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Identification of transmembrane domains that regulate spatial arrangements and activity of prokineticin receptor 2 dimers

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ABSTRACT

The chemokine prokineticin 2 (PK2) activates its cognate G protein-coupled receptor (GPCR) PKR2 to elicit various downstream signaling pathways involved in diverse biological processes. Many GPCRs undergo dimerization that can modulate a number of functions including membrane delivery and signal transduction. The aim of this study was to elucidate the interface of PKR2 protomers within dimers by analyzing the ability of PKR2 transmembrane (TM) deletion mutants to associate with wild type (WT) PKR2 in yeast using co-immunoprecipitation and mammalian cells using bioluminescence resonance energy transfer. Deletion of TMs 5–7 resulted in a lack of detectable association with WT PKR2, but could associate with a truncated mutant lacking TMs 6–7 (TM1–5). Interestingly, TM1–5 modulated the distance, or organization, between protomers and positively regulated $G_{\alpha s}$ signaling and surface expression of WT PKR2. We propose that PKR2 protomers form type II dimers involving TMs 4 and 5, with a role for TM5 in modulation of PKR2 function.

1. Introduction

G-protein-coupled receptors (GPCRs) comprise the largest family of transmembrane (TM) receptors in the human genome, responding to a plethora of signals that activate second messenger signaling cascade mechanisms via heterotrimeric G-proteins. Their diversity means they impact nearly every aspect of human physiology and pathophysiology and represent the most successfully exploited drug targets, due to their central role in pathogenesis of human disease. The linear model of G protein signaling cannot explain the diversity in GPCR function *in vivo*. This has driven our current understanding in the complex mechanisms that this superfamily of receptors can employ. One key mechanism is the ability of GPCRs to exist as dimers and/or higher order oligomers (Palczewski, 2010) which can both diversify and define receptor function including modulation of agonist and antagonist affinity, membrane trafficking and signal transduction specificity (Rivero-Müller et al., 2013).

The mammalian prokineticin family are involved in diverse biological processes, including neurogenesis, angiogenesis, carcinogenesis, circadian rhythm regulation, inflammation, immune system modulation, pain perception and GnRH ontogeny (Cheng et al., 2002; Giannini et al., 2009; LeCouter et al., 2001; Ng et al., 2005; Shojaei et al., 2007). Their importance in these pathways are underscored by the identification of mutations in prokineticin 2 (PK2) and prokineticin receptor 2 (PKR2) in patients affected with Kallmann syndrome (KS) and/or idiopathic hypogonadotropic hypogonadism, disorders characterized by delayed puberty and infertility (Cole et al., 2008; Dodé et al., 2006; Pitteloud et al., 2007). Binding of PK2 to its cognate GPCR, PKR2, is well known to activate $G\alpha_{q/11}$ signaling leading to the accumulation of inositol phosphate and the mobilization of intracellular Ca^{2+} (Lin et al., 2002a; Ngan and Tam, 2008). However, PKR2 can also activate other heterotrimeric G protein pathways including inhibition of cAMP accumulation through $G\alpha_{i/o}$ proteins, which in certain cell types also mediates mitogen-activated protein kinase signaling (Lin et al., 2002b), or even elevation of intracellular cAMP levels via $G\alpha_s$ (Chen et al., 2005).

We have previously demonstrated that PKR2 undergoes dimerization using the baker's yeast *Saccharomyces cerevisiae*, an accepted experimental system for characterizing human receptor pharmacology and signal transduction mechanisms (Dowell and Brown, 2002; Marsango et al., 2011). Dimerization of PKR2 was

Abbreviations: BRET, bioluminescence resonance energy transfer; CHO, Chinese hamster ovary; Cre-Luc, cyclic AMP response element-luciferase; GPCRs, G protein-coupled receptors; HEK, human embryonic kidney; KS, Kallmann syndrome; PK2, prokineticin 2; PKR2, prokineticin receptor 2; Rluc, *Renilla* luciferase; TM, transmembrane; WT, wild-type.

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sufficient for full receptor activity as co-expression of signal and binding deficient receptors was able to rescue PKR2 signaling via functional complementation. Importantly, we have demonstrated that endogenous PKR2 forms dimers in neutrophils, suggesting it is a relevant form of receptor activity *in vivo* (Marsango et al., 2011).

Despite the recognized importance of dimerization in receptor function, identifying which interfaces are involved and how an individual dimer is formed remains an unanswered question. Numerous molecular, biochemical and biophysical studies, including recent crystallography studies, suggest different TM segments are implicated in this process, depending on the specific receptor under investigation (Huang et al., 2013; Manglik et al., 2012; Palczewski, 2010; Wu et al., 2010; Zhu et al., 2013).

Therefore the aim of this study was to elucidate the dimer interface formed by PKR2 and thereby the organization of PKR2 protomers in the complex. Utilizing PKR2 deletion mutants in yeast and in mammalian cells, via both biochemical and biophysical approaches, we identify that PKR2 dimers form an interface with a prominent role for TM5 in modulating dimer organization. Importantly, we demonstrate that TM5 may play an unprecedented role in regulating receptor activity.

Table 1
Oligonucleotides used for construction of the various mutants.

Oligonucleotide	Sequence
R2 BamHI up	5′-ATA GGA TCC ATG GCA GCC CAG AAT GG-3′
R2 HindIII up	5′-ATA AAG CTT ATG GCA GCC CAG AAT GG-3′
TM1–6 dw	5′-CTC GAG CGG CCG CCA CGT GCT GGA TAT CTG CAG-3′
TM1–5 dw	5′-GTC GAC ACG AAC TCG ACA CCA AAG ATG-3′
TM1–4 dw	5′-TGG TCG ACA GGC CAG ATC TGG CCA CAG AAG ATC-3′
R2 SalI dw	5′-ATG TCG ACC TTC AGC CTG ATA CAG TCC-3′

2. Materials and methods

2.1. PKR2 constructs

The human PKR2 was used as a PCR template for all PKR2 constructs. Human PKR2 cDNA was amplified by PCR using as template human brain Marathon-Ready cDNA (Clontech) using the available PKR2 sequence (accession n. AL121755). The PKR2 mutants used in this study are illustrated in Fig. 1 and oligonucleotides used for construction of the various mutants in Table 1. ΔNterm-PKR2-HIS

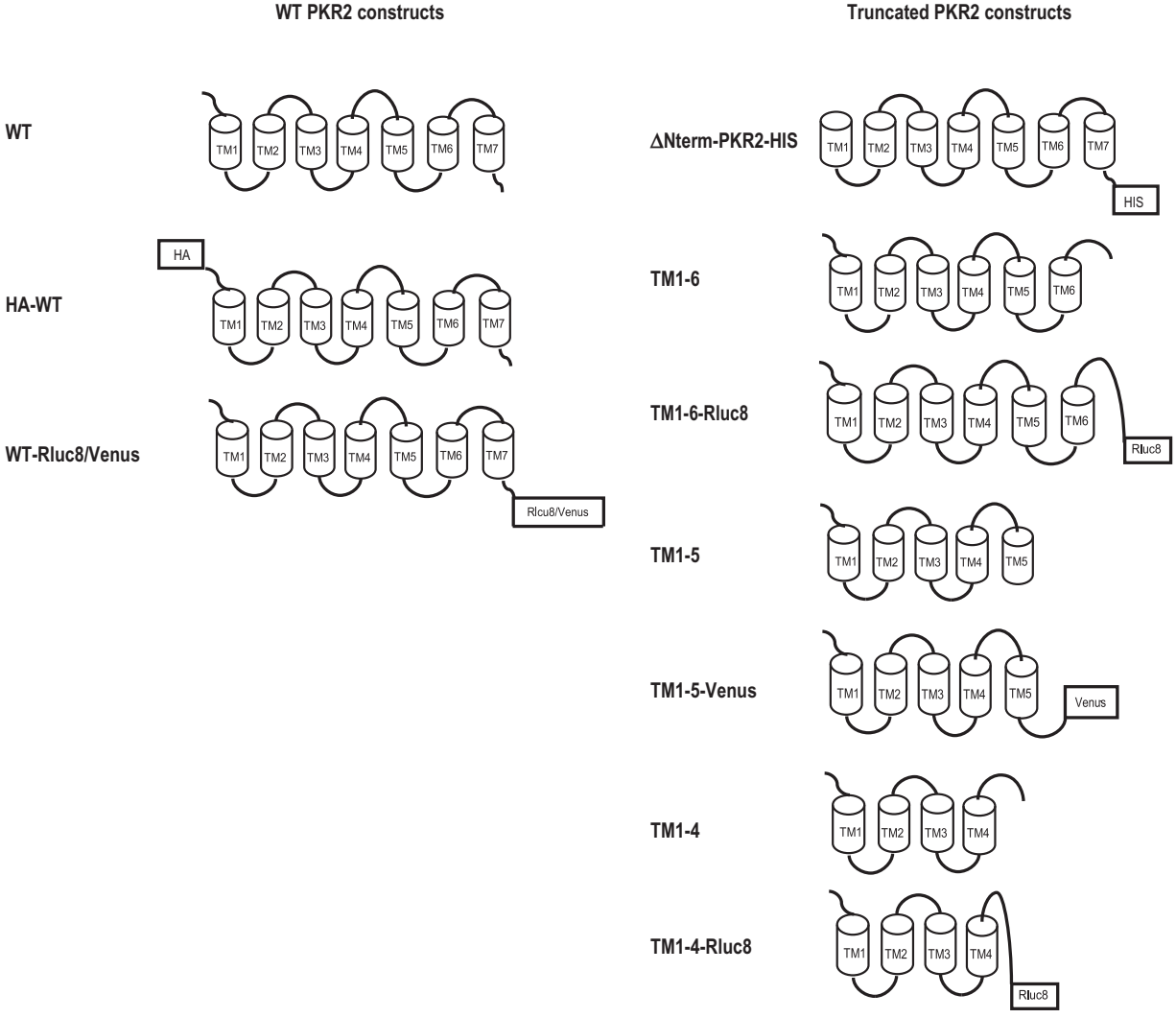

Fig. 1. Schematic representation of WT and truncated PKR2 constructs used in this study. WT PKR2 constructs include untagged WT, HA-WT, WT-Rluc8, WT-Venus (left panel). Truncated PKR2 mutants used in this study are ΔNter-PKR2-HIS, PKR2, TM1–6, TM1–6-Rluc8, TM1–5, TM1–5-Venus, TM1–4, TM1–4-Rluc8 (right panel).