

# Cell proliferation in the attainment of constant sizes and shapes: the Entelechia model

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**ABSTRACT** The Entelechia model is a generative model of morphogenesis where individual cells exhibit surface labels that express scalar difference and planar polarity along two orthogonal axes X and Y. The amount of surface label depends on the level of Martial (M) gene product within each cell. The model assumes that the confrontation of cells on both sides of compartment borders causes an increase in their level of M gene expression. The resulting disparity between the M value of border cells and that of their neighbors induces the latter to divide. After each division the daughter cells increase their own M value, and allocate to the best matching value position. The increase in M value at the borders therefore extends through the anlage in a cascade of proliferation. The Entelechia condition is reached when the border cells attain the species-specific maximal M values, and the value differences between adjacent cells become indistinguishable. Computer simulations reveal that this model accounts for a variety of observations made on imaginal discs, e.g., 1) each disc attains a constant size in terms of number of cells, independently of the growing conditions; 2) clonal restrictions separate populations of cells which proliferate by intercalary growth; 3) dissociated cells are capable of reconstructing original patterns upon reaggregation, and 4) genetic mosaics of morphogenetic mutations show local effects that may differ depending on the position of the mutant cells in the growing anlage.

**KEY WORDS:** *pattern formation, growth control, clonal analysis, compartment borders*

## Introduction

Morphogenesis, the attainment by developing organs of a constant size and shape, is associated with the interaction of two different processes, growth and pattern formation. The former is usually related to cell proliferation, the latter to spatial cell differentiation. So far, major experimental attention has been paid to understanding cell differentiation. We are starting to have an increasingly clearer picture of the role of cell-cell signaling, signal transduction pathways and transcriptional control in the differentiation of many different types of cells. Less understood are the processes leading to the patterned distribution of different cell types in space and in particular the relation of patterned cell differentiation to cell proliferation.

Ordered cell proliferation is obviously related to cell division, which is becoming well understood in molecular genetic terms. But how cell proliferation leads to the generation of morphogenetic space of constant species-specific dimensions is unknown. And yet this is the classical question of how organisms attain their completion or, in Aristotle's terms, their Entelechia. In this paper we present a formal model, the "Entelechia model", of how cells, through proliferation and cell-cell communication generate mor-

phological space of finite size and eventually shape. To that end, we shall make use of data of how cells of the wing disc of *Drosophila* proliferate and behave under normal and experimental conditions.

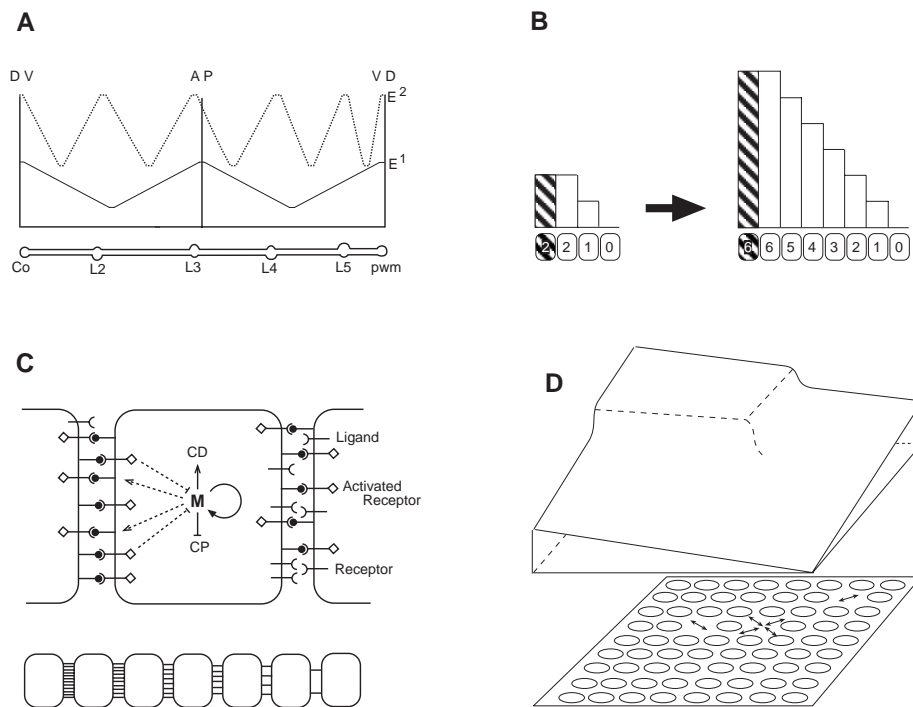
## Current views about pattern formation

There are two classical models currently dealing with patterned cell differentiation. In both models, individual cells acquire their specification (cell differentiation) from the system where they grow, i.e., they respond to an already grown or organized primordium.

In the first model, the structure of the primordium can be that of a morphogenetic field, as in the "prepattern" theory of C. Stern (1954). These fields are defined by centers, outwards from which the intensity of a signal (translated into "positional values") decreases, and to which cells respond, or not, depending on their genetic state. The positional values are in this model scalar values communicated between neighboring cells acting as relays. Fields expand from initial centers (maximal concentration of signals) and can be split into subfields.

This model has been applied to the morphogenesis of *Dictyostelium*, and to morphallactic (without cell proliferation) regeneration (Morgan, 1895). In genetic terms this model has

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**Fig. 1. The Entelechia model for the wing. (A)**

Upper panel, the generative gradients corresponding to the Entelechia condition for compartments ( $E^1$ ) and for vein sectors ( $E^2$ ) in either the dorsal or the ventral surface of the wing. Note that the slopes need not to be the same for each vein sector, as they may depend on local variations of the incremental value for  $E$ . A, anterior; P, posterior; D, dorsal; V, ventral. Lower panel: A/P section of the adult wing with the dorsal and ventral epithelia indicating the longitudinal veins (L2-6) and the wing margins (Co, costa; pwm, posterior wing margin).

**(B)** Two stages of the growth in which the increasing amount of  $M$  gene expression (ordinate: amplitude value of martial genes and their ligands) is correlated with position (numbers correspond to positional values). **(C)** The components of the Entelechia model, showing how cell communication controls cell division. The activated form of the receptors may be associated to specific adhesion molecules, not shown in the figure. The cell will enter CD, cell differentiation, or CP, cell proliferation, depending on the balance between  $M$  (martial) gene expression and  $M$  gene titration by the activated receptors. Bottom, a row of cells with different amounts of activated receptors, illustrating how the distribution of activated receptors polarizes each

cell. **(D)** Gradients of scalar positional values and scalar discontinuities in the X and Y axes of the epithelium. Cell division (arrows) is elicited whenever discontinuities are above the threshold for  $M$  disparity detection, and primarily at the position where there is a disparity in both X and Y axes.

proved to be very elusive, because in the dualism of prepattern and response to it, all known mutations turned out to affect only the latter. In genetic mosaics, mutant cells in a wildtype background cell-autonomously differentiate the mutant phenotype without affecting the surrounding ones [e.g., lack of chaetae in certain positions in *scute* mutations (García-Bellido and Santamaría, 1978, García-Bellido, 1979) or growth of homeotic tissue in *bithorax* mutations (Morata and García-Bellido, 1976)]. These results are interpreted as failures to respond to an invariant prepattern. Only very recently have genes operationally involved in these prepatterns been described (see below and discussion).

The second model, that of "positional information", states that cells differentiate according to their positional values, defined there as the concentration of a diffusible signal (morphogen or other) starting from a source and monotonically decreasing to a sink (Wolpert, 1969, 1971). Cells would respond to these signals independently of each other and differentiate various cell types or states depending on the distance from source and sink, thus formally ensuring pattern size invariance.

This model was first used to successfully explain epimorphic regeneration in hydra. It is now extended to explain patterning in other developing systems, such as early segmentation in the syncytial eggs of insects. Related models have been proposed to deal with epimorphic regeneration in appendages, like the "clock" (polar coordinates) model (French, *et al.*, 1976) which contemplates positional values along a proximo distal and a circular axis of reference. The discovery of clonal restriction boundaries in imaginal discs of *Drosophila* allows for the assignment of sources of signals to concrete entities in the developing organ. Several putative morphogens have been molecularly characterized (Lawrence and Struhl, 1996; Gómez-Skarmeta *et al.*, 1996). Again, this

model attempts to explain the patterning of cell differentiation in an already grown organ. These models hardly deal with the problem of growth, with the definition of the dimensions of the sources (because they rely on size invariance), with the acquisition of species specific final shapes and sizes, and with local pattern "accommodation" found in genetic mosaics (see below and discussion).

Both models invoke global supracellular properties impinging on individual cells, i.e., are systemic models. By contrast, the Entelechia model presented here relates to cell behavior (reflecting active genomes) and cell communication. Here the properties of the system result from the local properties of cells adding up to the whole. It aims, in the end, at understanding the mechanisms of the genetic control of size and shape via ordered cell proliferation, and to explain the attainment of species specific forms.

### Parameters of growth of the wing disc

The wing disc of *Drosophila* is the best characterized imaginal disc in terms of its cell proliferation parameters. The wing disc singularizes as a primordium of about 30 epidermal cells in the second thoracic segment of the embryo. At that time it is already clonally subdivided into two developmental compartments, an anterior (A) and a posterior (P) one. The primordium invaginates into the lumen of the embryo and after a quiescent period starts a cell division regime at the end of the first instar larval stage. Following cell proliferation new clonal restrictions separate dorsal from ventral, and proximal (notum/pleura) from distal (wing proper) compartments. At the end of the second instar, clonal restrictions begin to define the presumptive veins, and proliferation becomes restricted within intervein sectors. The wing disc evaginates at

pupation, and after one more round of cell divisions, at about 30 h of pupal development, cuticular differentiation is initiated. (García-Bellido and de Celis, 1992)

Cell proliferation is intercalar throughout the growth phase, with an average rhythm of 8.5 h. The grown disc contains about 50,000 adult cells. This final number remains constant, independent of disc growing conditions (García-Bellido, 1965). Certainly adult wings can be smaller (in terms of cell numbers) under restrictive nutritional conditions, but then they differentiate less than normal numbers of pattern elements such as chaetae (García-Bellido, 1972), i.e., their pattern is not size invariant.

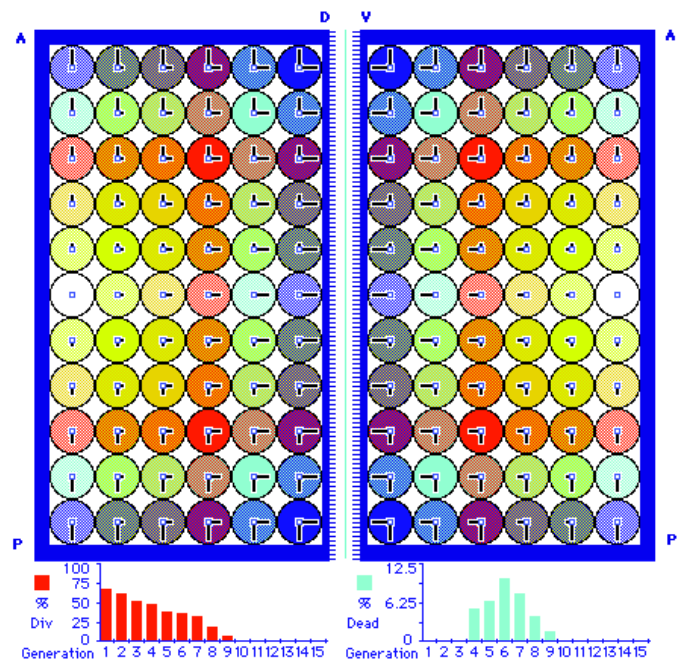
### Specific precedents indicating local control of cell proliferation

During the proliferative phase of the wing disc, the cells divide synchronously in clusters of a few non-clonally related cells. Mitotic orientation in these clusters is at random but the daughter cells subsequently allocate preferentially along the proximo-distal axis of the prospective wing (Milan, *et al.*, 1997). This postmitotic allocation is reminiscent of the capability of dissociated cells from mature imaginal discs to partially reconstruct patterned territories in mosaics, thanks to positional labels and polarities of the dissociated cells (García-Bellido, 1966).

Genetic mosaics have shown large local autonomy in cell proliferation. Thus wild type cells in a Minute background, growing faster, give rise to larger than normal clones, but allow the construction of an otherwise normally sized and patterned wing (Morata and Ripoll, 1975). *M/+* cells, which can give rise to whole organisms, fail to live in clones in a *+/+* background because they cannot compete with the pace of proliferation of the surrounding, normally growing cells. This competitive behavior reveals that *+/+* cells make the developmental decisions of dividing and generating morphogenetic space earlier than *M/+* cells (Simpson and Morata, 1981). Haploid cells (Santamaría and Gans, 1980, Santamaría, 1983) or cells harboring mutations that make them smaller (Díaz-Benjumea and García-Bellido, 1990), will however make patterns that are normally spaced in terms of cell numbers. Mosaics of these cells and normal cells show repetition of pattern elements along the mosaic border. Reciprocally, mosaics of larger than normal cells cause normally patterned outgrowths (Ferrus and García-Bellido, 1976; Held, 1979). Both results indicate that cells compute pattern distances in term of cell numbers, rather than responding to physical distances as postulated for morphogens.

Local autonomy in pattern formation is also shown by the offspring of isolated cells grown in a “feeder layer”; they form normally patterned territories that are fractions of the whole pattern. (García-Bellido and Nöthiger, 1976)

Genetic mosaics of *extramacrochaete* (*emc*) mutations that cause changes in cell differentiation to vein or intervein histotypes show differential cell behavior, their clones proliferating towards or away from veins, revealing local autonomy related to final positions of cells in the pattern (García-Alonso and García-Bellido, 1988; García-Bellido *et al.*, 1994; de Celis *et al.*, 1995). Mosaics of mutations such as *vein* (*vn*) or *nubbin* (*nub*), which cause reduced wings, also show local autonomy, causing reduced or absent vein sectors and consequently an “accommodation” of the remaining wing to match in size the mutant territory (García-Bellido *et al.*, 1994; Cifuentes and García-Bellido, 1997).

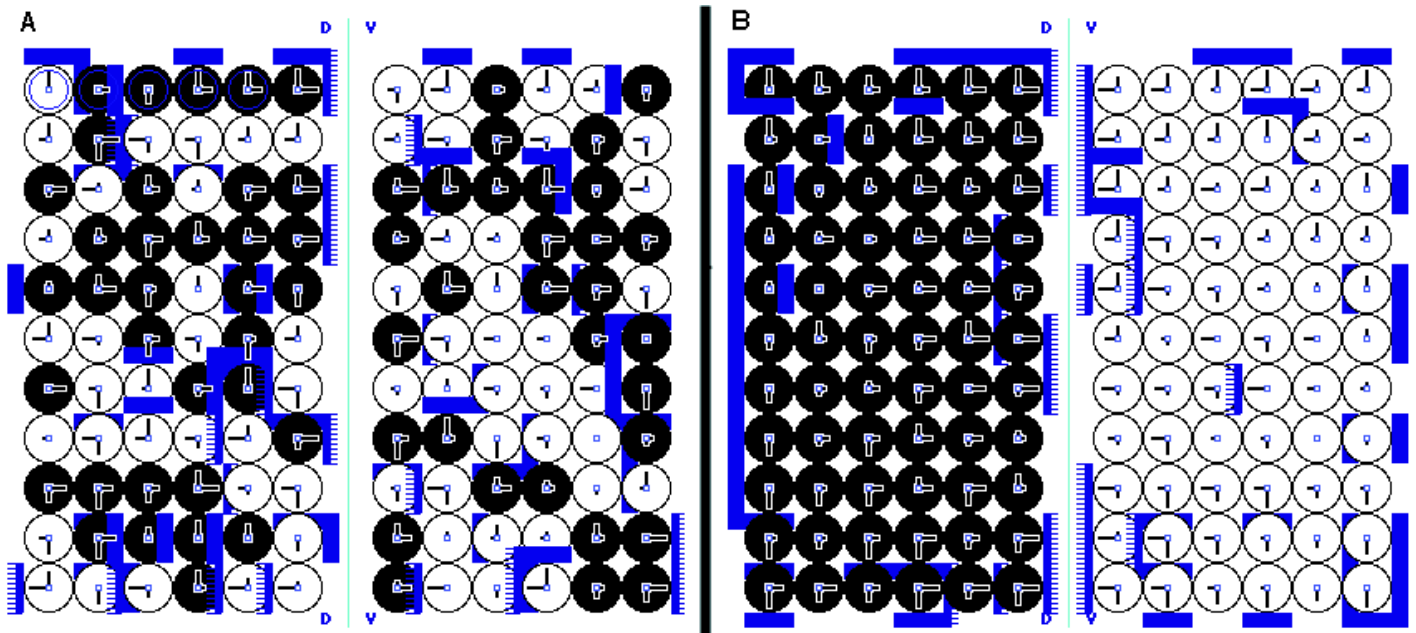


**Fig. 2. Intervein sector of the wing in its dorsal (D) and ventral (V) aspects in *Entelechia* condition.** Heavy lines correspond to anterior, posterior, and proximal restriction borders. The dorso ventral compartment boundary is indicated by the serrate line. Cells contain two variable vectors, corresponding in length to the martial (positional) value for the X and Y axes. Cells are asymmetric in the amount of signals received from their neighbors, with higher values in the cells closer to borders. [Notice the vector polarity (bars) in a double symmetry within dorsal or ventral sectors]. Highest values are at the A/P and D/V compartment boundaries. Scalar differences and their symmetries are represented in graded color. Bottom: percentage of cell division and cell death per generation.

Reciprocally, clonal ectopic expression of genes involved in the generation of compartment boundaries can, when present in the middle of compartments, cause duplication of wing territories in order to maintain the continuity of positional values of *engrailed* (*en*) and *hedgehog* (*hh*) (Basler and Struhl, 1994; Tabata *et al.*, 1995; Zecca *et al.*, 1995). These local effects depend on the position of the clones (far from or close to the compartment border), irrespective of clone size (i.e., of the size of the source). Accommodation in cell proliferation dynamics also follows apoptosis induced in given compartments or wing regions (Milan *et al.*, 1997). In all of these cases accommodating cell proliferation also affects cells of adjacent compartments, indicating that proliferation in the proximo-distal and anterior-posterior axes are interdependent variables.

### The entelechia model

Intercalar cell proliferation occurs within territories defined by clonal restriction boundaries. At the beginning of wing disc growth, cell proliferation takes place on both sides of the anterior/posterior (A/P) compartment boundary. This boundary is both an axis of symmetry and a reference for cell proliferation on both sides of it. The establishment of the dorso/ventral (D/V) clonal restriction



**Fig. 3. Dissociation and reaggregation of cells dissociated from discs that had attained the Entelechia condition. (A)** Random arrangement of cells following dissociation of a territory as in Figure 2. Dorsal and ventral cells, border and internal cells carrying their membrane labels are intermixed. **(B)** Cells reorganize following cell recognition of their membrane labels corresponding to selector identity (dorsal, black vs. ventral, white) first, and to positional value ( $X$  and  $Y$  vectors and polarity) subsequently, leading to local reconstruction of their original neighborhood relationships in groups of best matching cells.

boundary, after the disc has reached a given size in terms of cell numbers, generates a new axis of symmetry. From now on, these two boundaries become references for growth along two axes, anterior-posterior and proximo-distal, and subdivide the anlage symmetrically into four compartments. The model proposes that signals deriving from the confrontation of cell types across compartment boundaries elicit cell proliferation, and that intercalar cell division is triggered by signals deriving from the surrounding cells.

In the model, cells are assumed to have scalar values corresponding to their distance from the compartment borders, in addition to their specification by compartment selector genes (*engrailed* for posterior and *apterous* for dorsal). These scalar values are different for the two axes,  $X$  (for the anterior-posterior axis) and  $Y$  (for the proximo-distal one, which in the case of the wing sectors is the same as the dorso-ventral axis); they are highest in the cells forming the borders and lowest in the cells farthest away from them. In addition, these values are represented as cell recognition features (ligands and receptors, or adhesion molecules associated to the receptors). These recognition molecules are in principle symmetrically distributed in the cell membrane but become later asymmetrically active (Fig. 1D). Thus, according to the model, growing cells have not only recognition features controlled by selector genes, but also positional, scalar, values and polarities.

The essence of the model is that on both sides of compartment boundaries, exchange of signals between cells in the opposite compartments leads to the activation of nuclear genes (herewith called "martial",  $M$ ), one for each axis. Thus cells closer to the border have higher  $M$  values than those away from it. Martial genes activate specific ligands in amounts proportional to their own scalar

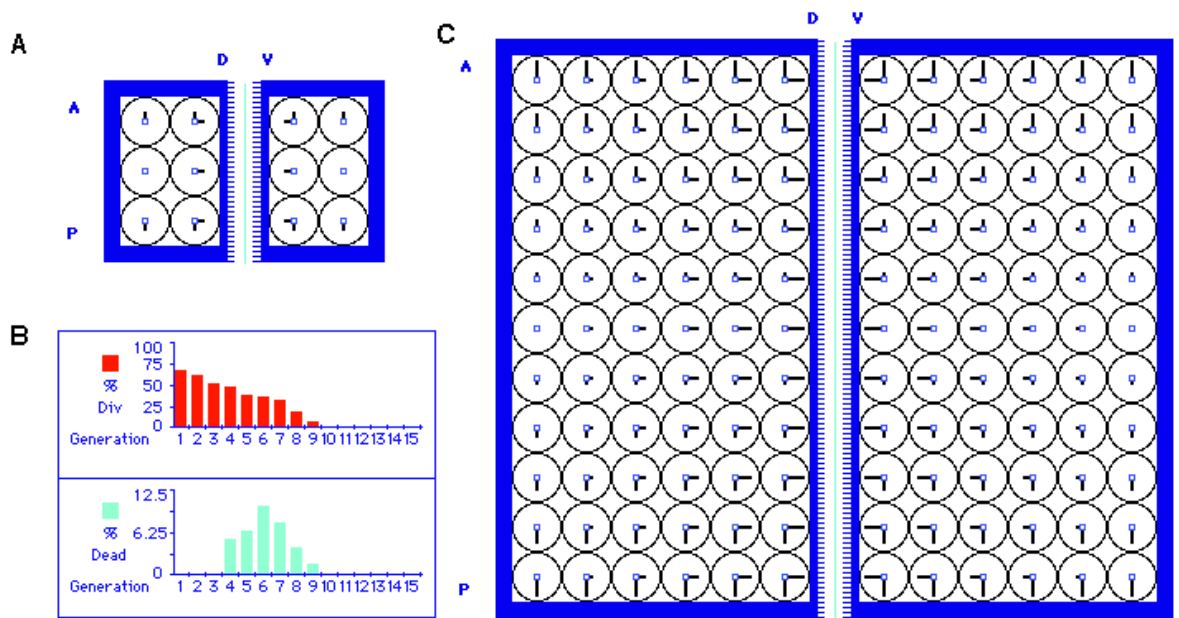
value. These ligands consequently activate the apposed receptors of neighboring cells. Ligands are limiting in amount, but receptors need not be. Although receptors and ligands are homogeneously distributed in the cell membrane, activated receptors will be asymmetrically distributed, being more abundant on the surface closer to the border than on the opposite one. Cells will therefore be polarized.

Each cell sums up the input of ligands from neighboring cells in the form of activated receptors, and enters cell division when the amount of activated receptors titrates the products of the corresponding martial gene down to a level which fails to maintain repression of the cell division machinery. Thus a cell will be pushed into division whenever one of its martial values (either  $X$  or  $Y$ ) is lower than that of its neighbors.

Following cell division, the daughter cells increase their martial values (by positive feedback of the  $M$  products on their rate of transcription) to match those of their neighbors. Thus, signals starting at the restriction borders elicit waves of cell divisions away from them. The system for comparing the  $M$  value of a cell to that of its neighbors ( $M$  disparity detection) has a threshold of sensitivity, called "increment value" in the model. This threshold, which reflects the efficiency in receiving and transducing the signals that titrate the martial gene products, determines the sensitivity of cells to differences of  $M$  values with neighboring cells. Cell division will continue in the primordium until border cells have reached maximal martial values (maximal  $M$  gene expression) while the differences between cells are below threshold. The compartment has then reached the Entelechia condition.

Cell proliferation in the disc uses borders of clonal restrictions as references. The wing proper has, in addition to the four





**Fig. 4. Two steps of the proliferation regime giving rise to the Entelechia condition.** (A) Initial conditions, six cells in each sector specified as to restriction borders. (B) Percentage of dividing cells (CP) and of dying cells (CD) along progressive rounds of cell proliferation. (C) The Entelechia condition after several computer rounds of cell division.

compartments mentioned above, sectors or intervein regions, separated by clonal restrictions running along the veins (González-Gaitán *et al.*, 1994). The model assumes that the same system of positional allocation is at the basis of the successive entelechia condition for smaller and smaller developmental subunits, starting from parasegments and ending (provisionally) with intervein regions. At each subdivision, new M genes may enter into function, or the same M gene may be used again in a new context. The model postulates that the process of ordered cell proliferation leading to the final wing is the same as that which occurs in the compartments of the early disc, but leads now to a final size and shape.

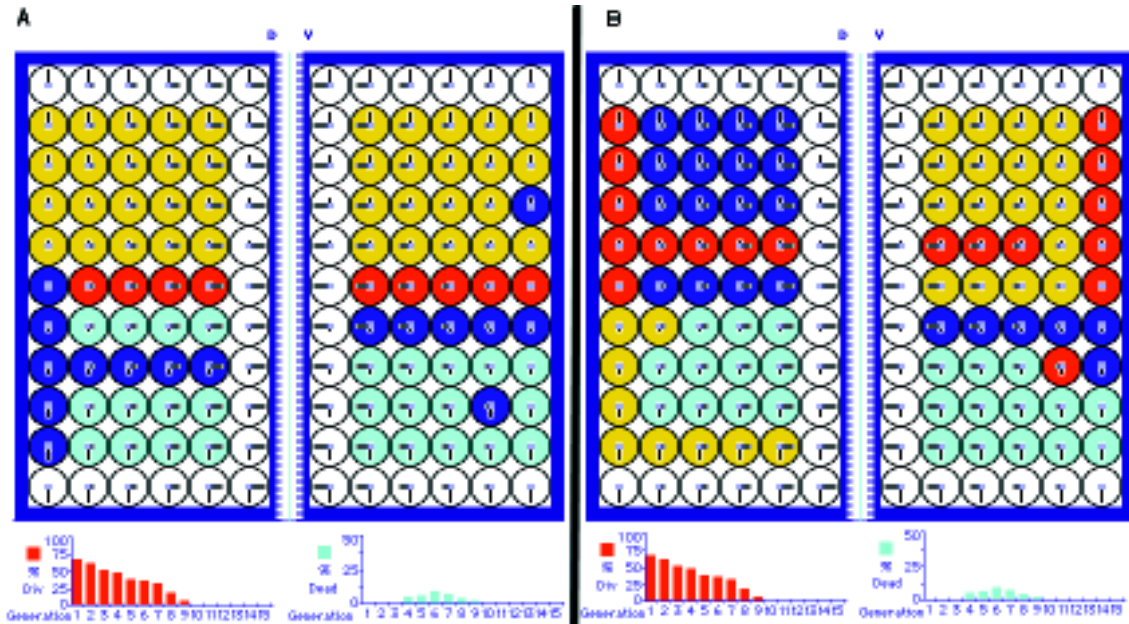
In this process, the spatial allocation of post-mitotic cells as seen *in vivo* (Milán *et al.*, 1996b), using the mechanisms revealed in dissociation/reaggregation experiments (García-Bellido, 1966), is crucial. We suggest that the activated receptors themselves, or associated adhesion molecules different in the two axes, asymmetrically label the cell surface with orientations orthogonal to each other. After cell division, which is at random in the planar axes of the epithelium, one of the daughter cells retains its martial value and position while the other, with intermediate martial values, allocates to a position best matching in values with those of neighboring cells. Occasionally a daughter cell might have identical values to those of one of the surrounding cells; those are assumed to enter apoptosis. The elongated shape of the wing then results from preferential allocation of postmitotic cells in the proximo-distal axis, perhaps because differences in positional values are smaller along that axis than along the antero-posterior axis in vein sectors.

Simultaneous discontinuities in martial values along the two axes, X and Y, may lead to larger clusters of mitotic cells, as observed *in vivo* (Milán *et al.*, 1996a; Fig. 1D). Cell proliferation in the anlage ends when the Entelechia condition is reached across the vein sectors (X axis) and along the proximo-distal (Y axis) are completed, and all the positional values are therefore filled (Fig. 2).

### Algorithms used in the computer simulation

We use a window of wing morphological space including the dorsal and ventral surfaces of an intervein sector (Fig. 2). The cells have both a selector label and a positional label. The latter distinguishes two parameters, corresponding to the two axes of computation, X and Y. The values in these axes are, for convenience, graded in intervals of ten. We represent cells as circles in the figures, although their outlines are hexagonal in the disc epithelia. The two martial values (X,Y) are represented as vectors, the size of which indicates the amount of M value, while the direction reflects cell polarities corresponding to the differential distribution of adhesors/receptors in their membranes. The polarity reflects, in the X axis, anterior vs. posterior, and in the Y axis, dorsal vs. ventral. The M value being maximal at the two borders, there is an inversion of polarity in the middle of the sectors, resulting in a symmetry line separating cells with opposite polarities at both sides of it (Fig. 2).

Cells respond with mitosis when either of the two axial signals arising from neighbor cells surpasses by 20% of the maximal M value any of its own martial values. Ensuing cell division is oriented at random. One of the daughter cells retains its martial value and position while the other computes the martial values of neighboring cells and increase its own value to average those of neighbors. The second cell which now has assumed intermediate martial values, moves to a position best matching in values with those of neighboring cells. If both X and Y values are identical to those of a neighbor cell, one of the two cells dies by apoptosis. For convenience every round or wave of divisions is initiated by an increase of 20% in martial value of the cells at either restriction border (X or Y). Cycles, or generations of cell divisions are discontinuous in the simulation (our computer lacks parallel multiprocessors), unlike the process *in vivo* where several divisions may occur in different places of the same sector at the same time. For convenience we have set the maximal martial value (maximal M gene expression) to 50 in each axis, and the increment value (threshold of M disparity detection) to 10.



**Fig. 5. (A,B) Examples of twin clones initiated in the same position in the second generation of cells.** (Offspring of grandmother cells in twin clones: yellow and red, blue and cyan). Notice split clones, clones crossing the A/P symmetry line within the sector, and clones of different sizes in terms of cell numbers.

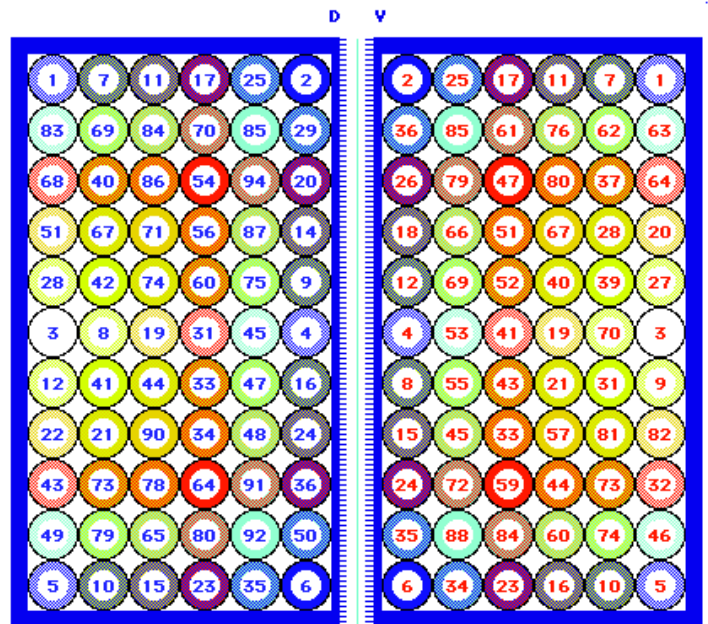
**Cell sorting out and pattern reconstruction**

In Figure 2 we represent the cells of a wing sector with the two dorsal and ventral aspects in the final Entelechia condition. In experiments of cell dissociation and reaggregation individual cells from different compartments sort out. However, cells of the same region of the disc are capable of reconstructing local patterns, recognizable by characteristic cuticular elements, such as chaetae or veins (García-Bellido, 1966,1972). These reconstructed patterns correspond to small regions of the complete pattern, an indication that in sorting out, cells first reaggregate by region and undergo finer allocation later. In this process of cell allocation, cell adhesion asymmetries in the cell membrane (both in the X and in the Y planar axes) play a major role. This leads to matching of cells not only with respect to position but also to polarity, because of the associated cell asymmetries (García-Bellido, 1966). We have modeled these processes by reaggregating dissociated cells of the final anlage (i.e., as in Fig. 2) in two steps. In the first step, cells sort out using the selector (dorsal vs. ventral) label (Fig. 3A). In a second step we allow cells with very different positional labels to sort out, in groups of similar values and then to allocate to final position by swapping places (positional values and polarity) with next neighbors. At the end of the process (Fig. 3B) groups of cells appear associated in clusters of matching values, but as expected from the experimental data the reagggregates show partial pattern reconstruction rather than the complete Entelechia condition. From here on, intercalary regeneration may follow driven on by the confrontation of cells with different positional values (not shown, but see below; García-Bellido, 1972).

**Cell proliferation to the entelechia condition**

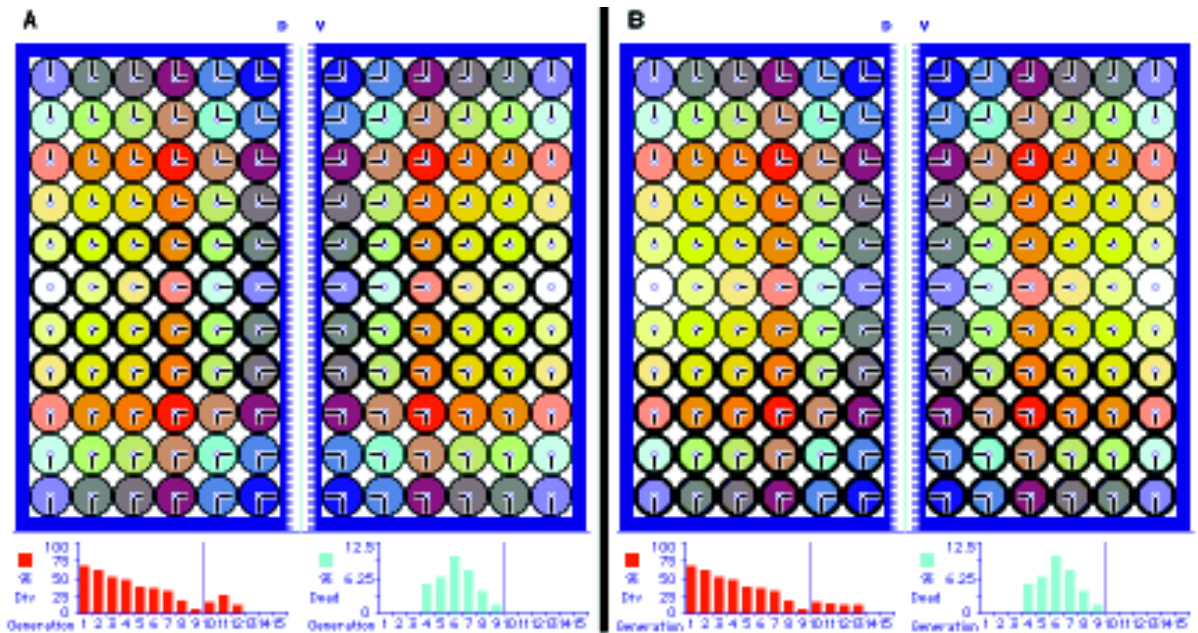
In Figure 4A, we present the starting condition of the growing anlage primordium with 6 cells on either side of the D/V restriction border. After 10 computer cycles of cell division the primordium reaches the Entelechia condition (Fig. 4C). The end result is

obviously the same in different runs of simulation, but differences appear between runs because the various steps are resolved by random decisions (Figs. 2,4,5 and 7). Thus the number of cell divisions, expressed as percentage of dividing cells, varies in each cycle, in part as an artifact of the computer simulation. However this percentage is more or less constant in the first cycles and decreases in the last ones (Fig. 4B), as occurs *in vivo* (Milán *et al.*, 1996a,b). Cell death varies in different runs of simulation but



**Fig. 6. Cells identified by order of appearance.** Low numbers correspond to early postmitotic cells, higher numbers to postmitotic cells of later generations (numbers in D and V correspond to two independent computer rounds). Notice that restriction borders and A/P symmetry line are associated to lower numbers than those of cells in intermediate regions.

**Fig. 7. Regeneration (A, intercalar; B, distal).** The new cells arising by cell proliferation, from cells at the border of the wound, are labeled with thicker cell membranes. Bottom: notice the extra rounds of cell proliferation (regeneration) with no associated cell mortality.



ranges between 5-10% cells per cycle, similar to the rates observed *in vivo* (2% of cells).

If we label the progeny of early cells in a simulation of clonal analysis (Fig. 5A,B), we observe a large variety of clone sizes and shapes for twin clones initiated in one of the 12 cells of Figure 4A. This is similar to the experimental observations. Clones appear compact and may cross the symmetry line within the sectors, again as *in vivo*, but not the D/V boundary. Cells along the restriction borders have exclusive clonal relationships, as we also find *in vivo* in clones running along veins. However, an algorithm modification that occasionally causes a border cell to leave the border and enter the rest of the population reproduces the *in vivo* situation even more accurately (not shown). In this computer simulation we can trace back the birth time of each postmitotic cell. As seen in Figure 6, the oldest postmitotic cells remain closer to the borders and in the middle line the youngest appear in intermediate regions. This indicates that clones grow from borders inwards, probably reflecting the *in vivo* process.

## Regeneration

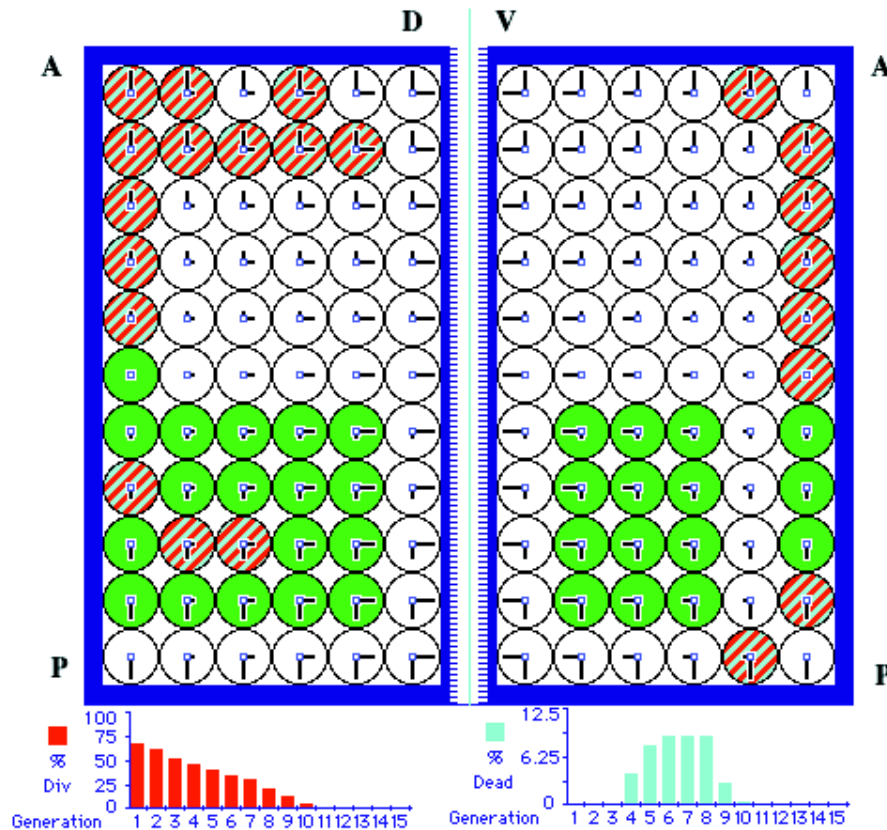
The same algorithms used to simulate cell proliferation under normal conditions can be used to follow processes of regeneration. We here simulate two types of regeneration, intercalar and distal. We model intercalar regeneration by removing several rows of cells of a final anlage (in Entelechia condition), and allowing cells at the border of the wound to come into contact and compute their positional values. Positional value differences elicit, as in normal development, cell division and cell allocation until the missing cells are finally regenerated, as indicated in Figure 7A (cells with thicker outlines). Both proximal and distal cells of the wound border are engaged in cell division, as is known to occur *in vivo* (Bohn, 1970). The same happens if the regeneration occurs in young, still growing primordia, which continue to grow later until they reach Entelechia condition (not shown).

In distal regeneration, where border cells are missing, following the joining of wound cells which belong to different compartments or sectors, cells exchange signals. This exchange leads to stepwise increase of their positional values, and to proliferation as in normal development (Fig. 7B). Alternatively, distal regeneration may be of the intercalar type if the signals that are generated upon joining generate immediately the maximal M value. In both cases the anlage reaches the Entelechia condition by local cell proliferation. It is not known which of the two modalities of distal regeneration takes place *in vivo*. Interestingly, apoptosis is not observed during the process of regeneration, contrary to the normal development (Fig. 7B, below).

## Cell behavior in genetics mosaics

The existence of cell positional values operating in the development of epithelia is corroborated by mutations that affect them. We model here the situation found in mosaics of several mutations which cause the preferential allocation of the mutant cells within the positional map of the growing anlage. This is the case of genes encoding for ligands, membrane receptors or martial genes (see discussion). For example, cells carrying loss of function alleles of *extramacrochaetae (emc)*, a nuclear gene, tend to grow in elongated clones towards and along veins, whereas gain of function alleles of the same gene tend to grow away from veins (García-Alonso and García-Bellido, 1988; de Celis *et al.*, 1995). Assuming that veins appear in the highest positional values in a sector, then the behavior of *emc* clones suggests that an insufficiency of *emc* function provides cells with higher than normal positional values, such that the daughter cells of the clone allocate to positions closer and closer to veins. If we provide dividing cells, in our model, with an incremented value in the X axis, clones duplicate the behavior observed *in vivo* (Fig. 8). If the *emc* clone is large (because initiated early) and all the cells of the sector are mutant, cell proliferation reaches the Entelechia con-





**Fig. 8. Clones of cells with intrinsically higher values in a martial gene.** (Due to mutation, for example, in *emc*). The mutant cells (striped) allocate to higher value positions (towards and along restriction borders) as cell proliferation proceeds. Notice that the twin clones (labeled cells) grow normally.

dition earlier, with less cells both in the X and in the Y axes, i.e., giving rise to smaller sectors (not shown), again duplicating the behavior of real *emc* clones.

## Discussion

The computer simulation of the process of cell proliferation leading to the Entelechia condition is based on cell behavior properties found in normal development and in mutant and experimental conditions. This includes 1) the role of differential gene expression at compartment boundaries (Blair, 1995; Lawrence and Struhl, 1996); 2) scalar differences revealed in cell recognition of dissociated cells with respect to position and polarity, within compartments and vein sectors (García-Bellido, 1972; García-Bellido and de Celis, 1992); 3) random mitotic orientations followed by preferential allocation of daughter cells along the primordium axes (Milán *et al.*, 1996b); 4) the attainment of constant sizes and shapes in imaginal discs grown under various experimental conditions (García-Bellido, 1965) and 5) the behavior of cells in clones in genetic mosaics (see below). The model postulates non-identical daughter cells with subsequent differential allocation to new positions, but we do not know how this is accomplished. Ensuing cell apoptosis, when new daughter cells and neighboring cells have identical M levels, is also an *ad hoc* proposition, but it could reflect insufficient cell signals as is the

case for apoptotic cells *in vivo* (Raff, 1992). However the low occurrence of apoptosis in the simulation compares well with the rare apoptosis found in normally growing discs (Milan *et al.*, 1997). The model postulates further an increase of M values after each cell division, a feature for which we have no observational data.

The algorithm used here reflects known biological features, but it is at best an analogy to the real situation. Its simplicity will hopefully help the search for properties operating *in vivo*. Although there are genetic candidates for the role of ligands (*vein*), receptors/adhesion molecules (*Notch*, *veinlet*, *top*) (Clifford and Schüpbach, 1989; García-Bellido, *et al.*, 1994; Schnepf *et al.*, 1996; Simcox *et al.*, 1996) and nuclear genes mobilizing the other signals (*blistered*, *extramacrochaetae*, Garrell and Modolell, 1990; de Celis *et al.*, 1995; Montagne *et al.*, 1996), we have no clear candidate for the role assigned to martial genes that could determine the X, Y positional values through scalar variations in their level, and trigger cell division following titration by activated receptors. *emc*, a nuclear HLH encoding gene, might control the expression of the corresponding martial gene, perhaps another HLH gene, for the X axis. Another candidate martial gene is *nubbin* (encoding a transcription factor) which causes, in the mutant condition or in genetic mosaics, the compaction of the wing in both the proximodistal and anterior posterior axis (Cifuentes and García-Bellido, 1997). The X and Y functions

are intimately related to each other, as seen in “accommodation” processes caused by mutations in genes at work at the clonal restriction boundaries (García-Bellido and Santamaría, 1972; Mohler and Vani, 1992; Diaz-Benjumea and Cohen, 1993; Basler and Struhl, 1994; Tabata *et al.*, 1995; Zecca, *et al.*, 1995). Thus, genes under the control of the selector *engrailed* and *apterous*, or those encoding the ligands and receptors mentioned above (García-Bellido *et al.*, 1994), may cause, in mutant flies or in genetic mosaics, reduction (or excess) of growth in both axes of the wing.

Clearly the Entelechia conditions in the wing must be different along the A-P and the P-D axis, for whereas value intervals between vein boundaries are small in number of cells, cell distances along the P-D axis are large without indications of clonal restrictions subdividing them. The adult wing has a lanceolate shape but except for the curved tip, the rest of the wing blade is rectangular, with a width that is constant in terms of cell numbers (González-Gaitán *et al.*, 1994). Cell distances between longitudinal veins, however, change from sector to sector, and thus the corresponding local differences have to be modulated, possibly by local changes in the cell communication parameters (ligands/receptors and/or transduction efficiency) reflected in the “increment” values. Similar changes may condition differences in wing size and vein sector width in other *Drosophila* species (García-Bellido, 1983) and even between larger insect taxa.



It seems reasonable to assume that the *Entelechia* function operating in early compartments, once completed, becomes substituted by new ones, corresponding to shorter distances between new borders which later will expand by cell division to fulfill new *Entelechia* functions (Fig. 1A). The *Entelechia* model may obviously apply to other imaginal discs as well. Patterning of cell types (chaetae, veins) will, like in the "prepattern" model, reflect cell responses to highest (or lowest) positional values. The *Entelechia* model generates patterns of constant sizes: it is not size invariant, as opposed to the "positional information" model. It only accounts for the generation of sheets of patterned cells. Conic appendages can also be considered, at the local level, as bidimensional patterns (Held, 1979). This model, however, cannot readily be extended to explain constant sizes and shapes in multilayered cell epithelia, muscles, or condensation of chondrocytes into bones, as found in vertebrate development.

#### Acknowledgments

We thank Antonio Baonza and other colleagues in our laboratory for helping with determination and preparation of the Figures. This work was supported by Grant PB92-0036 from the Dirección General de Investigación Científica y Técnica and an institutional grant from the Fundación Ramón Areces to the Centro de Biología Molecular Severo Ochoa.

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