Phenotypes of cytoskeletal mutants

Susan S. Brown

University of Michigan, Ann Arbor, Michigan, USA

Phenotypic studies continue to contribute to an understanding of the functions of cytoskeletal proteins. Many of these studies indicate some degree of functional redundancy within a family of cytoskeletal proteins. Some surprises have emerged, such as suggestions of unexpected relationships between the actin and microtubule cytoskeletons. Finally, phenotypic studies have provided evidence for a function of intermediate filaments.

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Introduction

Many laboratories are turning to studies of cytoskeletal mutants to gain an understanding of the *in vivo* role of proteins whose *in vitro* function has been well characterized in some cases [1,2]. This review covers the last year's progress in the study of non-muscle cytoskeletal proteins, and defines 'mutant' as any cell containing more or less than the normal amount of a cytoskeletal protein, as well as any cell containing altered protein, i.e. cells that have been micro-injected with protein or treated with antisense RNA. I have not included effects produced by micro-injection of antibody, as these studies have an additional level of uncertainty, due to questions of specificity, crossreactivity or interpretation ([3•] for example).

Actin

The single, essential actin gene in *Saccharomyces cerevisiae* has been mutagenized and the *in vivo* consequences examined. Johannes and Gallwitz [4•] have altered a number of highly conserved amino acid residues thought to be functionally important. Surprisingly, most of these substitutions are phenotypically neutral. Wertman *et al.* [5•] have taken a different approach; they avoided preconceived notions of which residues might be important by systematically replacing clusters of charged residues (which are mostly at the monomer surface) with alanines. They find that lethality results from changes in the nucleotide-binding pocket, in regions of predicted actin–actin contact in a filament, and in regions which may bind myosin or tropomyosin. Changes that cause temperature sensitivity occur where they might interfere with the interaction between actin and other proteins, whereas changes that are predicted to be buried at the interface between strands of the filament have no phenotypic effect. It has been found that the hydrophobic loop predicted to be involved in interstrand interactions is important to actin function (RK Cook, X Chen, PA Rubenstein, unpublished data). Cook *et al.* [6•] have found that altering the negatively charged amino acids near the amino terminus of actin produces significant *in vitro* effects but no dramatic phenotypic changes.

Actin relatives

Three distant relatives of actin have been identified. Whereas most actins are highly conserved and show greater than 90% identity, these actin-related proteins show approximately 50% identity to actins. The genes in *S. cerevisiae (ACT2)* [7•] and *Schizosaccharomyces pombe (act2)* [8•] are essential. Some divergence is found in regions that in actin have been implicated in interaction between monomers or in binding myosin, raising the possibility that these actin-related proteins do not function in the same way as other actins. Overexpression of *ACT2* causes morphological aberrations that might possibly suggest an effect on the actin cytoskeleton [7•]. Lees-Miller *et al.* [8•] make the interesting suggestion that Act2 protein could turn out to be an actin-capping protein. A third actin-like protein, actin-RPV (related protein, vertebrates) has been identified and shown to be part of a complex that activates dynein [9•]. Genes similar to each of these three actin-like proteins have been identified in *Caenorhabditis elegans*, indicating that they represent separate classes of proteins that can co-exist in a given organism (J Lees-Miller, D Helfman, unpublished data).

Abbreviations

CAP—cyclase-associated protein; ***ncd***—*non-claret disjunctional*; **PIP_2**—phosphatidylinositol bisphosphate.

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Profilin

Studies of mutants with defects in actin-associated proteins continue to provide clues to their function *in vivo*. This is important, because *in vivo* function is often difficult to infer from *in vitro* studies. For example, it is far from clear what profilin, a protein that binds actin and phosphatidylinositol bisphosphate (PIP_2) *in vitro*, actually does in the cell [10]. Studies of cyclase-associated protein (CAP) by Vojtek *et al.* [11•] suggest a relationship between profilin and the Ras and phosphatidyl inositol signalling pathways in yeast. CAP appears to be bifunctional; deletion of the amino-terminal portion causes defects in Ras activation of adenyl cyclase, whereas deletion of the carboxyl-terminal portion, like deletion of profilin, causes defects in the actin cytoskeleton. Profilin overexpression corrects these carboxyl-terminal defects. Although a correlation was observed between the ability of profilin to bind PIP_2 and to compensate for CAP, the situation may be more complicated, given recent findings that CAP may be an actin-binding protein [12] and that actin and PIP_2 binding appear to be intimately linked in mutagenesis studies of profilin (BK Haarer, SS Brown, unpublished data). Surprisingly, 10-fold overexpression of profilin in yeast has no obvious phenotypic effect (BK Haarer, SS Brown, unpublished data). If the function of profilin is to sequester PIP_2 and/or actin monomers, one would expect overexpression to be deleterious. Cao *et al.* [13•] have observed phenotypic effects when profilin concentration is increased by micro-injection of tissue culture cells. This treatment reduces the amount of filamentous actin located centrally in the cell, and reduces the formation of lamellipodia, but does not appear to affect cytokinesis or formation of focal contacts. (Different effects are obtained with other putative actin-sequestering proteins; thymosin β4, like profilin, reduces the filamentous actin concentration, but does not affect lamellipodia, whereas neither causes the cell retraction seen with DNase I or vitamin D binding protein [14•].) On the other hand, transfection experiments indicate that actin is stabilized by profilin overexpression (T Finkel, JA Theriot, KR Dise, GF Tomaselli, P Goldschmidt-Clermont, unpublished data). This apparent discrepancy may result from the different techniques used (micro-injection versus transfection). Cooley *et al.* [15•] report that *chickadee* in *Drosophila* encodes an ovary-specific profilin. The mutant phenotype indicates that profilin is needed for assembly of a population of actin filaments that appear to anchor nuclei and keep them from blocking cytoplasmic flow from nurse cells into the oocyte. This study is reminiscent of that by Cao *et al.* [13•], described above, in that central but not cortical actin appears to be affected.

Myosins

Conventional myosin has been implicated in cytokinesis but not mitosis. It was therefore unexpected to find that micro-injection of a constitutively active fragment of myosin light chain kinase into cultured cells has the opposite effect, affecting mitosis but not cytokinesis [16•]. Studies of an unconventional myosin, myosin IB in *Dictyostelium* [17•], have demonstrated that disruption of the gene that encodes it causes subtle differences in motility and intracellular particle movement. Studies of a very unconventional myosin, ninaC in *Drosophila* [18•], have advanced our understanding of its function. This myosin is odd by virtue of having an additional, amino-terminal, kinase-like domain. The larger of the two isoforms of ninaC is required for phototransduction, and is localized to the rhabdomeres (the microvillar structures where phototransduction takes place). This isoform has a minimyosin-like tail, and may form the actin-membrane linkage seen in rhabdomeres (like minimyosin in intestinal microvilli). The authors speculate that the myosin domain might move the kinase domain along the actin in the rhabdomere, or might be involved in membrane shedding.

Actin-crosslinking proteins

There is a family of actin-crosslinking proteins that are related by conserved stretches of sequence. Several studies indicate that these proteins are important not only for the arrangement of actin in the cell, but also for locomotion [19•–21•]. Witke *et al.* [22•], on the other hand, have found that simultaneous mutation of ABP-120 and α-actinin interferes with development but not with locomotion or other functions in *Dictyostelium*. This appears to contradict the work of Cox *et al.* [21•] on the same organism; perhaps differences in the way the mutants were produced can account for this discrepancy.

Microtubules

Sullivan and Huffaker [23•] describe a β-tubulin yeast mutant in which astral microtubules are preferentially affected at 18°. They show that the astral microtubules are required for mitotic spindle orientation and localization, but not for spindle elongation (anaphase B). Interestingly, a similar phenotype (both nuclei ending up in one of the two dividing cells) is seen in some of the cells that have a disrupted dynein gene (K Bloom, unpublished data).

Kinesin-related proteins

Genetics has played a major role in identifying proteins that are related to kinesin by virtue of sequence similarity in the motor domain (although motor activity has actually only been demonstrated in a couple of cases). Many of these proteins are involved in mitosis [24•–26•] or meiosis ([27•–29•]; reviewed in [30•]). This year, the family of such proteins identified in yeast either genetically or

by polymerase chain reaction has grown by four members [24•,25•,31•]: *KIP1* and *KIP2*, although expressed, can be deleted singly or in combination without altering phenotype [24•]. Deletion of *KIP1* does cause an abnormal phenotype in combination with mutation or deletion of another kinesin-related gene, *CIN8* [24•,25•]; the double mutants arrest with unseparated mitotic spindle poles. This phenotype, together with immunolocalization of these proteins to the mitotic spindle, suggests that they separate spindle poles to form the spindle by causing antiparallel sliding of microtubules. A similar defect has been reported for *bimC* mutants in *Aspergillus*, *cut7* mutants in *S. pombe*, and recently *urchin* in *Drosophila* (PT Wilson, MT Fuller, unpublished data). The proteins encoded by these genes, together with Eg5 from *Xenopus*, may constitute a structural as well as functional subgroup, as they share a higher degree of sequence similarity in the motor domain than other kinesin-related proteins. Interestingly, none of the members of the subgroup show much similarity in their tails. Thus, although *CIN8* and *KIP1* appear to have a similar function, the differences in tail sequences suggest that they might act in somewhat different ways. Deletion of *KAR3*, a gene that encodes a kinesin-related protein that does not fall in the subgroup, has effects in *cin8/kip1* mutants that are consistent with the idea that it is pulling the poles together while Kip1 and Cin8 proteins are pushing them apart [26•]. The fourth new kinesin-related gene in yeast, *SMY1*, has the intriguing ability, when overexpressed, to suppress defects in a gene encoding a myosin, *MYO2* [31•]. Deletion of *SMY1* has no apparent effect in otherwise wild-type yeast, but is lethal in a *myo2* mutant. This, plus the fact that the two proteins co-localize (SH Lillie, SS Brown, unpublished data), indicates that there is some degree of redundancy or interaction between proteins that one would have predicted from sequence to be motors that interacted with different cytoskeletal filaments.

Conventional kinesin

Conventional kinesin, unlike its cousins above, does not appear to be involved in mitosis or meiosis, but instead in vesicle or organelle transport. Gho *et al.* [32•] find that kinesin heavy chain mutations do not affect the number of synaptic vesicles seen in the nerve terminal, but do affect propagation of action potentials and neurotransmitter release. They hypothesize that a kinesin-related protein similar to UNC-104 protein in *C. elegans* may carry synaptic vesicles (as *unc104* mutant nerve terminals do have many fewer vesicles [33]), whereas kinesin itself may carry other membrane components (for instance, ion channels) down the axon. Ferreira *et al.* [34•] present evidence that kinesin antisense oligonucleotide does block transport of membrane components in cultured neurons, and also interferes somewhat with neurite outgrowth. It is possible that the latter effect is secondary to the former. However, as there is evidence that kinesin may have a second microtubule-binding site [35•], it is conceivable that it might cause microtubule sliding, which may in turn be involved in neurite outgrowth [36]. Microtubule crosslinking and stabilization may also be important in the formation of neurites, as overexpression of *tau* induced the formation of long processes in cultured cells [37•].

Intermediate filaments: keratin

The big news on intermediate filaments to come from mutant studies this year is the role of keratin in epidermolysis bullosa simplex (reviewed in [38•]). In this disease, the skin blisters as a result of lysis of the basal layer of the epithelium. Mutations causing the disease have been found in keratin genes that are expressed in this layer, confirming the conclusion reached from animal studies that intermediate filaments are required to strengthen cells against mechanical stress. Other studies have investigated the role of keratin filaments during development. Torpey *et al.* [39•] have found that keratin filaments are required for compaction (flattening) and for wound healing of the blastula epithelium, and also for its invagination during gastrulation in *Xenopus*. On the other hand, Baribault and Oshima [40•] have found that a different epithelium, mouse endoderm, does not require keratin filaments for its differentiation.

Redundancy

An interesting issue that keeps coming up in mutant studies is redundancy. Sometimes, deletion of a cytoskeletal gene appears to have no effect, and/or proteins appear to be able to substitute for one another [24•,25•,31•]. In some cases it has become clear that the redundancy is not complete; further study may reveal a subtle effect of a deletion [17•,21•], or identify a different set of conditions under which an effect can be seen. One can imagine that redundancy might be an inevitable feature of evolution; redundant proteins might arise by gene duplication, and then evolve into having increasingly different functions in the cell. However, apparent functional redundancy is also seen between proteins that appear unrelated. For example, deletions of *SAC6*, which encodes the actin crosslinker, fimbrin, and either *CAP2*, which encodes the capping protein subunit, or *ABP1*, a gene for actin-binding protein, have a synthetic phenotype; double but not single deletants are inviable or very sick (AEM Adams, JA Cooper, DG Drubin, unpublished data). Such proteins may have arisen separately during evolution, but may act in the same functional pathway.

Conclusions

Mutant studies are providing important clues to *in vivo* function. A number of the cytoskeletal proteins that have

been discussed here appear to be behaving as one would expect, confirming functions inferred or suspected from *in vitro* studies. Other studies, however, have provided surprises, such as the unanticipated relationship between proteins expected from sequence to be an actin- and a microtubule-based motor [31•]. We are learning more about how to interpret mutant phenotypes as results from different studies are compared. One lesson is that only with a complete deletion of a gene can one be certain that the phenotype observed is due to complete loss of function of that gene. This is an obvious reservation with antisense oligonucleotides, but less obvious complications can arise. For example, a partial deletion of one of the *Aspergillus* α-tubulin genes is lethal, whereas a more complete deletion of the gene is not [41]. In another example, a truncated keratin disrupts keratin filaments and interferes with the formation of an epithelium, whereas deletion of the gene encoding the other keratin required for formation of the same filament has no effect [40•]. It is likely in both these cases that the truncated product is interfering with function. In some cases, overexpression phenotypes may also be due to interference; for example, overexpression of yeast myosin genes (*MYO2* or *MYO4*) gives a phenotype that suggests interference with the function of another myosin (BK Haarer, SS Brown, unpublished data). A different kind of unexpected result cropped up in the work of Praitis *et al.* [42]. These authors found that equivalent point mutations gave quite different phenotypes in different organisms. As we continue to learn about such complexities, it is to be anticipated that mutant studies will become even more useful in understanding cytoskeletal function.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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SS Brown, Department of Anatomy and Cell Biology, Medical Science II Building, University of Michigan Medical School, Ann Arbor, Michigan 48109-0616, USA.