

# Heterologous expression of G-protein-coupled receptors: comparison of expression systems from the standpoint of large-scale production and purification

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**Abstract.** G-protein-coupled receptors (GPCRs) are of prime importance for cell signal transduction mechanisms and are the target of many current and potential drugs. However, structural data on these membrane proteins is still scarce because of their low natural abundance and the low efficiency of most of the expression systems currently available. This review presents the most important expression systems currently employed for heterolo-

gous expression of GPCRs; *Escherichia coli*, yeast, insect cells and mammalian cells. After briefly recalling the specificity, advantages and limitations of each system, particular emphasis is put on the quantitative comparison of these expression systems in terms of overall expression yield, and on the influence of various factors (primary sequence, origin, cell type, N- and C-terminal tags) on the results.

**Key words.** GPCRs; heterologous expression; membrane proteins; receptor; *Pichia pastoris*; NMR.

## Introduction

Very highly conserved through evolution and thereby expressed in nearly all organisms ranging from yeast to human beings; encoded by almost 1% of the human genome; involved in a wide spectrum of hereditary and somatic disorders and diseases from cancer to infertility; targets of more than 50% of the drugs nowadays used in therapeutics [1]; directly responsible for communication at the cellular level by their ability to be activated by a large number of very different extracellular signals such as photons, ions, lipids, peptides, nucleosides, nucleotides, hormones and neurotransmitters. Here are some of the main characteristics that describe and underline the fundamental roles of the superfamily of G-protein-coupled receptors (GPCRs). These membrane proteins are characterised by the presence of a common hydrophobic core composed of seven transmembrane spanning domains, and mediate external stimuli through a strong coupling with endogenous heterotrimeric G proteins [2, 3]. Because of their central role in biological systems, detailed understanding of the process that con-

trols the interaction of GPCRs with their ligands and associated G proteins is very challenging. This understanding would be greatly facilitated by high-resolution three-dimensional (3D) structures of these receptors. However, the only high-resolution structure of a GPCR currently available is that of bovine rhodopsin, which was recently determined by X-ray crystallography at 2.8-Å resolution [4]. This lack of structural data mainly originates in the membranous nature of these proteins and in their very low natural abundance. The comparison of the number of high-resolution 3D structures [obtained either by nuclear magnetic resonance (NMR) or X-ray crystallography] available for soluble (> 10,000) and membrane proteins (⊕30) in the Protein Data Bank clearly reflects the difficulties encountered with membrane proteins. The presence of a specific lipid environment essential to maintain functional conformation and their high molecular weight considerably complicate the determination of their 3D structures. Another hindrance to determining the structural biology of GPCRs is their intrinsic dynamic behaviour, which is characteristic of  $\alpha$ -helical proteins and directly linked to their activity of signal transduction through biological membranes. Finally, the two major techniques available to obtain high-resolution

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3D structures (NMR and X-ray crystallography) require high amounts of purified proteins (milligrams). Most GPCRs are naturally poorly expressed, and this renders impractical their direct purification from natural sources. Thus, their heterologous overexpression is an unavoidable step, and it is essential to develop highly efficient expression strategies. The objective of this article is to present and compare heterologous expression levels of GPCRs in the four main expression systems currently employed: *Escherichia coli*, yeasts, insects and mammalian cells.

In the context of structural biology experiments, the first objective is to be able to obtain high quantities of pure protein. In the literature, expression levels are given either in 'sites per cell' or in 'picomoles of receptor per milligram of membrane proteins', so that direct comparison is often difficult. A rapid conversion can be made using the approximate conversion rates proposed by Schertler [5] and Grisshammer and Tate [6] and summarized in table 1. The conversion factors presented in table 1 highlight that the real expression level efficiency is a combination of parameters such as the number of sites per cell, cell size and cell density. When expressed in picomoles of receptor per milligram of membrane protein the requirements for obtaining 1 mg of GPCR in 5 l of culture are respectively 13, 5 and 26 for *E. coli*, yeast and superior eucaryotic cells (insect or mammalian cells; these minimal expression levels have been calculated considering an average GPCR molecular weight of 45 kDa). Whenever the reported expression level was equal or superior to the minimal expression level previously defined, an 'overexpression factor' (O.F.) has been calculated (tables 2–5) and corresponds to the ratio between the reported expression level and what is required to produce 1 mg in 5 l of culture medium. For instance, in the case of the expression of the  $\beta$ -adrenergic receptor in *Saccharomyces cerevisiae* [7], the expression level is 115 pmol per milligram of membrane protein which corresponds to an O.F. of 23 (115/5).

Several very complete reviews are already available concerning the main advantages and drawbacks of all these expression systems and their use in expressing membrane proteins [5, 6, 8–13]. The present review is thus mainly focused on describing the results so far obtained, with only a brief recall of the specific characteristics of each system.

## *E. coli*

### Main characteristics of *E. coli*

The major advantages of this expression system are (i) low cost (ii) homogeneity of the recombinant proteins (no posttranslational modifications) and (iii) short generation time (20 min) and short delay of expression [6]. This sys-

tem offers the possibility to rapidly test a huge number of different genetic coding sequences [14, 15] and to optimise expression levels before undertaking the purification [15]. These characteristics render *E. coli* very appropriate to the constraints of structural biology. However, this system is not the best for functional studies of the receptor in vivo (signal transduction mechanism), since this bacteria does not contain any endogenous G protein. In particular, the lack of endogenous G protein can give rise to the absence of high-affinity binding sites for agonists [16]. However, the addition of purified G proteins to *E. coli* membranes expressing the 5HT<sub>1A</sub> receptor enabled restoration of the high-affinity binding properties [17]. Also, coexpression of G proteins and opioid receptors by gene fusion reveals high-affinity binding sites [16]. In some cases, the *E. coli* expression system is disadvantageous because the functionality of some GPCRs has been shown to depend on the presence of posttranslational modifications. For instance, it has been shown that suppression of a glycosylation site located in the N-terminus of rhodopsin perturbs the functional folding of this protein [18]. In the same way, phosphorylation of rhodopsin increases its proportion in the MII state, which activates G proteins [19]. Finally, the lipid composition of *E. coli* membranes is very different from that of eucaryotic cells, and this can greatly affect the binding properties of recombinant receptors. Indeed, certain receptors such as the oxytocin [20], the transferrin [21] and human  $\mu$ -opioid receptors [22, 23] strongly depend on the lipid environment of the protein. In addition, the reductive environment of the bacterial cytoplasm can considerably slow down the production of functional membrane proteins, particularly if the correct folding of the receptor in an active conformation requires formation of disulfide bridges. Fusion of *E. coli* periplasmic membrane proteins such as the maltose binding protein (MBP) to the GPCR of interest can overcome this problem by addressing the recombinant receptor in the periplasmic oxidative environment [24, 25]. *E. coli* can also be used for production of the heterologous protein in inclusion bodies [26]. These structures mainly contain the exogenous protein of interest and are very easy to purify, and this strategy can be very attractive. Moreover, the accumulation in these inclusion bodies of recombinant proteins protects them from proteolytic degradation by cytosolic, membranous and periplasmic proteases [27]. However, despite the fact that certain factors such as temperature modulation can favour the appearance of inclusion bodies, their formation is hard to control. Moreover, proteins accumulated in these structures are usually inactive, and functional refolding after purification is a required nontrivial step [26, 28]. This approach has already been employed for several soluble proteins, but much more rarely for membrane proteins [26, 28–31].

Table 1. Conversion factors required for the comparison of expression levels in *E. coli*, yeast, insect cells and mammalian cells.

Cell type	Average cell density	Conversion of the expression levels	Minimal expression levels necessary to obtain 1 mg of GPCR in 5 l of culture	
	(Cells/ml)	1 pmol/mg = X sites/cell, with X equal to:	Sites/cell	pmol/mg
<i>E. coli</i>	$1 \times 10^9$	200	$2.6 \times 10^3$	13
Yeast	$1 \times 10^8$	5000	$2.5 \times 10^4$	5
Insect $\cong$ mammalian cells	$1 \times 10^6$	$10^5$	$2.6 \times 10^6$	26

Minimal expression levels for the production of 1 mg of GPCR in 5 l of culture. On the basis of the values described by Schertler [5] and Grisshammer and Tate [6], the expression level necessary to obtain 1 mg of receptor in 5 l of culture was calculated for each expression system. An average molecular weight of 45 kDa for a GPCR is assumed. It should be noted that certain methylophilic yeasts, such as *P. pastoris*, can reach higher cell densities ( $1-3 \times 10^9$  cells/ml) so that the expression level required to produce 1 mg of recombinant receptor in 5 l of culture medium may be lower than indicated here for yeast in general.

Table 2. GPCRs expression in *Escherichia coli*.

Receptor type	Origin	Promoter	Fusion protein	Function	Receptor localisation	Expression level	Tags	Overexpression factor	Ref.
$\beta_2$ -Adrenergic	human	lac	$\beta$ -gal	+	M	0.4 pmol/mg	none	–	[32]
	human	T7	–	+	M	0.45 pmol/mg	none	–	[36]
	human	ptac12	LamB	+	M	220 sites/cell	none	–	[113]
	human	ptac12	LamB	+	M	0.5 pmol/mg	none	–	[34]
	human	ptac12	LamB	+	M	220 sites /cell	none	–	[114]
	human	lac	PhoA	ND	M	ND	c-myc Ct	–	[115]
$\beta_1$ -Adrenergic	human	ptac	MBP	+	M	6 pmol/mg	6-his Ct	–	[33]
	human	ptac12	LamB	+	M	ND	none	–	[116]
	human	ptac12	LamB	+	M	ND	none	–	[116]
	human	ptac12	LamB	+	M	50 sites/cell	none	–	[113]
	human	ptac12	LamB	+	M	1 pmol/mg	none	–	[34]
	turkey	ptac12	LamB	+	M	0.33 pmol/mg	none	–	[34]
Serotonin 5HT <sub>1A</sub>	human	MalE	MBP	+	M	120 sites/cell	none	–	[17]
Endothelin ETB	human	ptac	–	+	M	41 sites/cell	none	–	[117]
<b>Neurotensin</b>	<b>rat</b>	<b>ptac</b>	<b>MBP</b>	<b>+</b>	<b>M</b>	<b>15 pmol/mg</b>	<b>c-myc/5-his Ct</b>	<b>1.15</b>	<b>[14]</b>
<b>Neurotensin</b>	<b>rat</b>	<b>T5</b>	<b>*</b>	<b>+</b>	<b>ND</b>	<b>4–16 pmol/mg</b>	<b>c-myc/5-his Ct</b>	<b>1.23</b>	<b>[118]</b>
<b>Neurotensin</b>	<b>rat</b>	<b>ptac</b>	<b>MBP</b>	<b>+</b>	<b>M</b>	<b>15 pmol/mg</b>	<b>Bio Ct</b>	<b>1.15</b>	<b>[15]</b>
Neurotensin	rat	ptac	MBP	+	M	7.2 pmol/mg	c-myc and 5-his Ct	–	[37]
NK <sub>2</sub>									
Adenosine A <sub>1</sub>	human	lac	PhoA	+	M	0.4 pmol/mg	c-myc Nt	–	[115]
Adenosine A <sub>1</sub>	human	MBP	MBP	+	M	0.4 pmol/mg	none	–	[119]
Neuropeptide Y <sub>1</sub>	human	ptac	MBP	+	M	3.5 pmol/mg	FLAG and 6-his Ct	–	[120]
Olfactive OR5		T7	GST	–	IB	ND	6-his Ct	ND	[26]
<b>Adenosine A2a</b>	<b>human</b>	<b>ptac</b>	<b>MBP</b>	<b>+</b>	<b>M</b>	<b>10–20 nmol/l</b>	<b>10-his and Flag Ct</b>	<b>4.5</b>	<b>[122]</b>
Vasopressin V2	human	Trc	PhoA/-gal	–	M	ND	none	ND	[123]
Opioid $\mu$ , $\delta$ , $\kappa$ -	human	ptac	MBP	+	M	0.5 pmol/mg	none	–	[124]
Opioid $\mu$ -	human	ptac	MBP and G <sub>ao1</sub> or G <sub>oi2</sub> in Ct	+	M	$\approx$ 0.5 pmol/mg	none	–	[16]
M2 mACh	human	lac	MBP	+	M	6 pmol/mg	none	–	[125]
<b>TSH</b>	<b>human</b>	<b>T5</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>2.5 mg/l</b>	<b>6-his Ct</b>	<b>12</b>	<b>[126]</b>

MBP or MalE: maltose binding protein, inner membrane protein from *E. coli*; LamB: LamB protein, outer membrane protein from *E. coli*;  $\beta$ -gal:  $\beta$ -galactosidase, cytosolic protein from *E. coli*; PhoA: alkaline phosphatase, protein located in the bacterial cytoplasm; GST: glutathione S-transferase of *Schistosoma japonicum*; aa: amino acid; ND: not determined. All promoters are IPTG inducible, Nt: N-terminus; Ct: C-terminus; 5-his, 6-his, 10-his: penta-, hexa- and deca- histidines tags, respectively; Bio: Bio tag; M: membrane; IB: inclusion bodies. TSH: thyroid-stimulating hormone; mACh: muscarinic cholinergic; the 'overexpression factor' (boldface characters) represents the ratio between the expression level and the minimum required to obtain 1 mg (or more) of recombinant protein in 5 l of culture media (minimum expression level determined in *E. coli*, 13 pmol/mg of membrane protein, see table 1 and text).

\* Six different membrane proteins from phage.

Table 3. Expression of GPCRs in yeast.

Receptor	Origin	Yeast	Promoter	Signal/fusion peptide	Expression level	Ligand binding	G-protein coupling	Tags	Overexpression factor	Ref.
<b><math>\beta_2</math>-Adrenergic</b>	human	<i>S. cerevisiae</i>	GAL1	Ste2	<b>115 pmol/mg</b>	+	+	none	<b>23</b>	[7]
	human	<i>S. cerevisiae</i>	GAL1	Ste2	<b>36 pmol/mg</b>	+	ND	none	<b>7</b>	[47]
	human	<i>P. pastoris</i>	AOX1	pp $\alpha$ MF	<b>25 pmol/mg</b>	+	ND	Flag Nt Bio Ct	<b>5</b>	[66]
<b><math>\alpha_2</math>-C<sub>2</sub>-Adrenergic</b>	human	<i>Sz. pombe</i>	ADH	Ste2	<b>7.5 pmol/mg</b>	+	ND	none	<b>1</b>	[55]
	human	<i>S. cerevisiae</i>	GAL1	–	<b>7–70 pmol/mg</b>	+	ND	Histag Nt	<b>1.5–14</b>	[47]
	mouse	<i>P. pastoris</i>	AOX1	pp $\alpha$ MF, Pho1	<b>22 pmol/mg</b>	+	ND	c-myc Nt	<b>4</b>	[63]
	mouse	<i>S. cerevisiae</i>	PRB1	Bm	<b>16 pmol/mg</b>	+	ND	c-myc Nt	<b>3</b>	[64]
	mouse	<i>P. pastoris</i>	AOX1	pp $\alpha$ MF	<b>40 pmol/mg</b>	+	ND	Flag Nt/ Bio Ct	<b>8</b>	[65]
<b>Endothelin ETB</b>	human	<i>P. pastoris</i>	AOX1	pp $\alpha$ MF	<b>30–60 pmol/mg</b>	+	ND	Flag Nt Bio Nt	<b>6</b> <b>12</b>	[127]
Neurokinin NK2	human	<i>Sz. Pombe</i>	NMT1	Pho1	1.16 pmol/mg	+	–	none	–	[54]
Adenosine A2a	rat	<i>S. cerevisiae</i>	ADH1	–	0.45 pmol/mg	+	+	none	–	[208]
$\mu$ -Opioid	human	<i>P. pastoris</i>	AOX1	pp $\alpha$ MF	0.4 pmol/mg	+	–	none	–	[62]
$\mu$ -Opioid	human	<i>P. pastoris</i>	AOX1	pp $\alpha$ MF	4 pmol/mg	+	–	GFP Nt, c-myc, 6 his Ct	0.8	[68]
<b><math>\mu</math>-opioid</b>	human	<i>P. pastoris</i>	AOX1	pp $\alpha$ MF	<b>100 pmol/mg</b>	–	–	GFP Nt, c-myc, 6 his Ct	<b>20</b>	[69]
$\mu$ -Opioid	human	<i>S. cerevisiae</i>	GRAP1	17 aa Ste2	0.4 pmol/mg	+	–	none	–	[67]
m <sub>1</sub> -Muscarinic	human	<i>S. cerevisiae</i>	ADH	–	0.02 pmol/mg	+	ND	–	–	[44]
m <sub>5</sub> -Muscarinic	rat	<i>S. cerevisiae</i>	MF	pp $\alpha$ MF	0.13 pmol/mg	+	–	–	–	[128]
D <sub>2S</sub> -Dopaminergic	human	<i>S. cerevisiae</i>	PMA1	Ste2	1–2 pmol/mg	+	ND	–	–	[52]
D <sub>2S</sub> -Dopaminergic	human	<i>S. cerevisiae</i>	GAL10	Ste2	1–2 pmol/mg	+	ND	–	–	[45]
<b>D<sub>2S</sub>-Dopaminergic</b>	human	<i>Sz. pombe</i>	NMT1	Ste2	<b>14 pmol/mg</b>	+	ND	–	<b>3</b>	[52]
D <sub>1A</sub> -Dopaminergic	human	<i>S. cerevisiae</i>	GAP	Ste2	0.13 pmol/mg	+	ND	Flag+his Ct	–	[129]
D <sub>2</sub> -Dopaminergic	human	<i>Sz. Pombe</i>	NMT1	–	1 pmol/mg	+	–	none	–	[53]
Somatostatin	rat	<i>S. cerevisiae</i>	GAL1/10	–	0.2 pmol/mg	+	+	none	–	[40]
SSTR2										
<b>Rhodopsin</b>	bovine	<i>S. cerevisiae</i>	GAL1	–	<b>2 mg/10<sup>10</sup> cells</b>	+	–	none	<b>100</b>	[71]
<b>Rhodopsin</b>	bovine	<i>P. pastoris</i>	AOX1	Pho1	<b>0.3 mg/l</b>	+	–	none	<b>1.5</b>	[70]
GHRH	human	<i>S. cerevisiae</i>	GAL1/10	–	ND	+	+	none	ND	[130]
LysoPA Edg2/ Vzg1	human	<i>S. cerevisiae</i>	GAL	–	ND	+	+	none	ND	[131]
<b>Ste2</b>	<b>S. cer.</b>	<i>S. cerevisiae</i>	GAP	–	<b>350 pmol/mg</b>	+	+	Flag+his Ct	<b>70</b>	[72]

pp $\alpha$ MF:  $\alpha$ -mating factor prepeptide; AOX1: promoter of the alcohol oxydase 1 gene; Pho1: acid phosphatase signal peptide; Bm: Bacillus macerans signal peptide; GAP: promoter of the glyceraldehyde-3-phosphate dehydrogenase gene; NMT1; 'no message in thiamine': promoter inhibited in the presence of thiamine; PMA1: promoter of the plasma membrane ATPase; PRB1: promoter of the endopeptidase B gene; Ste2: receptor of the  $\alpha$ -factor; ND: not determined; Flag: Flag tag, 8 amino acid sequence: DYKDDDDK; Bio: biotinylation domain of the transcarboxylase from *Propionibacterium shermanii* [132]; GHRH: growth hormone releasing hormone; GAL: galactose inducible promoter; lysoPA: lysophosphatidic acid. Boldface characters correspond to the GPCRs for which the overexpression factor is > 1.

Table 4. GPCR expression in insect cells.

Receptor	Origin	Cell type	Expression level	Function	G Protein coupling	Tags	Overexpression factor	Ref.
<b><math>\beta_2</math>-Adrenergic</b>	hamster	Sf <sub>9</sub>	<b>30 pmol/mg</b>	+	ND	none	<b>1.25</b>	[133]
	human	Sf <sub>9</sub>	15 pmol/mg	+	ND	none	–	[84]
	turkey	Sf <sub>9</sub>	5 pmol/mg	+	+	none	–	[134]
	human	Sf <sub>9</sub>	<b>10–25 pmol/mg</b>	+	+	c-myc	<b>1</b>	[89]
	human	Sf <sub>9</sub>	<b>40 pmol/mg</b>	+	+	none	<b>1.7</b>	[135]
	human	Sf <sub>9</sub>	5–20 pmol/mg	+	ND	KT3	–	[207]
	human	Sf <sub>9</sub>	<b>24 pmol/mg</b>	+	+	c-myc Nt	<b>1</b>	[137]
	human	Sf <sub>9</sub>	2.5 pmol/mg	+	+	FlagNt/6-his Ct	–	[138]

Table 4. (continued).

Receptor	Origin	Cell type	Expression level	Function	G Protein coupling	Tags	Overexpression factor	Ref.
$\beta_2$ -Adrenergic	avian	Sf <sub>9</sub>	20 pmol/mg	+	+	none	–	[139]
$\alpha_{2c}$ -Adrenergic	human	Sf <sub>9</sub>	1.4 pmol/mg	+	+	none	–	[140]
Serotonin 1B	human	Sf <sub>9</sub>	1 pmol/mg	+	+	c-myc Nt	–	[141]
Serotonin 5HT <sub>1</sub>	human	Sf <sub>9</sub>	1 to 5 pmol/mg	+	+	none	–	[142]
Serotonin 5HT <sub>1A</sub>	human	Sf <sub>9</sub>	0.15 pmol/mg	+	+	none	–	[79]
<b>Serotonin 5HT<sub>1A</sub></b>	<b>human</b>	<b>Sf<sub>9</sub></b>	<b>5–34 pmol/mg</b>	+	–	<b>none</b>	<b>1.4</b>	<b>[143]</b>
Serotonin 5HT <sub>4A</sub>	mouse	Sf <sub>9</sub>	ND	+	ND	none	ND	[144]
Serotonin 5HT <sub>2C</sub>	rat	Sf <sub>9</sub>	1.106 sites/cell	+	+	none	–	[145]
Neurokinin NK <sub>2</sub>	human	Sf <sub>21</sub>	0.82 pmol/mg	+	+	none	–	[146]
Neurokinin NK <sub>1</sub>	rat	Sf <sub>9</sub>	1–2 pmol/mg	+	+	none	–	[136]
<b>Neurokinin NK<sub>1</sub></b>	<b>human</b>	<b>Sf<sub>9</sub></b>	<b>80 pmol/mg</b>	+	+	<b>none</b>	<b>3.33</b>	<b>[147]</b>
$\delta$ -Opioid	mouse	high <sub>5</sub>	1.4 pmol/mg	+	+	none	–	[148]
$\mu$ , $\delta$ , $\kappa$ -Opioid	human	Sf <sub>9</sub> /high <sub>5</sub>	1–2 pmol/mg	+	+	none	–	[149]
$\mu$ , $\delta$ , $\kappa$ -Opioid	human	Sf <sub>9</sub>	$\kappa$ : $5 \times 10^4$ sites/cell $\delta$ : $10^5$ sites/cell	+	–	none	–	[150]
			$\mu$ : $5 \times 10^5$ sites/cell and $10^5$ sites/cell	+	–	none	–	
$\mu$ -Opioid	human	Sf <sub>9</sub>	6–7.10 <sup>5</sup> sites/cell	+	–	6-his Nt	–	[151]
<b><math>\mu</math>-Opioid</b>	<b>human</b>	<b>high<sub>5</sub></b>	<b><math>5 \times 10^6</math> sites/cell</b>	+	–	<b>none</b>	<b>2</b>	<b>[151]</b>
$\mu$ -Opioid	human	Sf <sub>9</sub>	9 pmol/mg	+	–	6-his Ct	–	[152]
m <sub>3</sub> -Muscarinic	rat	Sf <sub>9</sub>	12–18 pmol/mg	+	ND	none	–	[153]
m <sub>1</sub> -Muscarinic	human	Sf <sub>9</sub>	5 pmol/mg	+	–	none	–	[134]
<b>m<sub>2</sub>-Muscarinic</b>	<b>human</b>	<b>Sf<sub>9</sub></b>	<b>30 pmol/mg</b>	+	–	<b>none</b>	<b>1.25</b>	<b>[134]</b>
m <sub>5</sub> -Muscarinic	rat	Sf <sub>9</sub>	ND	+	+	none	ND	[154]
m <sub>1</sub> -Muscarinic	human	Sf <sub>9</sub>	0.6 pmol/mg	+	ND	none	–	[81]
m <sub>2</sub> -Muscarinic	human	Sf <sub>9</sub>	4 pmol/mg	+	ND	none	–	[81]
m <sub>5</sub> -Muscarinic	human	Sf <sub>9</sub>	0.8 pmol/mg	+	ND	none	–	[81]
m <sub>3</sub> -Muscarinic	rat	Sf <sub>9</sub>	16 pmol/mg	+	ND	none	–	[81]
m <sub>4</sub> -Muscarinic	rat	Sf <sub>9</sub>	2.5 pmol/mg	+	ND	none	–	[81]
m <sub>3</sub> -Muscarinic	rat	Sf <sub>9</sub>	3.3 pmol/mg	+	ND	none	–	[85]
<b>m<sub>2</sub>-Muscarinic</b>	<b>human</b>	<b>Sf<sub>9</sub></b>	<b>36 pmol/mg</b>	+	<b>ND</b>	<b>6-his Ct</b>	<b>1.5</b>	<b>[155]</b>
D <sub>4</sub> -Dopaminergic	human	Sf <sub>9</sub>	5 pmol/mg	+	+	none	–	[156]
D <sub>2</sub> -Dopaminergic	human	Sf <sub>9,21</sub> /Tn <sub>5</sub>	5–8 pmol/mg	+	+	none	–	[157]
<b>D<sub>1</sub>-Dopaminergic</b>	<b>human</b>	<b>Sf<sub>9</sub></b>	<b>33 pmol/mg</b>	+	+	<b>c-myc Nt</b>	<b>1.4</b>	<b>[91]</b>
D <sub>2L</sub> -Dopaminergic	human	Sf <sub>9</sub>	2.6 pmol/mg	+	+	none	–	[91]
D <sub>2S,L</sub> -Dopaminergic	rat	Sf <sub>9</sub>	5–8 pmol/mg	+	+	none	–	[87]
D <sub>2S</sub> -Dopaminergic	human	Sf <sub>9</sub> /Tn	6 and 10 pmol/mg	+	ND	c-mycNt/6-his C	–	[158]
D <sub>2S</sub> -Dopaminergic	human	Sf <sub>9</sub>	10–19 pmol/mg	+	+	6-his Ct	–	[83]
LH/GH		Sf <sub>9</sub>	$4.5 \times 10^3$ sites/cell	+	+	none	–	[159]
FSH	human	Sf <sub>9</sub>	$7 \times 10^3$ sites/cell	+	ND	none	–	[160]
Cannabinoids	rat	Sf <sub>9</sub>	3.2 pmol/mg	+	ND	none	–	[161]
<b>N-formyl-peptide</b>	<b>human</b>	<b>Sf<sub>9</sub></b>	<b>27 pmol/mg</b>	+	–	<b>none</b>	<b>1</b>	<b>[162]</b>
Glutamate R1	rat	Sf <sub>9</sub>	1.5 pmol/mg	+	ND	none	–	[163]
<b>Glutamate R2,4</b>	<b>ND</b>	<b>Sf<sub>21</sub></b>	<b>15–30 pmol/mg</b>	+	<b>ND</b>	<b>none</b>	<b>1.2</b>	<b>[164]</b>
Glutamate R2	rat	Sf <sub>9</sub>	$2.5 \times 10^5$ sites/cell	+	ND	none	–	[165]
Oxytocin	human	Sf <sub>9</sub>	1.66 pmol/mg	+	ND	c-myc Nt	–	[20]
Olfactive Olp4	rat	Sf <sub>9</sub>	ND	ND	ND	6-his Ct	ND	[166]
Olfactive OR17-4	human	Sf <sub>9</sub>	ND	ND	ND	6-his Ct/Flag Nt	ND	[166]
<b>Rhodopsin</b>	<b>bovine</b>	<b>Sf<sub>9</sub></b>	<b>4 mg/l</b>	+	+	<b>6-his Ct</b>	<b>20</b>	<b>[167]</b>
CXCR4	mouse	Sf <sub>9</sub>	ND	+	+	HA Nt	ND	[168]
Substance P	rat	Sf <sub>9</sub>	26 pmol/mg	+	+	KT3 Ct	–	[169]
Substance P	human	Sf <sub>9</sub>	ND	+	ND	none	ND	[170]
Histamine H2	rat	Sf <sub>9</sub>	6 pmol/mg	+	+	6-his Ct	–	[171]
GRP	murine	Sf <sub>9</sub>	6 pmol/mg	+	+	none	–	[172]
<b>PACAP</b>	<b>human</b>	<b>Sf<sub>9</sub></b>	<b>80 pmol/mg</b>	+	–	<b>none</b>	<b>3</b>	<b>[173]</b>

PACAP: pituitary adenylate cyclase activating polypeptide; ND: not determined; HA: 'hemagglutinin epitope of the influenza virus'; FLAG: Flag epitope; FSH: follicle-stimulating hormone; LH/GH: lutropin/placental gonadotropin hormone; aa: amino acid; KT3: KT3 epitope TPPPEPET; GRP: gastrin releasing peptide.

Boldface characters correspond to the GPCRs for which the overexpression factor is > 1.

Table 5. GPCR expression in mammalian cells.

Receptor	Origin	Cell type	Expression level	Functionality	G Protein coupling	Tags	Overexpression factor	Ref.
$\alpha_{1B}$ -Adrenergic	ND	COS-7/SFV/CHO	4–5 pmol/mg	+	+	none	–	[174]
	<b>ND</b>	<b>BHK/SFV</b>	<b>24 pmol/mg</b>	+	+	<b>none</b>	<b>1</b>	[174]
$\alpha_2C_2$ -Adrenergic	human	CHO	1.2 pmol/mg	+	+	none	–	[175]
$\alpha_2C_2, C_4, C_{10}$ -Adrenergic	human	CHO	5 pmol/mg	+	+	none	–	[176]
<b><math>\alpha_2C_{10}</math>-Adrenergic</b>	<b>human</b>	<b>mouse C127</b>	<b>3–6 × 10<sup>6</sup> sites/cell</b>	+	+	<b>none</b>	<b>1.5</b>	[177]
<b><math>\beta_2</math>-Adrenergic</b>	<b>human</b>	<b>CHO</b>	<b>200 pmol/mg</b>	+	+	<b>none</b>	<b>8</b>	[98]
$\beta_2$ -Adrenergic	hamster	CHO	6 pmol/mg	+	+	none	–	[178]
Serotonin 5HT <sub>1B</sub>	mouse	NIH-3T3	19 pmol/mg	+	+	none	–	[179]
<b>Serotonin 5HT<sub>1F</sub></b>	<b>human</b>	<b>HEK-293</b>	<b>15–40 pmol/mg</b>	+	–	<b>none</b>	<b>1.6</b>	[180]
Serotonin 5HT-dro <sub>2A</sub>	<i>Drosophila</i>	COS-7	15 pmol/mg	+	+	none	–	[181]
Serotonin 5HT-dro <sub>2B</sub>	<i>Drosophila</i>	COS-7	9 pmol/mg	+	+	none	–	[181]
Serotonin 5HT <sub>1B</sub>	mammalian	L929	20 pmol/mg	ND	ND	none	–	[182]
Serotonin 5HT <sub>1B</sub>	mammalian	HEK-293	16 pmol/mg	ND	ND	none	–	[182]
Serotonin 5HT <sub>1F</sub>	mammalian	L929	9.5 pmol/mg	ND	ND	none	–	[182]
Serotonin 5HT <sub>1F</sub>	mammalian	HEK-293	3 pmol/mg	ND	ND	none	–	[182]
<b>Neurokinin NK<sub>1</sub></b>	<b>human</b>	<b>BHK/SFV</b>	<b>40 pmol/mg</b>	+	+	<b>none</b>	<b>1.5</b>	[183]
Neurokinin NK <sub>2</sub>	human	CHO	7 × 10 <sup>5</sup> sites/cell	+	ND	none	–	[184]
Adenosine A <sub>1</sub>	human	HEK-293	4 pmol/mg	+	+	6-his Nt/ Flag Ct	–	[185]
Adenosine A <sub>1</sub>	human	CHO	4–7 × 10 <sup>5</sup> sites/cell	+	+	none	–	[186]
Substance P	rat	COS	3 pmol/mg	+	+	none	–	[187]
Substance K	rat	COS	0.3 pmol/mg	+	+	none	–	[187]
Neuromedin K	rat	COS	0.16 pmol/mg	+	+	none	–	[187]
Substance K	human	NIH-3T3	9.7 × 10 <sup>3</sup> sites/cell	+	+	none	–	[188]
Substance P + K	rat	CHO	11 pmol/mg	+	+	none	–	[189]
Neuromedin K	rat	CHO	7.6 pmol/mg	+	+	none	–	[189]
Neurokinin A	hamster	MEL	0.7 pmol/mg	+	+	none	–	[146]
	human	MEL	2.2 pmol/mg	+	+	none	–	[146]
$\mu$ -Opioid	rat	COS	ND	+	+	none	ND	[190]
$\mu$ -Opioid	human	COS-7	0.23 pmol/mg	+	+	none	–	[191]
m <sub>1,2,3,4</sub> Muscarinic	human	HEK	1–3 × 10 <sup>5</sup> sites/cell	+	+	none	–	[192]
m <sub>1</sub> -Muscarinic	mouse	JEG-3	0.7–10 pmol/mg	+	+	none	–	[193]
m <sub>4</sub> -Muscarinic	chicken	JEG-3	1.7–27 pmol/mg	+	+	none	–	[193]
Glutamate mGlu <sub>2,3</sub>	rat	CHO/SFV	0.5 pmol/mg	+	+	none	–	[194]
Glutamate R8	human	CHO/SFV	10–13 pmol/mg	+	ND	none	–	[195]
<b>Neuropeptide Y1</b>	<b>human</b>	<b>HeLa/VV</b>	<b>1 mg/l</b>	+	<b>ND</b>	<b>none</b>	<b>5</b>	[196]
TRH	human	CHO	2–4 × 10 <sup>4</sup> sites/cell	+	+	none	–	[86]
<b>TRH</b>	<b>mouse</b>	<b>adenovirus</b>	<b>2 × 10<sup>6</sup> sites/cell</b>	+	+	<b>none</b>	<b>1</b>	[197]
<b>Glucagon</b>	<b>rat</b>	<b>BHK</b>	<b>78 pmol/mg</b>	+	+	<b>none</b>	<b>3</b>	[198]
Glucagon	rat	COS-7	2 × 10 <sup>5</sup> sites/cell	+	+	none	–	
Calcitonin	human	MEL	1.9 × 10 <sup>6</sup> sites/cell	+	ND	none	ND	[199]
Thromboxane A2	human	COS-7	24 pmol/mg	+	+	6 his Ct	–	[200]
Endothelin (ET-1)	porcine	COS	5.7 pmol/mg	+	+	none	–	[201]
<b>Histamine H2</b>	<b>rat</b>	<b>COS-7/SFV</b>	<b>80 pmol/mg</b>	+	+	<b>none</b>	<b>3</b>	[202]
Olfactive OR 17-40	human/rat	HEK-293/SFV	ND	+	+	none	ND	[203]
<b>Rhodopsin (synthetic)</b>	<b>bovine</b>	<b>COS-1</b>	<b>6 × 10<sup>6</sup> sites/cell</b>	+	+	<b>none</b>	<b>3</b>	[204]
<b>Rhodopsin</b>	<b>bovine</b>	<b>HEK-293S</b>	<b>10 mg/ml</b>	+	<b>ND</b>	<b>none</b>	<b>50</b>	[99]

TRH: thyrotropin releasing hormone; CHO: Chinese hamster ovary; HEK: human embryonic kidney; BHK: baby hamster kidney; JEG-3: human choriocarcinoma cells; COS: African monkey cells; MEL: murine erythroleukemia; L929: mouse fibrosarcoma; SFV: Semliki Forest virus; synthetic: synthetic gene; W: vaccinia virus; NIH3T3: mouse fibroblasts. Boldface characters correspond to the GPCRs for which the overexpression factor is > 1.

### Results obtained with *E. coli*

The most widespread strategy used to express GPCRs in *E. coli* is to fuse the receptor of interest with a bacterial protein. The different GPCRs expressed in *E. coli* and their associated expression levels are presented in table 2. The first GPCR expressed in this bacteria is the  $\beta_2$ -adrenergic receptor, fused in its N-terminus to the 279 initial residues of  $\beta$ -galactosidase, a cytosolic protein naturally expressed in *E. coli* [32]. A large number of attempts to improve the initial expression level have been performed, mainly by changing the promoter and by fusing the receptor to different partners. Fusion of the receptor to proteins belonging to the inner or to the outer membrane of *E. coli* such as the MBP [33] or LamB protein [32, 34, 35] have enable a 10-fold increase in the initial expression level of the  $\beta_2$ -adrenergic receptor. Nevertheless, no fusion protein has been used in the study by Breyer et al. [36] and the expression level of the 2-adrenergic receptor under the control of a strong isopropyl-1-thio- $\beta$ -D-galactopyranoside (IPTG)-inducible promoter (gene 10 of the T7 bacteriophage) is identical to those obtained for the same protein fused to the LamB or MBP genes [36]. Thus, the correct targeting of a GPCR to *E. coli* membrane does not strictly rely on its fusion to a membranous bacterial protein. However, fusion of the neurotensin receptor to the MBP resulted in a 40-fold increase in expression level [14]. This kind of difference suggests that the behaviour in terms of expression level is clearly receptor dependent. As shown in table 2, expression levels range from 0.2 to 16 pmol of receptor per milligram of membrane protein (equivalent to 15–450 sites per cell), and are quite low whatever the strategy employed (with the notable exception of the TSH receptor, see [126]). Moreover, within a given receptor family the results can be different even though the expression strategy employed is identical. For instance, the expression level of the  $\beta_1$ -adrenergic receptor is fourfold lower than that of the  $\beta_2$ -adrenergic receptor [113]. This clearly shows that for each GPCR, a variety of genetic constructs must be systematically employed because the results depend on the exact primary sequence [34]. This difference of behaviour could be explained by the presence of rare codons in higher quantities in certain particular sequences. For instance, the leukotriene B4 (LTB4) receptor was overexpressed in *E. coli* after an optimisation step consisting of replacing all the rare codons initially present in the sequence of the receptor [206]. Although the recombinant protein is chemically homogeneous, several populations of receptor can sometimes be observed. For instance, a population of both high- and low-affinity binding sites have been observed for the neurotensin NK<sub>2</sub> receptor [37]. The origin of these two populations is not clearly explained by the authors, but the hypothesis is that during the insertion of the receptor into the membrane, two different conformations could be generated [37]. Immuno-

detection experiments reveal high quantities of proteins where only low expression levels are observed using radiolabeled ligand-binding experiments, which again suggests the presence of different populations of receptor [14]. The size and topology of GPCRs can also influence their production. Thus, the work by Kiefer et al. shows that the presence of positively charged residues in the intracellular loop 1 (IL1), favours expression of the OR5 olfactive receptor by preventing its insertion into the bacterial membrane [26]. Indeed, if the protein is toxic for the bacteria, its insertion into the membrane (thought to increase toxicity) can limit its expression. The studies by Banères et al. [205] and by Kiefer et al. [26] are, to our knowledge, the only ones reported that describe the expression of GPCRs in inclusion bodies. Despite the fact that the expression level of the receptors produced has not been determined accurately because of lack of activity, these studies are particularly relevant because they were pursued up to the functional reconstitution step. Thus, even if the functional refolding of all GPCRs is not a guarantee, this approach is very promising.

### Yeast

#### Main characteristics of yeast

Yeast present short generation times (2 h), grow on very simple media, and their manipulation is easy and inexpensive. Moreover, the experience developed in particular by industries in the field of fermentation facilitates scaled-up production. Numerous plasmids are available for the expression of heterologous genes, and are either maintained under an episomal form or integrated in the genome of the selected yeast. In yeast, strong promoters are known and are either constitutive or inducible, such as PGK1 (constitutive) and GAL1 (galactose inducible) in *S. cerevisiae*, or AOX1 (methanol inducible) in *Pichia pastoris*. Contrary to bacteria, yeasts possess endogenous GPCRs and G proteins [38–41], and the eucaryotic nature of these microorganisms confers on them the capacity to perform posttranslational modifications [42, 43], which are sometimes essential for protein functionality [18, 19]. Nevertheless, the composition and quantity of *N*-glycans added by yeast are different from that of mammalian cells, and this can be problematical when precise oligosaccharide structures are essential to GPCR functionality [18]. The lipid composition of yeast membranes is also different from that of mammalian cells. Thus, yeast do not synthesise cholesterol like mammalian cells, but rather ergosterol, and this difference was shown to alter the functionality of some GPCRs [23]. Finally, yeast are surrounded by a cell wall that must be disrupted in order to extract membrane and intracellular proteins.

## Results obtained in yeast

The results obtained for the heterologous expression of GPCRs in yeast are presented in table 3.

### *S. cerevisiae*

The first yeast used for the production of recombinant proteins is *S. cerevisiae*. The most frequently employed promoters are galactose inducible, such as GAL1 and GAL10. It has rapidly been concluded that the fusion of the receptor, in its N-terminus end, to a yeast signal sequence favours its insertion into the membranes. Among the most used fusion sequences is that of the  $\alpha$ -mating factor prepropeptide (pp $\alpha$ MF), a sexual mating factor excreted by *S. cerevisiae*, which binds to the Ste2 receptor. The Ste2 N-terminus can also be used as a targeting sequence. The first attempts to express GPCRs in *S. cerevisiae* were those of the human  $\beta_2$ -adrenergic and  $m_1$ -muscarinic receptors [7, 44]. The  $m_1$ -muscarinic receptor was able to bind a variety of specific ligands, but its expression level (20 fmol/mg of membrane proteins) was extremely low [44]. On the contrary, the  $\beta_2$ -adrenergic receptor was very highly expressed: 115 pmol of receptor per milligram of membrane protein [7]. This result was obtained after the following handling: (i) replacement of the 5'-untranslated region and the 14 subsequent codons of the  $\beta_2$ -adrenergic gene by the corresponding region of the STE2 receptor gene; (ii) use of a strong promoter: GAL1 and coexpression of its transcriptional transactivator and (iii) induction of the  $\beta_2$ -adrenergic receptor expression in the presence of an antagonist which is likely to act as a structural stabilizer. In this study, the receptor coupling to the  $G_{\alpha s}$  subunit (also coexpressed) was demonstrated [7]. However, the use of an identical strategy (*S. cerevisiae*, GAL-type promoter, STE2 fusion) for the D2S-dopaminergic receptors resulted in a lower expression level (1–2 pmol of receptor per milligram of membrane protein) [45, 46]. This shows again that each GPCR behaves in a specific way and that the transposition of an efficient strategy from one to another is never guaranteed. Moreover, the transposition of batch-optimised culture conditions to the preparative scale (fermenters) can also result in modification of the expression level. For instance, the human  $\beta_2$ -adrenergic receptor, expressed under the conditions described by King et al. [7] but grown in fermenter, was 'only' produced at a level of 36 pmol per milligram of membrane protein [47]. The crucial factors for this study were the use of a selective medium without glucose, the control of pH between 7.2 and 7.5, and the presence of an antagonist in the induction medium. These conditions have been applied to the human  $\alpha_2$ -C2-adrenergic receptor fused in its C-terminus to a pentahistidine tag, but devoid of any fused sequence in its N-terminus. The production of the receptor, monitored by immunodetection using a receptor-specific antiserum and by radioactive ligand-binding experiments, has

reached levels between 7 and 70 pmol of receptor per milligram of membrane protein, depending on the experiment [47].

### *Schizosaccharomyces pombe*

Initially used to express bacteriorhodopsin [48–50], the yeast *Sz. pombe* has been successfully employed for GPCR expression. The strong *nmt1* (no message in thiamine) promoter, which is repressed by thiamine was generally used for expression [51]. The first GPCR expressed in *Sz. pombe* was the short form of the D<sub>2</sub>-dopaminergic receptor (D<sub>2S</sub>), placed under the control of the *nmt1* promoter and fused, in its N-terminus, to part of the Ste2 receptor [52]. In terms of selective ligand affinities, the recombinant receptor presented a pharmacological profile, typical of the D<sub>2S</sub>-dopaminergic protein, but the low affinities detected suggested a lack of coupling with endogenous yeast G proteins. The expression level of this receptor could be increased sevenfold from *S. cerevisiae* to *Sz. pombe*, using a strong promoter in both cases: GAL1 and *NMT1*, respectively. The expression of the 'long' form of the D<sub>2</sub>-dopaminergic receptor (D<sub>2L</sub>), has been realised under the control of the *NMT1* promoter. The expression level was 1 pmol per milligram of membrane protein with a pharmacological profile typical of a D<sub>2</sub>-dopaminergic receptor lacking coupling with the endogenous G proteins of *Sz. pombe* [53]. However, the neurokinin NK2 receptor was only produced at a level of 1.2 pmol per milligram of membrane protein [54], and the  $\beta_2$ -adrenergic at 7.5 pmol per milligram of membrane protein [55]. In the case of the NK2 receptor, the fusion sequence used was that of the signal sequence of the acid phosphatase *Pho1*, a yeast protein located at the cell surface. This sequence was essential in the case of NK2 receptor production, and no functional receptor was detected with a construct containing the receptor alone [54]. Like the pp $\alpha$ MF sequence, the *Pho1* sequence is supposed to favour the correct targeting to yeast membranes. For the NK2 receptor, the difference observed between the expression level detected on whole cells (430 sites per cell) and on membrane preparations (5500 sites per cell) suggests that only a small part of the protein is correctly targeted into the plasma membrane [54]. In the case of the  $\beta_2$ -adrenergic receptor, the alcohol dehydrogenase promoter (ADH) used was constitutive. Fusion to this GPCR of the Ste2 receptor in its N-terminus was required for expression of the protein, whether it was monitored by radiolabeled ligand binding experiments or by immunodetection [55].

### *Pichia pastoris*

Methylotrophic yeasts such as *P. pastoris*, *Hansenula polymorpha* and *Candida boidinii* have been used for



many years as hosts for the production of recombinant proteins [56–61]. The particularity of these yeast strains resides in their ability to use methanol as a single carbon source. Therefore, the methanol metabolism is completely central in these yeasts and the key enzymes involved in methanol assimilation are highly produced by the cells and can represent up to 80% of their total protein content. Thus, the use of strongly regulated and highly inducible (by methanol) promoters that control these enzymes expression, has allowed the production of a large number of recombinant proteins. These methylotrophic yeasts are particularly efficient, as they can grow rapidly, reach high cell densities, do not give rise to hyperglycosylation phenomena and also possess extremely efficient secretory machinery [43]. As shown in table 3, all the expression experiments of GPCRs in methylotrophic yeasts have focused on the use of *P. pastoris* by placing the heterologous gene under the control of the methanol-inducible alcohol oxidase 1 promoter (AOX1), and by using different signals/proteins fused to the receptor studied. In *P. pastoris*, only vectors leading to genome integration of the expression box are available, which is a guarantee of stable expression.

The expression levels of GPCRs vary from 0.4 for the human  $\mu$ -opioid receptor [62] to 40 pmol per milligram of membrane protein for the 5HT<sub>5A</sub>-serotonergic receptor [65]. For the 5HT<sub>5A</sub>-serotonergic receptor, this expression level is threefold superior to that previously obtained in *S. cerevisiae* [64]. Initially, two types of fusion proteins were used for expression of this receptor [65]: the signal peptide of the acid phosphatase (Pho1) of *P. pastoris*, and the prepropeptide of the  $\alpha$ -mating factor (pp $\alpha$ MF) of *S. cerevisiae*. The best results obtained for the 5HT<sub>5A</sub>-serotonergic receptor (22 pmol per milligram of membrane protein) were reached when using the pp $\alpha$ MF signal sequence and a particular yeast strain: SMD1163, deficient in two proteases. The selection of clones bearing several copies of the expression box inserted in the yeast's genome and the addition of ligand in the culture medium enabled an expression level of 40 pmol per milligram of membrane proteins [65]. For the human  $\beta_2$ -adrenergic receptor, the expression level in *P. pastoris* was 'only' of 25 pmol per milligram of membrane protein [66], in comparison with the 115 pmol per milligram of membrane protein previously reported in *S. cerevisiae* [7]. For the human  $\mu$ -opioid receptor placed under the control of the AOX1 promoter and fused to the pp $\alpha$ MF signal sequence, the expression level of 0.4 pmol per milligram of membrane protein [62] was rigorously identical to that reported in *S. cerevisiae* [67]. In *P. pastoris*, this expression level was not increased by the use of protease(s)-deficient strains such as SMD1163 or SMD1168, and the recombinant receptor presented a pharmacological profile for various opioid ligands typical of a  $\mu$ -opioid receptor lacking coupling with endoge-

nous yeast G proteins [62]. The same result (correct pharmacological profile but lack of G protein coupling) has been observed for this receptor in *S. cerevisiae* [67]. It is however interesting that G protein coupling can be restored in vitro. In addition, comparative quantification experiments of the total (fluorescence experiments) versus the active (binding experiments) proportions of the  $\mu$ -opioid receptor have shown that the majority of the receptor produced in *P. pastoris* is not detected by classical binding experiments [69]. Moreover, while the total amount of receptor produced is constant, the active proportion of this receptor can be increased fourfold by changing the induction conditions [68].

Bovine rhodopsin has also been expressed under the control of the AOX1 promoter and fused to the *Pho1* signal sequence in *P. pastoris* [70] and the reported expression level was  $3.7 \times 10^6$  sites per cell ( $2.4 \times 10^6$  in *S. cerevisiae* [71]). Thus, here again, the efficiency of a particular cell type for the production of a given GPCR is strongly dependent on the intrinsic nature of the protein considered. The highest GPCR expression level so far reported in yeast is that of the Ste2 receptor expressed at a level of 350 pmol per milligram of membrane protein in *S. cerevisiae* [72], but in the case of homologous expression. It has been observed that the recombinant receptors may or may not be properly coupled to G proteins in yeast. This hazardous coupling phenomenon can be explained by the difference in nature between yeast and superior eucaryotic cell G proteins. Expression of GPCRs in superior eucaryotic cells therefore appears to increase this coupling efficiency.

## The baculovirus/insect cells system

### Main characteristics of insect cells

The *Autographa californica* baculovirus is a double-stranded DNA virus surrounded by a lipid membrane (for review see [73]). This virus is able to selectively infect different insect cell types, among which the most frequently used are those of *Spodoptera frugiperda* (cell lines Sf<sub>9</sub> and Sf<sub>21</sub>) and of *Trichoplusia ni* (cell lines Hi<sub>5</sub> and MG<sub>1</sub>). These insect cells present relatively long generation times (up to 24 h) and require complex culture media. Their eucaryotic nature guarantees their ability to perform posttranslational modifications. The general principle of this system is to place the gene of interest in a plasmid between sequences of high homology for the baculovirus genome, under the control of a strong promoter, most often polyhedrin. Produced at a level of 50% of the total protein content of the cell, polyhedrin is one of the major components of the protective matrix in which the virus is subsequently embedded and is therefore responsible for virus survival after host cell death. Cotransfection of the plasmid and viral DNA in insect

cells allows insertion of the gene of interest into the viral genome *in vivo* by homologous recombination. The polyhedrin promoter is a 'late' promoter, and its activation occurs long after the infection process (8–24 h post-infection). This is an advantage when the recombinant protein is toxic for the cell. The drawback, however, is that receptor production occurs only at the end of the cell's life. In other respects, this system was initially established for transient expression, which is not optimum for large-scale production. The establishment of stable cell lines under the control of either a viral or insect promoter have subsequently been developed [74–78]. These stable cell lines offer an environment unperturbed by the viral cycle, which is ideal for functional studies. But the promoters are generally weaker than that of polyhedrin.

A large number of vectors and viral linearised DNA for transient expression are commercially available, and it is possible to obtain important virus stocks in a relatively short time (month scale).

### Results obtained with insect cells

Many GPCRs have been transiently expressed, but the attempts to express GPCRs in stable insect cell lines are still isolated [75–78]. The main results, presented in table 4, show that most of the time  $S_{B9}$  or  $S_{I21}$  cell lines were employed. In all cases, the GPCRs produced were able to bind radiolabeled ligands (agonists and antagonists) in a specific and saturable manner and to couple to endogenous G proteins. A detailed analysis of the results shows that the expression levels are very different from one GPCR to another: from 0.15 pmol/mg for human 5HT<sub>1A</sub>-serotonergic [79] to 80 pmol/mg for neurokinin NK1 receptors [80, 180]. Moreover, these expression levels are highly dependent on receptor subtype: using exactly the same expression strategy, the values obtained for each of the five muscarinic receptor subtypes were comprised between 0.6 and 16 pmol/mg [81]. Less variability was observed for the three opioid receptors subtypes, which were weakly expressed by this system [75, 76, 148–151]. Some parameters, such as the presence and the position of tags can also influence the expression level of a given receptor. For instance, fusion of a hexahistidine tail to the human  $\mu$ -opioid receptor N-terminus (hMOP1) diminished its expression level 10-fold, while it had no influence when fused to the C-terminus end of the receptor [151]. Positive charges brought by this polyhistidine tag could inhibit targeting of the receptor to the membrane. The addition of signal sequences such as that of pre-promelittin [158] or of hemagglutinin [82] can also improve the expression level of a receptor in the plasma membrane. However, a large proportion of the GPCR can be trapped in intracellular membranous compartments such as the Golgi apparatus or the endoplasmic reticulum [83]. One major drawback of this system from the per-

spective of structural biology experiments is that it often gives rise to heterogeneous populations of receptors (in terms of posttranslational modifications). For instance, glycosylation heterogeneity in the human  $\beta_2$ -adrenergic receptor N-terminus [84] and in the rat m<sub>3</sub>-muscarinic cholinergic receptor has been described [85]. In the case of the thyrotrophin receptor, despite high messenger RNA (mRNA) levels, no receptor molecule was revealed by binding experiments [86]. This underlines the importance of using an alternative means to detect the expressed protein, independent of radiolabeled ligand-binding experiments. Moreover, high-affinity binding sites for agonists are not always observed, probably due to an insufficient number of endogenous G proteins available to couple with the overexpressed GPCR [20, 87]. The very low abundance of cholesterol in insect cells can also explain the binding properties of certain GPCRs, as was observed for the oxytocin receptor [88]. Despite this phenomenon, a large number of studies have found functional coupling between endogenous G proteins and heterologously expressed GPCRs [89–91].

### Mammalian cells

#### Main characteristics of mammalian cells

The main advantages of mammalian cell expression system are that (i) these cells present the environment closest to the native tissues in which GPCRs naturally occur; (ii) they are able to perform complex posttranslational modifications and (iii) the lipid composition of their membranes is close to that of the native receptor. Especially, these cells can furnish G proteins able to interact with the GPCRs produced and secondary effectors for studying signal transduction. Thus, mammalian cells appear as the most appropriate for functional studies, which require an active protein, but not in very high amounts. There are two major approaches for heterologous expression of GPCRs in mammalian cells: transient and stable expression. Transient expression is performed under non-selective conditions. In this case, cells can be transfected with the gene encoding the GPCR of interest either with a plasmid or with a recombinant virus. Several virus types can be used, such as the vaccinia virus (for review see [92–94]) and the Semliki Forest virus (for review see [95]) for instance. Even if the "transfection" step using these viruses is very efficient, GPCR expression is transient since the cells die after several days. Thus, if high quantities of material are necessary in a repetitive way, establishment of stable cell lines is highly recommended. In the case of stable expression, the cells are grown under selective conditions thanks to the use of a selection marker cotransfected with recombinant DNA. The recombinant DNA can either be integrated in the host cell genome by using an appropriate vector or non-integrated in the case of vectors capable of episomal replication. In-

serted next to the gene encoding for the dihydrofolate reductase or the glutamine synthetase, the gene of interest can then be amplified. Selection of clones bearing multi-copy insertion events can then be realised using a high pressure of selection (high antibiotic concentration, for instance). Whatever the strategy employed, generation of an amplified stable cell line overexpressing recombinant GPCR is usually time-consuming (month-long delays).

### Results obtained with mammalian cells

A huge number of GPCRs have been expressed in mammalian cells, but this expression system has mainly been used to study the key residues involved in binding specific ligands and the possible corresponding structure of the active site of the receptor by associating transient expression and mutagenesis experiments (for review see [96, 97]). Rare are the GPCRs produced in mammalian cells that carry special tags for a subsequent purification step since they are most often dedicated to functional studies and not to overproduction (indeed, most of the “tagged” GPCRS have been used in the context of internalisation/desensitization or dimerisation studies for instance).

Table 5 shows some of the results obtained for expression of GPCRs in different mammalian cell lines, either by stable or transient expression. Indeed, these cells have been largely used for heterologous expression of GPCRs, particularly during their respective cloning step, in the case of the study of mutant or chimeric receptors or for studying GPCR oligomerisation and internalisation processes. Thus, the results summarised in table 5 are presented as an illustration and are not exhaustive. One can easily see that the expression level is highly variable from one GPCR to another. The highest production level reported is that of the human  $\beta_2$ -adrenergic receptor, which reached 200 pmol per milligram of membrane protein [98] and rhodopsin with 10 mg/l in culture of HEK-293 cells. In the latter case, purification to homogeneity was reported [99]. This result is very encouraging for other receptors even if scale-up of mammalian cell culture is likely to be difficult.

### Other systems

Aside from the major expression systems previously described, many other strategies have been used to express GPCRs. For example, two receptors were expressed with femtomolar expression levels in the Archea *Halobacterium salinarum*, the  $\beta_2$ -adrenergic receptor [101] and the yeast  $\alpha$ -mating factor receptor [100]. A caterpillar expression system was used to express, in a nonfunctional form, the extracellular part of the luteinizing hormone (LH) receptor, a member of the glycoprotein hormone family [102]. Heterologous expression in *Xenopus* oocytes was realised, but this system was mainly used to

study GPCR functional coupling since protein expression requires mRNA injection into each oocyte, which prevents large-scale production [103]. An original system was recently developed for the expression of GPCRs in fly eyes [104]. The *Drosophila melanogaster* metabotropic glutamate receptor was expressed in good yield (170  $\mu$ g/g fly heads), but heterologous expression of GPCRs is still a challenge in this system. Finally, cell free expression systems have many advantages, and numerous experiments have been reported with soluble proteins [105–109]. Although no GPCR has yet been expressed, these systems might become important in the following years, in particular in the context of structural genomics projects [110, 111].

### Discussion: factors influencing GPCR overexpression

Tables 2 to 5 (column ‘Overexpression factor’) clearly show that in general, GPCRs are only slightly overexpressed by the different heterologous systems presented above. The difficulty of all these systems in achieving overexpression of GPCRs finds its origin in the complex folding mechanism of membrane proteins, in host membrane insertion troubles, or in an inappropriate lipid environment and/or posttranslational modifications. Thus, only a better understanding of the in vivo folding mechanisms of these membrane proteins and detailed characterisation of the proteins and factors which participate in this folding process will lead to improvements in the use of these different expression systems. Today, systematic comparison of the expression of a given membrane protein in a particular system only generates an approximate and empirical guide to factors that can influence results. One should also keep in mind that negative results are often not published. Comparison of the different expression levels from one GPCR to another shows that it is difficult to predict whether a receptor will be efficiently overexpressed. Among a given expression system (for instance in insect cells), the expression level of different GPCRS can vary 100-fold. Similarly, the expression level for a given GPCR is significantly different from one system to another. Up to now, the highest expression level reached is that of the Ste2 receptor of *S. cerevisiae*, overexpressed in *S. cerevisiae* at a level of 350 pmol per milligram of membrane protein [72]. This example clearly illustrates the fact that the closer to the native environment of the receptor a system is, the higher the chance of achieving overexpression. The majority of heterologously expressed GPCRS are from murine and human origin, and this suggests that mammalian cells should present the more appropriate environment. However, and even if the data presented in table 5 are not exhaustive, mammalian cells are the least developed for high-scale production of recombinant proteins, and up to now have mainly been used for functional studies. It is therefore interesting to

notice that only a few of the GPCRs expressed in mammalian cells presented a tag enabling subsequent purification (e.g. Histags, Biotags, Flag tags). Whether they derive from mammals or insects, superior eucaryotic cells are able to perform posttranslational modifications that can be the source of heterogeneities and troubles during the purification step. In particular, N-glycosylations are very important for several proteins for their folding and/or stability.

The specific sequence of each GPCR is one of the key elements that controls its expression. Indeed, as shown in table 6, some receptors such as the  $\beta_2$ -adrenergic, are expressed very efficiently in almost every system, though the quantities vary from one to another. Other GPCRs, such as the human  $\mu$ -opioid receptor, are expressed at low levels whatever the system used (table 6), except in *P. pastoris* (100 pmol/mg), where 1% of the expressed receptor is active.

In conclusion, it seems that the only way to optimise the overexpression of a particular GPCR is to systematically test different expression systems and strategies. Several criteria can guide the researcher's choice, and particularly the ultimate goal of the work. Whenever the goal is high-resolution 3D structural data, the objective is simple: obtaining purified receptor, chemically and structurally homogeneous, in high quantities. To compare the efficiency of the different expression systems, Grishammer and Tate [6] have proposed a minimal production of 1 mg of recombinant protein in 5 l of culture medium. The corresponding minimal expression levels are reported in table 1 and are 13 pmol/mg of membrane protein in *E. coli*, 5 pmol/mg in yeast and 26 pmol/mg of membrane protein in superior eucaryotic cells. This rapid estimation indicates which receptors can be produced in high quantities

and in which cell types. These examples appear in boldface in tables 2–5. An initial rapid analysis of the four tables clearly shows that yeast is the system in which this objective was most frequently obtained. Of the 25 GPCRs expressed in this organism (table 3), 14 were expressed at a level higher than 5 pmol/mg of membrane protein. In *E. coli*, of 25 GPCRs expressed, only 4 exceeded the minimal expression level, and the numbers were 13 out of 60 in insect cells and 10 out of 55 for mammalian cells. The results presented in table 6 clearly show that this objective was easily reached for the  $\beta_2$ -adrenergic receptor whatever the system used, while it was possible only in *P. pastoris* for the human  $\mu$ -opioid receptor. In general, the term 'overexpression' is used to describe the production of 10–100 mg of protein per liter of culture. Indeed, for 3D crystallisation trials, several tens of milligrams of pure protein are needed, and an expression level of 1 mg in 5 l of culture media will rapidly be limiting, particularly when considering that the overall yield of the purification procedure is generally very low. In some case it is difficult to determine the expression level when the expressed GPCRs are not active in terms of ligand binding. For instance the OR5 olfactory receptor expressed in *E. coli* by Kiefer et al. [26] was expressed in inclusion bodies in an inactive form. It was then successfully purified and functionally reconstituted in proteoliposome. Thus, an initially inactive GPCR can be refolded into an active conformation, and the initial activity is not an absolute prerequisite [205, 206]. This strategy is greatly simplified if a detection test, independent of binding experiments, is available to quantify the GPCR expression level. In this regard, expression of a fusion protein between the receptor and green fluorescent protein may be of general interest [68, 69]. Progress in the in vitro re-

Table 6. Comparative heterologous expression levels of the human  $\beta_2$ -adrenergic and  $\mu$ -opioid receptors.

Receptor	Host cell	Strain	Expression level (pmol/mg)	Expression level (sites/cell)	Expression level necessary to obtain 1 mg of receptor in 5 l of culture media		Ref.
					pmol/mg	sites/cell	
Human $\beta_2$ -Adrenergic	<i>E. coli</i>		1	200 #	13	$2.6 \times 10^3$	[36]
	yeast	<i>S. cerevisiae</i>	115 #	$6 \times 10^5$	5	$2.6 \times 10^4$	[7]
	insect cells	<i>Sf<sub>9</sub></i>	10	$10^6$ #	26	$2.6 \times 10^6$	[84]
		<i>Sf<sub>9</sub></i> stable	3–4	$3.5 \times 10^5$ #	26	$2.6 \times 10^6$	[77]
		CHO stable	200 #	$2 \times 10^7$	26	$2.6 \times 10^6$	[98]
mammalian cells							
Human $\mu$ -Opioid	<i>E. coli</i>		0.4 #	80	13	$2.6 \times 10^3$	[124]
	yeast	<i>P. pastoris</i>	0.4 #	$2 \times 10^3$	5	$2.6 \times 10^4$	[62]
		<i>P. pastoris</i>	100 #	$5 \times 10^5$	5	$2.6 \times 10^4$	[69]
		<i>S. cerevisiae</i>	0.4 #	$2 \times 10^3$	5	$2.6 \times 10^4$	[67]
	insect cells	<i>Sf<sub>9</sub></i>	9 #	$10^6$	26	$2.6 \times 10^6$	[152]
			10	$10^6$ #	26	$2.6 \times 10^6$	[151]
	mammalian cells	COS-7	0.23 #	$2.3 \times 10^4$	26	$2.6 \times 10^6$	[191]

The # symbol represents the expression levels as they were given by the authors, while the other expression levels correspond to the conversions according to the values given in table 1.

folding of GPCRs would make overexpression in inclusion bodies in *E. coli*, or in any equivalent compartment in yeast, a strategy of choice for GPCRs, as it is already the case for many soluble proteins.

Whatever the strategy employed, once the overexpression step is reached, the protein must be extracted from the chosen organism and purified. To date (apart from the noticeable exception of rhodopsin), despite the numerous reports of the heterologous expression in GPCRs, only a single recombinant one has been solubilised, purified and subsequently used in a high-resolution NMR experiment [112]. This study has allowed the determination of the 3D structure of a small peptide ligand (PACAP), interacting with its corresponding GPCRs, using the transferred nuclear Overhauser effect approach [112]. It can be foreseen that the improvements observed recently in the field, and the numerous high-throughput initiatives for GPCR overexpression, will drastically change the situation in the near future.

- Flower D. R. (1999) Modelling G-protein-coupled receptors for drug design. *Biochim. Biophys. Acta* **1422**: 207–234
- Bockaert J. and Pin J. P. (1998) [Use of a G-protein-coupled receptor to communicate. An evolutionary success]. *C. R. Acad. Sci. III* **321**: 529–551
- Bockaert J. and Pin J. P. (1999) Molecular tinkering of G protein-coupled receptors: an evolutionary success. *EMBO J.* **18**: 1723–1729
- Palczewski K., Kumasaka T., Hori T., Behnke C. A., Moto-shima H., Fox B. A. et al. (2000) Crystal structure of rhodopsin: a G protein-coupled receptor. *Science* **289**: 739–745
- Schertler G. F. (1992) Overproduction of membrane proteins. *Curr. Opin. Struct. Biol.* **2**: 534–544
- Grisshammer R. and Tate C. G. (1995) Overexpression of integral membrane proteins for structural studies. *Q. Rev. Biophys.* **28**: 315–422
- King K., Dohlman H. G., Thorner J., Caron M. G. and Lefkowitz R. J. (1990) Control of yeast mating signal transduction by a mammalian beta 2-adrenergic receptor and Gs alpha subunit. *Science* **250**: 121–123
- Laage R. and Langosch D. (2001) Strategies for prokaryotic expression of eukaryotic membrane proteins. *Traffic* **2**: 99–104
- Waeber U., Buhr A., Schunk T. and Erni B. (1993) The glucose transporter of *Escherichia coli*. Purification and characterization by Ni<sup>2+</sup> chelate affinity chromatography of the IIBCGLc subunit. *FEBS Lett.* **324**: 109–112
- Sautel M. and Milligan G. (2000) Molecular manipulation of G-protein-coupled receptors: a new avenue into drug discovery. *Curr. Med. Chem.* **7**: 889–896
- Stanasila L., Pattus F. and Massotte D. (1998) Heterologous expression of G-protein-coupled receptors: human opioid receptors under scrutiny. *Biochimie* **80**: 563–571
- Wagner C. A., Friedrich B., Setiawan I., Lang F. and Broer S. (2000) The use of *Xenopus laevis* oocytes for the functional characterization of heterologously expressed membrane proteins. *Cell. Physiol. Biochem.* **10**: 1–12
- Tate C. G. and Grisshammer R. (1996) Heterologous expression of G-protein-coupled receptors. *Trends Biotechnol.* **14**: 426–430
- Grisshammer R., Duckworth R. and Henderson R. (1993) Expression of a rat neurotensin receptor in *Escherichia coli*. *Biochem. J.* **295**: 571–576
- Tucker J. and Grisshammer R. (1996) Purification of a rat neurotensin receptor expressed in *Escherichia coli*. *Biochem. J.* **317**: 891–899
- Stanasila L., Lim W. K., Neubig R. R. and Pattus F. (2000) Coupling efficacy and selectivity of the human mu-opioid receptor expressed as receptor-Galpha fusion proteins in *Escherichia coli*. *J. Neurochem.* **75**: 1190–1199
- Bertin B., Freissmuth M., Breyer R. M., Schutz W., Strosberg A. D. and Marullo S. (1992) Functional expression of the human serotonin 5-HT1A receptor in *Escherichia coli*. Ligand binding properties and interaction with recombinant G protein alpha-subunits. *J. Biol. Chem.* **267**: 8200–8206
- Kaushal S., Ridge K. D. and Khorana H. G. (1994) Structure and function in rhodopsin: the role of asparagine-linked glycosylation. *Proc. Natl. Acad. Sci. USA* **91**: 4024–4028
- Gibson S. K., Parkes J. H. and Liebman P. A. (1998) Phosphorylation stabilizes the active conformation of rhodopsin. *Biochemistry* **37**: 13910
- Gimpl G., Klein U., Reilander H. and Fahrenholz F. (1995) Expression of the human oxytocin receptor in baculovirus-infected insect cells: high-affinity binding is induced by a cholesterol-cyclodextrin complex. *Biochemistry* **34**: 13794–1380
- Nunez M. T. and Glass J. (1982) Reconstitution of the transferrin receptor in lipid vesicles. Effect of cholesterol on the binding of transferrin. *Biochemistry* **21**: 4139–4143
- Hasegawa J., Loh H. H. and Lee N. M. (1987) Lipid requirement for mu opioid receptor binding. *J. Neurochem.* **49**: 1007–1012
- Lagane B., Gaibelet G., Meilhoc E., Masson J. M., Cezanne L. and Lopez A. (2000) Role of sterols in modulating the human micro-opioid receptor function in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **275**: 33197–33200
- Abrahmsen L., Moks T., Nilsson B. and Uhlen M. (1986) Secretion of heterologous gene products to the culture medium of *Escherichia coli*. *Nucleic. Acids. Res.* **14**: 7487–7500
- Di Guan C., Li P., Riggs P. D. and Inouye H. (1988) Vectors that facilitate the expression and purification of foreign peptides in *Escherichia coli* by fusion to maltose-binding protein. *Gene* **67**: 21–30
- Kiefer H., Krieger J., Olszewski J. D., Von Heijne G., Prestwich G. D. and Breer H. (1996) Expression of an olfactory receptor in *Escherichia coli*: purification, reconstitution and ligand binding. *Biochemistry* **35**: 16077–16084
- LaVallie E. R. and McCoy J. M. (1995) Gene fusion expression systems in *Escherichia coli*. *Curr. Opin. Biotechnol.* **6**: 501–506
- Kiefer H., Maier K. and Vogel R. (1999) Refolding of G-protein-coupled receptors from inclusion bodies produced in *Escherichia coli*. *Biochem. Soc. Trans.* **27**: 908–912
- Efimov V. A., Fradkov A. F., Raskind A. B., Khristin M. S., Klimov V. V. and Chakhmakhcheva O. G. (1994) Expression of the barley psbA gene in *Escherichia coli* yields a functional in vitro photosystem II protein D1. *FEBS Lett.* **348**: 153–157
- Fiermonte G., Walker J. E. and Palmieri F. (1993) Abundant bacterial expression and reconstitution of an intrinsic membrane-transport protein from bovine mitochondria. *Biochem. J.* **294**: 293–299
- Matsuyama S., Fujita Y. and Mizushima S. (1992) Large-scale production of membrane proteins fused to a truncated SecA in *Escherichia coli*. *Biosci. Biotechnol. Biochem.* **56**: 1512–1514
- Marullo S., Delavier-Klutchko C., Eshdat Y., Strosberg A. D. and Emorine L. (1988) Human beta 2-adrenergic receptors expressed in *Escherichia coli* membranes retain their pharmacological properties. *Proc. Natl. Acad. Sci. USA* **85**: 7551–7555
- Hampe W., Voss R. H., Haase W., Boege F., Michel H. and Reilander H. (2000) Engineering of a proteolytically stable hu-

- man beta 2-adrenergic receptor/maltose-binding protein fusion and production of the chimeric protein in *Escherichia coli* and baculovirus-infected insect cells. *J. Biotechnol.* **77**: 219–234
- 34 Chapot M. P., Eshdat Y., Marullo S., Guillet J. G., Charbit A., Strosberg A. D. et al. (1990) Localization and characterization of three different beta-adrenergic receptors expressed in *Escherichia coli*. *Eur. J. Biochem.* **187**: 137–144
  - 35 Emorine L. J., Marullo S., Briend-Sutren M. M., Patey G., Tate K., Delavier-Klutchko C. et al. (1989) Molecular characterization of the human beta 3-adrenergic receptor. *Science* **245**: 1118–1121
  - 36 Breyer R. M., Strosberg A. D. and Guillet J. G. (1990) Mutational analysis of ligand binding activity of beta 2 adrenergic receptor expressed in *Escherichia coli*. *EMBO J.* **9**: 2679–2684
  - 37 Grisshammer R., Little J. and Aharony D. (1994) Expression of rat NK-2 (neurokinin A) receptor in *E. coli*. *Receptors Channels* **2**: 295–302
  - 38 Blumer K. J., Reneke J. E. and Thorner J. (1988) The STE2 gene product is the ligand-binding component of the alpha-factor receptor of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **263**: 10836–10842
  - 39 Kraakman L., Lemaire K., Ma P., Teunissen A. W., Donaton M. C., Van Dijck P. et al. (1999) A *Saccharomyces cerevisiae* G-protein coupled receptor, Gpr1, is specifically required for glucose activation of the cAMP pathway during the transition to growth on glucose. *Mol. Microbiol.* **32**: 1002–1012
  - 40 Price L. A., Kajkowski E. M., Hadcock J. R., Ozenberger B. A. and Pausch M. H. (1995) Functional coupling of a mammalian somatostatin receptor to the yeast pheromone response pathway. *Mol. Cell. Biol.* **15**: 6188–6195
  - 41 Stefan C. J., Overton M. C. and Blumer K. J. (1998) Mechanisms governing the activation and trafficking of yeast G protein-coupled receptors. *Mol. Biol. Cell.* **9**: 885–899
  - 42 Eckart M. R. and Bussineau C. M. (1996) Quality and authenticity of heterologous proteins synthesized in yeast. *Curr. Opin. Biotechnol.* **7**: 525–530
  - 43 Reilander H. and Weiss H. M. (1998) Production of G-protein-coupled receptors in yeast. *Curr. Opin. Biotechnol.* **9**: 510–517
  - 44 Payette P., Gossard F., Whiteway M. and Dennis M. (1990) Expression and pharmacological characterization of the human M1 muscarinic receptor in *Saccharomyces cerevisiae*. *FEBS Lett.* **266**: 21–25
  - 45 Sander P., Grunewald S., Bach M., Haase W., Reilander H. and Michel H. (1994) Heterologous expression of the human D2S dopamine receptor in protease-deficient *Saccharomyces cerevisiae* strains. *Eur. J. Biochem.* **226**: 697–705
  - 46 Sander P., Grunewald S., Maul G., Reilander H. and Michel H. (1994) Constitutive expression of the human D2S-dopamine receptor in the unicellular yeast *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* **1193**: 255–262
  - 47 Sizmann D., Kuusinen H., Keranen S., Lomasney J., Caron M. G., Lefkowitz R. J. et al. (1996) Production of adrenergic receptors in yeast. *Receptors Channels* **4**: 197–203
  - 48 Hildebrandt V. (1989) Regeneration and functional incorporation of bacteriorhodopsin in membranes of fission yeast but not in *E. coli*. *J. Protein Chem.* **8**: 345–346
  - 49 Hildebrandt M., Saur U. and Nellen W. (1991) Structure, expression and inactivation by gene disruption of the *Dictyostelium discoideum* prespore gene EB4. *Dev. Genet.* **12**: 163–169
  - 50 Hildebrandt V., Fendler K., Heberle J., Hoffmann A., Bamberg E. and Buldt G. (1993) Bacteriorhodopsin expressed in *Schizosaccharomyces pombe* pumps protons through the plasma membrane. *Proc. Natl. Acad. Sci. USA* **90**: 3578–3582
  - 51 Maundrell K. (1993) Thiamine-repressible expression vectors pREP and pRIP for fission yeast. *Gene* **123**: 127–130
  - 52 Sander P., Grunewald S., Reilander H. and Michel H. (1994) Expression of the human D2S dopamine receptor in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*: a comparative study. *FEBS Lett.* **344**: 41–46
  - 53 Presland J. and Strange P. G. (1998) Pharmacological characterisation of the D2 dopamine receptor expressed in the yeast *Schizosaccharomyces pombe*. *Biochem. Pharmacol.* **56**: 577–582
  - 54 Arkinstall S., Edgerton M., Payton M. and Maundrell K. (1995) Co-expression of the neurokinin NK2 receptor and G-protein components in the fission yeast *Schizosaccharomyces pombe*. *FEBS Lett.* **375**: 183–187
  - 55 Ficca A. G., Testa L. and Tocchini Valentini G. P. (1995) The human beta 2-adrenergic receptor expressed in *Schizosaccharomyces pombe* retains its pharmacological properties. *FEBS Lett.* **377**: 140–144
  - 56 Cregg J. M., Cereghino J. L., Shi J. and Higgins D. R. (2000) Recombinant protein expression in *Pichia pastoris*. *Mol. Biotechnol.* **16**: 23–52
  - 57 Faber K. N., Harder W., Ab G. and Veenhuis M. (1995) Review: methylotrophic yeasts as factories for the production of foreign proteins. *Yeast* **11**: 1331–1344
  - 58 Gellissen G. (2000) Heterologous protein production in methylotrophic yeasts. *Appl. Microbiol. Biotechnol.* **54**: 741–750
  - 59 Higgins D. R. and Cregg J. M. (1998) Introduction to *Pichia pastoris*. *Methods Mol. Biol.* **103**: 1–15
  - 60 Hollenberg C. P. and Gellissen G. (1997) Production of recombinant proteins by methylotrophic yeasts. *Curr. Opin. Biotechnol.* **8**: 554–560
  - 61 Romanos M. A., Scorer C. A. and Clare J. J. (1992) Foreign gene expression in yeast: a review. *Yeast* **8**: 423–488
  - 62 Talmont F., Sidobre S., Demange P., Milon A. and Emorine L. J. (1996) Expression and pharmacological characterization of the human mu-opioid receptor in the methylotrophic yeast *Pichia pastoris*. *FEBS Lett.* **394**: 268–272
  - 63 Weiss H. M., Haase W., Michel H. and Reilander H. (1995) Expression of functional mouse 5-HT5A serotonin receptor in the methylotrophic yeast *Pichia pastoris*: pharmacological characterization and localization. *FEBS Lett.* **377**: 451–456
  - 64 Bach M., Sander P., Haase W. and Reilander H. (1996) Pharmacological and biochemical characterization of the mouse 5HT5A serotonin receptor heterologously produced in the yeast *Saccharomyces cerevisiae*. *Receptors Channels* **4**: 129–139
  - 65 Weiss H. M., Haase W. and Reilander H. (1998) Expression of an integral membrane protein, the 5HT5A receptor. *Methods Mol. Biol.* **103**: 227–239
  - 66 Weiss H. M., Haase W., Michel H. and Reilander H. (1998) Comparative biochemical and pharmacological characterization of the mouse 5HT5A 5-hydroxytryptamine receptor and the human beta2-adrenergic receptor produced in the methylotrophic yeast *Pichia pastoris*. *Biochem. J.* **330**: 1137–1147
  - 67 Gaibelet G., Meilhoc E., Riond J., Saves I., Exner T., Liaubet L. et al. (1999) Nonselective coupling of the human mu-opioid receptor to multiple inhibitory G-protein isoforms. *Eur. J. Biochem.* **261**: 517–523
  - 68 Sarramegna V., Demange P., Milon A. and Talmont F. (2002) Optimizing functional versus total expression of the human mu-opioid receptor in *Pichia pastoris*. *Protein Expr. Purif.* **24**: 212–220
  - 69 Sarramegna V., Talmont F., Milon A. and Demange P. (2002) Green fluorescent protein as a reporter of human mu-opioid receptor overexpression in the methylotrophic yeast *Pichia pastoris*. *J. Biotechnology* **99**: 23–39
  - 70 Abdulaev N. G., Popp M. P., Smith W. C. and Ridge K. D. (1997) Functional expression of bovine opsin in the methyl-

- lotrophic yeast *Pichia pastoris*. *Protein Expr. Purif.* **10**: 61–69
- 71 Mollaaghababa R., Davidson F. F., Kaiser C. and Khorana H. G. (1996) Structure and function in rhodopsin: expression of functional mammalian opsin in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **93**: 11482–11486
  - 72 David N. E., Gee M., Andersen B., Naider F., Thorner J. and Stevens R. C. (1997) Expression and purification of the *Saccharomyces cerevisiae* alpha-factor receptor (Ste2p), a 7-transmembrane-segment G protein-coupled receptor. *J. Biol. Chem.* **272**: 15553–15561
  - 73 Fraser M. J. (1992) The baculovirus-infected insect cell as a eukaryotic gene expression system. *Curr. Top. Microbiol. Immunol.* **158**: 131–172
  - 74 Pfeifer T. A. (1998) Expression of heterologous proteins in stable insect cell culture. *Curr. Opin. Biotechnol.* **9**: 518–521
  - 75 Kempf J., Snook L. A., Vonesch J. L., Dahms T. E., Pattus F. and Massotte D. (2002) Expression of the human mu opioid receptor in a stable Sf9 cell line. *J. Biotechnol.* **95**: 181–187
  - 76 Bernard A. R., Kost T. A., Overton L., Cavegn C., Young J., Bertrand M. et al. (1994) Recombinant protein expression in a *Drosophila* cell line: comparison with the baculovirus system. *Cytotechnology* **15**: 139–144
  - 77 Kleymann G., Boege F., Hahn M., Hampe W., Vasudevan S. and Reilander H. (1993) Human beta 2-adrenergic receptor produced in stably transformed insect cells is functionally coupled via endogenous GTP-binding protein to adenylyl cyclase. *Eur. J. Biochem.* **213**: 797–804
  - 78 Millar N. S., Baylis H. A., Reaper C., Bunting R., Mason W. T. and Sattelle D. B. (1995) Functional expression of a cloned *Drosophila* muscarinic acetylcholine receptor in a stable *Drosophila* cell line. *J. Exp. Biol.* **198**: 1843–1850
  - 79 Mulheron J. G., Casanas S. J., Arthur J. M., Garnovskaya M. N., Gettys T. W. and Raymond J. R. (1994) Human 5-HT1A receptor expressed in insect cells activates endogenous G(o)-like G protein(s). *J. Biol. Chem.* **269**: 12954–12962
  - 80 Mazina K. E., Strader C. D. and Fong T. M. (1994) Expression and solubilization of a recombinant human neurokinin-1 receptor in insect cells. *J. Recept. Res.* **14**: 63–73
  - 81 Rinken A., Kameyama K., Haga T. and Engstrom L. (1994) Solubilization of muscarinic receptor subtypes from baculovirus infected Sf9 insect cells. *Biochem. Pharmacol.* **48**: 1245–1251
  - 82 Guan X. M., Kobilka T. S. and Kobilka B. K. (1992) Enhancement of membrane insertion and function in a type IIIb membrane protein following introduction of a cleavable signal peptide. *J. Biol. Chem.* **267**: 21995–21998
  - 83 Grunewald S., Reilander H. and Michel H. (1996) In vivo reconstitution of dopamine D2S receptor-mediated G protein activation in baculovirus-infected insect cells: preferred coupling to Gi1 versus Gi2. *Biochemistry* **35**: 15162–15173
  - 84 Reilander H., Boege F., Vasudevan S., Maul G., Hekman M., Dees C., et al. (1991) Purification and functional characterization of the human beta 2- adrenergic receptor produced in baculovirus-infected insect cells. *FEBS Lett.* **282**: 441–444
  - 85 Vasudevan S., Hulme E. C., Bach M., Haase W., Pavia J. and Reilander H. (1995) Characterization of the rat m3 muscarinic acetylcholine receptor produced in insect cells infected with recombinant baculovirus. *Eur. J. Biochem.* **227**: 466–475
  - 86 Harfst E., Johnstone A. P., Gout I., Taylor A. H., Waterfield M. D. and Nussey S. S. (1992) The use of the amplifiable high-expression vector pEE14 to study the interactions of autoantibodies with recombinant human thyrotrophin receptor. *Mol. Cell. Endocrinol.* **83**: 117–123
  - 87 Boudy V. A., Lu L. and Molinoff P. B. (1996) Differential coupling of rat D2 dopamine receptor isoforms expressed in *Spodoptera frugiperda* insect cells. *J. Pharmacol. Exp. Ther.* **276**: 784–794
  - 88 Gimpl G. and Fahrenholz F. (2000) Human oxytocin receptors in cholesterol-rich vs. cholesterol-poor microdomains of the plasma membrane. *Eur. J. Biochem.* **267**: 2483–2497
  - 89 Mouillac B., Caron M., Bonin H., Dennis M. and Bouvier M. (1992) Agonist-modulated palmitoylation of beta 2-adrenergic receptor in Sf9 cells. *J. Biol. Chem.* **267**: 21733–21737
  - 90 Nebigil C. G., Garnovskaya M. N., Casanas S. J., Mulheron J. G., Parker E. M., Gettys T. W. et al. (1995) Agonist-induced desensitization and phosphorylation of human 5-HT1A receptor expressed in Sf9 insect cells. *Biochemistry* **34**: 11954–11962
  - 91 Ng G. Y., O'Dowd B. F., Caron M., Dennis M., Brann M. R. and George S. R. (1994) Phosphorylation and palmitoylation of the human D2L dopamine receptor in Sf9 cells. *J. Neurochem.* **63**: 1589–1595
  - 92 Ausubel F. (1994). *Current Protocols in Molecular Biology*. Greene Publishing Associates and Wiley-Interscience, New York
  - 93 Moss B. (1991) Vaccinia virus: a tool for research and vaccine development. *Science* **252**: 1662–1667
  - 94 Mackett M. (1994) Expression of membrane proteins using vaccinia recombinants. In: *Membrane Protein Expression Systems: A User's Guide*, pp. 125-175, Gould G. W. (ed.), Portland Press, London
  - 95 Liljestrom P. and Garoff H. (1991) A new generation of animal cell expression vectors based on the Semliki Forest virus replicon. *Biotechnology* **9**: 1356–1361
  - 96 Schwartz T. W. (1994) Locating ligand-binding sites in 7TM receptors by protein engineering. *Curr. Opin. Biotechnol.* **5**: 434–444
  - 97 Strader C. D., Fong T. M., Tota M. R., Underwood D. and Dixon R. A. (1994) Structure and function of G protein-coupled receptors. *Annu. Rev. Biochem.* **63**: 101–132
  - 98 Lohse M. J. (1992) Stable overexpression of human beta 2-adrenergic receptors in mammalian cells. *Naunyn Schmiedeberg Arch. Pharmacol.* **345**: 444–451
  - 99 Reeves P. J., Kim J. M. and Khorana H. G. (2002) Structure and function in rhodopsin: a tetracycline-inducible system in stable mammalian cell lines for high-level expression of opsin mutants. *Proc. Natl. Acad. Sci. USA* **99**: 13413–13418
  - 100 Turner G. J., Reusch R., Winter-Vann A. M., Martinez L. and Betlach M. C. (1999) Heterologous gene expression in a membrane-protein-specific system. *Protein Expr. Purif.* **17**: 312–323
  - 101 Sohlmann P., Soppa J., Oesterheld D. and Lohse M. J. (1997) Expression of beta 2-adrenoceptors in halobacteria. *Naunyn Schmiedeberg Arch. Pharmacol.* **355**: 150–160
  - 102 Pajot-Augy E., Couture L., Bozon V., Remy J. J., Biache G., Severini M. et al. (1995) High-level expression of recombinant porcine LH receptor in baculovirus-infected insect cells or caterpillars. *J. Mol. Endocrinol.* **14**: 51–66
  - 103 Eidne K. A. (1994) Expression of receptors in *Xenopus* oocytes. In: *Membrane Protein Expression Systems: A User's Guide*, pp. 275–300, Gould G.W. (ed.), Portland Press, London
  - 104 Eroglu C., Cronet P., Panneels V., Beaufils P. and Sinning I. (2002) Functional reconstitution of purified metabotropic glutamate receptor expressed in the fly eye. *EMBO Rep.* **3**: 491–496
  - 105 Alimov A. P., Khmel'nitsky A., Simonenko P. N., Spirin A. S. and Chetverin A. B. (2000) Cell-free synthesis and affinity isolation of proteins on a nanomole scale. *Biotechniques* **28**: 338–344
  - 106 Baranov V. I. and Spirin A. S. (1993) Gene expression in cell-free system on preparative scale. *Methods Enzymol.* **217**: 123–142
  - 107 Kolb V. A., Makeyev E. V. and Spirin A. S. (2000) Co-translational folding of an eukaryotic multidomain protein in a prokaryotic translation system. *J. Biol. Chem.* **275**: 16597–16601

- 108 Martemyanov K. A., Shirokov V. A., Kurnasov O. V., Gudkov A. T. and Spirin A. S. (2001) Cell-free production of biologically active polypeptides: application to the synthesis of antibacterial peptide cecropin. *Protein Expr. Purif.* **21**: 456–461
- 109 Ryabova L. A., Morozov I. and Spirin A. S. (1998) Continuous-flow cell-free translation, transcription-translation and replication-translation systems. *Methods Mol. Biol.* **77**: 179–193
- 110 Yokoyama S., Hirota H., Kigawa T., Yabuki T., Shirouzu M., Terada T. et al. (2000) Structural genomics projects in Japan. *Nat. Struct. Biol.* **7 Suppl**: 943–945
- 111 Yokoyama S., Matsuo Y., Hirota H., Kigawa T., Shirouzu M., Kuroda Y. et al. (2000) Structural genomics projects in Japan. *Prog. Biophys. Mol. Biol.* **73**: 363–376
- 112 Inooka H., Ohtaki T., Kitahara O., Ikegami T., Endo S., Kitada C. et al. (2001) Conformation of a peptide ligand bound to its G-protein coupled receptor. *Nat. Struct. Biol.* **8**: 161–165
- 113 Marullo S., Delavier-Klutcho C., Guillet J. G., Charbit A., Strosberg A. D. and Emorine L. J. (1989) Expression of human beta1 and beta2 adrenergic receptors in *E. coli* as a new tool for ligand screening. *Bio/Technology* **7**: 923–927
- 114 Hill R. A. and Silence M. N. (1997) Improved membrane isolation in the purification of beta 2-adrenoceptors from transgenic *Escherichia coli*. *Protein Expr. Purif.* **10**: 162–167
- 115 Lacatena R. M., Cellini A., Scavizzi F. and Tocchini-Valentini G. P. (1994) Topological analysis of the human beta 2-adrenergic receptor expressed in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **91**: 10521–10525
- 116 Freissmuth M., Selzer E., Marullo S., Schutz W. and Strosberg A. D. (1991) Expression of two human beta-adrenergic receptors in *Escherichia coli*: functional interaction with two forms of the stimulatory G protein. *Proc. Natl. Acad. Sci. USA* **88**: 8548–8552
- 117 Haendler B., Hechler U., Becker A. and Schleuning W. D. (1993) Expression of human endothelin receptor ETB by *Escherichia coli* transformants. *Biochem. Biophys. Res. Commun.* **191**: 633–638
- 118 Hanninen A. L., Bamford D. H. and Grisshammer R. (1994) Expression in *Escherichia coli* of rat neurotensin receptor fused to membrane proteins from the membrane-containing bacteriophage PRD1. *Biol. Chem. Hoppe Seyler* **375**: 833–836
- 119 Jockers R., Linder M. E., Hohenegger M., Nanoff C., Bertin B., Strosberg A. D. et al. (1994) Species difference in the G protein selectivity of the human and bovine A1-adenosine receptor. *J. Biol. Chem.* **269**: 32077–32084
- 120 Munch G., Walker P., Shine J. and Herzog H. (1995) Ligand binding analysis of human neuropeptide Y1 receptor mutants expressed in *E. coli*. *Receptors Channels* **3**: 291–297
- 121 Herzog H., Munch G. and Shine J. (1994) Human neuropeptide Y1 receptor expressed in *Escherichia coli* retains its pharmacological properties. *DNA Cell Biol.* **13**: 1221–1225
- 122 Weiss H. M. and Grisshammer R. (2002) Purification and characterization of the human adenosine A2a receptor functionally expressed in *Escherichia coli*. *Eur. J. Biochem.* **269**: 82–92
- 123 Schulein R., Rutz C. and Rosenthal W. (1996) Membrane targeting and determination of transmembrane topology of the human vasopressin V2 receptor. *J. Biol. Chem.* **271**: 28844–28852
- 124 Stanasila L., Massotte D., Kieffer B. L. and Pattus F. (1999) Expression of delta, kappa and mu human opioid receptors in *Escherichia coli* and reconstitution of the high-affinity state for agonist with heterotrimeric G proteins. *Eur. J. Biochem.* **260**: 430–438
- 125 Furukawa H. and Haga T. (2000) Expression of functional M2 muscarinic acetylcholine receptor in *Escherichia coli*. *J. Biochem. (Tokyo)* **127**: 151–161
- 126 Busuttill B. E., Turney K. L. and Frauman A. G. (2001) The expression of soluble, full-length, recombinant human TSH receptor in a prokaryotic system. *Protein Expr. Purif.* **23**: 369–373
- 127 Schiller H., Haase W., Molsberger E., Janssen P., Michel H. and Reilander H. (2000) The human ET(B) endothelin receptor heterologously produced in the methylotrophic yeast *Pichia pastoris* shows high-affinity binding and induction of stacked membranes. *Receptors Channels* **7**: 93–107
- 128 Huang H. J., Liao C. F., Yang B. C. and Kuo T. T. (1992) Functional expression of rat M5 muscarinic acetylcholine receptor in yeast. *Biochem. Biophys. Res. Commun.* **182**: 1180–1186
- 129 Andersen B. and Stevens R. C. (1998) The human D1A dopamine receptor: heterologous expression in *Saccharomyces cerevisiae* and purification of the functional receptor. *Protein Expr. Purif.* **13**: 111–119
- 130 Kajkowski E. M., Price L. A., Pausch M. H., Young K. H. and Ozenberger B. A. (1997) Investigation of growth hormone releasing hormone receptor structure and activity using yeast expression technologies. *J. Recept. Signal Transduct. Res.* **17**: 293–303
- 131 Erickson J. R., Wu J. J., Goddard J. G., Tigyi G., Kawanishi K., Tomei L. D. et al. (1998) Edg-2/Vzg-1 couples to the yeast pheromone response pathway selectively in response to lysophosphatidic acid. *J. Biol. Chem.* **273**: 1506–1510
- 132 Cronan J. E. Jr (1990) Biotinylation of proteins in vivo. A post-translational modification to label, purify and study proteins. *J. Biol. Chem.* **265**: 10327–10333
- 133 George S. T., Arbabian M. A., Ruoho A. E., Kiely J. and Malbon C. C. (1989) High-efficiency expression of mammalian beta-adrenergic receptors in baculovirus-infected insect cells. *Biochem. Biophys. Res. Commun.* **163**: 1265–1269
- 134 Parker E. M., Kameyama K., Higashijima T. and Ross E. M. (1991) Reconstitutively active G protein-coupled receptors purified from baculovirus-infected insect cells. *J. Biol. Chem.* **266**: 519–527
- 135 Chidiac P., Hebert T. E., Valiquette M., Dennis M. and Bouvier M. (1994) Inverse agonist activity of beta-adrenergic antagonists. *Mol. Pharmacol.* **45**: 490–499
- 136 Kwatra M. M., Schwinn D. A., Schreurs J., Blank J. L., Kim C. M., Benovic J. L. et al. (1993) The substance P receptor, which couples to Gq/11, is a substrate of beta-adrenergic receptor kinase 1 and 2. *J. Biol. Chem.* **268**: 9161–9164
- 137 Loisel T. P., Ansanay H., St-Onge S., Gay B., Boulanger P., Strosberg A. D., et al. (1997) Recovery of homogeneous and functional beta 2-adrenergic receptors from extracellular baculovirus particles. *Nat. Biotechnol.* **15**: 1300–1304
- 138 Kobilka B. K. (1995) Amino and carboxyl terminal modifications to facilitate the production and purification of a G protein-coupled receptor. *Anal. Biochem.* **231**: 269–271
- 139 Parker E. M. and Ross E. M. (1991) Truncation of the extended carboxyl-terminal domain increases the expression and regulatory activity of the avian beta-adrenergic receptor. *J. Biol. Chem.* **266**: 9987–9996
- 140 Oker-Blom C., Jansson C., Karp M., Lindqvist C., Savola J. M., Vlask J., et al. (1993) Functional analysis of the human alpha 2C-C4 adrenergic receptor in insect cells expressed by a luciferase-based baculovirus vector. *Biochim. Biophys. Acta* **1176**: 269–275
- 141 Ng G. Y., George S. R., Zastawny R. L., Caron M., Bouvier M., Dennis M. et al. (1993) Human serotonin1B receptor expression in Sf9 cells: phosphorylation, palmitoylation, and adenylyl cyclase inhibition. *Biochemistry* **32**: 11727–11733
- 142 Parker E. M., Grisel D. A., Iben L. G., Nowak H. P., Mahle C. D., Yocca F. D. et al. (1994) Characterization of human 5-HT1 receptors expressed in Sf9 insect cells. *Eur. J. Pharmacol.* **268**: 43–53
- 143 Butkerait P., Zheng Y., Hallak H., Graham T. E., Miller H. A., Burris K. D. et al. (1995) Expression of the human 5-hydroxy-



- tryptamine1A receptor in Sf9 cells. Reconstitution of a coupled phenotype by co-expression of mammalian G protein subunits. *J. Biol. Chem.* **270**: 18691–18699
- 144 Ponimaskin E. G., Schmidt M. F., Heine M., Bickmeyer U. and Richter D. W. (2001) 5-Hydroxytryptamine 4(a) receptor expressed in Sf9 cells is palmitoylated in an agonist-dependent manner. *Biochem. J.* **353**: 627–634
- 145 Labrecque J., Fargin A., Bouvier M., Chidiac P. and Dennis M. (1995) Serotonergic antagonists differentially inhibit spontaneous activity and decrease ligand binding capacity of the rat 5-hydroxytryptamine type 2C receptor in Sf9 cells. *Mol. Pharmacol.* **48**: 150–159
- 146 Aharony D., Little J. et al. (1994) "Isolation and characterization of neurokinin A receptor cDNAs from guinea-pig lung and rabbit pulmonary artery." *J. Recept. Res.* **14**(6–8): 399–421
- 147 Mazina K. E., Strader C. D., Tota M. R., Daniel S. and Fong T. M. (1996) Purification and reconstitution of a recombinant human neurokinin-1 receptor. *J. Recept. Signal Transduct. Res.* **16**: 191–207
- 148 Wehmeyer A. and Schulz R. (1997) Overexpression of delta-opioid receptors in recombinant baculovirus-infected *Trichoplusia* in 'High 5' insect cells. *J. Neurochem.* **68**: 1361–1371
- 149 Obermeier H., Wehmeyer A. and Schulz R. (1996) Expression of mu-, delta- and kappa-opioid receptors in baculovirus-infected insect cells. *Eur. J. Pharmacol.* **318**: 161–166
- 150 Massotte D., Baroche L., Simonin F., Yu L., Kieffer B. and Pattus F. (1997) Characterization of delta, kappa, and mu human opioid receptors overexpressed in baculovirus-infected insect cells. *J. Biol. Chem.* **272**: 19987–19992
- 151 Massotte D., Pereira C. A., Pouliquen Y. and Pattus F. (1999) Parameters influencing human mu opioid receptor overexpression in baculovirus-infected insect cells. *J. Biotechnol.* **69**: 39–45
- 152 Wei Q., Zhou D. H., Shen Q. X., Chen J., Chen L. W., Wang T. L. et al. (2000) Human mu-opioid receptor overexpressed in Sf9 insect cells functionally coupled to endogenous Gi/o proteins. *Cell Res.* **10**: 93–102
- 153 Vasudevan S., Reilander H., Maul G. and Michel H. (1991) Expression and cell membrane localization of rat M3 muscarinic acetylcholine receptor produced in Sf9 insect cells using the baculovirus system. *FEBS Lett.* **283**: 52–56
- 154 Hu Y., Rajan L. and Schilling W. P. (1994) Ca<sup>2+</sup> signaling in Sf9 insect cells and the functional expression of a rat brain M5 muscarinic receptor. *Am. J. Physiol.* **266**: C1736–1743
- 155 Hayashi M. K. and Haga T. (1996) Purification and functional reconstitution with GTP-binding regulatory proteins of hexahistidine-tagged muscarinic acetylcholine receptors (m2 subtype). *J. Biochem. (Tokyo)* **120**: 1232–1238
- 156 Mills A., Allet B., Bernard A., Chabert C., Brandt E., Cavegn C. et al. (1993) Expression and characterization of human D4 dopamine receptors in baculovirus-infected insect cells. *FEBS Lett.* **320**: 130–134
- 157 Javitch J. A., Kaback J., Li X. and Karlin A. (1994) Expression and characterization of human dopamine D2 receptor in baculovirus-infected insect cells. *J. Recept. Res.* **14**: 99–117
- 158 Grunewald S., Haase W., Reilander H. and Michel H. (1996) Glycosylation, palmitoylation, and localization of the human D2S receptor in baculovirus-infected insect cells. *Biochemistry* **35**: 15149–15161
- 159 Narayan P., Gray J. and Puett D. (1996) Expression of functional lutropin/choriogonadotropin receptor in the baculovirus system. *Mol. Cell. Endocrinol.* **117**: 95–100
- 160 Christophe S., Robert P., Maugain S., Bellet D., Koman A. and Bidart J. M. (1993) Expression of the human follicle-stimulating hormone receptor in the baculovirus system. *Biochem. Biophys. Res. Commun.* **196**: 402–408
- 161 Pettit D. A., Showalter V. M., Abood M. E. and Cabral G. A. (1994) Expression of a cannabinoid receptor in baculovirus-infected insect cells. *Biochem. Pharmacol.* **48**: 1231–1243
- 162 Quehenberger O., Prossnitz E. R., Cochran C. G. and Ye R. D. (1992) Absence of G(i) proteins in the Sf9 insect cell. Characterization of the uncoupled recombinant N-formyl peptide receptor. *J. Biol. Chem.* **267**: 19757–19760
- 163 Kawamoto S., Onishi H., Hattori S., Miyagi Y., Amaya Y., Mishina M., et al. (1991) Functional expression of the alpha 1 subunit of the AMPA-selective glutamate receptor channel, using a baculovirus system. *Biochem. Biophys. Res. Commun.* **181**: 756–763
- 164 Keinänen K., Kohr G., Seeburg P. H., Laukkanen M. L. and Oker-Blom C. (1994) High-level expression of functional glutamate receptor channels in insect cells. *Biotechnology* **12**: 802–806
- 165 Kawamoto S., Hattori S., Oiji I., Hamajima K., Mishina M. and Okuda K. (1994) Ligand-binding properties and N-glycosylation of alpha 1 subunit of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA)-selective glutamate receptor channel expressed in a baculovirus system. *Eur. J. Biochem.* **223**: 665–673
- 166 Nekrasova E., Sosinskaya A., Natochin M., Lancet D. and Gat U. (1996) Overexpression, solubilization and purification of rat and human olfactory receptors. *Eur. J. Biochem.* **238**: 28–37
- 167 Klaassen C. H., Bovee-Geurts P. H., Decaluwe G. L. and De-Grip W. J. (1999) Large-scale production and purification of functional recombinant bovine rhodopsin with the use of the baculovirus expression system. *Biochem. J.* **342**: 293–300
- 168 Moepps B., Frodl R., Rodewald H. R., Baggolini M. and Gierschik P. (1997) Two murine homologues of the human chemokine receptor CXCR4 mediating stromal cell-derived factor 1alpha activation of Gi2 are differentially expressed in vivo. *Eur. J. Immunol.* **27**: 2102–2112
- 169 Schreurs J., Yamamoto R., Lyons J., Munemitsu S., Conroy L., Clark R., et al. (1995) Functional wild-type and carboxyl-terminal-tagged rat substance P receptors expressed in baculovirus-infected insect Sf9 cells. *J. Neurochem.* **64**: 1622–1631
- 170 Nishimura K., Frederick J. and Kwatra M. M. (1998) Human substance P receptor expressed in Sf9 cells couples with multiple endogenous G proteins. *J. Recept. Signal Transduct. Res.* **18**: 51–65
- 171 Beukers M. W., Klaassen C. H., De Grip W. J., Verzijl D., Timmerman H. and Leurs R. (1997) Heterologous expression of rat epitope-tagged histamine H2 receptors in insect Sf9 cells. *Br. J. Pharmacol.* **122**: 867–874
- 172 Kusui T., Hellmich M. R., Wang L. H., Evans R. L., Benya R. V., Battey J. F. et al. (1995) Characterization of gastrin-releasing peptide receptor expressed in Sf9 insect cells by baculovirus. *Biochemistry* **34**: 8061–8075
- 173 Ohtaki T., Ogi K., Masuda Y., Mitsuoka K., Fujiyoshi Y., Kitada C. et al. (1998) Expression, purification and reconstitution of receptor for pituitary adenylate cyclase-activating polypeptide. large-scale purification of a functionally active G protein-coupled receptor produced in Sf9 insect cells. *J. Biol. Chem.* **273**: 15464–15473
- 174 Scheer A., Bjorklof K., Cotecchia S. and Lundstrom K. (1999) Expression of the alpha 1b-adrenergic receptor and G protein subunits in mammalian cell lines using the Semliki Forest virus expression system. *J. Recept. Signal Transduct. Res.* **19**: 369–378
- 175 Fraser C. M., Arakawa S., McCombie W. R. and Venter J. C. (1989) Cloning, sequence analysis and permanent expression of a human alpha 2-adrenergic receptor in Chinese hamster ovary cells. Evidence for independent pathways of receptor coupling to adenylate cyclase attenuation and activation. *J. Biol. Chem.* **264**: 11754–11761

- 176 Eason M. G., Kurose H., Holt B. D., Raymond J. R. and Liggett S. B. (1992) Simultaneous coupling of alpha 2-adrenergic receptors to two G-proteins with opposing effects. Subtype-selective coupling of alpha 2C10, alpha 2C4 and alpha 2C2 adrenergic receptors to Gi and Gs. *J. Biol. Chem.* **267**: 15795–15801
- 177 Bresnahan M. R., Flordellis C. S., Vassilatis D. K., Makrides S. C., Zannis V. I. and Gavras H. (1990) High level of expression of functional human platelet alpha 2- adrenergic receptors in a stable mouse C127 cell line. *Biochim. Biophys. Acta* **1052**: 439–445
- 178 George S. T., Berrios M., Hadcock J. R., Wang H. Y. and Malbon C. C. (1988) Receptor density and cAMP accumulation: analysis in CHO cells exhibiting stable expression of a cDNA that encodes the beta 2-adrenergic receptor. *Biochem. Biophys. Res. Commun.* **150**: 665–672
- 179 Maroteaux L., Saudou F., Amlaiky N., Boschert U., Plassat J. L. and Hen R. (1992) Mouse 5HT1B serotonin receptor: cloning, functional expression and localization in motor control centers. *Proc. Natl. Acad. Sci. USA* **89**: 3020–3024
- 180 McAllister G., Charlesworth A., Snodin C., Beer M. S., Noble A. J., Middlemiss D. N. et al. (1992) Molecular cloning of a serotonin receptor from human brain (5HT1E): a fifth 5HT1-like subtype. *Proc. Natl. Acad. Sci. USA* **89**: 5517–5521
- 181 Saudou F., Boschert U., Amlaiky N., Plassat J. L. and Hen R. (1992) A family of *Drosophila* serotonin receptors with distinct intracellular signalling properties and expression patterns. *EMBO J.* **11**: 7–17
- 182 Van Craenenbroeck K., Vanhoenacker P., Leysen J. E. and Haegeman G. (2001) Evaluation of the tetracycline- and ecdysone-inducible systems for expression of neurotransmitter receptors in mammalian cells. *Eur. J. Neurosci.* **14**: 968–976
- 183 Lundstrom K., Mills A., Buell G., Allet E., Adami N. and Liljestrom P. (1994) High-level expression of the human neurokinin-1 receptor in mammalian cell lines using the Semliki Forest virus expression system. *Eur. J. Biochem.* **224**: 917–921
- 184 Turcatti G., Ceszowski K. and Chollet A. (1993) Biochemical characterization and solubilization of human NK2 receptor expressed in Chinese hamster ovary cells. *J. Recept. Res.* **13**: 639–652
- 185 Gao Z., Ni Y., Szabo G. and Linden J. (1999) Palmitoylation of the recombinant human A1 adenosine receptor: enhanced proteolysis of palmitoylation-deficient mutant receptors. *Biochem. J.* **342**: 387–395
- 186 Townsend-Nicholson A. and Shine J. (1992) Molecular cloning and characterisation of a human brain A1 adenosine receptor cDNA. *Brain Res. Mol. Brain Res.* **16**: 365–370
- 187 Ingi T., Kitajima Y., Minamitake Y. and Nakanishi S. (1991) Characterization of ligand-binding properties and selectivities of three rat tachykinin receptors by transfection and functional expression of their cloned cDNAs in mammalian cells. *J. Pharmacol. Exp. Ther.* **259**: 968–975
- 188 Kris R. M., South V., Saltzman A., Felder S., Ricca G. A., Jaye M. et al. (1991) Cloning and expression of the human substance K receptor and analysis of its role in mitogenesis. *Cell Growth Differ.* **2**: 15–22
- 189 Nakajima Y., Tsuchida K., Negishi M., Ito S. and Nakanishi S. (1992) Direct linkage of three tachykinin receptors to stimulation of both phosphatidylinositol hydrolysis and cyclic AMP cascades in transfected Chinese hamster ovary cells. *J. Biol. Chem.* **267**: 2437–2442
- 190 Wang J. B., Imai Y., Eppler C. M., Gregor P., Spivak C. E. and Uhl G. R. (1993) mu opiate receptor: cDNA cloning and expression. *Proc. Natl. Acad. Sci. USA* **90**: 10230–10234
- 191 Raynor K., Kong H., Mestek A., Bye L. S., Tian M., Liu J. et al. (1995) Characterization of the cloned human mu opioid receptor. *J. Pharmacol. Exp. Ther.* **272**: 423–428
- 192 Peralta E. G., Ashkenazi A., Winslow J. W., Ramachandran J. and Capon D. J. (1988) Differential regulation of PI hydrolysis and adenylyl cyclase by muscarinic receptor subtypes. *Nature* **334**: 434–437
- 193 Migeon J. C. and Nathanson N. M. (1994) Differential regulation of cAMP-mediated gene transcription by m1 and m4 muscarinic acetylcholine receptors. Preferential coupling of m4 receptors to Gi alpha-2. *J. Biol. Chem.* **269**: 9767–9773
- 194 Schweitzer C., Kratzeisen C., Adam G., Lundstrom K., Malherbe P., Ohresser S. et al. (2000) Characterization of [(3)H]-LY354740 binding to rat mGlu2 and mGlu3 receptors expressed in CHO cells using Semliki Forest virus vectors. *Neuropharmacology* **39**: 1700–1706
- 195 Malherbe P., Kratzeisen C., Lundstrom K., Richards J. G., Faull R. L. and Mutel V. (1999) Cloning and functional expression of alternative spliced variants of the human metabotropic glutamate receptor  $\alpha$ . *Brain Res. Mol. Brain Res.* **67**: 201–210
- 196 Walker P., Munoz M., Combe M. C., Grouzmann E., Herzog H., Selbie L. et al. (1993) High level expression of human neuropeptide Y receptors in mammalian cells infected with a recombinant vaccinia virus. *Mol. Cell. Endocrinol.* **91**: 107–112
- 197 Falck-Pedersen E., Heinflink M., Alvira M., Nussenzweig D. R. and Gershengorn M. C. (1994) Expression of thyrotropin-releasing hormone receptors by adenovirus-mediated gene transfer reveals that thyrotropin-releasing hormone desensitization is cell specific. *Mol. Pharmacol.* **45**: 684–689
- 198 Jelinek L. J., Lok S., Rosenberg G. B., Smith R. A., Grant F. J., Biggs S. et al. (1993) Expression cloning and signaling properties of the rat glucagon receptor. *Science* **259**: 1614–1616
- 199 Needham M., Egerton M., Millest A., Evans S., Popplewell M., Cerillo G. et al. (1995) Further development of the locus control region/murine erythroleukemia expression system: high level expression and characterization of recombinant human calcitonin receptor. *Protein Expr. Purif.* **6**: 124–131
- 200 Pawate S., Schey K. L., Meier G. P., Ullian M. E., Mais D. E. and Halushka P. V. (1998) Expression, characterization and purification of C-terminally hexahistidine-tagged thromboxane A2 receptors. *J. Biol. Chem.* **273**: 22753–22760
- 201 Elshourbagy N. A., Lee J. A., Korman D. R., Nuthalaganti P., Sylvester D. R., Dilella A. G. et al. (1992) Molecular cloning and characterization of the major endothelin receptor subtype in porcine cerebellum. *Mol. Pharmacol.* **41**: 465–473
- 202 Hoffmann M., Verzijl D., Lundstrom K., Simmen U., Alewijnse A. E., Timmerman H. et al. (2001) Recombinant Semliki Forest virus for over-expression and pharmacological characterisation of the histamine H(2) receptor in mammalian cells. *Eur. J. Pharmacol.* **427**: 105–114
- 203 Hatt H., Lang K. and Gisselmann G. (2001) Functional expression and characterization of odorant receptors using the Semliki Forest virus system. *Biol. Chem.* **382**: 1207–1214
- 204 Oprian D. D., Molday R. S., Kaufman R. J. and Khorana H. G. (1987) Expression of a synthetic bovine rhodopsin gene in monkey kidney cells. *Proc. Natl. Acad. Sci. USA* **84**: 8874–8878
- 205 Baneres J. L., Martin A. et al. (2003) “Structure-based analysis of GPCR function: conformational adaptation of both agonist and receptor upon leukotriene B4 binding to recombinant BLT1.” *J Mol Biol* **329**(4): 801–814
- 206 Baneres J. L. and Parelo J. (2003) “Structure-based analysis of GPCR function: evidence for a novel pentameric assembly between the dimeric leukotriene B4 receptor BLT1 and the G-protein.” *J Mol Biol* **329**(4): 815–829.
- 207 Kwatra M. M., Schreurs J. et al. (1995) “Immunoaffinity purification of epitope-tagged human beta 2-adrenergic receptor to homogeneity.” *Protein Expr Purif* **6**(6): 717-721
- 208 Price L. A., Strnad J. et al. (1996). “Pharmacological characterization of the rat A2a adenosine receptor functionally coupled to the yeast pheromone response pathway.” *Mol Pharmacol* **50**(4): 829–837