Chapter 9

PAXILLIN-ASSOCIATED ARF GAPS
Their Isoform Specificities and Roles in Coordination

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Abstract: Cell migration is a multifactorial process in which a number of distinct events occur simultaneously. Paxillin is an integrin-assembly adaptor protein. Here, properties of Arf GAPs bearing paxillin-binding capacity are described with their isoform specificity. Roles in linking Arf function with other intracellular events all need to be coordinately regulated during integrin-mediated cell adhesion and migration.

1. INTRODUCTION

Integrin-mediated cell movement is a multifactorial process in which a number of distinct intracellular events must occur simultaneously and coordinately. These include signal transduction, cytoskeletal remodeling, and membrane traffic and remodeling. These are required for proper adhesion, protrusion, traction and retraction, physical force generation and polarity formation. Cell migration is initiated by a variety of extracellular cues, such as by growth factors, cytokines, chemoattractants and extracellular matrix components. The intracellular biochemical events that are evoked by these stimuli (such as ion fluxes, protein activation, protein phosphorylation, and gene expression) have been extensively studied during the last two decades, and each has been well documented. The existence of cross talk between these events is also well known. However, several fundamental questions still remain largely unsolved. Prominent among these are the molecular mechanisms involved in the temporal and spatial coordination of distinct processes as well as the orchestration of distinct events occurring at different parts within a single cell, all of which result in a unified, efficient, directional migration. What is the principal or primordial
mechanism employed in such coordination and orchestration in integrin-mediated migration?

Arf GTPases are important factors regulating intracellular vesicle traffic and membrane remodeling. They are also important for actin-cytoskeletal remodeling. Recently, several Arf GTPase activating proteins (GAPs) have been found to bind paxillin, an integrin-assembly scaffolding/adaptor protein. In this chapter, the roles of a subset of cellular Arf GAPs in integrin-mediated cell adhesion and migration are discussed. We have focused on aspects of the mechanisms by which Arf activities coordinate the remodeling of membrane and actin-cytoskeleton in motile cells, together with the isoform specificity of the Arf GAPs involved. We will describe how paxillin-associated Arf GAPs differ structurally and functionally, and discuss the model in which different Arfs work with different subsets of Arf GAPs to effect changes in the actin cytoskeleton and membrane remodeling. The specificity of Arf GAPs for different Arfs, assessed both biochemically and in cell biological assays, will also be addressed. A hypothesis that explains the dual regulation of substrate specificities toward the different Arfs in vivo is proposed.

2. INTEGRINS, PAXILLIN AND CELL MIGRATION

Integrins are bi-directional, cell surface α/β heterodimeric signaling proteins. Integrins require assembly of several different types of proteins at their cytoplasmic tails to transmit their signals (Hynes, 2002). These integrin-assembly proteins also play a role in transmitting intracellular information into changes in the ability of integrins to bind extracellular ligands. Integrins exhibit dynamic properties during cell adhesion and migration. Newly synthesized integrins are transported to the plasma membrane via intracellular membrane/vesicle traffic. In actively migrating cells, subsets of integrins (including, e.g., fibronectin receptors) are recycled actively and can be brought to the leading edges of the cell periphery, perhaps coupled with their endocytosis at the trailing edge of the cell (Bretscher, 1989; Bretscher, 1992; Lawson and Maxfield, 1995). However, it appears that not all types of integrins can enter recycling endosomes (Bretscher, 1992; Roberts et al., 2001). In contrast, in cultured fibroblasts most of the β1 integrin molecules adhering to the extracellular matrix (ECM) at the rear of the cell are left behind as the cell body moves forward (Regan and Horwitz, 1992).

Most integrin-assembly proteins are recruited to integrins by the binding of integrin to extracellular environments. A hierarchical and step-wise regulation exists in the recruitment of integrin-assembly proteins (Miyamoto et al., 1995a; Miyamoto et al., 1995b). How then do such proteins assemble