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Grl1 Protein is a Candidate K Antigen in *Tetrahymena thermophila*

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In *Tetrahymena*, K antigens associate only with mature basal bodies and are expected to play important roles in the morphogenesis and function of the membrane skeleton around basal bodies, but these proteins have not been identified and their functions are unknown. Commercially available anti-human Rho GDP-dissociation inhibitor α (RhoGDIα) antibody (sc-33201) was accidentally found to show very similar immunofluorescence staining patterns to those of anti-K antigen antibodies, such as 424A8 and 10D12 mouse monoclonal antibodies, in *Tetrahymena*. A 40 kDa protein recognized by this antibody was partially purified and identified as granule lattice protein 1 (Grl1p) by matrix-assisted laser desorption/ionization-tandem time-of-flight mass spectrometry. In immunoblotting experiments this antibody was suggested to recognize endogenous Grl1p. The three-dimensional structure of proGrl1p protein predicted by I-TASSER was similar to a spectrin family protein. Grl1 may be a K antigen and a spectrin-like protein in *Tetrahymena*.

Key words: Grl1; K antigens; *Tetrahymena*; I-TASSER; RhoGDI.

Introduction

K antigens, a type of membrane skeleton protein, localize to the detergent-resistant membrane skeleton immediately adjacent to the basal body (Williams et al. 1990) and are used as a marker for mature basal bodies (Pearson et al. 2009; Shang et al. 2005). However, K antigens have not been identified and their functions are still unknown. During the course of a study on RhoGDI in mammalian cells it was accidentally found that in *Tetrahymena* the sc-33201 antibody raised against human RhoGDIα showed an immunofluorescence staining pattern very similar to that using an anti-K antigen antibody such as 424A8 in *T. pyriformis* (Williams et al. 1990) and 10D12 in *T. thermophila* (Pearson et al. 2009; Shang et al. 2005; Vonderfecht et al. 2011; Williams 2004). This study attempted to identify K antigens using the sc-33201 antibody. The results suggested that one of the K antigens was granule lattice protein 1 (Grl1) protein, and the predicted structure of proGrl1p revealed by I-TASSER (Roy et al. 2010) was very similar to a spectrin-like protein. These findings are useful to further understand the role and function of K antigens and Grl proteins.

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Results

Immunofluorescence Staining by the sc-33201 Antibody in *T. thermophila*

It was accidentally found that in *T. thermophila* an anti-human RhoGDI polyclonal antibody (sc-33201) stained rings around the ciliary basal bodies in somatic regions, and the anterior end of the ring was open (Fig. 1A). The diameter of rings was about 1 μm with a non-staining center of about 0.25 μm in diameter (Fig. 1B). The sc-33201 antibody stained the ring around existing basal bodies, but not newly formed basal bodies (Fig. 1C). These rings were localized just above the basal bodies (Fig. 1D). In the mature oral apparatus, the sc-33201 antibody stained along the clusters of ciliary basal bodies and the ribbed structure within the right wall of the oral apparatus (Fig. 1E). In dividing cells, basal bodies were formed at the oral primordium site, and the sc-33201 antibody did not stain around these basal bodies. The staining of rings immediately adjacent to this expanding field of basal bodies was not observed at this stage (Fig. 1F). These staining patterns were very similar to those obtained by the anti-K antigen monoclonal antibodies 424A8 in *T. pyriformis* (Williams et al. 1990) and 10D12 in *T. thermophila* (Pearson et al. 2009; Shang et al. 2005; Vonderfecht et al. 2011; Williams 2004). These staining patterns by the sc-33201 antibody were also similar to those in cells fixed with 35% ethanol/0.5% Triton X-100 which is the fixation method described by Williams et al. (Williams et al. 1990, 1992). These results strongly suggested that the sc-33201 antibody recognized K antigens. The cellular localization of the TtCdk1 protein examined by immunofluorescence staining is also similar to that of K antigens (Zhang et al. 2002). However, the anti-TtCdk1 antibody stained basal bodies of the developing oral primordium that were not stained with the anti-K antigen 424A8 antibody or sc-33201 antibody. Therefore, the sc-33201 antibody did not appear to recognize TtCdk1.

sc-33201 Antibody Does not Recognize TtRhoGDI

In whole cell lysates, the sc-33201 antibody recognized a ~40 kDa band (Fig. 2A) that was equivalent in size to bands (39–44 kDa) recognized by the anti-K antigen antibody 424A8 in *T. pyriformis* (Williams et al. 1990). This band was also abundantly detected in the insoluble fraction of 1% Triton X-100/150 mM NaCl, which was similar to the extraction buffer used in the immunofluorescence staining experiment, but not in the soluble fraction. The sc-33201 antibody was raised against human RhoGDIα. Therefore, to examine whether the sc-33201 antibody recognized TtRhoGDI, Xpress-tagged TtRhoGDI was immunoblotted by the sc-33201 antibody. The sc-33201 antibody specifically recognized human RhoGDIα, but not TtRhoGDI or human RhoGDIβ (Fig. 2B, middle panel). To further examine whether the 40 kDa protein recognized by sc-33201 was TtRhoGDI, a polyclonal antibody against TtRhoGDI was produced. This anti-TtRhoGDI antibody specifically recognized recombinant TtRhoGDI (Fig. 2B, lower panel) and a 24 kDa band in the *T. thermophila* cell lysate (Fig. 2C, first lane). The staining of this 24 kDa band was completely inhibited by absorption with the antigen peptide for this antibody (Fig. 2C, second and third lanes). These results indicated that the apparent molecular weight of endogenous TtRhoGDI was 24 kDa, and the 40 kDa band detected by the sc-33201 antibody was not TtRhoGDI.

Identification of the Protein Recognized by the sc-33201 Antibody

To identity the protein recognized by the sc-33201 antibody, the 40 kDa protein was partially purified as described in Methods, because this antibody did not work for immunoprecipitation analysis of the solubilized 40 kDa protein despite several attempts. The signal intensity of the 40 kDa band detected by the sc-33201 antibody was increased by about 10-fold in fraction 3 compared with the whole cell lysate (Fig. 3A). This 40 kDa band in the gel was excised, and the protein was analyzed by matrix-assisted laser desorption/ionization-tandem time-of-flight mass spectrometry (MALDI-TOF/TOF MS/MS) and identified as Grl1p of *T. thermophila*, which is the cleaved product of proGrl1p at lysine 188. *T. thermophila* Grl1p was the only protein with statistical significance of a peptide mass fingerprint match of less than 0.05.

Grl1p plays an essential role in the organization of the dense core granule (DCG) matrix (Chilcoat et al. 1996; Turkewitz et al. 1991), which is secreted in response to an extracellular stimulus such as dibucaine (Turkewitz et al. 2000). After stimulation of DCG secretion by dibucaine treatment, the intensity of the 40 kDa band detected by the anti-Grl1p antibody was clearly decreased in the treated cells and increased in the secreted fraction (Fig. 3B, upper panel). The change in signal intensity of the 40 kDa band recognized by the sc-33201 antibody (Fig. 3B, lower panel) was very similar to that by the