Activated STAT regulates growth and induces competitive interactions independently of Myc, Yorkie, Wingless and ribosome biogenesis

Aloma B. Rodrigues1, Tamara Zoranovic1, Aidee Ayala-Camargo1, Savraj Grewal2, Tamara Reyes-Robles1, Michelle Krasny1, D. Christine Wu3, Laura A. Johnston4 and Erika A. Bach1,∗

SUMMARY

Cell competition is a conserved mechanism that regulates organ size and shares properties with the early stages of cancer. In Drosophila, wing cells with increased Myc or with optimum ribosome function become supercompetitors that kill their wild-type neighbors (called losers) up to several cell diameters away. Here, we report that modulating STAT activity levels regulates competitor status. Cells lacking STAT become losers that are killed by neighboring wild-type cells. By contrast, cells with hyper-activated STAT become supercompetitors that kill losers located at a distance in a manner that is dependent on hid but independent of Myc, Yorkie, Wingless signaling, and of ribosome biogenesis. These results indicate that STAT, Wingless and Myc are major parallel regulators of cell competition, which may converge on signals that non-autonomously kill losers. As hyper-activated STATs are causal to tumorigenesis and stem cell niche occupancy, our results have therapeutic implications for cancer and regenerative medicine.

KEY WORDS: JAK/STAT, Myc, Cell competition

INTRODUCTION

During development, local interactions between cells influence their growth and their ability to contribute to the adult. The process of cell competition was first reported in studies in the Drosophila wing disc of Minutes (M), dominant mutations in ribosomal protein (Rp) genes that are homozygous lethal but when heterozygous yield viable animals that are slow to develop (Morata and Ripoll, 1975; Simpson, 1979; Simpson and Morata, 1981). Despite being viable in a homotypic environment, M/+ clones are not recovered in the adult when grown in the presence of wild-type cells. These seminal studies established that wild-type and M/+ cells compete for space within a compartment of the wing, that competition between them is local, and that death of M/+ cells is associated with proliferation of wild-type cells. However, the final size of the wing remained normal despite the fact that wild-type cells filled large parts of a compartment. Thus, the wild-type cells (termed ‘winners’) occupy the space of the M/+ ‘losers’, which they have eliminated through cell death. Recent work has established that M/+ losers die primarily through apoptosis and most death occurs at the clone boundary with wild-type cells (de la Cova et al., 2004; Moreno and Basler, 2004). Clonal growth assays in the Drosophila wing disc have revealed that cells with increased Myc become ‘supercompetitors’ that can kill losers at a distance – up to 10 cells away (de la Cova et al., 2004; Moreno and Basler, 2004). The fact that Myc supercompetitors lose their ability to out-compete wild-type cells when they are heterozygous for a Minute mutation [M(2)60E] (Moreno and Basler, 2004) suggests that the ability of Myc to act as a supercompetitor stems from its effects on ribosome function. Pathways that feed into Myc regulation, such as the Hippo pathway, have also been shown to be involved in competition (Tyler et al., 2007; Neto-Silva et al., 2010; Ziosi et al., 2010).

Recently, a Myc-independent mechanism of supercompetition has been reported. Cells lacking Wingless (Wg) signaling are out-competed but survive if they are placed in the context of cells that are growth impaired. Furthermore, cells with elevated Wg signaling eliminate their wild-type neighbors by secreting Notum, a phospholipase that inhibits Wg signaling in surrounding cells and as such become ‘supercompetitors’ (Vincent et al., 2011). This type of competition does not fit the classical definition as changes in Wg signaling lead to altered patterning of discs and appendages, and Wg loss- or gain-of-function cells cannot give rise to viable adults. However, this work is evidence that competitive events take place without directly involving ribosome function but as a consequence of developmental signaling pathway activation, and that cells are able to compare their ‘fitness’ to their neighbors by measuring relative signaling activity.

In the past decade, the field of cell competition has exploded, and while it is appreciated that several genes and pathways regulate the process of cell competition, a consensus on definitions for each
type of competitive interaction has not yet been achieved. For clarification, we will use the term ‘cell competition’ to mean the context-specific behavior of cells of a particular genotype: they are out-competed when surrounded by wild-type cells but viable when placed in the context of slower-growing cells (e.g. M/+). We will use the term ‘supercompetitor’ to mean a clone of cells overexpressing a particular factor that causes neighboring wild-type cells to experience a growth disadvantage.

It is not clear what the relationship between growth-promoting pathways and cell competition is. It is known that JAK/STAT signaling is a central regulator of growth/eye size in Drosophila, and we asked whether it was involved in competitive interactions as well. Stat92E, the sole STAT transcription factor in Drosophila, is a central regulator of eye size. In Drosophila, the cytokine Unpaired (Upd; Os – FlyBase) activates the receptor Domeless (Dome), which leads to the activation of the sole Janus kinase Hopscotch (Hop) and Stat92E, which translocates to the nucleus and modulates expression of its target genes (Arbouzova and Zeidler, 2006). Sustained activation of Stat92E causes dramatic overgrowth of the eye, fly leukaemia and germ-cell tumors (Hanratty and Dearolf, 1993; Kiger et al., 2001; Bach et al., 2003). Although these gain-of-function phenotypes implicate the JAK/STAT pathway in growth control and possibly in cell competition, the cell-autonomous requirement for Stat92E in these processes has not been elucidated. Here, we show that the JAK/STAT pathway is a crucial regulator of growth during early eye and wing development through competitive interactions: cells lacking Stat92E are out-competed by wild-type neighbors and eliminated by apoptosis. Furthermore, cells with sustained activation of Stat92E become winners, manifest supercompetitor characteristics and kill losers located several cell diameters away through non-autonomous induction of apoptosis. Finally, we provide strong evidence supporting the conclusion that JAK/STAT pathway activity does not require Myc, Yorkie (Yki), Wg or Decapentaplegic (Dpp) signaling or de novo ribosomatic biogenesis for its regulation of growth and competitive interactions between cells.

**Materials and Methods**

**Fly stocks**

These stocks are described in Flybase: Stat92E85C9, Stat92E397, dm4, lgl4, UAS-P35, UAS-hop, UAS-hopTun1, UAS-Myc, UAS-upd, en-gal4, fj-lacZ, ex-lacZ, hid-664, upd(f132)-gal4, dome-gal4, 10xSTAT-gfp, wg2 (wg-lacZ), UAS-Arm630 and UAS-TCP59. 

**Clonal analysis**

Two- to three-hour timed embryo collections were made on yeasted molasses food vials and raised at 25°C. Embryos were processed as described previously (Bach et al., 2007). Clones were induced by heat-shocking at specific times after egg deposition (AED) at 39°C and discs were dissected, fixed and stained at 115±2 hours AED or as noted. The MARCM technique was used to generate GFP-expressing Stat92E85C9 clones. The sibling clone was detected by CD2 as described (de la Cova et al., 2004). For the tub>Myc and null-Myc assays, 3-hour timed embryo collections were made, and clones were induced by heat-shocking at 48 hours AED at 37°C for 15 minutes. Wing discs were dissected at 68 hours after clone induction (AED). For the null-Myc assays, wing discs were dissected at 110 hours AED, dm4 FRT82B; tub>Myc y>gal4 UAS-gfp; UAS-hop48A/+ animals exhibited strong developmental delays. For the quantitative PCR assays, 2-hour timed embryo collections were made and clones were induced by heat-shocking at 102 hours AED at 39°C for 1 hour, and wing disc were dissected 16 hours AED. These stocks were used for clonal analyses: hs-flp122, FRT82B arm-lacZ/TM6c and hs-flp122, FRT82B Minute 96(C) ubi-gfp/TM6B. For the two-clone assay hs-flp122, tub-gal4, UAS-gfp; FRT82B tub-gal80 hs-Cd2 y>TM6B and hs-flp122, tub-gal4, UAS-gfp; FRT420 CD71 tub-gal80/Cyo; TM2/TM6B were used.

**Immunofluorescence**

We used antibodies specific for: β-galactosidase [1:50; Developmental Studies Hybridoma Bank (DHSB) or 1:2000; Cappel]; cleaved Caspase 3 (1:100; Cell Signaling); rat CD2 (1:500; Serotec); Fibrillin1 (1:500; EnCor); Stat92E (1:50); Myc (1:200; a gift from Gines Morata, Centro de Biologia Molecular Severo Ochoa, Madrid, Spain); phospho-JNK (1:100; Promega, v57938); Vestigial (Vg) (1:200; a gift from Sean Carroll, University of Wisconsin, Madison, WI, USA); Nubbin (Nub, 1:10); Teashirt (Tsh, 1:500); Engrailed (En, 1:100; DHSB); Wg (1:10; DHSB); Discs large (Dlg, 1:50; DHSB); Crumbs (Crb, 1:50; DHSB); Yorkie (Yki, 1:100); pSma(1:1250; a gift from Ed Lauffer, Columbia University, New York, NY, USA); Distal-less (Dll, 1:50); Sparse (1:300); and Homothorax (Hh, 1:2000). Alexa Fluor 546 Phalloidin was used 1:100 (Molecular Probes). Secondary antibodies (Molecular Probes and Jackson Immunologicals) were used at 1:200. Discs were fixed for 20 minutes in 4% formaldehyde (for β-galactosidase stains) or 4% paraformaldehyde (for all other stains) and were processed as described (Flaherty et al., 2010). Images were captured on an LSM510 Zeiss confocal microscope at 25×, 63× or 100×.

**Quantitation of clone areas**

The areas of clones were measured by ImageJ. y-axis error bars represent s.e.m. in Fig. 21, s.d. in Fig. 3D, Fig. 4C and Fig. 5C. Statistical significance was determined by the Student’s t-test.

**Quantitative real-time PCR**

Quantitative PCR was carried out on 20 wing discs in triplicate carrying clones misexpressing gfp, hop or Myc as described (Grewal et al., 2007). Primer sequences are as follows: Nop53 (F, AGGAGCTGATGCT-GCTTAT7; R, AATGTCACACTGTTACACT), Nop60B (F, CTTCTTG TAGGCGCTGGTG; R, GACTAAACCGTGCAACACT), Tif-1A (F, GTAGCGGAAGACGGCAAGG; R, AATGGCAATGTCCG-GTGT), dome (F, CCGAGTTCGGACTCTCACC; R, GATCGAC-TGCGAGATT) and chinino (F, CAGTGCCAATAGGCTAATG; R, TCAAGTTCTCCAGCTTAC).
act5c>stop>lacZ flip-out cassette (Struhl and Basler, 1993). Flp, expressed under the control of the upd locus, will excise the ‘stop’ from the flip-out cassette and allow expression of lacZ by the act5c promoter. Any cell that produces upd will be heritably marked with lacZ expression.

 upd-expressing cells populate nearly all regions of the disc, including the pouch (Fig. 1H,H'). Very few cells were labeled with β-galactosidase when the UAS-flp transgene was excluded from the analysis, indicating that there was no spurious activity of flp on the flip-out cassette (supplementary material Fig. S1A,A' and not shown). By contrast, cells expressing Nub or Tsh, other key transcription factors in wing development, display restricted lineages and populate only the pouch or notum/hinge, respectively (Zirin and Mann, 2007). Taken together, these data indicate that the JAK/STAT pathway is activated and functions broadly and evenly during early wing development (i.e. before 60 hours AED), when exponential growth occurs (Neto-Silva et al., 2009).
Stat92E is required for clonal growth during early disc development

As Upd and JAK/STAT signaling are induced early during eye and wing development (Fig. 1C) (Ekas et al., 2006), we sought to address the cell-autonomous requirement of Stat92E in clonal growth. We induced Stat92E clones and their wild-type sibling clones by mitotic recombination at 30, 36 and 48 hours AED in timed-collections and measured clone size as a percentage of the total clone area at 115 hours AED in wing and eye discs. As disc cells are epithelial and remain associated after mitosis, differences in clone size reflect differential growth rates (Neufeld et al., 1998). We used Stat92E85C9 and Stat92E397 (not shown), which are functionally amorphic alleles (Ekas et al., 2010), and obtained similar results for both. If Stat92E were not required for clonal growth, Stat92E clone areas would be ~50% of the total clone area. However, when induced early in wing development (at 30 and 36 hours AED), Stat92E85C9 clones displayed severe growth disadvantages when compared with their sibling clones regardless of clone location along the anterior-posterior (AP) or dorsal-ventral (DV) axes (Fig. 2A,B,D,E, arrowheads for Stat92E clones, arrows for sibling clones) and comprised only ~5% of the total clone area in the disc (Fig. 2I; supplementary material Fig. S2A). By contrast, control FRT82B wild-type clones and their sibling clones grew to equal sizes and were each ~50% of the total clone area (Fig. 2G-I; supplementary material Fig. S2A). The growth disadvantage of Stat92E clones persisted but weakened during development. Stat92E clones induced at 48 hours AED were only ~16% of the total clone area (Fig. 2C,F,I; supplementary material Fig. S2A,B). At the 48 hour time-point, Stat92E clones located anterior to the morphogenetic furrow or in the wing hinge (Fig. 2F,C) appeared to grow less well than clones located elsewhere in the same disc, but differences in clone sizes were not statistically significant (supplementary material Fig. S2B-D). We also noted that Stat92E clones in the notum induced at 48 hours AED tended to be larger than clones residing elsewhere in the disc (P<0.0073) (Fig. 2C; supplementary material Fig. S2B-D). Taken together, our results indicate that Stat92E has an essential growth requirement in all eye and wing cells in early disc development.

Cells lacking JAK/STAT pathway activity become losers

We found that poorly growing cells in Stat92E85C9 clones were apoptotic and basally extruded (Fig. 3A,A′,A″, green arrowheads; 3B,B′,B″, yellow arrowheads), in stark contrast to their faster-growing wild-type sibling clones (Fig. 3A,A′, red arrows). Expressing the caspase inhibitor P35 within Stat92E85C9 clones rescued their growth such that they grew to equal sizes as their

---

**Fig. 2. Stat92E is required for clone survival.**

(A-F) Stat92E85C9 clones (arrowheads) exhibit strong growth defects compared with their sibling clones (arrows). Stat92E85C9 clones were induced at 30 (A,D), 36 (B,E) or 48 (C,F) hours AED and analyzed in wing discs (A-C) and eye disc (D-F) at 115 hours AED. (G,H) Wild-type FRT82B control clones (arrowheads) and sibling clones (arrows), induced at 30 (G) or 48 (H) hours and analyzed in wing discs at 115 hours AED, grow to similar sizes. (A-H) Clones lack β-Gal and appear black, whereas sibling clones have two copies of β-Gal and appear white. (I) The area of Stat92E85C9 or FRT82B control clones expressed as a percentage of the total clone area (clone + sibling) per disc. Clones were induced at the times indicated and analyzed in eye and wing discs (for Stat92E clones) and in wing discs (for FRT82B controls). There were greater than 50 sibling-clone pairs for each time point. The entire sibling clone (+/+ or Stat92E or FRT82B (+/-) clone areas were measured in each disc and the standard error was calculated. Percentage area of sibling clones in eye discs (magenta bars) or wing discs (light-blue bars). Percentage area of Stat92E clones in eye discs (dark-purple bars) or wing discs (dark-blue bars). Percentage area of FRT82B sibling clones (light-green bars) or FRT82B control clones (dark-green bars) in wing discs.
siblings, confirming that the reduced growth of Stat92E85C9 clones was due to cell death (Fig. 3C,D). The cell death in Stat92E clones could be due to an autonomous requirement for Stat92E in cell viability or a competitive disadvantage in cells lacking Stat92E. To distinguish between these possibilities, we provided Stat92E clones with a growth advantage by inducing them in a Minute background. Stat92E85C9 clones induced at 30 hours AED in a Minute background grew well and filled large parts of the compartment in which they arose (Fig. 3E, arrowhead). We note that we cannot exclude the possibility that Stat92E+ cells competed against M/+ cells. Nevertheless, Stat92E-deficient cells exhibit the same context-specific behavior (i.e. that they are eliminated by apoptosis in normal tissue but are able to contribute to tissue growth when placed with poorly growing cells) that was observed for cell interactions induced by differences in Wg signaling (Vincent et al., 2011).

To date, few molecular markers have been reported for cell competition. In some instances, JNK-dependent death and Sparc expression have been described in loser cells (Moreno et al., 2002; Portela et al., 2010). We found that activated JNK is not detected in Stat92E85C9 clones (Fig. 3F), suggesting that they are eliminated through a JNK-independent pathway. By contrast, phospho-JNK was readily detected in lgl clones, consistent with a prior report (supplementary material Fig. S3A-B”) (Tamori et al., 2010). Sparc was reported to be a marker for a broad range of loser cells, including low-Myc cells and lgl clones (Portela et al., 2010). We did not observe Sparc in Stat92E85C9 clones (supplementary material Fig. S3C”). However, we also did not observe Sparc in low-Myc cells or lgl clones (supplementary material Fig. S3D-E”). These results suggest that Sparc is not a general marker for losers. Nevertheless, the fact that the growth of Stat92E clones is rescued by expression of P35, as is that of other loser cells (Li and Baker, 2007), suggests that cells lacking Stat92E are out-competed through the induction of apoptosis.

**Cells with hyper-activated Stat92E become supercompetitors**

To determine whether the cell-autonomous growth-promoting effects of Stat92E could lead to competition with neighboring cells, we used a clonal growth assay that serves as a direct measurement of cell competition and is predicated on the fact that wild-type cells neighboring supercompetitors will be at a growth disadvantage compared with the wild-type neighbors of control cells (de la Cova et al., 2004; Wu and Johnston, 2010). The two-clone assay was used to generate two different daughter cells from a single parent cell: a ‘Gal4 clone’ expressing a gene under the control of a UAS promoter and UAS-gfp, and a ‘sibling clone’ marked with CD2. We compared the growth rates of Gal4 clones, which overexpress only
clones were eliminated by apoptosis, we monitored activation of a severe growth disadvantage. To examine whether Hop Sibling clones neighboring Hop Gal4 clones suffer were ~50% smaller (\(P<10^{-6}\)) than that of the siblings of Control Gal4 clones (i.e. Control Sibling clones) and the growth rates of Hop Gal4 clones, marked by GFP (arrowhead) grow to equal sizes as Control Sibling clones, marked by CD2 in red (arrow). (B) GFP\(^{+}\) Hop Gal4 clones (arrowhead) grow to larger sizes than Hop Sibling clones in red (arrow). (C) Median area of Gal4 clones (green bars) and of sibling clones (red bars). The median areas in pixels\(^2\) were: 4951±294 (Control Gal4) and 4578±317 (Control Sibling), \(n=39\) clone pairs; 6276±676 (Hop Gal4) and 2339±369 (Hop Sibling), \(n=33\) clone pairs. Hop Gal4 clones are significantly larger than Control Gal4 clones (\(P<0.037\)). Hop Sibling clones are significantly smaller than Control Sibling clones (\(**P<10^{-6}\)). (D, D') Neither cells in Control Gal4 clone (green cells) nor neighboring wild-type cells contain activated Caspase 3 (Cas-3, magenta). (E, E') A Hop Gal4 clone (green cells) induces cell death (activated Caspase 3, magenta) in surrounding wild-type cells (E', red arrows). Mis-expression of Hop also induces apoptosis within the Hop Gal4 clone itself (E', green arrow). (F, F') A Upd Gal4 clone (green cells, outlined in F') induces cell death (activated Caspase 3, blue) in surrounding wild-type cells (F', arrow). Mis-expression of Upd also induces apoptosis within the Upd Gal4 clone itself (i.e. within the broken red line in F'). Engrailed (En, red) marks posterior cells.

\(UAS\text{-}gfp\) (called Control Gal4), with that of their sibling clones (called Control Sibling) and the growth rates of Hop Gal4 clones, which express a constitutively activated form of Hop \(UAS\text{-}hop\text{Tum-1}\) and \(UAS\text{-}gfp\), with their sibling clones (called Hop Sibling). The growth rates of Control Gal4 clones and Control Sibling clones were similar (Fig. 4A,C). By contrast, Hop Gal4 clones grew significantly faster and were ~25% larger than Control Gal4 clones (\(P<0.037\)) (Fig. 4B,C), consistent with a role for activated Stat92E in cell-autonomous growth. The increase in Hop Gal4 clone size is not due to an increase in cellular volume since cells overexpressing Hop were approximately the same size as cells overexpressing GFP (supplementary material Fig. S4A,B,E), a result that is in stark contrast to Myc, which increases cell size when misexpressed (not shown) (Johnston et al., 1999). These data suggest that hyper-activation of the JAK/STAT pathway increases clone size by augmenting both cellular growth and mitosis, a conclusion supported by cell cycle analysis. Posterior cells with sustained Stat92E activation (\(en>hop\)) had the same cell cycle distribution as posterior cells expressing only GFP (\(en>gfp\)) (supplementary material Fig. S4F). By contrast, mis-expressing Myc (\(en>Myc\)) led to an increased percentage of cells in G2, as previously reported (supplementary material Fig. S4F) (Johnston et al., 1999). These observations indicate that Stat92E is a balanced growth regulator that jointly accelerates cellular growth and proliferation, consistent with our previous findings in eye disc (Bach et al., 2003).

We predicted that if Hop Gal4 clones induce cell competition, the area of their sibling clones (called Hop Sibling) would be smaller than that of the siblings of Control Gal4 clones (i.e. Control Sibling clones). Indeed, we found that Hop Sibling clones grew poorly and were ~50% smaller (\(P<10^{-6}\)) than Control Sibling clones (Fig. 4A-C), indicating that sibling clones neighboring Hop Gal4 clones suffer a severe growth disadvantage. To examine whether Hop Sibling clones were eliminated by apoptosis, we monitored activation of Caspase 3. Activated Caspase 3 was observed in wild-type cells located several cell diameters away from the boundary with Hop Gal4 clones (Fig. 4E', red arrows), a distinguishing feature of cells undergoing competitive stress within the same compartment (de la Cova et al., 2004; Moreno and Basler, 2004). We did observe varying degrees of death in wild-type cells in the vicinity of Hop Gal4 clones (\(n=30\)). In ~50% of these discs, we observe a cluster of wild-type cells undergoing programmed cell death as in Fig. 4E. In about 30%, we see only a few Caspase 3-positive wild-type cells; in 20% of discs we observed little or no death in nearby wild-type cells (not shown). The variation in death of wild-type cells neighboring Hop Gal4 supercompetitors is consistent with Caspase 3 activation being a snapshot of a long process of elimination of losers from the epithelium. Apoptosis was also seen within Hop Gal4 clones (Fig. 4E', green arrow), a result that has been reported for other growth regulators and for \(axin\) mutant supercompetitor clones (de la Cova et al., 2004; Vincent et al., 2011). By contrast, Caspase 3 activation was not observed in Control Gal4 clones or in the cells surrounding them (Fig. 4D'). Additionally, clonal overexpression of the JAK/STAT ligand Upd, a noted mitogen, resulted in autonomous (Fig. 4F', inside red line) and non-autonomous (Fig. 4F', arrow) cell death, further validating that hyper-activation of the JAK/STAT pathway results in competitive interactions leading to death of neighboring wild-type cells.

To prove that the non-autonomous cell death observed in Hop Sibling clones is specifically due to the competitive stress exerted by neighboring Hop Gal4 clones, we mis-expressed Hop throughout the posterior compartment of the wing disc (\(en>hop\)) and monitored apoptosis in anterior cells. Anterior cells in \(en>hop\) discs should not be subjected to competition by posterior cells that have higher levels of Hop because compartment boundaries insulate cells from competition (Simpson and Morata, 1981; de la Cova et al., 2004). Consistent with these predictions, we did not detect activated Caspase 3 in anterior cells in \(en>hop\) discs (supplementary material Fig. S4C,D). Some apoptosis was observed within posterior cells in these discs, similar to apoptosis seen within Hop Gal4 clones (supplementary material Fig. S4D',...
Activated STAT is a supercompetitor

Activated Stat92E requires the pro-apoptotic gene hid to become a supercompetitor. (A) Control Gal4 clones marked by GFP (arrowhead) in a hidP1642/+ background and sibling clones marked by CD2 in red (arrow) in a hidP1642/+ background. (B) Hop Gal4 clones marked by GFP (arrowheads) in a hidP1642/+ background and sibling clones marked by CD2 in red (arrow) in a hidP1642/+ background. (C) Median areas of Gal4 clones (green bars) and sibling clones (red bars). The median areas in pixels² were: 5326±716 (Control Gal4) and 5056±666 (Sibling), n=23 clone pairs; 6192±650 (Hop Gal4) and 4943±564 (Hop Sibling), n=33 clone pairs. Hop Gal4 clones are no longer significantly larger than Control Gal4 clones (P<0.187). Hop Sibling clones are now similar in size to Control Sibling clones (P<0.338). (D-D‴) Neither Control Gal4 clones (green cells, arrowhead) nor their CD2⁺ sibling clones (red cells, arrows) contain activated Caspase 3 (blue) in a hidP1642/+ background. (E-E‴) Hop Gal4 clones (green cells, arrowhead) in a hidP1642/+ background do not induce cell death (activated Caspase 3, blue) in their sibling clones (arrowhead) or in surrounding wild-type cells.

Activated Stat92E supercompetitors require the pro-apoptotic gene hid to kill their neighbors

To test potential cooperation between Stat92E and Myc in cell competition, we examined the ability of activated Stat92E to rescue null-Myc cells (Fig. 6C,D). Null-Myc cells (i.e. dm¹ mutant cells) surrounded by ‘high-Myc’ cells expressing tub>MyC were not recovered (not shown) (Wu and Johnston, 2010). Remarkably, null-Myc clones overexpressing Hop survived even in the absence of the endogenous Myc gene, suggesting a role for activated Stat92E in growth and cell competition independent of Myc (Fig. 6G,G†). Consistent with this, we found that Myc levels were not elevated in Hop-expressing clones, which have hyper-activated Stat92E (Fig. 6H,H†), but were dramatically increased in Myc-expressing clones (supplementary material Fig. S5B,B). Conversely, Stat92E was not upregulated in Myc-expressing clones (Fig. 6I,I†) but was autonomously activated in Hop-expressing clones (supplementary material Fig. S5A,A†, arrowhead). Moreover, Myc transcripts were not elevated in posterior cells in en>hop wing discs (Fig. 6K). By contrast, Myc transcripts were dramatically reduced, cells neighboring Hop Gal4 winners are no longer at a growth disadvantage. Furthermore, our findings demonstrate that cells with hyper-activated Stat92E achieve winner status through non-autonomous, hid-dependent induction of apoptosis in losers, similar to cells with high Myc.
increased in posterior cells in en>hop wing discs (supplementary material Fig. S5D). The sense probe had low background in both genotypes (Fig. 6J; supplementary material Fig. S5C). Taken together, these data indicate that activated Stat92E does not affect Myc at the level of the protein or the gene. These results also indicate that Stat92E and Myc are not epistatic and that Stat92E probably acts independently of Myc in cell competition.

**Activated Stat92E does not upregulate Yki, Wg or Dpp signaling or ribosome biogenesis genes**

To test whether activated Stat92E induces targets of the Hippo pathway, we examined subcellular localization of Yki – the functional effector of the pathway – and expression of established target genes, expanded (ex) and four-jointed (fj) (Reddy and Irvine, 2008; Badouel et al., 2009), when the JAK/STAT pathway was ectopically activated. Inactive Yki is cytoplasmic, whereas activated Yki is nuclear (Oh and Irvine, 2008). In Hop-expressing clones, Yki was cytoplasmic (Fig, 6L,L'/H11032), suggesting that Yki is inactive despite ectopic activation of the JAK/STAT pathway. In addition, we observed no alteration in ex-lacZ or fj-lacZ in Hop-expressing clones (Fig. 6M-N'). Furthermore, we found no change in the expression of Crb (Fig. 6O,O'), an upstream regulator of the Hippo pathway (Grusche et al., 2010) that is a target of Stat92E in embryonic posterior
spiracles (Lovegrove et al., 2006). These data strongly suggest that JAK/STAT signaling does not lead to activation of Yki.

We also examined the activity of other pathways reported to regulate cell competition. Dpp signaling has been associated with winner status (Moreno et al., 2002; Moreno and Basler, 2004), but its activity, as assessed by phospho-Mad (pMad), was not upregulated in Hop-expressing clones (Fig. 7A, A′). Expression of wg-lacZ (red, white in B′) at the DV boundary is not altered in Hop flip-out clones (green). Hth is blue. (C, C′) Dll (red in C, white in C′) is not altered in Hop-expressing clones (green, asterisks). (D-D′) STAT activation (10xSTAT-gfp, green in D, white in D′) is not altered in clones misexpressing dominant-negative TCF (TCFDN, red in E, white in E′), which dominantly blocks Wg signaling. Clones expressing TCFDN grew poorly. (F, F′) Fibrillarin (red in F, white in F′), a nucleolar marker, is not increased in clones overexpressing Hop (green). (G) mRNAs for ribosome biogenesis genes (nop5, Nop60B, Tif-1A) are upregulated in wing discs overexpressing Myc (blue bars) but not in those overexpressing Hop (green) or in controls overexpressing only GFP (red). mRNA of the Stat92E target dome is increased in Hop samples, but not in Myc or controls.

Fig. 7. Activated Stat92E does not induce Dpp or Wg signaling or ribosome biogenesis genes, and Wg signaling does not alter JAK/STAT activity. (A, A′) Phospho-Mad (red in A, white in A′) was not increased in Hop-expressing clones (green in A and outlined in A′). (B, B′) Expression of wg-lacZ (red, white in B′) at the DV boundary is not altered in Hop flip-out clones (green). Hth is blue. (C, C′) Dll (red in C, white in C′) is not altered in Hop-expressing clones (green, asterisks). (D-D′) STAT activation (10xSTAT-gfp, green in D, white in D′) is not altered in clones misexpressing dominant-negative TCF (TCFDN, red in E, white in E′), which dominantly blocks Wg signaling. Clones expressing TCFDN grew poorly. (F, F′) Fibrillarin (red in F, white in F′), a nucleolar marker, is not increased in clones overexpressing Hop (green). (G) mRNAs for ribosome biogenesis genes (nop5, Nop60B, Tif-1A) are upregulated in wing discs overexpressing Myc (blue bars) but not in those overexpressing Hop (green) or in controls overexpressing only GFP (red). mRNA of the Stat92E target dome is increased in Hop samples, but not in Myc or controls.
Sustained Stat92E activation and supercompetitor status

We demonstrate that cells with activated Stat92E also achieve supercompetitor status and induce death of their wild-type neighbors up to several cell diameters away, which is similar to the non-autonomous death of wild-type cells induced by Myc or Wg supercompetitors. These results strongly suggest that non-autonomous cell death is a key feature of cell competition in response to local cellular differences in either STAT activity, Wg signaling or Myc. Moreover, we demonstrate that, like Myc (de la Cova et al., 2004), cells with activated Stat92E require the pro-apoptotic gene hid to kill surrounding neighbors and achieve supercompetitor status. Although these results suggest a link between Stat92E and Myc, to our surprise we found no link between JAK/STAT signaling and Myc mRNA or Myc protein or in targets of the Hippo pathway. Furthermore, we found no regulation of Wg signaling by activated STAT and no effect of Wg on STAT activity. Taken together, our results strongly suggest that activated STAT functions in parallel to Yki, Myc and Wg in growth and cell competition (Fig. 8).

Differences in ribosome activity between winners and losers appear to be crucial to Myc- and Minute-induced cell competition and may also be required by Myc for its supercompetitor activity. We find that activated STAT does not increase expression of an important set of ribosome biogenesis genes during late larval stages (Fig. 7G). We acknowledge that it is conceivable that JAK/STAT signaling might affect other ribosomal aspects not tested in this study. Assuming a similar relationship exists at earlier larval stages – when Stat92E is required for clonal growth – we favor the model that STAT-dependent cell competition is largely independent of de novo ribosome biogenesis. This would represent an important difference between JAK/STAT and Myc- or Minute-dependent cell competition (Fig. 8). Regardless, our results at the very least suggest the presence of multiple sensors of competitive situations and indicate that the way cells compare their fitness with one another is more complex than previously believed. Indeed, Myc- and ribosome-independent supercompetition appears to be a newly emerging paradigm in the field (this study) (Vincent et al., 2011). In conclusion, we find that differences in Stat92E activity reveal differences in cellular fitness that are in large part unrelated to Myc, ribosome biogenesis, Hippo, Wg or Dpp signaling activity. Moreover, given the conservation between the components of the Drosophila and mammalian JAK/STAT signaling pathway, our findings lead the way for further investigation of cell competition in mammals.

Acknowledgements
We thank our colleagues for flies and antibodies and Marc Amoyel and anonymous reviewers for helpful comments.

Funding
This work was supported by Canadian Institutes of Health Research [MOP86622 to S.G.] and by the National Institutes of Health [R01-GM078464 to L.A.J. and R01-GM085075 to E.A.B.]. Deposited in PMC for release after 12 months.

Competing interests statement
The authors declare no competing financial interests.

Supplementary material
Supplementary material available online at http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.076760/-/DC1

References
Activated STAT is a supercompetitor


