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# SOMATIC CELL GENETIC ANALYSIS OF THE INTERFERON SYSTEM

*(interferon genetics, interferon receptors, cell hybrids)*

**DORIS L. SLATE and FRANK H. RUDDLE**

Department of Biology
Yale University
P. O. Box 6666
New Haven, CT 06511

## SUMMARY

*The interferon system is amenable to somatic cell genetic analysis since the genes governing the synthesis of interferon and the cellular responses to interferon can be expressed in tissue culture cells. Limited species specificity exists in the interferon system, allowing interspecific hybrid cells to be used in mapping genes involved in interferon production and mechanism of action.*

*The synthesis of interferon and the attainment of the antiviral state following interferon treatment are separate genetic functions, governed by genes on different chromosomes. Three different human chromosomes have been implicated in the production of human fibroblast interferon based on studies using interspecific somatic cell hybrids. The recent cloning of multiple interferon gene sequences will provide probes for further gene mapping. Genes governing sensitivity of cells to interferon have been mapped to human chromosome 21 and mouse chromosome 16.*

*When human/mouse somatic cell hybrids are injected into mice of the same strain as the murine parent of the hybrid, antibodies directed against cell surface components coded by the retained human chromosomes are produced. Antibodies raised against human chromosome 21-coded cell surface determinants can block human interferon action on human fibroblasts, presumably by blocking a human interferon receptor. Monoclonal antibodies against this receptor can be produced and used to study the role of receptors in interferon action.*

## INTRODUCTION

Interferons comprise a group of proteins and glycoproteins

which exert a wide range of biological effects on target cells. They were first described by Isaacs and Lindenmann in 1957, and are best known for their ability to induce an antiviral state in sensitive cells. Cells in the antiviral state are unable to support growth and replication of a wide range of viruses. The synthesis of interferon can be induced by viral infection, exposure to double-stranded RNA, or by other stimuli, depending on the cells involved. Interferon is released from the producing cell and binds to cell surface receptors on other cells. This binding triggers a number of intracellular events in the responding target cell, including transcription and translation of new proteins, and the modification of some constitutive proteins (BAGLIONI 1979). Interferon also affects cell proliferation and can modulate the immune response (see STEWART 1979).

Interferon synthesis in response to various inducers and the attainment of the antiviral state following interferon treatment both require de novo RNA and protein synthesis. Messenger RNA isolated from cells after induction can be translated in cell-free systems, in Xenopus oocytes, or after microinjection into heterologous cells to give functionally active interferon. Several groups have cloned interferon sequences, both in the form of cDNA and genomic DNA (see DERYNCK et al. 1980, TANIGUCHI et al. 1980). Cloned sequences have been inserted into bacteria and can be expressed (see NAGATA et al. 1980).

Interferon is assayed by its biological activity, usually by quantitating its ability to protect cells from virus challenge. One unit is defined as that amount which reduces a virus growth parameter (for example, cytopathic effect on a cell monolayer or number of plaques) by 50 per cent compared with controls. Purified interferon has a specific activity of approximately $10^8 - 10^9$ units/mg protein. Given an average molecular weight of 20,000 daltons (range 15,000-35,000), interferon is active at concentrations as low as $10^{-13}$ or $10^{-14}$ M.

The mechanism of action of interferon is not definitively known, although numerous studies have revealed interesting biochemical changes in interferon-treated cells. Depending on the cells and viruses being studied, the inhibition of viral replication has been reported to occur at many levels: transcription, translation, and assembly or budding of mature virions (see STEWART 1979). Several possible pathways for translational inhibition in interferon-treated cells have been described: double-stranded RNA-dependent phosphorylation of an initiation factor (eIF-2) subunit which inhibits initiation complex formation; activation of an endoribonuclease by pppA(2'p5'A)n, a molecule synthesized by an enzyme dependent on double-stranded RNA, and degradation of the -CCA amino acid accepting end of transfer RNA molecules by a phosphodiesterase.

Within a given species, several types of interferon can be produced, depending on cell type and inducing stimulus. In the human interferon system, three major antigenic types of

interferon have been described: α (leukocyte), β (fibroblast), and γ (immune). The usual cells of origin for these three types are buffy coat leukocytes or established lymphoblastoid lines, fibroblasts, and T cells, respectively. A single cell type may produce more than one type of interferon, depending on what inducer is used to trigger interferon synthesis (HAVELL, HAYES & VILCEK 1978).

## SOMATIC CELL GENETIC STUDIES

Since interferon production and response are inducible phenotypes which can be studied in tissue culture cells, somatic cell genetic techniques can be employed to define and map loci involved in the interferon system. Limited species specificity (human interferons generally being more active on human cells than on mouse cells and vice versa) suggested that interspecific heterokaryons and hybrids could be used in the gene mapping studies.

Cells of two species can be fused with inactivated Sendai virus, polyethylene glycol, or other agents to form heterokaryons. After the genetic material from both parental cells is combined in a single nucleus, the resulting hybrid cell undergoes partial loss of one parental chromosome set. This "parasexual" system involving chromosome segregation permits gene mapping for the species whose chromosomes are lost. By correlating the presence of marker isozymes and an interferon phenotype, synteny assignments can be made for the genes involved. Identification of the specific chromosomes retained in a hybrid through banding and differential staining techniques is then used to assign genes to given chromosomes.

Early studies with human/chick heterokaryons (GUGGENHEIM et al. 1968) suggested that interferon structural genes from both parents could be expressed simultaneously. Heterokaryons and hybrids could also respond to the interferons of both parental types (GUGGENHEIM et al. 1969, CARVER et al. 1968).

Monkey/mouse hybrid cells segregating monkey chromosomes were used to make the first chromosome assignment for a gene governing the synthesis of monkey interferon (CASSINGENA et al. 1971). The monkey gene governing the establishment of the antiviral state was determined to be asyntenic to the gene for interferon production.

Experiments with human/mouse somatic cell hybrids segregating human chromosomes indicated a concordant segregation of the interferon-induced "antiviral protein" and indophenol oxidase B (cytoplasmic superoxide dismutase). These genes were assigned to human chromosome 21 (TAN, TISCHFIELD, & RUDDLE 1973, CHANY et al 1975). TAN et al. (1974) then examined the effect of chromosome 21 dosage on the expression of the human interferon induced antiviral state. The amount of interferon required by diploid human cell lines was 3 to 7 times higher than for trisomy 21 cell lines; monosomy 21 cells require even higher doses of interferon.

The product of the gene on chromosome 21 responsible for conferring sensitivity to human interferon has not been isolated, although a cell surface receptor for interferon is the most likely candidate. Different levels of interferon binding have been recorded for cells with different numbers of copies of chromosome 21 (WIRANOWSKA-STEWART & STEWART 1977). The gene governing human interferon sensitivity has been regionally mapped to the long arm of chromosome 21 (TAN & GREENE 1976, EPSTEIN & EPSTEIN 1976). Sensitivity to both leukocyte and fibroblast interferons appears to map to this region.

SLATE et al. (1978) demonstrated that a human/mouse somatic cell hybrid which retains chromosome 21 as the only detectable human genetic material could respond to human leukocyte and fibroblast interferons. The response was measured by inhibition of vesicular stomatitis virus yields and virus-induced cytopathic effects, and by assessing biochemical changes in extracts from interferon-treated cells. Human interferon could trigger mouse-specific elements of the antiviral response in this 21-only hybrid cell line.

Injecting mice with human/mouse somatic cell hybrids results in the production of antibodies which are directed against human cell surface components, provided that the mice are of the same strain as the murine parent of the hybrid. Antibodies raised against hybrid cells retaining human chromosomes 4, 21, and 22 could block the action of human interferon in diploid human fibroblasts (REVEL, BASH & RUDDLE 1976). This blocking activity was more pronounced in monosomy 21 cells and decreased as the number of copies of chromosome 21 increased. This is consistent with the gene dosage effect seen for human interferon sensitivity. Preabsorption of the antiserum on cells with chromosome 21 could remove its blocking activity, while preabsorption on cells retaining chromosomes 4 and 22 (but lacking 21) could not. These studies have been extended to antisera raised against human/mouse hybrids retaining human chromosomes 21 and 22 or just 21 alone with similar results (SLATE & RUDDLE 1978).

Although the usual pattern for chromosome segregation in human/rodent hybrid cells involves the preferential loss of human chromosomes, "reverse" segregant hybrids can be constructed by fusing primary mouse cells to transformed human cells. Using this approach, LIN et al. (1979) mapped the mouse gene responsible for conferring sensitivity to interferon to murine chromosome 16. COX et al. (1979) have also mapped the mouse interferon sensitivity gene to chromosome 16 using mouse/Chinese hamster hybrids segregating mouse chromosomes. Both groups found a syntenic relationship for the genes coding for mouse interferon sensitivity and cytoplasmic superoxide dismutase. This same syntenic relationship exists for the two corresponding human genes.

Although the classical somatic cell genetic analyses outlined above give clear-cut results for the chromosomal location of the genes governing interferon sensitivity, the location of

interferon structural genes is still debated. TAN, CREAGAN & RUDDLE (1974) found that human chromosomes 2 and 5 together were necessary for human interferon production in human/mouse somatic cell hybrids. More recent investigations have suggested that chromosome 5 alone (MORGAN & FALK 1977, TAN, TAN & BERTHOLD 1977) is responsible for human interferon production. SLATE & RUDDLE (1979) found evidence for separate fibroblast interferon loci on chromosomes 2 and 5. MEAGER et al (1979) have presented evidence for a single fibroblast interferon locus on human chromosome 9. With the recent discovery that interferons comprise a multigene family, it may be possible to reconcile these apparent disagreements (GOEDDEL et al. 1981, SEHGAL & SAGAR 1980).

## CURRENT RESEARCH EFFORTS

Our current research on the interferon system is focused on two major areas: mapping human interferon structural genes by a combination of classical somatic cell genetics and recombinant DNA techniques and generating monoclonal antibodies against the putative human interferon receptor. We will briefly outline the approaches we are taking in these projects.

As the number of human interferon genes which have been cloned by recombinant DNA technology increases, so does the number of probes which can be used to map these genes in somatic cell hybrids. The basic design of this mapping project is outlined in Figure 1. DNA is isolated from a number of human/rodent hybrid cells which have been carefully analyzed for their retention of human chromosomes. This DNA is digested with restriction endonucleases and electrophoresed in agarose gels to separate the digested DNA fragments by size. The DNA is then transferred onto nitrocellulose filters by Southern blotting and hybridized to a radioactively labelled interferon

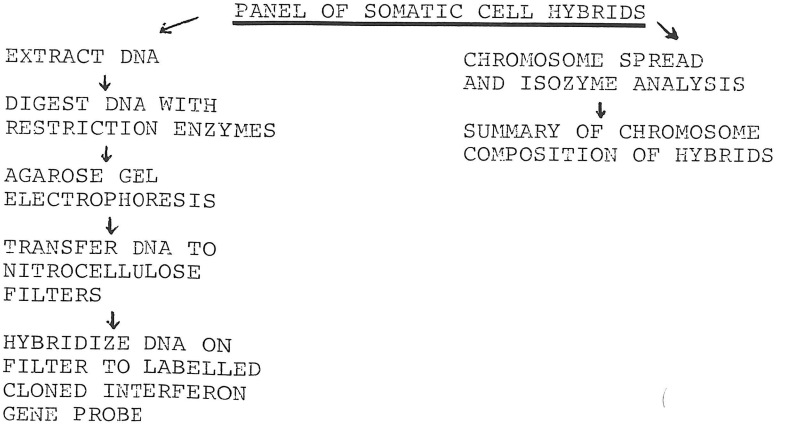


Figure 1. Scheme for identifying chromosomal location of cloned interferon gene sequence.

sequence probe. By comparing the hybridization pattern of the different hybrids to human and rodent DNA controls, it is possible to determine which hybrids have retained the given interferon sequence and to map the sequence to a particular human chromosome. Experiments of this type are being done in a number of different laboratories for both α and β interferon sequences. A major advantage of this mapping strategy is that it bypasses the need for expression of the interferon genes in a hybrid cell after induction.

Our second major area of interest in the interferon system is the development of monoclonal antibodies against the human interferon receptor. These studies are an extension of the work with mouse antibodies directed against chromosome 21-coded cell surface determinants discussed earlier. The basic strategy we employ is outlined in Figure 2. Spleen cells are removed from mice which have been injected with a human/mouse hybrid line retaining only human chromosome 21 and which are producing antibodies which block human interferon action. These cells are fused with an established mouse myeloma line to produce hybridomas which secrete antibodies into their tissue culture medium. The hybridomas are cloned, and the incubation

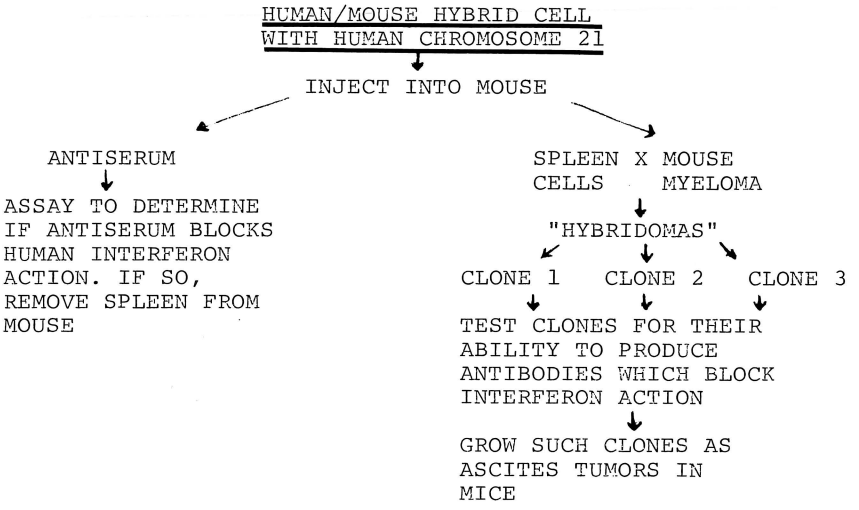


Figure 2. Strategy for identifying monoclonal antibodies directed against the human interferon receptor.

medium tested for the presence of antibodies which block interferon action. Once a line producing monoclonal antibody with good blocking activity is identified, it can be injected into mice and be grown as ascitic tumors.

## CONCLUSION

Somatic cell genetic techniques have permitted the

analysis of the genetic control of interferon production and response. Coupled with recent developments in recombinant DNA technology and immunology, these techniques should allow further mapping and a more detailed picture of the regulation of interferon gene expression.

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