Interferon Production and Action in Mouse, Hamster, and Somatic Hybrid Mouse-Hamster Cells

Abstract. *A hybrid mouse-hamster cell line was developed from a mouse cell line which produces a high titer of interferon and is sensitive to its action, and a hamster cell line which produces little interferon and is relatively insensitive to its action. Parental cell lines demonstrated complete species specificity with respect to interferon production and action. The hybrid cells produced interferon (or interferons) effective when tested on the mouse cell line and primary hamster cells; the hybrids were sensitive to the action of both mouse and hamster interferons. Hybrid cells produced ten times more hamster interferon than the parent hamster cell line and were eight times more sensitive to hamster interferon than the parent hamster cell line.*

The species specificity of interferons has been well documented (*1*, *2*). A somatic hybrid mouse-hamster cell line has been developed (*3*) from a mouse cell line and a hamster cell line. The mouse cell line produces a high titer of interferon and is sensitive to its action, and the hamster cell line produces little interferon and is relatively insensitive to its action. Therefore, the hybrid cells provide a unique opportunity for studying the expression of genes concerned with the production of interferon, as well as the genes concerned with the action of interferon.

Hybrid cells were isolated according to Littlefield's method (*4*), his drug-resistant mutant mouse and hamster cell lines being used as parents. A subclone of mouse L929, A_9 (*5*), which lacks hypoxanthine guanine phosphoribosyl transferase was mated in culture with B_1 (*6*), a Syrian hamster clone lacking thymidine kinase, derived from baby hamster kidney. These enzyme deficiencies prevent either parent cell from using hypoxanthine and thymidine in the medium to make purines and thymidylic acid when the endogenous synthesis of these nucleotides is blocked with aminopterin. The hybrid cells by complementation have both necessary enzymes and can be isolated since they can grow in a medium containing hypoxanthine, thymidine, and aminopterin which does not permit the growth of the parent cells.

The clone of hybrid cells used for these experiments, A_9B_1 clone No. 12, has been described (*3*); it has a hybrid karyotype and hybrid molecules for malate dehydrogenase, lactate dehydrogenase, and 6-phosphogluconate dehydrogenase on starch-gel electrophoresis (Fig. 1). The hybrid clone No. 12 used in these experiments continued to show hybrid molecules for all three enzymes during the entire period of these interferon studies.

Interferon production was induced in each case by infection with Newcastle disease virus (NDV) at a multiplicity of 5 plaque-forming units per cell, under conditions previously described (*7*). Extensive cytopathic changes were present in all three cell lines 48 hours after infection. Medium harvested from the cells 24 and 48 hours after infection was pooled, and the bulk of the NDV was sedimented by centrifugation at 59,000*g* for 3 hours. The supernatant fluid was acidified with 2*N* perchloric acid to *p*H 2 and placed at 4°C for 4 hours to inactivate any remaining viable virus and to precipitate some of the proteins other than interferon. The precipitate was sedimented by centrifugation at 12,000*g* for 30 minutes and discarded. The supernatant fluid was brought back to *p*H 7.2 by the addition of 5*N* sodium hydroxide and used as interferon.

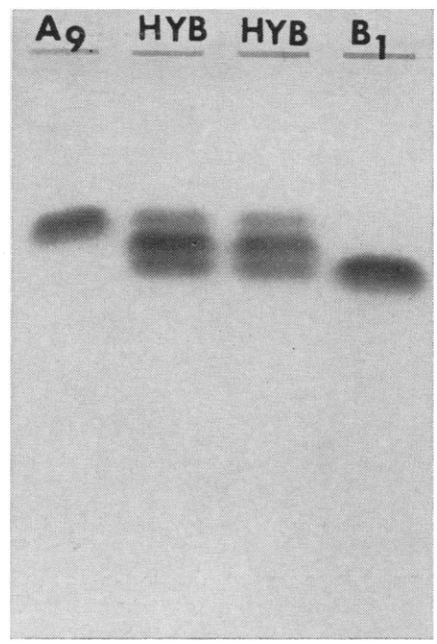

Fig. 1. Starch-gel electrophoretic enzyme patterns of 6-phosphogluconate dehydrogenase from parent (A_9 and B_1) and hybrid (HYB) cells. Note that the enzyme pattern for the hybrid cells consists of three bands: the mouse subunits, the hamster subunits, and between them the hybrid molecules made up by association of the parental subunits. Mixtures of A_9 and B_1 cell extracts consistently showed only the two parent types of subunits and failed to show the hybrid molecules. The hybrid cells also show hybrid molecules for lactate dehydrogenase and malate dehydrogenase (*3*).

Interferon activity was assayed by placing 3 ml of serial twofold dilutions of interferon on monolayers of cells in 60-mm plastic petri dishes. The cells were then incubated for 6 hours at 37°C. After removal of the interferon, a standard plaquing procedure (*7*) was carried out with vesicular stomatitis virus. Interferon titer was calculated by determining the greatest dilution which reduced by 50 percent the number of plaques found in virus control cultures which had not been treated with interferon (PR_{50} unit). In each experiment a single batch of interferon was assayed simultaneously on monolayers of the different species of cells.

The viral inhibitors under consideration had the following properties of interferons: They were not produced by noninfected cells; their production was inhibited in cells treated with actinomycin D; and they were not inactivated at *p*H 2, were not dialyzable, and were not active after treatment with trypsin.

The expected species specificity for the production and action of interferon was found for the parental mouse and hamster cell lines, as well as for primary human and hamster cells (Table 1). Interferon produced in a cell of one species conferred protection only on cells of the same species.

Mouse line cells produced interferon which protected mouse line cells but which conferred no protection on hamster line cells, primary hamster cells, or primary human cells. Hamster line cells produced so small an amount of interferon that it could be assayed only after tenfold concentration by vacuum dialysis, and then it protected only the primary hamster cells and not the relatively insensitive hamster line cells. Hamster line cells exhibited a relative lack of sensitivity to the action of hamster interferon since primary hamster cells were 16-fold more sensitive to the same batches of interferon.

The hybrid cells produced interferon (or interferons) which protected mouse line cells, primary hamster cells, and hybrid cells, but not the relatively insensitive hamster line cells or primary human kidney cells. They produced ten times more hamster interferon than the parental hamster line cells, since hamster cell line interferon which had been

Table 1. Assays of interferon titers (PR_{50} units) on different cells. Interferon titer was calculated by determining the greatest dilution which reduced by 50 percent the number of plaques found in virus control cultures which had not been treated with interferon (PR_{50} unit); NT, not tested.

Cells used for interferon production	Cells used for interferon assays				
	A_9 mouse line cells	B_1 hamster line cells	AB hybrid cells	Primary hamster cells	Primary human embryonic kidney cells
A_9 (mouse line)	32	0	64	0	0
B_1 (hamster line) (10× concentrated)	0	0	8	8	NT
AB (hybrid)	16	0	32	8	0
Primary hamster cells	0	32	256	512	0
Human (amniotic)	NT	NT	0	0	32

concentrated tenfold had the same 1 : 8 titer on primary hamster cells as did the unconcentrated hybrid cell interferon.

The hybrid cells were sensitive to interferons produced in mouse line cells, hamster line cells, hybrid cells, and primary hamster cells, but not to interferon produced in human cells. The hybrid cells were eight times more sensitive to interferon produced in primary hamster cells than the parental hamster line cells were.

The hybrid cells produced interferon (interferons) which protected both mouse and hamster cells and were sensitive to both mouse interferon and hamster interferon. Cellular production of interferon and sensitivity to its action are unrelated. Therefore, genetic determinants for both production of species-specific interferons and sensitivity to the action of species-specific interferons were contributed to the hybrid cells by both of the parental cell lines.

The hybrid cells produced ten times more hamster interferon than the hamster line cells. Thus, it appears that the presence of the mouse cell genome in hybrid cells in some way allowed for better expression of the information carried in the hamster cell genome concerning production of hamster interferon. The molecular basis for this is not now known.

Guggenheim *et al.* (*8*) recently reported that heterokaryons, created by Sendai-induced fusions of nucleated chick erythrocytes and human cells, produce low titers of chick interferon although nucleated chick erythrocytes alone do not produce any chick interferon. Heterokaryons contain separate nuclei from two different cells in the cytoplasm of a single cell and do not replicate, whereas the hybrid cells used in our experiments contain both parental genomes in a single nucleus and were propagated as a cell line.

It is interesting to speculate whether the hybrid cells produce three different interferons: mouse, hamster, and a hybrid interferon with mouse and hamster subunits. We do not know (*2*) whether the composition of an interferon includes only a single polypeptide chain or the multiple chains consistent with this hypothesis.

When tested for sensitivity to the action of hamster interferon, the hybrid cells were eight times more sensitive than the hamster line cells. Thus, we have another example of the presence of the mouse cell genome in the hybrid cell, allowing for better expression of information carried in the hamster cell genome.

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Visual Form Discrimination after Removal of the Visual Cortex in Cats

Winans (*1*) claims to have demonstrated form discrimination in cats following bilateral ablation of cortical areas 17, 18, and most of 19. If the claim could be substantiated it would be important, and surprising in view of the well-established microelectric findings on what may be termed a primary contour-coding system in these areas (*2*). However, it is not at all clear that Winans' claim is valid. Criticisms are offered on several grounds, the first and most important, hinted at in her report, being that in her situation a visual discrimination was possible which was not based on shape or pattern as such.

Consider the training stimuli used in her experiment; these were white isosceles triangles on black grounds, one with base horizontal (the positive shape) and the other rotated through 180° (the negative shape). Six sets of training shapes were used, each set consisting of the same pair of triangles, their sizes decreasing from one set to the next. In each case the same orientation was used for the positive shape. Since original training was on the largest pair, it is very possible that discriminative responding was based on a difference in brightness gradient between the pair, that is, that the cats learned to choose the pattern that was brighter at the bottom than at the top. Indeed, if the cats attended only to the bottoms (or tops) of the patterns, the original discriminative responding to the patterns could be based simply on a brightness difference between them. Since the training sequence consisted of the identical patterns reduced progressively in size, an initial bias toward responding in terms of differences in brightness gradients would be expected to transfer to other sets in the training sequence where the differences in gradient are not so obvious.

We argue, therefore, that Winans has not sufficiently demonstrated a true pattern or form discrimination in her experimental subjects. In order to do so it would be necessary, in the first place, to find a more adequate specification of pattern, and what one means by a pattern discrimination. This is not an easy matter (*3*), but at least one can define a pattern discrimination largely by exclusion; it must be a visual discrimination not based on differences in brightness, brightness gradient, or position. In