

RESEARCH REPORT

Growth control in the *Drosophila* eye disc by the cytokine UnpairedJannik Vollmer^{1,2}, Patrick Fried^{1,2}, Daniel Aguilar-Hidalgo^{3,*}, Máximo Sánchez-Aragón³, Antonella Iannini³, Fernando Casares^{3,†} and Dagmar Iber^{1,2,‡}

ABSTRACT

A fundamental question in developmental biology is how organ size is controlled. We have previously shown that the area growth rate in the *Drosophila* eye primordium declines inversely proportionally to the increase in its area. How the observed reduction in the growth rate is achieved is unknown. Here, we explore the dilution of the cytokine Unpaired (Upd) as a possible candidate mechanism. In the developing eye, *upd* expression is transient, ceasing at the time when the morphogenetic furrow first emerges. We confirm experimentally that the diffusion and stability of the JAK/STAT ligand Upd are sufficient to control eye disc growth via a dilution mechanism. We further show that sequestration of Upd by ectopic expression of an inactive form of the receptor Domeless (Dome) results in a substantially lower growth rate, but the area growth rate still declines inversely proportionally to the area increase. This growth rate-to-area relationship is no longer observed when Upd dilution is prevented by the continuous, ectopic expression of Upd. We conclude that a mechanism based on the dilution of the growth modulator Upd can explain how growth termination is controlled in the eye disc.

KEY WORDS: Eye disc development, Growth control, Upd, Dilution

INTRODUCTION

How organs measure their growth to control their final size is still an open question in biology. The primordia of the adult organs in *Drosophila*, the imaginal discs, present an attractive model system to address this question (Hariharan, 2015; Mirth and Shingleton, 2012). We recently found that the area growth rate of the *Drosophila* eye disc declines inversely proportionally to the increasing eye disc area (Vollmer et al., 2016). An inverse relationship between the growth rate and the total area could arise if a long-lived, diffusible, extracellular growth factor was diluted as the organ grows, because then dilution would reduce the concentration of this factor proportionally to the increase in eye disc area. The expression of such a growth factor would need to cease before the eye disc growth process starts to set an initial concentration. The factor would then need to be sufficiently long-lived to be diluted rather than degraded and the cellular response would need to be linearly related to the concentration. The factor would further need to be extracellular and

diffusible because the growth rate declines uniformly, while cell division patterns are non-uniform in the eye disc (Wartlick et al., 2014; Wolff and Ready, 1991). To avoid loss of the extracellular factor from the eye disc over developmental time, it would need to act on the apical side, which faces the closed luminal space of the disc. Finally, dispersal of the growth factor would need to be limited to an area close to the apical cell membrane, so that it would be diluted relative to the area.

Mutations in the JAK/STAT pathway, such as the loss or overexpression of its ligand Unpaired (Upd; Upd1 – FlyBase), are known to alter the size of the eyes without affecting eye disc patterning (Bach et al., 2003; Juni et al., 1996). Intriguingly, most of the above requirements for a factor controlling growth by dilution have already been reported for the cytokine Upd. Thus, Upd is expressed in the posterior margin of the eye disc in late second/early third larval stage, but its expression stops as soon as the differentiation of the posterior cells starts behind the differentiation front, called the morphogenetic furrow (MF) (Bach et al., 2007). In spite of the production stop, Upd as well as its intracellular response factor pSTAT (phosphorylated Stat92E) can still be detected with antibody staining 24–48 h after Upd production has ceased (Zhang et al., 2013). A GFP-tagged Upd has been shown to diffuse extracellularly (Tsai and Sun, 2004), and extracellular Upd and its downstream signalling factor, pSTAT, can indeed be detected uniformly in the entire wild-type eye and antenna discs by antibody staining (Zhang et al., 2013). Moreover, the JAK/STAT pathway responds approximately linearly to the Upd concentration in cell culture assays (Harrison et al., 1998; Wright et al., 2011). Finally, Upd has been found associated to the apical extracellular matrix (ECM), both in cell culture and in the eye disc (Harrison et al., 1998; Hombria et al., 2005; Zhang et al., 2013), and alterations of the ECM affect the Upd concentration in the ECM and its signalling capability (Zhang et al., 2013).

Here, we combine computational modelling, gene expression manipulations and quantitative measurements to test the Upd dilution-based mechanism. We find that the growth kinetics in Upd mutants are quantitatively consistent with the predictions of an area-dependent dilution mechanism, and that the stability of Upd is sufficient for a dilution mechanism. We conclude that a Upd-based dilution mechanism for organ growth control is plausible.

RESULTS AND DISCUSSION

We combined genetic perturbations and mathematical modelling to test the Upd-dependent dilution mechanism for growth control. To this end, we measured and simulated the effects of either lowering Upd availability or increasing Upd production ectopically and continuously, thereby counteracting dilution (Fig. 1A). As described before (Vollmer et al., 2016), we measured the total area, *T*, as well as the posterior, *P*, and anterior, *A*, areas (Fig. 1B) during eye disc development, using both 2D projections and 3D renderings of the imaged eye discs (Fig. S1; see Materials and

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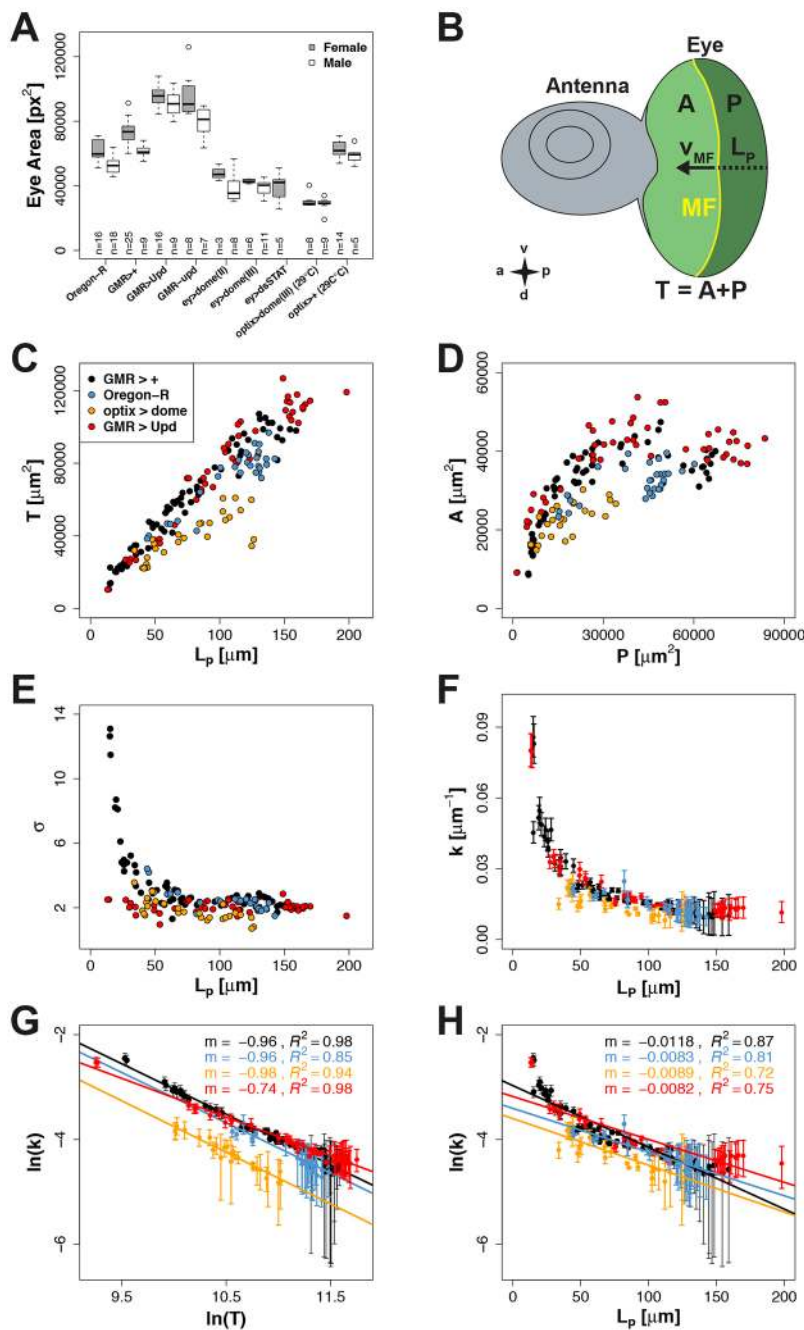


Fig. 1. Growth kinetics in differently sized *Drosophila* strains. (A) Boxplots of eye sizes in adult flies. Genotype (wild type or perturbations in the JAK/STAT signalling pathway), sex, and sample sizes are indicated. Full descriptions of genotypes are given in Materials and Methods. (B) Scheme of an eye-antenna imaginal disc with the characteristic measures. P, posterior area (dark green); A, anterior area (light green); T, total area; L_P , posterior length; MF, morphogenetic furrow (yellow); v_{MF} , speed of the MF. (C,D) Growth kinetics of eye imaginal discs in the different genotypes. (E,F) Axis ratio σ and area growth rate k (shown as mean \pm s.d.; Fig. S3) during eye disc development. (G,H) In-ln plot of the growth rate k versus total area T , and In-linear plot versus posterior length L_P (mean \pm s.d.). The data for Oregon-R and *GMR-GAL4* ('*GMR*>+') were reproduced from Vollmer et al. (2016).

Methods and supplementary Materials and Methods). Here, the posterior length L_P (Fig. 1B, dashed line) can be used as a measure of developmental progress (Vollmer et al., 2016; Wartlick et al., 2014).

Upd sequestration leads to slower growth, but maintains area dependency of growth rate

First, we reduced the concentration of available Upd in the developing eye by expressing a truncated form of its receptor *dome* (*dome* Δ CT), which sequesters Upd (Brown et al., 2001), or inhibited signal transduction by expressing a STAT-specific RNAi (see Materials and Methods for details) (Fig. 1A). All genetic combinations tested resulted in smaller adult eyes. As eye reduction was strongest in *optix-GAL4; UAS-dome* Δ CT ('*optix*>*dome*');

Fig. 1A), we continued with this genotype. Compared with *GMR*>+ and Oregon-R control discs (Vollmer et al., 2016), the total area growth, relative to MF advancement (as measured by L_P) is slower in *optix*>*dome* eye discs (Fig. 1C), and these discs have smaller anterior and posterior areas (Fig. 1D). A difference in the growth rate could, in principle, result from a difference in eye disc shape: for the same initial total area, more elongated eye discs grow to a smaller final size (Fig. S2). However, the *optix*>*dome* eye discs are, if anything, rounder than the control strains (Fig. 1E; supplementary Materials and Methods). Therefore, a shape difference cannot explain the observed difference in the growth rate.

We next checked whether the area growth rate would still decline inversely proportionally with the (more slowly) increasing eye disc area in the *optix*>*dome* mutant eye disc. As introduced previously

(Vollmer et al., 2016), the area growth rate can be determined as

$$k(L_P) = \frac{dT}{dL_P} / A. \quad (1)$$

Here, we assumed that the area growth rate, k , is linearly related to the Upd concentration, given the established linear relationship between the Upd concentration and the cellular STAT response (Harrison et al., 1998; Wright et al., 2011). As previously described (Vollmer et al., 2016), the derivative $\frac{dT}{dL_P}$ was determined by fitting the data in Fig. 1C with splines (Fig. S3). We could then use a diagnostic $\ln(k)$ versus $\ln(T)$ plot of the data to evaluate the plausibility of area-dependent growth control (P_A), i.e.

$$k(L_P) = k_0 \frac{T(0)}{T(L_P)} \quad (2)$$

for the different genotypes (Fig. 1G). To support an area-dependent growth law, the $\ln(k)$ versus $\ln(T)$ plots should be fitted by straight lines of slope minus one. As we showed before, the slope is indeed very close to minus one (-0.96) for the Oregon-R control strain (Vollmer et al., 2016). Intriguingly, the slope is very close to minus one (-0.98) also for the *optix>dome* strain, even though the eye discs of the *optix>dome* strain differ substantially in total size (Fig. 1C) and in the anterior area (Fig. 1D) over developmental time, and there are no correlated changes between $\frac{dT}{dL_P}$ and A (Fig. S4), thus pointing to distinct underlying growth kinetics in the different genotypes.

Sustained Upd expression leads to a slower decline in the growth rate

We next tested whether the cells in the anterior eye discs remain sensitive to changes in the Upd concentration throughout eye disc

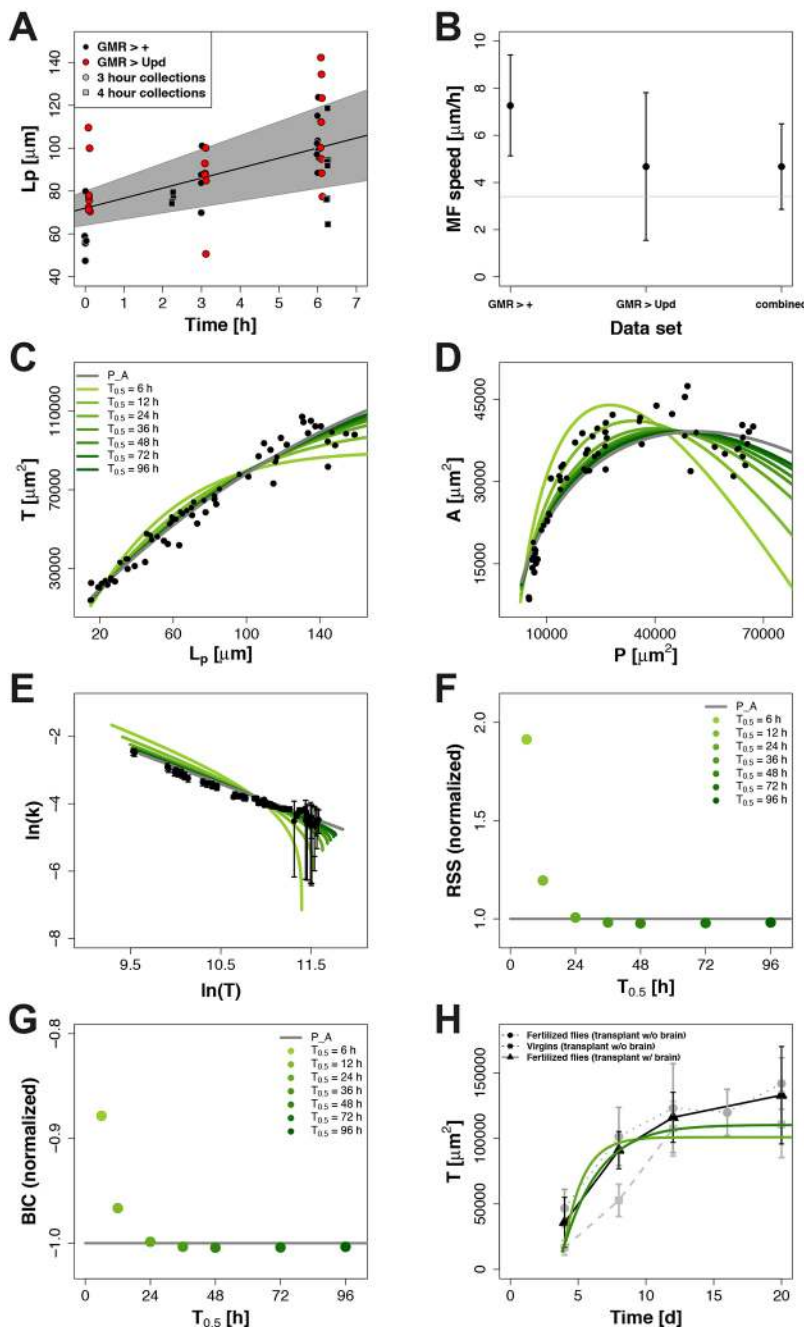


Fig. 2. Impact of Upd stability. (A) The position of the MF, i.e. the posterior length (L_P), at the indicated time intervals in $GMR>+$ (black) and $GMR>Upd$ (red) eye discs. Egg collection intervals were 3 h (circles) or 4 h (squares). The black line shows the linear fit of the combined data sets. The grey area indicates the 95% confidence interval. Egg collection and larval rearing was at 25°C; the last time point corresponds to the time of pupariation initiation (120 h after egg laying at 25°C). (B) Inferred MF speed in the $GMR>+$, the $GMR>Upd$, and the combined data set. Error bars indicate 95% confidence intervals. The grey line marks the previously inferred MF speed of 3.4 $\mu\text{m/h}$ (Vollmer et al., 2016; Wartlick et al., 2014). (C,D) Best fits of the model (Eqn. 4; supplementary Materials and Methods) with different half-lives to the measured total and anterior-posterior areas in $GMR>+$. P_A, area-dependent model (Eqn. 2). Parameter values are given in Table S1. (E) Comparison of the growth rates predicted by the model for the different half-lives (lines) to the data-inferred growth rate (dots, mean \pm s.d.) in $GMR>+$. (F) Deviation of the model from the datasets as measured by the residual sum of squares (RSS) versus the half-life used in the model. The values were normalized to the RSS obtained from fitting the pure dilution model (P_A; Eqn. 2). Parameters for the different models are given in Table S1. Grey line, RSS for a pure dilution model (P_A; Eqn. 2). (G) Bayesian information criterion (BIC) versus the half-life. Values were normalized to the BIC obtained for a pure dilution model (P_A; Eqn. 2). BIC was calculated as

$$BIC = n \cdot \ln\left(\frac{RSS}{n}\right) + k \cdot \ln(n),$$

where RSS is the residual sum of squares, n is the number of data points and k the number of parameters being estimated. Grey line, BIC for a pure dilution model (P_A; Eqn. 2). (H) The substantially slower growth kinetics of transplanted imaginal discs (supplementary Materials and Methods) (Garcia-Bellido, 1965) can be matched by the area-dependent growth model (Eqn. 4) with an Upd half-life of 24 h, and slightly better with 48 h. To reproduce the slow eye disc development, the MF speed v_{MF} had to be lowered to 5% of the value used for $GMR>+$. The initial growth rate, k_0 , had to be lowered to 28% or 18% for a half-life of 24 h or 48 h, respectively. Relative values are given with respect to

Table S1. Here, we note that the reported measurements are based on 2D measurements and include the entire eye-antennal disc (Garcia-Bellido, 1965). In our control strains, 2D and 3D measurements are very well correlated with $T_{3D}=1.6 \times T_{2D}$ (Fig. S1) (Vollmer et al., 2016) and the eye disc covers 60% of the entire eye-antennal disc such that the two effects cancel each other out.

development. To this end, we determined the area growth rate, k , in eye discs in which Upd was expressed ectopically in differentiating cells posterior to the MF ($GMR>Upd$; Fig. S3). Such overexpression has been shown before to result in large eyes (about 1.3-fold larger than control eyes, Fig. 1A) (Bach et al., 2003; Tsai and Sun, 2004). We find that the $GMR>Upd$ eye discs grow very similarly to $GMR>+$ control discs (Fig. 1C,D), except that the eye discs of $GMR>Upd$ larvae are rounder initially (Fig. 1E) and their area becomes larger eventually (Fig. 1C), demonstrating that the eye discs remain sensitive to the Upd concentration also at later stages.

The very good fit of a straight line with slope minus one to the control and $optix>dome$ data in the $\ln(k)$ versus $\ln(T)$ plot (Fig. 1G) strongly supported an area-dependent growth rate. Intriguingly, in case of the $GMR>Upd$ strain, the slope is -0.74 , i.e. $k(L_P) = k_0 \left(\frac{T(0)}{T(L_P)} \right)^{0.74}$ (Fig. 1G, red), which indicates a delayed reduction in the growth rate as $GMR>Upd$ eye discs grow. Importantly, consistent with model predictions, the initial decline in the growth rate is very similar in $GMR>+$ and $GMR>Upd$ eye discs. Thus, when we add Upd to a recently published model of eye disc development (Fried et al., 2016), the model predicts a similar initial decline in the Upd concentration in both control and $GMR>Upd$ eye discs (Fig. S5). This is so because in $GMR>Upd$ discs Upd production behind the MF is very low in early stages (when the number of GMR-expressing cells is still small), but the effect of dilution is strongest at early stages because the area fold-increase is fastest initially. We conclude that the growth rates that are obtained with a continued ectopic expression

of Upd behind the MF are consistent with growth control by dilution.

Finally, we note that the data in a $\ln(k)$ versus L_P plot are also fitted reasonably well by straight lines, which would correspond to an exponential decline in the growth rate

$$k(L_P) = e^{-\delta(L_P - L_P(0))}, \quad (3)$$

as could result from the linear decay of a growth factor (Fig. 1H) (Vollmer et al., 2016). However, the fit is consistently worse, particularly for early and late data points (Fig. 1H; Fig. S6). In conclusion, an area-dependent decline fits the data slightly better than an exponential decline.

A quantitative analysis of the required Upd stability and spreading

Independently of dilution, all proteins decay over time. As a result of Upd degradation and dilution, we then have for the area growth rate

$$k(L_P) = k_0 \frac{T(0)}{T(L_P)} e^{-\delta(L_P - L_P(0))}, \quad (4)$$

where $\frac{T(0)}{T(L_P)}$ represents the dilution effect, and $e^{-\delta(L_P - L_P(0))}$ incorporates turnover of Upd at rate δ . We can use the constant speed of the MF (Wartlick et al., 2014) to convert the degradation rate to time (Vollmer et al., 2016). We measured the speed of the MF in $GMR>+$ eye discs as $7.3 \pm 2.1 \mu\text{m/h}$ (mean \pm s.d.) and in $GMR>Upd$ discs as $4.7 \pm 3.1 \mu\text{m/h}$ (Fig. 2A,B), which is similar to

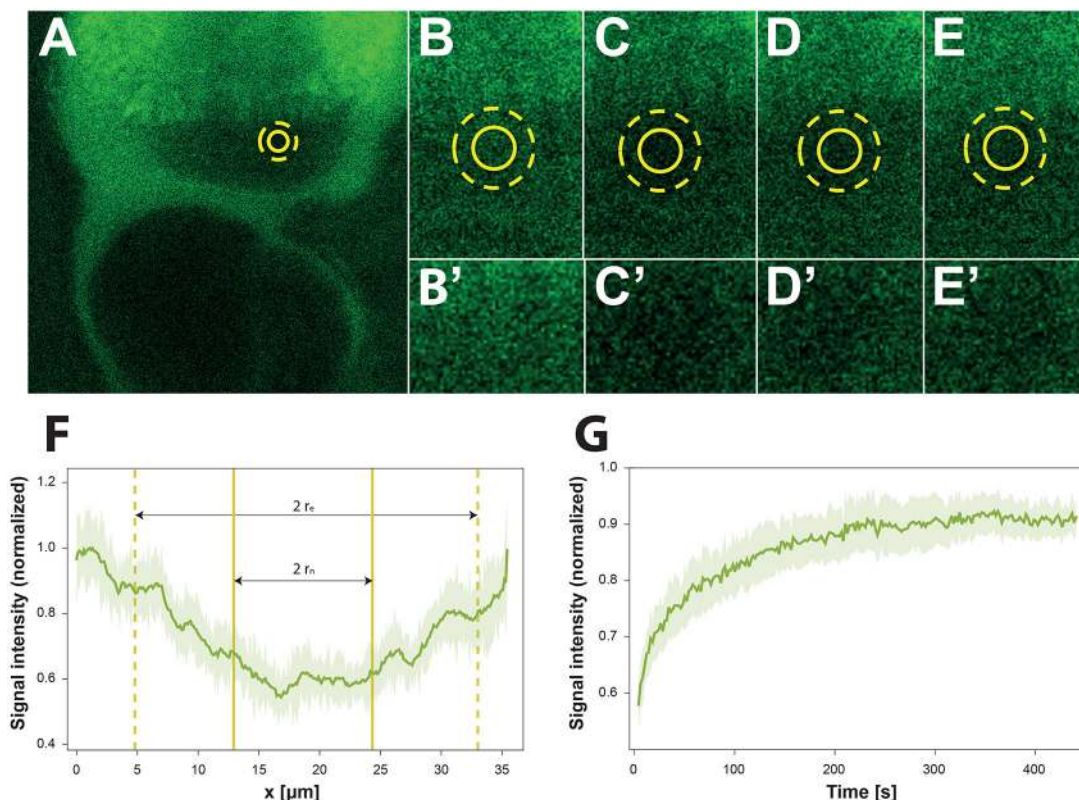


Fig. 3. The diffusion coefficient of Upd. (A) Eye imaginal disc from $GMR-GAL4; UAS-GFP:Upd$. The ROI selected for photobleaching is marked (yellow circle). (B-E') Magnification of ROI (photobleached area, solid circle in A) and the surrounding tissue (B-E) before photobleaching (B), directly after photobleaching (C), and at $t=100$ s (D) and $t=250$ s (E). Panels B'-E' show a magnification of the photobleached area (solid circles in panels B-E) at the same time points. (F) Normalized mean intensity of the bleached profile. $2r_n$, nominal diameter (ROI); $2r_e$, effective diameter. (G) Recovery dynamics of the mean GFP signal normalized to pre-bleached intensity. In F,G, shaded area shows s.e.m.

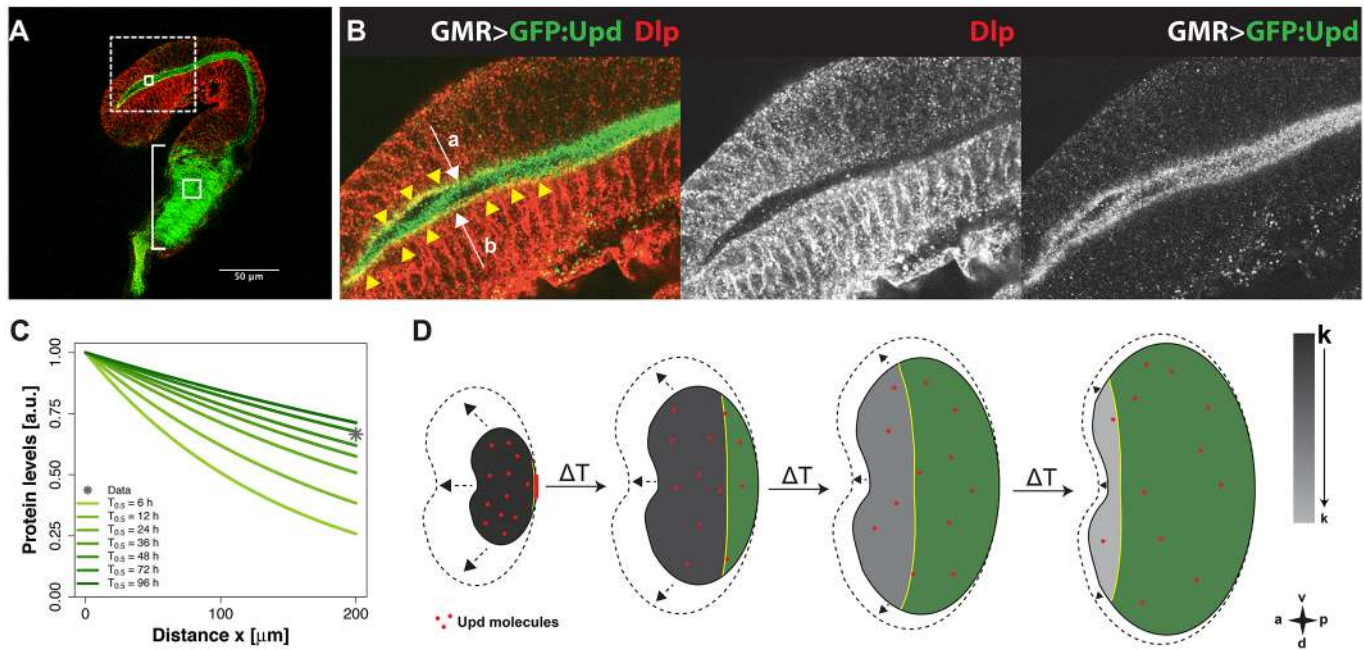


Fig. 4. Dispersal of Upd. (A) Confocal optical section of a *GMR>GFP:Upd* disc (GFP signal shown in green), additionally stained for the proteoglycan Dally-like protein (Dlp; red). The GFP:Upd-producing domain is indicated by the vertical bracket. The region marked by the dashed box is magnified in B. (B) Magnification of the disc shown in A. Arrows point from the basal (b) to the apical (a) sides of the epithelium. GFP:Upd is detected on the apical cell surface, colocalizing with Dlp (visualized as yellow signal; marked by yellow arrowheads), as well as in the apical luminal space. (C) Predicted steady-state gradients ($\frac{C}{C_0} = e^{-x/\lambda} = \sqrt{D/\delta}$) with the measured Upd diffusion coefficient, $D=0.7 \mu\text{m}^2/\text{s}$, for different Upd half-lives, $T_{0.5} [\delta=\ln(2)/T_{0.5}]$ (green lines). (D) Graphical summary of the dilution-based growth control mechanism. In the early stages of eye disc development, Upd molecules (red points) are produced at the posterior margin (red line) and spread over the small eye disc domain by diffusion. Upd production ceases at the onset of MF movement. As a result of the increase in the total eye disc area over time, the Upd concentration decreases by dilution. The growth rate, k , in the part anterior to the MF is directly proportional to the concentration of Upd (visualized from dark to light grey) and therefore declines inversely proportionally to the change in the total eye disc area. Thus, in the same time span, ΔT , the area increase is less, allowing the MF to catch up and terminate growth. Anterior is to the left, and posterior to the right. Green, posterior area; yellow, MF; dashed lines, growth within the next time step.

but slightly faster than previous reports (Wartlick et al., 2014). Because the linear movement of the MF can be explained with the dynamics of the Hedgehog (Hh)-Decapentaplegic (Dpp) patterning network (Fried et al., 2016), and *upd* mutants do not show patterning defects (Bach et al., 2003), MF movement was not expected to be strongly affected in *upd* mutants. With this MF speed, we require a Upd half-life, $T_{0.5} = \frac{\ln(2)}{\delta}$, of at least 24 h to obtain a good fit between measured and simulated eye disc growth (Fig. 2C–G). Growth was simulated using $\frac{dT}{dL_P} = k(L_P) \cdot A$ and the growth rate in Eqn. 4 (supplementary Materials and Methods) (Vollmer et al., 2016). For slower eye disc development, the required Upd half-life would be longer, and the minimal required Upd stability is therefore dictated by the slowest observed developmental progress. *Drosophila* eye disc development is substantially slowed down in eye discs that are grafted to adult female hosts, where they take almost 2 weeks to achieve their final size (Garcia-Bellido, 1965) (Fig. 2H). The dilution-based growth mechanism recapitulates the observed growth kinetics with an Upd half-life of 24 h reasonably well, but a better fit is obtained with a half-life of 48 h (Fig. 2H; supplementary Materials and Methods).

To establish the effective Upd turnover rate, δ , we measured the dispersion of GFP-tagged Upd (Tsai and Sun, 2004) and its effective diffusion coefficient, D , using fluorescence recovery after photobleaching (FRAP) in eye discs of *GMR>Upd-GFP* larvae. The FRAP-measured Upd diffusion coefficient, $D=0.7 \mu\text{m}^2/\text{s}$

(Fig. 3; see Materials and Methods and supplementary Materials and Methods for details), is higher than previously published FRAP-measured diffusion coefficients for other diffusible molecules in the ECM of *Drosophila* imaginal discs (0.04 – $0.1 \mu\text{m}^2/\text{s}$) (Kicheva et al., 2007). To determine a lower boundary on the Upd half-life, we compared the experimentally observed spreading when GFP:Upd is ectopically expressed in different parts of the eye disc (Fig. 4A,B; Fig. S7) with the expected steady state Upd concentration profiles for an effective diffusion coefficient of $0.7 \mu\text{m}^2/\text{s}$ and different half-lives (Fig. 4C). In agreement with previous reports (Zhang et al., 2013), we observe Upd-GFP to be essentially uniformly dispersed over a distance of about $100 \mu\text{m}$ from its expression domain and to decline to about two-thirds of its value in the source within $200 \mu\text{m}$ from its expression domain (Fig. 4A,C; Fig. S7). The observed shallow gradients all lie above the Upd gradient that would be expected with a half-life of 24 h, and the high Upd concentration at a long distance from the source is best approximated with a half-life of >60 h (Fig. 4C, star). Such an Upd half-life is sufficient to reproduce all measured growth data, including that obtained in *Drosophila* larvae (Fig. 2C–G), and for grafted eye discs (Garcia-Bellido, 1965) (Fig. 2H; supplementary Materials and Methods). It is also consistent with previous reports in which Upd was detected more than 24 h after its production had ceased (Zhang et al., 2013). We note that given the long half-life of the Upd protein, FRAP-based protein stability measurements must be expected to provide underestimates because of bleaching.

Finally, we note that the localization of Upd mainly in the apical ECM (Fig. 4B) (Harrison et al., 1998; Hombria et al., 2005; Zhang et al., 2013) also means that Upd will not be lost by diffusion out of the eye disc over time, as the apical cell side faces a closed luminal space. This would not be the case if Upd was secreted to the basal ECM.

Conclusion

We provide evidence that the cytokine Upd fulfils not only qualitatively, but also quantitatively, all requirements for area-dependent growth control of the eye disc based on its rate of dilution. Temporal and spatial changes in the expression of a Upd gene can modulate wing size in wasps (Loehlin and Werren, 2012), suggesting that Upd's role in controlling final organ size might be conserved beyond fruit flies. Variations in the initial amount of Upd could then explain the natural variation in eye size in different dipteran species. Open questions still remain. In particular, although declining growth rates are found throughout developing systems (Grunert et al., 2015; Ricklefs, 2010), the area-dependent growth law that explains the growth kinetics of the *Drosophila* eye discs does not fit the growth data available for the wing disc (Vollmer and Iber, 2016). Therefore, alternative growth control mechanisms need to have evolved as well.

MATERIALS AND METHODS

Drosophila strains

Oregon-R (Or-R) is a wild-type strain (FlyBase: <http://flybase.org>). GMR-Upd is a transgenic line in which the GMR-enhancer is linked directly to the *upd* cDNA (Bach et al., 2003). Gene expression manipulation was carried out using the GAL4/UAS system (Brand and Perrimon, 1993). The GAL4 strains used were: *GMR-GAL4* ('GMR>'; FlyBase identifier: FBgn0020433), *ey-GAL4* ('ey>'; FlyBase identifier: FBtp0012213), *optix2/3-GAL4* ('optix>'; Ostrin et al., 2006) and *dpp-GAL4* (FlyBase identifier: FBti0002123). UAS-strains used were: *UAS-Upd* ('>Upd'; Harrison et al., 1998), *UAS-GFP:Upd* ('>GFP:Upd'; Tsai and Sun, 2004), *UAS-domeΔCYT*, on either chromosome II or III ['>dome(II)' and '>dome(III)', respectively; Brown et al., 2001] and *UAS-dsSTAT92E* ('>dsSTAT'; VDRC stock: 43867). All flies were raised on standard media at 25°C unless stated otherwise. All data presented are from female flies/larvae if not stated otherwise.

Antibody staining, fixation and imaging

Eye imaginal discs were dissected and fixed according to standard protocols (Casares and Mann, 2000). Rabbit anti-aPKC (Abcam AB5813, 1:500), rabbit anti-GFP (Molecular Probes, A11122, 1:1000) and mouse anti-Dlp (13G8; Developmental Studies Hybridoma Bank, 1:5) were used as primary antibodies. Secondary antibodies used were Alexa Fluor 555-conjugated goat anti-rabbit, Alexa Fluor 488-conjugated goat anti-rabbit and Alexa Fluor 568-conjugated goat anti-mouse (Molecular Probes, A21428, A11034 and A11031, respectively; all 1:400). Stained discs were mounted with spacers to prevent flattening and were imaged using a Leica TCS SPE microscope.

Image analysis

Image analysis was performed as described by Vollmer et al. (2016). See supplementary Materials and Methods for details.

Computational analysis

Models were simulated and optimized in Matlab R2016a using a forward Euler scheme as described by Vollmer et al. (2016). See supplementary Materials and Methods for details.

Fluorescence recovery after photobleaching (FRAP)

We followed the same approach as Fried et al. (2016) and we provide a copy of the description in supplementary Materials and Methods. In short, the region of interest (ROI; Fig. 3A) was photobleached using a 488 nm argon

laser and recovery was recorded. Following Kang et al. (2012), the diffusion coefficient was calculated as $D_{Upd} = 0.67 \pm 0.19 \mu\text{m}^2 \text{s}^{-1}$.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

D.I. and F.C. conceived the study. All authors designed the experiments. F.C., J.V., D.A.-H., M.S.-A. and A.I. acquired experimental data. J.V., F.C., P.F., D.A.-H. and M.S.-A. analysed data. J.V., P.F. and D.I. developed and analysed the model. D.I., F.C. and J.V. wrote the manuscript.

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Supplementary information

Supplementary information available online at <http://dev.biologists.org/lookup/doi/10.1242/dev.141309.supplemental>

References

- Abramoff, M. D., Magalhães, P. J. and Ram, S. J. (2004). Image processing with ImageJ. *Biophotonics Int.* **11**, 36–42.
- Bach, E. A., Vincent, S., Zeidler, M. P. and Perrimon, N. (2003). A sensitized genetic screen to identify novel regulators and components of the *Drosophila* janus kinase/signal transducer and activator of transcription pathway. *Genetics* **165**, 1149–1166.
- Bach, E. A., Ekas, L. A., Ayala-Camargo, A., Flaherty, M. S., Lee, H., Perrimon, N. and Baeg, G.-H. (2007). GFP reporters detect the activation of the *Drosophila* JAK/STAT pathway in vivo. *Gene Expr. Patterns* **7**, 323–331.
- Brand, A. H. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401–415.
- Brown, S., Hu, N. and Hombria, J. C.-G. (2001). Identification of the first invertebrate interleukin JAK/STAT receptor, the *Drosophila* gene *domeless*. *Curr. Biol.* **11**, 1700–1705.
- Casares, F. and Mann, R. S. (2000). A dual role for homothorax in inhibiting wing blade development and specifying proximal wing identities in *Drosophila*. *Development* **127**, 1499–1508.
- Coleman, T. F. and Li, Y. (1996). An interior trust region approach for nonlinear minimization subject to bounds. *SIAM J. Optim.* **6**, 418–445.
- Fried, P., Sánchez-Aragón, M., Aguilar-Hidalgo, D., Lehtinen, B., Casares, F. and Iber, D. (2016). A model of the spatio-temporal dynamics of *Drosophila* eye disc development. *PLoS Comput. Biol.* **12**, e1005052.
- García-Bellido, A. (1965). Larvalentwicklung transplanterter Organe von *Drosophila melanogaster* im Adultmilieu. *J. Insect Physiol.* **11**, 1071–1078.
- Grunert, L. W., Clarke, J. W., Ahuja, C., Eswaran, H. and Nijhout, H. F. (2015). A quantitative analysis of growth and size regulation in *manduca sexta*: the physiological basis of variation in size and age at metamorphosis. *PLoS One* **10**, e0127988.
- Hariharan, I. K. (2015). Organ size control: lessons from *Drosophila*. *Dev. Cell* **34**, 255–265.
- Harrison, D. A., McCoon, P. E., Binari, R., Gilman, M. and Perrimon, N. (1998). *Drosophila* unpaired encodes a secreted protein that activates the JAK signaling pathway. *Genes Dev.* **12**, 3252–3263.
- Hombria, J. C.-G., Brown, S., Häder, S. and Zeidler, M. P. (2005). Characterisation of Upd2, a *Drosophila* JAK/STAT pathway ligand. *Dev. Biol.* **288**, 420–433.
- Juni, N., Awasaki, T., Yoshida, K. and Hori, S. H. (1996). The Om (1E) mutation in *Drosophila ananassae* causes compound eye overgrowth due to transposon-driven overexpression of a novel gene. *Genetics* **143**, 1257–1270.
- Kang, M., Day, C. A., Kenworthy, A. K. and DiBenedetto, E. (2012). Simplified equation to extract diffusion coefficients from confocal FRAP data. *Traffic* **13**, 1589–1600.
- Kicheva, A., Pantazis, P., Bollenbach, T., Kalaidzidis, Y., Bittig, T., Jülicher, F. and González-Gaitán, M. (2007). Kinetics of morphogen gradient formation. *Science* **315**, 521–525.

- Loehlin, D. W. and Werren, J. H.** (2012). Evolution of shape by multiple regulatory changes to a growth gene. *Science* **335**, 943-947.
- Mirth, C. K. and Shingleton, A. W.** (2012). Integrating body and organ size in *Drosophila*: recent advances and outstanding problems. *Front. Endocrinol.* **3**, 49.
- Neto, M., Aguilar-Hidalgo, D. and Casares, F.** (2016). Increased avidity for Dpp/BMP2 maintains the proliferation of progenitors-like cells in the *Drosophila* eye. *Dev. Biol.* **418**, 98-107.
- Ostrin, E. J., Li, Y., Hoffman, K., Liu, J., Wang, K., Zhang, L., Mardon, G. and Chen, R.** (2006). Genome-wide identification of direct targets of the *Drosophila* retinal determination protein Eyeless. *Genome Res.* **16**, 466-476.
- Ricklefs, R. E.** (2010). Embryo growth rates in birds and mammals. *Funct. Ecol.* **24**, 588-596.
- Staehling-Hampton, K., Jackson, P. D., Clark, M. J., Brand, A. H. and Hoffmann, F. M.** (1994). Specificity of bone morphogenetic protein-related factors: cell fate and gene expression changes in *Drosophila* embryos induced by decapentaplegic but not 60A. *Cell Growth Differ.* **5**, 585-593.
- Tsai, Y.-C. and Sun, Y. H.** (2004). Long-range effect of upd, a ligand for Jak/STAT pathway, on cell cycle in *Drosophila* eye development. *Genesis* **39**, 141-153.
- Vollmer, J. and Iber, D.** (2016). An unbiased analysis of candidate mechanisms for the regulation of *Drosophila* wing disc growth. *Sci. Rep.* **6**, 39228.
- Vollmer, J., Fried, P., Sánchez-Aragón, M., Lopes, C. S., Casares, F. and Iber, D.** (2016). A quantitative analysis of growth control in the *Drosophila* eye disc. *Development* **143**, 1482-1490.
- Wartlick, O., Jülicher, F. and Gonzalez-Gaitan, M.** (2014). Growth control by a moving morphogen gradient during *Drosophila* eye development. *Development* **141**, 1884-1893.
- Wolff, T. and Ready, D. F.** (1991). The beginning of pattern formation in the *Drosophila* compound eye: the morphogenetic furrow and the second mitotic wave. *Development* **113**, 841-850.
- Wright, V. M., Vogt, K. L., Smythe, E. and Zeidler, M. P.** (2011). Differential activities of the *Drosophila* JAK/STAT pathway ligands Upd, Upd2 and Upd3. *Cell. Signal.* **23**, 920-927.
- Zhang, Y., You, J., Ren, W. and Lin, X.** (2013). *Drosophila* glypicans Dally and Dally-like are essential regulators for JAK/STAT signaling and Unpaired distribution in eye development. *Dev. Biol.* **375**, 23-32.