Mini-Review

New Insights into the Interaction of Cytoplasmic Dynein with the Actin-related Protein, Arp1

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AMONG the most rewarding discoveries in modern cell biology are those that demonstrate convergence of results obtained using biochemical and genetic approaches. Scientists with a biochemical bent use in vitro assays that mimic complicated physiological processes to define participating molecules. Others exploit the power of genetics to rapidly identify and characterize genes that, when mutated, wreak havoc on normal cell physiology; the corresponding proteins are considered possible players in the process in question. When these two divergent experimental courses identify homologous proteins, even the most skeptical among us begin to believe that we have gained some insight into the protein's function. In recent years, the field of membrane traffic has benefitted enormously from the combined application of biochemical and genetic approaches, as proteins highlighted by in vitro studies have been found to have homologs in the *sec* proteins in the budding yeast, *S. cerevisiae*. The cell cycle is another area in which genetics and biochemistry have elegantly collided, as evidenced by the enhancement of our understanding of the M-phase-promoting factor cascade by yeast *cdc* mutants. As we continue to dissect and reconstitute some of the more complicated events that occur within cells it is certain that many more such intersections will occur. A series of recent papers, including two that appear in this issue (Clark and Meyer, 1994; Muhua et al., 1994; Plamann et al., 1994) report the discovery of a novel genes encoding the actin-related protein, Arp1, which was previously characterized using an in vitro, biochemical assay. It is now apparent that Arp1 participates in a number of distinct microtubule-based motility events powered by cytoplasmic dynein.

Cytoplasmic dynein is a ubiquitous, soluble microtubule-activated ATPase that transports objects toward microtubule minus ends. A wide variety of intracellular functions have been ascribed to the enzyme. Its subcellular localization and behavior in vitro suggest that cytoplasmic dynein drives minus end–directed movement of membranous organelles in the processes of retrograde transport in axons, basal-to-apical transcytosis in polarized epithelia, and traffic in the endocytic pathway of many cells (Schroer and Sheetz, 1991a; Walker and Sheetz, 1993). Dynein may also facilitate cytoskeletal rearrangements by causing movement of non-membranous cytoplasmic components such as f-actin or microtubules relative to microtubules. That the enzyme appears to be associated with kinetochores (Pfarr et al., 1990; Steuer et al., 1990; Wordeman et al., 1991) indicates a role in chromosome movement in mitosis. However, microinjection studies with dynein antibodies suggest that the process of spindle pole separation in prophase, not chromosome congression or anaphase movement, is most sensitive to loss of dynein function (Vaisberg et al., 1993).

Genetic analysis suggests yet other roles for cytoplasmic dynein. Disruptions of the *S. cerevisiae* dynein heavy chain gene (DYN1, Eshel et al., 1993; DHC1, Li et al., 1993) result in viable cells that are impaired for growth (Li et al., 1993), a defect that may result from an unusual and highly characteristic mitotic phenotype (Eshel et al., 1993; Li et al., 1993). The cells complete karyokinesis, undergoing chromosome congression and separation at normal rates, but spindle positioning and subsequent delivery of the daughter nucleus into the newly formed bud is perturbed. This results in a increased proportion of binucleate large-budded cells as well as anucleate, binucleate, and multinucleate daughter cells. The spindle orientation defect is exacerbated when cells are grown at a reduced temperature. Inhibition of delivery of the daughter nucleus to the bud may slow cell division, thus causing a reduction in growth rate, although it would appear that the cells possess a secondary mechanism for moving the nucleus into the bud which allows them to survive. Mutants in the cytoplasmic dynein heavy chain of the filamentous fungus *Aspergillus nidulans* (*nudA*, for *nu*clear *d*istribution) also exhibit defects in nuclear migration, in this case, into the elongating hypha (Xiang et al., 1994).

How might cytoplasmic dynein facilitate spindle orientation and nuclear migration? Spindle positioning depends on the astral microtubules that extend from the two ends of the elongated spindle into the mother cell and the bud (Sullivan and Huffaker, 1992). In the currently favored model, these microtubules anchor the spindle to the plasma membrane so that one end remains in the mother cell and the other is in the bud. As actin is also required for proper spindle orientation (Palmer et al., 1992), the site of anchorage may be a microfilament-rich cortical region. The cortical substructure of the mother cell is largely uncharacterized, but the bud surface contains concentrated patches of actin and actin-binding proteins that may serve as attachment sites. Cytoplasmic dynein associated with cortical structures positioned at opposite ends of the dividing cell might orient the spindle by

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walking towards the minus ends of the astral microtubules, effectively pulling the spindle into alignment. In the cytoplasmic dynein heavy chain mutants, astral microtubules are still seen to project into the bud, but apparently they are unable to become properly attached to the surface.

Nuclear migration in hyphae also requires intact microtubules (Oakley and Morris, 1980) which are aligned along the long axis of the filamentous structure. After microtubules are depolymerized, regrowth is initially observed at a site at the distal end of the hypha, suggesting that a microtubule-organizing center and associated microtubule minus ends are localized in this region (Hoch and Staples, 1985). Movement of nuclei toward the hyphal tip would therefore be predicted to be driven by cytoplasmic dynein associated with the nucleus.
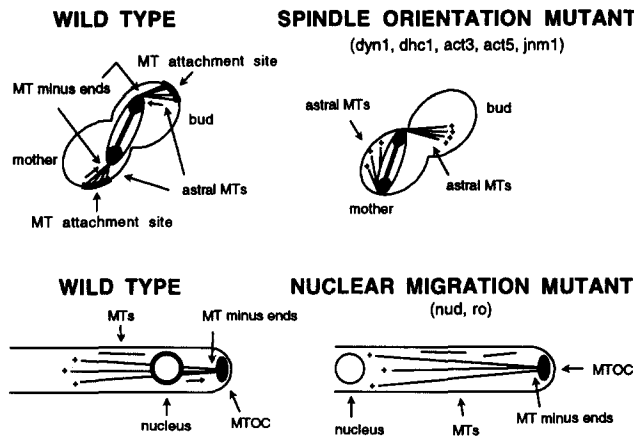
Of critical importance, then, is the mechanism of attachment of cytoplasmic dynein to either the nucleus or plasma membrane. Let us consider what is known about the association of this motor with other membranes. In vitro studies suggest that the functional interaction of dynein with intracellular membranes may be somewhat complicated, as other soluble factors besides the enzyme are needed. While purified dynein alone can adsorb to glass and plastic surfaces and produce microtubule-dependent movement, the dynactin (dynein activator) complex is required for dynein-powered movement of membrane vesicles (Gill et al., 1991; Schroer and Sheetz, 1991b). Dynactin complex, a 1.2-mD molecule (Schafer et al., 1994) that sediments at 20S (Gill et al., 1991), contains several subunits, including p160/p150 (Gill et al., 1991; Holzbaur et al., 1991) (the vertebrate homolog of the Drosophila glued protein; Swaroop et al., 1987), an actin-related protein (Lees-Miller et al., 1992; Paschal et al., 1993), conventional actin (Schroer and Sheetz, 1991b) and actin capping protein (Schafer et al., 1994) as well as 62-, 50-, 27-, and 24-kD subunits that have yet to be identified. The actin-related protein falls into the Arp1 class which includes actin-RPV (Lees-Miller et al., 1992), centractin (Clark and Meyer, 1992), and Actr 87C (Fyrberg et al., 1994), conserved proteins that are ∼55% identical to conventional actin. Arp1 comprises nearly 50% of the mass of the dynactin complex and contributes to its major structural domain, a 37-nm actinlike filament that also contains actin capping protein and, most likely, conventional actin (Schafer et al., 1994). Immunofluorescence studies indicate that, like dynein (Pfarr et al., 1990; Steuer et al., 1990; Gill et al., 1991; Vaisberg et al., 1993), dynactin complex has a diffuse, punctate distribution in cytoplasm (Gill et al., 1991; Clark and Meyer, 1992; Paschal et al., 1993) which is thought to reflect binding to membrane vesicles. In animal cells, dynactin complex may therefore serve to bind dynein to intracellular membranes to facilitate their movement on microtubules.

The recent identification of homologs of Arp1 in S. cerevisiae (Clark and Meyer, 1994; Muhua et al., 1994) and the filamentous fungus Neurospora crassa (Plamann et al., 1994), have made possible analysis of Arp1 function in a different context. Like cytoplasmic dynein, Arp1 appears to participate in spindle positioning and nuclear migration. Disruptions of the yeast gene (called ACT3 by Clark and Meyer, 1994; and ACT5 by Muhua and co-workers, 1994) result in a phenotype indistinguishable from that of the dynein heavy chain mutant. Spindle positioning is perturbed

even though astral microtubules still project into the bud, and binucleate mother cells and anucleate and binucleate daughters are observed with increased frequency. Despite these effects the gene is not essential for cell growth. Another hallmark of the dynein mutant phenotype is its exacerbation at reduced temperature, a phenomenon also seen in the Arp1 mutants. The nuclear migration defect is quite striking; in synchronized cultures, nearly half the cells are affected (Muhua et al., 1994). That the phenotype of the act5/dhc double mutant is indistinguishable from that of either single mutant provides further support to the conclusion that the two proteins have entirely overlapping functions (Muhua et al., 1994). Delivery of endocytic markers to the vacuole (a digestive compartment functionally equivalent to the lysosome) and vacuolar morphology are not inhibited (Clark and Meyer, 1994), suggesting that membrane traffic in the endocytic pathway is unaffected. That dynein and Arp1 appear not to be involved in the endocytic pathway is consistent with the fact that microtubule-based vesicle transport is thought not to play a prominent role in this organism owing to its small cell size and lack of an extensive microtubule cytoskeleton.

Unlike the budding yeast ACT3/ACT5 gene, which was sought deliberately, the discovery of the Neurospora Arp1 gene was serendipitous. The ropy (ro) mutants, described over 25 years ago (Garnjobst and Tatum, 1967), acquired their name because of their striking colony morphology. The growing hyphae do not extend in the typical elongated manner but instead have a curled, distorted appearance. Plamann and co-workers (1994) noted that suppressors of a protein kinase mutant (cot-1) also demonstrated the characteristic ropy morphology and were, in fact, novel ro alleles. The aberrant colony morphology correlates with a defect in hyphal nuclear migration; in ro mutants, nuclei continue to divide but remain clustered at the proximal end of the hypha, a phenotype reminiscent of that exhibited by Aspergillus nud mutants. Like nudA, the ro-1 gene encodes cytoplasmic dynein heavy chain, while ro-4 encodes Arp1. Once again similar phenotypes are exhibited by mutants in cytoplasmic dynein and Arp1, providing further support to the hypothesis that cytoplasmic dynein and the dynactin complex, two distinct components of the microtubule-based motility machinery, interact functionally within the cell. It will be interesting to determine whether the microtubule-based movement of other intracellular membranes (Steinberg and Schliwa, 1993) is affected in the ropy mutants.

The present studies do not address the question of whether, in yeast and the filamentous fungi, Arp1 forms a filament and/or participates in a structure analogous to the dynactin complex in vertebrate cells. The entire soluble pool of brain Arp1 takes the form of a 20S complex (Paschal et al., 1993), suggesting that this feature is relevant to the vesicle transport function of the protein. Elucidation of the physical properties of yeast and Neurospora Arp1 may not be straightforward, as these experimental systems are better suited to genetics than the pursuit of biochemistry. Moreover, yeast Arp1 (Act5p) is present in very low amounts (<0.00022% of total protein; Muhua et al., 1994) which will likely make its purification a challenge. But regardless of the form taken by Arp1, we can speculate on how cytoplasmic dynein and Arp1 might effect spindle orientation and nuclear migration (see Eshel et al., 1993; Li et al., 1993; McMillan and Tatchell, 1994; Muhua et al., 1994; and Plamann et al., 1994 for fur-

WILD TYPE   **SPINDLE ORIENTATION MUTANT**
(dyn1, dhc1, act3, act5, jnm1)

WILD TYPE   **NUCLEAR MIGRATION MUTANT**
(nud, ro)

Figure 1. (*Top*) Proposed model for spindle orientation in the budding yeast, *S. cerevisiae* (modified from Eshel et al., 1993; also discussed in Li et al., 1993; McMillan and Tatchell, 1994; and Muhua et al., 1994). In wild type cells, astral microtubules projecting from the spindle pole body interact with cortical attachment sites that contain dynein and/or Arp 1 (black arc) in the mother cell and the bud. Arp 1 may act to bind dynein to the attachment site. The spindle becomes aligned as dynein exerts force toward the minus ends of the two sets of astral microtubules (the direction of movement is indicated by *arrows*); dynein in the bud also pulls the elongated spindle into the bud. The mutants described here lack dynein heavy chain, Arp1 or Jnmlp, which causes a defect in the microtubule attachment site so that the astral microtubules no longer bind. The spindle does not become oriented properly between the mother and the bud and movement of the daughter nucleus into the bud is impaired. (*Bottom*) Proposed model for nuclear migration in filamentous fungi (modified from Plamann et al., 1994). In wild type cells, microtubules radiate from an organizing center (*MTOC*) at the tip of the extending hypha. Dynein and/or Arp1 are associated with the surface of the nucleus (surrounded by a thick line). Dynein walks toward the microtubule minus ends, causing the nucleus to migrate toward the tip. In nuclear distribution (*nud*) and ropy (*ro*) mutants, cytoplasmic dynein or Arp1 function is perturbed. Active dynein is no longer associated with the nucleus so it is unable to move toward the hyphal tip (the direction of movement is indicated by an *arrow*). In ropy mutants, the tip continues to grow in the absence of nuclear migration, but without nuclei and other associated components the hypha becomes curled.

ther discussion). In the model cartooned in Fig. 1, dynein and/or Arp1 are localized to cortical attachment sites in yeast and to the surface of nuclei in *Aspergillus* and *Neurospora*, an arrangement that may be analogous to their localization on internal membranes of animal cells. Arp1 may serve to anchor dynein to the membrane or it may interact with dynein that is itself tightly membrane associated. As both components must be present for dynein to drive movement toward the minus ends of astral or hyphal microtubules, loss of either will prevent spindle orientation and nuclear migration.

Another interesting form of nuclear motility occurs during embryonic mitoses in invertebrates such as the nematode *Caenorhabditis elegans*. After the first mitosis, one daughter nucleus rotates so that the next cell division will occur in a plane perpendicular to that of the other daughter cell. This reorientation is believed to require interaction between astral microtubules and the actin cortex (Hyman, 1989; Hyman

and White, 1987). Cortical attachment may use cytoplasmic dynein and dynactin complex in a manner similar to that proposed for yeast.

It is likely that our understanding of cytoplasmic dynein and dynactin complex function will continue to be enriched by the combined efforts of genetics and biochemistry. Recall that both dynein and dynactin complex are multisubunit proteins, each containing four or more distinct polypeptides. Since a defect in a subunit of either complex results in a similar phenotype, analysis of novel mutants exhibiting this same phenotype may permit identification of the other components. To date, several *ro* mutants have been isolated, one of which (*ro-3*) is a homolog of the p150/*glued* subunit of the dynactin complex (Plamann et al., 1994). The jnml (just nuclear migration) mutant of *S. cerevisiae* demonstrates a similar spindle orientation and nuclear migration phenotype to that observed in dynein heavy chain and Arp1 mutants (McMillan and Tatchell, 1994) and *jnml/dhc1* double mutants show no enhancement of phenotype, suggesting the two drive the same process. The primary sequence of Jnmlp reveals no similarity to any known subunit of dynein or dynactin complex, but as yeast dynein heavy chain and Arp1 are both highly divergent, Jnmlp may share little sequence identity with but still be the functional homolog of an identified component. JNM1 might also encode an as yet undefined element of the machinery responsible for spindle orientation. The JNM1 gene product (expressed as a β-galactosidase fusion protein) localizes to the spindle pole body, both in the bud of dividing cells and in nonmitotic cells, a location that does not readily fit our model. If Jnmlp is indeed a component of the anchoring machinery for astral microtubules, it would be expected to localize to the cell surface in cortical actin patches and to an analogous site in the mother cell. However, the appearance of Jnmlp at the spindle pole body may not be surprising, as both cytoplasmic dynein and Arp1 are found at the microtubule-organizing center in animal cells (Pfarr et al., 1990; Steuer et al., 1990; Gill et al., 1991; Clark and Meyer, 1992; Vaisberg et al., 1993). Because this localization can be abolished by disrupting cytoplasmic microtubules (Paschal et al., 1993, Schroer, T., unpublished observations) it is assumed that dynein and associated proteins accumulate at the microtubule-organizing center by walking to the minus ends of microtubules; Jnmlp may accumulate at the spindle pole body by a similar mechanism.

Additional insights into the interaction of cytoplasmic dynein and the dynactin complex should also result from further biochemical study. Both proteins are extremely well characterized; all the dynein subunits have been cloned (Vallee, 1993; Gill et al., 1994) as have most of the subunits of the dynactin complex and the remaining work is nearing completion. Progress is being made to understand the structure of both molecules (Vallee et al., 1988; Schafer et al., 1994). The major unanswered questions address the mechanisms of interaction of the two molecules with membranes and the precise mode by which dynactin complex regulates cytoplasmic dynein activity, areas that are ripe for investigation.

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