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The actin cytoskeleton coordinates the signal transduction and antigen processing functions of the B cell antigen receptor

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Abstract

The B cell antigen receptor (BCR) is the sensor on the B cell surface that surveys foreign molecules (antigen) in our bodies and activates B cells to generate antibody responses upon encountering cognate antigen. The binding of antigen to the BCR induces signaling cascades in the cytoplasm, which provides the first signal for B cell activation. Subsequently, BCRs internalize and target bound antigen to endosomes, where antigen is processed into T cell recognizable forms. T helper cells generate the second activation signal upon binding to antigen presented by B cells. The optimal activation of B cells requires both signals, thereby depending on the coordination of BCR signaling and antigen transport functions. Antigen binding to the BCR also induces rapid remodeling of the cortical actin network of B cells. While being initiated and controlled by BCR signaling, recent studies reveal that this actin remodeling is critical for both the signaling and antigen processing functions of the BCR, indicating a role for actin in coordinating these two pathways. Here we will review previous and recent studies on actin reorganization during BCR activation and BCR-mediated antigen processing, and discuss how actin remodeling translates BCR signaling into rapid antigen uptake and processing while providing positive and negative feedback to BCR signaling.

Keywords

actin cytoskeleton; endocytosis; signal transduction; receptor

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Compliance with ethics guidelines

Chaohong Liu, Margaret K. Fallen, Heather Miller, Arpita Upadhyaya, and Wenxia Song declare that they have no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

1.Introduction

Receptors on the cell surface are sensors of external cues. In response to the binding of specific ligands, receptors transmit ligand-receptor interactions into cascades of biochemical reactions in the cytoplasm or induce the internalization of ligand-receptor complexes, which can lead to the activation of intracellular signaling and ligand transport to endosomes. The B cell antigen receptor (BCR), which is responsible for surveying foreign substances or antigen in the environment, is unique in that it serves both as a signal transducer and as an antigen transporter. Both these functions of the BCR are essential for the immune function of B cells: generating antibody responses and regulating other immune branches. Signaling cascades induced by the BCR can lead to transcription and translation of a set of genes, which prepare this clone of B cells for proliferation (Niiro and Clark, 2002; Dal Porto et al., 2004). The uptake of antigen by the BCR into endosomes allows the antigen to be processed (antigen processing) into a form that is recognizable by T helper (Th) cells (Siemasko and Clark, 2001; Vascotto et al., 2007). Signals generated from both BCR-induced signaling cascades and Th cells that bind antigen presented on B cells are essential for the activation and differentiation of B cells into high affinity antibody secreting cells and memory B cells. BCR-mediated endocytosis of antigen not only initiates antigen processing, but also serves to remove the receptor from and attenuate its signaling at the cell surface. Therefore, the coordination between these two cellular events determines the duration of BCR signaling at the cell surface and the kinetics of antigen processing and presentation by B cells. Recent studies have demonstrated that signaling and membrane trafficking of surface receptors are regulated by their spatiotemporal organization, and that the actin cytoskeleton regulates the surface dynamics and organization of receptors (Galletta et al., 2010; Firat-Karalar and Welch, 2011; Kusumi et al., 2012a). In this review, we will highlight and discuss previous and recent studies on how actin remodeling induced by signaling activation in turn coordinates further signaling and antigen processing functions of the BCR.

2. B cell antigen receptor and its functions

B cells express clonally specific BCRs that have diverse specificities. The BCR is composed of membrane immunoglobulin (mIg) as the ligand binding subunit and Igα/Igβ heterodimer as the signaling subunit. Membrane Ig of the BCR can be any of the five isotypes depending on the development and differentiation stage of B cells (Reth, 1992; Brezski and Monroe, 2008). The antigen binding region of mIg is generated through VDJ gene recombination, and is thereby highly variable, which enables B cells to bind antigen with all possible chemical or physical configurations (Oltz, 2001; Bassing et al., 2002). Each of the Igα/Igβ cytoplasmic tails contains an immunoreceptor tyrosine-based activation motif (ITAM), which mediates signaling activation in the cytoplasm. Recent studies reveal an additional tyrosine-based signaling motif in the cytoplasmic tail of mIgG, but not in that of mIgM and mIgD, which enhances the signal transduction of mIgG BCR (Engels et al., 2009; Liu et al., 2010b). The binding of cognate antigen to the BCR triggers three major events at the B cell surface: formation of signaling microdomains, actin remodeling, and endocytosis of BCR-antigen complexes. Signaling activation and actin remodeling occur within seconds of antigen binding to the BCR (Tolar et al., 2005; Treanor et al., 2011; Liu et al., 2012a). While actin remodeling continues throughout the activation process, BCR endocytosis takes place

minutes after the initiation of signaling activation (Song et al., 1995; Brown and Song, 2001). BCR-triggered signaling cascades can induce the transcription activation of costimulatory molecules and cell cycle molecules, providing the first signals for B cell activation. The endocytosis of BCR delivers bound antigen to the endocytic system where protein antigens are fragmented and loaded onto MHC class II molecules. Recognition of antigenic peptide-loaded MHC class II and engagement of costimulatory molecules on B cells activate Th cells. Activated Th cells in turn provide additional signals for B cell activation. Both the signal transduction and antigen processing and presentation pathways of the BCR have been extensively studied (Siemasko and Clark, 2001; Dal Porto et al., 2004; Vascotto et al., 2007; Kurosaki, 2011). However, how these two BCR-centered cellular processes are spatially and temporally coordinated to optimize B cell activation remains unclear.

3. Early events of BCR activation

The activation of the BCR is initiated by antigen-induced receptor self-clustering. In vivo, B cells encounter antigen in either soluble or insoluble forms. Examples of insoluble antigens include antigens on the microbial surface and antigens acquired and presented by follicular dendritic cells and marginal zone macrophages (Gonzalez et al., 2009; Gonzalez et al., 2011). For soluble antigen, only multivalent forms can induce BCR self-clustering, which leads to the formation of receptor microclusters (Mongini et al., 1992; Tolar et al., 2005). For insoluble antigen, even monovalent forms can induce BCR clustering, such as those tethered to a membrane, likely due to the constraints of motion in the two dimensional plane of the membrane (Tolar et al., 2009a). Inhibition of BCR self-clustering, such as by stabilization of the cortical actin network, blocks signaling activation (Liu et al., 2012a). Advanced live cell imaging techniques, including fluorescence resonance energy transfer and total internal reflection fluorescence microscopy (TIRFM), have allowed for the analyses of BCR activation events at a molecular level (Tolar et al., 2008; Harwood and Batista, 2009; Tolar et al., 2009b; Harwood and Batista, 2010). Using these imaging techniques, Tolar et al. (Tolar et al., 2009a) show that the binding of surface BCRs to membrane-tethered antigens reduces receptor lateral mobility and induces receptor microcluster formation. The extracellular domain of the BCR, particularly the Cμ4 domain, has been suggested to mediate the mIgM-mIgM interaction, and this interaction domain is likely to be exposed through conformational changes of the BCR. BCR self-clustering also induces receptor interaction with lipid rafts and lipid raft-resident kinases, such as Lyn, that phosphorylate the ITAMs on the receptor (Sohn et al., 2006; Sohn et al., 2008). Phosphorylated BCRs in microclusters recruit signaling molecules, such as Syk (Tolar et al., 2005) and PLCγ2 (Weber et al., 2008), forming microsignalosomes and initiating signaling cascades. After self-clustering, BCR microclusters continue to grow over time as more receptors are recruited into the clusters and eventually the clusters coalesce with each other. In B cells activated by membrane-tethered antigen, BCR microclusters move to the center of the B cell membrane region that contacts antigen-tethered membrane (B cell contact zone) (Fleire et al., 2006; Tolar et al., 2009a; Liu et al., 2012a). In B cells activated by multivalent soluble antigen, BCR microclusters move to and coalesce at one pole of the cells (Schreiner and Unanue, 1977; Liu et al., 2012a). This eventually leads to the formation of a central

cluster, similar to immunological synapses formed between T cells and antigen presenting cells (Carrasco et al., 2004; Dustin, 2008) (Fig. 1A–1C).

The recruitment of proximal signaling molecules to and their phosphorylation at BCR microclusters (Tolar et al., 2005; Depoil et al., 2008; Weber et al., 2008; Liu et al., 2011) suggest that signaling activation occurs at the microcluster stage. In support of this hypothesis, BCRs with a relatively high affinity to an antigen can self-cluster and induce signaling faster and to higher levels than BCRs with a relatively low affinity to the same antigen (Fleire et al., 2006; Liu et al., 2010a). We have recently shown that inhibiting the merger of BCR microclusters into a central cluster, which extends the lifetime of BCR microclusters, enhances and prolongs BCR signaling (Liu et al., 2011). These data indicate that the lifetime of BCR microclusters is positively correlated with the magnitude and duration of BCR signaling, and that the merger of BCR microclusters into the central cluster is associated with signaling downregulation. These recent studies provide evidence that the spatiotemporal organization of surface BCRs controls signal transduction at the receptor.

4. Actin reorganization upon BCR activation

All mammalian cells possess actin rich structures inside them, and the cortical actin layer beneath the plasma membrane is the primary actin structure in B cells. Filamentous actin (F-actin) can organize into bundles and networks with the help of actin cross-linking proteins to support and control cell morphology and dynamics (Pollard and Cooper, 2009). These actin structures are linked to the plasma membrane by actin binding proteins, such as ezrin/radixin/moesin (ERM) that can directly interact with integral membrane proteins (Fehon et al., 2010; Neisch and Fehon, 2011). In B cells, ezrin has been shown to interact with a lipid raft-associated membrane protein, Csk binding protein (Gupta et al., 2006) (Table 1). On the basis of experiments detailing the biophysical, cell biological and dynamical aspects of the actin system, Kusumi et al. (Kusumi et al., 2012a, 2012b) proposed a picket fence model to describe the cortical actin network beneath the plasma membrane, where the actin cytoskeleton is the fence and ERM-associated integral membrane proteins are the pickets. In addition to providing structural support to the plasma membrane, the actin picket fence influences the lateral mobility of membrane proteins that extend their cytoplasmic tails into the cortical actin network. The interaction of the actin-bound ERM proteins with proteins in lipid rafts can further compartmentalize lipid rafts and lipid raft-associated molecules. Under these conditions, surface BCRs have to overcome the actin-imposed lateral diffusion barriers to be able to self-cluster, move and eventually undergo endocytosis.

The actin cytoskeleton is highly dynamic and is reorganized by polymerization and depolymerization in response to external and internal signals. Early studies have shown that B cells remodel their actin in response to cognate antigen (Schreiner et al., 1977; Braun et al., 1978; Bachvaroff et al., 1980). BCR activation induces the association of surface BCRs and its proximal signaling molecules with detergent insoluble cytoskeletal fraction of cells. BCRs can be released from the detergent insoluble fraction by treatment with the actin severing protein gelsolin (Braun et al., 1982; Hartwig et al., 1995; Jugloff and Jongstra-Bilen, 1997; Park and Jongstra-Bilen, 1997). Since lipid rafts also have the property of being detergent insoluble (Harder et al., 1998), these results suggest that BCR activation not only

induces actin remodeling, but also alters the physical relationship among BCRs, lipid rafts, and the actin cytoskeleton.

Using TIRFM, we and others have characterized the reorganization of the cortical actin in response to BCR activation. Upon binding to soluble or membrane-associated antigen, the cortical actin in B cells initially undergoes rapid disassembly (Freeman et al., 2011; Treanor et al., 2011). This disassembly of actin is likely initiated by the dephosphorylation of ezrin and cofilin (Fig. 1A). Dephosphorylation disassociates ezrin from lipid raft-anchor proteins, consequently detaching the cortical actin from the plasma membrane (Freeman et al., 2011; Treanor et al., 2011) (Table 1). Cofilin, a protein that can sever F-actin, is critical for antigen-induced actin depolymerization (Freeman et al., 2011) (Table 1). We have shown that cofilin is recruited to the vicinity of BCR clusters in a dephosphorylated active form that can bind and sever F-actin (Liu et al., 2012a). However, this initial depolymerization of actin is transient, similar to the dephosphorylation of ezrin, and is followed by polarized reassembly of actin filaments at and around BCR microclusters(Fig. 1B). Our studies have shown that sites of actin polymerization and F-actin accumulation first appear at the initial points of contact of B cells with the antigen presenting membrane before BCR microclusters become visible. Subsequently, nascent polymerization sites and F-actin appear to be in close association with newly formed BCR microclusters (Liu et al., 2011; Liu et al., 2012a). As the number of BCR microclusters increases, the level of F-actin, specifically in the B cell contact zone, increases over time until BCR clustering reaches a plateau. As BCR microclusters grow and merge with each other, both BCR and actin clusters move centripetally toward the cell center (Liu et al., 2012b). Upon the formation of the BCR central cluster, the level of cellular F-actin decreases while the sites of de novo actin polymerization and F-actin rich structures delocalize from BCR clusters and spatially organize around the outer edge of the BCR central cluster (Liu et al., 2011; Liu et al., 2012a) (Fig. 1C).

Such dynamic actin reorganization is mediated by rapid actin polymerization and depolymerization that are controlled by a number of actin regulators. We have shown that the hematopoietic specific Wiskott-Aldrich syndrome protein (WASP) (Table 1) and its ubiquitously expressed homolog N-WASP are critical for actin remodeling in B cells (Sharma et al., 2009; Liu et al., 2011; and our unpublished data). These two proteins are actin nucleation promoting factors and capable of activating de novo actin polymerization in response to signaling (Stradal and Scita, 2006; Thrasher and Burns, 2010). Gene deletion of WASP reduces antigen-induced actin reorganization, and the combined effect of WASP gene knockout plus B cell-specific N-WASP gene deletion almost abolishes actin remodeling in B cells (Liu et al., 2011 and our unpublished data).

Distinct from T cells, antigen-induced actin reorganization in B cells is largely independent of adhesion proteins. Adhesion proteins, which have been shown to facilitate the engagement of B cells with membrane-tethered antigens with low affinity or low concentrations (Carrasco et al., 2004), may regulate actin organization either directly or indirectly, and thereby enable a better contact of the B cell membrane with the antigen presenting surface.

5. Actin dynamics regulate BCR signaling

To initiate signaling, surface BCRs have to overcome the lateral diffusion barrier created by the cortical actin cytoskeleton in order to assemble into clusters. The existence of such a lateral diffusion barrier proximal to the B cell membrane and its inhibitory effect on signaling activation has been demonstrated by several studies. First, the steady-state lateral mobility of surface BCRs in F-actin and ezrin poor regions is much greater than that in F-actin and ezrin rich regions (Treanor et al., 2010; Treanor et al., 2011). Second, disrupting the actin cytoskeleton or deleting the cytoplasmic tails of the BCR that extend into the cortical actin network increases the lateral mobility of surface BCRs (Treanor et al., 2010). Third, depolymerization of F-actin by sequestration of G-actin induces spontaneous BCR aggregation and signaling in the absence of cross-linking antigen. However, stabilization of the cortical actin blocks BCR aggregation and signaling in response to both multivalent soluble and membrane-associated antigen (Liu et al., 2012a). While the binding of surface BCRs to multi-valent soluble antigens or insoluble antigens is energetically favorable and may help the receptor to overcome the lateral diffusion barrier imposed by the cortical actin cytoskeleton, the physical forces generated from actin remodeling are likely required for optimal signaling induction. It has been shown that the detachment and disassembly of the cortical actin network, as mediated by the dephosphorylation of ezrin and cofilin respectively, increases the lateral mobility of surface BCRs and subsequent self-aggregation of receptors (Treanor et al., 2010; Freeman et al., 2011; Treanor et al., 2011). Taken together, these results suggest that signaling-induced inactivation of ezrin and activation of cofilin lead to a transient disassembly of the cortical actin cytoskeleton, which reduces the actin diffusion barrier around antigen-bound BCR. Therefore, both the reduction in the energy barrier resulting from antigen binding and the reduced diffusion barrier resulting from the disassembly of cortical actin facilitate the clustering of surface BCRs and the initiation of signaling.

Antigenic stimulation induces morphological changes in B cells. B cells stimulated with multivalent soluble antigen form a protrusion at one pole of the cell where BCR microclusters merge (Unanue et al., 1972; Schreiner and Unanue, 1977). The formation of the cell protrusion may be due to a polarized disassembly of the cortical actin, which generates an F-actin poor region for BCR microcluster coalescence. However, the relationship between the formation of the membrane protrusion and BCR clustering and signaling initiation is unknown. B cells stimulated with membrane-associated antigens undergo a rapid spreading followed by contraction of the cell membrane (Fleire et al., 2006) (Fig.1B-1C). B cell spreading on antigen-tethered membrane increases the number of surface BCRs available to engage antigen and enhances the level of BCR self-clustering. Reciprocally, inhibiting B cell spreading reduces BCR clustering and signaling activation at the contact zone between the B cell membrane and antigen-associated membrane (Fleire et al., 2006; Liu et al., 2011; Liu et al., 2012a). The morphology of cells is largely controlled by the dynamic actin cytoskeleton. Cells spreading on a surface typically have two types of protrusions: flat broad lamellipodia that consist of short branched actin filaments and long thin filopodia that comprise of parallel actin bundles. The movement of both these types of protrusions is driven by rapid actin polymerization and F-actin treadmilling (Amann and

Pollard, 2001; Ahmed, 2011; Ridley, 2011). Both lamellipodia and filopodia were observed in stimulated B cells (Unanue et al., 1972; Fleire et al., 2006; Liu et al., 2011; Liu et al., 2012a), and BCR microclusters were found to form at the tips of filopodia, suggesting their role in antigen engagement and gathering. We have shown that active WASP, a hematopoietic specific actin nucleation promoting factor, and newly polymerized actin downstream of WASP are primarily localized at the leading edge of the lamellipodia of spreading B cells. Furthermore, WASP knockout causes significant decreases in B cell spreading, BCR self-clustering and signaling (Liu et al., 2011). These results suggest that WASP-mediated actin polymerization and formation of branched actin networks enhances BCR clustering and signaling by driving B cell spreading.

After the initial step of self-aggregation, BCR microclusters continue to grow over several minutes. It has been shown that BCRs with higher affinity to an antigen cluster faster resulting in more rapidly growing clusters than BCRs with a lower affinity to the same antigen, thus leading to higher levels of Syk recruitment and increased calcium flux (Liu et al., 2010a). These results indicate that the growth of BCR microclusters leads to an increase in signaling strength. BCR microclusters grow by gathering more receptors from the leading edge of the protruding membrane and by coalescence with other clusters (Liu et al., 2010a; Liu et al., 2011). The growth and merger of receptor clusters occur simultaneously with the lateral movement of BCR microclusters to one pole of the cell or to the center of the B cell contact zone. This centripetal movement of the BCR appears to be in coordination with actin reorganization in B cells, as actin polymerization sites get redistributed to the outer periphery of the region where BCR microclusters merge (Liu et al., 2011; Liu et al., 2012a). Actin polymerization at the outer rim and actin depolymerization at the interior of the B cell contact zone potentially create a centripetal flow of F-actin, which can drive the inward movement of BCR microclusters and deliver them to the central actin-poor region for merger. Perturbations of actin remodeling interfere with the merger of BCR microclusters and the formation of BCR central cluster (Liu et al., 2012a). Actin can thus regulate BCR signaling by controlling the growth and movement of BCR clusters.

The formation of the BCR central cluster occurs when the lamellipodia retract and the cell contact area decreases. We have demonstrated that the merger of BCR microclusters into the polarized central cluster is associated with the downregulation of surface signaling and is dependent on B cell contraction. This is supported by the following evidence. Tyrosine and Syk phosphorylation activities are primarily detected in BCR microclusters but significantly reduced in the BCR central cluster. Delayed B cell contraction is concurrent with a decrease in centripetal movement and merger of BCR microclusters into the BCR central cluster. Importantly, this inhibition of BCR central cluster formation is associated with enhanced and prolonged signaling (Liu et al., 2011). These data suggest that the contracting membrane of B cells facilitates the movement of BCR microclusters into the center of the contact zone for merger and may also help to stabilize the BCR central cluster by reducing the contact area available for BCR movement. While the underlying mechanism for the induction of B cell contraction is not known, membrane contractions in cells are typically mediated by acto-myosin dynamics (Vicente-Manzanares et al., 2009). Early studies found that non-muscle myosin II is recruited to BCR central clusters in response to multivalent soluble antigens and proposed a possible role for myosin in the formation of BCR clusters

(Schreiner et al., 1977). In T cells, non-muscle myosin II is important for the movement of TCR microclusters into the center of the contact zone and the formation of the immunological synapse between T cells and antigen presenting cells. Inhibition of non-muscle myosin IIA by blebbstatin or siRNA knockdown increases T cell spreading, reduces the inward movement of TCR microclusters, and destabilizes the immunological synapse (Ilani et al., 2009; Kumari et al., 2012). These findings support a role for actomyosin dynamics in B cell contraction and the merger of BCR microclusters into a polarized central cluster.

In summary, antigen-induced actin cytoskeleton remodeling can regulate BCR activation in multiple ways. Cortical actin disassembly can reduce the lateral diffusion barrier of surface BCRs, and thereby facilitate antigen-induced receptor self-clustering. B cell spreading, driven by rapid and polarized assembly of actin, allows B cells to gather more antigen and increases antigen engagement of the BCR. Rapid actin assembly and disassembly can generate actin centripetal flow, facilitating the inward movement of BCR microclusters and their merger into the central cluster. Actin-mediated B cell contraction drives the formation of the BCR central cluster, which downregulates BCR signaling activation (Fig. 1A–1C).

6. BCR signaling initiates and regulates actin reorganization

Actin remodeling in B cells is initiated by BCR signaling and mediated through interactions between a series of signaling molecules and actin regulators. Similar to T cells (Labno et al., 2003; Finkelstein and Schwartzberg, 2004), a Tec family kinase, Bruton's tyrosine kinase (Btk), has been found to be one of the key molecules that couple BCR signaling with the actin cytoskeleton (Sharma et al., 2009) (Fig. 1B). Btk is activated in the early stages of BCR signaling by binding to phosphatidylinositol-3,4,5-triphosphate [PdtIns(3,4,5)P_3] at the plasma membrane where it is activated by Lyn and Syk-mediated phosphorylation (Baba et al., 2001; Dal Porto et al., 2004). Btk can activate actin remodeling through multiple pathways. First, Btk activates Vav, a guanine nucleotide exchange factor (GEF) of Rac and Cdc42, by phosphorylation (Sharma et al., 2009). Second, Btk can increase PdtIns(4,5)P_2 production by recruiting and activating phosphatidylinositol-4-phosphate 5-kinases (PIP5Ks) to the B cell surface (Saito et al., 2003; Carpenter, 2004). Third, Btk can directly and indirectly phosphorylate actin regulators. The binding of Cdc42 and PdtIns(4,5)P_2 to WASP releases WASP from its auto-inhibitory conformation (Padrick and Rosen, 2010; Thrasher and Burns, 2010), and Btk-induced phosphorylation further stabilizes the open, active conformation of WASP (Blundell et al., 2009; Sharma et al., 2009) (Table 1). The conformational change enables WASP to activate the Arp2/3 complex which nucleates branched growth of actin filaments. The actin regulatory role of Btk is unlikely to be limited to activating WASP. We found that Btk deficiency had a much stronger effect on BCR-induced actin reorganization and actin-dependent B cell spreading and BCR clustering than WASP knockout (Sharma et al., 2009; Liu et al., 2011). Btk-activated Rho-family GTPases and PdtIns(4,5)P_2 production potentially regulate multiple actin regulators. PtdIns regulate the cellular localization and actin binding or regulatory ability of a variety of actin regulators, such as profilin that promotes the ATP/ADP exchange of G-actin, cofilin that destabilizes F-actin, gelsolin that severs F-actin and caps the bard end of F-actin (Van Troys et al., 2008; Bernstein and Bamburg, 2010), and ezrin that links the actin cytoskeleton to the

plasma membrane (Fehon et al., 2010; Neisch and Fehon, 2011). Cdc42, the GTPase downstream of Btk and Vav, is responsible for the polarization of both the actin cytoskeleton and the microtubule organization center toward to the BCR central clusters (Yuseff et al., 2011).

The importance of Btk in transmitting signals from the BCR to actin is further underscored by its unique position in the BCR signaling pathway. It not only functionally and/or physically interacts with multiple key components of the BCR signaling pathway, such PI3K, PLCγ2 and Vav, but is also a target of co-stimulatory and co-inhibitory receptors, such as CD19 and FcγRIIB respectively. CD19, when recruited to the BCR by antigenic stimulation and by complement factor C3d opsonized antigen, enhances Btk activation and lowers the signaling threshold for B cell activation (Fujimoto et al., 2002). FcγRIIB, when colligated with the BCR by immune complexes, inhibits Btk activation by activating SHIP-1, turning down antigen-induced BCR activation (Bolland et al., 1998; Brauweiler and Cambier, 2003; O'Neill et al., 2011). Based on the role of Btk in regulating actin reorganization, it can be predicted that CD19 activation will enhance, while FcγRIIB colligation with the BCR will inhibit antigen-induced actin reorganization by turning up and down Btk signaling respectively. Indeed, CD19 deficiency inhibits B cell spreading, BCR clustering and B cell spreading (Depoil et al., 2008). FcγRIIB colligation with the BCR inhibits BCR clustering and B cell spreading (Liu et al., 2010c). We have shown that B cell-specific deficiency of SHIP-1, which is activated by BCR-FcγRIIB colligation or BCR activation, not only increases Btk phosphorylation at the B cell surface, but also enhances antigen-induced WASP activation, actin polymerization and B cell spreading (Liu et al., 2011). These results further confirm the critical role of Btk in controlling actin remodeling in response to different external signals.

In addition to Rho-family GTPases and PtdIns, phosphorylation and dephosphorylation of actin regulators is another major mechanism by which actin dynamics is regulated (Table 1). The actin nucleation promoting activity of WASP is increased by phosphorylation at Y256, Y291, S242, S483 and S484 sites of WASP (Cory et al., 2002, 2003; Yokoyama et al., 2005), which stabilizes its open active conformation. We have shown that BCR activation increases the levels of phosphorylated WASP and recruits phosphorylated WASP to the membrane of B cells at the contact zone in a Btk-dependent manner (Sharma et al., 2009; Liu et al., 2011). However, whether Btk can directly phosphorylate WASP is not known. It has been suggested that WASP is a substrate of the Src family kinase Hck (Cory et al., 2002) and activated Cdc42-associated kinase 1 (ACK1) (Yokoyama et al., 2005), but whether these kinases are responsible for WASP phosphorylation in B cells and their relationship with Btk have not yet been determined. The actin binding ability of ezrin is controlled by phosphorylation at T567 (Table 1). The binding of ezrin to PtdIns(3,4)P_2 facilitates its phosphorylation and enables it to bind F-actin (Simons et al., 1998; Fievet et al., 2004). The actin binding activity of cofilin, an actin-severing protein, is inhibited by phosphorylation at S3 (Yang et al., 1998). BCR activation induces the dephosphorylation of cofilin which is dependent on Rap GTPase and Slingshot phosphatase (SSH) (Freeman et al., 2011) (Table 1). Dephosphorylated but not phosphorylated cofilin is preferentially recruited to BCR clusters (Liu et al., 2012a). Overexpression of cofilin S3A mutation, which blocks cofilin phosphorylation, enhances BCR-mediated actin reorganization, while Rap or SSH dominant

negative mutation inhibits BCR-induced actin remodeling and B cell spreading (Freeman et al., 2011). BCR activation also induces the phosphorylation of actin binding protein 1 (Abp1/HIP-55/SH3P7) at Y337 and Y347 (Larbolette et al., 1999; Onabajo et al., 2008). An in vitro study suggests Abp1 as a substrate of Src and Syk family kinases (Larbolette et al., 1999). The tyrosine phosphorylation of Abp1 is required for its recruitment to the BCR at the cell surface upon antigenic activation and for its function in BCR-mediated antigen processing (Onabajo et al., 2008) (Table 1).

As evident from the studies outlined above, the actin cytoskeleton is dynamically reorganized in response to BCR-antigen interaction. BCR signaling induces actin depolymerization and polymerization and regulates actin dynamics through its proximal signaling molecules, including Btk, Rho-family GTPase, and PtdIns metabolism, which control the activity and subcellular localization of actin regulators.

7. Actin dynamics in BCR internalization

Subsequent to signaling activation at the B cell surface, BCRs endocytose bound antigen into B cells. Internalized antigen-BCR complexes are targeted to late endosomes where protein antigens are fragmented by proteases and loaded onto MHC class II for antigen presentation at the B cell surface (Song et al., 1995; Siemasko and Clark, 2001; Boes et al., 2004; Vascotto et al., 2007). The processing and presentation efficiency of BCR-specific antigen is significantly higher than non-specific antigens. This high efficiency is likely due to the ability of BCR to capture specific antigens with high affinity and to target them to the antigen processing compartments (Casten et al., 1988; Song et al., 1995). BCRs are endocytosed primarily via the clathrin-mediated pathway (Guagliardi et al., 1990). The BCR undergoes constitutive endocytosis in the absence of antigen, suggesting that BCR signaling is not essential for BCR endocytosis. However, the binding of multivalent soluble antigen, which induces signaling, significantly increases receptor endocytosis (Song et al., 1995), and inhibition of BCR signaling reduces BCR endocytosis (Puré and Tardelli, 1992; Malhotra et al., 2009a, 2009b). While it is generally believed that both constitutive and antigen-induced BCR endocytosis utilizes the same clathrin-mediated pathway, this idea has not yet been directly confirmed. We have previously shown that actin remodeling is critical for signaling enhanced BCR endocytosis but is not required for the constitutive endocytosis of the BCR (Brown and Song, 2001), which suggests that BCR signaling can upregulate receptor internalization by inducing actin remodeling. In support of this hypothesis, signaling molecules that regulate actin dynamics have been shown to be required for BCR endocytosis and antigen processing. For example, Btk and Btk downstream signaling molecules Vav1/3 and Rac1/2 are required for the optimal efficiency of BCR-mediated antigen uptake, processing and presentation (Malhotra et al., 2009a, 2009b; Sharma et al., 2009). In addition, using B cells with a deficiency of the clathrin heavy chain, Stoddart et al. (Stoddart et al., 2005) show that 30% of BCR endocytosis is clathrin-independent but actin-dependent. These data provide evidence that BCR-induced actin remodeling facilitates both clathrin-dependent and independent BCR internalization, consequently increasing the kinetics and efficiency of antigen processing and presentation.

The actin cytoskeleton can potentially play multiple roles in BCR endocytosis, including (1) gathering surface BCRs into budding vesicles; (2) recruiting endocytotic machinery proteins to surface BCRs; (3) deforming the plasma membrane for the formation of budding vesicles; (4) detaching budding vesicles from the plasma membrane; and (5) moving nascent vesicles into the cytoplasmic interior of the cell. In previous sections, we have discussed the role of actin reorganization in promoting surface BCRs to aggregate into microclusters and merge into central clusters, which potentially facilitates the gathering of BCR-antigen complexes into budding vesicles, such as the clathrin-coated pit. However, a direct link between BCR clustering and endocytosis remains to be established. BCR signaling activation has been shown to increase the recruitment of clathrin and dynamin 2 to the cell surface and the phosphorylation of clathrin (Malhotra et al., 2009a; Stoddart et al., 2002). However, the recruitment of these endocytosis machinery proteins appears to be signaling-dependent, but not actin-dependent (Stoddart et al., 2002).

When B cells are treated with cytochalasin D, which inhibits signaling-activated BCR endocytosis, BCRs accumulate at deeply invaginated budding vesicles (Brown and Song, 2001). This result suggests that the actin cytoskeleton is involved in detaching BCR positive vesicles from the plasma membrane, but not in the membrane deformation during the formation of clathrin-coated pits. The role of actin in membrane fission has also been observed in other cell types (Collins et al., 2011; Mooren et al., 2012). We have identified Abp1 as the actin adaptor protein that brings F-actin to budding vesicles (Fig. 1D) (Table 1). Abp1 knockdown and knockout cause a decrease in BCR internalization and BCR-mediated antigen processing and presentation. Abp1 can directly interact with F-actin and dynamin 2, a GTPase that is responsible for constricting the neck of budding vesicles, through its actin depolymerizing factor homology domain and SH3 domain, respectively (Onabajo et al., 2008). Abp1 is recruited to the plasma membrane in response to BCR activation, and its recruitment depends on BCR-induced tyrosine phosphorylation of Abp1 as well as actin reorganization and the proline-rich domain of dynamin 2 (Onabajo et al., 2008). These results indicate that Abp1 links BCR-induced actin remodeling to the endocytosis process, whereby Abp1-mediated recruitment of F-actin to the neck of budding vesicles potentially provides an additional mechanical force for membrane fission. In addition, the actin cytoskeleton has been shown to reorganize away from BCR microclusters and the central cluster during receptor endocytosis (Liu et al., 2011; Liu et al., 2012a). Disassembly of cortical actin at receptor endocytosis sites disrupts the actin picket fence, enabling vesicles to bud into the cell interior. The colocalization of activated WASP with internalizing BCR and BCR-positive vesicles implicates a role for actin polymerization in driving the fusion and movement of BCR-containing vesicles (Sharma et al., 2009).

B cells uptake antigen in diverse physical forms for antigen processing and presentation. Internalization of membrane-associated or immobilized antigen likely requires additional physical forces in order to pull antigen from the presenting membrane and surface. Natkanski et al. have recently shown that non-muscle myosin II, which is recruited to the B cell contact zone at early times, is required for BCR-mediated endocytosis of membrane-associated antigen, but not soluble antigen. Myosin generates contractile force that pulls antigen with membrane into endocytosing vesicles (Natkanski et al., 2013). In addition, polarization of the cytoskeleton has been shown to be important for BCR-mediated

internalization of immobilized antigen but not soluble antigen. Following polarized actin reassembly at and around BCR microclusters and central clusters, the microtubule organization center is reoriented close to the BCR central cluster in response to latex bead-immobilized antigen, which leads to the polarization of late endosomes and lysosomes (Yuseff et al., 2011). When the polarization is inhibited, the sequestration, processing and presentation of immobilized antigen are reduced. The polarization of the microtubule organization center is dependent on BCR-induced activation of Cdc42 and its downstream atypical protein kinase C (Yuseff et al., 2011). Cdc42 is a universal regulator of cell polarity and is capable of translating polarized signaling cues into polarized organization of actin and microtubules (Etienne-Manneville, 2004). Whether the polarization of the microtubule organization center in B cells is dependent on the polarized reassembly of actin is not known. However, the coordination of actin and microtubules is likely to be required for the transport of antigen that is endocytosed through actin-dependent mechanisms to late endosomes and lysosomes.

The actin cytoskeleton specifically promotes BCR-mediated endocytosis of antigen and facilitates antigen transport to late endosomes/lysosomes. Signaling-activated actin remodeling likely facilitates the recruitment of surface BCRs to endocytosis sites and provides mechanical force for pulling antigen from associated membrane and for fission of budding vesicles (Fig. 1D). Furthermore, actin dynamics can drive vesicle movement and induce the polarization of the microtubule organization center and subcellular antigen processing compartments toward the BCR central cluster at the B cell surface.

Perspectives

Recent studies have clearly demonstrated critical functions for the actin cytoskeleton in BCR-mediated signal transduction and antigen processing processes. Early signaling of the BCR triggers actin reorganization as a series of sequential events, including a transient detachment and disassembly of the cortical actin network, subsequent polarized actin reassembly, and continuous remodeling synchronous with the dynamics of surface BCRs. These actin dynamics are regulated by a set of signaling-activated actin regulators. The signaling-dependent actin remodeling in turn regulates BCR signaling and antigen processing. Actin regulates BCR signaling by controlling lateral mobility of surface BCRs, the kinetics and magnitude of receptor clustering and central cluster formation. Furthermore, actin remodeling translates signaling activation to enhanced antigen processing and presentation. However, the mechanism by which the actin cytoskeleton transitions from driving B cell spreading and BCR clustering to endocytosis is not completely understood. Based on knowledge accumulated so far, we can speculate that actin may organize into different structures at different locations during the BCR signaling and endocytosis phases, which may be mediated by different actin regulators. We have identified Btk as the main signaling regulator of actin remodeling, and Btk-activated actin remodeling is important for both signaling amplification and antigen internalization. However, WASP, the down-stream actin regulator of Btk, is dispensable for both B cell spreading, BCR clustering and internalization, since WASP knockout only causes moderate reductions in these cellular events. This suggests that other actin regulators may play redundant or compensatory roles in these processes. We have demonstrated that Abp1 serves as an actin adaptor that links the

actin cytoskeleton with the endocytosis machinery. Our recent studies (unpublished data) found that the ubiquitously expressed homolog of WASP, N-WASP, play a much more dominant role in BCR internalization than WASP, suggesting that these two proteins have distinct roles in BCR signaling and endocytosis. Further, Abp1 has been shown to regulate actin dynamics by interacting with N-WASP. These results together indicate that WASP, N-WASP and Abp1 as well as non muscle myosin II cooperate with one another during BCR functional transition from surface signaling to endocytosis, and Btk-mediated signaling regulates this cooperative network. Therefore, what the functional relationships among these actin regulators are during the transition of BCRs from signaling to endocytosis, whether each of them modifies actin dynamics and organization in distinct ways, and how they are regulated by BCR signaling are interesting questions for future research.

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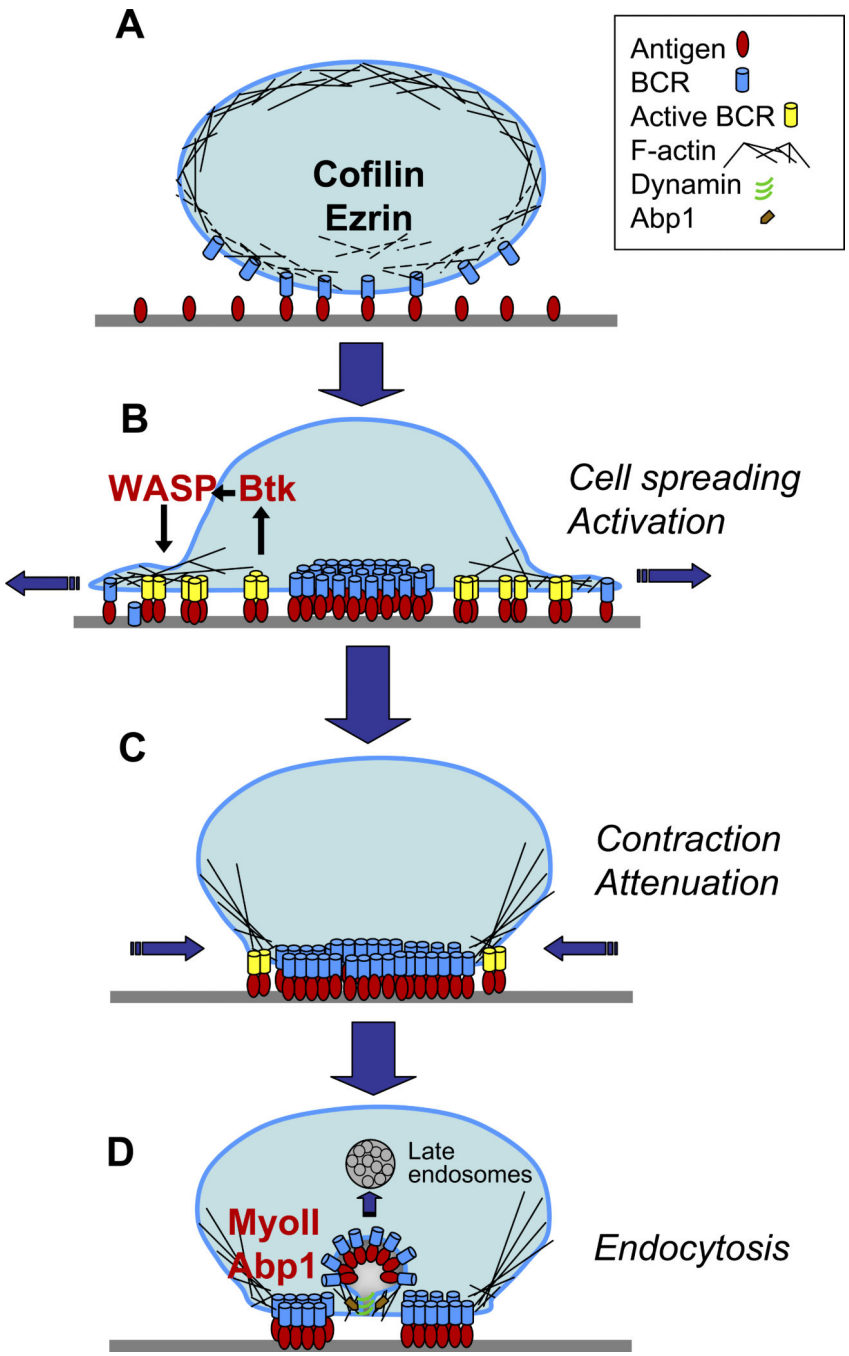

Figure 1.

Role of the actin cytoskeleton in coordination of BCR signaling and antigen processing functions. BCR signaling triggers actin reorganization as a series of sequential events. An initial transient detachment and disassembly of the cortical actin network is induced by dephosphorylation ezrin and cofilin (A), and a subsequent polarized actin reassembly is mediated in part by Btk-activated WASP (B). The actin remodeling facilitates BCR self-clustering and signaling induction in BCR microclusters. Actin-driven B cell spreading enhances BCR microclustering and signaling (A-B). The transition of actin-mediated cell

spreading to contraction promotes the coalescence of BCR microclusters and the formation of the central cluster, which leads to signaling attenuation (C). B cell contraction also likely helps to gather BCR-antigen complexes into endocytosing vesicles. The continuous actin remodeling, the actin adaptor protein Apb1 that couples F-actin with dynamin, and the actin motor non-muscle myosin II (MyoII) are required for the formation and fission of BCR containing budding vesicles from the plasma membrane (D).

Table 1

Actin regulators that are involved in BCR signaling and endocytosis

Full name	Abbreviation	Function in B cells	Activation mechanisms	Ref
Cofilin	Cofilin	Sever and depolymerize F-actin	Dephosphorylation by slingshot phosphatases	Freeman et al., 2011; Liu et al., 2012a
Ezrin	Ezrin	Detach the cortical actin from the plasma membrane	Dephosphorylation by unknown threonine phosphatase	Gupta et al., 2006; Treanor et al., 2011
Wiscott-Aldrich syndrome protein	WASP	Actin polymerization and B cell spreading	Btk-induced activation of Cdc42, PtdIn(4,5)P_2 production and phosphorylation	Sharma et al., 2009; Liu et al., 2011
Actin binding protein 1	Abp1/HIP-55/SH3P7	BCR endocytosis	Phosphorylation and actin remodeling	Larbolette et al., 1999; Onabajo et al., 2008