Published in final edited form as:

Nat Struct Mol Biol. 2009 May ; 16(5): 561–563. doi:10.1038/nsmb.1587.

The WAVE Regulatory Complex is Inhibited

Ayman M. Ismail, **Shae B. Padrick**, **Baoyu Chen**, **Junko Umetani**, and **Michael K. Rosen**

Howard Hughes Medical Institute and Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas 75390.

Abstract

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 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.

Members of the Wiskott-Aldrich Syndrome Protein (WASP) family play 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 guidance2–4. Aberrant WAVE signaling is important in tumor progression and metastasis5. In the cell, WAVE proteins are incorporated into a conserved, hetero-pentameric complex, referred to here as the WAVE Regulatory Complex (WRC), containing WAVE, Sra1, Nap1, Abi and HSPC3003,4,6. Sra1 binds to Rac, functionally linking the GTPase to Arp2/3 complex4,6. While the existence and importance of the WRC have been widely demonstrated4,6, the regulatory function of the complex 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 Sra13. Proof that a purified biochemical activity is attributed to the correct factors requires reconstitution. However, a subsequent reconstitution7 lead 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

Corresponding Author: Dr. Michael K. Rosen, Tel: (214)-645-6361, Fax: (214)-645-6291, Michael.Rosen@UTSouthwestern.edu.

Author Contributions

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

Note: Supplementary information is available on the Nature Structural and Molecular Biology website.

observations in cells8,9. Resolution of these two contradictory models is necessary to understand WAVE regulation and response to upstream signals.

One explanation would be an unidentified inhibitory factor in the initial purification. To examine this issue we reconstituted a highly homogeneous, recombinant human WRC in *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 to 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 (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 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, 2C and Supplementary Figs. 1B, 1C *online*). Thus, the inactivity of WRC-PreS, and by extension wild type WRC, is due to genuine inhibition, not 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 (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 did not express. But when we replaced the proline rich region with a $(GGS)_6$ 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 cleavage (Supplementary Fig. 2B *online*). Therefore several forms of the human and Drosophila WRC are inactive toward Arp2/3 complex.

Rac1 binds to Sra14,6, 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 GMPPNP (a GTP analog; Rac1-GMPPNP) activated the WRC, while Rac1-GDP did not (Fig. 2E). We estimate that the barbed ends here are over 10 fold greater than previously reported for activated WRC3,8. The dose dependence of activation suggests that Rac1 has micromolar affinity for WRC. Immobilized GST-Rac1-GMPPNP bound an Sra1:Nap1 heterodimer and all five components of a minimized WRC (MiniWRCΔVCA, Supplementary Table 1 *online*), while 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 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 due to aggregation. Indeed, we only obtained stable, reproducible activity of our WRC preparations 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 note that Rac may bind subcomplexes lacking WAVE with higher affinity than intact WRC, since 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 intra-complex interaction, perhaps to Sra1 and/or Nap1, since 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. Da Jia for assistance with WRC purification, Dr. Sanjay Panchal for assistance with ultracentrifugation experiments and Lynda Doolittle (University of Texas Southwestern Medical Center, Dallas, TX) for sharing reagents. Work was supported by the Howard Hughes Medical Institute and grants from the NIH (R01-GM56322) and Welch Foundation (I–1544). S. B. P. was supported by a fellowship from the NIH (1F32 GM06917902).

References

1. Takenawa T, Suetsugu S. Nat Rev Mol Cell Biol. 2007; 8:37–48. [PubMed: 17183359]
2. Billadeau DD, Nolz JC, Gomez TS. Nat Rev Immunol. 2007; 7:131–143. [PubMed: 17259969]
3. Eden S, Rohatgi R, Podtelejnikov AV, Mann M, Kirschner MW. Nature. 2002; 418:790–793. [PubMed: 12181570]
4. Soderling SH, Scott JD. Biochem Soc Trans. 2006; 34:73–76. [PubMed: 16417486]
5. Sanz-Moreno V, et al. Cell. 2008; 135:510–523. [PubMed: 18984162]
6. Stradal TE, Scita G. Curr Opin Cell Biol. 2006; 18:4–10. [PubMed: 16343889]
7. Innocenti M, et al. Nat Cell Biol. 2004; 6:319–327. [PubMed: 15048123]
8. Kim Y, et al. Nature. 2006; 442:814–817. [PubMed: 16862120]
9. Steffen A, et al. EMBO J. 2004; 23:749–759. [PubMed: 14765121]
10. Padrick SB, et al. Mol Cell. 2008; 32:426–438. [PubMed: 18995840]

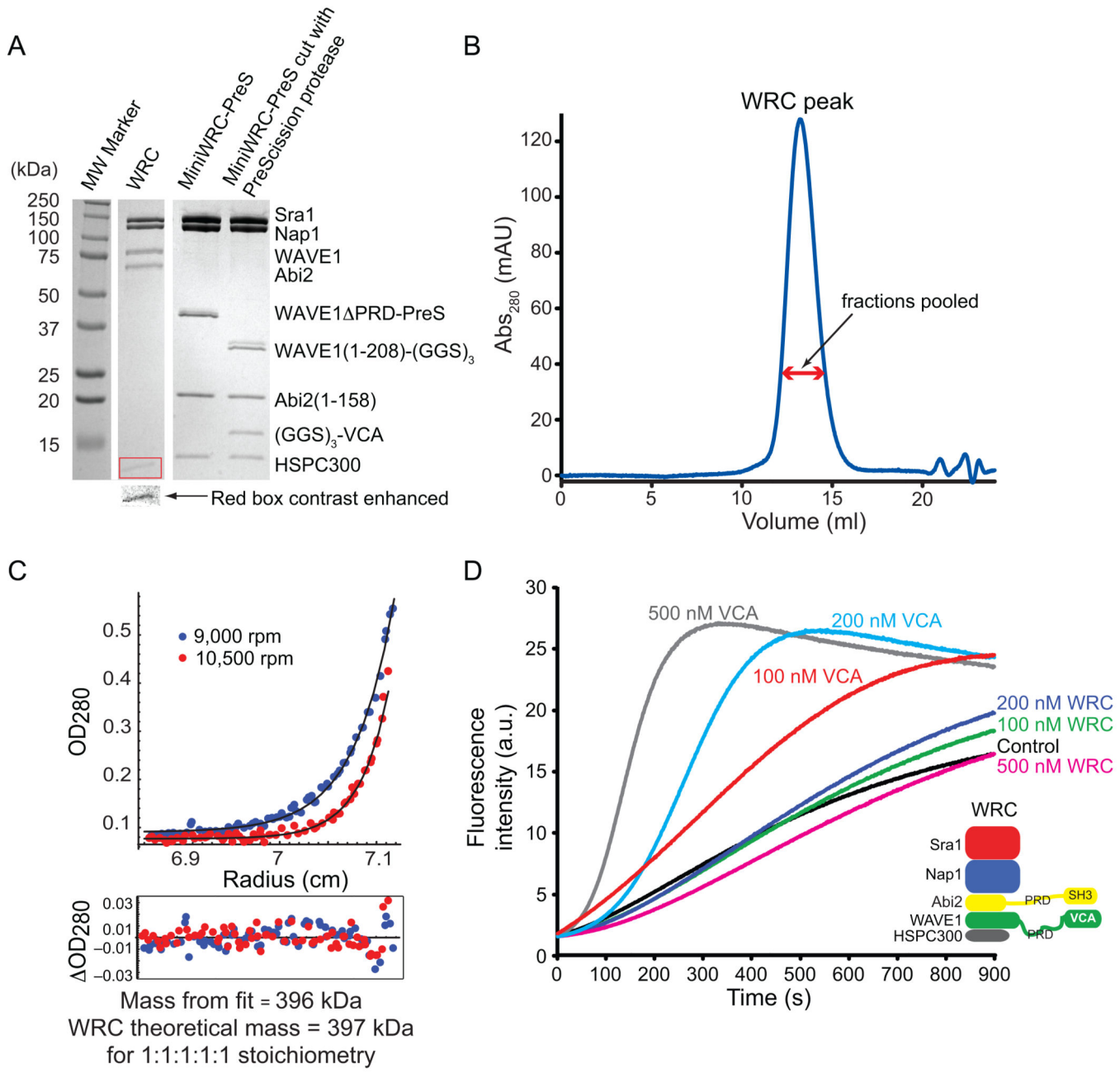

Figure 1.

WRC is inhibited. (A) Coomassie blue stained SDS-PAGE 8–20% 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. (C) Equilibrium ultracentrifugation absorbance traces for WRC with fits and residuals at indicated speeds. (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 *online*). PRD refers to proline rich domain in Abi2 and WAVE1. a.u: arbitrary unit.

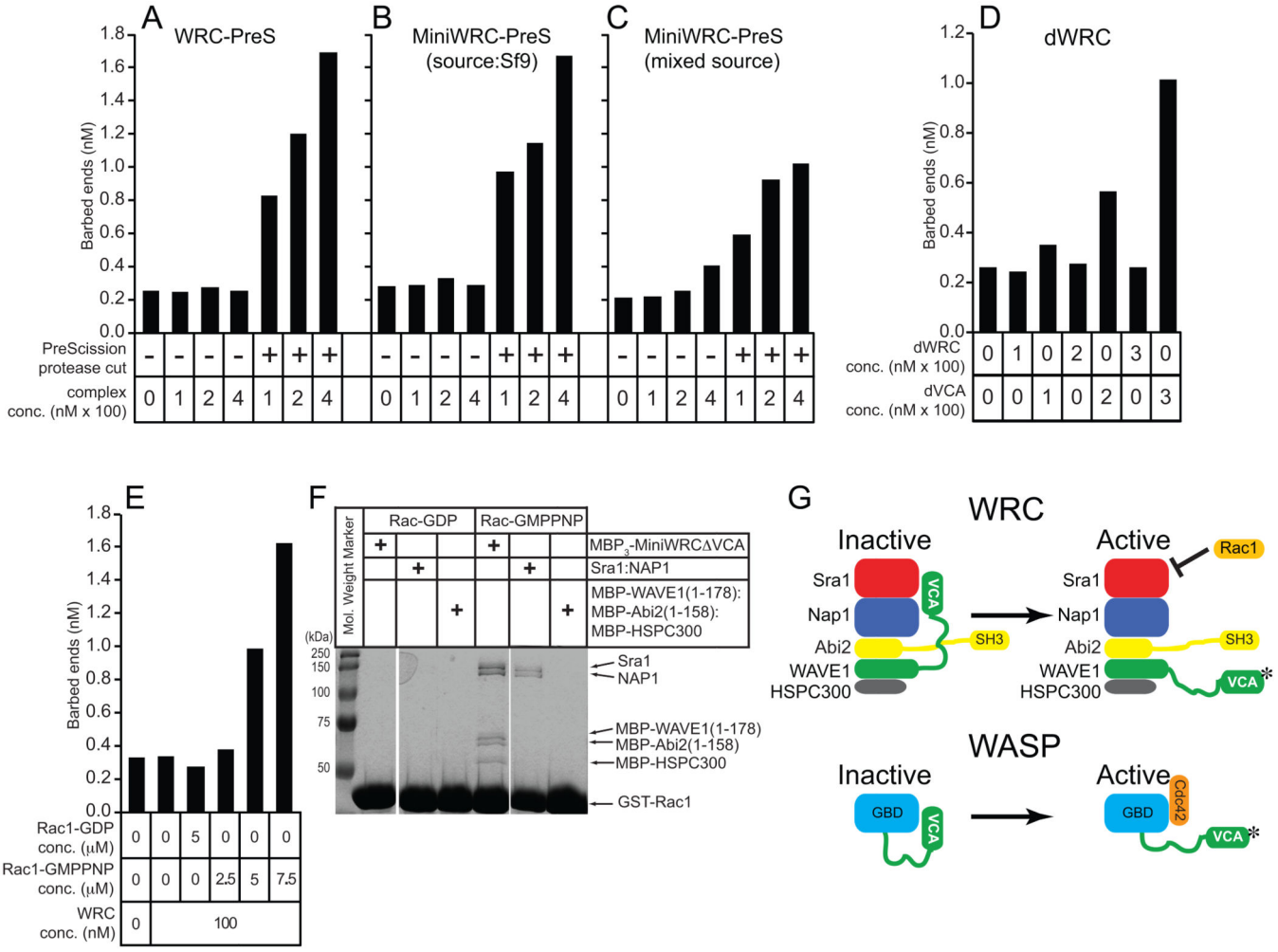

Figure 2.

Recombinant WRC can be activated. 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 same conditions as Fig. 1D. (F) GST-Rac pulldowns of WRC subcomplexes. Coommassie 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. Asterisk denotes active VCA.