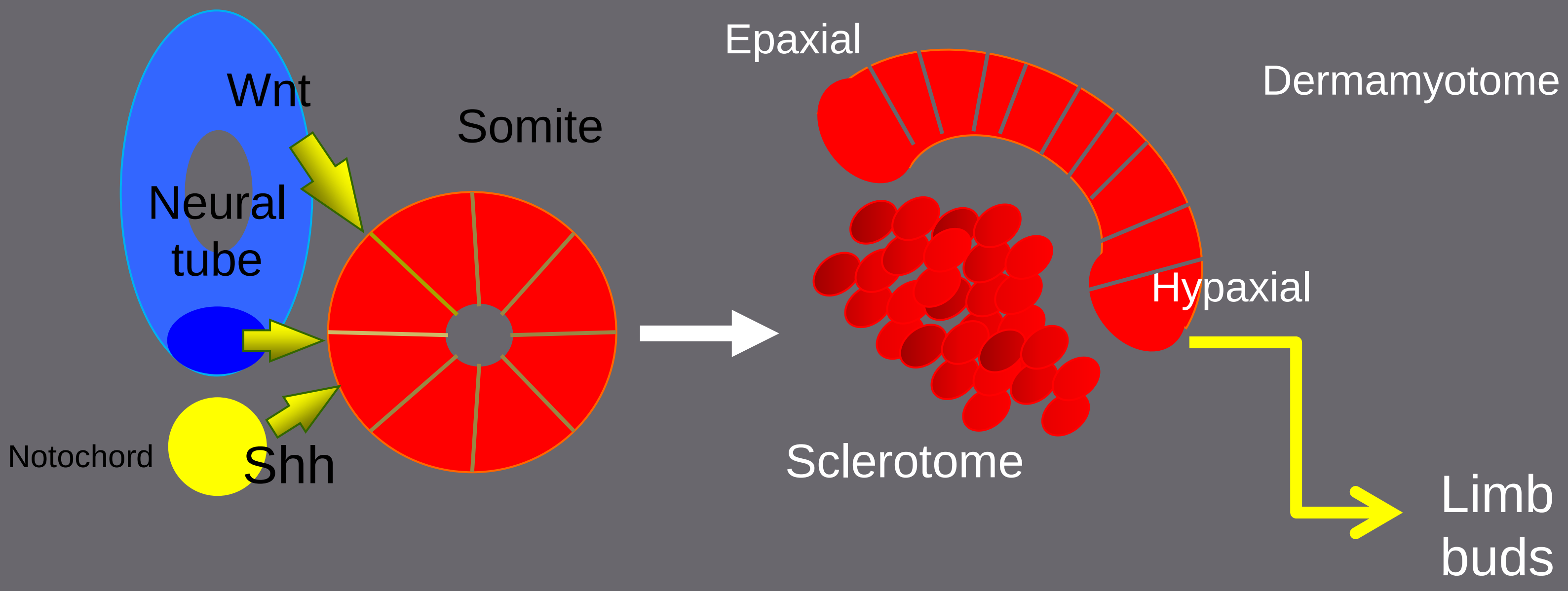
Ageing – preferential reduction of fast fibres in sarcopenia

Disease – preferential loss of fast fibres in Duchenne muscular dystrophy; complete absence of fast fibres in some nemaline myopathy patients.

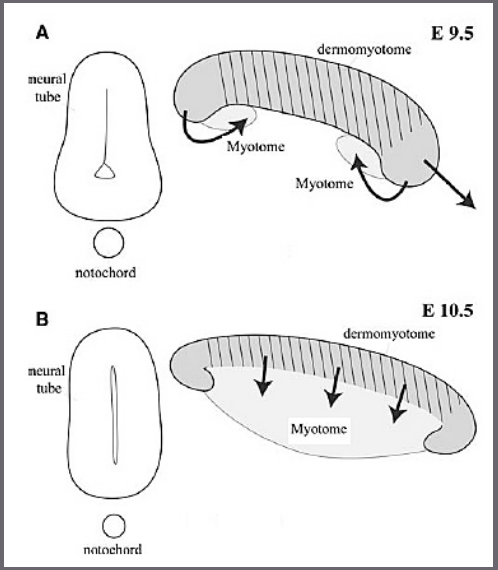
Atrophy responses – reduction of slow fibres in response to bed-rest, space flight and spinal cord injury.

The origin of embryonic myoblasts in the chick





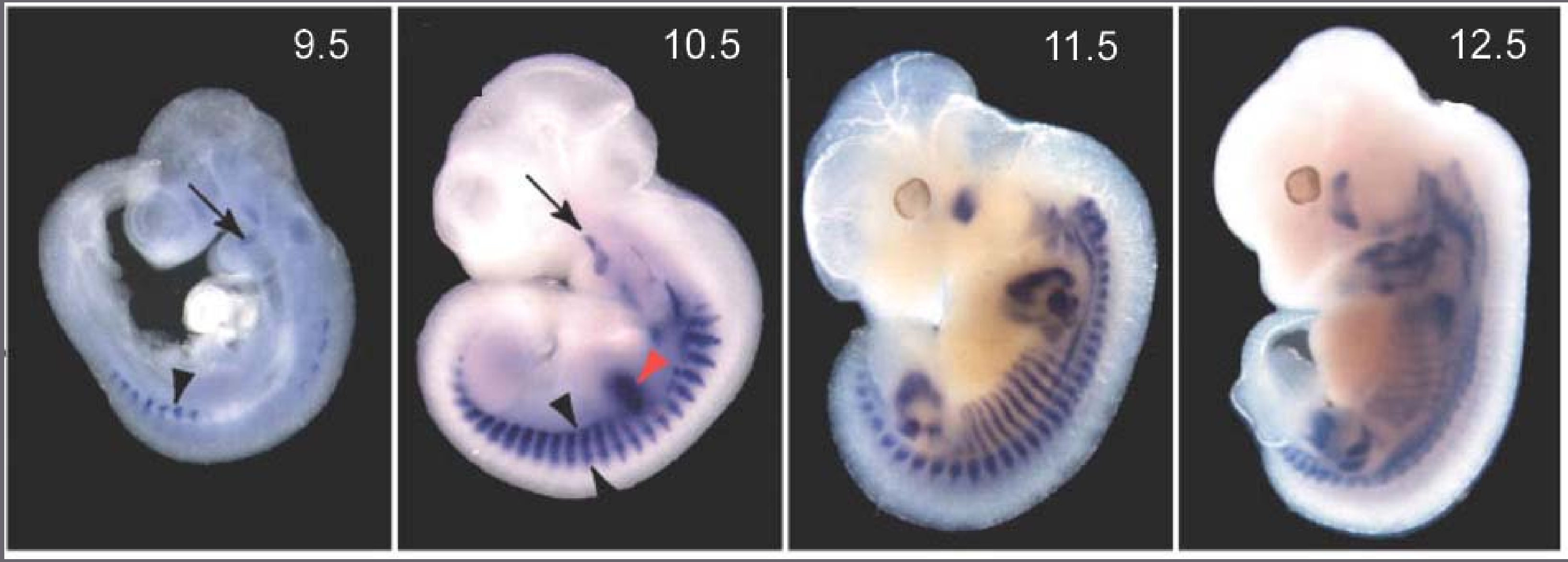
Myogenesis in the mouse



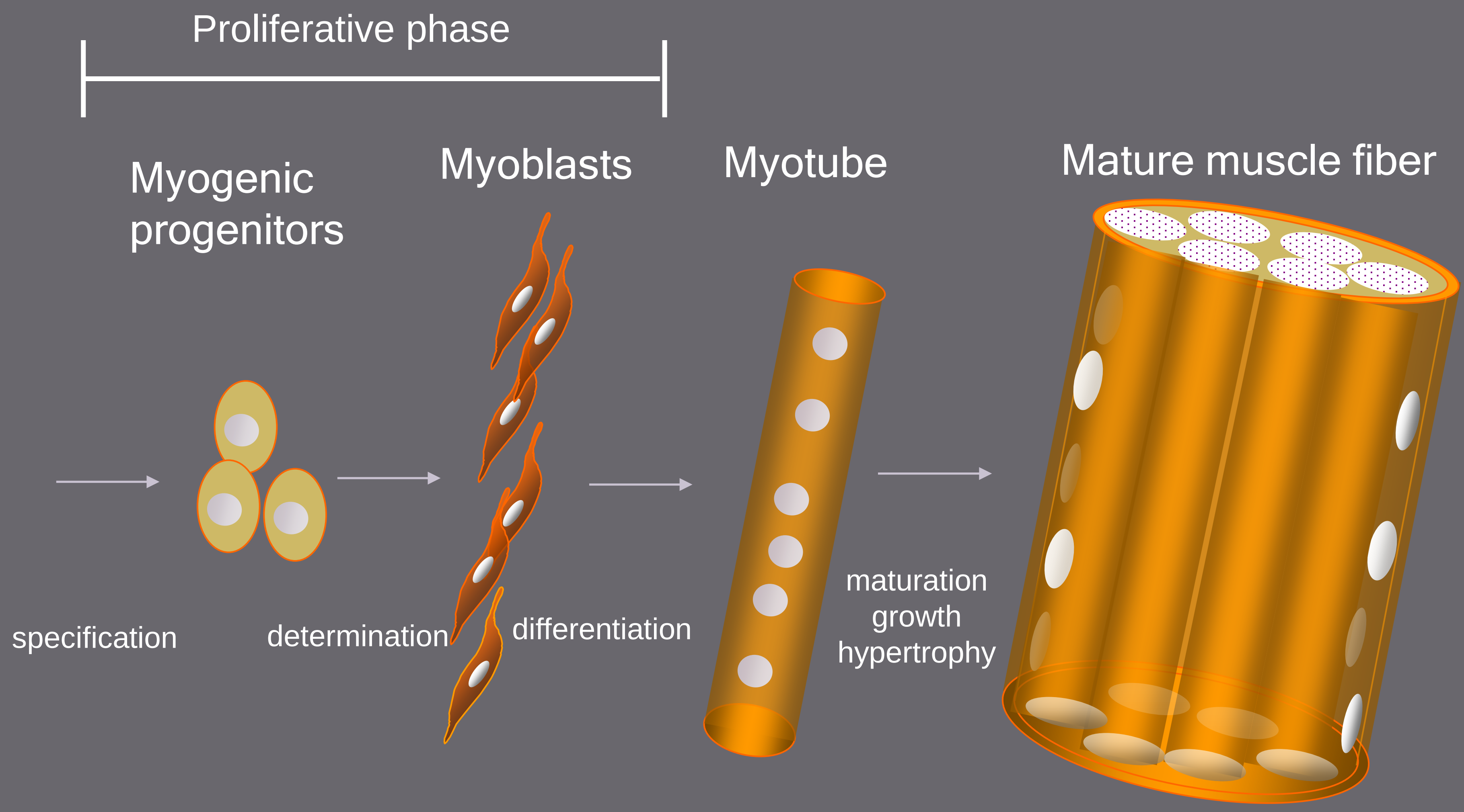
Formation of the myotome

Muscle progenitors delaminate from the edges of the dermamyotome to form the myotome. Some cells migrate into the limb buds. At E10.5 the dermamyotome disintegrates centrally and the main myotome is formed

Expression of the myogenic regulatory factor (MRF) gene MyoD



Myogenesis



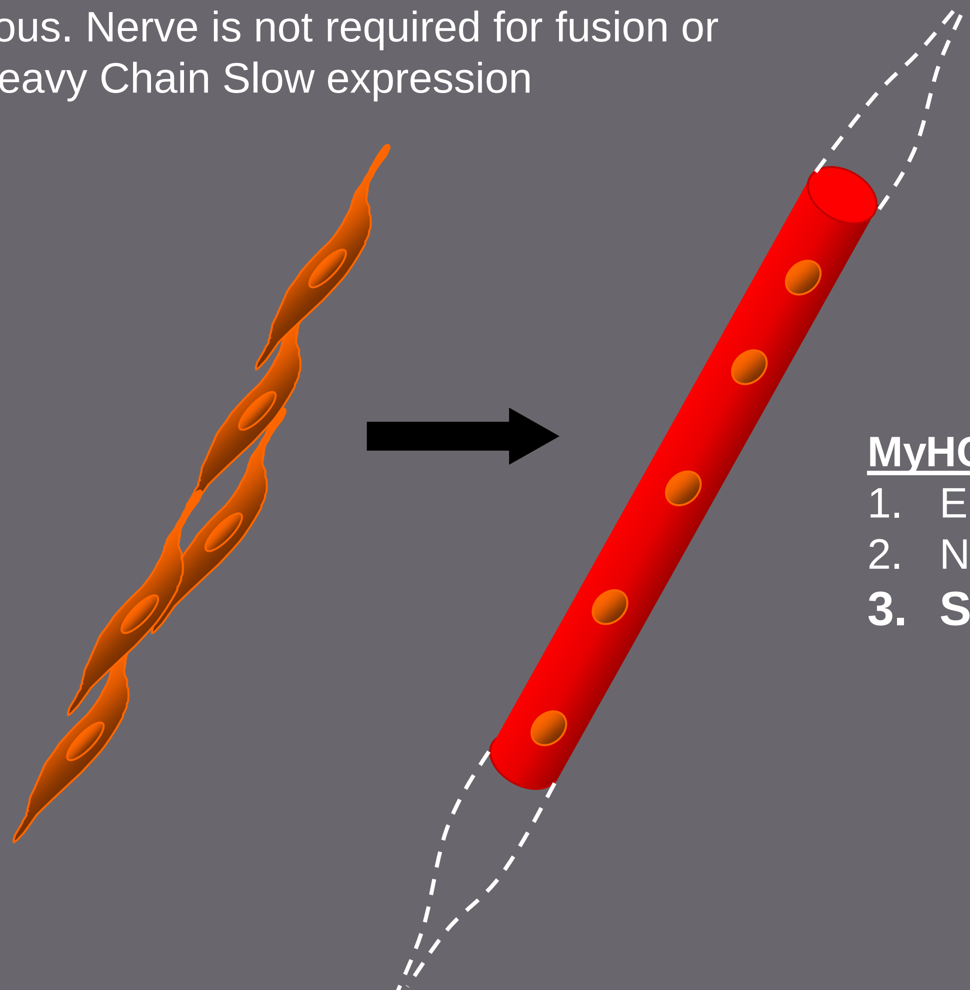
Proliferative phase
Myogenic progenitors
Myoblasts
Myotube
Mature muscle fiber
specification
determination
differentiation
maturation
growth
hypertrophy

Myoblast differentiation in culture

Myoblast $\xrightarrow{\textbf{Differentiation}}$ Myocyte $\xrightarrow{\textbf{Fusion}}$ Myotube

Differentiation of **primary** myotubes in the mouse hind-limb (12-14 dpc) and the beginning of fibre type patterning

Fusion of myoblasts is ordered and synchronous. Nerve is not required for fusion or Myosin Heavy Chain Slow expression



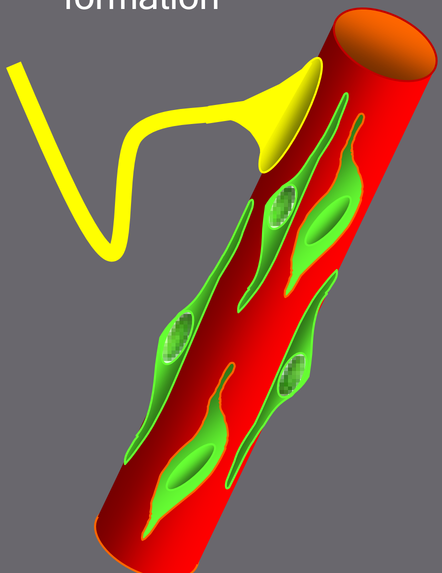
Tendon Attachment

MyHC expression

1. Embryonic
2. Neonatal
3. **Slow**

Secondary myotube formation - mouse hindlimb 14dpc - birth and continuing fibre type <u>patterning</u>

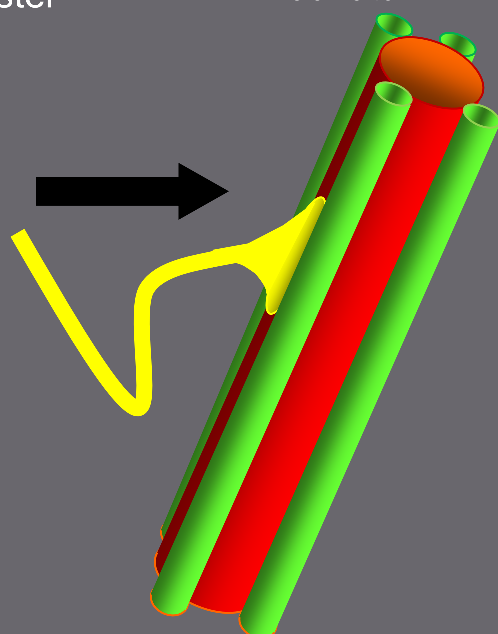
<u>14-16 dpc</u> - Pioneer motor axons contact primary myotubes. Necessary for survival of myotube and secondary myotube cluster formation



Secondary myotubes form in Clusters around primaries.

<u>MyHC gene expression</u>

1. Embryonic
2. Neonatal

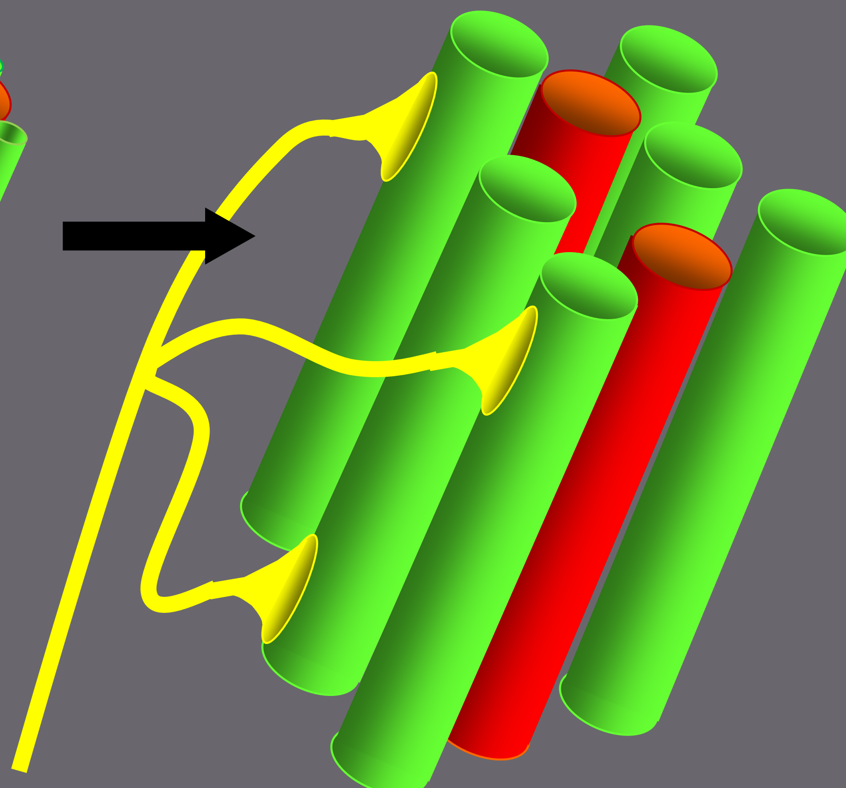


Late fetal stage- clusters disperse.

<u>MyHC gene expression</u>

Primaries - slow MyHC

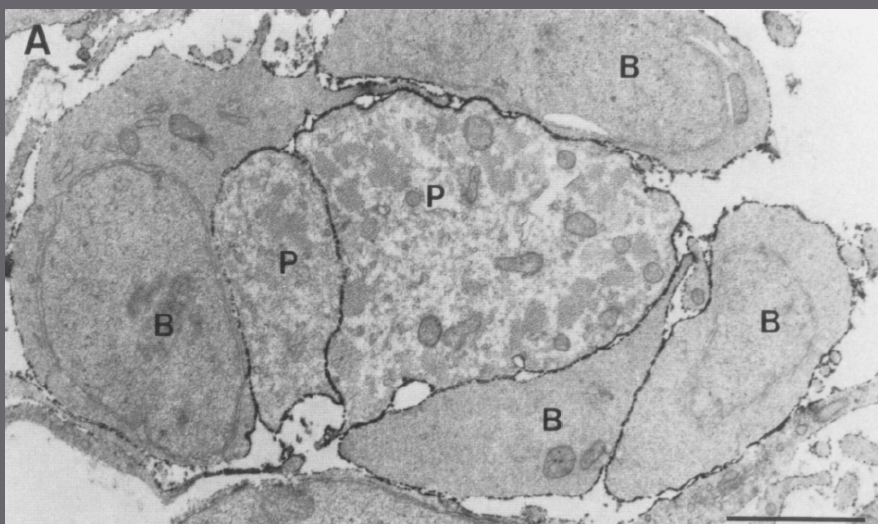
Secondaries - neonatal MyHC

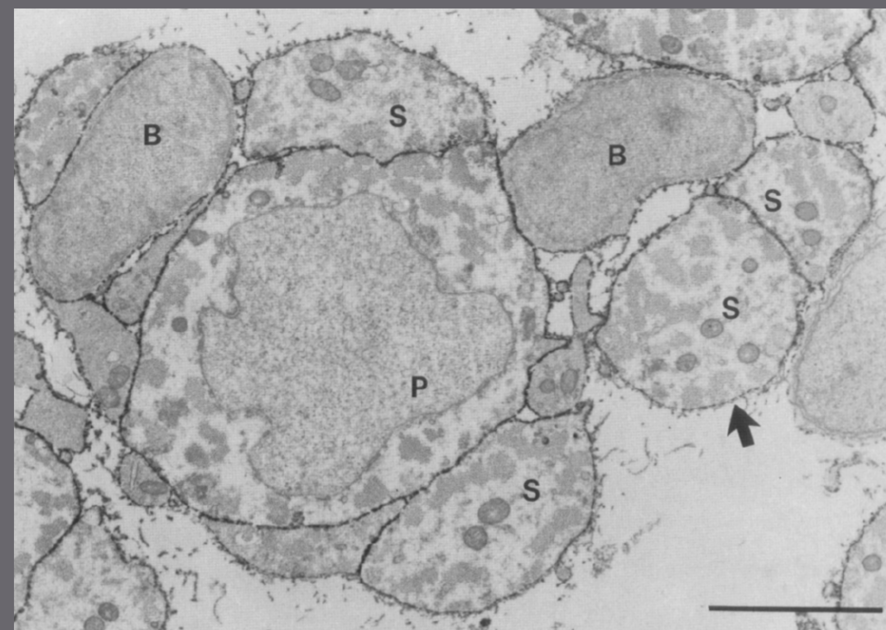


EM sections of developing iliofibularis muscle in chick embryos

Secondary myogenesis

Primary myogenesis

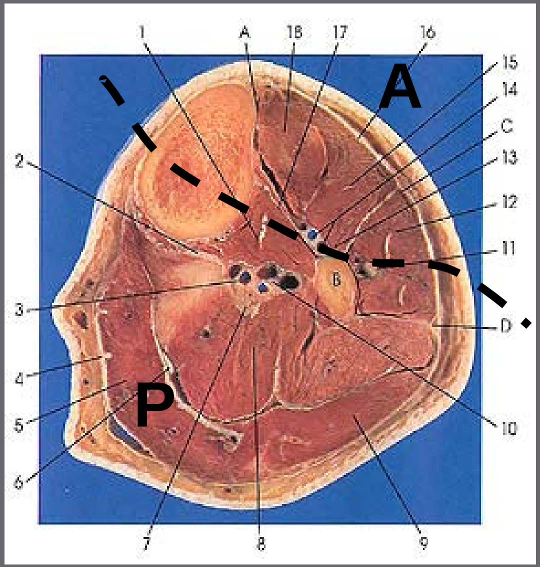




Barbara Fredette,* Urs Rutishauser,‡ and Lynn Landmesser*

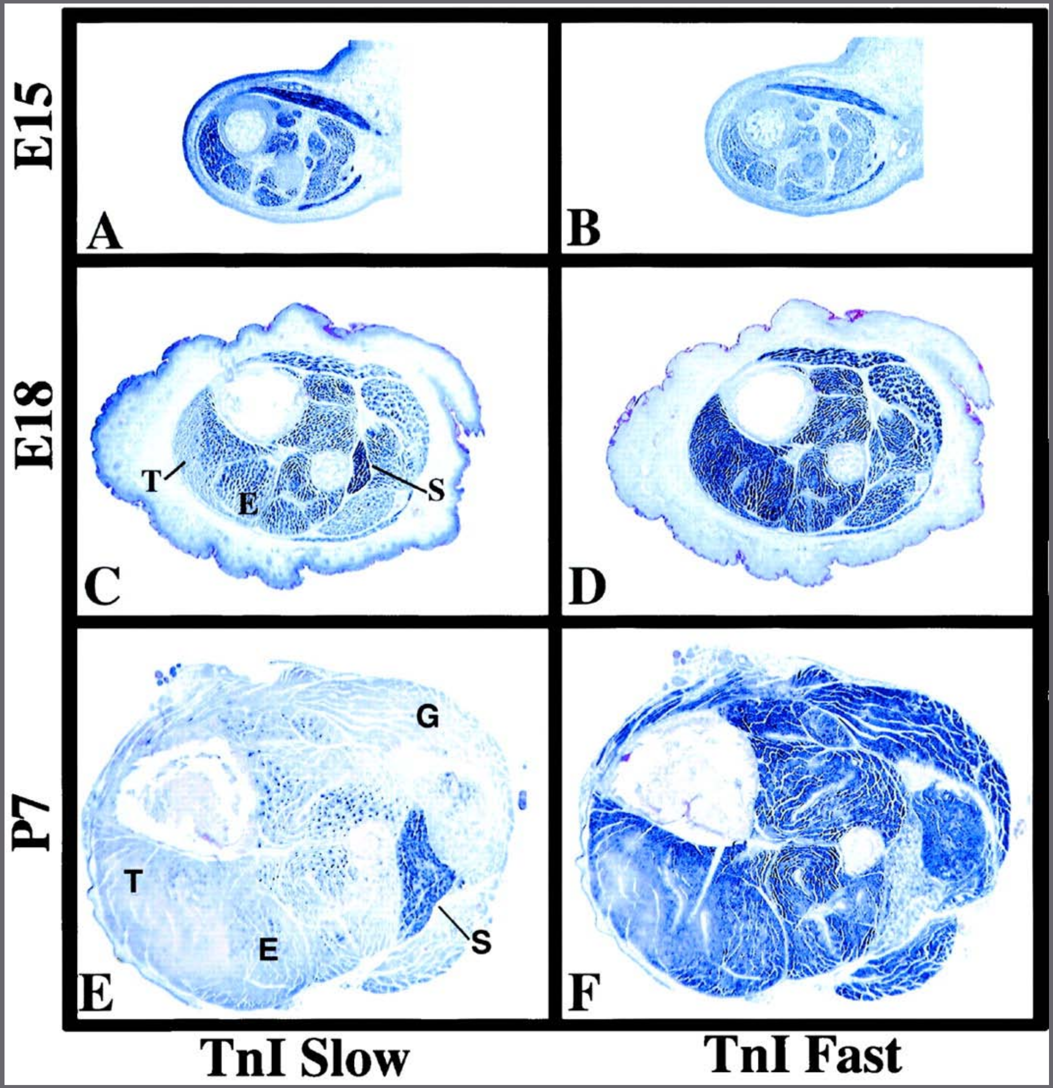
Studying muscles in the mouse as a model of human muscle development – the lower hind limb

Approx plane of section



18 Tibialis anterior
15 Extensor digitorum longus EDL
12 Peroneus brevis and longus
17 Tibialis posterior
8 Soleus
9 Gastrocnemius medial head
5 Gastrocnemius lateral head

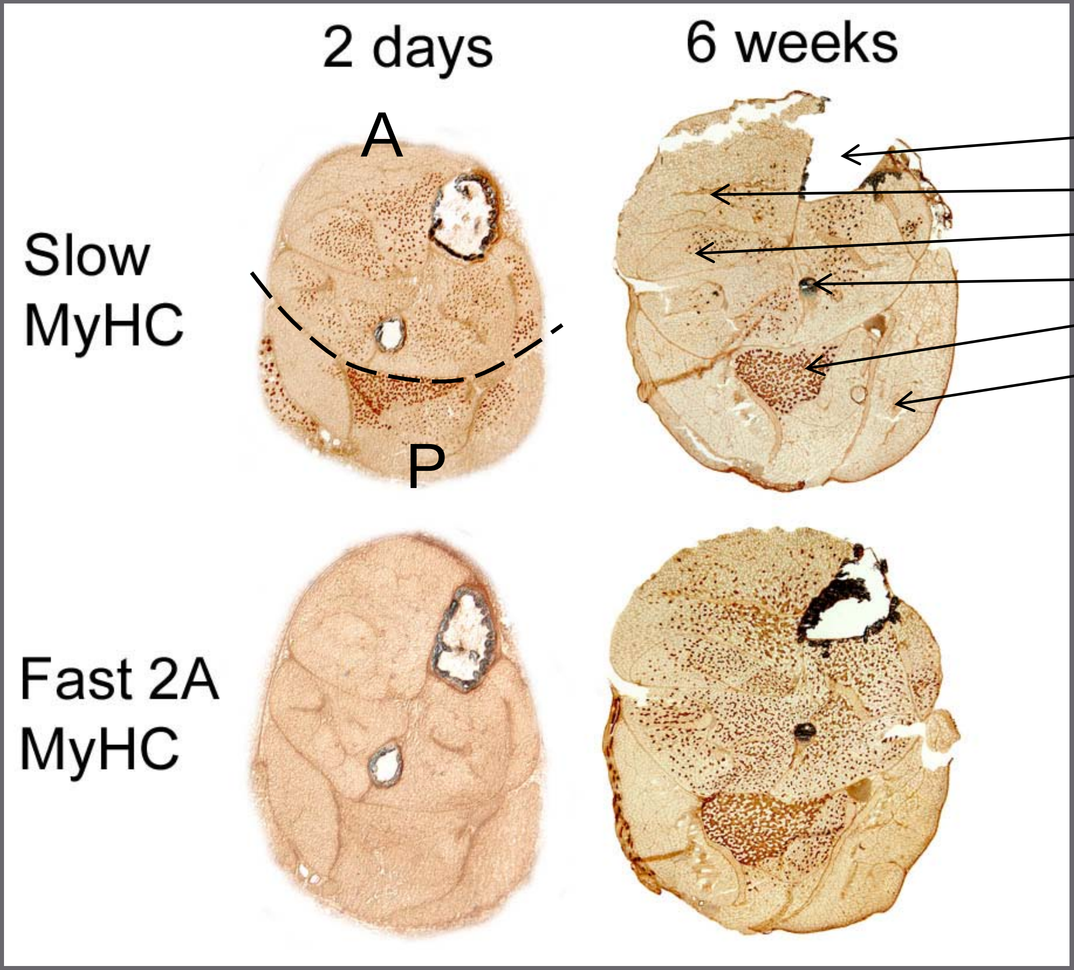
In situ hybridisation analysis of Troponin I isoforms in mouse crural sections



G = Gastrocnemius
S = Soleus
E = EDL
T = Anterior tibialis

Tnni1 is the gene that encodes the inhibitory subunit of the Troponin complex that is found in slow-twitch fibres.

Postnatal fibre conversion: slow fiber number declines and neonatal MyHC is replaced by the adult fast fibre MyHCs



Tibia
Tibialis anterior muscle
EDL muscle
Fibula
Soleus muscle
Gastrocnemius muscle

Transverse sections of hind-limbs from postnatal mice 2days and 6 weeks after birth – stained for Myosin heavy chain slow and Myosin heavy chain 2A

Plasticity of Muscle

Muscle Adaptation to Exercise Training

Adaptations to exercise training, particularly elevation in oxidative capacity of exercised muscle but also some myosin isoform changes mainly in fast subtypes.

Cross-Reinnervation

Buller *et al*. (1960) – Motor nerves supplying the (slow) soleus and (fast) FDL muscles swapped around. Contraction speed of soleus got faster, FDL slower.

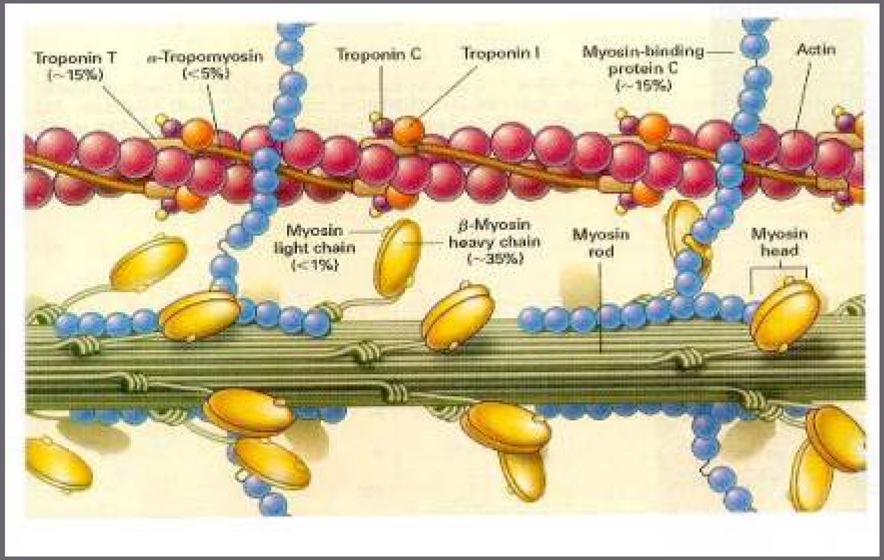
Chronic Low-Frequency Stimulation (CLFS)

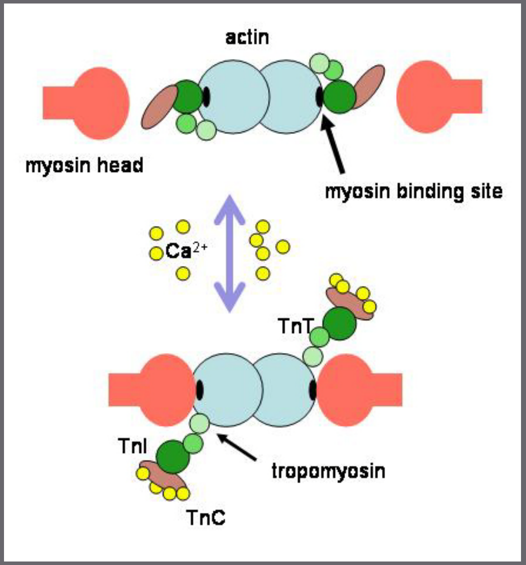
Artificial electrical stimulation of a nerve supplying a fast muscle with a tonic pattern mimics the impulse pattern of a slow nerve and induces fast to slow transformation Pette et al. (1973).

Pure Fibers, Hybrid fibers and the “Next-Neighbour Rule”

Analysis of myofilament isoforms in single fibers reveal the presence of “pure” and “hybrid” fibers containing, for example, MHC 2B and 2X. The percentage of hybrid fibers increases dramatically in transforming muscles <60% in rabbit CLFS experiment. Timing experiments reveal a gradual stepwise transition in the direction 2B->2X->2A->I. This finding is complimented by the fact that hybrids always contain a pair of “next-neighbour” isoforms.

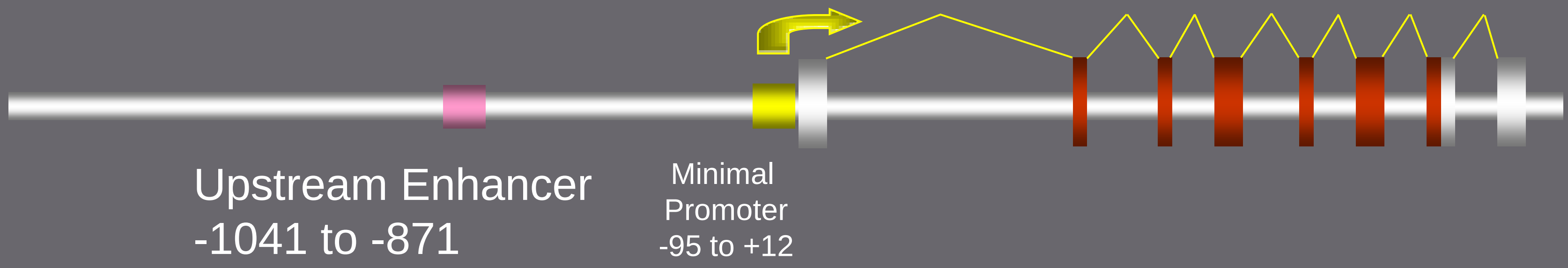
The *Troponin I* family of genes encode proteins essential for striated muscle contraction

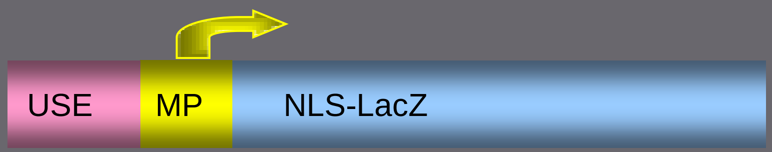




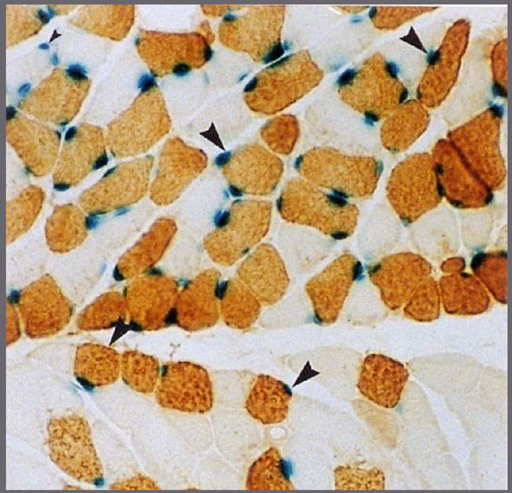
Gene name	Gene ID	Site of expression	Human gene location
Troponin I slow	*TNNI1*	skeletal muscle slow fibres	Chromosome 1
Troponin I fast	*TNNI2*	skeletal muscle fast fibres	Chromosome 11
Troponin I cardiac	*TNNI3*	heart muscle	Chromosome 19

Functional analysis of the *TNNI1* gene control region using transgenic mice





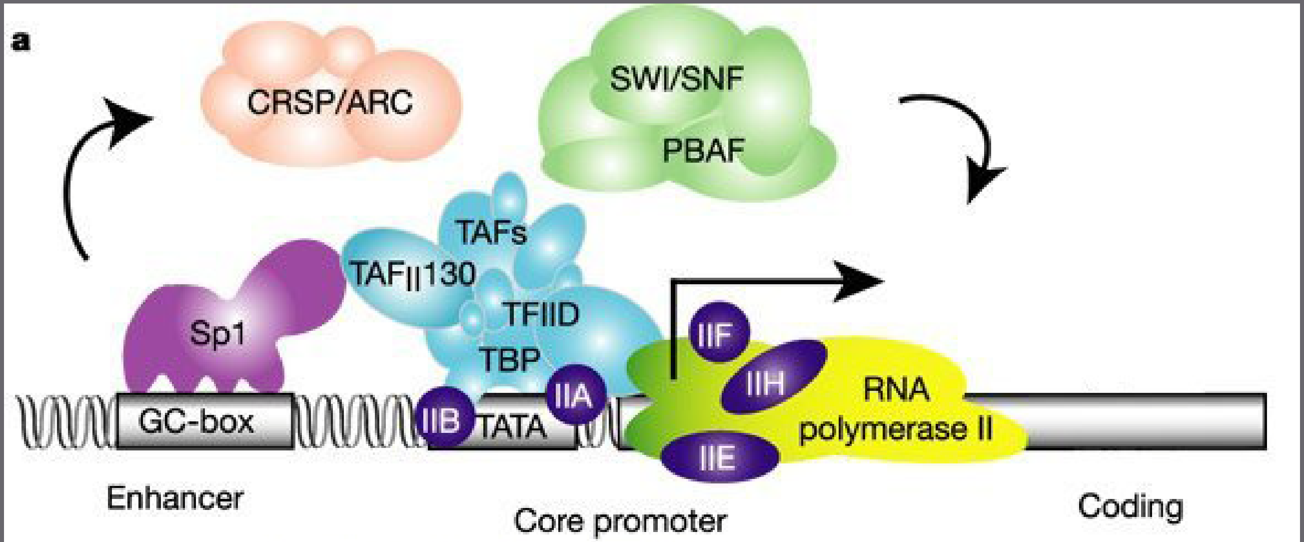
Slow fiber-specific transgene



Section of soleus muscle stained for Myosin heavy chain slow (brown) and nuclear-localized LacZ (blue)

Defenition of a promoter

A regulatory region a short distance upstream from the 5' end of a transcription start site that acts as the binding site for RNA polymerase II. A region of DNA to which RNA polymerase II binds in order to initiate transcription.



Defenition of an enhancer

A cis-regulatory sequence that can regulate levels of transcription from an adjacent promoter. Many tissue-specific enhancers can determine spatial patterns of gene expression in higher eukaryotes. Enhancers can act on promoters over many tens of kilobases of DNA and can be 5' or 3' to the promoter they regulate.

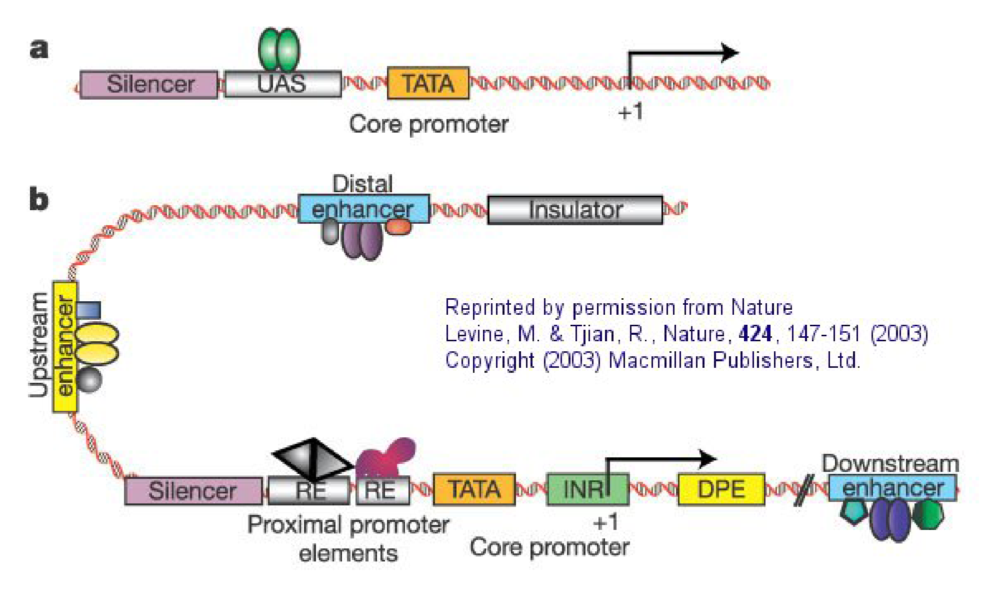


Figure 1 Comparison of a simple eukaryotic promoter and extensively diversified metazoan regulatory modules. **a**, Simple eukaryotic transcriptional unit. A simple core promoter (TATA), upstream activator sequence (UAS) and silencer element spaced within 100–200 bp of the TATA box that is typically found in unicellular eukaryotes. **b**, Complex metazoan transcriptional control modules. A complex arrangement of multiple clustered enhancer modules interspersed with silencer and insulator elements which can be located 10–50 kb either upstream or downstream of a composite core promoter containing TATA box (TATA), Initiator sequences (INR), and downstream promoter elements (DPE).

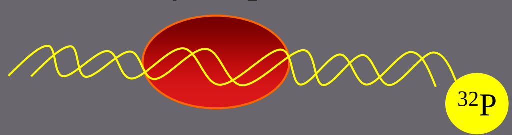
Finding proteins that bind to the upstream enhancer will lead to an understanding of how fibre type is regulated at the molecular level

Inr-like CCAC-Box MEF2 E-Box

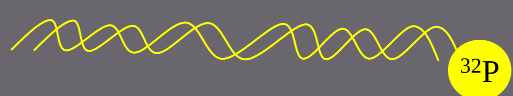
TGAGATGACAGACTATAATAGCCA**CAGGATTAA**CATAGCAGGCATTGTCTTTCTCTGACTATAGGGTGGGTATTATGGTTCATCAACCATCCTAAAAATACCGGTAAACAGGTGCAGCCCGGA

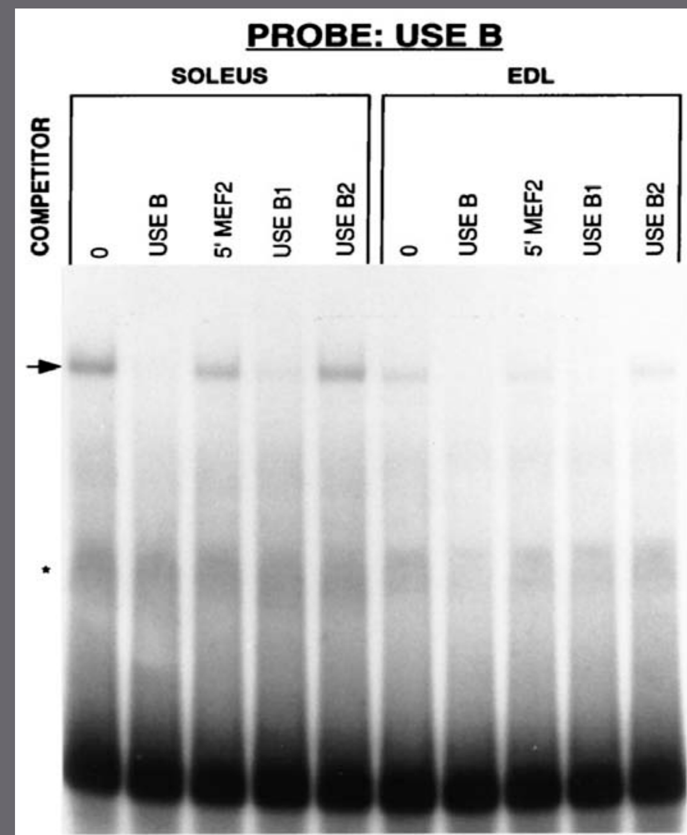
PROBE B

Shifted probe



Free probe





PROTEIN X?