Temporal lobe epilepsy (TLE) is a common seizure disorder, but the underlying molecular mechanisms are unknown. We reported previously that inactivation of the jerky gene in mice causes recurrent limbic seizures highly similar to TLE. Electrophysiological studies showed abnormal firing in hippocampal neurons in these mice, but it is not known how a deficiency in the Jerky protein leads to neuronal hyperexcitability. Here we show that Jerky is a brain-specific protein with a high expression level in neurons. Jerky binds mRNAs with high affinity, and it is a component of messenger ribonucleoprotein complexes in vivo. However, Jerky is not associated with ribosomes and actively translating mRNAs. These data suggest that Jerky may regulate mRNA use in neurons, and its deficiency could lead to perturbations in the regulated use of preexisting mRNAs.

Key words: Jerky; epilepsy; seizure; neuron; animal model; RNA binding protein; RNA–protein interaction; mRNA; translation

Temporal lobe epilepsy (TLE) is the most common seizure disorder in adults. The typical form of TLE is often severe and is associated with hippocampal atrophy. Members of families with strong history of febrile seizures have an increased susceptibility to TLE, but genetic factors are not known to play a major role in the development of TLE (Falconer et al., 1964). Autosomal dominant TLE and autosomal dominant lateral TLE are hereditary and nonlesional forms of TLE with a relatively benign disease course (Berkovic et al., 1994, 1996; Saenz et al., 1999; Gambardella et al., 2000; Ikeda et al., 2000; Picard et al., 2000). Genetic analysis found a linkage to chromosome 10q for autosomal dominant lateral TLE, but these studies did not lead to the cloning of a gene (Poza et al., 1999).

We reported previously that “jerky” mice have recurrent limbic seizures (Toth et al., 1995; Donovan et al., 1997) that are highly reminiscent of those seen in familiar TLE (Berkovic et al., 1994, 1996; Cendes et al., 1998; Saenz et al., 1999; Gambardella et al., 2000; Ikeda et al., 2000; Picard et al., 2000). First, the inheritance of the seizure disorder is autosomal dominant in both human and mouse. Second, penetrance of seizures in both human and mouse is partial. Third, the symptoms usually disappear spontaneously by age in both species.

Seizures in “jerky” mice are caused by the lack of the jerky gene (Toth et al., 1995; Donovan et al., 1997). The mouse Jerky protein is encoded by a single exon (Toth et al., 1995), consisting of 557 amino acid (aa) residues (GenBank accession number NM_008415). The similarity of Jerky to DNA transposons of the TC1/Pogo/Tigger family has been recognized (Toth et al., 1995). Although most DNA transposon copies are nonfunctional, transposon-like genes encoding proteins such as jerky and CENP-B (centromere binding protein-B) indicate that DNA transposons may have become fixed in the genome as functional genes (International Human Genome Sequencing Consortium, 2001). The human homolog of jerky (JRK/JHS) has also been cloned (Morita et al., 1998). A de novo nonconservative mutation to a potential glycosylation site in JRK/JHS has been described recently in an epileptic patient (Moore et al., 2001).

Based on its similarity to CENP-B, a possible nuclear regulatory function has been hypothesized for Jerky (Toth et al., 1995). CENP-B is an abundant nuclear protein localized on the centromere (Earnshaw and Rothfield, 1985; Earnshaw et al., 1987; Cooke et al., 1990; Sullivan and Glass, 1991) and is implicated in the assembly of centromeric DNA (Muro et al., 1992; Yoda et al., 1992). In contrast to CENP-B, Jerky is not exclusively localized to the nucleus (see Results) and may have a cytoplasmic function, in addition to a CENP-B-like nuclear role. Here we show that cytoplasmic Jerky is a constituent of translationally inactive messenger ribonucleoprotein (mRNP) particles in brain. Jerky may retain and mask mRNAs within mRNPs, and Jerky deficiency could lead to perturbations in the regulated use of preexisting mRNAs in neurons.

MATERIALS AND METHODS

Generation of glutathione S-transferase-Jerky fusion protein and polyclonal antibodies against Jerky. Recent sequence analysis of jerky mRNAs from brain indicated that the open reading frame (Toth et al., 1995) can be extended by 48 aa (sequence has been deposited to GenBank, accession number NM_008415), resulting in a protein of 557 residues. A glutathione S-transferase (GST)-tagged Jerky construct was generated by cloning a 1.65 kb cDNA, corresponding to full-length Jerky (1–557) in frame with an N-terminal GST tag. We first produced a jerky DNA fragment flanked by EcoRI sites by PCR using a 5′ primer (GGGAATTCGCAT GGCCTTCGAA GCAGGCTGCA) encoding an EcoRI site and the first

Wencheng Liu,1 Jeremy Seto,2 Gerald Donovan,3 and Miklos Toth1,2

1Department of Pharmacology, Weill Medical College and 2Graduate Program in Neuroscience, Weill Graduate School of Medical Sciences, Cornell University, New York, New York 10021, and 3Gene Expression, Progenics Pharmaceuticals, Tarrytown, New York 10591

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seven amino acids of Jerky, and a 3′ primer (C CGAATTCGTTGTACCTGCGAGTGAAGA) corresponding to an EcoRI site and the last seven amino acids of Jerky. Then, the EcoRI fragment was cloned into the expression vector pGEX-6P2 (Amersham Pharmacia Biotech, Piscataway, NJ). This plasmid was transformed into BL21 Escherichia coli, and protein expression was induced by 1 mM isopropyl-β-D-thiogalactopyranoside. GST-Jerky was purified as described by Guan and Dixon (1991).

To produce antibodies in rabbits, 4 mg of GST-Jerky was produced and sent to Strategic Biosolutions (Ramona, CA). These antibodies were affinity-purified with Jerky covalently bound to Sepharose beads (Amersham Pharmacia Biotech). The antibody recognized a 62 kDa protein in brain (see Fig. 1A). The size of this immunoreactive band is in a good agreement with the predicted molecular mass of 62.3 kDa of the Jerky protein. The antibody also recognized Jerky expressed in HEK 293 cells as a protein tagged with Flag (see Fig. 3C) and V5 (data not shown) epitope.

**RNA labeling.** Labeled mRNA fragments were obtained by using the RNA SELEX procedure of Dobbelstein and Shenk (1995), modified in our laboratory. Briefly, first-strand cDNA synthesis was initiated from mouse brain mRNA (Clontech, Palo Alto, CA) by using a primer with a random octamer at its 3′ end (SELEX 1, 5′-AGCAACAGCAA-GACTACGAGTGGANNNNNNNN3′). Second strand was generated by using a priming-containing random hexamer as a second strand primer (SELEX 2, 5′-GGGAGCTCAGAATAACGCTCACAGGCTCAA-NNNN3′). The second strand was PCR amplified with SELEX 1 primer without the random sequence (SELEX 1b, 5′-AGCAACAGCAAGACTACGAGTGGANNNNNNNN3′) and SELEX 2 primer without the random hexamer but with a 17-mer promoter sequence at the 5′ end (SELEX 2-7, 5′-GACAGCATTAATACGCTCTATATGGAGCTCAGATATAAAGCTCAA3′). The resulting PCR products were used to synthesize RNA probes by 17-mer polymerase in the presence of [γ-32P]ATP. Poly(A+)-purified RNA was isolated by centrifugation at 10,000 × g for 15 min. Supernatants were then collected and loaded onto 5–25% sucrose gradients, followed by centrifugation at 39,000 rpm for 100 min at 4°C. Twenty-three fractions were collected from each gradient. Each fraction was divided into two equal portions, one for RNA analysis and the other for protein analysis. For the RNA analysis, samples were phenol–chloroform extracted once, and the RNA was ethanol precipitated. The RNA was electrophoresed in a formaldehyde agarose gel, and the rRNA was visualized with ethidium bromide. For protein analysis, samples were first concentrated using Ultrafree-0.5 concentrators (Millipore). One-third of the concentrated sample was loaded onto 12.5% acrylamide gels and transferred onto PVDF membranes. Western analysis was performed as described above.

**Isolation of mRNA complexes by oligo-dT beads.** mRNA complexes were isolated as described by Feng et al. (1997). One mouse brain was homogenized in 1 ml of lysis buffer containing 20 mM Tris, pH 7.5, 100 mM KCl, 5 mM MgCl2, 0.5% NP-40, 200 U of rRNasin (Promega), and Complete Protease inhibitor cocktail (Roche). Post-mortem supernatants were isolated by centrifugation at 10,000 × g for 10 min. Aliquots (200 μl) were applied to 2 μl oligo(dT) beads (Millipore). Samples were incubated in binding buffer in the presence of either competitive poly(A+) (40 pmol), rRNaseA (30 U), and RNaseT1 (30 U), or ddH2O. After a 15 min incubation at 37°C, samples were centrifuged at 10,000 × g and washed three times in 500 μl of low-salt buffer. Samples were finally thrombo-eluted with preheated elution buffer. Aliquots, representing loaded, flow-through, combined washes, and eluate, respectively, were used for Western analysis.

**Isolation of Jerky-containing cytoplasmic complexes by immunoprecipitation.** Pull-down of Jerky complexes was performed essentially as described by Ceman et al. (1999). Briefly, 1 × 105 HEK 293T cells were transfected with a Flag-tagged Jerky construct. After 24 hr, cells were harvested and washed three times with 10 vol of PBS. Cells were then lysed mildly with lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 30 mM EDTA, and 0.5% Triton X-100 containing 1 μg/ml aprotinin, 40 μg/ml leupeptin, 1 mM DTT, and 30 U of RNasin (Promega), 0.5% Triton X-100) containing Complete Protease inhibitor cocktail for 45 min on ice. Nuclei were pelleted at 3000 × g for 10 min at 4°C. The cytoplasmic supernatant was precleared for 2 hr with 250 μl of Flag peptide (250 μg/ml)–anti-Flag antibody complex immobilized on agarose bead (Sigma). After centrifugation, the supernatant was immunoprecipitated with 200 μl of anti-Flag agarose bead for 3 hr. The immunoprecipitated material was recovered by centrifugation and washed twice with 1 ml of lysis buffer for 15 min at 4°C. Then, the material was washed again with lysis buffer containing 50 μl of RNase-free DNase I (Roche) in the presence of 200 U of RNasin (Promega). Finally, the immunoprecipitated material was washed with lysis buffer containing 200 U of RNasin and pelleted by gravity overnight. Protein–RNA complexes were eluted with a mixture of 150 μl of lysis buffer and 150 μl of Flag peptide (5 mg/ml) for 45 min. The supernatant containing the eluted complexes was recovered by centrifugation. Elution was continued with 500 μl of lysis buffer for 45 min. The eluates were combined, and 10% of this fraction was used for protein analysis. To recover the RNA content from the complexes, the rest of the sample was incubated with lysis buffer containing 100 μg of proteinase K (Life Technologies) and 200 U of RNasin at 37° for 15 min. The sample was then phenol–chloroform extracted, and the RNA was ethanol precipitated.

**Assays to test Jerky–RNA interaction.** Full-length GST-Jerky (0.5 μg) in RNA binding buffer (150 mM LiCl, 10 mM Tris, pH 7.5, and 1 mM EDTA) was bound onto poly(A), poly(U), poly(C), or poly(G) agarose columns (Sigma). The columns were washed with 20 column volume of RNA binding buffer. Bound Jerky was eluted twice with 500 μl of elution buffer (1 M NaCl, 10 mM Tris, pH 7.5, 1 mM EDTA, and 0.1% Triton X-100). Eluates were concentrated with Centricon concentrators (Millipore). Concentrated samples were subjected to SDS-PAGE and Western analysis using anti-Jerky antibodies as described above.

In other experiments, mouse brain mRNA (Clontech) was incubated with GST-Jerky immobilized on agarose beads for 20 min in RNA
Binding buffer at room temperature. Beads were washed twice with RNA binding buffer for 15 min. Bound mRNA was recovered by phenol-chloroform extraction, followed by ethanol precipitation, and was used for first-strand cDNA synthesis with oligo-dT primer in the present of [α-32P]dCTP. Also, labeled mRNA was interacted with Jerky immobilized on nitrocellulose membrane. GST-Jerky (0.2 μg) was applied per well onto a 0.45 μm nitrocellulose membrane in a slot blot system (BA85; Schleicher & Schuell, Keene, NH). Filters were incubated with 32P-labeled mRNA fragments (2 × 10^6 cpm/ml) in binding buffer (50 mM NaCl, 10 mM Tris, pH 7.5, 10 mM MgCl₂, 1 mM EDTA, 1 mM DTT, and 0.02% BSA) for 2 hr at 25°C. After incubation, filters were washed twice with binding buffer for 30 min each. Bound radioactivity was visualized by autoradiography.

Filter binding assays were performed as described by Hall and Kranz (1999). First, mouse brain mRNAs were preselected with GST-Jerky immobilized on glutathione beads, and the mRNA was labeled by the modified RNA SELEX procedure as described above. Labeled mRNA fragments were incubated with serial dilutions of purified GST-Jerky in a buffer containing 50 mM LiCl, 10 mM MgCl₂, 10 mM Tris, pH 7.5, 20 μg/ml BSA, and 1 mM EDTA for 30 min at room temperature. RNA-protein complexes were then filtered through a sandwich of a 0.45 μm nitrocellulose membrane (BA85; Schleicher & Schuell) on the top and a nylon membrane (Nytran; Schleicher & Schuell) on the bottom. Bound and unbound radioactivity on the nitrocellulose and nylon filters, respectively, was measured by a STORM 860 phosphorimage analyzer (Molecular Dynamics, Sunnyvale, CA), and K₀ was calculated as described by Hall and Kranz (1999).

RESULTS
The Jerky protein is exclusively expressed in brain
Antibody produced in rabbits against recombinant Jerky (see Materials and Methods) was used to determine the tissue-specific expression of Jerky in mice. Tissue distribution of Jerky was studied in both nuclear and cytoplasmic fractions of the tissues. Western blots showed a 62 kDa immunoreactive band in brain in both the cytoplasmic and nuclear fractions (Fig. 1A). The size of this immunoreactive band is in a good agreement with the predicted molecular mass of 62.5 kDa of the Jerky protein (GenBank accession number NP_032441). The antibody also recognized Jerky in rat brain (data not shown). Heart, liver, kidney, ovary, stomach, skeletal muscle, spleen, and pancreas showed no detectable immunoreactivity. In testis and lung, a weak immunoreactive band with an ~58 kDa mass was seen occasionally. It is not clear whether these represent a Jerky isoform or a cross-reactivity of the antibody in these tissues.

Figure 1. Expression of Jerky in mouse tissues and rat hippocampal cultures. A, Western analysis of cytoplasmic and nuclear fractions from mouse tissues by Jerky antibody. Br, Brain; H, heart; Lu, lung; Li, liver; K, kidney; T, testes; O, ovary; St, stomach; Sk, skeletal muscle; Sp, spleen; Pn, pancreas. B, Double-immunostaining of neuronal cultures by anti-Jerky (left panel; green on the merged image on the right) and anti-β-tubulin (middle panel; red on the merged image on the right) antibodies. Arrows and arrowheads indicate neuronal (β-tubulin-positive) and non-neuronal (β-tubulin-negative) cells, respectively.

Jerky is highly expressed in primary neurons
Jerky was immunolocalized with the polyclonal antibody in 1-week-old primary rat hippocampal cultures (Fig. 1B). Neurons were identified by staining with a β-tubulin antibody. Jerky immunostaining was strong in neurons and appeared to be granular. Consistent with the Western analysis shown in Figure 1A, Jerky was present in both the nucleus and cytoplasm (Fig. 1B). Immunostaining was also seen in the nucleus and the cytoplasm of non-neuronal (β-tubulin-negative) cells, but it was considerably weaker than in neurons.

Figure 2. Jerky comigrates with mRNP in 5–25% sucrose gradient. A, Jerky (top panel) and P0 (middle panel) immunoreactivity in gradient fractions derived from mouse brain cytoplasmic extracts. The bottom panel shows the distribution of RNA in the fractions. Analysis of the top 14 fractions of the total of 23 fractions is displayed. B, Jerky (top panel) and P0 (middle panel) immunoreactivity in gradient fractions derived from RNase-digested cytoplasmic extract. No intact 18 and 28S rRNAs were seen after RNase treatment (data not shown).

Jerky comigrates with mRNP complexes in sucrose density gradient
Because of the similarities between Jerky and the heterochromatin-associated CENP-B (see introductory remarks), the presence of Jerky in nuclear brain fraction and in the nuclei of neurons was not surprising. However, the presence of Jerky immunoreactivity in cytoplasmic brain fractions and in the cytoplasm of primary neurons was unexpected, and we further studied this unique feature of Jerky. Specifically, a possible association of Jerky with macromolecular complexes in brain cytoplasmic lysates was investigated by sedimentation analysis in 5–25% sucrose gradients (Fig. 2A, top panel). A significant portion of Jerky entered the gradient (fractions 1 and 2 represent soluble Jerky remaining on the top of the gradient) and was found in complexes with a sedimentation up to the 80S monosomes. The position of monosomes in the gradient was indicated by the presence of the 18 and 28S rRNAs, as well as the large ribosome subunit protein P0 (Fig. 2A, middle and bottom panels). Jerky was not detected in fractions containing polysomes (fractions 15–23; not shown in figure). Jerky was not found in association with polysomes either when polysomes were better resolved on 25–47% gradients (data not shown). A small fraction of the Jerky protein (Fig. 2A, top panel, fraction 7) cosedimented with the 40S small ribosomal subunit (indicated by the presence of the 18S ribosomal RNA in Fig. 2A, bottom panel). However, Jerky is probably not associated with the 40S ribosomal subunit because immunoprecipitation of
these complexes via S3 (a small ribosomal subunit protein) did not pull down Jerky (data not shown). We concluded that Jerky was positioned in the gradient in fractions that contain complexes characterized by a sedimentation of <8S. Typically, these fractions contain translationally inactive mRNP complexes but also other macromolecular complexes, such as proteasomes. We also concluded that Jerky is not associated with ribosomes.

Enzymatic treatment of brain cytoplasmic lysates with RNaseA before sedimentation resulted in a shift toward lower sedimentation of Jerky (Fig. 2, compare A, B), indicating that RNA is present and has a vital structural role in Jerky-containing macromolecular complexes. The RNaseA-induced leftward shift (2 fractions, representing 1 ml of the 11.5 ml gradient) of Jerky immunoreactivity was reproducible in three independent experiments. The level of P0 protein was increased in monosomal fractions in RNaseA-treated samples, indicating that polysomes were also disrupted by the enzyme (Fig. 1B, middle panel). Also, P0 protein appeared in soluble and low sedimentation fractions, indicating some disintegration of the monosomes and/or ribosome subunits.

Jerky is present in mRNA-containing cytoplasmic complexes

To further test the presence of Jerky in mRNPs, these particles were captured from brain extract (Fig. 3A). Although with low efficiency, mRNPs can be isolated by oligo-dT polystyrene latex beads (Fig. 2A). Preincubation of oligo-dT beads with poly(A^-) prevented the binding of Jerky-containing cytoplasmic complexes to the beads (Fig. 3A, lane 8), demonstrating that Jerky was captured via mRNA and not by another interaction. As expected, Jerky was present in the eluate when the brain lysate was pretreated with RNaseA and RNaseT1 (Fig. 3A, lane 16). These data indicate that Jerky is present in mRNP particles in mouse brain.

We also determined whether mRNAs can be detected in cytoplasmic Jerky complexes. HEK 293T cells were transfected with plasmids expressing Flag-tagged Jerky. Similar to the subcellular compartmentalization of Jerky in brain (Fig. 1A, Flag-Jerky showed an approximately equal distribution in 293T cytoplasmic and nuclear extracts (data not shown). As a control, an expression vector containing the Flag tag alone was used. Importantly, Jerky was not expressed in an exceedingly large amount in HEK 293T cells that could produce artificial interactions, because 16 μg of brain cytoplasmic extract contained more Jerky than 40 μg (normalized to the 40% transfection efficiency) of HEK 293T cell extract (Fig. 3B). Indeed, HEK 293T cells expressed Jerky at a relatively low level, especially when considering that neurons with high Jerky expression represent only a fraction of cells in brain. After transfection of HEK 293T cells, Flag-containing complexes were pulled down from cytoplasmic extracts by a monoclonal anti-Flag antibody. The presence of Jerky in Flag immunocomplexes derived from Flag-Jerky but not Flag-expressing cells was verified by polyclonal Jerky antibody (Fig. 3C). The mRNA content of these complexes was tested by reverse transcription initiated by oligo-dT primer in the presence of labeled dATP. As Figure 3D shows, mRNA was detected in Flag-Jerky but not in Flag immunocomplexes.

Jerky can directly bind mRNAs

Although the presence of Jerky in mRNP particles indicated an association between Jerky and mRNA, it was not known whether this interaction is direct or indirect. RNA binding of Jerky was assessed by incubating mouse brain mRNA with Jerky immobilized on agarose beads (Fig. 4A). Recombinant and purified Jerky (GST-Jerky) was used in these experiments to ascertain that RNA binding does not occur via contaminating proteins. Bound mRNA was detected by reverse transcription. As Figure 4A shows, labeled cDNA was obtained from Jerky but not control
of them performed in duplicate. GST-Jerky and labeled mouse brain mRNA fragments. GST-CRBP is the GST-Jerky preparation after PAGE is displayed on the Autoradiography of a representative set of (A)ous experiment (Fig. 4poly(G), and poly(U)) were used (Fig. 4poly(A), poly(C), poly(G), and poly(U)) were used (Fig. 4B). Similar to the previous experiment (Fig. 4A), mRNAs were retained on immobilized Jerky, suggesting again that brain mRNA can directly bind to Jerky. Homopolymers, except poly(C), were also retained by Jerky. CRBP, which was immobilized in comparable amounts as Jerky on the filters (Fig. 4B, Anti-GST), retained no RNA (Fig. 4B, right lanes).

Finally, we performed the reverse experiment when Jerky was in solution and the RNA was immobilized. Specifically, GST-Jerky was incubated with each of the four RNA homopolymers [poly(A), poly(C), poly(G), and poly(U)] covalently linked to agarose (Fig. 4C). At least 15% of Jerky was retained on poly(A) and poly(G) and somewhat more on poly(U) (15% of the eluates contained equal amounts or more Jerky than 1/10 of the loads). Binding to poly(C) was not detected. These data indicated that the affinity of Jerky to RNA homopolymers is U > G = A (Fig. 4C). GST-CRBP, used as a control, was not retained by poly(G) (Fig. 4C, bottom panel).

Although these experiments suggested that Jerky can directly bind mRNA, the tests used were not suitable to determine whether the affinity of the binding is high enough to be considered physiologically relevant. To address this question, filter binding assays were performed with labeled mRNA fragments and recombinant Jerky. Increasing amounts of GST-Jerky were incubated with constant amounts of 32P-labeled mRNA fragments in solution and filtered through a sandwich consisting of a nitrocellulose membrane that retained RNA–protein complexes [bound fraction (B)] and a nylon membrane that retained unbound [free (F)] RNA (Fig. 5, inset at top left). The purity of the Jerky preparation was assessed by staining the gel with Coomassie blue (Fig. 5, inset at bottom right), which showed an ~90 kDa protein (62 kDa Jerky fused to the 26 kDa GST) but not other proteins. Measuring both bound and free radioactivity allowed us to calculate binding affinity. As Figure 5 shows, the Kd of the binding by Jerky was ~5 nM, representing a high-affinity binding. This is comparable with the binding of other RNA binding proteins with known biological function in RNA processing, such as HIV-1 tat (Dingwall et al., 1990), HuR (Nabors et al., 2001), Sam68 (Lin et al., 1997), and many others.

**DISCUSSION**

**Jerky is a brain-specific protein, with a preferential expression in neurons**

In agreement with the neurological phenotype of the “jerky” mice, the Jerky protein is expressed in the brain. The brain-specific expression of the Jerky protein was unexpected because we found previously that, besides the brain, jerky mRNA was also detectable in various other mouse tissues by reverse transcription-PCR (Donovan et al., 1997). Northern analysis with poly(A+) mRNA confirmed the widespread expression of jerky mRNA in mouse tissues (data not shown). These data suggest that the brain-specific expression of the Jerky protein is controlled at the translational level. Additional studies will be needed to clarify the nature of this regulation. Expression of the Jerky protein in brain is primarily attributable to neuronal expression, although a lower expression was also detectable in non-neuronal cells. No other tissue expressed the 62 kDa Jerky protein in detectable amount, suggesting that the function(s) of the protein is limited to the nervous system.

Based on sequence similarity between Jerky and the heterochromatin-associated CENP-B, Jerky was expected to be an exclusively nuclear protein. Instead, we found that Jerky is approximately equally distributed between the nucleus and cytoplasm in hippocampal neurons. A similar distribution was found in HEK 293 cells expressing Flag-Jerky and V5-Jerky (data not shown). The presence of Jerky in the cytoplasm was not attributable to leakage from the nucleus during the immunostaining procedure because a nucleocytoplasmic distribution was also seen in vivo in GFP-Jerky-expressing HEK 293 cells (data not shown). Furthermore, Jerky showed a similar compartmentalization in brain in fractionation studies whether in the presence or absence of detergents. These data raised the possibility that Jerky may have a CENP-B-like function in the nucleus and an entirely new function in the cytoplasm.

CENP-B is believed to play a role in mitosis and/or meiosis. However, deletion of CENP-B in mice causes no apparent defect in mitosis and meiosis. Phenotypically, a moderate impairment in growth rate and disturbances in sexual functions have been found on some but not other genetic backgrounds (Hudson et al., 1998; Kapoor et al., 1998; Perez-Castro et al., 1998; Fowler et al. 2000). The mild phenotype of CENP-B knock-out mice is surprising because deletion of other centromeric proteins, such as Cenpa and Incenp, leads to embryonic lethality (Cutts et al., 1999; Howman et al., 2000). Interestingly, homozygote Jerky-deficient mice also show growth retardation and sexual dysfunction. These data would also be consistent with the idea of an overlapping CENP-B and nuclear Jerky function. A deficiency in Jerky, however, results in recurrent seizures, a unique phenotype not noticed in CENP-B knock-out mice. Moreover, this phenotype is related to a dosage-dependent (haplo-insufficient) function of Jerky because heterozygotes already display seizures. We reasoned that, whereas a deficiency in nuclear Jerky may be effectively compensated by CENP-B, an uncompensated loss of cytoplasmic Jerky could result in seizures. Although a role for nuclear
Jerky cannot be excluded in seizure induction, we first focused on the role of Jerky in the cytoplasm.

**Jerky is part of mRNP particles**

mRNAs in cells are either translationally active or inactive. Whereas translationally active mRNAs are associated with ribosomes, translationally inactive mRNAs are found in mRNPs. mRNP particles have a slower sedimentation than the 80S monosomes in sucrose gradients and are believed to represent stored mRNA or mRNA in transit to the ribosome. Several lines of evidence indicate that Jerky is part of mRNPs in brain. First, Jerky-containing macromolecular complexes cosediment with mRNP particles in sucrose gradients. Second, RNase treatment disrupts the integrity of these Jerky-containing particles. Third, mRNP complexes captured on oligo-dT beads contain Jerky. Together, these data indicate that Jerky is associated with mRNP particles containing translationally inactive mRNAs.

Although these data indicated the coexistence of mRNAs and Jerky in mRNPs, it was not known whether Jerky is directly involved in mRNA binding within these particles. Various in vitro binding assays with purified recombinant Jerky demonstrated that Jerky can bind mRNAs, as well as RNA homopolymers, with the exception of poly(C). Additional filter binding experiments with Jerky-preselected mRNAs showed a high-affinity interaction between Jerky and mRNA, suggesting that this interaction can be biologically relevant. The low nanomolar binding affinity of Jerky for selected mRNAs is comparable with or exceeds the binding affinity of known RNA binding proteins, such as HIV-1 tat protein, HuR, and Sam68, which associates with a subset of the global mRNA population and that have some sequence preference but no unique mRNA sequence recognition. Consistent with these data, Jerky has a high affinity to poly(U) and somewhat less to poly(G) and poly(A). Domain searches revealed no known RNA binding domain(s) involved in mRNA binding. Jerky may be a “group specific” mRNA binding protein (Keene, 2001) that associates with a subset of the global mRNA population and that have some sequence preference but not unique mRNA sequence recognition. Consistent with these data, Jerky has a high affinity to poly(U) and somewhat less to poly(G) and poly(A). Most domains revealed no known RNA binding domain motif within Jerky, and studies are under way to map the Jerky domain(s) involved in mRNA binding.

In summary, we describe a novel function for Jerky, a protein deficient in an animal model of inherited TLE. Specifically, Jerky is associated with translationally inactive mRNAs in the cytoplasm of neurons, and we hypothesize that a deficiency in Jerky leads to perturbations in the use of a currently unknown set of mRNAs. Even small changes in the use of mRNAs encoding channel and receptor proteins may result in hyperexcitability and seizures. Alternatively, mRNAs of developmentally important genes may be perturbed in Jerky-deficient mice resulting in the formation of intrinsically hyperexcitatable neuronal networks. Indeed, it has been shown that alterations in gene expression in the hippocampus precede and accompany the manifestation of seizures in human and animal models of TLE (Blumcke et al., 2000; Murray et al. 2000; Brooks-Kayal et al., 2001). Data described here implicate that perturbations in the use of a subset of mRNAs in neurons may be a disease mechanism in focal epilepsies.

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