# **Molecular Cell**

## The Gene-Silencing Protein MORC-1 Topologically Entraps DNA and Forms Multimeric Assemblies to Cause DNA Compaction

## **Graphical Abstract**



## Authors

HyeongJun Kim, Linda Yen, Somsakul P. Wongpalee, ..., John K. Kim, Joseph J. Loparo, Steve E. Jacobsen

## Correspondence

joseph\_loparo@hms.harvard.edu (J.J.L.), jacobsen@ucla.edu (S.E.J.)

## In Brief

MORC proteins in plants and animals regulate the compaction of chromatin, but the molecular mechanism is unknown. Kim et al. demonstrate that the *Caenorhabditis elegans* MORC-1 dimers can directly compact DNA by topologically entrapping DNA loops. Multiple MORC-1 dimers can also interact to form discrete foci of compacted DNA.

## Highlights

- Caenorhabditis elegans MORC-1 traps DNA loops
- Recruitment of additional MORC-1s cause further loop trapping and DNA compaction
- MORC-1 assemblages become topologically entrapped on DNA
- MORC-1 forms discrete foci *in vivo* and can phase transition *in vitro*





## The Gene-Silencing Protein MORC-1 Topologically Entraps DNA and Forms Multimeric Assemblies to Cause DNA Compaction

HyeongJun Kim,<sup>1,2,7,8</sup> Linda Yen,<sup>3,8</sup> Somsakul P. Wongpalee,<sup>3,4</sup> Jessica A. Kirshner,<sup>5</sup> Nicita Mehta,<sup>5</sup> Yan Xue,<sup>3</sup> Jonathan B. Johnston,<sup>6,7</sup> Alma L. Burlingame,<sup>6</sup> John K. Kim,<sup>5</sup> Joseph J. Loparo,<sup>1,\*</sup> and Steve E. Jacobsen<sup>3,7,9,\*</sup> <sup>1</sup>Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA

<sup>3</sup>Department of Molecular, Cell, and Developmental Biology, University of California, Los Angeles, Los Angeles, CA 90095, USA

<sup>4</sup>Department of Microbiology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

<sup>6</sup>Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA 94158, USA

<sup>7</sup>Howard Hughes Medical Institute, University of California, Los Angeles, Los Angeles, CA 90095, USA

<sup>8</sup>These authors contributed equally

<sup>9</sup>Lead Contact

\*Correspondence: joseph\_loparo@hms.harvard.edu (J.J.L.), jacobsen@ucla.edu (S.E.J.) https://doi.org/10.1016/j.molcel.2019.07.032

## **SUMMARY**

Microrchidia (MORC) ATPases are critical for gene silencing and chromatin compaction in multiple eukaryotic systems, but the mechanisms by which MORC proteins act are poorly understood. Here, we apply a series of biochemical, single-molecule, and cell-based imaging approaches to better understand the function of the Caenorhabditis elegans MORC-1 protein. We find that MORC-1 binds to DNA in a length-dependent but sequence non-specific manner and compacts DNA by forming DNA loops. MORC-1 molecules diffuse along DNA but become static as they grow into foci that are topologically entrapped on DNA. Consistent with the observed MORC-1 multimeric assemblies, MORC-1 forms nuclear puncta in cells and can also form phase-separated droplets in vitro. We also demonstrate that MORC-1 compacts nucleosome templates. These results suggest that MORCs affect genome structure and gene silencing by forming multimeric assemblages to topologically entrap and progressively loop and compact chromatin.

## INTRODUCTION

Microrchidia (MORC) proteins are members of a highly conserved family of GHKL (gyrase, HSP90, histidine kinase, MutL)-type ATPases that are found in prokaryotic and eukaryotic organisms (Dong et al., 2018; lyer et al., 2008). In *Arabidopsis thaliana, MORC1* and *MORC6* are required for silencing DNA methylated genes and for large-scale compaction of pericentromeric heterochromatin regions (Moissiard et al., 2012). In the mouse, MORC1 is involved in compacting and silencing transpo-

sons during male germline development (Pastor et al., 2014). In humans, MORC2 is part of the human silencing hub (HUSH) complex, which is also implicated in transposon silencing (Douse et al., 2018; Tchasovnikarova et al., 2017). In *Caenorhabditis elegans*, MORC-1 is required for transgene silencing and for maintaining the transgenerational silencing of small interfering RNA (siRNA)-targeted genes in the germline, and *morc-1* mutants show visible genome decondensation (Weiser et al., 2017). Despite these genetic and genomic studies demonstrating a role for MORCs in chromatin compaction processes (Douse et al., 2018; Harris et al., 2016; Moissiard et al., 2012, 2014; Tchasovnikarova et al., 2017; Weiser et al., 2017), the molecular mechanisms by which MORCs act to compact the genome are not understood.

MORCs generally share a similar domain arrangement, possessing N-terminal GHKL ATPase and S5 domains and a large unstructured region followed by a C-terminal coiled-coil domain that is thought to be important in homomer or heteromer formation (Douse et al., 2018; lyer et al., 2008; Li et al., 2016; Mimura et al., 2010; Takahashi et al., 2007). The ATPase cassette adopts a prototypical GHKL ATPase (Bergerat) fold and dimerizes upon ATP binding, much like other GHKL family members such as bacterial topoisomerase VIb (Corbett and Berger, 2005; Douse et al., 2018; Dutta and Inouye, 2000; Li et al., 2016). These properties have led to the proposal that MORCs may act as molecular clamps (Li et al., 2016; Mimura et al., 2010); however, very little is known about how MORCs interact with DNA or with chromatin.

Here, we demonstrate that MORCs bind and compact DNA by progressively trapping DNA loops, resulting in large protein assemblies that are topologically trapped on DNA. Compaction *in vitro* does not require ATP, although it is stimulated by the addition of ATP and especially by the addition of a non-hydrolyzable ATP analog. *C. elegans* MORC-1 is also found in nuclear bodies, and *in vitro* will undergo liquid-liquid phase separation. We also demonstrate that MORC-1 can compact DNA that has been assembled into chromatin. These observations provide

<sup>&</sup>lt;sup>2</sup>Department of Physics and Astronomy, University of Texas Rio Grande Valley, Edinburg, TX 78539, USA

<sup>&</sup>lt;sup>5</sup>Department of Biology, Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218, USA



## Figure 1. MORC-1 Is a DNA-Binding Protein

(A) Protein ([MORC-1] = 100, 200, 400, and 800 nM) was incubated with a 32P-labeled 250-bp DNA probe derived from  $\lambda$ -DNA and run on a 3% agarose gel, and then dried before exposure to a phosphor screen.

(B) MORC-1 at 200 nM was added to 0.5-nM 32P-labeled 250-bp  $\lambda$ -DNA that was premixed with cold 250-bp  $\lambda$ -DNA, 250-bp scrambled DNA, or a 50-bp  $\lambda$ -DNA probe (1, 10, 20, and 50 nM) and processed as described above. See also Figure S1.

mechanistic insight into how MORC family members function across eukaryotes to compact genomes and regulate gene silencing.

## RESULTS

## MORC-1 Binds DNA with Little Sequence Preference and Prefers Longer DNAs over Shorter DNAs

To study *C. elegans* MORC-1 in an *in vitro* reconstituted system, we purified bacterially expressed full-length MORC-1, containing both the conserved N-terminal GHKL ATPase domain known to dimerize upon ATP binding and the C-terminal coiled-coil domain thought to be important in multimerization (Figure S1A; Harris et al., 2016; Inoue et al., 1999; Iyer et al., 2008; Li et al., 2016; Mimura et al., 2010; Moissiard et al., 2012, 2014; Tchasovnikar-ova et al., 2017). Native mass spectrometry analysis of the purified protein (Figure S1B) revealed that it was well ordered and folded and that it was predominantly in equilibrium as a monomer or dimer, with minor populations of trimers and tetramers (Figure S1C), consistent with the observation that plant and mammalian MORCs exist as homomers or heteromers *in vivo* (Harris et al., 2016; Mimura et al., 2010; Moissiard et al., 2012, 2014).

Arabidopsis thaliana MORC1 was shown to bind DNA (Kang et al., 2012); we therefore used gel shift assays to test the DNA-binding activity of full-length C. elegans MORC-1. MORC-1 bound radiolabeled 250 bp double-stranded DNA (Figure 1A). Cold competitor DNA of the same sequence or a 250-bp DNA with a different sequence competed equally for binding to the labeled probe, suggesting that MORC-1 has little sequence specificity (Figure 1B). However, a 50-bp DNA derived from the original 250 bp sequence was unable to efficiently compete for binding, suggesting that MORC-1 prefers longer DNAs over shorter DNAs (Figure 1B). To confirm this observation, we performed gel shift assays with a DNA ladder ranging from 100 to 1,500 bp. At lower MORC-1 concentrations (<400 nM), only longer DNAs (sizes >1,000 bp) were shifted, but at higher concentrations (>800 nM) MORC-1 could also shift shorter DNAs (Figure S1D). Therefore, MORC-1 is capable of binding both long and short DNAs but exhibits a preference for longer DNAs under conditions in which MORC-1 is limiting. In addition, the observation that MORC-1 could bind ladder DNAs derived from multiple plasmids of varying DNA sequences again suggests that MORC-1 likely has little sequence preference, although we cannot rule out that MORC-1 may prefer particular DNA sequences not tested here.

## **MORC-1 Robustly Compacts DNA**

C. elegans morc-1 mutants exhibit X chromosome decondensation (Weiser et al., 2017). MORC mutants in other species also exhibit chromosome decompaction phenotypes (Moissiard et al., 2012). To directly test whether MORC-1 may be able to compact DNA, we imaged flow-stretched DNAs tethered to a functionalized coverslip via a 3' biotin neutravidin linkage and labeled at the 5' end with a guantum dot (Figure 2A; Graham et al., 2014; Kim and Loparo, 2016). The addition of MORC-1 into the flow cell induced DNA compaction, and the rate of compaction increased linearly with the MORC-1 concentration (Figures 2B and 2C). Compaction occurred in 150 mM NaCl but did not occur at NaCl concentrations >300 mM (Figure S2A). Compaction was largely reversible at a high salt concentration (500 mM NaCl) (Figure S2B), suggesting that the observed DNA compaction was not the result of non-specific protein aggregation.

MORCs are ATPases, and we therefore assayed the effect of adding ATP to the compaction assays. While the addition of ATP was not strictly required for compaction, the addition of ATP stimulated the rate of compaction by ~30% (Figures 2C, S2C, and S2D; Data S1). Moreover, the non-hydrolyzable ATP analog adenylyl-imidodiphosphate (AMP-PNP) further stimulated DNA compaction by ~2-fold relative to ATP (Figure 2D). ATP binding is known to induce GHKL head dimerization (Corbett and Berger, 2005; Douse et al., 2018; Li et al., 2016), and thus this observation suggests that it is ATP binding, rather than hydrolysis, that enables MORC-1 to most efficiently compact DNA.

## MORC-1 Uses a Loop-Trapping Mechanism to Compact DNA

We used a DNA motion capture assay to analyze the mechanism of MORC-1-mediated DNA compaction by following how segments along the DNA length were condensed (Graham et al., 2014; Kim and Loparo, 2016).  $\lambda$ -DNA was labeled at its five



## Figure 2. MORC-1 Compacts DNA Using a Loop-Trapping Mechanism

(A) A schematic of the flow-stretched DNA assay.

(B) A representative kymograph demonstrating compaction of a quantum dot (QDot)-labeled  $\lambda$ -DNA over time. Scale bars represent 10 s and 5  $\mu$ m. (C) Effect of ATP on MORC-1 compaction. Data were calculated from 48 different experiments, with a total of 1,199 different trajectories. n = 85, 48, 98, 122, 66, 84, 57, and 97 (without ATP), and n = 66, 94, 79, 52, 61, 76, 47, and 67 (with 2 mM ATP). Error bars: SE.

(D) AMP-PNP further stimulates DNA compaction. Compaction rates were from two independent experiments for each condition. Error bars represent the SE; n = 45, 46, and 56 for experiments with no nucleotide, 2 mM ATP, and 2 mM AMP-PNP, respectively.

(E) Left: schematic of DNA motion capture assay depicting the location of the five EcoRI-binding sites. Middle: plotted trajectories of the location of EcoRI-E111Q conjugated quantum dots over time. Bottom: representative kymograph. [MORC-1] = 40 nM, [ATP] = 1 mM.

See also Figures S2 and S3 and Video S1.

EcoRI-binding sites by quantum dot-labeled catalytically inactive EcoRI (EcoRI<sup>E111Q</sup>) (Figure 2E). Compaction initiated from the free end of the DNA and moved sequentially down to the tether point (Figure 2E), which is consistent with either a DNA loop-trapping mechanism, as demonstrated for bacterial Spo0J (ParB), or with a loop-extrusion mechanism, as shown for yeast condensin (Ganji et al., 2018; Graham et al., 2014). Proteins that compact DNA using either loop-forming mechanism are sensitive to the force applied to DNA by flow, and therefore preferentially initiate compaction from the free end of the DNA, which experiences substantially lower drag force compared to the DNA segments closer to the tether point (Graham et al., 2014; Kim and Loparo, 2016).

To directly test whether MORC-1 extrudes DNA loops, we tethered both ends of  $\lambda$ -DNA, allowing for slack in the DNA, and then flowed MORC-1 and ATP into the flow cell orthogonal to the direction of DNA tethering. DNA was imaged using the intercalating DNA stain SYTOX Orange. DNA compaction events occurred on both single- and double-tethered DNA in the same field of view. Single-tethered DNA was compacted down to the tether point, while double-tethered DNA became taut over time, showing that MORC-1 could compact both single- and



## Figure 3. MORC-1 Forms Bodies that Grow in Size with Concentration

(A) A representative kymograph showing Cy3 labeled MORC-1 cluster formation.

(B) At varying MORC-1 concentrations, foci intensities were quantified at a fixed time point (140 s after flowing in sample) and presented as boxplots. The number of clusters was n = 21, 34, 55, and 34for [MORC-1] = 2, 5, 10, and 40 nM, respectively. The red line inside each box corresponds to the median value, while the bottom and top edges of each box correspond to the 25th and 75th percentiles, respectively. A black dotted line connecting nearby median values was added for visual guidance and showed a strong linear correlation (Pearson correlation coefficient: r = 0.99996). See also Figure S4 and Videos S2 and S3.

double-tethered DNAs. In contrast to what was previously shown for condensin that acts via a loop-extrusion mechanism (Ganji et al., 2018), we did not see evidence for extruded DNA loops on MORC-1 compacted double-tethered DNA (Figure S3; Video S1). The absence of extruded loops implies that MORC-1 uses a loop-trapping mechanism rather than a loop-extrusion mechanism to condense DNA. This interpretation is also consistent with the observation that MORC-1 can compact DNA without the addition of ATP, since loop extrusion is an active ATP-requiring process driven by directional motor activity (Ganji et al., 2018).

## **MORC-1 Forms Discrete Foci as It Compacts DNA**

To investigate MORC-1 behavior as it compacts DNA, we nonspecifically labeled MORC-1 with Cy3, at an average of ~1 Cy3 per MORC-1 dimer. Fluorophore labeling did not perturb the ability of MORC-1 to compact DNA (Figure 3A). At concentrations <2 nM, where the majority of DNA-bound proteins were sinale MORC-1 dimers, we observed MORC-1 diffusing along DNA (Figure S4A). However, DNA compaction was not observed. Addition of higher concentrations of labeled MORC-1 (>10 nM) to the flow cell resulted in progressive DNA compaction down to the tether point, along with the formation of static MORC-1 foci on the DNA that grew brighter with time (Figures 3A and S4B; Video S2). In addition, the number of foci per substrate increased as a function of MORC-1 concentration (Figure S4C). We frequently observed collision events between mobile and static MORC-1 proteins, suggesting that the growth of static foci was in part due to the addition of DNA-bound MORC-1 proteins (Video S3). However, the relatively fast growth of MORC-1 foci suggests that more MORC-1 proteins were likely also added to the growing foci from the population in solution. As DNA compaction proceeded, static MORC-1 foci came into close proximity and merged into larger foci (Figure 3A; Video S2). There was a linear relation between the concentration of MORC-1 and the average number of MORC-1 molecules in each body, as estimated by Cy3 fluorescence (Pearson correlation coefficient >0.99) (Figures 3B and S4B-S4D). These results mirror the linear relation between MORC-1 concentration and DNA compaction rate and are consistent with a model in which MORC-1 molecules added to existing foci contribute to further DNA compaction. Furthermore, the growth of existing MORC-1 foci suggests that initial MORC-1-binding events seed additional MORC-1 binding in part via protein-protein interactions.

## MORC-1 Forms Discrete Nuclear Foci *In Vivo* and Forms Phase-Separated Droplets *In Vitro*

The MORC-1 foci observed on DNA during compaction are reminiscent of the in vivo nuclear bodies observed with plant MORCs (Harris et al., 2016; Moissiard et al., 2012). C. elegans MORC-1 was previously observed to be localized adjacent to dense areas of heterochromatin (Weiser et al., 2017). We used structured illumination microscopy (SIM) to visualize the nuclear distribution of C-terminal 3xFLAG-tagged MORC-1 expressed under its endogenous promoter in vivo (Weiser et al., 2017). We observed MORC-1 punctate bodies in both mitotically dividing germline stem cells and gonadal nuclei undergoing meiosis (Figure S5A). Consistent with previously published data in adult intestinal nuclei, MORC-1 bodies were mostly adjacent to but not overlapping with the most DAPI-dense areas corresponding to heterochromatin (Figure S5A; Weiser et al., 2017). Imaging these same cell types at a higher resolution using stochastic optical reconstruction microscopy (STORM) (Köhler et al., 2017) showed that in addition to large MORC-1 puncta, a series of smaller bodies could be seen throughout the nucleus (Figure 4A). These data are also consistent with observations that MORCs in multiple species form punctate bodies in vivo (Harris et al., 2016; Mimura et al., 2010; Moissiard et al., 2012). Together with the MORC-1 foci formation on flow-stretched DNAs, these results suggest that MORC-1 acts in part via higher-order oligomerization to cause DNA compaction.

Some proteins found in nuclear bodies such as the heterochromatic protein HP1 have properties of phase-separated condensates (Alberti et al., 2019; Boija et al., 2018; Larson et al., 2017; Sabari et al., 2018; Strom et al., 2017). MORC-1 contains a long predicted intrinsically disordered region (IDR) as determined by IUPred2A, and IDRs are known to be able to mediate phase separation (Figure S1A; Lin et al., 2015; Mészáros et al., 2018). We therefore tested whether purified MORC-1 protein may be able to form phase-separated droplets *in vitro*. Using both confocal and differential interference contrast (DIC) microscopy, we observed MORC-1 phase-separated droplets at 75 mM NaCl in





## Figure 4. MORC-1 Is Localized to Bodies In Vivo and Forms Condensates In Vitro

(A) MORC-1 is found in nuclear bodies of varying sizes *in vivo*. MORC-1 mitotic bodies (n = 96) are on average (mean) 229 nm; MORC-1 pachytene bodies (n = 53) are on average (mean) 170 nm. Scale bar represents 5 μm.

(B) MORC-1 undergoes liquid-liquid phase separation. Unlabeled protein was buffer exchanged into 75 mM NaCl and mixed with labeled protein to achieve a final population of 1%–2% labeling. Single color droplets were pre-formed by adding a final concentration of 10% PEG 3350. To image dual color droplets, pre-formed droplets were mixed together and incubated at room temperature before imaging. To image dual color MORC-1 on DNA, Cy5-MORC-1 was added to Cy3-MORC-1 pre-incubated with DNA. White represents areas of signal overlap. Scale bars represent 5 μm. See also Figure S5.

the presence of 10% polyethylene glycol 3350 (PEG 3350), a crowding agent that can mimic a crowded cellular environment (Lin et al., 2015; Protter et al., 2018; Figure 4B). We then tested for droplet fusion to confirm that these were phase-separated droplets (Lin et al., 2015; Protter et al., 2018). Cy3- or Cy5-labeled MORC-1 droplets were pre-formed in 10% PEG 3350, then mixed and observed to form droplets containing both Cy3 and Cy5 dye (Figure 4B). Furthermore, the addition of  $\lambda$ -DNA to MORC-1 in 75 mM NaCl without any PEG crowding agent caused MORC-1 to coalesce into fiber-like conformations that are also likely representative of a phase-separated state (Figure 4B; Lin et al., 2015). Thus, MORC-1 is able to form phase-separated droplets *in vitro*.

A subset of proteins found in cellular bodies with the properties of phase-transitioned condensates shows dispersion *in vivo* after exposure to 1,6-hexanediol, a solvent that can interfere with weak hydrophobic interactions (Alberti et al., 2019; Boija et al., 2018; Larson et al., 2017; Sabari et al., 2018; Strom et al., 2017). We thus subjected the same mitotic and meiotic germ cells to 5% 1,6-hexanediol treatment and quantified whether the MORC-1 bodies remained punctate or became diffuse upon treatment. We found that the proportion of nuclei containing punctate MORC-1 bodies remained largely unchanged upon 5% 1,6-hexanediol treatment, even though we observed that the peripheral localization of chromatin became disorganized and the ribonucleoprotein bodies that represented P granules became largely dispersed in the presence of 1,6-hexanediol (Updike et al., 2011; Wheeler et al., 2016; Figure S5B). Thus, while the *in vivo* MORC-1 bodies may represent intracellular condensates, these bodies are likely held together by additional complex interactions other than weak hydrophobic interactions (Kroschwald et al., 2015).

## **MORC-1 Topologically Entraps DNA**

The observation that MORC-1 nuclear bodies were largely 1,6hexanediol resistant in vivo suggested that like HP1, these MORC-1 bodies may engage chromatin to produce a more metastable phase-separated state in vivo. The presence of additional scaffolding materials such as nucleic acids can affect the physical properties of nuclear bodies (Kroschwald et al., 2015; Larson and Narlikar, 2018; Shin and Brangwynne, 2017; Strom et al., 2017; Wheeler et al., 2016). In addition, our in vitro phase transition experiments showed that MORC-1 formed granular fibrils upon incubation with  $\lambda$ -DNA (Figure 4B). Thus, a possible explanation for MORC-1 body resistance to 1.6-hexanediol in vivo is that it is stably engaged with DNA (Alberti et al., 2019; Larson and Narlikar, 2018). MORC ATPases have also been proposed to act like other GHKLs that can gate and encircle their substrates, and we therefore sought to test whether MORC-1 could topologically entrap DNA using single-molecule experiments (Corbett and Berger, 2005; Douse et al., 2018; Li et al., 2016; Mimura et al., 2010).

We observed that pre-formed, static Cy3-labeled MORC-1 bodies on flow-stretched DNAs without quantum dots became mobile when subjected to a high salt wash (500 mM NaCl). Rather than slowly disintegrating, they were frequently observed to move to the free end of DNA as a single unit before disengaging from the DNA and traveling in the direction of buffer flow (Video S4). This implies that MORC-1 may be topologically entrapping DNA, and thus needs to slide off the free end to be released. To test this hypothesis, we attached quantum dots to the free end of DNAs to determine whether this blocked the dissociation of MORC-1 foci. Quantum dot-labeled DNAs retained MORC-1 foci at a much higher rate (82% retained, n = 28) than unlabeled DNAs (16% retained, n = 32) (Figure 5; Videos S5 and S6). In addition, MORC-1 foci often accumulated at the free end when quantum dots were present (Figure 5). Static MORC foci could similarly block the movement of mobile foci (Figure S6A; Video S7). These observations are consistent with MORC-1's topologically entrapping DNA.

To further confirm topological entrapment, we incubated 8xHis-tagged maltose binding protein (MBP)-MORC-1 with plasmid DNAs of various topologies (supercoiled, open circular, or linear), captured MORC-1-DNA complexes with nickel resin, and then washed with either a low salt or high salt wash (Cuylen et al., 2011; Kanno et al., 2015; Murayama and Uhlmann, 2014). MORC-1 remained bound to all forms of DNA after low salt washes (150 mM NaCl) (Figures 1A, 6A, and 6B). However, upon high salt washes, MORC-1 retained open circular and supercoiled forms of DNA, but it did not retain significant amounts of linear DNA (Figures 6A and 6B). This occurred without added ATP, and the addition of ATP or AMP-PNP had only minor effects on retention preferences (Figures S6B–S6E).

We also confirmed the preference of MORC-1s for the retention of circular DNA by first incubating MORC-1 with nicked circular plasmid DNA and then linearizing the plasmid via restriction enzyme digest. We found that MORC-1 retained the circular plasmid but lost the linearized DNA (Figure 6B). These results indicate that MORC-1, like eukaryotic SMC proteins (Cuylen et al., 2011; Kanno et al., 2015; Murayama and Uhlmann, 2014), are able to topologically entrap DNAs.

## **MORC-1 Can Compact Nucleosomal DNA**

Because MORCs likely act on chromatin in vivo, we tested whether MORC-1 could compact a chromatinized template in our flow-based single-molecule experiments. We assembled nucleosomes on  $\lambda$ -DNA with a single biotinylated end using HeLa core histones, the histone chaperone NAP1, and the ATP-utilizing chromatin assembly and remodeling factor protein complex (ACF). The nucleosomal template was then tethered to the flow cell via the biotin linkage and visualized with SYTOX Orange staining. We observed that the length of the chromatinized substrates (mode =  $2.86 \mu m$ ) was approximately 3.5-fold shorter than mock-treated unassembled  $\lambda$ -DNA (mode = 10.08  $\mu$ m) (Figure 7A). This degree of DNA condensation is consistent with a previous report that showed that DNA with repeated positioning sequences with nucleosomes was lengthened by  $\sim$ 3.6-fold upon removal of the histone octamers (Brower-Toland et al., 2002). Introduction of 75 nM MORC-1 into the flow cell resulted in robust compaction (Figures 7B and 7C). Notably, independent of initial substrate length, and thus the amount of nucleosomal compaction, the chromatinized substrates compacted to  $<1 \mu m$  in length, demonstrating that MORC-1 can efficiently compact nucleosomal arrays (Figure 7C), although the mean compaction rate (43.5 nm/s at 75 nM MORC-1 with 2 mM ATP, n = 113) was 6- to 8-fold lower than with bare  $\lambda$ -DNA.

## DISCUSSION

Here, we demonstrated that MORC-1 can bind to, compact, and topologically entrap DNA. From single-molecule flow-stretched DNA experiments, we observed that after MORC-1 binds DNA, it seeds the formation of foci containing multiple MORC-1 proteins, which mirrors the MORC-1 nuclear puncta seen *in vivo*. MORC-1 is also able to compact DNA that has been assembled into nucleosome arrays.

The finding that MORC-1 compaction activity is stimulated more by AMP-PNP than by ATP is consistent with a model in which ATP binding by MORC-1, rather than ATP hydrolysis, promotes a conformation that can loop and compact DNA. MORC family members are known to dimerize through their N-terminal ATPase domain upon ATP binding (Douse et al., 2018; Li et al., 2016), and MORC-1 bound to AMP-PNP should therefore be constitutively dimerized at its N terminus. AMP-PNP likely promotes MORC-1 stability on DNA because it disfavors the opening of the ATPase dimers, preventing topologically entrapped DNA from dissociating from MORC-1. It was recently reported that mutations in human MORC2 that create constitutive dimerization within the N-terminal ATPase domain were also more efficient than wild type at silencing a transgene reporter, whereas mutations that disrupted ATP binding or dimer formation could



## With a quantum dot at the DNA free end





## Figure 5. MORC-1 Foci Topologically Entrap DNA

Representative kymographs of Cy3-MORC-1 foci movement on quantum dot-labeled DNA (top panel, [MORC-1] = 5 nM, [ATP] = 2 mM) and on bare DNA (bottom panel, [MORC-1] = 10 nM, [ATP] = 2 mM). Schematics below the kymographs illustrate the experiment. MORC-1 is applied to the flow cell and allowed to form foci; then it is subjected to a 500 mM NaCl and 2 mM MgCl<sub>2</sub> wash in the presence of 2 mM ATP. Scale bars represent 20 s and 5  $\mu$ m. SYTOX Orange-stained DNA is shown to the right of the kymographs to indicate the approximate location of foci on the DNA. Note that the quantum dot is not visible in any of the displayed spectral channels. At top, the top focus (magenta asterisk) contains approximately 2 MORC-1s and the bottom focus (lavender asterisk) contains approximately 7 MORC-1s. At bottom, the time gap between the two events as marked by the dashed line is 7.2 s. The top focus (orange asterisk) has approximately 7 MORC-1s. See also Figure S6 and Videos S4, S5, and S6.

not re-establish silencing (Douse et al., 2018). Together with our data, this supports a model in which MORCs use ATP metabolism to stimulate the conformational changes that regulate their stability and turnover on DNA.

MORC-1 demonstrates a preference for binding long DNAs over shorter DNAs. This preference can be explained by our observation that MORC-1 dimers can diffuse along DNA at low concentrations and forms assemblages of multiple MORC-1 molecules on DNA at higher concentrations. Thus, longer DNA substrates are able to, on average, load more MORC-1 molecules. The observation that MORC dimers can freely diffuse but then become static before growing into larger foci suggests that a conformational change occurs, likely coupled with initial DNA loop trapping, at the time that MORC-1 becomes statically localized on DNA. We propose that this conformational change also stimulates the interaction of MORC-1 with other freely diffusing MORC-1 molecules, recruiting them to static MORC-1 foci to initiate further DNA compaction. Both DNA compaction and the number of MORC-1 molecules per body scale linearly with MORC-1 concentration, suggesting an ordered process in which additional MORC-1 molecules recruited to static foci contribute to additional steps of DNA looping and



compaction. This process eventually leads to assemblages of MORC-1 molecules that stably compact DNA.

Although MORC-1 preferred longer over shorter DNAs, it appeared to display little preference for particular sequences, as it was able to bind to different DNA templates, including a DNA ladder composed of many different sequences. It therefore seems unlikely that MORC-1 would use DNA sequence as the basis for selecting its chromatin targets in vivo, which is consistent with the view that MORCs in different organisms act as epigenetic regulators that silence targets such as transposons that are composed of a diverse array of sequences (Moissiard et al., 2012; Pastor et al., 2014). It seems more likely that MORCs are guided to their chromatin targets by other proteins or by epigenetic marks. In this regard, one well-understood example is mouse MORC3, which contains a CW domain that binds to H3K4 trimethylation in vitro and which shows co-localization with H3K4 trimethylation in vivo (Li et al., 2016). C. elegans MORC-1 also contains a CW domain, but it is unknown whether it serves to bind methylated histones.

We observed that MORC-1 formed nuclear foci in vivo and that the MORC-1 protein contains a large, intrinsically disordered region and could form phase-separated droplets in vitro. Plant MORCs also form prominent nuclear bodies in vivo (Harris et al., 2016; Moissiard et al., 2012) and contain predicted intrinsically disordered regions, suggesting that these features may be generally important for the function of eukaryotic MORCs. Several chromatin regulators such as HP1 and Mediator have been shown to assemble into condensates to create an epigenetic nuclear microenvironment that enforces a particular epigenetic state (Boija et al., 2018; Larson et al., 2017; Sabari et al., 2018; Shin et al., 2018; Strom et al., 2017). Similarly, we propose that MORC-1 may first bind, topologically entrap, and locally compact its chromatin targets, but also form intracellular condensates to concentrate MORC-1 target loci into the observed in vivo MORC-1 bodies within nuclei.

## Figure 6. MORC-1 Selectively Retains Circular DNA after High Salt Washes

(A) MORC-1 was incubated with DNA of varying topologies and precipitated with magnetic resin, then washed twice before proteinase K elution. The retained DNA is visualized by resolving samples on a 1% 1× Tris-acetate-EDTA (TAE) agarose gel and post-stained with ethidium bromide.

(B) MORC-1 at 400 nM was incubated with 50 ng open circular DNA that was made by Nt.BsmAl digestion and precipitated with magnetic resin, and then washed twice with a high salt buffer and once with restriction enzyme (RE) buffer before diaestina with restriction enzymes to alter the topology. The sample was then washed using low or high salt and then eluted using proteinase K. The retained DNA is visualized by resolving samples on a 1% 1× TAE agarose gel and post-stained with ethidium bromide. Note that Pstl digestion was not complete, and there were trace amounts of open circular plasmid remaining. However, MORC-1 washed with high salt only retains the open circular form and not the linearized material. See also Figure S6.

In summary, the results of this study suggest that MORC-1 acts via a mechanism to trap a loop of DNA and then multimerize upon DNA binding to form topologically entrapped foci to cause stable chromatin compaction. Given that eukaryotic MORC proteins are highly conserved and evolved from ancient prokaryotic restriction-modification systems (lyer et al., 2008), it is likely that other plant and animal MORCs use a similar mechanism to enforce chromatin compaction.

## **STAR**\***METHODS**

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- LEAD CONTACT AND MATERIALS AVAILABILITY
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
  - Cloning and Purification of 8x-His-MBP-CeMORC
  - Gel shift assays
  - Native mass spectrometry data collection
  - DNA substrate preparation
  - Single-molecule flow-stretching assays
  - DNA motion capture assay
  - Double-tethered DNA assay
  - Chromatin assembly and compaction assay
  - Circular DNA binding assay
  - Protein labeling with fluorescent dyes
  - Liquid droplet sample preparation and imaging
  - Gonad dissection and immunostaining for SIM
  - O Structured illumination and image processing
  - STORM imaging
  - STORM image processing
  - Hexanediol treatment and imaging



### Figure 7. MORC-1 Compacts Chromatin

(A) Distribution of substrate lengths under flow. Mode of DNA substrates (n = 301) =  $10.03 \mu$ m; mode of chromatin substrates (n = 415) =  $2.84 \mu$ m. Substrates were flowed into the flow cell and subsequently visualized with  $3 \mu$ M SYTOX Orange.

(B) Kymograph depicting MORC-1 mediated compaction of a chromatinized template. Scale bars represent 7 μm and 3 s, respectively. [MORC-1] = 75 nM. (C) MORC-1 compacts chromatinized substrates of varying lengths. [MORC-1] = 75 nM was flowed into the flow cell, and compaction was allowed to proceed. Top: scatterplot of individual trajectories plotted by the initial and final lengths. Bottom: projection of the scatterplot, binning the trajectories by final DNA length.

#### QUANTIFICATION AND STATISTICAL ANALYSIS

- Native mass spectrometry data analysis
- Quantification of flow-stretching assays
- O Chromatin compaction data analysis
- STORM data analysis
- Hexanediol data analysis
- Quantification of circular DNA binding assay
- DATA AND CODE AVAILABILITY

#### SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j. molcel.2019.07.032.

## ACKNOWLEDGMENTS

We thank Michael Carey, Reid Johnson, Geeta Narlikar, Sridhar Mandali, and Elizabeth Thrall for discussion and advice; Sean Carney for assistance with reagent preparation; and Alison Frand and Sophie Katz for guidance and assistance with microscopy. The supercoiled pBR322 derivative was a gift from Sridhar Mandali and Reid Johnson. The MBP vector was a gift from Andrew Ah Young. This work was supported by NIH grant 1R35GM130272 and a grant from the W.M. Keck Foundation (both to S.E.J.); NIH R01 GM114054 and John and Virginia Kaneb Fellowship (both to J.J.L); the University of Texas Rio Grande Valley Start-up Grant and the University of Texas System's Rising STARs Award (both to H.J.K.); NIH grant P41GM103481 and a University of California, San Francisco, Research Resource Program (RRP) Shared Equipment Award (both to A.L.B.) for the purchase of the Exactive EMR mass spec-

trometer; a Ruth L. Kirschstein National Research Service Award (GM007185) and a University of California, Los Angeles, Dissertation Year Fellowship (both to L.Y.); and NIH grant R01 GM129301 (to J.K.K.). S.E.J. is an Investigator of the Howard Hughes Medical Institute.

## **AUTHOR CONTRIBUTIONS**

H.J.K., L.Y., J.J.L., and S.E.J. conceived of the study, designed the experiments, and wrote the manuscript. H.J.K., L.Y., S.P.W., and J.J.L. generated the reagents. H.J.K., L.Y., S.P.W., N.M., J.A.K., Y.X., J.B.J., and J.J.L. performed the experiments and analyzed the data. A.L.B., J.K.K., J.J.L., and S.E.J. supervised the project.

## **DECLARATION OF INTERESTS**

The authors declare no competing interests.

Received: November 6, 2018 Revised: May 28, 2019 Accepted: July 23, 2019 Published: August 22, 2019

#### REFERENCES

Alberti, S., Gladfelter, A., and Mittag, T. (2019). Considerations and challenges in studying liquid-liquid phase separation and biomolecular condensates. Cell *176*, 419–434.

Boija, A., Klein, I.A., Sabari, B.R., Dall'Agnese, A., Coffey, E.L., Zamudio, A.V., Li, C.H., Shrinivas, K., Manteiga, J.C., Hannett, N.M., et al. (2018). Transcription factors activate genes through the phase-separation capacity of their activation domains. Cell *175*, 1842–1855.e16.

Brower-Toland, B.D., Smith, C.L., Yeh, R.C., Lis, J.T., Peterson, C.L., and Wang, M.D. (2002). Mechanical disruption of individual nucleosomes reveals a reversible multistage release of DNA. Proc. Natl. Acad. Sci. USA *99*, 1960–1965.

Corbett, K.D., and Berger, J.M. (2005). Structural dissection of ATP turnover in the prototypical GHL ATPase TopoVI. Structure *13*, 873–882.

Cuylen, S., Metz, J., and Haering, C.H. (2011). Condensin structures chromosomal DNA through topological links. Nat. Struct. Mol. Biol. *18*, 894–901.

Dong, W., Vannozzi, A., Chen, F., Hu, Y., Chen, Z., Zhang, L., and Pritham, E. (2018). MORC domain definition and evolutionary analysis of the MORC gene family in green plants. Genome Biol. Evol. *10*, 1730–1744.

Douse, C.H., Bloor, S., Liu, Y., Shamin, M., Tchasovnikarova, I.A., Timms, R.T., Lehner, P.J., and Modis, Y. (2018). Neuropathic MORC2 mutations perturb GHKL ATPase dimerization dynamics and epigenetic silencing by multiple structural mechanisms. Nat. Commun. *9*, 651.

Dutta, R., and Inouye, M. (2000). GHKL, an emergent ATPase/kinase super-family. Trends Biochem. Sci. 25, 24–28.

Edelstein, A.D., Tsuchida, M.A., Amodaj, N., Pinkard, H., Vale, R.D., and Stuurman, N. (2014). Advanced methods of microscope control using  $\mu$ Manager software. J. Biol. Methods 1, e10.

Ganji, M., Shaltiel, I.A., Bisht, S., Kim, E., Kalichava, A., Haering, C.H., and Dekker, C. (2018). Real-time imaging of DNA loop extrusion by condensin. Science *360*, 102–105.

Graham, T.G., Wang, X., Song, D., Etson, C.M., van Oijen, A.M., Rudner, D.Z., and Loparo, J.J. (2014). ParB spreading requires DNA bridging. Genes Dev. 28, 1228–1238.

Graham, T.G.W., Walter, J.C., and Loparo, J.J. (2017). Ensemble and singlemolecule analysis of non-homologous end joining in frog egg extracts. In DNA Repair Enzymes: Cell, Molecular, and Chemical Biology, B.F. Eichman, ed. (Academic Press), pp. 233–270.

Harris, C.J., Husmann, D., Liu, W., Kasmi, F.E., Wang, H., Papikian, A., Pastor, W.A., Moissiard, G., Vashisht, A.A., Dangl, J.L., et al. (2016). Arabidopsis AtMORC4 and AtMORC7 form nuclear bodies and repress a large number of protein-coding genes. PLoS Genet. *12*, e1005998.

Inoue, N., Hess, K.D., Moreadith, R.W., Richardson, L.L., Handel, M.A., Watson, M.L., and Zinn, A.R. (1999). New gene family defined by MORC, a nuclear protein required for mouse spermatogenesis. Hum. Mol. Genet. *8*, 1201–1207.

lyer, L.M., Abhiman, S., and Aravind, L. (2008). MutL homologs in restrictionmodification systems and the origin of eukaryotic MORC ATPases. Biol. Direct 3, 8.

Kang, H.-G., Hyong, W.C., von Einem, S., Manosalva, P., Ehlers, K., Liu, P.-P., Buxa, S.V., Moreau, M., Mang, H.-G., Kachroo, P., et al. (2012). CRT1 is a nuclear-translocated MORC endonuclease that participates in multiple levels of plant immunity. Nat. Commun. *3*, 1297.

Kanno, T., Berta, D.G., and Sjögren, C. (2015). The Smc5/6 complex is an ATP-dependent intermolecular DNA linker. Cell Rep. *12*, 1471–1482.

Kim, H., and Loparo, J.J. (2016). Multistep assembly of DNA condensation clusters by SMC. Nat. Commun. 7, 10200.

Kim, H., and Loparo, J.J. (2018). Observing bacterial chromatin protein-DNA interactions by combining DNA flow-stretching with single-molecule imaging. Methods Mol. Biol. *1837*, 277–299.

Köhler, S., Wojcik, M., Xu, K., and Dernburg, A.F. (2017). Superresolution microscopy reveals the three-dimensional organization of meiotic chromosome axes in intact *Caenorhabditis elegans* tissue. Proc. Natl. Acad. Sci. USA *114*, E4734–E4743.

Kroschwald, S., Maharana, S., Mateju, D., Malinovska, L., Nüske, E., Poser, I., Richter, D., and Alberti, S. (2015). Promiscuous interactions and protein disaggregases determine the material state of stress-inducible RNP granules. eLife *4*, e06807. Larson, A.G., and Narlikar, G.J. (2018). The role of phase separation in heterochromatin formation, function, and regulation. Biochemistry 57, 2540–2548.

Larson, A.G., Elnatan, D., Keenen, M.M., Trnka, M.J., Johnston, J.B., Burlingame, A.L., Agard, D.A., Redding, S., and Narlikar, G.J. (2017). Liquid droplet formation by HP1 $\alpha$  suggests a role for phase separation in heterochromatin. Nature 547, 236–240.

Li, S., Yen, L., Pastor, W.A., Johnston, J.B., Du, J., Shew, C.J., Liu, W., Ho, J., Stender, B., Clark, A.T., et al. (2016). Mouse MORC3 is a GHKL ATPase that localizes to H3K4me3 marked chromatin. Proc. Natl. Acad. Sci. USA *113*, E5108–E5116.

Lin, Y., Protter, D.S.W., Rosen, M.K., and Parker, R. (2015). Formation and maturation of phase-separated liquid droplets by RNA-binding proteins. Mol. Cell *60*, 208–219.

Lu, J., Trnka, M.J., Roh, S.-H., Robinson, P.J.J., Shiau, C., Fujimori, D.G., Chiu, W., Burlingame, A.L., and Guan, S. (2015). Improved peak detection and deconvolution of native electrospray mass spectra from large protein complexes. J. Am. Soc. Mass Spectrom. *26*, 2141–2151.

Mészáros, B., Erdős, G., and Dosztányi, Z. (2018). IUPred2A: context-dependent prediction of protein disorder as a function of redox state and protein binding. Nucleic Acids Res. *46* (W1), W329–W337.

Mimura, Y., Takahashi, K., Kawata, K., Akazawa, T., and Inoue, N. (2010). Twostep colocalization of MORC3 with PML nuclear bodies. J. Cell Sci. *123*, 2014–2024.

Moissiard, G., Cokus, S.J., Cary, J., Feng, S., Billi, A.C., Stroud, H., Husmann, D., Zhan, Y., Lajoie, B.R., McCord, R.P., et al. (2012). MORC family ATPases required for heterochromatin condensation and gene silencing. Science *336*, 1448–1451.

Moissiard, G., Bischof, S., Husmann, D., Pastor, W.A., Hale, C.J., Yen, L., Stroud, H., Papikian, A., Vashisht, A.A., Wohlschlegel, J.A., and Jacobsen, S.E. (2014). Transcriptional gene silencing by Arabidopsis microrchidia homologues involves the formation of heteromers. Proc. Natl. Acad. Sci. USA *111*, 7474–7479.

Murayama, Y., and Uhlmann, F. (2014). Biochemical reconstitution of topological DNA binding by the cohesin ring. Nature *505*, 367–371.

Pastor, W.A., Stroud, H., Nee, K., Liu, W., Pezic, D., Manakov, S., Lee, S.A., Moissiard, G., Zamudio, N., Bourc'his, D., et al. (2014). MORC1 represses transposable elements in the mouse male germline. Nat. Commun. 5, 5795.

Protter, D.S.W., Rao, B.S., Van Treeck, B., Lin, Y., Mizoue, L., Rosen, M.K., and Parker, R. (2018). Intrinsically disordered regions can contribute promiscuous interactions to RNP granule assembly. Cell Rep. *22*, 1401–1412.

Sabari, B.R., Dall'Agnese, A., Boija, A., Klein, I.A., Coffey, E.L., Shrinivas, K., Abraham, B.J., Hannett, N.M., Zamudio, A.V., Manteiga, J.C., et al. (2018). Coactivator condensation at super-enhancers links phase separation and gene control. Science *361*, eaar3958.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., et al. (2012). Fiji: an open-source platform for biological-image analysis. Nat. Methods *9*, 676–682.

Schneider, C.A., Rasband, W.S., and Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675.

Shin, Y., and Brangwynne, C.P. (2017). Liquid phase condensation in cell physiology and disease. Science 357, eaaf4382.

Shin, Y., Chang, Y.-C., Lee, D.S.W., Berry, J., Sanders, D.W., Ronceray, P., Wingreen, N.S., Haataja, M., and Brangwynne, C.P. (2018). Liquid nuclear condensates mechanically sense and restructure the genome. Cell *175*, 1481–1491.e13.

Song, D., Graham, T.G.W., and Loparo, J.J. (2016). A general approach to visualize protein binding and DNA conformation without protein labelling. Nat. Commun. 7, 10976.

Strom, A.R., Emelyanov, A.V., Mir, M., Fyodorov, D.V., Darzacq, X., and Karpen, G.H. (2017). Phase separation drives heterochromatin domain formation. Nature *547*, 241–245.

Takahashi, K., Yoshida, N., Murakami, N., Kawata, K., Ishizaki, H., Tanaka-Okamoto, M., Miyoshi, J., Zinn, A.R., Shime, H., and Inoue, N. (2007). Dynamic regulation of p53 subnuclear localization and senescence by MORC3. Mol. Biol. Cell *18*, 1701–1709.

Tchasovnikarova, I.A., Timms, R.T., Douse, C.H., Roberts, R.C., Dougan, G., Kingston, R.E., Modis, Y., and Lehner, P.J. (2017). Hyperactivation of HUSH complex function by Charcot-Marie-Tooth disease mutation in MORC2. Nat. Genet. *49*, 1035–1044.

Updike, D.L., Hachey, S.J., Kreher, J., and Strome, S. (2011). P granules extend the nuclear pore complex environment in the C. elegans germ line. J. Cell Biol. *192*, 939–948.

Weiser, N.E., Yang, D.X., Feng, S., Kalinava, N., Brown, K.C., Khanikar, J., Freeberg, M.A., Snyder, M.J., Csankovszki, G., Chan, R.C., et al. (2017). MORC-1 integrates nuclear RNAi and transgenerational chromatin architecture to promote germline immortality. Dev. Cell *41*, 408–423.e7.

Wheeler, J.R., Matheny, T., Jain, S., Abrisch, R., and Parker, R. (2016). Distinct stages in stress granule assembly and disassembly. eLife 5, e18413.

Zhang, Z., and Marshall, A.G. (1998). A universal algorithm for fast and automated charge state deconvolution of electrospray mass-to-charge ratio spectra. J. Am. Soc. Mass Spectrom. *9*, 225–233.

## **KEY RESOURCES TABLE**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-digoxigenin, Fab fragments	Roche	Cat# 11214667001; RRID: AB_514494
Histidine tag antibody	Bio-Rad AbD Serotec	Cat# MCA1396; RRID: AB_322084
Anti-Flag M2 Antibody	Sigma-Aldrich	Cat# F1804; RRID: AB_262044
Anti-CSR-1 antibody	This paper	Peptide: N'-(CG) FDDWETLTTQLSYSTLDRLSKVRVV
Alexa Fluor 555 goat anti-mouse	Invitrogen	Cat# A21127; RRID: AB_141596
Alexa Fluor 488 goat anti-rabbit	Invitrogen	Cat# A11034; RRID: AB_2576217
Alexa Fluor 647 goat anti-mouse	Invitrogen	Cat# A21236; RRID: AB_2535805
Bacterial and Virus Strains		
BL21(DE3)Rosetta2	Novagen	Cat#71397
Chemicals, Peptides, and Recombinant Proteins		
Apyrase	New England Biolabs	Cat#M0398L
T4 polynucleotide kinase (PNK)	New England Biolabs	Cat#M0201S
Neutravidin	Thermo Scientific	Cat#31000
Nt.BSMAI	NEB	Cat#R0121S
PSTI-HF	NEB	Cat#R3140S
Cy3 NHS Ester	Lumiprobe	Cat#11020
Cy5 NHS Ester	Lumiprobe	Cat#13020
SyBr Gold	Invitrogen	Cat#S11494
γ-32-P EasyTide Adenosine Triphosphate	Perkin Elmer	Cat#BLU502Z250UC
Adenosine Triphosphate	Sigma	Cat#A2383
AMP-PNP	Sigma	Cat#A2647
Polyethylene Glycol 3350	Sigma	Cat#1546547
DAPI Solution	Thermo Fisher	Cat#62248
Vectashield	Vector Labs	Cat#H-1000
1,6-Hexanediol	Sigma-Aldrich	Cat#240117
Critical Commercial Assays		
In-Fusion HD Cloning Plus for DNA cloning	Takara	Cat#638920
Nucleosome assembly kit	Active Motif	Cat#53500
Qdot 605 antibody conjugation kit	Thermo Fisher Scientific	Cat#Q22001MP
Deposited Data		
Native mass spec	This paper, Mendeley Data	https://doi.org/10.17632/876gx33wm2.1
Microscopy Images	This paper, Mendeley Data	https://doi.org/10.17632/876gx33wm2.1
Kymographs	This paper, Mendeley Data	https://doi.org/10.17632/876gx33wm2.1
Experimental Models: Organisms/Strains		
morc-1::3xflag	Weiser et al., 2017	QK84
Oligonucleotides		
Biotinylated BL1-oligo: AGGTCGCCGCCC/BiotinTEG/	Kim and Loparo, 2016	N/A
Biotinylated BL2-oligo: GGGCGGCGACCT/BioTEG/	Kim and Loparo, 2016	N/A
Oligo with digoxigenin: AGGTCGCCGCCCAAAAA	Kim and Loparo, 2016	N/A
AAAAAA/Digoxigenin/		
Lambda probe, 50 bp Forward: ACGAACTCAGCCAGA	This paper	N/A
Lambda probe, 50 bp Reverse: TTGCATCAGATAGTTG	This paper	N/A

(Continued on next page)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Lambda probe, 250 bp Forward: GGGAGAAGGCGCA TGAGACTCGAA	This paper	N/A
Lambda probe, 250 bp Reverse: TCCCCGATACCTT GTGTGCAA	This paper	N/A
Scramble probe, 250 bp Forward: ATGGGCAGCA GCCATCACC	This paper	N/A
Scramble probe, 250 bp Reverse: TTGTGAACGGAAT CAACGAGCC	This paper	N/A
Recombinant DNA		
pRSF-8x-HIS-MBP-C. elegans MORC-1	This paper	Available upon request to lead contact
Software and Algorithms		
Fiji	Schindelin et al., 2012	https://imagej.net/Fiji
ImageJ	Schneider et al., 2012	https://imagej.nih.gov/ij/
MicroManager	Edelstein et al., 2014	https://micro-manager.org
HCImage	Hamatatsu	https://HCImage.com
Zeiss ELYRA PS.1 system	Zeiss	N/A
PCO Edge 4.2 camera	Zeiss	N/A
Zeiss LSM700	Zeiss	N/A
Zen SP5	Zeiss	https://www.zeiss.com/microscopy/us/products/ microscope-software/zen.html
Zen 2.1 SP3	Zeiss	https://www.zeiss.com/microscopy/us/products/ microscope-software/zen.html
Other		
Lambda DNA (N <sup>6</sup> -methyladenine-free)	New England Biolabs	Cat#N3013S
100 bp DNA ladder for gel shift assay (Figure S2)	New England Biolabs	Cat#N3231S
Micro Bio-Spin 30	Bio-Rad	Cat#732-6223
Ni-NTA Agarose	QIAGEN	Cat#30250
Ni-NTA Magnetic agarose beads	QIAGEN	Cat#36113
CutSmart Buffer	NEB	Cat#B7204S

## LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Steve E. Jacobsen (jacobsen@ucla.edu).

## **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

*C. elegans* were maintained using standard procedures at 20°C and fed OP50 *E. coli*. The *morc-1::3xflag* strain used in this study is listed in the Key Resources Table.

## **METHOD DETAILS**

## **Cloning and Purification of 8x-His-MBP-CeMORC**

*C. elegans* MORC-1 coding sequence (CDS) was cloned into the pRSF vector with an 8xHis MBP tag at the N terminus using In-Fusion cloning (Takara 638920). Plasmid was transformed into BL-21 (DE3) Rosetta2 cells (Novagen) and grown at 37°C, induced with 0.1 mM isopropyl β-D-1-thiogalactopyranoside and moved to 16°C for overnight expression. Cells were harvested then lysed by sonication in 20 mM Tris-Cl pH 7.5, 500 mM NaCl, 10 mM imidazole, 5 mM DTT, and protease inhibitors. Lysate was clarified by centrifugation and supernatant was applied to Ni-NTA agarose (QIAGEN). After binding, resin was washed with 20 mM Tris-Cl pH 7.5, 500 mM NaCl, 30 mM imidazole, 5 mM DTT, then treated with at least 2 units of apyrase (NEB M0398L) prior to elution except for the experiment shown in Figure S1D. Protein was eluted from the resin then dialyzed against 20 mM Tris pH 7.5, 150 mM NaCl, 5 mM DTT, 10% glycerol. Sample was then subjected to size exclusion chromatography (Superdex 200) in dialysis buffer. Fractions were pooled and concentrated in an Amicon Ultra-15 (Millipore) for storage.

## **Gel shift assays**

Probe was <sup>32</sup>P labeled with T4 PNK (NEB) and used at a final concentration of 0.45 nM unless otherwise indicated. Protein and probe were incubated at 23°C for 30 min in 20 mM Tris pH 7.5, 50 mM NaCl, 1 mM DTT, 1 mM MgCl<sub>2</sub>, 0.1 mg/ml BSA, then resolved on a 3% 0.5X TBE agarose gel and visualized by exposure to a phosphorscreen unless otherwise indicated. All images were collected with a Typhoon Imager 9000 (GE Amersham). For the ladder gel shift, a 100 bp double stranded DNA ladder (NEB N3231S) was used as substrate and visualized by SyBR gold staining (Invitrogen S11494).

## Native mass spectrometry data collection

Protein samples ( $\sim$ 0.5–1 µM) were buffer exchanged into 150 mM ammonium acetate, pH 7.5 using MicroBioSpin6 columns (Bio-Rad 7326221). Native mass spectrometry experiments were carried out on an Exactive Plus EMR mass spectrometer (Thermo Fisher Scientific), which was calibrated in the extended mass range with 5 mg/mL CsI prepared in water. Samples were sprayed from borosilicate capillaries (NanoES spray capillaries, borosilicate; Thermo Fisher Scientific) using a flow rate of 5–40 µL/min and analyzed in positive ion mode. Experimental parameters were optimized for each run but were generally: spray voltages, 0.8–1.5 kV; injection flatapole, 5; interflatapole lens, 5; bent flatapole, 5; transfer multipole, +4 to -4; C-trap entrance lens, -10 to +10; source DC offset, 25 V; fragmentation collision energy, 20–150 and collision-induced dissociation, 5–150; injection times, 50–200 µs; trapping gas pressure, 7.5; resolution, 17,500 arbitrary units; mass range, 500–20,000 *m/z*; capillary temperature, 250°C; S-lens RF value was set to 200 V; microscans, 10; and automatic gain control was set to 1e<sup>6</sup>.

### **DNA substrate preparation**

DNA substrates were prepared as previously described (Kim and Loparo, 2016). Briefly, to prepare a DNA substrate with a biotin at one end and a quantum dot at the other end, a biotinylated BL2-oligo (5'-ggg cgg cgg cct/BioTEG/-3') and an oligo with digoxigenin (5'-agg tcg ccg ccc aaa aaa aaa /Digoxigenin/-3') was annealed to each *cos* site of genomic bacteriophage  $\lambda$ -DNA (NEB N3013S). The biotin at one end of the substrate was used for surface-tethering of the DNA to a neutravidin coated coverslip while digoxigenin was used for labeling the DNA with anti-digoxigenin antibody-conjugated quantum dots (Thermo Fisher). The DNA substrate used in DNA motion capture experiments was constructed by annealing the BL1 oligo (5'-agg tcg ccg ccc/BiotinTEG/-3') to  $\lambda$ -DNA.

## Single-molecule flow-stretching assays

Coverglass passivation and flow cell construction and were performed as previously described (Kim and Loparo, 2016). For this study coverslips were functionalized with 4% biotinylated PEG. A home-built total internal reflection fluorescence (TIRF) microscope was used as described in Graham et al. (2017). DNA flow stretching experiments were performed by first preincubating the  $\lambda$ -DNA substrate with a biotin and a digoxigenin at each end with anti-digoxigenin antibody-conjugated quantum dot at room temperature (anti-digoxigenin antibody: Roche 11 214 667 001, Qdot 605 antibody conjugation kit: Thermo Fisher Scientific Q22001MP). 0.25 mg/mL neutravidin was applied to the flow cell and unbound neutravidin was washed from the flow cell after 5~10 min by EcoRI binding buffer (EBB buffer: 10 mM Tris, pH 8.0, 150 mM NaCl, 10 mM MgCl<sub>2</sub>) that contains 0.2 mg/mL BSA. Quantum-dot labeled biotinylated  $\lambda$ -DNA was flowed into the flow cell at a rate of 20-30 µL/min, and unbound excess DNA was washed away using MORC-1 sample buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 2 mM MgCl<sub>2</sub>). MORC-1 stock was diluted to the desired concentration by adding MORC-1 sample buffer either in the absence or presence of 2 mM ATP, and then flowed into the flow cell at a rate of 50 µL/min. The movies of flow-stretching assays were recorded using MicroManager (Edelstein et al., 2014).

## **DNA motion capture assay**

The assay was performed as previously described (Kim and Loparo, 2016, 2018). Briefly, catalytically-inactive EcoRI (6xHis-EcoRI E111Q; Graham et al., 2014) was incubated at room temperature for 30 min with anti-His-tag antibody-conjugated quantum dot 605 (Thermo Fisher Scientific Q22001MP) with a two-to-one ratio in 20 mM Tris, pH 7.5, 100 mM NaCl, and 0.1 mg/mL bovine serum albumin. Then, the mixture was incubated for 30 min at room temperature with  $\lambda$ -DNA where one end was biotinylated through a biotin oligo (BL1: 5'-agg tcg ccc/BiotinTEG/-3'). In parallel a surface-passivated flow cell was treated with 0.25 mg/mL neutravidin for 5~10 min and unbound excess neutravidin was washed out with EcoRI binding buffer (EBB buffer) that contained 0.2 mg/mL BSA. Subsequent steps were identical to those described above in the section entitled "Single-molecule flow-stretching assays" with the exception of site-specifically quantum-dot labeled  $\lambda$ -DNA.

## **Double-tethered DNA assay**

A flow cell was constructed by sandwiching a cross-shaped (1.8 mm and 2.5 mm widths, respectively) channel made out of doublesided tape (0.12 mm thickness) between a passivated coverglass and a quartz slide. PE60 tubes were used as inlet and outlet tubing for each channel. 0.25 mg/ml of neutravidin (Thermo Scientific 31000) was added to the flow cell using a gel loading pipet tip. After 6-7 min of incubation, EBB buffer (10 mM Tris pH 8.0, 150 mM NaCl, 10 mM MgCl<sub>2</sub>, 0.2 mg/ml bovine serum albumin) was flowed in using a syringe pump to wash away excess neutravidin. 180  $\mu$ L of the 400-fold diluted BL1-DNA substrate was flowed in at a rate of 30  $\mu$ L/min and allowed to incubate for 2 min in the absence of flow. Unbound DNAs were then washed away by flowing in 140  $\mu$ L of MORC sample buffer at a rate of 100  $\mu$ L/min. In order to tether the other end of DNA to the flow cell, 380  $\mu$ L of 100 nM BL2-oligo was flowed in at 30  $\mu$ L/min, and 120  $\mu$ L of MORC-1 sample buffer was immediately flowed in at 100  $\mu$ L/min to remove unbound BL2-oligo. DNA compaction was observed by adding 80 nM MORC-1 and 6 nM SYTOX-Orange to the flow cell using the channel perpendicular to DNA tethering in the presence of 2 mM ATP.

## Chromatin assembly and compaction assay

Chromatin compaction assays were imaged on a total internal reflection fluorescence microscope (Olympus). Biotinylated DNA was chromatinized using the Active Motif chromatin assembly kit (Active Motif 53500). Assembly was checked by MNAse digestion. Substrate was diluted into EBB+BSA and introduced into the flow cell using the parameters described above. 75 nM MORC-1 was diluted into MORC sample buffer as described above supplemented with 2 mM ATP, and introduced into the flow cell also using parameters described above, and visualized by 3 nM SYTOX Orange staining. Chromatin compaction movies were recorded.

#### **Circular DNA binding assay**

Unless otherwise noted, 2.4 µM protein was incubated for 30 min at room temperature with a pBR322 derivative of varying topologies in 20 mM Tris, pH 7.5, 150 mM NaCl, 1mM DTT). Topology was altered by restriction enzyme digest. Protein was precipitated using Ni-NTA magnetic agarose (QIAGEN 36113), then washed 2x with either a high salt (600 mM NaCl) or a low salt buffer (150 mM NaCl). Reactions were quenched with a stop buffer (20 mM Tris pH 8, 2% SDS, 1 mM EDTA) and samples were eluted by proteinase K digestion. For the re-linearization assay, 400 nM MORC-1 was bound to 50 ng total pBR322 pre-modified with Nt.BsmAl. Protein was precipitated as described above, then washed with 20 mM Tris pH 7.5, 500 mM NaCl, 1mM DTT, then buffer exchanged into a Restriction Enzyme (RE) buffer (New England Biosciences CutSmart Buffer, B7204) and digested for 30 min room temperature, then washed again with either a low salt buffer (150 mM NaCl) or a high salt buffer (500 mM NaCl.) For assays with nucleotide, all buffers were supplemented with 4 mM MgCl<sub>2</sub>. Reactions were loaded on a 1% 1xTAE gel and run overnight at room temperature, then post stained using ethidium bromide, and imaged using a Gel Logic 212 Pro (Carestream).

## Protein labeling with fluorescent dyes

Proteins were incubated with NHS-Cy3 or NHS-Cy5 dye (Lumiprobe 11020, 13020) at 4°C. Labeled protein was separated from free dye using Biospin 6 columns (Biorad). Cy-dye labeling efficiency was quantified using a Nanodrop; final concentrations were determined by averaging three readings.

## Liquid droplet sample preparation and imaging

Samples were buffer exchanged using Micro Bio-spin P-30 gel columns (Biorad 732-6223) into 20 mM Tris pH 7.5, 150 mM NaCl, 5mM DTT. Concentration was measured three times using a NanoDrop spectrophotometer (Thermo Fisher). Protein was diluted to the 75 mM NaCl. Final amounts: 10% PEG or 500 ng total, or 0.8 nM final,  $\lambda$ -DNA (NEB N3011S) with a final unlabeled protein concentration of 4.3 uM with 1%–2% Cy-labeled protein acting as tracer. Final reaction volume was 20 µL. Samples were imaged using a Zeiss LSM 880 with spectral imaging at 63X magnification. Signals from Cy3 and Cy5 were linear unmixed in Zen 9.0 (Zeiss).

## Gonad dissection and immunostaining for SIM

Gravid adult *C. elegans* were dissected in egg buffer (25 mM HEPES pH 7.4, 118 mM NaCl, 48 mM KCl, 2 mM EDTA, 0.5 mM EGTA), containing 15 mM sodium azide and 0.1% Tween-20. Samples were fixed in 1% formaldehyde in egg buffer for 10 s followed by freeze-cracking in liquid nitrogen, 1 min methanol fixation at  $-20^{\circ}$ C, and three washes in PBST. Slides were then incubated with primary mouse anti-FLAG antibody (1:100, Sigma F1804) in a humid chamber overnight at 4°C or 2 h at room temperature. Slides were washed three times in PBST and then incubated with secondary antibody (1:300, Invitrogen AlexaFluor 555 goat anti-mouse) in a humid chamber for 2 h at room temperature. Slides were washed three times in PBST, stained with 0.5 µg/mL DAPI in PBST for 15 min, washed in PBST, and mounted with Vectashield (Vectorlabs H-1000).

## Structured illumination and image processing

Structural Illumination Microscopy (SIM) was performed with Zeiss ELYRA PS.1 system (Carl Zeiss, Germany) equipped with a 100x/1.46 oil immersion objective and a PCO Edge 4.2 camera. Excitation wavelengths used were 405 nm and 561 nm. Images were acquired after 5 rotations and 5 standard shifts of the grating pattern. Image processing was carried out using Zen 2.1 SP3 software. For wild-type, n (mitotic) = 11, n (pachytene) = 12; For RNAi, n (mitotic) = 3, n (pachytene) = 4.

## **STORM** imaging

Immunofluorescence of extruded gonads was performed as previously described with minor modifications (Köhler et al., 2017). Briefly, germline extrusion was performed in egg buffer (25 mM HEPES pH 7.4, 118 mM NaCl, 48 mM KCl, 2 mM EDTA, 0.5 mM EGTA), containing 30 mM sodium azide and 0.1% Tween-20. Samples were fixed in 1% formaldehyde in egg buffer for 10 s followed by a 1 min methanol fixation at -20°C. Primary mouse anti-FLAG antibody used was 1:100 (Sigma F1804) in normal goat serum and

PBST and the secondary antibody was used at 1:100 (Invitrogen AlexaFluor 647 goat anti-mouse) in PBST. All washes and staining were performed in suspension. Germlines were mounted in imaging buffer (Tris·HCl, pH 7.5, containing 100 mM cysteamine, 5% glucose, 0.8 mg/mL glucose oxidase, and 40  $\mu$ g/mL catalase) and imaged immediately. At least n (mitotic) = 8 germlines and n (pachytene) = 11 germlines were imaged. Scale bar represents 5  $\mu$ m.

## **STORM image processing**

STORM was performed with a Zeiss ELYRA PS.1 system (Carl Zeiss, Germany) equipped with a 100x/1.46 oil immersion objective and a PCO Edge 4.2 camera. The 647-nm laser was used to excite fluorescence from antibody-labeled proteins after photobleaching them into the dark state. The 405-nm laser was adjusted during image acquisition. Images were acquired with 43.55 ms exposure time per frame for >13,000 frames. Events that emit fewer than 500 photons or that were not resolved to within 50 nm were discarded. Image processing was performed using Zen 2.1 SP3 software.

## **Hexanediol treatment and imaging**

Gravid adult *C. elegans* were dissected in egg buffer (118 mM NaCl, 48 mM KCl, 2 mM EDTA, 0.5 mM EGTA, 25 mM HEPES [pH 7.4]), containing 15 mM sodium azide and 0.1% Tween-20. Samples were incubated briefly in either 1x egg buffer or 5% 1,6-hexanediol in egg buffer then fixed in 1% formaldehyde in egg buffer for 10 s followed by a 1 min methanol fixation at  $-20^{\circ}$ C. Primary mouse anti-FLAG antibody (Sigma F1804) was used at 1:100 and rabbit anti-CSR-1 was used at 1:200 in normal goat serum and PBST. The secondary antibody was used at 1:300 (Invitrogen AlexaFluor 555 goat anti-mouse and 488 goat anti-rabbit) in PBST. All washes and staining were performed in suspension. Germlines were stained with DAPI (0.5  $\mu$ g/mL) then mounted with Vectashield (Vectorlabs H-1000). Images were acquired on a Zeiss LSM700 confocal microscope at 63x magnification. Image processing was performed using Zen SP5 software. Experiments were performed in biological triplicate. A total of n = 144 nuclei were quantified in 0% 1,6-hexanediol.

## **QUANTIFICATION AND STATISTICAL ANALYSIS**

## Native mass spectrometry data analysis

MagTran (Zhang and Marshall, 1998) and PeakSeeker (Lu et al., 2015) were used to process collected spectra. The *m/z* values were converted to charge state envelopes. The zero-charge state of each species was calculated from the charge state envelopes. Primary sequences were input into ExPASy ProtParam (https://web.expasy.org/protparam) to calculate average protein masses.

## **Quantification of flow-stretching assays**

DNA compaction trajectories were determined by fitting the positions of quantum dots using Gaussian fitting with customwritten MATLAB (MathWorks) codes that have provided in the previous publication (Kim and Loparo, 2016). From the trajectories, the positions and time points of the compaction initiation and completion were determined and its compaction rates were quantified.

## **Chromatin compaction data analysis**

MORC-1 mediated chromatin compaction was determined by measuring the change in the flow stretched DNA length using customwritten MATLAB (MathWorks) code (Song et al., 2016). Briefly, to improve the signal-to-noise ratio, five frames (corresponding to 0.5 s of the movie) were averaged. A region of interest (ROI) containing fluorescence signal was determined manually for each DNA molecule. For each averaged image, the pixels in the ROI were summed along the short axis of the DNA molecule to generate a fluorescence intensity line profile. The position of the free DNA end was then determined in each averaged image by applying a threshold of 50% of the maximum intensity after subtraction of the baseline; linear interpolation was used to determine this position to sub-pixel resolution. A similar procedure was followed to determine the position of the tethered end of the DNA, except that all line profiles were averaged and the position was determined from the resulting averaged profiles. Rates of compaction were determined from DNA length trajectories as described for quantum dot labeled DNAs.

## **STORM data analysis**

The longest diagonal distance of each MORC-1 punctate was measured by ImageJ (Schneider et al., 2012). Ninety six and fifty two random but discrete puncta of mitotic and meiotic puncta were measured respectively.

## **Hexanediol data analysis**

Quantification was performed in a binary fashion. Based upon the red signal (anti-FLAG), MORC-1 was categorized as either punctate (1) or diffuse (0) as determined by eye. To remove bias, in each germline, the six most proximal pachytene nuclei were used for quantification. The same nuclei were used for P granule quantification for consistency, which was also done in a binary fashion. If one P granule remained, that nucleus was designated as "P granules present."

## **Quantification of circular DNA binding assay**

Quantification was performed using Fiji (Schindelin et al., 2012). Intensities were averaged if there were replicates within the experiment, and normalized to the value obtained for open circular.

## DATA AND CODE AVAILABILITY

All codes are available upon request. Raw data have been deposited to Mendeley Data (https://doi.org/10.17632/876gx33wm2.1).