

Localization of the Human Mitogen-Induced GTP-Binding Protein Gem to Chromosome 8q22.3

Andreas Kapetanopoulos,* Bernard Vanhove,† Fritz H. Bach,*‡ and Rainer de Martin*¹

*Vienna International Research Cooperation Center, Brunnerstrasse 59, A-1235 Vienna, Austria; †Inserm U437, 30, Boulevard Jean Monnet, F-44035 Nantes, France; and ‡New England Deaconess Hospital, Harvard Medical School, 99 Brookline Avenue, Boston, Massachusetts 02215

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Gem, a member of the superfamily of GTP-binding proteins, has been isolated from the human T-cell line Jurkat by virtue of its inducibility in response to PHA and PMA (3) and from *abl* transformed H/P210 cells (1). It shows highest homology (60% on the amino acid level) to Rad, a gene overexpressed in skeletal muscle cells of type II diabetes-affected individuals (4). Both Gem and Rad differ from the ras consensus by a glycine to glutamic acid mutation in the G3 domain (corresponding to amino acid posi-

tion 60 in ras) that participates in binding and hydrolysis of the γ -phosphate of GTP. Furthermore, they lack a typical CAAX (where A is an aliphatic and X any amino acid) box at the carboxy terminus that in other GTP-binding proteins mediates membrane attachment. Gem and Rad are therefore regarded as a novel subfamily within the GTP-binding protein superfamily.

We have previously isolated the cDNA for the porcine homologue of Gem by differential screening of cytokine-activated versus resting porcine aortic endothelial cells and consecutively isolated the human homologue (Vanhove *et al.*, submitted for publication). A human Gem cDNA was used to analyze by Southern blotting a series of human/hamster and human/mouse somatic cell hybrid cell lines containing individual human chromosomes. Genomic DNAs (400 ng) from the cell hybrids were digested with *Pst*I, separated by agarose gel electrophoresis, and transferred onto a nylon membrane (Amersham Hybond-N). Using the Stratagene Quickhyb solution, hybridization was performed with a radiolabeled cDNA fragment containing 1.2 kb (positions 880–1990) of human Gem, and filters were exposed on a Phosphorimager (Molecular Dynamics) for 3 days. Under stringent conditions, weak cross-hybridization with the hamster and mouse genes that could be distinguished from the human gene by the different sizes of the *Pst*I fragments could be detected. The human Gem-specific band of approximately 8 kb was found exclusively in DNA containing human chromosome 8 (not shown).

¹To whom correspondence should be addressed. Telephone: 43-1-86634-620. Fax: 43-1-86634-623.

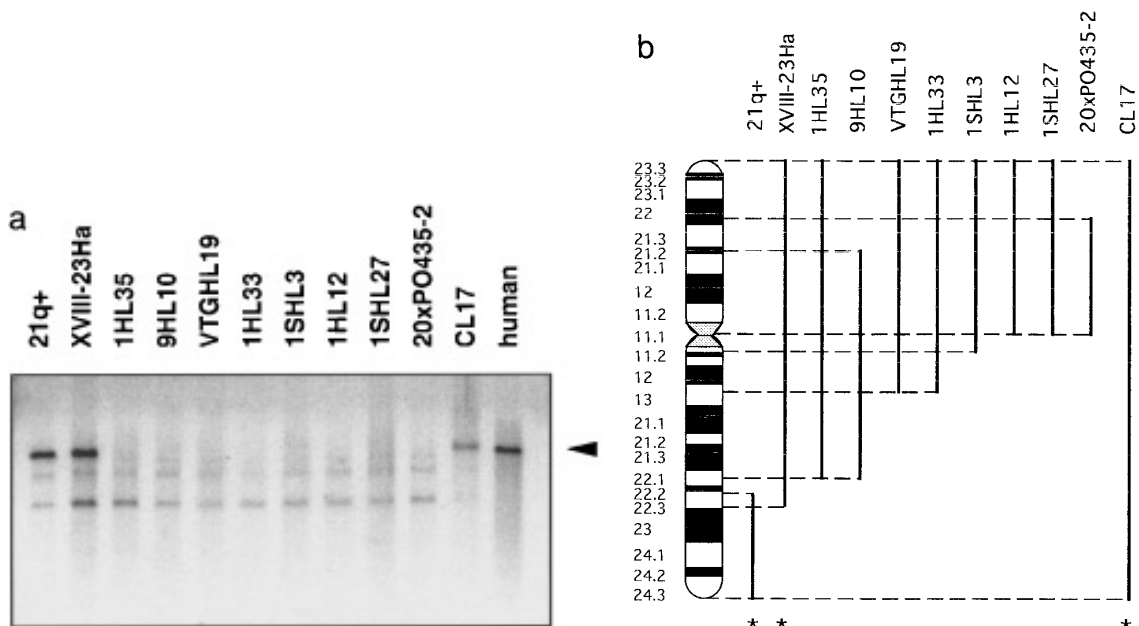


FIG. 1. Localization of Gem to 8q22.3. (a) Southern blot hybridization of Gem with DNAs from the chromosome 8 hybrid cell mapping panel. The position of the human Gem-specific band of 8 kb as determined in previous experiments is indicated by an arrowhead. (b) Schematic representation of the fragments of chromosome 8 present in the hybrid cell lines. As clones 21q+ and XVIII-23Ha give specific signals with the Gem cDNA probe, the corresponding locus could be assigned to 8q22.3. CL17 contains the entire human chromosome 8. "human," total human genomic DNA as control.

To determine more precisely the chromosomal localization of Gem, genomic DNAs from a hybrid cell mapping panel containing various chromosome 8 deletions (6) were hybridized under the same conditions as above. Two of these, 21q+ and XVIII-23Ha, which overlap in a short region of the long arm of chromosome 8, gave a human Gem-specific signal (Fig. 1).

The data presented here indicate that the gene encoding human Gem is located on chromosome 8q22.3. In this chromosomal region, ODF, the gene for human sperm outer dense fiber (2) has been located; furthermore, translocations affecting 8q22.3 are involved in congenital diaphragmatic herniae (5). In general, 8q22 is a site of chromosome fragility and appears to be the recombinogenic origin for several human malignancies. Most interestingly, Gem has been demonstrated to have growth-inhibitory function (3); therefore, deletion or mutation of this gene could lead to uncontrolled cell proliferation. Considering this and the role of GTP-binding proteins in intracellular signaling and oncogenesis, it is possible that functional deletion of Gem might be involved in the development of certain forms of human cancer.

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Localization of the Ileal Sodium-Bile Acid Cotransporter Gene (SLC10A2) to Human Chromosome 13q33

Melissa H. Wong,* P. Nagesh Rao,†
Mark J. Pettenati,† and Paul A. Dawson*¹

*Department of Internal Medicine-Gastroenterology and †Department of Pediatrics, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina 27157

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Bile acids are synthesized from cholesterol in the liver and secreted into the small intestine, where they facilitate absorption of fat-soluble vitamins and cholesterol (6). Rather than being excreted, the majority of bile acids are reabsorbed from the intestine and returned to the liver via the portal circulation. At the liver, bile acids are quantitatively extracted and resecreted into bile, thereby eliminating the need for substantial *de novo* hepatic bile acid synthesis. The ileum is the major site of active uptake of bile acids from the intestine, where a sodium-gradient-driven transporter has been identified in the ileal enterocyte (5). In light of the critical role of active ileal bile acid transport in the enterohepatic circulation of bile acids, mutations in the transporter or other ileal genes that participate in the transepithelial transport of bile acids are predicted to affect bile acid and cholesterol metabolism significantly. Examples of apparent familial defects in the enterohepatic circulation of bile acids have been described, including congenital defects in active ileal bile acid transport (3).

cDNAs encoding the ileal sodium-bile acid cotransporter have been isolated from hamster (8), rat (7), and human (9). In the course of cloning the human cDNA, a dysfunctional mutation was identified and characterized (9). As a first step toward establishing whether mutations in the ileal sodium-bile acid cotransporter gene underlie inherited disorders in bile acid and cholesterol metabolism, we have determined its chromosomal localization.

Studies using DNA from the NIGMS human/rodent cell hybrid mapping panel No. 1 localized the human ileal sodium-bile acid cotransporter gene (SLC10A2)² to autosome 13 (data not shown). To determine the precise location on human chromosome 13, we performed high-resolution fluorescence *in situ* hybridization (FISH) using a method for mapping genes directly on banded chromosomes (4). For these studies, a 4.5-kb SLC10A2 genomic clone (9) was labeled with biotin-14-dATP (BioNick; Life Technologies, Inc., Gaithersburg, MD). Peripheral blood lymphocyte cultures were synchronized with BrdU (250 μ g/ml), washed in RPMI 1640 medium, and exposed to thymidine (2.5 μ g/ml) for 6 h prior to harvest. The slides were stained with 0.5 μ g/ml Hoechst 33258 for 15 min, mounted in MacIlvaine's buffer, and irradiated with a 15-W black light source for 15 min at 50°C. Standard FISH methods were followed for hybridization and detection of the probe (2). The SLC10A2 probe was either hybridized singularly or cohybridized with a biotin-labeled Retinoblastoma gene probe (localized to 13q14; Rb1; Oncor, Gaithersburg, MD). The chromosomes were counterstained with 1 μ g/ml propidium iodide and observed under a Zeiss Axiophot fluorescence microscope. At least 20 metaphases were examined per hybridization. True images were captured and stored on the TOMS image analysis system (TOMS, Columbus, GA).

Localization of SLC10A2 to the long arm of chromosome 13 was confirmed by cohybridization of a Retinoblastoma

¹ To whom correspondence should be addressed at Department of Internal Medicine, Division of Gastroenterology, Bowman Gray School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157. Telephone: (910) 716-4633. Fax: (910) 716-6376.

² The HGMW-approved symbol for this locus is SLC10A2.