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AtTPR7 as part of the *Arabidopsis* Sec post-translocon

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Abbreviations: ER, endoplasmic reticulum; TPR, tetratricopeptide repeat; HSP, heat shock protein; SRP, signal recognition particle; BiFC, bimolecular fluorescence complementation

The secretory system in eukaryotic organisms ensures targeting of proteins to their place of function after they entered the endoplasmic reticulum either co- or post-translationally. Thereby proteins are translocated through the Sec translocon into the endoplasmic reticulum. In the *Arabidopsis* genome homologs for the three major components of the Sec translocon, the central pore Sec61α and the auxiliary proteins Sec62 and Sec63 are present. Phylogenetic analyses show Sec61α to be the most conserved subunit within the Sec translocon whereas Sec62 and Sec63 show less homology but contain the same functional domains among all organisms. We recently characterized a novel tetratricopeptide repeat domain containing protein, AtTPR7, as part of the *Arabidopsis* Sec translocon which is probably involved in chaperone assisted post-translational import. In this study we investigated the interaction of AtTPR7 with Sec62 as well as the cytosolic chaperones HSP70 and HSP90 not only in vitro but also in vivo to further strengthen the hypothesis of AtTPR7 being a chaperone docking protein of the Sec translocon for secretory preproteins in *Arabidopsis*.

The secretory pathway regulates proper targeting of proteins synthesized on cytosolic ribosomes to their destined compartments through the endoplasmic reticulum (ER) and the golgi apparatus as well as via the plasma membrane to the extracellular matrix. The first step in this pathway is the translocation of the newly synthesized proteins into the ER which can occur either co- or post-translationally.[1,2] During co-translational translocation elongation of the protein is arrested due to binding of the signal recognition particle (SRP) to the freshly synthesized nascent polypeptide chain appearing at the ribosome. Translation continues after binding of the SRP to the SRP receptor at the ER membrane and the polypeptide is translocated through the Sec61 channel into the ER.[3]

In the post-translational pathway proteins are first fully synthesized in the cytosol and therefore released from the ribosomes before being translocated. It was shown that predominantly small secretory proteins are translocated post-translationally into the ER in yeast and mammals. These small proteins are expected to be inefficiently recognized by the SRP at the ribosome since translation is completed only shortly after exposure of the N-terminal signal sequence.[4] This post-translational transport is typically facilitated by molecular chaperones such as the heat shock protein 70 (HSP70) in the cytosol.[5] In yeast post-translational translocation requires a heptameric Sec complex at the ER membrane, consisting of a tetrameric Sec62/63p complex (containing Sec62p, Sec63p, Sec71p and Sec72p) in addition to the heterotrimeric Sec61p complex (containing Sec61p, Sbh1p and Sss1p).[6] The Sec61 protein-conducting channel and Sec63p are involved in both, co- and post-translational translocation into the ER.[7] Sec62 as well as the two nonessential proteins Sec71p and Sec72p are specifically associated with the post-translational translocon in yeast. Sec62p is suggested to form a signal peptide receptor together with the proteins Sec71p and Sec72p.[8,9] Sec72p is a soluble protein which is anchored to the ER membrane via the integral membrane protein Sec71p.[6] Moreover, Sec72p contains a tetratricopeptide repeat (TPR) domain, facilitating interaction with HSP70 associated preproteins.[10] Homologs of the yeast Sec62p and Sec63p proteins are also present in mammals and it was shown that these proteins form complexes with each other as well as with Sec61.[11] However, mammalian Sec62 can additionally bind in close proximity to the ribosomal exit tunnel suggesting that it gained a function in comparison to the yeast homolog.[12] Sec71p as well as Sec72p are exclusively found in yeast.

Post-translational translocation into the ER in plants has not been investigated up to date, although homologs for the major components of the Sec translocon can also be identified in plant genomes. The *Arabidopsis thaliana* counterpart of the major channel subunit Sec61α is present as three isoforms, Sec62 occurs as a single gene in *Arabidopsis thaliana* whereas two isoforms of Sec63

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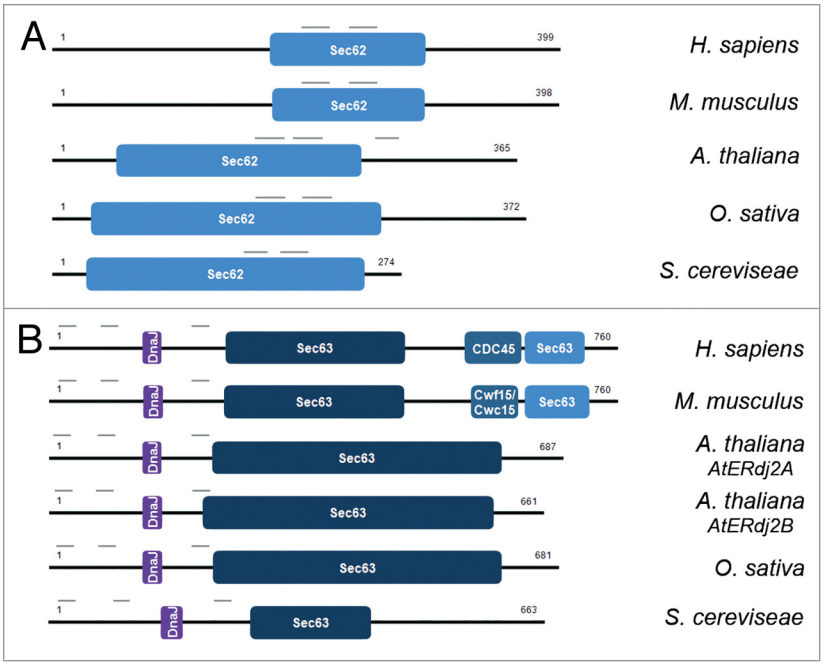

Figure 1. Domain structure of Sec62 and Sec63 in mammals, plants and yeast. (**A**) Comparison of the Sec62 protein of *Homo sapiens*, NP_003253.1; *Mus musculus*, NP_081292.1; *Arabidopsis thaliana*, At3g20920; *Oryza sativa*, Os02 g0435000; *Saccharomyces cereviseae*, NP_015231.2. (**B**) Comparison of the Sec63 protein of *Homo sapiens*, NP_009145.1; *Mus musculus*, NP_694695.3; *Arabidopsis thaliana*, At1g79940, At4g21180; *Oryza sativa*, Os04 g0307200; *Saccharomyces cereviseae*, NP_014897.1. Specific domains are indicated in colors. Predicted transmembrane domains are indicated as gray bars. Homolog genes were identified by "HomoloGene" (NCBI), conserved domains by "Conserved Domain Search" (NCBI) and transmembrane domains were predicted with the help of TMHMM (CBS).

have been identified, AtERdj2A and AtERdj2B.[13] Phylogenetic analyses revealed that the homology of the *Arabidopsis* Sec62 protein to the yeast and mammalian counterparts is comparatively low with a sequence identity of 12% and 15%, respectively. In contrast to yeast and mammals the plant Sec62 homologs show one additional hydrophobic stretch at the C-terminal part reaching from amino acids 254–271 which is predicted as a potential third transmembrane domain in *Arabidopsis* (**Fig. 1A**). In the *Arabidopsis* Sec62 protein the conserved domain of the Sec62 superfamily is reaching from amino acids 50–242 similar to the yeast homolog, whereas in the mammalian homologs this domain reaches only from amino acids 170–293 and is therefore significantly shorter. The two Sec63 isoforms in *Arabidopsis thaliana*, AtERdj2A and AtERdj2B, show 74% identity to each other and only 19% to the human and 22% to the yeast counterparts, respectively (**Fig. 1B**). Both, AtERdj2A and AtERdj2B have three predicted transmembrane domains within the N-terminal region and a J-domain located between the second and the third transmembrane domain, therefore showing the same characteristics as the yeast and mammalian homologs. All Sec63 homologs contain a domain of unknown function conserved in the Sec63 superfamily. The mammalian homologs have two additional domains at the C-terminus lacking in the plant Sec63 proteins, a CDC45-like domain in *Homo sapiens* replaced by a Cwf15/Cwc15 domain in *Mus musculus* and a Sec63 Brl domain which is known to be required for assembly of functional ER translocons[14] conserved in both. Deletion of AtERdj2A in *Arabidopsis* leads to a lethal phenotype and causes defects in pollen function, whereas AtERdj2B mutants grow normally without obvious developmental defect.[13] The β-barrel forming Sec61α subunit is the most highly conserved component of the Sec translocon with all three *Arabidopsis* isoforms bearing 67% identity to the human Sec61α subunit and 52% to the yeast Sec61p. The three *Arabidopsis* Sec61α isoforms share 89% identical amino acids among each other, indicating functional redundancy. Structural features as well as conserved domains such as the Plug- and the SecY-domain were compared between different organisms and showed no significant differences.

In our recent studies we analyzed the composition of the *Arabidopsis* Sec post-translocon and we identified a novel TPR domain containing protein, AtTPR7, which is interacting with AtERdj2A at the ER membrane.[15] Further analyses suggest Sec62 as additional interaction partner of AtTPR7 not only by in vitro pull down experiments but also in vivo performing a bimolecular fluorescence complementation assay (BiFC) (**Fig. 2**). For the pull down experiment recombinant AtTPR7 (amino acids 1–500) carrying a His-tag at the C-terminus replacing the transmembrane domain was purified as described previously.[15] By this deletion unspecific interactions between hydrophobic transmembrane domains of AtTPR7 and Sec62 are avoided. ATPR7 was incubated with radiolabeled Sec62 translated in reticulocyte lysate and recovered by Ni-NTA sepharose. As a control translated Sec62 was incubated with Ni-NTA sepharose without any AtTPR7-His construct and visualized by autoradiography. Sec62 specifically binds to AtTPR7-His and not to the empty beads (**Fig. 2A**). Hereby, we could show that AtTPR7 directly interacts with Sec62 in vitro.

In a second approach AtTPR7 and Sec62 were expressed in tobacco leaves, both linked to complementary parts of fluorescent tags only giving a signal at 515 nm when both fluorescent tags get in close proximity in the cell.[16] An ER mCherry marker protein was co-transformed to determine the location of the interaction.[15] As a negative control Venus^{N}-AtTPR7 was expressed together with the soluble C-terminal part of SCFP alone (empty vector), which should not be able to interact. This in vivo experiment revealed close proximity of AtTPR7 and Sec62 at the ER membrane (**Fig. 2B**), suggesting that AtTPR7 and Sec62 are part of the same protein complex. In combination with the in vitro results a direct interaction of AtTPR7 and Sec62 can be considered. An interaction of Venus^{N}-AtTPR7 with the soluble C-terminus of SCFP at the ER membrane could not be detected. This in vivo approach strengthens the idea that both, not only Sec62 but also AtTPR7, are part of the *Arabidopsis* Sec post-translocon, forming a similar complex to the yeast post-translocon.

Since Sec72 homologs are only present in fungi[17] AtTPR7 might functionally replace yeast Sec72p and its membrane anchor Sec71p in plants. The TPR domain of AtTPR7 seems to replace Sec72p whereas the transmembrane domain of AtTPR7 might compensate for Sec71p. AtTPR7 is a plant specific protein[15] not present in other higher eukaryotes. Among plants, however, AtTPR7 is conserved from the unicellular green algae *Chlamydomonas* to mosses, ferns, mono- and dicotyledonous plants.

TPR domain containing docking proteins are distributed among all cellular compartments associated with the membrane translocons and thereby facilitating post-translational translocation.[18] In yeast the TPR domain containing docking protein Sec72p has been described to bind HSP70 during post-translational translocation of preproteins into the ER.[10] Furthermore, Toc64, a TPR domain containing docking protein at the chloroplast outer envelope, plays a role in chloroplast import by recognizing HSP70/HSP90 bound preproteins.[19] In mammals and yeast Tom70 is the most prominent TPR domain containing receptor in the outer membrane of mitochondria. In yeast Tom70 binds to HSP70, whereas in mammals additional HSP90 binding was demonstrated. Furthermore, the mitochondrial membrane associated protein Tom34, which carries two TPR domains, is shown to interact with HSP70 and HSP90 suggesting a possible function as co-chaperone of HSP70 and HSP90 and thereby playing a role in mitochondrial preprotein delivery.[20,21] Tom70 seems to be functionally replaced by OM64 in plants, a close homolog to Toc64 in chloroplast.[22] We already investigated HSP70 and HSP90 binding to AtTPR7 in vitro in our recent study.[15] To strengthen our hypothesis of HSP70 as well as HSP90 involvement in ER preprotein delivery we performed an in vivo analysis using a BiFC approach (**Fig. 3**). As representative of the HSP70 family in *Arabidopsis* HSP70.1 was used for the interaction studies since it is demonstrated to be an abundant and constitutively expressed isoform.[23] A pull down experiment with all four cytosolic HSP90 isoforms of *Arabidopsis* revealed HSP90.2 to be the isoform with the strongest binding affinity[15] to AtTPR7 in vitro and therefore HSP90.2 was chosen as representative of the HSP90 family in *Arabidopsis* for the BiFC approach. We could show that AtHSP70.1 as well as AtHSP90.2 are interacting with AtTPR7 at the ER membrane in tobacco leaves in vivo since the signal at 515 nm was reconstituted. Expression of the soluble C-terminus of SCFP in the cytosol and Venus^{N}-AtTPR7 at the ER membrane shows no reconstitution of the fluorescent signal showing specificity of the AtTPR7-chaperone interaction. This additional in vivo data supports the idea of AtTPR7 functionally replacing yeast Sec71/72p in *Arabidopsis*. AtTPR7 is strongly assumed to be the docking protein for chaperone bound preproteins during post-translational translocation into the ER in plants. The TPR domain of yeast Sec72p is shown to cluster together with the HSP70 recognizing TPR domains (Hop1 and Hop2b) of the HSP70/HSP90 organizing protein (HOP) and not with the HSP90 binding one (Hop2a) and only HSP70 binding to its TPR domain was investigated so far.[17,24] Therefore, the involvement of HSP90 in addition to HSP70 adds more complexity to the plant translocon machinery in comparison to yeast. Moreover, since neither Sec71p/72p nor AtTPR7 are conserved in mammals, the question arises whether and how chaperone mediated post-translational translocation occurs in these organisms. As a next step it will be of high interest for us to investigate plant proteins as potential chaperone assisted post-translationally translocated preproteins of the ER to gain further insight into the function of AtTPR7 as part of the Sec post-translocon in plants.

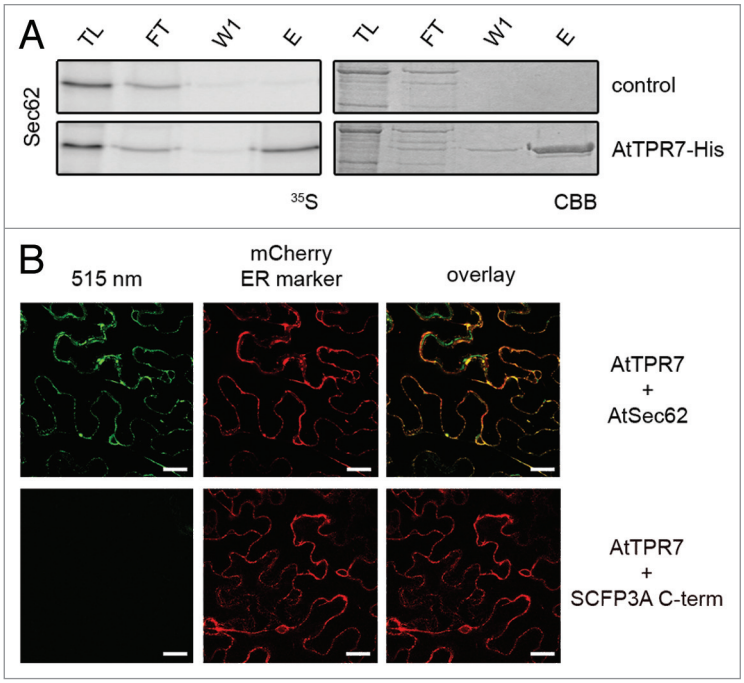

Figure 2. Interaction of AtTPR7 with Sec62. (**A**) Recombinant AtTPR7-His (20 μg) (At5g21990) was incubated for 1 h with 15 μl of radiolabeled Sec62 translation product in 300 μl 1× PBS buffer (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4; pH 7.3). AtTPR7-His was re-purified by incubation with 20 μl Ni-NTA sepharose for 1 h at RT. the Ni-NTA sepharose was washed for three times with 1 ml of 1× PBS buffer containing 50 mM imidazole and samples were subsequently eluted with 20 μl of 1× PBS containing 300 mM imidazole. Five percent of the translation (TL), 2% of the flow through (FT), 2% of the first washing step (W1) and the total eluates (E) were subjected on a 10% SDS-PAGE. Association of Sec62 was detected by autoradiography. A sample without AtTPR7-His constructs was used as a control. (**B**) For bimolecular fluorescence complementation experiments the N-terminal part of Venus was fused to the N-terminus of AtTPR7 and the C-terminal part of SCFP to the N-terminus of Sec62. The constructs were co-transformed with the ER mCherry marker (middle panel) and transiently expressed in tobacco leaves. As a control Venus^{N}-AtTPR7 was cotransformed with SCFP3A^{C} alone (empty vector) and the ER mCherry marker (bottom panels). Images of tobacco leaf cells were obtained by confocal laser scanning microscopy (Leica, Type: TCS SP5; objective lense: HCX PL APO CS; magnification: 63×; numerical aperture: 1.3; imaging medium: glycerol; software: Leica Application Suite/Advanced Fluorescence). Reconstituted fluorescence obtained by close proximity of the Venus and the SCFP parts was monitored at 515 nm (left panel). Overlay of the signal at 515 nm and the mCherry marker is shown (right panel). Scale bars: 10 μm.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

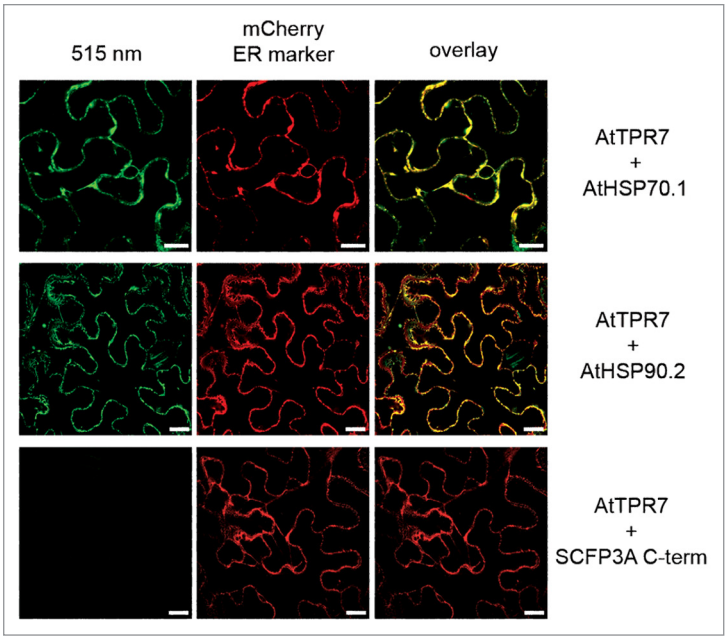

Figure 3. Interaction of AtTPR7 with AtHSP70.1 and AtHSP90.2. For bimolecular fluorescence complementation experiments the N-terminal part of Venus was fused to the N-terminus of AtTPR7 and the C-terminal part of SCFP to the N-terminus of AtHSP70.1 (At5g02500) and AtHSP90.2 (At5g56030), respectively. The constructs were cotransformed with the ER mCherry marker (middle panel) and transiently expressed in tobacco leaves. As a control $Venus^{N}$-AtTPR7 was cotransformed with $SCFP3A^{C}$ alone and the ER mCherry marker (bottom panels). Images of tobacco leaf cells were obtained by confocal laser scanning microscopy. Reconstituted fluorescence was monitored at 515 nm (left panel). Overlay of the signal at 515 nm and the mCherry marker is shown (right panel). Scale bars: 10 μm.

Acknowledgments

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References

1. Cross BC, Sinning I, Luirink J, High S. Delivering proteins for export from the cytosol. Nat Rev Mol Cell Biol 2009; 10:255-64; PMID:19305415; http://dx.doi.org/10.1038/nrm2657
2. Rapoport TA. Protein translocation across the eukaryotic endoplasmic reticulum and bacterial plasma membranes. Nature 2007; 450:663-9; PMID:18046402; http://dx.doi.org/10.1038/nature06384
3. Saraogi I, Shan SO. Molecular mechanism of cotranslational protein targeting by the signal recognition particle. Traffic 2011; 12:535-42; PMID:21291501; http://dx.doi.org/10.1111/j.1600-0854.2011.01171.x
4. Ast T, Schuldiner M. All roads lead to Rome (but some may be harder to travel): SRP-independent translocation into the endoplasmic reticulum. Crit Rev Biochem Mol Biol 2013; 48:273-88; PMID:23530742; http://dx.doi.org/10.3109/10409238.2013.782999
5. Dierks T, Klappa P, Wiech H, Zimmermann R. The role of molecular chaperones in protein transport into the endoplasmic reticulum. Philos Trans R Soc Lond B Biol Sci 1993; 339:335-41; PMID:8098538; http://dx.doi.org/10.1098/rstb.1993.0032
6. Harada Y, Li H, Wall JS, Li H, Lennarz WJ. Structural studies and the assembly of the heptameric posttranslational translocon complex. J Biol Chem 2011; 286:2956-65; PMID:20826819; http://dx.doi.org/10.1074/jbc.M110.159517
7. Brodsky JL, Goeckeler J, Schekman R. BiP and Sec63p are required for both co- and posttranslational protein translocation into the yeast endoplasmic reticulum. Proc Natl Acad Sci USA 1995; 92:9643-6; PMID:7568189; http://dx.doi.org/10.1073/pnas.92.21.9643
8. Lyman SK, Schekman R. Binding of secretory precursor polypeptides to a translocon subcomplex is regulated by BiP. Cell 1997; 88:85-96; PMID:9019409; http://dx.doi.org/10.1016/S0092-8674(00)81861-9
9. Plath K, Mothes W, Wilkinson BM, Stirling CJ, Rapoport TA. Signal sequence recognition in posttranslational protein transport across the yeast ER membrane. Cell 1998; 94:795-807; PMID:9753326; http://dx.doi.org/10.1016/S0092-8674(00)81738-9
10. Fang H, Green N. Nonlethal sec71-1 and sec72-1 mutations eliminate proteins associated with the Sec63p-BiP complex from S. cerevisiae. Mol Biol Cell 1994; 5:933-42; PMID:7841522
11. Meyer HA, Grau H, Kraft R, Kostka S, Prehn S, Kalies KU, et al. Mammalian Sec61 is associated with Sec62 and Sec63. J Biol Chem 2000; 275:14550-7; PMID:10799540; http://dx.doi.org/10.1074/jbc.275.19.14550
12. Müller L, de Escauriaza MD, Lajoie P, Theis M, Jung M, Müller A, et al. Evolutionary gain of function for the ER membrane protein Sec62 from yeast to humans. Mol Biol Cell 2010; 21:691-703; PMID:20071467; http://dx.doi.org/10.1091/mbc.E09-08-0730
13. Yamamoto M, Maruyama D, Endo T, Nishikawa S. Arabidopsis thaliana has a set of J proteins in the endoplasmic reticulum that are conserved from yeast to animals and plants. Plant Cell Physiol 2008; 49:1547-62; PMID:18718935; http://dx.doi.org/10.1093/pcp/pcn119
14. Jermy AJ, Willer M, Davis E, Wilkinson BM, Stirling CJ. The Brl domain in Sec63p is required for assembly of functional endoplasmic reticulum translocons. J Biol Chem 2006; 281:7899-906; PMID:16368690; http://dx.doi.org/10.1074/jbc.M511402200
15. Schweiger R, Müller NC, Schmitt MJ, Soll J, Schwenkert S. AtTPR7 is a chaperone-docking protein of the Sec translocon in Arabidopsis. J Cell Sci 2012; 125:5196-207; PMID:22899711; http://dx.doi.org/10.1242/jcs.111054
16. Gehl C, Waadt R, Kudla J, Mendel RR, Hänsch R. New GATEWAY vectors for high throughput analyses of protein-protein interactions by bimolecular fluorescence complementation. Mol Plant 2009; 2:1051-8; PMID:19825679; http://dx.doi.org/10.1093/mp/ssp040
17. Schlegel T, Mirus O, von Haeseler A, Schleiff E. The tetratricopeptide repeats of receptors involved in protein translocation across membranes. Mol Biol Evol 2007; 24:2763-74; PMID:17905998; http://dx.doi.org/10.1093/molbev/msm211
18. Kriechbaumer V, von Löffelholz O, Abell BM. Chaperone receptors: guiding proteins to intracellular compartments. Protoplasma 2012; 249:21-30; PMID:21461941; http://dx.doi.org/10.1007/s00709-011-0270-9
19. Qbadou S, Becker T, Mirus O, Tews I, Soll J, Schleiff E. The molecular chaperone Hsp90 delivers precursor proteins to the chloroplast import receptor Toc64. EMBO J 2006; 25:1836-47; PMID:16619024; http://dx.doi.org/10.1038/sj.emboj.7601091
20. Young JC, Hoogenraad NJ, Hartl FU. Molecular chaperones Hsp90 and Hsp70 deliver preproteins to the mitochondrial import receptor Tom70. Cell 2003; 112:41-50; PMID:12526792; http://dx.doi.org/10.1016/S0092-8674(02)01250-3
21. Faou P, Hoogenraad NJ. Tom34: a cytosolic cochaperone of the Hsp90/Hsp70 protein complex involved in mitochondrial protein import. Biochim Biophys Acta 2012; 1823:348-57; PMID:22178133
22. Chew O, Lister R, Qbadou S, Heazlewood JL, Soll J, Schleiff E, et al. A plant outer mitochondrial membrane protein with high amino acid sequence identity to a chloroplast protein import receptor. FEBS Lett 2004; 557:109-14; PMID:14741350; http://dx.doi.org/10.1016/S0014-5793(03)01457-1
23. Lin BL, Wang JS, Liu HC, Chen RW, Meyer Y, Barakat A, et al. Genomic analysis of the Hsp70 superfamily in Arabidopsis thaliana. Cell Stress Chaperones 2001; 6:201-8; PMID:11599561; http://dx.doi.org/10.1379/1466-1268(2001)006<0201:GAOTHS>2.0.CO;2
24. Feldheim D, Schekman R. Sec72p contributes to the selective recognition of signal peptides by the secretory polypeptide translocation complex. J Cell Biol 1994; 126:935-43; PMID:8051213; http://dx.doi.org/10.1083/jcb.126.4.935