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The WASp-based actin polymerization machinery is required in somatic support cells for spermatid maturation and release

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SUMMARY

WASp family proteins serve as conserved regulators of branched microfilament array formation via the Arp2/3 actin polymerization machinery. We have identified a specific role during spermatogenesis for the *Drosophila* WASp homolog (Wsp) and associated elements. Spermatogenesis within the fly testis is carried out in cysts, where a pair of somatic cyst cells encloses differentiating sperm. The final phase of the process involves the attachment of matured cysts to a specialized epithelium at the base of the testis, followed by release of individual motile spermatids into the adjoining seminal vesicle. *Wsp* mutant cysts contain fully mature sperm, but spermatid release does not occur, resulting in male sterility. Our data suggest that the Wsp-Arp2/3-based machinery acts in the cyst cells to influence proper microfilament organization and to enable cyst attachment to the base of the testis. Wsp activity in this context is mediated by the small GTPase Cdc42. Involvement of the cell surface protein Sticks and stones and the Wsp adapter protein D-WIP (Vrp1) is also crucial. In parallel, we demonstrate that N-WASp (Wasl), the major mammalian WASp family protein, is required in the somatic Sertoli cells of the mouse testis for sperm maturation. A requirement for WASp-based activity in somatic support cells therefore appears to be a universal feature of spermatogenesis.

KEY WORDS: Energy storage, Kinematics, Resilin, *Drosophila*

INTRODUCTION

Spermatogenesis constitutes a remarkable process of cell differentiation, during which small, immature spermatogonia are transformed into elongated and motile spermatids (Fuller, 1993; Roosen-Runge, 1977). A crucial feature of this process is the intimate association and cross-talk between the differentiating germ cells and specialized populations of somatic support cells. Studies in the *Drosophila* model system have underscored the significance of soma-germline interactions during spermatogenesis and have identified some of the key molecular mechanisms involved. Much of this analysis has focused on the establishment and maintenance of the germline stem cell niche and on the early phases of sperm differentiation (Fabrizio et al., 2003; Kiger et al., 2000; Leatherman and Dinardo, 2010; Schulz et al., 2002; Tulina and Matunis, 2001). Although the close germline-soma association persists throughout *Drosophila* spermatogenesis, much less is known about the impact of this association on more advanced stages of sperm differentiation.

Spermatogenesis in *Drosophila* commences at the apical tip of the testis, where the germline stem cells reside, and is initiated by a series of incomplete mitotic divisions and meiosis, producing a cyst with 64 interconnected haploid spermatids. A pair of somatic cyst cells, themselves generated from a separate apical stem cell population, enwraps the germline cyst from the outset, providing it with a thin outer lining (Fuller, 1993) (Fig. 1A). Upon completion of meiosis, the spermatocytes enter a prolonged phase of differentiation, during which they acquire the unique morphological and functional features of individual, elongated and motile spermatids. The differentiating spermatids remain enclosed within cysts throughout this process, as they journey progressively towards the basal end of the testis tube.

The pair of associated somatic cyst cells, which continue to line the differentiating cysts, accommodate the dramatic alterations to cyst size and organization through morphological changes of their own. One of the two cells, termed the tail cyst cell, spreads out so as to cover the elongating sperm tails, whereas the second, so-called head cyst cell, provides an enclosure for the bundled spermatid nuclei. Transfer of sperm from the testis and into the reproductive tract involves a complex series of events, which unfolds once the fully differentiated cysts reach the base of the testis tube (Fig. 1A). The head cyst cell becomes anchored in a terminal epithelium (TE) present at this location, whereupon the ~2 mm long and narrow cyst coils from its apical end to form a compact structure. Shortly thereafter, single, motile spermatids are released through the head cyst cell-TE interface and travel via the short testicular duct into the adjoining seminal vesicle (Miller, 1950).

Although this final phase of spermatogenesis has been the subject of detailed and elegant analysis by electron microscopy (Tokuyasu et al., 1972), identification and characterization of the underlying cellular features and molecular mechanisms are yet to be achieved. Here we describe the involvement of somatic support cells during the final phases of *Drosophila* sperm maturation, just prior to their release as freely motile cells into the reproductive tract. We present data suggesting that microfilament organization within the head cyst cell, mediated by the WASp-Arp2/3 branched actin polymerization machinery, is essential for cyst anchoring at the base of the testis and subsequent events.

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Polymerization of actin into branched microfilament arrays, mediated by the Arp2/3 protein complex, provides a mechanistic basis for a wide variety of basic cellular processes, including cell motility, the formation of cell-cell and cell-matrix adhesions, and endocytosis (Goley and Welch, 2006). Assignment of specific developmental tasks to general cellular machineries such as Arp2/3-based actin polymerization is problematic owing to their ubiquitous nature. One solution is to approach these issues by investigating pathway elements that play tissue-specific or developmental stage-specific roles. Activity of the Arp2/3 complex requires stimulation by nucleation-promoting factors (NPFs), such as WASp and SCAR/WAVE (Kurisu and Takenawa, 2009). The relatively restricted utilization of the *Drosophila* WASp homolog Wsp as an Arp2/3 NPF makes it a useful tool in this context (Zallen et al., 2002). Furthermore, the NPF activity of WASp family proteins depends upon its proper activation and localization by a variety of factors, which may be utilized only in specialized settings. For example, the restricted expression and function of D-WIP (also known as Vrp1), the *Drosophila* WASp-interacting protein (WIP) homolog, in developing embryonic muscles was key in revealing the involvement of the WASp-Arp2/3 pathway in myoblast fusion (Kim et al., 2007; Massarwa et al., 2007). Similarly, our discovery that activation of Wsp by the GTP-bound form of the signaling element Cdc42 occurs specifically during late spermatogenesis has now paved the way for the study of Wsp-Arp2/3 function in this particular developmental context.

We show here that Wsp, Cdc42 and the Arp2/3 complex are all required in the somatic cyst cells for the final phase of cyst maturation in the testis: attachment to the basal TE and the subsequent transfer of motile sperm out of the cysts and into the seminal vesicle. A similar requirement is demonstrated for the cell surface protein Sticks and stones (Sns) and for the adaptor element D-WIP, both of which are required, like Wsp, to promote embryonic myoblast fusion. Sns is expressed, along with a testis-specific isoform of D-WIP, in the head cyst cell, where Wsp, D-WIP and Cdc42 are shown to mediate microfilament organization. The concerted activity of Wsp and associated elements therefore appears to take place in the specific cell that associates with the TE and through which sperm 'escape' into the seminal vesicle. Finally, we demonstrate an analogous scenario in the mammalian testis, in that murine N-WASp (Wasl – Mouse Genome Informatics) is required in Sertoli cells, the major somatic support cells, for spermatocyte maturation. This suggests an evolutionary conserved function for WASp-based branched actin polymerization during spermatogenesis.

MATERIALS AND METHODS

Drosophila molecular genetics

Gene targeting designed to generate the *D-WIP*^{KO} allele was performed as described (Tsuda et al., 2010). For constructing the P[donor] plasmid, ~3 kb of both the upstream and downstream regions of the *D-WIP* locus were amplified and the amplified fragments were subcloned into the polylinker of pP[EndsOut2] (a gift from J. Sekelsky, University of North Carolina). The *mini-white* gene, a marker for transformation, was inserted between the upstream and downstream target sequences. Targeted lines were backcrossed to the *y w*^{67c23} *Df(1)w*^{67c23} stock for six generations. *D-WIP*^{testis} constructs were based on PCR amplification of clone AT07076 from an adult fly testis cDNA library (Stapleton et al., 2002). Amplified material was cloned into the appropriate *Drosophila* Gateway vectors (T. Murphy, Carnegie Institution of Washington) using the Gateway cloning system (Invitrogen). UAS-dsRNA constructs were commonly expressed in flies together with UAS-*Dicer2* to enhance RNAi activity (Dietzl et al., 2007). The following lines were used.

Mutant alleles: for *Wsp*, *Wsp*^{1} and Df(3R)3450 (Ben-Yaacov et al., 2001); for *D-WIP*, *D-WIP*^{D30} (Massarwa et al., 2007), *sltr*^{S1946} (Kim et al., 2007) and *D-WIP*^{KO} (this study).

GAL4 drivers: *arm*-GAL4 (ubiquitous) (Sanson et al., 1996); *eya*A3-GAL4 (cyst cells) (Leatherman and Dinardo, 2008); *PpY55A*-GAL4 (cyst cells) (Jung et al., 2007); T155-GAL4 (cyst cells) (Hrdlicka et al., 2002); *rp298*-GAL4 (*duf* gene) (Menon and Chia, 2001); and *Mef2*-GAL4 (myogenic mesoderm) (Ranganayakulu et al., 1996).

UAS-*Wsp* lines: UAS-*Wsp*, UAS-*Wsp*^{H242D} (Tal et al., 2002) and UAS-*Wsp-GFP* (Massarwa et al., 2007).

UAS-*D-WIP* lines: UAS-*D-WIP* (Massarwa et al., 2007), UAS-*D-WIP-GFP* and UAS-*D-WIP*^{testis}*-GFP* (this study).

Other UAS-based transgenes: UAS-RedStinger (nuclear marker) (Barolo et al., 2004); UAS-*Moesin*-GFP (microfilament marker) (Chihara et al., 2003; Dutta et al., 2002); and UAS-*Dicer2* (Dietzl et al., 2007).

UAS-dsRNA lines: *D-WIP* (KK102253), *Wsp* (KK108220), *Cdc42* (1253OR-2), *sns* (GD877), *Arp3* (GD35260) and *Sop2* (GD42172). GD and KK lines are from the VDRC (Vienna) collection and the *Cdc42* line is from NIG-FLY/DGRC (Kyoto).

Other lines: *don juan*-GFP (spermatid marker) (Santel et al., 1997) and *sns-GCN*-lacZ (*sns* reporter) (Zhuang et al., 2009).

Drosophila testis preparation, immunofluorescence and microscopy

Testes were derived from sexually mature (1- to 3-day-old) flies, which were further aged (where appropriate) for 3 days in isolation from females to allow sperm accumulation in the seminal vesicle. Testes (intact or ruptured to expose cysts) were dissected in TB (Arama et al., 2003) and fixed for 15 minutes at room temperature (RT) in 4% paraformaldehyde (PFA). A ~2-hour block at RT in blocking solution [phosphate-buffered saline (PBS) containing 0.1% Triton X-100 (PBT) plus 3% bovine serum albumin (BSA)] was followed by overnight incubation with primary antibodies, diluted in blocking solution, at 4°C in a humid chamber. Primary antibodies used were chicken anti-GFP (Aves; 1:500), guinea pig anti-D-WIP [(Eriksson et al., 2010) 1:500], rabbit anti-DsRed (Clontech; 1:500), rabbit anti-β-galactosidase (Cappel; 1:3000), mouse anti-polyglycylated tubulin [AXO 49 (Bre et al., 1996); 1:100] and mouse anti-Eya (Developmental Studies Hybridoma Bank, University of Iowa; 1:10). Following washes in PBT, testes were incubated for 1 hour with the appropriate fluorophore-conjugated secondary antibodies. Secondary antibodies (Jackson ImmunoResearch) were diluted 1:200 in blocking solution. Where used, phalloidin-TRITC (Sigma, 4 ng/μl) was added together with the secondary antibodies. Preparation and immunostaining of cryosectioned material were performed as described (Kaplan et al., 2010). All stained preparations were mounted in Vectashield/DAPI (Vector Laboratories). Images were acquired using Zeiss LSM 510 and LSM 710 confocal scanning systems.

Mouse genetics

N-WASp was disrupted in murine Sertoli cells by the following scheme. Mice bearing Dhh-Cre (Jaegle et al., 2003) were crossed to heterozygotes for the null allele *N-WASp*$^{-}$ (Snapper et al., 2001). Dhh-Cre/+; *N-WASp*$^{-}$/+ progeny (selected by tail PCR) were then crossed to mice homozygous for the conditional knockout allele *N-WASp*^{flox} (Cotta-de-Almeida et al., 2007), and Dhh-Cre/+; *N-WASp*$^{-/flox}$ males were identified among the progeny. Incorporation of the Scx-GFP marker (Pryce et al., 2007) was obtained by crossing mice bearing this transgene to Dhh-Cre/+; *N-WASp*$^{-}$/+, prior to the final cross to the *N-WASp*^{flox} homozygotes.

Mouse testis histochemistry and immunofluorescence

Hematoxylin and Eosin (H&E) histochemistry was performed on 10 μm paraffin sections, following fixation of dissected testes in Bouin's fixative. For immunofluorescence, dissected testes were commonly fixed in 4% PFA overnight at 4°C, washed with PBS and incubated in 30% sucrose overnight at 4°C, following which OCT blocks were prepared and cryosectioned (7 μm). Sections were permeabilized using 0.25% Triton X-100, 1% BSA in PBS (15 minutes at RT), blocked in 1% BSA and 5% goat serum in PBS (1 hour at RT) and incubated with chicken anti-GFP (Aves; 1:500) in a humid chamber overnight at 4°C. An alternative protocol used

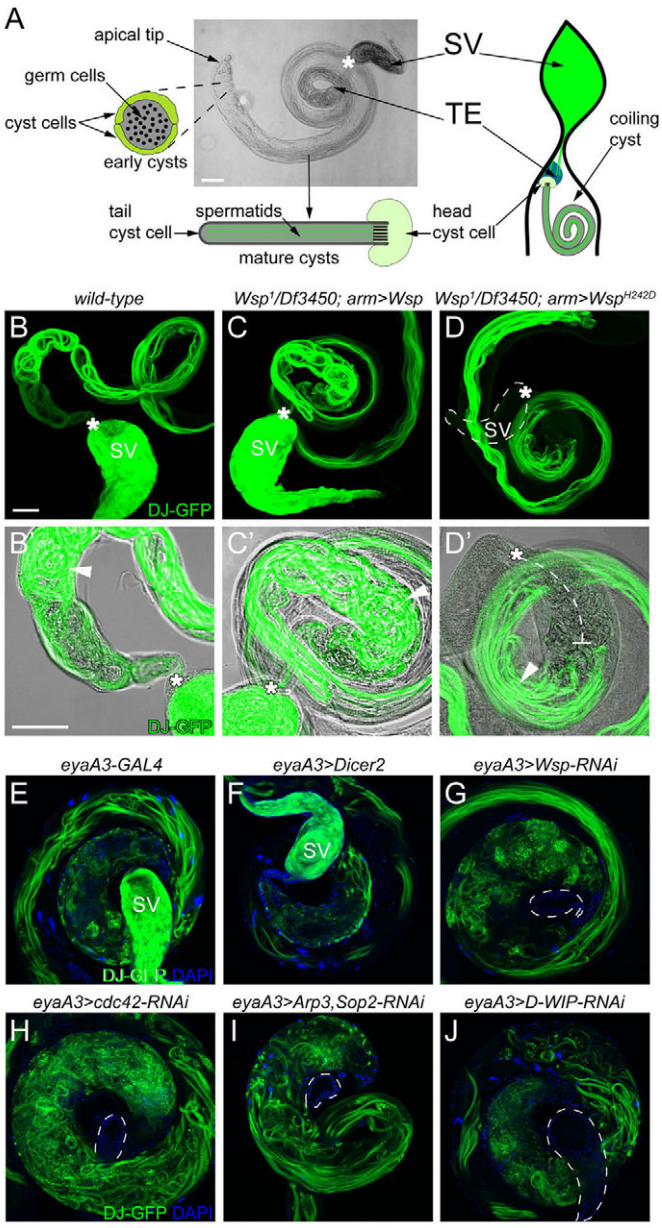

Fig. 1. Components of the Wsp-based actin polymerization machinery are somatically required for spermatid release. (**A**) Features of *Drosophila* spermatogenesis. Brightfield image of a *Drosophila* testis tube, flanked by schematics of an early meiotic-stage cyst (left), a mature elongated cyst (bottom) and of spermatid release and tissue organization at the base of the testis (right). SV, seminal vesicle; TE, terminal epithelium. Asterisks in all panels mark the interface between the basal tip of the testis and the SV. (**B-D**) Basal portion of testis tubes expressing the spermatid marker Dj-GFP (green). Both elongated and coiling cysts and an SV full of sperm are found in wild-type (B) and in Wsp^{1}/Df(3R)3450 flies rescued with *arm*-GAL4>UAS-*Wsp* (C), whereas the testis of a Wsp^{1}/Df(3R)3450 fly rescued with *arm*-GAL4>UAS-Wsp^{H242D} (D) contains elongated cysts but no coiling cysts are found and the SV (outlined) is empty of sperm. (**B′-D′**) Magnified views of the corresponding basal tip regions of the testes shown in B-D, on the background of a transmitted light image. The dashed line in D′ spans the basal area that is empty of cysts in the Wsp^{H242D}-rescued flies, and arrowheads indicate the highly coiled nature of basal cysts during normal spermatogenesis (B′,C′) and the lack of coiling that is characteristic of Wsp^{H242D}-rescued flies (D′). (**E-J**) Elongated but uncoiled cysts and empty SVs (outlined) are observed in testes of flies in which the cyst cell driver *eya*A3-GAL4 is used to express UAS-RNAi constructs directed against *Wsp* (G), *Cdc42* (H), the Arp2/3 subunits *Arp3* and *Sop2* (I) and *D-WIP* (J). Dj-GFP (green) and DAPI (blue) visualize spermatids and nuclei, respectively. A variable amount of cyst 'debris' is associated with the *eya*A3-GAL4 transgene, but coiling and spermatid release are unaffected (E). Incorporation of UAS-*Dicer2* for enhancement of RNAi efficiency was employed in all cases. Expression of *Dicer2* has no overt effect of its own (F). Scale bars: 100 μm in A; 50 μm in B,B′.

Bouin-fixed paraffin sections, which were deparaffinized, permeabilized and stained with anti-GFP as above, and then incubated at 37°C for 1 hour with undiluted rat IgM anti-Gcna1 (Enders and May, 1994). Following washes, all sections were incubated with secondary antibodies (1 hour at RT), washed and mounted in Vectashield.

RESULTS

Wsp activation through Cdc42 is essential for *Drosophila* spermatogenesis

Drosophila Wsp has been shown to contain a conserved and functional GTPase-binding domain that bears high affinity for the active (GTP-bound) form of Cdc42 (Ben-Yaacov et al., 2001). It was therefore surprising that previous analysis had failed to identify specific requirements for Cdc42 in the activation of Wsp within a developmental process. In these studies, Wsp^{H242D}, a construct encoding a Wsp variant that is incapable of binding GTP-Cdc42 (Tal et al., 2002), fully rescued the lethality of *Wsp* mutant flies, as well as the prominent neurogenic and myogenic *Wsp* phenotypes (Massarwa et al., 2007; Tal et al., 2002). Further analysis, however, revealed that *Wsp* mutant male flies, rescued using the Wsp^{H242D} construct, are completely sterile.

In order to assess possible spermatogenic defects in the Wsp^{H242D}-rescued flies, we made use of the spermatid marker Don Juan (Dj)-GFP (Santel et al., 1997). In wild-type testes (Fig. 1B), this marker visualizes the elongated cysts and the seminal vesicle, which is full of individualized, mature sperm. A similar arrangement of elongated cysts and a full seminal vesicle was observed when hemizygous Wsp^{1} mutant flies are rescued with an intact UAS-*Wsp* construct under the control of the ubiquitous *armadillo* (*arm*)-GAL4 driver (Fig. 1C). However, when Wsp^{1} flies were similarly rescued with the Wsp^{H242D} construct, the testis contained cysts that were full of elongated spermatids but no sperm was released into the seminal vesicle, which remained empty (Fig. 1D). Strikingly, the cysts of Wsp^{H242D}-rescued flies did not undergo coiling. Moreover, the extreme basal end of the testis tube, lined by the TE and leading up to the seminal vesicle, was free of cysts altogether (Fig. 1B′-D′).

We monitored several maturation markers in order to determine the differentiation stage of spermatids in Wsp^{H242D}-rescued flies. One marker is Dj-GFP itself, which is observed exclusively during late post-meiotic stages of spermatogenesis (Santel et al., 1997) and is robustly expressed by Wsp^{H242D}-rescued flies. We next followed sperm individualization, the process by which the 64 syncytial spermatids within each cyst are enclosed within distinct cell membranes. Dynamic cones of F-actin serve as established hallmarks of the individualization process (Noguchi and Miller, 2003). An examination of the individualization cones in the Wsp^{H242D}-rescued flies suggested that spermatid individualization in these mutants proceeds normally (see Fig. S1 in the

supplementary material). Finally, we stained testes from wild-type and Wsp^{H242D}-rescued flies with an antibody against polyglycylated tubulin, a post-translational modification that occurs at the onset of spermatid individualization (Arama et al., 2007; Bre et al., 1996), and obtained similar staining patterns in wild-type and mutant flies (see Fig. S1 in the supplementary material). Taken together, these data suggest that the *Wsp* mutant phenotype represents a late, post-individualization stage block in spermatogenesis, just prior to the onset of the cyst coiling process.

Wsp and key components of the Wsp-based actin polymerization machinery are required in the somatic cyst cells for spermatid release

As described above, cyst coiling and release of mature spermatids into the seminal vesicle are associated with a series of cellular events involving both the spermatids and the supporting somatic cyst cells, raising the question of the cell type in which *Wsp* is required. To address this issue we employed an RNA interference (RNAi) approach to disrupt Wsp activity specifically within the somatic cyst cells, using *eya*A3-GAL4 (Leatherman and Dinardo, 2008), a GAL4 driver based on the cyst cell factor *eyes absent* (*eya*) (Fabrizio et al., 2003), and a UAS-Wsp^{RNAi} construct. Knockdown of *Wsp* in this manner resulted in a male-sterile phenotype, characterized by spermatogenic arrest at the elongated cyst stage and empty seminal vesicles (Fig. 1G), similar to the phenotypes that we obtained following rescue of the Wsp^{1} mutant with the Wsp^{H242D} construct.

Identification of the somatic cyst cells as a site of *Wsp* activity now opened the door to evaluation of the requirement for Wsp-associated elements in mediating late-stage spermatogenesis and spermatid release. We first sought to complement the results obtained with the Wsp^{H242D} construct by directly demonstrating a requirement for Cdc42 in these processes. Indeed, cyst cell expression of RNAi directed against *Cdc42* led to the same phenotypes obtained with cyst cell *Wsp* RNAi and Wsp^{H242D} rescue, i.e. the testis contained mature cysts but the seminal vesicle was empty (Fig. 1H).

Wsp mediates actin filament nucleation through activation of the Arp2/3 complex. Knockdown of Arp2/3 complex activity was achieved using simultaneous *eya*A3-GAL4-based expression of RNAi constructs directed against *Sop2* and *Arp3* (*Arp66B* – FlyBase), which encode two subunits of the complex (Hudson and Cooley, 2002). Phenotypes similar to those obtained following *Wsp* and *Cdc42* disruption were again observed (Fig. 1I). Taken together, these observations suggest that Wsp activation of the Arp2/3 complex as mediated by GTP-bound Cdc42, which constitutes a fundamental molecular pathway for the generation of branched microfilament arrays (Carlier et al., 1999), is active in the somatic cells that enclose spermatogenic cysts and is essential for the final stages of cyst maturation and spermatid release.

D-WIP mutants display a late spermatogenic phenotype

Although WASp activation (in this case by GTP-Cdc42) and Arp2/3 stimulation are core features of the actin microfilament nucleation machinery, additional factors are commonly required to adapt this machinery to specific tissue and cell settings. The study of such factors is warranted, as they can shed light on the mechanistic properties and subcellular localization of the actin nucleation process. Although not capable of WASp activation, members of the conserved WASp-interacting protein (WIP) family have been shown to regulate WASp activity by various means, including stabilization and localization to sites of action (for a review, see Anton et al., 2007). We investigated whether D-WIP, the sole *Drosophila* WIP homolog, contributes to the final phases of spermatogenesis.

The primary developmental role of D-WIP is to promote embryonic myoblast fusion, which is achieved through its interaction with Wsp (Berger et al., 2008; Kim et al., 2007; Massarwa et al., 2007). This highly specific requirement allowed us to obtain viable flies by expressing a UAS-*D-WIP* construct (Massarwa et al., 2007) using the muscle-specific *Mef2*-GAL4 driver (Ranganayakulu et al., 1996) in a *D-WIP* null mutant background ($sltr^{S1946}$/$D\text{-}WIP^{D30}$). Such male flies, which are mutant for *D-WIP* in all non-muscle tissues, are completely sterile. These males showed a spermatogenic phenotype that was highly similar to that observed following rescue of the Wsp^{1} mutant with the Wsp^{H242D} construct (Fig. 2A,A′): the testis contained mature cysts that did not undergo appreciable coiling, cysts did not occupy the base of the testis tube, and the seminal vesicle was completely empty of released sperm.

A similar male-sterile phenotype was exhibited by flies homo- or hemizygous for $D\text{-}WIP^{KO}$, a hypomorphic and viable *D-WIP* allele (Fig. 2B,B′; see Materials and methods for details of $D\text{-}WIP^{KO}$ generation). Spermatids from both of these *D-WIP* mutant backgrounds expressed late-stage maturation markers, as observed for Wsp^{H242D}-rescued males (see Fig. S1 in the supplementary material). Furthermore, the characteristic late-stage arrest of spermatogenesis occurred following RNAi-mediated knockdown of *D-WIP* in cyst cells (Fig. 1J). Together, these results suggest that somatic regulation of sperm maturation constitutes a second cell-cell communication process, in addition to myoblast fusion, in which D-WIP and Wsp act in concert to mediate tissue differentiation during *Drosophila* development.

A novel testis-specific D-WIP isoform localizes to the head cyst cell

During embryonic myogenesis, D-WIP strongly localizes to sites of myoblast fusion (Berger et al., 2008; Kim et al., 2007; Massarwa et al., 2007), highlighting the cellular location where Wsp-mediated actin polymerization is active in this context. The essential involvement of D-WIP in late spermatogenesis is therefore significant, as it provides a potential tool for determining the location of Wsp pathway activity. We thus sought to localize D-WIP within developing spermatogenic cysts. This approach proved to be informative, as immunofluorescent detection using anti-D-WIP antisera revealed a specific accumulation of the protein within head cyst cells, in close proximity to the sperm nuclear bundle (Fig. 2C-C″). This localization pattern appears to be restricted to particularly mature cysts, which can be distinguished by the tight packing of the spermatid nuclei following individualization (Jayaramaiah Raja and Renkawitz-Pohl, 2005). D-WIP localization suggests, therefore, that the Wsp pathway is activated within the head cyst cell, a somatic support cell that physically associates with both mature spermatids and the TE at the basal end of the testis, near the time of spermatid release.

Since the available antibodies did not provide clear subcellular resolution, we sought to refine our observations of D-WIP localization using GFP-tagged forms. We generated transgenic flies harboring either UAS-*D-WIP-GFP*, a construct based on the major embryonic form of D-WIP (Kim et al., 2007), and UAS-$D\text{-}WIP^{testis}$-*GFP*, representing a novel, testis-specific variant that we had isolated (see Fig. S2 in the supplementary material). For expression of these constructs in the testis, we made use of the

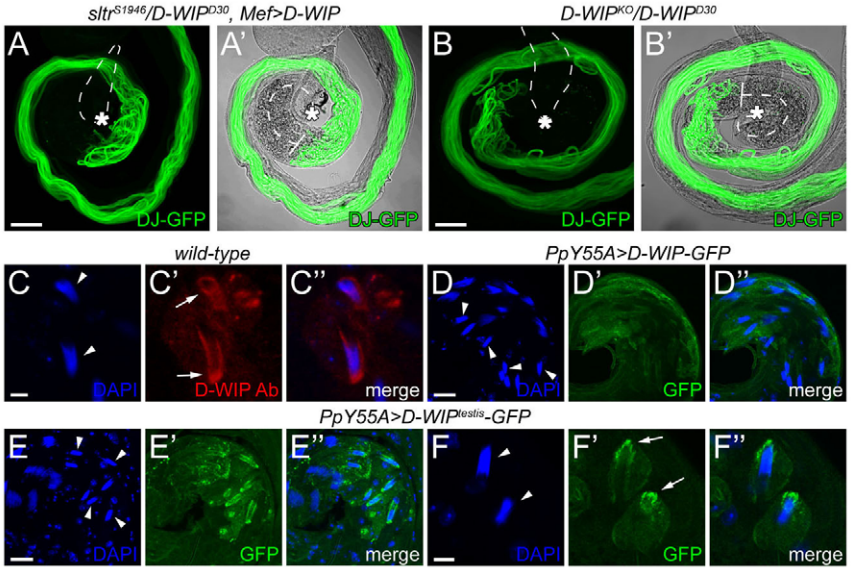

Fig. 2. D-WIP mediates spermatid release via a testis-specific isoform. (**A-B′**) Basal portion of testis tubes from *D-WIP* mutant *Drosophila* expressing the spermatid marker Dj-GFP (green). The elongated cysts in these testes do not coil and the SV is empty of sperm. Mutant genotypes shown are *sltr*^{S1946}/*D-WIP*^{D30}; *Mef2*-GAL4>UAS-*D-WIP* (A,A′) and *D-WIP*^{KO}/*D-WIP*^{D30} (B,B′). Asterisks in all panels mark the interface between the basal tip of the testis and the SV. Dashed lines outline the SV (A,B) or span the basal area that is empty of cysts (A′,B′). (**C-F″**) D-WIP localization patterns in mature cysts positioned at the testis basal end. In all cases, bundles of late-stage spermatid nuclei (arrowheads) are visualized with DAPI (blue). (C-C″) Testis of wild-type flies stained with an anti-D-WIP antibody (red). D-WIP is concentrated on the apical side of the nuclear array (arrows). (D-D″) Testis of flies bearing UAS-*D-WIP-GFP* (the major embryonic isoform) and the cyst cell-specific driver *PpY55A*-GAL4. D-WIP-GFP (green, anti-GFP) displays a diffuse GFP pattern. (E-F″) Testis of flies bearing UAS-*D-WIP*^{testis}*-GFP* and the cyst cell-specific driver *PpY55A*-GAL4. D-WIP^{testis}-GFP (green, anti-GFP) localizes adjacent to the nuclear bundle (arrows). Scale bars: 50 μm in A,B; 10 μm in C,F; 20 μm in D,E.

moderate cyst cell-specific driver *PpY55A*-GAL4 (Jung et al., 2007) to avoid possible overexpression artifacts and the cellular debris associated with the strong *eya*A3-GAL4 driver. Surprisingly, although the D-WIP-GFP fusion protein displayed a localization pattern that was highly similar to that of endogenous D-WIP when expressed in embryonic myoblasts (not shown), we did not detect any specific localization within late-stage cysts (Fig. 2D-D″). By contrast, D-WIP^{testis}-GFP displayed a prominent and specific localization within mature head cyst cells (Fig. 2E-E″), similar to the observations made using anti-D-WIP antibodies. Visualization of D-WIP^{testis}-GFP, which improved the subcellular resolution of the localization pattern, suggested that the protein localizes to the region of the head cyst cell in which the spermatid nuclei are embedded (Fig. 2F-F″). The localization pattern of D-WIP^{testis} suggests a discrete site within head cyst cells at which D-WIP and Wsp may interact to enable the final phases of spermatogenesis. We note, however, that attempts to rescue the D-WIP male-sterile phenotypes using D-WIP^{testis} and other isoforms have so far been unsuccessful, possibly owing to complexities related to alternative splicing of D-WIP transcripts or to inaccurate temporal expression patterns of rescuing constructs.

The cell adhesion molecule Sns is expressed in the head cyst cell and is necessary for spermatid release

The apparent involvement of both D-WIP and Wsp in sperm maturation raises the possibility that this process employs additional elements of the D-WIP/Wsp machinery that mediates myoblast fusion. Since cell-cell interactions are an essential aspect in both cases, we decided to focus on cell surface proteins as possible common mediating elements. Embryonic myoblast fusion is set in motion following heterotypic interactions between the transmembrane adhesion molecules Dumbfounded (Duf; also known as Kirre) and Sns (Abmayr et al., 2003). To assess whether these elements might be involved in late-stage spermatogenesis, we first used sensitive transcriptional reporters to attempt and detect expression in developing cysts. *duf* expression was monitored using *rp298*-GAL4, a GAL4 insertion in the *duf* locus (Menon and Chia, 2001), to drive UAS-RedStinger (Barolo et al., 2004), a bright nuclear RFP-based reporter. Although a strong signal was observed in the muscle sheath that wraps the testis, no indication of *duf* expression could be found in other cells of this tissue (Fig. 3A-A″). By contrast, a small population of cells located at the basal end of the testis was found to express *sns-GCN*-lacZ, a reporter in which an enhancer from the *sns* gene regulatory region is fused to β-galactosidase (Zhuang et al., 2009). These appeared to be mature head cyst cells, as they also expressed the nuclear cyst cell marker Eya and were closely associated with highly condensed spermatid nuclei (Fig. 3B-B‴).

The *sns* expression pattern corresponds well to the location and stage at which D-WIP and Wsp mediate sperm maturation. We therefore knocked down *sns* expression in the cyst cells, using *eya*A3-GAL4 and UAS-*sns*^{RNAi}, to assess the functional requirement for Sns. Strikingly, knockdown of *sns* in the cyst cells resulted in the same late-stage mutant phenotypes that we observed for the various components of the Wsp-based machinery (Fig. 3C). The expression and functional assays suggest that Sns contributes to D-WIP/Wsp regulation of spermatogenic cyst maturation, operating together with these elements within somatic head cyst cells.

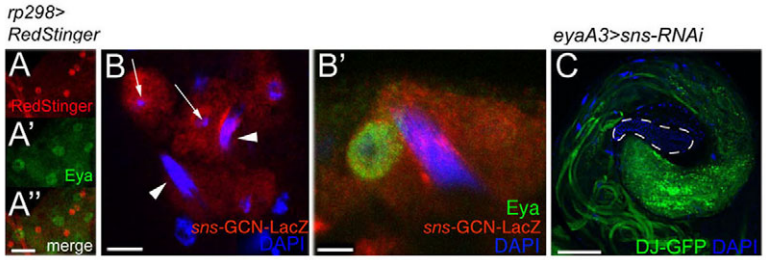

Fig. 3. Sns is expressed in the head cyst cell and is required for spermatid release. (**A-A″**) A portion of the basal region of the testis from a fly bearing the nuclear RFP marker UAS-RedStinger and *rp298*(*duf*)-GAL4. Duf-expressing nuclei (red, anti-DsRed) are restricted to the external muscle sheath and show no overlap with cyst cell nuclei (green, anti-Eya). (**B**) Portion of basal region from the testis of a fly bearing the cytoplasmic *sns* reporter *sns-GCN*-lacZ. Reporter expression (red, anti-β-galactosidase) is observed in head cyst cells. DAPI staining (blue) visualizes the adjacent arrays of needle-shaped spermatid nuclei (arrowheads) and the smaller, round cyst cell nuclei (arrows). (**B′**) Magnified view of a mature spermatid nuclear array embedded within a single (*sns-GCN*-lacZ-expressing) head cyst cell. Eya (green) is expressed in the head cyst cell nucleus. (**C**) Basal region of testis from a fly bearing Dj-GFP, *UAS-sns-RNAi* and the cyst cell driver *eya*A3-GAL4. Visualization of spermatids (anti-GFP, green) reveals elongated uncoiled cysts and the complete absence of spermatid transfer to the SV (outlined). Nuclei are visualized with DAPI (blue). Scale bars: 20 μm in A″; 10 μm in B; 5 μm in B′; 50 μm in C.

Wsp and D-WIP localize to a prominent head cyst cell actin structure at late stages of spermatogenesis

The phenotypes observed following disruption of the Wsp-based actin polymerization machinery during the final stages of *Drosophila* spermatogenesis imply a crucial role for microfilament arrays in mediating spermatid release. Consistent with previous reports (Desai et al., 2009) we observed a prominent F-actin structure (Fig. 4A), which, because of its late-stage appearance and positioning at the apical end of coiling cysts, adjacent to the spermatid nuclei, was likely to be involved in the final phases of sperm maturation within the testis. To identify the cell in which this structure is produced, we used the *PpY55A*-GAL4 cyst cell driver and UAS-*Moesin*-GFP, which encodes a GFP-tagged microfilament-binding protein (Chihara et al., 2003; Dutta et al., 2002). Moesin-GFP expressed in this fashion fully colocalized with the phalloidin-stained structure (Fig. 4B-B‴), demonstrating that the late-stage microfilament array is formed within the head cyst cell.

Inspection of isolated cysts and sectioned material was employed to examine structural features of the late-stage microfilament array. This approach revealed an apparent temporal progression, during which the array underwent a series of morphological transitions (Fig. 4C-E′). A dense microfilament ‘crown’ that resembled branched/dendritic microfilament networks in organization was observed to form, presumably within the head cyst cell, just beyond the tips of the spermatid nuclei, prior to their full condensation (Fig. 4C,C′). As the spermatid nuclei condensed, the microfilaments appeared to spread over and between them, eventually forming a closely intertwined structure (Fig. 4D,E′). Both lateral views (Fig. 4E,E′) and transverse sections (Fig. 4F,F′) of this structure suggest that the spermatid nuclei come to be encased within actin-lined projections that emanate from the head cyst cell.

Significantly, cyst cell-specific expression of D-WIP^{testis}-GFP and Wsp-GFP (Massarwa et al., 2007) via the *PpY55A*-GAL4 driver (Fig. 4G-H‴) demonstrated close spatial overlap of these proteins with the late-stage head cyst cell microfilament arrays.

D-WIP, *Wsp* and *Cdc42* are required for proper organization of late-stage head cyst cell microfilaments and spermatid nuclei

Prompted by these observations, we sought to assess the involvement of the Wsp-based actin polymerization machinery in the organization of head cyst cell microfilament structures at advanced stages of spermatogenesis (Fig. 5). Visualization of microfilaments and nuclei of late-stage cysts in mutant backgrounds in which *D-WIP* function was compromised revealed significant structural alterations (Fig. 5B-C″ and see Fig. S3 in the supplementary material). There was a consistent failure to achieve the intertwined and tightly packed organization of head cyst cell F-actin arrays and associated spermatid nuclei. The microfilament arrays appeared sparse and misshapen, and the normally tight and uniformly oriented bundles of nuclei were loosely packed and partially split. Similar phenotypes were also characteristic of late-stage cysts in *Wsp*^{H242D}-rescued flies (Fig. 5E-E″) and in flies in which RNAi targeting *Cdc42* was specifically expressed in the somatic cells of spermatogenic cysts (Fig. 5F-F″).

The abnormalities in microfilament organization are of particular significance because the genetic and protein localization data reported above imply that the functional defects associated with mutations in *Wsp*, *D-WIP* and *Cdc42* arise in the head cyst cell. In this context, we find it particularly noteworthy that the robust, transient network of head cyst cell microfilaments (Fig. 4C) was far less frequently observed in *Wsp* and *D-WIP* mutant testis preparations as compared with wild type [5% (n=59) versus 25% (n=40)]. Transverse sections through *D-WIP* (Fig. 5D-D″) and *Wsp* (not shown) mutant cysts demonstrated, however, that actin-based projections remained properly associated with individual spermatid nuclei at late stages of cyst maturation. Taken together, these observations suggest that the Wsp-based actin polymerization machinery profoundly influences tissue organization during the final stages of cyst maturation via the structuring of specific microfilament arrays within the head cyst cell.

N-WASp is required in the somatic Sertoli cells for murine spermatogenesis

Although the fly and mammalian testes differ substantially in overall structure and organization, spermatogenesis in these systems shares several common underlying principles. Sperm production in both cases is initiated from a stem cell population and proceeds within syncytial units, where the germ cells undergo meiosis, differentiation and individualization in tandem, prior to their release as distinct, mature spermatids. A second key feature is the intimate association of germline cells, throughout differentiation, with specialized somatic cell populations (cyst cells in *Drosophila* and Sertoli cells in mammals), which provide crucial

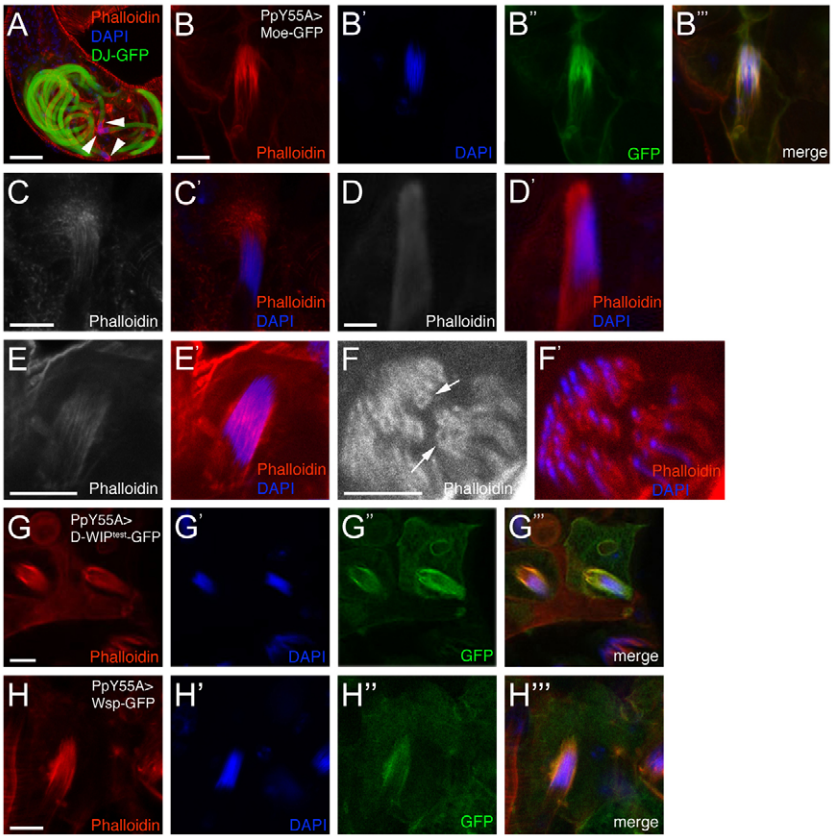

Fig. 4. Wsp and D-WIP localize to a head cyst cell actin structure that forms at late stages of *Drosophila* spermatogenesis. (**A**) Microfilament arrays (red, arrowheads) are closely associated with mature spermatid nuclei (blue) of coiling cysts (Dj-GFP, green) at the base of the testis. (**B-B‴**) Correspondence of the late-stage microfilament array (B) with the microfilament-binding protein Moesin-GFP (B″), when the latter is expressed via the cyst cell-specific driver *PpY55A*-GAL4, implying that the array forms within the head cyst cell. (**C-E′**) The head cyst cell array resolves from a dense apical network (C,C′) to projections overlapping the nuclei (D-E′). (**F**,**F′**) Sectioning of phalloidin-stained material reveals ring-like structures (arrows), suggesting that the microfilament array at this stage is composed of actin-lined projections that surround the spermatid nuclei. (**G-H‴**) Colocalization of the late-stage microfilament array with both D-WIP^{testis}-GFP (G-G‴) and Wsp-GFP (H-H‴) when they are expressed via the cyst cell-specific driver *PpY55A*-GAL4. Microfilaments are visualized with phalloidin (red or gray), nuclei by DAPI staining (blue) and GFP fusion proteins with anti-GFP (green). Scale bars: 25 μm in A; 10 μm in B,C,E,G,H; 5 μm in D,F.

guidance and support cues (Fuller, 1993; Griswold, 1998). Given the roles that we have identified for the *Drosophila* Wsp-based pathway in cyst cells, we sought to assess the somatic requirement for this conserved actin polymerization machinery during mammalian (murine) spermatogenesis.

Sertoli cells are the major somatic component of the seminiferous tubules, the site of spermatogenesis within the mammalian testis. Spermatogonial stem cells, which reside at the base of the Sertoli cell epithelium, give rise to syncytia of differentiating spermatids, which transverse the epithelium apically, and release individualized and mature spermatids to the tubule lumen (Fig. 6A-A″). The Sertoli cells communicate and maintain close contact with the spermatogenic lineage throughout this journey. To interfere with the branched actin polymerization pathway in these cells, we chose to disrupt N-WASp, the primary, ubiquitous mammalian WASp isoform (Kurisu and Takenawa, 2009), using a loxP-based conditional knockout (cKO) allele of murine *N-WASp* (*Wasl*) (Cotta-de-Almeida et al., 2007) and the Sertoli cell-specific driver desert hedgehog (*Dhh*)-Cre (Jaegle et al., 2003). We found that male mice in which *N-WASp* is disrupted in this fashion are viable and reach the age of sexual maturity, but are sterile.

We prepared and examined histological sections of seminiferous tubules to follow the progress of spermatogenesis in these mice as they mature (Fig. 6B,C,E,F,H,I,K,L). Tissue and cell type organization of the testis were properly established in young [post-natal day (P) 6] *Dhh*-Cre/*N-WASp*-cKO mice, with spermatogonial stem cells embedded near the basal portion of the circular Sertoli cell epithelium (Fig. 6B,C). At P20, however, the tubules of the mutant mice displayed morphological abnormalities, including an uneven distribution of spermatogonia and spermatocytes and the lack of an internal tubular lumen, which, by contrast, had begun to form in age-matched wild-type mice (Fig. 6E,F). Defective lumen formation and the associated failure to produce mature spermatids were strongly pronounced in older (P35 and P60) *Dhh*-Cre/*N-WASp*-cKO mice (Fig. 6H,I,K,L) and were coincident with a marked reduction in differentiating spermatogonia.

Close examination of seminiferous tubules from P35 testes, which normally contain germ cells at all stages of differentiation, suggested that spermatogenesis in *Dhh*-Cre/*N-WASp*-cKO testes does not progress beyond the final, round-spermatid stage of meiosis (Fig. 6N,O). In contrast to these germline defects, expression of the Sertoli-specific cytoplasmic marker scleraxis-GFP (Scx-GFP) (Pryce et al., 2007) did not reveal any overt morphological abnormalities or signs of degeneration in the Sertoli cell epithelium of either young or mature *Dhh*-Cre/*N-WASp*-cKO mice (Fig. 6D,D′,G,G′,J,J′,M,M′). Arrest of spermatogenesis in *Dhh*-Cre/*N-WASp*-cKO mice is therefore likely to be a consequence of specific defects in Sertoli cell communication with the differentiating spermatocytes, rather than a secondary result of abnormal Sertoli cell physiology.

DISCUSSION

Wsp and associated elements act in the *Drosophila* head cyst cell to mediate spermatid release

Several novel observations emerge from our study of the involvement of the branched actin nucleation machinery in *Drosophila* spermatogenesis. A central feature is the requirement for the NPF Wsp at advanced stages, just prior to the release of mature sperm from the testis into the reproductive tract. Our data suggest that Wsp is activated in this particular setting by GTP-bound Cdc42, and, in turn, stimulates the microfilament nucleating

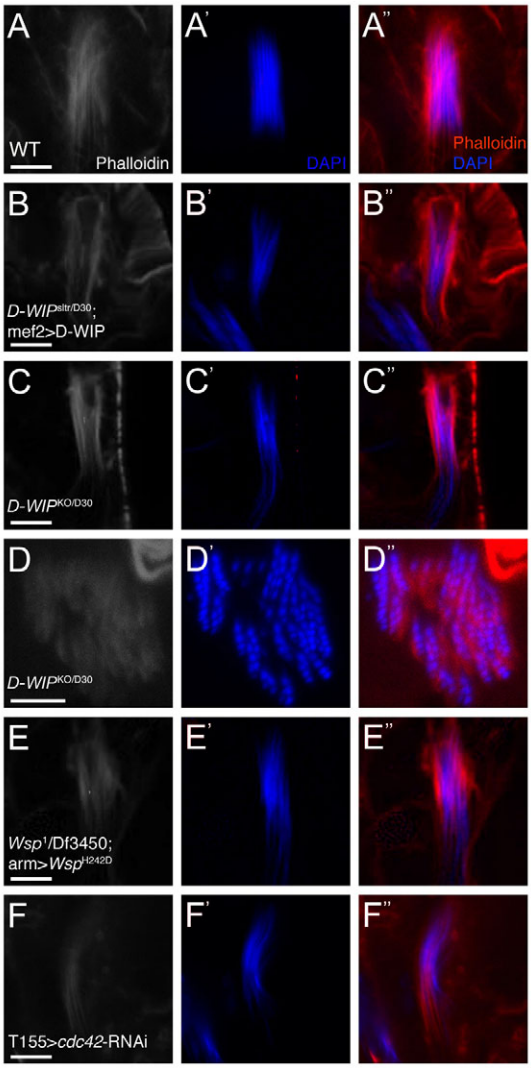

Fig. 5. *D-WIP*, *Wsp* and *Cdc42* are required at late stages for the proper organization of spermatid nuclei and the associated microfilament array. (**A-F″**) Organization of mature spermatid nuclei and associated microfilaments in testes from wild-type (A-A″), *D-WIP* mutant (B-D″) and Wsp^{H242D}-rescued mutant (E-E″) flies, as well as those in which an RNAi construct directed against *Cdc42* was expressed via the cyst cell driver T155-GAL4 (F-F″) (Hrdlicka et al., 2002). This driver was used to avoid obscuring 'debris' associated with *eya*A3-GAL4. D-D″ are from sectioned material, whereas all other images are of isolated cyst whole-mounts. Microfilaments are visualized with phalloidin (red or gray) and nuclei by DAPI staining (blue). Scale bars: 5 μm.

capacity of the Arp2/3 complex. Two additional contributing factors are the cell surface protein Sns and the adaptor D-WIP (acting via a testis-specific isoform), which were previously shown to interact with Wsp as mediators of embryonic myoblast fusion. Importantly, both the core actin nucleation machinery (Wsp and Arp2/3) and associated factors (Cdc42, Sns and D-WIP) are required in the somatic cyst cells for execution of the late spermatogenic process leading to spermatid release. The expression and localization patterns of Sns and D-WIP, respectively, further suggest that the coordinated activity of all elements takes place specifically within the head cyst cell, at a late stage of spermatogenesis. Correspondingly, D-WIP, Cdc42 and Wsp contribute to the proper organization of head cyst cell microfilaments, as evidenced by morphological abnormalities following functional disruption of these elements.

We suggest the following tissue-level scenario to account for these findings and link them to the mutant phenotypes (Fig. 7). Attachment of the head cyst cell to the TE present at the base of the testis is a crucial prerequisite for the release of motile sperm from the cyst into the seminal vesicle. Although the mechanism underlying the eventual 'escape' of sperm from the testis remains enigmatic, our data suggest that the morphology of the head cyst cell and the proper organization of its internal actin-based cytoskeleton influence the capacity of this cell to associate with the TE. We interpret the failure of mutant cysts to coil as a further indication of this defect in establishing cell-cell contacts: in the absence of a strong head cyst cell-TE association, coiling from the apical end of the cyst is likely to result in 'pulling away' at the basal end, accounting for the gap observed between the cysts and the base of the testis tube in all mutants examined.

As we report, prominent microfilament arrays form within the head cyst cell at stages leading up to spermatid release (see also Desai et al., 2009). The presence of these arrays at 'the right time and place' and the demonstrated requirement for Wsp machinery elements in their organization strongly suggest that they are key mediators of the crucial cellular associations that govern spermatid release. The identity of the actin structure involved is uncertain, as both a robust, albeit transient, cytoplasmic microfilament network as well as longer-lived actin-lined extensions are generated by the head cyst cell at late stages.

An additional open question is the nature of the molecular scenario underlying Wsp-Arp2/3 function in this particular context. Cdc42 is likely to play an activating role, whereas the basis for the contribution of Sns and D-WIP to this process remains to be elucidated. Models of myoblast fusion (Kim et al., 2007; Massarwa et al., 2007) have suggested that these elements might be part of a complex that determines the cellular site of Wsp-Arp2/3 activity. Although this scenario might apply in the current context as well, our analysis implies some clear distinctions. D-WIP does not appear to be used strictly for Wsp localization (see Fig. S3 in the supplementary material), suggesting a more complex role in mediating Wsp activity. Similarly, Sns would require an alternative binding partner to the myoblast surface molecule Duf, which does not appear to be expressed in spermatogenic cysts and adjacent cells; the Duf homolog Roughest (Bao et al., 2010) could potentially fulfill this role.

Regardless of the molecular details, a key issue is the means by which actin organization influences the relevant cell-cell interactions. One possibility is that head cyst cell microfilaments, possibly via tight association with the compact bundle of nuclei, dictate an overall morphology of the head cyst cell that impacts on its capacity to interact with other cells within the tissue. Alternatively, the well-established involvement of microfilaments in the generation and maintenance of cellular junctions (Hartsock and Nelson, 2008; Mege et al., 2006) might be at play, contributing to the adhesive capacity of the head cyst cell.

A somatic requirement for N-WASp during murine spermatogenesis

As described above, spermatogenesis is arrested in mice following specific disruption in Sertoli cells of *N-WASp*, which encodes the major mammalian WASp homolog. These findings generalize the significance of branched actin polymerization activity within

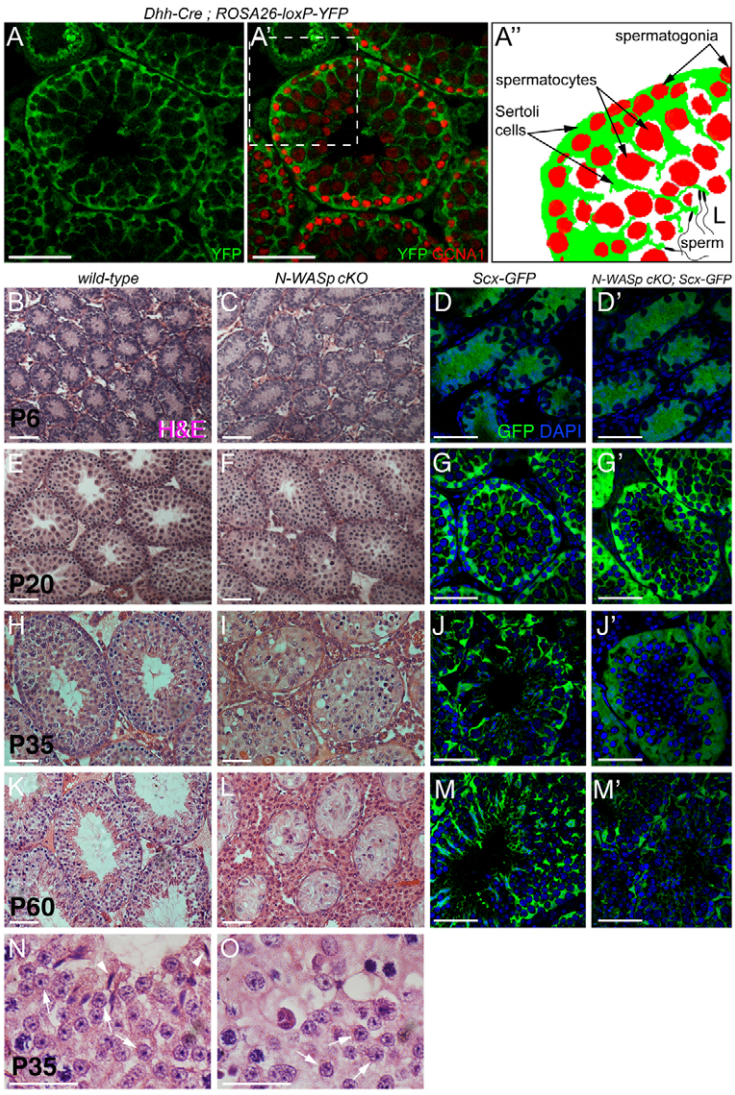

Fig. 6. N-WASp is required in somatic Sertoli cells for murine spermatogenesis. (**A-A″**) Structure and organization of murine seminiferous tubules. (A,A′) Sectioned tubules from a 20-day-old mouse bearing Dhh-Cre and ROSA26-loxP-YFP (Srinivas et al., 2001). Dhh-Cre-based recombination in the tubules is restricted to the somatic Sertoli cells, which then express YFP via the ubiquitous *ROSA26* promoter (anti-GFP, green), visualizing the circular epithelium of elongated Sertoli cells. All Sertoli cells are labeled, demonstrating the efficiency and specificity of Dhh-Cre. Nuclei of early-stage germ cells (spermatogonia and spermatocytes) are visualized using anti-Gcna1 (red). (A″) A schematic representation of the boxed region in A′, emphasizing the differentiating germ cell syncytia (red) and the supporting somatic Sertoli cells (green). The syncytia are derived from spermatogonial stem cells, which reside on the periphery of the seminiferous tubule, near the base of the Sertoli cell epithelium. Differentiation is accompanied by movement of the germ cell clusters through the epithelium, culminating in release of mature sperm into the tubule lumen (L). (**B**,**E**,**H**,**K**) Hematoxylin and Eosin (H&E)-stained histological sections of seminiferous tubules from wild-type (*N-WASp*^{flox}/*N-WASp*$^{-}$) mice at the indicated ages. Prominent features are the purple-stained germ cell nuclei and the expansion of the tubule lumen with age, as the tubules fill with mature sperm. (**C**,**F**,**I**,**L**) Similar preparations from age-matched *N-WASp*^{flox}/*N-WASp*$^{-}$ mice also bearing Dhh-Cre, leading to specific disruption of *N-WASp* in Sertoli cells. Tubule morphology and cellular organization appear normal in 6-day-old mutant mice (C), but tubules of older mutant animals fail to form a lumen and contain a diminishing germ cell population. (**D**,**D′**,**G**,**G′**,**J**,**J′**,**M**,**M′**) Seminiferous tubule preparations from age-matched wild-type (*N-WASp*^{flox}/*N-WASp*$^{-}$) and Dhh-Cre; *N-WASp*^{flox}/*N-WASp*$^{-}$ mice, which also bear Scx-GFP, marking the Sertoli cell cytoplasm. Visualization of Sertoli cells (GFP, green) and nuclei (DAPI, blue) shows an intact Sertoli cell epithelium at all ages examined. (**N**,**O**) Magnified views of the internal aspect of H&E-stained seminiferous tubules from 35-day-old wild-type (*N-WASp*^{flox}/*N-WASp*$^{-}$; N) and Dhh-Cre; *N-WASp*^{flox}/*N-WASp*$^{-}$ (O) mice. Meiotic stage spermatocytes and post-meiotic round spermatids (arrows) are found in both wild-type and mutant tubules, but mature spermatids (arrowheads) are present only in wild-type preparations. Scale bars: 50 μm in A-M′; 25 μm in N,O.

somatic support cells to the progress of spermatogenesis. Key issues that should now be addressed include determination of the N-WASp-dependent process through which Sertoli cells promote sperm differentiation, the nature of the molecular mechanism involved, and the degree to which parallels may be drawn between the *Drosophila* and mouse systems.

Microfilament arrays are associated with several unique Sertoli cell structures that mediate different stages of spermatocyte maturation (Vogl et al., 2008). Two of these, the apical ectoplasmic specialization (ES) and the blood-testis barrier (BTB), have recently been recognized as specific localization sites for Arp3, a subunit of the Arp2/3 complex, during key restructuring phases

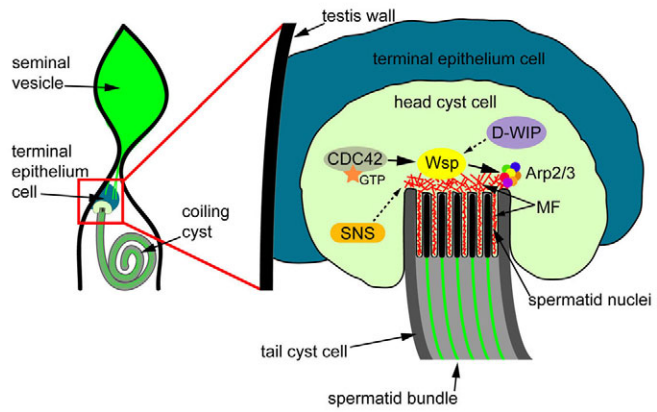

Fig. 7. Model for the involvement of the Wsp-based actin polymerization machinery in the final stages of spermatogenic cyst maturation. Overall tissue organization at the base of the *Drosophila* testis (left) and an enlargement of the cellular site where Wsp and related elements are active (right). Cdc42-GTP activation of Wsp stimulates the actin nucleation capacity of the Arp2/3 complex (solid arrows). Sns and D-WIP provide important contributions, although the molecular details of this are not fully established (dashed arrows). The concerted activity of the entire machinery governs the organization of microfilament (MF) arrays (red) within the head cyst cell. We propose that proper establishment of MF structures mediated by the Wsp-based machinery is a crucial aspect of head cyst cell morphology, influencing diverse aspects such as spermatid nuclear array organization and cyst association with the terminal epithelium prior to spermatid release.

(Lie et al., 2010). The apical ES is a Sertoli cell junctional structure to which spermatids are anchored during the mid and late phases of their maturation, whereas the BTB, which separates the basal and adluminal compartments of the seminiferous epithelium, is crossed by spermatocytes during meiosis (Yan et al., 2007). Arrest of spermatogenesis at the BTB is consistent with the *N-WASp* mutant phenotypes that we have described. Furthermore, such an arrest would mask a second, later requirement for apical ES construction, which might be more closely related to the role of Wsp during the late stages of *Drosophila* spermatogenic cyst differentiation. Interestingly, late-stage defects in spermatogenesis leading to partial male sterility have been reported for mice following targeted disruption of CR16 (Wipf3 – Mouse Genome Informatics), a member of the WIP protein family (Suetsugu et al., 2007), expanding the list of conserved elements of the WASp-based machinery that contribute to advanced features of sperm maturation.

In conclusion, our study describes an essential requirement for the WASp-Arp2/3 actin polymerization system during spermatogenesis in both flies and mice. The study highlights a fundamental role of actin nucleation within somatic support cells in orchestrating the intricate relationship between maturing sperm and the surrounding somatic support tissue. Future work should examine the specific cellular functions of WASp-Arp2/3 in each of the systems and explore the possibility of a conserved function, despite the morphological differences.

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Competing interests statement

The authors declare no competing financial interests.

Supplementary material

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