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Modulation of junction tension by tumor suppressors and proto-oncogenes regulates cell-cell contacts

Floris Bosveld§, Boris Guirao, Zhimin Wang, Mathieu Rivière*, Isabelle Bonnet‡, François Graner* and Yohanns Bellaïche§

INTRODUCTION

Tumor suppressors and proto-oncogenes play fundamental functions in cell proliferation, growth and apoptosis. The analyses of these functions have led to important advances in our understanding of tissue development and homeostasis as well as pathologies, including tumorigenesis (for reviews, see Zhao et al., 2011; Patel and Edgar, 2014; Baillon and Basler, 2014). In Drosophila epithelia, the tumor suppressors Fat (Ft) and Dachsous (Ds) regulate cell proliferation, tissue morphogenesis, planar cell polarity and junction tension. By analyzing the evolution over time of ft mutant cells and clones, we show that ft clones reduce their cell-cell contacts with the surrounding wt tissue in the absence of concomitant divisions and over-proliferation. This contact reduction depends on opposed changes of junction tensions in the clone bulk and its boundary with neighboring wt tissue. More generally, either clone bulk or boundary junction tension is modulated by the activation of Yorkie, Myc and Ras, yielding similar contact reductions with wt cells. Together, our data highlight mechanical roles for proto-oncogene and tumor suppressor pathways in cell-cell interactions.

KEY WORDS: Fat/Dachsous and Hippo pathways, Myc, Ras, Junction tension, Myosins, Clone shape

ABSTRACT

Tumor suppressors and proto-oncogenes play crucial roles in tissue proliferation. Furthermore, deregulation of their functions is deleterious to tissue architecture and can result in the sorting of somatic rounded clones minimizing their contact with surrounding wild-type (wt) cells. Defects in the shape of somatic clones correlate with defects in proliferation, cell affinity, cell-cell adhesion, oriented cell division and cortical contractility. Combining genetics, live-imaging, laser ablation and computer simulations, we aim to analyze whether similar or distinct mechanisms can account for the common role of tumor suppressors and proto-oncogenes in cell-cell contact regulation. In Drosophila epithelia, the tumor suppressors Fat (Ft) and Dachsous (Ds) regulate cell proliferation, tissue morphogenesis, planar cell polarity and junction tension. By analyzing the evolution over time of ft mutant cells and clones, we show that ft clones reduce their cell-cell contacts with the surrounding wt tissue in the absence of concomitant divisions and over-proliferation. This contact reduction depends on opposed changes of junction tensions in the clone bulk and its boundary with neighboring wt tissue. More generally, either clone bulk or boundary junction tension is modulated by the activation of Yorkie, Myc and Ras, yielding similar contact reductions with wt cells. Together, our data highlight mechanical roles for proto-oncogene and tumor suppressor pathways in cell-cell interactions.

Mutant clones are essential for unveiling how tumor suppressor and proto-oncogene activities modulate tissue proliferation, growth, cell-cell interactions and cell competition (for a review, see Wagstaff et al., 2013). In particular, the functions of tumor suppressors and proto-oncogenes in tissue organization and morphogenesis have often been recognized as their respective loss and gain of function leads to the formation of a rounded group of mutant cells (somatic clones) having a smooth boundary with the surrounding wild-type (wt) cells and thus reducing their contacts with neighboring wt tissue (Justice et al., 1995; Prober and Edgar, 2000, 2002; Baena-Lopez et al., 2005; Mao et al., 2006; Worley et al., 2013). This property is shared by the Ras and Myc proto-oncogenes as well as components of the Fat/Dachsous (Ft/Ds) and Hippo pathways (Justice et al., 1995; Adler et al., 1998; Johnston et al., 1999; Prober and Edgar, 2000, 2002; Garaó et al., 2000; Baena-Lopez et al., 2005; Mao et al., 2011; Worley et al., 2013).

Experimental and modeling approaches converged to show that the cell-cell contacts between two cell populations can be modulated by cell-cell adhesion, cell cortical contractility and cell division rate and orientation; in particular, the analysis of tissue or compartment boundary formation has provided important insights into the mechanisms modulating cell-cell contacts between two cell populations in response to cell signaling (for a review, see Fagotto et al., 2013). An increase in cell junction tension (which is larger when cortical contractility increases or adhesion decreases) at the interface between two tissues is known to favor a straight boundary between these two tissues or two compartments within a tissue and is essential for tissue development (Graner, 1993; Brodland, 2002; Käfer et al., 2007; Krieg et al., 2008; Hilgenfeldt et al., 2008; Landsberg et al., 2009; Monier et al., 2010; Alice et al., 2012; Röper, 2012; Fagotto et al., 2013; Calzolari et al., 2014; Umetzu et al., 2014). Theoretical analysis shows that randomly oriented cell divisions induce diffusive random cell displacements (Ranft et al., 2010), thereby adding a slight amount of disorder between two cell populations (Block et al., 2007; Radszewiet et al., 2009). Experimental analyses of cell division rate and orientation demonstrated that an increase in cell junction tension is necessary to prevent disorder introduced by cell divisions occurring near the anterior-posterior parasegment boundary of the Drosophila embryo (Monier et al., 2010). Although the role of cell division rate at the Drosophila dorsal-ventral boundary of the wing imaginal disc remains a matter of debate, cell division orientation has been shown to contribute to its shaping (Alice et al., 2012). Cell divisions oriented perpendicular to the boundary can increase the number of cell junctions at the boundary, and hence its raggedness, whereas cell divisions parallel to the boundary can decrease its raggedness. Notably, these effects of cell divisions can be reinforced or conversely weakened according to the number and orientation of cell rearrangements following the divisions.
We initially aimed to understand how Ft/Ds pathway, which modulates the activity of the myosin Dachs and of the Hippo/Yorkie pathway, promotes somatic clone rounding (Mao et al., 2006, 2011; Cho et al., 2006). The tumor suppressors Ft and Ds encode large proto-cadherins that interact in a heterophilic manner and regulate proliferation via the Hippo/Yki pathway or mitochondrial metabolism, planar cell polarity and tissue morphogenesis by promoting oriented cell divisions and cell-cell rearrangements (Mahoney et al., 1991; Clark et al., 1995; Matakatsu and Blair, 2004; Baena-Lopez et al., 2005; Cho et al., 2006; Silva et al., 2006; Willecke et al., 2006; Harvey and Tapon, 2007; Mao et al., 2011; Donougehe and DiNardo, 2011; Bosveld et al., 2012; Brittle et al., 2012; Marcinkevicius and Zallen, 2013; Lawrence and Casal, 2013; Matis and Axelrod, 2013; Degoutin et al., 2013; Sing et al., 2014).

Within tissues, opposing gradients of Ds and the Golgi resident kinase Four-jointed, which regulates Ft-Ds interaction, result in the polarization of Ft and Ds (Ishikawa et al., 2008; Brittle et al., 2010, 2012; Simon et al., 2010; Bosveld et al., 2012; Ambegaonkar et al., 2012). One of the effectors of Ft and Ds planar polarization is Dachs, which regulates cell division orientation, cell rearrangements, cell affinity, proliferation rate and junction tension (Mao et al., 2006, 2011; Bosveld et al., 2012; Brittle et al., 2012). Dachs membrane localization is promoted by the DHHC palmitoyltransferase Approximated (Matakatsu and Blair, 2008), and is inhibited by the interaction of the F-Box ubiquitin ligase Fbxl7 with Ft (Rodrigues-Campos and Thompson, 2014; Bosch et al., 2014). Conversely, the Ds intracellular domain can form a complex with Dachs in vitro and Dachs is proposed to be planar polarized in response to Ds polarization on the same cell junction (Bosveld et al., 2012; Brittle et al., 2012). However, it remains to be shown whether the polarization of Ds intracellular domain would be sufficient to polarize Dachs in vivo. The loss of Ft or Ds function in mutant clones leads to the formation of rounded clones minimizing their contact with neighboring tissues (Adler et al., 1998; Garoia et al., 2000; Mao et al., 2006). Loss of Ft activity induces apical cell constriction and results in the membrane accumulation of Dachs, and ft clone rounding depends on Dachs (Mao et al., 2006, 2011). Whether these changes in Dachs distribution are necessary for ft clone rounding remains to be determined.

Here, we investigated the role of loss of tumor suppressor and gain of proto-oncogene function in forming smooth clone boundaries in the Drosophila dorsal thorax (notum) epithelium where we could implement a time-lapse approach to follow the dynamics of clones. Initially focusing on the Ft/Ds pathway, we uncovered that ft clone rounding originates from two apparently opposed mechanical activities at the clone boundary and in the clone bulk. Extending the analysis to additional tumor suppressors and proto-oncogenes shows how distinct modulations of cell junction tension by tumor suppressors and proto-oncogenes could account for their common role in the regulation of cell-cell contacts.

**RESULTS**

**A time-lapse approach to follow the rounding of ft clones**

To understand the mechanisms of clone rounding we implemented a time-lapse approach to follow the dynamics of ftRNAi clones within the notum epithelium during pupal development (Fig. 1A). Using a combination of the flip-out and temperature-sensitive Gal4/Gal80ts systems (Basler and Struhl, 1994; McGuire et al., 2001), the clones marked by the expression of membrane-CherryFP (UAS-PH:ChFP) were generated in second instar larvae that were then kept at 18°C to avoid the expression of the UAS-ftRNAi. Following a temperature shift to 29°C at the end of larval development, we compared by time-lapse microscopy the proliferation and the circularity changes of a control group of cells and ftRNAi clones (Fig. 1B-D; Movie 1). As a reporter of Ft activity we imaged Dachs:GFP (D-GFP), membrane levels of which increase upon loss of Ft function (Bosveld et al., 2012). At 10 hours after pupa formation (hAPF), the control and ftRNAi clones had similar circularities and distributions of D-GFP. At 40 hAPF, whereas the control clones exhibited a reduced circularity and a D-GFP distribution similar to the surrounding cells, the ftRNAi clones had a much higher circularity and an increased D-GFP level at cell junctions (Fig. 1B-D). This shows that our live-imaging approach is suitable for understanding how Ft/Ds signaling regulates tissue organization. To investigate whether clone rounding depends on cell division, we compared the respective timing of cell division and clone shape changes as well as the rates of proliferation in control and ftRNAi clones (Fig. 1C). In both control and ftRNAi clones, a wave of cell divisions occurred between 15 and 20 hAPF. During this wave neither the control nor the ftRNAi clones rounded up. In fact, ftRNAi clone rounding was observed after cell divisions had ceased and once D-GFP had started to accumulate (Fig. 1B). Furthermore, the proliferation rates were similar in control and ftRNAi clones, showing that clone rounding can take place in the absence of over-proliferation (Fig. 1C). Together, these data show that rounding of ftRNAi clones, and thus the reduction of their contacts with neighboring wt cells, is not concomitant with cell divisions and does not require a change in proliferation rate.

**Dachs exhibits two distinct distributions in ft mutant clones**

Having found that D-GFP accumulates in ftRNAi clones during rounding and knowing that Dachs is necessary for ft clone rounding (Mao et al., 2006, 2011), we performed a detailed analysis of the distribution and the mechanisms of Dachs localization in ft clones. Ft signaling is known to prevent Dachs membrane accumulation (Mao et al., 2006, 2011; Bosveld et al., 2012; Brittle et al., 2012). Accordingly, D-GFP levels were increased in ft cells and sometimes segregated in distinct regions of the cell junctions (Fig. 2A; Figs S1, S2). We also noticed that D-GFP was strongly enriched all around the circumference of ft clones, i.e. at the interface between wt and ft cells, but reduced at the transversal junctions (Fig. 1B; Fig. 2A, yellow arrowheads). Quantitative analyses of the D-GFP distribution at clone boundary and transversal junctions revealed that D-GFP within mutant cells neighboring wt cells specifically accumulated at the clone boundary (hereafter referred to as ‘Dachs polarization at clone boundary’) (Fig. 2I). We therefore investigated the mechanisms leading to the polarization of D-GFP at the ft clone boundary. In agreement with the facts that the Ds intracellular domain forms a complex with Dachs in vitro, that Ds polarization promotes Dachs polarization and that Ds:GFP is polarized at the boundary of ft clones (Fig. 2B, yellow arrowheads; Strutt and Strutt, 2002; Ma et al., 2003; Matakatsu and Blair, 2004; Bosveld et al., 2012), D-GFP polarization was reduced at the boundary between wt and ft ds double mutant cells (Fig. 2C, white arrowheads; Fig. 2J). Whereas the overexpression of a full-length Ds transgene in ft ds clones (ds ft dsGFP) restores D-GFP polarization at the clone boundary (Fig. 2D, yellow arrowheads; Fig. 2J), overexpression of the Ds intracellular domain (Ds:intra) did not (Fig. 2E, white arrowheads; Fig. 2J), suggesting that the extracellular heterophilic Ds-Ft interaction is required to polarize Dachs. Accordingly, D-GFP polarization was reduced at the clone interface of ds ft dsGFP clones, which abutted a ft hypomorphic mutant tissue (compare Fig. 2D and 2F; Fig. 2J). Because loss of Ft activity inhibits Dachs membrane levels, D-GFP is also elevated in ft cells surrounding ds ft dsGFP clones. Together, our results support the hypothesis that Dachs is...
not only enriched in ft mutant clones, but is also polarized at the clone boundary due to Ds polarization.

Two independent and complementary results strengthen the proposal that the polarization of the Ds intracellular domain is sufficient to polarize Dachs at the ft clone boundary. First, in vitro analyses of D:GFP polarization using an S2-induced polarity assay (Johnston et al., 2009; Ségalen et al., 2010), in which the extracellular and transmembrane domains of the homophilic adhesion molecule Echinoid are fused to the intracellular domain of Ds (Ed:mCh:Ds:intra), showed that the Ed:mCh:Ds:intra polarity domain was sufficient for D:GFP recruitment and polarization, which colocalized with Ed:mCh:Ds:intra (Fig. 2K). Second, within the tissue, we found that the circumference of clones that overexpressed a chimera of the Ds intracellular and Ft extracellular domains (Casal et al., 2006) in ds ft clones can harbor a D:GFP polarization at the clone interface. At ~20 hAPF, D:GFP localization is similar inside and outside of the ftRNAi clone. At ~20 hAPF, D:GFP is enriched in the ftRNAi clone. Over time, D:GFP gradually accumulates and becomes polarized at the clone boundary. This clone becomes rounder over time (see C). Yellow arrowheads indicate cells where D:GFP is polarized.

Two complementary activities of Dachs contribute to ft clone rounding

The identification of two distinct changes in Dachs distribution in ft clones prompted us to investigate their respective contribution to clone rounding. We previously found that D:GFP polarization at cell junctions correlates with a higher junction tension (Bosveld et al., 2012). To investigate further the role of Dachs polarization in junction tension regulation, we first confirmed that Dachs polarization promotes an increase in junction tension, i.e. the force exerted by the junction parallel to it, which is considered positive when it reduces junction length and which can be estimated by the initial recoil velocity of the junction vertices upon its severing by laser ablation (Hutson et al., 2003). As Ds planar polarization is independent of Dachs function (Fig. 3A-C; Bosveld et al., 2012; Brittle et al., 2012), we compared the relaxation velocity of junctions showing high or low Ds:GFP signal in a dachs tissue, the junctions being labeled by Baz:mChFP. The loss of Dachs function abolishes the difference in relaxation velocity between junctions bearing high and low Ds:GFP (Fig. 3D). Because Ds and Dachs are
planar polarized at the same junctions, this indicates that Dachs activity is necessary to increase the tension of junctions harboring Ds and Dachs polarization.

Having confirmed that Dachs polarization is necessary to increase cell junction tension in wt tissue, we tested whether the Dachs polarization at the boundary of ft clones and the increase in cell junction tension (Fig. 4A,B; Bosveld et al., 2012) are sufficient to fully explain the higher circularity of ft clones relative to wt clones. The increased tension at ft clone boundaries was independent of the clone size and the orientation of the ablated junctions (Fig. S3). The loss of Ds activity in ft mutant clones is associated with a decreased D:GFP polarization at clone boundaries (Fig. 2C,J) and an average junction tension similar to that of wt (Fig. 4C,C’). Although the circularity of ds ft clones was lower than the circularity of ft clones, the circularity of ds ft clones remained higher than that of wt clones (Fig. 4A). As Dachs activity is essential to increase the ft clone boundary tension and the circularity of ft clones (Fig. 4A,B; Mao et al., 2006), this indicates that Dachs polarization at the clone boundary increases the circularity of ft clones and that an additional Dachs-dependent mechanism inside the clone contributes to ft clone rounding (Fig. 1; Fig. 4A).

To identify this additional mechanism, we probed the tension of junctions inside ft clones. Unexpectedly, we observed that inside ft clones the average cell junction tension was reduced (Fig. 4B). A
lower tension inside the clone was also measured in ds ft and in ds clones, showing that the tension decrease is independent of Ds function (Fig. 4C,D). Cell junction tension inside ft dachs, ds dachs and ds ft dachs clones as well as their circularities were similar to the wt, indicating that Dachs activity is necessary inside ft clones to reduce junction tension (Fig. 4B-D). The changes in junction tension were not associated with changes in the levels of the Drosophila Myosin II light chain (Spaghetti Squash, Sqh) at the boundary of or inside ft or ds clones (Fig. 4E,F; Fig. S4).

A common feature of ft, ds and double ds ft clones is to exhibit a higher level of Dachs (Mao et al., 2006; Brittle et al., 2012; Fig. 2A,1; Fig. S1A,H), which is reported to inhibit Wts (Cho et al., 2006) via Zyxin-dependent or -independent mechanisms (Rauskolb et al., 2011; Gaspar et al., 2015). We uncovered that loss of Wts activity (wts\textsuperscript{RNAi}) leads to clone rounding and to a decrease of junction tension similar to that measured inside ft, ds or ds ft clones (Fig. 4G,H). Furthermore, overexpression of Wts in ft clones (ft wts\textsuperscript{UP}) partially rescued the rounding of ft clones by reducing the polarization of Dachs and the tension at clone boundary junctions (Fig. 4G-J). Finally, the lower tension associated with Wts loss of function might be mediated by the activation of Yki as clones overexpressing Yki (yki\textsuperscript{UP}) are characterized by a junction tension similar to that of wts\textsuperscript{RNAi} (Fig. 4G). yki\textsuperscript{UP} clones display a rounder shape than wts\textsuperscript{RNAi} clones (Fig. 4H), suggesting that overexpression of Yki promotes clone rounding by both tension-dependent and tension-independent mechanisms. Together, these experimental observations indicate that Dachs has two apparently opposed mechanical activities: the polarized Dachs distribution at the boundary of the clone increases junction tension and the Dachs accumulation within the clone bulk decreases junction tension.

Two opposed mechanical activities cooperate to promote clone rounding

Next, we aimed to understand better the impact of differential junction tension, and more specifically to determine how both opposed mechanical activities might cooperate to determine the rounding and thus the segregation of developing clones. Based on a theoretical analysis (Graner, 1993; supplementary materials and methods), we analyzed the competition between the tension of cell junctions at the clone boundary $\gamma_b$ on the one hand, and the tensions of cell junctions within the clone $\gamma_c$, or in the surrounding wt cells $\gamma$ on the other hand (Fig. 5A). Modeling and experiments are independent of the biological origin of these tensions. This analysis shows that: (1) there is an energy cost associated with the length of the clone boundary when junction tensions $\gamma_b$, $\gamma_c$, or in the surrounding wt cells $\gamma$ on the other hand (Fig. 5A). Modeling and experiments are independent of the biological origin of these tensions. This analysis shows that: (1) there is an energy cost associated with the length of the clone boundary when junction tensions $\gamma_b$, $\gamma_c$, or in the surrounding wt cells $\gamma$ on the other hand (Fig. 5A). Modeling and experiments are independent of the biological origin of these tensions. This analysis shows that: (1) there is an energy cost associated with the length of the clone boundary when junction tensions $\gamma_b$, $\gamma_c$, or in the surrounding wt cells $\gamma$ on the other hand (Fig. 5A).

$$\sigma = 1/\gamma\left[\gamma_b - [(\gamma_c + \gamma)/2]\right].$$

(1)

It represents the energy cost per unit length of increasing the clone boundary by changing ‘homotypic’ junctions (wt-wt or mutant-mutant) into ‘heterotypic’ boundary junctions (wt-mutant), and should not be confused with the cell junction tensions themselves that are probed with laser ablations. An advantage of this description is that it takes into account junction tensions only with respect to their relative values, which are directly extracted from laser ablation experiments. The expression of $\sigma$ is general and includes the case where the tensions on both sides of a domain boundary are the same,
namely \( \gamma_c = \gamma_b \) (Landsberg et al., 2009). It emphasizes that the junction tensions around and within the clone act antagonistically to contribute to the clone boundary energy cost. We therefore expect that when \( \sigma \) is positive, the clone boundary becomes more regular and its circularity increases, thus reducing contact with neighboring wt cells. By contrast, when \( \sigma \) is negative, the heterotypic (wt-mutant) junctions are favored and clones would gradually become more scattered. In between, when \( \sigma \) is null, which corresponds to a wt clone in a wt tissue, we expect it to become slightly more ragged over time (partly as a result of cell division randomly oriented with respect to clone boundary). Importantly, this agrees with our experimental observations: (1) wt clone circularity decreases over time (Fig. 1C); and (2) \( \text{ftRNAi} \) clone circularity increases over time (Fig. 1C). This is illustrated in the graphs (Fig. 5B,D; Fig. S5B) showing the sign of \( \sigma \) versus the junction tensions at the clone boundary (\( \gamma_b \)) and inside the clone (\( \gamma_c \)) measured in wt, \( \text{ds ft} \), and \( \text{ds ft RNAi} \) clones (Fig. 4B-D,G). Using numerical simulations, we challenged more quantitatively this modeling by simulating the growth and proliferation of mutant clones using the cellular Potts model directly based on the ratios of experimental tension (Fig. 5C,D; Fig. S5; Movie 3). They showed that: (1) the experimental tension values in wt, \( \text{ds ft} \), \( \text{ds ft} \), and \( \text{ds ft RNAi} \) both at clone boundary and inside the clone agree with the rounding of \( \text{ft} \) clones and the lower circularity of \( \text{ds} \) and double \( \text{ds ft} \) clones (Fig. 5D); (2) the combination of an increase of junction tension at the clone boundary and a decrease of junction tension inside the clone leads to a higher circularity than an increase of junction tension solely at the clone boundary (compare \( \text{ft} \), simulated using tensions \( \gamma_b \) and \( \gamma_c \) with \( \gamma_b \) and \( \gamma_c = \gamma_b \); Fig. S5B); and (3) the dimensionless clone tension is relevant, and the observed clone circularity increases with \( \sigma \) similarly in experiments and in simulations (Fig. 5D; Fig. S5B). Our experimental observations and modeling of the \( \text{ft} \) clones uncovers how two distinct activities of the myosin Dachs associated with two distinct changes in its localization could induce clone rounding (reducing their contact with neighboring wt cells) by both increasing junction tension at the clone boundary and reducing it inside the clone.

Overexpression of proto-oncogenes modulates junction tension

Having found that the modulation of junction mechanical properties by the Ft/Ds and Hippo’Yki pathways promote clone rounding, we investigated whether the overexpression of proto-oncogenes known
GFP levels were normal inside the clones. We therefore turned our attention to the distribution of MyoII (Sqh::GFP). *rasV12UP* clones were associated with an increase in MyoII localization at the clone boundaries (Fig. 6F,H). Such an increased level of MyoII at the boundary of *rasV12UP* clone is in full agreement with the observed higher tension also reported for the MyoII distribution along the anterior-posterior compartment boundaries or during cell intercalation (Rauzi et al., 2008; Fernandez-Gonzalez et al., 2009; Monier et al., 2010; Umetsu et al., 2014). In contrast to *rasV12UP*, MyoII was globally reduced within *mycUP* clones (Fig. 6F,G). To determine whether the reduction of MyoII levels can account for the rounding of *mycUP* clones, we tested whether mimicking a reduction of MyoII levels using *sqhRNAi* was sufficient to decrease junction tension and to induce a rounded clone shape. Indeed, reducing MyoII levels led to a reduced tension inside *sqhRNAi* clones and promoted clone rounding (Fig. 6A,B).

## DISCUSSION

The segregation of tissues or of tissue compartments plays fundamental roles in the regulation of growth, patterning and morphogenesis (for a review, see Fagotto, 2014). Numerous tumor suppressors and proto-oncogenes share the common feature of promoting cell segregation apparent by the rounding of somatic clones upon their loss or gain of functions, respectively (Adler et al., 1998; Johnston et al., 1999; Prober and Edgar, 2000, 2002; Garoia et al., 2000; Baena-Lopez et al., 2005; Mao et al., 2006, 2011; Worley et al., 2013). Here, we show that loss of Fts and Hippo tumor-suppressor pathways as well as gain of function of the proto-oncogenes Myc, Ras and Yki lead to changes in cell junction tension. Although the changes are distinct and depend on distinct molecular mechanisms, taking into account both clone boundary and bulk tensions is sufficient to account for their segregation from surrounding wt tissue. Although previous works demonstrated that cell cortical contractility or cell adhesion at the interface between two compartments, tissues or clones can promote their separation or contribute to tissue invagination (Graner, 1993; Le Borgne et al., 2002; Brodland, 2002; Wei et al., 2005; Laplante and Nilson, 2006; Landsberg et al., 2009; Monier et al., 2010; Chang et al., 2011; Alice et al., 2012; Röper, 2012; Fagotto et al., 2013; Calzolari et al., 2014; Umetsu et al., 2014), our genetic and modeling findings show that...
both boundary and bulk cell junction tensions need to be considered to fully account for segregation. This point is illustrated by the analysis of the mechanisms of clone rounding in the case of the Ft/Ds pathway where loss of Ft activity leads to change in cell junction tension both at the clone boundary and within the clone bulk. We provide evidence that the rounding of \textit{ft} clones can result from two apparently opposed mechanical inputs that require Dachs myosin activity. On one hand, the polarization of Ds at clone boundaries promotes Dachs polarity and increases junction tension. On the other hand, loss of Ft or Ds activity in the bulk of the clone leads to elevated Dachs levels and a decrease in junction tension. Our genetic and modeling findings show how these two distinct mechanical inputs both contribute to \textit{ft} clone rounding. Importantly, increased levels of Dachs lead to an increased junction tension only at cell junctions where both Ds and Dachs are polarized, providing the first example of polarization and changes in the levels of a myosin leading to opposite changes in junction tension. The mechanism by which Dachs increases junction tension upon Ds-dependent polarization within tissues might be distinct from the one proposed for MyoII, because \textit{in vitro} recombinant Dachs does not bind F-Actin in an ATP-dependent manner, but rather modulates F-Actin organization by promoting the binding of Zyxin to F-Actin (Cao et al., 2014). The mechanisms by which Dachs reduces tension inside \textit{ft} and \textit{ds} clones are independent of Ft and Ds activity and correlate with an increase of Dachs level. Dachs is reported to inhibit Wts by both Zyxin-dependent and -independent mechanisms (Rauskolb et al., 2011; Gaspar et al., 2015) and loss of Wts activity is associated with a similar decrease of cell junction tension. Wts is localized at cell-cell junction and its activity correlates with its binding to distinct partners and with distinct apical-basal distributions (Rauskolb et al., 2014; Sun et al., 2015). Whether these changes in Wts binding partner at cell-cell junctions can account for the regulation of tension by Dachs remains to be analyzed. Finally, the Ft/Ds pathway controls the shape of mammalian tissue (Saburi...
et al., 2008), therefore it will be relevant to examine whether Ft and Ds regulate tissue shape in vertebrate systems through modulation of cell junction tension.

Our findings on the mechanisms of clone rounding provide information on cell competition and on a possible negative feedback between proliferation and tension regulation. In the context of cell competition, it has been recently reported that a clonal decrease of Myc levels, or a clonal increase of Myc levels in conjunction with an inhibition of apoptosis, promotes clone fragmentation and cell mixing; such cell-cell mixing increases cell-cell contacts and depends on reduced levels of F-actin, independently of changes in MyoII levels (Levayer et al., 2015). Our experimental results and previous findings show that Myc overexpression leads to clone rounding and a reduction of cell-cell contacts with neighboring wt cells (Prober and Edgar, 2002). Although we did not study Myc-mediated cell competition and our experimental setup was different from that of Levayer et al. (2015), it would be relevant to analyze whether Myc-mediated cell competition also depends on additional mechanisms associated with reduced cell-cell contact formation owing to a decrease of MyoII level. Lastly, our findings on Yki suggest the existence of a possible negative feedback between Yki activation and cell junction tension. Both experimental and modeling approaches converge to support the idea that mechanical tension is an important regulator of growth and proliferation (Shraiman, 2005; Hufnagel et al., 2007; Aegeerter-Wilsen et al., 2007, 2012; Mamamoto and Inger, 2010; Dupont et al., 2011; Sansores-Garcia et al., 2011; Aragona et al., 2013; Schluck et al., 2013; Rauskolb et al., 2014; Benham-Pyle et al., 2015). In particular, an increase in mechanical tensile stress leads to the activation of the Hippo/Yki (YAP/TAZ) pathway (Dupont et al., 2011; Sansores-Garcia et al., 2011; Rauskolb et al., 2014; Codella et al., 2014). We have provided evidence that ectopic activation of the Hippo/Yki pathway in the notum epithelial tissue decreases junction tension in the bulk of the clone. The decrease of junction tension upon activation of Yki might unveil the possible existence of negative feedback from Hippo and Yki signaling in response to tensile stress in Drosophila tissues. Although the exact molecular mechanisms remain to be better characterized, we propose that such negative feedback could be instrumental to prevent prolonged activation of the Hippo and Yki pathways in response to an increase of mechanical tissue tension associated with tissue development or with external stress. Finally, by demonstrating that other signaling pathways regulate clone bulk or boundary tensions, we foresee that junction tension regulation might support cross-talk between tumor suppressor/proto-oncogene pathways and the Hippo/Yki pathways to define the size and shape of tissues and organs.

**MATERIALS AND METHODS**

**Fly stocks**

_Drosophila_ stocks used were: ds^{65142}, ft^{69r}, ft^{b}, ds^{6013}, p^{219}, UAS-ft^{RNAi}, UAS-sqh^{RNAi}, UAS-wts^{RNAi}, UAS-yki, UAS-myc, UAS-raf^{152} (see Table S1 for full genotypes and references). Clones were generated using FLP/FRT, flip-out or mosaic analysis with a repressible cell marker (MARCM) techniques (Xi and Rubin, 1993; Basler and Struhl, 1994; Lee and Luo, 1999). Clones were induced in second instar larvae (20 min heat-shock at 37°C) and analyzed 3-4 days later in 18-22 hAPF pupa. Live-imaging was carried out on p^{RNAi} (hs-flp; ft^{69r}/E-Cad:GFP; tub-GAL80^{P1}; A-11122, Molecular Probes) and mouse anti-FasIII (1:50; rabbit) or gain (UAS-GFP, UAS-mRFP, UAS-PH:ChFP) of expression.

For immunohistochemistry, pupae were dissected, fixed and stained as described by Segalin et al. (2010). Primary antibodies used were rabbit anti-

**Quantification of clone circularity**

In the literature, the terms 'circularity', 'compactness', 'segregation' or 'separation' are used to express a size reduction of the contact with neighboring cells and can be quantified by measuring the clone area to tissue devoid of suppressor/proto-oncogene pathways and the Hippo/Yki pathways to define the size and shape of tissues and organs.

**Quantification of D:GFP polarity and junctional intensity of Sqh:**

_Drosophila_ stocks used were: ds^{65142}, ft^{69r}, ft^{b}, ds^{6013}, p^{219}, UAS-ft^{RNAi}, UAS-sqh^{RNAi}, UAS-wts^{RNAi}, UAS-yki, UAS-myc, UAS-raf^{152} (see Table S1 for full genotypes and references). Clones were generated using FLP/FRT, flip-out or mosaic analysis with a repressible cell marker (MARCM) techniques (Xi and Rubin, 1993; Basler and Struhl, 1994; Lee and Luo, 1999). Clones were induced in second instar larvae (20 min heat-shock at 37°C) and analyzed 3-4 days later in 18-22 hAPF pupa. Live-imaging was carried out on p^{RNAi} (hs-flp; ft^{69r}/E-Cad:GFP; tub-GAL80^{P1}; A-11122, Molecular Probes) and mouse anti-FasIII (1:50; rabbit) or gain (UAS-GFP, UAS-mRFP, UAS-PH:ChFP) of expression.

For immunohistochemistry, pupae were dissected, fixed and stained as described by Segalin et al. (2010). Primary antibodies used were rabbit anti-

fused to mCherry (Ed:mCh:Ds:intra). The Ds intracellular fragment used in the S2 cell polarity assay was cloned into pMT:Ed:mCherry with a five-amino acid linker (GGGGS) between the mCherry and the Ds intracellular domain. Dachs was cloned into pUWG (DRGC). Induced cell polarity assays were carried out as described previously (Johnston et al., 2009; Ségalen et al., 2010). The polarization of the GFP-tagged Dachs construct relative to the induced Ed:mCh:Ds:intra polarity interface was quantified from single confocal scans as follows. The mean GFP intensities all around the cell cortex (Icell-cortex) and at the Ed:mCh:Ds:intra interface (Ipolarity-domain) were quantified blindly using ImageJ. The D:GFP was scored as polarized when the Ipolarity-domain/Icell-cortex ratio was >1.1.

Theoretical analysis of clone shapes and numerical simulations

See supplementary materials and methods for theoretical analysis and numerical simulations.

Statistics

All error bars represent the standard deviation (s.d.). Statistical significance between experimental conditions was assessed using Student’s r-tests: distribution normalities were checked using Kolmogorov–Smirnov test. In cases where variables were different (F-test), significance was assessed using the unequal variance r-test. The one-way ANOVA Tukey’s test was used to assess significance between genotypes within the same dataset (circularity, D:GFP polarity). Significance between either clone boundary or clone bulk tensions was assessed using one-way ANOVA Dunnett’s test. By reporting significances based on one-way ANOVA, statistical differences between two different experimental conditions might have been missed.

Code availability

The code used for simulations based on the cellular Potts model is provided as a zip file in supplementary information along with the procedure to install and run it on MacOS.

Competing interests

The authors declare no competing or financial interests.

Author contributions

F.B. and Y.B. designed the project. F.B., I.B. and Z.W. performed experiments. Z.W. analyzed the data. B.G. and F.G. developed the theoretical analysis. B.G. performed simulations. F.B., B.G., F.G. and Y.B. wrote the manuscript.

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Supplementary information

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