ESSAY: AMERSHAM BIOSCIENCES AND SCIENCE PRIZE

PAS Domains and Metabolic Status Signaling

Jared Rutter

Amersham Biosciences and *Science* are pleased to present the prize-winning essay by Jared Rutter, a regional winner from North America, who is the 2002 Grand Prize winner of the Amersham Biosciences and *Science* Prize for young scientists.

AMERSHAM BIOSCIENCES & SCIENCE *Prize* FOR YOUNG SCIENTISTS

My thesis research began with the desire to understand the function of the PAS domain, a motif represented in a diverse array of proteins. PAS domains are used as sensor modules in bacterial two-component regulatory systems, where they sense a wide range of stimuli and regulate an associated histidine kinase (*1*). PAS domains are also found in a family of metazoan transcription factors, the basic helix-loop-helix (bHLH)–PAS proteins, where their role is less clear. My interest in the PAS domain led to the study of two very different PAS domain–containing proteins: PAS kinase and the transcription factor NPAS2, which regulates circadian rhythms.

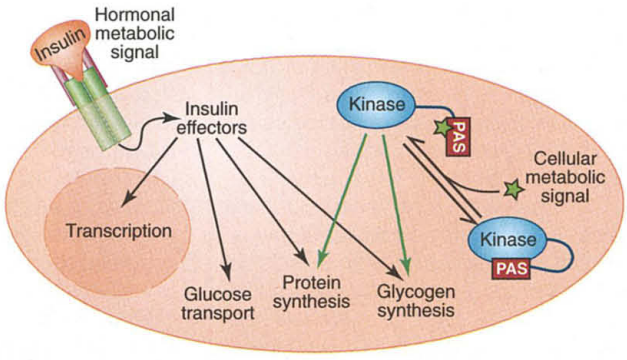
Much of the circadian or daily oscillation of human physiology is driven by the output from a transcriptional negative-feedback loop. The transcription factor composed of NPAS2 (or its paralog CLOCK) and BMAL1, both bHLH-PAS proteins, induces the expression of its own repressor, the CRY protein (*2*, *3*). The resultant cycle of transcriptional activation and repression oscillates with a period of about 24 hours. While elegant, this simple loop is insufficient to explain the ability of the circadian oscillatory device to adapt to changes in environmental and metabolic conditions (*4*). We identified two potential mechanisms whereby the NPAS2:BMAL1 transcription factor might mediate circadian rhythm adaptation.

First, we showed that NPAS2 binds a heme moiety in each of its two PAS domains and that this heme can bind gases such as CO (*5*, *6*). This observation raises the possibility of regulation by either redox changes or fluctuations in the cellular concentration of a specific gas. Second, we found that DNA binding of the NPAS2:BMAL1 transcription factor is stimulated by the reduced nicotinamide adenine dinucleotide phosphate NADPH and is inhibited by the oxidized nicotinamide nucleotide $NADP^+$ (*7*). Small fluctuations, therefore, in the cellular NADPH:$NADP^+$ ratio, which is coupled to cellular metabolic and energy status, might lead to substantial alterations in the ability of NPAS2:BMAL1 to bind DNA, thereby perturbing the timing of the transcriptional feedback cycle. Such effects might explain the ability of cellular metabolic activity and energy status to impinge upon the timing of the circadian rhythm (*4*).

The second project of my thesis research began with the identification of a gene in the DNA sequence database that was predicted to encode a protein containing both a serine/threonine kinase domain and a PAS domain, reminiscent of the bacterial two-component systems. This gene, named PAS kinase, is conserved from yeast to humans, and we used *Saccharomyces cerevisiae* to study its function. Our genetic study began with a screen for genes that, when overexpressed, would complement the growth defect of a strain lacking both PAS kinase genes. Of the 15 genes identified as high-copy suppressors, most (10 genes) were involved in promoting protein synthesis, while 3 were involved in sugar metabolism. The implication of PAS kinase in the positive control of translation was strengthened by the observation that PAS kinase suppresses the growth defect conferred by the loss of the translation factor eIF4B (*8*).

Understanding the biochemical basis for the genetic function of PAS kinase required identifying the proteins that it phosphorylates. Our biochemical approach to identifying PAS kinase substrates was simply to fractionate protein extracts into partially purified pools and analyze each fraction for proteins that became phosphorylated in the presence of active PAS kinase. This approach led to the identification of five PAS kinase substrates, which include three translation factors and two enzymes, Ugp1p and glycogen synthase, which catalyze the final two steps in the glycogen synthesis pathway (*8*). The amino acid that is phosphorylated in glycogen synthase was identified previously as a site of phosphorylation-mediated enzyme inhibition, but until now the responsible kinase was unknown (*9*). As predicted, elimination of the PAS kinase genes and the concomitant failure to properly regulate Ugp1p and glycogen synthase caused hyperaccumulation of glycogen (*8*).

Genetic analysis and biochemical identification of phosphorylation targets independently implicated PAS kinase in the control of protein synthesis and carbohydrate metabolism and storage. These two processes are tightly regulated by nutrient availability and metabolic status. Both effects of PAS kinase—stimulation of protein synthesis and inhibition of glycogen storage—are consistent with the functions of a regulatory system that is activated by nutrient availability. By analogy with the bacterial sensor kinases, we hypothesized that PAS kinase catalytic activity would be regulated by some cellular stimulus interacting with the PAS domain. This hypothesis is supported by our observation that the amino-terminal PAS domain of PAS kinase specifically interacts with and inactivates the kinase catalytic domain (*10*, *11*). Further, nuclear magnetic resonance–based studies of this domain show that it binds small organic compounds in a region analogous to the cofactor binding sites of two other kinase-regulating PAS domains, FixL and Phy3 (*11*). While still unidentified, the endogenous molecule that binds the PAS domain and activates PAS kinase is likely to be some nutrient essential for growth. When this nutrient is available, kinase activity initiates the cellular program appropriate for rapid growth by blocking energy storage and stimulating protein synthesis.



Responding to Metabolites. A schematic showing potential cross-talk between cell-autonomous PAS kinase signaling and global insulin signaling.

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Nutrient sensing and response in the unicellular yeast are cell autonomous. More complex organisms, however, must coordinate the metabolism of individual cells with the energy status of the organism as a whole. Insulin is one such global coordinating factor. It accumulates when circulating nutrient levels are adequate, and stimulates various sites in the body to behave accordingly. Each recipient cell must then integrate the global insulin signal with signals from its own local nutrient-sensing systems. PAS kinase might constitute just such a cell-autonomous sensory system that controls, as does insulin, protein synthesis and glycogen storage.

The two systems I have studied as a graduate student have an interesting commonality. Both appear to be regulated by cellular metabolism, and more specifically, by a specific intracellular metabolite or nutrient. Regulatory small molecules are typically considered to be restricted to the respective families of dedicated signaling molecules, including lipid and nucleotide second messengers and hormones. I believe that as more signaling networks are studied in biochemical, mechanistic detail, regulatory function will be found in a much larger and more diverse set of cellular metabolites.

References and Notes

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2002 Grand Prize Winner

Jared Rutter was born in a small Utah town in 1973, where he stayed until graduating from high school in 1991. After spending 2 years in Scotland, he attended Brigham Young University in Provo, Utah, and graduated in 1996 with a bachelor's degree in molecular biology. He went to Dallas, Texas, to pursue graduate studies at the University of Texas Southwestern Medical Center in the molecular biophysics graduate program. Under the guidance of Steve McKnight, Dr. Rutter studied the regulation and function of two proteins involved in sensing metabolic status and controlling cellular biology. After receiving his Ph.D., Dr. Rutter was appointed as the Sara and Frank McKnight Independent Fellow of Biochemistry at the University of Texas Southwestern. Dr. Rutter and his wife Deena, who is a successful artist, have three energetic young boys.

Jared Rutter

Regional Winners

Europe: Attila Toth, for his essay, "Cohesin and Monopolin: Two Major Determinants of Chromosome Segregation," based on his research in the laboratory of Kim Nasmyth at the Institute of Molecular Pathology, Vienna, Austria. Dr. Toth was born in 1973 in Nagykanizsa, Hungary. He studied biology and genetics at ELTE (Eötvös Loránd University) in Budapest, Hungary, from 1991 to 1996. He joined Dr. Nasmyth's group and received his Ph.D. from the University of Vienna in 2001. He continued his studies on chromosome segregation as part of a collaboration between the Nasmyth laboratory and the laboratory of John Kilmartin at the MRC Laboratory of Molecular Biology in Cambridge, UK. In 2002 he joined the laboratory of Azim Surani at Wellcome Trust and Cancer Research UK in Cambridge to study meiosis in mice.

The second European regional winner is Olivier Voinnet, for his essay, "Molecular Analysis of Post-Transcriptional Gene Silencing," reporting research carried out with David Baulcombe's group at the Sainsbury Laboratory, John Innes Centre, Norwich, UK. Dr. Voinnet was born in Paris, France. He has a master's degree in molecular and cellular biology from the University of Paris VI (Pierre et Marie Curie) and an engineering degree in Agronomy from the "grande école" Institut National Agronomique Paris. In 1996 he joined Dr. Baulcombe's group, where he continued as a postdoctoral fellow after obtaining his Ph.D. in 2001. He will continue to address aspects of RNA silencing in his own laboratory at the Institut de Biologie moléculaire des Plantes du CNRS.

North America: Wenying Shou, for her essay, "Want to Play the Finale of Mitosis? RENT Instruments from the Nucleolus First!" based on her doctoral research in the laboratory of Dr. Deshaies at Caltech. For another perspective, see the diary kept by the yeast cells. Dr. Shou was born in Hangzhou, China. She received her BA degree from Pomona College in Claremont, California, in 1993 and a Ph.D. from the California Institute of Technology in 2001. She is currently a Damon Runyon postdoctoral fellow at the Rockefeller University in New York City.

Japan: Mitsutoshi Setou, for his essay, "Cargo Binding Mechanisms of Molecular Motors," based on his Ph.D. research carried out in the laboratory of Dr. Nobutaka Hirokawa at Tokyo University. Dr. Setou was born in Kagawa, Japan. After graduating as valedictorian from the University of Tokyo School of Medicine, he joined Dr. Hirokawa's laboratory in 1996 and received his Ph.D. from the University of Tokyo Graduate School of Medicine in 2001. He is now a research associate in Dr. Hirokawa's laboratory and a PRESTO (Precursory Research for Embryonic Science and Technology) researcher of the Japan Science and Technology Corporation (JST) at the Mitsubishi Kagaku Institute of Life Science.

The second Japanese regional winner is Hiroshi Takayanagi, for his essay, "How Does the Immune System Break and Protect Bone? Molecular Cross-Talk in Osteoimmunology," based on his research at the University of Tokyo. Dr. Takayanagi was born in 1965 in Tokyo. He graduated from the Faculty of Medicine, University of Tokyo, in 1990. After 7 years as an orthopaedic surgeon in the University Hospital, he undertook doctoral research on the mechanism and regulation of bone destruction in arthritis in the Department of Orthopaedic Surgery and later the Department of Immunology, University of Tokyo, under the guidance of Dr. Taniguchi. Dr. Takayanagi obtained his Ph.D. from the University of Tokyo in 2001. He is currently an Associate in the Department of Immunology, Graduate School of Medicine, University of Tokyo, and a researcher in the PRESTO program of the JST.

All Other Countries: Raul Mostoslavsky, for his essay, "The Role of Chromatin Structure in the Establishment of κ-Chain Allelic Exclusion." Dr. Mostoslavsky was born in 1969 in Tucuman, Argentina, and received his M.D. degree in 1993 from the Faculty of Medicine at the National University of Tucuman. He pursued Ph.D. studies at the Hadassah Medical School, The Hebrew University, Jerusalem, under the supervision of Yehudit Bergman and Howard Cedar. Dr. Mostoslavsky received his Ph.D. degree in 2001. He currently holds a postdoctoral position in Professor Alt's laboratory at The Children's Hospital–Harvard Medical School, Boston.

For the full text of essays by the regional winners and for information about applying for next year's awards, see *Science* Online at www.sciencemag.org/feature/data/pharmacia/prize/winning.shl