Segregation of the germline is a fundamental event during early development. In *Drosophila*, germ cells are specified at the posterior pole of the embryo by the germplasm. As zygotic expression is activated, germ cells remain transcriptionally silent owing to the polar granule component (Pgc), a small peptide present in germ cells. Somatic cells at both the embryonic ends are specified by the torso (Tor) receptor tyrosine kinase, and in *tor* mutants the somatic cells closer to the germ cells fail to cellularize correctly. Here, we show that extra wild-type gene copies of *pgc* cause a similar cellularization phenotype, and that both excessive *pgc* and a lack of *tor* are associated with an impairment of transcription in somatic cells. Moreover, a lack of *pgc* partly ameliorates the cellularization defect of *tor* mutants, thus revealing a functional antagonism between *pgc* and *tor* in the specification of germline and somatic properties. As transcriptional quiescence is a general feature of germ cells, similar mechanisms might operate in many organisms to ‘protect’ somatic cells that adjoin germ cells from inappropriately succumbing to such quiescence.

Keywords: cellularization; germ cells; pole hole; torso RTK; transcription

INTRODUCTION

Segregation of the germline is one of the first events in early embryonic development. Germ cells and somatic cells develop in close proximity, suggesting that specific mechanisms exist to insulate germ cells from somatic differentiation and to protect somatic cells adjacent to germ cells from mechanisms that act within germ cells, such as transcriptional quiescence (see Strome & Lehmann, 2007).

*Drosophila* embryonic development starts with a series of syncytial nuclear divisions without cytokinesis (see Foe et al, 1993 for a review). As the number of nuclei increases, the soma becomes transcriptionally active, which is crucial for somatic cell formation (Wieschaus & Sweeton, 1988). By contrast, germ cells, which form at the posterior pole of the embryo, delay transcriptional activation and depend on a specialized, maternally synthesized cytoplasm—the germ plasm—for their formation (Lasko, 1992, Van Doren et al, 1998). It has been proposed that transcriptional quiescence is important for the segregation of the germline from the soma because it might make germ cells refractory to the activity of transcription factors that promote somatic fates.

In *Drosophila*, the torso (Tor) receptor tyrosine kinase becomes activated specifically at the embryonic poles of the blastoderm embryo where it triggers the developmental programme responsible for the most anterior and posterior terminal regions of the embryo (for a review, see Furriols & Casanova, 2003). Although it has been proposed that Tor signalling might be required for germ-cell development (Li et al, 2003), data indicate that Tor signalling is specifically downregulated in the germ cells to allow their development. First, two Tor target genes, *tailless* (*tlh*) and *huckebein*, are not expressed in the germ cells (Pignoni et al, 1990; Brünger & Jäckle, 1991). Second, germ cells have the capacity to reduce the level of Tor protein in their membranes (Martinho et al, 2004). Third, germ cells consistently show lower levels of mitogen-activated protein kinase (MAPK) phosphorylation than posterior somatic cells (Martinho et al, 2004) and the groucho protein is not phosphorylated in germ cells (Cinnamon et al, 2008). Finally, upregulation of Tor signalling causes defects in the formation and migration of germ cells (Li et al, 2003; Martinho et al, 2004). Taken together, these observations suggest that correct development of the germline requires inhibition or downregulation of Tor signalling. In posterior somatic cells, by
contrast, loss of Tor signalling leads to cellularization defects also known as the ‘pole–hole phenotype’ (Degelmann et al., 1986; Schüpbach & Wieschaus, 1986). Interestingly, in the absence of germ cells, the tor pole–hole phenotype is suppressed (Degelmann et al., 1986).

Previously, we have shown that the gene polar granule component (pgc) is required for germ-cell transcriptional repression (Martinho et al., 2004). Germ cells lacking pgc function inappropriately express somatic genes—for example, tll, zerknottl, and slow as molasses (slam)—that are required for cellularization and differentiation of posterior somatic cells. Transcriptionally repressed wild-type germ cells contain RNA polymerase type II lacking Ser 2 phosphorylation in its carboxy-terminal domain (CTD; Seydoux & Dunn, 1997), which is required in vivo for transcriptional activation (Proudfoot et al., 2002). Conversely, germ cells mutant for pgc show premature CTD Ser 2 phosphorylation, suggesting that pgc represses transcription at the elongation step (Martinho et al., 2004). Recently, it has been reported that the Pgc protein inhibits the chromatin recruitment of the positive transcription elongation-factor b (P-TEFB), which is responsible for CTD Ser 2 phosphorylation in vivo, providing a mechanism for pgc function (Hanyu-Nakamura et al., 2008).

Here, we show that upregulation of Pgc leads to cellularization defects that are similar to those observed in tor embryos. As pgc acts as a transcriptional repressor, and as zygotonic transcriptional activation is important for blastoderm cellularization (Wieschaus, 1996), this suggests that the pole–hole phenotype in both tor-mutant and pgc-upregulated embryos might be mediated by a failure of transcriptional activation. Consistent with this hypothesis, we observe a decrease in CTD Ser 2 phosphorylation and a concomitant impairment in the transcriptional activation of the cellularization gene slam in posterior somatic cells of tor-mutant and pgc-upregulated embryos. Taken together our results show that during the early stages of Drosophila embryogenesis, germ-line and somatic development are mutually antagonistic, partly due to distinct mechanisms of transcriptional regulation. We propose that Tor signalling ‘protects’ the somatic cells from the deleterious effect of germline specification mechanisms, in particular pgc-mediated transcriptional repression.

RESULTS AND DISCUSSION

To investigate the effect of pgc-dependent transcriptional repression on embryonic development, we increased pgc expression using six copies of the wild-type gene. Eggs laid by these flies develop embryos (hereafter referred to as 6x[pgc] embryos) that show higher levels of pgc RNA as assessed by whole-mount in situ hybridization (supplementary Fig S1 online). Surprisingly, we observed that these embryos showed a pole–hole phenotype (Fig 1C,H,M) similar to the cellularization defect previously observed in embryos from tor-mutant mothers (hereafter referred to as tor-mutant embryos; Degelmann et al., 1986; Schüpbach & Wieschaus, 1986; Fig 1B,G,L).

Before cellularization, nuclei divide synchronously and migrate towards the periphery of the embryo. Those reaching the posterior pole are incorporated into the germ cells at nuclear cycle 10, whereas somatic cells form during nuclear cycle 14 by an apical-to-basal ingestion of a cellularization furrow. Examination of tor-mutant and 6x[pgc] embryos showed that posterior nuclei fall into the yolk during the cellularization process (Fig 1G,H arrows). In some cases, cellularization furrows progressed partially but failed to enclose the nuclei basally (Fig 1M, arrowhead). As a consequence, many posterior somatic nuclei were not incorporated into elongated somatic blastoderm cells; instead germ cells were displaced inwards, leaving the impression of a hole in the posterior blastoderm (Fig 1L,M). Presumptive somatic nuclei are displaced throughout nuclear cycle 14 (Fig 2, arrowheads; supplementary Movie online). This defect in posterior blastoderm formation is not associated with apoptosis, as we did not detect activated caspase 3 or staining with acridine orange (data not shown). We also observed a very small number of nuclei displaced into the posterior yolk in wild-type embryos, but these did not cause a cellularization defect (Fig 2, arrows).

The similarity in cellularization phenotypes observed after the loss of Tor and overexpression of Pgc might suggest that Pgc interferes directly with Tor signalling (Deshpande et al., 2004). However, the results of three different experiments are more consistent with a global role of Pgc in transcriptional repression that is independent of Tor signalling (Martinho et al., 2004). First, we found that authentic Tor targets can be activated in germ cells even in the absence Tor signalling. In these experiments, we compared the expression of the Tor target gene tll in embryos mutant for pgc with embryos mutant for both tor and pgc. In single pgc-mutant embryos, tll, which is normally expressed in posterior blastoderm cells, was now also inappropriately activated in germ cells (Fig 3B). The tor,pgc double-mutant embryos lacked the Tor-dependent tll expression in posterior somatic cells but showed expression in germ cells (Fig 3D). Thus, in transcriptionally active germ cells, tll can be activated independently of Tor signalling. Second, Pgc overexpression affects the transcriptional activation of genes that are not Tor targets. We observed a decrease in the transcription of slam (Fig 4F, arrowhead; quantification in the supplementary information online), a gene required for the formation of all somatic cells (Lecuit & Wieschaus, 2000; Stein et al., 2002). Finally, overexpression of Pgc leads to a reduction in the activity of RNA polymerase type II. We found that CTD Ser 2 phosphorylation of RNA polymerase type II was reduced in posterior somatic cells from 6x[pgc] embryos compared with the wild type (Fig 4E). Taken together these results are consistent with a global role of pgc in transcriptional repression (Martinho et al., 2004; Hanyu-Nakamura et al., 2008). As active transcription is crucial for early blastoderm cellularization, this result further suggests that the pole–hole phenotype in 6x[pgc] embryos is due to general suppression of gene expression in posterior blastoderm nuclei.

Given the similarities of the pole–hole phenotype in 6x[pgc] and tor embryos, we next investigated whether transcription was similarly impaired in the posterior somatic cells of tor embryos. Indeed, we found a reduction in the levels of CTD Ser 2 phosphorylation (Fig 4C) and lower levels of slam messenger RNA (mRNA; Fig 4D; quantification in the supplementary information online) at the posterior pole of tor-mutant embryos. Thus, although Tor signalling is not specifically targeted by Pgc, Tor activity nevertheless seems to be required for normal transcription. As cellularization defects in tor mutants are restricted to the posterior pole—where germ cells form—this result further suggests that Tor might normally counteract the repressive effects of the germ plasm. Indeed, previous studies have
shown that the pole–hole phenotype of \textit{tor} embryos can be completely suppressed by loss of the germline (\textit{vasa} (\textit{vas}), \textit{tor} double mutants; Degelmann et al, 1986; Fig 1E,J,O). Therefore, we investigated whether the pole–hole phenotype of \textit{tor} embryos depends on \textit{Pgc}. We observed that the loss of \textit{pgc} partly suppressed the pole–hole phenotype of \textit{tor} embryos (Fig 1D,I,N) and rescued the transcription defects associated with the \textit{tor}-mutant phenotype (Fig 4G,H). These results suggest that the \textit{tor} pole–hole phenotype is, at least in part, due to inappropriate \textit{pgc} activity. Further supporting the antagonizing role of \textit{Tor} signalling and \textit{pgc} activity in somatic cells, we found that a \textit{tor} constitutive gain-of-function mutation can partly suppress the pole–hole phenotype of \textit{6x[pgc]} embryos (see supplementary information online).

Suppression of the pole–hole phenotype in \textit{tor,pgc} double mutants was significantly weaker than the one in \textit{vas,tor} double mutants. This difference in suppression efficiency might suggest that additional germ-plasm components other than \textit{pgc} could be responsible for causing the \textit{tor}-induced pole–hole phenotype (see below). In addition, the observation that six wild-type copies of \textit{pgc} have a much stronger effect on \textit{slam} expression in the posterior soma than the loss of \textit{tor} activity clearly strengthens the idea that the \textit{tor} pole–hole phenotype is not exclusively due to \textit{Pgc} and transcription-dependent mechanisms (see below). We favour the idea that the \textit{tor}-mutant cellularization defect arises as a consequence of distinct abnormalities, such as the \textit{pgc}-mediated impairment of \textit{slam} expression. This hypothesis also explains why in \textit{tor,pgc} double mutants \textit{slam}-transcription...
impairment is reverted to a greater extent than morphological cellularization defects.

Our results indicate that pgc, and probably other factors of the germ plasm, are deleterious for somatic-cell development and that Tor signalling antagonizes this deleterious effect. One possibility is that some germ-plasm components are not efficiently sequestered into the newly formed germ cells and remain in the cytoplasm of the somatic cells (Degelmann et al., 1986; data not shown). Indeed, we observed remnants of pgc mRNA, Pgc protein and other germ-plasm components in the posterior somatic cells of wild-type embryos (Fig 5A,B, arrows). Compared with wild-type or tor-mutant embryos, we detected consistently higher amounts of pgc RNA and Pgc protein in posterior somatic cells of 6x[pgc] embryos (see Fig 5). Thus, in the wild type, Tor signalling seems to be sufficient to antagonize the deleterious effect of pgc and other germ-plasm factors in posterior somatic cells but an increase of Pgc dosage seems to overwhelm this ‘protective’ capacity of Tor.

In 6x[pgc] embryos, only one aspect of germ plasm—transcriptional repressor activity—is increased, whereas other aspects of germ plasm are probably intact. In the wild type, the remnants of the germ-plasm components in posterior somatic cells might be present at near-threshold levels as even there a few nuclei fall into the interior, but without causing a pole–hole phenotype (Fig 2). Thus, the deleterious effect of germline determinants on somatic cells only becomes apparent when specific germ-plasm components are upregulated, as in 6x[pgc], or when Tor signalling is impaired.

How could Tor signalling antagonize pgc activity? Tor signalling is known to inactivate a transcriptional repressor activity, which involves the Cic transcription factor, through MAPK-dependent phosphorylation (Jiménez et al., 2000; Astigarraga et al., 2007). Thus, Tor signalling could similarly inactivate the remnants of pgc-mediated transcriptional repressor activity in somatic cells. In this regard, we note that mutants in all of the

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**Fig 2** Frames of time-lapse movies showing the development of wild-type, tor and 6x[pgc] embryos. Posterior embryonic poles of wild-type, tor and 6x[pgc] embryos with a His2avGFP transgene to label nuclei. Groups of nuclei from tor and 6x[pgc] embryos fall into the yolk at various time points during cellularization at nuclear cycle 14 (arrowheads). Occasionally, a few nuclei fall into the yolk in the wild-type embryo (arrows). Frames in the figure are separated by 12’30” intervals. GFP, green fluorescent protein; pgc, polar granule component; tor, torso; wt, wild type.

**Fig 3** The ectopic expression of till in the germ cells of pgc mutants is independent of tor activity. Posterior pole of cellular blastoderms hybridized with a till probe. (A) till is excluded from germ cells of wild-type embryos. (B) In pgc mutants, till is found in the germ cells. (C) In tor mutants, till is absent from the posterior cells. (D) In tor,pgc double mutants, till is absent from the posterior somatic cells but is expressed in the germ cells. pgc, polar granule component; till, tailless; tor, torso; wt, wild type.
genes examined in the tor-transduction pathway have a pole–hole phenotype, indicating that the ‘protective’ effect of these somatic cells is due to Tor signalling itself. However, we did not find any putative MAPK phosphorylation sites in the Pgc protein, suggesting that Pgc is not itself a direct target. In addition, we did not detect any obvious change in pgc RNA or protein levels in tor mutants (data not shown), suggesting that tor inactivates a pgc-dependent activity rather than pgc itself. Recently it has

Fig 4 | Transcriptional impairment associated with the pole–hole phenotype. (A,C,E,G) Posterior pole of cellular blastoderm labelled with an antibody that recognizes the active form of the RNA polymerase type II (pSer; green) and the germline specific Vas antibody (blue), and graphical representations of pSer levels in the posterior somatic nuclei (for exact numbers and quantification see the supplementary information online). Levels of pSer antibody staining are lower in the somatic cells next to the germ cells in tor (C) and 6x[pgc] (E), but similar to those in the other somatic cells in tor,pgc double mutants (G). (B,D,F,H) Posterior pole of blastoderm wild type, hybridized with a slam probe. A decrease in the signal can be detected in the somatic cells closer to the germ cells in tor (D) and 6x[pgc] (F) but not in tor,pgc double mutants (H). In (H), slam transcripts in the germ cells are due to the pgc mutation. pgc, polar granule component; slam, slow as molasses; tor, torso; wt, wild type.
been reported that Pgc represses transcription by inhibiting the recruitment of P-TEFb to chromatin (Hanyu-Nakamura et al, 2008), thus Tor could target components of the general transcriptional machinery. Further work will be required to establish the mechanism by which Tor signalling antagonizes Pgc activity.

In summary, our study provides evidence for a functional antagonism in the development of germline and somatic cells, and suggests a model for such an interaction. The function of Pgc is to repress global transcription in germ cells as such repression is important for the establishment of the germline fate, whereas Tor signalling allows transcription in the nearby somatic cells, which is required for their normal differentiation programme. Although the function of Pgc on transcription seems to be global and not specific to Tor targets, our results suggest that Tor signalling might interfere specifically with the ability of Pgc and possibly other germ-plasm components to inactivate the transcription in neighbouring somatic cells. Thus, pgc and tor have an important role in the ‘fate-protection’ mechanisms to shield each cell type from its ‘unwelcomed neighbour’ (Fig 5). Transcriptional quiescence is a common feature in early germ cells in many organisms to prevent them from differentiating as their somatic neighbours. Moreover, the mechanisms involved in maintaining such transcriptional quiescence typically depend on the segregation of cytoplasmic factors, such as the germ plasm, which can be an imperfect process (Cinalli et al, 2008). We propose that additional mechanisms exist to ‘protect’ nearby somatic cells from such remnants of germline-specifying components and that these might also operate in other organisms.

METHODS
All crosses were carried out at 25 °C under standard conditions. The slam and pgc antisense RNA probes were obtained as described in Martinho et al (2004). The tll DNA probe was obtained from pBluescript as described in Jiménez et al (2000). Antibodies used were mouse anti-neurotactin (Hybridoma bank BP106 clone, 1/5), rabbit anti-vasa (1/600), mouse anti-pSer2 CTD RNA polymerase type II (Covance, Princeton, NJ, USA; clone H5, 1/1000), rat anti-Pgc (A. Nakamura 1/100), 4’,6-diamidino-2-phenylindole (1/100) and wheat germ agglutinin (1/250). For additional information see the supplementary information online.
Supplementary information is available at EMBO reports online (http://www.emboreports.org).

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

REFERENCES