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Subcellular Localization of GTP Binding Protein in *Stentor coeruleus*

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The heterotrichous ciliate *Stentor coeruleus* shows a step-up photophobic response to visible light. In the previous paper, the existence of GTP-binding proteins was confirmed by using the antisera against the carboxy terminal decapeptide of transducin α subunit. The photoreceptor, stentorin, is localized in the pigment granule. If the immunoreactive G-protein directly interacts with the photoreceptor stentorin, the G-protein expected to be located in the pigment granule rather than plasma membrane. To elucidate the function of the immunoreactive G-protein, the localization of the G-protein in *Stentor coeruleus* was studied. The results suggest that this G-protein is located in the myoneme involved in the contraction and extension of the cell rather than in the pigment granule.

key words: *Stentor coeruleus*, GTP-binding protein, myoneme, photophobic response, stentorin.

INTRODUCTION

In animal and lower eucaryotes, heterotrimeric GTP-binding proteins (G-proteins) play key roles in signal-transducing pathways. The main function of G-proteins involves the relaying of signals such as hormone and light from various seven-transmembrane-domain receptors to intracellular effectors such as cGMP phosphodiesterase, ion channels, and adenylate cyclase [1].

Heterotrimeric G-proteins are made up of α, β, and γ subunits. The β and γ subunits form a tight complex. When the inactive GDP-bound G-protein interacts with an activated membrane receptor, the GDP is replaced by GTP and the $\beta\gamma$ complex dissociates from the GTP bound α subunit [2]. The α subunit interacts with the receptors and the effectors, but there are also evidence for a role of $\beta\gamma$ dimer in determining the specificity of G-protein function [3]. Termination of the signal is accomplished by the intrinsic GTPase activity of α subunit. The α subunit then reassoiciates with the $\beta\gamma$ dimer.

The heterotrichous ciliate, *Stentor coeruleus,* shows a step-up photophobic response to visible light [4]. The action spectrum for the photophobic response and the receptor potential are similar to the absorption spectra of both *Stentor* cell and photoreceptor, stentorin [5,6].

The blue green pigment granules are located in and just beneath the outer proteinaceous layer of the cortex, pellicle. The pigment granules are 0.3-0.7 μm in diameter [7]. The stentorin is present within the pigment granule. The chromophore structure of stentorin was thought to be a hypercin-derivative [8]. The chemical structure of the chromophore has now been elucidated as an octahydroxyl-diisopropylnaphthodianthrone [9].

Light stimulation generates a membrane potential depolarization (photoreceptor potential). This membrane potential of the *Stentor* cell membrane occurs in a few hundred milliseconds after the light stimulus. The delay of the membrane depolarization suggests the involvement of an initial biochemical process before the rise of the receptor potential [10]. However the biochemical events which lead to the membrane depolarization in *Stentor* cell is unknown. Results from physiological studies suggested the existence of GTP-binding proteins in *Stentor* [11]. The proteins from *Stentor* cell membranes were observed to cross-react with antisera against the carboxy terminal decapeptide of transducin α subunit [11]. As a first step to elucidate the function of this immunoreactive G-protein, the localization of the G-protein was investigated with the electron microscope. If the G-protein directly interacts with the photoreceptor stentorin, it is likely to be located in the pigment granule. We report here the localization of the G-protein in *Stentor coeruleus*.

MATERIALS AND METHODS

Cell culture

Stentor coeruleus cells were cultured in a buffered medium containing 0.5 mM $CaCl_2$, 1 mM $MgSO_4$, 1 mM $NaNO_3$, 0.1 mM KH_2PO_4, and 1 mM Tris-HCl (pH 7.8) at room temparature [11]. The cells were fed regularly (every 3-4 days) with axenically grown *Tetrahynema pyriformis* cells.

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Immunoelectron microscopy

Stentor cells were fixed with 3% glutaraldehyde in 100 mM sodium phosphate buffer, pH 7.2 for 1 h at room tempataure. The cells were dehydrated with an ethanol series. After dehydration, the cells were embedded in L.R. White resin (Polysciences) and sectioned. The sections were incubated with anti-α-transducin antibody (AS/7) (NEN) at dilutions between 1 and 10 μg/ml, overnight. After rinsing with 100 mM sodium phosphate buffer, pH 7.2, the sections were incubated for 2 h with goat anti-rabbit IgG (Amersham) to which 10 nm gold particles were attached. After rinsing, the grids were stained with 3% uranyl acetate for 5 min, and viewed with a Philips 201 microscope.

RESULTS AND DISCUSSION

Incubation of a thin section of the L.R. White-embedded *Stentor* cell with anti-α-transducin antibody AS/7 and subsequent detection by immuno gold revealed strong gold labeling in the myoneme (Figures 1 and 2) . No gold particles were found in the pigment granule (Figure 3) . Staining with AS/7 antibody also occurred in the kinetosome (km) fiber, but the extent of labeling is not as strong as in the myoneme.

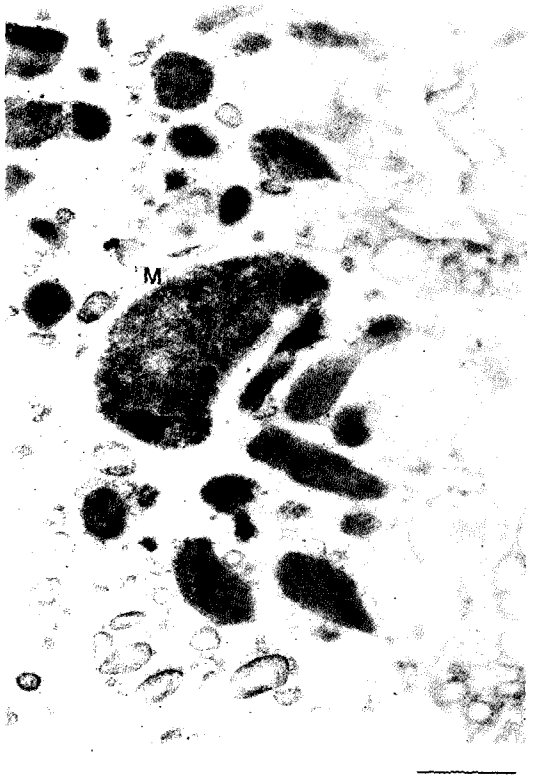

Figure 1. Immunogold labeling of the L.R. White-embedded *Stentor* cell section. The cell was fixed with 3% glutaraldehyde and embedded in L.R. White for sectioning. The section was incubated with the AS/7 antibody and subsequently with secondary antibody coupled to 10 nm gold particles. Note the strong labeling of the myoneme. No gold particles can be found in the pigment granule. M: Myoneme Bar is 1 μm

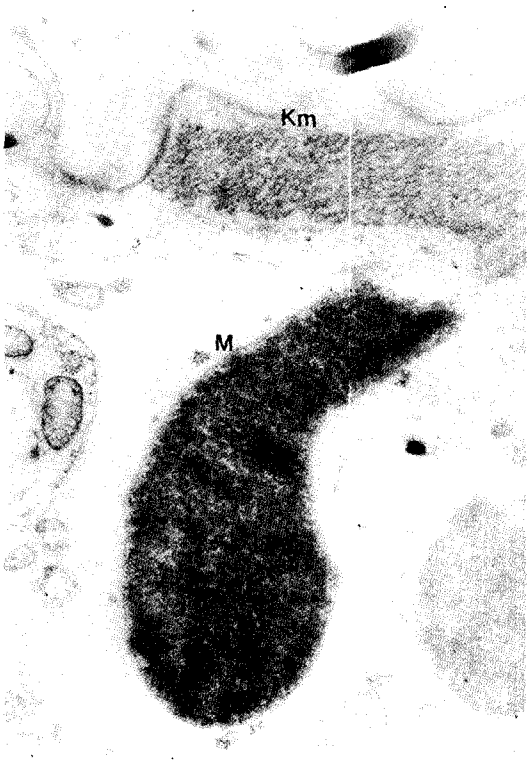

Figure 2. Immunogold labeling with the AS/7 antibody in the myoneme and the kinetosome fibre. Note the weak labeling in the kinetosome fibre compared to the labeling in the myoneme. M: Myoneme Km: Kinetosome Bar is 1 μm.

The myoneme and the kinetosome fiber are the main organelles responsible for generating rapid contraction and extension in *Stentor* cell [12]. The myonemes are organized with longitudinal fibers parallel to the length of the *Stentor* cells, whereas the km fibers are a longitudinal array of parallel ribbons or bundles of microtubules just below the cell surface [13-15].

We could confirm that membrane fractions of the Stentor cell possess transducin α subunit like protein by using AS/7 antibody [11]. The AS/7 antibody was raised against the carboxyl-terminal decapetide of the α subunit of transducin (KENLKDCGLF). The AS/7 antibody reacts strongly with the transducin α subunit and also with Gi class α subunits [15]. This cross-reactivity is due to the sequence similarity between the transducin α subunit and the Gi class of α subunits (KNNLKDCGLF) [16]. However, the AS/7 antibody does not react with Go class α subunits which have only 5 completely conserved amino acids in this 10 amino acids region [15].

The effects of pertussis toxin and fluoroaluminate on the photophobic response of *Stentor* cells suggested that heterotrimeric G- protein is involved in the light signal transduction [11]. However, these results do not provide evidence

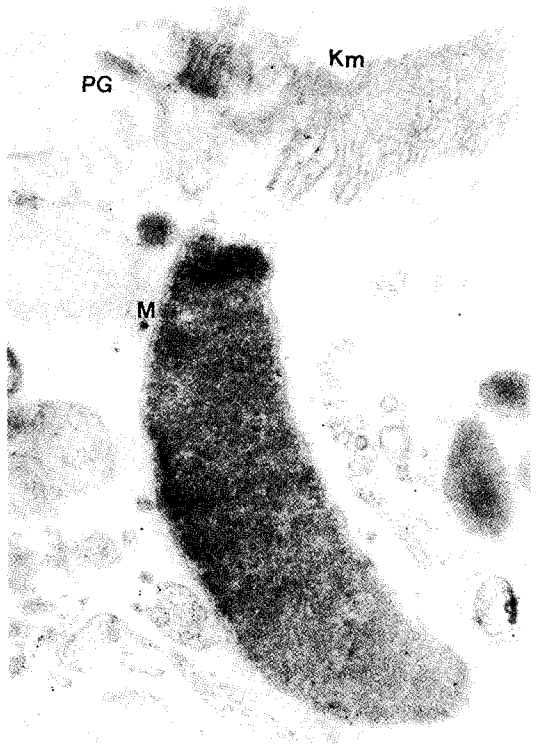

Figure 3. Immunogold labeling with the AS/7 antibody. The sample was prepared as in Figure 1. A pigment granule is seen on the upper left side of the photo, but no gold particles are found. Again note the strong labeling in the myoneme. M: Myoneme Km: Kinetosome PG: Pigment granule Bar is 1 μm.

for the direct stentorin-G-protein interaction. This Gα subunit can be a part of very complicated transduction pathway. It is also possible that another G-protein not detectable by the AS/7 antibody directly interacts with stentorin in the pigment granule.

The presence of Gα subunit in the myoneme and in the kinetosome fiber suggests that this G-protein may potentially be important in cell contraction and extension. The ability of generating rapid changes in cell length is one of the characteristics of some species of ciliates such as *Stentor* and *Vorticella*. The contraction can be generated by mechanical, chemical, and electrical stimuli. In *Stentor*, the fully extended cell length is around 1-2 mm, and upon light stimulus, the cell contracts to around one quarter of its original length at a maximum velocity of 10 to 20 cm/s [17].

The changes in the contraction of myonemes is dependent on calcium ions [2]. The motor force for cell contraction could come from the calcium-dependent macromolecular conformational change of the rubber-like protein. The light-induced cell elongation occurs in *Blepharisma*, a related heterotrich ciliate, and the light-induced cell response can be inhibited by membrane-permeable cGMP [18]. The light-induced cell elongation takes 5 to 15 min after light illumination, whereas the photophobic response takes place immediately after the light stimulation. However, both responses are inhibited by membrane-permeable cGMP. Thus, light perception by photoreceptors induces a decrease in cytoplasmic cGMP concentration. After lowering the cGMP concentration, the signal transduction pathway may diverge. One pathway induces cell elongation, which is mediated by the myoneme, and the other pathway elicits the photophobic response.

The *Stentor* cell can be contracted by mechanical, chemical, or electrical stimuli. The main organelle responsible for the cell contraction is the myoneme. Thus, the receptor that is coupled to the G-protein might be the converging point from the various signal transduction pathways. If the heterotrimeric G-protein is present only in the myoneme, and there is none in the pigment granules, the proton release from the activated stentorin might be a trigger for the activation of the receptor located in the myoneme. The G-protein α subunit then activates cGMP phosphodiesterase, and open calcium channels. The calcium influx causes the ciliary beat reversal in the photophobic response, as well as the cell contraction by the myoneme. To explore these possibilities, the dependence of cell contraction on cGMP may reveal if cGMP inhibits the cell contraction.

We cannot rule out the possibility that another G protein exists in the pigment granule and interacts directly with stentorin. *Stentor coeruleus* has a unique light signal transduction pathway. Thus, understanding this primitive system could help understand how the photoreceptor systems in invertebrates and vertebrates evolved.

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