CELL SIGNALING: The Double Life of a Transcription Factor Takes It Outside the Nucleus

Chan Young Park, *et al.*

Science **314**, 64 (2006);
DOI: 10.1126/science.1133757

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

CELL SIGNALING

The Double Life of a Transcription Factor Takes It Outside the Nucleus

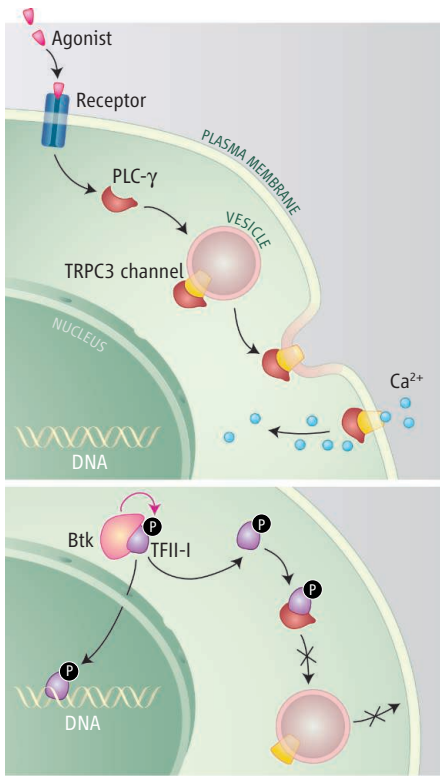
Although transcription factors had been thought to act only in the nucleus, new evidence that they can regulate calcium channels in the cytoplasm represents a mechanism for two-way communication within cells.

Chan Young Park and Richard Dolmetsch

It has long been known that calcium channels activate cellular signaling pathways that regulate transcription factors, but on page 122 of this issue, Caraveo *et al.* (*1*) turn this signaling paradigm on its head. They report that TFII-I, a ubiquitously expressed transcription factor, regulates the activity of TRPC3, a cell surface Ca^{2+} channel. Remarkably, the transcription factor affects the Ca^{2+} channel not through its effects on gene expression, but by competing with the channel for binding the enzyme phospholipase C (PLC). This mechanism of channel regulation reveals an entirely new way by which the TFII family of transcription factors can control cellular physiology and development. TFII-I thus joins a small group of transcription factors that function in both the nucleus and the cytoplasm.

TFII-I belongs to a family of general transcription factors, three of which are found in the 7q11.23 chromosomal region that is deleted in the congenital developmental disorder Williams-Beuren syndrome (*2*). Symptoms of this disease include hypersociability, mental retardation, and cardiac anomalies. The cardiac deficiencies are probably caused by deletion of the *elastin* gene (*3*), but the neurological and psychiatric symptoms might be due to deletion of neighboring genes such as *TFII-I* (*4*). TFII-I regulates transcription both by constitutively binding to the core sequence (Inr) found in the minimal promoter of most genes and by binding to other regulatory sites that lie in gene enhancers (*5*). Intriguingly, TFII-I is phosphorylated by Bruton's tyrosine kinase (Btk) (*6*), a protein that is important for B lymphocyte activation and which is mutated in X-linked agammaglobulinemia.

Agonist-induced activation of certain cell surface receptors is coupled to the activation of PLC, which leads to the release of Ca^{2+} from intracellular stores and Ca^{2+} influx across the plasma membrane (*7*). The resulting rise in intracellular Ca^{2+} is essential for many cell functions. Agonist-controlled Ca^{2+} entry depends on several types of ion channels including members of the transient receptor potential (TRP) family (*8*). TRPC3, the channel studied by Caraveo *et al.*, has been implicated in axon guidance (*9*) and in the development of cardiac hypertrophy (*10*).



Two jobs. (Top) Upon activation of specific receptors at the cell surface, PLC-γ binds to TRPC3 channels, causing insertion of the channels into the plasma membrane, where they mediate Ca^{2+} entry. **(Bottom)** The kinase Btk phosphorylates the transcription factor TFII-I, causing it to dissociate from Btk and bind to PLC-γ as well as enter the nucleus. By preventing PLC-γ from binding to TRPC3, TFII-I reduces the number of TRPC3 channels at the cell surface and inhibits agonist-controlled Ca^{2+} entry.

How PLC activates TRPC3 is somewhat controversial. PLC normally acts by cleaving phosphatidylinositol 4,5-bisphosphate to generate inositol trisphosphate and diacylglycerol. However, the authors previously reported that PLC-γ activates TRPC3 independently of its enzymatic activity (*11*) by binding to TRPC3 and increasing channel insertion into the plasma membrane. Among the five PLC subfamilies, PLC-γ members have a unique structure consisting of two separated halves of a pleckstrin homology (PH) domain, a conserved lipid-binding motif. Receptor activation causes the C-terminal half of the PH domain in PLC-γ to bind a PH-like "half domain" in TRPC3, thus increasing the plasma membrane insertion of TRPC3 and increasing Ca^{2+} influx (*12*).

Hints of a functional link between TFII-I and the TRPC3 channel came from the discovery that TFII-I binds the TRPC3 regulator, PLC-γ. When phosphorylated by Btk, TFII-I binds to a Src homology 2 (SH2) domain on PLC-γ. In addition, TFII-I binds to PLC-γ through an interaction between the partial PH domains in TFII-I and PLC-γ. Because the PH domain of PLC-γ is important for regulating TRPC3 at the cell surface, the authors investigated whether TFII-I expression regulates TRPC3 function. They found that reducing TFII-I protein levels increases Ca^{2+} influx whereas TFII-I overexpression reduces it, suggesting that TFII-I is a negative regulator of agonist-controlled Ca^{2+} entry. Deletion of a nuclear localization sequence did not affect the ability of TFII-I to reduce agonist-controlled Ca^{2+} entry, indicating that the transcription factor's function in the cytoplasm and nucleus are independent of each other.

Because PLC-γ regulates agonist-controlled Ca^{2+} entry by controlling the amount of TRPC3 at the cell surface, Caraveo *et al.* determined whether TFII-I regulates TRPC3 insertion into the plasma membrane by binding to PLC-γ. Reduced expression of TFII-I increased TRPC3 at the cell surface whereas overexpression of TRPC3 had the opposite effect. This depended on the partial PH domains in TFII-I and PLC-γ. Thus, cytoplasmic TFII-I can bind to the PH domain of PLC-γ and prevent PLC-γ from causing TRPC3 insertion in the plasma membrane.

So how does the interaction of TFII-I and PLC-γ regulate the physiology of cells? In Williams-Beuren syndrome, reducing the amount of TFII-I and its closely related family members might lead to overactivity of TRPC3, to errors in axon pathfinding, and to other developmental defects that depend on receptor-controlled calcium entry. Mutations of the voltage-activated L-type Ca^{2+} channel are associated with autism (*13*), suggesting that Ca^{2+} signaling can cause subtle effects on nervous system development that can result in cognitive disorders.

The study by Caraveo *et al.* raises several

The authors are in the Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305, USA. E-mail: ricardo.dolmetsch@stanford.edu

CREDIT: P. HUEY/SCIENCE

Published by AAAS

interesting questions. How is the relative abundance of TFII-I in the cytoplasm and nucleus determined? TFII-I phosphorylation causes TFII-I translocation into the nucleus (*14*), and yet this phosphorylated form of the protein also binds to PLC-γ in the cytoplasm. Understanding precisely how these two pools of TFII-I are regulated will reveal how the two functions of the molecule are controlled. PLC-γ also plays a key role in activating many signaling enzymes, including protein kinase C, and TFII-I may regulate many of these signaling events at the plasma membrane. The existence of proteins such as TFII-I and DREAM/KChIP (*15*, *16*) that regulate both transcription and ion-channel function support an emerging paradigm whereby proteins that function both in the nucleus and in the cytoplasm of cells coordinate the overall ability of a cell to respond to membrane stimuli and to activate gene expression.

References

1. G. Caraveo, D. B. van Rossum, R. L. Patterson, S. H. Snyder, S. Desiderio, *Science* **314**, 122 (2006).
2. Y. Franke, R. J. Peoples, U. Francke, *Cytogenet. Cell Genet.* **86**, 296 (1999).
3. A. K. Ewart *et al.*, *Nat. Genet.* **5**, 11 (1993).
4. S. K. Danoff, H. E. Taylor, S. Blackshaw, S. Desiderio, *Neuroscience* **123**, 931 (2004).
5. A. L. Roy *et al.*, *EMBO J.* **16**, 7091 (1997).
6. W. Yang, S. Desiderio, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 604 (1997).
7. G. S. Bird *et al.*, *Curr. Mol. Med.* **4**, 291 (2004).
8. P. D. Wes *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 9652 (1995).
9. Y. Li *et al.*, *Nature* **434**, 894 (2005).
10. H. Nakayama, B. J. Wilkin, I. Bodi, J. D. Molkentin, *Faseb J.* **20**, 1660 (2006).
11. R. L. Patterson *et al.*, *Cell* **111,** 529 (2002).
12. D. B. Van Rossum *et al.*, *Nature* **434**, 99 (2005).
13. I. Splawski *et al.*, *Cell* **119**, 19 (2004).
14. C. D. Novina *et al.*, *Mol. Cell. Biol.* **19**, 5014 (1999).
15. W. F. An *et al.*, *Nature* **403**, 553 (2000).
16. A. M. Carrion, W. A. Link, F. Ledo, B. Mellstrom, J. R. Naranjo, *Nature* **398**, 80 (1999).

10.1126/science.1133757

CHEMISTRY

Mass Spectrometry: Bottom-Up or Top-Down?

A novel approach to mass spectrometry involving fragmentation of intact proteins in the gas phase promises to greatly improve our ability to determine protein modifications.

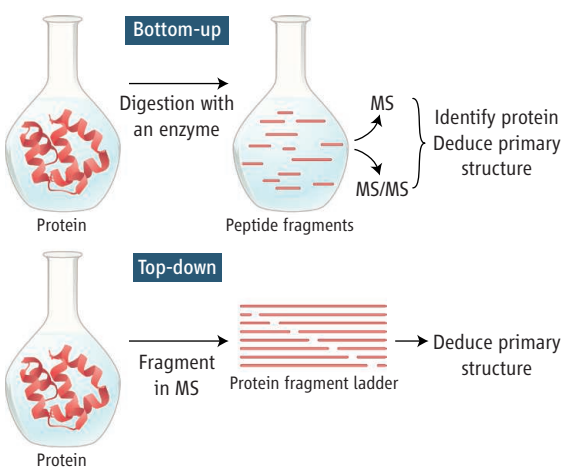
Brian T. Chait

The current revolution in proteomics and systems biology is driven by new analytical tools that are both fast and sensitive. Among these tools, mass spectrometry has become the method of choice for rapidly identifying proteins and determining details of their primary structures (*1*). Currently, there are two complementary lines of attack for the mass spectrometry analysis of proteins: the bottom-up and top-down approaches. On page 109 of this issue, Han *et al.* (*2*) extend the range of the top-down approach to proteins with molecular masses as high as 229 kD.

The bottom-up approach (see the figure, top panel) is widely used for identifying proteins and determining details of their sequence and posttranslational modifications (*1*). In this approach, proteins of interest are digested with an enzyme such as trypsin, and the resulting "tryptic peptides" are analyzed by electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI). These mass spectrometry techniques allow peptide and protein molecular ions to be put into the gas phase without fragmentation (*3*). The ESI– or MALDI–mass spectrometry analyses take place in two stages. First, the masses of the intact tryptic peptides are determined; next, these peptide ions are fragmented in the gas phase to produce information on their sequence and modifications.

The bottom-up approach is especially useful for identifying proteins, because tryptic peptides are readily solubilized and separated, tasks that are considerably more difficult for the parent proteins. In addition, many tryptic peptides can be readily analyzed by mass spectrometry analysis, providing useful fragmentation ladders (*4*) that often yield sufficient information to identify the parent protein. Unfortunately, only a small fraction of the tryptic peptides are normally detected, and only a fraction of these yield useful fragmentation ladders. The bottom-up approach is therefore suboptimal for determining modifications and alternative splice variants (*5*). It is a little like having a jigsaw puzzle, where many of the pieces are missing.

But even if we had all the pieces, the picture would still be incomplete, because—to produce a sufficient number of tryptic peptide ions to allow for their detection by mass spectrometry—it is currently necessary to examine the pieces of a billion or more copies of the protein of interest. So really we have a billion jigsaw puzzles, some of which are the same, but many of which are slightly different, because they correspond to copies of the protein containing different modifications. Thus, if the pieces are relatively small (as they usually are for tryptic peptides), we will lose



CREDIT: P. HUEY/*SCIENCE*

Dissecting the primary structures of proteins by mass spectrometry. In the widely used bottom-up approach (**top**), proteins of interest are digested in solution with an enzyme such as trypsin, and the resulting peptides are analyzed in the gas phase by mass spectrometry in two stages. In the first (labeled "MS"), the masses of the intact tryptic peptides are determined; in the second (labeled "MS/MS"), these peptide ions are fragmented to produce information on the identity and sequence of the protein as well as its modifications. In the top-down approach (**bottom**), intact protein ions are introduced into the gas phase and are fragmented and analyzed in the mass spectrometer, yielding the molecular mass of the protein as well as protein ion fragment ladders; this information can be used to deduce the complete primary structure of the protein. Both methods make extensive use of correlations of the mass spectrometric data with protein and whole-genome sequence databases.

The author is in the Laboratory for Mass Spectrometry and Gaseous Ion Chemistry, Rockefeller University, New York, NY 10021, USA. E-mail: chait@rockefeller.edu

Published by AAAS