Co-expression of the neurokinin NK2 receptor and G-protein components in the fission yeast *Schizosaccharomyces pombe*

Steve Arkinstall*, Mike Edgerton, Mark Payton, Kinsey Maundrell

Glaxo Institute for Molecular Biology, 14 Chemin des Aulx, 1228 Plan-les-Ouates, Geneva, Switzerland

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Abstract The fission yeast *Schizosaccharomyces pombe* has proven useful for studying molecular interactions between a range of signal transduction components. We now report the first co-expression of a mammalian seven-transmembrane receptor and G-protein components in *S. pombe*. We selected the human neurokinin NK2 receptor together with its G-protein-signalling partner G_q for this study. Yeast membrane fractions showed high levels of NK2 receptor-binding activity (1159 ± 534 (n = 3) fmol/mg protein) although initial experiments with intact cells revealed an absence of receptors at the cell surface. Using a construct comprising the NK2 coding sequence fused with the signal sequence from an endogenous phosphatase (*pho1*), we detected ~400 NK2 receptors/cell in unbroken yeast. Successful co-expression of the NK2 receptor with the G-protein subunits $G_{\alpha q}$, $\beta 1$ or $\beta 2$ and $\gamma 3$ failed to modulate agonist binding, suggesting the absence of functional interaction between these components. As an alternative test of $G_{\alpha q}$ function, we next expressed its downstream effector target phospholipase C-$\beta 1$ (PLC$\beta 1$) in *S. pombe*. Although PLC$\beta 1$ undergoes powerful in vitro activation by $G_{\alpha q}$ derived from baculovirus-infected Sf9 cells and mammalian cells, $G_{\alpha q}$ expressed in *S. pombe* is totally ineffective. Similar results were also achieved with the G-protein subunit $G_{\alpha 16}$. Together, these data suggest that seven-transmembrane receptors can be expressed in *S. pombe* at high levels and directed to the cell surface although their interaction with co-expressed G-proteins is undetectable. Production of inactive G_α-chains in *S. pombe* may account for these observations.

Key words: Schizosaccharomyces pombe; NK2 neurokinin receptor; G-protein; G_q; PLC$\beta 1$; Heterologous expression

1. Introduction

The seven-transmembrane superfamily of receptors mediate diverse cellular actions through interaction with heterotrimeric G-proteins and subsequent regulation of a range of downstream effector and second messenger systems [1,2]. One important second messenger response triggered by many G-protein-linked receptors is phospholipase C (PLC)-dependent hydrolysis of membrane phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate the second messengers inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) [3,4]. More than 20 distinct G-protein α-chains are currently identified and members of the G_q class play an critical role mediating increased PIP_2 hydrolysis following activation of PLCβ isoforms [2,5–7].

One important approach for demonstrating basic mechanisms for G-protein-dependent PIP_2 breakdown has involved heterologous expression of selected components in tranformed cell lines [8–11]. Unfortunately, a high background of endogenous regulatory elements in mammalian cells complicates interpretation of the precise and specific molecular interactions under study. For instance, although a single G-protein-linked receptor can couple to multiple effector and second messenger systems [12–17], it is often unclear whether these responses are direct or secondary to second messenger generation or kinase activation. An alternative approach is to express selected signal transduction components in a cellular enviroment with a limited complement of endogenous G-protein-linked receptors or associated signalling elements. One such system is the fission yeast *Schizosaccharomyces pombe* in which only two seven-transmembrane mating factor receptors and two endogenous G-protein α-chains have been identified [18–21]. The budding yeast *Saccharomyces cerevisiae* also expresses only a limited complement of G-protein components [1,22] and has been used as a host to express the human $\beta 2$-adrenergic receptor together with the G-protein α-chain $G_{\alpha s}$ [23]. Hitherto, no such studies have been reported using yeast for G-protein-linked receptors mediating PIP_2 hydrolysis.

The fission yeast *S. pombe* has emerged as a new and adaptable host cell system in which to study the function and molecular interactions of mammalian signal transduction components. So far, these have included PKC isoenzymes [24], Src and its negative regulator Csk [25,26], the PDGFb receptor and PLCγ [26], Raf-1 and MAP kinase kinase [27] as well as Ras and the phosphatidylinositol 3-kinase subunits p85α and p110α [28]. The advantages of this yeast system are due in part to the availability of range of expression vectors based on the tightly regulatable and powerful promoter *nmt* [29]. In this paper, we describe the first report of a mammalian seven-transmembrane receptor co-expressed with G-protein components in *S. pombe*. As a reflection of our interest in G-protein-dependent PIP_2 hydrolysis, we selected the neurokinin NK2 receptor together with G_q for these experiments.

2. Experimental

2.1. Strains, media and plasmid constructs

The host strain for *S. pombe* transformation, growth media and the procedure for protoplast transformation were as described [26]. The human NK2 receptor cDNA was obtained from Dr. E. Kawashima (Glaxo Institute for Molecular Biology, Geneva, Switzerland) while cDNAs encoding mouse $G_{\alpha q}$, bovine $G_{\beta 1}$ and human $G_{\beta 2}$ were provided by Professor M.I. Simon (California Institute of Technology, Pasadena, CA) and have been described previously [30–32]. Bovine $G_{\gamma 3}$ and PLC$\beta 1$ cDNAs were generous gifts from Dr. N. Gautam (Washington University, St Louis, MO) and Dr. P. Parker (Imperial Cancer Research Fund, London, UK), respectively, and were as reported [33,34]. Human $G_{\alpha 16}$ was provided by Dr. C. Power (Glaxo Institute for Molecular Biology). For expression in fission yeast, coding sequences from the above cDNAs were excised using appropriate restriction enzymes,

*Corresponding author. Fax: (41) (22) 794 69 65.
E-mail: sa7182@ggr.co.uk

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or in some cases, amplified by PCR, and subcloned into the *nmt1*-based expression vectors [29] as follows: The NK2 receptor was subcloned into the pREP1 (Leu^+) vector; G_α subunits were subcloned into the pREP4 (Ura^+) vector; $G_{\beta 1}$ and $G_{\beta 2}$ subunits were expressed from the pREP7 vector which contains the *S. pombe his5*$^+$ selectable marker (E. Leberer and K. Maundrell, unpubl. data); $G\gamma 3$ was expressed from pREP5 which contains the *ade6*$^+$ selectable marker; PLCβ1 was expressed from pREP3. With this series of constructs all three subunits of the heterotrimeric G-protein could be co-expressed with either its seven-transmembrane receptor or its downstream effector PLCβ1 with each plasmid being maintained in the cell by independent selection. For COS cell expression, the genes for $G_{\alpha q}$ and $G_{\alpha 16}$ were amplified by PCR using primers which placed a consensus eukaryotic translation initiation sequence [36] at the 5'-end of the gene and cloned into pSG5 or pBKCMV (Stratagene). pSG5/$G_{\alpha q}$, pBKCMV/$G_{\alpha 16}$ and the NK2 receptor expression vector pCDL/NK2 were transfected in COS-7 cells by electroporation [17,35].

2.2. NK2 receptor binding

For binding to intact yeast, $1–3 \times 10^9$ cells were harvested by centrifugation at $1000 \times g$ for 5 min, washed in 40 ml of 0.9% (w/v) NaCl, and resuspended in buffer N (25 mM Tris, 100 mM NaCl, 0.2% (w/v) BSA, pH 7.4) containing 3 mM $MnCl_2$. For binding to membrane fractions, yeast homogenates were prepared exactly as described [26] and diluted in buffer N. Saturation-binding experiments were performed by mixing aliquots of intact cells ($2–4 \times 10^7$ cells) or homogenates (50–100 μg protein) with the NK2 antagonist [^{3}H]SR48968 (26 Ci/mmol; Amersham, Zurich, Switzerland) at the concentrations indicated in a total volume of 1.0 ml for 120 min at room temperature. Non-specific binding was determined in parallel tubes using the NK2-selective antagonist GR94800 at 2 μM [37]. Competition binding was performed under identical conditions using 0.5–1.0 nM [^{3}H]SR48968 and antagonists or agonists at the concentrations indicated. GTPγS was included at 100 μM where indicated. Binding was terminated by rapid filtration through glass fibre filters (GF/B) presoaked with 10 mM Tris-HCl containing 0.5% (v/v) polyethylenimine (pH 7.4) followed by 3 washes of 4.0 ml with ice-cold buffer N containing 0.02% (w/v) BSA.

2.3. Measuring PLCβ1 activity with exogenous substrate

Crude homogenates of $G_{\alpha q}$-expressing baculovirus-infected Sf9 cells or transformed *S. pombe* were preactivated with GTPγS as described [38]. PLCβ1 was expressed in *S. pombe* (see above) and activation by $G_{\alpha q}$ was measured using [^{3}H]PIP_2 as substrate [38]. Where COS cell membranes were used as a source of $G_{\alpha q}$ or $G_{\alpha 16}$, membrane preparation as well as G-protein and PLCβ1 activation were performed as described previously [8]. In these experiments, *S. pombe* membranes containing $G_{\alpha q}$ or $G_{\alpha 16}$ were prepared in parallel under identical conditions.

2.4. Immunodetection of $G_{\alpha q}$, $G_{\alpha 16}$, $G_{\beta 1}$, $G_{\beta 2}$, $\gamma 3$ and PLCβ1

Preparation of membrane fractions, sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis and Western blotting were performed as described [26] using 10.0% (PLCβ1), 12% ($G_{\alpha q}$, $G_{\alpha 16}$, $G_{\beta 1}$ and $G_{\beta 2}$) or 20% gels ($G_{\gamma 3}$)). Polyclonal antibodies detecting $G_{\alpha q}$ were generated in house using keyhole limpet haemocyanin-conjugated peptides in rabbits as follows: GQ92 (EKVSAFENPYVDAIKS; $G_{\alpha q}$ 119–134) and GQ93B (CQLNLKEYNLV; $G_{\alpha q}$ 350–359). Antibody GQ93B displayed properties indistinguishable from CQ2 [39]. Other antibodies were from the following sources: $G_{\beta 1}$ and $G_{\beta 2}$ (SW/1; New England Nuclear; Regensdorf, Switzerland) and PLCβ1 (Upstate Biotechnology, Lake Placid, NY). Antibodies against $G_{\alpha 16}$ and $G_{\gamma 3}$ were kind gifts from Professor M.I. Simon (California Institute of Technology) and Dr. N. Gautam (Washington University), respectively.

3. Results and discussion

S. pombe transformed with NK2/REP1 and grown for 18 h under inducing conditions gave considerable displaceable binding of the NK2 receptor-selective antagonist radioligand [^{3}H]SR48968 in membrane fractions. No binding was detected in membranes derived from control cells (data not shown). Despite this, [^{3}H]SR48968 binding to intact cells expressing the

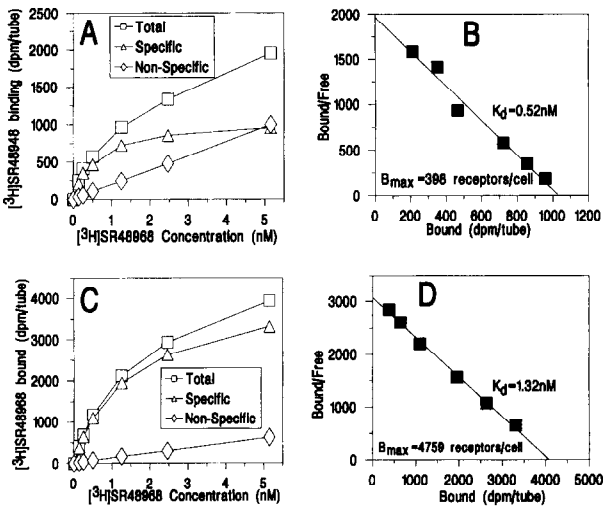

Fig. 1. NK2 receptor [^{3}H]SR48968 saturation binding and Scatchard analysis. Binding experiments were performed with *S. pombe* transformed with human NK2 receptor cDNA fused to a signal sequence derived from the yeast secreted phosphatase *pho1* and subcloned into pREP1. Fission yeast were grown for 18 h in the absence of thiamine and intact cells (A) or membrane fractions (C) incubated with increasing concentrations of [^{3}H]SR48968 in the absence (Total) or presence (Non-specific) of 2 μM GR94800 (see section 2). Non-specific binding was subtracted from total to give specific binding which was saturable and gave linear Scatchard transformation for both intact cells (B) and membrane fractions (D). Data points are the mean of triplicate determinations and are representative of six independent experiments.

NK2 receptor was indistinguishable from control cells (data not shown), suggesting inappropriate receptor trafficking and no translocation to the cell surface. To overcome this problem, the signal sequence derived from the endogenous yeast cell-surface phosphatase *pho1* [40] was fused to the 5'-end of the cDNA encoding the human NK2 receptor. Expression of this construct resulted in a clear increase in [^{3}H]SR48968 binding to intact cells which was both saturable and displaceable by the NK2 antagonist GR94800 (Fig. 1A). Scatchard transformation of this data was linear and gave a maximal binding capacity of 429 ± 315 NK2 receptors/cell ($n = 6$) and a $K_d = 0.52 \pm 0.23$ nM ($n = 6$) (Fig. 1B). To test the proportion of the total NK2 receptor population localized at the cell surface, parallel measurements were performed using crude homogenates containing total cell membranes. Binding to homogenates gave saturable binding (Fig. 1C) and linear Scatchard transformation (Fig. 1D) indicated a $K_d = 1.6 \pm 0.4$ nM ($n = 3$) and a total binding capacity of 5514 ± 1599 ($n = 3$) NK2 receptors/cell. This corresponds to 1159 ± 534 ($n = 3$) fmol NK2 receptor/mg of total cell protein. Together, these experiments indicate that $5.0 \pm 3.2\%$ ($n = 3$) of total cell NK2 receptor-binding activity is expressed at the cell surface.

To examine the pharmacological characteristics of the human NK2 receptor expressed in *S. pombe*, both intact cells and crude homogenates were incubated with the non-peptide antagonist [^{3}H]SR48968 in the presence of increasing concentrations of the natural agonist neurokinin A (NKA) or the NK2-selective antagonist GR94800 [37]. While GR94800 displayed similar high affinity in both whole cells ($IC_{50} = 5.5 \pm 1.8$ nM ($n = 6$)) and membranes (18.1 ± 3.7 nM ($n = 4$)), NKA showed a clear preference for binding to intact yeast cells where

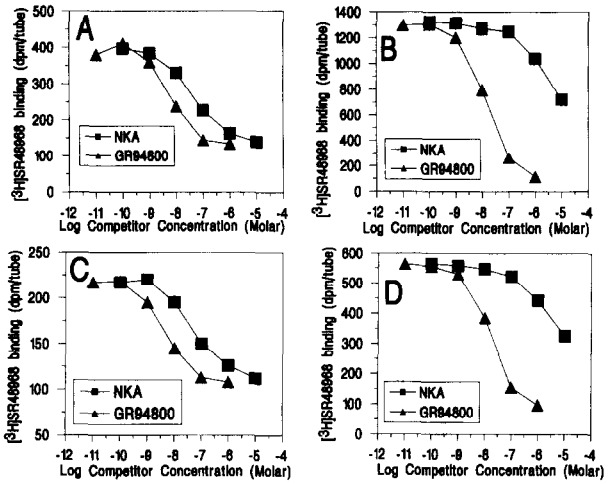

Fig. 2. Competition for NK2 receptor binding by NKA and GR94800. Competition binding was performed using *S. pombe* transformed with human NK2 cDNA fused to the signal sequence from yeast *pho1* and subcloned in pREP1. Fission yeast expressed either NK2 receptor alone (A,B) or co-transformed with $G_{\alpha q}$ subcloned in pREP4, $G_{\beta 2}$ in pREP7 and $G_{\gamma 3}$ in pREP5 (C,D). Yeast was grown for 18 h in the absence of thiamine and intact cells (A,C) or membrane fractions ((B,D) incubated with increasing concentrations of the natural agonist neurokinin A (NKA) or antagonist GR94800 in the presence of 0.75 nM [^{3}H]SR48968. Binding experiments performed as described in section 2. Data points are the mean of triplicate determinations and are representative of four to six independent experiments.

ts IC_{50} = 45.0 ± 6.0 nM (n = 4) (Fig. 2A,B). NKA concentrations at least 100-fold higher were required to inhibit [^{3}H]SR48968-binding crude cell homogenates (IC_{50} > 10 μM) Fig. 2B). This lower potency does not reflect hydrolytic breakdown of ligand as NKA preincubated as yeast homogenates nder normal assay conditions was unaltered in its ability to lisplace [^{3}H]SR48968 binding from intact cells with high affinty (not shown). Identical differential binding to intact and roken *S. pombe* cells was also observed with the NK2-selective gonist GR64349 [41] (data not shown). One potential explanaion for this observation is that the cell surface NK2 receptor s able to interact with an endogenous *S. pombe* heterotrimeric G-protein resulting in stabilization of a high-affinity agonist eceptor-binding state [42]. Currently, two endogenous G-protein α-chains, termed gpa1 and gpa2, have been identified molcularly in fission yeast [20,21] and both represent candidate omponents for such an interaction. However, while this is not xcluded, receptor theory [42] predicts that normal levels of guanine nucleotide within yeast cytosol should disrupt receptor-G-protein ternary complex and with it abolish high-affinity gonist binding in intact cells. An alternative possibility is that ompetition for [^{3}H]SR48968 binding by NKA in intact yeast ells is unrelated to a high-affinity NK2 receptor conformation s induced through G-protein interaction. Indeed, in chinese amster, ovary cells expressing the recombinant rat NK2 receptor, high-affinity agonist binding reflecting interaction with endogenous G-proteins is observed only when using radiolabeled NKA as ligand [43]. It seems that demonstration of guanine nucleotide-sensitive high-affinity displacement of [^{3}H]SR48968 binding by NKA may only be observed clearly in mammalian cells upon over expression of appropriate G-protein components, such as $G_{\alpha q}$ (see below; Fig. 4A). Rather than reflecting G-protein interaction, high-affinity NKA binding in intact yeast may, therefore, indicate that the fraction of NK2 receptors inserted within the plasma membrane undergo a posttranslational modification (e.g. glycosylation, phosphorylation or palmitoylation) distinct from that experienced by receptors localized in other membrane compartments. Selective cleavage of the *pho1* signal sequence in cell surface receptors does not account for this observation as wild-type NK2 receptor binding in membranes displays equally low-affinity NKA binding (data not shown).

To test whether the human NK2 receptor expressed in *S. pombe* was able to interact functionally with its natural G-protein G_q, mammalian $G_{\alpha q}$, $G_{\beta 1}$ or $G_{\beta 2}$ and $G_{\gamma 3}$ subunits were co-expressed following subcloning into the vectors REP4, REP7 and REP5, respectively. $G_{\gamma 3}$ has been reported previously to effectively form dimers with both $G_{\beta 1}$ and $G_{\beta 2}$ [44,45]. Following growth in the absence of thiamine, all G-protein

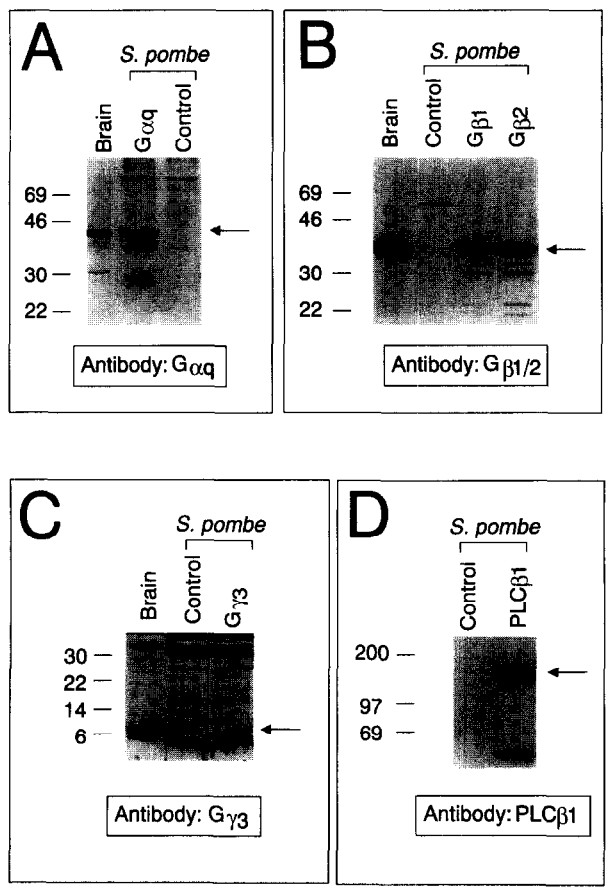

Fig. 3. Immunodetection of $G_{\alpha q}$, $G_{\beta 1}$, $G_{\beta 2}$, $G_{\gamma 3}$ and PLCβ1 expressed in *S. pombe*. Fission yeast co-transformed with NK2/*pho1* subcloned in pREP1 together with $G_{\alpha q}$ in pREP4, $G_{\gamma 3}$ in pREP5 and either $G_{\beta 1}$ or $G_{\beta 2}$ in pREP7 were used for immunodetection of G-protein components. Yeast transformed only with PLCβ1 expressed from pREP3 was also used for Western analysis. Cells were grown for 18 h in the absence of thiamine after which time homogenates were prepared by cell breakage using glass beads. Proteins were separated by SDS-PAGE, transferred to nitrocellulose paper and probed with antibodies specific for $G_{\alpha q}$ (A), $G_{\beta 1}$ or $G_{\beta 2}$ (B), $G_{\gamma 3}$ (C) and PLC$_{\beta 1}$ (D). Antibody binding was detected using HRP-conjugated second antibody or protein A/G together with ECL detection. Molecular weight markers and the position of immunodetected protein are indicated in each panel. Untransformed *S. pombe* and a rat brain crude homogenate were used as negative and positive controls, respectively.

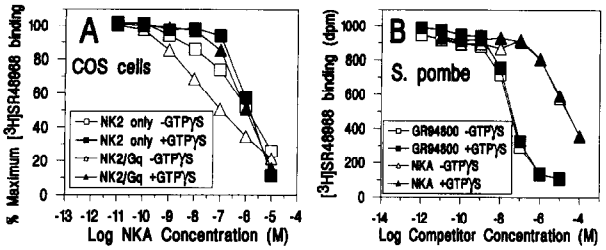

Fig. 4. Agonist binding to NK2 receptor is regulated by $G_{\alpha q}$ in COS cells but not *S. pombe*. (A) COS-7 cells expressing human NK2 receptor alone or together with $G_{\alpha q}$ were used to prepare membranes and [^{3}H]SR48968 binding measured in the presence of increasing concentrations of NKA. Compared with cells expressing NK2 receptor alone, radioligand binding was inhibited by lower concentrations of NKA upon co-expression with $G_{\alpha q}$ and this increased agonist affinity was abolished by co-incubation in the presence of 100 μM GTPγS. (B) Membrane fractions prepared from *S. pombe* co-expressing NK2/*pho1* together with immunodetectable $G_{\alpha q}$, $G_{\beta 2}$ and $G_{\gamma 3}$ were incubated with [^{3}H]SR48968 in the presence of NKA or GR94800 as indicated. Neither agonist- nor antagonist-binding profiles were altered upon co-incubation with 100 μM GTPγS. Data points are the mean of triplicate determinations and representative of three to six independent experiments.

components were immunodetectable in crude yeast homogenates migrating at 42 kDa ($G_{\alpha q}$; Fig. 3A), 35 kDa ($G_{\beta 1}$ and $G_{\beta 2}$; Fig. 3B) or 8 kDa ($G_{\gamma 3}$; Fig. 3C). These sizes are identical with those identified previously in native tissues and following expression in mammalian cells [2]. All G-protein components were localized predominantly in membrane fractions and soluble in the presence of 1% cholate (data not shown). When the NK2 receptor is co-expressed with $G_{\alpha q}$ in COS cells, guanine nucleotide-sensitive high-affinity agonist binding is increased considerably (Fig. 4A). Despite this, NK2 receptor expressed together with $G_{\alpha q}$, $G_{\beta 2}$ and $G_{\gamma 3}$ in *S. pombe* displayed essentially identical pharmacological characteristics as NK2 receptor expressed alone. Hence, the antagonist GR94800 inhibited [^{3}H]SR48968 binding potently in both intact cells and crude homogenates while NKA bound with high affinity only in unbroken cells (Fig. 2C,D). Neither antagonist or agonist binding to membranes was altered in the presence of 100 μM GTPγS (Fig. 4B) even though in parallel experiments GTPγS abolished totally $G_{\alpha q}$-dependent high-affinity NKA binding in COS cells (Fig. 4A).

One potential explanation for the absence of guanine nucleotide-sensitive high-affinity agonist binding to yeast membranes expressing NK2 receptor co-expressed with $G_{\alpha q}$, $G_{\beta 2}$ and $G_{\gamma 3}$ is misfolding, inactivation or inappropriate modification of mammalian G-protein components. As an additonal test of function for G_q from *S. pombe*, we established a system to assess G_q-dependent PLCβ activation as reported for purified mammalian components [46,47]. Bovine PLCβ1 [34] was subcloned into the REP1 and expressed in *S. pombe*. Following growth in the absence of thiamine, PLCβ1 was immunodetectable as a 150-kDa protein localized exclusively in membrane fractions (Fig. 3D). Consistent with expression of PLCβ1, yeast extracts hydrolysed exogenously supplied [^{3}H]PIP_2 and this activity was stimulated considerably by free Ca^{2+} at 10^{-5}M (Fig. 5A). Control membranes were inactive (data not shown). As reported recently [48], $G_{\alpha q}$ expressed recombinantly in baculovirus-infected Sf9 insect cells stimulates PLCβ1 activity following preactivation by GTPγS while GDPβS was totally inactive (Fig. 5B). In contrast, $G_{\alpha q}$ expressed in *S. pombe* was unable to mediate activation of PLCβ1 at any concentration of free Ca^{2+} between 10^{-8} to 10^{-5}M (Fig. 5C). $G_{\alpha 16}$ is an additional member of the G_q family of G-protein α-chains which is 56% identical in amino acid sequence to $G_{\alpha q}$ [5,49] and also mediates powerful activation of PLCβ isoenzymes [9,50]. Human $G_{\alpha 16}$ was expressed in both *S. pombe* and COS cells where it was immunodetectable as a protein migrating at 43 kDa (data not shown). While membranes from COS cells expressing $G_{\alpha 16}$ mediate powerful GTPγS-stimulated PLCβ1 activation (Fig. 5D), preparations from *S. pombe* expressing $G_{\alpha 16}$ were indistinguishable from control membranes (Fig. 5D). Together, these observations indicate that, although both $G_{\alpha q}$ and $G_{\alpha 16}$ are expressed in *S. pombe* at high levels with the expected molecular weight, they are inactive and unable to activate their downstream effector target PLCβ1 in response to GTPγS.

In summary, this is the first report of a mammalian seven-transmembrane receptor heterologously co-expressed with G-protein subunits in the fission yeast *S. pombe*. The NK2 recep-

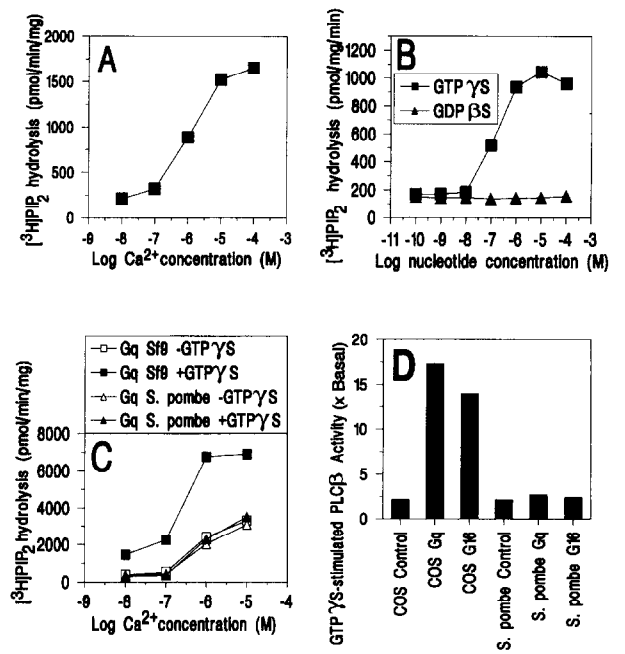

Fig. 5. Recombinant PLCβ1 produced in *S. pombe* is activated by $G_{\alpha q}$ and $G_{\alpha 16}$ from baculovirus-infected Sf9 cells and COS cells but not from fission yeast. *S. pombe* transformed with PLCβ1 subcloned in pREP3 was grown for 18 h in the absence of thiamine after which time homogenates were prepared by cell breakage with glass beads. (A) Ca^{2+}-stimulated hydrolysis of exogenous [^{3}H]PIP_2 by PLCβ1 expressed in crude homogenates of transformed fission yeast. Control cells gave undetectable phospholipid hydrolysis. (B) PLCβ1 is stimulated by $G_{\alpha q}$ produced in baculovirus-infected Sf9 cells preactivated in the presence of increasing concentrations of GTPγS. GDPbS preincubation did not activate $G_{\alpha q}$. Free Ca^{2+} concentrations were maintained at 10^{-7}M. (C) $G_{\alpha q}$ generated by baculovirus-infected Sf9 cells and preactivated by 100 μM GTPγS stimulates PLCβ1 at all free Ca^{2+} concentrations between 10^{-8}M and 10^{-5}M. $G_{\alpha q}$ expressed in *S. pombe* is totally inactive under identical conditions. Data points are the mean of triplicate determinations and representative of three independent experiments. (D) Membranes prepared from COS cells and *S. pombe* expressing either $G_{\alpha q}$ or $G_{\alpha 16}$ were incubated with PLCβ1 in the presence of GTPγS (100 μM) and [^{3}H]PIP_2. Only G-proteins overexpressed in COS cell membranes mediated PLCβ1 activation. Bars represent fold stimulation over basal activity by GTPγS and are the mean of triplicate determinations.

tor displays expected pharmacological characteristics and a proportion of these receptors can be expressed at the cell surface. The G-protein subunits $G_{\alpha q}$, $G_{\beta 1}$, $G_{\beta 2}$ and $G_{\gamma 3}$ were membrane associated and exhibited expected molecular weights. We also report the first expression in yeast of functional PLCβ1. While both receptor and effector molecules produced in *S. pombe* are biologically active, receptor-linked G-protein coupling breaks down at least in part because $G_{\alpha q}$ is inactive. Similar observations with $G_{\alpha 16}$ indicate functional inactivity for other members of the G_q family of G-proteins generated in *S. pombe*.

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References

[1] Dohlman, H.G., Thorner, J., Caron, M.G. and Lefkowitz, R.J. (1991) Annu. Rev. Biochem. 60, 651–688.
[2] Watson, S. and Arkinstall, S.J. (1994) The G-protein linked receptor factsbook. Academic Press, London, UK.
[3] Berridge, M.J. (1987) Annu. Rev. Biochem. 56, 159–193.
[4] Nishizuka, Y. (1992) Science 258, 607–614.
[5] Simon, M.I., Strathmann, M.P. and Gautam, N. (1991) Science 252, 802–808.
[6] Kaziro, Y., Itoh, H., Kozasa, T., Nakafuka, M. and Satoh, T. (1991) Annu. Rev. Biochem. 60, 349–400.
[7] Rhee, S.G. and Choi, K.D. (1992) J. Biol. Chem. 267, 12393–12396.
[8] Wu, D., Lee, C.H., Rhee, S.G. and Simon, M.I. (1992) J. Biol. Chem. 267, 1811–1817.
[9] Wu, D., Katz, A., Lee, C.H. and Simon, M.I. (1992) J. Biol. Chem. 267, 25798–25802.
[10] Aragay, A.M., Katz, A. and Simon, M.I. (1992) J. Biol. Chem. 267, 24983–24988.
[11] Conklin, B.R., Farfel, Z., Lustig, K.D., Julius, D. and Bourne, H.R. (1993) Nature 363, 274–276.
[12] Ashkenazi, A., Peralta, E.G., Winslow, J.W., Ramachandran, J. and Capon, D.J. (1989) Cell 56, 487–493.
[13] Cotecchia, S., Kobilka, B.K., Daniel, K.W., Nolan, R.D., Lapetina, E.Y., Caron, M.G., Lefkowitz, R.J. and Regan, J.W. (1990) J. Biol. Chem. 265, 63–69.
[14] Eistetter, H.R., Church, D.J., Mills, A., Godfrey, P.P., Capponi, A.M., Brewster, R., Schulz, M.-F., Kawashima, E. and Arkinstall, S.J. (1991) Cell Regul. 2, 767–779.
[15] Aramori, I. and Nakanishi, S. (1992) Neuron 8, 757–765.
[16] Aramori, I. and Nakanishi, S. (1992) J. Biol. Chem. 267, 12468–12474.
[17] Arkinstall, S., Emery, I., Church, D., Chollet, A. and Kawashima, E. (1994) FEBS Lett. 338, 75–80.
[18] Kitamura, K. and Shimoda, C. (1991) EMBO J. 10, 3743–3751.
[19] Tanaka, K., Davey, J., Imai, Y. and Yamamoto, M. (1993) Mol. Cell. Biol. 13, 80–88.
[20] Obara, T., Nakafuka, M., Yamamoto, M. and Kaziro, Y. (1991) Proc. Natl. Acad. Sci. USA 88, 5877–5881.
[21] Isshiki, T., Mochizuki, N., Maeda, T. and Yamamoto, M. (1992) Genes Dev. 6, 2455–2462.
[22] Marsh, L., Neiman, A.M. and Herskowitz, I. (1991) Annu. Rev. Cell Biol. 7, 699–728.
[23] King, K., Dohlman, H.G., Thorner, J., Caron, M.G. and Lefkowitz, R.J. (1990) Science 250, 121–123.
[24] Goode, N.T. and Parker, P.J. (1994) FEBS Lett. 340, 145–150.
[25] Superti-Furga, G., Fumagalli, S., Koegl, M., Courtneidge, S.A. and Draetta, G. (1993) EMBO J. 12, 2625–2634.
[26] Arkinstall, S., Payton, M. and Maundrell, K. (1995) Mol. Cell. Biol. 15, 1431–1438.
[27] Hughes, D., Ashworth, A. and Marshall, C.J. (1993) Nature 364, 349–352.
[28] Kodaki, T., Woscholski, R., Hallberg, B., Rodriguez-Viciana, P., Downward, J. and Parker, P.J. (1994) Curr. Biol. 4, 798–806.
[29] Maundrell, K. (1993) Gene 123, 127–130.
[30] Fong, H.K.W., Hurley, J.B., Hopkins, R.S., Miake-Lye, R., Johnson, M.S., Doolittle, R.F. and Simon, M.I. (1986) Proc. Natl. Acad. Sci. USA 83, 2162–2166.
[31] Fong, H.K.W., Amatruda, T.T., Birren, B.W. and Simon, M.I. (1987) Proc. Natl. Acad. Sci. USA 84, 3792–3796.
[32] Strathmann, M. and Simon, M.I. (1990) Proc. Natl. Acad. Sci. USA 87, 9113–9117.
[33] Gautam, N., Northup, J., Tamir, H. and Simon, M.I. (1990) Proc. Natl. Acad. Sci. USA 87, 7973–7977.
[34] Katan, M., Kriz, R.W., Totty, N., Philp, R., Meldrum, E., Aldape, R.A., Knopf, J.L. and Parker, P.J. (1988) Cell 54, 171–177.
[35] Edgerton, M., Chabert, C., Chollet, A. and Arkinstall, S.J. (1994) FEBS Lett. 354, 195–199.
[36] Kozak, M. (1987) Nucl. Acids Res. 15, 8125–8148.
[37] McElroy, A.B., Clegg, S.P., Deal, M.J., Ewan, G.B., Hagan, R.M., Ireland, S.J., Jordan, C.C., Porter, B., Ross, B.C. and Ward, P. (1992) J. Med. Chem. 35, 2582–2591.
[38] Arkinstall, S., Chabert, C., Maundrell, K. and Peitch, M. (1995) FEBS Lett. 364, 45–50.
[39] Mitchell, F.M., Mullaney, I., Godfrey, P.P., Arkinstall, S.J., Wakelam, M.J.O. and Milligan, G. (1991) FEBS Lett. 287, 171–174.
[40] Maundrell, K., Nurse, P., Schonholzer, F. and Schweingruber, M.E. (1985) Gene 39, 223–230.
[41] Palea, S., Dalforno, G., Gaviraghi, G., Hagan, R.M., Trist, D.G. and Pietra, C. (1993) Neuropeptides 24, 285–291.
[42] Lefkowitz, R.J., Cotecchia, S., Samama, P. and Costa, T. (1993) Trends Pharmacol. Sci. 14, 303–307.
[43] Rosenkilde, M.M., Cahir, M., Gether, U., Hjorth, S.A. and Schwartz, T.W. (1994) J. Biol. Chem. 269, 28160–28164
[44] Iniguez-Lluhi, J.A., Simon, M.I., Robishaw, J.D. and Gilman, A.G. (1992) J. Biol. Chem. 267, 23409–23417.
[45] Pronin, A.N. and Gautam, N. (1993) FEBS Lett. 328, 89–93.
[46] Smrcka, A.V., Helper, J.R., Brown, K.O. and Sternweis, P.C. (1991) Science 251, 804–807.
[47] Taylor, S.J., Chae, H.Z., Rhee, S.G. and Exton, J.H. (1991) Nature 350, 516–518.
[48] Helper, J.R., Kozasa, T., Smrcka, A.V., Simon, M.I., Rhee, S.G., Sternweis, P.C. and Gilman, A.G. (1993) J. Biol. Chem. 268, 14367–14375.
[49] Amatruda, T.T., III, Steele, D.A., Slepak, V.Z. and Simon, M.I. (1991) Proc. Natl. Acad. Sci. USA 88, 5587–5591
[50] Lee, C.H., Park, D., Wu, D., Rhee, S.G. and Simon, M.I. (1992) J. Biol. Chem. 67, 16044–16047.