Gene 583 (2016) 64–77

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Gene wiki review

Structure and function of the AAA+ ATPase p97/Cdc48p

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ARTICLE INFO

Article history:
Received 24 December 2015
Received in revised form 23 February 2016
Accepted 25 February 2016
Available online 3 March 2016

Keywords:
p97/CDC48
AAA+ ATPase
Segregase
Unfoldase
Protein quality control
ER-associated degradation
Chromatin-associated degradation
Mitochondria-associated degradation
Ribosome-associated degradation
Membrane fusion
Ubiquitin
Proteasome

ABSTRACT

p97 (also known as valosin-containing protein (VCP) in mammals or Cdc48p in *Saccharomyces cerevisiae*) is an evolutionarily conserved ATPase present in all eukaryotes and archaebacteria. In conjunction with a collection of cofactors and adaptors, p97/Cdc48p performs an array of biological functions mostly through modulating the stability of 'client' proteins. Using energy from ATP hydrolysis, p97/Cdc48p segregates these molecules from immobile cellular structures such as protein assemblies, membrane organelles, and chromatin. Consequently, the released polypeptides can be efficiently degraded by the ubiquitin proteasome system or recycled. This review summarizes our current understanding of the structure and function of this essential cellular chaperoning system.

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Abbreviations: VCP, valosin-containing protein; TER ATPase, transitional endoplasmic reticulum ATPase; AAA+, extended family of ATPases associated with various cellular activities; ER, endoplasmic reticulum; PQC, protein quality control; IBMPEF, Inclusion Body Myopathy associated with Paget's disease of the bone and Frontotemporal Dementia; ALS, amyotrophic lateral sclerosis; EM, electron microscopy; ATP, Adenosine triphosphate; ADP, Adenosine diphosphate; AMP-PNP, Adenylyl-imidodiphosphate; ERAD, ER-associated protein degradation; VAT, VCP-like ATPase.

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http://dx.doi.org/10.1016/j.gene.2016.02.042
0378-1119/Published by Elsevier B.V.

A genetic screen conducted three decades ago in *Saccharomyces cerevisiae* identified several alleles of *Cdc48* that affects cell growth at non-permissive temperatures due to a cell cycle arrest at the G2-M transition stage (Moir et al., 1982). The mammalian homolog of Cdc48p was later reported as a 97 kDa protein precursor for the small peptide valosin, and therefore named as valosin-containing protein (VCP) or p97 (Koller and Brownstein, 1987). Although it turned out that valosin is a purification artifact unrelated to p97 (Gill et al., 1989), the VCP nomenclature is still being used in the literature. In some species, the name transitional endoplasmic reticulum ATPase (TER ATPase) is used given the localization and function of a fraction of this enzyme at the endoplasmic reticulum (ER) (see below). In this review, we use p97 and Cdc48p for the mammalian and yeast homologs, respectively.

p97/Cdc48p belongs to a large ATPase family termed AAA+ (extended family of ATPases associated with various cellular activities) ATPase. Enzymes of this family function in all species from bacteria to humans, often as essential chaperones that promote protein folding or unfolding. p97/Cdc48p is a type II AAA+ ATPase because it has two AAA ATPase domains in tandem (named D1 and D2, respectively) (Fig. 1A). A short polypeptide linker (D1–D2 linker) connects the two ATPase domains and another linker (N–D1 linker) joins the D1 domain to a large amino-terminal domain (N-domain). The carboxyl-terminus of the D2 domain is appended with a short tail containing ~40 residues. Interaction of p97/Cdc48p with its partners is mostly mediated by the N-domain, although a few proteins bind p97/Cdc48p via the C-terminal tail (Buchberger et al., 2015; Ogura and Wilkinson, 2001). The D1 and D2 domains are homologous both in sequence and in structure. However, they have distinct functions. For example, the hexameric assembly of p97 only requires the D1 but not the D2 domain (Wang et al., 2003).

In mammalian cells, p97 is localized mainly to the cytoplasm with a fraction associated with the membranes of subcellular organelles such as the ER, Golgi, mitochondria, and endosomes (Acharya et al., 1995; Latterich et al., 1995; Rabouille et al., 1995; Ramanathan and Ye, 2012; Xu et al., 2011). The membrane localizations are probably mediated by membrane-associated receptors whose identity is largely unknown in most cases. A fraction of p97/Cdc48p is also present in the nucleus and serves essential roles in chromatin-associated events and nuclear protein quality control (PQC) (see below) (Madeo et al., 1998).

As one of the most abundant proteins in eukaryotic cells, p97 is ubiquitously expressed in multicellular organisms. In humans, the mRNA expression of p97 was moderately increased in certain types of cancer, and the expression level to some extent correlates with the sensitivity of cancer cells to a potent p97 inhibitor that is currently evaluated as a potential anti-cancer drug (Anderson et al., 2015).

Most known substrates of p97/Cdc48p are conjugated with ubiquitin chains and degraded by the 26S proteasome. Accordingly, many p97/Cdc48p cofactors/adaptors are capable of recognizing ubiquitin conjugates (Ye, 2006). It has been thought that the interplay between ubiquitin and the p97 system is critical for p97 functions, although the precise role awaits further elucidation. Some p97 cofactors are ubiquitin ligase or deubiquitinase that can process ubiquitin chains, but the vast majority serves as adaptors that link p97/Cdc48p to specific subcellular compartment or substrate.

More recently, genetic studies have linked mutations in p97 to several human diseases including IBMPFD (Inclusion Body Myopathy associated with Paget's disease of the bone and Frontotemporal Dementia) and amyotrophic lateral sclerosis (ALS). These findings have stimulated a flurry of investigations on the structure and function of p97/Cdc48p.

1. Structural organization of p97

1.1. Structural features of p97

Structural information on p97 was initially obtained with negatively stained samples examined by electron microscopy (EM). The results provided the basic shape of a ring-like hexameric molecule (Peters et al., 1990; Zhang et al., 1994). Higher resolution structures were later obtained by cryo-EM (Rouiller et al., 2000) and by crystallization of a N–D1 fragment (Zhang et al., 2000). These studies confirmed the hexameric assembly of p97, but also showed that unlike many bacterial AAA+ proteins, the assembly of p97 does not depend on the presence of nucleotide.

Subsequent high resolution structural studies were primarily carried out with X-ray crystallography (Table 1) focusing on full-length wild-type p97 (Davies et al., 2008; DeLaBarre and Brunger, 2003, 2005; Huyton et al., 2003) as well as several disease-associated mutants (Tang et al., 2010; Tang and Xia, 2012, 2013). More recently, three additional reports on the structure of full-length p97 appeared. One featured crystal structures of the full-length p97 bearing mutations in the D2 domain with nominal improvements in resolution (Hanzelmann and Schindelin, 2016b). The other two studies used the latest development in electron microscopic technology to obtain higher resolution structures of p97 (Banerjee et al., 2016; Schuller et al., 2016). The interactions of p97 with adaptors were also extensively studies with X-ray crystallography (Dreveny et al., 2004; Hanzelmann et al., 2011; Hanzelmann and Schindelin, 2011, 2016a; Kim et al., 2011; Kim and Kim, 2014; Lee et al., 2013; Qiu et al., 2010; Schaeffer et al., 2014; Zhao et al., 2007). These studies show that p97 forms two concentric rings (Fig. 1B and C); the N–D1 ring has a larger radius than the D2 ring owing to the laterally attached N-domain. Like other AAA+ ATPases, the AAA module of p97 features a highly conserved RecA-like domain and a characteristic helical domain (Fig. 1D). Each RecA-like domain in a protomer bears an active site, which is situated at the interface between two adjacent promoters in the hexameric assembly. The active site is formed by the classical Walker A (P-loop; G(x)4GKT) and Walker B motifs (hhhhDE, h for hydrophobic amino acid), responsible for nucleotide binding and hydrolysis, respectively. The configuration of the active site allows an arginine-finger residue (R359 for the D1 ring and R635 for the D2 ring) from an adjacent subunit to stimulate ATP hydrolysis.

1.2. Nucleotide-driven conformational changes in p97

It is generally believed that p97 undergoes dramatic conformational changes during the nucleotide hydrolysis cycle (Beuron et al., 2003, 2006; DeLaBarre and Brunger, 2005; Rouiller et al., 2002; Tang et al., 2010). Mechanical force generated by these conformational changes would be applied to substrate molecules to influence their stability and function. As a type II AAA+ ATPase, each p97 hexamer contains 12 ATPase domains and 6 N-domains. If each nucleotide-binding site were capable of producing 3 distinct conformations for apo-, ADP- and ATP-state, there would be a total of 3^{12} different conformations assuming each subunit operates independently. Reported structural conformations are far less than the theoretical possibilities due to inter-subunit communications and coordination. Nevertheless, the conformational dynamics of p97 is difficult to study because the six ATPase domains within each ring are not synchronized in ATP hydrolysis (see below).

The conformational changes driven by the nucleotide cycle of p97 have been sought by various biochemical and biophysical methods (Davies et al., 2005). Initially, low-resolution cryo-EM structures revealed that upon ATP hydrolysis moderate rotational movement occurs between the two ATPase rings, associated with either closure or opening of the D1 and D2 central pores (Rouiller et al., 2002), but subsequent studies suggested other modes of domain movement (Beuron et al., 2003). Due to resolution limitation, domain assignment in early EM-reconstruction studies has been unreliable, and therefore, it is not feasible to consolidate these structural data into a consistent model that explains the action of p97. Moreover, because insufficient resolution prevents accurate determination of the p97 nucleotide-binding state, the interpretation of the EM results relied on the assumption that all 12 ATP-binding sites are occupied by the added nucleotide in a