those from chitin isolated from yeast cell walls of *M. rouxii*.

To our knowledge, this is the first report on the formation of cell wall microfibrils in vitro. Our findings (i) demonstrate that the synthesis of chitin chains and their assembly into microfibrils can be attained with a soluble (*12*) enzyme preparation free of membranes (*21*); (ii) prove, unequivocally, that the Leloir (*22*) pathway of polysaccharide synthesis (glycosyl transfer from a nucleoside diphosphate sugar) operates in the formation of cell wall microfibrils; (iii) show that cell wall microfibrils can be formed in the absence of a living cell or its membranes (*23*); (iv) support the belief that the formation of cell wall microfibrils (polymer synthesis and fibril assembly) in the living cell takes place in the wall itself (*24*); (v) suggest that other wall components, such as matrix materials, are not indispensable for microfibril elaboration (*25*); and (vi) move us a step closer to the goal of understanding, and eventually duplicating in vitro, the assembly of a whole microfibrillar cell wall (*26*).

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Neuroblastoma: Drug-Induced Differentiation Increases Proportion of Cytoplasmic RNA That Contains Polyadenylic Acid

Abstract. *The production of cytoplasmic RNA that contains polyadenylic acid is increased, relative to total cytoplasmic RNA, in a neuroblastoma clone, NBE$^-$(A), after induction of differentiation by 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone, an inhibitor of adenosine 3′,5′-monophosphate phosphodiesterase. The amount of RNA that contains polyadenylic acid in cytoplasm may be greater in such differentiated neuroblastoma cells than in proliferating control cells.*

The presence of polyadenylic acid [poly(A)] sequence at the 3′ terminus of messenger RNA derived from a variety of sources has been reported (*1*). It is in the cytoplasm where RNA functions as a template in protein synthesis. Therefore we have examined the rate of appearance and amount of cytoplasmic RNA that contains poly(A) in a neuroblastoma cell line cultured in two widely differing states of genetic expression. It is possible to selectively separate the RNA's that contain poly(A) by utilizing their ability to bind to cellulose at high ionic strength (*2*).

The neuroblastoma clone NBE$^-$(A) which has choline acetyltransferase activity, but no tyrosine hydroxylase activity (*3*), was cultured and maintained as described (*4*). These cells have a doubling time of about 18 hours, and they produce tumors when injected subcutaneously into male A/J mice. Cells were plated in Falcon plastic flasks (75 cm^2); 4-(3-butoxy-4-methoxybenzyl) - 2 - imidazolidinone (RO20-1724, 200 μg/ml), a rather specific inhibitor of cyclic AMP phosphodiesterase (*5*), was added 24 hours after the plating. This drug results in an elevation of intracellular adenosine 3′,5′-monophosphate (cyclic AMP) and in increases of the enzyme activities of several enzymes, including some specifically associated with neural function (*4*). In addition, RO20-1724 induces many morphological indices of differ-

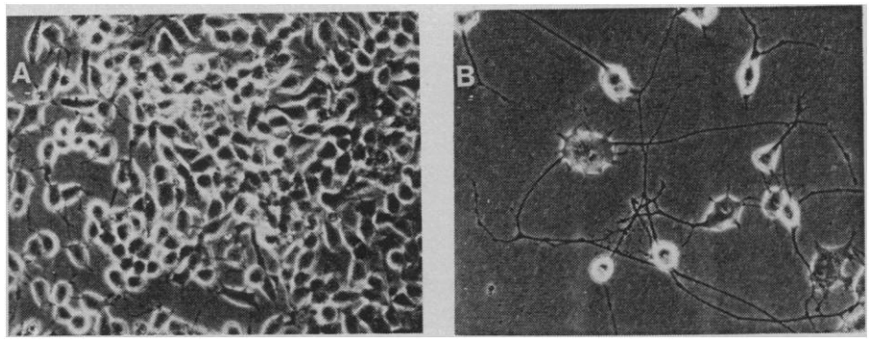

Fig. 1. Phase contrast micrographs of living neuroblastoma, NBE$^-$(A) culture. Cells were grown for 3 days in the absence (A) or presence (B) of RO20-1724. Cells grown in the presence of the drug have larger cell bodies and have formed extended neurites (× 160).

entiation in the neuroblastoma clone (Fig. 1). The drug and medium was changed 2 days after the plating, and the experiments on treated cells were performed after 3 days of treatment, because at this time the differentiated functions were irreversibly fixed (*4*). Control cells were treated similarly, except that no drug was added. The numbers of cells originally plated were such that, at the completion of the experiment, approximately the same number of cells would be present in drug-treated and control flasks (1.5×10^6 to 3.5×10^6 cells per sample). Fresh growth medium was changed 1 hour before addition of [2,8-^{3}H]adenosine (32.4 c/mmole). Cells were incubated in the presence of [^{3}H]adenosine (2 μc/ml) for a further time after which they were removed with 0.25 percent Viokase solution. The cell number was determined in a Coulter counter.

Each cell sample was homogenized in 5 ml of 0.32*M* sucrose together with 200 mg of chick brain (as a carrier), and the homogenate was centrifuged (1000*g* for 10 minutes) to remove the nuclei and intact cells. The cytoplasmic supernatant was then adjusted to contain 0.1*M* glycine, 0.1*M* NaCl, 0.01*M* EDTA, 1 percent sodium deoxycholate, *p*H 9.5, shaken with an equal volume of a mixture of chloroform and phenol (1 : 1, by volume) and centrifuged at high speed. To the aqueous supernatant, 1/20 its volume of 3*M* sodium acetate and 2 volumes of ethanol were added (*2*). The resulting precipitate, which contained the RNA, was centrifuged, taken up in 1 ml of water, and reprecipitated with 0.05 ml of 3*M* sodium acetate and 2.1 ml of ethanol. This final RNA precipitate was centrifuged, suspended in 0.5 ml of a high ionic strength buffer (0.1*M* tris·HCl, 0.3*M* NaCl, 0.001*M* EDTA, *p*H 7.0) and applied to a cellulose column (0.6 by 4.0 cm) previously washed with high ionic strength buffer. Chromatography was carried out at 37°C, and all media used were at this temperature. This was to ensure that only poly(A)-containing RNA would be retained by the column (*2*); 3.5 ml of high ionic strength buffer were then passed through the column to elute nonretained RNA. The column was then washed with 4 ml of water which removed poly(A)-containing RNA. No residual RNA was left on the column. Portions of the fractions were mixed with a compatible scintillation mixture (3a70, Research Products International), and the radioactivity was determined at an efficiency of 31 to 34 percent. The assayed samples ranged between 900 and 200,000 count/min.

To take nonspecific binding into account, ribosomal RNA was prepared free of messenger RNA from puromycin-incubated chick brain ribosomes (*6*). Of this RNA, 1.43 ± 0.05 percent was retained by cellulose columns equilibrated at high ionic strength. Accordingly, in order to determine the proportion of radioactivity in nonribosomal poly(A)-containing RNA, 1.4 percent was deducted from the percentage of RNA binding at high ionic strength.

After exposure of the cultures to [^{3}H]adenosine for 2 hours, the labeling of cytoplasmic RNA was much more extensive in the proliferating (control) cells than in the RO20-1724 treated, nondividing cells (Table 1). However, a greater proportion of the radioactive RNA from the RO20-1724 treated cells was retained by the cellulose column. This suggested that in the differentiated cells there was a greater production of cytoplasmic poly(A)-containing RNA relative to total cytoplasmic RNA. When the cultures were incubated with [^{3}H]adenosine for 18 hours, the total radioactivity within cytoplasmic RNA increased to approximately the same value in both differentiated and control cell cultures. In this case, the proportion of radioactive poly(A)-containing RNA was about eight times greater in the drug-treated cells than in control cells (Table 1).

The short-term labeling study may reflect rates of synthesis of various RNA species, while the longer studies may be more related to the stability and cytoplasmic concentration of RNA classes.

There is evidence that enzyme induction in eukaryotic cells is preceded by poly(A) synthesis (*7*). Reduction of cell proliferation and induction of enzymic and morphological differentiation can be effected in tumor cells by pharmacological elevation of intracellular cyclic AMP (*4*). These changes may be mediated by an increased emphasis on the production of poly(A)-containing RNA. Our data suggest that, while the expression of differentiated functions in neuroblastoma cells is accompanied by a reduced rate of total cytoplasmic RNA synthesis, there is a relatively increased rate of synthesis of poly(A)-containing cytoplasmic RNA. This results in an elevation of the proportion of cytoplasmic RNA that is messenger RNA.

Table 1. Incorporation of [^{3}H]adenosine into cytoplasmic RNA of control cells, and into cytoplasmic RNA of cells treated with RO-20-1724. After 3 days of incubation, in the presence or absence of the drug (200 μg/ml), cells were incubated again in medium containing [^{3}H]adenosine (2 μc/ml). Cytoplasmic RNA was then prepared from cells, and the proportion of radioactive RNA binding to cellulose columns at high ionic strength was determined. Means ± S.E.M. represent four or five independently cultured series.

Cell treatment	Time (hr)	[^{3}H]RNA per cell (10^3 count/min)	RNA that contains poly(A) (%)
Control	2	10.1 ± 0.9	2.0 ± 0.3
RO20-1724	2	1.65 ± 0.15*	5.0 ± 0.2*
Control	18	422 ± 31	0.3 ± 0.1
RO20-1724	18	403 ± 48	2.4 ± 0.3*

* Values significantly different in drug-treated cultures than control values ($P < .001$, 2-tailed *t*-test).

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Deuterium Micromapping of Biological Samples by Using the D(T,n)^{4}He Reaction and Plastic Track Detectors

Abstract. *A technique has been developed to micromap deuterium by using the D(T,n)^{4}He reaction and plastic track detectors. Labeling of cells with subpicogram quantities of deuterium was demonstrated. The technique was used to localize human lymphocytes transformed in vitro.*

The use of stable isotopes as tracers in biomedical and other fields is now of interest (*1*). In particular, the detection and localization of deuterium, the stable isotope of hydrogen, would be useful. Since nonexchangeable hydrogen is contained in most organic molecules, deuterated organic compounds could be used as tracers in vivo. We show here that the D(T,*n*)^{4}He reaction can be a sensitive indicator for the presence of deuterium and that plastic track detectors can be used to reveal the microscopic position of the deuterium (*2*). A general scheme for the localization of stable isotopes using nuclear reactions was proposed theoretically by Malmon (*3*).

Conventional autoradiography (ARG) of radioactive nuclides is one of the most valuable microlocalization techniques available for studying the incorporation and transport of molecules in living cell systems. The concomitant biological effect of the radiation on the living system under study may occasionally restrict the scope of information available from animal experiments, and certainly inhibits conventional ARG studies in human clinical investigation (*4*). For example, if one wished to trace autologous lymphocytes during biomedical studies of adoptive immunotherapy (*5*), it would be preferable to use a stable isotope rather than tritium to label the cells in vitro before reinjection. Tritiated lymphocytes could also be traced in the body after reinjection, but they might be damaged by chronic low-level beta radiation.

The deuterium is detected by bombarding the sample with a triton beam to produce the D(T,*n*)^{4}He reaction. The unique feature (*6*) of this reaction is a strong resonance with a peak total cross section of 5 barns at a low bombarding energy of 160 kev and a high total energy released in the nuclear reaction (*Q* value) of 17.5 Mev. The full width at half maximum of this resonance corresponds to 2.5 μm in tissue. The bombarding energy is so low that the yield of background alpha particles produced by triton bombardment of elements heavier than deuterium is negligible. An alpha particle emitted in the forward direction has an energy of 4.7 Mev. This means that samples up to several micrometers thick can be used with an absorber to stop the incident triton beam. The alpha particles, after passage through a 6-μm-thick Teflon (*7*) absorber, have an energy of about 2 Mev, which is suitable for detection in plastic track detectors.

In our experiments, an 8-μm-thick cellulose nitrate plastic track detector on a 100-μm Mylar backing for mechanical support (*8*) is mounted on an aluminum slide (2.54 cm by 7.62 cm). A 6-μm Teflon film is fastened, wrinkle-free, to the surface of the track detector. A drop of cells in suspension is evaporated to dryness on the Teflon. To minimize clumping of the cell specimens due to surface tension, the Teflon is coated with a layer of carbon 50 to 100 Å thick by vacuum evaporation to complete the detector assembly (see Fig. 1). The slide is mounted in thermal contact with a metal block cooled with air circulating through a Dry Ice–alcohol mixture during bombardment by a beam of 400-kev diatomic tritons (TT^+) at the Research Van de Graaff accelerator of Brookhaven National Laboratory. After bombardment, the detector is removed from the slide and etched in 6.25*N* NaOH at 40.0°C for 45 minutes to develop the alpha particle tracks as characteristic etched cones. The specimens and track detector can be examined and photomicrographed before and after bombardment.

Plastic track detectors (*9*) are very insensitive to other radiations, including photons, electrons, and neutrons produced during bombardment. The position and direction of the etched alpha particle tracks locate the spatial origin of the nuclear reaction to about 2 to 10 μm. The alpha particles which enter the track detector are detected with nearly 100 percent efficiency under these physical conditions.

The effective reaction cross section, $\bar{\sigma}$, taking into account the target thickness and efficiency of the track detector, is given by

$$\bar{\sigma} = \frac{1}{X_1} \int_0^{X_1} \sigma(\eta) G(\eta, X_1, X_2, X_3) d\eta \quad (1)$$

where X_1 and X_2 are the thicknesses of the sample and of the absorber, respectively, X_3 is the minimum detectable depth of alpha penetration into the detector, G is the geometrical efficiency, σ is the nuclear reaction cross section, and the integration is carried out over the particle penetration distance η (see Fig. 1).

In the materials used and for the energies encountered, the range of an alpha particle is approximately proportional to energy. With this approximation, the geometrical efficiency is (*10*)

$$G(\eta, X_1, X_2, X_3) = \frac{1}{2}\left[1 - \frac{X_1 - \eta}{R_1} - \frac{X_2}{R_2} - \frac{X_3}{R_3}\right] \quad (2)$$

where R_1, R_2, and R_3 are the calculated ranges (*11*) of reaction alpha particles in the sample, absorber, and detection plastic materials, respectively. The biological samples analyzed were typically 1 to 3 μm thick, the absorber was 6-μm Teflon, and the minimum detectable