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Minireview

Desensitization of herpesvirus-encoded G protein-coupled receptors

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Abstract

Members of the herpesvirus family, including human cytomegalovirus (HCMV) and Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8), encode G protein-coupled receptor (GPCR) homologs, which strongly activate classical G protein signal transduction networks within the cell. In animal models of herpesvirus infection, the viral GPCRs appear to play physiologically important roles by enabling viral replication within tropic tissues and by promoting reactivation from latency. While a number of studies have defined intracellular signaling pathways activated by herpesviral GPCRs, it remains unclear if their physiological function is subjected to the process of desensitization as observed for cellular GPCRs. G protein-coupled receptor kinases (GRK) and arrestin proteins have been recently implicated in regulating viral GPCR signaling; however, the role that these desensitization proteins play in viral GPCR function in vivo remains unknown. Here, we review what is currently known regarding viral GPCR desensitization and discuss potential biological ramifications of viral GPCR regulation by the host cell desensitization machinery.

Keywords: GPCR; Cytomegalovirus; KSHV; HCMV; MCMV; US28; M33; ORF74; GRK; Arrestin; Desensitization

Contents

Introduction

G protein-coupled receptors (GPCRs) form a diverse family of seven transmembrane spanning receptors that function in

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numerous cellular processes by activating signal transduction networks via heterotrimeric G proteins. Mammalian genomes encode ~1000 GPCRs that function to regulate physiological processes ranging from cardiac contractility to lymphocyte chemotaxis. GPCRs have also been found in numerous other organisms, including fungi and several viruses, and all appear to play important roles in the biology of their respective organism. Initiation of GPCR signaling typically occurs following the binding of agonist to the extracellular domains of the receptor (Fig. 1, left). The agonist-bound GPCR, via a series of conformational changes within its transmembrane domains, enables the receptor to catalyze GDP to GTP exchange on a Gα subunit of the heterotrimeric G protein complex. The G protein complex then dissociates generating a free GTP-bound Gα subunit and a free Gβγ heterodimer, both of which can modulate the activity of various downstream effectors including phospholipase C and adenylate cyclase to generate second messenger molecules. The GPCR superfamily can be subdivided into broad groups definable by the type of G protein(s) with which they activate; for example, $G_{q/11}$-coupled receptors activate the $G_{q/11}$ class of heterotrimeric G proteins to stimulate phospholipase C and generate the second messengers inositol triphosphate and diacylglycerol. Although this standard paradigm involves agonist-dependent activation of a receptor, there are a number of receptors that exhibit agonist-independent or "constitutive" signaling activity.

Desensitization of GPCR signaling

Given the diversity of the GPCR superfamily, the general process by which a cell regulates the magnitude and timing of GPCR signaling is surprisingly well conserved. This process, termed desensitization, is carried out by the sequential action of two families of proteins: the G protein-coupled receptor kinases (GRK), which phosphorylate intracellular serine and threonine residues of activated receptors, and the arrestins, which serve to uncouple phosphorylated GPCRs from heterotrimeric G protein complexes (Fig. 1, right). The concerted action of GRKs and arrestins ultimately results in rapid attenuation of G protein signaling and internalization of the stimulated receptor. It should be noted that second messenger dependent kinases such as protein kinase A (PKA) and protein kinase C (PKC) have also been shown to contribute to GPCR desensitization by phosphorylating serine and threonine residues for many different classes of activated GPCRs (reviewed in (Chuang et al., 1996)).

The roles played by the GRKs and arrestins in maintaining proper levels of GPCR signaling are critical on both a cellular and organismal level. Inappropriate levels of GPCR signaling in various GRK knockout and transgenic animals leads to a plethora of physiological and pathological phenotypes (reviewed in (Premont and Gainetdinov, 2007)). Moreover, reduced GRK expression has been observed in the immune cells of humans with rheumatoid arthritis and multiple sclerosis (Giorelli et al., 2004; Lombardi et al.,

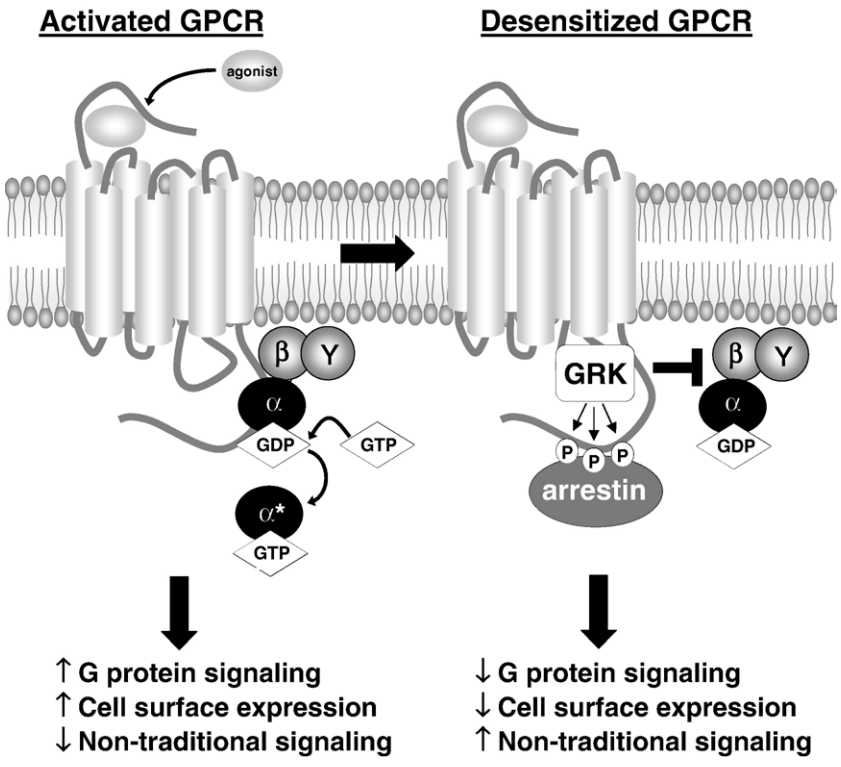

Fig. 1. Typical model of GPCR activation and desensitization. (Left) Agonist-bound GPCRs activate heterotrimeric G proteins through catalyzing GDP to GTP exchange on the Gα subunit. (Right) Following agonist binding, activated receptors are phosphorylated by GRKs, allowing for the binding of arrestin proteins to block further G protein signaling activity and facilitate receptor internalization. Arrestins also serve as scaffolding proteins to recruit various signaling molecules to activate non-traditional signaling pathways.

1999; Vroon et al., 2005). Conversely, elevated GRK activity resulting in decreased signaling from the β-adrenergic receptors in the myocardium has been associated with many cardiovascular diseases including hypertension, cardiac hypertrophy, and heart failure (Choi et al., 1997; Gros et al., 1997; Ungerer et al., 1993). Enhanced arrestin function has also been associated with diseased states, where a naturally occurring mutation within the vasopressin type II GPCR results in constitutive arrestin-mediated desensitization and the development of nephrogenic diabetes insipidus (Barak et al., 2001). Taken together, the data indicate that the desensitization process and the action of the GRKs and arrestin proteins play a crucial role in maintaining the appropriate level and duration of GPCR signaling activity.

GRK Proteins

The GRK family consists of seven members (GRK1-7), which display both tissue-specific expression and GPCR specificity (reviewed in (Ribas et al., 2007)). While GRKs 1 and 7 (retina) and GRK4 (testis) exhibit limited tissue distribution, GRKs 2, 3, 5, and 6 are ubiquitously expressed and thus regulate the majority of GPCRs within an organism. However, alternative protein domains contained within the various GRK isoforms alter their subcellular localization and protein partners and thus may differentially regulate members of the GPCR superfamily. GRKs 2 and 3 possess a carboxy-terminal pleckstrin homology (PH) domain, a PIP_2 binding domain that allows for association with the Gβγ heterodimer and targeting to the plasma membrane (DebBurman et al., 1996; DebBurman et al., 1995; Pitcher et al., 1992). In addition, GRK2 possesses an RH domain which interacts specifically with activated, GTP-bound $G\alpha_{q/11}$ proteins, suggesting a mechanism by which GRK2 can attenuate G protein signaling independent of receptor phosphorylation (Carman et al., 1999; Sallese et al., 2000). Subsequent experiments demonstrated that a kinase-deficient GRK2 mutant as well as the GRK2 RH domain alone can attenuate inositol phosphate (InsP) production from the metabotropic glutamate receptor 1 (mGluR1) in the absence of phosphorylation (Dhami et al., 2002; Dhami et al., 2004).

Arrestin proteins

Following GRK phosphorylation, arrestin proteins are rapidly recruited to the phosphorylated receptor. There are four members of the arrestin family; the retinal-specific arrestins, arrestin 1 and 2 (also termed rod arrestin and cone arrestin, respectively) and the more ubiquitous β-arrestins 1 and 2. The binding of arrestins to phosphorylated receptors serves several functions, the first of which is to form a steric block to uncouple the receptor from further stimulation of G proteins, thus "arresting" G protein signaling. Second, arrestin binding promotes receptor internalization through interactions with clathrin and other endocytic proteins such as AP-2 and *N*-ethylmaleimide-sensitive factor (NSF) (Laporte et al., 1999; McDonald et al., 1999). A third, and more recently appreciated role for arrestin function, involves the ability of arrestin-bound GPCRs to initiate a second wave of signal transduction independent of G protein signaling, also termed "non-traditional" signaling. In fact, arrestins have been shown to activate multiple MAPK pathways, including ERK, JNK, and p38, as well as the non-receptor tyrosine kinase c-Src and the E3 ubiquitin ligase Mdm2 (reviewed in (DeWire et al., 2007)). This scaffolding function of arrestins allows for the positioning of signaling proteins into close proximity of one another and remains a continually evolving area of G protein-independent signaling.

Herpesvirus encoded G protein-coupled receptors

Herpesviruses seem to have taken advantage of the utility of the GPCR signaling network as multiple family members encode proteins sharing sequence homology to cellular chemokine GPCRs (Table 1). The genes for these viral GPCR homologs are postulated as having been acquired from the host genome and maintained within the viral genome throughout its co-evolution with the host. Many of the herpesvirus-encoded GPCR homologs including US28 from the human cytomegalovirus (HCMV), M33 from the murine cytomegalovirus (MCMV), and ORF74 from the Kaposi's sarcoma-associated herpesvirus (KSHV) can initiate traditional G protein signaling cascades as well as other signaling networks involved in gene transcription, cytoskeletal rearrangement, and cell motility. Additionally, some of the viral GPCRs, including MCMV M33 for example, have been shown to affect viral pathogenesis in vivo (Beisser et al., 1998; Davis-Poynter et al., 1997).

While the cellular signal transduction pathways activated by US28, M33, and ORF74 have been characterized in some detail, the post-signaling regulation or desensitization of these viral GPCRs is relatively understudied and likely plays an important role in viral GPCR function. Recent studies suggest that US28,

Table 1
Herpesvirus-encoded GPCRs[a]

Herpesvirus	GPCR	References
Beta-		
HCMV	US27	(Chee et al., 1990), (Margulies and Gibson, 2007)
	US28	(Chee et al., 1990), (Gao and Murphy, 1994)
	UL33	(Chee et al., 1990), (Margulies et al., 1996)
	UL78	(Rigoutsos et al., 2003)
RhCMV	US28	(Hansen et al., 2003), (Penfold et al., 2003)
	UL33	(Hansen et al., 2003)
	UL78	(Hansen et al., 2003)
GPCMV	GP33	(Liu and Biegalke, 2001)
MCMV	M33	(Davis-Poynter et al., 1997), (Rawlinson et al., 1996)
	M78	(Oliveira and Shenk, 2001), (Rawlinson et al., 1996)
RCMV	R33	(Beisser et al., 1998), (Vink et al., 2000f)
	R78	(Beisser et al., 1999), (Vink et al., 2000)
HHV-6	U12	(Gompels et al., 1995), (Isegawa et al., 1998)
	U51	(Gompels et al., 1995), (Milne et al., 2000)
HHV-7	U12	(Nakano et al., 2003), (Nicholas, 1996)
	U51	(Nicholas, 1996), (Tadagaki et al., 2005)
Gamma-		
KSHV	ORF74	(Arvanitakis et al., 1997)
MHV-68	ORF74	(Virgin et al., 1997), (Wakeling et al., 2001)
EBV	BILF1	(Paulsen et al., 2005)
HVS	ECRF3	(Nicholas et al., 1992)

[a] Abbreviations used: HCMV (Human cytomegalovirus); RhCMV (rhesus cytomegalovirus); GPCMV (guinea pig cytomegalovirus); MCMV (murine cytomegalovirus); RCMV (rat cytomegalovirus); HHV-6 (human herpesvirus-6); HHV-7 (human herpesvirus-7); KSHV (Kaposi's sarcoma herpesvirus); MHV-68 (murine herpesvirus 68); EBV (Epstein–Barr virus); HVS (herpesvirus saimiri).

M33, and ORF74 can interact with the cellular GRK and arrestin proteins in vitro. These interactions appear to attenuate signaling, and thus may play an important role in the maintenance of an appropriate level of signal transduction. Moreover, the herpesvirus GPCRs may also interact with the GRK and arrestin proteins to initiate the "non-traditional" wave of signaling as described above. However, the biological impact of GRK and arrestin mediated regulation of viral GPCR signaling in vivo has not been addressed.

Cytomegalovirus

Cytomegaloviruses (CMVs) are large, double-stranded DNA viruses belonging to the beta herpesvirus family and typically establish a life-long, latent infection within their host. A ubiquitous opportunistic pathogen, the human cytomegalovirus (HCMV) is present in 50-90% of adults over age 50 worldwide yet infection remains largely asymptomatic within healthy individuals (reviewed in (Khanna and Diamond, 2006)). HCMV infection has been linked with acceleration of cardiovascular disease; however a true causal relationship between the virus and cardiovascular disease remains to be established. Perhaps more serious are the health risks of HCMV infection within immuncompromised hosts, including neonates and organ transplant recipients. For example, congenital HCMV infection afflicts 40,000 neonates per year in the USA, and in this situation is the leading infectious cause of hearing and vision loss, mental retardation, and encephalitis. HCMV infection has also been linked to the rejection of solid organ transplants in organ transplant recipients, as well as the development of pneumonia, retinitis and increased morbidity in HIV/AIDS patients (reviewed in (Khanna and Diamond, 2006)). Consequently, in 1999, the development of an HCMV vaccine was designated the highest priority by the Institute of Medicine of the National Academy of Sciences based on the potential economic impact and increase in quality of life (Stratton et al., 2000). Similar in their pathogenesis to HCMV, other mammalian CMVs including murine CMV (MCMV) and rat CMV (RCMV) have been useful in vivo models for understanding HCMV biology.

HCMV GPCR US28

Of the approximately 200kb within the HCMV genome there are fourteen ORFs which encode seven-pass transmembrane proteins: US27, US28, UL33, UL78, and the US12 family (US12-US21) (Chee et al., 1990; Gao and Murphy, 1994; Margulies and Gibson, 2007; Rigoutsos et al., 2003). US28 shares sequence identity with human CC and CXC chemokine receptors and is able to bind a number of CC chemokines including RANTES, MIP-1α/β, and MCP-1 (Gao and Murphy, 1994; Kuhn et al., 1995; Vieira et al., 1998). However, similar to most if not all viral GPCRs, ligand binding is not necessary for US28 signaling activity as US28 can couple to the $G_{q/11}$ signaling pathway to stimulate phospholipase C thereby increasing intracellular InsP and Ca^{2+}mobilization in an agonist-independent manner (Casarosa et al., 2001; Gao and Murphy, 1994; Miller et al., 2003; Minisini et al., 2003; Waldhoer et al., 2002). However, there have been reports suggesting that during HCMV infection in vitro, US28 promiscuously couples to members of the $G_{i/o}$, $G_{12/13}$, and G_{16} families of heterotrimeric G proteins (Billstrom et al., 1998; Melnychuk et al., 2004).

Functional studies performed in vitro have shed some light as to the role(s) US28 may play during HCMV infection. US28 can stimulate smooth muscle cell migration through activation of G_{12} proteins and Rho (Melnychuk et al., 2004; Streblow et al., 1999; Streblow et al., 2003). This ability of US28 to promote cellular migration may serve as a means for enhancing HCMV spread throughout the host as well as underlie the link between HCMV and the development of vascular disease. Additionally, US28 has been suggested to function as a viral oncogene, as fibroblasts expressing US28 can induce tumor formation when injected into nude mice (Maussang et al., 2006). This oncogenic effect of US28 involves the induction of proangiogenic and cell cycle factors and is dependent on G protein signaling as a US28 mutant that cannot engage G proteins is unable to stimulate cell cycle progression and promote tumor formation (Maussang et al., 2006).

The interaction between herpesviral GPCRs and the cellular desensitization machinery has been most thoroughly studied for US28. Like other viral and cellular GPCRs, US28 contains multiple serine and threonine residues within its cytoplasmic domains (Fig. 2). In the case of US28, the cytoplasmic tail undergoes constitutive phosphorylation mediated by both GRK2 and GRK5 and, as suggested by studies using pharmacological inhibitors, protein kinase C (PKC) and casein kinase 2 (Miller et al., 2003; Mokros et al., 2002). Mutational analysis identified specific serine residues within the carboxy terminal tail of US28 that, when mutated to alanine residues, results in attenuated US28 basal phosphorylation and stabilized US28 cell surface expression yet has no effect on US28-mediated NF-κB signaling (Mokros et al., 2002). In other studies, deletion of the US28 carboxy terminal tail, generating a truncated mutant termed US28(1-314), resulted in reduced US28 phosphorylation and higher levels of $G_{q/11}$ signaling, suggesting that serine and threonine residues within this tail region are targeted for both phosphorylation and attenuation of $G_{q/11}$ signaling (Miller et al., 2003). Desensitization of US28 is further supported by the finding that wild type US28 but not US28(1-314) can interact with β-arrestin 2, inducing its translocation to the plasma membrane (Miller et al., 2003). The US28(1–314) mutant is also defective in its activation of the MAP kinase p38 compared to wild type US28, suggesting that the interaction of US28 with β-arrestin initiates a second wave of signaling as described for many cellular GPCRs (Miller et al., 2003). Interestingly, β-arrestins do not appear to affect US28 trafficking as US28 surface expression and ligand binding is unaltered when US28 is expressed in β-arrestin knockout cell lines (Fraile-Ramos et al., 2003). However, the carboxy terminal tail can also interact with the receptor associated trafficking proteins including G protein coupled receptor-associated sorting protein (GASP), NSF, and sorting nexin 1 (SNX1) (Fraile-Ramos et al., 2001; Heydorn et al., 2004; Waldhoer et al., 2003). The functional importance of the interactions between US28 and desensitization/cell sorting proteins remain unclear and needs to be studied in the context of a HCMV infected cell. It is likely that these interactions will play important roles in signaling and trafficking of US28, thus impacting its function in vivo.

Viral GPCR	Carboxy terminal tail	Cellular Kinase(s)
HCMV US28	297 KFRQELHCLLAEFRQRLF**S**RDV**S**WYH**S**M**S**F**S**RR**SS**PSRRE**TSS**D**T**L**S**DEACRV**S**QIIP 354	GRK2, GRK5 (Miller et al., 2003); PKC, casein kinase 2 (Mokros et al., 2002)
MCMV M33	312 RDFNKRFMQCI**T**GKLF**S**RRRMLQERAGVR**S**P**ST**PHRAARGLAKIG**T**L**T**R**S**C**S**R**S**ELQR**S**A**S**APPPQ 377	GRK2 (Sherrill and Miller, 2006)
KSHV ORF74	311 PLIY**S**CLG**S**LFRQRMYGLLFQ**S**LRQ**S**FM**S**GA**TT** 342	GRK5, PKC (Geras-Raaka et al., 1998), (Bais et al., 1998)
MHV-68 ORF74	321 KKRMGE**S**VRRAVCRL**SS** 337	??
Cellular GPCR		
hCCR5	301 GEKFRRNYLLVFFQKHIAKRFCKCC**S**IFQQEAPERA**SS**VY**T**R**ST**GEQEI**S**VGL 352	GRK2,3,5,6 (Aramori et al., 1997); PKC (Oppermann et al., 1999)

Fig. 2. The carboxy terminal tails of viral GPCRs regulated by cellular kinases. The carboxy terminal tails of US28, M33, and ORF74 contain a number of Ser and Thr residues (bolded), which may serve as phosphorylation sites for cellular kinases previously shown to facilitate viral GPCR desensitization (Bais et al., 1998; Geras-Raaka et al., 1998; Miller et al., 2003; Mokros et al., 2002; Sherrill and Miller, 2006). Also shown for comparison is the carboxy terminal tail of the human CC-chemokine receptor 5 (CCR5) and its Ser residues (bolded) previously shown to be phosphorylated by GRKs and PKC (Aramori et al., 1997; Oppermann et al., 1999).

MCMV GPCR M33

The murine CMV GPCR homolog M33 belongs to the UL33 family of CMV GPCRs, sharing sequence homology and genome location with UL33, R33, and GP33 from human, rat, and guinea pig CMV, respectively (Davis-Poynter et al., 1997; Gruijthuijsen et al., 2002; Liu and Biegalke, 2001; Margulies et al., 1996). Interestingly, dissimilarities exist among the signaling activities within UL33 family members. In particular M33 and R33 appear to activate signaling pathways distinct from UL33 yet similar to those activated by US28. For example, M33 and R33 couple to $G_{q/11}$ to stimulate InsP accumulation and NF-κB activity in an agonist-independent manner (Gruijthuijsen et al., 2002; Sherrill and Miller, 2006; Waldhoer et al., 2002). Furthermore, M33, like US28, also stimulates smooth muscle cell migration which is suggested to be mediated through Rac-1, a Rho-like G protein (Melnychuk et al., 2005; Streblow et al., 2003). In vivo studies assessing their biological significance indicate that deletion of either M33 or R33 from their respective viral genomes decreases viral replication in animal models of infection. Both intraperitoneal and intraglandular inocculation of M33-deficient MCMV into mice yielded lower viral titers within the salivary gland compared to wild type MCMV, suggesting that M33 promotes both viral spread within the host and replication within tropic tissues (Davis-Poynter et al., 1997). Similarly, upon intraperitoneal inoculation in rats, R33-deficient RCMV produced lower salivary gland titer and higher survival rates than those infected with wild type RCMV (Beisser et al., 1998).

As with US28, M33 and R33 also appear to be desensitized by GRK2, as GRK2 can attenuate both M33-and R33-induced InsP accumulation. In cells transiently expressing R33, InsP accumulation is significantly decreased when GRK2 is co-expressed (Gruijthuijsen et al., 2002). Recent work from our laboratory demonstrated that M33 signaling through $G_{q/11}$ is diminished in the presence of overexpressed GRK2. Moreover, GRK2 overexpression significantly enhances M33 basal phosphorylation (Sherrill and Miller, 2006). Using point mutants of GRK2, it was demonstrated that both the kinase activity and the $G\alpha_{q/11}$-binding activity of GRK2 mediate M33 desensitization (Sherrill and Miller, 2006). Thus, similar to regulation of mGluR1 as described above, GRK2 can regulate viral GPCR signaling in both a phosphorylation-dependent and phosphorylation-independent manner. Preliminary studies from our lab also suggest that M33 can recruit β-arrestin, thus potentially enabling M33 to activate the NF-κB and ERK pathways in a G protein independent fashion (Melnychuk et al., 2005; Waldhoer et al., 2002). Future experiments aimed at defining the mechanism by which M33 induces NF-κB and ERK activation and the potential roles that these signaling pathways play in M33 function in vivo will be important as we continue to define the biological function of M33 in terms of viral pathogenesis.

Kaposi's sarcoma-associated herpesvirus

Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) is a member of the lymphotropic gammaherpesvirus family, which can cause life-long latent infection within certain lymphocyte populations. KSHV was first identified using representational difference analysis as the etiological agent of Kaposi's sarcoma (KS), a highly vascularized neoplasm of endothelial cell origin that is commonly observed in AIDS patients (Chang et al., 1994). In addition to causing KS, KSHV has been associated with the development of HIV/AIDS-related B-cell lymphomas within the peritoneal, pericardial, and/or pleural cavities and multicentric Castelman's disease within lymphoid organs (Cesarman et al., 1995; Dupin et al., 1999; Nador et al., 1996; Soulier et al., 1995).

KSHV GPCR ORF74

KSHV encodes a single GPCR termed ORF74, which is most closely related in sequence to the human CXC chemokine receptor for IL-8 and GRO-α. ORF74 induces high levels of InsP formation in an agonist-independent manner similar to US28 and M33 (Arvanitakis et al., 1997; Bais et al., 1998; Guo et al., 1997). This signaling activity was associated with tumor formation in mice transplanted with focus-derived NIH3T3 cells expressing ORF74, indicating that ORF74 can function as a viral oncogene (Bais et al., 1998). In addition, ORF74 can stimulate the production of various proangiogenic and proinflammatory factors, including ERK1/2, JNK/SAPK, p38, VEGF, and NF-κB (Bais et al., 1998; Guo et al., 2003; Pati et al., 2001; Smit et al., 2002). It also appears that these ORF74 signaling properties are increased in KSHV-positive PEL cells, further underscoring the biological significance of ORF74 as a contributor to KSHV-associated disease states (Cannon et al., 2006; Cannon et al., 2003).

While ORF74 stimulates tumor formation and InsP formation in the absence of agonist, this signaling activity can be enhanced or inhibited through the binding of agonists or inverse agonists, respectively. The CXC chemokine GRO-α acts as an agonist to augment ORF74 InsP signaling while other CXC chemokines, such as IP-10 and SDF-1α, function as inverse agonists to inhibit signaling (Rosenkilde et al., 1999). Ligand binding and G protien coupling to ORF74 apppear to be dictated by residues in the carboxy terminal tail, specifically those located proximal to the seventh transmembrane domain (Liu et al., 2004; Verzijl et al., 2006). The carboxy terminal tail also appears to serve as a determinant for ORF74 desensitization as it contains a number of serine and threonine residues that could serve as substrates for cellular kinases such as the GRKs (Fig. 2). In support of this hypothesis, PMA-induced protein kinase C (PKC) activation or overexpression of GRKs 4, 5, and 6, can block ORF74-induced InsP accumulation and inhibit foci formation in vitro (Bais et al., 1998; Geras-Raaka et al., 1998). Interestingly, overexpression of GRK2 is unable to block ORF74-induced InsP accumulation. These findings suggest that ORF74 signaling, like US28 signaling, can be regulated by kinases inside and outside of the GRK family and that specificity exists among GRK family members for viral receptors.

Murine herpesviruses as models for in vivo infection

The model systems for studying herpesvirus GPCR function include the exogenous expression of a single viral GPCR gene in transfected cells or expression of the viral GPCR in the context of

an entire viral genome. The transition from transfected cell models to infected cell models and eventually to animal models is necessary for defining the physiological function of viral GPCRs. Additionally, other viral gene products and/or their interaction with the host immune system may affect viral GPCR signaling. Unfortunately, the human herpesviruses, such as HCMV and KSHV, replicate only in human tissues, and therefore a detailed evaluation of the function of US28 and ORF74 in animal models is extremely difficult. To circumvent this problem, animal models of herpesvirus infection such as MCMV (a surrogate for HCMV) and murine herpesvirus-68 (MHV-68) (a surrogate for KSHV) provide essential tools to investigate the roles of US28 and ORF74 in vivo. The availability of whole viral genomes packaged within bacterial artificial chromosomes (BACs) and the ability to generate targeted mutations through DNA recombination are becoming useful tools to study viral GPCRs in this context. For example, the recent development of a BAC containing the full genome of the K181 strain of MCMV will allow for manipulation of the M33 ORF to dissect the signaling activities (G protein-dependent or G protein-independent) and the desensitization properties of M33 that might be involved in viral pathogenesis in vivo (Redwood et al., 2005). Given the functional similarities in signaling activity and regulation by GRK and arrestin proteins as discussed above, it is likely that M33 and US28 share related roles in the pathogenesis of their respective viruses. Thus, by studying MCMV and M33 in a relevant animal model, we should enhance our understanding of how US28 functions during HCMV infection. If US28 function is in fact critical to HCMV pathogenesis, a potential therapeutic avenue exists whereby targeting US28 expression or the desensitization machinery that regulates US28 signaling activity could be used to treat HCMV infection.

The genome of MHV-68 has also been cloned into a BAC and has been utilized as a model for studying KSHV infection (Adler et al., 2000; El-Gogo et al., 2007). MHV-68 encodes a GPCR homolog termed ORF74 that shares 25% sequence homology with the KSHV ORF74 and similarly induces foci formation in transfected NIH3T3 cells (Wakeling et al., 2001). MHV-68 ORF74 can also bind the CXC chemokines GRO-α, IL-8, KC, and MIP-2 to inhibit forskolin-induced CRE activation and InsP formation via $G_{i/o}$ activation (Verzijl et al., 2004). In delineating a functional role for MHV-68 ORF74 in pathogenesis, deletion of ORF74 from the genome of MHV-68 attenuates both viral replication and reactivation from latency (Lee et al., 2003; Moorman et al., 2003). This effect of MHV-68 ORF74 on viral replication is dependent on a Pertussis toxin-insensitive $G_{q/11}$ signaling pathway and involves the MEK and PI3K kinases (Lee et al., 2003). Perhaps targeting the G protein signaling pathways or downstream kinases activated by KSHV ORF74 would attenuate KSHV replication and/or blunt the receptor's oncogenic properties.

Role of desensitization in viral GPCR function in vivo

An important area of investigation with respect to the viral GPCRs is to determine what role (if any) the desensitization process plays in viral GPCR function in vivo. Based on the in vitro overexpression studies discussed above, it appears that viral GPCRs like US28, M33, and ORF74 are in fact regulated by GRKs and β-arrestin proteins similar to the cellular GPCRs. The attenuation of viral GPCR activity by cellular desensitization machinery in in vitro experiments raises a number of questions regarding the regulation of these receptors. First, are viral GPCRs truly regulated by cellular GRKs and β-arrestin in cells or animals infected with their respective herpesvirus? Mutagenesis of wild type viral GPCRs within the entire viral genome to produce epitope-tagged wildtype and phosphorylation-site deficient mutant receptors will greatly benefit this area of study. For example, receptor phosphorylation mediated by GRKs and receptor interaction with β-arrestin could be examined to determine if these cellular proteins govern viral GPCR desensitization during viral infection. In the case of M33, generation of a recombinant MCMV expressing a M33 mutant that is unable to engage the GRKs and β-arrestins would provide a useful tool for determining the effect of desensitization on M33 function in vivo.

Second, is the downregulation/attenuation of G protein signaling by the cellular desensitization machinery (or conversely, the activation of β-arrestin-dependent non-traditional signaling pathways) necessary for the proper function of viral GPCRs during viral replication and dissemination? Perhaps regulation by GRKs and β-arrestins is a mechanism used by viral GPCRs to achieve a balance between appropriate levels of intracellular second messengers and aberrantly high levels of G protein signaling. In mammalians, disruption of this balance can have deleterious effects as evidenced by the number of human pathologies associated with irregular levels of GPCR signaling (reviewed in (Smit et al., 2007)). For instance, it has been shown that GRK2-mediated desensitization of the metabotropic glutamate receptor 1A, a constitutively active GPCR associated with neurodegenerative diseases, is necessary to maintain appropriate levels of $G_{q/11}$ signaling and prevent cell death (Dale et al., 2000; Dhami and Ferguson, 2006). Therefore it seems likely that the herpesvirus GPCRs would have retained the ability to interact with the cellular desensitization machinery in order to prevent runaway levels of intracellular signaling that could lead to apoptosis or other unwanted disruptions of cellular homeostasis, both of which could negatively impact the success of the virus. The promise of therapeutic approaches regulating aberrant GPCR signaling and/or their desensitization properties has been raised in the treatment of cardiovascular diseases and the development of such treatment may also prove beneficial for treating HCMV and/or KSHV infection by altering viral GPCR function. This approach has already been demonstrated in vitro through the inhibition of ORF74-induced foci formation by overexpression of GRK5 and could be possibly tailored for other viral GPCRs (Bais et al., 1998).

Conclusion

The presence of viral GPCR homologs in several significant human herpesvirus pathogens such as HCMV and KSHV, combined with the findings that GPCR directed therapeutics have been extremely useful in the treatment of human disease, highlights the fact that a better understanding of herpesviral GPCR function is necessary. The availability of rodent models of herpesvirus infection suggests that it will be possible to define the function of these viral GPCRs using a combination of viral and

mouse genetics. Determining the effect of viral GPCR desensitization by GRKs and arrestins on viral replication in animal models will provide important clues to herpesviral GPCR function in the context of a natural herpesvirus infection.

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