WEB WATCH

In its own little world

- http://www.imb-jena.de/RNA.html

If you're looking for information about RNA, head for the RNA World Website. Set up and maintained by the Institute for Molecular Biology in Jena, Germany, this site does a sterling job of bringing together RNA-related web resources to make the search for relevant information much easier. The site has been updated fairly recently, although this is more than can be said for several of the entries that it links to — probably the only major disadvantage of the site. However, divided into six subcategories, the site should still have something for everyone.

Take, for example, the Books and Tutorials subcategory. For non-specialists, there are five tutorials on ribosomes that provide general structural and functional overviews using a combination of clear diagrams and text. Among other useful topics selected for tutorials are RNA splicing, RNA and DNA as catalysts, and RNA interference (RNAi). The timely RNAi tutorials provide not only an overview of this approach, but also offer informative protocols.

Valuable information is also available within the Software subcategory. Here, various software is available, including programs that predict RNA structures and RNA movies, which "create the impression of an RNA molecule exploring its own 2D structure space". Within 'Sequences, Secondary Structures and Other', you can gain access to the RNA Editing Web Site, whereas in 'Databases and Web Tools', you can learn all about the geometrical features of DNA — from A to Z.

Katrin Bussell

CYTOSKELETON

Branching out

The actin related proteins Arp2 and Arp3, together with five other protein subunits, form the Arp2/3 complex, which promotes polymerization of G-actin into F-actin and branching of these filaments to form an actin filament network. Pollard and colleagues have now determined the structure of the bovine Arp2/3 complex at 2.0 Å resolution, while Welch's team have reconstituted the human complex by expressing the subunits using a baculovirus expression system. Both studies give a clear insight into the nucleation and branching functions of the Arp2/3 complex.

The crystal structure shows that the seven subunits of the Arp2/3 complex are arranged into a 'kidney'-shaped flat ellipsoid. At the core of this complex, Arp2 and Arp3 are cradled by a C-shaped clamp formed by a stable heterodimer of the p20 and p34 subunits, which associate through their long carboxy-terminal α-helices. Welch and colleagues showed that this heterodimer is critical for the integrity of the complex. The crystal structure revealed that the p40 (bovine) subunit is a seven-blade WD40 β-propeller, which strengthens the top and bottom of the clamp, while p16 and p21 are globular subunits that are found at opposite edges of the complex.

The three-dimensional structures of Arp2 and Arp3 are similar to that of actin, being bilobed. However, in contrast to actin, ATP is absent, so their central clefts are open. Furthermore, Arp2 and Arp3 are rotated 180° around the filament axis relative to each other, compared with two neighbouring actin subunits. In this conformation, they are unable to nucleate a new filament.

So, Pollard and colleagues propose that activation of the Arp2/3 complex involves a rotation of the two halves of the complex to re-orient Arp2 and Arp3 so that they resemble an actin dimer in conformation, which favours nucleation. They also suggest that Wiskott–Aldrich Syndrome protein (WASP) — and its relatives — favours nucleation by promoting this active conformation. ATP binding to the Arp subunits might also contribute to activation.

In support of these findings, Welch's team showed that subcomplexes of the Arp2/3 complex that lack both Arp3 and p21 showed no nucleation activity, even when nucleation-promoting factors such as ActA and WASP were added. Subcomplexes missing only p21 retained nucleating activity in the presence of ActA or WASP, which is consistent with a crucial role of Arp3 as a nucleation template. Adding ActA or WASP to subcomplexes lacking p16 and p41 (human) only weakly stimulated nucleation, indicating that they have an important function in the structural organization of the nucleation site, inducing conformational changes during nucleation, or binding to ActA or WASP.

Does the crystal structure give us any clues as to how Arp2/3 promotes branching? The structure of branches shows multiple contacts between the Arp2/3 complex and the side of the 'mother' filament. Pollard's group suggest that the p40 (bovine) subunit — which contains a helix in a loop between two of its blades — might provide one of these anchors. Welch and colleagues showed that the purified p34/p20 heterodimer co-sedimented with F-actin, indicating that it also binds to actin filaments. Furthermore, they showed that p34/p20 could also crosslink actin filaments, although the structures did not fully resemble the typical 'Y' branches that the complete complex can form, implicating other subunits in branching. This is consistent with previous work showing that p34 can be chemically crosslinked to actin. So, further studies are needed to determine all of the subunits that are required for branching. Another challenge will be to fit depolymerizing factors, capping proteins and other actin-binding proteins into the equation.

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References and links

ORIGINAL RESEARCH PAPERS Robinson, R. C. *et al.* Crystal structure of Arp2/3 complex. *Science* **294**, 1679–1684 (2001) | Gournier, H. *et al.* Reconstitution of human Arp2/3 complex reveals critical roles of individual subunits in complex structure and activity. *Mol. Cell* **8**, 1041–1052 (2001)

FURTHER READING Volkmann, N. *et al.* Structure of Arp2/3 complex in its activated state and in actin filament branch junctions. *Science* **293**, 2456–2459 (2001)

WEB SITE

Matthew Welch's laboratory: http://mcb.berkeley.edu/labs/welch/