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Molecular Investigation on Encystation Process of *Giardia lamblia*

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Giardia lamblia is a pathogenic protozoan infecting humans via contaminated food and water. Besides its significance as a human pathogen, *G. lamblia* is an interesting organism in that it has nuclei, but does not have membraneous organelles such as mitochondria. In addition to this structural feature, the sequence data of 16S rDNA as well as other protein-coding genes of *G. lamblia* suggest that it is the most ancient eukaryote comprising the lowest branch in the evolutionary tree diverged from prokaryotic microorgnisms.

G. lamblia has a life cycle composed of two distinct stages, a trophozoite and a cyst. Cyst is an inactive, but infectious form to human, showing resistance to unfavored environments. Taken into the human host, it goes through a process called excystation, resulted in conversion to trophoziotes by action of acidic pH and digestive enzymes present in the host intestine. Trophozoite is a form able to multiply by binary fissions and to causes mal-adsorption and diarrhea by ubiquitous adherence to the intestinal epithelium. Before released in feces, some of trophozoites converted to cysts via a process called encystation. Little is known about the environmental or cellular signals that trigger these differentiation processes, encystation and excystation, or about molecular mechanisms underlying the differentiation processes of *G. lamblia*.

To develop the experimental tools to study *G. lamblia*, encystation process was reconstituted *in vitro* by inducing an axenic culture of trophozoites of *G. lamblia* into a cyst form with a high concentration of bile and alkaline pH condition. The reconstitution was confirmed by Northern analysis with the increased expression of the *cwp1* gene, which encodes the cyst wall protein 1. Examination with SEM (scanning electron microscopy) and TEM (transmission electron microscopy) clearly showed that the trophozoite was transformed to a cyst form delineated with filamentous wall. During encystation, we observed a disappearance of the flagella, an invagination of the adhesive disc, and an extensive formation of rER (rough endoplasmic reticulum) especially at 24 hours after the induction. Maintenance of viability of these *in vitro*-derived cysts was confirmed by vital staining with fluorescent diacetate (FDA) and propidium iodide (PI).

To identify the induced genes during encystation in *G. lamblia*, mRNA pools were searched by differential display reverse transcriptase-polymerase chain reaction. The isolated clones were putatively identified by homology search to be the genes encoding GAP2, CWP1, GLORF-C4, MYB, SEC24, etc (Table 1).

Table 1. list of cDNA clones induced during encystaion of *Giardia lamblia*.

Clone number	Gene or function
17A2	*sec24*
17A3	β -transducin
18A	*glorfC4*
22A, 23A	*cwp1*
24A	chaperon-t-complex
21C2	*hcp*
24C1	*gap2*
18G, 23G, 24G	*myb*
19G	unknown

One of the highest induced genes, *gap2*, encoding glyceraldehyde 3-phosphate dehydrogenase was investigated along with the other *gap* gene, *gap1*. While *gap1* was constitutively expressed, induction of Gap2 protein during encystation was confirmed. In complementation tests using an *Escherichia coli* defective in both *gapA* and *gapB*, only the cell containing the *gap1* gene was able to demonstrate glyceraldehyde 3-phosphate dehydrogenase activity and utilize glucose as a carbon source. Glyceraldehyde 3-phosphate dehydrogenase activities were found to be constitutive in *G. lamblia* extracts at various time points during encystation, suggesting that Gap2 is not a glycolytic enzyme, but its expression is increased during encystation.
A cDNA clone putatively encoding β-transducin homologous protein was also investigated. Since β-transducin is known as a member of cellular signaling pathways in other organisms, we initiated investigations on signaling pathway in *G. lamblia* by identifying the interacting proteins with β-transducin. Polyclonal antibodies specific to the recombinant β-transducin of *G. lamblia* was made and used as a ligand for an immuno-affinity column. A pool of proteins was obtained by passing crude extracts of *Giardia* trophozoite through this affinity column and used as antigens to immunize rats. Employing the resultant sera for immunoscreening of cDNA library of *G. lamblia*, we isolated cDNA clones encoding the immunopurified protein. Interestingly, all of the isolated cDNA clones were turned out to contain the gene for β-giardin, one of *Giardia*-specific proteins comprising cytoskeletal structures. The interaction between β-transducin and β-giardin was confirmed *in vivo* using the yeast two-hybrid system. In immunolocalization of these two proteins within *Giardia* trophozoite, β-transducin was present ubiquitously throughout the whole cell, whereas β-giardin was mainly located in an anterior half part of the trophozoite, possibly in adhesive discs. This result suggests that β-transducin homologous protein may modulate cytoskeletal components via direct interactions in *G. lamblia*.

Three out of the twelve isolated cDNA clones showing an increased transcription during encystation, were

identified to be the *myb* homologous gene encoding a well-known transcriptional factor involved in cellular development and differentiation. Amino acid sequences of Myb protein deduced from the isolated gene revealed that this Myb has two DNA binding domains at its carboxyl-terminus and a variable region at its amino-terminus. Induced expression and nuclear localization of Myb protein during encystations were observed *in vivo* using chimeric Myb-GFP. Recombinant Myb was used for a random site selection experiment in combination with a mobility shift assay to define the Myb binding site. The oligonucleotides selected by Myb binding contained a conserved sequence of GT_nG/CT_n, where n is 4 or 5.

Through investigation on the genes, induced during encystation process, we have gained tools to study transcription and signal transductions occurred in *G. lamblia.*