Eukaryotic cells package their genomes into a nucleoprotein form called chromatin. The basic unit of chromatin is the nucleosome, formed by the wrapping of ~147 bp of DNA around an octameric complex of core histones. Advances in genomic technologies have enabled the locations of nucleosomes to be mapped across genomes [1,2]. This has revealed a striking organisation with respect to transcribed genes in a diverse range of eukaryotes. This consists of a nucleosome depleted region upstream of promoters, with an array of well spaced nucleosomes extending into coding regions [2]. This observation reinforces the links between chromatin organisation and transcription. Central to this is the paradox that while chromatin is required by eukaryotes to restrict inappropriate access to DNA, this must be overcome in order for genetic information to be expressed. This conundrum is at its most flagrant when considering the need for nucleic acid polymerase’s to transit 1000’s of based pairs of DNA wrapped as arrays of nucleosomes.

Address
Wellcome Trust Centre for Gene Regulation and Expression, College of Life Sciences, University of Dundee, Dundee DD1 5EH, UK

Corresponding author: Owen-Hughes, Tom
t.a.owenhughes@dundee.ac.uk

Dissociative versus non-dissociative models for transcribing nucleosomes

In vitro a range of biochemical approaches indicate that RNA polymerase II (Pol II) can pass through a nucleosome without the need for complete dissociation of histone proteins (reviewed by [3]). However the distribution of Pol II pausing sites observed in vitro and in vivo differs [4,5], raising the awkward question of whether what has been observed in vitro accurately reflects what has taken place in vivo. Furthermore, changes to the experimental conditions used in vitro can result in increased histone dissociation. For example, closely packed polymerases are more effective in disrupting chromatin [6] and it has recently been reported that transcription rates in excess of 5 bp per second result in increasing levels of histone dissociation [7]. As elongation proceeds at 20 bp per second in yeast [8] and up to 830 bp per second in human cells [9], dissociation of nucleosomes is a possibility. Some support for the retention of histones during elongation stems from the observation that histones retain contact with DNA at moderately transcribed genes [10–13]. However, the majority of yeast genes are transcribed sporadically, approximately seven times per hour [14], making transient dissociation hard to detect. This problem is avoided at highly transcribed genes. In these cases substantial chromatin disruption is observed [15,16], but reassembly is rapid, occurring within 1 min of transcription ceasing [15]. This means that on a genome scale a correlation between histone association and Pol II occupancy could be interpreted as evidence for a transient dissociative mechanism. This is indeed what is observed [1,11–13]. What is not clear from these observations is whether Pol II is directly responsible for dissociation of histones or whether additional factors participate. Here we review the roles of some of the factors contributing to the pathway by which Pol II transits chromatin with emphasis on recent developments from studies in Saccharomyces cerevisiae which has proven to be an excellent model system.

The Pol II CTD and PAF complex: recruitment platforms

Key players in the orchestration of the interplay between chromatin and transcription are the C-terminal heptapeptide repeats (CTD) of the Rpb1 subunit of Pol II and the polymerase associated PAF complex (reviewed by [17–20]). The repeated sequence (YSPTSPS) within the C-terminus of Rpb1 is subject to differential phosphorylation during different phases of the transcription cycle. It is thought to be unphosphorylated upon recruitment to promoters facilitating interactions with initiation factors such as mediator. During the early stages of elongation the CTD is phosphorylated at serine 5 (SSP) by the Cdk7 subunit of TFIIH allowing recruitment of the mRNA capping complex. CTD SSP also destabilizes interactions with initiation factors and facilitates promoter escape and recruitment of the Brg1 kinase which phosphorylates the elongation factors Spt4 and Spt5. This in turn promotes recruitment of the PAF complex comprising the Paf1, Rtf1, Cdc73, Leo1 and Ctr9 proteins. PAF and Spt4/Spt5 assist the recruitment of Rad6 and Bre1 which ubiquitynlate H2B at K123 (H2BK123Ub). H2BK123Ub is in turn required for methylation of H3K4 and H3K79 by Set1 and Dot1, respectively. Set1 itself interacts with both the PAF complex and serine 5 phosphorylated CTD.
and the recruitment of Dot1 is also dependent on PAF. The Bur1 and Ctk1 (P-TEFb in humans) kinases are responsible for phosphorylation of the CTD at serine 2 (S2P). This marks the polymerase for progression to a fully elongation competent form. Phosphorylation at S2P suppresses the Sen1/Nrd/Nab3 termination pathway which may contribute to the large numbers of short non-productive transcripts observed at many genes [5**,18]. In combination, multiply phosphorylated CTD and PAF are responsible for the recruitment of the Set2 the enzyme that methylates histone H3 at K36.

**Modifications instruct modifications!**

One of the consequences of the pathway described above is the establishment of the characteristic distributions of histone H3 K4Me3 and H3 K36Me3 across coding regions (Figure 1). These modifications can in turn act as epitopes for the recruitment of chromatin binding proteins. For example, Eaf3 is a subunit of the histone acetyltransferase NuA4 [21] and the histone deacetylase Rpd3S [22,23]. Within Rpd3S the PHD domain of the Rco1 subunit together with the chromodomain of Eaf3 and interactions with the Pol II CTD phosphorylated at both S2 and S5

**Figure 1**

Typical distributions of histone and RNA polymerase modifications over coding regions. Genomic approaches have enabled the organisation of chromatin to be mapped at many genes. This has revealed a typical organisation for a transcribed gene involving a nucleosome depleted region upstream of the transcriptional start site followed by an array of nucleosomes. The strength of positioning decays towards the 3' ends of genes as indicated by the lighter shading in the schematic. Enrichment for selected factors is indicated by red shading on an idealized transcribed gene and illustrates some of the interplay between chromatin and transcription. Genes transcribed at low levels do not exhibit the same pattern of enrichment, possibly reflecting the difficulty in detecting low frequency events. Data adapted largely from [68,86,87*,88,89*].
direct the complex to transcribed chromatin where it removes acetylation preventing chromatin disassembly and inappropriate initiation from within coding regions [24,25,26*

An assortment of factors have been found to recognise histone H3 acetylated at lysine 4. These include the Sgf29 subunit of the SAGA complex [27**,28*]. The NuA5 HAT complex [29], human HBO1 HAT [30,31], BPTF subunit of the human NURF complex [31], the Set3c histone deacetylase complex [32] and human Chd1 [33].

The SAGA complex in addition to fulfilling a distinct function at promoters accompanies Pol II during elongation perhaps as a result of interactions between Sgf29 and H3K4Me3 and serine 5 phosphorylation of the Pol II CTD [27**,34,35]. This is especially prominent at highly transcribed genes such as GAL1 where the reduced acetylation observed in the absence of SAGA is associated with increased nucleosome occupancy in the coding region and decreased mRNA production [35] especially of long transcripts [36*]. There is evidence to suggest that acetylated nucleosomes are targeted for removal by bromodomain containing enzymes such as SWI/SNF and RSC [34,37*,38]. While both SWI/SNF and RSC have functions at promoters, there is also evidence linking both complexes to elongation [37*,39]. Furthermore, in vitro, the combined effect of histone acetylation and remodeling by RSC can facilitate transcription through nucleosomes [40*]. However, it remains possible that there are also modes of histone dissociation independent of histone acetylation [7**,16].

In addition to histone acetyltransferase activity, SAGA has a deubiquitinase (DUB) activity. As a H2B Ub is required for H3 K4Me3 which in turn recruits SAGA, this enzyme has the capability to destroy the H3K4Me3 messenger that summoned its recruitment. Furthermore, the removal of H2B Ub is required for recruitment of Ctk1 and phosphorylation of Pol II at serine 2 [41*]. As a result the recruitment of SAGA is not only required for efficient elongation, but its association is programmed to be transient. Feedback loops of this type are exactly what is required to generate a transient wave of destabilised chromatin during transit of Pol II.

The coupling of histone acetylation mediated nucleosome dissociation with transcription potentially initiates a destabilising positive feedback loop, which could drive further nucleosome depletion and faster elongation. While this may be an advantage at genes transcribed to high levels, at genes expressed at lower levels this provides an opportunity for transcription from cryptic promoters normally occluded by chromatin. To counter this effect histone acetylation is short lived as a consequence of coupling histone deacetylase activity with transcription as described above. Where nucleosomes have been removed, chromatin assembly pathways are required to reassemble nucleosomes.

**Histone chaperones: reassembly or dissociation**

Histone chaperones are prime candidates for a role in this chromatin assembly reaction [42]. Recent structural studies of the FACT chaperone complex indicate the presence of multiple domains capable of interactions with histones [43]. FACT interacts with H2A and H2B with high affinity but also interacts with H3–H4 [44], intact nucleosomes [44,45**] and is capable of directing the assembly of nucleosomes in vitro [46]. Functional studies of FACT are complicated due to the complex having distinct roles in replication and nucleosome removal at promoters. None the less, mutation of FACT results in histone depletion and increased histone exchange over coding regions and increased intragenic transcription over coding regions with no detectable change in elongation rate [47,48*,49]. These observations establish a role for FACT in chromatin reassembly following transcription by Pol II. This appears to contradict the original observations that FACT enhances elongation through nucleosomes in vitro [49]. One the one hand it could be that the original observations do not reflect the true function of FACT. Alternatively FACT may function in both the disassembly and reassembly of nucleosomes during transcription [50]. Although FACT is abundant (being present at approximately 1 copy per three nucleosomes), its action is targeted through physical interactions with the PAF complex and this requires CTD S5 phosphorylation. In addition, ubiquitin modification of H2BK123 has been observed to augment Pol II transcription through nucleosomes in the presence or absence of FACT in vitro [51*] and FACT function in chromatin reassembly in vivo [52*].

Spt6 acts similarly to FACT in the reassembly of chromatin following transcription [53*,54]. However, its interaction with RNA polymerase is mediated by interactions with the highly phosphorylated forms of the CTD [55–58]. Like yeast FACT, Spt6 interacts with nucleosomes only in the presence of the HMG box protein Nhp6 [45**,59].

The reassembly of chromatin following transcription is not restricted to coding mRNAs. Transcription of non-coding RNAs is also associated with chromatin assembly and in some cases this has been found to play regulatory roles [60–62].

**Re-phasing the template**

When nucleosomes are assembled in vitro, in the absence of other factors, the positions adopted by nucleosomes do not fully replicate those observed in vivo [63,64]. An ATP dependent activity has recently been found to be capable of directing this repositioning in yeast extracts [65**].
Prime candidates for this include the Isw1 remodelling enzyme that has been observed to influence nucleosome spacing in mid coding regions [66] and Chd1 which interacts with the Rtf1 subunit of PAF and FACT [67]. Both Isw1 and Chd1 are found to be enriched within the coding regions of highly transcribed genes [67,68]. The genetic interactions of CHD1 mutations with other elongation factors suggest that Chd1 acts to reduce the efficiency of elongation in a similar fashion to the Rpd3S histone deacetylase complex [69**]. As cryptic intragenic transcription is increased following mutation to components of either CHD1 or ISW1 it is possible that these proteins function with partial redundancy in chromatin assembly [69**]. Further support for this stems from the finding that the Chd1 and Isw1 ATPase share structurally related SANT and SLIDE accessory domains [70] and that deletion of these proteins results in an overall loss of nucleosome spacing over coding regions [68]. The establishment of regular nucleosome spacing may play an important role in stabilising the association of histones by firstly, influencing the ability of arrays of nucleosomes to form more compact structures, secondly, allowing for the association of abundant nucleosome binding proteins such as Nhp6 [71**] and thirdly, by simply preventing collisions between nucleosomes which can be destabilising [72]. In addition to an inhibitory effect on non-coding transcription, spaced chromatin may be less permissive to re-initiation events [73].

Some doubt remains as to how tightly coupled the nucleosome spacing reaction is to transcription. favouring close links to transcription are the observation that nucleosome spacing decays with distance from the +1 nucleosome whose positioning is likely to be established by other factors [2], and that there are strong functional ties linking both Isw1 and Chd1 to transcription. On the other hand the spacing reaction appears to proceed in the apparent absence of transcription in nuclear extracts [65**] and substantial organization is retained following inactivation of RNA polymerase [74*]. Possible explanations for these observations include spaced chromatin being sufficiently stable to persist once established, in the absence of ongoing transcription, and that there are sufficient spacing enzymes in nuclear extracts to organise chromatin in an untargeted fashion. Following inactivation of RNA polymerase a retrograde shift in the positioning of nucleosomes is observed involving many nucleosomes moving 10 bp towards the 3’ ends of coding regions [74*]. More recently, it has also been observed that the replacement of ancestral histones with nascent histones is slowest at the 5’ ends of long genes transcribed at low levels [75**]. The favoured explanation for these observations involve the net migration of nucleosomes against the direction in which RNA polymerase transcribes. As this behaviour is disrupted by deletion of the H4 tail [75**] which is required for the spacing activity of both Isw1 and Chd1, it is tempting to speculate that spacing in the wake of a transcribing polymerase is associated with a net movement of nucleosomes in a 5’ direction.

The problem with elongation

The very fact that Pol II moves across genes during elongation complicates studies of its localisation in comparison to activities which are recruited to fixed loci such as promoters. Furthermore, many genes are actively transcribed for relatively short periods of time, making it even harder to study factors involved in elongation using chromatin immunoprecipitation assays. Genetic studies of elongation factors are complicated as a result of them often having distinct roles in other processes such as chromatin reconfiguration at promoters (e.g. FACT, SAGA, SWI/SNF, RSC, NuA4, Chd1 and Isw1). Where a strong phenotype is conferred by one mode of action, for example promoter remodelling, it may confuse interpretation relating to transcriptional elongation. Another recurring issue is the presence of parallel pathways that confer partial redundancy which greatly complicates the interpretation of genetic interactions. For example, due to overlapping functions, multiple HDACs and chromatin remodelling enzymes must be removed to observe defects [26*,68].

The logic of the transcription cycle

Over the last decade important insights into many of the factors involved in transcribing through chromatin provide the opportunity to take a step back and consider the overall organisation of the pathway (Figure 2). The pathway involves branching and feedback connections that act to ensure process such as H2BK123Ub and histone acetylation not only occur during transcription, but are also transient. The overall logic of the process appears to be largely directed at ensuring the processes of chromatin disassembly and reassembly are tightly coupled with transcription in a fashion that is comparable with the cell cycle where multiple check points ensure regulated progression with the single outcome of duplication. Many of the chromatin related factors involved in transcriptional elongation have roles in chromatin reassembly following transcription. Their action may be directed at breaking the potentially dangerous positive feedback loop that could result if a pioneering polymerase disrupts chromatin so as to facilitate subsequent transcription events from both the coding and non-coding strands. This would be expected to result in correlated bursts of transcription, an effect that appears not to occur at typical yeast genes transcribed at moderate levels [8**]. However, this situation may differ at more highly regulated genes where short burst of transcription have been observed [76], and there may be a greater requirement for memory effects in organisms with more complex developmental programmes [77].

Highly transcribed genes are observed to be enriched for distinct patterns of histone modifications (Figure 1). The
different distributions of these modifications can largely be attributed to differences in the frequency with which RNA polymerase transcribes a gene. For example, at genes transcribed at low levels Pol II directed histone acetylation may be short lived as a result of coupled action of histone deacetylases. Indeed, chromatin reassembly following transcription has been estimated to occur within 1 min [15]. In contrast, the deacetylated and methylated state may be relatively stable. As yeast genes are transcribed at an average rate of seven transcripts per hour [14⁎], most genes would be expected to be reassembled as chromatin 90% or more of the time. However, at very highly expressed genes, the high frequency of polymerase passage would be expected to dramatically increase the proportion of time chromatin is disrupted. Overall, the application of this process to large numbers of genes provides a means of directing many of the observed patterns of histone modification across coding regions (Figure 1). Removal of methylation marks by histone demethylases is an area that requires further investigation. Evidence to date suggests that demethylases are likely to be involved, but are as yet difficult to place in the overall pathway [78⁎]. Furthermore dilution of methylation marks during replication may play a role in the removal of H3K4 methylation [78⁎]. This is in effect the reverse of the idea that histone marks are stably inherited from one generation to the next. As a result, it seems likely that a significant proportion of histone modifications that characterise the coding regions of yeast genes do not comprise an epigenetic signal, but are instead instructed by the frequency of transcription.

The above description is no doubt a simplification. There is considerable variation in the ways that different genes respond to the loss of different component’s of the transcriptional machinery [79]. There is also evidence that the elongation machinery is deployed in different ways at different yeast genes [26⁎,53⁎], and this is clearly deployed as a major point of regulation in higher eukaryotes [80–84]. However, many of the histone modifications associated with elongation appear to function in a similar way at large numbers of genes. As a result, they are not acting to specify a broad range of distinct downstream functions as proposed in the histone code hypothesis [85].

In summary, the process of transcription through chromatin is becoming clearer as a result of a huge effort to characterise each of the steps involved. While there are undoubtedly many aspects that still remain to be discovered, overall the process provides a means of ensuring that the dynamic events occurring at the site of transcription are restored. In this way a substantial proportion of the chromatin landscape can be considered as being directed towards discretely covering the tracks left by the passage of RNA polymerase.

Acknowledgements
We apologise to colleagues for not citing work due to space constraints. TOH and TG are supported by Wellcome Trust Senior Fellowship 095862. Would like to thank Jonathan Chubbi, David Dickerson and Vijender Singh for comments and discussion.

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as: • of special interest •• of outstanding interest

1. Lee W, Tillo D, Bray N, Morse RH, Davis RW, Hughes TR, Nislow C: A high-resolution atlas of nucleosome occupancy in yeast. Nat Genet 2007, 39:1235-1244.
2. Rando OJ, Chang HY: Genome-wide views of chromatin structure. Annu Rev Biochem 2009, 78:245-271.
3. Kulaeva OI, Studitsky VM: Mechanism of histone survival during transcription by RNA polymerase II. Transcription 2010, 1:85-88.
4. Bondarenko VA, Steele LM, Ujvari A, Gaykalova DA, Kulaeva OI, Polikanov YS, Luse DS, Studitsky VM: Nucleosomes can form a polar barrier to transcript elongation by RNA polymerase II. Mol Cell 2006, 24:469-479.
5. Churchman LS, Weissman JS: Nascent transcript sequencing •• visualizes transcription at nucleotide resolution. Nature 2011, 469:368.

This study developed a method to map Pol II nascent transcripts at nucleotide resolution in vivo and identify nucleosomes as major barriers to Pol II elongation. Moreover they observe widespread divergent transcription and identify Rpd3S as key element in control of antisense transcripts.

6. Jin J, Bai L, Johnson DS, Fulbright RM, Kireeva ML, Kashlev M, Wang MD: Synergistic action of RNA polymerases in overcoming the nucleosomal barrier. Nat Struct Mol Biol 2010, 17:745-1742.

An elegant series of biophysical measurements are used to show that two RNA polymerases in close proximity transcribe through nucleosomes more efficiently.

7. Bintu L, Kopaczynska M, Hodges C, Lubkowska L, Kashlev M, •• Bustamante C: The elongation rate of RNA polymerase determines the fate of transcribed nucleosomes. Nat Struct Mol Biol 2011.

(Figure 2 Legend) Systematic representation of selected events occurring during elongation through chromatin. The left panel summarises the series of alterations to chromatin occurring during the course of transcriptional elongation that are discussed in the text. In the right panel an attempt has been made to integrate these events with other events occurring during transcriptional elongation in the form of a flow diagram. Diamond shaped symbols represent decisions, rectangles represent processing steps, parallelograms input or output (normally recruitment of a complex), connected by lines with information flowing in the direction indicated by arrows. The colouring used in both panels is the same. The diagram is far from comprehensive as some aspects have been simplified or omitted to retain clarity. For example the diagram incorporates the SAGA, and Rpd3s HAT and HDAC complexes, but it is known that other HAT’s (e.g. NuA4 [34]) and HDAC’s (e.g. Set3C [26]) also function in elongation, and others may be as yet unidentified. Despite these limitations it is clear that the process involves parallel pathways and feedback loops. Similar features have been identified using systematic approaches [79,90]. For example H2B ubiquitination is required for H3K4 methylation, which in turn recruits SAGA which can remove the H2BK123Ub mark. Furthermore removal of H2BK123Ub is required for conversion of Pol II to the fully elongation competent form phosphorylated at serine 2. This form of RNA polymerase can act to recruit the Rpd3S histone deacetylase, which is capable of reversing histone acetylation deposited moments earlier by the SAGA (or NuA4) complexes. This logic is ideally suited to ensuring that destabilisation of chromatin during transit by Pol II is both tightly coupled to transcription and transient.
This study shows a correlation between the rate of transcription elongation in vitro and the level of nucleosomes following polymerase passage. They find a correlation between high rates of transcription elongation and remodelling of the nucleosomal template.

8. Larson DR, Zenklusen D, Wu B, Chao JA, Singer RH: Real-time observation of transcription initiation and elongation on an endogenous yeast gene. Science 2011, 332:475-478.

This study used single molecule, single cells analysis to measure transcription frequency, elongation and termination in living cells. No memory between initiation events is observed.

9. Mairu P, Knezevich A, De Marco A, Mazza D, Kula A, McNally JG, Marcello A: Fast transcription rates of RNA polymerase II in human cells. EMBO Rep 2011, 12:1280-1285.

10. Thiriet C, Hayes JJ: Replication-independent core histones dynamics at transcriptionally active loci in vivo. Genes Dev 2005, 19:677-682.

11. Dion MF, Kaplan T, Kim M, Buratowski S, Friedman N, Rando OJ: Dynamics of replication-independent histone turnover in budding yeast. Science 2007, 315:1405-1408.

12. Ruffle A, Jacques PE, Bhat W, Robert F, Nourani A: Genome-wide replication-independent histone H3 exchange occurs predominantly at promoters and implicates H3K36 acetylation and Asf1. Mol Cell 2007, 27:393-405.

13. Jamaa I, Imoberdorf RM, Strubin M: Continuous histone H2B and transcription-dependent histone H3 exchange in yeast cells outside of replication. Mol Cell 2007, 25:345-355.

14. Pelechano V, Chavez S, Perez-Oritz JE: A complete set of nascent transcription rates for yeast genes. Plos One 2010;5.

This study used a combination of genomic run-on and Pol II microarrays in combination with analysis of previously published datasets to construct a more accurate dataset of nascent transcription rates in yeast.

15. Schwabish MA, Struhl K: Evidence for eviction and rapid deposition of histones upon transcriptional elongation by RNA polymerase II. Mol Cell Biol 2004, 24:10111-10117.

16. Kristiyan A, Svejstrup JQ: Evidence for distinct mechanisms facilitating transcript elongation through chromatin in vivo. EMBO J 2004, 23:4243-4252.

17. Nechaev S, Adelman K: Pol II waiting in the starting gates: regulating the transition from transcription initiation into productive elongation. Biochim Biophys Acta-Gen Gene Regul Mech 2011, 1809:34-45.

18. Buratowski S: Progression through the RNA polymerase II CTD cycle. Mol Cell 2009, 36:541-546.

19. Li B, Carey M, Workman JL: The role of chromatin during transcription. Cell 2007, 128:707-719.

20. Jähning JA: The Paf1 complex: platform or player in RNA polymerase II transcription? Biochim Biophys Acta 2010, 1799:379-386.

21. Eisen A, Utley RT, Nourani A, Allard S, Schmidt P, Lane WS, Lucchesi JC, Coto J: The yeast NuaA1 and Dro sophila MSL complexes contain homologous subunits important for transcription regulation. J Biol Chem 2001, 276:3484-3491.

22. Carrozza MJ, Li B, Florens L, Suganuma T, Swanson SK, Lee KK, Shia WJ, Anderson S, Yates J, Washburn MP et al.: Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. Cell 2005, 123:581-592.

23. Keogh MC, Kurdistani SK, Morris SA, Ahn SH, Podolny V, Collins SR, Schuldiner M, Chin KY, Punna T, Thompson NJ et al.: Cotranscriptional Set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. Cell 2005, 123:593-605.

24. Li B, Gogol M, Carey M, Lee D, Seidel C, Workman JL: Combined action of PHD and chromo domains directs the Rpd3S HDAC to transcribed chromatin. Science 2007, 316:1050-1054.

25. Joshi AA, Struhl K: Eaf3 chromodomain interaction with methylated H3-K36 links histone deacetylation to Pol II elongation. Mol Cell 2005, 20:971-978.

26. Govind CK, Qiu HF, Ginsburg DS, Ruan C, Hofmeyer K, Hu CH, Swaminathan V, Workman JL, Li B, Hinnebusch AG: Phosphorylated Pol II CTD recruits multiple HDACs. Including Rpd3C(S), for methylation-dependent deacetylation of ORF nucleosomes. Mol Cell 2010, 39:234-246.

The phosphorylation state of the pol II CTD is shown to play an important role in the recruitment of HDAC complexes.

27. Bian CB, Xu C, Ruan JB, Lee KK, Burke TL, Tempel W, Baryshe D, Li J, Wu MH, Zhou BO et al.: Sgf29 binds histone H3K4me2/3 and is required for SAGA complex recruitment and histone H3 acetylation. EMBO J 2011, 30:2829-2842.

This paper studies the contribution of the Sgf29 subunit in recruitment of the SAGA complex to chromatin template. The authors show evidence for the structural basis of the Sgf29 tudor domains in selective binding to H3K4me2/3.

28. Vermeulen M, Eberle HC, Matarrese F, Marks H, Denissov S, Butler F, Lee KK, Olsen JV, Hyman AA, Stunnenberg HG et al.: Quantitative interaction proteomics and genome-wide profiling of epigenetic histone marks and their readers. Cell 2010, 142:967-980.

A combinatorial proteomics approach is used to study the proteins that interact with histone tri-methyl marks. The authors study the genomic distribution of the identified tri-methyl mark readers and assign them to complexes that elucidates the relationship between histone marks and gene regulation.

29. Tavernsa SD, Ilin S, Rogers RS, Tanny JC, Lavender H, Li HT, Baker L, Boyle J, Blair LP, Chat BT et al.: Yng1 PHD finger binding to H3 trimethylation at K4 promotes Nua3 HAT activity at K14 of H3 and transcription at a subset of targeted ORFs. Mol Cell 2006, 24:785-796.

30. Saksouk N, Avvakumov N, Champagne KS, Hung T, Doyon Y, Cayrou C, Paquet E, Ullah M, Landry AJ, Cote V et al.: HBO1 HAT complexes target chromatin throughout gene coding regions via multiple PHD finger interactions with histone H3 tail. Mol Cell 2009, 35:257-265.

31. Hung T, Binda O, Champagne KS, Kuo AJ, Johnson K, Chang HY, Simon MD, Kutateladze TG, Gozani O: ING4 mediates crosstalk between histone H3 K4 trimethylation and H3 acetylation to attenuate cellular transformation. Mol Cell 2009, 33:248-256.

32. Kim T, Buratowski S: Dimethylation of H3K4 by Set1 recruits the Set3 histone deacetylase complex to 5’ transcribed regions. Cell 2009, 137:259-272.

33. Flanagan JF, Mi LZ, Chruszcz M, Cymborowski M, Cines KL, Kim Y, Minor W, Rastinejad F, Khorasanizadeh S: Double chromodomains cooperate to recognize the methylated histone H3 tail. Nature 2005, 438:1181-1185.

34. Ginsburg DS, Govind CK, Hinnebusch AG: Nua4 lysine acetyltransferase Ess1 is targeted to coding regions and stimulates transcription elongation with Gcn5. Mol Cell Biol 2009, 29:6473-6487.

35. Govind CK, Zhang F, Qiu H, Hofmeyer K, Hinnebusch AG: Gcn5 promotes acetylation, eviction, and methylation of nucleosomes in transcribed coding regions. Mol Cell 2007, 28:31-42.

36. Tous C, Rondon AG, Garcia-Rubio M, Gonzalez-Aguilera C, Luna R, Aguilera A: A novel assay identifies transcript elongation roles for the Nup84 complex and RNA processing factors. EMBO J 2011, 30:1953-1964.

A new assay is developed to screen for factors affecting the rate of elongation. This shows that the SAGA and Nup84 complexes have roles in elongation.

37. Schwabish MA, Struhl K: The Swi/Snf complex is important for histone eviction during transcriptional activation and RNA polymerase II elongation in vivo. Mol Cell Biol 2007, 27:6987-6995.

This paper studies the recruitment of the Swi/Snf complex to both promoters and coding regions of active genes in yeast. The authors provide evidence that Swi/Snf can facilitate Pol II elongation functions.

38. Ferreira H, Fiaus A, Owen-Hughes T: Histone modifications influence the action of Swi/Snf family remodeling enzymes by different mechanisms. J Mol Biol 2007, 374:563-579.

39. Soutourina J, Floch VBL, Gendrel G, Flores A, Ducrot C, Dumay-Odetot H, Soulare P, Navarro F, Cairns BR, Lefevbre O et al.:...
Transcribing chromatin

Owen-Hughes and Gkikopoulos

Rsc4 connects the chromatin remodeler RSC to RNA polymerases. Mol Cell Biol 2006, 26:4920-4933.

40. Carey M, Li B, Workman JL: RSC exploits histone acetylation to abrogate the nucleosomal block to RNA polymerase II elongation. Mol Cell 2006, 24:481-487.

This study shows the combinatorial effect of histone acetylation and ATP-dependent chromatin remodelling in transcription elongation in vitro. RSC is found to relieve a Pol II pausing near a nucleosome in a histone acetylation dependent manner.

41. Wyce A, Xiao T, Whelan KA, Kosman C, Walter W, Eick D, Hughes TR, Krogan NJ, Strahl BD, Berger SL: H2B ubiquitylation acts as a barrier to Ctk1 nucleosomal recruitment prior to removal by Ubp8 within a SAGA-related complex. Mol Cell 2007, 27:275-288.

This study investigated further the role of ubiquitination in transcription regulation. The authors find that removal of H2B ubiquitylation is important for recruitment of Ctk1 and Pol II phosphorylation.

42. Das C, Tyler JK, Churchill MEA: The histone shuffle: histone chaperones in an energetic dance. Trends Biochem Sci 2010, 35:476-489.

43. Horende M, Ladurner AG: The chaperone-histone partnership: for the greater good of histone traffic and chromatin plasticity. Curr Opin Struct Biol 2011.

44. Winkler DD, Muthurajan UM, Hieb AR, Luger K: Histone chaperone FACT coordinates nucleosome rearrangement through multiple synergistic binding events. J Biol Chem 2011, 286:41883-41892.

45. Xin H, Takahata S, Blanksma M, McCullough L, Stillman DJ.

46. Formosa T: yFACT induces global accessibility of nucleosomal DNA without H2A-H2B displacement. Mol Cell 2009, 35:365-376.

This study suggests the FACT can alter nucleosomal DNA accessibility by a mechanism independent of H2A-H2B dimer displacement.

47. Belotserkovskaya R, Oh S, Bondarenko VA, Orphanides G, Studitsky VM, Reinberg D: FACT facilitates transcription-dependent nucleosome alteration. Science 2003, 301:1090-1093.

48. Mason PB, Struhl K: The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation in vivo. Mol Cell Biol 2003, 23:8323-8333.

49. Jamai A, Puglisi A, Strubin M: Histone chaperone Spt16 promotes redeposition of the original H3-H4 histones evicted by elongating RNA polymerase. Mol Cell 2009, 35:377-383.

This paper provides evidence that FACT is involved in regulating transcription-dependent histone occupancy in yeast. The authors show that deletion of Spt16 leads to a genome-wide loss of both H2B and H3 and that this requires active transcription.

50. Formosa T: The role of FACT in making and breaking nucleosomes. Biochim Biophys Acta (BBA) - Gene Regul Mech 2011 http://dx.doi.org/10.1016/j.bbagen.2011.07.009.

51. Stevens JR, O’Donnell AF, Perry TE, Benjamin JR, Barnes CA, Johnston GC, Singer RA: FACT, the Bur kinase pathway, and the histone co-repressor Hic1 have overlapping nucleosome-related roles in yeast transcription elongation. PLoS One 2011:6.

52. Pavi R, Zhu B, Li GH, Trojer P, Mandal S, Shilatifard A, Reinberg D: Histone H2B monoubiquitination functions cooperatively with FACT to regulate elongation by RNA polymerase II. Cell 2006, 125:703-717.

The authors using an in vitro reconstituted chromatin system to study the cooperative contribution of H2B ubiquitination, recruitment of the PAF and FACT complex in transcription elongation. The authors show evidence suggesting that H2B ubiquitination and recruitment of FACT prepare the template for Pol II elongation though chromatin templates.

53. Ivanovska I, Jacques PE, Rando OJ, Robert F, Winston F: Control of chromatin structure by Spt6: different consequences in coding and regulatory regions. Mol Cell 2011, 31:531-541.

This paper studies the role of Spt6 in regulation of chromatin over coding regions and promoters of yeast genes. The authors show that Spt6 has functions in transcription regulation that are distinct to its role in nucleosome occupancy over coding regions.

54. Kaplan CD, Laprade L, Winston F: Transcription elongation factors repress transcription initiation from cryptic sites. Science 2003, 301:1096-1099.

55. Close D, Johnson SJ, Sdano MA, McDonald SM, Robinson H, Formosa T, Hill CP: Crystal structures of the S. cerevisiae Spt6 core and C-terminal tandem SH2 domain. J Mol Biol 2011, 408:697-713.

56. Diebold ML, Loeliger E, Koch M, Winston F, Cavarelli J, Romier C: Noncanonical tandem SH2 enables interaction of elongation factor Spt6 with RNA polymerase II. J Biol Chem 2010, 285:38389-38398.

57. Sun M, Lariviere L, Dengl S, Mayer A, Cramer P: A tandem SH2 domain in transcription elongation factor Spt6 binds the phosphorylated RNA polymerase II C-terminal repeat domain (CTD). J Biol Chem 2010, 285:41897-41903.

58. Liu JP, Zhang JH, Gong QG, Xiong P, Huang HD, Wu B, Lu GW, Wu JH, Shi YY: Solution structure of tandem SH2 domains from Spt6 protein and their binding to the phosphorylated RNA polymerase II C-terminal domain. J Biol Chem 2011, 286:2918-29226.

59. McDonald SM, Close D, Xin H, Formosa T, Hint CP: Structure and biological importance of the Spt1-Spt6 interaction, and its regulatory role in nucleosome binding. Mol Cell 2010, 40:725-735.

60. Pinskiaya M, Gournvenec S, Morillon A: H3 lysine 4 di- and trimethylation deposited by cryptic transcription attenuates promoter activation. EMBO J 2009, 28:1697-1707.

61. Hainer SJ, Prunessi JA, Mitchell RD, Monteverde RM, Martens JA: Intergenic transcription causes repression by directing nucleosome assembly. Genes Dev 2011, 25:29-40.

62. Thebault P, Boutin G, Bhat W, Ruifange A, Martens J, Nourani A: Transcription regulation by the noncoding RNA SRG1 requires Spt2-dependent chromatin deposition in the wake of RNA polymerase II. Mol Cell 2011, 31:1298-1300.

63. Zhang Y, Moqtaderi Z, Rattner BP, Euskirchen G, Snyder M, Kadonaga JT, Liu XS, Struhl K: Intrinsic histone-DNA interactions are not the major determinant of nucleosome positions in vivo. Nat Struct Mol Biol 2009, 16:847-870.

64. Hainer S, Moore IK, Fondufe-Mittendorf Y, Gossett AJ, Tilloy D, Field Y, LeProust EM, Hughes TR, Lieb JD, Widom J et al.: The DNA-encoded nucleosome organization of a eukaryotic genome. Nature 2009, 458:362-372.

65. Zhang Z, Wippo C, Wal M, Ward E, Korber P, Pugh BF: A packing mechanism for nucleosome organization reconstituted across a eukaryotic genome. Science 2011, 332:977-980.

This study provides evidence for an ATP-dependent nucleosome organization mechanism as a major player that directs chromatin organisation with respect to transcription start sites.

66. Tiross I, Sigal N, Barkai N: Widespread remodeling of mid-coding sequence nucleosomes by Isw1. Genome Biol 2010, 11.

67. Simic R, Lindstrom DL, Tran HG, Reinick KL, Costa PJ, Johnson AD, Hartogz GA, Arndt KM: Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. EMBO J 2003, 22:1846-1856.

68. Gkikopoulos T, Schofield P, Singh V, Pinskiaya M, Mellor J, Smolle M, Workman JL, Barton GJ, Owen-Hughes T: A role for Snf2-related nucleosome-spacing enzymes in genome-wide nucleosome organization. Science 2011, 335:1739-1740.

69. Quan TK, Hartogz GA: Histone H3K4 and K36 methylation, Chd1 and Rpd3S5 impose the functions of Saccharomyces cerevisiae Spt4-Spt5 in transcription. Genetics 2010, 184:321-329.
This study provides genetic evidence for factors that negatively influence the elongation function of Spt4/S. The authors show that Spt5 suppressors can be found in chromatin modifying complexes like Rpd3S and Chd1 or in H3 mutants that provide a genetic link between transcription elongation and chromatin.

70. Ryan DP, Sundaramoorthy R, Martin D, Singh V, Owen-Hughes T: The DNA-binding domain of the Chd1 chromatin-remodelling enzyme contains SANT and SLIDE domains. *EMBO J* 2011, 30:2596-2609.

71. Celona B, Weiner A, Di Felice F, Mancuso FM, Cesarini E, Rossi RL, Gregory L, Baban D, Rossetti G, Giani P et al.: Substantial histone reduction modulates genomewide nucleosomal occupancy and global transcriptional output. *PLoS Biol* 2011:3.

A consequence of deleting HMGB1 proteins in yeast and human cells is found to be a global reduction in nucleosome occupancy. Reduced occupancy is found not to greatly affect the positioning of high occupancy nucleosomes.

72. Engholm M, de Jager M, Flaus A, Brench R, van Noort J, Owen-Hughes T: Nucleosomes can invade DNA territories occupied by their neighbors. *Nat Struct Mol Biol* 2009, 16:151-158.

73. Zhou BO, Zhou JQ: Recent transcription-induced histone H3 lysine 4 (H3K4) methylation inhibits gene reactivation. *J Biol Chem* 2011, 286:34770-34776.

74. Weiner A, Hughes A, Yassour M, Rando OJ, Friedman N: High-resolution nucleosome mapping reveals transcription-dependent promoter packaging. *Genome Res* 2010, 20:90-100.

This paper identifies differences in the susceptibility of nucleosomes to digestion with MNase. Following, inactivation of RNA polymerase, some changes to chromatin structure are detected, but substantial organisation is retained.

75. Radman-Livaja M, Verzijlbergen KF, Weiner A, van Welsem T, Friedman N, Rando OJ, van Leeuwen F: Patterns and mechanisms of ancestral histone protein inheritance in budding yeast. *Plos Biology* 2011, 9:e1001075.

An elegant recombination tagging system is used to compare the distribution of newly synthesized histones with those made in previous generations. The remarkable observation is made that old histones accumulate towards the 5' ends of genes, especially long genes transcribed at low levels.

76. Zenklusen D, Larson DR, Singh RH: Single-RNA counting reveals alternative modes of gene expression in yeast. *Nat Struct Mol Biol* 2008, 15:1263-1271.

77. Chubb JR, Treck T, Shenoy SM, Singh RH: Transcriptional pulsed of a developmental gene [see comment]. *Curr Biol* 2006, 16:1018-1025.

78. Radman-Livaja M, Liu CL, Friedman N, Schreiber SL, Rando OJ: Replication and active demethylation represent partially overlapping mechanisms for erasure of H3K4me3 in budding yeast. *PLoS Genet* 2010, 6:e1000837.

This study shows a role for the demethylase Jhd2 and DNA replication in removing H3K4 methylation. The finding that DNA replication acts to remove H3 methylation is contrary to the concept that histone modifications act as inherited signals.

79. Lenstra TL, Benschop JJ, Kim T, Schulze JM, Brabers NACH, Margaritis T, van de Pasch LAL, van Heesch SAAC, Brok MO, Koerkamp MJAG et al.: The specificity and topology of chromatin interaction pathways in yeast. *Mol Cell* 2011, 42:536-549.

80. Guenther MG, Levine SS, Boyer LA, Jaenisch R, Young RA: A chromatin landmark and transcription initiation at most promoters in human cells. *Cell* 2007, 130:77-88.

81. Muse GW, Gilchrist DA, Nechaev S, Shah R, Parker JS, Grissom SF, Zeitlinger J, Adelman K: RNA polymerase is poised for activation across the genome. *Nat Genet* 2007, 39:1507-1511.

82. Lee CY, Li XY, Hechmer A, Eisen M, Biggin MD, Venters BJ, Jiang CZ, Li J, Pugh BF, Gilmour DS: NELF and GAGA factor are linked to promoter-proximal pausing at many genes in *Drosophila*. *Mol Cell Biol* 2008, 28:3290-3300.

83. Nechaev S, Fargo DC, dos Santos G, Liu LW, Gao Y, Adelman K: Global analysis of short RNAs reveals widespread promoter-proximal stalling and arrest of Pol II in *Drosophila*. *Science* 2010, 327:335-338.

84. Core LJ, Waterfall JJ, Lis J: Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters. *Science* 2008, 322:1845-1848.

85. Henikoff S, Shilatifard A: Histone modification: cause or cog? *Trends Genet* 2011, 27:389-396.

86. Liu CL, Kaplan T, Kim M, Buratowski S, Schreiber SL, Friedman N, Rando OJ: Single-nucleosome mapping of histone modifications in *S. cerevisiae*. *PLoS Biol* 2005, 3:e328.

87. Mayer A, Lidschreiber M, Siebert M, Leike K, Sodding J, Cramer P: Uniform transitions of the general RNA polymerase II transcription complex. *Nat Struct Mol Biol* 2010, 17:1272.

A valuable resource characterising elongation factors into groups that associate with similar distributions over many genes.

88. Shieh GS, Pan CH, Wu JH, Sun YJ, Wang CC, Hsiao WC, Lin CY, Tung L, Chang TH, Fieming AB et al.: H2B ubiquitylation is part of chromatin architecture that marks exon-intron structure in budding yeast. *BMC Genomics* 2011, 12.

89. Schulze JM, Hentrich T, Nakashima S, Gupta A, Emberly E, Shilatifard A, Kobor MS: Splitting the task: Upb8 and Upb10 deubiquitinate different cellular pools of H2BK123. *Genes & Development* 2011, 25:2242-2247.

A recent study illustrating the distinct functions for deubiquitylases in regulating the chromatin landscape over coding regions.

90. Zheng JS, Benschop JJ, Shaels M, Kemmeren P, Greenblatt J, Cagney G, Holstege F, Li H, Krogan NJ: Epistatic relationships reveal the functional organization of yeast transcription factors. *Mol Syst Biol* 2010:6.