On the functionality of actin related proteins
Outline

• What are actin and actin related proteins?

• How are they related?

• Structural and evolutionary analysis

• What are the molecular dynamics of the Arp2/3 complex

• Proposed activation of the Arp2/3 complex
Actin

- Cytoskeletal protein, polymer
  many functions
  - cell motility
  - cell shape
  - cytokinesis
  - muscle

- Very well-conserved
- Ubiquitous in eukaryotes

Modified from Alberts, Molecular Biology of the Cell
Actin family
• Conventional actin
  - several “isotypes”
• “Actin-related proteins”
  - “ARPs”

Arp1: Part of “Dynactin Complex”:
  – necessary for function of microtubule motor dynein

Arps2/3: Part of “Arp2/3 Complex”
  – nucleate, branch actin polymerization

“Other” Arps...
Nucleation via Arp2/3

1. Extracellular stimuli
2. Produce active GTPases & PIP2
3. Activate WASp/Scar
4. Activate Arp2/3 complex to initiate new filaments
5. Barbed ends elongate
6. Growing filaments push membrane forward
7. Capping protein terminates elongation
8. Aging
9. ADF/cofilin severs & depolymerizes ADP-filaments
10. Profilin catalyzes exchange of ADP for ATP
11. Pool of ATP-actin bound to profilin
12. LIM-kinase inhibits ADF/cofilin

Pollard and Borsey 2003
Results:

• 4 expected subfamilies conserved human 🌌 yeast

• 4 additional (!) subfamilies humans 🌌 plants

 أشهر ALL well-supported by bootstrap analysis

 أشهر ALL new subfamilies have members implicated in nuclear functions

Hawse, Goodson 2002
Where to go from here???

Structure function analysis
- Identify regions of ARPs involved in functionally important interactions
- Predict specific ligands

Expect:
- Actin and ARPs have some common ligands
  - bind at a common face
  - amino acids on this face will be conserved between actin and the ARPs
- ARPs will have some subfamily-specific interactions
  - protein faces involved in these interactions will be well-conserved within subfamilies, divergent between subfamilies

Model ARPs onto actin crystal structure
Map conservation onto these models
Homology modeling

1. Obtain crystal structure
2. Align sequences
3. Energy minimize putative structure
Myosin

Actin

Arp 2

Arp 5

Arp 3

Arp 6

Arp 1

BAF53

Arp 8

Gelsolin

Multiple

Profilin
GNM results
Arp2/3 complex

(Robinson et al., 2001)
Fast modes

Mode 1

Mode 2

Mode 3
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