O., Lippert, C., et al. (2011). Nat. Genet. *43*, 956–963.

Chae, E., Bomblies, K., Kim, S.-T., Karelina, D., Zaidem, M., Ossowski, S., Martín-Pizarro, C., Laitinen, R.A.E., Rowan, B.A., Tenenboim, H., et al. (2014). Cell *159*, this issue, 1341–1351.

Coyne, J.A., and Orr, H.A. (2004). Speciation (Sunderland, Mass.: Sinauer Associates).

Maekawa, T., Kufer, T.A., and Schulze-Lefert, P. (2011). Nat. Immunol. *12*, 817–826.

Orr, H.A. (1991). Evolution *45*, 764.

Phadnis, N., and Orr, H.A. (2009). Science *323*, 376–379.

Spoel, S.H., and Dong, X. (2012). Nat. Rev. Immunol. *12*, 89–100.

Ueshima, R., and Asami, T. (2003). Nature *425*, 679.

A Split Personality for Nucleosomes

Daniel J. McKay[1] and Jason D. Lieb[2,*]

[1]Department of Biology, Department of Genetics, Integrative Program for Biological and Genome Sciences, The University of North Carolina at Chapel Hill, 250 Bell Tower Drive, Chapel Hill, NC 27599, USA

[2]Department of Human Genetics, The University of Chicago, 920 East 58th Street, Chicago, IL, 60637, USA

*Correspondence: jdlieb@uchicago.edu

http://dx.doi.org/10.1016/j.cell.2014.11.030

A high-resolution look at where histones touch DNA reveals a surprisingly intricate, dynamic, and modular nucleosome. Three advances in the study by Rhee et al. include unexpected interactions between the H3 tail and linker DNA, new evidence for existence of subnucleosomal particles, and asymmetric patterns of histone modification within a single nucleosome that correspond to the direction of transcription.

In eukaryotic cells, DNA is packaged into nucleosomes. A single nucleosome consists of a protein spool made of histones, wrapped by DNA. In addition to packaging DNA, nucleosomes also compete with other DNA-binding proteins and thereby influence access to the regulatory information that controls DNA-dependent processes such as transcription, replication, and DNA repair. In this issue of *Cell*, Pugh and colleagues (Rhee et al., 2014) apply a high-resolution mapping approach called ChIP-exo in yeast to examine the genome-wide position and organization of the individual histones that comprise nucleosomes. Their findings reveal surprisingly complex nucleosome substructures and dynamics that immediately bring to light an exciting set of new questions for the field, while at the same time evoking early models of the nucleosome (Weintraub et al., 1976).

Some background is required to set the stage for the three major advances derived from the results. The traditionally defined nucleosome core consists of an octamer of histone proteins, around which ~147 bp of DNA is wrapped. This octamer is composed of two copies each of the histones H2A, H2B, H3, and H4. More specifically, dimers of H3 and H4 interact to form a tetramer, which is flanked on each side by a dimer of H2A and H2B. Pugh and colleagues used ChIP-exo to determine the precise location of individual histone proteins across the yeast genome. ChIP-exo is a modified version of conventional chromatin immunoprecipitation (ChIP) that provides high-resolution identification of binding sites for proteins that interact with DNA. Like ChIP, the first step in ChIP-exo is to covalently crosslink proteins to DNA with formaldehyde. After sonication to shear the chromatin into smaller fragments and immunoprecipitation with antibodies that recognize the protein of interest, ChIP-exo then uses lambda exonuclease to digest DNA strands in the 5′ to 3′ direction. Digestion is blocked when the exonuclease reaches a protein-DNA crosslink. After high-throughput sequencing, pairs of 5′ ends on the forward and reverse strands (exonuclease stop points) thus represent the boundaries of a given protein-DNA interaction. ChIP-exo has previously been used to map binding sites for sequence-specific transcription factors (Rhee and Pugh, 2011), preinitiation complexes (Rhee and Pugh, 2012), and chromatin remodelers (Yen et al., 2012; Yen et al., 2013).

The first intriguing result of Rhee et al. (2014) concerns the amino-terminal tail of histone H3, which is heavily decorated with posttranslational modifications and has important regulatory functions. ChIP-exo results for H2B and H4 histones identified crosslinking points that closely correspond to the genomic locations expected from the crystal structure (Luger et al., 1997). On the other hand, ChIP-exo results for histone H3 showed an unexpected crosslinking pattern. In the crystal structure, most of the amino acids comprising H3 reside at the nucleosome midpoint (called the "dyad"), where they contribute substantially to DNA interactions (Luger et al., 1997). However, the predominant H3-DNA interaction determined by ChIP-exo was located in the linker DNA that separates adjacent nucleosomes, not at the nucleosme dyad. The authors speculated that this interaction may be mediated through the N-terminal tail of histone H3, and then tested their

hypothesis by performing ChIP-exo in a yeast strain lacking the first 28 amino acids of H3. While the crosslinking signal between H3 and linker DNA diminished in the H3Δ1-28 strain, a noticeable signal remained that was still greater than the H3 signal at the dyad. Since approximately ten amino acids of the H3 tail are still present in the H3Δ1-28 strain, the source of the residual H3-linker signal remains unresolved. The significance of this interaction has not yet been tested, but it is notable due to the known regulatory functions of the H3 tail and because interactions between histone tails and linker DNA may be important for higher-order chromatin organization.

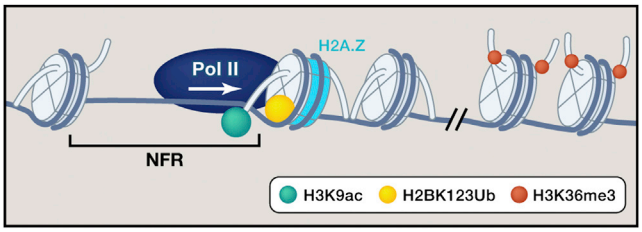

Figure 1. ChIP-Exo Reveals Asymmetric Features of Nucleosomes at Gene Promoters.

The histone variant H2A.Z is enriched at the +1 nucleosome, and is preferentially localized to the NFR-distal side of +1 nucleosomes with high turnover. At highly expressed genes, histone modifications linked to transcription (H2BK123ub and H3K9ac) are asymmetrically localized on the side of the +1 nucleosome closest to the NFR. H3K9ac is also associated with interactions between the H3 tail and DNA in the NFR. Away from promoters, histone H3 tails interact with linker DNA, except when H3K36 is methylated.

The increase in resolution afforded by ChIP-exo allowed the authors to make a second important finding: new evidence for semi-independent behavior of each half of the nucleosome and for the existence of subnucleosomal particles. The nucleosome crystal structure shows that the histones are roughly symmetrical about the dyad axis, with one H2A/H2B dimer and one H3/H4 dimer located on each half of the nucleosome. Therefore, one might expect that the histone occupancies on one half of the dyad would be tightly correlated with the histone occupancies on the other half of the dyad. While histone occupancies across the nucleosome are indeed correlated, the authors found that histone occupancies on a given half of the dyad correlated much more closely with each other than with the histone occupancies on the opposite half of the dyad. H2B in particular had pronounced differences in occupancy across the two halves of the nucleosome. These differences in histone occupancies could be explained by the presence of subnucleosomal-sized particles consisting of hexasomes (nucleosomes that lack one H2A/H2B dimer) and half-nucleosomes (nucleosomes containing one H2A/H2B dimer and one H3/H4 dimer). Multiple groups have provided evidence for subnucleosomal-sized particles previously (Floer et al., 2010; Henikoff et al., 2011; Kent et al., 2011). These subnucleosomal particles were interpreted to be partially unwound nucleosomes, "fragile" nucleosomes, and nonhistone transcription factor complexes. The ChIP-exo data presented in Rhee et al. is consistent with previous findings and extends them by providing strong evidence that subnucleosomal-sized particles smaller than hexasomes can consist of half-nucleosomes rather than H3/H4 tetramers.

The third important observation involves asymmetries in the posttranslational modifications and histone variants within nucleosomes. Since a given gene is transcribed predominantly in a single direction, the histone modifications and variants that contribute to different steps of the transcription cycle are typically deposited with a distinct polarity along the length of the gene. For example, H3K4me3 is primarily found at gene promoters, whereas H3K36me3 is enriched toward the 3′ end of genes. The authors asked if there was transcription-correlated polarity at the level of individual histone proteins within a nucleosome by performing ChIP-exo using antibodies that recognize H3K9ac, H2Bub, and the histone variant H2A.Z. Remarkably, the histone H3 crosslinks that remained following H3K9ac ChIP were enriched primarily on the NFR-proximal half of the +1 nucleosome. The +1 nucleosome is the first nucleosome in a gene immediately downstream of the transcriptional start site and is typically preceded by a nucleosome free region (NFR). ChIP-exo with antibodies to all forms of H3 revealed no crosslinking in the NFR, suggesting that the H3K9ac interaction occurred only in a small subset of the population or was very transient. Further investigation revealed that this signal was only found at highly transcribed genes. H2Bub also showed a similar pattern of occupancy on the NFR-proximal half of the +1 nucleosome of highly transcribed genes. The histone variant H2A.Z was also incorporated into the +1 nucleosome; however, it was asymmetrically localized to the other side of the nucleosome, on the NFR-distal half (Figure 1). This contrasts with previous ChIP-exo data in which H2A.Z showed symmetrical localization on the +1 nucleosome (Yen et al., 2013). Interestingly, the chromatin remodeler that deposits H2A.Z into chromatin (SWR-C) was reported to localize asymmetrically to the opposite (NFR-proximal) side of the +1 nucleosome. Therefore, how H2A.Z becomes enriched on the NFR-distal half of the +1 nucleosome remains to be determined. It is possible that an asymmetry exists in the proposed cycle of H2A.Z deposition and removal by chromatin remodelers (Yen et al., 2013).

The three principal findings described by Rhee et al., namely the interactions between histone H3 and linker DNA, evidence for existence of subnucleosomal particles, and the asymmetric patterns of histone modifications and variants, add to the evidence for a complex and dynamic nucleosome. Pugh and colleagues have discovered important details of nucleosome structure that will inform future investigations into the mechanisms by which histone organization, modification, and stability contribute to transcription and other DNA-dependent processes.

REFERENCES

Floer, M., Wang, X., Prabhu, V., Berrozpe, G., Narayan, S., Spagna, D., Alvarez, D., Kendall, J., Krasnitz, A., Stepansky, A., et al. (2010). Cell *141*, 407–418.

Henikoff, J.G., Belsky, J.A., Krassovsky, K., MacAlpine, D.M., and Henikoff, S. (2011). Proc. Natl. Acad. Sci. USA *108*, 18318–18323.

Kent, N.A., Adams, S., Moorhouse, A., and Paszkiewicz, K. (2011). Nucleic Acids Res. *39*, e26.

Luger, K., Mäder, A.W., Richmond, R.K., Sargent, D.F., and Richmond, T.J. (1997). Nature *389*, 251–260.

Rhee, H.S., and Pugh, B.F. (2011). Cell *147*, 1408–1419.

Rhee, H.S., and Pugh, B.F. (2012). Nature *483*, 295–301.

Rhee, H.S., Bataille, A.R., Zhang, L., and Pugh, B.F. (2014). Cell *159*, this issue, 1377–1388.

Weintraub, H., Worcel, A., and Alberts, B. (1976). Cell *9*, 409–417.

Yen, K., Vinayachandran, V., Batta, K., Koerber, R.T., and Pugh, B.F. (2012). Cell *149*, 1461–1473.

Yen, K., Vinayachandran, V., and Pugh, B.F. (2013). Cell *154*, 1246–1256.

How TriC Folds Tricky Proteins

Anastasia Zhuravleva[1,*] and Sheena E. Radford[1,*]
[1]Astbury Centre for Structural Molecular Biology, School of Molecular and Cellular Biology, University of Leeds, Leeds LS2 9JT, UK
*Correspondence: a.zhuravleva@leeds.ac.uk (A.Z.), s.e.radford@leeds.ac.uk (S.E.R.)
http://dx.doi.org/10.1016/j.cell.2014.11.029

How chaperonins orchestrate the successful folding of even the most elaborate of proteins is a question of central importance. In two recent studies in *Cell* by Joachimiak et al. and Freund et al., a new class of TRiC substrate is identified, and how the chaperonin exploits its different subunits to extend its substrate repertoire and direct productive folding is revealed.

For proper functioning, newly synthesized proteins must be correctly folded. This can be difficult to achieve, especially for large proteins with complex topologies. Misfolded proteins are not only inactive but can be toxic, creating a devastating imbalance of protein synthesis and folding that has been linked to many devastating diseases (Kim et al., 2013). Molecular chaperones interact with unfolded and partially folded proteins to facilitate folding and prevent misfolding and aggregation. To perform these functions, ATP-driven molecular chaperones, such as Hsp70s, Hsp90s, and the Hsp60 chaperonins, use the energy of ATP to control substrate binding and release and to promote correct folding (Kim et al., 2013).

Chaperonins are complex allosteric machines. They consist of two stacked rings of seven or more identical, or homologous, subunits that form a barrel-like structure used to encapsulate the folding substrate protein (Figure 1). The most well-studied group I chaperonin, bacterial GroEL, is formed from two rings, each with seven identical 60 kDa subunits. This homo-oligomeric chaperonin interacts with its substrate proteins predominantly via hydrophobic interactions (Figure 1, left). By contrast, the eukaryotic group II chaperonin, TCP-1 ring complex (TRiC), is a hetero-oligomeric chaperonin, and it recognizes its substrates via hydrophobic, electrostatic, and/or polar motifs (Dunn et al., 2001; Kalisman et al., 2013). The increased complexity of the hetero-oligomeric ring allows TRiC to promote folding of a very broad range of protein substrates. Indeed, about 5%–10% of all newly synthesized proteins require TRiC to fold (Yam et al., 2008). TRiC has also been shown to inhibit the aggregation of huntingtin, interacting with the tips of polyQ-containing fibrils, as well as smaller oligomers (Shahmoradian et al., 2013).

In this issue of *Cell*, Freund et al. (2014) report the discovery of a new TRiC substrate—the telomerase protein TCAB1—which is essential for trafficking of telomerase and small Cajal body RNAs required for telomere maintenance during cell division (Venteicher and Artandi, 2009). The authors performed a genome-wide RNA fluorescence in situ hybridization (FISH)-based siRNA screen for genes required for Cajal bodies' localization of a key telomerase enzyme, the telomerase RNA component (TERC), and telomerase protein TCAB1. Surprisingly, in addition to known telomerase assembly factors, the authors found that several TRiC subunits are required for TERC and TCAB1 localization in Cajal bodies. Depletion of TRiC results in a loss of TCAB1, mislocalization of telomerase and Cajal body RNAs, and failure of telomere elongation. TRiC, it turns out, is essential for TCAB1 folding. The results explain why mutations in TCAB1 can lead to severe diseases and suggest that a larger range of protein substrates than considered hitherto may require TRiC to fold.

In a second recent study in *Cell*, Joachimiak et al. (2014) shed exciting new light on the structural mechanism of substrate recognition by TRiC and how TriC is able to fold its broad range of protein substrates (Yam et al., 2008, Shahmoradian et al., 2013, Freund et al., 2014). Each ring of TRiC consists of eight homologous subunits (CCT1–CCT8) (Figure 1, right-hand, top), with the majority of the sequence variations between TRiC's subunits being found in their apical domains (Dunn et al., 2001). Like its GroEL homolog, substrates bind to the apical domains of TRiC, and it has been suggested previously that the sequence variations in these domains are important for substrate recognition (Dunn et al., 2001; Kalisman et al., 2013). However, how TRiC binds its broad repertoire of substrates and promotes their correct folding remained elusive.