Protist, Vol. 166, 349–362, July 2015
http://www.elsevier.de/protis
Published online date 28 May 2015

Protist

ORIGINAL PAPER

The TOM Complex of Amoebozoans: the Cases of the Amoeba *Acanthamoeba castellanii* and the Slime Mold *Dictyostelium discoideum*

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Submitted November 14, 2014; Accepted May 14, 2015
Monitoring Editor: Saul Purton

Protein import into mitochondria requires a wide variety of proteins, forming complexes in both mitochondrial membranes. The TOM complex (translocase of the outer membrane) is responsible for decoding of targeting signals, translocation of imported proteins across or into the outer membrane, and their subsequent sorting. Thus the TOM complex is regarded as the main gate into mitochondria for imported proteins. Available data indicate that mitochondria of representative organisms from across the major phylogenetic lineages of eukaryotes differ in subunit organization of the TOM complex. The subunit organization of the TOM complex in the Amoebozoa is still elusive, so we decided to investigate its organization in the soil amoeba *Acanthamoeba castellanii* and the slime mold *Dictyostelium discoideum*. They represent two major subclades of the Amoebozoa: the Lobosa and Conosa, respectively. Our results confirm the presence of Tom70, Tom40 and Tom7 in the *A. castellanii* and *D. discoideum* TOM complex, while the presence of Tom22 and Tom20 is less supported. Interestingly, the Tom proteins display the highest similarity to Opisthokonta cognate proteins, with the exception of Tom40. Thus representatives of two major subclades of the Amoebozoa appear to be similar in organization of the TOM complex, despite differences in their lifestyle.

Key words: TOM complex; mitochondria; *Acanthamoeba castellanii*; *Dictyostelium discoideum*; phylogenesis; translocase of the outer membrane; Amoebozoa.

Introduction

Mitochondria are essential for cell function and survival. The proper function of mitochondria depends on protein import, regarded as extremely challenging due to mitochondrial architecture; i.e. the

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http://dx.doi.org/10.1016/j.protis.2015.05.005

presence of the outer and inner membranes that form the borders for two aqueous compartments: the intermembrane space and matrix. As summarized by Schmidt et al. (2010), the import concerns all proteins of the outer membrane and the intermembrane space, as well as the majority of the inner membrane and matrix proteins. This results from the course of mitochondria formation during the evolution of an eukaryotic cell that consisted in gene transfer of an ancestor prokaryotic endosymbiont to the nucleus as well as in gene loss and emergence of new genes in the nucleus to control mitochondrial function (e.g. Cavalier-Smith 2010; Cavalier-Smith et al. 2014; Liu et al. 2011).

The protein import is mediated by a set of protein complexes located in both mitochondrial membranes (termed translocases) as well as in the intermembrane space and matrix but is initiated by the translocase of the outer mitochondrial membrane, known also as the TOM complex. The complex is regarded as a general entry gate for virtually all proteins imported into mitochondria (e.g. Becker et al. 2008; Dolezal et al. 2006; Hewitt et al. 2011; Lithgow and Schneider 2010; Mokranjac and Neupert 2009; Neupert and Herrmann 2007; Schmidt et al. 2010; Sokol et al. 2014; Varabyova et al. 2013; Walther et al. 2009). The translocase is a protein complex responsible for the imported protein recognition, translocation across or into the outer membrane, and for decoding of their targeting signals and subsequent sorting. Thus the TOM complex appears to play a fundamental role in the import process.

The subunit organization of the TOM complex, defined first for *Neurospora crassa* and *Saccharomyces cerevisiae* mitochondria (Ahting et al. 1999; Meisinger et al. 2001), is at present regarded as the canonical one. Moreover, available data allow the conclusion that the organization follows the scheme described for all translocases. It means that besides the subunit displaying channel-forming activity and being responsible for protein translocation, the complex contains subunits that regulate its structure and function. As summarized by Varabyova et al. (2013), in the case of the canonical TOM complex, the central subunit is Tom40, the channel-forming protein, whereas other proteins can be divided into two groups: (i) the core TOM subunits, which include the central receptor (Tom22) and the small Tom proteins (Tom5, Tom6, Tom7) regulating the complex dynamics; and (ii) the peripheral receptor subunits (Tom70, Tom20), which are more loosely associated with the complex and recognize different features and targeting signals within the imported proteins, which in turn initiate entry into a defined import pathway.

Interestingly, it has been proposed that mitochondrial protein import complexes, including the TOM complex, contain subunits formed by proteins common to all eukaryotes and additional subunits that have been added over time and regarded as common only to a particular eukaryotic lineage (Dolezal et al. 2006). Available data concerning representatives of different phylogenetic lineages indicate that only Tom40 is found in virtually all eukaryotes (Zarsky et al. 2012), whereas other subunits are definitely less conserved. In animals, the TOM complex subunit organization is very similar to the canonical one (e.g. Dolezal et al. 2006; Hewitt et al. 2011; Hoogenraad et al. 2002; Schneider et al. 2008), whereas in plants the complex does not contain orthologs of the canonical Tom20 and Tom70. Moreover, the plant ortholog of Tom22 is termed Tom9, while for Tom5 and Tom6 it is still discussed whether they are orthologous or analogous to the *S. cerevisiae* proteins (e.g. Murcha et al. 2014; Perry et al. 2008). In different protists, the TOM complex subunit organization usually differs distinctly from the canonical one but available data are not numerous and consistent.

Protists are a polyphyletic group of eukaryotic microorganisms defined in the past by exclusion of animals, fungi, and plants. In the recent classification proposed by the International Society of Protistologists (Adl et al. 2005), Eukaryota were divided into six supergroups: Chromalveolata, Excavata, Rhizaria, Amoebozoa (these four including protists only), Archaeplastida (including plants and some algae), and Opisthokonta (including animals, fungi and some protists). That classification was later discussed and refined by many authors (e.g. Adl et al. 2012; Cavalier-Smith et al. 2014; Keeling et al. 2005; Schilde and Schaap 2013). The Opisthokonta are most closely related to the Amoebozoa, which comprise a wide variety of amoeboid and flagellate organisms with single cells of various sizes that have adopted many different lifestyles and live in different environments. The Amoebozoa can be further be subdivided into the phyla Conosa, Lobosa, and probably Breviatea (e.g. Fiz-Palacios et al. 2013; Schilde and Schaap 2013).

Here we described the TOM complex of the amoeba *Acanthamoeba castellanii* and the slime mold *Dictyostelium discoideum*, representatives of the Lobosa and Conosa, respectively. Both species share properties with plant and animal cells and are known as particularly valuable research models for developmental biology and medicine (Annesley et al. 2014; Walker and Williams 2013). Previous