

ABSTRACT

Cells contain a complex system of filaments called the cytoskeleton that regulates a variety of cellular processes. In mammalian cells, these filaments can be categorized into three groups: actin filaments, microtubules and intermediate filaments. The work presented here focuses on actin filaments, which are predominantly found beneath the plasma membrane, where they play a central role in determining cell shape.

First, small molecules based on natural products were screened for their ability to perturb actin dynamics. One of these molecules (LGM235) was shown to influence the organization of actin in cells, even at extremely low concentrations (~85 pM). A fluorescently labeled analogue of LGM235 was found to be cell permeable and non-toxic, making it useful in studying static actin within living cells.

Next, a series of biosensors was developed in order to study the activities of three members (Rac1, Cdc42, and RhoA) of the Rho GTPase family of signaling molecules that are known to regulate the actin cytoskeleton. These biosensors were tested in combination with a photoactivatable Rac1 (PA-Rac1) construct to study the crosstalk between Rac1, Cdc42, and RhoA. Surprisingly, these experiments showed that the activities of Rac1, Cdc42, and RhoA all increase in response to acute photoactivation of Rac1. However, PA-Rac1 displayed an unexpected subcellular localization that is inconsistent with that of wild-type or constitutively active Rac1, making these crosstalk measurements difficult to interpret. Therefore, an optimized photoactivatable Rac1 construct was developed that localizes primarily to the plasma membrane.

As an alternative to photoactivation, a new and more generally applicable method of controlling Rho GTPase activity, based on chemically induced dimerization (CID), was developed. In this approach, a small molecule (referred to as a “dimerizer”) binds simultaneously to two distinct protein modules to form a heterodimeric protein complex. Because neither of the protein modules described here are endogenous to mammalian cells, this approach represents a novel bioorthogonal CID system that can be used to control protein function in cells. The C-terminal sequence from K-Ras was fused to one of these modules to ensure that it would localize to the plasma membrane. A constitutively active mutant of Rac1 lacking a prenylation site was fused to the other protein module and was observed largely in the cytosol. When dimerizer was added to cells expressing these two proteins, rapid recruitment of the constitutively active Rac1 mutant to the plasma membrane was observed. This recruitment, was accompanied by the formation of actin-based protrusions at the cell periphery that are typically observed upon activation of Rac1. And, similar experiments with constitutively active Cdc42 and RhoA mutants produced changes in the actin cytoskeleton and in cell morphology that are consistent with activation of these signaling molecules.

The tools described here can be used to study actin dynamics and its regulation by Rho GTPase signaling networks.