Profile of Marc R. Montminy

At any given moment in the human body, a typical cell encounters a barrage of environmental cues—hormones, nutrients, growth factors, and other chemicals—surging through the bloodstream, ready to influence the pattern of genes expressed in the cell by tickling receptors on the cell's surface. How an extracellular signal makes its way inside the cell to the nucleus, where it alters gene expression, is no simple task. But this molecular process is precisely what fascinates Marc Montminy, a professor at the Salk Institute for Biological Studies and a recently elected member of the National Academy of Sciences (NAS). Three decades ago, few might have guessed that his basic research findings would have major implications for the diagnosis and treatment of some of the most vexing health problems of our time, including diabetes and obesity. Yet he has tackled these and more.

Many extracellular signals do not enter the cell but, like runners in a relay race, pass their batons to so-called "second messengers," which are produced inside cells in response to specific environmental cues and are tasked with relaying messages carried by the extracellular signals. One industrious second messenger, cAMP, acts in response to a variety of hormones and growth factors in a way that regulates a striking number of biological processes, including metabolism, neurotransmission, and cell proliferation. Early in his career, Montminy cloned the founding member of a family of cAMP-regulated transcription factors, known as cAMP response element-binding protein (CREB) (1). Since then, he has provided fundamental insights into how CREB works at the molecular level; elucidated CREB's role as a nutrient sensor and regulator of energy metabolism; and revealed potential drug targets for insulin resistance, diabetes, and obesity.

Image courtesy of Joe Belcovson.

Marc R. Montminy.

Inspiring Leaders

Montminy was born and raised in the textile mill town of Lewiston, Maine. His mother, a school nurse, often brought home stethoscopes and blood pressure monitors, fostering an early interest in human anatomy and physiology. Later, as a high-school student, Montminy developed a taste for laboratory research. During the summer before his senior year, he attended a research program sponsored by the National Science Foundation geared toward students interested in biochemistry. During the 6-week program, Montminy and his teammates studied the different forms of an enzyme produced in the digestive tract. "We didn't get very far," Montminy recalls, but it was a valuable experience nonetheless. During the program, he met a young undergraduate attending Harvard University named Gary Nabel, who is now director of the Vaccine Research Center at the National Institutes of Health. "He was a very strong mentor," Montminy remembers. "In fact, he even got me interested in going to college at Harvard. I barely knew where Harvard was."

That summer experience compelled Montminy to apply to the undergraduate biochemistry program at Harvard University. As a freshman, he took a genetics course taught by Charles Thomas, an "active and inspiring" professor. An upper level course in cell biology taught by NAS member Anjana Rao, who was then a graduate student at Harvard University, "probably cemented my interest in doing science," Montminy says. "She was fantastic. She had a very rigorous approach to science."

Montminy graduated from Harvard University cum laude with his bachelor's degree in biochemistry in 1978 and enrolled in Tufts University School of Medicine, torn between pursuing medicine or research. During his third year of medical school, memories of his previous laboratory research experiences beckoned and he decided to pursue a dual MD/PhD degree program. "It turns out there were a lot of people in my class who were also interested in research," Montminy says. One of his classmates, Roderick MacKinnon, later won the Nobel Prize in Chemistry for his work on ion channels (2).

Montminy began casting about for a PhD mentor, and turned to Seymour Reichlin, the head of endocrinology at Tufts University School of Medicine, for advice. Reichlin recommended Richard H. Goodman, who was then a research fellow at Massachusetts General Hospital. Under Goodman's tutelage, Montminy started characterizing the structure of the gene that encodes a neuropeptide hormone called somatostatin (3). Montminy had such a good experience with Goodman that he remained a part of his laboratory when it relocated to Tufts University in 1983. "He was very good about giving me room to make mistakes but then also gave me a sense of how important it was to focus," Montminy says.

Multiple Pathways

Montminy received his MD degree, as well as a PhD degree in physiology, in 1984. He decided to forego clinical medicine and extended his research in Goodman's laboratory as a postdoctoral fellow, focusing on the regulation of somatostatin gene expression in cultures of rat neuronal cells. Montminy discovered that somatostatin mRNA levels increased in response to forskolin, a plant-derived chemical that stimulates cells to produce cAMP (4). He pursued the mechanism behind this phenomenon and soon made the seminal discovery of the cAMP response element (CRE), a short, 8-bp sequence of DNA upstream of the somatostatin gene that was required for the gene's response to cAMP (5). Montminy observed additional CREs upstream of other genes whose expression was also stimulated by cAMP and surmised that perhaps the element served as a binding site for a specific transcription factor that was regulated by cAMP.

Meanwhile, Montminy's work on the somatostatin gene caught the attention of Wylie Vale, an expert on neuropeptide hormones at the Salk Institute. Vale was interested in developing molecular biology expertise in his department and invited Montminy to establish his own laboratory there. Fortunately, protein purification happened to be one of the department's fortes. "When I set up my lab, they really provided a lot of core facilities that made it possible for me to focus on purifying and characterizing the protein that I was interested in."

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Shortly thereafter, Montminy purified a nuclear protein that bound to the CRE of the somatostatin gene and regulated somatostatin's expression (1). He named it CREB in homage to Edwin G. Krebs, the late biochemist who first discovered an enzyme called cAMP-dependent protein kinase (PKA), which mediates many of cAMP's actions in the cell. Montminy's discovery began to clarify how cAMP altered basic patterns of gene expression.

However, several key questions remained. How did cAMP induce CREB-dependent gene expression? It was well known that nearly all cAMP's effects were coordinated by PKA, which, when activated by cAMP, transfers phosphate groups to other proteins, thereby allowing cAMP-dependent signals to be propagated throughout a cell. Montminy's laboratory soon revealed that PKA phosphorylates CREB at a single, specific amino acid and, moreover, that phosphorylation at this site is required for CREB's activation (6, 7). "That was the first example that protein phosphorylation could regulate the activity of a transcription factor," Montminy explains. In 1990, the Endocrine Society acknowledged his fundamental contributions by awarding him the Richard E. Weitzman Award.

Second Signal

Although the findings revealed a clear basis for phosphorylation-dependent control of CREB's activity, more questions remained. "We and many other labs found that a number of other signals—growth factors, UV light, hypoxia—would trigger the phosphorylation of CREB at the same activating site as cAMP. Yet, those stimuli had only modest effects on gene expression. That led us to think that there was a second signal that was required in order for CREB to mediate changes in gene expression. That is, phosphorylation was one, but there was something else as well." Goodman, Montminy's former advisor, discovered a protein called CREB-binding protein (CBP), which bound only to the PKA-phosphorylated form of CREB (8). Further work revealed that CBP functions as a transcriptional coactivator, providing a crucial link between CREB and the general transcription machinery (9, 10). Collaborative studies with Peter Wright revealed the structural basis for how phosphorylation increases the binding of CREB to CBP (11).

However, it remained unclear how this widely expressed and readily activated transcription factor could elicit distinct effects on gene expression in different tissues. Montminy explored this mystery via a genome-wide analysis to characterize all the genes regulated by CREB in different human tissues (12). "Based on the fact that maybe 25 to 50 genes are upregulated by cAMP in any cell type, we expected that the number of CREB target genes would be fairly low, maybe a couple hundred at the most. We also knew that there were some genes with CREB binding sites, but those genes didn't turn on with cAMP, so we were curious as to whether those genes were occupied by CREB."

Surprisingly, Montminy found that up to 5,000 genes, nearly one-fourth of all mammalian genes, had conserved CREB binding sites that were occupied by CREB. Moreover, CREB was phosphorylated in response to cAMP at a majority of those sites, even though only about 100 of those genes are activated by cAMP in those cells. "We found that CBP was recruited to the subset of genes that were actually upregulated, so that suggested that there was some selecting principle that determined which genes would be active and which would not be activated. We are still confronting this question today."

Around the same time as the genome-wide analysis, Montminy's laboratory initiated a screening project to identify other proteins capable of activating CREB. The endeavor unveiled a second family of cAMP-regulated proteins that interacts with CREB and activates gene expression: the CREB-regulated transcription coactivators (CRTCs) (13). The CRTCs lie dormant in the cell until an increase in cAMP causes them to be stripped of their phosphate groups, thereby enabling the CRTCs to bind to CREB and stimulate gene expression (14).

Those studies, Montminy says, changed his working theory of how CREB works and provided further insight into why only a subset of genes is activated by cAMP, even though a large number of genes have CREB-occupied binding sites. Because CRTCs are only activated by cAMP, whereas many more signals can phosphorylate CREB, he now believes that the phosphorylation of CREB, together with the dephosphorylation of CRTCs, helps to determine which CREB-regulated genes are activated by cAMP.

Worthwhile Trip

Between 1996 and 1999, Montminy briefly relocated to Harvard University, a move primarily motivated by a desire to live near his family in New England. "It turned out to be a very worthwhile trip," Montminy notes. "At the time, we were primarily studying CREB activity in cell culture" and were not relating the >findings to physiological processes. However, influential studies by his colleagues and NAS members C. Ronald Kahn and Bruce Spiegelman at Harvard University's Joslin Diabetes Center compelled Montminy to investigate CREB's role in energy metabolism and diabetes. "Their interest in metabolic regulation really drove us to examine the role of the CREB pathway in this setting."

During short-term fasting, the liver switches on glucose-synthesizing genes to ensure a steady supply of fuel for glucose-dependent tissues, such as the brain and red blood cells. Montminy discovered that the activation of CREB and CRTC2 plays an important role in this process, known as gluconeogenesis (15, 16). Indeed, CRTC2 functions as a key regulator of blood glucose levels: It promotes glucose production during fasting; however, as glucose levels rise, the pancreas releases insulin, thereby shutting down glucose production via the inhibition of CRTC2 (17–19). Because liver glucose production is often increased in patients with insulin resistance and contributes to the development of type 2 diabetes, the findings suggested that drugs that attenuate the CREB-CRTC2 pathway may provide therapeutic benefits for patients with type 2 diabetes (20).

More recently, Montminy also turned his attention toward understanding the role of CREB and CRTCs in regulating energy metabolism in other tissues. For instance, CREB and CRTC3 appear to be involved in lipid metabolism in adipose tissue: Mice that lack CRTC3 resist obesity, whereas mutations in CRTC3 that increase its activity are associated with obesity in humans (21, 22). Montminy also found that CRTC1, which is almost exclusively expressed in the brain, might be a central mediator of energy balance and fertility, because mice that lack CRTC1 have increased appetites, become obese, and are infertile (23).

In his Inaugural Article, Montminy examined CREB signaling in the pancreas, focusing on the molecular switches that promote the viability and growth of β-cells, which secrete insulin (24). This work unveiled a surprising mechanism by which cAMP alters gene expression, which might hold clues for developing new treatments for both type 1 and type 2 diabetes.

Altogether, Montminy's work has detailed multiple mechanisms by which cAMP can modify gene expression. "The degree to which they contribute to the cells' response to hormones or other cues is still something we need to work out. Ultimately, I'd like to understand how the CREB pathway elicits different biological responses in distinct cell types, in part, by turning on distinct gene programs in response to cAMP. That should keep us busy for many years to come."

Nicholette Zeliadt, *Science Writer*

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