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Mammalian Runt-Domain Proteins and Their Roles in Hematopoiesis, Osteogenesis, and Leukemia

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Abstract Mammalian Runt-domain-containing factors are structurally and functionally similar and have essential roles in hematopoiesis and osteogenesis. These factors can act as either positive or negative transcriptional regulators of tissue-specific genes whose promoters or enhancers contain the consensus Runt-domain binding element, TGT/CGGT. This sequence is necessary but not sufficient to regulate the transcription of a wide variety of genes. Runt-domain factors are promoter organizers that cooperate with neighboring factors and recruit transcriptional co-activators or co-repressors to regulate expression of tissue-specific genes. *AML1* is required for hematopoiesis and is a frequent target of chromosomal translocations in acute leukemias. Fusion proteins generated by these translocations are dominant repressors of genes regulated by the Runt-domain factors. *AML3* may also be involved in leukemogenesis. In addition, *AML3* has an essential role in bone development, as it is required for osteoblast differentiation and is mutated in patients with cleidocranial dysplasia. J. Cell. Biochem. Suppls. 32/33:51–58, 1999.

Key words: AML1; AML2; AML3; CBFA; PEPB2α; ETO; leukemia; bone; osteoblasts; cleidocranial dysplasia; Runx

Mammalian cells contain three genes that encode for proteins that share structural and functional similarity with the *Drosophila* protein, Runt. Each gene was cloned in multiple laboratories and thus has several names (Table I). We use the acute myeloid leukemia (AML) nomenclature in this review. The mammalian Runt-domain factors have required roles as transcriptional regulators of hematopoiesis and osteogenesis. Because of alternative exon usage, multiple isoforms of each mammalian gene product exist. Nevertheless, the mammalian factors are more than 50% identical at the amino acid level and greater than 93% identical within a 100–120 amino acid domain. This region is called the runt homology domain (RHD) because it is almost 70% identical to a region in Runt (Fig. 1). The RHD of human AML-1 mediates binding to the DNA sequence, TGT/CGGT, hereafter called the Runt domain binding element. This site was identified as the binding sequence for the Moloney murine leukemia virus core binding factor (CBF) and for polyoma enhancer binding protein 2 (PEBP2), which stimulates viral replication [reviewed by Lutterbach and Hiebert, 1999]. The RHD not only binds DNA but also mediates association with CBFβ, a protein that does not bind DNA itself but increases the affinity of the Runt-domain factors for DNA [reviewed by Lutterbach and Hiebert, 1999]. CBFβ is the human homologue of the *Drosophila* proteins, Brother and Big Brother. Together with AML-1, CBFβ forms a transcription factor complex that is one of the most common targets of chromosomal translocations in acute leukemia (Fig. 2).

As a result of space constraints, we are unable to reference all original studies with these factors in this review. With apologies to the omitted authors, the reader is referred to other recent reviews for detailed descriptions of early studies and more complete lists of references [Lutterbach and Hiebert, 1999; Mundlos, 1999].

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TABLE I. Nomenclature of Mammalian Runt-Domain Factors

Acute myeloid leukemia	Core binding factor	Human genome organization (HUGO)	Polyoma enhancer binding protein 2	Other
AML-1	CBFα-2	Runx-1	PEBP2α-B	
AML-2	CBFα-3	Runx-3	PEBP2α-C	
AML-3	CBFα-1	Runx-2	PEBP2α-A	OSF-2, NMP-2
CBFβ	CBFβ		PEBP2β	

The AML nomenclature was derived from studies in human leukemic cells. CBF and PEBP2 specify the roles of these factors in murine studies with the Moloney murine leukemia virus and polyoma viral enhancer, respectively. OSF-2 is osteoblast stimulating factor 2 and NMP-2 is nuclear matrix protein 2.

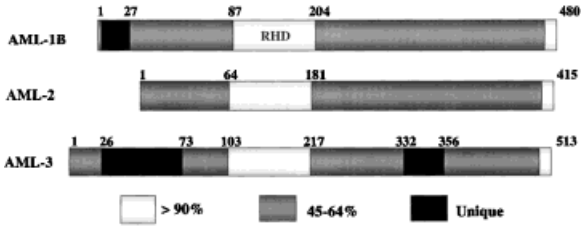

Fig. 1. The mammalian Runt-domain factors are highly conserved. The shaded regions indicate the amount of identity at the amino acid level. RHD denotes the runt homology domain. Numbers above each protein indicate a residue number.

AML-1 Is a Promoter Organizer That Regulates Hematopoiesis and Is Disrupted in Leukemias

AML1 was first cloned in humans as a target of the (8;21) chromosomal translocation. This rearrangement fuses the N-terminal half of AML-1 to Eight-Twenty-One (ETO), also known as myeloid translocation gene 8 (MTG8)) [Miyoshi et al., 1993]. *AML1* products are widely expressed in cells of the hematopoietic lineage and are transcriptional activators of numerous myeloid and lymphoid-specific genes [Lutterbach et al., 1998a; Westendorf et al., 1998, and references within]. DNA binding is mediated by the RHD and the C-terminus contains a transactivation domain [Meyers et al., 1995]. Nuclear localization requires a region distal to the RHD and nuclear matrix targeting is mediated by a domain in the C-terminus [Kanno et al., 1998; Zeng et al., 1997]. In transient transfection assays, AML-1B (also known as AML-1c), the largest transcriptionally active *AML1* isoform, is a relatively weak activator on its own; however, it cooperates with many factors to enhance transcription rates. Proteins identified as AML-1B binding partners, include C/EBP and Ets family members [Lutterbach and Hiebert, 1999; Lutterbach et al., 1998a; Westendorf et al., 1998]. Runt domain binding elements (TGT/CGGT) are often found within minimal promoter sequences of hematopoietic genes and are usually flanked by sites for C/EBP, Myb, AP-1, and Ets factors, suggesting that the cooperation is required for gene activation. *AML1* products also associate with transcriptional co-activators, including p300/CBP [Kitabayashi et al., 1998a], ALY [Bruhn et al., 1997], and YAP [Yagi et al., 1999]. These proteins do not bind DNA directly, but stimulate transcription by acetylating histones and/or by recruiting the RNA polymerase II transcription-initiating complex. Thus, AML-1 appears to aid in the organization of the promoter prior to transcriptional activation.

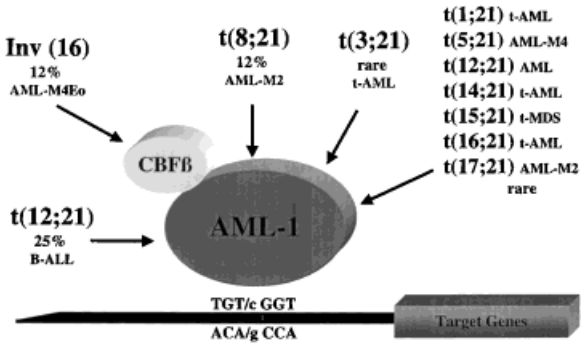

Fig. 2. The AML-1/CBFβ transcription factor complex is a frequent target of chromosomal translocations in acute leukemia. Ten translocations have been identified that fuse *AML1* sequences on chromosome 21 to other genes. *CBF*β is on chromosome 16 and is involved in a chromosomal inversion. These translocations are found in acute myeloid leukemias (AML), myeloid dysplasias (MDS), and B-cell acute lymphoblastic leukemias (B-ALL), some of which are therapy related (t-AML or t-MDS). The FAB subclassification (M2, M4) of leukemias with each translocation is indicated. The frequency of these translocations is shown as a percentage of leukemias with discernable translocations.

Although originally identified as transcriptional activators, *AML1* products are also transcriptional repressors [Lutterbach et al., 1999; Meyers et al., 1995]. In hindsight, this result is not surprising because Runt, the *Drosophila* homologue of AML-1, is a transcriptional repressor as well as an activator [Aronson et al., 1997, and references within]. AML-1B contains at least three domains that can contribute to transcriptional repression, suggesting that mul-

tiple regulatory controls exist. The final five amino acids, VWRPY, are conserved in Runt-domain proteins of all species. They are required for binding transducin-like enhancer of split (TLE) proteins, the mammalian homologues of the Groucho family of co-repressors [reviewed in Lutterbach and Hiebert, 1999]. This domain contributed to repression of a heterologous GAL4 promoter by GAL(1–147)-AML1 fusion proteins [Aronson et al., 1997]. However, it is not required for repression of the $p21^{Waf1/Cip1}$ promoter [Lutterbach et al., 1999]. This led to the discovery of a repression domain immediately C-terminal to the RHD (amino acids 208–237). This domain binds the co-repressor mSin3A in vivo [Lutterbach et al., 1999]. Another repression domain identified in the $p21^{Waf1/Cip1}$ promoter repression studies lies between amino acids 290–387 [Lutterbach et al., 1999]. The mechanism by which this domain mediates repression is unknown, but it may recruit other co-repressors.

The AML-1/CBFβ transcription factor complex is required for hematopoietic development [see Lutterbach and Hiebert, 1999, for references]. Deletion of *AML1* prevented differentiation of primitive hematopoietic stem cells in the fetal liver. Knock-out mice died during embryogenesis from central nervous system (CNS) hemorrhages, and $AML1^{-/-}$ embryonic stem cells failed to contribute to the hematopoietic system of chimeric mice. Moreover, the number of erythroid and myeloid progenitors was reduced in $AML1^{+/-}$ mice. Although CBFβ is ubiquitously expressed, $CBF\beta^{-/-}$ mice surprisingly exhibited the identical phenotype as $AML1^{-/-}$ mice. These results demonstrated that the AML-1/CBFβ transcription factor complex is required for hematopoiesis.

AML-2 Regulates Expression of Hematopoietic-Specific Genes

AML-2 is the least understood of the mammalian Runt-domain factors. It is expressed in hematopoietic cells and is at its highest levels in B lymphocytes and myeloid cells [Meyers et al., 1996]. Like related family members, AML-2 associates with CBFβ and activates transcription of genes containing the Runt domain binding element [Meyers et al., 1996]. Although it appears to be a slightly weaker activator on its own than AML-1B [Meyers et al., 1996; Westendorf et al., 1998], AML-2 cooperates with C/EBP-α to synergistically activate transcription [Westendorf et al., 1998]. AML-2 also binds to the mSin3A and TLE co-repressors [Levanon et al., 1998; Lutterbach et al., 1999]. Thus, AML-2 and AML-1B appear to have redundant transcriptional activities in transient transfection assays.

AML2 localizes to human chromosome 1p36.11–p36.13. The cDNA for human AML-2 (CBFα, PEBP2αC) was isolated from T and myeloid cell line libraries in low-stringency hybridization screens using murine PEBP2αA and/or PEBP2αB sequences as probes 11 [reviewed in Lutterbach and Hiebert, 1999]. AML-2 is smallest of the mammalian Runt-domain factors. While the RHD and VWRPY domains are highly conserved, AML-2 appears to lack an exon between amino acids 235 and 236 (Fig. 1). The absent sequence corresponds to amino acids 294–326 of AML-1B, which are within the boundaries of a transcriptional inhibitory domain and the p300 interaction site [Kanno et al., 1998; Kitabayashi et al., 1998a; Lutterbach and Hiebert, 1999; Lutterbach et al., 1999]. The functional consequence of these absent amino acids in AML-2 is unknown.

The only unique biological property that has been ascribed to AML-2 at this time is that its expression appears to be preferentially induced in splenic B cells and some B-cell lines by TGF-β1, which stimulates class switching to IgA [Shi and Stavnezer, 1998]. Although all mammalian Runt domain family members can activate transcription of the Ig-α promoter, AML-2 appears to be the major component of the TGFβ-inducible complex. The absolute requirement for AML-2 in development or in B-lymphocyte differentiation is not known as an *AML2* (*CBFα3/PEBP2αC*)-deficient mouse has not yet been described.

AML-3 Is Essential for Bone Development and Is Active in Hematopoietic Cells

The third mammalian Runt-domain factor (human AML-3 or murine CBFA-1/ PEPB2αA) is a required transcriptional regulator of osteoblast differentiation and bone formation [Ducy et al., 1997; Komori et al., 1997; Otto et al., 1997]. The recognition that a tissue-specific nuclear matrix protein, NMP-2, was related to AML-1 and that both of these factors could activate the osteocalcin promoter through Runt domain binding elements [Banerjee et al., 1996; Geoffroy et al., 1995; Merriman et al., 1995] were the first pieces of evidence that suggested

a role for Runt-domain factors in bone cells. Mice lacking *AML3* were smaller than normal and demonstrated a complete lack of both intramembranous and endochondral bone formation [Komori et al., 1997; Otto et al., 1997]. Cartilage development appeared to be normal. The lack of ossified ribs prevented newborn $AML3^{-/-}$ mice from breathing and caused death shortly after birth. The defect in bone formation resulted from the arrest of osteoblast maturation and the subsequent decreased expression of bone matrix proteins [Komori et al., 1997; Otto et al., 1997]. Other studies demonstrated that treatment of osteoblasts with *AML3* antisense oligonucleotides blocked the expression of several osteoblast-specific markers [Ducy et al., 1997]. Conversely, overexpression of an AML-3 isoform osteoblast-specific transcription factor 2 (OSF-2) in fibroblasts induced expression of bone-specific genes [Ducy et al., 1997]. Postnatal overexpression of a dominant negative form of AML-3 in differentiating osteoblasts induced an osteopenic phenotype [Ducy et al., 1999]. Thus, AML-3 is required for bone formation during and beyond embryonic development.

Like other mammalian Runt-domain proteins, AML-3 was first thought to play an important role in hematopoiesis. *AML3*, *CBFα1*, and *PEBP2αA* cDNAs were isolated from monocyte, thymus, and Ras-transformed fibroblast libraries, respectively [reviewed in Lutterbach and Hiebert, 1999]. *PEBP2αA* mRNA levels were high in T lymphocytes and low in B cells. AML-3 activates transcription of T-cell-specific genes, and cooperates with C/EBP-α to activate a myeloid-specific gene [Westendorf et al., 1998, and references within]. Surprisingly, few hematopoietic defects were seen in mice lacking *AML3* [Komori et al., 1997; Otto et al., 1997]. Liver and spleen hematopoiesis in $AML3^{-/-}$ embryos appeared to be normal through E17.5. At E18.5, $AML3^{-/-}$ embryos had more granulocytes and fewer B cells than were found in normal embryos. $AML3^{-/-}$ mice also demonstrated excessive extramedullary hematopoiesis in the spleen and liver due to the congenital absence of bone marrow [Deguchi et al., 1999].

AML-3 shares many sequence similarities, binding partners, and functional activities with other Runt domain family members (Fig. 1). The RHD is 93% identical to the corresponding domain in AML-1, and the last five amino acids, VWRPY, are 100% conserved. Like other family members, AML-3 binds to the mSin3A corepressor [Lutterbach et al., 1999]. Thus, AML-3 may act as a transcriptional repressor as well as an activator. The most noticeable structural difference between AML-3 and AML-1 and AML-2 is the presence of two unique domains in AML-3. A 41-amino acid glutamine/alanine (Q/A)-rich region is proximal to the RHD. An additional unique domain of 22 amino acids is inserted in the C-terminus (Fig. 1). The function of these domains is not clear. It was reported that the QA domain prevented association of the AML-3 isoform OSF-2 with CBFβ [Thirunavukkarasu et al., 1998]. However, heterodimerization with CBFβ increased the affinity of other AML-3 isoforms for DNA [Ogawa et al., 1993] and endogenous AML-3 migrates at a rate consistent with AML-3/CBFβ heterodimers in gel-shift assays [Meyers et al., 1996]. OSF-2 contains a unique N-terminal exon that is only present in mice [Xiao et al., 1998]. The absence of the OSF-2 isoform in humans suggests that the unique exon is not essential for AML-3 function [Xiao et al., 1998].

AML3 maps to human chromosome 6p21 and is mutated in cleidocranial dysplasia (CCD) patients [reviewed in Mundlos, 1999]. CCD is a rare autosomal dominant disease characterized by multiple skeletal abnormalities, including anaplastic or dysplastic clavicles, patent fontanelles, and supernumerary teeth. In CCD patients and in a radiation-induced mouse model for CCD, one *AML3*/Runx2 allele contains an insertion, deletion, or missense mutation in the RHD or C-terminal transactivation domain that creates transcriptionally inactive AML-3 proteins. Heterozygous loss of *AML3* in CCD patients and $AML3^{+/-}$ mice is also sufficient to produce the CCD phenotypes [Komori et al., 1997; Mundlos, 1999; Otto et al., 1997]. Thus, heterozygous loss of function of AML-3 causes CCD. The mechanism by which AML-3 haploinsufficiency induces this phenotype remains to be elucidated.

ROLES OF MAMMALIAN RUNT-DOMAIN PROTEINS IN LEUKEMIA

The AML-1/CBFβ transcription factor complex is one of the most frequent targets of chromosomal translocations in acute leukemias (Fig. 2). *AML1* maps to chromosome 21q22 and *CBF*β is found on chromosome 16q22. Thus far, these genes appear to be affected by as many as eleven chromosomal translocations, but the

breakpoints of many of these translocations have not yet been cloned [reviewed in Lutterbach and Hiebert, 1999]. The t(8;21)(q22;q22) is found in approximately 12% of AML with discernable chromosomal translocations and fuses the N-terminus and RHD of AML-1 to all but the first 30 amino acids of ETO. The t(16; 21)(q24;q22) and t(3;21)(q26;q22) translocations also fuse the amino terminus and RHD of AML-1 to an ETO-related protein, MTG16, and to the transcriptional repressor, EVI-I, respectively. These translocations are examples of relatively rare alterations in the *AML1* allele that are present in therapy-related AML. An additional 12–15% of AMLs contain the *inv(16)(p13;q22)* translocation, which fuses *CBFβ* to *MYH11*, the gene for a smooth muscle myosin heavy chain (SMMHC) [reviewed in Lutterbach and Hiebert, 1999]. The *t(12; 21)(p13;q22)* translocation joins the first 336 amino acids of translocation-Ets-leukemia (TEL) to nearly all of AML-1B [reviewed in Lutterbach and Hiebert, 1999]. It is found in up to 25% of pediatric acute B-lymphoblastic leukemias (B-ALL) with translocations. Thus, nearly a quarter of acute myeloid and B-lymphoblastic leukemias with discernable chromosomal translocations have an altered *AML1* or *CBF*β allele.

There are many functional consequences of these translocations. First, several of the translocations transform AML-1 into a constitutive transcriptional repressor of target genes [Lutterbach and Hiebert, 1999]. The fusion partners of AML-1 in the t*(8;21)* and *t(12;21)*, ETO and TEL, respectively, contribute multiple protein-protein interaction domains that bind components of the mSin3/N-CoR/HDAC co-repressor complex [Fenrick et al., 1999; Lutterbach and Hiebert, 1999; Lutterbach et al., 1998b]. These additional domains appear to stabilize the recruitment of co-repressor complexes to Runt domain binding elements. AML-1/ETO can block AML-1B, AML-2, and AML-3-induced transactivation of target genes [Westendorf et al., 1998]. AML-1/ETO can also block Ets-factor- and C/EBP-α-dependent activation [Lutterbach et al., 1998a; Westendorf et al., 1998]. However, all hematopoietic promoters are not affected by each of the fusion proteins in the same way [Fenrick et al., 1999]. For example, AML-1/ETO does not repress the basal activity of a defensin promoter, but does block transcription of the multi-drug resistance-1 promoter. By contrast, TEL/AML-1B represses both of these promoters. The different activities of the translocation fusion proteins may determine the leukemic phenotype.

Another possible consequence of the translocations is the disruption of normal hematopoietic cell differentiation. Overexpression of AML-1/ETO slows granulocyte-colony-stimulating factor (G-CSF)-induced differentiation of myeloid cells [Kitabayashi et al., 1998b; Westendorf et al., 1998]. *Inv(16)* transgenic mice show impaired neutrophil differentiation and develop granulocytic dysplasia [Kogan et al., 1998]. Primitive hematopoiesis is impaired in *AML1/ETO* and *CBF*β*/MYH11* heterozygous knock-in mice in a similar manner as in *AML1* and *CBF*β homozygous knock-out mice [reviewed in Lutterbach and Hiebert, 1999]. Although these knock-in mice do not develop leukemia, AML-1/ETO-expressing embryos contained dysplastic multilineage hematopoietic progenitors that had increased self-renewal.

Not only is *AML1* a frequent target of chromosomal translocations in acute leukemias, it is also mutated in 3% of AML patients. Single amino acid changes or frameshift mutations disable the DNA binding and transcriptional activities of AML-1 in these patients [Osato et al., 1999]. The frequency with which *AML1* is altered in leukemias is consistent with it being a key regulator of hematopoiesis.

AML-3 has not yet been directly associated with cancer, although several pieces of evidence hint that that it may have a role in tumorigenesis. The *AML3 (til-1/CBFA1/PEBP2aA)* locus was identified as the site of retroviral insertions in lymphomas from CD2-myc transgenic mice, suggesting that overexpression of some AML-3 isoforms may cooperate with c-myc to induce T cell lymphomas [Stewart et al., 1997]. In bone cells, AML-3 induces expression of several genes that may be associated with abnormal cell growth or metastasis, including the TGFβ-Type I receptor [Ji et al., 1998] and osteopontin [Sato et al., 1998].

FUTURE DIRECTIONS

Mammalian Runt-domain factors are key regulators of hematopoietic and osteoblast development. They appear to regulate these pathways by controlling the transcription of tissue-specific genes. Their ability to either activate or repress transcription suggests that other factors and signals transiently regulate mamma-

lian Runt-domain proteins. One prospect for future studies is the identification of switches that regulate the activation versus repression activities of Runt-domain proteins (Fig. 3). It is possible that post-translational modifications, such as phosphorylation and acetylation may affect the activity of these factors. For example, AML-1 may be capable of responding to extracellular signals transmitted by Erk-family kinases [reviewed in Lutterbach and Hiebert, 1999]. Cell and promoter specific factors may also affect the activities of Runt-domain family members.

The apparent redundancies between the Runt-domain family members at the transcriptional level is not surprising given the highly conserved nature of their DNA binding domain and their ability to act through the same DNA binding site. However, biological data from knock-out mice indicate that activities are not redundant. This may be explained by the expression patterns of the individual genes. Nevertheless, a second prospect for the future is delineating the unique properties of these factors. The overlapping transcriptional activities and expression pattern of Runt-domain factors suggests that their unique biological properties may be dependent on extracellular signals that specifically affect a given family member. Although it is highly probable that sequence differences outside of the highly conserved Runt homology domain may affect the responses of mammalian Runt-domain family members to cellular signals, it is also possible that their own expression is regulated by specific signals.

Haploinsufficiency of mammalian Runt-domain family members is emerging as a crucial element in hematopoietic and osteogenic development. Developmental defects in hematopoiesis and bone development in *AML1*$^{+/-}$ and *AML3*$^{+/-}$ mice, respectively, suggests that disruption of a single copy of these genes can have severe effects. Knowledge gained from molecular studies has been and will be useful in dissecting the mechanisms by which mutations modulate the functions of AML-1 and AML-3. Understanding the mechanisms by which heterologous loss of function affects the transcriptional activity of mammalian Runt-domain family members will provide insights into the development of leukemia and cleidocranial dysplasia.

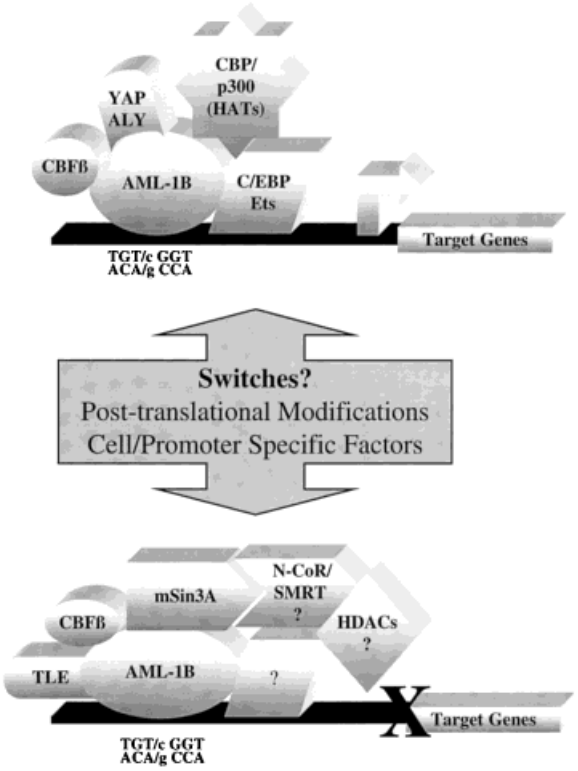

Fig. 3. AML-1B is an activator and repressor of transcription. To maximally activate transcription (top) the AML-1B/CBFβ complex associates with a variety of factors, including other DNA binding proteins (C/EBP and Ets family members) and co-activators (ALY, YAP, p300/CBP). Repression (bottom) is dependent upon direct association with TLE proteins and mSin3A. AML-1B may also associate either directly or indirectly with histone deacetylases (HDAC), Nuclear hormone co-repressor (N-CoR) or the silencing mediator of retinoid and thyroid hormones (SMRT). Potential molecular "switches" controlling these activities include post-translational modifications or cell and promoter-specific factors.

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REFERENCES

Aronson BD, Fisher AL, Blechman K, Caudy M, Gergen JP. 1997. Groucho-dependent and -independent repression activities of Runt domain proteins. Mol Cell Biol 17:5581–5587.

Banerjee C, Hiebert SW, Stein JL, Lian JB, Stein GS. 1996. An AML-1 consensus sequence binds an osteoblast-specific complex and transcriptionally activates the osteocalcin gene. Proc Natl Acad Sci USA 93:4968–4973.

Bruhn L, Munnerlyn A, Grosschedl R. 1997. ALY, a context-dependent coactivator of LEF-1 and AML-1, is required for TCRalpha enhancer function. Genes Dev 11:640–653.

Deguchi K, Yagi H, Inada M, Yoshizaki K, Kishimoto T, Komori T. 1999. Excessive extramedullary hematopoiesis in Cbfa1-deficient mice with a congenital lack of bone marrow. Biochem Biophys Res Commun 255:352–359.

Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G. 1997. Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation [see comments]. Cell 89:747–754.

Ducy P, Starbuck M, Priemel M, Shen J, Pinero G, Geoffroy V, Amling M, Karsenty G. 1999. A Cbfa1-dependent genetic pathway controls bone formation beyond embryonic development. Genes Dev 13:1025–1036.

Fenrick R, Amann JM, Lutterbach B, Wang L, Westendorf JJ, Downing JR, Hiebert SW. 1999. Both TEL and AML-1 contribute repression domains to the t(12;21) fusion protein. Mol Cell Biol 19:6566–6574.

Geoffroy V, Ducy P, Karsenty G. 1995. A PEBP2 alpha/AML-1-related factor increases osteocalcin promoter activity through its binding to an osteoblast-specific cis-acting element. J Biol Chem 270:30973–30979.

Ji C, Casinghino S, Chang DJ, Chen Y, Javed A, Ito Y, Hiebert SW, Lian JB, Stein GS, McCarthy TL, Centrella M. 1998. CBFa(AML/PEBP2)-related elements in the TGF-beta type I receptor promoter and expression with osteoblast differentiation. J Cell Biochem 69:353–363.

Kanno T, Kanno Y, Chen LF, Ogawa E, Kim WY, Ito Y. 1998. Intrinsic transcriptional activation-inhibition domains of the polyomavirus enhancer binding protein 2/core binding factor alpha subunit revealed in the presence of the beta subunit. Mol Cell Biol 18:2444–2454.

Kitabayashi I, Yokoyama A, Shimizu K, Ohki M. 1998a. Interaction and functional cooperation of the leukemia-associated factors AML1 and p300 in myeloid cell differentiation. EMBO J 17:2994–3004.

Kitabayashi I, Ida K, Morohoshi F, Yokoyama A, Mitsuhashi N, Shimizu K, Nomura N, Hayashi Y, Ohki M. 1998b. The AML1-MTG8 leukemic fusion protein forms a complex with a novel member of the MTG8(ETO/CDR) family, MTGR1. Mol Cell Biol 18:846–858.

Kogan SC, Lagasse E, Atwater S, Bae SC, Weissman I, Ito Y, Bishop JM. 1998. The PEBP2betaMYH11 fusion created by Inv(16)(p13;q22) in myeloid leukemia impairs neutrophil maturation and contributes to granulocytic dysplasia. Proc Natl Acad Sci USA 95:11863–11868.

Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, Shimizu Y, Bronson RT, Gao YH, Inada M, Sato M, Okamoto R, Kitamura Y, Yoshiki S, Kishimoto T. 1997. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts [see comments]. Cell 89:755–764.

Levanon D, Goldstein RE, Bernstein Y, Tang H, Goldenberg D, Stifani S, Paroush Z, Groner Y. 1998. Transcriptional repression by AML1 and LEF-1 is mediated by the TLE/Groucho corepressors. Proc Natl Acad Sci USA 95:11590–11595.

Lutterbach B, Hiebert SW. 2000. The role of the transcription factor AML-1 in acute leukemia and hematopoietic differentiation. Gene (in press).

Lutterbach B, Sun D, Schuetz J, Hiebert SW. 1998a. The MYND motif is required for repression of basal transcription from the multidrug resistance 1 promoter by the t(8;21) fusion protein. Mol Cell Biol 18:3604–3611.

Lutterbach B, Westendorf JJ, Linggi B, Patten A, Moniwa M, Davie JR, Huynh KD, Bardwell VJ, Lavinsky RM, Rosenfeld MG, Glass C, Seto E, Hiebert SW. 1998b. ETO, a target of t(8;21) in acute leukemia, interacts with the N-CoR and mSin3 corepressors. Mol Cell Biol 18:7176–7184.

Lutterbach B, Westendorf JW, Linggi B, Isaac S, Seto E, Hiebert SW. 2000. A mechanism of repression by acute myeloid leukemia-1, the target of multiple chromosomal translocations in acute leukemia. J Biol Chem (in press).

Merriman HL, van Wijnen AJ, Hiebert S, Bidwell JP, Fey E, Lian J, Stein J, Stein GS. 1995. The tissue-specific nuclear matrix protein, NMP-2, is a member of the AML/CBF/PEBP2/runt domain transcription factor family: interactions with the osteocalcin gene promoter. Biochemistry 34:13125–13132.

Meyers S, Lenny N, Hiebert SW. 1995. The t(8;21) fusion protein interferes with AML-1B-dependent transcriptional activation. Mol Cell Biol 15:1974–1982.

Meyers S, Lenny N, Sun W, Hiebert SW. 1996. AML-2 is a potential target for transcriptional regulation by the t(8;21) and t(12;21) fusion proteins in acute leukemia. Oncogene 13:303–312.

Miyoshi H, Kozu T, Shimizu K, Enomoto K, Maseki N, Kaneko Y, Kamada N, Ohki M. 1993. The t(8;21) translocation in acute myeloid leukemia results in production of an AML1-MTG8 fusion transcript. EMBO J. 12:2715–2721.

Mundlos S. 1999. Cleidocranial dysplasia: clinical and molecular genetics. J Med Genet 36:177–182.

Ogawa E, Inuzuka M, Maruyama M, Satake M, Naito-Fujimoto M, Ito Y, Shigesada K. 1993. Molecular cloning and characterization of PEBP2 beta, the heterodimeric partner of a novel *Drosophila* runt-related DNA binding protein PEBP2 alpha. Virology 194:314–331.

Osato M, Asou N, Abdalla E, Hoshino K, Yamasaki H, Okubo T, Suzushima H, Takatsuki K, Kanno T, Shigesada K, Ito Y. 1999. Biallelic and heterozygous point mutations in the runt domain of the AML1/PEBP2alphaB gene associated with myeloblastic leukemias. Blood 93: 1817–1824.

Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC, Rosewell IR, Stamp GW, Beddington RS, Mundlos S, Olsen BR, Selby PB, Owen MJ. 1997. Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development [see comments]. Cell 89:765–771.

Sato M, Morii E, Komori T, Kawahata H, Sugimoto M, Terai K, Shimizu H, Yasui T, Ogihara H, Yasui N, Ochi T, Kitamura Y, Ito Y, Nomura S. 1998. Transcriptional regulation of osteopontin gene in vivo by PEBP2alphaA/CBFA1 and ETS1 in the skeletal tissues. Oncogene 17: 1517–1525.

Shi MJ, Stavnezer J. 1998. CBF alpha3 (AML2) is induced by TGF-beta1 to bind and activate the mouse germline Ig alpha promoter. J Immunol 161:6751–6760.

Stewart M, Terry A, Hu M, O'Hara M, Blyth K, Baxter E, Cameron E, Onions DE, Neil JC. 1997. Proviral insertions induce the expression of bone-specific isoforms of PEBP2alphaA (CBFA1): evidence for a new myc collaborating oncogene. Proc Natl Acad Sci USA 94:8646–8651.

Thirunavukkarasu K, Mahajan M, McLarren KW, Stifani S, Karsenty G. 1998. Two domains unique to osteoblast-specific transcription factor Osf2/Cbfa1 contribute to its transactivation function and its inability to heterodimerize with Cbfbeta. Mol Cell Biol 18:4197–4208.

Westendorf JJ, Yamamoto CM, Lenny N, Downing JR, Selsted ME, Hiebert SW. 1998. The t(8;21) fusion product, AML-1-ETO, associates with C/EBP-alpha, inhibits C/EBP-alpha-dependent transcription, and blocks granulocytic differentiation. Mol Cell Biol 18:322–333.

Xiao ZS, Thomas R, Hinson TK, Quarles LD. 1998. Genomic structure and isoform expression of the mouse, rat and human Cbfa1/Osf2 transcription factor. Gene 214: 187–197.

Yagi R, Chen LF, Shigesada K, Murakami Y, Ito Y. 1999. A WW domain-containing yes-associated protein (YAP) is a novel transcriptional co-activator. EMBO J 18:2551–2562.

Zeng C, van Wijnen AJ, Stein JL, Meyers S, Sun W, Shopland L, Lawrence JB, Penman S, Lian JB, Stein GS, Hiebert SW. 1997. Identification of a nuclear matrix targeting signal in the leukemia and bone-related AML/CBF-alpha transcription factors. Proc Natl Acad Sci USA 94:6746–6751.