

The WAVE regulatory complex is inhibited

Ayman M Ismail, Shae B Padrick, Baoyu Chen, Junko Umetani & Michael K Rosen

The WAVE regulatory complex (WRC) transmits information from the Rac GTPase to the actin nucleator Arp2/3 complex. We have reconstituted recombinant human and *Drosophila* WRC in several forms and shown that they are inactive toward Arp2/3 complex but can be activated by Rac in a nucleotide-dependent fashion. Our observations identify core components needed for WAVE inhibition, reconcile contradictory existing mechanisms and reveal common regulatory principles for the WAVE/WASP family of proteins.

Members of the Wiskott-Aldrich syndrome protein (WASP) family have a central role in the transmission of extracellular signals to the actin cytoskeleton¹. These proteins use their VCA domain to stimulate the actin-nucleating activity of Arp2/3 complex in response to upstream signals from the Rho family GTPases Cdc42 and Rac.

The WAVE subgroup of the WASP family participates in numerous processes, including cell polarization and motility, T cell activation and neuronal guidance^{2–4}. Aberrant WAVE signaling is important in tumor progression and metastasis⁵. In the cell, WAVE proteins are incorporated into a conserved, heteropentameric complex, referred to here

as the WAVE regulatory complex (WRC), containing WAVE, Sra1, Nap1, Abi and HSPC300 (refs. 3,4,6). Sra1 binds to Rac, functionally linking the GTPase to Arp2/3 complex^{4,6}. Although the existence and importance of the WRC have been widely demonstrated^{4,6}, its regulatory function is poorly understood. Based on purification of the WRC from bovine brain, it was initially proposed that WAVE is inactive within the complex and that Rac binding activates it toward Arp2/3 complex, concomitant with the dissociation of Nap1 and Sra1 (ref. 3). Proof that a purified biochemical activity is attributed to the correct factors requires reconstitution. However, a subsequent reconstitution⁷ led to a very different model, in which WAVE is fully active within the WRC and Rac binding produces neither further stimulation toward Arp2/3 complex nor dissociation of the assembly, consistent with observations in cells^{8,9}. Resolution of these two contradictory models is necessary to understand WAVE regulation and response to upstream signals.

One explanation could be that there is an unidentified inhibitory factor in the initial purification. To examine this possibility, we reconstituted a highly homogeneous, recombinant human WRC in

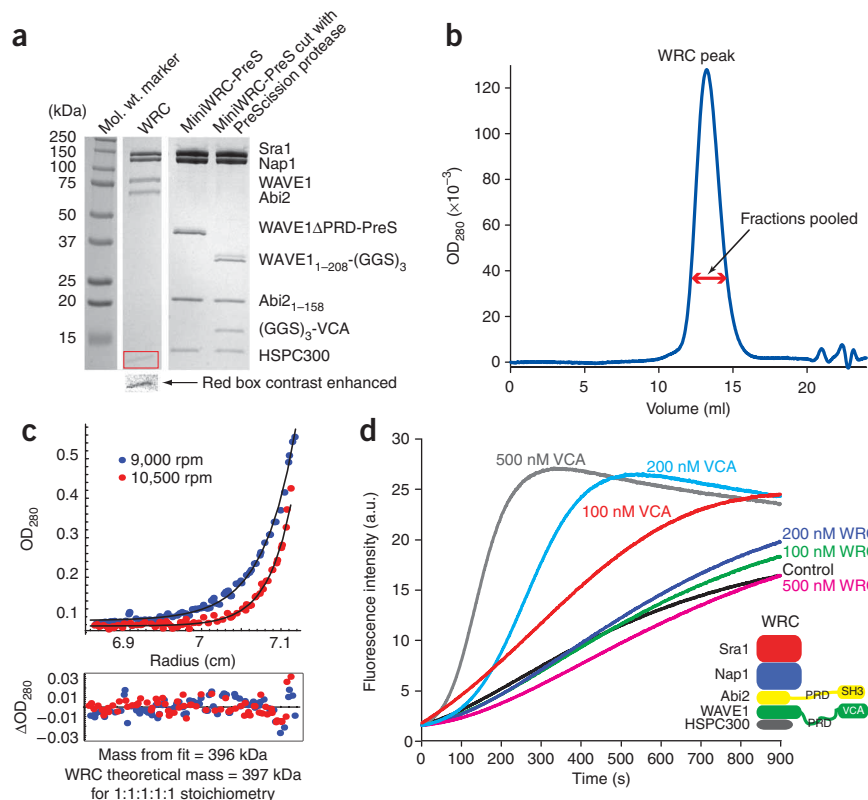


Figure 1 WRC is inhibited. **(a)** Coomassie blue-stained 8–20% SDS-PAGE gradient gel of the indicated purified complexes. Upper WAVE1 band in MiniWRC-PreS lanes corresponds to phosphorylation in the GGS linker (not shown). **(b)** Gel filtration chromatogram of human WRC. OD₂₈₀, optical density at 280 nm. **(c)** Equilibrium ultracentrifugation absorbance traces for WRC with fits and residuals at indicated speeds. OD₂₈₀, optical density at 280 nm. **(d)** Actin assembly assays of WAVE1 VCA and inhibited WRC performed with 10 nM Arp2/3 complex and 4 μM actin (5% pyrene labeled) in KMEI-G buffer (**Supplementary Methods**). PRD refers to proline-rich domain in Abi2 and WAVE1. a.u., arbitrary units.

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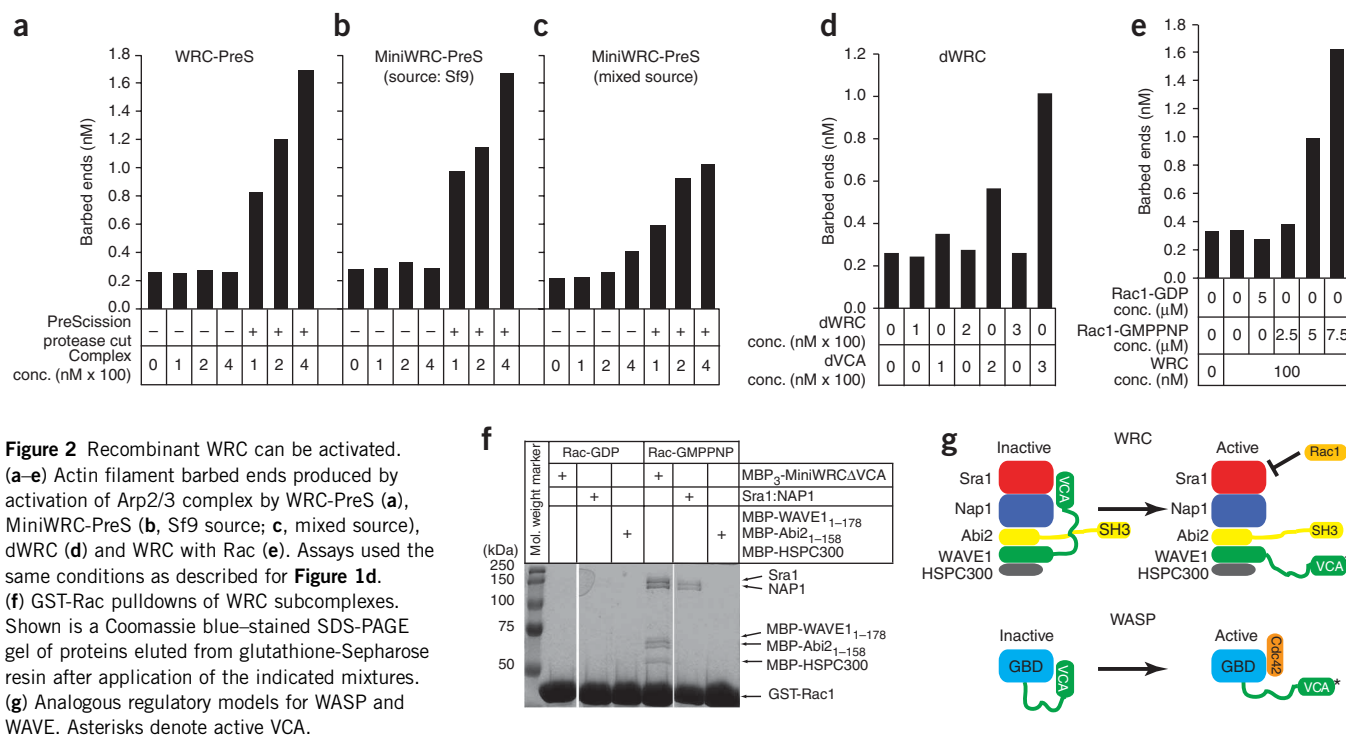


Figure 2 Recombinant WRC can be activated. (a–e) Actin filament barbed ends produced by activation of Arp2/3 complex by WRC-PreS (a), MiniWRC-PreS (b, Sf9 source; c, mixed source), dWRC (d) and WRC with Rac (e). Assays used the same conditions as described for Figure 1d. (f) GST-Rac pulldowns of WRC subcomplexes. Shown is a Coomassie blue-stained SDS-PAGE gel of proteins eluted from glutathione-Sepharose resin after application of the indicated mixtures. (g) Analogous regulatory models for WASP and WAVE. Asterisks denote active VCA.

Spodoptera frugiperda (Sf9) insect cells (Figs. 1a,b and Supplementary Table 1 online). Equilibrium analytical ultracentrifugation showed that the WRC contains one molecule of each subunit (Fig. 1c). WAVE1 VCA concentrations of 100–500 nM produced substantial activation of Arp2/3 complex in actin assembly assays, indicated by rapid filament assembly and a shorter initial lag time. The same concentrations of WRC produced virtually no activation (Fig. 1d). Thus, WAVE is inhibited within the recombinant, five-component WRC, ruling out the need for an additional inhibitory factor.

The VCA is at the immediate C terminus of all WASP proteins. Loss of even a few C-terminal residues substantially reduces VCA activity (data not shown). To determine whether the inactivity of our WRC preparations was due to *bona fide* inhibition or artifactual C-terminal proteolysis, we engineered a WRC in which the WAVE1 proline-rich region was replaced with a PreScission protease cleavage site (WRC-PreS). WRC-PreS was also inactive toward Arp2/3 complex (Fig. 2a). Treatment of this material with PreScission protease (GE Life Sciences) released the VCA from the other WRC components (Supplementary Fig. 1a online), allowing it to activate Arp2/3 complex (Fig. 2a).

We also created a WRC-PreS lacking the C-terminal proline-rich region and SH3 domain of Abi2 (MiniWRC-PreS). MiniWRC-PreS could be generated by coexpression of all five components in Sf9 insect cells (Fig. 1a). Alternatively, it was assembled from an Sra1–Nap1 heterodimer produced in Sf9 insect cells and a bacterially expressed WAVE–Abi–HSPC300 heterotrimer. Both versions of MiniWRC-PreS were inhibited and could be activated by PreScission cleavage, similar to WRC-PreS (Figs. 2b,c and Supplementary Figs. 1b,c). Thus, the inactivity of WRC-PreS, and by extension wild-type WRC, is due to genuine inhibition and not to inactivating modifications of the VCA.

These data imply that the intermolecular affinity of the VCA for the remaining components of the pentamer is low, consistent with our observations of only weak VCA inhibition by various WRC subcomplexes *in trans* (data not shown). Additionally, the proline-rich region

of WAVE and the C terminus of Abi are not necessary for inhibition. Finally, our two methods of generating MiniWRC-PreS demonstrate that inhibition within the WRC does not result from kinetic trapping produced during *in vivo* folding, but originates from the basic thermodynamics of the assembly.

To establish generality of this model, we also attempted to express the *Drosophila melanogaster* WRC (dWRC) in insect cells. Full-length dWAVE could not be expressed in these cells. But when we replaced the proline-rich region with a (GGG)₆ linker, expression increased substantially, enabling us to reconstitute dWRC. Like human WRC, dWRC was inactive toward Arp2/3 complex (Fig. 2d and Supplementary Fig. 2a online). A dWRC-PreS was also inactive and could be activated by PreScission protease (Supplementary Fig. 2b). Therefore, several forms of the human and *Drosophila* WRC are inactive toward Arp2/3 complex.

Rac1 binds to Sra1 (ref. 4,6), but it is not clear how this interaction affects the activity or integrity of the WRC. We found that in actin assembly assays, Rac1 loaded with the GTP analog GMPPNP (Rac1-GMPPNP) activated the WRC, whereas Rac1-GDP did not (Fig. 2e). We estimate that the barbed ends concentrations here are over tenfold greater than previously reported for activated WRC^{3,8}. The dose dependence of activation suggests that Rac1 has micromolar affinity for WRC. Immobilized glutathione-S-transferase (GST)–Rac1-GMPPNP bound an Sra1–Nap1 heterodimer and all five components of a minimized WRC (MiniWRCΔVCA, Supplementary Table 1), whereas GST-Rac1-GDP did not bind any component (Fig. 2f). Thus, Rac1 can activate WRC in a nucleotide-dependent fashion without dissociating the complex.

Our reconstitutions suggest that the source of the differences between previous models for WRC activity is not an unidentified factor in the WRC purified from natural sources but absent in the recombinant materials. Rather, the discrepancies appear to stem from differences in reconstituting and handling the assembly, and from the recently recognized fact that oligomerization of WASP proteins

substantially (>100-fold) increases their potency toward Arp2/3 complex¹⁰. In the report of active reconstituted WRC⁷, the complex was generated by mixing a Pir121–Nap1 subcomplex (Pir121 is a close homolog of Sra1) with a GST-WAVE2–Abi1 subcomplex on glutathione-Sepharose (and optionally adding HSPC300), incubating and washing away unbound materials before elution. This method would reject unbound Pir121–Nap1 subcomplex but retain uncomplexed GST-WAVE2–Abi1 material. We have found that various WAVE-containing subcomplexes of the pentamer have very high activity that increases over time as a result of aggregation. Indeed, we obtained stable, reproducible activity of our WRC preparations only when such subcomplexes were rigorously removed during purification (see **Supplementary Methods** online). These biochemical properties, plus the constitutive dimerization of GST, suggest that the high activity reported for the previous reconstitution of WRC resulted from contamination of WRC with aggregated, hyperactive subcomplexes. This high basal activity, coupled with the relatively low affinity of Rac for the WRC, could also explain the reported inability of the GTPase to stimulate activity further. A second protocol leading to active WRC involved a freeze-thaw cycle⁸, which activates the complex unless performed in the presence of >15% glycerol (**Supplementary Fig. 3** online). We cannot currently explain the reported WRC dissociation upon Rac activation³, but we note that Rac may bind subcomplexes lacking WAVE with higher affinity than intact WRC, as there would be less resistance to allosteric change in the former.

There are strong mechanistic parallels between the regulation of WASP and WAVE (**Fig. 2g**). In WASP, the VCA is inhibited by intramolecular contacts to the GTPase-binding domain (GBD)¹. WASP is activated by Cdc42 binding to the GBD, causing release of the VCA (which remains tethered to the GBD). In the WRC, the VCA is inhibited by an intracomplex interaction, perhaps to Sra1 and/or Nap1, as the pentamer is inactive but the WAVE1–Abi2–HSPC300 trimer is active¹⁰. The WRC is activated by Rac1 binding to Sra1,

which may release the VCA but does not cause dissociation of the complex. Thus, WASP and WAVE proteins are regulated by the same principles, achieved through different molecular details. It remains to be seen whether other WASP family members, such as WHAMM, WASH and Bee1, are regulated by analogous or distinct mechanisms.

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AUTHOR CONTRIBUTIONS

A.M.I. designed, performed and analyzed experiments and wrote the manuscript; S.B.P. performed equilibrium ultracentrifugation experiments, discussed and analyzed experiments and wrote the manuscript; B.C. performed pulldown experiment; J.U. maintained Sf9 cultures and assisted in cloning and baculovirus production; M.K.R. discussed experiments and wrote the manuscript.

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1. Takenawa, T. & Suetsugu, S. *Nat. Rev. Mol. Cell Biol.* **8**, 37–48 (2007).
2. Billadeau, D.D., Nolz, J.C. & Gomez, T.S. *Nat. Rev. Immunol.* **7**, 131–143 (2007).
3. Eden, S., Rohatgi, R., Podtelejnikov, A.V., Mann, M. & Kirschner, M.W. *Nature* **418**, 790–793 (2002).
4. Soderling, S.H. & Scott, J.D. *Biochem. Soc. Trans.* **34**, 73–76 (2006).
5. Sanz-Moreno, V. *et al. Cell* **135**, 510–523 (2008).
6. Stradal, T.E. & Scita, G. *Curr. Opin. Cell Biol.* **18**, 4–10 (2006).
7. Innocenti, M. *et al. Nat. Cell Biol.* **6**, 319–327 (2004).
8. Kim, Y. *et al. Nature* **442**, 814–817 (2006).
9. Steffen, A. *et al. EMBO J.* **23**, 749–759 (2004).
10. Padrick, S.B. *et al. Mol. Cell* **32**, 426–438 (2008).

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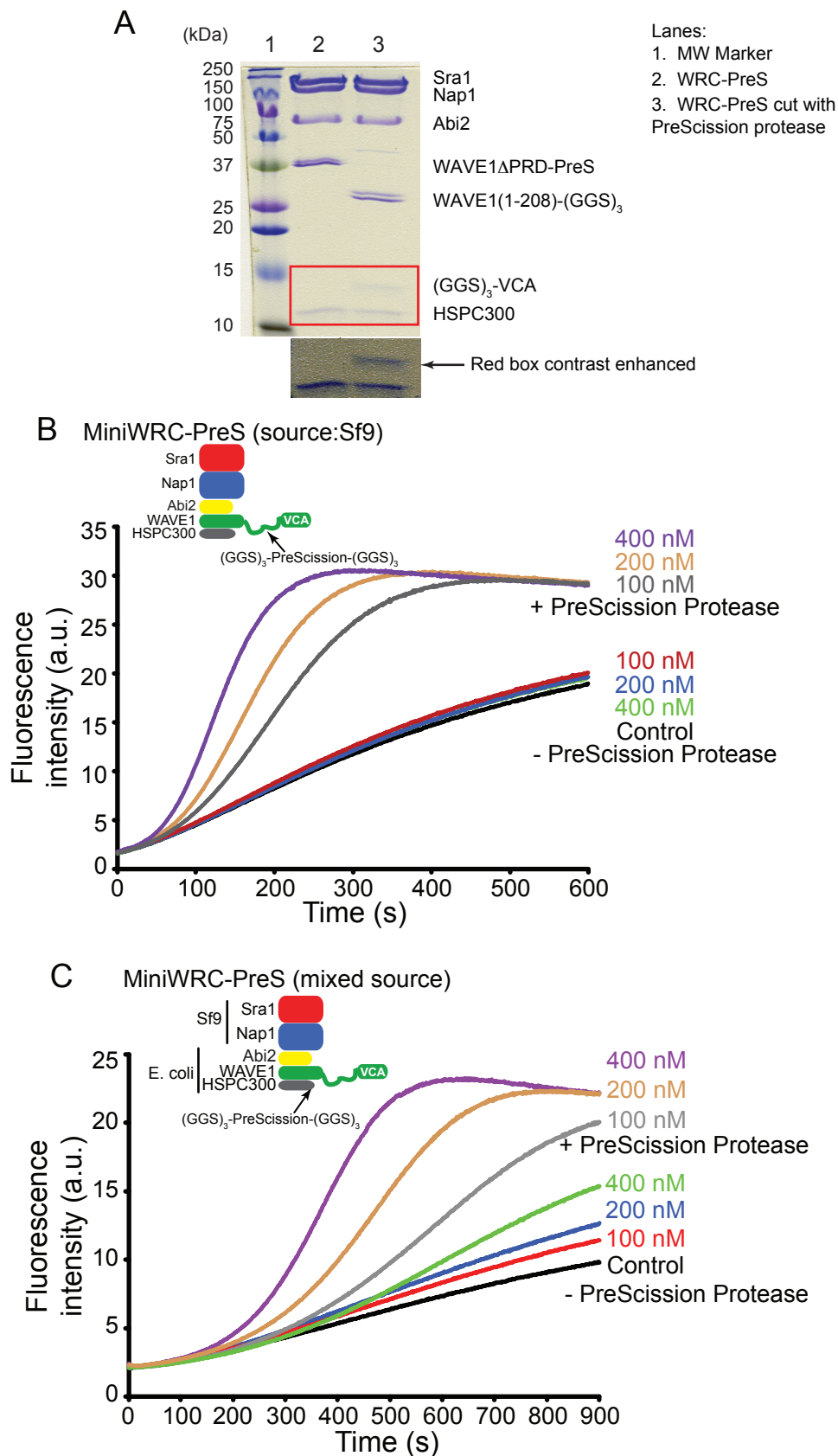
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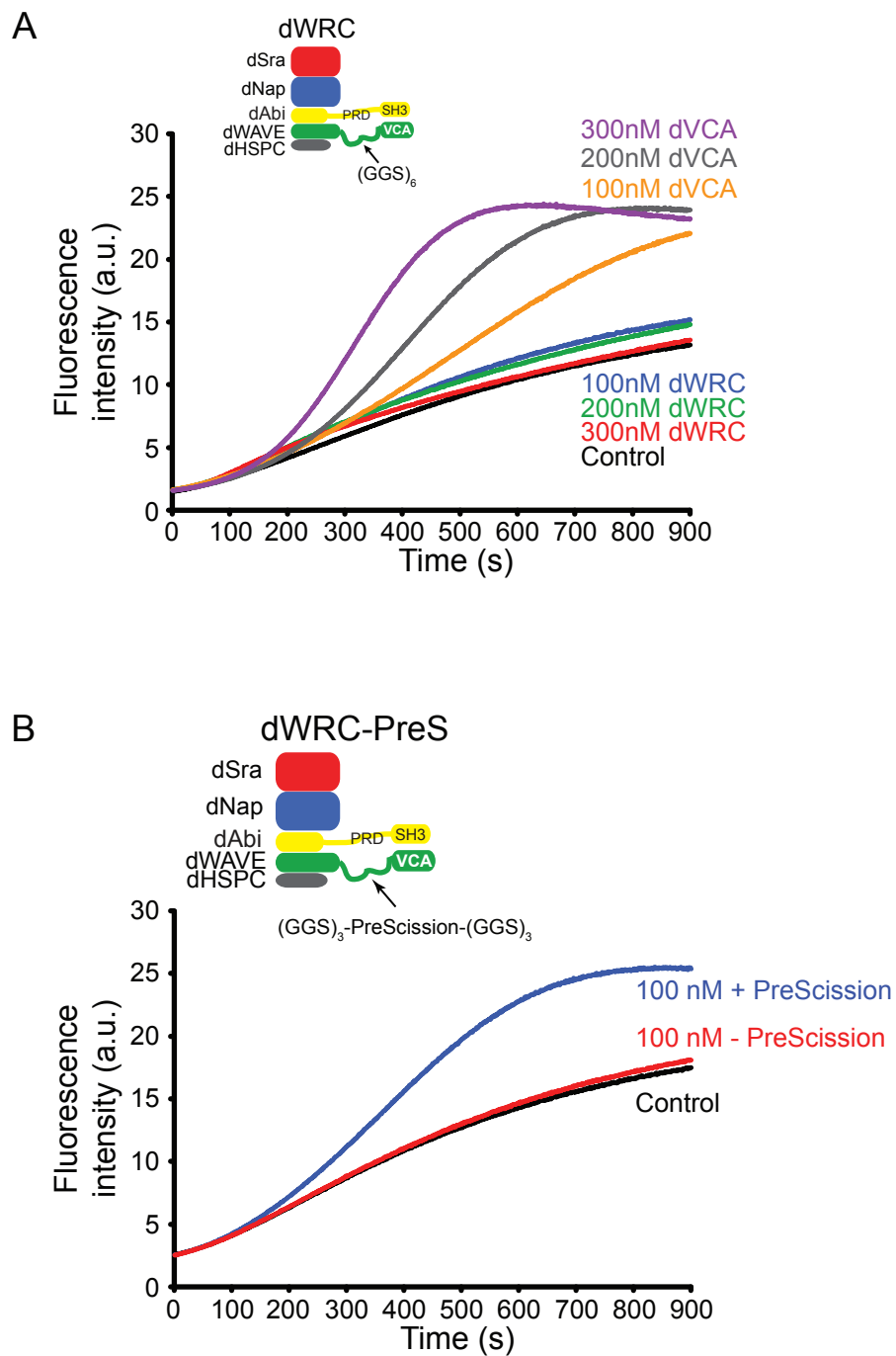
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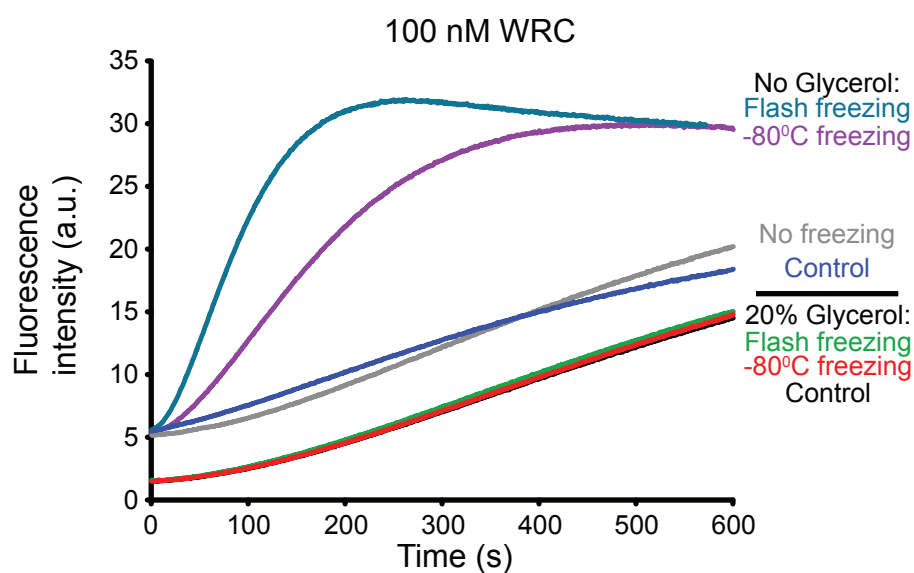
[Supplementary References](#)



Supplementary Figure 1. Recombinant WRC can be activated (A) Coomassie stained SDS-PAGE gel (15% acrylamide, gel in Figure 1A is 8-20% gradient gel) showing WRC-PreS before (lane 2) and after (lane 3) cleavage with PreScission protease. (B), (C) Actin polymerization assays with the indicated concentrations of MiniWRC-PreS expressed in Sf9 cells (B) or mixed Sf9/bacterial source (C) before and after treatment with PreScission protease.



Supplementary Figure 2. Recombinant *Drosophila* WRC is also inhibited. Actin polymerization assays with the indicated concentrations of dWRC and dVCA (A) or dWRC-PreS (B) before and after treatment with PreScission protease.



Supplementary Figure 3. WRC is activated by freezing in the absence of glycerol. For “20% glycerol” assays, WRC was frozen in 20% glycerol and used in pyrene actin assembly assays at 100 nM concentration as described in the methods. For “No glycerol” assays, WRC was frozen in 1.4% glycerol (w/v) as indicated, and used in pyrene actin assembly assays containing 1% glycerol (w/v). Fluorescence intensity offset between the two data sets reflects differences in glycerol concentrations between the two buffers.

	WRC	WRC-PreS	MiniWRC-PreS	MiniWRC Δ VCA
Sra1	+	+	+	+
Nap1	+	+	+	+
WAVE1	+			
WAVE Δ P-PreS		+	+	
WAVE1 (aa 1-178)				+
Abi2	+	+		
Abi2 (aa 1-158)			+	+
HSPC300	+	+	+	+

WAVE Δ P-PreS is WAVE1(aa 1-208)-GGSGGSGGS-LEVLFQGP-GGSGGSGGS-WAVE1(aa 484-559) where LEVLFQGP is the PreScission protease cleavage site.

Supplementary Table 1

Supplementary Methods

Cloning

All WRC components except human Nap1 were cloned into pFastBac vectors (Invitrogen) that were modified with the insertion of a 21 nucleotide promoter sequence from lobster tropomyosin before the start codon¹, with N-terminal, TEV cleavable His₆ (all components except HSPC300) or Glutathione S-Transferase (HSPC300) tags. Human Nap1 was cloned into pFastBacHtC. The constructs were used to generate baculoviruses used in Sf9 insect cells infections using the Bac-to-Bac system. In addition, WAVE1 Δ P-PreS (WAVE1 with residues 209-483 replaced by (GGG)₃-PreScission protease recognition site-(GGG)₃, WAVE1 (residues 1-178), Abi2(residues 1-158) and HSPC300 were cloned into a modified pMal-c2 vector (NEB, Tev protease recognition site added after MBP sequence) for expression in *E. coli*.

Protein expression and purification

Sf9 cells were co-infected with approximately equal amounts of the viruses expressing the different WRC components. Cells were harvested 60 hours post infection, resuspended in lysis buffer (LyB: 25 mM Tris pH 8.5, 20 mM imidazole pH 8, 150 mM NaCl, 20% glycerol (w/v), 5 mM β ME, protease inhibitors mix) and lysed using one freeze/thaw cycle. All subsequent handling was performed at 4°C.

WRC lysate was cleared by centrifugation (48,000 *g* for 45 min). WRC was adsorbed to NiNTA-agarose (Qiagen, six ml per liter of culture) in batch mode with nutation for 45 min in LyB. Tubes were completely filled with buffer to avoid excess aeration. Beads were washed in column mode three times with three column volumes (CV) of LyB, followed by three times with three CV of NiNTA wash buffer (NiWB: 25 mM Tris pH 8.5, 40 mM imidazole pH 8, 150 mM NaCl, 20% glycerol (w/v), 5 mM β ME) and eluted with three times one CV of NiNTA elution buffer (NiEB: 25 mM Tris pH 8.5, 400 mM imidazole pH 8, 150 mM NaCl, 20% glycerol (w/v), 5 mM β ME). Combined eluate was adsorbed to glutathione sepharose 4B (GS4B) resin (GE healthcare, three ml per liter of culture) in batch mode with nutation for 30 min in NiEB. Beads were washed in column mode four times with four CV of GS4B wash buffer (GSTWB: 25 mM Tris pH 8.5, 100 mM NaCl, 20% glycerol (w/v), 5 mM β ME) and eluted with three times one CV GS4B elution buffer (GSTEB: 100 mM Tris pH 8.5, 100 mM NaCl, 20% glycerol (w/v), 30 mM reduced glutathione, 5 mM β ME). Tags were cleaved by overnight incubation with TEV protease without agitation. Digest was diluted with an equal volume of Q1A (25 mM Tris pH 8.5, 20% glycerol (w/v), 5 mM β ME), applied to a two ml SOURCE15Q anion exchange column (GE Healthcare) and eluted using a 15 to 50% Q1B (25 mM Tris pH 8.5, 20% glycerol (w/v), 1 M NaCl, 5 mM β ME) gradient developed over 35 CV. A shallow gradient at this stage was necessary to resolve WRC from Nap1:Sra1 and WAVE1:Abi2:HSPC300 subcomplexes.

Pooled fractions were diluted with two volumes of S1A (20 mM HEPES pH 7, 20% glycerol (w/v), 1 mM DTT), applied to a two ml SOURCE15S cation exchange column (GE Healthcare) and eluted using a 8 to 30% S1B (20 mM HEPES pH 7, 20% glycerol (w/v), 1 M NaCl, 1 mM DTT) gradient developed over 44 CV. It is important to load the cation exchange column at a relatively high conductivity (about 130 mM NaCl) to prevent WAVE1 containing contaminants from binding to the column, thus separating WRC from free (active) WAVE1. WRC-containing fractions were concentrated using Amicon Ultra, 10000 MWCO (Millipore) and applied to 24 ml Superose6 gel filtration column (GE Healthcare) in (10 mM imidazole pH 7, 20% glycerol (w/v), 100 mM KCl, 1 mM EGTA pH 8, 1 mM MgCl₂, 1 mM DTT).

WRC-PreS, MiniWRC-PreS (Sf9 source), dWRC and dWRC-PreS were purified identically to WRC, except that the cation exchange step was unnecessary and omitted.

MiniWRC-PreS (mixed source) was reconstituted from two parts. First, a Nap1:Sra1 heterodimer was coexpressed in Sf9 cells and purified by NiNTA affinity, Source15Q anion exchange and gel filtration chromatographies as above. Second, a WAVE1 Δ P-PreS:Abi2(residues 1-158):HSPC300 heterotrimer was generated from bacterially expressed material. The three materials were expressed separately as TEV cleavable MBP fusions in BL21 (DE3) T1^R *E. coli*,

in LB (+ 2 g per L glucose). In each case, cells were grown to an OD₆₀₀ of 0.6-0.9 and induced with 1 mM IPTG, for 16 hours at 20°C. Cells were harvested, resuspended in (20 mM Tris pH 8, 200 mM NaCl, 2 mM EDTA, 2 mM DTT, 1 mM PMSF) and frozen. Cells were thawed in cool water and lysed using two passes of a cell disruptor (Avestin EmulsiFlex C5). Lysate was clarified by centrifugation (45,000 *g* for 45 minutes) and applied to a Amylose High Flow (N.E.B.) column in column mode. Column was washed with 2.5 CV of (20 mM Tris pH 8, 200 mM NaCl, 1 mM EDTA, 1 mM DTT), then with 4 CV of (20 mM Tris pH 8, 20 mM NaCl, 1 mM EDTA, 1 mM DTT) and eluted with 2.5 CV of (20 mM Tris pH 8, 20 mM NaCl, 1 mM EDTA, 1 mM DTT and 30 mM maltose). Equal concentrations of MBP fusions were mixed with 1% NP-40 and incubated overnight at 4°C to reconstitute a trimeric assembly. A long incubation and addition of NP-40 detergent were essential for complex formation. Complex was applied to an eight ml SOURCE15Q (equilibrated in Q2A: 20 mM Tris pH 8, 1 mM DTT) and eluted with a 0 to 50% gradient of Q2B (20 mM Tris pH 8, 1 M NaCl, 1 mM DTT) developed in 15 CV. Pooled fractions were concentrated and applied to a 320 ml Superdex200pg gel filtration column (GE Healthcare) and eluted with (20 mM Tris pH 8, 150 mM NaCl, 1 mM DTT). This purified trimer was then mixed with the Nap1:Sra1 dimer, in a 1.2:1 ratio. This mixture was incubated overnight and applied to an amylose column, washed three times with three CV of (20 mM Tris pH 8, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, 20% glycerol (w/v)), then eluted two times with three CV of (20 mM Tris pH 8, 200 mM

NaCl, 1 mM EDTA, 1 mM DTT, 20% glycerol (w/v), 30 mM maltose). Eluted complexes were cleaved with TEV protease at 22°C for six hours and applied to a 320 ml Superdex200pg column in KMEI plus 20% glycerol (w/v). Complete TEV cleavage is required at this point, as cleaved trimer is resolved from MiniWRC-PreS, while uncleaved trimer is not. MBP-WAVE1(residues 1-178):MBP-Abi2(residues 1-158):MBP-HSPC300 trimer and its complex with Nap1:Sra1 (MBP₃-MiniWRCΔVCA) were made exactly as above.

Rac1, actin, pyrene-actin and Arp2/3 complex were purified as previously described^{2, 3}.

Actin polymerization assay

All actin polymerization assays contained 4 μM actin (5% pyrene labeled) and 10 nM Arp2/3 complex in KMEI-G buffer, and were performed as described³. KMEI-G contains 15% (w/v) glycerol, 50 mM KCl, 1 mM MgCl₂, 1 mM EGTA, and 10 mM imidazole (pH 7.0). Barbed ends were calculated as described⁴, with the exception that the 15% glycerol present to stabilize the WRC materials reduces the barbed end actin on rate (k_{+B}) in a diffusion limited fashion⁵ which can be corrected for using literature values for the viscosity of glycerol solutions⁶.

Analytical ultracentrifugation

Equilibrium sedimentation experiments were performed in an XL-I ultracentrifuge (Beckman-Coulter) using sapphire-windowed, six-sectored cells in an An60-Ti rotor. After 22 hours at each speed (9,000, 10,500, 15,000 rpm), an absorbance profile was recorded using absorbance at 280 nm (in KMEI buffer plus 15% glycerol (w/v) at 20°C). Absorbance profile at 15,000 rpm was used to estimate the baseline. SEDPHAT was used to fit the data from both speeds simultaneously with baseline correction. Protein and buffer parameters were estimated using SEDNTERP⁷. Analyses of WRC yielded a mass of 396 kDa, consistent with the 397 kDa theoretical mass of a 1:1:1:1:1 stoichiometry pentamer. Error in the calculated mass was estimated as ~20 kDa, indicating that there could in principle be more than one HSPC300 subunit. However, we have also performed ultracentrifugation experiments on the MBP-WAVE1(residues 1-178):MBP-Abi2(residues 1-158):MBP-HSPC300 Figure 2F (not shown). In that case, precision was high enough to unambiguously establish 1:1:1 stoichiometry. Thus, the stoichiometry of the WRC is almost certainly 1:1:1:1:1.

GST pulldown experiments

GST-Rac1 is loaded with GDP when purified from bacteria. The protein was loaded with GMPPNP as described⁸. GST pulldown experiments were performed by mixing 400 pmol of GST-Rac1, 40 pmol of different WAVE

complexes, and 15 μ L glutathione sepharose 4B resin in one ml of pulldown buffer (PB: 20 mM Tris-HCl pH 8.5, 50 mM NaCl, 2 mM MgCl_2 , 5% glycerol (w/v) and 5 mM β ME). After gentle mixing at 4°C for 60 min, the resin was sedimented by gravity and washed three times with one mL of PB, followed by elution using 25 μ L of PB plus 30 mM reduced glutathione. Eluted proteins were resolved by SDS-PAGE and visualized with Coomassie blue.

Supplementary References

1. Sano, K., Maeda, K., Oki, M. & Maeda, Y. FEBS Lett 532, 143-6 (2002).
2. Aghazadeh, B., Lowry, W. E., Huang, X.-Y. & Rosen, M. K. Cell 102, 625-633 (2000).
3. Padrick, S. B. et al. Mol Cell 32, 426-438 (2008).
4. Leung, D. W., Morgan, D. M. & Rosen, M. K. Methods Enzymol 406, 281-96 (2006).
5. Drenckhahn, D. & Pollard, T. D. J Biol Chem 261, 12754-8 (1986).
6. Segur, J. B. & Oberstar, H. E. Ind. Eng. Chem 43, 2117-2120 (1951).
7. Laue, T. M., Shah, B. D., Ridgeway, R. M. & Pelletier, S. L. in Analytical Ultracentrifugation in Biochemistry and Polymer Science (eds. Harding, S. E., Rowe, A. J. & Horton, J. C.) (The Royal Society of Chemistry, Cambridge, UK, 1992).
8. Abdul-Manan, N. et al. Nature 399, 379-383 (1999).