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FISH mapping of three ammonia metabolism genes (*Glul, Cps1, Glud1*) in rat, and the chromosomal localization of GLUL in human and *Cps1* in mouse

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In recent years, the rat genes encoding glutamate dehydrogenase (*GLUD;* Das et al. 1993), glutamine synthetase (glutamate-ammonia ligase, *GLUL;* van de Zande et al. 1990) and carbamoyl-phosphate synthetase 1 (*CPS;* Van den Hoff et al. 1995) have been isolated. These enzymes have important functions in ammonia metabolism, and each of them is encoded by a single gene. *GLUD* (E.C. 1.4.1.3) catalyzes the reversible oxidative deamination of L-glutamate to 2-oxoglutarate and ammonia, using NAD+ or NADP+ as cofactor. *GLUL* (E.C. 6.3.1.2) catalyzes the synthesis of glutamine from glutamate, thereby hydrolyzing ATP to ADP. *CPS* (E.C. 6.3.4.16) is the first and rate-determining enzyme of the ornithine cycle and catalyzes the production of carbamoyl-phosphate from ammonia, bicarbonate and ATP. In the present communication, we mapped the position of these three genes in the rat by FISH and by somatic cell hybrids. In addition, we determined the chromosomal location of the human GLUL gene and of the mouse, *Cps1* gene by FISH.

For the FISH mapping of the human glutamine synthetase gene (also called glutamate-ammonia ligase, approved human gene symbol GLUL), a human cDNA (total 2738 bp in pBluescript; Van den Hoff et al. 1991) was used. As shown in Fig.1a, the gene could be unequivocally mapped to Chromosome (Chr) (HSA) 1, band q25.

The three genes were mapped in the rat both with a rat-mouse somatic cell hybrid panel (Szpirer et al. 1984; Klinga Levan et al. 1993) and with FISH. For the mappings with the cell hybrid panel, cDNA probes of approximately 1000 bp from the 3′ ends of the genes were used (*Glul* 1111 bp, van de Zande et al. 1990; *Glud1* 957 bp, Das et al. 1989; *Cps1* 883 bp, De Groot et al. 1986). The probes were labeled with radioactivity by use of α-^{32}P-CTP and the random priming method. They were subsequently hybridized to filters containing 15 μg of genomic DNA from each hybrid restricted with *Eco*RI or *Bam*HI. In each case the rat hybridizing fragments could be distinguished from the mouse bands, and the genes were assigned as follows: *Glud1* to rat Chr (RNO) 16, *Cps1* to RNO9, and *Glul* to RNO13.

In the rat, the FISH results corroborated and refined the findings from the somatic cell hybrid panel. Longer probes are preferred in FISH analysis, and for the regional mapping of the *Glul* gene with FISH, two genomic clones were used (pgGS2, 5000 bp including exons 2–6, and pgGS4, 4500 bp including exon 1; van de Zande et al. 1990). Both probes gave very clear signals at the same chromosomal location, and rat *Glul* could be sublocalized to RNO13q22 (Fig. 1b). The rat G-band nomenclature is according to Levan (1974); for an updated recent version of the idiogram, see RATMAP database (URL http://ratmap.gen.gu.se/ratmap/WWW Nomen/RNOIdiogrRev96new.GIF). For the mapping of rat *Glud1,* two clones of genomic DNA (pgGDH6d, 6700 bp containing exon 1, and pgGDH2u, 5800 bp containing exons 8–12; Das et al. 1993) were used. The results from each of the probes were the same, and *Glud1* could be sublocalized to RNO16p16 (Fig. 1c). Since RNO16 is a metacentric chromosome in which both chromosome arms have very similar stainability and banding pattern except in optimal metaphases, we wanted to check our conclusion with respect to which chromosome arm carried the *Glud1* gene. Sasaki and associates (1994) have published excellent pictures of FISH mapping of the *Atp7b* gene (Wilson Disease gene homolog), and convincingly assigned this gene to RNO16q12.3. We used the same probe (designated pWD4) in simultaneous hybridizations with the *Glud1* probe and could show that the two genes were located on opposite chromosome arms (Fig. 1d), thus verifying our conclusion that *Glud1* is at RNO16p16.

For the FISH mapping of the rat carbamoyl-phosphate synthetase gene (*Cps1*), a full-length cDNA probe (cCPSf.1, 5500 bp; De Groot et al. 1986) was used. The findings corroborated the previous hybrid panel mapping, and the *Cps1* gene could be sublocalized to 9q34 (Fig. 1e). Since the *Cps1* gene had not been mapped in the mouse, we attempted to map it by FISH with the rat cDNA probe. This worked out well, and the mouse *Cps1* gene could be assigned to mouse Chr (MMU) 1, band C3 (Fig. 1f).

Comparative mapping shows that the human GLUL gene is comprised in a region spanning bands HSA 1q22–1q42 and containing 11 human genes for which there are homologous rat genes on RNO13 (Table 1). Only seven of these genes have been mapped also in the mouse, but they are all situated distally in MMU1 (spanning about 26 cM from *C4bp* at map position 68 to *Atp1a2* at map position 94; mouse data from Mouse Genome Database, MGD). The human homolog of the rat *Glud1* gene is located at HSA 10q23.3, and the mouse homolog is on MMU14. *Glud1* is included in a group of three genes (also comprising *Rbp3* and *Sftp1*) that is conserved on these chromosomes. In contrast, the human and mouse genes homologous to the *Atp7b* gene on the long arm of RNO16 are on HSA13 and MMU8, respectively, as is the *Atp4b* gene, which is also located on RNO16. Thus, it looks as if RNO16 resulted from the fusion of two chromosome segments that are on separate chromosomes in both humans and mice, and, therefore, occurred after the separation of the rat lineage from human and mouse lineages. The human homolog of *Cps1* is on HSA2q33–36. In total, there are nine genes on HSA2q (spanning the segment 2q32–2q37) that have their homologs on RNO9 (Table 1). A corresponding segment in the mouse is located on MMU1 (spanning 31 cM from *Slc9a2* at map position 21 to *Ugt1a1* and *Akp3* at position 52). We have pointed out earlier that

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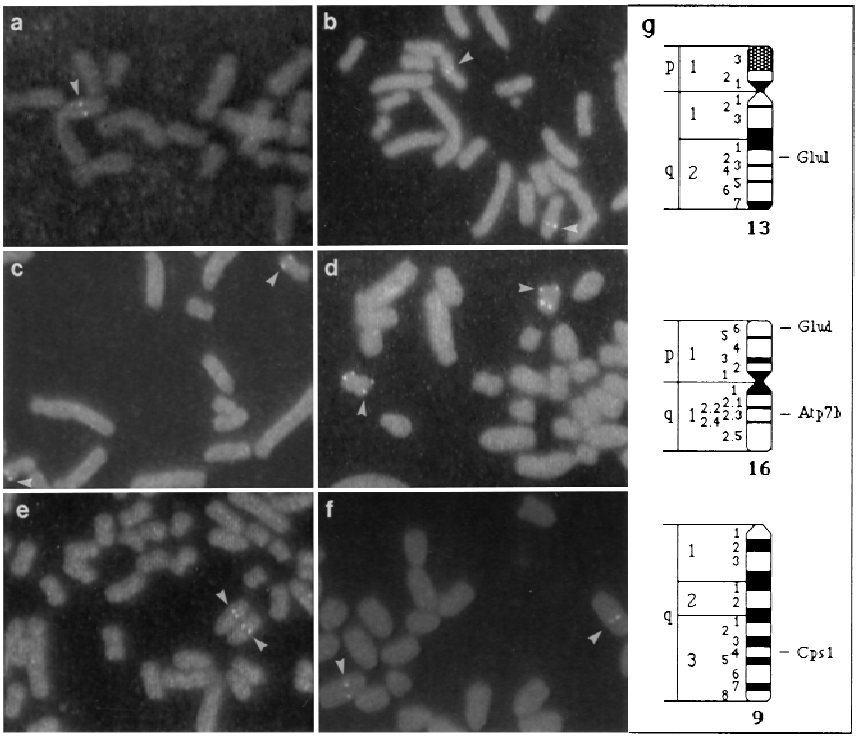

Fig. 1. For FISH mapping, the probes (0.5 μg) were labeled with biotin-14-dATP (Life Technologies) by nick translation (final size of probe fragments: 200–400 bp). Labeled probes were mixed in a hybridization buffer (50% formamide, 2 × SSC, and 10% dextran sulfate). The denatured hybridization mixture was placed on metaphase chromosome slides, which had been denaturated at 72°C for 2 min in 70% formamide, 2 × SSC. After hybridization for 48 h at 37°C in a humid box, preparations were washed for 15 min in 50% formamide, 2 × SSC, and blocked for 10 min in blocking buffer (3% BSA, 4 × SSC). The biotin-labeled probe molecules were detected with avidin-FITC (Oncor). The slides were washed for 15 min each in 1XPBD. Finally, chromosome spreads were counterstained with 0.5 μg/ml DAPI (4′,6-diamidino-2-phenylindole) and 1 μg/ml propidium iodide in an antifade solution (Vectashield, Vector Laboratories). Microscopy was performed with a Leitz DMRBE microscope. FITC signals were visualized through a band pass G/R filter (exciter, 490/20 nm). Metaphase chromosomes stained with DAPI were studied with a UV-A filter (exciter, 340–380 nm) for visualization of the banding patterns and identification of the individual chromosomes (data not shown). The images were captured with a Hamamatsu color chilled 3CCD camera C5810. **(a)** localization of the human GLUL gene to HSA 1q25; **(b)** localization of the rat *Glul* gene to RNO13q22; **(c)** localization of the rat *Glud1* gene to RNO16p16; **(d)** verification of the *Glud1* localization to RNO16p16 by simultaneous hybrization of the rat *Atp7b* (*Wd*) gene, previously mapped to RNO16q12.3 by Sasaki et al. (1994); **(e)** localization of the rat *Cps1* gene to 9q34; **(f)** localization of the mouse *Cps1* gene to MMU1, band C3. **(g)** Idiograms of RNO13, RNO16 and RNO9 with the locations of the mapped genes indicated.

Table 1. Comparative mapping of homologous genes showing the relation between rat Chr 13, human Chr 1q, and the distal part of mouse Chr 1 (top part), and the relation between rat Chr 9, human Chr 2q, and mouse Chr 1 (bottom part).

Rat gene symbol	Rat map position	Human gene symbol	Human map position	Mouse gene symbol	Mouse map position Chr (cM)
Atp1a2	13	ATP1A2	1q21–23	*Atp1a2*	1 (94)
F5	13	F5	1q21–25	*Cf-5*	1 (87)
Abl2	13	ABL2	1q24–25	*Abll*	1 (82)
Pla2c	13	PLA2G4	1q24–q25	—	—
Pepc	13	PEPC	1q25	*Pep3*	1D (71)
Glul	13q22	GLUL	1q31	—	—
Pfkfb2	13q24–25	PFKFB2	1q31	—	—
Ptprc	13	PTPRC	1q31–32	*Ptprc*	1 (74)
Ren	13	REN	1q32	*Ren*	1 (70)
C4bp@	13	C4BP	1q32	*C4bp*	1 (68)
Fh	13	FH	1q42.1	*Fh1*	—
Slc9a2	9	SLC9A2	2	*Slc9a2*	1 (21)
Gls	9	GLS	2q32–34	*Gls*	1 (26)
Idh1	9	IDH1	2q32–qter	*Idh1*	1 (30)
Cryg@	9	CRYG	2q33–35	*Cryg*	1 (32)
Tnp1	9	TNP1	2q34	*Tnp1*	1 (38)
Cps1	9q34	CPS1	2q33–36	*Cps1*	1C3 (−)
Inha	9	INHA	2q33–34	*Inha*	1 (42)
Ugt1a1	9q35–36	UGT1A1	2q37	*Ugt1a1*	1 (52)
Alp1	9	ALPI	2q37.1	*Akp3*	1 (52)

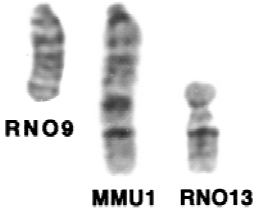

Fig. 2. Comparison of the G-banding patterns of mouse Chr 1 and rat Chrs 9 and 13. Banding patterns reveal that MMU1 basically represents the combination of the two rat chromosomes. Comparative mapping data fully support this conclusion (see Table 1).

MMU1 displays morphological similarities with RNO9 in its proximal part and with RNO13 in its distal part (Levan et al. 1991). This notion is then completely verified by the present analysis, and it appears clear that for these chromosomes not only morphology but also gene content and perhaps gene order have been conserved between the two species (Fig. 2).

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