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A novel functional activator of the Drosophila JAK/STAT pathway, unpaired2, is revealed by an in vivo reporter of pathway activation

M. Melissa Gilbert[a,b], Brian K. Weaver[b,1], J. Peter Gergen[c], Nancy C. Reich[b,*]

[a]*Graduate Program in Genetics, Stony Brook University, Stony Brook, New York, USA*
[b]*Department of Molecular Genetics and Microbiology, Stony Brook University, Nicolls Road, Stony Brook, NY 11794-5222, USA*
[c]*Department of Biochemistry and Cell Biology, Stony Brook University, Nicolls Road, Stony Brook, NY 11794-5212, USA*

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Abstract

Striking similarities continue to emerge between the mammalian and *Drosophila* JAK/STAT signaling pathway. However, until now there has not been the ability to monitor global pathway activity during development. We have generated a transgenic animal with a JAK/STAT responsive reporter gene that can be used to monitor pathway activation in whole *Drosophila* embryos. Expression of the *lacZ* reporter regulated by STAT92E binding sites can be detected throughout embryogenesis, and is responsive to the Janus Kinase *hopscotch* and the ligand *upd*. The system has enabled us to identify the effect of a predicted gene related to *upd*, designated *upd2*, whose expression initiates during germ band extension. The stimulatory effect of *upd2* on the JAK/STAT reporter can also be demonstrated in *Drosophila* tissue culture cells. This reporter system will benefit future investigations of JAK/STAT signaling modulators both in whole animals and tissue culture.

Keywords: STAT; JAK; Signal transduction; Embryogenesis; Unpaired; Gene expression; Transgene

1. Introduction

The Janus kinase (JAK) and Signal Transducer and Activator of Transcription (STAT) signaling pathway was first discovered in mammalian systems with the recombinant cloning of a STAT gene encoding a DNA binding factor, and identification of a JAK tyrosine kinase responsible for its phosphorylation (Fu et al., 1992; Schindler et al., 1992; Velazquez et al., 1992; Stark et al., 1998; Levy and Darnell, 2002). A link between JAK/STAT signaling and neoplasia was not apparent until studies with *Drosophila* revealed that a constitutively active JAK mutant caused tumor formation in animals (Perrimon and Mahowald, 1986; Hanratty and Dearolf, 1993; Luo et al., 1995). Studies with *Drosophila* can readily reveal physiological roles by offering a whole animal model system that can be evaluated with both loss-of-function and gain-of-function mutations within a short time span of reproduction. In addition, the *Drosophila* system allows the benefit of genetic manipulations to identify regulatory modulators and cross-talk between synergistic or antagonistic pathways.

In comparison to the four JAKs and seven STATs that have evolved in mammalian systems, *Drosophila* possess a single JAK gene (*hopscotch/hop*) and a single STAT gene (*stat92E*) (Perrimon and Mahowald, 1986; Binari and Perrimon, 1994; Hou et al., 1996; Yan et al., 1996). JAK molecules are associated with the intracellular domain of cell surface cytokine receptors and are activated in response to binding of extracellular ligand to the receptor (Darnell et al., 1994; Ihle et al., 1995; Taga and Kishimoto, 1995). The activated JAKs in turn tyrosine phosphorylate STAT molecules. Tyrosine phosphorylation of STATs promotes their dimerization via reciprocal phosphotyrosine and Src homology 2 (SH2) domain interactions. This conformational change can contribute to nuclear translocation, and it is essential for the ability of STATs to recognize specific DNA sequences. STAT dimers in humans or *Drosophila* bind to

* Corresponding author. Tel.: +1 631 632 8650; fax: +1 631 632 9797.
E-mail address: nreich@notes.cc.sunysb.edu (N.C. Reich).
[1] Present address: Washington University, St Louis, MO, USA.

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a conserved DNA sequence with dyad symmetry that can be present in multiple copies within promoters of responsive genes (Yan et al., 1996; Darnell, 1997). The STAT binding sequence is often referred to as an interferon-gamma activated site (GAS) since it was initially described in mammalian genes induced by the cytokine interferon-gamma.

The *Drosophila* JAK/STAT pathway has been found to play critical roles in varied functions such as embryo segmentation, proliferation, hematopoiesis, spermatogenesis, oogenesis, sex determination, immune response, and eye development (Luo and Dearolf, 2001; Hombria and Brown, 2002; Hou et al., 2002). To aide evaluation of these functions and those that remain to be discovered, we developed an in vivo reporter system to detect activation of the JAK/STAT pathway. We generated a STAT92E responsive reporter gene regulated by a conserved STAT binding site (GAS sequence) within minimal promoter sequences. This reporter was used to produce transgenic *Drosophila*, and in this study we describe pathway activation during embryogenesis.

The JAK/STAT pathway in *Drosophila* is activated via a functional receptor Domeless (Dome), also known as Master of marelle (MOM) (Brown et al., 2001; Chen et al., 2002). Dome shares some similarity with the extracellular ligand binding region of the mammalian interleukin-6 receptor family. The existence of an additional receptor component, CG14225, has been predicted based on a region encoding a protein with partial homology to Dome (Hombria and Brown, 2002; Hou et al., 2002). However, there have been no functional studies as yet with CG14225. The plethora of extracellular ligands that can stimulate mammalian STATs contrasts with the limited number of known ligands in the *Drosophila* system. The first ligand discovered to bind to the Dome receptor and activate the JAK/STAT signal pathway was the product of the gene *outstretched (os)*, also known as *unpaired* (*upd*) (Weischaus et al., 1984; Harrison et al., 1998). Upd is a secreted glycoprotein that has been shown to be associated with the extracellular matrix. Computer-based homology searches identified two predicted genes related to *upd* that are also on the X chromosome designated CG5988 (*upd2*) and CG15062/CG5963 (*upd3*) (Hombria and Brown, 2002; Hou et al., 2002). In this study we describe the first analysis of *upd2* expression during embryogenesis, and demonstrate its ability to stimulate activation of the JAK/STAT pathway.

2. Results

2.1. JAK/STAT activation is detected in a highly dynamic pattern during embryogenesis

To visualize global JAK/STAT pathway activation in the whole animal we generated a transgenic *Drosophila* line containing the *lacZ* gene regulated by STAT DNA binding sequences. Our construct included three STAT target sites (GAS) (Reich and Darnell, 1989) upstream of a minimal *Drosophila* heat shock promoter in the pCaSpeR.hsp.bas vector. We refer to this reporter gene and the *Drosophila* line as $(GAS)_3$-*lacZ*. Expression during embryogenesis is strikingly dynamic.

To evaluate expression of the reporter gene, in situ hybridization was performed to detect the *lacZ* mRNA transcript in homozygous $(GAS)_3$-*lacZ* embryos during various stages of development (Fig. 1). Expression of the *lacZ* gene is not detectable in syncytial blastoderm embryos (Fig. 1a). However, at the onset of cellularization, *lacZ* mRNA is detected throughout the embryo with strongest expression in the ventral region (Fig. 1b). Just prior to gastrulation, expression becomes more spatially restricted (Fig. 1c). At the onset of gastrulation, an intense *lacZ* signal is detected in a broad region anterior to the presumptive cephalic furrow and invaginating presumptive mesoderm (Fig. 1d). As germ band extension proceeds, expression is reduced (Fig. 1e), but reappears by early stage 9 in the head region and as a weak 14 stripe pattern (Fig. 1f). By stage 10 this pattern resolves into strong expression in 14 parasegments (Fig. 1g). The *lacZ* signal then recedes and is detected in small clusters of segmentally repeated cells (Fig. 1h). These data indicate that STAT binding sites within a promoter context can drive expression of a reporter gene in the *Drosophila* embryo. Homozygous *Drosophila* were also

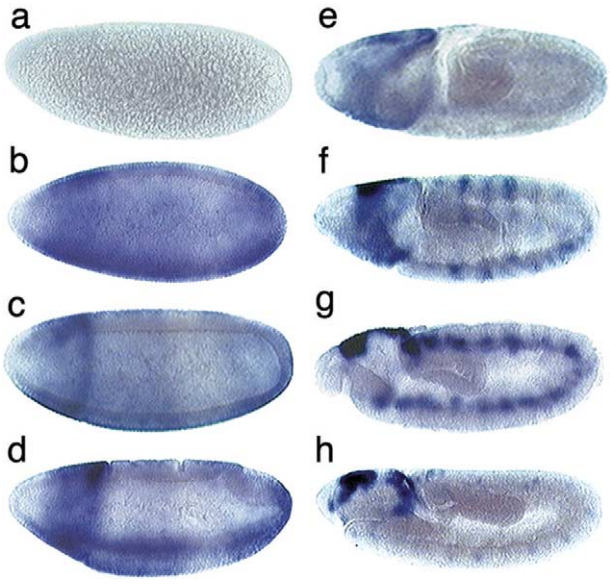

Fig. 1. Dynamic expression of the *lacZ* gene regulated by STAT DNA binding elements. In situ hybridization to *lacZ* mRNA during embryogenesis. Embryos are cross-section views oriented anterior to the left and dorsal to the top. (a) No maternal expression is detected in the syncytium. (b) Expression is first established ubiquitously at cellularization. (c) and (d) At the onset of gastrulation, expression becomes more spatially restricted and at early germ-band extension it is detected anterior to the presumptive cephalic furrow and in the presumptive mesoderm. (e)–(h) Expression decreases but is subsequently detected in a dynamic 14-stripe pattern that becomes restricted to small clusters of segmentally repeated cells.