bodies s^{-1}. (Experimental studies have yielded estimates of S in the range 0.5×10^{-1}–2×10^{4} antibodies s^{-1} (ref. 7).) All the parameters except S have been held fixed, including the various binding constants. Thus the antibody specificity is the same for the three plaques in Fig. 1. By increasing S the plaques change from partial to sombrero.

We have shown that differences in the antibody emission rate of a clone member can cause it to produce a plaque which differs in morphology from those plaques produced by other clone members. We have demonstrated transitions between partial and sombrero plaques. One other question to consider is whether changes in the antibody emission rate can cause progeny of a clear PFC to yield other than a clear plaque. If the antibody binding constants are identical for types 1 and 2 SRBC, a clear plaque will be produced for all antibody emission rates. A clear plaque, however, does not necessarily mean the antibody has identical binding constants for both types of SRBC. It may instead mean that the differences in specificity are not sufficient to produce a measurable difference in the plaque radii. Whether a large change in the emission rate could lead to two well defined plaque radii (a sombrero) is an open question.

BYRON GOLDSTEIN

Department of Physics,
Fairleigh Dickinson University,
Teaneck, New Jersey 07666

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DR CUNNINGHAM REPLIES—Plaque morphology has been used[1-5] as a simple way of distinguishing cells making different antibody specificities. Other interpretations of the meaning of plaque differences are possible, and Goldstein[6] has suggested that rate of antibody secretion is decisive. As with any complex assay system, it is impossible to discount such alternative interpretations absolutely. The question is not, 'are such artefacts conceivable?'—for evidently they are—but 'are they likely to occur under the experimental conditions?' Control experiments and arguments for the validity of plaque morphology as a specificity marker have been presented elsewhere[7]. Some of these are listed below.

Goldstein's argument requires that plaques with different morphologies should have different overall (largest) diameters. In fact, in many of our clones, the outer diameter of partial lysis was similar in most or all plaques, while the clear area of total lysis varied from one cell to the next. In effect, overall plaque size acts as a control against differences in rate of antibody production per unit time. His argument could, however, be rescued with more complicated assumptions, for example rapid fluctuations in rate of antibody release.

To eliminate this we watched plaques develop. Those which started as sombreros (after incubation for 15 min) always grew into larger sombreros with the same relative areas of clear and partial lysis at 1 h (ref. 7). Clear and partial plaques, (the 'extreme cases' of sombrero morphology), behaved similarly.

Individual plaque-forming cells, micromanipulated from one red cell monolayer to another, produced plaques of similar morphology at different temperatures[7]. That is, rate of antibody release did not seem to affect plaque morphology.

Clones grown *in vivo*[1,8] often contained several thousand antibody-forming cells all with exactly the same morphology on a mixed indicator monolayer. Others had varying proportions of two types, for example half were clear and half partial, with no intermediate forms. It is not an indisputable argument, but if plaque morphology depends critically on rate of antibody release, one would expect to find a spectrum of types in any collection of plaques. This observation of very large numbers of homogeneous plaques also serves to discount the suggestion that variation in rate of production is a rare event which our control experiments failed to detect.

Department of Microbiology,
John Curtin School of Medical Research,
Australian National University,
Canberra, Australia

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Temperature-sensitive growth of cells transformed by *ts-a* mutant of polyoma virus

ONE of the characteristics of the transformed state in culture in mammalian fibroblasts transformed by polyoma virus and Simian Virus 40 (SV40) is the lowered serum requirement for cellular growth[1-4]. The participation of a viral gene(s) in this attribute of transformation has been known for polyoma virus[5] and SV40 (refs 6 and 7). Recent experiments show that some of the other properties associated with the transformed state are also under the control of the same viral gene(s)[6-11]. The *ts-a* gene of polyoma virus controls the initiation of stable transformation in the hamster BHK 21 cell line[12], although it is usually accepted that the function of this gene is not required for the maintenance of the transformed state[12-14]. I report here the temperature-sensitive growth in low serum medium of some of the rat cell lines transformed by the *ts-a* mutant of polyoma virus, and suggest that this viral gene controls at least one aspect of the maintenance of transformed state in certain transformed cells.

Like other untransformed fibroblastic cells widely used in transformation experiments[1-3,18], 3Y1 rat cells require a high concentration of serum for growth and exhibit poor growth in medium containing low concentrations of serum, whereas 3Y1 cells transformed by polyoma virus or SV40 can grow well in low serum medium (Kimura and Kaneto, unpublished). To determine whether the *ts-a* gene of polyoma virus affects the growth properties of transformed cells, seven independent 3Y1 lines transformed by *ts-a* mutant at permissive temperature as well as three 3Y1 lines transformed by wild type (WT) virus were examined for their ability to grow in low (2%) and high (10%) serum medium at 33° C and 40° C, the permissive and non-permissive temperatures for the productive cycle of this mutant[15,19]. Figure 1 shows that the growth of untransformed 3Y1 cells was reduced in 2% serum at both 33° C and 40° C, compared with that in 10% serum. Growth in 2% serum of three of the 3 WT-transformed lines (Py-3Y1-3, 4 and 117) was also reduced at both temperatures, compared with that in 10% serum, but it was still much better than that of 3Y1 in 2% serum at both temperatures. A striking difference in cellular growth was observed in 3Y1 lines transformed by *ts-a* mutant. There was much less growth in four out of the seven *ts-a*-3Y1 lines (*ts-a*-3Y1-1, 2, 3 and 6) in 2% serum at 40° C than at 33° C. Two lines (*ts-a*-3Y1-3 and 6) further showed less growth in 10% serum at 40° C than 33° C. Three *ts-a*-3Y1 lines (*ts-a*-3Y1-4, 5 and 7) showed little or no detectable temperature-sensitivity for growth under the conditions, thus resembling WT-transformed 3Y1 cells. Figure 2 shows the growth curves of one of the *ts-a*-transformed 3Y1 lines (*ts-a*-

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