Arrestin makes T cells stop and become active

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T-cell activation requires signaling by T-cell receptors (TCRs) that bind antigen on the antigen-presenting cells (APCs) at the immunological synapse (IS). Sustained signaling requires continuous supply of new TCRs to the IS. In this issue of *The EMBO Journal*, Fernández-Arenas *et al* (2014) describe a novel role of β -arrestin-1 at the IS periphery: endocytosis of TCRs and chemokine CXCR4 receptors. Internalized TCRs are then delivered to the IS, where they engage antigen and support prolonged signaling, whereas CXCR4 internalization stops T-cell migration.

See also: E Fernández-Arenas *et al* (March 2014)

Development or prevention of an immune response involves an elaborate system of communications among different cell types involving long-range “messaging” via secreted cytokines and chemokines and direct cell-cell interactions (Xie *et al*, 2013). One of the cornerstones of a robust immune response is the activation of T cells specifically recognizing particular antigens. T-cell activation requires a tight contact with antigen-presenting cells (APCs), termed immunological synapse (IS) (Monks *et al*, 1998). T-cell receptors (TCRs) within the IS become activated by antigen presented on the surface of APCs. For full activation, T cells must remain in contact with APCs for a relatively long time, hence their motility needs to be suppressed. Furthermore, TCR signaling must continue for extended periods, which means that engaged TCRs in the IS, which become

desensitized and then internalized via a phagocytosis-like mechanism (Martínez-Martín *et al*, 2011), must be replaced by fresh unengaged TCRs transported from the cell surface outside of the IS (Huppa *et al*, 2003). The activity of TCR-stimulated PKC is necessary for T-cell activation (Monjas *et al*, 2004).

The work by Fernández-Arenas *et al* (Fernández-Arenas *et al*, 2014) revealed an elegant mechanism that connects PKC activation, inhibition of cell movement, and sustained delivery of fresh TCRs to the IS (Fig 1A). They showed that PKC, which is activated by “primed” TCRs that first bind the antigen, phosphorylates β -arrestin-1 at Ser163. This promotes β -arrestin-1 binding to CXCR4 and unengaged TCRs on the cell surface outside of the IS, whereupon both receptors are internalized, likely via clathrin-coated pits (Monjas *et al*, 2004). The removal of CXCR4 from the surface prevents stimulation of T-cell motility by CXCL12 acting via this receptor. Internalized unengaged TCRs are then delivered to the IS, thus renewing the supply of TCRs that ensures prolonged signaling. The authors show that both functions of β -arrestin-1 are critical for the activation of T cells. Although arrestins are known to be multi-functional signaling regulators (Gurevich & Gurevich, 2006), the central role of β -arrestin-1 in immune response convincingly established by Fernández-Arenas *et al* (Fernández-Arenas *et al*, 2014) was not expected.

Another surprising aspect of this study is an unconventional mechanism of β -arrestin-1 activation. In case of G-protein coupled

receptors (GPCRs), which are known to be internalized upon arrestin binding, arrestin activation occurs through global conformational change allowing for high-affinity receptor interaction. Receptor-attached phosphates bind key phosphate-binding residues (Fig 1B) and disrupt critical intramolecular interactions holding arrestin in its basal conformation (Gurevich & Gurevich, 2013). Arrestin transition into the high-affinity binding state requires the “melting” of the polar core, a group of interacting charged residues (Fig 1B), and detachment of the C-tail from the body of the molecule. Arrestin with a released C-tail promotes receptor endocytosis, since clathrin- and AP-2-binding sites, which play key roles in arrestin-dependent recruitment of bound receptors to clathrin-coated pits (Goodman *et al*, 1996), are localized to the C-tail and become accessible only when it is detached.

Fernández-Arenas *et al* (Fernández-Arenas *et al*, 2014) showed that β -arrestin-1 is activated by PKC phosphorylation of Ser163. Apparently, phosphorylated β -arrestin-1 binds GPCRs, such as CXCR4. Furthermore, β -arrestin-1 phosphorylated at Ser163 mediates internalization of both CXCR4 and TCR. This suggests that phosphorylation of β -arrestin-1 at Ser163 releases the C-tail. This serine resides next to the critical positive charges that bind receptor-attached phosphates (Fig 1B). From a structural perspective, one can envision a phosphate at Ser163 in β -arrestin-1 intruding upon phosphate-binding residues (Fig 1B) and disrupting ionic interactions, similar to the receptor-attached phosphates. Alternatively, the

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Different systems of arrestin names are used: arrestin-1 (a.k.a. S-antigen, 48 kDa protein, visual or rod arrestin), arrestin-2 (β -arrestin or β -arrestin1), arrestin-3 (β -arrestin2 or hTHY-ARRX), and arrestin-4 (cone or X-arrestin). Here arrestin-2 is called β -arrestin-1, as in the paper by Fernández-Arenas *et al* (Fernández-Arenas *et al*, 2014).

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Figure 1. PKC-phosphorylated β-arrestin-1 plays a central role in the function of the immunological synapse (IS) between T cell and antigen-presenting cell (APC) (Fernández-Arenas *et al*, 2014). (A) T cells are activated due to the binding of T-cell receptors (TCRs) to the cognate antigen (pink) associated with major histocompatibility complex (MHC). Arrestin binds “bystander” unengaged TCRs outside of the IS and promotes their internalization via clathrin-coated pits and subsequent delivery to the IS, which is critical for maintaining prolonged signaling. It also facilitates the internalization of chemokine CXCL12 receptors CXCR1, thereby suppressing T-cell motility. (B) Unconventional mechanism of arrestin activation by direct phosphorylation. The structure of β-arrestin-1 (PDB ID: 1G4R) showing as CPK model Ser163 phosphorylated by PKC (Fernández-Arenas *et al*, 2014), adjacent phosphate-binding residues Lys10, Lys11, Arg165, and the five residues of the polar core, which serves as the key sensor of receptor-attached phosphates: Arg169, Arg393, Asp26, Asp290, and Asp296 (Gurevich & Gurevich, 2013).

negative charge of the phosphate at Ser163 can repulse the negatively charged C-tail that resides at the same concave side of the arrestin N-domain, thus allowing receptor

binding and making clathrin and AP2 sites accessible.

The mechanism of arrestin activation by direct phosphorylation was never described

or even suspected before. Interestingly, serine in the same position is conserved in most vertebrate and invertebrate arrestins, and even in *C. elegans* this position is occupied by potentially phosphorylatable threonine (Gurevich & Gurevich, 2006). Thus, the finding of Fernández-Arenas *et al* (Fernández-Arenas *et al*, 2014) suggests the possibility that phosphorylation on this residue might be an earlier and unappreciated activation mechanism shared by multiple arrestin subtypes.

The work of Fernández-Arenas *et al* (Fernández-Arenas *et al*, 2014) not only establishes a critical role of β-arrestin-1 in the immune system, but also suggests an alternative mechanism of arrestin activation that might have general applicability.

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Author contributions

VVG and EVG wrote the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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