Abstract

Prohibitins, Phb1 and Phb2, are evolutionary conserved proteins with diverse cellular localization and different functions. In yeast, prohibitins form a high molecular weight complex in the inner mitochondrial membrane which physically interacts with the mAAA protease and affects the stability of non assembled proteolytic substrates. In the present work, I examined two possible roles of prohibitins in proteolysis: i) role as negative regulators of the mAAA protease (Steglich et al., 1999) and/or ii) function as molecular chaperones for the assembly of newly synthesized mitochondrial proteins (Nijtmans et al., 2000). To discriminate between both possibilities, the stability of proteolytic substrates of the mAAA protease was examined in cells harboring different levels of prohibitins. While overexpression of prohibitins did not stabilize non-native inner membrane proteins absence of prohibitins resulted in their rapid proteolysis. The results presented in this work suggest that prohibitins do not play a role as chaperones for the stabilization of mitochondrial proteins. Rather, prohibitins function by binding to the mAAA protease in a nucleotide independent manner and presumably modulating the accessibility of substrate to the protease or the activity of the protease.

The mAAA protease is recognized as a crucial component of the mitochondrial quality control system in yeast and in human (Arlt et al., 1996; Arlt et al., 1998; Nolden et al., 2005). In human, the mAAA protease is built up of paraplegin and Afg3l2 which are homologous to the yeast Yta10 and Yta12 proteins (Atorino et al., 2003). In mice, a third AAA subunit called Afg3l1 is expressed (Kremmidiotis et al., 2001) and, therefore, the subunit composition of the murine mAAA protease is unknown. Here, the complexes between paraplegin, Afg3l1 and Afg3l2 were examined in mitochondria from wild type and in SPG7-/- mouse strains. Immunoprecipitation experiments showed that paraplegin, Afg3l1 and Afg3l2 physically interact and are contained in a high molecular weight complex with prohibitins suggesting a conserved role of Phb1 and Phb2 for proteolysis in murine mitochondria. Co-immunoprecipitation and immunodepletion experiments using liver mitochondria from a SPG7-/- mouse strain showed that Afg3l1 and Afg3l2 can also form high molecular weight complexes in the absence of paraplegin. These complexes were additionally able to interact with prohibitins suggesting that
paraplegin is not essential for this interaction. Since paraplegin-specific antibodies are able to precipitate Phb1 and Phb2, it is conceivable that also paraplegin-Afg3l1 and paraplegin-Afg3l2 complexes will be able to bind prohibitins. Thus, mAAA proteases with different subunit composition appear to exist in the inner membrane of murine mitochondria. The physiological relevance of mAAA proteases with different subunit composition is discussed in view of differences in their substrate specificities and/or different tissue distribution.