Golgi 58K-like protein in pollens and pollen tubes of *Lilium davidii*

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Abstract In animal cells, Golgi apparatus is located near the microtubule organizing center (MTOC) and its position is determined partly by 58K protein. By sodium dodecyl-sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immuno-blotting methods, a 58K-like protein has been found in pollen grains and pollen tubes of *Lilium davidii*. Its molecular weight is very similar to that of the 58K protein of animal cells. By immunofluorescence labeling, under a confocal laser scanning microscope (CLSM), the animal 58K antibody revealed a punctate staining in pollen grains and pollen tubes, which is consistent with the distribution of Golgi apparatus in plant cells. In addition, immuno-gold labeling and transmission electron microscopy showed that the 58K-like protein bound mainly to the membrane of vesicles-like structure near Golgi apparatus. This is the first demonstration of the 58K-like protein in plant cells.

Keywords: Golgi apparatus, **Golgi 58K protein**, p**lant**, p**ollen.**

Golgi apparatus is an important organelle in eukaryatic cells. To modify and sort protein was one of its functions. Some special proteins have been found in Golgi apparatus of animal cells[1]. The 58K protein is one of cytoplasmically oriented, peripheral membrane proteins of the Golgi apparatus in animal cells[2]. Immunofluorescence microscopy demonstrated that 58K protein was localized on the Golgi apparatus[2—5]. Staining of the Golgi apparatus by anti-58K antibodies has since been observed in numerous cultured cell types[6, 7]. The protein was originally identified in a tissue extract based upon its microtubules binding activity. SDS-PAGE of purified 58K protein indicated that the protein is composed of a single type of ~58 ku subunit. Purified 58K protein also binds to microtubules. In addition, the Golgi membranes isolated from rat liver were found to contain stably bound 58K protein, and subfractionation of the Golgi membranes indicated that the 58K protein is a peripheral membrane protein facing the cytoplasm[2]. According to these properties, the function of 58K protein may be to anchor the Golgi apparatus to microtubules[3, 5].

Despite many differences, the structure and function of Golgi apparatus are similar between animal and plant cells[8, 9]. Some special protein homologues of Golgi apparatus in animal and yeast cells have been described from tomato and *Arabidopsis*[10]. The position of Golgi apparatus may also be related to the cytoskeleton in plant cells[11]. However, 58K protein has not been reported in plant cells.

1 Materials and methods

1.1 Materials

Pollens of *Lilium davidii* Duch. were collected from Lanzhou of Gansu Province, dehydrated for 24 h at room temperature, and then stored at −20℃.

1.2 Germination of pollen

The pollen was first equilibrated at −4℃ and room temperature in a moist chamber, and then germinated and grown at 24—26℃ on a rotator in an aqueous medium containing 0.03% $Ca(NO_3)_2$ 0.01% KNO_3 0.02% $MgSO_4$ 0.01% HBO_3 and 15% sucrose.

1.3 SDS-PAGE and immunoblotting

Acetone power of pollen was made according to Liu et al.[12]. SDS-PAGE was performed according to Laemmli[13]. Proteins were separated on 7.5% gels and stained with Coomassie Blue R250. Molecular-weight standards were purchased from Bio-Rad.

For immunoblotting, unstained duplicate gels were electrophoretically transferred to nitrocellulose sheets as described by Towbin et al.[14]. Then membranes were blocked for 2 h with 3% BTBS (2% BSA in Tris-buffered-saline (TBS: 20 mmol/L Tris-HCl, 500 mmol/L NaCl, pH 7.5)). The membrane was incubated with a monoclonal antibody of 58K protein (Sigma, dilution 1:500 in BTBS) for 1 h at 37℃, and then three 15-min washes with the BTBS solution were done. The second antibody (goat anti-mouse IgG, alkaline phosphatase-conjugated, Sigma) was then added at a dilution of 1:10 000 in BTBS and incubated for 1 h at 37℃. Strips were washed three times (15 min) with TBS. The chromogenic substrates for alkaline phosphatase were nitroblue tetrazoliun choride and 5-bromo-4-chloro-3-indolyl-phosphate. The reaction was stopped by rinsing the membranes several times with distilled water. In the controls the first antibody was omitted, and in that case, no staining was detected.

1.4 Immunofluorescence labeling and confocal observation

Pollen and pollen tube were processed for immunofluorescence labeling following the protocol reported by Baskin et al.[15]. Material was fixed for 1 h at room temperature in 4% paraformaldehyde in 50 mmol/L 1, 4-piperazinediethanesulfonic acid (Pipes) buffer, and then rinsed in three 10-min changes of 50 mmol/L Pipes. The dehydration and embedding procedure was modified as follows. Samples were passed through a graded ethanol series, and then through an ethanol-methacrylate series, 2:1, 1:1, 1:2 (*v*/*v*) ethanol: methacrylate, and then through three changes of 100% methacrylate, and left in the final change for at least 24 h. Samples were kept at 4℃ for the first two ethanol steps and then at −20℃ until embedding. The methacrylate mixture was 4:1 (*v*/*v*) buty-methacrylate to methylmethacrylate (Sigma), to which was added 0.5% (*w*/*v*) benzoin ethyl ether (Fluka).

Embedding was done at approximately 0℃, in which two long-wave ultraviolet (UV) lights

were placed 5 cm above sample height. The polymerization usually took 12—16 h.

Sections were cut at 1—2 μm and placed on small drops of water. To affix the sections to the slides, the slides were coated with high-molecular weight poly-L-lysine (0.1 mg/mL). The embedding medium was removed from sections by a 10-min incubation in acetone, followed by rehydration in phosphate-buffered saline (PBS, 137 mmol/L NaCl, 2.7 mmol/L KCl, 7 mmol/L Na_2HPO_4, 1.5 mmol/L KH_2PO_4, pH 7.3). Sections were then incubated for 1 h in a blocking solution (1% BSA), followed by treatment with primary antibody for 1 h at 37℃ and, after rinsing in PBS, treated with secondary antibody for 1 h at 37℃. The sections were then rinsed in PBS. The primary antibody was a monoclonal antibody to 58K protein (Sigma, diluted 1:60 in PBS). The secondary antibody was a goat anti-mouse FITC-conjugated antibody (Sigma, diluted 1:20 in PBS). In control the primary antibody was replaced by 3% BSA. In that case, no staining was detected.

The samples were observed with a Leica TCS-NT confocal laser scanning microscope (Leica Microscope and Scientific Instruments Group). Micrographs were made using Kodak Tmax 100 black-white film.

1.5 Immuno-gold labeling and electron microscopy observation

Pollen and pollen tube were processed for immuno-gold labeling following the protocol reported by Li et al.[16]. In brief, materials were fixed in 2% paraformaldehyde and 1% glutaraldehyde in 50 mmol/L potassium-phosphate buffer, pH 6.8, for 1 h at room temperature. Specimens were washed in phosphate buffer and post-fixed for 1 h in 1 % osmium tetroxide. Pollen and pollen tube were dehydrated in an ethanol series (10%, 30%, 50%, 70%, 95% and 100%) and embedded in L R White acrylic resin (London Resin Company). Polymerization of L R White was effected by heat-curing the resin at 45℃ for 24 h. Thin sections were collected on formvar-coated single pole gold grid.

Prior to labeling, sections on grid were treated with 5 % aqueous sodium *meta*-periodate for 20 min in order to overcome the masking of antigenic sites by osmium. Following the periodate pretreatment, sections on grids were placed in 3 % (*v/v*) BSA in PBS buffer, followed by primary antibody for 1 h, and, after rinsing in PBS, secondary antibody for 1 h. The sections were then rinsed in PBS. The primary antibody was a monoclonal antibody to 58K protein (Sigma, diluted 1:60 in PBS containing 0.3% fish gelatin). The secondary antibody was a goat anti-mouse 10 nm colloid gold-conjugated IgG (Sigma, diluted 1:20 in PBS containing 0.3% fish gelatin). The primary antibody was replaced by 3% BSA in control. The samples were observed and photographed under a JEM-100S electron microscope (voltage 80 kV).

2 Results

2.1 Immunoblotting identified the 58K-like protein in pollen

Coomassie blue-stained molecular weight markers (fig. 1, lane 1) and the gel loaded with pollen extracts (fig. 1, lane 2) were shown in the SDS-PAGE. Western blots of these protein frac-

tions probed with the anti-58K antibody are shown in fig. 1 (lane 3).

These results demonstrated that a single band was recognized (fig. 1, lane 1, arrow) by the anti-58K antibody in the pollen extracts and that this corresponds to a band of about 60 ku in the Coomassie blue-stained gel (fig. 1, lane 2).

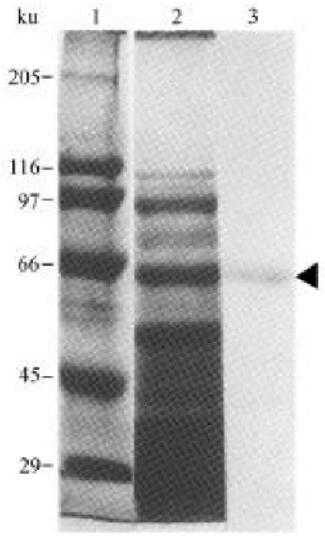

Fig. 1. SDS-PAGE and immunoblotting of pollen acetone powder of *Lilium davidii*. Lane 1, The standard molecular weight marker; lane 2, SDS-PAGE of pollen acetone powder; lane 3, immunoblotting of the 58K-like protein in pollen acetone powder. The 60 ku plant protein was recognized by the animal 58K antibody (arrow).

2.2 Immunofluorescence microscopy of the 58K-like protein in lily pollen and pollen tube

Lily pollens grown in the medium were processed for immunostaining. Discrete particles in the vegetative cytoplasm of lily pollen (fig. 2(a)) and pollen tube (fig. 2(b)) were detected by the 58K antibody, revealing a characteristic punctate staining that suggested the localization of the 58K-like protein on membranous organelles.

2.3 Immuno-gold labeling and electron microscopic observation

In the immuno-gold labeled sections of pollen tube, we could detect several gold particles associated with the membrane of vesicle-like structure near Golgi apparatus (fig. 3(a)). This is the first report about the precise distribution of 58K

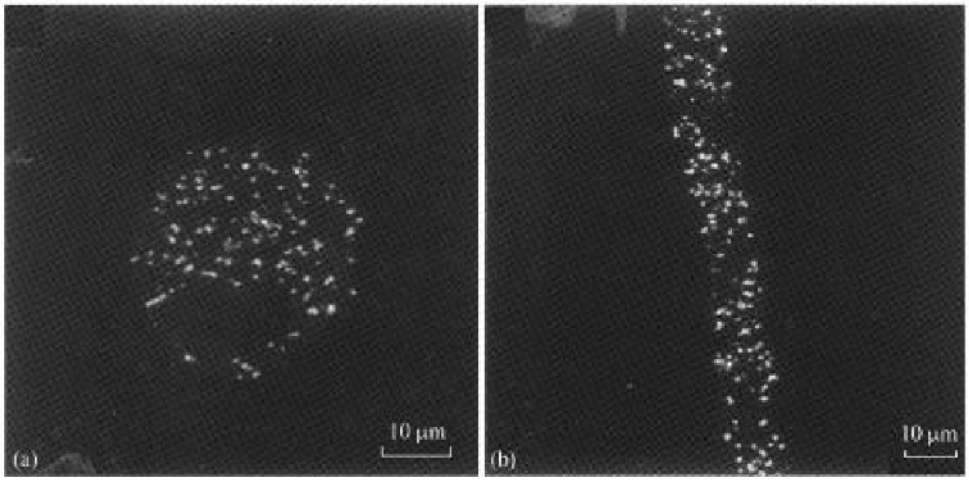

Fig. 2. Immunofluorescence localization of the 58K-like protein in pollen and pollen tube of *Lilium davidii*. Confocal microscopic observation revealed a characteristic punctate staining by the 58K antibody in pollen (a) and pollen tube (b).

protein in cells by immuno-gold labeling and electron microscopic observation. By serious observation, we found that immuno-gold particles were associated mainly with the surface of vesicles

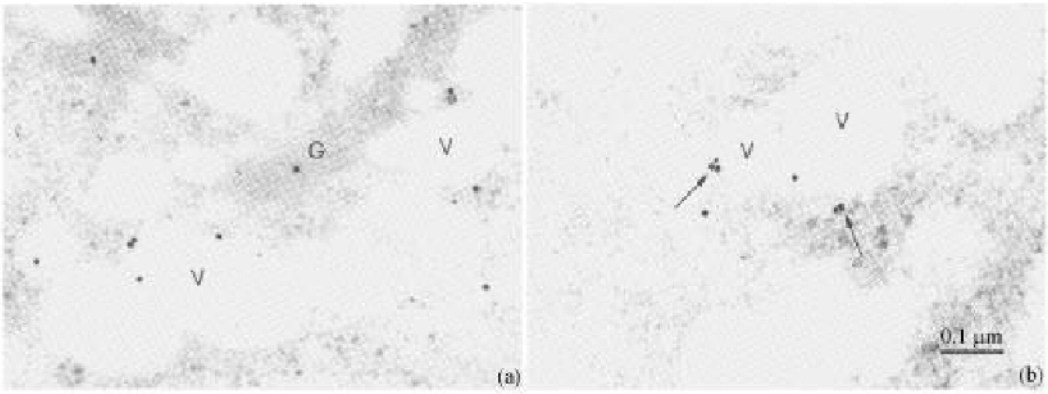

Fig. 3. Immuno-gold labeling and electron microscopic observation of the 58K-like protein in pollen tubes of *Lilium davidii*. The 58K-like protein is mainly associated with the membrane of vesicle-like structure near Golgi apparatus (a). Arrows indicate that 58K-like proteins are mainly present in the surface of these vesicles (b). G, Golgi apparatus; V, vesicles.

(fig. 3(b), arrows). This meant that 58K-like protein might be a cytoplasmically oriented, peripheral membrane protein of vesicle-like structure of Golgi apparatus in pollen tubes of *Lilium davidii*, which was consistent with the results determined by biochemical methods in animal cells. Control sections incubated in secondary antibody and omitting the primary antibody did not show any immuno-gold association with the Golgi apparatus, Golgi-derived vesicles or other cytoplasmic inclusions in pollen tube of lily (data no shown).

3 Discussion

By SDS-PAGE and immuno-blotting, we found that there was a protein band reacting with animal 58K antibody in pollen acetone powder of *Lilium davidii*, which has molecular weight of approximately 60 ku. The molecular weights of Golgi 58K protein in different animal cells were reported to be very similar[2—7]. We found that the 58K-like protein in pollen of *Lilium davidii* was consistent with those in animal cells. The result indicated that 58K protein should have core function in Golgi apparatus in both animal and plant cells.

In animal cells, the 58K protein was determined as a peripheral membrane protein of Golgi apparatus by biochemical methods. But the distribution of 58K protein was not observed directly with electron microscopy and its precise location in Golgi apparatus is not clear yet. By immuno-gold labeling and electron microscopic observation, we found that the 58K-like protein was a membrane protein of vesicles of Golgi apparatus in plant cell. The result not only provided further direct evidence to support the result obtained by biochemical methods about 58K protein in animal cells, but also showed that the 58K-like protein in plant cells might have some similar characteristics with those in animal cells.

There were many special antibodies and fluorescence probes for Golgi apparatus in animal

cells[9]. Hence, in animal cells, the distribution and dynamics of Golgi apparatus may be studied easily with immunofluorescence labeling under a fluorescence microscope or confocal laser scanning microscope. However, there are not so many special antibodies or fluorescence markers for plant Golgi apparatus, which is obviously a disadvantage for research. Horsley et al. obtained an antibody (JIM 84) from plant Golgi apparatus[17], and found that the antibody could label specifically the Golgi apparatus and plasma membrane in plant cells[18, 19]. But, the character of the antigen is still unclear. Because JIM 84 antibody is not a commercial product yet, it is difficult to apply it widely in plant cell research. On the other hand, the antibody of 58K protein was often used as a Golgi apparatus marker in animal cells[6, 7, 20]. But there has been no report on the protein in plant cells. Here, we have demonstrated that the animal 58K antibody could label specifically Golgi apparatus in pollen grains and pollen tubes. If the 58K antibody can become a commercial product, it is very possible that the 58K antibody would be used universally in plant cells as a plant Golgi apparatus marker.

Golgi 58K protein has since been found in numerous animal cells[6, 7]. But, whether Golgi 58K-like protein exists widely in plant cells needs to be further studied. 58K protein in animal cells has several identical properties of microtubule-associated proteins[2—5]. However, whether the 58K-like protein in plant cells has the same character remains to be determined.

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