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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*/Nkx2.5, 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 *utx-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; Grossniklaus and Paro, 2007). In addition to histone methylation, histone acetylation is also required for the maintenance of cell fates in multiple cell lineages of *Caenorhabditis elegans* (Shibata et al., 2010). By contrast, the role of histone variants in the maintenance of cell fates is unknown.

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In *C. elegans*, the AcH4-binding protein BET-1 maintains cell fates in multiple cell lineages including neural lineages and somatic gonad lineages, suggesting that it represents a fundamental mechanism for maintaining cell fates (Shibata et al., 2010). Subnuclear localization of BET-1 is regulated by two members of the MYST family of histone acetyltransferases (MYST HATs), MYS-1 and MYS-2, which are also required for the maintenance of cell fates (Shibata et al., 2010). BET-1 regulates the expression pattern of cell fate determinants to prevent the excessive production of certain types of cells. For example, BET-1 prevents the ectopic production of mechanosensory neurons through the repression of *mec-3*, which encodes a LIM homeodomain protein (Shibata et al., 2010).

BET-1 also prevents the production of extra distal tip cells (DTCs) (Shibata et al., 2010), although the transcription factor that induces extra DTCs in *bet-1* mutants is unknown. DTCs are produced from the most distal granddaughter cells of the Z1/Z4 cells after two rounds of asymmetric cell division (Kimble and Hirsh, 1979). The first asymmetric cell division is regulated by the Wnt/β-catenin asymmetry pathway, which induces the asymmetric expression of *ceh-22* in the daughter cells of the Z1/Z4 cells (Lam et al., 2006; Mizumoto and Sawa, 2007). However, the mechanisms that regulate the second round of asymmetric cell division, which produce the DTCs, are poorly understood, and the factors that induce DTC fate are currently unknown. In the progeny of granddaughter cells that do not produce DTCs, BET-1 maintains their fates to prevent the production of extra DTCs (Shibata et al., 2010).

BET-1 belongs to the BET family of proteins, which is evolutionarily conserved from yeast to humans (Florence and Faller, 2001). The yeast BET protein, BDF1, is a component of the SWR1 complex, which is required for the incorporation of yeast H2A.z, HTZ1 (Kobor et al., 2004). Throughout a range of organisms, including yeast, mammals and *Drosophila*, H2A.z associates with 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., 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

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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 Esa1, 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 and posterior 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.

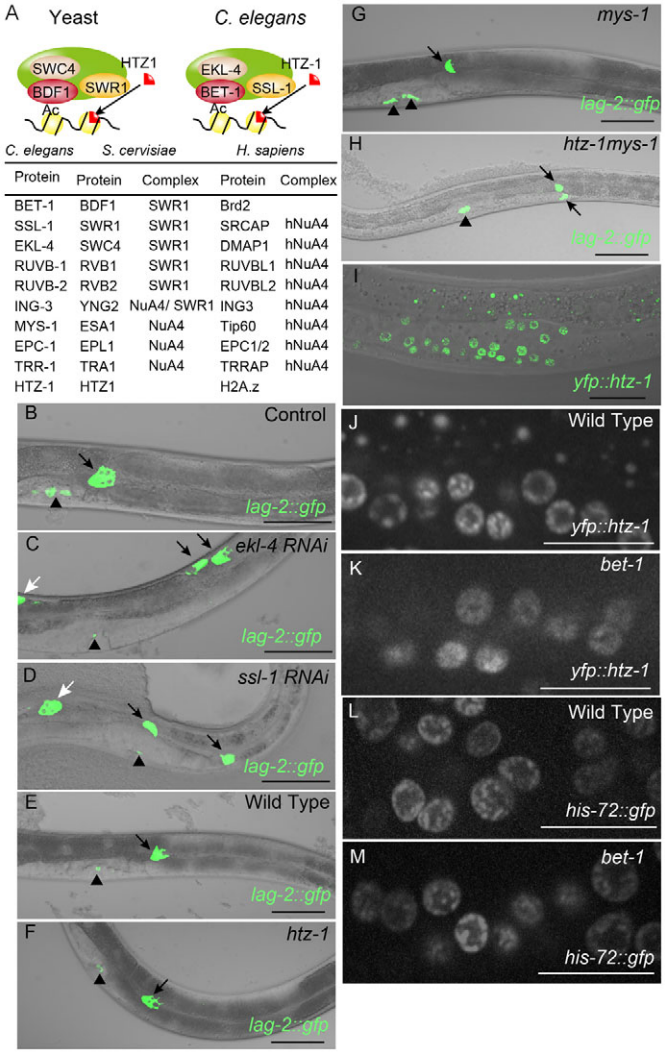

Fig. 1. Disruption of HTZ-1 causes the extra-DTC phenotype. (A) Schematics of the yeast SWR1 complex and HTZ1 (upper left) and *C. elegans* counterparts (upper right). Relevant homologs in *S. cerevisiae* and *H. sapiens* are listed (Doyon and Côté, 2004). SWR1, NuA4 and hNuA4 indicate the SWR1 complex, the NuA4 HAT complex and the human NuA4 HAT complex, respectively (bottom). (B-H) DTCs in control RNAi (B), *ekl-4* RNAi (C), *ssl-1* RNAi (D), wild-type (E), *htz-1*(*tm2469*) (F), *mys-1*(*n4075*) (G) and *htz-1 mys-1* (H) L4 animals. *lag-2::gfp* was used as a marker for DTCs. The black and white arrows indicate DTCs in posterior and anterior arms of the gonads, respectively. The arrowheads indicate *lag-2::gfp* expression in the cells of the vulva. (I) *yfp::htz-1* expression in SGCs in L4 animals. Dot-like signals in the upper half are auto-fluorescence of gut granules. (J-M) Subnuclear localization of YFP::HTZ-1 (J,K) and HIS-72::GFP (L,M) in SGCs of wild-type (J,L) and *bet-1*(*os46*) (K,M) L4 animals. Scale bars: 50 µm in B-I; 10 µm in J-M.

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

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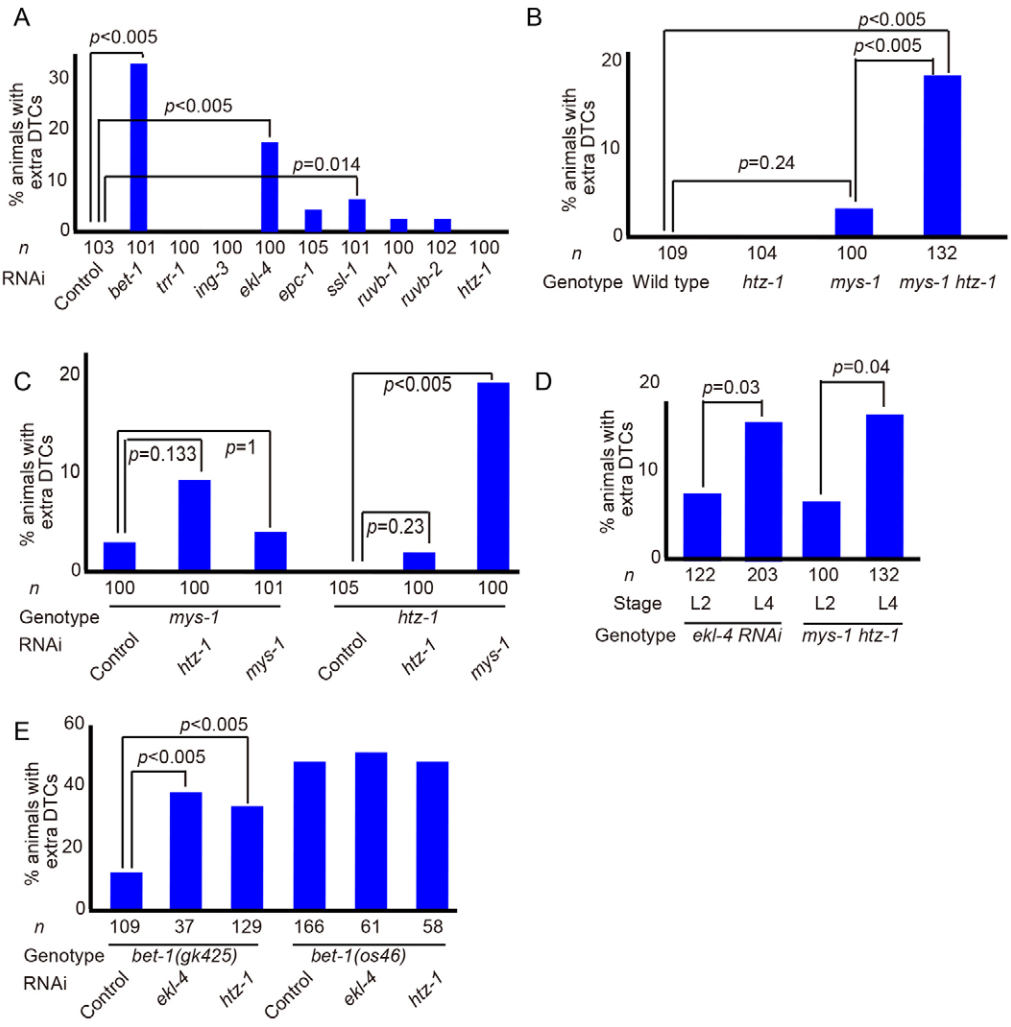

Fig. 2. HTZ-1 functions to maintain cell fate in the *bet-1* pathway. (A-E) Bar graphs show the percentage of adult and L4 (A,B,E), L3 and L4 (C) and L2 and L4 (D) animals with the extra-DTC phenotype. *n*, sample size.

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*, *htz-1* 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 was expressed in SGCs including DTCs (Fig. 1I; data not shown). In nine out of nine wild-type animals, puncta of 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-*

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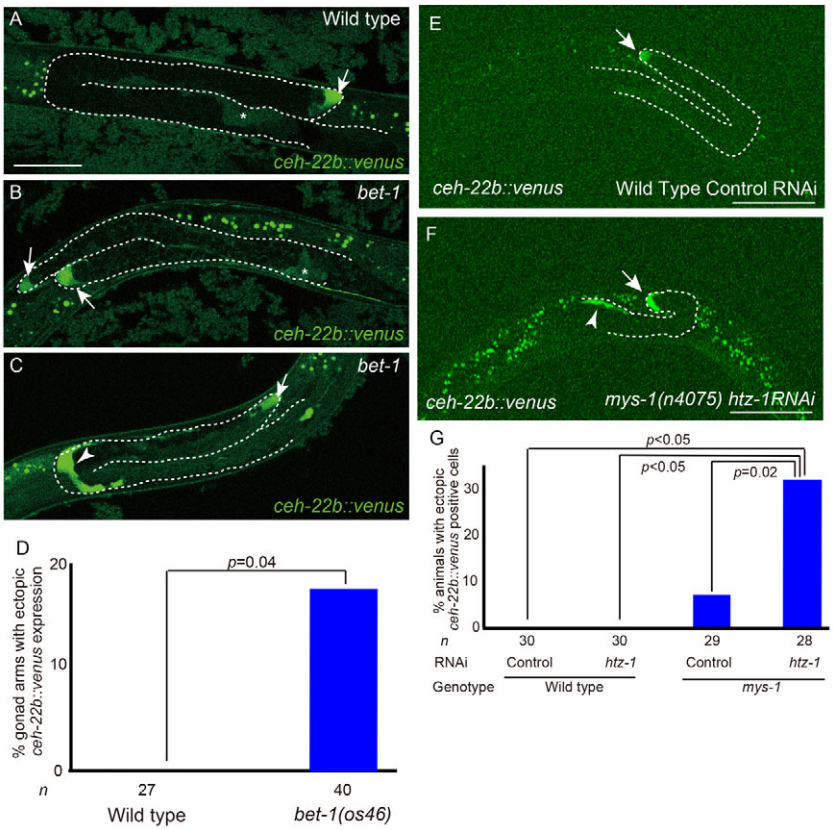

Fig. 3. Ectopic expression of *ceh-22b* in *bet-1* and *htz-1 mys-1* mutants. (A-C) *ceh-22b::venus* expression in the gonads of wild-type animals (A) and *bet-1*(*os46*) mutants (B,C) at L4. The arrows and arrowheads indicate DTCs and sheath cells, respectively, that strongly express *ceh-22b::venus* (A-C,E,F). The asterisks indicate weak *ceh-22b* expression in the proximal part of the gonad. (D,G) The bar graphs show the percentage of gonad arms with ectopic *ceh-22b::venus* expression at L4, when *bet-1* (D) or *mys-1* and *htz-1* (G) are depleted. (E,F) *ceh-22b::venus* expression in control RNAi (E) and *htz-1*(*RNAi*) *mys-1*(*n4075*) (F) animals. Scale bars: 50 µm in A-C,E,F. *n*, sample size.

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 mys-1* disruptants. We detected no ectopic expression 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

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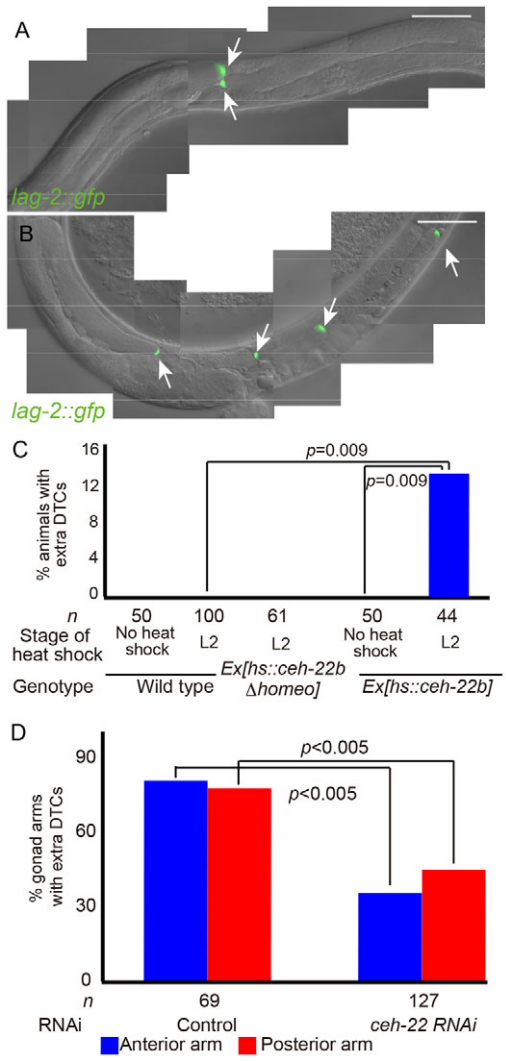

Fig. 4. Ectopic expression of *ceh-22b* induces extra DTCs. (A,B) DTCs in adult animals that have *hs::ceh-22b* in the absence (A) and presence (B) of heat-shock treatment at L2. Arrows indicate DTCs. *lag-2::gfp* was used as a marker for DTCs. Scale bars: 50 µm. (C,D) The bar graphs show the percentage of animals (C) and gonad arms (D) with the extra-DTC phenotype at the adult stage. *n*, sample size.

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 LacI::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 observed in 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 *htz-1 mys-1* double mutants. First, the extra-DTC phenotype of *htz-1 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 *htz-1 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 *htz-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, MYS-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*

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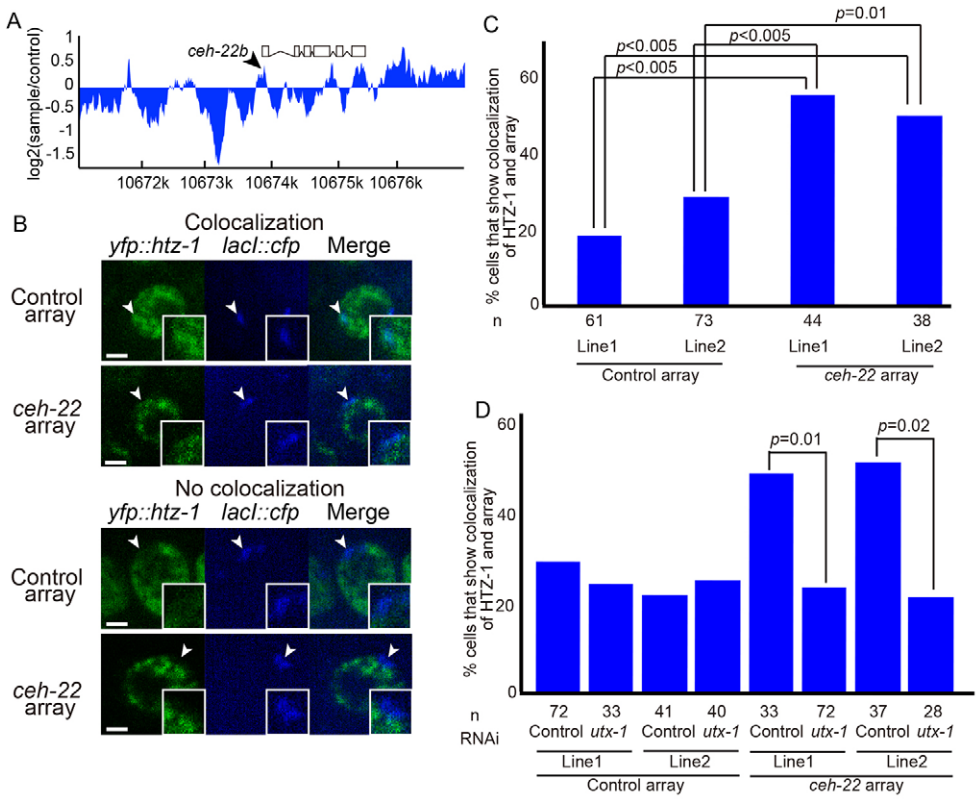

Fig. 5. Colocalization of HTZ-1 and the *ceh-22* locus. (A) Analysis of ChIP-seq data for HTZ-1 in L3 animals from modENCODE. Exons of *ceh-22b* are indicated by open boxes. Relative values of average signals for three samples against those for two controls are plotted. The graphs indicate the data from the 5′-upstream to 3′-downstream region of *ceh-22*. The position of *ceh-22b* TSS is indicated by the arrowhead. (B) Localization of YFP::HTZ-1 and extrachromosomal arrays in nuclei of SGCs at L4. Left, middle and right panels indicate *yfp::htz-1* (green), *lacI::cfp* (blue) and merged images, respectively. The arrowheads indicate LacI::CFP spots. Magnified images are shown in the insets. Pictures are of single focal planes. (C,D) The bar graphs indicate the percentages of samples that showed colocalization of YFP::HTZ-1 and LacI::CFP arrays in the wild-type (C) and the *utx-1* RNAi (D) backgrounds. Fisher's exact test was used to calculate the *P*-values. Scale bars: 1 µm. *n*, number of cells counted.

RNAi (Fig. 5D). The percentage of *ceh-22* array that showed colocalization with HTZ-1 decreased to the levels of the control arrays. These results show that H3K37 histone demethylase, UTX-1, enhances the colocalization between HTZ-1 and *ceh-22* locus.

DISCUSSION

Here, we have provided the first example of the requirement for a histone variant in the maintenance of cell fates. In the non-DTC lineage of SGCs, the histone variant HTZ-1 represses expression of *ceh-22b*, which would induce the formation of DTCs. Therefore, our results suggest that HTZ-1 and possibly its mammalian homolog, H2A.z, maintain cell fates through the repression of DNA-binding transcription factors. In addition, the antagonistic effects of *htz-1* and *utx-1*, the genome-wide analysis of the localization of HTZ-1 and H3K27me3, and the loss of colocalization between HTZ-1 and *ceh-22* locus by the depletion of *utx-1* suggest that HTZ-1 acts in the genomic region where H3K27 is methylated to a lesser extent.

The maintenance of cell fates by HTZ-1/H2A.z and MYS-1

We previously showed that BET-1 maintains cell fates in all four postembryonic lineages that we examined (Shibata et al., 2010). *htz-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 tumorigenesis (Conerly et al., 2010) as a result of the abnormal transformation of cell fates. The downregulation of Tip60, which is a mammalian counterpart of MYS-1, also causes 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 pluripotency 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,

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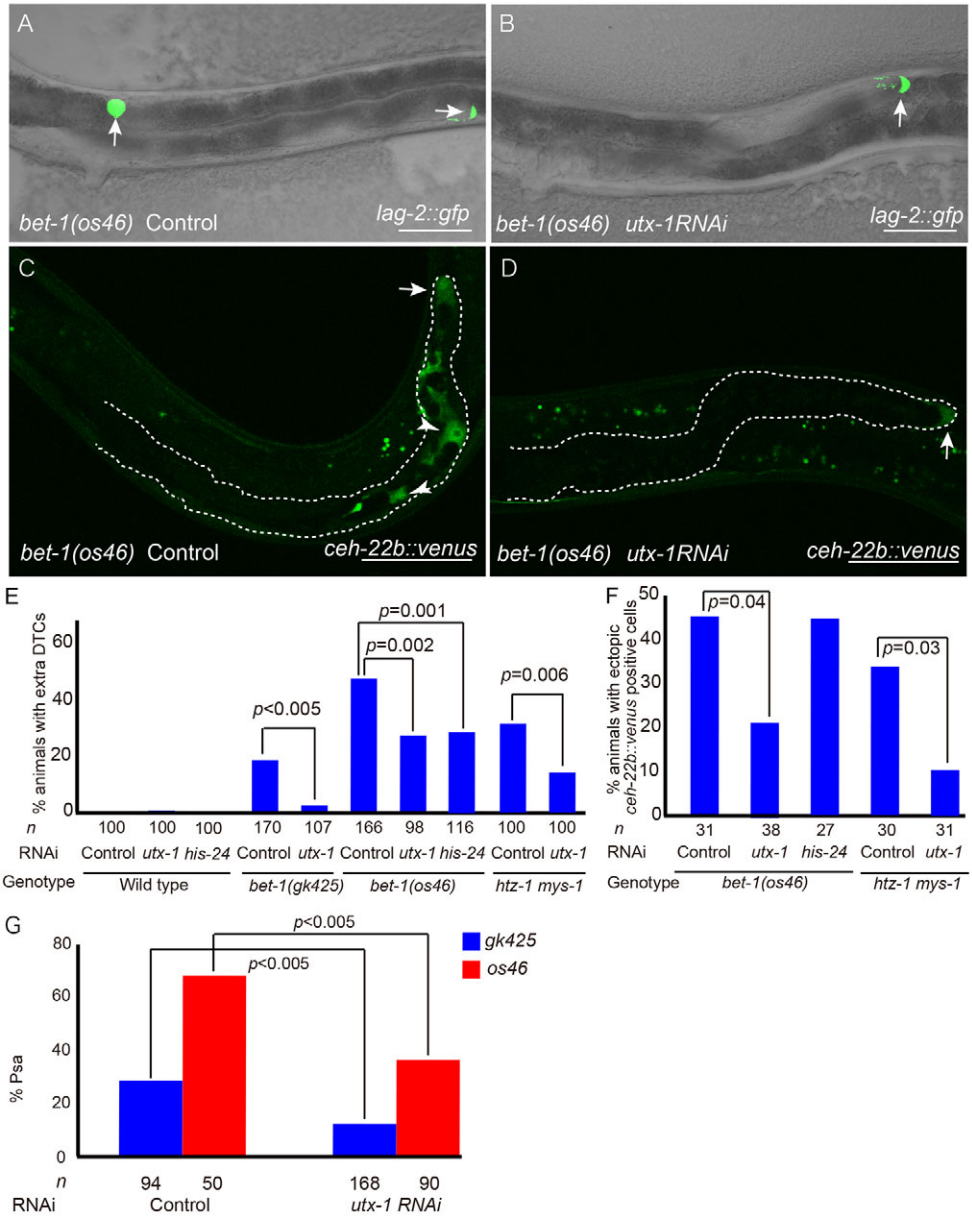

Fig. 6. Disruption of UTX-1 suppresses Bet-1 and Htz-1 Mys-1 phenotypes. (A-D) DTCs at the adult stage (A,B) and *ceh-22b::venus* expression at L4 (C,D) in *bet-1*(*os46*) (A,C) and *bet-1*(*os46*) *utx-1*(*RNAi*) (B,D) animals. *lag-2::gfp* was used as a marker for DTCs (A,B). (A,B) The arrows indicate DTCs. (C,D) The arrows and arrowheads indicate DTCs and the other *ceh-22b::venus*-expressing cells in the gonad, respectively. (E,F) The bar graphs indicate the percentage of L4 and adult animals with extra DTCs (E) and of L4 animals with ectopic *ceh-22b::venus* expression (F). (G) The bar graph indicates the percentage of samples with the Psa phenotype. *n*, sample size (E-G). Scale bars: 50 µm in A-D.

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-22b* expression in the non-DTC lineage of SGCs. The H3K27

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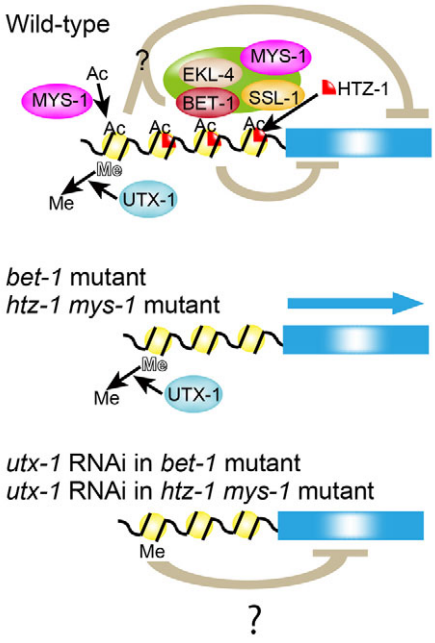

Fig. 7. A working model of the mechanism by which HTZ-1 maintains cell fates. Schematics of the regulatory states of the *ceh-22* locus in the non-DTC lineage of SGCs of wild-type (top), *bet-1*, *htz-1 mys-1* (middle), *bet-1 utx-1* and *htx-1 mys-1 utx-1* (bottom) animals.

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* (LGI), *mys-1* (LGV) and *htz-1* (LGIV) mutants are sterile and were thus maintained as heterozygotes over the *hT2*[*qIs48*] (LGI and III) or *nT1*[*qIs51*] (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*), *htz-1*(*tm2469*) and *mys-1*(*n4075*) 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: *qIs90* [*ceh-22b::venus*] (Lam et al., 2006), *qIs56* [*lag-2::gfp*] (Kostić et al., 2003), *pxEx214* [*yfp::htz-1, lacI::cfp*] (Updike and Mango, 2006) and *qEx556* [*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 (*Bam*HI-*Pst*I), yk1479b12 (*Bam*HI-*Pst*I), yk1360h08 (*Bam*HI-*Xba*I), yk1159b01 (*Bam*HI-*Pst*I), yk1258e11 (*Eco*RI), yk1650e12 (*Bam*HI-*Pst*I), yk1412b04 (*Bam*HI-*Xba*I) and yk733b08 (*Bam*HI-*Pst*I), 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-22bΔhomeodomain* PCR product consisted of an *hsp16-2* promoter that was amplified from pPD49.78 and a *ceh-22b* genomic fragment that lacks the region corresponding to amino acid residues 58-120.

Microscopy and statistical analysis

Expression of *ceh-22b::venus* was detected by confocal microscopy (LSM510 and Pascal, Zeiss, Jena, Germany) in larval stage (L)4 animals, because *ceh-22b::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 *htz-1*(*tm2469*) *mys-1*(*n4075*) double mutants, *ssl-1*(*RNAi*) animals, *ekl-4*(*RNAi*) animals, *mys-1*(*n4075*) mutants with *mys-1 RNAi* and *htz-1*(*tm2469*) mutants with *htz-1 RNAi*, because these animals did not grow to the adult stage. The subnuclear localization of *yfp::htz-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 spinning-disc confocal microscopy (CSU10 and CSUX1; Yokogawa, Tokyo, Japan) at 1-µm intervals. Colocalization spots were counted in each somatic gonadal cell in L3 and L4 animals. All nuclei having LacI::CFP signals in the captured images were analyzed. *ceh-22::mCherry* (50 µg/ml) was injected with *ttx-3::gfp* (50 µg/ml), *pUnc-76* (50 µg/ml) and *pSV2-dhfr8.32* (which contains 32 copies of the *lacO* sequence; 0.5 µg/ml) to make *ceh-22* arrays. Control arrays did not contain *ceh-22::mCherry*. Two independent lines were isolated for *ceh-22* and control arrays.

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).

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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

Supplementary material available online at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.090746/-/DC1

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