Localization of the β-Globin Gene by Chromosomal In Situ Hybridization

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SUMMARY

A 3.7-kilobase (kb) genomic clone of the human β-globin gene, including 1.5-kb upstream and approximately 0.5-kb downstream, was utilized in chromosomal in situ hybridization for precise mapping of the β-globin locus on peripheral blood lymphocyte-derived metaphases from a normal male, and for further evaluation of a clonal t(7;11)(q22;p15) translocation on bone marrow-derived metaphases from a 46-year-old male with erythroleukemia. Analyses of 205 midmetaphases from a normal male hybridized with the tritium-labeled β-globin probe and stained with quinacrine mustard dihydrochloride revealed approximately 12% of spreads to have silver-grain deposition over the p15 band of chromosome 11. Of the 365 silver grains observed to be located on or beside chromosomes, 25 (~7%) grains were localized in band p15. Karyotype analysis of a bone marrow specimen from the patient with erythroleukemia revealed hypodiploidy with various unidentified marker chromosomes as well as a presumably balanced translocation between 7q and 11p. Chromosomal in situ hybridization showed localization of silver grains at the junction between chromosomes 7 and 11 as well as to the normal chromosome 11, indicating that the β-globin locus had not been translocated in the chromosomal rearrangement. This case demonstrates the value of chromosomal in situ hybridization in the definition of chromosome rearrangements and provides further evidence for the localization of the β-globin gene to 11p15.

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The β-globin gene cluster has been assigned previously to several different human chromosomes including 2, 4, 5, and 11 ([1–16] and R. E. Magenis, D. Tomar, and T. A. Donlon, personal communication, 1983) by various mapping techniques including in situ hybridization, somatic cell hybrid studies, and chromosome sorting with DNA restriction enzyme analysis. In recent years, the assignment to 11p was confirmed; however, the regional assignment has been controversial.

The development of an improved technique of chromosomal in situ hybridization [16] utilizing 10% dextran sulfate to accelerate the rate of hybridization in addition to probes nick-translated to high specific activity with tritiated nucleotides has permitted the precise chromosomal assignment of multiple single- and low-copy gene sequences ([12–34] and R. E. Magenis, D. Tomar, and T. A. Donlon, personal communication, 1983). Furthermore, this technology has aided in the identification of gene sequences involved in chromosomal rearrangements and amplified regions [35–38]. It is now possible to use this technology to make predictions regarding gene sequences that may be involved in characteristic trans-

Fig. 1.—GTG-banded direct bone marrow preparation from a human male with erythroleukemia. Karyotype analysis revealed hypodiploidy, various unidentified marker chromosomes and double minutes, and a presumably balanced rearrangement between 7q and 11p, designated t(7;11)(q22; p15). The arrow points to the derivative chromosome 11.
locations as well as to gain information about the orientation (3' or 5') of these gene sequences with respect to translocation breakpoints.

Recent karyotype analysis in our laboratory of a direct bone marrow preparation from a 46-year-old male with erythroleukemia revealed a clonal t(7;11)(q22; p15) in addition to hypodiploidy and various marker chromosomes (fig. 1). Because of the demonstration of the involvement of immunoglobulin gene loci in the chromosomal translocations characteristic of malignant B-cell tumors [23, 39–42], we investigated the localization of the β-globin gene in relation to the translocation breakpoint in the malignant cells of this affected individual in comparison to the localization of the same probe in karyotypes derived from the lymphocytes of a normal male. Previous karyotype analyses of chromosome preparations from individuals with erythroleukemia have not suggested any characteristic rearrangements, although hypodiploidy and various unidentified marker chromosomes have been reported [43]. Further details of our karyotypic findings in this patient and in another patient with erythroleukemia will be reported elsewhere (manuscript in preparation).

MATERIALS AND METHODS

Radiolabeling of the β-Globin Probe

The recombinant plasmid used in the hybridization was a 3.7-kb genomic clone of the human β-globin gene that included 1.5 kb of 5' and approximately 0.5 kb of 3'-flanking sequences [44]. The probe was radiolabeled to a specific activity of 1.2 × 10^7 cpmp/µg with tritium by nick-translation using all four tritiated nucleotides as described [20].

Preparation of Midmetaphase Chromosomes

Metaphase chromosomes were obtained from peripheral blood lymphocyte cultures established from a normal human male according to a modification of Moorhead et al. [45]. Mitotic chromosome spreads from a direct bone marrow preparation were made from an adult male with erythroleukemia after a 15-min colcemid collection in RPMI 1640 medium (Gibco, Grand Island, N.Y.) at 37°C. Karyotype analysis of the bone marrow preparations by GTG-banding [46] revealed hypodiploidy with various marker chromosomes in addition to a presumably balanced clonal chromosome translocation: t(7;11)(q22;p15). All chromosome slides were aged for at least 2 months at room temperature prior to in situ hybridization.

In Situ Hybridization

In situ hybridization of the β-globin probe was performed as described [20] and is a modification of the procedure of Harper and Saunders [16]. Chromosome preparations were denatured in 70% (v/v) formamide in 2 × SSC (0.3 M NaCl-0.03 M sodium citrate), pH 7.0, at 70°C for 2 min and dehydrated in an ethanol series (70%, 80%, 90%, and absolute ethanol) for 2 min each. The tritium-labeled β-globin probe was denatured by heating at 70°C for 5 min at a concentration of 0.1 µg/ml in the hybridization solution, which consisted of 50% formamide in a 0.3 M NaCl-0.03 M sodium citrate-0.04 M sodium phosphate, pH 6.0, solution with 10% dextran sulfate (Pharmacia, Piscataway, N.J.) and 100 µg/ml sonicated herring sperm DNA. Hybridization was overnight (16–18 hrs) at 37°C. Slides were washed three times in 50% formamide in 2 × SSC, pH 7.0, at 39°C–40°C and five times in 2 × SSC, pH 7.0, at 39°C–40°C prior to dehydration in an ethanol series (70%, 80%, and 95%). The slides were then exposed to Kodak NTB2 nuclear-track emulsion for 18 days at 4°C and developed in Kodak D-19 developer at 15°C for 4 min.
Fig. 2.—Metaphase chromosome spreads from a normal human male hybridized with the β-globin probe. A, Chromosomes from peripheral blood lymphocytes stained with quinacrine mustard dihydrochloride and visualized under incident ultraviolet light. B, The same metaphase spread visualized under a combination of incident ultraviolet and transmitted visible light showing a silver grain at 11p15.

After fixation slides were rinsed extensively and dried at room temperature prior to staining in 0.005% quinacrine mustard dihydrochloride. Visualization of stained preparations was performed using incident ultraviolet light and a combination of incident ultraviolet and transmitted visible light that permitted data collection to be performed at the microscope. Silver-grain deposition was recorded with reference to a standard human chromosome idiogram at the 400 band stage [48].

RESULTS

Grain Distribution in Karyotypes from a Normal Human Male

Analyses of 205 metaphase preparations from a normal male hybridized with the tritium-labeled β-globin probe revealed approximately 12% of spreads analyzed to have silver-grain deposition either on or beside the p15 band of at least one chromosome 11 (fig. 2). Of the 365 silver grains observed to be located on or beside chromosomes, 25 (~7%) grains were localized in band p15 (fig. 3). Between one and five grains were present on or beside chromosomes per metaphase with an equivalent or lower background count of grains not located near any chromosome.
A secondary peak of hybridization was seen at 11p13, which represented approximately 3\% of all grains scored. Whether this secondary peak, which has almost twice the number of grains present at any site other than 11p15, represents true homology or a sampling artifact remains to be determined. Statistical analysis of these data by the Poisson distribution with the number of silver grains per chromosome adjusted for relative band length in a 400-band stage chromosome idiogram [48] revealed a highly significant distribution at 11p15 and 11p13 ($P < 10^{-3}$).

**Grain Distribution in Karyotypes from a Human Male with Erythroleukemia**

Seventeen metaphases selected for moderate to good chromosome morphology from a direct bone marrow preparation hybridized with the tritium-labeled β-globin probe were examined to determine whether hybridization was present on either the normal chromosome 11, the derivative chromosome 11, or the derivative chromosome 7. Silver grains were localized over the distal end of the short arm of the normal chromosome 11 in four metaphase spreads and in the region corresponding to the junction of chromosomes 7 and 11, in bands 7q22 and 11p15, in four metaphases (fig. 4). No hybridization was detected to the derivative chromosome 7.
Our assignment of the β-globin gene to 11p15 places this gene in the same chromosome band as the human insulin gene, which also has been localized to metaphase chromosomes by in situ hybridization [15, 18, 33, 34]. Furthermore, the c-Ha-ras-1 oncogene has been mapped to 11p14.1 on germline chromosomes [30] and 11p15 using both metaphase chromosomes [33] and gene-dosage determinations from patients with del(11)(p13)-associated predisposition to Wilms tumor, aniridia, genitourinary abnormalities, and mental retardation [48]. Additionally, in studies of somatic cell hybrids and linkage analyses, the parathyroid hormone gene has been assigned to chromosome 11 [49, 50]. Because DNA polymorphisms have been identified for these four genes, linkage analyses have been performed that are consistent with the assignment of the β-globin, insulin, and c-Ha-ras-1 genes to the distal end of 11p and suggest that the β-globin gene may be the most proximal locus of this group [33, 51].

**Fig. 4.** Metaphase spreads from a human male with erythroleukemia hybridized with the β-globin probe. A and C, Chromosomes from a direct bone marrow preparation stained with quinacrine mustard dihydrochloride and visualized under incident ultraviolet light. B, The same metaphase spread as (A) visualized under a combination of incident ultraviolet and transmitted visible light showing a silver grain in the region corresponding to the junction between chromosomes 7 and 11 as well as background grains over several chromosomes. D, The same metaphase spread as (C) under combination lighting showing a silver grain at 11p15.
Although our in situ assignment is in disagreement with an earlier in situ localization [13] that suggested a more proximal localization on 11p, it is in accord with the published photographs of in situ hybridization performed in a family study where the proposita had a deletion of the entire β-globin cluster [14] and a concomitant reduction in the number of silver grains localized on 11p in comparison to the parental hybridization frequencies. Additionally, studies of normal individuals ([15] and R. E. Magenis, D. Tomar, and T. A. Donlon, personal communication, 1983) and a patient with the aniridia-Wilms tumor syndrome who had an interstitial deletion of 11p11.3→15.1 demonstrated silver-grain deposition in 11p15, and in the latter case, excluded the β-globin gene from 11p11.3→15.1 (R. E. Magenis, personal communication, 1983).

Our analysis of the β-globin gene in situ hybridization in the t(7;11) karyotype has indicated that the β-globin locus has not been translocated to the 7q—chromosome. Whether this translocation, which may potentially involve the β-globin locus, will be found in other karyotypic analyses of individuals with erythroleukemia remains to be determined. However, the finding of an 11p15 chromosome rearrangement in the malignant cells of a patient with erythroleukemia in view of the link between the characteristic translocations involving immunoglobulin loci and the c-myc gene in Burkitt lymphoma [23, 39–42] is intriguing. Further investigation of this translocation and its possible relationship to the c-erb-B oncogene, which has been localized to chromosome 7 [52], is in progress.

The finding of a secondary peak of hybridization at 11p13 may indicate the presence of sequences homologous to β-globin at this site, but no other evidence supports this hypothesis because all sequences with homology to this locus are thought to have been cloned and to be within the β-globin gene cluster. This peak is of interest, however, as it is close to a previous assignment of the β-globin locus to 11p1205→1208 by studies of X-irradiation-induced deletions of 11p in Chinese hamster-human somatic cell hybrids [11] and assignment near the centromere of 11p by chromosomal in situ hybridization [13]. The possibility that this observation is an artifact of our in situ procedure remains to be determined as secondary peaks of hybridization have been detected in chromosomal in situ analyses of other probes (R. T., manuscript in preparation; and I. R. K., J. V. Ravetch, C. C. M., P. Hieter, G. F. Hollis, K. Nakahara, and P. Leder, manuscript in preparation). Additionally, we cannot exclude the possibility of a preference for the assignment of grains to light-staining regions; however, this observation and the data collection were performed independently by two investigators. This observation may also be due in part to differential chromosome contraction and/or packing.

Chromosomal in situ hybridization has become a powerful new technique in the precise localization of gene sequences. Our study has demonstrated the usefulness of this technique in the assignment of the β-globin gene to 11p15 in a normal human male and in a patient with a rearrangement of chromosome 11. Furthermore, we have demonstrated the value of chromosomal rearrangements in confirmation of gene assignments by in situ hybridization and the usefulness of this technology in the definition of chromosomal rearrangements.
LOCALIZATION OF THE β-GLOBIN GENE

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