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Global hypertranscription in the mouse embryonic germline

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Summary

Primordial germ cells (PGCs) are vital for inheritance and evolution. Their transcriptional program has been extensively studied and is assumed to be well-known. We report here a remarkable global upregulation of the transcriptome of mouse PGCs compared to somatic cells. Using cell-number normalized genome-wide analyses, we uncover significant transcriptional amplification in PGCs, including mRNAs, rRNA and transposable elements. Hypertranscription preserves tissue-specific gene expression patterns, correlates with cell size, and can still be detected in E15.5 male germ cells, when proliferation has ceased. PGC hypertranscription occurs at the level of nascent transcription, is accompanied by increased translation rates, and is driven by Myc factors n-Myc and l-Myc (but not c-Myc) and by P-TEFb. This study provides a paradigm for transcriptional analyses during development and reveals a major global hyperactivity of the germline transcriptome.

Graphical abstract

Percharde et al. find that mouse primordial germ cells are in a state of hypertranscription driven by Myc factors and P-TEFb.

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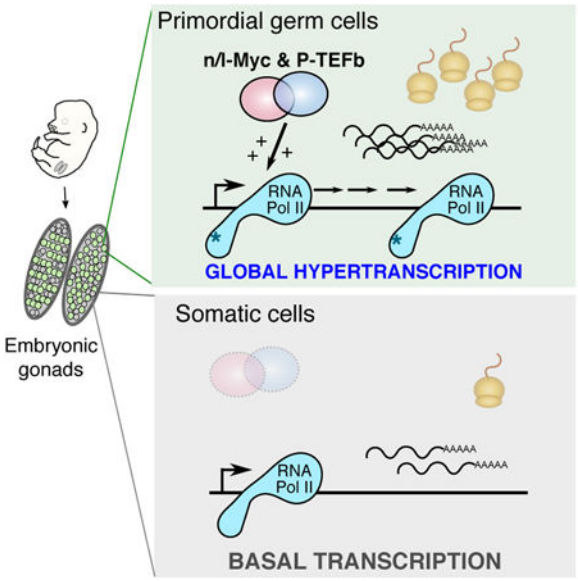
Author Contributions: MP and MR-S conceived of the project. MP and PW performed dissections, flow cytometry, nascent Click-iT assays, and immunofluorescence imaging and analyses. PW did cryosections, immunofluorescence, and flow cytometry analyses. MP designed and performed all other bench experiments and did the bioinformatics analyses. MP and MR-S wrote the manuscript with input from PW.

The authors declare that they have no conflict of interest.



Keywords

Hypertranscription; primordial germ cells; germline; myc; n-myc; l-myc; P-TEFb; cell competition

Introduction

The germline carries on to the next generation both developmental totipotency as well as genetic and epigenetic predispositions to disease. Primordial germ cells (PGCs), the embryonic precursors to mature oocytes and spermatozoa, are thus essential for ontogeny and species survival.

In mice, PGCs arise from a small group of epiblast cells that are first identifiable at embryonic day (E)7.25. From E7.75-E10.5, these cells migrate to the developing gonads where they proliferate and, from E12.5, commence sexual differentiation (Tam and Snow, 1981; Western et al., 2008). PGC development is accompanied by a remarkable level of epigenetic reprogramming, such as genome-wide DNA demethylation including the removal of genomic imprints, widespread changes to histone modifications and variants, and X-chromosome reactivation in females {reviewed in (Saitou et al., 2012)}. Significant insights have been gained into the unique chromatin reprogramming that PGCs undergo during their development. In parallel, several groups, including our own, have investigated the transcriptional profile of PGCs [e.g., (Grskovic et al., 2007; Kurimoto et al., 2008; Seisenberger et al., 2012; Sharov et al., 2003)]. These studies revealed that PGCs express a pluripotency program with high similarity to that of Embryonic Stem (ES) cells, along with unique modules specific to PGCs related to migration and sexual differentiation.

ES cells can exist in a state of hypertranscription, which involves a global nascent amplification of most of the transcriptome (Efroni et al., 2008; Guzman-Ayala et al., 2015; Percharde et al., 2017). This globally elevated transcriptional state has been proposed to

occur in several contexts of embryonic development. Chd1 promotes hypertranscription in ES cells and is essential for growth of the post-implantation epiblast (Guzman-Ayala et al., 2015) and for emergence of definitive hematopoietic stem/progenitor cells at midgestation (Koh et al., 2015). In addition, c-Myc-driven global transcriptional activation has been documented in ES cells, activated lymphocytes, and cancer cells (Lin et al., 2012; Nie et al., 2012) where it may drive rapid proliferation. The available data point to a critical role of global transcriptional elevation in embryogenesis. However, the genome-wide extent of hypertranscription has not been investigated during development in vivo (Percharde et al., 2017). Moreover, traditional methods of analyzing large sequencing datasets do not take into account global changes to gene expression. Thus, many cases of hypertranscription may have gone unnoticed.

In this study we focused on the developing germline, and asked whether PGCs can be distinguished from their neighboring somatic cells by their transcriptional output. Our results document a dramatic upregulation of the PGC transcriptome in male and female E13.5 gonads, inclusive of many genes associated with increased biosynthetic capacity and growth. Moreover, we find that PGC hypertranscription depends on the activity of Myc/Max and pTEFb, highlighting a potentially important role for these proteins in germ cell development. These results demonstrate that global hypertranscription occurs in vivo and reveal that the PGC transcriptome is much more dynamic than previously thought.

Results

Increased RNA Polymerase I and II transcripts in PGCs

To investigate the transcriptome of PGCs and neighboring somatic cells of the gonad (soma), we first took advantage of the Oct4/GFP transgenic mouse line (Yeom et al., 1996). PGCs and soma were profiled at E13.5, when sexual differentiation is readily apparent and cells are entering cell cycle arrest (Bowles and Koopman, 2007). Quantification of total RNA from equal numbers of sorted PGCs or soma revealed approximately 4-fold higher amounts of RNA in male PGCs than in soma, and 3-fold in female PGCs (Figure 1A). We next performed cell number-normalized (CNN) qRT-PCR for common housekeeping genes. Relative to soma, PGCs exhibit significantly higher levels of housekeeping genes typically used for qRT-PCR normalization, including *Gapdh, Ubb, Rpl7* and *H2A* (Figure 1B). Elevated gene expression is apparent throughout PGC development from E9.5 to E13.5 (Figure S1A-B), confirming that this phenomenon occurs over a wide window of PGC development. Moreover, PGC transcription is elevated in comparison to a range of E13.5 tissues, and is comparable to cultured ES cells (Figure S1C). Next, to rule out increased mRNA stability in PGCs compared to soma, the expression of both primary (unspliced) and mature RNA Polymerase (RNA Pol) I and II transcripts was profiled at E13.5. CNN qRT-PCR confirmed that pre-rRNA (Figure 1C), as well primary mRNAs (Figure 1D), are significantly upregulated in male and female E13.5 PGCs. Interestingly, both male and female PGCs are larger than somatic cells and similar to ES cells (Figure S1D-E). Taken together, these results indicate that both RNA Pol I and II transcripts are elevated in male and female PGCs.

Upregulation of nascent PGC transcription and translation

The increase in primary RNA transcripts in PGCs (Figure 1C-D) suggested that the rate of nascent transcription might be higher in the germline compared to soma. To test this, we performed nascent RNA labelling experiments in wild-type E13.5 embryos. Cells from dissociated male or female gonads were processed for 5-ethynyl-uridine (EU) incorporation, allowing quantification of nascent transcription by flow cytometry (Koh et al., 2015). We compared the EU incorporation of Ddx4/MVH-positive male or female PGCs with MVH-negative soma or limb (Figure S1F). This revealed a significant increase in nascent transcription in both male and female PGCs (Figure 1E-F). Moreover, the rate of nascent transcription in soma is very similar to E13.5 limb cells, confirming that hypertranscription is due to a specific elevation of transcriptional activity in PGCs, and not a soma-specific suppression (Figure 1E). Finally, we asked if PGC hypertranscription is linked to elevated protein synthesis. Nascent translation assays measuring incorporation of L-Homopropargylglycine (HPG) revealed that translation is also significantly higher in PGCs, relative to somatic cells (Figure 1G-H). Overall, these results reveal a significant elevation in RNA and protein synthesis in PGCs.

Preservation of hypertranscription in male E15.5 germ cells

Examples of hypertranscription in other developmental contexts are often associated with augmented proliferation (Guzman-Ayala et al., 2015; Koh et al., 2015; Nie et al., 2012). We next asked whether germ cells that are not proliferating still exist in a state of hypertranscription. We analyzed E15.5 embryos, when both male and female germ cells (GCs) have entered cell cycle arrest (Bowles and Koopman, 2007). EU incorporation experiments revealed that total nascent transcription is reduced in male and female E15.5 GCs compared to E13.5, and that there is more heterogeneity in the transcriptional output of GCs at E15.5 (Figure 2A-B). Nevertheless, male E15.5 GCs overall display levels of nascent transcription 1.5 to 2-fold higher than soma and limb (Figure 2A-B, Figure S2A). In agreement with these results, the levels of RNA Pol II Ser-2P, which marks elongating polymerase, are higher in PGCs of both sexes at E13.5 and remain higher in male GCs at E15.5 (Figure 2C and S2B-D). These results indicate that germ cell hypertranscription persists in male embryos even after proliferation has ceased.

Global analysis of hypertranscription by CNN RNA-seq in E13.5 PGCs

The EU data show that nascent transcription is globally elevated in PGCs but do not resolve which genes are transcriptionally amplified. Are most/all transcripts globally elevated in the developing germline, or do PGCs only express a subset of genes at very high levels? To answer this question, we performed cell number-normalized RNA-seq (CNN RNA-seq) at E13.5 (Table S1-2). Traditional RNA-seq experiments cannot provide accurate differences in expression per cell (Loven et al., 2012; Percharde et al., 2017), since libraries are created from equal amounts of RNA and data are normalized to read depth. For CNN analysis, we isolated RNA from equal numbers of male and female PGCs or soma from Oct4/GFP mice and added synthetic External RNA Controls Consortium (ERCC) RNA spike-ins according to cell number prior to library generation (Figure 3A) (Reid. and ERCC, 2005). Correlation analyses confirmed that PGCs and soma are transcriptionally distinct from each other and

are separated by sex, as expected (Figure S3A). We next asked whether the total number of expressed genes varies between PGCs and soma. Interestingly, all cell types express a similar number of genes (Figure 3B). In contrast, the number of highly expressed genes is higher in male and female PGCs than in soma (Figure 3B). In agreement, transcripts in PGCs show an increase in median gene expression compared to soma (Figure S3B), paralleling the total nascent transcription data (Figure 1E). These results indicate that PGCs express the same approximate number of genes as soma cells, but many genes are expressed at elevated levels.

To determine the expression patterns of individual genes, we generated heatmaps from read-depth normalized or CNN RNA-seq data. In contrast to traditional read-depth normalization, CNN analysis demonstrates that the vast majority of genes are expressed at higher levels in PGCs than soma, in both sexes (Figure 3C; Class 1) or in a sex-specific manner (Figure 3C; Class 2-3). In contrast, much fewer genes are upregulated in soma (Figure 3C; Class 4). Supporting this analysis, 36% and 59% of genes are significantly upregulated more than 4-fold in male and female PGCs respectively, but only 14% and 8% are upregulated in soma (Figure 3D, Table S1). Hypertranscription preserves tissue specificity, in that PGCs do not up-regulate somatic developmental regulators, such as Hox genes (Figure 3C and Figure S3C). Importantly, such global amplification of the transcriptome is not apparent without CNN analysis (Figure 3C, left), highlighting the need for appropriate normalization methods to probe global transcriptomic changes.

We next asked what types of genes are subject to hypertranscription in PGCs. Gene Ontology (GO) analysis of Class 1 genes includes many significant terms for ribosome biogenesis and rRNA processing (Figure S3D). Strikingly, all ribosomal protein genes are upregulated in male and female PGCs (Figure 3E). These results further highlight that the biosynthetic capacity of PGCs is overall elevated relative to soma, in agreement with their increased protein synthesis (Figure 1G-H), and cellular hypertrophy (Figure S1D-E).

Next, we investigated whether CNN analysis reveals distinct patterns of transposable element (TE) expression in PGCs. TEs are thought to be under tight control in the germline so as to preserve genomic integrity. Surprisingly, we found that nearly all TE families are more highly expressed in PGCs than soma (Figure 3F, Figure S3E), including many that appear suppressed or unchanged under read-depth normalization (Figure S3E). In contrast, the majority of TEs show similar expression levels between male and female PGCs (Figure 3F). TE upregulation is not specific to E13.5, as the levels of Line1, SineB2 and IAP elements are increased in PGCs over soma from E9.5-E13.5 (Figure S1B). Thus, the embryonic germline expresses a remarkably high level of TEs, with transposition presumably controlled at the post-transcriptional level (Weick and Miska, 2014). Taken together, these data document that transcripts for genes associated with growth and translation, as well as transcripts derived from most classes of TEs, are all present at higher levels per cell in PGCs relative to soma.

Myc proteins and P-TEFb drive PGC hypertranscription

Finally, we investigated potential drivers of PGC hypertranscription. The oncogene c-Myc promotes transcriptional amplification in cancer cells and lymphocytes (Lin et al., 2012;

Loven et al., 2012; Nie et al., 2012; Rahl et al., 2010). However, it has been reported that *Myc* expression is absent in PGCs and is only gained upon reprogramming to Embryonic Germ (EG) cells in culture (Durcova-Hills et al., 2008). Our RNA-seq data revealed that while *Myc* is not expressed in PGCs but is detected in soma, the Myc family members *Mycn* and *Mycl* show PGC-specific expression in both CNN and read-depth normalized data (Figure 4A, S4A). The expression pattern of *Myc* family genes and the Myc cofactor, *Max* was confirmed by CNN qRT-PCR (Figure 4B). We therefore set out to investigate whether Myc activity, mediated by n-Myc and l-Myc proteins, regulates hypertranscription in PGCs (Figure 4C). We performed EU incubations of dissociated gonads in the presence of 10058-F4, a potent Myc/Max inhibitor (Yin et al., 2003). These experiments revealed a striking reduction in nascent transcription in E13.5 PGCs after only 1.5 hours of Myc inhibition in both sexes (Figure 4D). In particular, nascent transcription in female PGCs decreases to levels nearly identical to those of soma. In contrast, Myc/Max inhibition has no effect on the rate of nascent transcription in soma of either sex (Figure 4D). Decreased EU incorporation is not due to decreased survival upon Myc inhibition, as no changes in cell death were detected in 10058-F4-treated samples relative to controls (Figure S4B). These data indicate that Myc activity promotes hypertranscription in PGCs.

Myc-dependent transcriptional amplification in cancer cells has been shown to involve RNA Pol II pause release mediated by P-TEFb/Cdk9 activity (Rahl et al., 2010). We investigated the role of P-TEFb in hypertranscription in PGCs by quantifying total nascent transcription in the presence of Flavopiridol, an inhibitor of P-TEFb/Cdk9 activity (Chao and Price, 2001). Mirroring Myc-inhibition, P-TEFb inhibition significantly reduces hypertranscription in both male and female PGCs without increased toxicity (Figure 4E, S4C). Taken together, these results indicate that n-Myc/l-Myc and P-TEFb are drivers of hypertranscription in the embryonic germline.

Discussion

Hypertranscription has been understudied but is likely pervasive in stem and progenitor cells during development (Percharde et al., 2017). Here, we provide transcriptome-wide documentation of hypertranscription in vivo. We show that the mouse embryonic germline sustains a remarkable level of hypertranscription relative to somatic cells at the same stage. This phenomenon can be uncoupled from cell proliferation and is correlated with increased translation and cell size. Importantly, the extent of hypertranscription in the germline only becomes clear after using CNN approaches. This work provides a set of experimental tools to investigate hypertranscription in vivo that will be of broad applicability in other contexts. The results raise several interesting questions as to the regulation and function of hypertranscription in the germline.

Among the most highly upregulated sets of genes in PGCs are those for ribosome biogenesis and translation. This suggests that increased transcription and translation may promote the increase in cell size in PGCs, in agreement with previous accounts of hypertrophy arising due to elevated biosynthesis (Kim et al., 2000). Indeed, an increase in nuclear size has been reported to occur in PGCs from E9.5 to E11.5 (Hajkova et al., 2008; Kagiwada et al., 2013). Moreover, feedback mechanisms whereby a larger cell volume reinforces elevated

transcription may exist and contribute to propagating a state of increased biosynthesis (Padovan-Merhar et al., 2015). Interestingly, while female E15.5 germ cells appear to have ceased hypertranscription, they are still larger than soma (Figure S2D). This likely reflects a very recent decrease in transcriptional activity, potentially coupled with entry into meiosis, which occurs in female but not male germ cells at this stage. A loss in hypertranscription may be due to reduced *Max* expression, which is already lower than in males at E13.5 (Figure 4B, S4A). In agreement, it has been proposed that a reduction in the level of Max is necessary for cultured female PGCs to enter meiosis (Maeda et al., 2013).

We show that hypertranscription is not limited to unique genes and rDNA, but includes TEs: on a per cell basis, transcripts from all major retrotransposon families are detected at higher levels in PGCs than in soma. This is surprising in light of studies of epigenetic repressive marks in PGCs, which imply that the expression of TEs is heavily suppressed in the germline [e.g., (Bourc'his and Bestor, 2004; Liu et al., 2014)]. Our data instead indicate that, while under the control of mechanisms that prevent transposition and preserve genomic integrity, TE RNAs are still abundant in PGCs. It will be of interest to investigate whether TE RNAs play novel regulatory roles in the germline (Gerdes et al., 2016).

Another important finding from this study is the role of Myc proteins in promoting PGC hypertranscription. This is likely driven by n-Myc and/or l-Myc, as c-Myc is repressed by Blimp1 in PGCs (Magnusdottir et al., 2013). The major mechanism by which Myc-stimulated transcriptional amplification occurs in cancer cells is by P-TEFb-regulated transcriptional pause release (Rahl et al., 2010), and our data suggest that the same mechanism is at play in PGCs. PGC-specific deletion of *Myc* genes, *Max,* or regulators of transcriptional pause release may shed light on the mechanisms that drive hypertranscription in the mouse germline.

In spite of a widespread amplification of the transcriptome, PGC hypertranscription maintains tissue-specific gene expression patterns, given that PGCs do not express transcriptional regulators of development of somatic lineages. The silencing of somatic genes in the context of PGC hypertranscription is all the more striking given the genome-wide loss of DNA methylation (Seisenberger et al., 2012), an epigenetic mark that often represses gene expression. It has been shown that the promoters of somatic developmental regulators are depleted in binding sites for activating transcription factors such as Myc (Ku et al., 2008) and are marked with H3K4me3/H3K27me3 bivalent chromatin in PGCs (Sachs et al., 2013). Thus, a combination of the architecture of the Myc transcriptional network with repressive histone marks such as H3K27me3 likely ensures tissue specificity in the context of PGC hypertranscription.

PGC hypertranscription is likely to be subject to strict temporal control. IF data from (Seki et al., 2007) suggest that RNA Pol II activity is suppressed in E8.5 PGCs but increases thereafter. Our own data indicate that PGC hypertranscription is ongoing between 9.5 and E15.5, peaking around E11.5, and has ceased, in females, by E15.5. Thus, hypertranscription in PGCs coincides with migration, colonization of the gonadal niche and early stages of differentiation in the gonads. Our results raise the question of how hypertranscription might regulate germline development. Migratory and early gonadal PGCs undergo rapid growth

and proliferation, which may depend on the ability of Myc factors to sustain high levels of hypertranscription and biomass accumulation. Another potential function of hypertranscription is to drive cell competition. The observed heterogeneity in the transcriptional and translational output of PGCs (Figures 1E-H and 2A) and the underlying role for Myc factors (Figure 4) raises the possibility that hypertranscription may mediate competition between PGCs (Percharde et al., 2017). There is evidence for competition between germ cells for the somatic niche in colonial ascidians (Laird et al., 2005), the Drosophila germline (Extavour and Garcia-Bellido, 2001) and postnatal mouse testis (Shinohara et al., 2002) Moreover, germ cell competition in the Drosophila ovary has been shown to be regulated by high levels of Myc (Rhiner et al., 2009), which may drive competition in the post-implantation epiblast (Claveria et al., 2013; Sancho et al., 2013). Selection of PGCs based on their biosynthetic output could be a conserved mechanism to ensure that the cells most fit to support embryonic development go on to contribute to the next generation. Overall, our data warrant future studies of hypertranscription during development of other lineages as well as dissection of its roles in germline biology.

Experimental Procedures

Animal work and PGC isolation

E13.5 or E15.5 embryos were derived from wild-type 6-8 week old ICR females mated either to ICR or Oct4/GFP B6 males (Yeom et al., 1996). Morning of the day of detection of copulatory vaginal plug was designated as E0.5. All animal experiments were in accordance with the guidelines of the UCSF Institutional Animal Care and Use Committee, protocol AN091331-03.

CNN qRT-PCR

E13.5 Oct4/GFP gonads were pooled and enzymatically digested in 0.5% Trypsin and 0.8 mg/mL DNase I (Worthington). PGCs and matched soma were isolated utilizing a FACS AriaII (BD Biosciences), according to GFP expression. Equal numbers of cells were sorted directly into RLT lysis buffer. RNA was purified, DNase treated, then used to generate cDNA for qRT-PCR.

Nascent transcription/translation assays

Gonads from wild-type ICR embryos were enzymatically dissociated and incubated with 1 mM 5-ethynyl-uridine (EU) or 50 μM L-Homopropargylglycine (HPG) at 37 °C for 1 h. Where indicated, DMSO, 10058-F4 (50 μM, Sigma F3055) or Flavopiridol (2 μM, Sigma F3680) was included 30 min prior to EU addition, for a total 90 min incubation. Nascent RNA was labeled with Click-iT RNA imaging kits (Thermo Fisher Scientific), followed by incubation with anti-MVH (Abcam, ab13840) to stain PGCs and GCs, as described previously (Wakeling et al., 2013).

CNN RNA-seq

10,000 PGCs or 30,000 soma were purified from pooled Oct4/GFP embryonic gonads and used to isolate RNA as above. 4 μL diluted ERCC control mix (Reid. and ERCC, 2005) (1:10,000, Thermo Fisher Scientific) was added per 10,000 cells to RNA prior to library

generation. Libraries were created from 8-10 ng DNase I-treated total RNA, using NEBNext ultra directional RNA library prep kit for Illumina (NEB), pooled at equimolar concentrations, and sequenced on an Illumina HiSeq4000. Three replicates were sequenced per condition, each yielding >10e^6 single-end 50 bp reads. Detailed bioinformatic methods are available in Supplemental Material online.

Statistical analyses

All statistical calculations were performed with GraphPad 7.0 software; details of individual tests are outlined within each figure legend.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Cell number-normalized methods reveal global hypertranscription in vivo
- mRNAs for biosynthesis, rRNA and transposon RNAs are amplified in mouse PGCs
- Hypertranscription correlates with cell size and translation
- Myc factors and P-TEFb promote PGC hypertranscription

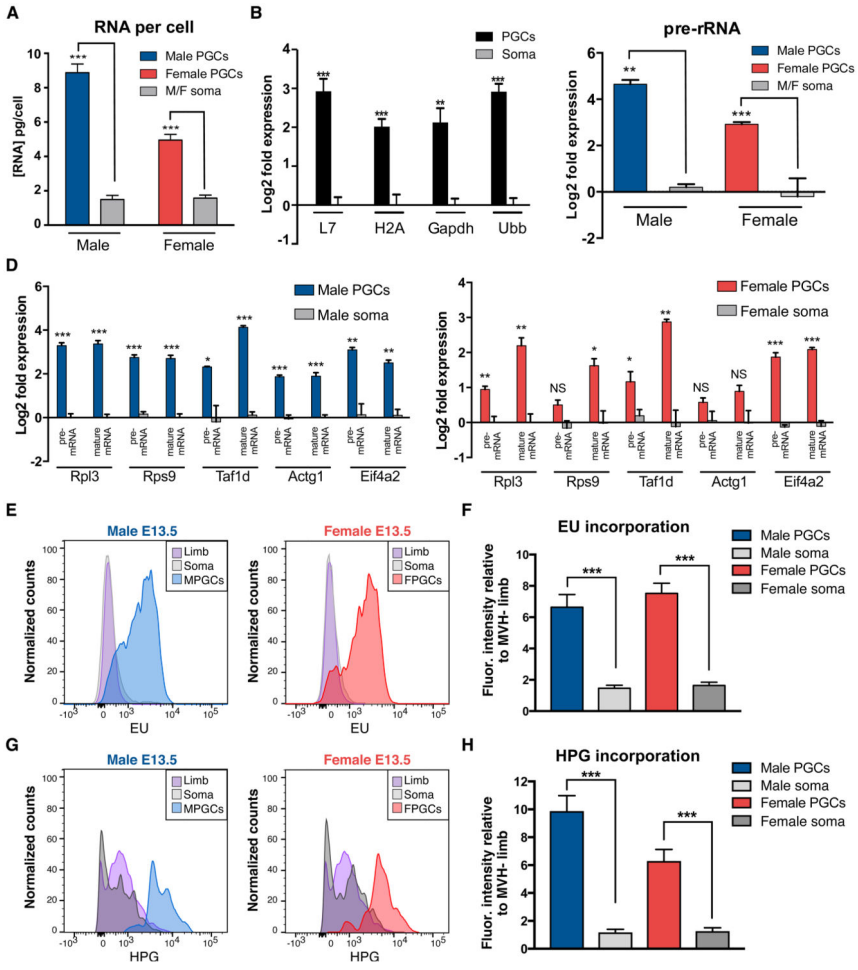

Figure 1. Transcriptional output of RNA Pol I and II is elevated in PGCs

(A) Quantification of total RNA per cell in PGCs or soma. (B-C) CNN qRT-PCR analysis of (B) housekeeping genes or (C) pre-rRNA in PGCs or soma. (D) Comparison of mature vs primary mRNA transcript expression in PGCs or soma. (E) Representative histograms of nascent transcription (EU incorporation) in E13.5 gonads. (F) Quantification of EU data, normalized to limb in each experiment. (G) and (H), as (E) and (F) respectively, but for nascent translation (HPG incorporation). All panels are representative of 3 or more biological replicates, data are mean +/- s.e.m. * P <0.05, ** P <0.01, *** P <0.001, ns: not significant, two-tailed Student's t-test. See also Figure S1.

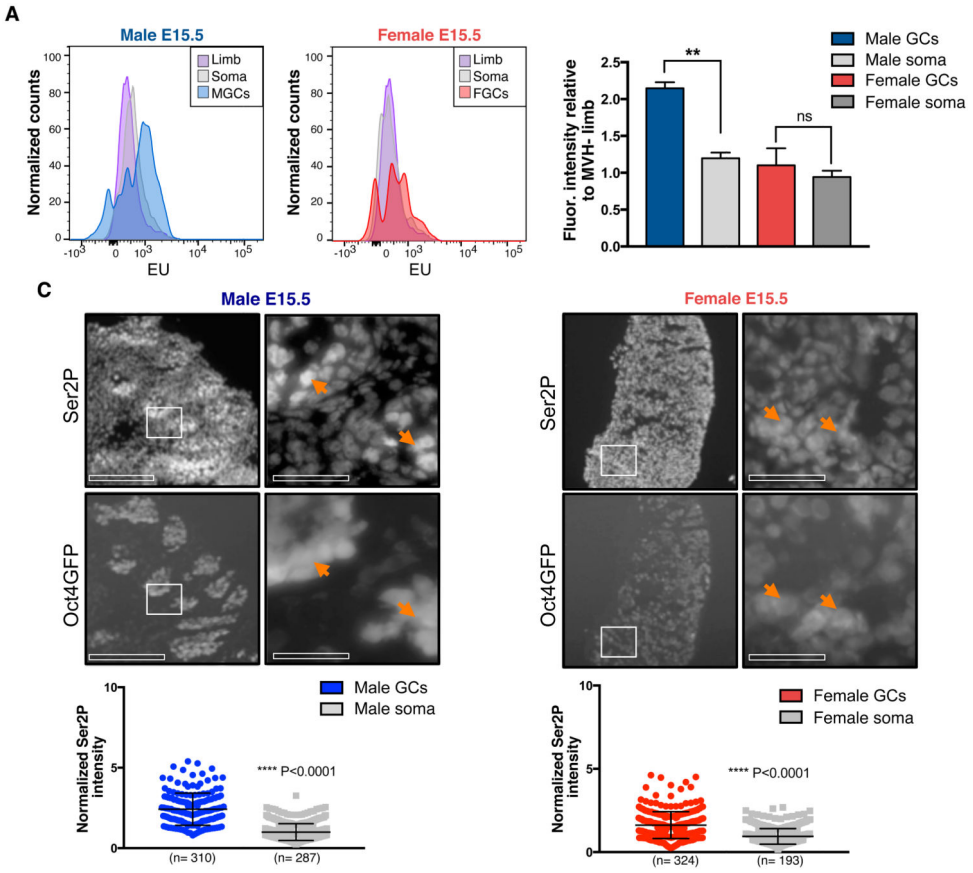

Figure 2. Elevation in transcriptional output persists in E15.5 male germ cells

(A) Representative histograms and (B) quantification of EU incorporation in E15.5 Germ Cells (GCs) or soma. Levels are normalized to EU incorporation in E15.5 limb in each experiment. (C) Representative images of RNA Pol II Ser-2P staining in Oct4/GFP-positive GCs or Oct4/GFP-negative soma, quantified below. Data were collected from 3 randomly selected fields per gonad in 3-4 gonads per sample, and are normalized to average intensity of each field. n, number of cells. Scale: 200μm, inset: 50μm. Data are mean +/- s.e.m from 3 biological replicates. ** P <0.01, **** P <0.0001, two-tailed Student's t-test. See also Figure S2.

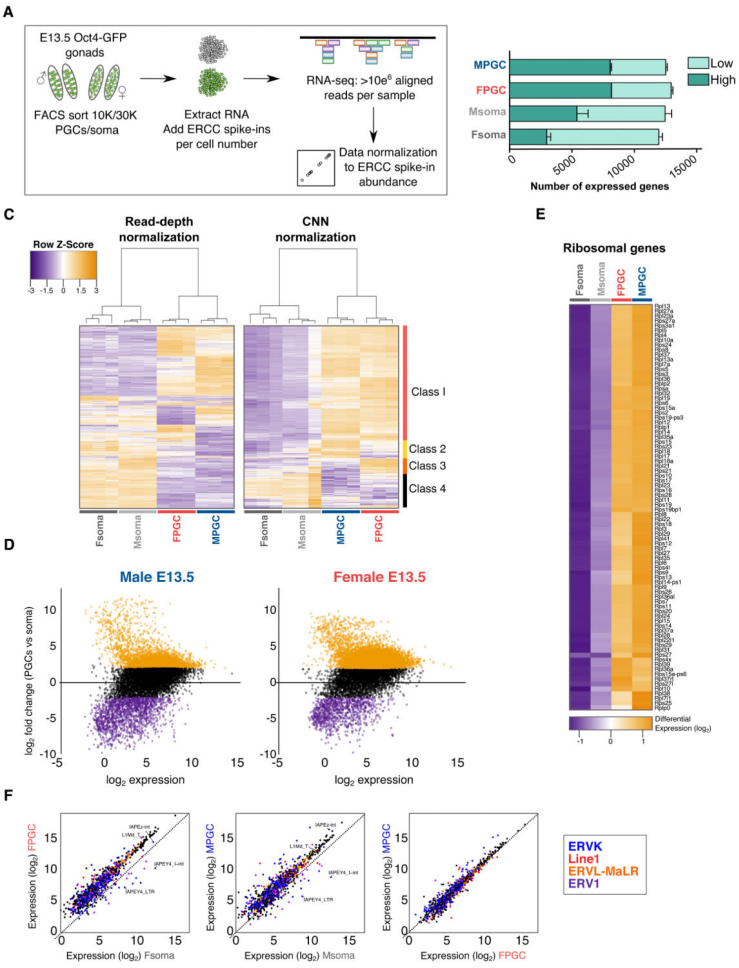

Figure 3. CNN RNA-seq reveals global PGC hypertranscription

(A) Schematic of CNN RNA-seq. (B) Quantification of the number of expressed genes (log_2 normalized reads ≥1) in male and female PGCs or soma. Expressed genes are further defined as having low expression (log_2 normalized reads <5), or high expression (log_2 normalized reads ≥5) in each sample. Error bars represent s.e.m. in 3 biological replicates. (C) Heatmaps of gene expression for all genes, in filtered and read-depth normalized (left), or cell number-normalized (CNN) (right) data. K-means clustering was used to identify four main classes of genes, based on their expression patterns. (D) MA plots of differentially expressed genes in PGCs vs soma for male or female embryos. Significantly altered genes are defined as those with FDR< 0.01 and log_2 fold-change >2 (orange) or <-2 (purple). (E) Heatmap of ribosomal protein gene expression in CNN RNA-seq data. (F) Scatter plots of average TE expression in male or female PGCs and soma. Data points are colored by TE family (see legend). Examples of up- or down-regulated TEs are indicated. See also Figure S3 and Tables S1 and S2.

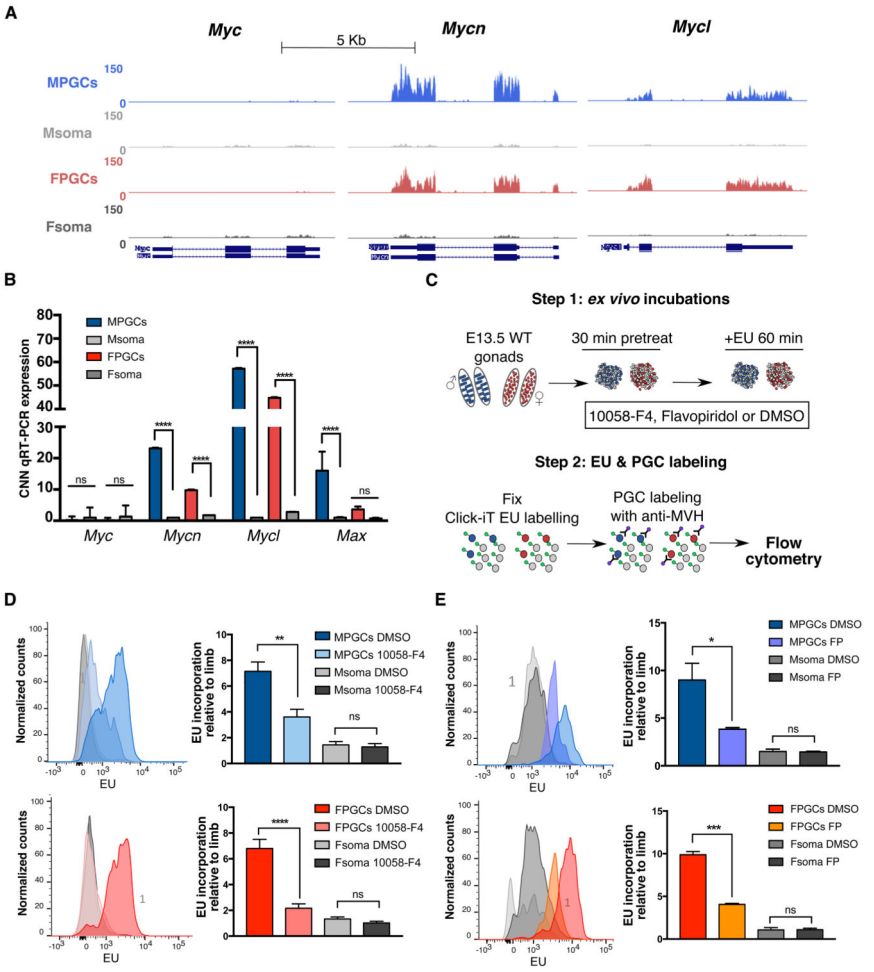

Figure 4. Myc factors and P-TEFb promote PGC hypertranscription

(A) Representative genome browser views of RNA-seq data for *cMyc, Mycn* and *Mycl* in E13.5 PGCs and soma. (B) CNN qRT-PCR validation of the expression of Myc factors in PGCs and soma. Data for each gene are normalized to its expression level in male soma, and represented as mean +/- s.e.m for 3 independent biological replicates. (C) Schematic of small molecule modulation of nascent transcription in dissociated gonads. (D) Representative histograms and quantification of EU incorporation in PGCs and soma incubated with DMSO or 50 μM 10058-F4, an inhibitor of Myc function. (E) Data as per (D), except that 2 μM flavopiridol (FP), an inhibitor of P-TEFb, was used instead of 10058-F4. Data for (D-E) are representative of 3-4 independent biological replicates. ** P<0.01, **** P <0.0001, ns: not significant, two-tailed Student's t-test. See also Figure S4.