SHORT COMMUNICATION

Differences in the UV-Crosslinking Patterns of the Poliovirus 5'-Untranslated Region with Cell Proteins from Poliovirus-Susceptible and -Resistant Tissues

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The restricted tissue tropism observed in poliovirus infection is not governed solely by the expression of the poliovirus receptor (PVR) gene, but might be controlled at stages beyond virus entry, such as translation, replication, or assembly. Translation of poliovirus RNA by a cap-independent mechanism requires interactions of the 5'-untranslated region (5'UTR) with cell proteins. To determine whether the patterns of these interacting proteins differ in HeLa cells and permissive and nonpermissive tissues, UV-crosslinking assays using the poliovirus 5'UTR and tissue extracts from PVR transgenic mice were performed. The results indicate a correlation between the presence of a 97-kDa UV-crosslinked protein and permissivity to poliovirus infection. Acquired poliovirus susceptibility in in vitro-cultured kidney cells also correlates with the presence of a 97-kDa crosslinked band. The interaction of the 97-kDa protein from HeLa cells and mouse brain with the poliovirus 5'UTR is stable and specific. Whether the 97-kDa protein plays a role in poliovirus translation and tissue susceptibility remains to be determined.

Poliovirus is an acute disease of the central nervous system caused by poliovirus, a human enteric virus of the Picornaviridae family (1). Most polioviruses have a distinct species tropism and only infect primates, where infection is transmitted by the oral–fecal route (2). After ingestion, poliovirus replicates first in the oropharyngeal and intestinal mucosa. Virus is shed into the gut lumen, leading to virus in the feces, and a transient viremia occurs. Disseminated virus then replicates in extraneuronal tissues, such as skeletal muscle and brown adipose tissue, which may be important in maintaining a persisting viremia (3, 4). Transmission of virus into the central nervous system (CNS), which takes place in approximately 1% of infections, may occur via peripheral nerves or ganglia by axonal transport or through the blood–brain barrier (5, 6).

Poliovirus replication is restricted to few tissues in primates. It has been suggested that the cellular poliovirus receptor (PVR) is the major determining factor in cell and tissue susceptibility, since bypassing the receptor binding step by transfection with poliovirus RNA permits one cycle of replication in many receptor-negative mammalian cell types (7). However, in PVR transgenic mice (TgPVR), which express PVR in a wide range of tissues, replication of poliovirus is detected only at a few locations, including neurons of the brain and spinal cord, skeletal muscle, and occasionally brown adipose cells (8, 9). Although viral replication is not detected in a variety of other tissues from the same animals such as thymus, adrenal gland, intestine, and kidney, these tissues can bind poliovirus (10). Despite expression of PVR RNA and poliovirus binding sites, many TgPVR mouse tissues remain refractory to poliovirus infection, indicating that tissue tropism in this animal model is not governed solely by expression of the PVR gene (9).

The nature of the internal block to poliovirus infection in nonsusceptible TgPVR mouse tissues, and in several established human blood cell lines in which viral replication differs depending on the differentiation stage and cell lineage (11), has not been identified. It has been suggested that poliovirus replication in those cells and tissues might be controlled at stages beyond virus entry, such as translation, replication, or assembly (12). The possibility that poliovirus mRNA translates efficiently only in certain cell types is consistent with results which show that translation of poliovirus RNA in U-937 cells [a histiocytic cell line that contains a number of poliovirus receptors equal to that of HeLa cells (13)] is considerably lower.
than that in HeLa cells (14). The limiting translation factors in some translation systems such as wheat-germ extracts, Xenopus oocyte extracts, and reticulocyte lysate suggest that the restriction in poliovirus translation might contribute in part to the tissue-specific replication of poliovirus (15). On the other hand, after cultivation of kidney in vitro, these cells become permissive to poliovirus infection (9). The basis of the acquired susceptibility to poliovirus infection in these cells is unknown, but might involve the induction of factors required in some of the viral processes mentioned above (9).

Translation of poliovirus RNA, which is one of the first steps in poliovirus replication, occurs by internal ribosome entry (16). This process is believed to require the canonical set of translation factors as well as other cell proteins that interact with the IRES (internal ribosome entry site) within the 5' untranslated region (5'UTR) (17–22). To determine whether there are differences in the interactions of such factors with the 5'UTR in permissive and nonpermissive tissues, UV-crosslinking assays were performed. To produce linear templates for runoff transcription, plasmids pFL-64 containing the complete cDNA sequence of the poliovirus type 3 5'UTR was cleaved with EcoRV (nucleotide nt 730), and pPV-275 containing the cDNA sequence of 275 to 672 with Ball (nt 628) and PvuII (nt 488). Heterologous unlabeled RNA was obtained after transcription of Bluescript cleaved with PvuII (nt 977). Labeled RNAs were prepared by in vitro transcription of pFL-64 and pPV-275 in the presence of [α-32P]UTP as described (17). Transcription reaction mixtures were treated with DNase RQ1 (Promega) at 37°C for 15 min, and unincorporated nucleotides were removed by centrifugation in Sepharose G50 columns.

In vitro cultivation of transgenic mouse kidney was performed as previously described (9). S10 extracts from HeLa and permissive kidney cultures were prepared as described (18). To prepare S10 extracts from tissues, 5-week-old TgPVR mice were sacrificed, and to the complete 5'UTR RNA nt 1–730 (lane 1) and a sequence containing the cDNA sequence of the poliovirus type 3 5'UTR nt 1–730 (lane 1). A similar pattern of crosslinked proteins was observed when a shorter RNA (nt 275–628), which contains the major element that confers cap-independent translation to poliovirus mRNA (nt 320 to 620) (19) was used (Fig. 1A, lane 2). The 80-, 72-, and 68-kDa proteins crosslinked at higher levels with the complete 5'UTR, while the 130- and 57/60-kDa proteins crosslinked at lower levels compared to the complete 5'UTR RNA (Fig. 1A, lanes 1 and 2). The variation in the crosslinking of these proteins might result from different conformations adopted by both RNAs. This result indicates that the same proteins bind to the complete 5'UTR and to sequences between nt 275 and 628, a region that also confers cap-independent translation of a reporter gene (19, 20). The nt 275–628 RNA was therefore used for subsequent experiments.

To determine whether there are differences in the interactions of cell proteins from permissive and nonpermissive tissues with the 5'UTR, UV-crosslinking assays were performed with extracts prepared from different TgPVR mouse tissues. Five proteins of molecular weights 97, 80, 68, 40, and 38 kDa from brain extract were cross-linked with the 32P-labeled RNA probe (Fig. 1B, lane 2). A doublet of 57/60 kDa was also observed after overexposure of the membrane. Five crosslinked proteins of 97, 80, 57, 52, and 42 kDa were detected when muscle extract was used (Fig. 1B, lane 3), while proteins of 70, 52, and 42 kDa were detected when kidney extract was used (Fig. 1B, lane 5). When thymus extracts were used, crosslinked proteins of 68, 56/54, 52, and 42 kDa were detected (Fig. 1B, lane 6).
These results show that a 97-kDa protein crosslinked poorly or not at all when extracts from the nonpermissive tissues kidney and thymus were used (Fig. 1B, lanes 5 and 6) while the 97-kDa protein crosslinked at significantly higher levels when extracts from HeLa cells, brain, muscle, and kidney cell culture, all of which are permissive for poliovirus infection, were used (Fig. 1B, lanes 1, 2, 3, and 4, respectively). Our results suggest a correlation between the presence of a 97-kDa protein that can be UV-crosslinked to the poliovirus 5′UTR and permissivity of TgPVR tissues to poliovirus infection.

To provide information on the binding site of the 97-kDa protein within the poliovirus 5′UTR and its specificity, competition experiments with homologous and heterologous RNAs were performed in HeLa extracts (Fig. 2). A 50-fold molar excess of homologous RNA from nt 1–730 or nt 275–628 strongly reduced the levels of all crosslinked proteins (Fig. 2, lanes 2 and 3), but a 50-fold molar excess of heterologous unlabeled RNA was an inefficient competitor (Fig. 2, lane 5), demonstrating that the crosslinking of all the observed proteins, including the 97-kDa protein, with nt 275–628 of the poliovirus 5′UTR is specific. To more precisely locate the 97-kDa protein binding site, an RNA consisting of nt 275–488 was used as a competitor (Fig. 2, lane 4). This RNA strongly reduced crosslinking of the 70-, 68-, and 57/60-kDa proteins, suggesting that they bind to the nt 275–488 sequence. The interaction of the 97- and 52-kDa proteins was also reduced but to a lesser extent, suggesting that they interact with a sequence within nt 275–488 and may have additional binding sites within nt 488–628.

The stability of the complexes was examined by incubating HeLa cell extracts with different salt concentrations prior to UV-crosslinking. Labeled RNA from nt 275–628 was incubated with 30 μg of HeLa S10 extract in the presence of 0, 300, 600, and 900 mM KCl (Fig. 3A, lanes 1, 2, 3, and 4, respectively). The interaction of the 110-, 68-, and 38-kDa crosslinked proteins was strongly reduced in the presence of 300 mM KCl, while the 57/60-kDa protein was reduced but to a lesser extent. None of the other interactions were disrupted. At 600 mM KCl, the interaction of the 97-, 70-, and 57/60-kDa proteins was significantly reduced. The 52-kDa protein was detected up to 900 mM KCl, although at lower levels. These results indicate that the interaction of the 97-, 70-, 57/60-, and 52-kDa proteins with the 5′UTR is stable in 300 mM salt. Similar experiments were also conducted to determine whether the interaction of the 97-kDa protein from mouse brain was also salt resistant (Fig. 3B). The interaction of the 80- and 68-kDa proteins was strongly reduced in the presence of 300 mM KCl, while none of the other protein interactions were disrupted (Fig. 3B, lane 2). The 97- and 38-kDa proteins from mouse brain tissue interacted with the poliovirus 5′UTR at 300 mM KCl. At 600 mM KCl, the interaction of all crosslinked proteins was disrupted (Fig. 3B, lane 3). Although these results do not show that the 97-kDa crosslinked proteins from different tissues and from HeLa cells are the same protein, they clearly share some characteristics, such as molecular weight, the ability to crosslink with nt 275–628 of poliovirus RNA, specificity of binding, and stability in 300 mM salt.

Cell proteins of 100 and 110 kDa (p100 and p110) have been detected crosslinked to the poliovirus 5′UTR (21). A protein of 97 kDa, which is specifically crosslinked to the rhinovirus 5′UTR, has been found to act synergistically with another cell protein, pPTB, to stimulate capped-independent translation directed by the rhinovirus IRES (22). Because of the similarities in primary sequences and in secondary structures within the IRES elements of poliovirus and rhinovirus, it is possible that they have similar requirements for initiation factors (18). In support of this hypothesis, RNA consisting of nt 275–628 from the poliovirus 5′UTR inhibits complex formation with nt 343–636 from HRV14, suggesting that some of the same cellular factors, including a 97-kDa protein, are bound by the two different viral sequences (18). Since p97 has not yet been identified, it is not known whether the 97-kDa protein detected in our study is identical to p97 that inter-
acts with the HRV 5'UTR, or p100/p110 that interacts with the poliovirus IRES. Antibodies to the 97-kDa protein from HeLa cells and mouse brain are currently being prepared.

Our results demonstrate that a 97-kDa protein, which may have a role in IRES function, was UV-crosslinked to the 5'UTR from permissive but not from nonpermissive tissues in a specific and stable manner as demonstrated by salt resistance and competition experiments. Extracts from kidney and thymus, which are not sites of poliovirus replication, did not contain p97 that could be crosslinked to the poliovirus 5'UTR. In contrast, p97 was detected in brain, muscle, and mouse kidney cell cultures, all of which are susceptible to poliovirus infection. If the 97-kDa protein is required for functioning of the poliovirus IRES, then its presence or absence might determine whether poliovirus can or cannot translate, and hence replicate, in specific tissues. Poliovirus translation in tissues might also be regulated by other cell proteins, in a positive or negative manner, which have not been detected by the UV-crosslinking used in these experiments. Further experiments are required to determine the role of p97 in poliovirus IRES function and whether p97 and other cell proteins regulate tissue and cell susceptibility to poliovirus infection.

REFERENCES