BIOINFORMATICS

pathFinder: A Static Network Analysis Tool for Pharmacological Analysis of Signal Transduction Pathways

Babru B. Samal[1*] and Lee E. Eiden[2]

Published 5 August 2008

The study of signal transduction is becoming a de facto part of the analysis of gene expression and protein profiling techniques. Many online tools are used to cluster genes in various ways or to assign gene products to signal transduction pathways. Among these, pathFinder is a unique tool that can find signal transduction pathways between first, second, or nth messengers and their targets within the cell. pathFinder can identify qualitatively all possible signal transduction pathways connecting any starting component and target within a database of two-component pathways (directional dyads). One or more intermediate pathway components can be excluded to simulate the use of pharmacological inhibitors or genetic deletion (knockout). Missing elements in a pathway connecting the activator or initiator and target can also be inferred from a null pathway result. The value of this static network analysis tool is illustrated by the predication from pathFinder analysis of a novel cyclic AMP–dependent, protein kinase A–independent signaling pathway in neuroendocrine cells, which has been experimentally confirmed.

Presentation Notes

Slide 1: Science Signaling logo

This presentation is provided by www.sciencesignaling.org.

Slide 2: pathFinder: A static network analysis tool for pharmacological analysis of signal transduction pathways

This talk focuses on the static network-generating online tool, pathFinder, which allows researchers to identify qualitatively all possible signal transduction pathways that connect any starting component and target from the set of possible two-component pathways (directional dyads) in its database. pathFinder is located at http://pathfinder.nimh.nih.gov.

Slide 3: The pathFinder home page

The home page of pathFinder provides access to the tool, which can be used to qualitatively identify all possible signal transduction pathways connecting any starting component to a target using a set of two-component pathways (also called directional dyads) present in the dyad database, also called the CClist. The PubMed references supporting the interaction between the components of a dyad are listed in the CClistRef and also can be accessed from the home page. Links to a tutorial, a glossary of terms, and other pathway generation tools are also provided.

Slide 4: Usefulness of pathFinder

The pathFinder program identifies potential signal transduction pathways between first, second, or nth messengers and targets within the cell. It identifies all possible signal transduction pathways connecting any starting component (the activated element) and target for a given set of possible two-component pathways in the pathFinder database. Any component can be excluded from the analysis in order to simulate the use of inhibitors or genetic knockout in an actual experiment. Thus, pathFinder can be used to discover novel pathways through which a signal could be transmitted in the presence of specific inhibitors. These predictions can then be empirically tested.

Slide 5: General architecture of pathFinder at NIMH

The pathFinder database consists of dyads, sets of activated elements (for example, first messengers) and target elements (for example, gene activation or cellular output). Any of the elements in this database can be excluded to mimic the use of inhibitors or gene knockouts in a laboratory experiment.

Slide 6: Target, activator, and coactivator along with the PMID reference

This slide displays a portion of the CClistRef, where the PubMed IDs (PMIDs) of references supporting the interaction between the two components is listed for each dyad. The PMIDs are hyperlinked for easy access to the abstracts. The dyads were manually curated from the literature describing the relevant signaling interactions between the molecules. In some cases, there is also a third component because some targets require coactivation. Any component (element) can be excluded from a given analysis.

Slide 7: Query format

A researcher can access the pathFinder program by selecting the "run pathFinder" from the home page. A pull-down menu displays the field to select one of the activated elements and a target element. Elements that are to be excluded are available in a scroll-down menu format. Multiple elements can be excluded by holding down the control key [personal computer (PC) keyboard] while clicking.

Slide 8: Pathways created from a query

The neuropeptide PACAP was selected as the activated element, and neurites were selected as the target element. PACAP activates the G protein–coupled receptor (GPCR) PAC1 on PC12 cells, which leads to neurite formation, a process called neuritogenesis [(*1*, *2*), and references therein]. This query reveals 165 potential pathways, of which only the first 23 are shown. Some redundancy is built into the analysis because of differing levels of specificity in the literature. For example, PACAP is thought to cause calcium mobilization (Ca^{2+}mob) through activation of G_q downstream of the PACAP receptor (PACAP-R) (*3*) but many reports document downstream effects of PACAP-induced Ca^{2+}mob, without mentioning either the specific PACAP receptor isoform or G protein involved in signaling (*4*) (compare lines 2 and 3 of output). There may be cells in which PACAP causes Ca^{2+}mob without the G_q-mediated activation of phospholipase C–β (PLC-β) (*5*). Likewise, activation of extracellular signal–regulated kinase (ERK) by the guanosine triphosphatase (GTPase) Rap1 through the cyclic AMP–regulated guanine nucleotide exchange factor cAMP-GEFII (also known as

[1]National Institute of Mental Health—Intramural Research Programs (NIMH-IRP) Bioinformatics Core, NIMH, NIH, Bethesda, MD 20892, USA. [2]Section on Molecular Neuroscience, Laboratory of Cellular and Molecular Regulation, 9000 Rockville Pike, Building 49, Room 5A-68, NIH, Bethesda, MD 20892, USA.

*Presenter and corresponding author. E-mail: samalb@mail.nih.gov

Epac) is incorporated as a series of dyads (lines 5 through 8 of output), even though in PC12 cells Rap1 activation by cAMP-GEFII does not lead to ERK activation (*1*).

Slide 9: The effect of excluding elements on pathway output

The power of pathFinder is that it provides a "working memory" that can be used to pare down the list of potential pathways to a list of likely pathways for a given cell type or process. In this example, the goal is to identify the possible pathways from PACAP to neuritogenesis in PC12 cells and to winnow the list by excluding components on the basis of available experimental data. Akt can be excluded, because inhibition of phosphatidylinositol 3-kinase (PI3K), which activates Akt, does not abolish PACAP-induced neuritogenesis in PC12 cells (*6*); this reduces the number of potential pathways from 165 to 141. Protein kinase C (PKC) can be excluded because inhibitors of PKC do not block PACAP-induced neuritogenesis (*7*), and this reduces the number of potential pathways to 108. Similar exclusions of the GTPase Ras and PLC were made on the basis of the failure of inhibitors of these enzymes to affect PACAP-induced PC12 cell neuritogenesis (*1*, *7*, *8*). Finally, cAMP-GEFII can be provisionally excluded as the cAMP sensor for PACAP neurite induction because an agonist of cAMP-GEFII, 8-(4-phenylchlorothio)-2?-O-methyl cAMP, although it supports neuritogenesis induced by canonical (PKA-dependent) cAMP signaling, has little or no effect on its own (*9*).

Protein kinase A (PKA) can be excluded on pharmacological grounds, because classical PKA inhibitors (such as H89 and KT5720) fail to inhibit PACAP-induced neuritogenesis, even though these agents have off-target effects on other kinases. (*7*).

Slide 10: Pathways remaining after inhibitor-based pathway exclusion

Many pathways are eliminated after exclusion, on pharmacological grounds, of Akt, PKC, Ras, PLC, cAMP-GEFII, and protein kinase A (PKA). Each of the 18 remaining pathways contains ERK. Because ERK inhibition completely blocks PACAP-induced neurite induction (*2*, *7*, *8*), it is appropriate that all remaining pathways shown are in fact ERK-dependent.

Of these remaining 18 pathways, 12 contain Egr1 and can be divided into two groups of six pathways each. The pathways shown in orange type include cdk5 as an element, and the ones in white include p53. Cdk5 is required for Egr1 to stimulate neuritogenesis (*10*). In the absence of a proven Cdk5-independent pathway from Egr1 to neuritogenesis (Egr1→p35→neurites), the six white pathways can be eliminated, leaving only the six orange and six blue pathways.

Blocking Egr1 function with small interfering RNAs (siRNAs) prevents PACAP-induced neuritogenesis (*7*). Because Egr1 is critical for neuritogenesis, all relevant independent pathways to neuritogenesis must be Egr1-dependent. Exclusion of Egr1 should therefore produce a null set. However, there are six Rsk1-containing pathways (in blue) that do not contain Egr1, so these can be eliminated. In general, if all of the candidate pathways can be eliminated by a "null set" criterion as above, it may be worthwhile to investigate the hypothesis that the process is indeed independent of that component. In this example, experimental demonstration that PACAP-induced neuritogenesis is actually Rsk1-independent would be warranted, especially because experimental evidence indicates that constitutive expression of Rsk1 induces neuritogenesis in PC12 cells (*11*). An experimental finding that abrogation of Rsk1 function blocks neuritogenesis induced by PACAP would justify addition of a Rsk1-Egr1 dyad to the pathFinder database.

After this last set of exclusions, we are left with the six orange pathways as candidates for transducing the neuritogenesis signal from PACAP.

Slide 11: Assigning biological relevance to the output

The six separate candidate pathways from PACAP to neurites that remain after the exclusions made in the preceding two slides can be split into two broad categories: one that involves calcium-dependent activation of adenylate cyclase (AC) (blue boxes) and another in which AC is activated through Gs (red boxes). Common to all six pathways is the critical dyad cAMP→Rap1 without the intermediates cAMP-GEFII or PKA (yellow box). By including reports of direct activation of Rap1 by cAMP (*12*) that were published before the discovery of either cAMP-GEFII (*13*, *14*) or Src-dependent activation of Rap1 (*15*), this potential third pathway for Rap1 activation by cAMP is uncovered. Thus, one function of pathFinder is to incorporate specificity of signaling pathways while preserving generality that might uncover important molecularly distinct redundancies across signaling nodes. The activation of Rap-like GTPases by cAMP that ultimately leads to activation of ERK is such an example.

Slide 12: A novel cAMP signaling pathway for differentiation

Through the use of inhibitors to block specific enzymatic pathways, Ravni *et al*. (*7*) confirmed that neuritogenesis induced by PACAP in PC12 cells is indeed cAMP-dependent (blocked by dideoxyadenosine), PKA-independent [not blocked by *N*-[2-(4-bromocinnamylamino)ethyl]-5-isoquinoline (H89)], and ERK-dependent [blocked by 1,4-diamino-2,3-dicyano-1,4-bis(methylthio)butadiene (U0126)].

Slide 13: Comparing pathFinder with other pathway tools

Many pathway tools are designed to assign pathways to gene products whose expression, as assayed by microarray analysis, changes significantly in response to a stimulus. On the other hand, pathFinder's unique function is to build pathways on the fly between an upstream activator and a downstream target using dyads (two-component pathways). It can also be used to interrogate the requirement for specific components in a given pathway, on the basis of known pharmacological perturbations of or genetic knockout data for the pathway.

pathFinder exclusively generates signal transduction pathways, where first messenger–initiated cellular events lead to the regulation of a specific gene(s) or cellular processes. pathFinder operationally defines a signal transduction pathway as an activator-initiated event that culminates in a discrete outcome, such as activation of a target by triggering a cellular output, a change in gene transcription, or a change in the enzymatic activity of a signaling protein.

Limitations of pathFinder are the lack of quantitative information and, especially, the limited number of entries. The latter disadvantage can be overcome if the user recognizes when information is missing. For example, if exclusion of elements known to be dispensable for the process (pathway) leads to display of no pathways (null set), then the components linking the activated and target elements remain to be discovered empirically or need to be added to pathFinder from the literature. In either case, this information can be encoded in pathFinder by simply adding this dyad to the pathFinder database without citations or intermediate dyads, pending further identification of intermediate signaling components.

Editor's Note: This contribution is not intended to be equivalent to an original research paper. Note, in particular, that the text and associated slides have not been peer-reviewed.

References

1. M. J. Gerdin, L. E. Eiden, Regulation of PC12 cell differentiation by cAMP signaling to ERK independent of PKA: Do all the connections add up? *Sci. STKE* **2007**, pe15 (2007).
2. A. Ravni, S. Bourgault, A. Lebon, P. Chan, L. Galas, A. Fournier, H. Vaudry, B. Gonzalez, L. E. Eiden, D. Vaudry, The neurotrophic effects of PACAP in PC12 cells: Control by multiple transduction pathways. *J. Neurochem.* **98**, 321–329 (2006).
3. S. Shioda, H. Ohtaki, T. Nakamachi, K. Dohi, J. Watanabe, S. Nakajo, S. Arata, S. Kitamura, H. Okuda, F. Takenoya, Y. Kitamura, Pleiotropic functions of PACAP in the CNS: Neuroprotection and neurodevelopment. *Ann. N.Y. Acad. Sci.* **1070**, 550–560 (2006).
4. K. Tanaka, I. Shibuya, T. Nagamoto, H. Yamasha, T. Kanno, Pituitary adenylate cyclase-activating polypeptide causes rapid Ca^{2+} release from intracellular stores and long lasting Ca^{2+} influx mediated by Na^{+} influx-dependent membrane depolarization in bovine adrenal chromaffin cells. *Endocrinology* **137**, 956–966 (1996).
5. K. Tanaka, I. Shibuya, Y. Uezono, Y. Ueta, Y. Toyohira, N. Yanagihara, F. Izumi, T. Kanno, H. Yamashita, Pituitary adenylate cyclase-activating polypeptide causes Ca^{2+} release from ryanodine/caffeine stores through a novel pathway independent of both inositol trisphosphates and cyclic AMP in bovine adrenal medullary cells. *J. Neurochem.* **70**, 1652–1661 (1998).
6. M. J. Gerdin, L. E. Eiden, personal communication.
7. A. Ravni, D. Vaudry, M. J. Gerdin, M. V. Eiden, A. Falluel-Morel, B. J. Gonzalez, H. Vaudry, L. E. Eiden, A cAMP-dependent, PKA-independent signaling pathway mediating neuritogenesis through Egr1 in PC12 cells. *Mol. Pharmacol.* **73**, 1688–1708 (2008).
8. P. Lazarovici, H. Jiang, D. Fink Jr., The 38-amino-acid form of pituitary adenylate cyclase-activating polypeptide induces neurite outgrowth in PC12 cells that is dependent on protein kinase C and extracellular signal-regulated kinase but not on protein kinase A, nerve growth factor receptor tyrosine kinase, p21ras G protein, and pp60c-src cytoplasmic tyrosine kinase. *Mol. Pharmacol.* **54**, 547–558 (1998).
9. A. E. Christensen, F. Selheim, J. de Rooij, S. Dremier, F. Schwede. K. K. Dao, A. Martinez, C. Maenhaut, J. L. Bos, H. G. Genieser, S. O. Døskeland, cAMP analog mapping of Epac1 and cAMP kinase: Discriminating analogs demonstrate that Epac and cAMP kinase act synergistically to promote PC-12 cell neurite extension. *J. Biol. Chem.* **278**, 35394–35402 (2003).
10. T. Harada, T. Morooka, S. Ogawa, E. Nishida, ERK induces p35, a neuron-specific activator of Cdk5, through induction of Egr1. *Nat. Cell Biol.* **3**, 453–459 (2001).
11. E. Silverman, M. Frodin, S. Gammeltoft, J. L. Maller, Activation of $p90^{Rsk1}$ is sufficient for differentiation of PC12 cells. *Mol. Cell. Biol.* **24**, 10573–10583 (2004).
12. D. L. Altschuler, S. N. Peterson, M. C. Ostrowski, E. G. Lapetina, Cyclic AMP-dependent activation of Rap1b. *J. Biol. Chem.* **270**, 10373–10376 (1995).
13. J. de Rooij, F. J. Zwartkruis, M. H. Verheijen, R. H. Cool, S. M. Nijman, A. Wittinghofer, J. L. Bos, Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature* **396**, 474–477 (1998).
14. H. Kawasaki, G. M. Springett, N. Mochizuki, S. Toki, M. Nakaya, M. Matsuda, D. E. Housman, A. M. Graybiel, A family of cAMP-binding proteins that directly activate Rap1. *Science* **282**, 2275–2279 (1998).
15. Y. Obara, K. Labudda, T. J. Dillon, P. J. Stork, PKA phosphorylation of Src mediates Rap1 activation in NGF and cAMP signaling in PC12 cells. *J. Cell Sci.* **117**, 6085–6094 (2004).

10.1126/scisignal.131pt4

Citation: B. B. Samal, L. E. Eiden, pathFinder: A static network analysis tool for pharmacological analysis of signal transduction pathways. *Sci. Signal.* **1**, pt4 (2008).