The zinc-finger transcription factor LSL-1 is a major regulator of the germline transcriptional program in Caenorhabditis elegans

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Abstract

Specific gene transcriptional programs are required to ensure the proper proliferation and differentiation processes underlying the production of specialized cells during development. Gene activity is mainly regulated by the concerted action of transcription factors and chromatin proteins. In the nematode Caenorhabditis elegans, mechanisms that silence improper transcriptional programs in germline and somatic cells have been well studied, however, how are tissue-specific sets of genes turned on is less known. LSL-1 is herein defined as a novel crucial transcriptional regulator of germline genes in C. elegans. LSL-1 is first detected in the P4 blastomere and remains present at all stages of germline development, from primordial germ cell proliferation to the end of meiotic prophase. loss-of-function mutants exhibit many defects including meiotic prophase progression delay, a high level of germline apoptosis, and production of almost no functional gametes. Transcriptomic analysis and ChiP-seq data show that LSL-1 binds to promoters and acts as a transcriptional activator of germline genes involved in various processes, including homologous chromosome pairing, recombination, and genome stability. Furthermore, we show that LSL-1 functions by antagonizing the action of the heterochromatin proteins HPL-2/HP1 and LET-418/Mi2 known to be involved in the repression of germline genes in somatic cells. Based on our results, we propose LSL-1 to be a major regulator of the germline transcriptional program during development.

Keywords: Caenorhabditis elegans; chromatin; transcription regulation; RNA-seq; ChiP-seq; germline; meiosis

Introduction

Sexual reproduction relies on the generation of functional gametes, which depends on the proliferation and differentiation of primordial germ cells (PGCs) into oocytes and sperms. To ensure proper gametogenesis gene activity must be tightly regulated from the birth of PGCs to the production of mature sperm and oocytes including proper progression through meiosis. In every organism studied to date, gene regulation mechanisms represent an intrinsic part of the germ cell specification process (Seydoux and Braun 2006; Strome and Updike 2015). Studies across species have been mainly focused on how transcription of the somatic program is silenced and propose 2 modes of transcription repression in PGCs. Initially, transcription is blocked by the inhibition of transcription elongation at the level of RNA polymerase II, and eventually, a chromatin-based transcription repression takes over later in development (Nakamura and Seydoux 2008; Updike et al. 2014; Strome and Updike 2015; Seydoux 2018). In the Caenorhabditis elegans germ line blastomeres, the PIE-1 protein sequesters the elongation factor P-TEFb. P-TEFb is a cyclin-dependent kinase that phosphorylates the CTD domain of polymerase II to allow transcription elongation (Ghosh and Seydoux 2008). Later, inhibition of the somatic transcriptional program switches to a chromatin-based repression. In C. elegans embryos, at about 100-cell stage, the P4 blastomere gives birth to PGCs Z2/Z3, PIE-1 disappears, and PGCs chromatin becomes depleted of di-methylated lysine 4 of histone H3 (H3K4me2)—a mark of active chromatin—and enriched in H3K9me—a mark of repressed chromatin (Seydoux and Dunn 1997; Schaner and Kelly 2006; Strome and Updike 2015). Loss of H3K4 methylation depends on the RNA binding proteins NOS-1 and NOS-2 (Schaner et al. 2003). However, PGCs Z2/Z3 are not completely transcriptionally silent. Zygotic expression of a few germline genes is detected: the P granules components (e.g. PGL-1), the germ cell fate maintenance RNA-binding protein NOS-1, or the chromatin-associated proteins XND-1 and OEF-1 (Kawasaki et al. 2004; Wang and Seydoux 2013; Mainpal et al. 2015; McManus and Reinke 2018). Although the H3K36 methyltransferase MES-4 is known to confer transcriptional competence to germline genes, the mechanism by which transcription is initiated in PGCs is not yet well understood (Rechtsteiner et al. 2010). Following the onset of transcription, chromatin continues to assume a protective role which is mediated by MES-2/3/6 proteins—the worm PRC2 complex—representing the somatic transcriptional program (Tursun et al. 2011; Patel et al. 2012). Finally, robust transcription is initiated when larvae start to feed after hatching of the embryo. At this stage,
PGCs start to proliferate and later, at the larval stage L3, these enter into meiosis and differentiate into sperm (at larval stage L4) and oocytes (at adult stage). For most germline specific genes studied in adult worms—with the exception of those active during spermatogenesis—promoters are permissive for transcription in all germ cells; proper patterning of gene expression requires the 3’ untranslated region (3’UTR). Specialized proteins FBF-1/2, GLD-1, and MEX-3 were identified as crucial for the posttranscriptional regulation at the level of the 3’UTR of mRNAs (Merritt et al. 2008).

Here, we report the functional characterization of LSL-1, a novel key transcription regulator of germline genes. LSL-1 protein is first detected in the P4 blastomere and maintained in PGCs and developing germ cells in the gonad. Absence of LSL-1 activity leads to chromosome pairing defects, high levels of apoptosis, and a very low production of functional gametes. Based on our transcriptome profiling experiments and ChIP-seq (chromatin immunoprecipitation followed by sequencing) analysis of data available from modERN (branched from modENCODE project), we propose that LSL-1 acts as a direct transcriptional activator of germline genes involved in different aspects of germline development, including meiotic prophase progression and genome stability. Furthermore, we found that the sterility of lsl-1 mutants is depending on the heterochromatin factors HPL-2/HP1 and LET-418/Mi2, involved in the silencing of germline gene transcription in somatic cells. Altogether, this lead us to propose that LSL-1 is an important player in the activation of the germline transcription program.

Materials and methods

Genetics

Worms were grown and maintained at 15°C and 20°C under standard conditions (Brenner 1974). Experiments were performed at 20°C unless otherwise stated. Caenorhabditis elegans var. Bristol (N2) was used as wild type. Standard genetic crosses were made to generate double mutants using strains previously backcrossed to N2 at least 4 times. A list of all strains used in this study is provided in the Supplementary Methods section.

Brood size, embryonic viability, and incidence of males

Synchronized L4 hermaphrodite worms were individually placed on NGM plates seeded with Escherichia coli OP50 and then transferred to new plates every 24 h until laying stopped. Total number of laid eggs, hatched larvae, progeny which reached adulthood, and males were scored. Each scoring experiment was performed at the indicated temperature with mutant strains and wild-type strain N2 running in parallel. Data were pooled from multiple rounds of experiments in each cytological analysis category; statistical comparison between genotypes was assessed using 2-tailed Student’s t-test with Welch’s correction, P-value ≤ 0.05.

Immunofluorescence

One-day adult hermaphrodite gonads were processed and immunostained as described by Phillips et al. (2009), with various modifications. Detailed protocol is included in the Supplementary Methods section.

Early-staged embryos were obtained from gravid hermaphrodite dissection and then processed as described in the Supplementary Methods immunofluorescence section. Embryos were also obtained by hypochlorite treatment of gravid hermaphrodites (50 mM NaOH + 1.25% NaOCl) (Lewis and Fleming 1995), thereby allowing the acquisition of late embryonic stages. Synchronized populations of worms for each larval stage were also collected in distilled water and fixed in the same manner as the bleached embryos, using a modified protocol from Finney and Ruvkun (1990), Miller and Shakes (1995), and Bettinger et al. (1996). Samples were fixed with 2% formaldehyde in 1x modified Ruvkun fixation buffer (MRFB) and immediately frozen in liquid nitrogen. These were then thawed and incubated on ice for 30 min with occasional inversion and washed 3 x 10 min in PBST with DAPI added between the second and third washes. Finally, slides were mounted with Vectashield H-1000 antifade mounting medium (Vector Laboratories; Burlingame CA, USA), stored at 4°C, and imaged. A list of antibodies used is available in the Supplementary Methods section.

DAPI-staining cytological analysis

At least 7 gonads of each genotype were stained with DAPI to determine the length extension of the mitotic to meiotic transition zone. Transition zone-like nuclei were identified based on their chromatin morphology and characteristic crescent shape. Length extension was measured in nuclei rows along the distal–proximal axis of the gonad according to Crittenden et al. (2006), defining its limits as the most distal and proximal rows where at least 2 nuclei exhibited the typical crescent shape.

At least 20 gonads of each genotype were stained with DAPI to quantify the number of DAPI-staining bodies in the diakinesis oocytes. The most proximal oocyte to the spermatheca (~1 oocyte) in each gonad was considered for the scoring. Slides were examined using a Nomarski and fluorescent Zeiss Axioplan 2 microscope to visualize the DAPI-staining bodies.

Data obtained from the different quantifications were pooled from multiple rounds of experiments in each cytological analysis category; statistical comparison between genotypes was assessed using 2-tailed Student’s t-test with Welch’s correction, P-value ≤ 0.05.

Fluorescence in situ hybridization

A probe was generated from the SS rDNA locus (located close to the pairing center region of chromosome V) by PCR (primer sequences) incorporating allyl-dUTP and labeled with the ATTO-488 NHS-ester fluorescent dye, as described in Sharma and Meister (2020). Fluorescence in situ hybridization (FISH) probe hybridization was adapted from Phillips et al. (2009) and is described in detail in the Supplementary Methods section.

Meiotic homologous chromosomes pairing dynamics analysis

To evaluate the progression of the chromosome pairing process, we monitored the localization of SUN-1::mRuby, HIM-8 (X-chromosome), and SS rDNA probe (chromosome V) signals. At least 4 gonads of 24 h post-L4 hermaphrodite worms of each genotype were analyzed. Germlines were divided in 7 equally long regions, from the distal tip to the proximal end of the pachytene stage, for statistical comparison between wild-type and lsl-1-mutant strains.

Length extension of the germline region containing nuclei with the presence of SUN-1::mRuby signal patches was measured in nuclei rows along the distal–proximal axis. Quantification of HIM-8 and SS rDNA foci involved scoring of the foci number observed per nucleus (n = 1: paired chromosomes; n > 1: unpaired chromosomes) in each germline region. Statistical comparisons...
were performed using 2-tailed Student’s t-test with Welch’s correction, P-value ≤ 0.05.

**Microscopy and image processing**

Imaging for the isl-1 expression pattern determination and germline cytological analysis was performed with a confocal microscope Leica TCS SPE-II DMS500Q. Images were collected using a 40x or 63x 1.3 NA objective (with 1.5x auxiliary magnification in embryos), and Z-stacks were set at 0.2 μm thickness intervals (0.5 μm for the cytological analysis of 1-day adult gonads). Embryos were staged by either morphology or number of blastomeres in early embryonic stages; larvae were staged by size or germline developmental phase from synchronized populations.

Images for meiotic chromosome pairing dynamics analysis were obtained using a pco.edge sCMOS camera attached to a Visitron Visiscope CSU-W1 spinning disk confocal microscope (Nikon Ti/E inverted microscope). Imaging was performed using a 100x 1.4 NA objective, and Z-stacks were set at 0.2 μm thickness intervals.

Total length of larval and 1-day adult gonads images were obtained as multiple Z-stacks due to their length and later merged to generate the complete final image using the ImageJ Stitching plugin (Preibisch et al. 2009) or Adobe Photoshop (2020). Images were processed using Fiji Imagej, background was subtracted, and contrast/brightness adjusted. Orientation of the images and final figure appearance were performed using Adobe Photoshop (2020) and Adobe Illustrator (2020).

**Germline apoptosis**

Apoptosis was determined using acridine orange (AO) staining in the germlines of 1-day-old hermaphrodite worms. Number of apoptotic corpses per gonad arm for wild-type and different single- and double-mutant strains was scored as in Shaham (2006). A detailed protocol is available in the Supplementary Methods.

**RNA extraction, cDNA library preparation, and sequencing**

Wild type and both isl-1(tm4769) and isl-1(jm1) mutant strains were synchronized and collected as young adult hermaphrodites 50-h postlarval hatching after hypochlorite treatment (at 20°C). Total RNA was extracted with TRIzol Reagent (Invitrogen; Carlsbad, CA, USA), and RNA was purified using the PureLink RNA Mini Kit (Invitrogen; Carlsbad, CA, USA) according to manufacturer’s instructions. cDNA library preparation and RNA sequencing were performed at the Next Generation Sequencing (NGS) Platform in Bern (https://www.nsg.unibe.ch/). Quality and concentration of each RNA sample and following cDNA libraries were determined with Qubit 2.0 fluorometer and the Fragment Analyzer CE12 AATI. cDNA libraries were built using the TrueSeq stranded mRNA library preparation kit (Illumina Inc.; San Diego, CA, USA). RNA sequencing (50bp paired-end reads) was performed on 3 biological replicates per sample with the Illumina NovaSeq 6000 Sequencing System, and cDNA libraries were multiplexed in a sequencing lane.

**RNA-seq data analysis**

The sequencing data were obtained from Bern NGS platform. Raw reads in .fasta format were then uploaded to the Galaxy web platform using the public server at https://usegalaxy.org (Afgan et al. 2016). Sequencing data analysis is described in more detail in the Supplementary Methods.

**Chromatin immunoprecipitation sequencing data**

We obtained the chromatin immunoprecipitation (ChIP) sequencing data analyzed in this study using the interface http://epic.gs.washington.edu/modERN/ that compiles the data from the model organism Encyclopedia of Regulatory Networks (modERN) consortium (Kudron et al. 2018)—branched from the model organism encyclopedia of DNA Elements (modENCODE) project (Gerstein et al. 2010)—available at: https://www.encodeproject.org/experiments/ENCSR969MX/. ChIP-seq data processing and analysis are described in detail in the Supplementary Methods section.

**Results**

**Isl-1 encodes a germ cell-specific zinc-finger transcription factor**

isl-1 (for lsy-2-like) was identified in a genome-wide RNAi screen as a suppressor of ectopic germline gene expression associated with mutations in let-418, which encodes an ATP-dependent chromatin remodeler (Erdelyi et al. 2017). Isl-1 is predicted to encode a 318 aa protein with at least 3 zinc-finger domains (Fig. 1a), which are homologous to the zinc-finger domains characterizing the SP/KLF family of transcription factors, a protein family with diverse functions in growth and development (Kaczynski et al. 2003; Pearson et al. 2008). In addition, 2 less conserved zinc fingers are located at the C terminal end of the protein (Fig. 1b). Along the entire length of the protein, LSL-1 shows 65% similarity to the LSL-1 paralog LSY-2—involved in ASE neuron specification and in the maintenance of germ–soma distinction—and 41% similarity to the zinc-finger domain of the human protein ZFPF57, which plays a role in the allelic expression of imprinted genes (Supplementary Figs. 1 and 2) (Alonso et al. 2004; Johnston and Hobert 2005; Lin et al. 2015; Liu et al. 2017). The C. elegans Mutant Consortium 2012, provided a 675 bp deletion allele, tm4769, that removes the first 3 exons and part of the promoter region (Fig. 1a, Table 1 and Supplementary Table 1), and we generated an additional allele, jm1, by inserting 2 consecutive stop codons 27 bp downstream the isl-1 translational initiation site (Fig. 1a). isl-1(tm4769) and isl-1(jm1) homozygous animals exhibit decreased brood size and embryo viability as well as a high incidence of males with respect to wild-type animals, indicating defects in meiotic prophase progression. Although both represent strong loss-of-function alleles, these exhibit a slightly different penetrance of the phenotype (Table 1). The present study was performed with the tm4769 allele and is supplemented with data on the jm1 allele (Supplementary material).

Using a LSL-1::GFP endogenous reporter, we examined the isl-1 expression pattern throughout development (Fig. 1b). CRISPR/Cas9 mediated knock-in of the GFP coding sequence in the endogenous isl-1 gene upstream of the stop codon did not interfere with the protein function (Table 1). LSL-1 is detected in cells marked by the presence of P granules, namely in the P4 blastomere and later on in PGCs Z2 and Z3 throughout embryogenesis (Fig. 1, c-g). During larval development and adult stage, LSL-1 is observed in proliferative germline nuclei and in pachytene and diplotene stage nuclei (Fig. 1, j and k). LSL-1::GFP signal disappears at the late diplotene stage and is barely detectable in oocytes and sperm (data not shown) (Fig. 1k). LSL-1 was not detected in somatic cells, including the gonadal sheath cells and the distal tip cell (Fig. 1k). These results indicate that isl-1 is specifically expressed in germ cells throughout development and is essential for the production of functional gametes.
LSL-1 is essential for normal progression of germ cells through meiotic prophase

To further investigate the function of LSL-1 in the germ cells, we inspected DAPI stained gonads which reveal the progression of nuclei through the different stages of meiotic prophase based on chromatin organization (Fig. 2a). By scoring the number of nuclei rows along the distal–proximal axis of the gonad, according to Crittenden et al. (2006), we observed an extended transition zone.
in *lsl-1(tm4769)* worms (32.9 ± 4.5 rows), with respect to the wild-type transition zone, comprised of 14.4 ± 2 rows in average (Fig. 2, a and b). Similar results were observed in *lsl-1(ljm1)* mutant gonads (Supplementary Fig. 3). These observations suggest that chromosome pairing might be perturbed in *lsl-1* mutants.

Closer examination of the DAPI-stained nuclei revealed an altered chromatin organization in the transition zone of *lsl-1* mutants. Chromatin appears to loop out of the otherwise normally clustered chromosomes (Fig. 2a, zone II and Supplementary Fig. 3a, zone II). The few pachytene stage nuclei in *lsl-1* mutants exhibit disorganized chromosomes, with thinner chromatin stretches which could represent unpaired regions of the chromosomes (Fig. 2a, zones V–VII and Supplementary Fig. 3a, zones V–VII).

In *lsl-1(tm4769)* mutant allele, we found less than 1.8% of *lsl-1* oocytes presenting the normal 6 DAPI-staining bodies, while the remaining 98.2% showed more than 6 DAPI-staining bodies [90% in *lsl-1(ljm1)* mutant allele] (Fig. 2c and Supplementary Fig. 3c). This indicates that a large portion of the chromosomes fail to undergo crossing over. These cytological defects are consistent with the high incidence of males and the decreased embryo viability observed in the progeny of *lsl-1* mutants, which likely result from chromosome missegregation at meiotic division I (Table 1 and Supplementary Table 1).

**PLK-2-dependent cell cycle delay is activated in *lsl-1* mutants**

The Polo-like kinase PLK-2 coordinates cell cycle delay and chromosome pairing (Fridkin et al. 2009; Harper et al. 2011). To determine whether the *lsl-1* extended transition zone depends on PLK-2 activity, we generated a *plk-2(ok1936) lsl-1(tm4769)* double mutant and scored the length of their transition zone. The double mutants *plk-2(ok1936) lsl-1(tm4769)* exhibit a transition zone length comparable to *plk-2(ok1936)* mutants, which is shorter than the wild-type transition zone (Fig. 2b). *plk-2(ok1936) lsl-1(tm4769)* worms are sterile and present a slightly higher number of univalents compared to *lsl-1(tm4769)* mutants (Table 2, Fig. 2c). These results suggest that PLK-2-dependent cell cycle delay is activated in the *lsl-1* mutant and might allow some level of pairing and recombination.

**Chromosome pairing is disrupted in absence of *LSL-1***

To test whether homologous chromosome pairing is perturbed in *lsl-1* mutants, we monitored the localization of SUN-1, which forms aggregates upon phosphorylation by checkpoint kinase CHK-2 and polo-like kinase PLK-2, following pairing initiation (Fridkin et al. 2009; Woglar et al. 2013). Using SUN-1:mRuby
transgenic worms, we observed SUN-1 aggregates at the beginning of the transition zone in lsl-1 and wild-type gonads (Fig. 3 and Supplementary Fig. 4). However, in lsl-1 mutants, SUN-1 patches were still detectable at the most proximal part of the germline, as far as zone VI, where no SUN-1 patches are detected in wild type (Fig. 3 and Supplementary Fig. 4). Thus, the idea that LSL-1 is involved in the proper progression of the pairing process.

To better identify the pairing defects in lsl-1 mutants, we monitored the localization of the X-chromosome Pairing Center (PC) protein HIM-8 using immunofluorescence (Fig. 3c and Supplementary Fig. 4). In wild-type animals, more than 90% of X chromosomes are paired from zone III to the most proximal regions of the germline and present 1 HIM-8 focus (Fig. 3c and Supplementary Fig. 4c). In lsl-1(tm4769) mutants, an increased number of single HIM-8 per nucleus is observed in zone II, suggesting that precocious X-chromosome pairing could occur (Fig. 3c and Supplementary Fig. 4c; zone II). In both wild type and lsl-1(tm4769) mutants, a significant decrease in the number of single HIM-8 foci is observed in mitotic zone (Fig. 3c; Supplementary Fig. 4). However, this observation could also be due to a slightly shorter mitotic region in lsl-1 mutants (Supplementary Fig. 4), affecting the localization of the X-chromosome Pairing Center (PC) protein HIM-8. To test pairing checkpoint activation, we measured the level of apoptosis in lsl-1 mutant germ lines lacking plk-2 activity. plk-2(ok1936) lsl-1(tm4769) double mutants show a decreased level of apoptosis compared to lsl-1 mutants, more similar to wild-type apoptosis level. This result is consistent with pairing defects triggering PLK-2 dependent apoptosis.

Table 2. Brood size, survival rate, and incidence of males (25°C).

| Genotype               | Mean brood size* | Viability (%) | Incidence of males (%) | nbδ |
|------------------------|------------------|---------------|------------------------|-----|
| Wild type              | 196.78 ± 42.22   | 94.46         | 0.09                   | 46  |
| lsl-1(tm4769)          | 0.05 ± 0.221     | 0.00          | n/a                    | 40  |
| plk-2(ok1936)          | 83.82 ± 35.42    | 16.81         | 21.94                  | 11  |
| plk-2(ok1936) lsl-1(tm4769) | 0.00 ± 0.00*** | n/a           | n/a                    | 12  |

*Data correspond to the mean ± SD of the total number of eggs laid per hermaphrodite parent. Statistical comparison between plk-2(ok1936) lsl-1(tm4769) double mutant and lsl-1(tm4769) genotype performed by 2-tailed Student’s t-test with Welch’s correction. n.s P-value > 0.05.

δTotal number of parental hermaphrodites per genotype. n/a, not applicable.

Absence of LSL-1 activity triggers elevated apoptosis levels

lsl-1 mutants lay a very limited number of embryos, suggesting that a high number of germline nuclei might be eliminated by apoptosis. Using acridine orange staining, we observed a significant increase in the number of apoptotic germ cells in lsl-1 mutants compared to wild-type worms (Fig. 4 and Supplementary Fig. 6). This elevated number of apoptosis could be due to the activation of pairing and/or DNA damage checkpoints (Harper et al. 2011; Kim et al. 2015; Mateo et al. 2016). To test pairing checkpoint activation, we measured the level of apoptosis in lsl-1 mutant germ lines lacking plk-2 activity. plk-2(ok1936) lsl-1(tm4769) double mutants show a decreased level of apoptosis compared to lsl-1 mutants, more similar to wild-type apoptosis level. This result is consistent with pairing defects triggering PLK-2 dependent apoptosis.

LSL-1 regulates transcription of germline genes

lsl-1 encodes a zinc-finger containing protein, which most functions as a transcriptional regulator. A transcriptome RNA-seq analysis revealed that a total of 978 genes were upregulated and 1100 downregulated in lsl-1(tm4769) mutants (q-value ≤ 0.01; ≥2 fold change ≥ 2) (Fig. 5a and Supplementary File 1). Tissue enrichment analysis (Angeles-Albores et al. 2016) showed that the vast majority of downregulated genes were associated with germline functions and the reproductive system. In addition, genes specific to male functions, to neurons, and to the epithelial system were also found to be deregulated (Fig. 5b and Supplementary File 2). Among the genes involved in germline functions, we identified genes involved in germ cell fate (nos-2 and zxd-1), in pairing and synapsis (pch-2, sun-1, syp-2, zim-1, and zim-3), in genome stability (chk-1, dph-2, and hsr-9), in P granules composition (gll-2, meg-4, mep-3, mep-6, oma-1, pgl-2, pie-1, and pos-1), or in the mitotic/meiotic transition (fbl-1 and glp-1). Overall, these data indicate that LSL-1 regulates genes involved in several germline processes.

The total number of DEGs in the lsl-1(ljm1) was lower (n = 496) than in the lsl-1(tm4769) allele (n = 2078) (Supplementary Fig. 7a and Supplementary File 1). However, 80% overlapped with the DEGs detected in lsl-1(tm4769) mutants (Supplementary Fig. 7b). Common DEGs appeared deregulated in the same direction (Supplementary File 3) and exhibited similar tissue enrichment patterns (Fig. 5b, Supplementary Fig. 7c and Supplementary File 2). These results are consistent with the difference in penetrance observed in the phenotypes associated with the 2 alleles.

LSL-1 binding sites are highly enriched on autosomes

To identify LSL-1 binding site to the genome, we analyzed ChIP-seq data available from the modERN consortium (Kudron et al. 2018). ChIP-seq was performed in worms carrying an LSL-1::TY1::EGFP::3xFLAG (wsls720) transgene, whose expression
matches the endogenously GFP-tagged LSL-1 (Fig. 1b, Supplementary Fig. 8).

A total of 3,896 significant peaks were identified as enriched by the ChIP-seq processing pipeline (SPP) (Kharchenko et al. 2008), IDR < 0.1%, and mapped corresponding to 3,078 genes in the *C. elegans* reference genome (version WS245) (Supplementary File 4). Peak distribution did not reveal marked intrachromosomal bias (Fig. 6a). However, LSL-1 is almost completely absent from the X-chromosome (n = 60) while highly enriched on chromosomes III and I (n = 901 and n = 869, respectively) (Fig. 6b). This distribution pattern resembles the chromosomal distribution of germline-specific genes (Reinke and Cutter 2009; Rechtsteiner et al. 2010; Kelly et al. 2014). Peaks were narrow, with an average size of 400bp (Fig. 6c), and 74% of them were preferentially associated

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**Fig. 3.** LSL-1 is required for the proper progression of homologous chromosome pairing. a,b) Representative confocal projection images of 1-day-old adult stage gonads of (a) wild type and (b) lsl-1(tm4769) animals, expressing SUN-1::mRuby and stained with DAPI. Dashed lines along the gonad delineate nuclei showing SUN-1::mRuby patches and extension of the transition zone. Vertical dashed lines mark the boundaries between the 7 equally long zones. (a’,b’) Each panel represents a magnification of the indicated zones. Arrows point to SUN-1::mRuby aggregates at the beginning of the transition zone (zones II/III) or to abnormal SUN-1::mRuby patches still detectable at the most proximal part of the germline in the lsl-1 mutant (zones IV and VI). c,d) Representative images of zone II/III and zone IV nuclei of the indicated genotypes: (c) immunostained with HIM-8 antibody; (d) hybridized with 5S rDNA FISH probe to monitor chromosome pairing and costained with DAPI. Arrows point to possible precocious paired chromosomes in late mitotic zone (zones II/III) or nuclei with unpaired signals (zone IV). c’,d’) Histograms showing the percentage of nuclei with paired (c’) HIM-8 and (d’) 5S rDNA signals, scored per zones of the indicated genotypes. P-value < 0.001 (**); P-value < 0.01 (**); P-value < 0.05 (*); P-value > 0.05 nonsignificant, by 2-tailed Student’s t-test with Welch’s correction. At least 3 gonads from independent experiments were scored for each genotype. Scale bars, 20 and 5 μm in whole gonad images and magnification panels, respectively.
with promoters (defined as 2 kbp upstream of a gene) (Fig. 6, d and e). Moreover, using MEME-ChIP tool (Machanick and Bailey 2011), motif enrichment analysis revealed a very significant enrichment for the motif TAC_GTA (Fig. 6f and Supplementary Fig. 9). This motif was described previously as highly enriched in upstream regions of germline genes (Narasimhan et al. 2015; Serizay et al. 2020). Altogether these observations reinforce the idea that LSL-1 could function as a transcriptional regulator of germline genes.

**LSL-1 is a transcriptional activator of germline genes**

To identify LSL-1 direct target genes, we cross-compared our RNA-seq data and the ChIP-seq analysis. 388 genes are bound by LSL-1, corresponding to 19% of the DEGs in isl-1(tm4769) mutant (n = 2,078) (Fig. 7a and Supplementary Table 2). Remarkably, most of these 388 genes were downregulated in isl-1 and mainly targeted at promoter regions (n = 296), which suggests that LSL-1 acts as a transcriptional activator (Fig. 7b, Supplementary File 5 and Supplementary Table 2). In addition, we performed a functional GO analysis using DAVID bioinformatics resources 6.8, NIAID/NIH tool with these potential LSL-1 direct targets (Huang et al. 2009). isl-1(tm4769) gene set was significantly enriched in GO terms (P-value ≤ 0.05), such as embryo development, P granules, 3’ UTR-mediated mRNA destabilization, germ cell development, or meiotic division (Fig. 7b); similar results were obtained from the isl-1(jmt1) dataset (Supplementary Fig. 10c).

Data presented herein indicate that LSL-1 could function as a direct transcriptional activator of germline genes involved in different processes, ranging from germ cell maintenance to pairing and synapsis processes, DNA stability, and P granules composition (Fig. 7c).
LSL-1 functions by antagonizing LET-418/Mi2 and HPL-2/HP1 heterochromatin proteins

Isl-1 was identified as a suppressor of developmental defects associated with mutations in let-418 (Erdélyi et al. 2017). To test whether isl-1 and let-418 were also genetically interacting in the germline, we generated isl-1(ljm1); let-418(n3536) double mutants. At the restrictive temperature of 22°C, embryo viability is partially restored in isl-1(ljm1); let-418(n3536) double mutants, indicating that LSL-1 might antagonize LET-418/Mi2 function in the germline (Table 3). LET-418/Mi2 is part of chromatin proteins known to repress transcription (Ahringer and Gasser 2018). To further investigate whether LSL-1 functions by antagonizing repressive chromatin formation, we investigated isl-1 interaction with heterochromatin factor coding genes hpl-1 and hpl-2, and histone H3K9 methyltransferase coding genes met-2 and set-25 (Ahringer and Gasser 2018). The H3K9 methyltransferases SET-25 and MET-2 are known to be responsible for most genomic H3K9 methylation (Towbin et al. 2012). We generated isl-1; met-2 set-25 triple mutants; however, loss of H3K9 methylation does not rescue isl-1 mutant phenotype, nor does absence of HPL-1 activity restore fertility. Interestingly, lack of HPL-2/HP1 activity partially restores fertility and confers viability to the embryos (Table 3). These overall genetic interactions suggest that LSL-1 could activate germline gene transcription by antagonizing repressive chromatin formation by LET-418/Mi2 and HPL-2/HP1.

Discussion

This study defines the C. elegans protein LSL-1 as a novel crucial transcriptional activator of germline genes. LSL-1 is present at all stages of germline development, from PGCs proliferation to differentiation through meiotic prophase progression. isl-1 loss-of-function mutants produce almost no functional gametes as a result of chromosome pairing defects, defective meiotic recombination, and genome instability. Transcriptomic analysis and ChiP-seq data show that LSL-1 binds germline gene promoters, acting mainly as a transcriptional activator. Furthermore, our genetic interaction analyses reveal that LSL-1 antagonizes the function of the heterochromatin proteins HPL-2/HP1 and LET-418/Mi2 to ensure production of viable progeny.

Isl-1 encodes a C2H2-type zinc-finger transcription factor closely homolog to LSY-2, that is present in all tissues and required for the specification of ASE neurons, proper vulva patterning, and repression of germline genes in somatic cells (Johnston and Hobert 2005; Lin et al. 2015). LSL-1, in contrast, appears to be expressed specifically in the germline. Both proteins are members of the SP1/KLF family of transcription factors, involved in growth and developmental processes (Supplementary Fig. 1) (Suske et al. 2005; Kim et al. 2017). These share a triple C2H2-type zinc-finger cluster and a less conserved double zinc finger at the C-terminus. The closest human homolog is ZFP57, which contains an additional KRAB domain interacting with the heterochromatin protein 1 (HP1) (Suske et al. 2005; Quenneville et al. 2011). Protein sequence and structure analysis suggest that LSL-1 is a DNA binding protein belonging to the SPI/KLF family of transcription factor, but not a true ortholog of the human protein ZFP57.

Worms bearing the 2 alleles, tm4769 and ljm1, exhibit very similar defects, but the penetrance of the phenotype is different despite repeated backcrosses to eliminate any additional mutations. A possible interpretation is that a cryptic translational initiation site (TIS) is used by the ribosome in isl-1(ljm1) mutants to produce a protein that still retains some functionality. Two AUG
codons downstream of the predicted TIS exhibit conserved nucleotides at position –3, –2, and +4 that could function as TIS consensus sequences (Hernández et al. 2019). Detailed comparison of the transcriptome of both mutant alleles (see below) is consistent with the interpretation that \textit{ljm1} represents a hypomorphic allele. Additional deregulated genes observed in \textit{lsl-1\textunderscore tm4769} with respect to \textit{lsl-1\textunderscore ljm1} are also enriched in germline genes, and most of them are bound by LSL-1 in their promoter region (Supplementary File 1).

LSL-1 is first detected in the P4 blastomere and could potentially represent the initial transcription factor that activates zygotic transcription of germline genes. The first zygotic germline transcripts, including LSL-1 targets, have been detected in Z2 and Z3 PGCs (Wang and Seydoux 2013; Lee et al. 2017). An interesting hypothesis would be that LSL-1 is part of the process that initiates the germline transcriptional program by interpreting the epigenetic memory of germline transcription. Germline genes are marked in the parental germline by the histone methyltransferase MES-4, which deposits H3K36 methyl marks while germline genes are transcribed (Tursun et al. 2011; Patel et al. 2012). These marks are transmitted and maintained in the embryos by MES-4 maternal contribution and therefore constitute an epigenetic memory of germline transcription. However, LSL-1 would function redundantly with other factors since its absence still leads to germ cell proliferation and a certain degree of germ cell differentiation.

LSL-1 appears to be one of the few transcriptional regulators functioning in the germline, LAG-1/CSL being another key transcription factor that controls germ cell fate in response to Notch signaling (Chen et al. 2020). To date, crucial studies have shown that proper patterning of germline gene expression requires the 3’UTR (Merritt et al. 2008). LSL-1 could function as a general activator of germline genes transcription followed by fine-tuning at the post-transcriptional level for proper patterning.

Monitoring of chromosome pairing by FISH or HIM-8, and SUN-1 localization showed that pairing dynamics are impaired in
lsl-1 mutants (Penkner et al. 2007; Woglar et al. 2013). Recombination also appears defective, as revealed by a high number of univalent in oocyte nuclei and an increased level of apoptotic germ cells, which depends on the recombination initiator protein SPO-11 (Dernburg et al. 1998). These observations are in agreement with our transcriptomic analysis. A large number of genes encoding essential meiotic proteins are downregulated in the absence of LSL-1, including the chromosomal axis component HTP-1, the synaptonemal complex proteins SYP-2 and SYP-4 (Martinez-Perez and Villeneuve 2005), or the pairing center binding ZIM proteins (ZIM-1, -2, -3, and HIM-8) (Phillips and Dernburg 2006). Genes involved in recombination, DNA repair, and genome stability, such as dsb-2, which plays a role in the control of meiotic DSB formation, or hsr-9 and chk-1, which are involved in the cell cycle checkpoints regulation in response to DNA damage, were also found to be downregulated in lsl-1 mutants (Rosu et al. 2013; Ryu et al. 2013; Zhang and Hunter 2014). These observations together with the germline expression pattern throughout development indicate a general role of LSL-1 in the transcription regulation of germline genes. However, all meiotic processes described above are significantly compromised but not completely abolished in the absence of LSL-1. A possible interpretation is that LSL-1 acts redundantly with other regulators and the stoichiometry of key factors involved in pairing, recombination and genome stability might be highly perturbed in the absence of LSL-1.

Defects associated with mutations in lsl-1 are mediated partially by the C. elegans HPL-2/HP1 heterochromatin protein and the chromatin remodeler LET-418/Mi2 (von Zelewsky et al. 2000; Bannister et al. 2001; Dialynas et al. 2008). LSL-1 was...
identified in a screen for suppressors of developmental defects associated with the absence of the chromatin repressor LET-418/Mi2. LSL-1 is required for ectopic localization of P granules in somatic cells of let-418 mutants (Erdelyi et al. 2017). Embryo viability is slightly restored in lsl-1(ljm1); let-418(n3536) double mutants (Table 3), and other lsl-1 associated defects, such as TZ extension and number of unrecombined chromosomes, are mildly suppressed in lsl-1; let-418 double mutants (Supplementary Fig. 3). The heterochromatin protein HPL-2/HP1 also contributes to lsl-1 phenotype. A global reorganization of chromatin could take place in the lsl-1 mutant germline, where HPL-2/HP1 plays a major role and LET-418/Mi2 a weaker one. Similarly to LET-418/Mi2, HPL-2/HP1 is known to act as a repressor of germline gene expression in the somatic cells (Coutham et al. 2006; Meister et al. 2011). This interaction of lsl-1 with hpl-2 and let-418 suggests that, in the absence of LSL-1, the germline chromatin adopts a conformation resembling the somatic one. However, no large set of somatic genes are upregulated in lsl-1 mutants (Supplementary File 2), indicating that downregulation of germline genes is not accompanied by somatic gene expression, at least not at the stage examined. Intriguingly, removing H3K9 methylase activities did not suppress lsl-1 sterility. Although HPL-2/HP1 binding onto the genome correlates with H3K9 methylation, hpl-2 mutants show more severe defects than mutants lacking H3K9 methylation which, furthermore, exhibit a relatively normal HPL-2/HP1 binding onto the genome (Garrigues et al. 2015). Altogether, this indicates that HPL-2/HP1 could perform functions independently of H3K9 methylation.

In conclusion, we characterize herein a new transcriptional regulator of genes that are involved in a wide range of germline processes. Since lsl-1 expression starts in the P4 blastomere, we propose that LSL-1 might initiate the germline transcriptional program and might be part of the process that interprets the epigenetic memory established in the parental germline by antagonizing HPL-2/HP1 and LET-418/Mi2 function, specifically in the germ cells. Identifying the mechanisms by which LSL-1 is recruited to the chromatin will contribute to understand how transcriptional programs are triggered in development and disease.

### Data availability

Sequencing files of the LSL-1 ChIP-seq experiment are accessible through the modERN website http://epic.gs.washington.edu/modERN/. Raw sequencing files of the RNA-seq experiment have been deposited in the ArrayExpress database at EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-11199. Strains and reagents used in this study are available upon request. Supplemental Materials consisting of supplemental methods, supplemental figures, supplemental tables, and supplemental files have been deposited at figshare portal https://doi.org/10.25386/genetics.18114410.

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### Table 3. Genetic interactions of lsl-1 with chromatin factor genes.

| Genotype | Mean brood sizea | Viability (%) | Incidence of males (%) | nb |
|----------|------------------|--------------|------------------------|----|
| Wild type | 301.79 ± 31.25 | 98.44 | 0.06 | 43 |
| lsl-1(tm4769) | 28.40 ± 23.95 | 0.12 | n/a | 59 |
| lsl-1(ljm1) | 58.26 ± 27.18 | 5.41 | 19.31 | 46 |
| hpl-1(tm1624) | 287.13 ± 31.73 | 94.43 | 0.05 | 16 |
| lsl-1(tm4769); hpl-1(tm1624) | 45.50 ± 29.07 | 0.00 | n/a | 12 |
| lsl-1(ljm1); hpl-1(tm1624) | 58.58 ± 42.29 | 2.42 | 41.18 | 12 |
| hpl-2(tm1489) | 248.85 ± 20.26 | 97.29 | 0.00 | 20 |
| lsl-1(tm4769); hpl-2(tm1489) | 128.82 ± 41.62 | 14.82 | 6.19 | 11 |
| lsl-1(ljm1); hpl-2(tm1489) | 153.64 ± 53.32 | 39.64 | 5.67 | 11 |
| met-2(n2456) set-25(n5021) | 276.62 ± 58.12 | 98.62 | 0.00 | 21 |
| lsl-1(tm4769); met-2(n2456) set-25(n5021) | 1.33 ± 3.47 | 0.00 | n/a | 12 |
| lsl-1(ljm1); met-2(n2456) set-25(n5021) | 53.73 ± 26.65 | 6.94 | 9.76 | 11 |
| Wild type22°C | 241.33 ± 46.45 | 98.62 | 0.07 | 6 |
| lsl-1(tm4769)22°C | 20.25 ± 19.71 | 0.00 | n/a | 12 |
| lsl-1(ljm1)22°C | 8.75 ± 20.42 | 10.48 | 9.09 | 12 |
| let-418(n3536)22°C | 147.33 ± 67.25 | 95.25 | 0.23 | 12 |
| lsl-1(tm4769); let-418(n3536)22°C | 0.58 ± 1.16 | 0.00 | n/a | 12 |
| lsl-1(ljm1); let-418(n3536)22°C | 17.31 ± 16.80 | 32.44 | 4.69 | 13 |

aData correspond to the mean ± SD of the total number of eggs laid per hermaphrodite parent. Statistical comparison between lsl-1 double or triple mutants and their corresponding lsl-1 genotype at the defined temperature, performed by 2-tailed Student’s t-test with Welch’s correction. **P-value ≤ 0.001, ***P-value ≤ 0.01, ***P-value > 0.05.
bTotal number of parental hermaphrodites per genotype.

Average number of hatched larvae in lsl-1(ljm1); let-418(n3536) (5.61 ± 0.75 hatched larvae) is significantly higher than in lsl-1(ljm1) mutant (0.91 ± 1.88 hatched larvae). *P-value ≤ 0.05.

n/a, not applicable.
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Conflicts of interest
None declared.

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