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REVIEW

Role of Epac Proteins in Mechanisms of cAMP-Dependent Immunoregulation

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Abstract—This review presents observations on the role of Epac proteins (exchange protein directly activated by cAMP) in immunoregulation mechanisms. Signaling pathways that involve Epac proteins and their domain organization and functions are considered. The role of Epac1 protein expressed in the immune system cells is especially emphasized. Molecular mechanisms of the cAMP-dependent signal via Epac1 are analyzed in monocytes/macrophages, T-cells, and B-lymphocytes. The role of Epac1 is shown in the regulation of adhesion, leukocyte chemotaxis, as well as in phagocytosis and bacterial killing. The molecular cascade initiated by Epac1 is examined under conditions of antigen activation of T-cells and immature B-lymphocytes.

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Adenosine-3′,5′-cyclophosphate (cAMP) is a secondary messenger through which effects of a number of hormones, neurotransmitters, and other signaling molecules are realized [1]. Receptors for interaction with ligands for activation of cAMP synthesis belong to the G-protein-coupled receptor (GPCR) family. Each member of this family interacts inside the plasma membrane with a member of another family of proteins capable of binding guanine nucleotides (G-proteins). All G-proteins (except small G-proteins of the ras family) are heterotrimers consisting of α-, β-, and γ-subunits. Every G-protein has a unique α-subunit, whereas the β- and γ-subunits are highly homologous. By now, 17 different types of α-subunits have been identified. The targeting of the G-protein effect is determined by the α-subunit type after which the whole G-protein is termed. Thus, G_s containing α_s stimulates adenylate cyclase (Ac), whereas G_i inhibits it, and G_q activates phospholipase C (PLC) [2].

Generation of cAMP is initiated if an extracellular primary messenger (neurotransmitter, hormone, chemokine, a lipid mediator, or a pharmaceutical preparation) binds with GPCR interacting with the stimulating α-subunit of the G-protein ($G\alpha_s$). This results in the replacement of GDP by GTP in the α_s-subunit of the G-protein and the subsequent dissociation of this subunit from the complex of β/γ-subunits possessing its own targets, whereas the α_s-subunit/GTP activates Ac, which converts ATP to cAMP and pyrophosphate [3]. There are

Abbreviations: Ac, adenylate cyclase; AKAP, A kinase anchoring protein; APC, antigen-presenting cell; BCR, B-cell receptor; cAMP, adenosine-3′,5′-cyclophosphate; CD, cluster of differentiation; C3G, CrkSrk homology 3 (SH3) domain guanine nucleotide exchanger; CR, complement receptor; DAG, diacylglycerol; Epac, exchange protein directly activated by cAMP; ERK, extracellular signal-regulated kinase; FoxO1, O-class forkhead transcription factor; GEF, guanine nucleotide exchange factor; GPCR, G-protein coupled receptor; IFN, interferon; IL, interleukin; IP_3, inositol-3-phosphate; Jak, Janus kinase (Just another kinase); JNK, *N*-terminal kinase of the Jun transcription factor; Lyn, tyrosine kinase of the Src family; LFA-1, leukocyte function associated antigen-1; MAPK, mitogen-activated protein kinase; MHC, major histocompatibility complex; PA, phosphatidic acid; PDE, phosphodiesterase; PI3K, phosphatidylinositol-3-kinase; PI-3,4,5-P_3, phosphatidylinositol-3,4,5-triphosphate; PKA, protein kinase A; PKB/Akt, protein kinase B; PKC, protein kinase C; PLC, phospholipase C; Rap, small GTPase; Rap-GAP, GTPase-activating protein; Rap-GRP, guanine nucleotide-releasing protein; RAPL, regulator of cell adhesion and polarization in the lymphoid tissue; ROS, reactive oxygen species; TCR, T-cell receptor; TEM, transendothelial migration; TGF, transforming growth factor; TNF, tumor necrosis factor.

nine types (isozymes) of membrane Ac and one soluble form of Ac, which are selectively expressed in different type cells [3]. Depending on the Ac type, its activity is regulated by both α_s- and α_i-subunits and by other proteins. Thus, Ac2, Ac4, and Ac7 are activated by β/γ-subunits of G-proteins in the presence of α_s-subunit. Ac1, Ac2, Ac4, Ac5, Ac6, Ac7, and Ac8 can be activated by protein kinase C (PKC). Ac1 and Ac8 are additionally activated by Ca^{2+}/calmodulin. The activity of Ac can also be inhibited by a set of different proteins and by Ca^{2+} [4, 5]. Note that immune system cells mainly express isozyme Ac7 [6].

The binding of cAMP with regulatory subunits of protein kinase A (PKA) results in phosphorylation of Ser and Thr residues on numerous target proteins including the cAMP transcription factor CREB (cAMP response element-binding protein) [7]. The free cAMP is converted by phosphodiesterases (PDE) to 5′-AMP [8, 9]. This process is regulated by 12 different members of a large family of tissue-specific PDEs [10]. Both Ac and PDE can be localized in different compartments of the cell [11]. Protein kinase A anchoring proteins (AKAP) determine the PKA and PDE localization in specific cellular microdomains and create discrete subcellular pools of cAMP and its effectors.

cAMP regulates differentiation, secretion, gene transcription, regulation of cell shape, restructuring of cytoskeleton, proliferation, apoptosis, adhesion, and migration [12]. Moreover, cAMP enhances growth effects due to interaction with Ras-mediated mitogen-activated protein kinase (MAPK) [13]. cAMP can cross-interact with the Ca^{2+}-dependent pathway of signal transmission [14] and with protein kinase B (PKB/Akt) [15]. In immune system cells cAMP modulates signal transmission by cytokines inhibiting the Jak/STAT pathway (Janus kinase/signal transmitter and transcription activator) [16].

Specific GPCR molecules inducing production of intracellular cAMP for various hormones, prostaglandins, and neurotransmitters are expressed on virtually all lymphomyeloid cells, and this is a molecular basis for the hormonal control of the immune system [17-19]. The main cell expressing specific hormonal receptors coupled with Ac are presented by neutrophils, antigen-presenting cells (APC), and T- and B-lymphocytes [17, 19]. An increase in the cAMP concentration in immune cells mainly results in disorders in the production of proinflammatory cytokines [20], in suppression of proliferation of T-lymphocytes [21, 22], a decrease in the chemotaxis of eosinophils [23], and in phagocytosis of macrophages [24]. An increase in cAMP decreases the activation of macrophages, inhibits expression of proinflammatory cytokines (Th1-cytokines) including tumor necrosis factor α (TNF-α), interleukins (IL-12 and IL-27), interferon γ (IFN-γ), and such chemokines as chemoattractant protein of monocytes 1 (MCP-1), the proinflammatory protein of macrophages 1β (MIP-1β), and IL-8 [25, 26], concurrently with stimulation of secretion of antiinflammatory cytokines (Th2-cytokines) IL-4 and IL-10 [17, 21, 27] and IL-23 [25].

The various functions of cAMP also include its ability to inhibit the growth, proliferation, and differentiation of T-cells [21, 28]. An increase in cAMP content leads to a decrease in the production of IL-2 by $CD4^+$T-cells and affects the transcription of proinflammatory Th1-cytokine genes. Concurrently, the transcription of IL-4 gene is activated, which is the major Th2 cytokine with immunosuppressive and antiinflammatory effects [21, 29]. All these events result in disorders in the proliferation and clonal expression of T-cells [21, 22].

PKA was earlier thought to be the main effector of cAMP in eukaryotic cells. But recently a protein family was identified that was termed Epac (exchange protein directly activated by cAMP) or cAMP-regulated factors of exchange of guanine nucleotides (cAMP-GEF) [30, 31]. These proteins bind with cAMP and activate small GTPases Rap1 and Rap2 of the Ras superfamily [32, 33]. PKA and Epac have been shown to be capable of inducing excessive, independent, and even opposite effects inside the same cell [34]. Moreover, activities of Ac and PDE are regulated by other signaling systems such as Ca^{2+} (via calmodulin, calmodulin kinase II, calmodulin kinase IV, and calcineurin), subunits of other G-proteins (such as G_i, G_o, and G_q), inositides (via activation of PKC), and tyrosine kinases [35].

Thus, reactions of cells to cAMP can depend on the level of its local production and degradation regulated by Ac and PDE [1, 8]; interaction of cAMP with PKA [36]; interaction of cAMP with various additional factors including Epac and cyclic nucleotide-derived channels [30, 31].

Epac PROTEINS – ACCEPTORS OF cAMP

Mammals have two Epac isoforms: Epac1 and Epac2, which are products of different genes [37]. Epac1 and Epac2 mediate cAMP-mediated signal transmission and are guanine nucleotide exchange factors for small GTPases (Rap1 and Rap2) [32, 38]. The Epac proteins were discovered during searches in the database of proteins that could be responsible for insensitivity of cAMP-induced activation of small GTPase Rap1 to PKA inhibitors [30, 32]. Concurrently, these proteins were identified as cAMP-GEFI (Epac1) and cAMP-GEFII (Epac2) on screening for the presence of brain-specific genes possessing motifs for secondary messenger binding [31]. While Epac1 is expressed in virtually all tissues, in particular in kidneys, ovaries, thyroid gland, and leukocytes [31, 39], Epac2 is characterized by more limited distribution and is expressed in adrenals, brain, and β-cells of the pancreas [30, 31, 37, 40]. Epac1 is expressed

in monocytes and macrophages [41, 42], B- and T-cells [42], eosinophils, neutrophils, platelets, and hematopoietic $CD34^+$ cells [39]. Epac molecules are localized in the plasma and nuclear membranes, perinuclear regions, mitochondria, and their subcellular redistribution depends on the cell cycle phases [43].

Epac1 and Epac2 are multidomain proteins different in structural organization (Fig. 1a). Thus, Epac2 has an additional *N*-terminal domain for cyclic nucleotide binding (CNB-A) [44]. Because only the Epac1 molecule is involved in the cAMP-dependent regulation of the immune system cells, just this molecule will be considered further.

Epac1 includes the *C*-terminal catalytic region, which has a CDC25-homologous domain (CDC25-HD) responsible for guanine nucleotide exchange of the Rap molecule, a Ras-associated domain (RA), and a Ras-exchanging motif (REM), which are necessary for stability of the catalytic region (GEF) [32]. The *N*-terminal region of Epac is a regulatory region that contains one cAMP-binding domain (CBD) or CNB domain and DEP (Dishevelled, Egl-10, and pleckstrin) domain responsible for Epac localization on the membrane [32, 45]. Moreover, the CNB domain of Epac1 interacts with microtubules [46], whereas the whole *N*-terminal region regulates the translocation of Epac to mitochondria [43].

The regulatory region has an auto-inhibitory ability, which is abolished on binding with a cAMP molecule [32]. In the inactive conformation the CNB domain is covalently bound with the catalytic CEF region through a separate polypeptide chain, whereas the intramolecular interaction of the CNB domain and CEF region sterically prevents the availability of Rap for the catalytic region (Fig. 1b). The CNB domain is bound with the catalytic site through REM, the so-called commutator (a pseudo-β-fold between the regulatory and catalytic regions). The REM domain is capable of binding with other regulatory proteins [47]. The CNB-domain acts as a molecular switch for reception of the intracellular cAMP content. Binding of cAMP with Epac results in conformational changes of Epac1 similar to movements in a hinge [48]. The interaction of cAMP with CNB provides for a reorientation of the CNB/DEP domains relative to the residual part of the molecule, which deprives the catalytic site of the CDC25-HD domain (GEF) from the CNB domain that promotes the binding with Rap leading to the replacement of GDP by GTP, i.e. to its activation [32, 33, 38].

The affinity for cAMP binding by the isolated CNB domain of Epac1 is lower than the affinity of its binding by CNB of PKA, i.e. PKA is activated by lower concentration of cAMP than Epac is [49]. However, a usual

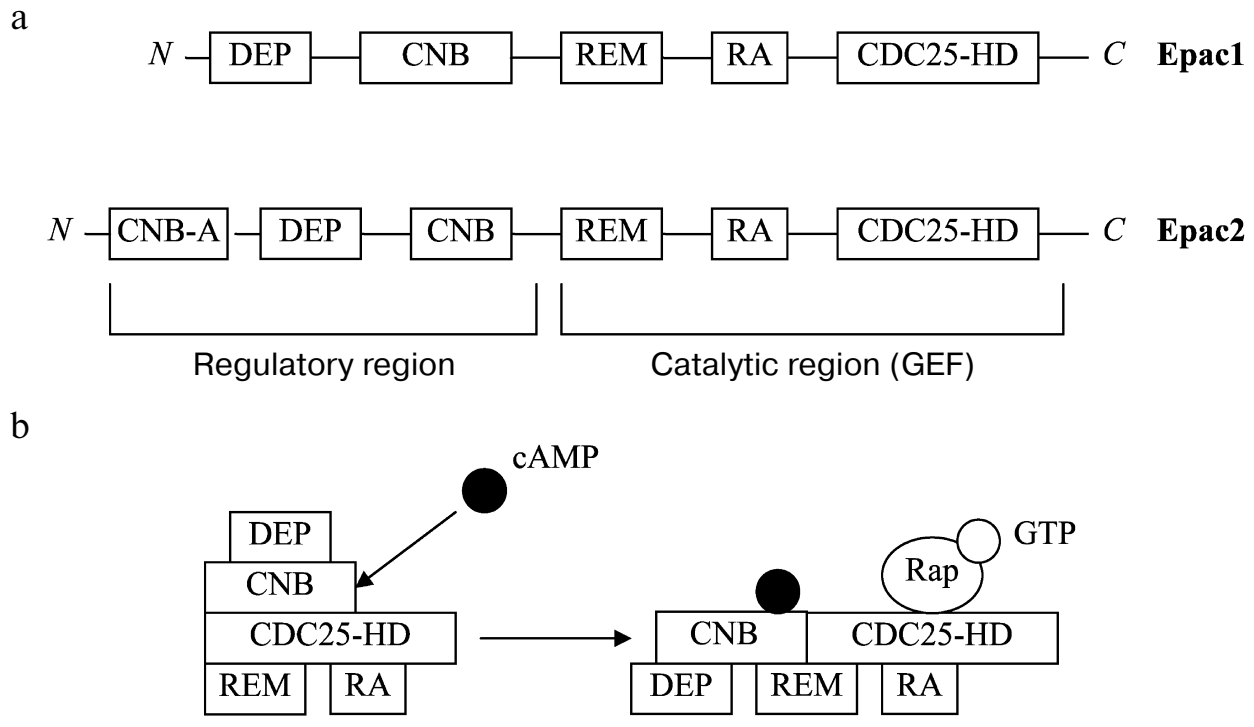

Fig. 1. Structural–functional organization of Epac molecules. a) Scheme of domain organization of Epac1 and Epac2. Epac2 is differentiated from Epac1 by an additional CNB-A domain capable of binding both cAMP and CNB domains. b) Scheme of domain restructuring of the Epac1 molecule upon interaction with cAMP. In the inactive form the regulatory region of the Epac1 molecule (DEP/CNB) acts as an auto-inhibitor, and this function is abolished upon binding with a cAMP molecule, which results in re-orientation of CNB/DEP domains associated with making the catalytic site of the CDC25-HD domain vacant for a Rap molecule.

increase in the intracellular concentration of cAMP is sufficient for activation of Epac1 [50] and recruiting the AKAP family proteins for participation in the process [51]. Epac proteins display their biological properties either as they are or in combination with PKA, whereas AKAP, which interact with PKA, PDE, and Epac, are responsible for coordinating the signal transmission to Epac and/or PKA molecules [40, 51].

The main catalytic function of Epac1 is to activate Rap1 and Rap2 [30, 32], which are main regulators of fundamental cellular processes such as proliferation, differentiation, migration, apoptosis, cytoskeleton restructuring, and contractility [52]. Small GTPases function as molecular switches shuttling between the inactive GDP-bound and the active GTP-bound states. Epac activates the replacement of GDP by GTP in the catalytic site of Ras-like GTPases, whereas proteins activating GTPase (Rap-GAP) accelerate GTPase activity of Ras-like GTPases causing their inactivation [53].

Figure 2 presents a general scheme of signal transmission via Epac proteins (the modulation of functions of integrins and ion channels are not shown). Epac via Rap1 modulates responses of the MAPK cascade through activation of the kinase regulated by extracellular signals (ERK)1/2 [29] and of the *N*-terminal kinase of the Jun transcription factor (JNK) independently of GEF activity [54]. In addition to activation of the MAPK system, Rap1 can also inhibit ERK1/2 depending on the cell type and its own compartmentalization [29]. Due to modulation of MAPK cascade activity, Epac is also involved in cell cycle regulation and in proliferation [55]. Moreover, Epac molecules can interact with microtubules and proteins bound with microtubules [56]. Microtubules act jointly with the actin cytoskeleton and regulate different

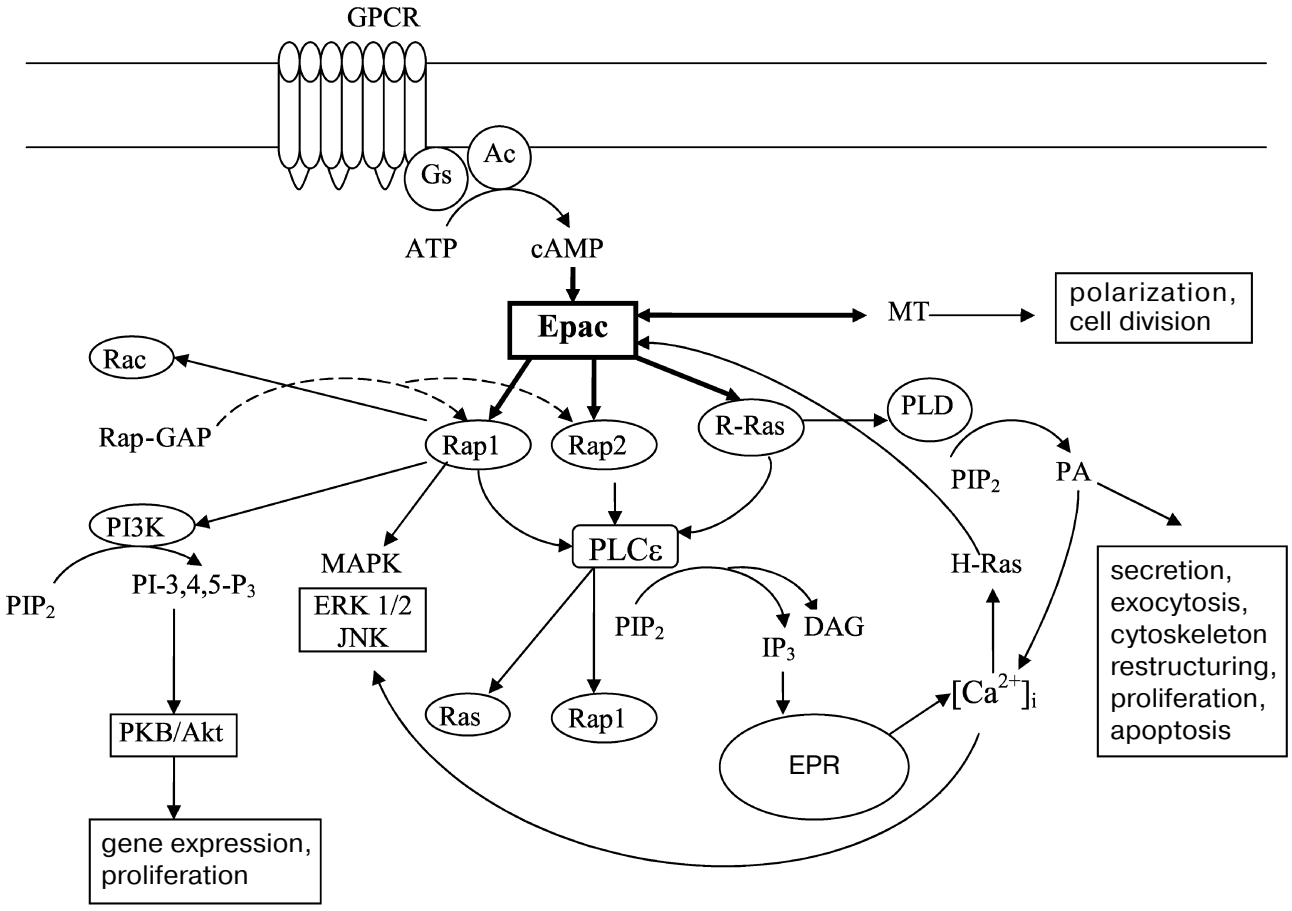

Fig. 2. Spectrum of intracellular effectors regulated by Epac. Epac1 directly regulates functions of microtubules (MT), which in their turn influence Epac1 activity. Rap1 and Rap2 molecules and also R-Ras are main intracellular effectors of Epac1. Rap-GAP is a negative regulator of Rap1 and Rap2. Jointly with R-Ras, activated Rap1 activates PLCε, which is separately activated by Rap2. Moreover, Rap1 activates Rac and PI3K and modulates activity of the MAPK cascade. The product of PI3K, PI-3,4,5-P_3, activates PKB/Akt, which similarly to MAPK regulates proliferation and gene expression. The activation of PLCε increases the concentration of intracellular Ca^{2+}, which additionally activates MAPK and H-Ras, thus modulating Epac1 function. The presence of CDC25-HD in the PLCε molecule allows it to independently activate Rap1 and Ras. R-Ras activated by Epac1, in addition to PLCε, activates PLD, and the product of its catalysis (PA) regulates $[Ca^{2+}]_i$ and associated cell functions (pathways of activation of ion channels and integrin molecules are not shown). Here and further EPR is endoplasmic reticulum, and solid and dashed arrows show, respectively, stimulation and inhibition.

reactions of cells, in particular polarization, division, and migration [57]. Dynamics of microtubules can play an important role in the regulation of signaling properties of Epac [58]. The stability of the microtubular network determines the ability of Epac molecules to activate Rap because the Rap proteins can associate with microtubules of the mitotic spindle [43].

Epac1 can activate both Rap1 and the Rap2 molecule, which is also localized on the plasma membrane and intracellular compartments, including the Golgi apparatus, lysosomes, endosomes, and the perinuclear space [30, 32]. In their turn, Rap2 and Rap1 together with R-Ras activate phospholipase C (PLCε) [59]. Activation of PLCε results in production of diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP_3), which increases the concentration of the intracellular Ca^{2+} ($[Ca^{2+}]_i$) [60]. The increase in $[Ca^{2+}]_i$ in turn activates H-Ras and ERK1/2 [61].

Thus, the Epac–Rap2–PLCε pathway can additionally induce activation of H-Ras and ERK1/2 mediating it through Ca^{2+}. The R-Ras-induced activation of PLCε also increases $[Ca^{2+}]_i$ in cells [62]. It seems that PLCε is a molecular commutator between universal secondary messengers Ca^{2+} and cAMP and members of the Ras superfamily of small GTPases comprising Ras, Rap, and Rho [63]. Note that a new member of the PLC family, PLCε, is similarly to Epac characterized by the presence of an *N*-terminal domain CDC25-HD possessing GEF activity to some Ras-like GTPases and two *C*-terminal RA-domains responsible for interaction with Ras-like GTPases [64]. The CDC25-HD domain of PLCε can directly activate Rap1, which provides for stable signal transmission [65]. Thus, PLCε acts in both forward and back directions relatively to Ras-like GTPases, and due to the CDC25-HD domain can simultaneously modulate cell responses independently of Ca^{2+}.

Epac also activates R-Ras, which increases the activity of PLD [66]. PLD hydrolyzes phosphatidylcholine of cell membranes to phosphatidic acid (PA), which is involved in a broad spectrum of early and late cellular responses, in particular in mobilization of Ca^{2+}, secretion, exocytosis, restructuring of cytoskeleton, cell proliferation, and apoptosis [67].

Another effector of the Epac–Rap system is PKB/Akt, which controls many vitally important processes, in particular cell proliferation and survival upon the modulation of gene transcription [68]. This occurs due to initiation by the Epac1–Rap1 system of the catalytic activity of phosphatidyl inositol-3-kinase (PI3K). Phosphatidyl inositol-3,4,5-triphosphate (PI-3,4,5-P_3), which is a product of PI3K, in turn regulates the activity of many proteins containing a PH-domain (pleckstrin-homologous domain), in particular, PKB/Akt and PLCγ2 [69]. Thus, Epac1 proteins can be involved in regulation of gene expression and of macrophage proliferation [70]. However, as in the case of ERK1/2, cAMP-activated Epac1 can either stimulate or inhibit PKB/Akt-phosphorylation [71], and this depends on both the cell type and on activators that determine the interaction of Epac1 and PKB/Akt [70].

Owing to their specific features, Epac proteins form a molecular axis between different members of the Ras superfamily [72]. They also interact with regulators of Ca^{2+}-induced exocytosis and with the network of microtubular cytoskeleton [56]. Epac molecules are involved in various cell reactions, in particular in secretion [72], integrin-mediated cell adhesion [58, 73], formation of intercellular associations [74, 75], apoptosis [76], proliferation [69, 70], and cell differentiation [41].

ROLE OF Epac1 IN ADHESION AND MIGRATION OF IMMUNE SYSTEM CELLS

The extrusion of leukocytes from the blood flow, or transendothelial migration (TEM), has a crucial importance under conditions of acute inflammation and induction of immune response. TEM is regulated by cytokines that activate the abilities of leukocyte for adhesion and migration. Inflammation is characterized by activation of leukocyte adhesion on the endothelium and by an increased vascular permeability induced by the cytokine TNF-α [77]. The chemokine-induced migration of leukocytes includes integrin-mediated adhesion of circulating leukocytes onto the endothelium, polarization of leukocytes in the direction of the chemokine source, and leukocyte migration across the endothelium [78].

The role of Epac1 in integrin-mediated adhesion was first detected in ovary carcinoma cells. In these cells Epac1 via Rap1 mediates $\alpha_5\beta_1$- and $\alpha_v\beta_3$-dependent (β_1-integrins) adhesion to fibronectin [73]. It has been established that cAMP controls the integrin-mediated adhesion of leukocytes mainly through the Epac1–Rap1 pathway [73, 79, 80]. An increased ectopic production of Epac1 induces a smoothed morphology and increases the ability of cells for adhesion [81, 82].

In T-lymphocytes Rap1 increases the avidity of molecules $\alpha_L\beta_2$ or LFA-1 (the antigen-1 associated with leukocyte function) of the β_2-integrin family, which bind with molecules of ligands ICAM-1 (intercellular adhesive molecule 1), which determines the LFA-dependent adhesion of the cells [83]. Epac1 activates Rap1 and thus stimulates the integrin-mediated adhesion of T-cells not only via LFA-1 but also via $\alpha_4\beta_1$ integrins [79]. In addition to T-cells, Epac1 stimulates the $\alpha_4\beta_1$- and $\alpha_5\beta_1$-integrin-mediated adhesion of U937 cells (a monocytic line) to fibronectin, also activating adhesion of normal monocytes to the vascular endothelium [39]. Moreover, Rap1 increases affinity of the $\alpha_{IIb}\beta_3$-integrin of megakaryocytes on their interaction with fibrinogen [84]. Similarly, the Epac1–Rap1 activation increases the β_2- and β_1-integrin-dependent adhesion of endothelial precursor cells to the

endothelial cell monolayer through ICAM-1 molecules [85] and of mesenchymal stem cells to fibronectin, respectively [73]. In hemopoietic $CD34^+$ precursor cells the cAMP-mediated activation of Rap1 also increases $\alpha_4\beta_1$ adhesion [86]. The activities of integrin are regulated by different pathways: by changing expression of the integrin molecules on the surface, by redistribution of membrane integrins accompanied by an increase in avidity, or by inducing conformational changes increasing their affinity [87]. The latter two pathways present a functional activation providing for the transformation of an inactive integrin into a ligand-binding heterodimer due to conformational changes in the extracellular domains of α- and β-chains [88]. Studies with specific antibodies revealed that Rap1 could regulate both avidity and affinity, but not the expression of integrin receptors on the cell surface [83, 89].

Thus, the Epac1–Rap1 pathway of signal transmission stimulates the adhesion of leukocytes through the redistribution of cells and activation and conformational changes in β_1- and β_2-integrins [59]. However, not all integrin-activating effects of cAMP–Epac1 are mediated through Rap1. Thus, Epac1 is shown to directly activate R-Ras [66], which regulates various processes of the integrin-mediated adhesion of cells and thus can complete the effects of Rap1 [90]. Moreover, a direct interaction of Epac1 with the receptor of the growth-transforming factor β1 (TGF-β1) has been shown [91]. TGF-β1 suppresses Epac1 transcription in the monocytic cell line U937, and this results in a decreased activation of Rap1 and $\alpha_M\beta_2$-integrins [92].

Note that agents increasing cAMP content, e.g. forskolin and 3-isobutyl-1-methylxantine, inhibit chemokine-induced adhesion and migration of monocytes [93]. But on the contrary, a urokinase-like plasminogen activator and relaxin, which also increase the cAMP content, stimulate the adhesion and migration of these cells [94, 95]. Additional studies have shown that this is associated with compartmentalization of cAMP molecules [96]. And an important role is played by specific PDEs preventing the diffusion of cAMP into the corresponding regions of the cells. This results in formation of cAMP "clouds" on limited regions that can be activated only by the most available local effectors. In one case cAMP will be activated by PKA and in another case by Epac1, with different effects of the activation [39]. This is confirmed by the observation that an increase in cAMP content in neutrophils inhibits their aggression and migration because they do not express Epac1 proteins [39].

Moreover, the cAMP–Epac1–Rap1 pathway regulates cell homing and adhesion to laminine-5 through $\alpha_3\beta_1$-integrin without involvement of $\alpha_6\beta_4$-integrin [80]. The constitutively active Rap1 induces lymphocyte polarization through its effector RAPL (regulator of cell adhesion and polarization in lymphoid tissue) independently of such stimuli as adhesion of the chemokine gradient [97]. Expression of constitutively active Rap1 in T-lymphocytes stimulates cell migration to immobilized molecules ICAM-1 and VCAM-1 (vascular cellular adhesion molecule-1) even in the absence of chemokines [97]. And conversely, Rap1 inhibition by Rap-GAP significantly suppresses the ability of B-lymphocytes to migrate towards α-chemokine CXCL12 (SDF-1) [98]. A selective activator of Epac1, 8-pCTP-2′O-Me-cAMP, increases the polarization and directed migration of the U937 cells [39], confirming the role of Epac1 in the regulation of leukocyte recruitment. Serotonin interacting with a specific receptor of the GPCR family activates the Epac1–Rap1 pathway, which also increases migration of monocytic U937 cells to the α-chemokine CXCL12 [39]. Moreover, the activation of Epac1 stimulates migration of monocytes to a monocytic chemoattractant, the β-chemokine CCL2 (MCP-1) [39]. Similarly, cAMP-increasing receptors of prostaglandin E2 (EP2/EP4) initiate the Epac1–Rap1 cascade in hematopoietic $CD34^+$ precursor cells that not only enhances increased TEM but also activates the homing of the $CD34^+$ cells to bone marrow [86]. This is also supported by data obtained on lymphocytes of RAPL-deficient mice characterized by a significant disturbance in chemokine-stimulated TEM and by the absence of homing to lymphoid tissues [99].

In polarized migrating leukocytes, Epac1 activates Rap1 in the perinuclear region, and as a result the activated Rap1 interacts with RAPL [39, 100]. GTP-bound or active Rap1 is localized at the cytoplasmic membrane of the anterior of the cell, whereas GDP-bound Rap1 is present in the perinuclear zone [101]. Stimulation of Rap1 by chemokines can induce translocation of RAPL from the perinuclear zone to the anterior where it is colocalized with $\alpha_L\beta_2$-integrin. In turn, the $\alpha_L\beta_2$-integrin can be present on the anterior of the cell only on the activation of Rap1 and RAPL [99, 100].

Thus, on the activation of Rap1 and RAPL the complex moves to the anterior of the cells and colocalizes integrins, which initiates the further polarization and migration of the cells. Extracellular ATP, due to interaction with $P2Y_{11}$ receptors equally activating Ac and PLC, strengthens the undirected migration of monocytes [102], and this enlarges the spectrum of intracellular transducers. Studies on knocked-out mice have shown the insufficiency of the PLC pathway for chemotaxis, recognizing, and response to chemoattractant gradient [103]. On the contrary, studies on neutrophils and peritoneal macrophages isolated from $PI3K^{-/-}$ mice revealed the importance of the PI3K content for chemotaxis [103, 104]. This may be associated with an increased accumulation of IP-3,4,5-P_3 and with PKB/Akt activation [105]. As a result, the pathway of Epac-dependent adhesion and homing of the immune system cells becomes significantly more complicated

(Fig. 3). cAMP activates Epac1, which activates Rap1/2. Then Rap1 can either interact with RAPL and activate integrins, or activate PLCε and PI3K. The activated PLCε catalyzes conversion of PIP_2 to DAG and IP_3, which increases $[Ca^{2+}]_i$ to be used further for activation of directed cell movement and for simultaneous CDC25-HD-mediated activation of Rap1 to promote its interaction with RAPL. The activated PI3K, due to its product IP-3,4,5-P_3, activates PKB/Akt, which is required for chemotaxis and simultaneously activates PLCγ that increases the Ca^{2+} signal. This can be responsible for fixing the PLC activation under the influence of ATP, but this activation seems to be not essential, whereas PI3K is more significant.

ROLE OF Epac IN REGULATION OF FUNCTIONAL ACTIVITY OF MONOCYTES/MACROPHAGES

Monocytes and macrophages are the first line of the organism's defense, which uses primitive systems of nonspecific recognition allowing them to phagocytize microbial agents and play an important regulatory role in the adaptive immune system as APC. They release specific proteins (cytokines) regulating other cells of the immune system by processing and presenting an antigen to T-cells.

Epac1 is established to function in both monocytes and monocytic U937 cells, where it regulates β_1-integrin-dependent adhesion, polarization, and chemotaxis [39]. The interaction of Epac proteins with matrix metallopro-

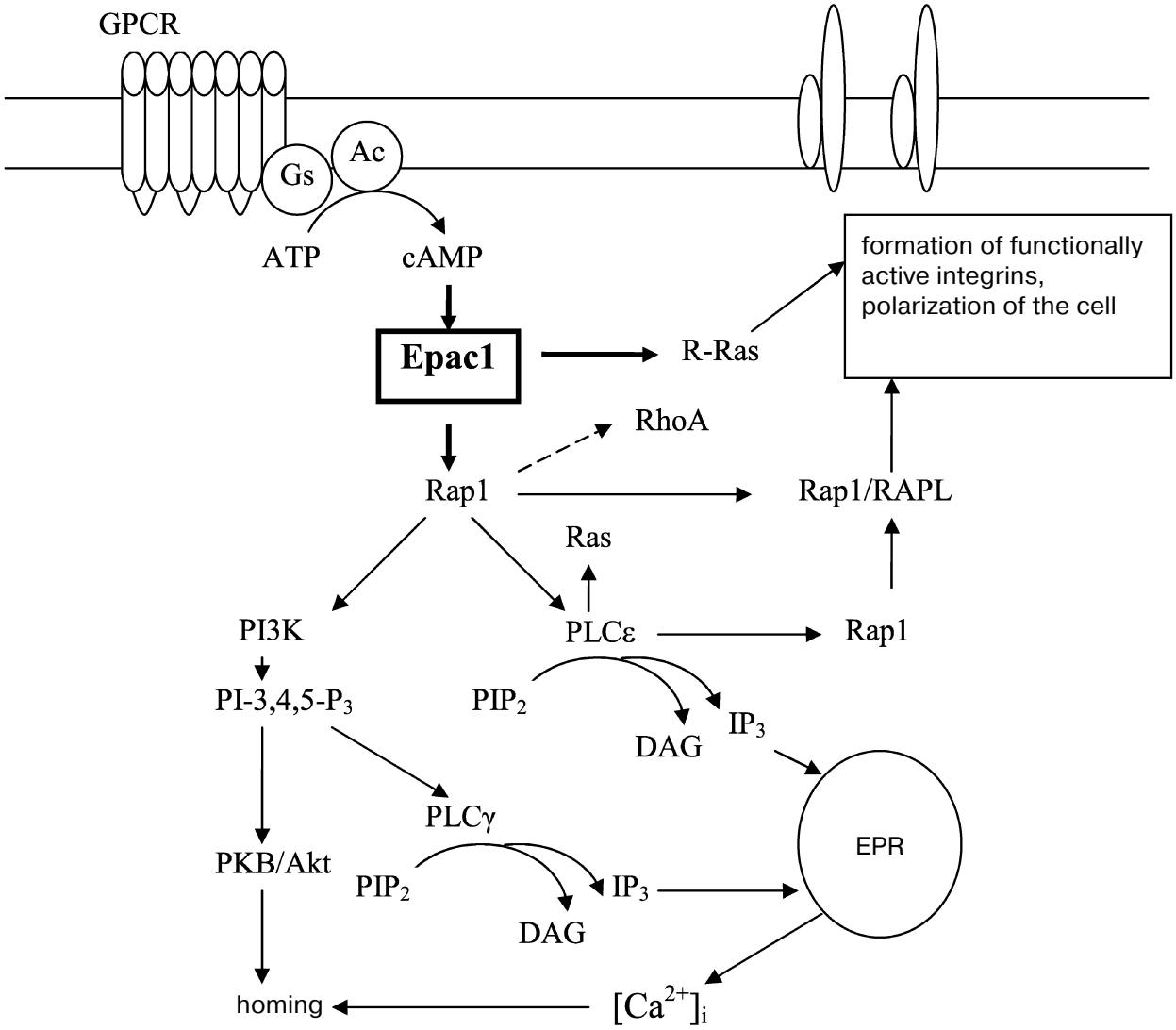

Fig. 3. Molecular mechanism of the regulation of adhesion and migration of leukocytes by Epac1. cAMP activates Epac1 through activation of Rap1, which activates integrins due to interaction with RAPL. Epac1 additionally activates R-Ras, which increases the cell adhesion potentiating the signal transmission from Rap1. Moreover, Rap1 activates PLCε and PI3K. PLCε catalyzes conversion of PIP_2 to DAG and IP_3, which increases $[Ca^{2+}]_i$ necessary for activation of the targeted movement of the cell. Rap1/RAPL is concurrently activated through CDC25-HD. Through its product IP-3,4,5-P_3, PI3K activates PKB/Akt, increasing chemotaxis, and PLCγ, which enhances the Ca^{2+} signal.

teinases suggests that the Epac1-mediated signal transmission can also be involved in tissue remodeling by macrophages [59].

Phagocytosis of monocytes/macrophages is regulated by a certain sequence of intracellular effectors responsible for cytoskeleton and membrane restructuring finally resulting in antigen uptake [106]. Some specialized molecules exposed on the surface of phagocytes can recognize microbial pathogens. These molecules include opsonin-dependent complement receptors (CR) and Fcγ receptors (FcγR) capable of binding constant IgG domains, as well as the A class opsonin-independent phagocytic receptors and mannose and dectin receptors. An increase in the intracellular content of cAMP suppresses phagocytosis mediated by both CR and FcγR and phagocyte receptors [107, 108].

cAMP inhibits phagocytosis by modulating the expression of phagocyte receptors. Thus, in the monocytic cell line U937 cAMP lowers expression of the stimulating receptor FcγRI and simultaneously increases expression of the inhibiting receptor FcγRIIb [108]. In the macrophage cell line PL8 the expression of FcγRIIa increases due to activation of the PKA–CREB pathway (cAMP response element-binding protein) [109]. However, only Epac1 but not PKA is involved in cAMP-dependent decrease in FcγR phagocytosis in alveolar macrophages [34, 110]. Both PKA and Epac1 can reciprocally regulate phagocytosis in different cell types. Although Epac1 is expressed in human peripheral monocytes, cAMP modulates the majority of functions through PKA and not through the Epac1–Rap1 pathway [41]. It seems that the contribution of PKA and Epac1 to the cAMP-induced inhibition of phagocytic activity depends on subpopulations of phagocytic cells and on their specialization.

In alveolar macrophages Epac1 inhibits FcγR-mediated phagocytosis, whereas a PKA-selective analog of cAMP, 6-Bnz-cAMP, does not display such influence [34, 110]. In this case the influence of Epac1 is mediated through activation of tyrosine phosphatase SHIP-1 [110]. In rat alveolar macrophages PKA and Epac1 play different roles in the suppressive activity of cAMP. Thus, cAMP-dependent inhibition of phagocytosis is mediated through Epac1, whereas PKA activation suppresses only production of TNF-α [111]. On the contrary, in circulating monocytes only PKA lowers the FcγR-dependent phagocytosis, whereas both Epac1 and PKA can inhibit this function in macrophages [41]. In peripheral blood monocytes [41] and neutrophils [112] cAMP has inhibitory effects only through PKA, whereas in microglia cells and in peritoneal macrophages myelin phagocytosis occurs with involvement of both Epac1 and PKA [107]. In monocytes/macrophages cAMP regulates the phagosomal oxidation and secretion of lysosomal proteinases and also production and secretion of reactive oxygen species (ROS) and nitric oxide (NO). These processes are, in particular, realized due to the ability of cAMP to inhibit two main stages of NADPH oxidase activation, such as phosphorylation and translocation of the cytosolic $p47^{phox}$-subunit to the cell membrane that leads to a decrease in ROS production [113]. In liver macrophages [114] and in alveolar macrophages [34, 41, 115] the suppression of pathogen-induced ROS production and of bacterial killing is mediated through Epac1. This seems to be due to a physical association of Epac1 proteins with phagosomes containing IgG-opsonized targets [116].

Although the activation of Epac1 has no influence on the pathogen-induced production of the majority of inflammatory cytokines by different leukocytes [115], this protein is involved in the suppression of endotoxin-induced production of IFN-β in macrophage cell lines [117]. Note that the Epac1 content increases threefold during the differentiation of monocytes into mature macrophages [41]. This is favorable for the Epac1-mediated suppressive effect of cAMP in mature macrophages [34]. Similarly, in the J774.A1 macrophage cell line the Epac1–Rap1 system inhibits phagocytosis of complement-opsonized targets through modulation of expression of FcγR molecules [111]. On consideration that Epac1 expression increases during monocyte differentiation into macrophages, the regulatory effects of cAMP in mature macrophages are preferentially mediated through Epac1. It may be that the cAMP-stimulating effect on macrophage proliferation is associated with Epac-dependent activation of PKB/Akt [70], which depends on both Rap1 and PI3K [69]. Figure 4 presents a possible mechanism of the Epac1-dependent regulation of functional activity of phagocytizing cells.

It should also be noted that the activation of Epac1 not only controls phagocytosis and ROS production and regulates monocyte differentiation into macrophages, but it also inhibits apoptosis of human leukocytes [118].

ROLE OF Epac IN REGULATION OF FUNCTIONAL ACTIVITY OF T-LYMPHOCYTES

The activation of T-cells that occurs on the interaction of T-cell receptors (TCR) with molecules of the main histocompatibility complex (MHC) containing an antigenic peptide is crucial for the adaptive immune system. As a rule, the antigen/MHC is presented to T-lymphocytes by dendritic cells, monocytes/macrophages, and B-lymphocytes of APC. The recognizable antigen/MHC complexes are expressed on the APC surface in limited number [119] and have a high dissociation rate and a low affinity for interaction with TCR [120]. For the activation of T-cells resulting in production of cytokines and proliferation, a steady signal transmission from TCR is required [121]. Therefore, the lymphoid cell and APC

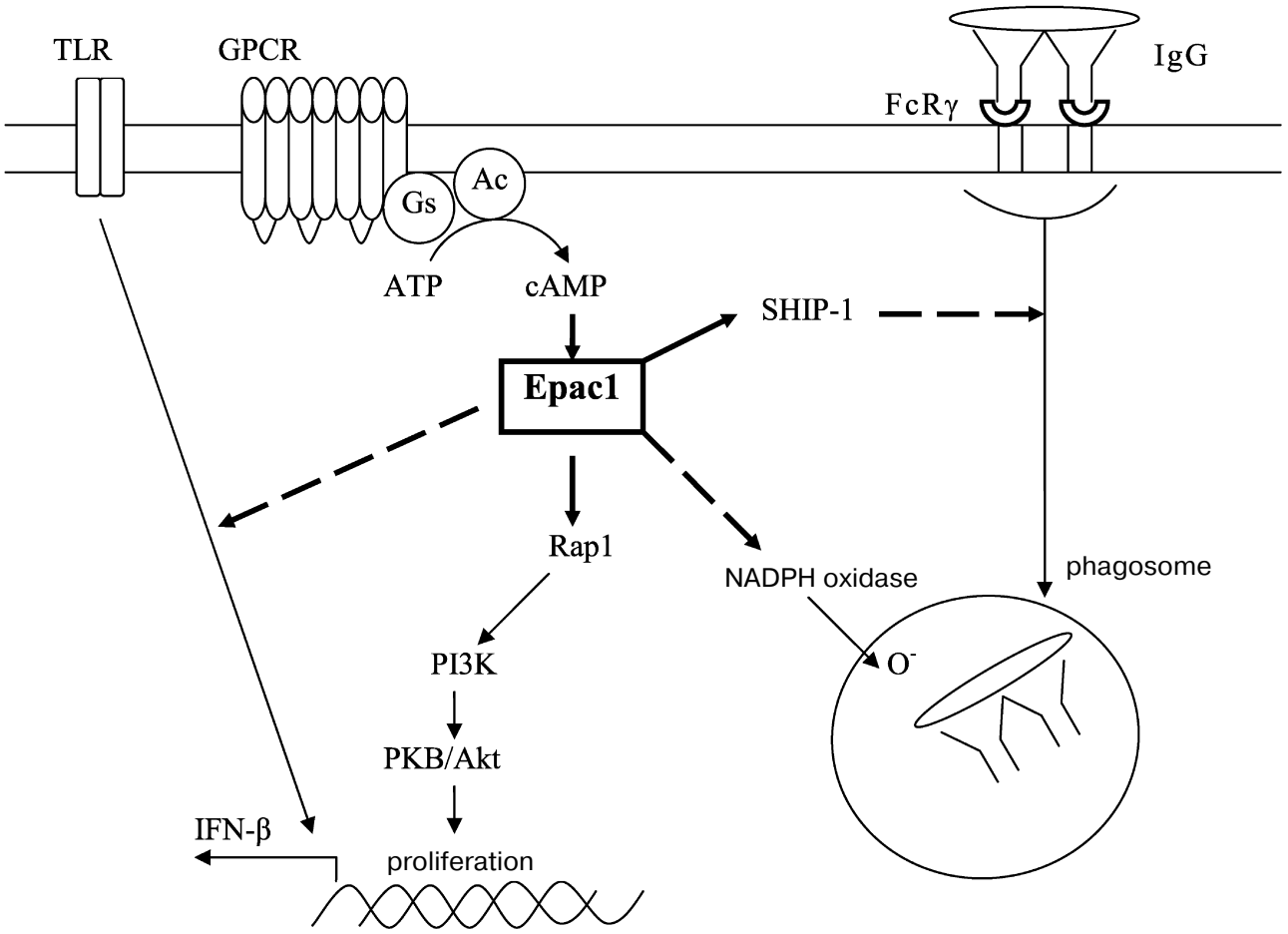

Fig. 4. A possible mechanism of the regulation of monocytes/macrophages by Epac1. Epac1 inhibits FcγR-mediated phagocytosis due to activation of tyrosine phosphatase SHIP-1. The cAMP-activated Epac1 proteins through their intracellular effectors affect the translocation of NADPH oxidase to membranes, which leads to a decrease in the production of ROS and bacterial killing. Another mechanism with involvement of Epac1 proteins includes activation of the Rap1–PI3K–PKB/Akt system that increases the proliferative activity of macrophages, and upon induction of IFN-β gene expression mediated through Toll-like receptor (TLR) molecules Epac1 inhibits this process (the mechanism is not considered).

have to be in direct contact through immunological synapses, which are supramolecular activation clusters [122]. An immunological synapse consists of a central cluster represented by the TCR/MHC complex encircled by LFA-1 molecules (leukocyte function-associated antigen-1; β_2-integrin) bound with ICAM-1 molecules and expressed on APC [123, 124]. The formation of these antigen-specific, spatially segregated contact zones correlates with proliferation of T-cells [125]. LFA-1 molecules mediate the low affinity adhesion that allows TCR to interact with the specific antigen/MHC complex. This interaction changes the TCR conformation and activates a cascade of intracellular messengers. Thus, LFA-1 plays the main role in the promotion of functional triggering of the TCR signal, notwithstanding the low density of the antigen presented on APC [126].

Coreceptor CD4 molecules (on T-helpers) or CD8 (on cytotoxic T-cells) that are directly involved in the initial stages of TCR interaction with, respectively, class I or II MHC, are associated with tyrosine kinase lck ($p56^{lck}$), which phosphorylates tyrosine-activated motifs (ITAM) and thus activates tyrosine kinase ZAP70 (ζ-associated 70-kDa protein). Then the enzymatic cascade includes LAT (membrane-associated adaptor molecule), PLCγ, Rac, and the MAPK cascade [127]. The activation of PLCγ and PKCθ triggers Rap1-GEF (Ras-GPR2) and C3G (CrkSrk homology 3 (SH3) domain guanine nucleotide exchanger) that transforms the inactive Rap1-GDP into the active Rap1-GTP. Then the active Rap1 interacts with its effector RAPL [100], which converts LFA-1 molecules into their high-affinity state [89, 128] resulting in formation of immunological synapses [129]. The T-cell then stops migration [130]. Note that the TCR-mediated activation of Rap1 is not involved in the signal transmission pathways leading to production of IL-2 [131], but is a messenger in signal transmission from

CD31 (the adhesive cellular molecule) [83]. CD31 strengthens the signal inducing T-cell adhesion through LFA-1 and VLA-4 (very late antigen-4; β_1-integrin). The binding of CD31 stimulates tyrosine phosphorylation of the cytoplasmic domain of the molecule, which results in interaction with various proteins, in particular, with PLCγ1 [83] due to strengthening the signal from TCR. Similarly, the early marker of T-cell activation, CD98, uses Rap1 for regulation of the LFA-1-mediated adhesion of T-lymphocytes [132].

Thus, Epac1, which is an exchange factor for Rap1/2, can be involved in the regulation of the antigen-specific activation of T-lymphocytes during the stage of activation of Rap1 proteins (Fig. 5). On the cell surface of T-lymphocytes a sufficient number of GPCR receptor molecules is expressed that are specific to various ligands, e.g. prostanoids and hormones increasing the intracellular content of cAMP [17, 133, 134] and, consequently, activating Epac1 molecules.

On one hand, the degree of Rap1 activation is shown to directly correlate with the level of formation of T-cells/APC conjugates [131], and the increased formation of the conjugates accelerates the signal transmission from TCR (an increase in the ERK1/2 activation and in the IL-2 production). However, the increased formation of the conjugates leads to the development of activating apoptosis [131]. It seems that the increased TCR transmission of the signal can be sufficient for inducing FasL

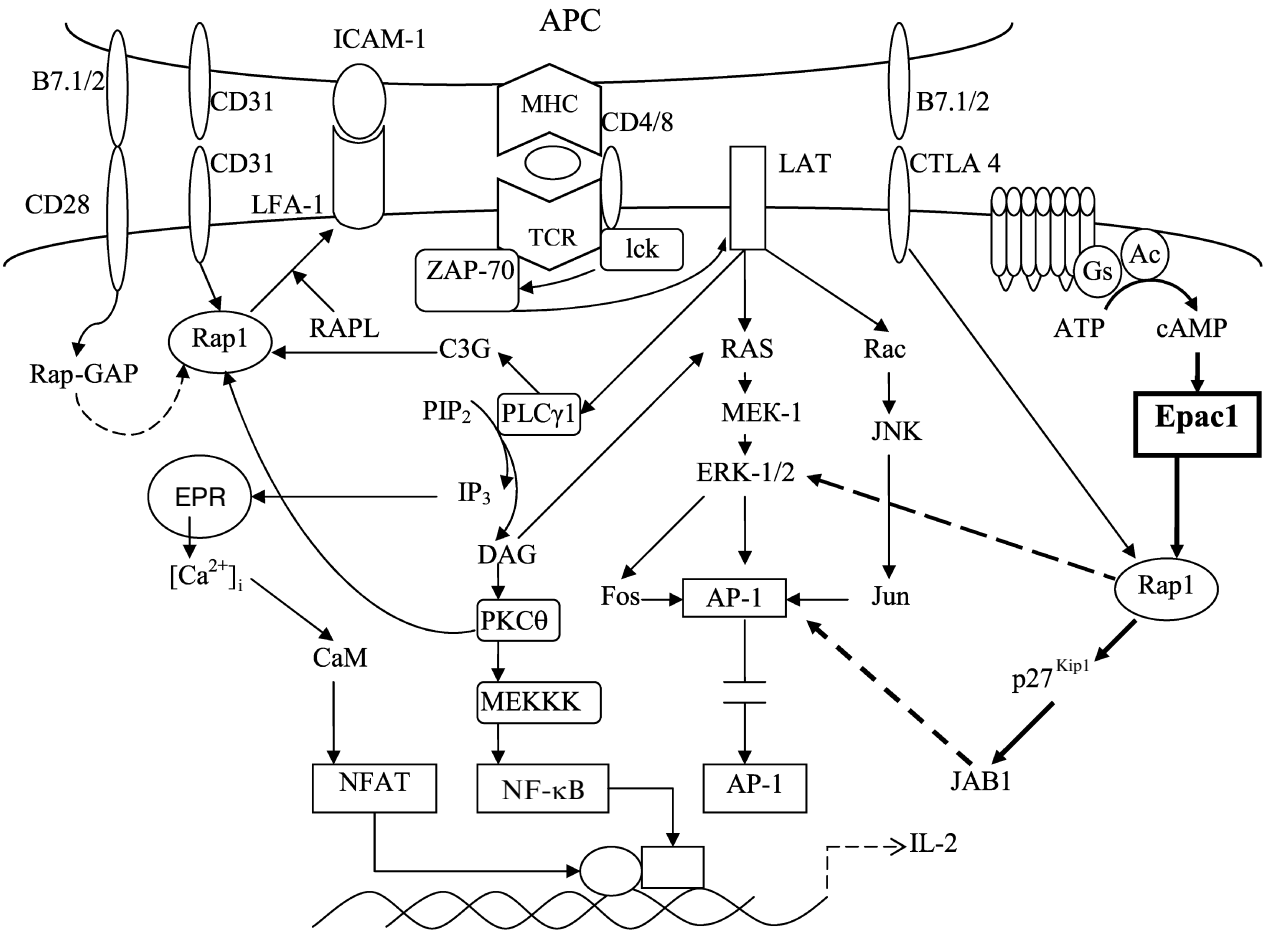

Fig. 5. Involvement of Epac1 in regulation of the signal transmission from TCR in T-lymphocytes. The interaction of TCR and coreceptor molecules CD4/8 with the antigen/MHC complex activates lck, which phosphorylates ZAP70. Then the enzymatic cascade includes a membrane-associated adaptor molecule (LAT), PLCγ, Rac, and the MAPK cascade. PLCγ1 and PKCθ activate Rap1-GEF – Ras-GRP2 (not shown) and C3G that transforms inactive Rap1-GDP into an active Rap1-GTP. The CD28 molecule, which induces Rap-GAP on interaction with B7.1/2, acts as a negative regulator of TCR-dependent activation of Rap1. The active Rap1 interacts with RAPL, which changes LFA-1 molecules into a high affinity state and forms immunological synapses. The homotypical binding of CD31 also results in Rap1 activation. The Rap1 activation by the Epac1 protein inhibits the pathway of signal transmission from TCR due to decrease in the antigen-dependent activation of ERK1/2 and accumulation of $p27^{Kip1}$. $p27^{Kip1}$ is associated with the c-jun co-activator JAB1, which results in a defective transactivation of AP-1 and transcription of the IL-2 gene. CTLA-4 additionally activates the pool of Rap1 molecules, which inhibits ERK1/2. CaM, calmodulin; JNK, *N*-terminal kinase of transcription factor Jun; MEK, MAPK/ERK kinase; MEKKK, MEKK kinase; NFAT, nuclear factor of activated T-cells; NF-κB, nuclear factor κB.

expression on APC on the activation of interactions of T-cells with APC; however, the apoptosis can also be activated due to the increased LFA-1/ICAM-1 adhesion [135]. In total, these data suggest that Rapl influence the intensity of activation of T-cells, regulating the strength of their interaction with APC.

On the other hand, the constitutive activation of Rap1 not associated with the TCR signal directly inhibits the signal transmission pathways from TCR (Rap1V12 transgenic mice). A hyperproduction of Rap1V12 in T-cells inhibits the antigen-dependent activation of ERK1/2 and results in accumulation of $p27^{Kip1}$ [131]. It is known that $p27^{Kip1}$ is a key negative regulator of the G_1 changing to the S-phase. It is associated with c-jun, which is a JAB1 co-activator in the cytoplasm [136], and increase in its concentration can cause the cytoplasmic translocation of JAB1 and result in a defective transactivation of AP-1 (the activating protein 1) and, as a consequence, in a disorder in the transcription of the IL-2 gene [137].

However, an active Rap1 molecule can bind but cannot activate Raf1 and thus can eliminate Raf1 from the Ras/ERK pathway. The TCR activation of Rap1 is inhibited by a CD28 molecule due to induction of the Rap1-GAP activity and is activated by a CTLA-4 molecule (cytotoxic T-lymphocyte antigen-4). However, CTLA-4 activates the pool of Rap1 molecules, which inhibits ERK1/2 [138] that decreases the activation of T-cells [139]. It seems that this mechanism also underlies antiproliferative effects of Rap1 in T-cells and determines the important role of Rap1 under conditions of anergy [140]. This hypothesis is supported, first, by imitation of the anergic phenotype of T-cells on the hyperexpression of an activated mutant of Rap1 (Rap1V12) [131] and, second, by the Rap1-stimulated production of the $CD4^+CD103^+$ regulatory T-cells (Treg) capable of suppressing immune responses [141]. It is also known that the interaction of ligands (B7.1/2) with CTLA-4 inhibits the functional activity of T-cells and suppresses the immune response [142]. It should be noted that ligands whose effects are associated with an increase in cAMP content lead to both induction of Treg [143] and blockade of the antigen-specific activation of T-cells [21].

However, in some works Rap1 was shown to induce a synergic effect with the Ras-mediated signal transmission and to activate ERK, in particular, due to binding by Rap1 of B-Raf that could activate ERK [144]. Note that while Epac1 activated the perinuclear pool of Rap1 and did not activate ERK [145], the Rap1 activation through C3G, on the contrary, activated ERK [146]. It is important that at the TCR-caused activation of T-cell C3G, as differentiated from Epac1, purposefully influenced Rap1 associated with the cytoplasmic membrane [145].

Thus, the ability of Rap1 to activate ERK depends on the Rap1-GEF used. The different localization of C3G and Epac1 suggests that each GEF can activate a separate pool of Rap1 and that these pools can differently regulate ERK. Thus, Rap1 is regulated by compartmentalized signal transmission [132]. It seems that Rap1 associated with the cytoplasmic membrane strengthens the LFA-1-mediated adhesion and activation of T-cells, whereas Rap1 associated with intracellular vesicles and activated by Epac1 counteracts the signal transmission through the Ras/MAPK pathway.

ROLE OF Epac IN FUNCTIONING OF B-LYMPHOCYTES

Signal transmission from the B-cell receptor (BCR) capable of recognizing an antigenic determinant is necessary for the normal functioning of B-lymphocytes. The interaction of BCR with the antigen or with polyclonal activators stimulates different biological effects, which depend on degree of B-cell differentiation, strength, and duration of the signal transmission, and also on the nature of co-stimulating molecules.

cAMP and its acceptors PKA and Epac play important roles in the regulation of functional activities of B-lymphocytes. The available data do not allow us to distinctly formulate the contribution of specific acceptors of cAMP to its effects, but they affirm that the effects of cAMP are preferentially realized through PKA in mature B-lymphocytes of human peripheral blood and mouse spleen and through Epac proteins in immature B-cells. The use of cAMP analogs (6-Bnz-cAMP) that can activate only PKA results in inhibition of ERK1/2 in mature B-cells in spleen independently of BCR, and this affects the function of B-lymphocytes and down-regulates the humoral immune response [147, 148]. As differentiated from mature B-lymphocytes, in poorly differentiated B-lymphocytes the cAMP effects are mediated by Epac. Thus, in the large-cellular B-lymphoma cAMP inhibits PKB/Akt independently of PKA [149], and Epac1 proteins display an antiapoptotic effect on the control of the BCR-induced apoptosis in B-cells of chronic lympholeukemia which are phenotypic analogs of memory B-cells [42, 150]. WEHI-231 cells were recently established to express both Epac1 and Epac2, and their activation by the Epac-specific analog of cAMP (8-pCPT-2′-O-Me-cAMP) resulted in increase in them of ERK1/2, PKB/Akt activity through Epac effectors Rap1 and H-Ras [151]. Moreover, it was shown in the same work that on BCR stimulation cAMP was produced, and Epac activation increased the inhibition of growth of immature B-cells and apoptosis initiated by ligands of BCR [151], thus supporting the negative selection. Thus, for the activation-induced apoptosis of immature B-lymphocytes and memory cells Epac1/2 were necessary, which increased proapoptotic signals from BCR, whereas in mature B-lymphocytes PKA

inhibited functional activity of B-cells independently of BCR.

On the interaction of BCR with an antigen, the growth of immature B-cells is inhibited in the G_1-phase of the cell cycle, and then these cells are subjected to apoptosis. This mechanism underlies the elimination of autoreactive B-cells [152, 153]. The WEHI-231 line of immature B-cells of lymphoma is widely used as a model for studies on negative selection during the development of B-cells [154, 155]. The development of the activation-induced apoptosis in immature B-cells is determined by activities of such proteinases as ERK1/2 and PKB/Akt [153]. The final response of immature B-lymphocytes to an antigen depends on the balance between the proapoptotic activity of ERK1/2 [156, 157] and the antiapoptotic activity of the PI3K-dependent PKB/Akt [158, 159].

The scheme of the role of Epac molecules during negative selection of immature B-cells is presented in Fig. 6. The interaction of BCR with ligands or with an antigen leads to activation of PLCγ2, which converts PIP_2 to IP_3, enhancing the release of intracellular Ca^{2+} and of DAG activating PKC. In turn, Ca^{2+} and DAG induce the appearance of activators of Ras exchange (Ras-GRP) and the PKC-mediated blockade of Ras inhibitors (Ras-GAP), which increases the BCR-induced apoptosis in WEHI-231 cells due to activation of Ras independently of ERK and PI3K [160]. Epac1 through its effectors Rap1/2-GTP and R-Ras additionally activates PLCε, which is expressed in WEHI-231 cells [151] and increases the effect of PLCγ2 [161]. Thus, Epac1 increases the BCR-induced activation of Ras1 and, as a consequence, of the MAPK cascade proteinases ERK1/2. Moreover,

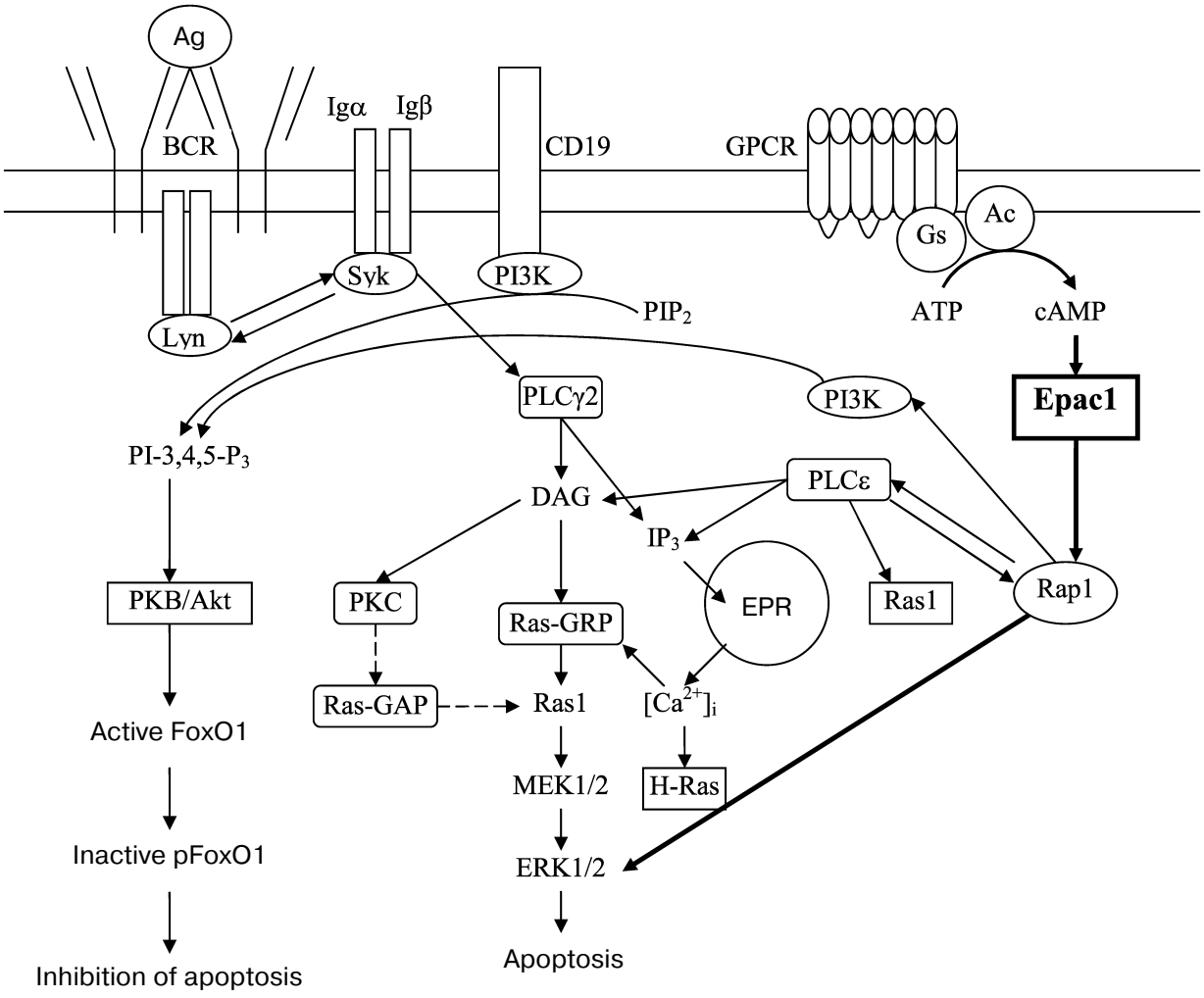

Fig. 6. Role of Epac1 in regulation of signal from BCR in immature B-cells. The interaction of BCR with an antigen activates PLCγ2, which converts PIP_2 to IP_3, increasing the release of intracellular Ca^{2+} and to DAG activating PKC. Ca^{2+} and DAG stimulate activators of Ras exchange (Ras-GRP) and through PKC inhibit molecules of Ras inhibitors (Ras-GAP). The activation of Ras1 leads to increase in the MAPK cascade. Epac1 via Rap1/2 and R-Ras (not shown) activate PLCε and increase the effect of PLCγ2. Moreover, Epac1 via Rap1 provides for additional activation of ERK1/2, resulting in increase of apoptosis. Ag, antigen; Syk, tyrosine kinase homologous to ZAP-70 of T-lymphocytes; Lyn, Src family tyrosine kinase; FoxO1, O-class transcription factor forkhead; pFoxO1, phosphorylated form of Fox01.

Epac1 activates Rap1 (Rap1-GTP), which provides for an additional activation of ERK1/2 and the PI3K-dependent PKB/Akt [162]. The above-described Epac-dependent mechanisms result in strengthening of apoptosis of WEHI-231 cells [151], which seems to indicate the domination of the ERK1/2 pathway despite the activation of PKB/Akt. Note that the Epac-specific cAMP as it is does not induce growth inhibition and apoptosis but only enhances them in response to activation of BCR molecules [151].

Data on cAMP induction during the BCR-mediated activation of cells [151] cannot be explained by classic schemes of signaling pathways of activation of B-lymphocytes. Most likely, the Ac activation is induced through GPCR; therefore, Epac acts in WEHI-231 cells as a modulator of late signaling events.

Epac-INDEPENDENT ACTIVATORS OF Rap

As mentioned above, the subgroup of Rap proteins is represented by two main molecules, Rap1 and Rap2, each of which has isoforms Rap1A, Rap1B and Rap2A, Rap2B (respectively, with 95 and 90% homology) [163, 164]. In the majority of cases the Epac proteins mediate the effects of cAMP through Rap1 and Rap2 molecules. An exception is the immediate activation of Epac1 by R-Ras [66], which regulates the integrin-mediated adhesion of cells and thus supplements the effect of Rap1 [165].

However, Rap1 is also activated by a number of extracellular agents or stimuli not associated with activation of the Epac proteins (Fig. 7). In particular, such agents include ephrin (through ephrin tyrosine kinases) [166], neurotransmitters (through receptors of N-

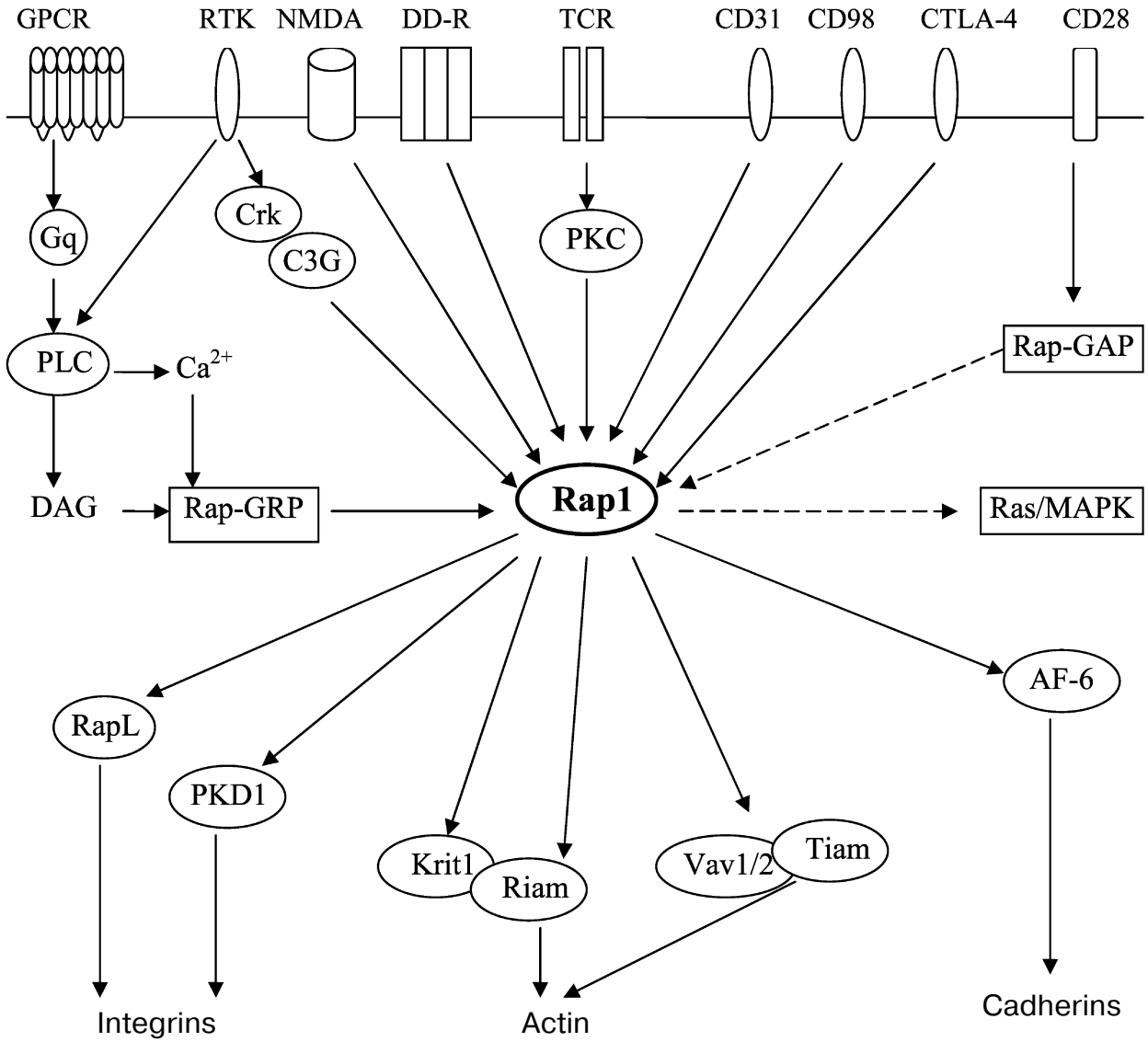

Fig. 7. Epac-independent activators of Rap1 and its effectors. RTK, receptor tyrosine kinase; AF-6, afadin.

methyl-D-aspartate (NMDA)) [167], TNF-α, IL-1, and a lipopolysaccharide (through receptors associated with death domains (DD-R)) [168, 169], an antigen integrated with MHC (through TCR) [131], the stroma cell factor-1 (SDF-1) [98], interaction of ligands with CD98 [170] and CD31 molecules [83]. In some cases Epac-independent activation of Rap is induced by GPCR ligands resulting in formation of such secondary messengers as Ca^{2+} and DAG (through Rap-GRP) [165].

In turn Rap1 activates some intracellular effector molecules mainly represented by scaffold proteins: AF-6 involved in formation of the intercellular binding [165]; RAPL and Riam [171] controlling cell adhesion, Krit1 [172], which is a protein binding with adhesion junction molecules, such as β-catenin [172]; Vav2 and Tiam1 which are Rac-GEF [173] and are necessary for restructuring of actin [174, 175]. It seems that this provides for the association between the majority of Rap1 functions with the cell adhesion and regulation of functions of integrins. Rap1 controls some specialized types of intercellular adhesion in the immune system, e.g. formation of immune synapses, which arise on the interaction of T-cell and APC [131] and binding of C3bi-opsonized objects of phagocytosis by $\alpha_M\beta_2$ integrins (CR3) [111]. Due to Rap1, inactive integrins, such as $\alpha_4\beta_1$ (VLA-4), $\alpha_5\beta_1$ (VLA-5), $\alpha_L\beta_2$ (LFA-1, CD11a/CD18), $\alpha_M\beta_2$ (CR3, CD11b/CD18), and $\alpha_{II}\beta_3$, are converted to ligand-binding heterodimers [83, 84, 111]. Moreover, Rap1 modulates responses of MAPK, in particular ERK1/2, inducing either stimulation or inhibition of these protein kinases depending on the cell type and their compartmentalization [29].

Thus, the Rap1 proteins are key regulators of external and intracellular signaling molecules, which act through effector molecules. In immune system cells Rap1 mainly controls adhesion processes that are involved in phagocytosis and formation of immune synapses and also chemotaxis. Depending on the GEF activating a particular pool of Rap1, compartmentalized Rap1 effectors will be activated leading to different effects. Besides, the spectrum of immune responses will be directly dependent on the cell type, the degree of its differentiation, the level and duration of the Rap1 activation [131], and also on the presence of Rap-GAP molecules.

Immune system cells expressing Epac1 proteins are regulated by cAMP via the Epac1–Rap1/2 pathway under conditions of PKA unavailability determined by AKAP proteins and concentration of cAMP. However, in some cases cAMP seems to be able to simultaneously activate both PKA and Epac1. Small GTPases Rap1 and Rap2 are the main intracellular effectors of Epac1. Effects of Rap molecules are mainly associated with appearance of functionally active β_1- and β_2-integrins and also with chemotaxis of immune system cells. In the case of monocytes/macrophages, Epac1 activation strengthens TEM and thus contributes to accumulation of the cells in the zone with a high concentration of specific chemokines. If Epac1 is activated in T-cells, the effect is realized through β_2-integrins (LFA-1), and this determines formation of the immune synapse, and, consequently, the degree of antigenic activation of T-lymphocytes. The strong interaction of T-cells with APC leads to apoptosis of lymphocytes due to a high Epac1-independent activation of Rap1, whereas the Epac1-dependent activation of Rap1 prevents the response of T-lymphocytes to antigen due to the suppressing influence on ERK1/2 and accumulation of protein $p27^{Kip1}$. Rap1 associated with the cytoplasmic membrane with the C3G-regulated activity increases the LFA-1-mediated adhesion and activation of T-cells, whereas Rap1 associated with intracellular vesicles and activated by Epac1 counteracts the Ras/MAPK pathway of signal transmission.

In addition to increasing the functional activity of integrin molecules in macrophages, Epac1 also triggers pathways for activation of intracellular effectors suppressing FcγR-mediated phagocytosis, and these pathways are accompanied by a decrease in ROS production and bacterial killing. However, under certain conditions the Epac1–Rap1–PI3K pathway can potentiate proliferation of these cells due to activation of PKB/Akt. The effects of cAMP can be realized via the Epac1–Rap1 pathway also in poorly differentiated B-lymphocytes. In these cells the activation of Epac1–Rap1 can increase the effect of the BCR-induced apoptosis, which underlies the negative selection of autoreactive cell clones.

Thus, the Epac proteins play an important role in the control of immune system cells increasing and/or reciprocally regulating cell responses realized through PKA. This double control broadens the range of cAMP influence. The intracellular localization of Epac molecules and their effectors are also important for a particular direction of cAMP action.

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