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Characterization of a Meiosis-Associated Heat Shock Protein 70

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Abstract

LIM18, originally identified as a lily gene specifically expressed in meiosis I of pollen mother cells, encodes a protein which is highly homologous to heat shock protein 70. The anti-LIM18 antibody raised against recombinant LIM18 protein recognized at least three proteins (68, 69 and 70 kDa). However, two dimensional PAGE analysis revealed at least six different proteins recognized by the antibody, suggesting the existence of multiple HSP70-like proteins in lily microsporocytes. Immunofluorescence microscopy of anther tissue revealed that LIM18 immunoreactive materials are localized both in the cytosol and in the nucleus of microsporocytes. A cell fractionation experiment also suggested that LIM18 or its immunologically-related molecules is present in the nucleus. Analysis of transgenic tobacco BY-2 cells expressing LIM18-GFP fusion protein indicated the nuclear translocation of LIM18 molecule. These results suggest that LIM18 is a meiosis-associated HSP70-like protein that may be involved in nuclear and/or chromosome events during meiosis I of microsporogenesis.

Abbreviations

DAPI, 4', 6-diamidino-2-phenylindole; GFP, green fluorescent protein; PAGE, polyacrylamide gel electrophoresis.

1. Introduction

All organisms respond to elevation of environmental temperature by synthesizing several families of proteins, known collectively as heat shock proteins (HSPs). Among these proteins, HSP70s are one of the most conservative proteins and are found in bacteria as well as humans. In eukaryotes, HSP70s are encoded by a multigene family and are classified into subgroups based on the difference of their expression pattern. Some of them are the heat shock cognate 70 (HSC70) proteins that are expressed constitutively and are involved in normal cellular processes such as protein folding, assembly and/or disassembly of macromolecular complex and intracellular targeting. Others are the inducible HSP70s which are produced under certain conditions of stress such as heat, cold and heavy-metal exposure, and are probably involved in the refolding of denatured or aggregated proteins (Mierynk *et al.*, 1992; Hendrick and Hartl, 1993; Fernandes *et al.*, 1994; Frydman *et al.*, 1994; Boston *et al.*, 1996; Hartl, 1996; Mierynk, 1997; Guy and Li, 1998; Schöffl *et al.*, 1998). On the other hand, the expression pattern of certain HSP70 has been shown to be developmentally regulated. For example, meiosis-associated accumulation of HSP70 mRNAs or proteins have been reported in yeast, mouse and rat (Kurtz *et al.*, 1986; Zakeri *et al.*, 1988; Wisniewski *et al.*, 1990; Rosario *et al.*, 1992; Wisniewski *et al.*, 1993). These HSP70s are thought to be involved in critical processes of the progression of meiosis.

Previously, 18 meiosis-associated cDNAs were isolated from microsporocytes of *Lilium longiflorum* and their corresponding genes designated as *LIM* (Lily messages Induced at Meiosis) genes. Among their gene products, LIM18 shows high similarity to other eukaryotic HSP70s, and its mRNA has been shown to be specifically expressed during meiosis I of microsporogenesis (Kobayashi *et al.*, 1994).

In the present study, we characterized HSP70-like molecules expressed during meiotic progression in higher plants. Although it is generally difficult to prepare samples for the study of meiosis in higher organisms, *L. longiflorum* has provided an excellent

system for the study of biochemical and cytological events that are correlated with the different stages of meiosis, because these stages are protracted and synchronized in anthers (Erickson, 1948). Taking advantage of this system, we carried out a series of experiments for the characterization of a meiosis-associated HSP70-like molecule, LIM18. In this study, in order to characterize the HSP70s in meiotic cells at the protein level, we generated anti-LIM18 antibodies and performed immunological analyses. Immunofluorescence microscopic observation and cell fractionation experiments indicated the presence of LIM18-related molecules in the cytoplasm as well as in the nucleus. In addition, using the green fluorescent protein (GFP) from *Aequorea victoria* as a protein tag, intracellular targeting of LIM18 molecule was also investigated. Analysis of transgenic tobacco cells expressing chimeric proteins suggested that the LIM18 is targeted to the nucleus.

2. Materials and Methods

2.1 Plant Materials

Protein samples from floral buds of lily (*Lilium longiflorum* cv. Hinomoto) were prepared as described previously (Mousavi *et al.*, 1999). Proteins for two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) were prepared by the phenol extraction method (Barent and Elthon, 1992).

2.2 Expression of recombinant LIM18 and antibody production

The pBluescript-LIM18 plasmid (Kobayashi *et al.*, 1994) was digested with the *Kpn*I, and the fragment containing a coding region was subcloned into *Kpn*I site of pQE30. Recombinant LIM18 was purified following the protocol for QIAexpress Expression System (Qiagen). Purified recombinant protein was used for immunizing rabbits (Japanese white rabbit) and mice (BALB/c mouse), and for affinity purification of the anti-LIM18 antibody.

2.3 Two-dimensional gel electrophoresis

Two-dimensional PAGE was performed by the method of O'Farrell (1975) using a Mini-PROTEAN II 2-D CELL system (Bio-Rad) according to the manufacturer's instructions. For the first dimension, the isoelectric focusing gel electrophoresis was carried out by combining Bio-Lyte 5/7 and Bio-Lyte 3/10 (Bio-Rad) in a ratio of 4 : 1. Second dimension SDS-PAGE was carried out using 10% acrylamide gels.

2.4 Cell fractionation

Nuclear and cytosol fractions of lily microspores were prepared based on the method described previously (Higashitani *et al.*, 1990). Pachytene microsporocytes from 30 to 50 buds were washed three times with buffer B containing 20 mM Tris-HCl (pH 7.5), 10% glycerol, 0.2 mM ethyleneglycol bis(2-aminoethyl ether) tetraacetic acid, 0.1 mM phenylsulfonylfluoride, 0.5 mM dithiothreitol, 0.3 M sucrose, and 10 mM $MgCl_2$. The cell suspension was homogenized in 4 m*l* buffer B with a glass homogenizer, and centrifuged at 4,000g for 10 min at 4 ℃. The supernatant was used as the cytosol fraction. The pellet was washed two times with buffer B and resuspended into 1 m*l* buffer B. Then the solution was overlaid onto 4 m*l* of 30% percoll in buffer B and centrifuged at 1,500g for 10 min at 4 ℃. The pellet was washed two times with buffer B and resuspended into buffer B without 0.3 M sucrose and 10 mM $MgCl_2$. The resultant cell suspension was used as the nuclear fraction. BY-2 cells were fractionated following the reported protocol with modification (Busk and Pagès, 1997). BY-2 cells were suspended in buffer A, (10mM HEPES pH 7.8, 10mM KCl, 10mM $MgCl_2$, 5mM EDTA, 1mM dithiothreitol, 0.2mM phenylsulfonylfluoride, 25% glycerol), and homogenized using a glass homogenizer. The homogenate was filtered through Miracloth (Calbiochem) by centrifugation and the resultant supernatant was used as the cytosolic fraction. Pellet was washed twice with buffer A and used as the nuclear fraction.

2.5 Western blotting and immunolocalization

Antibody detection of protein samples was done as described previously (Mousavi *et al.*, 1999). Anti-GFP monoclonal antibody (Clontech), anti-LIM18 polyclonal antibody, anti-LIM18 monoclonal antibody (7A3), anti-LIM13 polyclonal antibody, and anti-histone H2B antibody (generously gifted from Dr. Ichiro Tanaka) was used at dilution 1:2, 1:2000, 1:2, 1:500, and 1:2, respectively. The samples were immuno-stained as described previously (Mousavi *et al.*, 1999).

2.6 Reporter gene constructs and transformation of BY-2 cells

For preparation of the *LIM18-GFP* construct, C-terminal coding region of LIM18 cDNA was amplified by PCR using the following primers:

5'-ACCAGATCTACCTCCTCAATCTTCGGTCC-3' ; and

5'-TTGTGCCAGGGAATTGGTG-3'.

The amplified fragment was digested with *Bgl*II and then blunted by a fill-in reaction. The fragment

was digested with *Bst*XI and inserted into the *Xba*I and *Xho*I-blunted site of pGFP2 together with *Xba*I-*Bst*XI fragment of pBluescript-LIM18 plasmid (Kobayashi *et al.*, 1994). The CaMV 35S promoter-LIM18-GFP region was excised by digestion with *Sph*I and *Aat*II and blunted. Finally, the fragment was subcloned into the *Eco*RI-blunted and *Sma*I site of binary vector pBI101 (Clontech). For preparation of the *LIM13*::*GFP* construct, a *Xba*I/*Sac*I fragment of the original LIM13-GFP plasmid (Ogata *et al.*, 1999) was subcloned into the *Xba*I/*Sac*I site of pBI121 (Clontech). The plasmids were transformed into *Agrobacterium tumefaciens* LBA4404 by the electroporation method. BY-2 cells were transformed essentially as described by Matsuoka and Nakamura (1991).

2.7 Microscopy

Intracellular localization of GFP fusion proteins and FITC-stained samples were observed using a Olympus BHT fluorescence microscope and photographed with a PM-10ADS camera (Olympus, Tokyo, Japan) as described previously (Ogata *et al.*, 1999; Mousavi *et al.*, 1999).

3. Results

3.1 Production of recombinant LIM18 protein

To investigate the *LIM18* gene products in meiotic cells, we exploited the immunological approach using antibodies raised against recombinant LIM18 protein. A plasmid DNA for the overexpression of histidine-tagged LIM18 protein was constructed and introduced into *E. coli*. Induction of expression by the addition of 1 mM IPTG to the medium led to the production of a 69-kDa protein. The recombinant protein was affinity purified by Ni^{2+}-NTA column chromatography. In addition to the 69-kDa protein, a number of proteins were co-purified as resolved by SDS-PAGE gel. Because none of these proteins could be purified from IPTG-induced *E. coli* extracts which was transformed with the vector alone, these smaller proteins seemed to be degradation products and/or its translation were stopped in the coding region prior to the stop codon of LIM18. However, it is also possible that these extra bands are bacterial proteins that bind to LIM18 protein (**Fig. 1**). The purified fraction was used as an antigen for anti-LIM18 polyclonal and monoclonal antibodies.

3.2 Isoform composition of HSP70s in meiotic cells

In order to investigate the expression pattern of LIM18 and immunologically related HSP70 homologs, protein samples prepared from different tissues of *Lilium longiflorum* were separated by SDS-PAGE. Immunoblot analysis revealed that the anti-LIM18 antibody recognizes at least three protein bands with a molecular mass of 68-, 69- and 70-kDa, respectively. The 68- and 69-kDa bands appeared to be specific in the young anther, microsporocytes and immature/mature pollen. On the other hand, the 70-kDa protein was also detected in the leaf, stem and root (**Fig. 2**). To identify the immunologically-related proteins, we analyzed the HSP70-like proteins expressed during microsporogenesis by 2-D PAGE analysis. As shown in **Fig. 3**, six protein spots reacting against the anti-LIM18 polyclonal antibody were detected in extracts prepared from the pachytene stage microsporocytes (**Fig. 3**). Identical results were obtained from leptotene, zygotene and tetrad cell extracts

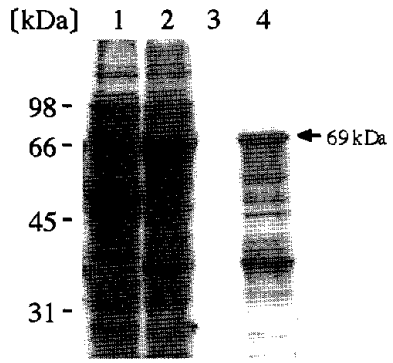

Fig. 1 Expression of LIM18 recombinant protein in *E. coli*. SDS-PAGE analysis of total protein extracts from IPTG treated *E. coli* cells carrying expression vector alone (lane 1), pQE30-LIM18 vector (lane 2), and affinity purified protein using Ni^{2+} resin from IPTG-treated *E. coli* cells carrying expression vector alone (lane 3), pQE30-LIM18 vector (lane 4). Protein molecular mass markers are indicated at the left in kilodaltons. Arrow indicates histidine-tagged LIM18.

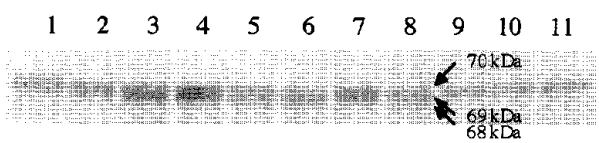

Fig. 2 Expression profile of HSP70s in *L. longiflorum*. Total protein (5 μg) was extracted from various tissues of *L. longiflorum* and separated on SDS-PAGE gel with 7.5 % acrylamide and immunostained using anti-LIM18 polyclonal antibody. Sample analyzed are follows; lane 1, young anther; lane 2, leptotene cell; lane 3, zygotene cell; lane 4, pachytene cell; lane 5, diplotene/diakinesis cell; lane 6, tetrad cell; lane 7, immature pollen; lane 8, mature pollen; lane 9, leaf; lane 10, stem; lane 11, root.

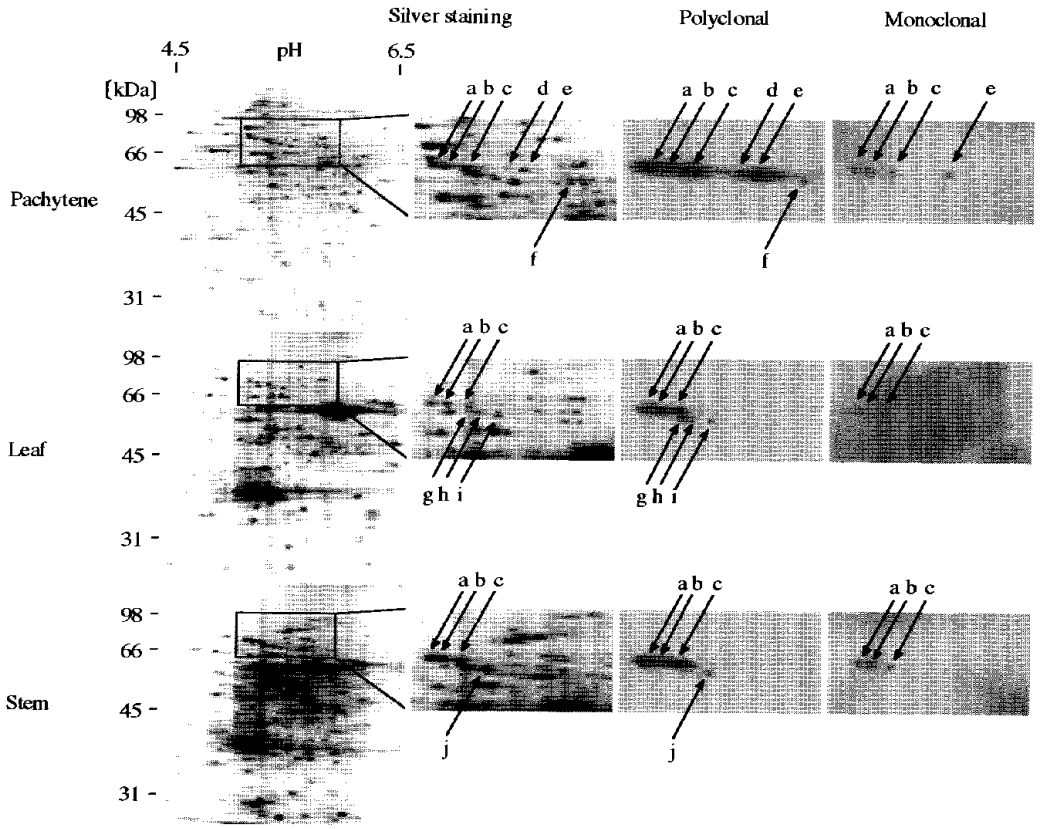

Fig. 3 Two-dimensional analysis of HSP70s in *L. longiflorum*. Total protein (5 μg) from pachytene stage microsporocytes, leaf and stem were separated by isoelectricfocusing gel electrophoresis followed by SDS-PAGE. Proteins were detected by silver staining and by immunostaining using anti-LIM18 polyclonal or anti-LIM18 monoclonal antibody (7A3). The magnified region enclosed with square is shown at right. The same spot is indicated with identical letter. Protein molecular mass markers are indicated at the left in kilodaltons.

(data not shown). Three of the six proteins (**Fig. 3** spots a, b and c) were also detected in the leaf and stem, while another three spots (**Fig. 3** spots d, e, f) were specifically found in microsporocytes. Of these three microsporocytes-specific spots, only one spot was recognized by the anti-LIM18 monoclonal antibody 7A3 (**Fig. 3** spot e). Together with the fact that the expression pattern of the protein coincided with stages at which the LIM18 mRNA has been known to be present (Kobayashi *et al.*, 1994), we identified the spot as the LIM18 gene product. Also, these results suggest the presence of at least three microsporocyte-specific HSP70-like proteins in *L. longiflorum*.

3.3 Immunolocalization of the LIM18 protein

To examine the spatial distribution and intracellular localization of LIM18 protein in lily, we conducted immunocytochemical experiments on sections of resin embedded anthers using an anti-LIM18 polyclonal antibody. As shown in **Fig. 4**, accumulation of LIM18-related protein is evident in metaphase I microsporocytes. Unlike the results

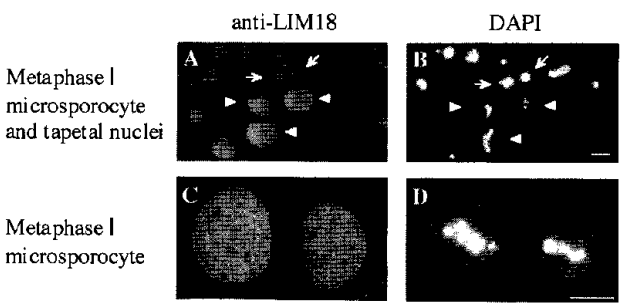

Fig. 4 Immunolocalization of HSP70s in meiotic cells. Resin embedded sections of metaphase I microsporocytes and tapetal nuclei were prepared and immunostained with anti-LIM18 polyclonal antibody (A and C). B and D correspond to the DAPI staining of A and C, respectively. Microsporocytes and tapetal nuclei are indicated with arrowhead and arrow, respectively. Scale bars = 50 μm in B and D.

obtained by western blot analysis, no strong signal was observed in sporophytic cells and tapetum (**Fig. 4, A and B**). The difference between the immunolocalization study and western blot analysis is

probably due to the difference in reactivity of the target protein. Signals from LIM18 immunoreactive materials were detected in the cytoplasm, but co-localization of FITC-and DAPI-derived signals indicated the presence of LIM18 or serologically related proteins within the nucleus and chromosomes (**Fig. 4, C** and **D**).

In order to confirm the results obtained by microscopic observation, we carried out cell fractionation of microsporocytes followed by protein gel blot analysis. The result shown in **Fig. 5** clearly demonstrates that the LIM18 or serologically related proteins are present in the nuclear fraction as well as in the cytosolic fraction. The LIM10 protein that showed high homology with cytoplasmic small heat shock proteins was detected exclusively in the cytosol. On the other hand, the histone H2B signals were detected only from the protein sample prepared from the nuclear fraction. The signals detected by polyclonal anti-LIM18 antibody had estimated molecular weights of 68, 69 and 70 kDa. The results obtained by cell fractionation were consistent with the intracellular localization of anti-LIM18 antibody-immunoreactive materials of microsporocytes.

3.4 Intracellular targeting of the LIM18-GFP fusion protein

To investigate the intracellular localization of LIM18 protein in the living cells, we fused cDNA encoding the full length LIM18 in-frame to the *Aequorea victoria* green fluorescent protein (GFP) gene. In order to obtain transgenic plant cells expressing the GFP fusion proteins, we used tobacco BY-2 cells because of the higher efficiency in Agrobacterium-mediated transformation. A meiosis-associated nuclear protein, LIM13, was used as a nuclear-localized marker protein (Ogata *et al.*, 1999). We generated transgenic tobacco BY-2 cell lines expressing GFP, LIM13-GFP and LIM18-GFP, respectively, under the control of cauliflower mosaic virus 35S promoter. In order to confirm the transgene expression, we carried out immunoblot analysis of protein extracts prepared from BY-2 transgenic cells. Antibodies against GFP, LIM18 and LIM13 proteins were used to detect the expression of transgene products. As shown in **Fig. 6**, the anti-LIM13 antibody specifically recognized a LIM13-GFP protein. With anti-LIM18 antibody, in addition to a 100-kDa band of LIM18-GFP fusion protein, lower molecular weight bands were also detected even from the wild type BY-2 cells. Using the anti-GFP antibody, we could detect GFP and LIM18-GFP expression specifically.

Observation of transformed BY-2 cells by fluorescence microscope revealed the nuclear localization of LIM13-GFP as compared with GFP alone (**Fig. 7**). While the LIM18-GFP protein appeared to be targeted to the nucleus, unlike LIM13-GFP, the fluorescence signal was also detected in the cytoplasm.

To confirm the localization of LIM18-GFP, we carried out cell fractionation experiment using

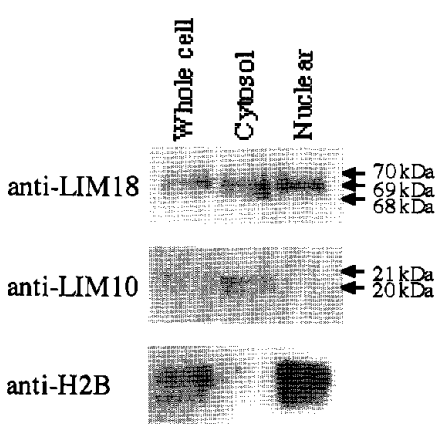

Fig. 5 Cell fractionation experiment of microsporocytes. Nuclear and cytosolic fractions were prepared from zygotene-stage microsporocytes. Immunoblotting analysis was carried out using anti-LIM18 polyclonal, anti-LIM10 monoclonal and anti-histone H2B monoclonal antibodies.

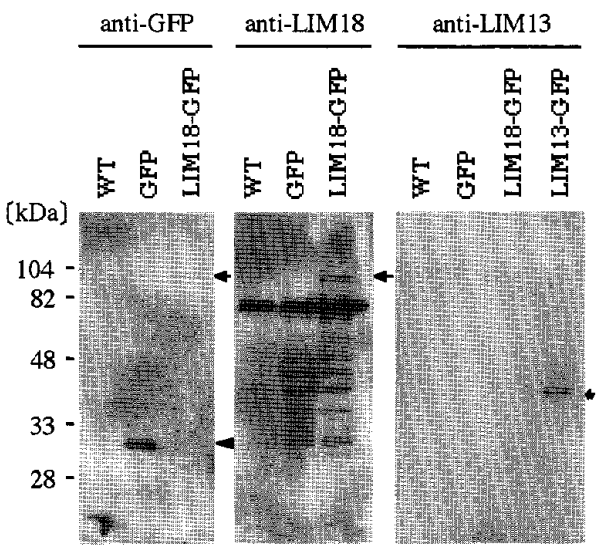

Fig. 6 Detection of GFP fusion proteins in transgenic BY-2 cells. Protein extracts from wild type and transformed BY-2 cells expressing *GFP* alone, *LIM18-GFP* and *LIM13-GFP* were analyzed by immunoblotting using anti-GFP monoclonal, anti-LIM18 polyclonal and anti-LIM13 polyclonal antibodies. Arrowhead, arrows and asterisk indicate GFP, LIM18-GFP and LIM13-GFP, respectively. Protein molecular mass markers are indicated at the left in kilodaltons.

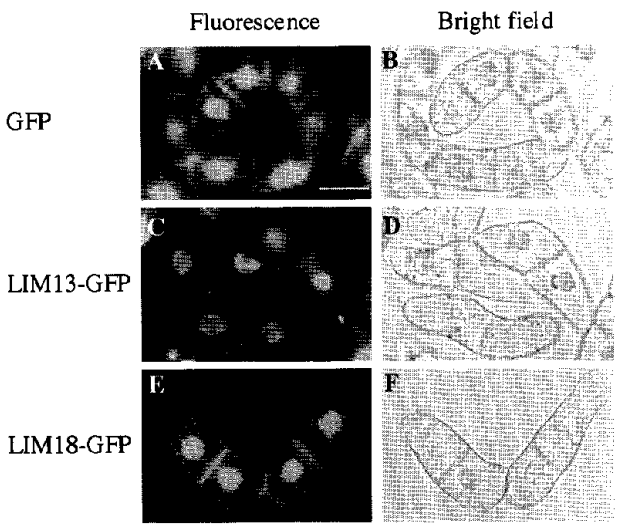

Fig. 7 Localization of GFP fusion proteins in transgenic BY-2 cells. Localization of GFP fusion proteins as visualized by fluorescent microscopy (A, C, E). The same cells observed with bright field are shown in B, D and E. Scale bar in (A) = 20 μm for (A) to (F).

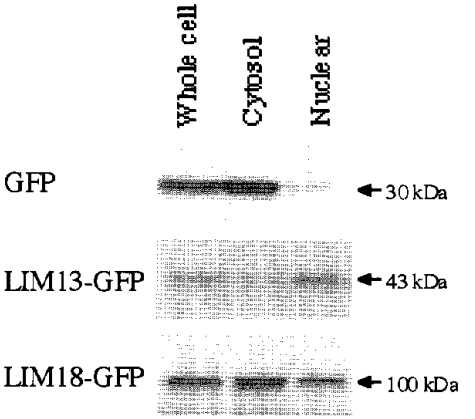

Fig. 8 Cell fractionation experiment of transgenic BY-2 cells. Nuclear and cytosolic fractions were prepared from transgenic BY-2 cells. GFP, LIM13-GFP and LIM18-GFP were detected by immunoblotting analysis using anti-GFP monoclonal, anti-LIM13 polyclonal and anti-LIM18 polyclonal antibodies, respectively. The molecular mass of the bands is shown at the right in kilodaltons

transgenic BY-2 cells. The result obtained by protein gel blot analysis was consistent with the microscopic observation. LIM13-GFP was detected from the nuclear fraction, while GFP alone was detected mainly from the cytosol. On the other hand, LIM18-GFP was detected both in the cytoplasmic and nuclear fraction **(Fig. 8)**.

These results strongly suggested that LIM18 is able to direct GFP into the nucleus. However, unlike conventional NLS-containing proteins, a certain amount of LIM18-GFP fusion protein was also detected in the cytoplasm. Taken together, we concluded that the LIM18 protein is dual-targeted to the nucleus and the cytosol.

4. Discussion

In higher plants, *HSPs* have been suggested to be involved in certain developmental processes. In addition to HSP70s, the expression of *HSP90s* and low molecular weight HSPs has been shown to be induced during embryogenesis, flowering and anther development (Bouchard, 1990; Dietrich *et al.*, 1991; Atkinson *et al.*, 1993; Marrs *et al.*, 1993; Tsukaya *et al.*, 1993; Duck and Folk, 1994; zur Nieden *et al.*, 1995; DeRocher and Vierling, 1995; Gagliardi *et al.*, 1995; Futamura *et al.*, 1999). These studies provided evidence for the gene expression of *HSPs* only at transcriptional levels. In the present study, we examined the protein levels of HSP70s to understand the function of meiosis-associated HSP70-like proteins, which was not obtained in earlier studies.

In order to obtain a specific antibody against LIM18 molecule, we used *E. coli* produced recombinant LIM18 protein as an antigen for the immunization of rabbit. However, we could not obtain a specific polyclonal antibody that could discriminate the LIM18 molecule from other HSP70-related proteins. We also tried to obtain monoclonal antibodies for the specific recognition of the LIM18 protein. As shown in **Fig. 3**, even with the monoclonal antibody, we were unable to distinguish the LIM18 protein from other molecules expressed in somatic tissues. These results suggest the existence of closely related multiple HSP70-like molecules in lily. Using 2D-PAGE analysis, we could detect up to six different spots reacting against the LIM18 antibody in meiocytes and three of them appeared to be microsporocyte specific. However, since phosphorylation of HSP70s has been observed in tomato cell cultures (Nover and Scharf, 1984), it is possible that these multiple protein spots detected in this study are the result of differences in the modification state of the protein. On the other hand, because at least three HSP70s were detected in mouse meiotic cells (Rosario *et al.*, 1992), it is also possible that redundant and/or distinct functions of immunologically-related HSP70s are involved in the maintenance of microsporocytes in lily.

The cells at meiosis and later stage haploid cells have a thermosensitivity much higher than that of somatic cells. In *L. longiflorum*, by heat-treatment onto microsporocytes, we could not observe any significant increase of HSP70s recognized by anti-LIM18 antibody (data not shown). Furthermore, no

consensus heat shock element was found in the 1.7-kb promoter region of *LIM18* (Minami *et al.*, unpublished data). Therefore, it seems likely that the expression of *LIM18* and related proteins are not regulated by the environmental stresses but by developmental factors to act in progression of meiosis and differentiation into gamete under non-stressed conditions. Developmental and organ-specific regulation of *HSP70* gene has also been described in other plants. For example, the expression of *Hsp70s* are induced during pollen development in maize and tomato (Duck and Folk, 1994; Gagliardi *et al.*, 1995), and cytoplasmic HSP70s are differentially expressed in vegetative and embryonic organs in pea (DeRocher and Vierling, 1995). Although more detailed investigations are necessary, comparative studies of these HSP70s with LIM18 will be interesting.

Indirect immunofluorescent microscopy and cell fractionation experiments of lily microsporocytes demonstrated the existence of proteins that are recognized by anti-LIM18 polyclonal antibody in the nuclei as well as in the cytosol (**Fig. 4, 5**). Interestingly, co-localization with chromosomes was also observed (**Fig. 4**). This intracellular localization feature in microsporocytes is quite similar to that of the HSP70-2 of mouse in spermatocytes (Allen *et al.*, 1996). HSP70-2 is a meiosis-specific member of HSP70 family and its structural feature also shows high similarity to LIM18 (74% identity at the amino acid level). HSP70-2 has been shown to be localized along the synaptonemal complex (S. C.) in mouse pachytene spermatocytes (Allen *et al.*, 1996), and the disassembly of the S. C. during diplotene / diakinesis phase is disrupted in *Hsp70-2*$^{-/-}$ mice (Dix *et al.*, 1996, 1997). In addition, Zhu *et al.* (1997) reported that HSP70-2 interacts with cdc2 and its transient interaction is necessary for activation of cdc2 in mouse testis. Consequently, in *Hsp70-2*$^{-/-}$ mice the association between HSP70-2 and cdc2 does not occurr, the progression of meiosis is stopped prior to diplotene / diakinesis, and the formation of normal sperm cells was not observed (Dix *et al.*, 1996, 1997; Eddy, 1999). Because of its high similarity to HSP70-2 at the amino acid sequence level and its expression pattern, it is highly possible that the LIM18 protein is also involved in critical processes of meiotic progression and chromosome behavior.

We examined the intracellular localization of LIM18 by GFP tag strategy. Using a microprojectile bombardment system, we introduced LIM18-GFP into onion skin cells. Fluorescent microscopic observation did not show any particular intracellular distribution of LIM18-*GFP* signal as compared with GFP alone (data not shown). However, using a transgenic BY-2 system we could observe differences in intracellular appearance of GFP-derived fluorescence. In addition, the result of cell fractionation experiments suggested the existence of LIM18-GFP in the nucleus. Although we could observe a relatively strong signal in the nucleus of the BY-2 cells expressing GFP alone, the result of cell fractionation experiment clearly show that the GFP is more abundant in the cytosol. On the other hand, the fluorescent signal derived from LIM18-GFP was more distinct in the nucleus than in the cytosol, which was confirmed by the cell fractionation experiment.

GFP is naturally dual-targeted to the cytosol and the nucleus, perhaps because its small size allows passive diffusion through the nuclear pore. Since the molecular mass of LIM18-GFP (approx. 100-kDa) is much higher than the estimated molecular cut off range of the nuclear pore, we concluded that the nuclear translocation of LIM18-GFP observed in this study is the result of active transport. The absence of nuclear localization signal and the presence of the EEDV signal suggest the intrinsic cytosolic localization of LIM18 (Hartl, 1996; Miernyk, 1997), but complexation with other nuclear targeted molecules may allow LIM18 molecules to be transported into the nucleus. This is probably the reason why LIM18-GFP is detected in the nucleus as well as in the cytosol. It will be interesting to know what kind of molecule interacts with LIM18 protein in BY-2 cells.

Previous studies on plant HSP70 revealed nuclear-localizing HSP70 immuno-reactive material in microspores of *Brassica napus* (Cordewener *et al.*, 1995). To our knowledge, however, LIM18 is the first nuclear localizing meiosis specific HSP70-like molecule in plants. Nuclear translocation of LIM18 is particularly important when considering the mechanisms involved in the meiotic nuclear and / or chromosome organization. Further research for the identification of interacting molecules will be necessary for the elucidation of the function of LIM18. Identification of molecules that interact with LIM18 will provide important information for the understanding of the progression and regulation of meiosis in higher plants.

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