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Neuronal properties of hybrid neuroblastoma × sympathetic ganglion cells

(somatic cell hybrids/neurotransmitters/tyrosine 3-monooxygenase/action potentials)

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ABSTRACT **Clonal mouse neuroblastoma cells without tyrosine 3-monooxygenase [EC 1.14.16.2; tyrosine hydroxylase; L-tyrosine, tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating)] activity were fused with normal cells from embryonic mouse sympathetic ganglia. One of the 37 hybrid cell lines obtained possesses high tyrosine 3-monooxygenase activity and synthesizes dopamine. These cells also have excitable membranes and generate action potentials in response to electrical stimuli. Thus hybrid cells, generated by fusion of neuroblastoma cells with normal cells from the nervous system, can acquire neural properties not found with the parental neuroblastoma cells.**

Although many murine neuroblastoma cell lines have been derived from tumor C1300, new cell lines with different neural phenotypes are needed. One approach has been to obtain somatic cell hybrids since cell lines with specific neural defects and cells that have acquired neural properties have been generated by cell fusion (1–5, ¶).

In this study, clonal mouse neuroblastoma cells lacking hypoxanthine phosphoribosyltransferase (EC 2.4.2.8; IMP: pyrophosphate phosphoribosyltransferase) were fused with sympathetic ganglion cells from embryonic mice. Sympathetic ganglia were chosen as a source of neurons because such ganglia contain only a few types of neurons which have been well characterized biochemically and electrophysiologically. Neuroblastoma cells were used as the other parent because they have neural properties and are related embryologically to sympathetic ganglion neurons. Our objective was to determine whether cell lines with interesting neural properties might be generated by the fusion of clonal neuroblastoma cells with normal cells from the nervous system.

In this communication, we describe a neuroblastoma × sympathetic ganglion hybrid cell line that has acquired the ability to synthesize dopamine.

MATERIALS AND METHODS

Cells and Media. Cells were grown in either F-14 (6) or the Dulbecco-Vogt modification of Eagle's minimal medium (DMEM, GIBCO cat. no. H-21) supplemented with fetal bovine serum in the ratio 95:5. Cultures were maintained in a forced-draft incubator at 37° in an atmosphere of 90% air–10% CO_2 saturated with water.

The neuroblastoma parent was N18TG2 (1, 2). This cell line is resistant to 1×10^{-4} M 6-thioguanine and lacks hypoxanthine phosphoribosyltransferase (HPRTase) activity. The cells do not multiply in media supplemented with 1×10^{-4} M hypoxanthine, 1×10^{-6} M aminopterin, and 1.2×10^{-5} M thymidine (HAT). The N18TG2 cells were grown in the presence of 1×10^{-4} M 6-thioguanine for at least four generations prior to use.

Primary cells were obtained from superior cervical and paravertebral sympathetic ganglia dissected from 12- to 14-day-old mouse embryos (C57BL/6) and dissociated with trypsin as described (7). Single cells were suspended in complete F-14 supplemented with 10 units/ml of 2.5S nerve growth factor. Cells were seeded into 100-mm plastic tissue culture dishes (Falcon, cat. no. 3003) and incubated for 4 hr at 37°. Attached cells, which consisted predominately of fibroblast-like cells, were discarded or were used for karyotype analysis. Floating cells and cells not firmly attached to the substratum were harvested by gentle washing and used immediately for hybridization. Aliquots of the cell suspension enriched with respect to neurons were seeded in separate dishes; after 36 hr 95% of the cells that attached resembled neurons in morphology.

Hybridization. Trypsinized neuroblastoma cells and normal cells from sympathetic ganglia were fused in suspension for 2 hr at 37° in 1 ml of F-14 supplemented with 10 units of nerve growth factor per ml in the absence of serum. The cell suspension contained 500 hemaglutinating units of Sendai virus inactivated with β-propriolactone, 5×10^5 N18TG2 neuroblastoma cells, and 2.5×10^5 cells from sympathetic ganglia (the fraction enriched with neurons). The cells were gently resuspended every 20 min during incubation. After incubation, cells were suspended again and were plated into 100-mm plastic culture dishes in complete F-14 medium supplemented with 10 units of nerve growth factor per ml at concentrations of 0.25, 1.25, and 5×10^4 cells per dish. After two days of incubation, the medium was changed to complete medium plus HAT. After 15–30 days in the selective medium, discrete colonies of greater than 100 cells were observed. The medium was removed from the plates and the colonies were individually isolated with cloning cylinders and harvested with the aid of glass pipettes with fine tips. After the cell lines were isolated and established in culture, the growth medium was changed to DMEM supplemented with 5% fetal bovine serum and HAT.

Characterization of Cell Lines. Karyotype analysis was performed by counting total numbers of chromosomes and

Abbreviations: HPRTase, hypoxanthine phosphoribosyltransferase (EC 2.4.2.8); DMEM, Dulbecco-Vogt modification of Eagle's minimal medium; dopa, 3,4-dihydroxyphenylalanine; dopamine, 3,4-dihydroxyphenylethylamine; HAT, 1×10^{-4} M hypoxanthine-1×10^{-6} M aminopterin-1.2×10^{-5} M thymidine.

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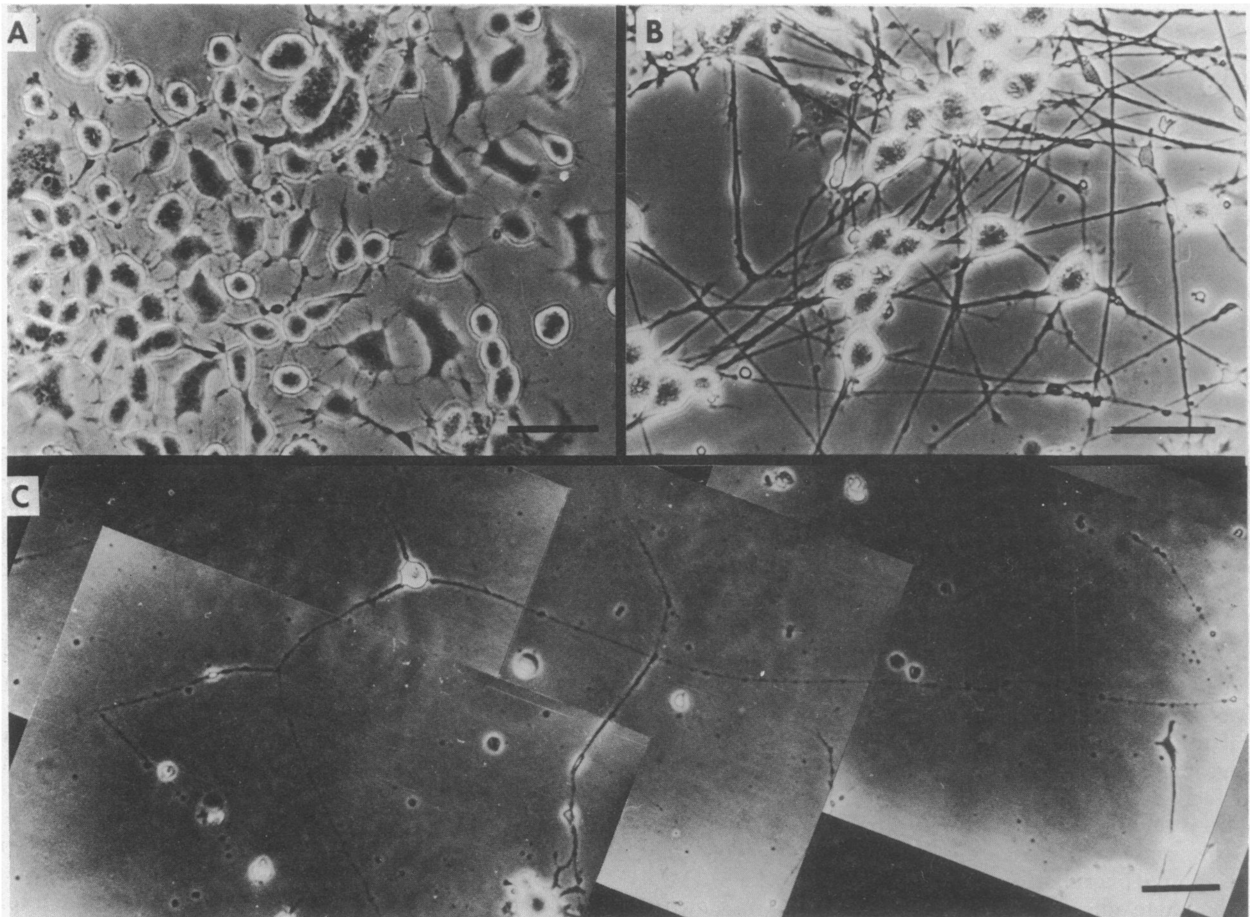

FIG. 1. Morphology of NX-31 hybrid cells. (A) NX-31 hybrid cells (logarithmic phase of growth) grown in complete DMEM. (B and C) NX-31 hybrid cells cultured for 6 days in complete DMEM supplemented with 1 mM dibutyryl cAMP. Bar represents 100 μm.

identifying and counting the large metacentric chromosomes typical of the N18TG2 parent. Chromosome spreads were prepared and stained with giemsa solution (8). Glucosephosphate isomerase (EC 5.3.1.9; D-glucose-6-phosphate ketol-isomerase) isozymes were separated by starch gel electrophoresis as described by DeLorenzo and Ruddle (9) using homogenates prepared from confluent cultures of NX-31, N18TG2, or from superior cervical ganglia or brain of fetal and weanling C57BL/6 mice (11, 13). Tyrosine 3-monooxygenase [EC 1.14.16.2; tyrosine hydroxylase; L-tyrosine, tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating)] and choline acetyltransferase (EC 2.3.1.6; acetyl-CoA:choline O-acetyltransferase) activities were measured as described (10–13). Protein was determined by a modification of the method of Lowry *et al.* (14). Monoamines synthesized by the cultured cells from tyrosine were identified by the procedures given in the legend of Table 3. The electrophysiological properties of the cultured cells were studied by techniques described (15). Spike rates of rise were measured by fixing the responses on the expanded scale of a storage oscilliscope (horizontal axis 1 msec/cm, vertical axis 20 mV/cm). Prior to assay for tyrosine 3-monooxygenase activity, catecholamine synthesis, or electrophysiological properties, the hybrid cells were cultured for at least two subcultures in DMEM supplemented with 5% fetal bovine serum, 1×10^{-4} M hypoxanthine, and 1.6×10^{-5} M thymidine.

RESULTS

Isolation of hybrid cells

Mouse neuroblastoma N18TG2 cells and cells from mouse sympathetic ganglia were fused as described in *Materials*

Table 1. Hybrid properties of NX-31 cells

	HPRTase	Glucosephosphate isomerase isozymes			Chromosomes		
	Growth in HAT medium	GPI-1A (A strain)	GPI-1AB (hybrid)	GPI-1B (C57BL/6)	Total (modal no.)	Metacentrics (modal no.)	*n*
N18TG2 clone	—	+	—	—	88	9	31
C57BL/6 primary	+*	—	—	+	40	0	15
X-31 hybrid	+	+	+	+	172	12	27

* Primary neurons have HPRTase activity, but are nonproliferating.

and Methods. Approximately 500 colonies, comprised of 1000 cells or more, were observed in medium containing HAT. The choice of culture medium and of parents favored the selection of hybrid cells because sympathetic neurons do not divide and the N18TG2 parent lacks HPRTase and consequently does not grow in HAT medium. In contrast, hybrid cells that grow well in HAT medium were selected because the sympathetic ganglion cell genome complements the N18TG2 HPRTase deficiency. Greater than 95% of the colonies contained cells with neuron-like morphology. About 100 colonies were isolated, and 37 were successfully passaged and maintained. Homogenates were prepared from each cell line and assayed for tyrosine 3-monooxygenase and choline acetyltransferase activities as described in *Materials and Methods*. None of the cell lines tested had appreciable choline acetyltransferase activity. One cell line, designated NX-31, had high tyrosine 3-monooxygenase activity. This line was therefore chosen for further characterization.

Hybrid nature of the NX-31 line

Since the reversion frequency of N18TG2 is less than 1×10^{-7} (1, 2) and since only 5×10^5 neuroblastoma cells were used for hybridization, it is likely that most of the colonies that were selected in the presence of HAT were hybrids of neuroblastoma and sympathetic ganglion cells.

The hybrid nature of the NX-31 line was verified by isozyme analysis (Table 1). The N18TG2 neuroblastoma cells, which originated from an A-strain mouse, and the sympathetic ganglion cells, which were obtained from C57BL/6 strain mice, have different electrophoretic variants of glucose phosphate isomerase (GPI) at the *Gpi-1* locus (9). NX-31 cells express the electrophoretic variants of both the parental cell strains (GPI-1A of N18TG2 neuroblastoma and GPI-1B of C57BL/6 sympathetic ganglia) as well as a third variant (GPI-1AB) of intermediate electrophoretic mobility which has been described in animals that are heterozygous at the *Gpi-1* locus (9). With NX-31 cells, the staining intensities of the two parental isozymes were almost equal and were approximately 50% that of the hybrid isozyme.

As shown in Table 1, the N18TG2 neuroblastoma and primary cells cultured from C57BL/6 mouse embryos have modal chromosome numbers of 88 (range 66–100) and 40, respectively. N18TG2 has a modal number of 9 large metacentric chromosomes (range 6–12). With NX-31 hybrid cells examined 20–40 generations after hybridization, the modal number of chromosomes was 172 (range 87–243). The hybrid cells also contained large metacentric chromosomes (modal number 12, range 4–16). The relatively high number of chromosomes in NX-31 cells suggests that the hybrid may have originated from the fusion of more than two cells. By the 16th subculture (120–150 generations after fusion), the NX-31 cell population was heterogeneous with respect to chromosome number. Chromosome numbers were clustered around modes of 142, 175, and 225.

Morphology

In the logarithmic phase of growth, most NX-31 cell bodies were round, and few cells extended processes (Fig. 1A). When the hybrid cells were cultured for several days in the presence of 1 mM dibutyryl cyclic AMP, they extended long, branching processes (Fig. 1B). Treatment with dibutyryl cyclic AMP has been shown to induce neurite outgrowth from neuroblastoma cells (16, 17). The morphology of NX-31 differs from that of N18TG2 parent and other neuroblastoma lines in that the processes of the hybrid cells tend to be finer, more branching, and more varicose. Occasionally, NX-31 cells were observed which closely resembled cultured primary sympathetic neurons in morphology (Fig. 1C).

Table 2. Tyrosine 3-monooxygenase activity of hybrid NX-31 cells

Cell	Generations	Tyrosine 3-monooxygenase specific activity (pmol/min mg protein)
N18TG2	100–200	1
NX-31	20–40	98
NX-31	45–65	95
NX-31	120–150	27
NX-31T	120–150 plus 10–13 after selection in tyrosine-free medium	394

Cells were grown to stationary phase in complete DMEM supplemented with 1×10^{-4} M hypoxanthine and 1.2×10^{-5} M thymidine. The preparation of cell homogenates and the method of assay for tyrosine 3-monooxygenase are described elsewhere (11, 20). Between 100 and 600 μg of protein were present in each reaction mixture. The final specific activity of L-[3,5-^{3}H]tyrosine was 7–10 cpm/pmol.

Tyrosine 3-monooxygenase activity

In contrast to the parent N18TG2 neuroblastoma, NX-31 hybrid cells had tyrosine 3-monooxygenase activity (Table 2). This enzyme, which is found in sympathetic neurons, catalyzes the conversion of tyrosine to dihydroxyphenylalanine (dopa), the first step in catecholamine synthesis. After four subcultures (30–50 generations after fusion), the specific activity of confluent cultures of NX-31 cells was about 100 pmol of dopa formed/min per mg of protein. By the 16th subculture (120–150 generations), the specific activity of the enzyme had fallen to about 30 pmol of dopa formed/min per mg of protein. Cells from subculture 16 were then selected in complete DMEM without tyrosine. The rationale for the selection process is that tyrosine 3-monooxygenase catalyzes the conversion of phenylalanine to tyrosine as well as the conversion of tyrosine to dopa; thus cells with tyrosine 3-monooxygenase grow in the absence of tyrosine (18). As shown in Table 2, the specific activity of tyrosine 3-monooxygenase found for NX-31 cells grown without tyrosine was approximately 400 pmol of dopa formed/min per mg of protein. The plating efficiency was 6×10^{-3}. When N18TG2 neuroblastoma cells were challenged with the same selection procedure, no colonies were found (plating efficiency less than 3×10^{-7}).

Characterization of catechols synthesized by NX-31 cells

Confluent cultures of NX-31 cells were incubated with [^{14}C]tyrosine and the ^{14}C-labeled products extracted from cell homogenates were characterized by thin-layer chromatography (see legend of Table 3 for methods). The results show that [^{14}C]compounds with the chromatographic mobilities of authentic dopa and dopamine were present with each of the four chromatographic systems used. Labeled norepinephrine, however, was not detected. No conversion

Table 3. Identification of dopa and dopamine synthesized from [^{14}C]tyrosine by hybrid NX-31 cells

Thin-layer chromatography solvent system	Radioactivity recovered as (cpm):		
	Tyrosine	Dopa	Dopamine
a	1227	597	404
b	1015	635	540
c	938	541	531
d	1080	555	432

NX-31 cells (subculture 14) in a 100-mm dish containing about 10^7 cells (10 mg of protein) were maintained for 11 days in complete DMEM containing 1 mM dibutyryl cAMP, 1×10^{-4} M hypoxanthine, and 1.2×10^{-5} M thymidine. The culture was then incubated for 17 hr in 3 ml of fresh medium which also contained 0.4 mM of uniformly labeled [^{14}C]tyrosine (1.25 mCi/mmol). After incubation, the medium was removed and the cells were washed three times with phosphate-buffered saline and extracted and homogenized in 0.5 ml of 0.2 M acetic acid. The extract was centrifuged at $5000 \times g$ for 10 min and the supernatant fraction was collected and evaporated under nitrogen; the final volume was approximately 50 μl. Aliquots of 10 μl were spotted on cellulose-coated thin-layer chromatography sheets (Eastman 6064) with 1 μg each of carrier dopa, dopamine, and norepinephrine. The four solvent systems listed below were used, and catechols and catecholamines were detected by the use of ferrocyanide-ethylenediamine reagent (19). The chromatograms were cut into 1.0×0.5 cm strips and each strip was assayed for radioactivity. A 5-μl aliquot of the incubation culture medium was also run in each system in order to establish the purity of the tyrosine and the counting efficiency (38%). Solvent systems used were: (a) isopropanol/pyridine/1 M acetic acid, 5:2:2; (b) butanol/ethanol/ammonium hydroxide/water, 10:1:2:4 (21); (c) ethyl acetate/acetic acid/water, 10:3:6 (22); and (d) butanol/ethanol/1 M acetic acid, 35:10:10 (23).

of tyrosine to dopa or dopamine was detected in similar experiments with N18TG2 cultures.

These results show that NX-31 cells synthesize dopamine and suggest that cells have both tyrosine 3-monooxygenase and aromatic-amino-acid decarboxylase (EC 4.1.1.28; aromatic-L-amino-acid carboxy-lyase) activities.

Electrical excitability

As shown in Fig. 2, NX-31 hybrid cells possess electrically excitable membranes and generate action potentials in response to electrical stimulation. Membrane excitability increased when cells were cultured for several days in the presence of 1 mM dibutyryl cyclic AMP similar to the behavior of other cell lines (15; A. Chalazonitis, J. Minna, D. Schoenberg, and M. Nirenberg, in preparation). Under these conditions, the active electrical properties of NX-31 hybrid cells resembled more closely those of primary sympathetic neurons than did those of N18TG2 (Fig. 2). The most striking difference was in the maximum rate of rise of the evoked spikes. For N18TG2 and NX-31 cells, the average maximum rates of rise were 24 and 85 V/sec, respectively. The latter value is similar to that of sympathetic neurons *in situ* (24, 25) and cultured *in vitro* (8). The electrical properties and chemosensitivity of NX-31 hybrid cells are described in detail elsewhere (26).

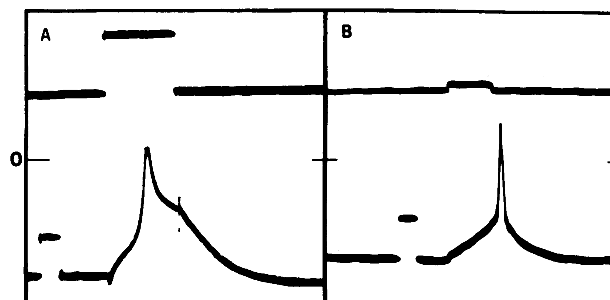

FIG. 2. Comparison of action potentials elicited from parental neuroblastoma N18TG2 and hybrid NX-31 cells. (A) Parental neuroblastoma N18TG2: Upper trace shows stimulation pulse 3 nA in intensity, 35 msec in duration. Lower trace shows cell membrane potential recorded at the cell resting potential of −55 mV. The cell had been cultured in the presence of 1 mM dibutyryl cAMP for 14 days. (B) NX-31 hybrid: Upper trace shows stimulation pulse 0.2 nA in intensity, 22 msec in duration. The record was obtained at the cell resting potential of −45 mV. Note the difference in the intensity of current needed to elicit action potentials in A and B. Note also the sharper rate of rise of the NX-31 spike, the greater overshoot amplitude, and more rapid repolarizing phase compared to the N18TG2 spike. The cell was cultured in the presence of 1 mM dibutyryl cAMP for 13 days. Both spikes are preceded by a calibration pulse 20 mV in amplitude and 10 msec in duration.

DISCUSSION

The results show that fusion of mouse neuroblastoma N18TG2, which lacks tyrosine 3-monooxygenase activity, with cells from mouse sympathetic ganglia that have this enzyme yields a somatic cell hybrid line that has high tyrosine 3-monooxygenase activity and synthesizes dopa and dopamine. Under appropriate culture conditions, the hybrid cells resemble neurons in morphology and possess highly excitable membranes that are capable of generating action potentials. Additional studies to be reported elsewhere show that these cells have nicotinic and possibly muscarinic excitatory acetylcholine receptors (26).

Both sympathetic ganglion neurons and interneurons have tyrosine 3-monooxygenase activity, which raises the possibility that at least part of the genome of the normal ganglion parent cell may be expressed in hybrid cells. On the other hand, certain neuroblastoma × glioma hybrid cell lines have neuronal properties not exhibited by either parent cell line, such as choline acetyltransferase activity (3, 5). Whether the neural properties of NX-31 hybrid cells are due to the expression of genes from the normal or the neoplastic cell, or to the genomes of both parents remains to be determined.

The neural properties of NX-31 cells are retained and are expressed many generations after fusion. For example, the electrical excitability of cell membranes was high 150 generations after hybridization (the oldest cells tested). Also, although the specific activity of tyrosine 3-monooxygenase in NX-31 cultures decreased after 16 subcultures, a subline with over 10-fold higher specific activity of this enzyme was obtained by growing the cells in selective medium lacking tyrosine. Such cells have been continuously grown in media lacking tyrosine for many generations with full retention of tyrosine 3-monooxygenase specific activity.

The evidence presented here shows that NX-31 cells synthesize dopamine, but not norepinephrine, which suggests that cells possess tyrosine 3-monooxygenase and aromatic amino-acid decarboxylase activities, but not dopamine β-monooxygenase (EC 1.14.17.1) activity. Thus NX-31 hybrid cells more closely resemble sympathetic ganglion interneurons which reportedly contain dopamine but not norepinephrine (27), than ganglion neurons, which synthesize norepinephrine.

In summary, fusion of neuroblastoma cells with normal

cells from the nervous system can yield hybrid cells with new neural properties. Such cell lines should be useful as model systems for studying synapse formation and other neural properties.

1. Minna, J., Nelson, P. G., Peacock, J., Glazer, D. & Nirenberg, M. (1971) "Genes for neural properties expressed in neuroblastoma × L cell hybrids," *Proc. Nat. Acad. Sci. USA* **68**, 234–239.
2. Minna, J., Glazer, D. & Nirenberg, M. (1972) "Genetic dissection of neural properties using somatic cell hybrids," *Nature New Biol.* **235**, 225–231.
3. Minna, J., Yavalow, J. & Coon, H. G. (1975) "Expression of phenotypes in hybrid somatic cells derived from the nervous system," *Genetics* **79** (Suppl.), 373–383.
4. Peacock, J. H., McMorris, F. A. & Nelson, P. G. (1973) "Electrical excitability and chemosensitivity of mouse neuroblastoma × mouse or human fibroblast hybrids," *Exp. Cell Res.* **79**, 199–212.
5. Amano, T., Hamprecht, B. & Kemper, W. (1974) "High activity of choline acetyltransferase induced in neuroblastoma × glia hybrid cells," *Exp. Cell Res.* **85**, 399–408.
6. Vogel, Z., Sytkowski, A. J. & Nirenberg, M. W. (1972) "Acetylcholine receptors of muscle grown *in vitro*," *Proc. Nat. Acad. Sci. USA* **69**, 3180–3184.
7. Chalazonitis, A., Greene, L. A. & Nirenberg, M. (1974) "Electrophysiological characteristics of chick embryo sympathetic neurons in dissociated cell culture," *Brain Res.* **68**, 235–252.
8. Hsu, T. C. (1973) in *Tissue Culture Methods and Applications*, eds. Kruse, P. F., Jr. & Patterson, M. K., Jr. (Academic Press, New York), pp. 764–767.
9. DeLorenzo, R. J. & Ruddle, F. H. (1969) "Genetic control of two electrophoretic variants of glucosephosphate isomerase in the mouse (*Mus musculus*)," *Biochem. Genet.* **3**, 151–162.
10. Schrier, B. K., Wilson, S. H. & Nirenberg, M. W. (1974) in *Methods in Enzymology*, eds. Fleisher, S. and Packer, L. (Academic Press, New York), Vol. 32B, pp. 765–783.
11. Richelson, E. & Nirenberg, M. (1974) in *Methods in Enzymology*, eds. Fleisher, S. & Packer, L. (Academic Press, New York), Vol. 32B, pp. 785–788.
12. Wilson, S. H., Schrier, B. K., Farber, J. L., Thompson, E. J., Rosenberg, R. N., Blume, A. J. & Nirenberg, M. W. (1972) "Markers for gene expression in cultured cells from the nervous system," *J. Biol. Chem.* **247**, 3159–3169.
13. Schrier, B. K. & Shuster, L. (1967) "A simplified radio-chemical assay for choline acetyltransferase," *J. Neurochem.* **14**, 977–985.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) "Protein measurement with the Folin phenol reagent," *J. Biol. Chem.* **193**, 265–275.
15. Chalazonitis, A. & Greene, L. A. (1974) "Enhancement in excitability properties of mouse neuroblastoma cells cultured in the presence of dibutyryl cyclic AMP," *Brain Res.* **72**, 340–345.
16. Prasad, K. N. & Hsie, A. W. (1971) "Morphological differentiation of mouse neuroblastoma cells induced *in vitro* by dibutyryl adenosine 3′:5′-cyclic monophosphate," *Nature New Biol.* **233**, 141–142.
17. Furmanski, P., Silverman, D. J. & Lubin, M. (1971) "Expression of differentiated functions in mouse neuroblastoma mediated by dibutyryl-cyclic adenosine monophosphate," *Nature* **233**, 413–415.
18. Breakefield, X. O. & Nirenberg, M. W. (1974) "Selection for neuroblastoma cells that synthesize certain transmitters," *Proc. Nat. Acad. Sci. USA* **71**, 2530–2533.
19. Schneider, F. H. & Gillis, C. N. (1965) "Catecholamine biosynthesis *in vivo:* An application of thin-layer chromatography," *Biochem. Pharmacol.* **14**, 623–626.
20. Amano, T., Richelson, E. & Nirenberg, M. (1972) "Neurotransmitter synthesis by neuroblastoma clones," *Proc. Nat. Acad. Sci. USA* **69**, 258–263.
21. Roser, R. & Tocci, P. M. (1972) "Detection of cathecholamines and related compounds with diazonium reagents by thin-layer chromatography," *J. Chromatogr.* **72**, 207–211.
22. Vahidi, A. & Sankar, D. V. S. (1969) "The application of paper and partition TLC to the separation of catecholamines and their metabolites," *J. Chromatogr.* **43**, 135–140.
23. Johnson, G. A. & Boukma, S. J. (1967) "A rapid method for separation of dopa, dopamine, and norepinephrine," *Anal. Biochem.* **18**, 143–146.
24. Nishi, S. & Koketsu, K. (1960) "Electrical properties and activities of single sympathetic neurons in frogs," *J. Cell. Comp. Physiol.* **55**, 15–30.
25. Blackman, J. G. & Purves, R. D. (1969) "Intracellular recordings from ganglia of the thoracic sympathetic chain of the guinea-pig," *J. Physiol. (London)* **203**, 173–198.
26. Chalazonitis, A., Greene, L. A. & Shain, W. (1975) "Excitability and chemosensitivity properties of a somatic cell hybrid between mouse neuroblastoma and sympathetic ganglion cells," *Exp. Cell Res.*, in press.
27. Bjorklund, A., Cegrell, L., Falck, B., Ritzen, M. & Rosengren, E. (1970) "Dopamine-containing cells in sympathetic ganglia," *Acta Physiol. Scand.* **78**, 334–338.