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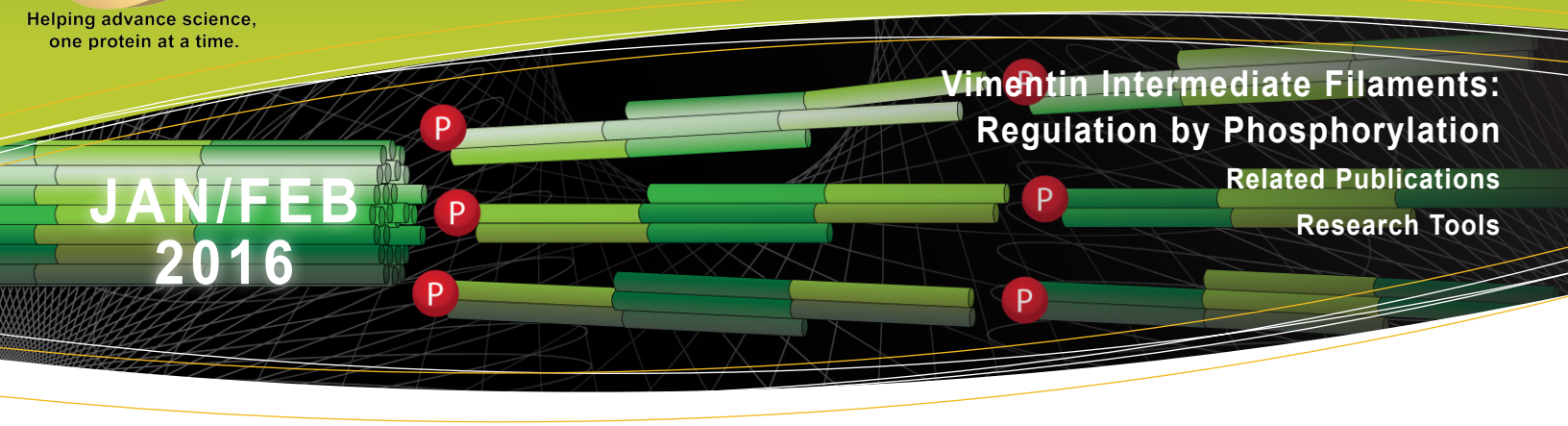

Vimentin Intermediate Filaments: Regulation by Phosphorylation

Introduction

Intermediate filaments (IFs) are one of three filament systems comprising the cytoskeleton of metazoan cells. IFs are highly dynamic structures essential for organizing the actin and tubulin filament systems and regulating cell signaling, motility, structure, and adhesion during interphase and mitosis. The function and localization of IFs are regulated by post-translational modifications (PTMs) such as phosphorylation, SUMOylation, ADP ribosylation, and O-GlcNAcylation[1-4]. The most common IF is vimentin. Vimentin is a type III IF protein found in cultured and tumor cells as well as the majority of cells derived from the mesenchyme and vimentin filaments are considered a marker of the epithelial to mesenchymal transition[5,6]. This newsletter focuses on vimentin and its regulation by phosphorylation. Phosphorylation regulates vimentin IF assembly/disassembly (i.e., dynamics), vimentin's binding partners, and vimentin's role in cell motility.

Phosphorylation and Vimentin Dynamics

Vimentin is modified by a variety of kinases, including, but not limited to, PKA[7], MAPKAP K-2[8], CaMKII[1-4] , RhoA kinase (ROCK)[9,10], Cdk1[11,12], Plk1[12], Akt1[13], PKC[7,14], Raf-1-associated kinases[15], PAK[16], PDGF[17], and Aurora B[18]. These kinases target Tyr[17,19,20] and Ser/Thr residues[2,3] (Table 1). Here, we discuss the Ser/Thr residues as they are well-characterized compared to Tyr residues.

Phosphorylation-mediated regulation of vimentin dynamics is integral for both mitosis and cell architecture/motility in interphase. Disassembly of vimentin IFs is a necessary step in cytokinesis. Mitotic-associated phosphorylation of vimentin IFs causes filament disassembly, allowing for the proper separation of vimentin between the two newly formed daughter cells[1-4,21]. Cyclin-dependent kinase 1 (Cdk1) phosphorylates Ser55 on vimentin from prometaphase to metaphase, inducing IF depolymerization[11,12,22]. Furthermore, this phosphorylation triggers direct binding of Polo-like kinase 1 (Plk1) to vimentin, leading to Plk1 activation and phosphorylation of vimentin on Ser82 which lasts from metaphase until the end of mitosis[12]. Plk1-mediated phosphorylation somewhat reduces the filament assembly of vimentin. Besides these kinases, vimentin is also phosphorylated by ROCK and Aurora B during mitosis. ROCK phosphorylates vimentin on Ser71 and Ser38[9], while Aurora B targets Ser72[18]. Both kinases phosphorylate vimentin at the cleavage furrow from anaphase to the end of mitosis, resulting in the necessary re-organization of vimentin filaments[9,18].

Other kinases that mediate vimentin dynamics are PAK and PKA. PAK phosphorylates vimentin on Ser56 which results in disassembly of vimentin filaments[16]. Two major PKA phosphorylation sites on vimentin are Ser38 and Ser72[7], though multiple other phosphorylation sites were identified (Table 1). Phosphorylation of these residues results in impaired assembly of vimentin filaments[7].

AA Residue	Kinase(s)	Reference(s)
Ser4	PKA, PKC	7
Ser6	PKA, PKC	7,14,21
Ser7	PKA, PKC	7
Ser8	PKA, PKC	7
Ser9	PKA, PKC	7
Ser33	PKC	9,14,21
Ser38 (or Ser39*)	PKC, CaMKII, ROCK, PKA, MAPKAP-K2, Akt*	7-9,13*,21
Ser41	PKA	7
Ser50	MAPKAP-K2, PKC	8,21
Ser55 (or Ser56*)	Cdk1, PKA, MAPKAP-K2, PAK*	8,9,11,12,16*
Ser71	PKA, ROCK	7,9,21
Ser72	PKA, Aurora B	7,18,21
Ser82	Plk1, CaMKII, MAPKAP-K2	8,9,12,21
Ser418	PKA	7
Ser429	PKA	7
Ser458	PKA	7
Thr457	PKA	7

Table 1. Vimentin phosphorylation sites. Numbering omits methionine starting amino acid, following convention of Ref. 7. Note that in some papers, methionone is counted. *Ser56 and Ser39 are the same residue as Ser55 and Ser38, respectively. For more information, see reviews[1-4].

Phosphorylation and Vimentin Binding

Vimentin phosphorylation also regulates binding of vimentin to other proteins. The 14-3-3 proteins bind the amino-terminal (head) domain of vimentin which prevents the binding and activation of 14-3-3 partner proteins involved in cell signaling and

Meetings and Sponsorships

Boston Area Mitosis and Meiosis (BAMM) Group Meeting
May 7th, 2016
Boston, MA, US

Front Range Cytoskeleton Meeting
May/June (tbd), 2016
Denver, CO, US

Gordon Research Conference - Signaling by Adhesion Receptors
June 18-19th, 2016
Lewiston, ME, US

European Cytoskeleton Forum - Cell Adhesion and Migration
June 20-23rd, 2016
Cambridge, UK

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cell cycle control, including oncogenic Raf[23]. Binding of 14-3-3 to vimentin requires phosphorylation of Ser/Thr residues on the amino-terminus of vimentin. Similarly, binding between Akt-phosphorylated Beclin 1 and vimentin is dependent upon phosphorylation of Ser39 on vimentin[24]. The Beclin 1/vimentin complex is involved in the inhibition of autophagy and Akt-mediated transformation[24].

Phosphorylation and Vimentin-mediated Motility

Vimentin phosphorylation also regulates processes underlying cell motility in normal and cancer cells. PKC mediates phosphorylation of vimentin (Ser6 and Ser33) that is associated with integrin-containing vesicles[14]. Integrins are needed for proper cell adhesion and motility and correct localization depends upon PKC-mediated phosphorylation of vimentin which allows proper integrin trafficking through vesicles to the plasma membrane[14]. Correspondingly, cell migration increases with PKC-mediated phosphorylation of vimentin. Vimentin phosphorylation is also integral in cellular responses to growth factors and Rho family GTPase activation. In serum-starved or non-motile fibroblasts, vimentin IFs are found throughout the cell periphery. Exposure to serum or photoactivatable Rac1 GTPase induces vimentin phosphorylation, IF depolymerization, and IF retraction from the cell periphery[25].

Vimentin phosphorylation also regulates cell motility in cancer cells. Upon activation of Akt, its tail region binds to vimentin's head region, resulting in phosphorylation of Ser39 on vimentin and a corresponding increase in cancer cell motility and invasion *in vitro* and tumor and metastasis growth *in vivo*. Increased motility is likely due to phosphorylation-induced disassembly of vimentin IFs. Furthermore, Akt1 phosphorylation of vimentin also offers a level of protection against caspase-mediate proteolysis[13].

Phosphate Sink

Phosphorylation of vimentin does not always affect vimentin dynamics. Mitogen-activated protein kinase-activated protein kinase-2 (MAPKAP-K2) phosphorylates Ser38, Ser50, Ser55, and Ser82[8], none of which alters vimentin filament assembly[8], prompting the suggestion that vimentin can function as a phosphate sink[8,26].

Summary

Vimentin IFs are modified by phosphorylation and at least three other PTMs (ADP ribosylation[27], SUMOylation[28], and O-GlcNAcylation[29,30]). Through these modifications, vimentin is spatially and functionally regulated. Despite a recent focus on the under-appreciated role vimentin IFs have in a myriad of cellular processes, much remains to be discovered, including the potential of PTM cross-talk[30].

Pathway Tools and Kits

Product	Cat. #	Amount
NEW **Phosphotyrosine Enrichment Kit**	BK160	30 assays
NEW **Ubiquitin Enrichment Kit**	BK161	30 assays
Phosphotyrosine Antibody Mouse Monoclonal Validated in WB, IP, IF	APY03-S APY03	1 x 25 µl 2 x 100 µl
Phosphotyrosine-HRP Antibody Mouse Monoclonal Validated in WB	APY03-HRP-S APY03-HRP	1 x 25 µl 1 x 100 µl
Anti-Phosphotyrosine Affinity Beads Validated in IP	APY03-beads	4 x 330 µl
Vimentin Protein Recombinant, Syrian Hamster	V01-A V01-C	2 x 50 µl 10 x 50 µl

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