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Abstract

This Podcast features a conversation with authors of a Research Resource published in the 25 October 2011 issue of *Science Signaling*. Although the extracellular signal-regulated kinase (ERK) pathway has been extensively studied, our understanding of all the regulatory interactions that modulate signaling is by no means complete. Adam Friedman and Norbert Perrimon discuss their group's strategy of combining functional and genomics approaches to identify common and specific regulators of ERK signaling in the fruit fly *Drosophila melanogaster*. The ERK pathway plays an important role in normal developmental and physiological contexts as well as in disease states, such as tumorigenesis.

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Technical Details

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Educational Details

Learning Resource Type: Audio

Context: High school upper division 11-12, undergraduate lower division 13-14, undergraduate upper division 15-16, graduate, professional, general public and informal education

Intended Users: Teacher, learner

Intended Educational Use: Learn, teach

Discipline: Biochemistry; bioinformatics, genomics and proteomics; cell biology; developmental biology; genomics; proteomics;

Keywords: *Science Signaling*, *Drosophila melanogaster*, ERK, extracellular signal-regulated kinase, fruit fly, MAPK, MAP kinase, mass spectrometry, mitogen-activated protein kinase, proteomics, Ras, RNAi, RNA interference, screen

Transcript

[Host – Annalisa VanHook] Welcome to the Science Signaling Podcast for October 25th, 2011. I'm Annalisa VanHook, and today I'm speaking with Adam Friedman and Norbert Perrimon about a study that combines functional and proteomics approaches to identify components of the Ras-ERK signaling network (1).

Linear models of signal transduction through a hierarchical cascade of protein-protein interactions are useful for understanding the basics of how a signal is passed through the core components of the pathway. In reality, however, signals are propagated through a large network of proteins with complex regulatory interactions, and many of these interactions are context- or cell type-specific. Approaches for identifying pathway components based on interfering with their function, such as mutagenesis screens or RNA interference screens, are great for identifying proteins that play a role in signaling, but they don't differentiate between proteins that play a direct role or an indirect role in propagating the signal. On the other hand, proteomics approaches may identify all of the protein-protein interactions that occur in a network, but they don't give any information about the directionality or hierarchy of the interactions within the network.

A team led by Perrimon and Friedman have used a combined functional and proteomics approach to identify components of the Ras-ERK signaling network that may have escaped detection in previous screens. Perrimon and Friedman spoke to me from Harvard University.

[Interviewer – Annalisa VanHook] This is Annalisa VanHook at Science Signaling, and on the phone with me is Adam Friedman and Norbert Perrimon. Welcome, Drs. Friedman and Perrimon.

[Interviewee – Norbert Perrimon] Glad to be here.

[Interviewee – Adam Friedman] Thank you very much. Glad to be here.

[Interviewer – Annalisa VanHook] Adam, for this analysis, you focused on the Ras-ERK pathway, which is a really intensively studied pathway that plays a role in development, homeostasis, and pathologies, such as cancer. What did you hope to learn about this pathway that wasn't already known?

[Interviewee – Adam Friedman] So the pathway, as you said, has been extensively studied for many years, and it's been really one of the canonical signaling pathways that have been described in development and disease. Our analysis is really fueled principally by a biological question, which is, "How complete is our understanding of the signaling output?" An underlying question that's been nagging the field is really how, how many regulators are out there, and how does this single pathway generate this kind of output in so many different contexts? So, to start to answer that question about specificity, you really have to basically get a roster of all of the genes and proteins involved in that pathway, and that's really where this started several years ago. So, you have to understand first, what are the components that regulate output of the pathway, and then how are they all connected—what is the hierarchy of all these connections? And then, once you have that information, then you can start to understand how do all of these components play a role in determining how the pathway is activated in these various contexts. The second question is really a secondary technical question that has been bothering the field for a number of years, which is, "How do you interpret functional screens—namely, RNA interference screens, where you knock down a gene—its transcript—and you determine what is the effect of knocking down that gene?" And one question from these studies have been what is the, the rate of false positives from this kind of analysis, or what are the significance of the genes that you identify from these kinds of analyses? Because you know that a number of the genes that you identify may be indirectly regulating the pathway through secondary or tertiary or

even, even more complex layers of regulation. Really, how direct is that regulation? So, we wanted to address that issue secondarily as a technical question.

[Interviewer – Annalisa VanHook] What model of ERK signaling did you use for your screen, and how did you combine the functional—or RNAi-based—approaches with a proteomics approach?

[Interviewee – Adam Friedman] So, we work in a Drosophila lab, and one of the great advantages of working in invertebrates, particularly Drosophila, are two. So, first is the wealth of knowledge that we already have about Ras and ERK signaling and Drosophila. It was one of the main model systems used to describe the core components of this pathway, and we know a great deal about the regulation of the pathway in Drosophila. And that also allows us technically to have a large number of reagents to study this pathway in Drosophila, as well as a number of different in vivo systems to study it, to be able to validate components that we find in cell culture. The other advantage of Drosophila is that it has much reduced redundancy compared to mammalian systems. So, there is a single ERK protein, for example, compared to multiple [ERKs] in mammalian systems. And that permits a degree of simplicity in the functional analysis when you're trying to knock down various components, you have a much greater likelihood of seeing an effect, as compared to when there might be feedback or regulation by other isoforms of the same protein.

[Interviewee – Norbert Perrimon] Actually, importantly as well, the, the RNAi methodologies that we are using—those long double-stranded RNAs—those are very effective reagents for doing knockdown in the Drosophila cells, so this technical aspect is very important to make sure that we generate accurate and very penetrant phenotypes.

[Interviewee – Adam Friedman] So, to address the question about the combining functional and proteomic approaches, we had previously published a genome-wide RNAi screen in Drosophila cells where we knocked down most of the genes individually in the Drosophila genome in cell culture and identified regulation of the ERK output through an immunofluorescence assay, measuring a phosphorylated or activated form of ERK. In order to expand upon this approach and address the questions that we had about how specific these regulators were and what is the complete size of the network regulating ERK, we combined that with a proteomic approach where we looked at all of the major canonical components of the pathway and generated tagged forms of these and used tandem mass spectrometry to identify all of the interactors with those core components. And we also expanded on our RNAi screens by conducting additional sets of RNAi screens under different stimuli in the same cell culture system. So, we had a total of six different screens, combined with our previous published screens, identifying regulators by functional approaches. And for a number of the canonical components, we then identified the interactors and then basically combined those two approaches after a series of a filtering of the proteomic analysis that our colleagues in Bonnie Berger's lab at MIT used SAINT methodology to filter out a number of different nonspecific regulators.

[Interviewee – Norbert Perrimon] The key aspect here is really that the, the intersection of the data sets from the proteomics and the RNAi, which really allows us to, to filter a lot of the noise on the, through both approaches and remove a lot of the false positives from which are inherent to each of the technology.

[Interviewer – Annalisa VanHook] And so, when you combined the data from these different approaches, what sorts of new pathway components or regulators did you identify?

[Interviewee – Adam Friedman] Before we did this work, a number of different investigators sort-of independently did either genome-wide unbiased functional screens or unbiased proteomic analysis to identify regulators through yeast two-hybrid or mass spectrometry. And the power of the approach is that we did both in the same system, because you can certainly start from one approach and then

perform the orthogonal approach on those regulators, but you really don't know how many things are you missing. And only by performing both approaches at the same time orthogonally do you identify really the totality of the regulators in that particular system. So, from this several hundred genes that were identified from both methodologies, we then proceeded to try to validate these both through additional RNAi screening and, importantly, through in vivo analysis. And we identified a number of different novel regulators. Some of these include a novel phosphatase family—the PPP6 complex—which may be involved in the regulation of Raf phosphorylation, which is known to be very important for its activation. And Raf is an upstream regulator of MEK and then ERK.

[Interviewer – Annalisa VanHook] Some of the genes fell out of both screens – they were identified by both the functional and the proteomics methods as regulating ERK signaling, but what about those genes that were only identified by one method or the other? Are there any of these genes that you're planning on studying any further?

[Interviewee – Norbert Perrimon] So, those are going to require a lot more validation to actually identify, characterize their function, whether or not they are real components of the network or whether or not they are simply false positives due to the technology that we are using. For example, from the mass spec analysis, many components may actually be really connected to the network but simply because we are using the phosphorylation of ERK as a readout for the activity of the network, they may not be validated using these assays simply because they may be feeding in a different biological process. So, clearly there is a lot more work which has to be done, and we need to really go and spend more time on those components that were identified with only one technology, as they may reflect a lot more biology, in fact, that we haven't really tapped into yet.

[Interviewer – Annalisa VanHook] Right. So, the fact that a gene may have only been identified through one approach or the other doesn't necessarily mean that it's not involved in regulating the pathway.

[Interviewee – Norbert Perrimon] That's correct. I mean, it could be [that] some components have a very subtle effect on the readout that we're using, which in this case again is phosphorylation of ERK MAP kinase, and it may be that their activity of those components is partially shunted by another component of the network. So, the type of follow-up analysis that we're starting to do on a large scale is to do combinatorial RNAi where we are going to sensitize the activity of the MAP kinase pathway and then ask whether or not those components which did not have a very significant RNAi score in the initial experiments, whether or not those in the sensitized background could have a major effect. So, clearly this is a...a lot of it is still work in progress, but it does provide a useful resource for follow-up studies.

[Interviewer – Annalisa VanHook] This work was done in the fly, where there's less redundancy and there's already a wealth of genetic and molecular tools for doing the analysis. Can you use this expanded network of Ras-ERK signaling in the fly to unravel the details of Ras-ERK signaling in other species, or would you need to repeat this sort of analysis in mammalian cells in a particular cellular context, such as a certain type of cancer or in a specific developmental event?

[Interviewee – Adam Friedman] So, we know that the Ras-ERK pathway is one of the most conserved signaling pathways, and that all of the core components of the pathway exist in mammalian systems and are quite important during mammalian development and, importantly, during cancer development. And aberrant signaling through the pathway is known to be responsible for a large number of cancers, including melanoma and pancreatic cancer. And, in fact, when we look at all of the proteins we identify, we, in fact, enrich for conserved proteins above the background. And so, we know that a large number of the genes we identify through both the functional and proteomic approaches are, in fact, conserved in mammalian systems, and we expect that a number of them will

also be part of the signaling network in mammalian systems. At the same time, one of the important points of our study is that we performed the analysis under several different signaling systems—so, we stimulated with an EGF ligand, we stimulated with insulin ligand, and we did the same for the proteomic studies—and we identified a large number that were specific to those particular conditions. And a number of the interactions were also dynamic in those particular conditions. And we also did the functional tests in different cell lines, as well, again identifying regulators that were specific to one particular cell line or the other. So, while we expect that a number of the components will be conserved and required in mammalian signaling, we also know how specific some of these regulators are to a particular cell type or stimulus. And determining which components are required under different signaling contexts in mammalian systems will require a great deal of downstream investigation, but the important feature is that we've essentially provided a roster of what the proteins might be from all of the proteins in mammalian systems to begin that kind of analysis. As another example of how these genes may be important mammalian systems, we know that our "hit list"—the genes that we identified that regulate signaling output—are, in fact, are enriched for genes that have been found to be mutated in human cancers from the number of whole exome sequencing projects that have been started in mammalian systems. So, for example, one of the genes we identified, *TepIV*, which is a homolog of CD109, in fact, is known to be mutated in 7% of colorectal cancers. It will require a great deal of additional studies to determine how this mutation leads to carcinogenesis and what its role is in cancer development. But one of the important implications of the study is that by identifying these genes that may be mutated in human cancers—and we also identified that they were regulators of Ras-ERK signaling—we can provide a clue as to how those genes may be involved in cancer development, perhaps through regulation of ERK output.

[Interviewee – Norbert Perrimon] I can also say, actually, that the data set that we are publishing is going to be very helpful for people which are doing very similar type of screen[s] in mammalian cells. So, I can imagine there are some people who will be doing basically the same type of screen that where we've done using phospho-ERK in different cell type[s] and so on. And as they generate their list of candidate hits, they may be able to use to filter directly their list with the list that we have and prioritize some candidates which may be more obscure—some genes of unknown functions—and really decide to focus the follow-up studies on those genes. Because one of the, the thing that we've learned all of the different RNAi screens over the years is that people tend to prioritize genes that they recognize—like a kinase or phosphatase or some transcription factor where some information is known—but there are a lot of genes, which are, where we know nothing about. And those usually are not on the top of everybody's list in term[s] of follow-up studies simply because they are a lot more risky because there is really nothing known about them—so, it's not clear exactly what kind of biochemical activity or function those things can have. So, I think that, long term, the data set that we provide might be a very powerful filter for the mammalian people to mine and then try to basically use that to prioritize what genes they should be following up on.

[Interviewer – Annalisa VanHook] Thank you, Dr. Friedman, Dr. Perrimon.

[Interviewee – Norbert Perrimon] Thank you very much.

[Interviewee – Adam Friedman] Sure. Thank you.

[Interviewer – Annalisa VanHook] That was Adam Friedman and Norbert Perrimon, authors of a Research article [Resource] published in this issue of Science Signaling. That paper, by Friedman and colleagues, is titled "Proteomic and Functional Genomic Landscape of Receptor Tyrosine Kinase and Ras to Extracellular Signal-Regulated Kinase Signaling" (1).

music

And that wraps up this Science Signaling Podcast. If you have any questions or suggestions, please write to us at sciencesignalingeditors@aaas.org. This show is a production of Science Signaling and of AAAS—Advancing Science, Serving Society. I'm Annalisa VanHook, and on behalf of Science Signaling and its publisher, the American Association for the Advancement of Science, thanks for listening.

Reference

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