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Comparison of seven different heterologous protein expression systems for the production of the serotonin transporter

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Abstract

The rat serotonin transporter (rSERT) is an *N*-glycosylated integral membrane protein with 12 transmembrane regions; the *N*-glycans improve the ability of the SERT polypeptide chain to fold into a functional transporter, but they are not required for the transmembrane transport of serotonin per se. In order to define the best system for the expression, purification and structural analysis of serotonin transporter (SERT), we expressed SERT in *Escherichia coli*, *Pichia pastoris*, the baculovirus expression system and in four different stable mammalian cell lines. Two stable cell lines that constitutively expressed SERT (Imi270 and Coca270) were constructed using episomal plasmids in HEK293 cells expressing the EBNA-1 antigen. SERT expression in the three different inducible stable mammalian cell lines was induced either by a decrease in temperature (cell line pCytTS-SERT), the addition of tetracycline to the growth medium (cell line T-REx-SERT) or by adding DMSO which caused the cells to differentiate (cell line MEL-SERT). All the mammalian cell lines expressed functional SERT, but SERT expressed in *E. coli* or *P. pastoris* was nonfunctional as assessed by 5-hydroxytryptamine uptake and inhibitor binding assays. Expression of functional SERT in the mammalian cell lines was assessed by an inhibitor binding assay; the cell lines pCytTS-SERT, Imi270 and Coca270 contained levels of functional SERT similar to that of the standard baculovirus expression system (250,000 copies per cell). The expression of SERT in induced T-REx-SERT cells was 400,000 copies per cell, but in MEL-SERT it was only 80,000 copies per cell. All the mammalian stable cell lines expressed SERT at the plasma membrane as assessed by [^{3}H]-5-hydroxytryptamine uptake into whole cells, but the V_{max} for the T-Rex-SERT cell line was 10-fold higher than any of the other cell lines. It was noticeable that the cell lines that constitutively expressed SERT grew extremely poorly, compared to the inducible cell lines whose growth rates were similar to the parental cell lines when not induced. In addition, the cell lines MEL-SERT, Imi270 and T-REx-SERT all expressed fully *N*-glycosylated SERT and no unglycosylated inactive protein, in contrast to the baculovirus expression system where the vast majority of expressed SERT was unglycosylated and nonfunctional.

Keywords: Membrane protein; Heterologous overexpression

1. Introduction

The initial problem that has to be addressed when studying the structure and function of membrane proteins is which heterologous protein expression system should be used for the production of the membrane protein of interest. Despite extensive experience from a wide variety of expression systems and expressed proteins, it is not easy to predict which system will be best for a given mammalian membrane protein [1]. 'Best' in this context could mean good cell surface expression, the presence of functional protein or high levels of functional protein

Abbreviations: BHK, baby hamster kidney; CMV, cytomegalovirus; DABCO, diazabicyclo[2.2.2]octane; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethylsulfoxide; EBNA, Epstein–Barr virus nuclear antigen; endo H, endoglycosidase H; FBS, foetal bovine serum; FITC, fluorescein isothiocyanate; GST, glutathione *S*-transferase; 5HT, 5-hydroxytryptamine (serotonin); HEK, human embryonic kidney; IPTG, isopropylthiogalactoside; MEL, mouse erythroleukaemia; NP40, nonidet P40; PAGE, polyacrylamide gel electrophoresis; PNGase F, peptide: *N*-glycosidase F; PBS, phosphate-buffered saline; RTI55, 2β-carbomethoxy-3β-(4-iodophenyl)tropane; SDS, sodium dodecyl sulfate; SERT, serotonin transporter; TBS, Tris-buffered saline

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production, or a combination of these. Published expression studies are often limited to only one or two expression systems, which makes it very difficult to draw meaningful conclusions about which is the best expression system for a particular membrane protein. We have decided to approach the expression problem by choosing one particular membrane protein and studying its expression in seven different systems. The membrane protein we chose was the rat serotonin transporter (rSERT) because it has a number of particular characteristics that make it both a user-friendly and challenging example of a mammalian membrane protein to be overexpressed.

Serotonin transporter (SERT) is expressed at the plasma membrane in many mammalian cell types including neurons, platelets and placenta, where it transports serotonin into the cell coupled to the inward movement of Cl^- and Na^+ down their concentration gradients [2,3]. There are predicted to be 12 transmembrane regions in SERT with the N- and C-terminus intracellular and a large extracellular loop between transmembrane regions 3 and 4 that contains two *N*-glycans. This model is supported by topological studies using antibody labelling techniques [4] and the binding of maleimides to SERT mutants with single-reactive cysteines [5]. To study the function of the *N*-glycans, a mutant SERT lacking both *N*-glycosylation sites was constructed; unglycosylated SERT transported [^{3}H]-5HT and bound the inhibitor [^{125}I]-RTI55 with identical kinetic parameters to native SERT, but functional expression levels were 20-fold lower compared to glycosylated SERT. This suggested that the *N*-glycans were not essential for SERT activity per se, but that they improved the folding and/or stability of SERT in the membrane [6]. The fact that the *N*-glycans on SERT are important in the interaction of SERT with the molecular chaperone calnexin lends further support to this hypothesis [7]. One characteristic of SERT that makes it particularly suitable for expression studies is that it binds antidepressant drugs and cocaine analogues with very high affinity. Binding assays can be performed on crude membrane preparations from whole cells, allowing the accurate determination of functional SERT molecules per cell, even if SERT is sequestered in the endoplasmic reticulum and therefore incapable of transporting serotonin into the intact cell [8]. Short epitope tags can also be placed at the C terminus without impairing the expression levels or the function of SERT [6,8,9]. Here we describe the production of SERT in seven different expression systems with analysis by four different criteria: (1) measurement of functional expression levels by inhibitor binding assays; (2) measurement of cell surface-expressed, functional SERT by serotonin uptake assays; (3) determination of the major cellular compartments containing expressed SERT by immunostaining of whole cells and confocal microscopy; (4) determination of the glycosylation status of the expressed SERT by Western blotting.

2. Materials and methods

2.1. Expression and analysis of SERT in Escherichia coli

The coding region of the cDNA for rSERT [10] was amplified by PCR using primers (5′–3′) TGAGAATTCGCAGCATGGAGACCACACCCTTAAATTC and GCTGAATTCTTACACAGCATT (initiator Met underlined). All PCR products were verified by DNA sequencing. The 1913-bp product was digested with *Eco*RI and the resulting 1901-bp fragment was ligated into *Eco*RI-digested pTrcHisA (trc promoter, N-terminal hexahistidine tag; Invitrogen), pRSETA (T7 promoter, N-terminal hexahistidine tag; Invitrogen) and pGEX-KG (tac promoter, N-terminal GST; modified form [11] of pGEX-2T from Amersham Pharmacia). See Fig. 1 for a diagrammatic representation of SERT fusions constructed. Expression experiments were carried out using freshly transformed *E. coli* BL21(DE3)pLysS, even when using the tac and trc promoters, to allow direct comparison with expression results using the T7 promoter. Cultures were grown in 2 × TY medium, either with osmotic stress (plus 1 M sorbitol and 2.5 mM betaine [12]) or without, to A_{600} of 0.6–0.7 and expression was induced by addition of IPTG to 1 mM. Following 1.5–2-h induction at 37 °C or room temperature, cells were harvested, and membranes were prepared as described in Ref. [13].

2.2. Expression and analysis of SERT in Pichia pastoris

*Eco*RI-digested PCR product (see *E. coli* methods above), encompassing the rSERT coding region and Kozak sequence, was ligated into *Eco*RI-digested pHIL-D2 *Pichia* vector (Invitrogen), to generate pHIL-D2-rSERT (Fig. 1). This

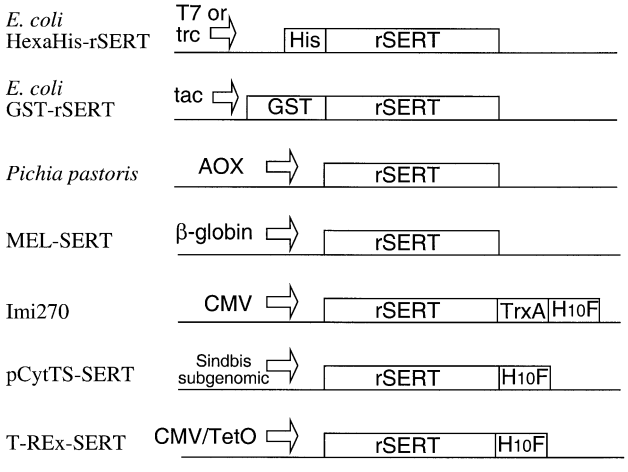

Fig. 1. Schematic diagram of SERT cDNAs expressed in each expression system. Boxes (not to scale) represent open reading frames encoding the rat serotonin transporter (rSERT) and various tags (His, hexa-histidine tag; GST, glutathione *S*-transferase; TrxA, thioredoxin; $H_{10}F$, deca-histidine tag followed by a FLAG tag). The arrows represent the promoter used to expressed SERT in each system. See Section 2 for further details.

vector was linearised with *Not*I and used to transform *P. pastoris* GS115 and SMD1168 as described previously [14]. SERT was integrated into the alcohol oxidase (AOX) locus in the *Pichia* genome, which resulted in the slow methanol utilising (Mut^s) phenotype. Recombinant clones were screened for their Mut^s phenotype by scoring growth on minimal dextrose medium (MD, 1.34% (w/v) yeast nitrogen base without amino acids, 4×10^{-5}% (w/v) biotin, 2% (w/v) glucose) and minimal methanol medium [MM, 1.34% (w/v) yeast nitrogen base without amino acids, 4×10^{-5}% (w/v) biotin, 0.5% (v/v) methanol] at 30 °C for 48 h.

Expression of the SERT was induced by transferring cells grown in MD, to MM medium for 48 h at 30 °C, 300 rpm. Crude membranes were then prepared as below. Cells were harvested by centrifugation at $9000 \times g$ for 10 min at 4 °C and were suspended in 50 mM Tris·HCl pH 7.4, containing 120 mM NaCl, 5 mM KCl, 1 mM EDTA and protease inhibitors (Complete™, Roche). Cells were lysed by passage through a French Pressure cell at 20,000 p.s.i. and then centrifuged at $10{,}000 \times g$ for 10 min at 4 °C to remove unbroken cells. The supernatant was ultracentrifuged at $120{,}000 \times g$ for 2 h at 4 °C. The resulting membrane pellets were suspended in the above buffer using an Ultra-Turrax homogeniser and stored at −80 °C.

2.3. Expression of SERT using the baculovirus expression system

Sf9 cells were grown and infected with a recombinant baculovirus that expressed SERT with a FLAG tag at its C terminus (bvSERT-TAG) exactly as previously described [8].

2.4. Construction of a 293-EBNA stable cell line expressing SERT from a constitutive promoter

In order to express an rSERT-TrxA-His10-FLAG fusion protein (Fig. 1) in mammalian cells, a *Not*I/*Xba*I cassette from plasmid pT43NTR-TrxA-H10F [15] containing the *E. coli* thioredoxin gene (*trxA*) followed by DNA encoding an in-frame His_{10} tag and a FLAG tag was ligated into *Not*I/*Xba*I-digested plasmid pCGT125 [6], making plasmid pCGT262. The *Eco*RV/*Xba*I cassette encoding rSERT-TrxA-His10-FLAG in plasmid pCGT262 was subsequently ligated into *Pvu*II/*Nhe*I-digested plasmid pREP4 (Invitrogen) to make plasmid pCGT270. The presence of TrxA at the C terminus of SERT was an attempt to improve the purification properties of SERT (Tate, unpublished results).

293-EBNA (Invitrogen) is a cell line derived from HEK293 cells that stably expresses the EBNA-1 antigen, which is essential for the episomal replication of plasmids such as pREP4 containing the EBV origin of replication, oriP. Cells were grown as an adherent line in DMEM supplemented with 10% FBS and 0.25 mg/ml geneticin at 37 °C in an atmosphere containing 5% CO_2. Cells were passaged every 2–3 days using standard tissue culture techniques. Cultures of 293-EBNA cells were grown in the absence or presence of the SERT inhibitors cocaine or imipramine (1 μg/ml in the media) for 1 week prior to transfection; these three cell lines were each transfected with plasmid pCGT270 by calcium phosphate precipitation. Two days after transfection, the cells were split into medium containing the selective antibiotic hygromycin (0.5 mg/ml). One week later, single colonies could be seen in the flasks containing inhibitors; the pools of cells were amplified before aliquots were stored in liquid nitrogen or assayed for the presence of SERT. Transfected cells grown in the absence of inhibitors did not yield any hygromycin-resistant colonies.

2.5. Inducible expression of SERT in mouse erythroleukaemic (MEL) cells

The rSERT cDNA was isolated as a *Sal*I/*Not*I fragment from plasmid pCGT109 (rSERT with no tag [6]) and ligated downstream from the β-globin promoter into the *Sal*I/*Not*I sites of plasmid pEV3 [16] to make plasmid pCGT216 (Fig. 1). MEL cells were grown as a suspension culture in DMEM containing 10% FCS and were grown in an atmosphere containing 5% CO_2. Cells (2×10^7 cells in 0.8-ml PBS) were electroporated with 50-μg pCGT216 (960 μF, 0.2 kV) in a 4-mm cuvette. Cells were added to 150 ml of complete medium and then split into 2-ml aliquots per well of a 24-well plate. Selection for the plasmid started 24 h after the transfection with 1 mg/ml G418. Ten clones were produced and amplified. To induce the expression of SERT, cells were split to 10^6 cells/ml in fresh medium and DMSO was added to a final concentration of 2%. Cells were grown for 4 days at 37 °C, harvested and resuspended in 1-ml PBS with protease inhibitors (Complete™, Roche) to about 2.5×10^4 cells/μl for analysis.

2.6. Tetracycline-inducible SERT expression using the T-REx system

The rSERT cDNA was transferred as an *Eco*RV/*Xba*I fragment from plasmid pCGT262 (see above) to plasmid pcDNA4/TO (Invitrogen) to make plasmid pCGT272 containing rSERT expressed from the tetracycline-controlled cytomegalovirus (CMV) promoter. The C-terminal TrxA-His10-FLAG tag at the end of SERT was replaced with a His10-FLAG tag by replacing the *Not*I/*Xba*I fragment with oligonucleotides encoding the amino acid sequence AA-AHHHHHHHHHHDYKDDDDKGG and a termination codon. Plasmid pCGT273 expressing SERT-His10-FLAG from the tetracycline-controlled CMV promoter (Fig. 1) was used to transfect an HEK293 cell line (T-REx-293; Invitrogen) that expressed the tetracycline repressor protein TetR.

The T-REx-293 cell line was grown in DMEM containing 10% FCS and 5 μg/ml blasticidin as an adherent cell culture. The cells were transfected using Lipofectamine 2000 according to the manufacturer's protocol (Life Tech-

nologies) and split into 24-well plates. One day after transfection, the selective antiobiotic Zeocin (Invitrogen) was added to 200 μg/ml to select for stable integrants. Clones growing in individual wells were tested for SERT expression by induction with tetracycline (1 μg/ml for 24 h), harvesting the cells and performing a dot blot probed with the anti-FLAG antiserum (see Western blotting below). The most highly expressing clones as judged from the dot blot were amplified and further characterized.

2.7. Cold-inducible expression of SERT using the pCytTS system

To allow the transfer of the rSERT cDNA into plasmid pCytTS, the *Eco*RV and *Xba*I restriction sites in plasmid pCGT273 were converted respectively to a *Pac*I site and an *Asc*I site. The oligonucleotide TTAATTAA was first ligated into *Eco*RV-digested plasmid pCGT273 and then the oligonucleotide CTAGGGCGCGCC was ligated into the resultant plasmid digested with *Xba*I, to make plasmid pCGT276. The *Pac*I/*Asc*I SERT insert from plasmid pCGT276 was then ligated into *Pac*I/*Asc*I-digested plasmid pCytTS [17] to make plasmid pCGT278 (Fig. 1).

The BHK-21 cell line was grown in DMEM supplemented with 10% FCS in an atmosphere containing 5% CO_2 as a suspension culture. The BHK-21 cells were transfected with plasmid pCGT278 using Lipofectamine 2000 (Life Technologies) according to the manufacturer's protocol. The transfected cells were split into 24-well plates with the antibiotic selection starting 1 day after transfection (10 μg/ml puromycin). Positive clones identified by induction and dot blotting (see below) were serially diluted to ensure a homogeneous cell population and then amplified. Cells were induced by placing a culture containing about 0.5×10^6 cells/ml in an incubator at 29 or 33 °C for 3 days.

2.8. Inhibitor binding assays on crude membrane preparations

A pellet of mammalian cells (10^7 cells) was resuspended in 1-ml PBS and transferred to a 1.5-ml microcentrifuge tube. The cells were pelleted (30 s, $14{,}000 \times g$) and resuspended in 0.1 × PBS containing protease inhibitors (Complete™, Roche). The cells were sheared by passaging seven times through a 26-gauge needle and the crude membranes were pelleted (10 min, $14{,}000 \times g$, 4 °C). The crude membranes were washed once with ice-cold PBS containing protease inhibitors (Complete™, Roche) and then resuspended in a final volume of 1 ml.

Single-point binding assays with the inhibitor [^{125}I]-RTI55 were performed exactly as previously described [6,8]. Briefly, 50-μl reactions were set up in triplicate which contained an aliquot of membranes, 2 nM [^{125}I]-RTI55 (DuPont NEN) and in the absence or presence of 10 μM cocaine to determine nonspecific binding. Membranes were pelleted in a microcentrifuge (10 min, $14{,}000 \times g$, 20 °C), the supernatant carefully removed and the radioactivity bound to the membranes determined on a gamma counter (1261 MultiGamma, Wallac). The amount of membranes added to the assay was sufficient to bind about 5% of the total [^{125}I]-RTI55 present.

2.9. [^{3}H]-5HT transport assays

Mammalian cells were plated onto poly-L-lysine-coated (0.1 mg/ml) 24-well plates. The 293-EBNA-Imi270 cell line was grown for 3 days prior to uptake experiments in medium that did not contain any imipramine. T-REx-SERT cells were grown for 24 h and then induced by the addition of 1.0 μg/ml tetracycline; the cells were grown for a further 16 h before uptake assays were performed. The pCytTS-SERT cell line was grown at 37 °C for 24 h and then transferred to 29 °C for 48 h to induce expression.

Just before the transport assay, the medium was aspirated and the cells were washed with warm TB buffer (10 mM Hepes pH 7.5, 150 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$). The assay volume was 0.4 ml which contained 10^5 cells. The assay was performed at room temperature and was started by the addition of [^{3}H]-5HT and terminated 3 or 6 min later by three washes of ice-cold TB containing 1 μM paroxetine. Radioactivity was released from cells using 2% SDS, which was then quantified by liquid scintillation counting. Nonspecific uptake was defined as the uptake in the presence of 10 μM paroxetine and this was subtracted from the total to obtain the specific uptake. Data were analysed using SigmaPlot 5.0 software package using the Michaelis–Menten equation; kinetic parameters were determined by nonlinear regression.

2.10. Fixing and staining cells for immunofluorescence analysis by confocal microscopy

Cells were grown on poly-L-lysine-coated coverslips in 24-well plates under standard culture conditions for each particular cell line as described above. Generally cells were seeded at about 20–50% confluency and allowed to grow for 1–2 days before use, or before induction. Coverslips were washed in 2-ml PBS in a 35-mm petri dish and then incubated in ice-cold 2% paraformaldehyde in PBS at 4 °C for 15 min. After aspiration, the remaining paraformaldehyde was quenched with 2-ml 50 mM NH_4Ac/PBS for 10 min at room temperature. If cells were permeabilized, nonspecific binding sites were blocked with 2-ml 10% goat serum/0.1% NP40 in TBS for 15 min at room temperature. The primary antibody was then added in 10% blocking buffer and incubated for 1 h at room temperature. Anti-FLAG m2 antibody (Sigma) was used at a dilution of 1:500. After washing three times for 5 min each in TBS/0.02% NP40 (TBS: 20 mM Tris pH 7.6, 137 mM NaCl), the secondary antibody Texas Red-conjugate (Molecular

Probes) was added at 1:500 dilution in 1% goat serum/TBS for 1 h at room temperature. The coverslips were then washed three times for 5 min each in TBS/0.02% NP40, rinsed briefly in water and blotted edge-on to tissue immediately before mounting in 10-μl polyvinylalcohol containing 2.5% DABCO. For specific staining of the plasma membrane, a 0.01 mg/ml solution of concanavalin A–FITC conjugate (Sigma) in PBS was added to the cells before blocking and incubated at 4 °C for 1 h; after washing in PBS, the cells were blocked and probed as above. Cells were visualised using a BioRad MRC1024 confocal microscope.

2.11. Western blotting

Protein samples were solubilized in GSB (20 mM Tris pH6.8, 1% SDS, 10% w/v glycerol, 10 mM 2-mercaptoethanol, 0.1% bromophenol blue) and incubated at 37 °C for 30 min. The proteins were separated on a 9% polyacrylamide gel and transferred to nitrocellulose (ECL, Amersham Biosciences) using standard techniques. Blots were probed with antibodies and developed using the ECL system (Amersham Biosciences). The anti-FLAG m2 monoclonal antibody (Sigma) and the anti-SERT CT-2 antibody [9] were used at a dilution of 1:1000. Treatment of membranes with endoglycosidase H and peptide:*N*-glycosidase F was performed at 37 °C for 1 h using the manufacturer's conditions (New England Biolabs).

3. Results

3.1. Expression of SERT in E. coli

Initial experiments under standard growth conditions showed that SERT could be expressed in *E. coli* both as GST-rSERT or HexaHis-rSERT fusion proteins, under the control of trc, tac and T7 promoters. However, induction of expression was found to be detrimental to cell growth, with low overall levels of expressions (100–200 μg/l of culture), as assessed by Western blotting, with most of the expressed protein found to be in the form of insoluble aggregates. In addition, the fusion protein was very susceptible to in vivo truncation, most likely due to proteolysis. Significant improvements in SERT expression were obtained by decreasing the induction temperature and by the modification of the growth medium (Fig. 2a). Growing *E. coli* in the presence of 1 M sorbitol with the inclusion of the osmoprotectant betaine [12] markedly improved expression levels, the level of proteolysis was significantly reduced, and induction of expression was no longer detrimental to cell growth. Full-length GST-rSERT and hexaHis-rSERT were now found to be associated with the bacterial membranes and expression levels obtained were 2–3 mg/l. The apparent molecular weight of GST-rSERT (80 kDa) and hexaHis-rSERT (64 kDa) on SDS-PAGE represented the full-length fusion protein despite the molecular weights being 95 and 75 kDa, respectively. The full-length nature of the fusion proteins was determined by the reactivity of antibodies that recognized the N-terminal tag and the C terminus of SERT (results not shown). The 175-kDa band

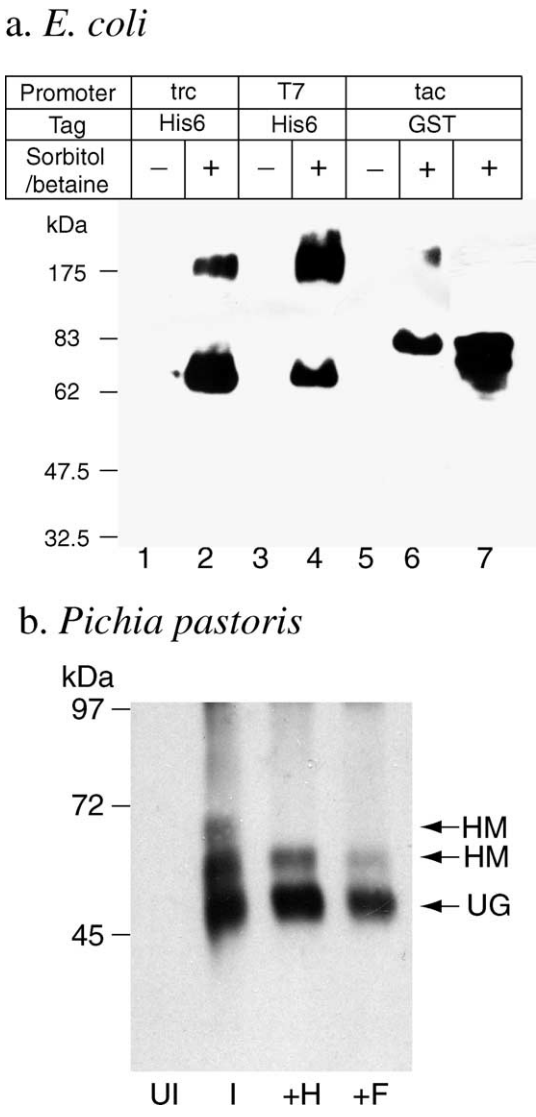

Fig. 2. Western blots of the serotonin transporter expressed in *E. coli* and *P. pastoris*. (a) The serotonin transporter was expressed in *E. coli* BL21(DE3)pLysS, with an N-terminal hexahistidine tag (lanes 1–4) or glutathione *S*-transferase tag (lanes 5–7), using plasmids pTrcHisA (trc promoter; lanes 1 and 2), pRSETA (T7 promoter, lanes 3 and 4) or pGEX-KG (tac promoter, lanes 5–7). Cells were grown to early log phase in 2 × TY medium with or without osmotic stress (1 M sorbitol and 2.5 mM betaine); expression was induced (1 mM IPTG) at room temperature for 2 h. Membrane preparations from the cultures were Western blotted with anti-serotonin transporter C-terminus antiserum (lanes 1–6), and also anti-GST (lane 7). (b) Membranes were prepared from both uninduced (UI) and induced (I) cultures of *P. pastoris* clone B119 (a derivative of strain SMD1168) that contained SERT cDNA under the control of the AOX1 promoter. *N*-Glycans were removed by the treatment of membranes with either endoglycosidase H (+H) or with peptide:*N*-glycosidase F (+F). The unglycosylated (UG) and high-mannose core glycosylated (HM) forms of SERT are indicated by arrows. Membrane preparations were probed with anti-serotonin transporter C-terminus antiserum.

represented an aggregated form of SERT also observed on SDS-PAGE analysis of insect cell membranes containing SERT produced using the baculovirus expression system [6].

Binding of [^{3}H]-imipramine and [^{3}H]-paroxetine to *E. coli*-expressed SERT was examined. In both cases, binding was found to be low affinity, with K_D values of approximately 1 μM (results not shown), compared to nanomolar values for the native transporter. [^{3}H]-Imipramine binding to the bacterial membranes could be displaced by a range of serotonin transporter ligands (at 10 μM) and, to a small extent, by serotonin (at 100 μM). This shows that the affinity of the transporter for serotonin and antidepressant drugs is lower than that of the native protein, suggesting one or more incompletely folded states.

3.2. Expression of SERT in P. pastoris

The rSERT cDNA was inserted into an expression cassette under the control of the inducible alcohol oxidase promoter. Following transformation of *Pichia* strains, the integration of the expression cassette into the yeast genome was confirmed by three methods: (1) selection screening of the slow methanol-utilisation phenotype (Mut^s) of individual recombinants; (2) PCR of *Pichia* genomic DNA with primers to the alcohol oxidase promoter; (3) Southern blotting of individual clones. These tests identified 36 independent clones that contained one to six copies of the SERT cDNA, 16 clones in strain GS115 and 20 clones in strain SMD1168. The *pep4* mutation in SMD1168 causes a deficiency in proteinase A activity, which is required for the proteolytic activation of a number of proteases in *P. pastoris*. SERT expression following methanol induction was examined by Northern blotting, [^{3}H]-imipramine binding and Western blotting of cell membrane preparations. A GS115 clone with the highest expression levels, as judged by Western blotting and [^{3}H]-imipramine binding, was found to have six copies of the expression cassette.

The *Pichia*-expressed SERT was found to be associated with cell membranes and, on Western blotting (Fig. 2b), was observed to migrate at 49, 58 and 65 kDa. The 49-kDa species represented full-length SERT. Treatment of membranes with endoglycosidase H and PNGaseF resulted in a decrease in the amount of the 65- and 58-kDa species but did not result in their complete removal. This is consistent with the presence of *N*-glycans on SERT that is misfolded, with consequently poor accessibility of the *N*-glycans to the glycosidases. The suggestion that expressed SERT was poorly or incompletely folded was substantiated by the absence of [^{3}H]-5HT transport into the rSERT-expressing *Pichia* clones. In addition, binding of [^{3}H]-imipramine and [^{3}H]-paroxetine to membranes was found to be of low affinity ($K_D \sim 1$ μM, results not shown).

3.3. Construction of four different mammalian cell lines for the production of SERT

Overexpression of integral membrane proteins often results in impaired cell growth and can result in positive selection against high expressing clones [1]. In an effort to prevent this happening during the selection of stable cell lines constitutively expressing potentially high levels of SERT, specific SERT inhibitors were added to the cell culture medium. The inclusion of either cocaine or imipramine (1 μg/ml) did not have any effect on the rate of growth of the 293-EBNA cells. Transfection of cells grown in the presence of either cocaine or imipramine led to the generation of hygromycin-resistant cell lines named 293-EBNA-Coca270 and 293-EBNA-Imi270, respectively. No antibiotic resistant colonies were found in transfections of cells grown in the absence of inhibitors. Both Imi270 and Coca270 cells grew very poorly with a doubling time of about 36 h. Characterisation of the cell lines is described in the following section.

The cell lines Imi270 and Coca270 were both grown in the presence of the SERT inhibitors imipramine and cocaine, respectively. Therefore, the poor growth characteristics of the Imi270 and Coca270 cell lines implied that it was the presence of SERT protein itself, and not its activity, that adversely affected cell growth. If this was the case, then by using an inducible mammalian expression system, stable cell lines would be developed in the absence of expressed SERT, and good expression could be produced on induction. Therefore, three different inducible systems were tested for the overexpression of SERT. The characteristics of each expression system are briefly outlined below.

(1) The MEL cell system [16] uses the ability of MEL cells to differentiate in the presence of DMSO to more erythroid-type cells; a consequence of this is the production of haemoglobin, which is under tight developmental control. Placing SERT cDNA under the control of the β-globin promoter allows the expression of the protein on addition of DMSO.

(2) The pCytTS system [17] uses temperature as the inducing agent. The system relies on a temperature-sensitive, non-cytopathic Sindbis virus replicase as the core component. A stable cell line is constructed that constitutively expresses the replicase and SERT in tandem on the same mRNA, which will result initially in the production of the replicase but not of SERT. At 37 °C the replicase is inactive, but if the temperature falls to 29–35 °C the replicase becomes active and produces minus strand replicas of the replicase-SERT mRNA; subsequently, SERT-encoding mRNA is produced from this template using the subgenomic Sindbis virus promoter upstream from SERT. The result is a massive production of SERT mRNA.

(3) The T-REx expression system (Invitrogen) is controlled using the bacterial control elements for expression of the tetracycline transporter. The repressor protein TetR prevents expression of the transporter in the absence of

tetracycline by binding to a specific DNA sequence, the Tet operator site (TetO), in the promoter region and thus prevents transcription. In the presence of tetracycline, the repressor protein is inactivated by tetracycline binding, thus releasing the blockage at the operator site, and hence transcription can proceed. In the T-REx system, cell lines stably producing the repressor protein TetR are stably transfected with DNA encoding SERT under the control of a CMV promoter, but which also contains two tandem TetO sites between the promoter and the SERT cDNA. SERT expression could be induced by the addition of tetracycline to the culture medium.

Stable cell lines for each of the three inducible expression systems were constructed as described in Section 2. The three cell lines were called MEL-SERT216, pCytTS-BHK-SERT278 and T-REx-293-SERT273, which for the sake of brevity will be referred to as MEL-SERT, pCytTS-SERT and T-REx-SERT, respectively (Fig. 1). It was noticeable that the inducible SERT-expressing cell lines grew at the same rate as untransfected cells, in contrast to the poor growth of the Coca270 and Imi270 cell lines that expressed SERT constitutively.

3.4. Comparison of SERT production in four mammalian cell lines

SERT production in the cell lines Imi270, MEL-SERT, pCytTS-SERT and T-REx-SERT was assessed using Western blotting, inhibitor binding assays on crude membranes, [^{3}H]-serotonin uptake into whole cells and confocal microscopy of immunostained cells. For each of the individual inducible cell lines, a time course of expression was performed to determine the optimum time for harvesting (results not shown). Consequently, the MEL-SERT cell line was induced for 4 days, the pCytTS-SERT cell line was induced for 3 days and the T-REx-SERT cell line was induced for 24 h.

Western blots of whole-cell extracts (Fig. 3) showed that SERT was expressed in all four cell lines. The mobility of the SERT-immunoreactive band varied between the expression systems and was due to different *N*-glycosylated states of the transporter; unglycosylated SERT has an apparent M_r of about 55 kDa on SDS-PAGE, except in the Imi270 cell line which expresses a SERT-TrxA fusion with apparent M_r of 70 kDa (Fig. 1). The apparent M_r of 55 kDa is consistent with the expression of full-length SERT [6] despite SERT having a calculated weight of 69 kDa. A comparison of the mobilities after treatment with endoglycosidase H (endoH), which removes high-mannose core *N*-glycans, and with peptide:*N*-glycosidase F (PNGase F), which removes all types of *N*-glycans, defined the glycosylation status of each species. The apparent mobility shifts on SDS-PAGE due to the *N*-glycans in each cell line are listed at the end of the results section in Table 2. SERT produced in the cell line pCytTS-SERT was a mixture of mainly unglycosylated and high-mannose core *N*-glycosylated protein, with no detectable complex *N*-glycosylation. This is very similar to SERT expressed in *Pichia* (Fig. 2b), although the yeast *N*-glycans were slightly larger than high-mannose *N*-glycans in mammalian cells, and in SERT produced using the baculovirus expression system. In contrast, the major SERT species in the cell lines MEL-SERT, Imi270 and T-REx-SERT was the complex *N*-glycosylated species with no detectable unglycosylated SERT. In addition, both T-REx-SERT and Imi270 cell lines contained SERT with some high-mannose core *N*-glycans.

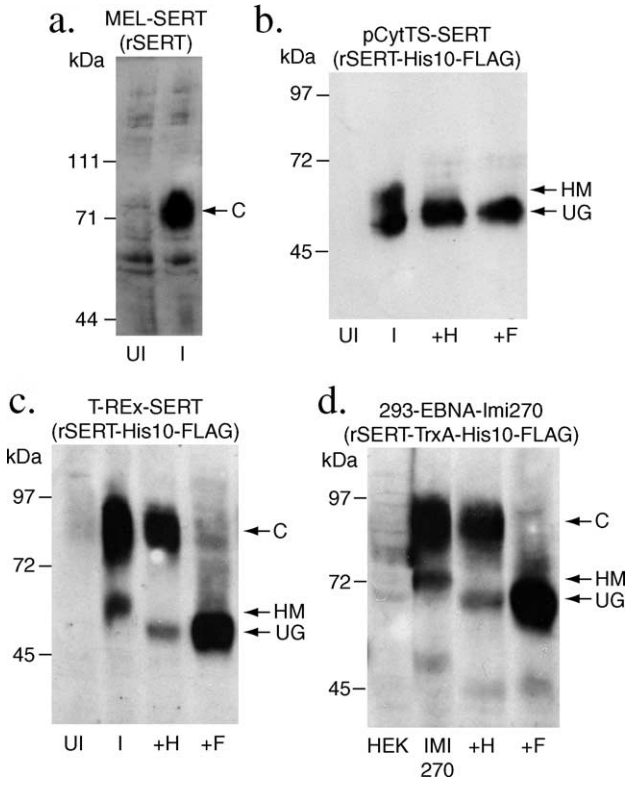

Fig. 3. *N*-Glycosylation of the serotonin transporter expressed in four mammalian cell lines. Crude membranes were prepared from uninduced (UI) and induced (I) cell lines and separated on 9% SDS-polyacrylamide gels which were subsequently transferred to nitrocellulose and probed with either anti-SERT CT-2 (a) or with an anti-FLAG antiserum (b, c, d). *N*-Glycosylation was removed from SERT by the treatment of membranes with either endoglycosidase H (+H) or with peptide:*N*-glycosidase F (+F). The different glycosylated forms of SERT are indicated: UG, unglycosylated; HM, high-mannose core *N*-glycans; C, complex *N*-glycans. The different tags on the expressed SERT are indicated in parentheses (see also Section 2).

The different types of *N*-glycosylation are indicators of the subcellular localisation of SERT. Unglycosylated SERT is predominantly nonfunctional and was found mainly in the ER where it was synthesized. Addition of the high-mannose core *N*-glycan is a co-translational event and only minor trimming of the oligosaccharide occurs in the ER (reviewed in Ref. [18]). Further trimming and elongation of the *N*-glycans occurs in the Golgi apparatus prior to transport of SERT to the cell surface. Thus, the high-mannose form is likely to be intracellular and the complex form is mainly

expressed at or near the plasma membrane. Confocal microscopy of cells stained for SERT using anti-FLAG antibodies (Fig. 4) confirmed the Western blotting results. The cell surface was labelled with FITC-conjugated concanavalin A and this co-localized with SERT in the Imi270 and T-REx-SERT cell lines. In contrast, the pCytTS-SERT cell line produced SERT that was mainly in internal membranes, which is identical for SERT expressed using the baculovirus expression system. The confocal microscopy also highlighted one of the major differences between SERT expressions in the T-REx-SERT and the Imi270 cell lines; only about 30% of the cells in the Imi270 cell line expressed SERT, in contrast to the T-REx cell line where at least 95% of cells expressed SERT. Efforts to improve the Imi270 cell line by cloning single cells after serial dilution were unsuccessful.

Uptake of [^{3}H]-5HT into whole cells showed that the cell surface-expressed SERT in the Imi270 and T-REx-SERT cell lines was fully functional (Fig. 5). However, there were marked differences in the levels of cell surface expression; Imi270 cells had a V_{max} of 28.1 ± 1.5 pmol/min/10^6 cells in comparison to induced T-REx-SERT cells that had a V_{max} of 212 ± 29 pmol/min/10^6 cells (Table 1). One parameter that changed markedly in the Imi270 and T-REx-SERT cell lines was the K_m for 5HT uptake, which varied between 204 nM in uninduced T-REx-SERT and 1854 nM in Imi270. This difference correlated with expression levels so that low copies of SERT per cell gave a low K_m (high affinity) and high copies per cell gave a high K_m (low affinity); note that the number of SERT copies per individual cell in Imi270 is actually higher than in induced T-REx-SERT (see below). This variation in K_m has been observed in the *Xenopus* oocyte expression system and was attributed to the lack of a SERT modulator on overexpression that is essential for high-affinity 5HT transport [19]. The MEL-SERT cell line also showed specific [^{3}H]-5HT uptake but this was not characterised further due to the overall very low SERT expression levels (Table 2). The pCytTS-SERT cell line showed specific [^{3}H]-5HT uptake in the uninduced state (V_{max} 1.62 ± 0.07 pmol/min/10^6 cells), which was double

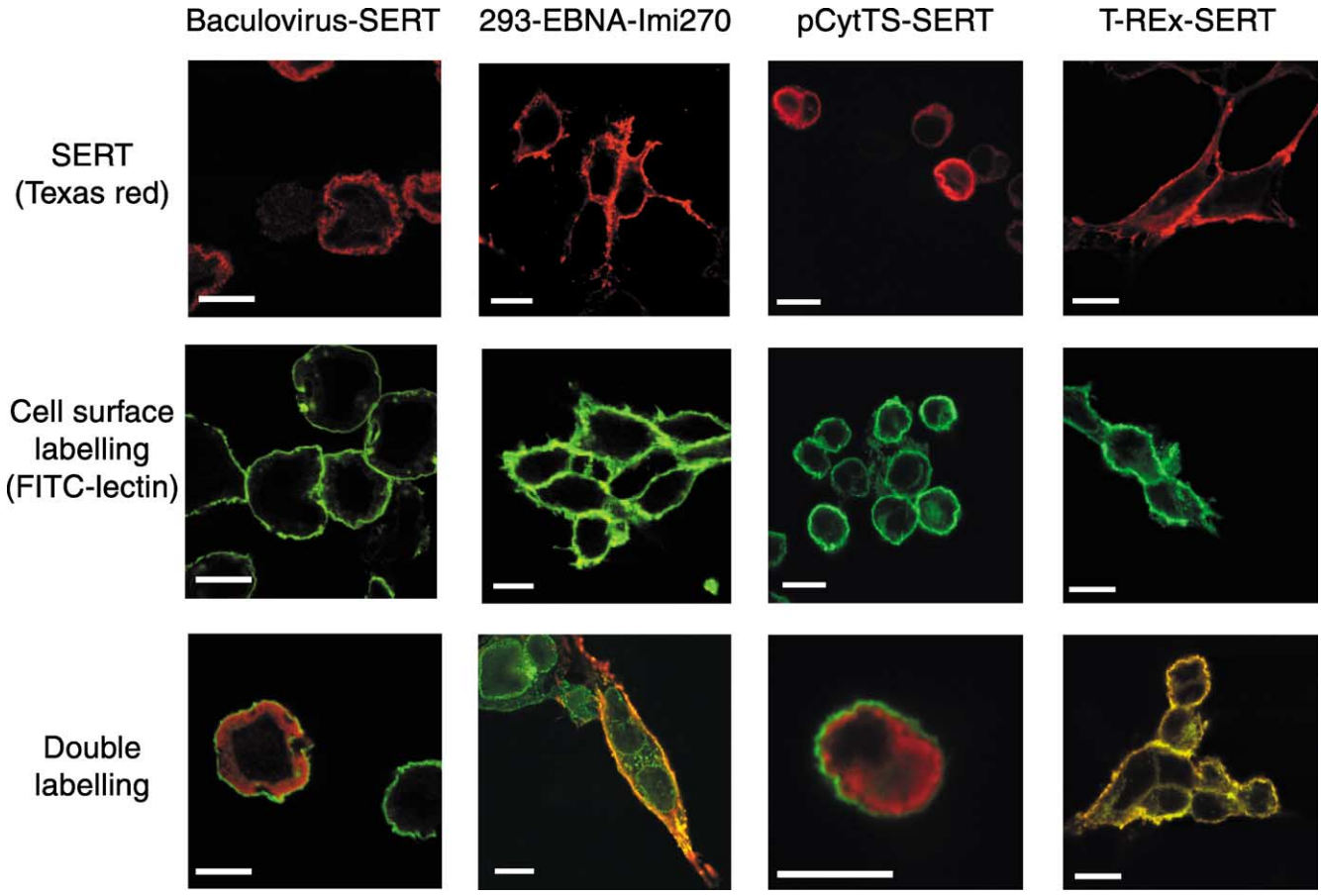

Fig. 4. Confocal microscopy of immunostained cells expressing the serotonin transporter. SERT was expressed in four different cell lines. The baculovirus expression system was used to express SERT in insect cells and the induction of SERT expression in the pCytTS-SERT and T-REx-SERT cell lines was performed as described in Section 2. These expression systems were compared to constitutive expression in the Imi270 cell line. Three different fields of view are given for each cell line (column of three panels). Staining was performed with the anti-FLAG antibody and an anti-mouse antibody conjugated with Texas Red on permeabilized cells to stain SERT (1st row). The cell surface was defined using FITC-conjugated concanavalin A, which was used to stain unpermeabilized cells (second row). Double labelling of the cells with both FITC-concanavalin A and anti-FLAG is shown in the third row (the Imi270 cell line was permeabilized during the concanavalin A treatment). The scale bar represents 10 μm.

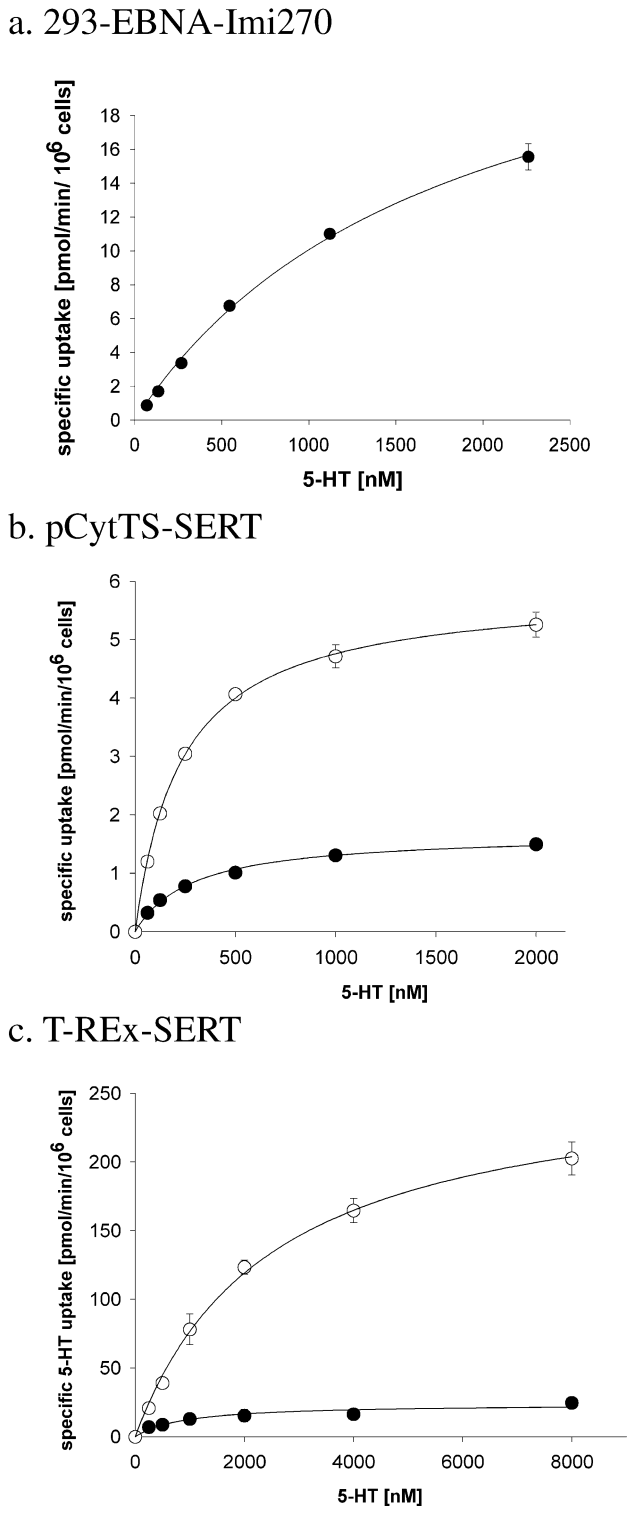

Fig. 5. Uptake of [^{3}H]-5HT into whole cells. Cell lines were grown and saturation kinetic experiments were performed as described in Section 2. (a) Imi270 cell line with constitutive expression of SERT. (b) The pCytTS-SERT cell line was either uninduced (filled circles) or induced at 33 °C for 48 h (open circles). (c) The T-REx-SERT cell line was either uninduced (filled circles) or induced with 1 μg/ml tetracycline for 24 h (open circles). Each data point was determined in triplicate and was plotted as mean value ± S.E. The saturation curve presented is a representative of three independent experiments, each giving similar results.

Table 1
Summary of [^{3}H]-5HT uptake data for different mammalian cell lines

Cell line	V_{max} [pmol/min/10^6 cells][a]	K_m [nM][a]
Imi 270	28.1 ± 1.5	1786.4 ± 173.2
CytTS-SERT uninduced	1.62 ± 0.07	232 ± 58
induced (48 h, 29 °C)	5.8 ± 1.1	159 ± 35
induced (48 h, 33 °C)	4.8 ± 1.6	192 ± 41
BHK cells untreated	0.89 ± 0.01	255 ± 10
48 h, 29 °C	0.45 ± 0.02	254 ± 39
T-REx-SERT uninduced	12.8 ± 2.2	204 ± 69
induced (10 ng/ml tet)	82.2 ± 6.7	1300 ± 100
induced (1 μg/ml tet)	212.3 ± 29.0	1610 ± 495

[a] 5-HT uptake assays were performed as described in Section 2. Data were fitted to the Michaelis–Menten equation using the nonlinear regression analysis tool of SigmaPlot. V_{max} and K_m values given are the mean ± S.E. of two to four independent experiments.

that seen in the parental BHK cells (V_{max} 0.89 ± 0.01 pmol/min/10^6 cells). Induction of the pCytTS-cells at 33 °C resulted in a small increase (Fig 5b) in the level of functional cell surface expressed SERT (V_{max} 4.77 ± 1.55 pmol/min/10^6 cells). However, [^{3}H]-5HT uptake activity in the pCytTS-SERT cell line decreased if cold induction was performed at 29 °C, as did the expression of the endogenous SERT transporter in the parental BHK cell line (Table 1). The very low levels of [^{3}H]-5HT uptake in the pCytTS-SERT cell line were consistent with the apparent absence of complex *N*-glycans on SERT seen in the Western blots (Fig.

Table 2
A comparison of expression systems used for the production of SERT

Expression system	Type[a]	Cell line	Nonfunctional expression levels (mg/l)[b]	Functional expression levels (copies/cell)[c]	Mass of *N*-glycans (kDa)
Escherichia coli	I	BL21 (DE3)	2–3	0	0
Pichia pastoris	I	GS115, SMD1168	2–3	0	9, 16
Baculovirus: SERT	I	Sf9, Sf21	~ 0.5	250,000	6
		Hi5, MG1	~ 1	250,000	6
Baculo: SERT + calnexin	I	Sf9	~ 0.5	750,000	6
EBNA-293	C	HEK293	minimal	280,000 (imi)	25, 4
			minimal	290,000 (coca)	
MEL-SERT	UI	MEL	–	1000	18
	I		minimal	80,000	
pCytTS-SERT	UI	BHK	–	12,500	6
	I		present	240,000	
T-REx-SERT	UI	HEK293	–	12,500	33, 6
	I		minimal	400,000	

[a] I, induced; UI, uninduced; C, constitutive.
[b] Estimated from band intensities on a Western blot. "Minimal" implies that all the expressed SERT is thought to be functional, but the presence of misfolded SERT cannot be definitely ruled out.
[c] Determined from binding assays using the inhibitor [^{125}I]-RTI55.

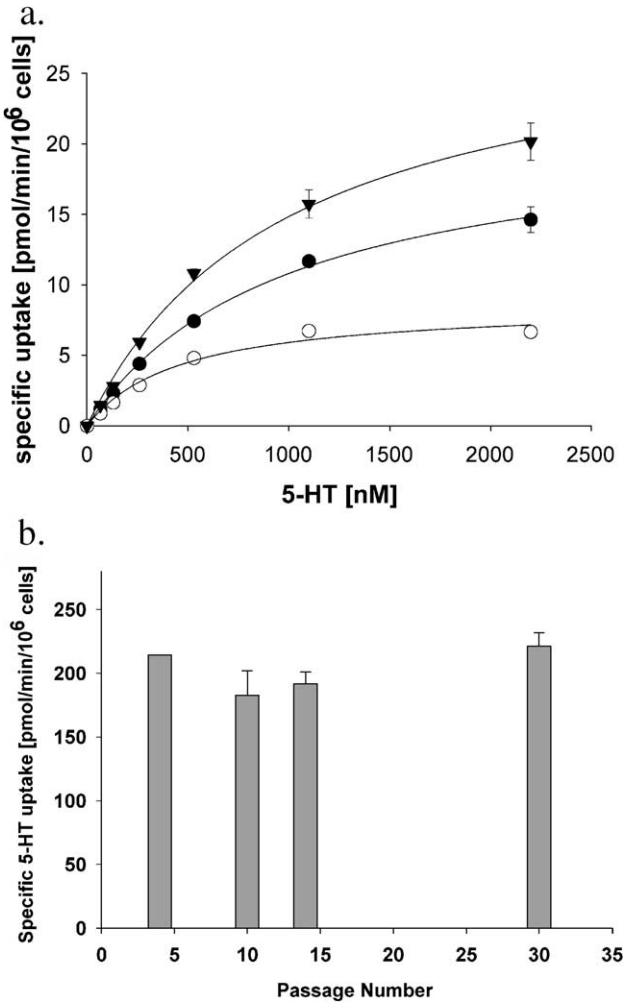

Fig. 6. Stability of [^{3}H]-5HT uptake activity in imipramine-deprived Imi270 and the T-REx-SERT cell lines over time. (a) Saturation kinetic experiments were perfomed on Imi270 cells grown in the absence of imipramine for 3 days (filled triangles), 12 days (filled circles) or 21 days (open circles); V_{max} values for the data are, respectively, 29.5 ± 1.3, 21.5 ± 0.7 and 8.6 ± 0.7 pmol/min/10^6 cells. Each data point was determined in triplicate and was plotted as mean value ± S.E. The saturation curve presented is a representative of two independent experiments, each giving similar results. (b) T-REx-SERT cells were passage two to three times a week as described in Section 2. For uptake assays, cells were seeded down into 24-well plates at the indicated passage number. Twenty-four hours later, SERT expression was induced with 1 μg/ml tetracycline. The uptake assay used a single concentration of [^{3}H]-5HT (5 μM) and was performed 20 h following induction as described under Section 2. Data presented are the mean ± S.E. of one experiment at each time point performed in triplicate.

3) and the lack of SERT immunostaining at the cell surface (Fig. 4).

For the purification of SERT, the most important parameter is how much functional SERT is expressed per cell. This was determined by single-point inhibitor binding assays on crude membrane preparations from whole cells, so that all functional molecules of SERT, regardless of their cellular location, were assayed. Binding assays were performed using the radiolabelled inhibitor [^{125}I]-RTI55 and showed (Table 2) that the T-REx-SERT cell line expressed the most functional copies of SERT (400,000 copies per cell). However, the confocal microscopy suggested that only 30% of the Imi270 cells expressed SERT, so therefore the SERT-expressing cells could contain up to a million copies of SERT per cell. The pCytTS-SERT cell line expressed similar levels of SERT to the baculovirus expression system (250,000 copies per cell).

3.5. Stability and inducibility of the cell lines

The stability of the inducible cell lines was considerably improved in comparison to the constitutive 293-EBNA cell lines Imi270 and Coca270, with no apparent increase in their doubling time after transfection. The T-REx-SERT cell line has been passaged continually for 3 months (30 passages) without any apparent reduction in the level of cell surface expression (Fig. 6). In contrast, the Imi270 cell line showed decreased expression on continuous passaging, especially if imipramine was removed from the medium (Fig. 6).

The inducibility of the various cell lines is apparent from the Western blots and activity data. The T-REx-SERT cell line expressed 12,500 copies per cell of SERT in the uninduced state (Table 2) and a trace of SERT can be seen on the Western blot (Fig. 3). One important observation was that the amount of tetracycline required for the induction of measurable SERT expression in the T-REx-SERT cell line was extremely low (10 ng/ml), which justified the use of bovine calf serum that was certified free of tetracycline to ensure reproducible results (Fig. 7). No SERT was apparent in the uninduced MEL-SERT cell line either from binding

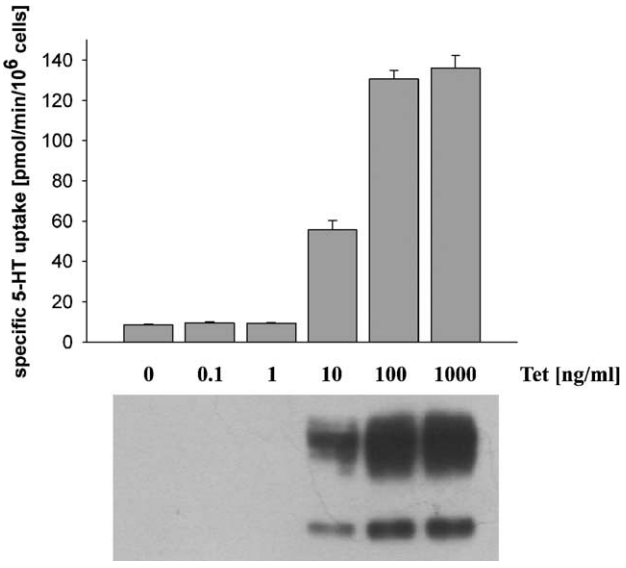

Fig. 7. Tetracycline-dependent induction of [^{3}H]-5HT uptake. T-REx-SERT cells were split into 24-well plates and 24 h later SERT expression was induced with the indicated concentration of tetracycline. An uptake assay using a single concentration of [^{3}H]-5HT (5 μM) was performed 20 h following induction as described under Section 2. Data presented are the mean ± S.E. of two independent experiments, each performed in triplicate.

data (Table 2) or Western blots (Fig. 3). The pCytTS-SERT cell line expressed low levels of functional SERT when uninduced (Table 2), which was only evident on Western blots after prolonged exposure (results not shown).

3.6. Are all the expressed SERT functional?

Crystallisation in either two or three dimensions requires milligrams of highly purified protein that is all in a single, functional conformation. It is, therefore, important to consider whether the heterologous expression system used to produce the protein is generating inactive protein in addition to the functional material. In the case of the baculovirus expression system it is clear that over 95% of expressed SERT is unglycosylated and mainly nonfunctional and, in addition, probably about 90% of the glycosylated SERT produced is also nonfunctional. The best way to get a feel for whether inactive SERT could be present is to use Western blotting of membrane samples containing the identical amount of functional SERT (Fig. 8). It is clear that the baculovirus system expressed considerably more SERT protein than the mammalian expression systems and, because the lanes contain identical amounts of functional SERT, this extra protein must be inactive. The high molecular weight SERT produced by the baculovirus was SDS-resistant aggregates and was not due to different glycosylated states [6]. The differences in blotting signal between the different mammalian cell lines were less pronounced when compared to the baculovirus system. Both the Imi270 and T-REx-SERT cell lines gave Western blot signals of similar intensity, suggesting that, for the same amount of functional SERT, they express the same amount of SERT polypeptide; this is indicative of fully functional SERT expressed in both cell lines. In comparison, the pCytTS-SERT cell line contained more SERT immunoreactive material in relation to functional SERT, suggesting that a proportion of SERT is misfolded and inactive. Careful optimisation of the induction temperature of the pCyt-TS cell line would inevitably reduce the amount of misfolded SERT, but unglycosylated SERT was still apparent after induction at 32 °C (results not shown).

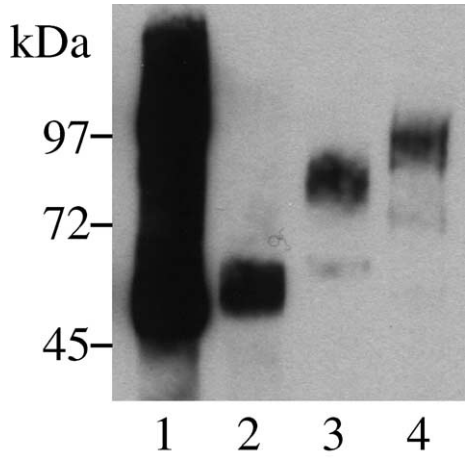

Fig. 8. Levels of inactive SERT in the stable mammalian cell lines compared to the baculovirus expression system. Whole-cell extracts containing equivalent amounts of functional SERT were loaded per lane, separated by SDS-PAGE and Western blotted. Lane 1, SERT expressed in Hi5 cells using the baculovirus expression system; lane 2, induced CytTS-SERT cells; lane 3, induced T-REx-SERT cells; lane 4, Imi270 cells.

4. Discussion

The most widely used expression systems for the production of integral membrane proteins are undoubtedly *E. coli* and the baculovirus expression system, and they have both been used successfully to overexpress a wide variety of transporters, receptors and channels [1]. *P. pastoris* has also been successfully used, for example, for the production of G protein-coupled receptors [20]. However, all these systems have their limitations for the expression of mammalian membrane proteins, especially if posttranslational modifications are required for the correct folding of a membrane protein into its fully functional state. The direct comparison of expressions of a particular membrane protein in many different expression systems has not been frequently performed, but the work described here will enable us to choose the best system for producing SERT for purification and crystallisation.

Heterologous expression of proteins in *E. coli* and yeast has two great advantages over the mammalian expression systems discussed here, namely the speed of producing overexpressing strains and the ease and cheapness of large-scale production. Expression of SERT in *E. coli* and *Pichia* was a real possibility when this work was performed because it had been shown that unglycosylated SERT was fully functional for both 5HT uptake and inhibitor binding [6]. However, both systems failed to produce functional SERT as assessed by uptake and binding experiments. This is undoubtedly due to the recent observation [21,22] that SERT has an obligatory requirement for cholesterol that cannot be mimicked by other sterols, such as ergosterol, that are found in yeasts. *E. coli* does not have any sterols in its membrane. It may be possible to manipulate the sterol composition of the SERT-expressing *Pichia* cell lines to contain cholesterol as has been performed for *Saccharomyces cerevisiae* [23], but in these experiments it is not clear whether the cholesterol can reach the ER where membrane protein synthesis occurs. In the absence of microbial expression systems that synthesize cholesterol in the ER, SERT will have to be produced in either insect or mammalian cells.

Prior to the work presented here, the best system for the production of reasonable quantities of SERT was the baculovirus expression system (Table 2). However, although the levels of expression were sufficient for purification, the major proportion of misfolded SERT produced in the baculovirus expression system probably contributed to the poor specific activity of the purified SERT [24]. The best

system for producing SERT in the stable mammalian cell lines generated here is undoubtedly the tetracycline inducible cell line T-REx-SERT. The level of functional expression was higher than using the standard baculovirus expression system, but not quite as high as baculovirus-mediated expression of SERT improved by the co-expression of the molecular chaperone calnexin (Table 2). However, the T-REx-SERT cell line has significant advantages over the production of SERT from recombinant baculoviruses. All the SERT expressed in the T-REx-SERT cell line are *N*-glycosylated, and probably the majority of them are functional as these are expressed at the cell surface. The T-REx-SERT cell line offers the opportunity to purify SERT that is probably all fully functional before the start of purification. Another advantage of the T-REx-SERT cell line is the high level of cell surface-expressed SERT, which is about 10 times higher than that obtained with previously published cell lines [25,26] and transient transfections [27]; this may facilitate future analyses of protein–protein and lipid–protein interactions of SERT in the plasma membrane. The only other case that we are aware of where a transporter (the human anion exchanger, band 3) was also expressed in an inducible mammalian system also led to stable, high expression levels of nearly 900,000 functional copies per cell [28]. However, these levels of expression would require large-scale growth of the cell lines; a 10-l culture of T-REx-SERT cells would yield about 1 mg of SERT before purification, assuming a cell density of 2×10^6 cells/ml.

The other two inducible cell lines characterised, MEL-SERT and pCytTS-SERT, were not as successful as the T-REx-SERT cell line, but probably for different reasons. The MEL-SERT cell line had very low levels of expression, perhaps due to the weakness of the β-globin promoter, but the expressed SERT was all fully *N*-glycosylated. In contrast, the pCytTS expression system is designed to produce a large amount of mRNA, just as with a viral infection, and this may have been the cause of the production of unglycosylated, nonfunctional SERT, just as in the baculovirus expression system. However, increasing the induction temperature from 29 to 33 °C to decrease the activity of the replicase, and hence reduce the intracellular mRNA levels, did not abolish the production of unglycosylated SERT (results not shown). Another possible reason for the production of unglycosylated SERT in the induced pCytTS-SERT cell line was that SERT folding could be less efficient at 29 °C than at 37 °C. This was suggested by the decrease in cell surface expression in induced pCytTS-SERT cells and in the parental BHK cells. It seemed unlikely that there was a global effect on protein expression in BHK cells at 29–33 °C, because this system has been very successful in producing large quantities of secreted proteins [17]. The pCytTS-SERT cell line does have advantages over the baculovirus expression system; the BHK cell line is very easy to grow in suspension to high cell densities and to induce during fermentation compared to the baculovirus system with its requirement of high-titre viruses. In addition, there is considerably less misfolded SERT expressed in the pCytTS-SERT cell line than in the baculovirus system. It is likely that the pCytTS system will prove successful for the expression of many receptors and ion channels that are not dependent on posttranslational modifications for efficient folding.

The comparison of four different mammalian expression systems may not be ideal because different parental cell lines were used in their construction. It is clear that the level of cell surface expression of a membrane protein may sometimes be dictated by the cell in which it is expressed [1], although the fact that SERT is found in various tissues (brain, placenta, platelets) may make this seem unlikely. However, the proteins required for the insertion of SERT into the ER in a functional form would be expected to be fully conserved between the mouse, human and hamster cell lines used in this study. Therefore, the levels of functional SERT expression per cell are probably dictated mainly by translational effects. A fuller understanding of how SERT expression is affected by cell type will require the use of different expression systems, such as the Semliki Forest Virus system.

5. Conclusion

This study showed that stable inducible mammalian cell lines produced levels of functional SERT similar to that of the baculovirus expression system. In addition, the majority of the expressed SERT was functional, whereas in the baculovirus expression system, the vast majority was non-functional. The levels of expression in the T-REx-SERT cell line are still not high enough to avoid the need for large-scale fermentation for the routine production of milligram quantities of functional SERT for crystallisation.

Acknowledgements

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