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Positioning and differentiation of veins in the *Drosophila* wing

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ABSTRACT Morphogenesis is the process by which structures with characteristic sizes, proportions and patterns of cell differentiation are generated during the development of multicellular organisms. How the elaboration of pattern is related to cell proliferation and growth control is a critical aspect of morphogenesis. The imaginal discs of *Drosophila* are a suitable model in which this can be investigated at cellular and molecular level, and recent genetic and developmental analysis has identified some of the key genes and mechanisms that participate in the regulation of their growth and patterning. This review will focus on the formation of the venation pattern in the adult wing, particularly on: 1) the subdivision of the wing blade into domains of gene expression that position the veins, and 2) the cell-cell signaling pathways that participate in the final differentiation of veins.

KEY WORDS: *imaginal development, vein differentiation, pattern formation*

The adult pattern of veins

The *Drosophila* wing is formed by dorsal and ventral surfaces which adhere tightly together through the basal cell membranes. The longitudinal veins run from basal trunks towards the wing margin, where they end at invariant positions (Fig. 1A). They have characteristic dorsal and ventral components formed by stripes of cells that are smaller and have more heavily pigmented cuticle than other wing cells (Fig. 1C-D). As in other pattern formation processes, the generation of the venation pattern can be divided in two stages: 1) determination of the positions in which the veins will differentiate and 2) differentiation of cells to form vein tissue. The allocation of veins to particular regions is determined by the genes and mechanisms that generate positional information in the growing wing disc. The differentiation of the veins then occurs by the combination of specific transcription factors and cell signaling processes conferring particular "vein" qualities to individual cells.

Compartment boundaries as major references for wing patterning

The adult wing arises from the dorsal mesothoracic imaginal disc, the wing disc. Imaginal discs are epithelial sacs that originate from embryonic cells that proliferate during larval development, and in a mature larva include a nearly complete inventory of the adult structures of the fly (Cohen, 1993). Wing disc cells are histologically similar, but they differ greatly in patterns of gene expression. Heterogeneous patterns of gene expression related to sensory organ (Campuzano and Modolell, 1992) or vein development (Sturtevant and Bier, 1995) are manifest in the mature third instar disc (Fig. 1B), and they are the result of a progressive process in which different parts of the disc acquire individual qualities. A major step in the understanding of imaginal disc development was the identification of lineage restrictions that subdivide the wing into compartments, such that the progeny of a group of cells (polyclone) is restricted to a particular part of the adult wing (García-Bellido *et al.,* 1973). The imaginal primordium is already divided into anterior and posterior cells during embryogenesis, and this is followed in the second larval instar by a further clonal segregation between dorsal and ventral cells (García-Bellido, 1975). The discovery of lineage compartments was followed by the realization that they correspond to domains of expression of specific genes, such as *engrailed* and *apterous* in posterior and dorsal cells, respectively (Kornberg *et al.,* 1985; Cohen *et al.,* 1992). It was postulated that this category of genes ("selectors") would confer a "genetic address" to groups of cells, determining their subsequent development (García-Bellido, 1975; Blair, 1995; Lawrence and Struhl, 1996). The subdivision of the disc into compartments has a second consequence that was foreseen on theoretical grounds: compartment boundaries can be considered as "organizing centres" with determining roles on growth and pattern within compartments (Meinhardt, 1982,1983). This theoretical prediction has been supported by the recent analysis of genes that mediate cell interactions between compartments and that have a major influence on the development of imaginal discs (Blair, 1995; Lawrence and Struhl, 1996). For

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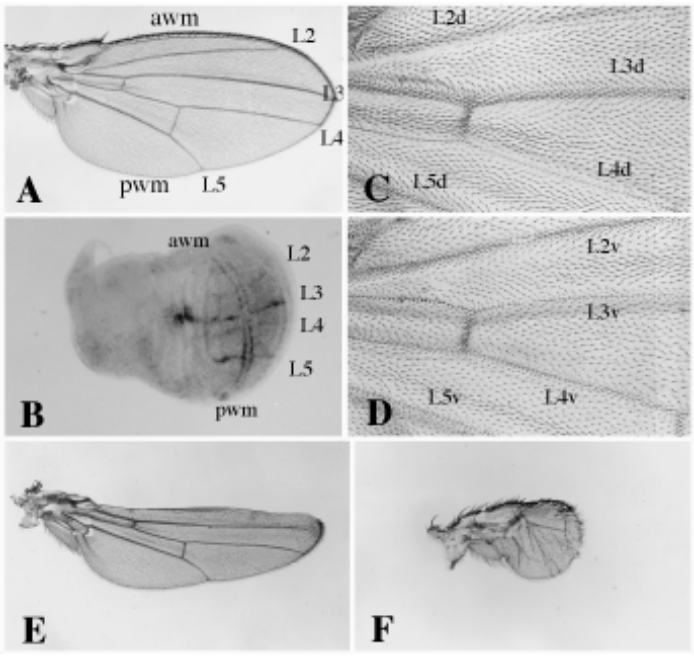

Fig. 1. Wild type wing and global effects of *dpp* and *Notch* activities
(A) *Wild type wing showing the regular spacing between longitudinal veins (L2 to L5).* **(B)** *Expression of ve in the primordia of all longitudinal veins (L2 to L5) and in two parallel stripes in the anterior and posterior wing margin (awm and pwm respectively).* **(C,D)** *Dorsal (C) and ventral (D) wing surfaces to show the differences in the dorsal and ventral components of each vein (d and v respectively).* **(E,F)** *Mutant phenotype of genes affecting dorso-ventral (E) and antero/posterior (F) patterning. (E) Loss of wing margin and adjacent tissue in an anterior Notch mutant clone. (F) Loss of wing growth and patterning characteristic of weak dpp disc alleles.*

example, at the anterior side of the anterior/posterior compartment boundary, the secreted protein Hedgehog activates transcription of *decapentaplegic*, a gene encoding a transforming growth factor beta protein homolog that has long range effects on promoting growth and organizing pattern (Fig. 1F). A different set of genes confers specialized qualities to cells forming the dorso/ventral boundary, where the local activation of the membrane receptor protein Notch directs the expression of several genes required for wing growth such as *vestigial* and *wingless* (Fig. 1E). The genetic interactions occurring at compartment boundaries have been the subject of several recent reviews (Blair, 1995; Lawrence and Struhl, 1996), and are only considered briefly in Figure 2.

Subdivision of the wing blade into domains of gene expression

The combined effects of *hedgehog* and *decapentaplegic* have a key role in organizing domains of gene expression along the antero-posterior axis of the wing disc. In addition to the activation of *dpp* (Basler and Struhl, 1994; Tabata and Kornberg, 1994; Felsenfeld and Kennison, 1995; Sanicola *et al.,* 1995), the hedgehog pathway is directly responsible for the anterior expression of the signaling molecule encoded by *vein (vn)* (Simcox *et al.,* 1996 and unpublished data), the transcription factors of the *iroquois* gene complex (Gomez-Skarmeta and Modolell, 1996; Gomez-Skarmeta *et al.,* 1996), and the anterior expression of *engrailed* (de Celis and Ruiz-Gomez, 1995; Guillen *et al.,* 1995; Mullor *et al.,* 1997). Dpp itself is required for the expression in broad domains centred on the A/P boundary of several genes encoding transcription factors, such as *spalt* and *spalt related (sal/salr)* (Barrio *et al.,* 1996) and *optomotor blind (omb)* (de Celis *et al.,* 1996; Grimm and Pflugfelder, 1996; Lecuit *et al.,* 1996; Nellen *et al.,* 1996) (Fig. 3). In addition, Dpp is also required for the expression of *vestigial (vg),* a gene expressed and required in all wing blade cells (Kim *et al.,* 1996,1997).

Individual genes are activated in response to Hh and Dpp at different times in the development of the disc. For example Hh directs *dpp* expression from the very beginning of disc development, whereas activation of *vn* and *iro* expression by Hh is only apparent mid way through the third larval instar (Gomez-Skarmeta and Modolell, 1996; Simcox *et al.,* 1996). Similarly, despite the early presence of Dpp, the expression of two of its downstream genes, *sal* and *omb*, starts later as thin stripes that expand during the third larval instar to form the broad domains characteristic of the mature disc (de Celis *et al.,* 1996; Grimm and Pflugfelder, 1996). The basis for the precise timing in the activation of downstream genes by *dpp* and *hh* is unknown, but indicates that additional factors are needed for individual genes to respond to these signaling molecules. The gene regulation cascade occurring at the A/P boundary leads to a complex landscape of transcription factor expression that links the organizing effect of Hh to the spatially restricted expression of other vein-forming genes to the veins.

Prefiguring the vein pattern

The transcription factors of the *iro* complex and the secreted protein Vn appear to be directly involved in the formation of veins. Thus the restricted expression of *iro* in the primordia of veins L3 (in response to Hh) and L5 (in response to unknown signals) could be directly activating the transcription of *veinlet (ve)*, a critical mediator of vein differentiation (Sturtevant *et al.,* 1993; Gomez-Skarmeta *et al.,* 1996). The expression of *vn* in response to Hh is also closely related to the differentiation of L4 even though this vein forms posterior to the domain of *vn* expression. Thus anterior clones of *vn* mutant cells non-autonomously prevent the differentiation of this posterior vein (García-Bellido *et al.,* 1994), presumably because secreted Vn protein is needed to activate the tyrosine kinase receptor homolog Torpedo (Top) in the domain where L4 will form (Simcox *et al.,* 1996). The *dpp* downstream genes *sal* and *salr* are also required in vein formation. Interestingly these genes are not necessary for the formation of L3 and L4, the veins included in their domain of high expression, but are required for the differentiation of L2 and the localization of L5 (de Celis *et al.,* 1996). These two veins form at different positions relative to the boundary between *sal/salr* expressing and non-expressing cells. The L2 vein is formed by cells that contain very low levels of Sal and Salr proteins, and the elimination of both *sal* and *salr* in genetic mosaics leads to the failure of L2 to differentiate (de Celis *et al.,* 1996; Fig. 4C). In contrast, elimination of only *sal* results in the displacement of L2 towards more posterior locations, where it is formed by *sal* mutant cells with a functional *salr* gene (de Celis *et al.,* 1996; Sturtevant *et al.,* 1997). The situation is very different in the case of L5, because this vein forms several cell diameters posterior to cells expressing Sal and Salr proteins. In spite of this, the position of L5 is shifted anteriorly

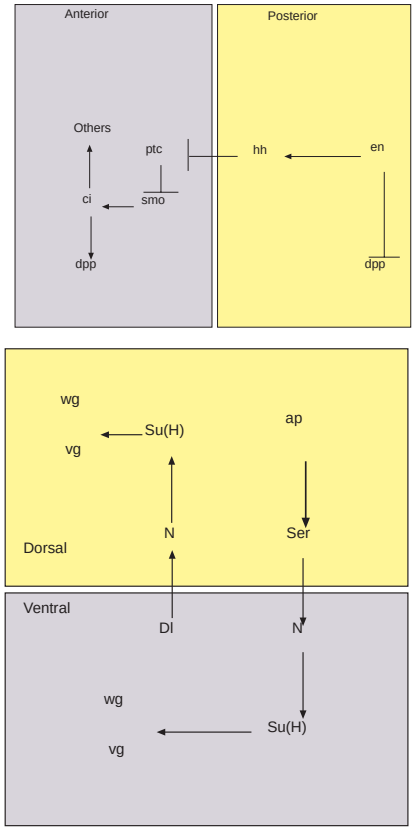

Fig. 2. Genetic interactions occurring at the antero-posterior and dorso-ventral compartment boundaries. (A) *Engrailed (en) represses the transcription of decapentaplegic (dpp) and allows the expression of hedgehog (hh) in posterior cells. In anterior cells Hh interacts with Patched (ptc), releasing the repression of Smoothened (smo). The activity of smo is transduced through a protein complex including the products of the genes fused, Costal2 and Cubitus interruptus (ci), a Gly-protein that might activate the transcription of dpp and presumably other genes (others) in anterior cells exposed to Hh.* **(B)** *The Notch ligands Serrate (Ser) and Delta (Dl) activate Notch (N) in ventral and dorsal cells respectively, although Dl is also required in dorsal cells. The expression of Ser is restricted during most of larval development to the dorsal compartment, under the regulation of the dorsal selector gene apterous (ap). Notch activity at the dorso-ventral boundary is mediated through Suppressor of Hairless [Su(H)], and participates in the transcriptional activation of the genes vestigial (vg) and wingless (wg) in dorsal and ventral cells that form the dorso-ventral boundary.*

when *sal* and *salr* activities are removed in the posterior compartment, implying that additional elements mediate the function of these genes in the formation of L5 (de Celis *et al.,* 1996; Fig. 4D). The critical influence of *sal* and *salr* levels in the formation of L2 and L5 veins is also seen when either of these genes is expressed ectopically throughout the wing: the veins L2 and L5 fail to differentiate, and the resulting wing is reduced in size (de Celis *et al.,* 1996; Fig. 4B). Ectopic and generalized expression of the *iro* genes also affects the size of the wing and the patterning of the veins (Gomez-Skarmeta *et al.,* 1996), indicating that restricted expression of all these genes to their normal domains is needed to generate the normal pattern of veins. The effects of manipulating expression of *sal, salr* and *iro* on vein formation suggest that these genes constitute the elements of a pre-pattern of transcription factors that prefigures the actual pattern of veins. Other genes that probably fall in a similar category include *radius incompletus (ri), aristaless (al)* and *abrupt (ab)*. These genes only affect individual veins, such as L2 *(ri, al)* and L5 *(ab)* (Diaz-Benjumea and García-Bellido, 1990a; Schneitz *et al.,* 1993; Hu *et al.,* 1995), and at least one of them, *al*, is exclusively expressed in a broad domain that includes the position of L2 (Campbell *et al.,* 1993).

In summary, the wing blade region of the mature wing imaginal disc is subdivided into domains of gene expression that form a landscape of transcriptional regulators (pre-pattern; Stern, 1954) required for the correct positioning of veins. It is not yet clear what mechanisms link this level of organization in broad domains with the restricted expression of genes directly involved in vein differentiation. The organization of the wing into domains of gene expression appears to be similar to both the subdivision of the leg discs in proximo/distal sectors of gene expression (Lecuit and Cohen, 1997), and the regionalization of the abdominal histoblast along the antero/posterior axes (Struhl *et al.,* 1997). The process of partitioning a growing epithelium into domains of gene expression has also strong resemblances with embryonic segmentation, where "gap" genes define broad domains that set the periodic patterns of expression of "pair rule" genes (Pankratz and Jackle, 1993). A major difference between subdivision of the blastoderm and imaginal discs is the involvement of cell proliferation in the second process. The appearance of domains of gene expression in discs starts before cell proliferation is completed, when the number of cells is only a fraction of the number in the mature disc. How the generation and maintenance of gene expression domains is associated with cell proliferation is therefore a critical issue in understanding the formation of an integrated pattern where each part has a characteristic size.

Cell proliferation patterns in the wing

Cell division in the wing disc is interstitial, with an average cell cycle of 8.5 h, and appears to have no obvious topographic relationship to compartment borders (Gonzalez-Gaitan *et al.,* 1994). Progression through the cell cycle is not related by lineage, but neither is it random because it occurs in clusters of synchro-

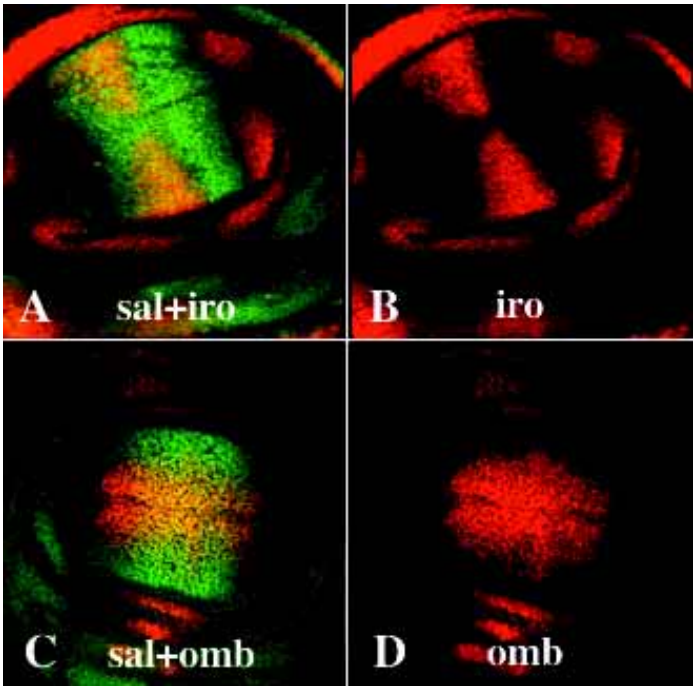

Fig. 3. Antero/posterior domains of expression in the wing blade. (A,B) *Expression of spalt (sal, green in A) and iroquois (iro, red in B).* **(C,D)** *Nested expression of sal (green in C) within a broader domain of optomotor blind (omb, red in D).*

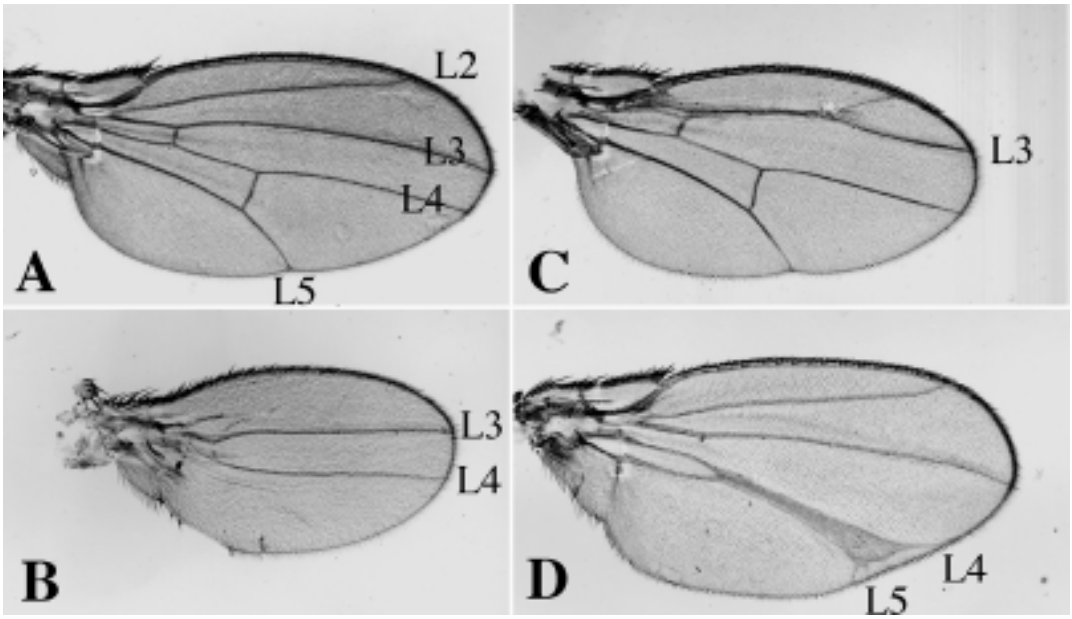

Fig. 4. Vein pattern modifications resulting from manipulating the expression of *sal* and *salr* genes. **(A)** *Wild type wing showing the normal position of veins (L2-L5).* **(B)** *Only the veins included in the domain of high sal/salr expression (L3 and L4) differentiate when salr is ectopically expressed throughout the wing blade.* **(C,D)** *Mosaic wings in which sal and salr expression is eliminated in the anterior (C) or posterior (D) compartment showing disappearance of L2 (C) and fusion of L4 and L5 (D).*

nized cells (Milan *et al.,* 1996a,b). At each developmental stage clusters of dividing cells are of similar size, and cluster formation must rely on the recruitment of competent neighboring cells by intercellular communication (Milan *et al.,* 1996a,b). Very little is known about the relationship between the spatial pattern of cell proliferation and the formation of constant-sized domains of gene expression. However, the observation that veins correspond to preferential clonal boundaries (Gonzalez-Gaitan *et al.,* 1994) suggests that there is a link between the positional information system that defines vein regions and the regulated cell proliferation that determines size (Diaz-Benjumea and García-Bellido, 1990a; García-Bellido and de Celis, 1992). Thus, it is conceivable that cellular proliferation is regulated in partially autonomous domains whose boundaries are pre-vein regions. Each of these domains would acquire its final size by intercalation of positional values between determined boundary regions (pre-veins), thereby spacing adjacent veins (García-Bellido and de Celis, 1992).

The interpretation that quantitative positional values are critical to direct cell proliferation between pre-vein boundaries predicts that there should exist mutations affecting intercalar cell proliferation and vein spacing simultaneously. One good candidate is *extramacrochaetae (emc),* because in genetic mosaics of *emc* alleles it appears that the extent of intercalar cell proliferation is related to the levels of *emc* activity. Cells where the function of *emc* is eliminated do not proliferate, presumably because they can not generate the intermediate positional values characteristic of intervein regions. In hypomorphic conditions, *emc* mutant cells do proliferate, but they grow almost exclusively along the proximodistal axis, forming extremely elongated clones that tend to be associated with veins (García-Alonso and García-Bellido, 1988; de Celis *et al.,* 1995a). In even weaker *emc* allelic conditions, *emc* mutant cells can grow extensively, filling entire inter-vein regions. However the size of mutant intervein territories is very reduced along the antero-posterior axis, resulting in the formation of adjacent veins (De Celis *et al.,* 1995a; Fig. 5). *emc* encodes a non-basic helix-loop-helix protein that is thought to antagonize the activity of several basic helix-loop-helix proteins, thus acting as a genetic repressor (Ellis *et al.,* 1990; Garrell and Modolell, 1990). The failure to generate normal-sized intervein territories in *emc* genetic mosaics suggests that a set of proteins whose activity is antagonized by Emc might be involved in generating intercalar positional values between clonal restriction boundaries.

The Entelechia model

The existence of clonal restrictions along veins, of synchrony in cell cycle progression between neighboring cells, and the behavior of mutant cells in genetic mosaics have led to a generative model to explain size control, the "Entelechia model". The model postulates that intercalar cell proliferation is driven by differences in positional values between neighboring cells. Cells would be able to communicate their positional values, and cell division will be triggered only when the difference in value between neighboring cells is above a certain threshold. According to this vision, the generation of territories with a characteristic size results from the resolution of all positional discontinuities by cell proliferation (García-Bellido and de Celis, 1992; García-Bellido *et al.,* 1994; Milan *et al.,* 1996a,b). The Entelechia model is similar to the polar coordinate model in that pattern formation is intrinsically associated with cell proliferation (French *et al.,* 1976), but differs in its emphasis on local cell interactions that occur without reference to global organizing centres (see also Bryant and Simpson, 1984).

Much work will be needed to elucidate the information that cells compute to drive cell proliferation, as well as the mechanisms that lead to growth termination once the positional landscape is completed. However, it appears that the organized deployment of position-specific transcription factors participates in regionalizing the growing ephitelium, conferring positional identities to broad wing sectors. Within these territories, cell intercalation might determine cell division patterns between reference boundaries. Intercalation to resolve positional discontinuities would lead to the construction of sectors of gene expression with constant sizes and whose edges would position vein territories. In this context it

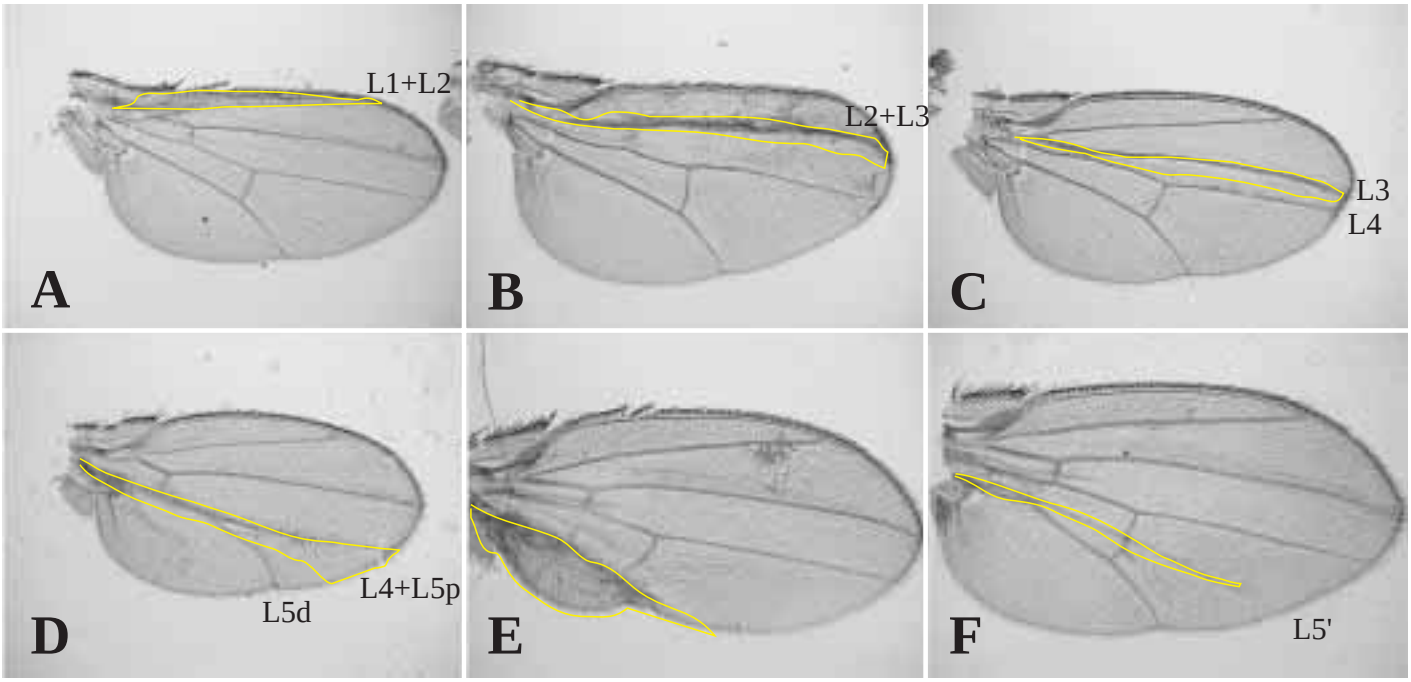

Fig. 5. Alterations in the spacing between veins in wings containing *emc* mutant clones. (A-D) *Clones of the weak allele emc[1] in different regions of the wing cause fusion of adjacent veins: L1 and L2 (A); L2 and L3 (B); L3 and L4 (C) and of L4 and proximal L5 (L5p D).* **(E,F)** *Clones of the stronger combination emc[1]/Df(3L)emc cause extreme reductions in the size of the intervein sector between L5 and the posterior wing margin (E) or grow as elongated stripes of cells that can differentiate ectopic veins (F). Clones in A-E are induced before the segregation between dorsal and ventral compartments. All clones are outlined.*

is possible that the global effects on wing size and pattern observed in *hh*, *dpp* or *Notch* alleles (Shellenbarger and Mohler, 1978; Posakony *et al.,* 1991; Basler and Struhl, 1994) are a consequence of the failure to initiate, early during disc development, positional discontinuities that will drive subsequent intercalar cell proliferation.

Signal transduction pathways that direct vein differentiation

The earlier stages in the construction of the venation pattern end when the expression of specific genes is allocated to presumptive vein regions. At this time we can consider the presumptive wing blade as being formed by alternating vein and intervein territories, which require different sets of genes to differentiate during pupal development. For example, two genes encoding transcription factors, *ventral veinless (vvl)* and *blistered (bs)* are expressed in vein and intervein territories respectively. The expression of *vvl* becomes restricted to the veins only after puparium formation, when Vvl protein is localized in pre-vein territories that are broader than the mature veins (De Celis *et al.,* 1995b). In contrast, the expression of *bs* is restricted to intervein regions during larval development, where it is required for them to differentiate appropriately (Fristrom *et al.,* 1994; Montagne *et al.,* 1996). During the later stages of larval development, and at least the first two days of pupal development, the subdivision of the wing blade into pre-vein and intervein territories is maintained by the activities of Bs and Vvl. In addition, cells in pre-vein regions will go through a cascade of signaling events that further restricts vein differentiation to the central region of the pre-vein.

The activities of two signaling pathways that function during imaginal and pupal development, Notch and Top, have a determining influence on vein differentiation (Fig. 6). Top and Notch pathways have antagonistic effects on vein development: Top activity promotes vein formation whereas Notch suppresses it. The contrasting effects of top and Notch are revealed in the phenotypes of mutations that alter (either increase or reduce) the activity of these pathways. Loss-of-function *top* alleles, or reduction in any of its downstream genes, results in the absence of veins (Clifford and Schupbach, 1989; Diaz-Benjumea and García-Bellido, 1990b; Diaz-Benjumea and Hafen, 1994), whereas increased or ectopic activation of the pathway results in the formation of both ectopic and thicker veins (Diaz-Benjumea and García-Bellido, 1990b; Baker and Rubin, 1992; Fig. 6A,D). In contrast, mutations which reduce *Notch* activity result in the formation of thicker veins, and increased or ectopic Notch activation represses vein differentiation (de Celis and García-Bellido, 1994; Fig. 6C,F). The places where the receptors Notch and Top are activated are complementary, Top being activated in vein cells and Notch in intervein cells (de Celis *et al.,* 1997; Gabay *et al.,* 1997). The existence of precise mechanisms that define the cells in which Top and Notch activities are initiated and maintained is a critical aspect in the formation of veins with a normal width . In the case of the Top pathway, the distribution of the putative ligand Vn is restricted to inter-veins (Simcox *et al.,* 1996), whereas expression of *ve*, a gene encoding a transmembrane protein that facilitates Top signaling, is restricted to veins (Sturtevant *et al.,* 1993; Fig. 8B). The expression of *ve* itself depends on *top* function (A. Baonza, personal communication), a regulatory relationship that could be utilized to maintain high levels of Top activity in vein cells.

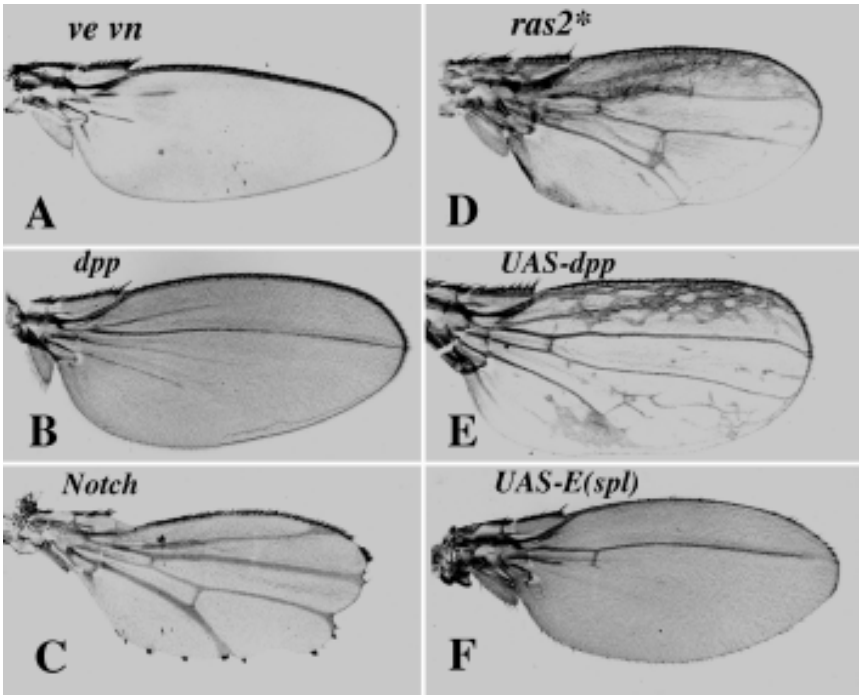

Fig. 6. Vein differentiation phenotypes resulting from modification in the activities of Top, Dpp and Notch signaling pathways. (A-C) *Loss of function of Top (A; ve vn wing) and dpp (B; dpp^{s4}/dpp^{s22} wing) prevent vein differentiation, whereas loss of function of Notch (C; fa^{nd} wing) results in the formation of thicker veins.* **(D,E)** *Ectopic activation of Top (D, activated ras2 wing) and Dpp (E, UAS-dpp/Gal4-580 wing) results in the formation of thicker and ectopic veins.* **(F)** *In contrast ectopic expression of the Notch-downstream gene E(spl)mb (UAS-E(spl)mb/Gal4-179 wing) results in the absence of veins.*

In the case of Notch, its ligand, the transmembrane protein Delta (Dl), is expressed at highest levels in the vein, whereas expression of Notch itself is higher in inter-veins (Kidd *et al.,* 1989; Fehon *et al.,* 1991; de Celis *et al.,* 1997; Fig. 7A,B). The heterogeneous distribution of Dl and Notch leads to the expression of the Notch downstream gene *Enhancer of split mβ (E(spl)mβ)* in the inter-veins, with maximal expression in the cells that separate each vein from the adjacent inter-vein (de Celis *et al.,* 1997; Fig. 7C). Once signaling is initiated in these cells, a feedback mechanism that links *Notch* activity with *Notch* transcription results in the accumulation of Notch protein in cells where Notch has been activated. This accumulation of Notch reinforces the polarity of signaling and maintains Notch expression and activity restricted to vein/intervein boundary cells during pupal development (de Celis *et al.,* 1997; Fig. 8D).

A consequence of Notch activity and subsequent *E(spl)mβ* expression is the transcriptional repression of *ve*. Thus, the differentiation of thicker veins in *Notch* loss of function mutants depends on *top* activity, and results from a failure to repress *ve* expression outside the vein (de Celis and García-Bellido, 1994; Sturtevant and Bier, 1995; de Celis *et al.,* 1997). The repression of *ve* by Notch implies that Notch function and Top signaling are intimately associated; Notch restricting the places where Top signaling is more effective. A second mechanism interrelates these two signaling pathways, since top activity is required for the accumulation of Dl in the veins (de Celis *et al.,* 1997). This links the establishment of

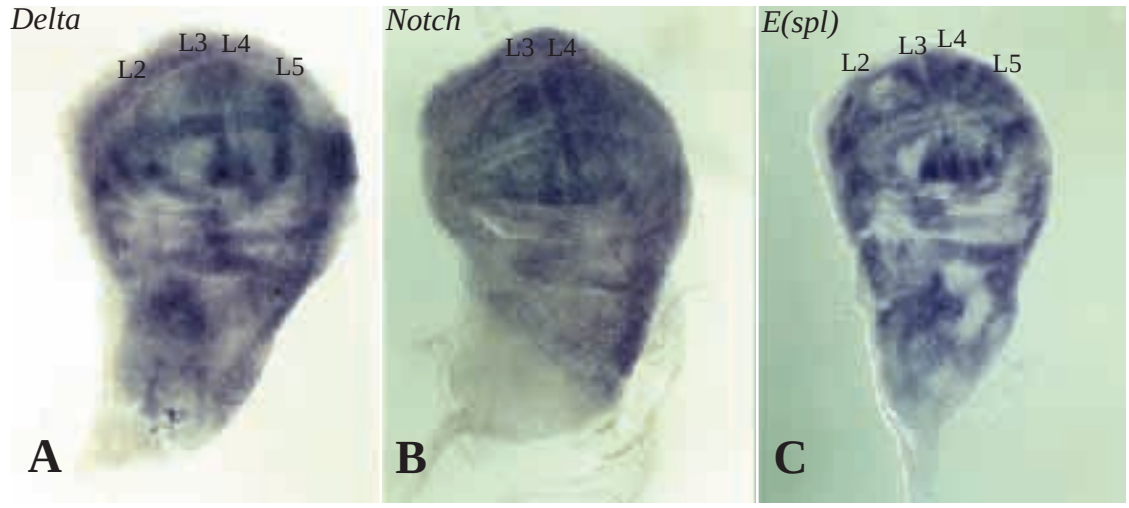

Fig. 7. Expression patterns in the wing disc of three members of the Notch pathway. (A) *Localized expression of the Notch-ligand Dl in vein territories, and* **(B)** *increased expression of Notch in intervein territories result in the preferential accumulation of E(spl)mβ in the interveins* **(C)**. *The presence of E(spl) in the interveins restricts the expression of veinlet, and consequently high levels of Top signaling, to the veins.*

the pro-vein territory by Top with Notch activity, and restricts vein formation within the pro-vein (Fig. 8).

The transcription factors that respond to Top in vein regions are unknown, but one outcome of their activity is the initiation of *dpp* expression in pro-veins. In addition to its role during the growth of the disc, *dpp* is also required for the differentiation of veins, and both loss or ectopic expression of *dpp* have dramatic effects on vein formation that are independent of its early function (Segal and Gelbart, 1985; de Celis, 1997). The expression of *dpp* is initiated in the veins during pupal development, and it is restricted to the places where *ve* is also expressed (Yu *et al.,* 1996; de Celis, 1997; Fig. 8C). *dpp* expression depends on Top activity, and it is required to maintain the vein differentiation program. Genetic epistasis experiments indicate that *dpp* function is needed downstream of *top* and *Notch* (de Celis, 1997). Thus, when *dpp* expression is driven by an exogenous promoter during pupal development it is able to trigger vein formation, even when *top* activity is severely compromised. In addition, once *dpp* is located in the veins it participates in maintaining *ve* expression, therefore connecting Dpp and Top signaling through feedback loops (de Celis, 1997). The expression of *dpp* in the veins is also under the regulation of the Dpp receptor *thick veins (tkv)*. Mutations that reduce, but do not eliminate, *tkv* function cause inappropriate ectopic expression of *dpp*, and result in the formation of thicker veins (Terracol and Lengyel, 1994; de Celis, 1997). Similarly, the expression of *tkv* is also dependent on its own activity, because *tkv* transcription is downregulated in response to Dpp (de Celis, 1997). The observed transcriptional relationships between *dpp* and *tkv* lead to the establishment, in each pre-vein territory, of a tripartite domain in which a central stripe of 2-3 cells wide that expresses *dpp* is bordered by cells with higher levels of *tkv* transcription (Fig. 8C). It is possible that a combination of both different levels of receptor expression and interactions between Tkv and other proteins belonging to Dpp-receptor complexes is responsible for the two different outcomes of *dpp* activity during vein development: activation of vein differentiation within the vein and repression of *dpp* transcription outside the vein.

During pupal development gene expression associated with veins is identical in the dorsal and ventral components of each vein. However, adult veins are distinct in their dorsal and ventral components, only one of them being corrugated. What determines in which vein surface the vein is corrugated is unknown, since genes affecting this process have not been identified. The establishment of surface-specific corrugation might involve interactions between both wing surfaces to ensure that only one vein component is corrugated (García-Bellido, 1977). The final stages of vein differentiation are characterized by the preferential accumulation of specific adhesion molecules by vein or intervein cells (Fristrom *et al.,* 1993; Murray *et al.,* 1995). Cell adhesion molecules, and presumably other cell differentiation genes, constitute the final targets in the vein differentiation program that is initiated in the imaginal disc with the establishment of vein competent territories. This category of genes would translate the activity of several signal transduction pathways into a simple cellular outcome, vein versus intervein differentiation.

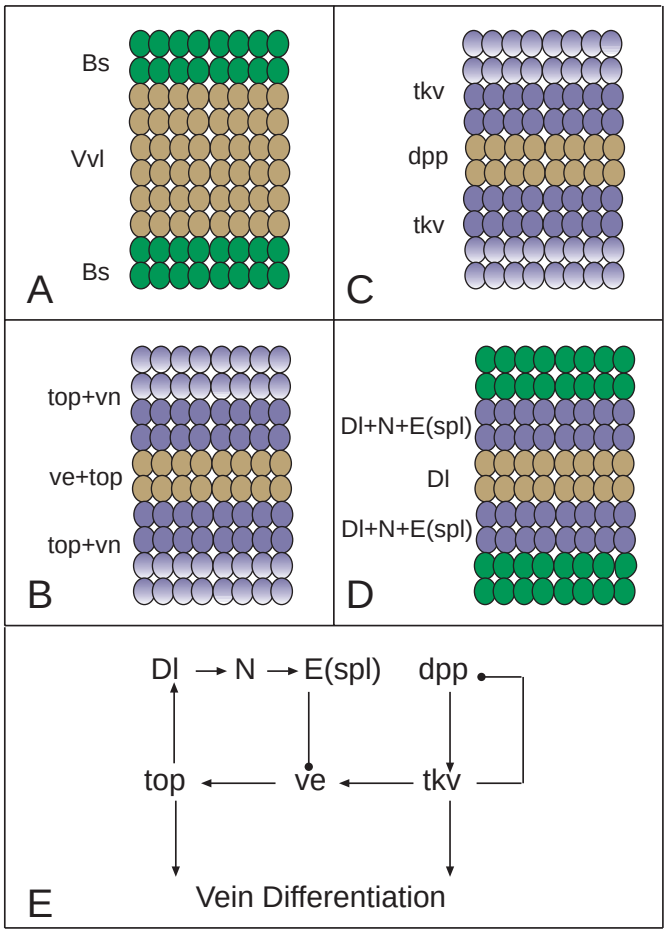

Fig. 8. Expression patterns and Genetic interactions during pupal development (30 h after puparium formation) of genes involved in vein differentiation. **(A)** *During pupal development the wing is subdivided in vein and intervein territories, characterized by the expression of the transcription factors Ventral veinless (Vvl, brown) and Blistered (Bs, green) respectively.* **(B)** *Localization of veinlet (ve, brown) to the vein, and preferential accumulation of top and vein (top and vn, purple) in boundary intervein cells within the pre-vein.* **(C)** *dpp is localized to the vein (dpp, brown), and one member of dpp-receptor complexes, thick veins (tkv. purple) accumulates in a pattern similar to top.* **(D)** *Localization of E(spl)mβ and Notch in boundary intervein cells within the provein (purple). Dl expression is localized to all pro-vein cells (purple and brown).* **(E)** *Genetic interactions, based on genetic and expression pattern analysis, of top, Notch and dpp signaling pathways. Arrows indicate activation and circles repression.*

Concluding remarks

A common theme in both imaginal disc development and vein formation is the generation of territories which have a precise size in terms of cell numbers. This involves the reiterative division of a growing ephitelium into domains of gene expression that are landmarks for positioning pattern elements. The first subdivisions are made with reference to compartment boundaries and rely on the non-autonomous effects of the signaling molecules Dpp and Hh. Expression domains might acquire their size through intercalar cell proliferation between reference boundaries, and determine the positions where a secondary category of genes expressed in smaller domains confer to cells the ability to enter a particular differentiation pathway. The final refinement from pre-veins into mature veins involves several signaling pathways linked through feedback loops, which ultimately determine the specialized characteristics of vein cells.

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