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Turnover of High-Molecular-Weight Cell Surface Proteins during Growth and Expression of Malignant Transformation[1]

M. Rieber, J. Bacalao, and G. Alonso

Center of Microbiology and Cell Biology, Instituto Venezolano de Investigaciones Cientificas, Apartado 1827, Caracas 101, Venezuela

SUMMARY

The turnover of cell surface proteins in normal rat kidney cells transformed by a temperature-sensitive Rous sarcoma virus has been studied by polyacrylamide gel electrophoresis and autoradiography using cell monolayers prelabeled by lactoperoxidase-catalyzed radioiodination. Labeling of serum-starved cells under conditions that are nonpermissive for the expression of transformation reveals most of the radioactivity in the 250,000 molecular weight region. Parallel labeling of cells simultaneously exposed to serum limitation, under conditions that are permissive for the expression of transformation, reveals some radioactivity in the same slow-migrating region, but most of the label appears in the two faster migrating regions. The relative turnover of such external proteins has been investigated by examining the relative alterations in iodinated proteins after addition of normal levels of serum to a medium of serum-starved cells.

There is a greater relative turnover of the high-molecular-weight external component under conditions in which the transformation phenotype is expressed, as compared with conditions that limit the expression of transformation.

INTRODUCTION

Recent comparisons between normal and transformed cells have established the presence of a slow-migrating glycoprotein with a tentative molecular weight of about 250,000 that exists in normal cells and is considerably decreased from the corresponding transformed counterparts (2, 3, 5, 7, 8, 12-16).

In addition to the transformation-mediated decrease or absence of such components, recent work has shown that such high-molecular-weight glycoprotein components do not vary only with transformation, but also vary depending on growth rate (2, 4, 12) and position in the cell cycle (2, 4, 9, 11).

We have now continued our studies on such transformation-sensitive components using the normal rat kidney cell system infected by a temperature-sensitive mutant of Rous sarcoma virus B77, cells that display the transformed character at 33° and revert to the normal phenotype at 37° (6, 12). In such a system, we have further investigated some aspects of the turnover of the transformation-sensitive slow-migrating glycoproteins, which are known to be external in several systems (3, 5, 7, 8, 16).

MATERIALS AND METHODS

Cell Cultures. The temperature-sensitive cell line used in this study was a clone (NT_3-KR) from an isolate of NRK cells infected with the ts 339 temperature-sensitive mutant of B77 virus. These cells exhibited the characteristics of transformed cells at 33° and of the normal phenotype at 37° when propagated in Dulbecco's medium (Grand Island Biological Co., Grand Island, N. Y.; Catalog No. H-16) supplemented with 10% fetal calf serum, 10% tryptose phosphate broth (Difco Laboratories, Detroit, Mich.), and 1% dimethyl sulfoxide (Sigma Chemical Co., St. Louis, Mo.) (6, 12). No significant change in the temperature-mediated phenotype was observed when the growth rate of cells was limited by seeding them in the above-described medium, supplemented with just 0.5% serum.

Iodination. This was carried out (8) with cultures, seeded at 2.5×10^4 cells/sq cm, that were inoculated in 9-cm Petri dishes (NUNC, Denmark). For most experiments, cells were iodinated after a 3-day exposure to a medium supplemented with 0.5% serum, except where indicated. However, in all cases the cultures were washed 3 times with medium without serum and were washed subsequently 3 times with PBS-G[1] that contained 5 mM glucose. Iodination was carried out in PBS-G, to which we added simultaneously an enzymic mixture of 50 μg lactoperoxidase (Calbiochem, Los Angeles, Calif.), and 1.25 units of glucose oxidase (Worthington Biochemical Corp., Freehold, N. J.), and 200 μCi sodium iodide ^{125}I-carrier-free for protein iodination (New England Nuclear, Boston, Mass.). After a 10-min iodination, cells were washed in PBI-G, which was identical in composition to the PBS-G, except that sodium iodide was substituted for sodium chloride to stop iodination. After 3 washes in PBI-G, cells were collected by scraping in PBI-G containing 2 mM FMSF to prevent proteolytic degradation and were harvested by centrifugation at 500 rpm for 10 min.

Glycosylation. Cells seeded as indicated above, were exposed to 50 μCi [^{3}H]glucosamine, 5 to 15 Ci/mmole, (New England Nuclear) in complete medium. Cells were

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[2] The abbreviations used are: PBS-G, phosphate-buffered saline, pH 7.2 (0.8% NaCl, 0.2% KCl, 0.115% Na_2HPO_4, 1% glucose, 1.68% $NaHCO_3$, 0.01% phenol red); PBI-G, phosphate-buffered iodide; FMSF, phenyl methyl sulfonyl fluoride.

washed 3 times with medium without label and 3 times with PBS-G before being harvested by scraping in PBS-G containing 2 mM FMSF and subsequent centrifugation.

Sodium Dodecyl Sulfate: Polyacrylamide Gel Electrophoresis. This was carried out by the high-resolution method of Laemmli (10) using slab gels in which the running gel contained 7.5% polyacrylamide and the stacking gel contained 3% polyacrylamide. Before electrophoresis, samples were dissociated by exposure for 3 min at 90° to a mixture of 2% sodium dodecyl sulfate, 0.1 M β-mercaptoethanol, 0.1 M Tris-HCl buffer (pH 6.5), and 0.002 M FMSF. After electrophoresis to 5 mm of the end of the gel, samples were fixed overnight in acetic acid:ethanol:water (7:30:63, v/v). Approximate molecular weights were estimated by comparison with the relative migration of standard polypeptides that were identically dissociated as described elsewhere (12).

Autoradiography. This was carried out for the iodinated samples by drying the gels onto filter paper under vacuum at a temperature of about 60° for 1 hr. Subsequently, the dried gels were placed in contact with RP-X-Omat medical X-ray film (Eastman Kodak Co., Rochester, N. Y.).

Fluorography. This was carried out for the samples labeled with [^{3}H]glucosamine for high-resolution detection of tritium radioactivity. Gels, fixed as described for iodinated samples, were exposed sequentially to dimethyl sulfoxide and then to 20% PPO, washed with H_2O, dried, and exposed to the RP-Royal-X-Omat film for 24 to 72 hr at −70° (1).

Densitometry. This was carried out with samples obtained from autoradiography by subsequently measuring their relative absorbance in a Gilford Model 240 spectrophotometer equipped with a linear transport accessory.

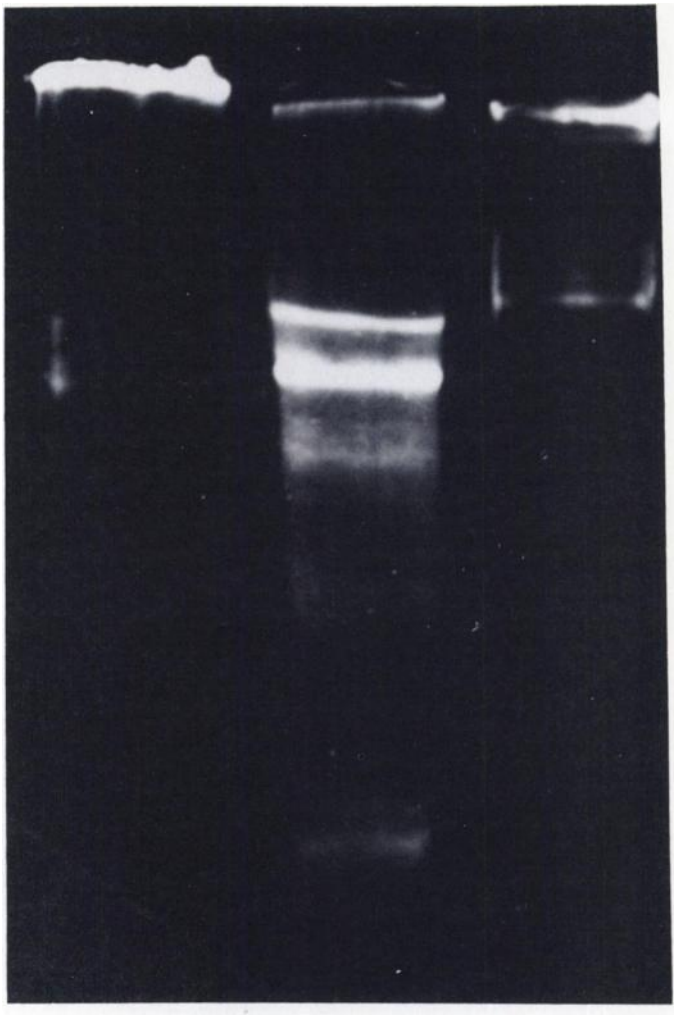

Chart 1. Comparative iodination of serum-restricted NT_3-KR cells. Cells were exposed to serum limitation for 72 hr and then were iodinated or glycosylated as described in "Materials and Methods." *Left*, cells kept at 37° before iodination; *middle*, cells kept at 33° before iodination; *right*, cells kept at 37° during glycosylation.

RESULTS

Our preliminary experiments investigated the relative labeling of external proteins from parallel cultures of NT_3-KR cells, which had been kept in serum-limited medium (0.5%) for 72 hr, under conditions that were permissive for the expression of transformation (33°) or at the nonpermissive temperature of 37°. Chart 1 shows that cells exposed for 3 days to 37° exhibit a significant part of the radioactivity appearing in 1 high-molecular-weight protein (apparent molecular weight of about 250,000), which barely enters the 7.5% running gel, and some iodination also appears in a faster migrating region. However, in contrast, cells seeded at the permissive temperature of 33° in low serum for 3 days showed some radioactivity in the high-molecular-weight region, but most of it appeared in faster migrating regions. That the major band iodinated in cells seeded at 37° is a glycoprotein is also suggested by the experiment in Chart 1; it shows an electrophoretogram of cells labeled with [^{3}H]glucosamine and subsequently exposed to fluorography in which a glycoprotein band appears at about the same position as the band that is preferentially iodinated in serum-starved cells seeded at 37°.

We then investigated whether there was a significant turnover of the external proteins detected by lactoperoxidase-mediated radioiodination and whether this turnover was selective and affected by conditions that allow the expression of transformation. Chart 2 shows that, after iodination of serum-starved cells seeded at 37° and after subsequent exposure to medium with 10% serum, there is some decrease in the intensity of the high-molecular-weight band and a concurrent increase in faster migrating components as compared with the pattern obtained in the cells examined immediately after iodination.

Although the high-molecular-weight iodinated component appears much less labeled in serum-starved cells seeded at 33°, nevertheless it was clearly detectable after 72 hr of exposure to 0.5% serum even at 33°. However, a study of its subsequent turnover demonstrated not only its decrease but also essentially its disappearance (Chart 3). A densitometry of the autoradiographies from Charts 2 and 3 in which the relative decreases of the slow-migrating iodinated component are compared to the amount of component present in freshly iodinated samples is presented in Charts 4 and 5. It can be seen that cells exhibiting the transformed phenotype

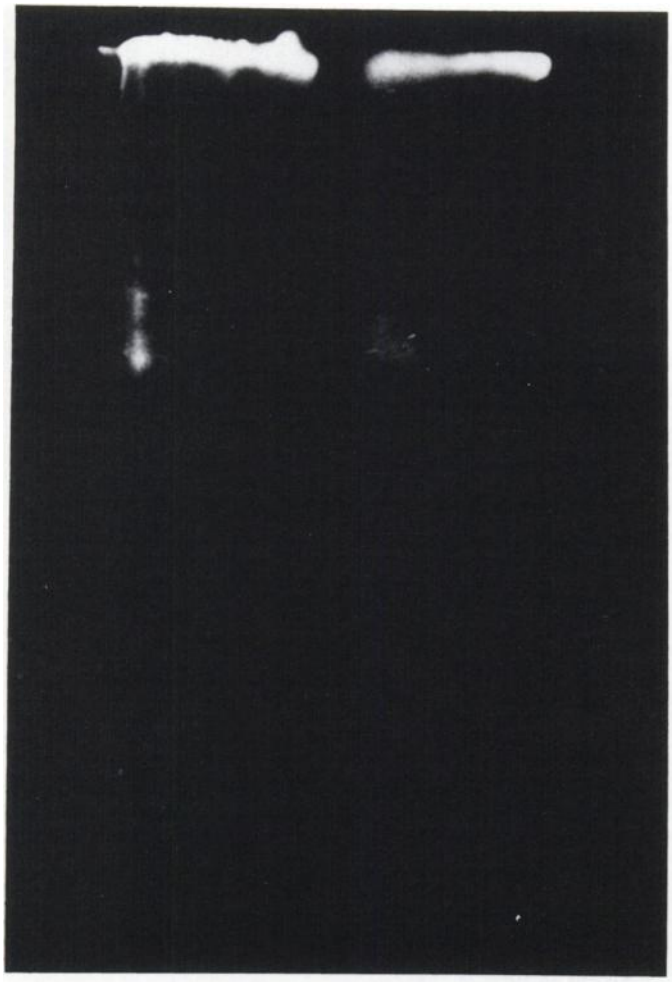

Chart 2. Relative decrease of the iodinated cell surface components under conditions that restrict the expression of transformation. *Left*, iodination pattern of cells labeled after serum limitation at 37° for 3 days and then immediately collected; *right*, iodination pattern of cells labeled as in *left* and subsequently exposed to medium supplemented with 10% fetal calf serum and collected after 16 hr.

at 33° (Chart 5) reveal a much more significant relative decrease of the slow-moving component than cells seeded at 37° (Chart 4).

An additional study in which NT_3-KR cells were seeded at 37° at a low density and then iodinated after 2.5 days before reaching confluence showed that, in contrast with growth-arrested serum-starved cells, the iodination pattern reveals a great proportion of iodination in well-defined fast-migrating regions, as well as some significant radioactivity in the slow-migrating region (Chart 6). However, 16 hr after iodination there is a preferential decrease in the 2 slow-migrating bands and in particular in the slowest iodinated component. Also, the same experiment (Chart 6) reveals an increase in most of the other components as well as 1 additional new component, changes that appear to originate from the turnover of the slow-migrating species.

DISCUSSION

As a result of exposure of the ts-NT_3-KR cells to a temperature of 33°, which allows the expression of transformation characteristics (6, 12), there is a clearly diminished exposure of a high-molecular-weight external component as indicated by the decreased iodination of such a slow, low-migrating component at 33° (Chart 1) as compared to the simultaneous labeling detectable in cells maintained at 37°, a temperature that limits the expression of transformation (6, 12). Such a decreased exposure could originate either from the masking or to a more accelerated turnover of such component in cells maintained at 33°.

The autoradiography experiments presented in Charts 2 and 3, and more clearly the corresponding densitometry

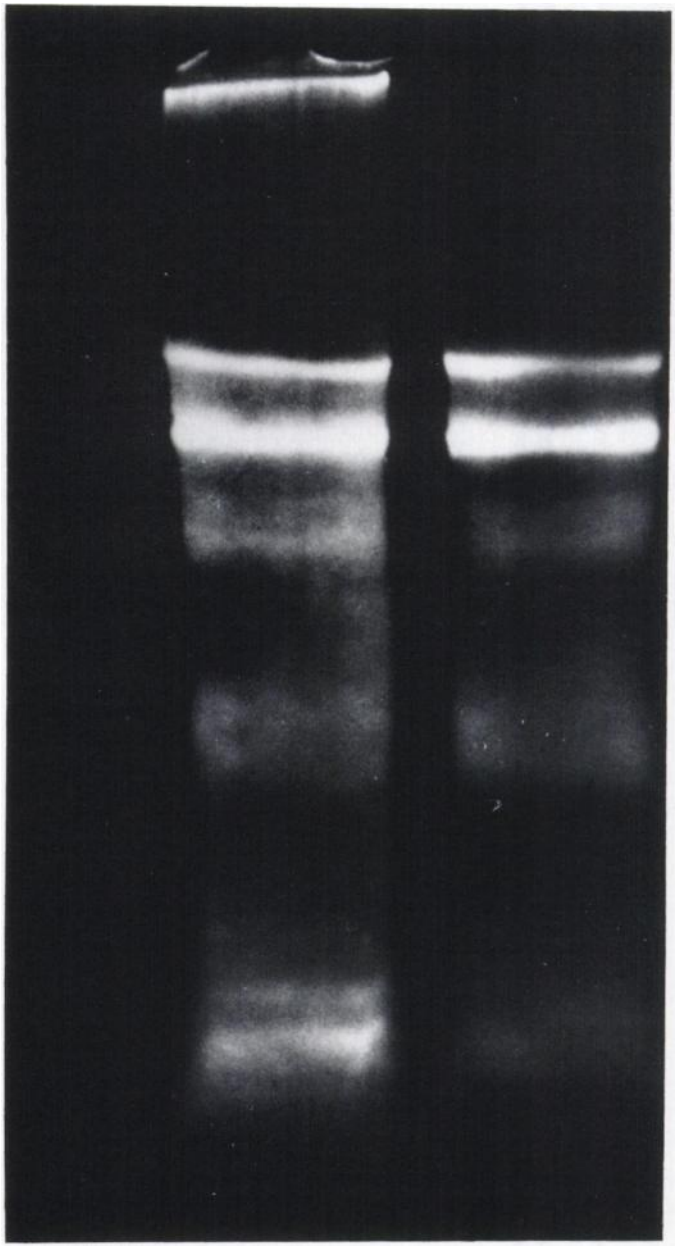

Chart 3. Relative decrease of the iodinated cell surface components under conditions that preferentially allow the expression of transformation. *Left*, iodination pattern of cells labeled after serum limitation at 33° for 3 days and then immediately collected; *right*, iodination pattern of cells labeled as in *left* and subsequently exposed to medium supplemented with 10% fetal calf serum and collected after 16 hr at 33°.

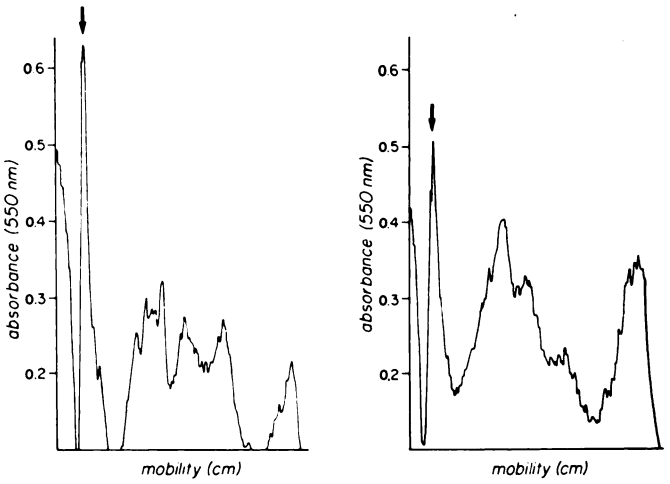

Chart 4. Densitometric estimation of the turnover of surface components iodinated at 37°. *Left*, profile from cells examined following iodination after a 72-hr exposure to serum limitation at 37°; *right*, profile from cells examined following iodination as in *left* and subsequent exposure for 16 hr at 37° to medium supplemented with 10% serum. *Arrow*, position of the high-molecular-weight iodinated component referred to in the text.

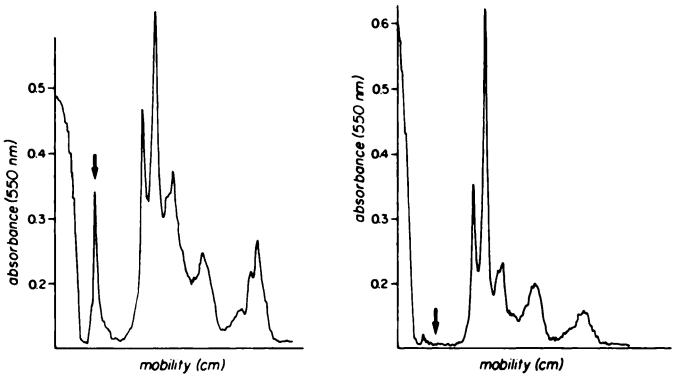

Chart 5. Densitometric comparison of the turnover of surface components iodinated at 33°. *Left*, profile from cells examined following iodination after a 72-hr exposure to serum limitation at 33°; *right*, profile from cells examined following iodination as in *left* and subsequent exposure for 16 hr at 33° to medium supplemented with 10% serum. *Arrow*, position of the high-molecular-weight iodinated component referred to in the text.

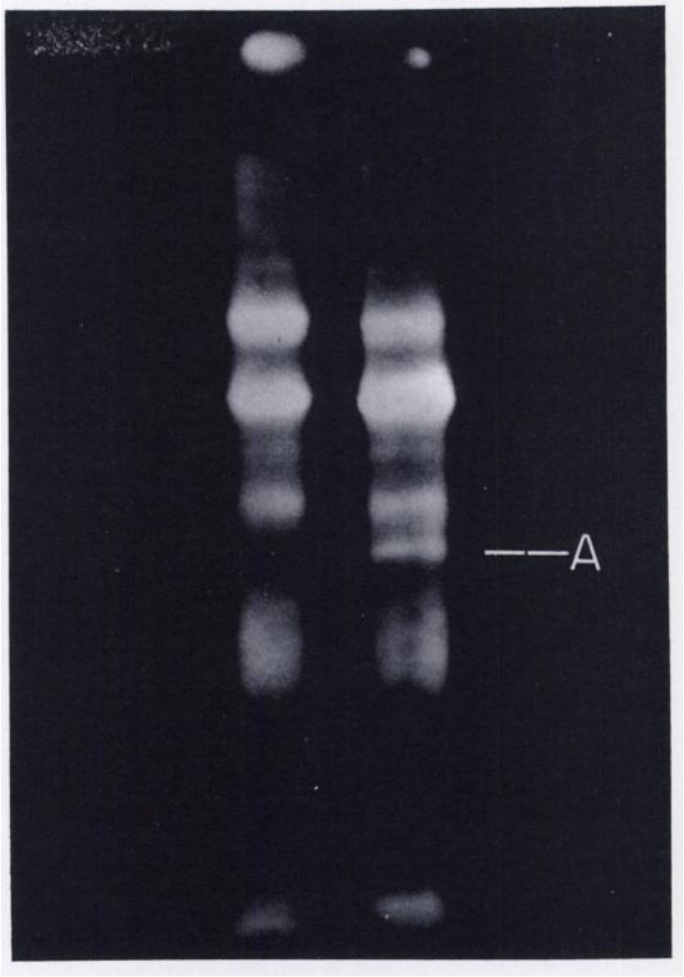

Chart 6. Relative labeling and turnover of external components in normal growing cells. Cells were grown for about 60 hr at 37° in complete medium supplemented with 10% fetal calf serum before iodination as indicated in "Materials and Methods." *Left*, pattern obtained from cells collected immediately after labeling; *right*, pattern obtained from iodinated cells subsequently exposed for 16 hours at 37° to a change of complete medium. *A*, position of the additional component appearing in *right* following iodination and subsequent growth.

presented in Charts 4 and 5, tend to favor the assumption that the diminished iodination of the high-molecular-weight surface component in cells grown at 33° is mainly due to a greater turnover of this component in cells that were maintained at 33°.

In experiments in which exponentially growing cells maintained at 37° were iodinated, it became apparent (Chart 6) that, in contrast with the pattern obtained by serum-starved cells also kept at 37°, rather than having most of the label in the slow-migrating region, the growing cells show a great deal of iodine label in faster migrating regions similar to those detectable in serum-starved cells kept at 33°. The immediate implication of the latter comparison is the similarity between iodination profiles of "normal" growing cells at 37° and "transformed" cells at 33° which although restricted in their growth rate in low serum cannot really become as quiescent as normal cells exposed to the same limiting conditions.

However, it seemed significant that, upon examination of the turnover of the external iodine label of exponentially growing cells kept at 37°, we could also detect a preferential decrease of the slow-migrating species concurrent with an increase in some of the intermediate and slower migrating components, and even the presence of a new fast-migrating component (Chart 6). The latter-quoted experiments also seem to strengthen the belief that 1 of the mechanisms, determining the limited iodination of the high-molecular-weight surface component during the expression of transformation and growth, is a rather greater turnover of such component in transformed and exponentially growing cells as compared with normal cells that were made quiescent by serum limitation (9, 11). However, normal cells exposed to a 3-day serum limitation at 37° and then preiodinated show a decrease in the iodination of the glycosylable high-molecular-weight slow-migrating component, not only when ex-

posed to complete medium supplemented with 10% serum, which permits DNA synthesis to start about 10 hr after serum stimulation, but also when simply given a change of medium with the same low levels of serum that do not stimulate significantly the progress of the cells through the DNA-synthetic phase of their cycle (observation not shown). All such observations seem to support the assumption that the high-molecular-weight surface components described above are normally subject to a significant turnover which may be accelerated during growth or transformation. However, such a turnover may well occur anyhow as part of a normal shedding mechanism.

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