Dimerization of TOC receptor GTPases and its implementation for the control of protein import into chloroplasts

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Pre-protein import into chloroplasts is facilitated by multiprotein translocon complexes in the envelope membranes. Major components of the TOC (translocon at the outer envelope membrane of chloroplasts) complex are the receptor proteins Toc33 and Toc159. These two receptors are related GTPases, and they are predicted to engage in homodimerization and/or heterodimerization. Although such dimerization has been studied extensively, its exact function in vivo remains elusive. In this issue of the Biochemical Journal, Oreb et al. present evidence that homodimerization of Toc33 prevents nucleotide exchange, thereby locking the receptor in the GDP-loaded state and preventing further activity. Pre-protein arrival is proposed to release this lock, through disruption of the dimer and subsequent nucleotide exchange. The Toc33-bound pre-protein is then able to progress to downstream steps in the translocation mechanism, with GTP hydrolysis defining another important control point as well as preparing the receptor for the next pre-protein client. These new results are discussed in the context of previous findings pertaining to TOC receptor dimerization and function.

Key words: chloroplast, dimerization, GDP-dissociation inhibitor (GDI)-displacement factor (GDF), G-protein activated by nucleotide-dependent dimerization (GAD), protein import, translocon at the outer envelope membrane of chloroplasts (Toc/TOC).

Approximately 90–95 % of the ~3000 proteins in chloroplasts are encoded in the nucleus and synthesized in the cytosol as pre-proteins, each one with an N-terminal targeting signal called a transit peptide. Thus a translocation machinery is needed to import the vast majority of chloroplast proteins from the cytosol. This machinery consists of two major translocon complexes, termed TOC (translocon at the outer envelope membrane of chloroplasts) and TIC (translocon at the inner envelope membrane of chloroplasts). Each translocon contains several membrane-bound protein components named after their predicted molecular masses, one of them being Toc34, which was first identified in pea (Pisum sativum). The orthologue of Toc34 in Arabidopsis thaliana, a major plant model system, is termed Toc33 [1].

Toc34 is a receptor protein that interacts with incoming pre-proteins at the cytosol–envelope interface. After recognition by Toc34, which acts in combination with another receptor protein Toc159, pre-proteins are transferred to the channel protein, Toc75, for translocation through the outer envelope membrane. Subsequently, pre-proteins engage the TIC complex before reaching their final destination inside the chloroplast. Interestingly, both of the receptors, Toc34 and Toc159, are GTPases, and so the pre-protein recognition step is thought to be controlled by a GTPase cycle [1]. Toc34 and Toc159 are related, belonging to the septin family of GTPases within the broader TRAFAC (translation factor-related) class [2].

GTPases are commonly employed by cells to regulate a wide variety of processes (e.g. protein transport, synthesis and signalling). Typically, they switch between GTP- and GDP-loaded states, with the former being the active condition. They share a common amino acid structure, albeit with variations on the theme, including five sequence motifs that control, for example, hydrolysis and nucleotide specificity. GTPases can be regulated by accessory proteins (modulators), such as GEFs (guanine-nucleotide-exchange factors) that promote the relatively slow replacement of GDP with GTP [3]. Once formed, the GTP-bound protein fulfills its function by interacting with downstream effectors to trigger a response. Such effector proteins can also be part of the regulation, like the modulators, as has been proposed for the pre-proteins recognized by Toc34 (see below). Inactivation of the process occurs when GTP is hydrolysed, which can be stimulated by another modulator, a GAP (GTPase-activating protein), which often uses a catalytic ‘arginine finger’ for its action [3].

Dimerization can also influence the GTPase cycle, as it does for instance in GADs (G-proteins activated by nucleotide-dependent dimerization), where the combination of individual monomers induces GTPase activity in the dimer, without a requirement for a GAP or GEF [3]. An arginine residue is often found at the dimerization interface, and this may act as an arginine finger (in effect, each monomer is a GAP to its partner). Ever since the crystal structure of Toc34 revealed a homodimeric configuration [4] (PDB code 1H65), such mechanisms have been proposed to play a role in chloroplast protein import, although the exact function of dimerization remains to be elucidated. Arginine residues exist at the TOC receptor dimer interface, but these have not been consistently shown to have classical arginine finger activity [5], and so may be important only structurally for dimer formation. Instead, pre-proteins were reported to induce receptor GTP hydrolysis [6,7], with one study suggesting that the transit peptide may function as a GAP [6].

An alternative possibility has been proposed in this issue of the Biochemical Journal by Oreb et al. [8], who present evidence

Abbreviations used: GAP, GTPase-activating protein; GDI, GDP-dissociation inhibitor; GDF, GDI-displacement factor; GEF, guanine-nucleotide-exchange factor; TIC, translocon at the inner envelope membrane of chloroplasts; Toc/TOC, translocon at the outer envelope membrane of chloroplasts.

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that an incident pre-protein actually stimulates GTP binding by Toc33. Using GDP-loaded Toc33, the rate of exchange for GTP was measured, with and without added pre-protein; in the presence of pre-protein, GTP binding was stimulated. By manipulating the extent of dimerization (through the alteration of receptor concentration, use of dimerization mutants or addition of a synthetic transit peptide), nucleotide exchange was shown to be considerably faster for monomers than dimers. Thus homodimerization of Toc33 was considered to be rate-limiting for GDP dissociation, which in turns limits the rate of GTP binding. The authors likened the effect of receptor dimerization to that of GDIs (GDP-dissociation inhibitors) on Ras-like GTPases, and compared the perturbing pre-protein to GDFs (GDI-displacement factors) [8]. The suggested ‘GDF-like’ role of the pre-protein (or, more specifically, of the transit peptide) is somewhat similar to an earlier proposal from Li et al. [9] that the pre-protein acts as a GEF. However, since the transit peptide was not shown to stimulate nucleotide exchange in the context of the monomeric form of Toc33, it was not considered to be a classical GEF [8].

Results supporting the functional significance in vivo of Toc34 dimerization were presented previously [5,10]. It is possible that Toc34–Toc159 heterodimerization, or even Toc159 homodimerization, also plays a role in pre-protein recognition, although experimental support for these possibilities is currently sparse [5,9,11]. There are numerous inconsistencies in the literature concerning TOC receptor action and, as a consequence, a consensus model has yet to emerge. In one model, Toc34 (perhaps in the homodimeric form) acts as the primary receptor, being the first point of contact for the transit peptide at the chloroplast surface. Upon ‘unlocking’ of the receptor by the pre-protein through nucleotide exchange, as discussed previously [8,9], it is possible that the Toc34–pre-protein complex engages Toc159 to form a receptor heterodimer. This would assure transfer of the pre-protein from Toc34 to Toc159, with GTP hydrolysis facilitating its onward passage into the Toc75 channel. Although such a model seems feasible, it should be borne in mind that GTPase- and/or dimerization-defective mutants of Toc34 and Toc159 retain in vivo functionality (i.e. upon expression, they efficiently complement corresponding knockout mutant plants) [5,10,12,13]. Such observations imply that events in the pre-protein recognition/binding process are not obligatorily coupled to defined steps in the GTPase cycles and that the receptors’ roles are quite permissive.

In an alternative model, Toc159 acts as the primary receptor. The Toc159 protein is structurally more complex than Toc34 in that it has an N-terminal acidic domain (as well as a large, atypical, membrane domain at the C-terminus) in addition to the GTPase domain. Recent evidence suggests that the acidic domain plays a critical role in determining pre-protein recognition specificity [14], supporting the notion that Toc159 is the point of initial binding. An earlier hypothesis that soluble Toc159 might collect pre-protein cargo in the cytosol before delivering it to the envelope now seems unlikely, as what was previously thought to be intact receptor free in the cytosol has since been identified as a fragment consisting of the acidic domain only [13,15]. Following initial binding, Toc159 might transfer the pre-protein to Toc34, which would in turn pass it on to the Toc75 channel. As in the previous model, receptor homo- and hetero-dimerization and the receptor GTPase cycles would control passage of the pre-protein through sequential steps.

It is abundantly clear that many unanswered questions remain in relation to TOC receptor action, and so various different possibilities and scenarios must be considered. For example, in addition to those models already discussed, it can be envisaged that Toc34 and Toc159 might act interchangeably as the primary receptor, depending perhaps on the conditions or the nature of the incident client, or that the two receptors might act simultaneously on occasion as co-receptors. Any model must take account of the aforementioned reports that receptors with disabled GTPase domains retain in vivo functionality [5,10,12,13], and the puzzling observation that Toc34, together with Toc75, is severalfold more abundant than Toc159 [16].

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