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총 설

Molecular chaperone as a sophisticated intracellular membership

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Abstract

Discovery of molecular chaperones has stimulate cell biologists and thus made it possible to re-examine the processes whereby proteins achieve and maintain their functional conformations within living cells. The term 'molecular chaperone' was first coined to describe one particular protein involved in the assembly of nucleosomes, but the term has now been extended to describe the function of a wide variety of proteins that assist protein assembly in a wide range of fundamental cellular processes. Besides protein synthesis, these processes include protein transport across membranes, folding of nascent polypeptide, the assembly and disassembly of oligomeric structures, and the recovery or removal of proteins damaged by various environmental stresses including heat shock. Progress of molecular chaperones research is still limited by the lack of 3-dimensional structural information and detailed interacts with target proteins in the cell. However, several laboratories around the world are attempting to extend our knowledge on the functions of molecular chaperone, and such efforts seem justified to finally provide the answers to the most burning questions shortly.

Key words : *Molecular chaperone, Folding, Assembly.*

History of the molecular chaperone concept

Up to a few years ago, scientists thought that polypeptides fold independently and spontaneously to achieve their functional 3-D shapes as bioactive proteins. However, they have recognized that this view is not incorrect since despite of all information is included in the amino-acid sequences, they are not perfectly refolded in the expected 3-dimensional protein. Recently, they have discovered that various classes of specialized proteins known as molecular chaperones are required to assist the folding of the other proteins *in vivo.* The term of molecular chaperone in the field of biochemistry, biophysics and cell biology was first used by Laskey[1] in 1978 to describe the properties of nucleoplasmin, which mediated the *in vitro* assembly of nucleosomes from isolated histones and DNA. This was based on the former Anfinsen's experiment in 1973 that denatured purified ribonuclease refolds spontaneously in the absence of other proteins into an active enzyme, which demonstrated first that newly synthesized polypeptide chain should be able to attain its functional conformation with no assistance from other cellular molecules[2].

Not serendipitously, the general concept of molecular chaperones developed over next several years as with results of the studies by Ellis and his colleagues on the biogenesis of the chloroplast enzyme ribulose bisphosphate carboxylase-oxygenase (rubisco) that fixes carbon dioxide in photosynthesis. In the respect of the assembly of the enzyme rubisco in chloroplasts isolated from hi-

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gher plants seems to require the transient assistance from other proteins that are not a final component of the assembled bioactive rubisco enzyme. This brilliant discovery indicated that rubisco large subunits, newly synthesized by isolated intact chloroplasts, are bound non-covalently to another abundant proteins before transfer to the holoenzyme and it was proposed that this binding might be an obligatory step in rubisco assembly [3]. However, this experimental result was not met with much enthusiasm by many life scientists. Fortunately at this time a speculative paper by Pelham[4] throw a light on the Ellis's pioneering idea. Although Pelham did not use the term 'molecular chaperone', he proposed that members of the heat shock protein family in animal and microbial cells are involved in the assembly and disassembly of proteins in the nucleus, cytosol and endoplasmic reticulum (ER). Pelham also suggested that these proteins have a role in normal protein folding and association in unstressed cells, and that they are required in increased amounts when proteins have been damaged by some stress to unscramble protein aggregates which could then refold correctly, and to prevent further damage by binding to exposed hydrophobic surfaces. After Pelham's speculation and Ellis's hypothesis, this more generalized proposal has been steadily extended to a growing range of proteins and cellular processes by Ellis [5], Rothman[6], Gething[7], Lorimer[8] and Hartl[9]. In addition, chaperonin family of molecular chaperones was identified by Hemmingsen[10] in 1988, and other chaperonins were discovered from the several kinds of intracellular organelles such as chloroplast, mitochondria and intracellular symbiont[11]. The chaperonins are now regarded as just one family within the wider class of molecular chaperones. The formal nomenclature of molecular chaperone was determined at Copenhagen meeting in 1987[12] and subsequently it was published in Nature by Ellis[13]. Over the past three years, researchers have refined the models of protein folding in prokaryotes and eukaryotes. These should be accelated by X-ray crystallography and NMR analysis of molecular chaperones to solve their 3-D structure *in vivo*.

Definition of molecular chaperone

Classically, a newly synthesized (nascent) polypeptide was thought to attain its functional conformation with no assistance from other intracellular molecules and with no further expenditure of bioenergy. These self-assembly and folding principle stem from the classic observations of Fraenkel-Conrat & Williams[14], who showed that reassembly of infectious TMV is completed by incubating together the well separated and purified virion components, RNA and capsids, and of Anson[15] and Anfinsen[2], who found that some purified denatured proteins regain their characteristic biological activities on removal of the denaturing agents in the absence of other macromolecules. However, Creighton[16] observed that in many cases the denaturation of proteins is not fully reversible in vitro, especially at physiological temperatures and at proper protein concentrations *in vivo*. This proposal should be supported by the growing number of instances where proteins will not assemble correctly at the rates and yields required *in vivo* unless other pre-existing proteins are present to assist them. This type of pre-existing proteins in cell are called as molecular chaperones.

Molecular chaperone was defined by Ellis[5] in 1989 as a family of unrelated classes of protein that mediate the correct assembly of other polypeptides in the cell, cytosol and ER. However in case of the chloroplast, mitochondria and intracellular symbiont, they was named especially chaperonin. That is not involved ultimately in the final bioactive productions. It is now simply defined that a molecular chaperone is any protein which binds another protein and has no function of its own. In 19 93, Hendrick[17] defined more fully a molecular chaperone as a protein which binds to and stabilizes an otherwise unstable conformer of another protein and, facili-

tates its correct fate *in vivo* by controlled binding and release of the substrate protein : be it folding, oligomeric assembly, between active/inactive conformations.

The concept of chaperone has been used in Europe since long time ago. A chaperon literally means a middle-aged woman who, for the sake of money, accompanies a young unmarried lady as a guider, teacher and protector in public, like a *"Joong-Mae-Jang-Ie (중매쟁이)"* in Korean. Thus a traditional role of the human chaperone, if described in cell biological terms, is to prevent improper interactions between potentially complementary surfaces. Moreover, human chaperones do not possess the steric information by which people interact nor are they present it during married life. Molecular chaperone in the biological field is thus a very appropriate term to describe proteins which prevent incorrect interactions between parts of other molecules, but they do not impart steric information or form part of the final functional structures. When molecular chaperone is based on the story described above, PDI (protein disulfide isomerase), one of the well known folding enzyme in ER, is of course not included in the family of molecular chaperones since it is an enzyme which has catalytic activity to change other molecules.

Functions of molecular chaperones in the cell

The term of protein folding and assembly covers the broad function of molecular chaperones not only during several cellular processes under normal conditions, but also limited damage by stresses such as heat shock and environmental stimulations. On the other hand, it is possible to view the stress response as an amplification of pre-existing molecular chaperone function which all cells require under normal growth conditions, rather than as a novel function induced by stress. However, in general the molecular chaperone function is defined as the prevention of incorrect interactions between transiently exposed surfaces by the binding of chaperone molecules to those surfaces. And protecting of cells against the damaging effects of various stresses is main function attributed to molecular chaperones.

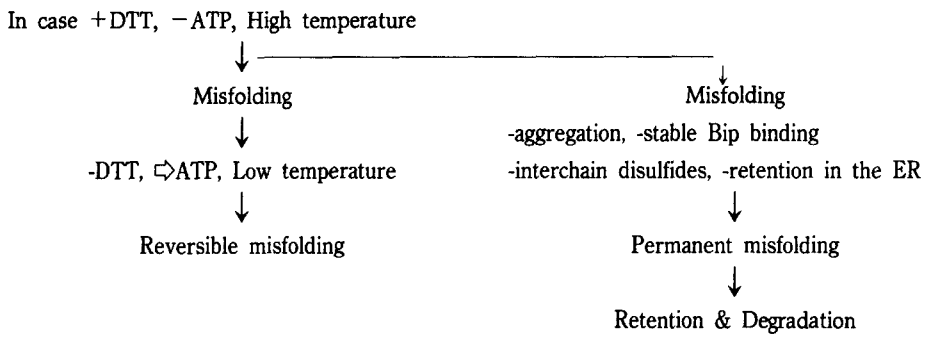
From the recent *in vivo* evidence[18] obtained from the *E. coli* RpoH mutant lacking most heat shock proteins overexpresses DnaK/DnaJ to prevent the aggregation of most nascent proteins, suggesting their function in the cell. Such a result has not been discovered yet in the eukaryotic cell, and then Kwon et. al.[19] has been trying the overexpression experiment of ER resident molecular chaperones such as Erp72, GRP94 and Bip. However, the result has demonstrated that these are only overexpressed for very short term (about 1-day) in the cultured cell, and that ultimately cells transfected by overexpression vector returned to the normal intracellular molecular chaperones, which result suggest that molecular chaperones are very important for interactions with other intercellular proteins for the normal cell physiology. when a kind of overexpression of a the molecular chaperone is not permitted for the cell survival because it make intracellular unbalance-conditions. The importance of molecular chaperones in the normal cell was also demonstrated by antisense RNA experiments against a kind of molecular chaperone mRNA. The result showed that all the cell transfected by antisense RNA were exterminated[20] for a week.

The emerging paradigm is that the binding and release of unfolded proteins are controlled through the regulation of chaperones by specific protein modulators (heat shock factors) and ATP hydrolyzing activities, and through the functional cooperation of different chaperones each other. As a result, newly made, unfolded, or partly folded proteins could be transferred in an orderly manner to the various polypeptide-handling system of the cell. An example of these another function is that a 73 kDa cytosolic protein called prp73 has been identified as a crucial component in the targeting for degradation of a protein containing KFERQ consensus peptide seque-

nce motif[21]. The prp73 is indistinguishable from mammalian hsp70 homolog in the biochemical functions, but the relationship between KFERQ binding and other functions of mammalian hsp70 is not known. If mammalian hsp70 recognizes these proteins for degradation, how does it interact with the machinery that transports them to and into the lysosome? In *E. coli,* abnormal proteins are rapidly degraded by the ATP-dependent La protease binding DnaK and GrpE proteins, together[22]. Intracellular proteolysis of puromycin-released peptides is reduced somewhat in DnaK mutants but is dramatically reduced in DnaJ mutants. It has been suggested that cooperation between the *in vivo* folding pathway and the protein degradation system would provide effective control over the level of abnormal proteins in the cell. From the author's experimental result, Erp72, a kind of ER resident molecular chaperone, degradates thyroglobulin that was demonstrated by methods of pulse-chase and 2-D gel[23].

Recently it is known that a number of intracelluar signaling molecules are found associated with molecular chaperones, which mediate the activity of various kinases and receptors. These suggest that modification of signaling molecules are manifest as changes in protein activities, oligomerization states, binding affinities, and signal channel properties[24-26]. In addition, it is also proposed that molecular chaperones may function as important regulators of gene expression by acting as switches in the formation of the appropriate transcription factor-transcription factor and transcription factor-DNA complexes. Such a mechanism would help ensure that complexes form at the right time and place in response to the correct signals. It is now clear that folding and assembly in the ER is a dynamic energy-driven process involving a host of cellular folding enzymes, proper environments, and various molecular chaperones. Following small summary shows the folding pathway in the ER as one of those functions.

Cotranslational folding	→	Posttranslational folding	→	Golgi
-folding begins		-folding of subdomains		
-signal sequence cleavage		-cycles of Bip binding and release		
-core glycosylation		-continued disulfide formation		
-disulfide formation		-folding intermediates		
-cycles of Bip binding and release		following next steps		
-orderly binding molecular chaperones		-subunit recognition and oligomerization		
		-carbohydrate trimming		
		-transport		



Future Aspects

The outline of the interaction of chaperones with newly synthesized proteins is known. However, many questions remain unsolved as to the mechanism of action of these ubiquitous proteins and as to the precise nature of their physiological roles *in vivo*. The answers will be unveiled by a sophisticated combination of biochemical analyses, molecular genetic *in vivo* and translational folding systems in vitro, which help powerfully to understand the chaperone-mediated protein folding and other components involved in these processes. Further insight for these functions will depend on the progress of several crystallographic laboratories in obtaining the necessary structural information on these fascinating components. And then eventually, by interdisciplinary approach involving cell biologists, biochemists, and biophysicists we may get detailed insight into how the protein has self-assembly and folding *in vivo* by chaperone-mediated interactions and how molecular chaperones work in a normal living cell, which are one of the most hot topics in cell biology and other biological fields. The stress response has begun to attract the attention of investigators interested in disease and medicine.

In medicine, the functions of molecular chaperones have to be fully understood at the molecular level in the near future, since some kind of irksome diseases are strongly associated with molecular chaperones. In pathogenic cells, correct folding and/or oligomerization of particular proteins fail to due to changes in their chaperone characterisity, and then an essential protein is not producted for the normal cell-life. It is known that some human viruses require host chaperones for their replication, assembly and folding of capsids. In addition, there is suggestive evidence that the molecular chaperones may represent important targets in a number of autoimmune diseases. Induction of the molecular chaperones has been observed in different tissues subjected to relevant traumas including ischemia / reperfusion. In ERSD (ER storage disease), misfolded or misassembled protein does not secret from the ER membrane and then it is accumulated in ER lumen. This result is also one of the demonstrations that molecular chaperones are associated with disease.

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So far known representive molecular chaperones[17), 27-32)].

Class name	Members	Proposed roles
TRiC family	TF55	binds thermally proteins, hydrolyzes ATP
	TRiC	promotes folding of actin, tubulin and luciferase
	TCP1	cytosolic equivalent of the chaperonin family
hsp60 family	GroEL	folding, refolding, stabilizes during heat stress
	hsp60	promotes folding/assembly, prevents aggregation
	hsp58	binds newly imported proteins
	Cpn60	required for assembly of rubisco
hsp70 family	DnaK	assembly/disassembly of replication complexes
	Bip/Grp78	binds unassembled/misfolded ER proteins
	Ssal-4p	stimulates protein transport into ER, mitochondria
	Ssc1p	promotes protein translocation into mitochondria
	ctHsp70	promotes insertion into thylakoid membrane
hsp90 family	hsp90	binds to steroid and dioxin receptor, aggregation
	hsp83	ATPase activity
	Grp94	binds to nascent ER protein after Bip
	hsp100	binds to actin and calmodulin
Nucleoplasmin	nucleoplasmin	nucleosome assembly and disassembly
	protein XLNO-38	ribosome assembly
	protein Ch-NO38	escort nascent r-protein
	nucleoplasmin S	
	GrpE	interaction with hsp70
	DnaJ	interaction with hsp70 and GrpE
	Lim	bacterial lipase folding
	Rb protein	binding of transcriptional factors
	prion	rogue molecular chaperone
	SecB	bacterial protein transport
	PapD	bacterial pilus assembly
	signal recognition particle	polypeptide transport
	subtilisin prosequence	improve the correct assembly
	α-lytic protease	help transportation of membrane
	ubiquitinated r-protein	ribosome assembly in eukaryotes
	trigger factor	posttranslational protein transport
	calnexin	recognition of misfolded protein in ER
	calreticulin	folding and assembly in ER
	PrtM/PrsA	folding of secreted bacterial protein
	Pro-sequences	protease folding
	PPIase	structural modification

In this paper, viral membrane proteins (viral molecular chaperones) are omitted, which are presented in detail at *Virology (1993), pp. 545-562* reviewed by Robert, R. W.

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초록 : 세포내인자로서의 정교한 기능을 하는 molecular chaperone.

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Molecular chaperone의 발견은 생명과학자들에게 살아있는 세포 내에서 어떻게 생체활성단백질이 만들어지고 유지되는지에 대한 자극과 함께 그것을 증명하기 위한 실험동기를 부여하였다. 초기에는 molecular chaperone이 nucleosomes의 assembly에 관여하는 단백질을 설명하기 위하여 사용되었으나, 지금은 기본적인 세포생리기능의 하나인 단백질의 folding과 assembly를 돕는 assistant protein으로 주로 사용된다. 단백질합성 뿐만 아니라 단백질수송, oligomeric structure의 assembly와 disassembly, heat shock을 포함한 각종 내, 외부스트래스에 의해서 변성된 단백질의 세포내분해와 회복에도 molecular chaperone이 관여하고 있다. 그러나 아직까지는 molecular chaperone들의 3차구조와 그들간의 상호작용에 관한 정보가 부족하여 크게 진전되지 못하고 있지만, 많은 연구자에 의한 정보축적으로 인하여 빠른 시일 내에 molecular chaperone의 세포내역할이 분명하게밝혀질것이다.