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PROSPECTS

Upstream and Downstream Targets of RUNX Proteins

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Abstract In recent years, the in vivo role of the three members of the RUNX family of transcription factors has in part been elucidated. While Runx1 is essential for mature haematopoiesis and Runx2 for osteochondrogenesis, Runx3 has a function in the nervous system. Translocations and mutations affecting the *RUNX1* gene are clearly implicated in leukemogenesis whereas recent data suggest that changed expression levels of *RUNX3* may be involved in gastric carcinogenesis. Germ line mutations in *RUNX2* have been identified in patients with an autosomal dominant skeletal disorder, cleidocranial dysplasia. While a number of pathways have been delineated that regulate RUNX activity, transcription factors binding to RUNX promoters are only beginning to be identified. A growing number of genes have been characterised that are being regulated in their transcriptional activity by different RUNX proteins. Whether a particular RUNX protein specifically targets a defined subset of downstream genes or whether there is some redundancy as to which RUNX protein activates which target promoter remains to be elucidated. J. Cell. Biochem. 89: 9–18, 2003.

Key words: RUNX; transcriptional regulation; transcription factor; AML

The RUNX family of transcription factor proteins consists of three known members (RUNX1–3) that share a high degree of sequence homology within most of their coding regions. The amino terminal part of these proteins comprises a region of 128 amino acids with conserved sequence homology to the *Drosophila* transcription factor runt. This so-called runt homology domain (RHD) binds to DNA in a sequence specific manner.

The finding that RUNX1 (aka AML1) as well as the RUNX-interacting protein CBFB are frequent partners in fusion proteins resulting from chromosomal translocations (the most frequent being t[8;21], inv[16], t[12;21]) in acute leukaemias and associated with specific leukaemia phenotypes (AML of FAB subtype M2, M4eo, ALL with B-cell precursor phenotype, respectively) with relatively good prognosis, has generated considerable interest in the haematological field. In recent years "knock-out" mice have been generated for all three *Runx* genes, and the analysis of their phenotypes has provided new insights into the in vivo function of RUNX proteins. Even though their expression patterns overlap in some organs, the phenotypes produced by the lack of the individual factors are distinct and point to key roles in the development of different cell lineages. Deficiency in Runx1 expression leads to an early block in haematopoietic differentiation. Therefore Runx1 knockout mice completely lack mature haematopoiesis [Okuda et al., 1996; Wang et al., 1996]. In contrast, homozygous null mutants for Runx2 (AML3) show an arrested osteoblast differentiation and thus are totally devoid of bone [Komori et al., 1997; Otto et al., 1997]. Recently, we and others have reported on the phenotype of Runx3 (AML2) deficient mice [Inoue et al., 2002; Levanon et al., 2002; Li et al., 2002]. While Li et al. [2002] described a hyperplasia of the gastric mucosa and suggested a link between lack of Runx3 expression and human gastric cancer, we did not find any evidence of gastrointestinal pathology in our

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mice. Both Runx3 deficient mouse strains suffer from severe limb ataxia due to a loss of dorsal root ganglia proprioceptive neurons.

In recent years the pathways in which the different RUNX proteins are involved are beginning to be elucidated.

SIGNALS UPSTREAM OF RUNX PROTEINS

Relatively little is known about the factors and pathways that control the expression of the three RUNX genes. It has become clear that each of the three genes is transcribed from two promoters [Ghozi et al., 1996; Xiao et al., 1998; Rini and Calabi, 2001]. The sequences of all six promoter regions contain several RUNX binding sites and indeed, auto regulation of RUNX expression by RUNX proteins has been shown [Drissi et al., 2000]. It is not clear, however, whether each RUNX protein acts mainly on its own promoter, for example, to stabilise the expression pattern or to prevent too high expression levels via negative feedback, or whether RUNX proteins bind to the promoters of the genes of the other two family members to inhibit their expression, in a kind of intrafamilial competition.

Promoter analysis proved transcriptional activity of both, the proximal (P2) and distal (P1) promoters of the *RUNX1* gene [Ghozi et al., 1996]. A more detailed analysis of the distal (P1) promoter of *Runx2* recently identified two distinct sites regulating transcriptional activity. While an NF1 site seems to bind NF1-A in non-osseous cells and suppresses *Runx2* gene activity, an AP1 site preferentially binds FosB to increase transcription in osteoblastic cells [Zambotti et al., 2002]. Furthermore the transcription factors Msx2, Bapx1, Hoxa-2 and PPARγ2 have been shown to regulate Runx2 expression, although no evidence for a direct interaction of these factors with the Runx2 promoters could be demonstrated so far [Kanzler et al., 1998; Lecka-Czernik et al., 1999; Tribioli and Lufkin, 1999; Satokata et al., 2000].

RUNX1, RUNX2 and RUNX3 transcription appears to be regulated by retinoids [Tanaka et al., 1995; Le et al., 1999; Jimenez et al., 2001]. Treatment with all-trans retinoic acid of U937 and HL-60 myeloid leukaemia cells, respectively, leads to an increase in expression levels of RUNX1 and RUNX3. RUNX3 expression in these cells could be augmented by vitamin D_3. Therefore RUNX3 expression may be regulated by retinoid/vitamin D nuclear receptors. RUNX2 transcription decreases when MC3T3 calvaria or ROS17/2.8 osteosarcoma cells are treated with vitamin D_3. Importantly, in ROS24.1 osteosarcoma cells lacking functional vitamin D receptors, no such effect is observed. The proximal RUNX2 promoter (P2) contains a vitamin D responsive element, and mutations in this element abolish vitamin D dependent regulation [Drissi et al., 2002]. Thus all three RUNX genes are transcriptionally controlled by retinoid/vitamin D nuclear receptors, albeit with the regulatory effect being positive for RUNX1 and RUNX3 and negative for RUNX2. Two other pathways involving nuclear receptors are directly or indirectly involved in RUNX2 regulation, since its expression can be increased by treatment of cells with tamoxifen and similar substances that act as estrogen receptor modulators, as well as with glucocorticoid hormone dexamethasone [Prince et al., 2001; Tou et al., 2001].

A number of cytokines have been shown to influence expression levels of RUNX2. TGFβ suppresses RUNX2 expression in ROS17/2.8 osteosarcoma cells, while in C2C12 myoblast precursor cells it induces RUNX2 transcription levels [Lee et al., 2000; Alliston et al., 2001]. The signal triggered by TGFβ is mediated through TGFβ receptors that recruit Smad factors. The different effects in the two cell lines on RUNX2 expression may therefore reflect the cellular context of accessory proteins involved in control of RUNX2 expression. Bone morphogenetic proteins (BMP) are members of the TGFβ superfamily and exert their effects also through Smad proteins. BMP-2 and BMP4/7 were shown to induce Runx2 expression [Tsuji et al., 1998; Lee et al., 2000; Banerjee et al., 2001]. Other cytokines that were shown to regulate RUNX2 expression are FGF and TNFalpha [Zhou et al., 2000; Gilbert et al., 2002].

Translation has been demonstrated to be a further level of control of RUNX expression [Pozner et al., 2000; Sudhakar et al., 2001]. In addition, post-translational events such as phosphorylation via MAPK pathway seem to play a role in the transactivating potential of RUNX proteins [Tanaka et al., 1996; Xiao et al., 2000]. These data providing insight into pathways governing RUNX expression are summarised in Figures 1 and 2. More work will have to be directed towards identifying the

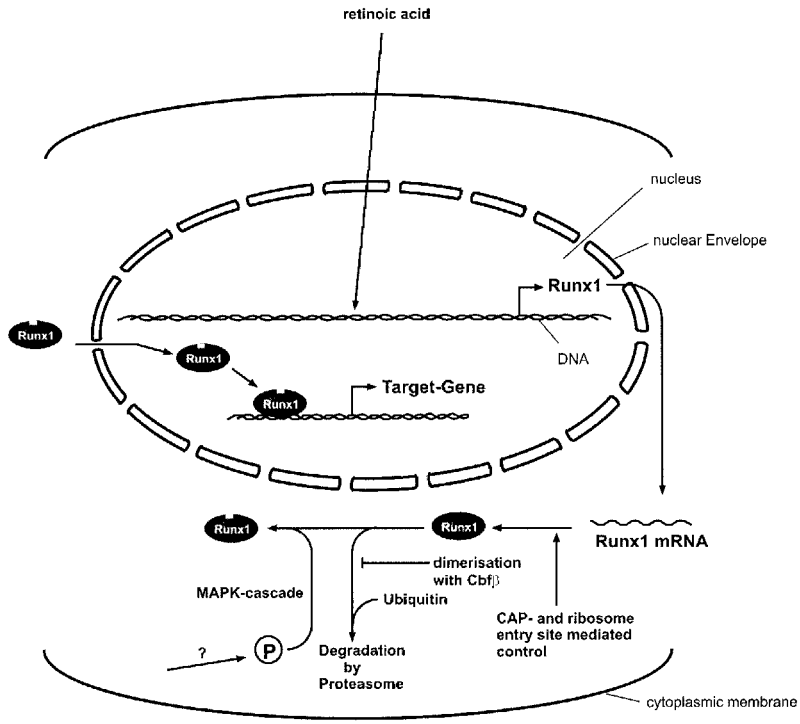

Fig. 1. Regulation of RUNX1 activity. White squares indicate phosphorylated proteins.

transcription factors immediately upstream of the different *RUNX* genes. Such studies will elucidate the basis for the evolution of different functions for the three genes that are so similar in structure and encoded proteins.

DOWNSTREAM TARGETS OF RUNX PROTEINS

RUNX proteins regulate the activity of their target genes by binding to the respective promoter or enhancer elements in a sequence-specific manner. The runt domain mediates sequence specificity for the consensus sequence that is cited as either 5′-PuACCPuCA-3′, or in reverse orientation, 5′-TG(T/C)GGT-3′. However, the sequence 5′-AACCACA-3′ seems to occur somewhat more frequently in proven or bona fide RUNX target promoters than other sequences also in agreement with the consensus. Table III lists putative or proven RUNX binding sites in the promoter regions of various target genes.

It is by now clear that RUNX proteins bind to their targets as part of multiprotein complexes. The cofactor CBFB that interacts with the RHD seems to be crucial for an effective interaction with the binding sites in the respective target promoters or enhancers. Cooperation with RUNX proteins at target promoters has also been described for the transcription factors c/EBPalpha, c/EBPbeta, c/EBPdelta, ets-1, MEF, PAX5 and Mi through physical interaction [Wotton et al., 1994; Petrovick et al., 1998; Libermann et al., 1999; Mao et al., 1999; Morii et al., 1999; Gutierrez et al., 2002]. Other proteins like ALY may stabilise such interactions [Bruhn et al., 1997].

RUNX proteins are able to either increase or actively inhibit the transcriptional activity of target genes, most likely depending on the specific cell type as well as the particular target gene. For transcriptional repression, RUNX proteins recruit transcriptional co-repressors like sin3A or members of the TLE protein family, the latter interacting with the carboxy-terminal VWRPY pentapeptide motif [Imai et al., 1998; Levanon et al., 1998; Wang et al., 1998; Javed et al., 2000].

Several genes have been characterised as RUNX targets (see Tables I and II for compilation). While for some of these (e.g., osteocalcin) there is very firm experimental proof that RUNX proteins bind to the respective promoter and modify transcriptional activity, for others there is sometimes less direct evidence (e.g.,

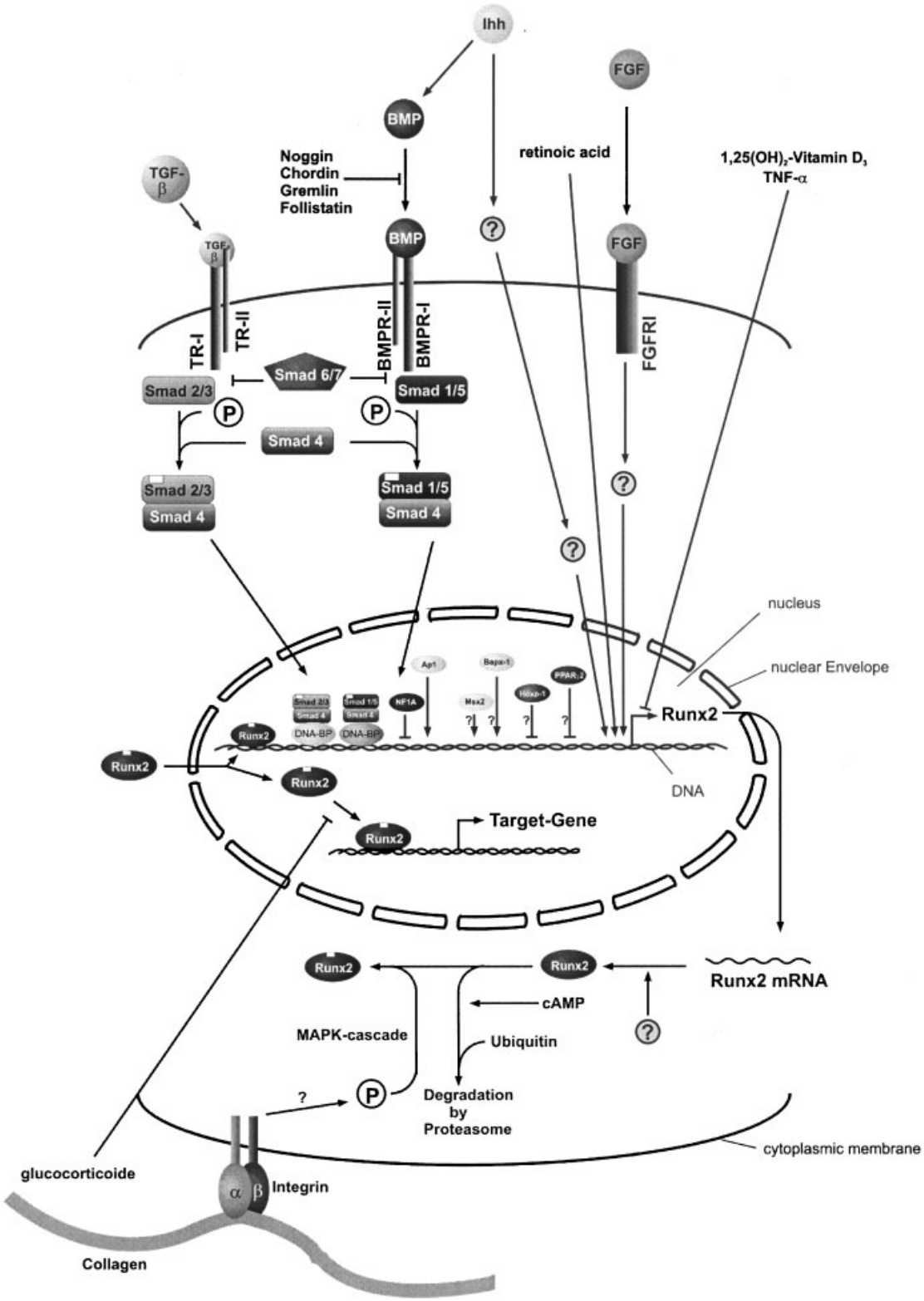

Fig. 2. Pathways involved in regulating the activity of RUNX2. White squares indicate phosphorylated proteins. Question marks indicate that for these factors a direct interaction with the RUNX2 promoter has not been shown.

TABLE I. Target Genes of RUNX Proteins in Haematopoietic Tissues

Tissue	Gene	Expressed in	Regulated by	Up/down	References
Lymphoid	Germ-line IgA1	B-cells	Runx1	Up	Pardali et al. [2000]
	Germ-line IgCα	B-cells	Runx3	Up	Hanai et al. [1999]
	B-cell specific tyrosine kinase (BLK)	B-cells	Runx1	Up	Libermann et al. [1999]
	TCR α,β,γ,δ-chains	T-cells	Runx1	Up	Hsiang et al. [1993]; Giese et al. [1995]; Hernandez-Munain and Krangel [1995]; Meyers et al. [1995]; Bruhn et al. [1997]
	IL-3	T-cells	Runx1		Mao et al. [1999]
	Granzyme B	T-cells	Runx1	Up	Wargnier et al. [1995]
	CD3	T-cells	Runx1	Up	Hallberg et al. [1992]
	GM-CSF	T-cells	Runx1 or Runx2	Up	Takahashi et al. [1995]
Myeloid	M-CSF receptor	Monocytes	Runx1	Up	Zhang et al. [1994]; Zhang et al. [1996]; Petrovick et al. [1998]
	MPO	Immature myeloid cells	Runx1	Up	Britos-Bray and Friedman [1997]; Nuchprayoon et al. [1994]
	$p14^{ARF}$	Multiple cell types	Runx1	Up	Linggi et al. [2002]
	$p21^{Waf1/Cip1}$	K562, NIH3T3 and others	Runx1	Up in K562, down in NIH3T3	Lutterbach and Hiebert [2000]
	Complement receptor 1	Haematopoietic cells	Runx1	Up	Kim et al. [1999]
	Defensin NP-3	Myeloid cells	Runx1,2,3	Up	Westendorf et al. [1998]
	Mast cell protease 6	Mast cells	Runx1	Up	Ogihara et al. [1999]
	Neutrophil elastase	Immature myeloid cells	Runx1	Up	Nuchprayoon et al. [1994]
	UBP43	Macrophage	AML1-Eto	Up	Liu et al. [1999]
	CD11a	Leukocytes	Runx1	Up	Puig-Kroger et al. [2000]
	CD36	Macrophage	Runx1	Up	Armesilla et al. [1996]
	CD53	Meukocytes, primitive myeloid cell line L-G	Runx1a	Down	Shimada et al. [2000]
	HERF1	Erythroid cells	Runx1	Up	Harada et al. [1999]
	MDR1	Leukemic cells	Runx1,2,3	Down	Javed et al. [2000]
	Art-1	Myeloid and erythroid cells	Runx1	Up	Harada et al. [2001]
	MRP14	Primitive myeloid cell line L-G	Runx1	Down	Shimada et al. [2000]
	Stefin 3	Primitive myeloid cell line L-G	Runx1	Down	Shimada et al. [2000]
	Uridine phosphorylase	Primitive myeloid cell line L-G	Runx1	Down	Shimada et al. [2000]
	Pim-2	Primitive myeloid cell line L-G	Runx1a	Down	Shimada et al. [2000]
	Mast cell carboxypeptidase A	Primitive myeloid cell line L-G	Runx1a	Up	Shimada et al. [2000]

changes in expression levels after transient transfection of RUNX in the presence of putative RUNX binding sites), and they may in fact only be a member of the same intracellular pathway.

Which RUNX protein regulates which of the target genes? This question may have a number of answers. First, the specificity of a RUNX protein for a given target may reflect the RUNX expression pattern. In some cell types only one of the three RUNX genes is expressed (e.g., in osteoblasts, RUNX2). However, in some tissues two or all three RUNX genes are expressed (e.g., cartilage) [Levanon et al., 2001; Stricker et al., 2002]. Second, the target promoter context may only allow a certain RUNX protein to bind. In vitro, the RUNX proteins seem to be interchangeable. Nevertheless, the situation may be quite different in vivo. However, positive experiments addressing this issue have not been published. A "knock-in" of one RUNX protein's cDNA into the locus of another RUNX gene might be able to discriminate between the two possibilities.

The large number of (putative or proven) RUNX targets that have been characterised in different tissues reflect the period of time that has passed since the discovery of a role for RUNX proteins in the respective cellular context and the impact of leukaemia and genetic studies importing upon the search for targets. Therefore most known targets are expressed in

TABLE II. Runx Target Genes in Skeletal Tissues

Tissue	Gene	Expressed in	Regulated by	Up/down	References
Bone	Osteocalcin	Osteoblasts	Runx2	Up	Geoffroy et al. [1995]
	Collagen α1(I)	Osteoblasts	Runx2	Up	Ducy et al. [1997]
	Bone sialoprotein	Osteoblasts	Runx2(1,3)	Up or Down	Ducy et al. [1997]; Javed et al. [2001]
	Ameloblastin	Odontoblasts	Runx2	Up	Dhamija and Krebsbach [2001]
	TGF-β receptor I	Osteoblasts	Runx2	Up	Chang et al. [1998]; Ji et al. [1998]
	C/EBPδ	Osteoblasts	Runx2	Up	McCarthy et al. [2000]
	Osteoprotegerin	Osteoblasts	Runx2	Up	Thirunavukkarasu et al. [2000]
	RANKL	Osteoblasts	Runx2	Up	Geoffroy et al. [2002]; Kitazawa et al. [1999]
	Osteopontin	Osteoblasts chondrocytes	Runx2	Up	Ducy et al. [1997]
	Collagenase 3	Osteoblasts, hypertrophic chondrocytes	Runx2	Up	Jimenez et al. [1999]
Cartilage	Osteopontin	Osteoblasts chondrocytes	Runx2	Up	Ducy et al. [1997]
	Collagenase 3	Osteoblasts, hypertrophic chondrocytes	Runx2	Up	Jimenez et al. [1999]
	Collagen X	Hypertrophic condrocytes	Runx2	Up	Leboy et al. [2001]
	VEGF	Hypertrophic chondrocytes	Runx2	Up	Zelzer et al. [2001]

cells of the haematopoietic and lymphoid compartment, others in bone and cartilage. To date no transcriptional targets have been described in other RUNX expressing tissues such as dorsal root ganglia, testis and endothelium. Generally most target genes studied encode proteins that are specific for and restricted to the given cell type. However, it cannot be ruled out that this may also reflect the specific interest of investigators rather than represent a feature of the respective RUNX protein.

The target gene that has clearly been studied in most detail with respect to transactivation by a RUNX protein is osteocalcin. Studies on the

TABLE III. Runx Binding Sites in Promoters of Runx Regulated Genes

Gene	Sequence	Position	References
Human RUNX1 (P1)	TGTGGAA	− 69	Ghozi et al. [1996]
Murine Runx2 (P1)	CACCACA	− 1024	Xiao et al. [2001]
	TACCACA	− 338	
	AGTGGTA	− 119	
	AACCACA	− 74	
	AACCACA	+ 29	
	AACCACA	+ 37	
	TGCGGTG	+ 47	
Human RUNX3 (P1)	AACCACA	− 4	Bangsow et al. [2001]
	AACCACA	+ 5	
Human IL-3	TGTGGT	− 139	Uchida et al. [1997]
	TGTGGG	− 52	
Murine myeloperoxidase	AACCACA	Enhancer	Nuchprayoon et al. [1994]
Neutrophil elastase	GGCCACA	− 72	Nuchprayoon et al. [1994]
M-CSF receptor	TGTGGTT	− 73	Fears et al. [1997]
Human granzyme B	CACCACA	− 92	Wargnier et al. [1995]
TCRα	TCCCGCA	Enhancer	Giese et al. [1995]
Complement receptor 1	TGTGGT	− 42	Kim et al. [1999]
Rat collagenase 3	AACCACA	− 132	D'Alonzo et al. [2002]; Jimenez et al. [1999]
Murine RANKL	AACCACT	− 207	Geoffroy et al. [2002]; Kitazawa et al. [1999]
Rat TGF-β receptor type I	TTCCGCA	− 1101	Ji et al. [1998]
	GGCCGCA	− 1077	
	AACCGCG	− 546	
	AGCCACA	− 313	
	AACCACG	− 251	
	GGCCGCG	− 81	
Murine osteopontin	AACCACA	− 136	Sato et al. [1998]
Murine osteocalcin	AACCACA	− 136	Ducy et al. [1996]
Murine ameloblastin	CACCAA	−248	Dhamija and Krebsbach [2001]
Chicken BSP	TGTGGAG	−1204	Javed et al. [2001]
	AACCACA	− 813	
	AGTGGTC	− 514	
	TGTGGTG	− 444	
	TGTGGTT	− 414	
	TGTGGTG	− 318	
C/EBPδ	AACCGCA	−165	McCarthy et al. [2000]

osteocalcin promoter provided the first evidence that a member of the RUNX family is an important regulator of osteoblast-specific gene expression [Geoffroy et al., 1995; Merriman et al., 1995]. This promoter contains three perfect RUNX binding sites, and a protein with immunoreactivity to RUNX1 antibodies was shown to bind to these sequences [Banerjee et al., 1996; Ducy et al., 1996]. Overexpression of RUNX1 in non-osseous cells was able to increase osteocalcin transcription [Banerjee et al., 1996]. Later RUNX2 was identified as a transcription factor with an osteoblast-specific expression pattern and shown to induce osteocalcin expression in this cell type [Ducy et al., 1997; Komori et al., 1997; Otto et al., 1997]. Although it is now widely accepted that RUNX2 is the RUNX protein that regulates osteocalcin expression, the earlier experiments involving RUNX1 point to potential pitfalls in the interpretation of such results. In in vitro experiments using forced expression of RUNX proteins to investigate the transactivation of potential target genes, any of the three RUNX proteins may be able to increase the target gene expression. Therefore this type of experiment may not tell us which RUNX protein regulates which target in a physiological context. Furthermore, a given target gene may in fact be regulated by different RUNX proteins in different tissues.

REFERENCES

Alliston T, Choy L, Ducy P, Karsenty G, Derynck R. 2001. TGF-beta-induced repression of CBFA1 by Smad3 decreases cbfa1 and osteocalcin expression and inhibits osteoblast differentiation. EMBO J 20:2254–2272.

Armesilla AL, Calvo D, Vega MA. 1996. Structural and functional characterization of the human CD36 gene promoter: Identification of a proximal PEBP2/CBF site. J Biol Chem 271:7781–7787.

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.

Banerjee C, Javed A, Choi JY, Green J, Rosen V, van Wijnen AJ, Stein JL, Lian JB, Stein GS. 2001. Differential regulation of the two principal Runx2/Cbfa1 n-terminal isoforms in response to bone morphogenetic protein-2 during development of the osteoblast phenotype. Endocrinology 142:4026–4039.

Bangsow C, Rubins N, Glusman G, Bernstein Y, Negreanu V, Goldenberg D, Lotem J, Ben-Asher E, Lancet D, Levanon D, Groner Y. 2001. The RUNX3 gene-sequence, structure and regulated expression. Gene 279:221–232.

Britos-Bray M, Friedman AD. 1997. Core binding factor cannot synergistically activate the myeloperoxidase proximal enhancer in immature myeloid cells without c-Myb. Mol Cell Biol 17:5127–5135.

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.

Chang DJ, Ji C, Kim KK, Casinghino S, McCarthy TL, Centrella M. 1998. Reduction in transforming growth factor beta receptor I expression and transcription factor CBFa1 on bone cells by glucocorticoid. J Biol Chem 273: 4892–4896.

D'Alonzo RC, Selvamurugan N, Karsenty G, Partridge NC. 2002. Physical interaction of the activator protein-1 factors c-Fos and c-Jun with Cbfa1 for collagenase-3 promoter activation. J Biol Chem 277:816–822.

Dhamija S, Krebsbach PH. 2001. Role of Cbfa1 in ameloblastin gene transcription. J Biol Chem 276:35159–35164.

Drissi H, Luc Q, Shakoori R, Chuva De Sousa Lopes S, Choi JY, Terry A, Hu M, Jones S, Neil JC, Lian JB, Stein JL, Van Wijnen AJ, Stein GS. 2000. Transcriptional autoregulation of the bone related CBFA1/RUNX2 gene. J Cell Physiol 184:341–350.

Drissi H, Pouliot A, Koolloos C, Stein JL, Lian JB, Stein GS, van Wijnen AJ. 2002. 1,25-(OH)2-vitamin D3 suppresses the bone-related Runx2/Cbfa1 gene promoter. Exp Cell Res 274:323–333.

Ducy P, Geoffroy V, Karsenty G. 1996. Study of osteoblast-specific expression of one mouse osteocalcin gene: Characterization of the factor binding to OSE2. Connect Tissue Res 35:7–14.

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

Fears S, Gavin M, Zhang DE, Hetherington C, Ben-David Y, Rowley JD, Nucifora G. 1997. Functional characterization of ETV6 and ETV6/CBFA2 in the regulation of the MCSFR proximal promoter. Proc Natl Acad Sci USA 94: 1949–1954.

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.

Geoffroy V, Kneissel M, Fournier B, Boyde A, Matthias P. 2002. High bone resorption in adult aging transgenic mice overexpressing cbfa1/runx2 in cells of the osteoblastic lineage. Mol Cell Biol 22:6222–6233.

Ghozi MC, Bernstein Y, Negreanu V, Levanon D, Groner Y. 1996. Expression of the human acute myeloid leukemia gene AML1 is regulated by two promoter regions. Proc Natl Acad Sci USA 93:1935–1940.

Giese K, Kingsley C, Kirshner JR, Grosschedl R. 1995. Assembly and function of a TCR alpha enhancer complex is dependent on LEF-1-induced DNA bending and multiple protein-protein interactions. Genes Dev 9:995–1008.

Gilbert L, He X, Farmer P, Rubin J, Drissi H, van Wijnen AJ, Lian JB, Stein GS, Nanes MS. 2002. Expression of the osteoblast differentiation factor RUNX2 (Cbfa1/AML3/Pebp2alpha A) is inhibited by tumor necrosis factor-alpha. J Biol Chem 277:2695–2701.

Gutierrez S, Javed A, Tennant DK, van Rees M, Montecino M, Stein GS, Stein JL, Lian JB. 2002. CCAAT/enhancer-binding proteins (C/EBP) beta and delta activate osteocalcin gene transcription and synergize with Runx2 at

the C/EBP element to regulate bone-specific expression. J Biol Chem 277:1316–1323.

Hallberg B, Thornell A, Holm M, Grundstrom T. 1992. SEF1 binding is important for T cell specific enhancers of genes for T cell receptor-CD3 subunits. Nucleic Acids Res 20:6495–6499.

Hanai J, Chen LF, Kanno T, Ohtani-Fujita N, Kim WY, Guo WH, Imamura T, Ishidou Y, Fukuchi M, Shi MJ, Stavnezer J, Kawabata M, Miyazono K, Ito Y. 1999. Interaction and functional cooperation of PEBP2/CBF with Smads. Synergistic induction of the immunoglobulin germline Calpha promoter. J Biol Chem 274:31577–31582.

Harada H, Harada Y, O'Brien DP, Rice DS, Naeve CW, Downing JR. 1999. HERF1, a novel hematopoiesis-specific RING finger protein, is required for terminal differentiation of erythroid cells. Mol Cell Biol 19:3808–3815.

Harada Y, Harada H, Downing JR, Kimura A. 2001. A hematopoietic-specific transmembrane protein, Art-1, is possibly regulated by AML1. Biochem Biophys Res Commun 284:714–722.

Hernandez-Munain C, Krangel MS. 1995. c-Myb and core-binding factor/PEBP2 display functional synergy but bind independently to adjacent sites in the T-cell receptor delta enhancer. Mol Cell Biol 15:3090–3099.

Hsiang YH, Spencer D, Wang S, Speck NA, Raulet DH. 1993. The role of viral enhancer "core" motif-related sequences in regulating T cell receptor-gamma and -delta gene expression. J Immunol 150:3905–3916.

Imai Y, Kurokawa M, Tanaka K, Friedman AD, Ogawa S, Mitani K, Yazaki Y, Hirai H. 1998. TLE, the human homolog of groucho, interacts with AML1 and acts as a repressor of AML1-induced transactivation. Biochem Biophys Res Commun 252:582–589.

Inoue K, Ozaki S, Shiga T, Ito K, Masuda T, Okado N, Iseda T, Kawaguchi S, Ogawa M, Bae SC, Yamashita N, Itohara S, Kudo N, Ito Y. 2002. Runx3 controls the axonal projection of proprioceptive dorsal root ganglion neurons. Nat Neurosci 5:946–954.

Javed A, Guo B, Hiebert S, Choi JY, Green J, Zhao SC, Osborne MA, Stifani S, Stein JL, Lian JB, van Wijnen AJ, Stein GS. 2000. Groucho/TLE/R-esp proteins associate with the nuclear matrix and repress RUNX (CBF(alpha)/AML/PEBP2(alpha)) dependent activation of tissue-specific gene transcription. J Cell Sci 113:2221–2231.

Javed A, Barnes GL, Jasanya BO, Stein JL, Gerstenfeld L, Lian JB, Stein GS. 2001. runt homology domain transcription factors (Runx, Cbfa, and AML) mediate repression of the bone sialoprotein promoter: Evidence for promoter context-dependent activity of Cbfa proteins. Mol Cell Biol 21:2891–2905.

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.

Jimenez MJ, Balbin M, Lopez JM, Alvarez J, Komori T, Lopez-Otin C. 1999. Collagenase 3 is a target of Cbfa1, a transcription factor of the runt gene family involved in bone formation. Mol Cell Biol 19:4431–4442.

Jimenez MJ, Balbin M, Alvarez J, Komori T, Bianco P, Holmbeck K, Birkedal-Hansen H, Lopez JM, Lopez-Otin C. 2001. A regulatory cascade involving retinoic acid, Cbfa1, and matrix metalloproteinases is coupled to the development of a process of perichondrial invasion and osteogenic differentiation during bone formation. J Cell Biol 155:1333–1344.

Kanzler B, Kuschert SJ, Liu YH, Mallo M. 1998. Hoxa-2 restricts the chondrogenic domain and inhibits bone formation during development of the branchial area. Dev Suppl 125:2587–2597.

Kim JH, Lee S, Rho JK, Choe SY. 1999. AML1, the target of chromosomal rearrangements in human leukemia, regulates the expression of human complement receptor type 1 (CR1) gene. Int J Biochem Cell Biol 31:933–940.

Kitazawa R, Kitazawa S, Maeda S. 1999. Promoter structure of mouse RANKL/TRANCE/OPGL/ODF gene. Biochim Biophys Acta 1445:134–141.

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. Cell 89:755–764.

Le XF, Groner Y, Kornblau SM, Gu Y, Hittelman WN, Levanon D, Mehta K, Arlinghaus RB, Chang KS. 1999. Regulation of AML2/CBFA3 in hematopoietic cells through the retinoic acid receptor alpha-dependent signaling pathway. J Biol Chem 274:21651–21658.

Leboy P, Grasso-Knight G, D'Angelo M, Volk SW, Lian JV, Drissi H, Stein GS, Adams SL. 2001. Smad-Runx interactions during chondrocyte maturation. J Bone Joint Surg Am Vol 83-A (Suppl 1):S15–S22.

Lecka-Czernik B, Gubrij I, Moerman EJ, Kajkenova O, Lipschitz DA, Manolagas SC, Jilka RL. 1999. Inhibition of Osf2/Cbfa1 expression and terminal osteoblast differentiation by PPARgamma2. J Cell Biochem 74:357–371.

Lee KS, Kim HJ, Li QL, Chi XZ, Ueta C, Komori T, Wozney JM, Kim EG, Choi JY, Ryoo HM, Bae SC. 2000. Runx2 is a common target of transforming growth factor beta1 and bone morphogenetic protein 2, and cooperation between Runx2 and Smad5 induces osteoblast-specific gene expression in the pluripotent mesenchymal precursor cell line C2C12. Mol Cell Biol 20:8783–8792.

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.

Levanon D, Brenner O, Negreanu V, Bettoun D, Woolf E, Eilam R, Lotem J, Gat U, Otto F, Speck N, Groner Y. 2001. Spatial and temporal expression pattern of Runx3 (Aml2) and Runx1 (Aml1) indicates non-redundant functions during mouse embryogenesis. Mech Dev 109:413–417.

Levanon D, Bettoun D, Harris-Cerruti C, Woolf E, Negreanu V, Eilam R, Bernstein Y, Goldenberg D, Xiao C, Fliegauf M, Kremer E, Otto F, Brenner O, Lev-Tov A, Groner Y. 2002. The Runx3 transcription factor regulates development and survival of TrkC dorsal root ganglia neurons. EMBO J 21:3454–3463.

Li QL, Ito K, Sakakura C, Fukamachi H, Inoue K, Chi XZ, Lee KY, Nomura S, Lee CW, Han SB, Kim HM, Kim WJ, Yamamoto H, Yamashita N, Yano T, Ikeda T, Itohara S, Inazawa J, Abe T, Hagiwara A, Yamagishi H, Ooe A, Kaneda A, Sugimura T, Ushijima T, Bae SC, Ito Y. 2002.

Causal relationship between the loss of RUNX3 expression and gastric cancer. Cell 109:113–124.

Libermann TA, Pan Z, Akbarali Y, Hetherington CJ, Boltax J, Yergeau DA, Zhang DE. 1999. AML1 (CBFalpha2) cooperates with B cell-specific activating protein (BSAP/PAX5) in activation of the B cell-specific BLK gene promoter. J Biol Chem 274:24671–24676.

Linggi B, Muller-Tidow C, van de Locht L, Hu M, Nip J, Serve H, Berdel WE, van der Reijden B, Quelle DE, Rowley JD, Cleveland J, Jansen JH, Pandolfi PP, Hiebert SW. 2002. The t(8;21) fusion protein, AML1 ETO, specifically represses the transcription of the p14(ARF) tumor suppressor in acute myeloid leukemia. Nat Med 8:743–750.

Liu LQ, Ilaria R, Jr., Kingsley PD, Iwama A, van Etten RA, Palis J, Zhang DE. 1999. A novel ubiquitin-specific protease, UBP43, cloned from leukemia fusion protein AML1-ETO-expressing mice, functions in hematopoietic cell differentiation. Mol Cell Biol 19:3029–3038.

Lutterbach B, Hiebert SW. 2000. Role of the transcription factor AML-1 in acute leukemia and hematopoietic differentiation. Gene 245:223–235.

Mao S, Frank RC, Zhang J, Miyazaki Y, Nimer SD. 1999. Functional and physical interactions between AML1 proteins and an ETS protein, MEF: Implications for the pathogenesis of t(8;21)-positive leukemias. Mol Cell Biol 19:3635–3644.

McCarthy TL, Ji C, Chen Y, Kim KK, Imagawa M, Ito Y, Centrella M. 2000. Runt domain factor (Runx)-dependent effects on CCAAT/ enhancer-binding protein delta expression and activity in osteoblasts. J Biol Chem 275: 21746–21753.

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.

Morii E, Ogihara H, Kanno T, Kim DK, Nomura S, Ito Y, Kitamura Y. 1999. Identification of the region of mi transcription factor which is responsible for the synergy with PEBP2/CBF. Biochem Biophys Res Commun 261: 53–57.

Nuchprayoon I, Meyers S, Scott LM, Suzow J, Hiebert S, Friedman AD. 1994. PEBP2/CBF, the murine homolog of the human myeloid AML1 and PEBP2 beta/CBF beta proto-oncoproteins, regulates the murine myeloperoxidase and neutrophil elastase genes in immature myeloid cells. Mol Cell Biol 14:5558–5568.

Ogihara H, Kanno T, Morii E, Kim DK, Lee YM, Sato M, Kim WY, Nomura S, Ito Y, Kitamura Y. 1999. Synergy of PEBP2/CBF with mi transcription factor (MITF) for transactivation of mouse mast cell protease 6 gene. Oncogene 18:4632–4639.

Okuda T, van Deursen J, Hiebert SW, Grosveld G, Downing JR. 1996. AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. Cell 84:321–330.

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. Cell 89:765–771.

Pardali E, Xie XQ, Tsapogas P, Itoh S, Arvanitidis K, Heldin CH, ten Dijke P, Grundstrom T, Sideras P. 2000. Smad and AML proteins synergistically confer transforming growth factor beta1 responsiveness to human germ-line IgA genes. J Biol Chem 275:3552–3560.

Petrovick MS, Hiebert SW, Friedman AD, Hetherington CJ, Tenen DG, Zhang DE. 1998. Multiple functional domains of AML1: PU.1 and C/EBPalpha synergize with different regions of AML1. Mol Cell Biol 18:3915–3925.

Pozner A, Goldenberg D, Negreanu V, Le SY, Elroy-Stein O, Levanon D, Groner Y. 2000. Transcription-coupled translation control of AML1/RUNX1 is mediated by cap- and internal ribosome entry site-dependent mechanisms. Mol Cell Biol 20:2297–2307.

Prince M, Banerjee C, Javed A, Green J, Lian JB, Stein GS, Bodine PV, Komm BS. 2001. Expression and regulation of Runx2/Cbfa1 and osteoblast phenotypic markers during the growth and differentiation of human osteoblasts. J Cell Biochem 80:424–440.

Puig-Kroger A, Lopez-Rodriguez C, Relloso M, Sanchez-Elsner T, Nueda A, Munoz E, Bernabeu C, Corbi AL. 2000. Polyomavirus enhancer-binding protein 2/core binding factor/acute myeloid leukemia factors contribute to the cell type-specific activity of the CD11a integrin gene promoter. J Biol Chem 275:28507–28512.

Rini D, Calabi F. 2001. Identification and comparative analysis of a second runx3 promoter. Gene 273:13–22.

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.

Satokata I, Ma L, Ohshima H, Bei M, Woo I, Nishizawa K, Maeda T, Takano Y, Uchiyama M, Heaney S, Peters H, Tang Z, Maxson R, Maas R. 2000. Msx2 deficiency in mice causes pleiotropic defects in bone growth and ectodermal organ formation. Nat Genet 24:391–395.

Shimada H, Ichikawa H, Nakamura S, Katsu R, Iwasa M, Kitabayashi I, Ohki M. 2000. Analysis of genes under the downstream control of the t(8;21) fusion protein AML1-MTG8: Overexpression of the TIS11b (ERF-1, cMG1) gene induces myeloid cell proliferation in response to G-CSF. Blood 96:655–663.

Stricker S, Fundele R, Vortkamp A, Mundlos S. 2002. Role of Runx genes in chondrocyte differentiation. Dev Biol 245:95–108.

Sudhakar S, Li Y, Katz MS, Elango N. 2001. Translational regulation is a control point in RUNX2/Cbfa1 gene expression. Biochem Biophys Res Commun 289:616–622.

Takahashi A, Satake M, Yamaguchi-Iwai Y, Bae SC, Lu J, Maruyama M, Zhang YW, Oka H, Arai N, Arai K. 1995. Positive and negative regulation of granulocyte-macrophage colony-stimulating factor promoter activity by AML1-related transcription factor, PEBP2. Blood 86: 607–616.

Tanaka K, Tanaka T, Ogawa S, Kurokawa M, Mitani K, Yazaki Y, Hirai H. 1995. Increased expression of AML1 during retinoic-acid-induced differentiation of U937 cells. Biochem Biophys Res Commun 211:1023–1030.

Tanaka T, Kurokawa M, Ueki K, Tanaka K, Imai Y, Mitani K, Okazaki K, Sagata N, Yazaki Y, Shibata Y, Kadowaki T, Hirai H. 1996. The extracellular signal-regulated kinase pathway phosphorylates AML1, an acute myeloid leukemia gene product, and potentially regulates its transactivation ability. Mol Cell Biol 16:3967–3979.

Thirunavukkarasu K, Halladay DL, Miles RR, Yang X, Galvin RJ, Chandrasekhar S, Martin TJ, Onyia JE. 2000. The osteoblast-specific transcription factor Cbfa1 contributes to the expression of osteoprotegerin, a potent inhibitor of osteoclast differentiation and function. J Biol Chem 275:25163–25172.

Tou L, Quibria N, Alexander JM. 2001. Regulation of human cbfa1 gene transcription in osteoblasts by selective estrogen receptor modulators (SERMs). Mol Cell Endocrinol 183:71–79.

Tribioli C, Lufkin T. 1999. The murine Bapx1 homeobox gene plays a critical role in embryonic development of the axial skeleton and spleen. Dev Suppl 126:5699–5711.

Tsuji K, Ito Y, Noda M. 1998. Expression of the PEBP2alphaA/AML3/CBFA1 gene is regulated by BMP4/7 heterodimer and its overexpression suppresses type I collagen and osteocalcin gene expression in osteoblastic and non-osteoblastic mesenchymal cells. Bone 22:87–92.

Uchida H, Zhang J, Nimer SD. 1997. AML1A and AML1B can transactivate the human IL-3 promoter. J Immunol 158:2251–2258.

Wang Q, Stacy T, Binder M, Marin-Padilla M, Sharpe AH, Speck NA. 1996. Disruption of the Cbfa2 gene causes necrosis and hemorrhaging in the central nervous system and blocks definitive hematopoiesis. Proc Natl Acad Sci USA 93:3444–3449.

Wang J, Hoshino T, Redner RL, Kajigaya S, Liu JM. 1998. ETO, fusion partner in t(8;21) acute myeloid leukemia, represses transcription by interaction with the human N-CoR/mSin3/HDAC1 complex. Proc Natl Acad Sci USA 95:10860–10865.

Wargnier A, Legros-Maida S, Bosselut R, Bourge JF, Lafaurie C, Ghysdael CJ, Sasportes M, Paul P. 1995. Identification of human granzyme B promoter regulatory elements interacting with activated T-cell-specific proteins: implication of Ikaros and CBF binding sites in promoter activation. Proc Natl Acad Sci USA 92:6930–6934.

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.

Wotton D, Ghysdael J, Wang S, Speck NA, Owen MJ. 1994. Cooperative binding of Ets-1 and core binding factor to DNA. Mol Cell Biol 14:840–850.

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.

Xiao G, Jiang D, Thomas P, Benson MD, Guan K, Karsenty G, Franceschi RT. 2000. MAPK pathways activate and phosphorylate the osteoblast-specific transcription factor, Cbfa1. J Biol Chem 275:4453–4459.

Xiao ZS, Liu SG, Hinson TK, Quarles LD. 2001. Characterization of the upstream mouse Cbfa1/Runx2 promoter. J Cell Biochem 82:647–659.

Zambotti A, Makhluf H, Shen J, Ducy P. 2002. Characterization of an osteoblast-specific enhancer element in the CBFA1 gene. J Biol Chem 277:41497–41506.

Zelzer E, Glotzer DJ, Hartmann C, Thomas D, Fukai N, Soker S, Olsen BR. 2001. Tissue specific regulation of VEGF expression during bone development requires Cbfa1/Runx2. Mech Dev 106:97–106.

Zhang DE, Fujioka K, Hetherington CJ, Shapiro LH, Chen HM, Look AT, Tenen DG. 1994. Identification of a region which directs the monocytic activity of the colony-stimulating factor 1 (macrophage colony-stimulating factor) receptor promoter and binds PEBP2/CBF (AML1). Mol Cell Biol 14:8085–8095.

Zhang DE, Hetherington CJ, Meyers S, Rhoades KL, Larson CJ, Chen HM, Hiebert SW, Tenen DG. 1996. CCAAT enhancer-binding protein (C/EBP) and AML1 (CBF alpha2) synergistically activate the macrophage colony-stimulating factor receptor promoter. Mol Cell Biol 16:1231–1240.

Zhou YX, Xu X, Chen L, Li C, Brodie SG, Deng CX. 2000. A Pro250Arg substitution in mouse Fgfr1 causes increased expression of Cbfa1 and premature fusion of calvarial sutures. Hum Mol Genet 9:2001–2008.