SYNTHESIS, MATURATION AND TRAFFICKING OF HUMAN Na^+-DICARBOXYLATE COTRANSPORTER NaDC1 REQUIRES THE CHAPERONE ACTIVITY OF CYCLOPHILIN B

Marc J. Bergeron, Marc Bürzle, Gergely Kovacs, Alexandre Simonin, and Matthias A. Hediger

Oligonucleotide sequences

wt huNaDC1 cDNA:

S primer: 5'(**BamHI**) CC**GGATCC**GCTCCACACCATGGCCACCTGC 3'

AS primer: 5'(**XbaI**) CC**TCTAGA**CTAGGGGCTTGGTGTGGTGGTG 3'

wt huNaDC1 with FLAG tag N-terminally:

S primer: 5'(**BamHI**) **GATCC**ATGGATTACAAGGATGACGACGATAAGATGGCCACCTGCTGGCAG**GCCCTGT** (**BglI**) 3'

AS primer: 3'(**BamHI**) **G**TACCTAATGTTCCTACTGCTGCTATTCTACCGGTGGACGACCGTC**CGGG** (**BglI**) 5'

huCypB without ER retention sequon (AIAKE):

S primer: 5'(**BclI**) **GATCA**TCGCAGACTGCGGCAAGATCGAGGTGGAGAAGCCCTTT**C** (**XhoI**) 3'

AS primer: 3'(**BclI**) **T**AGCGTCTGACGCCGTTCTAGCTCCACCTCTTCGGGAAA**GAGCT** (**XhoI**) 5'

Supplemental Table I. Synthetic oligonucleotides used for PCR reactions and to generate double strand DNA fragments. All oligos are written 5' to 3' excepted for the two complementary AS primers that are written 3' to 5'. Bold letters designate enzyme restriction sites in PCR oligos or cohesive compatible enzyme restriction sites. S = sense, AS = anti-sense. Note that S and AS primers for wt huNaDC1 with FLAG tag N-terminally and huCypB without ER retention sequon (AIAKE) are displayed hybridized to each other.

Composition of solutions

Modified Barth's Medium:
88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.66 mM $NaNO_3$, 0.75 mM $CaCl_2$, 10 mM Na-HEPES

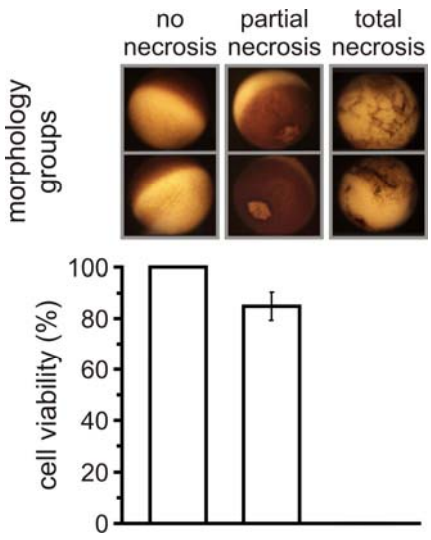
Regular flux medium for oocytes:
76 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1 mM Na_2HPO_4, 1 mM Na_2SO_4, 10 mM Na-HEPES

Regular flux medium for HEK293 cells:
135 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM Na_2HPO_4, 1 mM Na_2SO_4, 15 mM Na-HEPES

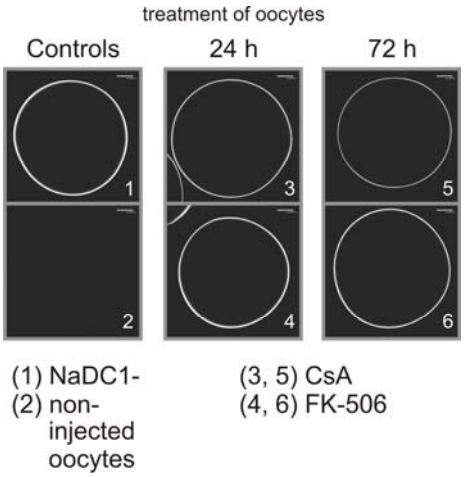
RIPA solution for oocytes:
50 mM Tris, 150 mM NaCl, 1% Triton-X100, 0.5% Na-deoxycholate, 0.1% SDS

RIPA solution for HEK293 cells:
50 mM Tris, 150 mM NaCl, 1% Triton-X100, 0.5% Na-deoxycholate, 0.1% SDS, 5 mM EDTA

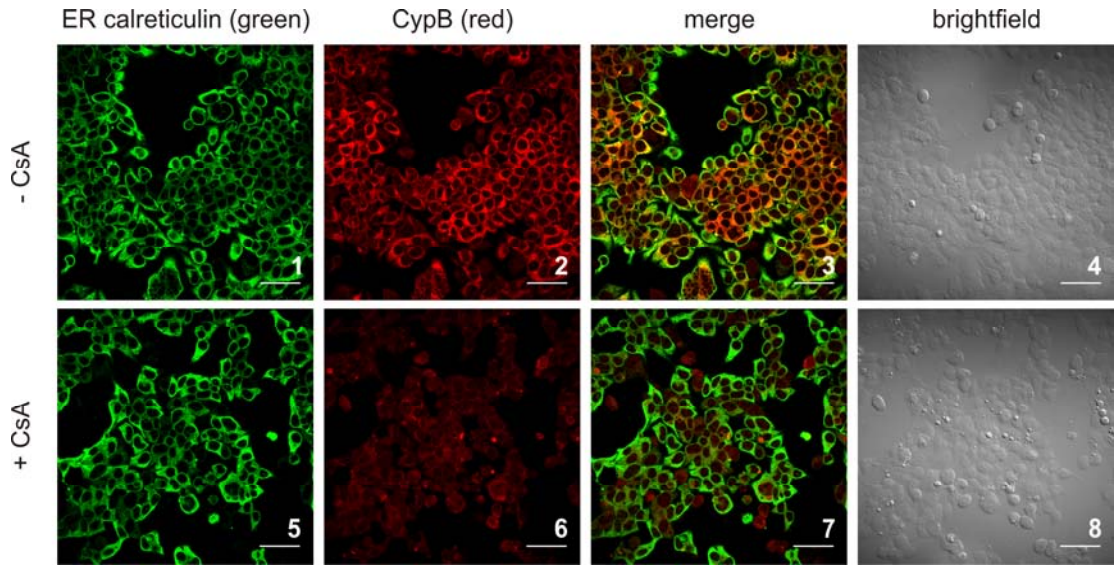
Supplemental Table II. Composition of solutions. The pH of all solutions was adjusted at 7.4.



Supplemental Figure 1. Validity of XTT cell viability assay in oocytes. Oocyte morphology groups were determined according to necrosis stage: 1) no necrosis or healthy oocytes, 2) partial necrosis (small spot on the animal side indicates the start of necrosis, and 3) total necrosis or dead oocytes (no difference between vegetal and animal poles). Two representative images for each morphology group are depicted. Images of oocytes were taken with a Nikon DIAPHOT 300 Inverted Phase Contrast microscope supplied with a camera. The oocyte viability was not significantly decreased in the partial necrosis group, while viability was completely abolished in the total necrosis group. Data were normalized to values obtained in the no necrosis group, expressed as %, and shown as averages (± S.E.) of 3 experiments.



Supplemental Figure 2. Immunofluorescence studies in *X. laevis* oocytes. NaDC1 distribution at the plasma membrane using mouse polyclonal anti-SLC13A2 antibodies shows a perfect fit with the cell surface biotinylation studies. NaDC1 signal was reduced by 50 µM CsA treatment for 24 h, and greatly reduced by 72 h treatment. Treatment with 5 µM FK-506 had no effect. Shown are representative 10X images of the 3-4 oocytes for each treatment. Scale bar: 150 µm.



Supplemental Figure 3. Further immunofluorescence studies in HEK293 cells. NaDC1-expressing HEK293 cells were not treated (- CsA) or treated with 10 µM CsA (+ CsA) for 24 h. Cells were first fixed and permeabilized as described in the *Experimental Procedures* section above, and sequentially incubated with a mixture of chicken polyclonal anti-calreticulin antibodies (dil. 1/1,000) and rabbit polyclonal anti-cyclophilin B antibodies (dil. 1/100; 5 h; RT) and a mixture of Alexa Fluor® 488-conjugated goat anti-chicken anti-IgG antibodies and Alexa Fluor® 594-conjugated goat anti-rabbit anti-IgG antibodies (Invitrogen) (dil. 1/3,000; 1 h; RT). The ER marker calreticulin (green; 1 and 5) and CypB (red; 2 and 6) were stained. CypB co-localized with calreticulin in the ER of non-treated cells (merge image 3) but not after CsA treatment (merge image 7). CsA had no specific effect on NaDC1-expressing HEK293 cells morphology (brightfield images 4 and 8). Images were taken with a Nikon C1 confocal laser microscope system. Shown are representative 40X images. Scale bars: 50 µm.