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Actin Methionine Oxidation: The Next Level of Dynamic Regulation

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Actin Methionine Oxidation: The Next Level of Dynamic Regulation

Actin PTM Background

Actin is a well-characterized, abundant, and essential cytoskeletal protein. Its dynamic properties allow it to shift between monomeric (G-actin) and polymeric (F-actin) states, which is vital for many cellular processes. Actin's dynamicity and function is regulated by many internal and external cues that are facilitated by actin binding proteins (ABPs), signal transducers, and other proteins. Additionally, several studies now indicate that actin itself is highly modified by post-translational modifications (PTMs); furthermore, intensive studies of specific actin PTMs have detailed their effect on actin dynamics, ABP interactions, and actin-dependent physiology[1,2]. For example, actin N-terminal acetylation, lysine acetylation, arginylation, SUMOylation, and ubiquitination have been studied. Here, current studies on physiologic oxidation of actin at methionine (Met)44 and Met47 are summarized.

Actin Oxidation: Focus on Methionine Sulfoxide (MetO)

The oxidation field in general has focused on pathological oxidation (oxidative stress) induced by naturally formed oxidants like H_2O_2, and while it was initially hypothesized that oxidation was "toxic", it is now known that oxidation is a signaling mechanism for both pathological and physiological events[3]. Most of this work related to reactive oxygen species (ROS) focused on thiol-based cysteine (Cys) modifications. This is also true in the case of actin, whereby, most of the early work on actin oxidation utilized H_2O_2 treatment and resulted in altered F-actin content and polymerization activity as defined by enhanced lag time, slower polymerization rate, and lower polymerization levels[4-6]. Further investigation showed that H_2O_2-induced oxidation of actin initially targeted Cys374, but also targeted several Mets, including, Met44, Met47, Met176, Met190, Met269, and Met355 *in vitro*[7]. MetO oxidation happens *in vivo* under normal or stress conditions; however, Manta and Gladsyshev suggested that ROS-induced MetO formation *in vivo* occurs very inefficiently relative to the kinetics of reducing enzymes like MsrA[8]. Thus, the question of whether actin's methionines can become oxidized enzymatically *in vivo* still remained unanswered. A seminal study by the Terman group identified a role for the enzyme, MICAL (molecule interacting with CasL), in mediating oxidation of Met44 and Met47 of actin *in vitro*[9]. These in vitro studies showed that MetO at Met44 was sufficient to promote both severing of filaments and decreased polymerization. Additionally, mis-regulation of actin MetO produced profound morphological consequences as overexpression of MICAL in Drosophila resulted in deformed bristle formation but was rescued with M44L actin mutants; thereby, defining its role *in vivo*[9,10]. A recent study identified a physiologic role for MICAL-1 oxidation of F-actin by determining that this mechanism is critical for depolymerizing actin during the terminal steps of cytokinesis[11]. These studies shed light on the physiologic importance that actin MetO has on actin's molecular, cellular, and morphologic functions.

Regulatory Mechanisms of Actin Oxidation

MICAL is an intracellular flavoprotein monoxygenase, conserved from insects to mammals, that functions as a catalyst for oxidation-reduction (redox) reactions[12]. There are three MICAL family members in mammals, and all of them can oxidize actin but with different kinetics and spatial localization and regulation[13]. Terman's group showed that MICAL interacts with actin and uses NADPH as a cofactor to oxidize actin at Met44 and Met47[9]. The Met44 residue resides in actin's D-loop of subdomain 2 which is critical for actin subunit contacts[14]. When oxidized, Met44 becomes negatively charged which interferes with actin monomer-monomer interactions; thus, promoting F-actin severing and depolymerization. Importantly, MICAL-mediated effects on actin do not occur through a diffusible oxidant like H_2O_2, as reductants like DTT did not alter MICAL activity, and MICAL needed to be in close proximity to actin[9].

Recent studies discovered that MICAL-mediated MetO of actin is reversed by the SelR/MsrB family of methionine sulfoxide reductase enzymes. Two groups independently identified SelR (MsrB) as the enzyme responsible for reduction of Met44 and Met47 and restoration of normal actin dynamics[15,16]. These studies determined that SelR/MsrB selectively reduced MICAL-oxidized actin, while another methionine sulfoxide reductase member, MsrA, did not. Since SelR/MsrB specifically reduces R-isomer MetO and MsrA specifically reduces S-isomer MetO, the groups concluded that MICAL stereo-specifically oxidized actin with MetO R-isomer. Collectively, these data describe a reversible, specific redox system that controls actin dynamics and cytoskeletal organization through regulation of a specific MetO of actin.

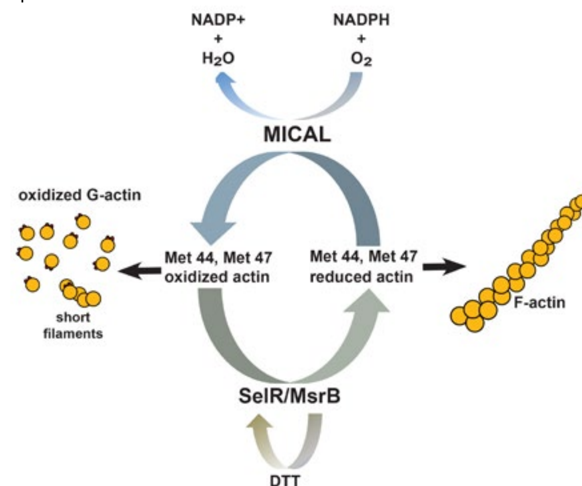

Figure 1. Actin Met44 and Met47 physiological redox system

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Actin Oxidation Physiology

Since MICALs appear to be expressed ubiquitously and the Met44 and Met47 residues of actin are highly conserved, it is likely that this mechanism of redox regulation may play a prominent role in modulating actin function in all tissue and cell types. Several studies highlight that MICAL proteins play a role both physiologically and pathologically in an array of tissues and organisms; however, whether or not these effects are facilitated through actin-dependent regulation have not been thoroughly investigated[17]. Following are a few examples where MICAL had a profound effect linked to its specific regulation of actin. One such study identified MICAL-2 as a regulator of nuclear G-actin levels, subsequent MRTF-A/SRF transcriptional regulation, and physiological regulation of heart development in zebrafish[18]. Another study investigated the effects that growth factors and chemo-repellents have on MICAL regulation of actin, and found paradoxically that effects of the chemo-repellent were amplified by growth factor signaling which had profound effects on physiological axon guidance regulation, as well as pathological tumor progression and response to treatment[19]. Finally, MICAL-1 is important for the development of hippocampal mossy fiber connections through F-actin regulation, and this physiological process may be important in neural disorders[20]. These studies and others highlight the profound effect that this small, physiologic MetO of actin can have on several distinct cellular processes.

Conclusions and Future Directions

These studies have undoubtedly laid significant groundwork towards advancing the actin PTM field through identification of this novel, reversible redox regulatory mechanism. Recent studies suggest that MICAL regulation of actin may be important for many actin-dependent cellular processes, and one must wonder how pervasive this redox mechanism is for actin biology. Along this same line of thinking, it will be interesting to determine how ABP regulation of actin works together and/or in opposition with critical PTMs like actin MetO. A recent study indicates that actin oxidation and cofilin synergize to disassemble actin[21], providing clear evidence ABP and actin PTM crosstalk does exist and warrants further investigation. As investigators decipher MICAL-oxidized actin's role in disease, it will be interesting to further define the interplay of ROS vs enzymatic actin MetO. Having useful MetO actin tools to address these types of questions will undoubtedly help researchers gain a better understanding of actin biology and whether or not MetO actin plays a role in their research models. Cytoskeleton, Inc. offers a variety of MICAL-oxidized (MOX) actin tools to help researchers incorporate this novel actin regulatory mechanism into their own research as they gain a better understanding of actin biology.

MOX Actin Products

Description	Amount	Cat. #
MICAL-Oxidized (Pyrene labeled) Actin Protein (95% pure) Rabbit Skeletal Muscle	2 x 250 ug	MXAP95
MICAL-Oxidized Actin Protein(>95% pure) Rabbit Skeletal Muscle	2 x 250 ug	MXA95
MICAL-1 Protein 6xHis	2 x 50 ug	MIC01
MsrB2 Protein 6xHis	2 x 50 ug	MB201
Actin Protein (pyrene labeled) Rabbit Skeletal Muscle	1 x 1 mg	AP05
Actin Protein (>99% pure) Rabbit Skeletal Muscle	4x 250 ug	AKL99
Actin Protein (>99% pure) Human Platelet	2 x 250 ug	APHL99

G-LISA Activation Assay Kits

Product	Reactions	Cat. #
G-LISA RhoA Activation Assay Biochem Kit (colorimetric format)	96	BK124
G-LISA RhoA Activation Assay Biochem Kit (luminescence format)	96	BK121

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Actin Biochem Kits

Actin Biochem Kit	Reactions	Cat. #
Actin Binding Protein Spin-Down Assay Biochem Kit rabbit skeletal muscle actin	30-100 assays	BK001
Actin Binding Protein Spin-Down Assay Biochem Kit human platelet actin	30-100 assays	BK013
Actin Polymerization Biochem Kit (fluorescence format) rabbit skeletal muscle actin	30-100 assays	BK003
G-Actin/F-actin In Vivo Assay Biochem Kit	30-100 assays	BK037

Tubulin Biochem Kits

Product	Reactions	Cat. #
Tubulin Polymerization Assay Biochem Kit (absorbance format), porcine tubulin	24-30	BK006P
Tubulin Polymerization Assay Biochem Kit (fluorescence format): 99% pure porcine tubulin	96	BK011P
Microtubule Binding Protein Spin-Down Assay Biochem Kit	50-100	BK029
Microtubule/Tubulin In Vivo Assay Biochem Kit	30-100	BK038