Regulation of WASp by phosphorylation
Activation or other functions?

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Abbreviations: WASp, wiskott-aldrich syndrome protein; N-WASP, neural WASP; PtdIns(4,5)P2, phosphatidyl inositol 4,5-bisphosphate; GBD, G protein binding domain; FRET, fluorescence resonance energy transfer; TCR, T cell receptor

Wiskott-Aldrich Syndrome protein (WASp) is an actin nucleation-promoting factor that regulates actin polymerisation via the Arp2/3 complex. Its mutation in human syndromes has led to extensive studies on the regulation and activities of this molecule. Several mechanisms for the regulation of WASp activity have been proposed, however, the role of tyrosine phosphorylation remains controversial, particularly due to inconsistencies between results obtained through biochemical and cell biological approaches. In this mini-review, we are addressing the major aspects of WASp regulation with an emphasis on the role of tyrosine phosphorylation on WASp activities.

Introduction

WASp is a haematopoietic-specific protein involved in actin-dependent events, such as cell motility, phagocytosis and immune synapse formation. Mutations associated with decreased WASp activity have been associated with Wiskott-Aldrich syndrome and X-linked thrombocytopenia, while activating mutations have been implicated in severe congenital neutropenia. Therefore, understanding the precise mechanisms by which WASp activity is regulated is clinically relevant as constitutive activation or inactivation of WASp result in phenotypically distinct diseases. Non-haematopoietic cells express neural (N)-WASP, which shows identical domain organisation and high primary sequence homology to WASp, even though several regions of divergence exist between the two molecules. Interestingly, several haematopoietic cells (e.g., platelets, monocytic lineage cells) express both WASp and N-WASP, and to what extend they may have unique or redundant functions remains unknown.

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and potentially Syk. S277 was phosphorylated by the dual kinase ACK1, along with Y291, and phosphorylation of this site contributed to increased actin polymerization in vitro, though its relevance in intact cells remains unexplored.

The effect of Y291 phosphorylation on WASp (and the equivalent Y253 in N-WASP) activity has been extensively studied in vitro using mainly actin pyrene assays as the output of activity. Collectively, these studies have uncovered a model whereby unfolding of WASp by Cdc42 binding activates WASp and exposes Y291 for phosphorylation. This results in enhanced Arp2/3-dependent actin polymerization as the affinity of the GBD domain towards the VCA domain is significantly reduced after phosphorylation thus favouring VCA-Arp2/3 interactions. Additionally, phosphorylation of WASp primes the molecule to activation by SH2 and/or tandem SH3 domain-containing proteins, and thus become active once again.

Figure 1. A model for the regulation of WASp by phosphorylation. The folded, inactive WASp molecule can be recruited to areas of receptor signaling via SH3 domain-containing adaptors (e.g., Nck) (A), were it can be activated by Cdc42 and PtdIns(4,5)P2, and phosphorylated by a kinase, such as Src (B). Once Cdc42 activity is terminated, the phosphorylated species (C) may be dephosphorylated by a phosphatase and go through further rounds of activation (A) or it may be proteolytically degraded (D). Alternatively, the phosphorylated species, may, in the absence of Cdc42 binding, refold and thus be “primed” (E) for activation by signals other than Cdc42, such as binding by SH2- and SH3 domain-containing proteins, and thus become active once again (F).

Regulation of WASp by phosphorylation. Both serine and tyrosine phosphorylation of WASp have been reported to take place in two distinct sites on the WASp molecule; serines 483 and 484 in the VCA domain (at the junction between the V and A regions), and serine 277, and tyrosine 291, in the GBD domain. The corresponding phosphorylation sites are also conserved in N-WASP, for which a separate tyrosine residue, Y175, has also been shown to be phosphorylated, albeit to a lesser extent, and its contribution to N-WASP activity and/or actin polymerization has not been assessed.

S483/484 phosphorylation, mediated by casein kinase II, is constitutive and perhaps associated with the maturation of competent species in vivo. Indeed, phosphorylation of these two serine residues increases affinity of WASp for Arp3, and is required for efficient actin polymerization by the full-length WASp in vitro and in vivo. Y291 on the other hand, is phosphorylated in a regulated manner and several stimuli have been shown to increase phospho-Y291 in cells, and a range of kinases has been proposed to phosphorylate WASp/N-WASP in vitro and in vivo (Table 1). These include Src family kinases, FAK, ACK1, Btk and potentially Syk. S277 was phosphorylated by the dual kinase ACK1, along with Y291, and phosphorylation of this site contributed to increased actin polymerization in vitro, though its relevance in intact cells remains unexplored.

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These elegant biochemical studies have shed light in the role of phosphorylation whereby actin polymerization is the direct
output of WASp activity. However the role of WASp phosphorylation in cells remains relatively unknown.

Regulation of phosphorylation in vivo. Table 1 summarizes the range of signals that can result in WASp phosphorylation in cells. Consistent with in vitro studies, Cdc42-independent activation and phosphorylation of WASp by Lyn results in actin polymerization following TCR ligation.22 However, this may be a result of a combination of SH3 domain and PtdIns(4,5)P₂ interactions driving WASp targeting and unfolding prior to phosphorylation. Our data using Cdc42 binding deficient WASp mutants or Cdc42-silenced macrophages, indicates a role for Cdc42 in phosphorylation. Our data using Cdc42 binding deficient WASp mutants, or indeed the other activating mutations, L272P and I294T,26 results in high basal phosphorylation of WASp in cells. Consistent with in vitro studies, Cdc42-independent activation and phosphorylation of WASp by Lyn results in actin polymerization, synapse formation,20,21,24 whereby Cdc42 provides a conformational relief of autoinhibition and accessibility of the GBD to kinases. Furthermore, overexpression of the activating mutation L270P, identified in patients with severe congenital neutropenia,25 resulted in increased resistance to disassembly by wiskostatin,29 a compound that inhibits WASp (and N-WASP) activity by stabilizing the autoinhibited conformation.30 These observations are consistent with increased activity of phosphorylated WASp in cells.

Interestingly, our data using a FRET-based WASp biosensor indicated that WASp conformation under basal conditions and in the podosomes of macrophages was similar between the Y291E and Y291F biosensor mutants.8,29 Changes in conformation may, as a result, not necessarily indicate an active species. It is, therefore, possible that WASp phosphorylation in vivo may not be exclusively associated with actin polymerization but may also influence the stability of actin filaments through decrease in depolymerisation. Accordingly, F-actin in macrophage podosomes regulated by Y291E WASp is more stable than in those regulated by Y291F WASp, even though both mutants result in apparent equal activity as evidenced by FRET.29

An important drawback in the use of the Y291E phosphomimetic, however, is that it may be insufficient in mimicking phosphorylation, while additionally it excludes the input of SH2 domain binding. This could explain why in some cases the Y291E mutation does not enhance actin-dependent events in vivo (e.g., actin tail length beneath Shigella pathogens31) as opposed to in vitro settings. Furthermore, it may also hinder processes whereby phosphorylation/dephosphorylation may be required for an obvious cellular phenotype, as our data have uncovered in the chemotaxis of macrophages to CSF-1.29

Additionally, our group and others have shown that FcγR-mediated phagocytosis in macrophages requires WASp Y291 phosphorylation.33,32 Importantly, our group has shown that overexpression of the constitutively active L270P WASp mutant, when coupled to the Y291F phospho-abolishing mutation, decreased phagocytosis in control macrophages.23 Furthermore, in Cdc42-silenced macrophages, the phagocytosis defect associated with loss of endogenous Cdc42 could be partially rescued by the activated L270P WASp mutant, but when the L270P mutation was coupled to the Y291F mutation, phagocytosis remained unaltered.23 This indicates that phosphorylation plays additional
roles other than simply shifting the balance towards the open conformation of WASp, and one speculation is that it may regulate the interaction with a SH2 domain containing protein. Consistently, the Y291E mutation only partially rescued phagocytosis in this setting.

The importance of WASp phosphorylation in vivo was recently highlighted in a study using mouse knock-ins of WASp phosphomutants (Y293E/F, the equivalent of Y291 in murine WASp), showing a requirement for tyrosine phosphorylation in several aspects of T cell development and cellularity in vivo, as well as red blood cell count. Therefore, WASp phosphorylation is required for events regulating immune system maturation in whole organisms, since in several aspects of leukocyte development the Y293F WASp knock-in mice were similar to the WASp knock-out ones.

The Role of Tyrosine Phosphorylation in WASp Turnover

Several studies have also demonstrated that instead of an activating signal, tyrosine phosphorylation may result in the proteolytic degradation of WASp/N-WASP, consequently resulting in inactivation. For example, in platelets, collagen-induced WASp phosphorylation was too transient to account for the “priming” model described above. Instead, phosphorylation of endogenous WASp was coupled to an almost instantaneous calpain-mediated cleavage, thus explaining signal, tyrosine phosphorylation may result in the proteolytic degradation of WASp/N-WASP, consequently resulting in inactivation. 

Consistently, the Y291F mutation has not been reported to result in unfolded Y291F biosensor was aberrantly localized to cell protrusions but not other areas in contact with the substrate. In contrast, the unfolded Y291F biosensor was aberrantly localized primarily because the use of glutamic acid substitutions may not completely mimic phosphorylation and prevent potential interactions with SH2 domain—containing proteins. Interestingly, N-WASP was found to be insensitive to calpain proteolysis in blood cells that express both WASp and N-WASP. Phosphorylation may therefore occur transiently following WASp activation, subsequently acting as an inactivation signal through degradation pathways. It is interesting however, to note that the L270P mutation has not been reported to result in proteolytic degradation of WASp even though it results in an unfolded molecule with high basal phosphorylation levels.

Conclusions

The many levels of WASp regulation highlight the importance of tightly controlling WASp activity in leukocytes. Tyrosine phosphorylation of WASp has been demonstrated in many cases, however, its precise functions in vivo remain unknown primarily because the use of glutamic acid substitutions may not completely mimic phosphorylation and prevent potential interactions with SH2 domain—containing proteins. Nevertheless, the extensive studies performed on patients with genetic syndromes and transgenic mice continue to shed light on this important molecule.

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References

1. Takenawa T, Suzuki S. The WASP-WAVE protein network: connecting the membrane to the cytoskeleton. Nat Rev Mol Cell Biol 2007; 8:57-48.
2. Kurisu S, Takenawa T. The WASP and WAVE family proteins. Genome Biol 2009; 10:226.
3. Pollitt A, Insall RH. WASP and SCAR/WAVE proteins: the drivers of actin assembly. J Cell Sci 2009; 122:2575-8.
4. Symons M, Derry JMJ, Karlak B, Jiang S, Lemahieu V, McCormick F, et al. Wiskott-Aldrich syndrome protein, a novel effector for the GTPase CDC42Hs, and CDC42. J Biol Chem 2009; 284:23302-11.
5. Cannon JL, Lahno GM, Boccia G, Sriv A, McGavin MHK, Simonovitch KA, et al. WASP recruitment to the T cell/APC contact site pects independently of Cdc42 activation. Immunity 2001; 15:249-59.
6. Higgs HN, Pollard TD. Activation by Cdc42 and PIP3 of Wiskott-Aldrich Syndrome protein (WASP) stimulates actin nucleation by Arp2/3 complex. J Cell Biol 2000; 150:1311-20.
7. Rohangi R, Ho HY, Kirchner MW. Mechanism of N-WASP activation by CDC42 and phosphatidylinositol 4,5-bisphosphate. J Cell Biol 2000; 150:1299-310.
8. Cammer M, Grevey J-C, Lorenz M, Dovas A, Condeelis J, Cox D. The mechanism of CSF-1 induced actin polymerization. J Cell Sci 2009; 122:426-38.
9. Rivera GM, Bricetto CA, Takehashi E, Snapper SB, Mayer BJ. Inducible clustering of membrane-tageted SH3 domains of the adaptors Nck triggers localized actin polymerization. Curr Biol 2004; 14:11-22.
10. Meng YS, Yamaga M, Zhu X, Wei Y, Sun H-Q, Wang J, et al. Essential and unique roles of PIP5K-γ and -α in Fce receptor-mediated phagocytosis. J Cell Biol 2009; 184:281-96.
11. Padrick SB, Cheng HC, Ismail AM, Panchal SC, Doolittle LR, Kim S, et al. Hierarchical regulation of WASP/WAVE proteins. Mol Cell 2008; 32:426-38.
12. Engstroem P, Lindberg U, Hall A. Two GTPases, Cdc42 and Rac, bind directly to a protein implicated in the immunodeficiency disorder Wiskott-Aldrich syndrome. Curr Biol 1996; 6:70-5.
14. Campellone KG, Cheng H-C, Robbins D, Siripala AD, McGhee EJ, Hayward RD, et al. Repetitive N-WASP-binding elements of the enterohemorrhagic Escherichia coli effector EspP synergistically activate actin assembly. PLoS Pathog 2008; 4:10000191.

15. Sallée NA, Rivera GM, Dubeir JE, Vaissescu D, Mullins RD, Mayer BJ, Lim WA. The pathogen protein EspF hijacks actin polymerization using mimicry and multivalency. Nature 2008; 454:1005-8.

16. Cory GOC, Cramer R, Blancholl L, Ridley AJ. Phosphorylation of the WASP-VCA domain increases its affinity for the Arp2/3 complex and enhances actin polymerization by WASP. Mol Cell 2003; 11:1229-39.

17. Yokoyama N, Loughedj J, Miller WT. Phosphorylation of WASP by the Cdc42-associated Kinase ACK1. DUAL HYDROXYAMINO ACID SPECIFICITY IN A TYROSINE KINASE. J Biol Chem 2005; 280:42219-26.

18. Baba Y, Nonoyoama S, Matsushita M, Yamadori T, Hashimoto S, Imai K, et al. Involvement of wiskott-aldrich syndrome protein in B-cell cytosplasmic tyrosine kinase pathway. Blood 1999; 93:2003-12.

19. Burton EA, Oliver TN, Pendergast AM. Abl kinases regulate actin comet tail elongation via an N-WASP-dependent pathway. Cell Biol 2005; 25:8834-43.

20. Torres E, Rosen MK. Contingent phosphorylation/dephosphorylation provides a mechanism of molecular memory in WASP. Mol Cell 2003; 11:1215-27.

21. Torres E, Rosen MK. Protein-tyrosine kinase and GTPase signals cooperate to phosphorylate and activate Wiskott-Aldrich Syndrome Protein (WASP)/Neuronal WASP. Biol Chem 2006; 281:3513-20.

22. Badour K, Zhang J, Shi F, Leng Y, Collins M, Siminovicth KA. Fyn and PTP-PEST-mediated regulation of Wiskott-Aldrich Syndrome Protein (WASP) tyrosine phosphorylation is required for coupling T cell antigen receptor engagement to WASP effector function and T cell activation. J Exp Med 2004; 199:99-111.

23. Park H, Cox D. Cdc42 Regulates Fcγ receptor-mediated phagocytosis through the activation and phosphorylation of Wiskott-Aldrich Syndrome Protein (WASP) and Neural-WASP. Mol Biol Cell 2009; 20:4500-8.