Fbw7 Targets Jun Heterodimers for Degradation

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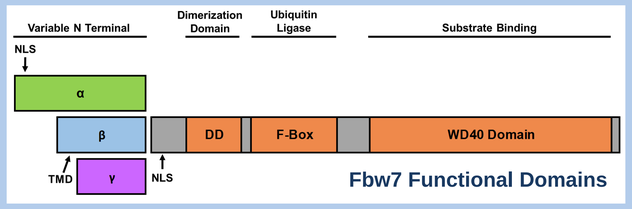
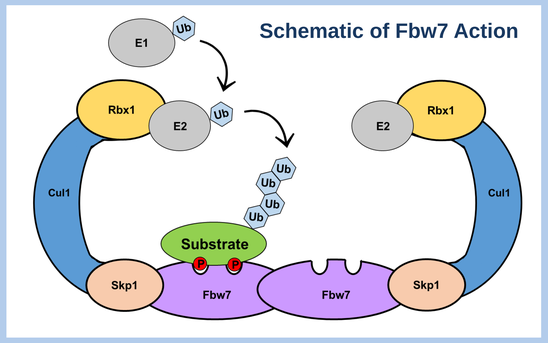
Introduction

- Fbw7 (F-box and WD repeat domain-containing 7) directs degradation of multiple oncogenic proteins through the proteasome pathway.



Fbw7 Functional Domains

- Fbw7 is a tumor suppressor and is mutated in about 10% of all human cancers.



- The Fbw7 SCF complex (dark borders above) acts as an E3 ligase and targets substrates for proteasome degradation via polyubiquitylation.

Cdc4 Phosphodegron (CPD):

ΦxΦΦΦ(T/S)PPx(T/S/E/D)

central phosphosite

+4 phosphosite or negative charge

Φ: hydrophobic amino acid
X: variable amino acid

- The substrate binding site of Fbw7 recognizes a CPD on the substrate. Phosphorylation of the CPD can regulate substrate degradation.
- Whereas substrates with an optimal CPD can bind to monomer Fbw7, we propose that substrates with weak CPDs may form homo/heterodimers and be targeted by dimerized Fbw7.
- Transcription factor Jun has a weak CPD that prevents Fbw7 from binding as a monomer. Jun binds to other proteins also containing weak CPDs.
- <u>Hypothesis:</u> Jun forms heterodimers which are targeted by the Fbw7 SCF complex.

Objective

To identify proteins that form heterodimers with Jun for targeting by the Fbw7 SCF complex.

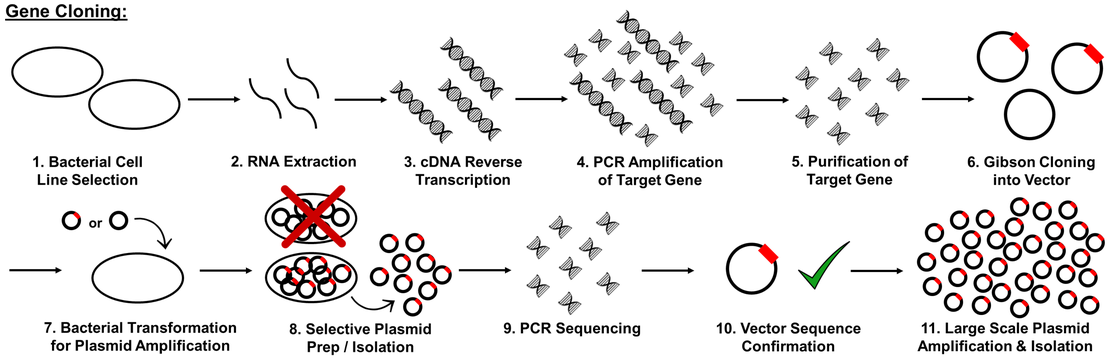
Proteins of Interest

Candidate proteins for Jun Heterodimers:

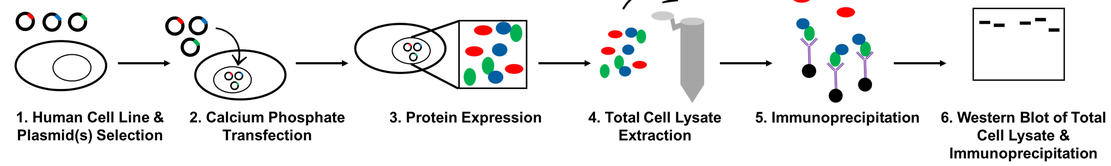
- ***Jun & Fos:*** Dimerize to form AP-1 Transcription Factor; cell cycle progression, apoptotic suppression, & differentiation.
- ***Creb3, Creb3L1, & Creb3L2:*** cAMP Responsive Element Binding Proteins; cell proliferation & ER stress response.
- ***ATF3 & ATF7.2:*** Activating Transcription Factors, immediate early stress response.
- ***AP4:*** Transcription factor, cell cycle progression.
- ***SMAD3:*** Transcription factor; proliferation, migration, & apoptotic response.

Methods and Materials

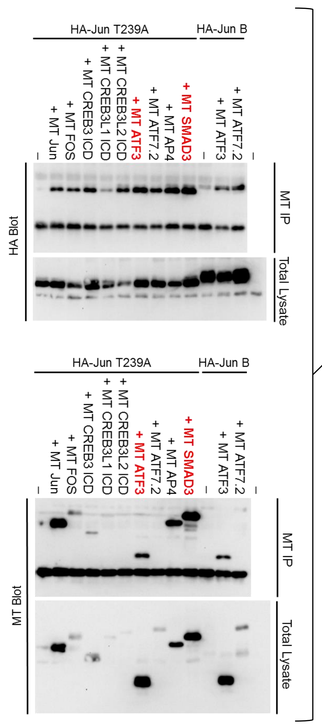
<u>Gene Cloning:</u>



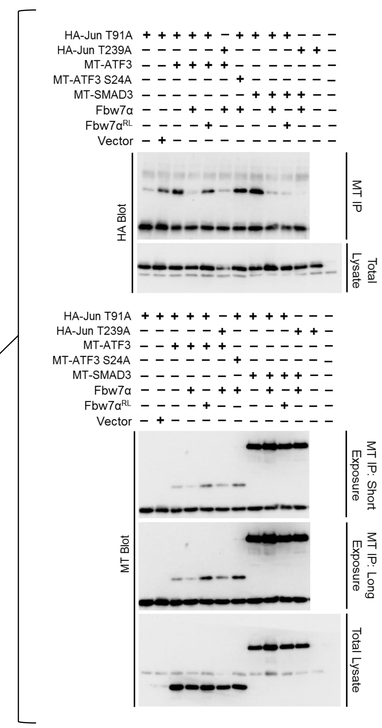
<u>Identification of Substrate Heterodimers:</u>



Results



- Pilot studies (left) indicate ATF3 & SMAD3 are likely candidates.
- ATF3 & SMAD3 show co-expression with Jun in Human Embryonic Kidney Cells (HEK) 293 A.
- ATF3 & SMAD3 co-immunoprecipitate (IP) with Jun, forming stable complexes.
- Preliminary results (right) indicate that the Jun-ATF3 heterodimer is likely targeted by Fbw7.
- In the presence of Fbw7α, the Jun-ATF3 heterodimer is degraded.
- In the presence of mutant Fbw7α^{RL}, the Jun-ATF3 heterodimer is not degraded.



Conclusions

- Jun forms stable heterodimers with weak CPD containing proteins, particularly ATF3 and SMAD3.
- Preliminary results suggest that the Jun-ATF3 heterodimer may be targeted by Fbw7 for degradation.
- Targeting of heterodimers could serve as a regulatory measure to modulate protein levels throughout the cell cycle.
- The immunoprecipitation protocol needs optimization to produce cleaner results. These results are a first look; they require replication and validation in future studies.

Acknowledgements

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Note: Figures were created by Lisa M Francomacaro.