Oncogenic Transcription Factors in the Human Acute Leukemias

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Chromosomal translocations in the human acute leukemias rearrange the regulatory and coding regions of a variety of transcription factor genes. The resultant protein products can interfere with regulatory cascades that control the growth, differentiation, and survival of normal blood cell precursors. Support for this interpretation comes from the results of gene manipulation studies in mice, as well as the sequence homology of oncogenic transcription factors with proteins known to regulate embryonic development in primitive organisms, including the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*. Many of these genetic alterations have important prognostic implications that can guide the selection of therapy. The insights gained from studies of translocation-generated oncogenes and their protein products should hasten the development of highly specific, and hence less toxic, forms of leukemia therapy.

The human acute leukemias arise from blood cell progenitors developing in the lymphoid or myeloid pathway or from primitive stem cells with multilineage potential. Careful analysis of clonal chromosomal abnormalities in blast cells from leukemia patients has had a profound impact on our understanding of the molecular changes involved in leukemogenesis (*1*, *2*). Most striking has been the finding of nonrandom, somatically acquired chromosomal translocations or inversions (hereafter grouped as translocations) in up to 65% of the acute leukemias (Fig. 1) (*3*). These structural rearrangements affect gene expression in ways that subvert normal programs of cell proliferation, differentiation, and survival, and they likely act in concert with other classes of genetic lesions (for example, those affecting tumor suppressors) in multistep pathways that culminate in leukemic transformation.

The most frequent targets of chromosomal translocations in the acute leukemias are genes that encode transcription factors, emphasizing the critical role of these "master" regulatory proteins in the control of blood cell development (*4*). The modular structure of transcription factors—including discrete DNA-binding, dimerization, and *trans*-effector domains—allows normally unrelated sequences from different chromosomes to be recombined into hybrid genes that encode fusion products with altered function (*2*). Activation of transcription factor genes by chromosomal translocations take two main forms (Fig. 2). In T- or B-lymphoid progenitors, such genes are frequently mobilized into the vicinity of genes encoding discrete chains of the T-cell receptor (TCR) or immunoglobulin (Ig) molecules, resulting in inappropriate expression of the translocated proto-oncogenes. More commonly, the coding exons of genes disrupted by a reciprocal translocation are incorporated into a single "fusion" gene, which generates a chimeric protein with unique properties.

Translocations that inappropriately activate transcription factor genes in acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) show remarkable specificity for hematopoietic cells blocked in defined stages of differentiation (Fig. 1). This property suggests that the different oncoproteins produced by these chromosomal changes specifically interfere with transcriptional networks that normally function in concert with growth factors and their receptors to regulate hematopoiesis. This hypothesis is reinforced by the results of gene manipulations in mice, which have demonstrated the profound and varied effects on normal hematopoiesis of translocation-targeted transcription factors, including *TAL1* (*SCL*), *LMO2*, *AML1*, and *CBFβ* (*4*).

The genes targeted by chromosomal translocations appear to stand near the top of evolutionarily conserved regulatory cascades, as indicated by their extensive sequence homology with genes controlling the earliest stages of embryonic development in primitive organisms (*5*). Only a few of the downstream responder genes regulated by these oncoproteins have been identified, however, leaving substantial gaps in contemporary models of leukemogenesis. Here, I will review recent progress in relating the activities of dysregulated transcription factors active in leukemic transformation to signal transduction pathways responsible for the control of normal hematopoiesis. I will also briefly describe emerging applications of molecular genetic findings in the clinical management of newly diagnosed patients with acute leukemia.

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Homeotic Genes as Targets of Oncogenic Transcription Factors

In most lymphoid leukemias of B- or T-cell origin, translocation-generated oncoproteins appear to transform committed progenitors whose stages of differentiation parallel those of the majority of cells in the leukemic clone. In many AML cases, by contrast, the translocations seem to aberrantly activate genes in primitive stem cells that have retained both multilineage and self-renewal capacity (*6*). These alterations are likely to affect pathways that are critical for normal proliferation and differentiation of hematopoietic progenitors. Emerging evidence from analysis of hematopoietic progenitor cells in mice and humans suggests that pathways centered around the regulation of the major clusters of homeobox-containing *HOX* genes can provide a conceptual framework for the actions of many of the oncogenic transcription factors shown in Fig. 1.

Human cells contain 39 major *HOX* genes, grouped in clusters (*HOX-A* to *HOX-D*) on four separate chromosomes. These genes share extensive homology with the *HOM-C* genes of *Drosophila* and play important roles in axial morphogenesis and patterning (*7*). The *HOX* genes are further divided into 13 paralog groups on the basis of their structural and functional identity with individual *HOM-C* genes (Fig. 3). Of immediate relevance to the origin of acute leukemia, the expression of individual *HOX* genes in blood cell progenitors follows tightly regulated programs that are specific for the stage and lineage of progenitor cell development, with universal down-regulation of *HOX* gene expression as the progenitors differentiate into mature blood cells (*8*). At least 22 of the 39 *HOX* genes are expressed by different subpopulations of $CD34^+$ human bone marrow progenitor cells. High levels of expression of the 3′ genes within the A and B complexes (*HOXB3*, for example) are found in the most primitive subsets of hematopoietic stem cells. These genes are subsequently down-regulated, and the *HOX* loci closer to the 5′ end are expressed as progenitor cells become committed to the myeloid or erythroid lineage (*8*).

In mice reconstituted with retrovirally infected murine bone marrow cells programmed to overexpress individual *HOX* genes, there are dramatic and highly lineage-specific effects on the proliferative and

self-renewal capacities of hematopoietic stem cells and committed progenitors. Each *HOX* gene appears to have a distinct effect. For example, *HOXB4* overexpression induces selective expansion of primitive hematopoietic stem cells that retain their capacity to differentiate, whereas *HOXB3* overexpression produces mice with small thymuses and increased numbers of immature thymocytes that cannot differentiate (*9*). Mice overexpressing *HOXB3* also have defects in early B-cell development and a myeloproliferative disorder characterized by splenomegaly and expansion of myeloid clonogenic progenitors. By contrast, overexpression of *HOXA10* leads to selective expansion of the megakaryocytic cell compartment with diminished numbers of monocytic and B-lymphoid progenitors. After a 5- to 8-month latency period, a substantial proportion of these mice develop AML (*10*). The leukemogenic potential of aberrantly expressed HOX proteins is also known from studies of transgenic mice (*11*) and of murine AML cells expressing endogenous *Hox* genes activated by retroviral insertion (*12*).

Figure 3B shows seven oncogenic transcription factors oriented according to their predicted roles in the regulation of *HOX* gene expression in myeloid and lymphoid progenitors. The gene *MLL*, the mammalian counterpart of the *trithorax* (*trx*) gene of *Drosophila*, has a major impact on human leukemogenesis, because it has been implicated in fusions with more than 25 other genes in both ALL and AML (*2*). In fruit flies, trx-G proteins have a positive role in the maintenance of cell type–specific patterns of *HOM-C* gene expression, apparently through epigenetic mechanisms that establish and sustain a receptive chromatin configuration (*13*). An analogous role for MLL is supported by biochemical studies (*14*) and phenotypic analysis of mice lacking or haploinsufficient for functional *Mll* genes (*15*). Thus, leukemogenic MLL fusion proteins are predicted to disrupt critical patterns of *HOX* gene expression in hematopoietic progenitor cells, in ways that selectively contribute to myeloid (for example, MLL-AF9) or lymphoid (for example, MLL-AF4) acute leukemias. Conversely, Bmi-1 is the murine ortholog of the *Drosophila* Psc protein, a member of the Polycomb (Pc-G) complex of proteins, which collectively oppose the actions of trx-G proteins and act as highly specific silencers of *HOM-C* gene expression (*13*). Gene disruption and biochemical studies support the prediction, based on sequence homology, that Bmi-1 functions in an analogous manner in murine embryonic development (*16*). Thus, when activated by retroviral insertion in murine B- and T-cell leukemias and lymphomas, Bmi-1 may down-regulate *Hox* target genes that are normally expressed by lymphoid progenitor cells.

Acting at the far end of the postulated cascade of *HOX* gene activation are the PBX proteins, the mammalian counterparts of the *Drosophila* extradenticle (exd) protein (*17*). Like exd, PBX1 forms complexes with specific subsets of HOX proteins (*18*). In a significant fraction of pre-B leukemias in children, *PBX1* is specifically rearranged with the *E2A* gene, and the small segment of PBX1 that mediates interactions with HOX proteins is critical for the transforming activity of the E2A-PBX1 chimera (*19*). This interpretation is supported by the finding that murine *Pbx*-like genes are frequently co-activated with *HOX* genes by retroviral insertion events that promote the devel-

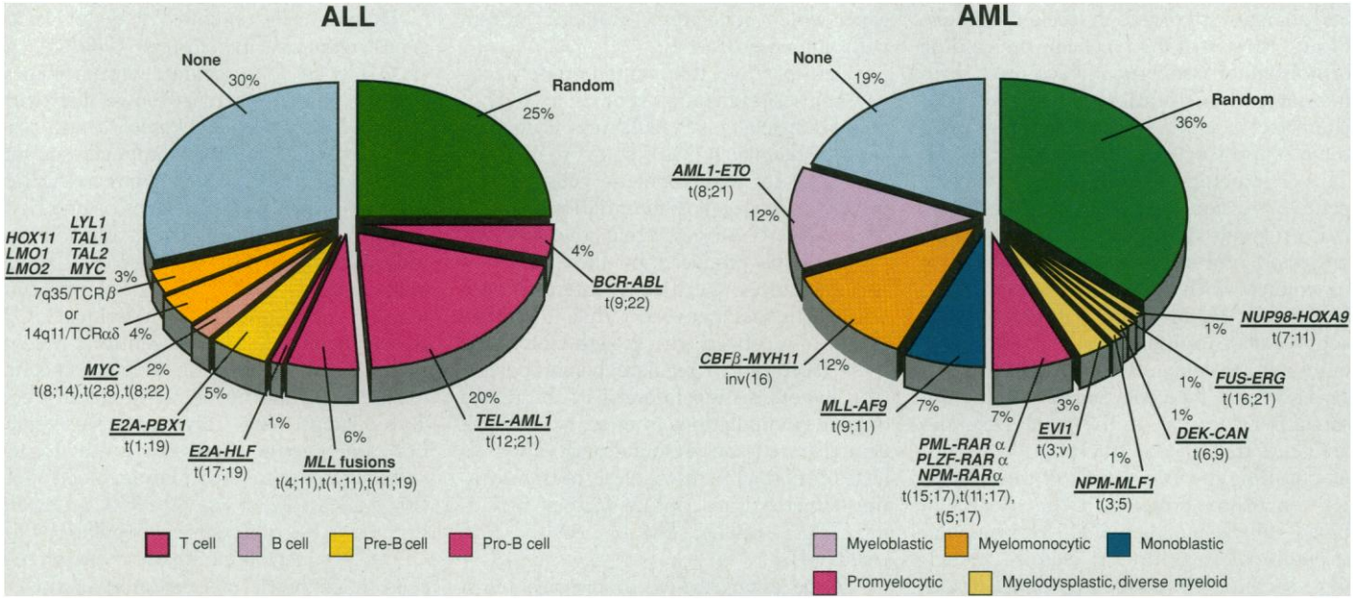

Fig. 1. Distribution of translocation-generated oncogenes among the acute leukemias of children and young adults (*1, 2*). The products of these aberrant genes are most often nuclear proteins active in transcription, with the notable exception of *BCR-ABL*, which encodes a cytoplasmic protein containing the activated ABL tyrosine kinase domain. In ALL, gene fusions tend to be specifically associated with one of the commonly recognized immunologic subtypes of the disease, as indicated by the color coding on the chart. The T-cell leukemias (orange) are characterized by translocations that dysregulate the expression of proto-oncogenes through the mechanism depicted in Fig. 2A. The *MYC* gene is also dysregulated through translocation into the vicinity of one of the *Ig* loci in B-cell leukemia (pink) and Burkitt's lymphoma. In the pre-B (yellow) and pro-B (magenta) immunologic subtypes of ALL, fusion genes encoding chimeric oncoproteins are produced by the mechanism outlined in Fig. 2B. The *BCR-ABL* and *MLL* gene fusions have also been identified in the chronic and acute myeloid leukemias, respectively, suggesting that they arise in primitive stem cells with multilineage potential. "Random" refers to sporadic translocations that have been observed only in leukemic cells from single cases. "None" refers to leukemias that lack identifiable gene abnormalities. In AML, the genes show specific associations with disease subtypes corresponding to the morphologic stages of normal myeloid cell development. Although both the *AML1-ETO* and *CBFβ-MYH11* fusion genes lead to alterations in the CBF transcription complex, they appear to disrupt hematopoiesis through distinct mechanisms, because they are associated with different morphologic subtypes of AML. Other rearrangements affect the *RARα* gene on chromosome 17 in promyelocytic progenitors, resulting in fusions with *PML* or, rarely, *PLZF* or *NPM*. Less frequent translocations give rise to fusion genes associated with myelodysplastic syndrome, progressing to AML (*NPM-MLF1*, *DEK-CAN*, and *NUP98-HOXA9*). [Adapted with permission from (*2, 34*)]

opment of murine AML (*12*). Regulators of *HOX* gene expression are aberrantly activated in human leukemias much more frequently than the *HOX* genes themselves, possibly because single *HOX* gene alterations are less potent than concerted dysregulation of subsets of these genes. It is clear, however, that at least one of the *HOX* genes, *HOXA9*, can serve as a target for chromosomal translocations, as demonstrated by the recent detection of *NUP98-HOXA9* fusion genes in cases of AML with the t(7;11) translocation (*20*).

A more speculative aspect of the model shown in Fig. 3B is the prediction that oncogenic fusion proteins involving RARα and the core binding factor (CBF) complex will ultimately be shown to act as upstream regulators of the *HOX* genes in leukemogenesis. Retinoic acid (RA), acting through its nuclear receptors (RARs), has profound effects on the patterns of *Hox* gene expression during embryogenesis (*7*). Individual *Hox* genes exhibit differential sensitivity to the concentration of retinoic acid: The 3′ paralog groups that regulate anterior structures of the embryo are sensitive to high levels of retinoic acid early in development, whereas the 5′ groups that regulate posterior structures act later and respond to lower levels. A reasonable prediction is that the RARα fusion products in human acute promyelocytic leukemia (APL) (for example, PML-RARα, PLZF-RARα, and NPM-RARα) disrupt myeloid cell development at the promyelocyte stage by altering the normal sequential pattern of *HOX* gene expression.

Similar arguments lead to the prediction that the AML1 protein (a close counterpart of the *Drosophila* pair-rule protein runt) and its physiologic binding partner, CBFβ (*21*), act as upstream regulators that participate in the initiation of specific patterns of *HOX* gene expression. Fusions involving *AML1* or CBFβ generate several hybrid proteins, primarily AML1-ETO, TEL-AML1, AML1-EVI1, and CBFβ-MYH11. The pair-rule proteins have major roles in initiating patterns of *HOM-C* gene expression early in *Drosophila* embryogenesis (*22*); by analogy, the runt-related leukemogenic fusion proteins may interfere with the ability of the endogenous CBF complex to establish the normal pattern of *HOX* gene expression during hematopoiesis. Amino acid motifs contributed by the different AML1 and CBFβ fusion partners appear to result in different alterations of *HOX* gene regulation in progenitors of different lineages. This would account for the association of AML1-ETO, AML1-EVI1, and CBFβ-MYH11 with distinct morphologic subtypes of AML across all age groups, whereas TEL-AML1 occurs only in pro–B cell ALL, predominately in children.

Little is known about the downstream targets of the vertebrate HOX proteins, making it difficult to expand the conceptual model in Fig. 3 beyond immediate regulation of the *HOX* genes themselves. Some clues have emerged from studies with *Drosophila*, which have demonstrated the importance of autoregulatory loops among the *HOM-C* genes and have identified additional HOM targets, such as the *decapentaplegic*, *Distal-less*, *teashirt*, and *wingless* genes (*7*). The ability of *HOX* and *HOM-C* genes to influence cellular identity and regional body structure during morphogenesis implies that targets of HOX regulatory pathways must ultimately include proteins that regulate cell proliferation, survival, adhesion, and migration—aspects of cell physiology that likely contribute to many of the recognized abnormalities of leukemic blast cells.

The pleiotropic potential of homeotic proteins is best illustrated by the orphan homeobox-containing HOX11 protein, which does not lie in the major *HOX* gene clusters mentioned thus far, and which contributes to T-cell ALL when its expression is dysregulated by chromosomal rearrangements. Murine Hox11 is required for spleen development, through a mechanism that appears to affect survival of splanchnic precursors (*23*). In addition, HOX11 interacts with phosphatases that normally function within a G2-phase checkpoint; thus, its dysregulation in T cells may interfere with this checkpoint and cause aberrant entry into mitosis (*24*). Overall, because the *HOX* genes appear to regulate multiple pathways that control cell fate, they provide a compelling focus for research on the proximal sites of action of many of the oncogenic transcription factors.

Subversion of Apoptosis in Leukemic Transformation

Aberrant regulation of *HOX* gene expression is by no means the sole mechanism through which oncogenic transcription factors transform cells. A crucial step in hematopoietic development is the elimination of B- and T-lymphoid progenitors that fail to rearrange their antigen receptor genes in a productive manner. Defective cells, estimated to include up to 75% of B-cell and 95% of T-cell precursors, are destroyed by apoptosis. This death program is thought to be triggered by damaged DNA or by the inability of cells to receive survival signals from antigen receptors expressed at the cell surface (*25*).

The idea that subversion of cell death pathways might lead to malignant transformation gained momentum with the discovery of the *BCL2* proto-oncogene at the site of a translocation between chromosomes 14 and 18 in human follicular lymphomas (*2*). Overexpression of *BCL2* has little or no effect on cell differentiation or proliferation, but rather prevents lymphocytes from initiating apoptosis in response to a number of stimuli (*26*). Ultimately, BCL2 was

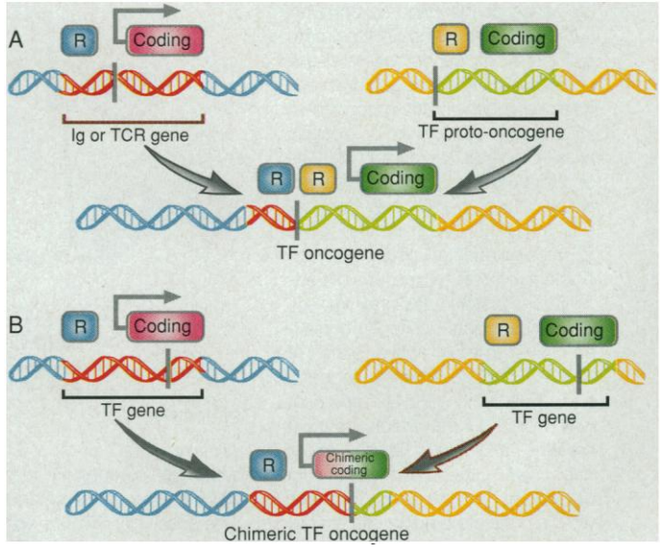

Fig. 2. Two distinct mechanisms by which chromosomal translocations aberrantly activate genes encoding transcription factors (TF). (**A**) Transcription factor proto-oncogenes that are silent or expressed at low levels in the progenitor cells of a particular lineage may be activated when placed under the control of potent enhancer elements within the regulatory region (R) of a gene that is normally highly expressed. Typically, the regulatory region in these cases is contributed by one of the *Ig* or *TCR* genes present in lymphoid progenitors of either the B or T lineage. (**B**) More commonly, chromosomal breakpoints occur within introns, between the coding sequences of each of two transcription factor genes on different chromosomes, producing a fusion gene that encodes a chimeric transcription factor with altered function. The regulatory sequences that drive expression of the hybrid gene are generally derived from the gene that contributes the amino-terminal amino acids to the chimeric protein; the carboxyl-terminal amino acids often derive from a gene that is not normally expressed in the progenitor cells in which the chimeric oncoprotein arises. Arrows indicate gene expression.

found to be a structural and functional homolog of CED-9, a dominant repressor of programmed cell death in the nematode *C. elegans* (*27*). Thus, by dysregulating a key anti-apoptotic protein in lymphocytes, the t(14;18) translocation promotes the accumulation of cells that otherwise would die. These cells then acquire additional mutations needed for full malignant conversion.

Although *BCL2* does not appear to be dysregulated by chromosomal rearrangement in the acute leukemias, investigations of the oncogenic E2A-HLF fusion protein, formed by the t(17;19) in pro-B lymphocytes, suggest that it disrupts an early step in a conserved cell death pathway that censors immature B lymphocytes. Dominant-negative inhibition of E2A-HLF in transformed lymphocyte progenitors induces apoptosis (*28*), suggesting that the chimeric protein increases the numbers of immature lymphoid cells by preventing their death. Consistent with this hypothesis, E2A-HLF blocks apoptosis in growth factor–deprived mouse pro-B cells and inhibits p53-mediated apoptosis triggered by ionizing radiation (*28*).

Sequence homology between the basic leucine zipper (bZIP) transcription factors HLF (hepatic leukemia factor) and CES-2, a cell death specification protein in *C. elegans*, suggests the model shown in Fig. 4 (*29*). In the developing nematode, loss-of-function mutations of *ces-2* are associated with increased activity of *ces-1*. This correlation leads to the aberrant survival of two superfluous serotonin-containing neurons (NSM sisters) through down-regulation of an apoptotic program that includes death effectors *ced-3* and *ced-4* or through up-regulation of the survival gene *ced-9*, each of which has counterparts in mammalian cell death programs (*27*, *30*). In human pro-B lymphocytes, E2A-HLF is postulated to compete with a mammalian CES-2–like protein for a common promoter binding site and to *trans*-activate (rather than repress) a *ces-1*–like gene, resulting in reduced apoptotic activity and prolonged survival of pro-B cells that otherwise would be targeted for destruction. The acquisition of additional mutations by these aberrantly surviving cells is probably required for the generation of a fully transformed leukemic clone.

At least one other oncogenic transcription factor, PML-RARα, has anti-apoptotic properties (*31*). Paradoxically, the MYC and E2A-PBX1 transcription factors, though clearly associated with the induction of human leukemias in vivo, promote apoptosis when overexpressed in experimental models (*32*). This result suggests that in lymphoid cells expressing these oncogenic proteins, additional mutations capable of disabling apoptotic pathways are needed to permit leukemic transformation.

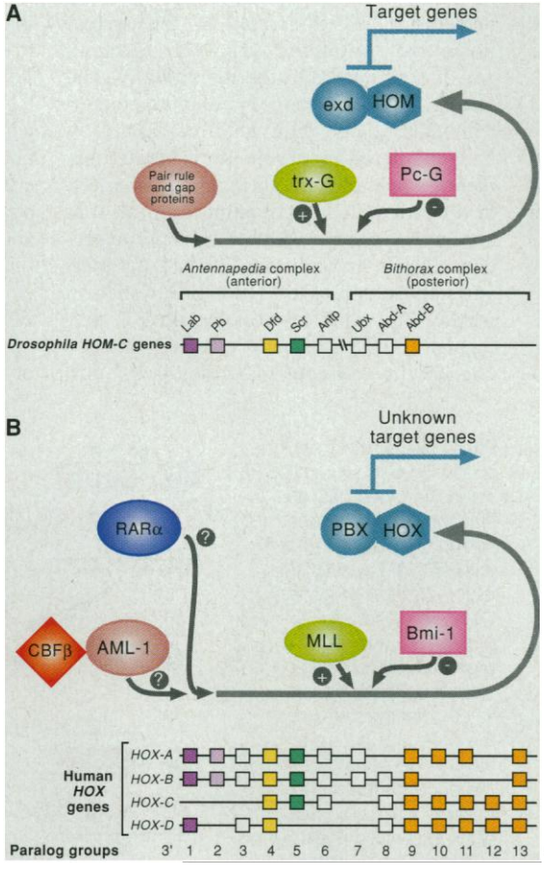

Fig. 3. A model based on conserved pathways of homeotic gene action that integrates the activities of seemingly diverse transcription factors in leukemogenesis. (**A**) Studies of *Drosophila* embryogenesis have established that the body plan of the fly is determined by a regulatory cascade centered around the control of homeobox gene expression within the *HOM-C* complexes (*7*). Genes at the 3′ end comprise the *Antennapedia* complex (*lab*, *pb*, *Dfd*, *Scr*, and *Antp*) and encode proteins that control the formation of anterior structures during embryonic development; more posterior segments are controlled by proteins encoded by genes of the *Bithorax* complex (*Ubx*, *abd-A*, and *Abd-B*). Upstream regulators of genes within these complexes include the *gap* and *pair-rule* genes, which act in concert to initiate the expression of specific *HOM-C* genes according to the spatial domains occupied by each embryonic progenitor cell. Continuous maintenance of *HOM-C* gene expression during *Drosophila* development requires two groups of transcription factors: trithorax (trx-g), whose members positively regulate and sustain the active state of *HOM-C* gene expression in cells, and Polycomb (Pc-G) proteins, which oppose the effects of trx-g proteins and continuously extinguish *HOM-C* gene expression in cells in which these genes are meant to remain inactive. The products of different *HOM-C* genes form complexes with other transcription factors, such as extradenticle (exd), which modulate binding-site and target-gene specificity. (**B**) The vertebrate *HOX* genes also dictate the body plan during embryogenesis. The human counterparts of the *Drosophila HOM-C* genes, found on a single chromosome in the fly, are designated *HOX-A* through *HOX-D* and are arranged on four separate chromosomes (four rows of colored squares). Genes within the *HOM-C* and *HOX* clusters show striking structural and functional conservation, as indicated by the color coding. Most of the *HOX* genes appear to have regulatory roles in normal hematopoiesis (*8*). In the model, seven translocation-associated proteins are predicted to regulate *HOX* gene expression, on the basis of the effects of their *Drosophila* homologs. The chimeric oncoproteins involved in leukemogenesis (Fig. 1) are postulated to act by disrupting the activity of their normal counterparts in *HOX* gene regulation.

Therapeutic Significance

Treatment of the acute leukemias has progressed from uniform strategies devised for large groups of patients to more refined protocols tailored to the risk of relapse in discrete subgroups (*33*). Although routinely recorded features, such as the blast cell immunophenotype and the presenting white blood cell count, provide useful criteria for risk assessment, molecular genetic changes appear to offer the most sensitive markers of potential leukemia cell aggressiveness and hence are the best guides to treatment (*33*, *34*).

In ALL, the *BCR-ABL* and *MLL-AF4* fusion genes are among the few prognostic markers indicating a requirement for hematopoietic stem cell transplantation in first remission (Table 1). Although the leukemias identified by these changes may respond well to standard treatment, they almost always acquire drug resistance within the first year or two after diagnosis, perhaps because the initial molecular lesion arises in pluripotent stem cells with an unlimited capacity for self-renewal (*35*). The *E2A-PBX1* chimera distinguishes an important subgroup of children with pre-B leukemia who have suboptimal responses to antimetabolite chemotherapy that is effective for most B-lineage leukemias but fare well when placed on more intensive regimens similar to those used for T-cell ALL and other high-risk cases (*36*). At the opposite end of the spectrum, the *TEL-AML1* fusion gene, found in ~20% of all children with pro-B–cell ALL, is associated with extended survival and probable cure in more than 85% of patients (*37*). Moreover, its prog-

nostic importance exceeds that of the age and white blood cell count of the patient at diagnosis, the gold standards of risk classification in this disease. Thus, by careful identification of *TEL-AML1*–positive leukemias at the time of diagnosis, one can administer well-tolerated antimetabolite-based treatments with a high likelihood of a favorable outcome. Indeed, this molecularly defined subgroup affords the opportunity to investigate new therapies that could reduce short- and long-term toxicities without jeopardizing high cure rates.

Although therapy for AML has become increasingly complex, more than half of the patients treated with chemotherapy alone can still be expected to relapse and succumb to their disease (*33*). Until recently, there were few prognostic markers that could identify AML patients who were likely to respond well to therapy. It now appears that the *AML1-ETO* and *CBFβ-MYH11* fusion genes will identify patients with a favorable prognosis when treatment consists of intensive chemotherapy including high-dose cytarabine (*38*), and *PML-RARα* distinguishes cases of APL that are particularly sensitive to all-*trans*-retinoic acid, a compound that induces differentiation of leukemic promyelocytes (*39*). When this agent is incorporated with anthracyclines into chemotherapy for APL, excellent long-term remission rates are achieved without hematopoietic stem cell transplantation (*40*). Chimeric messenger RNAs (mRNAs) transcribed from fusion oncogenes provide unique signatures for molecular detection of minimal residual leukemia after treatment (*33*), particularly in cases of APL harboring *PML-RARα* (*41*). However, the persistence of *AML1-ETO* or *CBFβ-MYH11* transcripts in the bone marrow and peripheral blood of AML patients in long-term remission after chemotherapy or bone marrow transplantation (*42*) raises questions about the general applicability of this strategy.

Thus far, the use of retinoic acid for therapy of APL affords the only example of effective treatment directed to a chimeric transcription factor. However, translocation-generated fusion proteins are truly "tumor-specific" and, as such, provide novel targets for therapy. One approach would be to use antisense oligonucleotides (*43*) or ribozymes (*44*), designed to inactivate the mRNAs encoding chimeric oncoproteins or their downstream effectors. An alternative form of targeted therapy may soon be possible, based on a new technology that permits the design of small molecules that repress the transcription of specific genes (*45*). A decided advantage of such a method would be the reduced likelihood of the outgrowth of resistant cells, a major liability of contemporary forms of cancer chemo-

Table 1. Therapeutic implications of commonly disrupted transcription factor genes in the acute leukemias of childhood.

Altered genes	Leukemia subtype* affected	Risk of treatment failure†	Recommended treatment‡
	Acute lymphoblastic leukemia		
TEL-AML1	Pro-B cell	Low	Well-tolerated chemotherapy (antimetabolites primarily)
E2A-PBX1	Pre-B cell	Intermediate	Intensive chemotherapy (genotoxic drugs and antimetabolites)
MYC	B cell	High	Intensive chemotherapy (rotation of genotoxic drugs)
MLL-AF4	$CD10^-$ pro-B cell	Very high	Allogeneic stem cell transplantation
BCR-ABL	Pro-B cell (predominantly)	Very high	Allogeneic stem cell transplantation
	Acute myeloid leukemia		
AML1-ETO	Acute myeloblastic leukemia with maturation (M2 morphology)	Intermediate	Intensive chemotherapy (including high-dose cytarabine)
CBFβ-MYH11	Acute myelomonocytic leukemia with eosinophils (M4Eo morphology)	Intermediate	Intensive chemotherapy (including high-dose cytarabine)
PML-RARα	Acute promyelocytic leukemia (M3 morphology)	Intermediate	Intensive chemotherapy (including all-*trans*-retinoic acid and anthracyclines)

*Subclassifications of AML are those of the French-American-British (FAB) group. †As determined in standard programs of chemotherapy (without hematopoietic stem cell rescue). Treatment failure refers either to remission induction or to remission maintenance, or both. The average rates of long-term, leukemia-free survival in children and adolescents with ALL or AML range from 60 to 70% and from 30 to 40%, respectively (*33*). ‡The choice of therapy is based on detection of the indicated fusion gene at diagnosis by cytogenetic analysis, Southern blotting, or RNA–polymerase chain reaction assays for chimeric mRNAs.

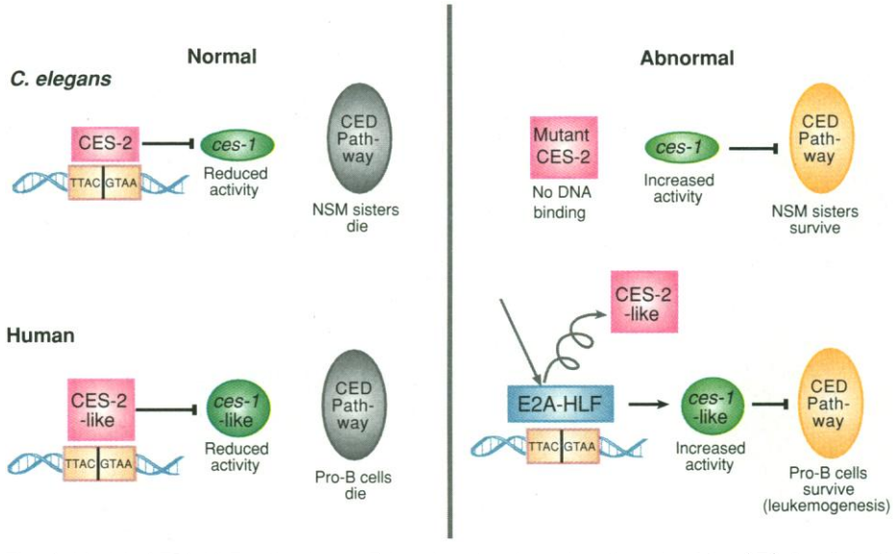

Fig. 4. Model of E2A-HLF action in pro-B lymphocytes, based on the role of the CES-2 cell death specification protein in *C. elegans*. The CES-2 basic leucine zipper (bZIP) transcription factor negatively regulates the (still uncloned) *ces-1* gene, leading to timely elimination of two serotonergic neurons (NSM sisters) during development. Loss-of-function mutations that inactivate *ces-2* lead to increased activity of *ces-1*, resulting in aberrant survival of these neurons in worms that survive to adulthood. The CED (cell death abnormal) proteins, including the CED-3 and CED-4 death effectors and the CED-9 survival protein, control the execution of CES-2–targeted neurons in *C. elegans* (*30*). By analogy, a similar cell death specification program may eliminate defective pro-B cells during development of the human immune system, through a process that appears to be disrupted by the E2A-HLF chimeric oncoprotein. The E2A (E12 and E47) proteins contain two domains with potent *trans*-activating activity (*46*), both of which are included in the amino-terminal portion of the E2A-HLF chimera. Because CES-2 and E2A-HLF bind to the same dyad-symmetric DNA sequence element (*29*), the hybrid protein is predicted to oppose the *trans*-repressor activity of a putative mammalian CES-2 ortholog, activating an evolutionarily conserved survival pathway in pro-B cells. [Adapted with permission from (*29*)]

therapy. In the not-too-distant future, it may be possible to link the different pathways controlling apoptosis, differentiation, and proliferative capacity in hematopoietic cells, providing a more comprehensive picture of the genetic basis of acute leukemia and perhaps new molecular targets for the design of effective therapy.

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Integrating Genetic Approaches into the Discovery of Anticancer Drugs

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The discovery of anticancer drugs is now driven by the numerous molecular alterations identified in tumor cells over the past decade. To exploit these alterations, it is necessary to understand how they define a molecular context that allows increased sensitivity to particular compounds. Traditional genetic approaches together with the new wealth of genomic information for both human and model organisms open up strategies by which drugs can be profiled for their ability to selectively kill cells in a molecular context that matches those found in tumors. Similarly, it may be possible to identify and validate new targets for drugs that would selectively kill tumor cells with a particular molecular context. This article outlines some of the ways that yeast genetics can be used to streamline anticancer drug discovery.

The recent remarkable progress in identifying molecular alterations in human tumor cells has unfortunately not been paralleled in the field of anticancer drug discovery. The shortage of effective anticancer drugs is due in part to the fundamental difficulties associated with the development of any safe effective drug. For example, it remains a formidable task to design small molecules that alter the function of macromolecules with both sensitivity and specificity (for example, an enzyme with a small active site). It is even more difficult to inhibit protein-protein interactions mediated over a large surface, or to restore function to a defective protein (such as an inactive tumor suppressor protein). Even when successful, massive efforts are required—often measured in years to decades—from dozens of chemists, biochemists, and toxicologists.

There are also many difficulties specific to anticancer drug discovery programs. An effective chemotherapeutic must selectively kill tumor cells. Most anticancer drugs have been discovered by serendipity, and the molecular alterations that provide selective tu-

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