Automation, Computation and the Single Cell: Illuminating Complete Protein Localization Networks

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Short Abstract* — Regulatory networks consisting of many inter-connected elements ensure reliable cell cycle progression and developmental transitions in bacterial cells. Notably, these networks rely on the dynamic three-dimensional deployment of signaling molecules, transcription factors and localization factors. We wish to understand the nodes, the topology, and the interactions of these networks in the bacterial model system *Caulobacter crescentus*. We developed a pipelined automated live cell microscopy procedure that included image acquisition, image processing, pattern recognition and statistical learning algorithms. Along with new techniques for absolute protein level quantification these results are being used to inform our systems level models in *Caulobacter crescentus.

Keywords* — systems biology, gene networks, protein localization, automated fluorescence microscopy, quantitative fluorescence microscopy, high content screening, asymmetric cell division, *Caulobacter crescentus.

I. Purpose

In prokaryotes, many important functions, including the establishment and maintenance of cell polarity, chromosome replication and segregation, cell division, and asymmetric positioning of polar organelles require dynamic localization of protein complexes [1, 2]. Despite advances in automated fluorescence microscopy and quantitative image analysis techniques, phenomena involving subcellular protein localization are still most commonly assessed in a non-quantitative way. Here we describe the design and application of automated quantitative fluorescence microscopy and an image acquisition and analysis platform optimized for screening hundreds of thousands of individual mutagenized bacterial cells to rapidly identify entire gene networks that affect the function and positioning of multiple target proteins.

II. Approach

We used *Caulobacter crescentus* as the target organism because localized proteins play a central role in its cell cycle regulation [3, 4]. Using an engineered Tn5 element we carried out conditional transposon mutagenesis. Custom software was used for full microscope automation and fast image acquisition. Automated image analysis was used to analyze several hundred individual cells per image field for several thousand mutants, and using statistical learning methods we identified those mutants belonging to protein distribution phenotypic classes distinctly different from unmutagenized controls.

III. Conclusions

Multidimensional hierarchical clustering of localization phenotypes allowed us to identify four statistically significant and systematically robust clusters of gene disruptions causing changes in the cell cycle timing and sub-cellular localization of reporter molecules. These results show that microscopy-based genetic screens can be radically accelerated through automation and computation. Epistasis experiments with this set of mutant strains allowed ordering of the protein localization network. This work shows that we can illuminate and properly order entire protein localization networks in microbial cells with a well-defined quantitative experimental procedure [5].

IV. Future

Having a technique to identify the nodes and topology of the gene/protein interaction network that effects the timing and placement of our localized reporter proteins is a first important step to understanding the complete network in a quantitative way. Now, in order to provide input to our models for cell cycle progression, we wish to have quantitative information on the abundance of the network proteins as a function of the cell cycle. Our preliminary data shows that we can measure the time varying absolute abundance of localized proteins in the living bacterial cell using an internal calibration exploiting the tetracycline Operator-Repressor system that sets the scale for the protein fluorescence signal.

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References

[1] Shapiro L, McAdams HH, Losick R (2002) Generating and exploiting polarity in bacteria. *Science* **298**, 1942–1946.

[2] Arigoni F, Pogliano K, Webb CD, Stragier P, Losick R (1995) Localization of protein implicated in establishment of cell type to sites of asymmetric division. *Science* **270**, 637–640.

[3] McAdams HH, Shapiro L (2003) A bacterial cell-cycle regulatory network operating in time and space. *Science* **301**, 1874–1877.

[4] Collier J, Shapiro L (2007) Spatial complexity and control of a bacterial cell cycle. *Curr Opin Biotechnol* **18**, 333–340.

[5] Christen B, Fero MJ, Hillson NJ, Bowman G, Hong S-H, Shapiro L, McAdams, HH (2010) High-throughput identification of protein localization dependency networks. *PNAS* **107**, 4681-4686.