# *Wasp*, the *Drosophila* Wiskott-Aldrich Syndrome Gene Homologue, Is Required for Cell Fate Decisions Mediated by *Notch* Signaling

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***Abstract.*** Wiskott-Aldrich syndrome proteins, encoded by the Wiskott-Aldrich syndrome gene family, bridge signal transduction pathways and the microfilament-based cytoskeleton. Mutations in the *Drosophila* homologue, *Wasp* (*Wsp*), reveal an essential requirement for this gene in implementation of cell fate decisions during adult and embryonic sensory organ development. Phenotypic analysis of *Wsp* mutant animals demonstrates a bias towards neuronal differentiation, at the expense of other cell types, resulting from improper execution of the program of asymmetric cell divisions which underlie sensory organ development. Generation of two similar daughter cells after division of the sensory organ precursor cell constitutes a prominent defect in the *Wsp* sensory organ lineage. The asymmetric segregation of key elements such as Numb is unaffected during this division, despite the misassignment of cell fates. The requirement for *Wsp* extends to additional cell fate decisions in lineages of the embryonic central nervous system and mesoderm. The nature of the *Wsp* mutant phenotypes, coupled with genetic interaction studies, identifies an essential role for *Wsp* in lineage decisions mediated by the *Notch* signaling pathway.

Key words: cytoskeleton • *Drosophila* • peripheral nervous system • signal transduction • Wiskott-Aldrich syndrome

## *Introduction*

Reorganization of the cytoskeleton is regarded as a crucial intermediary step in translation of extracellular cues to cellular responses. Members of the Wiskott-Aldrich syndrome protein (WASP)[1] family have risen into recent prominence, as key elements that link signal transduction pathways and the actin-based cytoskeleton. The identification of distinct structural domains in WASP proteins, coupled with in vitro functional studies, has led to the emergence of a comprehensive model for the cell biological roles performed by these elements (Svitkina and Borisy, 1999a; Mullins, 2000). According to this model, WASP proteins serve as a common platform, bringing together components of signal transduction pathways, with cellular machinery that promotes actin polymerization and microfilament reorganization. Execution of this program in the proximity of the cell surface can then lead to formation of protrusive, actin-based membrane structures in response to various cues. Signaling molecules with which WASP proteins associate include the activated, GTP-bound form of the CDC42 GTPase (Aspenstrom et al., 1996; Kolluri et al., 1996; Symons et al., 1996), membrane phosphoinositides (Miki et al., 1996), and Src homology 3 (SH3) domain proteins, which function in tyrosine kinase–based signaling (Banin et al., 1996; She et al., 1997). The cytoskeletal elements involved (Machesky and Insall, 1998) are monomeric actin and the Arp2/3 complex, an evolutionarily conserved complex of seven proteins (Machesky et al., 1994; Welch et al., 1997) that acts as a potent nucleator of nascent microfilaments and can bring about the formation of extensive dendritic microfilament networks (Mullins et al., 1998; Svitkina and Borisy, 1999b).

Mammalian species possess at least two closely related WASP homologues. In humans these include the prototype WASP, first described as the affected protein in the Wiskott-Aldrich syndrome (WAS) blood disorder (Derry et al., 1994), and the more generally expressed neuronal WASP (N-WASP) (Miki et al., 1996). A variety of studies have suggested key cellular roles for members of the WASP protein family. In addition to repeated demonstrations and analysis of their ability to relay CDC42-based signaling to the actin cytoskeleton (Symons et al., 1996; Miki et al., 1998; Rohatgi et al., 1999), WASP proteins

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[1]*Abbreviations used in this paper*: Ac, Achaete; APF, after puparium formation; CNS, central nervous system; Ct, Cut; Eve, Even-Skipped; GBD, GTPase binding domain; Kr, Kruppel; *N*, *Notch*; NGS, normal goat serum; N-WASP, neuronal WASP; PC, pericardial; PNS, peripheral nervous system; Pon, Partner of Numb; SOP, sensory organ precursor; Su(H), Suppressor-of-Hairless; Sv, Shaven; WAS, Wiskott-Aldrich syndrome; WASP, WAS protein; *Wsp*, *Wasp*.

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have been shown to participate in the actin-based motility of both intracellular pathogens (Frischknecht et al., 1999; Yarar et al., 1999) and endogenous membrane vesicles (Rozelle et al., 2000; Taunton et al., 2000). Assessments of WASP protein function in vivo, on the basis of mutations in the structural genes, have been possible in several settings. WAS and X-linked thrombocytopenia arise in individuals bearing a wide spectrum of mutations in the gene encoding human WASP (Derry et al., 1995). These potentially debilitating diseases result from malfunctioning of hematopoietic cells, particularly platelets (Rosen et al., 1995; Kirchhausen, 1998; Ochs, 1998). A generally similar disorder has been described for a mouse knockout model of WAS (Snapper et al., 1998). Structural abnormalities of the cell surface and underlying cortical cytoskeleton are commonly considered as primary causes of the various manifestations of WAS (Remold-O'Donnell et al., 1996). Mutations in *bee1/las17p*, which encodes a WASP-related protein in yeast, result in disruption of cortical actin patch formation (Li, 1997), upholding an evolutionarily conserved role related to proper organization of the cortical cytoskeleton. However, despite the considerable experimental data which have accumulated regarding the cellular functions WASP proteins can provide, clear in vivo roles have yet to be determined.

We report here on the identification of *Wasp* (*Wsp*), a WAS gene homologue in the fruit fly, *Drosophila melanogaster*, and on the isolation and characterization of mutations in this gene. The *Drosophila* homologue bears all the major structural features of mammalian WASP, making it a good candidate for functional studies of this intriguing protein family, via a genetic approach. We show that *Wsp* function is required during various stages of *Drosophila* development, for proper differentiation of sensory organs and other tissues. In particular, our results indicate that the *Drosophila* WASP homologue plays an essential role in lineage decisions involving asymmetric cell divisions, mediated by the *Notch* (*N*) signaling pathway.

# Materials and Methods

## Drosophila Genetics

*Wsp* germline clones were produced by heat-shock in hs-FLP; FRT82B *ovo*$^D$/FRT82B *Wsp*$^3$ larvae. The resulting adult females were crossed to Df(3R)3450/TM6B, P{iab-2(1.7)lacZ} males, allowing for detection of a wild-type paternal contribution on the basis of β-galactosidase expression (see Lindsley and Zimm, 1992; or Flybase [available at http://flybase.bio.indiana.edu/] for details concerning all genetic loci and fly stocks described throughout). Recombination of *neu*-GAL4 (Bellaïche et al., 2001) onto a Df(3R)3450 chromosome and of Pon-GFP (Lu et al., 1999) onto a *Wsp*$^3$ chromosome were carried out to enable time-lapse analysis of *Wsp* mutant pupae. *numb* head clones were produced in progeny of a cross between flies of the genotypes *ey*-FLP; *numb*$^2$ FRT40A/CyO (kindly provided by J. Knoblich, Research Institute of Molecular Pathology, Vienna) and *ey*-FLP; l(2)cl-L3$^1$ FRT40A/CyO (Newsome et al., 2000).

## Molecular Genetics

All experiments involving conventional use and manipulation of nucleic acids, including cloning and blot hybridizations, were performed according to standard protocols (Sambrook et al., 1989). The 12-kb genomic EcoRI fragment encompassing the *Wsp* gene was isolated during a chromosomal walk using a random-sheared phage library (Maniatis et al., 1978). A plasmid subclone of this fragment was used to isolate *Wsp* cDNAs from various libraries. *Wsp* cDNA clones and the genomic region encompassing the *Wsp* gene were sequenced in their entirety. Detection of DNA lesions in the *Wsp* mutant alleles was achieved by resequencing of genomic DNA derived from flies hemizygous for each of the three alleles. PCR-amplified material, based on primers corresponding to various *Wsp* sequences, was either sequenced directly or after subcloning into the pGEM-T vector (Promega). Each reported lesion was observed in at least three independent experiments. DNA sequencing was performed by the Weizmann Institute of Science DNA Sequencing Unit. The *Wsp* genomic rescue construct was obtained after subcloning of the 12-kb genomic EcoRI fragment into a CasPeR transformation vector (Pirrotta, 1988). A full-length *Wsp* cDNA was subcloned into the pUAST transformation vector (Brand and Perrimon, 1993). Germline transformation with these constructs was obtained by standard methods (Spradling, 1986). Multiple transgenic lines were established and used separately in the phenotypic rescue experiments. Phenotypic rescue of hemizygous *Wsp* flies was obtained using first and second chromosome insertions of the genomic rescue construct, or by driving the UAS-*Wsp* construct with the ubiquitous drivers *armadillo*-GAL4 and T80-GAL4, or with the neuronal *Elav*-GAL4 driver.

## Blot Overlay Assay

The blot overlay assays were performed as described previously (Symons et al., 1996). A *Wsp* cDNA fragment corresponding to residues 96–526 of the Wsp protein was subcloned into a pRSET plasmid expression vector (Invitrogen). Histidine-tagged Wsp fusion protein, partially purified on a Nickel− agarose bead affinity column, was electrophoresed and blotted onto nitrocellulose filters. The filters were incubated with 3 μg each of purified recombinant mammalian GTPases (kindly provided by D. Helfman, Cold Spring Harbor Laboratory, NY), previously labeled with [$\gamma$-$^{32}$P]GTP. Detection of recombinant Wsp was achieved using anti-Wsp rabbit polyclonal antisera, generated against the fusion protein.

## Preparation, Staining, and Examination by Microscopy of Adult and Embryonic Tissues

Adult cuticles were prepared by warming to 50°C for 10 min in 10% NaOH, to aid in removal of soft tissue, and mounted in Hoyer's medium. Dissected pupal retinas were fixed in 4% formaldehyde/PBS for 15 min. Dissected pupal nota were processed as described previously (Gho et al., 1999). Embryos were dechorionated in 50% sodium hypochlorite, permeabilized and fixed by rapid agitation for 20 min on the interface of a formaldehyde/PBS/heptane solution, followed by chemical "popping-off" of the vitteline membrane by rapid shaking on a methanol–heptane interface, and rehydration into PBS. All fixed samples were commonly incubated at room temperature for 1.5 h in 2% normal goat serum (NGS; Sigma-Aldrich), diluted in PBT (PBS/0.1% Triton-100), then stained with a primary antibody diluted in NGS/PBT at 4°C for 16–24 h. After washes, samples were incubated for 2–3 h at room temperature in 1:300 dilutions in NGS/PBT of goat-derived secondary antibodies (Jackson ImmunoResearch Laboratories), conjugated to fluorescent or peroxidase tags, and directed against the appropriate species. Primary antibodies and dilutions used in this study include: anti–β-galactosidase (rabbit, 1:2,000; Cappel); anti-Shaven (Sv, rabbit, 1:20; Fu et al., 1998); anti-Elav (mouse, 1:10; Developmental Studies Hybridoma Bank); anti-Achaete (Ac, mouse, 1:1; Developmental Studies Hybridoma Bank); anti-Cut (Ct, mouse, 1:20; Developmental Studies Hybridoma Bank); mAb 22C10 (mouse, 1:5; Developmental Studies Hybridoma Bank); anti–Suppressor-of-Hairless (Su[H], rat, 1:1,000; Gho et al., 1996); anti–Couch Potato (Cpo, rabbit, 1:2,500; Bellen et al., 1992); anti-Prospero (mouse, 1:5); anti–Even-Skipped (Eve, rabbit, 1:500; Frasch et al., 1987); anti-Kruppel (Kr, rabbit, 1:500; Gaul et al., 1987); anti-Numb (rabbit, 1:2,000; Rhyu et al., 1994); and anti–α-tubulin (rat, 1:1,000; Serotec).

Transmitted-light images were obtained using a ZEISS Axioplan microscope. Fluorescent images were collected using a Leica DMR-XA microscope or a Bio-Rad Laboratories MRC-1024 confocal system, using an argon/krypton mixed gas laser, and mounted on a ZEISS Axiovert microscope. Images were prepared for publication using Adobe Photoshop®. For time-lapse analysis, living pupae were mounted as described previously (Gho et al., 1999) and observed using an oil immersion 40× N.A. 1.25 lens. Images were acquired every 30–60 s by a 12 bits Micromax CCD camera (Princeton Instruments), mounted on a Leica DMR-XA microscope, and controlled using the Metamorph software (Universal Imaging Corp.). Time-lapse movies were assembled in Metamorph and annotated in Photoshop®.

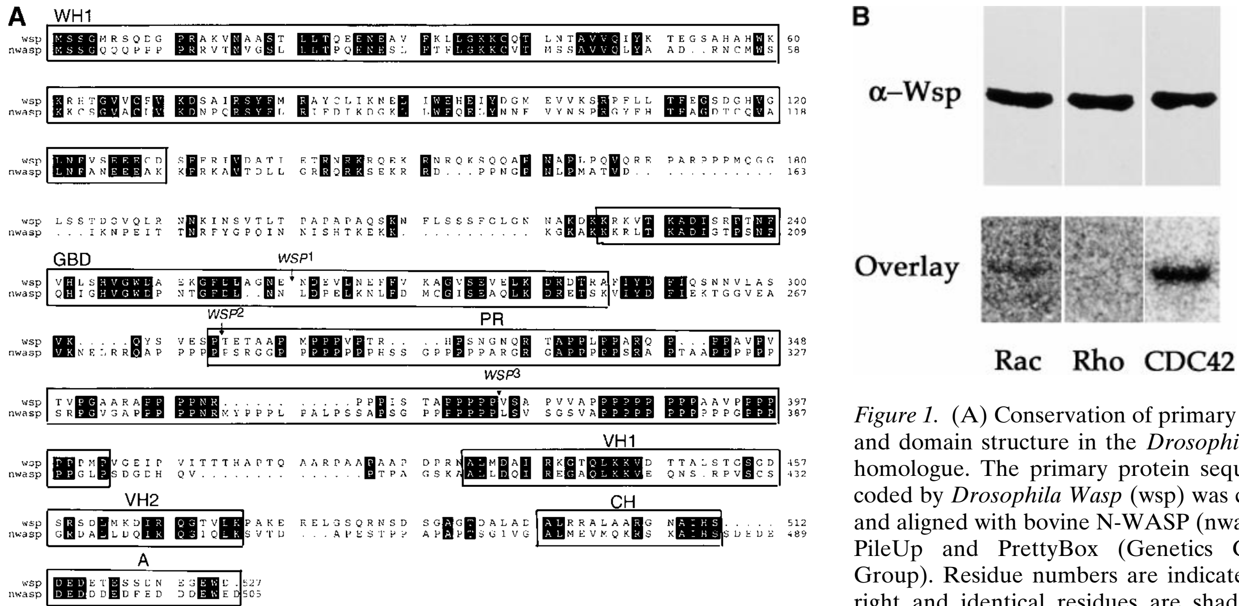



*Figure 1.* (A) Conservation of primary sequence and domain structure in the *Drosophila* WASP homologue. The primary protein sequence encoded by *Drosophila Wasp* (wsp) was compared and aligned with bovine N-WASP (nwasp) using PileUp and PrettyBox (Genetics Computer Group). Residue numbers are indicated on the right and identical residues are shaded black. The major structural and functional domains of WASP proteins are boxed. These include: the $NH_2$-terminal WH1 membrane-interacting domain; the CDC42 GTPase binding domain (GBD); the proline-rich SH3-binding domain (PR); monomeric actin-binding domains homologous to yeast verprolin (VH1 and VH2); and two COOH-terminal domains: a cofilin-homologous domain (CH) and an acidic tail (A) that are responsible for Arp2/3 complex binding. Domain structure generally follows Miki et al. (1996) and Symons et al. (1996). The GBD is defined as the minimal CDC42 high-affinity binding fragment (Rudolph et al., 1998). Arrows mark the positions of the frameshift mutations in the three *Wsp* alleles. (B) *Drosophila* Wsp binds the activated form of CDC42 in a blot overlay assay. A bacterially expressed recombinant fragment of Wsp (residues 96–526) was blotted onto nitrocellulose filters and incubated with [$\gamma$-$^{32}$P]GTP labeled recombinant mammalian GTPases (bottom). A strong interaction between Wsp and GTP-CDC42 and weak binding to GTP-Rac are observed. Binding to GTP-Rho could not be detected. This profile resembles that determined for mammalian WASP proteins (Aspenstrom et al., 1996; Kolluri et al., 1996; Symons et al., 1996). Reprobing of the filters with anti-Wsp antibodies was performed to ensure that equal amounts of the Wsp fragment were used in the assay (top).

## Results

### Identification of Mutant Alleles of Wsp, the Drosophila WAS Gene Homologue

We identified a WAS gene homologue within a chromosomal walk we performed in the region uncovered by the chromosomal deficiency Df(3R)3450, at cytogenetic division 98F of chromosome 3 of *Drosophila*. Comparison of genomic and cDNA sequences revealed that the transcription unit of this gene, which we have termed *Wsp*, is composed of seven exons, spread over ~6.5 kb. Conceptual translation of the single long open reading frame present in *Wsp* reveals that this gene encodes a 527-residue-long protein (Wsp), which is ~35% identical to mammalian WASPs (Fig. 1 A). Sequence similarity is particularly apparent within the recognized functional and structural domains of WASP proteins (Fig. 1 A). Indeed, we have found that Wsp binds both (GTP-bound) CDC42 (Fig. 1 B) and cytoskeletal elements (Tal, T., D. Vaizel-Ohayon, and E.D. Schejter, manuscript in preparation), implying conservation of biochemical function. We did not identify additional homologues in searches of the recently published sequence of the entire *Drosophila* genome (Adams et al., 2000), suggesting that *Wsp* is the sole bona fide WAS gene family homologue in *Drosophila*.

To identify mutant alleles of *Wsp*, we made use of a large collection of recessive lethal and female-sterile mutations which fail to complement Df(3R)3450 (Ahmed et al., 1998). Transgenic copies of an ~12-kb genomic fragment that includes *Wsp* were introduced into the background of hemizygous mutant flies from these lines. Three lethal mutant alleles, later shown to form a complementation group, were rescued to viability in this manner. Furthermore, the morphological phenotypes characteristic of *Wsp* mutant flies, described below, were fully ameliorated in the rescued flies. Phenotypic rescue of these alleles was also achieved after expression of a UAS-*Wsp* cDNA construct, under the control of various GAL4 drivers (Brand and Perrimon, 1993). Sequencing of PCR-amplified *Wsp* genomic DNA from hemizygous mutant animals revealed that all three alleles contain small (10–15 bp), distinct intragenic deletions, resulting in predicted frameshifts in the Wsp primary protein sequence (Fig. 1 A). In all three cases, the cytoskeleton-interacting COOH-terminal domain is lost, implying that protein function is severely compromised.

### Zygotic Mutations in Wsp Result in Cell Fate Transformations during Adult Sensory Organ Development

Hemizygous mutant *Wsp* flies from all three lines complete nearly all stages of imaginal development, and die as young adults. Most commonly, *Wsp* flies fail to fully eclose from the pupal case. Those that do can survive for a few days, but are lethargic and passive in their behavior. In general, *Wsp* flies do not display any gross morphological

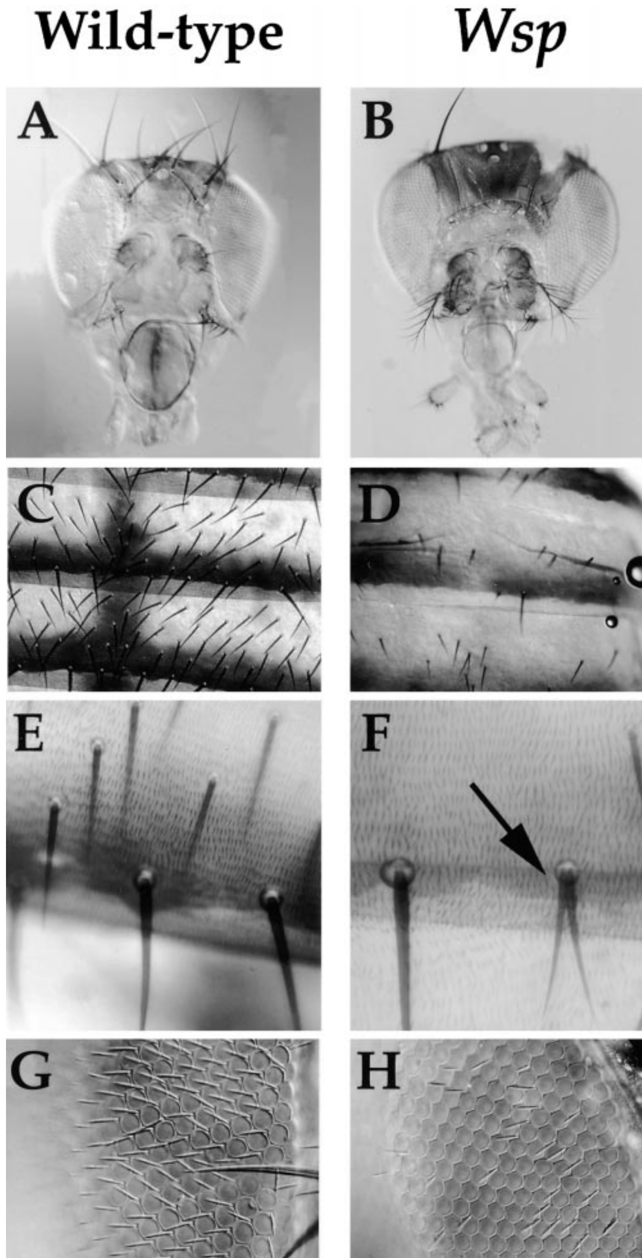
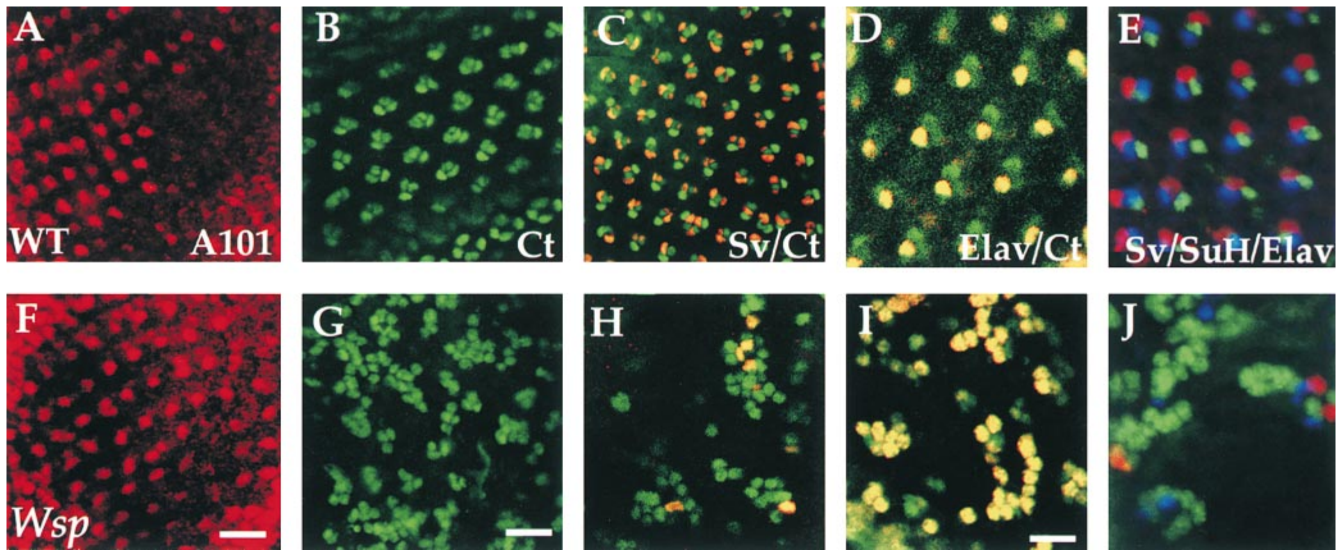



*Figure 2.* The bristle-loss phenotype of *Wsp* mutant flies. Panels show select portions of the external cuticle of adult wild-type (left) and *Wsp* (right) flies, which manifest large differences in bristle number. The mutant genotype in this and subsequent figures showing pupal and adult phenotypes is $Wsp^1$/Df(3R)3450. Comparative views of head capsules (A and B) and of the dorsal aspect of two abdominal segments (C and D) reveal cuticular regions that are nearly devoid of bristles in the mutant flies. Comparison of magnified portions of abdominal segments (E and F) demonstrates the loss of both bristle shafts and sockets (smooth cuticle phenotype), and the occasional appearance of duplicated bristles (arrow) in *Wsp* mutants. The strong interommatidial bristle-loss phenotype in the eye is readily apparent (G and H). Note that the mutation does not affect the ordered spatial pattern of the hexagonal eye facets, in keeping with the general normal morphology of tissues and organs of *Wsp* flies.

abnormalities. However, these flies exhibit a pronounced lack of neurosensory bristles, external manifestations of sensory organs stereotypically positioned just underneath the entire cuticle of the adult fly (Fig. 2). The bristle-loss phenotype is particularly apparent on the head capsule and abdomen (Fig. 2, A–D). Significant but less severe effects are observed on the legs and thorax of the mutant flies, where the smaller microchaete bristles are primarily affected (see below). The pattern of wing margin sensory bristles and wing blade nonsensory hairs is generally normal in *Wsp* mutant flies. Noticeable features of the *Wsp* phenotype, in addition to the marked reduction in bristle number, include loss of both the bristle shaft and bristle socket, occasional bristle duplications (Fig. 2, E and F), and a normal morphology of those bristles which do form in the mutant flies. These observations suggest impairments in sensory organ development, rather then defects in bristle formation per se, as a probable underlying cause for the *Wsp* phenotype. No major phenotypic distinctions were observed between flies hemizygous for the different alleles, or between hemizygous and transallelic combinations, suggesting that the phenotype described here approximates the full zygotic loss-of-function phenotype of *Wsp*.

The compound eye of the fly is composed of hundreds of individual facets (ommatidia), each of which is associated with a single cuticular sensory organ which forms during the first 2 d of development of the pupal retina and gives rise to a single bristle (Cagan and Ready, 1989). Loss of interommatidial bristles is a particularly penetrant and reproducible manifestation of the *Wsp* mutant phenotype (Fig. 2, G and H), therefore we chose to concentrate on sensory organ development in this tissue to follow the process in greater detail. Selection of single sensory organ precursor (SOP) cells from within a competent proneural cell cluster constitutes an initial step in development of *Drosophila* adult sensory organs (Ghysen and Dambly-Chaudiere, 1989; Campuzano and Modolell, 1992). We examined the SOP selection process at 3 h after puparium formation (APF) by staining dissected retinas for the A101 enhancer trap marker, which is expressed in SOPs immediately after their selection from within the proneural cluster (Huang et al., 1991; Blair et al., 1992). The A101 staining pattern of retinas derived from *Wsp* mutants fully resembles that of wild-type (Fig. 3, A and F), suggesting that events at the proneural stage are not affected by mutations in *Wsp* and that sensory organ development is properly initiated in the mutant animals.

Adult sensory organs are composed of clusters of four distinct cell types, which form after several rounds of asymmetric division from a single SOP (Hartenstein and Posakony, 1989; Gho et al., 1999; Reddy and Rodrigues, 1999) (Fig. 3 K). The SOP (also referred to as the pI cell) divides to produce the intermediary pIIa and pIIb cells. pIIa will give rise, upon division, to the bristle secreting trichogen and accompanying socket cell (tormogen), which form the externally visible portion of the sensory organ. Division of pIIb produces a third intermediary cell, pIIIb, which will divide again to generate the enervating neuron and supporting sheath cell (thecogen), both of which reside at a subepidermal level. The second product of the pIIb division is a glial cell, which moves away from the four-cell cluster as the sensory organ forms. Although the events surrounding precursor selection appear to proceed normally in *Wsp* mutants, a different picture emerges when mutant retinas are examined at 30 h APF, by which time the major stages of sensory organ development are completed. The transcription factor Cut (Ct) localizes to



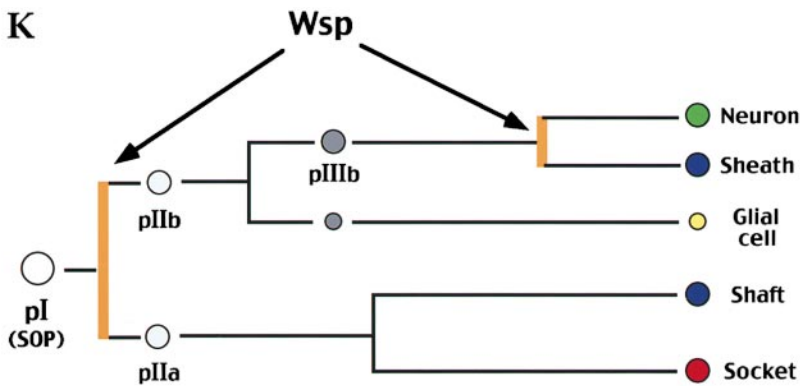




*Figure 3.* Abnormal differentiation pattern and spatial arrangement of sensory organ cells in the *Wsp* mutant retina. Confocal micrographs reveal the staining patterns of informative nuclear markers in sensory organ cells of developing wild-type (WT, A–E) and *Wsp* (F–J) pupal retinas. Most markers are also expressed by photoreceptor cell nuclei, which are at a slightly different focal plane and may appear at the edges and corners of the panels. The enhancer trap marker A101 drives β-galactosidase expression in SOP nuclei. A similar pattern of evenly spaced SOP nuclei is found in wild-type and mutant tissue at 3 h APF (A and F). In contrast, in mature sensory organs at 30 h APF (B and G) the wild-type four-cell formations (visualized with anti-Ct) give way to abnormal clusters in the mutant. Double labeling with Ct (green) and Sv (red), which stains sheath and bristle shaft cell nuclei, reveals minimal Sv staining in the mutant tissue (C and H), whereas double labeling with Ct (green) and Elav (yellow), which stains neuronal nuclei (D and I), demonstrates that nearly all sensory organ cells in the mutant retina express the neuronal marker. Triple labeling with the differentiation markers Sv (blue), Elav (green), and Su(H) (red), a socket cell–specific marker, underscores the preponderance of neurons and paucity of other cell types in mutant tissue (E and J). (K) A schematic representation of the cell division pattern within the adult sensory organ lineage (following Hartenstein and Posakony, 1989; Gho et al., 1999; Reddy and Rodrigues, 1999). Arrows point to the divisions (orange bars) where the mutant phenotypes indicate a requirement for *Wsp* function in specifying distinct cell fates, as discussed in the text. Bars: (F) 5 μm; (G) 9 μm; and (I) 10 μm.

all nuclei of external sensory organ cells (Blochlinger et al., 1993), including those that form in the pupal retina (Cadigan and Nusse, 1996). Sensory organ cells in *Wsp* mutant retinas properly express the Ct marker, but are abnormally distributed in large clusters, in contrast to the very regular four-cell formations seen in wild-type (Fig. 3, B and G). The availability of differentially expressed nuclear markers allows us to distinguish between the different cell types present in the developing sensory organ. To study the retinal differentiation pattern, we first used Shaven (Sv), a marker of both the sheath and bristle shaft cells (Fu et al., 1998). Double staining of retinas dissected 30-h APF revealed that Sv, which is normally expressed in half of the mature Ct-expressing sensory organ cells, is detected in only a small minority (<10%) of such cells in the mutant (Fig. 3, C, E, H, and J). A drastic reduction is also observed in the proportion of sensory organ cells that accumulate high levels of Suppressor-of-Hairless (Su[H]), a bristle socket cell marker (Gho et al., 1996) (Fig. 3, E and J). In contrast, the neuronal nuclear marker Elav (Robinow and White, 1991), which is normally restricted to the single neuron of each four-cell cluster, is found in the vast majority of mutant sensory organ cells (Fig. 3, D, E, I, and J). A similar phenotype of excess neurons and a near absence of bristle shaft, bristle socket, and sheath cells is observed during sensory organ development in the notum of *Wsp* mutant pupae as well (data not shown).

Taken together, these observations provide a basis for the bristle-loss phenotype of *Wsp* mutant flies. Although the program of sensory organ development is properly set in motion, execution of the sensory organ differentiation process is defective, leading to a predominance of neurons at the expense of nonneuronal cell types. Sensory organ phenotypes of this kind have been described for mutations in a variety of *Drosophila* genetic loci. In particular, elements of the signaling pathway involving the *N* receptor are thought to control cell fate decisions that assure proper differentiation of sensory organ cells into distinct cell types (for review see Posakony, 1994). The *Wsp* mutant phenotypes are consistent with a particular scenario of cell fate transformations during the asymmetric cell divisions which produce the mature sensory organ (Fig. 3

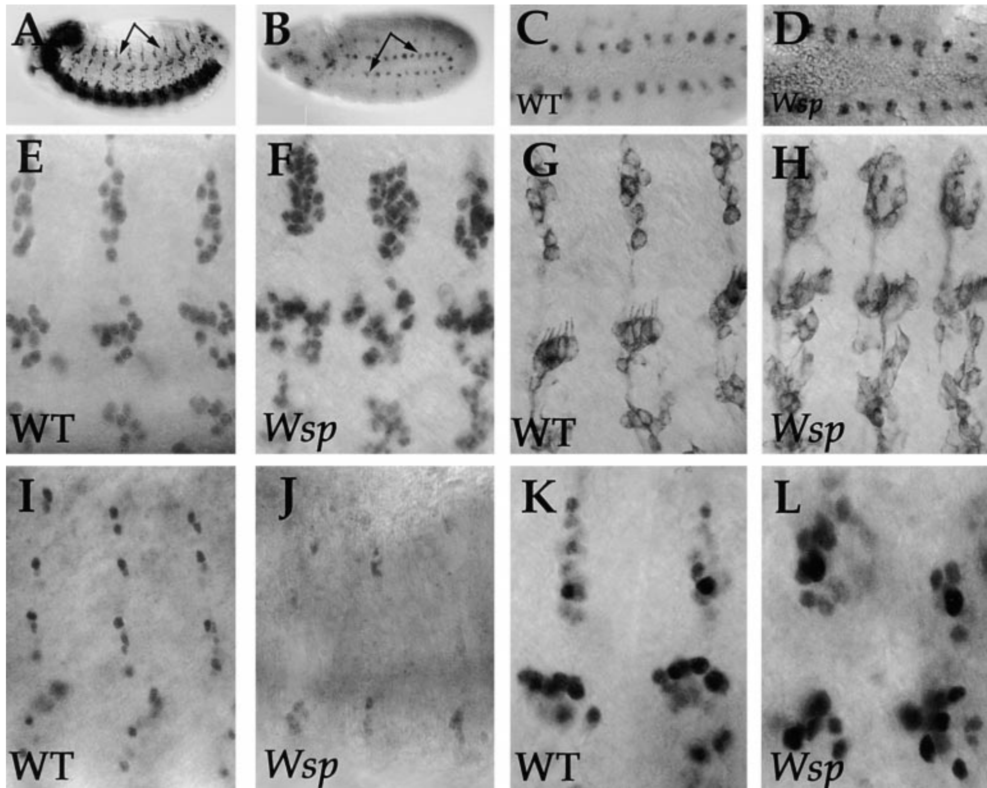



*Figure 4.* Excess of neurons and reduction of support cells in the PNS of $Wsp^{mat/zyg}$ embryos. Structure of the PNS of wild-type and $Wsp^{mat/zyg}$ embryos is revealed by staining with informative markers. In all panels the embryonic anterior is to the left and the dorsal aspect is up. Arrows in A point to the segmentally reiterated pattern of PNS sensory organs in a stage 14 wild-type embryo (WT) stained for Couch Potato (Cpo), which is expressed in all mature sensory organ cells (Bellen et al., 1992). The brain and ventral nerve cord are highlighted by this marker as well. Staining for the proneural marker Ac (B) shows the initial phases of SOP selection (arrows) in a stage 11, germband extended wild-type embryo. Magnified views of the Ac pattern in wild-type (C) and $Wsp^{mat/zyg}$ (D) embryos suggest that SOP selection proceeds normally in the mutant embryos. Excess numbers and ectopic positions of neurons in magnified portions of the mature (stage 15/16) $Wsp^{mat/zyg}$ PNS is revealed by staining with the neuronal nuclear marker Elav (E and F), and with the cytoplasmic/cell surface neuronal marker mAb 22C10 (G and H). A corresponding reduction in the number of $Wsp^{mat/zyg}$ PNS nonneuronal support cells is detected with the A1-2-29 (β-galactosidase–based) shaft/socket cell marker (I and J). Staining with the sheath cell marker Prospero reveals only a mild effect on sheath cell numbers in $Wsp^{mat/zyg}$ embryos (K and L).

K). Transformation of pIIa to a pIIb fate accounts for the absence of a bristle shaft/socket lineage, resulting in a smooth adult cuticle phenotype. Direct evidence for this cell fate change in *Wsp* mutant animals is provided below. In parallel, the apparent generation of two pIIb cells in each lineage, followed by a second, sheath-to-neuron transformation, constitutes a basis for the observed neuronal excess and vastly reduced numbers of sheath cells.

### *Wsp Is Required for Cell Fate Decisions during Sensory Organ Development in the Embryo*

The relatively late stage in development at which a zygotic *Wsp* mutant phenotype is observed raises the issue of whether *Wsp* function is essential only during metamorphosis and development of the adult fly. One possibility is that a maternal contribution of *Wsp* masks a requirement during embryogenesis. To address this matter, we employed the FLP-DFS technique (Chou and Perrimon, 1996) to produce $Wsp^-$ female germline clones, thereby eliminating any contribution of *Wsp* gene products from a maternal source. The fate of embryos derived from $Wsp^-$ germline clones is dependent on the genetic makeup of the paternal contribution. Embryos lacking both maternal and zygotic sources of *Wsp* (referred to herein as $Wsp^{mat/zyg}$ embryos) do not survive, indicating an essential requirement for *Wsp* during the course of embryogenesis. In contrast, eggs fertilized with $Wsp^+$ sperm develop normally, and give rise to viable and fertile adults, indicating that zygotic *Wsp* function can overcome the lack of a maternal contribution.

Cuticle preparations of $Wsp^{mat/zyg}$ embryos, which are completely devoid of *Wsp* function, are normal (not shown), implying that *Wsp* is not generally required for morphogenesis of the embryo. However, a more detailed examination reveals essential roles for *Wsp* in key cell fate decisions during *Drosophila* embryonic development. Based on the zygotic adult phenotype, we first chose to examine development of sensory organs in $Wsp^{mat/zyg}$ embryos. The sensory organs of the embryonic peripheral nervous system (PNS) form in the ectoderm during stages 10–13 of embryogenesis, in a segmentally reiterated pattern (Campos-Ortega and Hartenstein, 1985) (Fig. 4 A). Development of these structures, which are composed of single neurons and various nonneuronal support cells, follows the general guidelines of adult sensory organ development: selection of single SOPs from within a competent proneural cluster followed by a limited number of asymmetric divisions and *N*-dependent differentiation of distinct cell types (Bodmer et al., 1989; Guo et al., 1996).

The proneural marker Achaete (Ac) is transiently expressed in SOPs and their progeny during the initial stages of embryonic sensory organ determination (Ruiz-Gomez and Ghysen, 1993) (Fig. 4 B). The Ac staining pattern in $Wsp^{mat/zyg}$ embryos resembles that of wild-type (Fig. 4, C

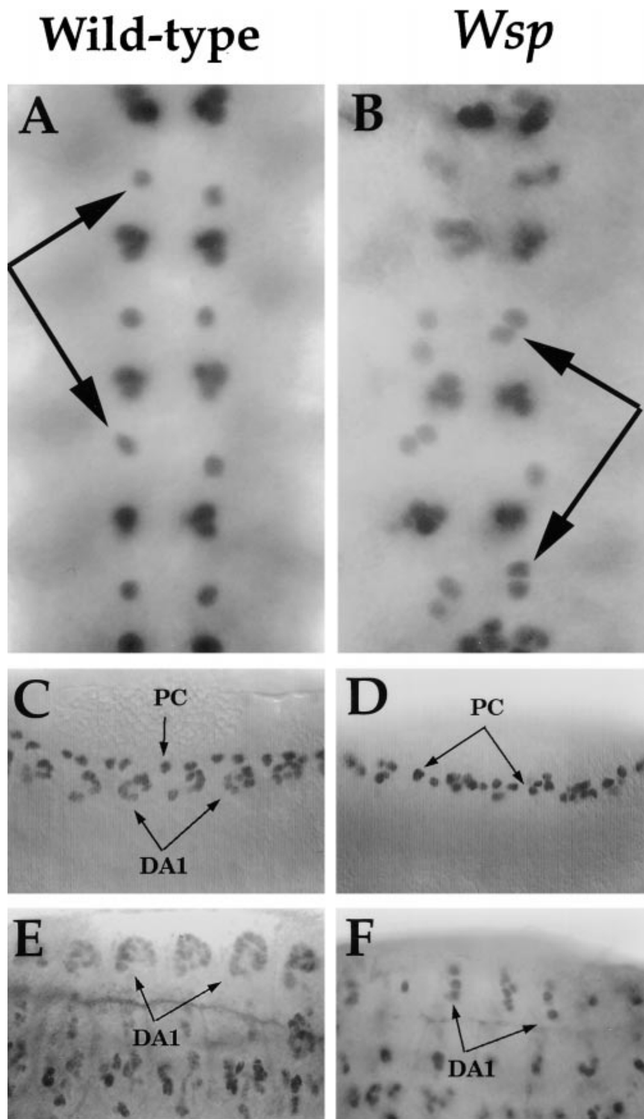



*Figure 5.* Cell fate transformations in the CNS and mesoderm of *Wsp*$^{mat/zyg}$ embryos. Panels show matched portions of the embryonic CNS (A and B) and mesoderm (C–F) of wild-type (left) and *Wsp*$^{mat/zyg}$ (right) embryos stained with informative markers. The pattern of stage 16 embryonic ventral nerve cord neuroblasts expressing the Eve marker appears in A and B. Anterior is up. Arrows point to the RP2 neuroblasts, which are duplicated in the mutant (B). In the dorsal mesoderm of stage 15 embryos, Eve expression in pericardial (PC) and muscle DA1 founders is observed in wild-type (C), whereas expression at the PC position predominates in the mutant (D). Correspondingly, the number of Kr-expressing DA1 cells in the wild-type dorsal mesoderm (E) is significantly reduced in *Wsp*$^{mat/zyg}$ embryos (F).

and D). Although this observation suggests that the early steps of sensory organ development proceed normally in the mutants, lack of *Wsp* function has a clearly deleterious effect on the subsequent maturation of embryonic sensory organs. When stained with anti-Elav or with mAb 22C10, which recognizes a neuronal membrane–associated antigen (Zipursky et al., 1984), later-stage *Wsp*$^{mat/zyg}$ embryos present an obvious excess of neurons (Fig. 4, E–H). Quantitative assessments by nuclear and cell counts suggest a near-doubling of neurons in the mutant embryos. Thus, for instance, as many as 25 neurons are commonly found in the combined l and d clusters of abdominal segments, which normally contain 14 neurons (Ghysen et al., 1986). As was observed in the developing adult retina, neuronal excess in the embryonic PNS comes at the expense of nonneuronal support cells. Far fewer cells express A1-2-29 (Fig. 4, I and J), a shaft and socket cell marker (Blochlinger et al., 1991; Hartenstein and Jan, 1992). Similar reductions are observed in the number of cells expressing Su(H), which specifically labels socket cells of external sensory organs (Gho et al., 1996; data not shown). These observations are readily explained by pIIa to pIIb cell fate transformations during embryonic sensory organ development, the suggested basis for the adult bristle-loss phenotype. However, not all nonneuronal cell types are affected to the same degree in *Wsp*$^{mat/zyg}$ mutants. Only mild reductions in staining of the sheath cell fate marker Prospero (Vaessin et al., 1991) are observed (Fig. 4, K and L), suggesting a lesser requirement for *Wsp* during the neuron/sheath cell fate decision in the embryonic PNS.

### *Wsp Participates in Additional, N-dependent Cell Fate Decisions during Embryogenesis*

We sought to determine whether a requirement for *Wsp* function existed in additional settings, in which execution of lineage and cell fate decisions had been shown to rely on the *N* pathway. We first examined this issue in an embryonic neuroblast lineage decision in the developing central nervous system (CNS). A pair of neurons designated RP2 develops in a specific position of each and every segment of the embryonic CNS (Thomas et al., 1984). The RP2 neurons are distinguishable from the RP2-sib pair, which derive from a common progenitor, by expression of markers such as the segmentation protein Even-Skipped (Eve) (Doe et al., 1988; Patel et al., 1989) (Fig. 5 A). Wild-type RP2 neurons express Eve in a persistent fashion, whereas RP2-sib neurons do so only transiently. Loss-of-function mutations in *N*, and in other genes that show *N*-like mutant phenotypes, result in a RP2-sib to RP2 fate transformation, so that in each segment all four neurons of this lineage express Eve (Buescher et al., 1998; Skeath and Doe, 1998). A similar duplication of persistent Eve-expressing neurons is characteristic of *Wsp*$^{mat/zyg}$ embryos (Fig. 5 B).

A second process we chose to study involves the *N*-dependent mesodermal lineage decision made between future pericardial (PC) and DA1 muscle founder cells, all of which derive from a common progenitor (Ruiz Gomez and Bate, 1997; Carmena et al., 1998; Park et al., 1998). In wild-type embryos, both cell types, which form in neighboring but distinct positions, express Eve (Fig. 5 C), but only the DA1 founders express the Kruppel (Kr) marker (Fig. 5 E). An apparent bias towards the PC cell fate in the mesoderm of *Wsp*$^{mat/zyg}$ embryos is observed after staining with these markers (Fig. 5, D and F). A marked reduction in the number of Eve- and Kr-expressing DA1 cells is coupled with an apparent increase in the number of Eve-expressing cells, present at the position normally occupied by PC cells. The mesodermal *Wsp* phenotype is exceptional, since it resembles *N* gain-of-function phenotypes observed in this tissue, adding a level of complexity to interpretations of *Wsp* function. In conclusion, the characterization of embryonic

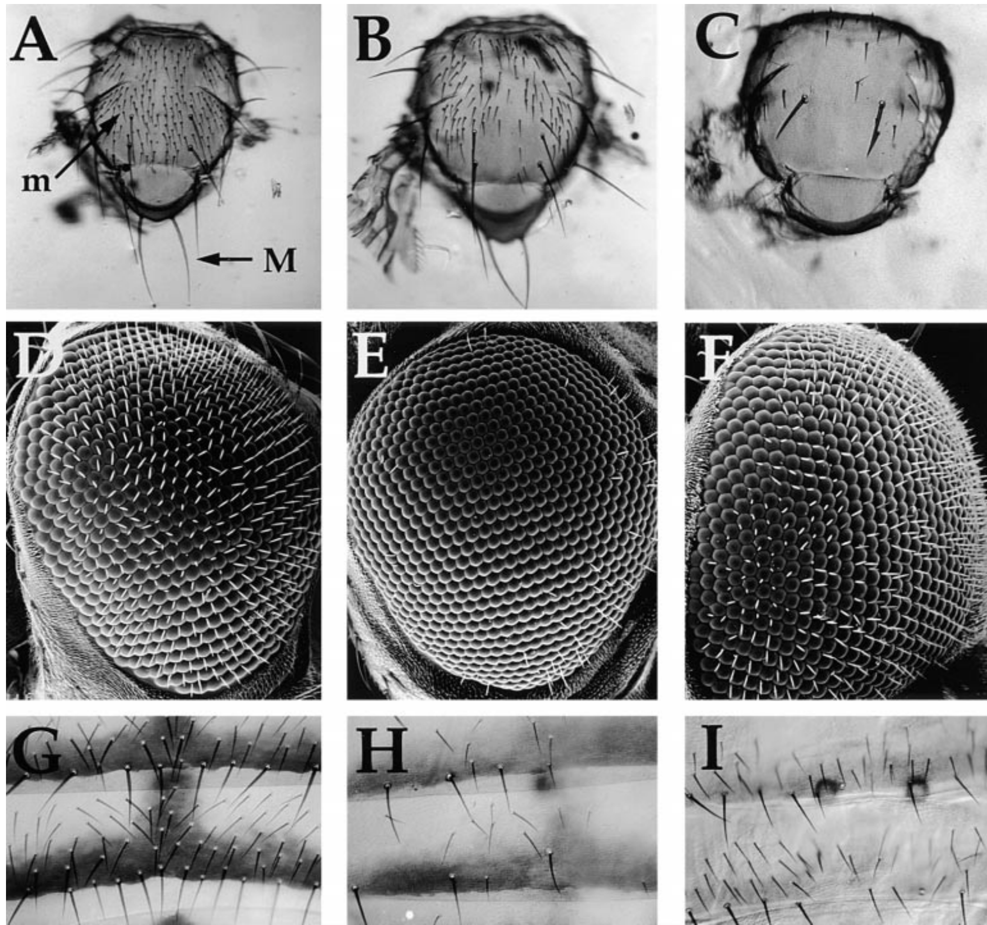



*Figure 6.* Enhancement and suppression of the *Wsp* bristle-loss phenotype by the *N* pathway. (A) Thorax of an $N^{ts1}$ fly raised at 25°C, showing a wild-type bristle pattern of both the larger macrochaetae (M) and the smaller and more numerous microchaetae (m). Reduction in microchaetae number on the thorax of a $Wsp^1$/Df(3R)3450 mutant fly (B) is readily apparent, whereas the macrochaete pattern is only mildly affected. The thoracic *Wsp* bristle-loss phenotype is strongly enhanced in an $N^{ts1}$; $Wsp^1$/Df(3R)3450 double mutant fly raised at 25°C (C). (D) Scanning electron microscope image of a wild-type eye. The eye of a $Wsp^1$/Df(3R)3450 mutant fly (E) is almost devoid of interommatidial bristles. Nearly full suppression of the *Wsp* phenotype is observed in a scanning electron microscope image of the eye of an $H^3$; $Wsp^1$/+; Df(3R)3450 fly (F). Significant restoration of the abdominal wild-type bristle pattern (G) is observed when comparing abdomens of a $Wsp^3$/Df(3R)3450 fly (H) to that of an $N^{int.hs}$; $Wsp^3$/Df(3R)3450 fly, raised at 29°C (I).

*Wsp* mutant phenotypes strongly implies an essential involvement of *Wsp* in various *N*-dependent lineage and cell fate decisions, throughout *Drosophila* development.

### *Genetic Interactions between Wsp and N Pathway Elements during Adult Sensory Organ Development*

The requirement for *Wsp* function in *N*-dependent cell fate decisions prompted us to search for genetic interactions between *Wsp* and *N* pathway elements, making use of the *Wsp* adult bristle-loss phenotype. Although the *N* pathway is involved in a wide variety of cell fate decisions during fly development, use of conditional mutant alleles has been successful in demonstrating that loss-of-function mutations in *N* itself and in its ligands result in PNS neuronal preponderance and associated phenotypes, in both embryos and adults, including the pIIa-to-pIIb and sheath-to-neuron transformations suggested for *Wsp* (Hartenstein and Posakony, 1990; Parks and Muskavitch, 1993; Guo et al., 1996; Zeng et al., 1998). We constructed a $Wsp^1$; *N* double mutant, using the temperature-sensitive $N^{ts1}$ allele (Shellenbarger and Mohler, 1978). At 25°C, $N^{ts1}$ flies display a wild-type morphology, including a normal array of neurosensory bristles (Fig. 6 A). Introducing this very mild *N* hypomorphic genotype into a *Wsp* mutant background results in a strong enhancement of the *Wsp* bristle-loss phenotype. Double mutant flies lack practically all bristles on regions of the cuticle such as the thorax, which is only partially affected by the *Wsp* mutation alone (Fig. 6, B and C).

In contrast to the enhancement achieved by reducing *N* function, significant suppression of the *Wsp* bristle-loss phenotype can be observed when activity of the *N* pathway is even moderately elevated. The neurosensory bristle pattern of *Wsp* mutant flies, which also lack one copy of the established *N* antagonist *Hairless* (*H*) (Bang and Posakony, 1992), is close to wild-type in appearance (Fig. 6, D–F). These flies eclose normally. Similarly, a significant, if somewhat less dramatic rescue of the *Wsp* phenotype is obtained using a gain-of-function allele of the *N* receptor itself. A transgenic construct ($N^{int.hs}$), in which the constitutively active, intracellular portion of *N* is expressed under the control of a heat-shock promoter (Struhl et al., 1993), was introduced into a *Wsp* mutant background. Mild (29°C) heat treatment of such flies, which has no noticeable effect on $N^{int.hs}$ flies on their own, leads to significant restoration of the bristle pattern, particularly in abdominal segments (Fig. 6, G–I). Sensitive genetic interactions can thus be demonstrated between *Wsp* and elements of the *N* pathway, raising the possibility of a common functional framework.

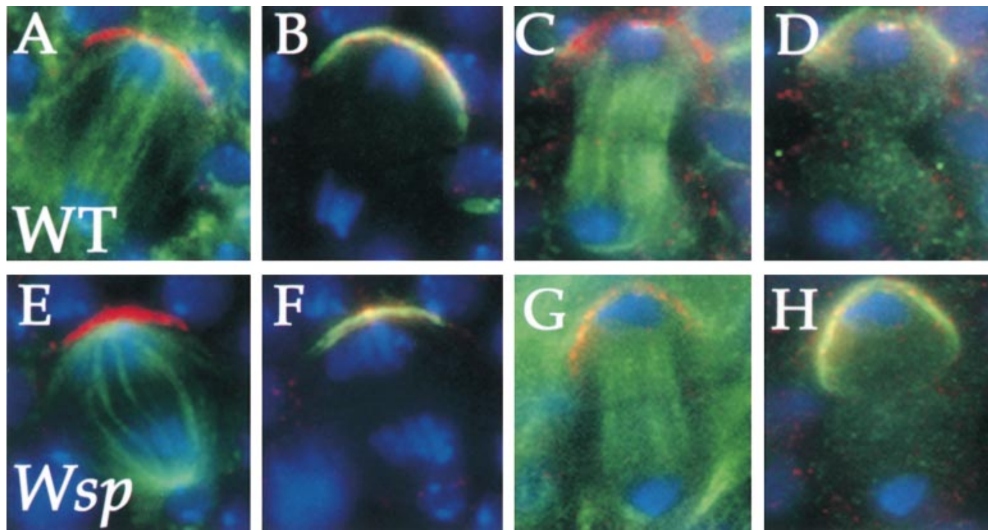



*Figure 7.* The unequal segregation of Numb and Pon-GFP is unaffected in *Wsp* mutant pI cells. Dissected nota from wild-type (WT, A–D) or *Wsp*[3]/Df(3R)3450 (E–H) mutant pupae were stained to reveal the localization of Numb (red throughout), Pon-GFP (green in B, D, F, and H), the orientation of the mitotic spindle (green in A, C, E, and G; visualized with antibodies to α-tubulin), and the condensation of the chromatin (blue throughout; visualized with DAPI). Anterior is up throughout. In both wild-type and mutant tissue, Pon-GFP colocalizes with endogenous Numb at the anterior cortex of pI from late prophase (not shown) to anaphase (B and F) and the two proteins segregate to the anterior pIIb cells at telophase (D and H). The mitotic spindles of wild-type (A and C) and *Wsp* mutant pupae (E and G) are similarly positioned within the plane of the epithelium and along the antero-posterior axis of the fly, and are aligned with the Numb/Pon-GFP crescent, enabling the strictly unequal segregation of Numb and Pon-GFP into the pIIb cell.

### *Wasp Is Not Required for the Asymmetric Distribution of Numb and Pon*

The established cellular roles of mammalian WASP proteins prompted us to consider instances of cytoskeletal involvement in *N*-based signaling, to try and reveal the mechanistic basis of *Wsp* function during *Drosophila* development. *numb* is considered a key regulator of sensory organ development, acting as an antagonist of *N* signaling in this tissue (Frise et al., 1996; Guo et al., 1996). During all cell divisions in the sensory organ lineage, Numb protein segregates into only one of the two progeny cells, thereby ensuring that the lateral inhibition mediated by N signaling is unidirectional, and providing a basis for assumption of distinct cell fates. Significantly, asymmetric distribution of Numb and other elements requires an intact microfilament-based cytoskeleton (Broadus and Doe, 1997; Knoblich et al., 1997; Lu et al., 1999), suggesting a possible site of action for Wsp and associated factors. We first addressed this possibility by determining and comparing the distribution and segregation of both Numb and the associated Partner of Numb (Pon) protein during division of the pI (SOP) cell in wild-type and mutant tissue (Fig. 7, A–H). In this study we used antibodies to follow endogenous Numb (Rhyu et al., 1994) and an ectopically expressed Pon-GFP chimera, previously shown to mimic the asymmetric distribution of the endogenous Pon protein during pI divisions (Lu et al., 1999; see below). During metaphase and anaphase of the wild-type pI division, which is aligned along the anterior–posterior axis of the fly (Gho and Schweisguth, 1998), Numb and Pon colocalize and form a crescent at the anterior cortex of the cell, directly above one of the poles of the mitotic spindle (Knoblich et al., 1995; Lu et al., 1998; Bellaïche et al., 2001) (Fig. 7, A and B). This asymmetric distribution ensures that the proteins segregate only to the anterior pIIb cell at telophase (Fig. 7, C and D). All aspects of the process are properly executed in *Wsp* mutant animals, including alignment of the spindle with the body axis, colocalization of Numb and Pon to an anterior crescent (Fig. 7, E and F), and strictly unequal segregation of Numb and Pon into the anterior pIIb cell (Fig. 7, G and H).

To further demonstrate that the cell fate transformations observed in *Wsp* mutant animals cannot be attributed to improper segregation and partitioning of Numb and Pon, we chose to follow both Pon distribution and the fate of the two cells derived from the asymmetric division of pI in living pupae. A sensory organ–specific GAL4 driver, *neu*-GAL4 (Bellaïche et al., 2001), was used to express a Pon-GFP chimeric protein (Lu et al., 1999) in pupal sensory organs, and time-lapse recordings of developing thoracic microchaete were carried out on both wild-type and *Wsp* mutant animals expressing this construct (Fig. 8). As shown above in fixed tissue, the pI cell of wild-type pupae divides within the plane of the epithelium to generate the anterior pIIb and the posterior pIIa cells, which are aligned along the fly's antero-posterior axis (Fig. 8, A–C). Pon-GFP forms a crescent at the anterior pole of pI, as reported previously (Bellaïche et al*.*, 2001), and subsequently segregates asymmetrically into pIIb. The pIIb cell divides perpendicularly to the plane of the epithelium to generate a small glial cell and the pIIIb cell (Gho et al., 1999). During this division, Pon-GFP forms a basal crescent and is asymmetrically distributed into the basal glial cell (Fig. 8, D and E). Finally, pIIa divides within the plane of the epithelium to generate two cells of equal size, the future bristle shaft and bristle socket cells (Fig. 8, F–J). In pIIa, as in pI, Pon-GFP forms an anterior crescent and segregates unequally into the anterior shaft cell.

In *Wsp* mutants, all aspects of the pI division match those seen in wild-type animals. The division generates an anterior–posterior pair of daughter cells, as Pon-GFP forms an anterior crescent within pI and segregates asymmetrically into the anterior cell (Fig. 8, K–M). However, from this stage on the events of sensory organ differentia-

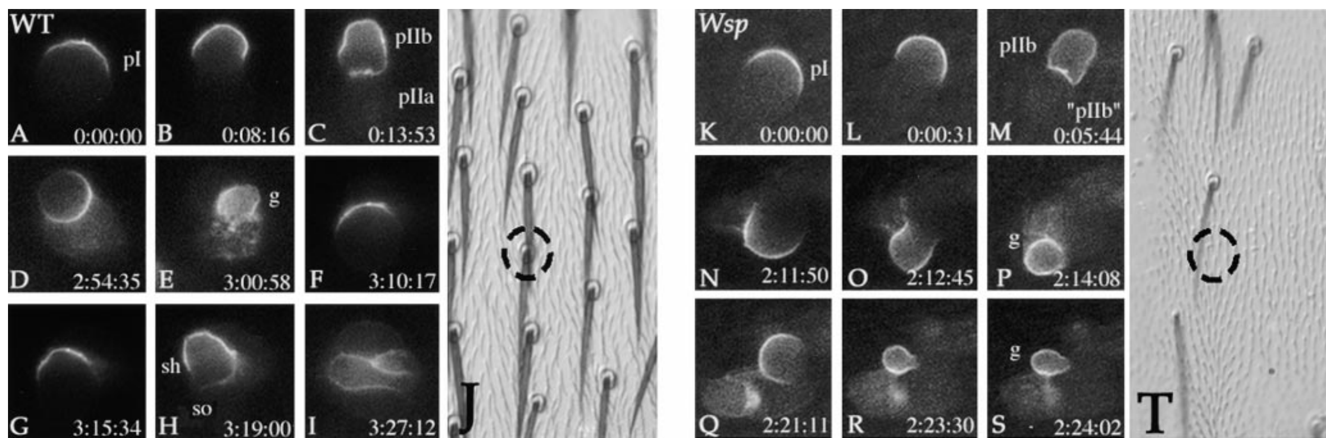



*Figure 8.* Dynamics of asymmetric cell division within the microchaete lineage, as revealed by the distribution of Pon-GFP. Time-lapse analysis of the first three divisions of this lineage are shown in living wild-type pupae (n*eu*-GAL4/UAS-Pon-GFP [A–I]) and *Wsp* mutant pupae (*neu*-GAL4; Df(3R)3450/UAS Pon-GFP; *Wsp*$^3$ [K–S]). Details are described in the text. Anterior is up throughout. Cell types indicated include pI (SOP); pIIa and pIIb, the progeny of the pI division; g, the glial cell progeny derived from the division of pIIb; sh (bristle shaft), and so (socket), progeny of the pIIa division (see also the legend to Fig. 3 K for description of this lineage). The positions of the sensory organs that were followed by time-lapse microscopy are denoted by dashed circles on images of cuticular preparations of the eclosed wild-type (J) and *Wsp* mutant (T) adults. The socket and shaft cells have differentiated normally in the wild-type fly (J), whereas both socket and shaft are missing at the recorded position in the mutant (T).

tion in *Wsp* differ substantially from those observed in wild-type. The first indication of an altered developmental progression is a randomization of the cell division pattern. In contrast to the strictly ordered sequence of divisions observed in the wild-type lineage, in which the anterior pIIb cell (which inherits Pon-GFP) always divides before the posterior pIIa cell, either of the two pI daughter cells in the mutant may divide after pI. In the time-lapse analysis presented here, the posterior ("pIIb") cell divides first (Fig. 8, N–P). A second, striking distinction from wild-type is that the divisions of both pI daughter cells are morphologically identical, and resemble the pattern seen in wild-type pIIb (Fig. 8, N–S). Both the anterior and posterior cell divisions are nonplanar, and generate two daughter cells of different sizes. In both "pIIb" cells, Pon-GFP forms a basal crescent and segregates into the small basal cell. These observations conclusively demonstrate that the two progeny of the pI division in *Wsp* mutant animals assume a similar, pIIb-like fate, but that this cell fate transformation cannot be attributed to improper partitioning and segregation of Numb and Pon.

### *Wasp Is Epistatic to Numb*

*Wsp* mutant phenotypes generally resemble those described for positive mediators of *N* signaling, whereas mutations in the *N* antagonist *numb* are distinct and opposite in character. Thus, adult sensory organ development in the absence of *numb* function leads to formation of multiple sockets, since both progeny of the pI division in this case assume a pIIa fate, and the subsequent division is characterized by shaft-to-socket transformations (Uemura et al., 1989; Rhyu et al., 1994). The opposite effects on cell fate provided us with an opportunity to determine an epistatic relationship between *Wsp* and *numb*. We examined this issue by producing clones of *numb* cells in a *Wsp* mutant background (Fig. 9, A–D). A powerful system for producing mutant clones in derivatives of the eye imaginal disc, which include the cuticle of the adult head capsule, has been recently described (Newsome et al., 2000). This system has been successfully adapted for the study of *numb* and other regulators of sensory organ formation (Török, T., D. Berdnik, and J. Knoblich, personal communication). Using this adaptation, we were able to consistently produce large *numb* head clones, in which the multiple socket phenotype characteristic of *numb* was observed throughout the head cuticle (Fig. 9 C). When such clones are made in animals hemizygous for *Wsp* alleles, multiple sockets are rarely observed, while the *Wsp* smooth head cuticle phenotype predominates (Fig. 9 D). These observations demonstrate that *Wsp* is epistatic to *numb*, i.e., a requirement for *Wsp* during adult sensory organ formation persists even in the absence of *numb* gene function. This finding is consistent with the normal segregation of Numb and Pon-GFP in *Wsp* mutants, with both observations suggesting that *Wsp* is not involved in localization of asymmetrically localized components, but rather provides a function further downstream.

## Discussion

Recent studies have identified WASP and WASP-related proteins as key components of the molecular mechanisms by which signaling information is conveyed to the cytoskeleton. The observations reported here establish specific roles for a member of the WAS gene family, in the context of a developing organism. The genetic analysis suggests an essential requirement for the *Drosophila* homologue, *Wsp*, in the execution of cell fate decisions underlying differentiation of sensory organs and other tissues. The nature of the *Wsp* mutant phenotypes, and the significant genetic interactions between *Wsp* and elements of the *N* pathway, lead us to suggest that the *Drosophila* WASP homologue influences cell fate decisions in the context of *N*-based signaling.

Abnormalities in the program of sensory organ differentiation are a primary consequence of mutations in *Wsp*. A variety of studies have led to the formulation of a gener-

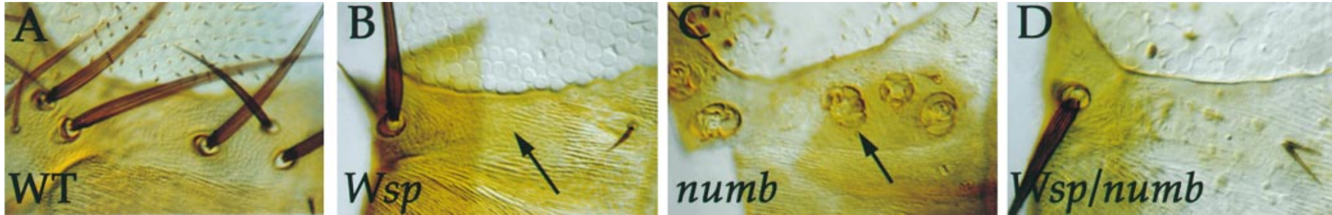



*Figure 9. Wsp* is epistatic to *numb*. Portions of the adult head cuticle adjacent to the eye from wild-type (WT, A), *Wsp*$^1$/Df(3R)3450 (B), *ey*-FLP; *numb*$^2$ FRT40A/l(2)cl-L3$^1$ FRT40A (C), and *ey*-FLP; *numb* FRT40A/l(2)cl-L3$^1$ FRT40A; *Wsp*$^1$/Df(3R)3450 (D) animals. The wild-type bristle pattern (A) gives way to multiple sockets when a *numb* clone is induced (arrow in C), whereas the smooth cuticle phenotype of *Wsp* mutants (arrow in B) is unaffected by such clones (D). The irregular eye facet pattern (C and D) is characteristic of *numb* head capsule clones and ensures that a large clone was indeed induced in the head of the *Wsp* mutant fly.

ally accepted model for sensory organ development in *Drosophila*. The model postulates a temporal progression, in which single SOPs, first selected from within a proneural cell cluster, inhibit neighboring cells from assuming a SOP fate, and then undergo several rounds of asymmetric division, establishing the distinct cell types from which sensory organs are comprised (Posakony, 1994; Ghysen and Dambly-Chaudiere, 2000; Lu et al., 2000). Mutations in *Wsp* lead to a predominance of neurons within sensory organs, at the expense of other cell types. However, expression of early markers of sensory organ differentiation appears unaffected and a general sensory organ hypertrophy, characteristic of breakdowns in the mechanism of lateral inhibition during the SOP selection phase, is not found. These observations suggest that *Wsp* function is required for establishing cell fate during the asymmetric cell division stage, subsequent to the initial determination of sensory organs. Indeed, by monitoring sensory organ development in living tissue, we have been able to conclusively demonstrate the transformation of the intermediate pIIa cell to a pIIb cell fate, and additional observations strongly imply a subsequent sheath-to-neuron cell fate transformation in this lineage. These findings imply a specific role for *Wsp* during sensory organ formation, in the context of cell fate determination via asymmetric division.

Lateral inhibition between neighboring cells, mediated by the *N* signaling pathway, governs the various stages of *Drosophila* sensory organ development (Simpson, 1997; Bray, 1998) and provides a molecular context for *Wsp* function. We have demonstrated significant genetic interactions between *N* pathway elements and *Wsp*, strengthening the case for a functional connection. The *N* pathway has been implicated in a wide variety of developmental processes and decisions in *Drosophila* (Artavanis-Tsakonas et al., 1999). A limited set of components, including the *N* receptor and its ligands, as well as elements such as the nuclear factors Su(H) and Enhancer-of-split, form the core of the pathway and are generally used to carry out its functions. Additional factors, usually cytoplasmic in nature, participate in more restricted sets of developmental events, for which *N*-based signaling provides a mechanistic basis. The data presented here, which identify specific requirements for *Wsp* function, suggest that the *Drosophila* WAS gene homologue is a member of the latter group of *N* pathway elements.

The challenge still before us is to elucidate the manner in which the established cellular functions of WASP proteins can be united with the role of *Wsp* in generation of cell fate diversity during *Drosophila* development. A possible hint comes from the particular developmental processes in which *Wsp* function is required. In addition to the requirement during a specific phase of embryonic and adult sensory organ development, we have identified roles for *Wsp* in cell fate decisions encompassing aspects of lineage determination in the embryonic CNS and mesoderm. This subset of *N*-dependent processes has been singled out previously due to significant functional requirements for the genes *sanpodo* (*spdo*) (Dye et al., 1998) and the *N* antagonist *numb* (Uemura et al., 1989; Frise et al., 1996). Mutations in *spdo* result in embryonic phenotypes highly reminiscent of *N* loss-of-function circumstances in these tissues, whereas impairments to *numb* lead to opposite phenotypic effects (Guo et al., 1996; Spana and Doe, 1996; Ruiz Gomez and Bate, 1997; Buescher et al., 1998; Carmena et al., 1998; Park et al., 1998; Skeath and Doe, 1998). The striking similarities in functional requirements lead us to propose that *numb*, *spdo*, and *Wsp* mediate *N* signaling within a common mechanistic framework. The nature of this framework is currently unclear and is a matter for speculation. *spdo* encodes a *Drosophila* homologue of vertebrate Tropomodulin, a microfilament pointed-end capping protein (Dye et al., 1998; Cooper and Schafer, 2000), suggesting a possible biochemical basis for cooperative function with *Wsp*. However, it should be noted that whereas mutations in both *spdo* and *Wsp* result in a bias towards a neuronal cell fate in the embryonic PNS and in duplication of RP2 neuroblasts, these mutations have opposite effects on the PC cell/DA1 muscle decision in the embryonic mesoderm, imparting a degree of complexity to the potential functional association between these elements. The requirement for an intact cellular microfilament array in establishing asymmetric localization of Numb and other factors (Broadus and Doe, 1997; Knoblich et al., 1997; Lu et al., 1999) suggested an attractive target for Wsp function. However, our data strongly argue against a role for *Wsp* in influencing the cytoskeletal basis of Numb localization, since both Numb and the associated factor Pon are properly localized in *Wsp* mutants. Therefore, the manner in which the presumed disruptions to cytoskeletal organization resulting from mutations in *Wsp* adversely affect the *N* pathway remains an open question. One avenue which should be considered, in light of recent findings, is the association of endocytosis with both *N*-based signaling and WASP cellular functions. Substantial genetic and biochemical evidence implies a crucial involvement of ligand-mediated endocytosis in *N* signal

transduction during various developmental processes, including sensory organ formation (Seugnet et al., 1997; Parks et al., 2000). Parallel studies have fostered a growing appreciation for WASP protein function in linking endocytic mechanisms with the microfilament-based cytoskeleton (for review see Qualmann et al., 2000), suggesting an intriguing cellular context in which *Wsp* may exert an influence over the *N* signaling pathway.

Finally, the involvement of *Wsp*, the *Drosophila* WASP homologue, in execution of cell fate decisions during fly development may well have implications for the manner in which mammalian WASP function is perceived. It is worthwhile to note in this context that roles for mammalian *N* homologues in lineage decisions of hematopoietic cells have been described (Deftos and Bevan, 2000). However, it is unclear whether the existing data support cell fate defects as an explanation for the human WAS phenotype. The full spectrum of hematopoietic cell types are found in the blood of WAS patients and the pleiotropic phenotypes described appear consistent with general abnormalities in cellular structure, rather than with defects in programs of tissue differentiation (Ochs et al., 1980; Remold-O'Donnell et al., 1996). Still, it may be too early to draw parallels between the invertebrate and mammalian systems, particularly since specific functional requirements for N-WASP, the ubiquitously expressed mammalian WASP, are yet to be described.

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