The BAH domain of ORC1 links H4K20me2 to DNA replication licensing and Meier–Gorlin syndrome

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The recognition of distinctly modified histones by specialized ‘effector’ proteins constitutes a key mechanism for transducing molecular events at chromatin to biological outcomes1. Effector proteins influence DNA-templated processes, including transcription, DNA recombination and DNA repair; however, no effector functions have yet been identified within the mammalian machinery that regulate DNA replication. Here we show that ORC1—a component of ORC (origin of replication complex), which mediates pre-DNA replication licensing2—contains a bromo adjacent homology (BAH) domain that specifically recognizes histone H4 dimethylated at lysine 20 (H4K20me2). Recognition of H4K20me2 is a property common to BAH domains present within diverse metazoan ORC1 proteins. Structural studies reveal that the specificity of the BAH domain for H4K20me2 is mediated by a dynamic aromatic dimethyl-lysine-binding cage and multiple intermolecular contacts involving the bound peptide. H4K20me2 is enriched at replication origins, and abrogating ORC1 recognition of H4K20me2 in cells impairs ORC1 occupancy at replication origins, ORC chromatin loading and cell-cycle progression. Mutation of the ORC1 BAH domain has been implicated in the aetiopathology of Meier–Gorlin syndrome (MGS)3,4, a form of primordial dwarfism, and ORC1 depletion in zebrafish morphants. Moreover, zebrafish depleted of H4K20me2 have diminished body size, mirroring the phenotype of orc1 morphants. Together, our results identify the BAH domain as a novel methyl-lysine-binding module, thereby establishing the first direct link between histone methylation and the metazoan DNA replication machinery, and defining a pivotal aetiological role for the canonical H4K20me2 mark, via ORC1, in primordial dwarfism.

The identification of protein modules that recognize the broad spectrum of modifications present on histone proteins is critical for understanding how chromatin dynamics influence fundamental nuclear processes. The BAH domain is an evolutionarily conserved chromatin-associated motif5. In budding yeast, ORC1—a component of ORC (origin of replication complex)—contains a bromo adjacent homology (BAH) domain that specifically recognizes the canonical H4K20me2 mark, via ORC1, in primordial dwarfism. In both of these yeast species, it is known that ORC1 binds directly to DNA sequences at origins of replication, whereas in metazoans, chromatin has a more significant role in directing ORC to replication origins2,13,14.

To understand the molecular basis of the ORC1 BAH domain recognition of H4K20me2, the crystal structure of the complex between the H4(14–25)K20me2 peptide and the ORC1 BAH domain of mouse ORC1 (Fig. 1f). H4K20me2 recognition is a common activity of BAH domains of ORC1 from several different metazoans. In contrast, the BAH domains of ORC1 from yeast Saccharomyces cerevisiae (scORC1BAH) and Schizosaccharomyces pombe lack H4K20me2-binding activity. In both of these yeast species, it is known that ORC1 binds directly to DNA sequences at origins of replication, whereas in metazoans, chromatin has a more significant role in directing ORC to replication origins2,13,14.

The preference of hORC1BAH for H4K20me2 over its me1/3 counterparts (see Fig. 1c) probably reflects the contribution from the hydrogen bond formed between the dimethylammonium proton and the side chain of an aromatic cage located at the mORC1 BAH surface, and composed of Tyr 63, Trp 87, Tyr 114 and Trp 119, and the dimethylammonium proton is hydrogen bonded to the side chain of GLu 93 (Fig. 2d), similar to H4K20me2 bound to the 53BP1 Tudor domain16 (Supplementary Fig. 2). The aromatic cage in mORC1BAH is absent in the published structure of scORC1BAH (Supplementary Fig. 3)15, explaining why scORC1BAH does not bind H4K20me2 (Fig. 1f).

The preference of hORC1BAH for H4K20me2 over its me1/3 counterparts (see Fig. 1c) probably reflects the contribution from the hydrogen bond formed between the dimethylammonium proton and the side chain carboxylate of Glu 93 (Fig. 2c). Indeed, previous studies demonstrated that a single Tyr-to-Glu substitution within an otherwise aromatic cage of the bromodomain and PHD domain transcription factor (BPTF) plant homeo domain (PHD) finger reverses the binding preference from trimethyl- to dimethyl-lysine17. Engineering a binding preference for H4K20me1 would probably require incorporation of a second carbonylate group to line the aromatic cage of hORC1BAH and facilitate hydrogen bond formation with both monomethylammonium protons. The high degree of specificity of mORC1BAH for the sequence surrounding H4K20 is conferred by a series of intermolecular...
are also observed (Fig. 2b, c). In this regard, acetylation at K16 slightly
bridge between Lys 16 of the H4 peptide and Glu 126 of mORC1 BAH,
hydrogen bond involving the imidazole ring of His 18, as well as a salt
interactions represent the majority of contacts, a water-mediated
channels of mORC1 BAH (Fig. 2b, c). Although backbone–backbone
hydrogen-bonding interactions involving Lys 16, His 18, Val 21 and
arginine 23 of the bound peptide and residues lining the peptide-binding
channel of mORC1 BAH (Fig. 2b, c). Although backbone–backbone
interactions represent the majority of contacts, a water-mediated
hydrogen bond involving the imidazole ring of His 18, as well as a salt
bridge between Lys 16 of the H4 peptide and Glu 126 of mORC1 BAH,
are also observed (Fig. 2b, c). In this regard, acetylation at K16 slightly
reduces the binding affinity of hORC1 BAH and mORC1 BAH for the
H4K16acK20me2 peptide (Supplementary Fig. 4).
To investigate the structural dynamics of H4K20me2 binding, we
determined the 1.70 Å crystal structure of mORC1 BAH in the free state
and using the indicated peptides. aa, amino acids.

hORC1 BAH binds with highest affinity to H4K20me2. ITC was used to
quantify the binding affinity of hORC1 BAH for the H4K20me2 peptide
with a Kd of 9.6 μM, with the binding affinity towards H4K20me1 and
H4K20me3 weaker by a factor of 2 to 3 (Kd = 32.7 μM and 17.4 μM,
respectively; Supplementary Fig. 6a). The mORC1 BAH domain shows
moderate sequence conservation through evolution, with relatively
high conservation of residues lining the H4K20me2-binding pocket
(Supplementary Fig. 6b); hORC1 BAH retains all the aromatic cage
residues that recognize H4K20me2, except for Trp 119 of mORC1 BAH,
which is substituted by Cys 120 in hORC1 BAH. Replacing mORC1 BAH
Trp 119 with a cysteine (human counterpart) resulted in increased
discrimination of H4K20me2 over H4K20me3 from twofold to three-
fold, approaching the eightfold discrimination observed for hORC1 BAH
(Supplementary Fig. 6a). However, given that this mutant could not fully
recapitulate the H4K20me2-binding specificity of hORC1 BAH (Sup-
plementary Fig. 6a), other residues probably contribute to H4K20me2
discrimination. Indeed, a C120A substitution in hORC1 BAH decreases
binding to all three methylation states of H4K20me2, with retention of
H4K20me2 specificity (Supplementary Fig. 6a).

ITC analysis also demonstrated that alanine substitutions of the
remaining residues lining the H4K20me2-binding pocket of hORC1 BAH
(Y64A, W88A, E94A and Y115A) abolished or largely diminished
H4K20me2 recognition (Fig. 2f). Similar results were observed with hORC1 BAH and mORC1 BAH cage mutants in peptide-
binding assays (Fig. 2g and Supplementary Fig. 6c) and in histone-
binding assays (Fig. 2h). These results further support the structural
analysis and establish the molecular basis for the H4K20me2–
ORC1 BAH interaction.

H4K20me2 is an abundant H4 modification18, and the BAH domain
of ORC1, although dispensable for ORC complex assembly, has been
shown to be important for loading of the complex onto chromatin in
humans cells19. We therefore postulated that the interaction between
ORC1 and H4K20me2 might regulate ORC stabilization at chromatin.
First, ORC components (ORC2, ORC3 and ORC5) affinity purified with
two structure-guided H4K20me2-binding mutants (hORC1(Y64A)
and hORC1(W88A)) with an efficiency equal to that observed with the
wild-type protein in both U2OS (Fig. 3a) and HT1080 (Supple-
mentary Fig. 7a) human cell lines, suggesting that H4K20me2 binding by
ORC1 is dispensable for ORC complex assembly. Next, analysis of lysates
biochemically separated into chromatin-enriched and soluble fractions
from cells stably expressing hORC1, hORC1(Y64A) or hORC1(W88A)
demonstrated that hORC1(Y64A) and hORC1(W88A) enrichment at
chromatin was considerably reduced in comparison to hORC1 (Fig. 3b
and Supplementary Fig. 7b). Moreover, chromatin association of the
ORC components ORC2, ORC3, ORC5 and ORC6 was compromised in
cells expressing the ORC1 H4K20me2-binding-pocket mutants
(Fig. 3c and Supplementary Fig. 7c). Thus, hORC1 BAH binding to
H4K20me2 is required for efficient stabilization of ORC1 and other
ORC components at chromatin.

Local chromatin structure is thought to have a role in the mech-
anism that determines mei zoan origins of replication13. In this con-
text, chromatin immunoprecipitation (ChIP) assays with a highly
specific H4K20me2 antibody in G1-synchronized U2OS cells (Sup-
plementary Figs 8 and 9) demonstrated an increase in the H4K20me2
signal at two defined human replication origins20–23 relative to adjacent
sequences (Fig. 3d and Supplementary Fig. 10). Moreover, like the pattern of H4K20me2, hORC1 occupancy peaked at origins relative to adjacent sequences (Fig. 3e and Supplementary Fig. 10). In contrast, hORC1(Y64A) and hORC1(W88A) enrichment at origins was not observed, even though the H4K20me2 peak at origins is present in these cell lines (Figs 3d, e and Supplementary Fig. 10). These data suggest that H4K20me2 may have a role at human origins by stabilizing ORC1 at the surface of mORC1BAH. The equivalent cage residues in hORC1BAH are labelled in parentheses. e, Structural overlay of the mORC1BAH K20me2-binding aromatic cage in the free (silver) and H4K20me2-bound states (salmon). The curved arrow indicates binding-induced structural shift. f, Mutations in the hORC1BAH K20me2-binding channel impair H4K20me2 recognition. ITC analysis of the indicated hORC1BAH mutants binding to H4K20me2 peptide; s.d. derived from nonlinear fitting. g, h, Mutations in the hORC1BAH dimethyl-lysine-binding cage abrogate H4K20me2 recognition. g, Binding assays as in Fig. 1b with the indicated hORC1BAH mutant proteins and biotinylated peptides. h, Top, western blot analysis of CTH-binding assays as in Fig. 1e with the indicated proteins and antibodies. PD, GST pull-downs. Bottom, Coomassie blue stain of input GST-fusion proteins and histones (20% of total). WT, wild type.

Figure 2 | The molecular basis of H4K20me2 recognition by ORC1BAH.

a–c, 1.95 Å crystal structure of mORC1BAH complexed with H4(14–25)K20me2 peptide. a, Ribbon representation of mORC1BAH bound to H4K20me2 peptide. The mORC1BAH (cyan) and the bound H4K20me2 peptide (yellow) are shown in ribbon and stick representations, respectively. Yellow indicates the hydrophobic contact. b, Details of intermolecular contacts in the mORC1BAH–H4K20me2 complex. mORC1BAH and H4K20me2 peptide residues are coloured in pink and yellow, respectively, with hydrogen bonds depicted as red dashed lines, and a water molecule (W) as a red sphere. c, Schematic representation of intermolecular contacts in the mORC1BAH–H4K20me2 complex. The residues from the H4K20me2 peptide and mORC1BAH are coloured in magenta and black, respectively. Yellow indicates the hydrophobic contact. d, Positioning of the K20me2 side chain within an aromatic cage of the indicated residues (red) on the surface of mORC1BAH. The equivalent cage residues in hORC1BAH are labelled in parentheses. e, Structural overlay of the mORC1BAH K20me2-binding aromatic cage in the free (silver) and H4K20me2-bound states (salmon). The curved arrow indicates binding-induced structural shift. f, Mutations in the hORC1BAH K20me2-binding channel impair H4K20me2 recognition. ITC analysis of the indicated hORC1BAH mutants binding to H4K20me2 peptide; s.d. derived from nonlinear fitting. g, h, Mutations in the hORC1BAH dimethyl-lysine-binding cage abrogate H4K20me2 recognition. g, Binding assays as in Fig. 1b with the indicated hORC1BAH mutant proteins and biotinylated peptides. h, Top, western blot analysis of CTH-binding assays as in Fig. 1e with the indicated proteins and antibodies. PD, GST pull-downs. Bottom, Coomassie blue stain of input GST-fusion proteins and histones (20% of total). WT, wild type.
test this hypothesis, an orc1 zebrafish morphant reconstitution system was established. Injection of two independent orc1-targeting morpholino oligonucleotides (MOs) resulted in growth retardation, recapitulating published results1 (Fig. 4d, e and data not shown). Co-injection of human ORC1 messenger RNA with zebrafish orc1-targeting MOs attenuated the dwarf phenotype observed in the orc1 morphant alone (Fig. 4d, e). In contrast, co-injection of hORC1(Y64A) and hORC1(W88A) mRNA failed to rescue orc1 morphants, moderately aggravating the growth retardation phenotype (Fig. 4d, e).

The H4K20me2/3 lysine methyltransferases (KMTs) Suv420h1 and Suv420h2 are conserved in zebrafish18. As shown in Fig. 4f, injection of MOs targeting Danio rerio suv420h1 and suv420h2 resulted in global depletion of H4K20me2 and H4K20me3, and an increase in H4K20me1; these changes in H4K20 methylation are similar to those observed in cells derived from Suvsuv420h1/h2 double-knockout mice18. Analysis of body size in the suv420h1/h2 morphants demonstrated

Figure 3 | ORC1–H4K20me2 interaction regulates ORC chromatin association and cell-cycle progression. a, Western blot analysis with the indicated antibodies of wild-type (WT) and H4K20me2-binding-pocket mutants (Y64A and W88A) affinity-purified Flag-tagged ORC1 complexes from U2OS cells. Control, empty vector control immunoprecipitate (IP), WCE, whole cell extract. b, The ORC1_BAH–H4K20me2 interaction is required for efficient ORC1 chromatin association. Western blot analysis of lysates biochemically separated into chromatin-enriched and soluble fractions from U2OS cells stably expressing the indicated ORC1 protein. Quantification of Flag–ORC1 levels is shown. Control, empty vector control lysates. Tubulin and H3 levels are shown as control for the integrity of fractionation. c, Disruption of ORC1 binding to H4K20me2 destabilizes ORC chromatin association. Western blot analysis of biochemically purified chromatin from U2OS cells as in b with the indicated antibodies. Total ORC protein levels in WCE are shown. d, H4K20me2 is enriched at DNA replication origins. H4K20me2 signal with the indicated antibodies and cell-cycle progression. a

Figure 4 | Disruption of the ORC1_BAH–H4K20me2 interaction leads to dwarfism in zebrafish. a–c, MGS-associated mutations F889 and E127G impair H4K20me2 binding by hORC1. a, Close-up view of mORC1_BAH bound to H4K20me2, with residues F88 and E126 (equivalent to F89 and E127 in hORC1, respectively) shown in stick representation. b, ITC analysis as in Fig. 1d of F89S and E127G hORC1_BAH mutants binding to H4K20me2 peptides. ITC data for wild-type hORC1_BAH from Fig. 1d. c, Binding assays as in Fig. 1b with the indicated hORC1_BAH mutant proteins and biotinylated peptides. d, e, The H4K20me2-recognition activity of hORC1 is required to rescue the dwarfism phenotype of orc1 morphants. Quantification of dwarf phenotype in zebrafish injected with MOs targeting the orc1 translation start site alone or MO co-injected with the indicated hORC1 mRNAs. Insert on left shows electrophoresis analysis of the indicated hORC1 mRNAs used for reconstitution. Control, uninjected embryos. Dwarfism was defined as a reduction of body length of ≏3 s.d. relative to the average size of the control zebrafish. Dwarfism analysed: control, 26; MO, 85; MO + hORC1(Y64A), 85; MO + hORC1(W88A), 83. P values are calculated with a two-tailed unpaired Student’s t-test. *P < 0.01, **P < 0.001. e, Representative images of zebrafish in 1 day post-fertilization (dpf). f, Depletion of H4K20me2/3 in suv420h1/suv420h2 morphants. Western blot analysis using the indicated antibodies of whole animal extracts 1 dpf from control zebrafish or zebrafish injected with MOs targeting the translation start sites of suv420h1 and suv420h2. Control, uninjected embryos. g, h, Dwarfism in suv420h1/suv420h2 morphants. Quantification of dwarfism in suv420h1/suv420h2 morphants relative to controls as described in d. Zebrafish analysed: control, 38; MO, 60. **P < 0.01. h, Representative images of zebrafish in 1 dpf. i, j, k, l, m, n, o, p, q, r, s, t, u, v, w, x, y, z.
metazoan ORC1 proteins. To our knowledge, these results provide the first direct link between histone methylation and the metazoan DNA replication machinery. The mechanism that determines metazoan origins of replication is thought to be dependent upon both the information encoded in the DNA sequence at origins, as well as the local chromatin architecture. Several histone modifications have been detected at origins, including H3K27me1, which regulates replication of DNA at heterochromatin in Arabidopsis. The mark H4K20me1 is present in early G1 at human origins and probably serves as the chromatin template for H4K20me2 catalysis. Because H4K20me1 is generated in a highly cell-cycle-regulated manner by the KMT PR-Set7 (also known as SET8), this modification may regulate licensing by governing the temporal and spatial availability of H4K20me2 at origins. We postulate that the recognition of H4K20me2 by ORC1 cooperates with other ORC chromatin-loading mechanisms in marking replication origins. On the basis of this model, during developmental phases requiring rapid cell division, inefficient pre-RC formation due to abrogation of the ORC–H4K20me2 interaction would result in delayed cell-cycle progression and insufficient cellular proliferation, a characteristic of proportional ‘hypocellular’ dwarfism disorders like MGS. Together, our findings reveal a new function for histone methylation signalling at chromatin in the regulation of DNA replication and organisinal growth.

METHODS SUMMARY

Materials, peptide microarray experiments, binding assays, structure analysis and crystallization conditions, and zebrafish experiments and data analysis are described in detail in Methods. For the zebrafish experiments, wild-type AB zebrafish (D. rerio) were maintained and raised using standard protocols. All zebrafish were treated in accordance with AAALAC approved guidelines at Stanford University (protocol number 10511).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions A.J.K. and P.C. performed the molecular biology, cellular and zebrafish studies; J.S. performed structural and binding affinity studies; S.Y. and J.K.C. advised on zebrafish experiments; S.I.-M. assisted in protein production and crystallization. A.J.K., P.C., J.S., D.J.P. and O.G. designed studies, analysed data and wrote the paper. All authors discussed and commented on the manuscript.

Author Information Atomic coordinates have been deposited with the Protein Data Bank under accession codes 4DOV (free structure) and 4DOW (H4K20me2-bound mORC1BAH) for the reported crystal structures. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to D.J.P. (pateld@mskcc.org) or O.G. (ogozani@stanford.edu).
METHODS
Materials and plasmids. Biotinylated peptides were synthesized at Stanford or Yale Protein and Nucleic Acid facilities as previously described. Antibodies used in this study: anti-histone H3 (Abcam), anti-Flag M5 (Sigma), anti-GST (Abcam), anti-tubulin (Upstate), anti-ORC2 (Upstate), anti-ORC3 (Abcam), anti-ORC5 (Sigma), anti-ORC6 (Abcam), anti-H4 (Abcam), anti-H4K20me1 (Abcam), anti-H4K20me2 (Abcam), anti-H4K20me3 (Abcam). ORC1 BAH domain was cloned into pGEX6P-1 for _in vitro_ binding experiments, pBabe-puro-3×Flag for generating cell lines stably expressing ORC proteins, pCDNA-HA-EGFP and pCAG-Flag ORC1 for transfection in WI-38 cells, pcDNA for producing _in vitro_ transcribed mRNA for zebrafish microinjection. Site-directed mutagenesis was performed to introduce point mutations (Stratagene), when further supplemented with 0.2 M 3-(1-pyridin)-1-propanol (Hampton Research). The crystals for the production of the crystals of free mORC1BAH even though the H4(14–25)K20me2 peptide was present in the crystallization solution. The crystals for both free and H4(14–25)K20me2-bound mORC1BAH domain were soaked in cryoprotectant made of mother liquor supplemented with 25% glycerol, before flash freezing in liquid nitrogen.

Structure determination. X-ray diffraction data sets for both free and H4(14–25)K20me2-bound mORC1BAH domain were collected at sodium peak wave-length on the 24-IDE NE-CAT beamline at the Advanced Photo Source, Argonne National Laboratory. The diffraction data were indexed, integrated and scaled using the HKL2000 program. The structure of free mORC1BAH was solved by the single-wavelength dispersion method with selenium atoms using the AutoSol procedure refined in PHENIX. The molecular replacement model for the free mORC1BAH domain was refined using PHENIX. The final model was refined to 1.70 Å resolution. The structure of H4K20me2(14–25)-bound mORC1BAH domain was solved by the molecular replacement method in Phaser using the free structure of the mORC1BAH domain as a search model. The H4(14–25)K20me2 peptide was then modelled in COOT and the structure of the H4(14–25)K20me2-mORC1BAH domain was refined using PHENIX. The final model of the complex was refined to 1.95 Å resolution. For both free and H4(14–25)K20me2-mORC1BAH domain structures, the B factors were data for refined collection and structural refinement for both free and H4(14–25)K20me2-bound mORC1BAH domain are summarized in Supplementary Table 1.

ITC measurements. Protein and peptide samples used for ITC measurements were subject to overnight dialysis against buffer containing 20 mM Tris-HCl, 100 mM NaCl, 2 mM DTT, pH 7.5. Before the measurement, the protein and peptide concentrations were adjusted to about 0.1 mM and 1 mM, respectively. The ITC experiment was carried out using a MicroCal iTC200 instrument at 5 °C. The titration curves were analysed using software Origin7.0 (MicroCal, ITC200).

Small-scale biochemical fractionation. Small-scale biochemical fractionation was performed from a protocol described previously. In short, 1 × 10^7 to 2 × 10^7 U2OS and HT1080 cells were collected, washed with PBS, and resupended in buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 1.5 mM MgCl2, 0.34 M sucrose, 10% glycerol, 1 mM DTT, complete protease inhibitor tablet (Roche)). Triton X-100 was added to a final concentration of 0.1%. Cells were incubated for 5 min, and nuclei were collected by centrifugation (1,300g, 4 °C, 5 min). The supernatant (S1) was clarified by centrifugation at 20,000 g for 5 min. The nuclei were washed once with buffer A and lysed in buffer B (3 mM EDTA, 0.2 mM EGTA, 1 mM DTT, complete protease inhibitor tablet) for 30 min. Chromatin was collected by centrifugation (1,700g, 4 °C, 5 min). The soluble fraction was combined with S1 and boiled at 90 °C with SDS sample buffer. Chromatin was washed with buffer B once and resuspended in SDS sample buffer, boiled at 90 °C for 10 min.

Immunoprecipitation. HT1080 and U2OS cells stably expressing Flag-ORC1 or Flag–ORC1 mutants were generated by retroviral transduction. Cells were lysed in cell lysis buffer containing 50 mM Tris-HCl, pH 7.4, 250 mM NaCl, 0.5% Triton X-100, 10% glycerol, 1 mM DTT, complete protease inhibitor tablet (Roche). Flag–ORC1 complexes were affinity purified by incubating anti-Flag M2 monoclonal-antibody-conjugated agarose beads (Sigma) in lysates overnight at 4 °C. Fractions were collected by centrifugation at 1,700g for 5 min. The nuclei were washed three times with cell lysis buffer and bound protein eluted into SDS sample buffer for western blot analysis.

ChIP analysis. ChIP was performed as previously described. The primer sequences used in this study were: MCM4, −5 kbp reverse, TCCAGTGCTAAAGCCCTGGGAT; MCM4 origin, reverse, TTGGTGTGCTTATCTGTGTTT; MCM4 origin, reverse, TTGGGGCTCAGTCTTGTGTTT; MCM4, +5 kbp forward, TTTTGAATCTTGTTAGC; MCM4, +5 kbp reverse, CAGCCTTTGTCGTTGCAAG; β-globin, −40 kbp forward, AGTCTCAGGGCTCTAAAGAGG; β-globin, −40 kbp reverse, CTGAGATCTCTTGAGTTGAG; β-globin, −40 kbp reverse, AGTCTCAGGGCTCTAAAGAGG; β-globin, −40 kbp forward, CAGCCTTTGTCGTTGCAAG; β-globin, −40 kbp reverse, CTGAGATCTCTTGAGTTGAG; β-globin, −40 kbp forward, CAGCCTTTGTCGTTGCAAG; β-globin, −40 kbp reverse, CTGAGATCTCTTGAGTTGAG; β-globin, −40 kbp forward, CAGCCTTTGTCGTTGCAAG; β-globin, −40 kbp reverse, CTGAGATCTCTTGAGTTGAG; β-globin, −40 kbp forward, CAGCCTTTGTCGTTGCAAG; β-globin, −40 kbp reverse, CTGAGATCTCTTGAGTTGAG; β-globin, −40 kbp forward, CAGCCTTTGTCGTTGCAAG; β-globin, −40 kbp reverse, CTGAGATCTCTTGAGTTGAG; β-globin, −40 kbp forward, CAGCCTTTGTCGTTGCAAG; β-globin, −40 kbp reverse, CTGAGATCTCTTGAGTTGAG; β-globin, −40 kbp forward, CAGCCTTTGTCGTTGCAAG; β-globin, −40 kbp reverse, CTGAGATCTCTTGAGTTGAG.

Zebrafish aquaculture and microinjection. Wild-type AB zebrafish (_Danio rerio_) were maintained and raised using standard protocols. All experiments were conducted in accordance with AALAC approved guidelines at Stanford University (protocol number 10511). Embryos obtained from natural matings were microinjected at the 1- to 2-cell stage with 1.5 nl of 0.18 mM sequence-specific orf-morpholino or 0.36 mM _suv420h1/h2_ combined morpholinos at 1:1 ratio targeting each gene's translation start site (GeneTools). For epistasis analyses, 1.5 nl of _suv420h1_ morpholino solution containing 0.18 mM _orc1_ morpholino and 0.36 mM _orc1_ morpholino was microinjected. The antisense morpholino for _orc1_ was used for microinjection. _orc1_, TCAGCTTGTGAGTATAGGCGCTCAT (described in ref. 4); _suv420h1_, ATCGGGCTCAGTCTTATTTCAT; _suv420h2_, CACTGCTTCACCCATCATC; _suv420h2_, CACTGCTTCACCCATCATC. The morpholino targeting the _orc1_ splice-site junction: ACAACCTCATTACTGCTACCCCTACG.
experiments, capped full-length ORC1 wild-type or H4K20me2-binding-pocket mutant mRNAs were transcribed \textit{in vitro} following the manufacturer’s manual (Ambion). Ten picograms of mRNA was co-microinjected with \textit{orc1} morpholino at the 1-cell stage. Embryos were cultured in E3 medium at 28.5 °C for 24 h before scoring phenotypes. Live fish images were captured for individual fish using a Leica M205FA stereomicroscope and body length was calculated by Leica Application Suite program. Percentage of dwarf fish (average for control $\pm 3\times$ standard deviation of control) in each experiment group was scored and \textit{P} values between groups were calculated by unpaired two-tailed Student’s \textit{t}-test.

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