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Cytoskeleton rotation relocates mitochondria to the immunological synapse and increases calcium signals

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ABSTRACT

Ca^{2+} microdomains and spatially resolved Ca^{2+} signals are highly relevant for cell function. In T cells, local Ca^{2+} signaling at the immunological synapse (IS) is required for downstream effector functions. We present experimental evidence that the relocation of the MTOC towards the IS during polarization drags mitochondria along with the microtubule network. From time-lapse fluorescence microscopy we conclude that mitochondria rotate together with the cytoskeleton towards the IS. We hypothesize that this movement of mitochondria towards the IS together with their functionality of absorption and spatial redistribution of Ca^{2+} is sufficient to significantly increase the cytosolic Ca^{2+} concentration. To test this hypothesis we developed a whole cell model for Ca^{2+} homoeostasis involving specific geometries for mitochondria and use the model to calculate the spatial distribution of Ca^{2+} concentrations within the cell body as a function of the rotation angle and the distance from the IS. We find that an inhomogeneous distribution of PMCA pumps on the cell membrane, in particular an accumulation of PMCA at the IS, increases the global Ca^{2+} concentration and decreases the local Ca^{2+} concentration at the IS with decreasing distance of the MTOC from the IS. Unexpectedly, a change of CRAC/Orai activity is not required to explain the observed Ca^{2+} changes. We conclude that rotation-driven relocation of the MTOC towards the IS together with an accumulation of PMCA pumps at the IS are sufficient to control the observed Ca^{2+} dynamics in T-cells during polarization.

1. Introduction

Organelle polarization is a fundamental biological process for many cellular functions [1–5]. Cytotoxic T lymphocytes (CTL) and natural killer (NK) cells are highly polarized during cell migration and immunological synapse (IS) formation with their cognate target cells like tumor cells or virus infected cells [6–8]. Polarity is often controlled by actin polymerization induced by Cdc42 [9] or Arp2/3-dependent actin nucleation [10]. The specificity of target cell killing is, among other mechanisms, guaranteed through polarized secretion of lytic granules (LG) containing perforin and granzymes at the IS [11–13]. The microtubule organizing center (MTOC, centrosome) is a key organelle involved in repositioning of LG towards the IS following actin depletion [14], and secretion of LG at the IS is one of the central polarization steps in CTL and NK cells [4,15]. The MTOC moves to the IS within several minutes of IS formation [13,16]. This directed movement requires the motor protein dynein [17] which according to the favored mechanism, mediates MTOC repositioning to the IS by generating force through microtubule binding at the outer ring of actin at the IS. This process is also referred to as cortical sliding mechanism. This view has recently been challenged by Yi et al. [18] who present a series of experiments favoring a mechanism that dynein mediates MTOC repositioning through a "microtubule end-on capture-shrinkage mechanism", by which dyneins act on microtubules docked at the center of the IS and not at the outer actin ring. Regardless of the model, the MTOC is considered a master regulator of T cell polarization [17] and it is most

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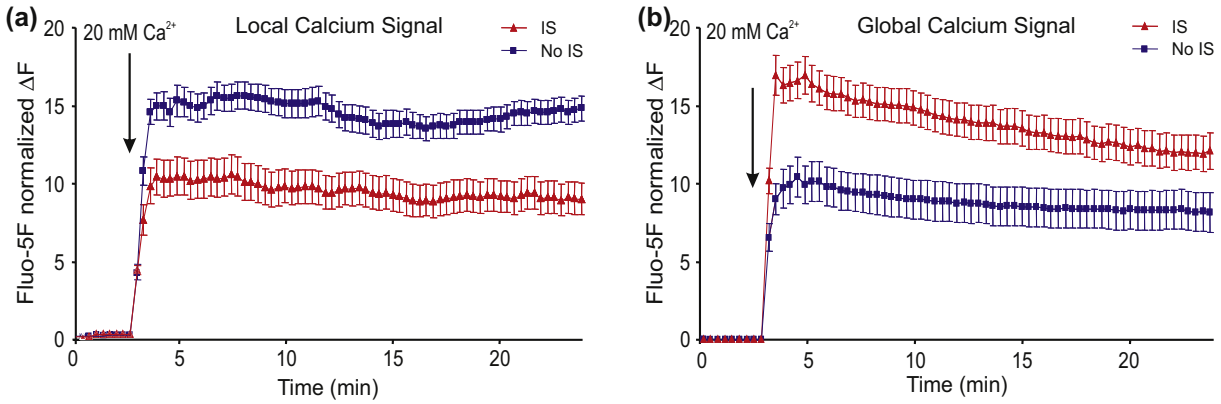

Fig. 1. Mitochondrial localization at the IS modulates local and global Ca^{2+} levels. Fluo-5F/AM loaded Jurkat E6.1 T-cells were settled either on anti-CD3 antibody-coated coverslips (inducing IS formation) or on poly-L-ornithine-coated coverslips (no IS formation). Ca^{2+} stores were depleted by 5–7 min thapsigarin (1 μM) pre-treatment in Ca^{2+} free solution and subsequently, cells were exposed to 20 mM Ca^{2+}. **(a)** Mean normalized fluorescence of Fluo-5F by TIRFM of 33 (with IS formation, red trace) and 37 (no IS formation, blue trace) cells over time (at 20 min, $p < 0.0001$) representing local Ca^{2+} signals at the plasma membrane. **(b)** Mean normalized fluorescence of Fluo-5F by epifluorescence microscopy of 17 (with IS formation, red trace) and 18 (no IS formation, blue trace) cells over time (at 20 min, $p = 0.036$) representing global Ca^{2+} signals. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

likely guiding other organelles like the Golgi apparatus [19,20], mitochondria [21–24] and LG [13] to the IS.

Several of the signaling steps governing CTL and/or NK cell dependent target cell death are Ca^{2+} dependent: (1) MTOC relocalization to the IS [18]; (2) mitochondria relocalization to the IS [24,25]; (3) secretion of LG at the IS [26–28]; (4) perforin-dependent lysis of target cells [29]. While the exact molecular mechanisms of how Ca^{2+} is involved in regulating these processes are not resolved yet, it is clear that Ca^{2+} influx through Orai (mostly Orai1) channels is the main Ca^{2+} source [28,30,31]. Ca^{2+} influx at the IS through Orai1 channels is among other factors controlled by mitochondrial positioning at the IS. There, mitochondria act as Ca^{2+} sinks, whereby they control Ca^{2+} dependent activity of Orai channels and local Ca^{2+} concentrations at the IS as well as global cytosol Ca^{2+} concentrations ($[Ca^{2+}]_{cyt}$) [23,32,33].

In this paper we show that cytoskeleton rotation relocates mitochondria to the immunological synapse. Unexpectedly, repositioning of mitochondria alone can modulate the global cytosolic Ca^{2+} concentration, independent of any influence of mitochondrial position on CRAC/Orai channel activity. We determine the geometric path that mitochondria take during relocation towards the IS and show that it is correlated with a rotation of the MTOC and microtubular network. We implement this rotation into a model for spatiotemporal Ca^{2+} dynamics in T-cells that we proposed recently [33] and present the predictions of this model about the dependence of the global and local Ca^{2+} concentration on the rotation angle of the cytoskeleton/mitochondria system.

2. Results

2.1. Mitochondria relocation correlates with Ca^{2+} increase

In 2011, we have shown that mitochondrial relocation to the IS decreases local cytosolic Ca^{2+} levels but increases global ones [23]. We confirm these findings by an independent set of experiments in human Jurkat T-cells (Fig. 1) similar to the experiments shown in Fig. 4C and D of our previous publication [23]. In case an IS is induced by anti-CD3 antibodies on the coverslip, mitochondria localize to the IS as shown by Quintana et al. [23] and the local Ca^{2+} signals at the plasma membrane are lower compared to conditions in which no IS was formed (Fig. 1a). In contrast the global Ca^{2+} signals are higher in case of IS formation (Fig. 1b). Localization of mitochondria at the IS thus decreases the local Ca^{2+} levels compared to experiments where no IS is formed but increases the global ones. In conclusion mitochondrial localization relative to CRAC channels at the IS determines local and global Ca^{2+} concentrations.

2.2. Mitochondria relocation correlates with cytoskeleton movement

Whereas it is undisputed that mitochondria relocalize to the IS, the exact mechanisms of the relocalization process have not been resolved. Mitochondrial fusion/fission [21] and cytoskeletal reorganization are very likely involved [3,5] but the exact mechanisms are not understood. Considering the MTOC relocalization to the IS [13,16,19,20] and its potential to guide other organelles there, it is reasonable to assume that the MTOC and mitochondrial movement could be correlated. We thus tested the hypothesis that mitochondria translocation and microtubule network reorientation towards the IS are correlated. We fluorescently labeled microtubules (with EMTB-3 × GFP) and mitochondria (with MitoTracker or DsRed2-Mito-7) in CTL. CTL were conjugated with target cells at 37 °C and translocation of microtubule network and mitochondria was visualized by time lapse microscopy. We found that mitochondrial localization was closely associated with microtubules (Fig. 2a). Following contact between CTL and a target cell, mitochondria were passively translocated to the IS along with microtubule network reorientation (Fig. 2a and Supplementary Movie 1). Moreover, we observed that occasionally mitochondria could actively move along microtubule tracks (Fig. 2b and Supplementary Movie 2). These results show that mitochondria relocation correlates with cytoskeleton rearrangement, especially microtubule network reorientation towards the IS.

2.3. Quantitative analysis of cytoskeleton and mitochondria rotation

For each time frame we extracted the three-dimensional positions of the MTOC, the IS, the cell center, and individual mitochondria using a higher time resolution for 3D pictures as described in the Materials and Methods section. The position data for all objects in a single frame were brought into a standard coordinate system by two rotations and one translation, such that MTOC, IS, and cell center lie in one plane, the x-z plane, and the rotation axis is parallel along the y-axis. We assume that the MTOC (in this case located at 90°) moves towards the IS. In Fig. 3a we sketch the movement of mitochondria that one expects in case mitochondria are attached to the microtubules: in the right half space they are rotated