

# Bazooka is required for localization of determinants and controlling proliferation in the sensory organ precursor cell lineage in *Drosophila*

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Contributed by Yuh Nung Jan, October 18, 2001

**Asymmetric divisions with two different division orientations follow different polarity cues for the asymmetric segregation of determinants in the sensory organ precursor (SOP) lineage. The first asymmetric division depends on *frizzled* function and has the mitotic spindle of the pI cell in the epithelium oriented along the anterior–posterior axis, giving rise to pIIa and pIIb, which divide in different orientations. Only the pIIb division resembles neuroblast division in daughter-size asymmetry, spindle orientation along the apical–basal axis, basal Numb localization, and requirement for *inscuteable* function. Because the PDZ domain protein Bazooka is required for spindle orientation and basal localization of Numb in neuroblasts, we wondered whether Bazooka plays a similar role in the pIIb in the SOP lineage. Surprisingly, Bazooka controls asymmetric localization of the Numb-anchoring protein Pon, but not spindle orientation, in pI and all subsequent divisions. Bazooka also regulates cell proliferation in the SOP lineage; loss of *bazooka* function results in supernumerary cell divisions and apoptotic cell death.**

The sensory organ precursor (SOP) cell lineage unfolds with each division exhibiting a stereotyped orientation and segregating Numb, a cell-fate determinant protein, to one of the two daughter cells (1), giving rise to a complete external sensory (ES) organ composed of a hair, socket, sheath, neuron, and glial cell. The pI and pIIa divisions occur along the anterior–posterior axis, whereas the pIIb and pIIIb divisions proceed along the apical–basal axis of the developing fly notum. Different polarity cues determine the asymmetric localization of cell-fate determinants in different divisions (2, 3). In pI, the correct spindle orientation and anterior localization of Numb crescent depend on *frizzled* function (3–5), which is also required for planar polarity in the surrounding pupal epithelium. The pI division generates a posterior daughter, pIIa, and an anterior daughter, pIIb. Although both pIIb and its daughter pIIIb divide along the apical–basal axis, only the pIIb division resembles the embryonic neuroblast division in its dependence on *inscuteable* for proper spindle orientation and Numb localization at the basal cortex (2, 3). Thus, *frizzled* affects the pI but not the pIIb division, whereas *inscuteable* is required for the correct orientation of the pIIb but not the pI cell division.

In embryonic mitotic neuroblasts, Inscuteable is localized to an apical crescent in a complex containing Pins, DaPKC, Dm-Par-6, and Bazooka (6–12). This apical complex is required for orienting neuroblast mitosis along the apical–basal axis and positioning cell-fate determinants at the basal cortex. Given the similar requirement for Inscuteable in the asymmetric divisions of embryonic neuroblasts and the pIIb cell of the SOP lineage, one might expect that Bazooka be involved in correct positioning of spindle and Numb crescent for the pIIb, the cell that divides in a neuroblast-like manner, but not for the pI. To our surprise, we found no Pon/Numb crescent formation during mitosis in the entire SOP lineage in *bazooka* mutant clones. In the absence of Bazooka the SOP lineage also exhibits ectopic mitosis, cell-fate transformation, and apoptosis of ES cell clusters.

## Methods

**Fly Lines and *in Vivo* Imaging.** *yw baz<sup>xi106</sup> P[mini-w<sup>+</sup>, FRT]<sup>9-2</sup>/FM6* (kindly provided by A. Wodarz, University of Düsseldorf, Germany) and *baz<sup>EH171</sup> P[mini-w<sup>+</sup>, FRT]<sup>9-2</sup>/FM6;pr pwn P[hsFLP]/Cyo* females were crossed to *P[w<sup>+</sup>, Gal80]*, *P[mini-w<sup>+</sup>, FRT]<sup>9-2</sup>;GAL4<sup>sca</sup> P[w<sup>+</sup>, UAS-Tau-GFP]*, and *P[w<sup>+</sup>, UAS-Tau-GFP]* (3). For antibody staining, *GAL4<sup>109-68</sup>* (a PNS-specific GAL4 line) *UAS-mCD8-GFP* (13) and *w<sup>-</sup>* were used. mCD8-GFP, a membrane marker, allowed us to stage SOP lineage cells before fixation. XZ images were acquired by using the vertical section scan mode on a Bio-Rad 1024 confocal microscope. Larvae were heat-shocked for 1 h at 37°C during first/second instar stages. Larvae were allowed to grow for 3–4 days until pupation and were mounted for live imaging or prepared for fixation (see below) 15 h after puparium formation (APF). Complete absence of Bazooka protein in *bazooka* mutant clones was confirmed by antibody staining with the Bazooka antibody (data not shown). Pupae were mounted and prepared for imaging as in ref. 3. Nota of pharate adults were fixed in 80% isopropanol, dissected, mounted in Hoyer's media, and observed in differential-interference contrast (DIC) optics.

**Immunohistochemistry and Terminal Deoxynucleotidyltransferase-Mediated dUTP Nick End Labeling (TUNEL).** Control and mutant clone pupae were fixed and stained by using standard protocols (14). Antibodies used were rabbit anti-Prospero (1/1,000), rabbit anti-Inscuteable (1/1,500; kindly provided by W. Chia, IMCB, Singapore), rabbit anti-Bazooka (1/1,500; kindly provided by A. Wodarz), rat anti-mCD8 (1/100; Caltag, South San Francisco, CA), guinea pig anti-Numb (1/1,000), and mouse anti- $\alpha$ -tubulin (1/1,000; Sigma). TUNEL staining was done by using the Apoptag kit (Intergen).

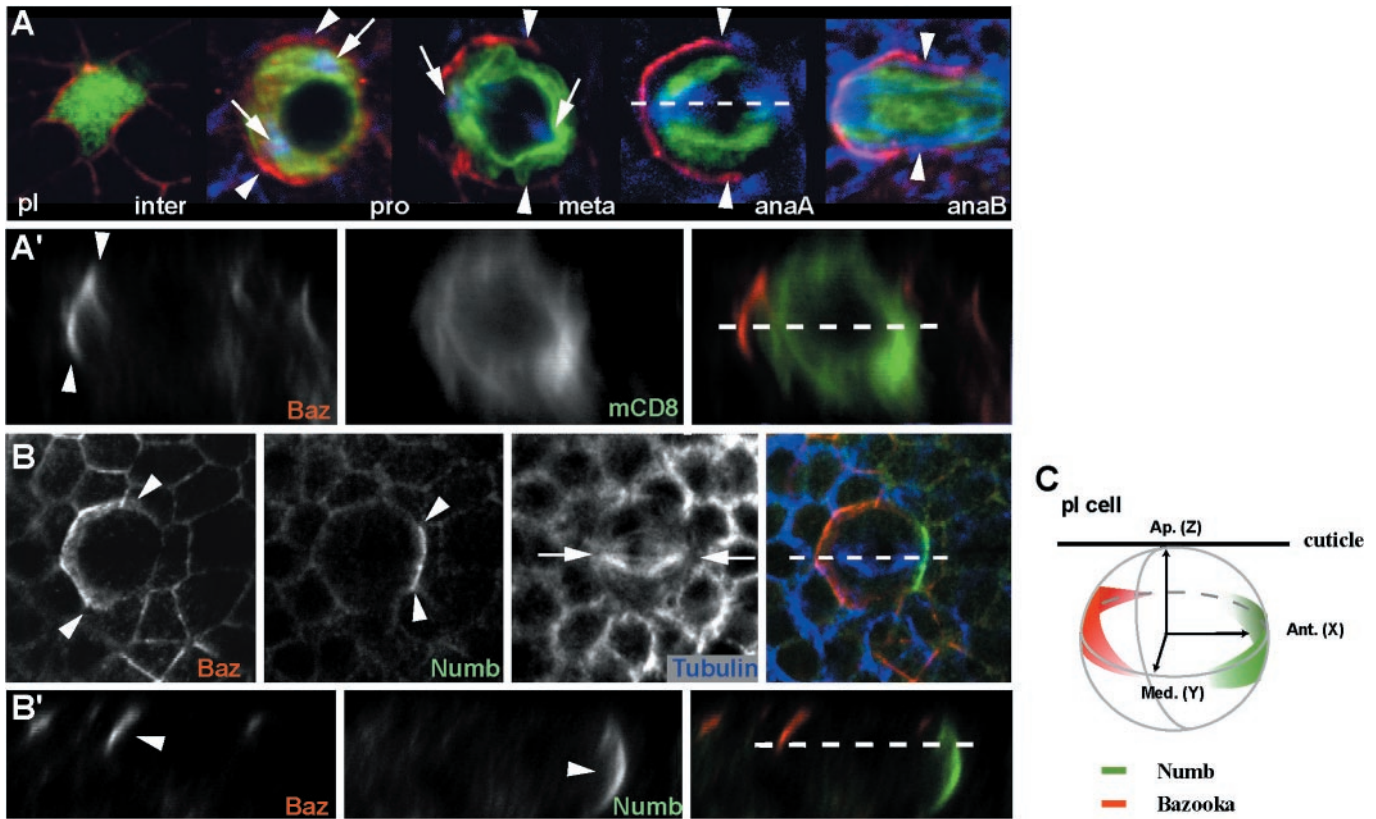
## Results and Discussion

For Bazooka to be involved in every asymmetric division of the adult SOP lineage one might expect Bazooka to be expressed in every precursor cell. Indeed, we found that Bazooka is asymmetrically localized in every dividing cell of the SOP lineage (Figs. 1 and 2). Starting with a strong accumulation at the apical surface of interphase pI cells, specifically at junctions with neighboring epithelial cells ( $n = 10$ , Fig. 1A, inter, which shows an apical section), Bazooka becomes enriched at the posterior cortex during mitosis ( $n = 12$ , Fig. 1A, pro and meta) and shows no overlap with the anterior Numb crescent at metaphase ( $n = 9$ , Fig. 1B and B'). By early anaphase, Bazooka forms a smooth posterior crescent (Fig. 1A, pro and anaA). At anaphase B, Bazooka is localized to the posterior cortex, although a signif-

Abbreviations: SOP, sensory organ precursor; ES, external sensory; APF, after puparium formation.

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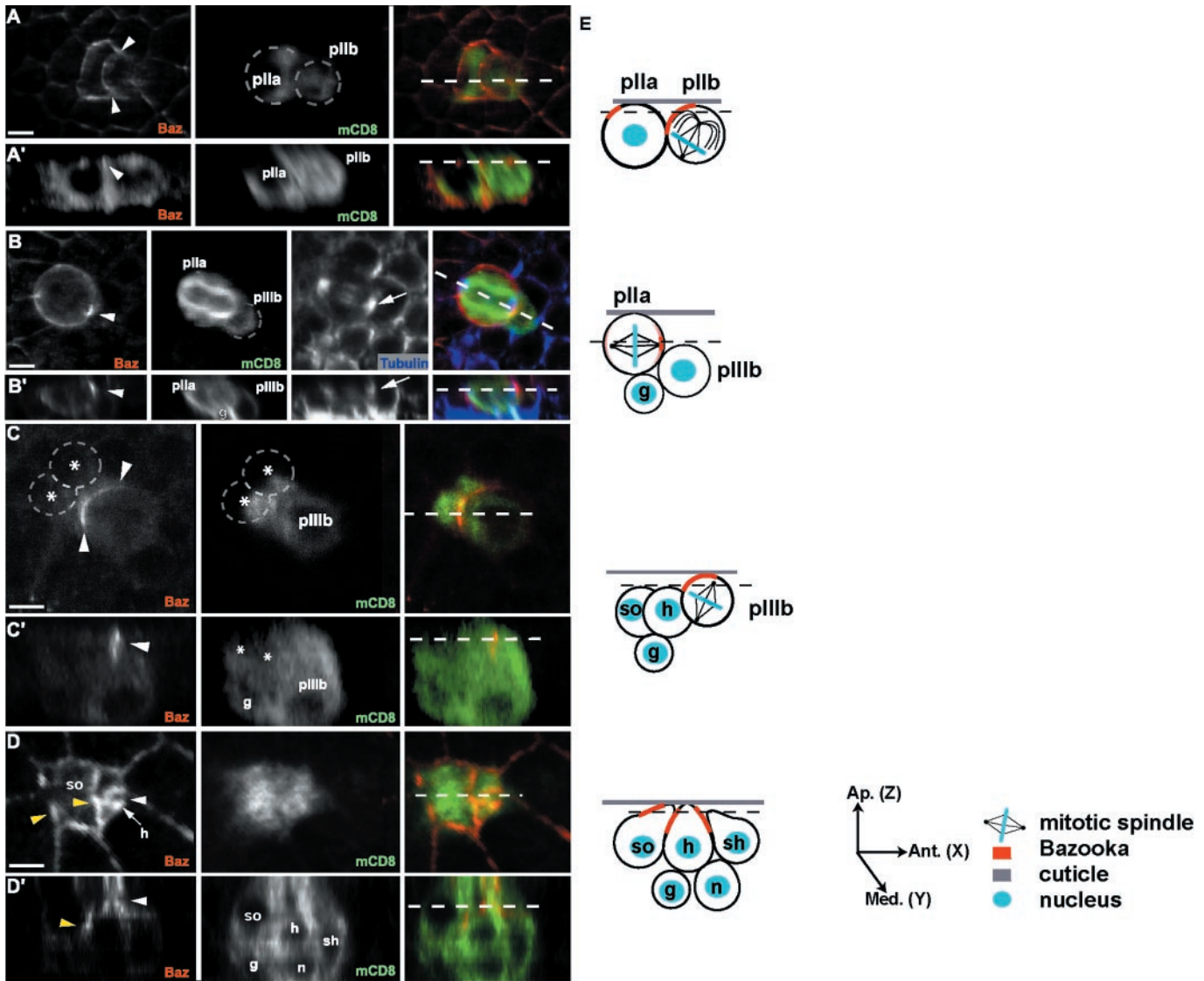


**Fig. 1.** (A and A') Bazooka protein in the dividing pI cell. Merges of Bazooka (red), mCD8 (green), and  $\alpha$ -tubulin (blue) in A and of Bazooka (Baz) and mCD8 in A'. Images are single XY sections extracted from a z-series (A) and a single XZ section (A') (see schematic in C for orientation; inter is an apical section, all others are equatorial sections). Dashed lines in A and A' indicate the plane of the XZ section in A' and the plane of the XY section in A, respectively, for the pI cell in early anaphase (anaA). mCD8-GFP accumulates in the cytoplasm in tube-like structures that resemble intracellular membrane compartments such as ER and Golgi. Anterior is to the right, medial is down in A, and basal is down in A'. In interphase (inter), Bazooka protein localizes to the apical cell border of the pI cell. At prophase (pro) the centrosomes (white arrows) straddle the nucleus, the pI cell rounds up, and Bazooka accumulates at the cell cortex (white arrowheads). By metaphase (meta), Bazooka is enriched at the posterior cortex (white arrowheads), but absent from the anterior cortex. At this stage, strong accumulations of mCD8-GFP surround the mitotic spindle (white arrows) but are excluded from the spindle mid-zone (dark region in the center of the cell). During early anaphase (anaA, A'), Bazooka forms a smooth posterior crescent (white arrowheads) that covers the entire posterior cortex. At late anaphase (anaB), Bazooka protein covers the entire posterior cortex and extends beyond the cleavage furrow (white arrowheads). (B and B') Bazooka and Numb localization (white arrowheads) in the pI cell at metaphase [Bazooka (red), Numb (green), and  $\alpha$ -tubulin (blue) in the merged images]. Images are single XY sections extracted from a z-series (B) and a single XZ section (B'). Dashed line in B indicates the plane of the XZ section in B'. Dashed line in B' indicates the plane of the XY section in B. Bazooka localizes to the posterior cortex at metaphase in the pI cell, Numb forms a crescent of the anterior cortex, and the two proteins do not colocalize. The mitotic spindle (arrows, blue) is aligned between the Bazooka and Numb crescent. Anterior is to the right, medial is down in B, and basal is down in B'. (C) Schematic representation of Bazooka (red) and Numb (green) localization in the pI cell at early anaphase, apical [Ap. (Z)] at the top, anterior [Ant. (X)] to the right, medial (Med. (Y)) is toward the reader from the plane of the paper. The pupal cuticle is at the apical surface of the pI cell.

icant amount remains anterior to the cleavage furrow ( $n = 4$ , Fig. 1A, anaB). The pI division is followed by division of the pIIb cell, which exhibits an apical-posterior crescent of Bazooka at mitosis ( $n = 6$ , Fig. 2A and A'). Subsequently, in the mitotic pIIa, Bazooka accumulates in the cell cortex and a strong patch of Bazooka is detected at the anterior cortex, a region that coincides with the position of the anterior-most centrosome of the mitotic spindle ( $n = 11$ , Fig. 2B and B'). Finally, in the mitotic pIIIb cell, Bazooka forms an apical-posterior crescent similar to the one observed in the pIIb cell ( $n = 8$ , Fig. 2C and C'). In comparing our observations to earlier work (1, 3, 5), we find that in the pI, pIIb, and pIIIb divisions, Bazooka is localized opposite the Numb crescent in mitosis; whereas in the mitotic pIIa cell, the anterior accumulation of Bazooka may colocalize with Numb. Following completion of these asymmetric divisions, Bazooka expression is enriched at the apical borders in cells of the developing es organ, specifically the hair and socket cells ( $n = 15$ , Fig. 2D and D'). Based on these observations, we conclude that Bazooka is expressed in all precursor cells within the SOP lineage; it is asymmetrically enriched during each cell division of

the SOP lineage, and its expression is maintained in the post-mitotic cells that will give rise to the external structures of the es organ, the hair and socket cells.

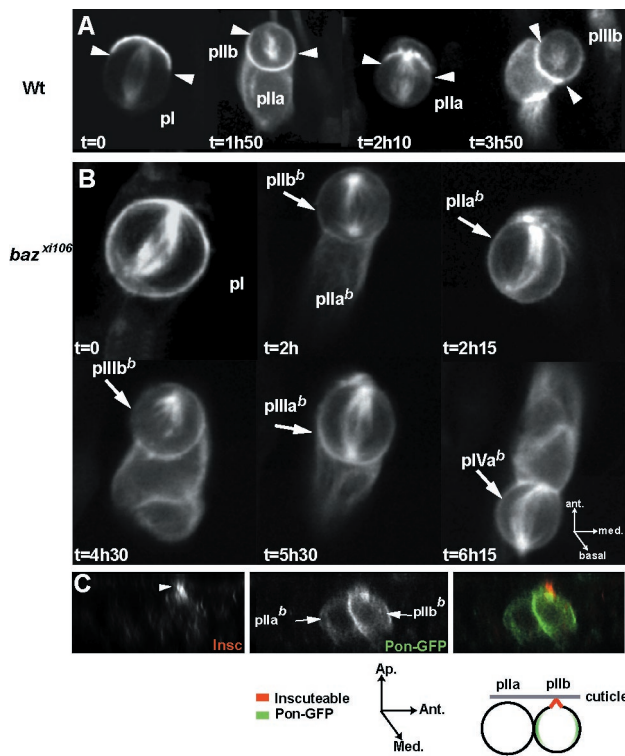
During embryonic neuroblast divisions, Bazooka is required not only to localize Inscuteable to the apical cortex and Numb, Miranda, Prospero, and Pon to the basal cortex, but also to orient the mitotic spindle along the apical-basal axis (7, 9, 15). To determine the requirement of *bazooka* in the asymmetric divisions of the adult SOP lineage, we used the MARCM system (13) to generate *baz* mutant clones expressing both Pon-GFP (as a reporter for Numb localization) and Tau-GFP (as a reporter for spindle orientation) under the control of *scaberous*-GAL4, which is strongly expressed in the SOP cell and in the SOP lineage (3). The movements of Pon-GFP and Tau-GFP were monitored in live tissue throughout all asymmetric divisions of the SOP lineage. In *baz<sup>xi106</sup>* or *baz<sup>EH171</sup>* null mutant clones, pI cells underwent mitosis at  $\approx 15$  h APF as in wild type. However, in all mutant pI cells observed ( $n = 12$ ), Pon-GFP remained uniformly distributed and never formed an anterior crescent as seen in dividing wild-type pI cells (Fig. 3A and B; see also Movies



**Fig. 2.** Bazooka protein localization in the cells of the SOP lineage including mitotic pIIb (A and A'), pIIa (B and B'), pIIIb (C and C'), and post-mitotic sensory organ cells (D and D'). Bazooka labeling (column 1, A–D') is red in the merged images. The cells of the SOP lineage are marked with mCD8-GFP expressed under the control of *GAL4<sup>109-68</sup>* (column 2; A–D') are green in the merged images, and  $\alpha$ -tubulin (column 3, B and B') in blue in the merged images, which are shown in column 3 (A and A', C and C', and D and D') and column 4 (B and B'). The XY sections in A–D are extracted from a z-series (anterior is to the right, medial is down), and images in A'–D' are single XZ sections of the same cells shown in A–D (anterior is to the right, apical is up). A–D are single XY sections (dashed lines indicate plane of the XZ section in A'–D'). Dashed lines in A–D indicate plane of the XZ section in A'–D'. Dashed lines in A'–D' and in schematic E indicate plane of the XY section in A–D. Dashed circles indicate outlines of cells that are out of the plane of focus. (Bar, 5  $\mu$ m.) (A and A') Bazooka forms an apical-posterior crescent (white arrowheads) in the dividing pIIb (small dashed circle), and is localized apically on the cortex of the pIIa cell (large dashed circle). Some cytoplasmic signal is also detected in basal portion of the pIIb and pIIa cells (A'). (B and B') In the dividing pIIa cell, Bazooka exhibits a strong localization to an anterior spot (white arrowhead), which closely coincides with the anterior centrosome at metaphase (white arrow), as well as uniform cortical localization. (C and C') Bazooka forms an apical-posterior crescent (white arrowheads) in the dividing pIIIb, similar to the pIIb. At this stage, Bazooka is not detected in the daughters of the pIIa (\*), or the basal-most glial cell (g). (D and D') Bazooka protein in the cells of the es organ following divisions (26 h APF). Cell types were inferred from their relative positions after the divisions (1). Bazooka is enriched in the apical portion of the es cluster composed of a hair (h), socket (so), sheath (sh), neuron (n), and glial (g) cell. A stalk of Bazooka is localized to the apical portion of the hair cell (white arrowhead). A ring of Bazooka forms around the apical boundary of the socket cell (yellow arrow). (E) Schematic representation of Bazooka localization in the mitotic pIIb, pIIa, and pIIIb, and in cells of the es organ; apical is at the top, anterior is to the right, and medial is above the plane of the paper. Hair (h), socket (so), sheath (sh), neuron (n), and glial (g) cell. Mitotic spindle orientation is based on refs. 1 and 3.

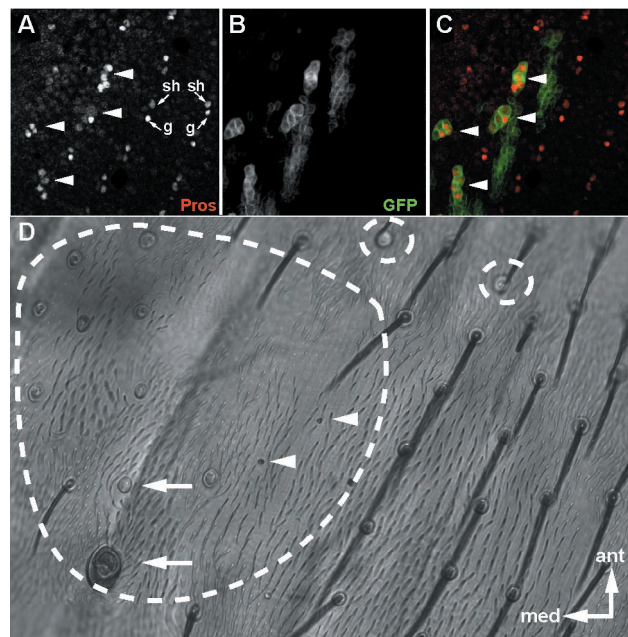
1–7, which are published as supporting information on the PNAS web site, [www.pnas.org](http://www.pnas.org). Nor did Pon-GFP crescents form in the subsequent divisions in the lineage ( $n = 9$ , Fig. 3B; see also Movies 4–7). Thus, although only the pIIb resembles the embryonic neuroblast in its orientation of division and requirement for Inscuteable (2, 3), Bazooka is required for the asymmetric Pon/Numb localization in the pI division, as well as all subsequent divisions.

Because Numb functions as an asymmetrically localized cell-fate determinant in the SOP lineage, the absence of Numb crescents in *baz* mutant clones could lead to cell-fate transformations in the daughters of the pI cell. Thus we will refer to the anterior daughter cell of the pI in *bazooka* mutant clones (the pIIb cell in the wild type) as pIIb<sup>b</sup>, and the posterior daughter cell as pIIa<sup>b</sup>. It is worth noting, however, that either loss-of-function or misexpression of *numb* only causes cell-fate trans-



**Fig. 3.** (A) Asymmetric divisions of wild-type SOP lineage cells revealed by Pon-GFP and Tau-GFP expressed under the control of *sca-Gal4*. Single plane confocal micrographs [anterior (ant.) is at the top, medial (med.) is to the right, and basal is above to the plane of the paper]. In the pi cell Pon-GFP forms an anterior crescent at metaphase (arrowheads;  $t = 0$ , time in h and min). In the pIIb cell Pon-GFP forms a basal crescent at metaphase (arrowheads), the mitotic spindle is positioned perpendicular to the crescent ( $t = 1$  h 50 min). In the pIIa cell Pon-GFP forms an anterior crescent at metaphase (arrowheads;  $t = 2$  h 10 min). In the pIIIb cell Pon-GFP forms a basal crescent at metaphase (arrowheads;  $t = 3$  h 50 min). In each mitotic precursor, the mitotic spindle is positioned perpendicular to the crescent. After the pIIIb division, no more mitoses are observed (not shown; ref. 1). (B) Pon-GFP and Tau-GFP in *bazooka*<sup>x106</sup> MARCM mutant clones. In each normal division within the lineage pi, pIIb<sup>b</sup>, pIIa<sup>b</sup>, and pIIIb<sup>b</sup> (arrows), Pon-GFP remains uniform in the cortex during mitosis. The mitotic spindle orientation is indistinguishable from the wild type (see A). In addition, the two-pIIa<sup>b</sup> daughter cells undergo an extra round of division (pIIa<sup>b</sup> and pIVa<sup>b</sup>; arrows) along the anterior–posterior axis to yield a total of seven cells. (C) Inscuteable localization in the dividing pIIIb<sup>b</sup> cell, MARCM clones of *bazooka*<sup>x106</sup> expressing Pon-GFP, labeled with Inscuteable antibody. Images are a single XZ section [apical (Ap.) is at the top, anterior (Ant.) is to the right, medial (Med.) is above to the plane of the paper; see schematic]. Inscuteable is expressed in the pIIIb<sup>b</sup> and localizes to an apical stalk (white arrowhead, red in the merged image) at mitosis. Pon-GFP (green in the merged image) is present in patches on the anterior and posterior cortex of the cell. (Right) A schematic representation of merged image in C of dividing pIIIb<sup>b</sup>, Inscuteable (red), and Pon-GFP (green). Apical (Ap.) is at the top, anterior (Ant.) is to the right, medial (Med.) is toward the reader from the plane of the paper. The pupal cuticle is at the apical surface of the pIIIb<sup>b</sup> cell.

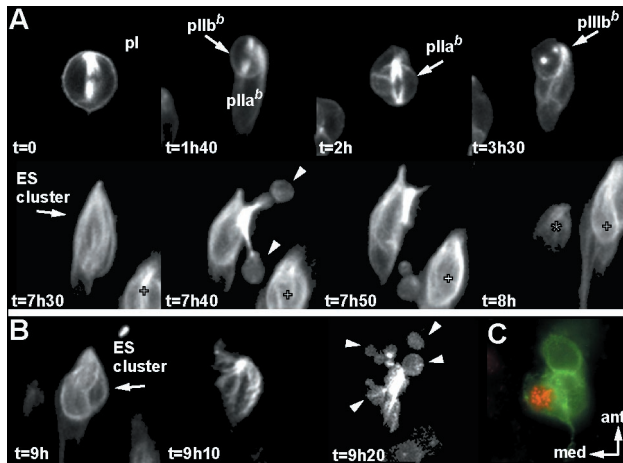
formation in a subset of sensory organs (16, 17), presumably because the Notch-mediated mutual inhibition may still allow the two daughter cells to adopt different cell fates, albeit without a bias set by the Numb crescent (17, 18). Transformation of pIIa to pIIb cell fate is known to alter the timing of mitosis of the transformed pIIa cell (19). We found that the timing of the pIIb<sup>b</sup>, pIIa<sup>b</sup>, and pIIIb<sup>b</sup> divisions was indistinguishable from wild-type pIIb, pIIa, and pIIIb cells. In addition, the pi and pIIa<sup>b</sup> spindles aligned along the A–P axis in all mutant clones ( $n = 9$ ). And in eight of the nine clones examined the pIIIb<sup>b</sup> spindles were oriented along the apical–basal axis as in wild type (the remaining pIIb<sup>b</sup> cell divided before the pIIa<sup>b</sup>, but had its spindle



**Fig. 4.** Cell-fate transformations within the SOP lineage in MARCM *bazooka* mutant clones. (A) Prospero (Pros) is expressed in the glial cell (g) and the sheath cell (sh) in the pIIb cell lineage. In wild-type SOP clusters, two cells (the glial cell and the sheath) express Prospero in each sensory organ cluster (arrows). In *bazooka* mutant clones (arrowheads), more than two Prospero-positive cells are typically found in sensory organ cell clusters. (B) GFP expression reveals MARCM *bazooka* mutant clones. (C) In this merge of A and B, ectopic Pros-positive cells (red) vary in number in clusters within the *bazooka* mutant clone (green, arrowheads). (D) Balding and missing hairs on the notum of pharate adult containing a *bazooka* mutant clone. Note the loss of external sensory structures, as well as sockets without hairs (arrows), and small bumps that may represent undifferentiated or aborted ES cells (arrowheads) within the clone (dashed region).

oriented along the anterior–posterior axis). Because an apically localized Inscuteable is required for mitotic spindle positioning in the pIIb cell (2, 3), we also examined Inscuteable localization in the pIIb<sup>b</sup> cell in *bazooka* mutant clones (Fig. 3C). We found that Inscuteable is localized to an apical stalk in pIIb<sup>b</sup>, similar to the wild-type pIIb ( $n = 12$ ). Thus we found that the great majority of pIIb<sup>b</sup> and pIIa<sup>b</sup> cells resemble wild-type pIIb and pIIa cells in their timing and orientation of division, as well as the expression of Inscuteable in the pIIb<sup>b</sup>. It therefore appears that these *bazooka* mutations did not cause detectable cell-fate transformation in most of the pIIb<sup>b</sup> and pIIa<sup>b</sup> cells, although it remains possible that there are partial transformations and cell-fate changes in a subset of these cells. In light of these observations, the complete loss of Pon-GFP crescents in every mitotic pIIb<sup>b</sup> and pIIa<sup>b</sup> cell examined strongly supports that Bazooka controls Pon/Numb asymmetric localization in not only pi but also pIIb and pIIa cells.

Some cell-fate changes apparently took place in the progeny of pIIa and pIIb. On adult nota, mutant clones contained patches of bald cuticle and regions with small bumps that may represent lost ES organs, and sockets without hairs (Fig. 4D). We also found ectopic Prospero-expressing cells in *baz* mutant clones (Fig. 4A–C), indicating that additional sheath and/or glial cells were present because of cell-fate transformations in the pIIb<sup>b</sup> lineage. Interestingly, in six of nine mutant SOP lineages examined, we observed ectopic divisions in the pIIa<sup>b</sup> cell lineage (Fig. 3B, pIIIa<sup>b</sup> and pIVa<sup>b</sup>). Whereas the wild-type pIIa cell divides only once to give rise to two external cells of the ES organ, the hair and socket cells (20), in *baz* mutant clones each daughter of



**Fig. 5.** (A) Clusters of ES cells undergo apoptosis following symmetric divisions in *bazooka* mutant clones. A MARCM *bazooka* clone undergoes four rounds of division ( $t = 0$ –3 h 30 min, arrows, beginning 17 h APF). Within 4 h following the divisions, several cells form apoptotic bodies that are rapidly cleared ( $t = 7$  h 30 min to 8 h, arrowheads). The remaining cells of the cluster drift out of the plane (\*,  $t = 8$  h). The neighboring mutant cluster (+) undergoes apoptosis 1 h later (not shown). (B) An entire cluster of ES cells ( $t = 9$  h, arrow) forms apoptotic bodies simultaneously ( $t = 9$  h 20 min, arrowheads). (C) TUNEL labeling (red) confirms presence of apoptotic nuclei in an ES cluster at 27 h APF within a *bazooka* mutant clone (marked with GFP, green).

the pIIa<sup>b</sup> cell underwent another round of division, causing the SOP lineage to produce a cluster of seven cells, as opposed to the normal five cells.

In the wild-type SOP lineage, shortly after the last division, of pIIIb, at  $\approx 24$  h after pupa formation (1), one of the pIIIb daughters forms a neuron and extends an axon. Within an hour after completion of the pIIIb mitosis, the small glial cell migrates away from the cluster along the axon (1). By following the development of the ES organs *in vivo* in *baz* mutant clones to observe their morphogenesis, we found no axon extension or glial cell migration in 16 of 20 ES organs examined (see Movies 8 and 9). Moreover, within 3–6 h after the last mitosis, clusters of cells underwent apoptosis in ten of twenty clones examined (Fig. 5; see also Movies 8 and 9). Apoptotic bodies formed and dispersed rapidly. We have confirmed apoptosis in clones by using immunohistochemistry and terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL) (Fig. 5C). Cell death of ES organ cells is specific to the *bazooka* mutants, because we have never observed apoptosis in wild-type ES cells (data not shown).

Bazooka and its homologue Par-3 in *C. elegans* are known to be required for asymmetric divisions, in embryonic neuroblasts in *Drosophila* (7, 9, 15) and in the zygote and early blastomeres of the worm embryo (21), respectively. In *Drosophila* neuroblasts, Bazooka forms a complex with Pins and localizes Inscuteable, which coordinates the asymmetric localization of Numb and spindle orientation (7–9, 12, 22). In the mitotic pI cell, an anterior Pins/Dlg complex has been shown to be required for Bazooka localization, and that Bazooka is required for Numb localization (23). Our study of the adult SOP lineage reveals several functions for Bazooka. First, we show that Bazooka is the first molecule to be required for the asymmetric localization of Pon, the adapter protein for Numb, in every division of the SOP lineage, even though only the pIIb division resembles embryonic neuroblast division both in its orientation along the apical–basal axis and its dependence on Inscuteable (2, 3). It thus appears that Bazooka may localize Pon and Numb in a pathway independent

of Inscuteable. Second, Bazooka is not required for proper spindle orientation in the asymmetric divisions of the SOP lineage. The function of Bazooka in the SOP lineage, therefore, concerns only determinant localization but not spindle orientation. Unlike the neuroblast, in the pIIb cell Inscuteable orients spindles along the apical–basal axis in the absence of Bazooka, indicating that pIIb cells may have a Bazooka-independent mechanism for Inscuteable localization. Third, although *bazooka* mutations did not cause detectable cell-fate transformation in most pIIb and pIIa cells, there is apparent cell-fate transformation occurring in the pIIb<sup>b</sup> lineage, and possibly partial cell-fate transformations of the pIIa<sup>b</sup> lineage leading to formation of cells of indeterminate cell fates, such as sockets without hairs or the bumps in *bazooka* mutant clones. Fourth, loss of Bazooka function leads to apoptosis. We cannot rule out the possibility that inadequate cell-fate specification results in apoptosis. However, the cell-fate transformations in the SOP lineage in various mutants reported thus far have not been associated with apoptosis, indicating that cell-fate transformation *per se* does not necessarily lead to apoptosis. The terminal fates of mutant es cell clusters are difficult to determine with certainty, because most undergo apoptosis rather than differentiation. Fifth, Bazooka appears to limit the number of cell cycles of the pIIa to one; the pIIa<sup>b</sup> daughters in *bazooka* mutant clones often proceed with mitosis instead of differentiating into hair and socket cells. Similarly, antiproliferative activity has been found in follicle cells of the ovary (24). Finally, loss of Bazooka function leads to failure of ES neuron axonal outgrowth and glial cell migration. These defects could reflect cell-fate changes in the pIIb<sup>b</sup> lineage or a requirement for *bazooka* in differentiation of ES organ cells.

In summary, we found Bazooka has a much broader spectrum of function in the SOP lineage than previously suspected. In *bazooka* mutant clones the Numb-anchoring protein Pon failed to form a crescent in every division of the SOP lineage, regardless of the requirement for Inscuteable. The function of Bazooka in the SOP lineage also differs from that in embryonic neuroblasts because Bazooka controls spindle orientation in neuroblasts but not in the SOP lineage. The pI, pIIb, and pIIa cells show little evidence of cell-fate transformation in *bazooka* mutant clones, and yet exhibit a total loss of Pon-GFP crescent formation. It thus appears that Bazooka controls Pon/Numb crescent formation in these precursors with different planes of division. Although in *bazooka* mutant clones there appears to be partial cell-fate transformation in later divisions in the lineage, it is striking that asymmetric localization of determinants is abolished in all divisions. The loss of Pon-GFP crescent is fully penetrant, in contrast to the variable and partially penetrant cell-fate transformation phenotype. These observations suggest that Bazooka is the general link between polarity cue and the localization of cell-fate determinants in all asymmetric cell divisions. Other previously uncharacterized functions uncovered in this study include the ability of Bazooka to restrict the number of divisions in the SOP lineage and to promote differentiation instead of apoptosis. It is worth noting that the function of Bazooka in the central nervous system (CNS) has been previously studied only for the neuroblast division. Based on our findings in the SOP lineage, it will be interesting to learn whether in the CNS Bazooka also has an Inscuteable-independent role in controlling asymmetry of subsequent divisions, as well as in regulating proliferation and apoptosis. Given that Bazooka/Par-3 is part of an evolutionarily conserved gene cassette (25), our findings of a myriad of previously uncharacterized functions of Bazooka in the sensory organ lineage raise the possibility that Bazooka/Par-3 may have a similarly wide range of functions in vertebrates.

We thank A. Wodarz, W. Chia, and the Bloomington Stock center for providing reagents. We thank J. Knoblich and D. Cox for helpful discussions and M. Rothenberg for critical reading of the manuscript. F.R. is supported

by the National Institutes of Health Neuroscience Training Grant and the Human Frontiers Science Program (HFSP) postdoctoral fellowship. L.Y.J. and Y.N.J. are Investigators at the Howard Hughes Medical Institute.

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