Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 5836–5840, September 1985
Genetics

Chromosome localization of the gene for human terminal deoxynucleotidyltransferase to region 10q23–q25

(terminal transferase/B and T cells/leukemias and lymphomas/chromosome rearrangement/genetics of neoplasia)

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Communicated by Peter C. Nowell, May 3, 1985

ABSTRACT **Complementary DNA clones representing the 3′ half, the 5′ half, and the entire coding region of the human terminal deoxynucleotidyltransferase gene (TdT; DNA nucleotidylexotransferase, nucleosidetriphosphate:DNA deoxynucleotidylexotransferase, EC 2.7.7.31) were used to screen a panel of mouse × human somatic cell hybrid DNAs to determine the chromosomal location of the human *TdT* gene. The results of the Southern transfer analysis of hybrid DNAs indicate that the gene for TdT is located on human chromosome 10. The *in situ* hybridization technique was then used to further localize the gene for TdT to region q23–q25 of human chromosome 10.**

Human terminal deoxynucleotidyltransferase (TdT; DNA nucleotidylexotransferase, nucleoside triphosphate:DNA deoxynucleotidylexotransferase, EC 2.7.7.31) is a 58-kDa protein (1) present in a major cell population of the thymus cortex and in a minor population of bone marrow lymphocytes (2). The normal biologic function of TdT is not fully understood and the reason for its presence in pre-T and pre-B cells remains an object of speculation. Recently, Desiderio *et al.* (3) have proposed that TdT might be responsible for inserting nucleotides (N regions) at the recombinational junctions of immunoglobulin heavy-chain genes during B-cell maturation. The insertion of N regions may also occur during recombinatorial joining of the T-cell-receptor genes (4). It is possible that the insertion of N regions reflects the increased levels of TdT in pre-T and pre-B cells. Approximately 100,000 TdT molecules per cell have been detected in cortical thymocytes and primitive bone marrow lymphocytes (1). Interestingly, we have detected N regions at the junction between chromosomes 11 and 14 in chronic lymphocytic leukemia of the B-cell type with the t(11;14) chromosome translocation (5). If the addition of N regions is the result of high TdT activity, this finding may suggest that the t(11;14) translocation may have occurred at the pre-B-cell stage of differentiation. TdT is also elevated in acute lymphoblastic leukemia and in cases of chronic granulocytic leukemia in blast crisis (6). Since the *TdT* gene is expressed in pre-B and pre-T cells and in certain human leukemias, this gene might have a role in oncogene activation in hematopoietic malignancies. In this study, human TdT cDNA clones isolated from a cDNA expression library derived from a pre-B-cell human leukemic cell line (7) have been used to determine the chromosomal location of the *TdT* gene in order to establish whether this gene is in a chromosome region subject to rearrangements in malignant T or B cells.

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MATERIALS AND METHODS

TdT cDNA Clones. During the course of these studies, three different cDNA clones were used: pT17, a pBR322 plasmid containing a 928-base-pair (bp) *Eco*RI (7) insert that represents the 3′ portion (3′ half) of full-length TdT cDNA; pT106, a 967-bp *Eco*RI insert in pUC8 that contains the 5′ half of the cDNA; and pT223, 1800 bp inserted in pUC19, which starts 3 bases from the start of the coding region and extends to the 3′ end of the cDNA. See Fig. 1 for a sketch of the relationship among these cDNA clones. The 3′ clone, pT17, was used for *in situ* hybridization. For analysis of segregation of TdT in mouse–human somatic cell hybrids, purified cDNA inserts from the 3′ or 5′ clones were usually used; total plasmid DNA from all three clones—3′, 5′, and total coding region (pT223)—were also used in Southern blot analysis, with results identical to those obtained by using the purified insert of the respective plasmid.

Southern Blot Analysis. DNAs from human cells, mouse cells, and mouse–human hybrids were extracted by cell lysis, proteinase K digestion, phenol extraction, and ethanol precipitation. Cellular DNA samples were digested with the appropriate restriction enzyme, sized in 0.8–1.0% agarose gels, and transferred to nitrocellulose as described by Southern (8). Hybridization was carried out in 50% formamide/4× NaCl/Cit (1× NaCl/Cit is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0)/0.2 mg of sonicated salmon sperm DNA per ml/1× Denhardt's solution (0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone) at 42°C for 15 hr or at 37°C for 24 hr. The probes were labeled by nick translation. After hybridization, the filters were washed and exposed to Kodak XAR-5 film with intensifying screens.

Cell Lines. Isolation, propagation, and characterization of parental cells and the somatic cell hybrids used in this study have been described (9–13).

All hybrids were studied for the expression of enzyme markers assigned to each of the human chromosomes (9). Some hybrid clones were karyotyped by trypsin/Giemsa and/or G-11 banding methods as described (9). In addition, the presence of specific human chromosomes in many of the mouse–human hybrids have been confirmed by DNA hybridization by using probes for genes assigned to specific human chromosomes (9–13).

***In Situ* Hybridization.** Metaphase spreads were prepared with normal human male phytohemagglutinin-stimulated (for 72 hr) *in vitro* lymphocyte cultures. Total plasmid (pT17) DNA containing the human TdT 928-bp *Eco*RI fragment was nick-translated with [^{3}H]dCTP (62 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear), [^{3}H]dGTP (39.9 Ci/mmol), [^{3}H]dTTP (100.1 Ci/mmol), and [^{3}H]dATP (51.9 Ci/mmol).

Abbreviations: TdT, terminal deoxynucleotidyltransferase; kbp, kilobase pair(s).

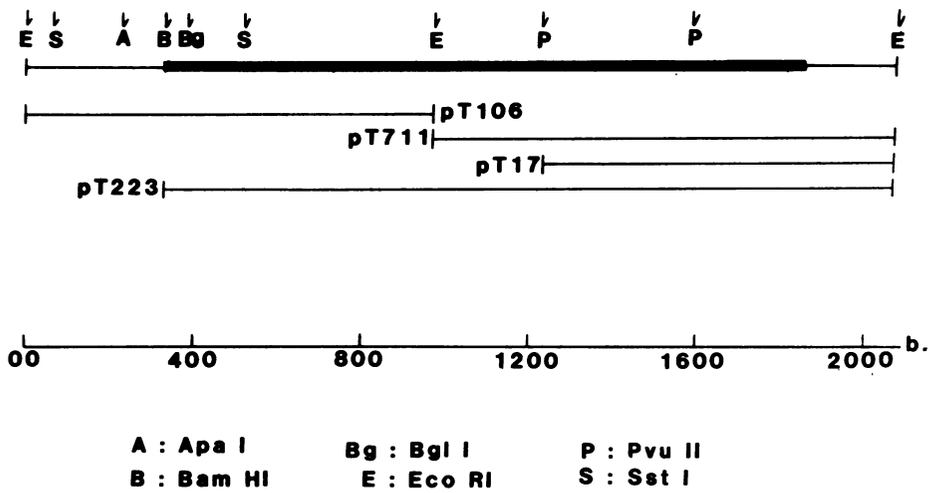

FIG. 1. Restriction maps of the TdT 5′, 3′, and total coding region cDNA clones.

The techniques used for *in situ* hybridization were essentially as described by Harper and Saunders (14). Chromosome preparations were treated with pancreatic RNase A (Sigma) and then denatured in 70% formamide in 2× NaCl/Cit (pH 7.0) at 70°C for 2 min. The chromosome preparations were then hybridized with ^{3}H-labeled pT17 DNA (specific activity, 3×10^7 cpm/μg) at a concentration of 140–280 ng/ml in 50% formamide/2× NaCl/Cit/10% dextran sulfate (Pharmacia), pH 7.0, for 20 hr at 37°C. A 300-fold excess of sonicated salmon sperm DNA was included as carrier. Slides were thoroughly rinsed in 50% formamide/2× NaCl/Cit at 39°C, exposed to Kodak NTB2 nuclear track emulsion for 16 days at 4°C, and developed with Kodak Dektol at 15°C. The chromosomes were then G-banded essentially as described by Cannizzaro and Emanuel (15) with a mixture of 6 parts of borate buffer (50 mM Na_2SO_4/2.5 mM $Na_2B_4O_7$, pH 9.2) to 1 part of Wright's/Giemsa stain solution (2.4 g of Wright's stain per liter/1.4 g of Giemsa stain per liter in methanol).

RESULTS

Chromosomal Location of the *TdT* Gene. DNA from a panel of mouse–human hybrid cells was analyzed for the presence of the human *TdT* gene by cleavage with restriction enzymes, followed by fractionation of DNA in agarose, transfer to nitrocellulose, and hybridization of nitrocellulose filters with either the 3′, 5′, or total TdT cDNA clones. In initial experiments, mouse and human DNA and mouse–human hybrid DNAs were cleaved with restriction enzyme *Hin*dIII and the nitrocellulose-bound DNA was hybridized to either the 3′ (pT17) or 5′ (pT106) TdT cDNA clones. An example of hybridization of *Hin*dIII-cleaved mouse, human, and hybrid DNAs to the 5′ (pT106) TdT clone is shown in Fig. 2. In *Hin*dIII-cleaved mouse genomic DNA, the 5′ TdT clone detects bands of 19.3 and 5.5 kbp (lane 2), although the mouse *TdT* gene is not reproducibly detected after *Hin*dIII cleavage and, as will be seen in later figures, is usually not seen at all after *Pst* I cleavage. The explanation for lack of detection of the mouse TdT is probably that small differences in stringency of hybridization and/or washing of filters can determine whether the mouse *TdT* gene is seen.

In *Hin*dIII-digested human DNA, the 5′ TdT probe routinely detects bands of 11.8 and 4.3 kbp (probably a doublet) and, occasionally, a 1.6-kbp band (Fig. 2, lane 1). In screening the mouse–human hybrid panel with the 5′ probe, it was found that the 11.8- and 4.3-kbp band segregated independently from a 4.1-kbp (lower band of 4.3 doublet) and the 1.6-kbp band (see lanes 3 and 4 versus lane 5). Since the 4.3- and 4.1-kbp *Hin*dIII fragments were not well separated in the human genome, *Pst* I-digested hybrid DNAs were used to analyze in more detail the independently segregated genes. *Pst* I-cleaved human and hybrid DNAs were blotted and hybridized to the 3′ probe, the 5′ probe, and the full-length probe (pT223).

After hybridization to the 3′ probe, three *Pst* I fragments (18.0, 8.5, and 4.9 kbp; data not shown) were detected in human DNA and in all hybrids showing positive hybridization; that is, with the 3′ half of the TdT coding region, all fragments segregated together and were thus on only one

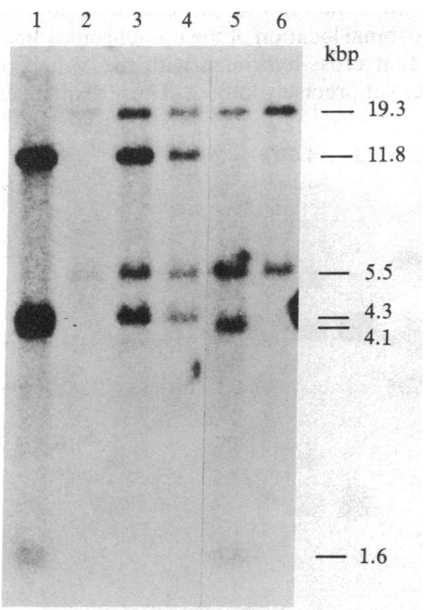

FIG. 2. Hybridization of the TdT 5′ cDNA clone to *Hin*dIII-cleaved parental and hybrid DNAs. Ten micrograms of cellular DNA from human (lane 1); mouse (lane 2); hybrid clone retaining human chromosomes 1, 3, 5, 6, 7, 8, 9, 10, 13, 14, 18, 20, 22, and X (lane 3); hybrid clone retaining human chromosomes 3, 5, 6, 9, 10, 13, 14, 18, 20, 22, and X (lane 4); hybrid clone retaining human chromosomes 2, 5, 12, and 19 (lane 5); and hybrid clone retaining human chromosomes 1, 3, 5, 14, 18, 22, and X (lane 6), was digested with an excess of restriction enzyme *Hin*dIII, sized on an agarose gel, transferred to nitrocellulose, and hybridized to ^{32}P-labeled TdT 5′ cDNA. Molecular sizes (in kbp) of human and mouse fragments hybridizing to this probe are shown on the right of the figure. The 11.8- and 4.3-kbp human fragments seen in lanes 1, 3, and 4 segregate with chromosome 10, and the 4.1- and 1.6-kbp fragments segregate independently of the other two bands as can be seen in lane 5.

chromosome, chromosome 10. *Pst* I-cleaved human DNA displayed three different fragments that hybridized to the 5′ TdT probe (>23, 10.0, and 7.5 kbp; Fig. 3, lane 2). The 10.0- and 7.5-kbp bands segregated together (as did the human *Hin*dIII 11.8- and 4.3-kbp bands; lanes 4–9) and were always found to correlate with the presence of human chromosome 10 in the hybrids.

Finally, to determine more precisely which part of the TdT cDNA has homology to the non-TdT sequences that segregate independently from chromosome 10 and to obtain an estimate of the minimal size of the *TdT* genomic locus, *Pst* I-cleaved parental and hybrid DNAs were hybridized to the TdT total coding region cDNA clone, pT223. As can be seen in Fig. 4 (lane 2, human DNA), the PT223 probe detects six bands, three of which are the >23-, 10-, and 7.5-kbp bands detected by the 5′ probe and three of which are the 18-, 8.5-, and 4.9-kbp bands detected by the 3′ probe. The hybrid DNAs represented in Fig. 4 are of three types, those that display all six *Pst* I bands (lanes 3, 4, and 8) and retain chromosome 10, those that display only the non-TdT, non-chromosome-10-linked >23-kbp band [lanes 5, 6, and 9 (lane 9 contains DNA from the same hybrid shown in lane 5 of Fig. 2)] and do not retain chromosome 10, and one that displayed only the five *Pst* I fragments [lane 7 (same hybrid seen in lane 3 of Fig. 2)] that are linked to chromosome 10. By summing the approximate molecular sizes of the five chromosome 10-linked *Pst* I fragments [18, 10, 8.5 (probably actually two unseparated fragments because this band is clearly heavier than the others), 7.5, and 4.9 kbp], it can be estimated that the *TdT* locus on chromosome 10 exceeds 50 kbp, while the non-chromosome-10-linked locus, which is homologous only to a part of the 5′ half of the TdT coding region, is >23 kbp. The chromosomal location of the non-chromosome-10-linked sequences that cross-hybridize with the 5′ half of the TdT cDNA is not yet precisely known. These sequences probably do not represent a *TdT* pseudogene because there is homology only with a part of the TdT coding region.

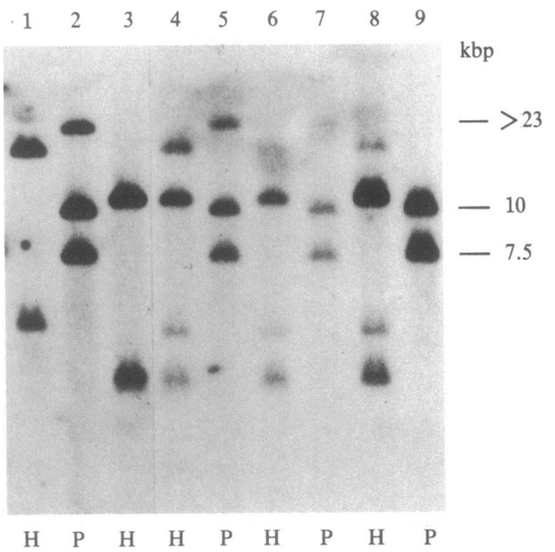

FIG. 3. Hybridization of the TdT 5′ cDNA clone to *Pst* I- or *Hin*dIII-cleaved hybrid and parental DNAs. Ten micrograms of cellular DNA from mouse (lane 1), human (lanes 2 and 3), hybrid A (lanes 4 and 5), hybrid B (lanes 6 and 7), and hybrid C (lanes 8 and 9) were cleaved with either *Hin*dIII (indicated by H below the lane) or *Pst* I (indicated by P below the lane), fractionated, transferred to nitrocellulose, and hybridized to ^{32}P-labeled TdT 5′ cDNA. Molecular sizes (in kbp) of human *Pst* I fragments that are detected with this probe are given on the right. Hybrids A, B, and C all retain chromosome 10 and two fragments detected by TdT 5′ cDNA; hybrid A also retains the >23-kbp fragment that segregates independently of chromosome 10.

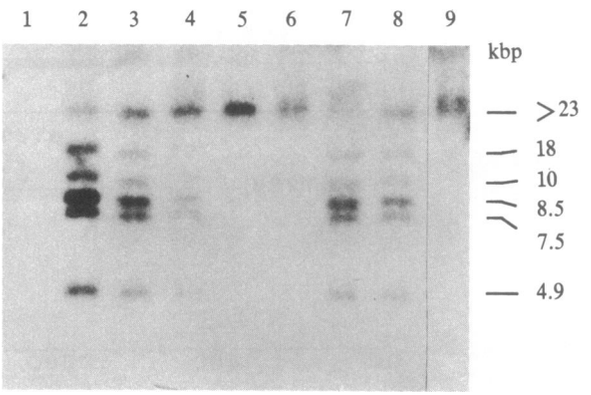

FIG. 4. Hybridization of the TdT total coding region cDNA clone to *Pst* I-cleaved hybrid and parental DNAs. Ten micrograms of cellular DNA from mouse (lane 1), human (lane 2), hybrid clone D retaining human chromosome 10 (lane 3), hybrid clone E retaining chromosome 10 (lane 4), hybrid clone F lacking human chromosome 10 (lane 5), hybrid clone G lacking human chromosome 10 (lane 6), hybrid clone H retaining human chromosome 10 (lane 7), hybrid clone I retaining human chromosome 10 (lane 8), and hybrid clone J lacking human chromosome 10 (lane 9), were processed as described in the legends to Figs. 2 and 3 and hybridized to ^{32}P-labeled pT223, the total TdT-coding region clone.

To recapitulate, after screening DNAs from 22 mouse–human hybrids retaining various subsets of human chromosomes, we have been able to assign the human *TdT* gene locus to chromosome 10; the chromosome correlation data are summarized in Table 1. The 5′ portion of the TdT coding region contains a region that is homologous to another locus in the human genome that is not located on chromosome 10.

Chromosome Localization of the Human *TdT* Gene by *In Situ* Hybridization. We have used the 3′ (pT17) ^{3}H-labeled human TdT cDNA clone in the plasmid pBR322 to screen human metaphase chromosomes by the *in situ* hybridization proce-

Table 1. Correlation of presence of TdT and specific human chromosomes in 22 mouse–human hybrids

Human chromosome	No. of hybrid clones, TdT/chromosome retention +/+	−/−	+/−	−/+	Number discordant
1	6	13	1	2	3
2	1	13	6	2	8
3	5	12	2	3	5
4	2	13	5	2	7
5	5	13	2	2	4
6	4	11	3	4	7
7	5	13	2	2	4
8	5	12	2	3	5
9	6	11	1	4	5
10	7	15	0	0	0
11	2	12	5	3	8
12	2	10	5	5	10
13	6	14	1	1	2
14	7	6	0	9	9
15	2	10	5	5	10
16	3	12	4	3	7
17	1	10	6	5	11
18	6	12	1	3	4
19	2	10	5	5	10
20	7	12	0	3	3
21	2	9	5	6	11
22	3	9	4	6	10
X	7	10	5	0	5

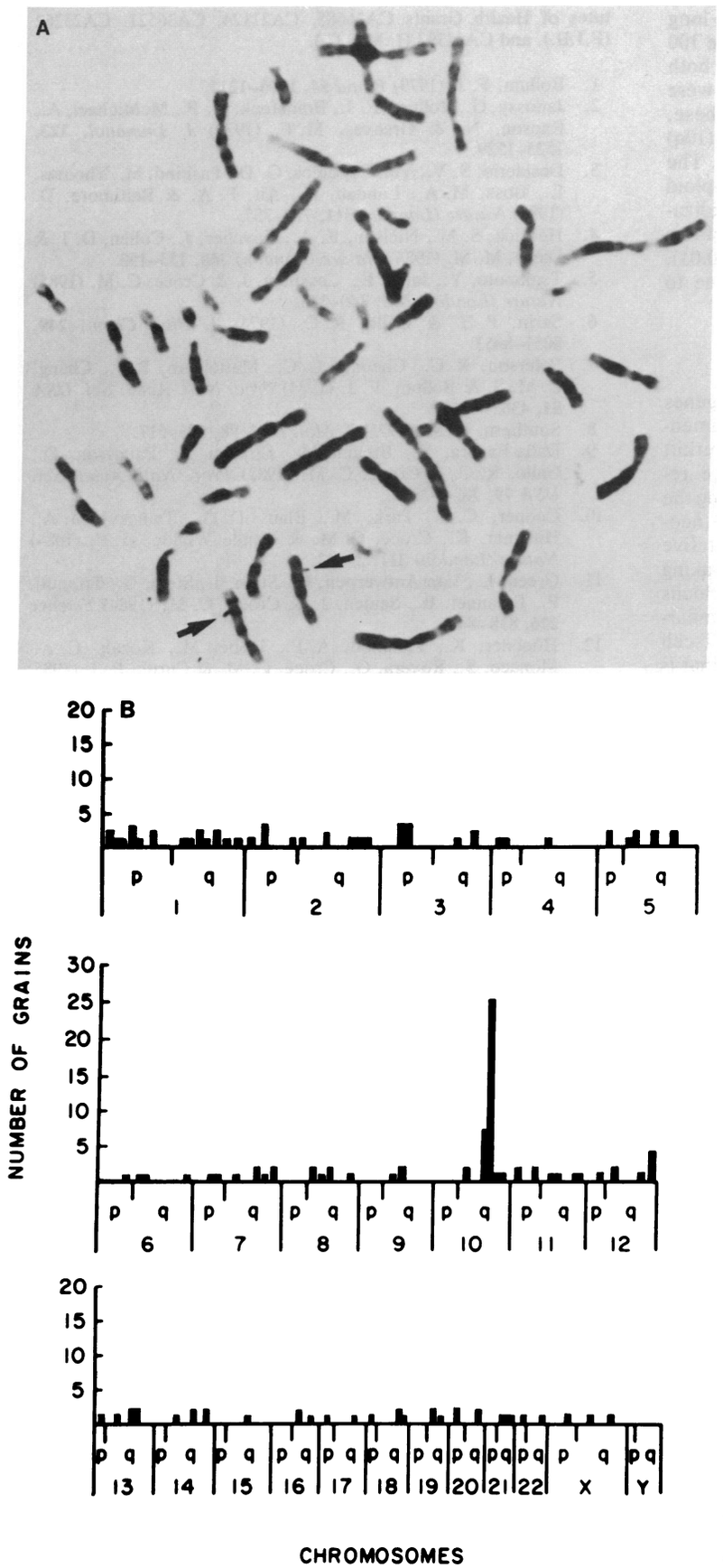

FIG. 5. Localization of the *TdT* gene in the human genome by *in situ* hybridization analysis. (*A*) Photograph of a G-banded lymphocyte metaphase spread hybridized with the human TdT probe pT17. Arrows indicate grains found in the distal portion of chromosome 10q. (*B*) Diagram showing the grain distribution in 100 metaphase chromosomes. Abscissa represents the chromosomes in their relative size proportion; ordinate shows the number of silver grains. The distribution of grains on 100 spreads was scored; 22% of all grains were located on the long arm of chromosome 10, with most grains at 10q24.

dure (14, 15). After autoradiography, the metaphase chromosomes were scored for grain localization. An example is shown in Fig. 5*A*. The distribution of labeled sites in 100 metaphase chromosomes is shown in Fig. 5*B*, where the ordinate represents the number of labeled sites and the abscissa represents the chromosome regions that were ex-

amined. As shown in Fig. 5*B*, the distal portion of the long arm of chromosome 10 was significantly labeled. Of the 100 cells examined, 33 (33%) exhibited labeling of one or both chromosomes 10 (Fig. 5*B*). About 22% of all grains were located on the long arm of chromosome 10 (Fig. 5*B*). Of these, ≈91% of the grains on the long arm of chromosome 10 (10q) were on region 10q23→q25, with most grains at 10q24. The long arm of chromosome 10 represents ≈3.22% of the haploid genome. Thus, our observation that >20% of the hybridization with the pT17 probe is localized on the distal half of this region of chromosome 10 is highly significant ($P << 0.01$). Thus, cytological hybridization localizes the TdT gene to region q23→q25 of human chromosome 10.

DISCUSSION

The chromosome localization of the immunoglobulin genes (16–18) and of cellular oncogenes (9, 19) has been instrumental to our understanding of the molecular basis of Burkitt lymphoma and Burkitt-type leukemia (20, 21). Since rearrangements involving the chromosomal region carrying the heavy-chain locus have also been observed in B-cell neoplasms of adults, it has also been possible to isolate putative human oncogenes involved in these malignancies by taking advantage of their close proximity to the heavy-chain locus due to specific chromosome translocations (21, 22). Similarly, our finding that the locus of the α chain of the T-cell receptor is located in a region of chromosome 14 (13) that is frequently involved in inversion and translocations in human T-cell neoplasms (23) suggests that this locus may have an important role in T-cell neoplasia. Since the *TdT* gene is extremely active in pre-B and pre-T cells, it might have a role in oncogene activation in human hematopoietic malignancies expressing increased levels of terminal transferase. Therefore, it was important to establish the chromosome location of the *TdT* gene in order to determine whether this chromosome region is involved in rearrangements in human hematopoietic malignancies.

The results of these studies indicate that the *TdT* gene is located on region q23→q25 of human chromosome 10. This finding might be of interest because recent reports describe rearrangements of this human chromosome in human T-cell neoplasms (23, 24). Analysis of these malignancies for rearrangements within or in proximity to the *TdT* locus should provide an answer to the question of whether the *TdT* locus plays a role in human leukemogenesis.

We thank Wendy Scattergood for skillful technical assistance and Kathy Reinersmann for preparation of the manuscript; we are also grateful to Josephine Romano and Dr. Pasquale Tripputi for some hybrid DNA samples. This work was supported by National Institutes of Health Grants CA16685, CA21124, CA36521, CA23262 (F.J.B.), and CA31393 (L.M.S.C.).

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