# Requirement for Pangolin/dTCF in *Drosophila* Wingless signaling

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**The Wingless (Wg) protein is a secreted glycoprotein involved in intercellular signaling. On activation of the Wg signaling pathway, Armadillo is stabilized, causing target genes to be activated by the transcription factor Pangolin (Pan). This study investigated the roles of Pan in the developing wing of *Drosophila* by clonal analysis. Three different aspects of wing development were examined: cell proliferation, wing margin specification, and *wg* self-refinement. Our results indicate that Pan function is critically required for all three of these processes. Consequently, lack of *pan* causes a severe reduction in the activity of the Wg target genes *Distalless* and *vestigial* within their normal domain of expression. Loss of *pan* function does not, however, lead to a derepression of these genes outside this domain. Thus, although Pan is positively required for the induction of Wg targets in the wing imaginal disk, it does not appear to play a default repressor function in the absence of Wg input. In contrast, lack of zygotic *pan* function causes a milder phenotype than that caused by the lack of *wg* function in the embryo. We show that this difference cannot be attributed to maternally provided *pan* product, indicating that a Pan repressor function usually prevents the expression of embryonic Wg targets. Together, our results suggest that for embryonic patterning the activator as well as repressor forms of Pan play important roles, while for wing development Pan operates primarily in the activator mode.**

Wingless (Wg) plays important roles in *Drosophila* development. It is required for patterning of the embryonic epidermis (1, 2), for the proper establishment of the embryonic nervous system (3–6), and also for the specification, growth, and cell-fate assignment of adult appendages, such as the wing and the leg (7, 8). In the developing wing imaginal disk, *wg* is first involved in the definition of the wing versus notum primordium (9, 10). Later, Wg is secreted at the dorsoventral (D/V) compartment boundary of the wing disk, where it directs the formation of wing margin structures (11) and from where it acts as a morphogen to organize gene expression (12, 13). Wg also plays a role in restricting its own expression to cells immediately adjacent to the D/V boundary, a phenomenon referred to as *wg* self-refinement (14).

Wg exerts most if not all effects on cell-fate specification by regulating the transcription of target genes in responding cells. The key regulatory event in the Wg transduction pathway appears to be the posttranscriptional up-regulation of the β-catenin homolog Armadillo (Arm). Arm, in turn, confers transcriptional activator activity to the lymphoid-enhancing factor (LEF)/T cell factor (TCF) homolog Pangolin (Pan)/dTCF (15, 16). LEF/TCF proteins belong to the family of high-mobility-group transcription factors that bind to specific DNA sequences. Because the loss-of-*pan*-function phenotypes resemble those caused by loss of Wg signaling, it is likely that Pan acts as a transcriptional activator for Wg target genes. It was reported that in the absence of Wg input, Pan also functions as a transcriptional repressor in the embryo, possibly via the corepressor protein Groucho (17). Consistent with this repressor role of Pan, cells up-regulate the activity of a *Ubx* midgut enhancer if its Pan binding sites have been mutated (18). In addition, when Pan-binding sites were mutated in the *even-skipped* mesodermal enhancer, ectopic gene expression was observed in the dorsal mesoderm (19). Therefore, it is likely that the net balance of the Wg-dependent activator and Wg-independent repressor levels of Pan determines whether Wg targets are induced or repressed.

Here we wanted to address the function of Pan in imaginal-disk development. Is Pan critical for Wg signaling in imaginal cells? If so, does it also play a dual role in activating and repressing the transcription of Wg targets? To answer these questions, we set out to study the function of Pan by clonal analysis and removed *pan* function genetically in subsets of cells of the wing imaginal disk. Our results demonstrate that Pan is involved in all aspects of Wg signaling in the developing wing and functions primarily as an activator in this tissue, whereas it plays a dual role as an activator and repressor during embryogenesis.

## Materials and Methods

**Fly Stocks and Genetics.** For $pan^{-/-}$ clonal analysis, homozygous $pan^2/pan^2$ null mutant animals (16) were rescued with a *P[tub-pan, w⁺]* insertion on the left arm of the second chromosome. An *arm-lacZ* and the *FRT40* transgenes were placed on the same chromosome arm by meiotic recombination. A *y w f hsflp* first and a *P[f⁺] ck⁻ FRT40* second chromosome were used to mark experimental clones with $ck^-$ and twin spots with $f^-$ in the adult wing (20). For $sgg^{-/-}$ $pan^{-/-}$ double-clonal analysis, a *P[tub-pan, w⁺]* insertion on the first chromosome was used. Larvae of the following genotype were generated for the induction of clones *y w P[tub-pan, w⁺] FRT19/y* $sgg^{D127}$ $f^{36a}$ *FRT19; hsflp/+;* $pan^2/pan^2$ or $pan^2/Dp(1;4)1021[y^+]$. For $pan^{-/-}$ germ-line clones, $pan^2/pan^2$ females were used that carried an $ovo^{D1}$ transgene on the left arm of the third chromosome (21) as well as a *P[tub-pan, w⁺]* rescue construct on the same arm (recombined by x-ray-induced male recombination; see below).

**X-Ray-Induced Male Recombination.** Third instar larvae were irradiated with 1,500 rad (Philips MG 160; 150 kV, 14 mA for 3 min with a 25-cm focus distance and a 2-mm Plexiglas filter).

**Induction of Clones.** $pan^{-/-}$ and $sgg^{-/-}$ $pan^{-/-}$ clones were induced by heat shock at 34°C for 30 min 1–4 days after egg deposition (by collecting eggs for 3 days, followed by a 1-day incubation before induction). Imaginal disks of third instar larvae were isolated for immunohistochemistry 1–3 days after *flp* induction. Germ-line clones were induced by applying heat shocks at late third instar or pupal stages.

**Immunohistochemistry.** Imaginal disks were stained as described (22). Antibodies against Dll (provided by S. Cohen, EMBL, Heidelberg), Vg (provided by S. Carroll, University of Wisconsin, Madison), and Wg (provided by S. Cohen) were diluted 1:500. Rabbit anti-β-Gal polyclonal antibody (Cappel) was used

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Abbreviations: Wg, Wingless; D/V, dorsoventral; Arm, Armadillo; Pan, Pangolin; Dll, Distalless; vg, vestigial; Sgg, Shaggy; Ci, Cubitus interruptus; Hh, Hedgehog; TCF, T cell factor.

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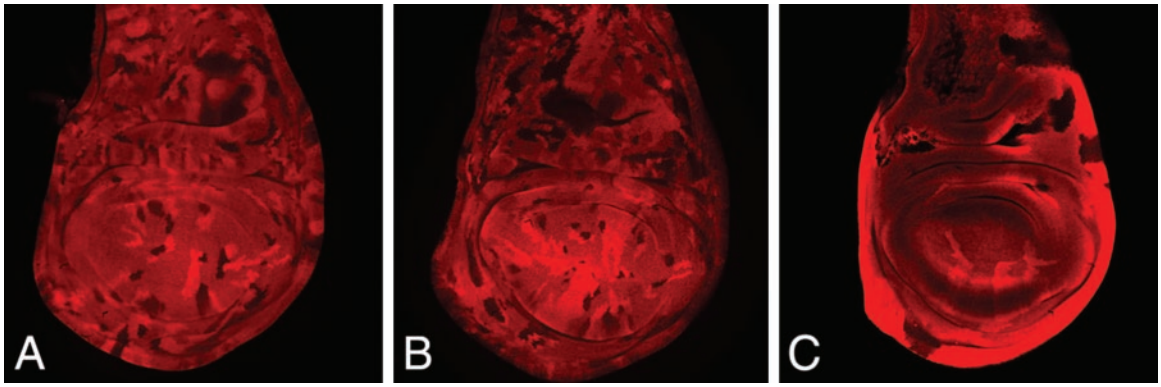


**Fig. 1.** Loss of Pan function leads to cell proliferation defects in the developing wing blade. $pan^{-/-}$ clones are marked by the absence of *lacZ* activity (shown in red), and their twin spots are marked by increased activity (bright red). The clones were induced at the second instar stage (*A*), at the first instar stage (*B*), or during late embryogenesis (*C*).

at 1:2,000 and mouse anti-$\beta$-Gal monoclonal antibody (Promega Z378A) was used at 1:1,000 to mark $pan^{-/-}$ clones.

**Wing Mounting.** Dissected wings were stored in a glycerin:ethanol 1:3 solution, then washed twice in 100% ethanol and mounted in Euparal.

**Cuticle Preparation.** Cuticle preparations for germ-line clones were performed by picking individual embryos onto slides, washing them with bleach, water, and methanol, and adding Hoyer's solution. After placing cover slips, the slides were heated at 65°C for 3–5 h.

## Results

**Pan Is Required for Proliferation and Survival of Wing Cells.** The *pan* gene is located on the small fourth chromosome, which does not usually undergo meiotic or mitotic recombination. Therefore, it is technically difficult to induce *pan* mutant cell clones. Here we used a *pan* transgene to circumvent this problem. The expression levels of *pan* appear to be critical for *Drosophila* development. High experimental Pan levels cause lethality, whereas low Pan levels fail to rescue *pan* mutant animals (not shown). Eventually, three insertions of a *pan*-rescuing transgene were recovered, in which the transcription of a full-length *pan* cDNA is driven by the ubiquitously active promoter of the *tubulin alpha1* gene. The insertion on chromosome arm 2L, which exhibits highest rescuing activity (100% larval, 70% adult), was used to generate an *arm-lacZ tub-pan[2L] FRT40* chromosome. By the use of an *hsp70-flp* transgene, marked clones were induced in a $pan^2$ null mutant background. Clones that lack *arm-lacZ* activity also lack *pan* activity; by contrast, sibling twin-spot clones inherit two copies of either transgene.

Induction of recombination during second larval instar resulted in *pan* mutant clones and twin spots of similar size throughout the wing imaginal disk (Fig. 1*A*). Earlier induction, during first larval instar, caused a reduced size of *pan* clones compared with their twin spots in the wing-blade primordium (Fig. 1*B*). When recombination was induced during late embryogenesis, only twin spots but no *pan* mutant clones were observed in the wing pouch (Fig. 1*C*). We interpret these observations as an indication that *pan* null mutant wing cells cannot proliferate normally and are lost from the wing epithelium once transient perdurance of Pan protein ceases in mutant cells. This behavior is reminiscent of that observed for clones mutant for other components of the Wg signaling transduction pathway, such as *arm*, *Dfrizzled*/*Dfrizzled2*, *legless*, or *pygopus* (13, 23, 24).

**Pan Acts as a Positive Regulator of Wg Target Genes.** Wg is expressed in the wing pouch of late third instar disks in a thin stripe of cells destined to form the presumptive wing margin (Fig. 2*A*). Wg emanating from this stripe acts at long range to activate the

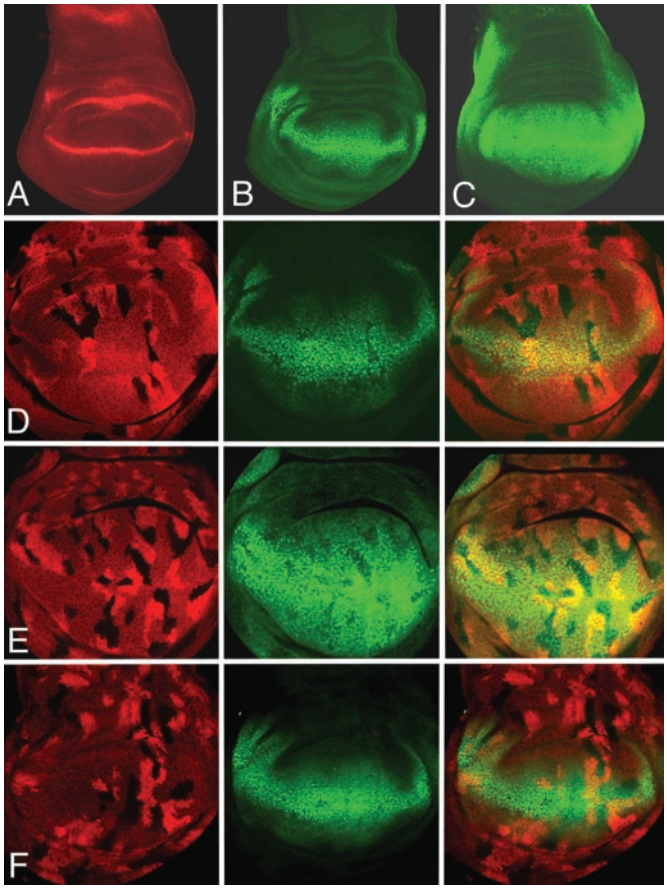


**Fig. 2.** Expression of Wg target genes in $pan^{-/-}$ and $groucho^{-/-}$ clones. In wild-type disks, *wg* is expressed (red) at the D/V compartment boundary. *Dll* (*B*) and *vg* (*C*) expression (green) straddles the stripe of *wg* expression in the wing pouch. Reduced expression of *Dll* (*D*, green) and *vg* (*E*, green) is observed in $pan^{-/-}$ clones [marked by the absence of *lacZ* activity (red in *D* and *E*)]. No ectopic expression is observed in $pan^{-/-}$ clones outside the normal *Dll* or *vg* expression domain. *groucho* mutant cells express normal levels of *Dll* expression (*F*). $groucho^{-/-}$ clones are marked by the absence of CD2 staining (red), whereas *Dll* expression is detected by antibody staining (green).

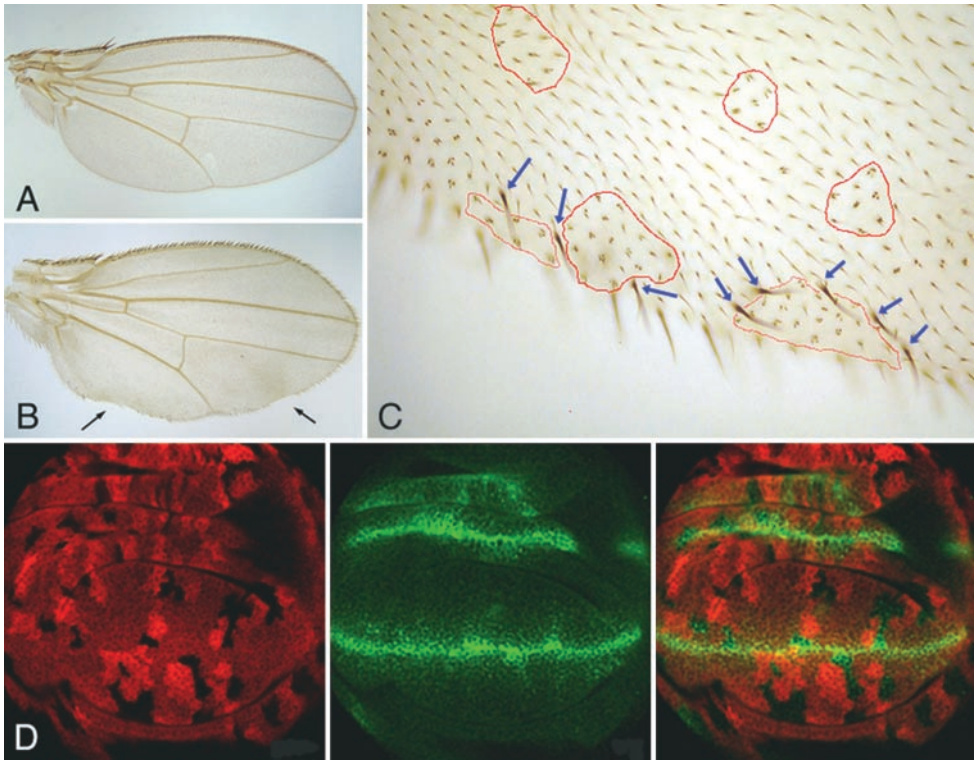


**Fig. 3.** *pan*$^{-/-}$ clones at the wing margin are associated with loss-of-Wg-signaling phenotypes. (*A*) Wild-type wing. (*B*) Small *pan*$^{-/-}$ clones show no abnormality within the wing blade, and clones at the wing margin (see arrows) lead to the loss of wing margin structures. *pan*$^{-/-}$ clones are marked with *crinkled*. The clones can be recognized under a high magnification (see red outlines). Ectopic bristles are observed surrounding *pan*$^{-/-}$ clones at the wing margin (see blue arrows). No effect was observed for *pan*$^{-/-}$ clones at the wing blade (red outlines). In wing imaginal disks, Wg is ectopically expressed in *pan*$^{-/-}$ clones near the D/V boundary (*D*). *pan*$^{-/-}$ clones are marked by the absence of *lacZ* activity (red). Wg expression is detected by anti-Wg antibody staining (green).

expression of a number of genes, such as *Distalless* (*Dll*) (Fig. 2*B*) and *vestigial* (*vg*) (Fig. 2*C*), which control wing development (12, 13, 25, 26). Although it has not been demonstrated that *Dll* and *vg* are direct target genes of Wg signaling, their expression levels serve as valuable readout for Wg transduction.

We generated *pan* mutant clones during the second larval instar and analyzed the expression of *Dll* and *vg*. *pan* mutant cells situated within the *Dll* and *vg* expression domain exhibit reduced expression levels of these genes (Fig. 2 *D* and *E*). *pan* mutant cells outside or straddling the outer limits of *Dll* and *vg* expression do not show a transcriptional up-regulation of these genes. These results indicate that Pan functions as a positive regulator for Wg signaling. Moreover, loss of Pan activity does not cause a derepression of target genes in cells receiving low or no Wg input.

It has previously been reported that Pan and Groucho function together to repress embryonic Wg targets in the absence of Wg signaling (17). To confirm our conclusion that Pan does not function as a transcriptional repressor in wing cells, we investigated the role of *groucho* in regulating Wg responsive genes. Loss of *groucho* function did not affect *Dll* expression in *groucho*$^{E48}$ homozygous cells (Fig. 2*F*).

By using the trichome and bristle marker *crinkled* we analyzed the adult fate of *pan* mutant cells in wing patterning. Peripheral *pan*$^{-/-}$ clones lead to the loss of wing margin structures, whereas clones in the central part of the wing blade exhibit no obvious defects (Fig. 3*B*). At higher magnification, we found that clones at the wing margin can cause the formation of ectopic wing margin bristles in adjacent wild-type territory (Fig. 3*C*), reminiscent of the behavior of *dishevelled* mutant clones (see below). Again, therefore, the phenotypes from loss of *pan*, which we have observed in *pan* mutant clones of developing and adult wings, resemble those from loss of functions of other positive regulators of the Wg signaling pathway. No obvious planar polarity defects were observed in *pan* mutant clones on adult wings. In sum, we interpret our results as an indication that Pan functions as a positive mediator of Wg outputs.

**Pan Is Required for *wg* Self-Refinement.** It was reported that Wg refines its own expression domain at the D/V boundary of third instar wing disks (14). The mechanism of this self-refinement, however, has not been definitely established. The original observation was made with *dishevelled* mutant cells, which ectopically express Wg if situated close to the D/V boundary. In a complementary study, Dishevelled was shown to interact molecularly with the intracellular domain of Notch (27). Because Notch signaling appears to function as prime inducer of *wg* transcription (28), these observations raised the possibility that an intersection of the Wg and Notch signaling pathways at the level of Dishevelled caused the attenuation of Notch signaling, and hence an autoregulatory reduction of *wg* transcription (14, 27). This conclusion implies that the distal components of the Wg pathway, such as Arm and Pan, would be dispensible for *wg* self-refinement.

Here we sought to test this hypothesis by analyzing the requirement for Pan in *wg* self-refinement. *pan*$^{-/-}$ clones in the wing pouch were analyzed for Wg expression. No ectopic Wg expression was detected in clones distant from the D/V boundary. However, *pan* mutant clones close to this boundary exhibit ectopic Wg expression (Fig. 3*D*), indicating that Wg refines its expression through a mechanism involving Pan, and hence the distal portion of the Wg signal transduction cascade. Consistent with this result we found that such clones can cause the formation of ectopic wing margin bristles in adjacent wild type cells

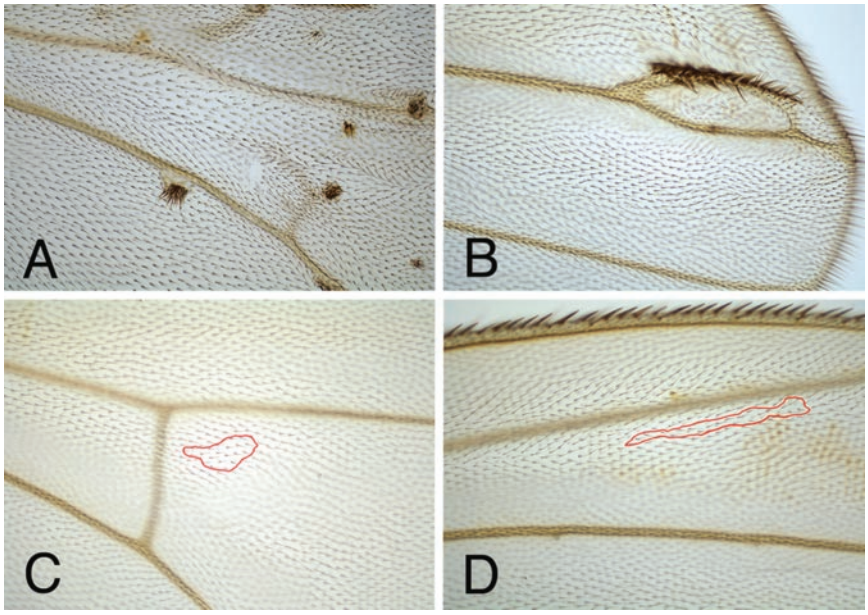


**Fig. 4.** Loss of *pan* function can revert the phenotype of $sgg^{-/-}$ clones. $sgg^{-/-}$ clones invariably form ectopic bristles (*A* and *B*), whereas $pan^{-/-}sgg^{-/-}$ clones never show ectopic bristles (*C* and *D*). Clones of $pan^{-/-}sgg^{-/-}$ double mutants are marked by *forked* bristles within the red outline.

(Fig. 3*C*). Because these structures are normally induced by high levels of Wg signaling, we interpret their ectopic presence to be a consequence of ectopic Wg production in the adjacent *pan* mutant cells. However, because we used an antibody to monitor Wg expression in this study, we cannot rule out an alternative possibility to explain the excess Wg protein observed in *pan* mutant cells; loss of Pan function presumably leads to the up-regulation of Dfrizzled2, which in turn may cause a stabilization of Wg protein at the cell surface (29).

**Evidence against Pan-Independent Outputs of Arm Signaling.** Having established a system to eliminate all *pan* function in the developing wing cells of *Drosophila*, we wanted to address the question of whether the Wg pathway is distally branched, or, in other words, whether the regulation of Pan activity is the sole output of Arm signaling. To activate Arm maximally without overexpression, we sought to remove the function of Shaggy (Sgg), an upstream negative regulator of the Wg signaling pathway. If at the same time Pan is also removed, we can ask what, if any, aspect of Arm signaling can bypass Pan.

Larvae of the following genotype were generated: *y w P[tub-pan] FRT19/y* $sgg^{D127}$ $f^{36a}$ *FRT19; hsp70-flp/+;* $pan^2/pan^2$ and their siblings which carry a wild-type copy of the *pan* gene *y w P[tub-pan] FRT19/y* $sgg^{D127}$ $f^{36a}$ *FRT19; hsp70-flp/+;* $pan^2/Dp[y^+]$. Clones double mutant for *sgg* and *pan* ($sgg^{-/-}$; $pan^{-/-}$) and *sgg* single mutant control clones ($sgg^{-/-}$; $pan^{+/-}$) were induced by a heat shock during first or second instar stage of these larvae and analyzed in the adult wing by means of their *forked* mutant phenotype. As reported previously (30), $sgg^{-/-}$ single mutant clones gave rise to tufts of ectopic bristles on the adult wing (Fig. 4 *A* and *B*). This attempt to form ectopic wing margin structures is typical for wing cells subject to a gain of Wg signaling. No ectopic bristles were observed when clones were double mutant for *sgg* and *pan* (Fig. 4 *C* and *D*). At the wing margin clones with this double mutant genotype exhibit phenotypes similar to those observed for *pan* single mutant clones (data not shown). These results indicate that the loss of Pan function causes a block in Wg signal transduction and argue against a Pan-independent branch downstream of Sgg. However, from these results we cannot exclude the possibility that Wg can signal independently of Pan during other developmental processes.

**Activator and Repressor Function of Pan in the Embryo.** The strong loss-of-Wg-signaling phenotype observed with *pan* null mutant cells is in apparent contradiction to the mild segment polarity defect of *pan* null mutant embryos. This difference could be accounted for by three explanations. First, it could be due to maternal *pan* product partially perduring to embryonic stages during which Wg signaling defines the cuticular pattern. Second, the loss of *pan* could lead to a partial derepression of Wg target genes if Pan exerts a repressor function in the absence of Wg signaling. Finally, part of the Wg signal could bypass Pan. The third possibility is unlikely given our findings in the wing disk, although we cannot formally rule out the existence of an embryonic pathway that is able to influence Wg target gene transcription in a Pan-independent manner. To discriminate between the more likely possibilities one and two, we sought to remove any potential maternal component of *pan*.

Female germ-line clones were generated by using the FLP recombination target (FRT)-FLP technique in combination with an FRT80 (3L) chromosome carrying both a $P[ovo^D]$ and a *P[tub-pan]* transgene. This chromosome was obtained by x-ray-induced recombination in males. We had to use a *P[tub-pan]* transgene insertion with lower rescuing activity than the one on 2L because, in contrast to the $P[ovo^D]$ on chromosome arm 3L, any single $P[ovo^D]$ on 2L fails to cause total sterility of females (21). Germ-line clones were induced in rescued $pan^{-/-}$ animals by means of a heat shock-driven *hsp70-flp* transgene. Embryos derived from resulting mosaic females and fertilized with *pan* mutant sperm lack both maternal and zygotic *pan* function.

Unfortunately, rescued mosaic females were rare, weak, and subfertile. To obtain 40 fertilized eggs that developed cuticle, 600 such females were needed. To our surprise, $pan^{-/-}$ mutants devoid of maternal contribution did not show phenotypes more severe than those that received maternal *pan* function (Fig. 5 *D–F*). To further examine the possibility that removing maternal contribution leads to embryonic death before the formation of cuticle, the ovaries of these females were examined by 4′,6-diamidino-2-phenylindole and phalloidin staining. All of the

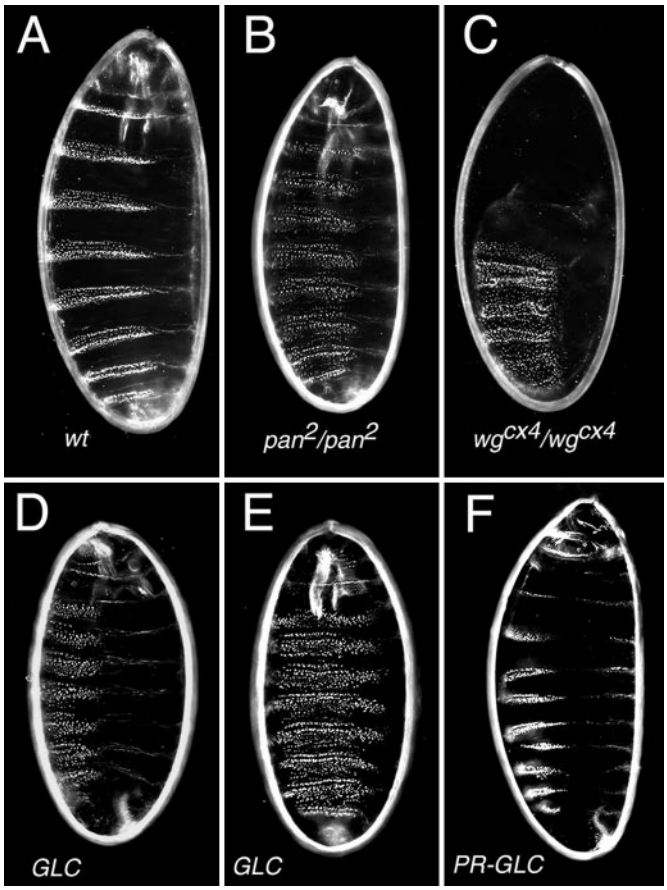


**Fig. 5.** Embryos maternally and zygotically mutant for *pan* display segment polarity phenotypes resembling those of zygotic *pan* mutant embryos. The alternation of naked cuticle and denticle belts in wild-type embryos (*A*) is replaced by a continuous lawn of denticles in $wg^{-/-}$ mutant embryos (*C*). The zygotic *pan* mutant phenotype is milder than that of *wg* mutants (*B*). Embryos derived from *pan* mutant germ-line clones (GLCs; *D* and *E*) do not show a more severe phenotype than the zygotic mutants. Paternal contribution can rescue the phenotype of $pan^{-/-}$ GLC-derived animals (PR-GLC; *F*).

stages examined during oogenesis appeared normal (data not shown). Hence, the most likely explanation for this weak segment polarity phenotype is a role for *pan* in repressing Wg-target genes in the absence of Wg activity. The analysis of embryos lacking *wg* function as well as maternal and zygotic *pan* function would provide an unequivocal answer. However, for technical reasons (see above) we failed to obtain such embryos. Instead we analyzed cuticles from embryos that only lacked zygotic *pan* and *wg* functions, and those resemble *pan* rather than *wg* mutants (data not shown). This observation is consistent with previous results from Cavallo *et al.* (17) and suggests a dual role for Pan during embryogenesis. Embryos devoid of maternal $pan^{+}$ product can be rescued paternally ($pan^{+}$ chromosome marked with $y^{+}$). Although the cuticular phenotype is restored to that of wild-type embryos, not all paternally rescued animals survived to adult stage (Fig. 5*F*). Together, our results indicate that, in contrast to the wing imaginal disk, the maternal and zygotic *pan* products function both as transcriptional activator and repressor of Wg targets.

## Discussion

Pan is the transcriptional mediator of the Wg signal transduction pathway. It is encoded by a gene located on the fourth chromosome. This location does not permit a functional analysis on the basis of mitotic recombination. Here we used a *pan* rescue construct to circumvent this problem. However, our transgene is driven by a foreign promoter and thus only partially effective. Rescued animals were not healthy and exhibited reduced fertility. Ideally, a genomic fragment with the endogenous regulatory regions should be used as rescue construct, as was done, for example, in the *cubitus interruptus* (*ci*) gene (31) which encodes the transcriptional mediator of the Hedgehog (Hh) signal transduction pathway. However, the *pan* gene is unusually large (>40 kb) and hence refractory to this approach. Below we discuss our findings and also indicate caveats and limitations of each conclusion.

**No Apparent Role of a Pan Repressor in Wing Disk Cells.** The genetic loss of *pan* function in wing imaginal disks causes a cell-autonomous reduction of *Dll* and *vg* expression. Similar observations were made with *dsh* and *arm* mutant clones (12). The pouch expression of the *Dll* and *vg* genes depends critically on Wg input (13); the apparent residual activity of these genes in *pan* mutant cells may therefore reflect perdurance, of either *pan* function or of their own products. It is unlikely that the low levels of *Dll* and *vg* products reflect a transcriptional derepression of their genes due to the removal of a Pan repression function, because $pan^{-/-}$ clones outside the normal realms of *Dll* and *vg* do not up-regulate these genes. This observation is surprising in the view of the dual roles that have been proposed for Pan in the embryo. Reduction of embryonic *pan* activity partially suppressed *wg* and *arm* mutant phenotypes, and led to the derepression of Wg-responsive genes (17). The apparent absence of a Pan repressor function in the wing primordium also contrasts with the situation in the Hh pathway. Genetic removal of *ci* results in a derepression of the Hh target gene *dpp* in wing disks (31, 32), indicating that Ci represses *dpp* transcription in the absence of Hh signaling. Thus, it appears that the contributions of Pan repressor and activator functions vary in different tissues and/or developmental stages.

Our analysis does not exclude, however, the following two possibilities in which a Pan repressor function may nevertheless play a role in wing development. First, in analogy to the Hh pathway where not all target genes are subject to repression by Ci (e.g., *ptc*; ref. 31), it is possible that some Wg targets other than *Dll* and *vg* are indeed derepressed in *pan* mutant cells. However, this is not very likely because *pan* mutant clones in adult wings only display loss-of-*wg* phenotypes. Second, it is possible that even low levels of *pan* expression may suffice to provide effective repressor function. As we argued above, wing pouch cells do not survive in complete absence of *pan* transcription. Thus, at the moment of analysis, $pan^{-/-}$ cells probably still contain at least some Pan protein. Our observation that *groucho* mutant cells do not up-regulate *Dll* expression renders this second possibility unlikely.

After completion of this study, Chan and Struhl (33) reported that membrane-tethered Arm can effectively transduce the Wg signal in an *arm* mutant background, and suggested that Arm may usually function by raising the nuclear ratio of activator to repressor forms of Pan in response to Wg, either by selectively exporting a Pan repressor form from the nucleus or by generating a Pan activator form in the cytoplasm. Although our inability to observe a derepression of Wg targets argues against the existence of a *constitutive* repressor function of Pan during wing development (see above), our experiments do not rule out a scenario in which Wg signaling regulates the balance of putative activator and repressor forms of Pan, mainly because the genetic removal of the *pan* gene would concomitantly affect the levels of both forms.

**An Absolute Requirement for Pan in Wg/Arm Signaling.** Several bifurcations appear to occur in the Wnt/Wg pathway downstream of Frizzled and Dishevelled, fuelling at least three

pathways with signaling activity, such as the canonical Wnt/β-catenin pathway, which activates target genes in the nucleus most likely through TCF/Pan; the planar cell polarity pathway, which involves JNK and cytoskeletal rearrangements; and the Wnt/$Ca^{2+}$ pathway, which activates phospholipase C and PKC (34). Here we wanted to address the linearity of the canonical Wnt/β-catenin pathway and asked whether all β-catenin/Arm signaling involves output by means of TCF/Pan proteins. Maximal stimulation of Arm signaling was obtained by removal of Sgg, the kinase that normally marks Arm for degradation. On the basis of our observations that *sgg pan* double-mutant clones behave like *pan* single-mutant clones, we concluded that Arm signaling cannot bypass Pan. In a similar but more comprehensive study, we have recently addressed the equivalent question for the Hh pathway (35): is there a branch of Hh signaling that bypasses Ci? The answer was unambiguously negative, despite many previous reports of apparent Ci/Gli-independent Hh outputs. For both pathways, the debate about this issue is nurtured in part by the different phenotypes of *ci* vs. *hh* and *pan* vs. *wg* mutant embryos, respectively, owing to a default repressor function of the nuclear mediators in these pathways.

It is important to point out, however, that, because of technical difficulties, we have not assayed the effect of the *sgg pan* double-mutant genotype in multiple developmental settings or with sensitive readouts. The *sgg pan* approach suffers from another deficit compared with the *ptc ci* analysis; whereas *ptc* appears to be a dedicated negative component of the Hh pathway, the kinase encoded by *sgg* plays multiple roles and its genetic removal may affect cells in ways beyond the constitutive activation of the Arm signaling pathway. On the other hand, our main finding in the adult wing is corroborated by our previous study in the embryo, where loss of Pan function can totally block the phenotype caused by constitutively activated Arm (15). Together, these results leave little room for the possibility that Arm can partially bypass Pan to regulate Wg targets in *Drosophila* development.

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