Dynamics Proteomics Using Fluorescent Labeled Protein Library in Living Human Cells

Alex Sigal, Ron Milo, Ariel Cohen , Naama Geva –Zatorsky, Yuvalal Liron, Tamar Danon, Natalie Perzov, Lydia Cohen, Uri Alon

Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 76100

A quantitative understanding of human protein networks requires the measurement of endogenous protein dynamics in living cells. We have constructed a tagged protein library in a high-throughput method in human cells. This technique labels genes by stably and randomly integrating into the genome a DNA sequence coding for a fluorescent tag - YFP. We studied the temporal variability of protein levels and the cell cycle-dependent changes in the proteome of human cells, using time-lapse microscopy. This approach to dynamic proteomics can aid in discovery and accurate quantification of the extensive regulation of protein concentration and localization in individual living cells.

single cell dynamics, yfp tagged proteins , variability ,memory ,cell cycle dependence of nuclear proteins.

—Purpose

A quantitative understanding of human protein networks requires the measurement of endogenous protein dynamics in living cells. An ideal measurement system would: (a) work at the protein level, because regulation of translation, localization and degradation is crucial in mammalian cells; (b) work at the level of individual cells, because experiments that average over cell populations can miss events that happen in only a subset of cells. Furthermore, averaging might miss all or none effects, and cell-cell variability; (c) follow cells over extended periods of time to reveal phenomena such as oscillations and temporal programs; and (d) make minimal perturbations to the state of the cells.

I. Results

We have constructed a tagged library in a high-throughput method in order to tag proteins expressed by a human cell. This technique labels genes by stably and randomly integrating into the genome a DNA sequence coding for a fluorescent tag. It is marked as an exon by flanking splice acceptor and donor sequences. If the tag integrates within an expressed gene, it is then spliced into the gene's mRNA and a fusion protein is translated. We studied the variability of protein levels as well as the temporal dynamics of this variability (1) and the cell cycle-dependent changes in the proteome of human cells by systematically measuring protein dynamics in individual living human cells (2). We used time-lapse microscopy to measure the dynamics of a random subset of 20 nuclear proteins, each tagged with yellow fluorescent protein (YFP) at its endogenous chromosomal location. We synchronized the cells in silico by aligning protein dynamics in each cell between consecutive divisions. We observed widespread (40%) cell-cycle dependence of nuclear protein levels and detected previously unknown cell cycle-dependent localization changes.

II. Conclusion

This study presents a system for dynamic proteomics in individual living cells, based on a library of tagged clones and automated microscopy combined with image analysis. It indicates that single-cell dynamics proteomic can complement present microarray and proteomic methods for high-resolution, quantitative view of protein networks in individual living human cells

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References

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