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Crucial Roles for Chromatin Dynamics in Cellular Memory

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Cellular memory is defined as a long-term maintenance of a particular pattern of gene expression through many rounds of cell division or even after cell division. It is critical for development and differentiation of multicellular organisms. Chromatin dynamics including histone modification, histone replacement and chromatin remodeling play key roles in cellular memory.

Key words: *Pc* group, *trx* group, position effect variegation, heterochromatin, histone H3.3.

Abbreviations: PHD, plant homeodomain; SP-Ring finger, Siz(septin-interacting protein)/PIAS(protein inhibitor of activated STAT)-Ring finger; PRC, Polycomb goup repressor complex; Su(z), Suppressor of zeste; Nurf, nucleosome remodeling factor; CBP, CREB(cyclic AMP response element-binding protein)-binding protein; SWI/SNF, mating type switch/sucrose non-fermenting; SET, Su(var)3-9/Enhancer of zeste/Trithorax; *bxd*, *bithoraxoid*; *Ubx*, *Ultrabithorax*; *Fab7*, *Frontabdominal 7*; *Abd-B*, *Abdominal-B*; TBP, TATA element-binding protein; FACT, facilitates chromatin transcription; SSRP1, structure specific recognition protein 1; SPT16, suppressor of ty element 16; HDAC, histone deacetylase; Su(var), suppressor of variegation; E(var), enhancer of variegation.

Chromatin can transmit a particular pattern of gene expression from one cell generation to the next. This phenomenon termed as cellular memory is critical for the development and differentiation of metazoans. For example, once an expression pattern of *Hox* genes along the anterio–posterior body axis has been established during the early embryogenesis, it is maintained throughout the life and governs formation of the body segments such as head, thorax and abdomen (*1*, *2*). Another example is the maintenance of the stem cell identity: a stem cell can self-renew while maintaining its totipotency (*3*).

Although cellular memory is evolutionarily conserved among metazoans, many pioneer works on the memory have been carried out by using the fruit fly *Drosophila melanogaster*. Two types of cellular memory have been studied extensively in *D. melanogaster*. One is the maintenance of *Hox* gene expression (*1*, *2*) and the other is the position effect variegation (PEV) (*4*, *5*). In this review, chromatin dynamics underlying the two types of cellular memory will be focused.

MAINTENANCE OF *HOX* GENE EXPRESSION

In 1947, P. Lewis isolated a *Polycomb* (*Pc*) mutant in which the sex comb, normally found only on the first leg of male, is also present on the second and the third legs. In embryos homozygous for the *Pc* mutation, many body segments showed characteristics of the posterior segments of wild-type. This phenotype cannot be explained by a mutation in any single *Hox* gene and E. Lewis has proposed that *Pc* can repress many *Hox* genes simultaneously (*6*). Since then many mutants with similar phenotypes have been isolated in different genes and these genes are classified into *Pc* group (*1*, *2*). Typical examples of *Pc* group are listed in Table 1.

Pc group genes do not appear to determine the initial patterns of *Hox* gene expression, but govern the maintenance of the repressed state of *Hox* genes once determined by the segmentations genes. As double mutations in different genes of *Pc* group cause more severe phenotypes than each single mutation does, *Pc* group gene products are thought to work cooperatively. In good agreement with this view, biochemical studies identified two complexes containing *Pc* group proteins. One is PRC1 complex including Pc, Ph, Psc and Ring (*7*). The other is PRC2 complex consisting of Esc, E(z), Su(z)12 and Nurf55 (*8*–*10*). E(z) in PRC2 methylates histone H3 at K27 and Pc in PRC1 binds to K27-methylated histone H3 through its chromodomain. Ring in PRC1 is responsible for ubiquitylation of histone H2A (*11*).

On the other hand, a mutant in any gene that is involved in the maintenance of the active state of *Hox* genes should show phenotypes with simultaneous loss of functions of many *Hox* genes. Based on this prediction, a *trithorax* (*trx*) mutant has been isolated (*12*). Subsequently many mutants have been isolated, which suppress the derepression of *Hox* genes in the *Pc* mutant (*13*). These mutants showed similar phenotypes as the *trx* mutant and hence, the corresponding genes are classified as *trx* group. Typical genes of *trx* group are listed in Table 1.

trx group functions to maintain the active state of *Hox* genes. Trx is a histone H3 methyltransferase at K4 and forms a complex containing a histone acetyltransferase CBP (*14*). *Trl* encodes the GAGA factor that binds to DNA through recognizing the GAGAG sequence (*15*). Brm is a catalytic subunit of the SWI/SNF-type

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Table 1. ***Pc* and *trx* groups.**

Drosophila	Human	Gene product
Pc group		
Polycomb(Pc)	*HPC1(M33), HPC2*	Chromodomain protein
Polyhomeotic(ph)	*HPH1(PAE28), HPH2*	Zn finger protein
Posterior sex comb(Psc)	*BMI1, MEL18*	Ring finger protein
Ring	*RING1, RING2*	Ring finger protein
Sex comb on midleg(SCM)	*SCM1, SCM2*	PRC1 subunit
extra sex comb(esc)	EED	WD domain protein
Enhancer of zeste[E(z)]	*EZH1, EZH2*	Histone H3 K27 methyltransferase
pleiohomeotic(pho)		
Polycomb like(Pcl)	*YY1*	DNA-binding protein
Enhancer of	*PHF1*	PHD finger protein
Polycomb[E(Pc)]	*EPC1, EPC2*	
Additional sex comb(Asx)	*ASXL1, ASXL2*	
trx group		
trithorax(trx)	*MLL*	Histone H3 K4 methytransferase
Trithorax like(Trl)		GAGA factor
brahma(brm)	*hBRM, BRG1*	Brm complex subunit
moira(mor)	*BAF155, BAF170*	Brm complex subunit
Snf5 related 1(Snr 1)	*hSNF5(INI)*	Brm complex subunit
absent, small or homeotic discs (ash 1)	*ASH 1*	SET domain protein
tonalli(tna)	*TNA*	SP-Ring finger protein
kismet(kis)		chromodomain/ATPase domain protein

chromatin remodeling factor Brm complex (*16*). Double mutants of *brm* and *Pc* suggest that Brm cannot prevent the *Pc*-dependent repression, but functions to activate transcription on genes that have escaped the *Pc* silencing. Ash1 carries a SET domain shared with many histone methyltransferases and forms a complex that is required for histone H3 K4 methylation (*17*), but its exact function is still unclear.

Pc group and *trx* group proteins occupy specific sites within the regulatory regions of *Hox* genes (*18*, *19*). These sites are termed Pc response element (PRE)/Trx response element (TRE). For example, the *bxd* region of *Ubx* and the *Fab7* region of *Abd-B* harbour PRE/TRE. Previously, it was thought that *Pc* group proteins occupy PRE/TRE in repressed cells while *trx* group proteins replace *Pc* group proteins at PRE/TRE in active cells. However, recent data ruled out this simple scenario. It turns out that PRC1, PRC2 and Trx are present at PRE/TRE in both active and repressed cells (*20*). Then how can *Pc* group proteins repress *Hox* genes in the presence of Trx, and how can *trx* group proteins maintain *Hox* gene expression in the presence of *Pc* group proteins? Surprisingly recent study using a model PRE-*hsp26* promoter construct has shown that *Pc* group silencing does not prevent the binding of TBP and RNA polymerase to the promoter (*21*). These data suggest that *Pc* group silencing is not due to block of accessibility of the transcription machinery, but is achieved through repression of transcription at the initiation and/or the early elongation step. Some component(s) in the PRC1 or PRC2 complex may affect the transcription preinitiation complex and inhibit the initiation or early elongation step.

Mechanisms of the *trx* group-dependent maintenance of *Hox* gene expression are also elusive at this moment, but two recent findings provide a clue for the issue. First, the GAGA factor recruits FACT, a heterodimer of SSRP1 and SPT16, to PRE/TRE, facilitates chromatin remodeling, and contributes to the maintenance of *Hox* gene expression (*22*). Second, non-coding RNAs are transcribed in the PRE/TRE regions in active cells but not in repressed cells, and this transcription is required for the maintenance of *Hox* gene expression (*23*). It is possible that the GAGA factor-dependent chromatin remodeling allows non-coding transcription in the PRE/TRE region, which in turn leads to release of the transcription machinery from the poised state through long-distance interactions between the regulatory region and promoter.

Although *Pc* and *trx* group proteins are displaced from mitotic chromosomes, these proteins should return back again soon after cell division to the original places on the interphase chromatin to maintain the repressed or active state. These processes are thought to be guided by histone modifications such as H3 K4, K9, K27, H4 K20 methylation and H3 K9, K27, H4 K16 acetylation but the precise mechanisms are still unknown. Clearly further studies are needed to elucidate the mechanisms underling *Pc*-dependent repression and its counteraction by *trx* group.

MAINTENANCE OF GENE EXPRESSION AGAINST HETEROCHROMATIN SILENCING

In 1930 H. Muller reported an important finding (*24*). When the *white* (*w*) gene that governs the eye color of the

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Table 2. **Modifiers of PEV.**

Gene	Map position	Gene product or feature
Suppressor		
Su(var)2-1		Butyrate (HDAC inhibitor) sensitive
Su(var)2-5	29A	HP1 (chromodomain protein)
Su(var)2-10		Butyrate (HDAC inhibitor) sensitive
Su(var)3-3		Histone H3 K4 demethylase
Su(var)3-6	87B	Protein phosphatase
Su(var)3-7	87E	Zn finger protein, binds to HP1
Su(var)3-9	88E	Histone H3 K9 methyltransferase
modulo	100F	DNA-binding protein
Enhancer		
Trithorax like	70F	GAGA factor
spt 16	62B	FACT subunit
E(var)3-4	89-93	
E(var)3-5	89-93	
E(var)3-6	86-89	
E(var)3-8	66	
E(var)3-12	86-89	
E(var)3-13	89-93	
E(var)3-93D	93D	BTB domain protein

fly is juxtaposed with pericentric heterochromatin by chromosome inversion such as w^{m4}, *w* expression becomes either silent or active in a stochastic manner. Once determined during embryogenesis, the silent or active state is maintained through cell divisions, which gives rise to variegated eye color. This is PEV. Genetic studies have identified many modifiers of PEV (*4*). Typical modifiers are shown in Table 2. Among suppressors of PEV, Su(var)2-5, Su(var)3-3, Su(var)3-7, and Su(var)3-9 encode heterochromatin protein 1 (HP1) (*25*), a histone H3 K4 demethylase (Gunter Reuter, personal communication), a Zn finger protein that interacts with HP1(*26*), and a histone H3 K9 methyltransferase (*27*), respectively. While molecular functions of most PEV enhancers remain unknown, *Trl* is known to encode the GAGA factor (*15*).

HP1 binds to a nucleosome by recognizing K9-methylated histone H3 through its chromodomain. HP1 also recruits Su(var)3-9 through protein–protein interactions. Then Su(var)3-9 methylates histone H3 at K9 in the neighboring nucleosome and another molecule of HP1 binds to the nucleosome. By repeating the process, heterochromatin has a tendency to spread into the neighboring regions (*5*, *28*) (Fig. 1A). However, heterochromatin is present only in the restricted regions of the genome (e.g. pericentric region). This means the presence of some mechanism that prevents the spreading of heterochromatin. As the spreading is mediated through suppressor functions, enhancers of PEV would be responsible for the blocking mechanism. Consistent with this expectation, recent study from this laboratory demonstrated that the GAGA factor-FACT complex and its binding site just downstream of *w* are crucial for PEV (*29*). Interestingly there are a peak of histone H3 K4 methylation and a dip of histone H3 K9 methylation at this site. The K9 methylation at d1 could be removed by histone replacement or demethylation. We considered replication-independent replacement of histone H3 by its variant H3.3 (*30*) rather than demethylation because histone H3.3 is a preferred target of K4 but not K9

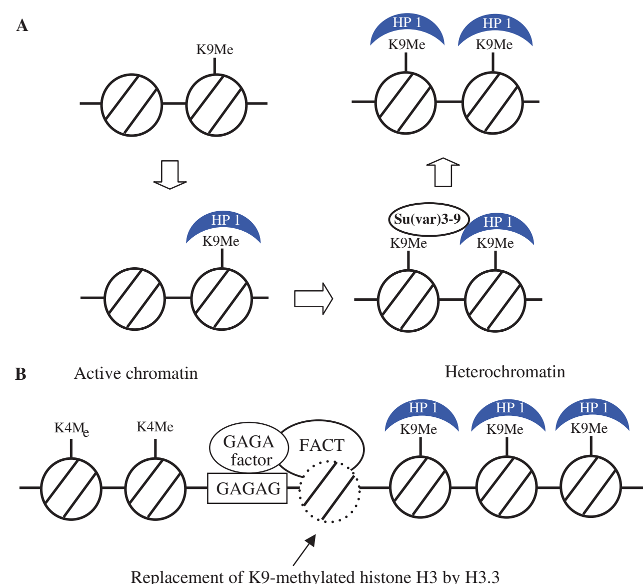

Fig. 1. **(A) Mechanisms of heterochromatin spreading.** In the heterochromatin, HP1 binds to a nucleosome by recognizing K9-methylated histone H3. HP1 recruits a histone methylase Su(var)3-9 through protein-protein interactions. Then Su(var) 3-9 methylates histone H3 at K9 of the neighboring nucleosome, which allows the binding of another molecule of HP1. By repeating the process, heterochromatin has a tendency to spead into the neighboring regions. (B) Histone H3.3 replacement prevents the spreading of heterochromatin. The GAGA factor and FACT direct replacement of a nucleosome containing K9-methylated histone H3 by a new nucleosome containing variant histone H3.3. This prevents the spreading of heterochromatin across the GAGA factor-binding site.

methylation (*31*) while a demethylated histone H3 could be a target of K9 methylation again. Indeed, we found that the GAGA factor and FACT direct replacement of K9-methylated histone H3 by histone H3.3 at this site, and prevent the spreading of heterochromatin (*29*) (Fig. 1B).

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The GAGA factor occupies PRE/TRE of *Hox* genes and hence, the above mechanism may operate not only in loci juxataposed with heterochromatin but also in the regulatory regions of *Hox* genes to prevent the *Pc* silencing. High levels of histone H3.3 have been also reported at the locus control region of the chicken folate receptor gene (*32*). This suggests evolutionary conservation of the barrier function against the chromatin silencing via histone H3.3 replacement from invertebrates to vertebrates.

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The data cited as Gunter Reuter, personal communication have been published: Rudolph, T., Yonezawa, M., Lein, S., Heidrich, K., Kubicek, S., Schäfer, C., Phalke, S., Walther, M., Schmidt, A., Jenuwein, T., and Reuter, G. (2007) Heterochromatin formation in *Drosophila* is initiated through active removal of H3K4 methylation by the LSD1 homolog SU(VAR)3-3. *Mol. Cell* **26**, 1–13

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