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Double-Stranded RNA Unwinding and Modifying Activity Is Detected Ubiquitously in Primary Tissues and Cell Lines

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A double-stranded RNA unwinding and modifying activity was found to be present in a wide range of tissues and cell types. The level of activity did not vary significantly with respect to the state of cell differentiation, cell cycle, or transformation. Thus, the unwinding and modifying activity, localized in the nucleus in somatic cells and capable of converting many adenosine residues to inosine, appears to be one of the housekeeping genes.

A double-stranded RNA (dsRNA) unwinding activity, originally found in *Xenopus* eggs (1, 22) and several mammalian tissue culture cell lines (23), converts up to 50% of adenosine residues to inosine in dsRNA (2, 24). Previous studies have suggested cell cycle and possibly developmental regulation of the activity. The physiological function as well as natural substrate RNAs for this novel activity are not yet understood. It has been postulated that the activity may be involved as a degradative pathway for dsRNAs or may be involved in naturally occurring antisense RNA regulatory pathways in eucaryotic cells. Indeed, one such example, the degradation of maternal basic fibroblast growth factor mRNAs mediated by antisense transcripts and the unwinding and modifying activity during *Xenopus* oocyte maturation, has been recently reported (12). In addition, the activity may be the cause of base-specific mutations that occur in several viral gene systems (15, 19). The most striking of these cases is that of defective measles virus genes derived from brain tissues of patients with subacute sclerosing panencephalitis and measles inclusion body encephalitis (3–5, 13, 24, 25). We have surveyed various primary tissues and cell lines to see whether the activity is expressed in a tissue-specific manner, especially in the human central nervous system (CNS), where the persistent infection of defective measles viruses occurs. We have found that the activity, localized in the nucleus, is detectable in all cells and tissues, including CNS, regardless of the differentiated, mitotic, or transformed state of the cells.

Comparison of activities from different tissues and cell lines. Although the unwinding-modifying activity has been shown to be present in *Xenopus* eggs and rapidly growing embryos (1, 22) and also in several transformed mammalian cell lines (23), it was not known whether the activity is also expressed in nontransformed primary tissues and cells. We prepared crude cell extracts from a variety of primary tissues and cells. Rat liver, kidney, spleen, testis, lymph node, and brain tissue extracts were all found to contain the unwinding-modifying activity (Fig. 1, lanes d to i). By using a quick-extract method, human natural killer (NK) and T cells (Fig. 1, lanes b and c), as well as chicken muscle cells (Fig. 1, lane j), were also found to express the activity. NK cells contained the highest activity, threefold higher than that of human lymphoblastoid GM1500 cells (Table 1), whereas the level from rat testis was the lowest among these primary tissues, though still detectable (Fig. 1, lane g; Table 1).

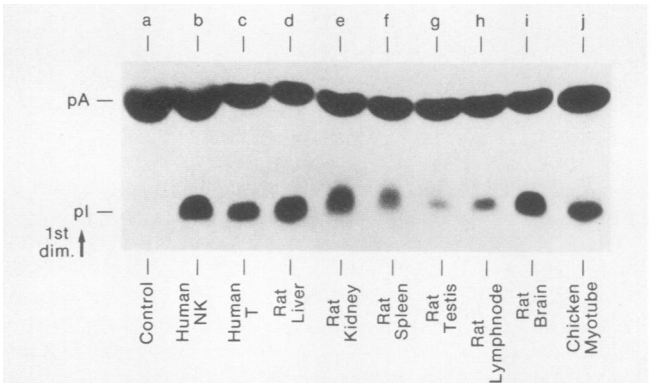

FIG. 1. Detection of unwinding-modifying activity in primary tissue and cells. Each assay was performed under conditions described previously (23, 24). The reaction mixture contained 10 fmol of [α-^{32}P]ATP-labeled mouse c-*myc* dsRNA and 20 μg of cell extract protein, except that 5 μg of NK cell extract protein was used (lane b). The unwound-modified RNAs were digested to 5′-mononucleotides by using P1 nuclease and were chromatographed on thin-layer cellulose chromatography plates in one dimension, using the following solvent: isobutyric acid-NH_4OH-water, 100:1.5:48.5 (vol/vol/vol). NK cells (lane b) and T cells (lane c) were prepared as described previously (16, 20). In lanes d to i, nuclear extracts were prepared from adult rat tissues according to the method described previously (8, 9). In lane j, chicken muscle cells prepared from the breast muscle of a chicken embryo were maintained as undifferentiated myoblasts in a growth medium containing Dulbecco modified Eagle medium (DEM) plus 20% fetal calf serum (FCS) and 0.5% chick embryo extract (GIBCO Laboratories). The formation of multinucleated, differentiated, postmitotic myotubes was induced with mitogen-poor fusion medium containing DME plus 2% horse serum. The quick-extract procedure (8) was used to prepare NK cells, T cells, and myotube extracts. The quick extract yields identical levels of modifying activity compared with the Manley whole-cell extract (16) and nuclear extract of HeLa and GM1500 lymphoblastoid cells (7, 9) (not shown).

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TABLE 1. Level of unwinding/modifying activity in various tissues and cell lines[a]

Tissue or cell type	Species	Cell line	Comments	[I/(A + I)] × 100 (%)[b]
Spleen	Rat	Primary		15 (N)
Lymph node	Rat	Primary		10 (N)
B lymphocyte	Mouse	P3xAg8	Plasmacytoma	30
	Human	Daudi	Burkitt lymphoma	25
	Human	GM1500	Lymphoblastoid, EB virus$^+$	40
T lymphocyte	Human	Primary	Mixed T cells	30
T4 helper	Human	Primary	CD4$^+$ CD8$^-$	20
T8 suppressor	Human	Prmary	CD4$^-$ CD8$^+$	20
T lymphocyte	Human	Molt-4	T lymphoma, CD4$^+$	40
	Human	SupT	T lymphoma, CD4$^+$ CD8$^+$	35
NK cell	Human	Primary		50
Liver	Rat	Primary		25 (N)
Kidney	Rat	Primary		25 (N)
	Monkey	CV-1		30
	Monkey	Cos 1	T antigen$^+$	30
	Human	CM03808	Nephroblastoma, Wilms tumor	30
Fibroblast	Mouse	L929		15
	Mouse	NIH 3T3	Quiescent	5
	Mouse	NIH 3T3	12 h + 10% FCS	15
Testis	Rat	Primary		5 (N)
Ovary	Hamster	CHO	DHFR$^-$	10
Muscle	Chicken	Primary	Myotube	15
	Mouse	C_2C_{12}	Myoblast	20
	Mouse	C_2C_{12}	Myotube	5
Epithelial	Human	HeLa	Adenocarcinoma	35

[a] Subsets of human T cells (T4 and T8) were purified to homogeneity from total T cells by negative antibody-selective fractionation (10). Daudi, GM1500, Molt-4, and SupT lines were grown in RPMI 1640 and 10% FCS; P3xAg8 cells were grown in DME plus 12.5% FCS and 1.5% calf serum; CV-1, Cos 1, and CHO cells were grown in DME and 10% FCS; HeLa cells were grown in Joklik S-MEM and 7% horse serum; GM03808 cells were grown in DME plus 15% FCS; L929 cells were grown in Joklik S-MEM and 5% FCS. Serum starvation and synchronization into quiescence of NIH 3T3 cells were carried out as described previously (23). Mouse myoblast C_2C_{12} cells were grown in DME plus 20% FCS and were induced to differentiate as described for chicken myoblasts in the legend to Fig. 1.

[b] Using conditions cited in the legend to Fig. 1, substrate conversion, $[I/(A + I)] \times 100$, was linear with respect to protein in the range of 5 to 40% inosine conversion. Any conversion greater than 40% was titrated back to the linear range by decreasing the amount of protein used. For instance, 20 μg of extract proteins from GM1500 and NK cells converted 40 and 50% of A to I, while 5 μg of extract proteins converted 10 and 30% of A to I, respectively. The percent inosine conversion was quantitated by using an LKB laser densitometer. (N), Nuclear extract instead of quick extract.

A variety of cell lines from various tissues were also analyzed. The levels of activity did not vary significantly among cell lines derived from B or T lymphocytes, kidney, fibroblast, ovary, or muscle cells (Table 1). As we reported previously (23), quiescent mouse fibroblast 3T3 cells increased their unwinding-modifying activity threefold after stimulation with serum (Table 1). In addition, the level of the activity decreased fourfold during in vitro induction of the mouse muscle cell line C_2C_{12} from myoblast to myotube, although the activity was still detectable in the differentiated postmitotic myotubes (Table 1). We also examined various conditions known to activate cellular antiviral mechanisms, including induction of (2′-5′) oligo adenylate synthetase, RNase L, and dsRNA-dependent protein kinase (14). Various interferons, treatment with synthetic dsRNA poly(I) · poly(C), and infection with dsRNA virus (reovirus) were found to have essentially no effect on the level of the unwinding-modifying activity (data not shown).

Detection of activity in the CNS and PNS. Recently, the possible involvement of the unwinding-modifying activity in highly biased hypermutation found in defective measles virus genomes has been suggested (3, 13, 24, 25). Persistent infection of such defective viruses is believed to induce progressive lethal disease of the CNS, e.g., subacute sclerosing panencephalitis and measles inclusion body encephalitis (3, 4, 13, 24, 25). Therefore, we next examined various tissues and cell lines derived from the CNS and also from the peripheral nervous system (PNS). Nuclear extracts were prepared from fetal human, newborn calf, and adult mouse brain tissue. The primary cells cultured in vitro, and transformed cells were prepared as a quick extract as described in the legend to Fig. 1. Similar to our finding with the whole rat brain extract (Fig. 1, lane i), the extracts of human, calf, or mouse brain contained high levels of the unwinding-modifying activity (Fig. 2A, lanes a to c). In addition, the quick extracts of human glioblastomas, astrocytomas, ependymomas, neuroblastomas, and retinoblastomas were found to contain high levels of the activity (Table 2).

The unwinding-modifying activity is localized in the nucleus in somatic cells. One apparent exception was a hamster glial cell line, HJC. With the quick extract made from these cells, an inosine spot was detectable (Fig. 2A, lane d), but the percentage of A-to-I conversion was very low (Table 2). The HJC extract did not include contaminating protease or inhibitors of the reaction, as indicated by the results of an experiment in which the GM1500 extract was mixed with the HJC extract before addition of the substrate RNA (Fig. 2A, lane j). The Manley extract (16) made from HJC cells, instead of quick extract, gave similar negative results (not shown). One additional rat glial cell line, C_6, and also rat schwannoma D6P2T, as well as several rodent primary cell cultures derived from the CNS or PNS, contained very low levels of the activity, similar to the HJC cells (Fig. 2A, lanes e to g and k to n). The low level of activity was not a general feature for all rodent cells derived from CNS and PNS or for all glial cells, since, for instance, the quick extracts of rat PC12 pheochromocytoma cells (Table 2) and bovine oligodendrocytes (Fig. 2A, lane o) contained detectable levels of the activity.

We were especially puzzled about the low levels of activity found in rat primary CNS cell culture extracts compared with the high levels of rat brain nuclear extracts,

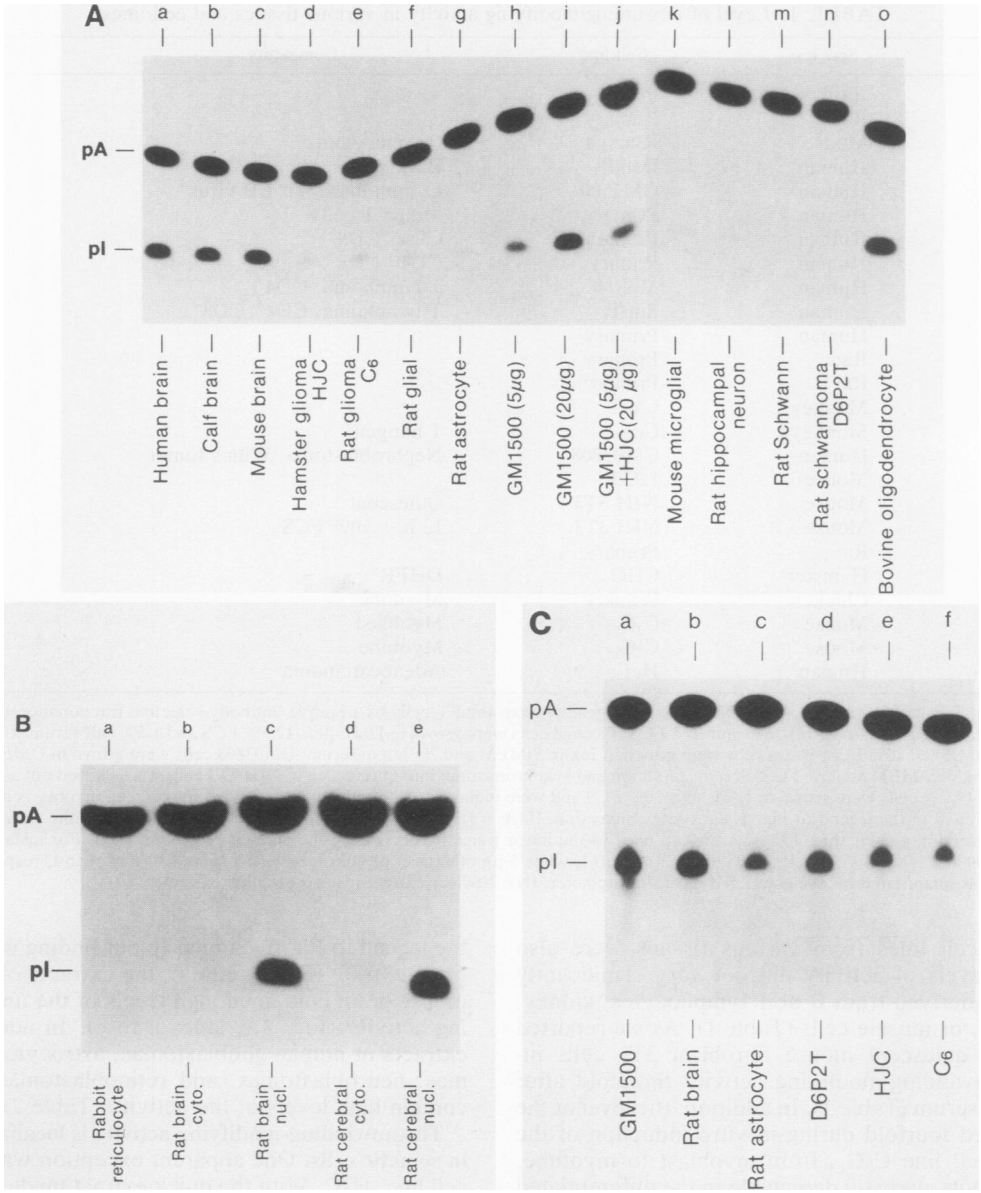

FIG. 2. Detection of unwinding-modifying activity in tissues and cell lines derived from CNS and PNS. (A) In lanes a to c, nuclear extracts were prepared from fetal human, newborn calf, and adult mouse brain tissue. The following cells were prepared as a quick extract as described for Fig. 1: HJC cells were maintained in DME and 10% FCS (lane d); rat primary glial cells were cultured from 1-day-old rat cerebra in OM-5 medium at either high (lane f, mixed glial cells: 65% astrocytes, 15% oligodendrocytes, and 25% precursor glial cells) or low (lane g, 90% astrocytes) cell density (17, 18). Each cell type was identified by immunostaining (17, 18). Rat glial tumor C_6 cells were grown in OM-5 medium (lane e); rat Schwann cells (lane m) were prepared from the sciatic nerve of neonatal rats and were grown in DME plus 10% FCS–2 μM forskolin–10 μg of glial growth factor per ml (21); rat hippocampal cortical neurons from 15-day-old embryos were grown in DME and 10% FCS for 7 days before harvesting (lane l). The cultures contained 50% neurons and 50% astrocytes, which were identified by morphology (6); microglial cells were dissected from newborn mouse brains, and the cells were maintained in vitro in DME and 10% FCS for 2 weeks. Microglia grew on top of the monolayer of astrocytes, were shaken for 16 h at 200 rpm for collection, and were immunostained for different markers (10). Microglia cells represented 77% of the shake-off culture (lane k). Bovine oligodendrocytes were purified to 97% homogeneity by directly trypsinizing minced cow brain and centrifuging the cells through a Percoll gradient (11). In lane j, 20 μg of HJC extract protein was mixed with 5 μg of GM1500 extract protein for 10 min at 37°C before the addition of substrate dsRNA. (B) Brains were removed from 15-day-old rats. Cytoplasmic and nuclear extracts of cerebral (lanes d and e) and noncerebral (lanes b and c) brain tissue were prepared (7). The lysate of enucleated rabbit reticulocytes was obtained from Bethesda Research Laboratories, Inc. (lane a). (C) Nuclear extracts (7) were prepared from rat 90% astrocyte cultures and from D6P2T, HJC, and C_6 cells. GM1500 and rat brain nuclear extracts were also included for comparison.

since the former culture was derived from cells of the latter. One possible explanation of this discrepancy is that the activity was exclusively localized within the nucleus and there was poor release of the activity during preparation of the whole-cell extracts. Therefore, we prepared cytoplasmic and nuclear extracts, instead of the quick extract, from 15-day-old rat cerebra and the rest of the brain (Fig. 2B, lanes b to e). Using a similar extract protocol, we prepared

TABLE 2. Level of unwinding/modifying activity in various tissues and cell lines derived from CNS and PNS[a]

Tissue or cell type	Species	Cell line	Comments	[I/(A + I)] × 100 (%)[b]
Brain	Human	Primary	Fetal	30 (N)
	Monkey	Primary	Adult	30 (N)
	Calf	Primary		30 (N)
	Mouse	Primary	2 day old	25 (N)
	Mouse	Primary	Adult	25 (N)
	Rat	Primary	Adult	30 (N)
Glial	Rat	Primary	90% astrocyte	10 (N)/1
	Rat	Primary	Mixed glial	1
	Rat	Primary	65% oligodendrocyte	1
	Bovine	Primary	97% oligodendrocyte	35
	Hamster	HJC	JC virus$^+$	10 (N)/1
	Rat	C_6	Glioma	10 (N)/1
Microglial	Mouse	Primary	77% microglial	1
Glioblast	Human	A172	Glioblastoma	30
	Human	HTB16	Glioblastoma	20
	Human	A1235	Astrocytoma	30
	Human	HTB14(U87)	Astrocytoma	30
	Human	HTB17	Astrocytoma	20
	Human	H4	Neuroglioma	35
Ependyma	Human	A2781	Ependymoma	30
Neuronal	Rat	Primary	50% hippocampal neurons	1
Neuroblast	Human	CHP100	Neuroblastoma	35
	Human	CHP126A	Neuroblastoma	30
	Human	CHP234	Neuroblastoma	45
Pheochromocyte	Rat	PC12	Pheochromocytoma	15
Retinoblast	Human	GM02718	Retinoblastoma, Rb$^-$[c]	15
	Human	Y79	Retinoblastoma, mutated Rb[d]	40
Schwann	Rat	Primary		1
	Rat	D6P2T	Schwannoma	15 (N)/1

[a] All assays and cell extract procedures were done as described in the legends to Fig. 1 and 2. Rat 65% oligodendrocyte cultures were prepared as a shake-off culture from 7-day-old cultures of day-old rat cerebra and were grown for 7 additional days in OM-5 medium on poly-L-lysine-coated plates (15, 16). HTB14, HTB16, and HTB17 cells were grown in DME and 10% FCS; A172, A1235, A2781, H4, CHP100, CHP126A, and CHP234 cells were grown in RPMI and 10% FCS; GM02718 cells were grown in DME and 15% FCS; PC12 cells were grown in RPMI 1640 plus 10% horse serum and 5% FCS; Y79 cells were cultured in RPMI 1640 plus 20% FCS. All other cells were grown and prepared as described in the legend to Fig. 2.

[b] See Table 1, footnote *b*.

[c] A portion of the Rb (retinoblastoma susceptibility) gene has been deleted.

[d] The Rb gene contains a point mutation making the protein nonfunctional.

cytoplasmic and nuclear extracts (Fig. 2C, lane c) from 14-day-old rat astrocyte cultures that were derived from day-old rat cerebra. In these preparations, the activity was detectable and localized exclusively in the nuclear fractions. In the case of the astrocyte cultures, there was greater than 50-fold enrichment of the activity in the nuclear extract compared with the standard quick-extract method. The rabbit reticulocyte lysate, commonly used for in vitro translation, was also found to contain no activity, confirming the nuclear localization of the unwinding-modifying activity (Fig. 2B, lane a). We next checked other cell lines that previously appeared to be depleted of the activity. D6P2T, HJC, and C_6 cells were all found to contain detectable levels of modifying activity in nuclear extract preparations (Fig. 2C, lanes d to f). Thus, we conclude that the unwinding-modifying activity, localized in the nucleus, can also be detected in rodent CNS and PNS cells if the extracts are properly prepared.

The results from this study have shown that the dsRNA unwinding-modifying activity is ubiquitously present in mammalian cell extracts of every tissue tested (Tables 1 and 2). Although the activity seems to be cell cycle regulated in some cell types (23), it can be detected also in postmitotic cells such as bovine oligodendrocyte and mouse and chicken myotube cells. The activity is also found in a wide range of species, including mammals, amphibian, avian, and *Drosophila* spp. (K. Nishikura and R. Wagner, unpublished results). Thus, the activity seems to be a typical housekeeping gene. The unwinding-modifying activity is localized in the somatic cell nucleus. This conclusion is in agreement with earlier work which suggested that the activity is localized in the nucleus of frog oocytes (B. Bass and H. Weintraub, personal communication). For an unknown reason, the activity is not released from the nuclei of some rodent CNS and PNS cells unless somewhat rigorous nuclear extraction procedures are used. This was not the case for all other cells tested, in which the activity was easily extracted into simplified whole-cell protein preparations. The reason for this difference is not clear, although it may ultimately provide a clue to the biological function of the activity. Finally, the fact that all human CNS cells contain the activity supports the possibility that measles viruses may be subject to rapid mutations by means of the unwinding-modifying activity in CNS cells.

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