Advantages of *Dictyostelium*

- Simple organism
- Rapid doubling time
- Contains many signaling networks found in larger eukaryotes
- Genetically pliable
- Genome is sequenced
- Differentiates and develops when starved
Dictyostelium life cycle is complete in 24 hours

- Unicellular eukaryote, social amoeba
- Vegetative condition: individual cell
- Starved condition: cells aggregate, fruiting body
Phosphorylation of Actin-related Protein (Arp2) Is Required for Normal Development and cAMP Chemotaxis in *Dictyostelium*

Choi, Thomason, Zaki, Insall and Barber
Making Phosphorylation Mutants of Arp2

A
Non-auxotrophic diploid heterozygous for ArpB
Thy+/-, Ura+/-, BSR+

Transform with Myc-ArpB plasmid (containing WT, double mutant or triple mutant ArpB allele)

Select colonies in axenic medium with G418

Haploid containing WT ArpB and Myc-ArpB
Thy- or Ura- BSR-

OR

Haploid containing Myc-ArpB only
Thy- or Ura- BSR+

B

Myc

Arp2
Chemotaxis Assay

WT

Phosphorylation mutant
Chemotaxis Assay

- **Speed**: rate of distance travelled over time; measured in \( \mu \text{m/min} \)

- **Directionality**: ability to stay on path; net distance traveled / total distance

\[
\text{Value} = 1 \\
0 > \text{Value} > 1
\]
Arp2 Phosphorylation Regulates Chemotaxis
F-actin

Prestimulus morphology → Cringe → Extension of new pseudopods → Resumed prestimulus morphology

0 30 60 90 120 150 180
Seconds after application of the stimulus
Arp2 Phosphorylation Regulates Actin Polymerization
Chemotaxis Assay

WT

Phosphorylation mutant
Arp2 Phosphorylation is Required for Proper Development
Arp2 Tyrosine Phosphorylation Increases During Development
Prolonged Development Rescues Chemotaxis Defect
Prolonged Development does not Rescue Actin Polymerization Defect
Arp2 Phosphorylation Controls Pseudopod Behavior
Arp2 Phosphorylation Plays Minor Role in Folate Chemotaxis

### Table 1

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Chemotactic index</th>
<th>Total speed of movement</th>
<th>Speed of movement up gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ax3</td>
<td>0.79 ± 0.25</td>
<td>11.9 ± 1.7</td>
<td>4.2 ± 3.2</td>
</tr>
<tr>
<td>Arp2&lt;sub&gt;WTR&lt;/sub&gt;</td>
<td>0.76 ± 0.23</td>
<td>11.3 ± 1.7</td>
<td>5.2 ± 1.8</td>
</tr>
<tr>
<td>Arp2&lt;sub&gt;T&gt;A&lt;/sub&gt;</td>
<td>0.84 ± 0.24</td>
<td>10.8 ± 2.7</td>
<td>5.7 ± 3.1</td>
</tr>
<tr>
<td>Arp2&lt;sub&gt;TY&gt;A&lt;/sub&gt;</td>
<td>0.77 ± 0.22</td>
<td>10.1 ± 1.6*</td>
<td>4.3 ± 2.2</td>
</tr>
</tbody>
</table>

*<sup>p < 0.05 with respect to Ax3 and Arp2<sub>WTR</sub>.</sup>
Arp2 Phosphorylation is not Important for Endocytosis

**TABLE 2**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Spot lifetime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ax3</td>
<td>20.8 ± 5.3</td>
</tr>
<tr>
<td>Arp2 WT</td>
<td>20.5 ± 4.0</td>
</tr>
<tr>
<td>Arp2 T-&gt;A</td>
<td>25.5 ± 6.1*</td>
</tr>
<tr>
<td>Arp2 TY-&gt;AF</td>
<td>20.5 ± 5.7</td>
</tr>
</tbody>
</table>

* p < 0.01 with respect to Ax3, Arp2 WT, and Arp2 TY->AF.
Arr2 Phosphorylation Regulates Chemotaxis Speed

**Table 3**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Chemotactic index</th>
<th>Total speed of movement</th>
<th>Speed of movement up gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µm/min</td>
<td>µm/min</td>
</tr>
<tr>
<td>Arp2(^{WTR})</td>
<td>0.98 ± 0.02</td>
<td>7.2 ± 1.7</td>
<td>5.5 ± 1.7</td>
</tr>
<tr>
<td>Arp2(^{T&gt;A})</td>
<td>0.97 ± 0.03</td>
<td>7.3 ± 1.6</td>
<td>5.5 ± 1.3</td>
</tr>
<tr>
<td>Arp2(^{TY&gt;AF})</td>
<td>0.95 ± 0.08</td>
<td>6.9 ± 1.4</td>
<td>4.3 ± 1.2(^a)</td>
</tr>
</tbody>
</table>

\(^a\) p < 0.01 with respect to Arp2\(^{WTR}\) and Arp2\(^{T>A}\).