12-23-1998

Drosophila Fascin Mutants are Rescued by Overexpression of the Villin-like Protein, Quail

Kelly Cant
Yale University

Brenda A. Knowles
Yale University

Shalina Mahajan-Miklos
Yale University

Matthew Heintzelman
Dartmouth College

Lynn Cooley
Yale University

Journal of Cell Science 111, 213-221 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
JCS9658

Drosophila fascin mutants are rescued by overexpression of the villin-like protein, quail

Kelly Cant[1,*,†], Brenda A. Knowles[1,*,‡], Shalina Mahajan-Miklos[1,§], Matthew Heintzelman[2] and Lynn Cooley[1,¶]

[1]Department of Genetics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510, USA
[2]Departments of Anatomy and Pathology, Dartmouth Medical School, Hanover, NH 03755, USA
*The first two authors contributed equally to this work
†Present address: Department of Medicine, Children's Hospital, Boston, MA 02116, USA
‡Deceased
§Present address: Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, USA
¶Author for correspondence (e-mail: lynn.cooley@yale.edu)

Accepted 3 November 1997: published on WWW 23 December 1997

SUMMARY

Actin bundle assembly in specialized structures such as microvilli on intestinal epithelia and *Drosophila* bristles requires two actin bundling proteins. In these systems, the distinct biochemical properties and temporal localization of actin bundling proteins suggest that these proteins are not redundant. During *Drosophila* oogenesis, the formation of cytoplasmic actin bundles in nurse cells requires two actin bundling proteins, fascin encoded by the *singed* gene and a villin-like protein encoded by the *quail* gene. *singed* and *quail* mutations are fully recessive and each mutation disrupts nurse cell cytoplasmic actin bundle formation. We used P-element mediated germline transformation to overexpress quail in *singed* mutants and test whether these proteins have redundant functions in vivo. Overexpression of quail protein in a sterile *singed* background restores actin bundle formation in egg chambers. The degree of rescue by quail depends on the level of quail protein overexpression, as well as residual levels of fascin function. In nurse cells that contain excess quail but no fascin, the cytoplasmic actin network initially appears wild type but then becomes disorganized in the final stages of nurse cell cytoplasm transport. The ability of quail overexpression to compensate for the absence of fascin demonstrates that fascin is partially redundant with quail in the *Drosophila* germline. Quail appears to function as a bundle initiator while fascin provides bundle organization.

Key words: Actin bundle, Oogenesis, Fascin, Villin

INTRODUCTION

The actin cytoskeleton is regulated by a large number of actin-binding proteins, some of which organize actin filaments into meshworks or bundles (Hartwig and Kwiakowski, 1991; Otto, 1994). Fascin, fimbrin, and villin belong to the family of F-actin bundling proteins. Different actin bundling proteins often co-exist in a single cell type, where they appear to be jointly required for actin bundle formation in specialized cell structures. In the microvilli of intestinal absorptive epithelia, for example, actin bundles contain two actin bundling proteins with distinct biochemical properties: villin and fimbrin (reviewed by Heintzelman and Mooseker, 1992; Louvard et al., 1992). Stereocilia of the ear contain actin bundles that have fimbrin and at least one other actin bundling protein (reviewed by Tilney et al., 1992). Actin bundles supporting *Drosophila* bristle extension contain fascin and another putative bundling protein, the product of the *forked* gene (Cant et al., 1994; Petersen et al., 1994; Tilney et al., 1995). In microvilli and in bristle formation, the two actin bundling proteins localize to actin bundles sequentially. The two-protein motif for bundle assembly and the distinct biochemical properties of actin bundling proteins argue that the two bundling proteins present in a given cell have non-redundant functions.

The analysis of cytoskeletal proteins in vivo continues to be aided by the use of genetic organisms like yeast, *Dictyostelium*, and *Drosophila*. We are using nurse cell cytoplasm transport during *Drosophila* oogenesis as a model system to examine actin bundle formation in vivo (reviewed by Cooley and Theurkauf, 1994; Knowles and Cooley, 1994; Robinson and Cooley, 1997). Late in oogenesis, nurse cell cytoplasm is transported rapidly into the oocyte by a process involving two actin filament networks. The subcortical actin network supports myosin-based nurse cell contraction that pushes nurse cell cytoplasm into the oocyte (Gutzeit, 1986; Wheatley et al., 1995; Edwards and Kiehart, 1996). The cytoplasmic actin bundles extend from the nurse cell plasma membrane and form a cage around the nucleus thus anchoring the nuclei away from intercellular bridges during rapid cytoplasm transport (Cooley et al., 1992; Cant et al., 1994; Mahajan-Miklos and Cooley, 1994). The actin bundles are made of a series of short modules that are laterally associated with their neighbors (Guild et al., 1997). During contraction of the nurse

cells, shortening of the actin bundles is accomplished apparently by sliding of these modules, thus collapsing into thicker bundles (Guild et al., 1997).

Formation of the cytoplasmic actin bundles requires the products of the *singed* and *quail* genes (Cant et al., 1994; Mahajan-Miklos and Cooley, 1994). Mutants in either gene do not form nurse cell actin bundles and the absence of bundles results in female sterility because untethered nurse cell nuclei become lodged in intercellular bridges and block nurse cell cytoplasm transport.

Singed protein is a functional homolog of the actin bundling protein, fascin, first isolated from sea urchins (Bryan et al., 1993; Cant et al., 1994; reviewed by Edwards and Bryan, 1995). Fascin acts as a monomer to crosslink actin filaments into a tightly packed, well organized, hexagonal array (Bryan and Kane, 1978). In vitro, tightly packed fascin-actin bundles exhibit an 11-12 nm cross banding periodicity by electron microscopy (DeRosier and Censullo, 1977; Bryan and Kane, 1978; DeRosier et al., 1981). In sea urchin, fascin is found on actin bundle structures supporting egg microvilli and coelomocyte filopodia (Otto et al., 1980; Otto and Bryan, 1981). In *Drosophila*, fascin is required for actin bundle assembly in developing bristles and in the nurse cell cytoplasm of egg chambers (Cant et al., 1994). Fascin is abundantly expressed in nurse cell and bristle cell cytoplasm during actin bundle formation. In these *Drosophila* (Overton, 1967; Riparbelli and Giuliano, 1995; Tilney et al., 1995; Guild et al., 1997) and sea urchin (Burgess and Schroeder, 1977; Spudich and Amos, 1979; Otto and Bryan, 1981) structures, the actin bundles that contain fascin exhibit an 11-12 nm cross-banding pattern by electron microscopy. In vertebrates, fascin has been found to be a component of a number of different actin based structures such as stress fibers, lamellopodia, and neural growth cones (Edwards and Bryan, 1995).

Quail protein shares 30% sequence identity with villin (Mahajan-Miklos and Cooley, 1994). In vitro analysis suggests that villin is a complex phosphoinositide and calcium-regulated actin binding protein (reviewed by Mooseker, 1985; Louvard, 1989; Friederich et al., 1990). At physiological calcium concentrations (10^{-7} M), villin bundles actin filaments, whereas at high calcium concentrations (10^{-5}-10^{-6} M) actin filaments are severed, capped or nucleated by villin. The protein has two major functional domains that each contain a single F-actin binding site: a 90 kDa core domain and a small, <10 kDa, carboxy-terminal domain termed the headpiece. The core domain is capable of binding to G-actin and F-actin, capping actin filaments, and severing actin filaments (see Fig. 1). The headpiece provides the second F-actin binding site that is necessary for villin's actin bundling activity.

The *quail* locus in *Drosophila* was shown to encode an ovary-specific villin-like protein that includes a carboxy-terminal headpiece domain (Mahajan-Miklos and Cooley, 1994). Quail is first expressed in midstage egg chambers where it is localized to the nurse cell subcortical actin network and cytoplasm. Then, as the nurse cell cytoplasmic actin bundles form in stage 10B, quail protein co-localizes with the cytoplasmic actin bundles (Mahajan-Miklos and Cooley, 1994). When quail is absent these cytoplasmic actin bundles fail to form. These results suggest that quail bundles cytoplasmic actin filaments in nurse cells.

Fascin and quail do not appear to be redundant in *Drosophila* since they are both genetically required for actin bundle assembly and they have distinct amino acid sequences and biochemical properties. In an effort to understand how different bundling activities are exploited in a single cell type, we have altered quail protein expression levels in *singed* mutant backgrounds. Although fascin function is ordinarily required for the production of viable oocytes, overexpression of the *quail* gene product can circumvent this requirement. We suggest these two actin bundling proteins act in concert to enhance the strength and stability of bundles, thereby ensuring the production of healthy oocytes.

MATERIALS AND METHODS

Drosophila stocks and genetics

Fly stocks were maintained at room temperature on standard corn meal medium. Three *quail* alleles were used in this analysis: qua^{PX42}, an EMS-induced null allele (Schüpbach and Wieschaus, 1991; Mahajan-Miklos and Cooley, 1994), qua^{HM14}, an EMS-induced weakly fertile allele (Schüpbach and Wieschaus, 1991; Mahajan-Miklos and Cooley, 1994) and qua^{1374}, a small deletion that uncovers the *quail* locus (Mahajan-Miklos and Cooley, 1994). *quail* null flies were qua^{PX42}/qua^{1374}. Canton S flies were used as the wild-type control. Embryos from w^{1118} females were microinjected for germline transformation. *singed* alleles sn^{G409E}, sn^{S289N}, and sn^{28} were generated by EMS mutagenesis (Cant and Cooley, 1996). sn^{G409E} is female fertile with a mild defect in nurse cell cytoplasm transport. Sequence analysis of sn^{G409E} revealed a single point mutation that changes glycine to glutamic acid. sn^{S289N} is a hypomorphic mutation and female sterile. Sequence analysis of sn^{S289N} revealed a single point mutation that changes serine to asparagine. sn^{28} is a *singed* null mutation.

Fertility was assessed by placing 10 females and 5 males in each of 3-5 vials and counting progeny at 15 days.

Germline transformation

A 3.3 kb *quail* full-length cDNA was cloned between the *Sal*I and *Eco*RI sites of pBS (Stratagene) to create pBSqua. The *Sal*I and *Eco*RI sites were converted to *Not*I sites by ligation of phosphorylated *Not*I linkers (New England Biolabs) at filled-in *Sal*I and *Eco*RI sites. The resulting *Not*I fragment was cloned into the *Not*I site of pGerm8 (Serano et al., 1994) to create P[quaWT]. The truncated *quail* cDNA lacking the headpiece domain was generated by PCR amplification. The 5′ PCR oligo spanned an internal *Bgl*II site and the 3′ PCR oligo converted the TCA first codon of the headpiece to a TGA stop codon followed by a *Bam*HI site. The *Bgl*II-*Bam*HI fragment cloned between the *Bgl*II and *Bam*HI (in the vector) sites in pBSqua to create pBSqua-hl. The *Sal*I and *Bam*HI sites of pBSqua-hl were converted to *Not*I sites and the *Not*I fragment was subcloned into pGerm8 to create P[quaHL]. *P[quaWT]* and *P[quaHL]* stable germline transformant lines were made using standard methods.

Western blot

Drosophila ovaries were ground in 1× Laemmli sample buffer and protein concentration was determined using the Bio-Rad protein assay (Bio-Rad Laboratories). Protein samples were separated by SDS-PAGE (10% acrylamide) (Laemmli, 1970) and transferred to Hybond membranes (Amersham). After transfer, filters were treated as described by Xue and Cooley (1993). Quail monoclonal antibody supernatant 6B9 was diluted 1:10 in Blotto. Singed monoclonal antibody supernatant 7C was diluted 1:10 in Blotto.

Immunofluorescence and microscopy

Ovaries were dissected in IMADS (Singleton and Woodruff, 1994), fixed and incubated with either rhodamine-conjugated phalloidin

(Molecular Probes) and/or concentrated quail monoclonal antibody 6B9 supernatant as described by Xue and Cooley (1993). Concentrated quail monoclonal antibody 6B9 was used 1:25 diluted in PBT. Scanning laser confocal images were collected using a Bio-Rad MRC600 system. Images were collected using a Zeiss ×25 lens with a numerical aperture of 0.8. Three optical sections were taken at 1-2 μm intervals and combined using the COMOS program.

For ultrastructural analysis, ovaries were dissected in IMADS (Singleton and Woodruff, 1994). Stage 10B and 11 egg chambers were collected and fixed 2% glutaraldehyde in phosphate buffer, pH 6.2, for 15 minutes at room temperature. Eggs were then transferred to 2% glutaraldehyde with 0.2% tanic acid for 1-2 hours at 4°C. After washing in phosphate buffer, egg chambers were placed in 1% OsO_4 and 1% glutaraldehyde, for 35 minutes at 4°C. Following washes in water, eggs were dehydrated in ethanol, embedded in EPON, and baked to obtain a block for sectioning. Thin sections were stained with 1% uranyl acetate and Reynold's lead citrate and then examined on either a Zeiss EM-10 CA or JEOL 100CX electron microscope at 80 kV.

RESULTS

Mutations in *quail* enhanced the *singed*^{G409E} oogenesis phenotype

To determine if fascin and quail act together to form actin bundles, second site non-complementation analysis was used. We combined the intermediate alleles *sn*^{G409E} and *qua*^{HM14} to simultaneously reduce the dose of wild-type fascin and quail. *sn*^{G409E} was a fertile allele caused by a missense mutation that changed glycine 409 to glutamic acid (Cant and Cooley, 1996). Although *sn*^{G409E} females were fully fertile (Table 1) and expressed nearly wild-type levels of fascin^{G409E}, nurse cell cytoplasm transport was incomplete and mutant egg chambers contained disorganized cytoplasmic actin bundles (Cant and Cooley, 1996). *qua*^{HM14} was a very weakly fertile allele producing 10% of viable eggs compared with wild type (Mahajan-Miklos, 1995). In *qua*^{HM14} egg chambers, the nurse cell cytoplasmic actin bundles were sparse and mature eggs are smaller than wild-type eggs. The small amount of residual quail protein in this allele was primarily localized to subcortical actin filaments (Mahajan-Miklos, 1995). In both *sn*^{G409E} and *qua*^{HM14} females, the decrease in nurse cell cytoplasm transport appeared to reflect disruption in actin bundle organization. Heterozygotes *sn*^{G409E}/+ and *qua*^{HM14}/+ and double heterozygotes *sn*^{G409E}/+;*qua*^{HM14}/+ had wild-type fertility.

The fertility of *sn*^{G409E} was reduced by the *qua*^{HM14} mutation (Table 1). While *sn*^{G409E} females were fertile, *sn*^{G409E};*qua*^{HM14}/+ females were weakly-fertile and produced only about 20% the amount of progeny produced by *sn*^{G409E} females. The fertility *qua*^{HM14} was not affected by heterozygous *sn*^{G409E}. The most striking effect was seen in egg chambers from females doubly homozygous for *sn*^{G409E} and *qua*^{HM14}. *sn*^{G409E};*qua*^{HM14} females were completely sterile.

Table 1. *quail* enhances the *singed* egg chamber phenotype

singed	*quail*	% Fertility
G409E	WT	100
WT	HM14/+	100
G409E	HM14/+	20
WT	HM14	10
G409E/+	HM14	10
G409E	HM14	0

The decrease in fertility appeared to result from a decrease in nurse cell cytoplasm transport that correlated with a disruption of cytoplasmic actin bundle formation. *sn*^{G409E} had a minimal defect in nurse cell cytoplasm transport. As fertility was reduced by the addition of the *qua*^{HM14} mutation, less nurse cell cytoplasm was transported. *sn*^{G409E};*qua*^{HM14} sterile females produced eggs that were only 50% the size of wild-type eggs due to a severe disruption of nurse cell cytoplasm transport (data not shown). Similar enhancement was seen with a second intermediate *qua* allele, *qua*^{WP614} (data not shown).

Full-length *quail* cDNA restored fertility in *quail* mutants

Nurse cell cytoplasmic actin bundle formation appeared to be sensitive to the level of quail protein expressed. To test whether increasing the level of quail protein expressed in egg chambers could circumvent the requirement for fascin we generated *quail* P-element transgenes. First, we used *quail* transgenes to confirm that quail expression could rescue *quail* mutants and to assess the importance of the quail headpiece domain in vivo. Two P-element transformation constructs were made that contain either a wild-type, full-length *quail* cDNA (*P[quaWT]*) or a truncated, 'headless' cDNA that terminated immediately after the quail core domain coding region and lacked the headpiece domain (*P[quaHL]*) (Fig. 1). Each construct was cloned into the pGerm8 transformation vector (Serano et al., 1994) that directs germline-specific expression in stage 6 and older egg chambers, making it an ideal choice to recapitulate the expression pattern previously determined for the *quail* gene (Mahajan-Miklos and Cooley, 1994). Transformant lines containing a single copy of *P[quaWT]* were crossed into a *quail* null background (*qua*^{PX42}/*qua*^{1374}) and tested for rescue of *quail* female sterility. Six out of nine independent *P[quaWT]* transformant lines analyzed restored fertility to *quail* homozygous females. Western blot analysis of ovarian protein extracts demonstrated that the extent of rescue observed was dependent upon the level of quail expression from the transgene. Rescued *quail* mutants with one copy of a *P[quaWT]* transgene expressed nearly wild-type levels of quail protein (Fig. 2, lanes 4 and 5). Transformant lines that did not rescue showed low or undetectable levels of protein expression.

In addition to restoring fertility to *quail* mutant females, actin bundle formation was also rescued in *quail* mutant egg

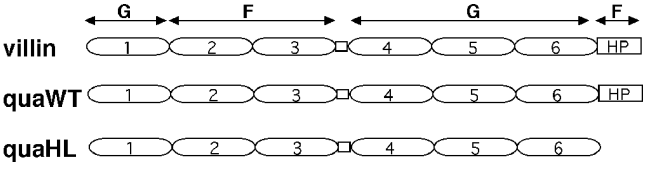

Fig. 1. Diagram of protein expressed from quail transgenes. Schematic representation of quail wild type (quaWT) and quail headless (quaHL) proteins that were expressed from transgenes as compared with villin protein. Villin and quail both contained six characteristic repeats and a carboxy-terminal headpiece domain (HP). Actin monomer (G) and actin filament (F) binding sites for chicken villin (reviewed by Weeds and Maciver, 1993) are indicated with arrows.

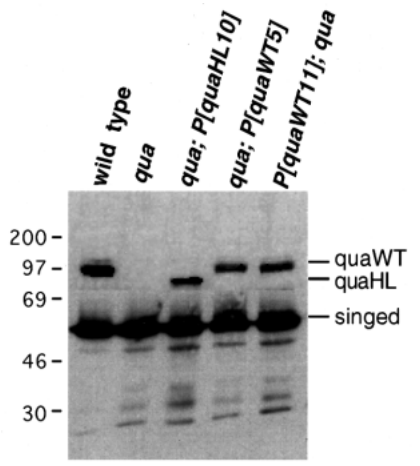

Fig. 2. Quail protein expression in transgenic lines. This western blot shows quail protein expressioin in ovary extracts from wild type, *quail* (*qua*) mutant, and *quail* mutants with a single copy of the P-element transgene indicated. Quail monoclonal antibody 6B9, which binds the core domain, detected a 97 kDa quail protein in wild-type ovary extracts (lane 1) that was absent in *quail* mutant ovaries (lane 2). A 97 kDa quail protein was expressed from transgene inserts *P[quaWT5]* on the third chromosome and *P[quaWT11]* on the first chromosome (lanes 4, 5). A truncated headless protein of approximately 90 kDa was expressed from the transgene insert *P[quaHL10]* (lane 3). Each transgene was present in a single copy and expressed an amount of quail protein that was only slightly less than the amount expressed in wild-type ovaries. Singed monoclonal antibody 7C detected a 57 kDa protein and was used as a loading control.

chambers carrying a *P[quaWT]* transgene. In wild-type stage 11 egg chambers, quail colocalized with the nurse cell subcortical actin network and cytoplasmic actin bundles (Fig. 3A,B; Mahajan-Miklos and Cooley, 1994). The absence of quail protein in nurse cells from *quail* mutants correlated with an absence of cytoplasmic actin bundles (Fig. 3B,C; Mahajan-Miklos and Cooley, 1994). Abundant cytoplasmic actin bundles were readily detected in rescued *P[quaWT5]* mutants (Fig. 3F). Quail protein expressed from the *P[quaWT5]* transgene colocalized with the nurse cell actin as seen in wild-type egg chamber (Fig. 3E).

Two of six independent *P[quaHL]* transformant lines expressed a 90 kDa headless protein at levels comparable to that of wild-type protein (Fig. 2, compare lanes 1 and 3). No rescue of *quail* sterility was obtained with headless transgenes. Homozygous mutant females that carried a *P[quaHL]* transgene laid few, undersized eggs and remained sterile as described for *quail*. Headless protein was readily detectable in stage 8 egg chambers where it localized to the subcortical cytoskeleton and cytoplasm (data not shown). However, beyond stage 10, the protein was most abundant in the nurse cell cytoplasm (Fig. 3G). This cytoplasmic staining correlated with the failure to form cytoplasmic actin bundles in late stage egg chambers (Fig. 3H). These results indicated that the headpiece domain of quail was required for formation of cytoplasmic actin bundles and suggested that the headpiece contains an actin binding domain. The headpiece domain was not required for the protein's initial localization to subcortical actin filaments.

Overexpression of quail suppressed the *singed* egg phenotype

Transformant lines carrying *P[quaWT11]* were used to overexpress quail protein in severe (sn^{S289N}), and null (sn^{28}) *singed* mutant backgrounds. Overexpression of quail, in otherwise wild-type egg chambers, did not disrupt cytoplasm actin bundles or nurse cell cytoplasm transport (data not shown). Egg chambers from sn^{S289N} females contained abundant $fascin^{S289N}$ in the nurse cell cytoplasm but cytoplasmic actin bundles failed to form (Fig. 4A) and mature eggs were 50% the size of wild-type eggs (Fig. 4B; Cant and

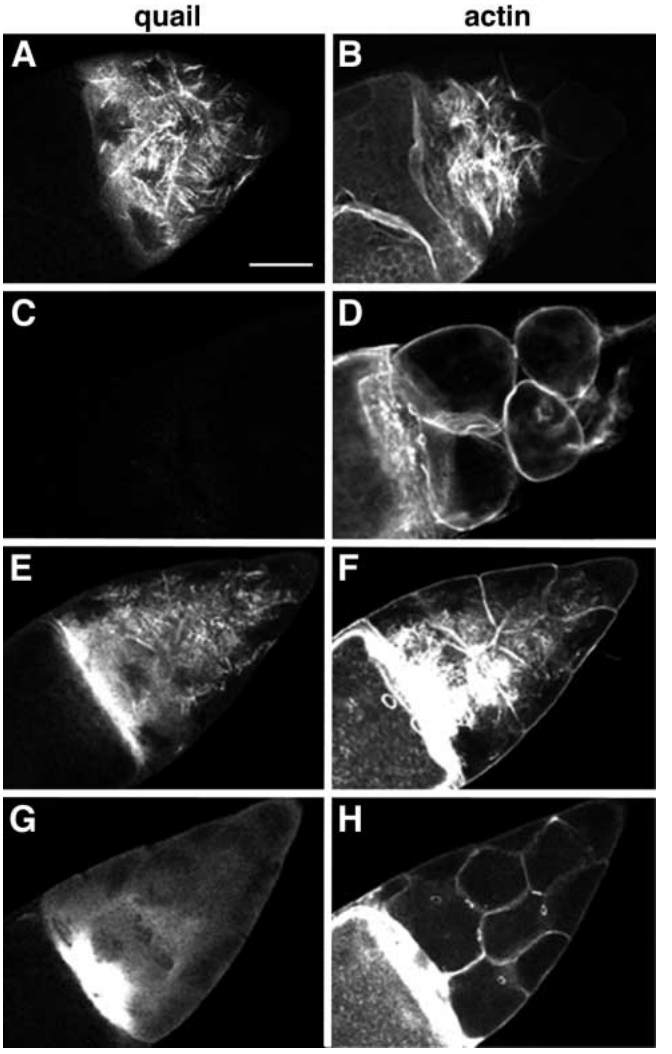

Fig. 3. Transgenic quail protein expression rescues *quail* mutants. Egg chambers from wild type (A,B), *qua* (C,D), *qua;P[quaWT5]* (E,F), and *qua;P[quaHL10]* (G,H) were double labeled with quail monoclonal antibody 6B9 (A,C,E,G) and rhodamine-conjugated phalloidin (B,D,F,H). In these stage 11 egg chambers, quail colocalized with the subcortical actin and the cytoplasmic actin bundles (A,B). In *quail* mutant egg chambers, quail protein (C) and the cytoplasmic actin bundles (D) were absent. In *qua;P[quaWT5]* egg chambers, quail protein expressed from the transgene localized to the subcortical actin and the cytoplasmic actin bundles and appeared wild type (E,F). At stage 11, quail headless protein localized primarily to the nurse cell cytoplasm (G) but the cytoplasmic actin bundles (H) were absent. Bar, 50 μm.

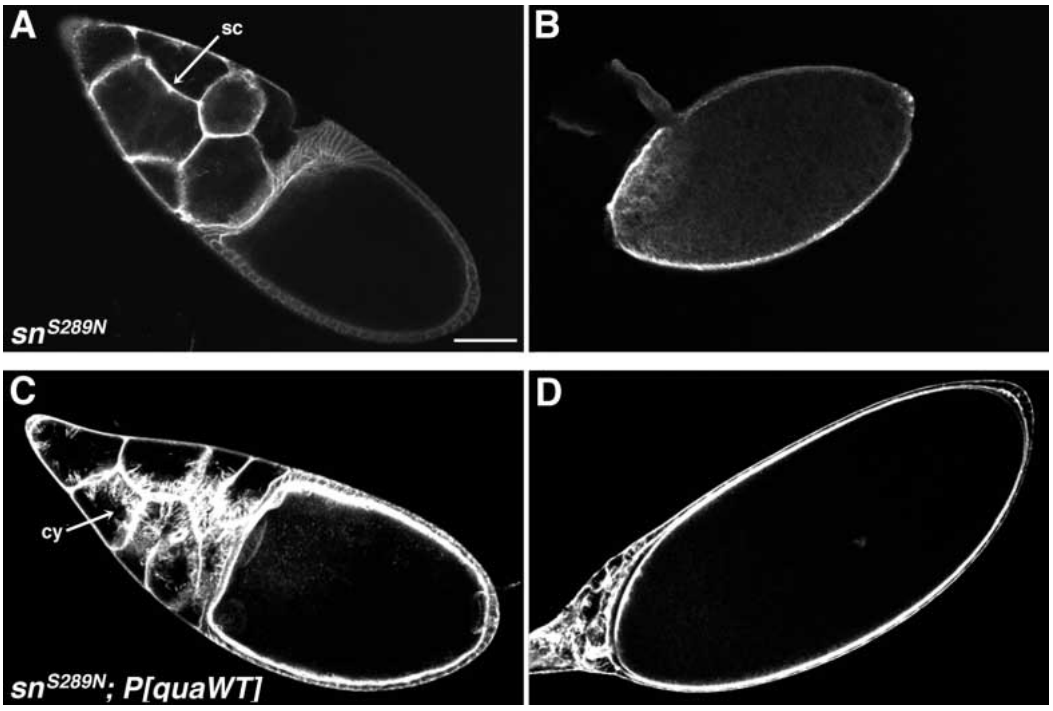

Fig. 4. Overexpression of quail rescued the sterility of sn^{S289N}. Two copies of the *P[quaWT11]* transgene were used to overexpress quail protein in sn^{S289N}. Stage 11 (A,C) and mature egg chambers (B,D) were stained with rhodamine-conjugated phalloidin. sn^{S289N} was a sterile allele in which the subcortical actin network (sc) was normal (A), the cytoplasmic actin bundles (cy) were absent (A), and mature eggs were only 50% the size of wild-type eggs (B). When four copies of quail (two endogenous and two transgenes) were expressed in the germline cells of sn^{S289N} mutants, nurse cell cytoplasmic actin bundles (cy) formed (C) and nurse cell cytoplasm transport was complete (D). Bar, 50 μm.

Cooley, 1996). sn^{S289N} females with three copies of *quail* (two endogenous and one transgene) were fertile, although the nurse cell cytoplasmic actin bundles appeared somewhat disorganized and mature eggs were only about 75% the size of wild-type eggs (data not shown). sn^{S289N} females with four copies of *quail* (two endogenous and two transgenic) were fertile and the cytoplasmic actin bundles appeared wild type (Fig. 4C, for wild type comparison see Fig. 5A), nurse cell cytoplasm transport was complete, and mature eggs were the same size as wild type (Fig. 4D, for wild type comparison see Fig 5C).

Overexpression of quail was also able to support cytoplasmic actin bundle formation in the absence of fascin. sn^{28} was previously described as a null allele that did not express *singed* transcript or protein (Cant and Cooley, 1996). In the absence of fascin, the nurse cell cytoplasmic actin bundles were nearly absent, the rapid phase of nurse cell cytoplasm transport was blocked, and mature eggs were only 50% the size of wild-type eggs (Fig. 5D,E,F). sn^{28} females with three copies of *quail* were semi-fertile and produced about 50% the number of eggs produced by wild-type females. Nurse cell cytoplasmic actin bundles were sparse and disorganized and mature eggs were only about 60-70% the size of wild-type eggs (data not shown). sn^{28} females with four copies of *quail* were fully fertile and cytoplasmic actin bundles initially appeared wild type (Fig. 5G). However, the nurse cell cytoplasmic actin bundles became disorganized during later stages (Fig. 5H) and mature eggs were only about 80% the size of wild-type eggs (Fig. 5I).

The ability of independent *P[quaWT]* transformant lines to rescue the *singed* oogenesis phenotype was correlated with the level of quail protein produced. Five independently derived *P[quaWT]* transformant lines that expressed quail protein and rescued *quail* sterility also rescued *sn* sterility. Since pGerm8 drives only germline-specific expression, *sn;P[quaWT]* transformants lines did not show rescue of the *singed* bristle phenotype.

The quail headpiece domain was required for *singed* rescue

In order to confirm that the quail rescue was dependent on quail bundling activity, we examined the ability of *P[quaHL]* transformant lines to rescue the *singed* oogenesis phenotypes in females with a wild-type *quail* background. Two copies of *P[quaHL10]* were not able to rescue the *sn* oogenesis phenotype of sn^{S289N} or sn^{28} (data not shown). In the *P[quaHL33]* transformant line, the truncated quail isoform was expressed abundantly from two transgene inserts on each second chromosome. Four copies of headless *P[quaHL33]* in addition to the two copies of endogenous full length quail did not rescue the sterility of *singed* alleles (data not shown). Rescue of the actin bundle defect and sterility of *singed* mutants required the headpiece of quail and therefore the bundling activity.

Ultrastructural analysis of actin bundles in rescued *singed* mutants

We used electron microscopy to evaluate the ultrastructure of actin bundles in *singed* mutant egg chambers rescued by the presence of excess quail. We analyzed actin bundles in wild type, sn^{S289N}*;P[quaWT]*, and sn^{28}*;P[quaWT]* egg chambers. Two types of wild-type actin bundles were seen: individual bundles (Fig. 6A) that were organized roughly in parallel to other bundles in the field and much thicker bundles that appeared to be composed of several individual bundles tightly packed next to one another (Fig. 6B). Based on the work of Guild et al. (1997), the individual bundles are likely to be modules present in stage 10 nurse cells before rapid transport while the much thicker ones are collapsed bundles in stage 11 nurse cells that are contracting. Actin bundles in sn^{S289N}*;P[quaWT]* egg chambers appeared wild type (data not shown). Individual actin bundles formed with only quail (sn^{28}*;P[quaWT]*) also resembled wild type (Fig. 6C). However, many of these actin bundles were more bent than wild type and they were often arranged at various angles to their

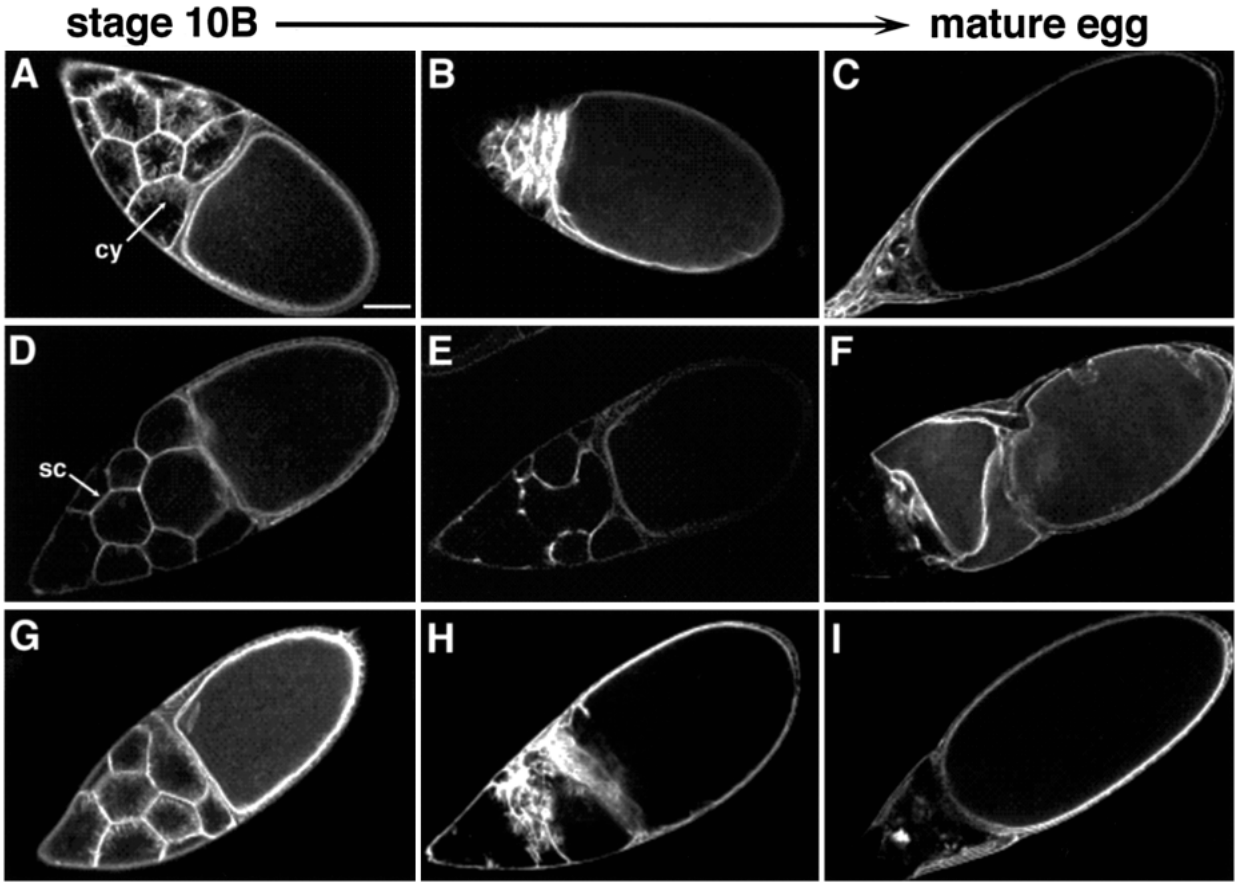

Fig. 5. Overexpression of quail partially rescued the *singed* null allele, sn^{28}. Wild type egg chambers stained with rhodamine-conjugated phalloidin illustrated that abundant nurse cell cytoplasmic actin bundles (cy) persisted from stage 10B until the end of oogenesis (A,B,C). In sn^{28} egg chambers, which do not contain fascin, the subcortical actin network (sc) was normal, the cytoplasmic actin bundles were absent, and eggs were only 50% the size of wild-type eggs (D,E,F). In the absence of fascin, abundant quail protein expressed from four copies of *quail* supported the initial formation of cytoplasmic actin bundles in stage 10B egg chambers (G) but was not able to maintain normal actin bundle organization in later stages (H). Although 4 copies of quail rescued the fertility of sn^{28}, the mature eggs were only about 80% the size of wild-type eggs (I). Bar, 50 μm.

neighbors (data not shown). In addition, there were groups of bundles loosely associated with neighbors (Fig. 6D) as if they failed to maintain their tight organization during nurse cell contraction. Immunofluorescence and ultrastructural analysis of actin bundles both suggest that although actin bundles can form without fascin they were disorganized and were probably not able to maintain a robust cage around nurse cell nuclei throughout the rapid phase of cytoplasm transport.

Electron micrographs of nurse cells sometimes show regions of cytoplasmic actin bundles that contain the 11 nm cross-banding pattern consistent with fascin crosslinks (Riparbelli and Giuliano, 1995; Guild et al., 1997). We were unable to consistently observe the 11 nm cross-banding pattern in wild-type bundles and we did not see any regular cross-banding pattern in quail rescued bundles.

DISCUSSION

Actin bundling proteins can influence the initiation, organization, or stabilization of an actin bundle; however, how they coordinate their activities in vivo is poorly understood. Actin bundling proteins typically have temporally distinct localization to actin bundles in vivo and have distinct biochemical properties in vitro; therefore, two bundling proteins present in a cell do not appear redundant. Supporting this idea, mutations that disrupt expression of fascin or quail inhibit cytoplasmic actin bundle assembly in *Drosophila* nurse cells (Cant et al., 1994; Mahajan-Miklos and Cooley, 1994). Furthermore, removal of villin expression with antisense mRNA disrupts the formation of microvilli in intestinal cell lines (Costa de Beauregard et al., 1995). By overexpressing quail in the *Drosophila* germline, we show that the function of quail is partially redundant with fascin in vivo.

Full-length quail protein is required for cytoplasmic actin bundle assembly

The ability of quail to bundle actin filaments is critical to nurse cell cytoplasm transport. Quail expression from the full length transgenes confirms that quail protein can rescue the actin bundle defect and restore fertility in *quail* females. The lack of rescue by the headless transgene indicates that the headpiece domain of quail is required for rescue. The quail headpiece is also required for quail's actin bundling activity in vitro (Mahajan-Miklos, 1995). The villin headpiece provides one of the two F-actin

binding sites necessary for villin's actin bundling activity. Deletion analysis and small peptide analysis first identified the headpiece sequence $K^{70}K^{71}E^{72}K^{73}$ as an essential actin binding motif (Friederich et al., 1992). Recently, thorough cysteine scanning mutagenesis of the chicken villin headpiece demonstrated that the F-actin binding activity of the 76 amino acid headpiece requires amino acids K^{38}, E^{39}, K^{65}, K^{70}, K^{71},L^{75}, F^{76} but does not require E^{72} and K^{73} (Doering and Matsudaira, 1996). Interestingly, all of these critical amino acids are conserved in quail except K^{38}, which is instead R^{38}. The quail headpiece appears to be required for actin bundling activity and this activity is necessary to rescue the *quail* phenotype.

The actin bundling proteins quail and fascin are partially redundant

Cytoplasmic actin bundle formation is sensitive to changes in protein levels of fascin and quail. The cytoplasmic actin bundle defect of the intermediate allele sn^{G409E} is enhanced by mutations in *quail*. Alternatively, overexpression of quail protein in nurse cells allows cytoplasmic actin bundle formation in the sterile allele sn^{S289N} and in the null allele sn^{28}. In the absence of fascin, four copies of quail can support the initial assembly and organization of cytoplasmic actin bundles. Our genetic data suggest that fascin and quail not only act together to stabilize actin filament bundles in nurse cells, but when quail is expressed in excess, the bundling activity of quail appears to be partially redundant with that of fascin.

The formation of stable, organized cytoplasmic actin bundles and the completion of nurse cell cytoplasm transport requires some fascin function. In sn^{28}*;P[quaWT]* females, quail-actin bundles lacking fascin do not maintain their initial organization and do not fully support the final stages of nurse cell cytoplasm transport. However, actin bundles in sn^{S289N} rescued by quail appear wild type by immunofluorescence and electron microscopy and are able to support nurse cell cytoplasm transport. This suggests that fascin^{S289N} has residual actin bundling activity and can stabilize the initial quail-actin bundles. These experiments provide evidence for both partially redundant and unique bundling functions of fascin and quail. While excess quail can compensate somewhat for the absence of fascin, quail and fascin are clearly providing distinct functions for the organization and stabilization of mature actin bundles and together these proteins promote the completion of oogenesis.

The appearance of quail-rescued actin bundles in *singed* mutants suggests that fascin bundling function is especially important for organizing modules. In wild type, short bundle modules composed of about 25 actin filaments are arranged serially to form the cytoplasmic actin bundles that reach up to 20 μm in length (Guild et al., 1997). In quail-rescued bundles, modules are present (Fig. 6C) but overall bundle organization is not rescued to wild type (Fig. 5H). Therefore, while quail protein by itself is sufficient to form modules, fascin could be indispensable for tethering the modules together. In wild type after nurse cell contraction, collapsed bundles have a 12 nm crossbanding pattern typical of fascin crosslinking that extends all across the bundle (Guild et al., 1997). In the absence of fascin, it appears that collapsed bundles are disorganized (Fig. 6D) suggesting that fascin is critical for maintaining bundle organization as modules slide past one another. However, the aberrant bundles do provide enough support for nurse cell nuclei

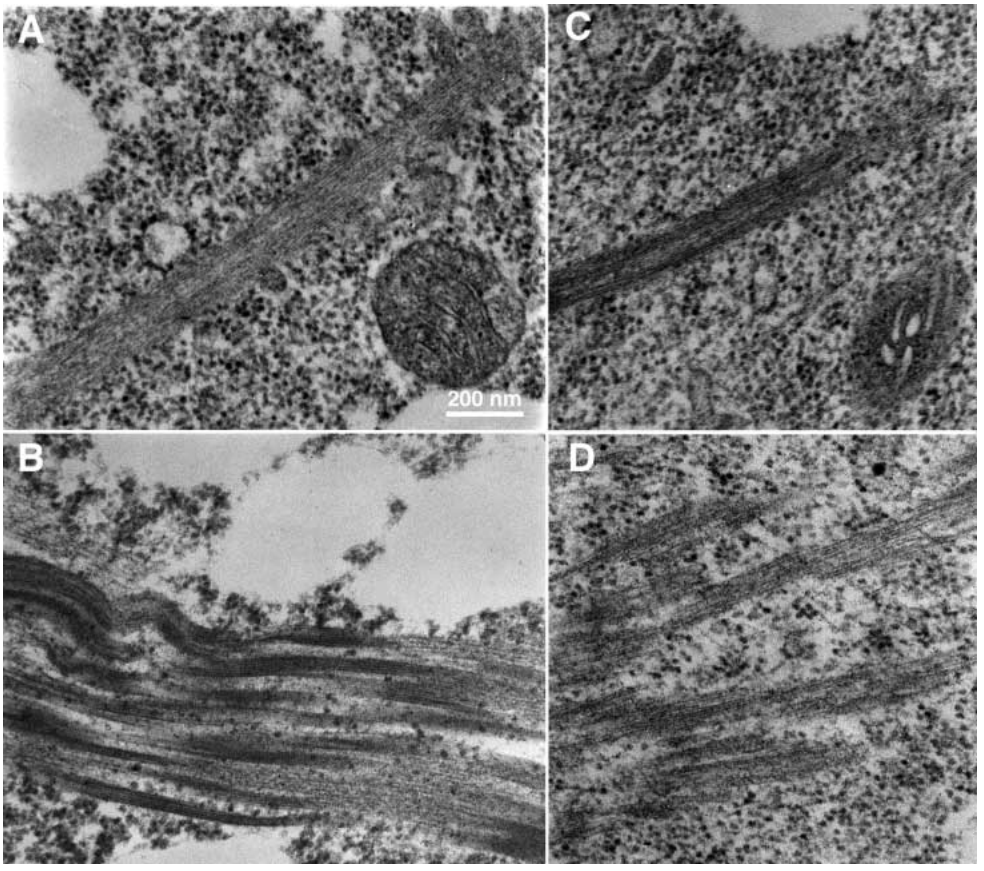

Fig. 6. Actin bundles in wild-type and *sn;P[quaWT]* egg chambers. These electron micrographs show actin bundles found in nurse cells of wild-type (A,B) and sn^{28}*;P[quaWT]* (C,D) females. Wild-type bundles were either thin individual bundles (A) or much thicker as if several smaller bundles were packed together (B). Individual actin bundles formed with excess quail and no fascin appeared wild type (C). However, larger aggregates of bundles were poorly organized (D). Bar, 200 nm.

that sufficient cytoplasm transport can take place producing viable eggs.

The ability of quail to compensate for the absence of fascin requires an increase in quail protein expression. In vitro, the organization and formation of actin bundles can reflect the concentration of the actin bundling protein with respect to F-actin. At ratios of 2:10 for villin to actin or 1:10 for fimbrin to actin, actin bundles consist of thin, loosely organized filaments (Bretscher, 1981; Matsudaira and Burgess, 1982). Increasing the ratio of villin or fimbrin to actin to 1:2 results in bundles that are large, straight, and organized (Glenney et al., 1981; Matsudaira and Burgess, 1982). The bundling properties of villin and fimbrin are additive (Glenney et al., 1981); at a villin:fimbrin:actin ratio of 1:1:10, actin bundles are large, organized and virtually indistinguishable from actin bundles formed at a ratio of fimbrin:actin or villin:actin of 1:2. In nurse cells, perhaps the concentrations of quail and fascin are limited such that both proteins are necessary to support actin bundle formation. In our rescue experiments we have increased the quail:actin ratio to a level that allows dense actin bundle formation.

Actin bundle assembly occurs through a two-step process of initiation and organization

Our data are consistent with a two-step model of actin bundle assembly in *Drosophila* nurse cells; one actin bundling protein, quail, initiates actin bundle assembly and then a second actin bundling protein, fascin, organizes the actin bundle allowing growth and stability of the bundle. This two-step theory was first proposed by Shibayama while studying intestinal development in chicken embryos (Shibayama et al., 1987), and has since been proposed to explain the formation of actin bundles in the intestinal microvilli in mouse (Ezzell et al., 1989), the stereocilia of the inner ear in chicken (Tilney and DeRosier, 1986), and *Drosophila* bristles (Tilney et al., 1995, 1996).

In microvillus actin bundles, the initiating and organizing actin bundling proteins are villin and fimbrin (reviewed by Heintzelman and Mooseker, 1992; Louvard et al., 1992). During chicken and mouse embryogenesis, villin is the first actin bundling protein to concentrate on the apical surface of developing intestinal epithelium at the time when the density of the microvilli on the apical surface is very low and the actin filaments appear unorganized (Shibayama et al., 1987; Ezzell et al., 1989). The timing of villin expression, and villin's ability to cap, sever and nucleate actin filaments in a calcium-dependent manner in vitro, make it an ideal protein to initiate the reorganization of the actin cytoskeleton necessary to form actin bundles. Indeed, ectopic expression of villin in cultured fibroblasts leads to the formation of microvilli-like structures and the reorganization of the actin stress fiber network (Friederich et al., 1989; Franck et al., 1990). Fimbrin localization to the apical surface occurs 2-3 days after villin in both chicken and mouse embryos and correlates with the appearance of a dense lawn of short microvilli containing organized actin bundle cores (Shibayama et al., 1987; Ezzell et al., 1989). Fimbrin appears to function similarly in stereocilia of the chick inner ear where actin bundles are initially disorganized but a few days later are hexagonally packed with a 12 nm periodicity (Tilney and DeRosier, 1986; Tilney et al., 1992).

In *Drosophila*, two actin bundling proteins are required for actin bundle assembly in both bristle development and oogenesis. In developing bristles, forked protein is present in the earliest stages of bristle extension (Petersen, 1994) before the accumulation of fascin (Wulfkuhle, 1995). While the biochemical properties of forked are not known, the protein is required for dense actin bundle formation and colocalizes with actin bundles in vivo (Petersen et al., 1994; Tilney et al., 1995). Forked could be providing the initial bundling activity that villin provides in microvilli. Fascin accumulates to very high levels in the cytoplasm and on actin bundles of extending bristles (Cant et al., 1994) after forked is detected (Wulfkuhle, 1995). Fascin is required for hexagonal packing of these actin bundles (Tilney et al., 1995). During *Drosophila* oogenesis, quail is present in nurse cells on the subcortical actin prior to the formation of cytoplasmic bundles and subsequently is present on cytoplasmic bundles (Mahajan-Miklos and Cooley, 1994). This localization, and the rescue of *singed* by overexpression of quail, support a function for quail as a bundle initiator. Fascin expression in nurse cells dramatically increases with cytoplasmic actin bundle assembly; however, its localization remains cytoplasmic (Cant et al., 1994) and therefore the timing of fascin localization relative to quail cannot be determined. Fascin is required for hexagonal packing in bristles and for maintenance of actin bundles in nurse cells suggesting that fascin is an organizer in both of these tissues.

The precise coordination of actin bundling proteins may reflect different affinities for F-actin among actin bundling proteins. The initiator protein should have a high affinity for F-actin and be able to pull together actin filaments that are in low concentration. The organizer protein would be of lower affinity and require that actin filaments be loosely juxtaposed to provide a high concentration of F-actin. Bacterially expressed *Drosophila* fascin appears to have a low affinity for F-actin in vitro (Cant et al., 1994). The abundant expression of fascin in developing bristles and nurse cells (Cant et al., 1994) may indicate that fascin also has a low affinity for F-actin in vivo.

This two-step theory suggests that the organizer proteins fimbrin and fascin would not be able to initiate actin bundle assembly. *P[singed]* transformants will be made to determine if overexpression of fascin can rescue *quail* mutants. However, fascin already appears to be expressed in vast excess in nurse cells since a 75% reduction in protein levels in weak *singed* alleles does not disrupt actin bundle formation (Cant et al., 1994). Fascin and fimbrin cannot cap, sever, or nucleate actin filaments and these properties may be important in villin's ability to initiate actin bundle formation. Therefore, *P[singed]* may not be able to rescue *quail*.

Nurse cell cytoplasm transport provides a powerful, sensitive genetic system for elucidating mechanisms of actin bundle formation and the coordination of multiple actin bundling proteins in vivo. Actin bundle assembly in nurse cells is sensitive to changes in actin bundling protein structure and expression levels. Subtle defects in actin bundle organization are visible by fluorescence microscopy and can result in a nurse cell cytoplasm transport defect that impairs female fertility. Work in this system should continue to complement biochemical characterization of actin binding proteins.

We are immeasurably grateful for the contributions Brenda Knowles made to our lives both scientifically and personally. The strength of character and optimism she brought to her life and her battle with leukemia remain inspirations to us. We thank Mark Mooseker for many valuable discussions and comments on the manuscript; Greg Guild for telling us about the work on nurse cell

actin bundle ultrastructure prior to publication; Kathleen Ayers for expert technical assistance; and Spyros Artavanas-Tsakonas and the Howard Hughes Medical Institute for the use of their confocal facility. This work was supported by grants from the NIH (GM43301) and Pew Charitable Trusts (L.C.), the Medical Scientist Training Program (GM07205) (K.C.), an NIH postdoctoral fellowship (B.A.K.), and a predoctoral fellowship from Bayer-Miles Pharmaceuticals (S.M.-M.).

REFERENCES

Bretscher, A. (1981). Fimbrin is a cytoskeletal protein that crosslinks F-actin in vitro. *Proc. Nat. Acad. Sci. USA* **78**, 6849-6853.

Bryan, J. and Kane, R. E. (1978). Separation and interaction of the major components of sea urchin actin gel. *J. Mol. Biol.* **125**, 207-224.

Bryan, J., Edwards, R., Matsudaira, P., Otto, J. and Wulfkuhle, J. (1993). Fascin, an echinoid actin-bundling protein, is a homolog of the *Drosophila singed* gene product. *Proc. Nat. Acad. Sci. USA* **90**, 9115-9119.

Burgess, D. R. and Schroeder, T. E. (1977). Polarized bundles of actin filaments within microvilli of fertilized sea urchin eggs. *J. Cell Biol.* **74**, 1032-1037.

Cant, K., Knowles, B. A., Mooseker, M. S. and Cooley, L. (1994). *Drosophila* singed, a fascin homolog, is required for actin bundle formation during oogenesis and bristle extension. *J. Cell Biol.* **125**, 369-380.

Cant, K. and Cooley, L. (1996). Single amino acid mutations in *Drosophila* fascin disrupt actin bundling function in vivo. *Genetics* **143**, 249-258.

Cooley, L., Verheyen, E. and Ayers, K. (1992). *chickadee* encodes a profilin required for intercellular cytoplasm transport during Drosophila oogenesis. *Cell* **69**, 173-184.

Cooley, L. and Theurkauf, W. E. (1994). Cytoskeletal functions during *Drosophila* oogenesis. *Science* **266**, 590-596.

Costa de Beauregard, M., Pringault, E., Robine, S. and Louvard, D. (1995). Suppression of villin expression by antisense RNA impairs brush border assembly in polarized epithelial intestinal cells. *EMBO J.* **14**, 409-421.

DeRosier, D., Mandelkow, E., Stillman, A., Tilney, L. and Kane, R. (1977). Structure of actin-containing filaments from two types of non-muscle cells. *J. Mol. Biol.* **113**, 679-695.

DeRosier, D. J. and Censullo, R. (1981). Structure of F-actin needles from extracts of sea urchin oocytes. *J. Mol. Biol.* **146**, 77-99.

Doering, D. S. and Matsudaira, P. (1996). Cysteine scanning mutagenesis at 40 of 76 positions in villin headpiece maps the F-actin binding site and structural features of the domain. *Biochemistry* **35**, 12677-12685.

Edwards, R. A. and Bryan, J. (1995). Fascins, a family of actin bundling proteins. *Cell Motil. Cytoskel.* **32**, 1-9.

Edwards, K. A. and Kiehart, D. P. (1996). *Drosophila* nonmuscle myosin II has multiple roles in imaginal disc and egg chamber morphogenesis. *Development* **122**, 1499-1511.

Ezzell, R. M., Chafel, M. M. and Matsudaira, P. T. (1989). Differential localization of villin and fimbrin during development of the mouse visceral endoderm and intestinal epithelium. *Development* **106**, 407-419.

Franck, Z., Footer, M. and Bretscher, A. (1990). Microinjection of villin into cultured cells induces rapid and long-lasting changes in cell morphology but does not inhibit cytokinesis, cell motility or membrane ruffling. *J. Cell Biol.* **111**, 2475-2485.

Friederich, E., Huet, C., Arpin, M. and Louvard, D. (1989). Villin induces microvilli growth and actin redistribution in transfected fibroblasts. *Cell* **59**, 461-475.

Friederich, E., Pringault, E., Arpin, M. and Louvard, D. (1990). From the structure to the function of villin, an actin-binding protein of the brush border. *BioEssays* **12**, 403-408.

Friederich, E., Vancompernolle, K., Huet, C., Goethals, M., Finidori, J., Vandekerckhove, J. and Louvard, D. (1992). An actin-binding site containing a conserved motif of charged amino acid residues is essential for the morphogenic effect of villin. *Cell* **70**, 81-92.

Glenney, J. R., Kaulfus, P., Matsudaira, P. and Weber, K. (1981). F-actin binding and bundling properties of fimbrin, a major cytoskeletal protein of the microvillus core filaments. *J. Biol. Chem.* **256**, 9283-9288.

Guild, G. M., Connelly, P. S., Shaw, M. K. and Tilney, L. G. (1997). Actin filament cables in *Drosophila* nurse cells are composed of modules that slide passively past on another during dumping. *J. Cell Biol.* **138**, 783-797.

Gutzeit, H. O. (1986). The role of microfilaments in cytoplasmic streaming in *Drosophila* follicles. *J. Cell Sci.* **80**, 159-169.

Hartwig, J. H. and Kwiakowski, D. J. (1991). Actin-binding proteins. *Curr. Opin. Cell Biol.* **3**, 87-97.

Heintzelman, M. B. and Mooseker, M. S. (1992). Assembly of the intestinal brush border cytoskeleton. *Curr. Top. Dev. Biol.* **26**, 93-122.

Knowles, B. A. and Cooley, L. (1994). The specialized cytoskeleton of the *Drosophila* egg chamber. *Trends Genet.* **10**, 235-241.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

Louvard, D. (1989). The function of the major cytoskeletal components of the brush border. *Curr. Opin. Cell Biol.* **1**, 51-57.

Louvard, D., Kedinger, M. and Hauri, H. P. (1992). The differentiating intestinal epithelial cell: establishment and maintenance of functions through interactions between cellular structures. *Annu. Rev. Cell Biol.* **8**, 157-195.

Mahajan-Miklos, S. and Cooley, L. (1994). The villin-like protein encoded by the *Drosophila quail* gene is required for actin bundle assembly during oogenesis. *Cell* **78**, 291-301.

Mahajan-Miklos, S. (1995). Characterization of *quail*, a gene required for actin bundle assembly during *Drosophila* oogenesis. Ph.D. thesis, Yale University.

Matsudaira, P. T. and Burgess, D. R. (1982). Partial reconstruction of the microvillus core bundle: Characterization of villin as a Ca^{++}-dependent, actin-bundling/depolymerizing protein. *J. Cell Biol.* **92**, 648-656.

Mooseker, M. S. (1985). Organization, chemistry, and assembly of the cytoskeletal apparatus of the intestinal brush border. *Annu. Rev. Cell Biol.* **1**, 209-241.

Otto, J. J., Kane, R. E. and Bryan, J. (1980). Redistribution of actin and fascin in sea urchin eggs after fertilization. *Cell Motil.* **1**, 31-40.

Otto, J. J. and Bryan, J. (1981). The incorporation of actin and fascin into the cytoskeleton of filopodial sea-urchin coelomocytes. *Cell Motil.* **1**, 179-192.

Otto, J. J. (1994). Actin-bundling proteins. *Curr. Opin. Cell Biol.* **6**, 105-109.

Overton, J. (1967). The fine structure of developing bristles in wild type and mutant *Drosophila melanogaster*. *J. Morphol.* **122**, 367-380.

Petersen, N. S. (1994). The forked protein is most abundant in the initial stages of actin fiber formation in *Drosophila* bristles. *Mol. Biol. Cell* **5**, 273a.

Petersen, N. S., Lankenau, D.-H., Mitchell, H. K., Young, P. and Corces, V. (1994). *Forked* proteins are components of fiber bundles present in developing bristles of *Drosophila melanogaster*. *Genetics* **136**, 173-182.

Riparbelli, M. G. and Giuliano, C. (1995). Cytoskeleton of the *Drosophila* egg chamber: new observations on microfilament distribution during oocyte growth. *Cell Motil. Cytoskel.* **31**, 298-306.

Robinson, D. N. and Cooley, L. (1997). Genetic analysis of the actin cytoskeleton in the *Drosophila* ovary. *Annu. Rev. Cell Dev. Biol.* **13**, 147-170.

Schüpbach, T. and Wieschaus, E. (1991). Female sterile mutations on the second chromosome of Drosophila melanogaster. II. Mutations blocking oogenesis or altering egg morphology. *Genetics* **129**, 1119-1136.

Serano, T. L., Cheung, H.-K., Frank, L. H. and Cohen, R. S. (1994). P element transformation vectors for studying *Drosophila melanogaster* oogenesis and early embryogenesis. *Gene* **138**, 181-186.

Shibayama, T., Carboni, J. M. and Mooseker, M. S. (1987). Assembly of the intestinal brush border: appearance and redistribution of microvillar core proteins in developing chick enterocytes. *J. Cell Biol.* **105**, 335-344.

Singleton, K. and Woodruff, R. I. (1994). The osmolarity of adult *Drosophila* hemolymph and its effect on oocyte-nurse cell electrical polarity. *Dev. Biol.* **161**, 154-167.

Spudich, J. A. and Amos, L. A. (1979). Structure of actin filament bundles from microvilli of sea urchin eggs. *J. Mol. Biol.* **129**, 319-331.

Tilney, L. G. and DeRosier, D. J. (1986). Actin filaments, stereocilia, and hair cells of the bird cochlea. *Dev. Biol.* **116**, 119-129.

Tilney, L. G., Tilney, M. S. and DeRosier, D. J. (1992). Actin filaments, stereocilia and hair cells: How cells count and measure. *Annu. Rev. Cell Biol.* **8**, 257-274.

Tilney, L. G., Tilney, M. S. and Guild, G. M. (1995). F actin bundles in *Drosophila* bristles I. Two filament cross-links are involved in bundling. *J. Cell Biol.* **130**, 629-638.

Tilney, L. G., Connelly, P., Smith, S. and Guild, G. M. (1996). F-actin bundles in *Drosophila* bristles are assembled from modules composed of short filaments. *J. Cell Biol.* **135**, 1291-1308.

Weeds, A. and Maciver, S. (1993). F-actin capping proteins. *Curr. Opin. Cell Biol.* **5**, 63-69.

Wheatley, S., Kulkarni, S. and Karess, R. (1995). *Drosophila* nonmuscle myosin II is required for rapid cytoplasmic transport during oogenesis and for axial nuclear migration in early embryos. *Development* **121**, 1937-1946.

Wulfkuhle, J. (1995). Fascins: identification of actin-binding domains and analysis of cellular functions. Ph.D. thesis, Purdue University.

Xue, F. and Cooley, L. (1993). *kelch* encodes a component of intercellular bridges in *Drosophila* egg chambers. *Cell* **72**, 681-693.