Invited Article

Photoregulation of Cytoplasmic Streaming: Cell Biological Dissection of Signal Transduction Pathway

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In mesophyll cells of Vallisneria gigantea Graebner, an aquatic angiosperm, induction and cessation of rotational streaming of the cytoplasm are under the control of light. In this article, I would like to outline our study on the functional elements implicated in this light-dependent phenomenon, namely, photoreceptor systems, motile apparatus, and ion transport systems across the plasma membrane, and on the modes of interactions among them, with emphasis on the regulatory role of the calcium ion.

Key words: Actomyosin — Calcium ion (Ca^{2+}) — Cytoplasmic streaming — Photosynthesis — Phytochrome — Plasma-membrane H^{+} pump

Phytochrome and Photosynthetic Pigment Cooperatively Regulate the Cytoplasmic Streaming

In most cases examined so far, light-dependent intracellular movements are under the control of blue-light photoreceptors (Senger 1980, 1984, Haupt 1982, Yatsuhashi 1996), although phytochrome (Haupt 1982, Dong et al. 1995, Yatsuhashi 1996) and photosynthetic pigment (Seitz 1979, Dong et al. 1995) have been shown to be involved in several cases. In the case of mesophyll cells of V. gigantea, whereas continuous irradiation with red light (650 nm, 10 \mu mol/m^{2}sec) most effectively induces the cytoplasmic streaming, far-red light (730 nm, 10 \mu mol/m^{2}sec) exhibits a specific inhibitory effect (Takagi and Nagai 1985). We first presumed that phytochrome exclusively functions as the photoreceptor in this response, that is, the cytoplasmic streaming is induced or inhibited, respectively, in the presence of the far-red light-absorbing form (Pfr) or the red light-absorbing form (Pr) of phytochrome. We confirmed that phytochrome is spectrophotometrically detectable in the crude extract obtained from the leaves (Takagi et al. 1990).

However, the observation that a brief irradiation with red light for less than a few minutes did not induce the cytoplasmic streaming at all led us to examine a possible involvement of other photoreceptors than phytochrome. We found that continuous irradiation with blue light (450 nm, 10 \mu mol/m^{2}sec) became effective to induce the cytoplasmic streaming only after a brief irradiation with red light (Takagi et al. 1990). The effect of a brief irradiation with red light was negated by a brief irradiation with far-red light applied immediately after the red-light irradiation, and the photoreversibility was repeatedly observed after alternating irradiation with red and far-red light. Inhibitors of photosynthesis, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and atrazine, substantially suppressed the induction of cytoplasmic streaming not only by continuous irradiation with blue light that followed a brief irradiation with red light but also by continuous irradiation with red light. The induction of cytoplasmic streaming seemed to occur only in the presence of Pfr and only when photosynthesis had taken place for several minutes. Although the apparently opposite effects of red and far-red light on the cytoplasmic streaming and the fluence of actinic irradiation required for the photoinduction of cytoplasmic streaming suggest that type II phytochrome is functional (Mandoli and Briggs 1981, Shinomura et al. 1996), we do not know how many and which molecular species of phytochrome (Furuya 1993) are involved in this phenomenon.

The Actomyosin System Drives the Cytoplasmic Streaming in a Ca^{2+}-Dependent Manner

Bundles of microfilaments, which are mainly composed of filamentous (F-) actin, serve as the tracks for the cytoplasmic streaming in the mesophyll cells of V. gigantea (Takagi and Nagai 1983), as is the case in various types of cytoplasmic streaming in plant cells (Shimmen and Yokota 1994). These bundles are aligned...
parallel to one another and run along the four anticlinal walls of the cell, namely, the two longer side walls and the two shorter end walls (Masuda et al. 1991, Ryu et al. 1995) (Fig. 1). This stationary organization of the bundles of microfilaments is maintained by the action of a presumed unidentified protein factor(s) in the cell wall (Masuda et al. 1992, Ryu et al. 1997).

As reported in several kinds of microfilament-dependent intracellular movement in plant cells (Nagai 1993), the calcium ion (Ca\(^{2+}\)) plays a critical role in the induction and cessation of cytoplasmic streaming. In the dark, when the cells were treated with solutions of various concentrations of Ca\(^{2+}\) in the presence of a divalent cation ionophore A23187, the cytoplasmic streaming was induced and maintained at the concentrations of Ca\(^{2+}\) lower than 10\(^{-6}\) M and inhibited at those higher than 10\(^{-6}\) M (Takagi and Nagai 1986). Since the inhibitory effect of Ca\(^{2+}\) on the cytoplasmic streaming was not mediated by disruption of the bundles of microfilaments, we sought the activity of a plausible motor protein(s) that could interact with F-actin. By the procedures developed for purification of myosin from lily pollen tubes (Yokota and Shimmen 1994), we succeeded in partial isolation of the activity from the leaves of \textit{V. gigantea} that supports the adenosine 5'-triphosphate (ATP)-dependent sliding movement of F-actin from skeletal muscle \textit{in vitro} (Takagi et al. 1995). Moreover, the activity exhibited a strict dependence on the concentration of Ca\(^{2+}\). The sliding movement of F-actin was completely inhibited in the presence of Ca\(^{2+}\) at concentrations higher than 10\(^{-6}\) M (Fig. 2). Thus we could confirm the existence of a Ca\(^{2+}\)-dependent motor activity that interacts with F-actin, and hence probably also a mechanochemical enzyme to generate the motive force for the cytoplasmic streaming in the mesophyll cells of \textit{V. gigantea}.

**Fluxes of Ca\(^{2+}\) Across the Plasma Membrane are Under the Control of Light**

From the findings described above, we supposed that red and far-red light regulate the cytoplasmic streaming through modulation of the content of calcium in the cytoplasmic matrix \textit{in vivo}, as reported in numerous physiological responses in plant cells (Hepler and Wayne 1985, Takagi and Nagai 1992). To ascertain this possibility, we fixed the cells under various light conditions for electron microscopy in the presence of antimony (Takagi and Nagai 1985), a precipitant specific for Ca\(^{2+}\) (Caswell 1979). As we anticipated, the amount of calcium-containing precipitate formed in the cytoplasmic matrix (Takagi and Nagai 1983) decreased under red light and increased under far-red light. In addition, in the presence of La\(^{3+}\), an ion used as an antagonist of Ca\(^{2+}\) channels in the plasma membrane of axons (Lettvin et al. 1964), the increase in the amount of precipitate observed under far-red light was no longer evident (Takagi and Nagai 1985). Concomitantly, the inhibitory effect of far-red light on the cytoplasmic streaming disappeared. The cessation of cytoplasmic streaming caused by irradiation with far-red light might be attributed to an influx of Ca\(^{2+}\) across the plasma membrane through Ca\(^{2+}\) channels.

We isolated protoplasts from the mesophyll cells by enzymatic digestion and further examined fluxes of Ca\(^{2+}\) across the plasma membrane (Takagi and Nagai 1988). Whereas the concentration of Ca\(^{2+}\) in a solution bathing the protoplasts increased under continuous irradiation with red light, it rapidly decreased upon irradiation with far-red light. The efflux of Ca\(^{2+}\) shared the same characteristics with the induction of cytoplasmic streaming in

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**Fig. 1.** Bundles of microfilaments provide the tracks for rotational cytoplasmic streaming in the mesophyll cells of \textit{V. gigantea}. After isolation by enzymatic digestion, single mesophyll cells were stained with fluorescein isothiocyanate-conjugated phalloidin as described by Masuda et al. (1991). A: Fluorescence microscopy. B: Phase-contrast microscopy.

**Fig. 2.** The motor activity isolated from \textit{V. gigantea} leaves supports the sliding movement of F-actin in a Ca\(^{2+}\)-dependent manner. The motor activity was partially isolated from homogenates of the leaves according to the procedures developed by Yokota and Shimmen (1994). The ATP-dependent sliding movement of F-actin from skeletal muscle, which had been labeled with rhodamine-conjugated phalloidin, was monitored in the presence of Ca\(^{2+}\) at 10\(^{-7}\) M (A and B) and at 10\(^{-4}\) M (C and D). The interval of the two serial micrographs, A and B, C and D, was 2 sec.