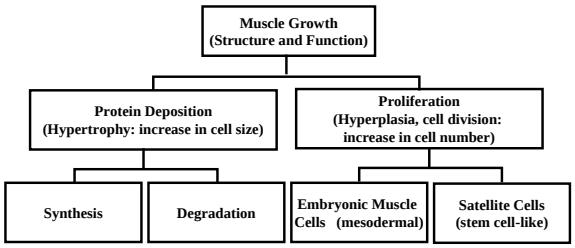
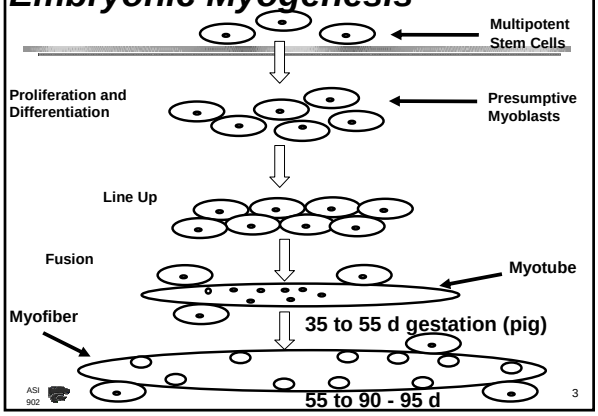
Myogenesis: A discussion with the end products in mind

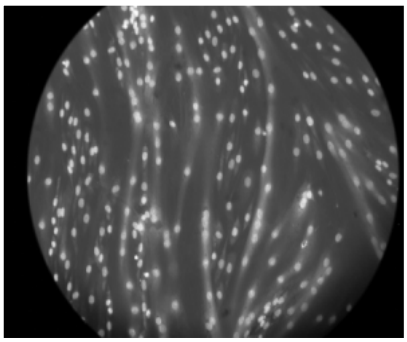


Embryonic Development of Skeletal Muscle

- **Somites, which are ball-like clusters of mesodermal cells, give rise to majority of skeletal muscle**
- **Muscle cells are derived from mesodermal germ layer→ muscle precursor cells or presumptive myoblasts (proliferating cells)**
- **Many cells at different stages of differentiation; in order to differentiate into muscle cells these cells require specific growth factors, receptors and transcription factors**

Embryonic Myogenesis





Myoblasts

- **Myoblasts –first identifiable muscle cell**
  - **withdrawn from the cell cycle**
  - **ability to synthesize myofibrillar proteins such as myosin and actin**
  - **fuse with other myoblasts to form multinucleated cells (myotubes)**

Myotubes

- **Myotubes (Primary and Secondary)**
  - **Multinucleated (1000's of nuclei per cell)**
  - **nuclei in myotubes are unable to divide (post-mitotic)**
  - **synthesize myofibrillar proteins (actin, myosin)**
  - **matures into a muscle fiber that can be found in postnatal muscle**
  - **fiber formation complete at birth, fibers cannot divide**

Therefore, FIBER # IS FIXED AT BIRTH
(HYPERPLASIA FIXED)

Terminal Differentiation

Terminal differentiation: cannot divide; post-mitotic

- Skeletal muscle as a model to study processes that regulate cell differentiation
  - Chosen as a model system because of two discernable steps:
    - 1. commitment of mesodermal progenitors to myoblasts (differentiation)
    - 2. subsequent differentiation of committed (withdrawn from cell cycle) myoblasts to contractile myotubes

Terminal Differentiation

- Proliferation and terminal differentiation of skeletal muscle cells are mutually exclusive
  - cell cycle arrest is prerequisite for myoblast to differentiate

Myogenic Regulatory Factors

- Myogenic Regulatory Factors (MRF's)
  - Myo D
  - Myf-5
  - Myogenin
  - MRF-4 (Myf-6, Herculin)
    - All are transcription factors (224-300 amino acids)
    - Basic/ helix-loop-helix-superfamily
    - found in cytoplasm, but translocate into nucleus with certain signals (heterodimers with E2 proteins)

MRFs

- [HLH] domain
  - allows for dimerization of these factors with ubiquitously expressed E-proteins, specifically E2A gene products – E12, E47
  - myogenic bHLH form heterodimers more efficiently with E12/E47 than homodimers

Terminal Differentiation

- Basic region
  - responsible for DNA binding
  - dimers bind to conserved DNA consensus sequence: CANNTG, where n = any base, also known as E-box
  - this sequence is found in promoter region of many muscle specific genes

MRFs

- MRF's
  - regulate cell-lineage specific transcription
  - transformation of other cell types into muscle

MRFs

- **Transformation**
  - **Constitutive over-expression (turn on genes) of MRF's in 10T1/2 mouse fibroblasts activate myogenic program as determined by morphological and biochemical criteria**
  - **All four MRF's (overexpressed, individually) results in muscle cells that are indistinguishable (morphologically and biochemically)**

MRFs

- **Gene/Gene product ablation?**
  - **Knock-out experiments (homozygous knock-out)**
  - **Myf-5 (-/-):**
    - **causes rib defect, die at birth due to respiratory failure, skeletal muscle appears morphologically normal and expresses muscle specific genes**
  - **Myo D (-/-):**
    - **similar consequences to Myf-5 ablation**
    - **significant increase in Myf-5 which appears to substitute for Myo D**
    - **double mutant Myo D/Myf-5 ablation causes complete absence of any skeletal muscle or mononucleated presumptive myoblasts**
      - **→ Myf-5 and Myo D have overlapping functions**

MRFs

- **Myogenin**
  - **initiates terminal differentiation**
  - **used as a marker for terminal diff. (myoblast → myotubes)**
  - **(-/-) homozygous knockout results in normal myoblast population but terminal differentiation of these myoblasts is absent**
- **MRF 4**
  - **causes maturation of myotubes into mature muscle fibers**
  - **(-/-) has not yielded much of an effect**

MRFs

- **Prerequisites of terminal differentiation?**
  - **Myo D can block proliferation, independent of its transcriptional activity of downstream muscle specific genes (Myo D cooperates with cell cycle inhibitors)**

MRFs and Proliferation

- **Myoblast proliferation →Serum (10%-20% fetal bovine)/Growth factors: enhance proliferation**
  - **immediate early gene products increased by serum/GF's: c-myc, c-fos, c-jun**
    - **transcription factors, some of first products of MAPK pathway, also known as proto-oncogenes**
    - **inhibit myogenesis – KEEP cells proliferating in cell cycle**

MRFs and Proliferation

- **Protein Id stimulated by presence of high serum**
  - **HLH protein but NO basic region → therefore, no DNA binding**
  - **Id = negative regulators of E-box mediated transcription through competitively binding E12/E47**
- **Cyclin D1**
  - **major job is to hyperphosphorylate retinoblastoma proteins (Rb), which keep cells in cell cycle →Myo D interaction with Rb (hypophosphorylated) forms a heterodimer**
    - **suppresses cell growth, withdrawal from cell cycle at G1**

Other factors

- **Other regulatory factors**
  - **MEF 2 transcription factors**
    - **obligatory cofactor**
    - **positively cooperates with MRF's**