The invertebrate phototransduction cascade is similar to that of mammalian hormonal receptor system

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The visual system of higher invertebrates is as highly developed as ours. The phototransduction cascade in the visual cell chemically converts light signals into electrical signals. Our biochemical investigation has revealed that in several invertebrates, light-stimulated rhodopsin activates Gq-type G-protein and the G-protein then activates phospholipase C. This is different from the mechanism of vertebrate phototransduction and is more similar to that found in mammalian hormonal receptor systems.

Introduction

Photons reaching a visual cell are converted into an electrical signal by sequential biochemical reactions: the phototransduction cascade. The converted signal is then transmitted to the nervous system. The visual cells in vertebrates and higher invertebrates (Crustacea, Insecta, and Cephalopoda) are well-developed light detectors that can detect single photons. Some insect visual cells have higher time-resolution than those of vertebrates, and this may be related to differences in the phototransduction cascade between these two types of animal. A number of previous studies have suggested that the invertebrate phototransduction cascade differs from that of vertebrates but the molecular mechanism in invertebrate is less well known.

Photoreception[1,2]

Vertebrates photoreceptors (rod and cone of the visual system) possess ciliary bodies and so are called 'ciliary-type photoreceptors'. The rod outer segment is a highly specialized structure for phototransduction and contains a stack of flattened membrane vesicles: the disks. Many invertebrates possess rhabdomeric photoreceptors which bear many microvilli in a structure called a 'rhabdomere'. The invertebrate rhabdomere is the specialized portion for phototransduction as equivalent to the vertebrate rod outer segment. Besides their differences in shape and structure, the electrophysiological response to light in vertebrate and invertebrate photoreceptor is quite different. Vertebrate ciliary-type photoreceptors hyperpolarize in response to light but invertebrate rhabdomeric photoreceptors depolarize. Light absorption by rhodopsin and related visual pigments triggers the biochemical events of phototransduction. The primary structures of various vertebrate and invertebrate opsins have been determined. Vertebrate bovine opsin is about 25% identical to higher invertebrate opsins. Among the invertebrates, crayfish opsin is 56% identical to fly opsin and 35% to squid opsin. In general, isomerization of rhodopsin chromophore, 11-cis-retinal, to the all-trans form on absorption of light triggers a conformational change in the opsin to generate an active form which then reacts with a heterotrimeric G-protein. In the vertebrate rod, photoactivated rhodopsin catalyzes the exchange of GTP for GDP on the α subunit of the G-protein transducin, forming activated transducin. This activated transducin α subunit interacts with the γ subunits of cGMP-phosphodiesterase (PDE) to produce active PDE, which hydrolyzes cGMP. The resulting decrease in cGMP concentration results in the closure of cGMP-gated cationic channels in the rod membrane. The rhodopsin/transducin/PDE cascade (left column in Fig. 1) is one of the best understood examples of signal transduction. Electrophysiological, biochemical, and molecular biological studies suggest that the phototransduction cascade of invertebrate rhabdomeric photoreceptors is different from that of vertebrates.

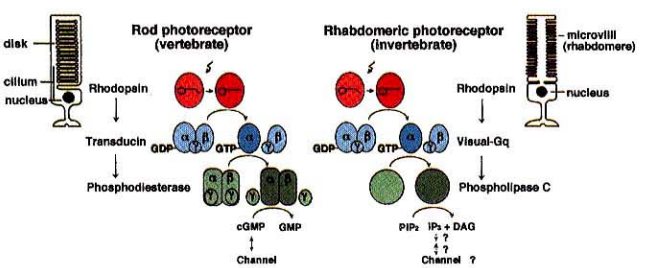

Fig. 1. Schematic representation of vertebrate and invertebrate phototransduction cascades.

Activation of Gq by rhodopsin in invertebrate[3,4]

The Crustacea, Insecta, and Cephalopoda are the major group of invertebrate for studies on phototransduction systems. Various investigators have reported the light-regulated ADP-ribosylation of G-protein in fly, squid, and octopus photoreceptors using bacterial toxins. Recently, a Gq-type of G-

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protein (Gq) has been identified in the fruitfly, crayfish, and squid visual photoreceptors. Gq is found in various kinds of tissues in vertebrates and is characterized as toxin-insensitive G-protein. The occurrence of toxin-sensitive and insensitive G-proteins in invertebrate photoreceptors raises the question of what kind of G-protein interacts with photoactivated rhodopsin and acts in the main pathway of phototransduction. We generated antibodies against consensus amino acid sequences of the ADP-ribosylation sites in transducin and analyzed their reactivity with G-proteins in squid and octopus photoreceptor membranes. The antibodies have crossreactivity with various subclasses of G-proteins because the amino acid sequences of ADP-ribosylation sites are well conserved among toxin-sensitive G-proteins. The antibodies did not detect any G-protein in the cephalopods but they showed strong reactivity with transducin of vertebrates. The results clearly show that the toxin-sensitive G-proteins are a minor component of invertebrate photoreceptor G-proteins. In contrast, the amount of toxin insensitive Gq is about 10% of rhodopsin content in crayfish, squid, and octopus photoreceptor membranes. This ratio is close to that of transducin to rhodopsin in vertebrate rod photoreceptor, suggesting that Gq plays a central role in invertebrate phototransduction. The next question is, "Is Gq actually activated by light-stimulated rhodopsin?" We have demonstrated the interaction between Gq and rhodopsin in the photoreceptor membranes of crayfish with an immunoprecipitation technique. The proteins which interact with rhodopsin were co-immunoprecipitated with rhodopsin on agarose beads crosslinked with monoclonal antibody against crayfish rhodopsin. The unirradiated and irradiated detergent-extracts of crayfish photoreceptor membrane proteins were incubated with the antibody-linked agarose. Immunoprecipitated proteins were analyzed with SDS-PAGE and immunoblotting. A 42 kDa protein was co-immunoprecipitated with rhodopsin in a light-dependent manner, and was identified as α subunit of Gq (Gqα) by partial amino acid sequencing and anti-Gqα-antibody reactivity. In the presence of GTPγS (a non-hydrolysable analog of GTP), Gq dissociated from activated rhodopsin. These results demonstrate that photoactivated rhodopsin activates Gq-type G-protein in the crayfish photoreceptor (right column in Fig. 1). A similar result was obtained in the squid visual photoreceptor system. Taken together with previous studies, it is now clear that Gq is activated by rhodopsin in the invertebrate systems studied so far. We also examined the localization of Gq in crayfish and squid visual cells by using anti-Gqα-antibodies to confirm the rhodopsin-Gq interaction. Gqα was localized to the rhabdomeric membranes, where rhodopsin is known to be located. Visual Gq must be a transducer in invertebrate rhabdomeric photoreceptors.

Activation of PLC by Gq in invertebrate[5)]

The Gq-type of G-protein is widely found in non-visual transduction systems, for example mammalian hormonal receptor systems, where Gq typically activates β-type phosphatidyl inositol-phospholipase C (PLC-β). We have shown that PLC-β is activated by GTP and light in squid photoreceptor membranes. Most recently, we have demonstrated the activation of PLC by visual Gq. As described above, light activates Gq to produce GTP-bearing Gqα and G$\beta\gamma$ in the photoreceptor membranes. We produced activated Gq in the squid photoreceptor membranes and purified the activated Gqα (bearing GTPγS) and G$\beta\gamma$ subunits by chromatography. PLC purified from the squid photoreceptor cells was reconstituted either with Gqα or G$\beta\gamma$ in lipid vesicles and PLC activity was measured. Gqα greatly enhanced PLC activity in a dose-dependent manner. However, G$\beta\gamma$ showed no enhancement of PLC activity. These results demonstrate that the activated Gqα (the GTP-carrying form) directly stimulates PLC in squid photoreceptor membranes. The PLC of squid is identified as a PLC-β4 or *Norp A* type by site-specific antibody binding. *Norp A* is a strong candidate as a key enzyme in the *Drosophila* fruitfly phototransduction cascade. In addition, it has been reported that light stimulates PLC activity in the photoreceptor of several other invertebrates. The mechanism that Gq activates PLC may be common among various invertebrate visual systems (see right column in Fig. 1).

Conclusion

Our *in vitro* biochemical studies have demonstrated the existence of a rhodopsin/Gq/PLC cascade in the invertebrate (arthropod and cephalopod) rhabdomeric photoreceptors. The sequential reactions are probably the main pathway of phototransduction, based on our data and genetic analyses of *Drosophila*, so the cascade of invertebrate phototransduction is clearly different from that of vertebrates, (i.e. rhodopsin/transducin/ phosphodiesterase cascade) and is more similar to some hormonal receptor systems. As shown in Fig. 1, the down-stream events following the activation of PLC are still uncertain. A possible light-sensitive ion-channel in *Drosophila* is known as *Trp* or *Trpl*. Identification and characterization of light-regulated ion-channel are the next key toward full elucidation of the molecular mechanism of phototransduction in invertebrates.

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