Behavior of Three Types of Ribovirus-Like Particles in Segregating Hamster × Mouse Somatic Hybrids

(electron microscopy/C-, R- and intracisternal A-type particles/mixed virions/loss of mouse chromosomes)

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Contributed by Boris Ephrussi, September 10, 1974

ABSTRACT An electron microscopic study of somatic hybrids resulting from a cross between hamster melanoma cells harboring R-type particles and mouse fibroblasts harboring intracisternal A-type and (mostly) immature C-type particles was performed. The major findings are: (a) hybrid cells that retain a large fraction of both hamster and mouse chromosomes produce all three types of particles; (b) the loss of mouse chromosomes is followed by that of intracisternal A- and C-type particles; (c) the continued production of A- and C-type particles is dependent on the retention of different mouse chromosomes; (d) the mixed intracellular population of R- and A-particles comprises a small number of complex structures in which the two types of virions are enclosed within a common envelope.

In the course of an electron microscopic study of melanogenesis in melanoma × fibroblast somatic hybrids (to be published elsewhere), the presence of virus-like particles in cells of both parental lines was noticed; in accordance with observations by others, Syrian hamster melanoma cells were found to harbor R-type particles (1) and mouse fibroblasts intracisternal A- and C-type particles (2). The fates of these three types of particles have been followed in the hybrid cells in order to establish whether their production is correlated with the loss of chromosomes from these hybrids. In this preliminary article we describe observations indicating the dependence of A- and C-type particle production on the retention of mouse chromosomes, as well as the occurrence of heterologous complexes containing R-type and intracisternal A-type particles within a common envelope. A detailed report will be published elsewhere.

MATERIALS AND METHODS

The parental cells, 2× Syrian hamster melanoma 3460-3 C1.4-6 and mouse fibroblasts LM(TK-) Cl.1D, as well as some properties of the hybrids obtained from their cross, have been described (3). It will be recalled that the karyotypes of the parental cells differ both by the total chromosome number and, more importantly, the proportion of acrocentric (A) and bi-armed (B) chromosomes (Table 1): thus losses from hybrid cells of acrocentric chromosomes, 1/4, of which are of mouse origin, result in lower A/B ratios.

Among the numerous hybrid clones isolated from this cross (3), three independent ones, and several subclones derived from one of them, were selected for the present study solely on the basis of decreasing chromosome numbers and A/B ratios, presumably reflecting the predominant loss of mouse chromosomes. The relationship between them is shown in Fig. 1.

A few hybrids from a different hamster melanoma × mouse fibroblast cross described in ref. 3 were also examined; electron micrographs of one of them (Figs. 7, 8, and 10) are included.

For electron microscopic examination, cell cultures were grown as described (3) but in the absence of selecting drugs. During late exponential growth, cultures were double-fixed in glutaraldehyde and osmium tetroxide and embedded in Epon. Ultrathin sections were double-stained with uranyl acetate and lead citrate prior to examination under a Hitachi HU 11-A electron microscope operating at 75 kV. The number of viral particles of each type was counted in all cell sections encountered by systematic displacement of the grids. The virus particles observed were classified as to type exclusively on the basis of structural criteria, as defined in refs. 4 and 5.

The number of particles of each type was recorded in 400 to 900 cell sections of each cell line, precautions being taken to minimize the repeated examination of sections of the same cell. Inspection of the "raw data" thus obtained showed the distribution of R- and, especially, A-type particles among sections of any one cell line to be nonrandom (the variance exceeding the mean) owing to both inter- and intracellular heterogeneity (occurrence of A-particles in clusters), as well as to variability of cell size and section area. To permit the comparison of the different hybrid clones with respect to average content of virus particles of each type and its limits of confidence, the "raw data" were recalculated in terms of the average percent of sections containing at least one particle: it is these values with their standard errors that are given in Table 1. Only conclusions thus found to be significant at the 5% level are given in the next section.

FIG. 1. Pedigree of the hybrid clones.
* Not examined.
Table 1. Relationship between karyotype and virus-like particles

<table>
<thead>
<tr>
<th>Cell line*</th>
<th>Mean number of chromosomes</th>
<th>Average % of cell sections containing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>A</td>
</tr>
<tr>
<td>3460 Cl.4–6 (10)</td>
<td>96  16  80  0.20</td>
<td>28.9 ± 2.3</td>
</tr>
<tr>
<td>LM(TK−)Cl.1D (21)</td>
<td>51  42  8  5.25</td>
<td>0</td>
</tr>
</tbody>
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Hybrids (expected):

|            | Total         | A     | B     | A/B | R     | A       | C1†     | Cm†     |

Bla (4) | 110  42  68  0.62 | 16.7 ± 2.4 | 56.5 ± 3.3 | 4.5 ± 1.4 | 14.8 ± 2.3 |

N4b (8) | 112  42  70  0.60 | 12.7 ± 1.8 | 28.7 ± 2.4 | 8.8 ± 1.5 | 8.4 ± 1.5 |

B2a-9 (7) | 120  42  78  0.54 | 11.7 ± 2.2 | 27.1 ± 3.1 | 4.5 ± 1.4 | 11.2 ± 2.2 |

B2a-8 (8) | 123  43  80  0.54 | 12.7 ± 2.3 | 45.7 ± 3.4 | 4.2 ± 1.4 | 13.0 ± 2.3 |

B2a-8-x (20) | 129  48  81  0.59 | 19.7 ± 2.7 | 51.7 ± 3.3 | 8.7 ± 1.9 | 24.5 ± 2.9 |

B2a-8-10 (19) | 94  30  64  0.47 | 25.0 ± 2.1 | 44.2 ± 2.4 | 1.6 ± 0.6 | 2.9 ± 0.8 |

B2a-8-10-EF (24) | 93  29  64  0.45 | 30.0 ± 2.5 | 36.3 ± 2.7 | 0.4 ± 0.2 | 0 |

B2a-8-10-EF5 (16) | 82  24  58  0.41 | 16.8 ± 1.9 | 53.0 ± 2.5 | 0.4 ± 0.2 | 0 |

B2a-8-7 (26) | 91  15  76  0.19 | 18.7 ± 2.0 | 0.3 ± 0.3 | 0 | 0 |

B2a-8-7-4 (15) | 86  15  71  0.21 | 11.4 ± 1.7 | 0 | 0 | 0 |

B2a-8-7-4d (12) | 80  13  67  0.19 | 7.3 ± 1.7 | 0 | 0 | 0 |

* In parentheses: number of metaphases examined.
† C1 and Cm designate immature and mature C-type particles, respectively.

RESULTS AND CONCLUSIONS

Virus Particles in Parental Cells. Fig. 2 shows several R-type particles, with their characteristic “wheel-like” internal structure (first described by Bernhard and Tournier, ref. 6), located in the cisternae of the endoplasmic reticulum of a melanoma cell. Figs. 3 and 4 show, respectively, intracisternal A-type particles within and a C-type particle budding from the surface of a mouse fibroblast. Like the majority of C-type particles present in the fibroblasts, the one seen in Fig. 4 is an immature form characterized by the presence of three distinct layers and a centrally located electron-lucid nucleoid (4); mature forms with electron-dense nucleoids (4) are very rare as can be seen in Table 1.

Virus Particles in Hybrid Cells. The karyotypes of the different hybrid populations, as determined at or near the time of their fixation for electron microscopic examination, are given in Table 1, where the clones are arranged so as to correspond to the fission of the hybrids shown in Fig. 1. It will be seen that, at this stage, all of the hybrids had total chromosome numbers considerably below the expected one (147), owing chiefly to the loss of acrocentric chromosomes, reflected, in all but one case, in A/B ratios lower than the one expected (0.61)*

As can be seen in Table 1, the first eight of the 11 hybrid populations listed contained all three types of virus particles: R, A, and C. However, since the percentages of sections containing each type of particle are always considerably below 100%, the data of Table 1 do not reveal the important fact that all three types of particles are frequently detected in the same cell section, i.e., are contained in the same hybrid cell. This is particularly true of the more numerous R- and A-type particles, which are often observed side by side, budding from the membrane of the same cisterna of the endoplasmic reticulum (Figs. 5 and 9). These observations thus show that (a) within these hybrid cells there is no compartmentalization of the formation of R- and A-type particles, and (b) in this series of hybrids, neither parental genome radically interferes with the formation of any one of the types of viral particles carried, prior to hybridization, by the other parent. [This absence of host restriction is especially noteworthy in the case of A- and R-type particles because the former have thus far never been described in hamster cells and the latter have been observed only exceptionally in mouse cells (7).] It must be pointed out, however, that, for the C particles, this statement is valid only so long as it is assumed that the particles observed in the hybrid cells and designated as C-type particles uniquely on the basis of their morphology are really identical with those harbored by the parental fibroblasts. In as much as this (unproven but not altogether unlikely) assumption is accepted, one will further observe that, in the first six hybrids of Table 1, the proportion of mature C-type particles released into the extracellular space (Fig. 6) is significantly higher than in the parental fibroblasts, i.e.: that the process of maturation of the C-particles present in the latter is enhanced in the hybrids by some kind of “helper mechanism” sensu lato. In the absence of criteria other than the purely structural ones used, it remains equally possible that what we see here is the induction, in the hybrids, of a different type C virus(es) (compare ref. 8).

Turning to the relationship between the karyotype of the hybrid cells and the production of the three types of virus particles, we observe first of all that R-type particles (even though their number is somewhat lower in most of the hybrids than in the parental cells†) are present in all 11 hybrids ana-

* Earlier karyological examination of the three independent hybrids (Fig. 1) gave the following mean total chromosome numbers and (in parentheses) A/B ratios: Bla: 143 (0.73); N4b: 140 (0.72); B2a: bimodal distribution; 140 (0.78) and 114 (0.69). As indicated in ref. 3, these hybrids had lost, to begin with, some bi-armed chromosomes.

† These, at first sight fortuitous, fluctuations of R particle content may well be correlated with qualitative karyological differences between hybrids with similar chromosomes numbers not revealed by the karyological technique used here, or with differences in growth phase at the time of fixation.
Virus Particles in Hamster × Mouse Somatic Hybrids

**Figs. 2–6.** ×100,000; the bar in Fig. 4 represents 100 nm. Fig. 2: R-type particles in a hamster melanoma cell. Fig. 3: Intracisternal A-type particles in a mouse fibroblast. Fig. 4: Immature C-type particle at the cell surface of a mouse fibroblast. Fig. 5: R-type (R) and intracisternal A-type (A) particles within the same cisterna of the endoplasmic reticulum (ER) of hybrid B2a-9. Fig. 6: Mature C-type particles released from hybrid B2a-8-x.

lyzed, whatever their karyotype (Table 1). In this respect, the behavior of A- and C-type particles is clearly very different: their production appears to be correlated with the retention of acrocentric (presumably mouse) chromosomes, since both types of particles disappear at the lowest A/B ratios (0.19–0.21) observed in the three sequentially derived hybrids B2a-8-7 → B2a-8-7-4 → B2a-8-7-4d (Table 1 and Fig. 1). The loss of A particles is, however, clearly independent of that of C particles since, in the parallel series of three sequential hybrids B2a-8-10 → B2a-8-10-EP → B2a-8-10-EP5, characterized by higher A/B ratios (0.41–0.47), the content of A-particles remains in the range of those of the more (chromosomally) complete hybrids, while the number of C particles rapidly declines and approaches 0. It thus appears that the persistence
of A and C particles depends on the retention of different mouse chromosomes.

Since, as can be seen in Fig. 1, both series of hybrids just discussed have a common origin in hybrid clone B2a-8, one may wonder whether the loss of type C particles in both of them is traceable to a single event: this appears to us unlikely because the two clones (B2a-8-10 and B2a-8-7) derived directly from B2a-8 are karyologically very different from it and from each other. We are, therefore, inclined to think that we are dealing with two independent instances of loss of C particles.

Be this as it may, it appears to us established that the continued production of both A- and C-type particles requires the presence of certain, different mouse chromosomes. These facts are compatible with the current idea of the integration of the viral genomes into the mouse chromosomes as well as with the hypothesis of the production by the latter of a factor necessary for the formation of A- and C-type particles.

Heterologous Complex Virions. In all eight hybrid clones producing large numbers of R and intracisternal A particles (first eight lines of Table 1) the mixed intracellular populations of these particles were found to comprise about 1–2% of heterologous complexes in which the two types of particles are enclosed within a common envelope. (The real frequency of these complexes must be considerably higher than that indicated, since these forms are detected only when cut longitudinally.) In addition to the most commonly observed duplex forms (Figs. 7, 10, and 11), triple, quadruple (Fig. 8), and quintuple forms were occasionally seen. Although many types of evidence have pointed to the occurrence of "hybrid" or "mixed particles" (see refs. in 9 and 10), this is, to the best of our knowledge, the first unambiguous electron microscopic demonstration of complex forms comprising different types of virions under a common viral coat. Their biological (antigenic, genetic, etc. . . . ) properties remain to be determined.

We thank Drs. W. Bernhard and Mary C. Weiss for critical review of the manuscript, Prof. G. Brun for help with the statistical analysis of the data, Mme A. Cassant for ultra thin sectioning and Mlle F. Ruiz for cell culture and karyological work. This study was conducted with the aid of grants from the Délégation Générale à la Recherche Scientifique et Technique and from the C.N.R.S. (A.T.P. "Différenciation Cellulaire").