

Molecular genetic approaches to understanding the actin cytoskeleton

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New tools in molecular genetics, such as genetic interaction screens and conditional gene targeting, have advanced the study of actin dynamics in a number of model systems. Yeast, *Dictyostelium*, *Caenorhabditis elegans*, *Drosophila*, and mice have contributed much in recent years to a better understanding of both the numerous functions and modes of regulation of the actin cytoskeleton.

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Abbreviations

ADF	actin depolymerizing factor
ES cells	embryonic stem cells
FH	Formin homology
GAP	GTPase activating protein
GEF	GDP exchange factor
GFP	green fluorescent protein
P	phosphate
PI	phosphatidylinositol
PI(4,5)P₂	PI 4,5-bisphosphate
REMI	restriction enzyme mediated integration
WASP	Wiskott-Aldrich syndrome protein

Introduction

In the past few years we have learned that the actin cytoskeleton is not simply important for cell motility and chemotaxis, but rather constitutes a central organiser of the cell, which is closely linked to a number of cellular functions ranging from cytokinesis and endocytosis to signal transduction and RNA localization. Hints of the multiple functions of the actin cytoskeleton have mainly emerged from genetic approaches aimed at defining essential genes for processes such as cytokinesis, endocytosis, and development. Independent findings from various systems are coming together and suggest that control of cytoplasmic organization and cell shape are necessary for almost any cell function.

In this review we focus on information obtained by molecular genetic studies of the actin cytoskeleton in yeast, *Dictyostelium*, *C. elegans*, *Drosophila*, and mouse. For each, we describe the relevant genetic tools and summarize recent examples from the literature in order to provide an overview of the powerful genetics that have been used to support and sometimes challenge current models of cytoskeletal function.

Yeast: no crawler – but an excellent tool to study the actin cytoskeleton

Although yeast is not motile like *Dictyostelium* or higher eukaryotic cells, its our advanced knowledge of its genetics make it a good model system in which to study many aspects of the actin cytoskeleton. Cell division, secretion and signaling are well studied in the yeast system. A useful and popular method to screen for genetic interactions is to look for ‘synthetic lethality’: cells defective in either of two genes are viable, but the doubly mutated cells are inviable. An alternative method is to look for a mild phenotype (caused by a known mutation) that is enhanced or suppressed by disruption of a second gene. Identification of mutated genes is facilitated by complementation — using plasmid libraries to identify clones that can rescue defects. With the complete sequence of the *Saccharomyces cerevisiae* genome now known, systematic knockout of every gene and analysis of phenotype is possible. Yeast also provides an important tool for studying interaction of proteins from a wide variety of organisms in the form of ‘two-hybrid’ technology.

Signaling and the central role of profilin partners

Small GTPases of the Rho subfamily are important signaling molecules for regulating the actin cytoskeleton and the identity and function of potential downstream effectors have recently been characterized. The yeast studies summarized here suggest that a chain, or possibly a complex, of interacting signaling molecules including small G-proteins, proteins of the formin family, and profilin may contribute to cytoskeletal organization.

In fission yeast, viable cells with Rho2 mutations exhibit irregular cell shape and cell wall defects [1], whereas Rho1 is essential for viability [2]. Expression of constitutively active Rho1 leads to delocalized actin patches suggesting a role for Rho1 in actin assembly [2]. In *S. cerevisiae*, Rho proteins and the Rho-related Cdc42 have been shown to interact with the formin homology (FH) proteins Bni1 and Bnr1 [3–5]. Other FH proteins, which include *Schizosaccharomyces pombe* Cdc12 and Fus1, *Aspergillus* SepA, *Drosophila* Cappuccino and Diaphanous, and the vertebrate Limb-deformity protein, influence actin remodeling in diverse processes such as establishment of cell polarity, cytokinesis, and limb development. Yeast lacking both Bni1 and Bnr1 show defects in polarization and actin organization resulting in defects in bud formation and cytokinesis [4].

FH proteins, may affect organization of the actin cytoskeleton via interactions with profilin [6]. Two-hybrid experiments using Bni1 and Bnr1 have revealed that they interact with the actin-binding proteins elongation factor EF1- α and profilin [4,5,7]. Synthetic-lethal interactions between the *S. pombe* FH protein cdc12p and

cdc3p (profilin) have also been observed, and loss of cdc12p causes defects in actin ring assembly and septum formation during cytokinesis [8]. Perhaps, distinct from its associations with FH proteins, profilin is in the same pathway as *S. cerevisiae* Sec3, an important component of vesicle maturation and exocytosis [9*].

Perspective

A picture emerges in which profilin is apparently a central mediator of several signaling pathways to the actin cytoskeleton. How profilin can act as a linker in different pathways, whether profilin is part of a dynamic multiprotein signaling complex (see [10]), and how the signals mediate the appropriate response of the actin cytoskeleton are important questions for the future.

Dictyostelium: crawling as the essence of life

Dictyostelium has a long history as a model system for the study of the role of the actin cytoskeleton in cell motility and chemotaxis. cAMP triggers chemotactic movement and aggregation of single amoebae followed by subsequent differentiation into a stalk-like fruiting body and spores. In many respects the motility of amoeba resembles neutrophils therefore providing an excellent model for this type mode of motility. *Dictyostelium* is haploid. This facilitates screens for mutations but also makes it difficult to study recessive mutations because of the amoeba's asexual life cycle. Cells can be easily mutagenized for large-scale screens, specific mutants can be generated using homologous recombination, and REMI (restriction enzyme mediated integration) was introduced recently as a useful genetic tool to generate random insertion libraries [11]. Several interesting *Dictyostelium* mutants for actin-binding proteins and signaling molecules have been generated recently and are discussed below.

Actin, myosin and cytokinesis

A number of amoeba with actin-binding protein mutations not only display motility defects but are also impaired in cytokinesis. Since cytokinesis requires a very precise temporal and spatial regulation of the actin cytoskeleton for the formation of the cleavage furrow, this process is more sensitive to subtle changes in the actin cytoskeleton than other actin-related functions.

Severe cytokinesis defects have been previously reported for cells with conventional myosin (myosin II) mutations growing in suspension, however, when adherent these cells undergo normal mitosis and cytokinesis. Recent work has shown that under normal 'stress-free' conditions myosin II does not localize to the cleavage furrow in *Dictyostelium* cells [12] raising the question whether — at least in *Dictyostelium* — a contractile ring is formed and needed to complete cytokinesis. Studies of myosin II null cells suggest that destabilization of the actin cortex in the cleavage furrow coupled with tension in the cell body might be sufficient for pinching off the daughter cells [13*]. Alternatively, one of the numerous unconventional

myosins (myosin I) in *Dictyostelium* might participate in the formation of the contractile ring.

Signaling to the actin cytoskeleton

Gene knockout experiments in *Dictyostelium* have further established the importance of signaling molecules — in particular small GTPases of the Rac and Rho family — for the integrity of the actin cytoskeleton. Inactivation of the racE gene in *Dictyostelium*, for example, results in abnormal actin aggregates and defects in cytokinesis suggesting a role for racE in the organization of the actin cytoskeleton [14]. Overexpression of RacC, another Rac isoform, has been shown to induce unusual actin rich blebs on the dorsal cell side; furthermore, phagocytosis was increased threefold while pinocytosis was concomitantly reduced threefold [15]. Mutation of RasG also suggests an important role of the Ras family for the regulation of the actin cytoskeleton. *Dictyostelium* with rasG mutations show normal growth but aberrant actin structures and defects in cytokinesis [16] — presence of F-actin deposits and lack of cell polarity suggests that actin turnover is disturbed.

GTPase activating proteins (GAPs) are another class of signaling molecules which interact with small G-proteins in order to increase their GTPase activity. Two members of this class have been identified in *Dictyostelium* — DGAP1 and GAPA. Strikingly, both proteins apparently have no GTPase-regulatory activity even though by amino-acid sequence they clearly belong to the GAP family. Elimination of DGAP1 results in an increase in cell motility, while overexpression leads to reduced motility and defects in cytokinesis [17*]. GAPA null cells grow as giant multinucleated cells, probably due to the formation of cleavage furrows which are frequently not completed [18].

In *Dictyostelium*, chemotactic cAMP signaling is a classical receptor, heterotrimeric G-protein coupled pathway. Disruption of the cAMP receptor gene leads to developmental arrest after aggregate formation. A mutation of SCAR (suppressor of cAMP receptor) — a Wiskott-Aldrich syndrome protein (WASP) homologue — was able to rescue the phenotype. Cells lacking SCAR have reduced F-actin levels and abnormal actin distribution during chemotaxis indicating a role for SCAR in regulating the actin cytoskeleton [19]. The genetic interaction with the cAMP receptor suggests a role for SCAR as a negative regulator of heterotrimeric G-protein coupled signaling. Inactivation of the single *Dictyostelium* G-protein β subunit causes defects in phagocytosis due to the perturbation of cytoskeletal reorganization that is required for the phagocytic cup formation [20].

Endocytosis and actin polymerization

Evidence suggests a close link between regulation of actin polymerization and endocytosis. Phosphatidylinositol (PI) phosphate products of PI-3 kinases appear to serve as second messengers for both processes and have been implicated as the key regulators. Disruption of two

Dictyostelium PI-3 kinase genes, DdPIP1 and DdPIP2, causes defects in the actin cytoskeleton as well as endocytosis. Mutant cells acquire an irregular cell shape and induce actin-rich filopodia. The endocytosis defects are specific for pinocytosis as phagocytosis of particles is not altered [21]. Reduced levels of the PI-3 kinase products PI(3,4)P₂ and PI(3,4,5)P₃ in DdPIP1 and DdPIP2 mutants have been correlated with the morphological abnormalities and the defects in the actin cytoskeleton [22]. A link between the actin cytoskeleton and the endocytic processes is also supported by results from *Dictyostelium* clathrin mutants generated by homologous recombination [23]. Cells with clathrin mutations are not only deficient in endocytosis but also fail to divide in suspension. The observed inability to recruit conventional myosin to the cleavage furrow suggests a defect in the formation of the contractile actin ring.

Perspective

Dictyostelium is a convenient system in which to study cell motility and other actin-related functions. Recent advances in applying the REMI technique has made it conceivable to mutate the *Dictyostelium* genome with plasmid DNA insertions and to screen the mutant population for specific defects in motility, chemotaxis and other actin-related functions. The tagged locus can then be isolated easily and the gene analyzed in more detail. In addition, *Dictyostelium* is an excellent cell type in which to explore and establish the use of green fluorescent protein (GFP) tagged proteins such as GFP-actin [24] or GFP-tubulin [25*] in order to study cytoskeletal dynamics.

Caenorhabditis elegans: worms on the move

The nematode *Caenorhabditis elegans* with its well-described invariant cell lineage has emerged as the definitive model organism for studying cell fate. With the sequence of its genome recently completed, coupled with efficient mutagenic and transgenic techniques, *C. elegans* is a useful model organism in which to apply 'functional genomics'. Extensive screens for mutants exhibiting defects in movement, touch sensitivity, axon guidance, and cell death have been performed and are beginning to yield surprising molecular clues into the mechanisms behind these events.

UNC mutations reveal regulatory actin-binding proteins

Nematode homologues of many actin-binding proteins, such as profilin, gelsolin, and alpha-actinin, have been identified in sequence databases but a few had already been identified by their mutant phenotype. For example loss of UNC-60 (UNC meaning uncoordinated), a member of the cofilin/actin depolymerizing factor (ADF) family, severely disrupts filament assembly in muscle cells [26]. Both unc-60 and unc-115 were isolated in a screen for an uncoordinated phenotype. UNC-115 is a homologue of the human actin-binding protein abLIM [27] and contains a villin-like domain thought to be responsible for binding to actin, and three LIM (lin-11, isl-1, mec-3) domains postulated to interact with other proteins.

Further molecular analysis of 'unc' alleles has revealed more actin regulators (see [28]).

The report [27] on the role of UNC-115 in axon guidance (see also [29]) illustrates the flexibility of nematode genetics, by demonstrating rescue of the unc-115 phenotype with a transgene encoding a GFP-UNC-115 fusion and utilizing mosaic analysis to pinpoint that the required function of UNC-115 is in neurons [27].

Signaling is a matter of life and death

Screens for cell migration and axon guidance defects have identified signaling components that link these processes to the actin cytoskeleton. Mutations in the Rho GTPase family member, MIG2, suggest that it functions in both cell movement and axon pathfinding [29,30*]. Expression of constitutively activated MIG2 transgenes in neuronal cells only leads to axon guidance defects, suggesting autonomous action of MIG2 in interpreting extracellular cues.

Signaling to the cytoskeleton is also important in the execution of the apoptotic pathway, many components of which have been identified in *C. elegans*. CED-5 is a homologue of human DOCK180, which can interact with the SH2/SH3 adaptor protein Crk and transduce signals from integrin-containing focal contacts [31,32*]. Nematodes with CED-5 mutations seem to undergo a normal caspase-mediated cell death program, but defects arise in the phagocytic engulfment of the 'corpses' [32*]. Because human DOCK180 has been detected in focal adhesions, a model has been proposed that CED-5 links detection of death signals and remodeling of the cytoskeleton. CED-5 mutants also display a cell migration defect that is not death-related, suggesting the protein may function in two distinct signaling pathways.

Roles of actin in C. elegans embryogenesis

Genetic screens for defects in embryo elongation and cytokinesis have also revealed potential regulators of the actin cytoskeleton. Migration of epidermal cell sheets, which allow 'closure' of the embryo, and cell shape changes, which drive embryonic elongation, are disrupted in worms with hmp-1 (α -catenin), hmp-2 (β -catenin), and hmr-1 (cadherin) mutations [33]. This supports the idea that communication between cell adherens-junctions and the actin network is crucial for early morphogenetic events. Defective cytokinesis is seen in mutants lacking CYK-1, a formin homologue. Cyk-1 mutants undergo normal mitosis and the cleavage furrow ingresses, but then regresses without a complete division [34]. Mutations of formin genes in other model organisms also lead to cytokinesis defects, suggesting a general role for formins in regulation of the actomyosin contractile ring.

Perspective

As more proteins are assigned to previously identified *C. elegans* mutant alleles, the pathways of many cellular and

developmental processes are being deciphered. Genetic interaction screens to suppress or enhance actin-related phenotypes and the use of GFP fusion proteins or GFP reporter genes promise to reveal additional actin regulators (e.g. [35]).

***Drosophila melanogaster*: rings and things made of actin**

For nearly a century, genetic experiments with the humble fruit fly have contributed a wealth of knowledge about many cellular processes, including cytoskeletal function. *Drosophila melanogaster* as a genetic tool is perhaps unique among higher eukaryotes because of the number of associated resources available, such as collections of small chromosomal deletions to map mutations and the method of P-element transformation for efficiently mutating the genome with tagged transposons or creating flies carrying exogenous transgenes. Of special interest for this review, because regulators of the actin cytoskeleton may have multiple roles throughout development, are various techniques available for creating mosaic animals.

Roles of actin in *Drosophila* gametogenesis

Both oogenesis and spermatogenesis require precise partitioning of cytoplasm and genetic material, mediated in part by actin microfilaments. In the developing egg chamber, specialized F-actin-containing structures, termed ring canals, are crucial for dumping of nurse cell contents into the oocyte. Similar structures are evident in sperm-producing cysts of the testis.

One well-documented case of an actin-binding protein with roles in gametogenesis is profilin, encoded by the *chickadee* (*chic*) locus [36]. Analysis of egg chambers from mutant mothers reveals that profilin is necessary for proper cytokinesis of the nurse cells, dumping of nurse cell contents into the oocyte, and possibly proper migration of border cells through the nurse cell cluster [36,37]. Profilin-mediated regulation of actin polymerization is important to prevent premature microtubule-based cytoplasmic streaming, which is thought to play a role in mixing and proper positioning of cytoplasmic determinants in the oocyte [38]. Cross-talk between the two networks is further illustrated in spermatogenesis where male-sterile *chic* mutants fail to properly assemble contractile actin rings and microtubule-based spindle bodies during spermatocyte meiosis [39]. Other potential actin regulators important for ring canal formation include Kelch (a protein containing scrui repeats, [40]), Hts (adducin-like protein, [41]), and Cheerio (protein identity unknown, [42]). Protein phosphorylation by Tec and Src kinases apparently plays a role in regulating growth of the assembled ring [43]. Expression of dominant-negative and constitutively active forms of *Drosophila* Rho, Rac, and Cdc42 in the ovary resulted in defective nurse cell dumping as a result of disrupted actin structures, while only Rac expression affected border cell migration [44].

Mutations in *twinstar*, which encodes a homologue of the cofilin/ADF family, result in defective spermatogenesis [45]. Hypomorphic alleles (which exhibit partial loss-of-function) cause late larval or early pupal lethality, and examination of the larval testis reveals failed cytokinesis and accumulations of F-actin. In oogenesis, germline mosaics using a *twinstar* null allele also lead to cytokinesis defects and over accumulation of F-actin in the nurse cells, perhaps nucleated at the site of ring canal assembly (Gunsalus K, personal communication).

Actin-bundling proteins such as quail (a villin homologue) and singed (a fascin homologue) are essential for assembly of a subclass of actin bundles in nurse cells [46]. In a recent report, transgenes were used to demonstrate that quail and singed may share some redundant functions, as overexpression of quail protein in sterile *singed* females can restore fertility, although actin organization itself is not completely restored [47].

Dynamic cell movements: roles for actin in embryogenesis

Drosophila embryogenesis begins as a series of rapid and synchronous nuclear divisions (without cytokinesis) followed by migration of the nuclei towards the plasma membrane. Individual nuclei are ‘pinched off’ by actomyosin-driven invaginations of membrane in a process called cellularisation. Later, morphogenetic movements of gastrulation are characterized by rapid changes in cell shape and migration, relying again on the actin cytoskeleton.

Nuclear fallout (*nuf*) is necessary for forming the hexagonal actin-based furrows that precede cellularisation [48]. The *nuf* gene encodes a novel phosphoprotein that cycles between the cytoplasm and the centrosomes, perhaps serving as a link between microfilaments and the centrosome [49]. The proteins Nullo [50] and Pebble [51] appear to stabilize the actomyosin network and direct assembly of the contractile actin rings necessary for complete cellularisation. Bottleneck and the gelsolin-like Flightless I protein, also directly or indirectly regulate the cytoskeletal network during cellularisation [52,53].

Gastrulation involves rapid changes of cell shape, such as those in ventral furrow formation, and large concerted movements of cell sheets, such as those in dorsal closure. Recently, two groups independently identified, a Rho-specific guanine nucleotide exchange factor, DRhoGEF2 and showed that *Drosophila* with mutations in this gene were unable to properly form ventral furrows [54*,55*]. Overexpression of a dominant-negative RhoA gives the same phenotypes as RhoGEF2 mutations, suggesting that a Rho-mediated pathway regulates cell shape changes. Also, mutations in components of the JNK (Jun amino-terminal kinase) signaling pathway (D-Jun, Basket, Hemipterous) result in ‘dorsal open’ phenotypes — the purse-string-like action of a contracting actomyosin network does not occur, leaving the cell sheets positioned

laterally and the dorsal surface exposed (reviewed in [56]). Identifying downstream effectors of Jun will be important to understand how transcriptional activation can lead to cytoskeletal changes.

Clues that focal adhesions may be utilized to effect dorsal closure come from study of the ‘dorsal open’ phenotype seen in *Drosophila* with *myoblast city* (*mbc*) mutations [57]. *Mbc* encodes a homologue of human DOCK180 (see above). There is also evidence that integrins are necessary for dorsal closure [58]; however, *Drosophila* lacking vinculin — a frequent component of focal contacts in mammalian cultured cells — develop normally [59].

Molecular scaffolding: roles of actin in bristle formation

Several mutations in bristle morphology in *Drosophila* are caused by a perturbed actin cytoskeleton. Wild-type neurosensory bristles contain a highly-ordered hexagonal array of actin filaments that is disrupted in *Drosophila* that lack the novel protein Forked, or the fascin homologue Singed, as a result of improper size and bundling of F-actin filaments [60]. Partial loss-of-function mutations of the β subunit of *Drosophila* capping protein also cause disorganized actin bundles and bent bristles, whereas stronger mutations cause early larval lethality [61]. These defects are probably due to unregulated barbed-end polymerization, although the exact actin-mediated mechanism of bristle outgrowth is unknown. Mutations in *chickadee* (profilin) and *twinstar* (cofilin/ADF) also cause gnarled and twisted bristles, suggesting an overaccumulation of actin bundles ([37]; Gunsalus K, personal communication).

Mutations such as *dishevelled*, cause random polarity of small actin-containing hairs on the *Drosophila* wing. Recent work suggests that Dishevelled triggers a JNK cascade [62] and that membrane localization of dishevelled may contribute to the oriented assembly of actin filaments and prehair initiation [63]. Dishevelled may in turn activate a parallel Rac/Rho cascade to induce actin filament assembly and polarized growth (reviewed in [64]).

Perspective

The development of new techniques to manipulate the genome of *Drosophila*, as well as new methods to alter *in vivo* expression levels of gene products within particular tissues or even single cells will facilitate study of cellular mechanisms. Table 1 lists some recent examples. The integration of two or more of these techniques should allow even more sophisticated analysis of cytoskeletal function. One strength of the *Drosophila* model is the ability to discern the effects of a mutation or protein overexpression in a complex multicellular organism. Although a number of cell culture techniques have been described [65], further development of new techniques for a larger variety of cell types will advance study of their wild-type and mutant phenotypes.

Mouse: a unique mammalian model system

The mouse is the only system that allows the study of the function of the actin cytoskeleton in mammals. Recent advances in mouse genetics offer tremendous potential not only for studying the actin network but also to better understand diseases related to cytoskeletal defects. Homologous recombination in embryonic stem cells (ES-cells), also known as ‘gene targeting’, has been a major breakthrough for the use of the mouse in molecular genetics. More recently techniques for conditional gene targeting have been established and this strategy is rapidly expanding into the field of mouse biology.

The gelsolin family of actin-binding proteins

The first targeted mutation of an actin-binding protein in mouse — gelsolin — resulted in homozygous mutants that were viable and fertile [66]. Detailed analysis of their cells revealed that specific aspects of cell motility were impaired although the overall capacity for motility was preserved. Neurons, for example, require the actin cytoskeleton not only to translate guidance signals into re-orientation of the extending growth cone but also to regulate ion channel activity. Gelsolin-null neurons show an increase in the steady-state content of F-actin that correlates with prolonged opening time of Ca²⁺ ion channels and hence an increased susceptibility to neurotoxic agents [67]. In addition, they show a delayed collapse of filopodia at the growth cone [68*]. Together with previous results this suggests that instead of being the master switch for actin breakdown, the function of gelsolin is rather to fine-tune actin disassembly in cells. Interestingly, the penetrance of the gelsolin-null mutation in mouse apparently depends on the genetic background. Backcross of the mutation into an inbred Balb/c background results in embryonic death late in gestation (Witke W, unpublished data).

The closest relative to gelsolin in mammals is capG — an actin-binding protein that does not sever but caps actin filaments. Ablation of capG in mouse results in disturbed macrophage ruffling activity and adhesion (Witke W, Southwick F, Kwiatkowski DJ, unpublished data). Gelsolin and capG appear not to have redundant functions as mice lacking both proteins have so far not revealed any synergistic effects (Witke W, unpublished data).

Villin, another member of the gelsolin family, is specifically expressed in gut epithelia and localizes to the F-actin bundles that sustain the shape of microvilli. Previous biochemical and transfection experiments suggested a critical function of villin for microvilli morphogenesis; however, mice with villin mutations display only minor alterations in the microvilli architecture that apparently do not affect the function of the brush border epithelium [69*]. Studies under stress conditions have not been carried out and partial compensatory effect by related molecules [70,71] were suggested to explain the mild phenotype.

Table 1

New genetic methods in *Drosophila melanogaster* that may facilitate the study of the actin cytoskeleton.

Techniques	Basic concept	Examples of use	References
FLP/FRT system	Adopted from yeast; Flp recombinase can catalyse recombination between two FRT sites causing excision or inversion of intervening sequences, or recombination between FRT-bearing chromosomes.	To create homozygous mutant clones in somatic and germline tissues by mitotic recombination between chromosomes. To activate overexpression of a protein in a clonal manner, for example a strong promoter and protein-coding region are juxtaposed after Flp-mediated excision of a 'stuffer' sequence. Targeted insertion of exogenous DNA into a genomic FRT site, may lead to consistent transgene expression levels between lines.	[78–81] [82]
Cre/loxP system	Adopted from bacteriophage P1; similar to FLP/FRT.	Not yet widely used in <i>Drosophila</i> due to lack of useful strains. In future, may be useful in conjunction with the FLP/FRT system for analysing the loss-of-function effects of two or more genes simultaneously.	
UAS/GAL4 system	Expression of yeast GAL4 in a tissue- or stage-specific manner can drive overexpression of another protein under control of UAS sequence.	Generation of a wide variety of GAL4 drivers by 'enhancer trapping' methods has increased utility of this technique. When combined with FLP/FRT system, GAL4 can be expressed in a clonal manner. When combined with FLP/FRT system, UAS-FLP allows mutant clones to be generated in patterns or tissues corresponding to GAL4 drivers. Random insertions of the UAS, in conjunction with a particular GAL4 driver of interest, allow systematic gain-of-function screens to be performed.	[83–85,86*]
TetR/tetO system	Expression of the bacterial Tet repressor protein and treatment of flies with tetracycline can repress a gene under control of the tetO; removal from tetracycline allows expression of the gene.	Used successfully in mammalian cell culture, and only recently introduced into flies May have the potential to finely-regulate levels of misexpression in response to tetracycline doses May serve as a complement to UAS/GAL4 system or the widely-used heat-shock inducible expression system to analyse the effects of overexpression of two or more proteins simultaneously	[87,88]
GFP (green fluorescent protein) fusions	Transgenic expression of a <i>Drosophila</i> protein fused to green fluorescent protein reveals <i>in vivo</i> localisation.	For example transgenic GFP–moesin flies reveal the localisation of moesin in a variety of tissue types. Work in <i>Drosophila</i> offers a good opportunity to test whether these fusions can rescue null alleles, demonstrating full functionality of the fusion protein. This may be useful in designing genetic screens for mutations that affect protein localisation.	[89,90]

Focal adhesion molecules and actin assembly

Ablation of vinculin — a component of focal adhesions serving as a link between integrins and the actin cytoskeleton — leads to severe heart and brain defects resulting in embryonic death around day 10 in mice [72]. Fibroblasts isolated from vinculin-null embryos show reduced adhesion correlating with a twofold increase in cell motility. Vinculin was shown to have numerous interaction partners hence multiple pathways might be affected by the vinculin mutation. One of the vinculin-binding proteins is another focal adhesion protein VASP (vasodilator stimulated phosphoprotein) [73] which is related to MENA (mammalian enabled). Both proteins are thought to recruit profilin into a complex that regulates actin assembly [74]. MENA binds to profilin with high affinity [75] and from its expression pattern it has been suggested that MENA plays an important role in actin assembly in neuronal tissues. Homozygous MENA-null mice are viable, however, they show specific neuronal defects (Gertler F, personal communication).

In contrast, profilin-null mice are not viable and embryos die before the blastocyst stage (Witke W, Kwiatkowski DJ, unpublished data). Interestingly, mice that are homozygous

for the MENA mutation and heterozygous for profilin I die *in utero* because failure in neural tube closure results in exencephaly — a phenotype which is observed in neither MENA-null mice or profilin-I-heterozygous mice alone (Gertler F, personal communication). This finding further corroborates the *in vivo* interaction of MENA and profilin and the importance of this complex for neural crest cell migration.

The Wiskott-Aldrich syndrome protein (WASP) is mutated in a human X-linked immunodeficiency. WASP is distantly related to the MENA/VASP family of proteins and has also been implicated in regulating the actin cytoskeleton. WASP-deficient mice are viable but show a reduced proliferation response of T cells upon CD3 ϵ stimulation whereas B-cell responses are apparently normal. Capping of CD3 ϵ -antibody complexes is greatly attenuated in T cells suggesting altered cytoskeletal function [76*], however, a specific defect in the actin cytoskeleton has not been reported.

Tensin is an SH2-domain-containing focal adhesion phosphoprotein that binds to F-actin. Tensin-null mice develop

Table 2**New genetic methods in mouse that may facilitate the study of the actin cytoskeleton.**

Techniques	Useful features	References
Cre/loxP system	When coupled with homologous recombination in embryonic stem cells, this system allows complex modification of the genome (removal, replacement, or inversion of genes or genomic regions). Mouse strains are available that express Cre recombinase under tissue- and developmental stage-specific promoters, increasing the versatility of the system.	[91–94]
FLP/FRT system	Recently introduced in embryonic stem cells and mice to allow modification of the genome alone or in coordination with the Cre/loxP system for sequential or simultaneous modifications. Active, thermostable Flp recombinases have been generated for use in embryonic stem cells or <i>in vivo</i> .	[95–97]
Tetracycline-controlled transactivator (tTA) system	First used in cultured cells as an inducible gene-expression system; recent reports show that it has successfully been introduced in mice. By altering tetracycline levels, offers a dose-dependent control of gene expression not available in the GAL4 system (although see 'inducible proteins'). Again, the goal is to create a collection of mice expressing tTA and rtTA in particular tissues.	[98,99]
UAS/GAL4 system	Under development in the mouse; more widely used in <i>Drosophila</i> . Goal is to create a collection of mice expressing tissue-specific GAL4–VP16, that would allow tissue-specific activation of any gene placed under UAS control.	[100]
Inducible proteins	Nuclear proteins (GAL4–VP16, Cre and Flp recombinases) have been temporally regulated by fusing them to hormone binding domains; addition of hormone leads to activity; placing them under regulation of tissue-specific promoters adds spatial control. Alternatively, proteins are placed under the control of inducible promoters; future combinations of Cre/Flp and the tTA system may also lead to spatial and temporal control.	[100–103]

normally and appear to be healthy; however, older mice tend to develop large kidney cysts resulting in renal failure [77]. It has been proposed that ablation of tensin leads to a weakening of focal adhesions specifically in kidneys.

Perspective

Recent advances in mouse genetics have allowed regulation of gene function at will. Using conventional gene targeting for genes such as vinculin or profilin I, the early stages of development have been the endpoint of analysis. Conditional gene targeting creates additional possibilities for the analysis of embryonic lethal mutations. Table 2 presents a summary of recently described mouse genetic techniques that will be useful for studying the actin cytoskeleton.

Conclusion

Genetics is a powerful tool to elucidate the functions of the actin cytoskeleton. Often the *in vivo* results are puzzling and seemingly contradict the functions suggested by *in vitro* biochemical findings. This emphasizes the importance of both types of studies for a deeper understanding of cytoskeletal regulation and concomitant revision of the current models. Each model organism discussed in this review offers a unique repertoire of advantages for the genetic analysis of the actin cytoskeleton and at the same time each model has its limitations. Yeast is an excellent system for 'interaction screens' and deciphering genetic pathways. The strength of *Dictyostelium* is the variety of motile responses one can study. The advantages of *C. elegans* and *Drosophila* are the more complex patterns of development, allowing the study of a broader spectrum of

actin-related functions. Although not chosen as a focus for this review, zebrafish may soon become a valuable asset for cytoskeletal study.

The mouse is currently the best mammalian model system where molecular genetics can be used, both for basic research and applied purposes — such as creating models of human disease. *In situ* studies as well as experiments on cultured primary cells will be paramount to reveal functions for individual actin-regulating factors.

Regardless of which model system is used to study the actin cytoskeleton, the described molecular genetic approaches paired with the advances in genome sequencing will certainly open new avenues to understand how a cell organizes the cytoplasm and controls cytoskeletal dynamics.

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References and recommended reading

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

- of special interest
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