Alternative Splicing of Staufen2 Creates the Nuclear Export Signal for CRM1 (Exportin 1)*

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Mammalian Staufen2 (Stau2), a brain-specific double-stranded RNA-binding protein, is involved in the localization of mRNA in neurons. To gain insights into the function of Stau2, the subcellular localization of Stau2 isoforms fused to the green fluorescence protein was examined. Fluorescence microscopic analysis showed that Stau2 functions as a nucleocytoplasmic shuttle protein. The nuclear export of the 62-kDa isoform of Stau2 (Stau262) is mediated by the double-stranded RNA-binding domain 3 (RBDD3) because a mutation to RBDD3 led to nuclear accumulation. On the other hand, the shorter isoform of Stau2, Stau259, is exported from the nucleus by two distinct pathways, one of which is RBDD3-mediated and the other of which is CRM1 (exportin 1)-dependent. The nuclear export signal recognized by CRM1 was found to be located in the N-terminal region of Stau259. These results suggest that Stau2 may carry a variety of RNAs out of the nucleus, using the two export pathways. The present study addresses the issue of why plural Stau2 isoforms are expressed in neurons.

The localization of mRNA to defined subcellular regions enables the spatial and temporal control of gene expression (1–4). In neurons, the dendritic transport of certain mRNAs and the subsequent local synthesis of proteins are thought to play a role in neuronal plasticity. Mammalian Staufen2 (Stau2),1 a dsRNA-binding protein, was shown to be a homolog of Droshophila melanogaster Staufen (dmStau) (5–7). It has been shown that dmStau anchors bicoid mRNA at the anterior of the oocyte and oskar mRNA to the posterior during early development (8, 9). Mammalian Stau2 is mainly expressed in the brain and is involved in mRNA transport in neurons (6, 7). Stau2 exists in the somatodendritic compartment of cultured hippocampal neurons. In dendrites, Stau2 associates with RNA granules that contain other RNA-binding proteins, ribosomal subunits, translation factors, and motor proteins (10, 11). RNA granules are transported in dendrites via microtubules (12). Therefore, RNA granules are thought to play an important role in the targeting of RNA in neurons, functioning as cellular trafficking machinery (13), although the formation process by which this macromolecular complex is formed is currently unknown. In addition, it is known that Stau2 has at least three splicing variants with molecular masses of 62, 59, and 52 kDa (7). However, the issue of whether these isoforms are functionally different has not been resolved.

Because of this, the questions arise as to why such Stau2 isoforms are present in cells and whether each isoform functions divergently. To answer these questions, we investigated the intracellular dynamic behavior of two isoforms of Stau2. In this report, we show that Stau2 functions as a nucleocytoplasmic shuttle protein. In addition, we demonstrate that these two isoforms are distinctly exported from the nucleus, suggesting that each isoform may carry different sets of RNAs out of the nucleus.

EXPERIMENTAL PROCEDURES

Cloning of Rat Staufen229—Using information on the expressed sequence tags, the rat Staufen229 was cloned from a rat brain cDNA library (Clontech) by PCR using the following primers: forward, 5′-C CGGACATGCTTCCAGATAATCAGTGTTTTCTGTCG-3′, and reverse, 5′-ATGGATCCTAGATGACCGACTTTGATTTCTTGCAGTCC-3′. The PCR product was digested with BamHI and cloned into the pEGFP-C1 vector (Clontech), and sequenced.

Constructions—Full-length rat Stau229 was cloned from a rat brain cDNA library by PCR using the following primers: forward, 5′-C CGGAT TTCTCTGAATATGAACGTTGTTTGAAATG-3′, and reverse, 5′-GTGGATCCAGCTTCCTCTCTAGAGCC-3′. The PCR product was digested with BamHI and cloned into the pEGFP-C1 vector. Using the following primers: forward, 5′-CGGAAATTCTTCCTCTGATGCT-3′, and reverse, 5′-TACGATCTCAGCGCTTCCTCTCTTGTGCT-3′, the PCR product was digested with BamHI and cloned into the pEGFP-C2 vector. The deletion mutant Stau229-(1–242) was constructed from Stau229-pEGFP-C1 by digestion with HpaI and EcoRI, and the fragment was cloned into the pEGFP-C2 vector. A fragment of Stau229-(1–242) was cloned into the pEGFP-C1 vector. The deletion mutant Stau229-(1–242) was constructed from Stau229-pEGFP-C1 by cutting out the fragments of XmaI-XmaI and ligation into the pEGFP-C1 vector. Stau229-(1–137) was constructed from Stau229-pEGFP-C1 by digestion with HpaI and EcoRI, and the larger fragment was ligated into the pEGFP-C2 vector. A fragment of Stau229-(1–242) was cloned into the pEGFP-C1 vector. The deletion mutant Stau229-(1–242) was constructed from Stau229-pEGFP-C1 by cutting out the fragments of EcoRI-BamHI and ligation into the pEGFP-C1 vector. Stau229-(1–242) was constructed from Stau229-pEGFP-C1 by digestion with HpaI and EcoRI, and the larger fragment was ligated into the pEGFP-C2 vector. A fragment of Stau229-(1–242) was cloned into the pEGFP-C1 vector. The deletion mutant Stau229-(1–242) was constructed from Stau229-pEGFP-C1 by cutting out the fragments of EcoRI-BamHI and ligation into the pEGFP-C2 vector.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank at EBI Data Bank with accession number(s) AY684759 and AY684790.

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‡These abbreviations are: Stau2, Staufen2; dsRNA, double-stranded RNA; GFP, green fluorescence protein; EGFP, enhanced GFP; RBDD, double-stranded RNA-binding domain; NES, nuclear export signal; MBP, maltose-binding protein; LMB, leptomycin B; PBS, phosphate-buffered saline.

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which the HindIII site had been previously cut and blunted. Stau2α × 2× (318–412) was constructed from Stau2α × 2× (318–571)–pEGFP-C1 by digestion with BamHI and EcoRI, and the larger fragment was blunted and self-ligated. Stau2α × 2× (318–571) was constructed from Stau2α × 2× (318–571)–pEGFP-C1 by digestion with EcoRI and BspEI, and the larger fragment was blunted and self-ligated. Stau2α × 2× (1–105) was constructed from Stau2α × 2× (318–571)–pEGFP-C1 by digestion with EcoRI and BspEI, and the larger fragment was blunted and self-ligated. The amino acid substitution mutants were created using the QuikChange site-directed mutagenesis kit (Stratagene). Stau2α × 2× RBD3* and Stau2α × 2× RBD3* were constructed from Stau2α × 2×–pEGFP-C1 and Stau2α × 2×–pEGFP-C1, respectively, using the following primers: forward, 5′-CCACATATGGAAGACCGGTGTTACCACCGGCGTTGC-3′, and reverse, 5′-GCACCCGGTTACCCGGGTGTC-3′; and forward, 5′-CTCACATATGAAGAGAGCGCTCTTCATATGTGG-3′, and reverse, 5′-CGACACCCGGGTAACGTGCTCACCGACAAAAGC-3′.

Fig. 1. Subcellular localization of Stau2 deletion mutants. A, the Stau2 deletion mutants prepared are listed. Numbers denote the amino acid position relative to the N terminus of rat Stau2α × 2×, and the gray boxes indicate the conserved dsRNA-binding domains. These mutants were fused to GFP and transiently transfected into HeLa cells. The dominant subcellular localization of these mutants is indicated on the right as follows: C, cytoplasm; N, nucleus. B, subcellular localization of these mutants in transiently transfected HeLa cells. GFP fusion proteins were visualized using a Zeiss Axiovert 200 fluorescent microscope. Full, full length; Scale bar, 10 μm.

Cell Cultures and Transfection—HeLa cells were incubated in Dulbecco’s modified Eagle’s medium (Sigma) supplemented with 10% heat-inactivated fetal bovine serum at 37 °C in 5% CO₂ atmosphere. Expression vectors were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol. At 24 h after transfection, neurons were fixed with 4% paraformaldehyde in PBS for 15 min at room temperature and then permeabilized with 0.2% Triton X-100 in PBS for 10 min at room temperature. The neurons were blocked with 3% skim milk in PBS for 1 h at room temperature followed by the same solution containing mouse monoclonal anti-MAP2 antibody (Sigma) for 1 h at room temperature. After washing three times with PBS, the neurons were incubated with Alexa 568-labeled goat anti-mouse IgG (Molecular Probes) in the blocking solution for 1 h at room temperature. The neurons were then washed five times with PBS and counterstained with 1 μg/ml Hoechst 33342.

RNA Interference—Short interfering RNA for depletion of CRM1 (siCRM1) was prepared as described previously (see also Ref. 17). Sense, UUGUGUGAAUUUGCUAUAC(dT)(T); and antisense, GUAUAGCAAAUCACCA(dT)(T), were obtained from Takara. Transfection was suspended in neurobasal medium (Invitrogen) supplemented with 25 and 0.5 mM l-glutamine and then plated at 3.0 × 10^5/mm² plate on polyethyleneimine-coated tissue culture dishes at 37 °C in a 5% CO₂ atmosphere. On day 10 after plating, neurons were transfected with expression vectors using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol. At 24 h after transfection, neurons were fixed with 4% paraformaldehyde in PBS for 15 min at room temperature and then permeabilized with 0.2% Triton X-100 in PBS for 10 min at room temperature. The neurons were blocked with 3% skim milk in PBS for 1 h at room temperature followed by the same solution containing mouse monoclonal anti-MAP2 antibody (Sigma) for 1 h at room temperature. After washing three times with PBS, the neurons were incubated with Alexa 568-labeled goat anti-mouse IgG (Molecular Probes) in the blocking solution for 1 h at room temperature. The neurons were then washed five times with PBS and counterstained with 1 μg/ml Hoechst 33342.

Fig. 2. The domain between RBD3 and RBD4 mediates the nuclear import of Stau2. HeLa cells were transfected with 2×MBP-GFP-Stau2α × 2× (243–317) or 2×MBP-GFP-Stau2α × 2× (318–412), and their localizations were visualized by fluorescence microscopy. Scale bar, 10 μm.
of HeLa cells with siCRM1 or mock (transfection reagents only) was performed in 60-mm dishes using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. The cells were harvested at 48 h after transfection and lysed. Then Western blot analysis was performed using a rabbit polyclonal anti-CRM1 antibody (Santa Cruz Biotechnology) or a mouse monoclonal anti-GAPDH antibody (Ambion).

Fluorescence Microscopy—All living and fixed cells were examined using a Zeiss Axiovert 200 fluorescent microscope (Carl Zeiss).

RESULTS

Stau2 Shuttles between the Nucleus and Cytoplasm—To better understand the dynamic behavior of Stau2 in cells, we initially investigated the subcellular localization of full-length Stau2 by transiently expressing a GFP fusion construct. As shown in Fig. 1, the full-length Stau2 was located primarily in the cytoplasm. However, when a variety of Stau2 deletion mutant constructs, fused to GFP, were transiently expressed in HeLa cells, we found that some mutants such as Stau2-(243–317) and Stau2-(273–373) were clearly localized in the nucleus (Fig. 1), indicating the possibility that Stau2 has the capability of entering the nucleus. Furthermore, the nuclear accumulation of both the Stau2-(243–317) and Stau2-(273–373) mutants suggests that the overlapping domain between RBD3 and RBD4 may mediate nuclear localization. In addition, the localization of Stau2-(318–571), Stau2-(318–412), and Stau2-(413–571) suggests that the domain just following RBD4 may also be involved in nuclear localization.

To exclude the possibility that these mutants might have passively diffused into the nucleus followed by the retention, they were fused to MBP (maltose-binding protein)-MBP-GFP (2×MBP-GFP). As shown in Fig. 2, 2×MBP-GFP-Stau2-(243–317) was localized predominantly in the nucleus, indicating that Stau2-(243–317) contains a nuclear localization signal. In contrast, 2×MBP-GFP-Stau2-(318–412) remained in the cytoplasm. These results indicate that the domain between RBD3 and RBD4 mediates the nuclear import of Stau2 but not the downstream domain of the RBD4.

On the other hand, the mutant Stau2-(208–272), which is equivalent to the RBD3, was predominantly localized in the cytoplasm, indicating that the RBD3 domain mediates the nuclear export of Stau2. These results indicate that Stau2 is capable of shuttling between the nucleus and cytoplasm.

Mutation of RBD3 Prevents the Nuclear Export of Stau2—To confirm that the RBD3 actually mediates the nuclear export of Stau2, we introduced a specific point mutation into the RBD3 that results in a reduction in its RNA binding activity. Based on previous findings on the structure of the double-stranded RNA-binding domain (14, 15), an RBD3 mutant of Stau2 (Stau2RBD3*) was created by replacing phenylalanine 239 with alanine, which has been reported to disrupt the
Stau2⁵⁹ is exported by two distinct pathways, a RBD3-mediated and a CRM1-dependent one. A, HeLa cells were transfected with GFP-Stau²⁵⁹ or GFP-Stau²⁵⁹RBD³*. GFP fusion proteins were visualized by fluorescence microscopy. Scale bar, 10 μm. B, HeLa cells were mock-treated or transfected with short interfering RNA for CRM1. At 48 h after transfection, GFP fusion proteins were visualized by fluorescence microscopy. (mock) siCRM1. D, HeLa cells were transfected with GFP-Stau⁵⁹ or GFP-Stau⁵⁹RBD³* together with (siCRM1) or without (mock) siCRM1. At 48 h after transfection, GFP fusion proteins were visualized by fluorescence microscopy. Scale bar, 10 μm.

Stau²⁵⁹ was also localized predominantly in the cytoplasm. Therefore, to test whether the nuclear export of Stau²⁵⁹ is mediated by RBD3, we created the RBD3-mutated Stau²⁵⁹ (Stau²⁵⁹RBD³*). Since the secondary structures of Stau²⁶² and Stau²⁵⁹ RBD3 are identical, the mutation was introduced in the same way as that for Stau²⁶². HeLa cells were transfected with GFP-Stau²⁵⁹RBD³*, and its localization was observed (Fig. 4A). Unexpectedly, in contrast to Stau²⁶²RBD³*, Stau²⁵⁹RBD³* showed no nuclear accumulation.

From these findings, we speculate that Stau²⁵⁹ is exported via an alternate export pathway, for example, a CRM1-dependent one. Therefore, HeLa cells transfected with GFP-Stau²⁵⁹RBD³* were treated with LMB. As shown in Fig. 4B, LMB treatment resulted in the nuclear accumulation of Stau²⁵⁹RBD³*. In contrast, wild type full-length Stau²⁵⁹ was localized predominantly in the cytoplasm, indicating that the RBD3-mediated nuclear export pathway, which is not sensitive to LMB, is also functional for Stau²⁵⁹. Thus, Stau²⁵⁹ appears to be exported from the nucleus by two distinct pathways; one is mediated by the RBD3, which is CRM1-independent, and the other is CRM1-dependent. To confirm this, we performed RNA interference targeting CRM1. A short interfering RNA duplex targeted to residues 90–108 of the human CRM1 open reading frame (siCRM1) was prepared according to the previous report (17) and transfected to HeLa cells. At 48 h after transfection, the cells were harvested, and the expression level of endogenous CRM1 was analyzed by Western blotting. As shown in Fig. 4C, this treatment specifically reduced the expression level of CRM1. To test the effect of the down-regulation of CRM1 on the cellular localization of Stau²⁵⁹, HeLa cells were transfected with siCRM1 together with GFP-Stau²⁵⁹ or GFP-Stau²⁵⁹RBD³*. As shown in Fig. 4D, whereas the down-regulation of CRM1 resulted in the predominant nuclear localization of GFP-Stau²⁵⁹RBD³*, GFP-Stau²⁵⁹ was primarily localized in the cytoplasm, which was consistent with the results obtained by using LMB.

Identification of a CRM1-dependent Nuclear Export Signal of Stau²⁵⁹ at the N-terminal Domain—Since the primary structure of Stau²⁵⁹ is different from that of Stau²⁶² only in the N-terminal domain (Fig. 5A), we hypothesized that the N-terminal domain of Stau²⁵⁹ mediates the CRM1-dependent nuclear export. For this, the deletion mutant Stau²⁵⁹(1–105) was constructed as a GFP fusion protein and transfected into HeLa cells. As shown in Fig. 5B, GFP-Stau²⁵⁹(1–105) was localized predominantly in the cytoplasm. In addition, LMB treatment promoted the nuclear localization of GFP-Stau²⁵⁹(1–105). On the other hand, the localization of GFP-Stau²⁶²(1–137) was similar to that of GFP alone and was not affected by LMB treatment. These results indicate that the N-terminal domain of Stau²⁵⁹ mediates the CRM1-dependent nuclear export.

It is well known that nuclear export signals (NESs) that are recognized by CRM1 are frequently composed of a stretch of characteristic amino acids such as leucine and isoleucine, as was first reported for the human immunodeficiency virus type 1 Rev (18) and protein kinase inhibitor (19) proteins. To test whether the hydrophobic amino acid-rich sequence INQMFSVQLSL in the N-terminal of Stau²⁵⁹ functions as the NES that is recognized by CRM1, HeLa cells were transfected with GFP-INQMFSVQLSL, and its localization was examined (Fig. 6A). GFP-INQMFSVQLSL was localized predominantly in the cytoplasm, and LMB treatment led to its nuclear localization, indicating that the sequence INQMFSVQLSL is recognized by CRM1.

To confirm that INQMFSVQLSL functions as the CRM1-dependent NES of Stau²⁵⁹, two point mutants of Stau²⁵⁹RBD³*...
FIG. 5. N-terminal domain of Stau2<sup>59</sup> is involved in the nuclear export by CRM1. A, schematic representation of Stau2<sup>62</sup> and Stau2<sup>59</sup>. Their amino acid sequences in N-terminal domain are different from each other. B, HeLa cells were transfected with GFP-Stau2<sup>62</sup>-(1–137) or GFP-Stau2<sup>59</sup>-(1–105), and the cells were then treated with 10 nM LMB for 4 h. GFP fusion proteins were visualized by fluorescence microscopy. Scale bar, 10 μm.

FIG. 6. Identification of the NES of Stau2<sup>59</sup> recognized by CRM1. A, amino acid sequences of the N-terminal domain of Stau2<sup>62</sup> and Stau2<sup>59</sup>. The NES recognized by CRM1 is highlighted in bold. Each underlined amino acid of Stau2<sup>59</sup> was changed to alanine. Asterisks denote identical amino acids between Stau2<sup>62</sup> and Stau2<sup>59</sup>. HeLa cells were transfected with GFP-INQMPSVQLSL, and the cells were then treated with 10 nM LMB for 4 h. Scale bar, 10 μm. B, HeLa cells were transfected with the NES and RBD3 double-mutated constructs as indicated. GFP fusion proteins were visualized by fluorescence microscopy. Scale bar, 10 μm. C, HeLa cells were transfected with GFP-Stau2<sup>59</sup>I4A or GFP-Stau2<sup>59</sup>L12A. GFP fusion proteins were visualized by fluorescence microscopy. Scale bar, 10 μm.
The export of Stau2 from the nucleus involves two distinct pathways: an RBD3-dependent one and a CRM1-dependent one. This was demonstrated using rat hippocampal neurons transfected with GFP-Stau259RBD3*, indicating that Stau259 is exported by CRM1. The CRM1-dependent nuclear export of Stau259 plays an important role in transporting dsRNA out of the nucleus.

**DISCUSSION**

Using Stau2 deletion mutant constructs, we report here that Stau2 is a nucleocytoplasmic shuttle protein. In addition, the findings show that the region between RBD3 and RBD4 functions as the nuclear localization signal of Stau2, consistent with a recent report by Macchi et al. (20), although the import factor that recognizes the nuclear localization signal remains to be determined.

We next analyzed the nuclear export of Stau2 in more detail. First, we showed that the nuclear export of Stau2 is mediated by CRM1. This is consistent with a recent report by Macchi et al. (20), who demonstrated that the RBD3 of Stau2 interacts with exportin 5 in an RNA-dependent manner. Moreover, they showed that the down-regulation of exportin 5 by RNA interference resulted in the nuclear accumulation of Stau2, indicating that exportin 5 is the nuclear export factor of Stau2. Furthermore, Brownawell and Macara (21) reported that the RBD of ILF3, Sprn, Staufen1, and protein kinase R bind to exportin 5, suggesting that exportin 5 participates in the nuclear export of dsRNA-binding proteins.

Interestingly, we report, for the first time, that a functional difference exists among the Stau2 isoforms. That is, Stau2 is exported from the nucleus only in a CRM1-mediated manner, whereas Stau2 is exported by two distinct pathways, a RBD-mediated one and a CRM1-dependent one. In addition, we identified the NES sequence at the N-terminal end of Stau2, indicating that alternative splicing creates a new functional domain for nuclear export. It is reasonable to speculate that both Stau2 isoforms, Stau2 and Stau2, are exported by the same two pathways because Stau2 is exported from the nucleus without any RNA, suggesting that exportin 5 participates in the nuclear export of dsRNA-binding proteins.

The question arises as to the nature of the biological significance of the export of Stau2 from the nucleus in a CRM1-dependent manner. Exportin 5 preferentially recognizes RNAs containing a minihelix motif, a structure that comprises a double-stranded stem of over 14 nucleotides with a base-paired 5' end and a 3–5-nucleotide protruding 3' end (22, 23). The RBD of Stau2 is a major dsRNA binding determinant. Therefore, when Stau2 is exported from the nucleus by exportin 5, Stau2 appears to bind to and, therefore, to carry restricted minihelix-RNAs from the nucleus. On the other hand, it is likely that Stau2 as well as Stau2, which are exported by CRM1, are able to transport other sets of dsRNAs that are unable to bind to exportin 5 or bind to the RBD3 in the absence of exportin 5. Considering this, along with the data that the expression of Stau2 is more abundant than Stau2 in the brain of adult rodents (7, 11), we propose the possibility that the CRM1-dependent nuclear export of Stau2 plays an important role in transporting various RNAs out of the nucleus. However, the possibility that Stau2 may be exported from the nucleus without binding any RNA cannot be excluded. Kress et al. (24) proposed a model for the vegetal RNA localization pathway in Xenopus oocytes in which Xenopus Staufen is
recruited into the core ribonucleoprotein complex in the cytoplasm that has been assembled in the nucleus. Further study will be required to determine whether Stau2 must be imported into the nucleus once to enter RNA granules.

REFERENCES

1. Kiebler, M. A., and DesGroseillers, L. (2000) Neuron 25, 19–28
2. Jansen, R. P. (2001) Nat. Rev. Mol. Cell. Biol. 2, 247–256
3. Saxton, W. M (2001) Cell 107, 707–710
4. Kloc, M., Zearfoss, N. R., and Etkin, L. D. (2002) Cell 108, 533–544
5. Buchner, G., Bassi, M. T., Andolfi, G., Baillio, A., and Franco, B. (1999) Genomics 62, 113–118
6. Tang, S. J., Meulemans, D., Vazquez, L., Celaco, N., and Schuman, E. (2001) Neuron 32, 463–475
7. Duchaine, T. F., Hemraj, I., Furic, L., Deitinghoff, A., Kiebler, M. A., and DesGroseillers, L. (2002) J. Cell Sci. 115, 3285–3295
8. St. Johnston, D., Beuchle, D., Nusslein, and Volhard, C. (1991) Cell 66, 51–63
9. Ferrandon, D., Ripichik, L., Nusslein, Volhard C., and St. Johnston, D. (1994) Cell 79, 1221–1232
10. Ohashi, S., Kunke, K., Omori, A., Ichinose, S., Ohara, S., Kobayashi, S., Sato, T. A., and Anzai, K. (2002) J. Biol. Chem. 277, 37804–37810
11. Mallardo, M., Deitinghoff, A., Muller, J., Goetz, B., Macchi, P., Peters, C., and Kiebler, M. A. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 2100–2105
12. Kohrmann, M., Luo, M., Kaether, C., DesGroseillers, L., Dotti, C. G., and Kiebler, M. A. (1999) Mol. Biol. Cell 10, 2845–2853
13. Knowles, R. B., Sabry, J. H., Martone, M. E., Dever, T. J., Ellisman, M. H., Bassell, G. J., and Kosik, K. S. (1996) J. Neurosci. 16, 7812–7820
14. Ryter, J. M., and Schultz, S. C. (1998) EMBO J. 17, 7505–7513
15. Ramos, A., Grunert, S., Adams, J., Micklem, D. R., Proctor, M. R., Freund, S., Bycroft, M., St. Johnston, D., and Varani, G. (2000) EMBO J. 19, 997–1009
16. Kudo, N., Matsumori, N., Taoka, H., Fujiwara, D., Schreiner, E. P., Wolff, B., Yoshida, M., and Horinouchi, S. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 9112–9117
17. Lund, E., Gutingher, S., Calado, A., Dahlberg, J. E., and Kutay, U. (2004) Science 303, 95–98
18. Fischer, U., Huber, J., Boelens, W. C., Mattaj, I. W., and Luhrmann, R. (1995) Cell 82, 463–473
19. Wen, W., Meinkoth, J. L., Tsien, R. Y., and Taylor, S. S. (1995) Cell 82, 463–473
20. Macchi, P., Brownawell, A. M., Grunewald, B., DesGroseillers, L., Macara, I. G., and Kiebler, M. A. (2004) J. Cell. Biol. 165, 53–64
21. Brownawell, A. M., and Macara, I. G. (2002) J. Cell. Biol. 155, 53–64
22. Gwizdek, C., Ossareh, Nazari, B., Brownawell, A. M., Doglio, A., Bertrand, E., Macara, I. G., and Dargemont, C. (2003) J. Biol. Chem. 278, 5505–5508
23. Gwizdek, C., Ossareh, Nazari, B., Brownawell, A. M., Evers, S., Macara, I. G., and Dargemont, C. (2004) J. Biol. Chem. 279, 884–891
24. Kress, T. L., Yoon, Y. J., and Mowry, K. L. (2004) J. Cell Biol. 165, 203–211
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