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The gene for human thioredoxin maps on the short arm of chromosome 3 at bands 3p11-p12

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Thioredoxin, a ubiquitous enzyme possessing an oxidoreductase activity, has recently been cloned in human. Using in situ chromosomal hybridization with a human thioredoxin cDNA probe, we have precisely localized the thioredoxin gene on chromosome 3 at bands 3p11-p12.

Thioredoxin; Gene mapping; (Human)

1. INTRODUCTION

Thioredoxin is the best representative enzyme of a group of proteins, widely distributed and possessing a dithiol-disulfide oxidoreductase activity [1,2]. This protein of 12000 Da is ubiquitous and found in many organisms, from plants and bacteria to mammalians. It has been identified originally in *Escherichia coli* as a hydrogen donor for ribonucleotide reductase and deoxyribonucleotide synthesis [1]. In higher organisms, the thioredoxin system (which includes NADPH as a proton donor, thioredoxin reductase and thioredoxin) seems to participate as a general dithiol-disulfide oxidoreductase in the cells. Multiple substrates for thioredoxin have been identified so far, such as ribonuclease [3], glucocorticoid receptor [4], and insulin [5]. Recently, a factor called acute T leukemia derived factor (ADF), almost identical to human thioredoxin, has been described [6]. It has been reported that ADF has an immunological role, such as induction of the p55 Tac interleukin-2 receptor subunit on a natural killer cell line called YT [6]. In the present study, using in situ hybridization on human prometaphase cells with a human thioredoxin (HTR) cDNA previously obtained in our laboratory [7], we report that that HTR gene is located on the short arm of chromosome 3 at bands p11–p12.

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Abbreviation: HTR, human thioredoxin

2. MATERIALS AND METHODS

DNA probe: cDNA clones encoding for human thioredoxin were isolated from the 3B6 EBV lymphoblastoid B cell line [7]. The HTR cDNA probe used for both Northern blot analysis and in situ hybridization was an *Eco*RI-*Dra*I 0.42 kb fragment excised from the PGEM3-HTR recombinant plasmid established by E.E. Wollman (fig.1a).

Northern blot analysis was performed as described previously [7]. Total RNA was extracted from the 3B6 lymphoblastoid B cell line, transferred on a nylon filter and hybridized with the HTR probe.

In situ hybridization was performed according to the procedure described by Lafage et al. [8]. The DNA probe was tritium labeled to a specific activity of 2×10^7 cpm/μg and hybridized on normal human male prometaphase spreads at a final concentration of 50 ng per ml of hybridization buffer. Autoradiographs were exposed at 4°C for 15 days.

3. RESULTS AND DISCUSSION

In order to ensure the specificity of our HTR

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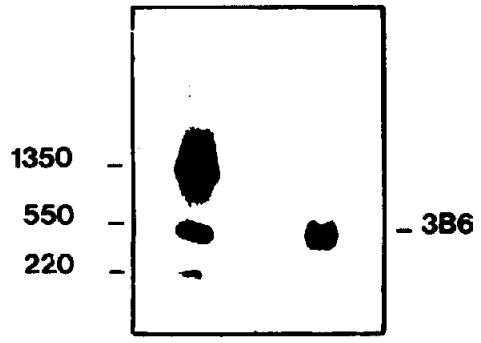

Fig. 1 b

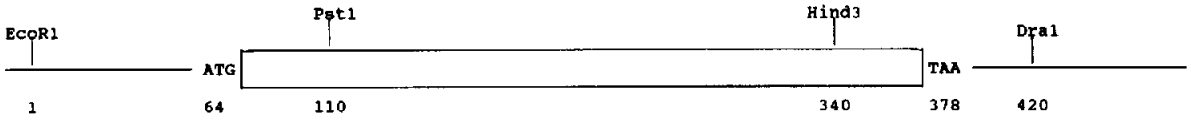

Fig.1b: Restriction map of the HTR gene. The coding region is boxed and restriction sites are shown with their base pair assignments. Fig.1a: Northern blot analysis of the 3B6 cell line with the HTR probe. First lane, RNA size markers synthesized by the SP6 RNA polymerase; second lane, RNA extracted from the 3B6 cell line.

probe, we performed a Northern blot analysis on total RNA extracted from the 3B6 lymphoblastoid B cell line (fig.1b) and we detected the single HTR mRNA species at the 550 base level [7].

Results of in situ hybridization with the HTR cDNA probe are shown in figs 2 and 3. 58 prometaphase cells were analyzed for silver grains associated with chromosomes. A total of 122 silver grains was scored (i.e., an average of 2.1 grains per cell), and 25 grains (20.5% of the total grains) were found to be associated with chromosome 3 (χ^2, $p < 10\text{-}8$) [9]. Of the 25 silver grains associated with chromosome 3, 18 grains (72%) mapped to region 3p1, the proximal region of the short arm of chromosome 3. More precisely, the maximal number of silver grains was observed at the 3p11-p12 bands (figs 3 and 4). There were no other highly significant clusters of silver grains in the other chromosomal regions (fig. 2). Consequently, we assigned the human thioredoxin gene to chromosome 3 at bands p11-p12.

Several genes have been mapped in a chromosomal region encompassing bands 3p11-p12 [10] such as the β_1-galactosidase gene at region 3p21-cen [11,12], the glutathione peroxidase 1 gene at region 3p13-q12 [11,13], and the protein

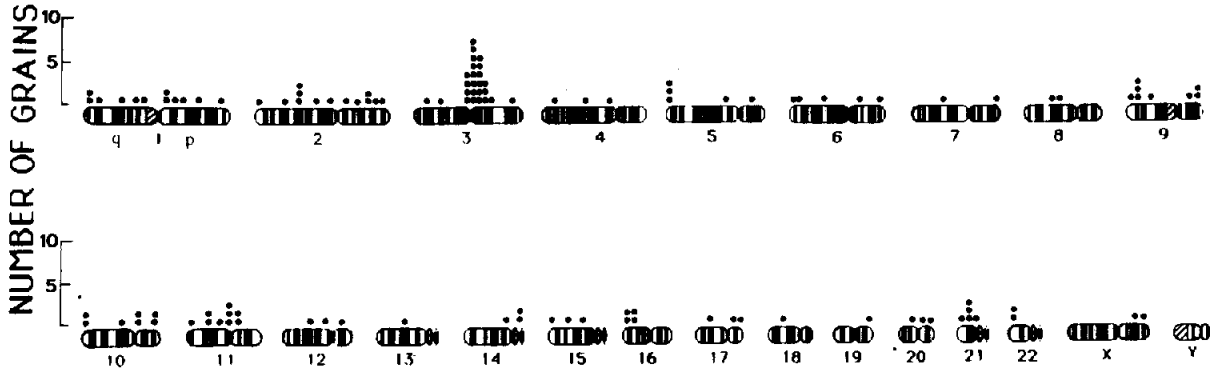

Fig.2. Distribution of silver grains scored over all chromosomes in 58 prometaphases after in situ hybridization with the HTR probe.

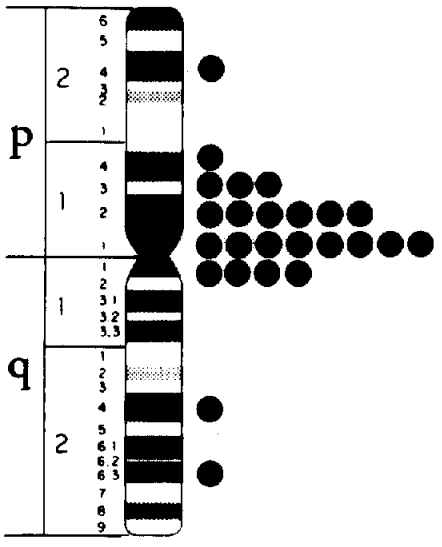

Fig.3. Diagram of G-banded chromosome 3 showing the detailed distribution of labeled sites.

S gene at region 3p11.1–q11.2 [14]. But so far, the HTR gene is the most precise gene marker of the 3p11–p12 region.

Moreover, chromosomal clonal abnormalities involving this region have been described [15] in some breast cancers [16–19] and in some cutaneous T cell lymphomas [20,21]. Whether the HTR gene is involved in these malignancies remains to be investigated.

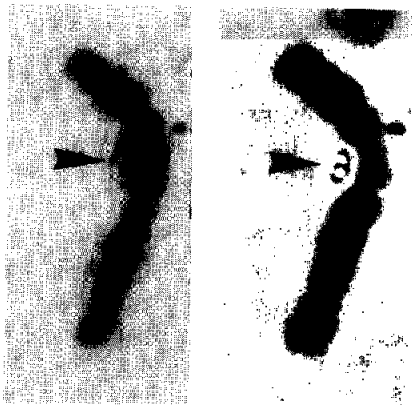

Fig.4. Partial representative prometaphase showing the specific location of the HTR gene on chromosome 3. Arrowheads indicate silver grains. Left, Giemsa staining; right, subsequent R-banding.

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