UCLA - 12 - 8 32

RECEIVED BY DTIC OCT 4 1971

UNIVERSITY OF CALIFORNIA, LOS ANGELES

MASTER

LABORATORY OF NUCLEAR MEDICINE AND RADIATION BIOLOGY

CONTRACT NO. AT (04-1) GEN-12

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

ISOLATION OF HIGH MOLECULAR WEIGHT RNAs FROM CULTURED
NEURAL TUMOR CELLS

Ross B. Edwards and Jean de Vellis

LABORATORY OF NUCLEAR MEDICINE AND RADIATION BIOLOGY
900 VETERAN AVENUE

UNIVERSITY OF CALIFORNIA, LOS ANGELES, CALIFORNIA 90024

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

These studies were supported in part by Contract AT(04-1) GEN-12 between the Atomic Energy Commission and the University of California

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED
Pey

ISOLATION OF HIGH MOLECULAR WEIGHT RNAs
FROM CULTURED NEURAL TUMOR CELLS

Ross B. Edwards and Jean de Vellis

Laboratory of Nuclear Medicine and Radiation Biology,
The Department of Anatomy and the Mental Retardation Center
University of California, Los Angeles 90024

SUMMARY

RNA extracted from cultured glial and neuronal tumor cells and analyzed by polyacrylamide gel electrophoresis contained material of molecular weights substantially greater than that of the heaviest ribosomal RNA precursor. Rat glial cells contained species of approximately 6×10^6 and 13×10^6 daltons while mouse neuronal cells contained only the 6×10^6 dalton species. RNA from intact whole rat brain had neither of the large RNA molecules. The origin of these RNAs is discussed, including the possibilities of viral contamination, endogenous viruses, tumor-specific RNA, adaptation to culture conditions and artifacts of analysis.

A number of workers have observed radioactively labeled RNAs larger than the 45S ribosomal precursor (1), but to our knowledge no such non-viral RNAs have been isolated in sufficient quantity to permit detection of discrete fractions visually or by optical instruments. This paper presents evidence for the existence, in two established cell lines of brain tumors, of two large RNA molecules in sufficient quantity to be observed visually in stained acrylamide gels.

Materials and Methods (2)

The Rat C-6 glial tumor cell line (3) and a mouse neuroblastoma cell line (4) were grown as previously described (5). Cells, or whole brains from three-month old male Wistar rats, were homogenized in ten volumes (but at least 5 ml) of extraction medium consisting of 0.1 M NaCl, 0.001 M EDTA, 0.001 M tris-HCl, 0.5%* sodium dodecyl sulfate (SDS), 0.5%

* All percentages are weight to volume in aqueous solutions unless otherwise indicated.

Filtrol 19 (Filtrol Corp., Los Angeles, California) and 0.033% (v/v) diethylpyrocarbonate (Eastman Kodak Co., Rochester, New York), using three passes of a Potter-Elvehjem apparatus with a clearance of 9 to 12×10^{-3} in. at 0 to 4°C . To each sample an equal volume of the following solution was added: 88% (v/v) freshly distilled phenol plus 12% (v/v) 0.1 M NaCl 0.001 M EDTA 0.01 M tris-HCl, subsequently made 0.1% in 8-hydroxyquinoline and adjusted to pH 6-7. The mixture was vigorously hand shaken for 60 seconds at 20°C , then shaken 30 minutes at 10°C and centrifuged for 10,000 g-min. The supernatant was removed and reextracted for 15 minutes with a half-volume of phenol solution at 10°C . The supernatant from a second centrifugation was made 0.3 M in NaCl followed by adding 2.3-2.4 volumes of cold 95% (v/v) ethanol and storing for 8 hours or overnight at minus 14°C . Nucleic acids were pelleted, washed once each with 95% ethanol, acetone and ether at 0°C , the pellet dissolved in DNase buffer (0.005 M MgSO_4 , 0.1 M sodium acetate, pH 5.0), the ether evaporated off and the DNA degraded by three successive five minute incubations of 20 $\mu\text{g}/\text{ml}$ deoxyribonuclease I (bovine pancreas, E.C. 3.1.4.5, ribonuclease-free; Worthington Biochemicals; Freehold, New Jersey) at 24°C . The sample was again extracted twice with phenol, precipitated with NaCl and ethanol and collected and washed as before, then dissolved in RNA sample buffer (0.02 M sodium acetate, 0.044 M tris, 0.002 M EDTA, 5% sucrose, pH 7,8) to give an approximate absorbance at 260 $\text{m}\mu$ of 130 (1 cm path). All solutions were then heated to 50°C for 2.5 minutes, then immediately chilled on ice, to reduce the possibility of RNA aggregation (6, 7). Flat gel electrophoresis was performed using the apparatus and general procedure of Reid and Bielecki (8). Acrylamide and methylenebisacrylamide recrystallization, gel solutions and electrophoresis buffer were prepared according to Loening (9). Addition of 0.8% agarose was as described by Uriel (10). Approximate molecular weights were estimated by using

endogenous mature ribosomal RNA as standards (11).

RESULTS

The figure shows glial cell RNA in wells 1 and 5, whole brain RNA in wells 2 and 6, control neuronal RNA in well 3 and RNA from neurons grown with growth hormone, thyroid hormone and cortisol in well 4. Arrows A and B indicate the positions of the high molecular weight RNAs. (See the legend for the identification of the other RNAs according to their positions indicated by the ruler). No RNA heavier than 45S is seen in either of the whole rat brain profiles; both glial cell profiles contain bands A and B, while both neuronal RNA preparations contain only band B. Molecular weights are estimated to be 13×10^6 (band A) and 6×10^6 (band B) daltons. (These are low estimates; including 45S and 32S RNA as standards gave values as high as 37 and 9×10^6 daltons for the respective bands, but the variation was quite large, whereas use of the 28S and 18S bands alone yielded results with a range of 10% or less). A duplicate gel was run (not illustrated) which gave the same results.

Comparison of the glial and whole brain RNAs on a 10% acrylamide gel (not illustrated) revealed two very faint bands of less than 10^5 daltons unique to the glial cells. Unlike the high molecular weight bands, these smaller RNAs were close to other bands present in all samples and were analyzed in a single run only.

Discussion

Although the black and white photographs make the high molecular weight bands appear very faint or invisible, observation of the actual gels reveals the unequivocal presence of the bands. Qualitatively identical profiles are seen for duplicate samples or for samples differing only in hormonal treatment. Identical results were obtained with a second gel. Also, the absence of the high molecular weight RNAs from rooster brain (not shown) (2) is further

evidence for the specificity of these RNAs to the cultured cells.

With present data it is impossible to specify the source of the high molecular weight RNAs. The samples were heated to 50°C in an attempt to break up aggregates of smaller RNA; however, aggregates such as those observed on acrylamide gels by Lovett and Leaver (12), which were disrupted by heating at 80°C, cannot be entirely ruled out. The possibility of viral contamination was reduced by using serum that had been serologically and electron microscopically screened for viral contamination by the supplier (Grand Island Biological Co., Grand Island, New York). Three separate bottles of medium were used for the three different cultures. It also seems unlikely that the three cultures, each distributed among from six to thirteen separate flasks, would be contaminated by the same virus (that with RNA band B) and would still remain viable and well attached to the flasks as they did.

If the high molecular weight bands are functionally associated with the cells the RNA could be related to recently observed cancer-associated viruses (13, 14). The neuronal cells, at least, contain particles resembling leukemia-type virus particles as seen by electron microscopy (15, 16). On the other hand the C₆ glial cell line does not appear to contain virus particle (17). The bands could also be tumor-specific but non-viral RNA. Another possibility is that the adaptation of the cells to culture conditions could have stabilized or induced new types of RNA; for example, rapidly turning over, heavier-than-45S heterogeneous nuclear RNA fractions (1) could be involved. Finally, the fact that both cultures contain band B while only the glial cells contain band A could relate to the similar yet different origins of the cells; both come from nervous tissue, but are of different specific cell type, and both are from the same taxonomic order but of different genera.

The lack of visible differences between the two neuroblastoma cultures

is of interest, since the hormones are known to induce the outgrowth of new neurites (5, 16) and to cause a dramatic increase in polysomes (apparently all of the same approximate size) (16). The fact that differences were not seen in the gel profiles suggests that even many-fold changes in cellular polysome concentration cannot necessarily be demonstrated visually on acrylamide gels.

Acknowledgements

We wish to thank Dr. Rod Bielecki for advise in the selection and use of suitable gel electrophoresis procedures, Dr. George Laties for providing the electrophoresis apparatus and Diane English for growing the cells. This research was supported by the United States Atomic Energy Commission under contract AT (04-1) GEN-12 between the A.E.C. and the University of California.

References

1. S. Penman, M. Rosbash and M. Penman, Proc. Natl. Acad. Sci. U.S. 67, 1878 (1970).
2. For a more detailed description of these methods, see R.B. Edwards, Ph.D. dissertation, UCLA; University Microfilms, Inc., Ann Arbor, Mich.; 30 (1971).
3. P. Benda, J. Lightbody, G. Sato, L. Levine and W. Sweet, Science 161, 370 (1968).
4. G. Augusti-Tocco and G. Sato, Proc. Natl. Acad. Sci. U.S. 64, 311 (1969)
5. J. de Vellis, D. English, R. Cole and J. Molson, in Influence of Hormones on the Central Nervous System, D. Ford, ed., Karger, Basel (in press).
6. L.E. Bosch, A. Bonnet-Smits and J. van Duin, Virology 31, 453 (1967).
7. J. J. Pene, E. Knight and J.E. Darnell, J. Mol. Biol. 33, 609 (1968).
8. M.S. Reid and R.L. Bielecki, Analyt. Biochem. 22, 374 (1968).
9. U.E. Loening, Biochem. J. 102, 251 (1967).
10. J. Uriel, Bull. Soc. Chem. Biol. 48, 969 (1966).
11. U.E. Loening, J. Mol. Biol. 38, 355 (1968).
12. J.S., Lovett and C.J. Leaver, Biochem. Biophys. Acta 195, 319 (1969).
13. H.M. Temin and S. Mizutani, Nature 226, 1211 (1970).
14. D. Baltimore, Nature 226, 1209 (1970).
15. D. Schubert, S. Humphreys, C. Baroni and M. Cohn, Proc. Nat. Acad. Sci. (USA) 64, 316 (1969).
16. J. de Vellis, D. English, J. Molson and G. Augusti-Tocco, Third Intern. Meet. of Intern. Soc. Neurochem. Abstracts p. 191, Budapest 1971.
17. J. de Vellis, D. English and F. Galey in "Cellular Aspects of neural growth and Differentiation" D. Pease Ed. UCLA Forum in Medical Sci. No.14 pp. 23-32 Univ. of Calif. Press (1971).

Figure 1. Gel electrophoretic profiles of RNA. Wells 1 and 5 contain cultured rat glial tumor cell RNA (2.1 A₂₆₀ units each). Wells 2 and 6 contain rat whole brain RNA (2.1 A₂₆₀ units each). Well 3 contains RNA from untreated cultured mouse neuroblastoma cells (0.5 A₂₆₀ units), and well 4 contains RNA from cultured mouse neuroblastoma cells treated for 10 days with growth hormone, thyroid hormone and cortisol (0.6 A₂₆₀ units). One A₂₆₀ unit is defined as the amount of RNA which gives an optical density of one when dissolved in 1 ml of distilled water and measured in a cuvette of 1 cm path length. Identification of the stained bands is as follows: band A at 14 mm on the ruler, 13×10^6 daltons; band B at 22-23 mm, 6×10^6 daltons; 27 mm, 45S ribosomal RNA (rRNA) precursor (4.4×10^6 daltons); 30-31 mm, 32S rRNA precursor (2.4×10^6 daltons); 33-35 mm, 28S rRNA (1.7×10^6 daltons); 42-44 mm, 18S rRNA (0.7×10^6 daltons); 77-78 mm, transfer RNA (0.025×10^6 daltons).