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Maintenance of cell fates through acetylated histone and the histone variant H2A.z in *C. elegans*

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Maintenance of cell fates is essential for the development and homeostasis of multicellular organisms and involves the preservation of the expression status of selector genes that control many target genes. Epigenetic marks have pivotal roles in the maintenance of gene expression status, as occurs with methylation on lysine 27 of histone H3 (H3K27me) for Hox gene regulation. In contrast, because the levels of histone acetylation decrease during the mitotic phase, acetylated histone has not been believed to contribute to the maintenance of cell fates. Because members of the bromodomain and extra terminal (BET) family bind to acetylated histones localized on mitotic chromosomes, it is possible that they may regulate the transcriptional status of genes throughout the cell cycle. In this commentary, we discuss the recent analyses of *C. elegans* BET family protein BET-1, which contributes to the maintenance of cell fates through the histone H2A variant HTZ-1/H2A.z. This mechanism represses transcription of selector genes in the genomic region where lysine 27 of histone H3 (H3K27) is demethylated by histone demethylase UTX-1. We discuss the possibility that BET-1 and HTZ-1 maintain the poised state of RNA polymerase II in the cell such that it is ready to respond to differentiation signals.

Keywords: cell fate maintenance, acetylated histone, BET-1, MYST family histone acetyltransferase, H2A.z, UTX-1, H3K27me, *ceh-22*, poised state

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The Maintenance of Cell Fates Through Histone Acetylation

Cell fate is determined by a combination of selector genes that encode transcription factors, which in turn, regulate many target genes.[1] Preserving the expression patterns of these selector genes is needed to maintain cell fates. Methylation on lysine 27 of histone H3 and Polycomb proteins maintain the repression of Hox genes.[2] In contrast to histone methylation, it is believed that histone acetylation does not function as an epigenetic mark that transmits cellular memory throughout the cell cycle, because the level of histone acetylation decreases during the mitotic phase.[3] Most of the acetylated histone-binding proteins do not associate with the mitotic chromosomes.[3] Exceptionally, BET family proteins that have two bromodomains do associate with mitotic chromosomes,[4-8] and therefore, they are expected to play a role in maintaining cell fates. BET family proteins are evolutionarily conserved from yeast to human.[6] Characteristic features of BET family proteins are two bromodomains that bind to acetylated histone and an extra terminal (ET) domain.[6] BET family proteins are divided into two subfamilies, the short subfamily and the long subfamily; members of the latter subfamily are found only in multicellular organisms (**Table 1**). We have previously shown that BET-1, a *C. elegans* BET family protein that binds to acetylated histone H4,[8] is involved in the maintenance of cell fate in multiple lineages, including ectodermal and mesodermal lineages.[8]

There has been recent and substantial progress in the study of BET family proteins with respect to medical research, especially in the fields related to cancer and viruses. For example, a fusion gene that consists of the mammalian BET family protein Brd4 and NUT (nuclear

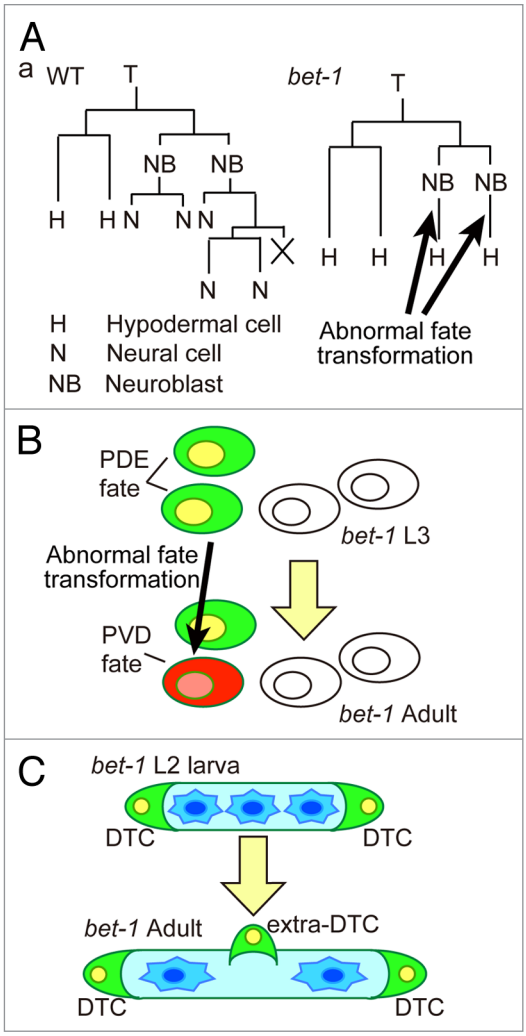

Figure 1. Phenotypes of *bet-1* mutants in *C. elegans*. (**A**) The T cell lineage in wild-type (WT) and *bet-1* mutants. The posterior granddaughter cells show abnormal transformation of cell fate from neuroblasts to hypodermal cells. (**B**) Schematic drawing of *bet-1* phenotype in the posterior lateral ganglia. In the wild-type V5.pa lineage, the PDE and PVD neurons express *osm-6::gfp* and *dop-3::rfp*, respectively. In *bet-1* mutants, abnormal transformation from the PDE fate to the PVD fate was observed. (**C**) In the Z1/Z4 lineage, which produces the somatic gonad, wild-type animals produce two distal tip cells (DTCs) at the late L1 stage. In *bet-1* mutants, abnormal transformation to the DTC fate occurs in the late larval stages, leading to the production of extra DTCs.

Table 1. BET family proteins

Species	Short subfamily	Long subfamily
C. elegans	BET-1	BET-2
S. cerevisiae	BDF1, BDF2	
H. sapiens	BRD2	BRD4
D. melanogaster	FSH-S	FSH-L

protein in testis) cause NUT midline carcinomas.[9] Constitutive expression of Brd2, another mammalian BET protein, also causes leukemia.[10] An inhibitor of BET proteins that interferes with binding of the bromodomain to acetylated histone has been studied as a drug for cancer therapy.[11,12] It has also been reported that some viruses utilize BET family proteins for their own transcriptional regulation.[13,14]

BET family proteins also have important roles during normal development. The loss of mouse Brd4 causes post-implantation lethality.[15] Mutations in *Drosophila fs(l)h* that encodes BET family proteins cause homeotic transformation.[16-18] Interestingly, homeotic transformation is also observed in mutants of Polycomb genes that are required for the maintenance of cell fates.[2] Therefore, the phenotype of *fs(l)h* mutants can be explained by the defect in the maintenance of cell fates. In addition, the role of BET family proteins as oncogenes suggests their role in the maintenance of cell fates.

In *C. elegans bet-1* mutants, abnormal cell fate transformation occurs in multiple cell lineages[8] (**Fig. 1A–C**). For example, transformation from the neural cell fate to the hypodermal cell fate was observed in the T cell lineage (**Fig. 1A**). Because *bet-1* RNAi causes embryonic lethality at the morphogenesis stage, BET-1 may function as a component of the fundamental mechanism that is required for the maintenance of cell fates. Interestingly, in *bet-1* mutants, cell fate transformation occurs even without cell division.[8] Therefore, BET-1 appears to be required for the maintenance of transcriptional status even after the final cell division. In addition, cells in *bet-1* mutants transform to become closely related cells in terms of cell lineage. Transformation to the fate of sister or cousin cells is frequently observed.[8] Thus, BET-1 maintains the difference between closely related cells, including sister cells.

In addition to BET-1, the MYST family histone acetyltransferases MYS-1 and MYS-2 are required for the maintenance of cell fates.[8] BET-1 appears to bind to regions that are acetylated by MYS-1 and MYS-2. Their involvement indicates the importance of histone acetylation in the maintenance of cell fates. The positive charge of lysine in the histone tail is important for the interaction between histone and DNA in the nucleosome.[19] Histone acetylation on lysine loosens the interaction between histones and DNA. Therefore, it has been believed that histone acetylation correlates with transcriptional activation. However, in *bet-1* mutants, ectopic expression of selector genes suggests that histone acetylation represses transcription through the activation of a BET-1-containing complex (see below).[20] Interestingly, genome-wide analyses in yeast suggest that each acetylation site appears to have a distinct role in transcriptional regulation, including transcriptional repression.[21] The mammalian BET family protein Brd4 is involved

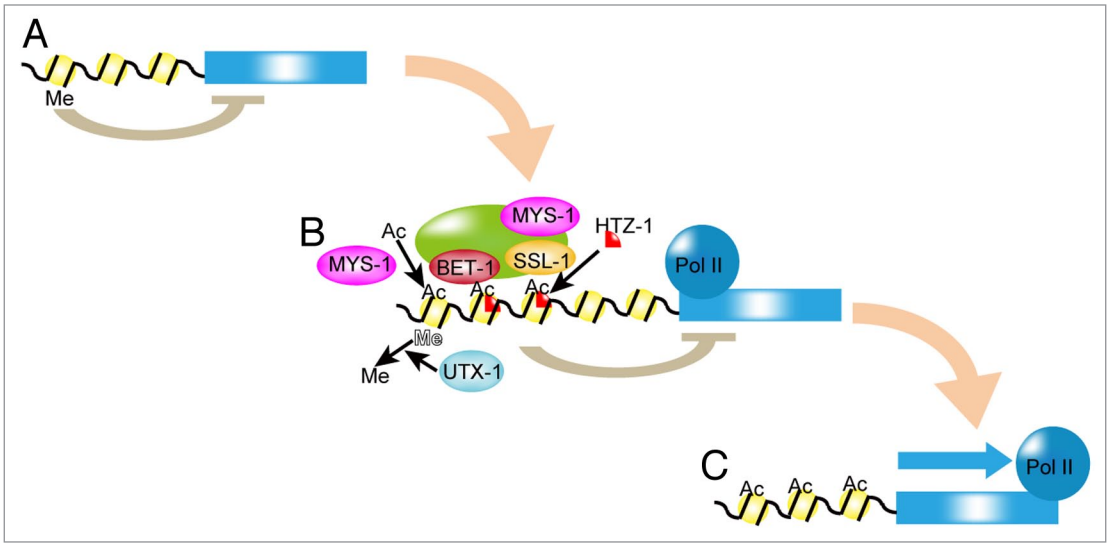

Figure 2. Working model for HTZ-1-dependent cell fate maintenance. (**A**) H3K27me silences the selector genes (blue box). Me, black line and yellow circle are methylation on H3K27, DNA, and histone, respectively. (**B**) UTX-1 demethylates H3K27me on selector gene loci. MYST histone acetyltransferases, including MYS-1 and MYS-2, acetylate the selector gene loci. Then, SSL-1 in the BET-1-containing complex deposits HTZ-1, which represses the transcription of selector genes. HTZ-1 may maintain the poised state of RNA polymerase II (Pol II). The transcription of selector gene(s) is ready to be activated. (**C**) When a cell receives a differentiation signal, repression by HTZ-1 is released and the gene is transcribed.

in repression of HPV (human papillomavirus) chromatin transcription.[14] Our results also suggests that BET-1 represses the transcription of *ceh-22*.[20] In addition, suppression of *bet-1* phenotypes by RNAi of *utx-1* that encodes H3K27 demethylase suggests that BET-1 and H3K27me has a similar effect in the maintenance of cell fates.[20] In addition, expression of *egl-15* and *sur-7* is upregulated in *bet-1* mutants.[22] Thus, growing evidence indicates that histone acetylation also acts in transcriptional repression.

Transcriptional Repression by Histone Acetylation Through the Histone H2A Variant H2A.z in the Maintenance of Cell Fates

Because BET-1 itself does not have a catalytic domain, BET-1 is likely to function as a complex. In yeast, the BET family protein BDF1 is a part of the SWR1 complex, which is required for the deposition of the histone H2A variant HTZ1/H2A.z.[23] In *C. elegans*, *htz-1*/H2A.z functions in the same genetic pathway with *bet-1* in the maintenance of cell fates.[20] The disruption of the SWR1 homolog SSL-1 causes the same phenotype that occurs in *bet-1* mutants. Disruption of HTZ-1 in the *mys-1* background also causes a defect in the maintenance of cell fates.[20] In addition, BET-1 regulates the subnuclear localization of HTZ-1.[20] Thus, in the maintenance of cell fates, BET-1 appears to function through the deposition of HTZ-1. HTZ-1 localizes on the transcription start site of the selector gene *ceh-22*, which is negatively regulated by HTZ-1 in the somatic gonad lineage, suggesting that BET-1-dependent HTZ-1 deposition directly regulates the transcription of selector genes. Thus, HTZ-1 appears to repress the transcription of selector genes in the maintenance of cell fates.

In *C. elegans*, genome-wide analysis of HTZ-1 indicates that the pattern of HTZ-1 occupancy on promoter regions is similar to that of RNA polymerase II.[24] There is, however, less of a correlation between HTZ-1 occupancy and transcriptional activity. Although H2A.z is implicated in transcriptional activation,[25-27] there is no simple correlation between HTZ-1 localization and transcriptional activation.[24] One of the attractive hypotheses is that HTZ-1 regulates the pausing of RNA polymerase II on the transcription start site (**Fig. 2**). This hypothesis is consistent with the colocalization of HTZ-1 and RNA polymerase II and transcriptional repression by HTZ-1. In the poised state, RNA polymerase II is stalled near the transcription start site.[28] Therefore, the poised state is transcriptionally inactive but easily activated. Because the poised state is ready-to-go for transcriptional activation, in cells that are ready to respond to developmental signals, selector gene(s) may be in the poised state for quick response to developmental signals. Interestingly, in *Drosophila*, RNA polymerase II localizes on promoter regions of many transcriptionally inactive genes including developmental genes.[29] Because cells transform to the fates of closely related cells in terms of lineage in *C. elegans* *bet-1* mutants, a transition to the poised state may also occur in sister or precursor cells of the cells that express selector genes. Maintenance of the poised state by HTZ-1 and BET-1 in the absence of a differentiation signal may prevent the over-production of specific cell types.

Histone acetylation recruits H2A.z to the selector gene loci through the action of BET-1 and SSL-1. However, how H2A.z maintains the resulting repression remains elusive. Transcriptional elongation by RNA polymerase II is promoted by phosphorylation at Ser2 on C-terminal domain (CTD).[28] Therefore, HTZ-1

might control the activity or recruitment of the P-TEFb complex that phosphorylates Ser2. Mammalian Brd4 associates with P-TEFb, suggesting that BET proteins may regulate C-terminal domain (CTD) phosphorylation at Ser2.[30]

The regulation of the silencing mark H3K27me is also important for the maintenance of cell fate. Genome-wide analysis indicates that genomic localization of HTZ-1 and genomic localization of H3K27me are inversely correlated, suggesting that HTZ-1 and H3K27me occupy distinct regions of the genome.[20] In *C. elegans*, methylation on H3K27 is regulated by the histone methylase MES-2 and the histone demethylase UTX-1.[31] RNAi screening has identified *utx-1* as a suppressor of the multiple *bet-1* mutant phenotypes, including expression of the HTZ-1 target gene *ceh-22*.[20] This suggests that histone acetylation and H3K27me have similar effects in the maintenance of cell fates. Therefore, the relationship between BET-1, MYS-1, HTZ-1, and UTX-1 appears to be conserved for multiple selector genes. We also showed that, when *utx-1* is disrupted, HTZ-1 localization to a target gene is decreased. Therefore, H3K27me appears to prevent the localization of HTZ-1 on the genome. In other words, HTZ-1 and BET-1 are recruited to the genomic region where H3K27 is demethylated by UTX-1. Thus, the HTZ-1-dependent mechanism appears to be an intermediate status between the H3K27me-related silenced state and transcriptional activation. Because the inverse correlation between H3K27me and H2A.z is also observed in differentiated mammalian cells,[32] this mechanism might be evolutionarily conserved.

Conclusion

Histone acetylation-dependent transcriptional repression of selector genes through H2A.z is important for the maintenance of cell fates. H2A.z may maintain the poised state of selector genes in the cell that are ready to respond to developmental signals. In contrast to transcriptional repression by H3K27me, the importance of transcriptional repression by histone acetylation and the poised state has been recognized only recently. Therefore, studying this mechanism should provide new insights into the transcriptional regulation that controls cell fates in animal development. *C. elegans* is advantageous for studying the maintenance of cell fate because of its invariant somatic cell lineage.[33] In addition, because the BET family protein Brd4 is known as a cancer-related gene, research in this field may also contribute to future cancer therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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