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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 a i- 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 a c a el e ic he e ch a i , i i k ha cell lacki g ATRX e hibi defec i H3.3 cc a c 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 a i 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 ied 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 a i 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-

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.

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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

17. Xu, M. *et al.* Partitioning of histone H3–H4 tetramers during DNA replication-dependent chromatin assembly. *Science* **328**, 94–98 (2010).
18. Campos, E.I. *et al.* The program for processing newly synthesized histones H3.1 and H4. *Nat. Struct. Mol. Biol.* **17**, 1343–1351 (2010).
Suggests that predeposition histone H3.1–H4 associates with multiple chaperones, aiding in histone synthesis or stability, modification and nuclear import.
19. Cook, A.J., Gurard-Levin, Z.A., Vassias, I. & Almouzni, G. A specific function for the histone chaperone NASP to fine-tune a reservoir of soluble H3–H4 in the histone supply chain. *Mol. Cell* **44**, 918–927 (2011).
20. English, C.M., Adkins, M.W., Carson, J.J., Churchill, M.E. & Tyler, J.K. Structural basis for the histone chaperone activity of Asf1. *Cell* **127**, 495–508 (2006).
Structural and functional analyses of Asf1–H3–H4 reveal that Asf1 forms a complex with the H3–H4 heterodimer through the H3 interface involved in the formation of a $(H3–H4)_2$ tetramer.
21. English, C.M., Maluf, N.K., Tripet, B., Churchill, M.E. & Tyler, J.K. ASF1 binds to a heterodimer of histones H3 and H4: a two-step mechanism for the assembly of the H3-H4 heterotetramer on DNA. *Biochemistry* **44**, 13673–13682 (2005).
22. Mizuguchi, G., Xiao, H., Wisniewski, J., Smith, M.M. & Wu, C. Nonhistone Scm3 and histones CenH3-H4 assemble the core of centromere-specific nucleosomes. *Cell* **129**, 1153–1164 (2007).
23. Dunleavy, E.M. *et al.* HJURP is a cell-cycle-dependent maintenance and deposition factor of CENP-A at centromeres. *Cell* **137**, 485–497 (2009).
24. Foltz, D.R. *et al.* Centromere-specific assembly of CENP-a nucleosomes is mediated by HJURP. *Cell* **137**, 472–484 (2009).
25. Hu, H. *et al.* Structure of a CENP-A-histone H4 heterodimer in complex with chaperone HJURP. *Genes Dev.* **25**, 901–906 (2011).
26. Zhou, Z. *et al.* Structural basis for recognition of centromere histone variant CenH3 by the chaperone Scm3. *Nature* **472**, 234–237 (2011).
Refs. 25,26 demonstrate that HJURP and its yeast counterpart Scm3 bind to the dimeric form of CenH3–H4 and prevent the spontaneous association of CenH3–H4 with DNA.
27. Jasencakova, Z. *et al.* Replication stress interferes with histone recycling and predeposition marking of new histones. *Mol. Cell* **37**, 736–743 (2010).
28. Driscoll, R., Hudson, A. & Jackson, S.P. Yeast Rtt109 promotes genome stability by acetylating histone H3 on lysine 56. *Science* **315**, 649–652 (2007).
29. Masumoto, H., Hawke, D., Kobayashi, R. & Verreault, A. A role for cell-cycle-regulated histone H3 lysine 56 acetylation in the DNA damage response. *Nature* **436**, 294–298 (2005).
30. Li, Q. *et al.* Acetylation of histone H3 lysine 56 regulates replication-coupled nucleosome assembly. *Cell* **134**, 244–255 (2008).
31. Tyler, J.K. *et al.* Interaction between the *Drosophila* CAF-1 and ASF1 chromatin assembly factors. *Mol. Cell. Biol.* **21**, 6574–6584 (2001).
32. Krawitz, D.C., Kama, T. & Kaufman, P.D. Chromatin assembly factor I mutants defective for PCNA binding require Asf1/Hir proteins for silencing. *Mol. Cell. Biol.* **22**, 614–625 (2002).
33. Donham, D.C. II., Scorgie, J.K. & Churchill, M.E. The activity of the histone chaperone yeast Asf1 in the assembly and disassembly of histone H3/H4-DNA complexes. *Nucleic Acids Res.* **39**, 5449–5458 (2011).
34. Winkler, D.D., Zhou, H., Dar, M.A., Zhang, Z. & Luger, K. Yeast CAF-1 assembles histone $(H3–H4)_2$ tetramers prior to DNA deposition. *Nucleic Acids Res.* **40**, 10139–10149 (2012).
35. Su, D. *et al.* Structural basis for recognition of H3K56-acetylated histone H3–H4 by the chaperone Rtt106. *Nature* **483**, 104–107 (2012).
Shows that Rtt106 binds $(H3–H4)_2$ tetramers and contains two histone-binding domains, an N-terminal oligomerization domain and tandem PH domains, which recognize H3 acetylated at lysine 56.
36. Zhang, W. *et al.* Structural plasticity of histones H3–H4 facilitates their allosteric exchange between RbAp48 and ASF1. *Nat. Struct. Mol. Biol.* **19**, aaa–bbb (2012).
37. Fazly, A. *et al.* Histone chaperone Rtt106 promotes nucleosome formation using (H3–H4)2 tetramers. *J. Biol. Chem.* **287**, 10753–10760 (2012).
38. Zunder, R.M., Antczak, A.J., Berger, J.M. & Rine, J. Two surfaces on the histone chaperone Rtt106 mediate histone binding, replication, and silencing. *Proc. Natl. Acad. Sci. USA* **109**, E144–E153 (2012).
39. Liu, Y. *et al.* Structural analysis of Rtt106p reveals a DNA binding role required for heterochromatin silencing. *J. Biol. Chem.* **285**, 4251–4262 (2010).
40. Quivy, J.P., Grandi, P. & Almouzni, G. Dimerization of the largest subunit of chromatin assembly factor 1: importance *in vitro* and during *Xenopus* early development. *EMBO J.* **20**, 2015–2027 (2001).
41. Nakano, S., Stillman, B. & Horvitz, H.R. Replication-coupled chromatin assembly generates a neuronal bilateral asymmetry in *C. elegans*. *Cell* **147**, 1525–1536 (2011).
42. Strahl, B.D. & Allis, C.D. The language of covalent histone modifications. *Nature* **403**, 41–45 (2000).
43. Sobel, R.E., Cook, R.G., Perry, C.A., Annunziato, A.T. & Allis, C.D. Conservation of deposition-related acetylation sites in newly synthesized histones H3 and H4. *Proc. Natl. Acad. Sci. USA* **92**, 1237–1241 (1995).
44. Alvarez, F. *et al.* Sequential establishment of marks on soluble histones H3 and H4. *J. Biol. Chem.* **286**, 17714–17721 (2011).
45. Kang, B. *et al.* Phosphorylation of H4 Ser 47 promotes HIRA-mediated nucleosome assembly. *Genes Dev.* **25**, 1359–1364 (2011).
Shows that phosphorylation of histone H4 Ser47 by Pak2 differentially regulates H3.1–H4 and H3.3–H4 deposition by promoting the association of HIRA with H3.3–H4 and inhibiting the association of CAF-1 with H3.1–H4.
46. Loyola, A. *et al.* The HP1α-CAF1-SetDB1-containing complex provides H3K9me1 for Suv39-mediated K9me3 in pericentric heterochromatin. *EMBO Rep.* **10**, 769–775 (2009).
47. Pinheiro, I. *et al.* Prdm3 and Prdm16 are H3K9me1 methyltransferases required for mammalian heterochromatin integrity. *Cell* **150**, 948–960 (2012).
48. Parthun, M.R., Widom, J. & Gottschling, D.E. The major cytoplasmic histone acetyltransferase in yeast: links to chromatin replication and histone metabolism. *Cell* **87**, 85–94 (1996).
49. Ejlassi-Lassallette, A., Mocquard, E., Arnaud, M.C. & Thiriet, C. H4 replication-dependent diacetylation and Hat1 promote S-phase chromatin assembly *in vivo*. *Mol. Biol. Cell* **22**, 245–255 (2011).
50. Zhang, H., Han, J., Kang, B., Burgess, R. & Zhang, Z. Human histone acetyltransferase HAT1 preferentially acetylates H4 molecules in H3.1–H4 dimers over H3.3–H4 dimers. *J. Biol. Chem.* **287**, 6573–6581 (2012).
51. Ye, J. *et al.* Histone H4 lysine 91 acetylation a core domain modification associated with chromatin assembly. *Mol. Cell* **18**, 123–130 (2005).
52. Yang, X. *et al.* HAT4, a Golgi apparatus-anchored B-type histone acetyltransferase, acetylates free histone H4 and facilitates chromatin assembly. *Mol. Cell* **44**, 39–50 (2011).
Shows that HAT4 is a new histone acetyltransferase that may be involved in replication-coupled nucleosome assembly in human cells.
53. Burgess, R.J., Zhou, H., Han, J. & Zhang, Z. A role for Gcn5 in replication-coupled nucleosome assembly. *Mol. Cell* **37**, 469–480 (2010).
54. Das, C., Lucia, M.S., Hansen, K.C. & Tyler, J.K. CBP/p300-mediated acetylation of histone H3 on lysine 56. *Nature* **459**, 113–117 (2009).
55. Shibahara, K. & Stillman, B. Replication-dependent marking of DNA by PCNA facilitates CAF-1-coupled inheritance of chromatin. *Cell* **96**, 575–585 (1999).
56. Zhang, Z., Shibahara, K. & Stillman, B. PCNA connects DNA replication to epigenetic inheritance in yeast. *Nature* **408**, 221–225 (2000).
57. Moggs, J.G. *et al.* A CAF-1-PCNA-mediated chromatin assembly pathway triggered by sensing DNA damage. *Mol. Cell. Biol.* **20**, 1206–1218 (2000).
58. Groth, A. *et al.* Regulation of replication fork progression through histone supply and demand. *Science* **318**, 1928–1931 (2007).
59. Franco, A.A., Lam, W.M., Burgers, P.M. & Kaufman, P.D. Histone deposition protein Asf1 maintains DNA replisome integrity and interacts with replication factor C. *Genes Dev.* **19**, 1365–1375 (2005).
60. Schulz, L.L. & Tyler, J.K. The histone chaperone ASF1 localizes to active DNA replication forks to mediate efficient DNA replication. *FASEB J.* **20**, 488–490 (2006).
61. Tan, B.C., Chien, C.T., Hirose, S. & Lee, S.C. Functional cooperation between FACT and MCM helicase facilitates initiation of chromatin DNA replication. *EMBO J.* **25**, 3975–3985 (2006).
62. Wittmeyer, J., Joss, L. & Formosa, T. Spt16 and Pob3 of *Saccharomyces cerevisiae* form an essential, abundant heterodimer that is nuclear, chromatin-associated, and copurifies with DNA polymerase alpha. *Biochemistry* **38**, 8961–8971 (1999).
63. Deal, R.B., Henikoff, J.G. & Henikoff, S. Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones. *Science* **328**, 1161–1164 (2010).
64. Szenker, E., Ray-Gallet, D. & Almouzni, G. The double face of the histone variant H3.3. *Cell Res.* **21**, 421–434 (2011).
65. Ray-Gallet, D. *et al.* HIRA is critical for a nucleosome assembly pathway independent of DNA synthesis. *Mol. Cell* **9**, 1091–1100 (2002).
66. Kappes, F. *et al.* The DEK oncoprotein is a Su(var) that is essential to heterochromatin integrity. *Genes Dev.* **25**, 673–678 (2011).
67. Sawatsubashi, S. *et al.* A histone chaperone, DEK, transcriptionally coactivates a nuclear receptor. *Genes Dev.* **24**, 159–170 (2010).
68. Ray-Gallet, D. *et al.* Dynamics of histone H3 deposition *in vivo* reveal a nucleosome gap-filling mechanism for H3.3 to maintain chromatin integrity. *Mol. Cell* **44**, 928–941 (2011).
Describes a SNAP-based assay to monitor H3.1 and H3.3 deposition in real time and shows that HIRA binds RNA polymerase II and DNA, aiding in the coordination of HIRA-mediated H3.3–H4 deposition and gene transcription.
69. Law, M.J. *et al.* ATR-X syndrome protein targets tandem repeats and influences allele-specific expression in a size-dependent manner. *Cell* **143**, 367–378 (2010).
70. Iwase, S. *et al.* ATRX ADD domain links an atypical histone methylation recognition mechanism to human mental-retardation syndrome. *Nat. Struct. Mol. Biol.* **18**, 769–776 (2011).
71. Katan-Khaykovich, Y. & Struhl, K. Splitting of H3–H4 tetramers at transcriptionally active genes undergoing dynamic histone exchange. *Proc. Natl. Acad. Sci. USA* **108**, 1296–1301 (2011).
72. Liu, C.P. *et al.* Structure of the variant histone H3.3–H4 heterodimer in complex with its chaperone DAXX. *Nat. Struct. Mol. Biol.* **19**, 1287–1292 (2012).
73. Elsasser, S.J. *et al.* DAXX envelops an H3.3–H4 dimer for H3.3-specific recognition. *Nature* **491**, 560–565 (2012).
Refs. 72,73 show that Daxx binds an H3.3–H4 heterodimer and describe how the histone-binding domain of Daxx recognizes H3.3 preferentially over H3.1.
74. Rufiange, A., Jacques, P.E., Bhat, W., Robert, F. & Nourani, A. Genome-wide replication-independent histone H3 exchange occurs predominantly at promoters and implicates H3 K56 acetylation and Asf1. *Mol. Cell* **27**, 393–405 (2007).

75. Williams, S.K., Truong, D. & Tyler, J.K. Acetylation in the globular core of histone H3 on lysine-56 promotes chromatin disassembly during transcriptional activation. *Proc. Natl. Acad. Sci. USA* **105**, 9000–9005 (2008).
76. Guillemette, B. *et al.* H3 lysine 4 is acetylated at active gene promoters and is regulated by H3 lysine 4 methylation. *PLoS Genet.* **7**, e1001354 (2011).
77. Bokoch, G.M. Biology of the p21-activated kinases. *Annu. Rev. Biochem.* **72**, 743–781 (2003).
78. Zee, B.M., Levin, R.S., Dimaggio, P.A. & Garcia, B.A. Global turnover of histone post-translational modifications and variants in human cells. *Epigenetics Chromatin* **3**, 22 (2010).
79. Jamai, A., Imoberdorf, R.M. & Strubin, M. Continuous histone H2B and transcription-dependent histone H3 exchange in yeast cells outside of replication. *Mol. Cell* **25**, 345–355 (2007).
80. Selth, L. & Svejstrup, J.Q. Vps75, a new yeast member of the NAP histone chaperone family. *J. Biol. Chem.* **282**, 12358–12362 (2007).
81. Andrews, A.J., Downing, G., Brown, K., Park, Y.J. & Luger, K. A thermodynamic model for Nap1-histone interactions. *J. Biol. Chem.* **283**, 32412–32418 (2008).
82. Mosammaparast, N., Ewart, C.S. & Pemberton, L.F. A role for nucleosome assembly protein 1 in the nuclear transport of histones H2A and H2B. *EMBO J.* **21**, 6527–6538 (2002).
83. Ito, T., Bulger, M., Pazin, M.J., Kobayashi, R. & Kadonaga, J.T. ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. *Cell* **90**, 145–155 (1997).
84. Andrews, A.J., Chen, X., Zevin, A., Stargell, L.A. & Luger, K. The histone chaperone Nap1 promotes nucleosome assembly by eliminating nonnucleosomal histone DNA interactions. *Mol. Cell* **37**, 834–842 (2010).
85. Belotserkovskaya, R. *et al.* FACT facilitates transcription-dependent nucleosome alteration. *Science* **301**, 1090–1093 (2003).
86. Winkler, D.D., Muthurajan, U.M., Hieb, A.R. & Luger, K. Histone chaperone FACT coordinates nucleosome interaction through multiple synergistic binding events. *J. Biol. Chem.* **286**, 41883–41892 (2011).
87. Stuwe, T. *et al.* The FACT Spt16 "peptidase" domain is a histone H3–H4 binding module. *Proc. Natl. Acad. Sci. USA* **105**, 8884–8889 (2008).
88. VanDemark, A.P. *et al.* The structure of the yFACT Pob3-M domain, its interaction with the DNA replication factor RPA, and a potential role in nucleosome deposition. *Mol. Cell* **22**, 363–374 (2006).
89. Xin, H. *et al.* yFACT induces global accessibility of nucleosomal DNA without H2A–H2B displacement. *Mol. Cell* **35**, 365–376 (2009).
90. Jamai, A., Puglisi, A. & Strubin, M. Histone chaperone spt16 promotes redeposition of the original h3-h4 histones evicted by elongating RNA polymerase. *Mol. Cell* **35**, 377–383 (2009).
91. Batta, K., Zhang, Z., Yen, K., Goffman, D.B. & Pugh, B.F. Genome-wide function of H2B ubiquitylation in promoter and genic regions. *Genes Dev.* **25**, 2254–2265 (2011).
92. Pavri, R. *et al.* Histone H2B monoubiquitination functions cooperatively with FACT to regulate elongation by RNA polymerase II. *Cell* **125**, 703–717 (2006).
93. Yuan, J., Adamski, R. & Chen, J. Focus on histone variant H2AX: to be or not to be. *FEBS Lett.* **584**, 3717–3724 (2010).
94. Heo, K. *et al.* FACT-mediated exchange of histone variant H2AX regulated by phosphorylation of H2AX and ADP-ribosylation of Spt16. *Mol. Cell* **30**, 86–97 (2008).
95. Zhang, H., Roberts, D.N. & Cairns, B.R. Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. *Cell* **123**, 219–231 (2005).
96. Jin, C. *et al.* H3.3/H2A.Z double variant-containing nucleosomes mark 'nucleosome-free regions' of active promoters and other regulatory regions. *Nat. Genet.* **41**, 941–945 (2009).
97. Mizuguchi, G. *et al.* ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science* **303**, 343–348 (2004).
98. Luk, E. *et al.* Chz1, a nuclear chaperone for histone H2AZ. *Mol. Cell* **25**, 357–368 (2007).
99. Straube, K., Blackwell, J.S. Jr. & Pemberton, L.F. Nap1 and Chz1 have separate Htz1 nuclear import and assembly functions. *Traffic* **11**, 185–197 (2010).
100. Costanzi, C. & Pehrson, J.R. Histone macroH2A1 is concentrated in the inactive X chromosome of female mammals. *Nature* **393**, 599–601 (1998).
101. Zhang, R. *et al.* Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. *Dev. Cell* **8**, 19–30 (2005).
102. Iles, N., Rulten, S., El-Khamisy, S.F. & Caldecott, K.W. APLF (C2orf13) is a novel human protein involved in the cellular response to chromosomal DNA strand breaks. *Mol. Cell. Biol.* **27**, 3793–3803 (2007).
103. Mehrotra, P.V. *et al.* DNA repair factor APLF is a histone chaperone. *Mol. Cell* **41**, 46–55 (2011).
104. Lorain, S. *et al.* Structural organization of the WD repeat protein-encoding gene HIRA in the DiGeorge syndrome critical region of human chromosome 22. *Genome Res.* **6**, 43–50 (1996).
105. Farrell, M.J. *et al.* HIRA, a DiGeorge syndrome candidate gene, is required for cardiac outflow tract septation. *Circ. Res.* **84**, 127–135 (1999).
106. Wilming, L.G., Snoeren, C.A., van Rijswijk, A., Grosveld, F. & Meijers, C. The murine homologue of HIRA, a DiGeorge syndrome candidate gene, is expressed in embryonic structures affected in human CATCH22 patients. *Hum. Mol. Genet.* **6**, 247–258 (1997).
107. Jiao, Y. *et al.* DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. *Science* **331**, 1199–1203 (2011).
108. Heaphy, C.M. *et al.* Altered telomeres in tumors with ATRX and DAXX mutations. *Science* **333**, 425 (2011).
109. Schwartzentruber, J. *et al.* Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature* **482**, 226–231 (2012).
Refs. 107–109 reveal mutations or alterations of the Daxx–ATRX–H3.3 deposition pathway in various cancers and describe the association of these mutations with alterations in telomeres and gene expression.
110. Wise-Draper, T.M. *et al.* Overexpression of the cellular DEK protein promotes epithelial transformation *in vitro* and *in vivo*. *Cancer Res.* **69**, 1792–1799 (2009).
111. Soekarman, D. *et al.* The translocation (6;9) (p23;q34) shows consistent rearrangement of two genes and defines a myeloproliferative disorder with specific clinical features. *Blood* **79**, 2990–2997 (1992).
112. Renella, R. *et al.* Codanin-1 mutations in congenital dyserythropoietic anemia type 1 affect HP1α localization in erythroblasts. *Blood* **117**, 6928–6938 (2011).
113. Ask, K. *et al.* Codanin-1, mutated in the anaemic disease CDAI, regulates Asf1 function in S-phase histone supply. *EMBO J.* **31**, 2013–2023 (2012).
114. Corpet, A. *et al.* Asf1b, the necessary Asf1 isoform for proliferation, is predictive of outcome in breast cancer. *EMBO J.* **30**, 480–493 (2011).
115. Polo, S.E. *et al.* Clinical significance and prognostic value of chromatin assembly factor-1 overexpression in human solid tumours. *Histopathology* **57**, 716–724 (2010).
116. Verreault, A., Kaufman, P.D., Kobayashi, R. & Stillman, B. Nucleosome assembly by a complex of CAF-1 and acetylated histones H3/H4. *Cell* **87**, 95–104 (1996).
117. Laskey, R.A., Honda, B.M., Mills, A.D. & Finch, J.T. Nucleosomes are assembled by an acidic protein which binds histones and transfers them to DNA. *Nature* **275**, 416–420 (1978).
118. Han, J., Zhou, H., Li, Z., Xu, R.M. & Zhang, Z. Acetylation of lysine 56 of histone H3 catalyzed by RTT109 and regulated by ASF1 is required for replisome integrity. *J. Biol. Chem.* **282**, 28587–28596 (2007).