PROTEOMICS

A proteome-wide view of thermal stability

Proteome thermal stability is probed using limited proteolysis and mass spectrometry.

Most organisms are adapted to life in a narrow temperature range. Too hot or too cold, and cells start to die. This process is thought to be triggered by the broad denaturation of proteins, those delicate canaries of the cell.

However, the factors responsible for heat-induced cell injury are not fully understood. Most studies of thermal stability have been performed with purified proteins. To dive more deeply into such questions, Paola Picotti of ETH Zurich and colleagues embarked on a systematic project to study protein thermal stability on a proteome-wide scale within the context of the cellular milieu.

A few years back, the Picotti group coupled limited proteolysis with mass spectrometry to monitor global structural changes to the proteome in response to a perturbation. In this approach, a proteome sample is treated for short time periods with a broad-specificity protease, which cleaves proteins at locally unstructured regions. The resulting fragments are then subjected to shotgun mass spectrometry analysis for identification. By comparing the proteolytic patterns of a sample perturbed by a ligand or growth condition (for example) with a control sample, the researchers were able to identify proteins, and the regions of these proteins, affected by the perturbation.

In their new work, Picotti and colleagues applied this limited proteolysis method to samples treated with increasing temperatures. Applying the approach to *Escherichia coli*, yeast, an extreme thermophilic bacterium, and to human cells, they obtained high-quality thermal denaturation profiles for approximately 1,000 proteins in each species.

Picotti's team drew some intriguing conclusions from this rich data set. They found that thermally stable proteins were generally shorter and enriched in lysine side chains and β-sheet structures. Thermally unstable proteins tended to be longer and enriched in aspartic acid and α-helices. The team also found that abundant proteins were on the whole more thermally stable than less abundant proteins, which supports the 'translational robustness' hypothesis that states that abundant proteins have evolved to tolerate translational errors that can cause misfolding and aggregation. Overall, they speculated that heat-induced cell damage is driven by the denaturation of a relatively small set of functionally essential 'hub' proteins.

This approach could be combined with other denaturation triggers to enable a deeper understanding of the determinants of proteome thermal stability.

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RESEARCH PAPERS

Leuenberger, P. *et al.* Cell-wide analysis of protein thermal unfolding reveals determinants of thermostability. *Science* **355**, eaai7825 (2017).