Nuclear envelopes show cell-type specific sensitivity for the permeabilization with digitonin.

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Method Article

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Abstract

Many protocols designed to analyze the intracellular distribution of antigens depend on detergents that permeabilize or semi-permeabilize membranes[1] . Whereas Triton X-100 or similar detergents are used to permeabilize all cellular membranes, digitonin can permeabilize preferentially the plasma membrane of mammalian culture cells under conditions that leave the nuclear envelope \(NE) intact[2] . Digitonin treatment of fixed cells has been important to distinguish between antigens on the nuclear and cytoplasmic side of the NE. Furthermore, incubation of unfixed cells with digitonin is a critical step to analyze nuclear import _in vitro_[3] . Macromolecules move in and out of the nucleus through nuclear pore complexes \(NPCs) which are embedded in the NE[4-6]. For the analysis of nuclear transport in a cell-free system it is therefore mandatory that the NE remains intact during the incubation period. Many in vitro nuclear transport studies were carried out with HeLa cells[3]; however, for some applications the use of other cell lines is preferable. Here, we compare the permeabilization of NEs in three mammalian cell lines that were treated with digitonin. Our protocol provides a guide to optimize the concentration of digitonin to permeabilize the plasma membrane, but not the NE, of mammalian culture cells.

Reagents

Bovine serum albumin \(BSA) Buffer B, adjusted to pH 7.3 with NaOH \[20mM Hepes; 110 mM potassium acetate; 5 mM sodium acetate; 2 mM magnesium acetate; 1 mM EGTA; add immediately before use: 2 mM DTT and protease inhibitors \(aprotinin, leupeptin, pepstatin; each at 1µg/ml)] Cell culture medium \(Gibco) Digitonin \(Sigma) DMSO \(Sigma) Formaldehyde Mounting medium; for example Vectashield \(Vector Laboratories) NaN_3 PBS, phosphate-buffered saline, pH 7.4 Triton X-100 Antibodies against a protein located in the nucleus; here: polyclonal anti-lamin B antibodies \(Santa Cruz Biotechnology, sc-6217) Cy3-labeled affinity-purified secondary antibodies \(Jackson ImmunoResearch) 4',6-diamidino-2-phenylindole \(DAPI; Sigma) **Cell lines** HeLa S3 \(human, cervix carcinoma) LLC-PK1 \(pig, kidney) NIH3T3 \(mouse, fibroblast)

Equipment

CO_2 incubator Laminar flow hood Humidity chamber Confocal microscope \(for example: Zeiss LSM510) or fluorescence microscope equipped with appropriate camera.

Procedure

CELL CULTURE Culturing of cells on poly-lysine coated coverslips has been described previously[7,8]. **INDIRECT IMMUNOFLUORESCENCE** All incubations are carried out in a humidity chamber at room temperature. 1. Dissolve digitonin in DMSO; prepare stock solutions in DMSO that are 1,000 fold concentrated. Immediately before use, dilute digitonin into buffer B. 2. Incubate cells for 3 min at room temperature and remove liquid. 3. Wash once with cold buffer B, rinse with PBS and fix with 3.7%

formaldehyde in PBS for 25 min. 4. Rinse with PBS; then move to step 5 or permeabilize with 0.1% Triton X-100 in PBS/2 mg/ml BSA/1 mM NaN_3 for 5 min. 5. Block with PBS/2 mg/ml BSA/1 mM NaN_3 \(blocking solution) for 1 h. 6. Incubate overnight with anti-lamin B antibodies \(0.5 µg/ml) in blocking solution. 7. Wash 3 times for 10 min with blocking solution, then incubate for 2 h with Cy3-labeled secondary antibodies \(3 µg/ml) in blocking solution. 8. Wash 3 times for 10 min with blocking solution, then incubate with 1 µg/ml DAPI in blocking solution for 2 min. 9. Mount specimen and seal with nail polish. **IMAGE ACQUISITION** Acquire images by confocal microscopy \(for example, Zeiss LSM510) using a 63x objective \(oil, NA = 1.4), pixel resolution of 0.65 µm, scan speed 5, four-line averaging[9]. **PROCESSING OF IMAGES** Process images with appropriate software such as Adobe Photoshop 8.0.

Timing

The procedures can be completed in 18 hours.

Troubleshooting

Nuclear envelope is permeabilized by digitonin treatment The concentration of digitonin as well as the incubation time with the detergent has to be optimized. For some applications it may be suitable to treat with digitonin on ice. **Intactness of NE cannot be tested by antibody staining** If it is not possible to test the barrier function of NEs by staining with antibodies against nuclear proteins, use alternative methods. For instance, the entry into the nucleus of large non-nuclear proteins, such as GFP-β-galactosidase, provides a method to detect leakiness of NEs without the need for immunostaining[10].

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Figures

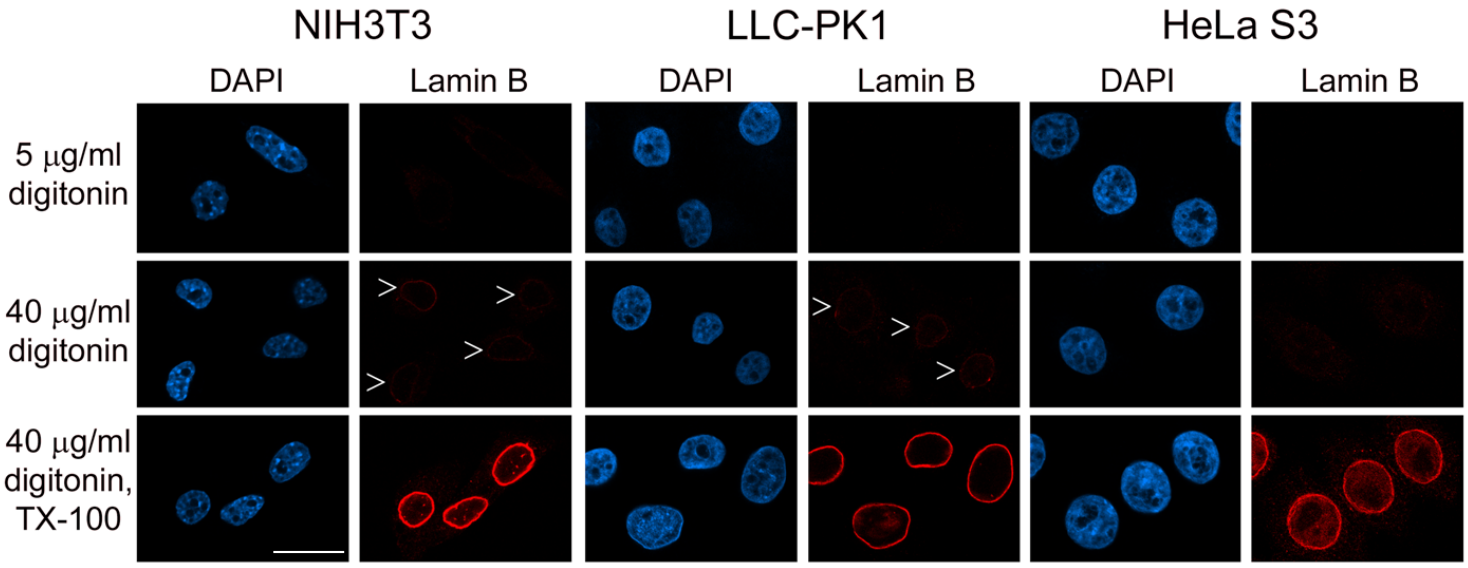

Figure 1

Effect of digitonin on the integrity of nuclear membranes in NIH3T3, LLC-PK1 and HeLaS3 cells. NIH3T3, LLC-PK1 and HeLaS3 cells were treated with 5 or 40 µg/ml digitonin as indicated. Cells were fixed and subsequently processed for indirect immunofluorescence with antibodies against lamin B; DNA was stained with DAPI. In control experiments, cells were incubated with Triton X-100 before the blocking step. Note that with 40 µg/ml digitonin weak staining with lamin B antibodies is observed for NIH3T3 and LLC-PK1, but not for HeLa S3 cells. Arrowheads indicate the staining of lamin B in NIH3T3 and LLC-PK1 cells that were incubated with 40 µg/ml digitonin. Size bar is 20 µm.