

Adherens junctions inhibit asymmetric division in the *Drosophila* epithelium

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Asymmetric division is a fundamental mechanism for generating cellular diversity. In the central nervous system of *Drosophila*, neural progenitor cells called neuroblasts undergo asymmetric division along the apical–basal cellular axis^{1,2}. Neuroblasts originate from neuroepithelial cells, which are polarized along the apical–basal axis and divide symmetrically along the planar axis. The asymmetry of neuroblasts might arise from neuroblast-specific expression of the proteins required for asymmetric division. Alternatively, both neuroblasts and neuroepithelial cells could be capable of dividing asymmetrically, but in neuroepithelial cells other polarity cues might prevent asymmetric division. Here we show that by disrupting adherens junctions we can convert the symmetric epithelial division into asymmetric division. We further confirm that the adenomatous polyposis coli

(APC) tumour suppressor protein is recruited to adherens junctions³, and demonstrate that both APC and microtubule-associated EB1 homologues^{3–5} are required for the symmetric epithelial division along the planar axis. Our results indicate that neuroepithelial cells have all the necessary components to execute asymmetric division, but that this pathway is normally overridden by the planar polarity cue provided by adherens junctions.

Drosophila neuroblasts delaminate from a polarized epithelial layer in the ventral neuroectoderm and divide asymmetrically along the apical–basal axis to produce larger apical neuroblasts and smaller basal ganglion mother cells. Previous studies identified Inscuteable (Insc) as a central protein in organizing neuroblast division^{6,7}. Insc provides positional information that couples mitotic spindle orientation with the basal localization of cell-fate determinants such as Numb and Prospero together with their respective adaptor proteins Partner of Numb (Pon) and Miranda^{8–10}.

The apical localization of Insc involves both a Baz-dependent initiation step and a maintenance step that requires Baz and Partner of Inscuteable (Pins)^{11–15}. The expression of Baz and Pins in both neuroblasts and neuroepithelial cells suggests that these cells share certain apical–basal polarity information. Consistent with this notion is the observation that, when Pon is expressed ectopically in epithelial cells it is localized to the basal cortex, as in neuroblasts¹⁰. Unlike neuroblasts, however, epithelial cells divide symmetrically along the planar axis and segregate ectopic Pon equally between the two daughter cells. These observations raise further questions: do epithelial cells have the ability to couple spindle orientation with protein localization, and segregate proteins asymmetrically between two unequally sized daughter cells? If so, what prevents them from executing this asymmetric division?

To characterize epithelial division by monitoring it in live embryos, we used transgenic embryos expressing Pon and tau proteins fused with green fluorescent protein (GFP). During epithelial cell cycle, tau–GFP-labelled mitotic spindle is formed along the planar axis of the embryo, and Pon–GFP is initially uniformly associated with the cortex and then localized to a basal crescent. The mitotic spindle remains orientated along the planar

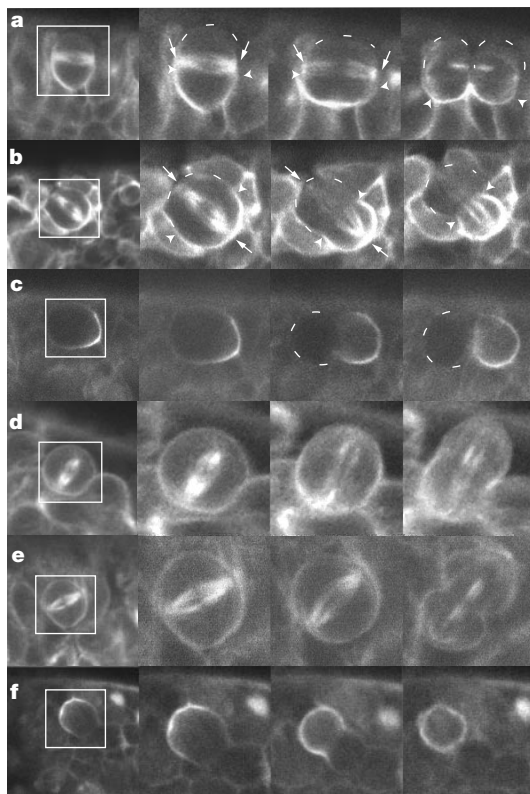


Figure 1 Epithelial divisions in wild-type and mutant embryos. **a**, Symmetric division of wild-type epithelial cells. **b**, **c**, **f**, Asymmetric epithelial divisions in *crb(RNAi)* (**b**), *UAS-Crb-intra* (**c**), and *crb^{11A22}/crb^{11A22}* (**f**) embryos. **d**, **e**, Symmetric epithelial division in *baz(RNAi)* (**d**) and *baz* and *crb* double RNAi (**e**) embryos. For each panel, the first image shows an epithelial cell under observation (square), followed by enlarged images of that cell during division. Arrowheads mark the Pon–GFP crescent, arrows mark tau–GFP-labelled spindles, and dotted lines mark the boundary of the Pon–GFP-negative daughter cells. Apical is up and basal down in all figures.

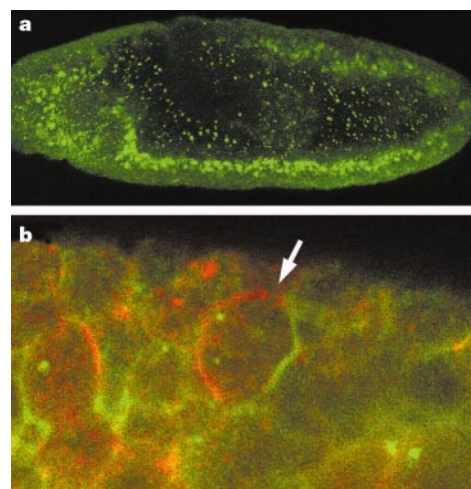


Figure 2 Mispositioning of Baz crescent and absence of ectopic neuronal marker expression in epithelial cells of *crb(RNAi)* embryos. **a**, *crb(RNAi)* embryos were allowed to develop to stages 10–12, and immunostained for Asense. Asense was expressed specifically in the developing central nervous system, and no ectopic expression in the epithelium was detected. **b**, Pon–GFP transgenic embryos injected with *crb* dsRNA were immunostained for Baz (red) and GFP (green). In the epithelial cell under observation (arrow), Baz is localized to a lateral crescent and Pon–GFP is localized as a crescent on the opposite side of the cortex.

axis throughout mitosis. After cytokinesis, the Pon–GFP crescent is bisected by the cleavage furrow and is equally distributed between two equally sized daughter cells (Fig. 1a). This *in vivo* analysis shows that the machinery for basal protein localization is intact in epithelial cells, but it is uncoupled from spindle orientation.

The uncoupling of spindle orientation with asymmetric protein localization in epithelial cells might be due to either a lack of such a coupling mechanism or the dominance of the coupling mechanism by yet another spindle-positioning mechanism. One of the hallmarks of epithelial cells is the adherens junction, which is composed of the cadherin–catenin complex and other associated proteins, is connected to the cytoskeleton, and is thought to be important in maintaining the planar organization of the epithelial monolayer. We therefore tested the possible role of adherens junction in orientating epithelial division. The formation of adherens junction requires genes such as *shotgun*, *crumbs* (*crb*) and *stardust*¹⁶. We used RNA interference (RNAi) to disrupt *Cr*b function and analysed the effect on epithelial division^{17,18}.

We injected double-stranded (ds) *crb* RNA into transgenic embryos expressing Pon–GFP and tau–GFP. In about 70% ($n = 200$) of *crb*(RNAi) embryos, we observed that the organization of the ectodermal epithelium was disrupted, with epithelial cells losing their columnar shape, adopting rounded morphology, and becoming separated from each other. Live imaging of epithelial divisions in these embryos revealed that nearly all the epithelial cells show a tight coupling between the positioning of Pon–GFP crescents and the orientation of the mitotic spindle. Pon–GFP crescents were found at basal and lateral positions and less frequently at apical positions on the cell cortex, and one of the spindle poles was positioned underneath the Pon–GFP crescent.

After cytokinesis, Pon–GFP was segregated to one of the two similarly sized daughter cells (Fig. 1b). Asymmetric segregation of Pon–GFP to one of two similarly sized daughter cells was also observed in *crb* zygotic mutant embryos (Fig. 1f). Immunostaining

of *crb*(RNAi) embryos with antibodies against Asense, Prospero and Insc indicated that epithelial cells do not express these neuronal markers, suggesting that the ability of these cells to undergo asymmetric division is not a result of cell-fate change (Fig. 2a; and data not shown).

Overexpression of the membrane-bound cytoplasmic tail of *Cr*b (*Cr*b-intra) causes similar disorganization of the epithelium as seen in *crb* mutants^{19,20}. We therefore examined the effect of overexpressing *Cr*b-intra on epithelial division. As observed in *crb*(RNAi) embryos, epithelial cells overexpressing *Cr*b-intra showed coupling of the mitotic spindle with the Pon–GFP crescent and asymmetric segregation of Pon–GFP to one of the daughter cells (60%, $n = 100$) (Fig. 1c). Thus, when the formation of the adherens junction is disrupted, epithelial cells switch from a symmetric to an asymmetric division pattern.

In addition to its function in localizing Insc and regulating division axis in the neuroblasts, *Baz* is also required for the formation of adherens junction and the maintenance of epithelial polarity²¹. We next investigated the function of *Baz* in epithelial division. The *baz*(RNAi) embryos showed overall disruption of epithelium organization similar to that observed in *crb*(RNAi) embryos. Unlike in *crb*(RNAi) embryos, however, epithelial cells in *baz*(RNAi) embryos divided in a symmetric fashion, with Pon–GFP distributed uniformly around the cell cortex throughout mitosis and the mitotic spindle orientated in random directions. After cytokinesis, two equally sized daughter cells were produced and Pon–GFP was equally distributed between them (90%, $n = 300$) (Fig. 1d).

As observed previously¹³, however, daughter cell size asymmetry in neuroblast division was largely unaffected in *baz*(RNAi) embryos (data not shown). We also observed that in *crb*(RNAi) epithelial cells *Baz* can still be localized into a crescent but the crescent is mispositioned and that Pon–GFP is always localized to the opposite side of the *Baz* crescent (Fig. 2b). This suggests that, although

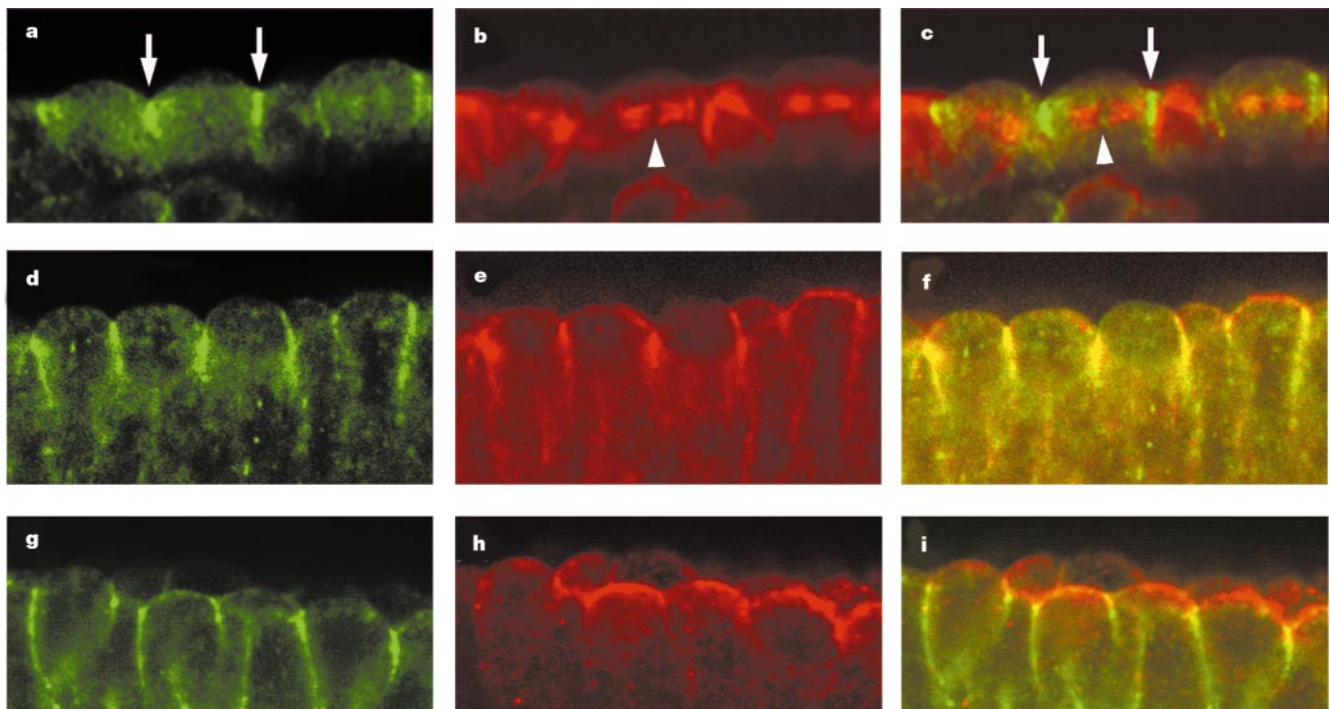


Figure 3 Localization of E-APC to the adherens junction and normal morphology of the adherens junction in epithelial cells in the PNR. **a–c**, E-APC (**a**) and tubulin (**b**) immunostaining. In dividing epithelial cells, the mitotic spindle (arrowhead) is orientated along the planar axis and E-APC is localized at cell–cell contacts (arrows). **d–f**, E-APC (**d**)

and E-cadherin (**e**) immunostaining. E-APC co-localizes with E-cadherin to the adherens junction. **g–i**, E-cadherin (**g**) and Insc (**h**) immunostaining. Epithelial cells within the PNR (Insc positive) exhibit normal adherens junction appearance compared with epithelial cells outside the PNR (compare **e** and **g**). **c**, **f** and **i** are merged images.

mispositioned, Baz is still functional in directing Pon–GFP localization in *crb(RNAi)* embryos. To test whether the coupling of Pon–GFP localization with spindle orientation observed in *crb(RNAi)* embryos is Baz dependent, we performed double RNAi by co-injecting a mixture of *baz* and *crb* dsRNAs. Epithelial divisions in the co-injected embryos looked similar to *baz* single-injected embryos, with Pon–GFP segregated equally between two equally sized daughter cells (80%, $n = 200$) (Fig. 1e). We therefore conclude that epithelial cells depend on Baz to couple spindle orientation with protein localization when the adherens junction is disrupted.

To investigate the molecular mechanism underlying the planar positioning of spindles by the adherens junction, we examined the function of proteins associated with the adherens junction. A ubiquitously expressed, epithelial-cell-enriched APC (E-APC) is localized to the adherens junction (Fig. 3a–f), and, in *shotgun* and *crb* mutants, this adherens junction localization of E-APC is disrupted^{3,22}. The human APC protein interacts with a microtubule-associated EB1 protein^{23,24}, and the yeast homologue of EB1 (Bim1), together with the cortical marker Kar9, has been implicated in a search-and-capture mechanism of spindle positioning^{4,5}. We therefore tested the function of E-APC in epithelial cell division.

In about 60% ($n = 300$) of *E-APC(RNAi)* embryos, we observed that the positioning of Pon–GFP crescent and orientation of mitotic spindle became tightly coupled during epithelial division. At cytokinesis, epithelial cells divided asymmetrically to produce two unequally sized daughter cells, and Pon–GFP was always segregated to the smaller daughter cell (Fig. 4a). The asymmetric segregation of Pon–GFP and the ability to undergo unequal cytokinesis all depend on Baz, because in *baz* and *E-APC* double RNAi embryos, Pon–GFP was equally segregated to two similarly sized daughter cells (data not shown). Therefore, in the absence of E-APC, epithelial cells divide asymmetrically in a Baz-dependent fashion. This suggests that adherens-junction-associated E-APC promotes spindle positioning along the planar axis and prevents the coupling of spindle positioning with asymmetric basal protein localization.

To test whether E-APC functions with EB1 to orientate the mitotic spindle, we performed RNAi on a closely related fly homologue of *EB1* (*dEB1*). In *dEB1(RNAi)* embryos, the epithelial divisions were also asymmetric, producing two unequally sized daughter cells, with Pon–GFP segregated to the smaller cell (Fig. 4b). We observed that the penetrance of *dEB1(RNAi)* phenotype (~20%, $n = 300$) is lower than that of *E-APC(RNAi)*. As there is strong maternal contribution of *dEB1*, the low penetrance might be due to a perdurance of maternal *dEB1* protein. Alternatively, it might be due to functional compensation by two other distantly related EB1 homologues in the fly genome (data not shown). It has been noted that E-APC lacks the carboxy-terminal domain that is required for interaction with EB1 (ref. 3), and we did not detect a direct interaction between E-APC and EB1 in our *in vitro* binding assays. It therefore remains to be determined whether the two are functionally linked together *in vivo* through some cofactor(s), or whether E-APC functions mainly to maintain adherens junction integrity³ and EB1 interacts with other unidentified molecules to orientate spindles.

Our results indicate that two sets of polarity cues exist for spindle positioning in epithelial cells: a planar polarity cue mediated by the adherens junction and an apical–basal polarity cue regulated by Baz. The division pattern of wild-type epithelial cells suggests that the planar polarity cue is normally dominant over the apical–basal polarity cue (Fig. 4c). Epithelial cells within the procephalic neurogenic region (PNR) that express endogenous *Insc* or epithelial cells outside of the PNR that express ectopic *Insc* are known to orientate their mitotic spindle along the apical–basal axis during division⁷. This suggests that the dominance of planar polarity over apical–basal polarity can be overcome by the expression of *Insc*. The normal appearance of the adherens junction in epithelial cells in the PNR (Fig. 3g–i), together with the observation that these cells divide along the planar axis and maintain their normal monolayer

organization in *insc* mutant⁷, suggests that *Insc* functions by strengthening the apical–basal polarity instead of weakening the planar polarity through changing the behaviour of the adherens junction.

When neuroblasts delaminate from the epithelium layer, they undergo morphological changes from columnar to round shape, lose their contacts with the surrounding cells and thus the adherens junction structures. This situation may be reminiscent of epithelial cells in adherens-junction mutants in which the planar polarity cue is lost. In both cases, the Baz-mediated polarity pathway takes over. That one polarity cue can dominate over another cue in orientating division axis may have its precedents in other organisms. Budding yeast can divide in either an axial or a bipolar pattern. Mutations in genes such as *AXL1*, *BUD3*, *BUD4* and *BUD10/AXL2* result in loss of polarity cue for axial bud formation and the cells divide in a

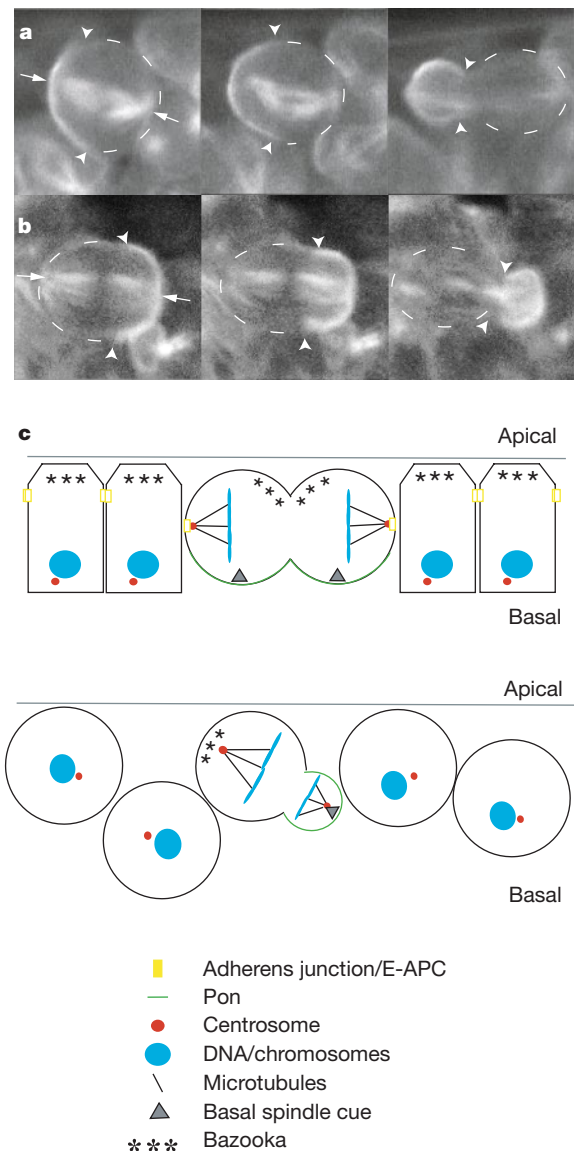


Figure 4 Epithelial divisions in *E-APC* and *dEB-1(RNAi)* mutant embryos. **a, b**, In an *E-APC(RNAi)* (**a**) and a *dEB-1(RNAi)* embryo (**b**), Pon–GFP forms a lateral crescent at mitosis, with the spindle coupled to it. At telophase, Pon–GFP is segregated asymmetrically to the smaller daughter cells. **c**, Model showing the competition between two polarity cues in controlling spindle positioning. In wild-type epithelial cells (top row), the planar polarity cue provided by adherens junction is dominant over the apical–basal polarity cue provided by Baz. When the planar polarity cue is lost, as in *E-APC*, *dEB1*, and *crb* mutant embryos (bottom row), the Baz-mediated asymmetry cue takes over.

bipolar fashion^{25,26}. This suggests that axial and bipolar cues coexist and that the axial cue is normally dominant over the bipolar cue. During mammalian cortical neurogenesis, neural progenitors switch from early symmetric divisions to later asymmetric divisions^{27,28}. It will be interesting to determine whether similar mechanisms and molecules are used to control this division symmetry switch in mammals. Together with some recent studies²⁹, our results on E-APC highlights the importance of tumour suppressors in regulating not only cell growth but also polarity and asymmetric division. □

Methods

Fly stocks and genetics

We used the *UAS-GAL4* system to express ectopically *UAS-Pon-GFP* and *UAS-tau-GFP* (a gift from A. Brand) constructs in epithelial cells under the control of a maternal *V32A-GAL4* driver (a gift from D. St Johnston). For the RNA interference experiment, virgin females from a homozygous recombinant line of *V32A-GAL4* and *UAS-Pon-GFP* were crossed to males from a homozygous *UAS-tau-GFP* line, and embryos were collected for injection. For the overexpression of Crb-intra, we crossed virgin females from a homozygous recombinant line of *V32A-GAL4* and *UAS-Pon-GFP* to males from a homozygous *UAS-Crb-intra* line (a gift from A. Wodarz). For characterization of epithelial cell division in *crumbs* mutant, *V32A-GAL4-UAS-Pon-GFP/+; crb^{11A22}/+* virgin females were crossed to males of the same genotype. Embryos produced from the above cross were aged to stages 9–10 and then processed for *in vivo* imaging study³⁰.

RNAi and *in vivo* imaging

Double-stranded RNAs were produced by *in vitro* transcription using polymerase chain reaction (PCR) products tagged at both ends with T7 RNA polymerase promoter sequences. The following PCR primers were used to generate the templates: dEB1, 5'-GGATCCTAATACGACTCACTATAGGGAGGAGCCAGGAATCATTTAGTTCCTCCG; dEB1, 3'-GGATCCTA ATACGACTCACTATAGGGAGGCGCTCTTTTCCAATCCCTCCAGG; E-APC, 5'-GGATCCTAATACGACTCACTATAGGGAGGAGTCCGGAGGGTG AGCCGCCGGGG; E-APC, 3'-GGATCCTAATACGACTCACTATAGGGAGGTGCTGC AACTTGTAAATAATTAAGCAGCTGGC; crb, 5'-GGATCCTAATACGACTCACTATAGGG AGGT GGAATGGACAACGACTACTGAAGCC; crb, 3'-GGATCCTAATACGACTCACTA TAGGGAGGTAAGTCCGCTATATAGGCATATAGG; baz, 5'-GGATCCTAATAC GACTACTATAGGGAGGCGCTAGTGTCTCCATGGCCCTCGGC. Double-stranded RNAs were injected into *Pon-GFP* and *tau-GFP* transgenic embryos as described¹⁷. Aged embryos were subjected to *in vivo* imaging analysis³⁰.

Immunohistology

For immunostaining of wild-type and *crb(RNAi)* embryos, we processed an overnight collection of mixed-stage wild-type embryos, or *crb* dsRNA-injected embryos aged to stages 10–12 as described¹⁰. We used the following primary antibodies: rabbit anti-EAPC (a gift from M. Bienz), mouse anti-Tubulin (Sigma), rat anti-E-cadherin (a gift from T. Uemura), guinea pig anti-Asense, rabbit anti-Insc (a gift from B. Chia) and mouse anti-GFP (Molecular Probes), rat anti-Bazooka (a gift from A. Wodarz). Images were recorded on a confocal microscope and processed with Photoshop.

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Complexes of MADS-box proteins are sufficient to convert leaves into floral organs

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Genetic studies, using floral homeotic mutants, have led to the ABC model of flower development. This model proposes that the combinatorial action of three sets of genes, the A, B and C function genes, specify the four floral organs (sepals, petals, stamens and carpels) in the concentric floral whorls^{1,2}. However, attempts to convert vegetative organs into floral organs by altering the expression of ABC genes have been unsuccessful^{3–5}. Here we show that the class B proteins of *Arabidopsis*, PISTILLATA (PI) and APETALA3 (AP3), interact with APETALA1

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