

Control of Protein Phosphorylation by Diffusion in Cellular Signaling Pathways

Daniel E. Zak
Daniel Baugh Institute
Thomas Jefferson University
Philadelphia, PA 19107
Department of Chemical
Engineering
University of Delaware
Newark, DE 19716
zak@che.udel.edu

Boris M. Slepchenko
Center for Biomedical Imaging
Technology
Department of Physiology
University of Connecticut
Health Center
Farmington, CT 06030
boris@photon.uhc.edu

Boris N. Kholodenko
Department of Pathology,
Anatomy and Cell Biology
Thomas Jefferson University
1020 Locust Street
Philadelphia, PA 19107
boris.kholodenko@mail.tju.edu

ABSTRACT

Multiple proteins involved in signal transduction cascades are phosphorylated and dephosphorylated at separate cellular locations. For instance, a protein kinase can be localized exclusively to the cell membrane, whereas a protein phosphatase can be delocalized throughout the cytosol. This suggests the possibility that there may be spatial gradients of the phosphorylated or unphosphorylated forms of such proteins. For example, if the protein is phosphorylated at the cell membrane and slowly diffuses into the cytosol where it is dephosphorylated, and the unphosphorylated form diffuses back to the membrane, then there may be a spatial gradient of the phosphorylated form, high close to the membrane and low within the cell. Using measured values of protein kinase and phosphatase activities, and protein diffusion rates, we recently demonstrated that spatial gradients of cellular phospho-proteins can be very large [1]. For a monocyclus cascade we estimated the extent to which a protein kinase, phosphatase and diffusion of signaling proteins control the protein phosphorylation flux and the phospho-protein gradient. We demonstrated that the control contribution of protein diffusion is potentially significant given the measured rates of protein kinases, phosphatases, and diffusion. For a spherical cell of radius $10 \mu\text{m}$, a protein diffusion coefficient of $10^{-8} \text{ cm}^2 \text{ s}^{-1}$, and rate constants of the kinase and phosphatase of about 1 s^{-1} , the control over the phosphorylation flux resides mainly on the phosphatase and protein diffusion, with approximately equal contribution from each of these [2]. The ratio of the phospho-protein concentrations at the cell membrane and cell center (the dynamic compartmentation of the phospho-protein) is shown to be controlled by the rates of the protein phosphatase and diffusion. The kinase can significantly contribute to the control of the absolute value of the phospho-protein gradient.

In the Ras-MAPK pathway, Raf (the kinase at the first MAPK cascade level) phosphorylates the downstream kinase, MEK, at the plasma membrane, whereas the specific MEK phosphatases are mainly distributed throughout the cytosol. In the cytosol, active MEK phosphorylates ERK (the terminal MAPK) and specific ERK phosphatases are localized to cytosol and nucleus. Spatial separation of protein kinases and phosphatases in the MAPK cascade may

result in the precipitous spatial gradients of activated MEK and ERK. We estimated that the concentration of active ERK near the plasma membrane can exceed its concentration in the perinuclear area by three orders of magnitude. Accordingly, the distance, over which the phosphorylation signal is attenuated to almost basal level, appears to be much smaller than the distance from the plasma membrane to the nucleus. This led us to the hypothesis that additional molecular mechanisms can control the relaying of signals through the MAPK cascades, from the plasma membrane to transcription factors in the nucleus.

REFERENCES

- [1] G. C. Brown and B. N. Kholodenko. Spatial gradients of cellular phosphoproteins. *FEBS Lett*, 457:452–454, September 1999.
- [2] B. N. Kholodenko, G. C. Brown, and J. B. Hoek. Diffusion control of protein phosphorylation in signal transduction pathways. *Biochem J*, 350(3):901–907, September 1999.