ISCI, Volume *17*

Supplemental Information

MORC3 Forms Nuclear Condensates through Phase Separation

Yi Zhang, Bianca Bertulat, Adam H. Tencer, Xiaojun Ren, Gregory M. Wright, Joshua Black, M. Cristina Cardoso, and Tatiana G. Kutateladze

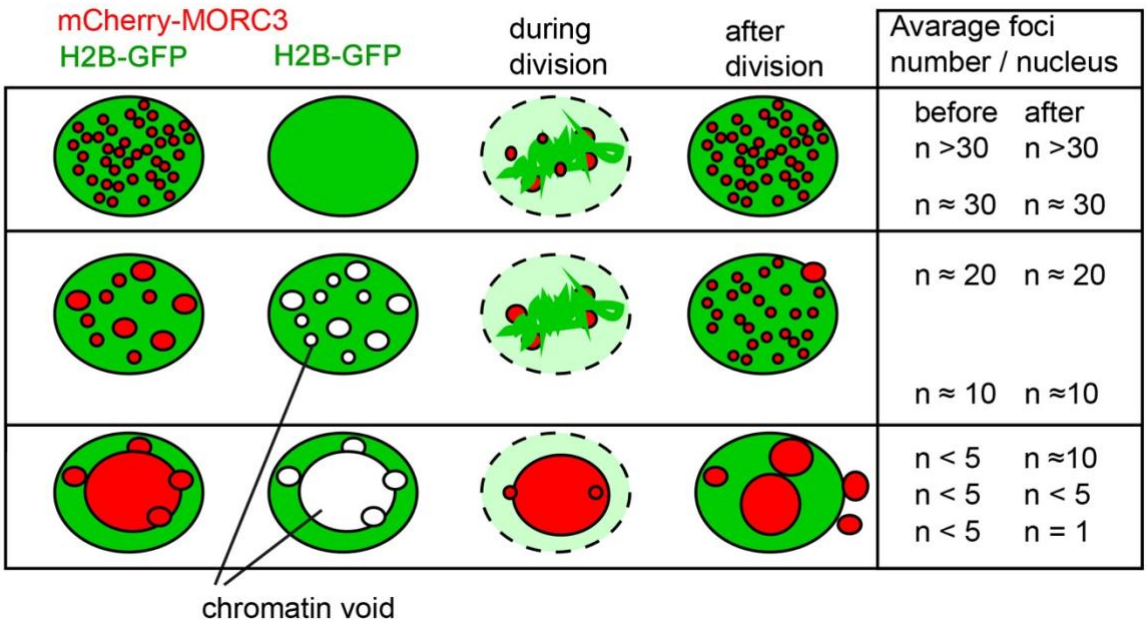

Figure S1. MORC3-NBs show distinct foci pattern in cells, Related to Figures 1 and 2. In over 8 independent transient transfection experiments, the depicted foci patterns were observed regularly in similar proportions. The vast majority of cells demonstrated more than 30 small foci per nucleus, followed by a large fraction of cells with medium and small foci and a minority of cells with less than 5 and 1-2 very large foci. Live cell DNA counterstaining experiments revealed an exclusion of DNA/chromatin and MORC3 signals in medium and large foci.

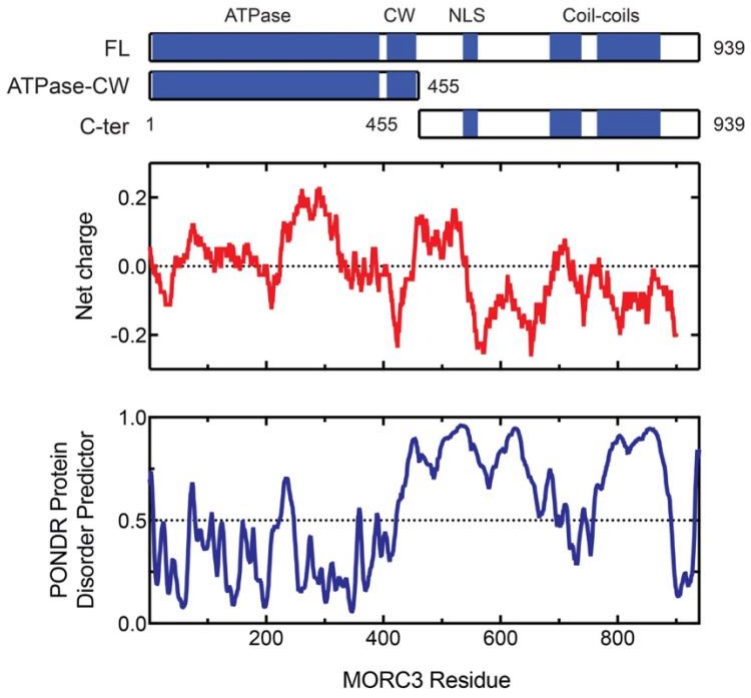

Figure S2. Domain structure, net charge, and disorder tendency of human MORC3 protein, Related to Figures 3 and 4. The net charge was calculated and plotted using a sliding window of 40 amino acids by EMBOSS. The disorder tendency was predicted using PONDR software. A score greater than 0.5 indicates intrinsically disordered regions.

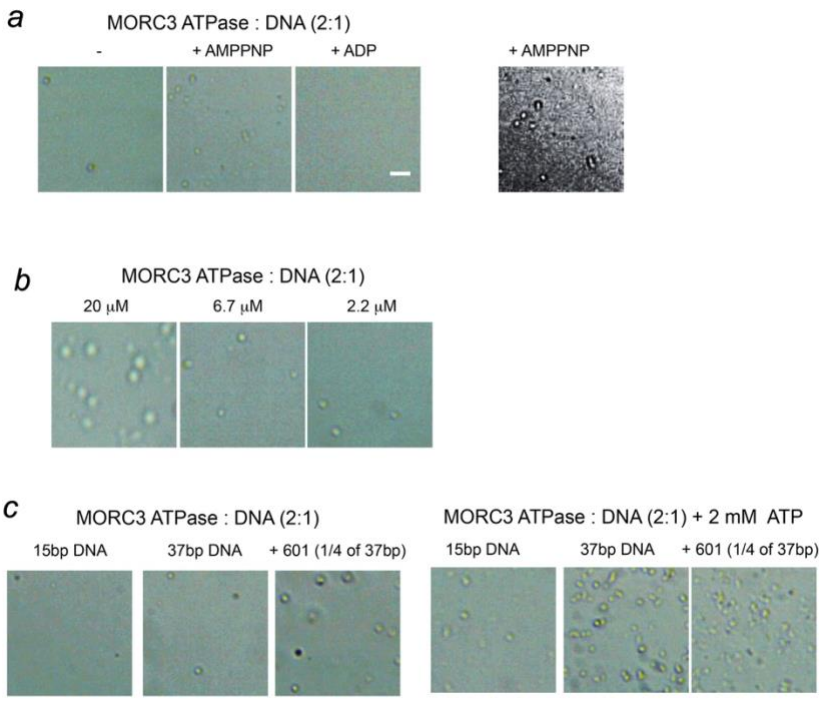

Figure S3. Representative images of MORC3-ATPase condensates, Related to Figure 3.

(a) Representative images of samples containing 6.7 μM ATPase protein and 3.3 μM 37 bp dsDNA in the absence and presence of 2 mM AMPPNP and ADP. Scale bar, 10 μm. A representative image processed by Photoshop for counting droplets is shown on the right. (b) Representative images acquired for indicated MORC3-ATPase concentrations with a fixed protein:DNA ratio of 2:1. (c) Representative images of samples containing 13.3 μM ATPase protein and 6.7 μM 15 bp dsDNA, 6.7 μM 37 bp dsDNA and 1.4 μM 601 DNA without or with 2 mM ATP.

TRANSPARENT METHODS

Cells, DNA Constructs and Transfection

Live cell experiments were performed with HeLa-H2B-GFP cells (Kanda et al., 1998), as previously described (Martin and Cardoso, 2010). Cells were cultivated using standard cell culture conditions (37 °C, 5% CO_2, humidified atmosphere) in D-MEM (Sigma-Aldrich) supplemented with 10% v/v FCS (Gibco), 2 mM glutamine (Sigma-Aldrich) and 50 µg/µl gentamycin (Sigma-Aldrich). If not stated otherwise, transient transfections with N-terminal mCherry-fused *MORC3* were performed with the Neon Transfection System (Invitrogen). The mCherry-MORC3 plasmid was generated using previously reported FLAG-tagged MORC3 plasmid by substituting the FLAG tag sequence with mCherry coding sequence through double digestion and T4 ligation (Andrews et al., 2016). HeLa-H2B-GFP cells were cultured in 10 cm petri dishes to 80% confluence, trypsin treated and harvested in PBS (centrifugation at 300 xg for 5 min). The resulting pellets were suspended according to manufacturer's instructions and transfected using 2-10 µg plasmid DNA and a 100 µl Neon tip. After electroporation, 100 µl were equally distributed to 3.5 mm glass bottom Ibidi dishes. Cells were allowed to recover at standard cell culture conditions (37 °C, 5% CO, humidified) overnight. The following day media was exchanged, and transfection efficiency was estimated by fluorescent light microscopy.

Image Acquisition and Analysis

Live cell time-lapse microscopy was performed using an UltraView VoX spinning disk system mounted on an inverted Nikon Ti-E microscope. Z-stack images were taken with a 60x plan apochromat NA 1.45 objective (Nikon; Tokyo, Japan) and a cooled 14-bit EMCCD camera (C9100-50, Hamamatsu Photonics K.K.; Hamamatsu City, Japan) yielding a voxel size of xyz 0.123 µm x 0.122 µm x 0.5 µm. If not stated otherwise, time lapse movies were generated with at a time interval of 10 min. Images and movies were analyzed and processed with the Perkin Elmer software "Volocity 6.3".

For imaging over time, individual nuclei were separated by cropping to a user defined region of interest. Quality parameters for nuclei selection were a completely covered nucleus in z and cell viability (checked in transmission light by general morphologic appearance) throughout the acquisition time. Within the selected regions of interest (ROIs), interphase nuclei were first segmented using H2B-GFP signals and potential holes inside the nucleus were filled to generate a solid chromatin volume object. MORC3-mCherry foci were identified and segmented either automatically or by intensity and assigned to tracked nuclei. The resulting measurement routine yielded MORC3 foci numbers per time point. All generated values were subsequently exported for processing in excel (Microsoft) and Prism 7 (GraphPad). For comparison of MORC3 foci numbers, first movie frames were assigned to landmarks such as metaphase entry and telophase and the respective MORC3 foci values were analyzed using Excel and Prism 7.

Fluorescence recovery after photobleaching (FRAP) experiments

WT mCherry-MORC3 plasmid were transfected into 2 million HeLa cells in 10-cm-diameter tissue culture dish by Lipofectamine 3000 (Life Technology, L3000-075) using manufacturer instructions. The next day after transfection, cells were trypsinized, harvested, seeded to a 35 mm gelatin-coated coverglass-bottom dish and cultured overnight. Cell culture medium was replaced with the live-cell imaging medium and maintained at 37 °C using a heater controller. FRAP imaging was performed using a Zeiss LSM 700 Observer as described previously (Tatavosian et al., 2019). Briefly, two images were taken before photobleaching and 20 images were taken with 10s intervals immediately after photobleaching. The images were analyzed using ImageJ. Fluorescence intensities were normalized to the signal before photobleaching to obtain the fluorescence recovery.

Protein expression and purification

The human MORC3 ATPase (aa 1-392) and ATPase-CW (aa 1-455) proteins were expressed and purified as described previously (Zhang, 2019). The C-terminus of MORC3 (aa 455-939) was cloned into a pDEST-17 vector and expressed and purified as the ATPase domain.

In vitro condensate formation

All in vitro condensate formation assays were performed in a buffer containing 20 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1 mM $MgSO_4$ and 1 mM DTT. All samples were prepared on ice and incubated for approximately 5 min before imaging on siliconized glass cover slides (Hampton). For MORC3 ATPase-CW, 20 μM protein samples were parallelly prepared, with or without 5 μM 37bp dsDNA, 200 μM histone H3K4me0 (1-12) or H3K4me3 (1-12) peptide and 10 μM reconstituted unmodified nucleosome. For MORC3 ATPase, unless otherwise stated, samples were prepared with or without 37 bp dsDNA at a protein:DNA ratio of 2:1 (see also figure 3 legends). Microscopy of the droplets was done using an M150C-I microscope (AmScope) equipped with a 10× objective and a MD35 digital camera (AmScope). A microscope camera calibration slide (OMAX, 0.01mm) was used to determine the scale. The number of condensates was counted in a 50 μm × 50 μm square area. Six non-overlapping square regions were counted for each sample and plotted with Prism 7.

To prepare MORC3 condensates that concentrate DNA, 13.3 μM ATPase or 20 μM ATPase-CW was mixed with 6.7 μM fluorescein (FAM)-labeled 37 bp dsDNA. For ATPase-CW, one sample was supplemented with 200 μM histone H3K4me3 (1-12) peptide. Confocal images were acquired on a Zeiss Observer.Z1 inverted microscope using a 40× oil objective and digitally captured. For the excitation and emission of FAM, a 488 nm laser was used. Images were processed and presented using ImageJ and Photoshop.

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