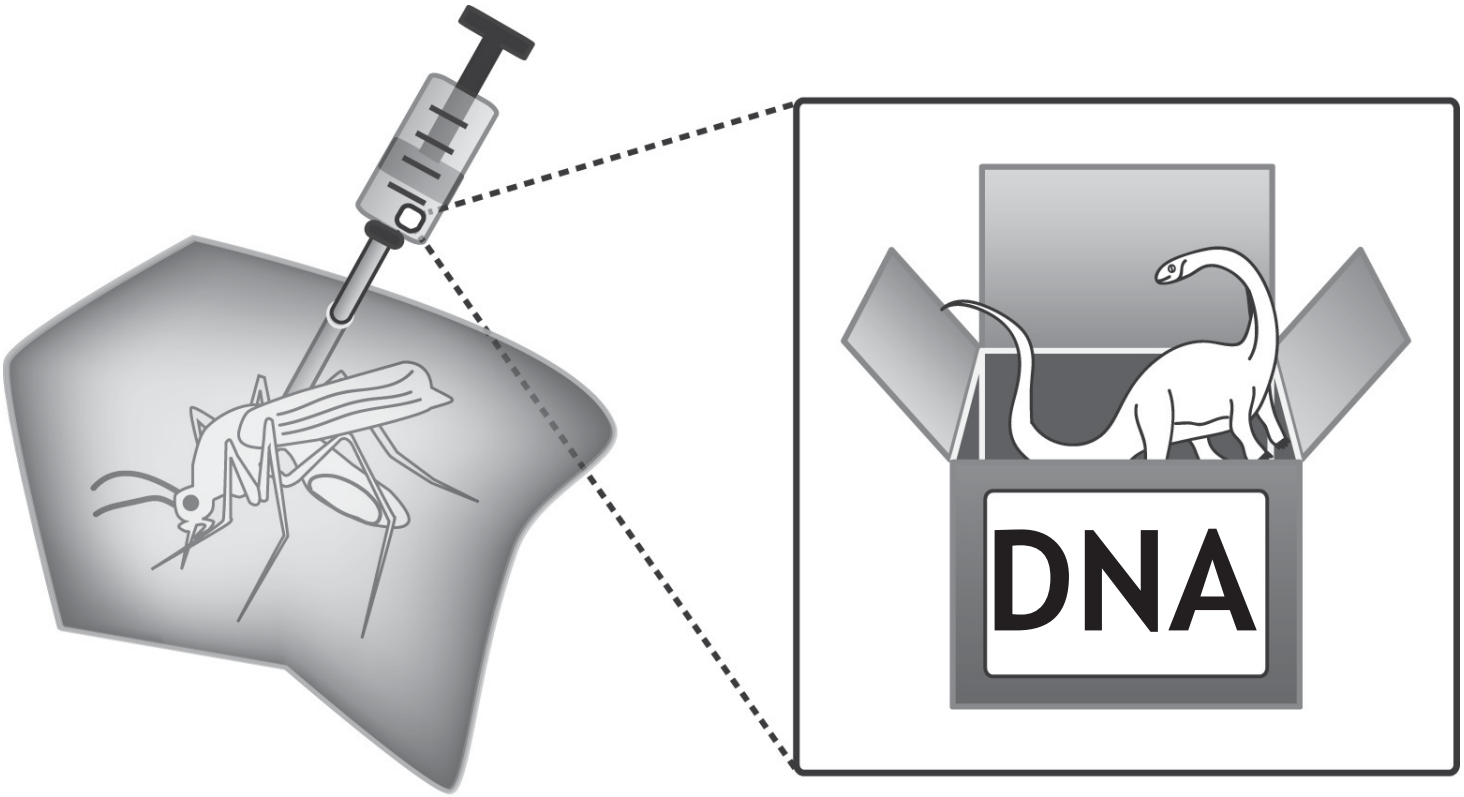
Packaging Your



By John Silva

In the dimly lit cave, an excavator called excitedly for his friends. While they huddled around him, a smile broke across his face as the light from his hardhat cut through the darkness, illuminating the petrified amber prison. An ancient mosquito had become trapped in sap after resting from a day's feed millions of years ago. Why was the find so special? Because inside the insect was a sample of dinosaur blood containing the DNA needed to resurrect dinosaurs from their extinction. While Steven Spielberg's world sensation movie Jurassic Park is fictitious, the movie helped make the general public well aware that DNA is the genetic material dictating animal development, that the sequence of base pairs contained within the double helix is the blueprint telling an animal how to grow from a tiny egg into a six metric ton voracious *Tyrannosaurus rex* – at least in Jurassic Park (1).

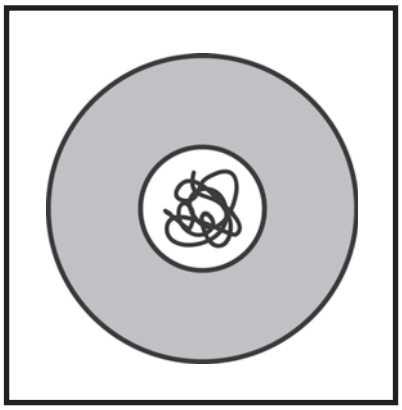
Although the importance of the DNA sequence is quite clear, the importance of packaging DNA inside the cell to regulating animal development is often overlooked. If DNA strands are compacted too much, certain factors are unable to access the condensed region. These activating factors are essential proteins responsible for promoting the copying of DNA into a complimentary strand whose sugar, unlike DNA's, is ribose, in a crucial process known as transcription. If the activating factors cannot reach the condensed region, the required single-stranded ribose-based copy that normally codes for the gene, known as mRNA, will consequently not be present. Thus, the cell's protein producing machine, the ribosome, will be unable to find the mRNA, and the ribosome will be incapable of converting the missing mRNA strand into its corresponding amino acid sequence.

Likewise, if the strands are not compacted enough, genes that should not be expressed may be (2). Since all cells in the body have the same DNA, what makes a brain cell different from a kidney cell, for instance, is the regulation of gene expression (3). One could imagine how catastrophic improper regulation could be. For example, in humans, altered gene regulation can result in several genetic disorders such as ICF Syndrome, an immunodeficiency marked by chronic respiratory and gastrointestinal

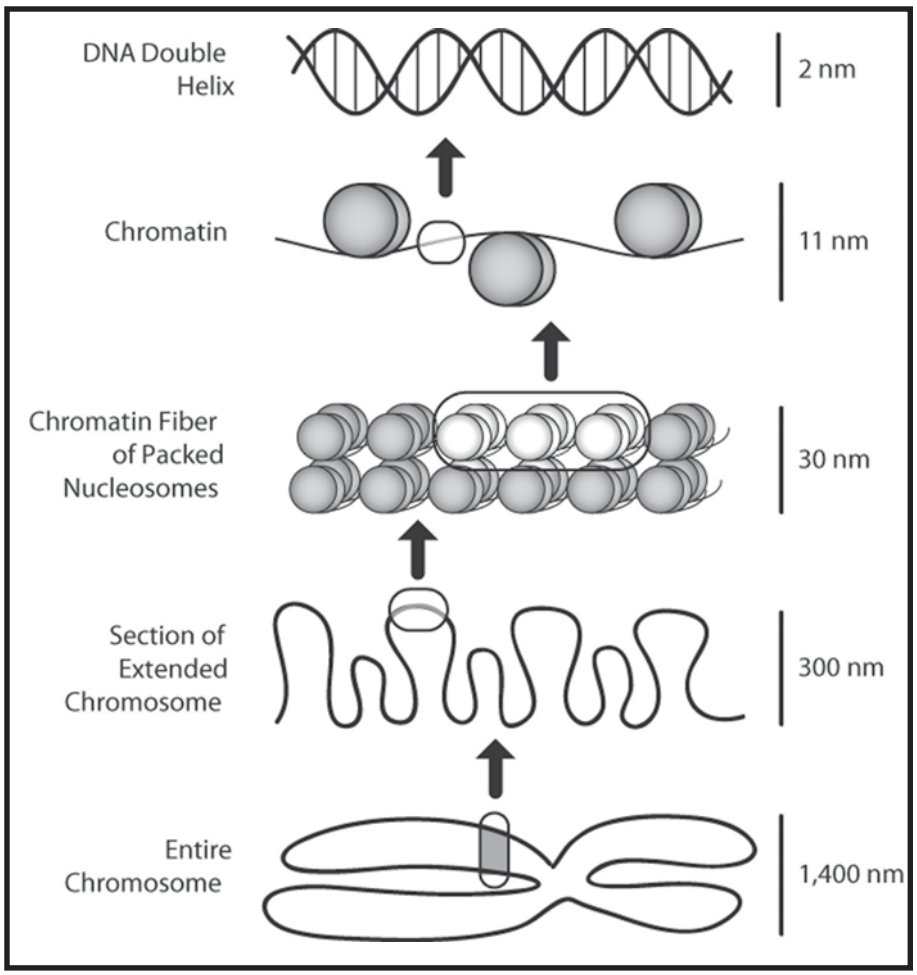
infections; Rett Syndrome, a severe neurological development disorder and the second highest cause of mental retardation amongst females; or Facioscapulohumeral Muscular Dystrophy, a disease leading to progressive muscle degeneration. Strikingly, extensive studies of these diseases have shown that their symptoms may be derived from abnormal organization patterns of stored DNA within the nucleus (4). Thus, there is a delicate balance maintained within cells ensuring the proper storage of DNA so that the right proteins are produced at the right time.

"Regulating gene expression through DNA methylation gives histone H1 a power not recognized before."

Most cells are only 10^{-6} to 10^{-4} meters in diameter, yet many are stuffed with DNA strands whose total lengths can be measured in meters! Such extreme storage is the result of protein-protein and protein-DNA interactions. Five proteins – H2A, H2B, H3, H4, and H1, the histone proteins – are responsible for compacting DNA. The first four constitute the core nucleosome proteins, which are a collection of proteins whose function is to aggregate in the nucleus, forming a cylinder called the nucleosome, around which DNA can wrap (Figure 1). H1 is special in that it serves as a linker histone grabbing nucleosomes and pulling them closer together into a 30 nanometer fiber. Linker DNA creates spaces between each nucleosome, giving the overall appearance of what looks like beads on a string. Zooming out to see the entire nucleus of the cell, one sees these fibers forming what looks like a big ball of entangled yarn (chromatin) comprised of DNA that is not fully condensed (Figure 2) (5).



▲ **Figure 2.** A generic eukaryotic animal cell containing uncondensed chromatin represented by the squiggly line within the nucleoplasm of the nucleus. In three dimensions, this "noodle soup" would look like a ball of yarn.



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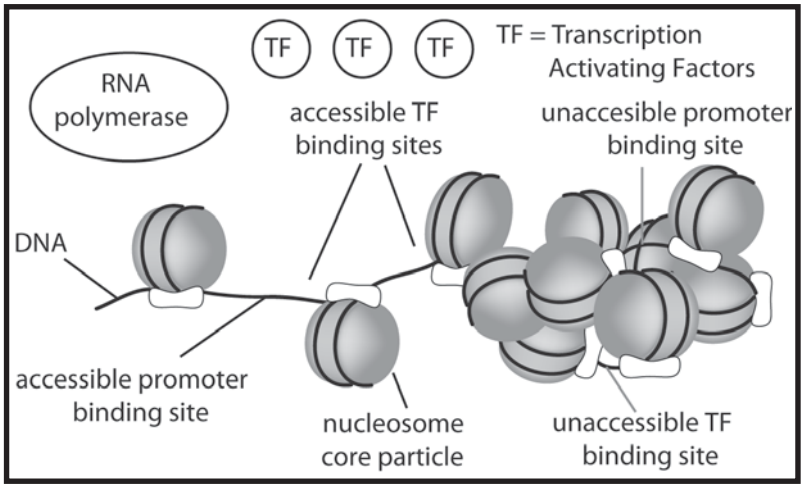
▲ **Figure 1.** Different levels of DNA compaction. Starting with the unbound double helix, various stages of condensation occur through association with the core histone proteins and the winding of DNA into more complex structures until the highly condensed metaphase chromosome superstructure at the bottom is achieved. Histone H1 protein is responsible for compacting the DNA from the "beads on a string" form of chromatin into the 30nm fiber.

Determining the Role of Histone H1

Not properly controlling how chromatin is stored can have serious consequences, which were not really appreciated by scientists until the last decade. In the late 1970s several papers presented evidence that H1 may be involved in coiling or condensing DNA, enough to form the higher order structures most people recognize as chromosomes (6,7). However, the matter was still up for debate; even into the early 80s, the role of H1 in chromatin condensing was still being speculated. Prominent investigators continued researching regulation of linker DNA length and its "possible relationship to histone H1" (8). By that point, biologists were well aware of the existence of the nucleosome, but the exact relationship between the nucleosome, the linker DNA length, and the H1 protein



◀ **Figure 3.** Position of the nucleosome cores along the length of DNA prevents the binding of transcription activating proteins. The uncondensed regions are clearly available for binding, whereas the transcription factor binding sites are not available because they lie within condensed regions. As an additional means of regulation, the promoter binding site for the enzyme RNA polymerase can also be positioned such that the enzyme cannot access the region. RNA pol copies DNA into a complimentary strand whose sugar unlike DNA's is ribose in a crucial process known as transcription. Without this complimentary strand the particular protein the gene encodes for cannot be made.

remained uncertain. As always in science, only the passage of time and piles of evidence allowed for a real consensus on this matter.

In 1983, the H1 protein was shown to promote stable replication of chromosomes in Chinese hamsters. Researchers reported no chromosome loss in over a year of continuous culture in replicating cells positive for H1 protein. The experiment showed that regulation of histone H1 by the chemical addition of inorganic phosphorous to the protein, a process known as phosphorylation, played a role in DNA condensation and replication. The scientists suggested that "the stable association of specific histones with DNA may encourage a specific pattern of gene expression," an insight that would later be verified (9). Thus, during the early 1980s, connections were being made between H1 protein regulation, chromosome superstructure, and the welfare of the studied organism.

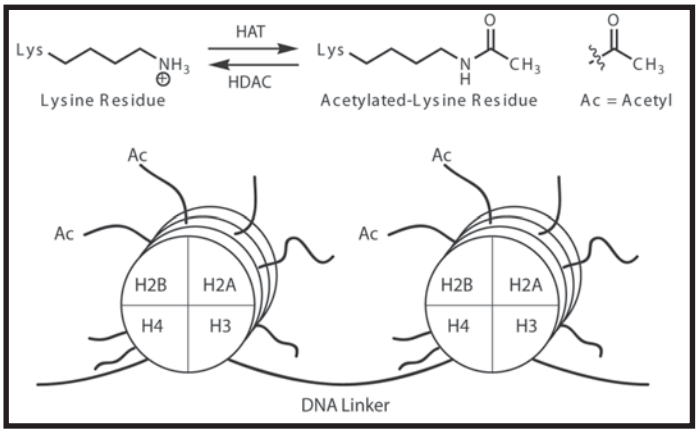
By the time Jurassic Park debuted, most biologists accepted H1's role in condensing DNA. In the late 90s, new evidence suggested that H1 protein controlled regulation of specific genes in animals, although these specific genes could vary across species. For example, in 1998, histone H1 in Xenopus frog eggs was shown to be a repressor of Xenopus oocyte 5s rRNA genes. rRNA is a molecule chemically similar to mRNA that comprises a permanent structural component of the ribosome. Thus, repression of the oocyte 5s rRNA genes by histone H1 impacts overall protein synthesis.

Scientists found H1 to be capable of binding to a nucleosome core in the 5s rRNA somatic gene, found in body cells and not in reproductive cells. Histone H1 was understood to change the density of chromatin at that location such that transcription factors were incapable of binding to the region (Figure 3). Evidence that histone H1 not only packaged DNA but also regulated expression of specific genes was finally available (10).

Nevertheless, exactly how H1 specifically regulated genes was not well understood until the coming of the new millennium (11). Appreciating the consequences of incorrectly packing DNA, specifically that caused by irregular H1 levels, requires an understanding of how DNA is packaged by the protein and how the packaging regulates gene expression. Biologists were well aware of the protein's repressive functions, but a big question loomed overhead: can H1 increase or initiate gene expression? After all, over-expression is just as detrimental as under-expression, and one way to understand the importance of how your DNA is packaged is to see what happens when the packaging materials are absent.

Recent Experimentation

In December 2005, researchers in the Department of Cell Biology at the Albert Einstein College of Medicine published a study in which they had depleted H1 levels in mouse cells in order to determine the importance of H1 protein in gene regulation. The researchers bred mice lacking three different genes H1c, H1d, H1e, all encoding a slightly different version of H1 (12). All three genes were knocked out because the investigators' previous study had shown that if at least 4 of the six subtypes of H1 remained, the mouse could develop normally. Expression of the remaining genes could be



▲ **Figure 4.** Two nucleosomes formed by histone octomers of the four core histone proteins H2B, H2A, H4, and H3 spaced out with linker DNA between them. The salient tails are the n-terminal protein tails targeted for enzymatic modification by histone acetyltransferaces (HATs), which can add the acetyl group (top right) to specific lysine residues in histone N-terminal domains (top left). Acetylation disrupts histone-to-histone interactions through neutralizing the positively charged amino acid lysine. When lysine amino acid residues on these tails are acetylated, the nucleosomes spread apart allowing for transcription factors to bind or the exposure of RNA polymerase promoter sites. Histone deacetylases (HDACs) can reverse the process of acetylation.

up-regulated in order to compensate for the loss (11).

The resulting triple H1-null embryo stem cells were collected, and despite their mutations in the H1 subtypes, the cells grew like normal cells. Even their size and shape were normal. However, the effects of such depletion eventually proved to be drastic: decreased nucleosome spacing, reduced chromatin compaction, and chemical modification of the four core histone proteins. These changes were fatal to the embryos.

What caused these effects? The reduction of H1 had caused a change in charge balance within the nucleus because more DNA, which has a negatively charged phosphorous backbone, was freely exposed to the nucleoplasm, the translucent fluid bathing the inside of the nucleus. To compensate, the linker DNA length was decreased, associating more DNA with the nucleosome. Although counterintuitive, the absence of H1 resulted in the nucleosomes being brought closer together in certain regions. In addition, the core histones were modified to balance charge so that the negatively charged acetyl groups were removed from the amino acid lysine, a component of the core histone proteins (Figure 4). Without H1, regions of the chromatin were condensed more than they would have been with H1 present!

Despite a global increase in condensation, some genes were highly up-regulated and therefore uncondensed. According to the researchers, "nearly one-third of the genes with altered expression in the H1-depleted cells are thought to be normally regulated by DNA methylation," the addition of CH_3 groups to bases in DNA (12). DNA methylation serves a variety of essential functions in cells ranging from the deactivation of genes or gene silencing, to embryonic development contributions. Therefore, regulating gene expression through DNA methylation gives histone H1 a power not recognized before in mammals.

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Looking Ahead

Many questions still remain. Of the twenty-nine genes with altered expression, nineteen up-regulated and ten down-regulated, four genes represent imprinted genes, or genes inside the cell that have one activated and one inactivated copy. In addition, the sex chromosome genes are over-represented; five of the twenty-nine genes are located on the X and Y chromosomes. Apparently, given the variety of genes with altered expression, complex processes are being initiated within the cell. There may even be a possible feedback mechanism involved as two of the nineteen up-regulated genes encode for linker histones. The arduous task of sifting through and elucidating these functions remains. Also, the applicability of these findings to other varieties of non-mammalian life such as plants needs to be determined. The answers to these questions may depend on many factors to be researched in the future (12).

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The value of this research, and of all research for that matter, is ultimately rooted in how such knowledge will improve the quality of human life. As a means of treating some diseases, the biopharmaceutical industry often employs proteins from one organism in another by means of genetic recombination. Unfortunately, these transgenic proteins frequently are rendered less effective by gene silencing via chromatin compaction. Because a large portion of the chromatin in a cell is dense, inactivated heterochromatin, when a transgene integrates into the genome of a target organism, it is more likely to insert into a deactivated region than an activated one. Therefore, further experimentation elucidating how histone H1 regulates specific gene expression and is related to DNA storage may help researchers discover methods of activating regions in which transgenes have been inserted (13). Future research on histone H1 will most likely lend itself to this task as scientists move from learning how DNA is packaged to doing the packaging themselves through chemical manipulations as this small science advances.

The experiments of the past decades illustrate important points about how your DNA is packaged and the importance of proper storage. DNA requires proteins for condensation into appropriate structures for accessing genes, silencing them, or allowing for chromosomal replication. H1 plays a complex species-dependent role in DNA compaction, condensing the molecule, repressing genes, or promoting up-regulation. Organisms with irregular H1 levels can suffer gross malfunctions in development, leading to the detriment of the animal. The systems in place for compensating for H1 loss and charge imbalance reveal how dynamic storage really is and to what lengths cells can go to ensure packaging is delivered. What can H1 do for you? H

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