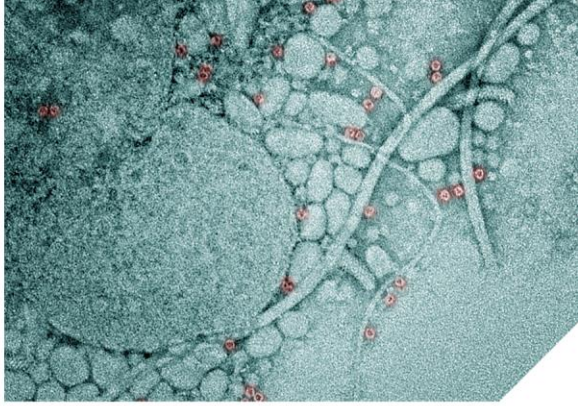
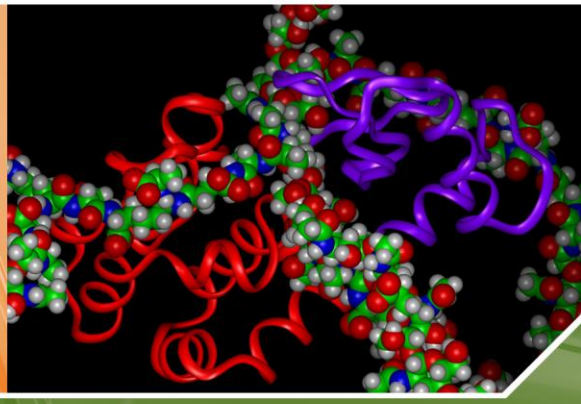


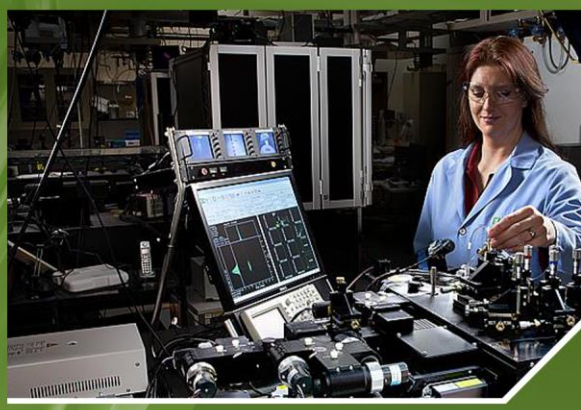
Biological Interactions and Dynamics

Science Theme Advisory Panel



June 14–15, 2012

Report: August 2012



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EMSL Biological Interactions and Dynamics—Science Theme Advisory Panel

S Kaplan
SE Baker

Report: November 2012

Prepared for the U.S. Department of Energy's Office of Biological and Environmental Research under Contract DE-AC05-76RL01830

Pacific Northwest National Laboratory
Richland, Washington 99352

Executive Summary

In early 2012, Dr. Scott Baker and Dr. Samuel Kaplan were tasked as co-chairs in overseeing fulfillment of the annual Science Theme Advisory Panel (STAP) meeting to discuss issues relevant to the Environmental Molecular Sciences Laboratory's (EMSL) Biological Interactions and Dynamics (BID) Science Theme. *Cell and Organellar Cycles and Their Underlying Roles within the Design of Biological Rhythms* became the meeting's focus (EMSL's BID-STAP agenda is featured in Appendix C).

The STAP's highest priorities were devoted to the following:

- Cutting-edge, high-quality science
- Ensuring that subject areas benefitted both EMSL and DOE biologically relevant areas, e.g., gene/phenotype, single-cell analyses, imaging, bioenergy, and synthetic biology
- Introducing an area of science not previously residing within EMSL's current scope.

The BID-STAP invitees' research interest areas, which primarily involve cell and organelle division as they affect or are affected by biological rhythms, have a basic commonality that readily supports and engages both EMSL and DOE's basic interests within relevant biological areas. Their research involves cellular processes that exist at the single-cell level, demonstrates genotype/phenotype, takes place in three-dimensional space in a temporal manner, and is the subject of intense scrutiny in the area of synthetic biology. Cell and organelle division and natural cyclical rhythms of biological systems are the controlling elements and cellular milieu where gene expression is embedded.

With these features and goals in mind, representatives from seven research laboratories were invited to EMSL to participate in the BID-STAP. The invitation (Appendix A) offered the motivation and described the meeting's goals. The topics discussed included: bacterial cell division, mitochondrial division, chloroplast division, organelle response to internal and external pressure, "clocks" in the cyanobacteria, and rhythms in fungi related to division and gene expression. Along with the invitees, EMSL's Capability Leads, its leadership, and both EMSL and Pacific Northwest National Laboratory (PNNL) staff were among the meeting participants (refer to Appendix B). In keeping with its open-forum premise, individual presentations were punctuated by a continuous stream of discussion among all participants. These discussions were lively, informative, and served to introduce EMSL to this branch of the scientific community. Notably, the invited speakers repeatedly expressed they typically would not attend each other's disciplinary meetings, making this advisory panel meeting a unique forum to cross fertilize their disparate areas.

Most apparent in these discussions was the fact EMSL has much to offer these areas of biological science. The scientists projected needs for future capabilities within EMSL that could serve cutting-edge research efforts for their programs and the community. The assembled group also expressed a series of underlying commonalities of research needs and questions that aligned their diverse interests and met the particular EMSL charge by providing a unique theme that could serve to unite their efforts and be a research focus for EMSL: *Organizational Principles of Biological Machines.* This descriptor elevates the study of biological machines to focus on defining the "design principles", not biologic-specific machines, that serve living systems in time and space and to define the machinery that accomplishes living functions. Such an effort would cut across phyla, genera, and species. If undertaken with EMSL's guidance and support, these studies could involve the breadth of the biologic world. Still, for practical considerations, they would be sharply focused to the kinds of studies provided within EMSL.

Acronyms and Abbreviations

BID	Biological Interactions and Dynamics
DOE	Department of Energy
EMSL	Environmental Molecular Sciences Laboratory
ER	endoplasmic reticulum
PNNL	Pacific Northwest National Laboratory
STAP	Science Theme Advisory Panel
ts	temperature sensitive

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1.0 Introduction

The 2012 Biological Interactions and Dynamics Science Theme Advisory Panel (BID-STAP) was orchestrated to engage a group of scientists to deliver their perspectives on a new, cutting-edge, and important science area. In consideration of said opportunity, several important considerations were kept at the forefront:

- The STAP's Science Theme must remain congruent with EMSL's capabilities and potential.
- Any Science Theme must support the U.S. Department of Energy's (DOE) mission in areas such as genotype/phenotype, single-cell analyses, imaging, biofuels, and synthetic biology.
- The Science Theme should be compatible with Pacific Northwest National Laboratory (PNNL) strategies.

Given these prerequisites, careful considerations were employed to evaluate a number of biological areas, resulting in pursuing research surrounding cell and organelle division, which both influence and are affected by cellular rhythms, or "circadian clocks."

The process of cell division within the cell cycle is fundamental to all living systems. Cell division marks a discrete event from which all temporal, spatial, and functional cellular activities can be measured. For example, in the area of synthetic biology, many researchers are investigating the deployment of "tool kits" that can be assembled in a particular host system. These tool kits enable production of a certain product or imbue the host organism with a specific behavior. As such, the "synthetic" bioproduct can possess a commercial or sensory value or become part of a larger, more complex biological consortium. Whatever the case, the "synthetic system" is the product of genotype enabling phenotype. Regardless of the experimental system, one particular principle must be maintained intact: the assembly of the synthetic tool kit and its usefulness must exist within a living cell capable of cell division. Unless the newly constructed cell can divide, the tool kit is useless. Thus, another aspect of this approach is simply not to understand the process of cell and organellar division but to develop these underlying genotypic/phenotypic parameters into an exportable tool kit in their own right.

To optimize the usefulness of a synthetic system, the system's functions within the dividing cell must be controllable. To maximize the opportunities offered by the deployment of the tools and outcomes associated with synthetic biology, fundamental questions must be addressed and resolved, including:

- Does the system function throughout the cell cycle or only at a certain point or time, i.e., are the genes involved always "on" or are they on only when certain other physiological function are enabled?
- How does any change in physiology, e.g., carbon and energy flow, affect the cell division process in time and space?
- Are the genes involved more likely to be expressed when certain internal environmental parameters are enabled, perhaps within an organelle?
- Are the genes involved more likely to be expressed only when certain environmental parameters are present during the light or dark cycle?

In bringing together scientists for EMSL's BID-STAP, careful considerations were expressed regarding the experimental systems that would be on display. These systems clearly would be at the forefront of their respective fields of research; exploitable at the single-cell level; and subjectable to *in situ* image analyses at the single-cell level, employing wild-type and mutant variations as the base subject matter for interrogation. They also would be capable of exploitation as a unique biologic entity in the form of a tool kit affected by the rhythmic oscillations that characterize living cells. Despite the

disparate nature of these experimental systems, the commonalities at the genotype/phenotype level provide a unifying theme to enable their consideration as a distinct and unique totality for further study.

Commonalities among speakers and attendees gave rise to a mutual research theme: *Organizational Principles of Biological Machines*, as each of the experimental systems could be characterized as a biological machine with more in common than different. Similarities in the systems at the genotypic and phenotypic design level and the fact that each is best studied through *in situ* imaging at the single-cell level, where individual experimental systems are steeped in spatial and temporal parameters, was especially notable. In addition, each system can be used to address the temporal nature of gene expression within the cell cycle. Finally, within the eukaryotic cell, each system is subject to rhythmic oscillations that affect genotype/phenotype, yet each imposes its own influence upon overall cell rhythmicity.

There was considerable interest in the science presented, as well as the role that EMSL could play in furthering pursuits these areas. At the BID-STAP's conclusion, it was strongly determined that there is a future for EMSL, PNNL, and DOE in understanding organizational principles of biological machines. The issues of how, when, and under what conditions was left to future considerations. The summary of this advisory panel workshop represents both the conclusion of a unique convergence of biological science and, simultaneously, the beginning of the next steps in this process.

2.0 Cell Division in Prokaryotes

All bacterial cells undergo cell division, and, in general, they use the same or a variation of similar division machinery. Division machinery studies have been enabled by modern, *in situ* cell imaging techniques and mutant organisms, some of which are temperature sensitive (ts). All of these systems have been characterized as composing a cell division machine that exists both spatially and temporally within the cell. Many (or certainly most) of the division machine components and their respective genes are known, their sequence of action has been ascertained, and their cellular residence visualized. Yet, the nature of their interactions, stoichiometries of these interactions, and intermediate assemblages during the construction of the machine, as well the structures of the components, are not understood.

2.1 Cell Division Machinery

Cell division in bacteria is termed "transverse binary fission" because at some point in the cell cycle, the bacterial cell appears to "pinch" in the middle and divide in half. It is now understood that the FtsZ ring forms mid-cell, resulting in a force for constriction generated by the Min system. The Min system prevents polar localization, causing localization by default at mid-cell with the daughter nucleoid and ultimately resulting in depolymerization of the ring. To demonstrate the ubiquity of this system, chloroplasts have three different FtsZ-like proteins, and mitochondria have two, with FtsZ present in almost all prokaryotes. However, it is not found in *Crenarchaea*.

In all systems, isolated FtsZ can be viewed as a polymeric structure. However, the details of the division machine are far more complex. At a point in the cell cycle, the synthesis of FtsZ is triggered (time of gene expression), which may have something to do with the nuclear cycle and increasing cell volume. Yet, the timing of this event depends on the status of the cellular physiology. As synthesis of FtsZ takes place, polymerization of FtsZ occurs on the cell membrane, and its localization to mid-cell is affected by the Min protein that oscillates back and forth between the two poles—preventing FtsZ localization from occurring elsewhere. As the localization of FtsZ occurs, additional proteins (approximately 10) are synthesized in a specified order (time) and localize to the developing Z-ring in a temporal sequence (spatial). During this 10-minute process, the Min system continues to oscillate between the cell poles. The precise function of each protein, the structure of each protein, the interactive forces and stoichiometry between the participating proteins, and the nature of each complex within the finished ring are all unknown. However, the tools and experience found in EMSL could be of substantial benefit in decoding these processes.

At some point, cell wall enzymes are recruited within the periplasm (outside the protoplast), resulting in the synthesis of a new septum mid-plane, which follows the constriction of the protoplast to give rise to two daughter cells. The genesis of the final constriction ring is under active study. The FtsA protein interacts with FtsZ and is an actin-like protein that binds adenosine triphosphate (ATP) and is an early contributor to final ring formation. It also may provide the constrictive force to protoplast division. The A protein is known to interact with the FtsN protein, which may help tether it to the cell membrane. At its cytoplasmic domain, FtsI also interacts with FtsA. Following cell division, the completed FtsZ ring structure falls apart. Several factors are known to participate in the process. Also, as this process nears completion, nucleoid partitioning occurs. Precisely how it is linked to protoplast division and septum formation is not well understood. Nonetheless, this process is so fundamental to cell biology that without a fuller understanding, harnessing gene expression for "product" development will not fully occur.

2.1.1 Defining Cell Division Machinery

Determining how EMSL could play a major role in defining the nature of these biological machines began with speaker Dr. William Margolin (University of Texas-Houston Medical School) describing cell division machinery of *E. coli*. Ultimately, there are numerous questions to pose and answer before the division machine is fully understood. However, there are some practical applications embedded within this process, such as how the use of conditional lethal mutants (ts) "freezes" the process, which can be fixed for some time before cells begin to die. Relief from the freeze results in a population of cells that are synchronized during subsequent rounds of cell division. Because the FtsZ protein (or others) has its homologue in other prokaryotic systems, the substitution of a mutant (ts) *E. coli* gene for its homologue may permit synchronization of any cell population. This population enables researchers to determine if a particular gene or gene complex is functioning throughout the cell cycle or, perhaps, at only a particular time in the cycle.

If the cell can be held for some period of time during the division process, it may be possible to increase the number of nucleoids per cell to a point where DNA sequencing of an individual cell becomes routine. Comparison of the DNA sequence of a series of individual cells in the cycle speaks to the heterogeneity of the chromosomal content within a cell population. Knowing the genes and proteins that compose the division machine heightens the possibility that these can be assembled into a functioning tool kit, which may be exported to different cell types in the design of a functional biologic processing machine. However, the machine has its own intrinsic design principles, e.g., the principles of localization, temporal expression, construction sequence, membrane association, and topological orientation, among many others—all principles yet to be defined. Such principles are extrinsic to the particular machine in question, but they could be exploited (if understood) to construct a purely synthetic machine and enable a new cellular function. Furthermore, in eukaryotes, organelle division both is influenced by and affects the biological clock or cellular rhythms, which govern gene expression both at the organismal level and within the organelle. Prokaryotes that have a defined clock, the cyanobacteria, are choice models for understanding the interactions between the clock and division cycle and, ultimately, to the optimal entrapment of light energy and carbon sequestration.

If DOE has a goal to harness and develop biologic systems in prokaryotes and eukaryotes, understanding and using these design principles are essential to all future efforts. Likewise, as seen in cell division of *E. coli*, EMSL's capabilities are precisely those required to unravel these design principles.

3.0 Organelle Division

Biologists are universally aware that cellular organelles, such as mitochondria and chloroplasts, have their origins in the capture of some primitive prokaryote by the nascent eukaryote cell type. Unlike animal or fungal systems, plants have both types of organelles. As such, the evolution of the plant or algal cell has served to accommodate each organellar type, and each organelle has evolved mechanisms essential to maintain both their individuality and cooperativity with one another and the nucleus. To exploit plant or algal systems for "product development," there must be an understanding of how the "cell cycle" of each organelle is manifest within the greater whole and how the machinery of the clock serves to integrate these disparate requirements to effect optimal cellular physiology.

3.1 Mitochondrial Division

As part of the BID-STAP, Dr. Jodi Nunnari (University of California, Davis) presented a discussion of mitochondrial division in *Saccharomyces cerevisiae*. Her studies reveal that what is true for yeast is, in principle, also true for higher eukaryotes. In yeast, the mitochondrion is a tubular structure surrounding the cortex. Although the DNA bodies are not discrete structures, they have their own DNA complement. The yeast mitochondrion imports most proteins, which are nuclear encoded. To study mitochondrial division in yeast, it is essential that yeast mating takes place, permitting mitochondrial division and mDNA segregation to happen.

When cells mate, the mitochondria fuse, but the DNA remains segregated. Mitochondrial fusion prevents the loss of mitochondria following cell division. Stress pushes mitochondrial division and potential loss, whereas starvation pushes mitochondrial fusion and preserves the mitochondria. Likewise, mitochondrial division proteins enhance the possibility of apoptosis and cell death, whereas mitochondrial fusion proteins limit apoptosis and promote cell survival. As such, many physiologic factors can effect mitochondrial division. Three gene/protein systems that participate in the mitochondrial division and fusion process have been recognized. These proteins are capable of self-assembly, require GTP binding proteins, and are membrane-associated. Dnm-1 localizes to the outer surface of the mitochondrion. *In vitro*, this protein exists as a dimer and, in the presence of GTP, can assemble and resemble the constriction site of a bacterium. This protein belongs to the family of dynamins. If the protein is added to a membrane in the presence of GTP, it will self-assemble into a constriction site. A second protein, Mdv1, also is located on the outer mitochondrial membrane and interacts with the protein, Fis1. Together, these two proteins recruit Dnm-1 to the mitochondrial outer membrane.

At this point, the endoplasmic reticulum, or ER, is recruited to bind to the outer mitochondrial membrane and aid in the constriction process. As in bacteria, the FtsZ proteins are likely to function internally to the mitochondrial division process. However, in the mitochondrial system in yeast, there is no wall septum formed to divide the structure as with bacteria, so it is likely the ER serves that function (Figure 1).

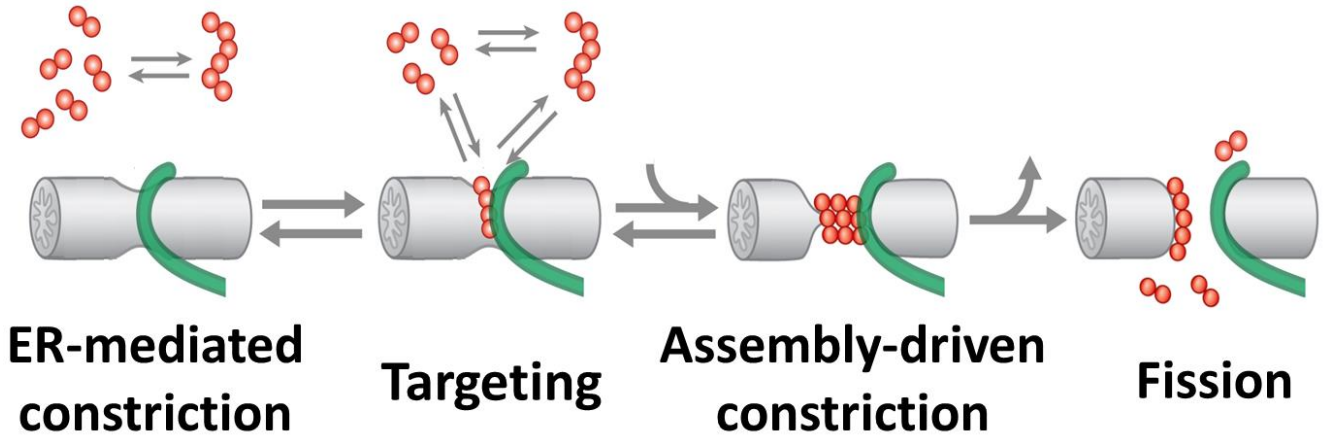

Figure 1. Proposed Model of Mitochondrial Division

Meanwhile, an FtsZ-like system is functioning to divide the mitochondrion from within. Because the mitochondrion represents the oxidative energy-generating system in eukaryotic cells, mitochondrial function and integrity are essential to any potential for exploitation in bioproduct design. In addition, the mitochondrion is essential for porphyrin biosynthesis, making its presence essential for most cellular activities, as well as in the filamentous fungi. The circadian clock also is a controlling factor in cellular metabolism and is involved in mitochondrial maintenance and physiology. For these reasons, mitochondrial division and maintenance are important processes necessary for using fungi in bio-processing activities to the maximal extent possible.

3.1.1 The Division Machine

There are striking similarities to the bacterial division process. In both time and space, a unique program of gene expression develops where distinct elements of the active machine are recruited in a defined order and interact with one another at a specific site within a spatial context. The structure of each component, the nature of the interactions, their stoichiometry, and their recruitment of the ER (at least, a specialized site on the ER) serve to complete the construction of that portion of the division machine. Internally to the mitochondrion, development of a second Fts-like machine would enable the internal constriction of the mitochondrion. These two machines must, in some way, coordinate their activities for the process to continue in an orderly manner. EMSL could play a unique and vital role in the study of mitochondrial division.

3.2 Chloroplast Division and Maintenance

While the workshop's goal was not aimed at developing a complete conceptualization of the existence of definable organizational principles involved in biologic machine structure and function, the importance of understanding chloroplast division and maintenance is evident from other studies. The process of thylakoid formation, light entrapment, and carbon dioxide sequestration are mission-relevant activities. However, chloroplast also is a site for amino acid biosynthesis, lipid synthesis, porphyrin synthesis, and other critical physiologic functions in plant-like systems. It exchanges information with the nucleus and the mitochondrion and plays a central role in the rhythmic day/night cycles of plants and algae. Because chloroplast has three FtsZ-like proteins, defining the complex cycle of chloroplast division and its integration into the cellular milieu is necessary. Currently, the machine where this process occurs is under study.

3.2.1 Mechanosensitization of the Chloroplast Membrane

Chloroplast sophistication, both functional and structural, became evident from the presentation by Dr. Liz Haswell (Washington University, St. Louis), which focused on the mechanosensitization of the chloroplast membrane; showed how both internal and external mechanical stimuli can effect chloroplast movement and osmotic pressure; and demonstrated the presence of homologues to the *E. coli* McsS, or mechanosensors. These mechanosensors give rise to ion channels, which enable membrane-mediated gating, and result in responsive activities of the chloroplast from both external and internal stimuli (Figure 2). These channels are responsible for the organelle's external shape and size. The chloroplast must protect itself from changes in osmolarity within the cytoplasm, where water and salt movements are in constant change relative to the cell's exterior. The movement of gases and solutes is unique in plant cells compared to animal systems. In this regard, chloroplast behaves like a true symbiont relative to the plant cell. In a mutant lacking the mechanosensitive channel, the chloroplast is enlarged, and multiple FtsZ rings can be found. Thus, chloroplast division must be highly sophisticated, and, in the process of division, the levels of internal membranes must be preserved, such as the mitochondrion. These membranes, or thylakoids, are the site of light entrapment, and both their density within the chloroplast and the numbers of chloroplasts are sensitive to light intensity, wavelength, and light versus dark cycles. As such, the division process must be integrated into all of these physiologic functions. To reap the full photosynthetic potential of any biologic system, chloroplast maintenance must be deciphered via an understanding of chloroplast division and thylakoid integrity.

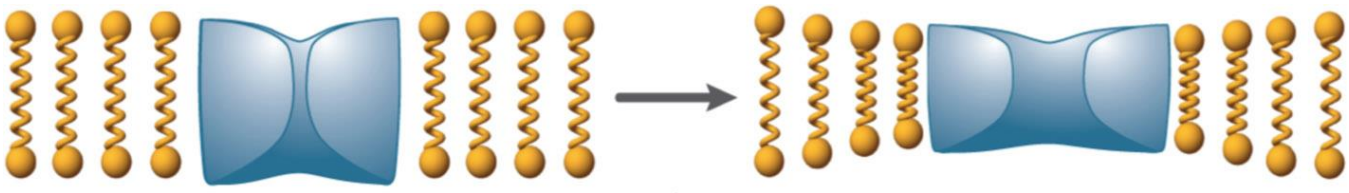

Figure 2. Cartoon Depicting Ion Channels' Influence on Organelle Shape and Size

Dr. Haswell's presentation, coupled with other studies, indicates there is sophisticated machinery that must exist for the orderly process of chloroplast division. Future works would involve both a temporal and spatial compartmentalization of this machine, which is likely to involve many protein components whose recruitment is in precise order and built upon some underlying framework. Unlike the mitochondrion, it is probable that both the chloroplast and nucleus contribute significant information to this machine. Determining how this machine marks the chloroplast DNA replication process and what final product must be elaborated to enable division requires further input. However, as previously observed, EMSL's basic capabilities can be important toward this process. Given the size of the chloroplast genome and its structural complexity, it is reasonable to state that, unlike the mitochondrion, chloroplast is a more coequal partner to the nucleus. As such, its coding capacity and regulatory totality must be deciphered.

4.0 Rhythms and Circadian Cycles

4.1 Circadian Cycles

To introduce circadian cycles, Dr. Susan Cohen (University of California, San Diego) spoke in detail regarding the test organism, *Synechococcus elongatus*, used in her circadian cycle studies. It has a genome size of ~2.7 Mbp, is genetically versatile, and has a Tn insertion library used to define "day" and "night" expressible genes. The structure of the oscillator protein involved in day/night cycling, Kai, is known and used by monitoring the cell's redox potential, which clearly differs under dark and light because it is a photosynthetic organism. Kai's output is used to regulate gene expression in a rhythmic fashion, as well as serving as a link to the cell division cycle. For example, if a mutant in Kai is no longer day-regulated, the cells are enlarged, and there are multiple FtsZ rings observed that are scattered, or not defined as they normally behave. Thus, there is a direct link between the day/night oscillator and cell division. To exploit cyanobacteria for a variety of productive activities, their intrinsic day/night rhythms and cell cycle regulation over a 24-hour span must be a continuing point of study. The FtsZ gene exists in a two-gene operon. By using the proper mutants, it is possible to separate the daily cyclical oscillations from the cell cycle. A second protein, CikA, has multiple domains, and it is possible to separate these domains following the construction of various mutant forms of the protein. These diverse constructs were exploited to analyze the relationship between rhythmicity and the cell cycle. To analyze their different forms, these mutant cells were separated using a microfluidics chamber and cell sorting programs—an area where EMSL has much to offer. These studies were conducted at the single-cell level, and, as indicated previously, imaging is at the heart of these analyses.

Under some conditions, FtsZ is located abnormally and results in double strand breaks in the DNA. In addition to the KaiA protein, there also is the KaiC protein. The use of fusion derivatives for microscopic analysis appears to have no effect upon their function, and the cells behave normally in terms of their rhythmic oscillations. KaiC is predominantly at the poles, and KaiA is slightly off-polar. To establish these observations are not the result of artifacts, both C- and N-terminal fusions were constructed and expressed from a neutral site within the genome. If KaiA is to localize properly, a third player, Pi, is essential. However, this is not true for KaiC. Employing this construction, chromosome partitioning and cell division are normal. The cells can become polyploidy with two, three, and four copies of the genome that remain discreet, and the ploidy process appears to be rhythmic. To follow cell division, chromosome behavior, and rhythmic cell oscillations, a variety of imaging techniques must be used and coupled with single-cell isolations.

4.2 Cell Rhythmicity and the Oscillator Protein

In relating his studies of the cyanobacteria clock, speaker Dr. Carl Johnson (Vanderbilt Kennedy Center) detailed a somewhat different approach. Although using the same organism, Dr. Johnson focuses on the oscillator, showing that the rhythms are persistent and independent of temperature, although cells can be trained to express different rhythmic patterns that are temperature-compensated. Even when cells are not dividing, they can express rhythmic patterns in gene expression.

Interestingly, when a cell cycle mutant was grown together with wild type under constant light, the cycle mutant was less competitive. Under others sets of conditions, a cycle mutant can become highly competitive. One especially important factor in cell rhythmicity is that carbon sequestration flux is greatly affected and subject to rhythmic controls. This is an important observation and indicates that using cyanobacterial systems for carbon sequestration or other bio-product designs must account for day/night oscillations and their relationship to the cell cycle.

There are a number of alternative explanations to describe how so many genes become affected in a rhythmic fashion, such as a common set of promoters and regulators subjected to oscillator output. Another possibility is that the level of DNA supercoiling is under oscillator control. Thus, entire sets of genes are affected by the degree of DNA supercoiling. There are certain implications, such as gene location, that would be subject to this hypothesis. Still, these are testable assertions, and it may be determined that a combination of reasons are responsible for the rhythmicity observed for the expression of many different sets of genes. Resolving this issue is important because the diurnal cycle is present in higher eukaryotes. As such, behavior in response to the day/night cycle is of considerable socioeconomic value. The three Kai proteins, A, B, and C, have been purified, and their structures determined. Together, they interact and oscillate in a rhythmic fashion. Given these observations, it is possible to use mutant forms of the proteins to follow structural changes and analyze how such changes might play out *in situ*. Furthermore, it is appropriate to ask if this is the "core" pacemaker for the rhythmic expression of all genes.

4.3 The Circadian Clock and Filamentous Fungi

Filamentous fungi are sophisticated organisms that serve as a model for higher-animal eukaryotic cell types. Speaker Dr. Deborah Bell-Pedersen (Texas A&M University), who studies the circadian clock in the filamentous fungus, *Neurospora crassa*, introduced the critical discussion regarding using fungal systems for bioprocess development—research integral to DOE's mission.

The existence of the clock in *Neurospora* was revealed some time ago via a simple set of experiments, i.e., through the use of the "race tube," where the linear growth of the organism can be followed over several day/night cycles from one end of the tube to the other. During the course of linear growth, aerial reproductive spores, called "conidia," are produced on aerial hyphae. Because these spores are orange (carotenoid, neurosporene), their presence is readily observed and can be monitored to show they are not produced continuously along the hyphal growth. Instead, they occur discontinuously in synchrony with the day/night cycle. The core components of the N. crassa clock are known and a high level model of the circadian oscillator is shown in Figure 3. Clock control of metabolism is important and relevant for the production of metabolites of interest as biofuels and bioproducts.

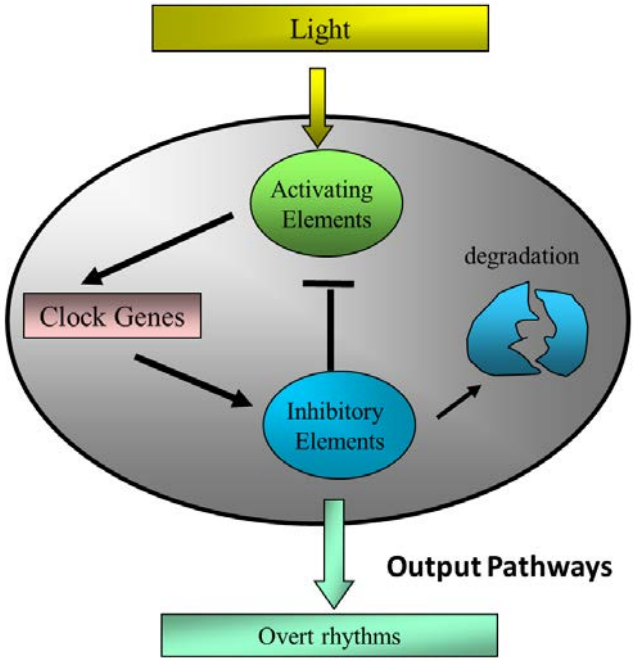

Figure 3. The Negative Feedback Model of a Circadian Oscillator

4.4 Future Course of Work

Rhythmic gene expression of cyanobacteria introduces many questions with obvious answers regarding the role that EMSL can take in future related studies. All of these studies emphasize the single cell. Gene and phenotype exist at the center of studies involving the oscillator's structure and function. Cell isolations and interrogations define the experimental approach, where the export of the oscillator and its derivatives can be further developed. Moreover, if the oscillator acts via changes in DNA, it is possible that gene/phenotype rearrangements can be directed to specific outcomes.

Cell rhythmicity and the circadian clock also have much in common with cell and organelle division. There is a significant relationship between the cell cycle, chromosome mechanics, and cell rhythms, particularly for cell organelles, and structural elements facilitating cell behavior are subcellular machines, evocative of the design principles inherent to all biological machines.

Despite the disparity of outcomes of the machines considered herein, there are numerous commonalities—enough to warrant investigating the potential of synthetic machines designed to accomplish new cellular functions or redirect existing ones.

5.0 Conclusion and Considerations

The BID-STAP focused on emphasizing common approaches employed in the researching biological machines and areas where scientific personnel and EMSL capabilities can unite in that endeavor. Discussions touched how each of these study areas possess a common thread in analyses methods, how the analyses represent EMSL's strengths, and how the outcomes of these studies are in congruence with the stated interests of both DOE and EMSL. In the extensive discussion sections, it became apparent that a unique and definable set of principles connected these different areas of research—namely, all were focused on the roles of unique biological machines that represented the core of the cell and organellar division processes and the circadian clock.

Among these discussions, it also became apparent that understanding how these machines worked and affected cellular function was at the center of DOE's mission and it stated interests. More importantly, as a DOE Office of Biological and Environmental Research-sponsored national user facility, there are logical and distinct advantages for pursuing biological machines studies in partnership with EMSL. To further this momentum and cultivate interest in this Science Theme, a future planning meeting, with some scrutiny toward future of EMSL capabilities, is under consideration with special attention to:

- Invited speakers and necessary attendees
- Funding options
- Building publicity for the proposed Science Theme and EMSL.

One program note: Based on an assessment of this BID-STAP, invited attendees requested that any tours of the EMSL facility proceed prior to the actual program.

Appendix A

Invitation to EMSL BID-STAP

Note: Invitations to the EMSL Biological Interactions and Dynamics Science Theme Advisory Panel meeting were sent via electronic mail.

Subject: Workshop Invitation on Cell Cycle and Rhythms

From: samuel.kaplan@uth.tmc.edu

We at EMSL (Environmental Molecular Sciences Laboratory) on the campus of the Pacific Northwest Regional Laboratory (PNNL) in Richland, Washington, are planning a two-day workshop relating to the replication (division) and circadian rhythms of microbes and cell organelles (Bacteria, Archea, Cyanobacteria, Mitochondria, Chloroplast). Our purpose is two-fold: first, the exchange of information in this exciting area of science, and second, to introduce you and your colleagues to EMSL. I invite you to give a presentation on your current research as part of this workshop.

EMSL is a state-of-the-art national user facility of the Department of Energy, which makes itself accessible to the broad scientific community in the areas of chemistry, physics, biochemistry, computation and biology. The capabilities offered by EMSL are exceptional. EMSL determines what new capabilities to purchase or develop through scientific workshops held with representation of the broader scientific community. This workshop will help us elicit the input and insights of what capabilities in biology could be pursued for the user facility community. We would like to take the initiative through this workshop of introducing you and your colleagues to EMSL and all that it has to offer for the conduct of your science, as well as bringing more "biology" into EMSL on an ongoing basis. For a more in-depth view of EMSL, we recommend the following links, where you will be able to view the display of facilities that are available and learn more about the facility.

http://www.emsl.pnl.gov/about/

http://www.emsl.pnl.gov/science/

http://www.emsl.pnl.gov/capabilities/

The workshop is scheduled for June 14-15, 2012. We propose that participants arrive the afternoon or early evening of June 13. The workshop will consist of approximately eight 35-40 minute presentations, with 10-15 minutes for discussion, on Thursday, June 14, and will include ample time for more informal discussions during the day of the meeting as well as during the evening activities. We would like your presentations to emphasize both your science as well as the tools you employ in the conduct of your science, and give thought to what "futuristic" tools you would find desirable. The following day, Friday, June 15, we will tour EMSL so that you may view firsthand how your science could benefit through collaborative interactions with the scientific leaders at EMSL, as well as what you would like to see available in the future. Following the tour there will be a one and one-half hour roundtable during which you can discuss how the EMSL facilities could enhance your scientific program and what further enhancements you would like to see available. The workshop will wrap up at noon for your departure..

EMSL will cover all of your travel expenses as well as providing a modest honorarium.

At this point in time, we would like to gauge your interest in participating. We have selected dates but can be flexible as we understand you may have other demands in this timeframe. I might add, Eastern Washington is delightful at this time of year and is a stellar wine-producing area.

Please respond to me at your earliest convenience, and I would be pleased to address any questions. My goal for EMSL in all of this is to provide superior facilities and technical expertise to the biological community. That is our mission, and this is a vehicle for getting input from the community.

Thanks for your attention and I look forward to your participation.

Samuel Kaplan, Professor Emeritus Microbiology, University of Texas Health Science Center/Houston

Scott E. Baker, Scientist, Chemical and Biologic Process Development Group, Interim Head for Biology, EMSL

Appendix B

EMSL BID-STAP Attendees

Invited Speakers

Deborah Bell-Pedersen, Texas A&M University
Susan Cohen, University of California, San Diego
Elizabeth Haswell, Washington University, St. Louis
Carl Johnson, Vanderbilt Kennedy Center
Sam Kaplan, University of Texas-Houston Medical School
William Margolin, University of Texas-Houston Medical School
Jodi Nunnari, University of California, Davis
*Katherine W. Osteryoung, Michigan State University

**Did not attend due to travel delays.*

EMSL and PNNL

Don Baer
Nancy Hess
Scott Baker
Bryan Linggi
Robby Robinson
Harvey Bolton
Blaine Metting
Mark Bowden
Dave Cowley
Bert DeJong
Scott Lea
Galya Orr
Lili Pasa-Tolic
Theva Thevuthasan
Erich Vorpagel
Nancy Washton
Jim Fredrickson
Allan Konopka
Joel Pounds
Bill Cannon
Katrina Waters
Karin Rodland
Mary Lipton
Josh Adkins
Jon Magnuson
Ken Bruno
Dave Culley
Karen Wahl
David Wunschel
David Koppenaal
Ray Teller
Bill Shelton
Karl Mueller
Bernd Kabius
Charity Plata
Laura Kuprat

Appendix C

Workshop Agenda

www.emsl.pnl.gov

Environmental Molecular Sciences Laboratory
902 Battelle Boulevard • P.O. Box 999 • Richland, WA 99352

EMSL BID STAP Meeting
June 14-15, 2012

AGENDA

June 14, 2012
EMSL 1077

7:15 am	**Badging - EMSL**
7:15 am	**Meet and Greet**
7:45 am	**Welcome, Plans for the Day** • Welcome – Allison Campbell • Introductions, plans for the day – Sam Kaplan, Scott Baker
8:00 am	**"Regulation of bacterial cell division from A to Z"** William Margolin, University of Texas, Houston Medical School
9:00 am	**"Molecular Basis of Mitochondrial Behaviors"** Jodi Nunnari, University of California, Davis
10:20 am	**"A Feeling for the Organelle: Multiple Roles for Mechanosensitive"** Elizabeth Haswell, Washington University, St. Louis
11:30 am	**EMSL Tour**
12:20 pm	**STAP discussions with Capability Leads** David Koppenaal & EMSL Capability Leads
1:30 pm	**"Elucidating the Relationship Between the Circadian Rhythm and Gene Expression"** Susan Cohen, University of California, San Diego
2:30 pm	**"As Time Glows By: Circadian Programs in Cyanobacteria from Molecules to Populations"** Carl Johnson, Vanderbilt
3:50 pm	**"Connections Between the Circadian Clock and Rhythmic Cellular Processes"** Deborah Bell Pedersen, Texas A&M
4:50 pm	**Closing Comments**
5:00 pm	**Return to Guesthouse**
6:00 pm	**Dinner at Anthony's –** by invitation only

Contact Information:
Laura Kuprat 509-371-6452
Scott Baker 509-372-4759

EMSL is located at PNNL

Pacific Northwest
NATIONAL LABORATORY

June 15, 2012
EMSL 1077

7:00 am	**Morning Refreshments-casual discussion**
8:10 am	**Organize for EMSL Tour**
8:30 am	**EMSL Tour**
10:20 am	**Round Table Discussion**
12:30 pm	**Adjourn**

Appendix D

EMSL Group Discussion: Core Questions

1. Have you seen any capabilities at EMSL that can positively affect your program?
2. What is the easiest way to access these capabilities?
3. Can you envision an active and ongoing collaboration with the lab? Would it include other labs?
4. What would you like to see in terms of capabilities at EMSL?
5. What "pie-in-the-sky" capabilities would you want to see?
6. What capability would move your personal research forward in a quantum leap?
7. What did or didn't we do right in this program?
8. What should we do to follow up this meeting?

How can EMSL reach out to the greater biological community?