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Translocations, Fusion Genes, and Acute Leukemia

Vaskar Saha,[1]* Bryan D. Young,[1] and Paul S. Freemont[2]

[1]Imperial Cancer Research Fund, Department of Medical Oncology, St Bartholomew's Hospital, London EC1A 6BQ, UK
[2]Molecular Structure and Function Laboratory, London WC2A 3PX, UK

Abstract Genes involved in chromosomal translocations, associated with the formation of fusion proteins in leukemia, are modular in nature and regulatory in function. It is likely that they are involved in the initiation and maintenance of normal hematopoiesis. A conceptual model is proposed by which disruption of these different genes leads to the development of acute leukemia. Central to this model is the functional interaction between the mammalian trithorax and polycomb group protein complexes. Many of the genes identified in leukemia-associated translocations are likely upstream regulators, co-participators or downstream targets of these complexes. In the natural state, these proteins interact with each other to form multimeric higher-order structures, which sequentially regulate the development of the normal hematopoietic state, either through *HOX* gene expression or other less defined pathways. The novel interaction domains acquired by the chimaeric fusion products subvert normal cellular control mechanisms, which result in both a failure of cell maturation and activation of anti-apoptotic pathways. The mechanisms by which these translocation products are able to affect these processes are thought to lie at the level of chromatin-mediated transcriptional activation and/or repression. The stimuli for proliferation and development of clinically overt disease may require subsequent mutations in more than one oncogene or tumor suppressor gene, or both. A more comprehensive catalogue of mutation events in malignant cells is therefore required to understand the key regulatory networks that serve to maintain multipotentiality and in particular the modifications which initiate and coordinate commitment in differentiating hematopoietic cells. We propose a model in which common pathways for leukemogenesis lie along the cell cycle control of chromatin structure in terms of transcriptional activation or repression. A clearer understanding of this cascade will provide opportunities for the design and construction of novel biological agents that are able to restore normal regulatory mechanisms. J. Cell. Biochem. Suppls. 30/31:264–276, 1998.

CHROMOSOMAL TRANSLOCATIONS INVOLVE TRANSCRIPTION FACTORS

The disease process in acute leukemia is characterized by the uncontrolled proliferation of immature white cells or blasts. For white cells to behave in this fashion, the transformed cell must undergo at least three definite changes. First, it must lose its capacity for differentiation. In acute lymphoblastic leukemia (ALL) for example, this arrest of maturation occurs in a progenitor cell that has already committed itself to an established lineage [Look, 1997]. By contrast, in acute myeloid leukemia (AML) transformation occurs in primitive stem cells that have retained both multilineage and self-renewal capacity [Bonnet and Dick, 1997]. The additional changes that must then occur for development of clinical disease are for the cell to escape apoptotic mechanisms, and to initiate proliferation. The cellular pathways responsible for these events are tightly controlled and regulated; clues as to how the cell undergoes these changes are being provided by the study of an increasing number of nonrandom chromosomal translocations identified in acute leukemias [Rabbitts, 1994].

Detailed molecular analysis of these translocations has identified a diverse groups of genes, apparently involved in the pathogenesis of clinically similar conditions. In general, these genes encode for transcription factors and in some cases they have been demonstrated to be critical for normal hematopoiesis [Shivdasani and Orkin, 1996]. As shown in Table I, the proteins encoded by these genes have a modular nature with distinctly defined domains. The most frequent result of leukemia-associated translocations, is the juxtaposition of parts of two such genes, so that a chimeric translocation product

*Correspondence to: Vaskar Saha, ICRF Department of Medical Oncology, St Bartholomew's and the Royal London Hospital School of Medicine, Charterhouse Square, London EC1M 6BQ, United Kingdom. E-mail: v.saha@mds.qmw.ac.uk

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TABLE I. Genes Involved in the Formation of Chimeric Fusions as a Result of Chromosomal Translocations in Acute Leukemia*

Gene	Localization	Domain retained in fusion product	Domain lost in fusion product	Other partners	Disease
MLL partners	*11q23*	*AT hook, methyltransferase*	*LAP/PHD fingers, SET*		
AF1q	1q21	Whole gene product	Unknown		AML
AF4	4q21	TA	Unknown		ALL
AF5	5q12	α-helix, coiled	LZ, acidic		AML
AF9	9p22	TA, α-helix	Unknown		AML/ALL
ENL	19p13.3	TA, α-helix	Unknown		AML/ALL
AF6	6q27	Kinesin, myosin, DHR	Ras activation		AML/ALL
AF10	10p12	LZ, Q-rich	LAP/PHD finger	CALM	AML
AF17	17q21	LZ, Q-rich	LAP/PHD finger		AML
AFX	Xq13	Unknown	forkhead, DNA binding		AML
AF6q21	6q21	Unknown	forkhead, DNA binding		Undiff
ELL	19p13.1	RNA polymerase II elongation factor	Unknown		AML
EEN	19p13	SH3, α-helix	Unknown		AML
ABI-1	10p11.2	SH3, HD	Unknown		AML
CBP	16p13	BRM, HAT, E1A, SRC-1	±CREB, LAP/PHD		AML
p300	22q13	BRM, Q-rich, LAP/PHD, TFIIb binding	CREB, ZF		AML
eps15	1p32	Crk binding, TK, EH	Unknown		AML
hCDCrel	22q11.2	CRE-like element	Unknown		AML
E2A partners	*19p13.3*	*TA, LH*	*bHLH*		
PBX1	1q23	HD	Unknown		ALL
HLF	17q22	LZ	Unknown		ALL
ETV6 partners	*12p13*	*HLH*	*DNA-binding, ETS*		
CBFA2	21q22	runt, TA		ETO, etc.	ALL
PDGFbR	5q33	TK, transmembrane	Ligand binding	CEV	CMML
ABL	9q34	TK		BCR	ALL, AML, CML
JAK2	9p24	TK			ALL, AML
MN1	22q11				AML
NTRK3	15q25	TK			FS*
Evi-1/MDS	3q26	ZF			AML
CBFA2 partners	*21q22*	*runt and TA with ETV6*	*loss of TA with ETO/EAP*		
ETV6	12p13	HLH	DNA-binding, ETS	Others	ALL
ETO	8q22	ZF			AML
EAP/EVI1	3q26	ZF			AML
MTG16	16q24	ZF			AML
CBFB	*16q22*	*CBFα binding*			*AML*
MYH11	16p13	Myosin heavy chain			
MOZ partners	*8p11*	*LPA/PHD, ZF, MYST, HAT*	*PAS?bHLH, RID*		
CBP	16p13	LAP/PHD, CREB, BRM, E1A, TFIIb, Q-rich		MLL	AML
TIF2	8q13	CID, HAT			AML
RARα partners	*17q21*	*B domain, DNA-binding, RAR*	*A domain, TA*		
PML	15q21	Ring, B-box, α-helix			APML
PLZF	11q23	ZF			APML
NPM	5q32	Nuclear localization		MLF1, ALK	APML
NuMA	11q13	Nucleosomal mitotic apparatus			APML

TABLE I. Genes Involved in the Formation of Chimeric Fusions as a Result of Chromosomal Translocations in Acute Leukemia*

Gene	Localization	Domain retained in fusion product	Domain lost in fusion product	Other partners	Disease
NUP98 partners	*11p15*	*GLGF motif*	*RNA binding*		
DDX10	11q22	RNA helicase domain	DEAD box		AML
HOXA9	7p15	HD			AML
CAN partners	*9q34*	*GLGF motif*			*AML*
DEK	6p23	DNA-binding			AML
SET	9p34	TAF-I activity			AML
TLS	*16p11*	*GYSQ*	*RNA binding*		*AML*
ERG	21q22	ETS DNA binding			

BRM, bromodomain; HAT, histone acetyltransferase; HD, homeodomain; HLH, helix-loop-helix; LZ, leucine zipper; TA, transactivation; TK, tyrosine kinase; ZF, zinc finger; FS*, infantile fibrosarcoma.
*Common genes are italicized; partners are in normal type.

is generated. The modular nature of the proteins involved, which can include DNA-binding, dimerization/oligomerization and *trans*-effector domains, thus allows normally unrelated sequences from different chromosomes to be recombined into hybrid genes that encode fusion products with altered function [Rabbitts et al., 1993]. We propose a conceptual model, shown in Figure 1, for the genesis of ALL and AML. It is our contention that the different genes involved in the pathogenesis of leukemia interact or influence each other as part of multimeric protein complexes that are involved in regulatory cascades which govern the normal development of hematopoietic cells. This regulation occurs at the level of transcriptional activation or repression, or both, through chromatin remodeling. Transformation and/or proliferation occur as a result of the aberrant activation or repression of specific genes, suggesting that ultimately there may be a common mechanism for all leukemogenesis.

LEUKEMIA-ASSOCIATED TRANSCRIPTION FACTORS ACT THROUGH HOX GENES

As shown in Table I, it is notable that the same gene products are involved in the pathogenesis of both ALL and AML. One explanation is that the genes targeted by chromosomal translocations are initiators of hematopoietic cell differentiation and proliferation via evolutionarily conserved regulatory cascades. Some evidence to support this comes from the extensive sequence homology between leukemic-associated genes and genes that control the earliest stages of embryonic development [Look, 1997]. This hypothesis is further reinforced by the results of gene manipulations in mice, which have clearly demonstrated the profound and varied effects on normal hematopoiesis of translocation targeted transcription factors [Shivdasani and Orkin, 1996]. Ongoing analyses of hematopoietic progenitor cells in mice and humans, suggests that leukemia-associated transcription factors are involved in pathways that regulate the major clusters of homeobox-containing *HOX* genes [Thorsteinsdottir et al., 1997]. Human cells contain 39 major *HOX* genes grouped in clusters (*HOX-A* to *HOX-D*) that share extensive homology with the *HOM-C* genes of *Drosophila* [Maconochie et al., 1996]. In the hematopoietic system, *HOX* genes are expressed in stage and lineage-specific patterns during early stages of development and hematopoietic differentiation. Expression of individual *HOX* genes in blood progenitor cells follow tightly regulated programs that are specific for the stage and lineage of progenitor cell development, with universal down-regulation of *HOX* gene expression as the progenitor differentiates into mature blood cells. This process is analogous to the expression of the *Drosophila HOM-C* complexes, which are responsible for the specification of the body segmental plan of the fly. In flies, genes at the 3′ end of the cluster comprise the *Antennapedia* complex and encode proteins that control the formation of anterior structures during embryonic development; more posterior body segments are controlled by genes of the *Bithorax* complex. These gene complexes are in turn regulated by two groups of transcription factors: trithorax (TrG) whose

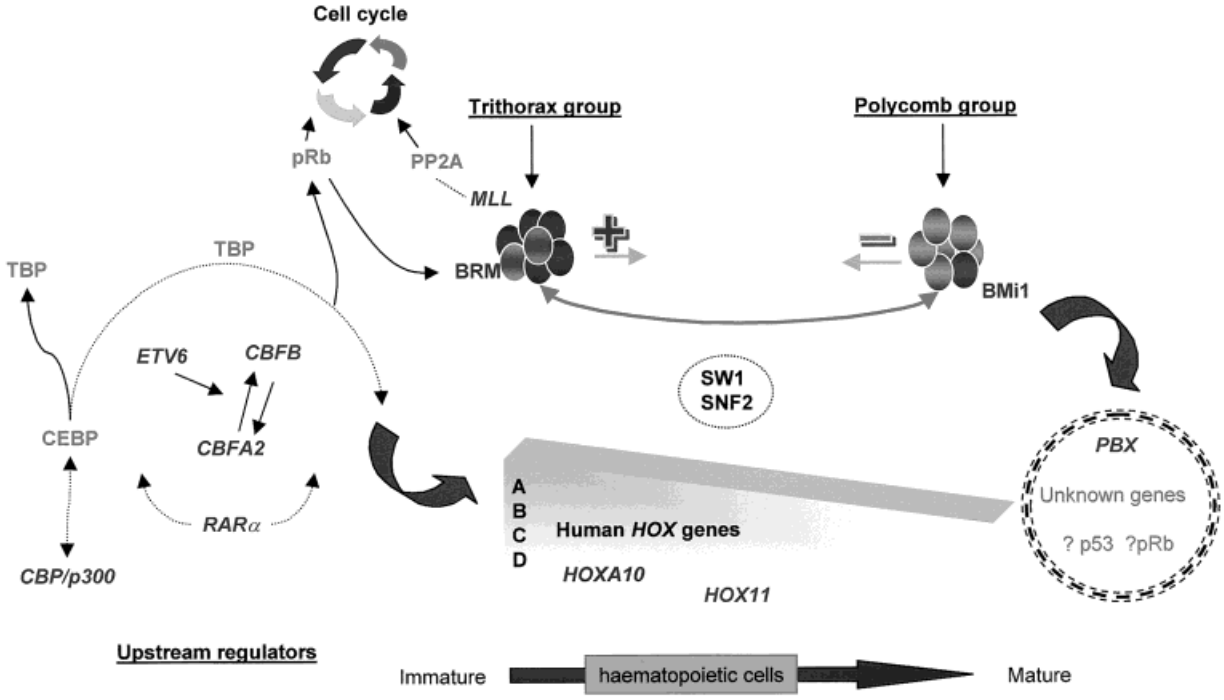

Fig. 1. Conceptual model showing leukemia-associated proteins that are probably involved in the regulation of *HOX* gene expression controlling the normal development of haematopoietic cells. At the centre of the model is the balanced interaction between the trithorax (TrG) and polycomb (PcG) group of proteins. Examples of TrG are MLL and BRM and of PcG is Bmi1. *HOX* gene expression decreases with progressive differentiation of the haematopoietic cell and this is regulated by a dose dependent interaction between mammalian TrG and PcG. TrG are required for maintenance (+) of differentiation and the PcG (−) for its repression. To the left of the diagram are genes that interact with each other and that are likely to be upstream regulators. By the nature of this model, the leukemic genes *ETV6, CBFA2, CBFB*, and *RARα* are dysregulated in uncommitted cells and are primarily involved in the development of acute myeloid leukemia. They may act on the HOX gene regulation pathways through the CCAAT/enhancer binding protein (CEBP), the retinoblastoma gene product (pRb) and members of the basal transcription complex such as the TATA binding protein (TBP). *Blue italics*, genes involved in translocation events; *red and black*, other important genes; *green*, interacting proteins. **Color plate on page 332.**

members positively regulate and sustain the active state of *HOM-C* gene expression, and polycomb (PcG) proteins, which oppose the effects of TrG proteins and continuously repress *HOM-C* gene expression [Simon, 1995].

HOX GENE EXPRESSION IS MAINTAINED BY INTERACTIONS BETWEEN TRG AND PCG

MLL, the human homologue of the *Drosophila trithorax* (*trx*) gene, has been implicated in translocated fusions with more than 25 other genes in both ALL and AML [Young and Saha, 1996]. *MLL* is an essential gene [Yu et al., 1995] and B- lineage lymphopenias occur in haploinsufficiency [Hess et al., 1997], suggesting a role in normal B cell development. Analogous to *trx*, *MLL* is implicated by genetic studies to function in the maintenance of *Hox* gene expression profiles during embryonic development [Look, 1997]. Consistent with this role, MLL and trx proteins contain C-terminal motifs known as *S*u(var)3–9/*e*nhancer of *z*este/trithorax (SET) domains which are present in a number of other proteins [Stassen et al., 1995; Tschiersch et al., 1994]. It appears that proteins, which contain SET domains, are involved in position-effect-variegation (PEV),a gene-silencing mechanism), telomeric and centromeric gene silencing, and possibly in determining chromosome architecture. Many of these proteins not only comprise the SET domain but contain additional sequence motifs/domains, including a cysteine-rich/zinc-finger or chromodomain [Jenuwein et al., 1998]. This would suggest that SET domain proteins act as multifunctional chromatin regulators with activities affecting both euchromatin and heterochromatin. In general, it appears that SET domain proteins can physically associate with chromatin, as part of multiprotein complexes like PcG or TrG, mediating long-term effects on chromatin structure and function. These effects

result in transcriptional activation or in repression of specific genes.

Central to the conceptual model proposed in Figure 1 is the decrease in *HOX* gene expression with progressive differentiation of the hematopoietic cell and that this is regulated by a dose-dependent interaction between the mammalian TrG and PcG complexes. The TrG complex is required for the maintenance of differentiation, whereas the PcG complex maintains repression of targeted genes. Unlike MLL, not a lot is known about the functions of other members of the mammalian TrG/PcG complexes [Brown et al., 1998]. Of the mammalian homologues of TrG proteins (other than MLL), hBRM has also been identified. It contains a conserved bromodomain and is a component of the coactivator SWI/SNF complex involved in the remodeling of chromatin during gene activation [Reyes et al., 1997]. The retinoblastoma gene product (pRb), also a cell cycle regulator, binds and represses hBRM activity. This finding suggests that pRb not only targets hBRM but also regulates its activity.

Recent studies have provided evidence for the existence of mammalian PcG genes and their involvement in repressing the transcription of *HOX* genes [Gould, 1997], although none has yet been shown to participate in leukemia-associated translocations. For example, mice lacking *bmi-1*, the murine and human homologue of the *Drosophila Posterior sex combs* (*Psc*) genes, display a progressive aplastic disease characterized by replacement of bone arrow space by adipocytes as well as a smaller spleen and thymus [van der Lugt et al., 1994]. Similarly, targeted disruption of *mel-18*, another mammalian *Psc* homologue, leads to B and T-cell developmental defects caused by insufficient response to interleukin-7 (IL-7) stimulation of the lymphoid precursors [Akasaka et al., 1997]. Progressive upregulation of most PcG genes has also been shown to be concomitant with differentiation of human bone marrow cells and supports a complex-constitutional model. Thus, silencing of *HOX* genes may be a two-step process requiring recognition of a small number of high-affinity PcG response elements, followed by recruitment of a larger number of dispersed lower-affinity sites by cooperative assembly. Newly synthesized PcG products would progressively interact with existing PcG protein complexes, favoring novel interactions with target sequences. This in turn permits gradual packaging or remodeling of DNA into a heterochromatin like structure and for *HOX* genes, this would involve a successive 3′ to 5′ closure of the clusters necessary for proper hematopoietic differentiation. Interestingly, overexpression of *HOX* genes in hematopoietic cells profoundly alters differentiation and proliferation [Lessard et al., 1998]. The emerging picture therefore, is that of a large multiprotein regulatory complex involving multiple protein-protein interactions which specifically alters the transcription of particular genes. Since leukemia-associated genes are modular in nature, it is not difficult to conceptualize that chimaeric translocation products will lead to dysregulation of this process perhaps by disrupting these interactions.

On another level, it is also possible that dysregulation is the result of aberrant signalling, for example the translocations involving MLL (Table I). More specifically, the anti-phosphatase SET binding factor 1(Sbf1) interacts with proteins containing SET domains, including *MLL* [Cui et al., 1998]. Anti-phosphatases protect active phosphorylated residues from dephosphorylation and thereby act to prolong the consequences of protein kinase action. The interaction of Sbf1 with SET domain proteins appears to be essential for normal growth regulation and differentiation of B-cell progenitors [De Vivo et al., 1998]. Disruption of this interaction results in loss of growth control, implying that Sbf1 functions as a SET domain-dependent positive regulator of cell growth. This strongly suggests that upstream signaling pathways that impinge on the SET domain, help to regulate the transcriptional effector functions of SET domain-containing proteins, such as MLL. Cytogenetic data, supported by gene transfer studies and knock-in mouse models, indicate that the critical product of translocations involving *MLL* is the der (11)-encoded fusion protein, which consists of the N-terminal ~1,200 amino acids of MLL fused to the carboxy-peptides of various species [Young and Saha, 1996]. As a consequence, the SET domain of MLL is deleted and replaced by sequences from a variety of heterologous proteins. Therefore it is likely that normal regulatory mechanisms can be circumvented either by heterologous protein fusion or interference with endogenous SET-Sbf1 interactions. In both cases, MLL could be uncoupled from upstream regulatory signals

with consequent inappropriate expression of subordinate genes.

FUSION PROTEINS MAY AFFECT APOPTOSIS

So far we have discussed processes which may lead to a disruption of the normal temporal pattern of HOX gene expression leading to cell maturation arrest. The same genes may also be involved in the abrogation of apoptosis thus immortalising the transformed cell. For example, the N-terminal A-T hooks of MLL, retained by the fusion product, are capable of binding DNA wrapped around nucleosomes. The N-terminus also interacts with at least two other proteins involved in chromatin assembly, namely the protein product of the *SET* gene, which is associated with leukemic translocations (Table I) and the serine-threonine phosphatase 2A catalytic subunit (PP2A) [Adler et al., 1997]. The protein product of *SET* is thought to be a nucleosomal assembly protein involved in the delivery of histones from the cytoplasm to the chromatin assembly machinery in the nucleus in a cell-cycle dependent manner [Adler et al., 1997]. PP2A activity on the other hand halts the progress of the cell cycle from G2 to M, which is possibly linked to its ability to dephosphorylate histone H1. Among known inhibitors of PP2A that allow progression from G2 to M are the orphan homeobox gene *HOX11*, the retinoic acid receptor (RAR), and the MLL-SET complex. PP2A is activated at the onset of apoptosis [Mills et al., 1998]; this activity can also be inhibited by HOX11 and the MLL-SET complex [Adler et al., 1997]. The fusion of the T-cell receptor α- or β-chain genes to the *HOX11* gene as seen in T-cell ALL [Look, 1997] may disrupt PP2A activity thus altering the regulation of the cell cycle leading to leukemogenesis. The fusion product of PML to the retinoic acid receptor a (RARα) seen in acute promyelocytic leukemia (APL) also has anti-apoptotic properties [Grignani et al., 1993], and this too could be mediated by interaction with PP2A. At least one other leukemic-associated transcription factor has anti-apoptotic properties, namely the E2A-HLF fusion protein formed by the t(17;19) translocation. E2A-HLF is thought to disrupt an early step in a p53 dependent cell death pathway, through activation of a *ces-1*-like gene, whose product then censors immature B lymphocytes [Altura et al., 1998]. It is notable that p53 activity can be influenced by PP2A activity [Takenaka et al., 1995]. Thus, the gene products that regulate development of hematopoietic progenitor cells may also participate in determining cell fate. It is plausible therefore, that cellular dysregulation by the chimaeric products of leukemic translocations could result not only in the arrest of differentiation, but ultimately in immortalization of a leukemic clone sa well, through a common anti-apoptotic pathway.

PROLIFERATION POTENTIAL OF THE TRG/PCG COMPLEX

Our conceptual model for hemopoietic development and leukemogenesis proposes that cellular dysregulation impinges on *HOX* gene expression, and there is some evidence to suggest that the same regulatory elements also participate in anti-apoptotic mechanisms. Therefore, this poses the questions: Are the downstream genetic/biochemical pathways controlling hematopoietic proliferation distinct from those involved in regulating embryonic *HOX* gene expression? In addition to their function in anteroposterior patterning, members of the TrG/PcG complexes are known to control several other developmental processes, including cell proliferation. There is however, some evidence to support separate pathways. For example, different domains of the PcG bmi-1 protein are required for lymphomagenesis and vertebral patterning. Furthermore mel-18, another PcG protein, may directly repress *c-myc* transcription, leading to a negative regulation of cell cycle progression [Lessard et al., 1998]. *MLL* too has been shown to upregulate the expression of *ARP1*, a member of a family of homeotic genes in hematopoietic cells [Arakawa et al., 1998]. In an alternative scenario, *HOX* genes could provide a common entry point for the control of cell fate and proliferation. They are well known in influencing hematopoietic cell fate and also in regulating proliferation during development. These interactions would therefore suggest a mechanism by which homeobox genes alter cell cycle progression [Kawabe et al., 1997]. Thus, the TrG/PcG complexes and associated proteins might have both *HOX*-dependent and -independent inputs into controlling the cell cycle.

REGULATION OF PCG AND TRG

If we stay with the assumption that events that lead to AML are initiated in the uncommitted stem cell, then genes involved in program-

ming the cell towards myeloid development are likely to be upstream regulators of the TrG/PcG protein complexes. Figure 1 (left) shows that some of the genes involved in myeloid development [Tenen et al., 1997] are also involved in leukemia-associated translocations, and are among possible candidates as regulators of the TrG/PcG complexes. Among the upstream regulators of the *Drosophila* HOM-C complex are the pair-rule and gap proteins. The *Drosophila* pair-rule protein runt is homologous to the central DNA binding domain of core binding factor A2 (CBFA2, also known as AML1). CBFA2 is the DNA-binding subunit of the CBFA2/CBFB transcription factor complex. The DNA-binding affinity of CBFA2 is increased through heterodimerization with CBFB, and both its DNA binding and interaction with CBFB are mediated through the central "runt" domain. Mice lacking either CBFA2 or CBFB are "bloodless" with the complete absence of definitive hematopoiesis [Castilla et al., 1996; Okuda et al., 1996; Wang et al., 1996] showing that both are required for normal hematopoiesis. In the model depicted in Figure 1, CBFA2 or CBFB would act as upstream regulators of hematopoiesis. CBFA2 is involved in two types of leukemic-associated chimaeric fusion products (Table I). In AMLs, the N-terminus of CBFA2, including the runt domain, is fused to both the carboxytermini of ETO (t(8;21)) and EAP/Evi-1 (t(3;21)). The C-terminus of CBFA2, which is capable of activating transcription and is able to induce cell differentiation, has been shown to interact with p300 and cyclic AMP response element binding protein (CREB)-binding protein (CBP). Over expression of p300 stimulates CBFA2-dependent transcription and the induction of cell differentiation [Kitabayashi et al., 1998]. p300/CBP are considered to be transcriptional co-activators that connect the basal transcriptional machinery to various DNA-binding transcriptional factors and, as shown in Table I, are disrupted in leukemia-associated translocations. p300/CBP interacts with histone acetyl transferase (HAT), and also possesses acetyltransferase activity itself, serving to regulate transcription through chromatin remodeling and recruitment of basal transcription factors. The fusion product of CBFA2-ETO in the t(8;21) translocation encodes a chimaeric protein comprising the N-terminus runt domain of CBFA2 and the C-terminus of ETO. The presence of the runt domain therefore allows this fusion product to interact efficiently with CBFB suggesting that a possible mechanism for leukemogenesis would be the neutralization of normal CBFA2/CBFB-mediated activity [Tanaka et al., 1998]. This explanation does not take into account the contribution of ETO. The zinc fingers on the C-terminus of ETO are retained in the fusion product and interact with the human nuclear receptor co-repressor (N-Cor). N-Cor forms a complex with mSin3 and histone deacetylase 1 (HDAC1), resulting in histone deacetylation and transcriptional repression [Wang et al., 1998]. Therefore, CBFA2-ETO fusion loses the transcriptional activation domain of CBFA2 but gains the repression domain of ETO. Interestingly, N-Cor also interacts with PLZF [Hong et al., 1997] the partner of RARα seen in APL associated with translocation t(11;17). These fusion products are therefore capable of generating signals, which would ultimately result in the production of dysplastic hematopoietic progenitors [Okuda et al., 1998]. If we postulate that these changes act upstream of the TrG/PcG regulation of homeobox genes (Fig. 1), the hematopoietic expansion will result in proliferation of primitive stem cells with multilineage and self renewal capacity, and thus lead to the eventual clinical development of AML.

The second type of fusion product that contains CBFA2 occurs in the t(12;21) translocation observed in ALL (Table I) [Golub et al., 1997]. In contrast to the other translocations involving *CBFA2*, this fusion product has the helix-loop-helix (HLH) motif of ETV6 (also known as TEL) at the N-terminus, fused to almost the entire *CBFA2* gene product, containing both the runt and transactivating domain at the C-terminus. The *ETV6-CBFA2* fusion is detected in about 20% of childhood ALL; in most such cases, the other *ETV6* allele appears to be lost [Golub et al., 1997]. In theory, CBFA2 retains its normal function and the "gain of function" is probably provided by the ETV6 HLH motif. It is possible that the ETV6 HLH motif homodimerizes with wild-type ETV6, acting in a dominant-negative manner, thus providing the "second hit" necessary to allow oncogenesis. It is notable that the ETV6-CBFA2 fusion protein results in the conversion of CBFA2 from a transcriptional activator to a transcriptional repressor [Fears et al., 1997]. However, not enough is known about the interactions and actions of ETV6 for us to clearly understand

why the *ETV6-CBFA2* fusion leads to ALL, and not to AML. We could speculate that CBFA2 is probably active further downstream in the development of progenitor cells committed to B-cell lineage [Shivdasani and Orkin, 1996]. Therefore, retention of the p300/CBP interactive C-terminus in the ETV6-CBFA2 fusion protein would be likely to play an important role in the genesis of pre-B ALL.

How does ETV6 fit into our conceptual model for hematopoiesis and leukemogenesis? The ets family of transcription factors, to which ETV6 belongs, is a large multigene family of transcription factors that share a conserved DNA-binding "ETS" domain and include several oncoproteins. Translocations other than the t(12;21) that involve ETV6, result either in the fusion of the ETV6 HLH domain to the tyrosine kinase domains of PDGFRb, ABL, and JAK2 or the aberrant expression of the ETV6 Ets DNA binding domain in the MN1-ETV6 fusion seen in myeloid leukemias [Golub et al., 1997]. ETV6 −/− mice die in utero with defective yolk sac angiogenesis and intrembryonic apoptosis of mesenchymal and neural crest cells [Wang et al., 1997]. Since ETV6 regulates apoptotic mechanisms in the developing mesenchyme, it is perhaps not surprising that fusion of the HLH domain of ETV6 to the tyrosine kinase receptor of the neurotrophin-3-receptor gene is implicated in the genesis of congenital fibrosarcoma [Knezevich et al., 1998]. ETV6 therefore appears able to transform target cells through diverse mechanisms. Further information is required about which genes are targeted by ETV6, although the creation of developmentally regulated or tissue-restricted knockouts will provide further insights into the mechanisms involved in ETV6-related leukemias.

Retinoic acid response elements (RAREs) are intimately involved in generating aspects of early embryonic *Hox* expression [Marshall et al., 1996]. The mammalian PcG system could, in principle, limit the accessibility of RAREs by controlling the progressive 3′ to 5′ opening of *HOX* complex chromatin [Core et al., 1997]. Alternatively, the PcG complex could act on individual RAREs within the complex in a more saltatory fashion, perhaps to antagonize transcriptional stimulation by retinoic acid receptors and their TrG co-activators. Since the involvement of the RARα is associated with the development of APL, it seems reasonable to suggest that in this case RARα fusion products disrupt committed myeloid progenitor cell development at the promyelocyte stage and does so by altering the normal sequential pattern of *HOX* gene expression.

DOWNSTREAM TARGETS

Coming back to our model described in Figure 1, little is known about the downstream targets of vertebrate HOX proteins. Some clues have emerged from studies with *Drosophila* which have demonstrated the importance of autoregulatory loops and have identified targets such as *extradenticle*, *decapentaplegic*, *Distal-less*, *teashirt*, and *wingless* genes [Lu and Kamps, 1996]. If the dysregulation of the *HOX* complex is crucial to the formation of leukemia, these regulatory pathways must ultimately include proteins that regulate cell proliferation, survival, adhesion, and migration. PBX proteins, the mammalian counterparts of *extradenticle*, form complexes with specific subsets of HOX proteins [Lufkin, 1997]. HOX proteins also bind DNA cooperatively with both PBX proteins as well as the chimaeric transcription factor E2A-PBX1 oncoprotein produced by the t(1;19) translocation in human pre-B-cell leukemia. This would suggest that leukemogenesis by E2A-PBX1 may occur by altering the transcription of cellular genes normally regulated by PBX-HOX motifs [Knoepfler et al., 1997]. Interestingly, mice lacking E2A die perinatally from unknown reasons and fail to produce mature B-cells. Thus, consistent with our model, the E2A-PBX1 fusion product could result in a maturation arrest in a committed B-lineage precursor thereby leading to the development of pre B-cell ALL.

THE LEUKEMIA CHROMATIN CONNECTION

Multiple lines of evidence suggest that components of nuclear architecture contribute both structurally and enzymatically to controlling gene expression. The nucleus is organized into domains that have defined relationships both with each other and with the surrounding genomic DNA. It is likely that the spatial organization of the subnuclear component is important for the temporal expression of genes, allowing protein products to be at the right place at the right time to mediate optimal biological control [Stein et al., 1998]. Several nuclear oncoproteins are organized in distinct subnuclear domains such as the PML oncogenic domains or nuclear bodies (PML NBs) that

contain the PML protein, the SP100 autoantigen and up to 15 other proteins [Hodges et al., 1998]. MLL, too, appears to be present within discrete nuclear domains which are distinct from those occupied by PML [Butler et al., 1997]. Strikingly, PML NBs are disrupted in APL associated with PML-RARα fusion. The subnuclear organization can be restored by the introduction of retinoic acid (RA) the ligand for RARα and correlates with the therapeutic response of RA [Hodges et al., 1998]. While the normal function of PML NBs remains unresolved, their number and size vary throughout the cell cycle, and there is evidence to suggest that nascent RNA, RNA polymerase II, CBP and pRb localize to PML NBs at different times. Recently, SP100 has been shown to bind to members of the heterochromatin protein 1 (HP1) families of nonhistone chromosomal proteins and that these complexes appear to concentrate in PML NBs [Seeler et al., 1998]. The SP100-HP1 complex behaves as a transcriptional repressor, and is thus likely to do so by chromatin re-modeling [Seeler et al., 1998].

The first step of transcription, as we understand it today, is the binding of upstream regulators and the downstream opening of chromatin. When chromatin is tightly packed, it impairs access of transcription factors to DNA binding elements, consequently this region of the genome is transcriptionally inactive. Active chromatin is "open," allowing transcription factors to bind and interact with the basal transcription machinery to achieve desirable levels of gene expression. As shown in Figure 2, activation can occur through the action of activator complexes on chromatin, which through mechanisms such as histone acetylation renders it more accessible to other regulatory or basal factors [Grunstein, 1997]. In the example, shown in Figure 2, histone deacetylase (HDAC1) complexes with pRb and the transcriptional

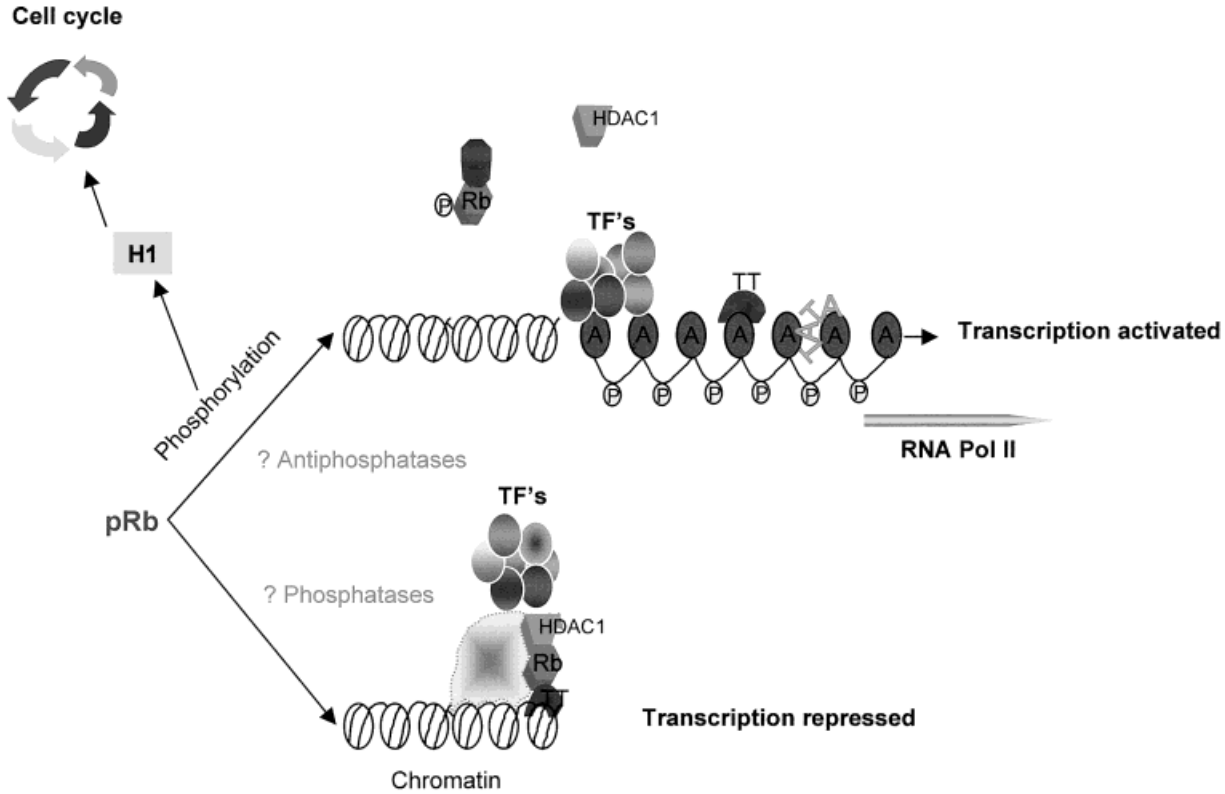

Fig. 2. Conceptual model for chromatin activation. Histone deacetylase (HDAC1) complexes with pRb and the transcriptional activator TT, removing highly charged acetyl groups from core histones, thereby causing tighter association of DNA and nucleosomes. This prevents transcription factors (TFs) from accessing DNA binding elements. Hyperphosphorylation by G1 cycle dependent kinases or binding by antagonists result in the release of pRb from TT and transcription, moving the cell from G1 to S. Phosphorylation of histone (H1) is associated with maintenance of the cell cycle and may reflect similar mechanisms. This activity can be terminated by phosphatases or histone acetyltransferases (HAT) and augmented by antiphosphatases such as Sbf-1. A, acetyl residue; P, phosphate. **Color plate on page 332.**

activator TT, removing highly charged acetyl groups from core histones and thereby causing tighter association of DNA and nucleosomes. This prevents transcription factors from accessing DNA binding elements. Hyperphosphorylation by G1 cycle-dependent kinases or binding by antagonists results in the release of pRb from TT and allows binding of transcription factors to DNA, thus initiating transcription. This response can be terminated by the action of phosphatases or HAT and augmented by the activity of anti-phosphatases such as Sbf-1.

Experimental results support a general recruitment model for gene activation, where the strength of interaction between the activator and a component of the basal transcription machinery correlates with the degree of transcriptional enhancement. Some DNA-binding factors, which themselves are unable to interact with the basal transcriptional machinery, may work through contacting non-DNA binding proteins (co-activators) to facilitate this interaction and recruitment. Indeed a number of co-activators such as the fusion partner CBP possess HAT activities. In addition, SWI/SNF or related complexes that use ATP hydrolysis to disrupt histone-DNA contacts have been found associated with some activators as well as RNA pol II holoenzyme. By contrast, transcriptional repressors may act either on the basal transcriptional machinery or on chromatin structure, or both. In this respect, a number of transcriptional repressors have recently been shown to recruit histone deacetylases, which are capable of altering chromatin structure by deacetylation of the amino termini of histones [Brehm et al., 1998; Chen et al., 1997; Grignani et al., 1998]. Conversely, HAT-mediated acetylation would destabilize nucleosomes thus increasing the accessibility of promoters.

In terms of leukemic-associated factors, HAT activity has been found in the fusion partners p300, CBP, MOZ, TIF2 (Table I) and the p300/CBP-associated factor P/CAF, a novel nuclear receptor co-activator ACTR and $TAF_{II}250$. Histones are not the sole target of acetyl transferases and deacetylases, as the protein p53 is acetylated both in vitro and in vivo by its co-activator p300. Acetylation of p53 results in the stimulation of its DNA binding activity, perhaps through conformational change. Another potential acetylation target is the RNA polymerase II machinery itself, as at least three initiation factors can function as acetyltransferase substrates. Recruitment of histone deacetylase has also been shown to be crucial to the transforming potential of the PML-RARα fusion proteins in APL, where the different effects of all *trans*-retinoic acid (ATRA) on the stability of the PML-RARα and PLZF-RARα co-repressor complexes determines the differential response of APLs to ATRA [Lin et al., 1998; Grignani et al., 1998].

As shown in Table I, many of the MLL partner gene products have been shown to have characteristics of bona fide transcription factors and others to be involved in transcriptional regulation. An emerging possibility is that they can interact with the RNA polymerase II transcription machinery and participate in chromatin activation/repression. Even though MLL has numerous partners, these partners appear to be very specific, since fusion of MLL to other proto-oncogenes such as c-*myc*, are not leukemogenic [Corral et al., 1996]. As is clear from Table I, the functions and interactions of these fusion partners are at present largely unknown. From what little we do know, we can speculate that *MLL* partners are probably part of normal signaling pathways involved in regulating TrG/PcG function. As illustrated by the Sbf1-SET interaction, the chimaeric nature of the modular fusion products could bypass normal regulatory mechanisms and alter downstream cellular activities. Clearly we require further information as to the nature of these gene products, their regulatory elements and their downstream targets.

FUSION PRODUCTS ARE NOT BOTH CAUSE AND EFFECT

We have presented an argument suggesting that fusion genes can corrupt regulatory pathways leading to dysmorphogenesis and inhibition of apoptosis. Cell proliferation too can be attributable the translocation event, acting through either HOX or HOX-independent pathways. However, the evidence suggests that for proliferation to progress to overt disease, additional events must take place. Studies in twins with leukemia for example, suggest that the translocation event occurs in utero and that there is a latency period before a clinical picture of leukemia evolves [Gale et al., 1997]. In an in vivo model of the *Mll-Af9* fusion, there is a long latency in tumor development in the *Mll-Af9* chimaeric mice [Corral et al., 1996]. Furthermore, translocated fusion transcripts are

now being detected in individuals with no evidence of disease and also from normal hematopoietic cells [Biernaux et al., 1995; Uckun et al., 1998; Yunis et al., 1987]. This finding suggests that the translocation events per se occur early in the disease process. Thus, development of leukemia, like solid tumors, is likely to be a multistep process that requires cooperative mutations in more than one oncogene and or tumor suppressor gene. It is increasingly apparent that cells with mutations typically found in leukemias and lymphomas frequently arise in normal individuals. Additional mutations are therefore necessary for progression to clinical malignancy and, given the rarity of disease, must ostensibly occur only in a subset of individuals.

Support for this idea comes as well from the therapeutic response to disease. Table II shows the prevalence and prognosis, on modern day chemotherapeutic regimens, in some of the leukemias discussed so far. In the management of children leukemia for example, those with a *ETV6-CBFA2* fusion in ALL have a good prognosis. However, although these children appear to have biologically identical fusion products, and 80% will be cured with conventional therapeutic regimens, 20% are refractory to treatment [Pui and Evans, 1998; Saha and Lilleyman, 1998]. Conversely, *MLL* involvement is associated with poor prognosis [Saha and Lilleyman, 1998], yet some children are cured with chemotherapy. The clues to these therapeutic conundrums must lie in events that occur subsequent to the translocation event. One question unlikely to be answered is whether there is an increased probability of secondary mutagenic events once specific leukemia-associated translocations have occurred. This would involve screening healthy populations for specific translocations, which would be difficult to justify on ethical and moral grounds. However, this type of information might allow the development of mass screening strategies and clinicians to predict likely disease development. In molecular terms, we still do not know why site-specific chromosomal translocations occur in the first place. Are these events rare and if so are they to do with local chromosomal sequences/structure?

The emphasis must now switch from focusing exclusively on translocations toward a more comprehensive characterization of the sequence of mutational events that occur during malignancy. Given the complexity of gene expression in multipotent cells, the future challenges will be to define those key regulatory networks that serve to maintain multipotentiality and the modifications that initiate and coordinate cellular commitment. Functional endpoints must be compared among multiple lineages in either cell culture or animal studies to understand how the signaling and expression pathways of different cell types influence the ultimate effect of the fusion product. Additional insights are also required in areas such as nuclear structure and organization, endogenous and exogenous DNA damaging events and recombination and repair pathways in different cell types. Ultimately the understanding obtained from these studies will allow us to determine whether the common origin of hematopoietic and mesenchymal lineages provides a link for the involvement of these genes in leukemias, lymphomas and sarcoma [Barr, 1998]. In summary, we propose a conceptual model, where common pathways for leukemogenesis lie along the cell cycle control of chromatin structure in terms of transcriptional regulation. A clearer molecular understanding of this cascade will provide opportunities for the design and construction of novel

TABLE II. Prevalence and Prognosis of Common Fusion Transcript-Associated Leukemias

Translocation	Disease	% Total	Survival (%)	Recommended treatment
ETV6-CBFA2	ALL	20–25	70–80	Chemotherapy (antimetabolites)
E2A-PBX1	ALL	10	70	Intensive chemotherapy (genotoxic drugs and antimetabolites)
BCR-ABL	ALL	5	~20	Allogeneic stem cell transplant
MLL-v	ALL	5	~20	Allogeneic stem cell transplant
CBFA2-ETO	AML	15–20	50–60	Intensive chemotherapy (with high-dose cytarabine)
CBFB-MYH11	AML	12	50–60	Intensive chemotherapy (with high-dose cytarabine)
PML-RARα	APL	7	50–60	Intensive chemotherapy (with all-*trans*-retinoic acid, anthracyclines)
MLL-v	AML, M4–M5	5–10	Variable	Intensive chemotherapy/allogeneic stem cell transplant

biological/therapeutic agents that would be able to restore normal regulatory mechanisms.

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