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Two separate molecular systems, Dachsous/Fat and Starry night/Frizzled, act independently to confer planar cell polarity

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Planar polarity is a fundamental property of epithelia in animals and plants. In *Drosophila* it depends on at least two sets of genes: one set, the Ds system, encodes the cadherins Dachsous (Ds) and Fat (Ft), as well as the Golgi protein Four-jointed. The other set, the Stan system, encodes Starry night (Stan or Flamingo) and Frizzled. The prevailing view is that the Ds system acts via the Stan system to orient cells. However, using the *Drosophila* abdomen, we find instead that the two systems operate independently: each confers and propagates polarity, and can do so in the absence of the other. We ask how the Ds system acts; we find that either Ds or Ft is required in cells that send information and we show that both Ds and Ft are required in the responding cells. We consider how polarity may be propagated by Ds-Ft heterodimers acting as bridges between cells.

KEY WORDS: *Drosophila*, Planar cell polarity, *dachsous*, *fat*, *four-jointed*, *starry night*, *frizzled*, Mosaic analysis, Abdomen

INTRODUCTION

Most organisms are built of epithelia consisting of cells that are both asymmetric in the apicobasal axis and within the plane of the cell sheet (Fanto and McNeill, 2004; Grebe, 2004). Planar cell polarity (PCP) is shown by the orientation of structures such as hairs in insects (Lawrence, 1966; Strutt, 2003; Saburi and McNeill, 2005), and cilia (Eaton, 1997) and stereocilia in vertebrates (Lewis and Davies, 2002). PCP is also implicated in convergent extension in vertebrate embryos (Wallingford et al., 2002). Genetic and molecular studies in *Drosophila* have identified proteins essential for PCP; these are generally conserved in vertebrates (Klein and Mlodzik, 2005). Here, we use *Drosophila* and build a new logical structure for PCP.

There are two sets of genes involved in PCP: the Stan system and the Ds system. The Stan system depends on a cadherin receptor-like molecule, Starry Night (Stan) (Chae et al., 1999; Usui et al., 1999) and Frizzled (Fz) (Adler et al., 1997), a receptor for Wnts (Wodarz and Nusse, 1998). Other proteins in the Stan system are Diego, Dishevelled, Van Gogh (Vang – FlyBase; also called Strabismus) and Prickle. There are several ideas about how the Stan system might function. A popular model proposes that PCP is determined by asymmetrically localised complexes of Stan system proteins in cell membranes (Strutt, 2002). This asymmetry, which has been observed in some epithelial cells, would be oriented by an unknown graded signal [‘factor X’ (Struhl et al., 1997a)]. Propagation of PCP would be driven by feedback between proteins, the asymmetrical arrangement of proteins in one cell affecting localisation in neighbouring cells (Tree et al., 2002; Amonlirdviman et al., 2005). We have argued (Lawrence et al., 2004) that this view is largely incorrect, and base our opinion mainly on two pieces of evidence. First, cells that completely lack the Fz protein can be polarised by their neighbours – yet, in the asymmetry model the orientation of each cell depends on the differential accumulation and activity of Fz (Tree et al., 2002). Second, flies that lack a crucial component of the feedback mechanism of the Stan system, Prickle, lose the asymmetric localisation of other core proteins – yet, in these flies, disparities in the amounts of Fz still propagate polarity from cell to cell (Lawrence et al., 2004). This result with *prickle* mutant flies has been confirmed in the wing and even extended to wings mutant for *dishevelled* (Strutt and Strutt, 2006).

Our alternative model for the Stan system has four main tenets (Lawrence et al., 2004): (1) Fz activity is normally gently graded from one cell to the next as a response to factor X; (2) a cell becomes polarised by comparing its own level of Fz activity with that of its various neighbouring cells, and pointing hairs towards neighbours with lower levels and away from neighbours with higher levels; (3) the level of Fz activity in any one cell is subject to feedback that adjusts its level to an average of its neighbours – this ‘averaging’ mechanism explains how and why experimentally induced disparities in Fz activity can induce changes in polarity that propagate for several cells; (4) cells perceive differences in their level of Fz activity relative to that of their neighbours through intercellular homodimers made by Stan – hence, Stan is required for cells both to send and to receive this information.

The second set of genes that acts in PCP, the Ds system, encodes two atypical cadherins, Dachsous (Ds) and Fat (Ft), as well as a resident Golgi protein, Four-jointed (Fj) (Strutt et al., 2004). The Ds system, like the Stan system, orients cellular outgrowths. However, unlike the Stan system, it also affects the orientation of cell divisions and organ shape, as well as having some input into growth (Bryant et al., 1988; Baena-López et al., 2005). In an important paper, Yang et al. (Yang et al., 2002) proposed that the polarity genes constitute a linear pathway in which morphogens, such as Wingless (Wg), orient the Ds system. In the eye, this system consists of opposing gradients of Fj and Ds controlled by Wg (Simon, 2004) with Fj first repressing Ds activity and Ds then repressing Ft activity. Yang and colleagues argued that Ft then activates Fz to polarise the Stan system. Thus, the graded activity of the Ds system constitutes factor X, and the Stan system transduces X to polarise cells. This single pathway model of PCP has become accepted and now prevails in the literature on PCP (Adler, 2002; Strutt and Strutt, 2002; Ma et al., 2003; Uemura and Shimada, 2003) (but see Klein and Mlodzik, 2005; Strutt and Strutt, 2005a; Strutt and Strutt, 2005b).

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Experiments in the *Drosophila* abdomen give comparable results with those in the eye. A morphogen, Hedgehog (Hh), appears to be responsible for activity gradients of Fj, Ds and Ft (Casal et al., 2002). As in the eye, the Stan system acts in PCP but there is no evidence as to whether there is a single pathway: Hh→Ds system→Stan system. Experimentally, the abdomen has some advantages over the eye. For example, in the eye, PCP is revealed in the arrangement of cells in entire ommatidia: each an ensemble of photoreceptors, lens and pigment cells. In the abdomen, the polarity of each cell is shown directly by the orientation of hairs produced by that cell alone. Here, we use this advantage to test whether the Ds and Stan systems act as part of a single linear pathway. Our main conclusion is that they do not and that each system deploys a different mechanism to polarise cells and to propagate polarity from cell to cell.

MATERIALS AND METHODS

Mutations and transgenes

Unless stated otherwise, FlyBase (Grumbling et al., 2006) entries of the mutations and transgenes referred in the text are as follows. CD2y$^+$: *Rnor\CD2$^{hs.PJ}$*. hs.FLP: *Scer\FLP1$^{hs.PS}$*. act.fz::GFP: *fz$^{P278L.Act5C.T:Avic\GFP-EGFP}$*.tub.Gal80: *Scer\GAL80$^{alphaTub84B.PL}$*. tub.Gal4: *Scer\GAL4$^{alphaTub84B.PL}$*. ptc.Gal4: *Scer\Gal4$^{ptc-559.1}$*. UAS.GFP: *Avic\GFP$^{Scer\UAS.T:Hsap\MYC,T:SV40nls2.}$*. UAS.fz: fz$^{Scer\UAS.cZa}$ and *fz$^{Scer\UAS.cSa}$*. UAS.ft: *ft$^{Scer\UAS.cMa}$*. UAS.ds: *ds$^{Scer\UAS.cTa}$*. UAS.fj: *fj$^{Scer\UAS.cZa}$*. UAS.Nrt::wg: *Nrt::wg$^{Scer\UAS.T:Ivir\HA1}$*. UAS.fz2DN: *fz2$^{GPI.Scer\UAS.T:Hsap\MYC}$*. UAS.Wnt2: *Wnt2$^{Scer\UAS.cSa}$*. UAS.Wnt4: *Wnt4$^{Scer\UAS.cSa.}$* UAS.Wnt6: *Wnt6$^{Scer\UAS.cSa}$*. UAS.Wnt8: *wntD$^{Scer\UAS.cSa}$*. UAS.Wnt10: *Wnt10$^{Scer\UAS.cSa}$*. fz$^-$: *fz^{15}* or *fz$^{21.}$ Df(3L)fz2*. fz2$^-$: *fz2^{C1}*. ds$^-$: *ds^{UA071}*. *ds^{38K}*. ft$^-$: *ft^{15}*. *ft^{12}*. fj$^-$: *fj^{d1}*. *fj^{N7}*. ptc$^-$: *ptc^{IIW}*. en$^-$: *Df(2)en^E*. FRT39: *P{FRT(w^{hs})}39*. FRT40: *P{neoFRT}40A*. FRT42: *P{neoFRT}42D*. FRT2A: *P{FRT(w^{hs})}2A*. FRT80: *P{neoFRT}80B*. The following are derivatives of *P{UAS-ds.T}* and *P{UAS-ft.M}* (Matakatsu and Blair, 2004), in which the amino acid sequence of the joins are UAS.ectoDs: …**FLFIHMRSRKPR**prp. UAS.ectoFt: …*LGSYVIYRFR*prprp. UAS.ectoDs::endoFt: …**FLFIHMRSRKPR***GKQEKIGSL*…. UAS.ectoFt::endoDs: …*LGSYVIYRFR***PRNAVKPHLAT**… (*ds* sequences are in bold, *ft* sequences are in italics, added sequences are in lower case and transmembrane sequences are underlined). UAS.endoFt: As in *P{UAS-wg.flu}* (Zecca et al., 1996), the *wg* signal peptide is followed by three copies of the HA1 epitope tag, joined to the Ft transmembrane and cytoplasmic domains. The amino acid sequence at the join is...[YPYDVPDYA]s*AAQVADPLSIGFTLVI*… UAS.endoDs: …[YPYDV-PDYA]s**AGGSSGGSIGDWAIGLL**… (the sequence in brackets corresponds to the last flu epitope; the beginning of the transmembrane domains of both proteins is underlined).

The *stan^3/stan^{E59}* allelic combination, the '*stan*$^-$' genotype, was chosen for the following reasons: the amorphic allele *stan^{E59}* is lethal homozygous, owing to a requirement for Stan activity in the nervous system. *stan^{E59}* mutant flies can be rescued by neural expression of the *stan* gene (Lu et al., 1999), but doing this was impractical with our complex genotypes and also open to the criticism that low level expression of the rescuing *UAS.stan* transgene in the epidermis might alleviate the PCP phenotype. We therefore used a hypomorphic allele, *stan^3*, in trans to *stan^{E59}*, a combination devoid of PCP activity in the abdomen (Lawrence et al., 2004) (see Fig. 2). For the key experiments where we generated *UAS.ft* and *ectoDs* clones in *stan*$^-$ flies (genotypes 4, 5, 12), the cells within the clones are *stan^{E59}/stan^{E59}* (the null genotype); only the surrounding cells are *stan^3/stan^{E59}* and yet polarisation still occurred. Nevertheless, under the same conditions, both *UAS.fz* and *UAS.fz UAS.stan* clones failed to repolarise (Lawrence et al., 2004; genotypes 2, 3). Finally, *stan^{E59} UAS.ft* and *stan^{E59} UAS.ectoDs* clones repolarise surrounding *stan^3/stan^{E59}*cells, even in flies that were also *fz*$^-$ (genotypes 18, 19) and indubitably lacking the Stan system.

Clones were induced by heat-shocking third instar larvae for 1 hour at 34, 35 or 37°C. Abdominal cuticles were dissected, mounted in Hoyer's and images captured with Auto-Montage (Syncroscopy) and processed with Adobe Photoshop (Adobe Systems).

Experimental genotypes

(1) *stan*$^-$ *fz*$^-$ clones: *y w hs.FLP; FRT42 act.fz::GFP CD2y$^+$/ FRT42 pwn stan^{E59} sha; fz*$^-$ *ri FRT2A/ fz*$^-$ *CD2y$^+$ ri FRT80*

(2) *tub.Gal4 UAS.stan UAS.fz* clones in *stan*$^-$: *y w hs.FLP; FRT42 stan^3 tub.Gal80 CD2y$^+$/ FRT42 pwn stan^{E59}; UAS.fmi UAS.fz/ tub.Gal4*

(3) *tub.Gal4 UAS.fz* clones in *stan*$^-$: *y w hs.FLP; FRT42 tub.Gal80 stan^3 CD2y$^+$/ FRT42 pwn stan^{E59}; UAS.fz/ tub.Gal4*

(4) *tub.Gal4 UAS.ft* clones in *stan*$^-$: *y w hs.FLP; FRT42 tub.Gal80 stan^3 CD2y$^+$/ FRT42 pwn stan^{E59}; UAS.ft/ tub.Gal4* and

(5) *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; FRT42 tub.Gal80 stan^3 CD2y$^+$/ FRT42 pwn stan^{E59}; UAS.ft/ +*

(6) *tub.Gal4 UAS.ft* clones: *y w hs.FLP/ w; FRT42 tub.Gal80 CD2y$^+$/ FRT42 pwn; UAS.ft/ tub.Gal4* and

(7) *y w hs.FLP tub.Gal4 UAS.GFP/y w hs.FLP; FRT42 pwn/ ds*$^-$ *FRT42 tub.Gal80 CD2y$^+$; UAS.ft/ +* and

(8) *y w hs.FLP; FRT42 pwn/ ds*$^-$ *FRT42 tub.Gal80 CD2y$^+$; UAS.ft/ tub.Gal4*

(9) *ft*$^-$ clones in *stan*$^-$: *y w hs.FLP; ft*$^-$ *stc FRT39 stan^{E59}/ CD2y$^+$ FRT39 stan^3*

(10) *tub.Gal4 UAS.ds* clones: *y w hs.FLP/ w; FRT42 tub.Gal80 CD2y$^+$/ FRT42 pwn; UAS.ds/ tub.Gal4*

(11) *tub.Gal4 UAS.ectoDs* clones: *y w hs.FLP; FRT42 tub.Gal80 CD2y$^+$/ FRT42 pwn sha; UAS.ectodDs/ tub.Gal4*

(12) *tub.Gal4 UAS.ectoDs* clones in *stan*$^-$: *y w hs.FLP tub.Gal4 UAS.GFP /y w hs.FLP; FRT42 pwn stan^{E59} sha/ FRT42 tub.Gal80 stan^3 CD2y$^+$; UAS.ectoDs/ +*

(13) *tub.Gal4 UAS.fj* clones in *stan*$^-$: *y w hs.FLP tub.Gal4 UAS.GFP; FRT42 tub.Gal80 stan^3 CD2y$^+$/ FRT42D pwn stan^{E59}; UAS.fj/ +*

(14) *tub.Gal4 UAS.ft* clones in *fz*$^-$: *y w hs.FLP tub.Gal4 UAS.GFP/ y hs.FLP; FRT42 tub.Gal80 CD2y$^+$; UAS.ft FRT42 pwn; fz*$^-$ *ri FRT2A/ fz*$^-$ *CD2y$^+$ ri FRT2A* and

(15) *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; FRT42 tub.Gal80 CD2y$^+$/ FRT42 pwn sha; fz*$^-$ *CD2y$^+$ UAS.ft/ fz*$^-$ *ri FRT2A*

(16) *ft*$^-$ clones in *fz*$^-$: *y hs.FLP; ft*$^-$ *stc FRT39/ CD2y$^+$ FRT39; fz*$^-$*/ fz*$^-$ *trc FRT2A*

(17) *tub.Gal4 UAS.ectoDs* clones in *fz*$^-$: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; FRT42 tub.Gal80 CD2y$^+$/FRT42 pwn sha; fz*$^-$ *CD2y$^+$ UAS.ectoDs/ fz*$^-$ *ri FRT2A*

(18) *tub.Gal4 UAS.ft* clones in *stan*$^-$ *fz*$^-$: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; FRT42 pwn stan^{E59} sha/ FRT42 tub.Gal80 stan^3 CD2y$^+$; fz*$^-$ *CD2y$^+$ UAS.ft ri FRT2A/ fz*$^-$ *CD2y$^+$ ri FRT80*

(19) *tub.Gal4 UAS.ectoDs* clones in *stan*$^-$ *fz*$^-$: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; FRT42 pwn stan^{E59} sha/ FRT42 tub.Gal80 stan^3 CD2y$^+$; fz*$^-$ *CD2y$^+$ UAS.ectoDs ri FRT2A/ fz*$^-$ *CD2y$^+$ ri FRT80*

(20) *fz*$^-$ clones in *ds*$^-$: *y w hs.FLP12; ds*$^-$ *FRT39/ In(2LR)bw^{V1}; fz*$^-$ *trc ri FRT2A/ CD2y$^+$ hs.GFP ri FRT2A*

(21) *tub.Gal4 UAS.fz* clones in *ds*$^-$: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; ds*$^-$ *FRT42 pwn/ ds*$^-$ *FRT42 Gal.80 CD2y$^+$; UAS.fz CD2y$^+$/ +* and

(22) *y w hs.FLP122 tub.Gal4 UAS.GFP/ y w hs.FLP122; ds*$^-$ *ck FRT40/ ds*$^-$ *tub.Gal80 FRT40; UAS.fz fz*$^-$ *fz2^{C1} FRT2A/ +*

(23) *tub.Gal4 UAS.stan* clones in *ds*$^-$: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; ds*$^-$ *FRT42 pwn/ ds*$^-$ *FRT42 tub.Gal80 CD2y$^+$; UAS.stan CD2y$^+$/ +*

(24) *tub.Gal4 UAS.fz* clones in *ft*$^-$: *y w hs.FLP; ft*$^-$ *FRT42 pwn sha/ft^{12} FRT42 tub.Gal80 CD2y$^+$; UAS.fz/tub.Gal4*

(25) *hh.Gal4 UAS.fz* in *ds*$^-$: *y w hs.FLP122; ds*$^-$ *ck FRT40/ In(2LR)bw^{V1}, ds*$^-$*; hh.Gal4/ UAS.fz fz*$^-$ *fz2*$^-$ *FRT2A*

(26) *2xfz$^+$ clones in ds*$^-$*/ ds*$^-$*; fz$^+$/fz*$^-$: *y w hs.FLP; ds*$^-$*; CD2y$^+$ trc ri FRT2A/ fz*$^-$ *Df(3L)fz2 FRT2A*

(27) *fz*$^-$ *tub.Gal4 UAS.ft* clones: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; UAS.ft FRT42 pwn; tub.Gal80 FRT2A/ fz*$^-$ *trc ri FRT2A*

(28) *tub.Gal4 UAS.fz* clones in *ds*$^-$ *fz*$^-$: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; ds*$^-$ *FRT42 sha/ ds*$^-$ *FRT42 tub.Gal80 CD2y$^+$; fz*$^-$ *CD2y$^+$ ri FRT2A UAS.fz/ fz*$^-$ *CD2y$^+$ ri FRT80*

(29) *tub.Gal4 UAS.ectoDs* in *ds*$^-$: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; ds*$^-$ *tub.Gal80 FRT40/ ds*$^-$ *ck FRT40; UAS.ectoDs/ +* and

(30) *y w FL122; ds⁻ CD2y⁺ FRT42 pwn sha/ ds⁻ FRT42 tub.Gal80 CD2y⁺; UAS.ectoDs/ tub.Gal4*
(31) *tub.Gal4 UAS.ds* in *ds⁻*: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; ds⁻ tub.Gal80 FRT40/ ds ck FRT40; UAS.ds/ +* and
(32) *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; ds⁻ FRT42 pwn/ ds⁻ FRT42 tub.Gal80 CD2y⁺; UAS.ds/ +*
(33) *tub.Gal4 UAS.ft* in *ds⁻*: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; ds⁻ tub.Gal80 FRT40/ ds⁻ ck FRT40; UAS.ft/ +* and
(34) *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; ds⁻ FRT42 pwn/ ds⁻ FRT42 tub.Gal80 CD2y⁺; UAS.ft/ +*
(35) *tub.Gal4 UAS.ectoDs* clones in *ft⁻*: *y w hs.FLP; ft⁻ FRT42 pwn sha/ft^{12} FRT42 tub.Gal80 CD2y⁺; UAS.ft/tub.Gal4*
(36) *tub.Gal4 UAS.ft* clones in *ft⁻*: *y w hs.FLP; ft⁻ FRT42 pwn sha/ft^{12} FRT42 tub.Gal80 CD2y⁺; UAS.ft/tub.Gal4*
(37) *tub.Gal4 UAS.fz UAS.ft* clones in *ds⁻*: *y w hs.FLP; ds⁻ CD2y⁺ FRT42 pwn sha/ ds⁻ FRT42 tub.Gal80 CD2y⁺; UAS.fz UAS.ft/ tub.Gal4*
(38) *tub.Gal4 UAS.fz UAS.ds* clones in *ds⁻*: *y w hs.FLP; ds⁻ CD2y⁺ FRT42 pwn sha/ ds⁻ FRT42 tub.Gal80 CD2y⁺; UAS.fz UAS.ds/ tub.Gal4*
(39) *tub.Gal4 UAS.ft UAS.fz* clones in *ft⁻*: *y w hs.FLP; ft⁻ FRT42 pwn sha/ft^{12} FRT42 tub.Gal80 CD2y⁺; CD2y⁺ UAS.ft UAS.fz/tub.Gal4*
(40) *stan⁻*: *y w hs.FLP/ +; stan^{3}/ ds⁻ CD2y⁺ FRT42 pwn stan^{E59} sha*
(41) *ds⁻*: *y w hs.FLP; ds⁻ tub.Gal80 FRT40/ ds⁻ CD2y⁺ FRT42 pwn stan^{E59} sha*
(42) *ds⁻ stan⁻*: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; ds⁻ tub.Gal80 FRT40 stan^{3}/ ds⁻ CD2y⁺ FRT42 pwn stan^{E59} sha*
(43) *ds⁻ ft⁻* clones: *y w hs.FLP/ y; ds⁻ ft⁻ stc FRT39/ CD2y⁺ FRT39*
(44) *ds⁻ ft⁻ tub.Gal4 UAS.ds* clones: *y w hs.FLP; ds⁻ ft⁻ stc FRT39/ tub.Gal80 CD2y⁺ FRT39; UAS.ds/ tub.Gal4*
(45) *ds⁻ ft⁻ tub.Gal4 UAS.ft* clones: *y w hs.FLP; ds⁻ ft⁻ stc FRT39/ tub.Gal80 CD2y⁺ FRT39; UAS.ft/ tub.Gal4*
(46) *ds⁻ tub.Gal4 UAS.ectoDs* clones: *y w hs.FLP; ds⁻ stc FRT39/ tub.Gal80 CD2y⁺ FRT39; UAS.ectoDs/ tub.Gal4*
(47) *ft⁻ tub.Gal4 UAS.ectoDs* clones: *y w hs.FLP; ft⁻ stc FRT39/ tub.Gal80 CD2y⁺ FRT39; UAS.ectoDs/ tub.Gal4*
(48) *ds⁻ ft⁻ tub.Gal4 UAS.ectoDs* clones: *y w hs.FLP; ds⁻ ft⁻ stc FRT39/ tub.Gal80 CD2y⁺ FRT39; UAS.ectoDs/ tub.Gal4*
(49) *tub.Gal4 UAS.ectoFt* clones: *y w hs.FLP; FRT42 pwn sha/ FRT42 tub.Gal80 CD2y⁺; UAS.ectoFt/ tub.Gal4*
(50) *ft⁻ tub.Gal4 UAS.ft* clones: *y w hs.FLP; ft⁻ stc FRT39/ tub.Gal80 CD2y⁺ FRT39; UAS.ft/ tub.Gal4*
(51) *ft⁻ tub.Gal4 UAS.ectoFt* clones: *y w hs.FLP; ft⁻ stc FRT39/ tub.Gal80 CD2y⁺ FRT39; UAS.ectoFt/ tub.Gal4*
(52) *ds⁻ ft⁻ tub.Gal4 UAS.ectoFt* clones: *y w hs.FLP; ds⁻ ft⁻ stc FRT39/ tub.Gal80 CD2y⁺ FRT39; UAS.ectoFt/ tub.Gal4*
(53) *tub.Gal4 UAS.ectoDs::endoFt* clones: *y w hs.FLP; FRT42 pwn sha/ FRT42 tub.Gal80 CD2y⁺; UAS.ectoDs::endoFt / tub.Gal4*
(54) *ds⁻ tub.Gal4 UAS.ectoDs::endoFt* clones: *y w hs.FLP; ds⁻ stc FRT39/ tub.Gal80 CD2y⁺ FRT39; UAS. ectoDs::endoFt/ tub.Gal4*
(55) *ft⁻ tub.Gal4 UAS.ectoDs::endoFt* clones: *y w hs.FLP; ft⁻ stc FRT39/ tub.Gal80 CD2y⁺ FRT39; UAS. ectoDs::endoFt/ tub.Gal4*
(56) *ds⁻ ft⁻ tub.Gal4 UAS.ectoDs::endoFt* clones: *y w hs.FLP; ds⁻ ft⁻ stc FRT39/ tub.Gal80 CD2y⁺ FRT39; UAS. ectoDs::endoFt/ tub.Gal4*
(57) *tub.Gal4 UAS.ectoFt::endoDs* clones: *y w hs.FLP; FRT42 pwn sha/ FRT42 tub.Gal80 CD2y⁺; UAS.ectoFt::endoDs/ tub.Gal4*
(58) *ds⁻ tub.Gal4 UAS.ectoFt::endoDs* clones: *y w hs.FLP; ds⁻ stc FRT39/ tub.Gal80 CD2y⁺ FRT39; UAS.ectoFt::endoDs/ tub.Gal4*
(59) *ft⁻ tub.Gal4 UAS. ectoFt::endoDs* clones: *y w hs.FLP; ft⁻ stc FRT39/ tub.Gal80 CD2y⁺ FRT39; UAS.ectoFt::endoDs/ tub.Gal4*
(60) *ds⁻ ft⁻ tub.Gal4 UAS. ectoFt::endoDs* clones: *y w hs.FLP; ds⁻ ft⁻ stc FRT39/ tub.Gal80 CD2y⁺ FRT39; UAS.ectoFt::endoDs/ tub.Gal4*
(61) *ptc.Gal4 UAS.endoDs*: *y w hs.FLP; Sp/ fj⁻ ptc.Gal4; UAS.endoDs/ +*
(62) *ptc.Gal4 UAS.endoFt*: *y w hs.FLP; Sp/ fj⁻ ptc.Gal4; UAS.endoFt/ +*
(63) *ds⁻ fj⁻*: *ds⁻ fj⁻/ ds^{38K} fj^{N7}*
(64) *tub.Gal4 UAS.fj* clones in *ds⁻*: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; ds⁻ tub.Gal80 FRT40/ ds⁻ ck FRT40 UAS.fj*
(65) *ft⁻ tub.Gal4 UAS.fj* clones: *y w hs.FLP; ft⁻ stc FRT39/ tub.G80 CD2y⁺ FRT39; UAS.fj/ tub.Gal4*
(66) *ds⁻ ft⁻ tub.Gal4 UAS.fj* clones: *y w hs.FLP; ds⁻ ft⁻ stc FRT39/ tub.G80 CD2y⁺ FRT39; UAS.fj/ tub.Gal4*
(67) *ds⁻ tub.Gal4 UAS.fj* clones: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; tub.Gal80 FRT40/ ds⁻ ck FRT40 UAS.fj*
(68) *tub.Gal4 UAS.fj UAS.ds* clones: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; FRT42 tub.Gal80 CD2y⁺/ FRT42D pwn UAS.fj; UAS.ds/ +*
(69) *tub.Gal4 UAS.fj UAS.ectoDs* clones: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; FRT42 tub.Gal80 CD2y⁺/ FRT42D pwn UAS.fj; UAS.ectoDs / +*
(70) *tub.Gal4 UAS.ft UAS.ds* clones: *y w hs.FLP122 tub.Gal4 UAS.GFP/ y w hs.FLP; FRT42 tub.Gal80/ FRT42 pwn; UAS.ft/ UAS.ds*
(71) *tub.Gal4 UAS.ft UAS.ectoDs* clones: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; FRT42 tub.Gal80/ FRT42D pwn sha; UAS.ft/ UAS.ectoDs*
(72) *tub.Gal4 UAS.ft* clones in *fj⁻*: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; FRT42 pwn fj⁻ / FRT42 tub.Gal80 fj⁻; UAS.ft/ +*
(73) *tub.Gal4 UAS.ectoDs* clones in *fj⁻*: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; FRT42 pwn fj⁻/ FRT42 tub.Gal80 fj⁻; UAS.ectoDs/ +*
(74) *tub.Gal4 UAS.ds* clones in *fj⁻*: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; FRT42 pwn fj⁻/ FRT42 tub.Gal80 fj⁻; UAS.ds/ +*
(75) *ptc⁻ en⁻* clones in *stan⁻*: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; FRT42 pwn ptc⁻ stan^{E59} en ⁻/ FRT42 tub.Gal80 stan^{3} CD2y⁺*
(76) *ptc⁻ en⁻* clones in *fz⁻*: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; FRT42 pwn cn ptc⁻ en ⁻/ FRT42 tub.Gal80; fz⁻ CD2y⁺ ri FRT2A/ fz⁻ ri FRT2A*
(77) *ptc⁻ en⁻* clones in *ds⁻*: *y w hs.FLP/ w; ds⁻ FRT42 CD2y⁺/ ds⁻ FRT42 pwn ptc⁻ en ⁻*
(78) *ptc⁻ en⁻* clones in *ds⁻ stan⁻*: *y w hs.FLP tub.Gal4 UAS.GFP/ y w hs.FLP; ds⁻ CD2y⁺ FRT42 pwn ptc⁻ stan^{E59} en ⁻/ ds⁻ FRT42 tub.Gal80 stan^{3} CD2y⁺*
(79) *tub.Gal4 UAS.wg clones* in *ds⁻*: *y w hs.FLP; ds⁻ ck FRT40/ ds⁻ tub.Gal80 FRT40; UAS.wg/ +* and
(80) *y w hs.FLP; ds⁻ FRT42 pwn sha/ds⁻ FRT42 tub.Gal80 CD2y⁺; UAS.wg/tub.Gal4*
(81) *tub.Gal4 UAS.Nrt::wg* clones in *ds⁻*: *y w hs.FLP; ds⁻ ck FRT40/ ds⁻ tub.Gal80 FRT40; UAS.Nrt::wg/ +*
(82) *tub.Gal4 UAS.fz2DN* clones in *ds⁻*: *y w hs.FLP; ds⁻ ck FRT40/ ds⁻ tub.Gal80 FRT40; UAS.fz2DN/ +*
(83) *tub.Gal4 UAS.Wnt2* clones in *ds⁻*: *y w hs.FLP; ds⁻ ck FRT40/ ds⁻ tub.Gal80 FRT40; UAS.Wnt2/ +* and
(84) *y w hs.FLP; ds⁻ FRT42 pwn sha/ds⁻ FRT42 tub.Gal80 CD2y⁺; UAS.Wnt2/tub.Gal4*
(85) *tub.Gal4 UAS.Wnt3* clones in *ds⁻*: *y w hs.FLP; ds⁻ FRT42 pwn sha/ds⁻ FRT42 tub.Gal80 CD2y⁺; UAS.Wnt3/tub.Gal4*
(86) *tub.Gal4 UAS.Wnt4* clones in *ds⁻*: *y w hs.FLP; ds⁻ ck FRT40/ ds⁻ tub.Gal80 FRT40; UAS.Wnt4/ +* and
(87) *y w hs.FLP; ds⁻ FRT42 pwn sha/ds⁻ FRT42 tub.Gal80 CD2y⁺; UAS.Wnt4/tub.Gal4*
(88) *tub.Gal4 UAS.Wnt6* clones in *ds⁻*: *y w hs.FLP; ds⁻ FRT42 pwn sha/ds⁻ FRT42 tub.Gal80 CD2y⁺; UAS.Wnt6/tub.Gal4*
(89) *tub.Gal4 UAS.Wnt8* clones in *ds⁻*: *y w hs.FLP; ds⁻ FRT42 pwn sha/ds⁻ FRT42 tub.Gal80 CD2y⁺; UAS.Wnt8/tub.Gal4*
(90) *tub.Gal4 UAS.Wnt10* clones in *ds⁻*: *y w hs.FLP; ds⁻ FRT42 pwn sha/ds⁻ FRT42 tub.Gal80 CD2y⁺; UAS.Wnt10/tub.Gal4*
(91) *ptc⁻ en⁻ stan⁻* clones in *ds⁻*: *y w hs.FLP; ds⁻ FRT42 pwn ptc⁻ en ⁻ stan^{E59}/ ds⁻ FRT42 tub.Gal80; tub.Gal4/ +*

RESULTS

The dorsal abdomen

The dorsal epidermis of the adult abdomen is segmented and divided into a chain of anterior (A) and posterior (P) compartments. The epithelium secretes pigmented plates (tergites), made by the A compartments and separated by strips of more flexible cuticle; most of the cells make cuticular hairs or bristles that point posteriorly. Cells in the P compartment secrete the morphogen Hh that controls cell polarity (and cell type) in the A compartment. Here, we focus on the A compartment (Fig. 1). The

vectors and extents of the gradients shown in Fig. 1 are derived from experiments with genetic mosaics: for example, just in *front* of a clone of *ds*$^-$ cells, wild-type hairs point the 'wrong' way (forwards). This, we argue, is because the normal grade of Ds activity (high at the back of the A compartment, low at the front), is locally reversed across the clonal border. At the back of the clone, the effects are concordant with the normal grade and therefore polarity is not altered. Similarly, clones of cells in which *ds* is overexpressed (henceforth called *UAS.ds* clones) make the hairs behind the clone point forwards, because, there, the normal grade of Ds activity is reversed. The corresponding experiments with *fj* and *ft* give similar results, except that the sign is opposite (*ft*$^-$ and *fj*$^-$ clones cause the polarity of wild-type cells to reverse behind the clones, and *UAS.fj* (Casal et al., 2002) and *UAS.ft* clones reverse in front of the clones). For the experiments described below, the genotypes are referred to by number (1-91; see also Fig. 8 for a summary of all results).

Is there a linear and causal relationship between the Ds and Stan systems?

If the linear relationship were correct, cells that lack the Stan system should not support propagation of polarity changes caused by disparities in the Ds system. Indeed, in the eye, the repolarising abilities of *fj*$^-$, *ds*$^-$ and *ft*$^-$ clones all appear to be blocked in the absence of *fz* (Yang et al., 2002) (but see Discussion). However, experiments in the abdomen lead to a different conclusion. Stan is required in both 'sending' and 'receiving' cells for the transmission of polarising information induced by differences in Fz activity: *stan*$^-$, *stan*$^-$ *fz*$^-$ and *stan*$^-$ *UAS.fz* clones do not repolarise their wild-type neighbours (genotype 1) (Lawrence et al., 2004) and neither *UAS.fz* nor *UAS.fz UAS.stan* clones repolarise surrounding *stan*$^-$ cells (genotypes 2, 3, Fig. 2B). These experiments show that, with respect to PCP, the Stan system is completely disabled by the *stan*$^-$ genotypes we have used (see Materials and methods). Nevertheless, we find *UAS.ft* clones in *stan*$^-$ flies reverse the polarity of cells anterior to the clone, particularly posteriorly within the A compartment (genotypes 4, 5, Fig. 2D), as they do in wild-type flies (genotypes 6-8, Fig. 3A). In addition, *ft*$^-$ clones in *stan*$^-$ flies (genotype 9) can reverse the polarity of cells behind the clone, as they do in wild-type flies (Casal et al., 2002). The repolarisations caused by gain or loss of Ft in clones have a similar range in both wild-type and in *stan*$^-$ flies, extending a few cell diameters away from the clones.

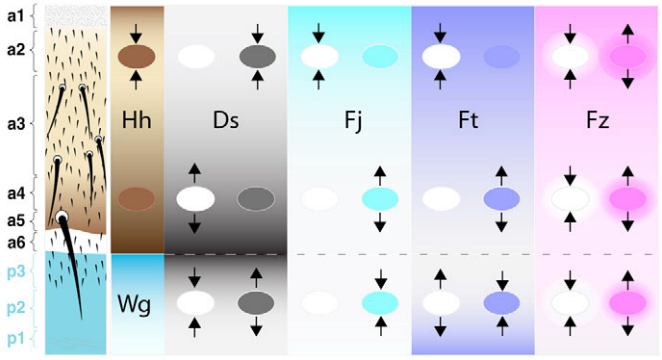

Fig. 1. A summary of polarising gradients in the abdomen. On the left are the types of cuticle in the A (black, a1-a6) and in the P compartment (blue, p3-p1). The compartments are patterned by morphogen gradients; Hh in A and Wg in P (Struhl et al., 1997a; Lawrence et al., 2002), which set up the Ds gradients and also the activity gradients of Fj and Ft (Casal et al., 2002). Clones (ovals) that lack or overexpress a gene affect the polarity of the surrounding wild-type cells (arrows). *ptc*$^-$ *en*$^-$ clones (brown) constitutively activate the Hh transduction pathway and produce reversal of the wild-type cells behind the clones (but only near the middle of the A compartment, where they cause a large discrepancy in the Hh transduction pathway between the clone and the surround). Loss of *ds* reverses the polarity of cells anterior to those clones located at the back of the A compartment (where the level of Ds activity is high) but has no effect on clones located at the front (where Ds activity is low). Overexpression of Ds has the opposite effects: repolarising only behind clones located near the front of the A compartment. The effects of clones involving Fj and Ft are opposite in sign to those involving Ds. In contrast to the other genes, clones involving Fz have similar effects wherever they are situated. We conjecture there is an alteration in Fz activity that spreads out from the clones as the surrounding wild-type cells readjust their levels of Fz activity by an averaging process (haloes) (Lawrence et al., 2004). This difference of clonal behaviours points to a distinction between the Ds and Stan systems.

We find comparable results for Ds: *UAS.ds* clones have only weak effects in wild-type flies (genotype 10). However, a form of Ds that lacks the cytosolic domain ('ectoDs') is more potent, so that *UAS.ectoDs* clones usually reverse the polarity of wild-type cells behind the clone, with a range of several cells (genotype 11, Fig. 3C). We have used ectoDs to test whether repolarisation caused by ectopic Ds activity depends on the Stan system, and find that it does not: in *stan*$^-$ flies, *UAS.ectoDs* clones reverse cell polarity strongly behind the clone (genotype 12, Fig. 2F). *UAS.fj* clones in *stan*$^-$ flies (genotype 13) also repolarise in front, as they do in wild-type flies. Thus, at least in the A compartment, signals coming from *UAS.ft*, *ft*$^-$, *UAS.ectoDs* and *UAS.fj* clones are effective and can propagate over several cell diameters through *stan*$^-$ territory. It follows that the Ds system has an intrinsic capacity to repolarise cells, even when the Stan system is incapacitated.

Our conclusion in the abdomen using *stan*$^-$ contrasts with results in the eye, using *fz*$^-$ (Yang et al., 2002). We therefore repeated the *UAS.ft*, *ft*$^-$ and *UAS.ectoDs* experiments described above in a *fz*$^-$ background (genotypes 14-17). For *UAS.ft* clones in *fz*$^-$ flies, we find that hairs in front are disturbed or reversed, although the effects are less consistent than in *stan*$^-$ flies. For *ft*$^-$ and *UAS.ectoDs* clones in *fz*$^-$ flies, hairs behind are reversed, as observed in *stan*$^-$ flies. We then made *UAS.ft* and *UAS.ectoDs* clones in *stan*$^-$ *fz*$^-$ flies (genotypes 18, 19) and, again, the clones repolarise nearby hairs in front and behind, respectively – the *UAS.ectoDs* clones have the strongest effects, reorienting the hairs around the clone over a long range (see Fig. S1 in the supplementary material). These results show that the Ds system can initiate and propagate PCP, even when the functions of both key components of the Stan system are abolished, indicating that the Ds system can confer and propagate PCP without the participation of the Stan system.

In the absence of the Ds system, cells are more responsive to the Stan system

If the two systems were independent, the extent of repolarisation caused by disparities in one system might be limited or overcome by the normal and opposing action of the other system. Indeed, in *ds*$^-$ wings, *fz*$^-$ clones repolarise surrounding cells over a longer range than they do in wild-type wings (Adler et al., 1998). Similarly, an ectopic gradient of Fz expression repolarises cells over an increased range when Ft is absent (Ma et al., 2003). In agreement, we find that in the abdomen, repolarisations induced by *fz*$^-$, *UAS.fz* or *UAS.stan* clones show a longer range in *ds*$^-$ (Fig. 2A; genotypes 20-23), and also when *UAS.fz* clones are made in *ft*$^-$ (genotype 24), than they do

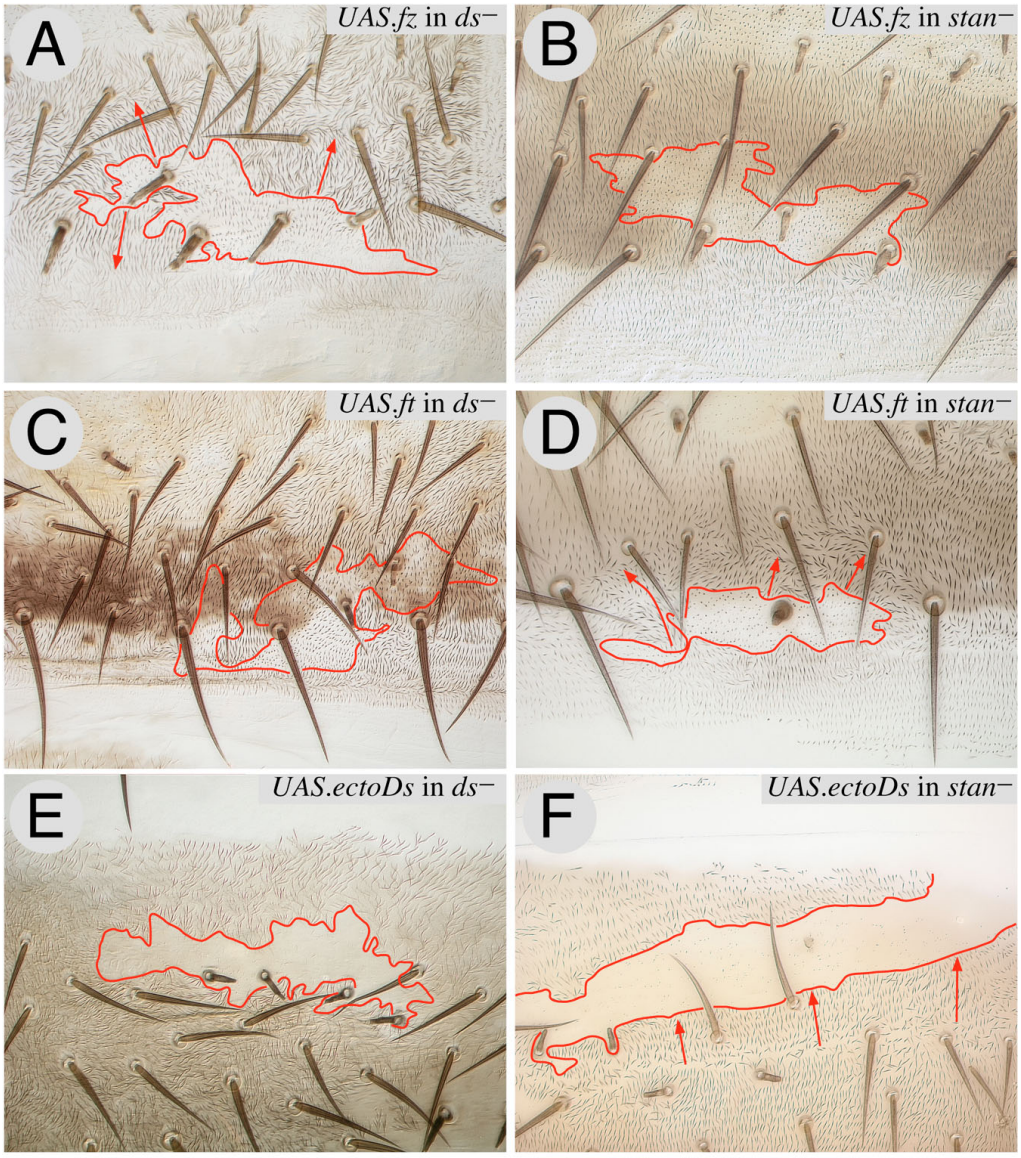

Fig. 2. The Ds and Stan systems are different and independent. Comparison of the effects of over-producing Fz, Ft and ectoDs (a particularly potent signalling form of Ds) in clones in flies lacking either the Ds (A,C,E) or the Stan (B,D,F) systems. (**A**) Clones overexpressing Fz (*UAS.fz*) reverse the polarity of wild-type cells over a short range (Lawrence et al., 2004) but they reverse polarity of *ds*$^{-}$ cells over a longer range. (**B**) *UAS.fz* clones have no effect in *stan*$^{-}$ flies. (**C**) *UAS.ft* clones reverse the polarity of wild-type cells in front of the clone (see Fig. 3A), but have no effect in *ds*$^{-}$ flies; (**D**) the same clones reverse polarity of *stan*$^{-}$ flies. (**E**) Clones overexpressing ectoDs reverse the polarity of wild-type cells behind the clone (see Fig. 3C), but have no effect in *ds*$^{-}$ flies. (**F**) These *UAS.ectoDs* clones reverse polarity in *stan*$^{-}$ flies. Clones are marked with *pwn* (A-D) and *pwn sha* (E,F). Anterior is towards the top, red lines outline the clone and red arrows indicate the polarity imposed on cells outside the clone.

in wild-type flies. In addition, if *UAS.fz* is driven in the entire P compartment (genotype 25), reversal at the back of the A compartment is greater in *ds*$^{-}$ than in wild-type flies. Finally, a weak disparity in Fz activity that does not repolarise cells in wild-type flies is sufficient to repolarise cells over several cell diameters in *ds*$^{-}$ flies (genotype 26, Fig. 4A). This same disparity can even induce a little repolarisation in *ds*$^{-}$/*ds*$^{+}$ flies (Fig. 4B).

Conflicts between the Ds and Stan systems can affect the sign or range of repolarisation.

Normally, *UAS.ft* clones in the A compartment reverse the polarity of cells in front of the clone and do so most strongly when located at the rear of the compartment, where endogenous Ft is least active. Conversely, *fz*$^{-}$ clones reverse the polarity of cells behind the clones, wherever they arise (Lawrence et al., 2004). Thus, in the A compartment, clones of *fz*$^{-}$ *UAS.ft* cells (genotype 27) will create opposing disparities in the Ds and Stan systems, and send conflicting outputs to the adjacent wild-type cells. We find that, at the front of the A compartment, they reverse posteriorly, behaving like *fz*$^{-}$ clones. At the back of the A compartment, however, *fz*$^{-}$ *UAS.ft* clones reverse anteriorly, as do *UAS.ft* clones. This can be explained as follows. For the Stan system, repolarisation is driven by the *difference* in Fz activity across the clone/background interface, which appears to be of similar strength all along the AP axis (Fig. 1). For the Ds system, the strength of the disparity in Ft activity between *UAS.ft* clones and the surround depends on position, being least at the front and greatest at the back of the A compartment (Fig. 1). Thus, in the anterior region, the repolarisation caused by Fz overcomes the weaker opposing influence of *UAS.ft*. At the rear of the A compartment, the effect caused by the Ds system is the stronger.

UAS.fz clones in wild-type flies reverse polarity in front of the clone, creating a conflict with the Ds system: this conflict appears to limit the range of repolarisation caused by such clones, as that range increases in *ds*$^{-}$ flies. In *fz*$^{-}$ flies, *UAS.fz* clones change the polarity of only the adjacent cells (Lawrence et al., 2004). If *UAS.fz* clones in *ds*$^{-}$ flies were using only the Stan system to drive long-range repolarisation, then *UAS.fz* clones in *ds*$^{-}$ *fz*$^{-}$ flies should behave exactly as they do in *fz*$^{-}$ flies, and they do: only one cell is repolarised (genotype 28).

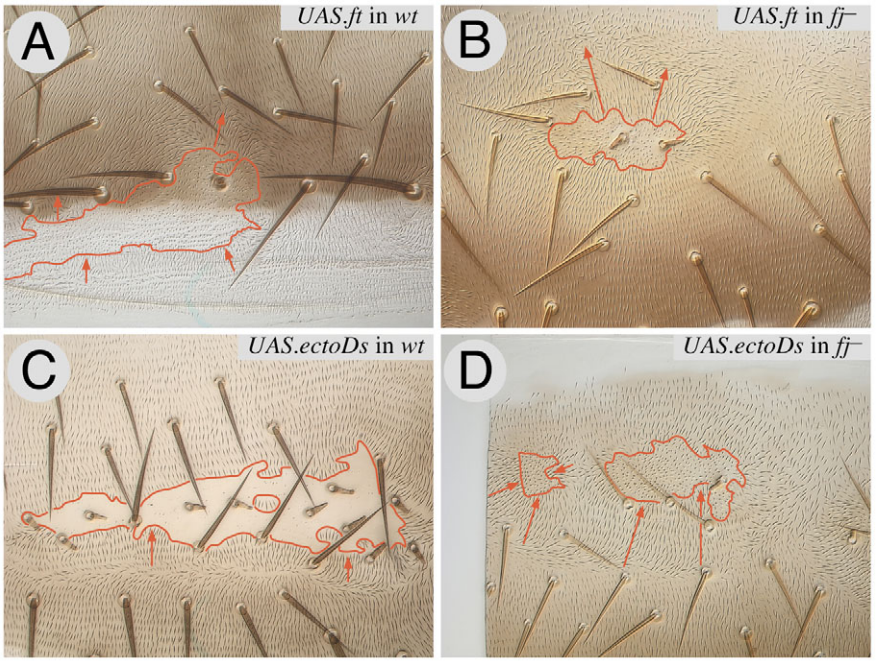

Fig. 3. The range of repolarisations caused by the Ds system is increased in *fj*⁻ flies. (**A-D**) Comparison of the effects of *UAS.ft* clones (reversing polarity in front of the clone in the A compartment and behind in the P compartment) (Casal et al., 2002) and *UAS.ectoDs* clones (reversing polarity behind) in wild-type flies (A,C) with the same types of clones in *fj*⁻ flies (B,D). The range in *fj*⁻ flies is increased. Clones marked with *pwn* (A,B,D) and with *pwn sha* (C). Anterior is towards the top, red lines outline the clone and red arrows indicate the polarity imposed on cells outside the clone.

Disparities in the Ds system do not bias the Stan system

The experiments above show that the Ds system can polarise cells independently of the Stan system. However, the Stan system might still be biased by the Ds system. To assess whether there is normally any input from the Ds system into the Stan system, we generated clones expressing *UAS.ectoDs*, *UAS.ds* or *UAS.ft* in *ds*⁻ flies (genotypes 29-34), and also clones expressing *UAS.ectoDs* or *UAS.ft* in *ft*⁻ flies (genotypes 35-36), and asked whether such clones repolarise surrounding mutant cells. The responding mutant cells are particularly sensitive to small disparities in activity of the Stan system (Fig. 4A); hence, if these types of clones were to bias the Stan system, either within the clone or across the border, they should repolarise the surround, in either *ds*⁻ or in *ft*⁻ animals. Nevertheless, they do not, not even changing the polarity of one cell in either *ds*⁻ (Fig. 2C,E) or in *ft*⁻ flies. We know that *UAS.ds*, *UAS.ectoDs* and *UAS.ft* are effective constructs even in the absence of endogenous Ds and Ft – when these constructs are expressed in *ds*⁻ *ft*⁻ clones, they repolarise surrounding wild-type cells (see below). As positive controls, we added *UAS.fz* separately to both *UAS.ft* and *UAS.ds* clones in *ds*⁻ flies (genotypes 37, 38) and then the long-range repolarisation normally induced by *UAS.fz* clones in *ds*⁻ flies (Fig. 2A) was seen. Likewise, when *UAS.fz* was added to clones expressing *UAS.ft* in *ft*⁻ flies, these clones again caused long-range repolarisation (genotype 39). Thus, the failure of *UAS.ds*, *UAS.ectoDs*, and *UAS.ft* clones to repolarise surrounding cells in *ds*⁻ or *ft*⁻ animals argues that the Stan system is not biased by the Ds system.

Cell polarity in the absence of both the Ds and Stan systems

If the Ds and Stan systems give independent inputs into PCP, the loss of either system might compromise polarity, but the loss of both systems should cause more damage. This is so: *stan*⁻ flies have almost normal hair polarities in the tergite, apart from near the front and near the rear (genotype 40; Fig. 5C); and in *ds*⁻ tergites, hair polarities are normal apart from whorls in the middle (genotype 41; Fig. 5A). The phenotype of *ds*⁻ *stan*⁻ flies is more extreme than in either *ds*⁻ or *stan*⁻, and hair and bristle polarity is randomised throughout the tergite (genotype 42, Fig. 5B). Similar results are observed for the ventral dentical pattern of the third instar larva: the double mutant condition is more severe than in either single mutant (Fig. 5E-G).

Polarisation depends on the balance of Ds and Ft activity in signal-sending cells

We now ask how the Ds system, when acting on its own, can affect PCP. The Ds system has three components and all appear to be graded in activity (Fig. 1). Either *ds*⁻ or *ft*⁻ clones can initiate polarity changes that spread into wild-type territory (Casal et al., 2002), but clones that lack both *ds* and *ft* do not cause repolarisations (genotype 43). Adding back either *UAS.ds* or *UAS.ft* to *ds*⁻ *ft*⁻ clones restores their ability to repolarise, with *UAS.ds* reversing polarity behind the clone and *UAS.ft* in front (genotypes 44, 45). These results suggest that an imbalance (from the normal ratio) of Ds and Ft proteins in the 'sending' cells changes polarity in the wild-type 'receiving' cells that then spreads further. The sending cell, in particular, does not need *both* Ds and Ft in order to repolarise nearby wild-type cells – the presence of either protein alone will do so.

Ds and Ft are both needed in the receiving cell

ds⁻ or *ft*⁻ clones both cause polarity changes in neighbouring wild-type cells. However, inside regions of such clones, the hairs are oriented in whorls, resembling small regions of entire *ds*⁻ or *ft*⁻ flies (Casal et al., 2002) (J.C., P.A.L. and G.S., unpublished), suggesting that the polarity outside the clone cannot propagate into territory lacking either Ds or Ft. Other experiments confirm this: as we have seen, *UAS.ds*, *UAS.ectoDs* and *UAS.ft* clones in *ds*⁻ flies all fail to repolarise, not even changing the polarity of those *ds*⁻ cells adjacent to the clone (Fig. 2C,E). Moreover, *UAS.ectoDs* and *UAS.ft* clones in *ft*⁻ flies also fail to repolarise any *ft*⁻ cells outside the clone. Together, these experiments show that cells need both Ds and Ft in order to receive and respond to a polarity signal initiated by the Ds system, even when that signal comes from immediate neighbours.

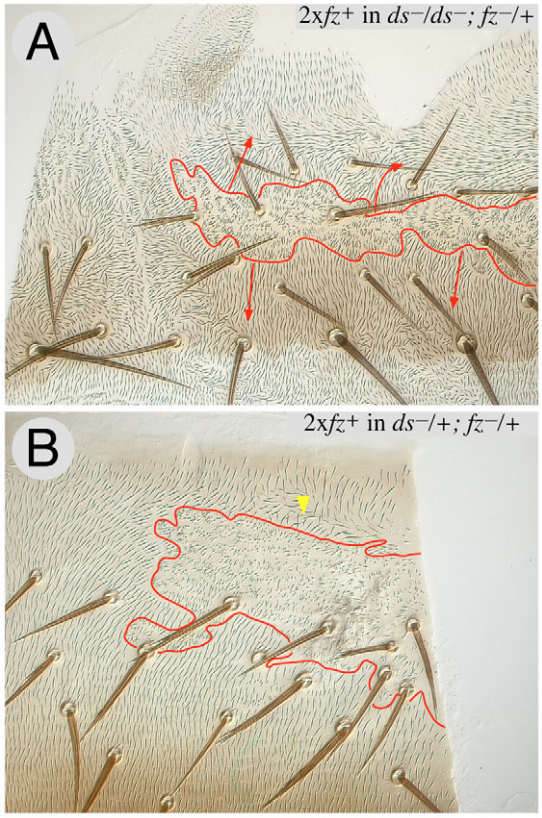

Fig. 4. Cells respond more to the Stan system in the absence of the Ds system. A twofold increase in the dose of the *fz* gene (between clone and surround) has no effect in wild-type flies (not shown) but, in *ds*⁻ flies, reverses polarity in front of the clone and imposes normal polarity behind the clone (**A**). Only a small effect (yellow arrowhead) is seen in a *ds*⁺/*ds*⁻ fly (**B**). Clones are marked with *trc*. Anterior is towards the top, red lines outline the clone and red arrows indicate the polarity imposed on cells outside the clone.

The ectodomains, not the endodomains, of Ft and Ds determine the sign of polarity

As described above, *UAS.ectoDs* clones repolarise surrounding cells as do *UAS.ds* clones (reversing behind), only more potently (Fig. 2F, Fig. 3C). The same is true for *UAS.ectoDs* clones that are also *ds*⁻, *ft*⁻ or *ds*⁻ *ft*⁻ and therefore lack one or both the endogenous proteins (genotypes 46-48) – presenting the Ds ectodomain on the surface of the sending cell is alone sufficient to change the polarity of the receiving cells. However, the Ft ectodomain cannot act alone: although *UAS.ectoFt* clones (genotype 49) behave similarly to *UAS.ft* and *ft*⁻ *UAS.ft* clones (genotype 50), *ft*⁻ *UAS.ectoFt* and *ds*⁻ *ft*⁻ *UAS.ectoFt* clones (genotypes 51, 52) behave, respectively, like *ft*⁻ or *ds*⁻ *ft*⁻ clones. Thus, the capacity of ectoFt to repolarise nearby cells also requires endogenous Ft in the sending cell, supporting suggestions that Ft may form cis-homodimers (Matakatsu and Blair, 2006).

Can the cytosolic domains influence the sign of the signal? We swapped them to make two chimaeric molecules, ectoDs::endoFt and ectoFt::endoDs and found the answer to be no. Clones expressing these proteins behaved as if they expressed the native protein with the same ectodomain, reversing hairs behind strongly (ectoDs::endoFt, genotypes 53-56) or in front (ectoFt::endoDs, genotypes 57-60), either when expressed in cells that were otherwise wild type, or were *ds*⁻, *ft*⁻ or *ds*⁻ *ft*⁻. However, the Ds and Ft endodomains are not always interchangeable: the endodomain of Ft cannot substitute for that of Ds in limiting the potency of the signal (*UAS.ectoDs* and *UAS.ectoDs::endoFt* clones repolarise strongly, whereas *UAS.ds* clones repolarise weakly). Nevertheless, the endodomain of Ds can substitute for the endodomain of Ft to allow the ectoFt protein to signal in the absence of endogenous Ft: *ds*⁻ *ft*⁻ *UAS.ectoFt::endoDs* clones reverse the polarity of cells in front of the clone, whereas *ds*⁻ *ft*⁻ *UAS.ectoFt* clones do not. We also made forms of Ds and Ft that lack the ectodomains (*UAS.endoDs* and *UAS.endoFt*). If endoDs or endoFt are expressed in wild-type cells (genotypes 61, 62), we see no alteration in polarity – however, some rescue of polarity was reported when endoFt was expressed in a *ft*⁻ mutant background (Matakatsu and Blair, 2006). The key finding is that Ds and Ft can each signal on their own, and that the nature of that signal is governed by the ectodomain.

Fj modulates the range of propagation due to the Ds system by acting through Ft

Fj acts in a graded fashion and appears to repress Ds and promote Ft activity (Zeidler et al., 1999; Casal et al., 2002; Yang et al., 2002). In the abdomen, *ds*⁻ *fj*⁻ flies (genotype 63) resemble *ds*⁻ flies, and *UAS.fj* clones have no effect on polarity in *ds*⁻ flies (genotype 64). *UAS.fj* clones in the tergite normally repolarise wild-type cells in front (Casal et al., 2002), but *UAS.fj* clones that are also *ft*⁻ or *ds*⁻ *ft*⁻ do not (genotypes 65, 66). These findings indicate that Fj works through Ds and/or Ft to polarise cells.

However, other results argue that Fj works specifically through Ft and not Ds: unlike *ft*⁻ *UAS.fj* clones, *ds*⁻ *UAS.fj* clones repolarise strongly in front (genotype 67), more strongly than clones that are simply *ds*⁻. In addition, *UAS.fj* clones behave like *UAS.ft* clones and reverse the polarity of cells in front, even when they co-express *UAS.ds* (genotypes 68,70) or *UAS.ectoDs* (genotypes 69,71). Thus, Fj appears to promote Ft to signal, irrespective of whether Ds is absent, or whether it is overexpressed.

To gain more insight into Fj, we made *UAS.ft*, and *UAS.ectoDs* clones in *fj*⁻ flies (genotype 72 and 73). The lack of Fj enhances the effects of both proteins: repolarisations can spread further than in any other situation we have seen, with a range of up to about 10 cells (Fig. 3B,D). By contrast, the action of *UAS.ds* clones is not enhanced in *fj*⁻ flies (genotype 74).

Dual control of the Ds and Stan systems by Hedgehog

According to the linear model of PCP, morphogens such as Hh in the abdomen or Wg in the eye, control polarity by establishing gradients of the Ds system, which then bias the Stan system. But, if the Ds and Stan systems are independent, we must now ask does Hh signalling bias both systems, or only one? To answer this, we used clones of *patched*⁻ (*ptc*⁻) cells in which the Hh transduction pathway is constitutively activated in all cells within the clone. Unfortunately, *ptc*⁻ clones can cause complex effects by ectopically inducing *engrailed* (*en*), leading to a Hh-secreting P compartment forming near the middle of the A compartment (Struhl et al., 1997b; Lawrence et al., 1999)! We avoided these problems by using *ptc*⁻ *en*⁻ clones (Lawrence et al., 1999; Lawrence et al., 2002). Such clones reverse the polarity of wild-type cells behind the clone, allowing us to test whether activation of the Hh transduction pathway can polarise cells via either, or both, the Stan and Ds systems.

ptc⁻ *en*⁻ clones cause reversal of polarity behind in *stan*⁻ (genotype 75, Fig. 6C), *fz*⁻ (genotype 76), and *ds*⁻ flies (genotype 77, Fig. 6A). However, *ptc*⁻ *en*⁻ clones do not reverse polarity in *ds*⁻

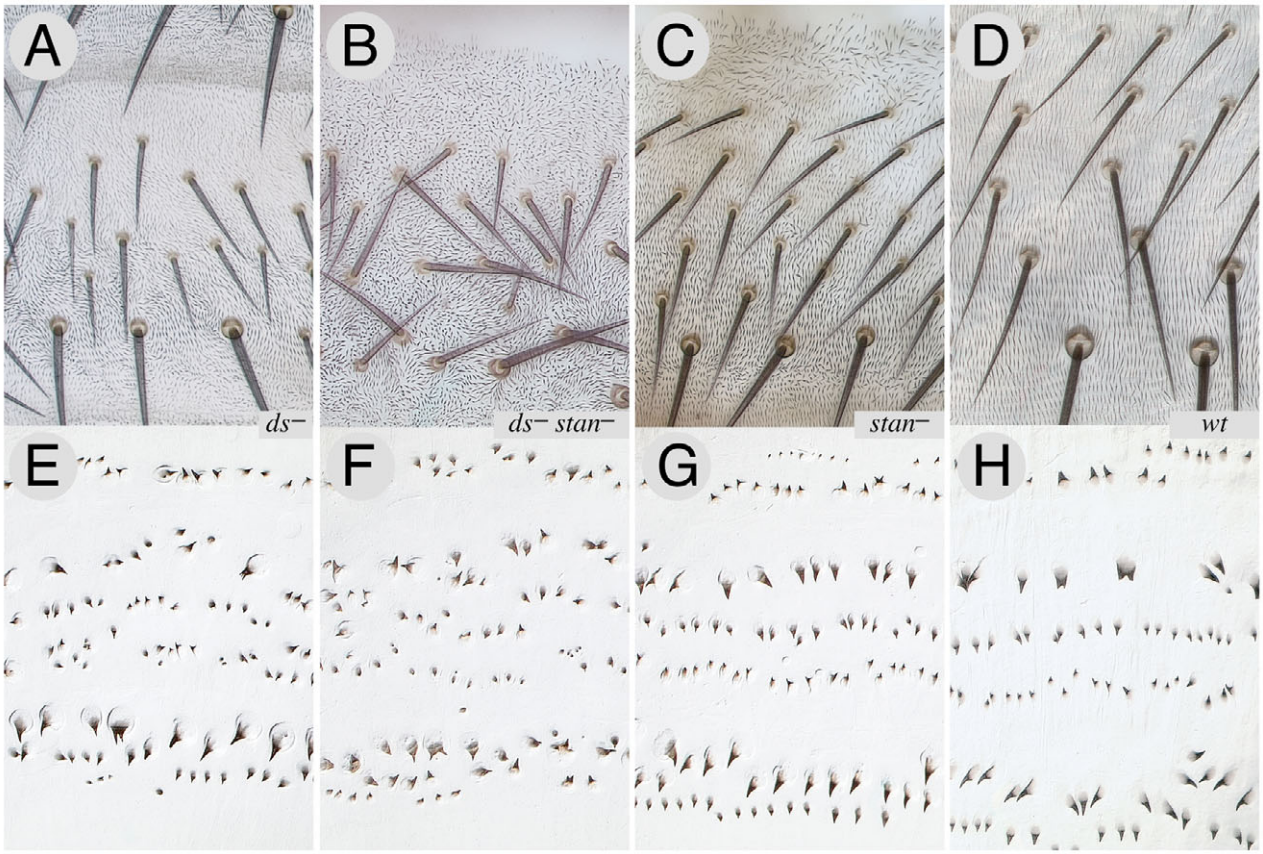

Fig. 5. The loss of one or both systems leads to different adult and larval phenotypes. (**A-D**) *ds*⁻ tergites have a whorly central area but the bristle pattern is near normal (A), whereas (C) *stan*⁻ tergites are dishevelled at the front and back in the A compartment, but near normal elsewhere. (B) In *ds*⁻ *stan*⁻ tergites, both the hairs and bristles are dishevelled everywhere. (D) A normal cuticle is shown for comparison. (**E-H**) In the 3rd instar larvae, *ds*⁻ have disturbed hairs in the anterior rows of the ventral denticles, but the most posterior rows 5 and 6 are normal (E). The *stan*⁻ larval denticle pattern (G), as far as we can see [compare with Price et al. (Price et al., 2006)] is like wild type (H), whereas the *ds*⁻ *stan*⁻ larvae (F) show randomised polarity. Note, for A-D, adult cuticles were mounted without squashing in order to preserve bristle orientation in its native state.

stan⁻ flies (genotype 78, Fig. 6B). It follows that Hh signalling polarises cells in the tergite largely, or only, via the Ds and Stan systems, and that it does so by means of two distinct inputs into PCP.

For the Ds system it seems that Hh governs cell polarity, at least in part, by driving the graded expression of the transcription factor Omb (Lawrence et al., 2002), which (probably) controls transcription of *ds*. For the Stan system, Hh presumably biases the activity of Fz (Lawrence et al., 2004) but it is not clear how it does so. It did not escape anyone's notice that Fz is a Wnt receptor and therefore many suggested that Wg or some other Wnt might be an intermediary. Several experiments argued against this possibility (Wehrli and Tomlinson, 1998; Lawrence et al., 2002), but they were all carried out in wild-type flies, where an active Ds system might have blocked any effect. Therefore, we made clones of cells that express *UAS.wg*, *UAS.Nrt::wg* (a membrane-tethered form of Wg), *UAS.fz2DN* (a membrane-tethered form of the Wg-binding domain of Fz2 to manipulate the distribution of Wg) and the remaining six *Drosophila* Wnts (*UAS.Wnt2*, *3*, *4*, *6*, *8* and *10*) in *ds*⁻ flies, but they induced no repolarisation (genotypes 79-90). These results argue against all known Wnt genes, notably Wg itself, as being polarising factors for the Stan system.

DISCUSSION

Many epithelia exhibit planar cell polarity (PCP), but examples from *Drosophila* have been studied in most depth (reviewed by Klein and Mlodzik, 2005). It was proposed long ago (Lawrence, 1966; Stumpf, 1966) that the vectors of a pervasive gradient orient PCP and here we examine how this is achieved. In the current and prevailing model, a morphogen gradient (for example, Hh or Wg) organises the expression of *fj* and *ds* to set up Ds system gradients (Casal et al., 2002; Simon, 2004). Then, small differences in Ds system activity from one cell to the next are thought to feed into Fz and bias the Stan system. The Stan system is then thought to act more directly on the cell to orient structures, such as ommatidia or hairs (Yang et al., 2002; Ma et al., 2003). Here, we test this model in the abdomen and find our results do not support the main part of it; instead they argue that the morphogen gradient acts separately on the Ds and Stan systems to generate two independent inputs into PCP.

The Ds system can polarise cells independently of the Stan system

The case for the Ds system polarising cells via the Stan system rested on epistasis experiments in the eye: disparities in the Ds system, such as clones of *ds*⁻ or *ft*⁻ cells, repolarise cells in wild-type flies, but not in *fz*⁻ flies. This requirement for Fz suggested that the Ds system

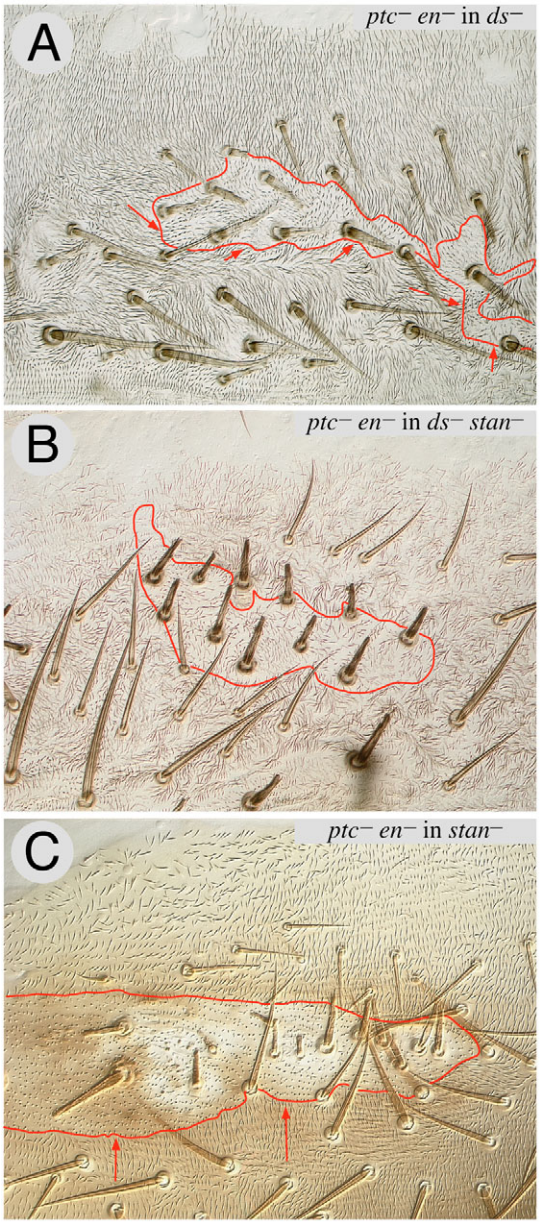

Fig. 6. *ptc*⁻ *en*⁻ clones in flies lacking one or both systems. (**A-C**) The Hh signal transduction pathway is maximally and constitutively activated in *ptc*⁻ *en*⁻ clones. Such clones reverse the polarity of hairs behind the clone both in *ds*⁻ flies (A) and in *stan*⁻ flies (C). However in *ds*⁻ *stan*⁻ flies, the *ptc*⁻ *en*⁻ have no discernable (consistent) effect on the surround (B) compared with A where there is a consistent effect: the hairs pointing inwards all around the clone. Clones marked with *pwn*. Anterior is towards the top, red lines outline the clone and red arrows indicate the polarity imposed on cells outside the clone.

might act via Fz (Yang et al., 2002). However, we find that, in the dorsal abdomen, the Ds system can polarise cells without the Stan system. We present several lines of evidence, but the most crucial is that clones of *UAS.ft* or *UAS.ectoDs* cells, both of which repolarise surrounding wild-type cells up to several cell rows away, also do so in *stan*⁻, *fz*⁻ or *stan*⁻ *fz*⁻ flies. It follows that the Ds system, acting alone and using Ds and Ft, can drive changes in the polarity of surrounding cells. This conclusion raises new questions: how does the Ds system produce and propagate polarising information without any involvement of the Stan system? What polarises the Stan system? How do cells integrate the two separate inputs from the Ds and Stan systems?

How does the Ds system produce and propagate polarising information?

The discovery that *fz*⁻ clones can change the polarity of nearby wild-type cells was important (Gubb and Garcia-Bellido, 1982; Vinson and Adler, 1987) and many attempts have been made to explain it: most models invoke feedback to amplify initial biases in Fz activity, within or between cells. Now we have shown that, independently of the Stan system, disparities in the Ds system can repolarise cells; yet the two systems employ fundamentally different molecules. How does the Ds system act?

First, morphogen gradients (Hh in A, Wg in P) (Lawrence et al., 2002) appear to polarise the Ds system by grading the amount and/or state of activity of three components of the system: Ds, Ft and Fj (Casal et al., 2002). Second, we find that cells can 'send' information by presenting either Ds or Ft to 'receiving' neighbours. Thus, both Ds and Ft appear to have ligand-like signalling activities that can repolarise surrounding cells. This signal appears to depend on the ratio of Ds to Ft in the sending cell (in the tergite, hairs made by the receiving cell point towards neighbours with a higher Ds/Ft ratio). It is not clear how this ratio is encoded but it presumably determines how much free Ds or Ft the sending cell presents to neighbours (Fig. 7). Third, we have shown that in order to respond to this signal by changing their polarity, the receiving cells need both Ds and Ft, indicating that Ds and Ft both have receptor-like and ligand-like properties and defying any simple categorisation of Ds as a ligand and Ft as a receptor. More relevant, perhaps, is the evidence that Ds and Ft can form trans-heterodimers that bridge adjacent cells both in culture and in vivo, and that Ds or Ft proteins become concentrated along cell interfaces in which the abutting cell presents only Ft or Ds, respectively (Strutt and Strutt, 2002; Ma et al., 2003). Furthermore, accumulation of either Ds or Ft along one cell surface, in response to excess Ft or Ds presented on the abutting surface, may lead to the depletion of Ds or Ft along the remaining surfaces of the same cell (Strutt and Strutt, 2002; Ma et al., 2003), localising and limiting the potential to form trans-heterodimeric bridges with other cells. These properties suggest a model in which Ds and Ft are required in the receiving cells both to respond to and to propagate polarising information (Fig. 7). For example, in *UAS.ft* clones, the more active Ft is presented by the sending cell, the greater amount of Ds would be drawn to the facing membrane of the receiving cell, leaving less Ds and more free Ft on the opposite face of the receiving cell (Fig. 7). Fourth, we ask how the amplitude of the signal is determined. The range depends on where (in the compartment) the clones are made, indicating that the degree of discrepancy between Ft and Ds levels in the clone and in the surrounding cells is a key factor. The range of repolarisation also depends on Fj, possibly acting on Ft to promote the formation of heterodimers. Thus, with *UAS.ft* clones in a *fj*⁻ background, in which heterodimers should be sparse because the activity of Ft is low, there would be a large discrepancy across the clone border that should produce a long-range effect, as observed. The same clones in a wild-type background should have a smaller discrepancy and therefore a shorter range (Fig. 7). In both wild-type and *fj*⁻ flies, excess ectoDs sends a much stronger signal than excess Ds, suggesting that the cytosolic domain may have an inhibitory function.

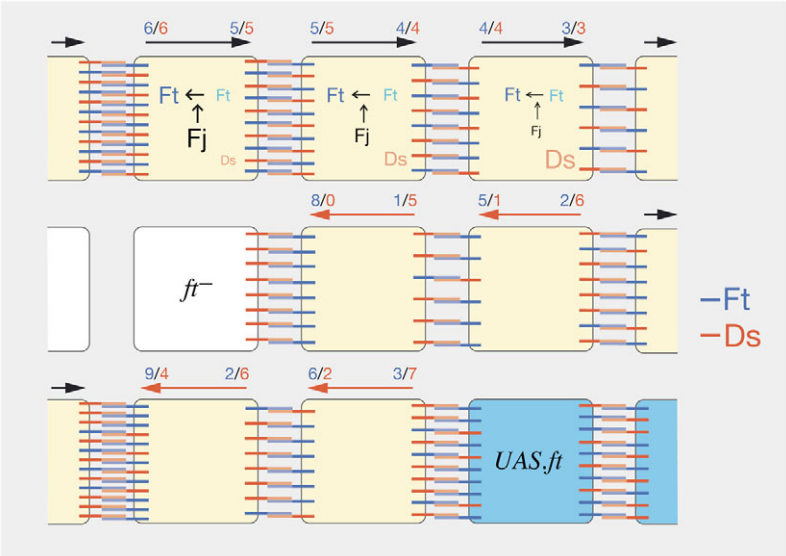

Fig. 7. A speculative model of the Ds system. The A compartment, anterior is towards the left. Ft is indicated in blue and Ds in red. The long arrows indicate the polarity of each cell: normal in black and reversed in red. In the wild type (top), there is evidence for a gradient of Ds (Ds, light red) increasing from anterior to posterior, and of opposing gradients of Fj and Ft activity (Casal et al., 2002), as indicated by the size of the letters. Although there is no gradient of Ft protein (Ft, light blue), we envisage a gradient of Ft activity (Ft, dark blue), driven by the action of Fj on Ft. Active Ft could become stabilised in the membrane of one cell so that it can form trans-heterodimers with Ds in the next cell (provided that sufficient Ds is present there). Only those molecules of Ft and Ds that form trans-heterodimers are shown; free Ft and Ds, as well as other possible forms of Ds and Ft (e.g. cis-complexes) are not shown, even though they may be in excess (the Ds protein gradient peaks posteriorly, but the gradient of Ds molecules engaged in trans-heterodimers peaks anteriorly). The polarity of a cell might depend on a comparison between the number of Ds molecules (red numbers above the cells) that are engaged in trans-heterodimers on the anterior and posterior faces of the cell, with the polarity of that cell pointing down the differential (from high to low, as shown). The probability of forming trans-heterodimers might depend on the availability of active free Ft, as well as on free Ds on abutting cell surfaces, which in turn could depend on graded Fj activity (driving the production of active Ft), on graded Ds protein accumulation, and even the possibility that Ds and Ft might form cis-heterodimers on the same cell surface. The middle row shows the effect of a *ft*⁻ cell, in which all Ds will be available to make trans-heterodimers with Ft on the facing (anterior) membrane of the wild-type cell on its right. Consequently, in this wild-type cell, Ft engagement in trans-heterodimers will be promoted along the anterior face. Conversely, the absence of Ft protein in the *ft*⁻ cell will deprive Ds on the surface of the abutting wild-type cell of binding partners, and allow abnormally high levels of Ds to be recruited into trans-heterodimers on the opposite (posterior) face. This excess of Ds molecules will then bind to Ft in the next most (more posterior) cell, and again, by depleting Ds from its anterior face, will repolarise it. This effect will weaken from cell to cell. The lower row shows a *UAS.ft* cell that will attract more Ds to the facing membrane (posterior) of the neighbour on its left, thereby polarising that cell, the effect spreading anteriorwards.

How do cells integrate the two separate inputs from the Ds and Stan systems?

At first sight, the tergites might seem exceptional, for here the Ds system can polarise cells in the absence of the Stan system – yet neither in the ventral pleura nor in the wing do *UAS.ft* or *UAS.ectoDs* clones repolarise cells that lack the Stan system. Thus, we now ask whether our results represent a fundamental property that is obscured in other places, or a special case that applies only to the tergite. Our results tell that the Ds system has an inherent capacity to confer and propagate PCP, and we rate this positive result as decisive, suggesting that the apparent failure of the Ds system to act independently in other parts of the fly could be explained in other ways. There are several possible explanations.

First, if cells normally integrate separate inputs from the Ds and Stan systems, the lack of one system might, in some places, interfere with the response to the other system. For example, in the pleura, as in the eye, polarity is randomised in the absence of the Stan system (Zheng et al., 1995; Wehrli and Tomlinson, 1998; Yang et al., 2002; Lawrence et al., 2004) and it may be impossible for the Ds system to reorganise polarity where there is such a strong requirement for the Stan system. Second, there are qualitative differences in the outputs of the two systems: the Ds system being involved in growth, cell shape and cell affinity (Bryant et al., 1988; Adler et al., 1998; Matakatsu and Blair, 2006); the Stan system not affecting these properties and instead possibly placing asymmetric structures, such as actin filaments. These differences might help explain why the Ds system can, even in the absence of the Stan system, reorient hairs in some tissues. Third, experiments that create conflicts between the Ds and Stan systems can lead to varying outcomes even in the tergite, depending on the location of the clones (e.g. *fz*⁻ *UAS.ft* clones, see Results). Perhaps cell polarity is a composite property (like height in humans!): the orientation of hairs being the deceptively simple outcome of diverse inputs. At the least our results show the linear pathway, Ds system→Stan system, is wrong in the tergite and challenge its universality.

The behaviour of *ptc*⁻ *en*⁻ clones is pertinent because they repolarise surrounding cells by means of both systems. In wild-type flies, these clones reverse behind in the A compartment. The type of cuticle made by *ptc*⁻ *en*⁻ clones corresponds to the back of the A compartment and it is here that we believe the Ds activity should normally peak and Ft activity should be minimal (Casal et al., 2002) – thus, it makes sense for *ptc*⁻ *en*⁻ clones to resemble *UAS.ectoDs* or *ft*⁻ clones. Similarly, as cells in the tergite make hairs that point towards neighbours with lower Fz activity, it makes sense that *ptc*⁻ *en*⁻ clones behave like *fz*⁻ clones: this is because all hairs in the wild-type A compartment point towards the back of the compartment, where Hh signalling peaks and where our model calls for Fz activity to be minimal (Lawrence et al., 2004).

The ability of *ptc*⁻ *en*⁻ clones to repolarise surrounding cells in *ds*⁻ flies provides an intriguing hint as to how Hh signalling might feed into the Stan system: we have made *ptc*⁻ *en*⁻ *stan*⁻ clones and these clones do not repolarise in *ds*⁻ flies (genotype 91), in contrast to *ptc*⁻ *en*⁻ clones. This result suggests that Hh might polarise the Stan system by acting via Ptc to regulate Fz activity, a mechanism that would depend on the *ptc*⁻ *en*⁻ cells communicating their altered level of Fz activity to their wild-type neighbours via Stan. If this were so, then Hh would be a component of the elusive Factor X!

Finally, we need to address why the Stan system proteins can be induced to form abnormal asymmetric distributions by manipulating the Ds system; for example, *ft*⁻ clones in the wing contain abnormally polarised cells that also show corresponding changes in the

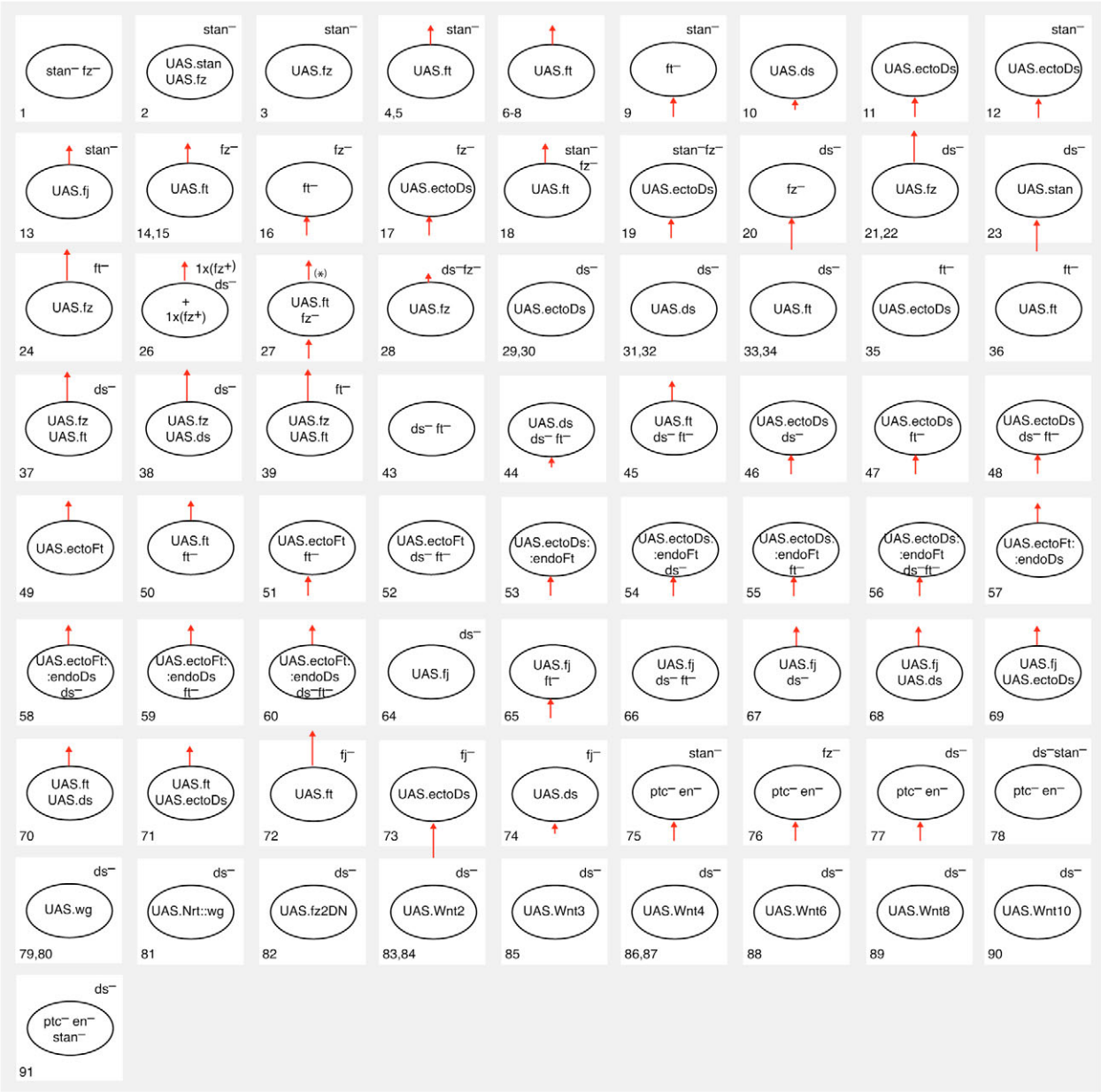

Fig. 8. A summary of the experiments. Results are shown for the tergite. Reversal of polarity is shown by arrows of different lengths, indicating the range, of one, several (two to four) or many cells (up to 10). The background genotype (e.g. *fz*⁻) is shown outside the clone but also applies to the clone itself. The numbers refer to the genotypes listed in the Materials and methods. The asterisk refers to *UAS.ft fz*⁻ clones that reverse polarity in front only when located at the posterior of the A compartment (see text).

distribution of Dishevelled (Strutt and Strutt, 2002; Ma et al., 2003). For us this presents no problem, as we have argued that the asymmetric accumulation of the Stan system proteins is an outcome not a cause of polarity (see Introduction) (see also Lawrence et al., 2004). Hence, if cells are reoriented by perturbing the Ds system, whatever polarity they adopt will show in both the asymmetric localisation of Stan system proteins and in the orientation of the hairs.

Registration of the Ds and Stan gradients

The Ds and Stan system gradients are not congruent – yet another argument that they are independent. The Ds system consists of two gradients with opposing slopes: the Ds activity peaking at the back of the A compartment, and declining forwards into the A compartment and backwards into P (Fig. 1) (Casal et al., 2002). By contrast, the Stan system appears to be a monotonic gradient of Fz activity with A and P cells both pointing down the gradient. An unsolved problem is the registration of a Fz activity gradient that presumably repeats once per metamere: do its borders coincide with segmental or parasegmental borders? We do not know, but two systems with different spatial registrations may solve the tricky problem of how cell polarity is maintained across boundaries.

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Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/133/22/4561/DC1

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