of peptide inhibitors can indeed be developed, although drug-resistant mutants have been an enduring theme in all anti-HIV therapies.

Only two precedents have been reported for inhibition of HIV-1 assembly by small molecules, and both compounds interact with CA. One, a derivative of betulinic acid, was originally discovered in a drug screen for compounds that interfere with virus replication in cell culture. The compound prevents proteolytic cleavage at the site that would become the C terminus of the mature HIV-1 CA[10,11], but the actual binding site has not been identified. Small changes in the amino acid sequence at the cleavage site render the virus drug resistant. The second compound emerged from an *in silico* screen for molecules that fit into a pocket in the region of Gag that forms the N terminus of the CA protein. However, it turned out that the compound binds to a different site in C-CA, inhibiting mature assembly of CA *in vitro* and in cell culture[12]. Other laboratories now also are using *in vitro* assembly systems to screen for additional HIV-1 assembly inhibitors.

Clearly there are many challenges involved in developing useful small-molecule analogs of the inhibitory peptide studied by Sticht *et al.*[5] and Ternois *et al.*[9], or of other peptides that may be mined from the phage display library. This work is important because it is the first systematic, molecular approach to specifically identify reactive surfaces in Gag or CA. It is far too early to predict whether these results will translate into useful drugs. Nevertheless, as for so many small molecules that do not reach the clinic, the inhibitory peptides and any future non-peptide-based analogs should be useful tools for further unraveling the principles of mature and immature HIV-1 assembly.

1. Ganser, B.K., Li, S., Klishko, V.Y., Finch, J.T. & Sundquist, W.I. *Science* **283**, 80–83 (1999).
2. Gross, I. *et al. EMBO J.* **19**, 103–113 (2000).
3. Li, S., Hill, C.P., Sundquist, W.I. & Finch, J.T. *Nature* **407**, 409–413 (2000).
4. Lanman, J. *et al. J. Mol. Biol.* **325**, 759–772 (2003).
5. Sticht, J. *et al. Nat. Struct. Mol. Biol.* **12**, 671–677 (2005).
6. Briggs, J.A.G., Wilk, T., Welker, R., Kräusslich, H.-G. & Fuller, S.D. *EMBO J.* **22**, 1707–1715 (2003).
7. Cairns, T.M. & Craven, R.C. *J. Virol.* **75**, 242–250 (2001).
8. Ivanov, D., Stone, J.R., Maki, J.L., Collins, T. & Wagner, G. *Mol. Cell* **17**, 137–143 (2005).
9. Ternois, F., Sticht, J., Duquerroy, S., Kräusslich, H.-G. & Rey, F.A. *Nat. Struct. Mol. Biol.* **12**, 678–682 (2005).
10. Li, F. *et al. Proc. Natl. Acad. Sci. USA* **100**, 13555–13560 (2003).
11. Zhou, J. *et al. J. Virol.* **78**, 922–929 (2004).
12. Tang, C. *et al. J. Mol. Biol.* **327**, 1013–1020 (2003).
13. Briggs, J.A.G. *et al. Nat. Struct. Mol. Biol.* **11**, 672–675 (2004).

A milestone in the odyssey of higher-order chromatin structure

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The recent crystal structure of tetranucleosomes supports zigzag-based models of chromatin fiber structure. The next challenge is to understand the modulation of chromatin structure in the nucleus, characterized by variability in DNA, histones and non-histone components.

In a recent issue of *Nature*, in what is likely to become a landmark paper in the long odyssey of the search for the principles of chromatin 'higher-order structure', Schalch *et al.*[1] report the X-ray structure of a tetranucleosome. In a sense, the journey began over 40 years ago when Joseph Gall showed that the contents of nucleated newt erythrocytes, when spread on a water surface and prepared for electron microscopy by metal shadowing or staining, appeared as highly flexible fibers of quite uniform diameter that he suggested were interphase chromosomes[2]. A decade later, after the discovery of the nucleosome, it became apparent that the fibers seen by Gall were composed of chains of nucleosomes that could be relaxed or compacted by varying the ionic strength of the medium, and the principal question became the arrangement of nucleosomes and linker DNA within them. During the next 30 or so years, the problem was attacked using a wide range of biochemical, biophysical and structural approaches. From this work, two recurrent themes emerged relating to the structural organization of the 30-nm chromatin fiber. In solenoidal arrangements, the DNA curvature established in the nucleosome continues in the linker DNA, leading to a 'stack of coins' that itself coils to form the fiber (**Fig. 1**). In contrast, in zigzag models linker DNA remains more or less straight and, in its simplest form, appears as a double row or stack of coins with linkers crisscrossing between the rows. Coiling or twisting of this two-start ribbon produces the chromatin fiber. Despite the many fundamental differences between these two architectures, it has been very difficult to obtain experimental evidence that unequivocally supports one or the other (or rules out both), although the weight of recent data from many different approaches has favored the zigzag[3].

The new work by Schalch *et al.*[1], reporting a tetranucleosome structure at a resolution of ~9 Å, builds on their earlier successes in determining the high-resolution structure of the nucleosome core particle, and on the more recent production and analysis of highly regular arrays of nucleosomes[4]. As with all these projects, the key to obtaining tetranucleosomes that can be crystallized is reconstitution with absolutely uniform components. The DNA component in the four-nucleosome arrays contained the '601' nucleosome positioning sequence selected on the basis of maximum affinity for histone octamers[5], along with *Xenopus laevis* core histones expressed in bacteria and devoid of post-translational modifications. Compaction and crystallization of the arrays was promoted by a high concentration of divalent cations. Although ~9-Å resolution is low by X-ray standards, it was sufficient to define the positions of the nucleosomes and linker DNA, allowing the high-resolution (1.9 Å) structure of the nucleosome core particle to be docked within the three-dimensional volume. The arrangement of nucleosomes in the tetramer unambiguously reveals a zigzag architecture, with two stacks of two nucleosomes and the three linker DNA segments passing between them. However, the detailed organization is surprisingly complex, with features that have not previously been suggested on the basis of models of generic zigzag structures. For example, the axes of the two stacks are not parallel, but rotated –71.3° with respect to each other, and there are many other subtle and unexpected nuances. Of the three linker DNA segments, the center one (between

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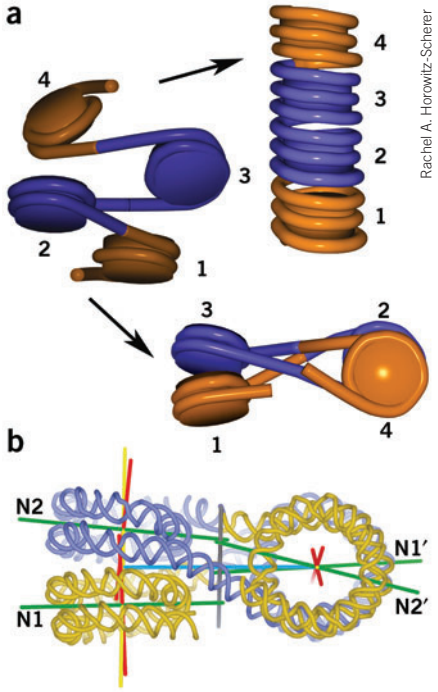

Rachel A. Horowitz-Scherer

Figure 1 Compacting chromatin to form the 30-nm fibers. (**a**) At left is a cartoon of a generic tetranucleosome in an extended mode, its configuration constrained only by the 1.75 turns of DNA establishing the entry and exit sites of the DNA and by the longitudinal and rotational flexibility (persistence length) of the linker DNA. Compaction by coiling of linker DNA leads to an arrangement of consecutive nucleosomes (numbered) in which nearest-neighbor relationships persist (upper right). In contrast, zigzag arrangements (one of which is shown at lower right) lead to alternate nucleosomes becoming nearest neighbors. To form a 30-nm solenoidal chromatin fiber, the uniseriate array coils, whereas zigzags could form fibers by coiling, twisting or a combination of both. (**b**) The tetranucleosome structure solved by Schalch *et al.*[1] has a zigzag architecture and features a complex arrangement of planes and axes. Nucleosomes N1 and N2 form one stack, N1′ and N2′ the other, with a crystallographic two-fold axis passing between them (reproduced from ref. 1 with permission). Blue, central nucleosomes; yellow, terminal nucleosomes. A video showing the rotating tetranucleosome in the supplemental material of Schalch *et al.*[1] is particularly helpful for visualizing the structural details.

nucleosomes 2 and 3 in **Fig. 1**) is straight and is bisected by the two-fold axis of the tetranucleosome; the other two linkers are quite bent. In a sense, the center linker and its two associated core particles are the most interesting portions of the structure, as they may be most representative of the pattern in a longer polynucleosome; the other two linkers and cores are terminal, so their locations and orientations could be influenced by end effects. A feature that will resonate with longtime chromatin aficionados is that the basic unit is the dinucleosome, matching a prediction that has been made many times over the past 25 years on the basis of nuclease digest patterns of chromatin.

Our current understanding of nucleosome core particle structure is limited by the uncertainty in the locations of the histone domains (primarily the N termini) that protrude from the core and, presumably because of their variable locations, are not seen in X-ray maps. Tantalizingly, some of these domains are known to participate in and be necessary for the compaction of nucleosomal arrays into chromatin fibers, and it will be important to discover whether they occupy fixed sites in the compact tetranucleosome and thereby contribute to the stability of the structure. That, however, will have to await higher-resolution data. Similarly, the much-debated location and molecular mechanism of linker histone H1, which is a key player in chromatin fiber formation in higher eukaryotes, remains to be determined. In this respect, the 167-bp nucleosome repeat length used by Schalch *et al.*[1] is close to the short repeats found in the yeast *Saccharomyces cerevisiae* and in the nuclei of mammalian (ox and rat) brain cortical neurons, both of which have a comparatively low H1 and nucleosome content, and considerably shorter than the ~180–190-bp repeat typical of most higher eukaryotic cells.

Schalch *et al.*[1] have given considerable thought to the implications of the tetra nucleosome structure for the chromatin fiber in general, modeling chromatin fibers by stacking tetranucleosomes. (In the crystals, fiber-like stacking does not occur.) The models gave a mass per unit length of ~6 nucleosomes per 11 nm of fiber, well within the range of measurements for chromatin fibers in solution (**Fig. 1**). Depending on the amount of twisting or coiling of the axis, the modeled fibers resemble those suggested in the mid-1980s on the basis of electron microscopy and X-ray scattering[6,7].

Can we expect to find chromatin fibers that are based on the tetranucleosome crystal structure in living nuclei, where dynamic variability rather than rigid uniformity rules the day? Native chromatin commonly shows variable linker length between nucleosomes, generally weak nucleosome positioning, modified DNA, core histone variants and numerous core histone modifications, as well as a plethora of bound non-histone chromatin proteins, all of which presumably contribute to the local functional state. Schalch *et al.*[1] estimate that the tetranucleosome architecture seen in the crystal could be maintained with a linker length variation up to ±5 bp. However, it seems likely that the native level of variability in both DNA and protein components will greatly restrict the occurrence of highly ordered chromatin. Indeed, many years of imaging sections of nuclei using both conventional and cryo techniques has failed to reveal ordered chromatin. In addition to variability in the components of native chromatin, there may well be hierarchical levels of folding above the 30-nm fiber, and local high concentrations of chromatin in nuclei demand a much greater compaction than would be produced from the close packing of 30-nm fibers[3]. In this sense, the crystallization of tetranucleosomes may represent the end of the beginning in the chromatin higher-order structure odyssey. Nevertheless, the new work[1], taken together with the zigzag ribbons reported by the same group last year[4] and the many previous observations supporting zigzag architectures[3], clearly establish this mode of folding as a primary basis of the molecular interactions underlying the complexities of nuclear chromatin. This constitutes a paradigm shift from models based on solenoidal architectures. It is to be hoped that it will now be possible to coax additional constructs to crystallize; the structures formed by oligonucleosomes with longer linker DNA, with specific core histone modifications or containing histone H1 would be particularly informative.

1. Schalch, T., Duda, S., Sargent, D.F. & Richmond, T.J. *Nature* **436**, 138–141 (2005).
2. Gall, J. *Science* **139**, 120–121 (1963).
3. Woodcock, C.L. & Dimitrov, S. *Curr. Opin. Genet. Dev.* **11**, 130–135 (2001).
4. Dorigo, B. *et al. Science* **306**, 1571–1573 (2004).
5. Lowary, P.T. & Widom, J. *J. Mol. Biol.* **276**, 19–42 (1998).
6. Williams, S.P. *et al. Biophys. J.* **49**, 233–248 (1986).
7. Woodcock, C.L., Frado, L.-L.Y. & Rattner, J.B. *J. Cell Biol.* **99**, 42–52 (1984).