New Species of Rapidly Hybridizing RNA in Contact-inhibited as Well as Transformed Hamster Cell Lines¹

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INTRODUCTION

The technique of competitive RNA-DNA hybridization has revealed significant changes in transcription in cells that have undergone major alterations in structure and function. New hybridizable RNA sequences are synthesized in the differentiating amphibian embryo, the developing embryonic mouse liver, the regenerating mouse liver, and the hybridizable RNA sequences detected in untransformed, contact-inhibited cell lines were similar to those present in transformed cells. Thus, primary hamster embryo cells appear to lack rapidly hybridizing RNA sequences that are transcribed in both transformed and untransformed cell lines. Moreover, these results indicate that the acquisition of oncogenicity is not necessarily correlated with the transcription of additional, rapidly hybridizing RNA sequences, since such new sequences may already be present in serially propagated, untransformed cells.

SUMMARY

The rapidly hybridizing RNA sequences transcribed in primary hamster embryo cells and in lines of serially propagated hamster cells were compared by the technique of RNA-DNA hybridization-competition. Such sequences are known to be transcribed from reiterated DNA sites. There were no rapidly hybridizing RNA sequences in primary hamster embryo cells that were not also present in transformed cell lines. However, in reciprocal experiments, unlabeled RNA from primary hamster embryo cells competed with only 62 to 66% of the hybridizable radioactivity present in RNA from transformed lines. In contrast, the rapidly hybridizing RNA sequences detected in untransformed, contact-inhibited cell lines were similar to those present in transformed cells. Thus, primary hamster embryo cells appear to lack rapidly hybridizing RNA sequences that are transcribed in both transformed and untransformed cell lines. Moreover, these results indicate that the acquisition of oncogenicity is not necessarily correlated with the transcription of additional, rapidly hybridizing RNA sequences, since such new sequences may already be present in serially propagated, untransformed cells.

Materials and Methods

Cell Lines. The cells examined included the following 3 lines derived from tumors produced by the 1st hamster passage of genetically homologous, hamster embryo cell lines which had previously been transformed in vitro (12): (a) line 1808, which was transformed by SV40⁴ and which contained the virus-specific surface (S) and nuclear (T) antigens, as well as virus-specific RNA and DNA; (b) line 1807, which was also transformed following exposure to SV40 but which contained only the S antigen and no detectable virus-specific RNA or DNA; and (c) spontaneously transformed line 1809, which had never been exposed to SV40 and which contained neither antigen (17, 18). All 3 lines exhibited loss of contact inhibition (reaching cell densities in excess of 3 X 10⁵ cells/sq cm in roller-bottle cultures) and induced tumors with s.c. inocula of less than 500 cells. The lines were SV40-free, and inocula of less than 500 cells. The lines were SV40-free, and included 3 lines transformed in vitro (12): (a) line 1808, which was transformed by SV40⁴ and which contained the virus-specific surface (S) and nuclear (T) antigens, as well as virus-specific RNA and DNA; (b) line 1807, which was also transformed following exposure to SV40 but which contained only the S antigen and no detectable virus-specific RNA or DNA; and (c) spontaneously transformed line 1809, which had never been exposed to SV40 and which contained neither antigen (17, 18). All 3 lines exhibited loss of contact inhibition (reaching cell densities in excess of 3 X 10⁵ cells/sq cm in roller-bottle cultures) and induced tumors with s.c. inocula of less than 500 cells. The lines were SV40-free, and no SV40 was induced by cell fusion or cocultivation with monkey cells (12, 17). Also used in the study were 2 cell lines, 1802 and 1804, which had been derived from the same pool of hamster embryo cells as were lines 1807, 1808, and 1809 but which were never exposed to SV40 and which did not become transformed during passage in vitro. These lines continued to

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⁴The abbreviations used are: SV40, simian virus 40; SDS, sodium dodecyl sulfate.
exhibit contact inhibition (reaching saturation densities of less than $1.5 \times 10^4$ cells/sq cm in roller-bottle cultures) and failed to induce tumors when $10^6$ viable cells were implanted in the Syrian hamster cheek pouch (12). A widely used continuous hamster cell line, BHK 21, was also studied. This line (obtained at Passage 172 from Microbiological Associates, Bethesda, Md.) retains certain characteristics of normal cells (28). Primary Syrian hamster embryo cells, prepared in our laboratories from 11-day hamster embryos or obtained from Microbiological Associates, were radiolabeled under conditions identical to those used for the continuous cell lines. Methods of propagation, media used, and harvesting techniques have been described (17). RNA from suspension cultures of mouse L1210 (leukemia) cells and human KB (epidermoid carcinoma) cells was used to determine heterologous competition.

Chemicals. Tritium-labeled uridine (28 Ci/mmole) was purchased from Schwarz BioResearch, Orangeburg, N. Y. DNase (electrophoretically pure) was purchased from Worthington Biochemical Corp., Freehold, N. J. Pancreatic RNase (Type XII-A) was obtained from the Sigma Chemical Co., St. Louis, Mo., and RNase T₁ (Grade B) was obtained from Calbiochem, Los Angeles, Calif. Subtilisin (Nagarse) was obtained from the Enzyme Development Corp., New York, N. Y., and SDS was a product of K and K Laboratories, Inc., Plainview, N. Y. Nitrocellulose HAWP filters (47 mm, 0.45-µm pore size) were purchased from The Millipore Corp., Bedford, Mass., and Liquifluor, a toluene phosphor, was obtained from the Nuclear-Chicago Corp., Des Plaines, Ill.

Preparation of Nucleic Acids. Paired preparations of radiolabeled and unlabeled RNA from each cell line were obtained. Cultures were refed when confluent and, 20 to 24 hr later, were radiolabeled for 6.5 hr with uridine-$\text{H}^3$, 100 µCi/ml. RNA was extracted from whole cells by a modified hot phenol : SDS procedure (17). Freshly excised adult Syrian hamster liver was used for the preparation of DNA. The tissue was minced and homogenized in 0.01 M Tris:0.25 M sucrose:0.003 M CaCl₂ buffer, and the homogenate was filtered through gauze and centrifuged at 600 X g. DNA was prepared from the pellet by the method of Marmur (19), as was Escherichia coli DNA.

RESULTS

Saturation of DNA. A 400-fold excess of whole-cell RNA was required to approach apparent saturation in hybridization reactions with 0.035 µg of hamster DNA. This was true for RNA from both primary hamster embryo and SV40-transformed cells (see Chart 1) and is consistent with many other observations that, in higher organisms, large excesses of whole-cell RNA are required to approach saturation of complementary sites in DNA (11, 15, 27, 31, 33). The percentage of input radioactivity hybridized at apparent saturation was similar in the case of RNA from primary hamster embryo cells and RNA from line 1808 (0.03 and 0.035%, respectively). The RNA from all other transformed and untransformed cell lines tested exhibited similar saturation kinetics. Reannealing of denatured hamster DNA, which might result in saturation at spuriously low RNA:DNA ratios, was not significant under the conditions used, since in previous experiments we have demonstrated that the percentage of input radioactivity hybridized at saturation is similar, whether the RNA is hybridized with DNA in solution or with DNA immobilized on nitrocellulose filters.

Heterologous Hybridization-Competition Reactions. Since

Chart 1. Hybridization of radiolabeled RNA from SV40-transformed hamster embryo cells (line 1808) and primary hamster embryo cells. Shown are the µg of line 1808 (●) and primary hamster embryo (○) RNA bound to hamster DNA at increasing inputs of RNA. Specific activities: line 1808 RNA, 2.5 x $10^4$ cpmp/µg; primary hamster embryo RNA, 12 x $10^4$ cpmp/µg. Hybridization was with 0.035 µg of hamster liver DNA. Points, average of duplicate reactions. The cpmp bound to 0.035 µg of E. coli DNA (the blank) at each RNA input have been subtracted (0 to 25 cpmp; < 10⁻³ % of input).
Heterologous competition was determined under the conditions used. When a saturating amount of radioactive RNA from SV40-transformed line 1808 was used in the hybridization reaction with hamster DNA, unlabeled line 1808 RNA competed in a manner consistent with that expected of an identical RNA (Chart 2A). Human (KB cell) RNA and mouse (L1210 cell) RNA competed to the extent of 10 and 30%, respectively (Chart 2A). This degree of heterologous competition agrees with other published observations on locus specificity (3–8, 13, 21, 22, 24, 29, 30).

Homologous Hybridization-Competition Reactions. Similar competition experiments were performed with radiolabeled line 1808 RNA and unlabeled competitor RNA from either untransformed line 1804 or primary hamster embryo cells. The unlabeled RNA from line 1804 competed completely with the radiolabeled RNA from SV40-transformed line 1808, indicating that there were no detectable, rapidly hybridizing RNA sequences transcribed in the transformed cells that were not also present in the untransformed, contact-inhibited cells. In contrast, unlabeled RNA from 2 different preparations of primary hamster embryo cells competed with only 62 to 66% of the hybridizable radioactivity present in RNA from line 1808 (Chart 2A). These results indicate that rapidly hybridizing RNA sequences that were not detectable in primary hamster embryo cells were transcribed in the SV40-transformed cells.

Reciprocal experiments were performed with radiolabeled RNA from primary hamster embryo cells and unlabeled competitor RNA from either untransformed line 1804 or primary hamster embryo cells. Unlabeled RNA from both sources competed equally well, and competition followed closely the theoretical curve for an identical RNA (Chart 2B), demonstrating that there were no rapidly hybridizing RNA sequences detectable in primary hamster cells that were not also present in the transformed cells. In addition, similar experiments were performed with radiolabeled RNA from untransformed line 1804 and with unlabeled RNA from either line 1804 or 1808. The results (Chart 2C) indicate that transformed line 1808 contained all hybridizable sequences detectable in untransformed line 1804. Thus, the rapidly hybridizing RNA’s from lines 1804 (untransformed) and 1808 (virus-transformed) appear to be identical. Consequently, one can infer from Chart 2A that the untransformed (line 1804) cells also contain sequences that were not present in primary hamster cells.

Experiments were also undertaken with RNA from another homologous untransformed cell line (1802), from a spontaneously transformed line (1809), from an SV40-exposed (but T-antigen-negative) transformed line (1807), and from a continuous hamster cell line (BHK 21). Competition experiments, performed with various combinations of radiolabeled RNA and unlabeled competitor RNA, revealed no differences between the rapidly hybridizable RNA’s from any of these serially propagated cell lines.

cpm/µg; net cpm hybridized without competitor, 517; C, competition with line 1804 RNA-3H (specific activity, 4.4 x 10⁴ cpm/µg; net cpm hybridized without competitor, 235).
DISCUSSION

These results indicate that the same techniques that have been utilized to demonstrate additional rapidly hybridizing RNA sequences in primary neoplastic cells (3, 4, 13, 21, 22, 29, 30) did not differentiate the RNA of transformed cell lines from that of homologous, untransformed controls. However, while hybridization of nucleic acids from higher organisms under the conditions of ionic strength, time, and temperature usually permitted the detection of RNA transcribed from reiterated DNA sequences (2, 7, 9, 14, 16, 20, 25, 26), these methods do not detect the RNA transcribed from unique DNA sequences, which comprise approximately two-thirds of the DNA in higher organisms (2, 7, 14, 20). Consequently, RNA's that are indistinguishable by the techniques used in the present study may or may not be identical. On the other hand, results that show incomplete competition between RNA's probably underestimate true differences. It is thus especially interesting that primary hamster embryo cells appear to lack rapidly hybridizing RNA sequences that are transcribed in both transformed and untransformed cell lines. These cell lines all were free of detectable mycoplasmas. Furthermore, the hamster DNA that was utilized was extracted from fresh hamster liver and would not be expected to have significant homology with the nucleic acids of an adventitious agent present in tissue culture. It thus seems probable that the additional rapidly hybridizing RNA sequences detected in the cell lines examined reflect biological differences between the cells of primary cultures and those of continuous lines (32). For example, primary embryo cell cultures consist of a mixture of cell types, whereas selective pressures result in the emergence of one or a few cell types during the establishment of continuous lines. Certain portions of the reiterated DNA might be further reduplicated in the cells that emerge in continuous culture, or regions of reiterated DNA that are inactive in the majority of the cells in primary cultures may be transcribed in these selected cells. The new, rapidly hybridizing RNA sequences reported in primary neoplasms (3, 4, 13, 21, 22, 29, 30) may reflect the occurrence of a similar selective process during oncogenesis in vivo. Alternatively, the more limited transcription of reiterated DNA in primary embryo cells might be the result of extrinsic restraints (e.g., hormones or intercellular relationships) which normally limit the extent of gene expression in vivo. The mediators of such restraints are probably still present in primary cultures but would certainly be diluted by subsequent passage in vitro. Their absence could thus explain the increased transcription observed in all of the continuous cell lines, both transformed and untransformed, that were examined. Similarly, the transcription of additional, rapidly hybridizing RNA sequences in primary neoplasms (3, 4, 13, 21, 22, 29, 30) may be only a reflection of the resistance to normal extrinsic controls that is characteristic of neoplastic cells arising in vivo.

Whatever the mechanisms underlying these differences may prove to be, the results of the experiments reported herein indicate that the acquisition of oncogenicity is not necessarily correlated with the appearance of new, rapidly hybridizing RNA sequences, since such new sequences may already be present in untransformed cells that are serially propagated.

REFERENCES

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