Phosphonucleotide Effects on MEK1-ERK2 Interaction

Phosphorylation States Alter MEK1-ERK2 Interaction

ATP Modulates MEK1-ERK2 Binding

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Nucleotide modulation of MEK1-ERK2 phosphorylation-interaction

Summary

By multiplexing AlphaScreen® and AlphaLISA® beads, we have recently observed in a single well the direct dissociation of MAP kinase ERK2 from MAP2K MEK1 upon phosphorylation. Furthermore, we have used this experimental approach to discriminate an allosteric inhibitor from an ATP competitor. Here, we use these tools to further study the protein-protein interactions between MEK1 and ERK2 with respect to phosphorylation state-dependent and -independent effects. We first determined that the dissociation between the two proteins is solely dependent on their activation state and does not involve a phosphorylation feedback loop. Second, by co- titration experiments on full-length recombinant proteins, we confirmed that phosphorylation of MEK1 (but not of ERK2) reduces their binding. However, this interaction is more sensitive to the phosphorylation of MEK1 than that of ERK2. Interestingly, the binding between unphosphorylated MEK1 and ERK2 is impaired by increasing ATP concentrations. This interaction modulation requires the intact catalytic domain of MEK1 but not that of ERK2. Other phosphonucleotides such as ADP, ATP-S, and UDP can also modulate MEK1-ERK2 binding, whereas AMP and GTP have no effect. Finally, ATP can modulate the interaction between ERK2 and its non-cognate MAP2K MK66 also in a phosphorylation-independent manner. The above results suggest that nucleotides can influence the conformation of MAP2Ks. These results affect MAP kinase binding and our Alpha technologies platform provided a sensitive approach to detect subtle modulation in protein-protein interactions. Moreover, multiplexing phosphorylation and interaction events allows screening for small molecules that modulate catalytic activity and enzyme-substrate interactions, either individually or together.

Materials & Methods

All recombinant protein kinases were purchased from Carna Bio except N-His-MEK1 from SignalChem and C-His-MEK1 (wt and K97R) from Millipore. Addition of detection reagents. Donor beads were used at 40 µg/mL and filters were from PerkinElmer.

All assays were performed in 384 well OptiPlates™ (PerkinElmer) at 23°C in Multilabel Plate Reader and filters were from PerkinElmer.

Technology. AlphaScreen and AlphaLISA beads as well as the EnVision® technology. AlphaScreen and AlphaLISA beads allowed simultaneous monitoring of substrate phosphorylation and enzyme binding.

Conclusion

AlphaScreen and AlphaLISA® beads allowed simultaneous monitoring of substrate phosphorylation and enzyme binding. AlphaScreen and AlphaLISA® beads were used interchangeably. ERK2 dissociates from MEK1 solely upon phosphorylation. MEK1-ERK2 interaction is performed by phosphorylation of ERK2 but not significantly by phosphorylation of MEK1.

ATP modulates MEK1-ERK2 binding by phosphorylation-dependent manner. This requires the kinase integrity of MEK1 but not of ERK2. MK66-ERK2 interaction is also ATP-sensitive. Our assays provide a sensitive platform to detect protein-protein interactions that can be modulated by small molecules such as nucleotides in a phosphorylation-dependent or -independent manner.