

● INVITED REVIEW

RACK1 regulates neural development

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Abstract

Receptor for activated C kinase 1 (RACK1) is an evolutionarily conserved scaffolding protein within the tryptophan-aspartate (WD) repeat family of proteins. RACK1 can bind multiple signaling molecules concurrently, as well as stabilize and anchor proteins. RACK1 also plays an important role at focal adhesions, where it acts to regulate cell migration. In addition, RACK1 is a ribosomal binding protein and thus, regulates translation. Despite these numerous functions, little is known about how RACK1 regulates nervous system development. Here, we review three studies that examine the role of RACK1 in neural development. In brief, these papers demonstrate that (1) RACK-1, the *C. elegans* homolog of mammalian RACK1, is required for axon guidance; (2) RACK1 is required for neurite extension of neuronally differentiated rat PC12 cells; and (3) RACK1 is required for axon outgrowth of primary mouse cortical neurons. Thus, it is evident that RACK1 is critical for appropriate neural development in a wide range of species, and future discoveries could reveal whether RACK1 and its signaling partners are potential targets for treatment of neurodevelopmental disorders or a therapeutic approach for axonal regeneration.

Key Words: RACK1; RACK-1; neural development; neurite outgrowth; axon outgrowth; axon guidance

Introduction

Receptor for activated C kinase 1 (RACK1) is an evolutionarily conserved scaffolding protein that can interact with multiple signaling molecules concurrently through its seven WD repeats. Although RACK1 interacts with many proteins, its most studied binding partners are protein kinase C (PKC) and Src tyrosine kinase. RACK1 binds and stabilizes the active conformation of PKC, and is both a substrate and inhibitor of Src. Other known binding partners of RACK1 include transmembrane receptors, receptor tyrosine kinases, ion channels, ribosomes, and integrins. Because RACK1 interacts with a wide variety of signaling molecules, it can regulate many functions within cells. These include apoptosis, cell migration, circadian rhythms, and development (Adams et al., 2011). More recent studies have shown that RACK1 is also involved in feeding behaviors (Fang et al., 2015) and memory maintenance (Liu et al., 2016). Alterations in RACK1 expression and signaling are implicated in various diseases including neural tube defects, cardiac dysfunction, renal failure, cancer, addiction, muscle atrophy, and faulty sperm development (Adams et al., 2011). In addition, changes in RACK1 expression levels have been observed in cases of bipolar disorder (Wang and Friedman, 2001), Down syndrome (Peyrl et al., 2002), Alzheimer's disease (Battaini et al., 1999; Battaini and Pascale, 2005), epilepsy (Xu et al., 2015), and addiction (McGough, 2004). Furthermore, changes in RACK1 localization have been observed in amyotrophic lateral sclerosis (ALS) patients (Russo et al., 2017) and there is increased interaction between huntingtin protein and RACK1 in a mouse model of Huntington's disease (Culver et al., 2012). Though, how RACK1 contributes to these conditions is still unclear.

Little is also known about the role of RACK1 in nervous system development. However, RACK1 is crucial for development, as mice lacking RACK1 are embryonic lethal (Volta et al., 2013) and RACK1 interacts with PTK7 to control neural tube closure (Wehner et al., 2011). RACK1 is also a member of messenger ribonucleoprotein complexes, which are involved in the transport and translational regulation of mRNAs. Specifically, RACK1 forms a complex with the mRNA binding protein, zipcode binding protein 1 (ZBP1), and β -actin mRNA. Within axonal growth cones, ZBP1 and RACK1 both regulate the local translation of β -actin mRNA following brain derived neurotrophic factor (BDNF) stimulation (Figure 1, Ceci et al., 2012). Local translation of β -actin mRNA is necessary for appropriate axon guidance (Yao et al., 2006). Thus, RACK1 may also regulate neural development via this mechanism (Ceci et al., 2012), as well as through its many other signaling functions (Adams et al., 2011).

RACK1 Regulates Axon Growth and Guidance

There is still much to be discovered about the role of RACK1 in neural development, but three studies have begun to elucidate how RACK1 regulates axon growth and guidance (Demarco and Lundquist, 2010; Dwane et al., 2014; Kershner and Welshhans, 2017). The first study to examine the role of RACK1 in axon guidance demonstrated that RACK-1, the *C. elegans* counterpart of mammalian RACK1, is required for axon pathfinding (Demarco and Lundquist, 2010). Another study in rat PC12 cells showed that RACK1 regulates neurite outgrowth via its ability to scaffold ANK repeat and PH domain-containing protein 2 (AGAP2) to focal adhesion kinase (FAK). When the RACK1 and FAK interaction is disrupted, or when AGAP2 expression is disrupted, neurite outgrowth

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is reduced (Dwane et al., 2014). Recently, we demonstrated that RACK1 is required in primary mammalian neurons for axon outgrowth and growth cone spreading (Kershner and Welshhans, 2017).

Demarco and Lundquist (2010) provided the first indication that RACK1 is involved in axon guidance. In this study, they investigated potential binding partners of UNC-115, which is homologous to the vertebrate actin binding LIM protein (abLIM) and a known regulator of lamellipodia and filopodia formation in *C. elegans* growth cones. A yeast two-hybrid screen revealed that UNC-115 binds to RACK-1, and subsequent co-immunoprecipitation experiments substantiated this result. Because RACK-1 interacts with Src, PKC, and UNC-115, all of which regulate axon pathfinding, it was hypothesized that RACK-1 would also regulate axon pathfinding.

The nervous system of *C. elegans* is well-characterized; thus, any defects in axon pathfinding are easily detected. Demarco and Lundquist (2010) used RNA interference (RNAi) to *rack-1*, and found that it caused defects in axon pathfinding of DD and VD motor neurons. These defects, which include aberrant axon guidance, branching, and premature termination, are very similar to those seen with *unc-115* perturbation. In addition to RNAi, an in-frame deletion of the *rack-1* locus, which eliminates part of the first, all of the second, and most of the third WD repeat (which contains the PKC binding site) was used. Similar to the RNAi experiments, the deletion allele also results in pathfinding defects of DD and VD motor neurons.

Finally, Demarco and Lundquist (2010) determined where RACK-1 is expressed by using a reporter transgene (RACK-1::GFP). RACK-1::GFP is present in most tissues, and is predominantly in the cytoplasm. Further, it is also expressed in the growth cones, axons, and cell bodies of VD commissural axons. As RACK-1 is expressed in most tissues, the next question was whether RACK-1 regulates axon pathfinding in a cell-autonomous manner. Expression of *rack-1* was driven in DD and VD neurons using the GABAergic neuron-specific *unc-25* promoter. Expression of this transgene significantly rescued lateral asymmetry and axon wandering defects in the *rack-1* deletion animals, confirming that RACK-1 cell-autonomously regulates axon pathfinding. The pathway by which RACK-1 regulates axon pathfinding was also investigated. RACK-1 acts downstream of CED-10/Rac and upstream of UNC-115 to regulate lamellipodia and filopodia formation as well as axon pathfinding. These results show that RACK-1 is an important regulator of *C. elegans* neural development.

A recent study investigated the role of RACK1 in a rat PC12 cell line, which can be differentiated into neuron-like cells (Dwane et al., 2014). Because RACK1 regulates cell protrusion via FAK in non-neuronal cells, it was suspected that a similar interaction may occur in neurons. Indeed, RACK1 coimmunoprecipitates with FAK in both rat hippocampal cells and PC12 cells. Next, PC12 cells were transfected with a plasmid containing RACK1-Y52F, a construct that prevents the interaction of FAK and RACK1. Cells expressing this

construct have significantly shorter neurites, suggesting the interaction between FAK and RACK1 is necessary for neurite outgrowth. Dwane et al. (2014) then investigated whether other RACK1 binding partners might be part of this pathway. Mass spectrometry of RACK1 immunoprecipitates from rat hippocampus identified AGAP2 as a RACK1 interacting protein. Interestingly, AGAP2 is a known regulator of FAK activity and focal adhesion assembly. Further experiments demonstrated that following RACK1 knockdown, the interaction of AGAP2 to FAK is decreased. AGAP2 knockdown was also shown to cause a 3-fold decrease in neurite length. Thus, RACK1, at least in part through its interactions with FAK and AGAP2, is a regulator of neurite extension in PC12 cells (Dwane et al., 2014).

We recently showed that RACK1 directly regulates axon growth of primary mouse cortical neurons. First, we performed a knockdown of RACK1 using shRNA (Kershner and Welshhans, 2017). Knockdown of RACK1 results in significantly shorter axons versus non-silencing control shRNA. Further, knockdown of RACK1 eliminates a BDNF-induced increase in growth cone area, suggesting that RACK1 plays an important role in the regulation of growth cone morphology. Next, we overexpressed wild-type RACK1. Interestingly, RACK1 overexpression also results in significantly shorter axons *versus* control conditions, suggesting that appropriate levels of RACK1 expression are necessary for axon outgrowth. Overexpression of RACK1 results in aberrant growth cone morphology and spreading as well. Under basal conditions, the growth cone area of RACK1 overexpressing neurons is significantly higher than control. Moreover, BDNF does not result in an increase in growth cone area. Thus, it appears that overexpression of RACK1 creates a ceiling effect whereby growth cones no longer respond to BDNF. In summary, we find that RACK1 is essential to appropriate axon length and growth cone morphology under both basal and BDNF-stimulated conditions.

RACK1 Regulates Local Translation and Point Contacts in Growth Cones

Taken together, these studies show a clear role for RACK1 in the regulation of neural development. RACK1 is a multifunctional scaffolding protein, and therefore could regulate neural development through multiple pathways. We focused our studies on the translational aspect of RACK1. RACK1 binds directly to ribosomes and is a member of the local translation complex (Ceci et al., 2012), thus it is highly likely that RACK1 mediated local translation regulates neural development. We further investigated the role of RACK1 in local translation, specifically examining where in the growth cone local translation occurs.

Multiple studies have demonstrated that point contacts, structures which adhere growth cones to the extracellular matrix, contribute to guidance cue mediated axon pathfinding (Short et al., 2016). Point contacts are somewhat similar to focal adhesions seen in other cell types, and are composed of adhesion proteins that link the extracellular matrix to the actin cytoskeleton. Within growth cones, retrograde flow is a

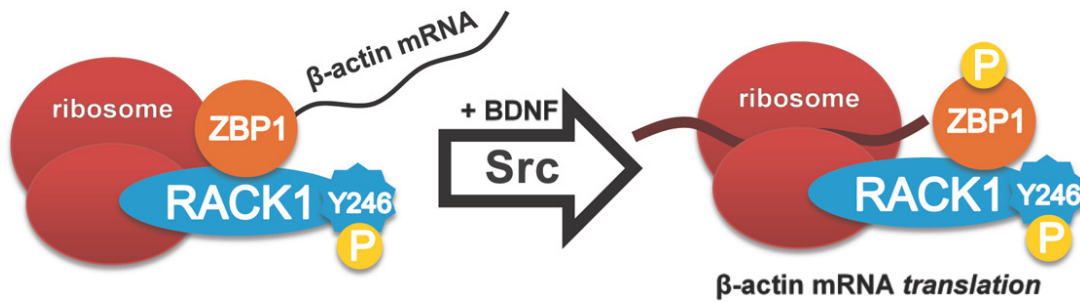


Figure 1 Model for the probable mechanism by which RACK1 facilitates local translation after BDNF induced phosphorylation by Src kinase. RACK1 is responsible for the recruitment of ZBP1 and β -actin mRNA to the ribosome. Following stimulation with BDNF, Src kinase is activated, resulting in the phosphorylation of ZBP1, subsequent release of β -actin mRNA and its local translation. BDNF: Brain derived neurotrophic factor; RACK1: receptor for activated C kinase 1; ZBP1: zipcode binding protein 1.

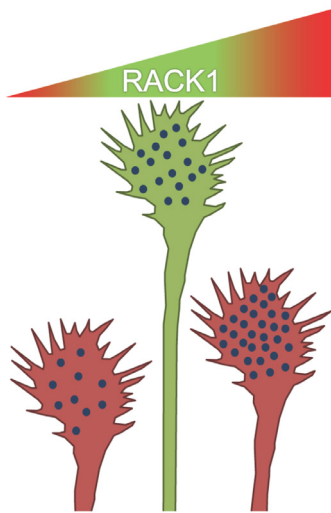


Figure 2 Appropriate levels of RACK1 are required for optimal point contact density and axon outgrowth.

Schematic figure showing the relationship between levels of RACK1, point contact density and axon length under basal conditions. Knockdown of RACK1 with shRNA results in lower point contact density and shorter axons (left growth cone), while overexpression of RACK1 results in higher point contact density and shorter axons (right growth cone). This suggests that there is an optimal level of point contacts that leads to maximal axon outgrowth (middle growth cone), and either an increase or decrease in point contact density from this optimal level results in decreased axon length. In this model, blue circles represent point contacts. RACK1: Receptor for activated C kinase 1.

facet of actin treadmilling whereby actin filaments flow from the distal (barbed) end back towards the proximal (pointed) end. “Clutching” of retrograde flow via focal adhesions in non-neuronal cells provides the force necessary for membrane protrusion and extension. A recent study has demonstrated that this “clutching” is accomplished in growth cones directly by point contact regulation of retrograde flow (Nichol et al., 2016). Thus, point contacts are essential to guidance cue mediated axon outgrowth and pathfinding, and appropriate neural development.

Because point contacts respond dynamically to guidance cues to regulate growth cone protrusion (Myers and Gomez, 2011; Nichol et al., 2016) and thus would be an optimal site for local translation, we investigated whether local translation occurs at point contacts. To address this question, we first examined whether RACK1, which is known to directly bind ribosomes, localizes at point contacts in growth cones (Kershner and Welshhans, 2017). We stained for two markers of point contacts, paxillin and vinculin, and are the first to show that RACK1 localizes at point contacts. However, this does not necessarily indicate that local translation occurs

at point contacts. Therefore, we stained for other members of the local translation complex, ribosomes and β -actin mRNA, and found that both of them reside at point contacts. A previous study demonstrated that BDNF stimulation results in an increase in the local translation of β -actin in growth cones (Yao et al., 2006). In our most recent study, we demonstrate that BDNF stimulation significantly increases the localization of RACK1 and β -actin mRNA at point contacts (Kershner and Welshhans, 2017). This provides additional support that point contacts are a site of local translation. Thus, point contacts act not only as adherence sites, but also as signaling centers for local translation within developing neurons.

Next, we examined how RACK1 expression levels affect point contact formation (Kershner and Welshhans, 2017). We found, in line with previous findings (Myers and Gomez, 2011), that BDNF results in an increase in point contact density in growth cones (Kershner and Welshhans, 2017). We also found that knockdown of RACK1 significantly decreases point contact density in growth cones under basal conditions and eliminates the BDNF-induced increase in paxillin-containing point contacts. Further, overexpression

of RACK1 causes a “ceiling effect” whereby point contact density is significantly increased under basal conditions and there is no additional increase following BDNF stimulation. Therefore, we conclude that appropriate RACK1 expression levels are required for point contact formation and, as a result, axon outgrowth (Figure 2). It is possible that separate pathways regulate point contact formation and axon outgrowth; however, the retrograde flow experiments by Nichol et al. (2016) demonstrate that point contact formation and growth cone protrusion are linked. Thus, we hypothesize it is the same pathway, and future experiments will help elucidate this mechanism.

Conclusions and Future Directions

From these studies, it is clear that RACK1 is vital for neurodevelopment given its regulation of axon growth and guidance, point contacts and local translation. RACK1 can regulate both point contact formation and local translation because it is a multi-functional ribosomal scaffolding protein. Thus, it can bind to components of the extracellular matrix, signaling molecules, RNA binding proteins, and ribosomes. Future studies in live cells using total internal reflection fluorescence (TIRF) microscopy, which permits visualization of proteins only at the membrane-substrate interface, will allow us to determine how RACK1 regulates aspects of point contact dynamics, such as formation, lifetime, and turnover. Further, the use of photoconvertible translation reporters in live cells will conclusively reveal whether local translation of β -actin mRNA occurs at point contacts. Increasing our understanding of this topic will not only provide foundational knowledge of the mechanism by which RACK1 regulates neural development, but could potentially reveal how disrupted RACK1 signaling may contribute to neurodevelopmental deficits. Thus, targeting RACK1 signaling may be a promising treatment for neurodevelopmental disorders and a therapeutic approach to axonal regeneration.

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References

Adams DR, Ron D, Kiely PA (2011) RACK1, A multifaceted scaffolding protein: Structure and function. *Cell Commun Signal* 9:22.

- Battaini F, Pascale A (2005) Protein kinase C signal transduction regulation in physiological and pathological aging. *Ann N Y Acad Sci* 1057:177-192.
- Battaini F, Pascale A, Lucchi L, Pasinetti GM, Govoni S (1999) Protein kinase C anchoring deficit in postmortem brains of Alzheimer's disease patients. *Exp Neurol* 164:559-564.
- Ceci M, Welshhans K, Ciotti MT, Brandi R, Parisi C, Paoletti F, Pistillo L, Bassell GJ, Cattaneo A (2012) RACK1 is a ribosome scaffold protein for β -actin mRNA/ZBP1 complex. *PLoS One* 7:e35034.
- Culver BP, Savas JN, Park SK, Choi JH, Zheng S, Zeitlin SO, Yates JR, Tanese N (2012) Proteomic analysis of wild-type and mutant huntingtin-associated proteins in mouse brains identifies unique interactions and involvement in protein synthesis. *J Biol Chem* 287:21599-21614.
- Demarco RS, Lundquist EA (2010) RACK-1 acts with Rac GTPase signaling and UNC-115/abLIM in *Caenorhabditis elegans* axon pathfinding and cell migration. *PLoS Genet* 6:e1001215.
- Dwane S, Durack E, O'Connor R, Kiely PA (2014) RACK1 promotes neurite outgrowth by scaffolding AGAP2 to FAK. *Cell Signal* 26:9-18.
- Fang L, Zhou J, Cheng S, Ying J, Yang Z, Yin L, Li S, Hou W, Wang Z (2015) High orexin-A neuron activity and RACK1 expression might be involved in the restricted feeding-entrained behaviors in mice. *Biol Rhythm Res* :1-11.
- Kershner L, Welshhans K (2017) RACK1 is necessary for the formation of point contacts and regulates axon growth. *Dev Neurobiol* doi:10.1002/dneu.22491.
- Liu L, Zhu J, Zhou L, Wan L (2016) RACK1 promotes maintenance of morphine-associated memory via activation of an ERK-CREB dependent pathway in hippocampus. *Sci Rep* 6:20183.
- McGough NN, He DY, Logrip ML, Jeanblanc J, Phamluong K, Kharazia V, Janak PH, Ron D (2004) RACK1 and brain-derived neurotrophic factor: a homeostatic pathway that regulates alcohol addiction. *J Neurosci* 24:10542-10552.
- Myers JP, Gomez TM (2011) Focal adhesion kinase promotes integrin adhesion dynamics necessary for chemotropic turning of nerve growth cones. *J Neurosci* 31:13585-13595.
- Nichol IV RH, Hagen KM, Lumbard DC, Dent EW, Go TM, Nichol RH, Hagen KM, Lumbard DC, Dent EW, Gomez TM (2016) Guidance of axons by local coupling of retrograde flow to point contact adhesions. *J Neurosci* 36:2267-2282.
- Peyrl A, Weitzdoerfer R, Gulesserian T, Fountoulakis M, Lubec G (2002) Aberrant expression of signaling-related proteins 14-3-3 gamma and RACK1 in fetal Down syndrome brain (trisomy 21). *Electrophoresis* 23:152-157.
- Russo A, Scardigli R, La Regina F, Murray ME, Romano N, Dickson DW, Wolozin B, Cattaneo A, Ceci M (2017) Increased cytoplasmic TDP-43 reduces global protein synthesis by interacting with RACK1 on polyribosomes. *Hum Mol Genet* 26:1407-1418.
- Short CA, Suarez-Zayas EA, Gomez TM (2016) Cell adhesion and invasion mechanisms that guide developing axons. *Curr Opin Neurobiol* 39:77-85.
- Volta V, Beugnet A, Gallo S, Magri L, Brina D, Pesce E, Calamita P, Sanvito F, Biffo S (2013) RACK1 depletion in a mouse model causes lethality, pigmentation deficits and reduction in protein synthesis efficiency. *Cell Mol Life Sci* 70:1439-1450.
- Wang HY, Friedman E (2001) Increased association of brain protein kinase C with the receptor for activated C kinase-1 (RACK1) in bipolar affective disorder. *Biol Psychiatry* 50:364-370.
- Wehner B, Shnitsar I, Urlaub H, Borchers A (2011) RACK1 is a novel interaction partner of PTK7 that is required for neural tube closure. *Development* 138:1321-1327.
- Xu X, Yang X, Xiong Y, Gu J, He C, Hu Y, Xiao F, Chen G, Wang X (2015) Increased expression of receptor for activated C kinase 1 in temporal lobe epilepsy. *J Neurochem* 133:134-143.
- Yao J, Sasaki Y, Wen Z, Bassell GJ, Zheng JQ (2006) An essential role for β -actin mRNA localization and translation in Ca^{2+} -dependent growth cone guidance. *Nat Neurosci* 9:1265-1273.