Regulation of zygotic gene expression in *Drosophila* primordial germ cells

Mark Van Doren, Anne L. Williamson* and Ruth Lehmann

Activation of the zygotic genome is a prerequisite for the transition from maternal to zygotic control of development. The onset of zygotic transcription has been well studied in somatic cells, but evidence suggests that it is controlled differently in the germline. In *Drosophila*, zygotic transcription in the soma has been detected as early as one hour after egg laying (AEL) [1]. In the germline, general RNA synthesis is not detected until 3.5 hours AEL (stage 8) [2] and poly(A)-containing transcripts are not observed in early germ cell nuclei [3]. However, rRNA gene expression has been demonstrated at this time [4]. Therefore, either there is a general, low level activation of the genome in early germ cells, or specific classes of genes, such as those transcribed by RNA polymerase (RNAP) II, are repressed. We addressed this issue by localizing the potent transcriptional activator Gal4–VP16 to the germline, and we find that Gal4–VP16-dependent gene expression is repressed in early germ cells. In addition, localization of germ plasm to the anterior reveals that it is sufficient to repress Bicoid-dependent gene expression. Thus, even in the presence of known transcriptional activators, RNAP II dependent gene expression is actively repressed in early germ cells. Furthermore, once the germ cell genome is activated, we find that *vasa* is expressed specifically in germ cells. This expression does not require proper patterning of the soma, indicating that it is likely to be controlled by the germ plasm.

Address: Skirball Institute and Howard Hughes Medical Institute, NYU Medical Center, 540 1st Avenue, New York, New York 10016, USA.

*Present address: Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, California 94304, USA.

Correspondence: Ruth Lehmann
E-mail: lehmann@saturn.med.nyu.edu

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Results and discussion

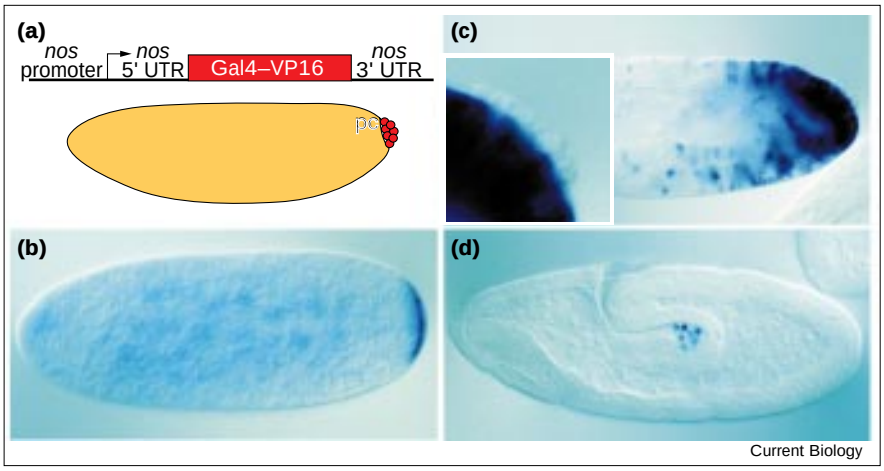
Repression of gene expression in the germline

To determine whether expression of genes transcribed by RNAP II is actively repressed in *Drosophila* germ cells, we asked whether a transcriptional activator could induce expression of a known target gene in these cells. As we are not aware of an endogenous transcription factor in *Drosophila* germ cells with a defined target gene, we localized the potent transcriptional activator Gal4–VP16 [5] to the germ cells and examined the expression of a Gal4–VP16-responsive target gene (Figure 1). We expressed RNA encoding Gal4–VP16 maternally using the *nanos (nos)* promoter and localized it to the germ cells using the untranslated region (UTR) of the *nos* RNA (Figure 1a). The *nos* gene is expressed during oogenesis [6], and the 3′ UTR of the *nos* RNA is sufficient to localize it to the posterior pole of the embryo [7] and restrict its translation to the posterior pole [8]. Nos protein forms a gradient with the highest levels at the posterior pole, and both the *nos* RNA and Nos protein are taken up into germ cells as they form [6]. By replacing the *nos* coding region with *Gal4–VP16*, we have localized RNA encoding Gal4–VP16 to the posterior pole (Figure 1b) and germ cells (not shown), which are highly active for translation at this time [2]. For a Gal4–VP16-responsive target, we used an upstream activation sequence (*UAS*)–*lacZ* transgene [9], which contains Gal4-binding sites upstream of a *hsp70* minimal promoter and the *lacZ* coding region.

As shown in Figure 1c, the *UAS–lacZ* transgene is expressed in somatic cells in the posterior region of the embryo. This is as expected if, like Nos, Gal4–VP16 is translated at the posterior pole to form a posterior to anterior concentration gradient. In contrast, the germ cells show no detectable expression of the *UAS–lacZ* target gene (Figure 1c inset), indicating that Gal4–VP16-dependent gene expression is repressed in these cells. We first see cytoplasmic accumulation of *lacZ* RNA in germ cells at ~3.5 hours AEL (stage 8–9; Figure 1d). This is ~2 hours after zygotic gene expression is first detected in somatic nuclei [1], in agreement with the time that Zalokar [2] observed an increase in total RNA synthesis in germ cells. We conclude that Gal4–VP16-dependent expression of the *UAS–lacZ* target gene is repressed in germ cells relative to somatic cells.

We next asked whether the repression of gene expression in the germ cells is conferred by the germ plasm and is downstream of *oskar (osk)*. In addition, we wanted to determine whether target genes for an endogenous *Drosophila* transcription factor would be repressed in the germline. Normally, *osk* RNA is localized to the posterior pole, where it is essential for germ plasm assembly. Localization of *osk* RNA to the anterior of the embryo using the *osk–bcd-3′UTR* transgene is sufficient to promote formation of anterior germ cells [10]. As the

Figure 1



Expression of a Gal4–VP16-responsive target gene is repressed in the germline relative to the soma. Anterior is to the left. (a) Diagram showing the *nos–Gal4–VP16* transgene and localization of Gal4–VP16 to the germ cells (pole cells, pc). (b) *In situ* hybridization of a pre-blastoderm embryo from a *nos–Gal4–VP16* transgenic mother using an antisense probe recognizing Gal4–VP16 but not *nos* sequences: *nos–Gal4–VP16* RNA is localized to the posterior pole. (c,d) *In situ* hybridization with an antisense *lacZ* riboprobe of embryos from *nos–Gal4–VP16* transgenic females crossed to *UAS–lacZ* transgenic males. (c) Early gastrula (stage 6, ~3 h AEL). Note that the posterior somatic cells express *lacZ* RNA whereas the germ cells do not (enlarged in inset). (d) Stage 9 embryo (~4 h AEL). The germ cells now express *lacZ* RNA. Similar results were obtained with two different *UAS–lacZ* transgenic lines, and no expression is seen from these lines in the absence of the *nos–Gal4–VP16* transgene. We have been unable to detect the Gal4–VP16 protein by whole-mount immunohistochemistry using a variety of antibodies, even in regions where Gal4–VP16 activity is clearly detectable. However, the *nos–Gal4–VP16* RNA disappears soon after the germ cells form (data not shown), indicating that the activity of the Gal4–VP16 protein observed later in the germ cells arises from translation of the *nos–Gal4–VP16* RNA at this early stage.

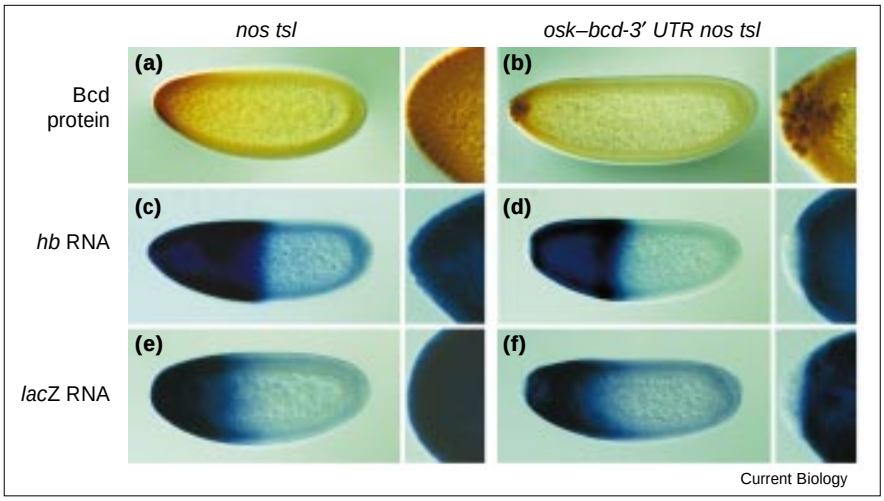
maternal transcription factor Bicoid (Bcd) normally forms an anterior to posterior protein gradient with the highest protein levels at the anterior pole (Figure 2a) [11], we could examine the expression of Bcd target genes in these anterior germ cells. It has been shown that Bcd is a direct transcriptional activator of *hunchback* (*hb*) [12,13]. Furthermore, synthetic reporter constructs with high-affinity Bcd-binding sites upstream of a *hsp70* minimal promoter and *lacZ* coding sequences (*Hb3Bcd3–lacZ*) are sensitive reporters of Bcd activity [14]. We tested the effect of germ plasm on the expression of *hb* and the *Hb3Bcd3–lacZ* reporter. To maximize Bcd activity, we performed these experiments in a genetic background that removes known translational repression of *bcd* by Nos [7,15] and post-translational regulation of Bcd by the terminal pathway (*torsolike*, *tsl*) [16]. In addition, this genetic background ensures that the anterior of the embryo is not transformed to a posterior identity by the ectopic *osk*, so that the anterior germ cells are truly in a somatic environment that is different from where they normally form.

In the absence of anterior *osk*, both the endogenous *hb* gene and the *Hb3Bcd3–lacZ* transgene are expressed at high levels in the anterior region, including the anterior pole (Figure 2c,e). When *osk* is localized to the anterior, maternal Bcd protein is present at high levels in the germ cells that form there (Figure 2b). However, neither the endogenous *hb* gene nor the *Hb3Bcd3–lacZ* transgene are expressed in the anterior germ cells, though they are expressed in anterior somatic cells (Figure 2d,f). Thus *osk*-nucleated germ plasm is sufficient to confer repression of Bcd-responsive target genes in anterior cells.

We have therefore observed repression of gene expression in the germline with the endogenous *Drosophila* transcription factor Bcd acting on its target gene *hb*, and also with the exogenous transcription factor Gal4–VP16 and synthetic target genes. Repression of an exogenous transcription factor and synthetic target genes suggests that this effect may be general in nature, rather than promoter specific, and may apply broadly to RNAP II dependent gene expression. Interestingly, expression of genes transcribed by RNAP I does not appear to be repressed in the *Drosophila* germline [4]. Therefore the mechanism is not so general as to affect transcription universally, as might be the case if the DNA was in a conformation incompatible with transcription.

Repression of gene expression in the germline has also been described in *Caenorhabditis elegans*. The *pie-1* gene is essential for repression of somatic gene expression in the *C. elegans* germline, and ectopic expression of PIE-1 in somatic cells is sufficient to repress gene expression in those cells [17]. As in *Drosophila*, RNAP I transcription is not affected in the *C. elegans* germline [4]. Although RNAP II is present in the germlines of *C. elegans* and *Drosophila* embryos, both lack a specific phosphoform of the enzyme [4] that may either cause or result from repression of RNAP II transcription. Interestingly, this phosphoform appears in the *Drosophila* germline at stage 7 (3 hours AEL [4]), which is just prior to when we first observe zygotic gene expression in these cells (3.5 hours AEL; Figure 1). Although it is possible that repression of gene expression in the germline is caused by decreased RNA stability, the changes in

Figure 2



Localization of *osk* RNA to the anterior pole results in repression of Bcd target gene expression. All panels show stage 5 embryos derived from *nos tsl* double mutant females crossed to males containing the *Hb3Bcd3–lacZ* transgene. For panels (b,d,f), the females also contained the *osk–bcd-3′UTR* transgene, which localizes *osk* RNA to the anterior pole and directs the formation of anterior germ cells. Anterior is to the left. **(a,b)** Embryos stained with anti-Bcd antibody. In (b), high levels of Bcd protein are present in the nuclei of germ cells formed at the anterior. **(c,d)** Embryos stained by *in situ* hybridization to *hb* RNA. **(e,f)** Embryos stained by *in situ* hybridization to *lacZ* RNA. In both (d) and (f), the anterior germ cells do not express the Bcd target genes but the neighboring somatic cells do.

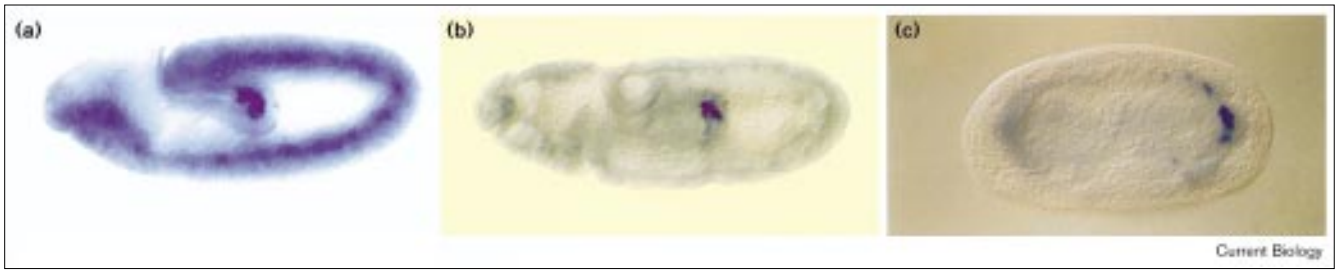
RNAP II suggest that the mechanism is more likely to be at the level of transcription.

Activation of germline-specific gene expression

The repression of germline gene expression must eventually be overcome so that germ cells can express the genes required for their unique developmental program. We therefore wanted to address how zygotic gene expression is activated in the germline and how germ cell specific gene expression is regulated. We found that the *vasa (vas)* gene [18,19] is expressed specifically in germ cells beginning at stage 9 (~4 hours AEL; Figure 3a). This is very soon after the time that germ cells become competent for expression of RNAP II target genes as judged by our experiments with Gal4–VP16 (Figure 1). To our knowledge, *vas* represents the earliest example of RNAP II dependent gene expression in the *Drosophila* germline.

Communication between somatic cells and germ cells is essential for proper germ cell migration and development (reviewed in [20]). Therefore, signals from somatic tissue might regulate *vas* expression in the germ cells. Because the germ cells are contained within the posterior midgut pocket at the time at which we first see *vas* expressed, we determined whether the correct identity of this tissue is required for *vas* expression. The *vas* gene is still expressed specifically in germ cells of mutants that lack a posterior midgut and hindgut (*tailless huckebein* and *tsl*; data not shown, but see Figure 3c). Another candidate for providing somatic signals to the germ cells is the mesoderm, as it lies adjacent to the midgut and is the target tissue for germ cell migration. However, *vas* expression is also unaffected in embryos that lack mesoderm (*twist snail*, *twi sna*; Figure 3b). Finally, we examined embryos in which all three systems for initiating anterior–posterior

Figure 3



Zygotic *vas* expression. All panels show *in situ* hybridization to *vas* RNA. **(a)** Stage 9 wild-type embryo (~4 h AEL). Zygotic expression of *vas* RNA is detected specifically in germ cells beginning at this time. The more general staining observed is still present in embryos of the genotype *Df(2L)A267/Df(2L)TE36 GW29*, in which both copies of the *vas* gene have been deleted, and therefore does not represent zygotic *vas* expression. Germ cell staining is absent from these embryos. **(b)** A *twi sna* double mutant embryo lacking all mesoderm. The germ cells still express *vas* RNA. **(c)** Embryo from a female *bcd nos tsl* triple mutant. These embryos develop no anterior–posterior pattern. The cells expressing zygotic *vas* RNA were identified as germ cells by the presence of Vas protein (data not shown), which is still localized maternally to the germ cells in this genetic background.

pattern have been removed (*bcd nos tsl*) and found that *vas* is still expressed specifically in germ cells (Figure 3c). Therefore, germ cell specific *vas* expression does not require proper specification of the endoderm or mesoderm and occurs even in the absence of anterior–posterior somatic patterning.

Implications for the germ plasm

Our results support the hypothesis that repression of germ cell gene expression, as well as its activation at the appropriate time and in a germ cell specific manner, are all controlled maternally by the germ plasm. In this model, at least one maternally supplied transcription factor would be localized to the germ cells, perhaps in a manner analogous to our *nos–Gal4–VP16* transgene, and would activate germline-specific targets once the germline is competent for gene expression. The *vas* gene is a candidate for being directly responsive to such a transcription factor. In addition, a mechanism for timing the onset of zygotic gene expression in the germline would also be provided maternally and be autonomous to the germ cells. Indeed, even germ cells isolated in culture appear to exhibit the normal program of germ cell specific repression and activation of RNA synthesis [21]. It is unlikely that the mechanism for activating the zygotic genome involves the nuclear to cytoplasmic ratio, as has been found for the soma [1], because germ cells stop dividing well before they initiate gene expression [22]. Finally, germ cells exhibit a variety of other behaviors prior to the time that they are competent for zygotic gene expression, including precocious cellularization [22], germ cell specific cell cycle regulation [22], translational regulation [23] and development of a migratory cellular morphology [24]. Thus, the germ plasm must contain a combination of factors controlling both the cellular behaviors of the early germ cells and the activation of primordial germ cell specific gene expression.

Materials and methods

The following stocks were used in this study: nos^{BN} tsl^{691}, nos^{L7} bcd^{E1} tsl^{146}, *Df(2L)A267* (*vas*$^{-}$), *Df(2L)TE36 GW29* (*vas*$^{-}$), tll^{9} hkb^{X179} (a gift from Herbert Jäckle), and twi^{IIH} sna^{IIG}. *UAS–lacZ* lines Bg4-1-2 and Bg4-2-46 were a gift from Norbert Perrimon [9], and *Hb3Bcd3–lacZ* line HSBGHbBcd3C was a gift from Nathalie Dostatni, Jessica Treisman and Claude Desplan [14]. To generate the *nos–Gal4–VP16* transgene, the *nos* coding sequences within a derivative of pHSXgnosb^{R} [7] were replaced with sequences encoding the FLAG epitope tag (IBI) and Gal4–VP16 [5], and the construct contains approximately 700 bp of the *nos* promoter, the *nos* 5′ and 3′ UTRs, and approximately 500 bp of genomic sequence 3′ of *nos* (further details available upon request). The transgenic line used shows no defects in germline development. *In situ* hybridization and whole-mount antibody staining of embryos were performed as described [7]. Mouse monoclonal anti-Bcd antibody was a gift from Wolfgang Driever.

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