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IQGAP1: A Regulator of Intracellular Spacetime Relativity

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Activating and inhibiting receptors of lymphocytes collect valuable information about their mikros kosmos. This information is essential to initiate or to turn off complex signaling pathways. Irrespective of these advances, our knowledge on how these intracellular activation cascades are coordinated in a spatiotemporal manner is far from complete. Among multiple explanations, the scaffolding proteins have emerged as a critical piece of this evolutionary tangram. Among many, IQGAP1 is one of the essential scaffolding proteins that coordinate multiple signaling pathways. IQGAP1 possesses multiple protein interaction motifs to achieve its scaffolding functions. Using these domains, IQGAP1 has been shown to regulate a number of essential cellular events. This includes actin polymerization, tubulin microrization, microtubule organizing center formation, calcium/calmodulin signaling, Pak/Raf/Mek1/2-mediated Erk1/2 activation, formation of maestrosome, E-cadherin, and CD44-mediated signaling and glyogen synthase kinase-3/adenomatous polyposis coli-mediated β-catenin activation. In this review, we summarize the recent developments and exciting new findings of cellular functions of IQGAP1. The Journal of Immunology, 2012, 188: 2057–2063.

Incessant communications of immune cells with their environment govern their development, trafficking, recognition of target Ags/cells, phenotypic conversion from effector to memory, or apoptotic death. Recent studies indicate the bygone era of linear signaling pathways and a paradigm shift to complex network circuits. Integration of “spacetime relativity” is obligatory to execute intended biological functions in lymphocytes. Multiple mechanisms have been described that facilitate the spatiotemporal coordination of signaling events in lymphocytes (1). Among these, scaffolding proteins play an important role. Scaffolding proteins are defined as proteins that can recruit, coordinate, and facilitate the spatiotemporal organization and the sequential activation of signaling molecules to achieve optimal functional outcomes. Cytoplasmic scaffolding proteins such as IQGAP, Carma1, KSR1, Ste5, MP1 Paxillin, or PSD-95 can function as processing centers of kinases and their substrates. IQGAP1 is one of the most evolutionarily conserved (>90%) scaffolding proteins and is present in a variety of organisms. There are three isoforms of IQGAPs (1, 2, and 3) that are described in human and mouse. Among these, IQGAP1 is ubiquitously found (2), whereas the expressions of IQGAP2 (liver, platelets, kidney, stomach, prostate, thyroid, and salivary glands) (3–6) and IQGAP3 (brain, lung, testis, small intestine, and colon) (3, 7) are restricted. Among lymphocytes, NK (8), B, and T cells (S. Malarkannan, unpublished observations) predominantly express IQGAP1. This review abridges the recent exciting information on IQGAP1 and its cellular functions (8).

IQGAP1 is a 190-kDa protein, and its functional domains indicate that it is a critical regulator of development and functions in multiple cell types. It was identified in 1994 as a widely expressed IQ domain-containing protein (9). This 1657-aa long scaffolding protein has been described to associate with >50 different protein partners (10). Knockout mouse for IQGAP1 has been generated, where the null mutants bred at normal frequencies and no other major physiologic defects could be identified except a significant onset of gastric hyperplasia later in their life (11). A possible explanation for these observations could be the presence of IQGAP2 and IQGAP3 isoforms. Multiple cell surface receptors have been described to directly recruit IQGAP1 through their cytoplasmic tails, including cadherins. The E- and N-cadherins recruit IQGAP1 to maintain their trans interactions in the tight and adherens junctions (12). E-cadherins are primarily expressed on epithelial cells but their ability to interact with receptors such as KLRG1 (13–18) that has been shown to play a critical role in the homeostasis of NK cells (19) further emphasize the potential roles of IQGAP1 in the immune system. Recent studies show that E-cadherin also interacts with integrin CD103 (20–22) that is...
expressed in specific T cell subsets (20, 23, 24) and CD11c-
high dendritic cells (25). These studies provide a functional
framework of how E-cadherin on mucosal epithelia or thymus
can influence the maturation and migration of CD103+ T
cells and dendritic cells. Additional future works are re-
quired to precisely determine the role of IQGAP1 in these
cell–cell interactions. In a recent study, IQGAP1 has been
shown to be part of a large cytoplasmic complex that con-
tained phosphorylated NFAT1, long intergenic noncoding
RNA, noncoding (RNA) repressor of NFAT (NRON), and
three of the kinases that are responsible for NFAT1 phos-
phorylation (26). This study further showed that the IQGAP1
preferentially interacted with phosphorylated NFAT1. Lack
of IQGAP1 increased the dephosphorylation of NFAT1, its
entry into the nucleus resulting in an augmented production
of IL-2 or IFN-γ from T cells after mitogenic activation (26).
These results, while defining the potential scaffold functions
of intergenic noncoding RNA, also emphasize the novel func-
tions mediated by IQGAP1 in immune cells.

IQGAP1 is composed of multiple protein recognition motifs
(Fig. 1A). As the name denotes, a unique domain in IQGAP1
contains sequence homology to the Ras GAP-activating
proteins (Ras-GAP). However, this domain lacks GAP activ-
ity and thereby is unable to regulate Ras-GTP or Rap-GTP
hydrolysis. The N-terminal calponin homology (CH) domain
of IQGAP1 binds to actin (27, 28), whereas the IQ domain
recruits calmodulin (29). The WW domain, with two highly
conserved tryptophans, is an interaction module for proline-
rich ligands (30) and binds to Erk1/2 (31). The functional role
of the coiled–coil domain is not known; however, it has a
presumptive α-helical domain with significant sequence ho-
mology to myosins.

The IQ domain is a tandem repeat of four IQ motifs
that mediates interactions with MEK1/2, myosin essential
light chains (9), S100B (a Zn2+- and Ca2+-binding protein)
(32), calmodulin (27, 29, 33), and calmodulin-related pro-
teins (Fig. 1A) (34). Pathmanathan et al. (35) demonstrated
that the first IQ domain recruited myosin essential L chain
Mlc1sa, whereas the first and the fourth interacted with my-
osome L chain, Mlcp1, from yeast. The first and second IQ
domains were responsible for interacting with S100B. The
C-terminal end of IQGAP1 engages with Cdc42-GTP (29),
Rac1-GTP (33), β-catenin (12), β-catenin (36), and ade-
nomatus polyposis coli (37). IQGAP1 also has the ability to
bind to Rap1a, B-Raf or C-Raf (39), Mek1/2 (40), and
Erk1/2 (31). Ras-GAP domain (GRD) interacts with small
GTPases such as Cdc42 (29, 33), Rac1 (33), and TC10 (41).
This GAP domain lacks the ability to hydrolyze the bound
GTP. Crystal structure of this region indicates that the
GRD domain of IQGAP family possesses a conserved three-
onine instead of the catalytic “arginine finger” described in
functional Ras GAPs that is obligatory for GAP hydrolysis
(42). The Ras-GAP C terminus domain interacts with the
microtubule-binding protein Clp170 (43), β-catenin (44), E-
cadherin (12), adenomatous polyposis coli (37). Other pro-
teins such as AKAP79 have been shown to regulate calcium
flux via PKA by directly binding to a C-
terminal domain of IQGAP1 (45, 46). Thus, IQGAP1 can
critically regulate cell polarization, transcription, actin and
microtubule function, MAPK cascade, and Ca2+/calmodulin
signaling (47, 48). Although these studies demonstrate the

![FIGURE 1. Protein structure and interacting partners of IQGAP1. (A) IQGAP1 is a 190-kDa protein that contains at least six distinct protein-
interacting domains. The calponin homology (CH) domain binds to poly-
merized F-actin. The function or the interacting partners of the coiled-coil
(CC) domain has yet to be defined. The two highly conserved tryptophan-
containing (WW) domains recruit Erk1/2. Isoleucine/glutamine-containing
(IQ) domain is a binding domain for multiple proteins including Rap1a, Rap1b, Mek1, Mek2, myosin ELC, Rafs, S100B, and Ca2+-independent
interaction of calmodulin and its related proteins. Ras-GAP domain (GRD)
interacts with small GTPases Cdc42 and Rac1. Ras-GAP C terminus domain
(GRCT) interacts with the microtubule-binding protein Clp170, β-catenin,
E-cadherin, Clasp2, and adenomatous polyposis coli. (B) The CH domain
of IQGAP1 interacts with polymerized F-actin. Furthermore, IQGAP1 can
delay the hydrolysis of Cdc42 and stabilize its interaction with WASp/Arp2/3
complex. IQGAP1 also plays important roles in linking actin meshwork with
plus-ends of microtubules through Clasp2. The role of IQGAP1 in the for-
mation and function of MTOC is not well understood. Both Clp-170 and
APC may facilitate the organization and multimerization of γ-tubulin
molecules that form the core of the centrosome and MTOC. Confocal images of
NK cells are shown. Original magnification ×100.](http://www.jimmunol.org/DownloadedFrom)
interacts with F-actin in a way that is critical to regulate the polymerization of actin (28, 52). It has been demonstrated that the purified IQGAP1 can directly bind to F-actin and cross-link the actin filaments into irregular, interconnected bundles that exhibit gel-like properties (85). In addition, IQGAP1 can also interact with various proteins that are involved in cytoskeletal reorganization (43, 52, 69). This includes Cdc42 and Rac1 (33, 49, 85), adenomatous polyposis coli (86), CLIP-170 (87), Clasp2 (88), and EB1 (89). Adenomatous polyposis coli that has been well characterized to regulate the polarized cell migration can also directly interact with IQGAP1 (37).

Cdc42 and Rac1 belong to the Rho family of small guanosine-3′,5′-diphosphates and play a significant role in regulating the cellular cytoskeleton (43, 90). In recent years, the requirement of IQGAP1 in Cdc42 and Rac1-mediated actin polymerization has been well established. Indeed, the GRD domain of IQGAP1 directly recruits small GTPases such as Cdc42 (29, 33), Rac1 (33), and TCTOP (41). Importantly, Cdc42-GTP and Rac1-GTP but not Rhod (6, 85) or Ras (33) have been shown to interact with the GRD domains of IQGAP1 and IQGAP2. Thus, it appears that the activated Cdc42-GTP will function as a linker between the WASp/Arp2/3 complex and IQGAP1/APC/Clp170/Clasp2 complexes. The ability of the N-terminal region of IQGAP1 (1–216 aa) to directly interact with F-actin (49) brings additional questions on the precise functional role played by IQGAP1. Is IQGAP1 critical for forming the actin meshwork using polymerized actin filaments? The ability of IQGAP1 to cross-link actin was augmented by guanosine 5′-O-(3-O-thio)triphosphate (GTPγS)-GST-Cdc42 but not by GDP·GST-Cdc42 (91). This augmentation occurred by a preferential oligomerization of IQGAP1 by GTPγS·GST-Cdc42. These findings reveal that the oligomerization of IQGAP1 is a crucial step mediated by Cdc42 in regulating the cross-linking of filamentous β or γ actin (49).

Another quantitative colocalization studies have shown that IQGAP1 also plays a significant role in the colocalization of N-WASP in close proximity to Arp2/3 complex in lamellipodial structures (84). In addition, communoprecipitation, pull-down and kinetic assays demonstrate that the C-terminal half of IQGAP1 activated N-WASP by interacting with its BR-CRIB domain similar to that of Cdc42, while the N-terminal half of IQGAP1 antagonizes this activation by association with a C-terminal region of IQGAP1 through intramolecular interactions (84). Thus, a structural change in the IQGAP1 protein can function as an autoregulatory switch that when turned “on” can activate N-WASP resulting in the stimulation of actin assembly in an Arp2/3-dependent manner. The shape and morphology of dendritic arbors of neurons depend on the plus-end tracking protein Clip-170 and IQGAP1 (81). This study demonstrates that a direct interaction of mTOR kinase with Clip-170 is required for the formation of the Clip-170/IQGAP1 complex. This complex is capable of regulating the actin/tubulin cytoskeletons in primary hippocampal and cortical neurons. Thus, IQGAP1 can function as a focal point for feedback interactions between the actin and microtubule cytoskeletal systems. One of the first evidences that IQGAP1 could play a major role in the reorganization of cytoskeleton came from Kaibuchi’s laboratory (43). This study showed that the activated Rac1/Cdc42, IQGAP1, and Clip-170 form a tripartite complex. Although these studies provide a mechanistic explanation of how IQGAP1 plays a critical role in the regulation of tubulin polymerization and microtubule remodeling, its unique role on microtubule organizing center (MTOC) is not well understood. Evidence on the role of IQGAP1 on MTOC reorganization came from studies by Watanabe et al. (37). Recent studies from the Malarkannan laboratory demonstrate that the size and shape of MTOC could be regulated by IQGAP1 via the small GTPase, Rap1b (8). Rap1b has been shown to directly interact with IQGAP1 (38). Lack of Rap1b did not affect the formation of the MTOC. However, the size and the length of MTOCs were proportionately much larger in NK cells that lacked Rap1b. Lack of Rap1b results in reduced ERK1/2 phosphorylation primarily because of an impairment in the sequential phosphorylation of B-Raf/C-Raf→MEK1/2→ERK1/2 signaling pathway that requires the presence of the IQGAP1 scaffold (8). Other studies by Kanwar et al. (92) demonstrate that the knockdown of IQGAP1 in the NK cell line YTS resulted in the inability of MTOC to reorient, whereas the ability of YTS cells to form conjugates with target cells was preserved. Lack of IQGAP1 did not grossly affect the development of T cells in the thymus. However, in the absence of IQGAP1, T cells fail to accumulate F-actin or polarize their MTOC toward anti-CD3-coated beads (93).

IQGAP1 provides the scaffold for the sequential phosphorylation of Pak→Raf→Mek→Erk1/2

Activation and phosphorylation of Erk1/2 constitute a key signaling event in all lymphocyte subsets. Erk1/2 phosphorylation regulates development and effector functions of T, B, and NK cells (8, 94, 95). MAPKs, in particular Erk1/2, have an important role in regulating the generation of IFN-γ, GM-CSF, MIP-1α, MIP-1β, and RANTES and cytotoxicity in NK cells (8, 96). MAPKs have also been shown to play a central role in the cytotoxic granule exocytosis from NK cells (97, 98). Therefore, their phosphorylation and subsequent subcellular compartmentalization has to be tightly regulated to achieve intended outcomes. Multiple scaffolding proteins including IQGAP1 (31, 99), KSR1 (100, 101), MP1 (102, 103), and PBD46 motif of Pak with very high affinity (109). Studies between the first α-helix and switch I region to bind the PBD46 motif of Pak with very high affinity (109). Studies have also indicated that Cdc42 exhibits differential binding
changes and phosphorylation of Ser338 (Raf-1) or Ser445 (B-  
quires the direct binding of Pak1 for inducing conformational  
quential activation on the IQGAP1 scaffold is Raf, which re-  
a synchronized fashion (112). The next substrate in the se-  
ning of IQGAP1 (and WASp). Synthetic peptide analogs from  
region (aa 84–120 and 157–191) were required for the bind-  
ning to IQGAP1. This demonstrates IQGAP1, Pak, and  
insert region" (111) and a part of the switch I domain (112)  
it has been shown that the activated Cdc42-GTP used the  
sequence in which small GTPases mitigate their autoinhibition  
H-Ras (116) or another Ras family member, Rap1b (8), can  
with its catalytic domain (115, 116). However, upon activation,  
Raf contain an N-terminal autoinhibitory domain that interacts  
with its catalytic domain (115, 116). However, upon activation,  
Raf-1 and B-  
require the direct binding of Pak1 for inducing conformational  
changes and phosphorylation of Ser338 (Raf-1) or Ser445 (B-  
iforms large macromolecular scaffolding structures. We name this IQGAP1-  
velop additional cellular paradigms. Recently, Awasthi et al.  
(8) have demonstrated that IQGAP1 can form a unique sig-  
nalosome in the perinuclear region of NK cells to coordinate  
the phosphorylation of Erk1/2 (Fig. 3). A significant quantity  
of phospho-ERK1/2 was colocalized with IQGAP1 in these  
averted NK cells. This novel IQGAP1-mediated signalosome  
forced abundant but transient phosphorylation of  
Erk1/2 and thereby the effector functions of NK cells. In  
nonstimulated NK cells, phospho-Erk1/2 was not detectable,  
and the IQGAP1 was distributed throughout the cytoplasm  
with slight accumulation around the perinuclear region. After  
15 min of activation, Erk1/2 phosphorylation was evident in  ​
NK cells, and after 30 min of activation, the distribution  
pattern of an IQGAP1 was drastically altered with a strong  
accumulation around the nucleus in NK cells.  
Both nonstimulated and NKG2D-activated NK cells con-  
tained comparable levels of total ERK1/2 proteins (8). It is also  
important to note that IQGAP1 can oligomerize to form  
macromolecular structures (118). This study demonstrates that  
IQGAP1 exists as a combination of monomers, dimers, and  
patterns to Pak1, WASp, and IQGAP1 (110). Independently,  
it has been shown that the activated Cdc42-GTP used the  
"insert region" (111) and a part of the switch I domain (112)  
to interact with IQGAP1.  
Specifically, the switch I domain (aa 29–55) served as the  
binding site for Pak1, whereas the determinants outside this  
region (aa 84–120 and 157–191) were required for the bind-  
ing of IQGAP1 (and WASp). Synthetic peptide analogs from  
the PBD46 motif of Pak partly prevented the ability of Cdc42  
binding to IQGAP1. This demonstrates IQGAP1, Pak, and  
WASP may form complexes that may interact with Cdc42 in  
a synchronized fashion (112). The next substrate in the se-  
quential activation on the IQGAP1 scaffold is Raf, which re-  
quires the direct binding of Pak1 to IQGAP1 has been described, it  
can bind to Rafs, and it is critical for the conformational change and activation of  
B- or C- Rafs. Rafs are recruited to the IQ domains of the IQGAP1. Activation of  
Rafs results in the recruitment and phosphorylation of Mek1/2 to the IQ  
domains. Activated Mek1/2 is essential for the recruitment and phosphorylation  
of Erk1/2 that have the ability to bind to the WW domains of the IQGAP1.  

FIGURE 2. IQGAP1 scaffold regulates Erk1/2 phosphorylation. Sequential activation of Cdc42-Pak1-B/C-Raf-Mek1/2-Erk1/2 occurs on the IQGAP1 scaffold. The GRD domain, the four IQ repeats, and the WW domain of the IQGAP1 are involved in the recruitment and phosphorylation of Cdc42, Rafs, and Mek1/2 and Erk1/2, respectively. Membrane proximal signaling from activation receptors activate the small GTPase Cdc42 that is recruited to the GRD domain of IQGAP1. This in turn results in the recruitment and activation of Pak1. Although no direct binding of Pak1 to IQGAP1 has been described, it can bind to Rafs, and it is critical for the conformational change and activation of  
B- or C- Rafs. Rafs are recruited to the IQ domains of the IQGAP1. Activation of  
Rafs results in the recruitment and phosphorylation of Mek1/2 to the IQ  
domains. Activated Mek1/2 is essential for the recruitment and phosphorylation  
of Erk1/2 that have the ability to bind to the WW domains of the IQGAP1.  

IQGAP1 forms a master signalosome  
Mechanistic insights of how IQGAP1 functions as a scaffold- 
folding protein during Erk1/2 activation or β-catenin–mediated  
gene transcriptions are fundamental in understanding  
signaling processes in lymphocytes. More importantly, the  
transient spatiotemporal organization of the signaling events  
by IQGAP1 will provide novel insights and will help to de-  
volve additional cellular paradigms. Recently, Awasthi et al.  
(8) have demonstrated that IQGAP1 can form a unique sig- 
inalsome in the perinuclear region of NK cells to coordinate  
the phosphorylation of Erk1/2 (Fig. 3). A significant quantity  
of phospho-ERK1/2 was colocalized with IQGAP1 in these  
averted NK cells. This novel IQGAP1-mediated signalosome  
forced abundant but transient phosphorylation of  
Erk1/2 and thereby the effector functions of NK cells. In  
nonstimulated NK cells, phospho-Erk1/2 was not detectable,  
and the IQGAP1 was distributed throughout the cytoplasm  
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NK cells, and after 30 min of activation, the distribution  
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accumulation around the nucleus in NK cells.  
Both nonstimulated and NKG2D-activated NK cells con-  
tained comparable levels of total ERK1/2 proteins (8). It is also  
important to note that IQGAP1 can oligomerize to form  
macromolecular structures (118). This study demonstrates that  
IQGAP1 exists as a combination of monomers, dimers, and  

FIGURE 3. Role of IQGAP1 in maestrosome formation. (A) IQGAP1 can compartmentalize and coordinate the signaling processes of multiple activation  
cascades that include the MAPK and β-catenin/TCF/LEF pathways. Evidence indicates that during the activation of MAPK pathway, IQGAP1 forms large macromolecular scaffolding structures. We name this IQGAP1- 
based master signalosome “maestrosome” to denote its macromolecular size. (B) Activation of NK cells through NKG2D and the resulting Erk1/2  
phosphorylation provide proof-of-principle for the formation of a maestro-
some in the perinuclear region of NK cells.  
(Er2 is predominantly recruited and phosphorylated via this sequential activation (31).
larger oligomers. The self-association region was mapped to aa 763–863 of IQGAP1 that contains the four IQ domains. Because IQGAP1 can bind to B-Raf (39), Mek1/2 (40), and Erk1/2 (31), a structured master signalosome is indispensable for the generation of transient but optimal functions of kinases. Such an ordered regulation of kinases could be an essential and integral part of the IQGAP1-mediated master signalosome. Existence and function of such IQGAP1-based signalosomes must be further investigated in T and B cells.

Conclusions
Recent studies have highlighted the central regulatory functions played by the IQGAP1 scaffold. Multiple protein partners have been identified and described to interact with IQGAP1; however, the molecular relevance of many of these interactions has yet to be defined. In addition, the fact that the knockout mice for IQGAP1 have only exhibited modest defects in the immune and cellular functions warrant more careful and detailed future analyses. The functional relevance of many of these interactions in immune cells is still under study. Activation of Erk1/2 through the IQGAP1 scaffold has been well established in multiple cell types. These studies also provide a molecular model that can be used to further explore the spatiotemporal kinetics of signaling events in lymphocytes. IQGAP1-based signalosomes are exciting molecular structures. Irrespective of recent studies focusing on its formation and possible functions, much of the biochemical basis and broader functional relevance of these signalosomes remains unknown. The precise spatiotemporal organization and recruitment of distinct signaling molecules to the IQGAP1 scaffold must be investigated in further detail. Future studies can identify drug targets in the IQGAP1 protein itself or among the myriad of IQGAP-interacting proteins that can be used in tumor treatments. This is of particular significance because IQGAP1 has also been reported to be over expressed in transformed cells.

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References


102. Sharma, C., T. Vomastek, A. Tarcsafalvi, A. D. Catling, H. J. Schaeffer,
99. Sbroggio, M., D. Carnevale, A. Bertero, G. Cifelli, E. De Blasio, G. Mascio,
98. Li, C., B. Ge, M. Nicotra, J. N. Stern, H. D. Kopcow, X. Chen, and
95. Yankee, T. M., and E. A. Clark. 2000. Signaling through the B cell antigen re-
92. Kanwar, N., and J. A. Wilkins. 2011. IQGAP1 involvement in MTOC and
89. V SV, M. D. Houslay, and J. B. Hemmings. 2009. A novel role for IQGAP1 in MAP