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Light-Dependent Interactions Between Rhodopsin and Photoreceptor Enzymes*

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Abstract. This short review summarizes recent results and hypotheses about the activation mechanism of photoreceptor enzymes via photoexcitation of rhodopsin.

Key words: Rhodopsin – GTP-binding protein – Phosphorylation – Photoreceptor membrane – Photoactivation

Photoexcited rhodopsin (R*) communicates the message of photon absorption to the rod cytoplasm by changing the concentration of cytoplasmic cGMP (Woodruff and Bownds 1978) via amplified activation of cGMP-phosphodiesterase (PDE) (Yee and Liebmann 1978). Early steps in this light-triggered enzyme activation cascade involve interactions between R* and GTP-binding protein (GBP). Figure 1 schematically summarizes recent results obtained from protein binding experiments, light-scattering and spectrophotometric measurements, light-induced phophorylation, nucleotide exchange, and PDE assays performed in a number of laboratories. (The reference list is not intended to be comprehensive.)

Part I of Figure 1

In the *dark-adapted* state, rhodopsin, the major intrinsic disk membrane protein, has no particular affinity for the kinase, GBP, or PDE. Bovine and frog rod outer segments contain on the order of 10 molecules of GBP and one molecule of PDE per 100 rhodopsin molecules (see Kühn 1981; C. Pfister, unpublished experiments); the amount of kinase is unknown but probably much smaller. Both the GBP and PDE are peripherally associated with the cytoplasmic surface of the disk membrane; their site of attachment is not yet determined. The kinase exists in both soluble and membrane associated form, most of it being soluble in isotonic saline in the dark (Kühn 1978, 1981).

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Part II of Figure 1

Light absorption leads to cis-trans isomerization of the retinal chromophore (Wald 1968) and to some conformational changes in the rhodopsin molecule including its cytoplasmic surface (Kühn et al. 1982). Binding sites for both GBP and kinase appear to become exposed upon light absorption, since both GBP and kinase (but not PDE) tightly bind to R* (Kühn 1978, 1980, 1981; Kühn and Hargrave 1981).

Competition between GBP and kinase for binding sites on R* has recently been demonstrated by studying phosphate incorporation into R* in disks in the presence and absence of added purified GBP (Kühn, manuscript in preparation): Conditions in which GBP forms a stable complex with R*, i.e., presence of GBP and absence of GTP, lead to inhibition of phosphorylation, whereas addition of micromolar concentrations of GTP, which dissociates GBP from R*, stimulates phosphorylation.

Binding of GBP to R* influences both GBP and R*. In GBP, the exchange of GTP for previously bound GDP on the G_α subunit (Fung et al. 1981) is enabled by its binding to R*. In R*, the equilibrium between Meta I and Meta II is shifted toward Meta II when GBP is bound (Emeis et al. 1982; Bennett et al. 1982). Similarly, the formation and decay of metarhodopsin III is highly perturbed by the binding of GBP to R* (Pfister, Kühn and Chabre, manuscript in preparation). Several lines of evidence indicate that the "active" photoproduct R* is identical with metarhodopsin II (Emeis et al. 1982; Bennett et al. 1982; Kühn et al. 1982).

Part III of Figure 1

Following nucleotide exchange, the G_α subunit in its GTP-binding form (G_α-GTP) dissociates from R* and from $G_{\beta\gamma}$ into the cytoplasm (Kühn 1981; Emeis et al. 1982). By diffusing within the inter-disk space to membrane-associated PDE, it will activate the PDE (Fung et al. 1981; Uchida et al. 1981). At physiological ionic strength, most of the $G_{\beta\gamma}$ stays membrane-associated (Kühn 1981; Emeis et al. 1982), whereas at low ionic strength, $G_{\beta\gamma}$ would be solubilized together with G_α-GTP (Kühn 1980, 1981). Biochemical (Fung and Stryer 1980; Fung et al. 1981) as well as light-scattering (Kühn et al. 1981) experiments at low bleaching extents have shown that one R* can sequentially interact with many (100–500) GBP molecules and catalyse GDP/GTP exchange on them. This corresponds well with the amplified activation of PDE first reported by Yee and Liebman (1978).

The kinetics of formation of the complex R*-GBP (in absence of GTP) and of its dissociation by GTP have been analyzed by recording flash-induced changes in light-scattering (Kühn et a. 1981), indicating that these interactions occur on a $\lesssim$ 100 ms time scale. Further studies on magnetically oriented rod outer segments provide more detailed insights into the physical nature of these light-scattering changes (Hofmann et al. 1981; Chabre et al. 1982).

Following dissociation of G_α-GTP from R*, the kinase has access to R* to bind to it and incorporate up to nine phosphate groups (Wilden and Kühn 1982) from ATP into serine and threonine residues of R*.

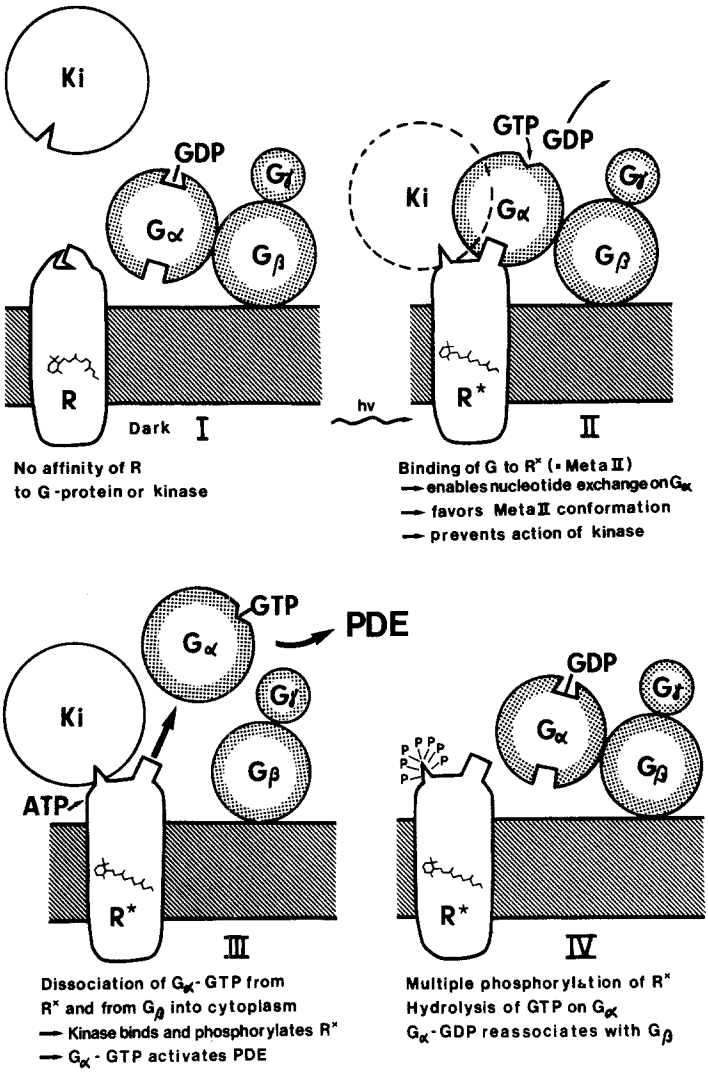

Fig. 1. Schematic presentation of proteins in the disk membrane. R, rhodopsin (molecular weight 41,000 dalton); R*, photoexcited rhodopsin; Ki, rhodopsin kinase (M_r 68,000 dalton; Kühn 1978), the enzyme that catalyzes phosphorylation of R* by ATP; G_α, G_β, G_γ, subunits of GTP-binding protein with approximate M_r values of 37,000–40,000, 35,000–37,000, and $\lesssim$ 10,000 dalton, respectively (Kühn 1980; Fung et al. 1981); PDE, cyclic GMP phosphodiesterase

Part IV of Figure 1

The GTP bound to G_α slowly gets hydrolyzed to GDP, and G_α-GDP then reassociates with $G_{\beta\gamma}$ on the membrane (Kühn, unpublished experiments). Rhodopsin undergoes slow further photoproduct decay which is apparently not influenced by its state of phosphorylation. It has been suggested that the ability of R* to activate the PDE is quenched by phosphorylation (Liebman and Pugh,

1980); this seems to imply that phosphorylated R* is unable to bind and activate GBP, a hypothesis which needs experimental confirmation. Rhodopsin finally returns to its dark-adapted state through a number of reactions including dephosphorylation by a phosphatase, and regeneration from opsin by 11-cis retinal.

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