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New gene in the homologous human 11q13–q14 and mouse 7F chromosomal regions

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Abstract. Alterations in the chromosomal region 11q13–11q14 are involved in several pathologies in which most of the key genes remain to be identified. In an effort to isolate as many candidates as possible, we are cloning genes from this region. We report here the mapping of a new sequence from 11q13.5–11q14. This sequence, designated D11S833E, putatively encodes a new gene, provisionally named *GARP*. We cloned its homologous sequence in the mouse and located it on Chromosome (Chr) 7, region F. The human and mouse genes belong to a conserved group of synteny. This, together with the similar conservation of the *FGF* and *TYR* genes, indicates that the human 11q13–q14 and mouse 7E–7F regions share homology.

Introduction

The q13–q14 region of human Chr 11 is involved in several genetic alterations associated with various pathologies. These include B-cell malignancies with a t(11;14) (q13;q32) translocation, several types of sporadic solid tumors with amplifications (Lidereau et al. 1988; Zhou et al. 1988; Theillet et al. 1989) or rearrangements (Arnold et al. 1989; Rosenberg et al. 1991) and multiple endocrine neoplasia type 1, a plurifocal familial malignant syndrome (Larsson et al. 1988) probably associated with alterations of an anti-oncogene. Growth control genes putatively involved in some of these proliferative diseases have been identified. Two FGF (historically meaning fibroblast growth factor; Goldfarb 1990) genes, *FGF3/INT2* and *FGF4/HST*, that map in this region (Casey et al. 1986; Adelaide et al. 1988) are separated by less than 40 kb on subband 11q13.3 (Nguyen et al. 1988). The *PRAD1* gene, also designated *D11S287* (Lammie et al. 1991) or *CYL* (Matsushime et al. 1991) encodes a cyclin-related protein (Motokura et al. 1991). *PRAD1* is linked to the *BCL1* locus (Lammie et al. 1991) that defines the breakpoint of the t(11;14) translocation (Tsujimoto et al. 1985), and it may be the oncogene activated by the translocation event, as well as one of the key genes of the 11q13 amplicons found in solid tumors (Lammie et al. 1991). In fact, an analysis of the 11q13 amplifications observed in breast carcinomas (Szepetowski et al., manuscript in preparation) showed that there might be several amplification units on 11q13–q14, and consequently, different oncogenes. In an effort to identify as many candidate oncogenes of the region as possible, we studied a new sequence that maps to 11q13.5–q14. This sequence received the designation D11S833E and contained a gene we provisionally (Klein 1988) named *GARP*.

Materials and methods

Hybrid cell lines

The 280-1C3-AG8-CE4 cell line (CE4 in text and figures) contains, in a murine genetic background, the 14q+ partner of the t(11;14) (q13;q32) translocation (corresponding to the *BCL1* locus) as the only source of human chr 11 sequences (Erikson et al. 1984). The CF52/46-5 cell line (CF52 in text and figures) is the same as CE4, except that the translocation is a t(11;16) (q13;p11), the breakpoint of which has not been defined precisely but has been localized telomeric to *BCL1* and *FGF3/FGF4* (Koeffler et al. 1981). DNAs from those cell lines were prepared and digested with *Eco*R I according to standard procedures.

Probes

Probes for the *FGF4/HST* locus were a 0.8 kb *Eco*R I-*Sst* I fragment derived from the pCSl plasmid (Adelaide et al. 1988) and a 0.6 *Pst* I fragment derived from cosmid clone cos 20 (D. Birnbaum, unpublished). Probes derived from the human *GARP* locus were: a 2.4 kb *Eco*R I fragment called PR 98 derived from cosmid clone cos 9 and

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the same fragment subcloned into a Bluescript (Stratagene) vector, pDB98, a 4.5 kb *Eco*R I fragment called D from cosmid cos 105, two 2.6 kb and 1.9 kb *Hin*d III subfragments called DU and DL and derived from D, and StuL, a 0.6 kb *Bam*H I-*Stu* I subfragment derived from DL. The Blur 8 clone was used as a probe for human *Alu* repetitive sequences (Jelinek et al. 1981). The mouse *Garp* probe was a 0.5 kb cDNA fragment, called BL, obtained after screening of a mouse embryo cDNA library with StuL.

Cosmid library

A cosmid library was constructed from *Mbo* I-partially digested DNA extracted from blood cells of a normal human male and inserted into the pWE 15 cosmid vector. Clones were screened without prior amplification by filter hybridization at 68°C in 6 × SSC, 1 × Denhardt's solution with ^{32}P oligolabeled DNA fragments. Cosmid DNAs were prepared as described (Noguchi et al. 1991).

Southern blot hybridization

Southern blot hybridizations were performed in standard conditions of stringency (either 68°C, 6 × SSC, 1 × Denhardt's or 42°C, 50% formamide, 6 × SSC, 1 × Denhardt's solution) using Nytran filters (Schleicher and Schuell).

RNA preparation and cDNA cloning

RNA from embryos and placentas were prepared by the guanidine isothiocyanate-cesium chloride method and poly(A)$^+$ were prepared by using oligo (dT) cellulose. The poly(A)$^+$ RNA were fractionated on 1% denaturing agarose gels containing 10% formaldehyde and transferred onto nylon membranes (Nytran, Schleicher and Schuell). Blots were hybridized as previously described (de-Lapeyriere et al. 1990). The cDNA library was constructed from 5 μg mRNA of 15.5 day old C3H/He mouse embryos by using cDNA synthesis and cloning kits (Amersham). The cDNA library was screened in the same way as the cosmid library.

Chromosomal mapping

In situ hybridizations were carried out on chromosome preparations obtained from phytohemagglutinin-stimulated human lymphocytes or from concanavalin-A stimulated lymphocytes from a WMP mouse in which all the autosomes except 19 are Robertsonian translocations, circumventing the problem of chromosomal identification. Lymphocytes were cultured for 72 h with 5-bromodeoxyuridine added for the final 6 h of culture. Plasmid probes were ^3H-labeled by nick translation and hybridized to final concentrations of 25–100 ng/ml, as previously described (Matteï et al. 1985). After coating with nuclear track emulsion (Kodak NTB2), the slides were exposed for 1–2 weeks. Chromosome spreads were stained with buffered Giemsa solution and metaphases photographed. R-banding was then performed by the F.P.G. method and metaphases rephotographed before analysis.

Results

Isolation of cosmids clones from the 11q13–q14 region

We previously reported a preliminary map of the 11q13.3 region containing *FGF3/INT2* and *FGF4/HST* with respect to *BCL1* (Nguyen et al. 1988; Hagemeijer et al. 1991). A putative HTF island (named HTF3) was identified at short distance from the *FGF* locus (Nguyen et al. 1988). Our primary aim was to identify the gene associated with this HTF and thus we began a cosmid walk from the *FGF4* gene towards HTF3, located 3′ to this gene. We screened a cosmid library with *FGF4*-specific probes (Adelaide et al. 1988; D. Birnbaum, unpublished) and obtained clones from the 3′ end of the gene. To ensure that the sequences present in these cosmid clones belonged to 11q13, they were systematically hybridized (not shown) to the DNA of the MDA-MB-134 mammary carcinoma cell line known to contain an 11q13 amplified region (Lafage et al. 1990). In the course of these cloning experiments we also co-isolated, as a contaminant of another cosmid clone, a cosmid (cos 9) that did not belong to the *FGF4* locus but, surprisingly, contained sequences amplified in the cell line. Indeed, this cosmid was shown to contain sequences from Chr 11q. Cosmid cos 9 was hybridized to Blur 8. No *Alu* repetitive sequences were detected in this cosmid. Probe PR 98, a 2.4 kb *Eco*R I fragment, was derived from cos 9 and subcloned into a pBluescript vector to give plasmid pDB 98. PR 98 was used to screen the cosmid library. A second series of cosmids, among which cos 105, was thus obtained.

Chromosomal mapping of the cosmid-derived sequences

Plasmid pDB 98 was used in chromosomal in situ hybrydization to determine the localization of PR 98. In the 100 metaphase cells examined after in situ hybridization, there were 195 silver grains associated with chromosomes and 61 of these (31.2%) were located on Chr 11 (Fig. 1). The distribution of the grains was not random: 53 of them (87%) mapped to the q13.3–q21 region of the long arm of Chr 11, with a maximum in the q13.5 and q14 bands. This result indicates that pDB 98 maps to the 11q13.5–q14 region of the human genome. This localization was confirmed by using Southern blot hybridizations of somatic hybrid cell lines DNAs (Fig. 2A). Two cell lines were used for this purpose, each containing the 11q13–qter part of a translocated human Chr 11 in a murine genetic background (see Material and methods). In CE4, the breakpoint in 11q13 is *BCL1* and is thus centromeric to *FGF3* and *FGF4* (Hagemeijer et al. 1991) while in CF52, the 11q13 breakpoint has been mapped telomeric to these two genes (Fig. 2B). As shown in Fig. 2A, the probe PR 98 hybridized to both cell lines, giving a positive signal the same size (2.4 kb) as in genomic human DNA (lanes Hu). In contrast, no hybridization was observed at this size with mouse DNA (lanes Mo). These results show that PR 98 lies telomeric to both *BCL1* and *FGF3/FGF4*, thus confirming the 11q13.5–q14 mapping. It was assigned the D11S833E symbol.

On long exposures of the blots shown in Fig. 2A, we observed that PR 98 hybridized to mouse DNA (4 kb band). It suggested that this sequence may present

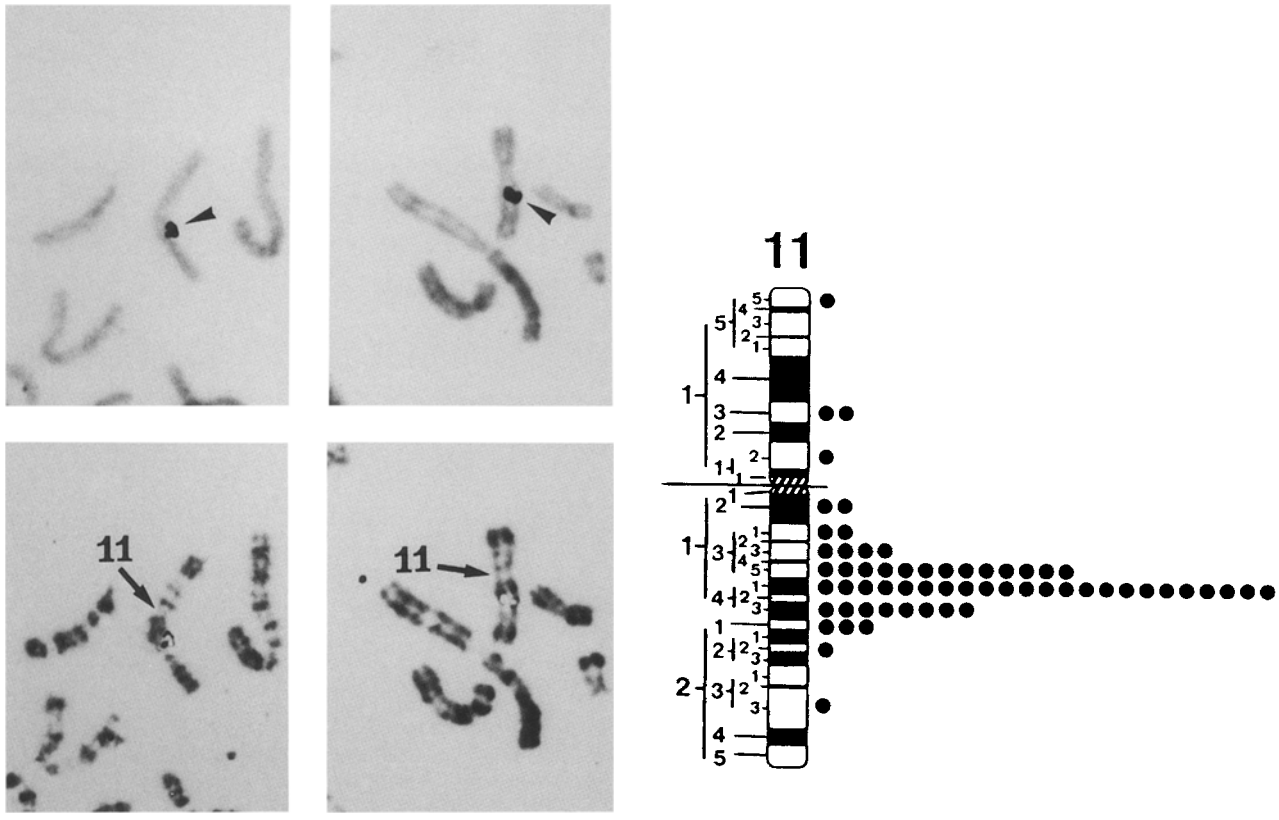

Fig. 1. Localization of cos 9 derived sequences (pDB 98) to human Chr 11, region q13.5-q14. **(A)** Two partial human metaphases showing the specific site of hybridization to Chr 11. **Top:** arrowheads indicate silver grains on Giemsa-stained chromosomes, after autoradiography. **Bottom:** chromosomes with silver grains were subsequently identified by R-banding (F.P.G. technique). **(B)** Diagram of the human G-banded Chr 11 illustrating the distribution of labeled sites for the pDB 98 probe.

some degree of conservation between human and mouse. This was further established by the results obtained in the next experiment.

A gene is located within the cloned sequences

*Eco*R I fragments from cos 105 were purified, labeled and hybridized to blot filters containing *Eco*R I digested human and mouse DNAs. Fragment D, a 4.5 kb *Eco*R I fragment from cos 105, hybridized to human DNA and to mouse DNA. This fragment was cleaved by *Hin*d III to yield two subfragments: DU and DL. Subfragment DL hybridized to mouse DNA. This is shown in Fig. 3A. This indicated that sequences contained in this fragment were conserved between human and mouse. These sequences, thought to correspond to a possible exon of a gene, were found located, by blot hybridizations, in a 0.6 kb *Bam*H I-*Stu* I fragment (StuL) which was subcloned and sequenced. This fragment was then used as a probe to isolate the corresponding mouse sequences. The symbol *GARP* is subsequently used to designate the putative gene.

The presence of coding sequences in the StuL fragment was further suggested by the result of a Northern blot hybridization of human and mouse RNAs (Fig. 3B). The StuL probe detected a transcript of 4.5 kb in poly $(A)^+$ RNA from human placenta (lane H). Cross-reactivity with a mouse transcript of 4.0 kb was also observed in the mouse placenta poly $(A)^+$ RNA (lane M) and in other tissues including 15.5 day old embryos (not shown). This transcript was interpreted as corresponding to the mouse *Garp* homolog. This indicated that a strong interspecies conservation exists between the human and mouse nucleotide sequences.

Isolation and chromosomal mapping of mouse Garp sequences

A cDNA library, constructed with poly $(A)^+$ RNA extracted from 15.5 day old C3H/He mouse embryos, was screened with the StuL probe. Two clones were obtained and analyzed. The BL probe, a 0.5 *Spe* I-*Kpn* I fragment cDNA was derived. Sequence analysis of human StuL and mouse BL fragments showed that the mouse cDNA clone indeed contained *Garp* sequences and could be used to determine the chromosomal localization of the mouse *Garp* sequences.

In the 100 metaphase cells examined after in situ hybridization, 371 silver grains were associated with chromosomes and 67 of these (18%) were located on Chr 7. The distribution of the grains was not random: 52/67 of them (77.6%) mapped to the E1-F2 region of Chr 7 with a maximum in the F1 band. This result is

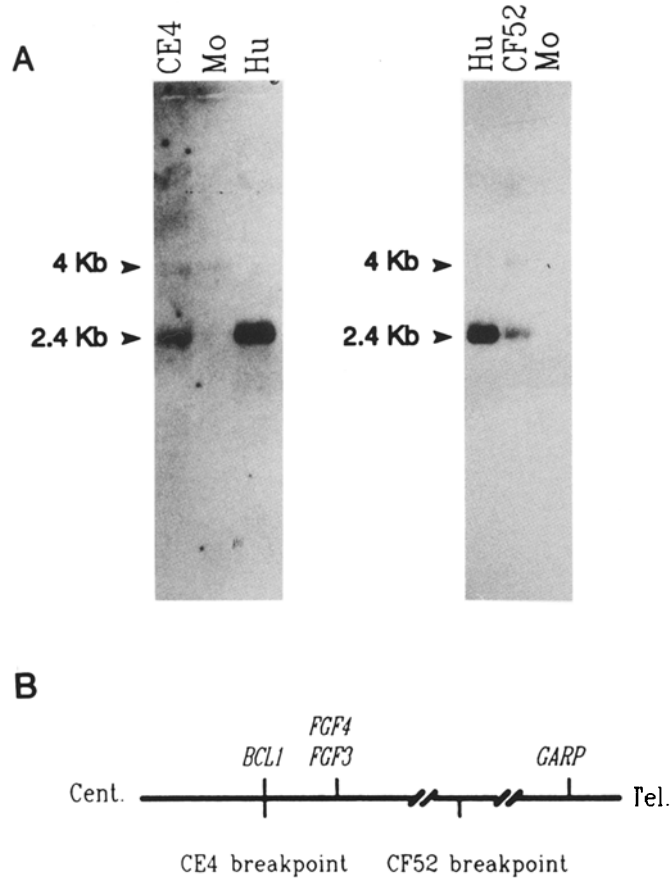

Fig. 2. On 11q, D11S833E (*GARP*) maps telomeric to *BCL1* and *FGF3/FGF4*. **(A)** Ten μg of mouse (lanes Mo), human (lanes Hu) and hybrid cell lines (lanes CE4 and CF52) DNAs were digested by *Eco*R I and run on agarose gels. Southern blot hybridizations with the human PR 98 probe shows bands of 2.4 kb in human and hybrids DNAs. Longer exposures of the blots detected a cross-reacting band of 4 kb in mouse and hybrids DNAs. **(B)** Diagram of human chromosome 11q13–q14 region showing the order of loci and breakpoints described in text.

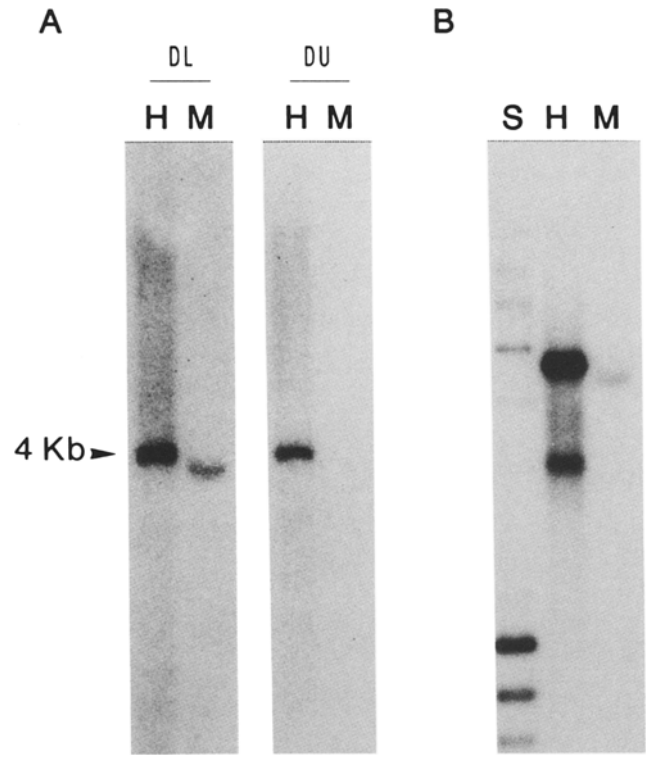

Fig. 3. Presence of a gene in the region defined by D11S833E. **(A)** Southern blot hybridization of human and murine DNAs with human probes derived from pDB98. Two DNA fragments, DL and DU, were used as probes to hybridize human (lanes H) and mouse (lanes M) DNAs, lighting up a 4 kb band in human (DL and DU) and in mouse (DL only). **(B)** Northern blot hybridization of human and murine RNA with a human *GARP* probe. The human StuL DNA fragment (see Material and methods) was used to hybridize human (lane H) and mouse (lane M) poly(A)$^+$ RNAs. Ten μg were loaded in each lane. Two human transcripts of 4.5 and 2.8 kb are detected in human and one of 4.3 kb in mouse, as determined using *Ava* I + *Nar* I fragments of phage λ and *Hae* III fragments of phage ϕ X as size markers loaded in lane S.

shown in Fig. 4. It allowed us to locate the putative *Garp* gene to the 7 E1-F2 region of the murine genome.

Discussion

The chromosomal region 11q13–q21 has been linked to several human pathologies, mostly malignant proliferations of various cell types and organs, including mammary glands (Lidereau et al. 1988; Theillet et al. 1989; Fantl et al. 1990), bladder (Proctor et al. 1991), squamous cells of head and neck (Berenson et al. 1989; Jin et al. 1990; Somers et al. 1990), oesophagus (Tsuda et al. 1989) and lung (Berenson et al. 1990), parathyroid, pituitary and pancreatic glands (Arnold et al. 1989; Rosenberg et al. 1991; Byström et al. 1990) and B-cells (Tsujimoto et al. 1985). Other types of diseases, including McArdle syndrome (Lebo et al. 1990), atopy (Cookson et al. 1989) cerebellar ataxia (Junien and McBride 1989), tuberous sclerosis (Smith et al. 1990) and major mental illness (StClair et al. 1990) are linked to loci mapping in a large region encompassing 11q12 to 11q21. Mapping and cloning of this region is thus paramount to the identification and characterization of genes involved in these diseases.

In an effort to study this region we cloned sequences mapping in 11q13.5–q14 and identified a gene we provisionally named *GARP*. This gene was also cloned in mouse. In this species, *Garp* is the fourth gene to belong to a conserved group of synteny, on Chr 7, including the *Tyr* gene encoding tyrosinase, and the *Fgf3* and *Fgf4* genes encoding FGF growth factors. In human, the *TYR*, *FGF3* and *FGF4* genes are located in 11q13.3 and 11q14–q21, all telomeric from *BCL1* (Hagemeijer et al. 1991). It thus appears that the region 11q13.3–11q21 in human and 7E–7F in mouse are homologous (Human Gene Mapping 10.5; Seldin et al. 1991; Szepetowski et al. 1992; this work). The localizations described here are therefore useful in the comparative mapping of these respective chromosomes (Fig. 5).

Whether *GARP* is involved in one of the pathologies associated with the 11q13–q14 region could be suspected only when its characterization is fully

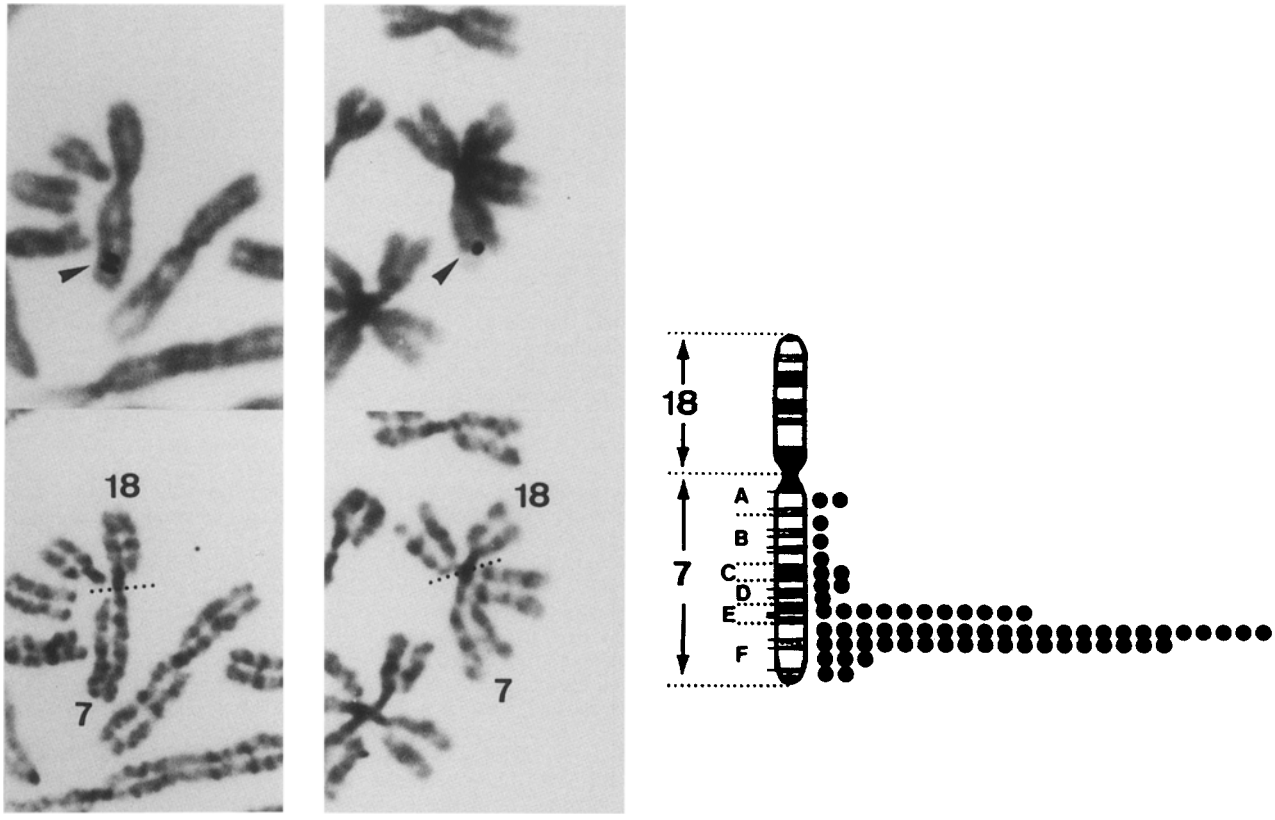

Fig. 4. Localization of the *Garp* gene to mouse Chr 7. **(A)** Two partial WMP mouse metaphases, showing the specific site of hybridization to Chr 7. **Top:** arrowheads indicate silver grains on Giemsa-stained chromosomes, after autoradiography. **Bottom:** chromosomes with silver grains were subsequently identified by R-banding. **(B)** Diagram of WMP mouse Rb(7;18) chromosome, indicating the distribution of labeled sites.

achieved. Work on breast tumor samples has analyzed the status of this locus and found that D11S833E can be amplified in a certain proportion of tumors without concomittant amplification of the *BCL1* locus (Szepetowski et al., manuscript in preparation). This indicates that either *GARP* or a closely linked gene can play a role in mammary carcinogenesis. Sequence analysis of the *GARP* gene and mapping of its regional environment will help in resolving this issue.

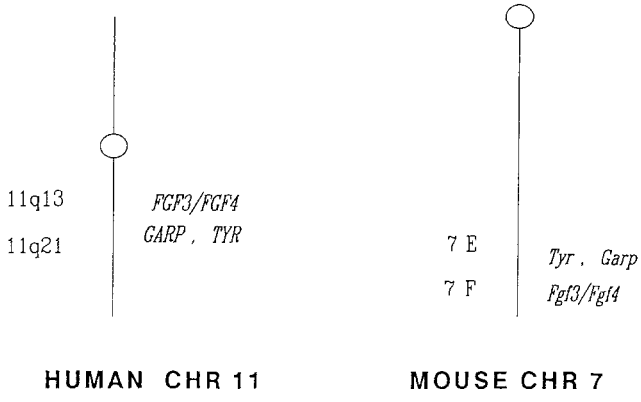

Fig. 5. Schematic representation of the human and mouse homologous regions containing *GARP*. Other loci of the conserved group of synteny are the *TYR* gene, located in human 11q14–q21 and mouse 7E, and the tandem of *FGF* genes, *FGF3/INT2* and *FGF4/HST*, located in human 11q13.3 and mouse 7F. The precise location of *GARP* with respect to these loci is unknown. However, due to the data obtained from the chromosomal in situ hybridization experiments, it may be that *GARP* is located in between the *FGF* and *TYR* genes.

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