A functional antagonism between the *pgc* germline repressor and *torso* in the development of somatic cells

José Manuel de las Heras^{1,2*}, Rui Gonçalo Martinho^{3,4,5*}, Ruth Lehmann^{3,4+} & Jordi Casanova^{1,2++}

¹Institut de Biologia Molecular de Barcelona (Consejo Superior de Investigaciones Científicas), and ²Institut de Recerca de Biomèdica, Barcelona, Spain, ³Howard Hughes Medical Institute, and ⁴Kimmel Center for Biology and Medicine of the Skirball Institute, Department of Cell Biology, New York University School of Medicine, New York, USA, and ⁵Instituto Gulbenkian de Ciência, Oeiras, Portugal

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

EMBO reports (2009) 10, 1059–1065. doi:10.1038/embor.2009.128

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* (*tll*) and *huckebein*, are not expressed in the germ cells (Pignoni *et al*, 1990; Brönnner & 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

¹Institut de Biologia Molecular de Barcelona (CSIC), and ²Institut de Recerca de Biomèdica, Barcelona, Spain

³Howard Hughes Medical Institute, and ⁴Kimmel Center for Biology and Medicine of the Skirball Institute, Department of Cell Biology, New York University School of Medicine, New York, USA

⁵Instituto Gulbenkian de Ciência, Oeiras, Portugal

*These authors equally contributed to this work

+Corresponding author. Tel: +1 212 263 8071; Fax: +1 212 263 7760; E-mail: lehmann@saturn.med.nyu.edu

++Corresponding author. Tel: +34 93 4034967; Fax: +34 93 4034979; E-mail: jcrbmc@cid.csic.es

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*, *zerknüllt*, 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 Ser2 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 Ser2 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 Ser2 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 zygotic 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 Ser2 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 ingression 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 Ser2 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 Ser2 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

Fig 1 | The pole-hole phenotype of *tor* and *6x[pgc]* embryos, and suppression of the *tor* pole-hole phenotype by *pgc* and *vas*. Posterior poles of wild type (A,F,K), *tor*⁻ (B,G,L), *6x[pgc]* (C,H,M), *tor*⁻,*pgc*⁻ (D,I,N) and *vas*⁻,*tor*⁻ embryos (E,J,O). Anti-neurotactin (Nrt; red) labels somatic but not germ cells; DAPI labels nuclei (blue); and in anti-Vas labels germ cells (green). Groups of nuclei fall into the yolk in *tor* and *6x[pgc]* mutants (arrows in G and H), and some cells fail to complete cellularization as shown by the lack of a basal membrane (see arrowhead in M) and many cells have lost the typical epithelial elongated shape. In (L) *tor* and (M) *6x[pgc]* embryos, germ cells are found in the 'hole' between the somatic cells. The pole-hole phenotype is partly suppressed in *tor*,*pgc* double mutants, whereas somatic nuclei still fall into the yolk (I), somatic cells are better arranged (D) and germ cells remain closer to the periphery (N). Suppression of the pole-hole phenotype is complete in *vas*,*tor* mutants (E,J,O); note that germ cells are not formed in these embryos because of the *vas* mutation (O). DAPI, 4',6-diamidino-2-phenylindole; *pgc*, polar granule component; *tor*, torso; *vas*, vasa; wt, wild type.

shown that the pole-hole phenotype of *tor* embryos can be completely suppressed by loss of the germline (*vasa* (*vas*),*tor* double mutants; Degelmann *et al*, 1986; Fig 1E,J,O). Therefore, we investigated whether the pole-hole phenotype of *tor* embryos depends on *Pgc*. We observed that the loss of *pgc* partly suppressed the pole-hole phenotype of *tor* embryos (Fig 1D,I,N) and rescued the transcription defects associated with the *tor*-mutant phenotype (Fig 4G,H). These results suggest that the *tor* pole-hole phenotype is, at least in part, due to inappropriate *pgc* activity. Further supporting the antagonizing role of Tor signalling and *pgc* activity in somatic cells, we found that a *tor* constitutive gain-of-function mutation can partly suppress the pole-hole phenotype of *4x[pgc]* embryos (see supplementary information online).

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

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 *tll* in the germ cells of *pgc* mutants is independent of *tor* activity. Posterior pole of cellular blastoderms hybridized with a *tll* probe. (A) *tll* is excluded from germ cells of wild-type embryos. (B) In *pgc* mutants, *tll* is found in the germ cells. (C) In *tor* mutants, *tll* is absent from the posterior cells. (D) In *tor,pgc* double mutants, *tll* is absent from the posterior somatic cells but is expressed in the germ cells. *pgc*, polar granule component; *tll*, tailless; *tor*, torso; wt, wild type.

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

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 blastoderms 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.

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 5 | *pgc* accumulates at the germ cells and surrounding cytoplasm. Posterior pole of a wild-type embryo hybridized with a *pgc* probe (A), stained with Pgc (B), and Vas antibodies (C), with WGA that labels the membranes (D) and a merge of WGA (blue) and anti-Vas (green) staining (E). *pgc* RNA accumulates in the germ cells and can also be detected in the surrounding somatic cytoplasm (arrows and inset with magnification). Similarly, Pgc protein accumulates in the germ cells and some traces can also be detected in cells that do not show staining for the germ-specific Vas protein (arrowheads and insets for more detail). We detected *pgc* RNA in the somatic cytoplasm surrounding the germ cells in 87% of wild-type embryos ($n=30$) and in 100% of $6x[pgc]$ embryos ($n=42$), and traces of Pgc protein in 30% of wild-type embryos ($n=27$) and 58% of $6x[pgc]$ embryos ($n=24$). (F) In germ cells, *pgc* represses gene transcription inhibiting the somatic fate and allowing the correct segregation of the germline. Other germplasm components probably participate in this process. Germ-cell fate is impaired by the overexpression of the Tor receptor (Martinho *et al* 2004). In the posterior somatic cells, the Tor pathway triggers the expression of the genes responsible for terminal fate and, in addition, counteracts the deleterious effect of *pgc* and other germplasm components in the somatic cellularization (asterisk). *pgc*, polar granule component; Tor, torso; Vas, vaso; WGA, wheat germ agglutinin; 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>).

ACKNOWLEDGEMENTS

We thank the colleagues in our laboratories for discussions and comments on the paper, A. Nakamura for the *Pgc* antibody and the *pgc* genomic constructs, and Bloomington stock collection for flies. J.M.H. was the recipient of the Formación del Personal Investigador fellowship. R.L. is a Howard Hughes Medical Institute Investigator. This work was supported by grants from the Fundação para a Ciência e Tecnologia [PPCDT/ DG/ BIA/ 82013/ 2006] to R.G.M. and the Ministerio de Educación y Ciencia and the Generalitat de Catalunya to J.C.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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