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Review

Phosphoregulation of the WAVE regulatory complex and signal integration

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ABSTRACT

The WAVE2 regulatory complex (WRC) induces actin polymerization by activating the actin nucleator Arp2/3. Polymerizing actin pushes against the cell membrane and induces dramatic edge protrusions. In order to properly control such changes in cell morphology and function, cells have evolved multiple methods to tightly regulate WRC and Arp2/3 activity in space and time. Of these mechanisms, phosphorylation plays a fundamental role in transmitting extracellular and intracellular signals to the WRC and the actin cytoskeleton. This review discusses the phosphorylation-based regulatory inputs into the WRC. Signaling pathways that respond to growth factors, chemokines, hormones, and extracellular matrix converge upon the WAVE and ABI components of the WRC. The Abl, Src, ERK, and PKA kinases promote complex activation through a WRC conformation change that permits interaction with the Arp2/3 complex and through WRC translocation to the cell edge. The neuron-specific CDK5 and constitutively active CK2 kinases inhibit WRC activation. These regulatory signals are integrated in space and time as they coalesce upon the WRC. The combination of WRC phosphorylation events and WRC activity is controlled by stimulus, cell type, and cell cycle-specific pathway activation and via pathway cross-inhibition and cross-activation.

Contents

1. Introduction

1.1. Structure and function of the WRC

The WAVE regulatory complex (WRC) activates the Arp2/3 actin nucleator to induce actin polymerization during cell motility and neurite extension. Actin monomers are abundant in the cytoplasm and join existing actin filaments to cause rapid polymerization. However, newly initiated actin dimers and trimers are

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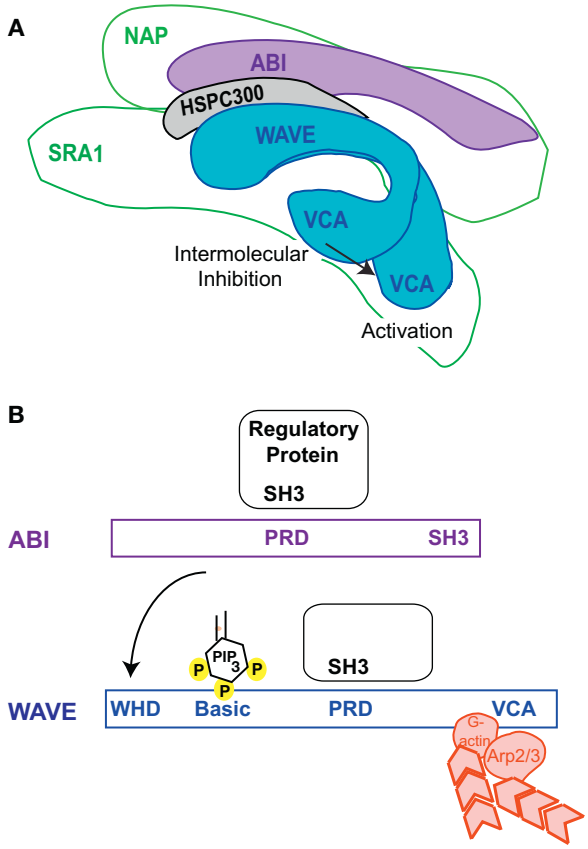

Fig. 1. Components and domains of the WRC. (A) The WRC is a stable heterocomplex of WAVE, ABI, NAP, SRA1, and HSPC300 isoforms. WAVE is also called SCAR, after its *Dictyostelium* homolog. NAP is also called hematopoietic protein (Hem). SRA1 is also called p53-inducible mRNA121 (PIR121) or cytoplasmic FMRP interacting protein (CYFIP). HSPC300 is also called BRICK. WRC activation involves release of the VCA domain from intermolecular interaction with the meander region of WAVE and SRA1. (B) WAVE and ABI interaction domains. WAVE interacts with ABI and HSPC300 through its WHD. WAVE interacts with negatively-charged phospholipids, such as phosphatidylinositol(3,4,5)-triphosphate (PIP3), through its basic region. Both WAVE and ABI interact with regulatory proteins through their polyproline rich domains (PRDs) binding to Src homology 3 (SH3) domains in the regulatory proteins. WAVE interacts with and activates the Arp2/3 complex through the WAVE VCA region.

unstable. The WRC-activated Arp2/3 complex acts as a catalyst to overcome this energy barrier and nucleates new actin filament branches at 70° angles from pre-existing filaments (for review, see [1,2]). The resulting crosslinked network of actin induces protrusion events necessary for cell movement during development, wound healing, immune responses, and cancer cell metastasis, and for new axon and dendrite formation during neuronal development.

The WRC is a stable pentamer of isoforms of the following five proteins: WASp-family verprolin homologous protein (WAVE), Ableson interacting protein (ABI), NCK-associated proteins (NAP), specifically Rac-associated 1 (SRA1), and hematopoietic stem progenitor cell 300 (HSPC300) (Fig. 1A) [3–7]. In this review, WAVE, ABI, and NAP refer to conclusions drawn from multiple isoforms of each protein that are considered to be universal. WAVE, ABI, and HSPC300 form a trimer with actin-polymerizing activity that rests upon a NAP:SRA1 dimer [4,5,7–9]. The recent WRC crystal structure revealed that SRA1 and NAP exhibit very similar domain organization, suggesting they belong to the same protein family even though they have minimal sequence identity [8].

WAVE proteins have four conserved domains: a WAVE homology domain (WHD, also called SCAR homology domain for the *Dictyostelium* homolog SCAR) at the N-terminus, a basic region, a polyproline-rich domain (PRD), and a VCA region (Fig. 1B). The WHD mediates complex formation with ABI, NAP, SRA1 and HSPC300 [5,8]. The polyproline-rich region binds Src-homology-3 (SH3) domains and WW domains in other regulatory proteins. ABI also has a PRD and an SH3 domain, suggesting WAVE and ABI may together contribute to WRC recruitment or oligomerization by SH3 or WW-domain containing proteins (Fig. 1B, reviewed in [10]). The VCA region is a 3-part domain at the WAVE C-terminus. The region is named for its verprolin homology domain (V), hydrophobic central or cofilin homology domain (C), and acidic domain (A). The function of the VCA region was elucidated in the related WASP proteins and is assumed to be similar for the WAVEs. The C–A regions bind the Arp2 and Arp3 subunits of the Arp2/3 complex, which are homologous to actin [11–13]. VCA binding to Arp2/3 induces changes in the tertiary and quaternary structure of the Arp2/3 complex so that the Arp2 and Arp3 subunits are in a "closed" conformation [14–16]. The V domain binds to G-actin and brings it in close proximity to the Arp2 and/or Arp3 subunits, thereby creating a pseudotrimer nucleus that grows into a new filament on its own [13,15,17].

1.2. Activation of the WRC

The WRC is a stable and intermolecularly inhibited complex. In many cell types, knockdown or knockout of any one component causes a reduction in the levels of the remaining components, indicating WAVE, ABI, NAP, SRA1, and HSPC300 are stabilized by their incorporation into the WRC [5,6,18–21]. Alone, the WAVE VCA region strongly stimulates actin nucleation [5,9]. The recent WRC crystal structure revealed that the VCA region is normally inhibited through interaction with the meander region of WAVE (WAVE1 residues 82–184) and SRA1 [8]. Upstream signals are needed to activate the WRC. However, these signals do not disrupt the WRC, but rather induce a conformational change that releases the VCA region so that it can bind Arp2/3 and actin [5,8,9,20,22]. Improper purification of the WRC, such as heating, freeze–thaw cycles, prolonged storage on ice, or lack of glycerol as a cryo-protectant can also lead to complex denaturation and errorneous actin polymerization activity [9,22–24]. Past conclusions from overexpression and *in vitro* studies need to be carefully reconsidered to determine if the WAVE was uncomplexed, since uncomplexed WAVE2 is artificially and constitutively active [5,9,19,22,24–27].

The WRC is a signal integrator. Signals from the Rac GTPase, phospholipids, and protein kinases that sense growth factor and substrate adhesion coalesce upon components of the WRC to induce WRC membrane localization and activation at the right time. Rac-GTP binds the WRC through SRA1 [8,28]. The binding competes with sequestration of the WAVE VCA region, so Rac-GTP binding makes the VCA region accessible to the Arp2/3 complex and actin [8].

WRC binding to negatively charged phospholipids contributes to WRC recruitment to the cell membrane and WRC activation of the Arp2/3 complex. Phosphatidylinositol(3,4,5)-triphosphate (PtdIns(3,4,5)P3), and to a lesser degree phosphatidylinositol(4,5)-diphosphate (PtdIns(4,5)P2), binds the basic region of WAVE2 [29]. Lipid binding is necessary for WAVE2 translocation to the leading edge and Rac-induced protrusion [29]. In addition, liposomes containing PtdIns(3,4,5)P3 are required to fully activate the *in vitro* actin polymerization activity of Rac-WRC [22]. Other negatively charged lipids can induce partial activity in a charge-dependent manner [22]. Activation of the WRC is highly cooperative and two VCA domains bind the Arp2/3 complex with greater affinity that a VCA monomer. Thus, phospholipid recruitment of the WRC to the cell membrane may increase activation of the Arp2/3 complex via WRC oligomerization [22,30].