Review

The dissociation activation model of B cell antigen receptor triggering

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ABSTRACT

To detect its cognate antigen, each B lymphocyte contains up to 120 000 B cell antigen receptor (BCR) complexes on its cell surface. How these abundant receptors remain silent on resting B cells and how they can be activated by a molecularly diverse set of ligands is poorly understood. The antigen-specific activation of the BCR is currently explained by the cross-linking model (CLM). This model predicts that the many BCR complexes on the surface of a B cell are dispersed signalling-inert monomers and that it is BCR dimerization that initiates signalling from the receptor. The finding that the BCR forms auto-inhibited oligomers on the surface of resting B cells falsifies these predictions of the CLM. We propose the dissociation activation model (DAM), which fits better with the existing body of experimental data.

1. Introduction

Mature B cells sense pathogens through the B cell antigen receptor (BCR), which is expressed in large numbers on their surface. The BCR is composed of the membrane-bound immunoglobulin (mIg) molecule and a heterodimer of the Igα (CD79a) and Igβ (CD79b) protein [1]. The evolutionarily conserved cytoplasmic tails of Igα and Igβ are essential for the signalling function of the BCR and both contain an immunoreceptor tyrosine-based activation motif (ITAM) [2]. A deletion or mutations in these tails render the BCR signalling-inert [3,4]. The two tyrosines of the ITAM sequence are targets of protein tyrosine kinases of the Src and spleen tyrosine kinase (Syk) family. Specifically, the Src-family kinase Lyn phosphorylates and interacts dominantly with the first ITAM tyrosine, whereas the Syk phosphorylates both ITAM tyrosines, thus creating docking sites for its two tandem Src homology 2 (SH2) domains [5,6]. Similar to Lyn, Syk is an allosterically regulated enzyme. The two tandem SH2 domains, the linker regions connecting the SH2 domains and the kinase domain (interdomain A and B) are involved in the inhibition of the Syk kinase activity [7]. The molecular details of this auto inhibition have been revealed by the crystal structure of the Syk family kinase Zeta-chain-associated protein kinase 70 (ZAP70) [8]. The auto inhibition of ZAP70 and Syk ensures that these kinases only become active once they bind a target protein that, in most cases, carries an ITAM sequence. When bound to the BCR, Syk remains active and phosphorylates several key components of intracellular signalling pathways, including the adaptor proteins SH2 domain-containing leukocyte protein of 65 kDa (SLP-65, also called BLNK, B-cell linker) and B-cell adapter for phosphoinositide 3-kinase (PI3K) (BCAP). In this way, BCR signals are connected to Ca^{2+} release and the PI3K pathways, respectively. Furthermore, Syk bound to one BCR complex can rapidly phosphorylate the ITAMs of neighboring BCR complexes, thus resulting in signal amplification [9]. However, this signal spreading seems to be possible only between BCRs of the same Ig class [10,11]. In general, we presently know more about the function of the BCR on activated B cells and this knowledge has been recently summarized in several excellent reviews [12–14]. However, without knowing more about the structure and regulation of the BCR on resting B cells, our view of the BCR activation process remains incomplete. We propose here that the BCR has an auto-inhibitory structure on resting B cells and that BCR signalling is regulated through the control of ITAM accessibility.

2. The problems with cross-linking model (CLM)

The CLM is one of most successful hypothesis in cell biology and is an integral part of many biological textbooks. It was formulated as a simple and universal mechanism for how receptors are activated on the cell surface, long before detailed knowledge about the structure and signalling mechanism of most receptors was obtained. This hypothesis states that receptors on the cell surface are dispersed monomers and that signalling occurs whenever two or more of these monomers are cross-linked together, for example by a bivalent ligand. One prominent class of receptors that are thought to be activated by cross-linking are members of the tyrosine kinase receptor family, such as the epidermal growth factor receptor (EGFR) and insulin receptor (IR). Indeed, it has been shown that the kinase domain of these receptors is activated by reciprocal trans-phosphorylation, fitting with the CLM [15]. One problem was, however, that some of the active ligands (i.e., EGF) are not really dimers. How could they then cross-link these receptors? One solution to this problem came from the analysis of the crystal structure of the ectodomain of these receptors. It was found that in addition to the inactive monomer form, EGFR are also existed as dimers that reside in an auto-inhibitory conformation [16]. Binding to EGF resulted in the stabilization of an altered conformation that brought the two kinase domains in close proximity to each other, thus allowing their reciprocal phosphorylation and activation [17]. In the meantime, IR was found to be a dimeric, allosteric membrane receptor. Inactive IR dimers are in equilibrium with active dimers. Insulin binding stabilizes the active dimeric state leading to activation [18]. Higher ordered structures (dimers, trimers, oligomers) have now been found to be the organizational principle of many classes of receptors on resting cells [15,19–21]. It is therefore thought that when cross-linking plays a role in the activation of these receptors, it is in most cases accompanied by conformational changes or reorganization of the receptor.

From the beginning, CLM was also an attractive model to explain the early events accompanying BCR activation. Firstly, it explained well the results of experiments using anti-receptor antibodies for BCR stimulation. Here, it was found that only bivalent F(ab)$_2$ antigen binding fragments but not monovalent Fab fragments of anti-receptor antibodies could activate signalling from the BCR [22]. Similar results where obtained from experiments with monovalent and polyvalent antigens although these results were not always clear-cut as sometimes a large monovalent antigen could also activate the BCR without apparent cross-linking [23–25]. These experiments were taken as proofs that cross-linking is the major mechanism of BCR activation, although other BCR model could explain these data as well (see below). Secondly, the analysis of the crystal structure of the antibody molecules revealed the existence of a flexible hinge region situated between the Fab and the constant parts (Fc) of this molecule [26]. It was discussed that this hinge should prevent the transfer of conformational alterations from the Fab to the Fc part of the antibody, thus excluding a role of conformational changes in BCR activation. The CLM did not require a conformational change of the BCR, thus taking care of this problem.

Upon closer examination, the CLM has several problems. First, from receptors that are activated by cross-linking, it is known that the spacing of the two receptors is highly critical for signalling. Therefore, these receptors often have precisely spaced dimerization structures that are activated by binding of their cognate ligands [27,28]. The BCR differs from these receptors in that it does not have one defined ligand. Rather the BCR can be activated by thousands of structurally different ligands. How this diverse library of ligands could always place two BCR-complexes at the right distance preferred for signalling is not clear and we referred to this as an unsolved problem of the clonal selection theory of Burnet [29].

Second, the CLM assumes that the reciprocal phosphorylation of ITAM-bound Syk is a critical step for BCR activation. However, this assumption is not supported by current data showing that Syk is already released from auto-inhibition and active just by binding to one ITAM [30,31]. A monomeric BCR is therefore perfectly able to activate Syk without cross-linking as long as its ITAM tyrosines are accessible. Furthermore, each monomeric BCR carries two ITAM sequences, namely that of Igα and Igβ, and thus could place two Syk molecules in close proximity to each other even without cross-linking.

Third, the CLM assumes that the numerous BCR complexes on the B cell surface are monomers but this assumption has never been rigorously proven and recent experiments do not support this assumption (see below).

Fourth, the CLM does not attribute any role to the evolutionarily highly conserved amino acids in the transmembrane (TM) region of the mIg molecule, apart from those involved in the binding of the Igα/Igβ heterodimer [32].

In spite of its original success in explaining the activation of the BCR in the context of the activation process of other receptors, in the case of the BCR, the CLM is a failing model that is no longer in line with the current experimental data on this receptor.

3. Dissociation activation model (DAM) and the oligomeric BCR on resting B cells

In an effort to study the structure of the BCR complex, we used blue native polyacrylamide gel electrophoresis (BN-PAGE), a method that was successfully employed to identify components of supramolecular protein complexes in the membrane of mitochondria [33]. In the BN-PAGE analysis, we found that under low-percentage detergent-lysate conditions, the BCR of class IgM and IgD runs as large macro-molecular complex [10]. Furthermore, we showed that in the case of the IgD–BCR, the size of these complexes was influenced by alterations of conserved amino acids in the TM region of the δm molecule, indicating that these TM amino acids play a role in BCR oligomerization. Based on these and additional functional data, we suggested that the BCR on resting B cells is an oligomer and that it is the opening of these oligomers that drives BCR activation [32]. This suggestion, which relied mainly on biochemical studies, was not well accepted by the research field and mostly regarded as an artifact of detergent lysis.

The challenge was then to study the behavior of the BCR on the surface of living cells. To take up this challenge, we tried several experimental approaches including chemical cross-linking and the Förster resonance energy transfer (FRET) methods using cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) tagged BCR components and the results were not clear-cut due to the limitations of the techniques. The FRET method was also used by Tolar et al. to study the organization of BCR on living cells [34]. However, while FRET can be a useful method to study the interaction inside a defined heterodimer, it could be quite problematic in studying a large protein complex, where CFP and YFP tagged components are not always next to each other and where the donor/acceptor ratio can be variable. Furthermore, FRET only allows for confirmation but does not rule-out a protein–protein interaction as there can be many reasons for a low FRET value apart from the absence of an interaction [35]. For example, if the two flourophores were oriented perpendicular to each other, there will be no FRET between CFP and YFP even if they are close enough.

To learn more about the organization of the BCR on living cells, we combined three different techniques [36]. One was a synthetic biology approach, namely, rebuilding the BCR on the surface of a

Drosophila Schneider cell (S2 cell) [9]. The second technique was a bifluorescence complementation assay (BiFC) allowing for the detection of BCR dimerization via the generation of fluorescent form of a complemented fluorescent protein [37]. The third method was based on the immunoprecipitation of multiprotein complexes detected by flow cytometry (IP-FCM), a technique that allows quantitative FACScan analysis of different components in a large protein complex following immunoprecipitation on beads [38]. For the BiFC experiments, we fused half-domains of YFP and CFP (YN and CC) to either Igα and Igβ or to two different Igα proteins. This allowed us to test either for Igα/Igβ heterodimerization or for BCR oligomerization. Once brought together, the YN or CC half-domains form a cYFP fluorophore, the generation of which can be monitored in living cells and quantified by IP-FCM. This analysis showed that BCR oligomerization is an efficient and spontaneously occurring process on the surface of living S2 and B cells. Having developed a readout for BCR oligomerization, we were able to identify an IgD–BCR mutant that is defective in this process. The monomeric BCR mutant carries alterations in the class-specific side of the TM-region of mIgD and lacks the disulfide bridge between Igα and Igβ. As previously shown by BN-PAGE [10], this indicates that the conserved amino acids at the TM-region of mIgD are involved in the oligomerization process. Furthermore, removing the disulfide bridge between Igα and Igβ may alter the conformation of this heterodimer and this could interfere with BCR oligomerization. This monomeric BCR mutant was found to be more active and less stably expressed on the B cell surface, suggesting that BCR complexes within oligomers are more ordered and that BCR activation may be driven by increasing entropy according to the second law of thermodynamic. Indeed we found that, compared to a BCR wild-type control, a BiFC-stabilized BCR-dimer is less well internalized and less active in signalling. From these experiments, we conclude that tight oligomers are the auto-inhibited form of the BCR and proposed with DAM a new model for BCR activation (Fig. 1). According to DAM, most BCR complexes forms auto-inhibited oligomers in the membrane of resting B cells. Right now, the nomenclature for "BCR oligomer" is not clearly defined. We thus suggest using the term "BCR oligomer" exclusively for the description of these auto-inhibited oligomers in the resting stage of the BCR. Upon antigen-binding, the BCR forms clusters of active monomers and we suggest to refer to these activated form of the BCR as active BCR clusters. It is feasible that within the BCR oligomer, both the conserved class-specific amino acids in the TM-region of mIgD and the ITAM tyrosines are engaged in interactions and thus difficult to access. A polyvalent antigen binding to mIg can interfere with the formation or promote the dissociation of the BCR oligo-

mer. Importantly, the dissociation process does not require a precise structure of the antigen. The BCR dissociation can thus have two consequences: it should make the Igα/Igβ tails more accessible for phosphorylation and it may expose the conserved class-specific amino acids of the TM region of the mIg molecule to the lipid environment. The latter event could result in changes of the lipid shell surrounding the BCR and help to target the receptor to other membrane areas, which are more prone to signalling, such as lipid rafts [39]. Such changes in the lipid environment of the BCR have recently been detected by a FRET study [40]. According to DAM, it is the dissociation of the BCR oligomer that drives activation. This dissociation may not only involve individual BCR complexes but also negative regulatory proteins that help the BCR to stay silent on resting B cells (see below).

4. Protein island and membrane receptor regulation

Many recent studies suggest that the plasma membrane is more structured than originally postulated by the fluid-mosaic-model [41]. In particular, signal-particle tracking techniques have shown that the lateral diffusion of membrane proteins is restricted to nanometer-scale confinement zones [42]. A membrane-skeleton fence (picket fence) model has been proposed to explain this confined diffusion [43]. According to this model, small membrane patches are surrounded by components of the cytoskeleton that limit the diffusion of associated membrane proteins to a nanometer space. These confinement zones could have different purposes. For example, they could contain lipid rafts and promote localized signalling events. Other confinement structures may be more associated with the signalling-inert receptor. In this respect, it is interesting that recent studies on the T cell antigen receptor (TCR) have suggested that on resting T cells, the TCR resides in protein islands and that, in these islands, the receptor is kept signalling-inert [44]. How the detected TCR oligomer [20] can be related to these structures requires further studies. Studies on the high-affinity IgE receptor (FcεRI) have also shown that the diffusion of this receptor is limited by actin containing cytoskeleton structures [45].

The group of Batista has found that members of the ezrin–radixin–moesin (ERM) protein family could restrict the diffusion of the BCR [46]. ERM proteins comprise an N-terminal FERM domain that can bind to adaptors or receptor tails, an alpha-helical connecting domain and an F-actin binding C-ERMAD domain [47]. These proteins can thus connect receptor or membranes to the actin cytoskeleton. In resting B cells, the ERM protein ezrin is associated with lipid rafts [48]. Upon BCR ligation, this protein is rapidly dephosphorylated on threonine 567 in the C-ERMAD domain. This
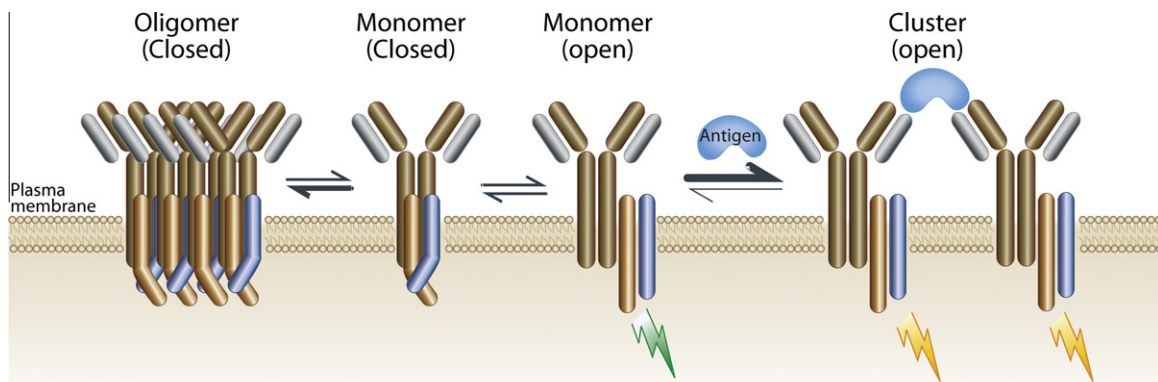

Fig. 1. Schematic drawing of the dissociation activation model (DAM). According to this model the BCR has an auto-inhibitory oligomeric structure on resting B cells. In this structure, the ITAM tyrosines are not accessible to kinase (closed). In the presence of antigen, the equilibrium between closed BCR oligomers and monomers is shifted towards the open clustered monomers. The dissociation of BCR oligomers leads to B cell activation.

results in detachment of ezrin from the cytoskeleton and apparently allows the BCR to move into lipid raft structures [49]. A similar process occurs in activated T cell where ERM protein dephosphorylation and the resulting cytoskeleton relaxation promote the formation of an immunological synapse between the T cell and the antigen presenting cell [50]. The ERM proteins may thus be part of the boarders that surround the nano-scale confinement zones of antigen- and Fc-receptors. Following detachment of the ERM proteins from the membrane, the antigen receptors could become more mobile and form the signalling-active micro-clusters that have been found on activated T and B cells.
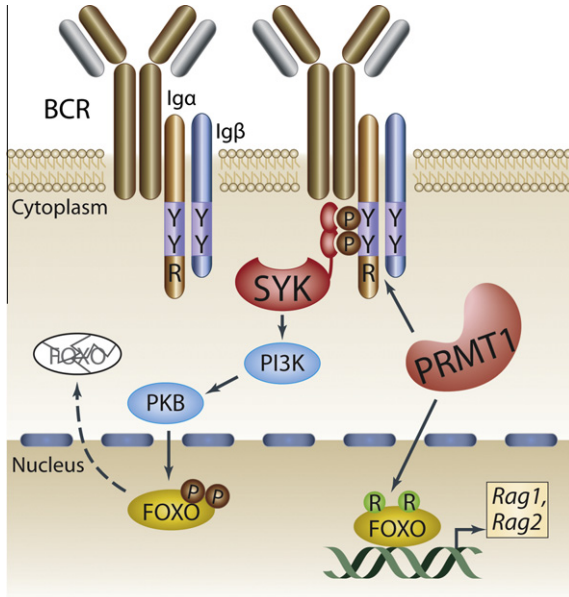
The model that the cytoskeleton guards receptors on resting cells is supported by the finding that signalling is induced after the treatment of cells with the F-actin inhibitor Latrunculin A (LatA) [51,52]. The group of Batista showed that LatA treatment results in an increased mobility of BCR complexes and associates BCR mobilization with increased signalling [46]. This is somewhat in conflict with data of Liu et al. that suggest that the degree of BCR immobilization is a readout for the strength of BCR signalling [53]. However, it is feasible that these opposite behaviors only reflect different phases of BCR signalling. After the dissociation of auto-inhibited BCR oligomers, BCRs may release from its cytoskeleton and move out of the confinement zone, results in an increased BCR mobilization at the beginning. At a later time point, the activated BCR may regain contact with the cytoskeleton and this may be important for the prolonged signalling of the receptor. In this context, it is interesting that the ERM protein ezrin carries two tyrosines in an ITAM like sequence that become phosporylated in the course of B cell activation [54]. Thus, it is feasible that tyrosine phosphorylated ezrin re-associates with the active BCR and promotes its immobilization.

5. A glimpse at the silenced BCR complex

How are the new findings of higher membrane organizations related to our model of an auto-inhibited BCR oligomer? If the numerous BCR complexes on the surface of resting B cells are indeed confined in nano-scale membrane patches, one could wonder how the many monomeric BCR that are predicted to exist by the CLM, are prevented from signalling in these crowded areas. The formation of auto-inhibited BCR oligomers, on the other hand, could be favored by BCR crowding in nano-scale confinement zones on the surface of resting B cells. The oligomeric structure of the resting BCR may also prevent the uncontrolled release of the receptors from theses confinement zones. Furthermore, the bordering cytoskeleton elements might be in direct contact with the BCR oligomer, thus controlling the accessibility of the ITAM-containing sequence of the receptor as discussed above. An emerging scheme is that, on resting lymphocytes, elements activating signalling are excluded from the antigen receptor confinement zone as has been found to be the case for LAT [55,56]. In addition, the confinement zones may promote the association of the antigen receptor with negative regulators. In this context, we have recently

Fig. 2. PRMT1 as an inhibitor and modulator of BCR signalling. In resting B cells, PRMT1 methylates a conserved arginine (R) at the tail of Igα. This modification interferes with the recruitment of the kinase Syk to the ITAM of Igα and inhibits signalling from the BCR. PRMT1 also methylates two R of the transcription factor FOXO1. This modification prevents the phosphorylation of FOXO1 by PKB that results in FOXO1 destruction. In this way, FOXO1 counteracts PI3 kinase signaling and promotes the differentiation of pre-B cells.
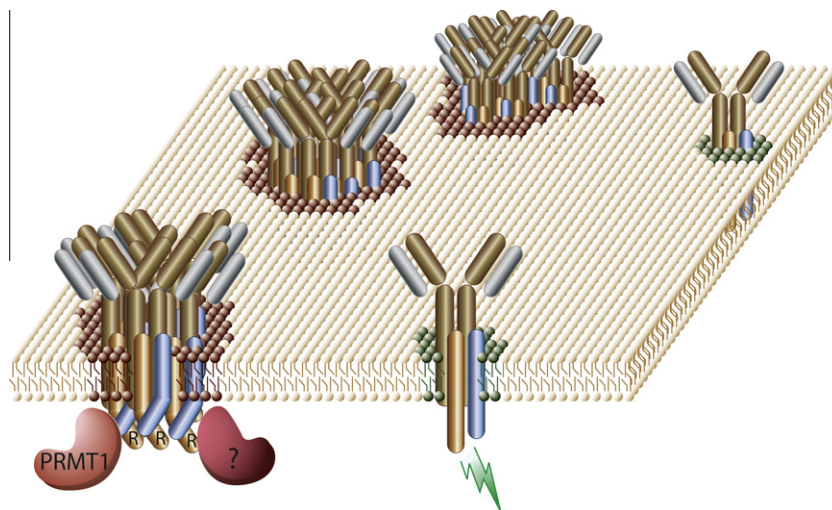

Fig. 3. Model of BCR silencing complexes on the membrane of B cells. According to the protein island model, most BCR complex on the surface of a B cells reside in nano-scaled confinement zones. In these areas, the autoinhibtory oligomeric BCR maybe surrounded by certain lipids and colocalized with negative regulatory proteins such as PRMT1. The dissociation from these inhibitory zones can result in a change of the lipid environment and BCR activation.

found that the protein-arginine-methyl-transferase1 (PRMT1) is co-localized with the resting BCR. The cytosolic tail of Igα carries a highly conserved arginine (R198), which is methylated by PRMT1 on resting B cells [57]. Interestingly, R198 methylation inhibits signalling from the BCR (Fig. 2). PRMT1 is known as a negative regulator in different microenvironments, both in the nucleus as well as in the cytosol. For example, by methylating arginines at tails of histones, PRMT1 inhibits gene expression. By methylating arginines of Forkhead box O1 (FOXO1), PRMT1 prevents protein kinase B (PKB) mediated phosphorylation and destruction of this differentiation factor via the PI-3 kinase pathway [58,59]. Upon BCR activation, PRMT1 dissociates from the BCR and R198 is rapidly demethylated. The R198 methylation is the first protein modification found to be associated with the resting BCR. We think that the identified BCR/PRMT1 association is only a first glimpse at BCR silencing complexes that maybe assembled in the confinement zones of resting B cells around the auto-inhibited BCR oligomers (Fig. 3).

In recent years, the focus of research was primarily on the active antigen receptors. As deregulated and hyperactive BCR antigen receptors are associated with several human diseases from autoimmunity to B lymphomas [60–65], it is clearly important to learn more about the structure and function of these receptors in its resting state. How to keep antigen receptors silent therefore, will be a major focus of research in years to come. Synthetic biology approaches and the new molecular tools provided by this scientific endeavor may be the key elements to shed more light on this aspect of B cell regulation.

Acknowledgements

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