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Characterization of a dominant-active STAT that promotes tumorigenesis in *Drosophila*

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ABSTRACT

Little is known about the molecular mechanisms by which STAT proteins promote tumorigenesis. *Drosophila* is an ideal system for investigating this issue, as there is a single STAT (Stat92E), and its hyperactivation causes overgrowths resembling human tumors. Here we report the first identification of a dominant-active Stat92E protein, Stat92E$^{\Delta N\Delta C}$, which lacks both N- and C-termini. Mis-expression of Stat92E$^{\Delta N\Delta C}$ *in vivo* causes melanotic tumors, while *in vitro* it transactivates a *Stat92E-luciferase* reporter in the absence of stimulation. These gain-of-function phenotypes require phosphorylation of Y^{711} and dimer formation with full-length Stat92E. Furthermore, a single point mutation, an R^{442P} substitution in the DNA-binding domain, abolishes Stat92E function. Recombinant Stat92E^{R442P} translocates to the nucleus following activation but fails to function in all assays tested. Interestingly, R^{442} is conserved in most STATs in higher organisms, suggesting conservation of function. Modeling of Stat92E indicates that R^{442} may contact the minor groove of DNA via invariant TC bases in the consensus binding element bound by all STAT proteins. We conclude that the N- and C- termini function unexpectedly in negatively regulating Stat92E activity, possibly by decreasing dimer dephosphorylation or increasing stability of DNA interaction, and that Stat92E^{R442} has a nuclear function by altering dimer:DNA binding.

Introduction

The Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway is evolutionarily conserved and is critical for numerous biological processes, including immunity and proliferation (reviewed in (Arbouzova and Zeidler, 2006; Levy and Darnell, 2002)). STATs are a family of latent cytoslic transcription factors that are activated by tyrosine phosphorylation, which allows the formation of an activated dimer through reciprocal phosphorylated tyrosine-Src Homology 2 (SH2) interactions between two STAT monomers. Studies in cultured cells have led to a model in which JAK non-receptor tyrosine kinases, constitutively associated with transmembrane receptors, are activated following ligand binding (Fig. 1A). JAK activation leads to the subsequent tyrosine phosphorylation of receptor sites to which unphosphorylated STAT dimers dock. STAT dimers are activated by JAK-dependent tyrosine phosophorylation and are then able to bind to consensus sequences in target genes and influence their transcription (Becker et al., 1998; Braunstein et al., 2003; Chen et al., 2003; Chen et al., 1998; Kretzschmar et al., 2004; Mao et al., 2005; Neculai et al., 2005; Novak et al., 1998; Schroder et al., 2004; Stancato et al., 1996). The activity of phosphorylated STAT dimers is transient, and these dimers are dephosphorylated in the nucleus and are exported to the cytoplasm (Mertens et al., 2006; Reich and Liu, 2006; Zhong et al., 2005).

Mammals have seven STAT proteins (STAT1-4, 5a, 5b, and 6) that share a similar conserved domain structure, including N-terminus, coiled-coil, DNA-binding, linker, SH2, and C-terminus (Fig. 1B and (Becker et al., 1998; Chen et al., 1998; Vinkemeier et al., 1998)). The N-terminal domain (residues ~1-130) is required for formation of tetramers as well as of non-phosphorylated dimers, for tyrosine dephosphorylation, for transcriptional activation and for protein-protein interactions (Chang et al., 2003; Chen et al., 2003; Murphy et al., 2000; Ota et al., 2004; Shuai et al., 1996; Vinkemeier et al., 1998; Xu et al., 1996). A helical coiled-coil domain beginning around residue 130 mediates interaction between several proteins including c-Jun (Zhang et al., 1999). The DNA-binding domain of STATs (DBD) (residues ~320 to 490) has limited contact with both the major and minor grooves of DNA (Chen et al., 1998). The linker domain (residues ~490 to 580) modulates the rate of STAT:DNA interactions, ultimately controlling transcriptional activation of STAT target genes (Yang et al., 2002). An SH2 domain (residues ~580-680) is required for the formation of an activated STAT dimer by mediating reciprocal interactions with a phosphorylated conserved tyrosine residue at position ~700 that exists in all STATs (Chen et al., 1998; Levy and Darnell, 2002). The phosphorylation of this tyrosine residue is

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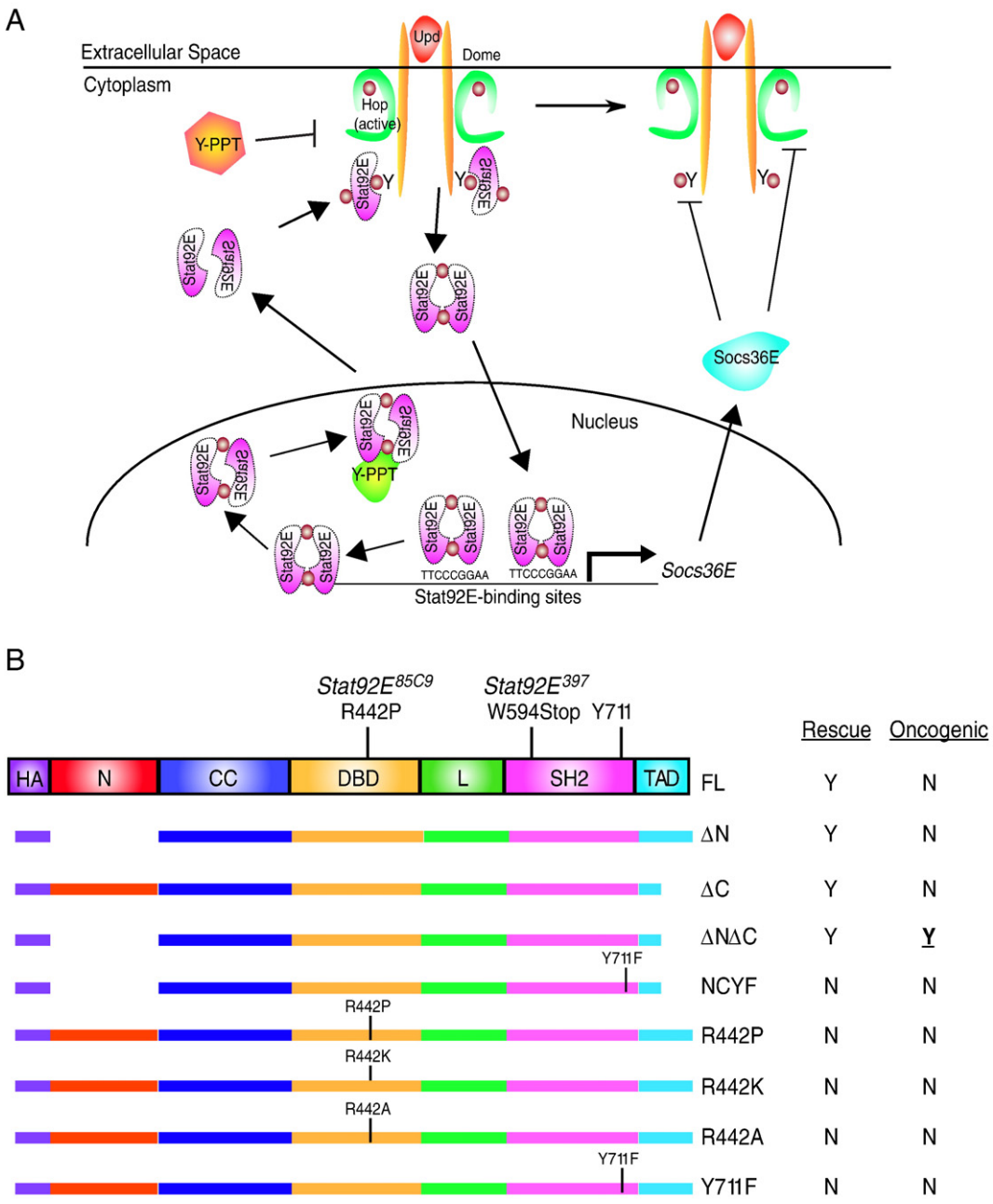

Fig. 1. Model of the *Drosophila* JAK/STAT pathway and *Stat92E* transgenes. (A) Model of the *Drosophila* JAK/STAT pathway. Hop is constitutively associated with Dome and is activated by Upd binding to a dimerized form of Dome (Brown et al., 2003). Activated Hop subsequently phosphorylates one or more tyrosine residues in the Dome cytoplasmic domain. Inactive Stat92E dimers dock at the phospho-tyrosine residues on the receptor. Stat92E dimers are then phosphorylated by the activated Hop proteins, assume an activated dimer conformation and translocate to the nucleus where they influence the transcription of target genes. Subsequently, activated Stat92E dimers no longer bind to Stat92E binding sites on DNA; they are dephosphorylated by an unidentified nuclear tyrosine phophatase (green Y-PPT); they are then exported as unphosphorylated inactive dimers to the cytoplasm. The basal phosphorylation of Dome, Hop and/or Stat92E is balanced by the actions of cytoplasmic tyrosine phosphatases (orange Y-PPT). (B) Stat92E domains include an N-terminal (N) (red), a coiled-coil (CC) (royal blue), a DNA binding (DBD) (yellow), a linker (L) (green), an SH2 (pink), and a C-terminal trans-activation (TAD) (turquoise). The critical tyrosine in Stat92E is located at residue 711. Deletions and substitutions were made to a *UAS-3HA-Stat92E^{FL}* transgene containing three N-terminal HA tags (purple). Constructs include deletion of residues 1-133 (ΔN); 725-761 (ΔC); 1-133 and 725-761 simultaneously (ΔNΔC). Substitution constructs include a mutation of the critical Y711 to F in ΔNΔC (ΔNΔCY711F); of R442 to P (R442P), R442 to K (R442K) and R442 to A (R442A); of the critical Y711 to F (Y711F). Constructs were tested in two *Stat92E* mutant backgrounds: *Stat92E^{85C9}*, which results from an R442P substitution, and *Stat92E^{397}*, which results from a premature stop at W594. "Rescue" refers to the abilty of a Stat92E variant to rescue *Stat92E* loss-of-function phenotypes *in vivo*. "Oncogenic" refers to the abilty of a Stat92E variant to cause overgrowths or melanotic tumors *in vivo*. Y = yes and N = no.

required for STAT function, which is abolished by its mutation to Phe in all species tested (Levy and Darnell, 2002). Lastly, the carboxy-terminal transactivation domain (TAD) varies in length from 38 to 200 amino acids and is required for transcriptional co-activation of mammalian STATs (Horvath, 2000).

Gain-of-function mutations in the *Drosophila* JAK *hopscotch* (*hop*) were the first to link the JAK/STAT pathway to cancer. These *hop* alleles result in hyperactive kinases that cause an over-proliferation of blood cells, leading to fly "leukemia" and lethality (Binari and Perrimon, 1994; Hanratty and Dearolf, 1993; Harrison et al., 1995; Luo et al., 1995). Similarly, sustained activation of the JAK/STAT pathway is a causal event in human leukemia and myeloproliferative disorders (Baxter et al., 2005; James et al., 2005; Jones et al., 2009; Kilpivaara et al., 2009; Lacronique et al., 1997; Levine et al., 2005; Olcaydu et al., 2009). Persistent activation of Stat3 is associated with a dozen types of human cancer, including all classes of carcinoma