# Tyrosine phosphorylation of WASP promotes calpain-mediated podosome disassembly

Lee Macpherson,[1] James Monypenny,[2] Michael P. Blundell,[3] Giles O. Cory,[4] Jessica Tomé-García,[1] Adrian J. Thrasher,[3,5] Gareth E. Jones,[2] and Yolanda Calle[1]

[1]Department of Haemato-oncology, King's College London, London; [2]Randall Division of Cell and Molecular Biophysics, King's College London, London; [3]Centre for Immunodeficiency, Molecular Immunology Unit, Institute of Child Health, University College London; [4]Peninsula College of Medicine and Dentistry, Exeter; and [5]Great Ormond Street Hospital NHS Trust, London, UK

## ABSTRACT

Podosomes are actin-based adhesions involved in migration of cells that have to cross tissue boundaries such as myeloid cells. The Wiskott Aldrich Syndrome Protein regulates *de novo* actin polymerization during podosome formation and it is cleaved by the protease calpain during podosome disassembly. The mechanisms that may induce the Wiskott Aldrich Syndrome Protein cleavage by calpain remain undetermined. We now report that in myeloid cells, tyrosine phosphorylation of the Wiskott Aldrich Syndrome Protein-tyrosine291 (Human)/tyrosine293 (mouse) not only enhances Wiskott Aldrich Syndrome Protein-mediated actin polymerization but also promotes its calpain-dependent degradation during podosome disassembly. We also show that activation of the Wiskott Aldrich Syndrome Protein leading to podosome formation occurs independently of tyrosine phosphorylation in spleen-derived dendritic cells. We conclude that tyrosine phosphorylation of the Wiskott Aldrich Syndrome Protein integrates dynamics of actin and cell adhesion proteins during podosome disassembly required for mobilization of myeloid cells during the immune response.

Key words: cell motility, WASP phosphorylation, dendritic cell, podosome, calpain.

*Citation: Macpherson L, Monypenny J, Blundell MP, Cory GO, Tomé-García J, Thrasher AJ, Jones GE, Calle Y. Tyrosine phosphorylation of WASP promotes calpain-mediated podosome disassembly. Haematologica 2012;97(5):687-691. doi:10.3324/haematol.2011.048868*



## Introduction

Complex patterns of adhesion and migration of leukocytes are required during the immune response. Cells of the myeloid lineage assemble characteristic highly dynamic actin-based adhesive structures termed podosomes that are thought to be involved in migration of cells that have to cross and invade tissue boundaries.[1-2] Podosomes cluster behind the extending leading edge of migrating myeloid cells and are required for polarization, persistent migration and chemotaxis.[3-5] We and others have identified the Wiskott Aldrich Syndrome Protein (WASP) and the WASP interacting protein (WIP) in the podosome actin core[4,6-8] where they play a major role in their formation and dynamics.[4,8] WASP is expressed in hematopoietic cells and belongs to the N-WASP/WAVE family of adaptor proteins that mediate *de novo* actin polymerization. Inactive WASP exists in a hairpin autoinhibited conformation provided by the interaction between the Cdc42-binding domain (CRIB domain) and the VCA domain[9] that can be relieved by binding to numerous upstream activators,[9] including tyrosine kinases that phosphorylate (human) WASP Y291.[10-11] Mutations in the gene coding for WASP lead to the Wiskott-Aldrich Syndrome (WAS), a hematologic disorder characterized by eczema, microthrombocytopenia, and immunodeficiency.[12] WASP-deficient leukocytes exhibit defects in the organization and regulation of the actin cytoskeleton including the loss of podosomes.[1-2] A point mutation (I294T) in WASP results in X-linked neutropenia[13] similarly to at least two other activating WASP mutations also localized within the CRIB domain.[13-14] The WASP I294T mutation resulted in enhanced actin polymerizing activity of WASP[13] and macrophages expressing WASP I294T displayed an increased number of podosomes with enhanced rate of turnover.[13] These results suggest that the active open conformation of WASP promotes actin polymerization as well as podosome disassembly. Although the idea that the same open conformation of WASP leads to both assembly and disassembly of podosomes may be counterintuitive at first, it is possible that for termination of podosomes actin polymerization and integrin recruitment have to be discontinued, and the same constituents of growing podosomes, such as WASP, may contribute to the disassembly process. We have previously shown that the rapid turnover of podosomes involves cleavage of WASP by the protease calpain,[15] further supporting a role of WASP in both podosome formation and disassembly.[1-2] However, the specific signaling mechanisms that make active WASP susceptible to cleavage by calpain leading to podosome disassembly remain unknown. In the present

*The online version of this article has a Supplementary Appendix.*

*Funding: this work has been supported by grants from the Burton Myeloma Programme (YC and LM), the Wellcome Trust (JM, AJT, GEJ, and YC) and the Medical Research Council (GEJ), Great Ormond Street Children's Charity (AJT).*
*Manuscript received on June 1, 2011 . Revised version arrived on November 11, 2011. Manuscript accepted on November 15, 2011.*
*Correspondence: Dr. Yolanda Calle, Telephone: +44.0.20.78485816; Fax:+44.0.20.77333877; E-mail: yolanda.calle@kcl.ac.uk*

study, we address the possible role of phosphorylation of WASP Y291 in the calpain-mediated cleavage of WASP during podosome turnover in migrating immature dendritic cells (DCs).

## Design and Methods

### Animals

Pathogen free C57 mice purchased from Harlan and WASP-null, WASP Y293E and WASP Y293F expressing mice on a C57 background were bred in our own animal facility in pathogen free conditions. All animals were handled in strict accordance with good animal practice as defined by UK Home Office Animal Welfare Legislation, and all animal work was approved by the Institutional Research Ethics Committee (Institute of Child Health, University College London, UK) and performed under project licence number 70/7024.

### Cell culture

DCs were generated from mouse spleens from wild-type, WASP-null, WASP Y293E and WASP Y293F expressing mice[16] as previously described.[8,15,17] THP-1 cells were obtained from the European Collection of Cell Cultures. Cells were infected using lentiviral vectors as previously described.[3,15]

### Immunofluorescence and analysis of live microscopy

Cells were fixed and processed for immunofluorescence labeling as previously described.[15] We used interference reflection microscopy (IRM) to visualize and analyze the adhesion-substratum interface of living cells using *Mathematica*™ 5.2 notebooks as previously described.[15,18]

Further details of methods used are described in the *Online Supplementary Appendix*.

## Results and Discussion

### Phosphorylation of WASP Y291 regulates the spatio-temporal dynamics of the podosome cluster

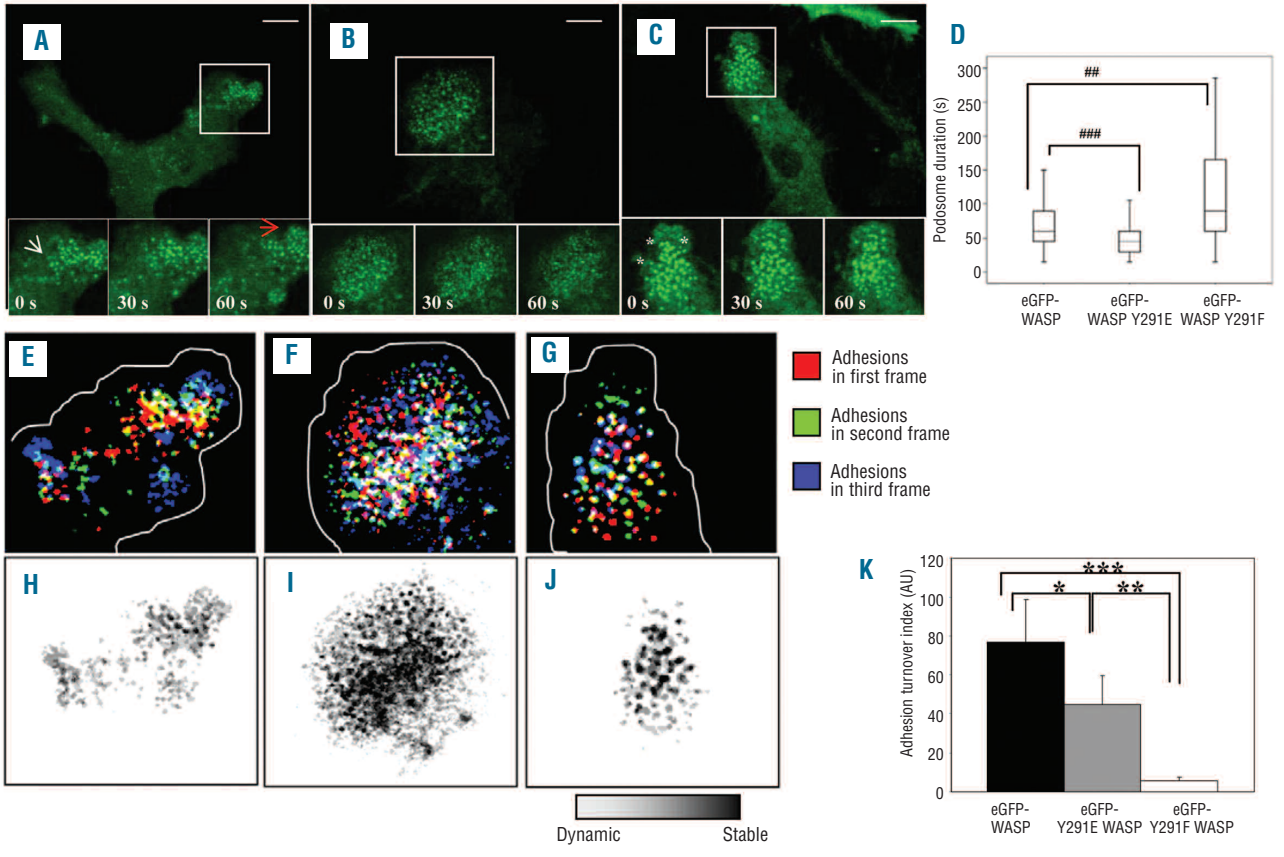
We have previously shown that expression of eGFP-tagged human WASP can recover the normal cytoskeletal organization and migratory properties of both human and mouse WASP null cells.[3,15] In order to study the role of tyrosine phosphorylation of WASP in the formation and dynamics of individual podosomes, we expressed human eGFP-WASP or WASP phosphovariants in WASP -/- mouse spleen derived DCs. As expected, podosome clusters in WASP -/- DCs expressing wild-type WASP displayed treadmill dynamics with podosomes located at the back disassembling over time[3,15] (Figure 1A, red pixels Figure 1E, light gray pixels, Figure 1H and *Online Supplementary Video 1*), newly formed podosomes assembled towards the margin of the leading edge (Figure 1A, blue pixels Figure 1E, light gray pixels Figure 1H) and a more stable central region that sustained the leading edge (yellow, pink and light blue pixels Figure 1E, dark gray and black pixels Figure 1H).[8,15] In DCs expressing WASP Y291E (human phosphomimetic mutant), podosomes were of smaller size with a shorter life span (Figure 1B and D, *Online Supplementary Video 2*). Assembly and breakdown of podosomes occurred more rapidly and also persistently in the same position (white, light blue and pink pixels Figure 1F) and newly assembled podosomes located more randomly within the podosome cluster (blue pixels Figure 1F). As a result, the spatial shift of the podosome cluster towards the margin of the leading edge was impaired resulting in stabilization of the overall cell contact to the substratum (dark gray and black pixels in Figure 1I and K).[16] Equivalent overall cell contact dynamics were observed previously in mouse cells expressing the mouse homologue mutants WASP Y293F/E.[16] The finding that phosphorylation of WASP Y291 reduces the life-span of podosomes suggests that relieving the closed conformation of WASP by phosphorylation of WASP Y291 promotes both the previously reported enhanced actin polymerization[10] as well as podosome disassembly similarly to the WASP I294T mutant.[13] DCs expressing WASP Y291F (human phosphodead mutant) formed podosomes at the front of the cluster as the leading edge extended (Figure 1C, green pixels Figure 1G and *Online Supplementary Video 3*). However, podosomes remained in the same position for a longer period of time while increasing in size (Figure 1D and G) resulting in delayed disassembly and stabilization of the overall adhesion[16] (dark gray and black pixels Figure 1J and K). Dynamics of eGFP-WASP proteins show that although both phosphomimetic and phosphodead mutations of WASP Y291 lead to stabilization of the overall cell adhesion, the actual dynamics of podosome cores (which are driven by WASP where it co-localizes with actin[8]) are different. This abnormal behavior of podosomes in cells expressing the WASP phosphomutants could explain the previously described abnormal matrix degradation, actin dynamics[19] and impaired chemotaxis.[16,19] Malfunction of these fundamental processes required for cells to trespass tissue barriers correlate with failure of mouse DCs expressing WASP phosphomutants to migrate across an endothelial cell monolayer similarly to WASP -/- cells (Figure 1L). Our results also show that WASP activation leading to podosome formation is independent of phosphorylation in Y291 in spleen-derived DC similarly to macrophages.[19] However, we have previously reported that in bone marrow derived DCs, expression of the phosphodead variant of WASP resulted in formation of smaller podosomes predominantly.[16] It is possible that the signaling mediated by specific posttranslational modifications of WASP may be dependent on the expression and/or activation of other pools of proteins capable of interacting with WASP expressed in specific cell subsets (myeloid cells from different sub-lineages or originated from different organs) or in activated cells.[20] These specific interactions may result in different cytoskeletal regulation, which may reflect cell-specific routes of migration *in vivo*.

In summary, our results show that phosphorylation of WASP Y291 is not a limiting factor for podosome formation and is tightly regulated during the spatio-temporal dynamics of the podosome cluster facilitating spleen-derived DC podosome disassembly.

### Phosphorylation of WASP Y293 promotes calpain mediated podosome disassembly

As expected, WASP Y293E (mouse phosphomimetic mutant) and WASP Y293F (mouse phosphodead mutant) spleen-derived DCs formed podosomes with smaller and larger actin cores, respectively, in comparison to WT cells (Figure 2A). In agreement with our previous results in DCs expressing wild-type WASP[15] (*Online Supplementary Figure S1A and D*), blocking calpain activity in WASP Y293E expressing DCs resulted in increased size of podosome

actin core and accumulation of vinculin (Figure 2A, *Online Supplementary Figure S1B and E*). The same treatments of WASP Y293F expressing DCs resulted in small increases in podosome size (Figure 2A, *Online Supplementary Figure S1C and F*) most likely as a result of inhibition of degradation of other podosomal components, such as talin, whose calpain-mediated cleavage also occurs during the turnover of podosomes in DCs.[15] Inhibition of calpain did not induce changes in the percentage of DCs with podosomes (Figure 2B) but enhanced podosome stability (Figure 2C).



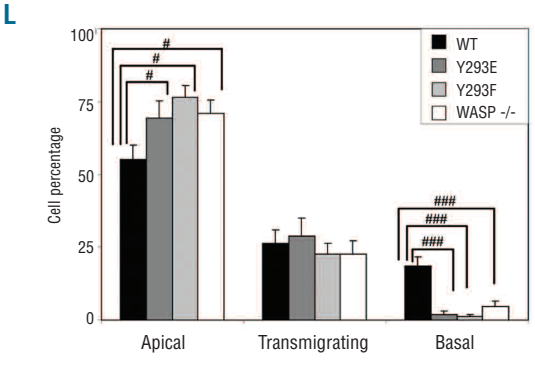




**Figure 1.** Phosphorylation of WASP is not required for podosome formation but promotes calpain mediated podosome turnover. WASP null DC infected with lentiviral particles carrying eGFP-WASP (A), eGFP-Y291E WASP (B) or eGFP-Y291F WASP (C) were plated on Poly-L-lysine coated coverslips overnight and mounted in viewing chambers to visualize WASP dynamics using confocal microscopy. Bar 10 m. Magnifications of the boxed areas at the bottom of the image show a series of 3 micrographs taken 30 s apart. (A) In DC expressing eGFP-WASP, podosomes at the back of the cluster progressively disassemble (white arrow head) and WASP locates in the core of nascent podosomes assembling at the margins of the leading edge (red arrow head). (B) eGFP-Y291E-WASP formed smaller cores and clustered predominantly in highly dynamic diffused clouds. (C) eGFP-Y291F WASP localized in large podosome cores with enhanced stability compared to eGFP-WASP. Asterisks point to examples of podosomes that persist throughout the course of the 60 s observation period. (D) Box and whiskers diagrams of podosome duration. The plots show the smallest value, the lower quartile, the median, the upper quartile and largest value of duration of podosomes that assembled in the first 30 s of initiation of the film. (E-G) Overlap using our pseudocolor method for analysis of adhesion turnover of the series of 3 images taken 30 s apart shown in (A, B and C), respectively. Adhesions in red represent adhesions breakdown after the first frame of the time course, whereas green or blue adhesions that were not associated with red adhesions would have formed in the second and third frame, respectively. Stable adhesions or where adhesions assemble and break down in the same region without spatial translocation are shown in white or pink. The white line shows the position of the margin of the leading edge. Notice that although the 2 blue clusters on the lower left and right side of the extension in (E) appear to be at the back of the cluster, in fact the leading edge extends at these positions (Online Supplementary video 1). (H-J) Composites for analysis of overall turnover of adhesion sites at the same 3 time points as the images used to generate (E, F and G), respectively. Areas of light gray color pixels represent dynamic adhesions whereas areas of dark gray and black color pixels represent increasingly stable adhesions. (K) Histograms represent the mean and SE of the adhesion turnover index of DCs. (L) Histograms indicate the average percentage and SE of DC transmigrated (basal), transmigrating or retained on the apical surface of a monolayer of LPS-activated endothelial cells after 1 h of co-culture. Significant differences were observed at **P*<0.05 ***P*<0.01 and ****P*<0.005 as indicated (Student's t-test) or #*P*<0.05 ##*P*<0.01 and ###*P*<0.005 as indicated (Mann-Whitney-Wilcoxon test's for not normal sets of data).

These results indicate that the small size and enhanced turnover of podosomes in DCs expressing phosphomimetic WASP involve calpain activity. Mouse spleen-derived DCs expressing WASP -Y293E showed very limited expression of WASP (Figure 2D). Inhibition of calpain recovered the levels of WASP in WASP Y293E expressing DCs (Figure 2E) indicating that in the case of DCs, degradation of phosphorylated WASP is mediated predominantly by calpain rather than the proteasome. We have also described the preferential degradation of WASP by calpain rather than the proteasome in spleen-derived DCs in the absence of WIP.[8] However, in other cells such as in splenocytes,[16] WASP levels are modulated by the proteasome and not calpain suggesting that WASP stability may be preferentially regulated by different degradation systems depending on the cell type.[16] Additionally, specific states of cell activation can also shift the pathway of cleavage/degradation of WASP. T cells, for example, degrade WASP via the proteasome under resting conditions whereas activation of T cells with PHA/IL-2 activates calpain and this pathway is preferentially used to degrade WASP.[20] In spleen-derived DCs, the increased levels of full length WASP Y293E and the reduced cleavage of talin as a result of calpain inhibition[15] (Figure 2E) may promote localized Arp2/3-mediated actin polymerization in the actin core and accumulation of paxillin and vinculin[21] in the podosome ring, respectively. This accumulation of components may stabilize the anchorage between F-actin and integrins, resulting in enhanced podosome structural stability and the observed decrease in podosome turnover (Figure 2C).

Human WASP Y291E was also more susceptible to degradation, as shown in experiments with the human monocytic cell line THP-1 infected with lentiviral vectors coding for eGFP-WASP/WASP phosphovariants and WIP-mCherry.[2] THP-1 cells expressed very low levels of eGFP-WASP alone (*data not shown*). The co-expresssion with

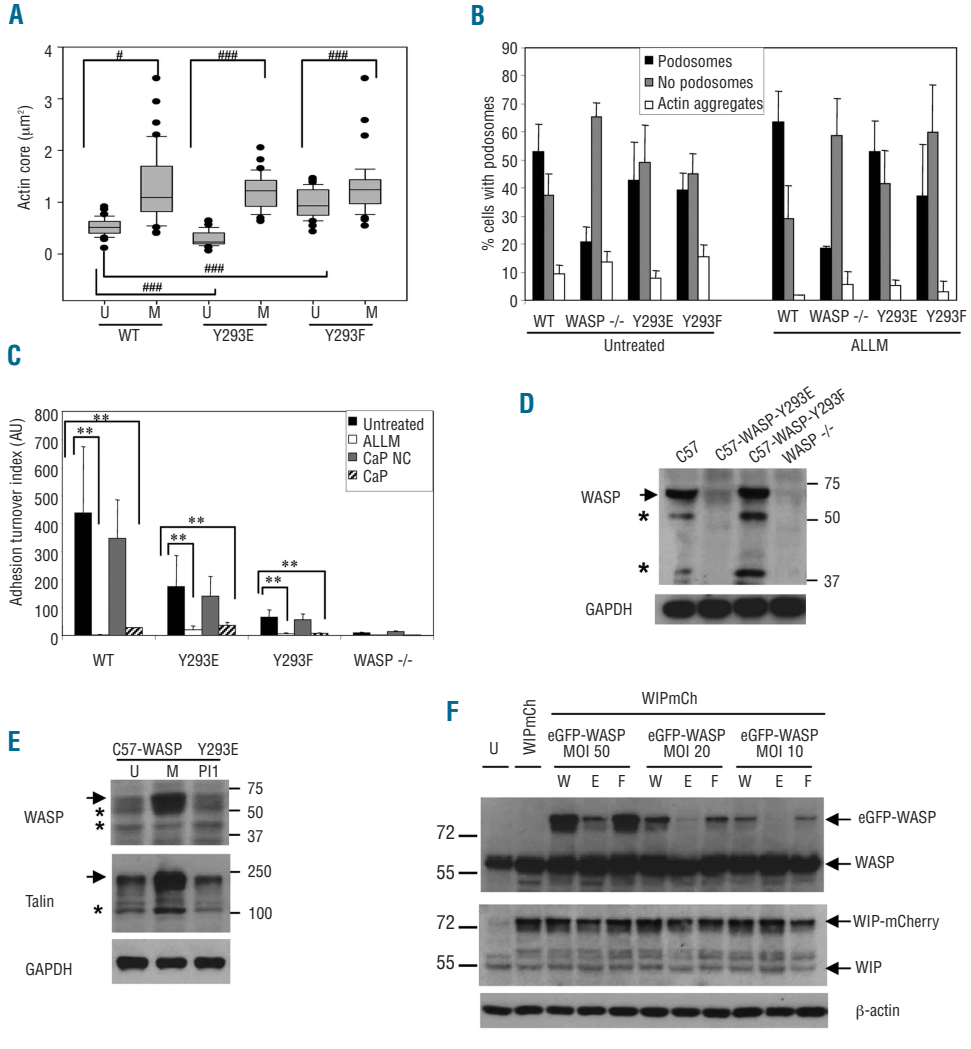



**Figure 2.** Phosphorylation of WASP results in calpain-dependant downregulation of WASP levels. (A) Box and whiskers diagrams showing the smallest value, the lower quartile, the median, the upper quartile and largest value of the area in μm² of actin cores of DCs left untreated (U) or treated with 50 μg/mL ALLM for 3 h (M). Outlying data are shown with dots. (B) Histograms show the percentage of DCs with or without podosomes or actin aggregates in cultures untreated or treated with calpain inhibitor ALLM (50 μg/mL, 3 h). (C) Histograms show the average adhesion turnover index in arbitrary units (AU) and SD of DCs left untreated or treated for 3 h with the calpain inhibitors ALLM (50 μM) or the active peptide of calpastatin (CaP) at 50 μM or the scrambled version of the peptide as negative control (CaP NC). N=15 cells per treatment group per experiment. Experiment was performed 3 times. Significant differences were observed at **P*<0.05 ***P*<0.01 and ****P*<0.005 as indicated (Student's t-test) or #*P*<0.05 ##*P*<0.01 and ###*P*<0.005 as indicated (Mann-Whitney-Wilcoxon's test for not normal sets of data). (D) Detection of WASP by Western blot in mice expressing different phosphovariants of WASP. Arrow points at full length WASP. Asterisks mark the position of previously determined calpain-mediated cleavage fragments of WASP 15. (E) Detection of WASP and talin cleavage by Western blot in C57 mice expressing eGFP-WASP Y293E untreated (U) or treated with 50 μM ALLM for 3 h (M) or the 50 μM proteasome inhibitor PS1 (PI1) for 3 h. Detection of GAPDH was used as loading control. (F) THP-1 cells already expressing WIP-mCherry were infected with human eGFP-WASP or eGFP-WASP Y291E or eGFP-WASP Y291F at MOI 10, 20 or 50. Detection of GAPDH or β-actin was used as loading control.

WIP-mCherry was required for THP-1 cells to express easily detectable levels of eGFP-WASP and phosphovariants. Expression of WIP-mCherry stabilized and increased levels of both endogenous WASP and eGFP-WASP (Figure 2F). In THP-1 cells expressing the same levels of WIP-mCherry, the measured levels of eGFP-WASP Y291E were always lower than levels of eGFP-WASP or eGFP-WASP Y291F, independently of the multiplicity of infection (MOI) used (Figure 2F). We conclude that tyrosine phosphorylation not only promotes a sustained WASP open conformation[10-11,16,22] but also enhances its susceptibility to calpain-mediated cleavage, thereby promoting disassembly of DC podosomes as the leading edge progresses.

The major role of WASP in myeloid cell biology is likely to be related to regulation of cell migration and other functions that require remodeling of the actin cytoskeleton rather than the control of cell differentiation. Although differentiation and proliferation of lymphoid cells is compromised by abnormal phosphorylation or lack of WASP,[16] numbers of myeloid cells *in vivo*[16,23] or expression of surface markers during DC maturation[24] remained unaltered suggesting that WASP is not involved in differentiation of myeloid cells. Previous work[15-16,24] and data shown in this study indicate a crucial role of WASP in organization of cell adhesion molecules and F-actin in DCs. These are fundamental processes also involved in phagocytosis,[16] which is essential during the immune response mediated by macrophages and DCs. Our current data also show that the tight regulation of WASP phosphorylation regulates the effective migration of DC across tissue barriers such as endothelial cells and may be involved in the control of specific routes of migration of myeloid cells during the immune surveillance.[23] Abnormal regulation of these functions in myeloid cells lacking WASP is thought to contribute to the phenotype of the Wiskott Aldrich Syndrome patients.

Taken together, our findings support the key role of WASP as a protein that integrates dynamics of actin and cell adhesion proteins during podosome disassembly and formation required for mobilization of myeloid cells during the immune response.

## Authorship and Disclosures

*The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.*

*Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.*

## References


1. Linder S. Invadosomes at a glance. J Cell Sci. 2009;122(17):3009-13.
2. Monypenny J, Chou HC, Bañón-Rodríguez I, Thrasher AJ, Antón IM, Jones GE, Calle Y. Role of WASP in cell polarity and podosome dynamics of myeloid cells. Eur J Cell Biol. 2011;90(2):198-204.
3. Blundell MP, Bouma G, Calle Y, Jones GE, Kinnon C, Thrasher AJ. Improvement of migratory defects in a murine model of Wiskott-Aldrich syndrome gene therapy. Mol Ther. 2008;16(5):836-44.
4. Linder S, Nelson D, Weiss M, Aepfelbacher M. Wiskott-Aldrich syndrome protein regulates podosomes in primary human macrophages. Proc Natl Acad Sci USA. 1999; 96(17):9648-53.
5. Tsuboi S. A complex of Wiskott-Aldrich syndrome protein with mammalian verprolins plays an important role in monocyte chemotaxis. J Immunol. 2006;176(11):6576-85.
6. Burns S, Thrasher AJ, Blundell MP, Machesky L, Jones GE. Configuration of human dendritic cell cytoskeleton by Rho GTPases, the WAS protein, and differentiation. Blood. 2001;98(4):1142-9.
7. Moreau V, Tatin F, Varon C, Genot E. Actin can reorganize into podosomes in aortic endothelial cells, a process controlled by Cdc42 and RhoA. Mol Cell Biol. 2003;23 (19):6809-22.
8. Chou HC, Antón IM, Holt MR, Curcio C, Lanzardo S, Worth A, et al. WIP regulates the stability and localization of WASP to podosomes in migrating dendritic cells. Curr Biol. 2006;16(23):2337-44.
9. Kim AS, Kakalis LT, Abdul-Manan N, Liu GA, Rosen MK. Autoinhibition and activation mechanisms of the Wiskott-Aldrich syndrome protein. Nature. 2000;404(6774): 151-8.
10. Cory GO, Garg R, Cramer R, Ridley AJ. Phosphorylation of tyrosine 291 enhances the ability of WASP to stimulate actin polymerization and filopodium formation. Wiskott-Aldrich Syndrome protein. J Biol Chem. 2002;277(47):45115-21.
11. Torres E, Rosen MK. Contingent phosphorylation/dephosphorylation provides a mechanism of molecular memory in WASP. Mol Cell. 2003;11(5):1215-27.
12. Bosticardo M, Marangoni F, Aiuti A, Villa A, Grazia RM. Recent advances in understanding the pathophysiology of Wiskott-Aldrich syndrome. Blood. 2009;113(25):6288-95.
13. Ancliff PJ, Blundell MP, Cory GO, Calle Y, Worth A, Kempski H, et al. Two novel activating mutations in the Wiskott-Aldrich syndrome protein result in congenital neutropenia. Blood. 2006;108(7):2182-9.
14. Devriendt K, Kim AS, Mathijs G, Frints SG, Schwartz M, Van Den Oord JJ, et al. Constitutively activating mutation in WASP causes X-linked severe congenital neutropenia. Nat Genet. 2001;27(3):313-7.
15. Calle Y, Carragher NO, Thrasher AJ, Jones GE. Inhibition of calpain stabilises podosomes and impairs dendritic cell motility. J Cell Sci. 2006;119(11):2375-85.
16. Blundell MP, Bouma G, Metelo J, Worth A, Calle Y, Cowell LA, et al. Phosphorylation of WASP is a key regulator of activity and stability in vivo. Proc Natl Acad Sci USA. 2009;106(37):15738-43.
17. West MA, Antoniou AN, Prescott AR, Azuma T, Kwiatkowski DJ, Watts C, et al. Membrane ruffling, macropinocytosis and antigen presentation in the absence of gelsolin in murine dendritic cells. Eur J Immunol. 1999;29(11):3450-5.
18. Holt MR, Calle Y, Sutton DH, Critchley DR, Jones GE, Dunn GA. Quantifying cell-matrix adhesion dynamics in living cells using interference reflection microscopy. J Microsc. 2008;232(1):73-81.
19. Dovas A, Gevrey JC, Grossi A, Park H, Abou-Kheir W, Cox D. Regulation of podosome dynamics by WASP phosphorylation: implication in matrix degradation and chemotaxis in macrophages. J Cell Sci. 2009;122(21):3873-82.
20. de la Fuente MA, Sasahara Y, Calamito M, Antón IM, Elkhal A, Gallego MD, et al. WIP is a chaperone for Wiskott-Aldrich syndrome protein (WASP). Proc Natl Acad Sci USA. 2007;104(3):926-31.
21. Franco SJ, Rodgers MA, Perrin BJ, Han J, Bennin DA, Critchley DR, Huttenlocher A. Calpain-mediated proteolysis of talin regulates adhesion dynamics. Nat Cell Biol. 2004;6(10):977-83.
22. Torres E, Rosen MK. Protein-tyrosine kinase and GTPase signals cooperate to phosphorylate and activate Wiskott-Aldrich syndrome protein (WASP)/neuronal WASP. J Biol Chem. 2006;281(6):3513-20.
23. de Noronha S, Hardy S, Sinclair J, Blundell MP, Strid J, Schulz O, et al. Impaired dendritic-cell homing in vivo in the absence of Wiskott-Aldrich syndrome protein. Blood. 2005;105(4):1590-7.
24. Olivier A, Jeanson-Leh L, Bouma G, Compagno D, Blondeau J, Seye K, et al. A partial down-regulation of WASP is sufficient to inhibit podosome formation in dendritic cells. Mol Ther. 2006;13(4):729-37.