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Brief Communication

Interferon-Regulated Human 2-5A Synthetase Gene Maps to Chromosome 12

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Abstract—*The low-molecular-weight human 2-5A synthetase gene has been assigned to chromosome 12 using rodent–human somatic cell hybrids and filter hybridization analysis of cell hybrid DNA. A cDNA probe representing almost all the coding sequences of the 2-5A synthetase gene hybridizes to four fragments of human DNA digested with the restriction enzyme EcoR1. By correlating the presence of these fragments in somatic cell hybrid DNA with the human chromosome content of the hybrids, the 2-5A synthetase gene can be mapped to chromosome 12. This contrasts with a previous assignment of this gene to chromosome 11 using an enzyme activity assay. The reason for this discrepancy remains unclear.*

INTRODUCTION

The interferons (IFNs) are a family of proteins which show a range of growth inhibitory and antiviral activities on responsive cells. The induction of the antiviral state has been correlated in some virus–cell systems with the activity of the enzyme 2-5A synthetase [ATP: (2′, 5′)oligio(A) adenyltransferase; EC 2.7.7.19; reviewed in ref. 1]. This enzyme, when bound to double-stranded RNA, synthesizes 2′-5′ linked oligomers of adenosine (2-5A) which activate a latent endoribonuclease resulting in degradation of RNA and inhibition of protein synthesis (2). Although virus replication is inhibited, the overall effects on the cell may be transient as the 2-5A is rapidly degraded by a 2′-phosphodiesterase (3, 4). 2-5A is produced naturally in interferon-treated virus-infected cells (5), and recent experiments with 2-5A analogs have provided direct evidence for a role for this 2-5A in inhibiting virus infection (6). There is a speculation and some limited evidence that the 2-5A system, with or without induction by IFN, may be involved in the control of cell growth and differentiation and in the response of cells to changing hormone status (7). The recent molecular cloning and characterization of the human synthetase gene (8–11) should allow determination of the contribution of the 2-5A system to the control of cellular processes.

Gene(s) which are involved in IFN sensitivity have been mapped previously to chromosome 21 (12). Results of [^{125}I]IFN binding studies on human–mouse somatic hybrids suggest this chromosome contains a gene which encodes the receptor for human IFN-alpha and beta (13). Using an enzyme assay, Shulman et al. reported assignment of low-molecular-weight 2-5A synthetase activity to human chromosome 11 (14). In this paper, we attempted to confirm the assignment of Shul-

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man et al. (14) by using a nearly full-length cDNA probe for low-molecular-weight 2-5A synthetase gene to screen a panel of human–mouse somatic hybrid DNAs by filter hybridization analysis. In contrast to the data of Shulman et al., our data allow the direct assignment of the low-molecular-weight gene to human chromosome 12 and not to chromosome 11.

MATERIALS AND METHODS

Hybrid Cell Lines. Human–rodent somatic cell hybrids were made by fusion of parental cells with polyethylene glycol and isolation in HAT selection medium (15). The hybrid series used in this study has been described previously (15–18). Human chromosome composition of hybrid lines was determined by trypsin–Giemsa banding (19) of metaphase chromosomes analyzed at the time of DNA preparation. The presence or absence of human chromosome 12 was confirmed in these studies by Cellogel electrophoresis (20) of lactate dehydrogenase B activity and/or by DNA restriction endonuclease analysis of DNA segment D12S7 (detected by probe pDL32B), known to be located on the long arm of chromosome 12 (21). The presence or absence of human chromosome 11 was confirmed by Cellogel electrophoresis of lactate dehydrogenase A activity and/or by DNA restrictions endonuclease analysis of the human catalase gene, known to be located on the short arm of chromosome 11 (22). In all cases, the results of the chromosome, isozyme, and DNA analyses were in complete agreement.

Filter Hybridization Analysis of Cell Hybrid DNA. DNA was isolated from parental and hybrid cells as described (23). Five to ten micrograms of each DNA sample were separated by agarose gel electrophoresis and transferred to nitrocellulose filters by the method of Southern (24). DNA probes were ^{32}P-labeled by nick translation and hybridized to filters using conditions described previously (16–18, 23). After washing, filters were air-dried and exposed to Kodak XAR-5 film with intensifying screens for 1–4 days at −70° C. The probe used in this study, pMS6-2, has been described previously (9, 11). Briefly, pMS6-2 is a 1386-bp cDNA that includes part of exon 3 and exons 4–8 of the 2-5A synthetase gene. It does not include the extension of exon 7 which is present in transcripts from the 2-5A synthetase gene in some cell lines as a result of alternative RNA processing (8, 9). This probe hybridizes to all the exon-containing EcoR1 fragments encompassing the complete 2-5A synthetase gene (9, 11).

RESULTS AND DISCUSSION

The isolation, mapping and sequencing of phage and cosmid genomic clones for the low-molegular-weight human 2-5A synthetase has indicated that this gene consists of eight exons contained within 12 kb of DNA. There are six EcoR1 fragments spanning this region and four of these (EcoR1 bands of 6.8, 4.0, 3.5, and 2.8 kb) contain all the exons of this gene (9, 10). Although the probe used in the study does not contain sequences representative of exons 1 and 2, exons 3 and 4 (which are encoded by this cDNA) are in the same 4.0-kb EcoR1 fragment (9).

To assign the gene for 2-5A synthetase to a particular chromosome, segregation of DNA sequences homologous to the pMS6-2 cDNA was examined in a series of 22 rodent–human somatic cell hybrids. DNAs were digested with EcoR1 and analyzed by Southern blotting using the 1386-bp insert of pMS6-2 as probe. As shown previously, this probe detects four EcoR1 bands in human DNA (Fig. 1). Under the hybridization conditions used, no homologous bands were detected in either mouse or Chinese hamster DNA. All four human bands cosegregated in the panel of hybrids examined and could be assigned to human chromosome 12 (Fig. 1, Table 1). All other human autosomes and the X chromosome were discordant in at least five

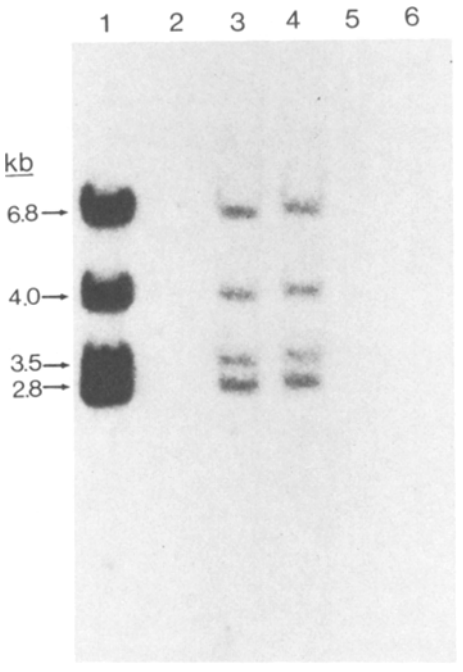

Fig. 1. Filter hybridization analysis of the human 2-5A synthetase gene. DNAs are from human (lane 1), mouse (lane 2), and mouse–human hybrids (lanes 3–6). DNAs were digested with EcoRI and Southern blot analysis performed with the pMS6-2 probe. The chromosome 11 and 12 composition of the hybrids in lanes 3–6 is as follows: lane 3, 11^-, 12^+; lane 4, 11^+, 12^+; lane 5, 11^+, 12^-; lane 6, 11^-, 12^-.

of the hybrids. The Y chromosome could be eliminated because all the hybrids were derived from female parental cells. Significantly, chromosome 11, to which a 2-5A synthetase activity was previously assigned by enzyme assay, was discordant in greater than 50% of the hybrids examined. This included five hybrids which contained chromosome 12 and 2-5A synthetase gene sequences in the absence of chromosome 11. Accordingly, from these data we conclude that the gene encoding 2-5A synthetase is located on human chromosome 12. Interestingly, the 2-5A synthetase gene is therefore syntenic with the human interferon gamma gene (27), although there is no evidence that these genes are coordinately regulated.

The previous assignment of a 2-5A synthetase activity to chromosome 11 (14) was based on a crude separation method in which the authors claimed to be able to distinguish between the small forms of mouse and human enzymes. Previous work had described two forms of synthetase in both human and mouse cells. In mouse Ehrlich ascites cells a large (predominantly nuclear) 85,000- to 105,000-dalton and a small (cytoplasmic) 20,000- to 30,000-dalton form are apparently encoded by two interferon-induced mRNAs of 3.8 and 1.5 kb, respectively (25). In human SV80 and HeLa cells oocyte injection and gel filtration experiments have indicated enzymes of 60,000–80,000 daltons and 30,000 daltons (26). In contrast, human Namalva cells appeared to have only the 30,000-dalton form of the enzyme. In the human system, although transcripts adequate in length to encode the 80,000-dalton protein have been described (8–11), all the evidence thus far suggests these represent unspliced transcripts of the low-molecular-weight synthetase gene (9, 10, and M.S. and B.R.G.W., unpublished data). However, there is a unique form of RNA processing of the 3′ end of this gene which can result in transcripts differing in size at the 3′ end (9, 10). Either one or both mRNAs may be present in different cell types. The deduced C terminus of the smaller (1.5- to 1.6-kb) mRNA appears to be hydrophobic, whereas that of the larger mRNA (1.7–1.8 kb) is acidic (10). This may lead to differences in chromatographic behavior or in interactions with other cellular proteins.

Several cDNA clones encoding the mouse low-molecular-weight 2-5A synthetase gene have been isolated (11). These hybridize to a single 1.7-kb mRNA from interferon-treated mouse JLSV9 cells (11). Although there is evidence in the mouse for a large mRNA species encoding 2-5A synthetase activity (25), this must represent either an unspliced precursor which is processed in *Xenopus* oocytes to give active synthetase mRNA or a transcript with only limited sequence homology to the small enzyme. The

Table 1. Segregation of Human 2-5A Synthetase Sequences with Human Chromosomes in Somatic Cell Hybrids

		Human chromosome[b]																						
Gene	Chromosome[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
+	+	4	4	9	4	5	3	8	5	1	4	4	11	4	10	3	5	5	6	6	5	7	2	8
+	−	7	7	2	7	6	8	3	6	10	7	6	0	7	0	8	6	6	5	5	6	4	9	2
−	+	2	3	3	5	7	2	6	1	0	3	5	0	2	6	3	1	5	4	3	7	6	3	9
−	−	9	8	8	5	4	9	5	10	11	7	6	11	9	5	8	10	6	2	8	4	4	8	1
% discordancy ($N = 22$)		41	45	23	57	59	45	41	32	45	48	52	0	41	29	50	32	50	41	36	59	48	57	55

[a]Chromosome scored "+" if present in >10% of metaphases scored.
[b]Chromosomes 4, 10, 11, 14, 21, and X were not scored in one hybrid each, due to X; autosome or autosome; autosome translocations present in human parental cells.

degree of homology (65% at the amino acid level) between the mouse and the human enzymes (11) and the finding that they are both encoded by mRNA of similar size (11) suggest that difficulties may arise in attempting to make chromosomal assignments based on data obtained from gel chromotography of crude or partially purified enzyme preparations. The complete characterization of the mouse 2-5A synthetase gene and its transcripts, and comparison with the human counterpart, should resolve the outstanding questions about the structure of the 2-5A synthetase gene and nature of its products.

Thus, there would appear to be perhaps two explanations for the discrepancy in the mapping results of Shulman et al. (14) and ourselves. First, the low-molecular-weight synthetase activity detected by Shulman et al. under the influence of human chromosome 11 may not be human in origin. Indeed, the considerations discussed above suggest that the species distinctions based on activity and chromotographic determinations alone need to be interpreted with caution. Second, the cDNAs described by Saunders et al. (9) and Benech et al. (8, 10), which are homologous to sequences on chromosome 12, may not represent the same gene as that being assayed by Shulman et al (14). In such a case, since the independent data from two groups (9–11) clearly support the conclusion that these cDNAs correspond to the human low-molecular-weight 2-5A synthetase gene, it is unclear what role a chromosome 11-encoded gene may play. In any event the data presented here provide direct evidence for the assignment of the human 2-5A synthetase gene to chromosome 12.

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