PERSPECTIVES: STRUCTURE

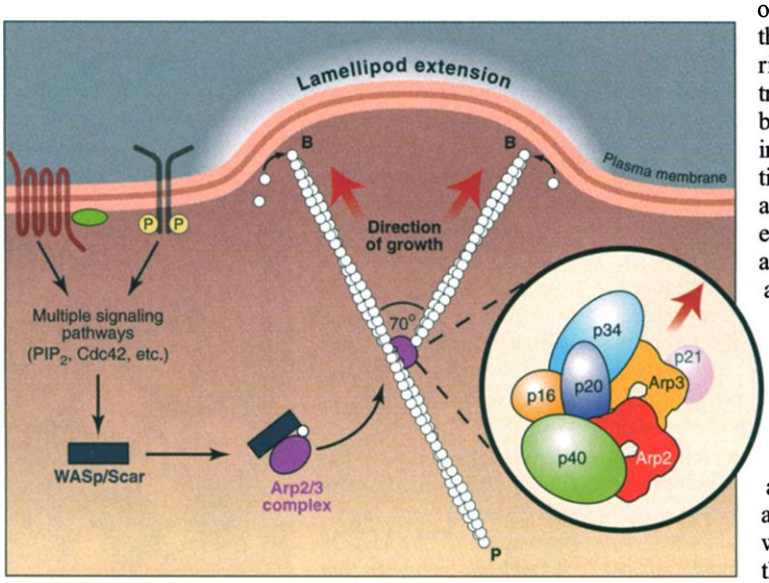
Action at the Y-branch

Alan Weeds and Sharon Yeoh

Cell motility in the form of locomotion, phagocytosis, and the extension of axons requires the polymerization of actin monomers (G-actin) into filaments (*1*). Protrusive activity at the cell's leading edge during motility is characterized by the formation of veils of cytoplasm (lamellipodia), which contain a meshwork of branching actin filaments. These filaments have their fast-growing ends (barbed ends) adjacent to the cell's plasma membrane. Extension of the filament meshwork depends on both addition of G-actin to existing barbed ends and the generation of new branches. Only one factor thus far has been shown to promote branched growth of actin filaments: the Arp2/3 complex. For those eagerly awaiting the solution of the three-dimensional structure of Arp2/3, they need look no further than the report by Robinson *et al.* (*2*) on page 1679 of this issue.

The Arp2/3 complex, first identified in single-celled organisms called protozoans, contains two actin-related proteins (Arp2 and Arp3) and five other protein components (*3*). Yeast genetically engineered to lack Arp2/3 either die or have a disrupted cytoskeleton. From electron microscopy and biochemical studies, it has become clear that Arp2/3 complexes participate in the formation of branched networks of actin filaments, and promote polymerization of G-actin monomers at the filament's barbed end (*4*). The requirement of Arp2/3 for cell motility has been confirmed in reconstitution experiments with bacteria such as *Listeria*, which hijack the motile machinery of host cells. In addition to actin, the Arp2/3 complex is one of only three factors essential for bacterial locomotion (*5*). The ability of the Arp2/3 complex to seed (nucleate) actin polymerization is regulated by a group of Wiskott-Aldrich Syndrome proteins (WASp/Scar), named after the discoverers of a rare immunodeficiency disease (*6*).

One key to understanding the function of a particular protein is to accurately pinpoint the location of that protein in the cell. Immunoelectron microscopy has localized the Arp2/3 complex to the Y-branch, the point where a daughter actin filament branches off from the parent filament (see the figure). This is consistent with the dendritic nucleation model of actin polymerization (*7*). Direct proof of dendritic nucleation has been elegantly provided by using parent actin filaments that fluoresce a different color from the growing daughter filaments (*8*). Furthermore, detailed electron microscopy has revealed the structure of the Arp2/3 complex at low resolution (*9*). It appears that Arp2/3 becomes attached to the side of the parent actin filament through the interaction of three of its five ancillary proteins (p16, p34, and p40) to three actin subunits. The ancillary proteins orient Arp2 and Arp3 such that the elongating daughter filament is at a 70° angle to the parent filament (hence the term Y-branch). These reconstructions provide a model for both isolated and filament-bound complexes and suggest a substantial conformational rearrangement of Arp2 and Arp3 within the complex once it has bound to the parent filament.



The world according to Arps. Members of the WASp/Scar protein family (black) integrate signals from multiple pathways to activate the Arp2/3 complex (purple). This complex binds to the sides of actin filaments (white) and seeds (nucleates) the formation of new actin filament branches, which elongate at their barbed ends (B). Red arrows indicate the direction of actin filament growth. This expanding network of branching actin filaments drives the protrusion of the plasma membrane and hence cell motility. **(Inset)** In the activated Arp2/3 complex, Arp2 and Arp3 are positioned with their five ancillary proteins such that they are able to direct barbed-end growth of daughter actin filaments (*2*). The clefts between the two lobes of Arp2 and Arp3 (partially obscured) point toward the bottom left of the inset.

The structure of the bovine Arp2/3 complex determined by Robinson *et al.* at 2.0 Å resolution not only reveals the disposition of its seven components, but also shows why the complex is inactive and how it can be activated. The overall shape of Arp2/3 resembles the "kidney bean" image from the electron micrograph reconstruction (*9*). Indeed, Arp2 and Arp3 have similar three-dimensional structures to actin, but in both proteins the central cleft between the two lobes is open (see the figure). In actin, this cleft binds to the energy-rich molecule adenosine 5′-triphosphate (ATP), whereas in both Arps ATP is absent. Most importantly, from the perspective of function, the two Arps are rotated 180° relative to each other compared with two adjacent actin subunits in a filament, such that filament nucleation is not possible. The Arps are cradled by the ancillary proteins p34 and p20, which self-associate loosely through extended carboxyl-terminal helices in antiparallel fashion. The p40 ancillary protein, a β propeller with seven blades, reinforces this clamp. The p21 and p16 components decorate opposite edges of the structure.

Reconstitution experiments by Gournier *et al.* (*10*) provide biochemical evidence for interactions among components in the complex that may direct nucleating activity and branch formation. The functions of the components are largely in agreement with those proposed in the Robinson *et al.* structure paper, although the part played by p40 in branch formation is contended. Gournier and colleagues provide evidence that the peripheral component p21 promotes efficient nucleation.

Robinson and co-workers propose that binding of ATP promotes the conformational changes necessary for complex activation, initially by closing the clefts in the two Arp subunits. A simple rotation of Arp2, p20, p40, and p16 of about 20° relative to the other subunits translates Arp2 about 3 nanometers relative to Arp3. This positions the two Arps in a similar orientation to that of an actin dimer in nucleation mode. The open nature of the structure, both in terms of the Arp

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subunits themselves and the interactions of associated proteins, suggests potential for substantial tightening of the conformation following rotation. Moreover, the carboxyl terminus of the activator WASp binds to both Arp2/3 and p21 to generate a cooperative system in which actin filaments (F-actin) enhance the affinity of WASp for the complex and further stimulate nucleation (*11*).

The structure of the Arp2/3 complex provides, for the first time, insights into how dendritic branches of actin filaments are assembled. After triggering by WASp, the elementary steps of branched filament growth are the assembly of Arp2/3 complexes and G-actin subunits on F-actin. Activation of WASp family members by various signaling pathways is an essential component of the regulatory process. Both phosphatidylinositol 4,5-bisphosphate and the small ras-related GTP-binding protein Cdc42 are essential for WASp activation, and bind to sequences in the amino-terminal part of this protein. Moreover, Cdc42 must be in its membrane-bound, GTP-containing form (*12*).

The Robinson *et al.* work provides a substantial springboard for further progress. It may be possible to gain structural information about the interplay among all of the components of the Y-branch complex, and about the mechanism that activates dendritic growth. A more ambitious project will be to elaborate the sequence of interactions involving actin depolymerizing factors, capping proteins, and profilin in the recycling of actin subunits. Current evidence suggests a complex series of steps, key to which is whether ATP or ADP is bound to actin (*13*). Future research will unravel how integration of signals at the cell surface mediates the processes that lead to the remodeling of the branched filament network and how this is translated into coordinated cell movement.

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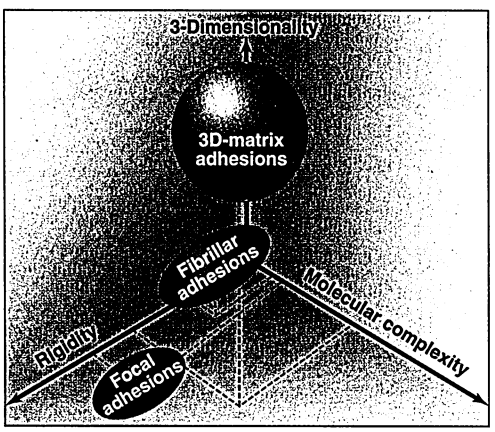
PERSPECTIVES: CELL BIOLOGY

Encounters in Space

Benjamin Geiger

At the end of nearly every talk about cell-matrix adhesions—the structures formed between cultured cells and the substratum on which they grow—someone in the audience invariably asks: "Beautiful pictures, but what is the physiological significance of these adhesions? Aren't they merely artifacts of tissue culture?" Slightly annoyed, the speaker usually mutters something about the general nature of model systems, and highlights similarities between adhesions formed in culture and those formed in vivo. Recent work points to a remarkable molecular heterogeneity in the adhesions formed by cultured cells as they attach to different substrates (*1*). Such studies, however, do not identify which adhesions are akin to those formed in vivo. On page 1708 of this issue, Cukierman and coworkers (*2*) directly address this question and reach some intriguing conclusions.

These investigators grew fibroblasts on shallow 3D matrices derived from other cultured cells or tissues. These 3D matrices are similar to the extracellular matrices produced by cells in vivo, yet are reasonably flat and thus can be readily analyzed by high-resolution fluorescence microscopy. The cultured cell-derived 3D matrix has been extensively used for growing cells under quasi-physiological conditions, yet its capacity to support specific molecular types of adhesions has not been determined (*3*, *4*). In their study, Cukierman and colleagues compare the characteristics of adhesions formed by cultured fibroblasts attached to a cell-derived 3D matrix, a 2D matrix, and a 3D collagen matrix. The rate at which fibroblasts attached to the cell-derived 3D matrix was 6- to 10-fold higher than that measured for all other surfaces. The adhesions formed with this matrix were elongated and morphologically distinct from the focal and fibrillar adhesions typical of cultured cells (*1*). When attached to the cell-derived 3D matrix, fibroblasts became spindle-shaped (like tissue fibroblasts in vivo) and lost their flat morphology. These spindle-shaped fibroblasts proliferated two to three times more rapidly than their counterparts attached to other surfaces.



Close encounters of the 3D kind. The molecular composition, rigidity, (pliability) and topography (three-dimensionality) of a matrix affects the formation of cellular adhesions. Focal adhesions are associated with rigid surfaces and are flat with limited molecular complexity. In contrast, fibrillar adhesions contain the fibronectin receptor and bind to pliable fibronectin fibrils. They are slightly 3D and are of moderate molecular complexity. The adhesions formed between cultured cells and a cell-derived 3D matrix are moderately pliable and probably highly complex, as well as being 3D.

The principal molecule associated with these fibroblast adhesions was integrin $\alpha5\beta1$, the major fibronectin receptor. Function-blocking antibodies against this integrin prevented the formation of 3D-matrix adhesions. The 3D-matrix adhesions contained large amounts of focal adhesion kinase (FAK), paxillin, and vinculin, thus resembling focal adhesions; yet, like fibrillar adhesions, they also contained the fibronectin receptor. Particularly intriguing was the low level of FAK phosphorylation in 3D-matrix adhesions, which contrasted with the highly phosphorylated FAK of focal adhesions.

What are the features of a 3D matrix that make it so different from other substrates? Cukierman and colleagues determined the specific contributions of matrix topography, molecular composition, and mechanical properties (pliability) to the ability of fibroblast adhesions to stimulate appropriate intracellular signaling pathways. They found that the topography (degree of three-dimensionality) of the matrix alone was not sufficient to activate adhesion-mediated signaling, because a 3D collagen matrix could not do the job. Nor was the "proper" molecular composition enough: If the 3D matrix was destroyed by "flattening" there was no signaling activity even with the correct molecular composition. Combining the correct topography and molecular composition was still insufficient, because reducing the

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