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Genetic Regulatory Networks:
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14. ABSTRACT The objective of this research is to understand how genetic regulatory circuits function. The behavior of genetic regulatory networks in bacterial cells is analyzed using computer-based simulations of molecular-level models, positional information from observation of GFP-labeled regulatory molecules, and gene expression data from microarray assays of wild-type and mutant Caulobacter cells. A new view of the cell cycle as a sequence of modular functions controlled by a few networked master regulatory proteins challenges former paradigms for how the cell works and how to model cell function.

15. SUBJECT TERMS

bacteria, genetic regulatory circuits, regulation

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FINAL REPORT

GRANT #: N00014-99-1-0563

PRINCIPAL INVESTIGATOR: Dr. Harley McAdams

INSTITUTION: Stanford University School of Medicine

GRANT TITLE: Genetic Regulatory Networks: Analysis and Simulation

AWARD PERIOD : 04 April 1999 - 30 September 2002 (with extension)

OBJECTIVE: The objective of this research is understand how genetic regulatory subsystems function and to elucidate the "engineering" design principles for biochemically based signal processing in individual cells and multicellular colonies.

APPROACH: We analyze the behavior of genetic regulatory networks comprised of coupled genetic and protein-level reactions using computer-based simulations of molecular-level models, positional information from observation of GFP-labeled regulatory molecules, and gene expression data from microarray assays of wild type and mutant Caulobacter cells. Experimental data for these studies is developed in close collaboration with (separately funded) experimentalists in the Shapiro laboratory at Stanford.

Several recent publications, including some originating in previous phases of this ONR project, have shown it is possible to model performance of relatively small regulatory circuits at a great level of detail. Examples include individual switches, sensory subsystems, or modules that execute a cellular function such as flagellar construction. To understand the overall functioning of cellular level regulation, however, we believe one has to take a systems perspective and consider performance of the circuit at a higher level of abstraction. In electronics this is termed analysis of the system architecture. But, biology is an experimental science, and experience shows that generalizations and abstract models that are not closely tied to observation are likely to be wrong. We have pursued several simultaneous experimental thrusts to develop a global picture of the regulatory circuitry that drives the cell cycle forward and implementing asymmetric

cell division. We analyze the results to extract the higher level system organization from the experimental details.

ACCOMPLISHMENTS:

We have developed bioinformatics tools and performed data analysis of gene expression microarray data to characterize the complex genetic circuit that regulates cell cycle progression and asymmetric cell division in *Caulobacter crescentus*. (This work has been in collaboration with the separately funded *Caulobacter* research program in the Shapiro laboratory at Stanford.) Analysis of time-sampled gene expression profiles for synchronized *Caulobacter* populations has identified 590 genes that are regulated in a cell cycle-dependent manner. We showed that nearly 25% of these are directly or indirectly regulated by a single global regulatory protein, CtrA, that thus plays a major role in coordinating cell cycle functions (5).

With additional funding from the DARPA Bio/Info/Micro program and the DOE Microbial Cell program, a concerted multi-disciplinary effort to identify all aspects of genetic regulation in *Caulobacter* has been initiated. This ONR project has been seminal the establishment of this undertaking. This effort is targeted to provide the most complete picture of cellular regulation available for any organism. Early results from this global regulatory analysis project are revolutionizing previous conceptions of bacterial cell cycle regulation.

The higher level architecture of the bacterial cell's control system organizes and coordinates the processive modular functions that do much of the work of the bacteria cell in order to execute the bacterial cell cycle. These modules include multiprotein machines, multiprotein signaling pathways, and co-regulated enzyme systems that implement metabolic pathways. We showed that these modules are created when and where they are needed in the cell and destroyed when they are not, and once they are launched they tend to act autonomously (e.g., modular metabolic pathways) or processively (e.g., the replisome, RNA polymerase, and the construction of structures such as flagella and pili) to finish their job (5,6,7).

We have shown that the *Caulobacter* cell cycle is an integrated system comprised of subsystems that perform

signal processing and control paradigms involving highly interconnected nets of nonlinear response elements. The view of the cell cycle as a sequence of modular functions controlled by a few networked master regulatory proteins provides a new paradigm for how the cell works and for modeling the cell.

PATENT INFORMATION: No patent applications were filed.

AWARD INFORMATION: Dr. McAdams was promoted to Associate Professor (Research) at Stanford during the period of this grant.

PUBLICATIONS AND ABSTRACTS (for total period of grant)

1. Hung, D., McAdams, H. H., Shapiro, L. "Regulation of the Caulobacter Cell Cycle". In Microbial Development, ed. Y. Brun, L. Shimkets. Washington, DC: ASM Press, (1999)

2. McAdams, H. H. and A. Arkin, "It's a noisy business! Genetic regulation at the nanomolar scale," *Trends in Genetics*. 15:65-69 (1999)

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4. Judd, E. M., M. T. Laub, H. H. McAdams, "Toggles and oscillators: New genetic circuit designs". *BioEssays*. 22:507-509 (2000)

5. Laub, M. T., H. H. McAdams, T. Feldblyum, C. M. Fraser, L. Shapiro, "Global analysis of the genetic network controlling a bacterial cell cycle". *Science*. 290:2144-2148 (2000)

6. Michael T. Laub, Lucy Shapiro, Harley H. McAdams, "Global Approaches to the Bacterial Cell as an Integrated System" in The Bacterial Chromosome, Ed. N. Patrick Higgins. ASM Press, Washington DC. In press.

7. Laub, M. T., Chen, S. L., Shapiro, L., McAdams, H. H., "Genes directly controlled by CtrA, a master regulator of the Caulobacter cell cycle," *Proc. Nat. Acad. Sci.*, USA. 99(7):4632-7 (2002)