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Aurora-A shines on T cell activation through the regulation of Lck

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Abstract

Different protein kinases control signaling emanating from the T cell receptor (TCR) during antigen-specific T cell activation. Mitotic kinases, e.g. Aurora-A, have been widely studied in the context of mitosis due to their role during microtubule (MT) nucleation, becoming critical regulators of cell cycle progression. We have recently described a specific role for Aurora-A kinase in antigenic T cell activation. Blockade of Aurora-A in T cells severely disrupts the dynamics of MTs and CD3z-bearing signaling vesicles during T cell activation. Furthermore, Aurora-A deletion impairs the activation of signaling molecules downstream of the TCR. Targeting Aurora-A disturbs the activation of Lck, which is one of the first signals that drive T cell activation in an antigen-dependent manner. This work describes possible models of regulation of Lck by Aurora-A during T cell activation. We also discuss possible roles for Aurora-A in other systems similar to the IS, and its putative functions in cell polarization.

Keywords

asymmetric cell division; Aurora A kinase; Golgi apparatus; immunological synapse; Lck tyrosine kinase; microvesicular traffic; T cell activation

Introduction

Immune synapse (IS) formation is an essential event during the development of the adaptive immunity. The IS consists of a highly organized molecular structure based on receptor and cytoskeleton segregation of an antigen-specific T cell triggered by an antigen-loaded antigen presenting cell (Ag-APC). The IS is necessary for the correct activation, proliferation, and differentiation of T cells, allowing them to carry out their effector function [1]. During this process, MTs become polarized, beginning with the translocation of the centrosome or microtubule-organizing centre (MTOC) to the IS. MTs also drive the polarization of the

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Conflict of interest

The authors have declared no conflict of interest.

Golgi apparatus (GA), the multivesicular bodies and mitochondria toward the APC. This crucial step helps the polarized secretion of cytokines and extracellular microvesicles [2]. These changes require the transmission of the activating signal from the T-APC contact to the cytoskeleton. Focusing on TCR-dependent signals, a crucial early event is TCR/CD3 phosphorylation, which mainly depends of the lymphocyte-specific kinase Lck, a member of the Src family (SFK). Its ability to trigger TCR/CD3 phosphorylation is regulated by the Lck inhibitory kinase Csk and the phosphatase CD45 [3, 4]. The activation of the TCR/CD3 complex upon scanning of the Ag-APC leads to its accumulation in signal-competent microclusters prior to the formation of the mature IS [5]. In fact, there is some controversy on whether the IS serves to potentiate or to down-regulate TCR activation [6]. These early signals are amplified by the phosphorylation of a number of cytosolic kinases, GTPases, and adaptors that propagate the activation signal [7]. Among the plethora of protein kinases involved in this process in the T cell, Aurora-A emerges as a novel effector that promotes proper T cell activation through the regulation of Lck [8].

Aurora-A is a serine/threonine kinase classically involved in cell cycle progression through centrosome maturation at the onset of mitosis. Aurora-A promotes MT growth by recruiting and activating diverse nucleation factors, e.g. NDEL1 [9]. In late G2, Aurora-A concentrates at the centrosomes and self-activates by autophosphorylation of residue Thr288 [10]. However, its proper activation and localization requires the help of the cofactor TPX2 [11]. Aurora-A is found at the IS in its active form during T cell interaction with Ag-APCs [8]. Active Aurora-A helps Lck activation and promotes MT nucleation from the centrosome, controlling the dynamics of microtubules and CD3z-bearing vesicles. However, actin cytoskeleton and cell spread are not affected by Aurora-A inhibition, which is consistent with the observed accumulation of the TCR/CD3 complex at the IS [8], by the tracking forces exerted by actin [12]. Accordingly, TCR conformational change upon activation is also conserved, as confirmed by Nck binding [8]. Remarkably, Aurora-A has been recently found as a potential molecule to target graft versus host disease (GVHD) in a transcriptome study searching for molecules controlling T cell activation, proliferation, and cytokine production [8, 13]. In this regard, inhibitors for Aurora-A have been used in clinical trials for the treatment of leukemias [14]. Therefore, the use of inhibitors to treat GVHD or leukemias should take into account a possible role of Aurora-A in the activation, proliferation, and differentiation of naïve and effector T cells to avoid undesired effects.

Here, we discuss several possible mechanisms by which Aurora-A may be activated during the first steps of T cell activation and by which it may exert its crucial function during the formation of the IS. Moreover, we postulate the possible role of Aurora-A in later stages of T cell activation, such as in the asymmetric cell division (ACD) under gone by naïve T cells [15].

Models of Lck regulation by Aurora-A

Direct regulation

During IS formation, Lck localizes near the TCR/CD3 complexes, which is required for Lck-mediated phosphorylation of CD3 immunoreceptor tyrosine-based activation motifs (ITAMs) [3]. Lck appears in specific membrane microdomains (rafts) containing MAL and

Caveolin-1 (Cav-1). These proteins are required for Lck clustering to the membrane during the IS, and MAL or Cav-1 depletion decreases the efficiency of CD3-ITAM phosphorylation [16, 17]. MAL is present in vesicles containing with the plasma membrane at the IS (Fig. 1A) [2]. Moreover, Lck plasma membrane targeting is also dependent on dual S-acylation on Cys 3 and 5 [18]. As Aurora-A gene depletion or pharmacological inhibition impedes the proper traffic of CD3z-bearing vesicles to the IS [8], we postulated that Lck bearing vesicles traffic may also be affected by the absence of Aurora-A activity. Interestingly, Aurora-A inhibition also prevented the rescue of CD3z phosphorylation in Lck-deficient cells (JCAM. 1) transfected with a full-length Lck fused to the ectodomain of CD4 [8]. CD4-Lck localizes at the IS in a vesicle-independent manner [19], suggesting that Aurora-A may control the enzymatic activation of Lck that is already bound to the plasma membrane, and not only its localization (Fig. 1A).

Lck and SFKs in general, are regulated reciprocally by phosphorylation and de-phosphorylation of two conserved tyrosine residues [3]. Lck is phosphorylated on residue Tyr394, which is used as a fiduciary marker of its activation. One study has shown that TCR activation does not control the phosphorylation level of Y394, suggesting that Lck is activated prior to T cell activation [20]. Another study showed Lck to be regulated through conformational changes, clustering, and spatio-temporal proximity to other proteins, e.g. Csk and CD45. Csk regulates the phosphorylation of Y505, which is inhibitory and CD45 dephosphorylates both Y505 and Y394 residues [21]. However, other authors have reported an increase of nearly 20% in Lck phosphorylation upon TCR priming, as measured by Western blot after immunoprecipitation, or through FLIM-FRET analysis with a biosensor for conformational changes [22]. Aurora-A inhibition decreases Y394 phosphorylation prior to TCR triggering. As Aurora-A is a serine/threonine kinase, Y394 is not expected to be its substrate. In fact, Y394 is an autophosphorylation site [3]. Based on these observations, a model can be drawn in which Aurora-A phosphorylates a yet unidentified residue in Lck, which may in turn influence Y394 autophosphorylation by affecting the conformation of Lck (Fig. 1A). Several Ser residues have been identified in Lck upon activation; mainly S59 at the unique N-terminal domain and S122, S158, S192, and S194 at the SH2 domain. S59 and S122 seem to Lck activity either in vivo or in vitro. In particular, S59 seems to act on the ability of the SH2 domain to interact with partners and it is phosphorylated during mitosis [23–26]. However, the fact that Aurora-A disruption decreases Lck activity [8] points to other residues as substrates for this enzyme. Interestingly, the consensus phosphorylation target site for Aurora-A (R-X-S/T-I/L/V [27]) was detected in Lck (RETL). Mass spectrometry analysis combined with the use of Aurora-A conditional knockout mice could help us to identify other putative phosphorylatable sites on Lck. Moreover, FLIM- FRET and super-resolution analyses would be a proper experimental strategy to assess the conformational changes that may occur in Lck protein and/or the possible partners of Lck during the IS.

Indirect regulation

It is feasible that additional protein(s) may serve as “interaction bridges” between Aurora-A and Lck. Csk and CD45 appear as suitable candidates. Phosphorylation of Lck at Y505 by Csk promotes the intramolecular interaction of the residue with the SH2 domain, favoring a

closed conformation of Lck (Fig. 1B) [28]. Hence, phosphorylation of Y505 prior to Y394 maintains Lck in a closed, inactive form, whereas phosphorylation of Y394 promotes Lck activation, even though Y505 is phosphorylated afterwards [3]. Moreover, CD45 dephosphorylates both residues in Lck and also CD3 ITAMs, although its effect is more noticeable in the ITAMs [4, 21, 29]. Aurora-A could phosphorylate and inactivate Csk, and thus Lck Y505 phosphorylation, which would maintain Lck in an active conformation (Fig. 1B). The described phosphorylation of Csk on S364 increases its activity, and therefore would not fit in this model [30]. Csk is regulated by the phosphoprotein associated with glycosphingolipid-enriched microdomains/Csk binding protein (PAG/Cbp) complex, which is phosphorylated in lipid rafts in resting T cells, thereby inducing Lck inhibition. Phospho-PAG/Cbp promotes the binding of Csk to Lck and the phosphorylation of Y505 (Fig. 1B) [31, 32]. After TCR triggering, dephosphorylation of PAG/Cbp in tyrosines leads to the dissociation of Csk from lipid rafts and allows Lck activation [33]. However, a recent study based on proteomic approaches has shown that an increase in PAG phosphorylation can be detected upon TCR activation, accompanied by Csk and SHIP1 and PTPN22 phosphatases interaction [34]. Hence, the absence of Aurora-A could reinforce the PAG/Cbp interaction with Csk, enhancing Lck inhibition due to the phosphorylation of the PAG/Cbp complex. Although Csk does not contain the Aurora-A target consensus sequence, PAG protein seems to contain a putative one (RQSV). Aurora A might be regulating PAG or the kinase that acts on it. These studies, together with the observed mislocalization of Lck at the IS in Aurora-A-inhibited cells and the fact that non-phosphorylatable Aurora-A cannot be properly recruited to the IS, suggest that Aurora-A may spatio-temporally regulate the localization of other proteins involved in TCR activation (Fig. 1B).

The spatial localization of the phosphatase CD45 is essential for TCR activation. When the eye-shaped IS forms, CD45 is sterically excluded from the T cell-APC proximal contact area to prevent the dephosphorylation of CD3 and Lck [35]. Interestingly, Aurora-A inhibition decreased both Lck phosphorylation at Y394 and that of the CD3 ITAMs [8]. However, although CD45 activity could explain the enhanced dephosphorylation of both residues in Aurora-A depleted cells, the sterical exclusion model for CD45 makes it unlikely for Aurora-A to regulate its activity directly.

Role of Aurora-A in ACD

We have shown that Aurora-A promotes MT growth and helps MT-dependent trafficking during T cell activation [8]. Upon IS formation, naïve T cells can undergo ACD, generating two pools of T cells: pre-effector and pre-memory (Fig. 2) [2, 15, 36]. Receptor clustering at the T cell-APC interface together with the polarization of the MTOC establishes an asymmetry axis that is maintained by the elongation of the mitotic spindle [36, 37]. After MTOC relocation to the IS, PAR3 and PKC θ also accumulate near the T cell-APC interface, which contribute to maintain T cell asymmetry (Fig. 2A) [38, 39]. Later, redistribution of PAR3-PAR6-aPKC, together with Numb at the distal pole and Scribble-Dlg complexes at the proximal pole would trigger ACD (Fig. 2B) [37].

From the observed accumulation of Aurora-A at the IS (either at the plasma membrane or the centrosome), which will give rise to the proximal pole, and due to its described role in

neural polarity [40], a model emerges in which: (i) Aurora-A gene targeting and chemical inhibition impair TCR/CD3 phosphorylation but neither polarization to the IS nor conformational changes. As a threshold of TCR activation is required for ACD [41], this would block the generation of different pools of daughter cells. (ii) Aurora-A controls the localization of other molecules involved in the onset or maintenance of T cell polarization. Some of these molecules could be PAR proteins, associated aPKCs, and their substrate Numb, which establish the orientation of the mitotic axis. In this view, Aurora-A would translate the T-APC contact into a sustained polarized signal that drives ACD in T cells from its initial steps of activation up to later events, such as the generation of the asymmetric polarity axis. This may be relevant in the process of determination of the T cell fate into memory or effector T cells due to unequal cell divisions, such as in CD8⁺ T cells during *Listeria monocytogenes* infection [42].

Regulation by GA of Aurora-A activation at the IS

During CD4⁺ and CD8⁺ T cell activation, the secretory elements, including the GA, ER (endoplasmic reticulum), endolysosomal system, and secretory vesicles are rapidly translocated with the centrosome to the IS [2, 43]. This specific localization promotes a fast and direct secretion of diverse molecules from the trans-Golgi network (TGN) and the endosomal compartment to the plasma membrane, regulating the polarized secretion of exosomes or IL-4 cytokine in CD4⁺ and perforin and granzymes from lytic granules in CD8⁺ T cells [43–45]. Several studies have proposed a relationship between GA and Aurora-A function and localization. During mitosis, the GA undergoes fragmentation and its inhibition at the G2 stage blocks the recruitment and activation of Aurora-A at the centrosomes [46]. Also, the GA participates in spindle formation. The GA matrix protein GM130 recruits importin α to the GA membranes, promoting the release of the spindle assembly factor TPX2. TPX2 activates Aurora-A, stimulating local MT nucleation (Fig. 3A) [47]. GM130 may control MT polymerization from the GA also in cells in interphase. To do this, it recruits AKAP450 to the cis-Golgi [48]. AKAP450 promotes tubulin nucleation through the binding to CGP subunits of the γ -Tubulin ring complex (γ -TURC) at the centrosome [49], which seems a shared mechanism for polymerization at the cis-Golgi. The structure of MTs is clearly different at the centrosome and the GA, as they show a radial array in the first organelle and a more tangential disposition at the GA [50]. In this regard, silencing of AKAP450 or over-expression of a dominant-negative, AKAP450-centrosomal domain prevents T cell activation and centrosome polarization to the IS [51]. Therefore, centrosome-resident AKAP450 is required for T cell activation, but the relative contribution of GA-resident AKAP450 remains to be determined. Although Aurora-A inhibition does not prevent centrosome polarization, tubulin nucleation is impaired. The possible tubulin-nucleation activity of the GA has not been determined in T cells. As Aurora-A is activated by TPX2 and this process is mediated by GM130 spindle formation in mitosis, these observations indicate that Aurora-A could be involved in MT polymerization from the GA during the IS, further supporting a conserved mechanism between cell division and IS formation (Fig. 3). A recent study showed that, during G2 phase, the GA fragmentation promotes Src protein activation at the TGN. This is relevant because Src phosphorylates Aurora-A on residue Y148; which is required for Aurora-A to localize to centrosomes and become active (Fig. 3A) [52]. Therefore, the translocation of the GA during the first step of

the IS might be involved in the activation of Aurora-A in this region. Some proteins that regulate Aurora A function in other systems, e.g. TPX2 or Src, which also participate in the formation of the IS, may form feedback loops that connect the regulation of different phenomena, e.g. activation of Aurora A by Src during mitosis and Lck regulation by Aurora A during the IS (Fig. 3B). Some works reveal a non-mitotic role for Aurora-A, such as ciliary disassembly, with different mechanisms of activation. This process requires interaction with Ca²⁺/calmodulin (CaM) [53]. IS formation has been compared with cilia formation, as a set of proteins are implicated in both processes, such as IFT (intraflagellar transport) proteins for transport of vesicles, AKAP450, or HDAC6, both regulators of microtubules [43]. During the IS, Ca²⁺ release-activated channels (CRAC) are opened after TCR activation, promoting proper IS formation by regulating actin organization [54]. Similarly, to the relationship between Ca²⁺ and Aurora-A activation in primary cilia formation, this interaction could imply a role of Ca²⁺ in Aurora-A activation during IS formation.

Conclusion

The functional relevance of Aurora-A in events of TCR pathway in T cell activation has been recently underscored; however, the molecular interactions and precise mechanism by which Aurora-A controls T cell activation remains undetermined. The interaction of Aurora-A with Lck, either direct or indirect, suggests the existence of a very complex regulatory network involving many kinases and phosphatases, with Aurora-A sitting at the core of such a network.

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Abbreviations

ACD	asymmetric cell division
APC	antigen presenting cell
aPKC	atypical protein kinase C
CaM	calmodulin
Cav-1	Caveolin-1
Cbp	Csk binding protein
CRAC	Ca ²⁺ release-activated channels
Csk	C terminal Src kinase

ER	endoplasmic reticulum
GA	Golgi apparatus
IFT	intraflagellar transport
IS	immune synapse
ITAM	immunoreceptor tyrosine-based activation motifs
Lck	lymphocyte-specific protein tyrosine kinase
MAL	myelin and lymphocyte
MT	microtubule
MTOC	microtubule-organizing centre
PAG	phosphoprotein associated with glycosphingolipid-enriched microdomains
SFK	Src family kinase
TCR	T cell receptor
TGN	trans-Golgi network

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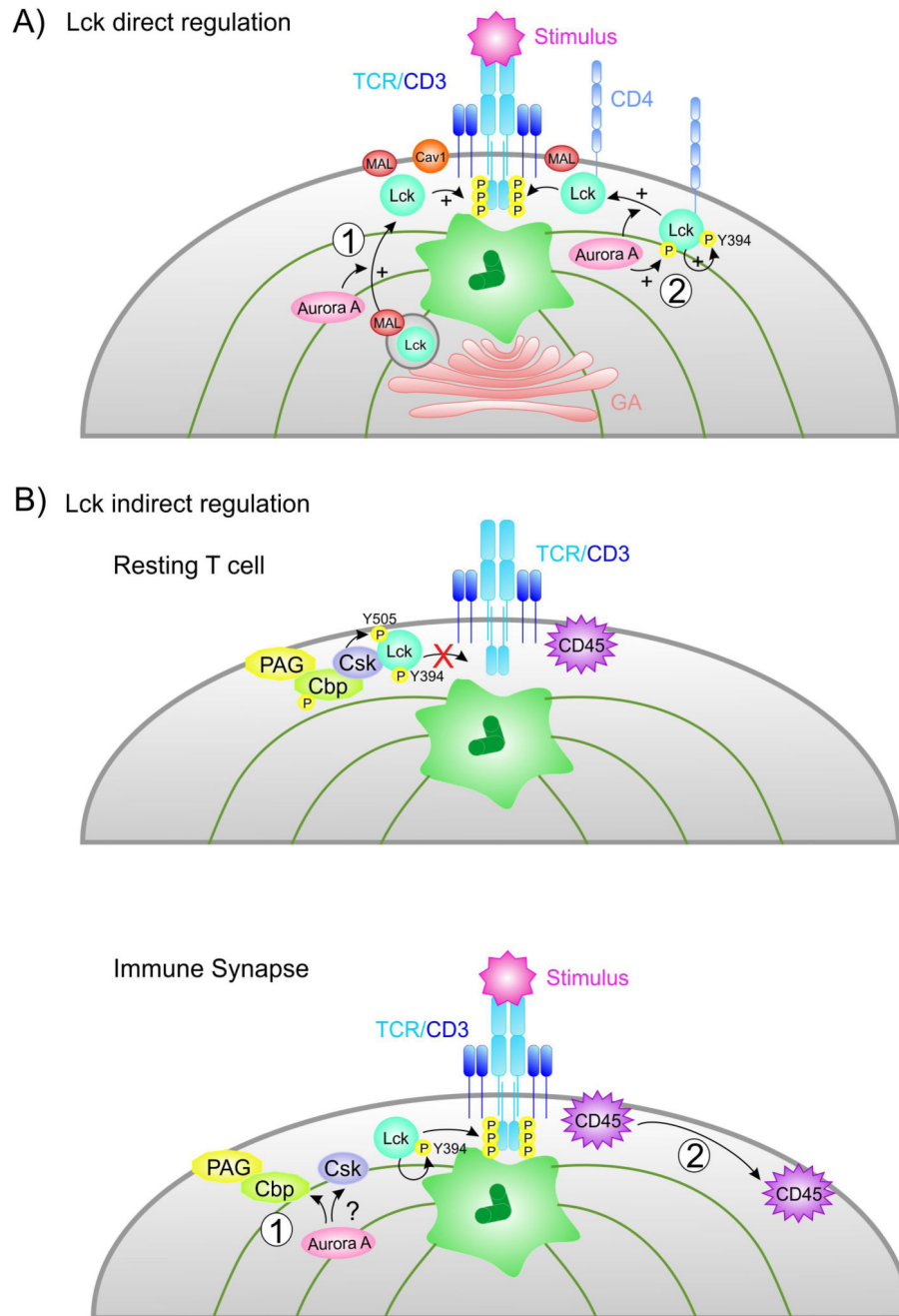


Figure 1.

Lck regulation by Aurora-A. A: Direct regulation of Lck by Aurora-A. (1) Aurora-A regulates the vesicular traffic of Lck through the microtubule network. (2) Possible phosphorylation of Lck by Aurora-A in an unknown residue promotes autophosphorylation in Y394 and proper translocation to the IS region. B: Indirect regulation of Lck by Aurora-A. In resting T cells, the PAG/Cbp complex bound to Csk promotes the binding of Csk with Lck, inhibiting it by the phosphorylation of Y505 residue. The phosphatase CD45 is located at the IS region. During T cell activation, (1) the PAG/Cbp complex promotes the release of

Lck by Csk, activating it. Proper Aurora-A localization at the IS could be regulating this mechanism through the phosphorylation of the PAG/Cbp complex or Csk directly. (2) After TCR triggering, the phosphatase CD45 is excluded from the IS. GA, Golgi apparatus; TCR, T cell receptor.

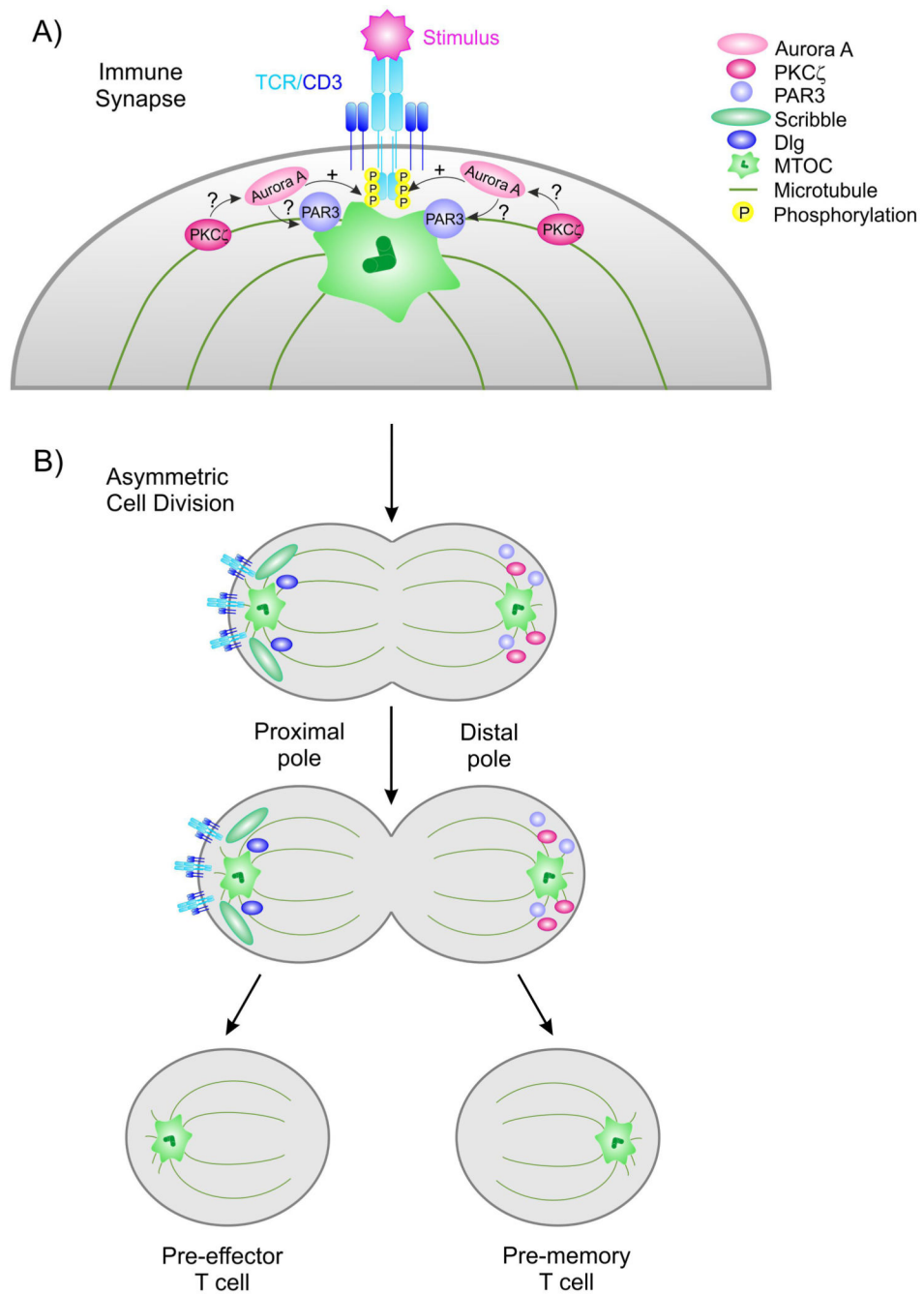
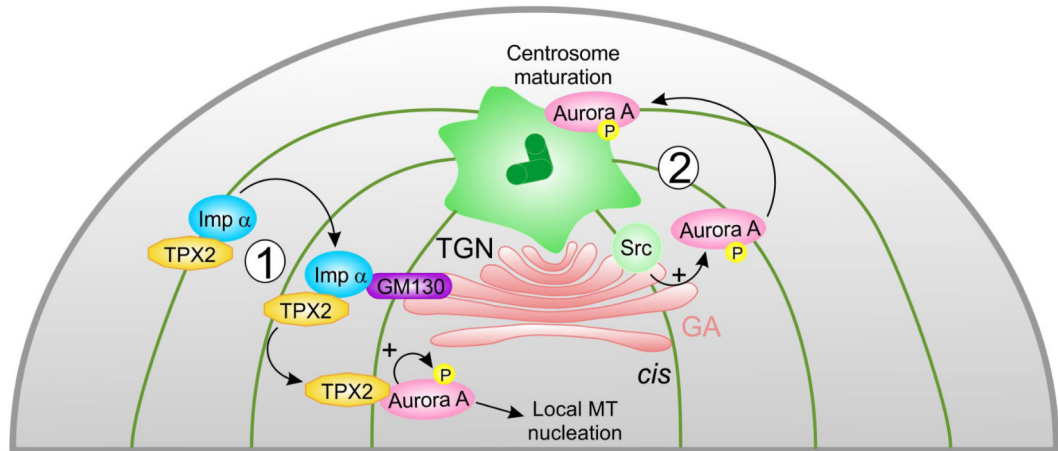
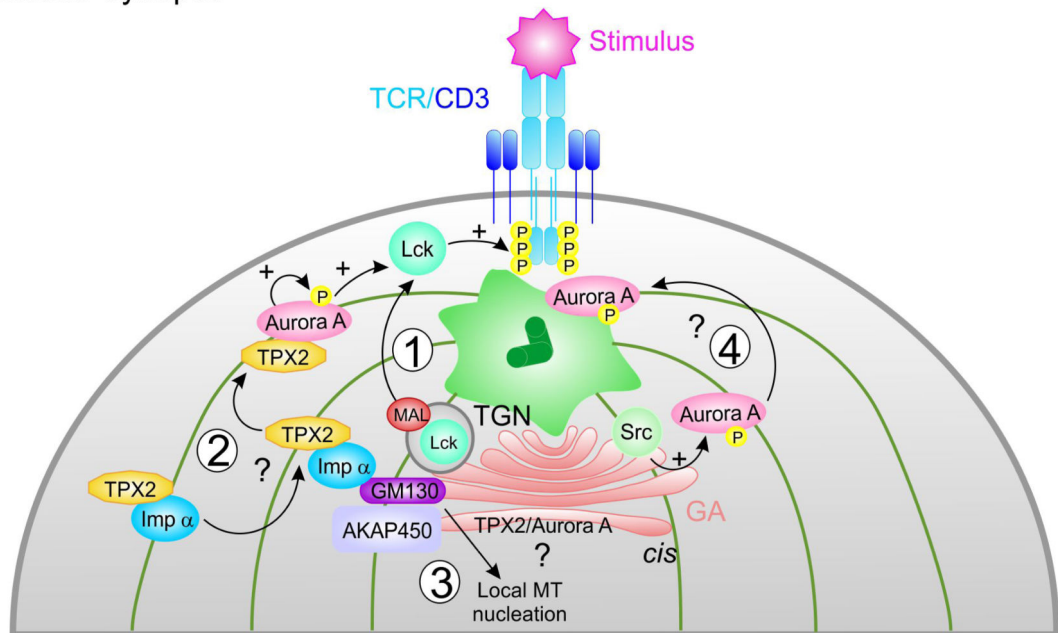


Figure 2. Possible role of Aurora-A in asymmetric T cell division. A: Localization of PAR3 and PKC θ during the IS. Possible regulation of Aurora-A by PKC θ and possible regulation of PAR3 by Aurora-A. TCR, T cell receptor. B: Localization of PAR3, PKC θ , Scribble, and Dlg during the asymmetric cell division.

A) G2 phase



B) Immune Synapse

**Figure 3.**

Activation of Aurora-A during the IS by proteins from the GA. A: Regulation of Aurora-A activity during G2 phase. (1) Interaction of GM130 with importin α promotes the release of TPX2 and the consequent Aurora-A autophosphorylation, promoting local MT polymerization. (2) Aurora-A phosphorylation by Src allows Aurora-A localization at the centrosome for proper centrosomal maturation. B: Possible model of Aurora-A regulation during the IS. (1) Translocation of vesicles containing Lck from the TGN to the membrane is directed by MAL. (2) Activation of Aurora-A by TPX2 release next to the IS area. (3)

Local MT polymerization from the GA through Aurora-A activation mediated by GM130 and AKAP450. (4) Activation of Aurora-A at the centrosome through Src activation. GA, Golgi apparatus; Imp a, importin a; MT, microtubule; TCR, T cell receptor; TGN, trans-Golgi network.