



Life Sciences Research Report 7

Hilary Koprowski

Editor

**Neoplastic Transformation:
Mechanisms and Consequences**

Dahlem Konferenzen

Viral and Cellular Contributions to Expression of the Transformed State

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INTRODUCTION

Our current understanding of growth control is limited by our inability to grow many normal cell types in culture. This inability blocks us from using our probe in vitro in all but the earliest steps of the progression from normal to malignant phenotype. It also blocks us from studying the cell types that most often become cancerous. Nevertheless, we can say now with some assurance that transcription and translation from one copy of an integrated viral gene is sufficient to disrupt the normal growth controls of rodent fibroblasts to such a degree that these fibroblasts become oncogenic. For the oncogenic DNA papovaviruses, virus infection with the gene itself needs integration, transcription, and translation to transform. The product of this gene, nuclear T antigen, is always present in fully transformed cells.

FIBROBLAST GROWTH CONTROL

Cancer can be the consequence of viral perturbation of normal, controlled cell growth. This perturbation may be thought of as a probe, albeit a blunt one, of the various mechanisms by which normal tissues maintain a constant cell mass. Even for the cell type most easily handled in vitro, the fibroblast, growth control is quite complex, and many sorts of oncogenic viruses are capable of generating a spectrum of transformed phenotypes (35). Serum deprivation, extensive cell-cell contact, and anchorage deprivation are each sufficient to trap a normal fibroblast in the G₁ phase of the cell cycle.

Serum

In its role in the body, the fibroblast proliferates to fill a wound. While normally all cells but endothelia are kept apart from serum, wounds fill with it. Thus, it is not surprising that fibroblasts need serum to grow in vitro. This serum requirement was thought to be quite high, but recent work has shown that serum can be reduced from 10% to 0.2% if the defined constituents of media are optimized (24). Serum can be replaced entirely by a mixture of polypeptide hormones, including insulin, transferrin, and fibroblast growth factor (16).

Viral transformation, the expression of a single gene, can result in a diminished requirement for serum (38), and therefore presumably for an altered hormone requirement for growth. Intracellular concentrations of cyclic AMP and cyclic GMP also change when cells are serum-transformed, suggesting the possibility that cyclic nucleotide production may be perturbed by the viral gene as well (26).

Density

Normal fibroblasts block in G_1 when they are in close contact with each other. A cluster of 4-5 cells seems to be the minimum necessary for this effect (29). Viral gene expression can lead to failure of fibroblasts to recognize each other, as a result of which they pile up in dense foci. Since this is a defect in cell-cell interaction, the external surface of a density-transformed fibroblast must differ from normal, and indeed this can be shown by direct biochemical analysis. The major change in proteins is the loss of a large, external transformation-sensitive (LETS) glycoprotein (17). This protein is exquisitely protease-sensitive, suggesting that the membrane change could be the consequence of local proteolysis. (42). Such proteolysis could occur by the action of a serum protease such as plasmin. Many density-transformants secrete a plasminogen activator (32). Since plasminogen activator is a normal secretion of endothelial cells (7) but not fibroblasts,

the viral gene in this case can be thought to be inducing an inappropriate differentiation as it transforms the fibroblasts.

Anchorage

Dividing tissue cells are, by and large, always in contact with each other or with collagen, and are spread out at all times except for mitosis. In culture as well, the normal cell becomes round only in mitosis, and then the two round daughter cells spread again, reestablishing an anchored configuration.

Normal fibroblasts must be able to anchor on a solid substrate at the end of mitosis, or they do not proceed through G_1 . Expression of the viral gene relaxes this specific requirement without at the same time preventing anchorage. Anchorage-transformants presented with a solid substrate will spread on it at all times except in mitosis, but need not do so in order to divide. Thus, in vitro, this cell-shape change is part of a normal growth control.

Cell Shape

Cell shape is the sum of the states of homo- and co-polymerization of a small number of cytoplasmic proteins, including actin, myosin, filamen, spectrin, tubulin, and intermediate filaments. The shape changes occurring in the division cycle of a fibroblast require a reversible polymerization-depolymerization of these proteins during each mitosis.

Normally, the fully spread, interphase fibroblast shows the most highly organized states of polymerization, in which proteins such as actin are organized in bundles large enough to be visualized by immunofluorescent light microscopy (14,20, 21,23,36,40). Microtubules are also abundant in such cells. Actin-containing bundles and the cytoplasmic microtubules are both gone from anchorage transformed fibroblasts, even well-spread ones (2,6,11,12,25,30,41). Thus, viral gene products are in some way capable of disrupting the ability of a cell to assemble its cytoarchitectural proteins in their most ordered

state. Proteases, especially plasmin, offer a possible mechanism here. Anchorage-transformed fibroblasts secrete plasminogen activator, and plasmin can specifically remove actin bundles from normal fibroblasts (31).

Tumorigenicity

Not all of the many changes in growth control that a viral gene causes need be directly related to the acquisition of the ability to grow as a tumor upon injection into a susceptible host. For the specific system of SV40-transformed rodent cells tested in athymic nude mice, a syndrome of phenotypic changes is most tightly correlated with tumorigenicity (37). This syndrome consists of loss of cytoarchitectural order, loss of anchorage requirement, and production of plasminogen activator. As expected, the syndrome is temperature-sensitive in cells transformed by virus mutants that are temperature-sensitive for transforming genes (11,30). This syndrome is one of necessity, not sufficiency, for tumorigenicity. Therefore, it is not surprising that viral mutants are capable of generating partial transformants which express the syndrome but are not tumorigenic. Nevertheless, these correlations do restrict the possible roles for the viral gene product to a set of cellular changes small enough to be studied extensively.

DIRECTIONS FOR FUTURE RESEARCH

Complete Mechanism of Viral Gene Action in Transformation

Expression of the A gene of SV40 seems to be necessary for the syndrome described above. We need to know which cellular molecules the A gene product interacts with to generate the syndrome. Cellular DNA is likely to be one immediate candidate: T antigen may be an inducer of host DNA synthesis, for it is needed for initiation of viral DNA replication. That is, the T antigen might force a cell from G₁ to S. A minimal hypothesis for the mechanism of this transformation would then be that the syndrome might be the consequence of the cell's traversal through the rest of the cycle, under its own regulation. To

show this, close analyses of the cell cycles of normal and SV40-transformed cells are necessary, and such analyses can best be done with the latest generation of cell sorters (19). Alternatively, the A gene product may interact structurally with cell membranes of any other cellular constituent, so the localization of all forms of SV40 T antigen within the cell remains a critical problem.

For other transforming viruses, the possibility is open for any order of viral gene regulation of the host cell cycle. The appropriate selection systems are now available to determine whether any viral genes exist to complement the presumed minimal function of the SV40 gene, that is, to direct the passage of a cell from S through M to the next G_1 . Such genes would be expected to yield products that would interact directly with host molecules involved in cell shape maintenance, since cell shape changes are most dramatic in the period around mitosis.

Host Mutagenesis

The bacterial viruses μ and P1 mutate their host genomes in the process of promiscuous integration. Is there a host-mutagenic component to viral transformation? The frequency of a double-recessive mutation in a mammalian cell is the square of the frequency of a single recessive mutation, suggesting that classical genetics operate in cultured cells (9). At least one tumor virus, SV40, integrates promiscuously in host chromosomes (3). Many mutagenic chemicals can transform cells in vitro, and almost all environmental carcinogens are bacterial mutagens (1,5). This suggests that it is time to determine whether a tumor virus is mutagenic for a recessive loss of gene function in an assay for changes in a totally gratuitous gene.

New Cell Types in Culture

The syndrome of transformation described above has been worked out only for fibroblastic cells. More than 90% of human tumors arise from nonfibroblastic cells. Some of these cells have

in the absence of the compound? Is tumor-secreted TAF tumor-specific, or is it identical to the factor(s) necessary for maintenance of capillary density in normal tissues? All these questions demand the courage to go beyond the limitations of single-cell-type cultures.

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