

Reactive oxygen species and hydrogen peroxide generation in cell migration

Dominika A Rudzka, Jenifer M Cameron, and Michael F Olson*

Cancer Research UK Beatson Institute; Garscube Estate; Switchback Road; Glasgow, UK

Directional cell migration is a complex process that requires spatially and temporally co-ordinated regulation of actin cytoskeleton dynamics. In response to external cues, signals are transduced to elicit cytoskeletal responses. It has emerged that reactive oxygen species, including hydrogen peroxide, are important second messengers in pathways that influence the actin cytoskeleton, although the identities of key proteins regulated by hydrogen peroxide are largely unknown. We recently showed that oxidation of cofilin1 is elevated in migrating cells relative to stationary cells, and that the effect of this post-translational modification is to reduce cofilin1-actin binding and to inhibit filamentous-actin severing by cofilin1. These studies revealed that cofilin1 regulation by hydrogen peroxide contributes to directional cell migration, and established a template for discovering additional proteins that are regulated in an analogous manner.

Keywords: actin, cofilin, cytoskeleton, chemotaxis, hydrogen peroxide, motility, ROS

© Dominika A Rudzka, Jenifer M Cameron, and Michael F Olson

*Correspondence to: Michael F Olson; Email: m.olson@beatson.gla.ac.uk

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Cell migration plays important roles in many normal biological and pathophysiological processes. In response to external signals, the actin cytoskeleton undergoes dynamic changes that are essential for cell migration. Information from these external signals may be transduced by pathways that involve direct protein-protein interactions, protein post-translational modifications or the generation of second messengers that propagate the signal within a cell, culminating in cytoskeletal responses. In recent years, it has become apparent that reactive oxygen species (ROS) are key intermediaries in many signal transduction pathways, acting as short-lived diffusible second messengers,¹ including pathways the culminate in

cytoskeletal responses and associated changes in cell motility.^{2,3}

As indicated by the name, ROS are chemically-reactive molecules containing oxygen, including superoxide anions or singlet oxygens, which may be by-products of biological reactions occurring in cell membranes, mitochondria, peroxisomes and endoplasmic reticulum catalyzed by oxidative enzymes.⁴ In mitochondria, ROS are produced through respiratory chain reactions when oxygen is prematurely and incompletely reduced to superoxide radicals. A significant source of ROS in migrating cells comes from the activity of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) Oxidase (NOX) complexes,⁵ and Molecules Interacting with CASL (MICAL) proteins.⁶ NOX and MICAL enzymes mainly produce superoxide, which can be reactive in its own right, but is usually converted to hydrogen peroxide by superoxide dismutases (SOD).⁷ The activity of NOX complexes can be triggered by various stimuli including chemotactic growth factors.⁸ Activators of MICAL are less well characterized, although a role of MICAL proteins in cellular responses to semaphorin/plexin signaling during axon guidance has been well established.⁶

There are 5 NOX complexes (NOX1–5) and 2 related DUOX enzymes (DUOX1–2), which are transmembrane ROS generators.⁹ NOX2 is the best characterized family member, and its activation is dependent on the formation of a 6 component protein complex composed of 5 phox units (gp91, p22, p40, 47 and p67) and GTP-bound Rac1 or Rac2. The requirement for Rac in the activation of NOX1 has also been clearly established but is less certain for other NOX complexes. In contrast, to NOX1 and NOX2, DUOX proteins are Rac-independent. The participation of Rac

GTPases in NOX2 complex formation is mediated via interaction with the activator p67 protein, while NOXA1 appears to be the mediator for Rac-induced NOX1 activation. The role of Rac proteins in promoting cell migration and ROS generation is strongly suggestive of a causal link between these activities.

ROS generation by MICAL enzymes has been linked directly to reorganization of filamentous actin structures¹⁰ via oxidation of both cytoskeletal¹¹ and nuclear actin.¹² MICAL enzymes contain 4 domains: an N-terminal flavin adenine dinucleotide (FAD) binding domain, a calponin homology (CH) domain, a Lin11, Isl-1 and Mec-3 (LIM) domain and a C-terminal coiled-coil (CC) domain where FAD is the redox enzymatic domain.⁶ In human cells there are 3 known isoforms, MICAL1, MICAL2 and MICAL3 and 2 MICAL homologs, MICAL-L1 and MICAL-L2.⁶ Although the function of MICAL proteins in regulating cell morphology and motility was first associated with neuronal axon guidance, all 3 MICAL proteins have been shown to regulate actin structures in non-neuronal cells as well.¹³ In addition to

their roles in cell migration, MICAL proteins are also involved with other cell biological functions including cell-cell adhesion,¹⁴ exocytosis and apoptosis.¹⁵

The main way that hydrogen peroxide regulates protein function is through oxidation of thiols on specific cysteines.¹⁶ The modification of thiol groups goes via production of unstable mono-oxidized sulfenic acid that may undergo further modifications to create the more stable sulfinic acid (2 oxygens) or sulfonic acid (3 oxygens). Thiol groups in the sulfenic acid stage may also be stabilised by the formation of intramolecular or intermolecular disulphide bonds. Although NOX and MICAL enzymes are significant ROS producers, and some proteins that become oxidized as a consequence (*e.g.* actin) have been identified, our knowledge of the wider spectrum of proteins modified downstream of NOX and MICAL has been limited by technical challenges presented by the robust identification of oxidized proteins.

In order to identify protein oxidation induced by cell migration, we used the cell permeable compound 5,5-dimethyl-1,3-cyclohexanedione (dimedone), which

irreversibly reacts with cysteine sulfenic acid, to trap newly oxidized proteins.¹⁷ Western blotting with an antibody that recognized dimedone-conjugated proteins¹⁸ revealed that migration increased protein oxidation, while mass spectrometry was used to identify peptides shifted by 138 Da rather than the 57 Da mass/charge shift produced by post-lysis iodoacetamide-mediated modification of cysteine thiols.¹⁹ In this way, we identified Cysteine 139 and Cysteine 147 on cofilin1 as being oxidized, and showed by immunoprecipitation and western blotting that cofilin1 oxidation was higher in migrating cells than in stationary cells. Further analysis indicated that the effect of these oxidation events was to decrease cofilin1-actin binding, reduce filamentous actin severing by cofilin, and alter directional cell migration. Our model shown in **Figure 1** indicates how the spatially-restricted inhibition of cofilin1 filamentous-actin severing activity could promote membrane protrusion and directional migration in response to a chemotactic stimulus. These observations raise further questions that will be addressed in the future, including, 1) What additional proteins that contribute to cell migration are regulated by oxidation. Two) What are the roles of NOX

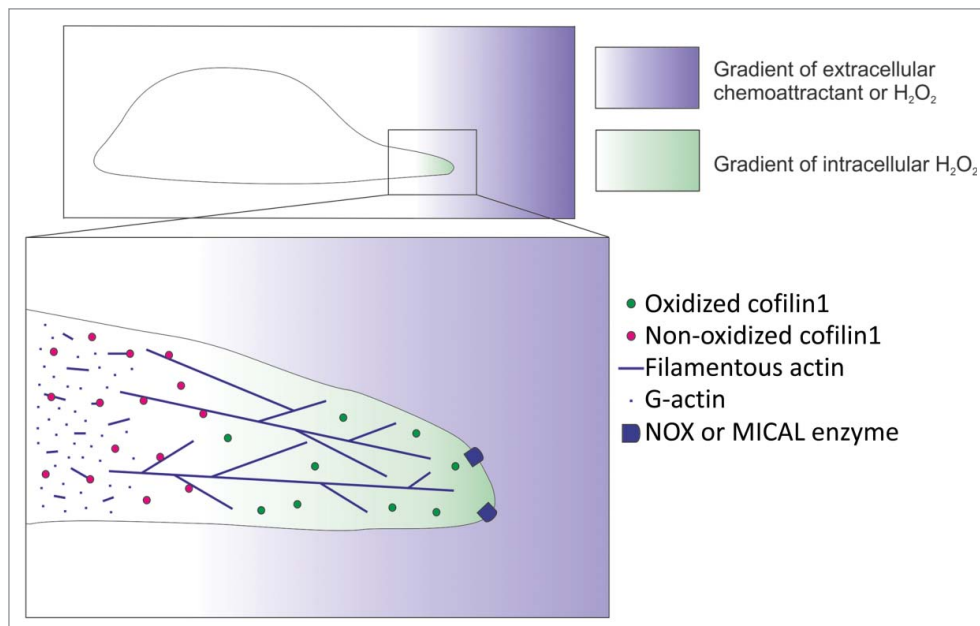


Figure 1. Model of cofilin oxidation in cell migration. Hydrogen peroxide (H_2O_2), acting directly as a chemoattractant or produced by activated NOX or MICAL enzymes, oxidizes cofilin1 at the leading edge of a cell. Consequently cofilin1 is prevented from binding to and severing or depolymerising filamentous actin, leading to net increases in actin polymerization. Meanwhile, cofilin1 further from the front of a protrusion remains active, supplying G-actin and polymerized actin barbed ends required to drive the continued growth of the protrusion. This localized control of cofilin1 activity facilitates cell migration by allowing protrusions to develop and extend.

and MICAL enzymes in the oxidation of cofilin and additional migration-regulating proteins?

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No potential conflicts of interest were disclosed.

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