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Comparison of the retinitis pigmentosa mutations in rhodopsin with a functional map of the C5a receptor

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Abstract

We compare the known retinitis pigmentosa (RP) mutations in rhodopsin with mutational data obtained for the complement factor 5a receptor (C5aR), a member of the rhodopsin-like family of G protein-coupled receptors (GPCRs). We have performed genetic analyses that define residues that are required for C5aR folding and function. The cognate residues in rhodopsin are not preferentially mutated in RP, suggesting that the predominant molecular defect in RP involves more than simple misfolding or inactivation. Energy calculations are performed to elucidate the structural effects of the RP mutations. Many of these mutations specifically disrupt the environment of the retinal prosthetic group of rhodopsin, and these do not correspond to essential residues in C5aR. This may be because a retinal group is present in rhodopsin but not in C5aR. Another subset of RP mutations is more generally important for receptor structure, and these mutations correlate with essential residues of C5aR.

Keywords: Rhodopsin; Retinitis pigmentosa; C5a receptor; Vision loss; Congenital disease; G protein-coupled receptor

1. Introduction

G protein-coupled receptors (GPCRs) are cell-surface receptors containing seven transmembrane helices separated by intra- and extra-cellular loops. Upon ligand binding, GPCRs trigger a cascade of downstream events (Neves, Ram, & Iyengar, 2002; Hamm, 2001). The estimated 948 GPCRs in the human genome (Takeda, Kadowaki, Haga, Takaesu, & Mitaku, 2002) include sensors for endogenous polypeptide and small-molecule hormones, environmental chemicals such as odorants, and light. As GPCRs cannot readily be reverse-engineered to determine their precise mechanism of action, a wide variety of structural and genetic studies have been used to explore this important class of proteins (see for example Wess (1999)).

The functioning of GPCRs relies upon a complex chain of events. The polypeptide must be synthesized and translocated into the endoplasmic reticulum, where it must be folded into the appropriate tertiary structure. For at least some receptors, including the C5a receptor (Floyd et al., 2003), GABA-A/B receptors (Balasubramanian, Teissere, Raju, & Hall, 2004), and angiotensin 2 type I receptor (Hansen, Theilade, Haunso, & Sheikh, 2004), oligomerization at this stage appears to be essential for further processing. Receptors must be transported to their site of action: the plasma membrane in most cases, or the outer segment discs in photoreceptors. The expressed receptor must exhibit a physiologically reasonable level of basal and pharmacologically triggered activity, and must also undergo appropriate downregulation, usually by phosphorylation and endocytosis, in order to terminate the signal

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(Tsao, Cao, & von Zastrow, 2001; Luttrell & Lefkowitz, 2002). Each of these steps has presented an opportunity for evolution to fine-tune the signaling cascades mediated by GPCRs, but each also presents an opportunity for failure or dysregulation. Therefore, analysis of nonfunctional or hyperfunctional receptors can provide insight into the mechanism of GPCR signaling. On the other hand, it is always difficult to discern a specific reason why a particular GPCR mutant shows aberrant function.

1.1. Mutational data from the congenital retinal dystrophies

The inherited retinal dystrophies provide a rich natural data set for understanding how receptors act as molecular switches. Retinitis pigmentosa (RP) is a hereditary progressive blindness syndrome with an incidence of 1 in 3500 individuals (Phelan & Bok, 2000). The mode of Mendelian inheritance of the condition—autosomal dominant or autosomal recessive—depends on the specific causative mutation. Retinitis pigmentosa is a genetically heterogeneous condition that has been linked to mutations in numerous components of the phototransduction cycle, including rhodopsin (Dryja et al., 1990a, 1990b), cGMP phosphodiesterase subunits (Huang et al., 1995; McLaughlin, Sandberg, Berson, & Dryja, 1993), the cGMP-gated cation channel (Dryja et al., 1995), and visual arrestin (Nakamachi, Nakamura, Fujii, Yamamoto, & Okubo, 1998; Nakazawa, Wada, & Tamai, 1998). The most common locus of mutation is, however, rhodopsin, which accounts for 1/3 of autosomal dominant cases (Phelan & Bok, 2000).

We became interested in investigating the structural information that can be derived from the rhodopsin mutations that give rise to retinal dystrophies. More than 100 different mutations in the opsin gene have been associated with RP (Farrar, Kenna, & Humphries, 2002; Phelan & Bok, 2000) or related milder diseases such as congenital stationary night blindness (CSNB) (Lem & Fain, 2004) and Leber congenital amaurosis (Woodruff et al., 2003). There have been multiple attempts to classify these mutations according to the behavior of the mutant rhodopsin; see for example Sung, Schneider, Agarwal, Papermaster, and Nathans (1991), Sung, Davenport, and Nathans (1993), Kaushal and Khorana (1994), Mendes, van der Spuy, Chapple, and Cheetham (2005) and references therein. Some classes of mutant rhodopsins are retained in the endoplasmic reticulum (ER), either because of failure to fold properly or because they are not transported to the outer segment. Other mutants have aberrant endocytosis, aberrant stability or post-translational modification, or enhanced coupling to transducin (Mendes et al., 2005). Importantly, the largest class of rhodopsin mutants is still the "unclassified" category, underscoring the fact that mutant classification relies on experimental data. Furthermore, the classes are not mutually exclusive, since a single point mutation could have complex effects on the protein's behavior.

Despite the genetic heterogeneity of RP, it is felt that many of the RP-associated alleles of rhodopsin are gain-of-function mutations (Rao & Oprian, 1996; Lem & Fain, 2004). Although formally this claim would have to be tested for each mutant on a case-by-case basis, some general remarks can be made. Opsin is constitutively active in the absence of retinal (Surya, Foster, & Knox, 1995; Woodruff et al., 2003), so mutants that fail to form a chromophore may activate transducin at a low (Melia, Cowan, Angleson, & Wensel, 1997) but unremitting level. This has been verified explicitly for several RP and CSNB mutants (Gross, Rao, & Oprian, 2003a; Dryja, Berson, Rao, & Oprian, 1993; Rao, Cohen, & Oprian, 1994; Robinson, Cohen, Zhukovsky, & Oprian, 1992). Certain RP mutants that associate poorly with retinal (Mendes et al., 2005) are retained in the ER (Stojanovic, Hwang, Khorana, & Hwa, 2003; Rajan & Kopito, 2005; Sung et al., 1993; Sung et al., 1991) or Golgi apparatus (Zhu et al., 2006), and at least some of these may show a certain level of basal signaling even though they fail to reach the cell surface. Although the ER is not a canonical site of GPCR signaling, the recently described membrane estrogen receptor is a GPCR that natively signals from the ER (Revankar, Cimino, Sklar, Arterburn, & Prossnitz, 2005), so this mechanism may become more widely appreciated in the future. In some mutant rhodopsins, the functional defect appears to be an alteration in the cascade of photointermediates or in the light sensitivity of the receptor (Bosch, Ramon, Del Valle, & Garriga, 2003; Ramon, del Valle, & Garriga, 2003). As further evidence for constitutive activity in the retinal dystrophies, we note that when retinal degeneration results from non-rhodopsin mutations in the photosignaling cascade, the mutation often mimics a state of constant light activation. For example, RP can occur when rod cGMP-gated Ca^{2+} channels are constitutively closed (Dryja et al., 1995; Lisman & Fain, 1995).

1.2. Random saturation mutagenesis of the C5a receptor

Complement factor 5a (C5a), a component of the mammalian complement system, serves as a chemotactic factor for neutrophils in the inflammatory response (Kohl, 2001). Its receptor, the C5a receptor (C5aR) (Gerard & Gerard, 1991), has been investigated as a target for pharmacotherapy in inflammatory states (Allegretti et al., 2005). Besides its intrinsic pharmacologic interest, the C5a receptor (C5aR) serves as a good model for family A GPCRs, which also include rhodopsin. Human C5aR and rhodopsin exhibit 19% amino acid identity, which is average for GPCRs, and there are several common points between these receptors that strongly suggest that they employ a similar mechanism.

These similarities are both structural and functional. The intracellular and extracellular loops of the two receptors are similar in length, and both contain a disulfide bond between the third transmembrane helix (TM3) and second extracellular loop (EC2). Both possess the canonical DRY