Possible Approaches to Therapy for Muscular Dystrophy

- Gene Replacement Therapy -

  Stem cells
  Viral delivery of dystrophin mini-gene
  Delivery of growth factors

- DNA/RNA manipulation

- Pharmacological agents -

  Drugs that will turn on the utrophin gene
  Drugs that will cause read-through of a premature stop codon
  Drugs/factors to promote muscle repair and/or decrease damage

Gene Replacement Therapy

Stem cells

- Muscle-derived (SP cells)
- Bone Marrow-derived SP cells
- Bone Marrow-derived Mesenchymal Stem Cells

Viral delivery of dystrophin mini-gene

- AAV / micro-dystrophin
- Lentivirus / mini-dystrophin

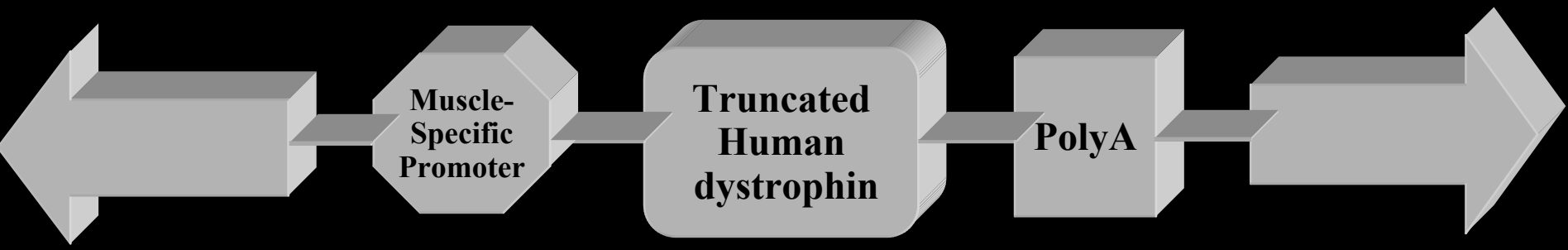
Viral or cellular delivery of growth factors to promote muscle repair

- IGF-I
- Anti-myostatin

Major Hurdles Facing Stem Cell-Based Gene Therapy for Muscular Dystrophy

- Culturing sufficient numbers of cells without losing their stem cell potential
- Delivery and targeting
- Commitment to become muscle

Schematic of a Dystrophin Mini-gene For Viral-Based Gene Therapy



Major Hurdles Facing Viral-Based Gene Therapy for Muscular Dystrophy

- Delivery
- Size limitation
- Immune response

DNA/RNA manipulation

DNA mutation correction

Chimeraplasts

-correction of point mutations or small deletions

RNA splicing modification

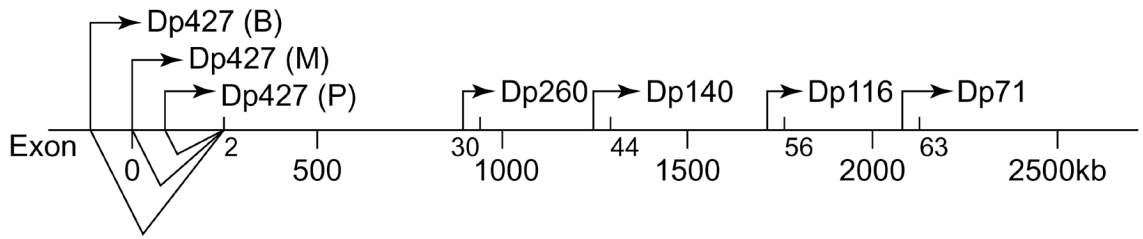
Oligonucleotides directed at splice junctions to produce exon slipping

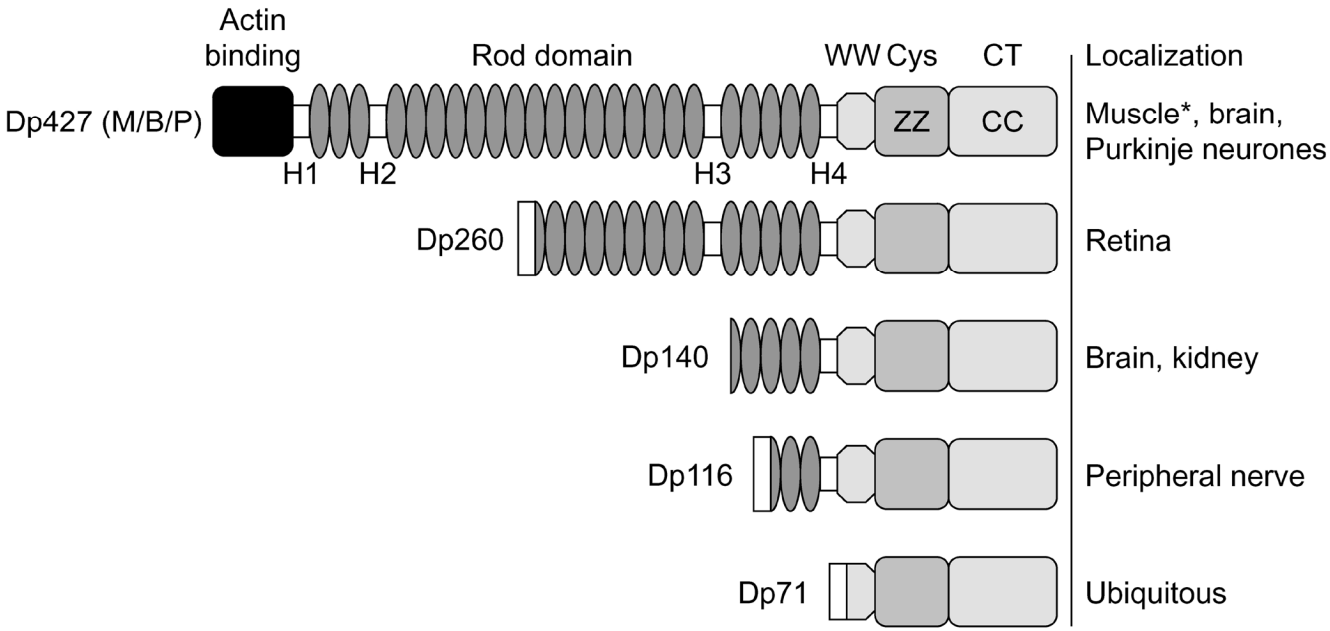
-production of truncated dystrophin

Major Hurdles Facing DNA/RNA Correction Therapies for Muscular Dystrophy

- Delivery
- Large deletions not correctable by chimeraplasts
- Unclear how functional all possible truncated dystrophins will be

Dystrophin Gene





Pharmacological agents

Factors that will turn on the utrophin gene

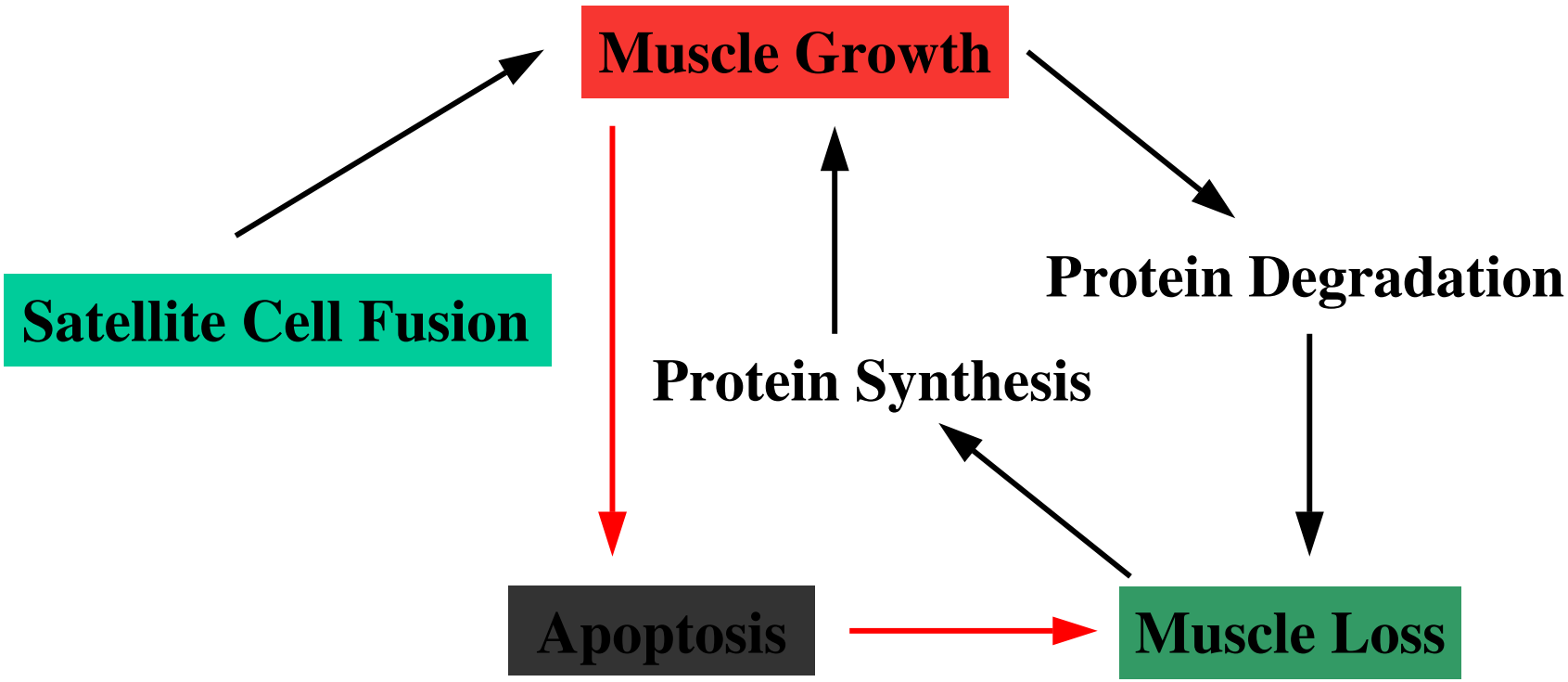
- Heregulin
- Screening for new compounds

Drugs that will cause read-through of a premature stop codon

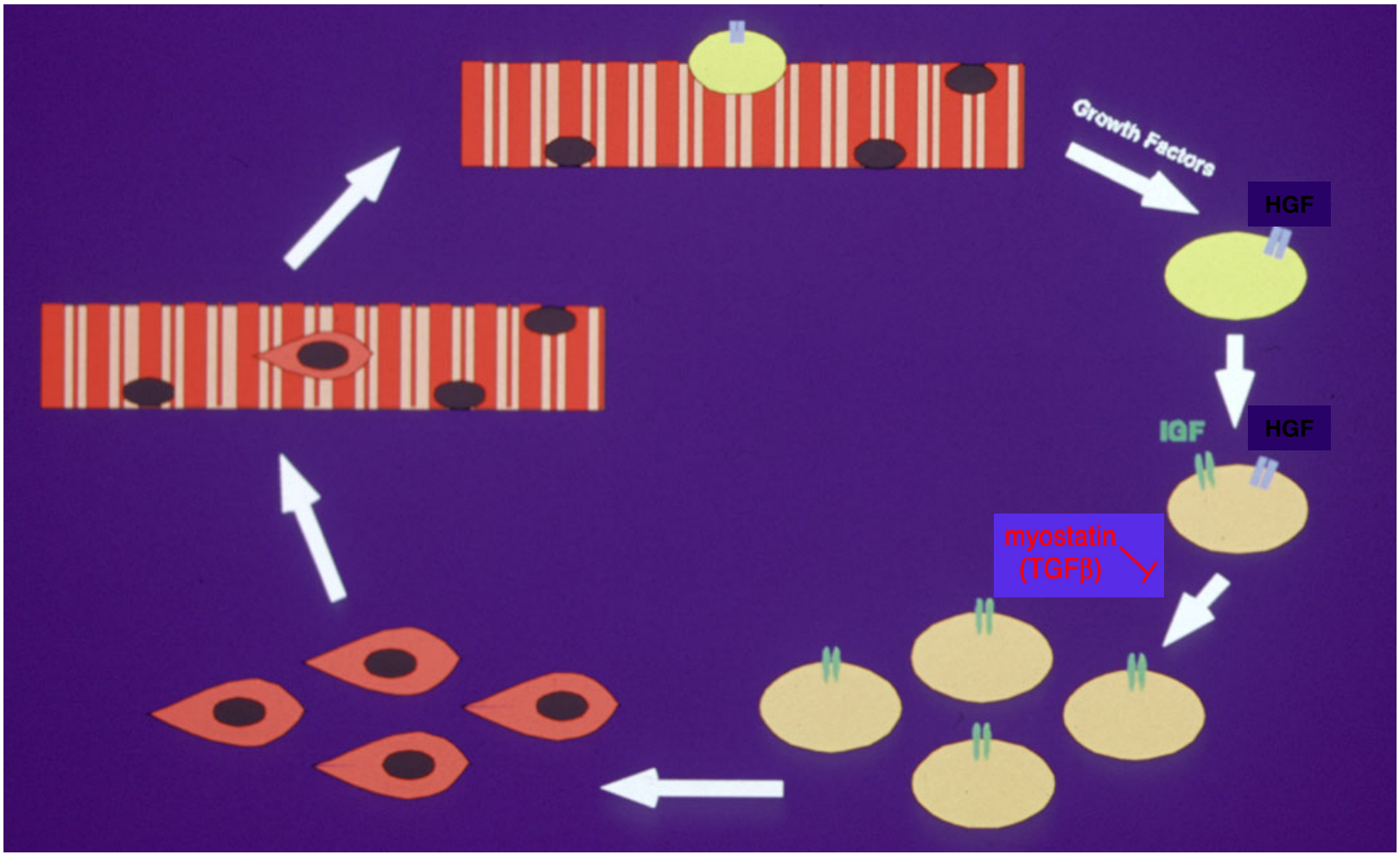
- Aminoglycosides
- Screening for new compounds

Drugs that will increase muscle regeneration and/or decrease susceptibility to damage

- Anti-myostatin
- Protease Inhibitors (Leupeptin, BBIC)


Muscle Growth
Satellite Cell Fusion
Protein Degradation
Protein Synthesis
Apoptosis
Muscle Loss

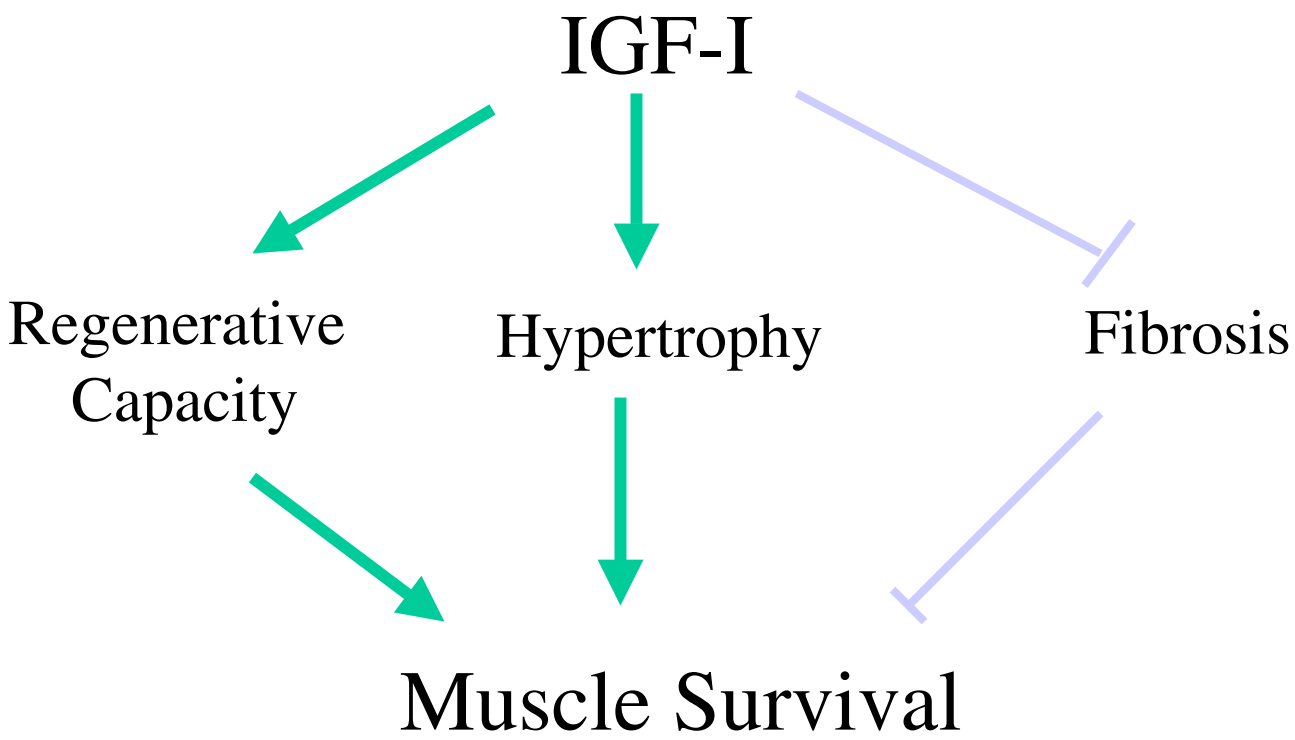
Muscle Growth and Regeneration



Factors to promote muscle repair

- IGF-I
- Anti-myostatin

Multiple benefits of increasing IGF-I or decreasing myostatin levels in dystrophic muscle



Major Question to be Answered

In a dog or a human, would this approach lead to depletion or sparing of satellite cells?

Administration of Protease Inhibitors to slow muscle breakdown

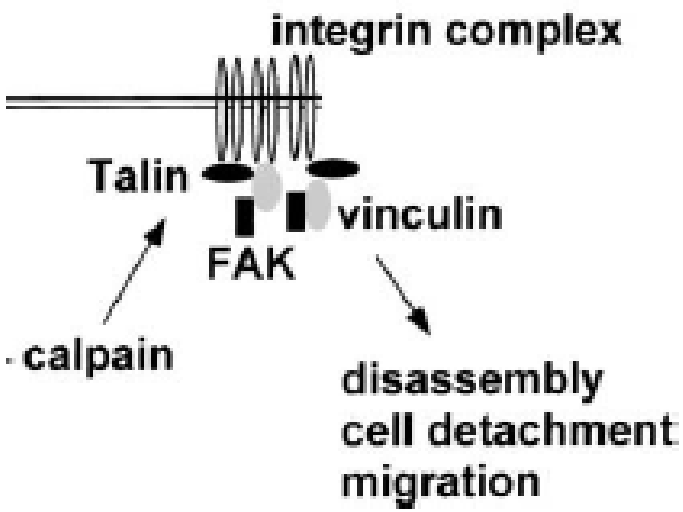
- Leupeptin (calpain inhibition)
- BBIC (proteosome inhibition?)

Protein Degradation Pathways in Muscle

Three primary pathways:

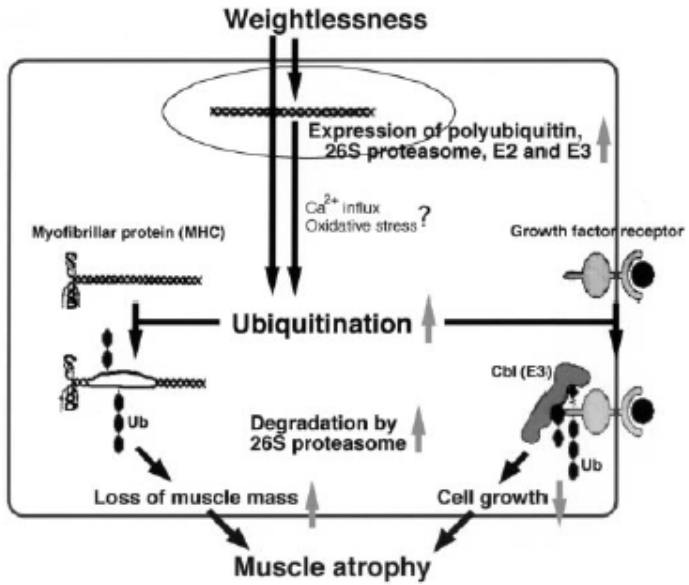
1. Lysosomal proteolysis pathway – endocytosed and membrane proteins
2. Ca^{2+}-dependent proteolysis pathway – soluble proteins, initial cleavage
3. ATP-dependent ubiquitin-proteasome pathway – 70% of soluble proteins

Ca^{2+}-dependent proteolysis



Perrin and Huttenlocher, 2002

ATP-dependent ubiquitin-proteasome



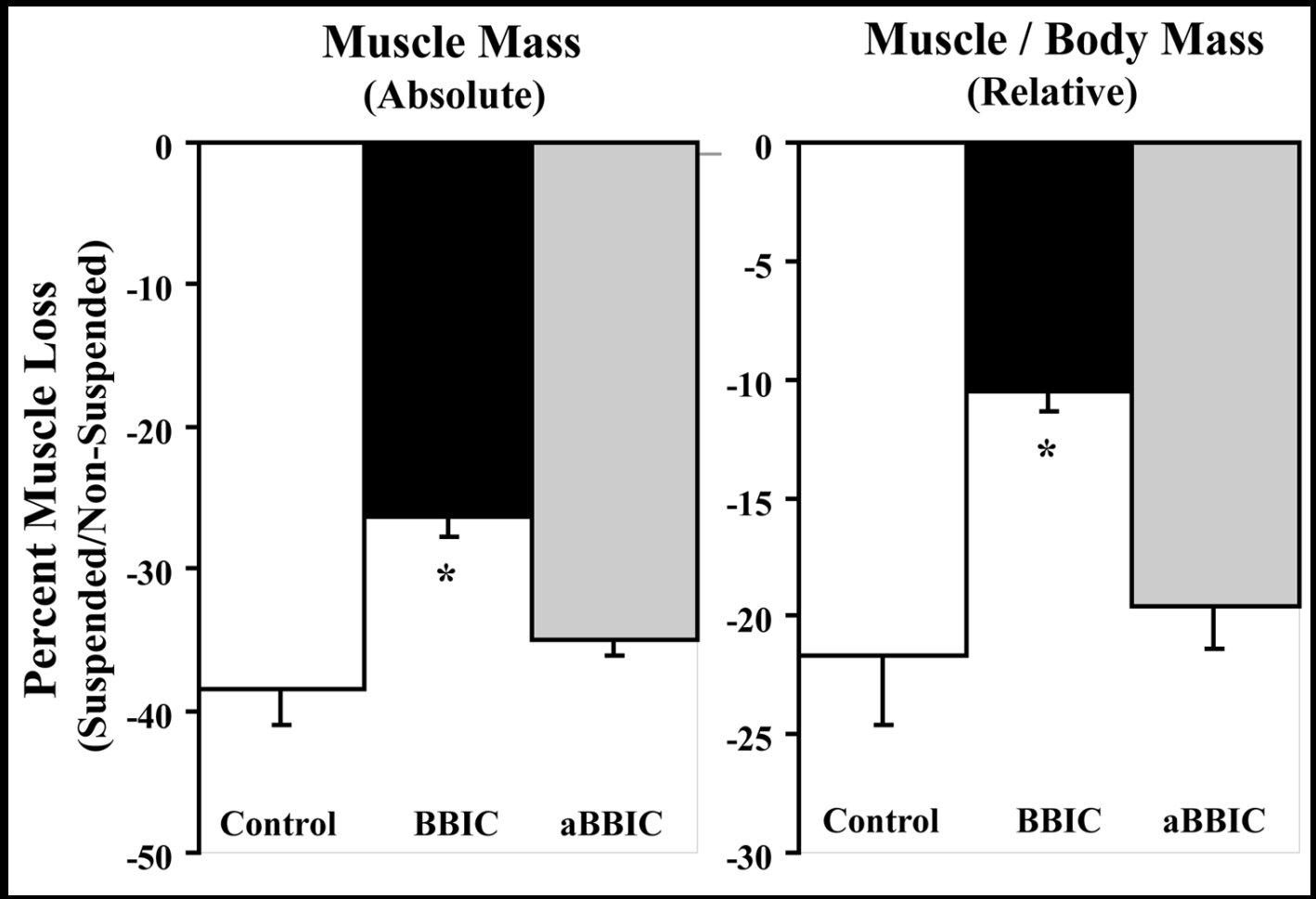
Ikemoto et al., 2002

Targeting Protein Degradation

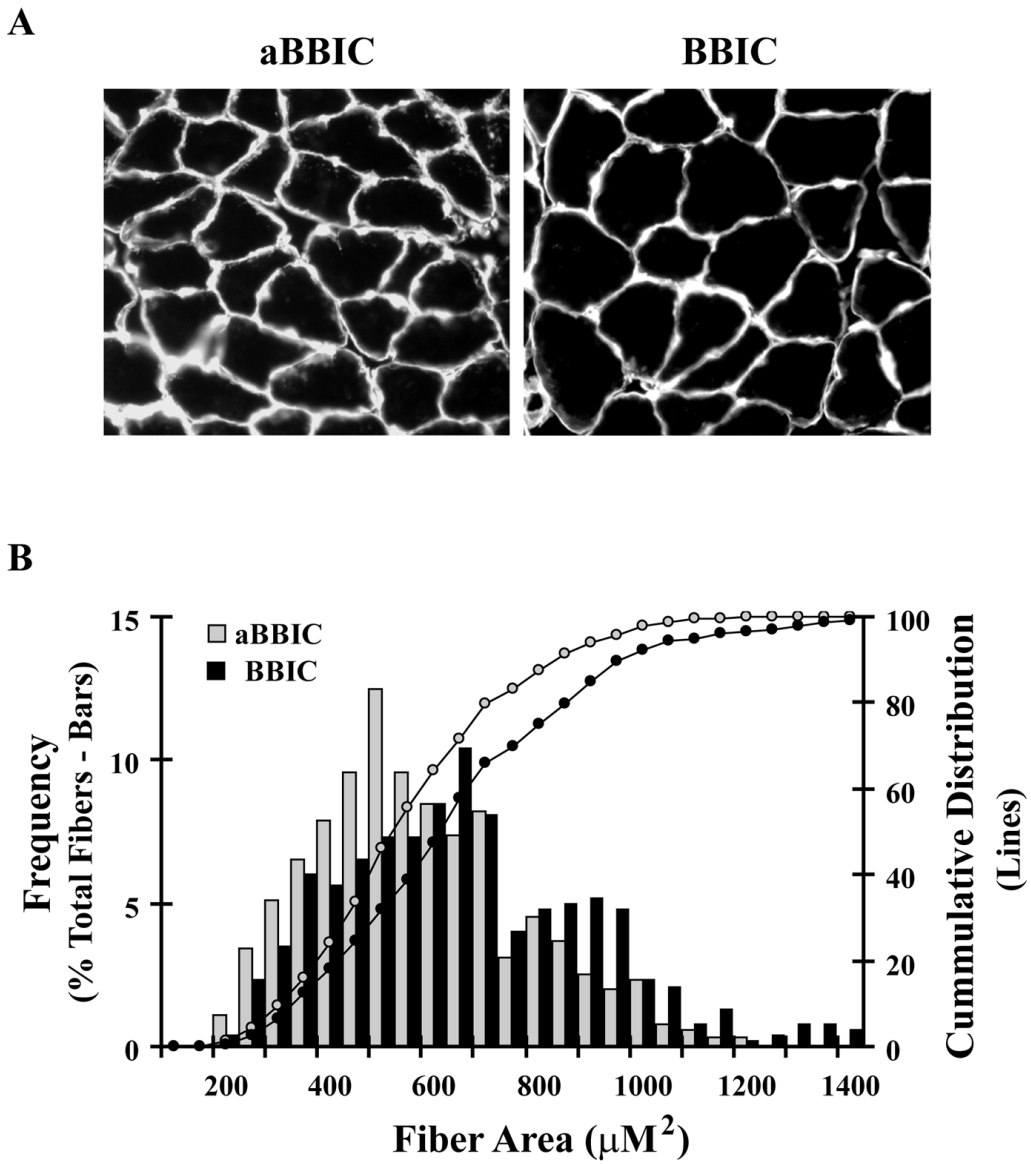
•Approach: Provide food supplemented with 1.0% Bowman-Birk's Inhibitor concentrate (BBIC)

- Bowman-Birk's Inhibitor (BBI)
  - Small 8 kD, soy-derived serine protease inhibitor
  - Dose-dependent inhibition of proteolysis
    - Lung, kidney, and liver
  - Increased life-span of mice consuming 1.0% BBI concentrate (BBIC)
  - Being evaluated as an anticarcinogenic agent in human trials

BBIC attenuates muscle loss associated with unloading



Fiber size loss attenuated by BBIC consumption



Major Question to be Answered

What are the long term effects of reduced protein turnover on function of the muscle?

Planned Steps Toward Clinical Trials for Muscular Dystrophy

What has happened to date?

- Phase 1 trial for AAV2/α-sarcoglycan (LGMD)
  - Halted prematurely at lower than efficacious dose
- Gentamicin trials for DMD with premature stop codons
  - CK levels dropped, but no dystrophin expression detected
- Phase 1 trial for CMV/dystrophin plasmid DNA
  - Low level expression seen in subset of patients

Planned Steps Toward Clinical Trials for Muscular Dystrophy

What is likely to happen over the next 5 years?

- Validation of new diagnostics -
  - MRI for non-invasive assessment of therapeutic correction
  - Improved strength testing for target muscles
- Phase 1 trial for myostatin inhibition
- Phase 1/2 trials for premature stop codon suppression
- Phase 1 trials for AAV/α- and γ-sarcoglycan (LGMD) and AAV/truncated dystrophin
- Evaluation of protease inhibitors
- Screens for new drugs

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