

Chromosomal localization of the human Thy-1 gene

(recombinant DNA/*in situ* hybridization/chromosomal hybrids)

JEANNE VAN RIJS*, VINCENT GIGUÈRE*, JACKY HURST*, TON VAN AGTHOVEN†, AD GEURTS VAN KESSEL†, SANNA GOYERT*‡, AND FRANK GROSVELD*§

*Laboratory of Gene Structure and Expression, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, United Kingdom; and
†Erasmus University, Postbox 1738, 3000 DR Rotterdam, The Netherlands

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ABSTRACT We have isolated the gene coding for human Thy-1. Introduction of this gene into HeLa cells by DNA-mediated transfer results in the expression of Thy-1 antigen on the cell surface. Chromosomal mapping of the Thy-1 gene by hybridization to metaphase chromosomes and Southern blots of DNA from hybrid cells indicate that the Thy-1 gene is located on the long arm of chromosome 11.

The Thy-1 antigen was first demonstrated by Reif and Allen (1) to be present on murine thymocytes, on T lymphocytes, and on neuronal cells. It has since been found in many species, including man and species as distantly related as squid (2). Its expression pattern varies in different species with the exception that it is always expressed in brain (2). There is no clear concept of the physiological role of Thy-1 (3–7), but it has been suggested that the Thy-1 gene represents a primordial domain from which the Ig superfamily of genes has been derived (2). The molecular weight of the Thy-1 antigen is 25,000 as determined by NaDodSO₄/PAGE, of which 30% is due to carbohydrate (8, 9). Recently, some of the Thy-1 genes have been cloned: the rat Thy-1 (10) and the mouse Thy-1.2 and Thy-1.1 (11, 30). In the two latter cases, the gene has been expressed in different cell types (ref. 11 and unpublished work). In this paper, we describe the cloning, expression, and chromosomal localization of the human Thy-1 gene.

MATERIALS AND METHODS

Thy-1 Recombinant Clones. A recombinant cosmid library was constructed from blood cell DNA by use of the vector pTCF according to standard procedures (12, 13). Colonies (600,000) were grown on four 22 × 22 cm nitrocellulose filters and processed for screening as described (14). Five positive colonies were detected after hybridization with a rat Thy-1 cDNA probe and colony-purified (GSE 505–509).

DNA-Mediated Transfection. The Thy-1 gene was inserted as an 8.2-kilobase (kb) *Eco*RI fragment into the expression vector pBSV (15). The resulting recombinant clone pBSHT1 was transferred into HeLa cells by the calcium phosphate coprecipitation procedure (16) followed by a glycerol shock (17). After 2 days in culture, the cells were scraped off the plates and analyzed for the presence of Thy-1 antigen. HeLa cells (2.5×10^5) or CHP100 (18, 19) cells (0.7×10^5) were used in a standard assay in microtiter wells with a mouse monoclonal antibody to human Thy-1 (gift of J. Fabre; ref. 20), a rabbit anti-mouse Ig (gift of B. Thomas), and ¹²⁵I-labeled protein A. The results are expressed as mean cpm per well

after subtraction of the background (same assay minus the mouse anti-human Thy-1 antibody).

In Situ Hybridization. Chromosomes were obtained from peripheral blood cells and prepared according to standard procedures (21) and hybridized according to Bartram *et al.* (22). RNase-treated chromosomes were denatured in 70% (vol/vol) formamide/2× NaCl/Cit (1× is 0.15 M NaCl/15 mM sodium citrate, pH 7) at 70°C for 2 min, rinsed three times in 2× NaCl/Cit, and dehydrated in ethanol. The Thy-1 *Bgl* II fragment was ³H-labeled by nick-translation to a specific activity of 10⁸ cpm/μg. The probe (2.5 ng per slide) was denatured at 70°C for 5 min in 1.5 M NaCl/0.15 M sodium citrate/0.2 M sodium phosphate/10% (wt/vol) dextran sulfate, pH 6.0, with a 1000-fold excess of denatured salmon sperm DNA, added (40 μl/22 × 40 mm coverslip) to the slides, and allowed to hybridize for 18 hr at 37°C. Slides were rinsed three times for 15 min in 50% formamide/2× NaCl/Cit at 39°C, five times in 2× NaCl/Cit at 39°C, and twice for 2 hr in 2× NaCl/Cit at room temperature. After dehydration in ethanol, the slides were dipped in Ilford (Warrington, PA) nuclear emulsion K2, exposed for 21 days, and developed. The specimens then were heat-denatured and reverse-banded with acridine orange. Grains then were correlated to chromosome bands directly by use of UV-light and normal-light exposures of the same metaphases in order to show the chromosomal bands and the grains, respectively.

Human Hybrid Cell Lines. The human–hamster a23 and e36 hybrid cell lines have been described (22). The human–mouse cell lines are described in ref. 23. All cell lines were analyzed for chromosomal content by identification of the chromosomes in at least 10 metaphase spreads (21). M11-X DNA was a gift from T. Ley (National Institutes of Health) and was isolated from a Friend cell line containing a single hybrid Xq-11p human chromosome. DNA (10 μg) from each of the hybrid cell lines was digested with *Bgl* II, using bacteriophage λ DNA as an internal control marker. The digested DNA was electrophoresed in 0.7% agarose gel, Southern blotted, and hybridized by standard procedures (24, 25).

RESULTS

Thy-1 Gene Cloning and Expression. The rat Thy-1 cDNA (10) was used to screen a human blood DNA library constructed with the cosmid pTCF (12). Five positive colonies were detected and purified by rescreening, and the DNA from each was isolated (14). Standard double-digestion restriction analysis resulted in the map shown in Fig. 1. It shows the two cosmids that contain the most flanking DNA

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Abbreviation: kb, kilobase(s).

‡Present address: Hospital for Joint Diseases, 301 East 17th Street, New York, NY 10010.

§To whom reprint requests should be addressed.

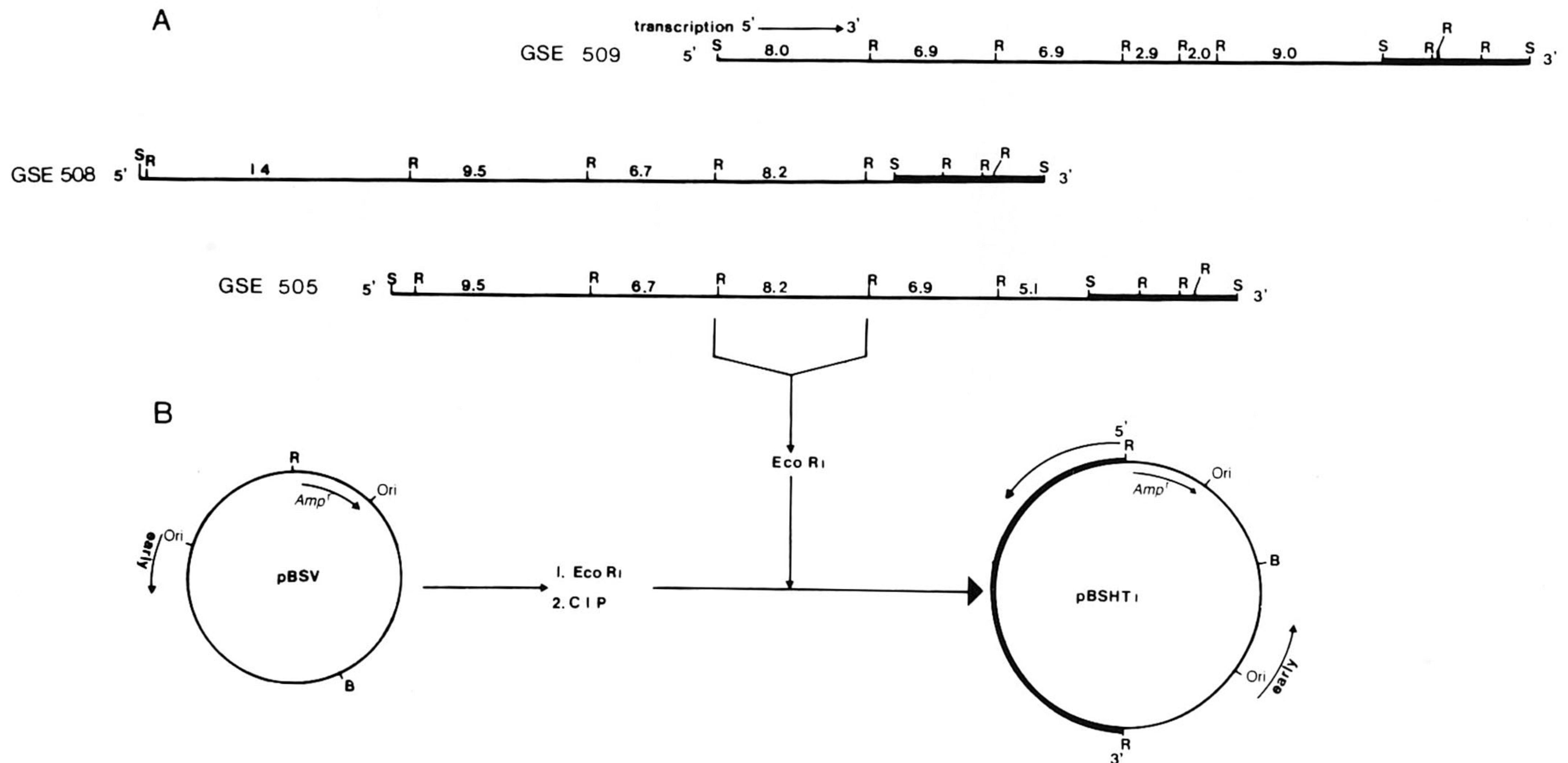


FIG. 1. (A) Restriction map of three cosmids containing the human Thy-1 genes. Thick lines represent the vector pTCF (12). Restriction sites: S, *Sal* I; E, *Eco*RI. Sizes of restriction fragments are given in kb. (B) Insertion of the 8.2-kb *Eco*RI fragment containing the human Thy-1 gene into the expression vector pBSV (15). Restriction sites: B, *Bam*HI; R, *Eco*RI. CIP, Calf intestinal phosphatase; Ori, origin; Amp^r, ampicillin-resistance gene.

in the 5' and 3' directions, respectively (GSE 509 and 508), and one of the remaining three overlapping cosmids (GSE 505). The Thy-1 gene is located on the central 8.2-kb *Eco*RI fragment, which is identical in size to the fragment identified by Southern blot analysis of total human DNA (not shown). Cross-blot hybridization and restriction enzyme fine mapping of the 8.2-kb *Eco*RI fragment showed that the human gene is highly homologous to the murine gene. It has the same organization as the mouse Thy-1 gene (30); i.e., it has four exons, rather than the published three exons (26), which correspond approximately to the functional domains of the protein, similar to the other members of the Ig superfamily (27). The exception is the 5' untranslated region of the mRNA, which is encoded by exon 1 and part of exon 2 (which also encodes the signal peptide). Cross-blot hybridizations between the complete human cosmid and the murine equivalent show that the 5' 6.7-kb and 3' 6.9-kb flanking fragments are homologous to the corresponding murine fragments. This indicates that a region larger than just the gene has been conserved during evolution, in agreement with existing protein data which indicate a high degree of conservation for the Thy-1 locus (2). The 8.2-kb fragment was subcloned in the expression vector pBSV (15), and the recombinant pBSHT1 was introduced into HeLa cells by calcium phosphate precipitation (16, 17). After 2 days, the cells were analyzed for the expression of Thy-1 antigen, using a mouse monoclonal antibody against human Thy-1 (20). Table 1 shows that the transfected HeLa cells are Thy-1-positive compared to untransfected HeLa cells and Thy-1-positive neuroblastoma cell line (CHP100) (18, 19). Consequently, both the hybridization and expression data indicate that the complete Thy-1 gene is present on the 8.2-kb *Eco*RI fragment.

Chromosomal Localization. To be able to map the Thy-1 gene to a particular chromosome, we used total human DNA as a probe and different restriction enzymes to localize unique and repetitive sequences on the 8.2-kb *Eco*RI fragment (data not shown). This allowed us to isolate a unique 3.2-kb *Bgl* II fragment which was used in two different mapping procedures: first, hybridization to Southern blots of

DNA isolated from a panel of human-hamster (a23 and e36) cell lines (22) and, second, *in situ* hybridization to fixed metaphase chromosomes. The hybridization to DNA from the panel of human-hamster (a23 and e36) cell hybrids showed, in addition to cross-hybridization with the hamster Thy-1 gene, a positive signal with the human Thy-1 gene in hybrids 16CB-18, E36-4.1.5, 17CB-15B, and 17CB-8B, when compared to the hamster (3.8-kb) and human (3.2-kb) control samples (Fig. 2, lanes 1, 2, 6, 8, 11, and 12, respectively). The human chromosome complement of these lines indicates that the Thy-1 gene must, therefore, be located on chromosome 11 (Table 2). Further Southern blot hybridization to DNA from a set of human-mouse hybrid cells containing a complex Philadelphia chromosome translocation (9q+;11q-;22q-) (23) showed the Thy-1-positive signal with hybrid WEGL1-3. This hybrid has the translocated 9q+ chromosome which contains the q12-qter region of chromosome 11. The other hybrids that lack chromosome 11 or contain the short arm (+11p) are negative. Consequently, the Thy-1 gene must be located on the long arm of chromosome 11. In the second procedure, we used the same Thy-1 DNA probe for *in situ*

Table 1. Expression of Thy-1 antigen

	¹²⁵ I-labeled protein A bound, cpm		
	Transfected HeLa	Nontransfected HeLa	CHP100
Mouse anti-human Thy-1	50	80	80
Rabbit anti-mouse Ig	—	140	100
Mouse anti-human Thy-1 plus rabbit anti-mouse Ig	814	180	580

In each assay, 2.5×10^5 HeLa cells, either nontransfected or transfected with pBSHT1, were incubated with a mouse anti-human Thy-1 monoclonal antibody, a rabbit anti-mouse Ig antibody, or a combination of both. Thy-1 expression was measured by ¹²⁵I-labeled protein A binding, given as an average ($n = 3$) cpm after subtraction of background (500 cpm). Human CHP100 cells (18, 19) were used as the positive control (0.7×10^5 cells, background 300 cpm).

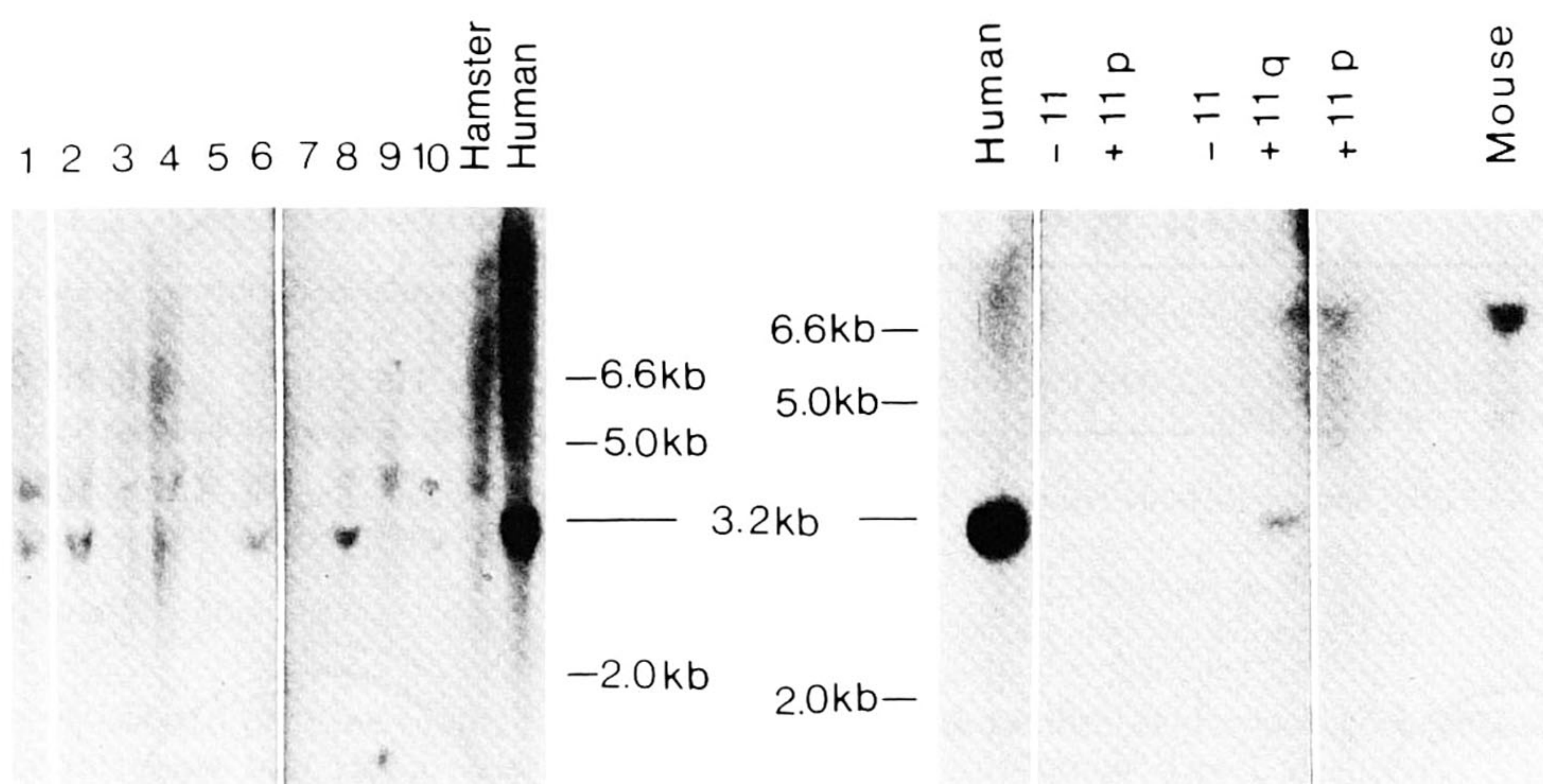


FIG. 2. Southern blot hybridization of a human Thy-1 probe to *Bgl* II-digested DNA from human-hamster cell hybrids. (A) The 3.2-kb *Bgl* II fragment was 32 P-labeled by nick-translation and hybridized to a blot containing DNA from hybrids 16CB18 (lane 1, overexposure of lane 10), E36-4.1.5 (lane 2), E36-2.9.5 (lane 3), 17CB3B (lane 4), 17CB2C (lane 5), 17CB15B (lane 6), 17CB8B (lane 8), and 16CB17 (lane 9), and from hamster (lane 1) and human (lane 12). See Table 1 for the human chromosomal complement in each hybrid. *Hind*III-digested λ DNA was used as size markers. (B) Hybridization as in A to DNA from the hybrids WESP-2A (-11), WEGL1-11 (+11p), WEGL1-9 (-11), WEGL1-3 (+11q), and M11-X (+11p) and from human and mouse.

hybridization to fixed metaphase chromosomes obtained from cultured blood cells prepared as described by Hagemeijer *et al.* (21). The probe was 3 H-labeled by nick-translation and hybridized to the RNase-treated and denatured chromosomes (22). After autoradiography and staining of the chromosomes (Fig. 3), 180 grains were counted on chromosomes. Significantly, 43, rather than random 9–10, of these were located on chromosome 11 ($P < 0.001$), and 25 of these grains were found near the tip of the q arm at position q23–q24 (Fig. 3). The 6 grains found at the tip of the short arm of chromosome 11 probably represent an artifact, since we have found a similar result with different probes (unpublished data). We conclude that the human Thy-1 gene is located on the long arm of chromosome 11, within position q23–q24.

DISCUSSION

The data show that we have isolated the gene coding for the human Thy-1 antigen by using cosmid recombinants. Although the 5' end of the human gene has not been localized

precisely, it is present on the 8.2-kb *Eco*RI fragment as shown by the expression data (Table 2) and mRNA analysis (not shown). However, preliminary data indicate that the exon–intron structure is different from the published gene organization (11) and that the gene contains at least four, rather than three, exons (unpublished results). Cross-hybridization with the murine gene shows a high degree of homology, including the 5' and 3' flanking regions and the introns (unpublished data), indicating an even higher degree of conservation than the previously described amino acid sequence homologies (2). The chromosomal mapping of Thy-1 by two different procedures shows that it is localized on the long arm of chromosome 11, a position different from any of the other known and localized members of the Ig superfamily. The hybridization to chromosomal panels indicates a position q13–qter (Fig. 2), similar to that described for α -glucosidase AB (28). The Thy-1 position is confirmed and defined more precisely by the *in situ* hybridization to position 11 q23–q24. The only other gene known to be in that particular region is that encoding uroporphyrinogen I synthase (29).

Table 2. Localization of the human Thy-1 gene

Cell line	Chromosome complement																						9q+	11q−	22q−	Thy-1	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22					X
16CB-18	−	±	+	+	−	−	±	−	+	−	+	−	−	+	+	+	−	−	−	+	±	+	+	−			+
E36-4.1.5	+	+	+	±	+	+	±	+	+	+	+	+	±	+	−	+	+	+	+	+	−	+	±	−			+
E36-2.9.5	+	+	+	+	−	+	−	±	−	+	−	+	−	+	+	+	−	+	+	−	+	+	+	−			−
17CB-3B	+	+	−	+	+	+	+	−	+	−	−	+	+	+	+	+	+	−	+	+	+	−	−	−			−
17CB-2C	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−			−
17CB-15B	−	±	+	+	+	−	−	−	−	+	+	+	+	−	−	+	+	±	±	+	+	+	+	−	+		+
17CB-22C	−	±	+	±	−	+	+	+	+	+	−	−	+	−	−	+	±	−	−	+	+	+	+	+	−		−
17CB-8B	−	−	−	+	+	+	−	+	+	+	+	+	±	−	−	+	+	−	+	±	+	+	±	−			+
16CB-17	+	−	+	+	−	−	−	+	−	−	−	+	−	+	−	+	+	+	+	−	+	+	−	−			−
WESP-2A	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
WEGL1-11	−	−	−	−	+	−	+	−	−	−	−	+	+	−	+	−	−	−	+	−	+	−	+	−	−	−	−
WEGL1-9	−	+	−	−	+	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
WEGL1-3	−	−	−	−	+	−	+	+	−	−	−	+	+	−	−	−	−	−	+	−	+	+	+	−	+	−	−
M11-X	t(11;X)																									−	

Human chromosome content of human-hamster (22) or human-mouse (23) cell hybrids as determined by karyotype analysis. Columns 1–Y: + and – indicate the presence or absence of chromosomes 1 to Y; ± indicates the presence of a chromosome but in <10% of the hybrid cells. The right-hand column shows the Thy-1 scoring of the cell lines by Southern blot hybridization (Fig. 2).

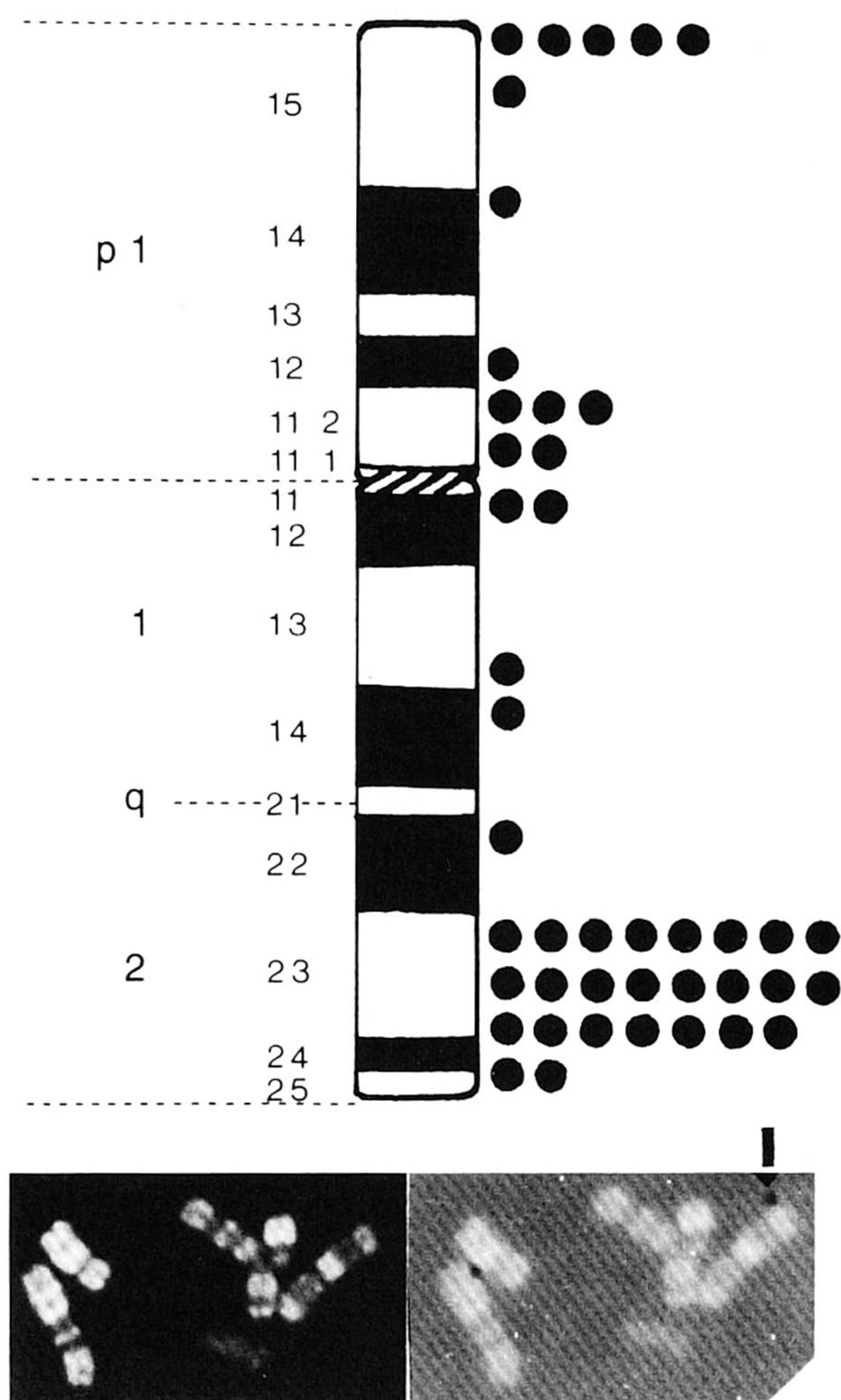


FIG. 3. *In situ* hybridization of the human Thy-1 probe to chromosome 11. (Upper) A map of chromosome-11 bands and the number of grains counted at each position (black circles). (Lower) UV light image (Left) and normal light image (Right) of a portion of a chromosomal spread (see *Materials and Methods*). The arrow indicates a grain at position 11q23-q24.

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