BRIEF REPORTS

The Novel Epithelial-Specific Ets Transcription Factor Gene ESX Maps to Human Chromosome 1q32.1

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We have recently isolated and characterized a novel member of the Ets transcription factor family, ESX (also known as ESE-1), the first Ets factor and one of only a few transcription factors exclusively expressed in epithelial cells (10). Since several members of this unique group of transcription factors have been directly implicated in human tumor-specific chromosomal translocations, we were interested in determining the specific chromosomal localization of ESX (1–3, 15). A human genomic clone encoding the ESX gene was obtained from Genome Systems after screening a PAC library with ESX-specific primers and further analyzed by subcloning and DNA sequencing (Barcinski *et al.,* manuscript in preparation). To determine the precise chromosomal location of ESX, we performed single-copy gene fluorescence *in situ* hybridization (FISH) to human male metaphase chromosome spreads (8). The ESX human genomic PAC–DNA insert was nick-translated using digoxigenin–dUTP (Boehringer Mannheim), and FISH was performed as detailed in Johnson *et al.* (4). Individual chromosomes were counterstained with 4′-6-diamidino-2-phenylindole-2HCl (DAPI), and color digital images, containing both DAPI bands and gene signal—detected with anti-digoxigenin-tagged rhodamine fluorescent label—were recorded using a triple-band pass filter set (Chroma Technology, Inc., Brattleboro, VT) in combination with a charged coupled-device camera (Photometrics, Inc., Tucson, AZ) and variable excitation wavelength filters (5). Images were analyzed using the ISEE software package (Inovision Corp., Durham NC). Nine digitized images were analyzed, most of which had a doublet signal characteristic of genuine hybridization on both homologs of chromosome 1 (Fig. 1). Doublet signal was not detected on any other chromosome. Detailed analysis of 14 individual chromosomes, using fluorescence banding combined with high-resolution image analysis, indicated that the ESX gene is positioned within band 1q32 (Fig. 1B), with the majority of signals appearing to cluster proximally in band 1q32.1.

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Chromosome 1q32.1 is of interest for a variety of diseases and especially for various types of cancer. Nonrandom structural abnormalities of chromosome 1q are frequently observed in human tumors and have been linked to many human cancers including ovarian, breast, cervical, lung, stomach, kidney, pancreas, retinoblastoma, testicular germ cell, melanoma, Wilms, medulloblastoma, hepatoblastoma, rhabdomyosarcoma, large bowel, head and neck squamous, glioma, tongue squamous, Ewing's sarcoma, bladder, colon, and myeloid leukemia (6, 7, 9, 11–14). Loss of heterozygosity for 1q32.1 was detected especially frequently in medulloblastoma, hepatoblastoma, collecting duct carcinoma of the kidney, cervical cancer, ovarian cancer, and testicular germ cell cancers, suggesting the presence of a putative tumor suppressor gene in that region (6, 7, 13, 14). Amplification of 1q32.1 has been frequently found in human gliomas, suggesting the location of a putative oncogene in that region (9). Localization of the ESX gene will enable us to explore the possibility that aberrations of the ESX gene are involved in human cancer formation.

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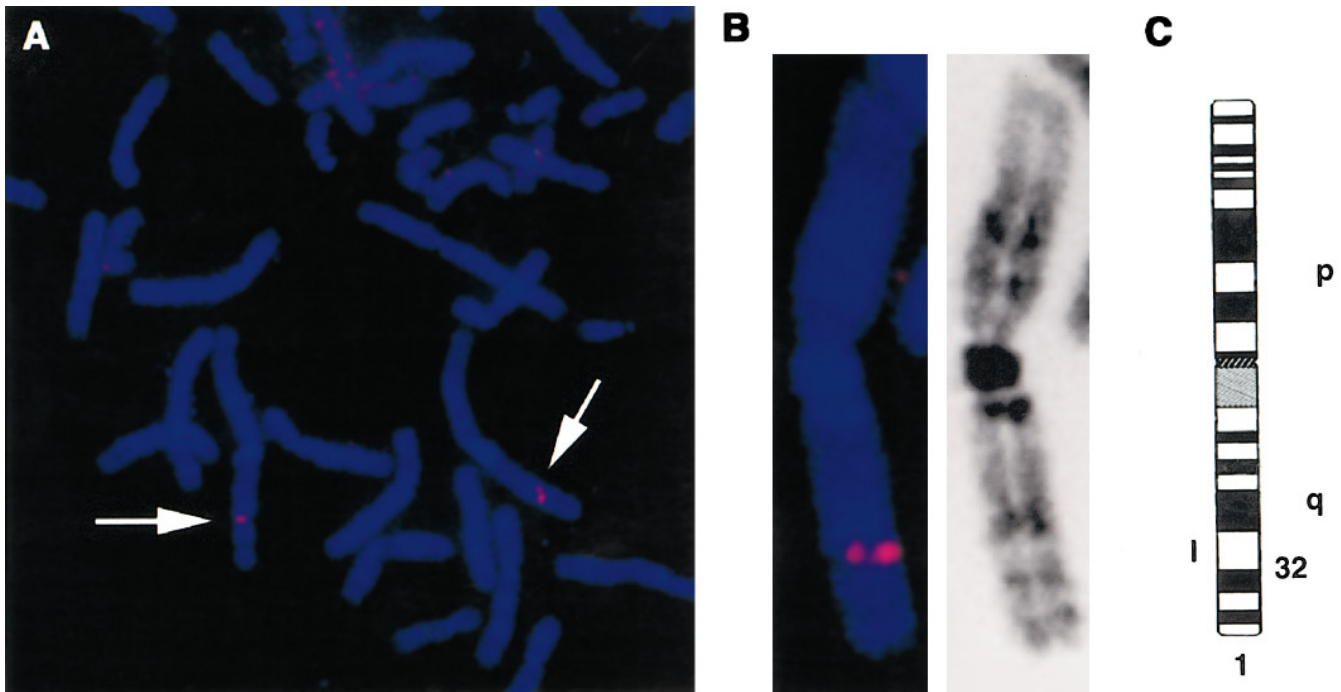
FIG. 1. Fluorescence *in situ* hybridization mapping of ESX (ESE-1). (**A**) A DAPI-stained metaphase spread, showing doublet gene signal (arrows) on both chromosome 1 homologs. (**B**) (**Left**) A single DAPI-banded chromosome 1 with hybridization signal. (**Center**) G-banded image converted from the same DAPI-stained chromosome. (**Right**) Idiogram of chromosome 1 marking the position of the gene (bar) to 1q32.

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Radiation Hybrid Mapping of Human ADAM10 Gene to Chromosome 15

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A variety of cell surface adhesion proteins and proteinases play important roles in the following events: tissue morphogenesis, placental implantation, and wound healing, as well as pathologies such as tumor cell invasion and cartilage degradation in arthritis. Cadherin, immunoglobulin superfamily members, selectins, and integrins are known as families of membrane-anchored cell surface adhesion molecules (6). Membrane-anchored cell surface proteinases include membrane-type matrix metalloproteinases (3). A family of proteins containing a disintegrin and metalloproteinase domain has been discovered and called the ADAM family. This family is a unique cell surface protein structure possessing both potential adhesion and proteinase domains (12). Amino acid sequence analysis on the ADAMs indicated that they contain

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pro-, metalloproteinase-like, disintegrin-like, cysteine-rich, EGF-like, transmembrane, and cytoplasmic domains.

Tumor necrosis factor-α (TNFα) is synthesized as a proinflammatory cytokine from a 233-amino-acid precursor. Conversion of the membrane-bound precursor to a secreted mature protein is mediated by an unidentified proteinase, termed TNFα convertase. Recently, it has been reported that ADAM10 among the ADAMs possesses a TNFα convertase activity (7). As it is well-known that TNFα is involved in a variety of diseases, it is possible that ADAM10 is related to the pathogenesis. We have, thus, mapped human the ADAM10 locus using a radiation hybrid mapping method covering whole human genomes (11) to elucidate whether the locus links to the disease susceptibility loci.

The nucleotide sequence of human partial ADAM10 cDNA was obtained from the GenBank/EMBL Data Bank under Accession No. Z48579 (2423 nucleotides). The primers used for the amplification of the gene were 5′-GGC ACT TTA AAG AGG AGG AG-3′ (sense, nucleotides 1981–2000) and 5′-TGG GTT CCT TTT CCA CCT-3′ (antisense, nucleotides 2272–2289 in the 3′-untranslated region).

Human genomic DNA purchased from Novagen was used. Chinese hamster genomic DNA was extracted from the liver: it was treated with proteinase K/ribonuclease A, and DNA was extracted with phenol/chloroform. The procedure used to amplify genomic DNA was conducted using Expand High Fidelity PCR System (Boehringer Mannheim), basically according to the manufacture's protocol. PCR conditions were 45 amplification cycles of 30 s at 94°C, 1 min at 60°C, and 2 min at 72°C, with a final extension of 10 min at 72°C (MiniCycler, MJ Research). The amplified PCR products were analyzed on 1.2% agarose (Gibco BRL)/ethidium bromide gel electrophoresis.

PCR amplication of the ADAM10 gene resulted in an ~300-bp product in the human, but not in the Chinese hamster. The human PCR product was sequenced to confirm its identity as the target fragment. It was subcloned into pT7Blue(R)T vector (Novagen) by TA-cloning, purified with QIAquick spin columns (Qiagen), and used as template for sequencing with the T7 or U19 primer, fluorescent terminators, and ABI Prism 377 DNA sequencer (Applied Biosystems). The sequence result of the product matched the published data completely.

A GeneBridge 4 radiation hybrid panel was obtained from Research Genetics, including a human cell clone and a hamster recipient cell clone and 93 radiation hybrid clones. The panel samples were subjected to PCR, the conditions of which were the same as described above. Produced fragments were separated on 1.2% agarose/ethidium bromide gel and typed. The typing data vector was 10010 11001 10001 10100 00000 10000 11000 11010 11110 11010 10010 00100 00101 12000 10100 00000 01101 11010 111. The results were sent through the World Wide Web at http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl, and the computed results were returned to us.

The radiation hybrid map around the ADAM10 locus is indicated in Fig. 1. ADAM10 was placed 12.44 cR$_{3000}$ from AFM321ZD5 on chromosome 15, linking with D15S121 and D15S159. From the database of the Whitehead Institute for Biomedical Research/MIT Center for Genome Research (http://www-genome.wi.mit.edu/cgi-bin/contig.phys_map), the ADAM10 locus can be assigned between 55 and 67 cM from the top on the genetic map of chromosome 15. Cytogenetically, it is likely that the locus is located on 15q2 (4).
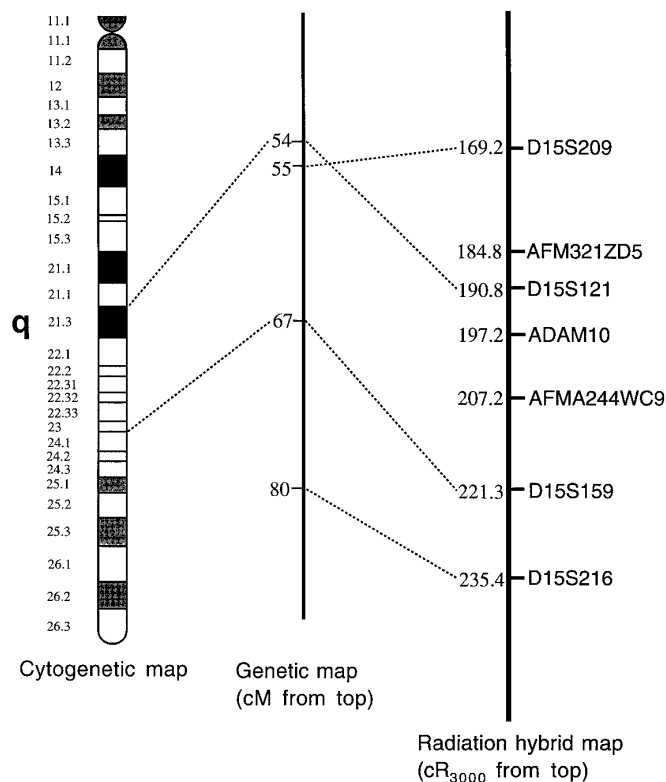

FIG. 1. Radiation hybrid map and genetic map around the ADAM10 locus. Numbers to the left of the radiation hybrid map and genetic map indicate distances from the top of human chromosome 15 (cR$_{3000}$ and cM, respectively). In addition, the idiogram of chromosome 15q is indicated at the left.

TNFα is implicated in the pathologies of inflammatory and autoimmune diseases because it plays an important role in the pathogenesis and progression of a chronic inflammatory disease, multiple sclerosis (MS) (2). MS has shown evidence of linkage to chromosomes 2, 3, 5, 6, 11, 17, and X in addition to the major histocompatibility complex on chromosome 6p (5, 9, 10). We believe that the ADAM10 gene should be studied more carefully in the MS pedigree because it contains a protein domain homologous to TNFα-converting enzyme (TACE) (1, 8). TACE is a new member of the ADAM family, which processes TNFα precursor, and may also be related to MS or rheumatoid arthritis. At the same time, the other ADAM10 domains may implicate this gene's role in other genetic diseases.

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Assignment of the Human Equilibrative Nucleoside Transporter (hENT1) to 6p21.1–p21.2

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An essential component of the action of nucleoside analog drugs used in anticancer therapies is the mediated uptake of the drug across the plasma membrane into the cell. This is achieved by integral membrane proteins known as nucleoside transporters. There are two major families of nucleoside transporters, the concentrative and the equilibrative (2). The concentrative nucleoside transporters appear to be restricted in their distribution within cells and tissues and also in their selectivity of nucleoside permeants. In contrast, the equilibrative nucleoside transporters appear to be widely distributed and have a broad substrate specificity. The latter group of transporters is the likely route of entry of many of the nucleoside analogs used in cancer chemotherapy. The recent cloning of the cDNA for the prototypic equilibrative transporter hENT1 (3) from human placenta has allowed us to determine the location of the corresponding gene.

The probe (named pES) used to identify the ENT1 locus was the ENT1 cDNA previously described (3). Fluorescence *in situ* hybridization (5) was used to map the probe to normal human lymphocyte chromosomes counterstained with propidium iodide and diamidinophenylindole (DAPI). As shown schematically in Fig. 1, the 1.7-kb cDNA probe mapped to chromosome 6p21.1–p21.2. The analysis of 20 well-spread metaphases determined the regional assignment of this cDNA probe. Positive hybridization signals of the cDNA at 6p21.1–p21.2 were noted in 10% of the cells. In addition, a 1.365-kb PCR-generated cDNA probe was used to obtain genomic clones by screening a P1-derived artificial chromosome (PAC) library. Two genomic PACs were isolated, each of which mapped to 6p21.1–p21.2. Signals were visualized in 95% of the cells and on both homologues in 90% of the positive spreads for the PAC probes. The band assignment was determined by measuring the fractional chromosome

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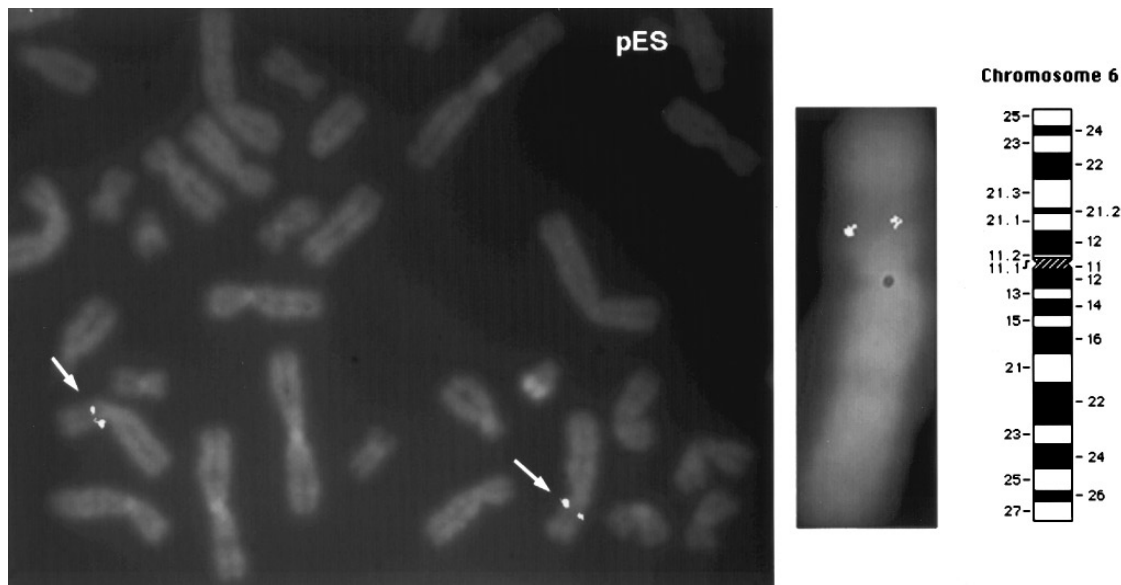
FIG. 1. Regional mapping of the ENT1 gene by fluorescence *in situ* hybridization to 6p21.1–p21.2 in normal human lymphocyte chromosomes counterstained with propidium iodide and DAPI. Avidin–fluorescein isothiocyanate (FITC) was used to detect the biotinylated probe. A thermoelectrically cooled charge-coupled camera (Photometrics, Tucson, AZ) was used to capture images of metaphase preparations, and separate images of DAPI-banded chromosomes (4) and of FITC-targeted chromosomes were obtained. These hybridization signals were acquired, merged using image analysis software, and then pseudocolored blue (DAPI) and yellow (FITC) as previously described (1). These images were then overlaid electronically. The gene was localized to chromosome 6 as shown.

length and by analyzing the banding pattern generated by the DAPI-counterstained image.

The equilibrative nucleoside transporter is a ubiquitously expressed transporter that is probably responsible for much of the uptake of adenosine in the body. A disruption in this gene could therefore have consequences in terms of the interaction of adenosine with its receptors, especially in the brain. A myoclonic juvenile epilepsy locus with a broad phenotype has been mapped to the same locus (6). However, further studies will be required to establish whether there is a link between mutations in the transporter and this condition.

The gene for the sodium-dependent, concentrative nucleoside transporter, hCNT1, was recently localized to 15q11.1–q11.2 (7). Despite their similar substrates, hENT1 and hCNT1 appear to be members of evolutionarily distinct families since the genes are located in disparate areas and there is no apparent homology between the cDNA sequences.

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Localization of the Gene for a Serine Protease with IGF-Binding Domain (PRSS11) to Human Chromosome 10q25.3–q26.2

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Insulin-like growth factors (IGFs) stimulate the proliferation and differentiation of a vast number of cell types. The actions of the growth factors are mediated and controlled by a complex system of components, including at least two different forms of IGF, two IGF receptors, seven different IGF-binding proteins (IGFBPs), and several proteases that cleave the IGFBPs (6, 8). Although heavily investigated, the whole system is far from being understood in detail due to its high complexity and to the still growing number of its individual components.

We have recently cloned the cDNA for a human protein termed L56 that seems to be part of the IGF signaling system (11). The novel protein shares a surprisingly high sequence similarity (58%) with the Htra family of serine proteases from bacteria (7). It is synthesized in a precursor form with a typical signal peptide, suggesting that it functions as a proteolytic enzyme after secretion. In contrast to the related bacterial proteases, the human protein contains an additional domain at its N-terminus that shows a striking sequence similarity to the IGF-binding domain conserved in all members of the IGFBP family. Furthermore, it corresponds in size (50 kDa) to a partially characterized protein from smooth muscle cells that has been demonstrated to cleave IGFBP-4 (9). We therefore speculated that the novel protein may represent one of the proteases specific for IGFBPs (11).

Initially we had isolated the cDNA clone for the L56 protease from a subtracted cDNA library that had been constructed during a systematic search for proteins whose synthesis was downregulated after oncogenic transformation (10). Clone L56 was expressed in most human tissues, but its synthesis was completely inhibited in SV40-transformed fibroblasts as well as in several spontaneous tumor cell lines. This inhibition is intriguing since it may cause a general disturbance in the IGF signaling system. A disturbance that ultimately leads to abnormal activation of the IGF receptor seems to be a prerequisite for the growth of most transformed cells (3).

The chromosomal localizations of the genes for the individual components of the IGF signaling system are known in detail (6, 8). We therefore determined the localization of the gene for the novel protease (PRSS11) using the technique of fluorescence *in situ* hybridization (FISH). FISH mapping was performed as described by Heng *et al.* (4, 5). Human blood lymphocytes were synchronized with BrdU. Metaphase spreads of chromosomes were prepared by conventional methods and fixed on glass
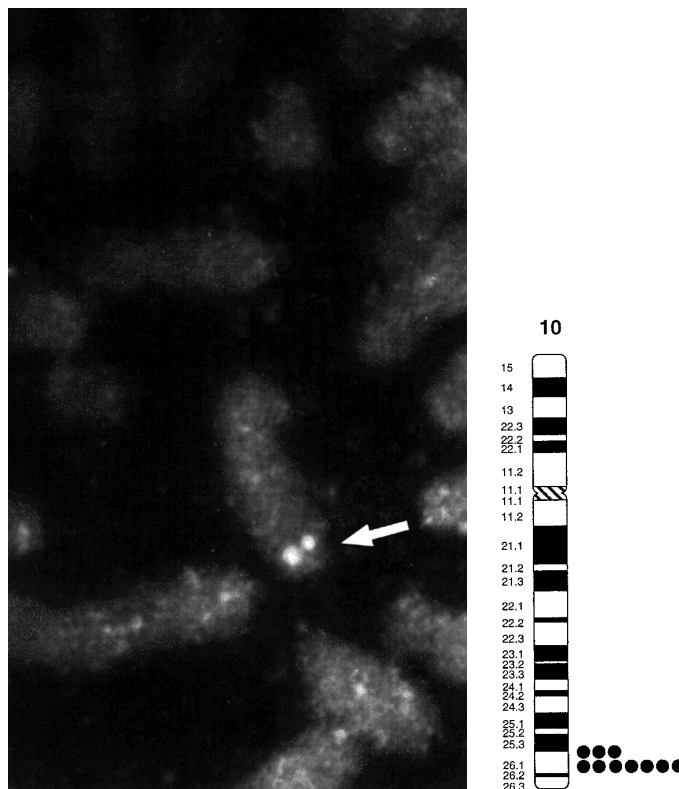
FIG. 1. Mapping of cDNA clone L56 to human chromosome 10. An example of the FISH signal is presented (**left**). The gene was assigned to region 10q25.3–q26.1 by superimposing the FISH signal and the DAPI banding pattern (**right**).

slides. The L56 cDNA (1934 bp) was labeled with biotinylated dATP and hybridized overnight at 37°C to the denatured chromatin in a buffer containing 50% formamide. After being washed, the slides were stained with fluorescein isothiocyanate-conjugated avidin and counterstained with 4,6-diamidino-2-phenylindole (DAPI). The FISH signal and the DAPI banding pattern were separately recorded.

Our probe hybridized specifically to the long arm of chromosome 10 (Fig. 1). Seventy-one percent of all the metaphase spreads examined ($n = 100$) showed a positive signal on one pair of chromosomes. The exact position, as determined by superimposing the FISH signal and the DAPI banding pattern, was region 10q25.3–q26.2. No additional locus was observed.

The gene for the L56 protease is thus situated at a single locus on human chromosome 10. No other gene for a component of the IGF system has so far been identified on this chromosome. However, it may be of interest to note that the gene for the insulin-degrading enzyme is located on chromosome 10 in region 10q23–q25. Similar to the L56 protease, which is related to the HtrA protease from bacteria, the insulin-degrading enzyme shows some sequence similarity to protease III from *Escherichia coli* (1, 2). The two bacterial proteases, however, are not related to each other. It is therefore possible that this region of human chromosome 10 harbors a number of conserved enzymes that may have evolved from bacterial genes.

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Assignment of the Mouse *Hsp25* and *Hsp105* Genes to the Distal Region of Chromosome 5 by Linkage Analysis

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In response to elevated temperatures, most cells substantially increase the synthesis of a specific set of proteins desig-

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nated heat shock proteins (Hsps). Widely conserved in all organisms, Hsps are subdivided into four groups based upon their approximate molecular weights: Hsp100, Hsp90, Hsp70, and the Hsp20s (see 13 for a detailed review). While Hsps were originally identified due to their induction following heat, most members are also constitutively expressed and play important roles in normal cell processes. In mice the Hsp20 group consists of single copies of three genes: Hsp25, αA crystallin, and αB crystallin. Overexpression of Hsp25 can protect cells from hyperthermia (11), chemotherapeutic drugs (6), and oxidative stress (11). The structure, expression, and functions of the Hsp100 genes is less well defined. At present only two members in mammals are known: Hsp105(14) and Hsp70RY(2). Hsp70RY was originally named due to a limited homology with the ATP binding domain present in all Hsp70 family members; however, it has little additional homology beyond that region and is more closely related overall to the Hsp100 family.

Genomic clones encoding the *Hsp25* and *Hsp105* genes were isolated by PCR screening of a 129/SvJ mouse P1 library (Genome Systems, St. Louis, MO). The *Hsp25* P1 gene clone was obtained by PCR screening using oligonucleotide primers based upon the published sequences at the 3′ end of the first exon and the 3′ end of the first intron (3). This primer arrangement ensured that only the functional and not the pseudogene copy would be isolated. From the resulting P1 clone, a 7.0-kb Nhe1 subfragment was obtained that contained the *Hsp25* gene based upon extensive restriction mapping and DNA sequence analysis (data not shown). Southern blot analysis was carried out with a 1.2-kb genomic *Bam*HI/*Sma*I DNA fragment isolated from the Nhe1 subclone that spanned the first exon and ended in the second intron of the *Hsp25* gene. This probe hybridized strongly to a single band in *Bgl*II restricted mouse genomic DNA of the same size as the corresponding fragment in the *Hsp25* P1 clone and very weakly to a second fragment. As the mouse genome is reported to contain only a single functional gene and one pseudogene (3), this latter fragment should represent the pseudogene and was not characterized further. The *Hsp105* probe was a 1.0-kb *Hin*dIII gene fragment that by DNA sequence analysis began in the last exon of the gene and ended 400 bp beyond the polyadenylation signal. The original clone containing the *Hsp105* gene was also isolated by PCR screening of the same P1 library utilizing oligonucleotide primers based upon the published 3′ sequence of an Hsp105 cDNA clone (14). This probe detected a single band in genomic Southern blots.

The chromosomal locations of the *Hsp25* and *Hsp105* genes were determined by analysis of two sets of multilocus crosses: (NFS/N or C58/J × *Mus musculus musculus*) × *M. m. musculus* (10) and (NFS/N × *Mus spretus*) × *M. spretus* or C58/J (1). DNAs from the progeny of these crosses have been typed for over 1000 markers distributed over the mouse genome including the chromosome (Chr) 5 markers *Gus* (glucuronidase, beta), *Zp3* (zona pellucida 3), and *Atrc1* (amino acid transporter cationic 1). These markers were typed as previously described (5, 7). Linkage was determined using the program LOCUS designed by C. E. Buckler (NIAID, Bethesda). Percentage recombination and standard errors
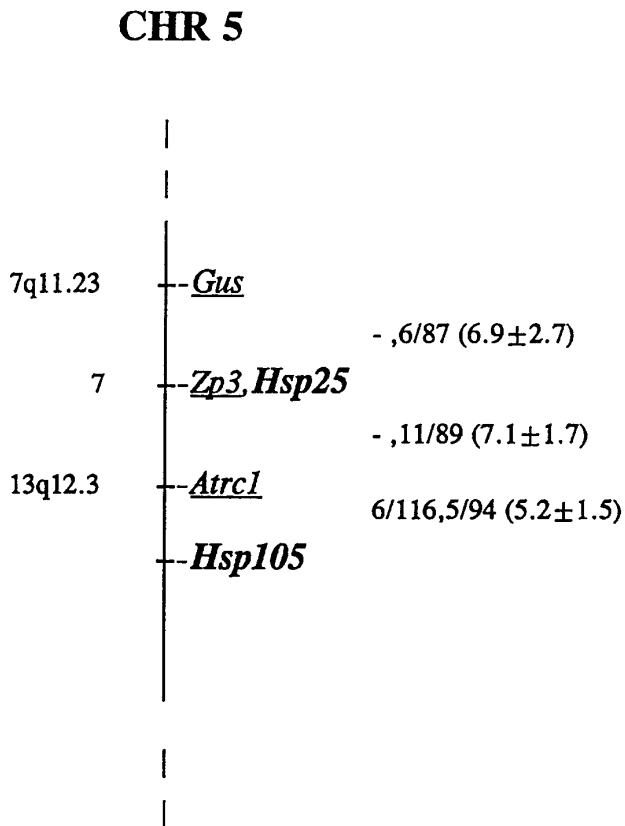
CHR 5


```
             |
             |
           --|--
7q11.23   --|--Gus
             |           - ,6/87 (6.9±2.7)
   7      --|--Zp3, Hsp25
             |           - ,11/89 (7.1±1.7)
13q12.3   --|--Atrc1
             |           6/116,5/94 (5.2±1.5)
           --|--Hsp105
             |
             |
           --|--
             |
             |
```

FIG. 1. Genetic map locations of *Hsp25* and *Hsp105* on mouse Chr 5. To the right of the map are given recombination fractions between adjacent loci, with the first fraction representing data from the *M. m. musculus* crosses and the second representing data from the *M. spretus* crosses. The numbers in parentheses represent percentage recombination and standard error. *Hsp25* was typed only in the *M. spretus* crosses. No double recombinants were identified within this Chr 5 interval. The map locations for the human homologs of the underlined genes are given to the left of the map.

were determined according to Green (4), and gene order was determined by minimizing recombinants.

For *Hsp105, Pvu*II digestion generated fragments of 5.0 kb in parental NFS/N and C58/J and 5.2 and 2.5 kb in *M. m. musculus. Bam*HI produced *Hsp105* fragments of 9.4 kb in NFS/N and C58/J and 12.4 kb in *M. spretus. Hsp25* was identified as a 2.8-kb *Hin*dIII fragment in *M. spretus* and a 2.9-kb fragment in NFS/N. No *Hsp25* polymorphisms were identified for the parental DNAs of the *M. m. musculus* crosses. Inheritance of the polymorphic fragments was followed in the progeny DNAs and compared with that of over 1000 markers previously typed and mapped. The loci defined by both probes mapped to different sites on distal mouse Chr 5 (Fig. 1). *Hsp25* was most closely linked to *Zp3* with no recombinants in 91 mice, indicating that these genes are within 3.2 cM at the upper limit of the 95% confidence level. *Hsp105* maps distal to *Atrc1,* and its position makes it the most distal expressed gene on this chromosome. The location of both genes to the same chromosome was also confirmed by *in situ* hybridization (data not shown). As indicated in Fig. 1, these regions of Chr 5 show homology to human chromosomes 7 and 13, suggesting map locations for the human homologs of these genes. The human *Hsp27* gene has, in fact, been mapped to chromosome

7 (12, 13), which is consistent with our location of the mouse *Hsp25* gene. The remaining members of the mouse *Hsp25* gene family, αA-crystallin (*Crya1*) and αB-crystallin (*Crya2*), are single-copy genes located on Chromosomes 17 and 9 (7, 9), respectively, indicating that there is no clustering of the *Hsp25* gene family members at a single location.

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