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Vesicularization of the endoplasmic reticulum is a fast response to plasma membrane injury

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ABSTRACT

The endoplasmic reticulum of most cell types mainly consists of an extensive network of narrow sheets and tubules. It is well known that an excessive increase of the cytosolic Ca^{2+} concentration induces a slow but extensive swelling of the endoplasmic reticulum into a vesicular morphology. We observed that a similar extensive transition to a vesicular morphology may also occur independently of a change of cytosolic Ca^{2+} and that the change may occur at a time scale of seconds. Exposure of various types of cultured cells to saponin selectively permeabilized the plasma membrane and resulted in a rapid swelling of the endoplasmic reticulum even before a loss of permeability barrier was detectable with a low-molecular mass dye. The structural alteration was reversible provided the exposure to saponin was not too long. Mechanical damage of the plasma membrane resulted in a large-scale transition of the endoplasmic reticulum from a tubular to a vesicular morphology within seconds, also in Ca^{2+}-depleted cells. The rapid onset of the phenomenon suggests that it could perform a physiological function. Various mechanisms are discussed whereby endoplasmic reticulum vesicularization could assist in protection against cytosolic Ca^{2+} overload in cellular stress situations like plasma membrane injury.

1. Introduction

The endoplasmic reticulum (ER) derives its name from its microscopic structure, as it forms a highly interconnected network of narrow membrane sheets and tubules. It is spread throughout the cell with in general a higher membrane density around the nucleus than in the cell periphery. Visualization of the ER in live-cell microscopy shows that tubules move, branch, fuse and form de novo [1]. This dynamic stability of the highly fluid ER membranes [2] is not surprising in view of its function in the initial steps of the secretory pathway and in various signaling events involving interactions with other cellular subcompartments [3,4]. For example, the ER is the major intracellular Ca^{2+} store. It is well known that the ER takes part in the generation of spatially and temporally complex cytosolic Ca^{2+} signals by preferentially releasing Ca^{2+} at specific sites and in response to specific messengers [5,6]. The normal ER morphology guarantees a very high surface to volume ratio, facilitating exchange of compounds with the cytosol, and increasing the chances of close apposition to other intracellular organelles in the crowded intracellular context. Various ER regions are located at strategic positions allowing mutual control between the ER membranes and other organelles [4,7,8]. The largest of these regions is the nuclear membrane, which is continuous with the ER. Peripheral ER microdomains form close contacts with the plasma membrane or its subdomains, allowing preferential interactions with specific ion channels, transporters and receptors. Close apposition of ER membranes to mitochondria may allow movement of Ca^{2+} between both organelles via a pathway that is shielded from the cytosol [3,9]. Therefore structural alterations of the ER may have profound effects on its function.

The most common structural alteration of the ER is a transition to a predominantly vesicular morphology. Probably the best known condition causing ER vesicularization is an excessive elevation of the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) by application of the ionophore ionomycin [10,11], by application of the ER Ca^{2+} pump inhibitor thapsigargin ([11]), or by overexpression of Ca^{2+} channels [12]. The observation that ER vesicularization may occur during the harsh treatment of cells necessary for their isolation [13], or also following 5 min of inhibition of glycolysis and mitochondrial respiration [14] may similarly be explained by an excessive increase of $[Ca^{2+}]_c$. Also more physiological increases of $[Ca^{2+}]_c$ have been reported to induce a transition of the ER from tubular structures to small vesicles [10,15]. In addition, there exist slower processes causing changes of ER morphology over hours or days, for example ER swelling associated with various forms of cell death [16] or with the overexpression of some specific proteins in cultured cells such as the chloride-iodide transporter pendrin [17] or the vanilloid receptor 1 [12].

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We here document that in cultured cells ER vesicularisation is a rapid response to membrane permeabilization either by saponin treatment or by mechanical injury and show that this response occurs independently of changes of cytosolic Ca^{2+}. The possibility is discussed that this structural change could help to counteract cytosolic Ca^{2+} overload and thereby promote cell survival.

2. Materials and methods

2.1. Cell culture and transfection

Cells of the HaCaT human keratinocyte cell line [18], HeLa cells and COS-1 cells were seeded into chamber slides (Nunc Intermed, Roskilde Denmark) at a density of 3×10^4/well and grown in a humidified incubator at 37 °C and 5% CO_2. The growth medium consisted of DMEM, supplemented with 10% fetal bovine serum, 3.5 mM L-glutamine, 88 U/ml penicillin and 88 μg/ml streptomycin.

After 1 day the cells were transfected with the cameleons YC3.3ER or GT-YC3.3 (gifts from R.Y. Tsien, University of California, San Diego) for imaging the endoplasmic reticulum and FRET measurements [19]. Transfection of the cameleons in the expression vector pcDNA3 was carried out using the transfection reagent GeneJuice (Novagen, WI, USA). The experiments were performed between 2 and 3 days after transfection.

2.2. Solutions and chemicals

Krebs solution contained (mM): 135 NaCl, 6 KCl, 1.2 $MgCl_2$, 1.5 $CaCl_2$, 12 glucose and 12 Na/Hepes (pH 7.3). High K^+ solution was obtained by replacing NaCl with KCl and Ca^{2+}-free conditions by replacing $CaCl_2$ by 2 mM EGTA. Saponin-was dissolved at 1 mg/100 ml in a solution containing (mM): 120 KCl, 30 imidazole, 2 EGTA, 2 $MgCl_2$, 1 ATP (solution S).

Saponin, ionomycin and ATP were purchased from Sigma (St. Louis, MO, USA). Fura-2 AM, TO-PRO3 and ER-Tracker Blue-White DPX were obtained from Molecular Probes (Invitrogen, Eugene, OR, USA).

2.3. Microscopy

Transfected live cells were imaged on an Olympus IX-81 inverted microscope operated by the Cell^R system. The whole setup, including the microscope, the solutions and the perfusion system were enclosed in a temperature-controlled Lucite chamber maintained at 37 °C. Fluorescence was recorded by a FView cooled CCD camera (Olympus), using a 40× UApo (NA 1.4) oil-immersion objective. For standard fluorescence observations we used a triple emission filter (460, 530 and 630 nm) and a 492/18 (for cameleons) or 572/23 nm (for TO-PRO3) excitation filter.

Mechanical stabbing of a single cell was done by briefly touching the cell with a 30G × 1/2" needle (Becton Dickinson, NJ) mounted on a micromanipulator.

FRET signals from the cameleon probe were determined by emission ratio measurements using a 436/15 excitation filter and simultaneous detection of emission at two wavelengths via a Dual-View™ imager (Optical Insights, Santa Fe, NM), via a 505 nm dichroic mirror and 480/30 and 535/40 emission filters. In selected regions of the image, the fluorescence ratio of both channels after background subtraction was then calculated using the Cell^R software.

For intracellular Ca^{2+} measurements, cells were loaded in Krebs containing 1 μM Fura-2 AM at room temperature for 30 min. After washing the excess of dye, the fluorescence ratio was recorded at 510 nm using 340 and 380 nm excitation filters. TO-PRO3, a red-fluorescent cell impermeant nucleic acid stain was used to record the loss of the permeability barrier of the plasma membrane. To visualize the ER in live non-transfected cells, ER-Tracker Blue–White DPX was applied at 1 μM for 30 min at 37 °C.

3. Results

3.1. Saponin permeabilization causes ER restructuring

Live cells expressing the ER-targeted cameleon YC3.3ER clearly reveal the structure of the ER, including a fine meshwork of narrow tubules that continuously moves and restructures at high resolution but whose general topology remains stable for several hours during standard observation conditions (superfusion with normal Krebs solution at 37 °C), as described previously [1].

Plasma membranes are typically enriched in cholesterol. Permeabilization of cells with cholesterol-complexing compounds like saponin or digitonin is a frequently used procedure to gain direct access to intracellular organelles by selective permeabilization of the plasma membrane while preserving the permeability barrier of the endoplasmic reticulum and mitochondria (see [20,21] for review). Cameleon-transfected HaCat cells were pretreated with Ca^{2+}-free Krebs solution and then exposed to Ca^{2+}-free, ATP-containing high-K^+ solution (S solution) supplemented with a low concentration of saponin to selectively permeabilize the plasma membrane. It is confirmed in Fig. 1 that this procedure conserves the functional integrity of the ER membrane barrier. As the YC3.3ER indicator [22] has a relatively high affinity for Ca^{2+} (K_d < 10 μM), the experiment demonstrates the complete Ca^{2+} depletion of the ER following the exposure to saponin. An increase

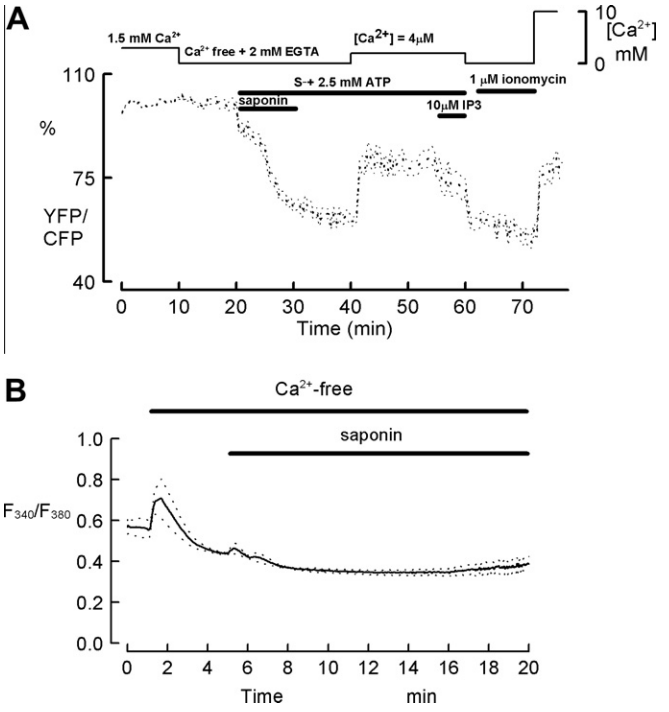

Fig. 1. Changes of the free Ca^{2+} concentration during saponin treatment. (A) FRET measurements of the ER-targeted YC3.3ER cameleon show that the permeability barrier of the ER and the function of its Ca^{2+} pumps are maintained despite the ER vesiculation. Cells were treated for 10 min with 10 μg/ml saponin in S solution with ATP. After addition of 4 μM free Ca^{2+} to the empty stores, the ER can take up Ca^{2+} again due to the SERCA pump activity. Addition of 10 μM IP_3 induced a partial Ca^{2+} release. At the end, minimum and maximum emission ratios were determined by application of ionomycin in EGTA or in 10 mM Ca^{2+}. The curve represents the mean of 6 cells. (B) Changes of $[Ca^{2+}]_c$ measured with Fura-2 in the same conditions as in (A). Changing to Ca^{2+}-free solution induced a rise of $[Ca^{2+}]_c$. Further addition of saponin had a negligible effect.