Developmental Biology 350 (2011) 169–182

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Evolution of Developmental Control Mechanisms

JAK-STAT signalling is required throughout telotrophic oogenesis and short-germ embryogenesis of the beetle *Tribolium*

Daniel Bäumer [1], Jochen Trauner [1], Dominik Hollfelder, Alexander Cerny [2], Michael Schoppmeier *

Department Biology, Developmental Biology Unit, Erlangen University, Staudtstr. 5, 91058 Erlangen, Germany

ARTICLE INFO

Article history:
Received for publication 24 February 2010
Revised 4 October 2010
Accepted 15 October 2010
Available online 23 October 2010

Keywords:
Telotrophic oogenesis
Tribolium castaneum
Epithelial patterning
Stalk cells
JAK-STAT signalling
Domeless
SOCS
Segment polarity
Pair-rule genes
Gap genes
Short germ
Segmentation

ABSTRACT

In *Drosophila*, the JAK-STAT signalling pathway regulates a broad array of developmental functions including segmentation and oogenesis. Here we analysed the functions of *Tribolium* JAK-STAT signalling factors and of *Suppressor Of Cytokine Signalling* (*SOCS*) orthologues, which are known to function as negative regulators of JAK-STAT signalling, during telotrophic oogenesis and short-germ embryogenesis. The beetle *Tribolium* features telotrophic ovaries, which differ fundamentally from the polytrophic ovary of *Drosophila*. While we found the requirement for JAK-STAT signalling in specifying the interfollicular stalk to be principally conserved, we demonstrate that these genes also have early and presumably telotrophic specific functions. Moreover, we show that the *SOCS* genes crucially contribute to telotrophic *Tribolium* oogenesis, as their inactivation by RNAi results in compound follicles. During short-germ embryogenesis, JAK-STAT signalling is required in the maintenance of segment primordia, indicating that this signalling cascade acts in the framework of the segment-polarity network. In addition, we demonstrate that JAK-STAT signalling crucially contributes to early anterior patterning. We posit that this signalling cascade is involved in achieving accurate levels of expression of individual pair-rule and gap gene domains in early embryonic patterning.

Introduction

The Janus kinase/signal transducer and activator of transcription (JAK-STAT) pathway is an evolutionarily conserved signalling system that plays essential roles in numerous biological processes in vertebrates and invertebrates (for review: Arbouzova and Zeidler, 2006; Denef and Schupbach, 2003). Insect oogenesis is one such example, where the JAK-STAT pathway regulates the differentiation of various cell types (Assa-Kunik et al., 2007; McGregor et al., 2002; Silver et al., 2005; Xi et al., 2003).

During early stages of *Drosophila* oogenesis, three follicle cell populations can be distinguished: (1) polar cells, which serve as key signalling centres, (2) stalk cells, which form interfollicular stalks that connect neighbouring egg chambers, and (3) main-body follicle cells, which build an epithelium overlying the germline cyst (for review: Bastock and St Johnston, 2008; Horne-Badovinac and Bilder, 2005; Spradling, 1993). Polar and stalk cells appear to differentiate from a common group of precursor cells in a stepwise manner (Tworoger et al., 1999). Notch as well as JAK-STAT signalling acts in subdividing the stalk/polar follicle cell primordium (Assa-Kunik et al., 2007; Xi et al., 2003): Polar cell fate requires the activation of Notch by the ligand Delta, which is produced by germline cells (Assa-Kunik et al., 2007; Lopez-Schier and St Johnston, 2001). These polar cells then express the JAK-STAT ligand *unpaired* (*upd*), which activates the JAK-STAT signalling pathway in neighbouring polar/stalk precursors. This induces the remaining precursors to differentiate as stalk cells (McGregor et al., 2002). The stalk cells intercalate to form the stalk and separate the egg chamber from the germarium. Accordingly, inactivation of JAK-STAT signalling results in the loss of the stalk and expansion of the polar follicle cell population, which results in fused egg chambers (compound follicles) (McGregor et al., 2002; Xi et al., 2003). Ectopic expression of Upd, on the other hand, results in the loss of polar cells and in the formation of supernumerary stalk-like cells (McGregor et al., 2002).

Oogenesis in *Drosophila* and in the beetle *Tribolium* follows two different modes of oogenesis (out of three to be found among insects), the polytrophic-meroistic (*Drosophila*) and telotrophic-meroistic (*Tribolium*) oogenesis (Trauner and Buning, 2007). Both have in common that germline cells can differentiate either in oocytes or in nurse cells. In polytrophic-meroistic ovaries, represented by *Drosophila melanogaster*, each germ cell cluster matures as one unit encased by somatic follicle cells. Exactly one cell of each cluster develops into an

* Corresponding author. Fax: +49 9131 85 2804.
E-mail address: mschoppm@biologie.uni-erlangen.de (M. Schoppmeier).
[1] Contributed equally.
[2] Current address: Institute for Physiology, University Hohenheim, Garbenstrasse 30, 70599 Stuttgart, Germany.

0012-1606/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2010.10.020

oocyte, while all others differentiate into nurse cells. In contrast, in telotrophic-meroistic ovaries as represented by the red flour beetle *Tribolium castaneum*, oocytes and nurse cells of a germ cell cluster separate such that each follicle contains only one germ cell, the oocyte. This oocyte remains connected to the tropharium – a syncytium of nurse cells – by a nutritive cord (Trauner and Buning, 2007). Although telotrophic ovary organogenesis has been studied at the morphological level in *Tribolium* (Trauner and Buning, 2007), virtually nothing is known about the molecular mechanisms underlying telotrophic oogenesis. In recent years *Tribolium castaneum* has developed into an advanced insect model system second only to *Drosophila melanogaster* (Richards et al., 2008), such that it is now possible to carry out functional studies of this mode of oogenesis. In order to gain insight into the patterning mechanisms underlying telotrophic oogenesis, we analysed the roles of JAK-STAT signalling including the *Tribolium* Suppressor of Cytokine Signalling (SOCS) orthologues for their functions in egg chamber formation and axes polarisation.

Also the mode of embryogenesis differs between *Drosophila* (long-germ mode) and *Tribolium* (short-germ mode). Therefore, we investigated the functions of JAK-STAT signalling throughout metamerisation of this beetle. In *Tribolium*—as in most other insects—only the anteriormost segments are patterned in the blastoderm stage, while all following segments are formed one by one from a posterior growth zone. While growth zone formation in *Tribolium* depends on the localised activity of the Torso-pathway (Schoppmeier and Schroder, 2005), it still is under debate, how blastodermal and growth zone patterning is achieved (Brown et al., 2001a; Bucher and Klingler, 2004; Cerny et al., 2005, 2008; Schoppmeier et al., 2009; Schoppmeier and Schroder, 2005; Schroder, 2003). It has been shown that early patterning in *Tribolium* does not involve *bicoid* and that gap genes have drastic different functions in *Tribolium* when compared to *Drosophila* (Brown et al., 2001a; Bucher and Klingler, 2004; Cerny et al., 2005, 2008; Kotkamp et al., 2010; Marques-Souza et al., 2008; Stauber et al., 1999, 2002). Also the regulation of pair-rule genes differs from *Drosophila*, as pair-rule interactions in the growth zone suggest that a segmentation clock is active (Choe and Brown, 2007; Choe et al., 2006). In contrast, at the level of segment-polarity genes studied so far, both expression and function appear to be conserved to a great extent (Bolognesi et al., 2008; Nagy and Carroll, 1994; Ober and Jockusch, 2006).

While we found conserved as well as divergent requirements for JAK-STAT signalling in patterning the telotrophic egg chamber, this signalling cascade apparently acts in the framework of the segment-polarity network during short-germ segmentation. In addition, we demonstrate that JAK-STAT signalling crucially contributes to early anterior (head) patterning, a function that we found to be related to regulatory input on gap and pair-rule gene domains.

Material and methods

Isolation of genes

The *Drosophila* JAK-STAT pathway components include the transmembrane receptor *domeless* (*dome*), one JAK (*hopscotch*, *hop*), one STAT (*Stat92E*), three Unpaired ligands (*upd 1 to 3*), and three SOCS-like genes that are thought to inhibit STAT function (Arbouzova and Zeidler, 2006). We used the *Drosophila* JAK-STAT components as query sequences in tBLASTn searches of the *Tribolium* genome (Richards et al., 2008) and identified a single *domeless* gene (*Tc-dome*, XP_001807060), one JAK (*Tc-hop*, XP_968564), one STAT (*Tc-STAT*, XP_969477), and four *Tribolium* SOCS paralogs, which we named *Tc-Socs-2* (XP_972490), *Tc-Socs-6* (TC003320), *Tc-Socs-16D* (XP_973720), and *Tc-Socs-36E* (TC003596) according to their closest *Drosophila* or vertebrate orthologs, respectively. We were not able, however, to identify any *unpaired* orthologs; this is most likely due to rapid sequence evolution of this gene family (Boulay et al., 2003).

Candidate genes were amplified from cDNA, cloned into pBluescript KS vector and sequenced to confirm their identity. Fragments of 900–1200 bp were used as template for dsRNA synthesis and digoxigenin labelled antisense RNA probes.

Immunohistochemistry and whole mount in situ hybridization

Female gonads were dissected as previously described (Trauner and Buning, 2007). Ovaries were fixed with 5% formaldehyde/PBS on ice for 60 min and washed in PBT (PBS with 0.1% Triton-X 100 and 3% BSA). To visualise the morphology, Hoechst 33258 and TRITC labelled Phalloidin, which labels the f-actin cytoskeleton were used.

For whole mount in situ hybridization, *Tribolium* ovaries were post-fixated for 15 min in 5% formaldehyde/PBT and rinsed four times in PBT followed by a proteinase K treatment (5 mg/ml) for 1 h on ice and an additional post-fix step (5% formaldehyde/PBS). Ovaries are again rinsed in PBT and pre-hybridised for 1 h at 65 °C (50% formamide, 5× SSC, 2% SDS). Hybridisation is performed overnight at 65 °C (50% formamide, 5× SSC, 2% SDS, 2% BBR (Boehringer Blocking reagent), 250 μg/ml tRNA, 100 μg/ml heparin) with 1–5 μl of a digoxigenin labelled riboprobe. After several washing steps, ovaries are rinsed with MAPT buffer (100 mM Maleic Acid, 150 mM NaCl, pH to 7.5 with NaOH, 0.1% Tween 20) and blocked for at least 2 h (BBR 0.5% in MABT). The alkaline phosphatase conjugated anti-dig antibody (Roche) is added at a concentration 1:2000 and incubated overnight at 4 °C. To remove unbound antibodies, ovaries are washed several times in MAPT buffer (for at least 2 h). Staining is performed in a standard AP buffer using FastRed as substrate (Roche). A detailed protocol is available from the authors.

To visualise the morphology after in situ hybridization, ovaries were counterstained by Hoechst 33258 (5 μg/ml) and an anti-α-tubulin antibody (mouse monoclonal, 1:1000; Sigma). Mitotically active cells were labelled by an anti-phospho-histone H3 antibody (rabbit polyclonal, 1:200; Upstate). The following secondary antibodies were used: Cy2 or Cy3 conjugated goat anti-rabbit (Jackson Immuno-Research, 1:50); Cy2 or Cy3-conjugated sheep anti-mouse (1:200, Sigma). All incubations were done at 4 °C overnight. Ovary images were captured on a Zeiss ApoTome.

Embryo fixation and embryo whole mount in situ hybridizations were essentially carried out as described before (Schoppmeier and Schroder, 2005).

RNAi and cuticle preparations

Pupal and adult RNA interference was essentially performed as described previously (Bucher et al., 2002; van der Zee et al., 2006). First instar offspring larvae were cleared in lactic acid/10% ethanol overnight at 60 °C. After washing once with lactic acid, cuticles were transferred to a drop of lactic acid on a slide. Cuticle autofluorescence images were captured on a Zeiss Axiophot and maximum projection images were generated from image stacks.

Results

Formation of interfollicular stalks

While in *Drosophila* several subpopulations of follicle cells can be distinguished by their specific cell morphologies (Horne-Badovinac and Bilder, 2005; Spradling, 1993) follicle cells in *Tribolium* form a rather uniform epithelium surrounding the developing oocytes (Fig. 1). In early pre-vitellogenic stages, the nuclei of the follicle cells are interlaced, which gives the epithelium a somewhat disorganised appearance (Figs. 1 C and D). During vitellogenic growth of the oocyte, follicle cell nuclei form a uniform epithelium (Fig. 1 A; compare stages III to IV follicle). In addition, interfollicular stalk cells become morphologically distinguishable (Figs. 1 E–J; stalk cells are defined as those

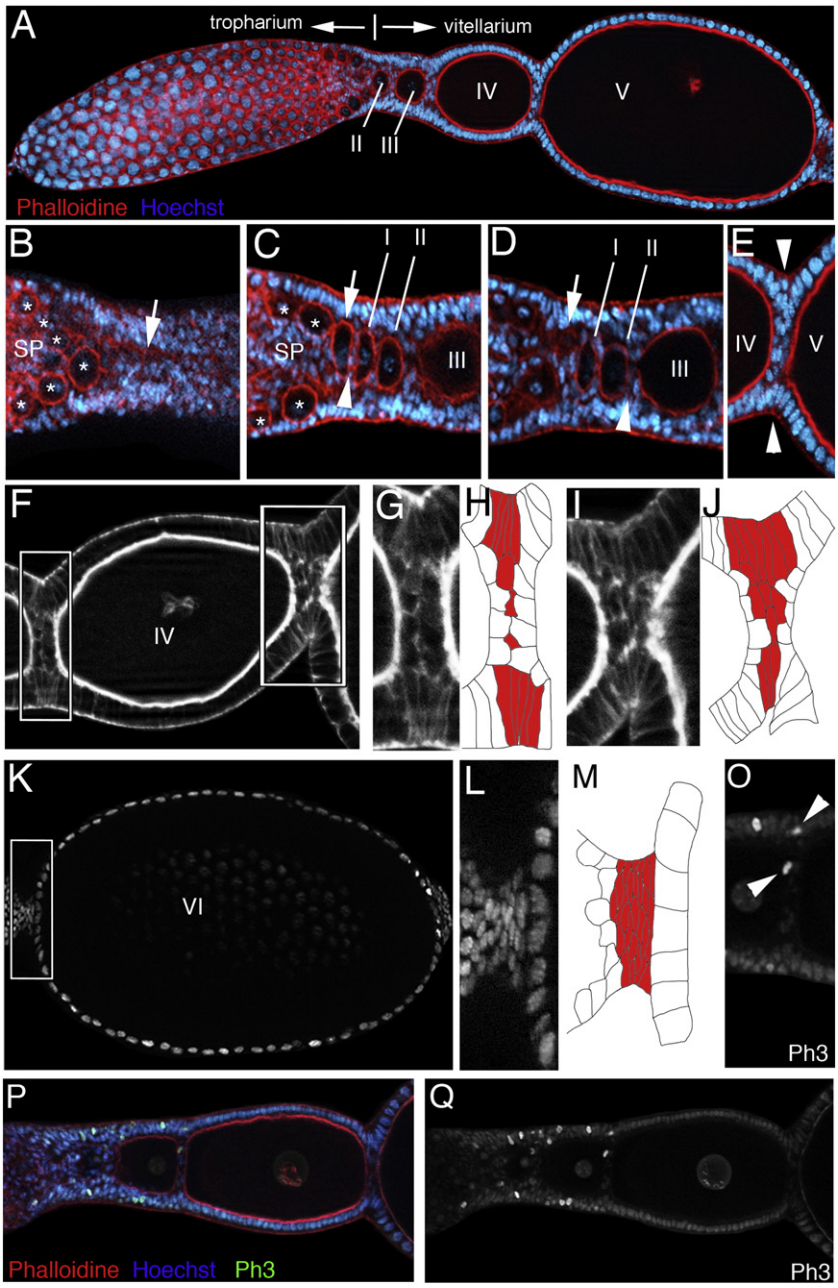

Fig. 1. Telotrophic oogenesis and follicle cell development. (A) *Tribolium* ovariole stained for f-actin with Phalloidin (red) and for DNA with Hoechst (blue). Telotrophic ovarioles can be subdivided in the anterior tropharium, which harbours the nurse cells and the posterior vitellarium, where oogenesis takes place. Roman numerals refer to egg chambers in different stages of oogenesis. (B–D) Different focal planes showing the transition zone between tropharium and vitellarium. (B) Arrested pro-oocytes (asterisks) are arranged around the somatic plug (SP). (C) Pro-oocytes that lose contact to somatic plug cells (arrow) enter the vitellarium and eventually become encapsulated by somatic follicle cells (arrowhead). During this process a pre-vitellogenic oocyte undergoes several shape changes: from round to a lens-like appearance oriented perpendicular to the axis of the ovariole (A–D). (D) Oocytes remain connected to the nurse cells by a nutritive cord (arrows in B and D). The arrowhead points to somatic cells that separate pre-vitellogenic (II) from early vitellogenic oocytes (III). (E) Close-up of the early stalk shown in (A). Interfollicular stalk cells do not contact the germline. (F–M) Stalk formation during telotrophic oogenesis. (F) Ovariole stained for f-actin with Phalloidin. (G, I) Close-ups of the stalks shown in (F) and (H, J) schematic representations, respectively. Individual follicles become subsequently separated by stalk cells (H, J, labelled in red), which do not contact the germline. (K) Late vitellogenic follicle, stained for DNA by Hoechst. During late stages of oogenesis, yolk is taken up by the oocyte from the hemolymph (vitellogenesis). The vitellogenic oocyte considerably increases in size, while follicle cells divide until an average number of 1050 cells per follicle to form a uniform epithelial sheath surrounding the oocyte (K). Subsequently, follicle cell nuclei become polyploid and—after completion of vitellogenesis—secrete the eggshell, i.e. the vitelline membrane and the outer chorion. (L) Close-up of the stalk shown in (K) and the respective schematic representation (M). Fully differentiated stalks consist in average of 34 cells (M, labelled in red). (O–Q) Cell division patterns during oogenesis. (O) Close-up of the ovariole shown in (P). Arrowheads point to mitotically active cells in early stalks. As judged by anti-phospho-histone (PH3) staining (labelling mitotically active cells (green)), in early stalks only a few mitotic cells can be recognised. (P) Ovariole stained for f-actin with Phalloidin (red), DNA with Hoechst (blue), and an antibody recognising phosphorylated Histone-3 (green). (Q) Anti-phospho-histone (PH3) channel only. Cell divisions are restricted to follicle cells and rather scattered than concentrated to distinct regions. Mitoses can be found in the posterior part of the tropharium during early follicle growth and also in those follicle cells that may contribute to stalk formation. All panels: anterior to the left. Roman numerals refer to egg chambers in corresponding stages of oogenesis.

follicle cells that do not contact the germline). In early stages, only a small number of such cells separate the egg chambers (Figs. 1 G–J). Eventually, the number of interfollicular stalk cells will rise to 34 cells on average. Differentiated stalk cells can be recognised by their position and by their characteristic cellular and nuclear morphology, i.e. disc-shaped (Figs. 1 K–M). Stalk formation does not seem to solely

depend on cell divisions, as only a small number of mitotic events can be detected in those regions of the ovariole (Figs. 1 O–Q). Most dividing cells are scattered among the follicle cell epithelium without being restricted to obvious domains, indicating that—as in *Drosophila* (Horne-Badovinac and Bilder, 2005; Spradling, 1993)—stalk formation during *Tribolium* oogenesis probably involves cell recruitment, i.e. migration of follicle cells to join the stalk.

Tribolium JAK-STAT and SOCS genes

In order to determine if—as in *Drosophila*—additional distinct follicle cell subpopulations can be identified within the *Tribolium* follicle epithelium, we cloned (see Material and methods) and analysed JAK-STAT pathway and SOCS orthologues. We were not able, however, to identify any *unpaired* orthologues, which is most likely due to rapid sequence evolution of this gene family (Boulay et al., 2003).

During *Drosophila* oogenesis, of the JAK-STAT signalling genes only *unpaired* activity is spatially restricted, while *Dm-dome and Dm-STAT* are expressed at low levels in all germline and somatic cells (Beccari et al., 2002; Ghiglione et al., 2002). Since we were not able to identify a *Tribolium unpaired* ortholog, we monitored *Tc-STAT* and *Tc-dome* expression during oogenesis. As in *Drosophila*, *Tc-STAT* is broadly expressed in the germline and in somatic tissues (not shown). *Tc-dome*, however, is expressed in a more distinct pattern, including the maturating oocytes and those follicle cells, which are about to separate individual follicles (Fig. S1). From the latter, *Tc-dome* mRNA extends into adjacent follicle cells. Given that *Drosophila dome* is a positive target of the JAK/STAT pathway in the embryo and imaginal discs (Flaherty et al., 2009; Lovegrove et al., 2006) and a negatively regulated target in follicle cells of the *Drosophila* ovary (Ghiglione et al., 2002), the *Tc-dome* expression pattern may indicate that JAK-STAT signalling is involved in the specification of the interfollicular stalk and adjacent follicle cells, some of which may later on contribute to the stalk cell population.

Of the four *Tribolium* SOCS paralogues, *Tc-Socs-6*, *Tc-Socs-16D*, and *Tc-Socs-36E* are expressed during oogenesis in largely identical patterns (Fig. S1), including pre-vitellogenic and vitellogenic oocytes and a subset of follicle cells, indicating that the *Tribolium* Socs genes may contribute to follicle cell patterning.

While *Tc-dome* and *Tc-STAT* are expressed ubiquitously throughout embryogenesis (not shown), *Tc-Socs-2* is the only *Tribolium* SOCS ortholog being expressed during embryonic stages. In the germ-rudiment stage, *Tc-Socs-2* expression is first visible in a posterior domain, which persists throughout early germ-band elongation. Subsequently, *Tc-Socs-2* becomes expressed in a segmental, likely neural fashion (Fig. S3).

Depletion of JAK-STAT signalling affects follicle cell patterning

During polytrophic *Drosophila* oogenesis, graded levels of STAT activation are necessary for the specification of several follicle cell subpopulations and for border cell migration. In addition, STAT activity is thought to play a role in germline stem cell maintenance (Lopez-Onieva et al., 2008; McGregor et al., 2002; Silver et al., 2005; Xi et al., 2003). To elucidate whether telotrophic oogenesis also requires the activity of the JAK-STAT pathway, we knocked down *Tribolium* JAK-STAT signalling components by RNAi and dissected ovaries at different time points after injection (days post injection, dpi). As we expected JAK-STAT signalling to be required for diverse processes during *Tribolium* oogenesis, we injected dsRNA into adults (adult RNAi), which in contrast to pupal RNAi (Bucher et al., 2002) allows to study late functions in oogenesis even if a gene to be investigated also affects earlier stages of oogenesis (or ovary organogenesis).

Tc-dome, *Tc-hop*, and *Tc-STAT* adult RNAi result in severe and basically identical phenotypes (Figs. 2 and S2). Two to three days after dsRNA injection, the somatic stalks, which in wildtype ovarioles separate individual vitellogenic follicles, are absent. Moreover, anterior and posterior follicle cells of adjacent vitellogenic egg chambers seem to maximise their area of contact. This probably causes the severe deformation of follicles (Figs. 2 and S2), which is accompanied by a block of oogenesis, as we observed the cessation of egg production in these females.

To elucidate whether RNAi affects the specification of putative stalk precursor cells already at even earlier stages, we analysed *torso-like* (*Tc-tsl*) mRNA expression (Fig. 3). We had shown previously that *Tc-tsl*, which is crucial for the spatial restriction of Torso-receptor signalling and, thus, important for the specification of terminal fates in the *Tribolium* embryo (Schoppmeier and Schroder, 2005) is expressed at both poles of late follicles in a subset of follicle cells. *Tc-tsl* expressing cells include the differentiated stalk cells as well as adjacent cells, which contribute to the separation of individual follicles (Fig. 3). These cells contact the oocytes, but are morphologically undistinguishable from the remaining follicle cells.

We found that in *Tc-dome* and *Tc-STAT* RNAi ovarioles, *Tc-tsl* expression is reduced (see Fig. 3 B for *STAT* RNAi): three days post injection only a few residual *Tc-tsl* expressing cells could be recognised (Fig. 3 B; arrowhead). Interestingly, *Tc-tsl* is entirely lost in those cells that initially separate very young oocytes, indicating a function of the JAK-STAT pathway in early separation of follicles.

These early phenotypes are reminiscent of phenotypes observed after depletion of JAK-STAT signalling in *Drosophila* ovarioles, where this pathway also is crucial for the separation of egg chambers and for stalk formation (McGregor et al., 2002). Hence, the requirement for JAK-STAT signalling during stalk cell determination may be conserved between telotrophic *Tribolium* and polytrophic *Drosophila* oogenesis. However, in contrast to *Drosophila*, we did not observe compound follicles (egg chambers comprising of two or more oocytes), as egg chambers remained to be separated by at least two layers of follicle cells (Figs. 2 and S2).

Interestingly, the depletion of *dome*, *hop*, or *STAT* also affects the alignment of pre-vitellogenic egg chambers. In wildtype ovarioles, early follicles become arranged in a single row as they are engulfed by somatic cells (Figs. 1 A–D). Upon *Tc-dome*, *Tc-hop*, or *Tc-STAT* RNAi, young oocytes still are encapsulated by follicle cells, but are not aligned in a linear row (Figs. 2 A–C, I–K and S2).

Seven days after injection, ovarioles display rather variable but severe morphological defects (Figs. 2 L and M). At that time, vitellogenic oocytes are found positioned side-by-side, indicating that miss-aligned early egg chambers have matured into vitellogenic stages. These oocytes then become arrested and fail to complete oogenesis.

In *Drosophila*, the activity of the JAK-STAT pathway crucially contributes to the polarisation of the oocyte and thus, to axis formation (McGregor et al., 2002; Steinhauer and Kalderon, 2006; Xi et al., 2003). To elucidate whether JAK-STAT signalling is also required for anterior–posterior axis formation during *Tribolium* oogenesis, we monitored the expression of *Tc-eagle* (*Tc-eg*) mRNA in *dome* depleted ovarioles (Fig. 4). In wildtype ovarioles, *Tc-eg* mRNA becomes subsequently localised to the anterior pole of vitellogenic oocytes (Figs. 4 and S1), indicating a polarisation event at that stage of oogenesis. Interestingly, upon *dome* RNAi, *Tc-eg* transcripts still become localised to the anterior pole of developing oocytes (Fig. 4) and embryos (not shown). Subsequently, we also observed *Tc-eg* transcripts at rather dorsal or ventral positions (Figs. 4 B and C). However, as *Tc-dome* RNAi eventually results in the displacement of entire egg chambers, we are confident that *Tc-eg* still is localised to the anterior pole and that the “asymmetric localisation” is rather due to the subsequent “rotation” of the entire egg chamber. Hence, while JAK-STAT signalling has a major impact on follicle cell patterning and stalk formation in *Tribolium*, this pathway may neither contribute to the initial polarisation nor to the maintenance of oocyte polarity.

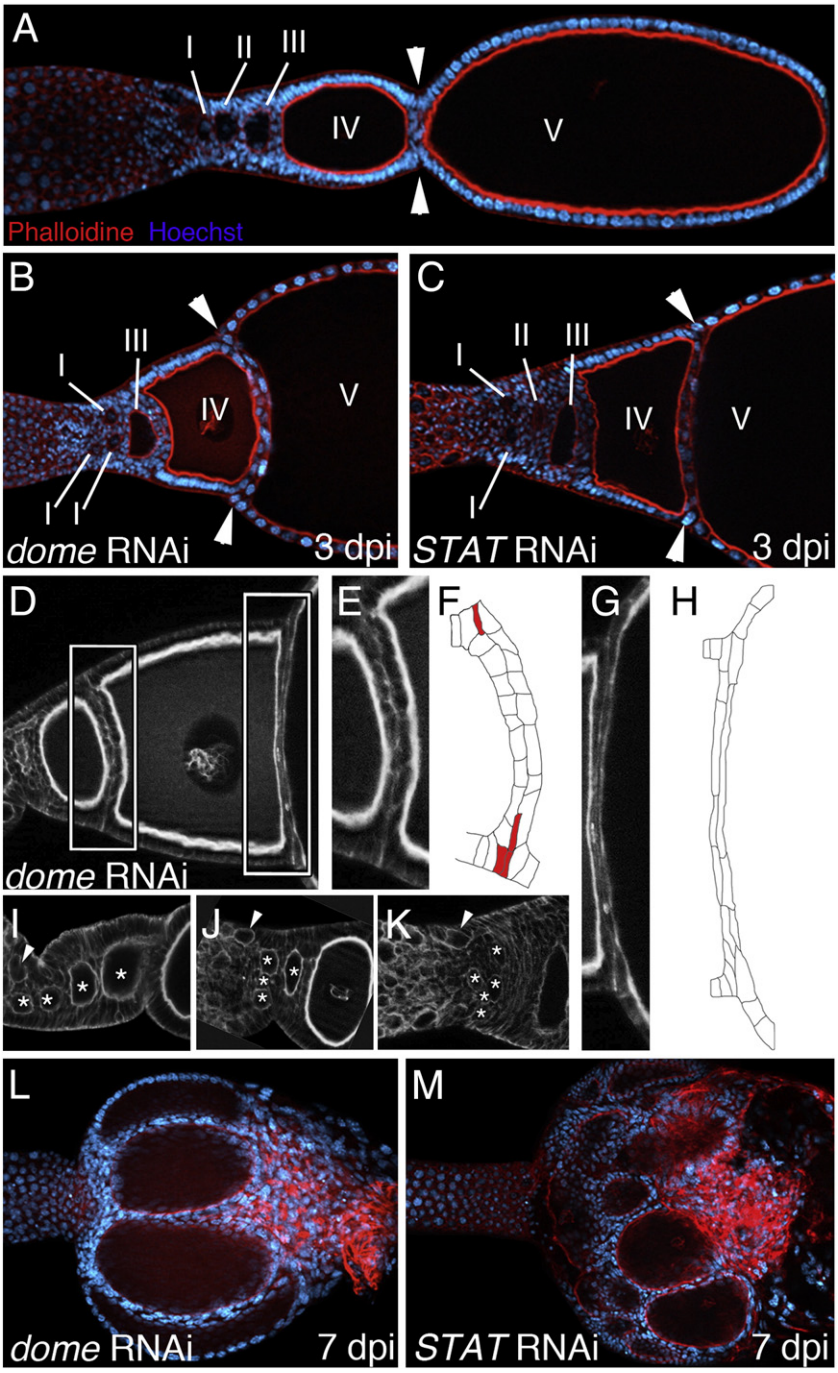

Fig. 2. *dome* and *STAT* RNAi ovariole phenotypes. (A) In the wildtype, vitellogenic egg chambers become separated by interfollicular stalk cells (arrowheads) that do not contact the germline. (B) *dome* RNAi and (C) *STAT* RNAi ovariole. Three days after dsRNA injection, the stalk between oocyte IV and oocyte V is absent. Instead, egg chambers remain directly attached to each other (arrowheads), which results in deformations of follicles. In addition, the alignment of pre-vitellogenic follicles (I, II) is disturbed. (D) In *dome* RNAi, follicles seem to maximise their area of contact to each other. (E, F) Close-up of the early (left) stalk shown in (D). Only a few residual stalk cells can be recognised (red cells in F, schematic representation). (G, H) Close-up of the later (right) stalk shown in D (H, schematic representation). No stalk cells are obvious. See also Fig. 1 for the wildtype situation. (I) In wildtype, pre-vitellogenic and early vitellogenic follicles are arranged in a row (asterisk). Upon (J) *dome* or (K) *STAT* RNAi, follicles (asterisk) are misarranged and positioned side-by-side. Arrowheads point to arrested pro-oocytes. (L, M) Seven days after dsRNA injection oogenesis is blocked completely. Egg chambers are miss-aligned and display severe, but rather variable morphological defects. Cell degeneration in posterior parts of the vitellarium is obvious. (A–C, L, M) Ovarioles stained with phalloidin for f-actin (red) and with Hoechst for DNA (blue). (D, E, G, I–K) Phalloidin channel. All panels: anterior to the left. Roman numerals refer to egg chambers in corresponding stages of oogenesis.

Tribolium Socs-6 RNAi results in compound follicles

The SOCS (Suppressor Of Cytokine Signalling) proteins are regulators of the JAK-STAT pathway that participate in a negative feedback loop (Arbouzova and Zeidler, 2006). Although two of the three *Drosophila* Socs genes (*Socs36E* and *Socs44A*) are expressed during oogenesis, their functions in early follicle cell patterning remain to be elucidated (Rawlings et al., 2004; Silver et al., 2005). During subsequent stages of *Drosophila* oogenesis, however, mis-sexpression experiments showed that SOCS36E is involved in achieving the precise level of STAT activity that is required for border cell migration (Silver et al., 2005). While *Drosophila Socs36E* is transcriptionally activated by JAK-STAT signalling, *Socs44* does not seem to participate in such an auto-regulatory negative feedback loop.

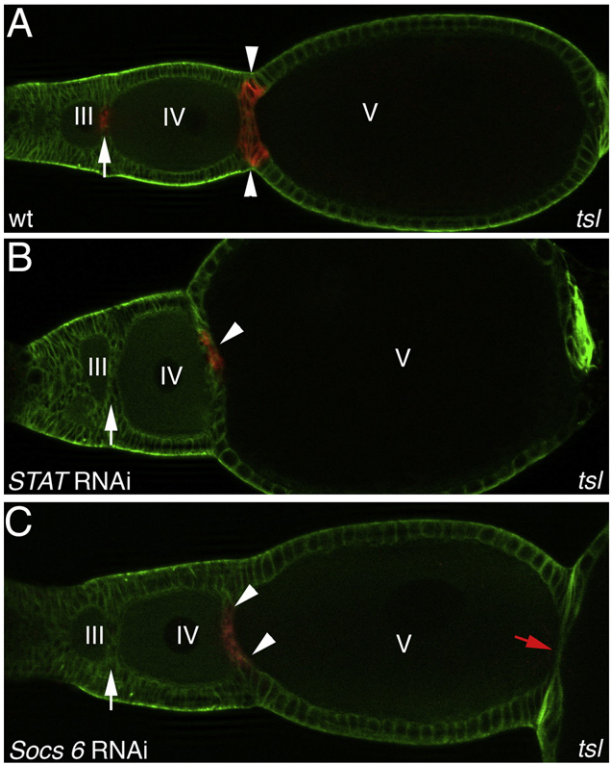

Fig. 3. *torso-like* expression in *STAT* and *Socs-6* RNAi. (A) Wildtype ovariole stained for tubulin (green) and *Tc-tsl* mRNA (red). *Tc-tsl* is expressed in the interfollicular stalk (arrowheads) and in putative stalk precursor cells (arrow). (B) *STAT* RNAi ovariole, stained for tubulin (green) and *Tc-tsl* mRNA (red). Three days after dsRNA injection the *STAT* phenotype is obvious, as the stalk is absent and egg chambers are directly attached to each other. *tsl* mRNA in stalk cells is severely reduced (arrowhead), while it is absent in the putative stalk precursors (arrow). (C) *Socs-6* RNAi ovariole, stained for tubulin (green) and *Tc-tsl* mRNA (red). Three days after dsRNA injection the initial *Socs-6* phenotype is obvious (arrowhead, red arrow). A subset of follicle cells that still separate vitellogenic egg chambers expresses the *tsl* marker. However, *tsl* expression in putative stalk precursors is lost.

Instead, *Socs44A* may function in modulating the EGFR pathway (Rawlings et al., 2004; Silver et al., 2005).

To gain insight into the function of SOCS proteins in insect oogenesis, we analysed three of the four *Tribolium Socs* genes by adult RNAi, i.e. *Tc-Socs-6*, *Tc-Socs-16D*, and *Tc-Socs-36E* (*Tc-Socs-2* is not expressed during oogenesis). Ovarioles were dissected and analysed for phenotypic effects three and seven days after injection. Interestingly, while oogenesis is affected upon *Tc-Socs-16D* and *Tc-Socs-6* RNAi (see the following discussion), *Tc-Socs-36E* RNAi had no obvious impact on oogenesis. To ensure that the RNAi was effective, we stained *Tc-Socs-36E* depleted ovarioles by in situ hybridization and found that *Tc-Socs-36E* mRNA is efficiently downregulated (not shown).

The knockdown of *Tc-Socs-16D* and *Tc-Socs-6*, on the other hand, resulted in identical phenotypes (see Fig. 5 for *Socs-6* RNAi), which were not further enhanced by the combined knockdown of both (or all three) *Tribolium Socs* genes (not shown). In these ovarioles, egg chambers fuse to form large compound follicles. Already three days after injection the number of both, the interfollicular stalk cells and the anterior and posterior terminal follicle cells of vitellogenic egg chambers are strongly reduced as judged by *Tc-tsl* expression and morphology (Figs. 3 and 5). Subsequently, terminal follicle and stalk cells are lost entirely, resulting in compound follicles comprising of vitellogenic oocytes (Figs. 5 E and F). Seven days after injection, even the fusion of pre-vitellogenic with vitellogenic follicles could be observed (Figs. 5 E and arrows in F). In these compound follicles, pre-vitellogenic oocytes remain in close contact to each other, and anterior and posterior terminal follicle cells and stalk cells that are normally found between older oocytes are completely absent. Thus, like JAK-STAT signalling, also *Tc-Socs-6* and *Tc-Socs-16D* are required for terminal follicle cell fate specification, but the loss of these genes results in an even stronger (or maybe qualitatively different) phenotype as these genes are also required for early oocyte separation and for maintaining those follicle cells that separate vitellogenic oocytes (Fig. 5).

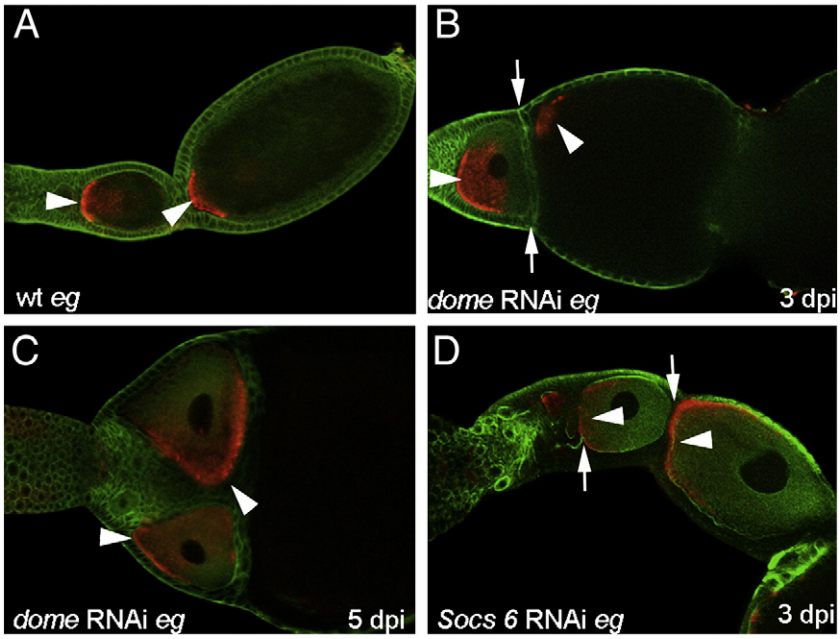

Fig. 4. *eagle* mRNA localisation in *Tc-dome* and *Tc-Socs-6* RNAi ovariols. (A) Wildtype ovariole stained for tubulin (green) and *Tc-eagle* mRNA (red). *Tc-eg* becomes restricted to the anterior part of the oocyte (arrowheads). (B) *dome* RNAi ovariole, stained for tubulin (green) and *Tc-eagle* mRNA (red). Three days after dsRNA injection the *dome* phenotype is obvious, as the stalk is absent and egg chambers are directly attached to each other (arrows). Still, *Tc-eg* mRNA becomes localised to the anterior pole of the oocytes (arrowheads). (C) Five days after dsRNA injection, *Tc-eg* remains localised to one pole of the oocyte. Note that the loss of stalk cells in *Tc-dome* RNAi results in the misalignment of egg chambers, which eventually displaces the entire follicle. Hence, in the upper egg chamber (C) *Tc-eg* is most likely still localised to the anterior pole and the "ventral localisation" is rather due to a subsequent rotation of the entire egg chamber. (D) *Socs-6* RNAi ovariole, stained for tubulin (green) and *Tc-eagle* mRNA (red). Three days after dsRNA injection the *Socs-6* phenotype is obvious (arrows). Still, *Tc-eg* mRNA becomes localised to the anterior pole of the oocytes (arrowheads).

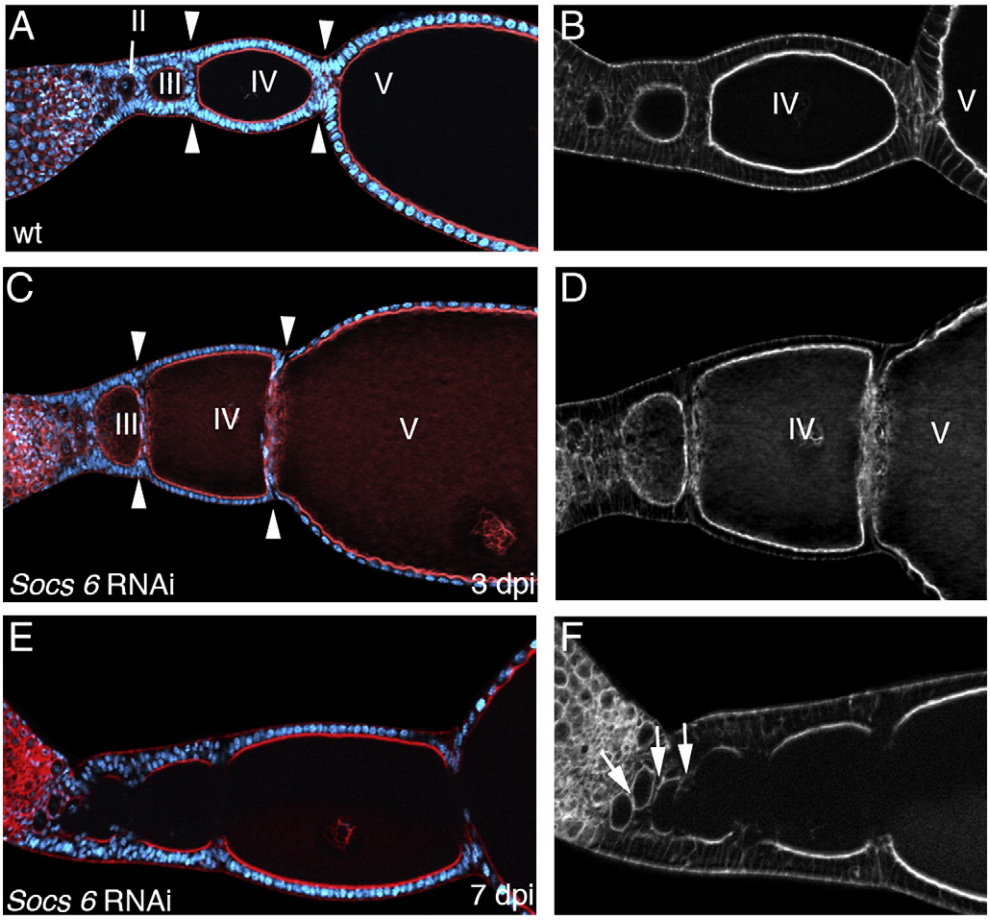

Fig. 5. *Socs-6* adult RNAi results in compound follicles. *Tribolium* ovarioles stained with phalloidin for f-actin (red) and with Hoechst for DNA (blue). (A) In the wildtype, growing follicles become subsequently separated by interfollicular stalk cells (III, IV, V, arrowheads). (B) Close-up of the wildtype ovariole shown in (A)—Phalloidin channel. (C, E) *Socs-6* adult RNAi ovarioles stained for Phalloidin (red) and Hoechst (blue). (C) Three days post injection of dsRNA not only the stalk cells are absent, but also terminal follicle cells (C, arrowheads) are severely reduced, ultimately resulting in a compound follicle. In addition, *Socs-6* RNAi affects the formation of egg chambers, as oocytes fuse with older follicles (F, arrows). (D) Close-up of the *Socs-6* RNAi ovariole shown in (C)—Phalloidin channel only. (E) Seven days after dsRNA injection all terminal follicle cells are absent, resulting in a single compound follicle. Note that individual oocytes are still separated by the plasma membrane, which can be visualised by cortical actin. (F) Close-up of the *Socs-6* RNAi ovariole shown in (E). Early egg chambers do not become separated properly, but remain attached to each other, and eventually will fuse (arrows). All panels: anterior to the left. Roman numerals refer to egg chambers in corresponding stages of oogenesis.

JAK-STAT signalling during embryonic patterning

In *Drosophila*, JAK-STAT signalling not only acts throughout oogenesis, but is also involved in early embryonic patterning (Binari and Perrimon, 1994; Brown et al., 2001b; Hou et al., 1996; Yan et al., 1996). Embryonic functions of JAK-STAT signalling can be investigated in *Tribolium* using adult RNAi despite the cessation of egg production a few days after the injection of dsRNA. Eggs that are laid a few days after dsRNA injection derive from oocytes that were already at an advanced stage of oogenesis at the time of treatment and these oocytes are able to complete their development. Embryos developing in such eggs display embryonic and larval phenotypes (Figs. 6–8).

In mildly affected *Tc-dome* RNAi larvae, the head as well as the first thoracic segment is absent, while the second and third thoracic segments as well as the abdominal segments are unaffected (Fig. 6 B). With an increasing RNAi effect, more posterior segments are also affected, ultimately resulting in cuticle balls from which often well-developed legs emerge (Figs. 6 C and D). However, while the head segments appear to be deleted entirely, the thoracic and abdominal segments are rather reduced and partially fused than absent, indicating that JAK-STAT signalling affects the maintenance of segment anlagen in *Tribolium*, i.e. acts in the framework of the segment-polarity genes (see the following discussion).

Given that *Tc-dome*, *Tc-hop*, and *Tc-STAT* RNAi result in largely identical oogenesis phenotypes, we expected also the embryonic phenotypes to be indistinguishable. Indeed, *Tc-hop* and *Tc-STAT* RNAi do result in severe segmentation phenotypes similar to those of *Tc-dome* (Figs. 6 B–H). Unexpectedly however, we only observe the head phenotype in more weakly affected *Tc-hop* RNAi, but not in *Tc-STAT* RNAi larvae (Figs. 6 G and H). While in strong *Tc-STAT* RNAi the loss of head segments might be obscured by the segment-polarity-like phenotype, in mildly affected *Tc-STAT* RNAi larvae head segments were clearly present (Fig. 6).

To further elucidate the embryonic patterning defects, we analysed the expression of the segmentation gene *wingless* (*wg*) in *Tc-dome* RNAi (Fig. 7). During differentiated blastoderm stages, *Tc-wg* is expressed in two ocular and a posterior domain (Fig. 7A). Subsequently, *Tc-wg* becomes expressed in a segmental fashion, as well as in a complex pattern in the head, including the labrum, ocular and antennal segments, as well as a domain in the stomodeum anlagen (Fig. 7 I) (Nagy and Carroll, 1994). In *Tc-dome* RNAi differentiated blastoderm stages, the ocular domains are severely reduced, whereas the posterior expression domain is unaffected. Noticeably, gnathal *Tc-wg* domains are not established during subsequent development (Figs. 7 C and G), thus reflecting the loss of the larval head. While *Tc-dome* RNAi anterior *Tc-wg* domains were not established, we could not

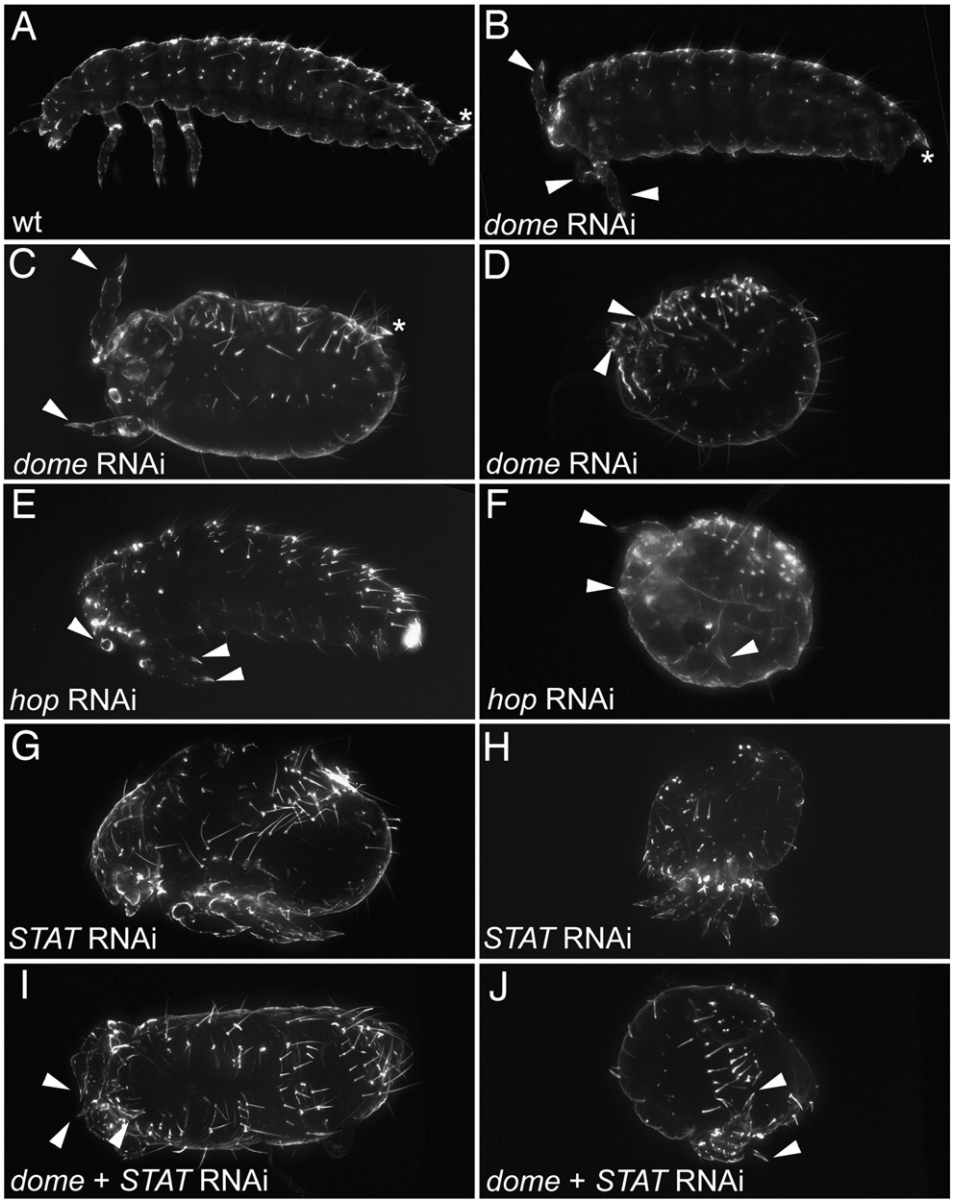

Fig. 6. Larval *dome*, *hop* and *STAT* RNAi phenotypes. (A) Cuticles of wildtype, (B-D) *Tc-dome*, (E, F) *Tc-hop*, and (G, H) *Tc-STAT* RNAi larvae. (I, J) *Tc-dome* and *Tc-STAT* double RNAi phenotypes. (B) Mildly affected *Tc-dome* RNAi larvae display head defects. (C, D) With increasing strength of the RNAi effect, segments seem to degenerate secondarily, as judged by the residual bristle and leg (arrowheads in D) patterns. (E) Also in mild *Tc-hop* RNAi, anterior segments are initially lost, while posterior segments form regularly. (F) Subsequently, segments degenerate and seem to fuse. (G) In mildly affected *Tc-STAT* RNAi larvae, the head pattern is unchanged, while posterior segments are reduced. (H) Ultimately, also in *Tc-STAT* RNAi, segments break down and seem to degenerate secondarily. Only legs are obvious. (I, J) The combined knockdown of *Tc-dome* and *Tc-STAT* does not enhance the single RNAi phenotypes any further, but show an additive effect, as in addition to the early *Tc-dome* head phenotype the posterior loss of segment boundaries is obvious. All panels: anterior to the left. Asterisks point to terminal structures and arrowheads point towards legs.

observe any obvious effects of *Tc-STAT* RNAi on the initial formation of pre-gnathal and gnathal *Tc*-wg domains (not shown).

Throughout subsequent germ-band elongation, abdominal *wg* domains are initially established correctly in *Tc-dome* RNAi (Fig. 7 J). This *Tc-wg* pattern, however, is lost during germ-band retraction, indicating a failure in segment or segmental boundary continuation (Fig. 7 K). Hence the (terminal) larval RNAi phenotype is reflected by the breakdown of the *wg* pattern. Corresponding phenotypes were also observed for late *Tc-hop* and *Tc-STAT* RNAi embryos (not shown). These phenotypes are reminiscent of those of the *Tribolium* segment-polarity genes (Bolognesi et al., 2008; Ober and Jockusch, 2006; Sulston and Anderson, 1996), suggesting a function for *Tribolium* JAK-STAT signalling in the segment-polarity network.

Since *Tc-Socs-2* is expressed throughout embryonic stages (Fig. S3), we also tested if the *Tribolium Socs* orthologues function in embryonic pattern formation. However, neither the single nor the combined knockdown of the four Socs genes yielded obvious segmentation phenotypes (not shown). Interestingly, also in *Drosophila*, a null allele of Socs36E, is viable and fertile (Issigonis et al., 2009; Singh et al., 2010). Hence, it remains to be tested whether *Tc-Socs-2* is activated by JAK/STAT signalling or if in *Tribolium* the function of JAK-STAT signalling in embryogenesis is independent of *Tc-Socs* genes.

Depletion of Tribolium dome affects early blastoderm patterning

While the terminal phenotypes of *Tc-dome*, *Tc-STAT*, and *Tc-hop* RNAi are probably due to a failure in segment maintenance, we found *Tc-dome* and *Tc-hop* to have additional roles in early anterior patterning that are likely *STAT* independent (Fig. 6). To visualise the emergence of gnathal segment primordia, we investigated the expression of the *Tribolium*

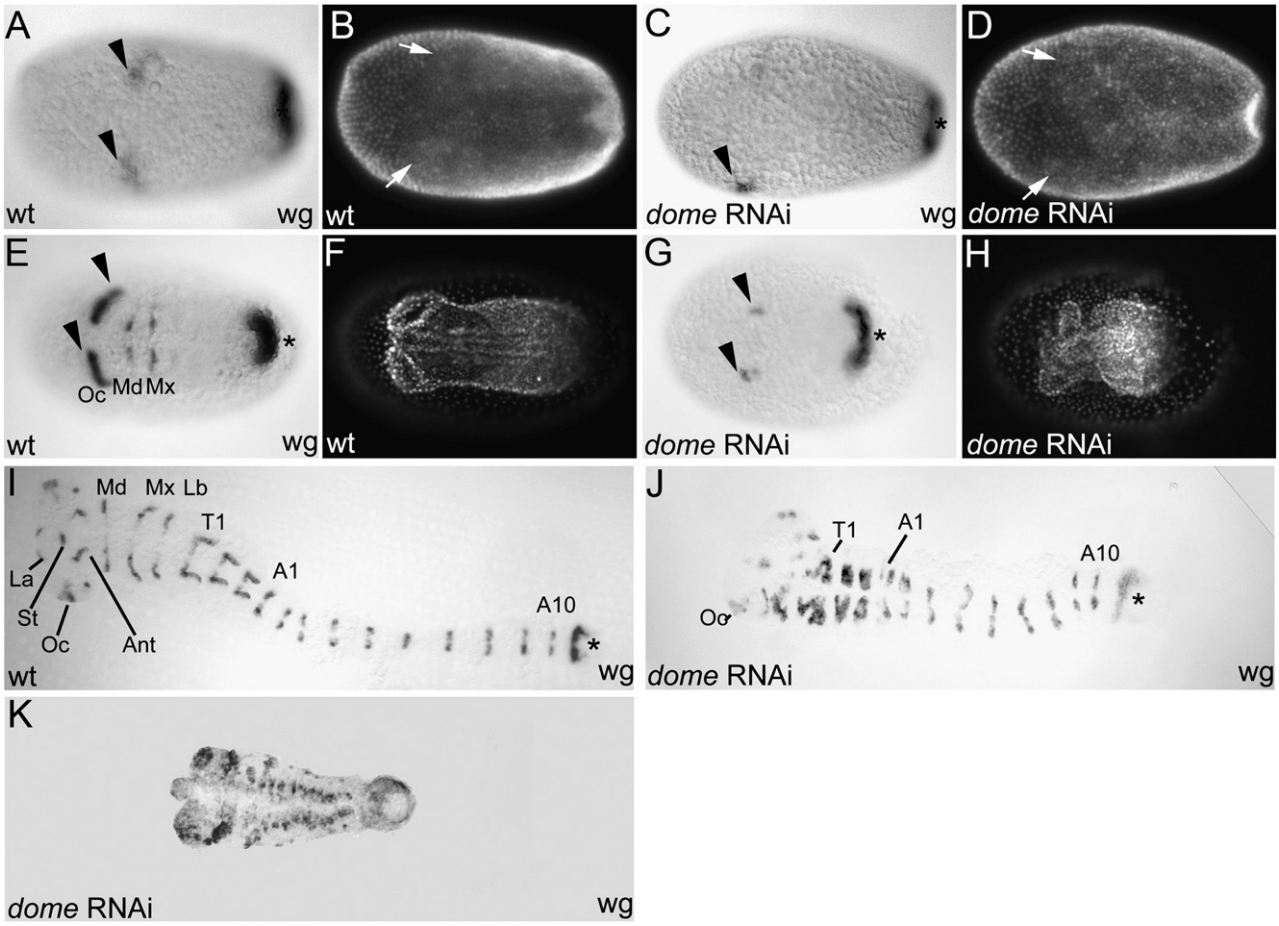

Fig. 7. *Tc-dome* is required for embryonic head formation and the maintenance of segmental boundaries. Wildtype (A, B, E, F, I) and *Tc-dome* (C, D, G, H, J, K) RNAi embryos stained for the segmental marker *wingless* (*Tc-wg*). Embryos in A, C, E, and G were counterstained for the nuclear marker Hoechst (B, D, F, H) to visualise morphology. (A, B) In wildtype differentiated blastoderm, *Tc-wg* is expressed in two ocular domains (arrowheads) and a posterior domain (asterisk). (C, D) Upon *Tc-dome* RNAi, the ocular domains are severely reduced, while posterior expression is unchanged. (E, F) During subsequent stages of wildtype development, gnathal *wg* domains become visible, which, however, (G, H) are not established in *Tc-dome* RNAi, indicating that the head anlagen in *Tc-dome* RNAi is indeed not formed properly. (I) In fully elongated wildtype germ-band stages, *Tc-wg* is expressed in a segmental fashion and in the head. (J) While in *Tc-dome* RNAi pre-gnathal and gnathal *Tc-wg* domains are largely absent and thoracic *Tc-wg* domains form only irregular, abdominal segment boundaries are initially established. (K) During germ-band retraction, the *Tc-wg* pattern becomes highly irregular, and the embryo gets smaller, indicating a segment boundary maintenance phenotype. All panels: anterior to the left, ventral views. Arrowheads point to ocular *Tc-wg* domains and asterisks to posterior *Tc-wg* domains, which persist throughout *Tribolium* segmentation. Arrows point to the head region. La: Labrum; St: Stomedeum; Oc: ocular domain; Ant: Antennae; Md: Mandibles; Mx: Maxillae; Lb: Labium; T: thoracic segment; A: abdominal segment.

pair-rule gene ortholog *even-skipped* (*Tc-eve*) in *Tc-dome* RNAi (Figs. 8 A and B). At the differentiated blastoderm stage, *Tc-eve* is expressed in three double segmental domains, labelling gnathal anlagen and the first thoracic segment primordial anlagen (Patel et al., 1994). Upon *Tc-dome* RNAi, we found the first primary *eve* stripe to be severely reduced, while more posterior *eve* domains (i.e. *eve* stripe 2 and *eve* stripe 3) were unaffected (Fig. 8 B). Next, we elucidated the impact of JAK-STAT signalling on the *Tribolium* gap genes *giant* (*Tc-gt*) and *knirps* (*Tc-kni*) (Bucher and Klingler, 2004; Cerny et al., 2008) (Figs. 8 C–F). *Tc-gt* forms an anterior domain comprising pre-gnathal and gnathal segments (Fig. 8C) (Bucher and Klingler, 2004). In *Tc-dome* RNAi anterior *gt* expression is considerably weaker and the head domain is reduced in size (Fig. 8 D).

The expression of *Tc-kni* (Cerny et al., 2008) arises at the undifferentiated blastoderm stage in a rather broad central domain, which refines to a wedge-shaped domain covering the mandibular and pre-gnathal region (Fig. 8 G). Like for *Tc-gt*, the depletion of *Tc-dome* results in a concomitant reduction of size and expression levels (Fig. 8 H). As expected, in STAT depleted embryos *Tc-gt* and *Tc-kni* expression was unchanged (not shown).

While some ventral *Tc-gt* and *Tc-kni* expression is preserved in *dome* depleted embryos, dorsal and lateral aspects are largely absent (Figs. 8 H and J). To elucidate whether this reflects regulatory input of JAK-STAT signalling on dorso-ventral (DV) patterning, we observed whether the boundary between embryonic and extraembryonic tissue is still oblique in JAK-STAT RNAi embryos. During wildtype development, the extraembryonic serosa expands dorsally towards the posterior pole. Concurrently, anterior embryonic anlagen become restricted to a ventral position and the boundary between embryonic and extraembryonic tissue is no longer straight but oblique. This dorsal tilt of the embryo–serosa boundary is under control of the DV patterning system (i.e. dorsal Dpp activity; e.g. van der Zee et al., 2006). As monitored by Hoechst staining, in *dome* depleted embryos the embryo–serosa boundary is still oblique (Figs. 8 F and J), indicating that JAK-STAT signalling has no impact on DV axis formation in *Tribolium*.

Our data confirm that *Tc-dome* RNAi affects the patterning of pre-gnathal and gnathal anlagen already during early embryonic stages. Subsequently, however, the loss of head segments is obscured by the segment-polarity-like phenotype.

Discussion

JAK-STAT signalling is required throughout Tribolium embryogenesis

In this work, we found that JAK-STAT signalling is involved in *Tribolium* not only in oogenesis but also in embryonic segmentation.

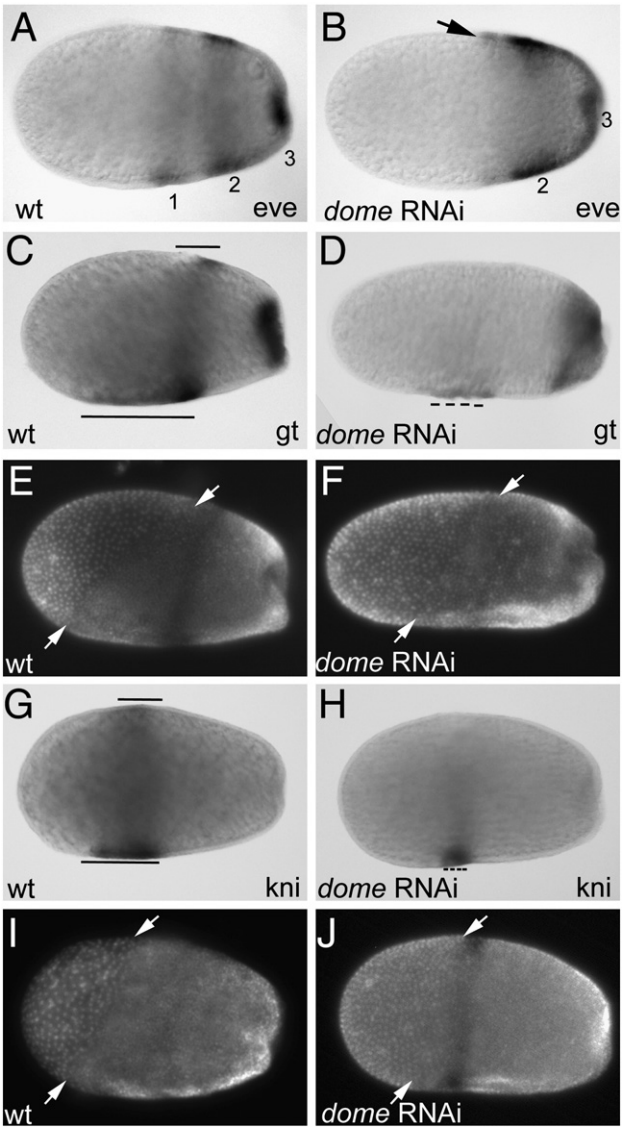

Fig. 8. *Tc-dome* RNAi affects blastodermal expression of pair-rule and gap genes. Stage matched differentiated blastoderm wildtype (A, C, G) and *Tc-dome* RNAi (B, D, H) embryos, stained for the pair-rule gene *Tc-eve* (A, B) and for the gap genes *Tc-gt* (C, D) and *Tc-kni* (G,H). Embryos in C, G, D, and H were counterstained with Hoechst 33342 (E, F, I, J). (A) At that stage of development, *Tc-eve* is expressed in three primary domains (1–3). (B) Upon depletion of *Tc-dome*, the first *Tc-eve* domain is severely reduced (arrow). (C, E) *Tc-gt* is expressed in a wedge-shaped domain, covering pre-gnathal and gnathal anlagen (lines). The posterior part of the anterior domain corresponds to the maxillary segment anlagen. (D, F) In *Tc-dome* RNAi, this anterior domain is hardly visible (dashed line). The serosa–embryo boundary (arrows), however, is still oblique, indicating that dorso-ventral axis formation is unaffected. (G, I) In the differentiated blastoderm, *Tc-kni* is expressed in a domain covering mandibular and pre-gnathal anlagen (lines). (H, J) Again, upon *Tc-dome* RNAi this domain is severely reduced (dashed line). All panels: anterior to the left, lateral views. Arrows point to the serosa–embryo boundary.

This is reminiscent of *Drosophila*, where the inactivation of *hop*, *Stat92E*, *upd1*, or *dome* results in largely identical phenotypes, namely in the loss of the fifth abdominal segment and the posterior mid-ventral portion of the fourth segment. Additional variable defects occur in other segments: reduction of the second thoracic and eighth abdominal segments, and fusion of the sixth and seventh abdominal segments (Binari and Perrimon, 1994; Small et al., 1996; Yan et al., 1996). Initial defects appear to occur at the level of pair-rule genes and only later extend to a subset of *wingless* and *engrailed* stripes and thus, are probably related to STAT activity in the early blastoderm.

In contrast to *Drosophila*, the terminal phenotypes of *Tc-dome*, *Tc-STAT*, and *Tc-hop* deprived larvae (Fig. 6) suggest that in *Tribolium* this pathway functions at the level of the segment-polarity genes. Initially, *wg* expression domains are established precisely in RNAi embryos and are only lost secondarily, indicating a failure in segment boundary maintenance rather than in segment boundary formation, which is reminiscent of the depletion of segment-polarity genes in *Tribolium* (Bolognesi et al., 2008; Ober and Jockusch, 2006). While we observed this terminal segment-polarity phenotype for all members of the JAK-STAT signalling pathway analysed in this study, early patterning defects were restricted to *Tc-dome* and *Tc-hop* embryos (Figs. 6–8). In such RNAi embryos, pre-gnathal and gnathal anlagen did not form properly, which seems to be correlated to regulatory input on anterior gap gene and pair-rule gene domains.

It is still under debate, how anterior patterning in *Tribolium* is realised (Kotkamp et al., 2010; Schoppmeier et al., 2009). While Bicoid (Bcd) is restricted to higher dipterans (Brown et al., 2001a), Orthodenticle (Otd) and Hunchback (Hb) were believed to substitute for Bcd as anterior morphogens in non-dipteran insects (Lynch et al., 2006; Schroder, 2003). However, recent work in *Tribolium* suggests that neither Hb nor Otd provides concentration dependent information (Kotkamp et al., 2010; Marques-Souza et al., 2008). Thus, it is likely that anterior patterning in *Tribolium* involves additional—as yet unknown—segmentation genes, which may act as morphogenes.

We now demonstrate that *Tribolium dome* and *hop* crucially contribute to early anterior blastodermal regionalization, as in RNAi embryos pre-gnathal and gnathal anlagen were already lost early in embryogenesis (Figs. 7 and 8). During *Drosophila* oogenesis, Upd generates a morphogen gradient along the anterior–posterior axis of the follicular epithelium surrounding the egg chamber (Xi et al., 2003). This raises the possibility that JAK-STAT signalling provides positional information during *Tribolium* cellular blastoderm stages. We consider it, however, unlikely that JAK-STAT signalling fulfils Bcd-like functions in head patterning, as the first *eve* stripe as well as the anterior *kni* and *gt* domains were rather reduced than absent (Fig. 8) and thus, are not obliterated as they are in *Drosophila bicoid* mutants (Driever and Nusslein-Volhard, 1988; Hulskamp et al., 1990; Kraut and Levine, 1991; Rivera-Pomar et al., 1995; Tautz, 1988). Moreover, we found *dome*, *STAT*, and *hop* mRNAs to be expressed ubiquitously, rather than in distinct (anterior) domains. To reveal the spatial and temporal activity of JAK-STAT signalling during early *Tribolium* patterning, it will be necessary to identify the *Tribolium* ligand of the receptor Domeless.

We propose that patterning of pre-gnathal and gnathal anlagen during early *Tribolium* development requires some basal JAK-STAT activity to ensure proper expression levels of gap and pair-rule genes. This is comparable to *Drosophila*, where JAK-STAT signalling is necessary to achieve distinct levels of expression of particular stripes of pair-rules genes, rather than setting pair-rule stripe boundaries (Binari and Perrimon, 1994). Hence, during early insect development, JAK-STAT signalling may have a conserved role as a general activation system for transcription. Subsequently, however, *Tribolium* JAK-STAT functions in the frame of the segment-polarity network. The segmental expression of *Drosophila upd* genes in the extended germ-band stage may thus represent an evolutionary relict.

Early patterning function may be mediated by alternative Hop targets

Unexpectedly, *Tc-STAT* RNAi did not yield in any obvious early patterning phenotypes. As monitored by in situ hybridisation, *dome* mRNA was absent in *dome* RNAi embryos (not shown). While we thus consider it unlikely that the RNAi effect was only incomplete, we hypothesise that early embryonic patterning in *Tribolium* may involve an additional STAT-like gene. Hence, JAK-signalling throughout *Tribolium* oogenesis and during late embryogenesis (i.e. the segment-polarity function) may employ *Tc-STAT*, whereas the early patterning function may require another (as yet unidentified) STAT-like transcription factor.

While in mammalian systems, several STAT genes were reported, which fulfil different functions during embryogenesis (Stephanou and Latchman, 2005), in *Drosophila*, it is still unclear how STAT92E elicits both proliferative and anti-proliferative effects (Mukherjee et al., 2005). However, neither for *Drosophila* nor for *Tribolium* a second evident STAT-homolog was recognised in the genome, indicating that —at least in *Tribolium*—JAK-STAT signalling may involve alternative (i.e. STAT independent) transcription factors, which would serve as functional STAT-orthologues. Still, even though there is evidence for non-canonical JAK-STAT signalling in *Drosophila* (Shi et al., 2006), thus far there is no support for an involvement of transcription factors other than STAT and it also remains to be elucidated whether Hop indeed can phosphorylate alternative targets. Hence, additional studies are required to uncover the divergent roles of *Tc-dome* and *Tc-STAT* in early embryonic patterning of *Tribolium.*

Function of JAK-STAT signalling in follicle patterning

During *Drosophila* oogenesis, JAK-STAT signalling is essential for the subdivision of a population of polar/stalk precursor cells into pairs of polar cells and 8–10 stalk cells. Expression of the ligand UPD is restricted to the polar follicle cells and induces the remaining cells of the precursor group to adopt a stalk cell fate. Differentiated stalk cells then intercalate to form the stalk and thereby separate the egg chamber from the germarium (McGregor et al., 2002). Inactivation of JAK-STAT signalling results in supernumerary polar cells at the expense of stalk cells, and consequently in fused egg chambers (compound follicles) (McGregor et al., 2002; Xi et al., 2003). The situation in *Tribolium*, however, is less clear, as we currently have no markers for polar and terminal cells (if such cell populations indeed exist in *Tribolium*). Thus, we currently cannot exclude the possibility that an earlier step in follicle cell specification (e.g. specification of a stalk/polar cell precursor group) is disrupted in ovarioles lacking STAT activity, or that STAT is required later on for cell proliferation or recruitment of additional stalk cells from adjacent follicles. Although stalk cells become morphologically distinguishable only during advanced stages of vitellogenesis, there is evidence for an early stalk precursor population, as the *Tc-tsl* gene is expressed in those follicle cells that separate individual follicles early in oogenesis (Fig. 3). Interestingly, *Tc-tsl* expression in this region is severely reduced upon *Tc-dome* or *Tc-STAT* RNAi, suggesting that these cells already might have acquired a stalk precursor identity, and that the specification of this identity depends on JAK-STAT signalling. Therefore, we deem it likely that the loss of the stalk in JAK-STAT depleted ovarioles is due to the mis-specification of follicle cell populations, i.e. stalk precursors. In the wildtype, these cells may arise—analogously to *Drosophila* (McGregor et al., 2002; Xi et al., 2003)—in a stepwise (and JAK-STAT-dependent) manner from a common terminal/stalk cell precursor population (Fig. 9).

JAK-STAT signalling and oocyte alignment

While the requirement for JAK-STAT signalling during stalk formation may well be conserved between polytrophic *Drosophila* and telotrophic *Tribolium* oogenesis, there are indications that in *Tribolium* JAK-STAT signalling has additional functions at a very early step during oogenesis, as the loss of *Tc-dome*, *Tc-hop*, or *Tc-STAT* activity affects the positioning of young pre-vitellogenic follicles (Figs. 2 and S2). Upon pro-oocyte maturation, these cells separate from the somatic plug and enter the vitellarium, where they become encapsulated by somatic cells (Fig. 1). As these early follicles develop, they become aligned in a single row. After knockdown of JAK-STAT signalling, oocytes fail to align in this manner (Fig. 2).

The molecular mechanism for egg chamber alignment is not known, but could involve a JAK-STAT-based relay mechanism signalling from older to younger follicles, similar to the way follicle polarity is transmitted in *Drosophila* from one cyst to the next younger one (Torres et al., 2003)—although this relay in *Drosophila* depends on Notch rather than on STAT activity (Assa-Kunik et al., 2007; Grammont and Irvine, 2001; Lopez-Schier and St Johnston, 2001). In *Tribolium*, a JAK-STAT based relay mechanism could result in differential expression of adhesion molecules directing oocyte migration or, alternatively, JAK-STAT signalling might function to prevent dormant oocytes from entering oogenesis, resulting in follicles developing prematurely and in parallel if this signal is removed.

Whatever the exact role of JAK-STAT signalling during *Tribolium* oocyte alignment may be, this clearly is a function that at this early stage of oogenesis is not known from *Drosophila*, where this signalling cascade appears to be required in subsequent steps of follicle development, i.e. the induction of interfollicular stalk cells or (even later) the migration of border cells (Assa-Kunik et al., 2007; McGregor et al., 2002; Silver et al., 2005; Xi et al., 2003). Thus, even though also in *Drosophila* strong JAK-STAT phenotypes can eventually lead to impaired arrangements of egg chambers (due to the formation of compound follicles or the subsequent breakdown of the stalk) this early function of JAK-signalling in egg chamber alignment could be specific for telotrophic oogenesis.

Polarisation of the oocyte appears to be independent of JAK-STAT signalling

In *Drosophila*, JAK-STAT mediated induction of terminal follicle cell fates is crucial for axis formation. Secretion of Upd by the polar cells at each end of the egg chamber generates a morphogen gradient along the anterior–posterior axis (Xi et al., 2003). This gradient distinguishes terminal follicle cells from main-body follicle cells and establishes a symmetrical pre-pattern of the follicular epithelium. This (AP) symmetry is broken when the oocyte nucleus lies close to the posterior pole of the oocyte, where EGF signalling to the overlying posterior follicle cells, and subsequent back signalling, lead to the repolarisation of the oocyte cytoskeleton and to the localization of maternal *bicoid* and *oskar* mRNAs at the poles (Gonzalez-Reyes et al., 1995; Roth et al., 1995).

The localization of *Tc-eg* mRNA to at least one pole of the oocyte during *Tribolium* oogenesis (Figs. 4 A and S1), leads to significant implications for AP axis specification during *Tribolium* oogenesis: The sole presence of an mRNA localization mechanism suggests that asymmetric RNA distribution might be relevant also for patterning the *Tribolium* embryo (as it is the case in *Drosophila*). Although *Tc-eg* has no obvious function (Bucher et al., 2005), it is likely that other gene products localised by the same machinery can provide essential patterning functions in *Tribolium*. Interestingly, upon *Tc-dome*, *Tc-STAT*, or *Tc-Socs-6* RNAi *Tc-eg* transcripts still become localised to the anterior pole of developing oocytes (Fig. 4). We also found that in the absence of JAK-STAT signalling *Tc-eg* mRNA still becomes localised to the anterior pole of the embryo (not shown). Hence, while EGF signalling is required for proper axes patterning (Lynch et al., 2010), JAK-STAT signalling may neither contribute to the initial polarisation nor to the maintenance of oocyte polarity.

The process of *Tc-eg* localization (*Tc-eg* mRNA is distributed ubiquitously at the beginning of oogenesis but subsequently becomes localised to one pole) suggests that the anterior–posterior axis of the oocyte is established early during oogenesis. Further studies will be required to reveal if this polarity arises in response to the spatial arrangement of nutritive chords or requires interactions of germline and soma.

Tribolium SOCS proteins are required for early follicle patterning

Of the four *Tribolium* Suppressors of Cytokine Signalling (Socs) orthologues we identified in the genome of this beetle, only *Tc-Socs-6* and *Tc-Socs-16D* appear to have a function during telotrophic oogenesis. Knocking down either one or both of these genes causes the loss of

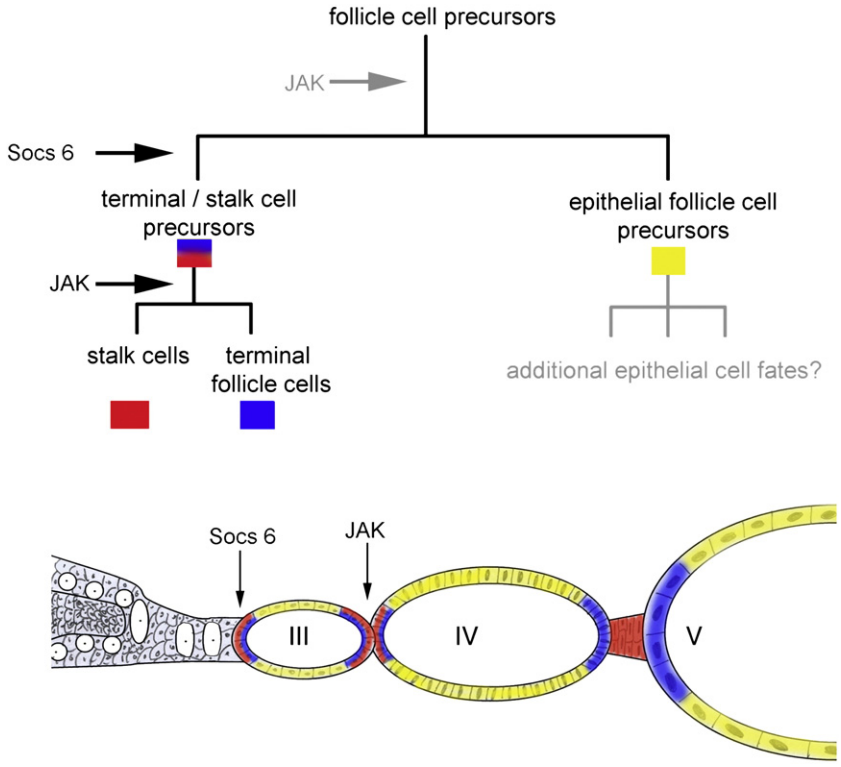

Fig. 9. Model for early follicle cell patterning. During telotrophic *Tribolium* oogenesis, follicle cell populations become specified in a stepwise manner. After encapsulation, individual follicles become aligned in a single row, a process likely to depend on JAK-STAT signalling (grey arrow). However, it is still unknown whether this process already involves the specification of follicle cell subpopulations. Subsequently, follicle cells become subdivided into a terminal/stalk precursor (red + blue) and an epithelial population (yellow), which involves Socs-6 (and Socs-16D). SOCS proteins are likely required for the specification of terminal/stalk precursor cells. The determination of stalk precursor cells then depends on JAK-STAT signalling, as in the absence of this signalling pathway the stalk is lost (see text for details). Roman numerals refer to egg chambers in corresponding stages of oogenesis.

separating stalk cells and of terminal (i.e. anterior and posterior) follicle cells (Fig. 5), which could be due to a general role of SOCS proteins in cell maintenance (Arbouzova and Zeidler, 2006; Croker et al., 2008). Still, as lateral epithelial follicle cells are unaffected, the *Tribolium* SOCS proteins more likely contribute to the early specification of follicle cell populations (see Fig. 9).

Originally, SOCS proteins were identified as negative regulators of cytokine signalling that function in suppressing JAK-STAT signalling (Arbouzova and Zeidler, 2006; Croker et al., 2008). They are themselves target genes of this pathway and are expressed in response to STAT activation, thereby forming negative feedback loops to downregulate pathway activity. Given that during telotrophic *Tribolium* oogenesis SOCS proteins are involved in negatively regulating JAK-STAT signalling, we expected *Socs*-RNAi to cause overactivation of JAK-STAT signalling, which could result in enlarged stalks. However, depleting *Tc-Socs-6* or *Tc-Socs-16D* leads to even more severe phenotypes than JAK-STAT RNAi. Still, as we currently cannot generate JAK/STAT gain-of-function phenotypes for *Tribolium*, it remains to be elucidated whether the depletion of SOCS resembles JAK-STAT de-repression phenotypes. Alternatively, *Tribolium* SOCS proteins may not act through the JAK-STAT pathway but independent of, or in parallel to it.

In *Drosophila*, *upd* mutations abolish *Socs36E* expression, consistent with a role of *Socs36E* in JAK-STAT signalling (Karsten et al., 2002). During spermatogenesis, SOCS36E was reported to act in a negative feedback loop to inhibit JAK-STAT signalling (Issigonis et al., 2009; Singh et al., 2010). However, neither for *Tc-Socs-6* nor for *Tc-Socs-16D* we observed changes in expression levels or expression pattern upon *dome* or *Stat* RNAi (not shown), strengthening our interpretation that *Tc-Socs-6* and *Tc-Socs-16D* function independent of JAK-STAT signalling.

There is growing evidence for SOCS proteins being involved in the regulation of signalling pathways other than cytokine receptors, including EGFR signalling (Croker et al., 2008). The *Drosophila* SOCS44A protein, for instance, has been shown to upregulate EGFR pathway activity (Rawlings et al., 2004) and also SOCS36E modulates EGFR signalling in the imaginal wing disc (Callus and Mathey-Prevot, 2002). During oogenesis, the *Drosophila* EGFR signalling pathway is required for axes formation (Nilson and Schupbach, 1999) and border cell migration (Silver et al., 2005). Initially, however, EGFR is required for proper egg chamber formation, and impairing this pathway results in compound follicles (Goode et al., 1996; Sapir et al., 1998) resembling the phenotype we observe after depleting *Tribolium Socs* genes. Thus, it is tempting to speculate that the SOCS proteins may modulate EGFR signalling rather than JAK-STAT signalling during *Tribolium* oogenesis. While the exact molecular role of SOCS proteins in telotrophic oogenesis remains to be elucidated, it is interesting to note that we demonstrate here for the first time an unquestionable requirement for *Socs* genes in insect oogenesis, since thus far available *Drosophila Socs36E* alleles are apparently fertile (Issigonis et al., 2009; Singh et al., 2010) and beyond that only gain-of-function experiments have been reported (Rawlings et al., 2004; Silver et al., 2005).

A framework to interpret early follicle patterning in Tribolium

This study reports our initial attempts to genetically analyse the mechanisms underlying telotrophic oogenesis in *Tribolium*. Given that our data mainly concern one out of several pathways that are likely involved, we obtained first hints rather than a full picture as to how follicle development proceeds in *Tribolium* (summarised in Fig. 9).

The first here identified regulatory step in follicle differentiation takes place in the anterior part of the vitellarium. During or shortly after encapsulation, pre-vitellogenic egg chambers become arranged in a single row in a JAK-STAT dependent manner. At that stage of *Tribolium* oogenesis an initial distinction of terminal/stalk precursor cells (red + blue in Fig. 9) versus epithelial follicle cells (yellow in

Fig. 9) may take place, as the linear arrangement of follicles probably requires differential adhesion characteristics for those follicle cells contacting two oocytes and those contacting only one. In contrast to *Drosophila* (Grammont and Irvine, 2001; Lopez-Schier and St Johnston, 2001), this early step already involves JAK-STAT signalling. That such an early terminal/stalk precursor population indeed exists is supported by the *Tc-Socs-6* and *Tc-Socs-16D* phenotypes, in which both, the anterior and posterior follicle cells—and as a consequence—also the stalk cells are absent. As indicated by the differences between the *Tc-Socs-6/Tc-Socs-16D* and the *Tc-dome/Tc-STAT* RNAi phenotypes, where only stalk cells are lost, this function of SOCS proteins probably is quite independent of JAK-STAT signalling.

A subsequent step in *Tribolium* oogenesis then again involves JAK-STAT signalling and may result—interpreted in analogy to the situation in *Drosophila*—in the determination of stalk precursor cells (red in Fig. 9) out of a common terminal/stalk precursor population. Then the interfollicular stalk is formed, mostly by migration and intercalation of these stalk precursor cells.

In *Drosophila*, JAK-STAT signalling later on mediates the specification of additional follicle cell fates, namely the border cells, the stretched follicle cells, and the centripetal cells (Xi et al., 2003). Given the less complex egg chamber architecture in telotrophic ovaries, it is unclear, whether additional subpopulations of follicle cells differentiate in *Tribolium*. Generating new molecular markers including enhancer traps (see Trauner et al., 2009), and identifying new players in insect oogenesis through genome-wide RNAi screens should allow rapid progress in understanding the genetic basis of *Tribolium* oogenesis.

Supplementary materials related to this article can be found online at doi:10.1016/j.ydbio.2010.10.020.

Acknowledgments

We are grateful to Martin Klingler for continuous support. We thank Martin Klingler and Ralph Rübsam for critical reading of the manuscript. The work of MS was funded through grants by the German Research Foundation (DFG).

References

Arbouzova, N.I., Zeidler, M.P., 2006. JAK/STAT signalling in *Drosophila*: insights into conserved regulatory and cellular functions. Development 133, 2605–2616.
Assa-Kunik, E., Torres, I.L., Schejter, E.D., Johnston, D.S., Shilo, B.Z., 2007. Drosophila follicle cells are patterned by multiple levels of Notch signaling and antagonism between the Notch and JAK/STAT pathways. Development 134, 1161–1169.
Bastock, R., St Johnston, D., 2008. *Drosophila* oogenesis. Curr. Biol. 18, R1082–R1087.
Beccari, S., Teixeira, L., Rorth, P., 2002. The JAK/STAT pathway is required for border cell migration during *Drosophila* oogenesis. Mech. Dev. 111, 115–123.
Binari, R., Perrimon, N., 1994. Stripe-specific regulation of pair-rule genes by hopscotch, a putative Jak family tyrosine kinase in *Drosophila*. Genes Dev. 8, 300–312.
Bolognesi, R., Farzana, L., Fischer, T.D., Brown, S.J., 2008. Multiple Wnt genes are required for segmentation in the short-germ embryo of *Tribolium castaneum*. Curr. Biol. 18, 1624–1629.
Boulay, J.L., O'Shea, J.J., Paul, W.E., 2003. Molecular phylogeny within type I cytokines and their cognate receptors. Immunity 19, 159–163.
Brown, S., Fellers, J., Shippy, T., Denell, R., Stauber, M., Schmidt-Ott, U., 2001a. A strategy for mapping bicoid on the phylogenetic tree. Curr. Biol. 11, R43–R44.
Brown, S., Hu, N., Hombria, J.C., 2001b. Identification of the first invertebrate interleukin JAK/STAT receptor, the *Drosophila* gene domeless. Curr. Biol. 11, 1700–1705.
Bucher, G., Klingler, M., 2004. Divergent segmentation mechanism in the short germ insect *Tribolium* revealed by giant expression and function. Development 131, 1729–1740.
Bucher, G., Scholten, J., Klingler, M., 2002. Parental RNAi in *Tribolium* (Coleoptera). Curr. Biol. 12, R85–R86.
Bucher, G., Farzana, L., Brown, S.J., Klingler, M., 2005. Anterior localization of maternal mRNAs in a short germ insect lacking bicoid. Evol. Dev. 7, 142–149.
Callus, B.A., Mathey-Prevot, B., 2002. SOCS36E, a novel *Drosophila* SOCS protein, suppresses JAK/STAT and EGF-R signalling in the imaginal wing disc. Oncogene 21, 4812–4821.
Cerny, A.C., Bucher, G., Schroder, R., Klingler, M., 2005. Breakdown of abdominal patterning in the *Tribolium* Kruppel mutant jaws. Development 132, 5353–5363.
Cerny, A.C., Grossmann, D., Bucher, G., Klingler, M., 2008. The *Tribolium* ortholog of knirps and knirps-related is crucial for head segmentation but plays a minor role during abdominal patterning. Dev. Biol. 321, 284–294.
Choe, C.P., Brown, S.J., 2007. Evolutionary flexibility of pair-rule patterning revealed by functional analysis of secondary pair-rule genes, paired and sloppy-paired in the short-germ insect, *Tribolium castaneum*. Dev. Biol. 302, 281–294.
Choe, C.P., Miller, S.C., Brown, S.J., 2006. A pair-rule gene circuit defines segments sequentially in the short-germ insect *Tribolium castaneum*. Proc. Natl Acad. Sci. USA 103, 6560–6564.
Croker, B.A., Kiu, H., Nicholson, S.E., 2008. SOCS regulation of the JAK/STAT signalling pathway. Semin. Cell Dev. Biol. 19, 414–422.
Denef, N., Schupbach, T., 2003. Patterning: JAK-STAT signalling in the *Drosophila* follicular epithelium. Curr. Biol. 13, R388–R390.
Driever, W., Nusslein-Volhard, C., 1988. The bicoid protein determines position in the *Drosophila* embryo in a concentration-dependent manner. Cell 54, 95–104.
Flaherty, M.S., Zavadil, J., Ekas, L.A., Bach, E.A., 2009. Genome-wide expression profiling in the *Drosophila* eye reveals unexpected repression of notch signaling by the JAK/STAT pathway. Dev. Dyn. 238, 2235–2253.
Ghiglione, C., Devergne, O., Georgenthum, E., Carballes, F., Medioni, C., Cerezo, D., Noselli, S., 2002. The *Drosophila* cytokine receptor Domeless controls border cell migration and epithelial polarization during oogenesis. Development 129, 5437–5447.
Gonzalez-Reyes, A., Elliott, H., St Johnston, D., 1995. Polarization of both major body axes in *Drosophila* by gurken-torpedo signalling. Nature 375, 654–658.
Goode, S., Morgan, M., Liang, Y.P., Mahowald, A.P., 1996. Brainiac encodes a novel, putative secreted protein that cooperates with Grk TGF alpha in the genesis of the follicular epithelium. Dev. Biol. 178, 35–50.
Grammont, M., Irvine, K.D., 2001. fringe and Notch specify polar cell fate during *Drosophila* oogenesis. Development 128, 2243–2253.
Horne-Badovinac, S., Bilder, D., 2005. Mass transit: epithelial morphogenesis in the *Drosophila* egg chamber. Dev. Dyn. 232, 559–574.
Hou, X.S., Melnick, M.B., Perrimon, N., 1996. Marelle acts downstream of the *Drosophila* HOP/JAK kinase and encodes a protein similar to the mammalian STATs. Cell 84, 411–419.
Hulskamp, M., Pfeifle, C., Tautz, D., 1990. A morphogenetic gradient of hunchback protein organizes the expression of the gap genes Kruppel and knirps in the early *Drosophila* embryo. Nature 346, 577–580.
Issigonis, M., Tulina, N., de Cuevas, M., Brawley, C., Sandler, L., Matunis, E., 2009. JAK-STAT signal inhibition regulates competition in the *Drosophila* testis stem cell niche. Science 326, 153–156.
Karsten, P., Hader, S., Zeidler, M.P., 2002. Cloning and expression of *Drosophila* SOCS36E and its potential regulation by the JAK/STAT pathway. Mech. Dev. 117, 343–346.
Kotkamp, K., Klingler, M., Schoppmeier, M., 2010. Apparent role of *Tribolium* orthodenticle in anteroposterior blastoderm patterning largely reflects novel functions in dorsoventral axis formation and cell survival. Development 137, 1853–1862.
Kraut, R., Levine, M., 1991. Spatial regulation of the gap gene giant during *Drosophila* development. Development 111, 601–609.
Lopez-Onieva, L., Fernandez-Minan, A., Gonzalez-Reyes, A., 2008. Jak/Stat signalling in niche support cells regulates dpp transcription to control germline stem cell maintenance in the *Drosophila* ovary. Development 135, 533–540.
Lopez-Schier, H., St Johnston, D., 2001. Delta signaling from the germ line controls the proliferation and differentiation of the somatic follicle cells during *Drosophila* oogenesis. Genes Dev. 15, 1393–1405.
Lovegrove, B., Simoes, S., Rivas, M.L., Sotillos, S., Johnson, K., Knust, E., Jacinto, A., Hombria, J.C., 2006. Coordinated control of cell adhesion, polarity, and cytoskeleton underlies Hox-induced organogenesis in *Drosophila*. Curr. Biol. 16, 2206–2216.
Lynch, J.A., Brent, A.E., Leaf, D.S., Pultz, M.A., Desplan, C., 2006. Localized maternal orthodenticle patterns anterior and posterior in the long germ wasp Nasonia. Nature 439, 728–732.
Lynch, J.A., Peel, A.D., Drechsler, A., Averof, M., Roth, S., 2010. EGF signaling and the origin of axial polarity among the insects. Curr. Biol. 20, 1042–1047.
Marques-Souza, H., Aranda, M., Tautz, D., 2008. Delimiting the conserved features of hunchback function for the trunk organization of insects. Development 135, 881–888.
McGregor, J.R., Xi, R., Harrison, D.A., 2002. JAK signaling is somatically required for follicle cell differentiation in *Drosophila*. Development 129, 705–717.
Mukherjee, T., Hombria, J.C., Zeidler, M.P., 2005. Opposing roles for *Drosophila* JAK/STAT signalling during cellular proliferation. Oncogene 24, 2503–2511.
Nagy, L.M., Carroll, S., 1994. Conservation of wingless patterning functions in the short-germ embryos of *Tribolium castaneum*. Nature 367, 460–463.
Nilson, L.A., Schupbach, T., 1999. EGF receptor signaling in *Drosophila* oogenesis. Curr. Top. Dev. Biol. 44, 203–243.
Ober, K.A., Jockusch, E.L., 2006. The roles of wingless and decapentaplegic in axis and appendage development in the red flour beetle, *Tribolium castaneum*. Dev. Biol. 294, 391–405.
Patel, N.H., Condron, B.G., Zinn, K., 1994. Pair-rule expression patterns of even-skipped are found in both short- and long-germ beetles. Nature 367, 429–434.
Rawlings, J.S., Rennebeck, G., Harrison, S.M., Xi, R., Harrison, D.A., 2004. Two *Drosophila* suppressors of cytokine signaling (SOCS) differentially regulate JAK and EGFR pathway activities. BMC Cell Biol. 5, 38.
Richards, S., Gibbs, R.A., Weinstock, G.M., Brown, S.J., Denell, R., Beeman, R.W., Gibbs, R., Bucher, G., Friedrich, M., Grimmelikhuijzen, C.J., Klingler, M., Lorenzen, M., Roth, S., Schroder, R., Tautz, D., Zdobnov, E.M., Muzny, D., Attaway, T., Bell, S., Buhay, C.J., Chandrabose, M.N., Chavez, D., Clerk-Blankenburg, K.P., Cree, A., Dao, M., Davis, C., Chacko, J., Dinh, H., Dugan-Rocha, S., Fowler, G., Garner, T.T., Garnes, J., Gnirke, A., Hawes, A., Hernandez, J., Hines, S., Holder, M., Hume, J., Jhangiani, S.N., Joshi, V., Khan, Z.M., Jackson, L., Kovar, C., Kowis, A., Lee, S., Lewis, L.R., Margolis, J., Morgan, M., Nazareth, L.V., Nguyen, N., Okwuonu, G., Parker, D., Ruiz, S.J., Santibanez, J.,

Savard, J., Scherer, S.E., Schneider, B., Sodergren, E., Vattahil, S., Villasana, D., White, C.S., Wright, R., Park, Y., Lord, J., Oppert, B., Brown, S., Wang, L., Weinstock, G., Liu, Y., Worley, K., Elsik, C.G., Reese, J.T., Elhaik, E., Landan, G., Graur, D., Arensburger, P., Atkinson, P., Beidler, J., Demuth, J.P., Drury, D.W., Du, Y.Z., Fujiwara, H., Maselli, V., Osanai, M., Robertson, H.M., Tu, Z., Wang, J.J., Wang, S., Song, H., Zhang, L., Werner, D., Stanke, M., Morgenstern, B., Solovyev, V., Kosarev, P., Brown, G., Chen, H.C., Ermolaeva, O., Hlavina, W., Kapustin, Y., Kiryutin, B., Kitts, P., Maglott, D., Pruitt, K., Sapojnikov, V., Souvorov, A., Mackey, A.J., Waterhouse, R.M., Wyder, S., Kriventseva, E.V., Kadowaki, T., Bork, P., Aranda, M., Bao, R., Beermann, A., Berns, N., Bolognesi, R., Bonneton, F., Bopp, D., Butts, T., Chaumot, A., Denell, R.E., Ferrier, D.E., Gordon, C.M., Jindra, M., Lan, Q., Lattorff, H.M., Laudet, V., von Levetsow, C., Liu, Z., Lutz, R., Lynch, J.A., da Fonseca, R.N., Posnien, N., Reuter, R., Schinko, J.B., Schmitt, C., Schoppmeier, M., Shippy, T.D., Simonnet, F., Marques-Souza, H., Tomoyasu, Y., Trauner, J., Van der Zee, M., Vervoort, M., Wittkopp, N., Wimmer, E.A., Yang, X., Jones, A.K., Sattelle, D.B., Ebert, P.R., Nelson, D., Scott, J.G., Muthukrishnan, S., Kramer, K.J., Arakane, Y., Zhu, Q., Hogenkamp, D., Dixit, R., Jiang, H., Zou, Z., Marshall, J., Elpidina, E., Vinokurov, K., Oppert, C., Evans, J., Lu, Z., Zhao, P., Sumathipala, N., Altincicek, B., Vilcinskas, A., Williams, M., Hultmark, D., Hetru, C., Hauser, F., Cazzamali, G., Williamson, M., Li, B., Tanaka, Y., Predel, R., Neupert, S., Schachtner, J., Verleyen, P., Raible, F., Walden, K.K., Angeli, S., Foret, S., Schuetz, S., Maleszka, R., Miller, S.C., Grossmann, D., 2008. The genome of the model beetle and pest *Tribolium castaneum*. Nature 452, 949–955.

Rivera-Pomar, R., Lu, X., Perrimon, N., Taubert, H., Jackle, H., 1995. Activation of posterior gap gene expression in the *Drosophila* blastoderm. Nature 376, 253–256.

Roth, S., Neuman-Silberberg, F.S., Barcelo, G., Schupbach, T., 1995. cornichon and the EGF receptor signaling process are necessary for both anterior-posterior and dorsal-ventral pattern formation in *Drosophila*. Cell 81, 967–978.

Sapir, A., Schweitzer, R., Shilo, B.Z., 1998. Sequential activation of the EGF receptor pathway during *Drosophila* oogenesis establishes the dorsoventral axis. Development 125, 191–200.

Schoppmeier, M., Schroder, R., 2005. Maternal torso signaling controls body axis elongation in a short germ insect. Curr. Biol. 15, 2131–2136.

Schoppmeier, M., Fischer, S., Schmitt-Engel, C., Lohr, U., Klingler, M., 2009. An ancient anterior patterning system promotes caudal repression and head formation in ecdysozoa. Curr. Biol. 19, 1811–1815.

Schroder, R., 2003. The genes orthodenticle and hunchback substitute for bicoid in the beetle Tribolium. Nature 422, 621–625.

Shi, S., Calhoun, H.C., Xia, F., Li, J., Le, L., Li, W.X., 2006. JAK signaling globally counteracts heterochromatic gene silencing. Nat. Genet. 38, 1071–1076.

Silver, D.L., Geisbrecht, E.R., Montell, D.J., 2005. Requirement for JAK/STAT signaling throughout border cell migration in *Drosophila*. Development 132, 3483–3492.

Singh, S.R., Zheng, Z., Wang, H., Oh, S.W., Chen, X., Hou, S.X., 2010. Competitiveness for the niche and mutual dependence of the germline and somatic stem cells in the *Drosophila* testis are regulated by the JAK/STAT signaling. J. Cell. Physiol. 223, 500–510.

Small, S., Blair, A., Levine, M., 1996. Regulation of two pair-rule stripes by a single enhancer in the *Drosophila* embryo. Dev. Biol. 175, 314–324.

Spradling, A.C., 1993. Germline cysts: communes that work. Cell 72, 649–651.

Stauber, M., Jackle, H., Schmidt-Ott, U., 1999. The anterior determinant bicoid of *Drosophila* is a derived Hox class 3 gene. Proc. Natl Acad. Sci. USA 96, 3786–3789.

Stauber, M., Prell, A., Schmidt-Ott, U., 2002. A single Hox3 gene with composite bicoid and zerknullt expression characteristics in non-Cyclorrhaphan flies. Proc. Natl Acad. Sci. USA 99, 274–279.

Steinhauer, J., Kalderon, D., 2006. Microtubule polarity and axis formation in the *Drosophila* oocyte. Dev. Dyn. 235, 1455–1468.

Stephanou, A., Latchman, D.S., 2005. Opposing actions of STAT-1 and STAT-3. Growth Factors 23, 177–182.

Sulston, I.A., Anderson, K.V., 1996. Embryonic patterning mutants of *Tribolium castaneum*. Development 122, 805–814.

Tautz, D., 1988. Regulation of the *Drosophila* segmentation gene hunchback by two maternal morphogenetic centres. Nature 332, 281–284.

Torres, I.L., Lopez-Schier, H., St Johnston, D., 2003. A Notch/Delta-dependent relay mechanism establishes anterior-posterior polarity in *Drosophila*. Dev. Cell 5, 547–558.

Trauner, J., Buning, J., 2007. Germ-cell cluster formation in the telotrophic meroistic ovary of *Tribolium castaneum* (Coleoptera, Polyphaga, Tenebrionidae) and its implication on insect phylogeny. Dev. Genes Evol. 217, 13–27.

Trauner, J., Schinko, J., Lorenzen, M.D., Shippy, T.D., Wimmer, E.A., Beeman, R.W., Klingler, M., Bucher, G., Brown, S.J., 2009. Large-scale insertional mutagenesis of a coleopteran stored grain pest, the red flour beetle *Tribolium castaneum*, identifies embryonic lethal mutations and enhancer traps. BMC Biol. 7, 73.

Tworoger, M., Larkin, M.K., Bryant, Z., Ruohola-Baker, H., 1999. Mosaic analysis in the drosophila ovary reveals a common hedgehog-inducible precursor stage for stalk and polar cells. Genetics 151, 739–748.

van der Zee, M., Stockhammer, O., von Levetzow, C., Nunes da Fonseca, R., Roth, S., 2006. Sog/Chordin is required for ventral-to-dorsal Dpp/BMP transport and head formation in a short germ insect. Proc. Natl Acad. Sci. USA 103, 16307–16312.

Xi, R., McGregor, J.R., Harrison, D.A., 2003. A gradient of JAK pathway activity patterns the anterior–posterior axis of the follicular epithelium. Dev. Cell 4, 167–177.

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