ABSTRACT

Among the molecular modulators of cytoskeletal organization are the Abl family tyrosine kinases c-Abl and Arg (Abl1 & Abl2) that regulate actin dynamics by phosphorylating cytoskeletal proteins and through direct F-actin binding. In addition to a catalytic domain, they possess SH3, SH2 and actin binding domains. The c-Abl tyrosine kinase maintains tissue homeostasis through its ability to regulate apoptosis and actin dynamics. In vivo, c-Abl activity is stringently regulated and mechanisms involved are not fully understood.

The intrinsic kinase activity of c-Abl is strictly regulated through intra- and intermolecular interactions as well as tyrosine phosphorylation of critical residues. The catalytic activity of c-Abl is increased by multiple physiological stimuli like DNA damage, oxidative stress, entry into S-phase, integrin activation and platelet-derived growth factor stimulation. c-Abl kinase activity is negatively regulated by PTPs and by ubiquitin-dependent degradation. Overexpression of c-Abl induces apoptosis and c-Abl null cells are more resistant to apoptotic stimuli. It functions through phosphorylation of target proteins in a context dependent manner and integrates signals to actin remodelling. Morphological changes that occur during apoptosis, such as detachment, shrinkage and membrane blebbing are dependent on actin cytoskeletal modification.

C3G (RapGEF1) is an ubiquitously expressed guanine nucleotide exchange factor (GEF) that targets small GTPases Rap1, Rap2, R-Ras and TC10. C3G has been implicated in pathways triggered by growth factors, cytokines, G-protein coupled receptors and adhesion receptors. C-terminus of C3G is responsible for target G-protein activation and proline-rich sequences in its central region bind SH3 domains of Crk, Cas and Hck. The catalytic activity of C3G is regulated by Crk binding and tyrosine phosphorylation at Y504. Src family kinases phosphorylate C3G and Y504 phosphorylated C3G localizes to the Golgi and subcortical actin cytoskeleton. When co-expressed in mammalian cells, interaction of Hck with C3G results in activation of an apoptotic pathway, which is independent of the catalytic activity of C3G. Non-catalytic sequences of C3G are responsible for suppression of transformation induced by oncogenes. C3G plays
a role in cytoskeletal reorganization and filopodia formation. Knock-out of C3G in mice causes embryonic lethality and mutant fibroblasts show impaired cell adhesion and enhanced cell migration.

The cytostatic effects of c-Abl have been attributed to its ability to induce apoptosis. Kinase activity of c-Abl is required not only for its transforming activity, but also for apoptosis. It has therefore been important to identify Abl activation mechanisms as well as effector substrates involved in apoptosis. Our recent work showed the requirement of C3G in c-Abl induced filopodia formation in response to integrin stimulation indicating a functional association of these two molecules in a common pathway towards actin cytoskeletal remodelling. This prompted us to investigate the interaction between these molecules, the ability of c-Abl to phosphorylate C3G and determine functional consequence of this interaction/phosphorylation with respect to c-Abl signalling.

The main objectives of this work have been:
1) To determine whether C3G is an interacting partner and substrate of c-Abl kinase.
2) To analyse the mechanisms of regulation of c-Abl activity towards phosphorylation of C3G
3) To characterize the consequence of interaction and phosphorylation of C3G by c-Abl.

Chapter 1 provides information on the properties and functions of c-Abl and C3G pertinent to the current study.

Chapter 2 provides the description and source of reagents used and a detailed description of the methodologies used in this study.

Chapter 3 describes results, which identify C3G as a substrate and effector of c-Abl tyrosine kinase. C3G interacts with and is a substrate of c-Abl both in vivo and in vitro. Ectopic expression of c-Abl induced phosphorylation of endogenous
C3G on Y504 in cells undergoing apoptosis. p-C3G showed polarized distribution due to restricted c-Abl activation in regions rich in F-actin dependent on cellular F-actin dynamics. Compared to C3G or c-Abl, p-C3G showed stronger binding to the actin cytoskeleton. Localized C3G phosphorylation, and coincidence with cells undergoing apoptosis was dependent on F-actin binding domain of c-Abl. Oxidative stress but not cisplatin activates cellular Abl to phosphorylate C3G. c-Abl activity is repressed in live cells due to PTP activity. Inhibition of C3G expression and function using RNAi or dominant negative approaches inhibited c-Abl mediated apoptosis. These findings identify C3G as a novel target of c-Abl activated in response to physiological stimuli such as oxidative stress and during apoptosis.