found that in vivo and in vitro inactivation of Hsp90 caused dissociation of the 26S proteasomes into their constituents. Conversely, these dissociated constituents reassembled in Hsp90-dependent fashion both in vivo and in vitro. These processes were ATP-dependent and were suppressed by geldanamycin, an Hsp90 inhibitor. These results strongly suggest that the ATPase activity of Hsp90 is essential to the assembly and maintenance of the 26S proteasome and that Hsp90 plays some regulatory roles on the UPS pathway through assembly and disassembly of the 26S proteasome (Fig. 1). At this point of view, the complete dissociation of the 26S proteasome after severe heat shock might be worth mentioning. Our data showed that during 4-hour after incubation at 50°C, the activities and the amount of the 26S proteasome were repressed during this period and were partially suppressed by overexpression of Hsp90. Such suppression is rational, considering that the 26S proteasome presumably requires heat-shock protein, Hsp90, for its biogenesis. Hsp90s are also required for heat-damaged proteins and might be busy after thermal insults. The disappearance of the 26S proteasome is also reasonable because while cells must acquire vital proteins without protein synthesis, they have to refold heat-denatured proteins, which usually might be degraded by the 26S proteasome. In this regard, the regulation of the 26S proteasome by Hsp90 is important for cell viability under severe stress conditions, which might form part of a fundamental survival mechanism. Considering this regulatory role of Hsp90, impairment of the UPS pathway caused by protein aggregation[57] might be partially brought about by the collapse of this regulation; the protein aggregates deprived of Hsp90 in cells.

PERSPECTIVES

Although recent studies have revealed that multiple steps of interactions between molecular chaperones and the UPS pathway enable cells to survive stressful environments, our understanding of these interactions is not fully satisfactory. In the endoplasmic reticulum (ER) quality control system, the fate of unfolded proteins is regulated by two transcriptional programs to induce ER chaperones[58] and ER-associated degradation (ERAD)-components,[59] depending on the quality and/or quantity of unfolded proteins accumulated in ER. Since the degradation of unfolded proteins in both ER and cytosol is responsible for the cytosolic UPS pathway, more highly organized mutual interaction between cytosolic molecular chaperones and UPS might be required to give versatility to cells; cells have to change the proportions of unfolded proteins to be refolded or to be degraded in response to environmental conditions. Further identifications should be made to define these interactions and their physiological significance.

One of the unanswered fundamentally critical issues in the protein quality control system is how unfolded proteins are designated to either refolding or degradation in the cell. How does the cellular machinery know the degree of protein impairment? Is there a pathway for severely unfolded proteins incapable of refolding by the chaperone team to be processed through the UPS pathway for their degradation? Even though molecular chaperones and UPS are principal players that work jointly in this pathway, the possibility that as-yet-unidentified molecule(s) handles refolding and destruction of unfolded proteins cannot be excluded. Whether the cell can indeed manage such a balance awaits future study.

In this review, we focused on the Hsp70 and Hsp90 as chaperone molecules responsible for the quality control system, but we should keep in mind that ubiquitin is also a member of the heat shock family proteins. During the last decade, it has become evident that cells have at least two or more polyubiquitin genes encoding multiple ubiquitins in a tandem fashion,[6] and expression of the polyubiquitin gene is up-regulated in response to various stresses. This elegant way devised evolutionarily to produce ubiquitin efficiently means that large amounts of ubiquitin are required for cell survival under environmental stressful conditions. In fact, two polyubiquitin genes are not necessary in normally proliferative budding yeast, but they become essential under stress conditions. Thus, it is worth emphasizing that not only the refolding machine but also the degrading machinery is also stress-inducible. Intriguingly, inhibition of the UPS pathway induces heat shock-response,[60-62] and increased ubiquitin-mediated proteolysis can replace the essential requirement for the heat shock protein induction.[63] Moreover, molecular chaperones, such as Hsp70 and Hsp90, are responsible for the maintenance of functional states of the UPS pathway, particularly the 26S proteasome as mentioned above.[56] These observations uncover a strong functional link between UPS and molecular chaperones.

Various diseases are caused by failure of proper protein folding. Accumulation of protein aggregates, which are cytotoxic, is tightly linked to neurodegenerative diseases,[2] and the instability caused by misfolding is associated with cystic fibrosis, maple syrup urine disease, cancer,[64] myotonic dystrophy,[65] immunodeficiency,[66] and type 2 diabetes.[67] These facts indicate that regulation of intracellular balance between refolding and degradation is a critical issue for cells. We speculate that not only mutations of each protein, but also the deficiency of the chaperone or the UPS system may cause protein misfolding or aggregation. It is noteworthy to point out that proteasome inhibitors increase the frequency of ubiquitin-positive intracellular inclusions that carry the genes of many neurodegenerative disorders.[68,69] Therefore, one could assume that a critical aspect of various neuronal degenerative diseases is failure of protein quality control mediated by molecular chaperones and/or UPS. There is a great interest in the interaction between some putative protein folding diseases and the chaperone system or the UPS pathway. Further studies are required to clarify this issue molecularly.

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