DOI: 10.1534/genetics.105.046904

# Identification of Drosophila Genes Modulating Janus Kinase/Signal Transducer and Activator of Transcription Signal Transduction

**Tina Mukherjee, Ulrich Schäfer and Martin P. Zeidler**[1]

*Department of Molecular Developmental Biology, Max Planck Institute for Biophysical Chemistry, 37077 Göttingen, Germany*

Manuscript received June 15, 2005
Accepted for publication December 9, 2005

ABSTRACT

The JAK/STAT pathway was first identified in mammals as a signaling mechanism central to hematopoiesis and has since been shown to exert a wide range of pleiotropic effects on multiple developmental processes. Its inappropriate activation is also implicated in the development of numerous human malignancies, especially those derived from hematopoietic lineages. The JAK/STAT signaling cascade has been conserved through evolution and although the pathway identified in Drosophila has been closely examined, the full complement of genes required to correctly transduce signaling *in vivo* remains to be identified. We have used a dosage-sensitive dominant eye overgrowth phenotype caused by ectopic activation of the JAK/STAT pathway to screen 2267 independent, newly generated mutagenic *P*-element insertions. After multiple rounds of retesting, 23 interacting loci that represent genes not previously known to interact with JAK/STAT signaling have been identified. Analysis of these genes has identified three signal transduction pathways, seven potential components of the pathway itself, and six putative downstream pathway target genes. The use of forward genetics to identify loci and reverse genetic approaches to characterize them has allowed us to assemble a collection of genes whose products represent novel components and regulators of this important signal transduction cascade.

DEVELOPING cells *in vivo* are influenced by, and interact with, their surroundings via multiple mechanisms central to which are the signal transduction cascades. Activation of such cascades by extracellular ligands generally converts signals into changes in the gene expression profile of a responding cell. Although only a relatively limited number of such pathways have been identified, these signaling cascades have generally been conserved throughout evolution and are often active at multiple stages of development where they exert a wide range of pleiotropic effects, including cellular growth, proliferation, and differentiation.

The *Ja*nus *k*inase (JAK)/*s*ignal *t*ransducer and *a*ctivator of *t*ranscription (STAT) pathway is one such signaling cascade. The pathway was first identified in mammals where extensive analysis has led to the development of a canonical model for JAK/STAT signaling in which nonreceptor JAK tyrosine kinases are associated with the intracellular portion of *trans*-membrane receptors. Following ligand binding to dimerized cytokine receptors the associated JAK molecules become active and auto- and *trans*-phosphorylate one another and their receptors. The resulting phospho-tyrosine residues are recognized by the SH2 domain of normally cytosolic STAT proteins, which are recruited to these docking sites before being themselves phosphorylated on a C-terminal tyrosine residue by the JAKs. The activated STATs form homo- and hetero-dimers and translocate to the nucleus, bind to a palindromic DNA recognition sequence, and activate the transcription of pathway target genes (Zeidler *et al.* 2000a; Bromberg 2002; Kisseleva *et al.* 2002).

In mammals, four Jak molecules have been identified: Jak1, Jak2, Jak3, and tyrosine kinase 2 (tyk2). STATs compose a family of seven structurally and functionally related proteins: Stat1–4, Stat5a, Stat5b, and Stat6. Stat proteins play a central role in transmitting cell surface cytokine signals into the nucleus and in induceing cellular proliferation, differentiation, and survival signals in multiple hematopoetic cell types. Under normal circumstances, ligand availability and negative feedback mechanisms tightly regulate the cytokine-mediated activation of Stats (Bowman *et al.* 2000). However, constitutive activation of multiple Jaks and Stats is associated with diverse leukemias and lymphomas (Friedmann *et al.* 1996; Lacronique *et al.* 1997; James *et al.* 2005), resulting in a radical alteration of the gene expression and ligand-independent survival/proliferation of these transformed cells (Sternberg and Gilliland 2004). Mechanisms required for the regulation of the JAK/STAT pathway and candidate downstream target genes, however, are comparatively poorly understood. The redundancy present in the human system with multiple activating cytokines, Jaks and Stats, makes a genetically

[1]*Corresponding author:* Department of Molecular Developmental Biology, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany. E-mail: mzeidle@gwdg.de

based approach to identifying pathway interacting genes difficult (Levy and Darnell 2002). By contrast, the JAK/STAT pathway in Drosophila represents a less complex and genetically tractable system with which the pathway can be studied.

The canonical pathway in Drosophila consists of the homologous secreted ligands Unpaired (Upd), Upd2, and Upd3 (Harrison *et al.* 1998; Agaisse *et al.* 2003; Hombria *et al.* 2005) and a cytokine-like transmembrane receptor, Domeless (Dome; Brown *et al.* 2001), with homology to gp130 and the leukemia inhibitory factor receptor (Hombria and Brown 2002). Dome genetically interacts with *stat92E* and has been shown to associate with Upd when coexpressed in mammalian cells (Brown *et al.* 2001; H. W. Chen *et al.* 2002). A JAK tyrosine kinase called Hopscotch (Hop; Binari and Perrimon 1994) bearing 27% identity to human JAK2 and a single STAT-like transcription factor, STAT92E (also known as Marelle; Hou *et al.* 1996; Yan *et al.* 1996), with 37% identity to human STAT5b, are also encoded by the fly genome. Drosophila homologs of other components of the mammalian JAK/STAT pathway have also been identified. These include *p*rotein *i*nhibitor of *a*ctivated *S*TAT (PIAS), *s*uppressor *o*f *c*ytokine *s*ignaling (SOCS), and *s*ignal *t*ransducer *a*daptor *m*olecule-like molecules. dPIAS [also known as *suppressor of variegation 2-10 (Su(var)2-10)* and *zimp*] interacts genetically and biochemically with the JAK/STAT pathway (Chung *et al.* 1997; Mohr and Boswell 1999; Betz *et al.* 2001; Hari *et al.* 2001). The Drosophila genome encodes three SOCS genes and the expression of *socs36E*, a member of the vertebrate SOCS4/5 class, is regulated by JAK/STAT pathway activity and functions to suppress JAK activity (Karsten *et al.* 2002; Rawlings *et al.* 2004), while SOCS44A, a member of the vertebrate SOCS6/7 class, is independent of JAK pathway activity but capable of repressing JAK-induced signaling (Rawlings *et al.* 2004).

In vertebrate systems biochemical approaches and gene-targeting experiments have identified a requirement for the pathway in diverse processes, including embryonic development, neuronal survival, development of the immune system, and hematopoesis (Levy and Darnell 2002; Boulay *et al.* 2003). In addition, STAT3 and STAT5 appear to represent the major STATs involved in promoting oncogenesis (Bowman *et al.* 2000) while Stat1 induces antiproliferative responses and functions as a potential tumor suppressor (Platanias 2005).

Many of these processes are mirrored in Drosophila. In the adult, the pathway is involved in stem cell renewal in the male germline (Kiger *et al.* 2001; Tulina and Matunis 2001) as well as in border cell migration and stalk cell development in oogenesis (Beccari *et al.* 2002; McGregor *et al.* 2002). In early stages of embryonic development, JAK/STAT signaling plays an important role in sex determination (Sefton *et al.* 2000) and regulates embryonic segmentation by controlling the expression of the pair-rule genes *even-skipped*, *runt*, and *fushi tarazu* (Binari and Perrimon 1994; Hou *et al.* 1996; Yan *et al.* 1996; Harrison *et al.* 1998). At later embryonic stages, roles in tracheal and posterior spiracle formation have been identified in *dome* mutants (Brown *et al.* 2001; H. W. Chen *et al.* 2002) along with a requirement in both fore- and hind-gut development (Johansen *et al.* 2003; Josten *et al.* 2004). During larval development, the JAK/STAT pathway is also required for hematopoiesis (Luo *et al.* 1997), ommatidial rotation in the eye (Zeidler *et al.* 1999a), and cellular proliferation in the wing disc. In this tissue, STAT92E exerts both early proproliferative and late antiproliferative functions (Mukherjee *et al.* 2005). To achieve this degree of complexity, it is probable that Drosophila JAK/STAT signaling is influenced by a range of both environmental and physical interactions, which act to modulate the consequences of its activation during different developmental processes.

A recent genetic screen to identify chromosomal regions interacting with the JAK/STAT pathway *in vivo* has identified a number of novel regulators and components of the pathway (Bach *et al.* 2003). However, such a screen allows only the identification of specific genes via a candidate approach and caveats associated with the availability of alleles and varying genetic backgrounds apply. By contrast, the ability to identify potentially interacting loci from among mutations generated by random mutagenesis represents a potentially more stringent approach. We therefore employed the *P{w*$^+$*, GMR-upd*$\Delta$*3′}* transgenic strain previously described by Bach *et al.* (2003). In this stock a *trans*-gene containing multimerized binding sites for the eye-specific transcription factor Glass (Ellis *et al.* 1993) is used to drive expression of the pathway ligand Upd. Expression of Upd posterior to the morphogenetic furrow by the *g*lass *m*ultimerised *r*esponse (GMR) promoter results in increased levels of JAK/STAT pathway activity as shown by upregulation of the pathway target *socs36E* (Karsten *et al.* 2002) and increased levels of cellular proliferation shown by staining with the mitosis-specific marker phospho-Histone3 (Bach *et al.* 2003). Increased cellular proliferation occurs primarily in a region ahead of the morphogenetic furrow and corresponds to the first mitotic wave (Bach *et al.* 2003; Tsai and Sun 2004). The additional cells that result appear to differentiate normally and give rise to a greatly enlarged adult eye with overgrowth particularly apparent in dorsal regions (Figure 1, A and B). As required of a dosage-sensitive assay, the degree of eye overgrowth caused by *P{w*$^+$*, GMR-upd*$\Delta$*3′}* is dependent on the ability of downstream JAK/STAT pathway components to transduce the overactivation and is significantly suppressed following the removal of a single copy of the *stat92E* locus (Figure 1C).

Here we present our analysis of mutants identified in an $F_1$ genetic screen to identify potential modifiers of the Drosophila JAK/STAT pathway. We have screened 2267 independent autosomal *P{Mae-UAS.6.11}* (Crisp

and Merriam 1997) insertions for their interaction with the *P{w+, GMR-updΔ3′}*-induced eye overgrowth phenotype (Bach *et al.* 2003). In addition to the initial identification, we further validated interacting loci using a combination of reverse genetic RNA interference (RNAi)-based approaches and *in vivo* expression studies. The screen identified 23 potential pathway interacting loci, including members of the Dpp and Notch signaling pathways, seven potential pathway components defined by RNAi knockdown, and six novel pathway target genes whose expression patterns appear to be modulated by changes in the JAK/STAT pathway activity.

## MATERIALS AND METHODS

**Genetic interactions:** For genetic interaction assays, *y,w, P{w+,GMR-updΔ3′}/FM7, P{w+,Ubq-GFP}* (Bach *et al.* 2003) virgins were crossed to males of the indicated genotypes (Tables 1 and 2). Each batch of interaction assays was grown at 25°, the "average" eye overgrowth in adult progeny of the next generation was scored in relation to positive and negative/neutral controls crossed to *stat92E*$^{06346}$ mutants and "wild type" (Ore-R or $w^{1118}$) lines, respectively. In general, lack of interaction (±) results in somewhat variable eye sizes while increasing levels of suppression or enhancement (indicated by − or +, respectively) are more uniform. The *Ten-m* alleles were a gift of Ron Wides and the details of other alleles used are available at http://flybase.bio.indiana.edu/. To screen for GMR modifiers, *P{w+ GMR-rho}* flies were crossed to males with genotypes listed in Tables 1 and 2 and, as controls, *P{w+ GMR-rho}* virgins were crossed to wild-type (Ore-R) males and *stat92E*$^{06346}$ males.

For interaction with the loss-of-function $os^{1}$ allele (Verderosa and Muller 1954), homozygous females were crossed to Ore-R, $w^{1118}$, and *stat92E*$^{06346}$ as negative and positive controls, respectively. Crosses to potentially interacting alleles were set up in parallel with controls. Hemizygous $os^{1}$ mutant males were scored for an enhancement of their eye-size reduction in the next generation.

**Inverse PCR:** Inverse PCR was performed essentially as described on the BDGP web page (http://www.fruitfly.org/). The PCR-amplified DNA was sequenced, and the resulting sequences were aligned to release 3.0 and release 4.0 genomic DNA using BLAST searches (Adams *et al.* 2000).

**RNA interference:** Double-strand (ds)RNA targeting the various candidate genes was prepared from 400- to 500-bp PCR products, amplified from genomic DNA using primers containing a 5′ T7 promoter (GAATTAATACGACTCACTATAGG GAGA). The 18- to 20-bp gene-specific portion of primers was directed against single exons of the 18 candidate genes. Further details are available on request. PCR products were used as direct templates for *in vitro* transcription using the T7 RNA polymerase. To obtain dsRNA, *in vitro*-transcribed RNA was heated to 95° for 1 min and then allowed to cool slowly to room temperature.

For the paracrine assay, $5 \times 10^6$ $Kc_{167}$ cells were seeded in 6-well dishes 1 day before transfection. Cells were batch transfected using Effectene (QIAGEN, Chatsworth, CA). For "signaling cells" (Figure 2B), 600 ng of *pAct5c-upd-GFP* was transfected per well in a 6-well dish, and for "receiving cells," 500 ng of *6x2xDraf*Luc(wt) reporter and 25 ng of *pAct5c-RL* was transfected. Plasmids are described in Müller *et al.* (2005). Following transfection, cells were grown for 24 hr, and subsequently the media were removed and the cells were mixed at a ratio of 1:1 in serum-free Schneider's Drosophila medium. A total of 25,000 cells were then aliquoted into 96-well plates containing 20–50 nm dsRNA/well. Following dsRNA treatment, the cells were grown for 72 hr and then lysed. Twenty microliters of the lysate was used to carry out the dual luciferase measurements. Firefly and *Renilla* luciferase activity was measured by dual-luciferase reporter assay (Promega, Madison, WI) on Wallac Victor Light 1420 luminescence counter (Perkin-Elmer, Norwalk, CT). Relative reporter activity is shown as a ratio between the firefly luciferase readout and that of the *Renilla* luciferase. Reporter activation values in the experimental samples were normalized to mock transfected cells.

**Statistics:** Statistical analysis of data sets was undertaken using Microsoft Excel (mean and standard deviation measurements) and *U*-tests (see http://faculty.vassar.edu.html).

**Histology:** *In situ* hybridization was carried out as described in Lehmann and Tautz (1994). To prepare sense and antisense probes for the candidate genes, direct PCR products amplified from genomic DNA with T7-containing primers were used as templates for *in vitro* transcription using the digoxygenin labeling kit (Roche). Wild-type and *P{w+,GMR-updΔ3′}* eye discs were prepared and stained in parallel and the color reaction was developed for the same amount of time. The discs were dissected and mounted in 70% glycerol and photographed using a Zeiss Axioskop2 MOT microscope.

## RESULTS

**Design of a sensitized screen:** To identify dominant modifiers of *P{w+, GMR-updΔ3′}*, we generated new independent autosomal insertions of the *P{Mae-UAS.6.11} P* element using the crossing scheme in Figure 1F. In this scheme, the mutagenic transposon was mobilized from within the *CyO* balancer using the *{Δ2-3}99B* transposase source. Single, *yellow*+ males were selected in the $F_2$ generation to ensure unique transposition events. In the $F_3$ generation, the chromosome containing the reintegrated *P* element was identified, with those inserted in the X chromosome used for a genomics project (Beinert *et al.* 2004) and those present on autosomes crossed to *P{w+, GMR-updΔ3′}* females. The eye size of the resulting *P{w+, GMR-updΔ3′}/+; P{Mae-UAS.6.11}/+* females was compared to controls out-crossed to the Ore-R or $w^{1118}$ "wild type" strains and the strong *stat92E*$^{06346}$ allele (Hou *et al.* 1996). Interaction strength was graded according to a scale in which those equivalent to the *P {w+, GMR-updΔ3′}/+; stat92E*$^{06346}$*/+* control (Figure 1C) were classified as strong suppressors while those comparable to *P{w+, GMR-updΔ3′}; +/+* (Figure 1B) were defined as no effect (±). Using this scoring system, *P{Mae-UAS.6.11}* insertions that modify the *P{w+ GMR-updΔ3′}*-induced eye overgrowth were identified. Although the degree of overgrowth induced by the *P{w+ GMR-updΔ3′}* transgene varies within any population of $F_{4a}$ progeny, it was consistently observed that the range of variance within a population was reduced in backgrounds showing specific interactions and this phenomena was also used to identify potentially interacting loci.

In crosses showing probable interaction with *P{w+, GMR-updΔ3′}*, sibling males carrying the *P{Mae-UAS.6.11}*

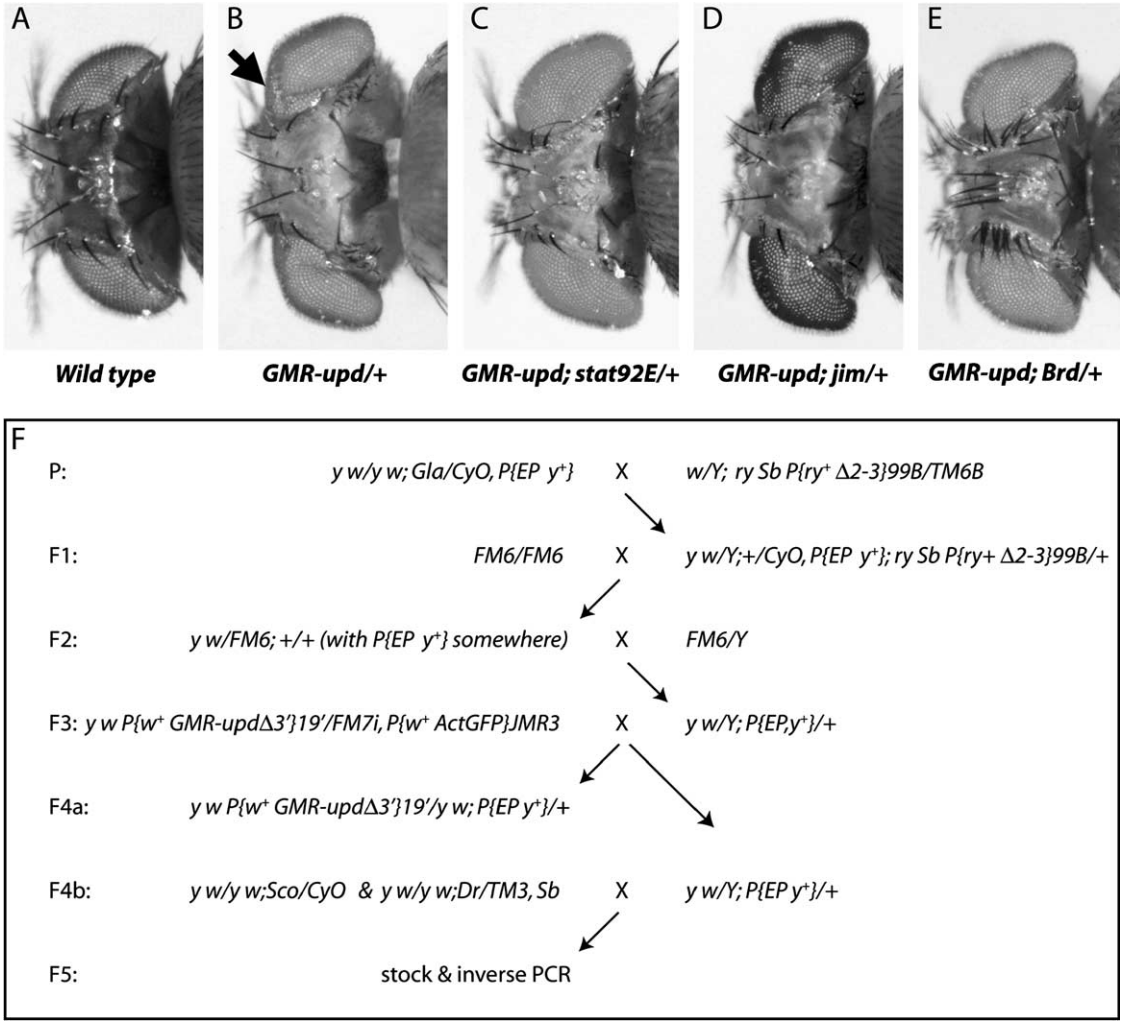


Figure 1.—The *P{w+, GMR-updΔ3′}* sensitized screen. One copy of the *P{w+, GMR-updΔ3′}* transgene inserted on the first chromosome results in overgrowth of the adult eye and the formation of dorsal folds (arrow in B). Removal of one copy of *stat92E* suppresses the enlarged-eye phenotype (C). Removal of one copy of *jim* results in a mild suppression (D) and loss of one copy of *Brd* results in a good suppression of the overgrown-eye phenotype (E). Note that the dorsal folds in B are missing in these eyes. (F) Generation of independent autosomal insertions of *P{Mae-UAS.6.11} P*-element (abbreviated as *P{EPy+}*). Genotypes: WT (Ore-R) eye (A); P{w+, GMR-updΔ3′}′/+ ; +/+ (B); P{w+, GMR-updΔ3′}′/+ ; stat92E$^{06346}$/+ (C); P{w+, GMR-updΔ3′}/+ ; jim$^{BG01625}$/+ (D); P{w+, GMR-updΔ3′}/+ ; Brd$^{1}$/+ (E).

insertion were recovered and balanced. The resulting stocks were then retested over multiple rounds to ensure that the interaction was consistent and segregated with the $y^+$ marked chromosome. Examples of representative eyes showing interaction between *P{w+, GMR-updΔ3′}* and the interacting mutations identified include the weak/moderate suppression shown by *jim* alleles (Figure 1D) and the strong suppression caused by the removal of a single copy of *Bearded* (Figure 1E).

We tested a total of 2267 independent, primarily autosomal insertions for interaction. During the initial round of screening, we retained a total of 91 (4%) interacting loci for further analysis and, of these, 23 (1%) candidates passed all subsequent rounds of retesting and were classified as potential interactors (Table 1).

Although the genetic interaction induced by the 23 *P{Mae-UAS.6.11}* insertions was reconfirmed during multiple rounds of rescreening with *P{w+, GMR-updΔ3′}*, it remains possible that the effect observed may result from a modulation of the strength of the GMR promoter rather than from any influence on the JAK/STAT pathway itself. We therefore crossed candidate insertions to a stock misexpressing the Rho GTPase within the developing eye under the control of the GMR promoter (Rebay and Rubin 1995; Häcker and Perrimon 1998). Insertions that interact with both *P{w+, GMR-updΔ3′}* and the dominant rough-eye phenotype induced by *P{w+, GMR-rho}* are likely to represent nonspecific interactions. Although most insertions showed interactions specific for only *P{w+, GMR-updΔ3′}* (Table 1), one insertion, subsequently identified as representing a

**TABLE 1**

**Interacting candidate genes from the *P{w+, GMR-updΔ3'}* screen**

| Allele | *P* element | | EP | Cytology | Disrupted gene | Interaction | | Comments/references |
|---|---|---|---|---|---|---|---|---|
| | Position | Location | | | | *GMR-upd* | *GMR-rho* | |
| *F332.1* | 2.8 kb 5′ of first transcriptional start site | 200,515 in AE003699.3 | N | 87E8-E9 | *diminished discs (did/alt1)* | −− | +/− | Defects in cell cycle and chromosome condensation (Gatti and Baker 1989) |
| *P7.7/03* | 8.4 kb 5′ of first transcriptional start site | 101,671 in AE003544.4 | N | 68C12-13 | *Mob1* | − | +/− | Required for exit from mitosis in yeast (Luca *et al.* 2001) |
| *P7.9/02* | 13 bp 3′ of first transcriptional start site | 73,903 in AE003591.4 | N | 77C1 | *tribbles (trbl)* | −− | +/− | Negative regulation of mitosis (Seher and Leptin 2000) |
| *F389.1* | In 1st intron | 56,888 in AE003581.4 | N | 23B1-2 | *lilliputian (lilli)* | − | −− | Regulation of cell size (Wittwer *et al.* 2001) |
| *F393.1* | 204 bp 5′ of first transcriptional start site | 224,892 in AE003833.4 | N | 45F1-F3 | *mir-14* | −− | +/− | Negative regulation of cell death (Xu *et al.* 2003) |
| *P1.3/03* | In 2nd intron | 11,985 in AE003720.3 | N | 90D1-6 | *couch potato (cpo)* | − | +/− | RNA binding (Bellen *et al.* 1992; Lasko 2000) |
| *H469.2* | 7.9 kb 5′ of first transcriptional start site | 32,337 in AE003599.2 | N | 80A3 | *jim* | + | +/− | Zinc-finger TF activity (Drysdale *et al.* 2005) |
| *F4.24/03* | In 2nd intron | 86,857 in AE003493.3 | N | 12A9-B2 | *NFAT* | ++ | +/− | RNA polymerase II transcription factor activity (Drysdale *et al.* 2005) |
| *F3.28/03* | In 1st intron | 245,143 in AE003808.4 | N | 52F5-7 | *CG8443* | − | +/− | Translation initiation factor activity (Drysdale *et al.* 2005) |
| *P10.3/12* | In 2nd exon | 36,214 in AE00–3721.3 | N | 90F6-7 | *Sequence-specific single-strand DNA-binding protein (Ssdp)* | −− | +/− | Transcription regulator activity (L Chen *et al.* 2002) |
| *H74.3* | In 1st intron | 84,204 in AE003800.4 | N | 55C1 | *poly (A)-binding protein (pAbp)* | − | +/− | RNA binding (Lasko 2000) |
| *F464.1* | 1 bp 5′ of first transcriptional start site | 95,124 in AE003698.3 | N | 85B10 | *C- terminal Binding Protein (CtBP)* | − | +/− | Negative regulation of transcription from Pol II promoter (Alifragis *et al.* 1997) |
| *H186.1* | 33 bp 5′ of first transcriptional start site | 434,6686 in AE03481.3 | N | 64B2-4 | *Cip4* | − | +/− | Rho interactor activity (Drysdale *et al.* 2005) |
| *P2.3/03* | In 3rd intron | 60,887 in AE003451.3 | N | 9D2 | *Rin1/Sprint (Spri)* | + | +/− | Ras interactor activity (Szabo *et al.* 2001) |
| *P1.24* | In 3rd intron | 109,964 in AE003636.4 | N | 33E5-9 | *bunched (bun)* | −− | +/− | Component of *dpp* signalling (Dobens *et al.* 2000) |
| *F716.1* | 305 bp 5′ of first transcriptional start site | 50,764 in AE003532.3 | N | 71A4 | *Bearded (Brd)* | − | +/− | Negative regulator of notch pathway (Lai *et al.* 2000) |

(*continued*)

**TABLE 1**

**(Continued)**

| Allele | *P* element | | EP | Cytology | Disrupted gene | Interaction | | Comments/references |
|---|---|---|---|---|---|---|---|---|
| | Position | Location | | | | *GMR-upd* | *GMR-rho* | |
| *F29.6* | 783 bp 5′ of first transcriptional start site | 2,649 in AE003467.3 | N | 61A5 | *mth-like8 (mthl8)* | − | +/− | G-protein-coupled receptor activity (Brody and Cravchik 2000) |
| *F411.1* | 350 bp 5′ of first transcriptional start site | 284,254 in AE003597.4 | Y | 79D2 | *Tenascin-major (Ten-m)* | + | +/− | Secreted and transmembrane protein (Baumgartner *et al.* 1994) |
| *F463.1* | 14 kb 5′ of first transcriptional start site | 139,258 in AE003680.4 | N | 85B1 | *CG8351* | −− | +/− | ATPase activity, coupled, involved in protein folding (Drysdale *et al.* 2005) |
| *F595.1* | In 1st exon | 5,907 in AE003689.2 | N | 86C7 | *Sorbitol dehydrogenase-2 (Sodh-2)* | −− | +/− | Carbohydrate metabolism (Luque *et al.* 1998) |
| *P10.1/12* | In 1st intron | 30,952 in AE003781.5 | N | 39E2 | *CG3305* | − | +/− | Contains Lamp/CD68 domains (Drysdale *et al.* 2005) |
| *F294.3* | In 2nd intron | 46,839 in AE003820.3 | N | 49D4-6 | *CG17574* | −− | +/− | Unknown, no homologs |
| *F422.1* | 300 bp 5′ of first transcriptional start site | 112,391 in AE003520.4 | Y | 75C6 | *CG4306* | − | +/− | Unknown, no homologs |
| *A1* | 11 kb 5′ of first transcriptional start site | 192,436 in AE003623.3 | N | 30A2 | *CG32982* | −− | +/− | Pleckstrin-like protein domains (Drysdale *et al.* 2005) |

The degree of interaction observed was classified under "*GMR-upd*" and "*GMR-rho*" as +/−, no effect; +, mild enhancer; ++, moderate enhancer; −, mild suppressor; −−, moderate suppressor. EP indicates if the UAS sequence within the *P* element is oriented to drive expression of the associated gene. Cytology is computationaly derived.

putative *lilli* allele, also modified the *P{w+, GMR-rho}* rough-eye phenotype. This finding is consistent with previous studies that identified *lilli* as a transcriptional regulator of the GMR promoter (Tang *et al.* 2001).

**Identification of interacting genes:** Having identified the *P{Mae-UAS.6.11}* insertions specifically interacting with *P{w+, GMR-updΔ3′}*, we then set out to determine the genes associated with these mutations. Genomic DNA flanking the *P*-element insertion site was therefore recovered by inverse PCR (Beinert *et al.* 2004), sequenced, and aligned to release 3.0 and release 4.0 genomic DNA (Adams *et al.* 2000; Celniker *et al.* 2002) using BLAST searches. Single, unambiguous *P*-element insertion positions could be determined for all interactors. The position of the insertion relative to the putatively mutated genes, the absolute position within AE clones of the Drosophila euchromatin release 4.0 sequence, and the direction of potential misexpression from the upstream activation sequences (UAS) present within the *P{Mae-UAS.6.11}* transposon are given in Table 1.

As expected from a genetic screen of this type, candidate interacting genes of various classes were identified. These include proteins poposed to be involved in the regulation of the cell cycle (*did*, *Mob1*, *tribbles*), transcription factors (*jim*, *NFAT*), DNA- and RNA-binding proteins (*Ssdp*, *CG8443*, *couch potato*, *pAbp*, *CtBP*), members of other signal transduction pathways (*Cip4*, *Bearded*, *bunched*, *sprint*, *mth-like 8*) as well as the cell adhesion protein *Tenascin-M*. In addition, a number of uncharacterized genes of unknown function were also identified (*CG3305*, *CG4306*, *CG17574*, *CG32982*).

Although no examples of multiple *P*-element insertions were detected by inverse PCR, it remains possible that the interacting mutations may be independent of the *P{Mae-UAS.6.11}* transposons characterized. In addition, the identity of loci potentially mutated by transposons inserted at a distance from currently annotated transcription units is not always unambiguous. To address these limitations, we therefore set out to test the interaction of other available alleles of the candidate genes. Using mutations obtained from the Bloomington stock center, as well as lines from individual labs, we were able to retest independently generated alleles of all 23 putatively interacting loci for their ability to modulate *P{w+, GMR-updΔ3′}*-induced eye overgrowth (Table 2).

The ability to validate putative mutations in this manner was particularly helpful in the case of the $did^{F332.1}$ and $Mob1^{P7.7/03}$ alleles where the putatively mutagenic *P*-element insertion was mapped between 2.8 and 8.4 kb upstream of the first annotated transcriptional start site (Table 1). Despite the separation between gene and transposon, independently generated alleles of each of these loci all demonstrated consistent interaction with *P{w+, GMR-updΔ3′}* (Table 2). It therefore seems likely that the *P* elements originally identified represent *bona fide* alleles and may affect enhancer regions, differential splice forms, or as-yet-unannotated 5′ exons.

Although most genes tested showed interactions consistent with those originally identified, a subset of the alleles that we identified, including $Ten\text{-}m^{F411.1}$, $NFATF^{4.24/03}$, and $jim^{H469.2}$, shows interactions opposite to those produced by independently generated loss-of-function alleles (Table 2). Given the presence of UAS sequences within the *P{Mae-UAS.6.11}* transposon, and potential cryptic promoters present within the long terminal repeats of the *P* element, it is possible that the mutations identified represent gain-of-function alleles whose misexpression may explain the converse interactions observed.

On the basis of our ability to consistently identify interactions with independently generated alleles, we subsequently focused our efforts on 18 candidate genes (underlined in Table 2) on the basis that these are most likely to represent *bona fide* interacting loci. These candidate modulators of JAK/STAT signal transduction form the basis of our further studies.

**Interaction testing with $os^1$:** Having defined a set of loci that interact with the *P{w+, GMR-updΔ3′}* gain-of-function phenotype, we then tested for potential genetic interaction with a complementary loss-of-function phenotype. Previous reports have shown that the hypomorphic loss-of-function $os^1$ allele of the pathway ligand Upd results in a reduction in the size of the adult eye (Verderosa and Muller 1954), a phenotype that can be rescued by exogenous pathway activation (Bach *et al.* 2003; Tsai and Sun 2004). In addition, the degree of eye-size reduction caused by $os^1$ is significantly enhanced when an individual is simultaneously mutant for the downstream pathway components *hop* or *stat92E* (Tsai and Sun 2004).

Although the enhancement of the $os^1$ small-eye phenotype caused by the removal of one copy of *stat92E* is relatively subtle, a distinct and reproducible genetic interaction is observed (not shown). We therefore used this interaction to screen alleles of the genes previously identified as modifiers of *P{w+, GMR-updΔ3′}* and tested for enhancement of the eye-size reduction that may indicate an interaction between the tested loci and endogenous JAK/STAT signaling (Table 2).

Although not observed in all cases, many of the loci tested modify not only the gain-of-function, but also the loss-of-function eye phenotypes (Table 2) and are therefore likely to represent genes that are required for both ectopically activated and endogenous levels of JAK/STAT pathway signaling.

**Characterization of modifiers—RNAi-based assays:** The modification of the *P{w+, GMR-updΔ3′}*-induced phenotype may be the consequence of mutations in genes encoding components of the JAK/STAT pathway, direct and indirect regulators of the pathway, or downstream target genes required to elicit the biological phenotype used in the initial screen—namely cellular overproliferation in the developing eye.

**TABLE 2**

**Secondary validation of the *P{w⁺, GMR-updΔ3′}* candidate genes**

| Gene | Allele | *GMR-upd* | *os*[1] |
|---|---|---|---|
| *did/alt1* | *F332.1* | −− | ND |
| | *EY03597* | −− | Y |
| | *l(3)05137* | −−− | Y |
| *CG8443* | *F3.28/03* | − | N |
| | *KG02346* | −− | N |
| *Ssdp* | *P10.3/12* | −− | Y |
| | *KG03600* | −−− | Y |
| *Mob1* | *P7.7/03* | − | Y |
| | *KG00128* | −− | Y |
| | *KG05879a* | −−− | Y |
| | *KG05765* | −−− | N |
| *jim* | *H469.2* | + | ND |
| | *BG01625* | −− | ND |
| | *GE23777* | − | N |
| | *GE16615* | −− | N |
| *trbl* | *P7.9/03* | −− | N |
| *NFAT* | *F4.24/03* | ++ | ND |
| | *GE02173* | −− | N |
| *pAbp* | *H74.3* | − | ND |
| | *EP310* | +/− | Y |
| | *k10109* | −−− | ND |
| *CtBP* | *F464.1* | − | ND |
| | *87De-10* | +/− | Y |
| | *03463*[a] | + | N |
| | *KG07519* | −− | Y |
| *mir-14* | *F393.1* | −− | ND |
| | *k10213* | − | Y |
| *cpo* | *P1.3/03* | − | ND |
| | *l(3)01432* | +/− | N |
| *Cip4* | *H186.1* | − | ND |
| | *EY11321* | −−− | Y |
| *sprint* | *P2.3/03* | + | ND |
| | *KG07279* | −− | Y |
| *bun* | *P2.7/03* | −− | Y |
| | *P1.24* | −− | Y |
| | *00255* | −− | Y |
| | *KG00392* | −− | Y |
| | *KG00456* | −− | Y |
| *Brd* | *F716.1* | − | Y |
| | *1* | −−− | Y |
| | *BG02319* | −− | Y |
| *mthl8* | *F29.6* | − | Y |
| *Ten-m*[b] | *F411.1* | + | N |
| | *odz3* | −− | ND |
| | *odz1* | −−− | Y |
| | *odz29* | −− | ND |
| *CG8351* | *F463.1* | −− | Y |
| | *KG01477* | −− | Y |
| *Sodh-2* | *F595.1* | −− | Y |
| *CG3305* | *P10.1/12* | − | N |
| | *KG04055* | −−− | Y |
| *CG17574* | *F294.3* | −− | ND |
| | *EY01345* | −− | Y |
| *CG4306* | *F422.1* | − | ND |
| | *EY06480* | −−− | Y |
| *CG32982* | *A1* | −− | N |

(*continued*)

The Upd ligand is a secreted glycoprotein (Harrison *et al.* 1998) capable of activating the JAK/STAT pathway in cells located at some distance from the source of expression (Zeidler *et al.* 1999b; Tsai and Sun 2004). To fulfill this function, Upd must be post-translationally modified, secreted into the extracellular space, and must interact with receptors on the receiving cell. Given that the Upd expression domain is physically separate from the region of increased cellular proliferation (Tsai and Sun 2004), it is possible that genes identified in our screen may represent loci required for these upstream processes.

We therefore set out to devise a model with which such paracrine signaling can be mimicked in a tissue-culture-based system using an assay with which JAK/STAT pathway activity can be determined (Müller *et al.* 2005). The reporter used consists of a firefly luciferase gene upstream of 12 copies of a STAT92E binding site originally identified in the promoter of the pathway target gene *Draf* (Figure 2A; Kwon *et al.* 2000). To generate a paracrine model of pathway signaling, we transfected an Upd-expressing vector into one population of $Kc_{167}$ (Cherbas *et al.* 1977) "signaling cells" and transfected the reporter and transfection control into another population of $Kc_{167}$ "receiving cells" (Figure 2B). These two cell types were then mixed and co-cultured in sextuplicate parallel experiments carried out in 96-well plates before measurement of reporter gene activity. In this scenario, receptor stimulation must result from Upd originally expressed in another cell (Figure 2B) with the *6x2xDraf*Luc reporter showing a robust induction in response to this form of paracrine activation (Figure 2C; Müller *et al.* 2005).

Given the effectiveness of the reporter system, we then set out to use RNAi-mediated knockdown (Clemens *et al.* 2000) to identify the candidate genes that might represent pathway components or modulators.

Under the experimental conditions used, Upd-dependent paracrine stimulation, in the absence of dsRNA, results in luciferase activity ~60 times higher than that of unstimulated controls (Figure 2C, columns 1 and 2). This level of reporter activation was defined as 1 unit of luciferase activity and is not affected by dsRNA-targeting *Rhodopsin-5* or *lacZ* used as negative controls. However, addition of dsRNA-targeting *stat92E* was

**TABLE 2**

**(Continued)**

The degree of interaction was classified as +/−, no interaction; +, mild enhancer; ++, moderate enhancer; −, mild suppressor; −−, moderate suppressor; −−−, good suppressor. For *os*[1]: Y, enhancement of the small-eye phenotype observed; N, no effect; ND, not determined.

[a] Previously identified as a mild enhancer (Bach *et al.* 2003).

[b] A deficiency removing this locus was previously identified as a suppressor (Bach *et al.* 2003).

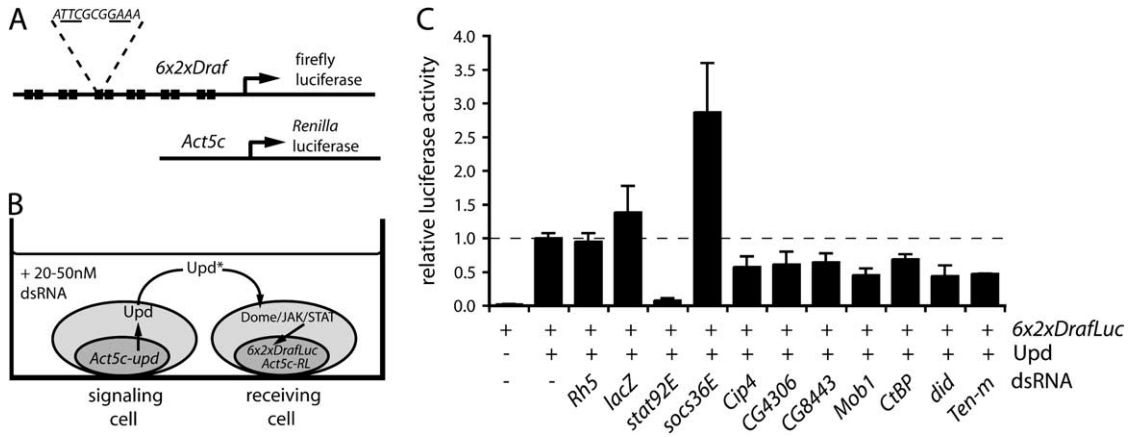


FIGURE 2.—Identification of pathway modifiers using a cell-based RNAi assay. (A) Diagrammatic of the *6x2xDraf* Luc STAT reporter construct containing 12 STAT92E binding sites (shown with essential residues underlined) and *pAct5C-RL* used as transfection control. (B) Outline of the paracrine assay system. (C) The effect of dsRNA treatment on the paracrine mode of pathway activation. JAK/STAT pathway activity in each case is shown as fold luciferase activity, and mock transfection corresponds to the basal level of pathway activity with full reporter activity defined as 1 (dashed line). dsRNA-targeting *Rh5* and *lacZ* show no effect, *stat92E* dsRNA treatment results in an almost basal level of reporter activity, and dsRNA treatment knocking down *socs36E* results in threefold activation. The seven candidate genes identified in this screen show statistically significant reduction in reporter activity: *cip4*, $P = 0.0002$; *CG4306*, $P = 0.001$; *CG8443*, $P = 0.0007$; *Mob1*, $P = 0.001$; *CtBP*, $P = 0.05$; *did*, $P = 0.015$; and *Ten-m*, $P = 0.012$.

sufficient to reduce reporter activity to almost basal levels. Conversely, knockdown of the negative regulator *socs36E* boosted reporter activity almost threefold (Figure 2C). These results serve to validate the assay and suggest that potential positive and negative regulators of the pathway can be identified using this technique.

We therefore synthesized dsRNA targeting the 18 candidate genes selected for further analysis (underlined in Table 2) and tested these in the assay described above. These assays identified seven dsRNAs, which statistically significantly ($P < 0.05$) reduced the level of reporter activity (Figure 2C), a result consistently reproduced in multiple experiments. Given this interaction, it is likely that the seven identified interacting dsRNAs target the transcripts of genes encoding potential pathway components and/or regulators. The remaining 11 loci for which no consistent effect was observed (not shown) may represent genes functioning at other levels.

Although this tissue-culture-based assay gives a clear result for interacting loci, lack of interaction is not sufficient to disprove a potential role. For example, it is possible that the noninteracting loci may constitute pathway components that are not required or expressed in $Kc_{167}$ cells but are necessary for full activity in the developing eye disc. Alternatively, it is also possible that the dsRNA treatment used did not adequately knock down the activity of all targeted transcripts.

**Characterization of modifiers—*in situ* hybridization:** To represent credible candidates, the identified genes must be expressed within the developing eye imaginal disc during the stages when additional *P{w⁺, GMR-updΔ3′}*-induced overproliferation occurs. In addition, it is possible that the screen has identified pathway target genes whose expression is either directly or indirectly modulated by STAT92E activity and whose activity is required for the proliferative cellular response. To address both questions, we examined the expression pattern of the genes identified by *in situ* hybridization to their mRNAs in both wild type and *P{w⁺, GMR-updΔ3′}/+* late third instar eye-antennal imaginal discs (Figures 3 and 4).

As expected for genuinely interacting genes, antisense RNA probes used for *in situ* staining showed that all identified candidates are expressed in wild-type eye-imaginal discs. Although some interacting genes are expressed only at low levels, a large proportion are upregulated in or ahead of the morphogenetic furrow (Figures 3 and 4). By contrast, control sense RNA probes showed no or only low-level background staining (not shown). Of the loci examined, 12 are expressed at essentially equivalent levels in both wild-type and *P{w⁺, GMR-updΔ3′}/+* eye discs (Figure 3). However, the expression of *CtBP*, *trbl*, *mthl-8*, *CG3305*, *Ten-m*, and *Mob1* is clearly modified by ectopic JAK/STAT signaling identifying them as potential pathway target genes (Figure 4). Of this group, expression of *CtBP*, *trbl*, *mthl-8*, and *CG3305* were upregulated in *P{w⁺, GMR-updΔ3′}* eye discs (Figure 4, A–H), an effect consistent with the known role of STAT92E as a transcriptional activator. By contrast, both *Ten-m* and *Mob1* show a clear and consistent decrease in expression associated with *upd* misexpression (Figure 4, I–L). Although this apparent negative regulation is unexpected, it is possible that it may represent an indirect effect of pathway signaling.

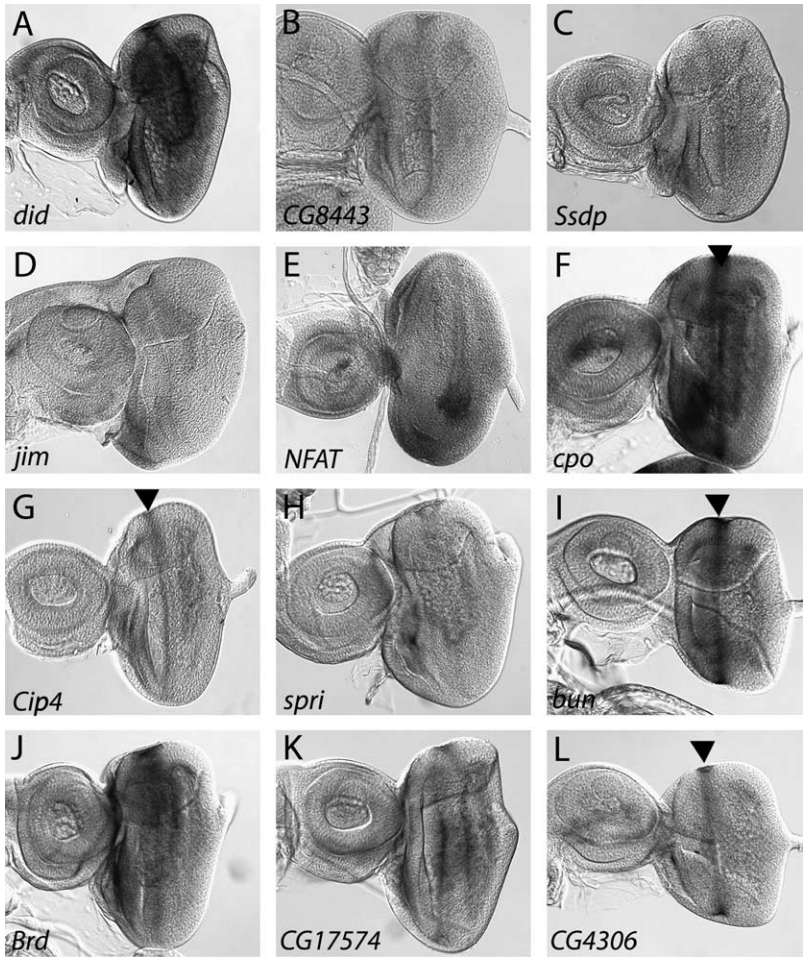


Figure 3.—Expression of candidate genes in wild-type third instar larval eye-imaginal discs. All the genes are expressed in wandering third instar eye discs; in some, expression is also detected in the morphogenetic furrow (MF; arrowheads). The expression pattern of these genes does not change in *P{w⁺, GMR-updΔ3′}* eye discs (data not shown). Discs are shown with anterior at the left and dorsal at the top. *In situ* antisense probes: *did* (A); *CG8443* (B); *Ssdp* (C); *jim* (E); *NFAT* (E); *cpo* (F); *Cip4* (G); *Sprint* (H); *bun* (I); *Brd* (J); *CG17574* (K); *CG4306* (L).

It should, however, be noted that the ability of *P{w⁺, GMR-updΔ3′}* to alter the expression pattern of the six interacting genes does not prove that these loci are normally the target of endogenous pathway activity.

***P {w⁺, GMR-updΔ3′}* and signal transduction pathways:** One intriguing aspect of the genes identified was the interaction with *bearded*, a component of the *Notch* (*N*) pathway, and *bunched*, a positive regulator of the *decapentalplegic* (*dpp*) signal transduction pathway. These results suggest that cellular proliferation induced by ectopic *upd* expression is also sensitive to inputs from other signal transduction pathways. Such effects may result from direct interaction or may be the result of coregulation of common pathway target genes involved in cellular proliferation. While a genetic interaction between the *P{w⁺, GMR-updΔ3′}* eye overgrowth phenotype and Dpp pathway members has already been observed (Bach *et al.* 2003), the interaction with Notch signaling components has not been previously described. We therefore tested other members of the Notch pathway to determine if mutations in these components show similar interactions. Consistent with our original identification of an allele of *bearded*, mutations in the Notch receptor and Delta ligand also suppress eye overgrowth, although the Serrate ligand does not appear to interact (Table 3). Although this finding is consistent with reports from vertebrate systems in which activation of STAT3 by Notch has been demonstrated (Kamakura *et al.* 2004), it is also possible that the coregulation of common target genes may explain this interaction. One precedent for such coregulation is the expression of *four-jointed* in the developing eye disc, which not only requires JAK/STAT pathway activity but also integrates Notch and Wingless signaling (Zeidler *et al.* 2000b).

***P{w⁺, GMR-updΔ3′}* and cell cycle components:** Given the increase in cellular proliferation associated with the eye overgrowth phenotype used in the screen, we were intrigued that no mutations in components of the core cell cycle machinery had been identified. However, given the nonsaturating nature of our mutagenesis, it is possible that such alleles were not included in the collection of mutated chromosomes screened. To address this question, we therefore tested for potential interactions with alleles of known cell cycle components (Table 4). Although weak interaction was observed for some alleles, the majority of mutations removing *diminutive*, *string*, *CyclinA*, *CyclinB*, *CyclinB3*, *CyclinD*, *CyclinE*, *E2f transcription factor*, *roughex*, *p53*, *dacapo*, *gigas*, *Dichaete*, or the cyclin-dependent kinase *cdc2* showed no consistent

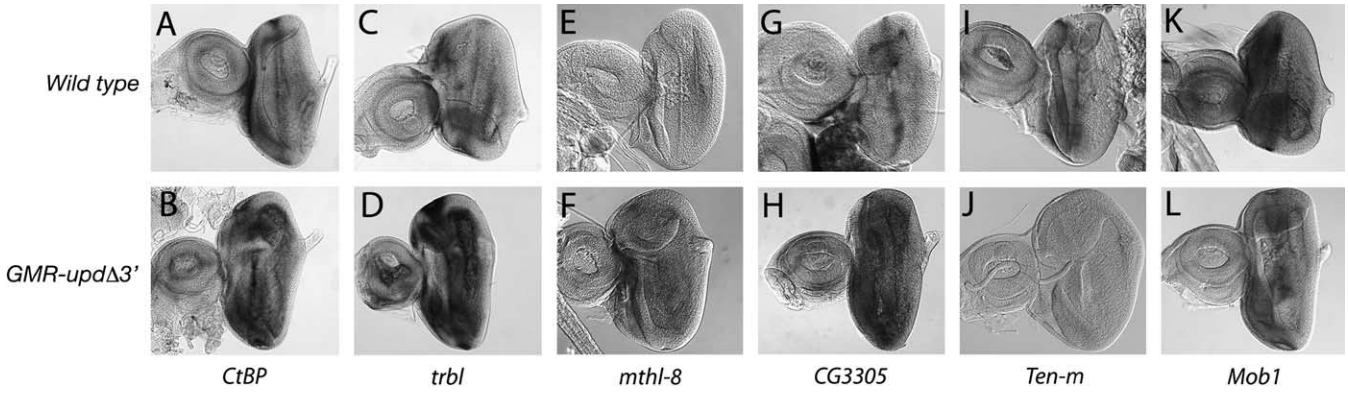


FIGURE 4.—Potential JAK/STAT pathway target genes. Expression patterns of candidate genes in wild-type (top row) and *P{w⁺,GMR-updΔ3′}*/+ (bottom row) eye discs identified six potential pathway target genes. *CtBP*, *trbl*, *mthl-8*, and *CG3305* are expressed in wild-type eye discs and are upregulated in *P{w⁺, GMR-updΔ3′}*/+ eye discs (A and B, C and D, E and F, G and H). *Ten-m* is expressed uniformly in a wild-type eye disc with stronger staining in the MF (I); by comparison, expression in *P{w⁺, GMR-updΔ3′}*/+ eye discs is below detectable levels (J). *Mob1* expression is also detected in wild-type eye discs with higher levels of expression ahead of the MF (K). The expression ahead of the MF is strongly reduced in *P{w⁺, GMR-updΔ3′}* eye discs (L). Anterior is at the left; dorsal is at the top. All experimental pairs of wild-type and *P{w⁺, GMR-updΔ3′}*/+ eye discs were stained for the same time under identical conditions.

interaction with *P{w⁺, GMR-updΔ3′}* (Table 4). Furthermore, although the *cdk4³* allele previously reported as interacting with STAT92E (CHEN *et al.* 2003) was classified as a weak suppressor, other independently generated *cdk4* mutations produced no consistent interactions (Table 4).

## DISCUSSION

We have used a genetic approach to identify regulators of the Drosophila JAK/STAT signal transduction pathway. Using an *in vivo* eye overgrowth assay, we screened 2267 independent *P*-element insertions and identified 23 loci responsible for the modification of the overgrown eye phenotype associated with *P{w⁺, GMR-updΔ3′}*. Using a quantitative cell-based STAT92E activity assay, we have determined that seven of these candidates are likely to be potential pathway components/regulators. In addition, *in situ* hybridization was used to show that expression of six of these genes is modulated in response to pathway activity and is likely to represent direct or indirect pathway target genes.

The loci identified represent new components and modulators of the JAK/STAT signal transduction pathway, most of which have not previously been identified as such. Our analysis of the genes, in conjunction with their known biological roles, allow the candidates to be subdivided into a number of classes.

**Cell cycle proteins:** The eye overgrowth induced by *P{w⁺, GMR-updΔ3′}* results from additional rounds of mitosis in eye-imaginal disc cells anterior to the morphogenetic furrow (BACH *et al.* 2003). Despite the ectopic JAK/STAT pathway activation caused by the misexpression of *upd*, these cells are patterned essentially normally and go on to form an increased number of ommatidia in the *P{w⁺, GMR-updΔ3′}* eye disc (BACH *et al.* 2003). Despite this proliferation-dependent phenotype, core cell cycle regulatory proteins failed to show consistent interactions when assayed as part of a candidate approach (Table 4). While unexpected, this result suggests that the core cell cycle regulatory proteins do not represent components that become rate limiting in the proliferative environment tested.

Despite the lack of interaction with core cell cycle components, alleles of *did*, *trbls*, and *Mob1* were identified as modifiers of the overgrown eye phenotype. Indeed, homozygous *did* mutants have been described as having small imaginal discs (GATTI and BAKER 1989), and a phenotype similar to that is observed in *hop^M13* mutant third instar larval discs (PERRIMON and MAHOWALD 1986; MUKHERJEE *et al.* 2005). While not central to cell cycle progression, these loci appear to be involved

**TABLE 3**

***P{w⁺*, GMR-updΔ*3′}* and Notch signaling pathway components**

| Gene | Allele | *GMR-upd* | Comments |
|---|---|---|---|
| *stat92E* | *06346* | −−− | Positive control |
| *Notch* (*N*) | *I1N* | −− | Temperature-sensitive allele raised at partially permissive temperature (25°) |
| *Delta* (*Dl*) | *9P39* | −−− | |
| | *3* | −−− | |
| | *RevF10* | +/− | |
| *Serate* (*Ser*) | *Bd-3* | +/− | |
| *Bearded* (*Brd*) | *F716.1* | − | Allele identified in this report |
| | *1* | −−− | |
| | *BG02319* | −− | |

+/−, no interaction; −, weak suppressor; −−, moderate suppressor; −−−, strong suppressor.

**TABLE 4**

***P{w+, GMR-updΔ3′}* and cell cycle components**

| Gene | Allele | *GMR-upd* | Comments/ references |
|---|---|---|---|
| *diminutive* (*dm*) | *1* | − | |
| *cdc2* | *2* | +/− | |
| | *3* | − | |
| *string* (*stg*) | *4* | +/− | Drosophila Cdc25 Homolog |
| | *01235* | +/− | |
| *CyclinA* (*CycA*) | *03946* | + | |
| | *C8LR1* | +/− | |
| *CyclinB* (*CycB*) | *2* | +/− | |
| *CyclinB3* (*CycB3*) | *2* | +/− | |
| *CyclinD* (*CycD*) | *KG04817* | +/− | |
| *Cyclin E* (*CycE*) | *AR95* | +/− | |
| | *05206* | +/− | |
| | *k05007* | +/− | |
| *Cyclin-dependent kinase 4* (*Cdk4*) | *3* | − | *cdk4* has been previously reported as interacting with *stat92E* (Chen *et al.* 2003) |
| | *k06503* | +/− | |
| | *s4639* | +/− | |
| *DP transcription factor* (*Dp*) | *49Fk-1* | +/− | |
| *E2F* | *07172* | +/− | |
| *roughex* (*rux*) | *1* | +/− | |
| | *2* | − | |
| *p53* | *5A-1-4* | +/− | |
| *dacapo* (*dap*) | *4* | +/− | |
| | *04454* | +/− | |
| | *07309* | − | |
| *gigas* (*gig*) | *109* | +/− | Cell-size regulator |
| *Dichaete* (*D*) | *r8* | +/− | |

+/−, no interaction; +, mild enhancer; −, mild suppressor.

in its regulation and may imply that the interaction between JAK/STAT signaling and cellular proliferation is indirect.

Of particular interest are the inconsistent interactions observed between *Cdk4* alleles. Although *cdk4* represents the only Drosophila component of the cell cycle machinery proposed to interact with the JAK/STAT pathway (Chen *et al.* 2003), our assay identified only one of the three alleles tested as a weak suppressor of the eye overgrowth phenotype (Table 4). Previous studies did not utilize loss-of-function experiments but rather utilized the converse approach. When misexpressed by a *P{w+, GMR-Gal4}* driver, the coexpression of *P{w+, UAS-CycD}*, *P{w+, UAS-Cdk4}*, and *P{w+, UAS-upd}* dramatically enhanced the eye overgrowth phenotype over that mediated by *P{w+, UAS-upd}* or *P{w+, UAS-CycD}* and *P{w+, UAS-Cdk4}* alone (Chen *et al.* 2003). Although it is possible that loss of a single copy of the *cdk4* locus does not reduce protein levels below a rate-limiting threshold, the inconsistency of interactions produced by multiple *cdk4* alleles is puzzling and true existence or nature of any potential interaction between JAK/STAT signaling and endogenous Cdk4 remains to be established.

**Transcription factors and coregulators:** We have identified a number of transcription factors as interacting loci in our screen. One of these is the Drosophila homolog of the *n*uclear *f*actor of *a*ctivated *T*-cells (NFAT), a locus originally identified as an inducer of cytokine gene expression (Shaw *et al.* 1988). Intriguingly, it has been shown that human NFAT, in conjunction with NF-κB, AP-1, and STATs, represents factors involved in mediating cytokine and T-cell-receptor-induced interferon-γ signaling (Malmgaard 2004). Intriguingly, activation of these transcription factors results in the production of numerous intrinsic antiviral factors in the vertebrate system, a role that has also been shown to depend on JAK/STAT signaling within Drosophila fat-body cells (Agaisse *et al.* 2003). Although further analysis of this interaction is required, this is the first report that suggests an evolutionarily conserved link between NFAT and JAK/STAT signaling in Drosophila.

*C-terminal binding protein* (*CtBP*), a transcriptional corepressor previously characterized as an enhancer of the Drosophila JAK/STAT pathway (Bach *et al.* 2003), has also been identified in our screen. While not all alleles of *CtBP* show consistent interaction with *P{w+, GMR-updΔ3′}* (Table 2), cell culture assays utilizing dsRNA-mediated knockdown imply that *CtBP* is a component of the JAK/STAT pathway, which acts as a positive regulator of signaling (Figure 2C). In addition, an independent genomewide RNAi-based screen for JAK/STAT pathway interactors also identified dsRNAs targeting *CtBP* as a suppressor of pathway signaling (Müller *et al.* 2005). Finally, an upregulation of *CtBP* transcript is observed in *P{w+, GMR-updΔ3′}* eye discs compared to wild-type eyes (Figure 4, A and B). Given the results from cell-based assays and *in situ* analysis, it appears most likely that CtBP does indeed represent a positive regulator of JAK/STAT pathway activity. This finding is particularly surprising, given the previously identified role for CtBP as a transcriptional repressor, which, in combination with the Groucho corepressor, is involved in repressing Su(H)-mediating expression of Notch pathway target genes (Barolo *et al.* 2002). The significance of our result, however, remains to be determined and it is conceivable that the observed interaction with the eye overgrowth phenotype represents an indirect effect, possibly via interaction with Notch pathway signaling activity.

**Extracellular proteins:** One aspect of the screen undertaken is the paracrine mode of Upd signaling required for cellular overproliferation. In the *P{w+, GMR-updΔ3′}* eye, the region of *upd* expression is spatially separate from the domain in which increased levels of cellular proliferation are observed (Bach *et al.* 2003;

Tsai and Sun 2004) and the ligand must therefore be able to move to and activate the pathway in neighboring cells. Although it has been shown that Unpaired represents a secreted extracellular signaling molecule that is both post-translationally glycosylated and able to associate with the extracellular matrix (ECM) (Harrison *et al.* 1998), very little is known regarding the mechanisms regulating these processes.

One class of molecules previously shown to be involved in the extracellular trapping and movement of signaling ligands is the heparan sulfate proteoglycans (HSPGs) Dally, Dally-like, Perlecan, and Syndecan (Principalle and de Agostini 2002). These molecules, and their extensive post-translational modifications, not only play important roles in providing shape and biomechanical strength to organs and tissues, but also have been shown to be required for the transduction of signaling by the Wingless, Hedgehog, and the FGF-like ligands Heartless and Breathless (Lin *et al.* 1999; The *et al.* 1999; Baeg *et al.* 2004). Despite the significance of HSPGs for the transduction of these ligands, mutations in the HSPGs themselves, as well as mutations in the HSPG-modifying enzymes *sugarless* and *sulphateless*, do not appear to interact with the eye overgrowth phenotypes associated with *P{w*$^{+}$*, GMR-upd*$\Delta$*3′}* (not shown; E. Selva, personal communication) and suggest that Upd is likely to interact with the ECM via different mechanisms. One potential component of this alternative mechanism identified in our screen is *Tenascin-major* (*Ten-m*). *Ten-M*, also known as *odd Oz* (Dgany and Wides 2002), encodes an extracellular adhesion molecule that was also classified as a component of the JAK/STAT pathway in the tissue-culture-based paracrine signaling assay (Figure 2C). Although the tissue culture results imply a direct function of the molecule in pathway signaling, further analysis of the role of *Ten-m* in controlling the secretion and/or movement of Upd remains to be determined *in vivo*.

**Signaling pathways:** The Drosophila eye is dispensable in a laboratory environment and sensitized genetic screens that compromise its function have proven to be powerful tools for the identification of signal transduction pathway components (Dickson *et al.* 1996; Karim *et al.* 1996; Bach *et al.* 2003). Drosophila eye development is, however, a complex process involving multiple signal transduction pathways including EGFR, Hh, Notch, Dpp, and Wingless. A number of examples of interactions between these pathways and JAK/STAT signaling have been described. For example, a gradient of *four-jointed* in the developing eye disc is determined by the coordinated activities of Notch, Wingless, and JAK/STAT pathways (Zeidler *et al.* 1999a). Also, at the posterior dorso/ventral border of the eye, *Notch* and *eye gone* (*eyg*) have been shown to cooperatively induce expression of *upd*, which then acts to promote cell proliferation (Chao *et al.* 2004). Consistent with these complex interactions, our screen has identified *Bunched* (*bun*), a member of the Dpp signal transduction pathway (Dobens *et al.* 2000), and *Bearded* (*brd*), a member of the Notch signaling pathway (Lai *et al.* 2000). *bunched* is a transcription factor that genetically interacts with *dpp* (Treisman *et al.* 1995; Dobens *et al.* 2000). Strikingly, Dpp pathway components have previously been reported as modulators of the *P{w*$^{+}$*, GMR-upd*$\Delta$*3′}* eye phenotype, with hypomorphic alleles of *dpp* and *Mothers against dpp* (*Mad*) representing strong suppressors of eye overgrowth (Bach *et al.* 2003). Similar interactions in mammalian systems have identified the synergistic activity of STAT3 and Smad1 in the differentiation of astrocytes from their progenitor cells. These proteins, however, do not physically interact, but bind to p300/CBP to promote the transactivation of target genes (Nakashima *et al.* 1999).

Finally, our screen also identified *mth-like8*, a seven-pass *trans*-membrane protein with predicted G-protein-coupled receptor activity. Although expression of *mth-like8* changes in response to JAK/STAT pathway activation (Figure 4), an in-depth analysis of its interaction remains to be undertaken.

**Summary:** As is no doubt the case for all signaling pathways required during development, the JAK/STAT cascade does not function in isolation, and cross-talk between multiple interacting loci is likely to be involved in generating developmental responses by this apparently "simple" pathway. However, the identity of many of these interacting partners is as yet largely unknown. Although traditional forward genetic analysis using transposon-mediated mutagenesis is almost impossible to drive to saturation, the rapidity with which mutated genes can be identified makes this approach appealing. In particular, when combined with reverse genetic approaches such as RNAi, candidate interactions can be rapidly validated. The combination of forward and reverse genetic techniques used here has identified a number of diverse loci involved in transducing and regulating JAK/STAT signaling *in vivo*. Given the significance of the pathway during development and its implication in human malignancies, it is hoped that a future detailed analysis of these gene products will provide the foundation for a better understanding of this signal transduction cascade.

We are grateful to Erika Bach, the Bloomington Stock Center, Alan Spradling, Ron Wides, and Pernilla Roth for fly stocks. Iris Plischke, Meike Werner, and Sabine Häder provided valuable technical assistance. M.P.Z. is supported by a Deutsche Forschungsgemeinschaft Emmy Noether fellowship, U.S. received funding from the Deutsches Human Genome Project grant to H. Jäckle, and T.M. is funded by a Max Planck Society predoctoral fellowship.

## LITERATURE CITED

Adams, M. D., S. E. Celniker, R. A. Holt, C. A. Evans, J. D. Gocayne *et al.*, 2000 The genome sequence of *Drosophila melanogaster*. Science **287:** 2185–2195.

Agaisse, H., U. M. Petersen, M. Boutros, B. Mathey-Prevot and N. Perrimon, 2003 Signaling role of hemocytes in *Drosophila*

JAK/STAT-dependent response to septic injury. Dev. Cell **5:** 441–450.

Alifragis, P., G. Poortinga, S. M. Parkhurst and C. Delidakis, 1997 A network of interacting transcriptional regulators involved in *Drosophila* neural fate specification revealed by the yeast two-hybrid system. Proc. Natl. Acad. Sci. USA **94:** 13099–13104.

Bach, E. A., S. Vincent, M. P. Zeidler and N. Perrimon, 2003 A sensitized genetic screen to identify novel regulators and components of the Drosophila JAK/STAT pathway. Genetics **165:** 1149–1166.

Baeg, G. H., E. M. Selva, R. M. Goodman, R. Dasgupta and N. Perrimon, 2004 The Wingless morphogen gradient is established by the cooperative action of Frizzled and heparan sulfate proteoglycan receptors. Dev. Biol. **276:** 89–100.

Barolo, S., T. Stone, A. G. Bang and J. W. Posakony, 2002 Default repression and Notch signaling: Hairless acts as an adaptor to recruit the corepressors Groucho and dCtBP to Suppressor of Hairless. Genes Dev. **16:** 1964–1976.

Baumgartner, S., D. Martin, C. Hagios and R. Chiquet-Ehrismann, 1994 *Tenm*, a *Drosophila* gene related to *tenascin*, is a new pair-rule gene. EMBO J. **13:** 3728–3740.

Beccari, S., L. Teixeira and P. Rorth, 2002 The JAK/STAT pathway is required for border cell migration during *Drosophila* oogenesis. Mech. Dev. **111:** 115–123.

Beinert, N., M. Werner, G. Dowe, H. R. Chung, H. Jackle *et al.*, 2004 Systematic gene targeting on the X chromosome of *Drosophila melanogaster*. Chromosoma **113:** 271–275.

Bellen, H. J., H. Vaessin, E. Bier, A. Kolodkin, D. D'Evelyn *et al.*, 1992 The Drosophila *couch potato* gene: an essential gene required for normal adult behavior. Genetics **131:** 365–375.

Betz, A., N. Lampen, S. Martinek, M. W. Young and J. E. Darnell, Jr., 2001 A *Drosophila* PIAS homologue negatively regulates *stat92E*. Proc. Natl. Acad. Sci. USA **98:** 9563–9568.

Binari, R., and N. Perrimon, 1994 Stripe-specific regulation of pair-rule genes by *hopscotch*, a putative Jak family tyrosine kinase in *Drosophila*. Genes Dev. **8:** 300–312.

Boulay, J. L., J. J. O'Shea and W. E. Paul, 2003 Molecular phylogeny within type I cytokines and their cognate receptors. Immunity **19:** 159–163.

Bowman, T., R. Garcia, J. Turkson and R. Jove, 2000 STATs in oncogenesis. Oncogene **19:** 2474–2488.

Brody, T., and A. Cravchik, 2000 *Drosophila melanogaster* G-protein-coupled receptors. J. Cell Biol. **150:** F83–F88.

Bromberg, J., 2002 Stat proteins and oncogenesis. J. Clin. Invest. **109:** 1139–1142.

Brown, S., N. Hu and J. C. Hombria, 2001 Identification of the first invertebrate interleukin JAK/STAT receptor, the *Drosophila* gene *domeless*. Curr. Biol. **11:** 1700–1705.

Celniker, S. E., D. A. Wheeler, B. Kronmiller, J. W. Carlson, A. Halpern *et al.*, 2002 Finishing a whole-genome shotgun: release 3 of the *Drosophila melanogaster* euchromatic genome sequence. Genome Biol. **3:** RESEARCH0079.0071–0079.0014.

Chao, J. L., Y. C. Tsai, S. J. Chiu and Y. H. Sun, 2004 Localized Notch signal acts through *eyg* and *upd* to promote global growth in *Drosophila* eye. Development **131:** 3839–3847.

Chen, H. W., X. Chen, S. W. Oh, M. J. Marinissen, J. S. Gutkind *et al.*, 2002 *mom* identifies a receptor for the *Drosophila* JAK/STAT signal transduction pathway and encodes a protein distantly related to the mammalian cytokine receptor family. Genes Dev. **16:** 388–398.

Chen, L., D. Segal, N. A. Hukriede, A. V. Podtelejnikov, D. Bayarsaihan *et al.*, 2002 Ssdp proteins interact with the LIM-domain-binding protein Ldb1 to regulate development. Proc. Natl. Acad. Sci. USA **99:** 14320–14325.

Chen, X., S. W. Oh, Z. Zheng, H. W. Chen, H. H. Shin *et al.*, 2003 Cyclin D-Cdk4 and cyclin E-Cdk2 regulate the Jak/STAT signal transduction pathway in *Drosophila*. Dev. Cell **4:** 179–190.

Cherbas, P., L. Cherbas and C. M. Williams, 1977 Induction of acetylcholinesterase activity by beta-ecdysone in a *Drosophila* cell line. Science **197:** 275–277.

Chung, C. D., J. Liao, B. Liu, X. Rao, P. Jay *et al.*, 1997 Specific inhibition of Stat3 signal transduction by PIAS3. Science **278:** 1803–1805.

Clemens, J. C., C. A. Worby, N. Simonson-Leff, M. Muda, T. Maehama *et al.*, 2000 Use of double-stranded RNA interference in *Drosophila* cell lines to dissect signal transduction pathways. Proc. Natl. Acad. Sci. USA **97:** 6499–6503.

Crisp, J., and J. Merriam, 1997 Efficiency of an F1 selection screen in a pilot two-component mutagenesis involving *Drosophila melanogaster* misexpression phenotypes. Dros. Inf. Serv. **80:** 90–92.

Dgany, O., and R. Wides, 2002 The *Drosophila odz/ten-m* gene encodes a type I, multiply cleaved heterodimeric transmembrane protein. Biochem J. **363:** 633–642.

Dickson, B. J., A. van der Straten, M. Dominguez and E. Hafen, 1996 Mutations modulating Raf signaling in Drosophila eye development. Genetics **142:** 163–171.

Dobens, L. L., J. S. Peterson, J. Treisman and L. A. Raftery, 2000 *Drosophila bunched* integrates opposing DPP and EGF signals to set the operculum boundary. Development **127:** 745–754.

Drysdale, R. A., M. A. Crosby, W. Gelbart, K. Campbell, D. Emmert *et al.*, 2005 FlyBase: genes and gene models. Nucleic Acids Res. **33:** D390–D395.

Ellis, M. C., E. M. O'Neill and G. M. Rubin, 1993 Expression of *Drosophila* Glass protein and evidence for negative regulation of its activity in non-neuronal cells by another DNA-binding protein. Development **119:** 855–865.

Friedmann, M. C., T. S. Migone, S. M. Russell and W. J. Leonard, 1996 Different interleukin 2 receptor beta-chain tyrosines couple to at least two signaling pathways and synergistically mediate interleukin 2-induced proliferation. Proc. Natl. Acad. Sci. USA **93:** 2077–2082.

Gatti, M., and B. S. Baker, 1989 Genes controlling essential cell-cycle functions in *Drosophila melanogaster*. Genes Dev. **3:** 438–453.

Häcker, U., and N. Perrimon, 1998 *DRhoGEF2* encodes a member of the *dbl* family of oncogenes and controls cell shape changes during gastrulation in *Drosophila*. Genes Dev. **12:** 274–284.

Hari, K. L., K. R. Cook and G. H. Karpen, 2001 The *Drosophila Su(var)2–10* locus regulates chromosome structure and function and encodes a member of the PIAS protein family. Genes Dev. **15:** 1334–1348.

Harrison, D. A., P. E. McCoon, R. Binari, M. Gilman and N. Perrimon, 1998 *Drosophila unpaired* encodes a secreted protein that activates the JAK signaling pathway. Genes Dev. **12:** 3252–3263.

Hombria, J. C., and S. Brown, 2002 The fertile field of *Drosophila* Jak/STAT signalling. Curr. Biol. **12:** R569–R575.

Hombria, J. C., S. Brown, S. Häeder and M. P. Zeidler, 2005 Characterisation of Upd2, a *Drosophila* JAK/STAT pathway ligand. Dev. Biol. **288:** 420–433.

Hou, X. S., M. B. Melnick and N. Perrimon, 1996 *marelle* acts downstream of the *Drosophila* HOP/JAK kinase and encodes a protein similar to the mammalian STATs. Cell **84:** 411–419.

James, C., V. Ugo, J. P. Le Couedic, J. Staerk, F. Delhommeau *et al.*, 2005 A unique clonal *JAK2* mutation leading to constitutive signalling causes polycythaemia vera. Nature **434:** 1144–1148.

Johansen, K. A., D. D. Iwaki and J. A. Lengyel, 2003 Localized JAK/STAT signaling is required for oriented cell rearrangement in a tubular epithelium. Development **130:** 135–145.

Josten, F., B. Fuss, M. Feix, T. Meissner and M. Hoch, 2004 Cooperation of JAK/STAT and Notch signaling in the *Drosophila* foregut. Dev. Biol. **257:** 181–189.

Kamakura, S., K. Oishi, T. Yoshimatsu, M. Nakafuku, N. Masuyama *et al.*, 2004 Hes binding to STAT3 mediates crosstalk between Notch and JAK-STAT signalling. Nat. Cell Biol. **6:** 547–554.

Karim, F. D., H. C. Chang, M. Therrien, D. A. Wassarman, T. Laverty *et al.*, 1996 A screen for genes that function downstream of Ras1 during Drosophila eye development. Genetics **143:** 315–329.

Karsten, P., S. Hader and M. P. Zeidler, 2002 Cloning and expression of *Drosophila* SOCS36E and its potential regulation by the JAK/STAT pathway. Mech. Dev. **117:** 343–346.

Kiger, A. A., D. L. Jones, C. Schulz, M. B. Rogers and M. T. Fuller, 2001 Stem cell self-renewal specified by JAK-STAT activation in response to a support cell cue. Science **294:** 2542–2545.

Kisseleva, T., S. Bhattacharya, J. Braunstein and C. W. Schindler, 2002 Signaling through the JAK/STAT pathway, recent advances and future challenges. Gene **285:** 1–24.

Kwon, E. J., H. S. Park, Y. S. Kim, E. J. Oh, Y. Nishida *et al.*, 2000 Transcriptional regulation of the *Drosophila raf* proto-oncogene by *Drosophila* STAT during development and in immune response. J. Biol. Chem. **275:** 19824–19830.

Lacronique, V., A. Boureux, V. D. Valle, H. Poirel, C. T. Quang *et al.*, 1997 A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia. Science **278:** 1309–1312.

Lai, E. C., R. Bodner, J. Kavaler, G. Freschi and J. W. Posakony, 2000 Antagonism of Notch signaling activity by members of a novel protein family encoded by the *bearded* and *enhancer of split* gene complexes. Development **127:** 291–306.

Lasko, P., 2000 The *Drosophila melanogaster* genome: translation factors and RNA binding proteins. J. Cell Biol. **150:** F51–F56.

Lehmann, R., and D. Tautz, 1994 *In situ* hybridisation to RNA, pp. 576–598 in *Drosophila melanogaster: Practical Uses in Cell and Molecular Biology*, edited by L. S. B. Goldstein and E. A. Fryberg. Academic Press, San Diego.

Levy, D. E., and J. E. Darnell, Jr., 2002 Stats: transcriptional control and biological impact. Nat. Rev. Mol. Cell Biol. **3:** 651–662.

Lin, X., E. M. Buff, N. Perrimon and A. M. Michelson, 1999 Heparan sulfate proteoglycans are essential for FGF receptor signaling during *Drosophila* embryonic development. Development **126:** 3715–3723.

Luca, F. C., M. Mody, C. Kurischko, D. M. Roof, T. H. Giddings *et al.*, 2001 *Saccharomyces cerevisiae* Mob1p is required for cytokinesis and mitotic exit. Mol. Cell. Biol. **21:** 6972–6983.

Luo, H., P. Rose, D. Barber, W. P. Hanratty, S. Lee *et al.*, 1997 Mutation in the *Jak* kinase JH2 domain hyperactivates *Drosophila* and mammalian Jak-Stat pathways. Mol. Cell. Biol. **17:** 1562–1571.

Luque, T., L. Hjelmqvist, G. Marfany, O. Danielsson, M. El-Ahmad *et al.*, 1998 Sorbitol dehydrogenase of *Drosophila*. Gene, protein, and expression data show a two-gene system. J. Biol. Chem. **273:** 34293–34301.

Malmgaard, L., 2004 Induction and regulation of IFNs during viral infections. J. Interferon Cytokine Res. **24:** 439–454.

McGregor, J. R., R. Xi and D. A. Harrison, 2002 JAK signaling is somatically required for follicle cell differentiation in *Drosophila*. Development **129:** 705–717.

Mohr, S. E., and R. E. Boswell, 1999 *Zimp* encodes a homologue of mouse *Miz1* and *PIAS3* and is an essential gene in *Drosophila melanogaster*. Gene **229:** 109–116.

Mukherjee, T., J. C. Hombria and M. P. Zeidler, 2005 Opposing roles for *Drosophila* JAK/STAT signalling during cellular proliferation. Oncogene **24:** 2503–2511.

Müller, P., D. Kuttenkeuler, V. Gesellchen, M. P. Zeidler and M. Boutros, 2005 Identification of JAK/STAT signalling components by genome-wide RNAi. Nature **436:** 871–875.

Nakashima, K., M. Yanagisawa, H. Arakawa, N. Kimura, T. Hisatsune *et al.*, 1999 Synergistic signaling in fetal brain by STAT3-Smad1 complex bridged by p300. Science **284:** 479–482.

Perrimon, N., and A. P. Mahowald, 1986 *l(1)hopscotch*, a larval-pupal zygotic lethal with a specific maternal effect on segmentation in *Drosophila*. Dev. Biol. **118:** 28–41.

Platanias, L. C, 2005 Mechanism of type-I and type-II-interferon-mediated signalling. Nat. Rev. Immunol. **5:** 375–386.

Princivalle, M., and A. de Agostini, 2002 Developmental roles of heparan sulfate proteoglycans: a comparative review in *Drosophila*, mouse and human. Int. J. Dev. Biol. **46:** 267–278.

Rawlings, J. S., G. Rennebeck, S. M. Harrison, R. Xi and D. A. Harrison, 2004 Two *Drosophila* suppressors of cytokine signaling (SOCS) differentially regulate JAK and EGFR pathway activities. BMC Cell Biol. **5:** 38.

Rebay, I., and G. M. Rubin, 1995 Yan functions as a general inhibitor of differentiation and is negatively regulated by activation of the Ras1/MAPK pathway. Cell **81:** 857–866.

Sefton, L., J. R. Timmer, Y. Zhang, F. Beranger and T. W. Cline, 2000 An extracellular activator of the *Drosophila* JAK/STAT pathway is a sex-determination signal element. Nature **405:** 970–973.

Seher, T. C., and M. Leptin, 2000 Tribbles, a cell-cycle brake that coordinates proliferation and morphogenesis during *Drosophila* gastrulation. Curr. Biol. **10:** 623–629.

Shaw, J. P., P. J. Utz, D. B. Durand, J. J. Toole, E. A. Emmel *et al.*, 1988 Identification of a putative regulator of early T cell activation genes. Science **241:** 202–205.

Sternberg, D. W., and D. G. Gilliland, 2004 The role of signal transducer and activator of transcription factors in leukemogenesis. J. Clin. Oncol. **22:** 361–371.

Szabo, K., G. Jekely and P. Rorth, 2001 Cloning and expression of *sprint*, a *Drosophila* homologue of *RIN1*. Mech. Dev. **101:** 259–262.

Tang, A. H., T. P. Neufeld, G. M. Rubin and H. A. Muller, 2001 Transcriptional regulation of cytoskeletal functions and segmentation by a novel maternal pair-rule gene, *lilliputian*. Development **128:** 801–813.

The, I., Y. Bellaiche and N. Perrimon, 1999 Hedgehog movement is regulated through *tout velu*-dependent synthesis of a heparan sulfate proteoglycan. Mol. Cell **4:** 633–639.

Treisman, J. E., Z. C. Lai and G. M. Rubin, 1995 Shortsighted acts in the Decapentaplegic pathway in *Drosophila* eye development and has homology to a mouse *TGF-beta-responsive* gene. Development **121:** 2835–2845.

Tsai, Y. C., and Y. H. Sun, 2004 Long-range effect of Upd, a ligand for Jak/STAT pathway, on cell cycle in Drosophila eye development. Genesis **39:** 141–153.

Tulina, N., and E. Matunis, 2001 Control of stem cell self-renewal in *Drosophila* spermatogenesis by JAK-STAT signaling. Science **294:** 2546–2549.

Verderosa, F. J., and H. J. Muller, 1954 Another case of dissimilar characters in Drosophila apparently representing changes of the same locus. Genetics **39:** 999.

Wittwer, F., A. van der Straten, K. Keleman, B. J. Dickson and E. Hafen, 2001 Lilliputian: an AF4/FMR2-related protein that controls cell identity and cell growth. Development **128:** 791–800.

Xu, P., S. Y. Vernooy, M. Guo and B. A. Hay, 2003 The *Drosophila* microRNA *mir-14* suppresses cell death and is required for normal fat metabolism. Curr. Biol. **13:** 790–795.

Yan, R., S. Small, C. Desplan, C. R. Dearolf and J. E. Darnell, Jr., 1996 Identification of a *Stat* gene that functions in *Drosophila* development. Cell **84:** 421–430.

Zeidler, M. P., N. Perrimon and D. I. Strutt, 1999a The *four-jointed* gene is required in the *Drosophila* eye for ommatidial polarity specification. Curr. Biol. **9:** 1363–1372.

Zeidler, M. P., N. Perrimon and D. I. Strutt, 1999b Polarity determination in the *Drosophila* eye: a novel role for *unpaired* and JAK/STAT signaling. Genes Dev. **13:** 1342–1353.

Zeidler, M. P., E. A. Bach and N. Perrimon, 2000a The roles of the *Drosophila* JAK/STAT pathway. Oncogene **19:** 2598–2606.

Zeidler, M. P., N. Perrimon and D. I. Strutt, 2000b Multiple roles for *four-jointed* in planar polarity and limb patterning. Dev. Biol. **228:** 181–196.

Communicating editor: T. Schüpbach