Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy

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PNAS, Vol. 98, No. 25, Dec 4 2001, pg. 14440-14445

- Muscle packed with proteins
- Serves as a primary reserve of amino acids for
  - Hepatic gluconeogenesis
  - Energy production
- Fasting >>> increase in the Tx of ubiquitin proteolysis related genes >>> increased catalysis of muscle >>> muscle atrophy

www.yamagiku.co.jp/pathology/case/case109.htm
The point?

- What are the factors to affect the acceleration of muscle proteolysis in catabolic mode of the organism?
- Establish a model of transcriptional adaptations during muscle atrophy that are responsible for the activation of muscle breakdown.
How did they go about identifying those factors?

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See http://www.u.arizona.edu/~gatts/azcc/flowchart.gif.
• Isolation of gene, whose Tx significantly increased in atrophying muscle

• The protein it encodes fro was cloned + its properties described

• Analogous to E3 ligases of the SCF class but specific to striated muscle
Atrogin-1 protein

- F-Box present at aa 228-267. Does it bind Skp1 protein?
- Should act as an adaptor between the proteins targeted for ubiquitination and the rests of the SCF complex.
- NLS present. Does it target nuclear proteins? TF?
- Does not contain leucine-rich regions or WD40 domains, BUT has PDZ interacting motif at its C-terminus to bind proteins with PDZ domains.

To test the possibility Atrogin-1 is an SCF component:

- GST pull-down assay to purify GST-atrogin-1
- Immunoblot against Myc >>> Interaction with Skp1

- GST-atrogin-1 extract immunoblotted against FLAG and HA to confirm interaction of atrogin with Cul-1, Roc-1 and Skp1, respectively

- No interaction occurred with any of the F-box mutants

Northern blot analysis of atrogin mRNA

- After fasting, the levels of 3 transcripts increased

- The 7-fold increase specific exclusively to striated muscle...

- …although some atrogin mRNA detectable in the cardiac control tissue also

Could atrogin expression induce atrophy?

- Cause or result of atrophy?

- mRNA levels of atrogin starts to increase as early as 16 hours after food removal….

- …with max at 24 hours…

- … and high atrogin mRNA levels maintained up to 72 hours after food removal.

• Muscles weighed prior to mRNA extraction.

• Muscle mass only decreasing 30 hours after food removal.

• Activation of Atrogin-1 gene important in the development and progression of muscle protein loss in fasting.
Why should identifying this gene be important?

- Atrophy associated with several human diseases:
  - Diabetes
  - Cancer
  - Renal failure

- In all cases, increase in Atrogin expression observed

Why should identifying this gene be important?

The atrophy symptoms of several diseases could be eliminated by finding a drug that would either inhibit the Tx of Atrogin-1 gene, Tl of its mRNA or inhibit its function as an E3 ligase.
Future focus

• Is Atrogin involved in degradation of TFs?

• Does it act in the nucleus?

• If so, how is it transported into the nucleus?