[Frontiers in Bioscience 8, s206-222, May 1, 2003]

E2A BASIC HELIX-LOOP-HELIX TRANSCRIPTION FACTORS IN HUMAN LEUKEMIA

David P. LeBrun

Department of Pathology, Queen's University, Kingston, Ontario

TABLE OF CONTENTS

1. ABSTRACT

The gene *E2A* on chromosome 19 is involved in recurrent chromosomal rearrangements associated with pediatric acute lymphoblastic leukemia. The resulting fusion of 5' *E2A* sequences with 3' portions of other genes leads to the expression of two well-characterized fusion proteins: E2A-PBX1 and E2A-HLF. Since the E2A, PBX1 and HLF proteins all appear to function as transcription factors, it appears likely that the oncogenic fusion proteins contribute to leukemia development by causing abnormal transcriptional regulation of key target genes. Furthermore, since the E2A portion of the fusion proteins contains transcriptional activation domains, and the PBX1 and HLF portions contain DNA binding domains, leukemogenesis may be due, at least in part, to excessive transcriptional induction of target genes defined by PBX1 or HLF. However, recent findings suggest that this model is simplistic and possibly incorrect. In this article, I review the evidence pertaining to leukemogenesis by the well-characterized E2A-fusion proteins and consider its mechanistic implications.

2. INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common form of childhood cancer. In roughly 45% of ALL cases, the leukemia cells contain somatic, non-random chromosomal translocations. Translocations involving specific breakpoints on specific chromosomes have consistently been associated with particular leukemia subtypes, as defined by morphology, immunophenotype or clinical features. This association has suggested that non-random translocations play a causal role in leukemia pathogenesis. Abundant work over the last quarter century has validated this idea (1).

Non-random translocations promote leukemogenesis by altering the function of genes located near the involved chromosomal breakpoints. In most instances, such translocations lead to the expression of chimeric proteins comprising polypeptide elements that are normally encoded by separate genes residing on separate chromosomes. This occurs because the chromosomal breaks that develop during translocation occur within introns of the genes involved on each chromosome. Transcription and pre-mRNA splicing of the resultant, fused genes lead to in-frame fusion of portions of the open reading frames on the novel, aberrant transcripts.

The gene *E2A*, at chromosome band 19 p13.3, is involved in at least three structural chromosomal rearrangements that occur in pediatric ALL. Translocation 1;19 is detectable in roughly 5% of cases (2). It fuses a 5' portion of *E2A* to the gene *PBX1* on chromosome 1, leading to expression of the chimeric protein E2A-PBX1 (3). Translocation 17;19, which is relatively rare, fuses an equivalent portion of *E2A* to the gene *HLF*, leading to expression of the protein E2A-HLF (4, 5). More recently, a rearrangement of chromosome 19 was described that fuses *E2A* to the gene *FB1*, thus apparently generating several E2A-FB1 fusion proteins (6).

My objective in this review is to outline some postulated mechanisms by which *E2A*-fusion genes contribute to leukemogenesis, and consider features of the

related wild-type proteins that may pertain to oncogenesis. Most of the discussion will focus on *E2A-PBX1*, since it is most commonly encountered clinically and best characterized functionally.

3. STRUCTURE AND FUNCTION OF THE E2A GENE PRODUCTS

The *E2A* gene products, called E12 and E47 (or "E2A proteins", for simplicity), may be considered amongst the founding members of the basic helix-loop-helix (bHLH) family of transcription factors since it was the original characterization of these proteins by Murre, McCaw and Baltimore that led to the recognition of the bHLH motif (7). In the bHLH domain, two amphipathic helices separated by a flexible "loop" structure mediate homo- or heterodimeric interactions between bHLH domains on separate protein molecules, whereas an adjacent group of basic amino acids immediately amino-terminal to the helix-loop-helix (HLH) portion mediates contacts with nucleotide bases in the major groove of DNA. DNA binding by bHLH proteins requires protein dimerization and generally occurs at sites, called "E-boxes", that contain the remarkably simple consensus sequence CANNTG. The crystal structure of the E47 bHLH domain bound to the sequence -CACCTG- has been reported (8). Hundreds of bHLH proteins have been identified in diverse organisms, including yeast and humans, where they appear to play fundamental roles in cell type specification (9).

E12 and E47 belong to "class I" of the bHLH family, which also includes HEB and E2-2 in mammals and Daughterless in Drosophila. Because their DNA binding is limited to elements containing the E-box consensus, class I proteins are also called "E-proteins" (9). E-proteins are expressed widely in tissues and are generally believed to promote tissue-specific differentiation through heterodimerizing with "class II" proteins, expression of which is restricted to particular tissues. For example, the E2A proteins promote myogenic differentiation through heterodimerizing with the myogenic class II proteins MyoD, MRF4, Myf-5 and myogenin, which are preferentially expressed in cells committed to skeletal muscle differentiation (10). The transcriptional and developmental functions of E2A proteins can be antagonized by a group of "class V" proteins called Id. Id1, Id2, Id3, and Id4 lack a basic region adjacent to their HLH domain and therefore appear capable of sequestering E-proteins in non-DNA binding heterodimers.

E2A generates both the E12 and E47 proteins through differential pre-mRNA splicing of a single exon. The two proteins are highly identical, with the differences between them largely limited to the bHLH domain, located near the C-terminus, within which there is 80% identity (Figure 1). Like other E-proteins, the E2A proteins are believed to function as transcriptional activators of specific target genes, generally binding DNA as heterodimers with class II bHLH proteins. Mapping studies using transient co-transfection of portions of E2A proteins experimentally fused to heterologous DNA binding domains have defined two activation domains, called AD1 and AD2, capable of mediating transcriptional activation of reporter genes (11, 12). AD2 is located near the middle of the protein. Its trans-activating activity appears limited to cells derived from pancreatic beta cells. In contrast, AD1, encompassing the first 99 amino acid residues at the extreme N-terminus of the protein, can function in a broad range of cell types, including B-lymphoid cells. Many DNA-binding transcription factors induce transcription in part by recruiting multi-protein co-activator complexes that, in many instances, are associated with acetyltransferase (AT) enzymatic activity (13). This results in acetylation of histones and other proteins in the neighborhood of the DNA-bound transcription factor, presumably enhancing accessibility of the locus to the general transcriptional apparatus and recruiting other chromatin-modifying proteins. Recently published findings indicate that AD1 contains an "LDFS motif" that is conserved in bHLH proteins from diverse species, including yeast. This domain, which is required for transcriptional activation by AD1, mediates interaction with an AT-associated co-activator complex called SAGA in yeast (14). While mammalian counterparts to SAGA exist, their interaction with AD1 has not been reported. E2A proteins can also interact with p300, a large protein with intrinsic AT activity (15, 16).

E2A proteins were originally identified through their ability to bind E-boxes within the prototypic intronic enhancer elements associated with the immunoglobulin heavy (*IGH*) and kappa light chain genes (7). E-boxes are also present in a number of non-lymphoid-specific genes and E2A proteins are implicated in non-lymphoid regulatory functions including, as already mentioned, myogenesis. However, several observations suggest a particular requirement for *E2A* in the ontogeny and function of lymphoid cells. Enforced expression of E12 or E47 can induce immunoglobulin gene rearrangement and transcription of several B-lineage-specific genes in non-B-lineage cells (17-19). These include two genes, early B-cell factor (*EBF*) and *Pax-5*, that are required in the earliest stages of B-lineage commitment and appear to function concurrently with, or downstream of, *E2A*. Perhaps more compellingly, homozygous *E2A*-null mice manifest complete blockade of B-lineage differentiation at the pro-B cell stage, prior to *IGH* gene rearrangement, along with profoundly disordered thymocyte maturation and a strong propensity to develop T-lymphoblastic lymphomas (20-22). Non-lymphoid lineages appear unaffected or affected in more subtle ways. Additional evidence points to roles for E2A proteins at later stages of B-cell development, including *IGH* class switching (23-26). At least some of the evident specificity for B-lymphocytes may be explained by the observation that E47 can form DNA-binding homodimers exclusively in B-lineage cells; DNA binding by E12 homodimers is inhibited by a domain N-terminal to the bHLH (27). Therefore, while the role of E2A proteins in heterodimers in other tissues may be filled by other proteins in the absence of E2A, the DNA-binding E47 homodimers formed in B-lineage cells may be irreplaceable. Furthermore, although expression of *E2A* has been considered ubiquitous, surveying primary human tissues has indicated preferential expression in B-

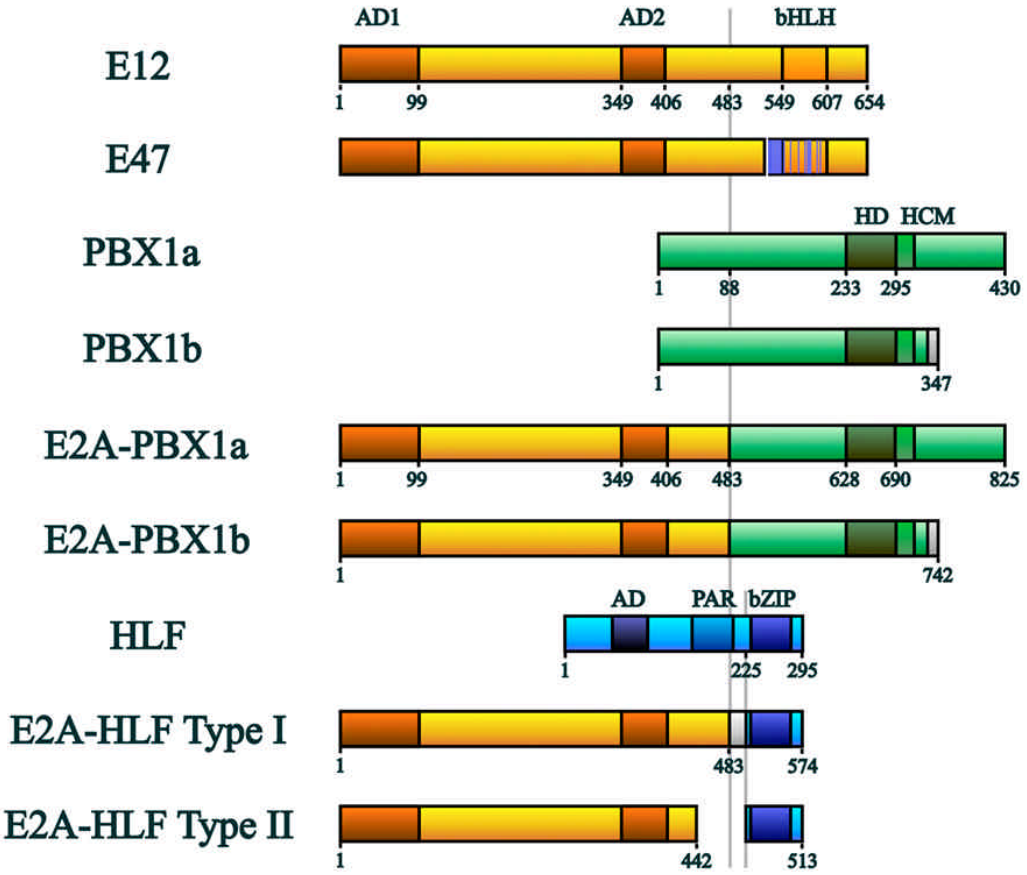

Figure 1. Domain structures of E2A proteins and fusion partners. The numbers indicate the first and last amino acid positions of the various proteins or domains. For E2A, PBX1 and E2A-PBX1, the numbers 483 and 88 refer to the amino acid positions on the N-terminal side of the fusion points. For HLF, the number 225 refers to the amino acid position on the C-terminal side of the fusion point. The vertical blue lines in E47 indicate non-identical amino acids relative to E12. AD, activation domain; bHLH, basic helix-loop-helix domain; HD, homeodomain; HCM, HOX cooperativity domain; PAR, PAR domain; bZIP, basic leucine zipper domain.

lymphocytes, particularly those in the "dark zone" of germinal centers (23, 25).

Considering their roles in transcriptional regulation, immunoglobulin gene rearrangement, isotype switching, and their interaction with ATs, it has been suggested that E2A proteins may function to regulate accessibility of specific loci to both the general transcriptional and recombination machinery (24).

4. CHROMOSOMAL TRANSLOCATION 1;19 AND *E2A-PBX1*

4.1. Clinical Features

Using standard cytogenetic techniques, t(1;19)(q23;p13.3) is detectable in roughly 5% of pediatric ALL cases, making it the second most frequently-encountered non-random translocation in this disorder, after t(12;21) (28). Its prevalence among adult cases appears similar (29). Translocation 1;19 is associated with ALL cases displaying a pre-B immunophenotype, as defined by the presence of cytoplasmic, but not surface, immunoglobulin μ heavy chain protein, and is detectable in some 20-25% of such cases (30, 31). The translocation has also been described in occasional cases of adult ALL, T-ALL, malignant lymphoma and acute myelogenous leukemia (AML) (29, 32-36). Prognostically, t(1;19) has been associated as an independent variable with an increased risk of relapse after treatment for pediatric ALL using protocols that were in general use in the 1980s (2). However, the translocation does not appear to represent an adverse prognostic factor in the context of current, more intensive therapeutic regimens (37).

In over 90% of cases, t(1;19) is associated with rearrangement of the *E2A* gene, as detected using genomic Southern blots or reverse transcriptase PCR (RT-PCR) (38, 39). Southern blots represent a sensitive means of detecting *E2A* involvement in t(1;19) since chromosomal breakpoints are consistently clustered within a 3.5 kb intron located between exons 13 and 14 of this gene (40, 41). Reagents have been developed for the detection of *E2A* translocation by fluorescence *in situ* hybridization, an assay that should

prove clinically useful (42). In the vast majority of cases, molecular rearrangement of *E2A* is associated with creation of an *E2A-PBX1* fusion gene, which may be inferred in clinical samples from the presence of aberrant E2A-PBX1 transcripts detectable by either Northern blots or RT-PCR. The observation that a small number of cases with cytogenetically detectable 1;19 translocations are not associated with rearrangement of *E2A* suggests involvement of other loci at the molecular level.

The *E2A-PBX1* fusion gene resides on the derivative chromosome 19. As a reciprocal translocation, t(1;19) is expected to result also in a derivative chromosome 1 harboring a *PBX1-E2A* fusion gene. However, in most cases of t(1;19) ALL, the translocation is unbalanced in most neoplastic cells. More specifically, apparent nondysjunction results in the loss of the derivative chromosome 1 and reduplication of the normal chromosome 1. The loss of the derivative chromosome 1 appears to take place during tumor progression, since both balanced and unbalanced translocation-containing cells are present in some cases. Controversy exists as to the clinical implications of these observations with some, but not all, studies suggesting an adverse prognosis in association with predominantly balanced 1;19 translocations (31, 43). From a biological perspective, the consistent retention of the derivative chromosome 9 and frequent loss of the derivative chromosome 1 in neoplastic cells, as well as the lack of expression of *PBX1-E2A* even in cases in which the derivative chromosome 1 is retained, supports a particular requirement for the *E2A-PBX1* fusion gene in leukemogenesis.

4.2. Structure and Function of PBX1

PBX1 was first identified as the gene fused to *E2A* as a consequence of the 1;19 translocation in leukemia (3). PBX1, its protein product, contains a homeodomain, a DNA-binding module related to the helix-turn-helix domains of bacterial DNA-binding proteins (Figure 1) (44). The homeodomain consists of 3 alpha helices packed around a central hydrophobic region.

Homeodomain proteins were first identified in Drosophila as the products of genes found to harbor mutations in strains of flies that display “homeotic” developmental abnormalities (i.e., those resulting from miss-assignment of cellular or body segment identity during embryogenesis) (45). These *HOM* genes are clustered together in the Drosophila genome in a linear sequence that correlates with that of their anatomical fields of influence on the anterior-posterior axis of the developing fly embryo. Their mammalian orthologs consist of 39 *HOX* genes distributed in four clusters within which the genes may be aligned into 13 paralogous groups (46). The HOX proteins encoded by these genes contain a relatively conserved, prototypic homeodomain of 61 amino acids. A large body of work, including gene targeting in mice, has confirmed the fundamental role of HOX proteins in mammalian embryonic development and cell fate determination.

Low-stringency cDNA library screening uncovered two genes closely related to *PBX1*, called *PBX2* and *PBX3*, and database searching recently uncovered *PBX4* (47, 48). The PBX proteins are expressed widely in tissues. Differential expression patterns of PBX1 in embryonic tissues have implied a role in organogenesis (47, 49, 50). Of relevance to leukemia, PBX1b and PBX3a are expressed by hematopoietic progenitors during the earliest stages of intra-embryonic hematopoiesis (51). Interestingly, however, PBX1 is not expressed in lymphoid cells.

The PBX proteins belong to the TALE (three amino acid loop extension) family of homeodomain proteins. These are encoded by genes that reside outside of the HOX clusters and are distinguished in part by the presence of three additional amino acids between the first and second helices of their homeodomains (52). Strong genetic evidence from Drosophila originally suggested that *EXD*, the fly ortholog of the mammalian *PBX* genes, cooperates with *HOM* genes during fly embryogenesis (53). Consistent with this observation, the PBX1 protein can interact directly with the products of most *HOX* genes (i.e., those from paralogous groups 1 to 10) such that the affinity and sequence specificity of DNA binding by the PBX1/HOX heterodimers is enhanced relative to the monomeric or homodimeric proteins (Figure 2) (54-59). Optimal cooperative DNA binding by PBX1/HOX heterodimers requires both homeodomains and interaction of a pentapeptide module (YPWMX) on the HOX protein with a hydrophobic pocket created within the PBX1 homeodomain by the three “additional” TALE amino acids (60). Furthermore, DNA-binding in the ternary complex is stabilized by a short (roughly 20 amino acids), conserved, C-terminal extension of the PBX1 homeodomain that has been referred to as the “HOX cooperativity motif” (HCM, Figure 1) (60, 61).

Besides the PBX proteins, the TALE family includes another group of related, non-clustered genes whose protein products include MEIS1 and PREP1 (also known as PKNOX1) (52, 62). These “MEIS-like” proteins are homologous to one another within their homeodomains and within a domain near their N-termini called the “HM domain”. MEIS-like proteins can heterodimerize with PBX proteins in an interaction involving the N-terminal HM domain of the MEIS-like factor and an evolutionarily conserved N-terminal domain of PBX (63-65). At least in nuclear extracts from some cell types, PBX1 exists largely in complexes with MEIS-type proteins even in the absence of DNA binding, raising the possibility that the principal functional unit in cell nuclei is the PBX-MEIS dimer (64). Nuclear localization of PBX1 appears to depend on its interaction with a MEIS protein, which prevents active nuclear export. Such control of PBX1 nuclear localization appears to play a regulatory role *in vivo*, at least during embryonic development (66-68). The MEIS-like proteins can also bind DNA cooperatively as heterodimers with some HOX proteins (paralogous groups 9 to 13) (69). Since PBX1 employs separate interaction surfaces to interact with HOX- and MEIS-type proteins (i.e., the homeodomain and N-terminal region, respectively), the potential exists for the formation of MEIS-PBX-HOX trimers (Figure 2). Such ternary complexes have indeed been observed (70, 71).

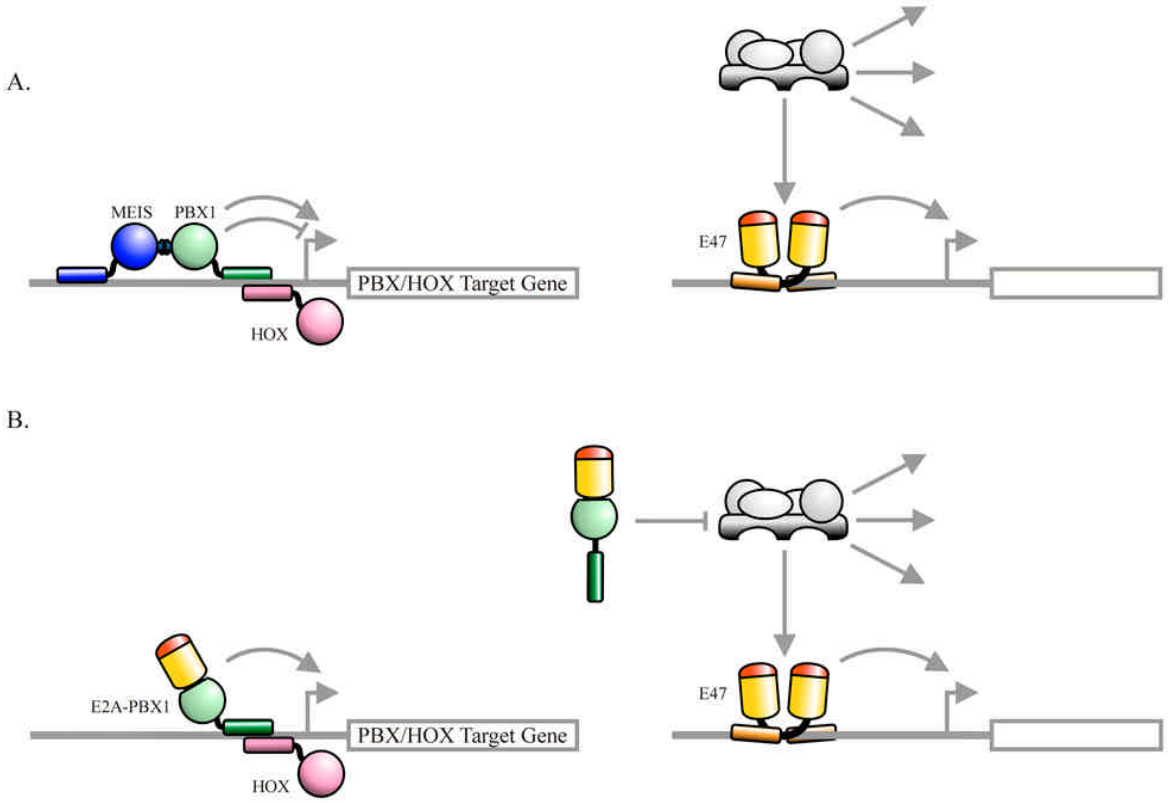

Figure 2. Models of transcriptional regulation and deregulation. A. Transcriptional regulation by wild-type proteins. In the diagram on the left, PBX1 binds to a tripartite DNA element in cooperation with MEIS and HOX proteins to repress or induce a hypothetical target gene. Transcriptional co-regulatory and other interactive transcription factors are not illustrated but probably involved. In the diagram on the right, E47 expressed in B-lymphoid cells binds an E-box element as a homodimer and induces target gene transcription by recruiting a co-activator complex. The latter may also be required for other DNA-binding transcription factors to fulfill their roles in maintaining a physiological gene expression profile. B. Models of transcriptional deregulation by E2A-PBX1. The diagram on the left illustrates induction by E2A-PBX1 of a hypothetical target gene normally regulated by PBX/HOX/MEIS-containing complexes. In this model, transcriptional deregulation is dependent on DNA binding by E2A-PBX1 mediated by an intact PBX1 homeodomain and HOX-interactive elements. In the diagram on the right, E2A-PBX1 impairs the function of a co-activator complex required in physiological gene regulation by E47 or other, hypothetical DNA binding transcription factors some of which could function normally as tumor suppressors.

Since the PBX, MEIS and HOX families each contain multiple members that can interact in different combinations, the theoretical potential exists for the formation of a considerable number of multi-protein complexes that differ with respect to their sequence preferences on binding to DNA (reviewed in reference 72). PBX1-PBX1 homodimers favor the sequence TGATTGAT, a direct repeat of the sequence TGAT. This consensus was determined using multiple rounds of selection by binding of random oligonucleotides (73-75). Although such PBX1 homodimers can form *in vitro*, it remains unclear what role they may play biologically (76). In *in vitro* DNA binding studies, PBX-HOX heterodimers favor the bipartite consensus sequence TGATNNAT(g/t)(g/a), within which the underlined portion is recognized by PBX and the remainder by the HOX partner (72, 77). The identity of the nucleotides at the variable positions varies according to the particular HOX partner involved. Sequences corresponding to this consensus have been identified within a number of naturally occurring regulatory elements (78). Those within elements that direct spatially restricted expression of Hoxb1 and Hoxb2 in embryonic mice have been particularly well characterized. In these studies, the requirement for intact PBX/Hox binding sites for directing the expression of reporter genes in normal anatomical distributions in transgenic mice supports the relevance of *in vitro* DNA binding studies to the *in vivo* setting (78-80). For example, a sequence element recognized by the homeodomain of MEIS-type proteins resides near, but not contiguous with, the single PBX/HOX binding site in the Hoxb2 enhancer. This MEIS binding site can stabilize DNA binding by a MEIS/PBX1/Hox complex and is required for spatially appropriate reporter gene expression in transgenic animals (71, 80).

The manner in which PBX1-containing hetero-oligomeric complexes influence target gene transcription once they are bound to their cognate DNA sequences is poorly understood. Transiently transfected PBX1 can

repress transcription of reporter constructs. This function maps to a central region of the protein (amino acids 39-232) and to the variably spliced C-terminus of PBX1a (81-83). Interactions with the co-repressor proteins HDAC1, HDAC3, mSIN3B, N-CoR and SMRT have also been mapped to these domains. In contrast, HOX proteins can demonstrably function as activators or repressors; some proteins can carry out either function, depending in part on the context of their binding site and, presumably, interactions with partner proteins, including PBX (84, 85). Accordingly, the downstream effects of DNA-bound PBX1/HOX hetero-oligomers appear quite variable. Experiments in which PBX1 was co-transfected with a large number of HOX proteins and PBX/HOX binding site combinations mostly failed to show trans-activation (86). Nonetheless, some HOX proteins appear to contain transcriptional activation domains and can induce transcription when transfected alone or with PBX- or MEIS-type proteins (87-89). Most convincingly, studies of the Hoxb1 and Hoxb2 enhancers document the potential of MEIS/PBX/HOX complexes to induce reporter gene expression in transgenic animals as well as on transient transfection of cultivated cells (71, 80, 90, 91). Recent findings suggest that most HOX proteins can interact with CBP or p300 by means of their homeodomains (86). Surprisingly, the interaction with CBP appears to impair both DNA binding by HOX and the intrinsic AT activity of CBP, suggesting that the HOX-CBP interaction may function to repress, rather than induce, transcription. In some instances, the transcriptional activity of DNA-bound HOX proteins or PBX/HOX complexes appears to be modulated by afferent signals originating from the cell surface (72). For example, Ras signaling induces vulva development in the nematode *Caenorhabditis elegans*. It appears that the HOX gene lin-39 is required to specify the outcome of Ras signaling by selectively activating vulva-specific genes in cells of the central body region (92). In a separate study using cultured cells, PBX/HOX complexes could be switched from transcriptional repressors to activators on the same promoter by cell aggregation or the activity of protein kinase A, a downstream mediator of signals transmitted through cAMP (83). Therefore, the downstream transcriptional output from key cis-acting regulatory elements may be determined through the integration of information specifying cell identity or anatomical positional, conveyed by DNA-bound PBX/HOX complexes, with signals from the cell surface, conveyed by signal-responsive transcription factors that bind nearby DNA.

Results of gene targeting studies in mice support the developmental requirement for *PBX1*. *PBX1*-/- animals manifest late embryonic lethality associated with hypoplasia or aplasia of multiple organs (93, 94). The abnormalities include extensive skeletal defects, some of which are distributed anatomically in a pattern consistent with HOX misfunction. Since these occur despite unperturbed expression levels of the pertinent HOX proteins, the findings are generally consistent with the proposed role for PBX1 in modulating HOX function at the protein level.

4.3. E2A-PBX1 is an Oncogenic Transcriptional Activator

In the vast majority of cases, t(1;19) results in expression of E2A-PBX1. E2A-PBX1 comprises the N-terminal two thirds of E2A fused to most of PBX1 (Figure 1). The portion of the E2A proteins usually included in the fusion protein (amino acids 1-483) is identical in E12 and E47. Differential splicing of the PBX1 portion continues to occur in the fusion pre-mRNA, so two fusion proteins are produced: E2A-PBX1a, which contains amino acids 89 to 430 of PBX1a, and E2A-PBX1b, containing the shorter C-terminus of PBX1b. Since no functional differences between these differential splice products have been consistently demonstrated, I will refer to them collectively as E2A-PBX1. In a minority of leukemia cases, the commonly encountered fusion transcripts are detectable in association with variant transcripts in which an additional 27 nucleotides, presumably originating from a differentially spliced exon, are present at the fusion point (95).

The oncogenicity of E2A-PBX1 has been demonstrated convincingly using a number of experimental models. Enforced expression in fibroblasts using retroviral vectors results in growth deregulation with the formation of transformed foci in confluent cell monolayers, anchorage-independent growth in soft agar, or tumor formation on injection into nude mice (96). Lethally irradiated mice reconstituted with primary murine bone marrow retrovirally transduced with E2A-PBX1 develop primitive myeloid neoplasms manifesting as acute myeloblastic leukemia (AML) or its solid tumor counterpart, granulocytic sarcoma (97). This apparent ability of E2A-PBX1 to block myeloid differentiation has also been shown in tissue culture: retrovirally transduced murine marrow propagated in the presence of GM-CSF or IL-3 gives rise to immortal, cytokine-dependent cells with morphological, cytochemical and immunophenotypic features of primitive myeloid blasts (98). Interestingly, marrow transduction with E2A-PBX1 fused to the ligand binding domain of the estrogen receptor protein gives rise to populations of primitive progenitors that are critically dependent on the presence of high concentrations of estradiol for continued propagation in tissue culture and maintenance of their relatively undifferentiated state; estrogen withdrawal leads to granulocytic or myeloid differentiation and death (99). Finally, transgenic mice in which lymphoid expression of *E2A-PBX1* is driven by an *IGH* enhancer die of primitive, largely solid lymphoid neoplasms at an early age (100). Curiously, these tumors are of T-lineage and arise primarily in the thymus with morphological and immunophenotypic features corresponding to those of T-lymphoblastic lymphoma.

The molecular mechanisms by which E2A-PBX1 contributes to neoplastic transformation remain uncertain despite having been the focus of considerable research. Normal hematopoiesis requires the intricate functional coordination of numerous transcription factors. Since the wild type E2A and PBX1 proteins are transcription factors with documented roles in hematopoiesis, the notion that E2A-PBX1 may function through disturbing the transcriptional regulation of key target genes is widely

favored. The cellular phenotypes observed in naturally occurring and experimental neoplasms induced by E2A-PBX1 suggest that these target genes play roles in lineage commitment, cellular maturation , and the regulation of cell proliferation.

The 1;19 translocation results in replacement of the C-terminal one third of E2A proteins, including the bHLH domain, with most of PBX1, including the homeodomain (Figure 1). Since the homeodomain of PBX1 is believed to be the only DNA binding module on the molecule, E2A-PBX1 might induce leukemia through the abnormal transcriptional activation of target genes defined by the PBX1 portion of the oncoprotein (Figure 2). Consistent with this model, wild-type PBX proteins fail to activate appropriate reporter plasmids, but E2A-PBX1 is potently trans-activating (73-75). Furthermore, structure-function comparisons have indicated a general correlation between the trans-activation of PBX-responsive reporters and neoplastic transformation (101, 102). Forced expression of wild-type E2A or PBX proteins is not transforming. As originally conceived, the "trans-activation of PBX1 target genes" model envisioned DNA binding by E2A-PBX1 as being mediated simply by the homeodomain of PBX1. But more contemporary ideas must take into account potential interactions between the PBX, HOX and MEIS classes of proteins.

4.4. Mechanisms of Oncogenesis: Contributions of the PBX1 Portion

Both clustered and non-clustered homeoproteins are implicated in normal hematopoiesis. For example, expression of genes in the HOXA and HOXB clusters is regulated differentially in hematopoietic cells in a differentiation stage- and lineage-dependent manner (103, 104). Enforced expression of HOXB4 by retroviral transduction of primary bone marrow results in a striking expansion of hematopoietic stem cells (105). And, experimental germline disruption of murine homeobox genes has resulted in hematopoietic perturbations in whole animals including, in the case of the non-clustered gene Hox11, asplenia (106-109). *Pbx1*-null animals manifest severe embryonic anemia associated with a reduction in the numbers and colony-forming ability of hematopoietic stem cells (51).

Homeodomain proteins are also involved in the abnormal hematopoiesis implicit in leukemia. More particularly, a number of homeoproteins besides PBX1 are structurally altered or miss-expressed as a result of direct somatic mutations in leukemic cells. For example, at least 5 homeoproteins (HOXA9, HOXD13, PMX1, HOXA11 and HOXA13) are fused to a portion of the nuclear pore complex protein NUP98 as a result of translocations associated with human myeloid neoplasms (110-113). HOX11 is transcriptionally up-regulated as a result of t(10;14) in cases of T-ALL (114). Neoplastic transformation by at least some oncogenic HOX proteins appears to require interaction with PBX proteins (115).

But what role might interactions with HOX proteins play in leukemogenesis by E2A-PBX1? The HOX-interactive portion of PBX1 (i.e., the homeodomain and adjacent HCM) is retained in E2A-PBX1 and, like wild-type PBX1, the fusion protein is capable of cooperating with HOX proteins in binding to DNA *in vitro* (Figure 1) (54, 55, 59). Furthermore, co-expressed Hoxa9 can synergize with E2A-PBX1 in transforming cells in primary murine bone marrow (116). However, since Hoxa9 and E2A-PBX1 are each independently capable of transforming primary murine hematopoietic cells, the apparent cooperativity could reflect the combined functional effects of separate oncogenic pathways, rather than direct physical interactions between the proteins. Wild-type PBX1 neither transforms primary hematopoietic cells nor enhances transformation by Hoxa9, suggesting that PBX1 availability is non-limiting in HOX-mediated transformation (117).

Pertinent to the role of PBX-HOX interactions are the PBX1 domain requirements in E2A-PBX1 leukemogenesis. Neither wild-type E2A nor PBX1 is oncogenic. Deleting the entire PBX1 portion from E2A-PBX1 abrogates focus formation in fibroblasts, indicating that truncation of E2A is not sufficient. Curiously, focus formation can be reconstituted by adding back any one of several non-overlapping portions of PBX1, including the PBX1 homeodomain alone, PBX1 lacking the homeodomain, or the portion of PBX1 lying between the fusion point and the homeodomain (61, 101). Perhaps most surprisingly, deletion of the homeodomain, the only DNA binding module on the E2A-PBX1 molecule and a component required for interacting with HOX proteins, impairs neither focus formation in cultured fibroblasts nor lymphoma induction in transgenic mice (101). In contrast to the findings in fibroblasts, immortalization and differentiation blockade in primary murine bone marrow require both the homeodomain and the HCM (102). Collectively, these results suggest that, while DNA binding, and perhaps HOX interactions, are dispensable for growth deregulation in the fibroblast assays, both may be required for immortalization and differentiation blockade in primary bone marrow cells. However, since technical differences between the assays could explain these discrepant results (for example, differential stability of mutant proteins in different cell types), the roles of the PBX1 homeodomain and HOX interactions in human leukemia remain undetermined.

Ultimate resolution of this issue will require the identification of transcriptional target genes whose deregulation by E2A-PBX1 contributes to leukemogenesis. Several labs have used representational difference analysis (RDA), a PCR-based technique, to identify transcripts affected by expression of E2A-PBX1 in cultured cells. Some studies have identified genes induced upon enforced expression of exogenous E2A-PBX1 in cultured fibroblasts or hematolymphoid cells (118-121). Others have identified transcripts differentially expressed in ALL cell lines with t(1;19), as compared with ALL cells lacking this translocation (122, 123). These two approaches are complementary: the former determines dependency of candidate target gene expression on E2A-PBX1, and the latter determines the transcriptional correlates of

endogenous E2A-PBX1 in the context of its "naturally occurring" gene expression profile and genetic background. Collectively, these experiments identified 27 candidate E2A-PBX1 target genes, some of which appear plausible as oncogenic mediators. No targets of E2A-PBX1 repression were identified. Some candidate target genes were inducible by E2A-PBX1 in one cell type (for example fibroblasts) but not another (for example myeloid cells), and a subset were inducible by mechanisms independent of DNA binding by E2A-PBX1. Importantly, these observations suggest that target genes may be regulated downstream of E2A-PBX1 through multiple distinct mechanisms involving a variety of cofactors. Do the candidates genes unearthed to date mediate E2A-PBX1-induced leukemia? Several are not expressed in t(1;19)-positive leukemic cells. Of those that are, neither the nature of their regulatory relationship with E2A-PBX1, nor a critical role in oncogenesis have been demonstrated.

The portion of PBX1 that interacts with the MEIS-like proteins is contained within the first 80 amino acids and therefore lost on fusion to E2A (Figure 1). As a result, E2A-PBX1 cannot interact directly with the MEIS-like factors and is unlikely to participate in functional complexes with them. The MEIS-like proteins could still play some role in E2A-PBX1-mediated leukemogenesis, however. Nuclear localization of PBX proteins appears to depend on the MEIS interaction (66-68). Nuclear localization signals located within the PBX1 homeodomain become functional only on interaction of an N-terminal domain with the corresponding region of a MEIS-type protein. Whereas transiently transfected PBX1 assumes a pan-cellular or cytoplasmic distribution in some cells, E2A-PBX1 appears exclusively nuclear (124). Nuclear localization of the fusion proteins seems to be mediated by constitutive nuclear localization signals within the E2A portion of the molecule, but loss of the MEIS-interactive domain of PBX1 could also contribute by exposing the nuclear localization signals in the PBX1 homeodomain. This hypothesis is supported by the observation that a PBX1 mutant with a deletion of the N-terminal 80 amino acids is predominantly nuclear on transient transfection in some cell types (68). Also noteworthy is that transiently transfected E2A-PBX1 is not distributed uniformly in nuclei but rather concentrated within spherical nuclear domains that do not correspond to known structures or functional subdomains (124). This unique localization pattern is not shared by either the wild type PBX1 or E2A proteins and could conceivably contribute to the transforming properties of E2A-PBX1.

4.5. Mechanisms of Oncogenesis: Contributions of the E2A Portion

Deletion of the transcriptional activation domain AD1 abrogates the ability of the oncoprotein to induce focus formation in fibroblasts or differentiation arrest in primary myeloid cells (101, 102). The same deletions also abrogate trans-activation of PBX1-responsive reporter plasmids in transient transfection experiments. These results suggest that fusion to PBX1 of one or more activation domains from E2A promotes oncogenesis through aberrant transcriptional induction of PBX1/HOX target genes. While this concept is attractive, additional findings raise the possibility of complementary or alternative oncogenic mechanisms.

In the induction of transformed foci in fibroblasts, the PBX1 portion of E2A-PBX1 can be supplanted by heterologous dimerization/DNA binding domains derived from the yeast transcription factors GAL4 or GCN4 (125). Furthermore, transduction of fibroblasts with an amino terminal portion of E2A (aa 1-483, the portion present in E2A-PBX1) fused to two tandem FKBP domains, which mediate homodimerization only in the presence of a synthetic ligand, induces focus formation exclusively in the presence of the synthetic ligand. These results are consistent with the ability of separate, non-overlapping portions of PBX1 to reconstitute focus formation when fused to E2A 1-483. They suggest that the PBX1 portion could function by promoting oligomerization of the oncoprotein or juxtaposition of multiple E2A moieties. Effects on growth regulation could be mediated through enhanced binding of oligomerized E2A-fusion proteins to key nuclear cofactors. Although E2A-PBX1 can homodimerize on certain DNA binding sites, the biological relevance of this interaction remains unclear (76).

How might E2A-PBX1 promote leukemogenesis independently of DNA binding? As we have argued previously, E2A-PBX1 could exert dominant-inhibitory effects on wild-type E2A or related proteins (125). Diverse observations suggest that E2A could function as a tumor suppressor. E2A proteins can promote cellular differentiation and exert anti-proliferative effects. For example, heterodimers formed by E2A with tissue specific, myogenic bHLH proteins promote muscle differentiation associated with cell cycle arrest (126). In fibroblasts, forced expression of E47 delays progression through the G1 phase of the cell cycle (127). E2A can directly trans-activate the CDK inhibitor p21/WAF1/CIP1 (128). Enforced expression of E47 or E12 in E2A-deficient lymphoma cells induces apoptosis (129). Conversely, Id proteins, which function as naturally occurring dominant inhibitors of E2A, promote proliferation and a transformed phenotype in transfected cells (130, 131). SCL/TAL1, a bHLH protein that can sequester E2A in transcriptionally inactive multimeric complexes, is an oncoprotein whose over-expression contributes to human T-ALL (132-134). Finally, some features of *E2A-PBX1* transgenic mice are also observed in *E2A*-null animals, including disordered thymocyte maturation, a paucity of B-lymphocytes and a strong propensity to develop T-lymphoblastic lymphoma (20, 22, 100).

Additional evidence for E2A-PBX1 interference with E2A function in leukemia induction derives from retroviral mutagenesis experiments. *E2A-PBX1* transgenic mice infected with Moloney murine leukemia virus (MMuLV) develop lymphoma after a shorter latency period than uninfected animals (135). In 88% of these neoplasms, retroviral insertion involves the *Notch 1* locus. All of the disrupted *Notch1* alleles encode truncated proteins predicted to increase signaling through downstream pathways. The Notch proteins are transmembrane receptors

that play fundamental, if pleiotropic, roles in cell fate determination. Notch signaling interferes with E2A function and early B-cell development (136). In fact, mice that express activated Notch share several features with E2A-null animals, including B-cell deficiency and a tendency to develop T-lineage neoplasms (22). Increased signaling from Notch1 has been implicated in primary human lymphoid neoplasms, including T-ALL and malignant lymphomas (137, 138). Therefore, the observation that gain of function mutations in *Notch1*, a known inhibitor of E2A, can synergize with *E2A-PBX1* in generating lymphoid neoplasms in mice supports the notion that impaired E2A function may contribute to E2A-PBX1 leukemogenesis.

The activation domains of DNA binding transcription factors generally induce transcription from nearby promoters by recruiting co-activator proteins through protein-protein interactions (reviewed in reference 13). The E2A transcriptional activation domain AD1 is consistently required for neoplastic transformation by E2A-fusion proteins, regardless of DNA binding competency. According to the "trans-activation of PBX1 target genes" model of E2A-PBX1 leukemogenesis, the oncogenic requirement for AD1 is in the recruitment of co-activators that induce transcription of PBX/HOX target genes. However, AD1 could also function to sequester cofactors, including co-activators, required for wild-type E2A to carry out tumor suppressor functions. Moreover, the adverse consequences of interaction between E2A-PBX1 and co-activator complexes need not be limited to E2A. In theory, a large number of different transcription factors could be influenced by virtue of a common requirement for functional interactions with co-activators affected by E2A-PBX1 (Figure 2).

The proteins CBP and p300 are close paralogs in mammals and possess intrinsic AT activity. They function as transcriptional co-activators, interacting with a large number of DNA binding transcription factors (reviewed in reference 139). Heterozygous germline disruption of CBP predisposes to malignancy in humans and mice (140, 141). CBP/p300 interact with a complex that contains the AT protein p/CAF. E1A is an adenovirus oncoprotein that promotes host cell cycle progression by interacting with the pocket protein pRb. However, the full transforming function of E1A also requires interaction with CBP/p300. Although the mechanism by which the E1A-CBP/p300 interactions contribute to neoplastic transformation is not completely understood, disruption of the interaction between CBP/p300 and p/CAF seems important (142). Could E2A-PBX1 function in a manner analogous to E1A, by altering the function of CBP/p300 or of other transcriptional co-regulatory factors?

E2A proteins can interact with CBP/p300, and AD1 of E2A has been shown to interact with the SAGA complex in *S. cerevisiae* (14-16). SAGA is the yeast counterpart of several mammalian HAT complexes, including the p/CAF complex. Although no published evidence supports direct interaction between E2A-PBX1 and the SAGA-related mammalian HAT complexes, one compelling possibility is that such complexes may represent tumor suppressors whose functions are compromised through physical interaction with E2A-PBX1. Furthermore, PBX1 can interact with transcriptional co-repressors, including some with intrinsic HDAC activity (82, 83). Could HDACs bound to the PBX1 portion of E2A-PBX1 modify the function of co-activator complexes tethered to the E2A portion of the oncoprotein?

5. CHROMOSOMAL TRANSLOCATION 17;19 AND *E2A-HLF*

5.1. Structural Features of E2A-HLF and Evidence of Oncogenicity

Translocation (17;19)(q22;p13), the other well characterized translocation involving *E2A* in pediatric ALL, occurs in 0.5 to 1% of cases (143). Although the rarity of this translocation has made it difficult to establish clinical correlations, most patients have been older children or adolescents (144). Many have manifested at diagnosis a blood clotting abnormality called disseminated intravascular coagulation (DIC), usually rare in children presenting with standard ALL. Hypercalcemia is also relatively common. Immunophenotypically, t(17;19)-associated leukemic cells are generally of the "common", B-cell precursor variety without cytoplasmic immunoglobulin heavy chain expression. Most patients with t(17;19) died of leukemia, suggesting that this translocation portends an adverse clinical outcome.

In 1992, molecular cloning of the chromosomal breakpoints established that in most cases, cytogenetically detected t(17;19) is associated with fusion of *E2A* to a novel gene on chromosome 17 called *HLF* (hepatic leukemia factor) (4, 5). *HLF* encodes a transcription factor of the basic leucine zipper (bZIP) family. Functionally analogous to the bHLH of E2A, the bZIP domain consists of a region rich in basic amino acids immediately N-terminal to an amphipathic alpha-helical domain. The basic region contacts DNA in the major groove, favoring the nearly palindromic consensus sequence 5'-GTTACGTAAT-3', and the zipper domain mediates homo- or heterodimeric interactions with other bZIP proteins (145, 146). Sequence conservation within the bZIP domain, and in a more C-terminal region called the "proline and acidic amino acid-rich" (PAR) domain, identifies HLF as a member of a subgroup of bZIP factors called "PAR proteins" (Figure 1). HLF can bind DNA either as a homodimer or a heterodimer, with the choice of heterodimeric partners limited to other PAR proteins. HLF can trans-activate reporter genes in transient co-transfection experiments, and an activation domain has been mapped to a region within the N-terminal half of the protein (147). HLF is expressed preferentially in the liver, kidneys and some central nervous system neurons, with little or no expression in hematopoietic cells (4, 148).

In a general sense, t(17;19) resembles t(1;19) with respect to the structural features of its fusion protein: the C-terminus of E2A, including the bHLH domain, is lost and replaced by a C-terminal portion of HLF that includes the bZIP domain to create the chimeric protein E2A-HLF

(Figure 1). Two mechanisms serve to maintain the reading frame across the fusion points in E2A-HLF mRNAs (149). In Type I translocations, exon 13 of *E2A* is fused to exon 4 of *HLF*, which is in a different reading frame. In the fused, processed mRNA molecule, E2A and HLF exons are separated by a cryptic exon which contains intronic nucleotides from *E2A* at its 5' end, intronic nucleotides from *HLF* at its 3' end, and a short intervening run of non-templated nucleotides presumably added by terminal deoxynucleotidyl transferase (TdT) at the time of translocation. In this manner, the reading frame is maintained, and the *E2A*- and *HLF*-encoded portions of the resulting protein are separated by polypeptide sequences that do not occur in either of the wild-type proteins. In Type II translocations, exon 12 of *E2A* is fused directly to exon 4 of *HLF* in the processed mRNA. No frame shift occurs across the fusion point since these exons are in the same reading frame. Two inferences may be drawn from these structural considerations. In Type I translocations, the convoluted manner in which the reading frame is maintained suggests that mere truncation of E2A is insufficient for oncogenesis and that the HLF portion makes a positive contribution. Secondly, the absence from Type II *E2A-HLF* of *E2A* exon 13, present in both *E2A-PBX1* and Type I *E2A-HLF*, indicates the dispensability of this exon for oncogenesis.

E2A-HLF induces foci, anchorage-independent growth, and tumorigenicity in NIH 3T3 fibroblasts; in transgenic mice E2A-HLF induces lymphoid malignancies (150-152). Forced expression of a dominant-inhibitory mutant of E2A-HLF in cells with t(17;19) (expressing endogenous E2A-HLF) produces apoptotic cell death (153). Conversely, forced expression of exogenous E2A-HLF blocks apoptosis of IL-3-dependent Ba/F3 cells upon growth factor deprivation . Therefore, leukemogenesis by E2A-HLF may involve aberrant escape from physiological cell death.

Two groups have generated mice in which expression of an *E2A-HLF* transgene is directed by immunoglobulin gene-derived enhancers (151, 152). The thymuses in these animals are small, showing disordered thymocyte maturation and increased apoptosis. A high rate of T-lineage lymphoblastic neoplasms was noted. One mouse model, but not the other, manifests B-lineage lymphopenia apparently resulting from blockade of B-lineage maturation at an early stage. The pro-apoptotic effect of E2A-HLF in the thymuses of these animals appears incongruent with the activation of anti-apoptotic signaling pathways in tissue culture models.

5.2. Transcriptional Target Genes of E2A-HLF

The obvious structural parallels with E2A-PBX1 have suggested that E2A-HLF might contribute to leukemogenesis in a similar manner. As with E2A-PBX1, a prevailing model portrays leukemogenesis by E2A-HLF as mediated by abnormal transcriptional regulation of key target genes regulated by HLF. Unlike PBX1, however, wild-type HLF is a strong trans-activator of artificial target genes, and the potency of HLF as a trans-activator is not significantly enhanced on fusion with E2A (145, 147). Furthermore, E2A-HLF binds DNA with lower affinity than wild-type HLF, especially on non-optimal binding sites, due to the loss of the PAR domain and of other elements that stabilize the interaction of HLF with DNA. In support of the prevailing model, on the other hand, E2A-HLF can trans-activate reporters in a broader range of cell types than wild-type HLF, and the altered DNA-binding properties associated with E2A-HLF could alter the repertoire of genes targeted for activation (146). The E2A activation domains could function in a qualitatively different manner than the HLF activation domain, despite their apparent equivalency in co-transfection assays. Along these lines, a powerful activation domain from the Herpes simplex protein VP16 fails to supplant the E2A-derived portion of E2A-PBX1 in deregulating growth in fibroblasts (125). Alternatively, E2A-HLF induction of HLF target genes in lymphoid progenitor cells, which do not normally express wild-type HLF, could be oncogenic simply as a result of ectopic function.

Dr. Thomas Look and his colleagues have used RDA to identify genes expressed downstream of E2A-HLF in IL-3-dependent lymphoid cells. In the nematode *Caenorhabditis elegans*, CES-2 is a bZIP transcription factor that negatively regulates *ces-1* which, in turn, negatively regulates cell death-promoting genes. An analogous pathway appears to exist in mammals. E2A-HLF is analogous to CES-2 and can induce expression of *SLUG*, a zinc finger transcription factor homologous to CES-1 (154). Forced expression of SLUG promotes survival in hematopoietic cells, possibly through repression of pro-apoptotic genes. Thus, E2A-HLF could impair physiological cell death through aberrant transcriptional repression of pro-apoptotic genes mediated indirectly through the induction of *SLUG*. Accordingly, expression of *SLUG* in leukemic cell lines appears largely restricted to those expressing E2A-HLF. Although a critical requirement for signaling through *SLUG* in E2A-HLF leukemogenesis remains to be demonstrated, the involvement of this pathway in regulation of cell survival fits well with the anti-apoptotic phenotype in E2A-HLF-expressing cells.

Dang et. al. reported recently that forced expression of E2A-HLF in FL5.12 cells, an IL-3-dependent pro-B line, induces the expression of genes from the *Groucho* family and represses *AML1* (also called *RUNX1*) (155). The *Groucho* genes encode co-repressors capable of interacting with DNA-binding transcription factors to down-regulate target gene transcription. *AML1* is the gene most frequently involved by chromosomal translocations in human leukemia. For example, t(12;21), the most frequent translocation in pediatric ALL (20-25% of cases), fuses most of the AML1 protein to a portion of the ets-type transcription factor TEL (reviewed in reference 156). AML1 is a DNA-binding transcription factor required for the establishment of definitive hematopoiesis (157). Powerful evidence suggests AML1-fusion proteins promote leukemogenesis through dominant-inhibitory effects on AML1-containing protein complexes. Furthermore, Groucho family proteins (called Grg and TLE proteins in mice and humans, respectively) can interact with AML1 to

convert it from a transcriptional activator to a repressor. Therefore, the findings of Dang et. al. raise the possibility that E2A-HLF leukemogenesis may be mediated, at least in part, through transcriptional repression of AML1 target genes. Potential exists for a novel mechanistic convergence downstream of *E2A* and *AML1* translocations.

5.3. E2A-HLF Structure/Function Correlations: Parallels with E2A-PBX1 and E2A-FB1

Structure-function studies of E2A-HLF have generated interesting and sometimes surprising results. Deletion of either of the E2A activation domains (AD1 or AD2) or of the HLF leucine zipper abrogates growth deregulation in fibroblasts (150). Interestingly, neither the basic nor ZIP domains of HLF are required for the anti-apoptotic effect of E2A-HLF in FL5.12 cells (158). In fact, amino acids 1-483 of E2A unfused to any polypeptide suffice to block apoptosis in this system. However, one or both of the E2A activation domains are required, depending on the functional status of the HLF DNA-binding domain. Full length, wild-type E12 or E47 do not inhibit apoptosis. Therefore, simple deletion of a C-terminal portion of E2A that includes the bHLH domain appears to unmask some latent anti-apoptotic function associated with the N-terminal two thirds of the protein. Notably, for transcriptional induction of *Groucho* family genes and repression of *AML1*, DNA binding by E2A-HLF is also dispensable, and the AD1 activation domain of E2A is required (155). As with E2A-PBX1, the oncogenic requirements for various sub-domains of the HLF portion of E2A-HLF are variable, depending on the particular experimental system employed, while the requirement for at least one of the E2A activation domains seems constant.

Most striking in the animal models is the similarity of the *E2A-HLF* transgenic mice to both the *E2A-PBX1* transgenic and *E2A*-null animals; each of these models manifests disordered thymocyte maturation, a paucity of peripheral B-lymphocytes, and a high rate of T-lymphoblastic malignant neoplasms (20-22, 100, 151, 152). A parsimonious explanation might invoke loss of function of *E2A*-dependent signaling pathways in the *E2A-HLF* and *E2A-PBX1* transgenic animals.

According to a recent report, in some cases of childhood leukemia a rearrangement of chromosome 19 results in fusion of *E2A* to a novel gene called *FB1* (6). In most cases analyzed, the translocations result in out of frame fusions with *FB1*, theoretically leading to truncation of the chimeric protein products within the *FB1*-encoded portion. No functional studies have been published. However, based on this report, leukemogenesis by E2A-FB1 fusion proteins would appear not to rely on induction of specific, FB1-defined target genes; expression of a truncated E2A protein may be more important.

The evidence cited in this section suggests that the three E2A-fusion proteins characterized so far contribute to leukemogenesis through a shared mechanism involving the E2A portion, with the heterologous C-terminal additions playing lesser roles. On the other hand, the oncogenic mechanisms of E2A-HLF may diverge significantly from those of E2A-PBX1. Whereas E2A-HLF is anti-apoptotic in hematopoietic cells, E2A-PBX1 is pro-apoptotic; expression profiles have uncovered divergent transcriptional programs downstream of the two oncoproteins; and, perhaps most impressively, divergent clinical and immunophenotypic features occur in the human leukemias associated with these two fusion proteins (121, 159). While these differences await a definitive mechanistic explanation, the potential role played by differential effects on transcriptional target genes defined by the PBX1 or HLF portions of the proteins merits continued, vigorous investigation.

6. PERSPECTIVE

The molecular characterization of oncogenic E2A-fusion proteins has blossomed from what initially appeared as the relatively straightforward documentation of transcriptional induction of PBX1- or HLF-defined target genes by heterologous E2A activation domains into diverse lines of investigation. These studies have shed considerable light, for example, on transcriptional aspects of lymphopoiesis and lymphocyte function, as well as transcriptional mechanisms by which HOX proteins function in the determination of cellular identity. Although exciting, our expanded awareness of potential leukemogenic mechanisms has made a full understanding of the mechanisms by which E2A-fusion proteins contribute to the development of leukemia in children seem farther away than a decade ago. The high priority work remaining to be done falls into four categories: 1) identification and characterization of key protein-protein and protein-DNA interactions involving E2A-fusion proteins; 2) identification of biologically relevant target genes of E2A-fusion proteins; 3) identification and characterization of functionally important post-translational modifications of E2A-fusion proteins; and, 4) development of a B-lymphoid experimental model of E2A-fusion protein leukemogenesis that can be used to determine which molecular findings are actually relevant to leukemogenesis. It seems likely that such studies will eventually elucidate functional relationships between E2A-fusion proteins and known or yet to be uncovered signaling pathways and, eventually, contribute to the development of new leukemia drugs that are more effective and less toxic than those currently in use.

7. ACKNOWLEDGMENT

I thank Dr. Stephen Hunger, Dr. Adam Goldfarb and Ms. Brandy Hyndman for thoughtful comments on the manuscript.

8. REFERENCES

1. Rowley, J. D.: The role of chromosome translocations in leukemogenesis. *Semin Hematol* 36: 59-72 (1999)
2. Crist, W. M., A. J. Carroll, J. J. Shuster, F. G. Behm, M. Whitehead, T. J. Vietti, A. T. Look, D. Mahoney, A. Ragab, D. J. Pullen & *et al*: Poor prognosis of children with pre-B acute lymphoblastic leukemia is associated with the t(1;19)(q23;p13): a Pediatric Oncology Group study. *Blood* 76: 117-122 (1990)

3. Nourse, J., J. D. Mellentin, N. Galili, J. Wilkinson, E. Stanbridge, S. D. Smith & M. L. Cleary: Chromosomal translocation t(1;19) results in synthesis of a homeobox fusion mRNA that codes for a potential chimeric transcription factor. *Cell* 60: 535-545 (1990)
4. Hunger, S. P., K. Ohyashiki, K. Toyama & M. L. Cleary: Hlf, a novel hepatic bZIP protein, shows altered DNA-binding properties following fusion to E2A in t(17;19) acute lymphoblastic leukemia. *Genes & Dev* 6: 1608-1620 (1992)
5. Inaba, T., W. M. Roberts, L. H. Shapiro, K. W. Jolly, S. C. Raimondi, S. D. Smith & A. T. Look: Fusion of the leucine zipper gene HLF to the E2A gene in human acute B-lineage leukemia. *Science* 257: 531-534 (1992)
6. Brambillasca, F., G. Mosna, M. Colombo, A. Rivolta, C. Caslini, M. Minuzzo, G. Giudici, L. Mizzi, A. Biondi & E. Privitera: Identification of a novel molecular partner of the E2A gene in childhood leukemia. *Leukemia* 13: 369-375 (1999)
7. Murre, C., P. S. McCaw & D. Baltimore: A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and myc proteins. *Cell* 56: 777-783 (1989)
8. Ellenberger, T., D. Fass, M. Arnaud & S. C. Harrison: Crystal structure of transcription factor E47: E-box recognition by a basic region helix-loop-helix dimer. *Genes Dev* 8: 970-980 (1994)
9. Massari, M. E. & C. Murre: Helix-loop-helix proteins: regulators of transcription in eucaryotic organisms. *Mol Cell Biol* 20: 429-440 (2000)
10. Dias, P., M. Dilling & P. Houghton: The molecular basis of skeletal muscle differentiation. *Semin Diagn Pathol* 11: 3-14 (1994)
11. Quong, M. W., M. E. Massari, R. Zwart & C. Murre: A new transcriptional-activation motif restricted to a class of helix-loop-helix proteins is functionally conserved in both yeast and mammalian cells. *Mol Cell Biol* 13: 792-800 (1993)
12. Aronheim, A., R. Shiran, A. Rosen & M. D. Walker: The E2A gene product contains two separable and functionally distinct transcription activation domains. *Proc Natl Acad Sci USA* 90: 8063-8067 (1993)
13. Naar, A. M., B. D. Lemon & R. Tjian: Transcriptional coactivator complexes. *Annu Rev Biochem* 70: 475-501 (2001)
14. Massari, M. E., P. A. Grant, M. G. Pray-Grant, S. L. Berger, J. L. Workman & C. Murre: A conserved motif present in a class of helix-loop-helix proteins activates transcription by direct recruitment of the SAGA complex. *Mol Cell* 4: 63-73 (1999)
15. Qiu, Y., A. Sharma & R. Stein: p300 mediates transcriptional stimulation by the basic helix- loop-helix activators of the insulin gene. *Mol Cell Biol* 18: 2957-2964 (1998)
16. Eckner, R., T. P. Yao, E. Oldread & D. M. Livingston: Interaction and functional collaboration of p300/CBP and bHLH proteins in muscle and B-cell differentiation. *Genes & Dev* 10: 2478-2490 (1996)
17. Kee, B. L. & C. Murre: Induction of early B cell factor (EBF) and multiple B lineage genes by the basic helix-loop-helix transcription factor E12 . *J Exp Med* 188: 699-713 (1998)
18. Schlissel, M., A. Voronova & D. Baltimore: Helix-loop-helix transcription factor E47 activates germ-line immunoglobulin heavy-chain gene transcription and rearrangement in a pre-T-cell line. *Genes & Dev* 5: 1367-1376 (1991)
19. Choi, J. K., C. P. Shen, H. S. Radomska, L. A. Eckhardt & T. Kadesch: E47 activates the Ig-heavy chain and TdT loci in non-B cells. *EMBO J* 15: 5014-5021 (1996)
20. Bain, G., E. C. Robanus Maandag, D. J. Izon, D. Amsen, A. M. Kruisbeek, B. C. Weintraub, I. Krop, M. S. Schlissel, A. J. Feeney, M. van Roon, M. van der Valk, H. P. J. te Riele, A. Berns & C. Murre: E2a proteins are required for proper B cell development and initiation of immunoglobulin gene rearrangements. *Cell* 79: 885-892 (1994)
21. Zhuang, Y., P. Soriano & H. Weintraub: The helix-loop-helix gene E2A is required for B cell formation. *Cell* 79: 875-884 (1994)
22. Bain, G., I. Engel, E. C. Robanus Maandag, H. P. te Riele, J. R. Voland, L. L. Sharp, J. Chun, B. Huey, D. Pinkel & C. Murre: E2A deficiency leads to abnormalities in $\alpha\beta$ T-cell development and to rapid development of T-cell lymphomas. *Mol Cell Biol* 17: 4782-4791 (1997)
23. Goldfarb, A. N., J. P. Flores & K. Lewandowska: Involvement of the E2A basic helix-loop-helix protein in immunoglobulin heavy chain class switching. *Molecular Immunology* 33: 947-956 (1996)
24. Quong, M. W., D. P. Harris, S. L. Swain & C. Murre: E2A activity is induced during B-cell activation to promote immunoglobulin class switch recombination. *EMBO J* 18: 6307-6318 (1999)
25. Rutherford, M. N. & D. P. LeBrun: Restricted expression of E2A protein in primary human tissues correlates with proliferation and differentiation. *Am J Pathol* 153: 165-173 (1998)
26. Kee, B. L., M. W. Quong & C. Murre: E2A proteins: essential regulators at multiple stages of B-cell development. *Immunol Rev* 175: 138-149 (2000)
27. Shen, C. P. & T. Kadesch: B-cell-specific DNA binding by an E47 homodimer. *Mol Cell Biol* 15: 4518-4524 (1995)
28. Pui, C. H. & W. E. Evans: Acute lymphoblastic leukemia. *New England Journal of Medicine* 339: 605-615 (1998)
29. Groupe Francais de Cytogenetique Hematologique: Cytogenetic abnormalities in adult acute lymphoblastic leukemia: correlations with hematologic findings outcome. A Collaborative Study of the Group Francais de Cytogenetique Hematologique. *Blood* 87: 3135-3142 (1996)
30. Carroll, A. J., W. M. Crist, R. T. Parmley, M. Roper, M. D. Cooper & W. H. Finley: Pre-B cell leukemia associated with chromosome translocation 1:19. *Blood* 63: 721 (1984)
31. Pui, C.-H., S. C. Raimondi, M. L. Hancock, G. K. Rivera, R. C. Ribeiro, H. H. Mahmoud, J. T. Sandlund, W. M. Crist & F. G. Behm: Immunologic, cytogenetic, and clinical characterization of childhood acute lymphoblastic leukemia with the t(1;19) (q23; p13) or its derivative. *J Clin Oncol* 12: 2601-2606 (1994)
32. Hunger, S. P., T. Sun, A. F. Boswell, A. J. Carroll & L. McGavran: Hyperdiploidy and E2A-PBX1 fusion in an adult with t(1;19)+ acute lymphoblastic leukemia: case

report and review of the literature. *Genes Chromosomes Cancer* 20: 392-398 (1997)
33. Miyamoto, K., N. Tomita, A. Ishii, H. Nonaka, T. Kondo, T. Tanaka & K. Kitajima: Chromosome abnormalities of leukemia cells in adult patients with T-cell leukemia. *J Natl Cancer Inst* 73: 353-362 (1984)
34. Wlodarska, I., M. Stul, C. De Wolf-Peeters, G. Verhoef, C. Mecucci, J. J. Cassiman & H. Van den Berghe: t(1;19) without detectable E2A rearrangements in two t(14;18)- positive lymphoma/leukemia cases. *Genes Chrom Cancer* 10: 171-176 (1994)
35. Vagner-Capodano, A. M., M. J. Mozziconacci, H. Zattara-Cannoni, A. M. Guitard, I. Thuret & G. Michel: t(1;19) in a M4-ANLL . *Cancer Genet Cytogenet* 73: 86-88 (1994)
36. Troussard, X., R. Rimokh, F. Valensi, D. Leboeuf, O. Fenneteau, A. M. Guitard, A. M. Manel, F. Schillinger, C. Leglise, A. Brizard & *et al*: Heterogeneity of t(1;19)(q23;p13) acute leukaemias. French Haematological Cytology Group . *British Journal of Haematology* 89: 516-526 (1995)
37. Raimondi, S. C., F. G. Behm, P. K. Roberson, D. L. Williams, C. H. Pui, W. M. Crist, A. T. Look & G. K. Rivera: Cytogenetics of pre-B-cell acute lymphoblastic leukemia with emphasis on prognostic implications of the t(1;19). *J Clin Oncol* 8: 1380-1388 (1990)
38. Hunger, S. P., N. Galili, A. J. Carroll, W. M. Crist, M. P. Link & M. L. Cleary: The t(1;19)(q23;p13) results in consistent fusion of E2A and PBX1 coding sequences in acute lymphoblastic leukemias. *Blood* 77: 687-693 (1991)
39. Privitera, E., M. P. Kamps, Y. Hayashi, T. Inaba, L. H. Shapiro, S. C. Raimondi, F. Behm, L. Hendershot, A. J. Carroll, D. Baltimore & *et al*: Different molecular consequences of the 1;19 chromosomal translocation in childhood B-cell precursor acute lymphoblastic leukemia. *Blood* 79: 1781-1788 (1992)
40. Mellentin, J. D., J. Nourse, S. P. Hunger, S. D. Smith & M. L. Cleary: Molecular analysis of the t(1;19) breakpoint cluster region in pre-B cell acute lymphoblastic leukemias. *Genes Chrom Cancer* 2: 239-247 (1990)
41. Cleary, M. L.: Transcription factors in human leukaemias. [Review]. *Cancer Surveys* 15: 89-104 (1992)
42. Boomer, T., M. Varella-Garcia, L. McGavran, L. Meltesen, A. S. Olsen & S. P. Hunger: Detection of E2A translocations in leukemias via fluorescence *in situ* hybridization. *Leukemia* 15: 95-102 (2001)
43. Secker-Walker, L. M., R. Berger, P. Fenaux, J. L. Lai, B. Nelken, M. Garson, P. M. Michael, A. Hagemeijer, C. J. Harrison, Y. Kaneko & .: Prognostic significance of the balanced t(1;19) and unbalanced der(19)t(1;19) translocations in acute lymphoblastic leukemia. *Leukemia* 6: 363-369 (1992)
44. Gehring, W. J., M. Affolter & T. Burglin: Homeodomain proteins. *Annual Review of Biochemistry* 63: 487-526 (1994)
45. Scott, M. P. & A. J. Weiner: Structural relationships among genes that control development: sequence homology between the Antennapedia, Ultrabithorax, and fushi tarazu loci of Drosophila. *Proc Natl Acad Sci U S A* 81: 4115-4119 (1984)
46. Maconochie, M., S. Nonchev, A. Morrison & R. Krumlauf: Paralogous Hox genes: function and regulation. *Annu Rev Genet* 30: 529-556 (1996)
47. Monica, K., N. Galili, J. Nourse, D. Saltman & M. L. Cleary: PBX2 and PBX3, new homeobox genes with extensive homology to the human proto-oncogene PBX1. *Mol Cell Biol* 11: 6149-6157 (1991)
48. Wagner, K., A. Mincheva, B. Korn, P. Lichter & H. Popperl: Pbx4, a new Pbx family member on mouse chromosome 8, is expressed during spermatogenesis . *Mech Dev* 103: 127-131 (2001)
49. Roberts, V. J., M. A. van Dijk & C. Murre: Localization of Pbx1 transcripts in developing rat embryos. *Mech Dev* 51: 193-198 (1995)
50. Schnabel, C. A., L. Selleri, Y. Jacobs, R. Warnke & M. L. Cleary: Expression of Pbx1b during mammalian organogenesis. *Mech Dev* 100: 131-135 (2001)
51. DiMartino, J. F., L. Selleri, D. Traver, M. T. Firpo, J. Rhee, R. Warnke, S. O'Gorman, I. L. Weissman & M. L. Cleary: The Hox cofactor and proto-oncogene Pbx1 is required for maintenance of definitive hematopoiesis in the fetal liver. *Blood* 98: 618-626 (2001)
52. Burglin, T. R.: Analysis of TALE superclass homeobox genes (MEIS, PBC, KNOX, Iroquois, TGIF) reveals a novel domain conserved between plants and animals. *Nucleic Acids Res* 25: 4173-4180 (1997)
53. Rauskolb, C. & E. Wieschaus: Coordinate regulation of downstream genes by extradenticle and the homeotic selector proteins. *EMBO J* 13: 3561-3569 (1994)
54. Lu, Q., P. S. Knoepfler, J. Scheele, D. D. Wright & M. P. Kamps: Both Pbx1 and E2A-Pbx1 bind the DNA motif ATCAATCAA cooperatively with the products of multiple murine Hox genes, some of which are themselves oncogenes. *Mol Cell Biol* 15: 3786-3795 (1995)
55. Chang, C. P., W. F. Shen, S. Rozenfeld, H. J. Lawrence, C. Largman & M. L. Cleary: Pbx proteins display hexapeptide-dependent cooperative DNA binding with a subset of Hox proteins. *Genes & Dev* 9: 663-674 (1995)
56. Phelan, M. L., I. Rambaldi & M. S. Featherstone: Cooperative interactions between HOX and PBX proteins mediated by a conserved peptide motif. *Mol Cell Biol* 15: 3989-3997 (1995)
57. Neuteboom, S. T., L. T. Peltenburg, M. A. van Dijk & C. Murre: The hexapeptide LFPWMR in Hoxb-8 is required for cooperative DNA binding with Pbx1 and Pbx2 proteins. *Proc Natl Acad Sci USA* 92: 9166-9170 (1995)
58. Knoepfler, P. S. & M. P. Kamps: The pentapeptide motif of Hox proteins is required for cooperative DNA binding with Pbx1, physically contacts Pbx1, and enhances DNA binding by Pbx1. *Mol Cell Biol* 15: 5811-5819 (1995)
59. van Dijk, M. A., L. T. Peltenburg & C. Murre: Hox gene products modulate the DNA binding activity of Pbx1 and Pbx2. *Mech Dev* 52: 99-108 (1995)
60. Piper, D. E., A. H. Batchelor, C. P. Chang, M. L. Cleary & C. Wolberger: Structure of a HoxB1-Pbx1 heterodimer bound to DNA: role of the hexapeptide and a fourth homeodomain helix in complex formation. *Cell* 96: 587-597 (1999)
61. Chang, C. P., i. DeVivo & M. L. Cleary: The Hox cooperativity motif of the chimeric oncoprotein E2a-Pbx1 is necessary and sufficient for oncogenesis. *Mol Cell Biol* 17: 81-88 (1997)
62. Nakamura, T., N. A. Jenkins & N. G. Copeland: Identification of a new family of Pbx-related homeobox genes. *Oncogene* 13: 2235-2242 (1996)
63. Knoepfler, P. S., K. R. Calvo, H. Chen, S. E. Antonarakis & M. P. Kamps: Meis1 and pKnox1 bind

DNA cooperatively with Pbx1 utilizing an interaction surface disrupted in oncoprotein E2a-Pbx1. *Proc Natl Acad Sci USA* 94: 14553-14558 (1997)
64. Chang, C. P., Y. Jacobs, T. Nakamura, N. A. Jenkins, N. G. Copeland & M. L. Cleary: Meis proteins are major *in vivo* DNA binding partners for wild- type but not chimeric Pbx proteins. *Mol Cell Biol* 17: 5679-5687 (1997)
65. Berthelsen, J., V. Zappavigna, F. Mavilio & F. Blasi: Prep1, a novel functional partner of Pbx proteins. *EMBO J* 17: 1423-1433 (1998)
66. Pai, C. Y., T. S. Kuo, T. J. Jaw, E. Kurant, C. T. Chen, D. A. Bessarab, A. Salzberg & Y. H. Sun: The Homothorax homeoprotein activates the nuclear localization of another homeoprotein, extradenticle, and suppresses eye development in Drosophila. *Genes Dev* 12: 435-446 (1998)
67. Berthelsen, J., C. Kilstrup-Nielsen, F. Blasi, F. Mavilio & V. Zappavigna: The subcellular localization of PBX1 and EXD proteins depends on nuclear import and export signals and is modulated by association with PREP1 and HTH. *Genes Dev* 13: 946-953 (1999)
68. Saleh, M., H. Huang, N. C. Green & M. S. Featherstone: A conformational change in PBX1A is necessary for its nuclear localization. *Exp Cell Res* 260: 105-115 (2000)
69. Shen, W. F., J. C. Montgomery, S. Rozenfeld, J. J. Moskow, H. J. Lawrence, A. M. Buchberg & C. Largman: AbdB-like Hox proteins stabilize DNA binding by the Meis1 homeodomain proteins. *Mol Cell Biol* 17: 6448-6458 (1997)
70. Shen, W. F., S. Rozenfeld, A. Kwong, L. G. Kom ves, H. J. Lawrence & C. Largman: HOXA9 forms triple complexes with PBX2 and MEIS1 in myeloid cells. *Mol Cell Biol* 19: 3051-3061 (1999)
71. Jacobs, Y., C. A. Schnabel & M. L. Cleary: Trimeric association of Hox and TALE homeodomain proteins mediates Hoxb2 hindbrain enhancer activity. *Mol Cell Biol* 19: 5134-5142 (1999)
72. Mann, R. S. & M. Affolter: Hox proteins meet more partners. *Curr Opin Genet Dev* 8: 423-429 (1998)
73. van Dijk, M. A., P. M. Voorhoeve & C. Murre: Pbx1 is converted into a transcriptional activator upon acquiring the N-terminal region of E2A in pre-B-cell acute lymphoblastoid leukemia. *Proc Natl Acad Sci USA* 90: 6061-6065 (1993)
74. Lu, Q., D. D. Wright & M. P. Kamps: Fusion with E2A converts the Pbx1 homeodomain protein into a constitutive transcriptional activator in human leukemias carrying the t(1;19) translocation. *Mol Cell Biol* 14: 3938-3948 (1994)
75. LeBrun, D. P. & M. L. Cleary: Fusion with E2A alters the transcriptional properties of the homeodomain protein PBX1 in t(1;19) leukemias. *Oncogene* 9: 1641-1647 (1994)
76. Calvo, K. R., P. Knoepfler, S. McGrath & M. P. Kamps: An inhibitory switch derepressed by pbx, hox, and Meis/Prep1 partners regulates DNA-binding by pbx1 and E2a-pbx1 and is dispensable for myeloid immortalization by E2a-pbx1. *Oncogene* 18: 8033-8043 (1999)
77. Chang, C. P., L. Brocchieri, W. F. Shen, C. Largman & M. L. Cleary: Pbx modulation of Hox homeodomain amino-terminal arms establishes different DNA-binding specificities across the Hox locus. *Mol Cell Biol* 16: 1734-1745 (1996)
78. Popperl, H., M. Bienz, M. Studer, S. K. Chan, S. Aparicio, S. Brenner, R. S. Mann & R. Krumlauf: Segmental expression of Hoxb-1 is controlled by a highly conserved autoregulatory loop dependent upon exd/pbx. *Cell* 81: 1031-1042 (1995)
79. Maconochie, M. K., S. Nonchev, M. Studer, S. K. Chan, H. Popperl, M. H. Sham, R. S. Mann & R. Krumlauf: Cross-regulation in the mouse HoxB complex: the expression of Hoxb2 in rhombomere 4 is regulated by Hoxb1. *Genes & Dev* 11: 1885-1895 (1997)
80. Ferretti, E., H. Marshall, H. Popperl, M. Maconochie, R. Krumlauf & F. Blasi: Segmental expression of Hoxb2 in r4 requires two separate sites that integrate cooperative interactions between Prep1, Pbx and Hox proteins. *Development* 127: 155-166 (2000)
81. Lu, Q. & M. P. Kamps: Selective repression of transcriptional activators by Pbx1 does not require the homeodomain. *Proc Natl Acad Sci USA* 93: 470-474 (1996)
82. Asahara, H., S. Dutta, H. Y. Kao, R. M. Evans & M. Montminy: Pbx-Hox heterodimers recruit coactivator-corepressor complexes in an isoform-specific manner. *Mol Cell Biol* 19: 8219-8225 (1999)
83. Saleh, M., I. Rambaldi, X. J. Yang & M. S. Featherstone: Cell signaling switches HOX-PBX complexes from repressors to activators of transcription mediated by histone deacetylases and histone acetyltransferases. *Mol Cell Biol* 20: 8623-8633 (2000)
84. Capovilla, M. & J. Botas: Functional dominance among Hox genes: repression dominates activation in the regulation of Dpp. *Development* 125: 4949-4957 (1998)
85. Pinsonneault, J., B. Florence, H. Vaessin & W. McGinnis: A model for extradenticle function as a switch that changes HOX proteins from repressors to activators. *EMBO J* 16: 2032-2042 (1997)
86. Shen, W. F., K. Krishnan, H. J. Lawrence & C. Largman: The HOX homeodomain proteins block CBP histone acetyltransferase activity. *Mol Cell Biol* 21: 7509-7522 (2001)
87. Vigano, M. A., G. Di Rocco, V. Zappavigna & F. Mavilio: Definition of the transcriptional activation domains of three human HOX proteins depends on the DNA-binding context. *Mol Cell Biol* 18: 6201-6212 (1998)
88. Li, X., C. Murre & W. McGinnis: Activity regulation of a Hox protein and a role for the homeodomain in inhibiting transcriptional activation. *EMBO J* 18: 198-211 (1999)
89. Penkov, D., S. Tanaka, G. Di Rocco, J. Berthelsen, F. Blasi & F. Ramirez: Cooperative interactions between PBX, PREP, and HOX proteins modulate the activity of the alpha 2(V) collagen (COL5A2) promoter . *J Biol Chem* 275: 16681-16689 (2000)
90. Berthelsen, J., V. Zappavigna, E. Ferretti, F. Mavilio & F. Blasi: The novel homeoprotein Prep1 modulates Pbx-Hox protein cooperativity. *EMBO J* 17: 1434-1445 (1998)
91. Vlachakis, N., S. K. Choe & C. G. Sagerstrom: Meis3 synergizes with Pbx4 and Hoxb1b in promoting hindbrain fates in the zebrafish. *Development* 128: 1299-1312a (2001)
92. Maloof, J. N. & C. Kenyon: The Hox gene lin-39 is required during C. elegans vulval induction to select the outcome of Ras signaling. *Development* 125: 181-190 (1998)
93. Selleri, L., M. J. Depew, Y. Jacobs, S. K. Chanda, K. Y. Tsang, K. S. Cheah, J. L. Rubenstein, S. O'Gorman & M. L.

Cleary: Requirement for Pbx1 in skeletal patterning and programming chondrocyte proliferation and differentiation. *Development* 128: 3543-3557 (2001)
94. Kim, S. K., L. Selleri, J. S. Lee, A. Y. Zhang, X. Gu, Y. Jacobs & M. L. Cleary: Pbx1 inactivation disrupts pancreas development and in Ipf1-deficient mice promotes diabetes mellitus. *Nat Genet* 30: 430-435 (2002)
95. Izraeli, S., H. Kovar, H. Gadner & T. Lion: Unexpected heterogeneity in E2A/PBX1 fusion messenger RNA detected by the polymerase chain reaction in pediatric patients with acute lymphoblastic leukemia. *Blood* 80: 1413-1417 (1992)
96. Kamps, M. P., A. T. Look & D. Baltimore: The human t(1;19) translocation in pre-B ALL produces multiple nuclear E2A-Pbx1 fusion proteins with differing transforming potentials. *Genes & Dev* 5: 358-368 (1991)
97. Kamps, M. P. & D. Baltimore: E2A-Pbx1, the t(1;19) translocation protein of human pre-B-cell acute lymphocytic leukemia, causes acute myeloid leukemia in mice. *Mol Cell Biol* 13: 351-357 (1993)
98. Kamps, M. P. & D. D. Wright: Oncoprotein E2A-Pbx1 immortalizes a myeloid progenitor in primary marrow cultures without abrogating its factor-dependence. *Oncogene* 9: 3159-3166 (1994)
99. Sykes, D. B. & M. P. Kamps: Estrogen-dependent E2a/Pbx1 myeloid cell lines exhibit conditional differentiation that can be arrested by other leukemic oncoproteins. *Blood* 98: 2308-2318 (2001)
100. Dedera, D. A., E. K. Waller, D. P. LeBrun, A. Sen-Majumdar, M. E. Stevens, G. S. Barsh & M. L. Cleary: Chimeric homeobox gene E2A-PBX1 induces proliferation, apoptosis, and malignant lymphomas in transgenic mice. *Cell* 74: 833-843 (1993)
101. Monica, K., D. P. LeBrun, D. A. Dedera, R. B. Brown & M. L. Cleary: Transformation properties of the E2a-Pbx1 chimeric oncoprotein: fusion with E2a is essential, but the Pbx1 homeodomain is dispensable. *Mol Cell Biol* 14: 8304-8314 (1994)
102. Kamps, M. P., D. D. Wright & Q. Lu: DNA-binding by oncoprotein E2a-Pbx1 is important for blocking differentiation but dispensable for fibroblast transformation. *Oncogene* 12: 19-30 (1996)
103. Sauvageau, G., P. M. Lansdorp, C. J. Eaves, D. E. Hogge, W. H. Dragowska, D. S. Reid, C. Largman, H. J. Lawrence & R. K. Humphries: Differential expression of homeobox genes in functionally distinct CD34+ subpopulations of human bone marrow cells. *Proc Natl Acad Sci U S A* 91: 12223-12227 (1994)
104. Giampaolo, A., P. Sterpetti, D. Bulgarini, P. Samoggia, E. Pelosi, M. Valtieri & C. Peschle: Key functional role and lineage-specific expression of selected HOXB genes in purified hematopoietic progenitor differentiation. *Blood* 84: 3637-3647 (1994)
105. Antonchuk, J., G. Sauvageau & R. K. Humphries: HOXB4-Induced Expansion of Adult Hematopoietic Stem Cells *Ex vivo*. *Cell* 109: 39-45 (2002)
106. Kappen, C.: Disruption of the homeobox gene Hoxb-6 in mice results in increased numbers of early erythrocyte progenitors. *Am J Hematol* 65: 111-118 (2000)
107. Shimamoto, T., Y. Tang, Y. Naot, M. Nardi, P. Brulet, C. J. Bieberich & K. Takeshita: Hematopoietic progenitor cell abnormalities in Hoxc-8 null mutant mice. *J Exp Zool* 283: 186-193 (1999)
108. Lawrence, H. J., C. D. Helgason, G. Sauvageau, S. Fong, D. J. Izon, R. K. Humphries & C. Largman: Mice bearing a targeted interruption of the homeobox gene HOXA9 have defects in myeloid, erythroid, and lymphoid hematopoiesis. *Blood* 89: 1922-1930 (1997)
109. Roberts, C. W., J. R. Shutter & S. J. Korsmeyer: Hox11 controls the genesis of the spleen. *Nature* 368: 747-749 (1994)
110. Nakamura, T., D. A. Largaespada, M. P. Lee, L. A. Johnson, K. Ohyashiki, K. Toyama, S. J. Chen, C. L. Willman, I. M. Chen, A. P. Feinberg, N. A. Jenkins, N. G. Copeland & J. D. Shaughnessy, Jr.: Fusion of the nucleoporin gene NUP98 to HOXA9 by the chromosome translocation t(7;11)(p15;p15) in human myeloid leukaemia. *Nat Genet* 12: 154-158 (1996)
111. Raza-Egilmez, S. Z., S. N. Jani-Sait, M. Grossi, M. J. Higgins, T. B. Shows & P. D. Aplan: NUP98-HOXD13 gene fusion in therapy-related acute myelogenous leukemia. *Cancer Res* 58: 4269-4273 (1998)
112. Nakamura, T., Y. Yamazaki, Y. Hatano & I. Miura: NUP98 is fused to PMX1 homeobox gene in human acute myelogenous leukemia with chromosome translocation t(1;11)(q23;p15). *Blood* 94: 741-747 (1999)
113. Fujino, T., A. Suzuki, Y. Ito, K. Ohyashiki, Y. Hatano, I. Miura & T. Nakamura: Single-translocation and double-chimeric transcripts: detection of NUP98-HOXA9 in myeloid leukemias with HOXA11 or HOXA13 breaks of the chromosomal translocation t(7;11)(p15;p15). *Blood* 99: 1428-1433 (2002)
114. Dube, I. D., S. Kamel-Reid, C. C. Yuan, M. Lu, X. Wu, G. Corpus, S. C. Raimondi, W. M. Crist, A. J. Carroll, J. Minowada & *et al*: A novel human homeobox gene lies at the chromosome 10 breakpoint in lymphoid neoplasias with chromosomal translocation t(10;14). *Blood* 78: 2996-3003 (1991)
115. Krosl, J., S. Baban, G. Krosl, S. Rozenfeld, C. Largman & G. Sauvageau: Cellular proliferation and transformation induced by HOXB4 and HOXB3 proteins involves cooperation with PBX1. *Oncogene* 16: 3403-3412 (1998)
116. Thorsteinsdottir, U., J. Krosl, E. Kroon, A. Haman, T. Hoang & G. Sauvageau: The oncoprotein E2A-Pbx1a collaborates with Hoxa9 to acutely transform primary bone marrow cells. *Mol Cell Biol* 19: 6355-6366 (1999)
117. Kroon, E., J. Krosl, U. Thorsteinsdottir, S. Baban, A. M. Buchberg & G. Sauvageau: Hoxa9 transforms primary bone marrow cells through specific collaboration with Meis1a but not Pbx1b. *EMBO J* 17: 3714-3725 (1998)
118. Fu, X. & M. P. Kamps: E2a-Pbx1 induces aberrant expression of tissue-specific and developmentally regulated genes when expressed in NIH 3T3 fibroblasts. *Mol Cell Biol* 17: 1503-1512 (1997)
119. McWhirter, J. R., M. Goulding, J. A. Weiner, J. Chun & C. Murre: A novel fibroblast growth factor gene expressed in the developing nervous system is a downstream target of the chimeric homeodomain oncoprotein E2A-Pbx1. *Development* 124: 3221-3232 (1997)
120. de Lau, W. B., J. Hurenkamp, P. Berendes, I. P. Touw, H. C. Clevers & M. A. van Dijk: The gene encoding the granulocyte colony-stimulating factor receptor is a target for deregulation in pre-B ALL by the t(1;19)- specific oncoprotein E2A-Pbx1. *Oncogene* 17: 503-510 (1998)

121. Rutherford, M. N., G. R. Bayly, B. P. Matthews, T. Okuda, W. M. Dinjens, H. Kondoh & D. P. LeBrun: The leukemogenic transcription factor E2a-Pbx1 induces expression of the putative N-myc and p53 target gene NDRG1 in Ba/F3 cells. *Leukemia* 15: 362-370 (2001)
122. Fu, X., S. McGrath, M. Pasillas, S. Nakazawa & M. P. Kamps: EB-1, a tyrosine kinase signal transduction gene, is transcriptionally activated in the t(1;19) subset of pre-B ALL, which express oncoprotein E2a-Pbx1. *Oncogene* 18: 4920-4929 (1999)
123. McWhirter, J. R., S. T. Neuteboom, E. V. Wancewicz, B. P. Monia, J. R. Downing & C. Murre: Oncogenic homeodomain transcription factor E2A-Pbx1 activates a novel WNT gene in pre-B acute lymphoblastoid leukemia. *Proc Natl Acad Sci U S A* 96: 11464-11469 (1999)
124. LeBrun, D. P., B. P. Matthews, B. J. Feldman & M. L. Cleary: The chimeric oncoproteins E2A-PBX1 and E2A-HLF are concentrated within spherical nuclear domains. *Oncogene* 15: 2059-2067 (1997)
125. Bayly, R. & D. P. LeBrun: Role for homodimerization in growth deregulation by E2a fusion proteins. *Mol Cell Biol* 20: 5789-5796 (2000)
126. Neuhold, L. A. & B. Wold: HLH forced dimers: tethering MyoD to E47 generates a dominant positive myogenic factor insulated from negative regulation by Id. *Cell* 74: 1033-1042 (1993)
127. Peverali, F. A., T. Ramqvist, R. Saffrich, R. Pepperkok, M. V. Barone & L. Philipson: Regulation of G1 progression by E2A and Id helix-loop-helix proteins. *EMBO J* 13: 4291-4301 (1994)
128. Prabhu, S., A. Ignatova, S. T. Park & X. H. Sun: Regulation of the expression of cyclin-dependent kinase inhibitor p21 by E2A and Id proteins. *Mol Cell Biol* 17: 5888-5896 (1997)
129. Engel, I. & C. Murre: Ectopic expression of E47 or E12 promotes the death of E2A-deficient lymphomas. *Proc Natl Acad Sci U S A* 96: 996-1001 (1999)
130. Barone, M. V., R. Pepperkok, F. A. Peverali & L. Philipson: Id proteins control growth induction in mammalian cells. *Proc Natl Acad Sci USA* 91: 4985-4988 (1994)
131. Deed, R. W., S. M. Bianchi, G. T. Atherton, D. Johnston, M. Santibanez-Koref, J. J. Murphy & J. D. Norton: An immediate early human gene encodes an Id-like helix-loop- helix protein and is regulated by protein kinase C activation in diverse cell types. *Oncogene* 8: 599-607 (1993)
132. Goldfarb, A. N. & K. Lewandowska: Inhibition of cellular differentiation by the SCL/tal oncoprotein: transcriptional repression by an Id-like mechanism. *Blood* 85: 465-471 (1995)
133. Hofmann, T. J. & M. D. Cole: The TAL1/Scl basic helix-loop-helix protein blocks myogenic differentiation and E-box dependent transactivation. *Oncogene* 13: 617-624 (1996)
134. Park, S. T. & X. H. Sun: The Tal1 oncoprotein inhibits E47-mediated transcription. Mechanism of inhibition. *J Biol Chem* 273: 7030-7037 (1998)
135. Feldman, B. J., T. Hampton & M. L. Cleary: A carboxy-terminal deletion mutant of Notch1 accelerates lymphoid oncogenesis in E2A-PBX1 transgenic mice. *Blood* 96: 1906-1913 (2000)
136. Pui, J. C., D. Allman, L. Xu, S. DeRocco, F. G. Karnell, S. Bakkour, J. Y. Lee, T. Kadesch , R. R. Hardy, J. C. Aster & W. S. Pear: Notch1 expression in early lymphopoiesis influences B versus T lineage determination. *Immunity* 11: 299-308 (1999)
137. Ellisen, L. W., J. Bird, D. C. West, A. L. Soreng, T. C. Reynolds, S. D. Smith & J. Sklar: TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell* 66: 649-661 (1991)
138. Jundt, F., I. Anagnostopoulos, R. Forster, S. Mathas, H. Stein & B. Dorken: Activated Notch1 signaling promotes tumor cell proliferation and survival in Hodgkin and anaplastic large cell lymphoma. *Blood* 99: 3398-3403 (2002)
139. Blobel, G. A.: CBP and p300: versatile coregulators with important roles in hematopoietic gene expression. *J Leukoc Biol* 71: 545-556 (2002)
140. Petrij, F., R. H. Giles, H. G. Dauwerse, J. J. Saris, R. C. Hennekam, M. Masuno, N. Tommerup, G. J. van Ommen, R. H. Goodman, D. J. Peters & .: Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. *Nature* 376: 348-351 (1995)
141. Kung, A. L., V. I. Rebel, R. T. Bronson, L. E. Ch'ng, C. A. Sieff, D. M. Livingston & T. P. Yao: Gene dose-dependent control of hematopoiesis and hematologic tumor suppression by CBP. *Genes Dev* 14: 272-277 (2000)
142. Schiltz, R. L. & Y. Nakatani: The PCAF acetylase complex as a potential tumor suppressor. *Biochim Biophys Acta* 1470: M37-M53 (2000)
143. Raimondi, S. C., E. Privitera, D. L. Williams, A. T. Look, F. Behm, G. K. Rivera, W. M. Crist & C. H. Pui: New recurring chromosomal translocations in childhood acute lymphoblastic leukemia. *Blood* 77: 2016-2022 (1991)
144. AT Look: Genes altered by chromosomal translocations in leukemias and lymphomas. In The genetic basis of human cancer. Eds: Vogelstein B, Kinzler KW, McGraw-Hill, NY 109-142 (1998)
145. Hunger, S. P., R. Brown & M. L. Cleary: DNA-binding and transcriptional regulatory properties of hepatic leukemia factor (HLF) and the t(17;19) acute lymphoblastic leukemia chimera E2A-HLF. *Mol Cell Biol* 14: 5986-5996 (1994)
146. Inaba, T., L. H. Shapiro, T. Funabiki, A. E. Sinclair, B. G. Jones, R. A. Ashmun & A. T. Look: DNA-binding specificity and trans-activating potential of the leukemia-associated E2A-hepatic leukemia factor fusion protein. *Mol Cell Biol* 14: 3403-3413 (1994)
147. Hunger, S. P., S. Li, M. Z. Fall, L. Naumovski & M. L. Cleary: The proto-oncogene HLF and the related basic leucine zipper protein TEF display highly similar DNA-binding and transcriptional regulatory properties. *Blood* 87: 4607-4617 (1996)
148. Hitzler, J. K., H. D. Soares, D. W. Drolet, T. Inaba, S. O'Connel, M. G. Rosenfeld, J. I. Morgan & A. T. Look: Expression patterns of the hepatic leukemia factor gene in the nervous system of developing and adult mice. *Brain Res* 820: 1-11 (1999)
149. Hunger, S. P., P. E. Devaraj, L. Foroni, L. M. Secker-Walker & M. L. Cleary: Two types of genomic rearrangements create alternative E2A-HLF fusion proteins in t(17;19)-ALL. *Blood* 83: 2970-2977 (1994)

150. Yoshihara, T., T. Inaba, L. H. Shapiro, J. Y. Kato & A. T. Look: E2A-HLF-mediated cell transformation requires both the trans- activation domains of E2A and the leucine zipper dimerization domain of HLF. *Mol Cell Biol* 15: 3247-3255 (1995)
151. Honda, H., T. Inaba, T. Suzuki, H. Oda, Y. Ebihara, K. Tsuiji , T. Nakahata, T. Ishikawa, Y. Yazaki & H. Hirai: Expression of E2A-HLF chimeric protein induced T-cell apoptosis, B-cell maturation arrest, and development of acute lymphoblastic leukemia. *Blood* 93: 2780-2790 (1999)
152. Smith, K. S., J. W. Rhee, L. Naumovski & M. L. Cleary: Disrupted differentiation and oncogenic transformation of lymphoid progenitors in *E2A-HLF* transgenic mice. *Mol Cell Biol* 19: 4443-4451 (1999)
153. Inaba, T., T. Inukai, T. Yoshihara, H. Seyschab, R. A. Ashmun, C. E. Canman, S. J. Laken, M. B. Kastan & A. T. Look: Reversal of apoptosis by the leukaemia-associated E2A-HLF chimaeric transcription factor. *Nature* 382: 541-544 (1996)
154. Inukai, T., A. Inoue, H. Kurosawa, K. Goi, T. Shinjyo, K. Ozawa, M. Mao, T. Inaba & A. T. Look: SLUG, a ces-1-related zinc finger transcription factor gene with antiapoptotic activity, is a downstream target of the E2A-HLF oncoprotein. *Mol Cell* 4: 343-352 (1999)
155. Dang, J., T. Inukai, H. Kurosawa, K. Goi, T. Inaba, N. T. Lenny , J. R. Downing, S. Stifani & A. T. Look: The E2A-HLF oncoprotein activates Groucho-related genes and suppresses Runx1. *Mol Cell Biol* 21: 5935-5945 (2001)
156. Lorsbach, R. B. & J. R. Downing: The role of the AML1 transcription factor in leukemogenesis. *Int J Hematol* 74: 258-265 (2001)
157. Okuda, T., J. van Deursen, S. W. Hiebert, G. Grosveld & J. R. Downing: AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. *Cell* 84: 321-330 (1996)
158. Inukai, T., T. Inaba, S. Ikushima & A. T. Look: The AD1 and AD2 transactivation domains of E2A are essential for the antiapoptotic activity of the chimeric oncoprotein E2A-HLF. *Mol Cell Biol* 18: 6035-6043 (1998)
159. Smith, K. S., Y. Jacobs, C. P. Chang & M. L. Cleary: Chimeric oncoprotein E2a-Pbx1 induces apoptosis of hematopoietic cells by a p53-independent mechanism that is suppressed by Bcl-2. *Oncogene* 14: 2917-2926 (1997)

Key Words: leukemia, cancer, transcriptional regulation, oncogenes, E2A, PBX1, HLF, hematopoiesis, Review

Send correspondence to: David P. LeBrun, M.D., Department of Pathology, Queen's University, Kingston, Ontario K7L 3N6, Canada, Tel: 613-548-1307, Fax: 613-533-2907, E-mail:lebrun@cliff.path.queensu.ca