MOLECULAR BIOLOGY

# A Higher Order of Silence

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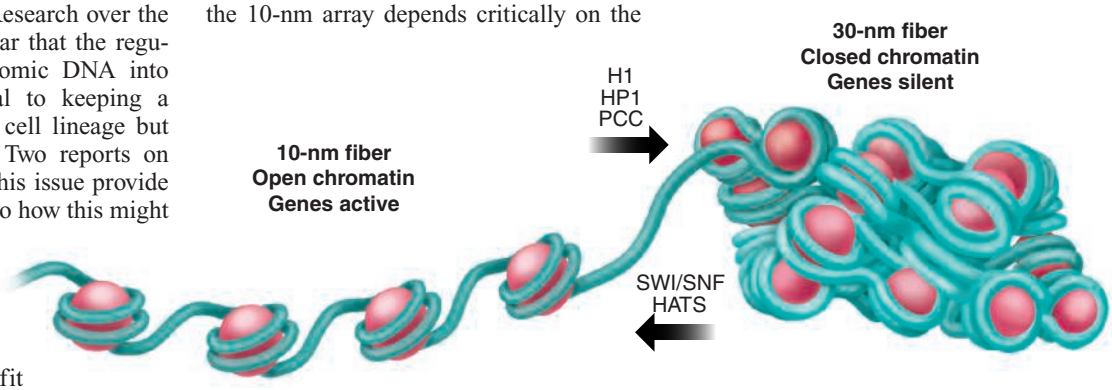
During the development of multicellular organisms, a single fertilized egg gives rise to a plethora of specialized cell types, which are the building blocks of distinct tissues. Because virtually all the cells in our body contain an identical genome, it is the discriminative reading of the genetic information that determines whether a cell is a muscle, skin, or nerve cell. In order to have the "right cell" at the "right place," it is essential that a chosen cellular gene expression program be maintained throughout cell division. Failures in cellular memory or epigenetic control can lead to serious developmental defects and diseases such as cancer. Research over the past decade has made clear that the regulated compaction of genomic DNA into chromatin is fundamental to keeping a gene turned "on" in one cell lineage but turned "off" in another. Two reports on pages 1571 and 1574 of this issue provide intriguing new insights into how this might be achieved (*1*, *2*).

The packaging of DNA into chromatin allows the DNA of human cells (about 2 m in length if stretched out) to fit into a nucleus with a diameter of only 10 μm. The basic repeat element of chromatin is the nucleosome, which consists of 147 base pairs (bp) of DNA wrapped 1.7 times around an octamer of histone proteins (two copies each of core histones H2A, H2B, H3, and H4). Core histones contain a trihelical histone fold domain that mediates histone-histone and histone-DNA binding, as well as unstructured amino-terminal tail domains that are subjected to extensive covalent modifications. Nucleosomes, connected by about 20 to 60 bp of linker DNA, form a 10-nm "beads-on-a-string" array, which can be compacted further into a "30-nm" chromatin fiber (see the figure) (*3*, *4*). Whereas the three-dimensional structure of the nucleosome is known in exquisite detail (*5*), the structure of the higher order 30-nm chromatin fiber is poorly understood.

One basic issue is the arrangement of the nucleosomes within the 30-nm fiber. Two classes of model have been proposed: (i) the "one-start helix" in which nucleosomes, connected by bent linker DNA, are arranged linearly in a higher order helix; and (ii) the "two-start helix" in which nucleosomes, connected by straight linker DNA, zigzag back and forth between two adjacent helical stacks. To distinguish between these two competing models of higher order chromatin folding, Dorigo and co-workers (*1*) developed an ingenious experimental approach using a fully defined in vitro system to generate regular nucleosomal arrays. Further compaction of the 10-nm array depends critically on the base of the histone H4 amino-terminal tails, believed to contact the histone H2A/H2B dimer of the neighboring nucleosome. Indeed, disulfide cross-links between a pair of cysteine residues that replaced selected amino acids in histone H4 and H2A stabilized the higher order chromatin structure. Next, Dorigo *et al*. digested the linker DNA connecting adjacent nucleosomes within the cross-linked compacted chromatin. Analysis of the length of the nucleosome stacks, now solely connected by internucleosomal cross-links, revealed a two-start rather than a one-start organization. This conclusion was corroborated by electron microscopy. In addition to important structural insights, this study shows that local interactions between nucleosomes can drive self-organization into a higher order chromatin fiber.

But what is the physiological relevance of higher order chromatin? Notably, the buffer conditions promoting formation of a 30-nm chromatin fiber reflect the in vivo environment better than do those that yield a 10-nm fiber. One basic premise of chromatin regulation is that genes are silenced through compaction of chromatin, which reduces the accessibility of DNA. In contrast, gene expression may require the "opening up" of chromatin. The Polycomb group (PcG) of gene repressors and the trithorax group (trxG) of gene activators are two antagonistic classes of proteins that may act through modulation of chromatin structure (*6*–*8*). Together, these factors maintain the gene expression patterns of key developmental regulators and hence are crucial players in cellular differentiation, stem cell renewal, and cancer. The trxG group includes members of the SWI/SNF family of adenosine triphosphate (ATP)–dependent chromatin remodeling factors, which use energy derived from ATP hydrolysis to open up chromatin. Conversely, in vivo studies suggest that PcG repression reduces DNA accessibility, but how this is achieved remains unclear (*6*–*9*).

In their study, Francis *et al*. (*2*) used electron microscopy to visualize the compaction of a nucleosomal array promoted by a core polycomb complex, named PCC. It will be of interest to determine whether PCC-induced compacted chromatin forms a bona fide two-start 30-nm fiber. One

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**Regulated chromatin folding directs gene expression.** A parsimonious model illustrating the transition from a 10-nm "beads-on-a-string" open chromatin formation to the next level of chromatin organization: the compacted 30-nm chromatin fiber. Depicted is one possible form of the chromatin fiber produced by a "two-start helix." Folding or unfolding of the chromatin fiber affects the accessibility of DNA to regulatory factors, which control gene expression. Whereas gene silencing factors such as the PCC complex, HP1, and H1 stabilize higher order chromatin folding, gene activators such as the SWI/SNF remodeling complexes and histone acetyl transferases (HATS) initiate chromatin unfolding.

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PCC complex compacts about three nucleosomes, which suggests that each complex might contact multiple nucleosomes and bring them closer together. Removal of the unstructured histone tails by the protease trypsin did not affect chromatin compaction by PCC; hence, these tails may not be required. Histone tail modifications may, however, contribute to the recruitment of PCC in vivo (*10*). Furthermore, it remains possible that the base of the H4 tail, which is important for internucleosome association, was not completely removed by trypsin treatment. One subunit of PCC, named PSC, appears to be particularly critical; a region of PSC that is essential in vivo is also important for chromatin compaction in vitro.

The term "higher order chromatin" is frequently used, or abused, to explain epigenetic effects on gene expression, but what it refers to in molecular terms has not been well defined. The Dorigo *et al*. study provides a first glimpse of chromatin folding at the next level beyond the nucleosomal array. Meanwhile, the Francis *et al*. findings support the notion that PCC creates compacted chromatin domains that silence genes. These studies emphasize that higher order folding is an intrinsic attribute of the nucleosomal array used by gene regulatory factors. Silencing factors such as PCC, HP1, or the linker histone H1 appear to act, albeit through different mechanisms, by stabilizing the internucleosome interactions that drive higher order folding. Conversely, gene activation by SWI/SNF chromatin remodelers and histone acetyl transferases is likely to involve destabilization of the 30-nm fiber. The dissection of the diverse mechanisms by which chromatin folding is regulated will be central to understanding the molecular basis of cellular memory.


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PHYSICS

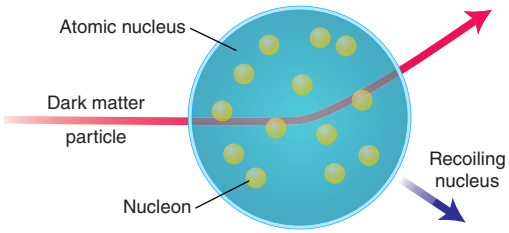
# What Is Dark Matter Made Of?

**Konstantin Zioutas, Dieter H. H. Hoffmann, Konrad Dennerl, Thomas Papaevangelou**

Astrophysical observations reveal that galaxies and clusters of galaxies are gravitationally held together by vast halos of dark (that is, nonluminous) matter. Theoretical reasoning points to two leading candidates for the particles that may make up this mysterious form of matter: weakly interacting massive particles (WIMPs) and axions. Particle accelerators have not yet detected either of the two particles, but recent astrophysical observations provide hints that both particles may exist in the universe, although definitive data are still lacking. Dark matter need not consist exclusively of only one of these two types of particles.

Precise measurements of the cosmic microwave background have shown that dark matter makes up about 25% of the energy budget of the universe; visible matter in the form of stars, gas, and dust only contributes about 4%. However, the nature of dark matter remains a mystery. To explain it, we must go beyond the standard model of elementary particles and look toward more exotic types of particles.

One such particle is the neutralino, a WIMP that probably weighs as much as 1000 hydrogen atoms (henceforth, we refer to the neutralino as a generic WIMP). Neutralinos are postulated by supersymmetric models, which extend the standard model to higher energies. To date, no neutralinos have been created in particle accelerators, but in the future they may be produced in the world's most powerful particle accelerator, the Large Hadron Collider currently being built at CERN. A recent precise measurement of the magnetic dipole moment of the muon favors the existence of new particles such as neutralinos.




**Detection of neutralinos.** Neutralinos can be detected directly with underground detectors through their elastic scattering on nuclei. The energy deposited by the recoiling nucleus (large circle) is expected to provide the direct signature.

Another possibility for the direct detection of neutralinos is to seek evidence for the tiny nuclear recoils produced by interactions between neutralinos (created when the universe was very young and very hot) and atomic nuclei (see the first figure). Because such interactions are rare and the effects small, they can only be detected in experiments that are conducted underground, where the high-energy cosmic radiation is suppressed by several orders of magnitude.

Astrophysical observations could provide indirect evidence for neutralinos. On astrophysical scales, collisions of neutralinos with ordinary matter are believed to slow them down. The scattered neutralinos, whose velocity is degraded after each collision, may then be gravitationally trapped by objects such as the Sun, Earth, and the black hole at the center of the Milky Way galaxy, where they can accumulate over cosmic time scales. Such dense agglomerates could therefore yield an enhanced signal for the postulated neutralinos of cosmic origin.

Another possible signal may come from collisions between two neutralinos, which are believed to result in pairwise annihilation of the neutralinos in dense condensates of such particles. This process would be highly energetic, with energies of billions of electron volts (eV)—much higher than the energy of solar neutrinos, which does not exceed tens of millions of eV. The neutrinos resulting from neutralino annihilation should carry a distinct signature that could be observed with neutrino telescopes designed to search for dark matter of this kind. Neutrinos (for example, from annihilating neutralinos deep within the solar core) are the only particles associated with neutralino annihilation or decay that are likely to escape from their place of birth.

Recently, the gamma-ray spectrometer on the European Space Agency's INTEGRAL satellite has provided evidence for a "fountain" of antimatter electrons (that is, positrons) that are being ejected from some object near the galactic center, presumably a black hole. The data indicate that some

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