A Systems Genetics Approach to the Sporulation Control Network in *Physarum polycephalum*

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ABSTRACT

In this paper, we describe a Systems Genetics approach to the sporulation control network in *Physarum polycephalum* based on the time-resolved analysis of signaling events in individual plasmodial cells. This work was presented at PhysNet2015.

Categories and Subject Descriptors

J.3 [**Life and Medical Sciences**]: Biology and genetics

Keywords

Physarum polycephalum, cell differentiation, systems genetics, Waddington epigenetic landscape.

1. INTRODUCTION

Cell differentiation is a widely-spread phenomenon among the eukaryotes and its regulatory control is causally involved in development, health, and disease [1]. While cells specialized through differentiation stick together and execute specific functions within well-organized tissues and organs of animals or plants, in many unicellular eukaryotes, specialized cells of different shape, function, and molecular composition occur in temporal order in the course of a life cycle instead of cooperating as building bricks of a body [9]. From humans to lower eukaryotes, differentiation events are often triggered by or depend on (environmental) signals that are sensed by the cell via specific receptor proteins and processed through a regulatory network of interacting biomolecules [1; 9] that displays the functional characteristics of a complex system [12].

The *Physarum polycephalum* plasmodium is part of a sophisticated life cycle involving active (amoeba, flagellate, plasmodium) and dormant (cysts, spherules, sclerotia, spores) cell types. Because of the natural synchrony of the plasmodial population of nuclei in cell cycle and differentiation and because of the experimental manipulations which the plasmodium allows, plasmodial sporulation was put forward as a model system of cell differentiation [10] and cell cycle control [8]. Together with the well-established classical genetics [2], the availability of next generation sequencing technologies for transcriptome sequencing, the genome sequence with its genome-wide repertoire of annotated genes [11], the *Physarum* plasmodium now at last has the chance to become a competitive model for the systems biology of cell differentiation at the single cell level.

2. APPROACH AND METHODOLOGY

The general concept is to follow the response and relaxation of the system to static and/or transient experimental perturbations by measuring the activity of biomolecules representing multiple nodes and targets of the regulatory network. Static perturbations are introduced through mutations in genes that influence or alter the function of the sporulation control network [5]. The gene dosage and hence the concentration of each wild type gene product tagged by mutation can be arbitrarily fine adjusted by fusion of wild type and mutant plasmodia in appropriate relative mass ratio.

Transient perturbations are performed by stimulating the phytochrome photoreceptor with a brief pulse of far-red light. At different time points after this photo-perturbation, samples are repeatedly taken from the same plasmodial cell to generate a single cell time series data set capturing the response to transient perturbation and eventually the relaxation of the system [6]. To analyse and evaluate the experimental data sets, we have purposefully developed a scalable computational framework for reverse engineering [3; 4], for modeling, and for simulation [7] of the structure and the functional dynamics of the regulatory network.

3. DEVELOPMENTAL TRAJECTORIES OF INDIVIDUAL PLASMODIAL CELLS

Sporulation of competent plasmodial cells was triggered by a brief pulse of far-red light. Samples were taken from each plasmodium at different time points after the pulse and the expression pattern of the set of 35 genes used as marker for commitment and sporulation was analysed with a multiplex RT-PCR assay. Principal component analysis of the data set revealed that individual plasmodia take different trajectories from the plasmodial to the sporulated state. The location of starting and end points of the trajectories of sporulating plasmodia and of plasmodia that could not sporulate because the stimulus intensity was too low mapped within specific regions of the PCA plot [6]. This supports the idea that the plasmodial and the sporulated differentiated state are the result of alternative cellular attractors within a quasi-potential landscape of the multistable system that regulates cell differentiation [12]. It also suggests that individual cells indeed take different trajectories through this Waddington-type landscape [6].

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Comparative analysis of the expression kinetics of pairs of genes revealed different patterns of co-regulation showing how programs of differential regulation vary between individual plasmodial cells [6]. Mutations in genes that impact the ability of the plasmodium to sporulate in response to stimulation qualitatively changed the pattern of gene co-regulation [6], suggesting that mutations may alter the topology of the quasi-potential landscape while creating new attractors that are reached through altered programs of differential regulation.

4. CONCLUSIONS

The *Physarum* plasmodial cell provides ample homogeneous material for system-wide and time-resolved single cell studies on cell differentiation in a genetic model organism. The results obtained so-far offer a straight-forward way to reverse engineer the topology of the regulatory network controling the developmental decision to sporulation and to infer those structural elements that determine the functional dynamics of this complex system.

Orthologs of many regulators that are involved in differentiation control of mammalian cells, stem cells, or germ cells are present in the *Physarum* genome [11]. Hence, analysis of the sporulation control network may finally contribute to a systems level understanding of analogous processes in mammalian cells. Studies on *Physarum* may also provide experimental evidence contributing to a general understanding of the impact of mutations on the dynamic behavior of regulatory networks in the context of a Waddington-type quasipotential landscape. As pharmacological compounds are reagents that modulate the activity of individual nodes or of multiple nodes of a network (like mutations do), the relevance of such insight may not remain purely academic.

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6. REFERENCES

[1] Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P., 2008. *Molecular Biology of the Cell*. Garland Science, New York.

[2] Dee, J., 1987. Genes and development in *Physarum*. *Trends Genet. 3*, 208-213.

[3] Durzinsky, M., Wagler, A., and Marwan, W., 2011. Reconstruction of extended Petri nets from time series data and its application to signal transduction and to gene regulatory networks. *BMC Systems Biology 5*, 1 (Jul 15), 113.

[4] Marwan, W., Sujatha, A., and Starostzik, C., 2005. Reconstructing the regulatory network controling commitment and sporulation in *Physarum polycephalum* based on hierarchical Petri net modeling and simulation. *J. Theor. Biol. 236*, 349-365.

[5] Rätzel, V., Ebeling, B., Hoffmann, X.K., Tesmer, J., and Marwan, W., 2013. *Physarum polycephalum* mutants in the photocontrol of sporulation display altered patterns in the correlated expression of developmentally regulated genes. *Develop. Growth Differ. 55*, 247-259.

[6] Rätzel, V. and Marwan, W., 2015. Gene expression kinetics in individual plasmodial cells reveal alternative programs of differential regulation during commitment and differentiation. *Develop. Growth Differ. 57*, 408–420.

[7] Rohr, C., Marwan, W., and Heiner, M., 2010. Snoopy--a unifying Petri net framework to investigate biomolecular networks. *Bioinformatics 26*, 7 (Apr 1), 974-975.

[8] Rusch, H.P., Sachsenmaier, W., Behrens, K., and Gruter, V., 1966. Synchronization of mitosis by the fusion of the plasmodia of *Physarum polycephalum*. *J. Cell Biol. 31*, 204-209.

[9] Russo, V.E.A., Cove, D.J., Edgar, L.G., Jaenisch, R., and Salamini, F., 1999. *Development: Genetics, Epigenetics and Environmental Regulation*. Springer.

[10] Sauer, H.W., Babcock, K.L., and Rusch, H.P., 1969. Sporulation in *Physarum polycephalum*. A model system for studies on differentiation. *Exp. Cell Res. 57*, 319-327.

[11] Schaap, P., *et al.*, 2015. The *Physarum polycephalum* genome reveals extensive use of prokaryotic two-component and metazoan-type tyrosine kinase signaling. *Submitted.*

[12] Zhou, J.X. and Huang, S., 2011. Understanding gene circuits at cell-fate branch points for rational cell reprogramming. *Trends Genet 27*, 2 (Feb 1), 55-62.