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Oxysterol-binding protein related-proteins (ORPs) 5 and 8 regulate calcium signaling at specific cell compartments

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ABSTRACT

Oxysterol-binding protein related-protein 5 and 8 (ORP5/8) localize to the membrane contact sites (MCS) of the endoplasmic reticulum (ER) and the mitochondria, as well as to the ER-plasma membrane (PM) MCS. The MCS are emerging as important regulators of cell signaling events, including calcium (Ca^{2+}) signaling. ORP5/8 have been shown to interact with phosphatidylinositol-4,5-bisphosphate (PIP_2) in the PM, and to modulate mitochondrial respiration and morphology. PIP_2 is the direct precursor of inositol trisphosphate (IP_3), a key second messenger responsible for Ca^{2+}-release from the intracellular Ca^{2+} stores. Further, mitochondrial respiration is linked to Ca^{2+} transfer from the ER to the mitochondria. Hence, we asked whether ORP5/8 would affect Ca^{2+} signaling in these cell compartments, and employed genetically engineered aequorin Ca^{2+} probes to investigate the effect of ORP5/8 in the regulation of mitochondrial and caveolar Ca^{2+}. Our results show that ORP5/8 overexpression leads to increased mitochondrial matrix Ca^{2+} as well as to increased Ca^{2+} concentration at the caveolar subdomains of the PM during histamine stimulation, while having no effect on the cytoplasmic Ca^{2+}. Also, we found that ORP5/8 overexpression increases cell proliferation. Our results show that ORP5/8 regulate Ca^{2+} signaling at specific MCS foci. These local ORP5/8-mediated Ca^{2+} signaling events are likely to play roles in processes such as mitochondrial respiration and cell proliferation.

1. Introduction

Members of the oxysterol-binding protein related-protein (OSBP-related, or ORP) family are involved in the regulation of lipid transport at membrane contact sites (MCS) [1]. MCS are crucial for inter-organellar communication and lipid transfer as well as for the regulation of second messenger molecules, including the calcium ion (Ca^{2+}) [2]. ORPs, including ORP5 and ORP8 (ORP5/8), are involved in oncogenesis, possibly through the regulation of membrane lipids and the related downstream signaling events [3]. ORP5/8 have been characterized as countertransporters of phosphatidylinositol-4-phosphate (PI4P) and phosphatidylserine at the interface of the endoplasmic reticulum (ER) and the plasma membrane (PM) [4]. ORP5/8 were also shown to bind to phosphatidylinositol-4,5-bisphosphate (PIP_2) and modulate its transport from the PM to the ER [5]. Also, it was shown that ORP8 may not bind to P14P and PIP_2 with high affinity [6]. Interestingly, it was recently reported that ORP8 is not strongly associated with the PM in resting conditions but becomes recruited to the PM upon induction of PIP_2 production [7]. Moreover, ORP5/8 were recently found to localize at the ER-mitochondria contact sites where they modulate mitochondrial morphology and function [8]. Approximately 80% of the ORP5 and ORP8 sequences overlap, they both insert into the ER membrane through a C-terminal transmembrane (TM) domain, contain a lipid transfer domain (ORD) and interact with the PM through a pleckstrin homology (PH) domain [5,9]. The mitochondrial interactions of ORP5/8 depend on the ORD domain that binds to the mitochondrial outer membrane protein PTPIP51 [8].

Ca^{2+} is a ubiquitous second messenger that regulates several cellular functions including proliferation, migration and respiration. Intracellular Ca^{2+} concentrations are tightly regulated and multiple organelles and cellular compartments are involved in the handling and sensing of Ca^{2+} signals. Ca^{2+} is stored in the ER from where it can be released to the cytosol upon different stimuli, such as activation of the inositol trisphosphate receptor (IP_3R) [10]. ER Ca^{2+} release can be directed towards the mitochondria through the molecular interactions at contact sites designated as mitochondria-associated membranes

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(MAMs). Mitochondria take up Ca^{2+} through the mitochondrial calcium uniporter (MCU) to activate the respiratory enzymes, whereas mitochondrial Ca^{2+} overloading can lead to apoptosis [11,12]. MCU is tightly controlled by accessory proteins, the threshold Ca^{2+} concentration for MCU activation is high, and hence, ER-mitochondria tethering is essential for the generation of high Ca^{2+} microdomains that allow for MCU activation [12–17]. Also, local, spatiotemporally restricted Ca^{2+} signaling events may take place at cytoplasmic leaflets of the PM and at the lipid-rich PM compartments, caveolae, where many molecules related to Ca^{2+} signaling, such as PIP_2, have been shown to localize [18,19]. Importantly, MAMs and caveolae-ER contacts are implicated in oncogenesis, and Ca^{2+} transfer trough the IP_3R to the mitochondria is essential for cancer cell metabolism [20,21]. Further, ORP5/8 reside at the MAMs, which have been recognized to play a role in cardiovascular pathophysiology in part through disturbed mitochondrial Ca^{2+} signaling [22]. Also, the possible involvement of ORP5/8 in Ca^{2+} signaling has been recently reviewed [23].

Considering that ORP5/8 are involved in the physical interactions and lipid transfer between the ER, the PM and the mitochondria, we hypothesized that this might also affect the Ca^{2+} handling at these membrane interfaces. Further, ORP5/8 modulate the PM levels of PIP_2 [5], which is the substrate for phospholipase C (PLC) that hydrolyzes PIP_2 to IP_3 and diacylglycerol (DAG). IP_3 binds to and activates the IP_3R in the ER, and DAG activates many of the PM Ca^{2+} channels. The ORP5/8 mediated modulation of PIP_2 transport might thus affect intracellular Ca^{2+} signaling. Therefore, we set out to characterize the importance of ORP5/8 in regulating Ca^{2+} specifically in the mitochondria, at the caveolar compartments of the PM, and in the overall cytoplasm.

2. Methods

2.1. Cell culture and plasmid and siRNA transfections

HeLa cells were used in all experiments of this study. The cells were routinely cultured in DMEM (#D6046, Sigma-Aldrich) medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Life Technologies). The cells were grown in a humidified incubator at 37 °C and 5% CO_2. The GFP-ORP5 expression plasmid is described in [8]. The human ORP8 ORF was inserted in the XbaI site of pEGFP-C1 (Clontech/TakaraBio, Mountain View, CA). ORP5 and −8 specific Silencer® Select siRNAs (s377 and s41692, respectively) and a non-targeting control siRNA (Cat n:o 4390846) were purchased from ThermoFisher Scientific (Waltham, MA). HeLa cells were transfected for 24 h with the GFP-ORP5 or −8 plasmids, or plain pEGFP-C1 as a negative control. TurboFect (#R0531, Thermo Scientific) transfection reagent and OptiMEM media (#31985-070, Life Technologies) were used for all plasmid transfections. siRNA transfections were carried out using HiPerfect transfection reagent (Qiaqen).

2.2. Intracellular calcium measurements by employing aequorin

The luminescent Ca^{2+} binding recombinant protein, aequorin, was employed to measure Ca^{2+} concentrations in the cytoplasm, in the mitochondria, and at the caveolae as previously described in [24,25]. Briefly, 5000–10,000 HeLa cells were seeded on to a 96-well cell culture plate for measurements when a plate reader setup was employed (HIDEX Sense plate reader, HIDEX corp., Turku, Finland). Alternatively, when employing an in-house built single-tube luminometer setup with a perfusion chamber, 75 000–100,000 HeLa cells were seeded on to 13-mm poly-L-lysine coated coverslips in a 12-well plate. The cells were grown overnight and transfected with the desired aequorin plasmids. The measurements were conducted on the following day. Before every measurement, the cells were incubated with 5 μM coelenterazine (SynChem) for one hour in HEPES-buffered saline solution (HBSS: 118 mM NaCl, 4.6 mM KCl, 10 mM glucose, 20 mM HEPES with 1 mM CaCl2 or, alternatively, 150 μM EGTA, when Ca^{2+} free buffer was used). The cells were stimulated with 100 μM histamine in Ca^{2+} containing or in Ca^{2+} free HBSS depending on the experimental setup. The cells were primed for store-operated calcium entry (SOCE) by treating the cells with 1 μM thapsigargin (TG) for five minutes in the presence of 150 μM EGTA whereafter the SOCE was induced by perfusing the cells with HBSS buffer containing 1 μM TG and 1 mM Ca^{2+}. At the end of each experiment, the cells were lysed with 100 μM digitonin in the presence of 10 mM Ca^{2+} to obtain the maximal light production values of the sample. The obtained luminescence raw data values were analysed and transformed as described in [24]. The cytoplasmic and mitochondria targeting aequorin constructs were a kind gift from professor Paolo Pinton (University of Ferrara, Italy).

2.3. Calcium measurements by employing fura-2 AM

For Fura-2 AM measurements 5000–10,000 cells were seeded on to 96-well plates. The cells were grown overnight and transfected with the desired constructs. On the following day, they were washed twice with HBSS and incubated with Fura-2 AM (2 μM, Life Technologies) for 30 min at room temperature. Then, the cells were washed twice and incubated for 15 min in HBSS at room temperature, washed twice and transferred to the HIDEX plate reader. The excitation filters were set to 340 and 380 nanometers, respectively, and the emission was read at 510 nanometers. The fluorescence signal was recorded at one second intervals and the ratios of the two excitation wavelengths were calculated for each time point. These values were used for analysis of the data.

2.4. Western blot analysis

Cell lysates were prepared by washing the cells three times with PBS, whereafter Laemmli sample buffer was added to the samples. The lysates were boiled for 5 min and separated by SDS-PAGE, whereafter the proteins were transferred to a nitrocellulose membrane. The membranes were subjected to the desired primary or secondary antibodies in a phosphate buffered saline (PBS) solution containing 1% bovine serum albumin. The ORP5 antibodies were from Sigma-Aldrich and the ORP8 antibodies are described in [26]. HSC70 antibody was purchased from Enzo life sciences. The dilutions for the primary antibodies were 1:1000 and 1:3000 for the secondary antibodies (anti-rabbit HRP-conjugated antibody, BioRad; anti-rat HRP-conjugated antibody, Cell Signaling Technology). The protein bands were visualized by chemiluminescence (ECL, Perkin Elmer). Anti-Xpress and Anti-Xpress-HRP antibodies (Invitrogen) were employed for the detection of epitope-tagged ORP5/8 constructs that were used in the IP_3 production measurements.

2.5. Measurement of IP_3 production

IP_3 was measured using the HitHunter IP_3 Fluorescence Polarization Assay Kits (DiscoverRx Tech, Fremont, CA, USA). Briefly, 10,000 cells transfected with pcDNA4 HisMax C, ORP5 or ORP8 vector in 96-well plates (Corning, USA) were treated with 10 μM histamine for the designated times, and the cellular reaction was terminated by adding 0.2 N perchloric acid. The plate was shaken at 650 r.p.m. for 5 min. Then 20 ul mixture buffer from 96-well plates were tansferred into black 384-well plates (Greiner, Germany). The IP_3 tracer was subsequently added to each well, and the IP_3 binding protein was added to the plate. The polarized fluorescence from the IP_3 tracer was read on a Microplate Reader (CLARIOstar, BMG LABTECH). The IP_3 concentration was calculated from the IP_3 standard curve.

2.6. Proliferation assay

Fifty-thousand HeLa cells were plated on 6-well plates and grown

overnight. Thereafter the cells were transfected with plasmid constructs carrying GFP, GFP-ORP5 or GFP-ORP8, respectively. The cells were grown for 48 h and subjected to ^{3}H-thymidine (0.4 μCi/ml) for the final four hours. Thereafter the cells were washed three times in ice-cold PBS on ice, followed by a 10-min incubation in 5% trichloric acetic acid, and then incubated for 10 min with 0.1 M NaOH. Then the suspension was mixed 1:5 with Optiphase Hisafe 3 scintillation liquid (PerkinElmer). Radioactivity was measured using a Wallac 1410 liquid scintillation counter. ^{3}H-thymidine was from PerkinElmer.

2.7. Confocal microscopy

The cells were plated on poly-L-lysine coated coverslips, grown overnight and transfected with plasmid constructs carrying GFP, GFP-ORP5 or GFP-ORP8, respectively. The cells were then grown overnight, washed in PBS, fixed using 4% paraformaldehyde and mounted with Mowiol mounting medium. Microscopy was conducted by employing a Leica SP5 (Wetzlar, Germany) confocal microscope.

2.8. Statistics

The results are shown as the mean ± S.E.M. Statistical analysis of the data was conducted by unpaired Student's *t*-test when two means were compared or by One-Way Anova with Tukey's post-hoc test when three or more means were compared. The GraphPad Prism 5 program (GraphPad Software Inc., San Diego, CA) was used for the statistical analyses. A P-value below 0.05 was considered statistically significant.

3. Results

To assess the importance of ORP5/8 in regulating Ca^{2+} in intracellular compartments, we employed the luminescent calcium indicator protein, aequorin. Upon transient expression, wild-type aequorin localizes to the cytosol, but aequorin has been genetically engineered to target different cell compartments such as the mitochondria and the caveolae [24,25]. First, we set out to measure cytoplasmic and mitochondrial Ca^{2+} upon overexpression of GFP-ORP5 or −ORP8 in the presence of extracellular Ca^{2+}. To induce the intracellular Ca^{2+} fluxes we used the G-protein coupled receptor (GPCR) agonist, histamine. Our results show that the cytoplasmic Ca^{2+} was not affected in cells overexpressing either ORP5 or ORP8 when stimulated with 100 μM histamine, whereas ORP5 overexpression resulted in increased mitochondrial Ca^{2+} uptake upon histamine stimulation (Fig. 1A). To further explore the possible involvement of ORP8 in the ER-mitochondria Ca^{2+} flux, we excluded the extracellular Ca^{2+} from the experimental setting. In this condition, ORP8 overexpression significantly increased mitochondrial Ca^{2+} uptake upon stimulation with histamine (Fig. 1B). Treating the cells with 10 μM BAPTA-AM for 1 h completely abolished the mitochondrial Ca^{2+} responses (Supplementary Fig. 1A). Overexpression of the GFP-ORP5/8 constructs was confirmed by microscopy (Fig. 1C). However, knock-down of ORP5 and −8 separately or simultaneously did not affect the stimulation-evoked mitochondrial Ca^{2+} uptake (Fig. 2A). Efficient knock-down of ORP5/8 was confirmed by western blotting (Fig. 2B)

As mentioned above, ORP5 and −8 bind to PIP_2 and are localized to the ER-PM contact sites, and caveolae are enriched in PIP_2 [5,19]. Therefore, we tested whether the caveolar microdomain of Ca^{2+} might be affected by ORP5/8 overexpression or silencing. We found that ORP8 overexpression slightly but significantly increased the histamine-induced Ca^{2+} concentration at the caveolae ($[Ca^{2+}]_{cav}$) in the presence of extracellular Ca^{2+} (Fig. 3A) This effect was more pronounced in the absence of extracellular Ca^{2+} (Fig. 3B). Also ORP5 overexpression significantly augmented the histamine-induced $[Ca^{2+}]_{cav}$ (Fig. 3C). In contrast, knockdown of ORP5/8 did not affect $[Ca^{2+}]_{cav}$ (Fig. 3D).

Caveolae may harbour molecules that are involved in the regulation of the store-operated Ca^{2+} entry (SOCE) [18]. Hence, we investigated whether ORP5/8 would affect $[Ca^{2+}]_{cav}$ during thapsigargin (TG)-induced SOCE. We found that overexpression of ORP5 or −8 was without an effect on SOCE at the caveolar microdomain (Fig. 4A&B). Interestingly, cytoplasmic Ca^{2+} was slightly but significantly inhibited by ORP5 overexpression during TG-induced SOCE (Fig. 4C). Further, ORP5 overexpression inhibited the cytosolic Ca^{2+} flux in an experimental setting where Ca^{2+} was present in the extracellular milieu during the TG treatment (Supplementary Fig. 1B).

Even though we could not detect an effect of ORP5 or −8 on the agonist-induced cytoplasmic Ca^{2+}, we found it interesting to test whether the overexpression of these proteins might modify the release of IP_3 by PLCs. Therefore, the cellular IP_3 concentration was measured upon histamine stimulation. We found that ORP5 overexpression significantly augments IP_3 production whereas ORP8 overexpression was without an effect (Fig. 5).

ORP proteins have been implicated in the control of cell proliferation. It has been shown that ORP4 silencing halts cell proliferation and that ORP4L modulates proliferation through Ca^{2+} and the nuclear factor of activated T cells (NFAT) pathway [27,28]. Also, it is well established that intracellular Ca^{2+} plays key roles in controlling cell proliferation [10]. Hence, we used the ^{3}H-thymidine incorporation assay to quantify the proliferation of GFP, GFP-ORP5 or GFP-ORP8 transfected HeLa cells. Interestingly, we found that the ORP5/8 overexpressing cells showed a significant increase in proliferation as compared to the GFP-transfected controls (Fig. 6).

4. Discussion

In this study we present evidence that elevated cellular levels of the OSBP-related proteins ORP5 or −8 modify Ca^{2+} homeostasis in an organelle-specific fashion. Experiments with caveolae- or mitochondria-targeted aequorin Ca^{2+} probes demonstrated that while neither ORP5 nor −8 overexpression had a significant effect on the cytoplasmic Ca^{2+} concentration in histamine-stimulated cells, ORP5 and −8 elevated the Ca^{2+} concentrations both in the mitochondrial matrix as well as at the caveolar sub-compartments of the PM. The effect of ORP5 on mitochondrial Ca^{2+} was more pronounced than that of ORP8, the effect of which was only detectable when extracellular Ca^{2+} was chelated. Modulation of mitochondrial calcium by ORP5/8 is consistent with the reported disturbance of mitochondrial respiratory function observed in ORP5/8 depleted cells [8], considering that mitochondrial calcium, as well as the proximity of mitochondria to the intracellular Ca^{2+} stores, are crucial for the activity of key mitochondrial machineries responsible for oxidative energy production [21,29,30].

In contrast to what has been reported for ORP4L in T-cell acute lymphoblastic leukemia cells [31], we detected no significant changes in the cytoplasmic Ca^{2+} levels during histamine-stimulation in cells overexpressing ORP5/8. This finding underlines the role of ORP5/8 as regulators and coordinators of local Ca^{2+} signaling events specifically at the MCS at the ER-mitochondria and at the ER-PM/caveolae interfaces. Previously, ORP4L has been reported to augment IP_3 production in T-cells [31]. In our study, we found that ORP5 overexpression leads to increased cellular IP_3 levels during histamine stimulation, whereas ORP8 overexpression did not affect the histamine-induced IP_3 generation. Hence, ORP5 may modulate the detected local Ca^{2+} signaling events in part through IP_3-dependent mechanisms whereas the effect of ORP8 is attributed to other factors. We find it possible that ORP5 overexpression augments mitochondrial Ca^{2+} uptake in part through increased apposition of ER and mitochondria, and that the resulting increase in mitochondrial Ca^{2+} buffering capacity masks the cytoplasmic Ca^{2+} effects that would otherwise be expected due to the ORP5-mediated increase in the IP_3 production. Further, we speculate that ORP5-mediated interactions at the ER-caveolae MCS may allow for the generation of local caveolar Ca^{2+} hotspots at these sites even in the presence of high mitochondrial Ca^{2+} buffering.

As noted above, the effect of ORP8 overexpression on mitochondrial

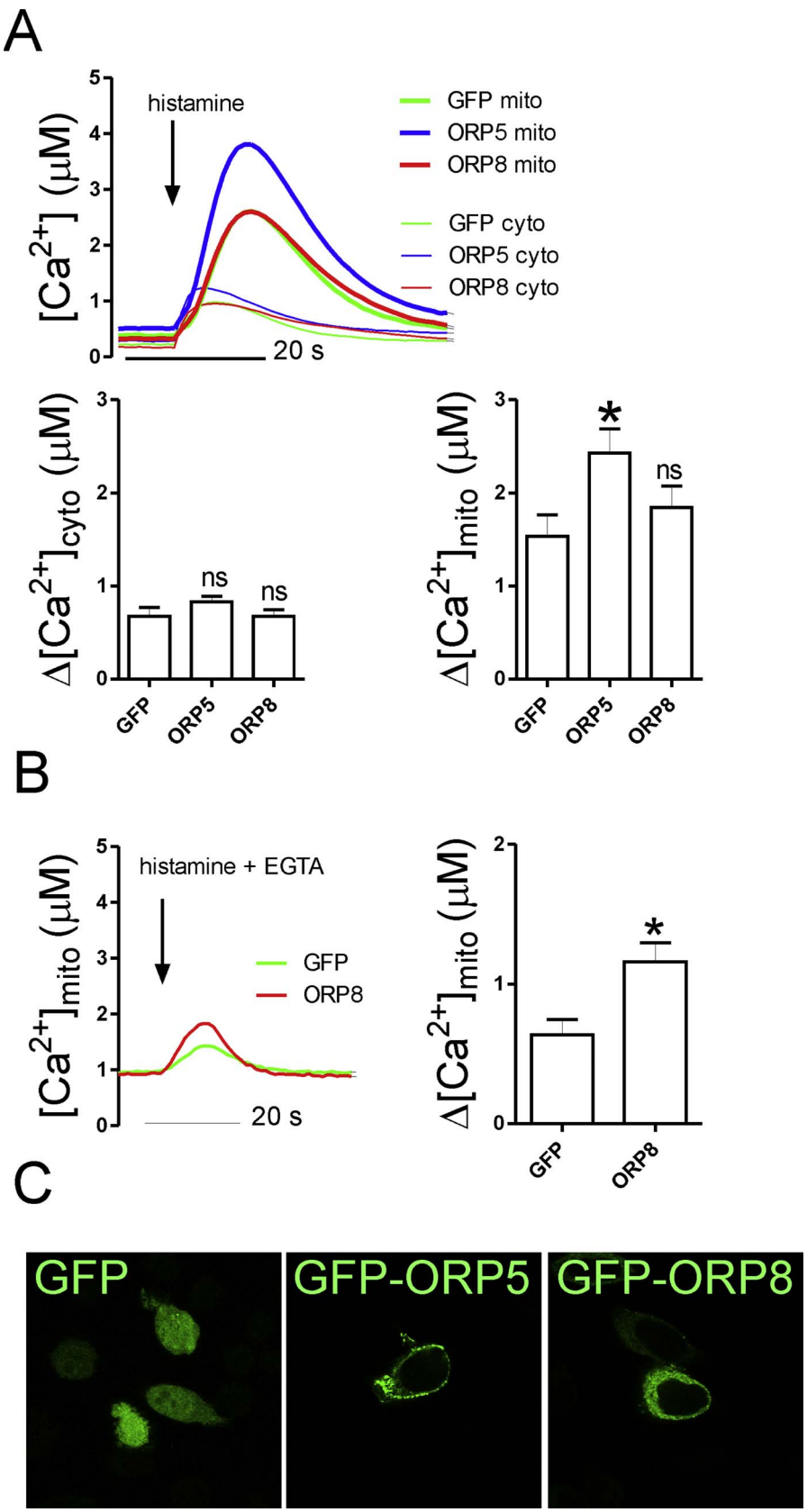

Fig. 1. Mitochondrial, but not cytosolic, $[Ca^{2+}]$ is increased by ORP5/8 overexpression upon 100 μM histamine stimulation. A) Representative traces and quantification of cytoplasmic and mitochondrial Ca^{2+} fluxes during histamine stimulation in the presence of 1 mM Ca^{2+}. B) Mitochondrial Ca^{2+} flux during histamine stimulation in the presence of 150 μM EGTA. C) Confocal microscopy images showing HeLa cells expressing GFP, GFP-ORP5 and GFP-ORP8 constructs, respectively. The bars represent the mean ± SEM of the change in the calcium concentration during the experiment (maximal value – basal value). *, $p < 0.05$; ns = not significant. N = 3–6.

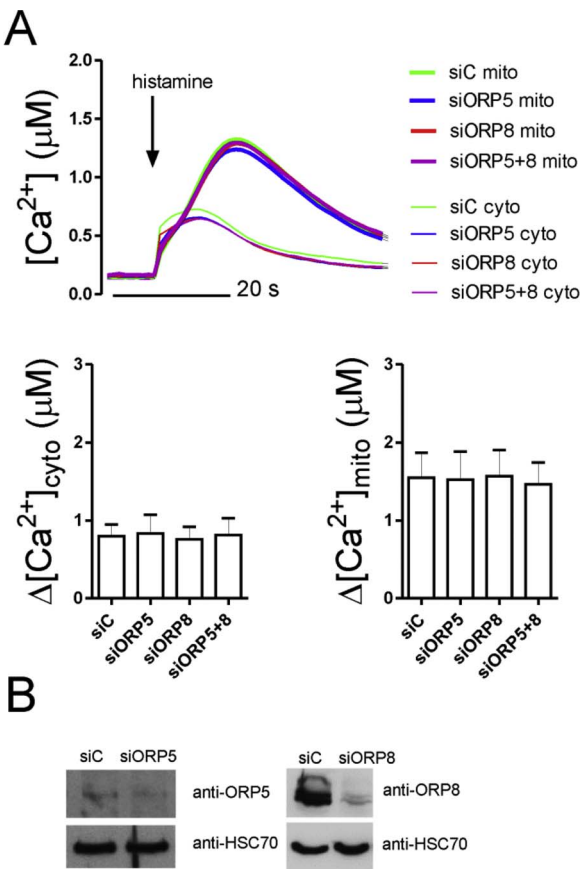

Fig. 2. Downregulation of ORP5/8 does not affect mitochondrial calcium upon 100 µM histamine treatment. A) HeLa cells transfected with control siRNA or siRNAs targeting ORP5, ORP8 or ORP5 and −8 were stimulated with 100 µM histamine in the presence of 1 mM Ca^{2+}. The bars represent the mean ± SEM of the change in the calcium concentration during the experiment (maximal value – basal value). N = 3. B) Representative western blot images showing the effect of ORP5 (left panel) and ORP8 (right panel) targeting siRNAs as compared to the control siRNAs (siC).

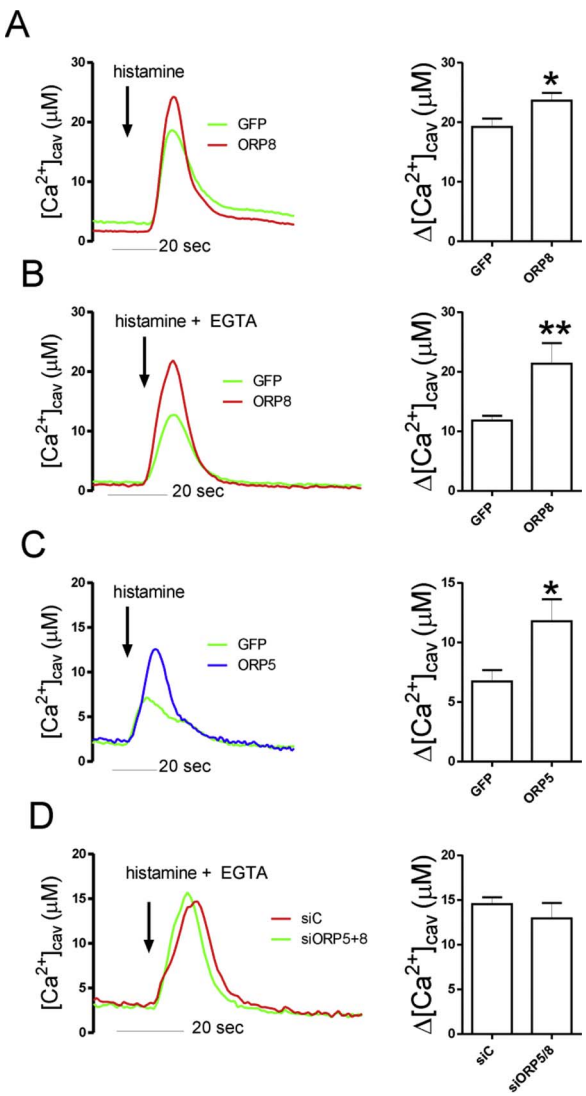

Fig. 3. Histamine-induced Ca^{2+} flux at the caveolar micro-domain is augmented by ORP5/8 overexpression whereas knock-down of ORP5/8 was without an effect. A) ORP8 overexpression increases $[Ca^{2+}]_{cav}$ during 100 µM histamine-stimulation in the presence of 1 mM Ca^{2+}. B) The effect of ORP8 overexpression on $[Ca^{2+}]_{cav}$ is more pronounced in the presence of 150 µM EGTA. C) ORP5 overexpression augments $[Ca^{2+}]_{cav}$ during histamine-stimulation in the presence of 1 mM Ca^{2+}. D) Simultaneous knock-down of ORP5 and −8 is without an effect on $[Ca^{2+}]_{cav}$ during histamine-stimulation in the presence of 150 µM EGTA. The traces are representative and the bars represent the mean ± SEM of the change in the calcium concentration during the experiment (maximal value – basal value). *, $p < 0.05$; **, $p < 0.01$. N = 3-8.

calcium was only detectable in the absence of extracellular Ca^{2+}. This may be explained by the previous finding that ORP8 is more evenly distributed throughout the ER membranes whereas ORP5 is more specifically localized to the MAM MCS [4,8]. Thus, the effect of ORP8 overexpression on mitochondrial Ca^{2+} uptake was unmasked when the extracellular Ca^{2+} was chelated and the IP_3-releaseable ER Ca^{2+} was the primary source of Ca^{2+}. The data suggests that ORP5/8, and ORP5 in particular, has the ability to organize and expand an ER-mitochondrial membrane contact domain at which Ca^{2+} transport is active. The physical association of ORP5/8 with PTPIP51 is consistent with this notion, considering that the mitochondrial outer membrane protein PTPIP51 in a complex with the ER protein VAPB is also reported to control the ER-mitochondria associations [8,32]. Further, ORP5/8 are involved in the modulation of mitochondrial morphology and the integrity of the mitochondrial network, key aspects which are known to affect the regulation of mitochondrial Ca^{2+} [8,33].

It has been reported that ER and PM can form junctions at the caveolae [34]. Also, the interaction of IP_3R1 and −3 with the Ca^{2+}-conducting transient receptor potential canonical cation channels 1 and 3 (TRPC1, −3), respectively, is coordinated at the caveolae [35,36]. Further, caveolae are considered as important signaling hubs that control a wide range of cellular events such as migration, adhesion and invasion, and these processes are modulated by Ca^{2+} signals [18,34]. Our results showing that ORP5/8 modulate Ca^{2+} at the caveolae may thus in part offer new mechanistic insight to the previously reported ORP5/8-mediated regulation of invasion and migration [3,37].

We did not observe disturbances in mitochondrial or caveolar calcium upon siRNA-mediated knock-down of ORP5, −8 or both. This is consistent with the previous observation that inter-organelle MCSs rarely depend on a single tethering component. For example, in yeast the abolishment of the ER-PM contact sites required the genetic disruption of six MCS components [38]. We also find it possible that knocking down the ORPs involved in the vital MCS calcium transport processes will lead to compensatory responses in order to maintain organelle Ca^{2+} homeostasis, which may lead to a dampening of the effect of ORP5/8 knock-down on mitochondrial or caveolar Ca^{2+}. Also, the partial knock-down of ORP5/8 proteins achieved by siRNA treatment may not be sufficient to induce significant effects in the robust Ca^{2+} fluxes that were studied. Further, we found that ORP5/8 overexpression did not affect SOCE at the caveolar microdomain of the plasma membrane. However, cytoplasmic Ca^{2+} was slightly reduced in ORP5 overexpressing cells during TG-induced SOCE. Interestingly,SOCE proteins STIM1 and Orai1 are translocated from the PIP_2-

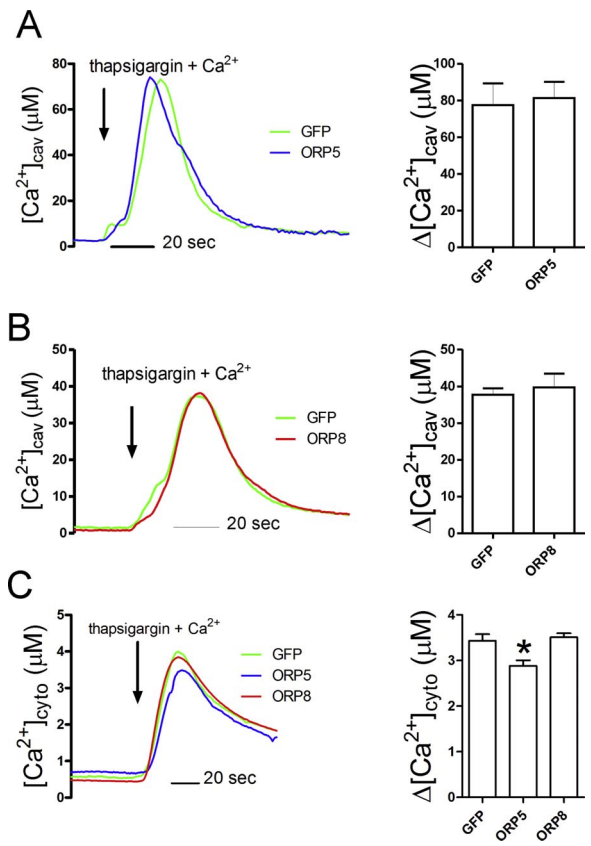

Fig. 4. Store-operated calcium entry at the caveolar sub-compartment is not affected by ORP5 (A) or ORP8 (B) overexpression whereas cytoplasmic Ca^{2+} is reduced by ORP5 overexpression (C). The cells were treated for 5 min with 1 μM TG in HBSS buffer containing 150 μM EGTA and then perfused, as indicated by the arrows, with HBSS buffer containing 1 μM TG and 1 mM Ca^{2+} to induce SOCE. The traces are representative and the bars represent the mean ± SEM of the change in the calcium concentration during the experiment (maximal value – basal value). N = 4–8, *P < 0.05.

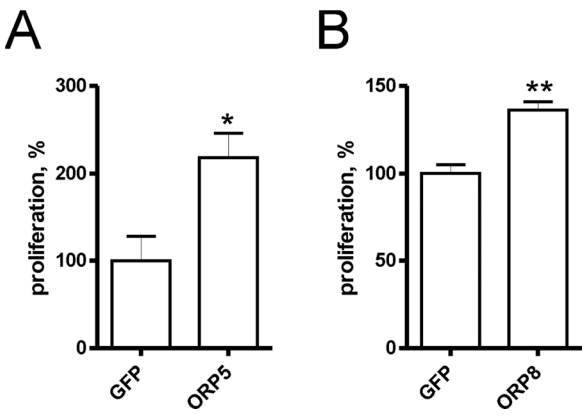

Fig. 6. Overexpression of ORP5 (A) or ORP8 (B) increases cell proliferation. The proliferation of Hela cells transfected for 48 h with plain GFP (control) or the GFP-ORP constructs was determined by ^{3}H-thymidine incorporation during 4 h. The bars represent the mean ± SEM of 3–4 experiments. *p < 0.05, **p < 0.01.

rich PM domains to the PIP_2-poor PM domains upon ER Ca^{2+} depletion, whereas ORP5/8, and especially ORP5, interact with PIP_2 at the PM [5,7,39]. Intriguingly, we find that ORP5 augments histamine-induced Ca^{2+} fluxes whereas cytoplasmic Ca^{2+} during SOCE is reduced upon ORP5 overexpression. These results are not contradictory as histamine-induced Ca^{2+} fluxes and SOCE recruit different proteins and signaling pathways, even if these pathways are interconnected (e.g. SOCE following the IP_3-mediated Ca^{2+} release). Hence, there are kinetic differences in the activation of the histamine-induced Ca^{2+} fluxes and SOCE, SOCE being activated through STIM and ORAI oligomerization in response to the IP_3-mediated ER emptying [40,41].

We also found that ORP5/8 overexpression increases the proliferation of HeLa cells. This is in line with the similar findings regarding ORP4L [28]. Further, ORP5 overexpression is associated with tumor cell invasion [3] and proliferation (unpublished observations disclosed in ref. [3] and published during the revision of the present article [42]). In contrast to our finding, ORP8 has been linked to inhibition of cell growth in gastric cancer cells through induction of ER stress and to initiation of apoptosis in hepatocellular cancer cells through the Fas-pathway [43,44]. Interestingly, Ca^{2+} is essential for the initiation of Fas-mediated apoptosis [45], and ER stress, as well as the regulation of apoptotic events are associated with ER-mitochondria Ca^{2+} transfer [46]. On the other hand, Ca^{2+} handling at the ER-mitochondria interfaces is involved in the regulation of energy metabolism and cell proliferation [47]. Therefore, we find it possible that ORP8-mediated changes in mitochondrial Ca^{2+} homeostasis may modulate cell fate in a context-dependent manner.

In conclusion, our results provide evidence for the importance of ORP5/8 in regulating Ca^{2+} in the mitochondrial matrix as well as at the caveolar sub-compartments of the PM. These findings corroborate the role of ORP5/8 as MCS proteins facilitating organelle interactions at specific membrane interfaces.

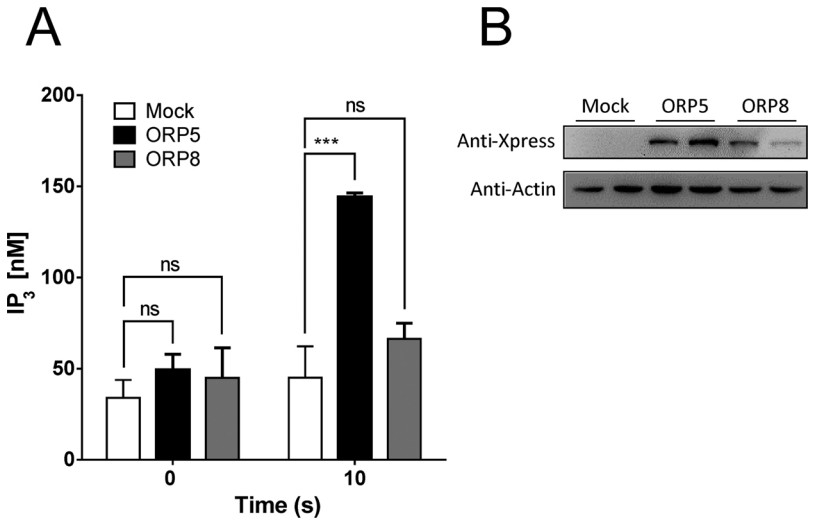

Fig. 5. Overexpression of ORP5 increases cellular IP_3 levels during histamine stimulation whereas ORP8 overexpression is without an effect. The IP_3 concentration was measured from unstimulated cells (time point 0), and at 10 s after histamine stimulation (A). The expression of epitope-tagged recombinant ORP5 and ORP8 was confirmed by western blot using anti-Xpress epitope antibodies (B). ***P < 0.001, N = 3.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ceca.2018.03.001.

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