Cell Cycle 10:5, 731-732; March 1, 2011; © 2011 Landes Bioscience

Plugging PKA into ERK scaffolds

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Abbreviations: ERK, extracellular-signal regulated kinase; MAPK, mitogen-activated protein kinase; AKAP, A-kinase anchoring protein; PKA, cAMP-dependent protein kinase; KSR, kinase suppressor of Ras

Cancers often arise in part through derangements in protein kinase signaling. A striking example of this is the finding that approximately 30% of human tumors have mutations in Ras or B-Raf, leading to aberrant ERK kinase activation. Kinase signaling networks are often organized by scaffolding and anchoring proteins that help shape the dynamics of signal processing. AKAP-Lbc associates with the ERK scaffold protein KSR-1 to organize a growth factor and cAMP responsive signaling network. AKAP-Lbc also directs PKA phosphorylation of KSR-1 on a critical residue to ensure maximal signaling efficiency.

Cancer is a group of diseases in which transformed cells display uncontrolled cell division and growth beyond normal limits. This promotes invasive movement of cells to advance the destruction of adjacent tissues.[1] Certain aspects of these adverse pathophysiologies are sometimes a consequence of aberrant or indiscriminant protein phosphorylation events catalyzed by protein kinases. Therefore, tight control of kinase signaling is essential for maintaining cellular integrity.[2]

One way to achieve this level of control is to efficiently manage the progression of signals through preassembled kinase complexes. This not only improves the spatial resolution of phosphorylation events, but also speeds up the transmission of information through signal transduction pathways.[2] Prototypic examples are the three-tier Mitogen Activated (MAPK) or Jun N-terminal (JNK) Protein Kinase cascades. For example, the ERK1/2 cascade couples signals from growth factors to cell proliferation through mobilization of the GTPase Ras. Active Ras stimulates Raf kinase, which in turn phosphorylates and activates MEK. This intermediary enzyme relays the signal by phosphorylating the terminal kinase, ERK. ERK then acts on downstream targets, resulting in changes in enzyme activity, protein localization and transcription and translation of key effector proteins.[3] Research interest in this pathway has been heightened by clinical evidence that up to one third of human tumors display mutations in Ras or B-Raf. As a result, small molecule drugs, such as the Raf inhibitor sorafenib, are being used to combat renal and hepatic cancers.[4]

Several investigators have suggested that further fine-tuning of ERK cascades can be achieved upon the recruitment of additional regulatory enzymes. This involves the establishment of larger multiprotein networks that are nucleated by scaffolding and anchoring proteins.[2,3] In mammalian cells, scaffold proteins such as Kinase Suppressor of Ras (KSR) and MEK Partner-1 (MP1) fulfill this role by orienting ERK kinases in relation to each other and in proximity to key cellular effectors.[3] We recently discovered that KSR-1 and the A-Kinase Anchoring Protein (AKAP)-Lbc form the core of a larger ERK network that incorporates protein kinase A (PKA) and integrates signals from the second messenger, cAMP (**Fig. 1**).[5] The implications of this finding are fascinating, especially when one considers that cAMP elevation antagonizes growth factor dependent signaling to ERK kinases in some cell types, yet augments signal relay through the Raf/MEK/ERK trio in other cells.[6] One explanation for this may lie in the actions of PKA and its substrates within the confines of the KSR-1/AKAP-Lbc signaling network.

The major target of inhibitory action is likely to be c-Raf, as PKA can phosphorylate c-Raf on multiple sites to disrupt activation by the small GTPase Ras.[6,7] It is less clear how PKA can sustain activation of the ERK cascade, though several possibilities were proposed.[6,8]

Another target may be KSR itself. This scaffolding protein has several predicted PKA sites and is phosphorylated within the context of the AKAP-Lbc/KSR-1 complex.[5] We focused our studies on a site at the C-terminus of KSR-1, serine 838. Several lines of inquiry point towards the functional significance of Ser 838 phosphorylation. This PKA site is conserved in vertebrate and invertebrate KSR orthologs. Mutation of Ser 838 to alanine impairs growth factor mediated ERK1/2 activation. Finally, re-expression of KSR-1 S838A in knockout MEFs (or overexpression in HEK293 cells) fails to support normal ERK1/2 activation in response to growth factors and cAMP. Taken together these observations imply that phosphorylation of serine 838 of KSR-1 by anchored PKA is required to maximally stimulate this ERK signaling network.

So how does phosphorylation of KSR-1 by PKA modulate signaling to ERK? Clues may come from the region surrounding

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Submitted: 01/18/11; Accepted: 01/19/11
DOI: 10.4161/cc.10.5.14902
Comment on: Smith FD, et al. Nat Cell Biol 2010; 12:1242–9.

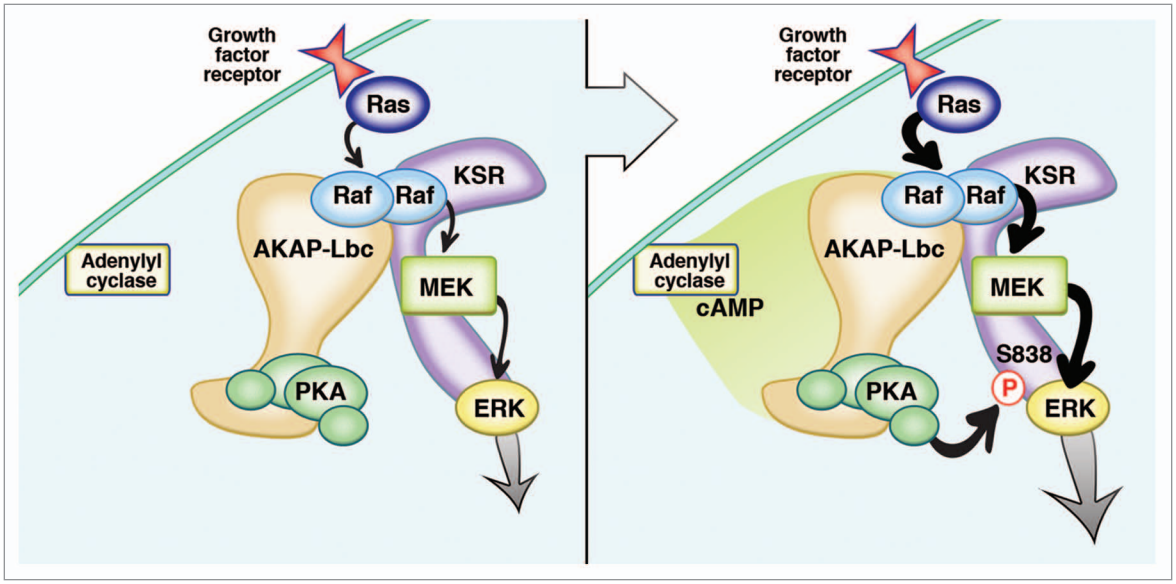

Figure 1. A schematic representation of the AKAP-Lbc/KSR-1 signaling network and the integration of growth factor and cAMP signals. AKAP-Lbc associates with the MAPK scaffold KSR-1 and positions PKA to respond to cAMP by phosphorylating KSR-1 on serine 838. We propose that this phosphorylation event is necessary to support efficient ERK signaling. Furthermore, the stoichiometry of different components in the complex may determine whether cAMP stimulates or inhibits ERK activation in different cell types.

S838 on KSR-1, which resembles a corresponding sequence on Raf (c-Raf S621 or B-Raf S729). Raf activity is regulated when this phosphorylated serine binds to 14-3-3 proteins. This does not seem to be the case for KSR-1, as ablation of S838 has no effect on 14-3-3 binding (ref. 9 and Smith FD et al., unpublished). Another possibility is that S838 somehow regulates association with MEK as nearby mutations, such as the C809Y allele (analogous to a loss of function mutation identified in *C. elegans*[10]), disrupt MEK binding and impair ERK1/2 signaling. Additionally PKA phosphorylation of S838 could provide a conformational element that constrains the integrity of the KSR-1/AKAP-Lbc core complex. The answers to these questions should be forthcoming when structural studies on this complex are complete.

Nonetheless, our study highlights the complexity of interactions between the Ras/Raf/MEK/ERK and the cAMP signaling pathway and uncovers an unexpected mode of PKA regulation of the scaffold KSR-1. Furthermore, we believe it will be informative to determine the ratio of B-Raf to c-Raf associated with AKAP-Lbc and whether tumor-specific changes in expression levels or dysregulation of other elements of this multiprotein network influence the progression of various cancers.

Acknowledgements

F.D.S. and J.D.S. are supported in part by DK54441 and funds from foundation Leducq.

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