Characterization of a Functional Bacterial Homologue of Sodium-dependent Neurotransmitter Transporters*

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Andreas Androusellis-Theotokis‡, Naomi R. Goldberg§, Kenji Ueda¶, Teruhiko Beppu, Matthew L. Beckman‡, Shonit Das‡, Jonathan A. Javitch§, and Gary Rudnick‡

From the ‡Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06520-8066, §Center for Molecular Pharmacology, Departments of Psychiatry and Pharmacology, Columbia University College of Physicians and Surgeons, New York, New York 10032, and the ¶Department of Applied Biological Sciences, Nikon University, 1866 Kameino, Fujisawa-shi, Kanagawa 252-8510, Japan

The tnaT gene of Symbiobacterium thermophilum encodes a protein homologous to sodium-dependent neurotransmitter transporters. Expression of the tnaT gene product in Escherichia coli conferred the ability to accumulate tryptophan from the medium and the ability of other amino acids. Tryptamine and serotonin were weak inhibitors with Ki values of 200 and 440 μM, respectively. By using a T7 promoter-based system, TnaT with an N-terminal His6 tag was expressed at high levels in the membrane and was purified to near-homogeneity in high yield.

Transporters responsible for reuptake of neurotransmitters across the plasma membrane of neurons and glia fall into two gene families (1). The majority of small neurotransmitters, including glycine, γ-amino butyric acid (GABA),1 dopamine, norepinephrine, and 5-hydroxytryptamine (5-HT, serotonin), are transported by proteins belonging to the family designated the neurotransmitter:sodium symporter (NSS) family 2.A.22 by Saier (2). Glutamate, however, is transported by a family of mono- and dicarboxylic amino acid transporters, the dicarboxylate/amino acid:cation symporters family (2). Proteins in both families play important roles in brain function as indicated by the profound behavioral effects of drugs that influence their activity, such as cocaine and amphetamines, which interact with amine transporters in the NSS family (3–12), and many antidepressant drugs that inhibit serotonin and norepinephrine transporters (13–17).

Among the sequences found to be homologous to the NSS family of transporters are a number of "orphan" transporters, for which no function is known. These orphans include v7-3 (18), NTT4 (19, 20), inebriated (21), blot (22), and NTT5 (23), among others. The largest number of orphan sequences in this family is found in prokaryotic organisms. Although these orphan sequences are highly similar to those encoding functional transporters, it is possible that these proteins fulfill other functions. For example, within the ATP-binding cassette family of transporters are the sulfonyleurea receptor (24) and the cystic fibrosis transmembrane regulator chloride channel (25). In the dicarboxylate/amino acid:cation symporters neurotransmitter transport family is EAAT4, a ligand-gated ion channel (26); SGLT3, a member of the sodium:solute symporter (SSS) sugar transporter family, also is not a transporter but rather a glucose-gated ion channel. Moreover, some proteins, such as adenylyl cyclase (28) and patched (29) also have 12 transmembrane segments but no known transport function. For the orphan transporters in the NSS family, it is important to know if any of the newly discovered prokaryotic sequences actually encode functional transporters.

Symbiobacterium thermophilum is a symbiotic thermophile, the growth of which is dependent on co-culture with an associated Bacillus strain (30, 31). The 16S rDNA-based taxonomy showed that S. thermophilum occupies a novel phylogenetic branch in the Gram-positive group without clustering with any other genus (30). This bacterium produces a thermostable tryptophanase directed by the tna1 gene which, when cloned (32), was found to be part of a tna operon with an unusual gene organization. The operon differs from the conserved structure among enterobacteriaceae in that it consists of three open reading frames (33). Furthermore, in the region downstream from the tryptophanase gene, this unique bacterium appears to encode a transporter, TnaT, that belongs, based on sequence homology, to the NSS family. In this communication, we demonstrate that the tnaT gene encodes a Na1-dependent tryptophan transporter and that this transporter can be expressed in the cell membrane and purified in high yield.

MATERIALS AND METHODS

Plasmid Preparations—The nucleotide sequence of the tna gene cluster of S. thermophilum was submitted to the DNA Data Bank of Japan under accession number AB010832. For the construction of the pET28c(+)-expression plasmid (Novagen, Madison, WI), the tnaT sequence was amplified by standard PCR with the following primers: TnaT-N (‘-CATCATATGGAGGCACAGCGC (corresponding to

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CAAGAAAGCTGGGTACTAGCCCACCTCCCCGCCGG (antisense). By CGAGGCACAGCGCGATCAGTGG (sense) and GGGACCACTTTGTA-
the following primers: GGGGACAAGTTTGTACAAAAAAGCAGGCTC-
except that 0.15 mM IPTG was used for 2 h. After induction, cells were transformed with this plasmid and selected with ampicillin and kana-
ment encoding a 6xHis-TnaT (see below) was subcloned into pQE82

For expression of TnaT in the Escherichia coli strain CY15212 (mtr-, araP-, toliT), a construct was prepared that would allow constitutive expression of the transporter proteins. The constitutively active pro-
mer for the β-lactamase gene (Amp’) was amplified by PCR from a pBluescript II KS(+) plasmid (Stratagene, La Jolla, CA) using the following primers: TATAAGCTTAGGGCCTTCTCCGCCGGAAAATG, which contains a BglII site, and ATATCTAGAATCTCTCTTTTAA-
TAGCAGTGG (sense) and GGGACCACTTTGTA-
edIII-digested plasmid (Novagen catalogue number 69744-3) between the single restriction sites for BglII and XhoI. tnaT was removed from the pET22b(+) vector and inserted downstream of the promoter, using restriction sites for NdeI and HindIII.

For inducible expression of TnaT in CY15212, an EcoRI/XhoI frag-
ment encoding a 6xHis-TnaT (see below) was subcloned into pQE32 (Qiagen), a plasmid with an inducible T5 promoter. CY15212 cells were transformed with this plasmid and selected with ampicillin and kana-
mycin. A pool of colonies was grown and induced as described below, except that 0.15 mM IPTG was used for 2 h. After induction, cells were prepared for uptake or membranes were prepared as described below. For immunoblotting, samples were resolved by SDS-PAGE, transferred to nitrocellulose, and blotted with an anti-His antibody (Santa Cruz Biotechnology, Santa Cruz, CA) followed by a horseradish peroxidase-conjugated secondary antibody. Chemiluminescence was detected and quantitated on a FluorChem 8000 (Alpha Innotech Corp., San Leandro, CA) followed by a horseradish peroxidase-

To analyze tryptophan transport resulting from expression of the S. thermophilum tnaT gene, we used E. coli strain CY15212, obtained from the Yale E. coli Stock Center (CGSC 7672). This strain is inactivated in the three genes, mtr, tnaB, and araP, that encode tryptophan transporters (38). As shown in Fig. 2 (squares), this strain is incapable of accumulating [3H]tryptophan. However, transformation of CY15212 with an expression plasmid encoding the S. thermophilum tnaT gene led to robust [3H]tryptophan uptake, as shown in Fig. 2 (filled circles).

A consequence of tryptophan uptake by cells expressing TnaT is that the transporter facilitated growth of cells on tryptophan-containing minimal media. Fig. 3 shows the dra-
matic increase in growth by BL21 cells transformed with pET26-TnaT over the first 12 h of incubation (filled circles) in contrast to the relatively slow growth in Trp-free medium or by control cells lacking the tnaT insert. The increase in cell growth indicates that tryptophan taken up by cells is accumulated within the cell where it can be metabolized and is not merely bound to the cell surface.

Trp transport by TnaT was saturable, as shown in Fig. 4. Under the conditions used, the Vmax for transport was 242 ± 9 pmol per min per mg of cell protein, and the Km was 145 ± 14 nm. The inset of Fig. 4 shows an Eadie-Hofstee (39) transforma-
tion of the transport rate data. The rate shows simple saturation with tryptophan concentration. To test the specificity of tnaT-encoded tryptophan transport, we measured the initial rate of transport in the presence of 100 μM concentrations of the 20 naturally occurring amino acids and also cystine, trans-proline, tryptamine, and serotonin. The results are shown in Fig. 5. Aside from tryptophan, none of the

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amino acids tested significantly inhibited tryptophan influx. A small inhibition was observed with tryptamine and serotonin, and the concentrations of these amines that inhibited influx by 50% were found to be 200 μM and 440 μM, respectively (not shown). With one exception, inhibitors of mammalian biogenic amine transporters also failed to block TnaT-mediated tryptophan transport. The following compounds failed to inhibit at 100 μM (not shown): imipramine, desipramine, fluoxetine, citalopram, nomifensine, mazindol, GBR-12909, GBR-12935, amphetamine, and 3,4-methylenedioxymethamphetamine. Cocaine (1 mM) also did not inhibit (not shown).

Sertraline, a serotonin reuptake inhibitor with nM affinity, inhibited tryptophan uptake with a \(K_I\) of 98 μM (not shown).

In TM1 of all amino acid transporters in the NSS family, there is a glycine residue that is replaced by aspartate in the biogenic amine transporters. In TnaT, the corresponding residue is a glycine at position 24. Mutation of this residue to an aspartate led to a transporter that was unable to transport tryptophan (Fig. 6A), serotonin, or 1-methyl-4-phenylpyridinium (not shown). This was not due to a block in expression because the His\(_6\)-tagged G24D mutant was expressed in the membrane of CY15212 cells at 69 ± 15% (n = 3) of the level of wild type His\(_6\)-tagged TnaT (Fig. 6B).

A defining characteristic of the sodium-dependent neurotransmitter transporter family is the requirement for sodium ions. In almost all of the family members studied, sodium is required, and the transmembrane sodium gradient provides a driving force for solute accumulation. In many of the transporters in this family, chloride ion is also required, and the Cl\(^-\) gradient also provides part of the driving force. The data in Fig. 7 show that TnaT absolutely requires Na\(^+\) but not Cl\(^-\) for transport. Transport was minimal below 0.1 mM Na\(^+\) and was half-maximal at ~1 mM Na\(^+\). The inset shows that influx was essentially the same in NaCl and sodium isethionate medium but was not detectable in medium in which N-methyl-D-gluca-
mine-Cl replaced NaCl. Thus, tryptophan transport catalyzed by the tnaT gene product was Na\(^+\)-dependent but not Cl\(^-\)-dependent.

Neurotransmitter transport utilizes transmembrane ion gradients. Within the NSS family, the coupled inward movement, or symport, of Na\(^+\) and neurotransmitter molecule is an almost universal feature. However, at least one member, the K\(^+\)-coupled amino acid transporter, catalyzes substrate symport with K\(^+\) as well as Na\(^+\) (40). Most of the known transporters in the NSS family also require Cl\(^-\), which is symported with substrate in the cases that have been examined (41). Among bacterial transport systems, some are energized directly by ATP, whereas others are coupled to transmembrane ion gradients.

As an approach to determine the driving force used by TnaT to accumulate tryptophan, we used 2,4-dinitrophenol (DNP), a proton ionophore, to dissipate the transmembrane electrochemical potential for H\(^+\) (\(\Delta\mu\)H\(^{\text{H}}\)). As representatives of transporters coupled to ATP or to ion gradients, we used the histidine transporter, which is in the ATP-binding cassette family and known to be driven by ATP hydrolysis (42), and the proline transporter, a Na\(^+\)-coupled symporter (43, 44). Because dissipation of \(\Delta\mu\)H\(^{\text{H}}\) can deplete ATP supplies by allowing futile H\(^+\) pumping through the F\(_{0}\)F\(_{1}\)-ATPase (45), we also included N,N\(^{\prime}\)-dicyclohexylcarbodiimide (DCCD) to inhibit the ATPase. The results in Table I demonstrate that, in both the presence and the absence of DCCD, DNP strongly inhibited both proline and tryptophan accumulation while having virtually no effect on histidine transport. These results strongly suggest that TnaT-mediated tryptophan transport, like the endogenous proline transport of E. coli, is coupled to the transmembrane electrochemical Na\(^+\) potential, which depends on \(\Delta\mu\)H\(^{\text{H}}\).

For purification of the TnaT protein, the gene was tagged at the N terminus with 6 histidine residues and expressed in BL21(DE3)/pLysE using a T7 promoter-mediated expression system as described under “Materials and Methods.” After induction by IPTG, the cells were disrupted; membranes were isolated, and the membrane proteins were extracted with do-
decyl maltoside. The His-tagged TnaT protein was purified using nickel chromatography and analyzed by SDS-PAGE. The purified protein migrated as a single band of ~45 kDa relative to the predicted molecular size of the tagged construct of 57,258 kDa (Fig. 8). In prokaryotic proteins the N-terminal Met is often cleaved, which would give a predicted mass of 57,127 kDa. Preliminary matrix-assisted laser desorption ionization mass spectrometry analysis of the purified TnaT gave a molecular mass of 57,012 ± 44 kDa (mean ± S.D., n = 2) or 99.8% of the predicted mass, suggesting that the protein is full-length and unmodified. From an initial culture of 1 liter, we obtained ~0.5 mg of highly purified protein.

**DISCUSSION**

The tnaT gene is typical of an increasing number of prokaryotic sequences with striking homology to the NSS family of Na⁺-coupled neurotransmitter and amino acid transporters. To illustrate this observation, a BLAST search (46) of GenBank was performed using a highly conserved portion of the consensus sequence (37) for the NSS family. At the time the search was performed, it yielded, in addition to tnaT, more than 40 other sequences from bacteria and Archaea, although no significant matches were found in sequences from yeast, fungi, or plants. The sequence similarities between the predicted prokaryotic proteins, including TnaT, and mammalian members of the NSS transporter family are extensive (Fig. 1). For the 30 prokaryotic sequences with the greatest sequence similarity to the NSS family, the MEMSAT2 transmembrane topology prediction method (47) found 12 well aligned transmembrane domains (TM) in 12 of these sequences, including TnaT, 11 TMs in 16 other sequences, and 10 TMs in 2 sequences. The sequences containing fewer than 12 TMs lacked the last one or two TMs and were homologous with the 12 TM sequences through the first 10 or 11 TMs.

The apparent variability in the lengths of these transporter-like sequences highlights the uncertainty regarding their function. Without an unequivocal demonstration that these sequences encode functional transporters, we cannot rule out the possibility that they are responsible for other membrane functions. The characterization of TnaT as a functional Na⁺-coupled tryptophan transporter opens up this large family of orphan transporters to experimental study. We hope that these studies will shed light also on the structure and function of eukaryotic NSS neurotransmitter transporters. For example, if we find that those proteins with 10 or 11 TMs are functional as transporters, it will help to define which TMs are required for substrate binding, ion coupling, and other functions.

Like almost all of the transporters in the NSS family, TnaT required Na⁺ for its function, although unlike many other NSS transporters, we found that Cl⁻ was not required (Fig. 7). Moreover, TnaT-mediated Trp influx saturates at relatively low concentrations (Fig. 4) and is highly selective, similar to other NSS transporters. This high degree of functional and sequence similarity between TnaT and the mammalian members of the NSS family is even more remarkable in light of the complete absence of homologous sequences in yeast, fungi, or higher plants. This situation was found also in the sodium: solute symporter family (2), which also has many members in prokaryotes and animals but almost none in yeast, fungi, or higher plants (48).

One possible reason for the restricted distribution of this family to some prokaryotes and animals lies in the Na⁺ dependence of the NSS family. Prokaryotic and animal cells are known to maintain transmembrane Na⁺ gradients that are used for driving metabolite accumulation. Yeast and plants, however, extrude Na⁺ but rarely use solute-Na⁺ symport for metabolite accumulation (49, 50). If the coupling of substrate transport to Na⁺ is an integral property of NSS transporters, and they exclusively function, therefore, only as Na⁺ symporters, the restriction of their distribution to organisms that utilize a transmembrane Na⁺ gradient for metabolite transport would be understandable. This may also explain the distribution among prokaryotes. For example, among bacilli, *Bacillus halodulans*, an alkaliphilic species that uses the Na⁺ gradient as a driving force, has an NSS homologue (51), whereas *Bacillus subtilis*, a neutralophile, does not (52). A consequence of this explanation, however, is that all bacterial NSS proteins should catalyze Na⁺ symport, a prediction that we are currently testing.

There is a high degree of sequence identity between TnaT and mammalian transporters for serotonin (21%), and dopamine (24%), and the consensus NSS sequence from the Conserved Domains data base (37) (30%). A similar degree of identity was found with mammalian GABA, betaine, and taurine transporters. In particular, the identity is strongest in the region beginning before TM1 and ending in TM2, where there is also strong homology among previously known members of the NSS family. The most striking difference in the primary structure is in EL2, between TM3 and TM4, which is much shorter in TnaT than in other NSS family members. Two cysteine residues thought to exist as a disulfide in SERT (53) and DAT (54) are absent. In fact, the entire TnaT sequence contains only one cysteine, near the beginning of TM5.

The TnaT sequence contains a glycine residue at position 24 in TM1. This position corresponds to the location of a glycine in all of the NSS amino acid transporters and an asparagine in all of the NSS biogenic amine transporters. In SERT, this residue

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**Table I**

The effect of 2,4-dinitrophenol on transport of histidine, proline, and tryptophan.

| Condition | L-[2,5-3H]Histidine | L-[U-14C]Proline | L-[5-3H]Tryptophan |
|-----------|---------------------|-----------------|-------------------|
| Control   | 100 ± 5             | 100 ± 7         | 100 ± 10          |
| DCCD      | 103 ± 10            | 103 ± 8         | 92 ± 7            |
| DNP       | 102 ± 10            | 7.0 ± 0.1       | 15 ± 5            |
| DCCD → dNP| 91 ± 2              | 7.5 ± 0.6       | 15 ± 10           |

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**Fig. 8.** Coomassie-stained SDS-PAGE of nickel chromatography purification of TnaT. TnaT was prepared as described under "Materials and Methods." Lane 1 is the flow-through that did not bind to the nickel column. Lanes 2 and 3 are sequential fractions from the 20 mM imidazole elution. Lanes 4 and 5 are fractions eluted with 40 and 250 mM imidazole, respectively. The molecular masses of standards in kDa are shown on the right by arrows.
has been implicated in the recognition of substrates and inhibitors (55) and was proposed to interact with the ligand amino group. Consistent with the lack of an aspartate at this position, the amines tryptamine and 5-HT (serotonin) were poor inhibitors of TnaT-mediated Trp transport (Fig. 5). Likewise, cocaine, an inhibitor of all NSS amine transporters, was ineffective as an inhibitor, as were a variety of other NSS amine transporter inhibitors. Mutation of Gly-24 to Asp as found in SERT, NET, and DAT ablated tryptophan transport but did not bestow upon TnaT the ability to transport 5-HT or 1-methyl-4-phenylpyridinium, consistent with the existence of multiple determinants of substrate specificity.

In TM3, several residues have been implicated in the binding and transport of substrates by NSS transporters. A tyrosine at position 176 in SERT, conserved as Tyr-102 in TnaT, has been implicated as a residue site in SERT (56) and GAT-1 (57) and is conserved in all NSS transporters. Also conserved is an isoleucine at position 105 of TnaT that corresponds to Ile-172 of SERT and Ile-155 of NET. These residues were proposed to form part of the gate that closes to prevent access to the substrate-binding site in the internal facing form of the transporter (58). An isoleucine at position 172 in SERT (proposed to be in proximity to the substrate site) is conservatively replaced by Val-98 in TnaT. This residue is a valine also in DAT but is not particularly conserved throughout the NSS family. Additional TM3 residues that are conserved between SERT, DAT, and TnaT are Ile-106, Trp-108, Leu-110, Tyr-112, and Leu-113.

The majority of ion-coupled bacterial transporters utilize H+ symport to accumulate metabolites within the cell, although Na+ influx is coupled to solute uptake by some. The primary driving force for both types of transport system is the respiratory chain that, in E. coli, pumps H+ ions out of the cell, creating a transmembrane pH difference and an electrical potential that together comprise the electrochemical H+ potential, $\Delta \Psi_H^+$. An electrogenic Na+/H+ exchange transporter, or antiporter, known as NhaA (59), uses the $\Delta \Psi_H^+$ to expel Na+ ions from the cell, creating a driving force for Na+-solute symport.

The Na+ requirement for TnaT-mediated transport is likely to reflect Na+-Trp symport, although the results presented here do not rule out a possible H+-Trp symporter with an external Na+ requirement. The sensitivity to uncoupling by DNP suggests that the $\Delta \Psi_H^+$ is an intermediate in the bioenergetic coupling of TnaT, as it is with the Na+-proline symporter PutP (44). It will be important to determine, using isolated bacterial membrane vesicles (60), whether the Na+ gradient is indeed an immediate driving force for transport and also to measure the stoichiometric coupling of Na+ to Trp flux.

As targets for major therapeutic drugs as well as drugs of abuse, neurotransmitter transporters in the NSS family are an important focus of research in neuroscience. Structural and mechanistic studies of the NSS transporters have revealed the ion coupling stoichiometry and some mechanistic aspects of these proteins (61, 62). Their topological orientation has been studied extensively (63–69). Some residues have been identified as potential sites of substrate and inhibitor binding (55–57, 70, 71), and others have been shown to change their accessibility or responsiveness in response to ligand binding or conformational changes that accompany transport (58, 67–69, 72, 73).

Nevertheless, many questions about the structure and mechanism of the NSS family remain unanswered. Part of the problem lies in the relatively low abundance of these proteins and their liability during purification. Among transporters in this family, only the GABA transporter has been purified in a reconstitutively active form (74), and attempts with other members of the family have met with difficulty (27). The discovery that a bacterial homologue of these proteins is a functional transporter with similar characteristics to the mammalian transporters represents an important step toward addressing questions about their structure and mechanism. Our demonstration that TnaT can be expressed in the cell membrane and purified in high yield (Fig. 8) will allow biochemical and structural approaches that have not been available previously for understanding the structure and function of the NSS family of neurotransmitter transporters.

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43. Ramos, S., and Kaback, H. R. (1977) Biochemistry 16, 854–859
44. Cairney, J., Higgins, C. F., and Booth, I. R. (1984) J. Bacteriol. 160, 22–27
45. Hugenholtz, J., Hong, J. S., and Kaback, H. R. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 3446–3449
46. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) Nucleic Acids Res. 25, 3389–3402
47. Jones, D. T., Taylor, W. R., and Thornton, J. M. (1994) Biochemistry 33, 3038–3049
48. Turk, E., and Wright, E. M. (1997) J. Membr. Biol. 159, 1–20
49. Blumwald, E., Aharon, G. S., and Apse, M. P. (2000) Biochim. Biophys. Acta 1465, 140–151
50. Versaw, W. K., and Metzenberg, R. L. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3884–3887
51. Takami, H., Nakasone, K., Takaki, Y., Maeno, G., Sasaki, R., Masui, N., Fuji, F., Hirama, C., Nakamura, Y., Ogasawara, N., Kubara, S., and Horikoshi, K. (2000) Nucleic Acids Res. 28, 4317–4331
52. Kunst, F., Ogasawara, N., Moszer, I., Albertini, A. M., Alloni, G., Azevedo, V., Bertero, M. G., Bessieres, P., Bolotin, A., Borchert, S., Borris, R., Boursier, L., Brans, A., Braun, M., Brignell, S. C. B., Bren, S., Brouillet, S., Bruschi, C. V., Caldwell, B., Caspano, N. M., Choi, S. K., Codani, J. J., Conrert, I. F., Danchin, A. et al. (1997) Nature 390, 249–256
53. Chen, J. G., Liu-Chen, S., and Rudnick, G. (1997) Biochemistry 36, 1479–1486
54. Wang, J., Moriwaki, A., and Uhl, G. (1995) J. Neurosci. 19, 416–419
55. Barker, E. L., Moore, K. R., Rakhshan, F., and Blakely, R. D. (1999) J. Neurosci. 19, 4705–4717
56. Chen, J. G., Sachpatzidis, A., and Rudnick, G. (1997) J. Biol. Chem. 272, 28321–28327
57. Bismuth, Y., Kavanagh, M. P., and Kanner, B. I. (1997) J. Biol. Chem. 272, 16996–16102
58. Chen, J. G., and Rudnick, G. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 1044–1049
59. Padan, E., and Schuldiner, S. (1994) J. Exp. Biol. 196, 443–456
60. Kaback, H. R. (1974) Science 186, 882–892
61. Rudnick, G. (2002) in Neurotransmitter Transporters, Structure, Function, and Regulation (Reith, M. E. A., ed) 2nd Ed., pp. 25–52, Humana Press Inc., Totowa, NJ
62. Kanner, B. I. (2002) in Neurotransmitter Transporters, Structure, Function, and Regulation (Reith, M. E. A., ed) 2nd Ed., pp. 235–254, Humana Press Inc., Totowa, NJ
63. Bennett, E. R., and Kanner, B. I. (1997) J. Biol. Chem. 272, 1203–1210
64. Olivares, L., Aragon, C., Gimenez, C., and Zafra, F. (1997) J. Biol. Chem. 272, 1211–1217
65. Clark, J. A. (1997) J. Biol. Chem. 272, 14695–14704
66. Chen, J. G., Liu-Chen, S., and Rudnick, G. (1998) J. Biol. Chem. 273, 12675–12681
67. Ferrer, J. V., and Javitch, J. A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 9238–9243
68. Androutsellis-Theotokis, A., Ghassemi, F., and Rudnick, G. (2001) J. Biol. Chem. 276, 45933–45938
69. Androutsellis-Theotokis, A., and Rudnick, G. (2002) J. Neurosci. 22, 8370–8378
70. Barker, E. L., Perlm, M. A., Adkins, E. M., Houlihan, W. J., Pristupa, Z. B., Niznik, H. B., and Blakely, R. D. (1998) J. Biol. Chem. 273, 19439–19468
71. Adkins, E. M., Barker, E. L., and Blakely, R. D. (2001) Mol. Pharmacol. 59, 514–523
72. Golovanevsky, V., and Kanner, B. I. (1999) J. Biol. Chem. 274, 23020–23026
73. Ni, Y. G., Chen, J. G., Androutsellis-Theotokis, A., Huang, C. J., Moczydlowski, E., and Rudnick, G. (2001) J. Biol. Chem. 276, 30942–30947
74. Radian, R., Bendahan, A., and Kanner, B. I. (1986) J. Biol. Chem. 261, 15437–15441
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Additions and Corrections

Vol. 278 (2003) 15461–15464

The mammalian target of rapamycin (mTOR) partner, raptor, binds the mTOR substrates, p70 S6 kinase and 4E-BP1, through their TOR signaling (TOS) motif.

Hiroki Nojima, Chiharu Tokunaga, Satoshi Eguchi, Noriko Oshiro, Sujuti Hidayat, Ken-ichi Yoshino, Kenta Hara, Noriaki Tanaka, Joseph Avruch, and Kazuyoshi Yonezawa

Page 15461, Abstract, line 9 should read: Herein we demonstrate that raptor binds to p70S6k and 4E-BP1 through their respective TOS (conserved TOR signaling) motifs, a short conserved segment previously shown to be required for amino acid- and mTOR-dependent regulation of these mTOR substrates in vivo. The phrase “a short conserved segment previously shown” was inadvertently omitted.

Vol. 278 (2003) 18330–18335

Kinetics control preferential heterodimer formation of platelet-derived growth factor from unfolded A- and B-chains.

Carsten Müller, Susanne Richter, and Ursula Rinas

Page 18332, Equation 5: This equation was printed incorrectly. The correct equation is shown below:

\[ k = A \exp\left(\frac{\Delta S}{R}\right) \exp\left(-\frac{\Delta H}{RT}\right) \]  

(Eq. 5)

This does not affect the results of the paper.

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The grant support for Matthew L. Beckman was inadvertently omitted.

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