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GFP reporters detect the activation of the *Drosophila* JAK/STAT pathway *in vivo*

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Abstract

JAK/STAT signaling is essential for a wide range of developmental processes in *Drosophila melanogaster*. The mechanism by which the JAK/STAT pathway contributes to these processes has been the subject of recent investigation. However, a reporter that reflects activity of the JAK/STAT pathway in all *Drosophila* tissues has not yet been developed. By placing a fragment of the Stat92E target gene *Socs36E*, which contains at least two putative Stat92E binding sites, upstream of *GFP*, we generated three constructs that can be used to monitor JAK/STAT pathway activity *in vivo*. These constructs differ by the number of Stat92E binding sites and the stability of GFP. The *2XSTAT92E-GFP* and *10XSTAT92E-GFP* constructs contain 2 and 10 Stat92E binding sites, respectively, driving expression of enhanced *GFP*, while *10XSTAT92E-DGFP* drives expression of destabilized *GFP*. We show that these reporters are expressed in the embryo in an overlapping pattern with Stat92E protein and in tissues where JAK/STAT signaling is required. In addition, these reporters accurately reflect JAK/STAT pathway activity at larval stages, as their expression pattern overlaps that of the activating ligand *unpaired* in imaginal discs. Moreover, the *STAT92E-GFP* reporters are activated by ectopic JAK/STAT signaling. *STAT92E-GFP* fluorescence is increased in response to ectopic *upd* in the larval eye disc and mis-expression of the JAK kinase *hopscotch* in the adult fat body. Lastly, these reporters are specifically activated by Stat92E, as *STAT92E-GFP* reporter expression is lost cell-autonomously in *stat92E* homozygous mutant tissue. In sum, we have generated *in vivo* GFP reporters that accurately reflect JAK/STAT pathway activation in a variety of tissues. These reporters are valuable tools to further investigate and understand the role of JAK/STAT signaling in *Drosophila*.

Keywords: STAT; JAK; Unpaired; *Drosophila*; *In vivo* reporter; Eye; Wing; Antennal and leg imaginal discs; Embryogenesis; Larva; Gene expression; Transgene; Signal transduction

1. Results and discussion

The Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway is an evolutionarily conserved signaling system that plays essential roles in numerous biological processes in vertebrates and invertebrates, including immunity, hematopoiesis and proliferation (reviewed in Levy and Darnell, 2002). Since *Drosophila* is highly amenable to genetic manipulations, it has served as an excellent model organism for studying this pathway (reviewed in Hombria and Brown, 2002; Hou et al., 2002). Genetic studies in *Drosophila* have identified several components of the JAK/STAT pathway, including three cytokine-like Unpaired (Upd) molecules (Upd, Upd2 and Upd 3) (Agaisse et al., 2003; Gilbert et al., 2005; Harrison et al., 1998; Hombria et al., 2005); the transmembrane receptor

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Domeless (Dome) (also called Master of Marelle), which is distantly related to the mammalian gp130 cytokine receptor (Brown et al., 2001; Chen et al., 2002); the JAK Hopscotch (Hop) (Binari and Perrimon, 1994), which is most similar to mammalian Jak2; the STAT Stat92E (Hou et al., 1996; Yan et al., 1996), which is homologous to mammalian Stat3 and Stat5; and *Socs36E*, a member of the SOCS/CIS/JAB family of JAK/STAT negative regulators (Alexander and Hilton, 2004; Callus and Mathey-Prevot, 2002; Karsten et al., 2002). The JAK/STAT signaling cascade is initiated when Upd binds to Dome, causing the receptor to undergo a conformational change. Hop molecules, which are constitutively associated with the Dome cytoplasmic domain, are then able to phosphorylate one another, as well as specific tyrosine sites on the receptor. Cytosolic Stat92E is recruited to these activated receptor sites and is subsequently phosphorylated on a specific tyrosine residue (Y711) by the associated Hop proteins (Yan et al., 1996). Activated Stat92E molecules dimerize and accumulate in the nucleus where they alter the transcription of target genes, such as *dome* and *Socs36E*, by binding to specific DNA sequences (consensus TTCNNNGAA) (Bach et al., 2003; Ghiglione et al., 2002; Karsten et al., 2002; Yan et al., 1996).

The *Drosophila* JAK/STAT pathway regulates many developmental processes, including sex determination, stem cell maintenance, oogenesis, border cell migration, embryonic segmentation, gut development, tracheal development, hematopoiesis, immunity, and eye development (Agaisse et al., 2003; Bach et al., 2003; Beccari et al., 2002; Binari and Perrimon, 1994; Brown et al., 2001; Johansen et al., 2003; Kiger et al., 2001; Sefton et al., 2000; Silver and Montell, 2001; Sorrentino et al., 2004; Tulina and Matunis, 2001; Xi et al., 2003). The contribution of JAK/STAT signaling to these processes has been the subject of recent investigations. However, an *in vivo* reporter to monitor the spatial and temporal activation of the *Drosophila* JAK/STAT pathway at multiple developmental stages is lacking.

A number of tools have been developed previously to visualize the activity of the *Drosophila* JAK/STAT pathway. These include the βlue-βlau technique that detects homo-dimerization of the Dome receptor in *Drosophila* embryos (Brown et al., 2003), reagents to visualize Stat92E activation such as a Stat92E-GFP fusion protein that exhibits nuclear translocation in cultured cells upon activation (Karsten et al., 2006), and an antibody specific for the tyrosine phosphorylated form of activated Stat92E (Li et al., 2003). In addition, Gilbert and colleagues recently generated an *in vivo* reporter to monitor JAK/STAT pathway activity (Gilbert et al., 2005). In their reporter called $(GAS)_3$-*LacZ*, *LacZ* is driven by multimerized Gamma Activated Site (GAS) elements, to which mammalian Stat1 dimers bind with optimal affinity (Decker et al., 1997; Reich et al., 1989). The $(GAS)_3$-*LacZ* reporter accurately detects pathway activation in the embryo. However, no data was presented on the expression of this reporter at later developmental stages. Although useful for some studies, these reagents have some limitations. Here, we present a characterization of *in vivo* GFP reporters, generated by placing Stat92E binding sites from a Stat92E target gene (*Socs36E*) upstream of enhanced or destabilized GFP, that accurately reflect activity of the *Drosophila* JAK/STAT pathway. These reporters allow us to examine, for the first time, the spatial and temporal activity of the JAK/STAT pathway at all developmental stages in *Drosophila*.

1.1. Generating Drosophila JAK/STAT pathway in vivo reporters

One of the few characterized JAK/STAT target genes in *Drosophila* is *Socs36E*, which is transcriptionally activated by JAK/STAT signaling (Karsten et al., 2002). Socs36E acts as a negative regulator of this pathway, presumably by either blocking Hop activation, or by competing with Stat92E for activated receptor sites (Alexander and Hilton, 2004; Callus and Mathey-Prevot, 2002). The first intron of *Socs36E* contains a 441 bp fragment with at least two potential Stat92E binding sites (Karsten et al., 2002). We used tandem repeats of this fragment to drive expression of enhanced or destabilized GFP *in vivo*. We recently employed a similar strategy to generate a luciferase reporter to monitor JAK/STAT pathway activity *in vitro* (Baeg et al., 2005). Specifically, we generated a *2XSTAT92E-GFP* reporter and a *10XSTAT92E-GFP* reporter by placing one or five tandem repeats, respectively, of this 441 bp fragment upstream of a minimal heat-shock promoter (*hsp*) and a cDNA encoding enhanced *GFP* (Fig. 1A). We also generated a *10XSTAT92E* reporter driving expression of destabilized GFP (called *10XSTAT92E-DGFP*). While enhanced GFP is stable for more than 24 h, the destabilized form is only stable for ~8 h, and is therefore a better temporal marker of transcriptional activity than enhanced GFP (Li et al., 1998).

The accuracy of the *2XSTAT92E-GFP* and *10XSTAT92E-GFP* reporters was confirmed by their embryonic expression patterns. Activation of the *Drosophila* JAK/STAT pathway results in increased levels and/or stability of the Stat92E protein (Chen et al., 2002; Johansen et al., 2003; Read et al., 2004). In wild type stage 10 embryos, Stat92E protein is detected in stripes (Fig. 1B), which is consistent with Upd and Upd2 expression domains (Gilbert et al., 2005; Harrison et al., 1998; Hombria et al., 2005). Both the *2X*- and *10XSTAT92E-GFP* reporters are expressed in a similar striped pattern in stage 10 embryos and specifically overlap with Stat92E protein (Fig. 1B′, B″ and data not shown). Previous work has demonstrated that JAK/STAT pathway activity is important for the development of polar cells and border cells in the ovary (Beccari et al., 2002; Silver and Montell, 2001), as well as that of posterior spiracles (Brown et al., 2001), hindgut (Johansen et al., 2003), and pharynx (Hombria et al., 2005) in the embryo. We therefore examined the expression of the *10XSTAT92E-GFP* reporter in these tissues. In the ovary, *upd* is expressed specifically in polar cells and in border cells (Beccari et al., 2002; Silver and Montell, 2001). The *10XSTAT92E-GFP* reporter is