MICROFILAMENT BUNDLES, LETS PROTEIN AND GROWTH CONTROL IN SOMATIC CELL HYBRIDS

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SUMMARY

Hybrid cell lines between normal rat embryo fibroblasts and TA3B mouse tumour cells, or between TA3B and BI hamster sarcoma cells, have been examined for the expression of the cell surface large external-transformation-sensitive (LETS) protein and the organization of microfilament bundles. LETS protein was detected by lactoperoxidase-catalysed radioiodination and microfilament bundles were visualized by indirect immunofluorescence with antibodies directed against actin or myosin. Hybrids which exhibited normal growth control characteristics had high levels of LETS protein and extensive microfilament bundles. Neoplastic transformation appears to be suppressed in these hybrids. Hybrids which had the growth control characteristics typical of transformed cells had reduced or zero levels of LETS protein and few microfilament bundles. These results confirm previous studies on the expression of the transformed phenotype in these hybrids and demonstrate that there is a good correlation between normal growth control and the presence of LETS protein and microfilament bundles. However, the changes in cell surface LETS protein and in the organization of microfilament bundles often appeared to be quantitative reductions rather than all-or-none effects. The magnitude of the alterations in levels of LETS protein and in the organization of microfilaments appeared to correlate with the range of transformed characteristics exhibited by the cells. One transformed hybrid in particular, selected for growth in agar, had some surface LETS protein, some microfilament bundles and retained density-dependent inhibition of growth.

INTRODUCTION

We have previously shown that 3 out of 4 hybrid cell lines between TA3B mouse mammary tumour cells and normal rat embryo fibroblasts (REF) have normal growth control as demonstrated by the cells exhibiting density dependent inhibition of growth and failing to grow in agar suspension (Marshall & Dave, 1978). The transformed phenotype, as detected by tests for growth control, therefore appears to be suppressed in these 3 hybrid lines. Suppression also occurs in all 5 hybrid lines between TA3B and BI hamster sarcoma cells. However suppression in the BI x TA3B hybrids is less stable and variants which grow in agar and lack density-dependent inhibition of growth can be selected by culturing hybrid cells that grow in agar or form tumours in nude mice. In order to examine whether suppression extends to characteristics of the transformed phenotype which are not detected by alterations in growth control
we have examined hybrids for microfilament bundles and cell surface LETS (large external-transformation-sensitive) protein.

As discussed by Harris (1975) the suppression of malignancy by cell fusion followed by the segregation of malignant variants provides a genetic system to determine whether any putative marker of neoplastic transformation correlates with malignancy since it must show a precisely defined pattern of expression in the suppressed hybrids and their segregants. We have adapted this approach to examine whether the expression of microfilament bundles and LETS protein correlates with growth control in suppressed and variant hybrids.

Immunofluorescent staining with antibody directed against actin or myosin demonstrates that normal spread fibroblasts contain fluorescent staining cables that run the length of the cell at the adherent surface (Lazarides & Weber, 1974; Weber & Groschel-Stewart, 1974). These cables or sheaths appear to consist of bundles of microfilaments (Buckley, 1975; Goldman, Lazarides, Pollack & Weber, 1975; Goldman, Yerna & Schloss, 1976). Microfilament bundles detected by immunofluorescence are absent or less frequent in cells of most transformed lines (Goldman, Chang & Williams, 1974; Pollack, Osborn & Weber, 1975). The organization of the cytoskeleton therefore appears to be different in normal and transformed cells.

One of the most striking differences between normal and transformed cells is the reduction or absence in transformed and tumour cells of a major cell surface glycoprotein of approximate mol. wt. 240000, called Z protein, soluble fibroblast antigen (SFA), cell surface protein (CSP) or LETS protein (reviewed in Hynes, 1976). This extracellular glycoprotein may be detected either by external labelling procedures (Gahmberg & Hakomori, 1973; Hynes, 1973) or with antisera directed against cell surface proteins (Ruoshlati, Vaheri, Kuusela & Linder, 1973) or cold-insoluble globulin (Chen, Gallimore & MacDougal, 1976). Some neoplastically transformed cell lines express LETS protein at the cell surface (Pearlstein, Hynes, Franks & Hemmings, 1976) but the amounts appear to be reduced and the organization on the cell surface differs from normal cells (Chen et al. 1976). The role of LETS protein remains to be fully clarified but it is significant that it can behave as an agglutinin (Yamada, Yamada & Pastan, 1975), is especially concentrated at cell contacts (Chen et al. 1976) and the surface levels are highest in G1 and lowest at mitosis (Hynes & Bye, 1974). Addition of isolated LETS protein to transformed cells restores some of the properties of normal cells including microfilament bundles and adhesiveness (Ali, Mautner, Lanza & Hynes, 1977; Willingham et al. 1977).

MATERIALS AND METHODS

Cells and cell culture

The BI, TA3B, REF and their hybrids have been previously described (Marshall & Dave, 1978). In some cases subclones of the transformed hybrids were isolated by seeding 100 cells into agar and picking well isolated colonies. Cells were routinely grown at 37 °C in Dulbecco's medium with 10% foetal calf serum (DF10) (Gibco, Paisley; Scotland) and passaged when confluent. For the localization of microfilament bundles cells were seeded at 1/8 or 1/4 confluent density onto 11-mm coverslips in DF10 and fixed after about 48 h. BI, TA3B cells and
their hybrids for the detection of LETS protein were allowed to reach confluence and then incubated for a further day in Dulbecco's medium containing 0.25% foetal calf serum. TA3B, REF cells and their hybrids were allowed to reach confluence in DF10 and then kept for 1 further day in DF10 before iodination.

**Immunofluorescence staining**

Coverslips for immunofluorescence with antibody against actin were processed as described previously (Pollack & Rifkin, 1975). In early experiments cells were grown in London, fixed and sent to Stony Brook to be stained, in later work cells were grown at Stony Brook, fixed and stained immediately. Coverslips for immunofluorescence with antibody against myosin were rinsed in PBS, fixed in acetone at −20 °C for 30 s, dried and then stained first with rabbit antimyosin for 1 h at 37 °C and then with fluorescein isothiocyanate-conjugated goat anti-rabbit serum (Miles). At least 100 cells were examined for each cell line and classed as positive if they possessed structures which ran the length of the cell. LETS protein was detected by immunofluorescence with antibody against cold-insoluble globulin on cells fixed in 75% formaldehyde for 15 min. Coverslips were examined under epi-illumination using a Zeiss photomicroscope.

**Lactoperoxidase catalysed radio-iodination and SDS gel electrophoresis**

These techniques were essentially those described by Hynes & Humphryes (1974) except that carrier free 125I (Radiochemical Centre, Amersham, U.K.) was used at a concentration of 500 μCi/ml. Since we found that there was some day-to-day variation in the efficiency of iodination, suppressed hybrids, their transformed variants and parental cells were always iodinated at the same time. A trypsin-sensitivity control to confirm the identity of LETS protein (Hynes & Humphryes, 1974) was run on most cell samples by incubating iodinated cells for 10 min in PBS containing 10 μg/ml trypsin (Sigma). To serve as a positive control NIL cells (Hynes, 1973) were always iodinated in parallel with the hybrids and parentals and a sample of iodinated NIL cells applied to one track of the slab gel. Protein concentration was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951).

**RESULTS**

**Microfilament bundles**

Fig. 1A shows that well spread REF contain many microfilament bundles, as has been previously described (Pollack et al. 1975). In our experimental conditions about 70% of REF were positive for structures. Less than 1% of mouse TA3B tumour cells possess microfilament bundles which can be visualized by immunofluorescence. A TA3B cell stained with antibody to actin is shown in Fig. 1B.

Table 1 demonstrates that a large fraction of cells with microfilament bundles are found only in those TA3B X REF hybrids which show normal growth control because they fail to grow in agar and exhibit density-dependent inhibition of growth. The expression of microfilament bundles is further evidence that neoplastic transformation is suppressed in these hybrids. A typical suppressed hybrid, TREF 765, with many microfilament bundles stained with antibody against actin is shown in Fig. 1C. In contrast TREF 13.5B a hybrid which grows in agar, lacks density-dependent inhibition of growth and is tumorigenic in nude mice, contained less than 15% of cells with microfilament bundles detectable by immunofluorescence. Subclones of TREF 13.5B selected by growth in agar also contain very few cells with microfilament bundles.
Fig. 1. Indirect immunofluorescent staining of TA3B, REF, BI cells and their hybrids with antibody directed against actin (A) or myosin (M). All photographs at same magnification of approximately × 530. A, REF cells (M); note many microfilament bundles. B, TA3B cells (A); diffuse staining no microfilament bundles. C, TA3B x REF hybrid TREF 765 (A); note many microfilament bundles. D, TA3B x REF hybrid TREF 13.5B (A); diffuse staining. E, BI cells (A); diffuse staining. F, BI x TA3B hybrid BIT 4.5 (M); note many microfilament bundles. G, BI x TA3B hybrid BIT 4.5 AC3/10 (M); diffuse staining. H, BI x TA3B hybrid BIT 3.4 AC1 (M); note some microfilament bundles.
Table 1. Growth control and presence of cell surface LETSP and microfilament bundles in TA3B × REF (TREF) cell hybrids

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>% colony-forming efficiency in agar</th>
<th>Density-dependent inhibition of growth</th>
<th>% cells with microfilament bundles</th>
<th>Level of surface LETSP</th>
</tr>
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<tbody>
<tr>
<td>REF</td>
<td>Non-transformed parental</td>
<td>0*</td>
<td>No</td>
<td>71</td>
<td>High</td>
</tr>
<tr>
<td>TA3B</td>
<td>Transformed parental</td>
<td>2.3</td>
<td>No</td>
<td>&lt;1</td>
<td>Absent</td>
</tr>
<tr>
<td>Hybrids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TREF 765</td>
<td>Non-transformed hybrid</td>
<td>0*</td>
<td>Yes</td>
<td>75</td>
<td>High</td>
</tr>
<tr>
<td>TREF 765/SC1</td>
<td>Clone of hybrid 765</td>
<td>0</td>
<td>Yes</td>
<td>40</td>
<td>High</td>
</tr>
<tr>
<td>TREF 765/SC2</td>
<td>Clone of hybrid 765</td>
<td>0</td>
<td>Yes</td>
<td>59</td>
<td>High</td>
</tr>
<tr>
<td>TREF 3.2</td>
<td>Non-transformed hybrid</td>
<td>0</td>
<td>Yes</td>
<td>42</td>
<td>High</td>
</tr>
<tr>
<td>TREF 3.31</td>
<td>Non-transformed hybrid</td>
<td>0</td>
<td>Yes</td>
<td>59</td>
<td>High</td>
</tr>
<tr>
<td>TREF 13.5B</td>
<td>Transformed hybrid</td>
<td>1.0</td>
<td>No</td>
<td>15</td>
<td>Absent</td>
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<tr>
<td>TREF 13.5B AC1/1</td>
<td>13.5B cloned in agar</td>
<td>3.0</td>
<td>No</td>
<td>3</td>
<td>Absent</td>
</tr>
<tr>
<td>TREF 13.5B AC2/2</td>
<td>13.5B cloned in agar</td>
<td>30.0</td>
<td>No</td>
<td>1</td>
<td>Absent</td>
</tr>
<tr>
<td>TREF 13.5B AC2/5</td>
<td>13.5B cloned in agar</td>
<td>10.4</td>
<td>No</td>
<td>4</td>
<td>Absent</td>
</tr>
<tr>
<td>TREF 13.5B AC3/2</td>
<td>13.5B cloned in agar</td>
<td>16.7</td>
<td>No</td>
<td>1</td>
<td>Absent</td>
</tr>
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</table>

* No colonies were obtained in agar for these cell lines.
<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>% colony-forming efficiency in agar</th>
<th>Density-dependent inhibition of growth</th>
<th>% cells with microfilament bundles</th>
<th>Level of cell surface LETSP</th>
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</thead>
<tbody>
<tr>
<td>BIT</td>
<td>Transformed parental</td>
<td>33.0</td>
<td>No</td>
<td>&lt; 1</td>
<td>Low</td>
</tr>
<tr>
<td>TA3B</td>
<td>Transformed parental</td>
<td>2.3</td>
<td>No</td>
<td>&lt; 1</td>
<td>Absent</td>
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<tr>
<td>Hybrids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIT 4.5</td>
<td>Non-transformed hybrid</td>
<td>0.7</td>
<td>Yes</td>
<td>96</td>
<td>High</td>
</tr>
<tr>
<td>BIT 4.5 AC3/10</td>
<td>Transformed derivatives of</td>
<td>66.0</td>
<td>No</td>
<td>8</td>
<td>Absent</td>
</tr>
<tr>
<td>BIT 4.5 AC14/7</td>
<td>4.5 cloned in agar</td>
<td>67.0</td>
<td>No</td>
<td>NT*</td>
<td>Absent</td>
</tr>
<tr>
<td>BIT 3.4</td>
<td>Non-transformed hybrid</td>
<td>0.05</td>
<td>Yes</td>
<td>80</td>
<td>High</td>
</tr>
<tr>
<td>BIT 3.4 AC1</td>
<td>Transformed derivatives</td>
<td>6.9</td>
<td>Yes</td>
<td>49</td>
<td>Reduced compared to 3.4</td>
</tr>
<tr>
<td>BIT 3.4 AC13</td>
<td>of 3.4</td>
<td>4.3</td>
<td>Yes</td>
<td>NT</td>
<td>to 3.4</td>
</tr>
<tr>
<td>BIT 4.2</td>
<td>Non-transformed hybrid</td>
<td>0.13</td>
<td>Yes</td>
<td>31</td>
<td>High</td>
</tr>
<tr>
<td>BIT 4.2 AC2/3</td>
<td>Transformed derivatives of</td>
<td>42.0</td>
<td>No</td>
<td>8</td>
<td>Reduced compared to 4.2</td>
</tr>
<tr>
<td>BIT 4.2 AC2/7</td>
<td>4.2 cloned in agar</td>
<td>39.0</td>
<td>No</td>
<td>8</td>
<td>Absent</td>
</tr>
<tr>
<td>BIT 4.2 AC4/5</td>
<td></td>
<td>25.0</td>
<td>No</td>
<td>NT</td>
<td>Absent</td>
</tr>
<tr>
<td>BIT 4.2 AC4/6</td>
<td></td>
<td>1.1</td>
<td>No</td>
<td>NT</td>
<td>Reduced compared to 4.2</td>
</tr>
<tr>
<td>BIT 897/A</td>
<td>Nude tumour from hybrid 4.2</td>
<td>43.0</td>
<td>No</td>
<td>2</td>
<td>Absent</td>
</tr>
<tr>
<td>BIT 901/B</td>
<td>Nude tumour from 4.2 AC2/3</td>
<td>34.0</td>
<td>No</td>
<td>18</td>
<td>Similar to 4.2 AC2/3</td>
</tr>
</tbody>
</table>

* NT = not tested.
Microfilaments and LETS protein in hybrid cells

Like TA3B less than 1% of B1 hamster sarcoma cells contain microfilament bundles. As Table 2 shows three hybrid cell lines between B1 and TA3B (BIT 4.5, 3.4, 4.2), which show normal growth control, contained many cells with extensive microfilament bundles. A BIT 4.5 hybrid cell is shown in Fig. 1F. Therefore like the TA3B × REF hybrids suppression of neoplastic transformation following cell fusion results not only in normal growth control but also in extensive microfilament bundles in a large fraction of the cells. Examination of the results presented in Table 2 shows that there was some variation in the number of positive cells between different lines of suppressed hybrids. While part of this variation is possibly due to differences between the lines, the relatively low number of positive cells recorded for BIT 4.2 probably arises from keeping the cells fixed for prolonged periods before staining.

Variants can be isolated from suppressed BIT hybrids by the culture of cells that grow in agar or in nude mice. These variants grow in agar and usually lack density dependent inhibition of growth (Marshall & Dave, 1978). It can be seen from Table 2 that these variant hybrids contained far fewer cells with microfilament bundles. These results demonstrate a correlation between the altered growth control typical of transformed cells and the reduction in number of cells positive for microfilament bundles. A typical variant stained with antibody against myosin is shown in Fig. 1G. No microfilament bundles are visible in this cell. Variant BIT 3.4 AC1 contained a considerable number of cells with microfilament bundles although the cells grew in agar. However, subjective evaluation of the positive cells suggested they contained fewer microfilament bundles than the original BIT 3.4. This phenotype was also found in a subclone of BIT 3.4 AC1. These results suggest that a quantitative reduction in microfilament bundles rather than a complete loss may sometimes accompany anchorage independent growth. Furthermore, BIT 3.4 AC1 differed from the other transformed variants in that it possessed density dependent inhibition of growth and considerable levels of LETS protein.

LETS protein

Lactoperoxidase-catalysed iodination followed by SDS gel electrophoresis shows that there are large amounts of LETS protein on the surfaces of REF cells but none on TA3B tumour cells. The autoradiograph of a gel illustrated in Fig. 2 shows that only those TREF hybrids which show normal growth control (TREF 765, 765 SC1, 765 SC2, 3.2, 3.31) have LETS protein on their surfaces. The hybrid TREF 13.5B which grows in agar and is tumorigenic and its subclones (13.5B AC1/1, AC2/2, AC2/5, AC3/2) did not have detectable cell surface LETS protein. Fig. 2 also shows that the levels of LETS protein on suppressed hybrids appear to be higher than on the normal REF cells because there is more radioactivity in the LETS protein bands even though each sample applied to the gel contained the same number of cells.

Unlike TA3B cells radio-iodination of B1 cells detects some cell surface LETS protein. However, Fig. 3A shows that when B1 cells are examined by immuno-fluorescence with antibody against cold-insoluble globulin which detects LETS protein, that staining is restricted to the edges and cell-cell contacts of B1 cells. LETS protein on B1 cells is rarely found as fibrils which run across the surface of normal
cells (Chen et al. 1976). The autoradiograph shown in Fig. 4 shows that the levels of iodinated LETS protein on the surface of the suppressed hybrid BIT 4.5 are higher than on BI cells. The identity of the iodinated material was further confirmed by showing that it was removed by incubation in 10 μg/ml trypsin for 10 min (Hynes & Humphries, 1974). Furthermore, immunofluorescent staining illustrated in Fig. 3c shows that LETS protein on BIT 4.5 is arranged in the fibrillar structures typical of normal cells. Variants of BIT 4.5, 4.5 AC3/10 and 4.5 AC14/7 isolated by their ability to grow in agar and lacking density-dependent inhibition of growth do not have any cell surface LETS protein detectable by iodination.

The suppressed hybrid BIT 3.4, like BIT 4.5 has high levels of LETS protein demonstrable by iodination. Variants of BIT 3.4 selected for growth in agar (BIT 3.4 AC1, 3.4 AC13) still retain considerable levels of LETS protein. But Fig. 5 illustrates that the amount per cell is reduced compared to BIT 3.4. This is especially clear when samples are compared by applying to the gel equal amounts of protein, and by implication, equal numbers of cells. To complete the correlation between the levels of LETS protein and the expression of neoplastic transformation we investigated whether variants selected for tumour production showed reduced levels of LETS protein. Fig. 6 shows that cultures from 897/A, a tumour produced by inoculation of BIT 4.2 into nude mice, completely lack LETS protein. BIT 4.2, the suppressed hybrid with normal growth control, has high levels of LETS protein. BIT 4.2 AC2/7, a variant of BIT 4.2 selected for growth in agar, also lacked LETS protein. BIT 4.2 AC2/3 a variant of BIT 4.2 selected by growth in agar still produced some LETS protein although unlike BIT 3.4 AC1 it did not show density-dependent inhibition of growth. It was of interest to determine whether growth of BIT 4.2 AC2/3 in nude mice would select for cells which completely lack LETS protein. This was not found to be the case, since Fig. 6 shows that 901B, a nude mouse tumour from BIT 4.2 AC2/3, still produced similar amounts of LETS protein to BIT 4.2 AC2/3.

DISCUSSION

We have shown that hybrid cells which do not grow in agar and possess density-dependent inhibition of growth, contain extensive microfilament bundles and have high levels of extracellular LETS protein. These hybrids therefore resemble normal

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Fig. 2. Autoradiograph from SDS gel electrophoresis (7% acrylamide) of lactoperoxidase-catalysed iodinated cell surface proteins of REF and its hybrids with TA3B tumour cells. Samples were loaded for 3 x 10⁶ cells in each track. Track A, REF, 0.37 x 10⁶ cpm; B, TREF 765 SC1, 1.34 x 10⁶ cpm; C, TREF 765 SC2, 2.4 x 10⁶ cpm; D, TREF 2/3.2, 1.3 x 10⁶ cpm; E, TREF 2/3.31, 1.74 x 10⁶ cpm; F, TREF 13.5B, 0.96 x 10⁶ cpm; G, Nil cells as a marker of LETS protein, 10⁵ cpm.

Fig. 3. Indirect immunofluorescence staining for LETS protein with antibody directed against cold-insoluble globulin. All photographs are of a magnification of approximately ×340. A, BI cells, note fluorescence around edges of cells and cell contacts. B, same field of BI cells phase-contrast, note the large number of cells in the field. C, BIT 4.5, note fluorescent fibres which run over the surface of the cells. D, same field BIT 4.5 phase-contrast, note far fewer cells compared with BI, cells are also more spread.
Microfilaments and LETS protein in hybrid cells

Fig. 6. Autoradiograph from SDS gel electrophoresis of iodinated cell surface proteins of BI, TA3B cells, the suppressed hybrid BIT 4.2 and its derivatives selected for tumour production (897/A, 901/B) or growth in agar BIT 4.2 AC2/3, BIT 4.2 AC2/7. The arrowheads mark the position of LETS protein. Samples with equal amounts of radioactivity (10^4 125I cpm) were added to tracks A-E, G, I, K and the remaining tracks received samples with equal amounts of protein (25 μg). Track A, Nil, 10^4 cpm; B, TA3B, 10^5 cpm; C, BI, 10^6 cpm; D, BIT 4.2, 10^6 cpm; E, BIT 4.2 AC2/3, 10^6 cpm; F, BIT 4.2 AC2/3, 0.5 x 10^5 cpm; G, BIT 4.2 AC2/7, 10^6 cpm; H, BIT 4.2 AC2/7, 0.5 x 10^5 cpm; I, 897/A, 10^5 cpm; J, 897/A, 0.5 x 10^5 cpm; K, 901/B, 10^6 cpm; L, 901/B, 0.82 x 10^6 cpm.

Fig. 4. Autoradiograph from SDS gel electrophoresis of iodinated cell surface proteins of BI, TA3B cells, the suppressed hybrid BIT 4.5 and its transformed variants 4.5 AC3/10, 4.5 AC14/7. The arrows mark the position of LETS protein. Samples with equal amounts of radioactivity (5 x 10^4 125I cpm) were added to tracks A, B, D, F, H, J and L. Samples of cells which had been iodinated and then treated with trypsin were run in the other tracks, the samples in these tracks contained the same amount of protein as the non-trypsinized samples. Track A, Nil; B, TA3B; C, TA3B+trypsin; D, BI; E, BI+trypsin; F, BIT 4.5; G, BIT 4.5+trypsin; H, BIT 4.5 AC3/10; I, BIT 4.5 AC3/10+trypsin; J, BIT 4.5 AC 14/7; K, BIT 4.5 AC 14/7+trypsin; L, Nil.

Fig. 5. Autoradiograph from SDS gel electrophoresis of iodinated cell surface proteins of BI, TA3B cells, the suppressed hybrid BIT 3.4 and its transformed variants BIT 3.4 AC1, BIT 3.4 AC13. The arrows mark the position of LETS protein. Samples with equal amounts of radioactivity (10^5 125I cpm) were applied to tracks A-F and L. The other samples (G-I) were loaded for 26 μg of protein. Track A, Nil, 10^5 cpm; B, TA3B, 10^6 cpm; C, BI, 10^6 cpm; D, BIT 3.4, 10^6 cpm; E, BIT 3.4 AC1, 10^6 cpm; F, BIT 3.4 AC13, 10^6 cpm; G, TA3B, 0.18 x 10^6 cpm; H, BI, 0.2 x 10^6 cpm; I, BIT 3.4, 10^6 cpm; J, BIT 3.4 AC1, 0.5 x 10^6 cpm; K, BIT 3.4 AC13, 0.5 x 10^6 cpm; L, Nil, 10^5 cpm. Note that comparing the transformed variants BIT 3.4 AC1, 3.4 AC13 with BIT 3.4 on the basis of equal amounts of protein (≈ equal numbers of cells) shows that the transformed variants have decreased amounts of LETS protein.
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cells not only in their growth control (Marshall & Dave, 1978), but also in other markers of the non-transformed phenotype. Therefore by all criteria we have examined neoplastic transformation appears to be suppressed in these hybrid cells. Hybrid cells which show the alterations of growth control characteristic of transformed cells have disorganized microfilament bundles and have zero or small amounts of LETS protein. The transformed hybrids therefore have the properties of typical transformed cells.

These observations on two hybrid cell systems support the general observation (e.g. Pollack et al. 1975) that normal growth control correlates with extensive microfilament bundles while the anchorage-independent growth of transformed cells (as shown by growth in agar) correlates with the loss of bundles. However, exceptions to anchorage-independent growth correlating with the complete loss of microfilament bundles were found (e.g. BIT 3.4 AC1). Exceptions have been observed before (e.g. Willingham et al. 1977). But it remains to be determined whether such exceptions arise due to alternative pathways of transformation or whether the loss of microfilament bundles is not a direct result of neoplastic transformation. However, the consistent appearance of both microfilament bundles and LETS protein in all the hybrids with normal growth control and their absence in most of the transformed hybrids argues that reduction in microfilament bundles and LETS protein are events closely related to the transformation event that results in altered growth control. The results with BIT 3.4 AC1 which grew in agar and had less extensive arrays of microfilament bundles than the non-transformed BIT 3.4 suggest that a decrease rather than complete loss of microfilament bundles may be a sufficient condition for anchorage independent growth.

The level of LETS protein expressed by a transformed cell appears to be correlated with the range of transformed cell characteristics that it shows. BIT 3.4 AC1 and 3.4 AC13 produced considerable amounts of LETS protein, retained density-dependent inhibition of growth and some microfilament bundles although they grow well in agar. The observation that all hybrids which retained microfilament bundles also had LETS protein supports other evidence that there is a close correlation between cell surface LETS protein and the organization of microfilament bundles (Ali et al. 1977; Willingham et al. 1977).

Our observations of a good correlation between growth control and the levels of LETS protein are in disagreement with previous studies with hybrid cells (Marciani, Lyons & Thompson, 1976). In these experiments the correlation appeared to be broken because LETS protein was detected on hybrid cells irrespective of whether they had normal growth control. However, it has recently become clear that some transformed cell lines have cell surface LETS protein (Chen et al. 1976; Pearlstein et al. 1976) and that neoplastic transformation may result in a decrease rather than a complete absence of LETS protein. It is therefore important to have an estimate of the relative amounts of LETS protein on a transformed cell and its normal counterpart. The data of Marciani et al. (1976) do not provide this information since their transformed and non-transformed hybrids arose from independent fusion events. Our transformed hybrids which possessed LETS protein were derivatives of sup-
pressed hybrids and by comparison of an equal number of cells could be shown to possess less LETS protein.

The ability to isolate hybrids in which neoplastic transformation is suppressed and from which transformed variants can be isolated is a useful system for determining whether putative indicators of the transformed phenotype show a pattern of expression which correlates with growth control. The suppressed and transformed hybrids form pairs of non-transformed and transformed cell lines which should be very closely related genetically. Any indicator of the transformed phenotype should show a strictly defined pattern of expression in the suppressed hybrids and their transformed derivatives. Azarnia & Loewenstein (1977) have also demonstrated the usefulness of such an experimental approach in another hybrid cell system in which the relationship of gap junctions to normal growth control was examined. A potential application of this approach may be in the study of neoplastic transformation of cells from epithelia. Because normal epithelial cells cannot be grown extensively in vitro, it is hard to characterize the differences between the transformed and normal cells. Suppressed hybrids between the transformed epithelial cells and normal cells could then provide the ‘normal’ counterpart for characterizing the differences between the transformed and ‘normal’ cells.

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