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

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(Image removed due to copyright considerations.)

See Case 109, Tetanus, at Pathology of Infectious Diseases, CD-Rom English Version, by YUTAKA TSUTSUMI, M.D. (<http://www.yamagiku.co.jp/pathology/photo/photo109-2.htm>).

Background

- 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

The point?

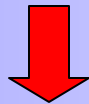
- What are the factors to affect the **acceleration of muscle proteolysis** in catabolic mode of the organism?
- Establish a model of **transcriptional adaptations** during muscle atrophy that are responsible for the **activation of muscle breakdown**.

How did they go about identifying those factors?

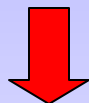
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See <http://www.u.arizona.edu/~gwatts/azcc/flowchart.gif>.

- Isolation of gene, whose Tx significantly increased in atrophying muscle

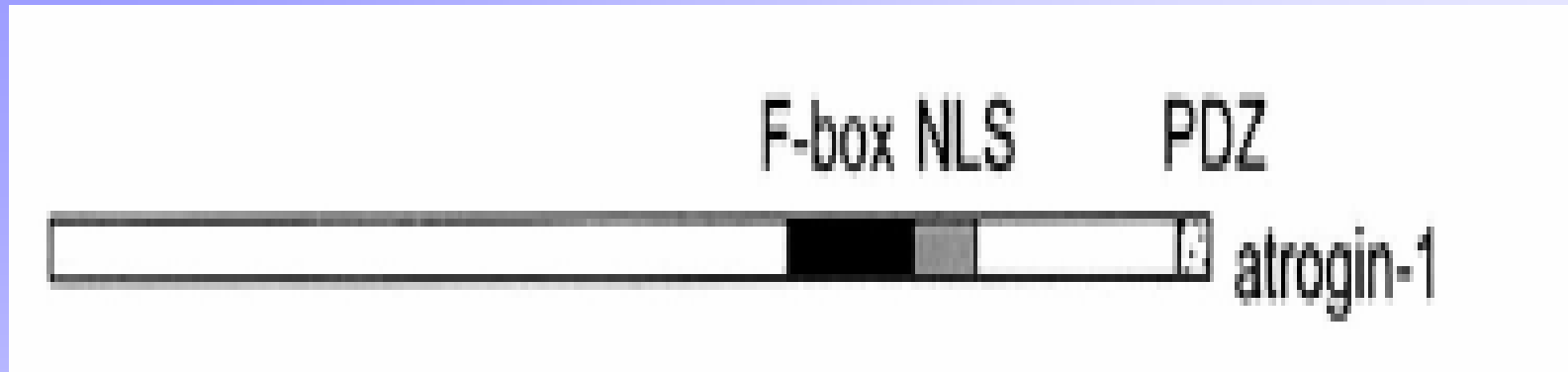


- The protein it encodes for was cloned + its properties described



- Analogous to E3 ligases of the SCF class but specific to striated muscle

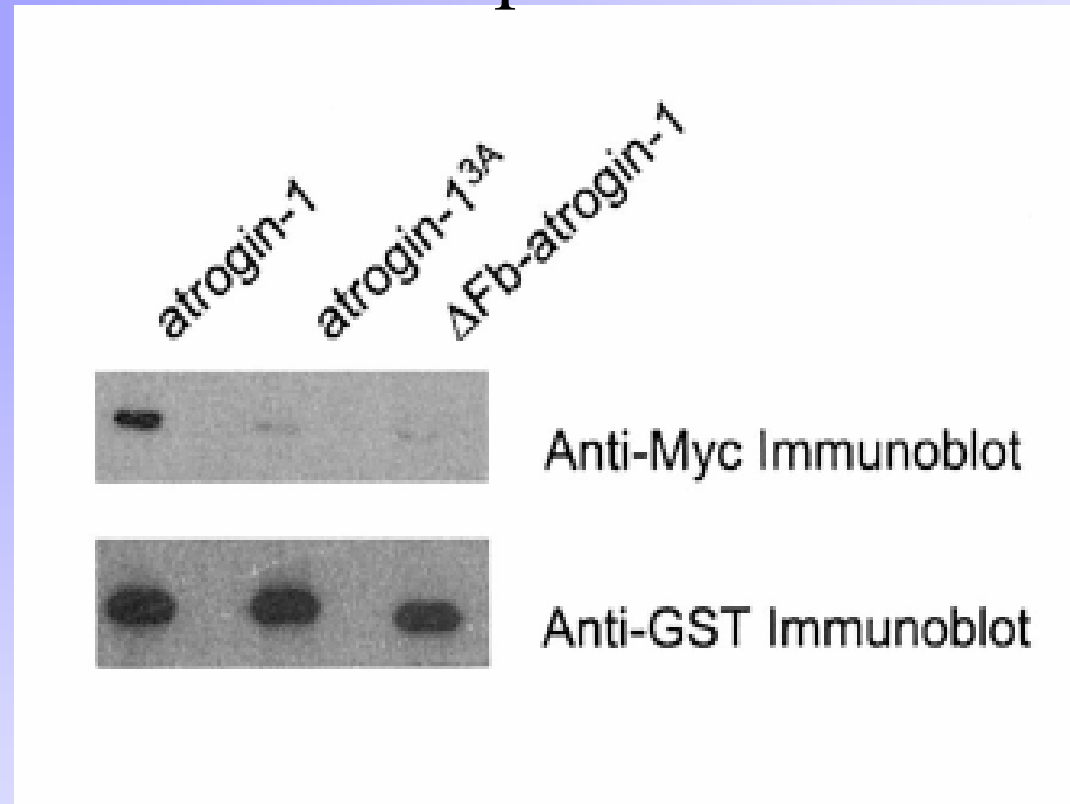
Atrogin-1 protein



Source: Figure (1B), Gomes, M.D. et al. "Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy." *Proc Natl Acad Sci U S A.* 2001 Dec 4;98(25):14440-5. Epub 2001 Nov 20. Copyright 2001 National Academy of Sciences, U.S.A. Used with permission.

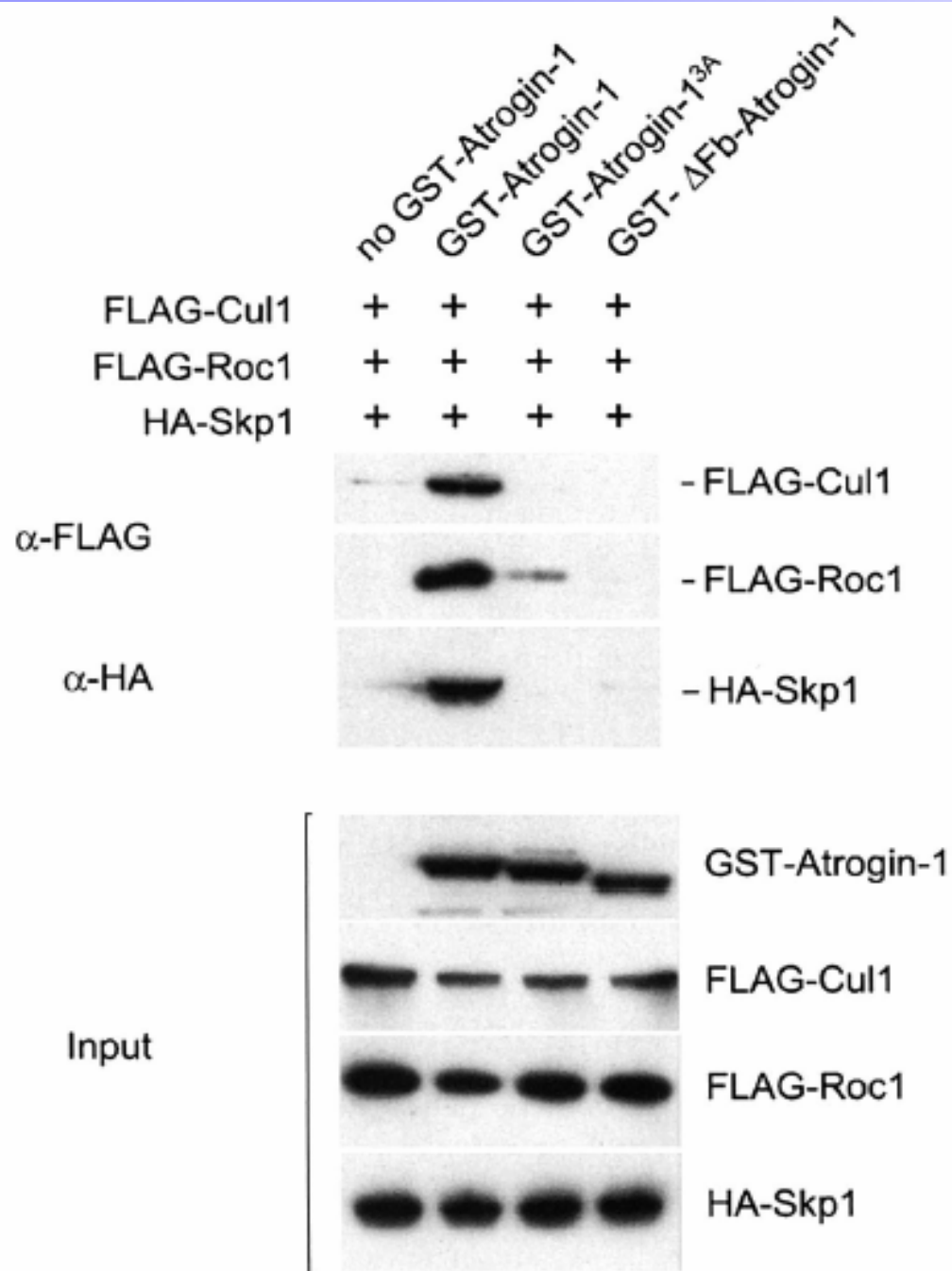
- F-Box present at aa 228-267. Does it bind Skp1 protein?
- Should act as an adaptor between the proteins targeted for ubiquitination and the rest of the SCF complex.
- NLS present. Does it target nuclear proteins? TF?
- Does not contain leucine-rich regions or WD40 domains, BUT has PDZ interacting motif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

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



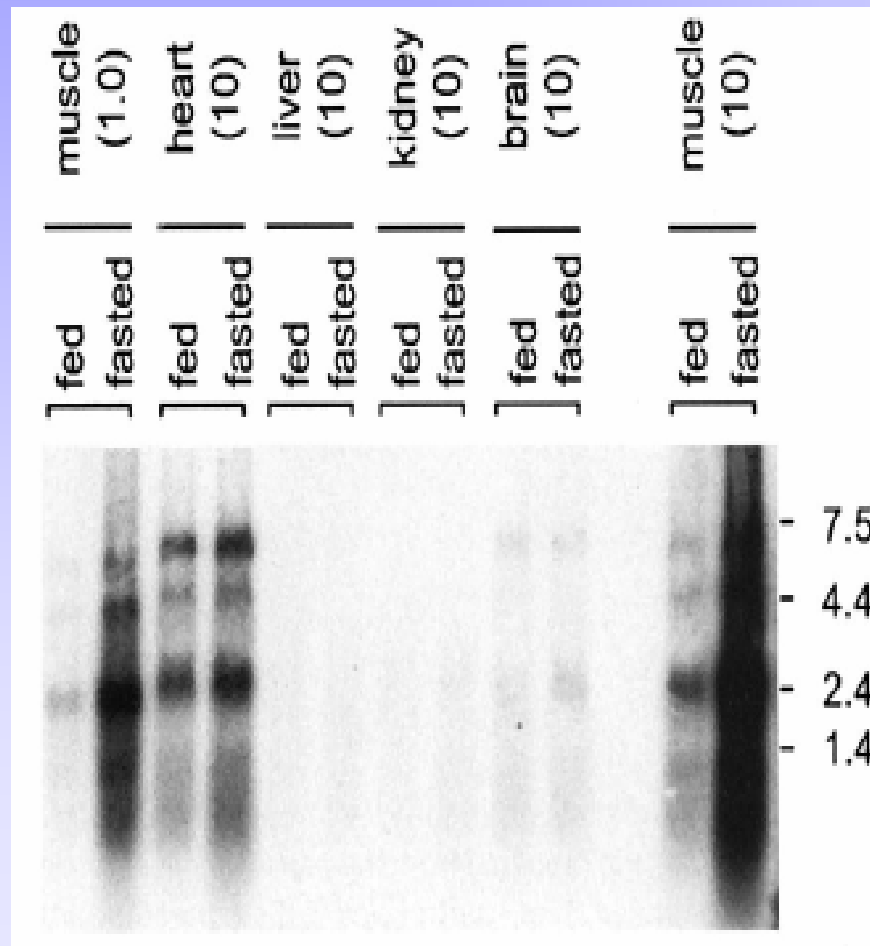
Source: Figure (1E), Gomes, M.D. et al. "Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy." Proc Natl Acad Sci U S A. 2001 Dec 4;98(25):14440-5. Epub 2001 Nov 20. Copyright 2001 National Academy of Sciences, U.S.A. Used with permission.

- 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 atrogenin mRNA

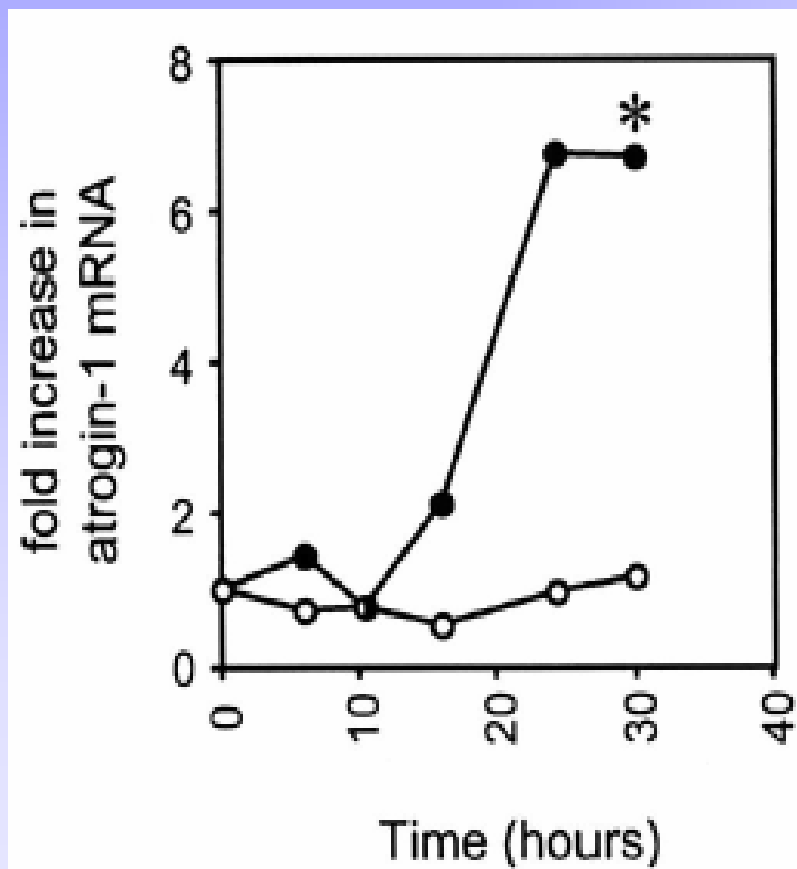


- After fasting, the levels of 3 transcripts increased
- The 7-fold increase specific exclusively to striated muscle...
- ...although some atrogenin mRNA detectable in the cardiac control tissue also

Source: Figure (3A), Gomes, M.D. et al. "Atrogenin-1, a muscle-specific F-box protein highly expressed during muscle atrophy." Proc Natl Acad Sci U S A. 2001 Dec 4;98(25):14440-5. Epub 2001 Nov 20.

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Could atrogenin expression induce atrophy?



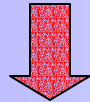
- Cause or result of atrophy?
- mRNA levels of atrogenin starts to increase as early as 16 hours after food removal....
- ...with max at 24 hours...
- ... and high atrogenin mRNA levels maintained up to 72 hours after food removal.

Source: Figure (3B), Gomes, M.D. et al. "Atrogenin-1, a muscle-specific F-box protein highly expressed during muscle atrophy." Proc Natl Acad Sci U S A. 2001 Dec 4;98(25):14440-5. Epub 2001 Nov 20.

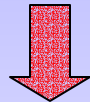
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...

- Muscles weighed prior to mRNA extraction.

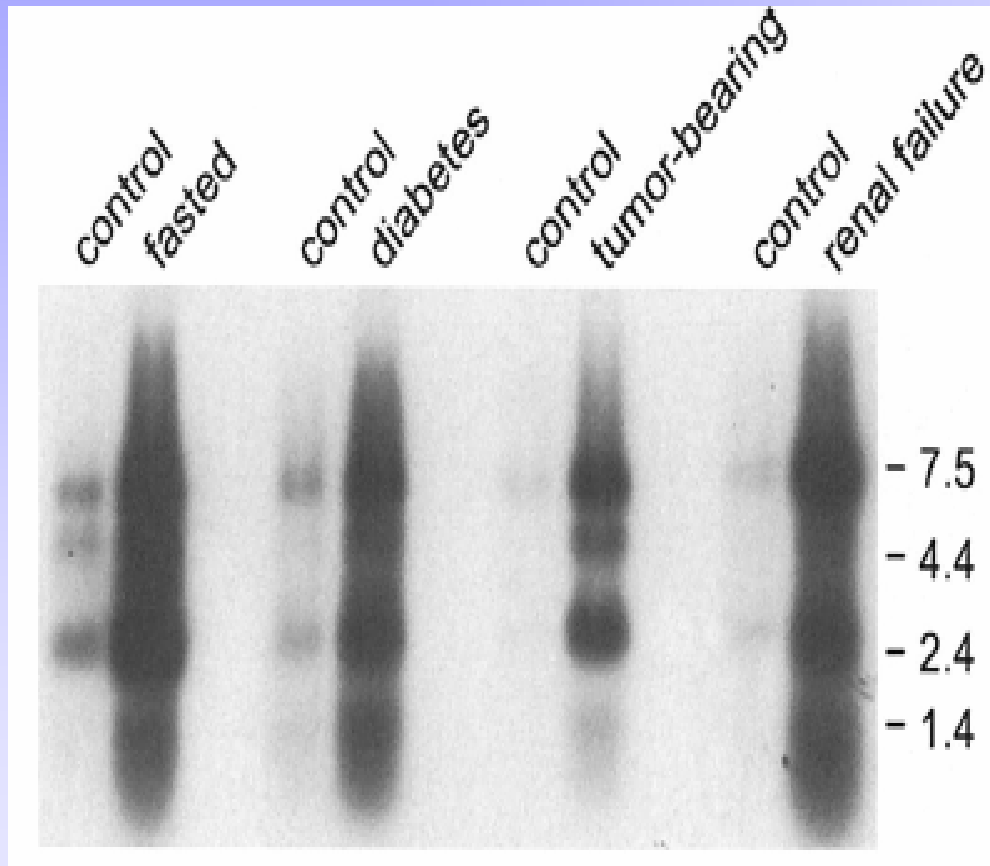


- Muscle mass only decreasing 30 hours after food removal.



- **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

Source: Figure (4), Gomes, M.D. et al. "Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy." *Proc Natl Acad Sci U S A.* 2001 Dec 4;98(25):14440-5. Epub 2001 Nov 20. Copyright 2001 National Academy of Sciences, U.S.A. Used with permission.

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 Atrogen-1 gene, T1 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?