band (Fig. 1). To our knowledge, this is the first human chromosomal localization of an enzyme metabolizing $InsP_3$. A screening of the Genome DataBank and Online Mendelian Inheritance in Man from April 1992 failed to reveal any candidate disease that might be explained in single terms by mutation of the gene. The data clearly establish that despite their sequence similarity (ca. 65%) indicating a common evolutionary origin, hh39R and hh3 clones encoding two $InsP_3$ 3-kinase isozymes are associated with different genes on different chromosomes.

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Mapping of the Gene Encoding the α-Subunit of the Human H^+, K^+-ATPase to Chromosome 19q13.1 by Fluorescent *in Situ* Hybridization

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H^+,K^+-ATPase is the enzyme responsible for ATP-dependent exchange of H^+ for K^+ across plasma membranes (reviewed in (3)). This enzyme, which is a member of the P-type transport ATPases that include Na^+,K^+-ATPase and Ca^+-ATPase, differs from other proton pumps such as the F_1F_0 mitochondrial ATP synthase and the organellar V-type H^+-ATPase. The H^+,K^+-ATPase consists of two subunits, an α-subunit, which exhibits a catalytic function during active transport, and a β-subunit, the function of which remains to be defined. H^+,K^+-ATPase is expressed at a very high level in mammalian gastric parietal cells (1, 4), where it functions primarily as a proton pump, transporting H^+ in exchange for K^+ into secretory canaliculi and, from there, into the gastric lumen. In addition to parietal cells, H^+,K^+-ATPase may be expressed in a variety of other cells, including colonic enterocytes and renal tubular cells. Using polymerase chain reaction, we have detected H^+,K^+-ATPase mRNA in brain, testis, and ovary (Song and Yamada, unpublished observations).

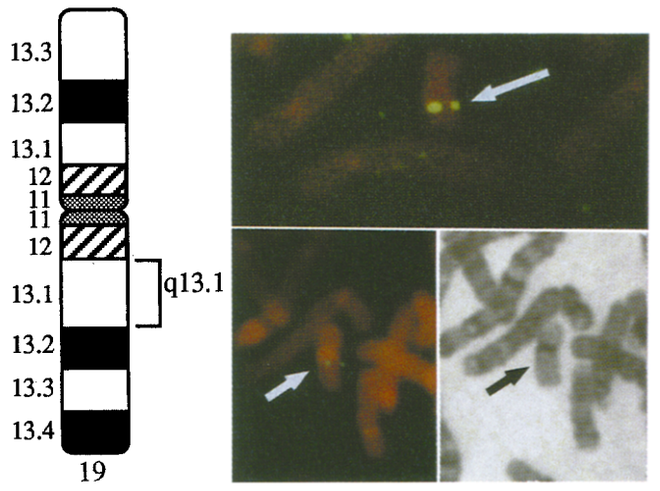

FIG. 1. (**Left**) Idiogram of human chromosome 19 indicating the location of the H^+,K^+-ATPase gene to 19q13.1. (**Right**) Examples of FISH using a H^+,K^+-ATPase α probe (HG53-1-1a). (**Top**) Double-fluorescent signals over the proximal long arm of chromosome 19 following FISH. (**Bottom**) A double-fluorescent signal (**left**) and the identical cell G-banded (**right**).

We have recently cloned and sequenced the cDNA of the canine H^+,K^+-ATPase α- and β-subunits (Song and Yamada, unpublished; GenBank Accession No. M36978 for α and M76486 for β) as well as the genomic DNA for the canine, rat (Song and Yamada, unpublished; GenBank Accession No. M36979), and human α-subunits.

To identify the chromosomal loci encoding this gene, 1 μg of a clone of the human H^+,K^+-ATPase α-subunit (designated HG53-1-1a), which contains the entire 22 exons (approximately 13 kb), was labeled with biotin and hybridized to human metaphase chromosomes as previously described (2). Of 29 metaphase cells examined, 28 had double-fluorescent signals, one on each chromatid over the mid-long arm of chromosome 19. Only chromosomes in which both chromatids had a signal were included for analyses, making the background hybridization extremely low. The same cells hybridized for FISH were previously G-banded (using Wright's stain) and photographed to allow direct comparison of the results. The results demonstrate that the chromosomal loci of H^+,K^+-ATPase α can be localized to 19q13.1 (Fig. 1).

It is of considerable interest that this localization is adjacent to that for the Na^+,K^+-ATPase αIII gene (19q13.2) in view of the 70% cDNA homology between the genes for the two enzymes. This suggests the possibility that one of the two genes may have arisen from the other by tandem gene duplication during the process of evolution.

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Assignment, by *in Situ* Hybridization, of the Growth Arrest-Specific Gene, *Gas-1*, to Mouse Chromosome 13, Bands B3–C2

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Growth arrest-specific (Gas) genes were cloned from mRNAs unique to quiescent, serum-starved NIH3T3 mouse fibroblasts (10). Six cDNA clones that detected mRNAs preferentially expressed when the cells were growth-arrested and down-regulated by activation with serum were isolated. It was proposed that these genes may have a functional role in sustaining growth arrest or may be related to the control of differentiation. The genes have since been further characterized, but their functions remain unknown. The expression of one of these genes, *Gas-1*, is transcriptionally regulated (2). Sequencing of Gas-1 cDNA is currently in progress, but little else is known of the gene (Sorrentino, personal communication, 1991). Since the expression of *Gas-1* is cell cycle-regulated, implying a possible role in maintaining the quiescent state, it may also be a tumor-suppressor gene. Preliminary evidence suggests that *Gas-1* is abnormal in mouse and human tumors, possibly due to alterations in the methylation state of the gene (Cowled *et al.*, unpublished data).

In this article, we report the mapping, by *in situ* hybridization, of *Gas-1* to mouse chromosome 13, bands B3–C2. A 1.3 kb fragment of Gas-1 cDNA, cloned at *Eco*RI sites in the plasmid Bluescript, was labeled by nick translation with tritiated dATP, dCTP, and dTTP to a specific activity of 5.9×10^7 cpm/μg. The labeled probe was hybridized to spreads of mitotic chromosomes prepared from splenic lymphocytes of male BALB/c mice as previously described (13,14). Since the specific activity of the probe was low, the slides were not acetylated and, following unsuccessful exposure for 7 days, the slides were exposed to Ilford L4 emulsion for 3 months. Individual grains were scored onto standard idiograms of G-banded mouse chromosomes (9). The scorer (G.C.W.) had no previous knowledge of the location of *Gas-1* in the murine genome.

In approximately 100 cells, 50% of the grains were located over Chromosome 13, and the background grains on the other chromosomes showed no subsidiary peaks (Fig. 1). Of the 101 grains over Chromosome 13, 86 were in the four tall peaks over bands B and C. The two tallest peaks, containing 62 grains, were located over the bands 13B3 and 13C1–C2. This region constitutes the most precise localization of *Gas-1* possible using the present data.

Most recently Colombo *et al.* (4) have shown linkage of *Gas-1* to the following markers on Chromosome 13: *Xmv-13* (xenotropic murine leukemia virus 13), no recombination; DNA segment *D13Pas2*, approximately 8% recombinants; and *Dhfr* (dihydrofolate reductase), approximately 17% recombinants. These data had been incorporated into the map of mouse Chromosome 13 of Justice and Stephenson (6), which shows *Gas-1* at 45 map units and physically aligned with band C1, which is the central band of the three to which we have allocated the gene. From all of these sources of data, it seems likely that *Gas-1* is in band 13C1 in the mouse, an assignment that supersedes that of Colombo *et al.* (3), who assigned *Gas-1* to Chromosome 12 on the basis of the pattern of inheritance of an *Ava*I restriction fragment length polymorphism.

The location of *Gas-1* on Chromosome 13 has considerable significance when the synteny and order of homologous human genes are considered. Mouse Chromosome 13 contains eight loci within a 30-cM segment that is conserved in human chromosome arm 5q, mainly in 5q11–q14 (6). *Gas-1* is close to the IL-9 gene in the mouse, one of the loci in the segment conserved on human chromosome 5. On this basis, it is predicted that the human homologue of *Gas-1* should map to chromosome arm 5q. This region of the genome is highly significant as a site of a number of potential tumor suppressor genes

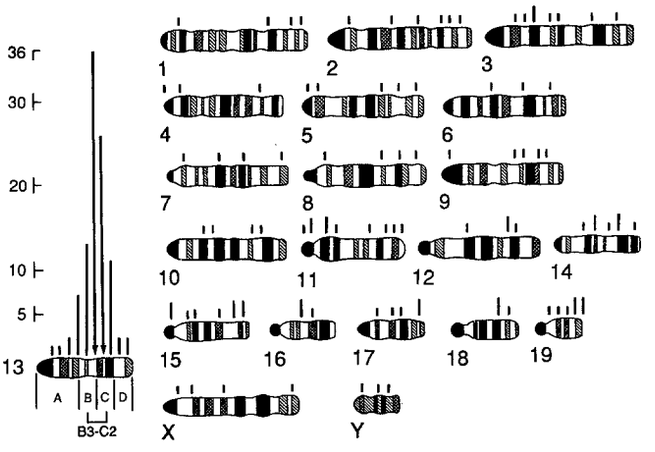

FIG. 1. Localization of grains over the chromosomes in approximately 100 metaphase spreads following hybridization with the tritiated Gas-1 cDNA probe. Band B2 (5) of Chromosome 13 is shown as a double line. The three bands under the two tallest peaks of grains (arrowed) are marked as the most precise possible localization of *Gas-1*.

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