Assignment of Two Human Autoantigen Genes—Isoleucyl-tRNA Synthetase Locates to 9q21 and Lysyl-tRNA Synthetase Locates to 16q23–q24

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Protein synthesis is initiated by the attachment of amino acids to cognate tRNAs by aminoacyl-tRNA synthetases (aaRS). Five of twenty human aaRS (histidyl-RS, threonyl-RS, alanyl-RS, glycyl-RS, and isoleucyl-RS) have been identified as targets of autoantibodies in the autoimmune disease polymyositis/dermatomyositis (PM/DM; Ref. 18). Autoantibodies to human lysyl-RS, a sixth autoantigenic aminoacyl-RS, were recently identified (19). The genes for histidyl-RS and threonyl-RS have been localized to chromosome 5 (4), and we recently reported that the genes for alanyl-RS and glycyl-RS localize to chromosomes 16 and 7, respectively (12). To understand the genesis of autoimmune responses to aaRS better, we have used PCR-based screening of somatic cell hybrid panels and fluorescence *in situ* hybridization (FISH) to assign the genes for isoleucyl-RS and lysyl-RS.

Isoleucyl-RS (gene symbol *IARS*) and lysyl-RS (gene symbol *KARS*) are the only autoantigenic aaRS that are known to associate with the aaRS high-molecular-weight complex (HMWC), a 1.1-MDa complex that includes half of the 20 aaRS (7). Recently the cDNA sequence of *IARS* was reported (13, 16). Partial sequence determination of human *KARS* (Q. Ge and I. N. Targoff, unpublished data) was used to identify a putative human *KARS* sequence in the Gen-Bank database (Accession No. D31890). The predicted amino acid sequence from the ORF of the putative human *KARS* was compared by BESTFIT (GCG; Ref. 5) to other sequences identified using BLAST. The human sequence was most homologous (94.0% identity; 96.5% similarity) to an unpublished sequence from hamster (Gen Bank Accession No. P37879)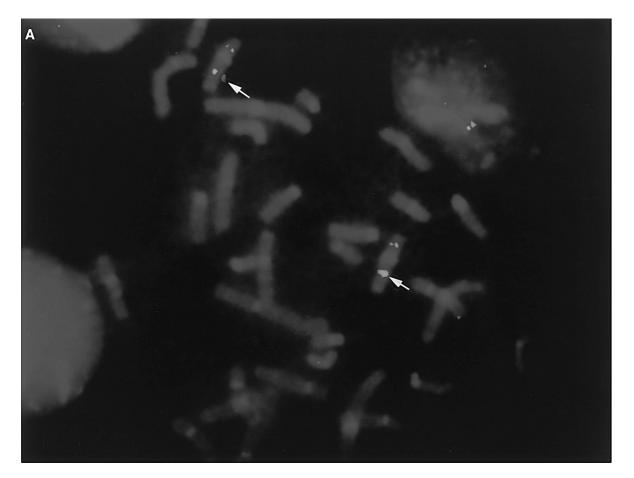. The human sequence also showed very strong homology to published sequences for lysyl-RS from another eukaryote, *Saccharomyces cerevisiae* (57.6% identity; 73.0% similarity; GenBank Accession No. A92695; Ref. 11), and from prokaryotes, *T. thermophilus* (45.9% identity; 63.9% similarity; GenBank Accession No. P41255; Ref. 3), *Escherichia coli lysU* (45.1% identity; 64.8% similarity; GenBank Accession No. JS0400; Ref. 8), *E. coli lysS* (43.7% identity; 63.2% similarity; GenBank Accession No. JS0401; Ref. 8), *C. jejuni* (42.9% identity; 64.4% similarity; Gen-Bank Accession No. P14825; Ref. 2), and *M. hominis* (41.0% identity; 63.2% similarity; GenBank Accession No. X74912; Ref. 15). From this we conclude that the human sequence is *KARS*, and this sequence was used for chromosomal localization.

To localize the genes encoding *IARS* and *KARS*, PCR screening was performed with primer pairs (*IARS:* sense, 5′-tagcatgtacttatcaatgttgttcggtca; antisense, 5′-atatctgag-tttgtttattgttctgattt; *KARS:* sense, 5′-aagacaagaaggagaatg-tagcaaccactga; antisense, 5′-aacaggattaaaaagaaattttta-attcc) synthesized using 3′-UT nucleotide sequences of the respective genes. PCR was performed under stringent conditions ((i) 95°C, 120 s; (ii) 95°C, 30 s, 55–65°C, 30 s, 72°C, 60 s for 35 cycles; (iii) 72°C, 300 s) using a Coriell rodent/human somatic cell hybrid panel (Mapping Panel 2, Coriell Cell Repositories, Camden, NJ). With *IARS* primers a product of the correct size (467 nt) was generated using the hybrid containing chromosome 9. With *KARS* primers a product of the correct size (230 nt) was generated using the chromosome 16 hybrid. The identity of the PCR products was confirmed by Southern hybridization with internal ^{32}P-radiolabeled oligonucleotide probes (*IARS:* 5′-cac-acatgaacacactgaagatatttcc; *KARS:* 5′-ctgtctagaaaataataa-ttgcaagttgta; data not shown). Control experiments were conducted by PCR screening and by Southern analysis: human genomic DNA was positive, and mouse and hamster genomic DNA controls were negative. A BIOS rodent/human somatic cell hybrid panel (Polychromosomal PCRable DNA Somatic Cell Hybrid Panel, BIOS Laboratories, New Haven, CT) was used to confirm chromosomal assignments (data not shown).

Fine chromosomal localization of *IARS* and *KARS* by FISH utilized DNA purified from P1 clones. PCR screening of a P1 library was performed as previously reported (17) by Genome Systems, Inc. (St. Louis, MO) with primer pairs specific for the 3′-UT regions (see above for sequences). FISH analysis was performed by hybridizing digoxigenin-labeled P1 DNA to normal metaphase chromosomes from PHA-stimulated peripheral blood lymphocytes. The results of *IARS* localization were confirmed by cohybridizing with a previously characterized clone from 9p (Ink4A; Ref. 6). FISH analysis of *KARS* was confirmed by cohybridization with a chromosome 16-specific centromere probe. Each locus was measured specifically as the average percentage distance from the centromere to the teleomere on 20 chromosomes. The results of FISH analyses are shown in Fig. 1. The *IARS* locus (Fig. 1A) was assigned to 9q21, and *KARS* (Fig. 1B) was assigned to 16q23–q24.

Because another autoantigenic aaRS, alanyl-RS (gene symbol *AARS*), has been assigned to 16q22 (12), we de-

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FIG. 1.   *In situ* hybridization of human metaphase chromosomes and PCR screening of chromosome 16 partial hybrids. (A) Digoxigenin-conjugated DNA from an *IARS*-specific P1 clone was cohybridized with a 9p-specific probe (Ink4A). Fluoresceinated anti-digoxigenin antibody staining shows the *IARS* probe on 9q (arrow) and the Ink4A probe on 9p. (B) Digoxigenin-conjugated DNA from a *KARS*-specific P1 clone was cohybridized with a chromosome 16 centromeric probe. Fluoresceinated anti-digoxigenin antibody staining shows that the *KARS* probe (arrow) and centromere probe are located on the same chromosomes. (C) An idiogram of chromosome 16 shows the results of PCR screening (+ or −) using *AARS* or *KARS* primers with chromosome 16-specific hybrids.
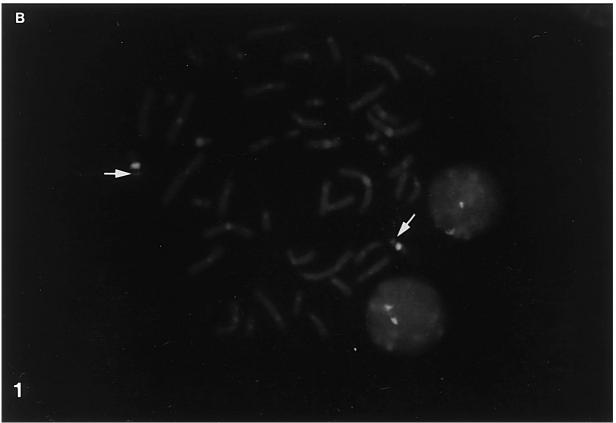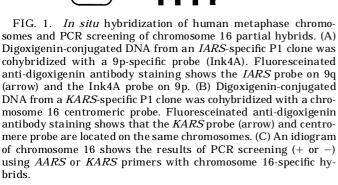
cided to evaluate the linkage between *AARS* and *KARS* on chromosome 16. By PCR screening with chromosome 16-specific mouse/human somatic cell hybrids (D. F. Callen and M. J. Siciliano, unpublished data; Ref. 1, 9), we previously determined (12) that *AARS* locates to 16q22. When these experiments were repeated with *AARS* primers, no PCR product was produced when the hybrid lacking the centromeric portion of 16q22 (CY5) was used as template. This hybrid gave positive results with *KARS* (Fig. 1C). Hybrid controls containing 16q23–q24 (CY6 and CY7) were positive by PCR screening with *KARS* primers, and hybrid controls specific for chromosome 16 but not containing 16q23–q24 (CY2, 92-2, and I-E) were negative. In addition, dual FISH analysis with *KARS* and *AARS* was performed using metaphase chromosomes. Although quantitative distance measurements of the *KARS* and *AARS* loci were not achieved due to diffuse staining of chromosome-specific repetitive elements on chromosome 16 with the *AARS* P1 DNA probe (see 12), the *AARS* locus could be positioned centromeric to the *KARS* locus. These results demonstrate that the gene for *KARS* is not directly linked to the gene for *AARS* on chromosome 16.
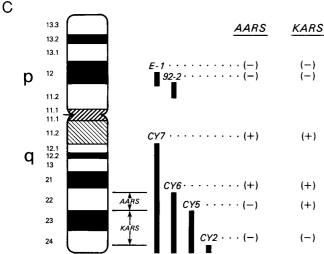
Autoantigenic aaRS are critical to understanding disease pathogenesis because autoantibodies to aaRS arise prior to the onset of disease. Since aaRS have also been shown to drive the immune response (10), it is important to understand the regulation of these gene products. Recently O'Hanlon *et al.* identified a gene in head-to-head orientation with human histidyl-RS, and analysis of the predicted protein sequence strongly suggests that the new gene is the mitochondrial analogue of histidyl-RS (14). Although the importance of gene structure in the regulation

of histidyl-RS and other autoantigenic aaRS is not known, the finding that the gene for *KARS* maps close to the gene for *AARS* suggested that the two genes could be directly linked. We now report that the map site for the *KARS* gene is close to but distinct from the map site of *AARS.* We also report here that the two HMWC-associated autoantigenic aaRS genes, *IARS* and *KARS,* map to different chromosomes.

REFERENCES

1. Callen, D. F., Doggett, N. A., Stallings, R. L., Chen, L. Z., Whitmore, S. A., Lane, S. A., Nancarrow, J. K., Apostolou, S., Thompson, A. D., Lapsys, N. M., Eyre, H. J., Baker, E. G., Shen, Y., Holman, K., Phillips, H., Richards, R. I., and Sutherland, G. R. (1992). High-resolution cytogenetic-based physical map of human chromosome 16. *Genomics* 13: 1178–1185.

2. Chan, V. L., and Bingham, H. L. (1992). Lysyl-tRNA synthetase gene of Campylobacter jejuni. *J. Bacteriol.* 174: 695–701.

3. Chen, J., Brevet, A., Lapadat-Tapolsky, M., Blanquet, S., and Plateau, P. (1994). Properties of the lysyl-tRNA synthetase gene and product from the extreme thermophile Thermus thermophilus. *J. Bacteriol.* 176: 2699–2705.

4. Cruzen, M. E., Bengtsson, U., McMahon, J., Wasmuth, J. J., and Arfin, S. M. (1993). Assignment of the cysteinyl-tRNA synthetase gene (CARS) to 11p15.5. *Genomics* 15: 692–693.

5. Genetics Computer Group (1991). Program Manual for the GCG Package, Version 7.

6. Kamb, A., Gruis, N. A., Weaver-Feldhaus, J., Liu, Q., Harshman, K., Tavtigian, S. V., Stockert, E., Day, R. S., III, Johnson, B. E., and Skolnick, M. H. (1994). A cell cycle regulator potentially involved in genesis of many tumor types. *Science* 264: 436–440.

7. Kisselev, L. L., and Wolfson, A. D. (1995). Aminoacyl-tRNA synthetases from higher eukaryotes. *Prog. Nucleic Acid Res. Mol. Biol.* 48: 83–142.

8. Leveque, F., Plateau, P., Dessen, P., and Blanquet, S. (1990). Homology of lysS and lysU, the two Escherichia coli genes encoding distinct lysyl-tRNA synthetase species. *Nucleic Acids Res.* 18: 305–312.

9. Liu, P., Siciliano, J., White, B., Legerski, R., Callen, D., Reeders, S., Siciliano, M. J., and Thompson, L. H. (1993). Regional mapping of human DNA excision repair gene ERCC4 to chromosome 16p13.13–p13.2. *Mutagenesis* 8: 199–205.

10. Miller, F. W., Waite, K. A., Biswas, T., and Plotz, P. H. (1990). The role of an autoantigen, histidyl-tRNA synthetase, in the induction and maintenance of autoimmunity. *Proc. Natl. Acad. Sci. USA* 87: 9933–9937.

11. Mirande, M., and Waller, J. P. (1988). The yeast lysyl-tRNA synthetase gene. Evidence for general amino acid control of its expression and domain structure of the encoded protein. *J. Biol. Chem.* 263: 18443–18451.

12. Nichols, R. C., Pai, S. I., Ge, Q., Targoff, I. N., Plotz, P. H., and Liu, P. (1995). Chromosomal localization of two human autoantigens by PCR screening and *in situ* hybridization—Gly-

cyl-tRNA synthetase locates to 7p15 and alanyl-tRNA synthetase locates to 16q22. *Genomics* 30: 131–132.

13. Nichols, R. C., Raben, N., Boerkoel, C. F., and Plotz, P. H. (1995). Human isoleucyl-tRNA synthetase: Sequence of the cDNA, alternative mRNA splicing, and the characteristics of an unusually long C-terminal extension. *Gene* 155: 299–304.

14. O'Hanlon, T. P., Raben, N., and Miller, F. W. (1995). A novel gene oriented in a head-to-head configuration with the human histidyl-tRNA synthetase (HRS) gene encodes an mRNA that predicts a polypeptide homologous to HRS. *Biochim. Biophys. Res. Commun.* 210: 556–566.

15. Ozkokmen, D., Birkelund, S., and Christiansen, G. (1994). Characterization of a Mycoplasma hominis gene encoding lysyl-tRNA synthetase (LysRS). *FEMS Microbiol. Lett.* 116: 277–282.

16. Shiba, K., Suzuki, N., Shigesada, K., Namba, Y., Schimmel, P., and Noda, T. (1994). Human cytoplasmic isoleucyl-tRNA synthetase: Selective divergence of the anticodon-binding domain and acquisition of a new structural unit. *Proc. Natl. Acad. Sci. USA* 91: 7435–7439.

17. Smoller, D. A., Petrov, D., and Hartl, D. L. (1991). Characterization of bacteriophage P1 library containing inserts of Drosophila DNA of 75–100 kilobase pairs. *Chromosoma* 100: 487–494.

18. Targoff, I. N. (1992). Autoantibodies in polymyositis. *Rheum. Dis. Clin. N. Am.* 18: 455–482.

19. Targoff, I. N., Trieu, E. P., and Miller, F. W. (1993). Reaction of anti-OJ autoantibodies with components of the multi-enzyme complex of aminoacyl-tRNA synthetases in addition to isoleucyl-tRNA synthetase. *J. Clin. Invest.* 91: 2556–2564.

Localization of the Importin-*β* Gene to Mouse Chromosome 11D and Rat Chromosome 10q32.1

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Importin has been identified as among the cytosolic proteins forming the nuclear pore targeting complex of the nuclear localization sequence-mediated (NLS-mediated) nuclear protein import machinery. The selective import of proteins into the cell nucleus occurs in two steps, both of which require the presence of an NLS. The first step is binding to the cytoplasmic surface of the nuclear pore complex (NPC), which forms channels for diffusion and active transport across the double membrane bilayer of the nuclear envelope (1, 2). This step requires NLS and soluble factors, namely importin-α (M_r 60 kDa), importin-β (M_r 90 kDa), and Ran/TC4. The complex consisting of importin-α, by which the NLS of nuclear proteins is primarily recognized, and importin-β binds the import substrate in the cytosol, which binds to the NPC. This step does not require energy. The second step is the energy-dependent translocation through the NPC. The Ran/TC4 molecule mediates the energy-dependent process. Then, finally, the import substrates and importin-α are transported into the nucleus. In contrast, importin-β accumulates at the nuclear envelope, but not in the nucleoplasm. In detailed analyses, it was revealed that the binding of the nuclear pore targeting complex to NPC is mediated by importin-β (3).

The chromosomal localization of the importin-β gene to mouse and rat chromosomes was performed using direct R-banding fluorescence *in situ* hybridization (FISH) using a mouse cDNA fragment as a biotinylated probe. Preparation of R-banded chromosomes and FISH was performed as described previously (6, 7). Mitogen-stimulated lymphocyte cultures were synchronized by thymidine block, and differential replication staining was performed using 5-bromodeoxyuridine incorporation in the late replication stage and by using UV exposure of chromosomal slides after staining with Hoechst 33258. The murine importin-β gene is transcribed at 4.3 or 5.6 kb (manuscript in preparation).

The mouse importin-β 5.3-kb cDNA *Sal*I–*Bam*HI fragment, isolated from a murine cDNA library and inserted into pBluescript II, was labeled by nick-translation with biotin-16–dUTP (Boehringer Mannheim), following the manufacturer's protocol. The labeled fragment was hybridized to the denatured chromosomal slide, and then the slide was incubated overnight at 37°C.

The amplification method was used for staining the slides. After the slides were washed, they were incubated under coverslip with goat anti-biotin antibody (Vector Laboratories) at a 1:500 dilution in 1% BSA/4× SSC for 1 h at 37°C. The slides were washed with 4× SSC, 0.1% NP-40 in 4× SSC, 4× SSC for 5 min each and then stained with fluorescein-anti-goat IgG (Nordic Immunology) at a 1:500 dilution for 1 h at 37°C. After washing following the above-mentioned protocol for 10 min each on the shaker and draining the excess liquid from the slides, they were stained with 0.75 μg/ml propidium iodide.

As shown in Fig. 1, the signals were localized to the proximal end of mouse chromosome 11D and to the q32.1 band of rat chromosome 10 (4–6, 8, 9). This result suggests that the mouse and rat importin-β genes map to a region with conserved linkage homology (10) and provides additional evidence that mouse chromosome 11 and rat chromosome 10 evolved from an ancestral mammalian chromosome.