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

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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 ‘losers’) 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; Li and Baker, 2007).

This type of ‘classical’ cell competition has also been documented in the case of Myc, a transcription factor that regulates expression of genes controlling proliferation, cellular growth and ribosome biogenesis (de la Cova et al., 2004; Moreno and Basler, 2004; de la Cova and Johnston, 2006). Animals with hypomorphic mutations in Myc [encoded by the diminutive (dm) gene] are viable but grow more slowly and are smaller than wild-type flies. However, when dm mutant cells are placed in apposition to wild-type cells, the dm cells are out-competed (Johnston et al., 1999; 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 (Arbozova and Zeidler, 2006). Sustained activation of Stat92E causes dramatic overgrowth of the eye, fly leukemia 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 ribosome biogenesis for its regulation of growth and competitive interactions between cells.

MATERIALS AND METHODS

Fly stocks

These stocks are described in Flybase: Stat92E85C9, Stat92E157, dml, lgl4, UAS-P35, UAS-hop, UAS-hopDm1, UAS-Myc, UAS-upd, en-gal4, fl-lacZ, ex-lacZ, hid864, upd(E132)-gal4, dome-gal4, 10xSTAT-gfp, wg2 (wg-lacZ), UAS-Armb40 and UAS-TCP590. actin5c-stop-lacZ was obtained from Gary Struhl (Columbia University, New York, NY, USA).

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-shock at specific times after egg deposition (AED) at 39°C and discs were dissected, fixed and stained at 151±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-shock at 48 hours AED at 37°C for 15 minutes. Wing discs were dissected at 68 hours after clone induction (ACI). For the null-Myc assays, wing discs were dissected at 110 hours ACD, dm4 Frt80 Y; tub>Myc y>gal4 UAS-upd, UAS-hop4Ra/+ animals exhibited strong developmental delays. For 10xSTAT-gfp reporter (Bach et al., 2007). In wild-type early stage 15 embryos, cells in the wing disc primordium, marked by Vg, express low levels of upd mostly in the anterior domain (Fig. 1A-A’), and there was little or no expression of 10xSTAT-gfp at this time (Fig. 1B-B’). These data indicate that the JAK/STAT pathway is not strongly activated during the earliest stages of wing development. However, in a mid-second instar disc (~60 hours AED), Stat92E activity is detected at higher and equal levels in nearly all cells, including those in the pouch (Fig. 1C-C’, arrow). These results indicate that there is no graded activation of Stat92E at this stage, and suggest that upd is expressed broadly and early in wing development. Subsequently, upd expression and Stat92E activation become reduced in the pouch and concentrated in the hinge (Fig. 1D-E’E’,F’, arrows).

We used lineage tracing to determine the fate of Upd-producing cells, using upd-gal4, UAS-flp and an
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 large parts of the wing disc, including the pouch (Fig. 1G,G’). Most cells in a mid-second instar wild-type wing disc at ~60 hours AED have equivalent activation of Stat92E as assessed by 10xSTAT-gfp (green). wg-lacZ is red and Tsh is blue. (D–F) Stat92E activation (10xSTAT-gfp, green) is reduced in the pouch during mid-to-late second instar but is maintained in the hinge. Wg protein is red. The pouch is marked by an arrowhead in C–E. (F–F’) upd (upd-GFP, green) is localized to the hinge during mid-to-late second instar. Nub, which marks the wing pouch, is red. Tsh is blue. (G,G’) Lineage-tracing experiments with upd-gal4, UAS-gfp; act5c>stop>lacZ/UAS-flp indicate that most cells in the larval wing disc expressed the upd gene. upd-gfp is green, β-Gal is red and Nub is blue. The notum is marked by an arrow in G’. (H,H’) Lineage-tracing analysis of dome-gal4, UAS-gfp; act5c>stop>lacZ/UAS-flp indicates that most cells in the disc express dome. β-Gal is red and Dig is green. In all panels, dorsal is upwards and anterior is towards the left.
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 Stat92E<sup>85C9</sup> and Stat92E<sup>397</sup> (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), Stat92E<sup>85C9</sup> 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. 2; 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 in the disc (Fig. 2I; 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 Stat92E<sup>85C9</sup> clones were apoptotic and basally extruded (Fig. 3A,A'/H11032, A'/H11630, green arrowheads; 3B,B'/H11032, B'/H11630, yellow arrowheads), in stark contrast to their faster-growing wild-type sibling clones (Fig. 3A,A'/H11033, red arrows). Expressing the caspase inhibitor P35 within Stat92E<sup>85C9</sup> clones rescued their growth such that they grew to equal sizes as their...
siblings, confirming that the reduced growth of Stat92E85C9 clones was due to cell death (Fig. 3C,C,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 expression (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-F′), 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-C′′). 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

Fig. 3. Cells lacking Stat92E become losers and are killed by induction of apoptosis. (A-A′) A GFP+ Stat92E85C9 MARCM clone (A,A′,A″, arrowheads) grows poorly compared with its CD2+ (red) sibling clone (A′,A″, arrows). Cells in the Stat92E85C9 clone have activated Caspase 3 (blue) (A,A′,A″, arrowheads). (B-B″) x-z sections of images in A-A″ reveal that Stat92E85C9 clones are basally extruded (arrowheads). (C-C″) Inhibiting cell death by expression of P35 prevents the death of Stat92E85C9 clones in green (broken line), which now grow to similar sizes as sibling clones in red (solid line). Cells in the Stat92E85C9 + P35 clone do not have activated Caspase 3 (blue). (D) The areas of 19 Stat92E85C9 MARCM clones expressing P35 (green bar) and their CD2-positive sibling clones (red bar). The average area of a Stat92E + P35 clone was 5276±708 pixels², whereas that of the CD2-positive sibling clone was 5484±744 pixels². (E) Stat92E85C9 clones (arrowhead) induced at 30 hours AED in a Minute background grow to large sizes. Stat92E clones lack GFP and appear black, whereas heterozygous Minute/+ clones have one copy of GFP and appear white. (F-F″) A GFP+ Stat92E85C9 clone (broken line in F) does not have phospho-JNK (F′,F″, arrowheads). The CD2+ sibling clone (solid line in F) is marked by an arrow (F′).
UAS-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-hopTum-1 and UAS-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. Prior clones 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 and for axin (Hop Gal4) and 2339±369 (Hop Sibling), Control 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.

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', **P<10^-6). (D,D') Neither cells in Control Gal4 clone (green cells) nor neighboring wild-type cells contain...
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Fig. 5. Activated Stat92E requires the pro-apoptotic gene hid to become a supercompetitor. (A) Control Gal4 clones marked by GFP (arrowhead) in a hid\(^{P1642}\)/+ background and sibling clones marked by CD2 in red (arrow) in a hid\(^{P1642}\)/+ background. (B) Hop Gal4 clones marked by GFP (arrowheads) in a hid\(^{P1642}\)/+ background and sibling clones marked by CD2 in red (arrow) in a hid\(^{P1642}\)/+ background. (C) Median areas of Gal4 clones (green bars) and sibling clones (red bars). The median areas in pixels\(^2\) were: 5326±716 (Control Gal4) and 5056±666 (Control 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\(^\prime\)) Neither Control Gal4 clones (green clones, arrowhead) nor their CD2\(^+\) sibling clones (red cells, arrows) contain activated Caspase 3 (blue) in a hid\(^{P1642}\)/+ background. (E-E\(^\prime\)) Hop Gal4 clones (green clones, arrowhead) in a hid\(^{P1642}\)/+ background do not induce cell death (activated Caspase 3, blue) in their sibling clones (arrowhead) or in surrounding wild-type cells.

Activated Stat92E rescues null-Myc cells from competition

Given the similarities between our findings and those reported for the competitive growth regulatory functions of Myc, we investigated potential epistasis between Stat92E and Myc. We used the tubulin/tub\(->\)Myc competition assay to assess whether activated Stat92E could rescue wild-type cells that would normally be eliminated by neighbors expressing higher levels of Myc (Fig. 6A,B) (de la Cova et al., 2004; Moreno and Basler, 2004). Clones of wild-type cells, called ‘low-Myc’, have lower levels of Myc than the surrounding tub\(->\)Myc-expressing cells and are eliminated from the wing disc due to cell competition imposed by their neighbors (Fig. 6E). By contrast, low-Myc clones expressing Hop were frequently recovered (Fig. 6F), indicating that if these losers have hyper-active Stat92E they suffer less competitive stress and/or have increased survival.

To test potential cooperativity 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. dm4\(^{-}\)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,6\(\prime\)). Consistent with this, we found that Myc levels were not elevated in Hop-expressing clones, which have hyper-activated Stat92E (Fig. 6H,6\(\prime\)), but were dramatically increased in Myc-expressing clones (supplementary material Fig. 5B,5\(\prime\)). Conversely, Stat92E was not upregulated in Myc-expressing clones (Fig. 6I,6\(\prime\)) but was autonomously activated in Hop-expressing clones (supplementary material Fig. 5A,5\(\prime\), arrowhead). Moreover, Myc transcripts were not elevated in posterior cells in en\(->\)hop wing discs (Fig. 6K). By contrast, Myc transcripts were dramatically

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

To investigate the mechanism by which activated Stat92E induces death in its neighbors, we performed the two-clone cell competition assay in a background in which one copy of the pro-apoptotic gene hid was inactivated, which severely impedes Myc-dependent cell competition (de la Cova et al., 2004; Neto-Silva et al., 2010; Wu and Johnston, 2010). Our results demonstrate that cells within a growing tissue that acquire increased levels of activated Stat92E become supercompetitors and kill their wild-type neighbors. Furthermore, they respect compartment boundaries, another hallmark of competition.

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arrow). These data demonstrate that wild-type cells adjacent to Hop Gal4 clones become losers and are eliminated from the epithelium. The increased size of Hop Gal4 clones when compared with Control Gal4 clones, and the reduced size of Hop Sibling clones when compared with Control Sibling clones, suggests that Hop Gal4 clones acquire winner status and Hop Sibling clones become losers and undergo apoptosis, features that are hallmarks of cell competition (de la Cova et al., 2004; Neto-Silva et al., 2010; Wu and Johnston, 2010). Our results demonstrate that cells within a growing tissue that acquire increased levels of activated Stat92E become supercompetitors and kill their wild-type neighbors. Furthermore, they respect compartment boundaries, another hallmark of competition.

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increased in posterior cells in en>MyC 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 (green in D, white in E), which dominantly blocks Wg signaling. Clones expressing TCF(DN) grew poorly. (E,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 (TCF(DN), red in E, white in E'), which dominantly blocks Wg signaling. Clones expressing TCF(DN) 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.
**DISCUSSION**

This study establishes a role for JAK/STAT signaling in cell competition between somatic cells that contribute to the adult organism. We show that wing disc cells lacking Stat92E activity suffer from competitive stress exerted by their wild-type neighbors and undergo apoptosis. However, when these same cells are placed with growth-disadvantaged cells (i.e. M/+) they are viable. This context-dependent behavior of cells (i.e. viable when homotypic but disadvantaged when in apposition to more robust cells) is a hallmark of cell competition (Johnston, 2009). Interestingly, the growth of Stat92E clones can be rescued by inhibition of apoptosis (Fig. 3C,D). By contrast clones lacking Myc or ribosomal genes such as Rpl135 cannot grow even when death is inhibited (Johnston et al., 1999; Grewal et al., 2005). This may represent an important difference between activated Stat92E and Myc function in losers.

Despite these differences, activated Stat92E does in fact share distinguishing features of cell competition with Myc: winners with activated Stat92E (1) induce programmed cell death in losers at a distance and (2) require hid to do so (this study) (de la Cova et al., 2004). In addition, our observation that cells null for Myc can be at least partially rescued by autonomous activation of Stat92E is noteworthy because autonomous expression of Yki does not rescue these cells (Neto-Silva et al., 2010). We propose that the partial rescue of null Myc cells by activated Stat92E is probably not due to an increase in ribosome activity or in expression of Myc target genes that drive ribosome assembly as activated Stat92E does not induce a subset of ribosomal genes during third instar (Fig. 7G).

**Stat92E is required early in development for growth of the wing disc**

We demonstrate that the JAK/STAT pathway plays an obligate role in growth of all cells in the young wing disc (30-48 hours AED). During this period of exponential growth, we show that imaginal cells lacking Stat92E are less competitive and are subjected to stress imposed by their wild-type neighbors and they are ultimately killed by hid-dependent apoptosis. We did not observe any regional effects of Stat92E at early time points: Stat92E clones grew poorly regardless of their position on the AP and DV axes when induced at 30 or 36 hours AED. The results from the Stat92E clonal analyses presented here are consistent with but stronger than those published by another group (Mukherjee et al., 2005). This discrepancy may be due to their use of a weaker allele, Stat92E06346 (Ekas et al., 2010).

**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.

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**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


