RESEARCH ARTICLE SUMMARY

STRUCTURAL BIOLOGY

Structural insights into histone binding a nucleosome assembly by chromatin assem

Chao-Pei Liu*†, Zhenyu Yu†, Jun Xiong†, Jie Hu†, Aoqun Song†, Dongbo Ding Mingzhu Wang, Juan Yu, Peini Hou, Kangning Zeng, Zhenyu Li, Zhuqiang Zhan Wei Li, Zhiguo Zhang, Bing Zhu*, Guohong Li*, Rui-Ming Xu*

INTRODUCTION:

. D A -

D A. H

. C -1 (CAF-1)

H3 H4

D A. A -

CAF-1 -

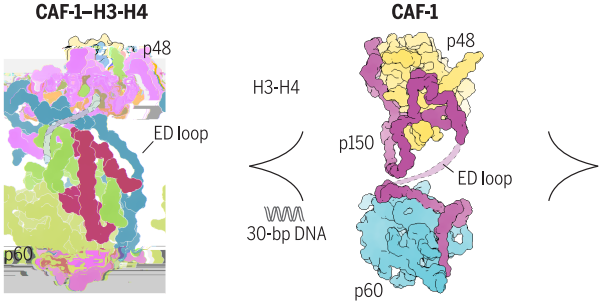
RATIONALE:

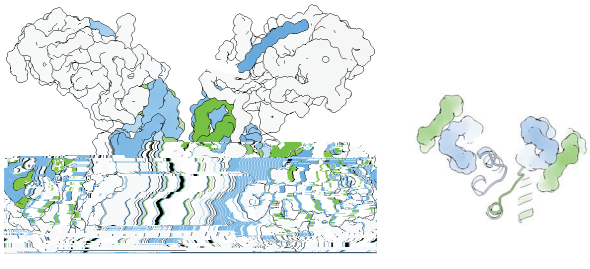
(-E)

CAF-1

(F)

RESULTS:





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RESEARCH ARTICLE

STRUCTURAL BIOLOGY

Structural insights into histone binding and nucleosome assembly by chromatin assembly factor-1

Chao-Pei Liu[1]*†, Zhenyu Yu[1]†, Jun Xiong[1]†, Jie Hu[1]†, Aoqun Song[1]†, Dongbo Ding[1]†, Cong Yu[1,2], Na Yang[3], Mingzhu Wang[4], Juan Yu[1], Peini Hou[1], Kangning Zeng[1,5], Zhenyu Li[6], Zhuqiang Zhang[1], Xinzheng Zhang[1,5], Wei Li[1,5], Zhiguo Zhang[7], Bing Zhu[1,5,8]*, Guohong Li[1,5]*, Rui-Ming Xu[1,2,5]*

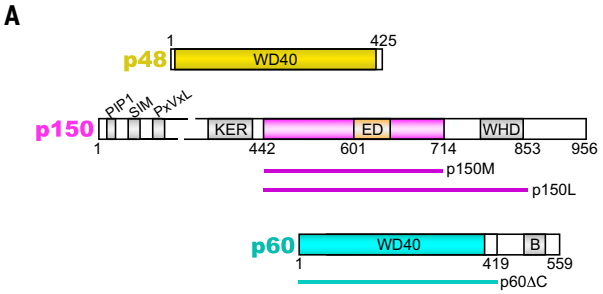
Chromatin inheritance entails de novo nucleosome assembly after DNA replication by chromatin assembly factor-1 (CAF-1). Yet direct knowledge about CAF-1's histone binding mode and nucleosome assembly process is lacking. In this work, we report the crystal structure of human CAF-1 in the absence of histones and the cryo–electron microscopy structure of CAF-1 in complex with histones H3 and H4. One histone H3-H4 heterodimer is bound by one CAF-1 complex mainly through the p60 subunit and the acidic domain of the p150 subunit. We also observed a dimeric CAF-1–H3-H4 supercomplex in which two H3-H4 heterodimers are poised for tetramer assembly and discovered that CAF-1 facilitates right-handed DNA wrapping of H3-H4 tetramers. These findings signify the involvement of DNA in H3-H4 tetramer formation and suggest a right-handed nucleosome precursor in chromatin replication.

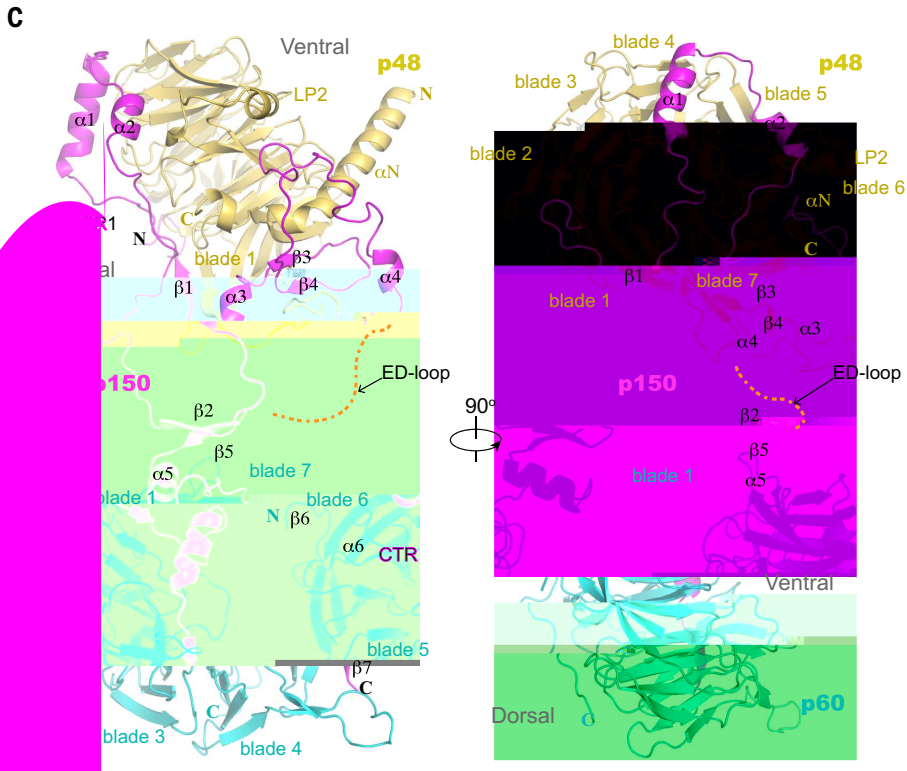
T _ _ - _ (C), 147 _ _ ( ) D A _ _ _ _ H3, H4, H2A,_ H2B _ -_ (*1*). D D A _ , _ _ _ _ _ _ - _ D A _ (*2*, *3*). H _ - _ _ _ ,_ _ _ D A , H3-H4 _ (*4*–*6*). _ - _ _ _ D A _ _ _ , H3_ H4_ - D A _ _ , _ H2A-H2B C . _ _ -1 (CAF-1) D A _ – _ , _ _ (*7*–*9*). D , _ - CAF-1, ,_ _ . CAF-1 _ _ _ _ 40 D A _ (*7*). H3.1-H4 _ _ _ _ _ - _ _ H3-H4 _ A F1_ - _ _ _ (C A) (*10*–*13*). H _ CAF-1 _ _ - _ _ (*14*), - _ (*15*, *16*). _ _ _ _ - _ _ _ (*12*, *13*, *17*–*19*). A - _ , CAF-1 _ _ _ _ _ – D A _ - _ (*20*, *21*)_ _ _ _ _ _ (*2*, *22*). H _ CAF-1 150 (CHAF1A), 60 (CHAF1B),_ 48 (BB 4/ A 48) - (*9*, *23*). 150 _ _ _ _ _ _ _ , D A- E _ HD _ _ _ ED _ _ - (*21*, *24*, *25*) (F . 1A). - _ 150 _ _ , _ _ C- _ _ - _ _ 60, - (*9*). B 60_ 48 _ D40- _ _ _ _ β- _ (F . 1A). 60 _ - _ D A _ _ _ _ _ _ _ - _ (*26*–*28*). _ _ C- _ A F1- _ (B _) _ _ _ _ _ H3-H4 CAF-1 (*29*). - _ , 60 H3-H4_ CAF-1' _ _ (*9*, *30*). 48 _ _ H3-H4 _ - _ - (*23*, *31*). _ _ _ CAF-1' _ - _ _ _ (*32*, *33*). , _ _ _ CAF-1 _ _ _ - (*30*, *34*–*36*). , _ - _ _ CAF-1 _ _ (-E) CAF-1 H3-H4. , _ _ CAF-1 _ -_ H3-H4 - _ , _ _ _ .

Results

Overall structure of the CAF-1 complex

_ CAF-1 - 150 _ _ _ 150 , _ _ _ ED _ -_ C- _ _ (_ C ,),_ 150 , _ _ C- _ (F . 1A). - _ _ _ (F . 1B), 150 , 60 _ C- _ 140- (60ΔC),_ - 48, CAF1- C _ _ - . _ (C222$_1$, C2,_ 2$_1$)_ 3.4- 3.6-





0 _ 48. 150 _ _ _ _ _ 48 β , (F . 1C). - _ , _ 463 _ 1, _ (β1), α (α1 α2), _ . 1 _ , _ _ _ 48 _ - _ . 48- _

150 , _ 2,_ - _ _ 548 606_ - 1 _ 15- _ _ 48 60. 2 _ α (α3_ α4)_ _ β _ (β3_ β4). 2 - _ 48 _ _ β _ β3_ _ 48' _ 7,_ _ β3-β4_ β4-α4 , _ _ _ _ _ _ , C- _ α 48 (F . 1C). _

1_ 2 _ _ 150 48. 60 _ - _ β _ _ - _ β-_ β4 _ 4_ β1 _ 5 _ _ ,_ β- β1_ β2 _ 6,_ _ C- _ _ _ _ _ β (F . 1C). C 150 _ 60 α (α5_ α6)_ β _ (β6_ β7), _ β5 _ _ _ _ β _ β2 . α5 150 C _ 60 _ _ 60 _ _ β - . β6-α6-β7 _ 150 C _ _ _ 5_ 6 60 β , β6_ β7 _ _ _ β _ β4 _ _ 6_ _ 5 60, , _ α6 _ _ - _ _ 6 (F . 1C).

_ _ _ CAF-1 _ _ _ _ _ , _ _ _ 48_ 60 _ CAF-1' _ _ _ .

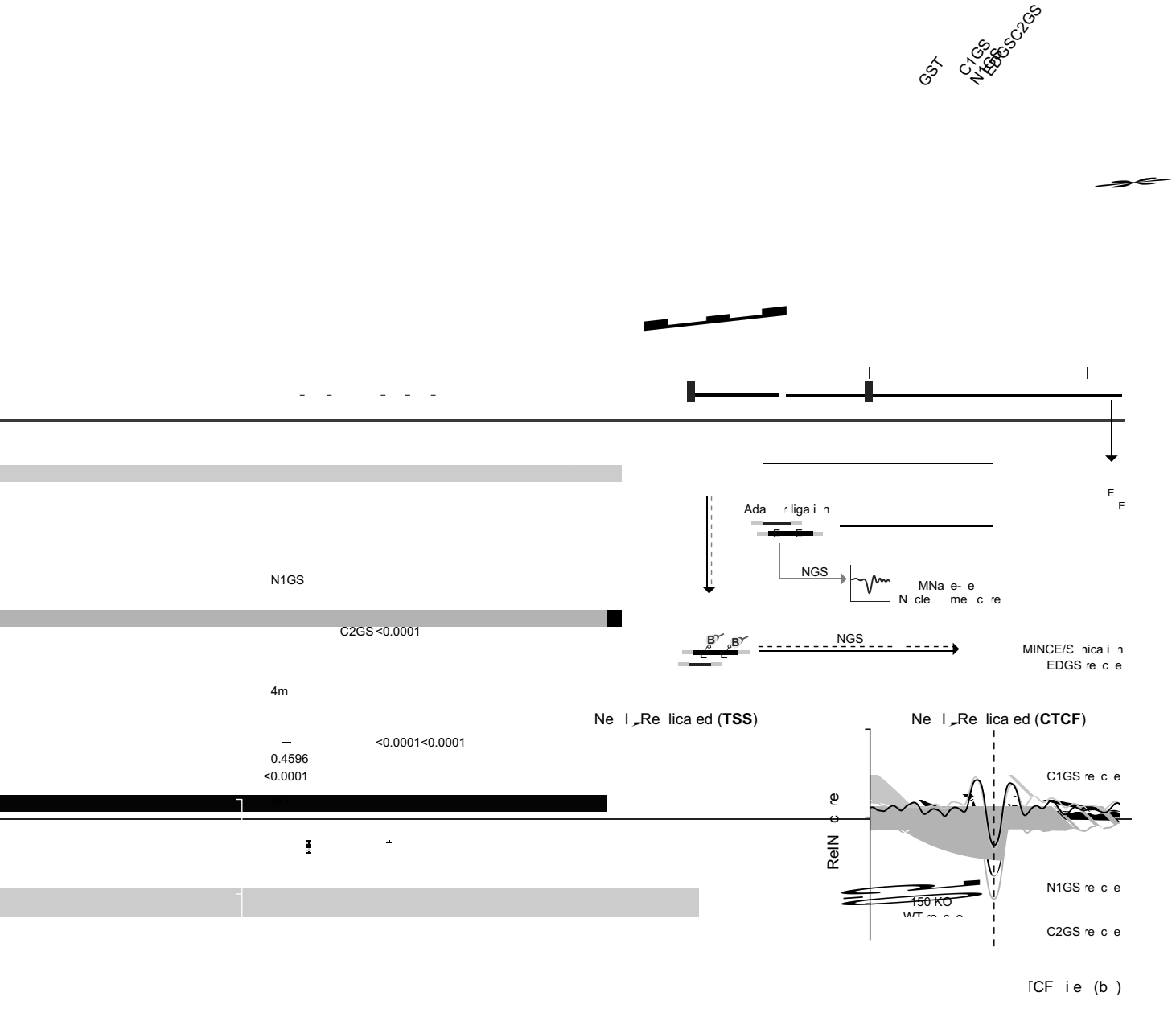
Structure of CAF-1 bound to histone H3-H4

E _ _ _ H3-H4 - _ _ _ CAF-1, G - _ D A _ _ (. 2), . -_ -E _ _ - CAF-1 (CAF1-FC)_ CAF1- C,_ CAF-1 150 _ HD _ , - 48,_ 60ΔC (F . 1B_ . 2B), _ H3-H4_ 3.6-_ 3.8- - , (. 3_ 4_ _ 2_ 3). _ E _ _ 3.8- _ . 5. _ _ _ _ _ _ ; E _ _ - _ _ 0.976,_ _ _ _ _ 3.5 (. 4_ _ 3). H , _ - - CAF-1 (CAF1-FC) _ CAF-1 (CAF1- C), _ CAF-1 _ , . , 150,_ - _ _ 491 713,_ D40 _ 60 (1 392) _ 48 (11 411)_ (F . 2, A B), _ CAF-1 _ . _ CAF-1 _ _ , _

_ _ _
CAF-1 _ _ _
H3-H4, _ ,_ _ _ / -
. _ _ 30-
D A (D A_{30}), _ _ _ _
_ _ _ -
_ _ -_ _ -
E _ _ _ .
A CAF-1_
_ H3-H4
(F . 2, A_ B). C _ _
, 48_ 60
_ , _ _ _ _
_ (F . 2C_
1). 60
_ _ , 48 _ _
_ _ _ 60 _ -
_ _ H3-H4.
F _ , 150 α4 -
_ _ 70 _ _ (F . 2C_
1). _ _ _ -
_ _ H3-H4 , 1
150 _ _ 48 -
_ , _
α1 _ .
1_ 2 _ , 2
48 _ _ _ _

(F . 2B). 50- -
_ C 150,
_ _ ED ,
H3-H4 (F . 2A_

ED- _ _ _ ,
_ _ H3-H4 _ _
_ _
_ H3-H4 ,
_ α1 H3_
H4 _ _ _ D A
(F . 3A). _ -
_ ED 150_ H3-H4
_ _
_ _ C 2_ H3-
H4 (. 6A) (*37–39*), _ _
_ _ _ H3-H4 -
_ D A D A
_ . _ _ _ _
CAF-1_ C 2 H3-H4 _
_ _ _ _ -
_ H3-H4 , (*40*).
_ - _ 32- ,
_ - ED
-E _ , _ - _ -
_
_ ED _ -
H3-H4 (. 5B). -
, _ _
ED _ _ .
-_ C- _ _ ED ,
ED- 1 (601 617)_ ED-C1 (-
618 632),_ _ C- _ _ -
, ED-C2 (617 646) _
α5A, _ _ - G ,
_ _ _ - _ -
_
_ (F . 3A). H α5A _
α1-α2 H3_ α2-α3 H4 (F .
2D). G - (F . 3B)_ -
(F . 3C_ . 6B)_ _
_ ED-C1G _ ED-C2G
_ _
_ _ CAF-1, _ _
_ ED- _
(EDG), _ ED- 1G -
_ _ _ . A _ -
_ _
ED-C1 4 , E628
(G $^{628}\rightarrow$), D629 , E630 ,_ D632G -
_ _ G -
_ _ _ (F . 3, B_ C).
F , _ ED- 1 _ _
H4_ ED-C1_ ED-C2
H3 (F . 3A), _ _
H3_ H4 _ . A_ -
ED – _ A ^{63},
A ^{69},_ A ^{83} H3 (H3-3A)_
A ^{35}, A ^{39},_ ^{44} H4 (H4-3A)
_ ,_ H3-3A_ 8 -.0303(63) /F4.6(4( ) 6.2(A))-6(33)-362.( )32.4(3)-3.9(376.222( ).5((-6(35.)-485(3)6) (3)8.7((33)9)


GST
C1GS
N1GS
EDGSC2GS
Ada ligai n
NGS
MNa e- e
N cle me c re
NGS
MINCE/S nica i n
EDGS re c e
N1GS
C2GS <0.0001
4m
Ne l Re lica ed (TSS)
Ne l Re lica ed (CTCF)
<0.0001<0.0001
0.4596
<0.0001
C1GS re c e
RelN c re
N1GS re c e
150 KO
C2GS re c e
TCF ie (b)

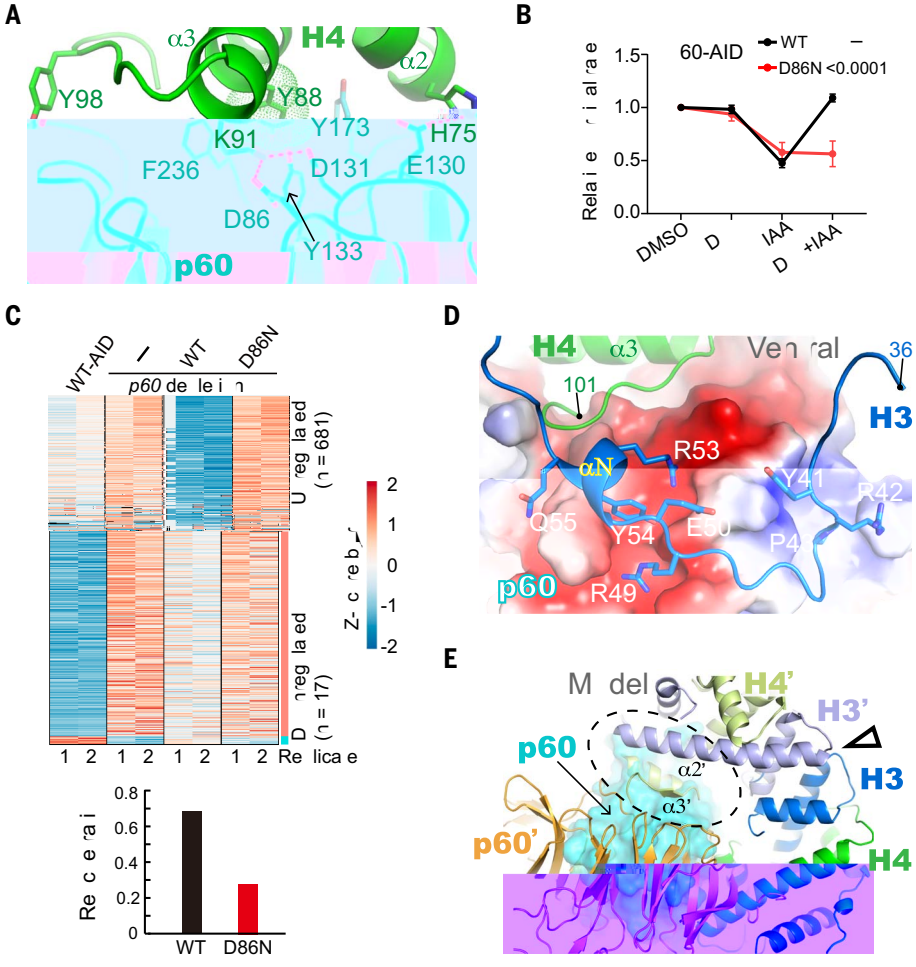


Fig. 4. Interactions between p60 and histone H3-H4. (**A**) Histone H4–p60 interaction at the center of the ventral surface of p60. Involved residues are shown in a stick model. Magenta dashed lines indicate potential hydrogen bonds. Tyr[88] of H4, which is situated in the central basin of the ventral face of p60, is superimposed with a dots model. F, Phe; P, Pro; Q, Gln; Y, Tyr. (**B**) Cell proliferation activity of WT p60 and the D86N mutation assayed in the same was as in Fig. 3D. Error bars represent SEM from three biological replicates. (**C**) Heatmaps showing differentially expressed genes (DEGs) in p60-depleted and rescued cells versus WT-AID HAP1 cells. Two biological replicates were performed for each experiment. The bottom panel shows the rescue ratios of transcriptional changes, which is defined as 1 − [#(*A*∩*B*)]/[#(*A*)], where *A* is the set of DEGs from p60 depletion and *B* is the set of DEGs from rescue expression of WT or D86N p60 constructs. The # function gives the number of genes in the set. (**D**) Interaction between histone H3 (dark blue) and p60. Histones H3-H4 are drawn in a cartoon model, with involved H3 residues depicted in a stick model. p60 is shown in a surface model colored according to electrostatic potential (negative, red; neutral, white; positive, blue) with the display range of −3 to +3 k_BT/e, where k_B is the Boltzmann constant and T and e are the temperature and electron charge in SI units, respectively. (**E**) Docking of a second CAF-1–H3-H4 complex (p60′, orange; H3′, light blue; H4′, light green) to model the formation of a H3-H4 tetramer between the H3′-H4′ heterodimer and the H3-H4 heterodimer from the first CAF-1–H3-H4 complex, which is colored as in Fig. 2B, results in steric clashes between H4′ and p60 (indicated with a dashed oval), and p60′ with p60 and H4. The black triangle indicates the H3-H3′ dimerization interface. p150 and p48 have been removed for viewing clarity.

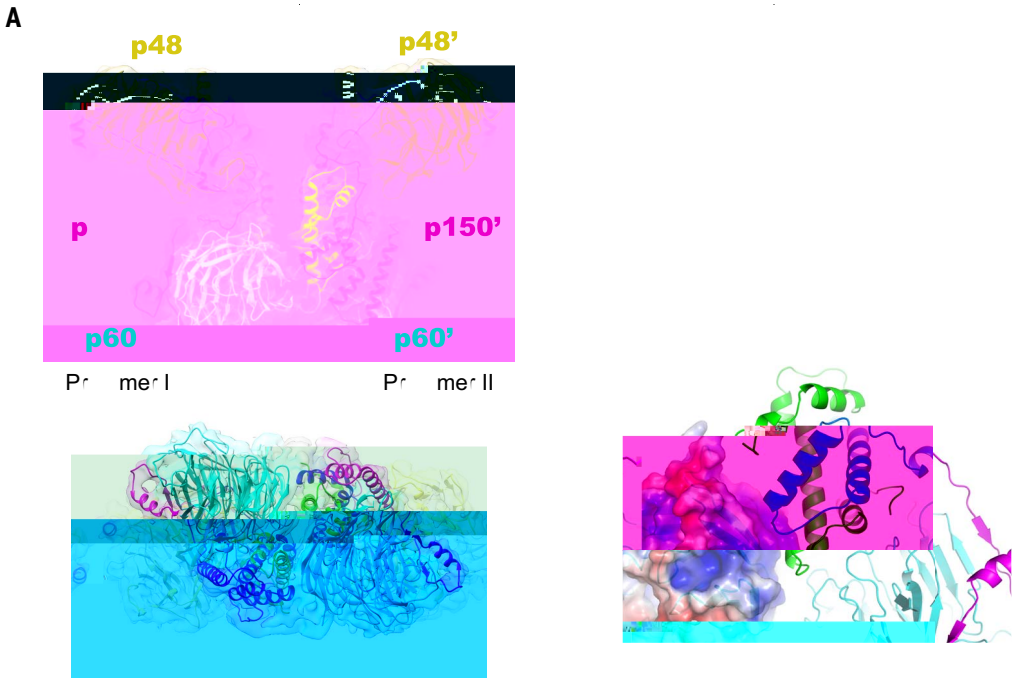
H3-H4 , H3-H4 H3-H4 (*51*). A H3-H4 - , - H3-H4

CAF-1 (*24*). CAF-1 H3-H4 - E C - CAF-1 H3-H4 (. 3 4). -E CAF-1 C FC 4.6 ,

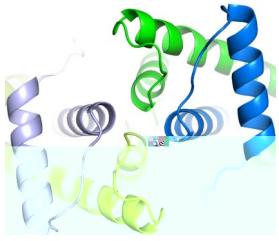
CAF-1–H3-H4 (F . 5A . 3 4). A EC- A ( ). 30- D A, -E , - CAF-1–H3-H4 - , C, C, FC CAF-1 (F . 5B . 8). A -E CAF-1–H3-H4 , D A . - CAF-1–H3-H4 . , 60 , 60 , C- β4 1 2 β3-β4 3, C- α2 H4 (F . 5C). H3 H3-H4 (F . 5D). H3-H4 - “ ” , 60. A 60- , D A, H3-H4 , CAF-1 (*8*, *35*, *52*).

CAF-1 facilitates the assembly of a right-handed ditetrasome

D H3-H4 D A CAF-1 D A H3-H4 . , CAF1- C, H3-H4, 147- 601 D A 2 50 C , 1 2, -E G F (. 9A). F 1 , CAF-1– - 3.5- 3.8- , - (. 9, B F; 2). 2 CAF-1 , 5.6 (. 10 2). - H3-H4 (*53*); , . -E - CAF-1 (. 11 3). C -E CAF-1 3.8- 6.6- , . B



–CAF-1– –CAF-1– C FC CAF-1 (. 11, E F). , - CAF1- C - . CAF-1– - - , 108- D A - H3-H4 1.5 - - (F . 6A . 12). E H3-H4 D A - , , , α2-α3 H4 (F . 6B). A , - H3 α2 H4 α3 H3-H4 - H3-H4 (F . 6B). - H3-H4 - CAF-1– , 45 D A - - (F . 6A). H3-H4 - 60 C- H4 α2 β , 2 3 (F . 6C). A , 60 D A H4 , 3 4. D A 60 - (E A) (. 13A). F - , α H3 H3-H4 D A, H4 α1 , H4 α2 H3- H4 (. 13B). - -



H3-H4 . H , D A. 60 β6-α6-β7 150 C , 60 CAF-1 CAF-1 H3-H4; , 150 48 (F . 6A). CAF-1 H3-H4 D A. A 5.6- -E 2 - - (. 10 2), 60- 150 - (. 10E). F , 2 48 60 D40 , (. 10C). A , , CAF-1, D A, H3-H4 (*8*). CAF-1– - D A . - , (F) D A 50 C . - , *et al.* (*54*),

- - - - - D A CAF-1.

Materials and methods

Plasmid construction

CAF-1 F B 1( ). - G 150 C- 6 H 60 48 . A G 150 . F - CAF-1 , D A - 150 - - A , D A 60 48 . A A 150 . - H3.1 H4 - CDFD 1 ( ). 150 60 H3.1 H4 - D- - (B), -101) , . A D A . A D A D2 (*44*), 1 D A 232 (C 1- , A , . 72834). 1(F74G) D A AA 1- CAG-F -E 2 (A , . 68460) C (B) F -E 2 . *AAVS1* C 2 (A , . 52961) . A A D- 2A- - *p150 p60* C -C 9- . *p150*-A D - (*59*), *p60*-A D - . *p150 p60* C 2. F 150 60 , *p150 p60* D A C - C 9 (A , . 50661) C C 9 , CA - 64 B (A , . 61425). 150 60 - (C).

Protein expression and purification

CAF-1 21 B - -B ( ). CAF-1 , . - CAF-1 293F (HE 293F) . A 500 HE 293F 0.5 CAF-1 (1:1:1) 2 . 48 37 C. CAF-1 - (CAF1- C) G - 150 (442 714), 60ΔC-H , 48-H , - 20 -HC , H 7.5, 500 C , 5 , 1 - (F) - , . C , A 30 4 C, . 500 . - , 1 ED A (D) - A , (G H) (GE H -) A-500 (20 -HC , H 7.5, 500 C , 1 ED A, 1 D) 2 . G H A-500, 30 G H. G - 100 C , (GE H) 0.1 2 C . E D - (D - AGE), CAF1- C - H 16/60 200 (GE H) A-500. E - CAF1- C - 20 / -80 C . CAF-1 (CAF1- C) - 150 (442-853) , HD , - , - . F - CAF-1 (CAF1-FC) - G (G) 2 , A-500. , A-500. - 6 10/300 G (GE H) A-2000, 2 C . C- 6 H - 48 60ΔC - A, - H , H 16/60 200 . H3.1-H4 - CDFD 1- H3.1-H4 *Escherichia coli* B 21 C (DE3) . - 0.5 -β-D- (G) 37 C 4 . H 5- (GE H), - 2 C .

Crystallization

- - - - CAF1- C - - 20 C 1.0 μ (7 10 /) 1.0 μ 8% (/) , H 6.0, 20% (/) EG 3350. - - 7% , H 6.0, 22% (/) EG 3350, 0.12 , 0.05 - 4 C. - - (3 5 /) 15 25% (/) EG3350 0 0.2 . - , 7% , H 6.0, 18% (/) EG 3350, 0.12 , 0.13 3 5 . C - - 15% .

X-ray diffraction data collection, structure determination, and refinement

D F (F) - B 17 315 CCD (AD C) 0.97915 B 19 6 0.97853 . D - H 2000 (*60*). $C222_1$, 2_1 C2 . $C222_1$, 2_1, C2 3.5-, 3.6-, 3.4- , . $C222_1$ ( ) HA E (*61*) 48/ BB 4 D B (DB) D 3GFC D40- C 1(DB D 3F 0). .

150 48 60. A CAF1- C C (62), HE (63). R R 0.225 0.257, , 96.8 3.2% , . 463 713 150, 1 394 60, 7 411 48. 150 607 657, , 100 111 60 . C222$_1$. F , (64). D 1.

DNA preparation

A 30- - D A (D A_{30}) (H C)- D A (: 5′-G AA CCCC GGCGG AAAA-CGCGGGGG-3′; : 5′-CCCCCGCG -AACCGCCAAGGGGA AC-3′) (10 HE E , H 7.5, 100 C , 5 C $_2$). 20 µ 95 4 C 0.1 C/ . 147- 601 (601) D A *E. coli* - (65, 66). B , 147- 601 D A EGF - 1 (A), . 147- D A - E , (EG) , . 147- 601 D A 5′-C GGAGAA CCC-GG GCCGAGGCCGC CAA GG CG AGACA-GC C AGCACCGC AAACGCACG ACGCG-C G CCCCCGCG AACCGCCAAGGGG-A AC CCC AG C CCAGGCACG G CAC-A A A ACA CC G -3′.

Cryo-EM sample preparation

CAF-1 150 HD (CAF1- C) H3-H4 1:2.5 A-500, 30 , H 16/60 200 - , 10 / . CAF1-C–H3-H4 D A_{30} 1:1.5 A-500, B (20 HE E , H 7.5, 50 C , 1 D). 4 C 36 . CAF1-FC–H3-H4-D A30 - CAF1- C , 6 10/300 G (GE H) . CAF-1, H3-H4, 601 D A (D A_{147}) CAF-1, H3-H4, D A_{147} 1:2.5:1 A-2000 B 4 C 36 . , 1 / 200 µ , - G F (67). , - 12- 10 30% - B 0 0.15% E - (-A). A 4 C 16 38,000 40 (B C), - 25 500 µ . 8% - , - - E . A-50, A-500 C 50 , -E 300 2/1 C /A (C , .) CAF1- C–H3-H4–D A_{30}, CAF1-FC–H3-H4–D A_{30}, CAF1- C–H3-H4–D A_{147} 300 2/1 CAF1- C–H3-H4–D A_{147} . G 950 A $_2$/A 60 . A 3-µ 0.4 / - . A - 10 , (55 , , GE H -) 3 4.5 , - FE (F). A -E 10 C 100% .

Cryo-EM data acquisition, image processing, and 3D reconstruction

C -E FE A G F 200 130,000. G B - 2 . - 20 . - - 1- (0.5- -). –1.0 –1.5 µ . E 5 , 50 $^-$/ ^{2} (, 8.9 $^-$/ /) 32 . A CAF1- C–H3-H4 - D A_{30} . 3. A 23,996 A C (68) - , - - (C F) A C G (69). - , 19,607 - . A C - 2D - - - . A C, 19,430,612 . 2D , 2,269,295 ( ) . , CAF1- C–H3-H4 . A ( ), - , . F - , 927,863 -3.0 (70) 2 . (71) 3D . 400,801 3D . 304,368 A C , 3.9- . - 3.8 . F , 53,708 -3.0 3D , 24,904 , - 7.2 CAF1- C–H3-H4 . F C2 6.1- . F CAF1-FC–H3-H4–D A_{30} , . 4, 15,893 A C - , C F A C. , 14,532 - . A C - - 2D - . A 4,999,183

. 2D
1,199,544
688,535 , 3.6- . B
CAF1-FC–H3-H4 CAF1-C–H3-H4 (0.976),
(130)
977,978 .
A
: CAF1-H3-H4.
CAF1-H3-H4 552,289
530,555 .
3.5 . , 77 23%
FC C ,
CAF1-FC 168,932
CAF1- C
53,708 ,
130- .
A 197,148
50,508
CAF1-H3-H4
6.3 . F C2
5.9-
CAF1-H3-H4 .
C1 101,016 ,
4.63- ( )
4.58- ( ) .
,
C -F - HE .
F
(F C) A C,
4.6-
CAF1-H3-H4 .
A
1 CAF1- C–H3-H4–D A_{147}
. 9. D -
30-
D A . F , 11,206
10,316
C F A C.
B -
,
, 10,865,604
2D 1,088,125
: CAF1-
-3.0
3D .
F - ,
459,354
3D . 335,568
,
3.5 . F
CAF1- , 628,771
3D . 178,734
,
3.8 .
F 10
2 . F
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. A
1,568,202
. A 243,993
2D
. 126,187
-3.0
3D .
8190
18 .
76,910
CAF1-
5.6 6.3 C2
,
A
CAF1-FC–H3-H4–D A_{147}
. 11. F , 8801
8002
. A 3,199,346 .A
947,480
2D
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CAF1-FC-
. F CAF1-FC- ,
67,319
,
CAF-1-
3.8 . 13,993
CAF1-FC-
,
CAF-1-
6.6 .
A
- F C 0.143.
A C.

Model building and refinement of the cryo-EM structures

C CAF1- C
H3.1-H4 A -
A F1–H3.1-H4 (DB D 7 6)
CAF1- C–H3-H4
-E
C F C (72). 1:1
CAF1- C–H3-H4
2:2
. F -
601 D A
(DB D 3 0) H3.1-H4 -
-E . F
CAF-1- ,
CAF1- C
H3.1-H4 ,
601 D A
. A CAF1- C
-
CAF-1 .
HE
C .
HE . F -
, 4.6- 6.1- CAF-1-
H3-H4 5.6- 6.6-
CAF-1- ,
.
HE
. A ,
3.8- CAF-1-
, - 60
150
. 2 3.
C , C (73),
(C, ,).

Topological assay for nucleosome assembly

D A
(74)

_ . _ D A _ _ -
_ ϕ 174 F D A (EB,
3021)_ 100 _ , D A
_ (_ _ _ , 2240A) _ -
10 -HC , H 7.5, 125
_ C , 2 C

, A- EB D A (E7645) , . A - (-), E - D A (CE- -) - : 15 -HC , H 7.5, 0.5 - EG- (B), 0.1 C $_4$ (-A), 0.5 H A (-A), 10 (-A) 45 . D A A (1.1) 10 -HC , H 7.5. , D A C1 ( ). μ 1 B & (5 -HC H 7.5, 0.5 ED A, 1 C , 0.05% -20) 2 B & . 1:1 D A 45 . , 1 B & , 1 E 0.05% -20, 10 -HC , H 7.5 10 μ H_2. 98 C 10 , 16 C - D A . - C - - 1 A . , , . - - , *Homo sapiens* (38) B 2 (*79*) . . DA (*80*) 120 180 . 10- . - CE- - . CE- D A . , CE- - , - - D A E . A C CF- - - 1000- . C CF - (C -) G E (GE) (G 4640493) (*81*)

Molecular weight determination by SEC-MALS

-D A - EC- A DA HE E 18- - ( ) A H C 6 10/300 G - (GE H). 20 -HC , H 7.5, 125 C , 2% - 12 . B A - 1 / . F CAF-1 CAF-1–H3-H4 - D A_{30} 1 / 0.5 / . D A A .

Single-molecule FOMT analysis

A - 159- D A C D A (543) *Sty* . 147- 601 D A *Bsa* - , - . , (2 μ , D) 150 C 8 , . , (-A , 2) 5 - 100 μ (0.1 / ,) B 4 4 C. - 100 μ - 10 / B A, 1 ED A, 10 , H 8.0, 10 / F127 (-A , 2443), 3 $_3$ 4 C . F (*54*, *82*), F . 6D, (*83*). F 8- D A (D 1, , 65601) , (*82*, *84*), 0.45 1.8 . A - 1 F (50 C , 25 HE E , H 7.5, 0.1 ED A, 0.025% EG, 0.025% H, 1 / B A), 100 μ - D A (3 /μ) F 20 - 200 μ F . (1) F - - 20 . , 200 μ F - 1-H3H4 (200) CAF1-FC–H3-H4 (50) (50 C , 25 HE E , H 7.5, 0.1 ED A, 0.25% EG, 0.25% H, 1 / B A) 4 C 30 . - 100 . A G -E CCD - 500 H 4 (A 60 , A 1.42,). - (*x*, *y*, *z*) 0.7 . (x , y) R , , , : $$_{x,y,R} = \sum_i (x_i - x)^2 + (y_i - y)^2 - R^2 \quad (1)$$ x , y , R , (x_i, y_i), A $\vec{b}, \vec{x}$: $$A = \begin{matrix} x_1 & y_1 & 1 \\ x_1 & y_2 & 1 \\ \cdots & \cdots & \cdots \\ x_n & y_n & 1 \end{matrix}, \quad \vec{b} = \begin{matrix} x_1^2 + y_1^2 \\ x_2^2 + y_2^2 \\ \cdots \\ x_n^2 + y_n^2 \end{matrix}, \quad \vec{x} = \begin{matrix} 2x_c \\ 2y_c \\ r^2 - x_c^2 - y_c^2 \end{matrix} \quad (2)$$ $$A\vec{x} = \vec{b} \quad (3)$$ $$\vec{x} = A\,A^{-1}A\,\vec{b} \quad (4)$$ A x , y , R , (x , y). B , (x_i, y_i) $\vec{r}_i$, $\vec{r}_i$ $\vec{r}_{i+1}$ $$\Delta\theta_{i,i+1} = \frac{\vec{r}_i, \vec{r}_{i+1}}{\vec{r}_i \ \vec{r}_{i+1}} \quad (5)$$ $$\theta_k = \sum_{i=1}^{k-1} \begin{matrix} \Delta\theta_{i,i+1}, & \Delta\theta_{i,i+1} \\ 0, & k = 1 \end{matrix} \quad (6)$$ (r_i, ϕ_i)

$$r_i = \sqrt{(x_i - x_c)^2 + (y_i - y_c)^2} \quad (7)$$

$$\phi_i = \frac{y_i - y_c}{x_i - x_c} \quad (8)$$

F (*85*).

EMSA

E A (20 µ), 0.5 µ 147- D A 0.5, 1, 2.5, 5, 10 µ 60ΔC 48, 20 -HC , H 7.5, 50 C , 1 D , 30 , 6% 120 50 0.5 BE - . B G (F) G D E (B -).

Quantification and statistical analysis

- A C (*68*). - (*64*). D - , - (A A) p . D A-

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