

## Chapter 2

# Historical Sketch of the Discovery and Recognition of the Function of Chaperonins

**Abstract** The history of chaperonin research goes back to the 1970s when the first representatives of these proteins have been described, in the first place with no clues as to their general molecular activity. A series of breakthrough studies have paved the way to our current understanding of chaperonin biology.

The history of chaperonin research goes back to the 1970s when the first representatives of these proteins have been described in the first place with no clues as to their general molecular activity-assisting other proteins in folding. A time line with the major discoveries and findings regarding chaperonin research is given in Table 2.1. The *Escherichia coli* *groEL* and *groES* genes encoding the GroEL and GroES proteins made the start in the early 1970s. The GroEL and GroES proteins encoding the large and small subunits of the chaperonin complex were found to be essential for the growth of bacteriophage T4 by sophisticated genetic screens (Georgopoulos et al. 1972; Takano and Kakefuda 1972). Electron microscopy and biochemistry experiments established that *E. coli* GroEL formed complexes consisting of two stacked seven-meric rings (Fig. 2.1; Hendrix 1979).

The RuBisCO-binding protein of plant chloroplasts saw the light of scientific publication in the early 1980s and in the end of the 1980s/beginning of the 1990s. The RuBisCO-binding protein was found to be a protein associated with the large subunit of RuBisCO (ribulose 1,5-bisphosphate carboxylase/oxygenase), a highly abundant protein in plant leaf cells. RuBisCO is the photosynthetic CO<sub>2</sub> fixing enzyme. The RuBisCO large subunit is synthesized inside chloroplasts whereas the small subunit is synthesized in the cytosol and imported posttranslationally. These first reports were unaware of the general function of chaperonin proteins in all cellular systems and more insight into the structural properties and mechanistic principles for the functioning of these proteins was first emerging after years of research. In the end of the 1980s, the availability of DNA sequencing technology showed that the plant chloroplast and *E. coli* proteins are evolutionarily related (Hemmingsen et al. 1988). Using an antibody directed against a 58 kDa protein from the ciliate protozoan *Tetrahymena thermophile* showed that it cross-reacted with proteins of approximately 60 kDa mass present in the mitochondria of a range of organisms such as from yeast to humans (McMullin and Hallberg 1988)

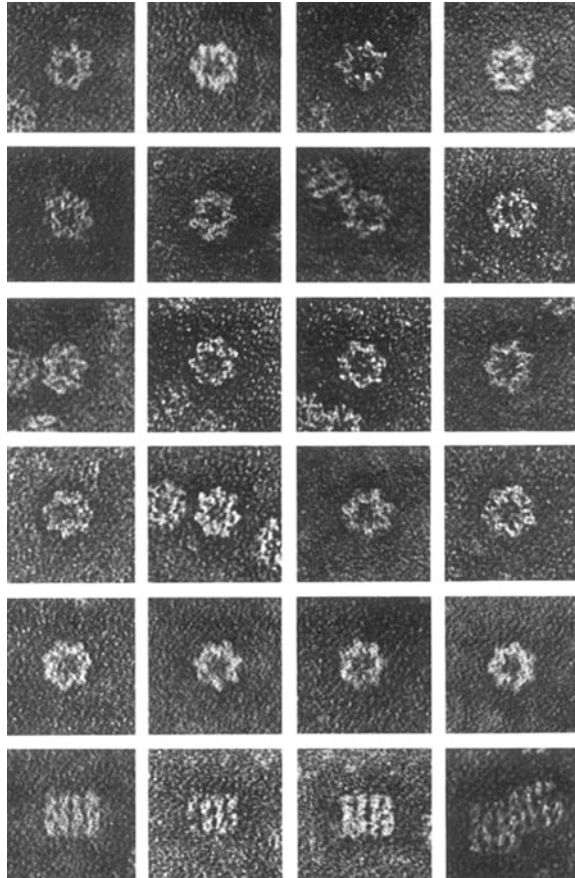
**Table 2.1** Timeline of major discoveries and findings regarding chaperonin research

Year	Finding	Reference
1972	Mutations in <i>E. coli</i> abolishing propagation of $\lambda$ and T4 phage later turning out to map to the <i>groE</i> locus	Georgopoulos et al. (1972); Takano and Kakefuda (1972)
1977	Indications for a large protein complex binding the large subunit of RuBisCO	Ellis (1977)
1979	The products of the <i>E. coli</i> <i>groE</i> operon encodes a complex composed of two stacked rings of seven-mer subunits	Hendrix (1979)
1988	Nucleotide sequences of <i>E. coli</i> GroEL and GroES and plant RuBisCO-binding protein	Hemmingsen et al. (1988)
1989	Folding of a model protein imported into yeast mitochondria involves interaction with the mitochondrial Hsp60 chaperonin and is ATP dependent	Ostermann et al. (1989)
1989	GroEL and GroES promote assembly of heterologous ribulosebiphosphate carboxylase	Goloubinoff et al. (1989)
1989	The GroES and GroEL proteins are essential for the growth of <i>E. coli</i> cells	Fayet et al. (1989)
1992	Protein folding in the cell; <i>seminal review on molecular chaperones and folding catalysts</i>	Gething and Sambrook (1992)
1993	Model for the GroEL/GroES reaction cycle	Martin et al. (1993)
1994	Crystal structure of the symmetric GroEL complex	Braig et al. (1994)
1997	Crystal structure of the asymmetric GroEL/GroES complex with bound nucleotides	Xu et al. (1997)
2002	Neurodegenerative disease caused by mutation in the gene encoding Hsp60	Hansen et al. (2002)
2005	Substrate spectrum of the <i>E. coli</i> GroEL/GroES complex	Kerner et al. (2005)
2010	Hsp60 knockout mice	Christensen et al. (2010)
2015	Crystal structure of the human Hsp60/Hsp10 complex	Nisemblat et al. (2015)

establishing the importance of this protein family in all organisms. Around at the same time, this was further emphasized by observations that knocking out the GroEL gene in *E. coli* (Fayet et al. 1989) or growing yeast cells with temperature-sensitive mutants of the chaperonin genes were not compatible with cell viability (Cheng et al. 1989). The fact that the *Tetrahymena thermophiles* representative of the family increased in expression by approximately 2–3 fold (McMullin and Hallberg 1987) together with the approximate size of 60 kDa has coined the nomenclature of Hsp60 for the mitochondrial chaperonins in all eukaryotic organisms.

As a further addition to the family, a protein found in the eukaryotic cytosol and assembling to a structure termed the TCP-1 ring complex (TRiC) was found to be evolutionarily distantly related to the Hsp60/Hsp10 and GroEL/GroES chaperonins.

**Fig. 2.1** Electron microscopic pictures of purified GroEL. Negatively stained GroEL complexes seen in top view or side view (*bottom panel*). Reprinted from: Hendrix (1979) with permission from Elsevier



The eukaryotic TRiC chaperonin is evolutionarily rooted from Archaea, a domain of prokaryotes, whose chaperonin is termed thermosome (Trent et al. 1991). The two groups, bacterial GroEL/GroES, mitochondrial Hsp60/Hsp10, and chloroplast Cpn60/Cpn10 on one side and TRiC and thermosome on the other were then categorized as type I and type II chaperonins, respectively (Hemmingsen 1992).

In the late 1980s, more and more observations suggested that ATP-hydrolyzing heat shock proteins are involved in folding of proteins in the cell (Pelham 1986). Anfinsen's pioneering work had shown that for some proteins, folding is solely determined by the amino acid sequence. It had long been known that for some proteins, two types of helper enzymes can promote folding to the native state: (i) protein disulfide isomerases that assist in the formation of the correct disulfide bond formation, and (ii) peptidyl-prolyl-cis-trans-isomerases that mediate fast switching of the covalent bond fixing the *cis* or *trans* form of proline, which otherwise limits the mobility of polypeptide chains undergoing folding. However, it was now emerging that in vivo, the self-assembly of protein structures was facilitated by folding helper proteins that were subsequently termed molecular

chaperones (Ellis and Hemmingsen 1989). Molecular chaperones bind and interact with proteins undergoing folding but are not part of the final native structure. The exact mechanisms describing how the chaperone–client interaction promoted folding were on those days still largely elusive.

The finding that the *E. coli* GroEL and GroES proteins together with ATP could reconstitute active dimeric RuBisCO in an in vitro experiment (Goloubinoff et al. 1989b) was a milestone that suggested that these proteins had a very general role for cellular function. In the ensuing years, this triggered an avalanche of experimental investigations exploring chaperonin structure, function, and molecular mechanisms. Once the basic concept of a chaperone system that formed peculiar ring complexes and was able to promote folding and assembly of proteins had been established, the door was wide open for addressing the structures, mechanisms, and functions of chaperonin complexes.

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