

High –Throughput Mapping of a Dynamic Signaling Network in Mammalian Cells

Barrios-Rodiles et al 2005
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Represented by
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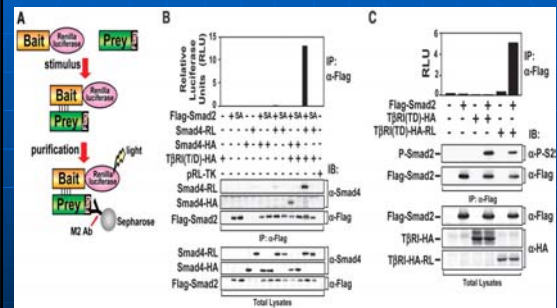
Protein-Protein Interactions (PPI)

- Key for cell signaling
- Dictate timing and intensity of network outputs
- High-throughput LUMIER is developed to systematically map PPIs in mammalian cells.

LUMIER

- Uses Renilla Luciferase enzyme
- Luciferases: enzymes that emit light by catalyzing the production of light in the reaction between Luciferin and ATP

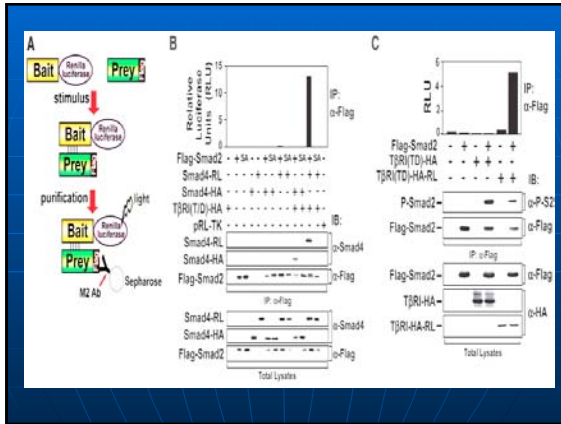
LUMIER



TGF beta

- Superfamily of extracellular morphogens
- Regulate biological precesses in metazoans
- Signals through ligand-induced heteromeric receptor complexes: I and II

- RL fused to Smad4 (Smad4-RL) co-expressed with :
- Flag-Smad2
- or Flag-Smad2(2SA) which does not bind Smad4

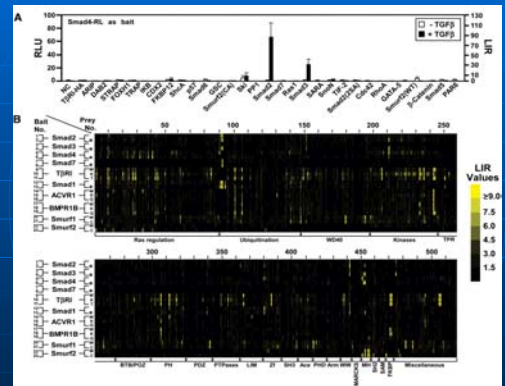


Results

- In the absence of TGF beta signaling: Smad4-RL no associated with Smad2
- In the presence of signaling: Smad4-RL was strongly associated with Smad2
- No Smad2(2SA) association was detected

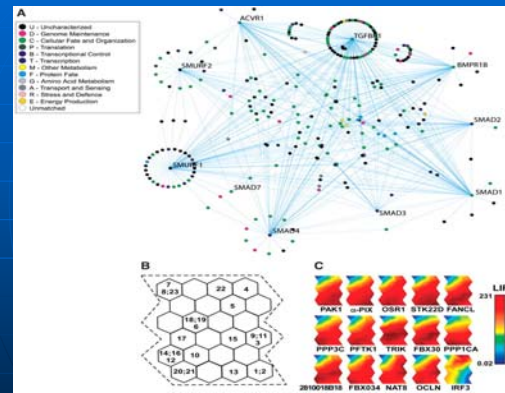
Pilot-screen of Smad4-RL against 30 different cDNAs

- In presence of Absence of TGF beta
- Results: detected signal dependent interactions between Smad4-RL and Flag-Smad2 and Flag-Smad3 and Flag-ski



Identifying novel targets of the TGF beta pathway

- Algorithm that analyzed the interaction of each RL-tagged bait with library of Flag-tagged proteins (preys)



Results

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