The Role of the Arp2/3 Complex in Actin Organization and Dynamics of Neuronal Growth Cones

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Abstract
The neuronal growth cone is a highly motile sensor at the tip of neuronal processes. A key factor in growth cone motility is the cytoskeletal protein actin. As actin polymerizes to form filaments, the cell gains mechanical support as well as enough force to generate directional movement (1-4). One of the main actin nucleation factors that ultimately lead to actin polymerization and filament branching in cells is a seven subunit protein complex referred to as Arp2/3. Even though much is known about Arp2/3 in controlling actin-based motility of non-neuronal cells (1-5), there has been a controversy about its function in growing neuronal cells (6-7). The large growth cones elaborated by Aplysia Californica neurons in culture provide an excellent model system to gain better insights in the localization and function Arp2/3 in growth cone motility. Using immunostaining technique in combination with fluorescence microscopy I plan to localize the Arp2/3 complex in Aplysia Californica growth cones and to correlate the localization of Arp2/3 with actin as well as with cortactin, a regulator of Arp2/3 activity. Furthermore, I will apply a chemical inhibitor to Arp2/3 and study its effects on the actin cytoskeleton. These findings will provide new insights into actin-based growth cone motility and may likely have an impact on future treatments for numerous neurological disorders.

Background
Actin and Cellular Dynamics
Actin plays a vital role in various cellular processes. Cells use a rich array of actin-binding proteins that regulate nucleation, elongation, branching, severing and bundling of actin filaments and thereby produces different types of actin superstructures such as lamellipodia and filopodia (1-4).

Arp2/3 Complex
Arp2/3 produces new actin filaments by anchoring them to pre-existing mother filaments (Fig. 1-2).

Evidence suggests that Src tyrosine kinase substrate protein contactin is a key player in this chain reaction (5).

Results

Arp3

Figure 4. Arp3 sequence alignment. Primary antibody used for Arp3 detection was a Mouse monoclonal antibody against amino acids 1-110 of Arp3 of human origin (Millipore 07-227).

Figure 3. Aplysia Califormica (http://pods.asu.edu/openpedal/Aplysia_california )

Aplysia californica (Fig. 3) as a model system offers large neurons that yield large growth cones in comparison to other neurobiological model systems.

Materials and Methods
Aplysia californica Sequence Analysis
Known sequences were collected from NCBI. In determining conservation of subunits, sequences used were from C. elegans, Drosophila melanogaster, Homo sapiens, and Mus musculus. Using blastn and conserved regions of the proteins, we identified potential Arp2/3 sequences in the Aplysia californica genome. These sequences were then aligned with known sequences using Vector NTI software.

Western Blot Assays
Western blotting was performed using standard protocols after running 10% SDS-PAGE. Primary antibody used for p34-Arc detection was a Rabbit polyclonal antibody against amino acids 285-298 of human Arc (Millipore 07-227).

Identification of subunit sequences for Aplysia californica

Arp2/3 subunits in databases

Evidence suggests that Src tyrosine kinase substrate protein contactin is a key player in this chain reaction (5).

Select antibodies

Project Map

Figure 2. Structure of the Arp2/3 7 subunit complex (Bochziokowska et al., Structure 2008).

Future Experiments

• Immunostaining with both p34-Arc and Arp3 antibodies in cultured cell the cytoskeleton.

• Repeat Western blots and immunostaining with new rabbit anti-Arp3 antibody (H-110; Santa Cruz sc-15390).

• Use of Arp2/3 inhibitor CK-666 to investigate effects on F-actin.

• Study Arp2/3 complex in the context of Src/cortactin signaling.

Discussion

• Database searches with conserved sequences yielded between 30-100% of the sequences for 6 of the 7 Aplysia Arp2/3 subunits

• A mouse anti-Arp-3 antibody detected no signal in Aplysia CNS protein lysate.

• A rabbit anti-p34 antibody showed several bands but with incorrect molecular weight in Aplysia CNS lysate.

References


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