Patterned Anchorage to the Apical Extracellular Matrix Defines Tissue Shape in the Developing Appendages of Drosophila

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SUPPLEMENTAL INFORMATION

Supplementary Figures

Figure S1. Morphometrics on the \textit{nw} phenotype, relating to Figure 5A-F.
Landmark data from wild type and mutant wings were collected for 15 positions across the wing blade (A) (blue: hinge; red: wing blade). A principle component analysis revealed that 77\% of the phenotypic variance arising from the \textit{nw} allele phenotypes, and 85\% of the variance arising from the RNAi phenotypes were associated with a single principle component (PC1) (B). The value of PC1 is correlated with the severity of the mutant phenotype (C), and the shape warp is a stretch of the wing blade along the P-D axis with landmarks along the anterior and posterior margins shifting toward the midline of the blade, and the distal landmarks shifting outward (D).

Figure S2. Genetic and molecular characterization of \textit{nw}, relating to Figure 5G,H.
(A) Deficiency mapping of \textit{nw} to division 54B-C. Of all available deficiencies in the region, only \textit{Df(2R)BSC355} and \textit{Df(2R)BSC406} fail to complement alleles of \textit{nw}. (B) The interval between the distal breakpoint of \textit{Df(2R)BSC161} and the distal breakpoint of \textit{Df(2R)BSC406} includes 14 genes, and, of these, only one, \textit{CG43164}, produces the \textit{nw} phenotype when silenced by RNAi. (C) The \textit{CG43164} gene encodes two related proteins, which we refer to as \textit{Nw-S} and \textit{Nw-L}, from a series of differentially spliced transcripts. The two P-element insertions KG02048 and G18887, fail to complement all \textit{nw} alleles, and are inserted at different locations within the 5'-UTR. Three different RNAi hairpins (blue boxes) are associated with the gene, 49678, which targets an exon common to all transcripts, and 12800 and 50712 which target the final exon which is only in some of the transcripts. (D) The \textit{Nw-S} and \textit{Nw-L} isoforms share a common N-terminal domain which includes a single C-Type Lectin Domain (CTLD, in green) which has five Cysteine residues (red bars). \textit{Nw-L} has an additional large C-terminal domain that contains no identifiable motif and is highly variable in sequence even between closely related species. The dominant alleles, \textit{nw}^B and \textit{nw}^D are associated with lesions in the \textit{Nw} protein, \textit{nw}^B is a 3 aa deletion of the signal peptide cleavage site, and \textit{nw}^D a missense mutation S34>R. \textit{nw}^{Drs5}, a revertant of \textit{nw}^D, is associated with an additional missense mutation G103>C (see Table S1).

Figure S3. Genetic and molecular characterization of \textit{ta} and \textit{ll}, relating to Figure 5I.
(A) Characterization of \textit{ta}. The \textit{ta} locus was deficiency mapped to a short interval between the distal breakpoints of \textit{Df(2R)BSC298} and \textit{Df(2R)BSC158}, which contains eight genes including
the \textit{Pal1} locus (red). Sequencing of the \textit{ta} allele revealed a nonsense mutation (Q57>STOP) in the first coding exon of the gene, truncating the protein well before the catalytic core domain (green). \textbf{(B)} Characterization of \textit{ll}. The \textit{ll} locus was deficiency mapped to a short interval between the distal breakpoints of \textit{Df(2R)BSC610} and \textit{Df(2R)BSC601}, which includes 9 genes including \textit{Phm}. The \textit{Phm} locus is essential and loss-of-function alleles, such as the P-element insertion k07623, are lethal. The two classical alleles \textit{ll}\textsuperscript{1} and \textit{ll}\textsuperscript{2} are associated with the same lesion, a 10 bp deletion in the poorly conserved C-terminus of the protein that follows the catalytic core (green). This induces a frame shift that deletes 22 aa and appends 79 aa in \textit{ll}\textsuperscript{1} and 80 aa in \textit{ll}\textsuperscript{2}. The difference between the two alleles is due to a number of polymorphisms including a 3 bp insertion in the middle of the appended domain (Table S1). Given that neither \textit{ll} allele is lethal, this additional domain must compromise, but not eliminate protein function.

\textbf{Figure S4.} \textit{Dp is required for the localization of Nw-GFP to the aECM in the pupal wing, relating to Figure 6.}

Pupal wings stained with Phalloidin to highlight cellular Actin (red) and Nw-GFP (green) under control of the \textit{nub-Gal4} driver. \textbf{(A,B)} Wild type wings at 19h APF (A) and 24h APF (B) showing the localization of Nw-GFP. As in Figure 6, Nw-GFP is localized to the margin at 19 h APF and in a diaphanous network overlying the wing epithelium at 24h APF. \textbf{(C,D)} In the \textit{dp-RNAi} mutant wings, evident from the change in wing shape, Nw-GFP is completely absent from the wing blade, showing that in the absence of Dp, Nw does not localize to the aECM. \textbf{(E,F)} Scanning electron micrographs showing the dorsal surface of the wing blade of \textit{nub-Gal4>Nw-GFP} (E) and \textit{nub-Gal4>dp-RNAi} (F) wings in the intervein between L3 and L4 at 24h APF (scale bar 5 microns). In the mutant wing, the aECM is absent, thus Dp is essential for aECM assembly.

\textbf{Figure S5.} \textit{Vertex model adapted to the simulation of pupal wing development, relating to Figures 3,4, and 6.}

\textbf{(A)} Schematic of cells and of Dumpy anchorage to the cuticle as implemented in our vertex model. \textbf{(B)} Example of T1 transition (neighbor exchanges). \textbf{(C)} Removal of a vertex from the network. \textbf{(D)} Example of T2 transition (cell extrusion). \textbf{(E)} Initial conditions used in the simulations. Wing blade cells are shaded red, hinge cells light blue. L2-L5: longitudinal veins. Green thick lines denote the zones of the hinge which positions are fixed. Thin vertical green lines show the boundaries between the four zones of the blade for which a specific value for the spring constant ($\beta_k^{(x)}$) is attributed. Edges at the vein-intervein interface with increased line tension are marked in red.
Figure S1. Morphometrics on the nw phenotype
Figure S2. Genetic and molecular characterization of nw

A

54B  54C  54D  54E

100 kb

Df(2R) Exel6066  Df(2R) Exel7149  Df(2R) Exel7152

Df(2R) BSC154  Df(2R) BSC355  Df(2R) BSC347

Df(2R) BSC161

Df(2R) BSC406

B

10 kb

Df(2R) BSC161

Df(2R) BSC355

Df(2R) BSC406

C

1 kb

kg02048  g18887

Nw-RC

Nw-RE

Nw-RA/B

Nw-RD

D

\textit{nw}^{0}  \Delta \text{CAA}  S34+R  \textit{nw}^{PSS}  G103>C

Nw-S

SP  CTLD

Nw-L

Variable
Figure S3. Genetic and molecular characterization of \textit{ta} and \textit{ll}

A

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figureA.png}
\end{figure}

B

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figureB.png}
\end{figure}
Figure S4. Dp is required for the localization of Nw-GFP to the aECM in the pupal wing.
Figure S5. Vertex model adapted to the simulation of pupal wing development
### Supplementary Tables

**Table S1.** Molecular lesions associated with alleles of *nw*, *ta* and *ll*, relating to Figures 5, S2 and S3.

| Allele | DNA lesion | Protein lesion | Details of lesion |
|--------|------------|----------------|-------------------|
| *nw*²  | insertion? | none           | Rearrangement breakpoint in the first intron |
| *nw*⁵  | Insertion into 5'-UTR | none | P-element insertion G18887 affecting RC and RA/B transcripts |
| *nw*K⁵ | Insertion into 5'-UTR | none | P-element insertion G18887 affecting only RC transcript |
| *nw*⁸  | 9 bp in frame deletion | ΔCAA | Deletion of part of signal peptide cleavage site, residues 15-17 |
| *nw*³  | AGC>AGA | S34>R | |
| *nw*³₅⁵ | AGC>AGA | S34>R | The G>C transition is induced on the *nw*³ chromosome |
|        | GGT>TGT | G103>C | |
| *ta*¹  | CAG>TAG | Q57>STOP | Nonsense mutation in second coding exon deleting all functional domains |
| *ll*¹  | 10 bp deletion | | Frame shift after S339 + 79 aa |
| *ll*²  | 10 bp deletion | | Frame shift after S339 + 80 aa polymorphic 3 bp insertion after natural stop codon |

*a The *nw*⁵ and *nw*K⁵ insertions lie in different parts of the 5'UTR. *nw*² lies in part of the UTR common to all transcripts, while *nw*K⁵ lies upstream in a part of the UTR specific to the RC transcript. See Figure 7C. 

*b* *ll*¹ and *ll*² share the identical 10 bp deletion but differ in a number of polymorphisms in the region that follows the stop codon (see Figure 8B) which may account for the different phenotype produced by the two mutations.*
Supplementary Movies

Simulations with the vertex model of late pupal wing development under the different patterns of Dumpy anchorage obtained in our experiments. Wing blade cells are shaded red, hinge cells light blue and vein cells dark blue. White dots: cuticle outline; blue spheres: areas of the cuticle with Dumpy attachments; green lines depict the springs used to simulate dumpy anchorage. Vertices of the hinge margin with fixed positions are colored in green.

Movie S1. Simulations of a wild-type wing and of a nub-Gal4 > dp-RNAi wing, referring to Figure 3

Movie S2. Simulations of the phenotypes produced by driving dp-RNAi with sal-Gal4, hh-Gal4, dpp-Gal4, and brk-Gal4, referring to Figure 4.

Movie S3. Simulations of a wild-type wing and of a Tub-Gal4>nw-RNAi wing, referring to Figure 6
SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Drosophila strains and genetics

To generate new recessive nw mutations, a mutagenesis was performed to revert nw<sup>D</sup>. For this screen, nw<sup>D</sup>/CyO males were crossed to isogenic pr cn females, and the nw<sup>D</sup>/pr cn male progeny were treated with 30 mM Ethyl Methane Sulfonate (Sigma). The mutagenized males were backcrossed to pr cn and the pr+ cn+ male progeny were scored for reversion of the nw phenotype. Candidate revertants were crossed to Sp Bl L/CyO virgin females, and the nw<sup>D</sup>+/Sp Bl L progeny scored for transmission of the revertant allele. 25,700 chromosomes were scored in the screen, and a single revertant, nw<sup>D</sup>-rS5, was recovered.

Molecular cloning

To generate pUASt-Nw, the 2.4 kb Nw-RB cDNA was excised from clone LD10058 (Drosophila Genome Resource Center) by digestion with EcoRI and XhoI and directionally cloned into pUASt. For GFP-tagged Nw-S (Nw-GFP), the cDNA LD10058 was used as a template in a PCR reaction using primers Nw.TOPO.5’ and Nw.TOPO.3’ (CACCGGGCCAAATCGTACTCTTCTGCC; CTGGCCGAGCAGAGGGGGATGC), which amplify the region between the start and stop codons and are designed for directional TOPO cloning. The resulting 584 bp fragment was cloned into the Gateway entry vector pENTR using the pENTR Directional TOPO Cloning Kit (Invitrogen). The resulting plasmid pENTR-Nw-Sc was used as a donor for cloning into the Drosophila Gateway vector pPWG using the LR Clonase system (Invitrogen).

As there was no available cDNA for the C transcript (Figure S2), which gives rise to Nw-L, total RNA was isolated from 10 mg of 0-12 hour embryos from an isogenic w strain using the RNAeasy Mini Kit (Qiagen). 500 µg of total RNA were used in a reverse transcription reaction using the Accuscript Enzyme (Aligent Technologies) according to the supplier’s recommendation, followed by PCR using the primers Nw.RT.5’Long and Nw.RT.3’Long (CACCCCTGAAGCGAGGTCTGATCG; GTCATCCATGGCAGACTGGTTTGC). The reaction produced two bands of 1.9 and 2.4 kb corresponding to the B and C forms of the transcript, and the smaller band (corresponding to the C-transcript) was gel purified and cloned into pENTR as described above to produce pENTR-Nw-L. To make the GFP-tagged version, pENTR-Nw-L was used as a template in a PCR reaction with the primers Nw.TOPO.5’ and Nw.TOPO.3’Long and
the 1.4 kb amplified fragment was cloned into pENTR. This clone, pENTR-Nw-Lc, was used as a donor for Gateway cloning into pPWG as described above to generate pP-Nw-Lc-GFP.

To produce 3xFlag-tagged and HA-tagged versions of the short and long protein isoforms for tissue culture experiments, pENTR-Nw-Sc and pENTR-Nw-Lc were used as donors for Gateway cloning into the pAWF and pAWH vectors, respectively. The resulting clones, pA-Nw-Sc-Flag, pA-Nw-Sc-HA, pA-Nw-Lc-Flag, and pA-Nw-Lc-HA were used in tissue culture experiments.

Mutant sequencing

Mutant alleles were sequenced from PCR amplified fragments from genomic DNA derived from mutant homozygotes or hemizygotes using pairs of primers flanking each of the exons (Nw.Ex1.F AAGCTA ACTGCTTTCGGCACCAAC; Nw.Ex1.R GGCTTGAAGTTACTTAGCGCTTCCG; Nw.Ex2-3.F CCACTTGTGGTACCATCATATGACC; Nw.Ex2-3.R TTCCTCTCCAATCAGTGCTC; Nw.Ex4.F TGTCATATTGCCTGCGTGCTAGAC; Nw.Ex4.R GGATAACACCTCGACCCAAACACAC). For \( nw^D \), \( nw^B \), and \( nw^{D-rS5} \), the chromosome was balanced over the green balancer CyO-GFP, and homozygous mutant larvae were collected based on the absence of the GFP marker. Genomic DNA was isolated from 10 mutant larvae and the fragments amplified with Phusion Taq Polymerase (Invitrogen), and purified using a QIAquick PCR Purification Kit (Qiagen). For \( nw^2 \), the original chromosome was embryonic lethal as a homozygote, so genomic DNA was isolated from homozygous \( nw^2 \) adults produced from a recombinant chromosome. For this chromosome, no lesion was detected in either coding or non-coding exons, but it was not possible to amplify the fragment between Nw.Ex1.F and Nw.Ex1.R. Further studies localized the lesion to the 5' end of the first intron, and as this junction could not be bridged by PCR it is either a large insertion or an inversion breakpoint.

Morphometrics

For the morphometric analysis, fifteen landmarks (Figure S1) were digitized from wing images using the Fly Wing Kit plug-in for ImageJ (provided by C. Klingenberg) and the principal component analysis was performed using MorphoJ (Klingenberg, 2011). Shape information for each genotype was extracted by Procrustes superimposition and outliers removed. Data for different genotypes were combined and a covariance matrix was generated that was used in a principal component analysis to quantify shape change. The value “Relative PC1” was obtained taking the absolute value of the difference between the PC1 score (\( PC1 \)) and the mean PC1
score for the wild type \((X_{OR})\) and adding 1 to make all values greater than 0, i.e. \(\text{RelPC1} = 1 + (PC1_i - X_{OR})\).

**Computational Modeling**

To model the effects of global forces on whole wing morphology, we built a computational vertex model based on the existing computational framework originally developed by Honda et al. (Honda et al., 2004), and further adapted by other authors for the study of developmental processes in epithelial tissues of *Drosophila* (Aegerter-Wilmsen et al., 2012; Canela-Xandri et al., 2011; Farhadifar et al., 2007; Mao et al., 2011). The model includes cell adhesion, cell intercalation, cell growth, apoptosis, cell contraction and cell division. Here, we extended the vertex model to simulate the development of the pupal wing by incorporating hinge contraction and attachment of the margin to a fixed position. This models the period from 18h APF to 24h APF. The model was implemented in gfortran, using openGL to visualize the outputs. The simulations runs were executed on the computer cluster at the Research Computing Center, Florida State University. The parameter names, symbols and values are shown below:

| Parameter name                                           | Symbol          | Value   |
|----------------------------------------------------------|-----------------|---------|
| Range of vertex movement                                 | \(\delta d_{\text{min}} - \delta d_{\text{max}}\)   | 0 - 0.02|
| Probability of acceptance of a non favourable vertex movement (noise) | \(P_{\text{accept}}\) | 0.05    |
| Line tension (blade)                                     | \(\Lambda_{ij}\)  | -0.02   |
| Line tension (hinge)                                     | \(\Lambda_{ij}\)  | -0.01   |
| Line tension at vein/intervein boundaries (hinge)        | \(\Lambda_{ij}\)  | 7.0     |
| Line tension at vein/intervein boundaries (blade)        | \(\Lambda_{ij}\)  | 1.0     |
| Line tension at the tissue boundary                      | \(\Lambda_{ij}\)  | 4.0     |
| Perimeter contractility blade                            | \(r_{ij}\)      | 0.03    |
| Perimeter contractility hinge                            | \(r_{ij}\)      | 0.06    |
| Critical distance to undergo T1 transition               | \(d_{\text{min}}\) | 0.1     |
| Critical area to undergo T2 transition                   | \(A_{\text{min}}\) | 0.01    |
| Preferred area (initial conditions)                      | \(A^{(0)}\)     | 10      |
| Preferred area (final)                                   | \(A^{(0)}\)     | 5.0     |
**Vertex model**

In the vertex model, only the apical sides of the cells were considered. Cells were represented as 2D polygons, made of vertices connected by edges (Figure S5). The vertices could move over developmental time as a result of intra- and inter-cellular mechanical forces. The movement of the vertices was implemented by comparing the mechanical energy of a vertex in its current position \((x, y)\) with the energy of a randomly chosen point nearby \((x + \delta d, y + \delta d)\). When the energy in the new position was smaller, then the movement was accepted as the new vertex location. When the energy is bigger, the movement was accepted with probability \(P_{\text{accept}} = 0.05\) in order to introduce stochastic fluctuations. The model was run by iteratively moving a randomly chosen vertex and by updating its energy.

The energy \((E)\) of a vertex \(i\) is given by

\[
E(R_i) = \sum_{\alpha} \frac{K_\alpha}{2} (A_\alpha - A_\alpha^{(0)})^2 + \sum_{i,j} \Lambda_{i,j} \cdot l_{i,j} + \sum_{\alpha} \frac{\Gamma_\alpha}{2} \cdot L_\alpha^2
\]

Equation (1)

where \(R_i = (x_i, y_i)\) is the position of the vertex \(i\). The first and the third summations are over all the cells \(\alpha\) in which the vertex \(i\) is present, and the second summation is over all the cell edges \(\{i, j\}\) in which the vertex \(i\) is present. \(A_\alpha\) is the apical area of the cell \(\alpha\) and \(K\) is the area elasticity modulus, which is assumed to be equal for all the cells in our simulations. \(A_\alpha^{(0)}\) is the preferred area of the cell \(\alpha\), considered equal for all cells \((A_\alpha^{(0)} = A^{(0)})\). The distance and the line tension between the pairs of vertices \(\{i, j\}\) are denoted \(l_{i,j}\) and \(\Lambda_{i,j}\), respectively. The third term includes the perimeter of the cell \(\alpha\) (\(L_\alpha\)) and the perimeter contractility coefficient \((\Gamma_\alpha)\). By choosing \(\sqrt{A^{(0)}}\) as a unit of length and \((K A^{(0)})^2\) as a unit of energy (as in (Farhadifar et al., 2007)), dividing both sides of Eq. 1 by \((K A^{(0)})^2\) results in the following dimensionless equation:

\[
\tilde{E}(R_i) = \sum_{\alpha} \frac{1}{2} \left( \frac{A_\alpha}{A^{(0)}} - 1 \right)^2 + \sum_{i,j} \tilde{\Lambda}_{i,j} \cdot \frac{l_{i,j}}{\sqrt{A^{(0)}}} + \sum_{\alpha} \frac{\tilde{\Gamma}_\alpha}{2} \cdot \frac{L_\alpha^2}{A^{(0)}}
\]

Equation (2)
Where \((A_\alpha/A(0))\), \((l_{ij}/\sqrt{A(0)})\) and \((l_{\alpha}^2/A(0))\) are, respectively, dimensionless area, bond length and perimeter. This model is characterized by dimensionless line tension \((\bar{\Lambda}_{ij} = \Lambda_{ij} / K(A(0))^{3/2})\) and dimensionless perimeter contractility \((\bar{\Gamma}_{\alpha} = \Gamma / KA(0))\).

Rearrangements of the topology of the vertices in the network were allowed when two vertices \(i, j\) were located less than a minimum distance \(d_{\text{min}}\) apart, and a movement of one of the vertices was energetically favorable such that the distance between the vertices decreases. If the two vertices had three neighbors each, then a T1 transition occurred (Figure S5B). If one of the two vertices had only two neighbors (Figure S5C), then the two vertices were merged and the edge between them vanished. If a triangular cell became smaller than a threshold area \(A_{\text{min}}\), then the cell was eliminated, simulating extrusion from the epithelium by a T2 transition (Figure S5D).

**Cell cycle, growth and division**

To simulate the cell cycle, we implemented an internal clock in each cell. Initially, the cells started at a random point in the cycle. After the end of its cycle is reached a cell starts to increase its preferred area \(A_\alpha(0)\) by a fixed amount until it reaches twice its initial value. This increase in the preferred area leads to a progressive increase in cell area through the movement of vertices (as described by equations [1-2]). If \(A_\alpha\) was 1.85 times (Canela-Xandri et al., 2011) the original area at time \(t=0\), or larger, the cell was divided by adding a new edge passing through the center of mass of the cell. After division, the two daughter cells re-initiated the cycle from zero and \(A_\alpha(0)\) was reset to the value that the mother cell had before entering into the mitotic process. Note that cell division depends on the cell cycle and on the local conditions a cell was exposed to: when a cell reached the end of its cycle, it may divide, but only if its area was above the 1.85 fold threshold. The length of the clock and the rate of progression of the cell cycle are parameters of the model, but whether a cell manages to increase its size by 1.85 fold is an outcome of the model and depends on the local conditions each cell is exposed to (e.g. the pressure exerted by neighboring cells). We allowed a doubling of the cell number in the blade, which corresponds to the amount of proliferation observed during the late pupal stage (Aigouy et al., 2010). All of the mitotic events occurred in the first half of the simulation. During the second half of the run, only T1 and T2 events could lead to topological rearrangements of the network. These conditions reproduce the two phases of the pupal wing development described previously (Aigouy et al., 2010).
Orientation of cell divisions

In the pupal wing blade, there is an association between cell shape and the orientation of division (Aigouy et al., 2010). Accordingly, we implemented the Hertwig rule for the orientation of cell division, an empirical rule that is observed in many different cell types (Minc and Piel, 2012): the direction of cell division was perpendicular to the longest axis of the cell. The longest axis was defined as the vector between the cell’s center of mass and the farthest vertex relative to the center.

Initial conditions

We started with an initial tissue of ~1800 cells arranged to mimic a pupal wing at 18hrs APF (Figure S5E). Although real pupal wings have more cells, it is necessary to work with a lower cell number to reduce the computational time of the simulations. Four cell types were considered: blade intervein cells, blade vein cells, hinge intervein cells and hinge vein cells. The initial cell area was of ~10 arbitrary units.

Longitudinal veins

Longitudinal veins were added at positions resembling the pattern observed in early pupal wings (Figure S5E). To avoid intermixing of vein and intervein regions (Resino et al., 2002), we implemented differential adhesion between vein and intervein cells as reported by (O'Keefe et al., 2012). This was done by increasing the line tension at the vein-intervein boundaries, relative to the line tension at vein-vein and intervein-intervein boundaries (see parameter values above). This is a common way to implement a separation between populations of cells (e.g. Canela-Xandri et al., 2011).

Tissue constriction in the hinge and blade

During the late pupal stages, the wing cells undergo a reduction of their apical area due to a cuboidal to columnar transition (R.P.R., unpublished). To simulate this, we forced all the cells to reduce their area during the run, by decreasing the \( A^{(0)} \) parameter for all the cells by a factor of two relative to the initial cell area.

It has been reported that from 15-18h APF, a patterned constriction of the hinge pulls at the blade generating P-D tension (Aigouy et al., 2010). This was simulated by manipulating the cortical contractility and the line tension parameters. The values of these two parameters were set to be two-fold higher in the hinge than in the blade cells. These conditions are sufficient to
contract the hinge relative to the blade, but they produce an isotropic contraction. In order to simulate the anisotropic contraction of the hinge oriented in the PD axis, the line tension increase at the vein-intervein interface edges was higher in the hinge than in the blade (see parameter values above).

Margin conditions

To maintain a smooth wing margin, we assumed a greater stiffness of the cell edges located at the external boundary of the wing margin (i.e., edges facing the exterior). To implement this, we assumed that the line tension for the external edges \( (\Lambda_{\text{ext}}) \) was higher than that of the internal edges (see parameter values above). Without that requirement, any local anisotropies in the growth or direction of cell division in the cells close to the margin caused buckling and folding of the margin, which is never observed in actual wings. In order to simulate the attachment of the hinge to the notum, we fixed the position of vertices at the anterior part of the proximal margin of the hinge. The positions of the vertices located at the anterior margin of the hinge were fixed (Figure S5E). This creates asymmetry of hinge contraction along the anterior-posterior axis.

Attachment to the cuticle by Dumpy

The attachment of the epithelium to the overlying cuticle by Dp was modeled by anchoring vertices of the wing margin to a fixed position with a spring (Figure S5A). This requires an additional term to the equation (2) such that:

\[
E(R_i) = \sum_a \frac{1}{2} \left( \frac{A_a}{A^{(0)}} - 1 \right)^2 + \sum_{(i,j)} \Lambda_{ij} \cdot \frac{l_{ij}}{\sqrt{A^{(0)}}} + \sum_{\alpha} \frac{r_{\alpha}}{2} \cdot \frac{L^2_{\alpha}}{A^{(0)}} + \beta_k^{(x)} \cdot \frac{l_{ik}}{\sqrt{A^{(0)}}}
\]

Equation (3)

where \(i\) indexes the vertex and \(k\) the fixed point it is anchored to (Figure S5A). The length of spring is \(l_{ik}\), and its spring constant is \(\beta_k^{(x)}\). We model changes in Dumpy concentration by varying \(\beta_k^{(x)}\). We divided the margin of the blade into four regions along the proximal-distal axis (Figure S5E), and allowed the spring constant to vary among these regions: relatively low in the most proximal part of the wing \( (\beta_k^{(1)}) \); intermediate in the middle part of the wing \( (\beta_k^{(2)} \) and \( \beta_k^{(3)} \); and high in the most distal part of the wing \( (\beta_k^{(4)}) \):
To simulate the genotypes sal-Gal4, hh-Gal4, dpp-Gal4 and brk-Gal4, Dumpy anchorage was removed from the zones of the margin indicated in Figure 4B-E.

| Dumpy spring constant 1 | β_k (1) | Dumpy spring constant 2 | β_k (2) | Dumpy spring constant 3 | β_k (3) | Dumpy spring constant 4 | β_k (4) |
|-------------------------|---------|-------------------------|---------|-------------------------|---------|-------------------------|---------|
| (proximal)              | 0.35    | (medial/proximal)       | 0.55    | (medial/distal)         | 1.55    | (distal)                | 2.0     |
| Dumpy spring            |         |                         |         |                         |         |                         |         |
| constant 1              |         |                         |         |                         |         |                         |         |
| Dumpy spring constant 2 |         |                         |         |                         |         |                         |         |
| (medial/proximal)       | 0.35    | (medial/distal)         | 0.55    | (distal)                | 1.55    | (distal)                | 2.0     |
| constant 2              |         |                         |         |                         |         |                         |         |
| Dumpy spring constant 3 |         | (medial/distal)         | 0.55    |                         | 1.55    |                         | 2.0     |
| (medial/distal)         |         |                         |         |                         |         |                         |         |
| Dumpy spring            |         |                         |         |                         |         |                         |         |
| constant 3              |         |                         |         |                         |         |                         |         |
| (medial/distal)         |         |                         |         |                         |         |                         |         |
| Dumpy spring constant 4 |         |                         |         |                         |         |                         |         |
| (distal)                |         |                         |         |                         |         |                         |         |
| Dumpy spring            |         |                         |         |                         |         |                         |         |
| constant 4 (distal)     |         |                         |         |                         |         |                         |         |
| β_k (4)                 |         |                         |         |                         |         |                         |         |

The choice of such a step gradient makes the assumption that the differences in the strength of the Dumpy anchorage between neighboring vertices are negligible with respect to differences between the different areas of the margin. To simulate the pattern of anchorage observed in the Tub-Gal4>nw-RNAi wing, the values of the constants β_k (1) and β_k (2) were set to zero, and the value of β_k (3) was decreased, leaving anchorage only at the most distal part of the wing. To simulate the phenotypes produced by driving dp-RNAi with sal-Gal4, hh-Gal4, dpp-Gal4, and brk-Gal4, the anchorage was removed from the zones of the margin indicated in Figure 3B-E).

The genotype nub-Gal4>dp-RNAi was simulated by setting all the β_k (x) terms to zero. The maximum length of the springs was fixed to be of ~ 2.5 cell diameters. When a l_k went above this threshold value, then the spring was removed, simulating the breakage of a Dp attachment.
SUPPLEMENTAL REFERENCES

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