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Microfilament dynamics during HaCaT cell volume regulation

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Abstract

Cell volume is an important parameter in many physiological processes, and is closely regulated in many cell types. In those cells, swelling induced by hypotonic media is followed by an ion-driven regulatory volume decrease. In many cell types, this regulatory volume decrease requires an intact actin cytoskeleton. Therefore, we investigated the changes in the structure and polymerization state of the actin cytoskeleton in HaCaT keratinocytes during cell swelling and regulatory volume decrease. Disruption of the actin cytoskeleton by 2 μM cytochalasin D inhibits regulatory volume decrease in HaCaT cells. Cells swollen in the presence of low concentrations of cytochalasin D (0.8 μM, 305–250 mosM) keep the elevated volume even after cytochalasin D removal. A further decrease of tonicity (250–200 mosM) is again counteracted by regulatory volume decrease reaching the volume, which has been established at 250 mosM. In contrast, no visible changes occurred in actin cytoskeleton morphology of EGFP–actin-transfected HaCaT cells during swelling or regulatory volume decrease. However, biochemical analysis showed an increase in total F-actin levels 90 s after the onset of hypotonicity. The ratio of Triton-soluble to -insoluble actin also increased after hypotonic shock, suggesting that the measured increase in F-actin is primarily due to de novo polymerization and formation of short actin filaments, i.e., actin oligomers. These results show that a rapid reorganization of the actin cytoskeleton takes place after hypotonic treatment. This reorganization can influence signaling in response to hypotonicity either indirectly by means of sequestering or releasing actin-associated proteins, or directly by the interaction of short actin filaments with plasma membrane ion channels, and may be involved in determining a new volume set point.

Keywords: Cell volume regulation; Actin; RVD; Cytochalasin; Keratinocytes

Introduction

A fundamental property of many animal cells is the ability to regulate their volume under osmotic perturbation and during cell shape change. In a hypotonic environment, after initial swelling, cells undergo a slower, compensatory shrinkage through the loss of intracellular solutes, mainly K^+ and Cl^-, along with osmotically obliged water, a process known as regulatory volume decrease (RVD, reviewed, e.g., in Hallows and Knauf, 1994; Hoffmann and Dunham, 1995; Okada and Maeno, 2001). The effectors of this process, plasma membrane ion channels and transporters, have been well characterized in the past (Stutzin and Hoffmann, 2006; Wehner, 2006). The nature of the volume sensor, however, is still under debate. Attempts to elucidate the

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volume sensor transduction pathway for volume regulation thus far have revealed evidence for the involvement of intracellular Ca^{2+} concentration, protein phosphorylation, arachidonic acid and phosphoinositide turnover, as well as mechanical sensors, such as stretch-activated channels and the cytoskeleton (for review see Alexander and Grinstein, 2006; Hoffmann and Pedersen, 2006). Certainly, different mechanisms may be operative in different cell types, and presumably several of these elements could interact to produce an integrated volume regulatory response.

The cytoskeleton is a highly dynamic system, which can mediate and modulate extracellular signals as well as provide mechanical support and flexibility to control and alter cell shape and function. Thus, many studies have been performed to elucidate the involvement of the cytoskeleton in cell volume regulation (Moustakas et al., 1998; Pedersen et al., 2001). Mainly microfilaments have been studied so far, and microscopic and biochemical analyses showed a change in actin organization and/or polymerization state in several cell types after hypotonic challenge (Bibby and McCulloch, 1994; Hallows et al., 1996; Mountian et al., 1998; Pedersen et al., 1999; Pritchard and Guilak, 2004). Consistently, disruption of the actin cytoskeleton by cytochalasins impairs or abolishes RVD in some cell types (Downey et al., 1995; Ebner et al., 2005; Pritchard and Guilak, 2004; Shen et al., 1999). Possible functions for the actin cytoskeleton in RVD are the direct activation of transport proteins, modulation of transporter turnover or sensing tension/force transmittance (Hoffmann and Mills, 1999; Jakab et al., 2002; Wehner et al., 2003). However, the changes in the actin cytoskeleton are quite diverse in different cell types and some cell models are reportedly indifferent to microfilament disruption (Downey et al., 1995; Hallows et al., 1996).

This study was undertaken to assess the possibility of microfilament involvement in volume regulatory responses of HaCaT keratinocytes. We investigated the effect of microfilament disruption by cytochalasin D (CD) on swelling and RVD. The organization of the actin cytoskeleton during swelling and RVD was studied by confocal laser scanning microscopy, and volume-dependent shifts in intracellular G- and F-actin contents were measured by biochemical analysis. The data presented here provide evidence that sensing of volume changes requires an undisturbed actin fibrillar system, which is involved in setting the point of "normal" volume.

Material and methods

Cell culture and transfection

HaCaT cells (Boukamp et al., 1988) of passages 30–60 were used and grown in keratinocyte culture medium supplemented with 10% fetal calf serum and 10 mM HEPES buffer (medium and supplements from Gibco, Glasgow, UK). Cells were passaged 1:6 twice a week. For experiments, cells were grown in tissue culture Petri dishes (NUNC, Wiesbaden, Germany) or Petri dishes with cover glasses (fluorescence imaging) for 24–48 h. Full medium was replaced by serum-free medium 12 h prior to the experiment. Cells were transiently transfected with pEGFP–actin (Clontech, Heidelberg, Germany) using Effectene transfection reagent (Qiagen, Hilden, Germany) according to the manufacturer's recommendation and used for imaging 48–72 h after transfection.

Confocal imaging

The time-lapse imaging of the EGFP–actin-transfected cells was done with a TCS SP5 confocal microscope (Leica, Wetzlar, Germany) using an HCX PL Apo objective (63 × /1.3 N.A. Glyc. 37 °C). Excitation was done using the 488-nm line of an Argon laser and emission was collected in the range of 495–530 nm. Image stacks were deconvolved using AutoDeblur X1.4.1 (Media-Cybernetics, Silverspring, USA), and maximum intensity projections and cross-sections were generated with Imaris 5.0.3 (Bitplane, Zürich, Switzerland).

The volume determinations of untransfected cells were performed using a Leica TCS 4D confocal laser scanning microscope fitted with the appropriate filters and PL Fluotar objective (100 × /1.3 N.A.) that was controlled by the SCAN Ware 5.10 software (Leica, Wetzlar, Germany). Live-cell experiments were performed at 37 °C and 5% CO_2 in a humidified chamber.

Cell volume measurements

Cell volume was determined in two independent ways. The relative cell volume was determined from the *xz*-cross-sectional area of cells stained with the plasma membrane marker Vybrant DiI (Molecular Probes, Leiden, The Netherlands) imaged with a confocal microscope. Single *xz*-sections were recorded with a frequency of 0.1 Hz for 2 min before and 15 min after osmolarity change. The relative volume is expressed as the ratio of the cell cross-section at a given time (A_t) to the cross-section before osmolarity change (A_0). Since adherent cells predominantly change in height during volume changes, the cross-section serves as a reliable measure for cell volume (Korchev et al., 2000; Maric et al., 2001).

The absolute cell volume was measured by reflection interference scanning acoustic microscopy. This technique generates interference fringes, which delineate the surface topology of an adherent cell, thus allowing