A rise of the intracellular Ca\(^{2+}\) concentration has multiple signaling functions. Sustained Ca\(^{2+}\) influx across plasma membrane through calcium release-activated calcium (CRAC) channels is required for T-cell development in the thymus, gene transcription, and proliferation and differentiation of naïve T-cells into armed effector cells. Intracellular Ca\(^{2+}\) signals are shaped by mitochondria, which function as a highly dynamic Ca\(^{2+}\) buffer. However, the precise role of mitochondria for Ca\(^{2+}\)-dependent T-cell activation is unknown. Here we have shown that mitochondria are translocated to the plasma membrane as a consequence of Ca\(^{2+}\) influx and that this directed movement is essential to sustain Ca\(^{2+}\) influx through CRAC channels. The decreased distance between mitochondria and the plasma membrane enabled mitochondria to take up large amounts of inflowing Ca\(^{2+}\) at the plasma membrane, thereby preventing Ca\(^{2+}\)-dependent inactivation of CRAC channels and sustaining Ca\(^{2+}\) signals. Inhibition of kinesin-dependent mitochondrial movement along microtubules abolished mitochondrial translocation and reduced sustained Ca\(^{2+}\) signals. Our results show how a directed movement of mitochondria is used to control important cellular functions such as Ca\(^{2+}\)-dependent T-cell activation.

Ca\(^{2+}\) entry through calcium release-activated calcium (CRAC) channels, which are opened following depletion of Ca\(^{2+}\) stores, is necessary for T-cell activation (1–4). The sustained activity of CRAC channels is needed for transcription of early genes (5), for T-cell development in the thymus (6), and for the control of antigenic responsiveness and tolerance (7). Very recently, several molecules in this signal transduction pathway were identified. These include ORAI1 (also called CRACM1), which is a necessary component for CRAC channel activity in T-cells (8, 9), STIM1, which is probably the long-sought Ca\(^{2+}\) sensor that links store depletion to CRAC opening (10–12), and WAVE2, which is also involved in CRAC channel activation, probably through an interaction with the cytoskeleton (13).

Apart from the major role of mitochondria as cellular energy sources and their role in cell death (14, 15), mitochondria have been identified as regulatory elements for CRAC channels (2, 16, 17). Mitochondria can reduce Ca\(^{2+}\)-dependent inactivation of CRAC channels in submembranous Ca\(^{2+}\) microdomains by quickly removing the inflowing Ca\(^{2+}\), highlighting the importance of organelle localization within a cell. Mitochondrial localization and movements are determined by microtubules (18–21) and microfilaments (20, 22). Recently, Ca\(^{2+}\) signaling and mitochondrial movements have been linked through an unknown Ca\(^{2+}\) sensor molecule that translates Ca\(^{2+}\) signals into the microtubular motor protein-based mitochondrial movements (20, 23). These data suggest that mitochondrial movements may not always be random but could be directed to certain cellular domains and have specific localization-dependent functions.

### EXPERIMENTAL PROCEDURES

**Cells**—Human Jurkat T-cell lines were isolated and grown as described previously (24, 25).

**Reagents**—All chemicals and antibodies not specifically mentioned were from Sigma (highest grade). Other reagents used in our experiments included Fura-2/AM, thapsigargin (TG), MitoTracker® Green FM, BAPTA (all from Molecular Probes), mouse anti-human CD3-RPE-conjugated mAb (DakoCytomation), mouse anti-human CD45-Alexa Fluor®488 mAb (Sorex), di-8-aminophenylhexylpyridinium (Invitrogen), and BTP2 (Altana Pharma).

**Fluorescence Microscopy and Ca\(^{2+}\) Imaging**—Imaging experiments were done as previously described (26). A 40× (Uplan/Apo, numerical aperture 1.0) or 100× (Uplan/Apo numerical aperture 1.35, oil immersion) objective were used. Ca\(^{2+}\) Ringer’s solution contained (in mM): 155 NaCl, 4.5 KCl, 1 CaCl\(_2\), 2 MgCl\(_2\), 10 d-glucose, and 5 Hapes (pH 7.4 with NaOH). CaCl\(_2\) was substituted by 1 mM EGTA to prepare Ca\(^{2+}\)-free Ringer’s solution.

To visualize mitochondria, cells were incubated with 100–200 nM MitoTracker® for 30 min at 22–23°C. Excess dye was removed by washing twice. Cells were illuminated at 490 nm. DCLP 500 (UV) was used as a dichroic mirror and LP 515 as an emission filter.

**Bead Stimulation**—As described previously (27), we followed the standard procedure for absorbing proteins on polystyrene microparticles (size >0.5 µm) established by Polysciences Europe.
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GmbH Company (www.polysciences.com). Two alterations in the procedure were required to optimize our results. In step 1, we employed 100 μl of a 2.5% suspension of beads (∼2.1 × 10⁷ beads), and in step 7, we added 50 μg of the protein to be absorbed. Chemical composition and pH of buffers were the same as recommended in the protocol. Azid-free anti-human CD3 mAbs (Euroclone) were passively coupled to microparticles (diameter = 5.83 μm). They were stored at 4 °C in the specified storage buffer until use. Beads were washed twice with phosphate-buffered saline before resuspending them in the Ringer’s solution used for the experiments.

Confocal Microscopy—For confocal imaging, a Nipkow disc-based scanning head (QLC-100, VisiTech International) was attached to an upright microscope (Eclipse 600, Nikon) equipped with a 100× water lens (numerical aperture 1.1, Nikon). The light source was a 488-nm solid-state laser (Sapphire 488–30, Coherent). A dichroic mirror between the microscope and pinhole disc reflects the emission light that passes a 500-nm long-pass barrier filter. For detection, a charge-coupled device camera (OrcaER, Hamamatsu Photonics) in the 2 × 2 binning mode, resulting in a 672 × 512 pixel resolution, was used. Acquisition was controlled by the VoxCellScan Software (VisiTech International).

Two-dimensional time lapse data at a maximum rate of 20 images/s were analyzed using ImageJ (Wayne Rasband, National Institute of Mental Health). Stacks were deconvolved using AutoDeblur X (AutoQuant).

2-Photon Microscopy—The same experimental setup as previously described was used (28). The emission beam splitter contained a dichroic mirror at 565 nm followed by a channel 1 band pass at 610/75 nm and a channel 2 long pass at 500 nm. The 2-photon excitation setting was 800 nm for dyes with 1-photon excitation near 488 nm. Complete pictures of the sample were taken every 5 min with a resolution of 512 × 512 or 2048 × 2048 pixels.

To stain mitochondria, cells were loaded with 200 nM MitoTracker® for 1 h. The plasma membrane was stained with a mouse anti-human CD45-Alexa Fluor®488 mAb. Cells were incubated for 30 min with antibodies.

Electrophysiology—Patch clamp experiments were done exactly as described previously (26). The standard pipette solution for whole-cell patch clamp recordings contained (in mM): 0.05 InsP₃, 5 × 10⁻⁸ TG, 140 Cs-aspartate, 2 MgCl₂, 5 Mg-ATP, 0.5 Tris-GTP, 2.5 malic acid, 2.5 Na-pyruvate, 1 NaH₂PO₄, and 10 Hapes (pH 7.2 with CsOH). This solution was either supplemented with 0.66 CaCl₂ and 1.2 EGTA ([Ca²⁺]ᵣ, approximately 5 mM) to weakly buffer [Ca²⁺], or with 10 mM EGTA to strongly buffer [Ca²⁺]. The extracellular solution contained (in mM) 101.66 NaCl, 3 KCl, 20.66 CaCl₂, 1.22 MgCl₂, 6.66 d-glucose, and 3.33 Hapes (pH 7.4 with NaOH).

Electroporation of Antibodies—Electroporation was carried out as described previously (27) with RPMI 1640 medium containing 0.1 mg/ml anti-kinesin 5B mAb or 0.1 mg/ml anti-hu-
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Results

Ca²⁺ influx through CRAC channels controls the translocation of mitochondria to the plasma membrane. Data were analyzed using TILL Vision (TILL Photonics), Igor Pro (Wavemetrics), and Microsoft Excel. All values are given as mean ± S.E.; n = number of cells. In case data points were normally distributed, a paired or unpaired two-sided Student’s t test was used. If normal distribution could not be confirmed, a non-parameterized test (Mann-Whitney) was carried out. Levels of significance are indicated in Figs. 1–5 (* refers to p < 0.05, ** refers to p < 0.01, and *** refers to p < 0.001).

**FIGURE 2.** Ca²⁺ influx through CRAC channels controls the translocation of mitochondria to the plasma membrane. A, statistical analysis of the mitochondrial localization close to the plasma membrane between the two different concentric circles (as in Fig. 1B) at different times after initiation of Ca²⁺ influx by 1 μM TG as shown in the inset. B, statistical analysis of the mitochondrial localization close to the plasma membrane under resting conditions (open bars) and at the end of the Ca²⁺ plateau (shaded bars) after stimulation with (in μM) 1 TG (see inset in A) in 0.1 (9 cells), 0.25 (9 cells), 0.5 (9 cells), 1 (13 cells), and 20 (10 cells) mM Ca²⁺ Ringer’s solution. C, statistical analysis of the mitochondrial localization close to the plasma membrane under resting conditions, at the end of the Ca²⁺ plateau, and 15 min after removing extracellular Ca²⁺ solution in 12 cells. D, statistical analysis of the mitochondrial localization close to the plasma membrane after loading the cells for 30 min with (in μM) 20 (26 cells), 50 (24 cells), and 100 (22 cells) BAPTA/AM. E, statistical analysis of the mitochondrial localization close to the plasma membrane in the presence of 100 nM BAPTA under resting conditions, after TG-induced store depletion, and at the end of the Ca²⁺ plateau in 17 cells. F, confocal and 2-photon analysis of mitochondrial movement. Confocal fluorescence images from single MitoTracker®/AM/di-8-aminonaphthylethylpyridinium co-labeled Jurkat T-cells before and after inducing Ca²⁺ influx by the re-addition of 1 mM Ca²⁺ Ringer’s solution in TG pretreated or nonpretreated cells. The statistical analysis of the fraction of mitochondria located in a ring <1 μm away from the plasma membrane is depicted at the bottom. Averaged were 23 (resting) and 12 (for the other conditions) cells. For 2-photon measurements, cells were labeled with MitoTracker®/AM and mouse anti-human CD45-Alexa Fluor®488 MAb (to stain the plasma membrane). The statistical analysis of the fraction of mitochondria located in a ring < 300 nm away from the plasma membrane is depicted at the bottom. Averaged were 38 cells for each condition.

Data Analysis and Statistics—Data were analyzed using TILL Vision (TILL Photonics), Igor Pro (Wavemetrics), and Microsoft Excel. All values are given as mean ± S.E.; n = number of cells. In case data points were normally distributed, a paired or unpaired two-sided Student’s t test was used. If normal distribution could not be confirmed, a non-parameterized test (Mann-Whitney) was carried out. Levels of significance are indicated in Figs. 1–5 (* refers to p < 0.05, ** refers to p < 0.01, and *** refers to p < 0.001).
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In parallel, mitochondrial movement toward the plasma membrane was also observed after bead stimulation, as evident from Fig. 1. [Ca\(^{2+}\)] signals and the amount of mitochondrial movement were comparable with the cells stimulated with TG. In the analysis, we only included cells that did not change their shape following bead contact. Because Ca\(^{2+}\) depletion did not induce any mitochondrial translocation toward the plasma membrane, we tested mitochondrial movement in the following experiments by inducing Ca\(^{2+}\) influx through TG in 1 mM Ca\(^{2+}\) Ringer’s solution, as shown in the inset of Fig. 2A. A translocation of mitochondria toward the plasma membrane was already measured 2 min after the onset of Ca\(^{2+}\) entry, suggesting the existence of a fast Ca\(^{2+}\)-dependent mitochondrial movement mechanism (Fig. 2A). The dependence of mitochondrial movement on Ca\(^{2+}\) influx was supported by the correlation between the translocation of mitochondria toward the plasma membrane and the increase of the extracellular Ca\(^{2+}\) concentration (Fig. 2B), which enhances the driving force for Ca\(^{2+}\) entry, as previously reported (24). Even in the presence of 0.1 or 0.25 mM external Ca\(^{2+}\), which generates only very little Ca\(^{2+}\) entry through CRAC channels, a small movement of mitochondria toward the plasma membrane was observed. The movement appears to be saturated with 1 mM Ca\(^{2+}\). Another argument for the dependence of the mitochondrial movement on Ca\(^{2+}\) influx can be gathered from the observation that mitochondrial translocation toward the plasma membrane was completely reversed after removing the extracellular Ca\(^{2+}\) solution (Fig. 2C) and was abolished in T-cells loaded with the Ca\(^{2+}\) chelator BAPTA (Fig. 2D). Because Ca\(^{2+}\) influx in T-cells depends strictly on the activity of CRAC channels (24, 30), we analyzed the intracellular localization of mitochondria in T-cells treated with the CRAC channel blocker 3,5-bis(trifluoromethyl)pyrazole derivate BTP2 (31). 100 nM BTP2 significantly reduced mitochondrial movement toward the plasma membrane (Fig. 2E). BTP2 did not completely prevent the movement, which was expected, because in the presence of 100 nM BTP2, Ca\(^{2+}\) entry through the CRAC channel is reduced to a level similar to

toward the plasma membrane was also observed in CD3\(^{+}\) peripheral blood lymphocytes during the Ca\(^{2+}\) plateau (data not shown). We next analyzed the complete mitochondrial population by spacing five concentric circles within the cells with a constant distance of 0.99 \(\mu\)m (Fig. 1D). The average distance between mitochondria and the plasma membrane did not change during store depletion compared with resting conditions; however, during the Ca\(^{2+}\) plateau, the fluorescence between the outer circles was significantly increased. In parallel, the Mitotracker\textsuperscript{®} fluorescence decreased between the inner circles, indicating that the complete mitochondrial population had moved toward the plasma membrane.

To analyze whether mitochondrial movement toward the plasma membrane also occurred in T-cells in the case of T-cell receptor stimulation, we used anti-CD3-coated beads to stimulate the cells. Fig. 1E shows a typical bead experiment in which the contact between bead and cell induces a rise of the intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]), as previously reported (27).
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**A** Control

![Graph](image1.png)

**B** Nocodazole incubation (No mitochondrial movement)

![Graph](image2.png)

**C**

![Graph](image3.png)

**D**

![Graph](image4.png)

**E**

![Graph](image5.png)

**FIGURE 4.** Patch clamp analysis of CRAC channel activity reveals its dependence on mitochondrial movement toward the plasma membrane. Examples of CRAC channel recordings at −80 mV are shown with 0.66 mM Ca\(^{2+}\)/1.2 mM EGTA ± nocodazole (A and B) or 10 mM EGTA ± nocodazole (C and D) in the pipette solution. E, statistical analysis of the CRAC current 3 min after establishing the whole-cell configuration as a fraction of the maximal current for the conditions shown in A–D.

conditions in which 0.1–0.25 mM are present in the external solution (compare Ref. 31 and Fig. 2B). Directed mitochondrial movement toward the plasma membrane in T-cells following Ca\(^{2+}\) influx through CRAC channels was also confirmed by confocal microscopy and by 2-photon microscopy (Fig. 2F). In both cases, the mitochondrial population was translocated to areas close to the plasma membrane after the Ca\(^{2+}\) signal reached the plateau following Ca\(^{2+}\) influx induced by the re-addition of 1 mM Ca\(^{2+}\) solution in TG-pretreated T-cells, an effect not observed after the re-addition of 1 mM Ca\(^{2+}\) solution without TG pretreatment. In addition, we also confirmed mitochondrial movement toward the plasma membrane in T-cells transfected with mitochondrial green fluorescent protein (data not shown). The results shown in Figs. 1 and 2 demonstrate that the directed movement of mitochondria toward the plasma membrane depends on Ca\(^{2+}\) influx through CRAC channels in T-cells.

Mitochondria Translocation toward the Plasma Membrane

Mitochondria Translocation toward the Plasma Membrane Is Required for Sustained Ca\(^{2+}\) Signals—The mitochondrial movement toward the plasma membrane following the activation of Ca\(^{2+}\) influx through CRAC channels was almost completely abolished by pre-incubation of the cells with nocodazole, a microtubule inhibitor (Fig. 3, A and B) but only slightly with latrunculin B, a microfilament inhibitor (data not shown). The inhibition of mitochondrial movement toward the plasma membrane by nocodazole greatly reduced the sustained Ca\(^{2+}\) plateau compared with control conditions (Fig. 3, C and D). The disruption of microtubules induced by nocodazole is time-dependent, because the percentage of subplasma membrane MitoTracker\(^{®}\) fluorescence between the two concentric circles as well as the sustained Ca\(^{2+}\) signal decrease with the nocodazole incubation time (Fig. 3, C and D). The Ca\(^{2+}\) re-addition protocol usually generates very high Ca\(^{2+}\) influx signals, because the slow up-modulation of the plasma membrane Ca\(^{2+}\) ATPases cannot prevent the “overshoot” of the intracellular Ca\(^{2+}\) concentration (32). To avoid this [Ca\(^{2+}\)]\(_i\) overshoot, we also compared the effect of nocodazole to control conditions by directly activating Ca\(^{2+}\) influx in the presence of external Ca\(^{2+}\). Under these conditions, mitochondrial movement toward the plasma membrane was completely abolished by nocodazole (Fig. 3E) and the concomitant [Ca\(^{2+}\)]\(_i\) rise was also drastically reduced (Fig. 3F). Nocodazole did not interfere with mitochondrial Ca\(^{2+}\) uptake or plasma membrane Ca\(^{2+}\) ATPase activity (supplemental Fig. 1). We therefore postulate that the sustained high [Ca\(^{2+}\)]\(_i\), which depends on Ca\(^{2+}\) influx through CRAC channels, is maintained through stimulation-dependent mitochondrial movement toward the plasma membrane. A testable prediction of this model is that inhibition of mitochondrial movement into the vicinity of the plasma membrane should decrease CRAC current amplitudes.

Mitochondrial Movement toward the Plasma Membrane Controls CRAC Channel Activity—To analyze CRAC channel activity, we used the whole-cell configuration of the patch clamp technique. CRAC channels were activated through depletion of the endoplasmic reticulum Ca\(^{2+}\) store using a combination of InsP\(_3\) and TG. In a first series of experiments, we compared CRAC channel inactivation using a small concentration of the Ca\(^{2+}\) chelator EGTA in the pipette, which is not sufficient to prevent Ca\(^{2+}\)-dependent inactivation of CRAC channels (16). Under these conditions, mitochondrial Ca\(^{2+}\) uptake close to the channels is required to maintain CRAC channel activity by reducing local accumulation of Ca\(^{2+}\) near
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Kinesin motor proteins control both mitochondrial translocation toward the plasma membrane and sustained Ca\(^{2+}\) signals. A, MitoTracker\(^{\text{GM/AM-loaded/anti-kinesin 5B or anti-IgG mAb}}} transfected T-cells before and after stimulation with 1 \(\mu\text{M TG}} in 1 \text{mM Ca}\(^{2+}\) Ringer's solution. MitoTracker\(^{\text{GM/AM-loaded/anti-kinesin 5B or anti-IgG mAb}}} and infrared images were taken in parallel before (resting) and at the end of the Ca\(^{2+}\) plateau (at 1000 s after cell stimulation). B, statistical analysis of the mitochondrial localization close to the plasma membrane in transfected resting T-cells (n = 29) and at the end of the Ca\(^{2+}\) plateau in anti-IgG mAb-transfected T-cells (n = 14) and at the end of the plateau in anti-kinesin 5B mAb-transfected T-cells (n = 41). C, identical experiments as the one presented in Fig. 1A, except that cells were transfected with anti-IgG mAbs as the control or anti-kinesin 5B mAbs. The [Ca\(^{2+}\)], at 900–1000 s was analyzed for anti-IgG mAbs (865 ± 31 nM, 457 cells) and anti-kinesin 5B mAb (414 ± 17 nM, 320 cells)-treated cells. IR, infrared image; Mito, MitoTracker\(^{\text{GM/AM-loaded/anti-kinesin 5B or anti-IgG mAb}}} image.

FIGURE 6. Model for the mitochondrial movement-dependent, efficient T-cell activation. Mitochondrial translocation toward the plasma membrane is essential for a sustained [Ca\(^{2+}\)] signal and subsequent transcription factor activity. This directed and Ca\(^{2+}\)-influx-dependent mitochondrial relocalization allows efficient T-cell activation, most likely by initiating a closer contact between mitochondria and CRAC channels. This close localization facilitates a larger and more sustained Ca\(^{2+}\) influx by reducing the Ca\(^{2+}\)-dependent CRAC channel inactivation, which subsequently leads to higher [Ca\(^{2+}\)], and a concomitant change in the transcription factor profile from NFAT-AP1 to NFAT, NFAT-AP1, and NF\(\kappa\)B (see also Refs. 3 and 7).

Kinesin Controls Mitochondrial Translocation toward the Plasma Membrane and Sustained Ca\(^{2+}\) Signals—Kinesin motor proteins control the anterograde movement of different organelles and cargos along microtubules (33). Because the translocation of mitochondria toward the plasma membrane is an anterograde motion, we introduced 0.1 mg/ml anti-kinesin 5B mAb into T-cells using electroporation and analyzed in-parallel mitochondrial translocation and [Ca\(^{2+}\)], signals following TG stimulation. Anti-kinesin 5B mAb
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...Ca\textsuperscript{2+} accumulation of mitochondria close to the plasma membrane. In turn, CRAC is activated through depletion of Ca\textsuperscript{2+} stores; Ca\textsuperscript{2+} influx through activated CRAC channels triggers mitochondrial translocation to the plasma membrane, which in turn keeps CRAC channels active. There are numerous examples of such double positive feedback loops; for example, InsP\textsubscript{3} activates InsP\textsubscript{4}-R, which releases Ca\textsuperscript{2+}, which binds to InsP\textsubscript{4}-R, which in turn releases more Ca\textsuperscript{2+}.

According to a model proposed by Hajnoczky and co-workers (20), the high submembranous [Ca\textsuperscript{2+}], (in our case generated through CRAC activity) would decrease mitochondrial motility thereby immobilizing them close to the plasma membrane. There are, however, some differences between the model of Hajnoczky and co-workers (20) and our data. Hajnoczky and co-workers show that mitochondrial motility is reduced in a myoblast cell line (H9c2) after Ca\textsuperscript{2+} release and recovered by 50% when the Ca\textsuperscript{2+} signal reaches a plateau. They conclude that mitochondria are immobilized close to microdomains of high Ca\textsuperscript{2+} near the endoplasmic reticulum. In T-cells, we do not observe such a high Ca\textsuperscript{2+} release, and T-cell activation is largely determined by Ca\textsuperscript{2+} influx through CRAC channels. Ca\textsuperscript{2+} release in T-cells is usually initiated in large areas throughout the cytosol. Therefore, there would probably be no preferential direction for mitochondria movement. Hence, in the "release" case, Ca\textsuperscript{2+}-dependent mitochondria movement toward the endoplasmic reticulum would be extremely hard to measure, because such movement would almost not differ from random movement. In the "influx" case, there is a preferential direction for a Ca\textsuperscript{2+}-dependent movement, namely toward the plasma membrane, where they would be finally immobilized by the high [Ca\textsuperscript{2+}], microdomains close to CRAC channels. Therefore, the data by Hajnoczky and co-workers (20) and our data may be reconcilable, because after an initial movement due to Ca\textsuperscript{2+} signals (which may not be distinguishable from random movement in case the endoplasmic reticulum is the Ca\textsuperscript{2+} source), the subsequent high [Ca\textsuperscript{2+}], may immobilize mitochondria (in our case at the plasma membrane). In addition, in flat cells, such as adherent myoblasts, there may be no directed movement of mitochondria to the plasma membrane, because mitochondria are already close to the influx channel (in z dimension).

Because Ca\textsuperscript{2+} influx through CRAC channels increases the accumulation of mitochondria close to the plasma membrane and local this localization near CRAC channels sustains CRAC activity, we propose that CRAC channels and mitochondria control each other in a double positive feedback loop. The concomitant increase in global [Ca\textsuperscript{2+}], shifts the transcription factor profile away from mostly NFAT-AP1 to a combination of NFAT, NFAT-AP1, and NF\textsuperscript{κB} (Fig. 6; see also Refs. 3 and 7). Consequently, this transcriptional profile change leads to a differential protein expression. Thus, a small change in mitochondrial localization is likely translated into a large effect on T-cell activity.

REFERENCES

1. Lewis, R. S., and Cahalan, M. D. (1995) Annu. Rev. Immunol. 13, 623–653
2. Parekh, A. B., and Putney, J. W. Jr. (2005) Physiol. Rev. 85, 757–810
3. Quintana, A., Griesemer, D., Schwarz, E. C., and Hoth, M. (2005) Pflugers Arch. 450, 1–12
4. Gallo, E. M., Cante-Barrett, K., and Crabtree, G. R. (2006) Nat. Immunol. 7, 25–32
5. Dolmetsch, R. E., Xu, K., and Lewis, R. S. (1998) Nature 392, 933–936
6. Bhakta, N. R., Oh, D. Y., and Lewis, R. S. (2005) Nat. Immunol. 6, 143–151
7. Macian, F., García-Cozar, F., Im, S. H., Horton, H. F., Byrne, M. C., and Rao, A. (2002) Cell 109, 719–731
8. Feske, S., Gwack, Y., Prakriya, M., Sririkant, S., Puppel, S. H., Tanasa, B., Hogan, P. G., Lewis, R. S., Daly, M., and Rao, A. (2006) Nature 441, 179–185
9. Vig, M., Peinelt, C., Beck, A., Koomoa, D. L., Rabah, D., Koblan-Huberson, M., Kraft, S., Turner, H., Fleig, A., Penner, R., and Kinet, J. P. (2006) Science 312, 1220–1223
10. Liou, J., Kim, M. L., Heo, W. D., Jones, J. T., Myers, J. W., Ferrell, J. E. Jr., and Meyer, T. (2005) Curr. Biol. 15, 1235–1241
11. Zhang, S. L., Yu, Y., Roos, J., Kozak, J. A., Deerinck, T. J., Ellisman, M. H., Stauderman, K. A., and Cahalan, M. D. (2005) Nature 437, 902–905
12. Spassova, M. A., Soboloff, J., He, L. P., Xu, W., Dziedzak, M. A., and Gill, D. L. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 4040–4045
13. Nolz, J. C., Gomez, T. S., Zhu, P., Li, S., Medeiros, R. B., Shimizu, Y., Burkhardt, J. K., Freedman, B. D., and Billadeau, D. D. (2006) Curr. Biol. 16, 24–34
14. Newmeyer, D. D., and Ferguson-Miller, S. (2003) Cell 112, 481–490
15. Duchan, M. R. (2000) J. Physiol. (Lond.) 529, 57–68
16. Hoth, M., Button, D. C., and Lewis, R. S. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 10607–10612
17. Gütensch, M. D., Bakowski, D., and Parekh, A. B. (2002) EMBO J. 21, 6744–6754
Mitochondria Localization and CRAC Activity

18. Ball, E. H., and Singer, S. J. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 123–126
19. Knowles, M. K., Guenza, M. G., Capaldi, R. A., and Marcus, A. H. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 14772–14777
20. Yi, M., Weaver, D., and Hajnoczky, G. (2004) J. Cell Biol. 167, 661–672
21. Varadi, A., Johnson-Cadwell, L. I., Cirulli, V., Yoon, Y., Allan, V. J., and Rutter, G. A. (2004) J. Cell Sci. 117, 4389–4400
22. Morris, R. L., and Hollenbeck, P. J. (1995) J. Cell Biol. 131, 1315–1326
23. Yoon, Y. (2005) Science’s STKE 2005, pe18
24. Fanger, C. M., Hoth, M., Crabtree, G. R., and Lewis, R. S. (1995) J. Cell Biol. 131, 655–667
25. Schwarz, A., Tutsch, E., Ludwig, B., Schwarz, E. C., Stallmach, A., and Hoth, M. (2004) J. Biol. Chem. 279, 5641–5647
26. Philipp, S., Strauss, B., Hirnet, D., Wissenbach, U., Mery, L., Flockerzi, V., and Hoth, M. (2003) J. Biol. Chem. 278, 26629–26638
27. Quintana, A., and Hoth, M. (2004) Cell Calcium 36, 99–109
28. Tutsch, E., Griesemer, D., Schwarz, A., Stallmach, A., and Hoth, M. (2004) Eur. J. Immunol. 34, 3477–3484
29. Zweifach, A., and Lewis, R. S. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6295–6299
30. Feske, S., Prakriya, M., Rao, A., and Lewis, R. S. (2005) J. Exp. Med. 202, 651–662
31. Zitt, C., Strauss, B., Schwarz, E. C., Spaeth, N., Rast, G., Hatzelmann, A., and Hoth, M. (2004) J. Biol. Chem. 279, 12427–12437
32. Bautista, D. M., Hoth, M., and Lewis, R. S. (2002) J. Physiol. (Lond.) 541, 877–894
33. Vale, R. D. (2003) Cell 112, 467–480
34. Varadi, A., Cirulli, V., and Rutter, G. A. (2004) Cell Calcium 36, 499–508
35. Hirokawa, N., and Takemura, R. (2005) Nat. Rev. Neurosci. 6, 201–214
36. Yildiz, A., Tomishige, M., Vale, R. D., and Selvin, P. R. (2004) Science 303, 676–678