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## Serine-threonine kinases in TCR signaling

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### Abstract

T lymphocyte proliferation and differentiation are controlled by signaling pathways initiated by the T cell antigen receptor. Here we explore how key serine-threonine kinases and their substrates mediate T cell signaling and coordinate T cell metabolism to meet the metabolic demands of participating in an immune response.

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T lymphocyte proliferation and differentiation are controlled by signaling pathways initiated by the T cell antigen receptor (TCR). The initial events in TCR signaling are mediated by cytosolic tyrosine kinases and adaptors that function to couple the TCR to a network of serine-threonine kinases that propagate the signal from the cell membrane to the nucleus and determine the transcriptional changes that support effector T cell differentiation. In this context, the immune activation of T lymphocytes is a metabolically demanding process, and emerging evidence suggests that serine-threonine kinases that control the metabolic programs that determine T cell function are themselves regulated by extracellular nutrient availability and the cytokine milieu. Here we describe key serine-threonine kinases that mediate TCR signaling and explore how serine kinase networks can coordinate T cell metabolism to meet the metabolic demands of participation in an immune response.

### Diacylglycerol-regulated serine-threonine kinases in T cells

A key event in the TCR signaling cascade is phospholipase C- $\gamma$  (PLC- $\gamma$ )-mediated hydrolysis of phosphatidylinositol-(4,5)-bisphosphate (PtdIns(4,5)P<sub>2</sub>) at the cell membrane, which generates the second messengers inositol-(1,4,5)-trisphosphate (Ins(1,4,5)P<sub>3</sub>) and polyunsaturated diacylglycerols (DAGs) (Fig. 1). T lymphocytes express multiple DAG-binding serine-threonine kinases in the protein kinase C (PKC) family<sup>1,2</sup>. The importance of these enzymes for T cell activation was first recognized after experiments showing that phorbol esters, pharmacological mimics of DAG that activate PKCs, could regulate cytokine production and TCR expression in T cells<sup>3,4</sup>. Another observation that drew the attention of

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immunologists to PKCs was that the PKC- $\theta$  isozyme selectively polarized to the contact area between a T cell and an antigen-presenting cell (APC) loaded with antigen. This formed the basis for the concept of the now well-studied immunological synapse. In this context<sup>5,6</sup>, it is now recognized the recruitment of PKC- $\theta$  to the immune synapse is regulated by other PKC isozymes, notably PKC- $\epsilon$  and PKC- $\eta$ <sup>7</sup>. It is also now well known that PKCs are not the sole targets for DAG signaling in T cells. Other TCR-regulated DAG binding proteins in T cells are guanine nucleotide-exchange factors (RasGRPs)<sup>8</sup> and the protein kinase D (PKD) serine-threonine kinases<sup>9</sup>.

DAG probes have been used to define the spatial dynamics of DAG production in T lymphocytes responding to antigen presented by major histocompatibility complex (MHC) molecules on the surface of APCs<sup>10–13</sup>. When T cells make contacts with antigen-loaded APCs, a sharp gradient of DAG rapidly develops, resulting in an accumulation of this lipid in a focused area of T cell membrane, at the point of contact with the APC. This polarized concentration of DAG permits the localized recruitment and activation of PKCs and PKDs<sup>7,11,13</sup>. The rate-limiting step for PKC activation by TCR triggering is thus production of DAG and the localization of PKCs to the plasma membrane. PKC activity is also dependent on the phosphorylation of a conserved T-loop motif within the catalytic domain of the enzyme by phosphoinositide-dependent protein kinase 1 (PDK1)<sup>2</sup>. However, there is high basal activity of PDK1 in T cells, and the T-loop phosphorylation of PKCs is a priming phosphorylation that occurs before TCR engagement<sup>14</sup>. In addition, the PKC  $\alpha$ ,  $\beta$ I and  $\beta$ IIm isoforms have a calcium-binding site, but the concentrations of calcium found in quiescent T cells (70–100 nM) are sufficient to support enzyme catalytic activity. Mature T loop-phosphorylated PKCs and/or calcium-bound PKCs are thus dependent on DAG and phospholipid binding at membranes to achieve full catalytic function.

Several substrates identified for PKCs explain why these kinases are important for TCR signal transduction (Fig. 1). For example, PKC- $\theta$  phosphorylates caspase recruitment domain-containing MAGUK protein 1 (Carma1), allowing this scaffolding protein to bind to signaling protein Bcl-10 and the I $\kappa$ B kinase IKK $\gamma$ , promoting the assembly of the IKK signaling complex and coupling the TCR to the activation of the transcription factor NF- $\kappa$ B<sup>15,16</sup>. PKCs also have an essential role in effector cytotoxic T lymphocytes (CTLs) for antigen receptor-induced reorientation of the centrosome<sup>13,17</sup> and the polarized exocytosis of the cytolytic granules that kill target cells<sup>18</sup>. The relevant PKC substrate for centrosome polarization is the myosin regulatory light chain, which controls the localization of the nonmuscle myosin motor complex<sup>17</sup>. PKCs also allow the TCR to control T cell adhesion and cell motility. The phosphorylation of the guanine nucleotide exchange protein RapGEF2 by PKC- $\theta$  thus triggers activation of the guanine nucleotide-binding protein Rap1 that in turn activates the integrin LFA-1 (ref. 19) (Fig. 1). PKCs also phosphorylate the  $\beta$ -chain of LFA-1 on sites that regulate the interaction of LFA-1 with cytoskeletal proteins and controls LFA-1-mediated cell adhesion<sup>20</sup>. DAG-PKC signaling cascades thus regulate cell adhesion and cell migration at multiple levels and control T cell positioning within lymphoid tissues, T cell transmigration across endothelial membranes and T cell contacts with APCs.

Another DAG-binding serine-threonine kinase expressed abundantly in T cells is PKD2. This kinase is rapidly activated by TCR engagement in a response that is initiated directly by

antigen receptor–regulated increases in DAG binding to an N-terminal regulatory domain in PKD2 (ref. 21). The activation of PKD2 is then completed and stabilized by PKC-mediated phosphorylation of Ser707 and Ser711 within the activation loop of the PKD2 catalytic domain<sup>22,23</sup>. The allosteric regulation of PKD2 by PKC phosphorylation affords a mechanism for this molecule to act as a signal amplifier for PKC. PKDs are substrates for most classical and novel PKCs and are thus regulated by antigen receptors irrespective of the repertoire of PKC isoform expression in a particular cell<sup>9</sup>. Importantly, PKC-phosphorylated PKD2 is catalytically active in the absence of DAG binding and hence does not need to be localized to the plasma membrane to remain active. Indeed, during the sustained response to antigen receptor engagement, phosphorylated and active PKD2 molecules are found localized in the cytosol<sup>11</sup>. The allosteric activation of PKD2 by PKC-mediated phosphorylation thus allows this kinase to transduce TCR-mediated increases in DAG from the cell membrane to the cytosol. The importance of PKD2 as a PKC substrate and an effector of DAG-PKC signaling in T cells has been demonstrated *in vivo* through T cell–mediated immune responses in mice expressing PKD2 variants that cannot be phosphorylated by PKC (S707A and S711A in PKD). These PKD-mutant mice showed a striking reduction in the ability of the TCR to induce the production of proinflammatory cytokines such as interleukin 2 (IL-2) and interferon- $\gamma$  (IFN- $\gamma$ )<sup>24,25</sup> (Fig. 1).

## Ras-regulated serine-threonine kinase pathways

Triggering of the TCR is known to activate serine-threonine kinase signaling cascades regulated by Ras guanine nucleotide–binding proteins. TCR-mediated DAG production controls Ras activity by regulating the intracellular localization and PKC-mediated phosphorylation of RasGRPs<sup>8</sup>. Ras rapidly cycles between GDP-bound (inactive) and GTP-bound (active) states. When active, Ras proteins bind to the serine-threonine kinase Raf-1, initiating the mitogen-activated protein kinase (MAPK) cascade that phosphorylates and activates the serine-threonine kinases Erk1 and Erk2 (Fig. 2). The Ras-Raf-Erk1-Erk2 pathway is important for TCR function during positive selection in the thymus and in peripheral T cells<sup>26,27</sup>. The importance of Erk1 and Erk2 in T cells is partially explained by their ability to phosphorylate transcription factors and thus regulate their activity. For example, Erk1 and Erk2 activate the ternary complex factor subfamily of ETS-domain transcription factors Elk-1, SAP-1 and SAP-2, which control expression of immediate early genes (such as those encoding c-Fos, Egr1 and Egr3) in T cells<sup>28</sup>. The Erk-mediated regulation of c-Fos expression contributes to the activator protein-1 (AP-1) transcription complex formed by the transcription factors c-Fos and Jun<sup>29</sup>. The ternary complex factors also control the transcription of dual-specificity (threonine/tyrosine) MAPK phosphatases that initiate a negative regulatory feedback pathway that acts to limit the duration and magnitude of Erk activity<sup>30</sup>. Erk1 and Erk2 also have a role in regulating microtubule remodeling through the phosphorylation and regulation of stathmin<sup>31</sup>. However, it should be emphasized that Erk1 and Erk2 kinases are not the endpoint of the kinase signaling cascade. In this context, the 90-kilodalton ribosomal protein S6 kinase (RSK) is an Erk1 and Erk2 substrate that has been implicated in cell cycle progression and cytokine production in T cells<sup>32</sup>. The initiating step for RSK activation is thus Erk1- and Erk2-mediated phosphorylation of Ser369, Thr365 and Thr577 in its C-terminal catalytic domain. The

activated C-terminal catalytic domain of RSK then autophosphorylates its Ser386 to create a docking site for PDK1, which then phosphorylates Ser227 in the N-terminal RSK kinase domain, thereby activating the enzyme<sup>33</sup>. In addition, Erk1 and Erk2 phosphorylate and activate the MAPK signal-integrating kinases 1 and 2 (MNK1 and MNK2). Erk1 and Erk2 phosphorylate regulatory sites outside the T activation loop in MNK1 and MNK2 that are required for efficient activation<sup>34,35</sup>. When active, MNK1 and MNK2 regulate protein translation through the phosphorylation of the eukaryotic translation initiation factor eIF4E<sup>35,36</sup>. The ability of DAG to couple the TCR to diverse serine-threonine kinases is thus a mechanism that allows the TCR to coordinate the regulation of key processes for T cell activation such as transcription, translation and proliferation (Figs. 1 and 2).

### The PtdIns-(3,4,5)P<sub>3</sub>-regulated serine-threonine kinase Akt

One other lipid second messenger that couples the TCR to pathways of serine-threonine phosphorylation is phosphatidylinositol-(3,4,5)-trisphosphate (PtdIns(3,4,5)P<sub>3</sub>)<sup>37</sup>. In naive T cells the intracellular abundance of PtdIns(3,4,5)P<sub>3</sub> is low, but it increases rapidly after triggering of the TCR<sup>38,39</sup>. T cells can sustain production of large amounts of PtdIns(3,4,5)P<sub>3</sub> for many hours in a response that requires continual engagement of the TCR, costimulatory molecules such as CD28 and continual activation of class 1 phosphatidylinositol-3-OH kinase (PI(3)K) p110<sup>38,40,41</sup>. One crucial function for PtdIns(3,4,5)P<sub>3</sub> is that of regulating the activity of the serine-threonine protein kinase B, also known as Akt. A rate-limiting step for Akt activation is phosphorylation of Thr308 within the Akt catalytic domain by PDK1. PtdIns(3,4,5)P<sub>3</sub> binding to the Akt pleckstrin homology (PH) domain causes a conformational change that allows PDK1 to phosphorylate Thr308 within the Akt catalytic domain and activate the enzyme<sup>42</sup>. A key step in Akt activation is the colocalization of PDK1 and Akt, and two mechanisms ensure that this happens efficiently. One is mediated by high-affinity PtdIns(3,4,5)P<sub>3</sub> binding to the PDK1 PH domain, which is not essential for PDK1 catalytic activity but promotes translocation of PDK1 to the plasma membrane where it can colocalize with Akt<sup>43</sup> (Fig. 3a). The second mechanism is controlled by Akt phosphorylation on its C-terminal Ser473 by the kinase complex mTORC2, which allows phosphorylated Akt to dock to a substrate-docking motif on PDK1 termed the PIF pocket<sup>44</sup> (Fig. 3a).

mTORC2 comprises the mTOR serine-threonine kinase in complex with RICTOR, MLST8 and mSIN1 (ref. 45). The signals that control the activity of mTORC2 in T cells are not understood, but the PI(3)K sensitivity of TCR-induced phosphorylation of Akt at Ser473, its mTORC2 site, suggests that mTORC2 function is regulated downstream of PI(3)K. Thus mTORC2 has a vital role in controlling the hydrophobic motif phosphorylation of other members of the AGC family of protein kinases (protein kinase A, protein kinase G and PKC), which need to be colocalized and phosphorylated by PDK1 (ref. 14). For example, mTORC2 can couple the TCR to serum- and glucocorticoid-induced protein kinase 1 (SGK1)<sup>46</sup>, and this is important for the differentiation of type 2 helper T (T<sub>H</sub>2)<sup>47</sup> and T<sub>H</sub>17 cells<sup>48,49</sup> (Fig. 3b).

How important is mTORC2 for Akt activation in T cells? In T cells lacking mTORC2 complexes, there is no TCR-induced phosphorylation of Ser473 in Akt; however,

phosphorylation of Thr308 in Akt is lower but not ablated, which suggests that phosphorylation of this residue is not obligatory for Akt activation<sup>50</sup>. Akt activity is also low but not ablated in T cells that express a PDK1 mutant that cannot bind PtdIns(3,4,5)P<sub>3</sub> (ref. 51). Collectively, these results support the argument that there are two mechanisms by which T cells can colocalize PDK1 and Akt: one is dependent on mTORC2 and the PDK1 PIF pocket, and the other is dependent on PtdIns(3,4,5)P<sub>3</sub>-mediated colocalization of PDK1 and Akt (Fig. 3a).

## The role of Akt in TCR signal transduction

The paradigmatic role of Akt in many cells is that it functions to control cell metabolism. Numerous experiments using PI(3)K inhibitors in different T cell populations support the idea that PI(3)K, and hence Akt, are important for glucose uptake, protein synthesis or both<sup>52–54</sup>. However, many of the PI(3)K inhibitors used in these studies, such as LY294002 and wortmanin, have off-target effects on other kinases, such as mTOR and PIMs<sup>55–57</sup>. Moreover, there is no direct evidence in mature T cells that Akt functions to couple the TCR to key metabolic programs. Indeed, PI(3)K and Akt direct the transcriptional program of T cells, but that they are not required for TCR-mediated initiation of glucose uptake or glycolysis in naive T cells<sup>58</sup>. There is also no evidence that Akt is essential for peripheral T cell survival or proliferation, which is inconsistent with the idea that this kinase links the TCR to metabolic programs<sup>58</sup>.

A major role for Akt in the context of TCR signaling is to control the phosphorylation and localization of the Foxo transcription factors, including Foxo1, Foxo3a and Foxo4. The Foxo transcription factors are nuclear and active in quiescent T cells, but when phosphorylated by Akt they exit the nucleus and form a complex with 14-3-3 proteins in the cytosol, thereby terminating their transcriptional activity<sup>59</sup> (Fig. 3b). Foxos target genes in naive T cells, including genes encoding cytokines, cytokine receptors and transcription factors<sup>60–62</sup>. For example, Foxos drive expression of the  $\alpha$ -chain of the receptor for IL-7, an essential homeostatic cytokine for T cells<sup>61,63</sup>. Foxo1 also induces expression of the transcription factor KLF2, which directly regulates the transcription of genes encoding chemokine receptors and adhesion molecules that control T cell trafficking<sup>51,61,64–66</sup>. TCR triggering causes T cells to activate Akt, which results in phosphorylation and inactivation of Foxos. The loss of Foxo transcriptional activity immediately causes loss of expression of KLF2 and of its target genes encoding L-selectin (CD62L), the chemokine receptor CCR7 and sphingosine 1-phosphate receptor S1P<sub>1</sub> (refs. 61,67). CD62L is an adhesion molecule that is crucial for the transmigration of T cells across high endothelial venules, CCR7 controls lymphocyte entry and retention into secondary lymphoid tissues, and S1P<sub>1</sub> controls T cell egress from lymph nodes<sup>68</sup>. Consequently, the loss of Foxos activity in TCR-triggered T cells changes the normal trafficking program of T cells<sup>61,69</sup>.

The role of Akt in T cells is not limited to controlling T cell trafficking. For example, a key role for Akt in CD8<sup>+</sup> T cells is to promote effector cell differentiation by driving expression of cytolytic effector molecules and controlling the cytokine receptor profile of effector T cells<sup>58</sup>. Akt-regulated genes in CD8<sup>+</sup> T cells include those encoding multiple granzymes, Fas ligand, the cytolytic effector perforin and IFN- $\gamma$  (*Ifn $\gamma$* ). It is also notable that Akt

positively regulates expression of genes encoding the IL-12 receptor  $\beta$ -chain while negatively regulating expression of genes encoding IL-6 receptors and the CD70 coreceptor CD27 (refs. 58,70). Akt can thus act to couple the TCR to the transcriptional programs that control effector T cell differentiation and, indeed, is necessary for the TCR to initiate expression of key effector molecules in CTLs. For example, inhibition of Akt blocks TCR-mediated induction of IFN- $\gamma$  expression in both naive CD8<sup>+</sup> T cells and effector CTLs<sup>58</sup>. The molecular mechanism for the diverse effects of Akt on T cell transcriptional programs resides in the ability of nonphosphorylated Foxos to both transactivate and repress gene expression. For example, Foxos act as positive regulators in the context of *Klf2* (encoding KLF2) and *Ii7r* (encoding IL-7R $\alpha$ ), whereas in the context of *Irfg*, Foxos are repressive. Accordingly, in TCR-activated T cells, Akt-regulated nuclear export of Foxo transcription factors is required for IFN- $\gamma$  production. Thus, in the context of TCR signaling, Akt regulates expression of cytolytic effector molecules and determines the repertoire of cytokine receptors, adhesion molecules and chemokine receptors expressed by T cell populations<sup>58,61,69</sup>.

### Serine-threonine kinases control T cell metabolism

T cells use serine-threonine kinases to modulate the key metabolic programs that meet the demands of participating in an immune response. The metabolic reprogramming of immune-activated T cells is triggered by antigen receptors but sustained by costimulatory signals and inflammatory cytokines. TCR engagement initiates increases in glucose uptake and a glycolytic switch whereby activated T cells swap from metabolizing glucose primarily through oxidative phosphorylation to using the glycolytic pathway<sup>71</sup>. The activation of the Erk1 and Erk2 initiates this switch in glucose metabolism by regulating the expression of c-Myc, a key transcription factor for this process<sup>72–74</sup> (Fig. 2). T cell activation also increases cellular amino acid uptake to support high levels of protein synthesis<sup>75</sup>. These TCR-regulated increases in amino acid uptake are not only required for protein synthesis; glutamine can be diverted into glutaminolysis. It is also important that T cells control the intracellular supply of leucine, because this controls the activity of the serine-threonine kinase complex mTORC1 (ref. 76). In this context, glutamine metabolism in T cells is mediated through Erk1 and Erk2, Akt and mTORC1 control of c-Myc expression<sup>72–74</sup>, whereas the uptake of leucine is mediated through system L amino acid transporters whose expression is controlled by calcium- and calcineurin-mediated signals<sup>75</sup> (Figs. 2 and 4).

One kinase that is crucial for T cell metabolism is mTORC1. This protein complex is composed of mTOR and RAPTOR, MLST8, PRA40 and DEPTOR<sup>45,77</sup>. The mTORC1 complex is activated in response to TCR engagement and has two known direct substrates: p70 S6-kinase 1 and eIF4E-binding protein 1, which have selective roles in regulating mRNA translation, protein synthesis and ribosome biogenesis<sup>78,79</sup> (Fig. 4). mTORC1 signaling also activates the transcription factors SREBP-1 and SREBP-2, which induce the expression of enzymes involved in *de novo* fatty acid and sterol biosynthesis<sup>79,80</sup>. Moreover, mTORC1 has a key role in regulating the translation of the hypoxia-inducible factor 1 (HIF-1) transcription factor complex. The HIF-1 pathway functions to couple mTORC1 to a diverse transcriptional program that sustains expression of glucose transporters and multiple rate-limiting glycolytic enzymes<sup>81</sup>. The mTORC1–HIF-1 pathway

is thus not required to initiate glucose metabolism in TCR-activated cells, but it is required to sustain glucose metabolism and glycolysis in activated T cells<sup>74,81</sup>. HIF-1 also controls effector T cell differentiation by coupling mTORC1 to multiple transcriptional programs that control expression of inflammatory cytokines, cytolytic effector molecules and essential chemokine and adhesion receptors that regulate T cell trafficking<sup>81–83</sup>.

## Nutrient-sensing serine-threonine kinases in T cells

The discussion above focuses on how pathways of serine-threonine phosphorylation control T cell metabolism. However, there are key serine-threonine kinases that respond to cellular energy demands to modulate T cell responses. In simple terms, these kinases sense rates of nutrient transport into T cells, and the activity of these nutrient sensors affects T cell activation and function. For example, evolutionarily conserved pathways whereby mTORC1 activity is regulated by glucose and amino acid availability<sup>45,84</sup> exist in T cells<sup>75,85,86</sup>. T cells also express the AMP-activated kinase AMPK- $\alpha$ 1, which stimulates the conservation and production of ATP in response to metabolic stress<sup>87,88</sup>. AMPK- $\alpha$ 1 is activated in response to TCR triggering via a pathway mediated by calcium and calcium-calmodulin-dependent protein kinase kinase 2 (CAMKK)<sup>87</sup>. Moreover, AMPK- $\alpha$ 1 can also be phosphorylated and activated in T cells by liver kinase B1 (LKB1) in response to changes in glucose metabolism that increase cellular AMP/ATP ratios<sup>89,90</sup>. The LKB1-AMPK- $\alpha$ 1 pathway thus functions as a sensitive sensor of glucose transport in T cells and one of its functions is to regulate mTORC1 activity<sup>90</sup>.

Triggering of the TCR activates mTORC1, and the molecular basis for this process is not fully understood but is known to be dependent on TCR regulation of the expression of system L amino acid transporters, which ensure the effective transport of leucine into activated T cells<sup>75</sup>. How does leucine control mTORC1 activity in mammalian cells? The current model holds that amino acid sensing regulates the recruitment of mTORC1 to lysosomal membranes<sup>76</sup>. A crucial step is amino acid-dependent activation of the guanine nucleotide exchange activity of the Ragulator complex. This process results in the accumulation of active GTP-bound Rag GTPases, which then recruit mTORC1 to the lysosomal surface, where the complex interacts with Rheb-GTP, a potent direct stimulator of mTORC1 kinase activity<sup>76</sup>. In T cells, the rate of intracellular leucine uptake in T cells is determined by TCR control of system L amino acid transporter expression; that is, the TCR has ‘assumed’ control of the evolutionarily conserved pathway whereby amino acids control mTORC1 activity by controlling the transport of leucine into the T cell<sup>75</sup>. In a similar context, the activity of mTORC1 is controlled by glutamine availability, and the TCR also controls mTORC1 activity by regulating the expression of the glutamine transporter ASCT2 and hence controlling the cellular uptake of glutamine<sup>86</sup>.

Most models of lymphocyte signal transduction invariably link mTORC1 activation to PI(3)K and Akt. The reason for this link is that Akt can phosphorylate tuberous sclerosis complex 2 (TSC2), a Rheb GTPase-activating protein (GAP) that negatively regulates the accumulation of the Rheb-GTP complexes required to activate mTORC1 (refs. 45,77). It was originally proposed that phosphorylation of TSC2 by Akt inhibited its GAP activity and allowed the accumulation of Rheb-GTP. It was also proposed that Akt phosphorylation

promoted TSC2 binding to 14-3-3 proteins to cause the sequestration of TSC2 in the cytosolic fraction away from lysosomes and, hence, away from Rheb<sup>91,92</sup>. It is clear that deletion of TSC complex in T lymphocytes makes these cells hyper-responsive for TCR-mediated activation of mTORC1 (ref. 93). However, although TSC2 phosphorylation frequently correlates with an increase in mTORC1 activation, it is not yet proven that Akt-mediated phosphorylation of TSC2 leads to its repression<sup>94</sup>. Hence there is no direct biochemical evidence that the regulation of phosphorylation of TSC2 by Akt suppresses its GAP activity toward Rheb. Similarly, the regulation of TSC2 localization to the lysosome by Akt has yet to be proven to be a key regulatory event in T cells. Indeed, selective inhibition of PtdIns(3,4,5)P<sub>3</sub> production and activation of Akt does not compromise mTORC1 activation in T cells<sup>81</sup>. The GTP and GDP loading of Rheb, which is controlled by the TSC complex, is thus essential for mTORC1 activity in T cells<sup>50</sup>, and the TSC complex acts as a negative regulator of mTORC1 activation<sup>93</sup>. However, TCR control of amino acid signaling to mTORC1 seems to dominate mTORC1 regulation rather than TCR control of TSC via Akt<sup>75,81</sup>.

Another key regulatory input that controls mTORC1 is mediated by AMPK- $\alpha$ <sup>145,77</sup>. AMPK $\alpha$ 1 acts as a crucial glucose sensor in activated effector T cells and monitors T cell energy status to restrict mTORC1 activity to metabolically sufficient T cells<sup>90</sup>. AMPK- $\alpha$ 1 can regulate mTORC1 activity because it can phosphorylate and activate TSC2, the Rheb GAP<sup>95</sup>. Accordingly, the TCR will be unable to trigger mTORC1 activity unless it can induce expression of glucose transporters and allow sufficient glucose supply for cells to maintain cellular AMP/ATP ratios to restrict AMPK $\alpha$ 1 activity and allow the accumulation of active Rheb-GTP. The restraint of mTORC1 activity by AMPK- $\alpha$ 1 seems to be important in the effector-to-memory transition of T cells. Effector T cells need to revert from a metabolically active state to a quiescent catabolic state to produce memory T cells. The regulation of mTORC1 activity by AMPK- $\alpha$ 1 seems to be a pivotal control switch for this transition<sup>90,96</sup>.

## Complexities and conclusions

Here we present a relatively linear, simplistic view of how a few conserved serine kinases and their substrates are activated by the TCR and regulate T cell function. However, high-resolution quantitative mass-spectrometry experiments are beginning to reveal the real scale and complexity of the TCR-regulated serine-threonine phosphoproteome. Phosphoproteomic experiments have been performed using clonal cytotoxic T cell populations triggered with cognate peptide-MHC complexes and primary and transformed human T cells polyclonally activated with antibodies to the TCR complex<sup>97–99</sup>. These experiments have identified thousands of serine-threonine-phosphorylated proteins in T cells and shown that triggering of TCR complexes induces increases or decreases in the phosphorylation of ~20% of the serine-threonine phosphoproteome of a T cell population<sup>97,99</sup>. One key insight from these studies is that there is a complicated web of basal protein phosphorylation in T cells before TCR engagement, including links between serine-threonine kinases and chromatin regulators, that maintains the transcriptional programs permissive for TCR function. These data sets also afford insights about the scope of TCR-regulated phosphorylation. The TCR controls phosphorylation of proteins that control all the significant events associated with T



cell activation, such as transcriptional regulation, cytoskeleton reorganization, the cell cycle, survival and cell growth<sup>97</sup>. It is noteworthy that transcription factors and chromatin regulators are overrepresented.

The power of unbiased global approaches to study protein phosphorylation is that they unequivocally identify which protein isoforms or splice variants are expressed in a T cell population and which isoforms are regulated by TCRs. Unbiased global phosphoproteomic approaches can yield new insights about known TCR signaling pathways, but the real power resides in their potential to identify TCR-regulated phosphorylations that can direct new targeted, hypothesis-driven experiments to dissect molecular mechanisms controlling T cell function. TCR-regulated phosphoproteomic data sets available in the literature<sup>97–99</sup> contain highly valuable information about previously unknown phosphorylation sites on key transcription factors for T cell function and links between TCR-regulated serine-threonine kinases and transcriptional regulators<sup>97–99</sup>. They show that current knowledge of how serine-threonine kinases mediate TCR signal transduction is at a nascent stage, but they highlight that understanding the function of TCR-regulated serine-threonine phosphorylations is essential for an understanding of how the TCR regulates the essential epigenetic, transcriptional and metabolic programs that control T cell participation in an immune response.

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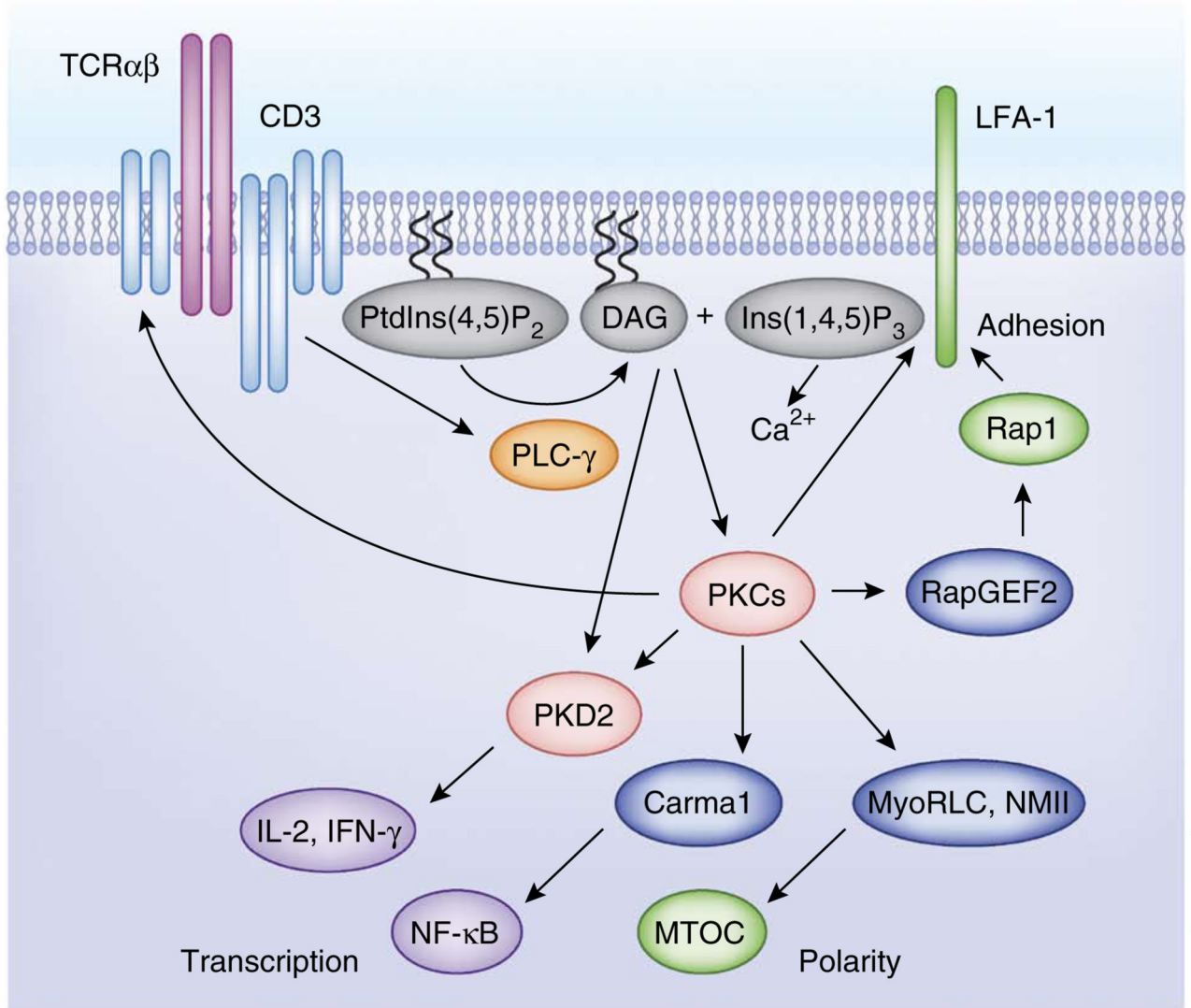
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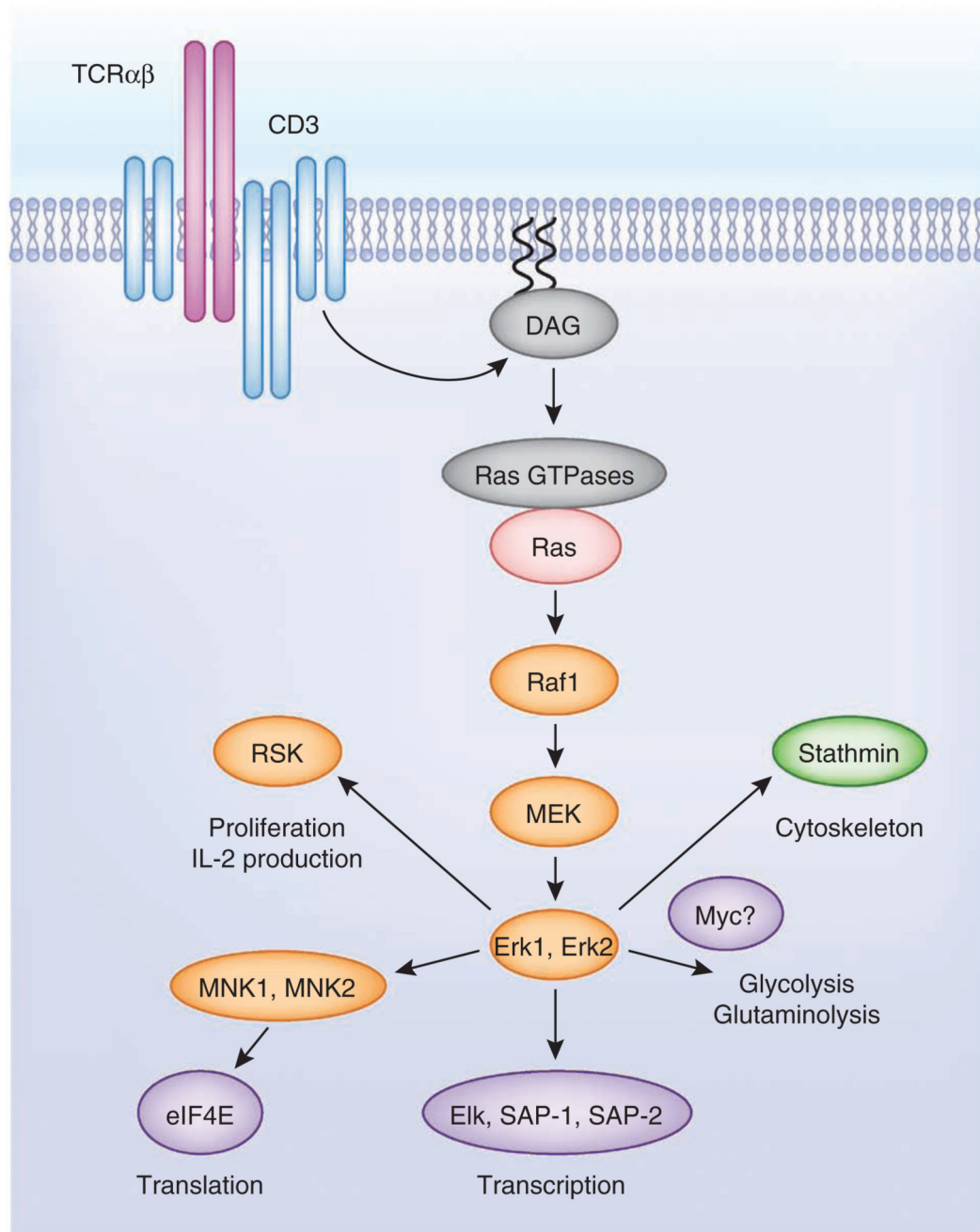
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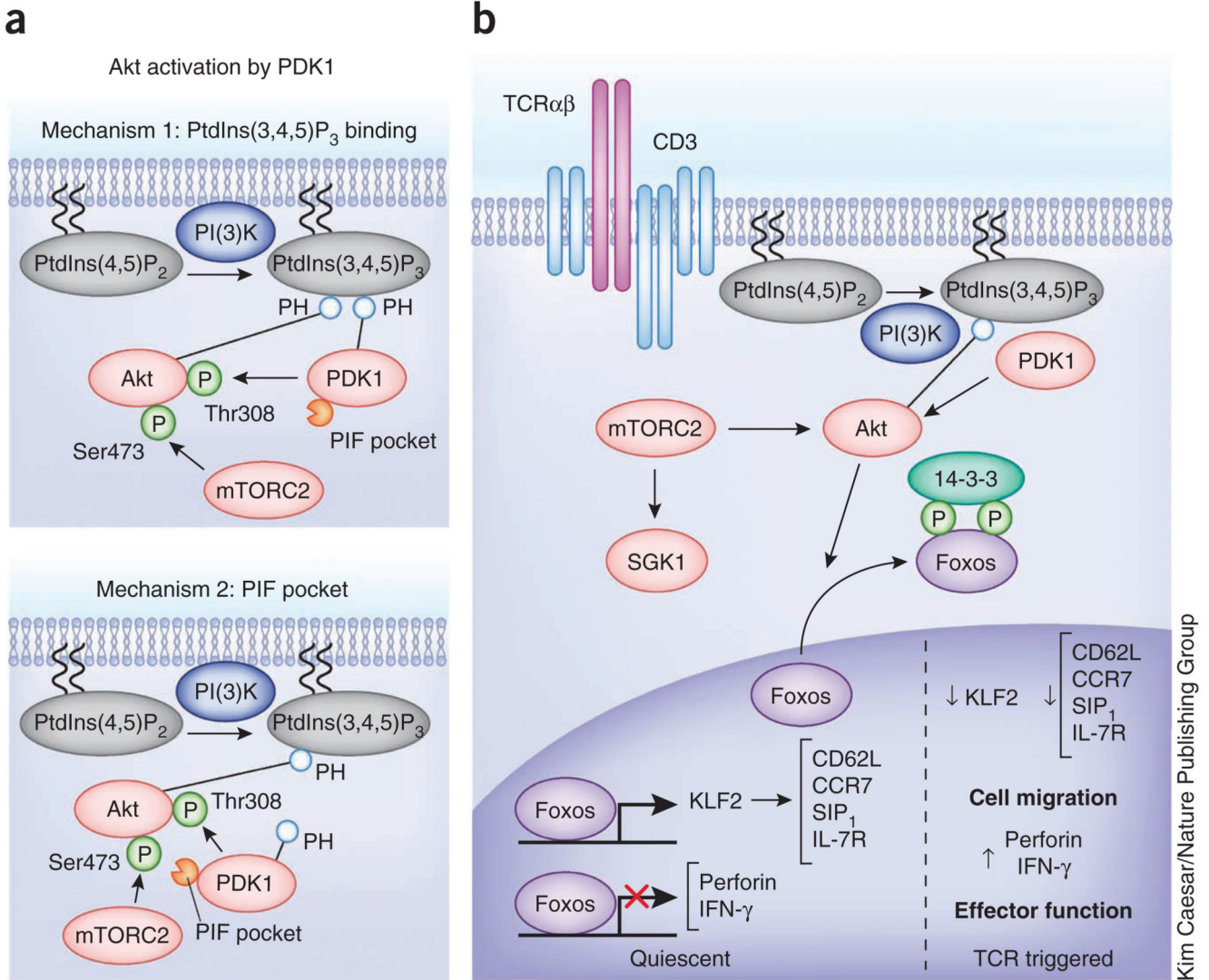
**Figure 1.** DAG-regulated serine-threonine kinases in T cells. TCR triggering activates PLC- $\gamma$ , which cleaves PtdIns(4,5)P<sub>2</sub> to generate two key second messenger molecules, DAG and Ins(1,4,5)P<sub>3</sub>. The accumulation of DAG in the plasma membrane mediates the activation kinases PKC and PKD. PKC substrates regulate different aspects of the T cell activation process, such as cell adhesion, establishment of polarity and transcription. MTOC, microtubule organizing center; MyoRLC, myosin regulatory light chain; NMII, nonmuscle myosin.



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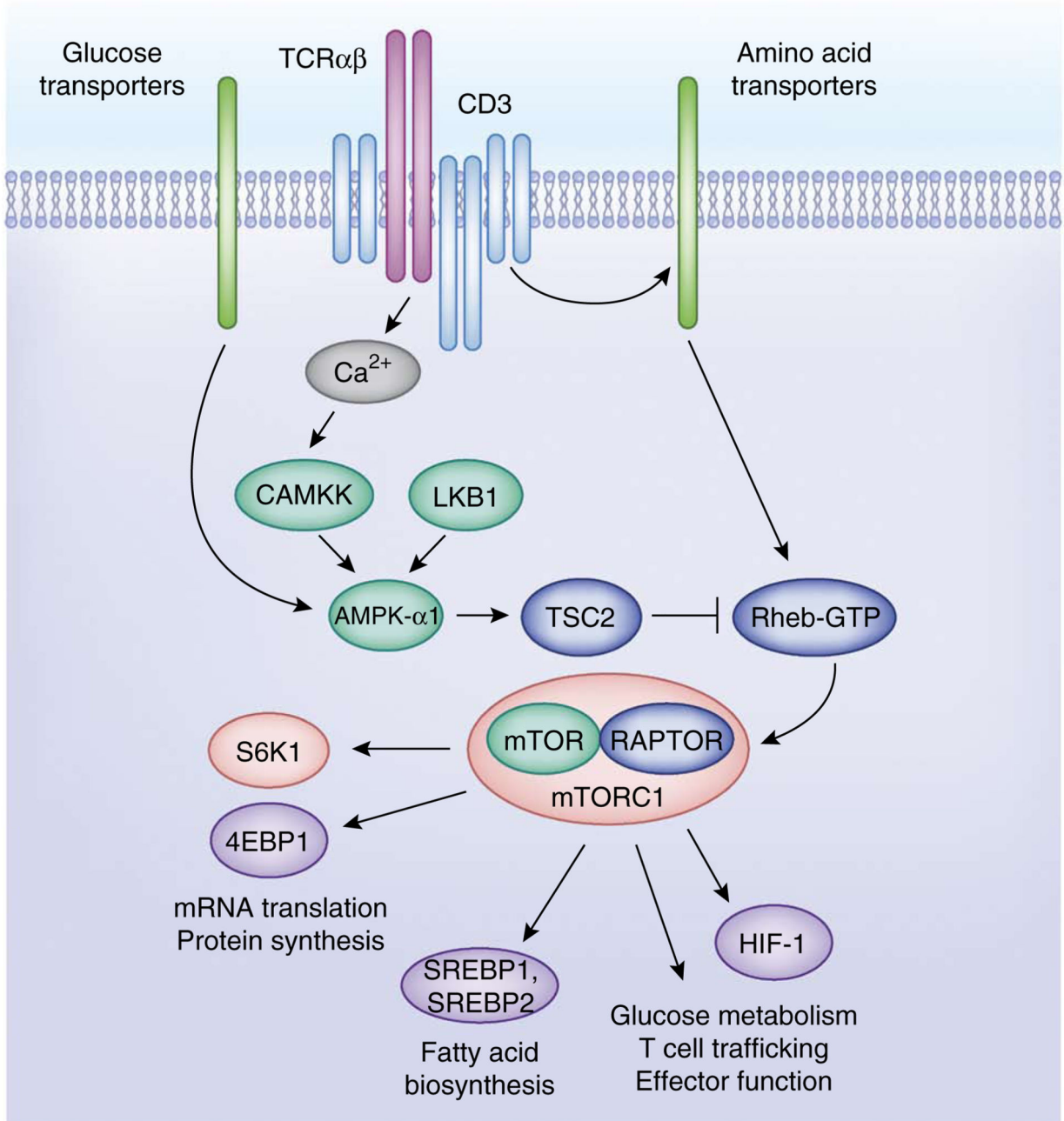
**Figure 2.** Ras-regulated serine-threonine kinase pathways. TCR-mediated DAG generation activates Ras GTPases, which promote Ras activation and start the MAPK signaling cascades required for Erk1 and Erk2 activation. Erk1 and Erk2 kinase activity controls T cell activation through different substrates that control cytoskeleton remodeling, cell metabolism, transcription, translation and proliferation.





**Figure 3.**

The PtdIns(3,4,5)P<sub>3</sub>-regulated serine-threonine kinase Akt. **(a)** Mechanisms of Akt activation by PDK1. Two described mechanisms for colocalization of Akt and PDK1; one is dependent on PDK1 binding to PtdIns(3,4,5)P<sub>3</sub> and the other is dependent on PDK1 recruitment to Akt via mTORC2-mediated Ser473 phosphorylation. **(b)** Role of Akt in TCR signal transduction. Akt activation by PDK1 has a key role in T cell activation, regulating the subcellular localization and function of the Foxo family of transcription factors. Foxos regulate the expression of key molecules that control T cell trafficking and effector functions.



**Figure 4.** Nutrient-sensing serine-threonine kinases in T cells. Cartoon depiction of the complex regulation of T cell metabolic pathways by different nutrient sensing serine-threonine kinases such as AMPK- $\alpha$ 1 and the mTORC1 complex. Through the regulation of the expression or function of different transcription factors, mTORC1 controls key aspects of T cell function such as protein and fatty acid biosynthesis, glucose metabolism, T cell trafficking and effector function.