ON THE REDUCED INTERCELLULAR ADHESIVENESS OF VIRALLY TRANSFORMED BHK21 CELLS

J. G. EDWARDS,* J. McK. DYSART, D. H. EDGAR† AND R. T. ROBSON‡
Department of Cell Biology, University of Glasgow, Glasgow G12 8QQ, Scotland

SUMMARY

Baby hamster kidney fibroblasts (BHK21 cells) transformed by polyoma or Rous sarcoma viruses aggregate less than the untransformed parental cells when incubated in growth medium in a gyratory shaker for 18-24 h. This difference can be measured by electronic particle counting, or by filtering aggregated suspensions of 35S-labelled cells through bolting fabric. The aggregation of transformed derivatives is not enhanced by the presence, during aggregation of ε-aminocaproic acid, an inhibitor of plasmin activation. Some lines of transformed BHK21 cells do not appear less adhesive than untransformed cells in a short-term aggregation assay, and none adheres markedly less well when seeded onto homotypic cell sheets. The decreased aggregation of transformed cells is consistent with suggestions that LETS protein is involved in intercellular adhesion of fibroblasts as well as in attachment of cells to non-cellular substrates. If so, the short-term aggregation of freshly trypsinized cells may depend on secretion of LETS from an intracellular pool.

INTRODUCTION

Using micromanipulation, Coman (1944) showed that certain carcinoma cells could be separated from each other by lower distractive forces than the epithelial cells presumed to have given rise to them. Since then however, workers defining or measuring intercellular adhesiveness in various ways have not found malignant cells in general to be less adhesive than their normal counterparts. The discovery of the fibroblast surface or matrix protein, known inter alia as large, extracellular transformation-sensitive protein (LETS protein), fibronectin, and from chick cells as cell-surface protein, CSP (reviewed by Hynes, 1976) has stimulated interest in the adhesive properties of cultured fibroblasts. It seems clear that this protein is very effective in promoting attachment and spreading of fibroblasts on culture substrates (Yamada, Yamada & Pastan, 1976; Ali, Mautner, Lanza & Hynes, 1977) and several authors have suggested it may also be involved in intercellular adhesion. Viral transformation of established fibroblastic lines commonly leads to a reduction of LETS protein at their surfaces (Hynes, 1976). If this protein is indeed involved in intercellular

* To whom requests for reprints should be addressed.
‡ Present address: Bioscience Group, Corporate Laboratory, ICI Ltd, The Heath, Runcorn, Cheshire, England.
adhesion, there should be conditions under which its concentration at the surface
determines intercellular adhesiveness. Under these conditions, transformed cells
should adhere less well to each other than do their untransformed counterparts.

In an earlier brief report from this laboratory, Edwards, Campbell & Williams
(1971) described some lines of BHK21 cells, newly transformed by polyoma virus,
which aggregated much less than the parent cells in a short-term assay in serum-free
medium. It was already clear that at least one line of transformed cells did not show
this reduction, and meanwhile other exceptions, one of which will be described below,
have come to light. It has also emerged that transformed cells (including cells from
stocks known to exhibit reduced aggregation) adhere just as well as untransformed
cells when seeded onto cultured sheets of cells of like type. This seems to be a matter
of the relative sensitivity of the different method of assay.

There are conditions however, as we shall now describe, under which all transformed
BHK21 cells which we have tested, including various stocks of lines transformed by
both polyoma and Rous sarcoma viruses, adhere less well to each other than do cells
of the parent clone. In a long-term (rotation mediated) assay, aggregation of the un-
transformed cells progresses considerably beyond that of transformed cells, regardless
of whether the latter show ‘normal’ aggregation in the first hour after dispersal from
culture. As described previously by Edwards, Dysart & Hughes (1976) the behaviour
of ricin-resistant lines of BHK21 cells generally resembles that of transformed cells
in this respect. Although it is not yet clear if these findings will prove to be applicable
to any other set of fibroblastic cell lines, they are consistent with the involvement of
LETS protein in the adhesion of BHK21 cells, under the particular conditions we
have used. BHK21 cells and their various derivatives thus offer a favourable oppor-
tunity to test the role of this and other surface components and of their glycosylation,
in intercellular adhesion.

METHODS

Cells

We have used various sources of BHK21 clone 13 (C13) cells, with identical results: C13
(Glasgow) from a very low passage stock originally held in the Institute of Virology; C13
(racin parental), cells used by Meager, Ungkitchanukit & Hughes (1976) for selection of ricin-
resistant derivatives (see also Edwards et al. 1976), and C13 (Flow) obtained direct from Flow
Laboratories, Irvine. Lines transformed by polyoma virus were PyCl (one of the lines described
by Edwards et al. 1971); PyY (Stoker & Macpherson, 1964) recloned by us; PyY (Flow)
un-recloned, from Flow Laboratories; PyY AA/AAR/TG (Subak-Sharpe, 1965), resistant to
various purine analogues, from Professor Subak-Sharpe. (See also Edwards & Campbell, 1971.)
Lines transformed by Rous sarcoma virus (Macpherson, 1965, 1966) were supplied to us by
Dr M. G. Vicker (Bryan strain) and Dr J. D. Pitts (Schmidt–Ruppin strain). Growth medium
was Glasgow-modified Eagle's 8 vol., tryptose phosphate broth, 1 vol., and either calf serum,
1 vol. (ECT) or foetal bovine serum, 1 vol. (EFT). For aggregation and attachment experi-
ments, cells were dispersed from cultures by the 'low trypsin' method previously described
(which uses trypsin and EDTA simultaneously); (Edwards, Campbell, Robson & Vicker, 1975).
For detachment without trypsin, trypsin-EDTA was replaced with 0·35 mM EDTA in
phosphate-buffered saline, pH 7·2.
Mycoplasma

Mycoplasmal contaminants of cell cultures frequently grow attached to cell-surfaces (reviewed by Barile, 1973) and clearly could grossly affect intercellular adhesive behaviour. We described earlier (Edwards et al. 1975, 1976) variant stocks of C13 cells which had lost the ability to aggregate in the short-term. Such stocks we now know to be contaminated by mycoplasma (species unknown), which we assume to be responsible for their altered properties. The findings reported in this and earlier articles have been obtained or confirmed with cells judged free of mycoplasma both by measurement of the incorporation of \([\text{PH}]\text{uridine and PHTIuracil into RNA}\) (Schneider, Stanbridge & Epstein, 1974) and by staining with the DNA-binding fluorochrome Hoechst 33258 (Russell, Newman & Williamson, 1975).

Intercellular adhesion

Long-term (rotation-mediated) aggregation and short-term aggregation were as described previously (Edwards et al. 1976; Edwards & Campbell, 1971). To measure the extent of aggregation, 0.1-ml samples were diluted in 20 ml 0.9% (w/v) sodium chloride and counted in a Coulter counter \(Z\text{a}\), using a 200-\(\mu\)m aperture (Edwards, 1973). Alternatively aggregation was measured by using cells stably prelabelled with \(\text{H}^\text{P}\): cells were grown for 24 h in medium minus tryptose-phosphate (EF) containing 2 \(\mu\)Ci \(\text{H}^\text{P}\)-phosphate/ml, which was then replaced with unlabelled EF for a further 18 h of growth. Duplicate or triplicate aliquots (0.25 ml) of aggregated suspensions were pipetted onto disks (3 cm diameter) cut from Nitex bolting fabric of appropriate pore size. The Nitex disks were supported on the base ring of an inverted 15-ml funnel of a Millipore microanalytical filter assembly, and held in place by a film of Vaseline. Each sample was rinsed on the filter with 10 ml Eagle's medium, delivered from a graduated dropping-funnel, and transferred without further processing to a scintillation phial for measurement of radioactivity. To measure total cell-bound \(\text{H}^\text{P}\), aliquots were also collected on 0.22-\(\mu\)m Millipore filters.

For measurement of cell attachment to confluent cell-sheets, 0.25 \(\times\) 10\(^6\) cells were plated on 13-mm-diameter circular glass coverslips placed individually in 18-mm-square compartments of Sterilin Repli 103 25-compartment boxes. The cell-sheets were grown for 18–24 h in 2 ml EFT/compartment. Coverelips bearing cell sheets were rinsed in Hanks' medium, pH 7.2, buffered with 0.01 M HEPES. Coverslips were drained immediately before use, then received 10\(^6\) \(\text{H}^\text{P}\)-labelled cells in 0.1 ml Hanks' HEPES. After incubation at 37 °C in a humid atmosphere, the coverslips were rinsed in Hanks' HEPES, drained, and transferred to scintillation phials. The 'fraction of cells retained' was the mean cpm retained on duplicate or triplicate coverslips expressed as a fraction of total cpm in 0.1 ml of cell suspension.

RESULTS

The reduced aggregation of transformed cells in a long-term assay

In order to follow aggregation of BHK21 cells in suspension for much longer times than previously, we adopted conditions similar to those originally described by Moscona (1961) for the study of 'histogenetic reaggregation'. Thus we resuspended cells in complete growth medium, as opposed to a simple salts medium, and incubated the suspension in a gyrating, rather than a reciprocally shaking, incubator. Incubated overnight under such conditions, BHK21 clone 13 (C13) cells yield firm spherical aggregates, many of which are approximately 100 \(\mu\)m in diameter. Various different stocks of transformed cells, including all those tested so far, aggregate less. Moreover the aggregates which do form in suspensions of transformed cells give the impression of being less stable, for instance they seem to redisperse to some extent when the flask containing them is shaken by hand for removal of aliquots. Fig. 1 shows the marked
Reduced adhesiveness of transformed BHK21 contrast between the appearance of aggregated suspensions of C13 cells and of 2 transformed derivatives. The simplest method we have found to measure this difference is to use a Coulter counter to count the total number of 'particles', i.e. single cells, and aggregates regardless of size, counted as one (Edwards, 1973). Summaries of 3 sets of data obtained by this method for various different stocks of C13 and transformed derivatives (as it happens in 3 consecutive calendar years) appear in Table 1 and are also shown in Fig. 2.

---

Fig. 2. Rotation-mediated aggregation of BHK21 cells, measured by Coulter counting. (Graphic display of same data as in Table 1.) •, various stocks of untransformed C13 cells; ▲, polyoma virus-transformed cells; ■, Rous sarcoma virus-transformed cells.

Data from the derivative PyY/AA/AAR/TG are included in Table 1a because they show that the transformed phenotype defined by this assay has been retained through the long sequence of passages necessary to accumulate consecutive salvage pathway mutations.

There are possible objections to this method of assessing aggregation: first, since it measures only a reduction in particle number, the difference could be due to preferen-
Table 1. Rotation-mediated aggregation of BHK21 cells measured by Coulter counting

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Mean (N_{te}/N_0)</th>
<th>Standard deviation</th>
<th>No. of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) C13 (Glasgow)</td>
<td>0.15</td>
<td>0.08</td>
<td>14</td>
</tr>
<tr>
<td>PyCl</td>
<td>0.55</td>
<td>0.22</td>
<td>10</td>
</tr>
<tr>
<td>PyY AA/AAR/TG</td>
<td>0.78</td>
<td>0.27</td>
<td>7</td>
</tr>
<tr>
<td>(b) C13 (Glasgow)</td>
<td>0.20</td>
<td>0.07</td>
<td>12</td>
</tr>
<tr>
<td>(Ricin parental)</td>
<td>0.14</td>
<td>0.04</td>
<td>7</td>
</tr>
<tr>
<td>PyY (sub-clone)</td>
<td>0.92</td>
<td>0.24</td>
<td>9</td>
</tr>
<tr>
<td>RSV (Bryan)</td>
<td>0.65</td>
<td>0.22</td>
<td>6</td>
</tr>
<tr>
<td>RSV (Schmidt-Ruppin)</td>
<td>1.04</td>
<td>0.22</td>
<td>7</td>
</tr>
<tr>
<td>(c) C13 (Flow)</td>
<td>0.15</td>
<td>0.05</td>
<td>7</td>
</tr>
<tr>
<td>PyY (Flow)</td>
<td>1.03</td>
<td>0.40</td>
<td>6</td>
</tr>
<tr>
<td>RSV (Schmidt-Ruppin)</td>
<td>0.68</td>
<td>0.33</td>
<td>5</td>
</tr>
</tbody>
</table>

\(N_{te}/N_0\) is the ratio of the Coulter counts \(L_1-\infty\) measured in duplicate before and after aggregation, using 0.1-ml aliquots of cell suspensions.

Fig. 3. Rotation-mediated aggregation of BHK21 cells measured by filtration through Nitex bolting fabrics. Cells were stably labelled with \(^{32}P\) by growth in medium containing inorganic \(^{32}P\) phosphate, followed by a chase period in unlabelled medium containing tryptose phosphate broth. Ordinate, the radioactivity retained on Nitex of each pore size, expressed as a fraction of the retention on an 0.22-\(\mu\)m Millipore filter, which collects all intact cells. ●, untransformed C13 cells (Glasgow stock); ▲, polyoma virus-transformed cells (sub-clone of PyY); ■, Rous sarcoma virus-transformed cells (Bryan strain). Points show mean, standard deviation and number of experiments.
Reduced adhesiveness of transformed BHK21

Potential loss of untransformed cells from suspension (by cell lysis, or more likely by adhesion to the aggregation flask); second, since cells of the transformed lines can divide in suspension (Stoker, O'Neill, Berryman & Waxman, 1968) they could yield a higher count solely by virtue of cell division. The appearance of suspensions, routinely photographed, nevertheless indicates that there are substantial differences in the distribution of cells among size classes of aggregates. To confirm this we have used cells stably prelabelled by incorporation of $^{32}$P to measure the proportion of cells in aggregated suspensions which are retained on bolting fabric filters of various pore-sizes (See Fig. 1). Data obtained by this method (Fig. 3), which overcomes both objections, show clearly that suspensions of C13 cells aggregate more extensively than those of transformed cells. There is a minor effect of cell division on the particle count, however, since suspensions of transformed cells which have aggregated only slightly at 18 h sometimes show a small increase (e.g. $1.2-1.4$ times) instead of the expected decrease in count.

Fig. 4. Rotation-mediated aggregation of EDTA-resuspended BHK21 cells assessed by retention on Nitex bolting fabric, pore size 50 µm. Symbols as in Fig. 3. ○, untransformed clone 13 cells; ▲, polyoma virus-transformed cells; ■, RSV-transformed cells.

The aggregation difference is not dependent on exposure to trypsin

If we omit trypsin from the initial resuspension procedure, the cells can only poorly be separated, and only then by an extent of shearing in a Pasteur pipette which causes extensive cell lysis. Using a dispersal procedure based on EDTA alone, and gentle aspiration, transformed cultures yield reasonably single cell suspensions, untransformed a population of ragged aggregates. If these rather different initial suspen-
Adhesions formed in suspension between BHK21 cells are readily redispersed, at least at early times, by low levels of proteolytic enzymes such as trypsin (Edwards & Campbell, 1971). In view of the evidence that some aspects of the transformed phenotype may result from activation of serum plasminogen by a cellular protease (Ossowski et al., 1972) it seemed possible that the less extensive aggregation of transformed cells might result from a higher degree of plasmin activation in the aggregating suspensions of these cells. To test this, we added e-aminocaproic acid (EACA) to cell suspensions. EACA inhibits plasminogen activation (Alkjaersig, Fletcher & Sherry, 1959) and fibrinolysis by cultured fibroblasts (Unkeless et al., 1973; Chou, Black & Roblin, 1974). At the concentration used (10 mg/ml) EACA partly inhibits the growth of C13 cells but has little effect on transformed derivatives (Edgar, unpublished). Fig. 5A shows that the presence of EACA did not restore 'normal' aggregation to polyoma-transformed cells. Somewhat surprisingly, however, significant stimulation of aggregation was found if transformed cells were both grown and aggregated in the presence of EACA (Fig. 5B). Thus if differential plasmin activation is involved in the
Reduced adhesiveness of transformed BHK21

difference in aggregation, it must affect the cells before they are dispersed from culture for aggregation.

Some transformed lines show 'normal' short-term aggregation

Data summarized in Table 2 show that various stocks of polyoma-transformed BHK21 cells (Py-BHK) aggregate much less than C13 cells in the first hour from trypsin dispersal, as originally described by Edwards et al. (1971). This result is however not universal for transformed BHK21 cells. In addition to the line adapted to growth in suspension which we used in earlier work to show that short-term aggregation does not require exposure to trypsin (Edwards & Campbell, 1971) we have found, in agreement with Vicker (1976) that cells transformed by the Bryan strain of Rous sarcoma virus (RSV(B)-BHK) also aggregate in the short-term assay. The same cells nevertheless show 'transformed' behaviour in the long-term assay described above.

Table 2. Short-term aggregation of BHK21 cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Mean $N_{45}/N_0$</th>
<th>Standard deviation</th>
<th>No. of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>C13 (Glasgow)</td>
<td>0.46</td>
<td>0.07</td>
<td>16</td>
</tr>
<tr>
<td>C13 (Flow Laboratories)</td>
<td>0.57</td>
<td>0.09</td>
<td>6</td>
</tr>
<tr>
<td>PyCl</td>
<td>0.91</td>
<td>0.05</td>
<td>5</td>
</tr>
<tr>
<td>PyY (sub-clone)</td>
<td>0.93</td>
<td>0.13</td>
<td>10</td>
</tr>
<tr>
<td>PyY (Flow laboratories)</td>
<td>0.93</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>RSV (Bryan strain)</td>
<td>0.66</td>
<td>0.10</td>
<td>5</td>
</tr>
<tr>
<td>RSV (Schmidt-Ruppin)</td>
<td>0.87</td>
<td>0.09</td>
<td>5</td>
</tr>
</tbody>
</table>

Cells were aggregated for 45 min in a reciprocating shaker either in growth medium or in HEPES-buffered Hanks'. $N_{45}/N_0$ is the ratio of the Coulter counts, $L_{1-\infty}$ measured in duplicate before and after aggregation, using 0.1-ml aliquots of cell suspensions.

Table 3. Adhesion of BHK21 cells to homotypic cell sheets

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Fraction of cells adherent after 45 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>C13 (Glasgow)</td>
<td>0.74 ± 0.12 (Mean of 10 values)</td>
</tr>
<tr>
<td>PyY (Sub-clone)</td>
<td>0.68, 0.65, 0.41, 0.54</td>
</tr>
<tr>
<td>RSV (Bryan strain)</td>
<td>0.91, 0.85, 0.74, 0.76</td>
</tr>
</tbody>
</table>

Transformed cells are not less adhesive to sheets of themselves

When we use a different type of assay of intercellular adhesiveness, in which suspended cells are seeded onto confluent sheets of cells of like type, none of the transformed lines shows a marked reduction compared with C13, in ability to adhere. Indeed under these conditions RSV(B)-BHK cells sometimes adhere slightly better than untransformed cells (Table 3 and Fig. 6). Similar results have been reported by Allen & Minnikin (1975) using an attachment assay similar in principle, although different in details.
Fig. 6. Attachment of BHK$_{21}$ cells to confluent cell sheets. 0.10 ml containing $5 \times 10^4$ $^{32}$P-labelled cells in HEPES-buffered Hank's medium, was pipetted onto each of a series of 13-mm coverslips on which a confluent cell sheet had been grown. Coverslips were washed after incubation for the times indicated, and residual adherent cells measured by scintillation counting. Ordinate is retained radioactivity as a fraction of total, and measures the fraction of cells retained. ●, C$_{13}$ cells adhering to C$_{13}$ sheets; ▲, PyY (recloned) adhering to PyY; ■, RSV (B)-BHK cells adhering to RSV(B)-BHK sheets.

DISCUSSION

These experiments establish that in rotation-mediated aggregation transformed BHK$_{21}$ cells adhere consistently less well to each other than do untransformed. Some lines also show a parallel deficit in short-term aggregation, as first reported by Edwards et al. (1971). Reduced aggregation of Py-BHK cells has also been observed by O'Neill (1973), by Whur, Koppell, Urquhart & Williams (1977) and under certain conditions by Urushihara, Hakura & Okada (1976). The reduced aggregation of transformed BHK$_{21}$ cells seems to parallel the greater ease with which cells from confluent transformed cultures can be separated from each other to yield single cell suspensions (O'Neill & Burnett, 1974; Whur et al. 1977). Untransformed BHK$_{21}$ cells in confluent sheets seem to be bound together by a trypsin-sensitive linkage which is redeployed during aggregation, and which is less well developed in cultures of transformed cells.

Possible involvement of LETS protein in determining aggregation behaviour of BHK cells

There have been several suggestions that in addition to adhesion to culture substrates, LETS protein may be involved in intercellular adhesion (Weston & Roth, 1969; Yamada, Yamada & Pastan, 1976; Zetter, Chen & Buchanan, 1976; Mautner & Hynes, 1977).

Evidence was presented earlier (Edwards et al. 1975) that aggregation of BHK$_{21}$ cells depends on the integrity of a protein (then designated $P_a$) which is synthesized by cells, rather than bound from medium, and which is external, in the sense of being
Reduced adhesiveness of transformed BHK21

accessible to trypsin acting on intact cells. There is some circumstantial evidence that P_a could be LETS: the aggregation of trypsinized BHK cells increases with culture density (Edwards & Campbell, 1971; Urushihara et al. 1976) as does the amount of LETS detected by iodination (Pearlstein & Waterfield, 1974) or by galactose oxidase labelling (Gahmberg, Kiehn & Hakomori, 1974); the reduced aggregation of transformed BHK cells described above could be a response to the decrease in LETS (Hynes, 1973; Gahmberg et al. 1974; Pearlstein & Waterfield, 1974). It may be objected that a highly trypsin-sensitive species can hardly be involved in short-term aggregation, which occurs with no detectable lag (Edwards et al. 1975). However we previously noted that whereas early formed adhesions are very sensitive to dispersal by proteolytic enzymes, much more extensive exposure to trypsin as pretreatment is needed to abolish short-term aggregation. We therefore suggested that the latter may depend on emergence of P_a (LETS protein?) from a shielded state, presumably on its extrusion from an intracellular pool.

Since viral transformation of established lines commonly results in diminution of LETS it must be asked why corresponding reduction of intercellular adhesiveness seems not generally to have been observed in cells other than BHK21. Variables which may be responsible, or have been held responsible, include identity of the cells, methods of dispersing cells from cultures and methods of measuring intercellular adhesion.

There seems to be no reason a priori why the adhesive properties of cells of different established lines should be similar, and respond similarly to transformation. The ability of cells to adhere and otherwise interact to form a tissue may be retained to a greater or lesser degree in established lines, many of which are not even approximately diploid. Moreover, even though lines may preserve some histogenetic characteristics of the cells which gave rise to them, the identity of these progenitors is not always clear. For example, a number of studies of intercellular adhesiveness have used Balb 3T3 cells (Dorsey & Roth, 1973; Walther, Ohman & Roseman, 1973; Cassiman & Bernfield, 1975; Vosbeck & Roth, 1976) which may be derivatives of vascular endothelial cells (Porter, Todaro & Fonte, 1973; Boone, 1975).

We have explained above that we cannot avoid using trypsin to disperse C13 cells from confluent cultures, the state in which they seem to be most adhesive. Cassiman & Bernfield (1975), discussing short-term aggregation, have pointed out that the use of trypsin is likely to remove adhesive factors from the cell surface, or otherwise modify surface components involved in adhesion. This could hardly apply with greater force than if our hypothesis of LETS involvement is correct. We have outlined above in terms of an intracellular pool of a trypsin-sensitive component, one way in which it is possible that the aggregation of briefly trypsinized cells may yet reflect a genuine difference in adhesiveness. Using Balb 3T3 and WI 38 cells, however, Cassiman & Bernfield (1975) found that EDTA-resuspended transformed cells aggregated faster than corresponding untransformed lines, as did Wright, Ukena, Campbell & Karnovsky (1977) for a series of transformed lines (although for the opposite result, see Thompson, Elligsen & Frey, 1975). O'Neill & Burnett (1974) also reported a decrease in aggregation of EDTA-resuspended Balb 3T3 cells with culture density.
Presumably a different mechanism of adhesion operates in these experiments, in which transformation-sensitive surface components are not limiting.

Our results serve to emphasize that the term 'cell adhesiveness' has meaning only in relation to a particular method of assay. Lines such as RSV(B)-BHK, aggregate much as do untransformed cells in the short-term but fall behind at longer times. It is not difficult to explain this in general terms: the short-term assay measures the rapid formation of small clusters of cells; formation of much larger aggregates in the long-term assay may require greater strength of adhesion between individual cells, or altered cell shape, and may depend on a broader spectrum of cellular activities, including perhaps the synthesis and accumulation of extracellular matrix. In specific terms of LETS protein it is necessary to assume that the residual LETS of RSV(B)-BHK is sufficient for short-term aggregation, but not in the longer-term. More difficult to explain is the finding that Py-BHK cells which adhere minimally to each other in suspension, adhere extensively on a similar time-scale to confluent sheets of themselves. One possibility is that Py-BHK cells adhere by finding exposed culture-substrate, since sheets of criss-crossed polyoma-transformed cells undoubtedly do present some gaps. Perhaps more likely is the possibility that in this type of attachment assay, even when the system is shaken, many of the seeded cells are in stationary contact with the cell sheet, allowing relatively long contact time before the adhesion is challenged by washing.

Decreased adhesiveness of transformed cells may be a secondary consequence of altered growth control

O'Neill (1973) has shown that the adhesiveness of trypsinized BHK cells in a short-term aggregation assay decreases when the cells are released from growth inhibition by infection with polyoma virus (abortive transformation) or by addition of serum. This correlation with growth state could account for our earlier finding, confirmed by Urushihara et al. (1976), of increased aggregation with increasing culture density. The reduced aggregation of transformed cells can then be seen as a reflexion of their different growth state, either in the cultures dispersed for aggregation, in the aggregating suspensions or both. (In suspension the untransformed cells may be at least partially inhibited by anchorage-dependence (Stoker et al. 1968) whereas the transformed cells escape this limitation.) As discussed by Hynes & Bye (1974) in relation to levels of LETS protein, a cell property correlated in this way with growth state may be interpreted either as having a causal role in growth control, or as being an aspect of the differentiated state of the cells which is enhanced in cells in G1 growth arrest. In view of the variability in intercellular adhesive properties of established lines subject to growth control, we suspect adhesiveness to be an example of the latter.

If this interpretation is correct, our experiments have no bearing on ways in which surface changes could control growth. They nevertheless suggest that as a consequence, however remote, of viral gene action, components involved in intercellular adhesion are in some way deficient in suspensions of virally transformed BHK cells, an observation which may contribute to the identification of species concerned.
Reduced adhesiveness of transformed BHK21

We thank Gordon Campbell and Andrew Hart for excellent technical assistance. This work is supported by the Cancer Research Campaign.

REFERENCES


(Received 4 July 1978)