VIEWPOINT

Signaling Pathways for PC12 Cell Differentiation: Making the Right Connections

D. Vaudry,[1] P. J. S. Stork,[2] P. Lazarovici,[3] L. E. Eiden[1]*

A key issue in signal transduction is how signaling pathways common to many systems—so-called canonical signaling cassettes—integrate signals from molecules having a wide spectrum of activities, such as hormones and neurotrophins, to deliver distinct biological outcomes. The neuroendocrine cell line PC12, derived from rat pheochromocytoma, provides an example of how one canonical signaling cassette—the Raf → mitogen-activated protein kinase kinase (MEK) → extracellular signal-regulated kinase (ERK) pathway—can promote distinct outcomes, which in this case include neuritogenesis, gene induction, and proliferation. Two growth hormones, epidermal growth factor (EGF) and nerve growth factor (NGF), use the same pathway to cause PC12 proliferation and differentiation, respectively. In addition, pituitary adenylate cyclase–activating polypeptide (PACAP), a neurotransmitter that also causes differentiation, uses the same canonical cassette as NGF but in a different way. The Connections Map for PC12 Cell Differentiation brings into focus the complex array of specific cellular responses that rely on canonical signal transduction systems.

The PC12 cell line was derived from rat pheochromocytoma, a tumor arising from chromaffin cells of the adrenal medulla. It is a useful model for studying cell signaling for at least two reasons: (i) There are few growth factors, neurotrophins, and hormones to which it does not respond; and (ii) distinct responses of differentiation, proliferation, and survival can all be assessed independently. Differentiation (halted proliferation and neurite outgrowth) of PC12 cells by NGF was described in the first report on the cell line (*1*). NGF signaling through the receptor tyrosine kinase (RTK), TrkA, causes differentiation (*2*). The paradoxical finding that the *src* and *ras* oncogene products enhanced rather than blocked NGF-induced differentiation led to the identification of signaling pathways involving both Ras and Src as part of the total differentiation response to NGF (*3*). A closely related RTK activated by EGF stimulates proliferation, rather than differentiation, of PC12 cells (*4*). The responses to NGF and EGF both require ERK, a mitogen-activated protein kinase (MAPK). Neurite outgrowth stimulated by PACAP, an adrenomedullary neurotransmitter, also occurs through ERK activation, in a process similar to but distinct from NGF signaling (*5*).

These studies put into focus a fundamental question of signal transduction: How are canonical signaling cassettes, such as Raf → MEK (i.e., MAPK kinase) → ERK, accessed by hormones and neurotrophins and differentially integrated into the signaling network (*6*) of PC12 cells to promote distinct outcomes, including neuritogenesis, gene induction, and proliferation (*7*)?

The duration of signaling through ERKs may hold the key to the very different outcomes of EGF and NGF stimulation. EGF induces rapid and transient Ras- and Rap1-dependent ERK phosphorylation, whereas NGF stimulation of ERK is both rapid and sustained, with sustained activation dependent on signaling to ERK through Rap1 (*8, 9*) (Fig. 1). Differential recruitment of phosphatidylinositol 3-kinase (PI3K) and scaffolding components (such as the adaptor FRS2) to activated TrkA, but not to the EGF receptor complex, may be the explanation for sustained Rap1-mediated B-Raf activation by TrkA, but not by the EGF receptor (*8–10*). PI3K also activates the c-Jun NH_2-terminal kinases (JNKs), which, through activation of c-Jun, can promote differentiation or apoptosis, depending on the cell's history of exposure to NGF (*11*). Thus, differentiation, survival, and proliferation may involve a balance among MAPK signaling pathways that depends, in turn, on the combination of neurotrophins and other first messengers present in the cellular milieu.

G protein–coupled receptor (GPCR) activation can also stimulate some aspects of differentiation, especially neurite outgrowth. PACAP signals through the GPCR type 1 PACAP-preferring receptor (PAC1) in PC12 cells (*12, 13*). Both NGF and PACAP cause robust neurite outgrowth, which requires activation of ERK (*14*). NGF requires both Ras- and Rap1-dependent B-Raf activation to stimulate neurite outgrowth, whereas PACAP signaling is Ras-independent (*5*). Does PACAP stimulate a second pathway that substitutes for Ras in neuritogenic signaling? PACAP-stimulated neurite extension is blocked by RpcAMPS, a cyclic adenosine 3′,5′-monophosphate (cAMP) antagonist (*15*). Elevation of cAMP activates ERK through protein kinase A (PKA)–dependent activation of Rap1, which stimulates B-Raf (*16*). However, PACAP-stimulated neuritogenesis is not blocked by the PKA inhibitor H89 (*5, 14*), which suggests that another cAMP sensor besides PKA mediates activation of ERK by PACAP. Finally, cAMP response element (CRE)–mediated transcription is a convergence point for multiple

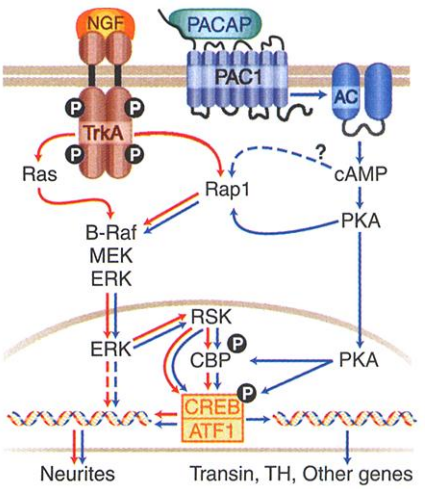

Fig. 1. Signaling pathways for PACAP- and NGF-dependent PC12 cell differentiation. PACAP-dependent signaling is coded in blue, NGF-dependent signaling in red. Arrows are meant to convey major features of information flow through signaling pathways activated differentially by NGF and PACAP. Differences in intensity, duration, and synergy of signaling through a given node or set of nodes, although not indicated, contribute to qualitative differences in PACAP and NGF actions. For example, Ras- and Rap1-dependent signaling are thought to account for immediate and sustained effects, respectively, of NGF mediated through ERK. Rap1-dependent B-Raf activation may also differ in intensity and duration in a stimulus-dependent fashion, perhaps accounting for PKA-dependent and PKA-independent aspects of signaling through ERK. Thus, although the TrkA and PACAP pathways activate several common cellular signaling components, their ultimate effects on gene transcription and cellular phenotype differ substantially. Abbreviations: AC, adenylate cyclase; ATF1, activating transcription factor 1; CBP, CREB binding protein; CREB, cAMP response element–binding protein; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase kinase; NGF, nerve growth factor; PAC1, type 1 PACAP-preferring receptor; PKA, protein kinase A; RSK, ribosomal S6 protein kinase; TH, tyrosine hydroxylase.

[1]Section on Molecular Neuroscience, Laboratory of Cellular and Molecular Regulation, National Institute of Mental Health, Bethesda, MD 20892, USA. [2]Vollum Institute, Oregon Health Sciences University, Portland, OR 97201, USA. [3]Department of Pharmacology, School of Pharmacy, Hebrew University of Jerusalem, Jerusalem 91120, Israel.

*To whom correspondence should be addressed. E-mail: eiden@codon.nih.gov

pathways initiated by GPCR and RTK activation in PC12 cells, through both CRE-binding protein (CREB) and CREB-binding protein (CBP) (Fig. 1) (*17, 18*).

Transcription of CRE-dependent genes in PC12 cells that is required for differentiation may also depend on additional cis elements in CREB-responsive genes besides the CRE itself. For example, NGF increases expression of the c-*fos* gene through both a CRE and a serum response element (SRE) (*19*). PACAP also increases expression of neuropeptide genes through separate cAMP and calcium signaling pathways (*20*). Thus, combinatorial specificity is a fundamental element in the signaling equation at all levels of transduction, from receptor-stimulated signal generation to transcriptional responses.

PC12 cells continue to be an important model system by which to study how hormones, neurotransmitters, and neurotrophins initiate multiple signaling pathways that converge on specific cellular targets to execute complex processes, such as neurite extension and competence for neuronal excitability. Understanding this complexity will require a broader view of signal transduction, in which pathway specificity is created by the integration of unique combinations of canonical signaling cassettes within a specific cell. The PC12 Cell Differentiation Pathway (http://stke.sciencemag.org/cgi/cm/CMP_8038) (*21*) in the STKE Connections Maps should facilitate this effort as it applies to neuronal differentiation.

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VIEWPOINT

The Jasmonate Pathway

Robin Liechti and Edward E. Farmer*

Plants are faced with many of the same problems as animals—a need for regulation of metabolic processes and reproduction and for defense against enemies. Jasmonates in plants serve key roles in gene and metabolic regulation, defense, responses to trauma, reproduction, and possibly communication. Some remarkable features of plant responses, such as production of repellent volatiles as a defense against herbivorous insects, or the massive transcriptional reprogramming that occurs in response to wounding, are under the control of the jasmonate pathway. Details of the jasmonate signaling pathway are currently at the center of active research that is generating exciting results. The Jasmonate Biochemical Pathway at the STKE Connections Maps is designed to present and keep pace with these developments.

Jasmonates (*1*, *2*) are potent lipid regulators in plants that mediate responses to both mechanical trauma and pathogenesis (*3–5*), and play pivotal roles in reproduction (*6*) and metabolic regulation (*7*).The biological activities of these lipid-derived molecules are reminiscent of some of the roles of well-known mediators in animals, most notably prostaglandins. This parallel between the molecules is strongly reinforced when one considers their structures. Whereas prostaglandins are formed from the 20-carbon eicosanoid precursor arachidonic acid, jasmonates are derived principally from the 18-carbon fatty acid linolenic acid. Jasmonate synthesis, at least in reproductive tissues, begins with phospholipase A_1-release of linolenic acid (*8*). Oxygenation of linolenic acid is catalyzed by 13-lipoxygenase. The resultant 13-hydroperoxide is dehydrated by allene oxide synthase to an unstable allene oxide intermediate (*9*) before cyclization, guided by allene oxide cyclase, to the cyclopentenone ring-containing 12-oxo-phytodienoic acid (OPDA) (Fig. 1). OPDA can be further metabolized, by reduction of the ring double bond catalyzed by OPDA reductase 3, yielding a cyclopentanone intermediate. This intermediate is then subjected to three rounds of β-oxidation, which yield the best-known jasmonate family member, the 12-carbon regulator jasmonic acid (JA) (*2*). Both OPDA and JA have several fates within plant tissues; both can be conjugated by esterification to other molecules, galactolipids in the case of OPDA (*10*) and various amino acids or simply a methyl group in the case of JA (*2*).

One of the remarkable features of the jasmonate pathway is that its members have different biological activities. Whereas JA synthesis is required for male fertility in the model plant *Arabidopsis*, its precursor OPDA can play an important regulatory role in defense along with JA (*11*). Another remarkable feature of the jasmonate pathway is that the activity of some of its members is not confined within the plant. Volatile jasmonate family members, such as the JA metabolite *cis*-jasmone (cJ), help regulate the behavior of some insects—for example, repelling herbivorous species and attracting their predators—and may act as an indicator of JA metabolism in damaged leaves, thereby signaling leaf quality to herbivores (*12*). Some evidence suggests that certain volatiles of the jasmonate pathway may enable communication among plants (*12*).

Jasmonate concentrations are tightly regulated in plants; the relative basal amount of each jasmonate family member may differ from tissue to tissue. In the case of mechanical wounding, rapid jasmonate biosynthesis occurs with differential control over the concentration of each compound (*11*). Massive transcriptional reprogramming under the control of jasmonates is a feature of wounding (*11*) and a response to certain pathogens (*13*). Upon wounding, the expression of large numbers of genes is increased or decreased in a jasmonate-dependent manner, and those that are increased include front-line defense-related genes encoding wound-related and pathogenesis-related proteins. It is not yet known which jasmonate-regulated genes are necessary for male fertility in *Arabidopsis*.

With a basic understanding of the biochemistry in place, the task now turns to understanding the proteins and genes that are involved in jasmonate signaling. The jasmonate perception

Gene Expression Laboratory, Ecology Institute, University of Lausanne, 1015 Lausanne, Switzerland.

*To whom correspondence should be addressed: edwardelliston.farmer@ie-bpv.unil.ch