RESEARCH ARTICLE

HTZ-1/H2A.z and MYS-1/MYST HAT act redundantly to maintain cell fates in somatic gonadal cells through repression of ceh-22 in *C. elegans*

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ABSTRACT

The stable maintenance of acquired cell fates is important during development and for maintaining tissue homeostasis. Although histone modification is one of the major strategies used by cells to maintain their fates, the mechanisms by which histone variants maintain cell fates are not well understood. In *C. elegans*, the acetylated-histone-H4 (AcH4)-binding protein BET-1 acts downstream of the MYST family histone acetyltransferases MYS-1 and MYS-2 to establish and maintain cell fates in multiple cell lineages. Here we show that, in the bet-1 pathway, the histone H2A variant HTZ-1/H2A.z and MYS-1 are required for the maintenance of cell fates in a redundant manner. BET-1 controlled the subnuclear localization of HTZ-1. HTZ-1 and MYS-1 maintained the fates of the somatic gonadal cells (SGCs) through the repression of a target, ceh-22/Nix2.5, which induced the formation of the leader cells of the gonad. H3K27 demethylase, UTX-1, had an antagonistic effect relative to HTZ-1 in the regulation of ceh-22. Nuclear spot assay revealed that HTZ-1 localized to the ceh-22 locus in SGCs in an utb-1-dependent manner. We propose that HTZ-1 and MYS-1 repress ceh-22 when UTX-1 removes its silencing mark, H3K27 methylation on the ceh-22 locus, thereby maintaining the fates of SGCs.

KEY WORDS: H2A.z, UTX, BET, *C. elegans*

INTRODUCTION

The maintenance of cell fates is accompanied by the establishment and maintenance of stable chromatin structures that are involved in the continuous expression or repression of cell type-specific genes. Histone modifications have important roles in establishing and maintaining stable chromatin structures after DNA-binding transcription factors activate and/or repress cell type-specific genes. For example, Polycomb group genes and H3K27 methylation are required for the maintenance of cell fates through the repression of *Hox* genes (Ringrose and Paro, 2004). These findings indicate positive roles for H2A.z in transcriptional regulation. By contrast, H2A.z is also found in pericentric heterochromatin, suggesting that H2A.z functions to establish and/or maintain heterochromatin (Hardy et al., 2009). Thus, H2A.z has been linked to both gene activation and repression. In multicellular organisms, but not in yeast, H2A.z is essential for the genomic regions that flank transcriptional start sites (Raisner et al., 2005; Barski et al., 2007; Mavrich et al., 2008). In *Tetrahymena thermophila*, H2A.z is enriched in transcriptionally active chromatin (Allis et al., 1980). Yeast HTZ-1/H2A.z prevents the spreading of silenced chromatin in the subtelomeric region (Meneghini et al., 2005; Barski et al., 2007; Mavrich et al., 2008). In *Tetrahymena thermophila*, H2A.z is enriched in transcriptionally active chromatin (Allis et al., 1980). Yeast HTZ-1/H2A.z prevents the spreading of silenced chromatin in the subtelomeric region (Meneghini et al., 2003). These findings indicate positive roles for H2A.z in transcriptional regulation. By contrast, H2A.z is also found in pericentric heterochromatin, suggesting that H2A.z functions to establish and/or maintain heterochromatin (Hardy et al., 2009). Thus, H2A.z has been linked to both gene activation and repression. In multicellular organisms, but not in yeast, H2A.z is essential for viability (van Daal and Elgin, 1992; Faast et al., 2001; Whittle et al., 2008). Interestingly, genome-wide studies in mouse and *C. elegans* show that the targets of H2A.z are enriched in genes connected to developmental processes, including genes that encode transcription factors (Creyghton et al., 2008; Whittle et al., 2008). These facts...
suggest that one of the important roles of H2A.z is the transcriptional control of developmentally regulated genes.

In *C. elegans*, the depletion of HTZ-1 causes embryonic lethality and sterility (Whittle et al., 2008). HTZ-1 facilitates the transcriptional activation of the PHA-4 targets during foregut development (Updike and Mango, 2006). In addition, HTZ-1 restricts the dosage compensation complex that downregulates gene expression by ~50% along the X chromosome, suggesting positive transcriptional roles for HTZ-1 (Petty et al., 2009). A negative role for HTZ-1 in transcriptional regulation has not yet been reported.

Here, we show that *C. elegans* HTZ-1 is required for the maintenance of cell fate in the BET-1 pathway, indicating that AcH4 and HTZ-1 cooperate to maintain stable cell fates. Because of the resulting defect in the maintenance of cell fates, *htz-1 mys-1* double mutants produced extra DTCs, which are the leader cells for gonad development. In the somatic gonadal cells (SGCs), ectopic expression of *ceh-22/Nkx2.5* induced extra DTCs. Through the repression of *ceh-22*, BET-1, MYS-1 and HTZ-1 prevented the production of extra DTCs from the non-DTC lineage of SGCs. From RNAi screening for suppressors of *bet-1* mutants, we identified *utx-1*, which encodes H3K27 demethylase. We found that UTX-1 and HTZ-1 have antagonistic effects to the expression of *ceh-22*. Therefore, our results strongly suggest that one of the important developmental roles for H2A.z is the maintenance of cell fates through the repression of transcription factors.

RESULTS

htz-1 and mys-1 repress the DTC fate

The yeast homolog of BET-1 is BDF1, a component of the SWR1 complex, which deposits HTZ1/H2A.z on the genome, and that of MYS-1 is Esal, a component of the NuA4 HAT complex (Fig. 1A) (Doyon and Côté, 2004; Kobor et al., 2004). In humans, these two complexes appear to form a larger complex, the hNuA4 HAT complex, which contains the counterparts of the SWR1 complex and the NuA4 HAT complex (Doyon and Côté, 2004). To examine whether the *C. elegans* counterparts of the SWR1 complex components and the NuA4 HAT complex repress the DTC fate as did BET-1, we performed RNAi experiments for *trr-1*, *ing-3*, *ekl-4*, *epc-1*, *ssl-1*, *ruvb-1* and *ruvb-2* using animals that express the DTC marker *lag-2::gfp*. In the wild-type gonad, each anterior gonad arm has a single DTC (Fig. 1B). Feeding RNAi experiments for *ekl-4* and *ssl-1* caused partial embryonic lethality, as did *bet-1* RNAi, and the escapers showed the extra-DTC phenotype, as did *bet-1* mutants (Fig. 1C,D; Fig. 2A). The extra DTCs, which expressed *lag-2::gfp*, had a cup-like shape, as was observed for normal DTCs (data not shown). In addition, the extra DTCs were positioned at the tip of the extra gonad arms (data not shown), suggesting that they acted as leader cells for elongation of the gonad arms as the normal DTCs do (Kimble and Hirsh, 1979). *ssl-1* and *ekl-4* encode homologs of SWR1 and SWC4, respectively, which are components of the SWR1 chromatin remodeling complex in yeast (Ceol and Horvitz, 2004; Rocheleau et al., 2008). SWR1 mediates incorporation of HTZ1 into nucleosomes (Kobor et al., 2004). The mammalian EKL-4 homolog DMAP1 (DNA methyltransferase-associated protein) forms a complex with the chromatin remodeling factor SRCap (Fig. 1A) (Doyon and Côté, 2004). Although DMAP1 also forms a complex with the DNA methyltransferase DNMT1 (Rountree et al., 2000), there is no DNMT1 homolog in *C. elegans* (data not shown). Therefore, *ssl-1* and *ekl-4* are likely to regulate the deposition of HTZ-1/H2A.z on the genome to repress the DTC fate.

We performed *htz-1* feeding RNAi but did not observe the extra-DTC phenotype (Fig. 2A). Then we examined the effect of *htz-1* depletion in a sensitized *mys-1* mutant background. MYS-1 and MYS-2 have redundant functions in the repression of the DTC fate (Shibata et al., 2010). We observed *htz-1 mys-1* double mutants and found that the *htz-1* mutation significantly enhanced the extra-DTC phenotype of *mys-1* mutants (Fig. 1E-H; Fig. 2B), indicating that HTZ-1 represses the DTC fate. Because we used homozygous progeny from heterozygous hermaphrodites, the progeny may have maternal *htz-1* and *mys-1* gene products. To examine whether these
maternal products contribute to the suppression of the extra-DTC phenotype in *htz-1* mutants, we performed *htz-1* RNAi in *htz-1* mutants. *mys-1* RNAi, but not *htz-1* RNAi, led to the extra-DTC phenotype in the *htz-1* background (Fig. 2C). *mys-1* RNAi also had no effect in *mys-1* mutants. These results indicated that *mys-1* and *htz-1* act redundantly in the repression of DTC fate.

**HTZ-1 maintains the fate of SGCs in the bet-1 pathway**

We previously reported that the extra-DTC phenotype of *bet-1* mutants is more severe in adults than in L2 animals because BET-1 maintains cell fates (Shibata et al., 2010). *ekl-4* (RNAi) and *htz-1* *mys-1* double mutants also showed a more severe phenotype in L4 animals than in L2 animals (Fig. 2D). These results suggest that *HTZ-1* and *EKL-4* maintain the fate of SGCs.

Next, we examined whether *htz-1* and *ekl-4* function in the *bet-1* pathway. RNAi of either *htz-1* or *ekl-4* enhanced the extra-DTC phenotype of the weak *bet-1*(*gk425*) allele. By contrast, when the activity of *bet-1* was completely lost in the null allele, *os46* RNAi and *ekl-4* RNAi did not enhance the extra-DTC phenotype (Fig. 2E). These results indicated that *htz-1* and *ekl-4* maintain the fate of SGCs in the *bet-1* pathway.

**BET-1 controls subnuclear localization of HTZ-1**

We observed the expression of YFP::HTZ-1 and found that YFP::HTZ-1 were observed in the nuclei of SGCs (Fig. 1J). If BET-1 regulates HTZ-1 deposition on the genome, the *bet-1* mutation may alter the subnuclear localization of HTZ-1. Indeed, the *bet-1* mutation obscured the puncta of YFP::HTZ-1 in five out of eight animals, although YFP::HTZ-1 still localized to the nuclei of SGCs in all eight animals (Fig. 1K). We also observed the localization of the histone H3 variant HIS-72/H3.3, which was normal even in the *bet-1* mutant background (Fig. 1L,M), suggesting that the erratic localization of HTZ-1 in *bet-1* mutants is not because of the defective assembly of the chromatin, but rather is probably caused by the defective deposition of HTZ-1 into the chromatin.

**BET-1 and HTZ-1 maintain non-DTC fates through the repression of ceh-22**

Because extra DTCs in *bet-1* mutants (Shibata et al., 2010) and *htz-1* *mys-1* mutants (data not shown) have characteristics typical of normal DTCs, including their cell shape, competence to migrate and ability to express *lag-2::gfp*, we expected that BET-1 and HTZ-1 regulate the transcription factor that specifies the fate of DTCs.

We thus examined the expression of the NK-2 family homeodomain factor CEH-22. *ceh-22* produces two kinds of transcripts, *ceh-22a* and *ceh-22b*, which are required for pharyngeal and gonadal development, respectively (Okkema et al., 1997; Lam et al., 2006). *ceh-22b* is required for the asymmetry between daughter cells of the Z1/Z4 cells and is expressed in distal daughters and granddaughters of the Z1 and Z4 cells until L3 (Lam et al., 2006). We observed ceh-
22b::venus expression by confocal microscopy and found that ceh-22b::venus was expressed in DTCs until the middle of L4 (Fig. 3A). Weaker ceh-22b::venus expression was also observed in the proximal part of the gonad (Fig. 3A). In the bet-1 L4 animals, strong ceh-22b::venus expression was observed in all DTCs, including extra DTCs (Fig. 3B). We also found that, in addition to DTCs, some SGCs expressed ceh-22b::venus at the same levels as did the DTCs (Fig. 3C). In wild-type animals, only DTCs expressed ceh-22b::venus strongly (Fig. 3A,D). These results indicate that BET-1 represses the expression of ceh-22b in the non-DTC lineage of SGCs.

To determine whether HTZ-1 represses the expression of ceh-22b in the non-DTC lineage of SGCs, we observed ceh-22b::venus in htz-1(RNAi) animals and rare ectopic expression below statistical significance in mys-1 mutants. htz-1 RNAi in the mys-1 background significantly enhanced the phenotype of ectopic ceh-22b::venus expression (Fig. 3E-G). Extra DTCs in htz-1 mys-1 disruptants also expressed ceh-22b::venus (data not shown). These results suggest that HTZ-1 represses the expression of ceh-22b in the non-DTC lineage of SGCs.

The progenitor cells of DTCs, Z1/Z4, undergo two rounds of asymmetric cell division at the L1 stage to produce the Z1.aa/Z4.pp cells, which differentiate into DTCs. Ectopic expression of ceh-22 in the L1 stage causes the loss of asymmetry between daughter cells of the Z1/Z4 cells, resulting in the production of extra DTCs (Lam et al., 2006). We examined the effect of ectopic expression of ceh-22b in L2 or L3 animals after completion of these asymmetric cell divisions. ceh-22b was driven by a heat-shock promoter (hs::ceh-22b). In the case of no heat shock, animals did not show the extra-DTC phenotype (Fig. 4A,C). Heat-shock treatment of L2 or L3 animals caused the extra-DTC phenotype (Fig. 4B,C; data not shown). These extra DTCs attached to the gonad arms, indicating that they were formed from the non-DTC lineage of SGCs (Shibata et al., 2010). CEH-22 that lacked a homeodomain could not induce extra DTCs (Fig. 4C), suggesting that DNA-binding activity of CEH-22 is important for the induction of DTCs. These results indicate that ectopic expression of ceh-22b during the middle and late larval stages can induce extra DTCs.

To determine whether the extra-DTC phenotype is dependent on ceh-22, we performed ceh-22 RNAi in bet-1 mutants. Although ceh-22 is required for the differentiation of the Z1.a and Z4.p cells, which produce DTCs in wild-type L1 animals, ceh-22 and control RNAi in bet-1 background showed a loss of DTCs phenotype in only 13.2% and 8.8% animals, respectively, at the L2 larvae (data not shown). Thus, we could analyze the effect of ceh-22 RNAi on the extra-DTC phenotype in the later stages. We scored the percentage of gonad arms that had extra DTCs and found that ceh-22 RNAi suppressed the extra-DTC phenotype in bet-1 mutants (Fig. 4D). These results indicated that BET-1 and HTZ-1 maintain the non-DTC fates of SGCs through repression of ceh-22.

**Colocalization of HTZ-1 with the ceh-22 locus in SGCs**

To examine whether HTZ-1 associates with ceh-22 in SGCs, we analyzed chromatin immunoprecipitation sequencing (ChIP-seq) data for HTZ-1 in L3 larvae from modENCODE (Gerstein et al., 2010). The modENCODE data indicated that there was no significant peak corresponding to the transcription start site (TSS) of ceh-22b (data not shown). However, because ectopic ceh-22 expression and the production of extra DTCs occurred only in the SGCs, which comprise ~10% of all cells in L3 larvae, it is possible that the number of cells in which HTZ-1 binds the ceh-22 locus is too small to generate significant signals during ChIP-seq analysis. In modENCODE, there were two control and three sample data sets. Indeed, weak positive signals around the TSS of ceh-22b were detected when we compared the average data for three samples with...
that for two controls (Fig. 5A). The positive signals were also observed when each of the three samples was individually analyzed (supplementary material Fig. S1), suggesting that these signals are significant.

Next, to examine the relationship between HTZ-1 localization and the ceh-22 locus in non-DTC lineage of SGCs, we analyzed colocalization of YFP::HTZ-1 and the ceh-22 locus with the nuclear spot assay (Gonzalez-Serricchio and Sternberg, 2006). We used a strain that expresses YFP::HTZ-1 and LacCl::CFP, which binds the lac operator (lacO) (Updike and Mango, 2006). Into this strain, the extrachromosomal array that contains multiple copies of lacO DNA and ceh-22 (ceh-22 array) was introduced. This ceh-22 array also contained ttx-3::gfp and unc-76, which are expressed in neural cells but not in SGCs, as transgenic markers. An extrachromosomal array that contained lacO, ttx-3::gfp and unc-76 but not ceh-22 (control array) was used for control experiments. Both the ceh-22 array and the control array colocalized with HTZ-1 (Fig. 5B), but a statistical analysis indicated that colocalization was more frequently observed in cells that had the ceh-22 array (Fig. 5C). This result indicates that HTZ-1 colocalizes with the ceh-22 locus in SGCs, where HTZ-1 maintains cell fates through the repression of ceh-22.

**utx-1 RNAi suppresses multiple phenotypes of bet-1 mutants**

During RNAi screening for bet-1 suppressors that used the chromatin subset of the Ahringer library (Kamath et al., 2003), we found two suppressor genes, his-24/H1.1 and utx-1, which encodes histone H3K27 demethylase (Fisher et al., 2010) (Fig. 6A,B,E). If UTX-1 and/or HIS-24 regulate the same target genes as BET-1, the ectopic expression of ceh-22b in the bet-1 background should be suppressed by RNAi for either utx-1 or his-24. The ectopic expression of ceh-22b::venus was in observed only 21% of bet-1(os46) mutants treated with utx-1 RNAi compared with its expression in 45% of bet-1(os46) mutants treated with control RNAi. By contrast, his-24 RNAi had no effect on ceh-22b expression in bet-1 mutants. This result indicated that BET-1 and UTX-1, but not HIS-24, have an antagonistic effect on the expression of ceh-22 (Fig. 6C,D,F).

We also examined whether utx-1 RNAi can suppress other phenotypes of bet-1 mutant. In bet-1 mutants, neuroblasts that produce phasmid socket cells fail to maintain that cell fate and transform into hypodermal cells (Shibata et al., 2010), producing the phasmid socket absent (Psa) phenotype. The Psa phenotype of bet-1 mutants was suppressed by utx-1 RNAi (Fig. 6G), indicating that utx-1 RNAi can suppress multiple phenotypes of bet-1 mutants. Thus, bet-1 and utx-1 have an antagonistic role in multiple cell lineages.

**UTX-1 has an antagonistic effect with respect to HTZ-1**

We also examined the effect of utx-1 RNAi in hts-21 mys-1 double mutants. First, the extra-DTC phenotype of hts-21 mys-1 mutants was suppressed by utx-1 RNAi (Fig. 6E; supplementary material Fig. S2A,B). Second, the ectopic expression of ceh-22b::venus in the SGCs of hts-21 mys-1 mutants was also suppressed by utx-1 RNAi (Fig. 6F; supplementary material Fig. S2C,D). These results, together with those in the bet-1 background, suggest that hts-1, mys-1, bet-1 and utx-1 regulate the expression of ceh-22b, such that the effect of UTX-1 is antagonistic to that of HTZ-1, MY5-1 and BET-1.

The genetic interaction with UTX-1 suggests that HTZ-1 functions in the region where H3K27 is methylated to a lesser extent. To test this hypothesis, we compared the genomic localization of HTZ-1 and H3K27me3 in L3 animals (supplementary material Fig. S3A). As expected, stronger signals for HTZ-1 were observed in the region where H3K27 is less methylated, and vice versa. A scatter plot revealed that HTZ-1 and H3K27me3 showed a negative correlation (supplementary material Fig. S3B). HTZ-1 and H3K27me3 are negatively correlated for all chromosomes in L3 and in embryonic stages (supplementary material Fig. S3C). Although the other repressive mark, H3K9me3, also showed a negative correlation with HTZ-1 in L3 and embryonic stages, this correlation was weaker than that of HTZ-1 and H3K27me3.

**UTX-1 enhances the colocalization between HTZ-1 and ceh-22 locus**

As there is a negative correlation between HTZ-1 and H3K27me3, we wondered whether depletion of UTX-1 affects the colocalization between HTZ-1 and ceh-22 locus. In the case of control arrays, utx-1 RNAi had no effect on the colocalization between HTZ-1 and ceh-22 locus. By contrast, the colocalization was disrupted by utx-1.
H3K27me3, and the loss of colocalization between HTZ-1 and utx-1, the genome-wide analysis of the localization of HTZ-1 and locus by the depletion of 22 cell.

fundamental mechanism that maintains cell fates in many types of cell fates. Therefore, we suggest that HTZ-1 is a part of the morphogenesis stage can result from a defect in the maintenance of not the only possible explanation, embryonic lethality in the morphogenesis stage after most of the embryonic cells have been htz-1 phenotype was not as high (Y. S. and K. N., unpublished). In addition, mutants, although the penetrance of the Psa bet-1 1 mys-1 mutants showed Psa and extra-DTC phenotypes that are also observed in bet-1 mutants, although the penetrance of the Psa phenotype was not as high (Y. S. and K. N., unpublished). In addition, htz-1 RNAi and bet-1 RNAi cause embryonic lethality in the morphogenesis stage after most of the embryonic cells have been specified (Whittle et al., 2008; Shibata et al., 2010). Although it is not the only possible explanation, embryonic lethality in the morphogenesis stage can result from a defect in the maintenance of cell fates. Therefore, we suggest that HTZ-1 is a part of the fundamental mechanism that maintains cell fates in many types of cell.

As HTZ-1 and MYS-1 function redundantly in the bet-1 pathway, each of them can individually maintain cell fates. We previously showed that MYS-1 and MYS-2 are required for the localization of BET-1 (Shibata et al., 2010). Here, we show that BET-1 is required for the subnuclear localization of HTZ-1; therefore, it is likely that MYS-1 and MYS-2 are important for the deposition of HTZ-1. In the mys-1 mutants that show normal subnuclear localization of BET-1 (Shibata et al., 2010), the BET-1-containing complex probably recruits HTZ-1 to its targets in a MYS-2-dependent manner. In the htz-1 mutants, the histone acetyltransferase activity of MYS-1 and MYS-2 is likely to be enough to maintain cell fates. Acetylated histone may mediate the repression that is independent of the deposition of HTZ-1. Although histone acetylation is frequently correlated with transcriptional activation (Shahbazian and Grunstein, 2007), our results suggest that it can also act in transcriptional repression.

In mammals, although the relationship between H2A.z and the maintenance of cell fates is not known, H2A.z is implicated in mammary epithelial cells in response to the differentiation signal, indicating that H2A.z is implicated in tumorigenesis (Conerly et al., 2010) as a result of the abnormal transformation of cell fates. The downregulation of Tip60, which is involved in tumorigenesis (Gorrini et al., 2007), suggesting that mammalian H2A.z may also maintain cell fates. However, because the disruption of H2A.z causes peri-implantation lethality (Faast et al., 2001), investigation of the developmental roles of H2A.z remains elusive. By contrast, disruption of H2A.z in embryonic stem cells (ESCs) causes a defect in response to the differentiation signal, indicating that H2A.z is involved in the pluriptotency of ESCs (Creyghton et al., 2008). Therefore, H2A.z could be involved in the acquisition of differentiated cell fates rather than the maintenance of cell fates in ES cells. H2A.z thus appears to have distinct roles in ESCs and differentiated cells in mammals.

The regulation of developmental genes by H2A.z
The involvement of HTZ-1 in the maintenance of cell fates suggests that HTZ-1 stabilizes the expression pattern of developmental genes,
including the genes that encode DNA-binding transcription factors. Consistent with this, HTZ-1 localization is frequently observed on the promoters of developmental genes (Whittle et al., 2008). In mouse ESCs, H2A.Z also localizes to the promoters of developmental genes (Creyghton et al., 2008). Because it is present in a wide range of organisms, the regulation of developmental genes that encode DNA-binding transcription factors may be an evolutionarily conserved role for H2A.Z, which leads to the maintenance of cell fates.

**HTZ-1, MYS-1 and UTX-1 cooperatively regulate gene expression**

Genome-wide analysis in *Saccharomyces cerevisiae* indicates that H2A.Z marks genes in the euchromatin (Raisner et al., 2005). Even in the euchromatin, there are active and inactive genes. H2A.Z has been implicated in both transcriptional activation and repression (Allis et al., 1980; Meneghini et al., 2003; Hardy et al., 2009). However, in contrast to its role in transcriptional activation, the roles of H2A.Z in transcriptional repression remain to be explored. Our results suggest that the repressive effect of H2A.Z in euchromatin is required for the maintenance of cell fates. Interestingly, it is reported that H2A.Z causes stabilization and destabilization of nucleosomes (Li et al., 1993; Abbott et al., 2001; Jin and Felsenfeld, 2007; Ishibashi et al., 2009). Thus, it might be possible that H2A.Z controls transcriptional activation and repression via affecting the stability of nucleosomes.

In this study, we showed a negative correlation between HTZ-1 and H3K27me3 in terms of genome-wide localization, suggesting that HTZ-1 functions in the euchromatin, where H3K27 is methylated to a lesser extent. The obstruction of colocalization between HTZ-1::YFP and the ceh-22 locus in utx-1 RNAi indicates that the subnuclear localization of HTZ-1 is regulated by histone H3K27 demethylase, UTX-1.

Based on the present study, we propose the following model for the regulation of transcription of the *ceh-22* gene in SGCs (Fig. 7). In addition to being the direct target of HTZ-1, the *ceh-22* locus may be a target of UTX-1. HTZ-1, AcH4 and H3K27me3 repress *ceh-22* expression in the non-DTC lineage of SGCs. The H3K27
demethylase UTX-1 may belong to a complex that is distinct from the SSL-1 complex based on studies of its mammalian counterpart (Issaeva et al., 2007; Fisher et al., 2010). Because UTX-1 regulates HTZ-1 localization, H3K27me3 prevents the deposition of HTZ-1. In the wild-type non-DTC lineage of SGCs, UTX-1 and MYST HATs catalyze the demethylation of H3K27me3 and the acetylation of histone H4, respectively, at the promoter region of the ceh-22 locus. The SSL-1 complex binds AcH4 through BET-1 and incorporates HTZ-1 into the promoter region of ceh-22, thereby maintaining the repression of ceh-22. Acetylation of H4 in the neighboring nucleosomes by MYS-1 in the BET-1/MYS-1/SSL-1 complex may contribute to extending the domain that incorporates HTZ-1. AcH4 may also have an HTZ-1-independent function that represses ceh-22. In bet-1 and htz-1 mys-1 mutants, the promoter region of ceh-22 has neither HTZ-1, AcH4 nor H3K27me3. Therefore, the absence of these repressors causes ectopic expression of ceh-22 in the non-DTC lineage of SGCs. When utx-1 is disrupted in bet-1 and htz-1 mys-1 mutants, the ceh-22 locus does not contain HTZ-1 and AcH4, but it may be silenced by the H3K27me-dependent mechanism.

How does H2A.z control developmental genes?

Interestingly, H2A.z enrichment is not correlated with transcriptional rates across multiple organisms (Raisner et al., 2005; Whittle et al., 2008). Genome-wide analyses have revealed that the distribution of H2A.z on the promoter region is, however, correlated with that of RNA polymerase II (Pol II), although the regions are not always transcriptionally active. Thus, for some HTZ-1 targets, Pol II may be poised near the transcription start site in a ready-to-go state (Whittle et al., 2008). Transcriptional reactivation is likely to be easier when a locus is repressed by HTZ-1 (through pausing of Pol II) compared with when it is repressed (silenced) by H3K27me3. Therefore, HTZ-1 presumably maintains cell fates through the repression of DNA-binding transcription factors in the cells that are ready to respond to developmental signals. Thus, in multiple cell lineages of mutant animals, the ectopic expression of determinants without developmental signals appears to cause cell fate transformation. Our results suggest that HTZ-1 and H3K27me3 play important roles in the transcriptional regulation of determinants. However, the mechanism of transcriptional regulation is still unclear. One possible explanation is that because the Z1/Z4 cells begin to express CEH-22B::VENUS at low levels shortly before their division (Lam et al., 2006), UTX-1 can remove H3K27 methylation to allow ceh-22b expression to occur. HTZ-1, and possibly AcH4, could repress ceh-22 expression by pausing RNA Pol II at the promoter region in the Z1/Z4 descendants that do not produce DTCs, whereas in the descendants that produce DTCs, ceh-22 expression is allowed by Pol II-dependent transcription. In this model, we hypothesized that UTX-1 acts before BET-1, AcH4 and HTZ-1. It is, however, also possible that UTX-1 acts simultaneously with BET-1, AcH4 and HTZ-1 to maintain the demethylated state of H3K27 on the ceh-22 locus. As bet-1 mutants do not produce extra DTCs in the adult stage, an HTZ-1-independent mechanism, for example H3K27me3, may repress ceh-22 to an extent to confer resistance to differentiation signals.

Recent work from genome-wide analyses has revealed the relationship between H2A.z and other chromatin marks (Zilberman et al., 2008; Jin et al., 2009). The role of H2A.z in development had, however, been largely unknown. Our findings demonstrate that H2A.z is involved in the maintenance of cell fate during development. Because BET-1 is required in multiple cell lineages, HTZ-1 appears to function in a fundamental mechanism that maintains cell fates. Conservation of H2A.z and related molecules in multiple organisms suggests that the molecular machinery for H2A.z-dependent cell fate maintenance could also operate in other organisms.

MATERIALS AND METHODS

Strains, culture and RNAi

N2 Bristol was used as the wild-type strain (Brenner, 1974). The animals were cultured at 22.5°C or 20°C. The bet-1 (LG1), mys-1 (LGIV) and htz-1 (LGIV) mutants are sterile and were thus maintained as heterozygotes over the rt2[qls48] (LG1 and HI) or nt1[qls51] (LGIV and V) balancer. The phenotypes of homozygotes generated from the heterozygous hermaphrodites were analyzed. bet-1(os46) is a nonsense mutation (Shibata et al., 2010). bet-1(gk425), bet-1(tm2469) and mys-1(lw4075) are deletions (Ceol and Horvitz, 2004; Whittle et al., 2008; Petty et al., 2009; Shibata et al., 2010), gk425 is a weak allele, because its phenotype is weaker than that of the null allele, os46 (Shibata et al., 2010). Because tm2469 removes one-third of the htz-1 coding region and n4075 removes the part of the mys-1 coding region that corresponds to the chromo-domain and the HAT domain, they are both likely to be null or strong loss-of-function alleles. The following transgenes were used: qls90 [ceh-22::venus] (Lam et al., 2006), qls56 [lag-2::gfp] (Kostić et al., 2003), pEx214 [yfp::htz-1, lacI::cfp] (Updike and Mango, 2006) and qEx536 [hs::ceh-22b] (Lam et al., 2006).

The bet-1, ceh-22 and utx-1 RNAi constructs were described previously (Kamath et al., 2003; Shibata et al., 2010). For the trr-1, ing-3, ekl-4, epc-1, ssl-1, ruvb-1, ruvb-2 and htz-1 RNAi constructs, the restriction fragments from yk1328h02 (BanHi-Psil), yk1479b12 (BanHi-Psil), yk1360h08 (BanHi-Xbal), yk1159b01 (BanHi-Psil), yk1258e11 (EcorI), yk1650e12 (BanHi-Psil), yk1412b04 (BanHi-Xbal) and yk733h08 (BanHi-Psil), respectively, were inserted into the L4440 vector plasmid (Timmons and Fire, 1998). Feeding RNAi experiments were performed as described (Kamath et al., 2001). RNAi screening was performed using the C. elegans RNAi chromatin library (Source BioScience, Nottingham, UK). The hs::ceh-22b homedomain PCR product consisted of an hsp16-2 promoter that was amplified from pdP49.78 and a ceh-22b genomic fragment that lacks the region corresponding to amino acid residues 58-120.

Fig. 7. A working model of the mechanism by which HTZ-1 maintains cell fates. Schematics of the regulatory states of the ceh-22 locus in the non-DTC lineage of SGCs of wild-type (top), bet-1, htz-1 mys-1 (middle), bet-1 utx-1 and htz-1 mys-1 utx-1 (bottom) animals.
Microscopy and statistical analysis

Expression of ceh-22::venus was detected by confocal microscopy (LSM510 and Pascal, Zena, Germany) in larval stage (L4 animals, because ceh-22::venus in DTCs was not detectable in adults (data not shown). Expression of lag-2::gfp in DTCs was detected by epifluorescence microscopy (Axioskop2plus and Axioplan2; Zeiss, Jena, Germany). The number of DTCs was scored at L3 and L4 in hzt-1(tm2469) mys-1(n4075) double mutants, slr-1(RNAi) animals, mys-1(n4075) mutants with mys-1 RNAi and hzt-1(tm2469) mutants with hzt-1 RNAi, because these animals did not grow to the adult stage. The subnuclear localization of yfp::hzt-1 was detected by spinning-disc confocal microscopy (CSU10 and CSUX1; Yokogawa, Tokyo, Japan) in L4 animals. The Psa phenotype was analyzed as described (Sawa et al., 2000).

Heat-shock treatment

To obtain synchronized animals, newly hatched larvae were collected for 1 hour. Then, animals were cultured for 16 hours to L2 at 22.5°C. L2 animals were transferred to plates that had been pre-heated to 33°C and were maintained at 33°C for 30 minutes before being transferred to 22.5°C. The number of DTCs was then scored at the adult stage.

Nuclear spot assay

The nuclear spot assay was performed as described (Updike and Mango, 2006), with the following modifications. Images were acquired with a laser scanning confocal microscope (LSM510 and Pascal, Zeiss, Jena, Germany) at 1-2006, with the following modifications. Images were acquired with a laser scanning confocal microscope (LSM510 and Pascal, Zeiss, Jena, Germany) in larval stage (L)4 animals, respectively; Shigeo Hayashi, members of the Nishiwaki and Sawa laboratories for technical assistance. (which contains 32 copies of the ceh-22::venus

Expression of H3K27me3 (modENCODE_2400 and modENCODE_3171) and H3K4me3 (modENCODE_3290 and modENCODE_3291) was detected using custom-written, semi-automated scripts in Matlab (MathWorks, Natick, MA, USA).

Analysis of HTZ-1 and trimethylated H3K27 (H3K27me3) and H3K9 (H3K9me3) data from modENCODE

We downloaded data for HTZ-1 (modENCODE_43 and GSE28762), H3K27me3 (modENCODE_2400 and modENCODE_3171) and H3K9me3 (modENCODE_188 and modENCODE_691) from GEO (http://www.ncbi.nlm.nih.gov/geo/) and modENCODE (http://www.modencode.org/), respectively. Correlation was calculated by using custom-written, semi-automated scripts in Matlab (MathWorks, Natick, MA, USA).

Competing interests

The authors declare no competing financial interests.

Author contributions

Y.S., H.S. and K.N. designed the research and analyzed data. Y.S. and K.N. wrote the paper. Y.S. performed experiments.

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Supplementary material

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References


