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A human c-*erbA* oncogene homologue is closely proximal to the chromosome 17 breakpoint in acute promyelocytic leukemia

(somatic cell hybrids/myeloid leukemia/chromosomal translocations/*in situ* hybridization)

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ABSTRACT **A human cDNA library was screened for sequences homologous to the *erbA* gene of avian erythroblastosis virus (AEV). One such clone, cHerbA-1, was used to map the chromosomal location of highly homologous human sequences that were found to be present on chromosome 17 as judged by Southern blot screening of a panel of mouse–human hybrid cell lines segregating human chromosomes. cHerbA-1 was hybridized *in situ* to metaphase chromosomes from a normal male subject and from a female patient with an acute promyelocytic leukemia (APL) having the typical t(15;17) translocation. The results localized the cellular c-*erbA* sequences on chromosome 17 to the q21–q24 region of normal chromosomes and indicated that the c-*erbA* sequences remained on the 17q$^-$ chromosome in the APL cells, suggesting that they could be assigned to the 17(q21–q22) region. For additional data, we hybridized human neoplastic cells derived from a poorly differentiated acute leukemia carrying a t(17;21) translocation with thymidine kinase (TK)-deficient LMTK$^-$ mouse cells. A resulting hybrid, containing only the 21q$^+$ chromosome, did not have human c-*erbA* sequences. Since the breakpoint on 17q in this translocation was similar to that in the APL t(15;17) translocation, this supported the assignment of c-*erbA* to the q21–q22 region of chromosome 17. The apparent close proximity of the c-*erbA* sequences to the chromosomal breakpoints in these two leukemias suggests a possible role for this oncogene homologue in the development of these neoplasms.**

Retroviral oncogenes derive from normal cellular counterparts, which can be activated by any of several mechanisms often involving juxtaposition with viral or cellular activators (promoters/enhancers, "active" chromatin regions) (1–7). To date, three retroviruses are known to contain two separate genes of cellular origin: E26, with *myb* and *ets* (8, 9); MH2, with *myc* and *mil/mht* (10, 11); and avian erythroblastosis virus (AEV), with viral genes v-*erbA* and v-*erbB* (12–15). Of these three, AEV is the most extensively characterized (15).

The v-*erbA* gene, which is expressed as a 75-kDa gag-erbA fusion protein, plays a distinct, though probably ancillary role in effecting transformation (16). The v-*erbB* gene appears to be the predominant effector of transformation (17–19). It is a member of the *src* gene family of protein kinases (16) and has considerable homology to peptides of the epidermal growth factor receptor (20).

One c-*erbB* and two c-*erbA* cellular genes have been isolated from human genomic libraries. Hybridization studies strongly suggest the existence of at least one additional human c-*erbA* (21). To investigate the roles (if any) played by *erbA*- and *erbB*-related sequences in the causation of human cancer, our laboratories have undertaken a general study of such sequences in the human genome. Here we report evidence supporting the location of *erbA*-related sequences on human chromosome 17 in the q21–q22 region, proximal and close to the breakpoint on chromosome 17 in acute promyelocytic leukemia.

MATERIALS AND METHODS

Probes. Molecular subclones of the AEV provirus were originally made by A. J. Kung and were kindly provided by William A. Haseltine. The v-*erbA*-specific probe used in these studies is the 500-base-pair (bp) *Pst* I fragment indicated in Fig. 1 and corresponds to probe 3 described by Jannsson *et al.* (21).

DNA Sequence Determination. Probes were sequenced by the method of Biggin *et al.* (22) as indicated in Fig. 1.

Cell Lines and Hybrid Clones. The cell lines and hybrid clones used for the initial mapping of c-*erbA* sequences have been extensively described in previous studies (23, 24).

Hybrid clone CMC275S was derived from fusion of cells from a patient with undifferentiated acute leukemia with thymidine kinase (TK)-deficient mouse LMTK$^-$ cells. The leukemic cells carried a t(17;21)(q21–q22;q22) chromosome translocation (see Fig. 5 *Upper*), and clinical details of the case have been published elsewhere (25). Within the technical limits of the material, the breakpoint on chromosome 17 (q21–q22) was considered identical with that of the characteristic t(15;17) chromosome translocation of acute promyelocytic leukemia (26). The human chromosomes present in this hybrid were identified by a combination of trypsin/Giemsa and G-11 banding techniques, as described (27).

Labeling of Probes. All probes for Southern blots and library screening were labeled by minor modifications of standard nick-translation procedures (28, 29) with [α-^{32}P]dNTPs to a specific activity of 0.5–2.0 $\times$ 10^9 cpm/μg. Probes for *in situ* hybridization were labeled by similar methods with [^{3}H]dCTP and [^{3}H]dTTP (New England Nuclear) to a specific activity of 1 $\times$ 10^7 cpm/μg.

Restriction Analysis. Cellular DNA was digested for 4–5 hr at 37°C with 4–5 (60-min) units of enzyme per μg of DNA in the buffer recommended by the manufacturer (New England Biolabs) and was analyzed by the Southern technique (30).

Filter Hybridizations. Southern blot and library screening hybridizations were at 37°C in 4× NaCl/Cit (1× NaCl/Cit = 0.15 M NaCl/0.015 M Na citrate, pH 7) containing 0.2% NaDodSO$_4$, 1× Denhardt's solution (31), and 50% formamide with or without 10% dextran sulfate (31, 32). Washes no more stringent than at 68°C with 2× NaCl/Cit were suffi-

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Abbreviations: AEV, avian erythroblastosis virus; APL, acute promyelocytic leukemia; TK, thymidine kinase; v- and c-*erb* genes, viral and cellular *erb* genes; bp, base pair(s); kb, kilobase(s).

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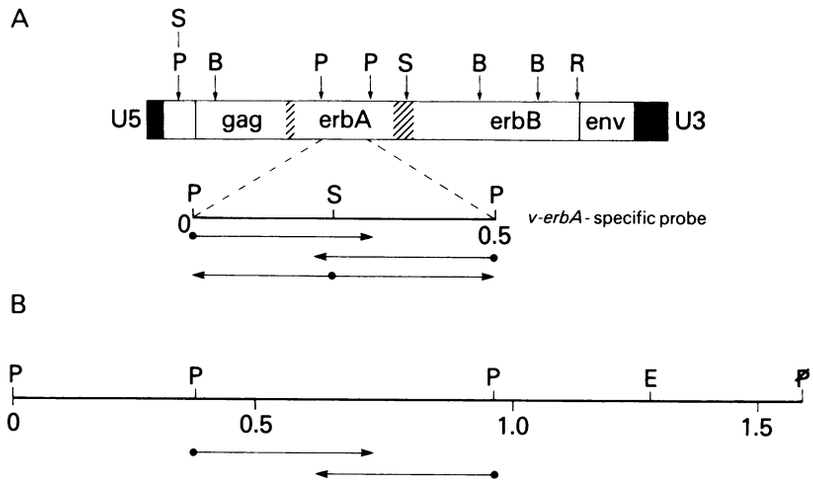


FIG. 1. (*A*) Restriction map of the AEV provirus [adapted from Jansson *et al.* (21)]. The 500-bp v-*erbA*-specific *Pst* I fragment has been expanded. (*B*) Restriction map of probe c-HerbA-1. Arrows indicate the strategy used to determine the sequence of the two homologous fragments.

cient to allow visualization of human bands hybridizing directly with the viral v-*erbA*-specific probe. These wash and hybridization conditions were used both for early mapping studies with the hybrid cell lines and for screening the human cDNA library. When hybridizing probes of human origin to human or hybrid DNA, the same hybridization conditions were used, but the final wash was at 68°C in 0.2× NaCl/Cit.

***In Situ* Hybridizations.** The cHerbA-1 probe was hybridized *in situ* to metaphase chromosomes from peripheral blood lymphocytes of a normal male subject and from bone marrow of a female APL patient with the typical t(15;17) (q22–q25;q21–q22) translocation (26). The quality of the control metaphases permitted us to analyze them by our standard methods (33). The technically poor quality of the leukemic material, however, limited the analysis. In these metaphases, chromosomes with overlying grains were assigned to one of the seven chromosome groups (A–G), and the grains were mapped only to general chromosomal regions. The 17q$^-$ chromosome often could not be distinguished from the normal *F* group chromosomes and so was grouped with them.

Library Screening. A total of 84,000 colonies from a K562 cDNA library constructed by Watt *et al.* (34) were screened by standard methods (35) with the v-*erbA*-specific probe.

RESULTS

Isolation of a Human c-*erbA* Clone from a cDNA Library. From the human cDNA library one clone, cHerbA-1, was obtained that strongly hybridized with the v-*erbA*-specific probe. Fig. 1 diagrams the location of the v-*erbA*-specific probe in the AEV provirus and the restriction map of the cHerbA-1 insert. Sequence analysis by the diagrammed strategy (Fig. 1) indicated that the v-*erbA*-specific probe and the 500-bp *Pst* I fragment of cHerbA-1 were 82% homolo-

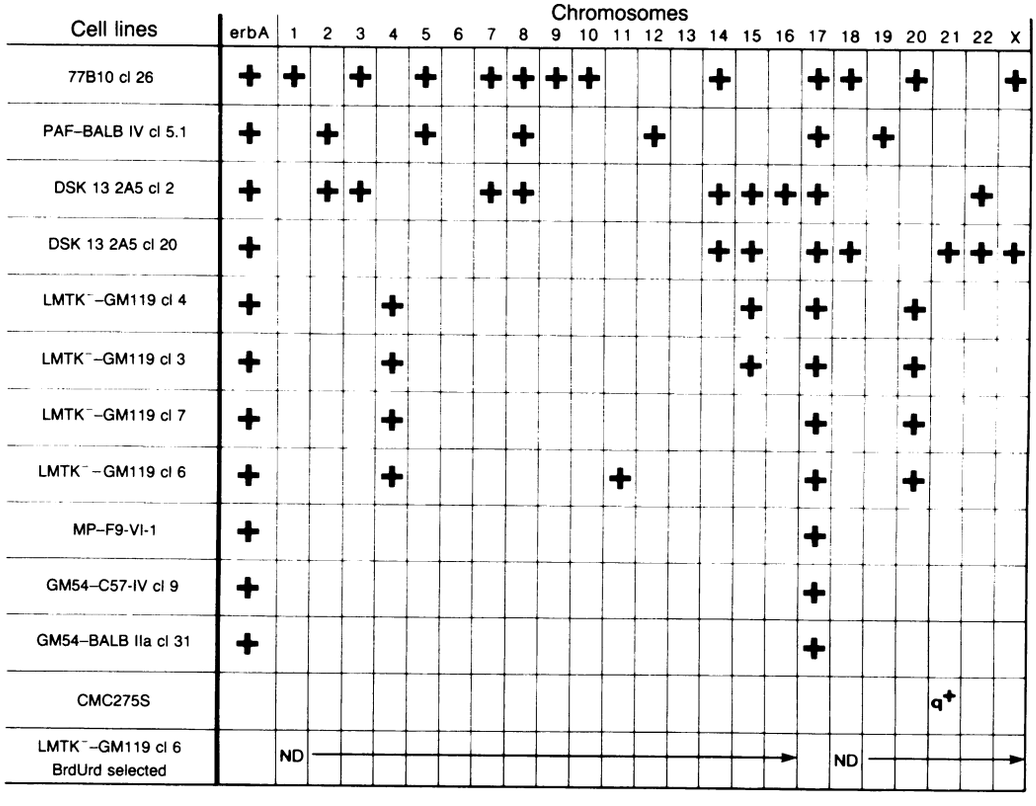


FIG. 2. Chromosomal mapping of c-*erbA*. The indicated hybrids were screened with the v-*erbA*-specific probe or with cHerbA-1. ND, not determined.

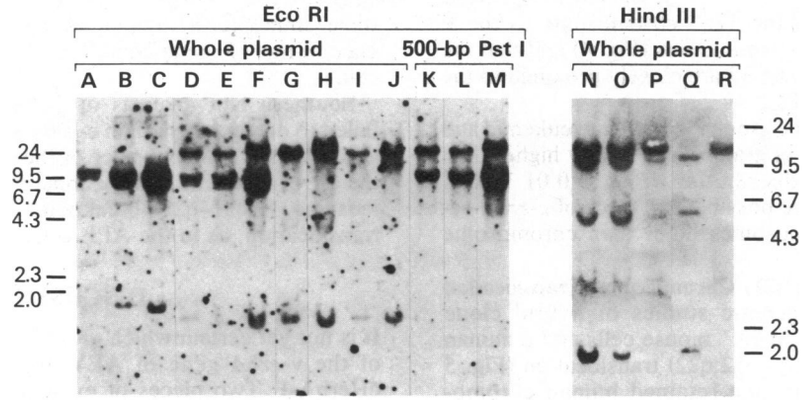


FIG. 3. Southern blot of selected hybrid DNAs. DNA was cut with the indicated enzymes and probed with either the whole cHerbA-1 plasmid or with the 500-bp *Pst* I fragment of cHerbA-1. Lanes: A, normal human peripheral blood leukocytes, 10 μg; B, GM607, 20 μg; C, PAF, 30 μg; D, MP-F9-VI-1, 15 μg; E, GM54-BALB IIa cl 31, 15 μg; F, GM54-C57-IV cl 9, 30 μg; G, CMC275S, 20 μg; H, CMC275S, 30 μg; I, LMTK$^-$, 20 μg; J, LMTK$^-$, 30 μg; K, MP-F9-VI-1, 15 μg; L, GM54-BALB IIa cl 31, 15 μg; M, GM54-C57-IV cl 9, 30 μg; N, GM54-C57-IV cl 9, 20 μg; O, GM54-BALB IIa cl 31, 20 μg; P, MP-F9-VI-1, 15 μg; Q, GM607, 10 μg; R, LMTK$^-$, 20 μg. GM607 (lanes B and Q) and PAF (lane C) are "normal" human control cell lines. LMTK$^-$ (lanes I, J, and R) is a mouse cell line used for fusions.

gous at the nucleic acid level and 89% homologous at the amino acid level.

Consistent with this high degree of homology is the finding that, of the three human *Hin*dIII fragments detected by cHerbA-1 at 2.0, 5.6, and 11 kilobases (kb) (see Fig. 3, lane Q), both the 2.0- and the 5.6-kb fragments were also detected by the v-*erbA*-specific probe (data not shown).

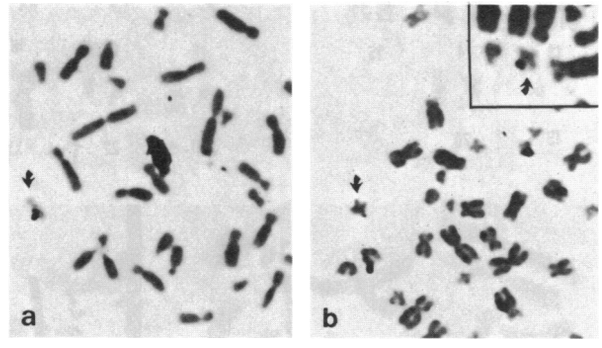
Human c-*erbA* Sequences Are on Chromosome 17. Fig. 2 summarizes the chromosomal mapping data obtained from screening a panel of mouse–human hybrid cell lines in which human chromosomes had segregated. The presence of human chromosome 17 correlates with the presence of human sequences homologous to *erbA*.

Fig. 3 shows the restriction patterns of DNA from selected mouse–human hybrids probed either with cHerbA-1 or the 500-bp *Pst* I fragment isolated from cHerbA-1. Normal human DNA cut with *Eco*RI (lanes A, B, and C) has two strongly hybridizing bands at 9.5 and 8.9 kb, both of which are well resolved from mouse-specific bands (lanes I and J). A human band is also seen at 1.6 kb, but its proximity to the mouse band and its weak hybridization to the probe make it unreliable for mapping. Additional human bands exist at 2.5 kb (lane A) and 5.4 kb (lanes B and C), but they are faint and possibly polymorphic.

The clear presence of the human 9.5- and 8.9-kb *Eco*RI bands in MP-F9, GM54-BALB IIa cl 31, and GM54-C57-IV cl 9, which contain only human chromosome 17 in a mouse chromosome background (lanes D, E, and F) confirms the presence of c-*erbA* sequences on chromosome 17. These hybrid lines also contain the weakly hybridizing 5.4-kb *Eco*RI human fragment (lanes K, L, and M) and the three strongly hybridizing *Hin*dIII human fragments (lanes N, O, and P) detected by cHerbA-1.

To delimit more accurately the location of c-*erbA* sequences, the cHerbA-1 probe was hybridized *in situ* to metaphase chromosomes from a normal male. A total of 213 chromosomally localized grains from 85 metaphases were analyzed. Seventeen grains (8%) were present over the 17(q21–q24) region, consistent with the Southern blot localization of c-*erbA* sequences to chromosome 17 (Fig. 4). Seventeen grains were also present over the paracentromeric region of chromosome 16 (16p11–q11). No other chromosomal region of comparable length had more than 10 grains, although there appeared to be some localization over the telomeric region of each of the D group chromosomes.

Relation of c-*erbA* to the t(15;17) Translocation Seen in Acute Promyelocytic Leukemia. The cHerbA-1 probe was also hybridized *in situ* to metaphase chromosomes from leukemic cells with a t(15;17)(q22–q25;q21–q22) translocation. A total of 212 grains were counted. The results of the hybridizations to both normal and leukemic cells are summarized in Fig. 4. The normal F group chromosomes had no more grains than predicted by a random distribution. The leukemic "F group" chromosomes (which included the 17q$^-$ chromosome) had almost twice the number of grains predicted by a random distribution after correction for the additional member of the group ($\chi^2 = 10.3$, $P << 0.01$). Nearly all of these grains were located over telomeric regions. This sug-



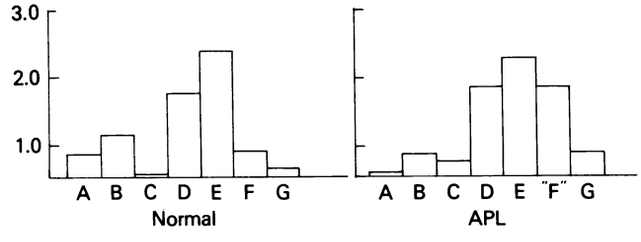


FIG. 4. (*Upper*) *In situ* hybridization of cHerbA-1 showing localization of grains to the 17(q21–q24) region of normal metaphase chromosomes (arrow) (*a*) and metaphase from an APL patient with the t(15;17) translocation (*b*). In *b*, the arrow indicates a grain over the telomeric region of a chromosome of the "F group" (which included the 17q$^-$ chromosome), and the *Inset* shows similar grain localization (arrow) from another metaphase. (*Lower*) Histograms of the number of grains observed over each chromosome group in normal and APL metaphases divided by the number predicted by a random distribution. Ratios significantly less than 1.0 were not interpreted.

gests that the addition of the $17q^-$ chromosome to the F group is the source of the excess grains (Fig. 4) and that the c-*erbA* genes on chromosome 17 are closely proximal to the breakpoint observed in APL.

The D and E chromosome groups in both the leukemic and normal cells had numbers of grains significantly higher than predicted by a random distribution ($P << 0.01$ for all groups), consistent with the possible existence of c-*erbA*-related sequences on chromosomes other than chromosome 17.

Relation of *erbA* to a t(17;21) Chromosomal Translocation in Leukemia Cells. Cytogenetic studies of hybrid clone CMC275S, derived from $LMTK^-$ mouse cells and a human leukemia with a t(17;21)(q21–q22;q22) translocation (Fig. 5 *Upper*) demonstrated only one retained human chromosome, the $21q^+$ chromosome resulting from the translocation (Fig. 5 *Lower*). Isozyme data on this clone were consistent with this finding, being positive only for the gene for superoxide dismutase (soluble), *SOD1*, located on chromosome 21 at q21.1 (36). The growth of these hybrids in HAT (hypoxanthine/aminopterin/thymidine) medium required the presence of the TK gene-containing $21q^+$ chromosome in every cell.

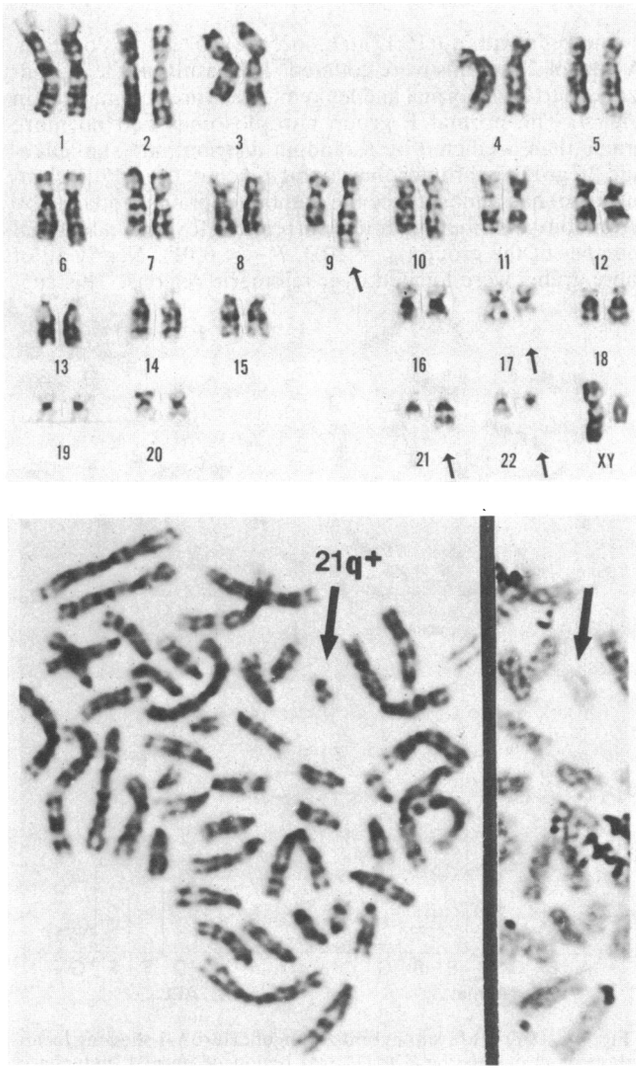


FIG. 5. (*Upper*) Karyotype of parental leukemic cell with two translocations (arrows); t(17;21)(q21–q22;q22) and t(9;22)(q34;q11). Additional details are provided in ref. 25. (*Lower*) Portion of a trypsin/Giemsa-banded metaphase from hybrid clone CMC275S containing the $21q^+$ chromosome (arrow) derived from the t(17;21) translocation as the only human chromosome. G-11 staining of the same metaphase (*Inset*) indicates the human origin of the $21q^+$ chromosome (27).

Southern blot analysis of DNA from this hybrid clone failed to detect any human c-*erbA* sequences (Fig. 3, lanes G and H) despite the presence of the $21q^+$ chromosome. Thus, the c-*erbA* sequences were apparently proximal to the chromosome 17(q21–q22) breakpoint involved in this t(17;21) translocation, as in the APL cells.

DISCUSSION

It is not yet certain which of the human c-*erbA* homologues of the v-*erbA* gene of AEV are detected by our probe, cHerbA-1. Two pieces of evidence suggest that cHerbA-1 corresponds to the λhe-A1 described by Jansson *et al.* (21). First the "9.5-kb" *Eco*RI band detected by cHerbA-1 is probably the same as the "9.7-kb" *Eco*RI band detected by λhe-A1. Second, with *Hin*dIII-digested DNA, cHerbA-1 hybridizes predominantly to a 14-kb mouse band (Fig. 3, lane R), as does λhe-A1. On the other hand, neither the λhe-A1 nor λhe-A2 cloned by Jansson *et al.* (21) accounts for either the 5.6- or 1.9-kb *Hin*dIII bands homologous to both c-HerbA-1 and the v-*erbA*-specific probe (21). It may be that either or both of the two genomic clones stop just short of including these fragments, though we consider this unlikely. Jansson *et al.* (21) report the probable existence of other human c-*erbA* sequences distinct from the ones they have cloned. For instance, a probe from λhe-A1 hybridizes to a 4.3- and a 7-kb *Eco*RI band not accounted for by any of their genomic clones. Possibly we have cloned a new member of a multigene family. Only sequence analysis will provide an unambiguous answer. Whatever the case, the isolation of cHerbA-1 from a cDNA library ensures that the human c-*erbA* gene we have cloned is an actively transcribed one.

The findings presented here support the localization of this gene to the q21–q22 region of human chromosome 17, thus extending a recent report (37) mapping *erbA-1* to the p11–q21 region of chromosome 17. The presence of c-*erbA* sequences on chromosome 17 is indicated by the somatic cell hybrid data in Fig. 2 and by the results of the *in situ* hybridization of cHerbA-1 to normal metaphase chromosomes, with the grains localized over the distal two-thirds of the q arm of chromosome 17(q21–q24).

The *in situ* hybridization data also suggest that there may be c-*erbA* sequences elsewhere in the genome, particularly in the paracentomeric region of chromosome 16. This is of potential interest in view of the recent description of a chromosome abnormality involving this region in a variant of acute monomyelocytic leukemia (38); but it is also surprising because all of the *Hin*dIII and *Eco*RI bands detected in normal human cells were also detected in hybrids containing only human chromosome 17. Either the sequences present on chromosome 16 have identical *Hin*dIII and *Eco*RI fragments or the stringency of the *in situ* hybridization conditions permit the detection of sequences not seen at the high stringencies used for the Southern blots. The significance of the weak localization of grains to the telomeres of the D group chromosomes is also not clear.

The preponderance of grains over the five "F group" chromosomes in the *in situ* hybridization study of metaphases from an APL patient with the t(15;17) translocation appears to derive from the presence of the $17q^-$ chromosome. This suggests that c-*erbA* lies proximal to the breakpoint on chromosome 17(q21–q22) observed in this characteristic translocation (26), in agreement with the study of Sheer *et al.* (39).

The findings from the hybrid clone between human leukemic cells with a t(17;21) translocation and mouse cells further suggest that c-*erbA* sequences lie proximal to the q21–q22 breakpoint on 17q seen in this latter translocation and to

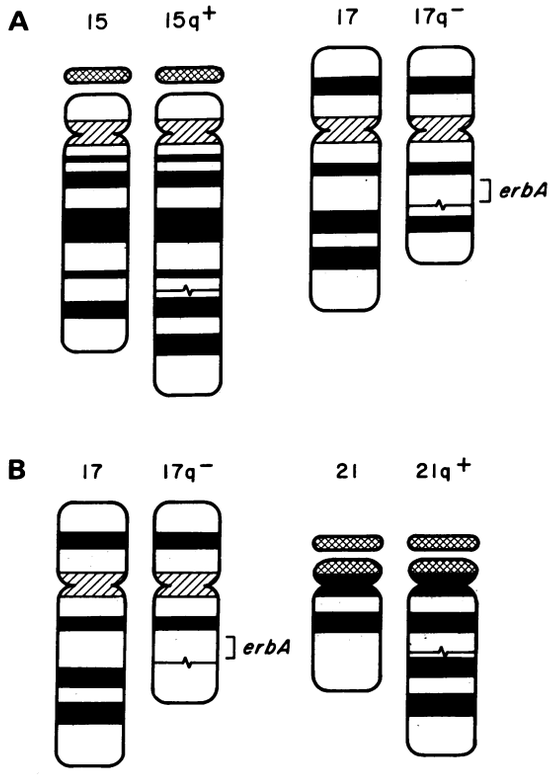


FIG. 6. The postulated relation of c-*erbA* to the chromosomal breakpoints seen in a case of APL with the typical t(15;17)(q22–q25;q21–q22) translocation (*A*) and a case of acute undifferentiated leukemia with a t(17;21)(q21–q22;q22) translocation (*B*).

the TK locus. The hybrid with only the $21q^+$ chromosome did not contain the c-*erbA* gene, whereas the TK gene had to be present within the translocated segment of 17q (25). The technical quality of the original karyotypic studies on this t(17;21) leukemia did not permit precise localization of the breakpoint on 17q (25), but it appeared to involve the distal portion of band 17q21 (Fig. 5), close to band q22, and therefore was quite similar to the 17q breakpoint in APL (26). The breakpoint on chromosome 21 seen in this t(17;21) translocation was in band q22, a location similar to that in the characteristic t(8;21)(q22;q22) translocation observed in some cases of acute myelogenous leukemia in which activation of the c-*mos* oncogene on chromosome 8 by juxtaposition with "activating" sequences from 21q has been suggested (40).

Taken together, the present data from both normal cells and from two cases of leukemia are consistent with the view that a human homologue of the *erbA* oncogene is located in the q21–q22 region of chromosome 17 (Fig. 6) and is, therefore, a candidate for activation, after chromosomal translocation events, by DNA sequences brought adjacent to it.

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