Cytoskeleton signalling

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Laura Machesky, recent winner of the Women in Cell Biology Junior Career Achievment Award and new *Biochemical Journal* editor.

Laura Machesky was invited by the University of Leicester to present a seminar at a meeting sponsored by the Biochemical Society in November. "Laura was involved in discovering actin-related proteins (Arps)", says host Jim Norman introducing her achievements. "She also elucidated the key mechanism by which actin polymerization is controlled."

Phagocytosis, in which cells internalize particles, is central to immunity. Particles labelled with IgG or C3bi (part of the complement system) can be internalized by cells expressing either the Fcg receptor (FcgR) or the complement receptor. After ingestion, the process of phagocytosis is actin-dependent. "The complex of proteins that makes up Arp2/3 is involved in the control of cell motility, as well as in the organization of actin in the cytoskeleton," explains Laura, "and multiple signalling pathways lead to actin polymerization." Laura has shown that the Arp2/3 complex localizes to lamellipodia of crawling cells[1] and that it localizes to FcgR- and CR3-mediated phagosomes[2].

"The seven proteins that make up the Arp2/3 complex act like an actin dimer to assist in cross-linking: they nucleate actin filament assembly. The complex binds to actin filaments and makes dendritic branches on the sides or barbed ends, permitting further filaments to bind."

"Proteins of the WASP family (Wiskott–Aldrich syndrome protein family) are responsible for activating the Arp2/3 complex[3]. "The syndrome is an X-linked deficiency in these proteins and is associated with small platelets and the need for bone marrow transplants." A second family, the Scar proteins, are also involved in activating the Arp2/3 complex.

Laura is investigating several questions surrounding the control of actin assembly. These include: why do cells have multiple WASP family proteins?; is there receptor/GTPase/signal mediator specificity in these pathways?; and, how universal and essential is the WASP Arp2/3 pathway?

"During myeloid differentiation, cell-type specifies the distribution of Scar1 and WASPs. HL-60 cells, which differentiate into either neutrophils or macrophages, were used. The precursor cells have high levels of Scar1 and low levels of WASP, neutrophils have low Scar1 and high WASP levels, while macrophages have low levels of both. Studies in mice showed that Scar1 is expressed in the brain, spinal chord, heart and testes. Scar1–3 and the WASP proteins are regulated differently, and are tissue specific," says Laura. "Scar1 is constitutively active, while WASP isn't. This points to the conclusion that they are involved in different signalling pathways."

Laura uses the phagocytosis pathway as a model system for analysing signalling pathways. Phagocytosis involves specific receptors and leads to actin assembly. The FcgR requires Rac and CDC42, while the CR3 requires Rho. Therefore, although the GTPase requirement for these receptors and the mechanism of ingestion is different, both require actin assembly. Using a phagocytic assay, Laura has shown that the Arp2/3 complex localizes to FcgR-mediated phagosomes in macrophages, and that delocalizing the Arp2/3 blocks FcgR-mediated phagocytosis.

Attempts are also being made to analyse the process downstream of Rho: "Could mDia or Rho-associated kinase (ROK) and myosin II be involved?" Using a catalytically dead ROK, or Y27632 inhibitor, CR3 internalization can be inhibited. Furthermore, inhibitors of myosin II have demonstrated that this enzyme is important, as has its localization to the phagosome during CR3- and FcgR-mediated phagocytosis.

Laura postulates a model where the complement receptor activates Rho, which activates ROK, which activates myosin II and leads to an accumulation of Arp2/3 and eventually actin assembly — the particle is then engulfed. The link between myosin II and Arp2/3 remains unclear. It could be a direct coupling event or an indirect actin filament recruitment process. FcgR-mediated phagocytosis uses a different mechanism where myosin II is not required for Arp2/3 complex formation or actin recruitment. It is, however, required for particle engulfment.

by Gary Burd
(Executive Editor)

References

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