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Interactions between Wingless and Notch during the assignation of cell fates in *Drosophila*

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ABSTRACT A large number of observations suggest that, during *Drosophila* development there are close functional interactions between the activity of Notch receptor and that of a signaling molecule encoded by *wingless* gene. In this essay, I summarize these interactions and discuss the possibility that Wingless acts as a ligand for Notch as part of a switch that is iteratively involved in the assignation of cell fates during development.

KEY WORDS: *Wingless, Notch, pattern formation*

Introduction

During development cells divide and become different with defined tempos and various modes. Cells become different in groups to give rise to tissues or "histotypes" e.g., mesoderm, which then is subdivided into different subpopulations, or neural tissue that will also give rise to different cellular populations. Within any of these groups, cells have individual identities which contribute to the patterning of the different tissues. For example within the nervous system individual neurons have particular properties which allow them to establish specific connections and act as specific relays for signals, or within the epidermis cells differentiate features that shape the palm of the hand or the tip of a finger. An organism results from the coordination of these processes and Antonio García Bellido has devoted a large part of his energy to understanding the fundamental problem posed by this natural process of coordination. In the course of this effort he has resurrected the classic Aristotelian concept of *Entelechia* (García Bellido and de Celis, 1992; García Bellido *et al.,* 1994). A free translation of *Entelechia* from the Greek, would be "perfection", although its etymology bears a more fitting meaning of its biological use: that which bears the end in itself (Driesch, 1908). Antonio has used it to embody the inner natural tendency of developing systems towards their final form in a manner that is reproducible. This notion was first applied to modern embryology by Hans Driesch (1908), not as something precise but rather in despair for lack of insight into the mechanics of developmental systems. Antonio García Bellido has made a more defined use of the term as the condition (Entelechia condition), towards which the organism tends, much as the state of lowest developmental energy. The challenge that he has set in this conceptualization is to find the molecular rules and mechanisms which drive cells towards these conditions.

Here I would like to discuss a series of functional relationships between a ligand encoded by the *wingless (wg)* gene and a receptor encoded by the *Notch (N)* gene, in *Drosophila*. My contention will be that these molecules and the interactions they engage in represent essential elements of a process that is at the heart of most cell fates decisions in developing systems.

Wingless

The *wg* gene of *Drosophila* is a member of the *Wnt* gene family and encodes a secreted glycoprotein involved in cell interactions (Nusse and Varmus, 1992). Flies homozygous for the wg^{1} allele lack wings due to a loss of *wg* function during adult development (Couso *et al.,* 1993; Fig. 1A,B), a phenotype that christens the locus. The complete absence of the gene results in lethality at the end of embryogenesis and the resulting first instar larvae secretes a cuticle with the normal number of segments but an aberrant pattern of each of these units (Baker, 1987; Fig. 1C,D). Because a hallmark of this phenotype is a mirror image duplication of the pattern of cuticular secretions, *wg* has been classified as a "segment polarity" gene (Nüsslein Volhard and Wieschaus, 1980).

In the course of the last ten years, studies of conditional gain and loss of function of Wingless have yielded a detailed catalog of the requirements for *wg* function during *Drosophila* development. For example, making use of the temperature sensitive allele wg^{IL114} (Nüsslein Volhard *et al.,* 1984) it has been possible to remove *wg* function at particular times and places and thus identify when, where and what for Wingless is needed (see for example Bejsovec and Martinez Arias, 1991; Couso *et al.,* 1993). In this manner requirements have been uncovered for gene expression, cell behavior and patterning as well as for the large scale organization of the wings and legs of *Drosophila*. The targeted gene expression

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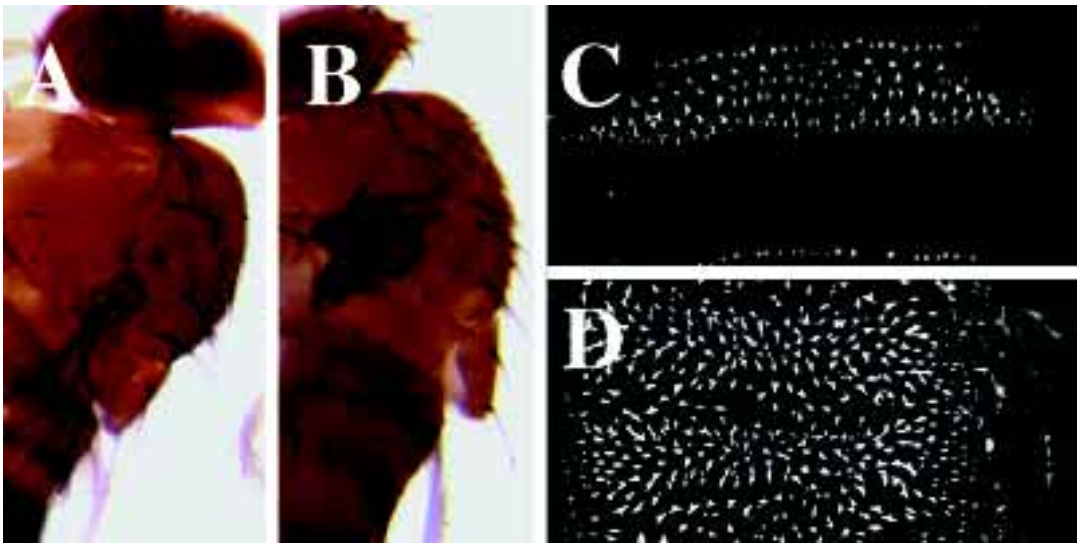

Fig. 1. Pattern defects in *wingless (wg)* mutants. (A) *Lateral view of a wildtype fly showing the notum and a patterned outgrowth on its flank that is the wing (arrow).* **(B)** *View similar to (A) but of a wg^{1} homozygous fly. Notice that instead of the wing, the flank of the notum contains an outgrowth that mimics notal tissue. This is usually referred to as wing to notum transformation.* **(C)** *Dark field image of the cuticle of an abdominal segment of a wildtype larva. The anterior region is decorated by several rows of denticles and the posterior region is naked.* **(D)** *Same view as (c) but of two segments of a wg null allele (wg^{CX4}), showing that the posterior region is now covered with denticles and that there are clear problems with the polarity of the denticles (for details of these patterns see review in Martinez Arias, 1993).*

system of Brand and Perrimon (1993), has enabled the complementary experiment, i.e., to explore the consequences of expressing Wingless at the wrong time or/and in the wrong place. Altogether these experiments reveal seemingly disparate functions which range from the regulation of the expression of genes like *engrailed (en)*, *gooseberry (gsb)* or *achaete (ac),* to the definition of the appearance of single cell precursors for muscles, neuroblasts, sensory organs or the primordia of wings and the legs. These processes must have a common denominator in some molecular event and various attempts have been made to capture its essence. In this spirit it has been suggested that Wingless is a morphogen (Zecca *et al.,* 1996), an organizer (Diaz Benjumea and Cohen, 1995) or even a glue (Sampedro *et al.,* 1993). However, although some of these notions are full of meaning and tradition, none provides a substantial insight into what it is that Wingless does at the molecular level.

The regulation of *en* expression by Wingless during embryogenesis provides a useful ground to test some of the concepts that have been applied to Wingless. In the blastoderm, *en* expression is initiated by the pair rule genes in one stripe per segment, but the maturation of this pattern and its maintenance in the early rounds of proliferation requires certain levels of Wingless from adjacent cells (Fig. 2A-C; DiNardo *et al.,* 1988; Martinez Arias *et al.,* 1988; Bejsovec and Martinez Arias, 1991). In the absence of *wg*, Engrailed expression although initiated normally, decays. Making use of a temperature sensitive allele of *wg* which results in a nonsecreted protein at the restrictive temperature (Gonzalez *et al.,* 1991), it is possible to modulate the amount of Wingless protein that is secreted by the cells. At the permissive temperature the expression of Engrailed is similar to that of wildtype embryos but, as the temperature of the experiment is increased, and the amount of Wingless secreted by the cells decreases, the width of the stripes of *en* expression also decreases until at 25°C Engrailed expression is not maintained (Fig. 2D-F). This shows that the concentration of Wingless plays a role in wingless signaling, however it does not appear to be instructive. Local or global ectopic expression of *wg* in wildtype or *wg* mutant embryos (Bejsovec and Martinez Arias, 1991; Baylies *et al.,* 1995), evokes stripes of *en* expression that can be slightly broader than the wild type, but still stripes.

These experiments suggest that Wingless "enables" cells to do what they want to do. The fact that Wingless can elicit stripes of Engrailed expression in a *wg* mutant, and that these stripes are in the right place suggests that the cells are "programmed" to express *en* in stripes and that all Wingless is doing is evoking this response. The concentration determines how far from the source this response is stabilized.

Wingless and the assignation of cell fates

Further evidence for a role of Wingless in similar permissive processes is derived from its requirements in the specification of cell fates in the mesoderm and the nervous system. In the wildtype, the sensory organs and the muscles are derived from precursors which arise from clusters of cells that express members of the Achaete Scute Complex (ASC) (Ghysen and Dambly Chaudière, 1988; Carmena *et al.,* 1995). These genes are under the control of *wg* in the embryo (M. Ruiz Gomez personal communication and AMA unpublished observation) and the adult (Phillips and Whittle, 1993; Couso *et al.,* 1994) and perhaps as a result, most elements of the peripheral nervous system (PNS) (Fig. 3C,D), many neuroblasts (Hartenstein *et al.,* 1994) and many muscles (Baylies *et al.,* 1995) do not appear or differentiate in *wg* mutants. In the mesoderm, for example, the expression of *S59* (Baylies *et al.,* 1995 and Fig. 3A,B) and *eve* (Lawrence *et al.,* 1995; Wu *et al.,* 1995; Park *et al.,* 1996) in precursors of specific mesodermal derivatives have been the subject of in depth analysis. In both cases, loss of *wg* function leads to the loss of expression of these genes and the associated precursors. As in the case of *en*, ubiquitous expression of *wg* (or wingless signaling) in *wg* mutants results in the rescue of the expression of *S59* and *eve* in a pattern similar to that of the wild type (Fig. 4; Baylies *et al.,* 1995; Park *et al.,* 1996).

These observations reinforce the conclusion that Wingless does not determine what happens, rather it enables cells to do

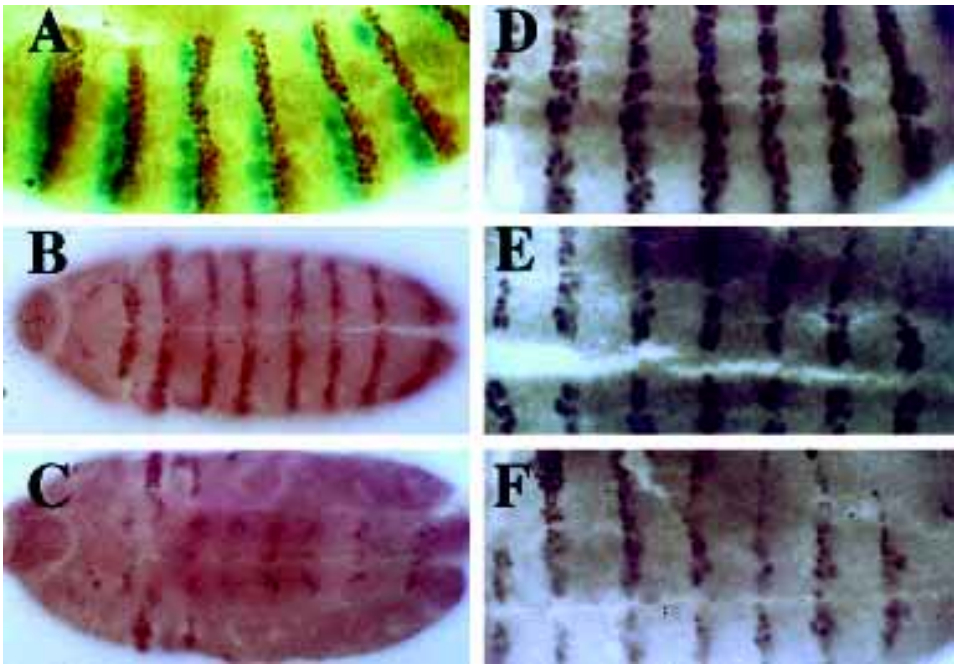

Fig. 2. Expression of Engrailed protein in stage 10 wildtype (A,B) and wg mutant embryos (C-F). (A) *Lateral view of a late stage 10 wild type embryo showing that in the ectoderm, Engrailed is expressed in a stripe two to three cells wide (brown nuclei), adjacent to a row of cells that express wingless (blue).* **(B)** *Ventral view of a stage 10 wildtype embryo showing the expression of Engrailed in one stripe per segment.* **(C)** *Ventral view of a stage 10 wg^{cx4} mutant embryo showing that the expression of Engrailed has disappeared from the ectoderm. The clusters that remain represent gnathal segments (arrows) and cells in the developing nervous system (arrowheads).* **(D-F)** *Examples of Engrailed expression in the ectoderm of stage 10 embryos mutant for the temperature sensitive wingless allele wg^{IL114} grown at different temperatures. At 17°C (D) the expression of Engrailed is indistinguishable from wild type, but as the temperature is increased to 20°C (E) or 22°C (F), the width of the stripes progressively decreases. At every temperature there is a range of phenotypes, but the pictures shown are representative of the majority classes.*

what they are programmed to do anyway. In a sense it acts as a photographic developer; the image is already there, how much of it comes up being determined by the time of exposure to wingless or its concentration. In the case of the embryo, the mechanism that defines the "image" or the pattern must lie in the developmental program. In the case of *en*, this must be the activity of the pair rule genes; for *S59* and *eve*, more complex mechanisms which might have an input from the pair rule genes.

Wingless signaling

A variety of genetic studies have identified a group of genes with null phenotypes similar to that of *wg* (reviewed in Martinez Arias, 1993). These genes fall into two classes depending on whether they are required for the expression or the function of Wingless. Members of the first class, which includes the secreted protein Hedgehog and the transcription factor Ci, are involved in defining the spatial and temporal domains of wingless expression. Members of the second class determine whether Wingless signals or not and can be further subdivided into two classes: those encoding proteins that ensure that Wingless can signal, i.e., those involved in regulating its secretion or interactions with molecules in the ECM, and those encoding proteins involved in the transduction of the Wingless signal. The latter are essential for wingless signaling and their defects cannot be rescued by excess wingless function.

Three genes, *dishevelled (dsh), armadillo (arm)* and *pangolin (pan)/dTCF*, are prototypes of the signal transduction class (Cavallo *et al.,* 1997). The first of these genes, *dsh*, might be the first relay in wingless signaling at the plasma membrane and encodes a small cytoplasmic protein without enzymatic activity but with motifs present in proteins which interact with transmembrane receptors. The *arm* gene encodes a *Drosophila* homolog of beta-catenin, a protein involved in junctional complexes at the plasma membrane mediated by cadherins. Armadillo has been shown to have a second function in signaling which can be separated from that involved in cell adhesion (Orsulic and Peifer, 1996). Finally, the *pan/dTCF* gene encodes a homolog of the HMG nuclear protein LEF1/TCF and recent work shows that this protein interacts with Armadillo to implement wingless function. Absence of any of these three molecules leads to absence of wingless signaling, endogenously or exogenously.

In addition to genes with *wg* mutant phenotypes, genetic studies have identified mutations with a phenotype that mimics many aspects of excess wingless signaling. One of them, *shaggy (sgg)/ zeste while 3 (zw3),* results in constitutive wingless signaling in a *wg* independent manner (Siegfried *et al.,* 1992). The *sgg/zw3* gene

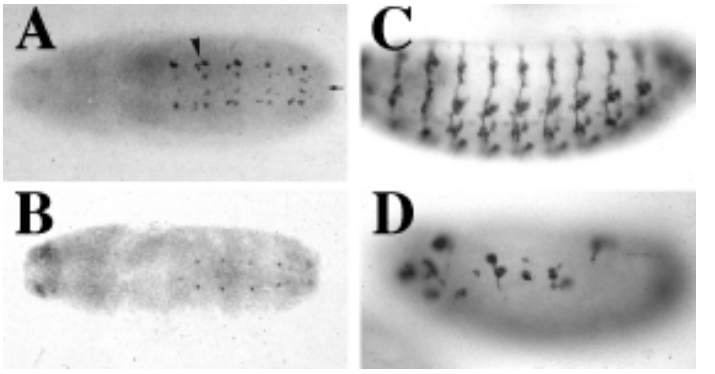

Fig. 3. Patterns of muscle precursors (A, B) and peripheral nervous system elements (C,D) in wild type and wg^{cx4} mutant embryos. (A) *Wildtype; S59 gene expressed in some muscle precursors and cells in the developing nervous system.* **(B)** *In wg mutant embryos, the only expression that is detected is in a few cells of the nervous system.* **(C)** *In wild type embryos, the monoclonal antibody 22c10 reveals the complement of elements of the peripheral nervous system which disappear almost completely in the absence of wg function.*

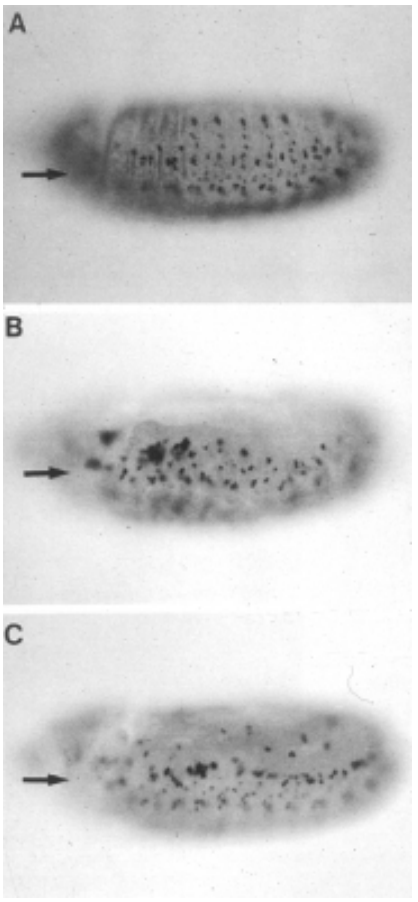

Fig. 4. Pattern of S59 expression in wildtype (A) and wg^{cx4} mutant embryos rescued by ubiquitous expression of Wingless in the mesoderm (B) or the ectoderm (C). *Notice that patterns are very similar and that S59 expression is in precise positions despite the global supply of the wingless signal.*

encodes a *Drosophila* homolog of the Ser/Thr kinase GSK3 which has been shown to regulate the stability of Armadillo.

Genetic epistases indicate that Wingless, through Dsh, inactivates Sgg and that, as a consequence of this activity Armadillo is stabilized and allowed to interact with Pan/dTCF to mediate wingless signaling (Fig. 5). All elements of this cascade have been found in vertebrates where they are thought to operate in the same manner as in *Drosophila* (Moon *et al.,* 1997). These comparative studies have also suggested that a protein encoded by the APC gene, known to regulate the stability of Armadillo in certain instances, is involved in wingless signaling. This suggestion stems from the study of colorectal cancers in which mutations in either APC or Armadillo result in very stable Armadillo which can interact with LEF1(Morin *et al.,* 1997; Rubinfeld *et al.,* 1997). However, mutations in the *Drosophila* homolog of APC have no effect on wingless signaling (Hayashi *et al.,* 1997) which suggests that perhaps APC is the first of many tissue specific regulators of wingless signaling.

Recently, members of a family of seven transmembrane receptors encoded by *frizzled (fz)* genes, have been shown to bind Wingless in tissue culture assays (Bhanot *et al.,* 1996) . This, together with some circumstantial evidence from naturally occurring dominant negative variants of these receptors in vertebrates (Leyns *et al.,* 1997; Wang *et al.,* 1997), has led to the suggestion that *fz* genes are receptors for Wingless. This is likely to be the case, however, to date there is no evidence that this binding elicits a signal alone. In *Drosophila*, there are two fz genes. Mutations in *fz*, also called *fz1*, have no effect on wingless signaling and this has led to the suggestion that D*fz2* might encode the Wingless receptor (Banhot *et al.,* 1996). However, in the presence of Wingless, S2 cells expressing *Dfz2* under the control of an inducible promoter will stabilize Armadillo, but this effect is weakened upon induction of the *Dfz2* gene (Banhot *et al.,* 1996) suggesting that there is more than Dfz2 to Wingless signaling.

In any event, it is worth emphasizing that the chain of events accepted to exist between all these gene products, simply reflects genetic "epistases" upon which functional links have been painted by making use of some presumed biochemical properties of the components. The resulting picture should be taken as a framework to think about wingless signaling and not as a "wingless signaling pathway".

Notch

A few years ago, in an attempt to identify genes required for wingless signaling, together with S. Bishop, I undertook a screen for dominant enhancers of *wg* function during wing development. The screen was performed in a variety of ways and revealed multiple hits in three genes that now we know are closely associated with *wg* during the growth and patterning of the wing: *vestigial*, *Serrate* and *Notch*. *Notch* was, by far, the most commonly hit enhancer for *wg* and this led us to explore in more detail the interactions between *Notch* and *wingless*.

Notch has a well characterized function, not in wingless signaling but rather in the process of lateral inhibition (Greenwald and Rubin, 1992; Artavanis Tsakonas *et al.,* 1995). Our current knowledge of *Notch* function is derived from extensive analysis of its role during neurogenesis in *Drosophila*. In this process, groups of cells in defined positions of the ectoderm or the epidermis acquire the potential to become neural. However, usually only one of the cells adopts the neural fate and in the process inhibits surrounding cells from adopting the same fate. This process of cell fate restriction is known as "lateral inhibition" and has been shown to occur in other cell types and processes (Hartenstein *et al.,* 1992; reviewed in Simpson, 1994). The outcome of this inhibitory interaction is not simply that cells will not adopt a particular fate but rather that they will be able of engaging in other decisions later in development i.e., they remain developmentally "naive" (Artavanis Tsakonas *et al.,* 1995).

The *Notch* gene encodes the receptor for lateral inhibition and the *Delta* gene the ligand. The Notch receptor is a single span transmembrane receptor with complex extracellular and intracellular domains (Artavanis Tsakonas *et al.,* 1995). This complex

Wg → R (Fz/x) Dsh ⊣ Sss/Zw3 → Arm → Pan/dTCF

Fig. 5. Summary of the current view of Wingless signaling. *Wingless, (wg); Receptor, (R); frizzled, (Fz); dishevelled, (Dsh); zeste white 3/shaggy, (zw3/sgg); armadillo, (arm); pangolin/dTCF, (pan/dTCF).*

molecular structure, together with the pleiotropy of the Notch mutant phenotype has often led to the suggestion that Notch might act as a multifunctional receptor. However, to date, the only ligands that have been identified are encoded by the *Delta* and *Serrate* genes which bind to the same extracellular region and appear to have redundant function (Simpson, 1994; Artavanis Tsakonas *et al.,* 1995).

At first sight, it is not clear what lateral inhibition might have to do with wingless signaling. However, a comparison of the mutant phenotypes of *Notch* and *wg*, suggests a connection. The function of *Notch* during lateral inhibition is the opposite of that which we have described above for *wingless*: whereas wingless signaling "encourages" the adoption of particular cell fates, Notch signaling tends to inhibit this process (Fig. 6). In this context, it is interesting to note that genetic analysis suggest that there are two functions encoded in Notch and that one of these functions is related to Wingless (Brennan *et al.,* 1997).

Notch is required for Wingless signaling

The different phenotypes of *Notch* and *wg* mutants raise the possibility that the requirement for Notch in wingless signaling during wing development represents a case of a special interaction and that there is no other functional connection between these molecules. This appears not to be the case. Developmental analysis indicates a close correlation between the functions of these two molecules (Couso and Martinez Arias, 1994; Hing *et al.,* 1994; Couso *et al.,* 1995). For example, *Notch* is required for the patterning of the larval segment: in the absence of Notch, the ventral cuticle of *Notch* mutant larvae displays a segment polarity phenotype (Fig. 7A-C) and a loss of *en* and *gsb* expression. In addition to the effects on wing development already described, loss of *Notch* function during the development of the adult also mimics defects in wingless signaling. Thus, loss of *Notch* function in the second instar leads to wing to notum transformations and leg defects that are similar to those caused by loss of *wg* function. These effects are exaggerated by antimorphic alleles of *wg* (Couso and Martinez Arias, 1994).

Another example of a connection between *Notch* and *wingless* is provided by some members of a class of *Notch* alleles, the *Abruptex* class. These mutations display losses of Sensory Organ Precursors that are similar to those observed in the loss of *wg* function and these defects are very sensitive to the amount of wingless signaling (Couso and Martinez Arias, 1994; Brennan *et al.,* 1997). Although these alleles are often referred to as gain of function (Simpson, 1994), genetic analysis indicates that some of them are loss of function mutations closely associated with the function of Wingless (Brennan *et al.,* 1997). They cluster in a region of EGF repeats that maps far from those required for Delta function and therefore might identify a region that is required for a hypothetical function of Notch in wingless signaling.

A direct requirement for Notch function in wingless signaling has been further highlighted by experiments in which a phenotype is created in the eye by expression of *wg* in the R7 photoreceptor under the control of the *sevenless (sev)* promoter (Cadigan and Nusse, 1996). Interestingly, this phenotype is suppressed by simultaneously reducing *Notch* function. This observation, however, has been undermined by the observation that loss of *Delta* function also suppresses the effects of ectopic expression of *wg* in

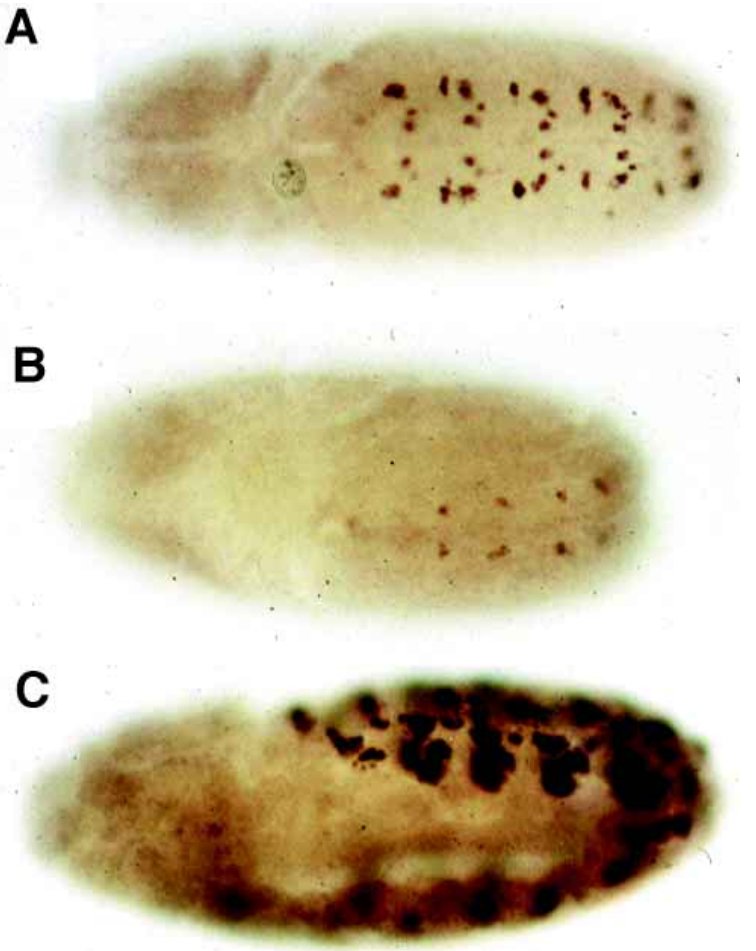

Fig. 6. Expression of S59 in wildtype (A), *dsh*^{v26} (B) and *N*^{55e11} maternal and zygotic mutant embryos (C).

R7. This result can still be consistent with a requirement for Notch in wingless signaling. A variety of studies have shown that there are functional feedbacks between *Notch* and *Delta* (Simpson, 1994) and these would indicate that reducing the concentration of Delta will lead to a reduction in the concentration of *Notch* which, in turn would account for the suppression of the sev*Wg* phenotype.

Altogether, some of these observations led us to suggest that the requirement for *Notch* in wingless signaling might reflect a ligand receptor interaction (Couso and Martinez Arias, 1994). If we are to contemplate this possibility, we would have to view *Notch* as a dual receptor whose activity depends on the ligand that is bound to it. There are precedents for such situations: multifunctional receptors are well known in the immune system where certain cytokine receptors bind more than one ligand and can form part of more than one receptor complex. The particular complex that forms depends on the relative concentration of the competing ligands (e.g., reviewed in Mehler and Kessler, 1997).

There are two experiments which raise some caveats over the notion that Wingless might use Notch to signal *in vivo*. Ectopic expression of *wg* leads to ectopic expression of *en* in the embryo and *ac* in the adult and these effects do not appear to require Notch (Rulifson and Blair, 1995; Cadigan and Nusse, 1996). While it is true that, at first sight, these observations contrast with the require-

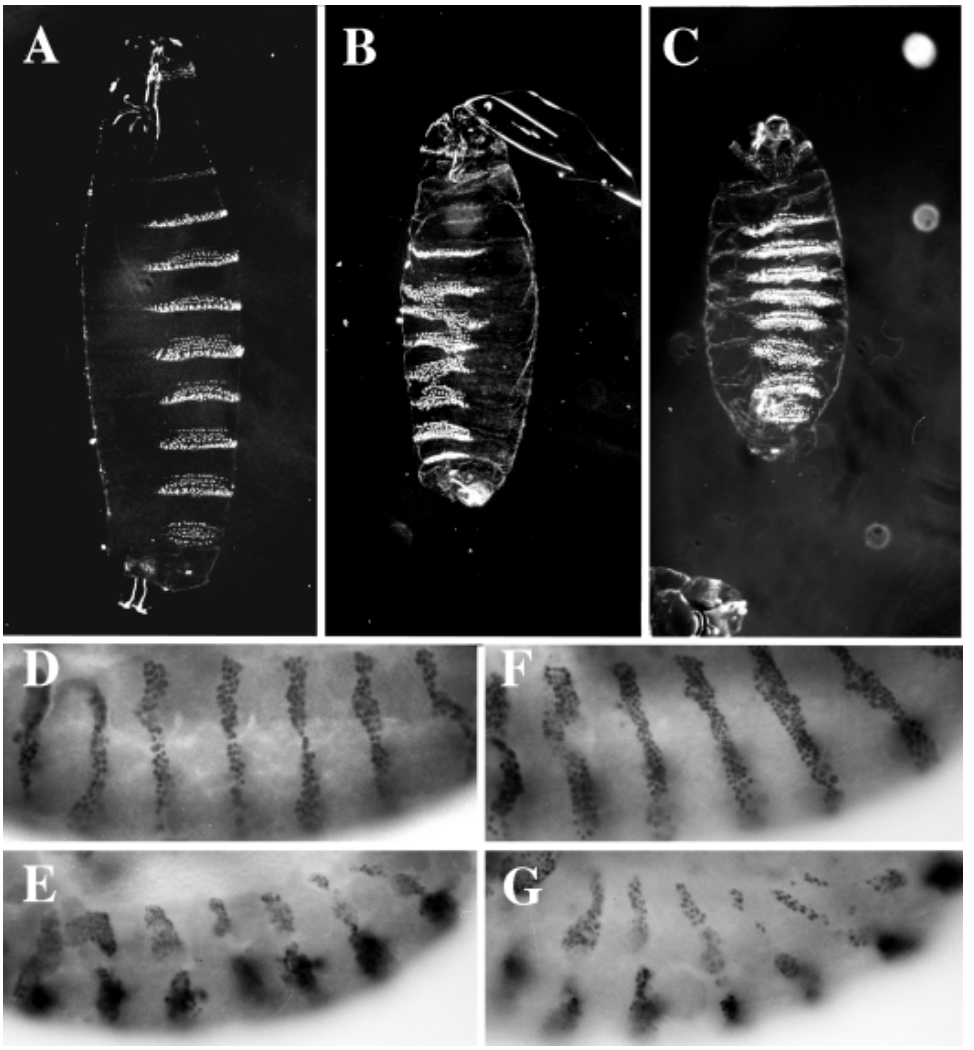

Fig. 7. Effects of removal of *Notch* function on the pattern of the cuticle (dark field images in a-c) and the expression of *engrailed*. (A) *Cuticle of a wild type larva.* **(B)** *Cuticle of a N^{55e11}/N^{ts1} larva grown at 17°C until early stage 11 and then shifted to 30°C for the rest of development. Notice that the larva is shorter than the wildtype and displays segment fusions similar to segment polarity mutants (compare with C.* **(C)** *Cuticle from a wg^{lL114} larva treated as B. It shows a weak wg mutant phenotype similar to that of the N mutant embryo shown in (B).* **(D,F)** *Expression of Engrailed in early (D) and late (F) stage 11 zygotically rescued embryo derived from a N^{55e11} germ line clone. Overall the expression of Engrailed is similar to wild type.* **(E,G)** *Expression of Engrailed in early (E) and late (G) stage 11 embryo lacking Notch function, derived from a N^{55e11} germ line clone. In both instances the ventral region is neurogenic and shows enlarged clusters of Engrailed expression which represent cells in the developing nervous system. On the dorsal epidermis, though, the expression of Engrailed is weaker and with much fewer cells than in the wild type. This is not due to a neurogenic effect in this region because the boundary of the neurogenic region is not altered in the absence of Notch and reflects a requirement for Notch in Engrailed expression.*

ments for Notch in wingless signaling summarized above, they do not invalidate them. Rather they highlight that there is a complexity to wingless signaling beyond our present understanding of the situation. One possibility to account for these results is derived from the observation that, in the absence of Notch, engrailed expression is not abolished (except in the ventral epidermis, where it is abolished; AMA unpublished observation) but reduced which suggests that Notch is only one component of a Wingless receptor. The possibility that there is a receptor complex would thus suggest that perhaps, in the absence of Notch, excess Wingless might work through a partial receptor (see also Couso and Martinez Arias, 1994 and Brennan *et al.,* 1997 for discussions of this issue).

Dishevelled as a link between Notch and Wingless

The functional interactions between Notch and wingless signaling are complemented with reports of interactions between Notch and Dishevelled. In particular, although physical interactions between Wingless and Notch remain to be proven, a physical interaction has been described between the intracellular domain of Notch and a specific region of Dishevelled in a yeast two hybrid system (Axelrod *et al.,* 1996).

A function for this interaction can be deduced from genetic experiments. Whereas loss of *dsh* function leads to the absence of neural precursors, excess Dishevelled leads to an excess of neural precursors which is enhanced by lowering the activity of Notch or Delta. Although a simple interpretation of these interactions is that Notch is involved in the transduction of the wingless signal, it has been suggested that they reflect a role for Dishevelled in antagonizing the function of Notch during lateral inhibition. This is surprising because when Dishevelled is overexpressed, the extra bristles are perfectly spaced, a phenotype very different from that of Notch mutants, in which the bristles are duplicated or multiplied. In addition, it is worth remembering that during wing development Dishevelled works with rather than against Notch signaling. Another argument that questions this proposal is that mutations in Notch that delete the region that binds Dishevelled e.g., N^{60g11}, do indeed result in the loss of neural precursors. However, these mutations are not gain of function but rather loss of function in a hereto unknown proneural function of Notch (Brennan *et al.,* 1997).

For these reasons, the existing observations are equally compatible for a role in which Dishevelled acts as a link between Notch and other Wingless receptors to stabilize a signaling complex which forms upon Wingless binding each of its constituents.

Notch as an element of a developmental switch

Altogether the results summarized above suggest: 1) that there is a close relationship between Notch and Wingless signaling during *Drosophila* development; 2) that these interactions cannot be explained away by arguing simply for parallel signaling pathways or by interactions of Dishevelled with Notch which do not involve Wingless; and 3) that the nature and outcomes of these interactions are complex.

The possibility that Notch and Wingless interact directly (Young and Wesley, 1997) and that Notch might thus be a truly multifunctional receptor, i.e., one with multiple ligands which elicit different responses, suggests a view of Notch as the central piece of a molecular device involved in the assignation of cell fates during development (Fig. 8). It might be that, when cells face a fate choice, the decision making process depends on the state of Notch activity: if more Wingless than Delta is bound to Notch, cells adopt that fate, but if more Delta than Wingless is bound to them they do not. That is, whether a cell adopts or forfeits a fate that is offered to it by running programs of gene expression might be determined by the relative concentrations of Delta and Wingless. This suggestion would account for the observation that *Notch* and *wg* mutants have opposing mutant phenotypes in the process of cell fate assignations in the mesoderm and the nervous system and views of *Notch* as a substrate for competition by different ligands which would take it into different receptor complexes.

The two aspects of Notch function discussed above: its ability to be part of more than one receptor complex and to be a substrate for competition, could allow Notch to operate as a developmental switch. In addition, they might help explain the observation that reducing the amount of Delta or Notch by half when increasing the amount of Wingless or Dishevelled, leads to a dramatic increase in Wingless signaling (Axelrod *et al.,* 1996 and unpublished observation). It might be that the stoichiometries of Notch are different in the different receptor complexes in which it participates, and that the number of Notch molecules in a Wingless receptor complex is lower than the number of Notch molecules in a Delta receptor complex. For example Notch might contribute dimers to the Delta receptor complex but only monomers to the Wingless one. Under these conditions, simple kinetic considerations indicate that lowering the amount of Notch by half will lead to a moderate increase in wingless signaling which will be dramatically enhanced by increasing the amount of Wingless or Dishevelled (unpublished observation), as it is observed (Axelrod *et al.,* 1996 and unpublished observation). This possibility should lead to a re-examination of the results of Axelrod *et al.* (1996) and, perhaps, of the *N* haploinsufficient phenotype itself. The increases in sensory organs observed in both cases might not be simply due to negative effects of wingless signaling on lateral inhibition, but to increased signaling in a concomitant proneural signaling pathway which requires also Notch (Couso *et al.,* 1994; Brennan *et al.,* 1997).

During development, cells are offered choices of developmental fate which they can either accept or forfeit. In the latter event, they will remain uncommitted and can undergo a different choice later in development. If they accept that fate they will be funnelled down a program of gene expression that will lead to similar but qualitatively different choices later in development. It is possible that the switch proposed above is a central element in this iterative process. In particular, if the active forms of each of the signaling components is under the control of the system, it might be possible to conceive a simple homeostatic device that is used over and over to influence the cell fate assignations that characterize the development of multicellular organisms. During the cell fate decisions the amount of Notch at the surface would be depleted by its interactions with the different ligands and, as a result of the process, Notch might be restored to the surface of the cells for the next round of decisions. The qualitative nature of the decisions i.e., what is being decided in terms of expression of transcription factors, would depend on the internal developmental program, the switch being only involved in whether or not a cell accepts or forfeits a decision.

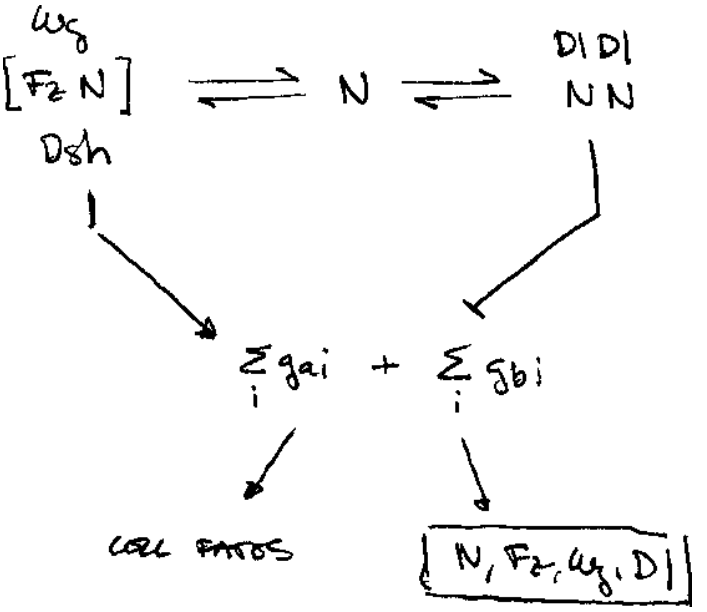

Fig. 8. Notch as the central element of a switch during cell fate assignation. *As discussed in the text, Notch might be a substrate for competition between Delta and Wingless and the outcome of this competition is the specification of cell fates. If more Notch exists in the Wingless receptor complex, the cell will adopt a fate that is offered to it. Whereas if there is more Notch in the Delta complex, it will not. The genes regulated by both systems are the same and include genes directly involved in the process of cell fate assignation (ga) and others involved in the regulation of the system (gb). The latter are probably involved in regulating the levels of expression of the basic machinery of the system. As shown it is likely that Notch contributes a monomer to the Wingless receptor and a dimer to the Delta one.*

The essence of the mechanism outlined here is very akin to that suggested for Entelechia by Antonio García Bellido (1992,1994). This model seeks to understand relationships between growth and pattern in the wing disc of *Drosophila* and envisions ordered fluctuations in the ratios of signals, receptors and responding genes as the basis of the molecular engine for these processes. Furthermore, it was proposed that Notch and Delta are key players in these regulatory interactions and that, as long as there are unbound receptors and free ligands in the cell surface, the system will continue to compute information and thus continue to grow and, by extension, to be patterned. In the Entelechia condition, this stops because ligands and receptors balance each other out (García Bellido and de Celis, 1992).

Similarly, in the model proposed here, cell fate assignations are driven by fluctuations in the concentration of Notch and will continue as long as Notch and the ligands are in the surface of the cells. If the outcome of adopting a cell fate is to suppress the expression of Notch or of the competing ligands, this might signal the initiation of differentiation. The model presented here makes a series of predictions that can be tested experimentally.

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