How B cells capture, process and present antigens: a crucial role for cell polarity

Maria-Isabel Yuseff, Paolo Pierobon, Anne Reversat and Ana-Maria Lennon-Duménil

Abstract | B cells are key components of the adaptive immune response. Their differentiation into either specific memory B cells or antibody-secreting plasma cells is a consequence of activation steps that involve the processing and presentation of antigens. The engagement of B cell receptors by surface-tethered antigens leads to the formation of an immunological synapse that coordinates cell signalling events and that promotes antigen uptake for presentation on MHC class II molecules. In this Review, we discuss membrane trafficking and the associated molecular mechanisms that are involved in antigen extraction and processing at the B cell synapse, and we highlight how B cells use cell polarity to coordinate the complex events that ultimately lead to efficient humoral responses.

Immunological synapse
The interface between an antigen-presenting cell and a lymphocyte. The hallmark of this structure comprises two concentric regions: one region that is referred to as the central supramolecular activation cluster (cSMAC), where immune receptors are enriched, and another region that is referred to as the peripheral SMAC (pSMAC), which contains adhesion molecules such as lymphocyte function-associated 1 (LFA1) bound to its ligand intercellular adhesion molecule 1 (ICAM1).

[1]*Inserm U932, Institut Curie, 12 rue Lhomond, 75005 Paris, France.*
Correspondence to A.-M.L.-D.
e-mail:
Ana-Maria.Lennon@curie.fr
doi:10.1038/nri3469

The ability of B cells to capture external antigens and to present them as peptide fragments on MHC class II molecules to $CD4^+$ T cells is a crucial step in the adaptive immune response. This communication between B cells and T cells — known as T cell–B cell cooperation[1] — is required for B cells to form germinal centres, to differentiate into high-affinity antibody-producing plasma cells and to develop into memory B cell populations. The presentation of antigens by B cells on MHC class II molecules is a complex process that involves several stages: first, external antigens are recognized and captured by B cells through their B cell receptor (BCR); second, the antigen is processed by degrading the antigen in internal compartments within the B cell and then the corresponding peptide fragments are loaded on MHC class II molecules; and third, MHC class II–peptide complexes are presented to $CD4^+$ T cells.

As described below, the recognition by B cells of antigen that is tethered at the cell surface of specialized antigen-presenting cells (APCs) leads to the formation of an immunological synapse[2]. This dynamic platform coordinates signalling with antigen extraction and processing, and is crucial for B cells to become fully activated. In this Review, we describe each of the key stages involved in the processing and presentation of antigens by B cells, and we discuss the molecular mechanisms that govern each process. Special emphasis is given to the role of cell polarity in coordinating the formation of the immunological synapses that enable B cells to efficiently respond to antigenic challenges and to carry out their immune effector functions.

Antigen encounter and the role of APCs

Encounter of B cells with antigen in SLOs. In mammals, B cells are generated in the bone marrow and, after going through several developmental checks[3], they migrate to the spleen where they differentiate into mature naive B cells. Mature B cells then recirculate through the bloodstream and migrate to secondary lymphoid organs (SLOs), such as the spleen and the lymph nodes, which provide the necessary structural and chemical microenvironment for B cells to encounter antigens and to become fully activated[4]. In the lymph nodes, B cells migrate from high endothelial venules towards the B cell zone (which is found in the lymph node cortex), where they are organized into cell aggregates known as follicles. This directed migration is mediated by a chemokine gradient of CXC-chemokine ligand 13 (CXCL13), which is produced by a network of follicular stromal cells[5]. The recognition of CXCL13 by CXC-chemokine receptor 5 (CXCR5), which is expressed by B cells, generates signals that promote the migration of B cells, thus enabling them to constantly monitor their environment for antigens.

B cells can encounter soluble or large particulate antigens that are attached to the surface of neighbouring cells, such as macrophages, follicular dendritic cells (FDCs) or dendritic cells (DCs)[6–9]. Small soluble antigens gain access to the lymph nodes through the afferent lymph vessels and might directly pass into the B cell follicle independently of cell-mediated antigen presentation[10]. The mechanisms by which low-molecular-mass

antigens access the B cell zone remain controversial, but it has been suggested that their entry is facilitated by small pores located in the subcapsular sinus region of the lymph nodes[11] or that it might occur by simple diffusion[12].

Despite the ability of follicular B cells to rapidly gain access to soluble antigens, there is convincing evidence from *in vivo* studies that larger antigens (with a molecular mass that is greater than 70 kDa) — such as viral aggregates, immune complexes or antigen-coated microspheres — which have limited access to the follicle, can reach the B cell zone and can trigger B cell activation[13]. Such antigens are found tethered to the surface of specialized APCs and are particularly efficient at inducing B cell responses, even at low densities[2]. B cells are localized in defined environments in SLOs, which favour their encounter with antigens; for instance, in the cortex of the lymph nodes, B cell follicles are strategically positioned beneath the subcapsular sinus, where B cells can continuously sample antigens that are presented by subcapsular sinus macrophages[6,7]. In addition, B cell follicles contain FDCs, which are also capable of presenting antigens to B cells[8]. The interaction of B cells with antigens that are presented on the surface of neighbouring cells in the SLOs triggers the formation of an immunological synapse that facilitates the efficient extraction and processing of membrane-tethered antigens (discussed in detail below). For a more detailed overview of the different cell types and surface molecules that are involved in antigen presentation to B cells, we refer the reader to a recent review[13].

The establishment of immunological synapses must be coupled to an arrest in B cell migration, which enables antigens to be acquired. Interestingly, the presence of CXCL13 enhances B cell activation during antigen recognition, which suggests that there is interplay between BCR and CXCR5 signalling. Indeed, CXCL13 promotes membrane ruffling and lymphocyte function-associated antigen 1 (LFA1; also known as αLβ2 integrin)-supported adhesion during antigen recognition (the formation of kinapses) through a mechanism that requires the actomyosin network. Furthermore, the strength of BCR signalling alters CXCL13-mediated B cell migration[14], which shows how B cells couple antigen acquisition to their migratory capacity.

Formation of the B cell immunological synapse. It is well established that B cells form an immunological synapse upon engagement of their BCR with antigens that are bound to the surface of specialized APCs. This dynamic structure that B cells form shows the classical features of the immunological synapse that was originally described in T cells[15,16], where the BCR forms a central cluster surrounded by a ring of adhesion molecules, including LFA1. Integrins promote the adhesion of B cells to APCs, and their engagement can lower the threshold for B cell activation when antigen avidity is low[17].

The BCR comprises a plasma membrane immunoglobulin coupled to a signalling module that is formed by the Igα–Igβ dimer, containing immunoreceptor tyrosine-based activation motifs (ITAMs), in which tyrosine residues rapidly become phosphorylated by SRC family kinases upon antigen engagement[18,19]. This results in the recruitment and activation of SYK, followed by the induction of calcium signalling, which is involved in initiating the gene transcription required for B cell function[20]. The early events of B cell activation induce a rapid actin-dependent membrane-spreading response at the antigen-contact site, which helps to increase the number of BCR–antigen encounters and that is required for the formation of signalling microclusters[21,22]. Following this, antigens are gathered into BCR microclusters, in which co-receptors (such as CD19)[23], as well as cytosolic signalling components (such as LYN, SYK and VAV1[24,25]) are recruited to form 'microsignalosomes'. This is followed by a contraction phase in which antigen–BCR complexes converge into a central cluster. This contraction is caused by the concerted actions of rearrangements of the cortical actin cytoskeleton, which are mediated by ERM proteins (ezrin, radixin and moesin proteins; which link plasma membrane proteins to the actin cytoskeleton[26]), and the microtubule-based motor protein dynein[27]. The spreading response that is shown by B cells is tightly coupled to the signalling capacity of the cell, as cells that recruit fewer signalling molecules to microclusters show deficient spreading responses to membrane-bound antigens[23]. This has a direct effect on the amount of antigens that are accumulated at the synapse and, therefore, that are essential for efficient antigen extraction. These results show that signalling events are tightly coordinated during synapse formation and that they are crucial for B cell activation (FIG. 1).

Role of the actin cytoskeleton at the B cell synapse. As mentioned above, B cells undergo dynamic changes in their actin cytoskeletons to induce cell spreading following synapse formation, which helps to promote the gathering and the extraction of membrane-tethered antigens (FIG. 1). Several downstream BCR effectors that regulate actin cytoskeletal rearrangements have been identified. These include the RHO GTPases RAC1 and RAC2, which promote actin polymerization and are important for cell spreading upon BCR stimulation. In particular, RAC2 (REF. 28), which functions with RAP1 GTPases[29], is required for LFA1-mediated membrane spreading during synapse formation. Conversely, RAP1 GTPases also promote B cell spreading in the absence of LFA1 engagement, by generating fast rearrangements of the actin cytoskeleton through the activation of the actin-severing protein cofilin[30]. Indeed, BCR engagement was shown to initially induce a fast de-polymerization of the actin cytoskeleton, followed by a polarized re-polymerization[30,31]. Furthermore, the severing of F-actin, which is controlled by RAP GTPases, regulates the formation and the mobility of BCR microclusters, thereby affecting B cell signalling and antigen gathering[30].

It has been suggested that the severing of the cortical actin cytoskeleton might be required to remove the barriers that restrict receptor diffusion[32], thus promoting BCR microcluster formation. Such a mechanism could be coupled to the inactivation of ERM proteins

Cell polarity
The asymmetric organization of both functional and structural cell components, which are crucial to coordinate diverse biological functions ranging from directional cell migration and asymmetric cell division to the maintenance of tissue integrity.

CXC-chemokine ligand 13 (CXCL13). A chemokine belonging to the CXC-chemokine family that functions as a chemoattractant for B cells by binding to CXC-chemokine receptor 5 (CXCR5).

Lymphocyte function-associated antigen 1 (LFA1). An integrin that is formed by the α-integrin (also known as CD11a) and β-integrin (also known as CD18) chains, It is present in diverse cell types of the immune system, such as lymphocytes, macrophages and neutrophils. LFA1 binds to its ligand intercellular adhesion molecule 1 (ICAM1), which is present on the cell surface of antigen-presenting cells. In B cells, it promotes cell adhesion and antigen gathering during immunological synapse formation, thereby facilitating B cell activation.

Kinapses
Motile adhesive interactions between lymphocytes and antigen-presenting cells. They differ from synapses because the interactions can be transitory.

ERM proteins
A family of three closely related proteins formed by ezrin, radixin and moesin that connect actin filaments with the plasma membrane. They possess a FERM (protein 4.1, ezrin, radixin and moesin) domain that mediates interactions with proteins in the plasma membrane and a charged carboxyl terminus that interacts with actin filaments.

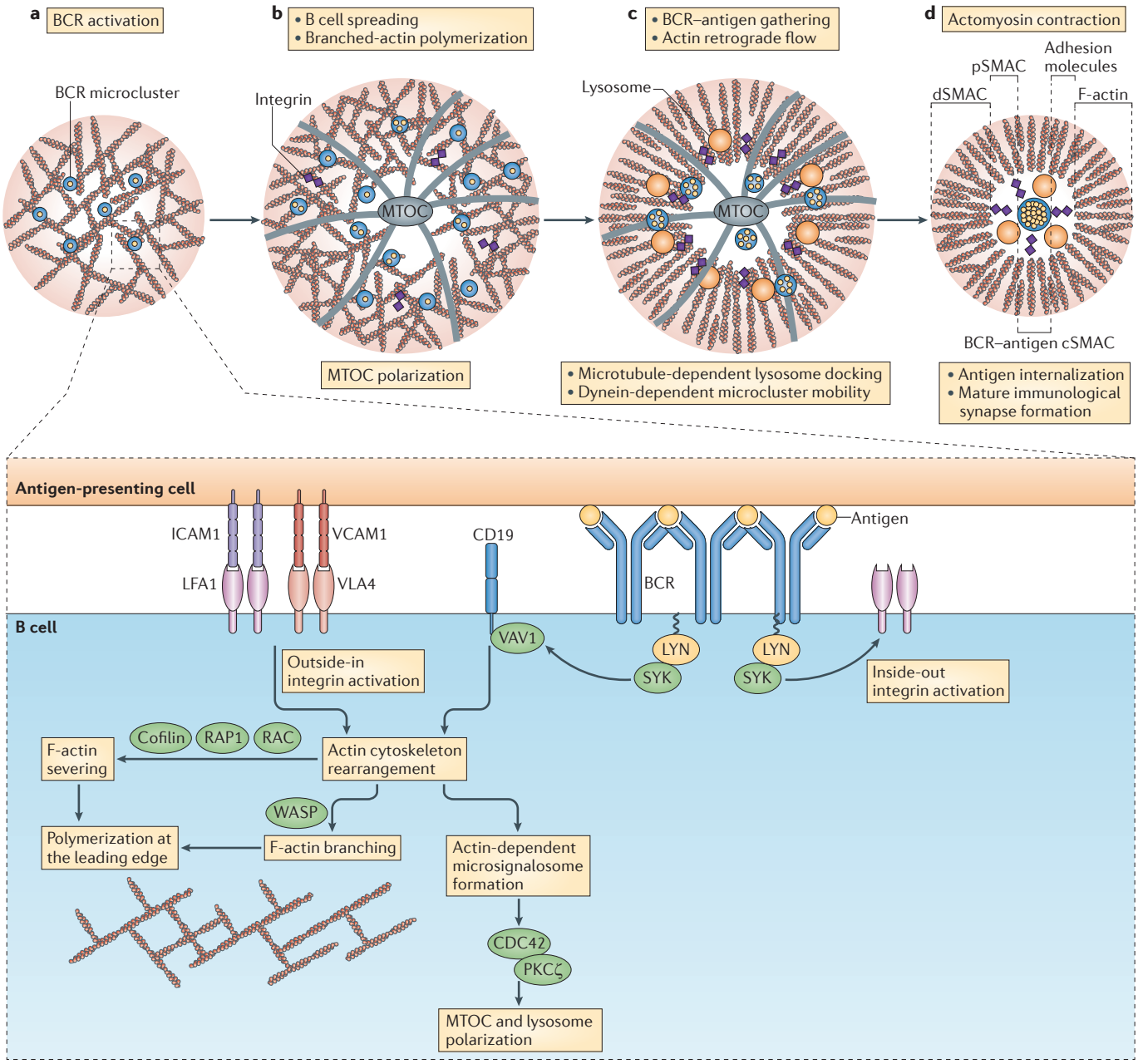


Figure 1 | **B cell immunological synapse formation.** **a** | B cell receptor (BCR) engagement by an antigen triggers the formation of BCR oligomers, which converge to form signalling platforms called microclusters. CD19 is transiently recruited to these microclusters, leading to the activation of signalling molecules such as SYK, LYN and VAV1. The activation of the integrins lymphocyte function-associated antigen 1 (LFA1) and very late antigen 4 (VLA4) promotes B cell adhesion to the surface of the antigen-presenting cell, thereby lowering the threshold of activation (shown in inset). The downstream signalling events that these integrins initiate promote RAC-dependent actin polymerization as well as F-actin severing by the activation of RAP1 and cofilin. **b** | The actin cytoskeletal rearrangements are essential for the spreading and contraction response that is required for antigen gathering by B cells. Concomitantly, the microtubule network is organized towards the immunological synapse where the minus-end molecular motor dynein drives the concentration of microclusters at the synapse. **c** | The polarization of the microtubule-organizing centre (MTOC) is also essential for the local recruitment and secretion of MHC class II$^+$ lysosomes that promote antigen extraction. **d** | Together, these dynamic events lead to the formation of a mature immunological synapse that is characterized by concentric regions: the central supramolecular activation cluster (cSMAC) in which BCRs that are engaged with antigens are concentrated, the peripheral SMAC (pSMAC), which contains adhesion molecules such as LFA1, and the distal SMAC (dSMAC) in which actin is enriched. This structure is essential for B cells to coordinate cell signalling with antigen extraction. CDC42, cell division control protein 42; ICAM1, intercellular adhesion molecule 1; PKCζ, protein kinase C ζ-type; VCAM1, vascular cell adhesion molecule 1; WASP, Wiskott–Aldrich syndrome protein.

Stochastic simulations
A system of particles can be described by its equations of motion. In a system that is subject to thermal fluctuations it is necessary to include a stochastic (random) term that accounts for these fluctuations. By using stochastic simulations a numerical integration of these equations can be generated. Quantities that are experimentally measurable are obtained by averaging several realizations of the process.

Microtubule organizing centre
(MTOC; also known as the centrosome in animal cells). A major site of microtubule nucleation that is enriched in α-tubulin. This dynamic structure organizes the mitotic and meiotic spindle and basal bodies that are associated with cilia.

Lysosomes
The central degradative compartments of the cell. The lysosomes contain an acidic pH (4.6–5.0) and they are where lysosomal hydrolases are concentrated.

— driven by BCR stimulation — which also leads to a transient increase in BCR mobility and is required for efficient microcluster formation, for membrane spreading and to gather antigens at the synapse[26]. In addition, the proteins that regulate actin cytoskeleton dynamics are essential to promote the development and the activation of B cells.

Wiskott–Aldrich syndrome protein (WASP)-deficient B cells show aberrant cell surface clustering of LFA1 downstream of BCR engagement during the formation of B cell synapses, as well as showing defects in spreading and migration[33].

Overall, the control of receptor diffusion dynamics by actin cytoskeletal rearrangements is crucial for B cells to 'tune' BCR signalling and to promote antigen uptake during B cell synapse formation.

Microtubule-dependent trafficking of molecules in synapse formation. Stochastic simulations of the dynamics of BCR and LFA1 molecules suggest that the formation of the synapse occurs only if BCR mobility is enhanced by directed motors[34–36]. Recent work has highlighted a role for the microtubule network in B cell synapse formation. It was shown[27] that dynein — a microtubule minus-end motor protein that is involved in the transport of intracellular cargo — is required to concentrate BCR microclusters at the synapse. Moreover, and similarly to observations that were originally made in immunological synapses established by natural killer (NK) and cytotoxic T cells[37], B cells were found to rapidly relocate their microtubule organizing centre (MTOC) to the site of antigen encounter[38]. In B cells, the repositioning of the MTOC at the immunological synapse occurs at the same time as the repositioning of MHC class II-containing lysosomes, which could control the local concentration of MHC class II molecules required to process incoming antigens[38]. In addition, the local secretion of MHC class II-containing lysosomes at the synapse is required to promote the extraction and the processing of tethered antigens[38].

The role of MTOC polarization in lysosome transport to the B cell synapse has been assessed by carrying out laser ablation of the MTOC and subsequently analysing the effect on lysosome distribution (FIG. 2). It was shown that lysosomes remained dispersed and that they did not cluster at the immunological synapse when the MTOC had been disrupted. This shows that the microtubule network guides lysosome trafficking to the synapse that forms upon BCR engagement with immobilized antigen. The molecular mechanisms behind MTOC repositioning have not been fully resolved, but they have been suggested to be dependent on forces generated by synapse-associated dynein in T cells[39]. This could also involve the coupling of microtubules to the actin network at the synapse by proteins such as cytoplasmic linker protein 170 (CLIP170), IQ-containing RAS GTPase-activating protein 1 (IQGAP1) and CLIP-associating protein 1 (CLASP1)[40,41]. In addition, the MTOC is reorientated in migrating fibroblasts as a result of the rearward movement of the nucleus that is generated by the actomyosin network[40]. Whether MTOC polarization to the synapse of B cells is also promoted by dynein and whether it is accompanied by nuclear movement will be interesting to investigate. Interestingly, microtubule polarization in B cells uses the conserved atypical protein kinase C ζ-type (PKCζ)–cell division control protein 42 (CDC42) polarity machinery[38], which is shared by many other biological systems and which modulates cell polarity during directed cell migration, tissue development and asymmetric cell division[41]. Accordingly, recent studies show that B cells also undergo asymmetric cell division following antigen stimulation (see below).

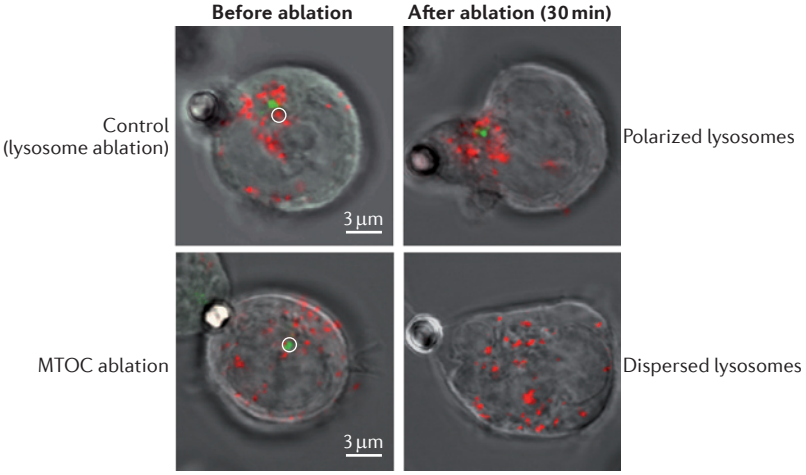


Figure 2 | **Polarization of the MTOC directs the trafficking of lysosomes towards the immunological synapse.** The figure shows microscopy images of a B lymphoma cell line expressing red fluorescent protein (RFP)-tagged cathepsin D and green fluorescent protein (GFP)-tagged centrin. The cells are engaged with an anti-IgG-coated bead before and after a two-photon laser-mediated ablation of either the microtubule organizing centre (MTOC) (lower panel) or a lysosome (upper panel), as a control. White circles show ablated regions. In the control cell there is recruitment and clustering of lysosomes towards the immunological synapse, whereas in the MTOC-ablated cells, the lysosomes remained dispersed. Scale bar represents 3 μm.

Antigen processing by B cells

Lysosome secretion at the immunological synapse. The immunological synapse is a dynamic platform where both endocytic and exocytic processes take place. Indeed, T helper and cytotoxic T cells were shown to secrete effector molecules, such as cytokines and lytic granules, through the immunological synapse[42,43], thereby providing a selective way in which to activate or to destroy interacting target cells. Exocytosis at the immunological synapse in T cells was found to occur in subdomains that have low levels of polymerized actin[44], which could facilitate the local secretion of molecules[45]. Cortical actin was shown to function as a fusion barrier that impedes exocytosis at the plasma membrane of epithelial cells[46]. In the case of B cells, the recruitment of lysosomes to the synaptic interface was shown to occur using total internal reflection fluorescence microscopy (TIRFM). The recruited lysosomes became progressively immobile, which suggests that vesicle docking at the plasma membrane was taking place. In addition, these studies showed that lysosomes were being locally secreted at the synapse, as shown by measuring the local

acidification of the extracellular synaptic space and by directly monitoring exocytic events using TIRFM. Whether lysosome exocytosis at the B cell synapse is associated with regions that have a low density of actin remains to be investigated.

The accurate docking and fusion of specialized vesicle carriers at the immunological synapse relies on complex cellular machinery that includes tethering factors from the RAB family of small G proteins, as well as factors from the SNARE (soluble *N*-ethylmaleimide-sensitive factor accessory protein receptor) family[47,48]. For instance, the delivery of recycling endosomes carrying T cell receptors at the immunological synapse is dependent on the SNARE proteins vesicle-associated membrane protein 2 (VAMP2) and VAMP3, and is accompanied by the clustering of the target SNAREs syntaxin 4 and synaptosomal-associated protein 23 (SNAP23) at the plasma membrane[49]. The exocytosis of secretory lysosomes has been well documented in many cell types[50], and in cytotoxic T cells it is regulated by RAB27A[51], which was recently shown to interact with synaptotagmin-like protein 1 (SLP1; also known as SYTL1) and SLP2 (REF. 52). The molecular regulators of lysosome exocytosis in B cells have not yet been identified. However, our unpublished data suggest that VAMP7 could be involved, as this SNARE protein is recruited with lysosome-associated membrane protein 1 (LAMP1)-expressing lysosomes to the B cell synapse following BCR engagement with immobilized antigen (A.-M.L.-D., M.-I.Y. and J. Diaz, unpublished observations).

Role of proteases in antigen extraction at the B cell synapse. Lysosomes are transported towards the site of antigen encounter, where they undergo exocytosis. This suggests that the activity of extracellular proteases that are locally released at the immunological synapse is required to free membrane-tethered antigens on antigen-bearing cells[38]. Interestingly, an analogous mechanism has been described in macrophages, in which lysosome secretion in an enclosed acidic extracellular zone is used to promote the digestion of aggregated lipoproteins by lipases and proteases[53]. Whether the B cell synapse that forms when the cell is in contact with membrane-tethered antigens is also a tightly enclosed structure is unknown. In addition, an uninvestigated aspect of B cell biology is the possibility that lysosomal lipases that are secreted at the immunological synapse have a role in promoting the acquisition of membrane-bound antigens. Alternatively, B cells could extract membrane-bound antigens through a process known as trogocytosis, which involves the intercellular exchange of membrane fragments[8,54,55]. It is probable that such a mechanism would also depend on cytoskeletal rearrangements to generate the force that would be required to extract the antigens and/or to facilitate the local secretion of hydrolases and possibly lipases.

The nature and amount of proteases that are secreted at the B cell synapse — an environment that is different from that of the lysosomal compartments — could also determine the size of the peptide antigen that will be presented to T cells. In addition, antigenic peptides that are generated in the extracellular space might also be directly loaded on cell surface MHC class II molecules, independently of the intracellular compartments. Indeed H2-DM, which is the chaperone that catalyses peptide loading on MHC class II molecules, has also been detected on the cell surface of both B cells and immature DCs[56,57], where extracellular antigen processing and peptide loading was shown to take place. Interestingly, and similarly to DCs, B cells contain much lower levels of lysosomal proteases than macrophages[58]. This favours the presentation of antigenic peptides on MHC class II molecules by limiting the complete destruction of the peptide determinants[59].

BCR internalization and signalling. Antigens that are acquired by the BCR in either a soluble or a tethered form induce receptor endocytosis and signalling events, which are both equally important for B cells to become fully activated. The local secretion of hydrolases at the immunological synapse promotes the extraction of membrane-bound antigens[38], which suggests that antigen degradation occurs, at least partially, in the extracellular space and probably at the same time as the main intracellular pathway. Indeed, BCR engagement induces the maturation of late endosomal and lysosomal compartments, into which receptors are rapidly internalized and antigens are degraded[60] and processed to form MHC class II–peptide complexes (FIG. 3). BCR internalization is clathrin-dependent[61] and relies on the ubiquitylation of the immunoglobulin α-chain[62] and the immunoglobulin heavy chain[63] of the BCR complex. Ubiquitylation of antigen–BCR complexes was recently shown to occur downstream of SYK-dependent signalling[64], which highlights that endocytic trafficking and signalling of the BCR are tightly linked. These events are also influenced by the nature of the antigen that binds to the BCR. Indeed, oligomeric antigens, such as those that are membrane-bound, were shown to trigger stronger BCR-mediated signalling and to promote more efficient endocytic trafficking of BCR–antigen complexes than monovalent antigens[65]. Interestingly, BCR signalling continues within endocytic compartments, where it leads to the sequential phosphorylation of kinases that control the transcription of genes required for B cell activation[66].

Recent work has highlighted the importance and complexity of the endomembrane machinery that is involved in this process. Beige mice, which are deficient in endosome biogenesis, show delayed transport of the endocytosed antigen–BCR complexes to their lysosomes and are consequently less efficient at presenting antigens to T cells[67]. However, these B cells show more sustained BCR signalling, which suggests that the delayed delivery of the BCR–antigen complex to the lysosomes facilitates continuous signalling from early endosomal compartments. Thus, the subcellular localization of the BCR, which changes following antigen recognition, not only facilitates the transport of antigens to be processed but also regulates the signalling effector functions that affect B cell fate *in vivo*.

Atypical protein kinase C ζ-type
(PKCζ). An atypical member of the PKC family that does not require either calcium or diacylglycerol for its activation. It associates with partitioning defective 3 (PAR3) to regulate cell polarity in diverse cell types.

Cell division control protein 42
(CDC42). A RHO-GTPase that controls diverse cellular functions including cell morphology, migration and cell division. By interacting with Wiskott–Aldrich syndrome protein (WASP), CDC42 regulates actin polymerization.

Asymmetric cell division
A cell division in which the organelles and proteins do not distribute equally, giving rise to two daughter cells with different properties and fates. This is required for cell specialization and mainly relies on asymmetry in the spindle position during the prophase stage of mitosis. Notably, stem cells can divide asymmetrically to give rise to two distinct daughter cells: one cell that is a copy of themselves and one cell that is programmed to differentiate into another cell type.

Total internal reflection fluorescence microscopy
(TIRFM). A fluorescence microscopy technique that involves illuminating and observing a thin layer of the specimen (about 200 nm) close to the cover slip using an 'evanescent wave' (which is formed when a laser encounters the glass surface above the critical angle in such a way that it is 'totally reflected'). It combines the speed and the resolution of the usual fluorescence microscopy (being an example of widefield microscopy) with the possibility of excluding excitation and emission from unwanted planes, providing a high signal to noise ratio.

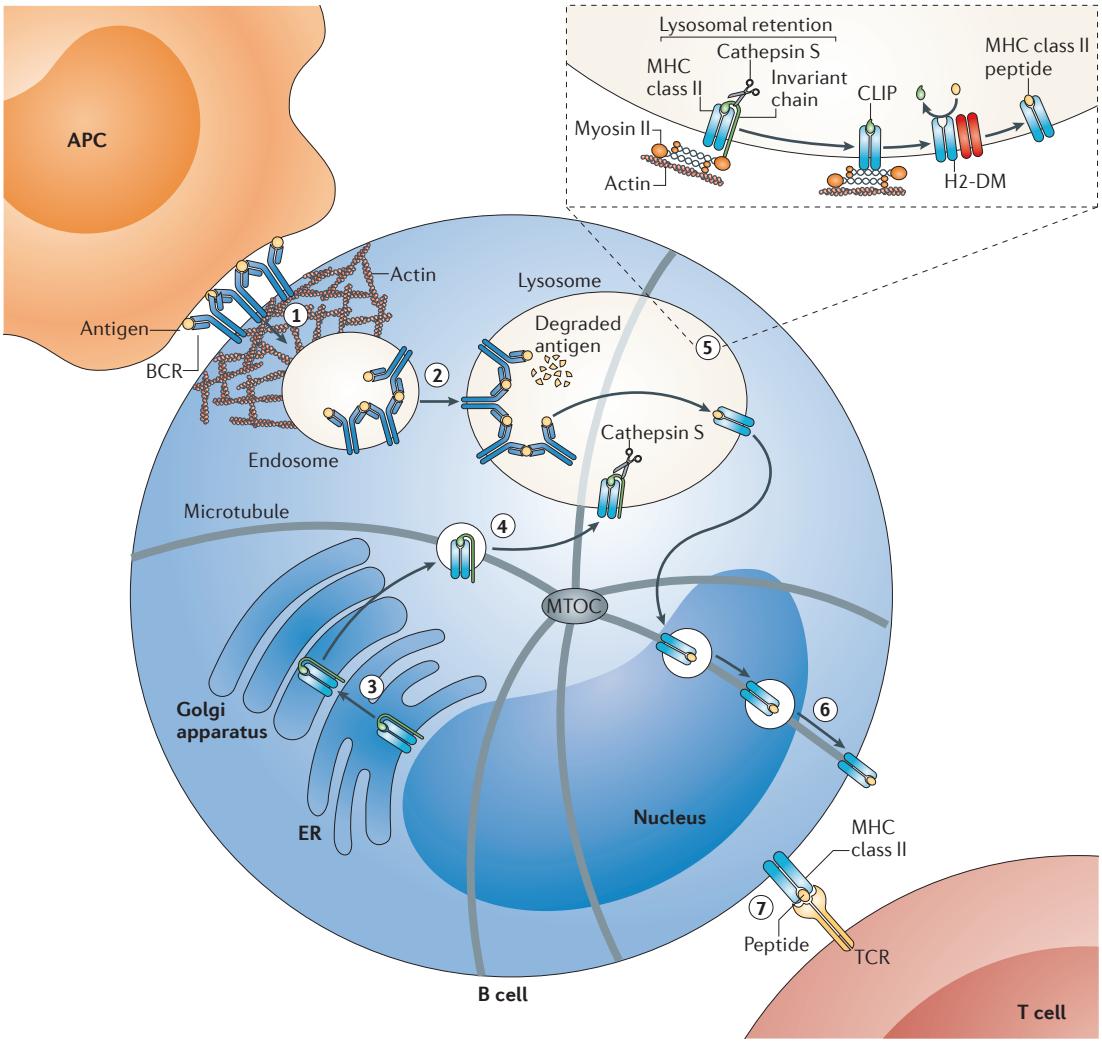


Figure 3 | **Membrane trafficking events required for antigen processing in B cells.** Antigen recognition is mediated by the B cell receptor (BCR). The engagement of antigen and BCR induces receptor oligomerization and downstream signalling that promotes dynamic actin cytoskeletal rearrangements. This enables efficient BCR–antigen internalization into late endosomal compartments (step 1). Concomitant with its endocytosis, the interaction of the BCR with antigen also promotes the biogenesis of the antigen-processing compartment in which both the antigen and the accessory molecules, such as MHC class II molecules, H2-DM and proteases, converge[60,109,110] (step 2). MHC class II molecules associate with their chaperone — the invariant chain in the endoplasmic reticulum (ER) — which has three essential functions: it assists in the folding of MHC class II molecules, it prevents early antigen binding and it directs the trafficking of MHC class II molecules through the *trans*-Golgi network to the endo-lysosomes[111] (step 3). This occurs as a result of leucine-based sorting motifs that bind the adaptor complexes AP1 and AP2 (step 4). The invariant chain also promotes the interaction with myosin II, which is required for the convergence of MHC class II molecules and BCR–antigen complexes in lysosomes that process antigens. In the antigen-processing compartment, proteolysis of both the antigen and the invariant chain takes place to produce antigenic peptides and to free the peptide-binding groove of MHC class II molecules, respectively. The last step of invariant chain cleavage requires the activity of the cysteine protease cathepsin S and is followed by peptide exchange, which is catalysed by the chaperone H2-DM[112,113] (step 5). This also removes the endosomal retention motif in the cytosolic tail of the invariant chain and enables mature MHC class II-peptide complexes to be exported to the cell surface (step 6). These complexes can then interact with their intended T cell (step 7). APC, antigen-presenting cell; CLIP, cytoplasmic linker protein; MTOC, microtubule organizing centre; TCR, T cell receptor.

Beige mice
Members of a mouse strain typified by beige hair that carry the lysosomal trafficking regulator (*Lyst*) mutation.These mice have an autosomal recessive disorder that is characterized by hypopigmentation and immune cell dysfunction. Beige mouse abnormalities result from aberrant lysosomal trafficking and are similar to those of patients with Chediak–Higashi syndrome.

Role of the actin cytoskeleton in the trafficking of BCR–antigen complexes. The molecular mechanisms responsible for the reorganization of the endocytic pathway that is triggered by BCR stimulation are not completely understood. Several lines of evidence suggest that a tight coupling of both BCR signalling and dynamic actin cytoskeletal reorganization is required. Indeed, BCR engagement triggers tyrosine phosphorylation of actin-binding protein (ABP1). This, in turn, promotes dynamin recruitment and actin rearrangements that enable efficient receptor internalization into late endosome compartments, where antigen processing takes place[68]. Other downstream BCR molecules that link receptor signalling to the actin cytoskeleton include

Bruton tyrosine kinase (BTK), which was recently shown to control the WASP-dependent actin dynamics that are required for the internalization and the processing of BCR–antigen complexes[69]. In addition, the tyrosine kinase SYK regulates actin remodelling, on which the transport of MHC class II$^+$ lysosomes towards incoming antigens depends[70]. Actin remodelling by SYK could involve its downstream effectors protein tyrosine kinase 2β (PYK2; also known as PTK2B) — which regulates the activity of the actin severing and capping protein gelsolin[71] — or VAV1 (which is a GTP exchange factor for RHO GTPases); both are known to be modulators of actin dynamics[72]. Indeed, VAV1 is recruited to BCR-signalling microclusters and is crucial for the propagation of B cell spreading in response to antigen stimulation[73].

Furthermore, through a mechanism that also requires the actin cytoskeleton, internalized BCR–antigen complexes are stored within non-terminal lysosomal compartments in which antigen degradation is limited. This therefore facilitates the prolonged production and the cell surface expression of antigenic peptide–MHC class II complexes, thereby favouring the presentation of antigens to T cells[74]. Finally, the actin-associated motor myosin II — which is activated following BCR engagement — was shown to regulate the polarized transport of MHC class II molecules towards internalized antigens[75]. This highlights that BCR-dependent actin remodelling also controls the trafficking of MHC class II molecules, promoting the formation of the antigen-processing compartment (FIG. 3).

Antigen presentation to T cells: T cell–B cell cooperation. A pivotal step in B cell activation is the presentation of processed antigens to CD4$^+$ T cells, which enables B cells to receive the necessary stimuli to become fully activated[1]. After antigen encounter, B cells must migrate towards the T cell boundary, where contacts with cognate T helper cells can be established[76]. The receptors that support this directed B cell migration are CC-chemokine receptor 7 (CCR7; which recognizes CC-chemokine ligand 19 (CCL19) and CCL21 that are produced by T stromal cells[77]) and Epstein–Barr virus-induced G protein-coupled receptor 2 (EBI2; also known as G protein-coupled receptor 183), which promote the migration of B cells to the outer follicle during the early stages of activation[78]. Importantly, the interaction between B cells and T cells also leads to the formation of an immunological synapse, where bidirectional activation signals are exchanged between the cells. Local secretion was shown to be promoted at the immunological synapse in both cell types by the generation of a polarized phenotype and the re-orientation of their MTOC, together with their Golgi apparatus, towards the synaptic interface[79]. In addition, in the same study it was shown that B cells also polarize MHC class II compartments towards the contact site with T cells, which the authors suggest could facilitate the local delivery of antigenic ligands for TCR stimulation[79]. After interacting with T cells, B cells migrate to the interfollicular region where they proliferate and differentiate into short-lived plasmablasts. These cells contribute to the primary immune response by generating antibodies that are of a relatively low affinity[80]. Another group of activated B cells, expressing high levels of CXCR5, concomitantly migrates into the B cell follicle, where they continue to proliferate and to form germinal centres. In the B cell follicle, B cells undergo affinity maturation and differentiate into antibody-producing plasma cells or long-lived memory B cells[81,82]. The signals that determine the differentiation of B cells are poorly understood, but the context of the early activation events, such as antigen recognition and presentation to T follicular helper cells[83] (see below), probably influences their final outcome.

Cell polarity in B cell activation

Cell polarity has been extensively studied in a wide range of systems from budding yeast to immune cells (BOX 1), in which the interpretation of spatial cues initiates the formation of landmarks that are progressively reinforced to form a specialized domain[84]. In this section, we review the role of B cell polarity during the key stages of B cell activation: antigen encounter and differentiation in germinal centres.

B cell polarity and antigen acquisition. The cellular contacts that occur between B cells and antigen-bearing cells, such as FDCs or macrophages, can generally last between 20 and 30 minutes[6,8]. Therefore, the immediate establishment of cell polarity could be a mechanism that is used by B cells to rapidly acquire antigen and to optimize their activation during these short encounters. Indeed, it has been shown that B cells rapidly polarize their MTOC, together with MHC class II$^+$ lysosomes, towards the antigen contact site — a process that relies on the small GTPase CDC42 and its downstream effector protein PKCζ. Impairment of MTOC and lysosome polarization by silencing CDC42 or PKCζ compromises the ability of B cells to extract, process and present immobilized antigen to T cells[38]. Importantly, genetic evidence also shows the involvement of these polarity proteins in B cell functions. Mice with a conditional deletion of CDC42 show defects in B cell lymphogenesis, whereas PKCζ-deficient mice show impaired humoral immune responses[85,86].

Studies *in vivo* using two-photon microscopy to observe where and how B cells acquire particulate antigens, have shown that internalized antigens are frequently concentrated at the uropod of B cells that are migrating to the T cell zone[6]. This suggests that following antigen acquisition, B cells continue to show a polarized phenotype. Furthermore, recent studies have shown that this asymmetric distribution of antigens within B cells is maintained throughout cell division, which leads to asymmetric antigen segregation among daughter cells; this, consequently, provides the daughter cells with differential capacities for antigen presentation[87]. The daughter cells that have inherited larger amounts of antigens are more effective at stimulating cognate T cells, which might give them an advantage in competing for the limited T cell help that is available. Whether the generation of these unequal populations ultimately changes the effector fates of B cells *in vivo* remains to be elucidated.

Uropod
Protrusion of the plasma membrane that forms at the rear end of migrating cells.

B cell polarity in germinal centres. The mechanisms that determine the differentiation of B cells into long-lived memory cells or plasma cells in germinal centres have not yet been resolved, but they are probably influenced by antigen affinity, by extracellular cues that are produced by other cell types and by the time of activation[88]. The selection of high-affinity B cell clones in the germinal centres is thought to be dependent on signals generated either by BCR crosslinking with antigens that are tethered to the surface of FDCs or by germinal centre T helper cells that have been stimulated by high-affinity B cell clones presenting uptaken antigen on MHC class II molecules[89]. Recent studies using intravital microscopy imaging combined with *in situ* photoactivation to label germinal centre B cells have provided strong evidence that T helper cells in the germinal centre are the limiting factor in affinity-based selection[90].

The ability of B cells to rapidly polarize their antigen-processing machinery in response to defined extracellular cues could determine their different cellular fates. It has been shown that mutations in the RHO or RAC guanine nucleotide exchange factor dedicator of cytokinesis 8 (DOCK8) leads to deficient intercellular adhesion molecule 1 (ICAM1) clustering at the B cell synaptic interface, thereby impairing peripheral supramolecular activation cluster (pSMAC) formation. This defect results in impaired affinity maturation of B cells and deficiencies in the mature antibody responses[91]. Importantly, DOCK8 is a CDC42-specific guanine-nucleotide exchange factor and was recently shown to control the activity of CDC42 that is present at the leading edge of migrating DCs. DOCK8-deficient DCs show impaired CDC42 activation at the leading-edge membrane, which results in an inability to extend long protrusions in the direction of migration[92]. Whether the DOCK8-dependent defects that are observed in B cells also result from a failure to locally activate CDC42 and, consequently, to efficiently polarize the cell during synapse formation is an interesting possibility to investigate.

Activated B cells move with a highly polarized morphology within germinal centres, forming extensions such as filopodia at the leading edge and uropods at the trailing edge. In addition, stationary B cells have extending protrusions to contact FDCs in order to continue to sample their microenvironment[93]. Whether B cells also use conserved polarity proteins to establish this specialized morphology when scanning for antigens remains to be addressed. The recent findings about the influence of polarity in B cell asymmetric cell division[94] suggest that the first antigen encounter could provide the extracellular cue that the cell uses to successfully carry out its differentiation programme through a highly conserved polarity mechanism. Therefore, it is essential to define the molecules that regulate early B cell polarization.

In addition to its role in promoting efficient antigen processing and presentation, PKCζ-dependent B cell polarization is also required for the later stages of B cell activation. In germinal centres, activated B cells asymmetrically segregate PKCζ, along with the transcriptional regulator B cell lymphoma 6 (BCL-6) and the interleukin-21 receptor (IL-21R), which are unequally distributed among daughter cells. This promotes diversity among the daughter cells and could ensure that two cells with different fates are generated, which are destined either to accomplish effector functions or to develop into memory cells, thereby providing a mechanism for self-renewal[94]. However, the fate of daughter cells *in vivo* remains to be determined. Interestingly, this asymmetric cell division requires polarity cues from the microenvironment, as B cells that have defects in the adhesion molecule ICAM1 fail to divide asymmetrically[94].

Models of cell polarity: perspectives and analogies. From a physical point of view, cell polarity is an interesting example of symmetry breaking[95], in which the system starts with a homogeneous distribution of molecules and finishes by forming a defined pattern, such as a single patch of molecules at the pole of the cell[96]. Although cell polarity in B cells is not as well studied as cell polarity in yeast and in the model organism *Caenorhabditis elegans*, several key features of cell polarity that are specific to B cells are beginning to be identified, and some computational models have been created[97,98]. First, the synapse comprises a signalling platform that breaks cell symmetry and guides the formation of a polarized phenotype (BOX 1). At the immunological synapse, microtubule molecular motors such as dynein are required for the coalescence but not for the formation of small signalling BCR microclusters[27]. Second, the activation of CDC42 and its downstream effector PKCζ are required to establish and to maintain B cell polarity[38]. Third, B cells develop actomyosin contractions that might have a number of functions: they might create an intracellular flow towards the synapse as in *C. elegans*[99]; they might gather BCR–antigen complexes at the central SMAC (cSMAC)[21,75]; or they might generate the forces that are required for antigen internalization. Fourth, integrins such as LFA1 have been shown to enhance the signal that is delivered following BCR engagement[17]. Whether integrin-mediated mechanosensing affects cell polarity remains to be established. Interestingly, it has recently been shown that B cells discriminate between antigens that are associated with substrates of different rigidities, which indicates that they are competent for mechanotransduction[100].

Data that have been gathered from theoretical models and genetics tools that are available in yeast are consistent with the idea that there is a general mechanism for cell polarity, which consists of a combination of positive and negative feedback loops[101–103]; the positive feedback loops function swiftly and amplify small fluctuations in signalling, whereas the negative feedback loops confine the actions of the initial positive feedback loops using a long-range inhibitor mechanism. In the case of B cells, positive feedback loops could correspond to the positive signals that control proteins involved in the actomyosin dynamics downstream of BCR, such as RAC[28], RAP1 (REF. 29) or BTK, which activate the actin nucleator WASP[69], or to pathways that are induced by co-stimulatory receptors such as CD19 (REF. 23). The inhibitory mechanisms might simply arise from the limited levels of proteins, or from the involvement

Filopodia
Dynamic actin-rich filamentous protrusions that extend from cells.

Symmetry breaking
The process by which a system switches from a disordered or a uniform state to a state in which an ordered shape, direction or pattern is established; for example, proteins that are normally uniformly distributed will concentrate at a single spot after receiving a certain stimulus.

Mechanosensing
The capacity of cells to sense mechanical stimuli, such as deformation of the membrane, the cortex, the nucleus and other structures, or to sense changes in the adhesive properties of the substrate.

Box 1 | **Establishing polarity: the roles of PAR, actin and microtubules**

Cell polarity is fundamental for the development of multicellular organisms as well as for the functional responses of individual cells. Cell polarity is coordinated by conserved proteins belonging to the partitioning defective (PAR) polarity complex, namely Scribble and Crumbs. PAR proteins function downstream of cell division control protein 42 (CDC42) to regulate actin polymerization and they are dynamically associated with the cell cortex and the cytoskeleton. Relevant models of cell polarity are described below.

Yeast

In the budding yeast *Saccharomyces cerevisiae*, Cdc42 is the main polarity activator that functions downstream of Bud1 (which is the yeast homologue of Rap1). In its active GTP-bound form, Cdc42 localizes to sites of active growth (the bud) where it regulates actin polymerization via formins and Wiskott-Aldrich syndrome protein (Wasp). Actin cytoskeletal rearrangements and the microtubule network coordinate the delivery of proteins to the polarized bud (see the figure).

***Caenorhabditis elegans* oocyte**

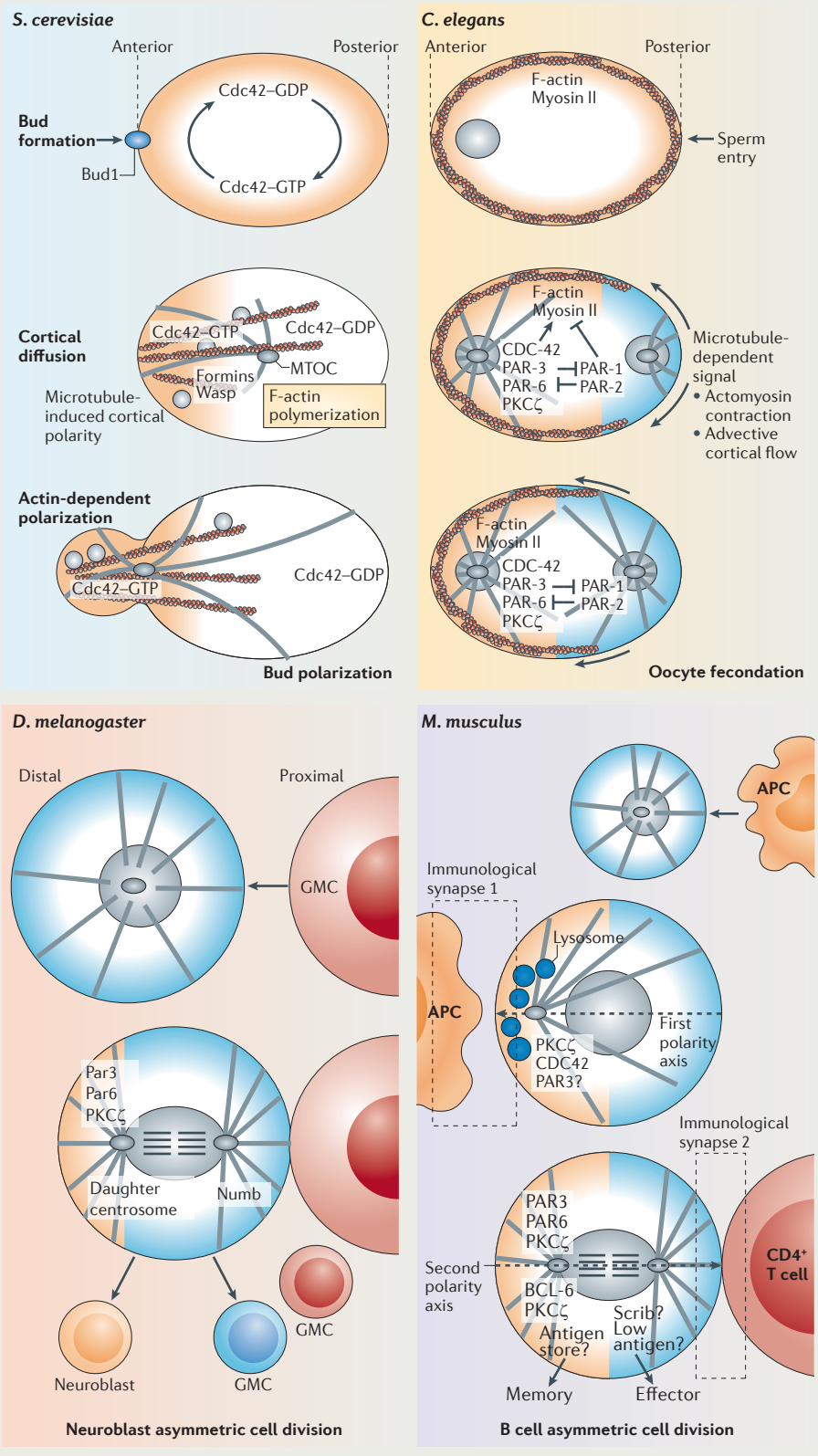
PAR is crucial for generating the anterior–posterior body axis in *C. elegans* embryos. The PAR-3–PAR-6– protein kinase C ζ-type (PKCζ) domain forms at the anterior pole, opposite to the entrance of the sperm. Depletion of these proteins on the posterior side causes an accumulation of PAR-1 and PAR-2 via cross-inhibition mechanisms. Anterior PAR proteins promote actomyosin contraction, which is inhibited on the opposite side by PAR-1. In addition, there is a diffusive signal from the microtubule organizing centre (MTOC) to the local cortex that inhibits myosin II contraction. As a result CDC-42 and anterior PAR accumulate away from the sperm MTOC (see the figure).

Neuroblast progenitor cells in *Drosophila melanogaster*

The mitotic spindle orientates following contact with the ganglion mother cell (GMC) and the daughter centrosome is positioned at the distal pole. During this process, Par proteins are sequestered at the distal pole, whereas the fate determinant Numb remains at the proximal pole. This induces the formation of two different daughter cells: a neuroblast and a GMC (see the figure).

Polarity in B cells in *Mus musculus*

During B cell activation, several axes of polarity are established. First, interaction with antigen-presenting cells (APCs) induces MTOC polarization to the immunological synapse by a mechanism that is dependent on CDC42 and PKCζ. This promotes the polarized recruitment and secretion of lysosomes at the immunological synapse, which is required for antigen extraction. Second, when B cells present antigens to T helper cells they receive a polarity cue that leads to asymmetric cell division in germinal centres. B cells asymmetrically segregate PKCζ, the transcriptional regulator B cell lymphoma 6 (BCL-6) and the interleukin-21 receptor, which are unequally distributed among daughter cells (see the figure). Question marks indicate proteins that are thought to be involved but for which further evidence is required.



Membrane tension
A measure of how stretched the cell membrane is to compensate for the osmotic pressure of the cytoplasm. It can be modified by changing the osmotic pressure of the medium and can be measured by micromanipulation techniques measuring the force necessary to pull tubes of membrane.

Partitioning defective 3
(PAR3). Together with PAR6 these proteins form the PAR polarity complex and are both scaffolding proteins that are implicated in cell polarity. PAR3 and PAR6 bind to each other via their PDZ (PSD95, DLGA and ZO1 homology) domains. They localize at the plasma membrane via atypical protein kinase C ζ-type, cell division control protein 42 (bound to PAR6) or via their PDZ domains.

Table 1 | **Effect of polarity-related proteins on B cell functions**

Protein	Functions shown *in vitro*	Functions shown *in vivo*	Refs
DOCK8	Required for spreading and synapse formation	Required for mature antibody responses and germinal centre formation	91
CDC42	Required for antigen extraction, processing and presentation	Required for early and late B cell development, and B cell proliferation, survival, and signalling. Absence of CDC42 leads to impaired antibody production	38,85
PKCζ	Required for antigen extraction, processing and presentation	Required for BCR signalling, cell proliferation and survival; knockout mice show defects in immune responses	38,86
Dynein	Involved in the gathering of antigen during immunological synapse formation	Data not available	27
RAP1	Required for membrane spreading and synapse formation, and for BCR signalling in response to particulate antigen	RAP1B-knockout mice have defects in the development of marginal zone B cells and reduced homing of B cells to the lymph nodes	29,114,115
RAC	RAC1 and RAC2 regulate B cell spreading during immunological synapse formation	RAC1 and RAC2 are required for B cell development, signalling and survival	28,114,115
WASP	Required for B cell spreading and migration	WASP deficiency leads to a reduction in mature peripheral B cell subsets. Mice that are double-deficient for both WASP and NWASP have reduced B cell immune responses to T cell-independent and T cell-dependent antigens	33,116

BCR, B cell receptor; CDC42, cell division control protein 42; DOCK8, dedicator of cytokinesis 8; NWASP, neural Wiskott–Aldrich syndrome protein; PKCζ, protein kinase C type-ζ.

of negative regulators, such as phosphatase and tensin homologue (PTEN)[104] or SH2 domain-containing protein tyrosine phosphatase 1 (SHP1; also known as PTPN6)[105]. In addition, the mechanical properties of the membrane, such as membrane tension, might help to determine a polarity axis by restricting the diffusion of positive signals[106].

It is worth noting that the process of polarization should be understood in a stochastic manner; in many cases the axis of polarity is randomly established and, for the most part, it is only stabilized following signalling that induces cytoskeletal rearrangements[107] (BOX 1). This supports the idea that, in order to quickly respond to a signal, cells can maintain an unstable state. It is tempting to speculate that, in B cells, contact with immobilized antigens breaks the symmetry of the cell, and that ancestral polarity proteins, such as CDC42, PKCζ and partitioning defective 3 (PAR3), amplify the initial signals, thereby reinforcing the selected polarity axis. Testing the robustness of B cell polarity by activating the BCR at two different cell poles should help to determine whether this hypothesis is correct. Super resolution microscopy, which has so far been implemented to study the static synapse substructures[108], coupled with physical modelling, should shed light on the dynamics and the spatial regulation of polarity proteins.

Conclusions

Recent work has provided new insights into how B cells coordinate complex cellular pathways in order to achieve their immune functions. Studies that have been carried out both *in vitro* and *in vivo* have highlighted the dynamic cellular changes that B cells undergo while they are scanning for antigens in the lymph nodes, as well as following antigen extraction, processing and presentation. It is becoming increasingly clear that B cells are dependent on cell polarity as a central mechanism to coordinate these functions and that alterations in key polarity proteins can affect the outcome of B cell immune responses (TABLE 1). Therefore, the identification of new intracellular proteins that regulate B cell polarity during synapse formation and during their directed cell migration in germinal centres could provide insights into how B cells efficiently achieve these immune functions. Special focus should be given to extracellular proteins in the lymphoid microenvironment, such as chemokines, extracellular matrix proteins and galectins. These proteins could modulate different aspects of cell polarity, such as cytoskeletal rearrangements and lysosome trafficking, and could thereby affect the ability of B cells to respond to antigens. Proteins that modulate B cell polarity could represent valuable candidates to modulate B cell responses *in vivo*.

1. Mitchison, N. A. T-cell–B-cell cooperation. *Nature Rev. Immunol.* **4**, 308–312 (2004).
2. Batista, F. D., Iber, D. & Neuberger, M. S. B cells acquire antigen from target cells after synapse formation. *Nature* **411**, 489–494 (2001).
3. Bertrand, F. E. *et al.* Microenvironmental influences on human B-cell development. *Immunol. Rev.* **175**, 175–186 (2000).
4. von Andrian, U. H. & Mempel, T. R. Homing and cellular traffic in lymph nodes. *Nature Rev. Immunol.* **3**, 867–878 (2003).
5. Cyster, J. G. B cell follicles and antigen encounters of the third kind. *Nature Immunol.* **11**, 989–996 (2010).
6. Carrasco, Y. R. & Batista, F. D. B cells acquire particulate antigen in a macrophage-rich area at the boundary between the follicle and the subcapsular sinus of the lymph node. *Immunity* **27**, 160–171 (2007).
7. Junt, T. *et al.* Subcapsular sinus macrophages in lymph nodes clear lymph-borne viruses and present them to antiviral B cells. *Nature* **450**, 110–114 (2007).

References 6 and 7 highlight roles for subcapsular macrophages in the lymph nodes in the presentation of large antigens to B cells *in vivo*.

8. Suzuki, K., Grigorova, I., Phan, T. G., Kelly, L. M. & Cyster, J. G. Visualizing B cell capture of cognate antigen from follicular dendritic cells. *J. Exp. Med.* **206**, 1485–1493 (2009).
9. Qi, H., Egen, J. G., Huang, A. Y. & Germain, R. N. Extrafollicular activation of lymph node B cells by antigen-bearing dendritic cells. *Science* **312**, 1672–1676 (2006).
References 8 and 9 use two-photon microscopy to visualize *in vivo* how B cells capture antigen from FDCs in primary follicles or from DCs upon entry to the lymph nodes.
10. Pape, K. A., Catron, D. M., Itano, A. A. & Jenkins, M. K. The humoral immune response is initiated in lymph nodes by B cells that acquire soluble antigen directly in the follicles. *Immunity* **26**, 491–502 (2007).
11. Clark, S. L. Jr. The reticulum of lymph nodes in mice studied with the electron microscope. *Am. J. Anat.* **110**, 217–257 (1962).
12. Gretz, J. E., Norbury, C. C., Anderson, A. O., Proudfoot, A. E. & Shaw, S. Lymph-borne chemokines and other low molecular weight molecules reach high endothelial venules via specialized conduits while a functional barrier limits access to the lymphocyte microenvironments in lymph node cortex. *J. Exp. Med.* **192**, 1425–1440 (2000).
13. Batista, F. D. & Harwood, N. E. The who, how and where of antigen presentation to B cells. *Nature Rev. Immunol.* **9**, 15–27 (2009).
14. Saez de Guinoa, J., Barrio, L., Mellado, M. & Carrasco, Y. R. CXCL13/CXCR5 signaling enhances BCR-triggered B-cell activation by shaping cell dynamics. *Blood* **118**, 1560–1569 (2011).
15. Grakoui, A. *et al.* The immunological synapse: a molecular machine controlling T cell activation. *Science* **285**, 221–227 (1999).
16. Yuseff, M. I., Lankar, D. & Lennon-Dumenil, A. M. Dynamics of membrane trafficking downstream of B and T cell receptor engagement: impact on immune synapses. *Traffic* **10**, 629–636 (2009).
17. Carrasco, Y. R., Fleire, S. J., Cameron, T., Dustin, M. L. & Batista, F. D. LFA-1/ICAM-1 interaction lowers the threshold of B cell activation by facilitating B cell adhesion and synapse formation. *Immunity* **20**, 589–599 (2004).
18. Cambier, J. C., Pleiman, C. M. & Clark, M. R. Signal transduction by the B cell antigen receptor and its coreceptors. *Annu. Rev. Immunol.* **12**, 457–486 (1994).
19. Reth, M. & Wienands, J. Initiation and processing of signals from the B cell antigen receptor. *Annu. Rev. Immunol.* **15**, 453–479 (1997).
20. Baba, Y. & Kurosaki, T. Impact of Ca^{2+} signaling on B cell function. *Trends Immunol.* **32**, 589–594 (2011).
21. Fleire, S. J. *et al.* B cell ligand discrimination through a spreading and contraction response. *Science* **312**, 738–741 (2006).
This paper describes the two-phase spreading and contraction response exerted by B cells that are in contact with membrane-bound antigens. This response promotes antigen gathering into a central cluster at the synapse.
22. Harwood, N. E. & Batista, F. D. Early events in B cell activation. *Annu. Rev. Immunol.* **28**, 185–210 (2010).
23. Depoil, D. *et al.* CD19 is essential for B cell activation by promoting B cell receptor-antigen microcluster formation in response to membrane-bound ligand. *Nature Immunol.* **9**, 63–72 (2008).
24. Depoil, D. *et al.* Early events of B cell activation by antigen. *Sci. Signal.* **2**, Pt1 (2009).
25. Tolar, P., Sohn, H. W. & Pierce, S. K. Viewing the antigen-induced initiation of B-cell activation in living cells. *Immunol. Rev.* **221**, 64–76 (2008).
This review highlights experiments using live-cell imaging technologies that can be used to show the early spatiotemporal signalling events that occur downstream of BCR engagement.
26. Treanor, B., Depoil, D., Bruckbauer, A. & Batista, F. D. Dynamic cortical actin remodeling by ERM proteins controls BCR microcluster organization and integrity. *J. Exp. Med.* **208**, 1055–1068 (2011).
27. Schnyder, T. *et al.* B cell receptor-mediated antigen gathering requires ubiquitin ligase Cbl and adaptors Grb2 and Dok-3 to recruit dynein to the signaling microcluster. *Immunity* **34**, 905–918 (2011).
28. Arana, E. *et al.* Activation of the small GTPase Rac2 via the B cell receptor regulates B cell adhesion and immunological-synapse formation. *Immunity* **28**, 88–99 (2008).
29. Lin, K. B. *et al.* The rap GTPases regulate B cell morphology, immune-synapse formation, and signaling by particulate B cell receptor ligands. *Immunity* **28**, 75–87 (2008).
30. Freeman, S. A. *et al.* Cofilin-mediated F-actin severing is regulated by the Rap GTPase and controls the cytoskeletal dynamics that drive lymphocyte spreading and BCR microcluster formation. *J. Immunol.* **187**, 5887–5900 (2011).
31. Hao, S. & August, A. Actin depolymerization transduces the strength of B-cell receptor stimulation. *Mol. Biol. Cell* **16**, 2275–2284 (2005).
32. Treanor, B. *et al.* The membrane skeleton controls diffusion dynamics and signaling through the B cell receptor. *Immunity* **32**, 187–199 (2010).
33. Meyer-Bahlburg, A. *et al.* Wiskott–Aldrich syndrome protein deficiency in B cells results in impaired peripheral homeostasis. *Blood* **112**, 4158–4169 (2008).
34. Tsourkas, P. K., Baumgarth, N., Simon, S. I. & Raychaudhuri, S. Mechanisms of B-cell synapse formation predicted by Monte Carlo simulation. *Biophys. J.* **92**, 4196–4208 (2007).
35. Tsourkas, P. K., Longo, M. L. & Raychaudhuri, S. Monte Carlo study of single molecule diffusion can elucidate the mechanism of B cell synapse formation. *Biophys. J.* **95**, 1118–1125 (2008).
36. Tsourkas, P. K. & Raychaudhuri, S. Modeling of B cell synapse formation by Monte Carlo simulation shows that directed transport of receptor molecules is a potential formation mechanism. *Cell. Mol. Bioeng.* **3**, 256–268 (2010).
37. Stinchcombe, J. C. *et al.* Centriole polarisation to the immunological synapse directs secretion from cytolytic cells of both the innate and adaptive immune systems. *BMC Biol.* **9**, 45 (2011).
38. Yuseff, M. I. *et al.* Polarized secretion of lysosomes at the B cell synapse couples antigen extraction to processing and presentation. *Immunity* **35**, 361–374 (2011).
This paper was the first to show how B cells use conserved polarity mechanisms to locally recruit and secrete lysosomes at the immunological synapse in order to extract and present immobilized antigens on MHC class II molecules.
39. Combs, J. *et al.* Recruitment of dynein to the Jurkat immunological synapse. *Proc. Natl Acad. Sci. USA* **103**, 14883–14888 (2006).
40. Gomes, E. R., Jani, S. & Gundersen, G. G. Nuclear movement regulated by Cdc42, MRCK, myosin, and actin flow establishes MTOC polarization in migrating cells. *Cell* **121**, 451–463 (2005).
41. Iden, S. & Collard, J. G. Crosstalk between small GTPases and polarity proteins in cell polarization. *Nature Rev. Mol. Cell Biol.* **9**, 846–859 (2008).
42. Stinchcombe, J. C., Bossi, G., Booth, S. & Griffiths, G. M. The immunological synapse of CTL contains a secretory domain and membrane bridges. *Immunity* **15**, 751–761 (2001).
43. Poo, W. J., Conrad, L. & Janeway, C. A. Jr. Receptor-directed focusing of lymphokine release by helper T cells. *Nature* **332**, 378–380 (1988).
44. Stinchcombe, J. C., Majorovits, E., Bossi, G., Fuller, S. & Griffiths, G. M. Centrosome polarization delivers secretory granules to the immunological synapse. *Nature* **443**, 462–465 (2006).
45. Chemin, K. *et al.* Cytokine secretion by $CD4^+$ T cells at the immunological synapse requires Cdc42-dependent local actin remodeling but not microtubule organizing center polarity. *J. Immunol.* **189**, 2159–2168 (2012).
46. Xu, J. *et al.* Mechanism of polarized lysosome exocytosis in epithelial cells. *J. Cell Sci.* **125**, 5937–5943 (2012).
47. Jordens, I., Marsman, M., Kuijl, C. & Neefjes, J. Rab proteins, connecting transport and vesicle fusion. *Traffic* **6**, 1070–1077 (2005).
48. Weber, T. *et al.* SNAREpins: minimal machinery for membrane fusion. *Cell* **92**, 759–772 (1998).
49. Das, V. *et al.* Activation-induced polarized recycling targets T cell antigen receptors to the immunological synapse; involvement of SNARE complexes. *Immunity* **20**, 577–588 (2004).
50. Andrews, N. W. Regulated secretion of conventional lysosomes. *Trends Cell Biol.* **10**, 316–321 (2000).
51. Stinchcombe, J. C. *et al.* Rab27a is required for regulated secretion in cytotoxic T lymphocytes. *J. Cell Biol.* **152**, 825–834 (2001).
52. Holt, O. *et al.* Slp1 and Slp2-a localize to the plasma membrane of CTL and contribute to secretion from the immunological synapse. *Traffic* **9**, 446–457 (2008).
53. Haka, A. S. *et al.* Macrophages create an acidic extracellular hydrolytic compartment to digest aggregated lipoproteins. *Mol. Biol. Cell* **20**, 4932–4940 (2009).
54. Aucher, A., Magdeleine, E., Joly, E. & Hudrisier, D. Capture of plasma membrane fragments from target cells by trogocytosis requires signaling in T cells but not in B cells. *Blood* **111**, 5621–5628 (2008).
55. Joly, E. & Hudrisier, D. What is trogocytosis and what is its purpose? *Nature Immunol.* **4**, 815 (2003).
56. Arndt, S. O. *et al.* Functional HLA-DM on the surface of B cells and immature dendritic cells. *EMBO J.* **19**, 1241–1251 (2000).
57. Santambrogio, L. *et al.* Extracellular antigen processing and presentation by immature dendritic cells. *Proc. Natl Acad. Sci. USA* **96**, 15056–15061 (1999).
58. Delamarre, L., Pack, M., Chang, H., Mellman, I. & Trombetta, E. S. Differential lysosomal proteolysis in antigen-presenting cells determines antigen fate. *Science* **307**, 1630–1634 (2005).
59. Lennon-Dumenil, A. M. *et al.* Analysis of protease activity in live antigen-presenting cells shows regulation of the phagosomal proteolytic contents during dendritic cell activation. *J. Exp. Med.* **196**, 529–540 (2002).
60. Lankar, D. *et al.* Dynamics of major histocompatibility complex class II compartments during B cell receptor-mediated cell activation. *J. Exp. Med.* **195**, 461–472 (2002).
61. Stoddart, A. *et al.* Lipid rafts unite signaling cascades with clathrin to regulate BCR internalization. *Immunity* **17**, 451–462 (2002).
62. Zhang, M. *et al.* Ubiquitinylation of Igβ dictates the endocytic fate of the B cell antigen receptor. *J. Immunol.* **179**, 4435–4443 (2007).
63. Drake, L., McGovern-Brindisi, E. M. & Drake, J. R. BCR ubiquitination controls BCR-mediated antigen processing and presentation. *Blood* **108**, 4086–4093 (2006).
64. Katkere, B., Rosa, S. & Drake, J. R. The Syk-binding ubiquitin ligase c-Cbl mediates signaling-dependent B cell receptor ubiquitination and B cell receptor-mediated antigen processing and presentation. *J. Biol. Chem.* **287**, 16636–16644 (2012).
65. Kim, Y. M. *et al.* Monovalent ligation of the B cell receptor induces receptor activation but fails to promote antigen presentation. *Proc. Natl Acad. Sci. USA* **103**, 3327–3332 (2006).
66. Chaturvedi, A., Martz, R., Dorward, D., Waisberg, M. & Pierce, S. K. Endocytosed BCRs sequentially regulate MAPK and Akt signaling pathways from intracellular compartments. *Nature Immunol.* **12**, 1119–1126 (2011).
67. Chatterjee, P., Tiwari, R. K., Rath, S., Bal, V. & George, A. Modulation of antigen presentation and B cell receptor signaling in B cells of beige mice. *J. Immunol.* **188**, 2695–2702 (2012).
This paper shows that BCR signalling that is initiated at the plasma membrane continues in intracellular compartments, initiating a sequential phosphorylation of kinases.
68. Onabajo, O. O. *et al.* Actin-binding protein 1 regulates B cell receptor-mediated antigen processing and presentation in response to B cell receptor activation. *J. Immunol.* **180**, 6685–6695 (2008).
69. Sharma, S., Orlowski, G. & Song, W. Btk regulates B cell receptor-mediated antigen processing and presentation by controlling actin cytoskeleton dynamics in B cells. *J. Immunol.* **182**, 329–339 (2009).
70. Le Roux, D. *et al.* Syk-dependent actin dynamics regulate endocytic trafficking and processing of antigens internalized through the B-cell receptor. *Mol. Biol. Cell* **18**, 3451–3462 (2007).
71. Wang, Q. *et al.* Regulation of the formation of osteoclastic actin rings by proline-rich tyrosine kinase 2 interacting with gelsolin. *J. Cell Biol.* **160**, 565–575 (2003).
72. Hornstein, I., Alcover, A. & Katzav, S. Vav proteins, masters of the world of cytoskeleton organization. *Cell. Signal.* **16**, 1–11 (2004).
73. Weber, M. *et al.* Phospholipase C-γ2 and Vav cooperate within signaling microclusters to propagate B cell spreading in response to membrane-bound antigen. *J. Exp. Med.* **205**, 853–868 (2008).
74. Gondre-Lewis, T. A., Moquin, A. E. & Drake, J. R. Prolonged antigen persistence within nonterminal late endocytic compartments of antigen-specific B lymphocytes. *J. Immunol.* **166**, 6657–6664 (2001).

75. Vascotto, F. *et al.* The actin-based motor protein myosin II regulates MHC class II trafficking and BCR-driven antigen presentation. *J. Cell Biol.* **176**, 1007–1019 (2007).
76. Pereira, J. P., Kelly, L. M. & Cyster, J. G. Finding the right niche: B-cell migration in the early phases of T-dependent antibody responses. *Int. Immunol.* **22**, 413–419 (2010).
77. Reif, K. *et al.* Balanced responsiveness to chemoattractants from adjacent zones determines B-cell position. *Nature* **416**, 94–99 (2002).
78. Pereira, J. P., Kelly, L. M., Xu, Y. & Cyster, J. G. EBI2 mediates B cell segregation between the outer and centre follicle. *Nature* **460**, 1122–1126 (2009).
79. Duchez, S., Rodrigues, M., Bertrand, F. & Valitutti, S. Reciprocal polarization of T and B cells at the immunological synapse. *J. Immunol.* **187**, 4571–4580 (2011).
80. Cunningham, A. F. *et al. Salmonella* induces a switched antibody response without germinal centers that impedes the extracellular spread of infection. *J. Immunol.* **178**, 6200–6207 (2007).
81. Allen, C. D., Okada, T. & Cyster, J. G. Germinal-center organization and cellular dynamics. *Immunity* **27**, 190–202 (2007).
82. MacLennan, I. C. Germinal centers. *Annu. Rev. Immunol.* **12**, 117–139 (1994).
83. Crotty, S. Follicular helper CD4 T cells (T_{FH}). *Annu. Rev. Immunol.* **29**, 621–663 (2011).
84. Drubin, D. G. & Nelson, W. J. Origins of cell polarity. *Cell* **84**, 335–344 (1996).
85. Guo, F., Velu, C. S., Grimes, H. L. & Zheng, Y. Rho GTPase Cdc42 is essential for B-lymphocyte development and activation. *Blood* **114**, 2909–2916 (2009).
86. Martin, P. *et al.* Role of ζ-PKC in B-cell signaling and function. *EMBO J.* **21**, 4049–4057 (2002).
87. Thaunat, O. *et al.* Asymmetric segregation of polarized antigen on B cell division shapes presentation capacity. *Science* **335**, 475–479 (2012).
88. Shlomchik, M. J. & Weisel, F. Germinal center selection and the development of memory B and plasma cells. *Immunol. Rev.* **247**, 52–63 (2012).
89. Victora, G. D. & Nussenzweig, M. C. Germinal centers. *Annu. Rev. Immunol.* **30**, 429–457 (2012).
90. Victora, G. D. *et al.* Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter. *Cell* **143**, 592–605 (2010).
91. Randall, K. L. *et al.* Dock8 mutations cripple B cell immunological synapses, germinal centers and long-lived antibody production. *Nature Immunol.* **10**, 1283–1291 (2009).
This work shows that DOCK8 mutations impair the accumulation of the integrin ligand ICAM1 in the B cell immunological synapse. This leads to B cell-deficient signalling and highlights how humoral immunodeficiency is dependent on the organization of the B cell immunological synapse.
92. Harada, Y. *et al.* DOCK8 is a Cdc42 activator critical for interstitial dendritic cell migration during immune responses. *Blood* **119**, 4451–4461 (2012).
93. Hauser, A. E. *et al.* Definition of germinal-center B cell migration *in vivo* reveals predominant intrazonal circulation patterns. *Immunity* **26**, 655–667 (2007).
Using multiphoton *in vivo* microscopy, this work describes the migration patterns of B cells between the dark and light zones of the germinal centre, detailing the morphological changes that B cells undergo during this movement.
94. Barnett, B. E. *et al.* Asymmetric B cell division in the germinal center reaction. *Science* **335**, 342–344 (2012).
This study describes how, following cell division, germinal centre B cells asymmetrically segregate molecules that are involved in B cell fate, as well as the ancestral polarity protein PKCζ, to generate unequal daughter cells.
95. Li, R. & Bowerman, B. Symmetry breaking in biology. *Cold Spring Harb. Perspect. Biol.* **2**, a003475 (2010).
96. Mogilner, A., Allard, J. & Wollman, R. Cell polarity: quantitative modeling as a tool in cell biology. *Science* **336**, 175–179 (2012).
97. Hat, B., Kazmierczak, B. & Lipniacki, T. B cell activation triggered by the formation of the small receptor cluster: a computational study. *PLoS Comput. Biol.* **7**, e1002197 (2011).
98. Tsourkas, P. K., Liu, W., Das, S. C., Pierce, S. K. & Raychaudhuri, S. Discrimination of membrane antigen affinity by B cells requires dominance of kinetic proofreading over serial engagement. *Cell. Mol. Immunol.* **9**, 62–74 (2011).
99. Goehring, N. W. *et al.* Polarization of PAR proteins by advective triggering of a pattern-forming system. *Science* **334**, 1137–1141 (2011).
100. Wan, Z. *et al.* B cell activation is regulated by the stiffness properties of the substrate presenting the antigens. *J. Immunol.* **190**, 4661–4675 (2013).
101. Wedlich-Soldner, R., Altschuler, S., Wu, L. & Li, R. Spontaneous cell polarization through actomyosin-based delivery of the Cdc42 GTPase. *Science* **299**, 1231–1235 (2003).
This is an important paper, showing the role of CDC42 in cell polarity. In this paper, experiments that are corroborated by a mathematical model show that a stable axis of polarity arises in yeast from a positive feedback mechanism that amplifies a random concentration of activated CDC42. This work suggests that spontaneous polarization might occur and could be stabilized by external cues.
102. Gierer, A. & Meinhardt, H. A theory of biological pattern formation. *Kybernetik* **12**, 30–39 (1972).
103. Jilkine, A. & Edelstein-Keshet, L. A comparison of mathematical models for polarization of single eukaryotic cells in response to guided cues. *PLoS Comput. Biol.* **7**, e1001121 (2011).
This paper is a detailed survey of the different models of cell polarity. The authors identify the general features of a polarizing cell that are common to all systems, they highlight the advantages and the disadvantages of different mathematical models and they test the response of the different models to a given stimulus.
104. Baracho, G. V., Miletic, A. V., Omori, S. A., Cato, M. H. & Rickert, R. C. Emergence of the PI3-kinase pathway as a central modulator of normal and aberrant B cell differentiation. *Curr. Opin. Immunol.* **23**, 178–183 (2011).
105. Liu, C. *et al.* A balance of Bruton's tyrosine kinase and SHIP activation regulates B cell receptor cluster formation by controlling actin remodeling. *J. Immunol.* **187**, 230–239 (2011).
106. Houk, A. R. *et al.* Membrane tension maintains cell polarity by confining signals to the leading edge during neutrophil migration. *Cell* **148**, 175–188 (2012).
107. Wedlich-Soldner, R. & Li, R. Spontaneous cell polarization: undermining determinism. *Nature Cell Biol.* **5**, 267–270 (2003).
108. Mattila, P. K. *et al.* The actin and tetraspanin networks organize receptor nanoclusters to regulate B cell receptor-mediated signaling. *Immunity* **38**, 461–474 (2013).
109. Cheng, P. C., Steele, C. R., Gu, L., Song, W. & Pierce, S. K. MHC class II antigen processing in B cells: accelerated intracellular targeting of antigens. *J. Immunol.* **162**, 7171–7180 (1999).
110. Forquet, F. *et al.* Presentation of antigens internalized through the B cell receptor requires newly synthesized MHC class II molecules. *J. Immunol.* **162**, 3408–3416 (1999).
111. Wolf, P. R. & Ploegh, H. L. How MHC class II molecules acquire peptide cargo: biosynthesis and trafficking through the endocytic pathway. *Annu. Rev. Cell Dev. Biol.* **11**, 267–306 (1995).
112. Villadangos, J. A. *et al.* Proteases involved in MHC class II antigen presentation. *Immunol. Rev.* **172**, 109–120 (1999).
113. Watts, C. Antigen processing in the endocytic compartment. *Curr. Opin. Immunol.* **13**, 26–31 (2001).
114. Brezski, R. J. & Monroe, J. G. B cell antigen receptor-induced Rac1 activation and Rac1-dependent spreading are impaired in transitional immature B cells due to levels of membrane cholesterol. *J. Immunol.* **179**, 4464–4472 (2007).
115. Walmsley, M. J. *et al.* Critical roles for Rac1 and Rac2 GTPases in B cell development and signaling. *Science* **302**, 459–462 (2003).
116. Westerberg, L. S. *et al.* Wiskott–Aldrich syndrome protein (WASP) and N-WASP are critical for peripheral B-cell development and function. *Blood* **119**, 3966–3974 (2012).

Acknowledgements
The authors kindly thank C. Hivroz, Y. R. Carrasco, D. Obino and D. Lankar for discussions and critical reading of the manuscript. This work was funded by grants from the European Research Council to A.-M.L.-D. and the L'Agence nationale de la recherche Jeunes Chercheuses et Jeunes Chercheurs programme to P.P.

Competing interests statement
The authors declare no competing financial interests.

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