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I e ka ic cell , ch a i e c de e ige e ic i f a i a d f c di c i h ca ical a d a ia hi e a ede - g e ge e abili [1,2]. H e ige e icall de e i ed ch a- i a e a e aga ed da gh e cell d i g i i , i a ce e ed e ige e ic i he i a ce, i e f he challe gi g e i i he ch a i a d e ige e ic field[3,4]. O e ke ce c ib i g e ige e ic i he i a ce i a e bl f he cle e, he ba ic e ea i g i f ch a i . The cle e c i f 145 147 ba e ai f DNA a ed a d a hi e c a e c ai i g e hi e $(H3\ H4)_2$ e a e a d hi e H2A H2B di e [5]. A cle e e a ba ie f DNA- ela ed ce e , he fi be di a e bled all DNA e lica i , DNA e ai a d a - c i i achi e ie acce he DNA. F ll i g DNA e lica- i d i g S ha e, cle e a e a e bled, i c a i g b h a e al hi e a d e l he i ed hi e , i a ce called e lica i -c led cle e a e bl . N cle e a e bl d - i gge e a c i i a d hi e e cha ge cc h gh he cell c cle i a e lica i -i de e de a e [1,2].

Ea l die gge ed ha cle e a e bl cc i a e i e a e : he hi e $(H3\ H4)_2$ e a e , i cl di g b h ld a d e H3 H4, i de i ed fi , a d hi i a idl f ll ed b de i i f H2A H2B di e [6]. S i g hi del, - cle al i e - edia e c ai i g $(H3\ H4)_2$ e a e a d DNA, called e a e , a e f ed he hi e a e i c ba ed i h DNA i he e e ce f hi e cha e e *in vitro*[7]. Hi e cha e e a e ke ei ha f c i a l i le e f cle e f a i (**Box 1**; **Table 1**). Ca ical hi e H3 (hich, i highe e ka ic cell , efe H3.2 a d H3.1, hich diffe b e a i acid i h a) i de i ed DNA b he hi e cha e e CAF-1 d i g DNA e lica i c led cle e a e bl (**Fig. 1a**,**b**). The hi e H3 a ia H3.3, diffe i g f ca ical H3 b f fi e a i acid , i de i ed, al g i h hi e H4, b he hi e cha e e HIRA a d Da i e lica i -i de e de cle e a e bl [8–10]. I hi e ie , e

New H3–H4 dimers bind various histone chaperones. Ne l - he i ed H3 H4 lec le a ea f di i c ei c le e h l f ll i g hei he i i he c la . P ifica i f h a ca ical hi e H3.1 f HeLa c lic e ac , f l- l ed b e a a i f he ei c le e b ch a g a h , gge ed ha e H3.1 a cia e i h he ei cha e e H c70 bef e bei g a e bled i a la ge c le c ai i g hi- e cha e e -NASP, hi e H4 a d ei cha e e H 90 (ef. 18). H3 H4 he a cia e i h he l i e ace l a fe a e Ha 1 RbA 46, f ace la i , a d hi e cha e e A f1 a d i i -4 bef e clea i [18]. M e ece l , i a b e ed ha de le i f NASP e l i ed ced a f f ee hi e H3 H4 a d ha NASP ec hi e f deg ada i b cha e e- edia ed a hag , h gh i hibi i f H 90 a d H c70 ac i i [19]. Th , e H3.1 H4 f a i c le e i h diffe e hi e cha e e eg la e f ee hi e ab da ce a d clea i , hich babl affec he de i i f e H3 H4 e lica i g DNA.

How are new $(H3–H4)_2$ tetramers formed? O ce b d A f1, e H3 H4 i i ed f he c la he cle . Va i die ha e h ha e lec le f A f1 bi d a H3 H4 he e di e f a he e i e ic c le [14,20], i h A f1 bi d- i g he H3 i e face i l ed i f a i f a $(H3 H4)_2$ e a e [21] (**Fig. 2a**,**b**). Si ila l , i ha bee h ha HJURP (Sc 3 i ea), he cha e e f he ce e ic hi e H3 a ia CENP-A [22 24], bi d he CENP-A i e face i l ed i e a e f a i [25,26] (**Fig. 2c**). Th , A f1 a d HJURP e e e a cla f H3 H4 cha e - e ha bi d he di e ic f f H3 H4.

O e ke e l ed e i i h $(H3 H4)_2$ e a e a e f ed f e H3 H4 di e c le ed i h A f1. E ide ce f a i die a del i hich H3 H4 f he A f1 H3 H4 c le i a fe ed he hi e cha e e , ch a CAF-1 a d R 106, f cle e a e bl . Fi , i h a cell , A f1 eg la e he l f H3 H4 a ailable CAF-1 d i g e lica- i e [27]. I b ddi g ea , A f1 i e e ial f ace la i f H3 l i e 56 (H3K56ac)[15,28], a a k f e l he i ed H3 (ef. 29). I a l , A f1 a d H3K56ac a e e i ed f he efficie a cia- i f H3 H4 i h R 106 a d CAF-1 *in vitro* a d *in vivo*[30]. Fi all , A f1 di ec l i e ac i h he h a 60 (ea Cac2) b i f CAF-1 (ef . 31,32). *In vitro*, A f1 bi d H3 H4 i h i ila affi i a CAF-1 R 106 bi di g H3 H4 (ef . 33 35), hich ai e he e i f h H3 H4 ca be a fe ed f A f1 he hi- e cha e e . A ece d i dica e ha RbA 48, a b i f CAF-1, bi d he e di e ic H3 H4 a d ha A f1 ca a cia e i h he RbA 48 H3 H4 c le . I e e i gl , he affi i f A f1 f RbA 48 H3 H4 i l e ha ha f H3 H4 (ef. 36), hich g- ge ha he i e ac i be ee A f1 a d H3 H4 i eake ed ce he A f1 H3 H4 c le a cia e i h he hi e cha e e . T ge he , he e e l gge ha he i e ac i be ee A f1 a d he hi e cha e e a facili a e he a fe f H3 H4 f he A f1 H3 H4 c le he hi e cha e e .

H3K56ac i l ca ed fa a a f he H3 i e face i l ed i $(H3 H4)_2$ e a e f a i [5], hich gge ha R 106 a d CAF-1 ad a diffe e de f i e ac i i h hi e c - a ed ha f A f1 (**Fig. 2b**). I deed, ece die i dica e ha $(H3 H4)_2$ e a e a e babl f ed R 106 a d CAF-1 bef e de i i f H3 H4 lec le a he e lica i f k. R 106 c - ai a di e i a i d ai a he R 106 N e i a d a d ble leck i h l g (PH) d ai ha i c i ical f ec g i i f H3K56ac[35,37 39] (**Fig. 2d**). *In vitro*, b h he R 106 di e i a i d ai a d he a de PH d ai bi d H3 H4, i h he R 106 di e i a i d ai bi di g ace la ed H3 H4 a d he a de PH d ai ec g i i g H3K56ac[35]. I addi i , R 106 bi d a $(H3 H4)_2$ e a e *in vitro* a d *in vivo*[35,37]. Th , R 106 a -

Table 1 Histone chaperones and their functions during nucleosome assembly

Histone chaperone	Histone cargo	Function during nucleosome assembly	Key references
Anti-silencing factor 1 (Asf1)	H3–H4	Histone import; histone transfer to CAF-1 and HIRA; regulation of H3K56ac	14,20,30
Chromatin assembly factor 1 (CAF-1)	H3.1–H4	H3.1–H4 deposition; $(H3–H4)_2$ formation	8,12,34,116
Death domain–associated protein (Daxx)	H3.3–H4		

H3.1 H4 d e i i h e H3.1 H4 f i ed cle - e d i g S ha e f he cell c cle[17].

H3 and H4 modifications regulate replication-coupled nucleosome assembly. Hi e ei a e a ked, b hi e- dif i g e e, i h - a la i al difica i , ch a ace la i , e h la i , h h la i a d bi i la i . The e a k ha e di i c f c i a d eg la e a be f cell la ce e [42]. Ne H3 H4 i dified - a la i all , ch ha i i di i g i hable f a e al hi e H3 H4 (ef . 27,29,43). Rece die i dica e ha difica i e H3 H4 affec e lica i -c led cle - e a e bl i a i a , i cl di g he eg la i f hi e ei f ldi g a d ce i g[18,27], hi e clea i [44] a d he i e ac i be ee hi e a d hi e cha e e [30,45].

M e h la i f hi e H3 l i e 9 (H3K9 e1) i a ea l a k b e ed e l he i ed hi e H3 i a alia cell .

Al h gh lec la i igh i he f c i f hi difica i i cle e a e bl i ill lacki g, H3K9 e1 a be i l ed i hi e ce i g f ll i g hi e he i a d/ he c - e i f e H3K9 e1 i e h la ed H3 l i e 9 (H3K9 e3), a a k he e ch a i [18,46]. S i g he la e idea, a- i i H3K9 e1 l i e e h l a fe a e ha e bee f d affec he e ch a i i eg i [47].

Diace la i f hi e H4 a l i e 5 a d 12 (H4K5,12ac), ca al ed b Ha 1 RbA 46 (ef . 43,48), i de ec ed e l - he i ed hi e H4 f ea a d h a cell a d i likel be a ea l difica i cc i g e H3 H4 (ef. 18). Hi e H4 a ha b i g a i a H4K5 a d H4K12 a e i ed le efficie l i he cle ha a e ild- e hi e [49]. M e e , Ha 1 RbA 46 a d H4 K5,12ac eg la e he a cia i

ha e h ha Da , hich f ac le i h he ch ai- e deli g fac ATRX, i a H3.3 hi e cha e e[9,10]. Al h gh i e ai be de e i ed he he Da eg la e H3.3 cc ac a el e ic he e ch ai , i i k ha cell lacki g ATRX e hibi defec i H3.3 cc ac a el e e a d e ice ic DNA egi [10], hich gge ha Da ATRX i i l ed i H3.3 de- i i a el e ic egi . I addi i HIRA a d Da , he h a h l g f *D. melanogaster* DEK i babl a he H3.3 hi e cha e e i h a le i ai ai i g he e ch ai i eg i , i a , h gh i e ac i i h HP1α (ef . 66,67). T ge he , he e die i dica e ha H3.3 i de i ed a diffe e ch a i egi b di i c hi e cha e e .

Wha fac aid i he ec i e f H3.3 hi e cha e e c le e diffe e ch ai l ci? HIRA bi d d ble- a ded DNA a d RNA l e a e, hich ide a ible echa i he eb HIRA- edia ed cle e a e bl f H3.3 i li ked ge e a c i i [68]. The Da bi di g a e ATRX bi d e e i i e DNA e e ce [69], a d he ADD d ai f ATRX ec g i e hall a k ch a i ig a e f he e ch a i , ch a H3K9 e3, MeCP2 a d HP1α (ef. 70). Th , i i ible ha ATRX ec i Da el e ic he e ch a i f H3.3 de i i . T ge he , he e d- ie gge ha HIRA a d Da a e ec i ed di i c ch a i l ci h gh diffe e echa i , eg la e H3.3 cc a c a de i ed ch a i l ci.

I e H3.3 H4 de i ed a a di e e a e? I i k ha d i g S ha e, a all f ac i f a e al $(H3.3\ H4)_2$ e a- e li i di e f H3.3 H4 a d f i ed cle e c ai i g b h e a d ld H3.3 H4; hi i i c a a e al H3.1 H4 lec le , hich a el li [17]. I b ddi g ea , i ed cle e a e i a il l cali ed highl a c ibed egi eg la ele e [71]. The ef e, i c a e H3.1 H4 lec le ha a e likel be de i ed i a e a e ic f , e H3.3 H4 a be de i ed i b h di e ic a d e a e ic f . T ece i de e de die ha e h ha he hi e- bi di g d ai (HBD) f Da f a c le i h he H3.3 H4 he e di e [72,73]. Re a kabl , H3.3- ecific e id e , Gl 90 a d Ala87 f H3.3, a e i ci al de e i a f Da ' efe e ial ec g i i f H3.3 e H3.1. Ala87 i ec g i ed b a hall h d- h bic cke f Da , he ea Gl 90 bi d a la e i e ha di c i i a e agai Me 90 f H3.1 (ef. 72). The c e f he Da HBD H3.3 H4 c le al e eal ha Da HBD H3.3 H4 c e e i h DNA f hi e bi di g. I fac , like f ll-le g h Da , he Da HBD H3.3 H4 c le e ca f e a e [73], hich gge ha he b e ed c e f Da HBD H3.3 H4 c le e de g aj c f a i al cha ge d i g he a e bl f H3.3 H4 i cle e . F e die a e eeded de e i e he he HIRA e a i ila echa i ec g i e H3.3 H4 a d el cida e h HIRA a d Da e f a i f H3.3 H4 c ai i g cle e .

Histone modifications in replication-independent assembly. Ace la i a k e l he i ed hi e a e i a , l f he eg la i f e lica i -c led cle e a e- bl b al f e lica i -i de e de cle e a e bl . F e a le, i addi i i le i e lica i -c led cle e a e bl , H3K56ac e hi e e cha ge a d e i b d- di g ea [74,75]. R 109 a d Gc 5, e e ca al i g ace la i f e H3 (ef . 30,53), ha e bee h ace la e hi e H3 l i e 4 (H3K4ac), a a k c ela ed i h a c i i al ac i a i [76]. Th , ace la i e e e H3 affec b h e lica i -c led a d e lica i -i de e de cle e a e bl . Beca e e f he e difica i eg la e hi e hi e cha e e i e ac- i i e lica i -c led cle e a e bl , i i ible ha i ila echa i a e ed eg la e e lica i -i de e de cle e a e bl .

I addi i ace la i , he difica i babl affec he de i i f H3.3 H4. F e a le, h h la i f hi e H4 e i e 47 (H4S47 h), ca al ed b he 21-ac i a ed ki a e 2 (Pak2), i e e hi e H4 ha c- ifie i h A f1a a d A f1b i a- alia cell . H4S47 h e cle e a e bl f H3.3 H4 a d i hibi cle e a e bl f H3.1 H4 b i c ea i g he bi d- a[illegible]S a83 7( )- 3 2(e)-74 2713( )-13(H)-2714

edia ed ai l h gh S 16, he ea SSRP1 efe e iall bi d H3 H4 (ef. 86). I b ddi g ea , he N e i f S 16 ha bee h bi d H3 H4 *in vitro*[87], a d P b3, he SSRP1 h l g, c - ai a de PH d ai [88], a if al f d i he H3 H4 cha e - e R 106 (ef . 35,38,39). Th , FACT a f c i a a cha e e f b h H3 H4 a d H2A H2B.

M a i i c da i -1 a e a cia ed i h c ge i al d e h - ie ic a e ia e I (CDAI), a a e di de . E a i a i f e h c e f CDA1 a ie e ealed defec i he e ch a i c e a d HP1 l cali a i [112]. Rece l , c da i -1 a f d c - if i h A f1a a d A f1b (ef . 45,113). C da i -1 bi d A f1 h gh he a e A f1 face a d HIRA a d CAF-1, hich i lie c e i i i h HIRA a d CAF-1 f A f1 bi di g[113]. C da i -1 e id e a ed i CDAI a ie a e fa e ed f he A f1 bi di g i e, e c da i -1 a ei ha b i g he e a- i e hibi ed defec i A f1 bi di g[113]. The e e l gge ha CDAI a be ca ed b al e a i i cle e a e bl a d highligh he i a ce f e eg la i f di i c e f cle e a e bl .

Fi all , al e a i i hi e cha e e e e i ha e bee d c e ed a e ial g ic a ke f diffe e ca ce . A f1b, e f he i f f A f1 i a alia cell , i e i ed f cell life a i , a d highe A f1b i a cia ed i h i c ea ed e a a i a d h e i al f b ea ca ce a ie [114]. High CAF-1 60 c ela e i h ad e e c e i e al, e d e ial a d ce ical ca ce [115]. Beca e A f1b a d CAF-1 a e i l ed i cell life a i , i c ea ed ei ab da ce f he e fac i ca ce cell c ld be d e he e ha ced life a i a f ca ce cell . Al e a i el , i c ea ed a f he e cha e e a al e cle - e a e bl , e l i g i ge e i abili a d he i f ige e i . F he i e iga i i eeded de e i e hee e hich he al e ed ab da ce f hi e cha e e b e ed i h a ca ce i he c e e ce he ca e f ige e i .

Concluding remarks

G ea ide ha e bee ade i de a di g h e lica i - c led a d e lica i -i de e de cle e a e bl a h- a a e eg la ed b hi e cha e e a d hi e difica i . I addi i , c ec i be ee defec i cle e a e bl

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