((Commentary))

Heat Shock Protein Reports on Proteome Stress[†]

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Abstract

Proper regulation of protein homeostasis (proteostasis) is essential to maintain cellular fitness. Proteome stress causes imbalance of the proteostasis, leading to various diseases represented by neurodegenerative diseases, cancers, and metabolic disorders. The biosensor community recently embarked on the development of proteome stress sensors to report on the integrity of proteostasis in live cells. While most of these sensors are based on metastable mutants of specific client proteins, a recent sensor takes advantage of the specific association of heat shock protein 27 with protein aggregates and exhibits a diffusive to punctate fluorescent change in cells that are subjected to stress conditions. Thus, heat shock proteins can be also used as a family of sensors to monitor proteome stress.

The maintenance of proper protein homeostasis (proteostasis) is an essential function for all cells to survive [1]. Compromised proteostasis primarily occur under insults from either endogenous or exogenous stress conditions. These conditions include but not limited to temperature changes, chemical toxins, pathogen infections, and genetic mutations. The consequence of a compromised proteostasis involves primarily accumulation of protein aggregates in varying cellular compartments. In these compartments, cells have evolved sophisticated protein machineries that act as a proteostasis network to counteract loss of proteostasis by refolding misfolded proteins, disaggregating protein aggregates, and degrading these abnormal proteins when they cannot fold properly [2-4]. Loss of proteostasis has been associated with an increasing number of diseases, represented by neurodegenerative disorders, metabolic disorders, several types of cancer, and specific cardiovascular diseases.

To probe the biological mechanisms and to better develop therapeutic approaches for these diseases, new strategies are highly desired to report on cellular proteostasis under various stress conditions. Several protein-based sensors have been developed to detect stress-induced protein aggregation in live cells. Most of these sensors rely on metastable protein as a protein reporter. Within a compromised proteostasis, metastable protein sensors aggregate and produce measurable signal for detection. Examples are provided by firefly luciferase, a *de novo* designed retroaldolase, Halo-Tag, and barnase [5-9]. Thus, most present proteome stress sensors use aggregation of a specific metastable protein to flag proteome stress. This approach arrives with a primary limitation: Metastable proteins may not represent the entire proteome.

This limitation is overcome in a recent report wherein heat shock protein 27 (Hsp27) is used as a reporter to flag proteome stress [10]. Hsp27 (HspB1) is a small heat shock protein, whose main function is to provide support of cell survival under stress conditions[11]. The way that Hsp27 achieves this function is to stabilize misfolded proteins and inhibit their subsequent aggregation. Due to its association with protein

aggregates, Hsp27 has been regarded as a biomarker for stress granule and aggresomes [7, 12]. In non-stressed conditions, Hsp27 exhibits rapid diffusion constants in cells, thus showing diffusive fluorescence signal. When Hsp27 binds to stress-induced protein aggregates, the diffusion constants can be dramatically reduced, thus displaying granular and punctate fluorescent structure. Replying on this fluorescent signal change, the authors creatively use a Hsp27-GFP chimeric fusion protein to report proteome stress. Using HeLa cells as a cellular model, they demonstrate that the diffusive fluorescent signal of Hsp27-GFP effectively converts to fluorescent foci under several stress conditions, including an oxidative stressor Arsenite and a proteasome inhibitor MG132. Interestingly, incubating cells with aggregates formed by Aβ-peptide also induced numerous fluorescent foci in both cytoplasm and nucleus. These observations demonstrate that the Hsp27-GFP sensor is able to detect a wide range of proteome stress-induced protein aggregation in live cells.

Heat shock protein is a large family of proteins that can interact with misfolded proteins and their aggregates. Potentially, many members of this family can be used as reporter for proteome stress. Genetically fused fluorescent protein as described in this work is one approach that requires transfection. Chemically, it is feasible to conjugate fluorophores to molecules that specifically bind to heat shock proteins. Treating cells with these molecules would result in fluorescent heat shock proteins whose signal can be used as proteome stress sensors without the need of transfection. Thus, this work demonstrates heat shock proteins as an additional family to expand the design and use of proteome stress sensors.

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Figure 1: Heat-shock proteins can be used as proteome stress sensors. Fluorescent images of cells expressing the Hsp-GFP fusion chimeric proteins would exhibit signal changes from diffusive to punctate structure. Formation of the foci indicates is due to the high specificity of the heat shock proteins to stress-induced protein aggregates.

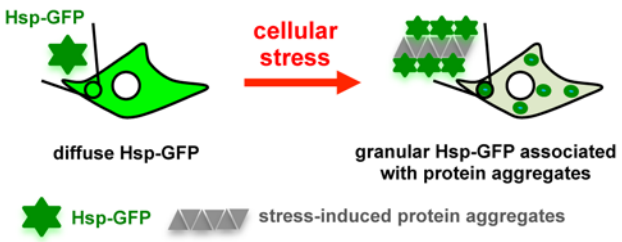

Figure 1