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*Review*

# Heat Shock Protein Magic in Antigen Trafficking within Dendritic Cells: Implications in Antigen Cross-presentation in Immunity

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**Dendritic cells (DCs) take up soluble- or cell-associated antigens and digest them, delivering fragments to the MHC class I pathway to display antigenic peptides to $CD8^+$ T cells, a process known as cross-presentation. The pathway requires that, in order to be degraded by proteosomes, the extracellular antigens must have access to the cytosol across the endosomal membranes. Although the cross-presentation phenomena was first identified in the 1970s, the molecular mechanism responsible for the translocation is still not fully understood. In this context, we have recently found that cytosolic heat shock protein (HSP)90 translocates internalized antigen to the cytosol in DCs. Our results revealed the important role that cytosolic HSP90 plays in cross-presentation by pulling out endosomal antigen to the cytosol.**

**Key words:** heat shock protein 90, dendritic cells, cross-presentation, proteasome, cytotoxic T cell immunity

$CD8^+$ and $CD4^+$ T lymphocytes recognize antigenic peptides in the context of MHC class I and class II molecules, respectively. The peptides presented by MHC class I molecules consist of 8–11 amino acids which are degradation products of endogenous cellular proteins by the proteasome. The peptide-binding groove formed by the $\alpha1/\alpha2$ domain of MHC class I molecules creates a $\beta$-sheet floor surrounded by 2 long $\alpha$-helices, yielding pockets to which anchor residues bind, and typically a peptide binds in the groove in an extended linear conformation [1]. Peptides associated with the MHC class II peptide-binding groove consist of 10–34 amino acids and are mostly derived from exogenous proteins internalized via phagocytosis/endocytosis. Thanks to the open ends of the peptide-binding groove of MHC class II molecules, the bound peptides are not limited to a certain length.

The heat shock proteins (HSPs) are molecular chaperones whose expression is elevated by stresses such as heat, in what is called the "heat shock response" [2]. HSPs are also referred to as stress proteins, whose expression is increased by conditions that cause protein denaturation or unfolding in the cells. Thus, HSPs are engaged in (i) promoting protein folding/refolding and preventing aggregation of misfolded proteins; (ii) targeting misfolded proteins for degradation by the proteasome; and (iii) facilitating protein transport. We proposed that the unique feature of HSPs—*i.e.*, binding to a wide array of peptides/proteins—is essential in the trafficking and degradation of antigens within cells, and is thus linked to antigen processing and presentation. In this review, we provide an overview of the antigen processing pathway - endogenous and exogenous antigen processing/presentation pathway, and consider how HSPs are

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integrated into the pathway. Our own recent results in this field are included in the presentation.

## Endogenous Antigen Processing

$CD8^+$ T cells recognize naturally processed peptides presented by MHC class I molecules [3]. Peptides associated with MHC class I molecules are mainly products of cellular proteins degraded by the ubiquitin-dependent proteasome system [3]. During *de novo* protein synthesis by the ribosome, nearly 3% of the proteins undergo misfolding, which occurs mainly due to mutational changes in the encoding gene, transcriptional and translational mistakes, and unsuccessful protein folding. These misfolded proteins are called defective ribosomal products (DRiPs) and are implicated as the main source of antigen peptides presented by MHC class I molecules [4] (Fig. 1).

DRiPs are prone to aggregation, there becoming good targets of the molecular chaperones HSP70/HSP40 and HSP90. These molecular chaperones hold DRiPs while recruiting the E3 ubiquitin ligase,

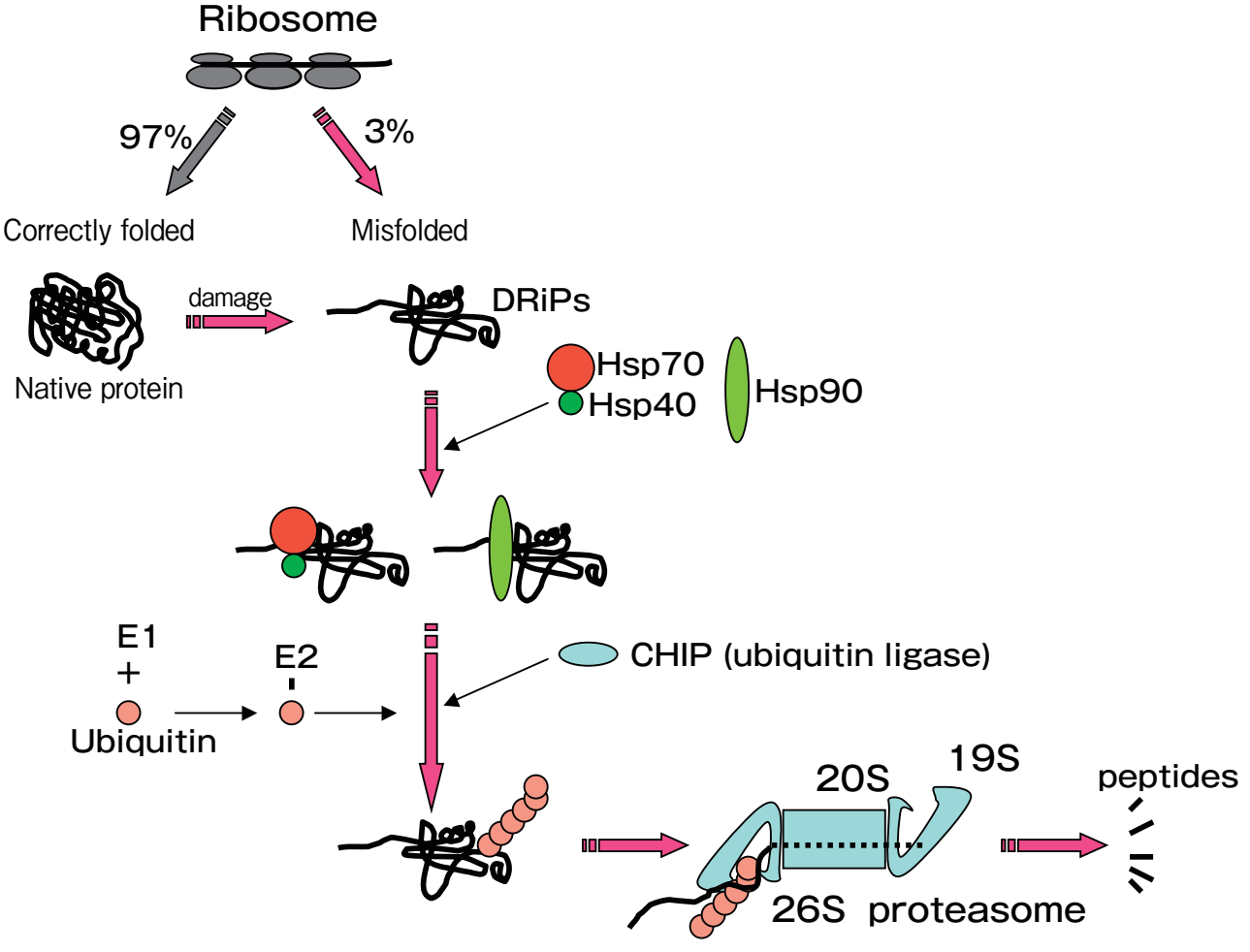


Fig. 1 Nearly 3% of newly synthesized proteins (either self or foreign antigens) on the ribosome undergo misfolded status, and are called DRiPs. The aggregation-prone DRiPs are dangerous because the aggregates eventually cause cell death. To prevent this unfortunate scenario, Hsp70/Hsp40 and Hsp90 sense and hold DRiPs, while simultaneously recruiting E3-ubiquitin ligases such as CHIP to polyubiquitinate for their degradation by the 26S proteasome. Just a few percentage points of the total amount of the produced peptides will enter the ER to associate with the newly synthesized MHC class I heavy chain-$\beta$2m complex. In the case of DRiPs, it appears that these wasted proteins are not truly wasted, because they are the main source of peptides presented by MHC class I molecules to $CD8^+$ T cells. Thus, imperfect ribosomal function makes perfect sense in terms of immune surveillance.

CHIP (carboxyl terminus of HSC70 interacting protein), to polyubiquitinate, which eventually causes degradation of DRiPs by the proteasome [5] (Fig. 1). This idea has not been formally demonstrated. Importantly, MHC class I expression is not downregulated in CHIP-deficient cells, indicating that additional unknown E3 ubiquitin ligase(s) are involved redundantly in the degradation of DRiPs. A novel E3 ligase should be identified so that its involvement in the MHC class I antigen processing pathway would be fully understood.

The peptides generated by the proteasome in the cytosol are ferried through transporter associated with antigen processing (TAP) molecules into the endoplasmic reticulum (ER), where they associate with the MHC class I-$\beta$2m complex to form stable trimolecular complexes [1]. These peptides are displayed on the cell surface in the context of MHC class I molecules to activate $CD8^+$ T cells.

## Exogenous Antigen Processing

Phagocytes such as dendritic cells (DCs) and macrophages take up extracellular antigens in the endosome and/or phagosome and digest them with lysosomal enzymes. Hence, the proteolytic fragments (peptides) are loaded onto MHC class II molecules that have been sorted with the guidance of invariant chain (Ii) from the ER, and MHC class II-peptide complexes are moved up on the cell surface to display those peptides to $CD4^+$ T cells [6], thereby stimulating B cell-mediated humoral immunity. Thus, internalized antigens undergo proteolysis in the endosome/lysozome and the resulting peptides are presented to $CD4^+$ T cells. Should this be the only pathway for presentation of exogenous antigen by antigen presenting cells (APCs), $CD8^+$ T cells specific to viral or tumor antigens would never be activated. To overcome this problem, DCs have the unique ability to forward even extracellular antigens from the endosome to the cytosol, where they are further forwarded to the proteasome. The exceptionally specialized machinery seems to be utilized to secure the priming of $CD8^+$ T cells after internalization of exogenous antigens [7], as described below.

## Cross-presentation Is Essential for Activation of $CD8^+$ T Cells by Exogenous Antigens

DCs are the most powerful APCs, able to prime naïve T cells to give rise to protective immunity of cytotoxic T cells ($CD8^+$ T cells) against cancers and infectious agents [7]. During viral infection or malignant transformation, any intracellular antigenic alterations in non-APCs must be presented to $CD8^+$ T cells, which is essential in fighting virus-infected cells and cancer cells [8]. However, cancer cells cannot act by themselves as professional APCs to prime T cells, although they express tumor antigens on the cell surface. In addition, DCs do not become infected by certain viruses, and thus cannot present such viral antigens directly to $CD8^+$ T cells. Therefore, DCs must internalize those neighboring tumor or infected cells, digesting them, and then present antigen peptides to $CD8^+$ T cells in the context of MHC class I molecules. This pathway is called cross-presentation [9], and is believed to be specific to DCs, not other types of APCs such as macrophages or B cells. In DCs, significant proportions of exogenous antigens within the endosome are translocated to the cytosol [10], and degraded by the 26S proteasome to render the peptides presentable by MHC class I molecules. The produced peptides enter the ER through TAP molecules to associate with MHC class I molecules, and move up on the cell surface to activate $CD8^+$ T cells.

## Mechanism of Antigen Translocation from the Endosome to the Cytosol

In cross-presentation, the pathway through which exogenous antigens can access MHC class I molecules is not fully understood. The delivery of exogenous proteins within the endosome to the cytosol might require the same protein complex machinery that is responsible for the dislocation of misfolded proteins in the ER. ER-misfolded proteins dislocated to the cytosol are polyubiquitinylated for degradation by the 26S proteasome, a process known as the ER-associated degradation (ERAD) pathway [11, 12]. A mechanistic explanation of the cross-presentation can be found in the indirect observation that the ER membranes may fuse with the nascent phagosome, referred to as the ER-phagosome fusion [13]. ER-phagosome fusion

allows internalized exogenous antigens to dislocate to the cytosol, followed by degradation by the proteasome, resulting in the production of antigen peptides presentable by MHC class I molecules [14, 15]. If this is true, the pore structure for translocation of the antigen to the cytosol is the Sec61 complex, which is utilized for dislocation of ER-misfolded proteins to the cytosol by the ERAD pathway. *Pseudomonas aeruginosa* Exotoxin A, an inhibitor for retro-translocation of ER-misfolded proteins, substantially blocked cross-presentation [10]. However, there is still controversy as to whether the Sec61 is required for cross-presentation, because the toxin has been reported to bind AAA ATPase cdc48 (in yeast)/p97 (in mammals) but not the Sec61 complex [10].

Exogenous proteins in the endosome must be relatively unfolded before being translocated to the cytosol, since the size of the membrane pore is not large enough for escape in keeping its native form [16, 17]. Such unfolded proteins are reminiscent of the DRiPs. If the unfolded antigen is similar to DRiPs, it must first be recognized by HSPs, both in the endosome and cytosol, for its smooth translocation and then refolded or degraded by the proteasome. In this context, HSPs might play critical roles that will be discussed in the next section.

## Indispensable Role of HSP90 in Antigen Cross-presentation

In considering how HSPs are involved in cross-presentation (in terms of translocation of endosomal antigens to the cytosol), there might be 2 distinct mechanisms. First, HSPs direct CHIP-mediated polyubiquitinylation of retro-translocated unfolded proteins for proteasomal degradation. Second, HSPs direct retro-translocation of proteins from the endosome to cytosol, a mechanism that is supported by evidence that Grp78 and cytosolic HSP70/HSP90 drive polypeptide into the ER and the mitochondria, respectively. Our recent observations clearly indicate that HSPs play a pivotal role in antigen trafficking, which links to antigen cross-presentation.

We recently found that molecular chaperones such as HSP90 play important roles in this antigen cross-presentation [18, 19]. The main results we obtained are as follows.

1. We generated HSP90$\alpha$-deficient mice by the homologous recombination method. Cross-presentation was significantly downregulated in bone-marrow-derived dendritic cells (BMDC) of HSP90$\alpha$-deficient mice, although endogenous antigen processing/presentation was only marginally affected. Also, cross-priming of HSP90$\alpha$-deficient mice after immunization with cell-associated antigen was downregulated. We originally expected that HSP90$\alpha$ deficiency would be embryonic lethal, since HSP90$\alpha$ is highly expressed in mouse ES cells, which suggests it would play an essential role in fetal development. Surprisingly, however, we found that the mice survived and grew normally although male mice had azospermia in the testis. The HSP90$\alpha$-deficient mice allowed us to analyze the role of HSP90$\alpha$ in the translocation of extracellular antigen into the cytosol.

2. Image stream analysis suggested that internalized extracellular antigen was translocated to the cytosol in an HSP90-dependent manner.

3. Chemical inhibitors of HSP90 blocked the association of extracellular antigen with cytosolic HSP90 and translocation of antigen into the cytosol, and thereby blocked cross-presentation.

4. An antigen translocation assay with purified endosomes containing extracellular antigen revealed that HSP90 is sufficient to pull antigen out of the endosome and into the cytosol.

5. As previously demonstrated, *in vivo* administration of cytochrome c induces apoptosis of $CD8^+$ DCs, because this DC subset is mainly involved in antigen cross-presentation. Extracellular cytochrome c is internalized and translocated from endosome to cytosol, resulting in induction of apoptosis. Importantly, the reduction of $CD8^+$ DC numbers is eliminated in HSP90$\alpha$-deficient mice, indicating that the translocation of cytochrome c into the cytosol is impaired in these mice.

A schematic incorporating our findings in a possible mechanism of the cross-presentation of extracellular antigen is depicted in Fig. 2.

## Concluding Remarks

For cross-presentation, extracellular antigen uptake into endosomes is followed by translocation of the internalized antigen from endosomes to the cytosol for proteasomal degradation. A major unresolved issue, which has been a mystery since the discovery of the

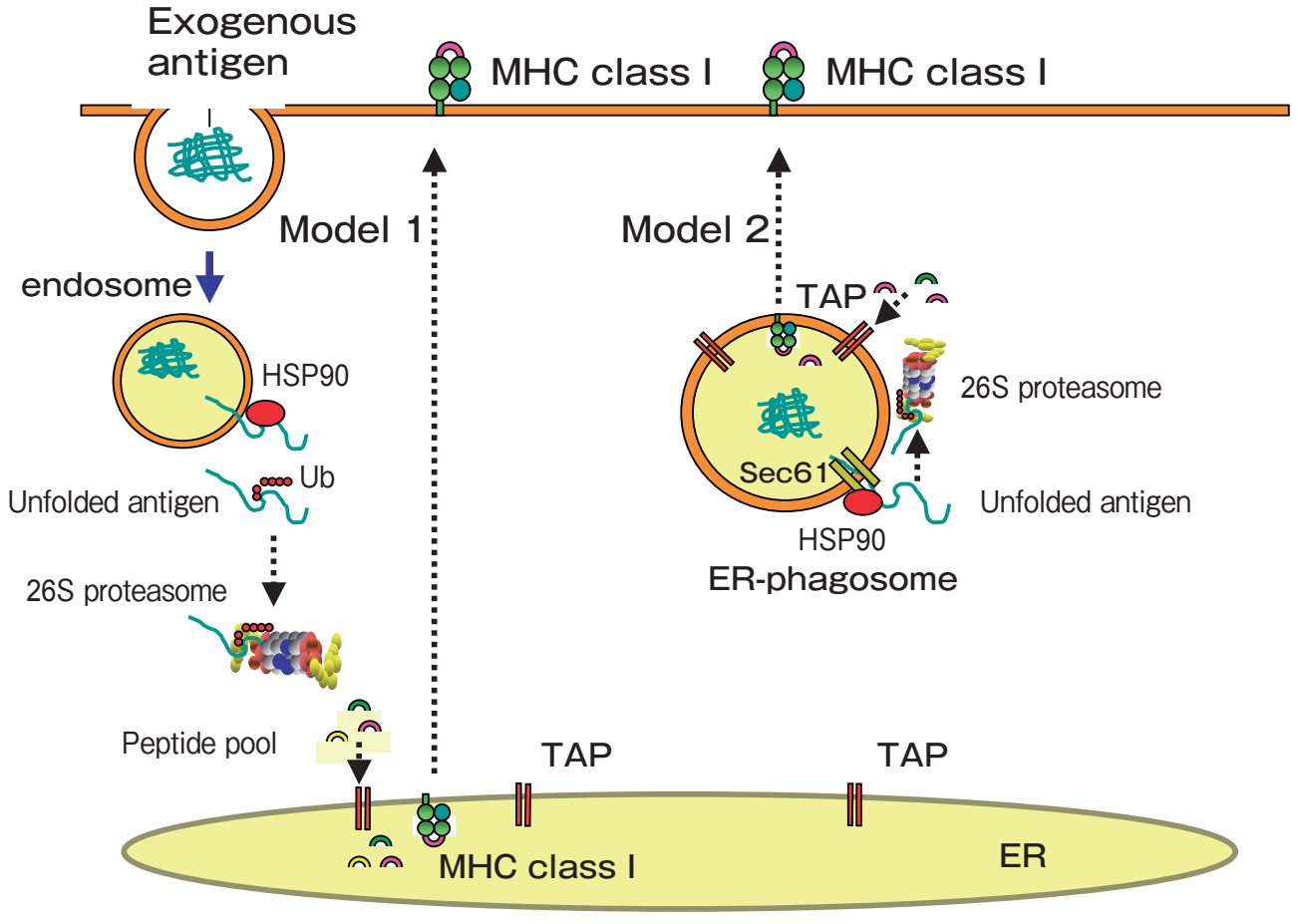


**Fig. 2** Models for cross-presentation of extracellular antigen by DCs after its translocation into the cytosol by HSP90. DCs uptake extracellular antigen through the endosome/phagosome pathway. Cytosolic HSP90 binds to the relatively unfolded antigen as it emerges across the endosomal membranes through a putative translocon (Sec61 complex ?) and promotes its translocation into the cytosol. Once the extracellular antigen appears in the cytosol it can be processed by the conventional MHC I pathway: degraded by the proteasome into short peptides that are loaded onto MHC I molecules in the ER. $CD8^+$ T cells then recognize the MHC class I-epitope complex on the cell surface. In model 2, the proteasome is attached on the cytosolic face of the ER-phagosome and immediately degrades the translocated antigen into short peptides. The peptides then re-enters into the ER-phagosome from which they are translocated.

cross-presentation phenomena in the 1970s, is how the antigen within endosome/phagosomes can be transferred across the membranes of these organelles for proteolysis in the cytosol. We believe that our findings, at least in part, have resolved the decades old issue.

Finally, growth factors such as fibroblast growth factor (FGF)-1/2 [20] and certain toxins like diphtheria toxin appear to utilize HSP90 for their translocation into the cytosol in non-antigen-presenting cells [21, 22]. In light of these evidences, the HSP90-mediated antigen translocation system might have developed as a common mechanism in a variety of cells, causing physiological outcomes such as transcriptional activation [20], intoxication [21, 22], and cross-presentation [18, 19].

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