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Identification of a yeast artificial chromosome clone encoding an accessory factor for the human interferon γ receptor: Evidence for multiple accessory factors

(chromosome 21/class I major histocompatibility complex antigens/signal transduction)

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ABSTRACT **Human chromosomes 6 and 21 are both necessary to confer sensitivity to human interferon γ (Hu-IFN-γ), as measured by the induction of human HLA class I antigen. Human chromosome 6 encodes the receptor for Hu-IFN-γ, and human chromosome 21 encodes accessory factors for generating biological activity through the Hu-IFN-γ receptor. A small region of human chromosome 21 that is responsible for encoding such factors was localized with hamster–human somatic cell hybrids carrying an irradiation-reduced fragment of human chromosome 21. The cell line with the minimum chromosome 21-specific DNA is Chinese hamster ovary 3x1S. To localize the genes further, 10 different yeast artificial chromosome clones from six different loci in the vicinity of the 3x1S region were fused to a human–hamster hybrid cell line (designated 16-9) that contains human chromosome 6q (supplying the Hu-IFN-γ receptor) and the human *HLA-B7* gene. These transformed 16-9 cells were assayed for induction of class I HLA antigens upon treatment with Hu-IFN-γ. Here we report that a 540-kb yeast artificial chromosome encodes the necessary species-specific factor(s) and can substitute for human chromosome 21 to reconstitute the Hu-IFN-γ-receptor-mediated induction of class I HLA antigens. However, the factor encoded on the yeast artificial chromosome does not confer antiviral protection against encephalomyocarditis virus, demonstrating that an additional factor encoded on human chromosome 21 is required for the antiviral activity.**

Human interferon γ (Hu-IFN-γ) is a protein that induces a variety of biological responses such as antiviral, antiproliferative, and immunomodulatory activities in sensitive cells (1–5). Immunoregulatory functions induced by Hu-IFN-γ, such as induction of class I and class II human HLA antigens, activation of macrophages, regulation of immunoglobulin class switching, and up-regulation of Fc receptor expression are involved in modulating a variety of other host defense mechanisms (5–10). The first event in inducing these responses is the specific binding of IFN-γ to its cell-surface receptor encoded on human chromosome 6 (11) or mouse chromosome 10 (12). Human chromosome 6 also encodes the human class I major histocompatibility complex (MHC) antigens (13). However, human chromosomes 6 and 21 (14, 15) and mouse chromosomes 10 and 16 (16) are required for sensitivity to IFN-γ, as measured by the induction of class I MHC antigens, which indicates that the binding of IFN-γ to the receptor is not sufficient to induce these antigens. Interaction between the extracellular domain of the Hu-IFN-γ receptor and the species-specific factors that leads to class I HLA antigen induction upon treatment with Hu-IFN-γ was suggested by experiments with chimeric receptors (17–19). Furthermore, a 5-amino acid sequence (YDKPH) of the intracellular domain of the Hu-IFN-γ receptor is required for this activity (20, 21). Moreover different accessory factors are involved in encephalomyocarditis virus (EMCV) and vesicular stomatitis virus antiviral activity (20). However, little is known about how the binding of Hu-IFN-γ to its receptor initiates this diverse array of functional changes.

The region of chromosome 21 necessary for sensitivity to Hu-IFN-γ, as assayed by the expression of class I HLA antigens, was further localized within a region of ≈1–3 Mb of chromosome 21q with irradiation-reduced somatic hybrid cells (22). The cloned Hu-IFN-γ-receptor cDNA (23, 24) was expressed in hybrid cells that have human chromosome 21q as the sole human chromosome to reconstitute a biologically active Hu-IFN-γ receptor for HLA class I antigen induction (25) and EMCV antiviral protection activity (20). Thus, human chromosome 21q appeared to encode all necessary factors for both activities. Also a recent study with mouse–human hybrid cells transfected with the Hu-IFN-γ receptor cDNA showed that human chromosome 21 is sufficient for induction of class I MHC antigens, EMCV antiviral activity, and induction of 2′,5′-A oligoadenylate synthetase in response to Hu-IFN-γ (26).

The cloning of DNA into yeast artificial chromosomes (YACs) has allowed isolation of much larger DNA fragments than was previously possible (27). Also, development of PCR-based YAC screening methods has facilitated the identification of specific YAC clones (28). These large cloned segments have potential applications for the isolation of functional domains from chromosomes (29). To assay for the biological functions of genes included in YACs, yeast spheroplasts can be fused to cultured mammalian cells. Experiments to demonstrate the functional expression of genes carried on YACs have been done with glucose-6-phosphate dehydrogenase, hypoxanthine phosphoribosyltransferase, and phosphoribosylglycinamide formyltransferase (GART) YAC clones (30–32). To allow selection for fused cells in these cases, a gene cassette that confers resistance to antibiotic G418 in mammalian cells can be introduced into the YAC by homologous recombination targeted to human genomic sequences (33, 34). We obtained 10 YACs that were screened by various primer pairs generated from six different markers located in the vicinity of the 3x1S region of human chromosome 21q. After integration of the neomycin (G418)-resistance gene into the YACs, the yeast spheroplasts were

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Abbreviations: YAC, yeast artificial chromosome; PFGE, pulsed-field gel electrophoresis; IFN, interferon; Hu-IFN-γ, human IFN γ; CHO, Chinese hamster ovary; GART, phosphoribosylglycinamide formyltransferase; EMCV, encephalomyocarditis virus; MHC, major histocompatibility complex; neo^r, neomycin resistance.

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fused to hamster cells containing human chromosome 6q and the human *HLA-B7* gene (14, 15) to determine which YACs contain genes that confer sensitivity to Hu-IFN-γ in terms of class I HLA antigen induction and protection from EMCV infection. This report describes the identification of a YAC clone exhibiting the chromosome 21 accessory factor activity that confers class I MHC antigen induction, but does not confer EMCV antiviral protection.

MATERIALS AND METHODS

Cells. The 16-9 cell line, derived from parental line 836-3A, is a Chinese hamster ovary (CHO-K1) hybrid containing a translocation of the long arm of human chromosome 6 and a transfected human *HLA-B7* gene obtained from S. Weissman (Yale University, New Haven, CT) (refs. 14, 15, 35, 36; A. Rashidbaigi, personal communication). The 3x1S cells are CHO-K1 cells that contain a small 1- to 3-Mb region of human chromosome 21 (22).

Ten YAC clones were obtained through the Chromosome 21 Joint YAC Screening Effort (directed by David Patterson, Eleanor Roosevelt Institute, Denver). YAC clones were maintained and grown in AHC medium (37). The addresses of the YAC clones used in this study are 525 B119G7, 525 A165D7, 525 C4C10, 525 C14B2, H8(517) A222A12, GART D142H8, 518 A234B10, 518 B134B9, SOD D112A5, and IFNAR B49F1. All YACs were maintained in *Saccharomyces cerevisiae* AB1380 (*Mata, ura3, trp1, ade2-1, can1-100, lys2-1*, and *his5*) (27).

Construction of Integrating Plasmid. A 5.4-kb *Hin*dIII restriction fragment containing the *S. cerevisiae LYS2* gene (from Jules O'Rear, Robert Wood Johnson Medical School, Piscataway, NJ) was inserted into the *Hin*dIII site of pMC1neo Poly A (Stratagene) to yield the plasmid pMC-LYS2. A 0.3-kb *Alu* family repeat in plasmid pBP47 (33) (from Roger H. Reeves, Johns Hopkins University School of Medicine, Baltimore) was modified by inserting a *Cla* I linker into the *Eco*RI site to form plasmid pBP47-*Cla*I. The modified *Alu* fragment was cut from plasmid pBP47-*Cla*I with *Bam*HI, treated with mung bean nuclease, and ligated to the *Sal* I site of pMC-LYS2 after filling-in with the Klenow fragment of DNA polymerase I, yielding plasmid pJS1, the integrating plasmid.

Fusion. To construct YACs containing the antibiotic G418 marker, yeast spheroplast transformation was done with 5 μg of linearized plasmid pJS1 (37). Each of the YAC clones containing the neomycin-resistance (neo^r) gene was fused to 16-9 cells as described (33). Thirty-six hours later, plates were washed with phosphate-buffered saline to remove dead cells and yeast, then refed with complete F-12 medium containing G418 at 450 μg/ml (GIBCO). Cells were fed as necessary, and resistant colonies typically appeared after 10–14 days. The G418-resistant colonies were pooled and expanded for further analysis.

Electrophoresis, Hybridization Procedures, and Assays. Agarose plugs were prepared as reported (38). The agarose plugs were analyzed by pulsed-field gel electrophoresis (PFGE) (CHEF, OWL Scientific Plastics, Cambridge, MA). The DNAs were blotted onto Nytran (Schleicher & Schuell) by standard Southern blot procedures (24, 25). DNA probes were labeled with [α-^{32}P]dCTP (NEN, 3000 Ci/mmol; 1 Ci = 37 GBq) with random hexadeoxynucleotides as primers (39). Cytofluorographic analysis of cells for expression of the HLA-B7 surface antigen was done as described (15, 25), except that antibiotic G418 was omitted because it was found that its presence inhibited MHC induction (T.M.M., unpublished observation). Transfected cells were assayed for resistance to EMCV by a cytopathic-effect inhibition assay (40).

RESULTS

3x1S Region YACs. The 3x1S somatic cell hybrid contains DNA that encodes one component of the human type I IFN receptor (22, 41–43) as well as the accessory factors responsible for class I HLA antigen induction upon binding of Hu-IFN-γ to its receptor (14, 15, 22, 44). The segment of human chromosome 21 in 3x1S cells is referred to as the 3x1S region, which is ≈1–3 Mb in size, depending on the estimation method (ref. 44; R.J.D., unpublished data). To identify the gene(s) for accessory factor(s) for the Hu-IFN-γ receptor system, Jung (44) prepared a cosmid library from 3x1S DNA and screened the library with labeled total human DNA so that cosmid clones containing highly repetitive human sequences (*Alu, Kpn*, etc.) could be selected. More than 200 cosmid clones were isolated, which theoretically covered the 3x1S region two to six times. The resultant cosmids were transfected into CHO cells that had been stably transfected with both the *HLA-B7* gene and Hu-IFN-γ-receptor cDNA in an attempt to reconstitute a biologically active Hu-IFN-γ-receptor system capable of inducing human class I HLA antigen expression. This approach was not successful, possibly because this region may contain several genes necessary for activity that cannot be cloned into a single cosmid, the gene is too large to be accommodated in a single cosmid vector, or there was a bias during screening with human repetitive DNA so that the particular region of DNA was not represented in the cosmid clones positive for human DNA. For these reasons, we chose to use YAC clones to carry out a similar experiment to that described above because YAC clones can maintain >1 Mb of DNA as an exogenous fragment (27, 45). Through the Chromosome 21 Joint YAC Screening Effort, we obtained 10 YAC clones screened with various primer pairs derived from six different loci that are scattered in the vicinity of the 3x1S region as shown in Fig. 1 and Table 1. The superoxide dismutase (*D21SOD1*), *D21S58*, GART (*D21PRGS*), *D21S65*, H8 (*D21S17*), and IFNAR (*D21IFNAR*) loci were known to be in the 3x1S region (22). Although the *D21S55* locus was not in the 3x1S region, it was close enough to the 3x1S region to be tested. The YAC derived from the *D21S58* locus was not available in time to be included in this study. The 525 B119G7 YAC and IFNAR B49F1 YAC are chimeric YACs that have noncontiguous human DNA fragments (David Patterson, personal communication). The SOD D112A5 YAC clone contains two

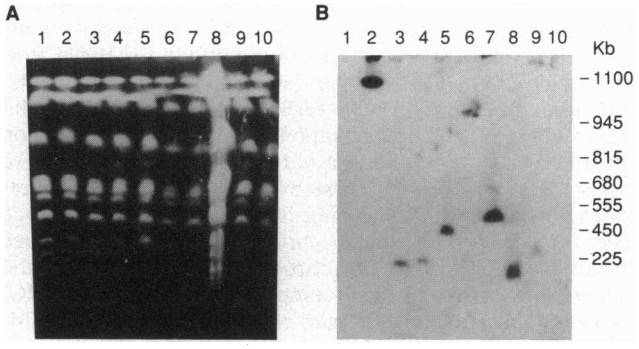

FIG. 1. PFGE of various YACs and hybridization to total human DNA. (*A*) PFGE analysis of nine YAC clones from the 3x1S region of human chromosome 21q. Lanes: 1, *S. cerevisiae* AB1380; 2, 525 B119G7 (1200 kb); 3, 525 A165D7 (210 kb); 4, 525 C4C10 (220 kb); 5, 525 C14B2 (450 kb); 6, H8(517) A222A12 (220 kb); 7, GART D142H8 (540 kb); 8, 518 A234B10 (210 kb); 9, 518 B134B9 (225 kb); and 10, SOD D112A5 (180 kb, 120 kb). IFNAR B49F1 (150 kb) is not shown here. (*B*) Southern hybridization of the PFGE gel. The blot from *A* was probed with total human DNA labeled by random priming. The size of each YAC is shown above in parentheses. Numbers at right of the blot indicate the size of various markers in kilobase pairs (Kb).

Table 1. Functional assay of YAC clones

Locus	Probe name	Address of YAC	Size of YAC, kb	YAC.neo^r	G418-resistant colonies, no.	MHC response
D21S65	525	B119G7	1200	525B119G7.neo.1	2	−
				525B119G7.neo.18	0	NA
		D165D7	210	525A165D7.neo.12	0	NA
				525A165D7.neo.16	0	NA
		C4C10	220	525C4C10.neo.3	0	NA
				525C4C10.neo.20	0	NA
		C14B2	450	525C14B2.neo.2	0	NA
				525C14B2.neo.19	0	NA
D21S17	H8 (517)	A222A12	220	H8(517)A222A12.neo.6	0	NA
				H8(517)A222A12.neo.13	3	−
D21PRGS	GART	D142H8	540	GARTD142H8.neo.3	4	+
				GARTD142H8.neo.16	1	−
				GARTD142H8.neo.18	Many	+
D21S55	518	A234B10	210	518A234B10.neo.2	0	NA
				518A234B10.neo.15	0	NA
		B134B9	225	518B134B9.neo.8	0	NA
D21SOD1	SOD	D112A5	180		0	NA
			120		0	NA
D21IFNAR	IFNAR	B49F1	150	IFNARB49F1.neo.32	5	−
				IFNARB49F1.neo.35	0	NA

The MHC response of the last column represents human class I HLA antigen induction upon treatment with Hu-IFN-γ: −, no response; +, positive response; NA, not applicable. Transfected cells were tested as pools of G418-resistant colonies. The SOD D112A5 YAC was not tested for HLA induction because a neo^r-positive YAC was not obtained from the transformation. The dish of transformants with GARTD142H8.neo.18 (YAC-JS2) contained many small colonies.

different YACs, yet we do not know which one is the SOD YAC. Until we know how the clones overlap, it is difficult to estimate how much of the 3x1S region is covered by these YAC clones. However, if we assume no overlap between the YACs from different loci, except the chimeric 525 B119G7 and IFNAR B49FI YACs, then five different YAC clones (525 C14B2, 517 A222A12, GART D142H8, 518 B134B9, SOD D112A5) may cover ≈1.5 Mb of the 3x1S region, which represents at least 50% of the region.

Integration of the Neo^r Gene into YACs. To test the function of these YACs, the neo^r gene was introduced into the YACs by targeting homologous recombination to *Alu* repeat sequences. A similar procedure was described (33) for *S. cerevisiae* YPH252. Because all of the 3x1S region YACs were derived from *S. cerevisiae* AB1380, which has a genotype different from that of *S. cerevisiae* YPH252, another integrating plasmid, pJS1, containing a modified human *Alu* family member BLUR13·ClaI, the yeast *LYS2* gene, and the neo^r gene was designed as described under *Materials and Methods*. Digestion of pJS1 with *Cla* I produces a linear 10-kb molecule with two free ends homologous to *Alu* sequences. The *Cla* I-linearized plasmid should then target integration to *Alu* sequences on the YAC by homologous recombination. This event introduces the *LYS2* gene (and neo^r gene) into the YAC clones so that Lys2$^+$ transformants can be selected on yeast minimal medium lacking lysine. Twenty Lys2$^+$ transformants were selected from each of 10 different YAC clones transformed with integrating plasmid pJS1. To check for integration of the neo^r gene into yeast DNA, each of the 20 Lys2$^+$ transformants was screened by colony hybridization with the neo^r gene as a probe (data not shown). The frequency of a neo^r gene in the Lys2$^+$ transformants varied from 5% to 80%. It seems that in some cases only the *LYS2* gene was selectively integrated during recombination. To confirm that the neo^r gene was integrated into the YAC rather than the endogenous yeast chromosomes, two neo^r positive clones from each YAC were selected for analysis by PFGE and hybridization with probes for the neo^r gene (Fig. 2*A*). About 76% of the neo^r-positive clones were targeted to the YAC rather than yeast chromosome II, which contains a mutated *LYS2* gene (data not shown). Because the *LYS2* gene in *S. cerevisiae* AB1380 is almost intact, it is possible that the *LYS2* gene in the integrating plasmid was targeted to the endogenous yeast *LYS2* sequence in some cases. We note that the GART D142H8.neo.18 clone had a smaller YAC than the original one (Fig. 2*A*). In this case, it is possible that two distant *Alu* sequences were targeted, and the internal DNA fragments between them were lost during recombination (46). To test whether the neo^r gene cassette is integrated into the YAC intact, total yeast chromosomal DNA from targeted YAC clones were digested with *Bam*HI and *Xho* I, and the blot was probed with labeled neo^r gene. As shown in Fig. 2*B*,

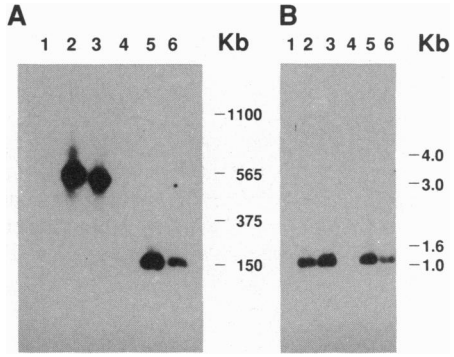

FIG. 2. Southern hybridization. (*A*) Agarose plugs from neo^r Lys2$^+$ transformants derived from each YAC clone were analyzed by PFGE, and the blot was probed with the labeled neo^r gene to determine whether or not the neo^r gene was targeted to the YAC. Lanes: 1, GART D142H8; 2, GART D142H8.neo.16; 3, GART D142H8.neo.18; 4, IFNAR B49F1; 5, IFNAR B49F1.neo.32; and 6, IFNAR B49F1.neo.35 YACs are shown here as example. The GART D142H8.neo.18 (lane 3) clone contains smaller YACs than the original one (GART D142H8), possibly resulting from deletions generated by homologous recombination between two *Alu* sequences on the original YAC during the integration process. (*B*) Yeast chromosomal DNAs from the same YAC clones as in *A* were digested with *Bam*HI and *Xho* I to release the neo^r gene cassette, and the blot was probed with the labeled neo^r gene. Each targeted YAC clone shows a 1.1-kb fragment hybridizing to the probe, suggesting that the neo^r gene cassette was integrated intact into the YAC.

1.1-kb fragments corresponding to the neo^r gene were apparent, indicating that the neo^r gene was integrated without disruption of the coding sequence. In some cases (one out of five), DNA fragments of a different size were detected.

Fusion and Cytofluorographic Analysis. Spheroplasts from the neo^r gene-containing YAC clones were fused to 16-9 cells. After selection with G418 for 2 weeks, resistant colonies were obtained from several of the YACs (Table 1). Not all fusions generated G418-resistant colonies, possibly due to disruption of the neo^r gene during the targeting process. Colonies from the individual fusions were pooled and analyzed for IFN-dependent class I HLA antigen induction by cytofluorography. The parental 16-9 cells displayed very good class I HLA induction upon treatment with Hu-IFN-αA/D, a human IFN active on hamster cells (47) (Fig. 3*A*), and a slight reduction in HLA expression in response to Hu-IFN-γ at 1000 units/ml (Fig. 3*B*). The 16-9 cells transformed by fusion with GART D142H8.neo.18 (henceforth called YAC-JS2) also exhibited a good response to Hu-IFN-αA/D (Fig. 3*C*). In addition, they also showed good HLA induction upon treatment with Hu-IFN-γ at 1000 units/ml (Fig. 3*D*). Treatment of these transformed 16-9 cells with Hu-IFN-γ at 200 units/ml also gave a response similar to that for treatment with 1000 units/ml (data not shown). The results shown in Fig. 3 with 16-9 cells fused to YAC-JS2 were performed on several occasions with pools of clones and with individual clones with similar results. It is interesting to note that a colony derived from the GART 142H8.neo.16 YAC was negative for HLA induction by Hu-IFN-γ. This YAC clone may have a deletion of $\approx$10 kb because the apparent YAC size is similar to the original GART YAC, even after integration of the 10-kb plasmid (Fig. 2*A*, lane 2). It is difficult to conclude definitively that the negative response resulted from the deletion of a portion of the gene(s) that encodes the Hu-IFN-γ accessory factor(s) because only one colony, which may not have incorporated the entire YAC (31), was assayed. However, if we screen a sufficient number of neo^r-positive YACs that do not respond to Hu-IFN-γ, we may be able to identify the gene(s) disrupted during integration of the pJS1 plasmid.

Antiviral Activity of 16-9 Cells Transformed with YAC-JS2. It was reported that hamster–human somatic hybrid 153B7-8 cells (carrying chromosome 21q as its sole human chromosome) transfected with the Hu-IFN-γ-receptor cDNA are protected against EMCV infection and display an increase in class I HLA antigen expression upon treatment with Hu-IFN-γ (20, 25). Furthermore, because the 3x1S region of chromosome 21q is sufficient to complement the Hu-IFN-γ receptor for class I HLA induction (22, 44), we tested whether this region also encodes the accessory factor that confers protection against EMCV infection in response to Hu-IFN-γ. The results indicate that the 3x1S region of chromosome 21q encodes the accessory factors for EMCV antiviral protection (Table 2). However, 16-9 cells transformed with YAC-JS2 (expressing the accessory factors for class I HLA antigen induction) do not show any protection against EMCV. This result indicates that there are at least two accessory factors on human chromosome 21q: one is required for induction of MHC class I antigens, and a second is required for protection against EMCV.

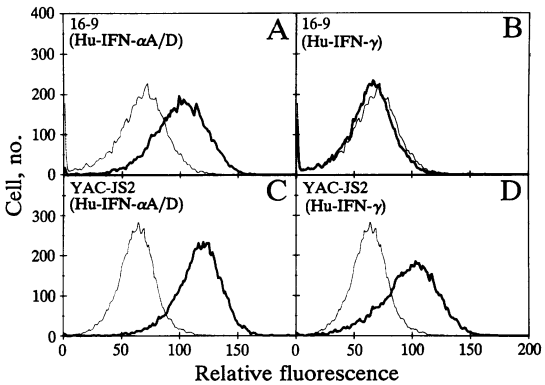

FIG. 3. Induction of HLA-B7 surface antigen in 16-9 cells fused with YAC-JS2. The HLA-B7 antigen was detected by treatment of cells with mouse anti-HLA monoclonal antibody (W6/32) followed by treatment with fluorescein isothiocyanate-conjugated anti-mouse IgG. The cells were analyzed by cytofluorography. (*A* and *B*) 16-9 cells. (*C* and *D*) Pools of 16-9 cells fused to YAC-JS2, the GART D142H8.neo.18 YAC clone. The thin line indicates cells not treated with IFN, and the heavy line indicates treatment with the indicated Hu-IFN at 1000 units/ml. *A* and *C* show treatment with Hu-IFN-αA/D, and *B* and *D* show treatment with Hu-IFN-γ. Fluorescence values shown are not linear but represent the fluorescence detection channels (of which there are 256) of the cytofluorograph. The real value of the *x* axis shown spans approximately three decades on the logarithmic scale.

DISCUSSION

Although expression of proteins has been demonstrated with YACs containing known genes (30–32), this study demonstrates a specific biological function from an undefined gene in a YAC expressed in eukaryotic cells. To introduce the G418 antibiotic-resistance gene into the YACs used in this study, an integrating plasmid (pJS1) was constructed for *S. cerevisiae* strain AB1380 that has been widely used for construction of YAC libraries. In conjunction with yeast and eukaryotic cell-fusion techniques, the integrating plasmid was used to introduce the neo^r gene into the YACs so that the functional activity of the YACs could be determined. Furthermore, the integrating plasmid can also be used to localize the region of the YAC responsible for functional activity through its ability to produce insertional inactivation of genes and deletions. Recombination with plasmid pJS1 can produce deletions of various size within a YAC. We have observed deletions of up to 350 kb for the GART D142H8 YAC.

Our study showed that the chromosome 21 accessory factor gene necessary for class I HLA antigen induction by Hu-IFN-γ is located in the 540 kb of human DNA in the GART D142H8 YAC. This observation is consistent with data that indicated that the gene for the accessory factor is located in the distal portion of band 21q22.1, near markers *D21S58* and GART (21, 43).

We previously demonstrated that the accessory factors required for vesicular stomatitus virus protection are different from those leading to EMCV protection after Hu-IFN-γ treatment (20). The observation that a small segment of human chromosome 21q (3x1S region) was sufficient to induce class I HLA antigen expression and EMCV protection with Hu-IFN-γ suggested that the same accessory factor was involved

Table 2. Antiviral effects of Hu-IFN-γ on transfected CHO cells

Cell line	DNA transfected	IFN	ED_{50}, units/ml
153B7-8	Hu-IFN-γR cDNA	Hu-IFN-γ	2
3x1S	Hu-IFN-γR cDNA	Hu-IFN-γ	14
16-9	YAC-JS2	Hu-IFN-γ	>10,000

Transfected cells were assayed for resistance to EMCV by a cytopathic-effect inhibition assay (40). The 153B7-8 cells are CHO-K1 cells containing a transfected *HLA-B7* gene and human chromosome 21q (15, 24). Irradiation-reduced somatic cell hybrid 3x1S cells containing a 1- to 3-Mb fragment of human chromosome 21q were described (21). The somatic cell hybrid 16-9 cells contain human *HLA-B7* gene and human chromosome 6q encoding the Hu-IFN-γ receptor. The ED_{50} represents the concentration of IFN-γ (units/ml) that provided 50% protection of cells against EMCV. All cells were protected from EMCV infection by Hu-IFN-αA/D(Bgl) in control experiments. Each of these cell lines was derived from the same parental CHO-K1 cells.

in both activities (20). However, the observation that 16-9 cells transformed with YAC-JS2 were not protected against EMCV challenge is evidence that another accessory factor is necessary for this antiviral activity or that both the antiviral factor and the class I MHC induction factor are required for this antiviral activity. Thus, our data indicate that the distinct additional factor responsible for the EMCV antiviral effect present in the 3x1S region is not included in the GART D142H8 YAC. One might argue that the factor for antiviral activity is in the GART D142H8 YAC but the gene was disrupted due to targeting of the integration plasmid. However, because two separate pools of 16-9 cells fused with different neo^r gene-targeted YACs (GARTD142H8.neo.3 and YAC-JS2; Table 1) showed MHC class I induction but no protection against EMCV when treated with Hu-IFN-γ, the argument for disruption of the same gene is less convincing.

The identification of a YAC that encodes the species-specific accessory factor for class I HLA induction by Hu-IFN-γ should eventually lead to identification of the cDNA for the protein. This information will help us to understand the interactions between the Hu-IFN-γ receptor and the accessory factors. To locate the genes and to find cDNAs for the accessory factors, various approaches such as YAC fragmentation (46), exon-trapping (48), hybrid selection of cDNA with the YAC (49), and direct screening of the cDNA library with the YAC (50) can be done. We have mapped the GART D142H8 YAC with some restriction endonucleases (*Not* I and *Asc* I). The mapping data suggest that the GART D142H8 YAC is similar to yGART2 (600 kb) described by Gnirke *et al.* (32) because both YACs have two *Not* I sites and the GART gene is located between two *Not* I sites (170 kb). Our results are consistent with and extend the results of Chumakov *et al.* (51), who reported a physical map of human chromosome 21 constructed with the use of overlapping YACs. Our current efforts are directed at defining the specific gene or genes on the GART D142H8.neo.18 YAC that contribute the chromosome 21 accessory function required for activity of the Hu-IFN-γ receptor. This study and our previous studies (20, 44) show that accessory factor functions for class I MHC induction, protection of cells against EMCV infection, and protection against vesicular stomatitis virus infection are mediated by different factors or a different combination of factors. We thus conclude that a family of accessory factors that mediate specific intracellular signals is associated with the IFN-γ receptor. These factors are probably qualitatively and quantitatively different in various tissues, a hypothesis that explains the individual responses of different cells and tissues to IFN-γ.

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