I. Please provide an updated abstract of your proposal (a paragraph or two) and a brief biographical sketch (no more than one page).

Exploring the role of inhibition by unfair competition in the interaction between PP1-GADD34 and phosphorylated Inhibitor-1

Abstract

Cdk1-Cyclin B (also known as M phase-promoting factor, or MPF) is the key molecule that drives a cell’s transition from interphase into mitosis (M phase). MPF is a kinase, an enzyme that adds phosphate groups to certain target proteins. Phosphorylation by MPF activates some target proteins and deactivates others, as required for their regulation during mitosis. The enzymatic counterparts of MPF are the phosphatases that remove the phosphate groups from target proteins at the end of M phase. The action of these phosphatases, particularly one called protein phosphatase 2A-B55 (PP2A-B55), is essential to allow cells to transition from M phase back into interphase. Proper progression of the cell cycle requires that phosphatases such as PP2A-B55 become inactivated during the interphase-to-M phase transition but then reactivated during the subsequent M phase-to-interphase transition. It has been found that a small, heat-resistant protein known as Endosulfine (Endos) is critical for the regulation of PP2A-B55 during the cell cycle. During entry into M phase, the activity of MPF results in phosphorylated Endos (pEndos), which binds tightly to PP2A-B55 and prevents it from interacting with its usual substrates. However, PP2A-B55 is able to remove very slowly the phosphate group from pEndos, ultimately resulting in dephosphorylated Endos that no longer inhibits the phosphatase. At the end of M phase, inactivation of MPF prevents further production of pEndos, enabling PP2A-B55 to inactivate all the pEndos in the cell. PP2A-B55 can thus recognize its usual substrates, and this drives the cell out of M phase. Note that pEndos acts both as a substrate and an inhibitor of PP2A-B55; this mechanism is called inhibition by unfair competition.

We believe that inhibition by unfair competition is a widespread phenomenon underlying the regulation of many phosphatases that participate in many biological processes. I will focus on a complex of protein phosphatase 1 (PP1) and GADD34 (growth arrest and DNA damage-inducible protein 34). It has been hypothesized that the phosphorylated version of a molecule called Inhibitor-1 (plnhibitor-1) can bind to and inactivate PP1-GADD34 in a manner similar to the interaction between PP2A-B55 and pEndos. I will purify and characterize PP1-GADD34, one of the normal substrate of this phosphatase called eIF2α, and plnhibitor-1; I will then use these reagents to test the idea that PP1-GADD34 is
regulated by the inhibition by unfair competition mechanism. The findings of these studies may help in the understanding of intracellular phosphorylation in general. Furthermore, these investigations may also be of interest to the cellular response to stress, because eIF2α phosphorylation is induced when cells are subjected to stress, and this lowers the rate of protein synthesis in stressed cells.

Biographical Sketch

In tenth grade, as part of a school policy, I participated in the science fair. With a partner, I dreamed up a rather unimaginative hypothesis, crafted a predictable set of experiments to test it, and ended up with a decent poster to present in the school gymnasium. It was my first exposure to research of any kind, and while my results turned out to be thoroughly unexciting, I found that my interest had been piqued. I had a fantastic time sharing my hard work and trying to convince each person passing by my table to pause and listen to me rave about bacterial growth and the scientific method.

When I arrived at Cornell, I knew that at some point I wanted to become involved in research. However, that stint at the science fair was the extent of my prior experience, and while I had enjoyed myself, I had to admit that my time in the lab itself had been far from thrilling. Time had dulled my memories of the fun I had had presenting my work, and I was worried that I would find “real” research itself dull and repetitive. I had visions of endlessly pouring liquids from test tube to test tube... despite all this, I searched for opportunities and, before I knew it, I was officially involved in research.

I discovered soon enough that while research does involve a good amount of repetition, it is far from boring. The first lab that I joined taught me techniques that I had read about in my biology textbooks, and being able to perform them myself was beyond exciting. It also placed me under the guidance of a mentor who showed me that in addition to scientific knowledge, research also involves critical thinking and problem-solving abilities, as well as a substantial amount of creativity. After spending a summer working in the lab, I gained an increased appreciation for the thought process behind research.

Last semester, after taking genetics with Dr. Goldberg, I discovered that I was very interested in the techniques and ideas presented in the course. Deciding to try something new, I joined his lab, where I have had the chance to apply many of the skills and concepts I learned about in class. Starting again in a different lab has been both exciting and challenging as I try to acquaint myself with a new field. This summer, I hope to use the Tanner Dean's Research Grant to support my staying in Ithaca to continue the work that I have begun in Dr. Goldberg’s lab.

II. Statement of purpose (3-5 pages). Please describe your project in detail: explain the significance of your research and your reasons for wishing to engage in it, and tell us where you will carry out this research and what resources you will use. Discuss briefly how your project relates to existing research in your field on the same subject. Be sure to include a bibliography.

Statement of Purpose

The cell cycle is a tightly regulated series of events guiding a cell through the processes of growth, DNA replication, and division, eventually producing two identical daughter cells. It can be separated into interphase, which consists of the G₁, S, and G₂ phases, and mitosis (M phase). During M phase, replicated DNA is faithfully segregated between the two daughter cells, a complex task that
involves the coordination of many regulatory proteins (1). Mechanisms exist within the cell to ensure that M phase occurs at the proper time, because inappropriate entry or exit from M phase can cause diseases such as cancer. An essential component of this cell cycle regulation is the activity of cyclin-dependent kinases (CDKs), molecules that drive the different phases, and phosphatases, which counter the activity of kinases and allow exit from phases (2).

CDK activity is dependent upon the level of cyclins, proteins whose levels rise and fall with time due to repeated cycles of synthesis and degradation. After binding to cyclin, the CDK-cyclin complex is able to add a phosphate group to a serine or threonine residue on certain target proteins, which can either activate or deactivate the targets. Various CDK-cyclin complexes exist, and different ones are responsible for entry into and advancement through different phases of the cell cycle. Cdk1-Cyclin B (also known as M phase-promoting factor, or MPF) is the complex that drives the transition from interphase into M phase.

Phosphatases serve as the enzymatic counterparts to kinases, because phosphatases remove the phosphate groups that kinases add to target proteins. One of the most important of these phosphatases is protein phosphatase 2A-B55 (PP2A-B55), whose substrates include a subset of the phosphate groups that were added to target proteins during M phase by MPF. It is imperative that PP2A-B55 should be de-activated during M phase entry; if this were not the case, PP2A-B55 would prematurely remove the phosphates added by MPF, resulting in a cell unable to enter M phase and a self-defeating regulatory mechanism. It is equally important that PP2A-B55 be reactivated when cells exit mitosis so that the phosphate groups can be removed at the proper time.

Newly found biochemical circuitry mediates the inverse relationship between MPF and PP2A-B55. When MPF is activated during M phase entry, it phosphorylates another kinase called Greatwall (Gwl). In turn, Gwl adds a phosphate group to a small, heat-resistant protein called Endosulfine (Endos). This phosphorylation causes Endos to bind to and thus inactivate PP2A-B55. The interaction between
PP2A-B55 and Gwl-phosphorylated Endosulfine (pEndos) is known as ‘inhibition by unfair competition’ because of its extraordinarily small $K_m$, which allows the active site of PP2A-B55 to bind pEndos more tightly than any other substrate for this phosphatase. In this way, pEndos acts as a competitive inhibitor of PP2A-B55 (3). However, pEndos is also a target of PP2A-B55’s catalytic activity. The phosphatase very slowly removes the phosphate group from pEndos, eventually resulting in a dephosphorylated Endos that no longer inhibits PP2A-B55. Because MPF is inactivated by the degradation of its corresponding cyclin at the end of M phase, Gwl is also inactivated at this time. Without additional phosphorylation of Endos, PP2A-B55 activity depletes the remaining supply of pEndos. Once pEndos is gone, PP2A-B55 is free to bind to and dephosphorylate its regular substrates, thus driving the cell out of M phase.

While ‘inhibition by unfair competition’ is a recently discovered mechanism, we believe that it may be an underlying basis for many other biological interactions. One possibility is that early in the evolution of eukaryotic organisms, a kinase ancestral to Gwl developed the ability to phosphorylate and inactivate a small protein such as Endos, which in turn was an inhibitor of a phosphatase ancestral to PP2A-B55. The implications of such a model are vast, as reversible phosphorylation by kinases and phosphatases is one of the most important signaling and regulatory mechanisms in living organisms (4). This hypothesis is made even more significant by the fact that a surprisingly large number of gaps still remain in our current understanding of many phosphatases and their regulation.

For my project this summer, I hope to purify and characterize several different protein phosphatases of the same general class as PP2A-B55. Using these enzymes, I will be able to explore whether inhibition by unfair competition is an important component of their regulation. To provide just one example, the phosphatase PP1 is known to associate with a regulatory subunit called GADD34 (growth arrest and DNA damage-inducible protein 34) that directs PP1 to the substrate eukaryotic initiation factor eIF-2α. The dephosphorylation of eIF-2α plays a key role in determining the rate of protein synthesis in cells. It has been proposed that PP1-GADD34 is inhibited by a small, phosphorylated
molecule called pInhibitor-1, which would form an inactive heterotrimeric complex (5). Our hypothesis is that this system works similarly to that involving PP2A, its regulatory subunit B55 and pEndos. However, to date no one has been able to verify that pInhibitor-1 inhibits PP1-GADD34. I will test this idea by purifying PP1-GADD34 from tissue culture cells transfected with a tagged version of GADD34, assaying the activity of PP1-GADD34 on its substrate eIF-2α, and then adding pInhibitor-1 to see if it in fact inhibits PP1-GADD34. If this is the case, I will determine whether the unfair competition mechanism described above is responsible for the interactions of these three proteins. These results will help us understand the function and regulation of phosphatases, and may also provide information about the mechanisms by which cells control the rate of protein synthesis.

This work will take place in Dr. Goldberg's lab here at Cornell, where I will have access to the necessary supplies and equipment. I will purify plasmid DNA containing genes of interest such as GADD34 and eIF-2α, and then amplify the coding regions of these DNAs via polymerase chain reaction to create fragments suitable for cloning into vectors that will allow expression of these proteins in E. coli cells or in transfected human tissue culture cells. I will then be able to purify the desired proteins with affinity chromatography and use them to further study the nature of various interactions among the protein phosphatases and their regulatory subunits. In addition, I will use the bioinformatics software Lasergene to help me design the proper PCR primers and to analyze data. Last year, I was able to present the results of my summer research at the OUB’s SILS Undergraduate Research Symposium. With the support of the Tanner Dean’s Summer Research Grant, I hope to spend another productive summer in the lab, immersing myself more fully in research and continuing to learn about the concepts and practical techniques that my courses have taught me to love.

References

