Mammalian somatic cell hybrids and their susceptibility to viral infection

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Somatic hybrids between cells of different mammalian species have been used to study the dependence of viral functions on host cell genes. In the case of human-mouse hybrids, polio virus infection depends on the presence of the human gene for the polio receptor. Hybrids containing partial human genomes may be permissive or nonpermissive depending on which human genes (chromosomes) are included. Hybrids may therefore be used to make a chromosome assignment for this gene and possibly for any human gene upon which the multiplication of a virus is dependent. In hamster-mouse hybrids, the proportion of mouse and hamster chromosomes may be varied over a broad range. Polyoma virus multiplication in such hybrids depends on the presence of the mouse genome. Provided this requirement is satisfied, early viral function is carried out without regard to the size of the hamster genome, but late viral functions, including viral DNA synthesis, are suppressed when the hamster chromosome complement is too large. A cellular gene product which suppresses multiplication of viruses of foreign species might have a role in preventing the spread of these viruses across species lines.

When nonpermissive cells transformed by SV40 are fused with cells permissive for the virus, viral multiplication can often be detected in the heterokaryons (14, 15, 25). This means that the presence in the heterokaryon of the genome of the nonpermissive parental cell does not prevent any of the stages of viral multiplication. The same situation appears to hold for hamster Rous virus transformants fused with permissive chicken cells (22). Some degree of dominance of the permissive state has also been demonstrated more directly by infection of heterokaryons between cells of permissive and nonpermissive species; monkey-mouse heterokaryons, for example, supported the multiplication of SV40 (25) which depends on the monkey genome, and human-hamster heterokaryons gave evidence of synthesis of adenovirus type 12 (26) which depends on the human genome.

The events following infection of a hybrid (mononucleate) cell line with a virus may not be identical to those following infection of a heterokaryon between the same two cell types for a number of reasons:

1) The heterokaryon must contain all the genetic material present in the two parental lines, but the hybrid line may not, depending on whether chromosome loss has been extensive enough to eliminate all homologues of any chromosome. Even if the process of chromosome loss does not proceed that far, it may alter the chromosome balance in a hybrid cell in such a way as to affect viral multiplication. There may also occur in the hybrid a doubling of the chromosome complement of one of the participating species. This probably occurs shortly after fusion, when the nucleus of one species may replicate its DNA once before synchronization with the nucleus of the other species (11). This appears to be especially common for hybrids between human cells and the mouse line 3T3-4E (17). Chromosome balance in a polykaryocyte should be affected only by the proportion in which cells of each type combine to form the fusion product.

2) The cellular proteins present in a freshly constituted heterokaryon were made in the parental cells and mixed in a common cytoplasm after fusion. On the other hand all of the components in a hybrid cell many generations after the fusion event were synthesized after the hybridization, and may differ from those which were present immediately after fusion. For example, hybrid cells contain a number of hybrid enzymes composed of subunits deriving from the genomes of the two participating species (3, 6, 18, 27). Freshly constituted heterokaryons should not contain hybrid enzymes, for these...
appear to be formed only at the time the subunit polypeptides are synthesized. With time, continuing protein synthesis in the heterokaryon would be expected to lead to the accumulation of hybrid enzymes (19), but under the usual conditions, virus-induced heterokaryons have a short life-span, undergo little growth, and by the time they are studied may not have accumulated appreciable amounts.

3) If any interaction occurs between the genomes within the nucleus of a hybrid cell but not between separate nuclei in a heterokaryon, this may result in differences in composition between the two types of fusion products. For example, if such an interaction results in cessation of transcription of any cellular gene, then the dilution and destruction of the transcribed RNA would result in disappearance of the corresponding protein from the hybrid.

As can be seen from the above, the possibility exists that viral infection in hybrid cells may be followed by events different from those occurring in freshly constituted heterokaryons. The greater homogeneity of cloned hybrid cell populations also permits more exact analysis of the infectious process. Two main categories of effect have been examined in interspecies hybrid lines: 1) The dependence of any viral function on cellular genes. Elimination of chromosomes of the permissive species, if it leads to loss of permissiveness, will establish the necessity of a cellular gene for the support of some viral function. 2) In the presence of a complete complement of chromosomes from the permisssive species, any diminution in the degree of permissiveness of the hybrid cell indicates inhibition of a viral function by a gene product from the nonpermissive species. Both types of effect occur, and examples will be given below.

HUMAN-MOUSE HYBRIDS AND SUSCEPTIBILITY TO POLIO VIRUS

All human (and other primate) culture lines are susceptible to polio virus, but mouse cells are not, as they lack the surface receptor necessary for viral attachment (9, 10). Human–mouse hybrid lines containing only partial human chromosome complements have been isolated and found to be susceptible to polio virus (20) because they possess the human viral receptors (24). Figure 1 shows the process of viral multiplication in a monkey line BSC-1, two human diploid strains FA and WI-38, and in a hybrid line WE-2. The course of viral multiplication and the viral yields are similar. It was concluded that the human viral receptors are made and function normally in hybrids containing preponderantly mouse genetic material. The receptor activity of the hybrids, measured as ability to adsorb labeled virus at 4°C is usually not as great as that of the purely human cells, which presumably contain more homologues of the chromosome bearing the receptor gene (Table 1); but the receptor activity is sufficient so that, at least at moderately high multiplicity, the virus is able to initiate an infection in virtually all the cells. The mouse genome does not prevent the synthesis of the receptor, and as expected (9) does not inhibit any stage in the subsequent multiplication process (20).

When human–mouse hybrid lines are carried serially in culture, human chromosomes are eliminated. During this process progeny cells become polio resistant (24), presumably due to loss of the chromosome bearing the receptor gene. Even in a recently cloned culture of sensitive hybrids, there are some cells which have become resistant in this way, and they can be obtained selectively by viral infection, which eliminates all the susceptibles. The surviving hybrids, such as WE-2/Po, are thoroughly
resistant to reinfection (Fig. 1), and do not contain significant amounts of receptor activity (Table 1). As an alternative to chromosome loss, resistance might arise through mutation of the retained human receptor gene. No purely human cell line completely resistant to polio virus has been obtained (5), even using selective methods (23). Though there is reason to believe that such a mutant may not be impossible to prepare (21), mutational loss of the polio receptor gene must be very rare and may be confidently excluded as the cause of polio resistance in the hybrid cells.

If chromosome loss is the cause of the resistance, comparison of the chromosomes of different polio-sensitive hybrids with those of the corresponding virus-resistant sublines should make it possible to learn what human chromosome is (or is not) associated with the presence of the viral receptor. Examination of a number of such hybrids showed no association with any biarmed chromosome and suggested that one of the human acrocentrics is responsible for the receptor gene (16).

Human–mouse hybrids able to support the multiplication of polio virus were infected with other viruses which can multiply in human cells but not in mouse cells—the Echo viruses, SV40, and the human adenoviruses. Some of the Echo viruses were able to grow in the hybrids. SV40 was able to express early viral functions (T-antigen synthesis) but not late ones (capsid antigen synthesis) (20). Adeno types 2 and 12 did not synthesize either T-antigen or capsid antigen (20). It is clear that a hybrid which can support polio multiplication may be partially or completely unable to support functions of other primate viruses. This may be due to either of the factors described earlier. For example, human genes not present in the polio-sensitive hybrids may be required for adsorption, penetration, or the multiplication of the other viruses. As the polio-sensitive hybrids often contain far less than the haploid human chromosome complement, this seems quite likely, and precisely for this reason it is not possible to say whether the presence of the mouse genome contributes anything to the failure of the infectious process. Only where all chromosomes of the permissive parental cell are present in the hybrid can failure of a viral function be ascribed to an inhibitory effect by a product originating from the genome of the nonpermissive species.

HAMSTER–MOUSE HYBRIDS AND SENSITIVITY TO POLYOMA VIRUS

Somatic cell hybrids of hamster and mouse are not like those of the human and mouse in that there is not a strong tendency to the elimination of chromosomes of one parental type (6, 18). Probably mouse chromosomes are eliminated more frequently than hamster chromosomes (18), but it is quite possible to obtain hybrids which possess approximately parental-sized complements from both species, or which possess an excess of either mouse or hamster chromosomes (2).

The mouse cell 3T3-4E is permissive for polyoma virus and the hamster cell BHK T6A is not. Each line possesses a selective marker, and following cocultivation, a number of independent clones of hybrids of the two lines were isolated. The response of these hybrids to polyoma infection was studied in detail.

The following applies to those of the hybrids which possessed a mouse chromosome complement in the diploid range or higher. All hybrids supported early viral functions (T-antigen synthesis) as efficiently as did the mouse parental cells. At moderate viral multiplicity virtually all the cells participated, compared with less than 1% for the nonpermissive hamster parental line. This was the case independent of the size of the hamster chromosome complement of the hybrid. It can be concluded that T-antigen synthesis was a fully dominant function (2).

With respect to the subsequent events of the infectious process, there were important differences between the same hybrids, depending on the proportions of mouse and hamster genetic material. If the hybrid cell contained a hamster complement in the diploid range, then

![Graph showing inhibition of polyoma DNA synthesis in hamster–mouse hybrids.](image)

**FIG. 2.** Inhibition of polyoma DNA synthesis in hamster–mouse hybrids. (Adapted from 2.)

**TABLE 2.** Inhibition of viral capsid synthesis in mouse-hamster hybrids by the genome of the nonpermissive species

<table>
<thead>
<tr>
<th></th>
<th>Number of Chromosomes</th>
<th>Cells Making Capsid Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mouse</td>
<td>Hamster</td>
</tr>
<tr>
<td>BHK</td>
<td>0</td>
<td>44</td>
</tr>
<tr>
<td>4B</td>
<td>39</td>
<td>95</td>
</tr>
<tr>
<td>7B</td>
<td>115</td>
<td>48</td>
</tr>
<tr>
<td>3T3-4E</td>
<td>68</td>
<td>0</td>
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the virus could usually multiply as efficiently as in the mouse parental cell, and the yields of virus were comparable. However, if the hamster chromosome complement was larger (2–3 times the diploid number) then viral multiplication was seriously affected, the yield dropping to less than 1% that of more permissive hybrids. Whenever the yield of virus was reduced, it could be shown that viral DNA synthesis had been inhibited also. Figure 2 shows the relation between the amount of viral DNA synthesis occurring in a hybrid and the proportion of its chromosomes derived from the two species. It can be seen that an increase in the ratio of hamster to mouse chromosomes was accompanied by reduced viral DNA synthesis. The inhibition was very small in hybrids whose chromosome ratio was less than 0.6 but very marked at a ratio of 3.6. Hybrid 10A, an unusual hybrid in a number of respects (2), showed more inhibition of DNA synthesis than would be expected from its chromosome constitution. As all of these hybrids contained a mouse chromosome complement in the diploid range or higher and very probably a complete set of mouse genes, the interference with the transition from early viral function to viral DNA synthesis may be ascribed to an inhibitory effect originating in the hamster genome and becoming very marked where the ratio of hamster to mouse gene products is high.

It would be quite interesting to know whether the multiplication of other viruses might also be inhibited by the genome of a nonpermissive species. Preliminary experiments suggest that they are. Some of the same mouse–hamster hybrids which had been examined with respect to polyoma virus were infected with adenovirus type 2. The BHK hamster cell is moderately permissive for this virus and the 3T3 mouse cell is not, just the reverse of the polyoma situation. Examination of capsid antigen synthesis by fluorescence showed that the mouse genome exerted a suppressive effect analogous to that of the hamster genome on polyoma virus (Table 2). The suppression of capsid antigen synthesis was marked in hybrid 4B, which possesses an approximately diploid number of mouse chromosomes, and complete in hybrid 7B, which possesses a much larger number of mouse chromosomes, and is fully permissive for polyoma virus.

POSSIBLE SIGNIFICANCE OF A CELLULAR INHIBITORY MECHANISM FOR VIRUSES OF FOREIGN SPECIES AS A FACTOR LIMITING CROSS-SPECIES CONTAGION

Among the animal viruses, some are able to infect successfully only a very narrow host range, while for others the host range is much wider. One example of a mechanism of host restriction has already been mentioned, that due to the absence of receptor substance necessary for infection by polio (and other enteroviruses). This relation presumably indicates a late evolution of the polio virus in association with the primates. Cells of other species, lacking the receptor, need no other form of protection against this virus but are susceptible if the viral genetic material is introduced by special means (7, 10).

Most RNA viruses are conspicuous with respect to the variety of species whose cultured cells will support multiplication. This includes the oncogenic ones (4, 8). In nature the spread of these viruses may be limited to certain species by other mechanisms of resistance (such as immunological) which the viruses can sometimes circumvent. One reason that RNA viruses can grow in the cells of many species may be that, possessing their own polymerases, they are freed from any restriction imposed by those of the host. Large DNA viruses (pox group) similarly are able to grow in the cells of many species, and possibly for the same reason, as they possess their own DNA and RNA polymerases (12, 13).

Members of the papova and adenovirus groups, while they can cross species barriers for transformation in culture or tumor production in vivo, are much more species specific for the support of multiplication, as they often grow in cells of only the native species or closely related ones. This restriction is not due to receptor problems or to limitation of early events in the infectious cycle since attachment, penetration, uncoating, and early functions may occur even in nonpermissive cells.

In hamster–mouse hybrid cells, the hamster genome inhibits polyoma multiplication. The inhibition could result from any of the following:

1) The presence of a product of the hamster genome which interferes with the replication of the viral DNA.

2) The presence of competing homologous gene products (polymerases?) specified by each of the two genomes, the product of mouse origin being suitable for viral DNA replication, and that of hamster origin unsuitable. A high hamster–mouse chromosome ratio would result in an excess of the unsuitable product and in inefficient replication. This would be especially likely if the product were an enzyme made of subunits deriving from the two species, and for this reason inactive.

3) Host-controlled restriction involving breakdown of viral DNA, as has been shown in bacteriophage infection (1). Such a process does not appear to have been demonstrated for animal viruses, and seems very unlikely to be the cause of the inhibition of polyoma virus multiplication because of the normal expression of at least one viral function (T-antigen synthesis).

Whatever the cause of this inhibition, it is not (at least for polyoma virus) as complete in hamster–mouse hybrids as it is in hamster cells. Under such conditions serial passage of a virus in a hybrid may permit selection for spontaneous viral mutants able to grow well in the presence of the inhibitory condition, and in this way the virus may acquire the ability to grow in the nonpermissive parental cell. Such an opportunity for the evolution of a virus to growth in a foreign species might never arise under natural conditions. For this reason serial cultivation in hybrid cells containing human genes of viruses not able to grow in human cells might lead to the creation
of new viruses pathogenic for the human. It would therefore seem best to avoid such experiments on hybrids of human cells, until the evolutionary possibilities become clearer from studies of hybrids of other species.

REFERENCES