RIC-3 has been identified as a molecule essential for the recruitment of functional nicotinic acetylcholine receptors composed of α7, but it exhibits inhibitory effects on α4β2 or α3β4 receptors. In this study, we investigated the role of RIC-3 in the recruitment of 5-hydroxytryptamine type 3A (5-HT3A) receptors to the cell surface. Although RIC-3 is not essential for the surface transport of 5-HT3A receptors, we found that its presence enhances both receptor transport and function in a concentration-dependent manner. RIC-3 is localized to the endoplasmic reticulum, as evidenced by co-localization with the chaperone molecule, binding protein (BiP). RIC-3 is not detected at significant levels on the cell surface when expressed alone or in the presence of 5-HT3A. RIC-3 and 5-HT3A show a low level interaction that is transient (<4 h). That RIC-3 can interact with an endoplasmic reticulum-retained 5-HT3A construct, combined with the transient interaction observed and lack of significant surface-expressed RIC-3, suggests that RIC-3 may play a role in 5-HT3A receptor folding, assembly, or transport to the cell surface.

The most remarkable morphological feature of the brain is not the highly complex, interconnected neuronal pathways but the enormous number (~10^15) of potentially distinct synaptic connections involved in information transfer between neurons. Moreover, synapses are not static and passive translators of information but can change their efficiency of synaptic transmission (1). This process is termed "synaptic plasticity" and is thought to lie at the heart of the capacity of the brain for learning and memory. Synaptic plasticity may arise pre- or postsynaptically (or both), modulating neurotransmitter release or neurotransmitter receptor responses, respectively.

A fundamental question in neurobiology is how receptor biosynthesis is orchestrated. A vast array of receptor-interacting proteins have been identified as participating in ligand-gated ion channel trafficking and localization (1) and have lead to dramatic advances in our knowledge of synaptic plasticity. The 5-HT3 receptors belong to the Cys loop superfamily of ligand-gated ion channels that includes the nicotinic acetylcholine, GABAA, and glycine receptors. The structural relationship (2–5) of the members of this group suggests that their folding and assembly may involve similar posttranslational chaperone-mediated events (6–8).

The forward transport of these receptors requires the appropriate assembly of specific subunits and release from the endoplasmic reticulum (ER) (6,7). Specific assembly signals have been identified in receptors for GABAA (9), glycine (10), and acetylcholine (11). The export of receptors from the ER represents a critical checkpoint for surface expression, with quality control within the lumen of the ER being performed by the resident chaperone proteins (9, 12). In addition, cytoplasmically exposed ER retention signals within the receptors have been identified as elements that control protein export from the ER (13). The failure to pass these quality control checks results in ER-associated degradation by the proteasome (12, 14, 15).

In addition to the generalized chaperone molecules found in the ER, a growing list of selective (to varying degrees) chaperone molecules has been discovered over the last few years. These molecules are responsible for the recruitment of the members of the Cys loop superfamily of ligand-gated ion channels. Critical control steps include receptor folding and assembly, surface transport, synaptic targeting/clustering, and stability. Molecules implicated in receptor folding and assembly include cyclophilin (ACHR and 5-HT3 receptor) (16, 17), RIC-3 (ACHR) (18, 19), Plic-1 (GABAA receptor) (14), and possibly GRIF-1 (20). Molecules implicated in receptor transport include rapsoyn and 14-3-3eta (ACHR) (21, 22), GABAA receptor-associated protein and Plic-1 (GABAA receptor) (14, 23, 24), and gephyrin (glycine receptor) (25). Finally, selective molecules involved in synaptic targeting, clustering, and surface stability include rapsoyn and neuregulin (ACHR) (26, 27), gephyrin and collybistin (glycine receptor) (28, 29), and Plic-1, gephyrin, Huntington-associated protein 1, and GABAA receptor-associated protein (GABAA receptor) (30–33).

Remarkably, despite the size of this growing list and apart from an implication for cyclophilin (based on modulation of receptor function) (16), no molecule has been implicated in the recruitment of 5-HT3 receptors. As RIC-3 has been proposed to inhibit 5-HT3 receptor expression (19), we addressed the role of RIC-3 on 5-HT3A transport to the cell surface. RIC-3 was identified in a genetic screen for molecules that are required to maintain AChR function (18). Characterization of RIC-3 confirmed that it is required for the production of functional AChRs (18, 19). The predominant localization of RIC-3 to the ER and not at synapses, combined with the observation that AChRs failed to exit the neuronal cell body in the absence of RIC-3 (19), is highly suggestive of a role in AChR transport.
RIC-3 promotes 5-HT$_3$ receptor expression

RIC-3 promotes 5-HT$_3$ receptor expression. However, a contradictory study (34) reports that RIC-3 is not involved in the transport of AChR α7 receptors to the cell surface. Instead, the function of RIC-3 appears to be at the level of protein folding and is essential for the generation of the α7 ligand (α-bungarotoxin) binding site. Furthermore, RIC-3 was found at the cell surface associated with α7 (34), raising the question regarding the location of RIC-3 chaperone activity.

We investigated the role of RIC-3 on the trafficking of 5-HT$_3$A receptors and discovered a significant role in the enhancement of receptor recruitment to the cell surface. The interaction of RIC-3 with 5-HT$_3$A occurs within the ER and is transient, and RIC-3 is not detected at the cell surface. We propose that RIC-3 plays a role in the transport of 5-HT$_3$A receptors, possibly by enhancing protein folding and/or stabilization.

MATERIALS AND METHODS

Cell Culture and Transfection—Simian COS-7 cells (ATCC CRL 1651) and human embryonic kidney (HEK293) cells were maintained in DMEM (Invitrogen) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 μg/ml streptomycin, and 100 units/ml penicillin in an atmosphere of 5% CO$_2$. Exponentially growing cells were transfected by electroporation (400 V, infinity resistance, 125 farads, Bio-Rad Gene Electropulser II). 10 μg of DNA was used for each transfection (2 × 10$^6$ cells) with equimolar ratios of expression constructs. Cells were analyzed 12–48 h after transfection.

DNA Constructs—Human 5-HT$_3$A subunit cDNAs were expressed from the mammalian expression vector pGWI (8). The 5-HT$_3$A-Myc and 5-HT$_3$A-HA were tagged with the Myc epitope (EQKLISEEDL) or the hemagglutinin (HA) epitope (YPYDVPDYA) between amino acids 5 and 6 (Thr$^5$-tag-Thr$^6$ in 5-HT$_3$A) by site-directed mutagenesis as reported previously (8, 13). Human RIC-3 was a kind gift from M. Treinin (Hebrew University) and was subcloned into pGWI by PCR (In-HindIII/ XbaI sites).

Antibodies—Anti-HA and anti-Myc monoclonal antibodies were used directly as supernatant (20 μg/ml) or purified on immobilized protein A. Antiserum to RIC-3 was raised in sheep using either an extracellular/ luminal (depending on whether RIC-3 is expressed on the cell surface) epitope (RIC-3b, SDGQPTGARFQHSL) or an intracellular epitope (RIC-3a, KAYTGSLMKRRNF) as the antigen. RIC-3b was used for immunofluorescence/ELISA, and RIC-3a was used for immunoprecipitations. Neither RIC-3 antibody produced a significant signal by immunofluorescence on rat neurons (hippocampal). The secondary antibodies, goat anti-mouse Alexa Fluor 488/568 and donkey anti-sheep Alexa Fluor 488, were purchased from Molecular probes and used at 1 μg/ml.

The secondary antibody, sheep anti-mouse horseradish peroxidase (HRP), was purchased from Amersham Biosciences and used at 1/1000.

Immunofluorescence—COS7 cells were fixed in 3% paraformaldehyde (in PBS), washed twice in 50 mM NH$_4$Cl (in PBS), and blocked (10% FBS, 0.5% bovine serum albumin in PBS) for 30 min. Subsequent washes and antibody dilutions were performed in PBS containing 10% FBS and 0.5% bovine serum albumin for 30 min. Subsequent washes and antibody dilutions were performed in PBS containing 10% FBS and 0.5% bovine serum albumin. Following surface immunofluorescence, cells were permeabilized by the addition of 0.5% Triton X-100 (10 min), and the immunofluorescence protocol was repeated from the NH$_4$Cl step. Where applicable, the fluorophores, Alexa 488 and Alexa 568, were used to detect surface or total receptor populations, respectively. Cells were examined using a wide-field imaging system (Imprisision).

Quantification of Cell Surface Expression—HEK293 cells were plated into 96-well dishes. Eight transfections (2 μg of 5-HT$_3$A-HA and 0–8 μg of RIC-3, total DNA maintained at 10 μg using GABAA receptor α-1 cDNA) were used in each dish (with each condition in sextuplet). Cells were incubated in 3% paraformaldehyde (in PBS). Cell surface detection was performed in the absence of detergent, and total expression levels were determined following Triton X-100 treatment (0.5% for 15 min). Cells were washed twice in 50 mM NH$_4$Cl (in PBS) and blocked (10% FBS, 0.5% bovine serum albumin in PBS) for 1 h. Subsequent washes were performed in blocking buffer. Immunofluorescence was determined using an HRP-conjugated secondary antibody and assayed using 3,3′,5,5′-tetramethylbenzidine (Sigma) as the substrate, with detection at 450 nm after 30 min following the addition of 0.5 mM H$_2$O$_2$. The reaction rate was determined to remain linear for up to 1 h.

Membrane Potential Assay—HEK293 cells were plated into 96-well dishes (Molecular Devices). Eight transfections (as above) were used for each dish. After 24 h, cells were incubated in Red Membrane Potential assay solution diluted 1:10 (Molecular Devices) for 1 h at room temperature. A range of concentrations of 5-HT (in Hanks’ solution) was added automatically by the Flexstation HIB apparatus and responses recorded over 90 s using SoftMax Pro 4.6.16 to analyze the results and plot dose-response curves according to the manufacturer’s instructions. Each point is the average of at least 6 wells monitored over a total area of ~6 mm$^2$.

Immunoprecipitation—HEK293 cells were l-methionine-starved for 30 min before being labeled with [35S]methionine (0.2 μCi/cm$^2$ dish, Translabel ICN/Flow) for 4 h. Where appropriate, cells were chased in DMEM/FBS in the presence of 100 μg/ml cycloheximide for time indicated. Cells were lysed in 10 mM sodium phosphate buffer containing 5 mM EDTA, 5 mM EGTA, 50 mM sodium fluoride, 50 mM sodium chloride, 1 mM sodium orthovanadate, 5 mM sodium pyrophosphate, 2% Triton X-100, 0.5% deoxycholate, 0.1 mM phenylmethylsulfonyl fluoride, 10 mM mg/ml leupeptin, 10 mg/ml antipain, 10 mg/ml pepstatin, and 0.1 mg/ml aprotinin (lysis buffer). Immunoprecipitations were performed as described previously (8) and analyzed by SDS-polyacrylamide gel electrophoresis followed by autoradiography.

Cell Surface Biotinylation—Cells were washed three times in cold PBS, and cell surface proteins were biotinylated using 0.5 mg/ml sulfo-NHS-LC-biotin (Pierce) for 30 min on ice followed by quenching in cold PBS containing 100 mM glycine. Cells were lysed in PBS containing 1% Triton X-100, 0.5% deoxycholate, and 1 mM protease inhibitors overnight using NeutrAvidin-agarose (Pierce) at 4 °C. Beads were washed five times and bound protein eluted in SDS-PAGE sample buffer.

RESULTS

To examine the subcellular distribution of 5-HT$_3$A receptors and RIC-3, heterologous expression in COS-7 cells was utilized. COS-7 cells were chosen because of their flattened phenotype, offering higher morphological resolutions of intracellular structures. To facilitate biochemical and morphological analyses, the 5-HT$_3$A subunit was tagged using the epitopes of HA or Myc. These epitope tags were added to the amino terminus of 5-HT$_3$A between amino acids 5 and 6 of the mature polypeptide (downstream from the predicted signal sequence cleavage site) to create 5-HT$_3$A-HA or 5-HT$_3$A-Myc. No functional effects of these tags were evident (8).

It has been reported previously (8) that 5-HT$_3$A-HA can access the cell surface and function as a homomeric 5-HT$_3$ receptor. In support of this assertion, surface immunofluorescence of the HA epitope can be detected in the absence of detergent, where it is evenly distributed in a punctate pattern (Fig. 1B). Intracellular staining is also evident (Fig. 1A). In higher expressing cells (not shown), receptors often exhibited localization to filopodial structures, as observed elsewhere (32). Upon permeabilization, the majority of intracellular receptors are observed in an ER-like pattern (8). In addition, small punctate spots are observed, indicative of their presence in transport vesicles (36). Upon the co-expression of 5-HT$_3$A-HA and RIC-3, a more robust cell surface expression of 5-HT$_3$A-HA is observed (Fig. 1D). Moreover, a striking localization to filopodia upon co-expression is most likely a result of increased expression rather than a distinct targeting mechanism. In addition to increased surface staining, a significant increase in immunofluorescence is observed in permeabilized cells (Fig. 1C).

Given the apparent increase in cell surface levels of 5-HT$_3$A, we endeavored to explore the subcellular localization of recombinantly expressed RIC-3 using the antibody raised against the extracellular/luminal (depending on localization to the surface/intracellular compartments, respectively) region. It should be noted that although earlier analysis programs predicted two transmembrane domains (18), more recent data bases such as those for SignalP 2.0 (34) and SignalP 3.0 suggest that the first transmembrane region is a signal sequence. Regardless, the epitope used in this study is predicted to be extracellular/luminal in either model. In the presence of detergent, a high level of RIC-3 is detected, showing a highly clustered distribu-
RIC-3 Promotes 5-HT<sub>3</sub> Receptor Expression

Fig. 1. Immunocytochemical localization of 5-HT<sub>3A</sub> receptor and RIC-3 in COS-7 cells. A and B, COS-7 cells expressing 5-HT<sub>3A</sub>-HA were probed with antibodies against the extracellular/luminal HA epitope in the absence of detergent followed by secondary antibodies conjugated to Alexa Fluor 488 to label surface receptors (B). The same cells were then permeabilized and processed as above, using secondary antibodies conjugated to Alexa Fluor 568 to label total receptors (A). C and D, COS-7 cells expressing 5-HT<sub>3A</sub> and RIC-3 (1:1 ratio) were processed as for A and B to detect total (C) and surface (D) 5-HT<sub>3A</sub>. E and F, COS-7 cells expressing RIC-3 were probed with antibodies against the extracellular/luminal region of RIC-3 in the presence (E) or absence (F) of detergent followed by secondary antibodies conjugated to Alexa Fluor 488. G and H, COS-7 cells expressing RIC-3 were probed with antibodies against the extracellular/luminal region of RIC-3 (G) and an antibody against BiP (H) in the presence of detergent followed by secondary antibodies conjugated to Alexa Fluor 488 (RIC-3) or Alexa Fluor 568 (BiP). The scale bars represent 20 μm.

Fig. 2. Quantification of 5-HT<sub>3A</sub>-Myc expression levels in the presence of increasing amounts of RIC-3. HEK293 cells transfected with 2 μg of cDNA encoding 5-HT<sub>3A</sub>-Myc and varying (0–8 μg) amounts of RIC-3 are shown. GABA<sub>A</sub> receptor α-1 cDNA was included to ensure that equal amounts of cDNA (10 μg) were used in all transfections. Surface receptor levels were detected using antibodies to Myc followed by secondary antibodies conjugated to HRP in the absence of detergent. Total receptor levels were determined as above, in the presence of detergent. Surface/total ratios were derived from this data set. Each value represents the mean ± S.E. of at least six determinants in at least three independent experiments. * significant difference from the control without RIC-3 (p < 0.001, t test).

HA, positive signals are apparent for cell surface receptors (mock-transfected cell values have been subtracted) (Fig. 2). Increasing amounts of RIC-3 cDNA (2–8 μg) were co-transfected with 2 μg of 5-HT<sub>3A</sub>-HA. In support of a role in the recruitment of 5-HT<sub>3A</sub> receptors to the cell surface by RIC-3, cell surface levels of 5-HT<sub>3A</sub>-HA were enhanced as RIC-3 was increased. The most significant enhancement occurred at equimolar or 1.2 ratios, with diminishing additional effects at higher ratios. An examination of the ratio between surface and total receptor levels supported this observation and suggests that the transport pathway may become limiting for some other factor. Interestingly, a small increase in total receptor expression was observed, becoming significant when higher amounts of RIC-3 were co-transfected. This result suggests that RIC-3 may be exerting a stabilizing effect on 5-HT<sub>3A</sub>.

To assess the impact of RIC-3 on 5-HT<sub>3A</sub> receptor activation, we utilized a membrane potential reagent and analysis using a Flexstation ("mini FLIPR") apparatus (Molecular Devices). 5-HT<sub>3A</sub> was co-transfected with increasing amounts of RIC-3 (0–8 μg). Maximal responses to a range of 5-HT concentrations were plotted against log 5-HT concentration to yield a dose-response curve for each ratio of 5-HT<sub>3A</sub>:RIC-3. In support of the ELISA results, increasing maximal responses were observed with increasing ratios of co-transfected RIC-3 (Fig. 3). However, the EC<sub>50</sub> for 5-HT responses was unaltered for the different ratios, ranging from 1.85 to 2.4 μM, as reported previously for 5-HT<sub>3A</sub> (35). Thus, these results are consistent with an increase in cell surface receptor number and a lack of change in the apparent potency of 5-HT.

To determine the existence of direct protein interactions between 5-HT<sub>3A</sub>-HA and RIC-3, we performed co-immunopre-
and residence at, the plasma membrane.

interaction can occur within the ER. However, it is possible
cDNA for RIC-3. The Hill coefficients are not significantly different,
control without RIC-3 (Fig. 4)

transfections to ensure equal amounts of cDNA (10 μg). After 24 h, cells were
loaded with the membrane potential reagent and exposed to varying concentrations of 5-HT, and responses were recorded for 1 min
agarose and probed for the existence of RIC-3 by Western

To address whether the 5-HT3A-HA and RIC-3 interaction is

Because 5-HT3 receptor assembly occurs within the ER, and
because this is the predominant location for RIC-3, it seems likely that their interaction would at least initially occur within
this compartment. To confirm that these two molecules are able to interact within the ER, we repeated the experiment using a 5-HT3A construct with an engineered ER retention
signal, 5-HT3A CRAR7, that has been shown to be retained in the
ER (13). The results of this experiment (Fig. 4B) are identical
to those for the wild-type 5-HT3A-HA, confirming that the
interaction can occur within the ER. However, it is possible that the interaction is maintained throughout its transport to,
and residence at, the plasma membrane.

To address whether the 5-HT3A-HA and RIC-3 interaction is
transient, we radiolabeled HEK293 cells expressing 5-HT3A-HA
and RIC-3 with [35S]methionine, and then radiolabel was
removed and cells were chased in the presence of excess cold
methionine (DMEM/FBS) for 0–4 h (Fig. 4C). Over this period
of time, we found that the 5-HT3A-HA expression level was
stable. However, the amount of RIC-3 co-immunoprecipitating
was reduced significantly, suggesting that the interaction between 5-HT3A and RIC-3 is transient, lasting just hours.

Given the recent report of RIC-3 at the cell surface when
co-expressed with the nicotinic acetylcholine α7 subunit in
A7–3 cells (34), we addressed whether the same might be true
for 5-HT3A and RIC-3. The cell surface of HEK293 cells
expressing RIC-3 or RIC-3 and 5-HT3A-HA were biotinylated. The
biotinylated surface proteins were purified using NeutrAvidin-
arose and probed for the existence of RIC-3 by Western
blotting. These results show that RIC-3 was not present at the

DISCUSSION

RIC-3 was identified in a genetic screen in Caenorhabditis
elegans for suppression of a dominant mutation in the acetyl-
choline receptor subunit DEG-3 (18). The DEG-3 mutation
leads to the production of a non-desensitizing channel that is
responsible for causing necrotic death to neurons. In this
screen, mutations in RIC-3 were found to suppress this toxicity,
implying an essential role for RIC-3 in acetylcholine receptor
function. This hypothesis was confirmed by electrophysiologi-
al analysis, in which responses to acetylcholine, nicotine, and
levamisole, but not GABA, were eliminated in ric-3 mutant

cells (18). Furthermore, RIC-3 was localized to neuronal cell
bodies, as were DEG-3 receptors in ric-3 mutants, suggesting
an essential role for RIC-3 in the ER on the formation of
nAChR.

Later analysis extended this role of RIC-3 to human nACh α7 receptors (19). Remarkably, an inhibitory effect of RIC-3 was
observed on the function of nACh αβ2 and αβ4 and 5-HT3
receptors, with no effect on glycine receptors. In contrast to the
apparent requirement for RIC-3 on DEG-3 transport from the
cell body (18), Williams et al. (34) identified no role for RIC-3 in
the transport of nAChR α7 receptors to the cell surface. Instead
these authors determined a role in the generation of the ligand
(α-bungarotoxin) binding site. Although this role might occur
within the ER and be a prerequisite for export of nACh α7
receptors to the cell surface, the findings of this study suggests
that surface nACh α7 occurs in the absence of exogenous RIC-3
and that the nACh α7-RIC-3 interactions were present on the
cell surface.

Given the requirement for RIC-3 in the transport of DEG-3
(18) and the folding of α7 (34), the inhibitory function of RIC-3
on nACh αβ2 and αβ4 and 5-HT3 receptors (19), and the lack
of effect of RIC-3 on GABAα receptors or glycine receptors (18, 19), we
decided to investigate the role of RIC-3 on the surface transport of GABAAα
receptor transport to the cell surface. First, in agreement with
earlier findings for GABAA receptor function (18), we found no
modulatory role for RIC-3 on the surface transport of GABAAα
β2 receptors (results not shown). In contrast to the inhibition
of RIC-3 on 5-HT3 receptor function, we observed a robust
enhancement of 5-HT3A receptor surface expression and
function. It is not clear why our findings are contradictory, but they
may reflect differences in the expression system (oocytes versus
HEK293/COS-7 cells) or species differences of the 5-HT3A
cDNA (murine versus Homo sapien).

Regardless, we observed clear increases in cell surface
immunofluorescence in the presence of RIC-3, leading to the
dramatic localization of 5-HT3α to filopodia. This apparent
increase in filopodial association is most likely the result of an
increased surface expression rather than specific re-localiza-
tion because of RIC-3, because a similar distribution in the
same cell type has been reported previously when expression at
higher levels was evident (32). The ER is a critical checkpoint
in the transport of cell surface proteins. In keeping with a role
for RIC-3 within this compartment, RIC-3 exhibits co-localiza-
tion with the ER chaperone, BiP. Furthermore, we could not
detect significant levels of RIC-3 at the cell surface by either
immunofluorescence or cell surface biotinylation. This finding
appears to contradict the results observed previously (34).
However, an important difference is that in the previous study,
cells co-expressed nACh α7 receptors. Thus RIC-3 may be
stabilized at the cell surface by α7 receptors. However, this is not

Fig. 3. Determination of receptor activation by 5-HT in the presence of increasing amounts of RIC-3. HEK293 cells trans-

fected with 2 μg of cDNA encoding 5-HT3A-Myc and varying amounts (0–5 μg) of RIC-3 are shown. GABAα receptor α-1 cDNA was used in all
transfections to ensure equal amounts of cDNA (10 μg). After 24 h, cells were

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the case for 5-HT₃A receptors, in keeping with the low level, transient interaction that can be detected. Furthermore, significant levels of surface RIC-3 could not be determined when co-expressed with 5-HT₃A. The low level of detectable interactions between 5-HT₃A and RIC-3 is consistent with a transient or low affinity interaction. Further studies are required to confirm and map the interaction sites to address the mechanism of action of RIC-3 on 5-HT₃A receptors.

Using a cell-based ELISA method to quantify 5-HT₃A receptor recruitment, we observed that the increase in 5-HT₃A receptor surface expression related to the level of RIC-3, a result supported by our functional analysis. Interestingly, an increase in total receptor levels was consistently observed, particularly when RIC-3 was present in excess. This is unlikely to be because of a specific transcriptional regulation, because all flanking regions of the 5-HT₃A have been removed, and receptor expression is driven from the vector promoter. Furthermore, GABA<sub>A</sub> receptor expression from the same vector is unaffected by RIC-3 expression (results not shown). Rather, this would be consistent with a role for RIC-3 in stabilizing 5-HT₃A receptor subunits by a mechanism such as promoting receptor folding (as observed for nACh α7 receptors) (34) or permitting more receptors to avoid degradation and thus, traffic to the cell surface (in contrast to the mechanism observed previously for nACh α7 receptors) (34). When we examined the ratio of surface to total receptors, it appeared that the enhancement in cell surface levels became saturated at low levels (1:2 ratio for 3A to RIC-3) of co-transfected RIC-3, suggesting the limitation of some other factor(s) in receptor transport.

Given the effect of RIC-3 on both surface and total 5-HT₃A receptor levels, the ER localization of RIC-3 (and absence from the cell surface) and the transient, low level interaction with 5-HT₃A receptors suggest that RIC-3 promotes some posttranslational modification that is a prerequisite for protein structure/stability and transport to the cell surface. In contrast to DEG-3 in C. elegans, RIC-3 is not an absolute requirement for the surface expression of 5-HT₃A in COS-7/HEK293 cells, as it is not endogenously expressed (34). An attractive yet highly speculative possibility to explain the striking variability in the promotion or inhibition of receptor function (18, 19, 34) is that RIC-3 may play a role in defining receptor composition by promoting the folding of certain receptor subunits into a particular conformation that is either a prerequisite for or a barrier to assembly with another subunit. The nAChR α7 receptor may be able to traffic in either conformation (37, 38).

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