

Minireview

## Distinct functions for ERKs?

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### Abstract

The Ras/Raf/MEK/ERK signaling pathway is one of the best understood signal routes in cells. Recent studies add complexity to this cascade by indicating that the two ERK kinases, ERK1 (p44<sup>ERK1</sup>) and ERK2 (p42<sup>ERK2</sup>), may have distinct functions.

An important intracellular signaling module leads from the small GTPase Ras through a cascade of protein kinases - Raf, MEK, and the extracellular signal regulated kinase ERK. Signaling through the Ras/Raf/MEK/ERK pathway has been implicated in many cellular processes, including proliferation, differentiation, survival, metabolism and morphology. Deregulation of the pathway has been associated with various pathologies, most notably with proliferative disorders such as cancer but also, more recently, with specific developmental abnormalities [1,2]. It is becoming increasingly clear that the temporal regulation of this pathway is critical for determining the signal output. A well known example of this is seen in pheochromocytoma-derived PC12 cells, in which a transient activation of the pathway is associated with proliferation, whereas sustained activation drives differentiation into neuronal-like cells [3]. Further evidence, from a detailed study of *Drosophila* eye development, has indicated that different thresholds of ERK activity result in distinct outcomes *in vivo*; low and high levels of Ras/Raf/ERK activity are associated with the promotion of cell survival and differentiation, respectively [4]. There are many other such examples in other cell types and diverse organisms [5-7]. Preliminary insights into how signal dynamics can be 'read' by a cell have started to become apparent, but much is not understood, and this remains one of the challenges of cell biology [8,9].

The two ERKs, ERK1 (also called p44<sup>ERK1</sup>) and ERK2 (also called p42<sup>ERK2</sup>), have numerous substrates, many of which are nuclear and participate in the transcriptional regulation of a range of cellular processes. The two kinases are very similar in sequence and have tended to be thought of interchangeably [10]. The activity of the pathway is often represented by the levels of MEK-directed phosphorylation of ERK1 and ERK2, as detected by phospho-specific antibodies; the double bands on electrophoresis gels (phospho-ERK1 and phospho-ERK2) that appear in response to numerous stimuli are usually taken together as representing total ERK activity. Likewise, the importance of this signaling pathway in various cellular processes tends to be deduced from the effects of well-characterized inhibitors such as U0126, which inhibit the upstream kinase MEK and so would not distinguish between the activity of the two ERKs. Recent studies, however, including a report by Vantaggiato and Formentini *et al.* [11] in this issue of *Journal of Biology*, present compelling evidence that the ERK kinases are not functionally redundant but might have very different roles.

The two ERK proteins are coexpressed in most tissues but stark differences in their relative abundance were the first possible hints of a differential function [12]. More intriguingly, the knockout phenotypes turned out to be very different: ERK2 knockout mice die early in development, showing

that ERK1 cannot compensate for ERK2 [13]. By contrast, ERK1-deficient mice are viable, and have only minor defects, such as a deficit in thymocyte maturation - and there is no detectable compensatory upregulation of ERK2 levels [14]. The current study from Riccardo Brambilla's laboratory [11] along with a previous study from the same group [15] provide the most convincing evidence to date that the two kinases might have distinct roles. In these reports, an important finding has been that loss of ERK1 can result in a mouse with apparently 'improved' functions. ERK1<sup>-/-</sup> mice were found to have an increased rate of learning and better long-term memory than wild-type controls - processes in which ERK signaling has previously been shown to be important [15]. ERK2 levels were not higher in the brains of these animals, but in primary neurons isolated from the knock-out animals, enhanced ERK2 activation was observed, despite an equivalent activation of the upstream kinase MEK. Furthermore, an enhancement of long-term potentiation was observed in brain slices isolated from the ERK1<sup>-/-</sup> animals - an improvement in function apparently attributable to enhanced ERK2 activity. These findings appeared inconsistent with a simple redundancy of function but are more consistent with an inhibitory effect of ERK1 on ERK2 signaling.

In the more recent study, Vantaggiato and Formentini *et al.* [11] investigated the role of the specific ERKs in cellular proliferation and Ras-induced oncogenic transformation - processes in which total ERK signaling has well-established roles. Again, it was found that loss of ERK1 appeared to result in a 'gain of function' suggestive of an inhibitory role for ERK1 in these particular cellular outputs. Mouse embryo fibroblasts (MEFs) isolated from ERK1 knockout mice seemed to proliferate faster than control cells. ERK2 levels were unperturbed in these cells, but activation, monitored by phosphorylation status, was found to be elevated and more sustained in the ERK1 knockouts compared with wild-type controls; this resulted in a more persistent induction of downstream immediate-early genes such as *c-fos* and *zif-268*. Adaptation issues can sometimes be associated with knock-out animals, with secondary changes masking the original phenotype. To avoid these problems, the authors [11] also specifically repressed ERK1 and ERK2 expression in MEFs using lentiviral vectors expressing short hairpin RNAs directed to each isoform. The ERK1 knockdown reproduced the effects seen in the ERK1 knockout cells, with an enhanced activation of ERK2 associated with the more rapidly proliferating cells. Conversely, the ERK2 knockdown cells proliferated poorly. These experiments seem to indicate that the proliferative signal is mediated by ERK2, whereas ERK1 has some type of inhibitory function.

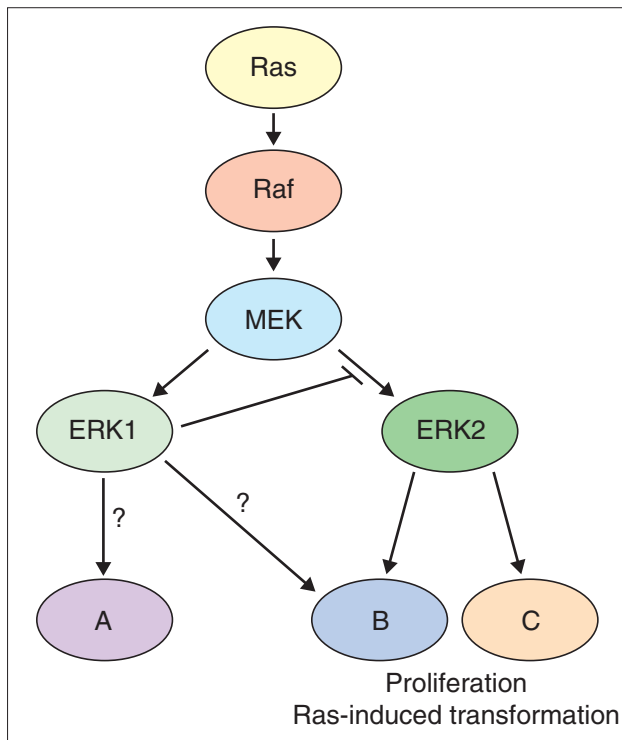
ERK signaling has been shown to be important for Ras-induced proliferation and transformation in many cell

systems [1,16,17]. In NIH 3T3 cells, which express apparently similar levels of ERK1 and ERK2, Vantaggiato and Formentini *et al.* [11] found that Ras-induced colony formation was inhibited by knock-down of ERK2, whereas loss of ERK1 had no effect, indicating that the transforming activity of Ras requires ERK2 activity but not ERK1. Furthermore, overexpression of ERK1 inhibited Ras-induced transformation whereas overexpression of ERK2 did not, despite the two proteins being expressed to similar levels. Significantly, this inhibition did not require ERK1 kinase activity, as a kinase-dead mutant had a similar effect.

One possible interpretation of these results is that ERK1 competes with ERK2 for the upstream kinase MEK, but that the targets, and thus the function, of the two kinases are different. In this case it would appear that the proliferative signal is mediated solely by ERK2. The authors argue that this is the case and show that in the absence of ERK1, increased association of ERK2 with MEK can be detected. The hypothesis that the kinases can compete in this way is backed up by the observation that a kinase-dead form of ERK2 can also block Ras-induced transformation. Together, these data provide compelling evidence for a distinct role for the two kinases. The opposing effects of the knock-downs on proliferation and cellular transformation strongly argue for opposing functions. Likewise, the ability of ERK1 but not ERK2 to block Ras transformation, and the fact that the ERK2 kinase-dead mutant blocks Ras transformation efficiently, suggests that the two kinases do not function interchangeably.

So, ERK2 appears to be the mediator of the proliferative signal in MEFs and is required for Ras transformation of NIH 3T3 cells, whereas ERK1 appears to have an antagonistic function (Figure 1). But does this mean that they have a distinct set of substrates or just different affinities for the same substrates? In other words, does ERK1 really do something different, or does it just do the same thing less well? The experiments published so far do not formally distinguish between these possibilities; clarification will require a comparative analysis of ERK substrates in cells knocked down for each ERK. Genetic evidence from knock-ins of each ERK into each other's locus could also be invaluable.

Whatever the mechanism, these results demonstrate that the relative levels of ERK1 and ERK2 can have profound effects on the readout from the ERK pathway, resulting in distinct cellular outcomes. It will be important to reassess how ERK levels change during various biological processes that have been shown to be regulated by ERK signaling and to determine the contribution of each ERK to the response. The subtle phenotype of the ERK1 knockout mouse might argue against a fundamental role for ERK1, although adaptation



**Figure 1**

A diagram of the Ras/Raf/MEK/ERK pathway, showing the possible distinct functions for ERK1 and ERK2. A, B and C represent hypothetical downstream effectors. Proliferation and Ras-induced transformation require ERK2 but not ERK1. ERK1 appears to act antagonistically to ERK2 to compete for MEK, resulting in a dampening of ERK2 signaling. It is currently not known whether ERK1 and ERK2 have different substrates.

should be considered. It will be of great interest, however, to determine whether the phenotypes in the ERK1 knockout mouse result from a change in the dynamics of ERK2 signaling. These defects include a reduction of proliferation of some cell types and problems with the differentiation of others [5,12,14,18,19]. Is this the result of loss of ERK1-specific targets, a lowering of total ERK signaling, or a change in the dynamics of signaling through ERK2? In all cases reported so far, it appears that loss of ERK1 is associated with increased activation and/or a more sustained activation of ERK2 following an identical stimulus. How this might affect output may be difficult to predict. It is well established that many cells are dependent on ERK signaling for proliferation but that enhanced signaling can result in an exit from the cell cycle [3,20-23]. In some cases this is understood at the molecular level. In NIH 3T3 cells, low levels of ERK signaling induce cyclin D1 to maximal level and stimulate proliferation, whereas higher levels of ERK signaling induce the cyclin-dependent kinase inhibitor

p21<sup>Cip1</sup>, which causes cell-cycle arrest [21,24]. It would thus be easy to envisage how phenotypes of the ERK1 knockout animals, even including reduced cell proliferation could be the result of enhanced signaling through ERK2 rather than being due to a loss of ERK1-specific targets or a general decrease in ERK activity. A careful analysis of the signaling pathways involved should allow a distinction between these two possibilities to be made.

Evidence is mounting that ERK1 and ERK2 have distinct functions. Future studies need to take these findings on board and assess how the interplay between the two kinases affects the signaling dynamics of this pathway and how this can contribute to the cellular response.

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