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Review

Important roles of reversible acetylation in the function of hematopoietic transcription factors

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Abstract

Hematopoiesis is a very complex process whose proper functioning requires the regulated action of a number of transcription factors. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) play significant roles in the regulation of hematopoietic transcription factors activity. Transcription factors such as GATA-1, EKLF, NF-E2, GATA-1, PU.1 recruit HATs and HDACs to chromatin, leading to histone acetylation and deacetylation, that affect chromatin structure and result in gene expression changes. On the other hand, transcription factors themselves can be acetylated and deacetylated by HATs and HDACs, respectively. Consequently, some important functions of these transcription factors are influenced, including DNA binding, transcription activation, repressor activity and protein-protein interactions. The regulation of hematopoietic transcription factors activity by HATs and HDACs may serve as a good model for studying how tissue-specific and lineage-specific gene expression is controlled through acetylation/deacetylation of histone/nonhistone proteins.

Keywords: histone acetyltransferases - histone deacetylases - hematopoietic transcription factor

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Introduction

Hematopoiesis is a very sophisticated process involving many factors, including hormones, hormone receptors, signal transducers, transcription factors and cell cycle regulators. These factors are finely tuned by endogenous and exogenous signals during hematopoietic development. Among them, transcription factors may play a central role in lineage choice and differentiation since they determine the correct expression of the appropriate genes at the right times. Such factors include GATA-1, FOG (friends of GATA-1), EKLF, NF-E2, PU.1, C-myb, TAL-1/SCL and GATA-2. The functioning and regulation of these transcription factors have been the subject of intense research, thus a good deal of information is available on the way extracellular signals regulate transcription factors activity by reversible phosphorylation through signaling pathways. In recent years, however, protein activity control through other forms of reversible modifications, including acetylation and deacetylation, attracted more and more attention. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) were first identified as enzymes able to acetylate and deacetylate histones, but an increasing number of studies revealed that they could also regulate other proteins as well, including transcription factors. Indeed, reversible acetylation plays significant role in the functional regulation of hematopoietic transcription factors. On one hand, transcription factors are able to recruit HATs and HDACs to acetylate and deacetylate chromatin histones, thus changing its structure. On the other hand, transcription factors themselves can be acetylated and deacetylated by HATs and HDACs. This review summarizes the regulatory role reversible acetylation plays in the function of hematopoietic transcription factors.

General characters of HATs and HDACs

HATs and HDACs have been found in all eukaryotes and some members are highly conserved from yeast through human. Even in prokaryotes, there are some proteins showing high homology to eukaryotic HDACs [1]. Therefore one can infer that HATs and HDACs may have significant biological roles. HATs catalyze the transfer of acetyl groups from acetyl-CoA to lysine residues of target proteins, while HDACs act to remove these acetyl groups. The acetylation status of proteins is thus determined by the relative activity of these two kinds of enzymes. For histones, lysine residues targeted for acetylation reside within their N-terminal tails. When acetylated, histones have less positive charges due to the loss of ε-NH^{2+} groups on lysine residues, consequently the interaction of the DNA backbone phosphorous groups with histones is weakened, the structure of chromatin becomes relatively lax and genes can be transcribed more easily. When histones are deacetylated, the opposite occurs. For other proteins than histones, functions can change according to the acetylation status (*e.g.* the DNA binding, transcription activation and repressor activity of transcription factors).

HATs

Histone acetylation was discovered 30 years ago, but in 1996 the first histone acetylase related to transcriptional regulation was identified [2]. In fact, HATs have been studied for quite a long time as transcription factors, co-activators or even components of RNA polymerase. It is now clear that through acetylating histones and other proteins, they participate in the whole transcription process. These HATs are recruited to promoter or enhancer regions by sequence specific DNA binding proteins, but at least one HAT, activating transcription factor -2 (ATF-2), has sequence-specific DNA binding capacity [3], a property whose physiological significance needs further study. Increasing numbers of HATs target proteins, other than histones, have been identified, including transcription factors such as p53, GATA-1, EKLF (erythroid Krüppel-like factor), HNF-4 (hepatocyte nuclear factor 4), structural proteins such as tubulin, and other proteins such as nuclear-import proteins. Certainly this list will become longer as a result of future research.

Seemingly homogenous HATs can be classified into different families showing a high within-family homology and low to no homology between fami-

Table 1: Classification of HDACs in yeast and human

	Class I	Class II	Class III
Yeast	RPD3	Hda 1	Sir2
		HOS1	
		HOS2	
		HOS3	
Human	RPD3	HDAC4	SIRT1
	HDAC1	HDAC5	SIRT2
	HDAC2	HDAC6	SIRT3
	HDAC3	HDAC7	SIRT4
	HDAC8	HDAC9	SIRT5
		HDAC10	SIRT6
			SIRT7

lies. Some human HATs have counterparts in yeast through mammals while others have not [4].

HDACs

According to the homology degree to their yeast counterparts, HDACs are divided into three classes [5] (Table 1). HDACs 1, 2, 3 and 8 are members of Class I and the closest homologs of the yeast protein RPD3. Class II contains HDACs 4, 5, 6, 7, 9 and 10, more similar to the yeast protein Hda1. The yeast silencing regulator protein Sir2 that plays an important role in gene silencing, is the founder member of class III HDAC. In humans, there are seven members of this class, named SIRT 1-7. The activity of class I and class II HDACs can be inhibited by some common small molecules such as trichostatin A (TSA) and n-butyrate. Class III HDACs are not sensitive to TSA or n-butyrate, but to nicotinamide, a physiological product of deacetylation reactions catalyzed by class III HDACs. Class III HDACs are collectively termed sirtuins and their functions are largely unknown. Recent work shows the major protein targets of sirtuins are transcription factors including p53, FOXO (forkhead transcription factors) family members, NF-κB (nuclear factor κB) and Ku70 [6]. Due to their substrate and activity specificity, it is assumed that sirtuins may modulate cell fate under specific stress and metabolic conditions. Another characteristic of class III HDACs is that their activity is dependent on NAD^+. Therefore, HDACs can also be classified according to NAD^+ requirement into two groups: "classical" HDACs, whose activity is NAD^+-independent, including Class I and Class II, and NAD^+-dependent HDACs comprising sirtuins.

Regulation of hematopoietic transcription factors by reversible acetylation

GATA-1

GATA-1 is expressed in erythroid cells, megakaryocytes, eosinophils, mast cells and Sertoli cells of the testis [7, 8]. GATA-1 protein is a zinc finger transcription factor required for the survival and maturation of erythroid precursor cells. GATA-1 null mice show anemia resulting from impaired differentiation and maturation of erythroid cells [9]. Important

GATA-1 binding sites are found at the globin gene promoters, the DNase I-hypersensitive sites (HSs) in the locus control region (LCR) of β-globin gene cluster, as well as in the regulatory sequences of many other erythroid-specific genes [10]. The highly conserved N-terminal zinc-fingers in GATA-1 are required for interacting with many other protein factors such as FOG (friends of GATA), EKLF, LMO2 (a member of the LIM-only family of transcription factors) and CREB binding protein (CBP) [11].

Boyes *et al.* first reported that GATA-1 could be acetylated by the transcriptional cofactor CBP/p300 *in vivo* and *in vitro* and the acetylation directly stimulated GATA-1-dependent transcription [12]. CBP/P300 acetylates GATA-1 at conserved lysine-rich domains near the C-terminal tails of both zinc fingers, and acetylation is precluded *in vitro* when these domains are mutated or deleted. CBP-mediated acetylation of murine GATA-1 did not change its DNA-binding activity, nor did mutations at the major acetylation site, although the latter impaired its biological activity. Given the above results, Hung *et al.* demonstrated that acetylation in either motif does not regulate nuclear localization, DNA binding, or protein stability [13]. One possibility is that acetylation leads to a conformational change of GATA-1, increasing the accessibility of transcriptional regulatory proteins to the chromatin template. At the same time, acetylation motifs might represent docking sites for co-activators and repressors, therefore acetylation might positively or negatively regulate their binding affinity.

GATA-1 acetylation may lead to a tissue-specific histone acetylation pattern, which is permissive for high levels of globin gene expression [14]. GATA-1 stimulates acetylation of both histone H3 and H4 at the LCR and the β-major-globin gene promoter. Time course experiments further demonstrated that GATA-1 occupancy of GATA elements induced recruitment of CBP, resulting in histone acetylation and globin gene expression. Thus, GATA-1 directly contributes to the recruitment of acetyltransferases to β-globin locus. This finding supports data showing a low level of globin gene expression in the absence of GATA-1, when a substantial amount of NF-E2 is already present at HS2, but hyperacetylation at HS2 still needs GATA-1 [15]. Furthermore, both NF-E2 and EKLF proteins are present in cells lacking GATA-1 (proerythroblast-like G1E cells, derived from GATA-1-null murine embryonic stem cells) and do not undergo substantial increase upon GATA-1 activation [16]. Thus, GATA-1 appears to be the limiting factor for acetyltransferase recruitment.

Some studies demonstrated that GATA-1 interacts with HDACs 1, 3, 4 and 5, although GATA-1 binds in vitro to HDACs 3, 4 and 5 but not HDAC1. Among these histone deacetylases, HDAC5 is present at high level in the nuclear extract of mouse erythroleukemia (MEL) cells, and represses the transcriptional activity of GATA-1. The amino-terminal zinc finger of GATA-1 has been found, using a pull-down assay, to be essential for GATA-1 interaction with HDAC5, and is also the binding site of FOG-1. These observations suggest that the amino-terminal zinc finger of GATA-1 is essential for normal erythropoiesis. These data also indicated that HDAC5 may hinder normal erythropoiesis by restraining the transcriptional activity of GATA-1, consistent with the significant decrease in GATA-1 association with HDAC5 over the course of MEL cells differentiation [17].

NF-E2 (nuclear factor E2)

NF-E2 plays a critical role in erythroid and megakaryocytic gene expression. This basic-zipper (b-Zip) transcription factor is a heterodimer consisting of p45NF-E2 and a wider expressed small subunit p18, which is a member of the small Maf proteins family [18, 19]. p45 contains the trans-activation domain and p18/Maf protein comprises the DNA-binding motif. Functional NF-E2 binding sites are found in a variety of regulatory elements controlling erythroid-specific gene expression, including the cis-acting elements within the human α-and β-globin loci as well as in regulatory modules of genes whose products are involved in iron metabolism [18].

In vitro binding studies suggested a direct physical interaction between the N-terminal activation domain of p45 NF-E2 and CBP. However, a recent study showed that interaction between NF-E2 and CBP leads to potent acetylation of Maf but not p45. The acetylation sites are four conserved lysine residues in the Maf DNA-binding domain. Maf acetylation stimulates DNA binding activity and transcriptional activation by NF-E2 [20]. Other interesting findings demonstrated that high levels of histone H3 and H4 acetylation at HS2 and HS3 of the β-globin LCR were independent of p45 NF-E2, but histone acetyla-

tion at the β-globin promoter was reduced in p45/NF-E2 null erythroleukemia CB3 cells [21]. Thus it is possible that LCR-bound NF-E2 might interact with the promoter indirectly through other promoter-bound transcription factors or *via* an LCR-coupled looping mechanism [22]. Murine p45/NF-E2 contains two WW domain-binding motifs, PPXY-1 and PPXY-2, PPXY-1 being required for β-globin transcription activation [23]. This PPXY-1 function may rely on the interaction with a co-regulator distinct from CBP/P300 because the PPXY-1 mutant does not impair the recruitment of CBP/P300 to HS2 [24]. Thus PPXY-1 might mediate essential chromatin remodeling events. Hence we hypothesize that interaction between NF-E2 and CBP might have two functions: modulation of NF-E2 activity and regulation of chromatin structure.

It is important to point out that NF-E2 activity on chromatin remodeling cannot be completely attributed to HAT. A study showed that NF-E2 could trigger chromatin remodeling at HS2 by recruiting an ATP-dependent chromatin-remodeling complex *in vitro* [25].

EKLF (erythroid Krüppel-like factor)

EKLF is expressed in erythroid, megakaryocytic and mast cells [26]. This protein includes three C_2H_2 type zinc finger at its C-terminus [27]. EKLF binds to CACCC elements located in the proximal promoter region of the adult expressed β-globin in mice and human. Its activity is crucial for consolidating the switch to high levels of adult β-globin expression during erythroid ontogeny. Absence of EKLF induced by gene inactivation in mice leads to lethal β -thalassemia. EKLF activation of the β-globin gene is dramatically enhanced by the LCR HS2 locus and the β-globin promoter, suggesting an important role for EKLF in regulating chromatin structure [28]. EKLF can interact with PCAF (p300/CBP associated factor) and CBP/P300 but only CBP/P300 acetylates EKLF and stimulates its transcriptional activity. EKLF acetylation occurs at two lysine residues, one in the activation domain (lysine 288), the other in the zinc-finger domain (lysine 302). Directed mutagenesis of Lys-288 decreased the ability of EKLF to transactivate the β-globin promoter *in vivo*, while mutagenesis of Lys-302 did not [29, 30]. Acetylation did not affect the DNA-binding activity of EKLF, but stimulated SWI/SNF chromatin remodeling complex recruitment, resulting in the formation of ERC-1 (EKLF co-activator remodeling complex-1), chromatin remodeling and transcriptional activation of β-globin. There are three possible explanations of these data. First, histone acetylation and transcriptional activation are associated; second, β-like globin locus is enriched in acetylated histones in erythroid cells; third, HATs utilize multiple mechanisms to serve as bridging molecules between activators and the basal transcription machine, allowing the access of other DNA-binding proteins. EKLF can also function *in vivo* as a transcription repressor and play an additional role in regulating erythroid gene expression and differentiation. Chen *et al.* found that EKLF could interact with the co-repressors mSin3A/HDAC1 through its zinc finger domain; EKLF repressive activity could be reverted by the HDAC inhibitor TSA. This function depends on formation of a protein-protein complex that effectively recruits co-repressors and/or HDACs to a target promoter [31].

GATA-2

GATA-2 is abundantly expressed in immature erythroid progenitors and declines over the course of maturation. It is also found in other cell types [32, 33]. Studies on GATA-2 deficient mice have shown that it plays an important role during the early stages of erythropoiesis. Suppression of GATA-2 in later stages of differentiation is required for normal erythropoiesis [34].

GATA-2 can be acetylated *in vitro* by p300 and GCN5 leading to an increase in its DNA binding activity. Furthermore, GATA-2 displayed a transcriptional synergism with p300 that was impaired by mutation of each acetylation site. This suggests that acetylation provides multiple control points for the regulation of GATA-2 function [35]. GATA-1 and GATA-2 both are acetylated. It is conceivable that as GATA-1 levels rise, GATA-1 recruits CBP/P300 away from factors required for proliferation of precursor cells, such as GATA-2, using them for the activation of differentiation-specific genes. GATA-1 triggers GATA-2 repression by means of disrupting positive autoregulation, followed by establishment of a domain-wide repressive chromatin structure [36]. Ozawa *et al* observed that HDACs, HDAC3 in par-

Table 2. The roles of acetylation & deacetylation in the function of hematopoietic transcription factors

Hematopoietic transcription factors	HAT	Effect of acetylating transcription factor itself	DNA binding activity	Site of histone acetylation	HDAC	Effect of deacetylation
GATA-1	CBP/P300	β-globin gene expression ↑ erythroid differentiation ↑	not changed	β-globin LCR	HDAC4/5	erythroid differentiation ↓
NF-E2	CBP/P300	transcriptional activation	increase	β-globin promoter	-	-
EKLF	CBP/P300	transcriptional activation	not changed	β-globin LCR & promoter?	mSin3A/HDAC1	transcription repression
	PCAF	transcriptional activation	not changed	-	-	-
GATA-2	CBP/P300	erythroid differentiation ↓	increase	-	HDAC3	erythroid differentiation ↑
TAL-1/SCL	P300	erythroid differentiation ↑	increase	-	mSin3A/HDAC1	erythroid differentiation ↓
	PCAF	erythroid differentiation ↑	increase	-	-	-
c-Myb	P300	transcriptional activation	not changed	-	-	-
	hGCN5	transcriptional activation	not changed	-	-	-
PU.1	keeps the balance between cell proliferation and maturation presumably by regulating the acetylation status of hematopoietic transcription factors and histones					

ticular, interact with GATA-2 to repress its transcriptional activity. So both acetylation and deacetylation are important for the function of this transcription factor [37]

TAL-1/SCL (stem cell leukemia factor)

TAL-1/SCL is a transcriptional factor with a basic helix-loop-helix (bHLH) motif [38, 39]. TAL-1/SCL binds to E-box (CAGGTG) DNA elements as a heterodimer with the products of other bHLH genes, including E12 and E47 [40]. These heterodimers also participate in a transcription complex including GATA-1, LMO2, Ldb-1 [41]. TAL-1/SCL is expressed in early hematopoietic progenitors and in more mature megakaryocytes, erythroid and mast cells. TAL-1/SCL expression is high in erythroid cells and plays important roles in their differentiation [42]. Abnormal expression of TAL-1/SCL within T-lymphocytes is associated with acute T-cell leukemia. TAL-1/SCL function is also associated with acetylation and deacetylation. For example, TAL-1/SCL can be acetylated by p300 and PCAF (P300/CBP-associated factor) *in vitro* and *in vivo* [43, 44]. The PCAF acetylation sites of TAL-1/SCL map to a lysine-rich motif in the bHLH region. In differentiated erythroleukemia cells, increased TAL-

1/PCAF interaction, TAL-1 acetylation and TAL-1 DNA binding activity are observed. Another study found that p300 coimmunoprecipitates with TAL-1 in extracts from DMSO-induced MEL cells. The amino-terminal sequence of p300 and the bHLH domain of TAL1 interact *in vitro*, but the interaction does not affect the DNA-binding activity of TAL-1. The TAL-1/P300 complex may have an important role in erythroid differentiation [43]. In 2000, Huang *et al.* showed that a corepressor complex including mSin3A and HDAC1 interacts with TAL-1 and restricts its function in erythroid differentiation [45]. Recent studies also demonstrated that TAL-1/SCL induces leukemia by repressing E47 expression and by recruiting the mSin3A/HDAC1 co-repressor complex to target loci, such as the CD4 gene [46]. TAL-1/SCL-induced tumors are sensitive to pharmacological inhibition of HDAC and undergo apoptosis. Thus the association of TAL-1/SCL function with acetylation and deacetylation is very complex and will provide us a clue for cancer therapy. SCL acetylation increases during MEL cells differentiation, paralleling PCAF protein levels. At the same time, acetylation by PCAF also leads to reduced affinity of SCL for the co-repressor mSin3A. Thus acetylation of SCL is highly regulated during erythroid cell differentiation and might contribute to a switch from repressor to activator function. This seems illustrative for the importance of acetylation-deacetylation balance in gene expression and cellular differentiation.

C-myb

The c-myb proto-oncogene product is a 75kDa sequence-specific DNA-binding protein that functions as a transcriptional activator. c-Myb protein is expressed at high levels in immature hematopoietic cells and regulates their proliferation and differentiation. Moreover, its forced expression inhibits erythroid differentiation [47]. c-Myb contains three functional domains: a DNA-binding domain (DBD), a transcription activation domain (TA), and a negative regulatory domain (NRD) [48]. CBP is able to acetylate c-Myb lysine residues within the NRD both *in vitro* and *in vivo*. Studies indicated that acetylation of c-Myb NRD enhanced its affinity for CBP and had two functional consequences for c-Myb activity: increased DNA binding activity and increased transactivating capacity [49]. *In vitro* acetylation assays showed that c-Myb could also be acetylated by hGCN5. The histone acetyltransferase activity of hGCN5 is significantly lower than that of p300, while c-Myb acetylation by hGCN5 is higher than by p300 [50]. c-Myb expression is the highest in progenitor cells of the myeloid, erythroid and lymphoid lineages. Forced expression of c-myb blocks differentiation of erythroid and myeloid precursor cells. Expression of a dominant interfering form of c-Myb results in enhanced erythroid differentiation. Thus, c-myb functions in maintaining hematopoietic precursor cells in a proliferative state. A recent report indicated that acetylation of the c-Myb NRD could also serve to inhibit the binding of an uncharacterized inhibitor to c-Myb [49]. This observation can partly explain the above phenomenon. Another explanation could be that differentiation factors compete proliferation-stimulating factors for CREB-binding protein (CBP) activity, depending on their expression levels during cellular maturation, or on cellular signals able to regulate their interaction with CBP and p300 [51].

PU.1

The Ets family oncoprotein PU.1 is normally expressed in myeloid and lymphoid cells [52]. PU.1 expression in erythroid progenitors can induce erythroleukemia in mice [53]. Transformation by PU.1 is achieved by maintaining erythroid precursor cells in an undifferentiated proliferative state. The molecular mechanism of this phenomenon is poorly understood. Recent researches showed that PU.1 could specifically and efficiently inhibit CBP-mediated acetylation of several hematopoietic transcription factors, including GATA-1, NF-E2 and EKLF. In addition, PU.1 blocks histones acetylation at the β-globin gene promoter and LCR, and it interferes with acetylation-dependent transcriptional events [54]. These results indicate that histone acetylation is sensitive to PU.1 levels and suggest that one of the mechanisms by which PU.1 inhibits erythroid differentiation is inhibition of histones and transcription factors acetylation. However, PU.1 is essential for myeloid and lymphoid cell development. This is paradoxical in respect to the above phenomenon. There are two possible explanations: either PU.1 function depends on the promoter context; or it depends on its expression level. At the

same time, tissue-specific expression of PU.1 is also regulated by chromatin structure. In multiple cell lines treated with TSA, loss of PU.1 is observed at both the mRNA and the protein levels. This loss of PU.1 expression is correlated with a significant increase in H4 acetylation at the PU.1 promoter region [55]. We can though infer that PU.1 functions in keeping the balance between cell proliferation and maturation by regulating the acetylation status of hematopoietic transcription factors and histones.

Concluding remarks

Much has been revealed on the interaction between transcription factors and cis elements over the past years, and the attention of scientists has recently turned to the interaction between protein factors. Transcription factors can induce chromatin changes that facilitate transcription through interactions with other protein factors among which HATs and HDACs are prominent. HATs and HDACs can reversibly acetylate or deacetylate transcription factors and/or histones thus modulating transcriptional activation, and changing the chromatin structure. The final effects are gene expression modulation and changes of cell physiological functions according to exogenous or endogenous signals (Table 2).

Mechanisms that control the activity of hematopoietic transcription factors through the interplay of HATs and HDACs could be a good model for studying how tissue-specific and lineage-specific gene expression are controlled in the context of acetylation/deacetylation of histone/nonhistone proteins.

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References

1. **Buck S.W., Gallo C.M., and Smith J.S.,** Diversity in the Sir2 family of protein deacetylases, *J. Leukoc. Biol.*, **75**: 939-950, 2004
2. **Brownell J.E., Zhou J., Ranalli T., Ranalli T., Kobayashi R., Edmondson D.G., Roth S.Y., Allis C.D.,** Tetrahymena histone acetyltransferases A: a homolog to yeast Gcn5p linking histone acetylation to gene regulation, *Cell*, **84**: 843-851, 1996
3. **Kawasaki H., Schiltz L., Chiu R., Itakura K., Taira K., Nakatani Y., Yokoyama K.K.,** ATF-2 has intrinsic histone acetyltransferases activity which is modulated by phosphorylation, *Nature*, **405**: 195-200, 2000
4. **Marmorstein T., Roth S.Y.,** Histone acetyltransferases: function, structure, and catalysis, *Curr. Opin. Gene. Dev.*, **11**: 155-161, 2001
5. **de Ruijter A.J.M., van Gennip A.H., Caron N., Kemp S., van Kuilenburg A.B.P.,** Histone deacetylases (HDACs): characterization of the classical HDAC family, *Biochem. J.*, **370**: 737-749, 2003
6. **Blander G., Guarente L.,** The Sir2 family of protein deacetylases, *Annu. Rev. Biochem.*, **73**: 417-435, 2004
7. **Zon L.I., Tsai S., Burgess S., Matsudaira P., Bruns G., and Orkin S.H.,** The major human erythroid DNA-binding protein (GF-1): primary sequence and localization of the gene to the X chromosome, *Proc. Natl. Acad. Sci. USA*, **87**:668-672, 1990
8. **Yamamoto M., Takahashi S., Onodera K., Muraosa Y., Engel J.D.,** Upstream and downstream of erythroid transcription factor GATA-1, *Genes. Cell.*, **2**: 107-115, 1997
9. **Weiss M.J., Keller G., Orkin S.H.,** Novel insights into mouse erythroid development revealed through *in vitro* differentiation of GATA-1-embryonic stem cells, *Genes. Dev.*, **8**: 1184-1197, 1994
10. **Weiss M.J., Orkin S.H.,** GATA transcription factors: key regulators of hematopoiesis, *Exp. Hematol.*, **23**:99-107, 1995
11. **Trainor C.D., Omichinski J.G., Vandergon T.L., Gronenborn A.M., Clore G.M., Felsenfeld G.,** A palindormic regulatory site within vertebrate GATA-1 promoters requires both zinc fingers of the GATA-1 DNA-binding domain for high-affinity interaction, *Mol. Cell. Biol.*, **16**: 2238-2247, 1996
12. **Boyes J., Byfield P., Nakatani Y., Vasily O.,** Regulation of activity of the transcription factor GATA-1 by acetylation, *Nature*, **396**: 594-598, 1998
13. **Hung H.L., Lau J., KIM A.Y., Weiss M.J., Blobel G.A.,** CREB-binding protein acetylates hematopoietic transcription factor GATA-1 at functionally important sites, *Mol Cell Biol.*, **19**: 3496-3505, 1999
14. **Letting D.L., Rakowski C., Weiss M.J.,** Formation of a tissue-specific histone acetylation pattern by the hematopoietic transcription factor GATA-1, *Mol. Cell. Biol.*, **23**: 1334-1340, 2003
15. **Johnson K.D., Grass J.A., Boyer M.E., Kiekhaefer C.M., Blobel G.A., Weiss M.J., Bresnick E.H.,** Cooperative activities of hematopoietic regulator recruit

RNA polymerase II to a tissue-specific chromatin domain, *Proc. Natl. Acad. Sci. USA.,* **99**: 11760-11765, 2002

16. **Kiekhaefer C.M., Grass J.A., Johnson K.D., Boyer M.E., Bresnick E.H.,** Hematopoietic-specific activators establish an overlapping pattern of histone acetylation and methylation within a mammalian chromatin domain, *Proc. Natl. Acad. Sci. USA.,* **99**: 14309-14314, 2002
17. **Watamoto K., Towatari M., Ozawa Y., Miyata Y., Okamoto M., Abe A., Naoe T., Saito H.,** Altered interaction of HDAC5 with GATA-1 during MEL cell differentiation, *Oncogene*, **22**: 9176-9184, 2003
18. **Andrews N.C., Erjument-Bromage H., Davidson M.B., Tempst P., Orkin S.H.,** Erythroid transcription factor NF-E2 is a haematopoietic-specific basic-leucine zipper protein, *Nature*, **362**: 722-728, 1993
19. **Andrews N.C., Kotkow K.J., Ney P.A., Erjument-Bromage H., Tempst P., Orkin S.H.,** The ubiquitous subunit of erythroid transcription factor NF-E2 is a small basic-leucine zipper protein related to the v-maf oncogene, *Proc. Natl. Acad. Sci. USA.*, **90**: 11488-92, 1993
20. **Hung H.L., Kim A.Y., Hong W., Rakowski C. Blobel G.A.,** Stimulation of NF-E2 DNA binding by CREB-binding protein (CBP)-mediated acetylation, *J. Biol. Chem.*, **276**: 10715-10721, 2001
21. **Johnson, K.D., Christensen, H.M., Zhao B., Bresnick E.H.,** Distinct mechanism control RNA polymerase II recruitment to a tissue-specific locus control region and a downstream promoter, *Mol. Cell.* , **8**: 465-471, 2001
22. **Sawado, T, Lgarashi K, Groudine, M.,** Activation of beta-major globin gene transcription is associated with recruitment of NF-E2 to the beta-globin LCR and gene promoter, *Proc. Natl. Acad. Sci. USA.*, **98**: 10226-10231, 2001
23. **Mosser E.A., Kasanov J.D., Forsberg E.C., Kay B.K., Ney P.A., Bresnick E.H.,** Physical and functional interactions between the transcription domain of the hematopoietic transcription factor NF-E2 and WW domains, *Biochemistry*, **37**: 13686-13695, 1998
24. **Kiekhaefer C.M., Boyer M.E., Johnson K.D., Bresnick E.H.,** A WW domain-binding motif within the activation domain of the hematopoietic transcription factor NF-E2 is essential for establishment of a tissue-specific histone modification pattern, *J. Biol. Chem.,* **279**: 7456-7461, 2004
25. **Armstrong J.A., Emerson B.M.,** NF-E2 disrupts chromatin structure at human β-globin locus control region hypersensitive site 2 *in vitro*, *Mol. Cell. Biol.*, **16**: 5634-5644, 1996
26. **Turner J, Crossley M.,** Basic Krüppel-like factor function within a network of interacting haematopoietic transcription factors, *Int. J. Biochem. Cell. Biol.*, **31**: 1169-1174, 1999
27. **Perkins A.,** Erythroid Krüppel like factor: from fishing expedition to gourmet meal, *Int J. Biochem. Cell. Biol.*, **31**:1175-1192, 1999
28. **Donze D, Townes T.M., Bieker J.J.,** Role of erythroid Krüppel-like factor in human gamma- to beta-globin gene switching, *J. Biol. Chem.*, **270**: 1955-1959, 1995
29. **Zhang W.J., Kadam S., Emerson B.M., Bieker J.J.,** Site-specific acetylation by p300 or CREB binding protein regulates erythroid Krüppel-like factor transcriptional activity *via* its interaction with the SWI-SNF complex, *Mol. Cell. Biol.*, **21**: 2413-2422, 2001
30. **Zhang W, Bieker J.J.,** Acetylation and modulation of erythroid Krüppel-like factor (EKLF) activity by interaction with histone acetyltransferases, *Proc. Natl. Acad. Sci. USA.*, **95**: 9850-9860, 1998
31. **Chen X.Y., Bieker J.J.,** Unanticipated repression function linked to erythroid Krüppel-like factor, *Mol. Cel. Biol.*, **21**: 3118-3125, 2001
32. **Dorfman D.M., Wilson D.B., Bruns G.A.P., Orkin S.H.,** Human transcription factor GATA-2; evidence for regulation of preproendothelin-1 gene expression in endothelia cells, *J. Biol. Chem.,* **267**: 1279-85,1992
33. **Nagai T., Harigae H., Ishihara H., Motohashi H., Minegishi N., Tsuchiya S., Hayashi N., Gu L., Andres B., and Engel J.D.,** Transcription factor GATA-2 is expressed in erythroid, early myeloid and CD34+ human leukemia-derived cell lines, *Blood*, **84**: 1074-84, 1994
34. **Briegel K., Lim K.C., Plank C., Beug H., Engel J.D., Zenke M.,** Ectopic expression of a conditional GATA-2/estrogen receptor chimera arrests erythroid differentiation in a hormone-dependent manner, *Genes. Dev.*, **7**: 1097-109, 1993
35. **Hayakawa F, Towatari M, Ozawa Y, Tomita A, Privalsky ML, Saito H.,** Functional regulation of GATA-2 by acetylation, *J. Leukoc. Biol.*, **75**:529-40, 2004
36. **Grass J.A., Boyer M.E., Pal S., Grass J.A., Boyer M.E., Pal S., Wu J., Weiss M.J., Bresnick E.H.**, GATA-1-dependent transcriptional repression of GATA-2 *via* disruption of positive autoregulation and domain-wide chromatin remodeling, *Proc. Natl. Acad. Sci. U S A.*, **100**: 8811-6, 2003
37. **Ozawa Y., Towatari M., Tsuzuki S., Hayakawa F., Maeda T., Miyata Y., Tanimoto M, Saito H.**, Histone deacetylase 3 associates with and represses the transcription factor GATA-2, *Blood*, **98**: 2116-2123, 2001
38. **Hershfield M.S., Kurtzberg J., Harden E., Moore J.O., Whang-Peng Je., and Haynes BF.,** Conversion of a stem cell leukemia from a T-lymphoid to a myeloid phenotype induced by the adenosine deaminase inhibitor 2'-deoxycoformycin, *Proc. Natl. Acad. Sci. USA.*, **81**: 253-257, 1984
39. **Begley C.G., Visvader J., Green A.R., Aplan P.D., Metcalf D., and Gough N.M.,** Molecular cloning and chromosomal localization of the murine homology of the human helix-loop-helix gene SCL, *Proc. Natl. Acad. Sci. USA.*, **88**: 869-873, 1991
40. **Shivdasani R.A., Orkin S.H.,** The transcriptional control of hematopoiesis, *Blood*, **87**: 4025-4039, 1996
41. **Wadman I.A., Osada H., Grutz G.G., Agulnick A.D., Westphal H., Forster A., Rabbitts T.H.,** The LIM-only protein Lmo2 is a bridging molecular assembling an erythroid, DNA-binding complex which includes the TAL1, E47, GATA-1 and Ldb1/NLI proteins, *EMBO. J.*, **16**: 3145-3157, 1997.
42. **Aplan P.D., Nakahara K., Orkin S.H., Kirsch IR.,** The SCL gene product: a positive regulator of erythroid differentiation, *EMBO. J.*, **11**: 4073-81, 1992
43. **Huang S., Qiu Y., Stein R.W., Brandt S.J.,**. p300 functions as a transcriptional coactivator for the TAL1/SCL

oncoprotein, *Oncogene,* **18**:4958-4967, 1999
44. **Huang S., Qiu Y., Shi Y., Xu Z., Brandt S.J.,** P/CAF - mediated acetylation regulates the function of the basic helix-loop-helix transcription factor TAL1/SCL, *EMBO. J.,* **19**: 6792-6803, 2000
45. **Huang S., Brandt S.J.,** mSin3A regulates murine erythroleukemia cell differentiation through association with the TAL (or SCL) transcription factor, *Mol. Cell. Biol.*, **20**: 2248-2259, 2000
46. **O Neil J., Shank J., Cusson N., Murre C., Kelliher M.,** TAL1/SCL induces leukemia by inhibiting the transcriptional activity of E47/HEB, *Cancer Cell*, **5**: 587-596, 2004
47. **Shivdasani R.A., Orkin S.H.,** The transcriptional control of hematopoiesis, *Blood*, **87**: 4025-4039, 1996
48. **Sakura, H, Kanei-Ishii, C, Nagase, T, Nakagoshi H, Gonda T.J., Ishii S.,** Delineation of three functional domains of the transcriptional activator encoded by the c-myb protonocogene, *Proc. Natl. Acad. Sci. USA.*, **86**: 5758-576, 1989
49. **Sano Y.J. Ishii S.,** Increased affinity of c-myb for CREB-binding protein (CBP) after CBP-induced acetylation, *J. Biol. Chem.*, **276**: 3674-3682, 2001
50. **Tomita A., Towatari M., Tsuzuki S., Hayakawa F., Kosugi H., Tamai K., Miyazaki T., Kinoshita T., Saito H.,** c-Myb acetylation at the carboxyl-terminal conserved domain by transcriptional co-activator p300, *Oncogene*, **19**: 444-451, 2000
51. **Gerd A., Blobel G. A.,** CREB-binding protein and p300: molecular integrators of hematopoietic transcription, *Blood*, **95**: 745-755, 2000
52. **Scott E. W., Simon M. C., Anastasi J., Singh H.,** Recruitment of transcription factor PU.1 in the development of multiple hematopoietic lineages, *Science*, **265**: 1573-1577, 1994
53. **Ben-David, Y., Bernstein, A.,** Friend virus-induced erythroleukemia and nultistage nature of cancer, *Cell*, **66**: 831-834, 1991
54. **Hong W., Kim A.Y., Ky S., Rakowski C., Seo S.-B., Chakravarti D., Atchison M., Blobel G.A.,** Inhibition of CBP-mediated protein acetylation by the Ets family oncoprotein PU.1, *Mol. Cell. Biol.*, **11**: 3729-3743, 2002
55. **Laribee R.N. Klemsz M.J.,** Loss of PU.1 expression following inhibition of histone deacetylation, *J. Immun.*, **167**: 5160-5166, 2001