Developmental Biology 379 (2013) 152–166

Contents lists available at SciVerse ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Socs36E attenuates STAT signaling to optimize motile cell specification in the Drosophila ovary

Amanda J. Monahan, Michelle Starz-Gaiano *

Department of Biological Sciences, University of Maryland, Baltimore County, 1000 Hilltop Circle Road, Baltimore, MD 21250, USA

ARTICLE INFO

Article history:
Received 10 January 2013
Received in revised form
22 March 2013
Accepted 23 March 2013
Available online 10 April 2013

Keywords:
Socs36E
Drosophila
Border cells
JAK/STAT
Apontic
Cell motility
Cell fate

ABSTRACT

The Janus kinase/Signal transducers and activators of transcription (JAK/STAT) pathway determines cell fates by regulating gene expression. One example is the specification of the motile cells called border cells during Drosophila oogenesis. It has been established that too much or too little STAT activity disrupts follicle cell identity and cell motility, which suggests the signaling must be precisely regulated. Here, we find that *Suppressor of cytokine signaling at 36E* (*Socs36E*) is a necessary negative regulator of JAK/STAT signaling during border cell specification. We find when STAT signaling is too low to induce migration in the presumptive border cell population, nearby follicle cells uncharacteristically become invasive to enable efficient migration of the cluster. We generated a genetic null allele that reveals *Socs36E* is required in the anterior follicle cells to limit invasive behavior to an optimal number of cells. We further show *Socs36E* genetically interacts with the required STAT feedback inhibitor *apontic* (*apt*) and APT's downstream target, *mir-279*, and provide evidence that suggests APT directly regulates *Socs36E* transcriptionally. Our work shows Socs36E plays a critical role in a genetic circuit that establishes a boundary between the motile border cell cluster and its non-invasive epithelial neighbors through STAT attenuation.

Introduction

During normal development, coordinated genetic circuits instruct cells to respond to fate-determining signals. In pathological events, the genes involved in these circuits can become ectopically activated, or regulatory components of endogenous signaling can break down, leading to undesirable outcomes. Thus, decoding how genetic circuits are regulated is important to understanding both development and disease. The Janus kinase (JAK) and Signal transducers and activators of transcription (STAT) proteins are key components in a highly conserved pathway that allows cells to convert extracellular cues into intracellular responses by regulating gene expression (Arbouzova and Zeidler, 2006; Bromberg and Chen, 2001; Levy and Darnell, 2002). Originally discovered for its role in promoting cytokine-induced gene expression, the JAK/STAT pathway has since been implicated in animal development, including regulation of cell proliferation, stem-cell maintenance, cell differentiation, immune system regulation, and cell migration (Arbouzova and Zeidler, 2006; Bromberg and Darnell, 2000; de Cuevas and Matunis, 2011; Hombría and Brown, 2002; Levy and Darnell, 2002; Luo and Dearolf, 2001). Hyper- and hypo-activation of the pathway, however, has been linked to numerous disorders, including various cancers (Bromberg et al., 1999; Chen et al., 2012a; Levy and Darnell, 2002). The requirement for precise JAK/STAT signaling underscores the importance of studying the regulatory components of the pathway.

The border cells of the *Drosophila melanogaster* ovary require JAK/STAT signaling for their specification and characteristic migration, and have provided some insight into the function and regulation of this pathway (Hombría and Brown, 2002; Montell et al., 2012). The fly genome encodes a single STAT (Stat92E), one JAK (Hopscotch/Hop), and one receptor (Domeless/Dome), as opposed to the numerous orthologs found in mammals; thus the study of the pathway in Drosophila eliminates many issues with redundancy found in vertebrates (Arbouzova and Zeidler, 2006; Devergne et al., 2007; Ghiglione et al., 2002; Hombría and Brown, 2002; Hou et al., 2002; Luo and Dearolf, 2001). The Drosophila ovary is comprised of a procession of egg chambers undergoing oogenesis, which is divided into 14 stages (King, 1970). Each egg chamber is composed of 16 germline cells – one oocyte and 15 nurse cells – surrounded by a monolayer of somatic epithelial cells, the follicle cells (King, 1970; Spradling, 1993). At stage 8, two specialized cells, the anterior polar cells, secrete the cytokine-like molecule Unpaired (Upd), causing graded activation of the JAK/STAT pathway in the 9–12 closest epithelial cells (Montell

* Corresponding author. Fax: +1 410 455 3875.
E-mail addresses: monahan2@umbc.edu (A.J. Monahan), starz@umbc.edu (M. Starz-Gaiano).

0012-1606/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ydbio.2013.03.022

et al., 2012; Van de Bor et al., 2011). By stage 9, cells that initially had low STAT pathway activation switch it off entirely, thereby reducing the number of follicle cells with STAT activity to 4–6. Cells with high STAT activity assemble around the non-migratory polar cells to form the border cell cluster. The cluster detaches from the epithelium and migrates along the nurse cells to arrive at the oocyte by stage 10, where it is required to form a fertilizable egg (Montell, 2003; Montell et al., 2012).

STAT controls the specification of border cells through modulation of gene expression. Two essential downstream targets required for normal border cell specification and migration are encoded by the genes *slow border cells* (*slbo*) and *apontic* (*apt*) (Montell et al., 1992; Silver et al., 2005; Silver and Montell, 2001; Starz-Gaiano et al., 2008). SLBO, the C/EBP transcription factor, promotes border cell specification and represses APT, while APT negatively feeds back on the circuit, repressing STAT and SLBO. APT levels are similar between follicle cells directly adjacent to the polar cells and those more distal at stage 9 (Starz-Gaiano et al., 2008). In contrast, both STAT activity and SLBO expression are graded, decreasing proportionally with distance from the polar cells, and initially detected in a greater number of anterior follicle cells than will eventually become border cells. Mutants that fail to reduce the initial STAT-positive/SLBO-positive population to an appropriate number of cells display abnormal cell invasion and delay, while those disrupting STAT-regulated gene expression result in too few motile cells and loss of migration (Montell et al., 2012; Van de Bor et al., 2011). Thus, optimal border cell migration requires the specification of a precise number of motile cells enclosing the polar cells.

Genetic and expression analyses, along with the finding that loss of *apt* expands the range and magnitude of SLBO expression, have led to the current genetic circuit paradigm. This states that follicle cells that maintain high levels of STAT activity sustain an above-threshold level of SLBO, which inhibits APT and promotes border cell fate. In contrast, lower levels of activated STAT yield higher signaling via APT than SLBO, establishing cells that remain in the surrounding epithelium as the nurse cell-associated stretch cells, which shut off STAT signaling entirely (Montell et al., 2012; Starz-Gaiano et al., 2009, 2008). In follicle cells with lower STAT activity, APT directs STAT attenuation, in part, by promoting the expression of *mir-279,* which targets the *stat* messenger RNA (Yoon et al., 2011). Loss of *mir-279,* however, results in a less penetrant mutant phenotype than loss of *apt*, indicating APT is either capable of repressing STAT directly or that it must control the expression of another STAT regulator.

The *Suppressor of cytokine signaling* (*Socs*) gene family is composed of well-conserved inhibitors of numerous signal transduction pathways, including JAK/STAT, (Alexander, 2002; Cooney, 2002; Croker et al., 2008; Krebs and Hilton, 2001), making members of this family candidates to be additional regulators in border cell specification. Mammals contain eight *Socs* genes (*Socs 1–7* and *CIS*), while Drosophila have only three, named after their cytological locations—*16D, 36E,* and *44A* (Arbouzova and Zeidler, 2006; Callus and Mathey-Prevot, 2002; Karsten et al., 2002; Rawlings et al., 2004). While the mammalian SOCS family is divided into two classes – those with a short N-terminus (CIS and SOCS1-3) and those with a long N-terminus (SOCS4-7) – the fly proteins fall only in the latter class (Alexander, 2002; Callus and Mathey-Prevot, 2002; Croker et al., 2008; Karsten et al., 2002; Rawlings et al., 2004). *Socs16D* and *44A* are orthologous to mammalian *Socs6* and *7*, while *Socs36E* is most similar to *Socs5*. A hallmark of SOCS proteins is their conserved architecture near the carboxy terminus – an SH2 domain and a SOCS box – which is essential for their role in ubiquitination (Alexander, 2002; Croker et al., 2008; Rawlings et al., 2004). Through ubiquitin-based attenuation, SOCS proteins are able to fine-tune STAT signaling.

Several studies have demonstrated functional conservation between Drosophila and vertebrate SOCS proteins. In specific contexts, *Socs36E* has been reported to repress precise receptor tyrosine kinases, including Sevenless during eye development and the epidermal growth factor receptor (EGFR) in the epithelium during wing development (Almudi et al., 2009; Herranz et al., 2012). In the developing wing, Socs36E was also determined to be a negative regulator of the JAK/STAT pathway (Callus and Mathey-Prevot, 2002; Rawlings et al., 2004). These studies also provided evidence that the SH2 and SOCS box domains are essential for Socs36E function in eye and wing development (Almudi et al., 2009; Callus and Mathey-Prevot, 2002). Further, *Socs36E* has been characterized in the Drosophila testes as an essential negative regulator of JAK/STAT signaling (Issigonis et al., 2009; Singh et al., 2010).

We have determined that *Socs36E* plays a critical role in specifying the optimal number of border cells. We generated a genetic null allele of *Socs36E* and found that flies homozygous for this mutation incorrectly specify motile cells, which results in an additional invasive cell phenotype. The phenotypes observed when *Socs36E* expression was either heightened or lost are consistent with loss of function or gain of function of STAT activity, respectively (Beccari et al., 2002; Silver et al., 2005; Silver and Montell, 2001; Starz-Gaiano et al., 2008; Yoon et al., 2011). We did not observe any phenotypes associated with dorsally-directed migration, which is mediated by EGFR (Duchek and Rørth, 2001; McDonald et al., 2006), suggesting that Socs36E does not regulate EGFR during border cell movement. We determined that *Socs36E* genetically interacts with *apt* and its downstream target *mir-279,* and that APT can bind to a site in the *Socs36E* enhancer. Our work indicates APT regulates the expression of both *Socs36E* and *mir-279*, which are each independently required to limit STAT activity and establish a discrete boundary between the motile border cells and their non-motile neighbors.

Materials and methods

Expression and over-expression assays

We crossed the *P[GawB]Socs36E^{NP5170}* and *P[GawB] Socs16D^{NP7149}* ((Brand and Perrimon, 1993), Kyoto stock center) lines to *w-; UAS-mCD8-GFP* ((Lee and Luo, 1999), Bloomington stock center) to determine the expression pattern of *Socs36E* and *Socs16D,* respectively. Over-expression experiments were performed at 25 °C to generate the following genotypes: *P[GawB] c306* (expressed in anterior follicle cells and referred to as *AFC-Gal4* in text, (Manseau et al., 1997)); *UAS-mCD8-GFP, c306-Gal4; UAS-Socs36E* ((Callus and Mathey-Prevot, 2002), Bloomington stock center)), *upd-GAL4* (expressed in polar cells; (Khammari et al., 2011), Bloomington stock center)*; UAS-mCD8-GFP, upd-GAL4; UAS-Socs36E, Socs36E-Gal4/UAS-mCD8-GFP, Socs16D-Gal4 (P [GawB]Socs16D^{NP7149}); UAS-mCD8-GFP,* and *Socs16D-Gal4; UAS-Socs36E.*

Generation of novel Socs36E alleles

We obtained *y^{1}w^{67c23}; P[EPgy2]Socs36E^{EY06665}* ((Bellen et al., 2004; Singh et al., 2010), Bloomington stock center) and outcrossed it to a dominantly marked stock to allow modifiers to be recombined away from the insertional allele. We established a homozygous viable stock, and then isogenized the second chromosome. This generated a "cleaned up" hypomorphic allele of *Socs36E ^{EY06665}*. PCR analysis confirmed the P-element was still present at the *Socs36E* locus. To excise the P-element, we crossed the cleaned-up *Socs36E^{EY06665}* allele to the transposase-bearing stock *w*; Sp/CyO; ry^{506},Dr, Δ2-3/TM6,* and re-balanced the