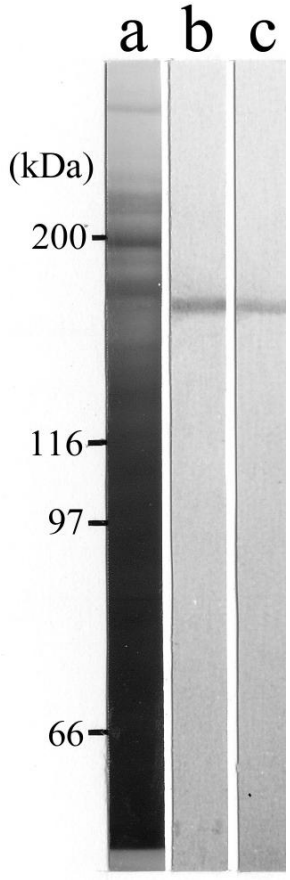
A subclass of Myosin XI Is Responsible for the Translocation of Endoplasmic Reticulum in Tobacco Cultured BY-2 Cells. *Etsuo Yokota*[*]*, Shunpei Ueda, Kentaro Tamura, Hidefumi Orii, Satoko Uchi, Seiji Sonobe, Ikuko Hara-Nishimura, and Teruo Shimmen*

SUPPLEMENTARY DATA

Supplementary Figure S1.

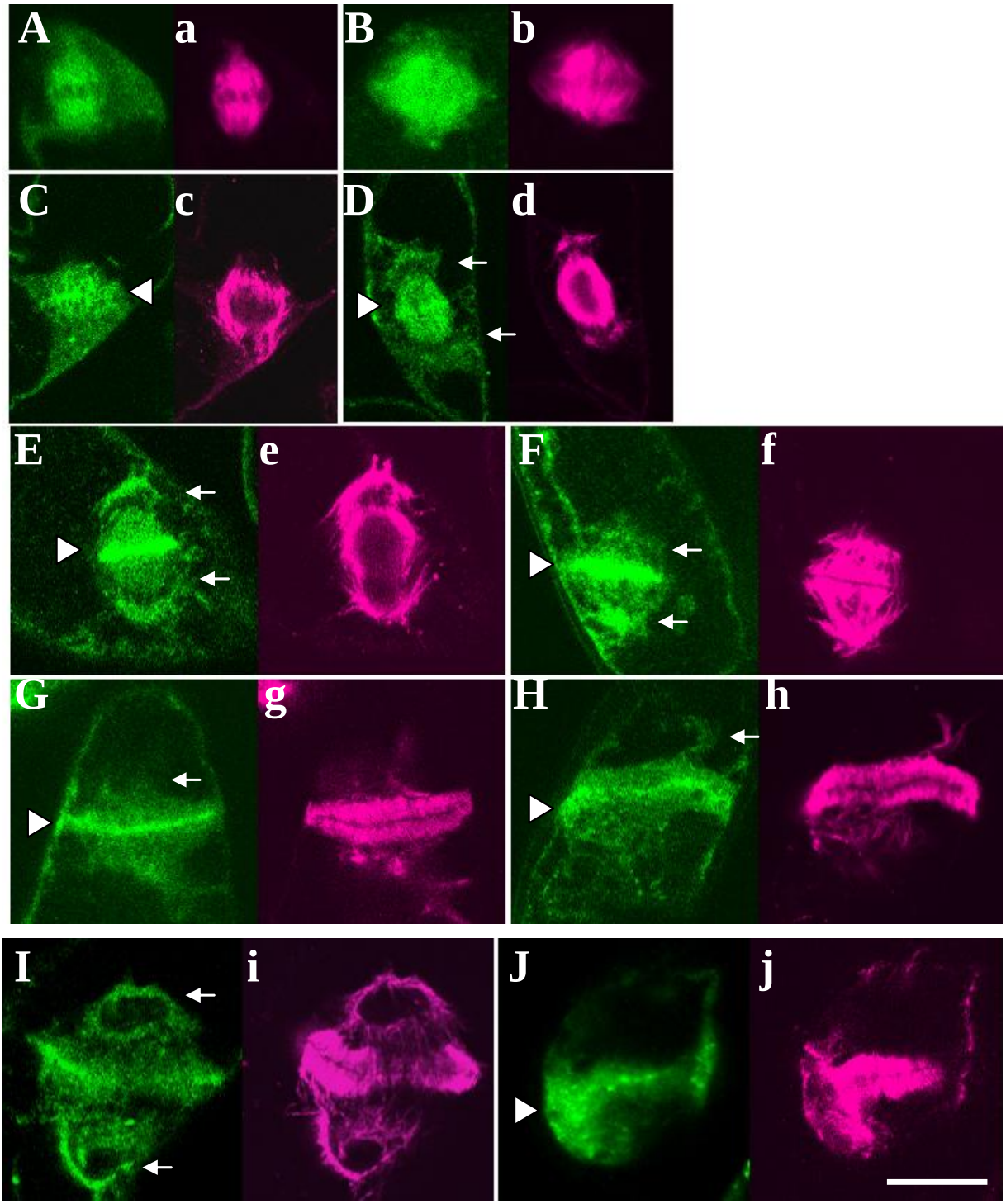
Immunoblotting of crude extract of BY-2 cells with an antibody against 175-kDa myosin heavy chain.



a, CBB staining gel of crude extract. b, Immunoblotting with an antiserum against 175-kDa myosin heavy chain. c, Immunoblotting with an affinity purified antibody isolated from the serum. The molecular masses of standard proteins are indicated on the left in kilodaltons.

Supplementary Figure S2.

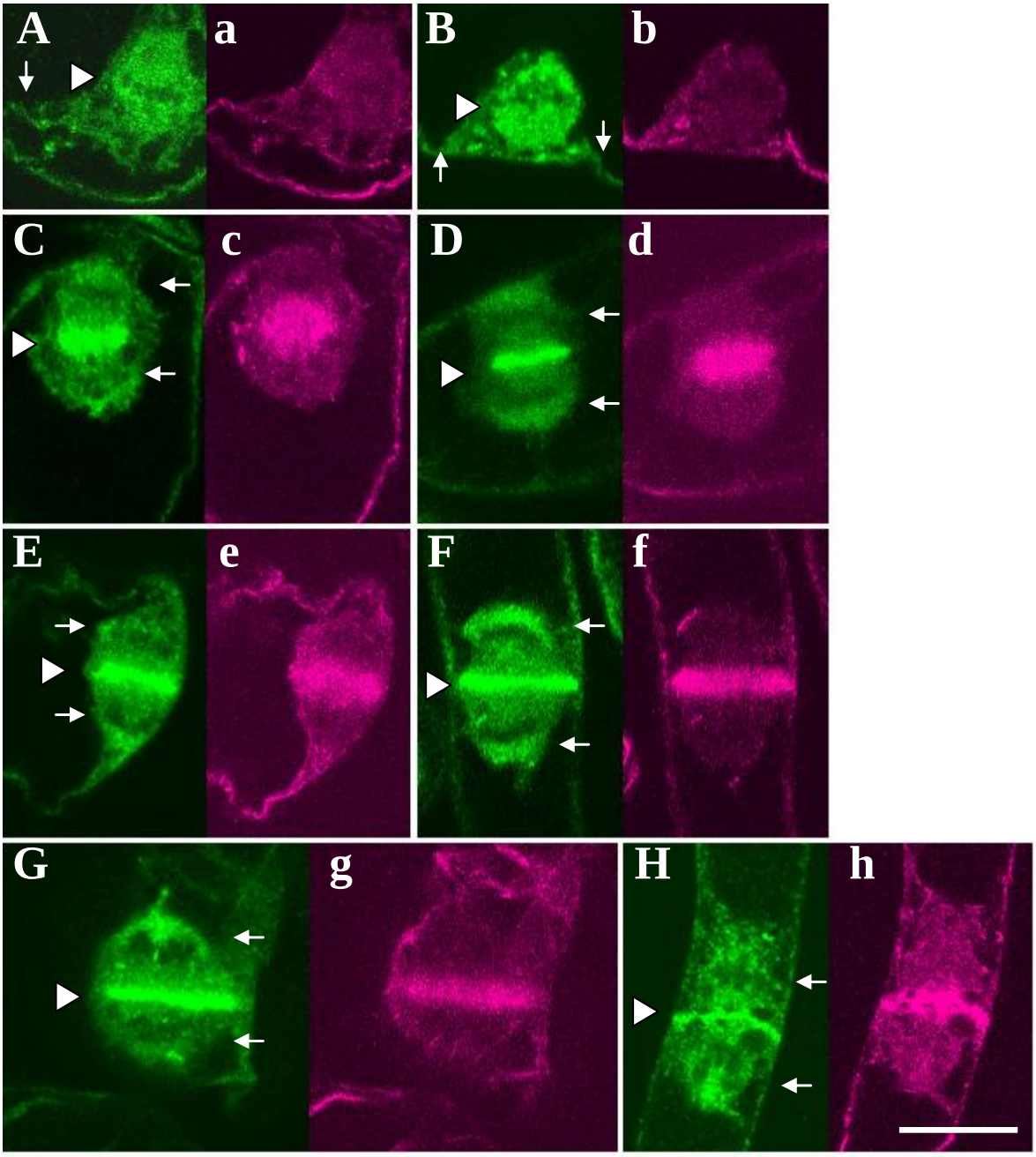
Localization of 175-kDa myosin and microtubule in mitotic BY-2 cells.



Double immunofluorescence staining was carried out using antibodies against 175-kDa myosin heavy chain (capital letters) and tubulin (small letters). A and B, Metaphase. C and D, Anaphase and early telophase. E and F, Mid telophase. G and H, Mature phragmoplasts. Triangles indicate the accumulation of 175-kDa myosin in the equatorial planes of phragmoplasts. Arrows indicate the position of daughter nuclei. I and J, Late telophase. Triangle and arrows indicate the diffusion of 175-kDa myosin from the equatorial plane and the accumulation of this myosin around daughter nuclei, respectively. Bar represents 20 μm.

Supplementary Figure S3.

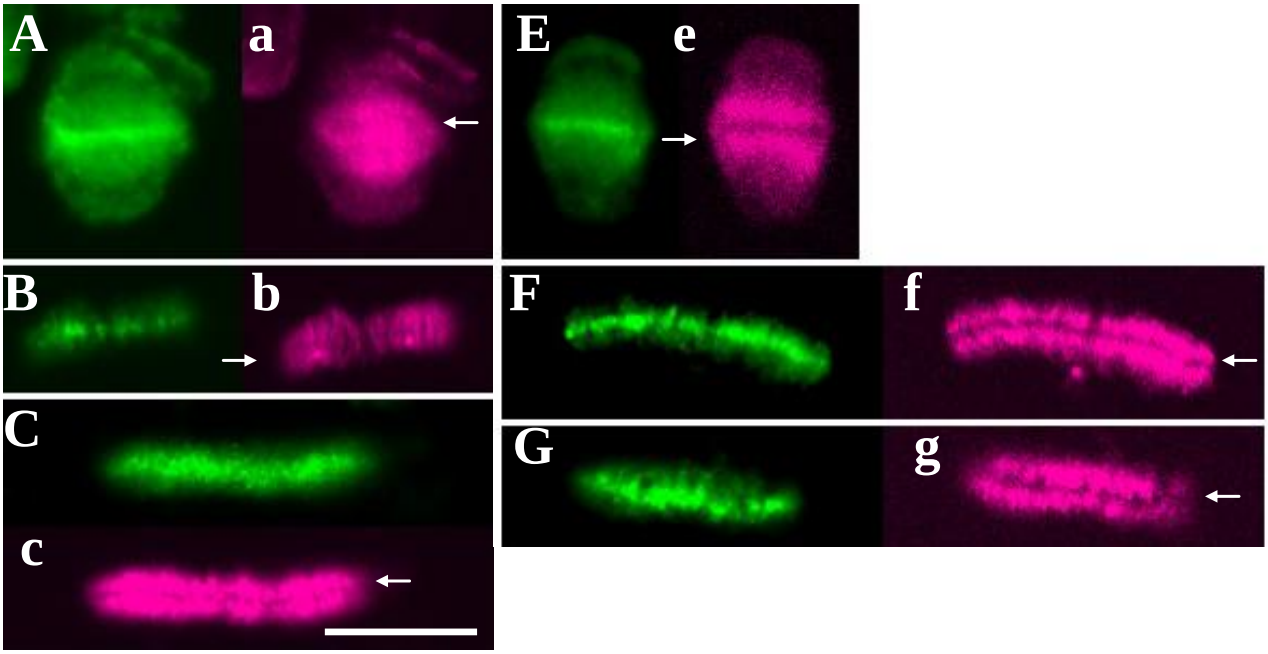
Localization of 175-kDa myosin and actin filament in mitotic BY-2 cells.



Double fluorescence staining was carried out using the antibody against 175-kDa myosin (capital letters) and RP (small letters). A and B, Metaphase. Triangles and arrows indicate the position of aligned chromosomes in the mid planes and cytoplasmic stands, respectively. C, D and E, Early telophase. F, G and H, Mature phragmoplasts. Triangles and arrows indicate the 175-kDa myosin accumulating in the equatorial planes and surrounding daughter nuclei, respectively. Bar represents 20 μm.

Supplementary Figure S4.

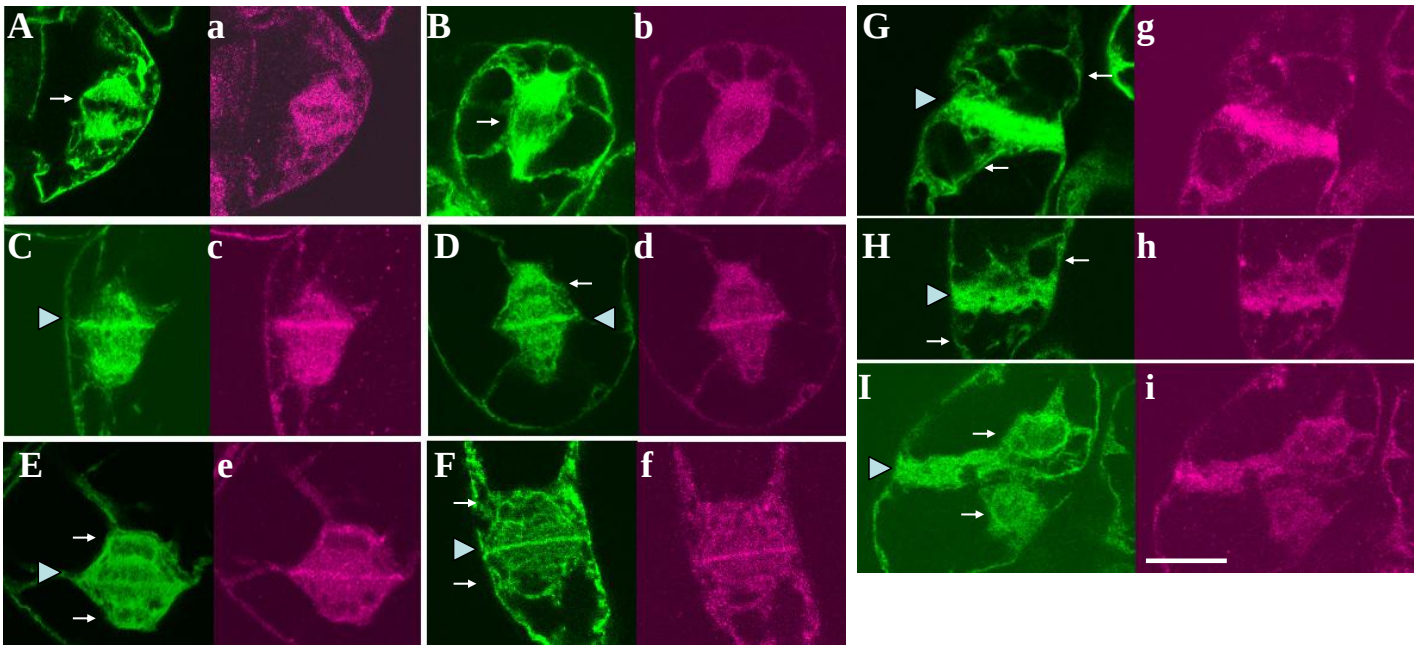
Localization of 175-kDa myosin in isolated phragmoplasts.



Double fluorescence staining of isolated phragmoplasts was carried out with the antibody against the 175-kDa myosin (capital letters) and RP (a, b and c) or the anti-tubulin antibody (e, f and g). Arrows indicate the boundary between the opposite actin filament bundles (a, b and c) or microtubule bundles (e, f and g). Bar represents 20 μm.

Supplementary Figure S5

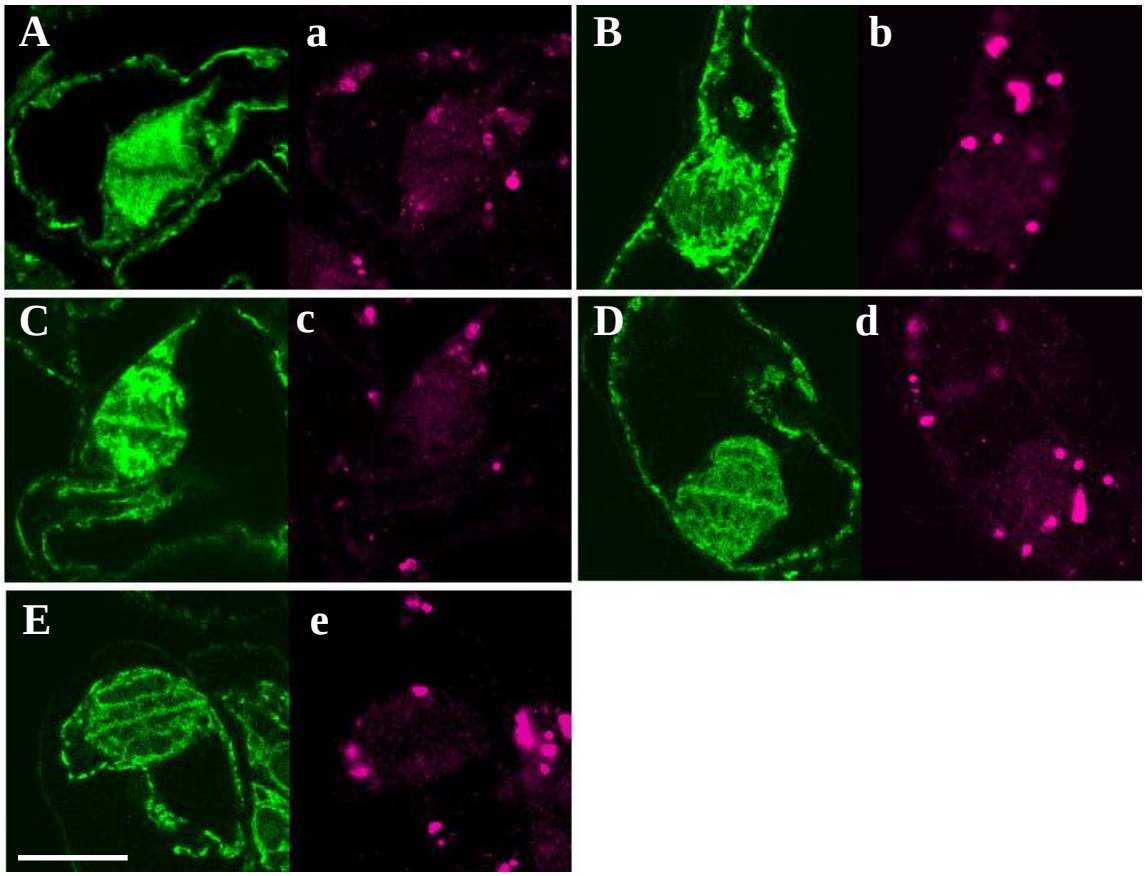
Positional relation of GFP-ER with 175-kDa myosin in mitotic BY-2 cells.



Immunofluorescence staining with the anti-175-kDa-myosin antibody was carried out for cells expressing GFP-ER. Capital letters, GFP-ER. Small letters, 175-kDa myosin. A and B, Metaphase. Arrows indicate aligned chromosomes in spindles. C, D and E, Early and mid telophase. F, Mature phragmoplast. Triangles and arrows indicate the GFP-ER accumulating the equatorial planes in phragmoplasts and daughter nuclei, respectively. G, H and I, Late telophase. Triangles and arrows indicate the diffusion of GFP-ER from phragmoplasts and the accumulation around daughter nuclei, respectively. Bar represents 20 μm.

Supplementary Figure S6.

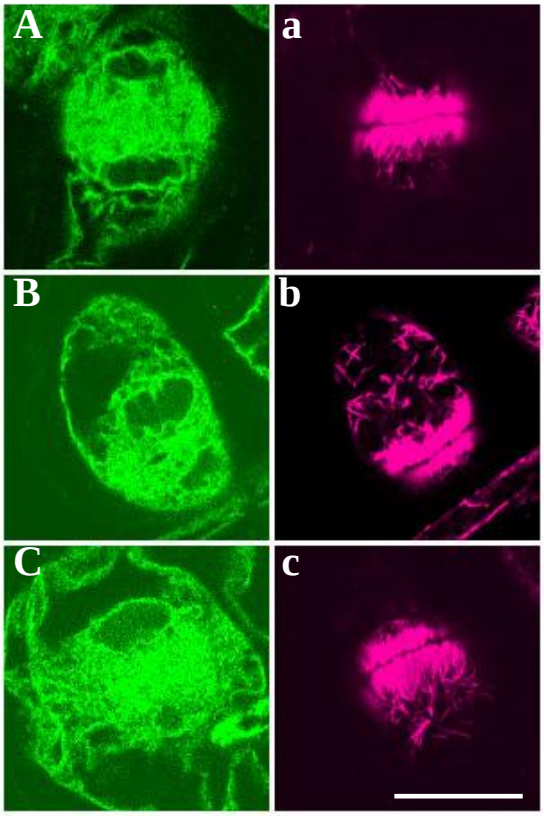
Localization of 170-kDa myosin in mitotic BY-2 cells.



BY-cells expressing GFP-ER were immunostained with an antibody against 170-kDa myosin heavy chain. Capital letters, GFP-ER. Small letters, Immunostaining with the anti-170-kDa myosin antibody. A and B, Metaphase. C and D, Mid telophase. E, Mature phragmoplast. Bar represents 20 μm.

Supplementary Figure S7.

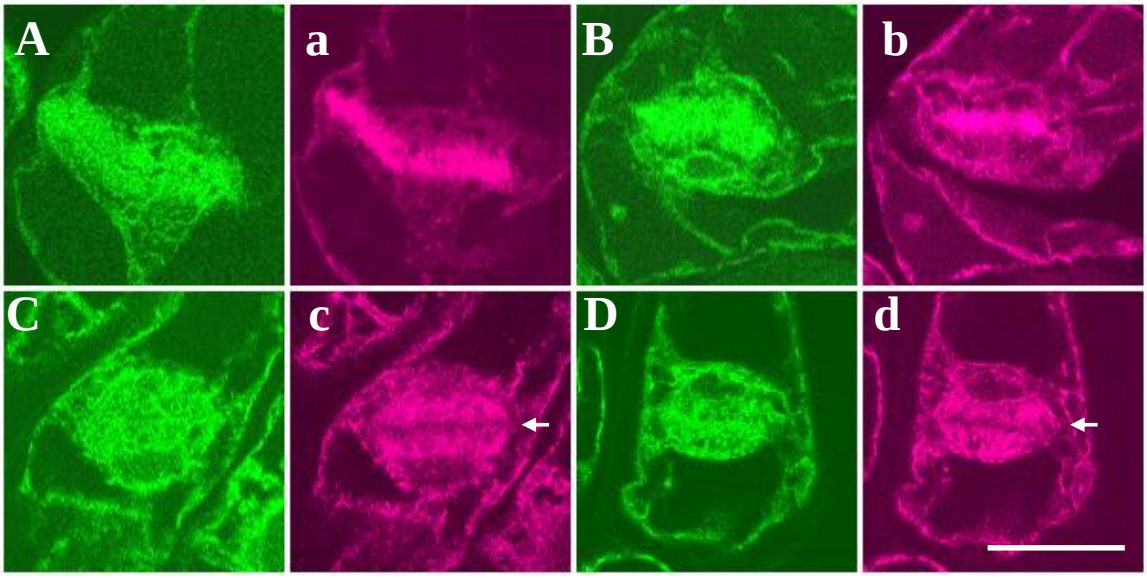
Organization of microtubule in phragmoplasts in BDM-treated BY-2 cells.



BY-2 cells expressing GFP-ER were treated with BDM, and then were immunostained with the anti-tubulin antibody. Capital letters, GFP-ER. Small letters, microtubules. The accumulation of GFP-ER in the equatorial planes of phragmoplasts was suppressed, although the organization of microtubule in phragmoplasts was similar to that of control cells. Bar represents 20 μm.

Supplementary Figure S8.

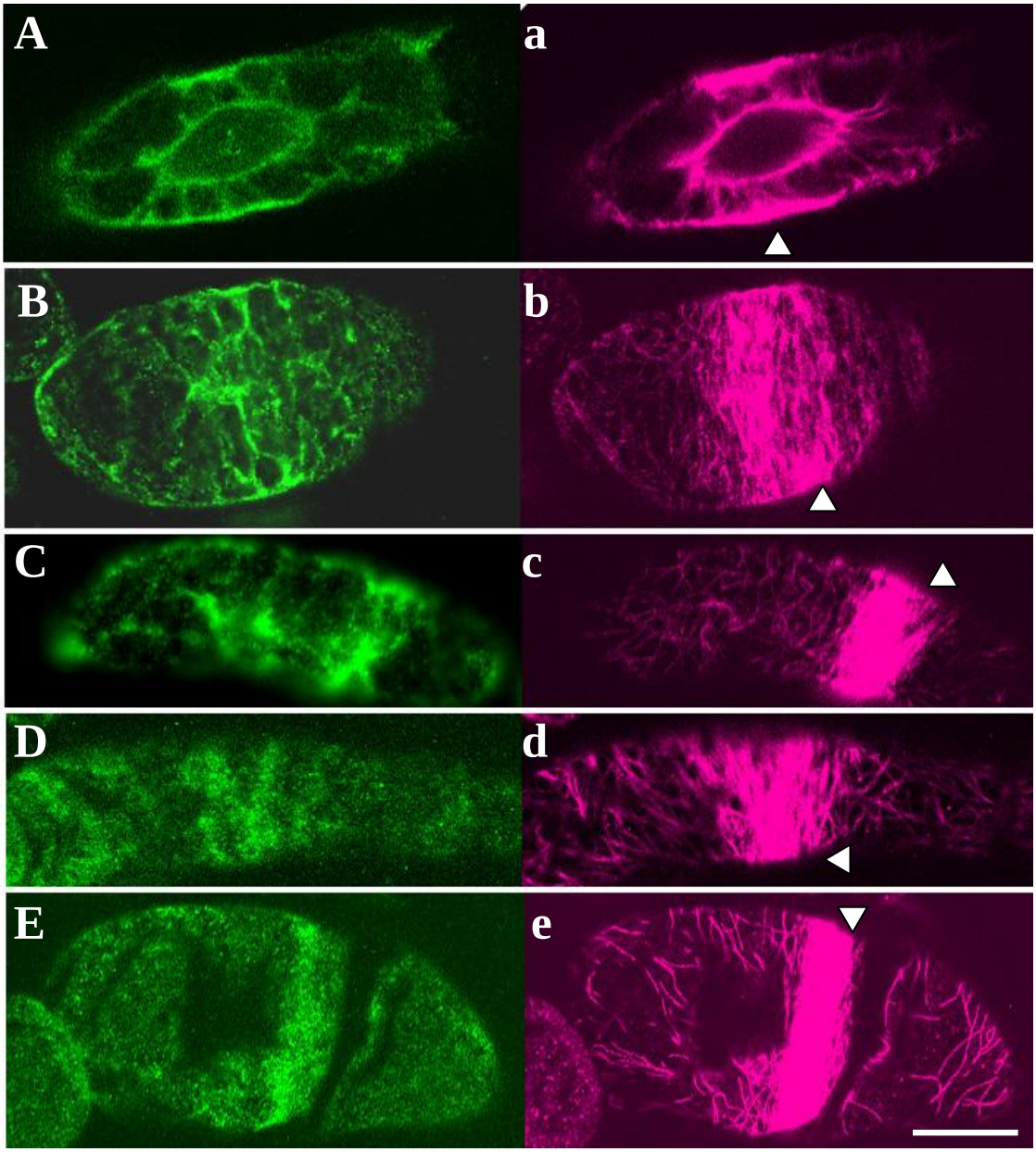
Organization of actin filament in phragmoplasts in BDM-treated BY-2 cells.



BY-2 cells expressing GFP-ER were treated with BDM, and then were stained RP. Capital letters, GFP-ER. Small letters, actin filaments. Two types of actin filament organization in the phragmoplast were observed in similar frequency to each other. One type (a and b) was similar to that observed in untreated cells with BDM. In second type (c and d), the boundary region of opposite actin filament bundles became clear (arrows), maybe because the expansion of this region. However, the accumulation of GFP-ER in the equatorial planes of phragmoplasts was suppressed in both types. Bar represents 20 μm.

Supplementary Figure S9.

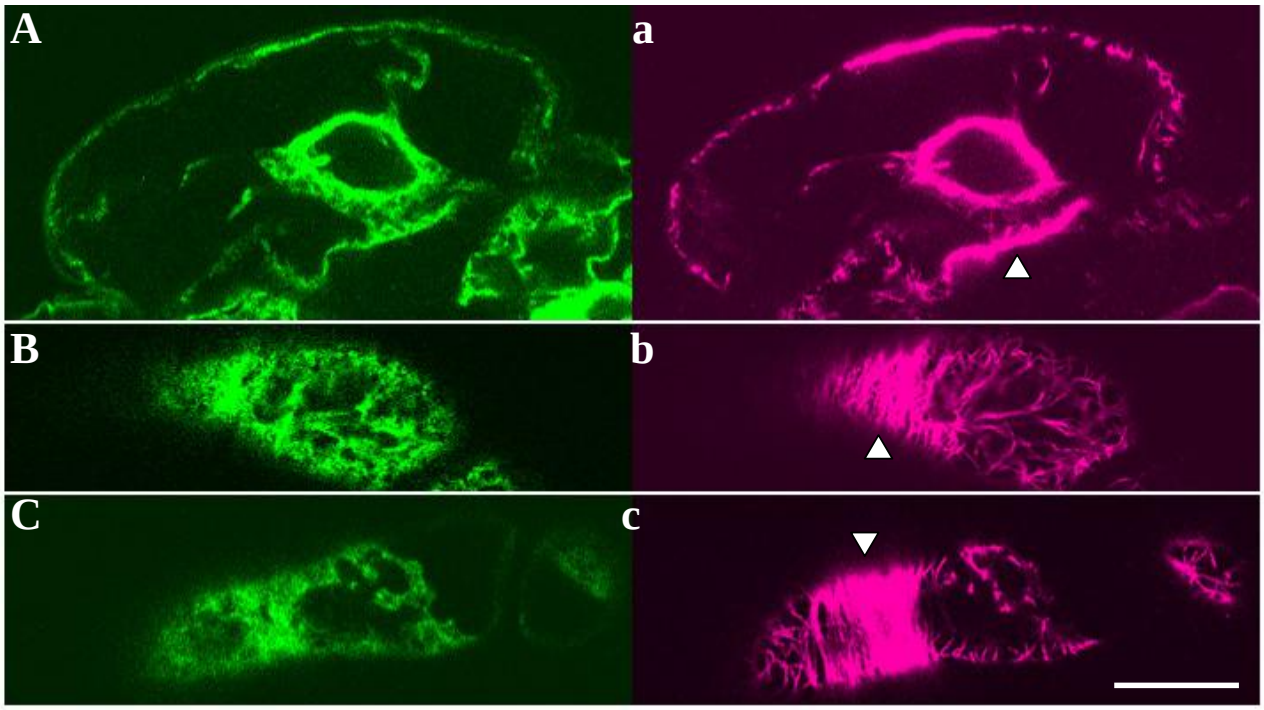
Localization of 175-kDa myosin in PPB region.



Double immunofluorescence staining was carried out using antibodies against 175-kDa myosin (capital letters) and tubulin (small letters). Triangles indicate microtubule bundles in PPB regions. Bar represents 20 μm.

Supplementary Figure S10.

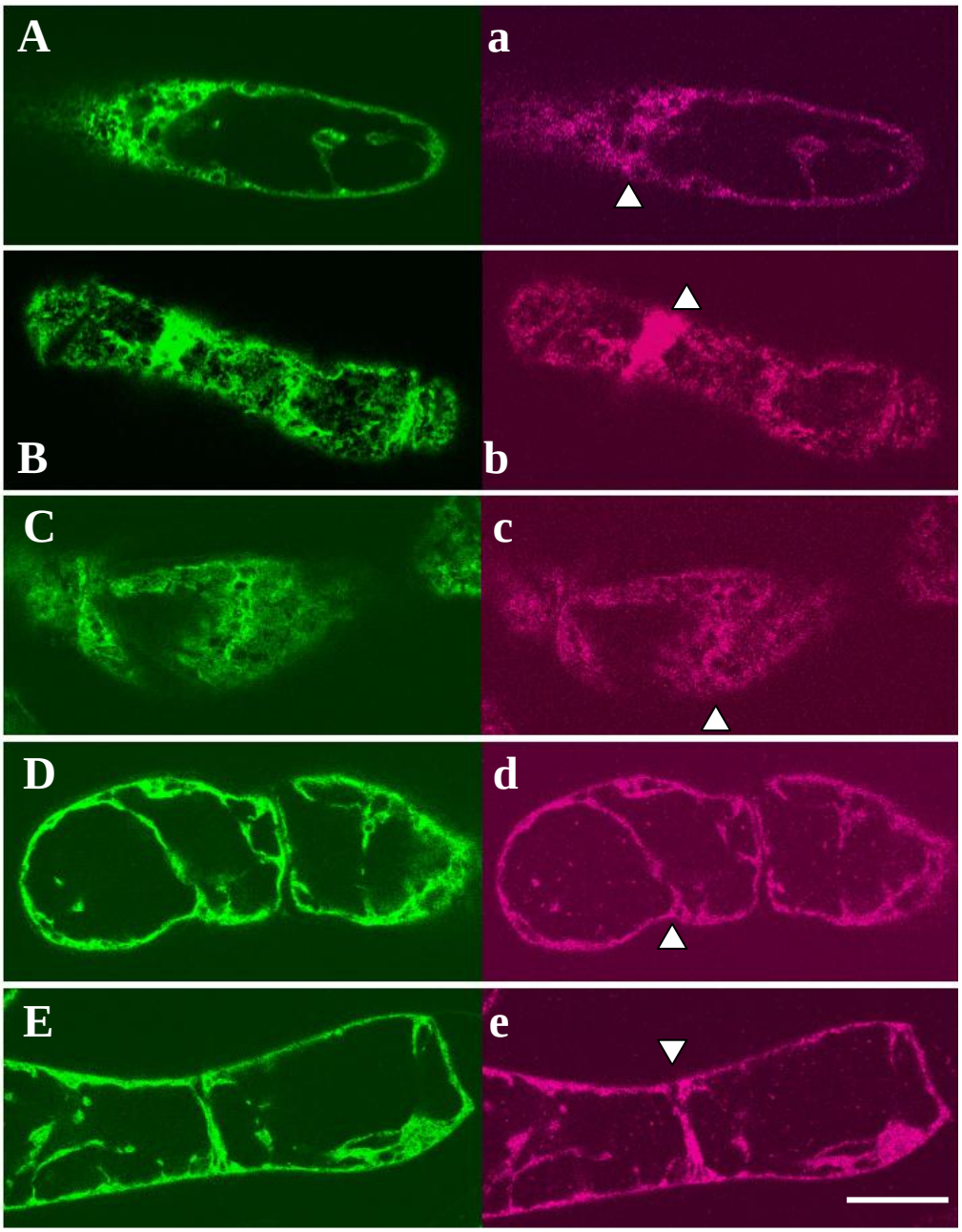
Localization of GFP-ER in PPB region.



Immunofluorescence staining with the anti-tubulin antibody was carried out for cells expressing GFP-ER. Capital letters, GFP-ER. Small letters, microtubules. Triangles indicate microtubule bundles in PPB regions. Bar represents 20 μm.

Supplementary Figure S11.

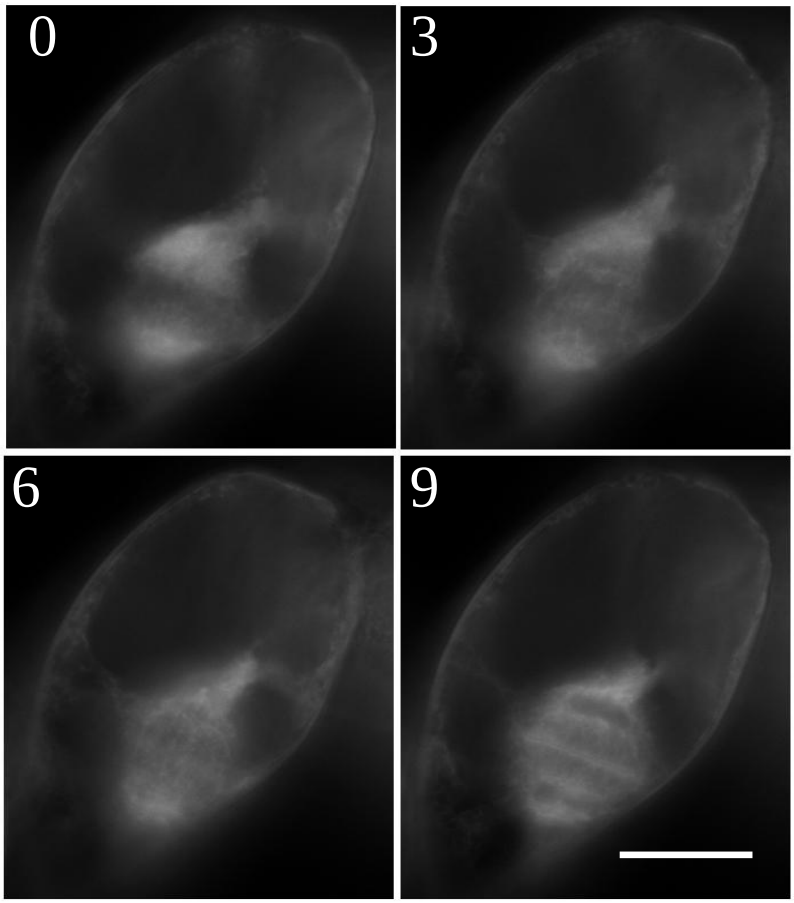
Positional relation of GFP-ER with the 175-kDa myosin in PPB region.



Immunofluoresence staining with the antibody against 175-kDa myosin was carried out for cells expressing GFP-ER. Capital letters, GFP-ER. Small letters, 175-kDa myosin. Triangles indicate PPB regions. Bar represents 20 μm.

Supplementary Figure S12.

Time course of GFP-ER accumulation into the equatorial plane of phragmoplast in a living BY-2 cell.



Numbers on each panel indicate the time (in minutes) from metaphase. 0, spindle. 3, anaphase. 6, early telophase. 9, mid telophase. Bar represents 20 μm.