CHAPTER 4

RAS AND THE RAF/MEK/ERK CASCADE

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Abstract: The Raf/MEK/ERK protein kinases constitute a key effector cascade used by Ras to relay signals regulating cell growth, survival, proliferation, and differentiation. These kinases are activated in a sequential manner through direct phosphorylation. Raf is the initiating kinase that interacts with membrane-localized GTP-bound Ras. The signal is then transduced from Raf to MEK and from MEK to ERK, ultimately resulting in the phosphorylation of critical cellular targets by activated ERK. In addition to the core enzymes of the cascade, various scaffolding proteins and signaling modulators have been identified that affect the efficiency and level of signaling through this important kinase cascade. An emerging concept is that these factors contribute to the spatiotemporal control of Ras/ERK signaling, allowing sensitive activation and deactivation of the pathway in response to diverse extracellular cues.

Keywords: Signal transduction, protein kinase, phosphorylation

1. INTRODUCTION

Signal transduction is the process whereby cells translate extracellular signals into specific biological responses. In many cell types, modules of sequentially activating protein kinases, such as the MAPK cascades, are essential for this process, functioning as a relay route from the cell surface to the nucleus and as central integrators of the signaling inputs. Signal transduction mediated by the RasGTPase is no exception and in higher eukaryotic organisms, the kinase module used by Ras is the MAPK cascade comprised of the Raf/MEK/ERK kinases, also known as the ERK module (Pearson et al., 2001). The first kinase in this module, Raf, is a direct effector of Ras that binds specifically to active GTP-bound Ras. This interaction recruits the cytoplasmic Raf protein to the plasma membrane where it becomes activated. Raf then phosphorylates and activates MEK, which in turn phosphorylates and activates ERK. The cascade culminates when activated ERK...
phosphorylates critical cytoplasmic and nuclear substrates required for a specific cellular response.

In this chapter, we will examine how signal transmission through the Raf/MEK/ERK cascade is regulated. First, we will review the molecular mechanisms that control the activity of the core kinase components Raf, MEK, and ERK. Then we will discuss the scaffolding proteins and signaling modulators that affect the efficiency, spatiotemporal dynamics, and level of signaling through this major kinase cascade.

2. THE RAF KINASES

Members of the Raf serine/threonine kinase family are the initiating enzymes in the three-tiered ERK kinase cascade. In mammalian cells, there are three Raf proteins, Raf-1, A-Raf, and B-Raf (Hagemann and Rapp, 1999). Invertebrate organisms such as Drosophila melanogaster and Caenorhabditis elegans encode a single Raf kinase, but no homolog is present in yeast. The Raf kinases were first discovered when the \textit{raf-1} gene was identified as the cellular counterpart of the murine retroviral oncogene, \textit{v-raf} (Rapp et al., 1983). Raf-1 is the most widely expressed member of the three mammalian Raf kinases with significant protein levels detected in all cell types examined (Storm et al., 1990). Expression of the other family members is more limited with A-Raf expression highest in urogenital tissues and B-Raf expression highest in neuronal tissues, testis, and haematopoietic cells. Determining how Raf kinase activity is regulated has been a daunting task that has challenged investigators for years – due largely to the complexity of the process. In this section, we will examine the molecular mechanisms involved in Raf regulation. Our discussion will focus primarily on Raf-1, the most extensively studied Raf protein; however, distinct regulatory features of the other Raf family members will also be described.

2.1 Regulation of Raf by Autoinhibition

All Raf proteins contain three conserved regions, CR1, CR2, and CR3, and can be divided into two functional domains – an N-terminal regulatory domain and a C-terminal catalytic domain (Figure 1). The N-terminal regulatory domain contains both CR1, which consists of a Ras binding domain (RBD) and a cysteine-rich domain (CRD), and CR2, a region rich in serine and threonine residues, whereas the C-terminal catalytic domain comprises the CR3 (Daum et al., 1994). The first indication that the Raf N-terminus serves a regulatory role came from the observation that this domain is absent in the oncogenic v-Raf protein (Rapp et al., 1983). Subsequently, it was shown that deletion of this domain converts any of the mammalian Raf proteins into constitutively active kinases capable of inducing cell transformation (Heidecker et al., 1990; Stanton et al., 1989). The N-terminus thus functions as a repressor, inhibiting the activity of the catalytic domain through intramolecular interactions (Chong and Guan, 2003; Cutler et al., 1998), and for Raf