Histone Structure

III. A possible model for chromosome structure

By Ken Biegeleisen, M.D., Ph.D.

Transcript of narration

SLIDES 1-2
No audio (title page, TOC)

SLIDE 3

Introduction

Before we proceed, a word about the screen. If you haven't downloaded this PowerPoint presentation to your computer, then you're still online, and you're looking at a Flash export of the PowerPoint, courtesy of a lovely little company called iSpring.

There are a number of toggle switches in the iSpring Flash Player you're looking at now, but I only know about three of them.

First of all, if you click this little thing in the upper right-hand corner, you'll toggle back-and-forth to-and-from full-screen mode. If you want an even larger image, you can get rid of the slide strip on the left by clicking this thing in the lower right. If you do this, you'll still be able to navigate, at least from section-to-section, because I've inserted a "Table of Contents" hyperlink on nearly every slide in this presentation.

And finally, you can adjust the audio volume with this volume button down here. There are several other controls on the Flash Player, but I have no idea what they do. If you've got lots of time on your hands, you can experiment with them.

SLIDE 4

Hello. I'm Dr. Ken Biegeleisen. I'm going to show you a hypothetical new model for chromosome structure. Because our knowledge of chromosome structure is still in its infancy, this model is, by the very nature of the subject, considerably more speculative than the models proposed in the other slide shows on this web site, wherefore I have segregated it out as a separate presentation.

Nevertheless, this new model does indeed follow logically, and almost-automatically from the nucleosome model proposed in Part II of this slide series on histone structure, which, in turn, follows logically from the protamine-DNA structure also posted on this web site.

I presume that you are coming here from Part II of the series, and that you are therefore familiar with our newly-proposed histone model. If not, I think it unlikely that anything here will make much sense to you, and in that case, you are strongly advised to go back and watch Part II. Moreover, if you are not *very* familiar with currently-accepted concepts of histone structure, as portrayed in the world's textbooks of molecular biology, then you really need to watch part I of the trilogy as well, wherein that subject is extensively covered.

The object in the upper left-hand corner of this slide is the starting point for our new chromosome structure. Shown here is one instance of our model of the histone octamer plus its associated DNA; a model which is elaborated upon extensively in Part II of this slide series. The view is axial; we're looking at a cross-section of a column of DNA and protein which extends for a very long distance along the z-axis, which is perpendicular to the plane of the computer screen.

As explained -- when this view was introduced in the previous slide presentation -- each of the eight DNA double-*non*-helices extends upward for a long distance, as illustrated by the *not*-so-long exemplary segment...

SLIDE 5

...shown here. When this segment reaches the proper point, it will curve around...

SLIDE 6

...as I have attempted to illustrate with this somewhat clumsy curved ladder cartoon.

SLIDE 7

Then the strand simply goes back the way it came, giving rise to a non-helical tetraplex of mutually-intercalated duplexes.

SLIDE 8

In Part II of this slide series, I settled on this structure as the most likely candidate for the 30-nm fiber. This, then, is the motif we shall employ today, and from which we shall build up to an entire chromosome.

I pointed out that this structure is by no means "carved in stone", and that numerous alternative structures are possible. The fundamental principles we shall employ, however, in the creation of a model for higher-order chromatin structure, are almost completely general, and not specific to any particular one of the various alternative models which could be proposed.

Before proceeding with higher-order chromatin structure, however, I wish to backtrack momentarily.

SLIDE 9

I wish to backtrack to this; our most simple model for a 30-nm fiber; one which I rejected in Part II. The reason I rejected it was that I was put off by the hole in the middle, which I regarded as the molecular biological equivalent of a "vacuum" of sorts. I referenced the famous saying of Aristotle, "Nature abhors a vacuum", and, although I made mention of the possibility that the hole might actually represent a channel for the passage of enzymes, I rejected that hypothesis, because I imagined that the enzymes would be too large for the space available.

Well, at that time I was thinking of large enzyme structures such as the DNA polymerase III holoenzyme complex. But, when I considered the matter in more detail later, it occurred to me that many polymerases are smaller than that, and that the smaller ones would actually fit. To illustrate that, let's gray out our 30-nm fiber model, and bring in some polymerases.

SLIDE 10

Here's a representative large polymerase, from bacteriophage ϕ29; its long dimension is just under 200 Å, which is far too large to fit into the 100 Å channel. But it *would* fit if we were to rotate it 90º about the y-axis.

Here, however, is a representative small enzyme, which fits very nicely. This is our old 1950s friend DNA polymerase I; this version, from Bacillus stearothermophilus; is only 84 Å in its largest dimension; if we rotate it about the y-axis it shrinks to a mere 65 Å. An enzyme this size could probably shoot down this channel like a cannonball.

Consequently, since I formally ruled out this structure in Part II, I am now formally *un*-ruling it out; it's back under consideration. Nevertheless, I still favor the 6-octamer model I showed a few slides up. I will, however, use this simpler 4-octamer model to illustrate some basic principles.

SLIDE 11

Fortunately for me, with respect to the countless hours of work I did developing a chromosome model, the number of histone octamers in the cross-section of the 30-nm fiber, as to whether it's 6, as in my currently-preferred model, or 4, as shown here, doesn't affect the outcome nearly as much as one might think. Neither the manner of association between adjacent 30-nm fibers, nor the ratio of protein:DNA in the whole chromosome, are at all affected by altering the octamer count.

The manner of association between adjacent 30-nm fibers we'll look at in a moment; as for the chromosomal protein:DNA ratio, the number we gave in Part II was determined solely by the protein:DNA ratio in a single octamer; the 3-dimensional arrangement of multiple octamers played no part in the calculation.

The only important effect of going from a 4-octamer model with a channel, to a closely-packed 6-octamer model, is that the former, being less compact, will presumably occupy a larger fraction of the nuclear space. The difference is not likely to be critical, however, because, taking the human eukaryotic cell as an example, the entire genome probably doesn't occupy more than about 20% of the nuclear space in any event.

With respect to the manner of association of adjacent 30-nm fibers, it's precisely the same as the manner of association of adjacent octamer cores within the individual fiber. This statement may seem confusing now, but it will become clear presently.

In all the candidates for 30 nm fiber structure that I looked at, there were precisely two types of interactions that could be invoked, to impart stability to the structure. These are the very same interactions we have looked at several times previously, in both the protamine-DNA and histone-DNA models. In Part II, these interactions were described in Slides 244-253, which are the source of the images that follow:

First, there were the rectangular arrays of charge interactions between the negatively-charged DNA phosphate groups, and the positively-charged Lys and Arg residues in the N-terminal β-strands.

Secondly, there were peptide backbone hydrogen bonding interactions between adjacent β-strands; exactly the sorts of interactions found in β-sheets such as keratin.

Here's the close-up view of the charge-charge interactions again.

SLIDE 12
(No audio – transitional motion slide)

SLIDE 13

Please note the square charge array involving two negative charges from DNA phosphates, diagonally-situated from one another, and two positive charges from lysine or arginine R-groups, also diagonally-situated. Let’s bring in a little box to mark these charges. This is precisely the square-shaped charge array we "borrowed", so-to-speak, from the protamine-DNA model, and is one of the key features of that structure. The principle here is quite the same; adjacent columns of DNA tetraplexes and protamine β-strands can align as shown, with the respective positive and negative charges lying at the corners of a square.

Since the diagonals of a square are 1.41 times the lengths of the sides, and since charge interactions vary with the square of the distance, the attractive forces in the sides of these squares are approximately twice the magnitude of the repulsive forces at the diagonals, making this a very stable structure where it can be found.

SLIDE 14

Only 1/3 of the amino acid residues at the N-terminal regions of each of the 8 histone subunits are Lys or Arg. Therefore, 2/3 of the time, the complete square charge array shown here will not be realized, but rather, one or the other of the positively-charged amino acid residues will be replaced by a non-basic residue at the opposite diagonal. This will most often be an amino acid with a hydrophilic R-group, which we may represent by the letter "H", which, while not being able to form a salt bridge, can still form a hydrogen bond with DNA phosphate.

SLIDE 15

The other species of interaction between adjacent 10 nm nucleosome strands is a classic sort of hydrogen-bonding between the peptide backbones of their respective N-terminal β-

strands, shown here in axial cross-section. We cannot see the hydrogen bonds in this view, however. We're going to have to rotate to a longitudinal view.

SLIDE 16
(Movie; no audio)

SLIDE 17

This is about the correct ribbon spacing for 3Å hydrogen bonds between the antiparallel peptide backbones. We have thus joined this pair of nucleoprotein complexes via hydrogen-bonding of their N-terminal β-strands.

Here's a reminder of the manner of hydrogen bonding in an anti-parallel β-sheet-type structure.

In all the higher-order chromatin forms we shall look at, the basis of the interaction between adjacent structures will be either this type of β-sheet interaction, or the salt bridge interaction shown in the previous slides.

SLIDE 18

This slide, also borrowed from Part II, reviews the two distinct orientations of protein and DNA in the 4 corners of the histone octamer. These two different orientations arise because of the peculiar spatial arrangements found in the Kornberg-Luger crystal structure, which our model has attempted to follow as closely as possible. Thus, the two H3-H4 corners are approximately aligned with the x-axis, which facilitates formation of β-sheet-like structures with adjacent octamers, whereas the two H2A-H2B corners seem to be radially arrayed with respect to the octamer core, which facilitates the formation of the square-shaped charge arrays.

SLIDE 19

Here again is my "preferred" model. It's 30 nm in its long dimension, but a little less in the short dimension. The multiple salt bridges and hydrogen bonds in its horizontal midline would render it a very stable structure.

I'd like to show the pathway which I imagine DNA might take in negotiating a structure like this one. We're going to start in the lower right-hand corner, now highlighted. The X-shaped structure is a tetraplex, consisting of two mutually-intercalated, non-helical DNA duplexes, as discussed several times previously.

SLIDE 20

We've also previously looked at this, a somewhat-awkward axial view of a DNA duplex rising up from an octamer corner like the one in the previous slide, looping around, then returning to complete the intercalated tetraplex.

Now let's look at this from a more distant perspective.

SLIDE 21

Here's our 30-nm fiber candidate, which I've now tilted forward, so I can demonstrate the path of the DNA through it. Since I'm going to render the DNA as being perfectly vertically-aligned with the y-axis, the protein should really be in the x-z plane, but then you'd be looking at the protein edge-on, and you wouldn't be able to see very much of it, wherefore I've added the tilt.

I should perhaps re-emphasize that this is a narrow slice; that is, a cross-section; through a structure that proceeds up and down the y-axis, the direction of which runs from the floor to the ceiling of the room you're sitting in. The DNA runs along the y-axis for very considerable distances. This I've attempted to indicate by the heavy reddish-brown arrow on the left.

The green line now threading its way through the octamer cross-section is not a structure, but merely a path. It's the path of the DNA duplex which forms the left-hand side of the X-shaped tetraplex in the lower right-hand corner.

When the DNA reaches the top of this loop, it curves around, then returns from whence it came, completing, in the process of returning, the tetraplex structure.

In case it's not absolutely clear, this is merely a side-view of the structure we previously viewed from the top:

SLIDE 22

The DNA goes up...

SLIDE 23

...it loops around...

SLIDE 24

...and returns to intercalate with itself, giving rise to the tetraplex at the octamer corner.

SLIDE 25

OK, back to our former view.

The DNA column actually rises much farther than shown here, because, as we shall see very shortly, as it rises, it traverses numerous 6-histone complexes like this one, before it loops around for the return journey. To put this in an approximately-accurate macroscopic scale, relative to the size of the image on your computer monitor, you should imagine the loop at the top of the green path as actually being approximately at the level of the ceiling of the room you're sitting in right now.

I'm presuming, of course, that you're in a fairly ordinary room with 8-10 foot ceilings. If you live in a palace with hundred-foot ceilings and chandeliers, all bets are off.

SLIDE 26

This completes our first DNA loop, which occupies the lower right-hand corner of our 30-nm fiber cross-section. I'm going to presume that the DNA will wind its way circumferentially around the 2-nucleosome pair on the right side of the figure, in the direction indicated by the large red-brown arrow. The DNA doesn't *have to go* in this direction, of course; numerous alternative pathways can be imagined, but I'll presume it to be this direction for the sake of illustrating the basic principle of this structure, which is that the DNA, as we shall see, never gets twisted or tangled, and can be replicated without topological difficulty.

So next, the DNA path will curve upward for the second loop. This second loop will occur at the next stop along the red-brown path, which is the upper right-hand corner of the same octamer. In order to keep the picture intelligible, I'll fade out the first loop before drawing in the second:

SLIDE 27

OK, this represents the path taken by the DNA through the second corner of the octamer, passing through the cross-sectional point indicated by the green arrow. Then we move on to the next octamer.

SLIDE 28

This completes our 3rd loop. Let's look at the rest of them, fading out the previous ones almost completely, so that the picture doesn't get too cluttered.

SLIDES 29-32
(DNA loops – no audio)

SLIDE 33

This completes the journey of the DNA around the first of *3 pairs* of nucleosome cores which constitute this 30-nm fiber model. Next...

SLIDE 34

...the DNA swings over to the next pair of nucleosome cores to start the journey over again. In the end...

SLIDE 35

...after winding its way through the remaining histone octamers of the 30-nm fiber, you'll have something like this – an extensive collection of DNA loops, all of which constitute a single DNA duplex strand, which enters and exits at the points shown.

In case it's not completely obvious, the perspective of this drawing is purposefully-distorted, with the DNA pathways being perfectly upright with respect to the y-axis, but the plane of the protein being tilted towards us, so that we can see its upper surface. In a more realistic drawing, the protein would be viewed edge-on, perpendicular to the y-axis, but then I'd have no way to portray the threading of the DNA through the 6 histone octamers.

By way of preview, let me say now that I'm going to show you a full-scale model of this structure a few slides down, and in that scale, much of the detail shown here will be lost. There are 24 individual looped DNA strands in this slide, which are arranged as 6 groupings of 4 looped DNA structures each, now enumerated at the top of the slide. In the full-scale drawing, to be shown a few slides down, it will be impossible to portray each of the 4 looped structures separately, and, as you'll see, each of the 6 groups will appear to be six individual thick green strands. This will become clear when we get to that slide.

It's important to note that there are *no twists* in this mass of precisely-positioned DNA loops. Let's return momentarily to the cross-sectional view to make sure we're properly considering this all-important aspect of the structure.

SLIDE 36

Let's return to the cross-section of DNA at the lower right-hand quadrant of this structure, to see what happens to its torsional state as it rotates through the six histone octamer cores which constitute this 30-nm fiber model. We start by *highlighting* this corner of the model.

SLIDE 37

Note that it rotates a bit when it moves to the next position.

SLIDE 38

There's not much rotation in the next step, but in the subsequent step…

SLIDE 39

...it rotates about 60º. These rotations continue in the subsequent steps.

SLIDES 40-43
(No audio)

SLIDE 44

Now, by the time we've reached the final position, in this portion of the journey, we've rotated about -290º, almost a full twist. We therefore must reverse this rotation.

SLIDE 45

This we propose to do in the process of transitioning to the next portion of the 30-nm fiber. As an aside, if you check this with a rubber band, you'll be able to confirm quickly that this maneuver confers a small right-handed helical twist upon the DNA, which is the direction in which DNA naturally twists anyway; it would hardly even require an enzyme to facilitate this, other than to limit the amount of twisting to no more than that which is needed at this point.

SLIDE 46

Here's the artist's representation of this angular maneuver, which completely restores the torsional state of the column of DNA to that of the starting position.

SLIDE 47

Thus does the DNA wind its way through the 6 octamer subunits which constitute this model of the 30-nm fiber, which winding we've represented here in the manner of an electrician's power cord. This we've done because electricians have discovered something long ago which remains unknown in molecular biology even to the date of this presentation.

SLIDE 48

The power cord on the left is coiled up, as virtually all current DNA models portray DNA to be coiled up, into a lovely circular twisted helical conformation. It's great for packaging, because it looks neat and orderly, but if you've ever tried to open a package containing something like this, you know perfectly well what will happen when you try to unwind it – you get a horrible tangled mess, which takes quite a bit of effort to untangle. If the cord is very long, you also need a lot of space to do the unwinding. That's why professional electricians *don't* wind up their long power cords as shown in the picture on the left, but rather as shown in the picture on the right, showing the beginnings of a back-and-forth winding, rather than a twisted helical winding. A power cord folded that way can be immediately unfolded in part or in total, any time, and without fighting a topological battle.

SLIDE 49

So as our DNA loops its way through the 30-nm fiber, it will follow a path something like this. The path of the individual DNA duplexes, represented by the green loops, is, topologically speaking, un-twisted, and the higher-order path of the whole loops, represented by the orange power cord, is also topologically untwisted.

SLIDE 50

This rather clumsy set of drawings attempts to illustrate the following principle: If one were to grasp the ends of the DNA strands which constitutes this structure, and to pull, the DNA would emerge in a perfectly linear fashion, with no twists or knots.

I don't know if these crude graphics really illustrate this point adequately, but, to re-state the case verbally, in this model, the cell no longer needs to waste time on massive and now-unnecessary winding and unwinding operations, so that the energies of the cell may be focused on the process of replication only.

SLIDE 51
Title slide
(No audio)

Completion of the chromosome
I. 30 nm fiber to 300 nm fold

SLIDE 52

Here's a compaction chart, quantitating the degree of compaction to be achieved by the folding of our new structure into a chromosome.

This chart assumes the human full-genome count of $6x10^9$ bp. In current textbooks, the figure for the total length of all the DNA, in all 46 chromosomes, is usually given as being approximately 2 meters, assuming, of course, the Watson-Crick structure, with its 3.4 Å base-pair spacing. This would give rise to an average length, for each one of the cell's 46 chromosomes, of about 44 mm.

Since, however, our model employs not the Watson-Crick structure, but the Wu straight-ladder structure, which has 6.8 Å spacing, twice that of Watson-Crick DNA, we therefore see, in Line 1 of the chart, a figure which is commensurately twice as large, namely 88 mm, for the ***average*** length of a completely extended human chromosome.

Therefore, relative to the dimensions of our Wu straight-ladder structure, the 88 Å length represents a compaction of "1", *i.e.,* no compaction at all.

Now, in our new histone structure, there is a Wu tetraplex, *i.e.,* a pair of intercalated duplexes, in each of its 4 corners. We therefore have 2x4=8 Wu duplexes per nucleosome, so we immediately have an 8-fold compaction.

Next we assemble 6 histone octamers into a 30-nm fiber, which provides an additional 6-fold compaction, or 48-fold in all. The time has now come to consider the 300-nm fold, widely-presumed to be the next stage of higher-order chromatin structure.

SLIDE 53

Here is the model for a 300-nm fold, which arises naturally from our histone structure. It may not be clear how it so arises, so I'll switch back to a previous slide in which it was depicted in partially-schematic form, in a scale which was enormously compressed in the axial direction.

SLIDE 54

Here's the previous slide. You may recall me saying, at that time, that the 6 groupings of 4 looped DNA strands each, would *not* be possible to portray in the full-scale structure. Well, here are those 6 groupings of DNA again. As I also said, in a scale model based upon the size of the histone octamer on your computer monitor, the actual lengths of these 24 DNA loops would be about the height of the ceiling in an average room. In order to appreciate this scale, let's put a man in the room.

SLIDE 55

There's our man. That's Newton, my favorite scientist, not because he was smart, but because he didn't get paid for his work; he did it because it interested him, and for no other reason. He had no lab, no research grant and no co-workers; he worked alone in his father's barn, because Cambridge was closed due to a plague epidemic.

Here, Newton is staring with amusement and puzzlement at an accurately-scaled macroscopic model of our 300-nm fold. As I said before, the 6 groups of 4 looped DNA strands each, have now taken on the appearance of 6 thick linear strands, because the individual DNA strands and their connecting loops are too small to be seen, especially at this viewing angle. In the bottom of the structure, however, where the directions of the DNA loops are more favorable for viewing at this angle, I have been able to show at least some of them.

The structure looks like a 25-story tower, where each story is a single instance of the 30-nm, 6-histone structure we have been looking at. Let's bring in a magnified sample cross-section, so that we may keep this in mind.

This is the cross-sectional structure we have been examining for a quite a while, although previously only from the axial perspective, looking from the top down. Here it's being shown obliquely. In the tower in the middle of the slide, however, we only see its front edge. Each of the 25 stories of the tower is a 6-octamer plane exactly like this one. Because the view is partially-obscured by the DNA, represented by the thick green vertical lines, only a little bit of each of the three most-anterior of the six histone octamers are visible at each of the 25 levels, as indicated by the yellow arrows.

The number of protein levels, 25, is merely the length of the entire strand, which is 300-nm, divided by the inter-octamer "beads-on-a-string" spacing of 120 Å, or 12 nm. This 12 nm inter-octamer spacing, which I proposed in Part II of this slide series, is not carved in stone; as I admitted there, I have no way of establishing an exact spacing between octamers, because of two uncertainties: I cannot logically define an exact length for linker DNA segments between octamers, and I cannot assign an exact role for histone subunit H1, which therefore has been excluded from our model. Either one of these undetermined features, if they were to suddenly become determined with certainty, could alter our current 12 nm inter-octamer spacing significantly.

In spite of all these uncertainties and inexactitudes, I believe that the model shown here, or one very similar to it, is a reasonable approximation of what chromatin structure will eventually prove to be. This, then, is my proposed structure for a 30-nm fiber, folded at 300 nm intervals.

Hopefully it's now clear what I meant before when I said that the green loops of DNA, in the earlier slides, extended much farther than indicated. An exemplary green loop is now

indicated by a yellow arrow. The actual longitudinal extent of the DNA column may now be seen to be represented as being the height of the tower in the middle of the slide, which reaches about to the ceiling of the room within which Sir Isaac is sitting.

Each of the green lines in the tall column is therefore not a distinct linear strand, but a conglomeration of 4 strands, which loop from one to the next at the tops and bottoms of the column. The entirety of the DNA is therefore a single long looping pathway, with a single entry point and a single exit point from the 25-story structure. (If you look carefully at the base of the structure, you'll see the entry and exit points). This single long "beads-on-a-string" strand is carefully wound, as shown in previous slides, so that there are no net helical twists in the DNA between the entry and exit points.

In this scale, which is almost exactly 10,000,000-fold magnified, the tower is about 1 foot wide and 10 feet tall. There are about 150 histone octamer cores in the structure, of which only half are visible here because of the angle of viewing. The individual octamer cores, in this magnified model, would be about the size of tennis balls, and would be longitudinally-separated by a space about the same size. The structure would contain a little over 21,000 base-pairs, which, after intercalation, would be spaced about an eighth of an inch, or 3 mm apart.

What happens when you approximate two such towers? How do adjacent 300-nm segments interact chemically? Let's remove Sir Isaac and the other props and consider this.

SLIDE 56

We now introduce a second 300-nm tower. Note that there's still only one entry and one exit point, because, although I've portrayed these structures as being fused together from separate instances of them, I've only done that for dramatic effect: the DNA strand is in fact continuous between them, as the loop indicated by the arrow exits the tower on the right and immediately enters the tower on the left.

A word is in order concerning the zig-zag structure often portrayed in review articles. Here's one such illustration, taken from a typical article on the subject of chromosome structure:

SLIDE 57

This is a current view of chromosome structure. The blue arrow indicates the part of the drawing showing the 300-nm zig-zag folding which is seen in electron micrographs of actual chromosomes, and often assumed to be the next higher stage in chromatin folding after the 30-nm fiber. The model of chromosome structure I present in the current slide show would not give a zig-zag appearance quite as literal as that shown here, but it might produce an EM picture something like it. But what do electron micrographs of chromosomes actually show? Let's look at one.

SLIDE 58

Here's an electron micrograph which I've seen cited several times. The area in question is the one indicated by the two long, thin parallel arrows in the photo on the left. On the right is a close-up of that area. The original caption says: "The arrows indicate 200-300 nm fiber that appears to follow a coiled or zigzag course." If you take a close look at this picture, you'll see that there's quite a bit of leeway in the interpretation thereof.

In a chromosome with a structure such as the one I propose in our current slide show, electron microscopy would likely disclose regions of zig-zag appearance, arising from random to-and-fro motion of adjacent 300-nm segments, although I wouldn't expect it to be as *regular* as portrayed in the drawing in the previous slide.

In order to achieve the zig-zag configuration, we need only to assume that the DNA loops connecting adjacent 300-nm segments are long enough to allow some to-and-fro motion between them. We might then see something like this, ...

SLIDE 59

...where random to-and-fro motion coincidentally gives rise to a zig-zag appearance. Although I would expect that a geometrically-perfect alteration of a zig-zag nature would be less often seen than a string of approximately-parallel strands, the latter would still be consistent with the EM picture above, within which one cannot distinguish between a zig-zag or a coil, which are two very different structures.

Therefore, for lack of published data supporting any major alternative, I shall proceed with the assumption that the next step up in higher chromatin structure, after the 30-nm fiber, is a 300-nm tall, relatively flat ribbon, created by extending this structure indefinitely to the right and left as shown.

The next question is: How do adjacent 300-nm segments interact?

SLIDE 60

Adjacent 30-nm fibers can interact with one another in the same two ways we have seen in all the protamine-DNA, and histone-DNA interactions previously, namely by either the peculiar square-array patterns of salt bridges between positively-charged basic residue R-groups in protein, and negatively-charged phosphate groups in DNA, or, by hydrogen bonding of β-sheet peptide backbones.

If two adjacent 30-nm fibers encounter one another along their long axes, this model predicts a salt-bridge interaction between the first and second fiber which is precisely the same sort of charge-charge interaction as the one seen between the histone octamer cores inside the same fibers. If the two fibers encounter one another along their short axes, there are two interactions possible, both the hydrogen bonding of β-sheet peptide backbones, and, with only a slight conformational change which I strongly suspect is very possible, the square-array salt bridges. The point is that our 30-nm fiber model, in addition to arising fairly effortlessly from our histone octamer model, also readily gives rise to a 300-nm fold, since 300-nm lengths of 30-nm fiber can be folded and aligned with adjacent folds according to the principles shown here.

SLIDE 61
Title slide
(No audio)

Completion of the chromosome
II. 300 nm fold to 700 nm fold

SLIDE 62

I'm going to present my 700-nm fold *molecular* model side-by-side with my *macroscopic* model of it, because I myself couldn't form a clear picture of these foldings without a life-sized model. We'll start with our 300-nm fold. The life-sized model we have been looking at, with the help of our friend Sir Isaac, is enlarged 10,000,000 times, so that the 300 nm becomes 300 cm, which is almost exactly 10 feet; just a little taller than the average New York City apartment ceiling.

The 300-nm fold is simply a length of 30-nm fiber, folded back on itself every 300 nm. In the 10,000,000-times-enlarged model, the 30 nm width becomes 30 cm, which is almost exactly 1 foot. So, in this slide, we are representing a single segment of the 300-nm folded structure as a tower, with dimensions 1 ft wide by 10 feet in height. Inside the 300-nm tower, as we have seen, is a stack of 25 planar structures, sort of like a 25-story building, each of which contains a 6-histone-octamer array, which can conveniently be thought of as being composed of 6 tennis balls, of which only 3 are visible in this view. There's a total of approximately 150 histone octamers in the 25 stories combined. Through this tower of histones a single, long DNA duplex winds its way, giving the appearance of multiple longitudinal strands, but there's really only one, with a single entry point and a single exit point.

How much DNA is in this tower? Remember that we're employing the Wu straight-ladder DNA structure, with base pairs spaced at 6.8 Å, so each vertical strand, or more properly "loop" of DNA contains 300nm/6.8Å = 441 bp. The six histone octamers of each story of the tower are set in a particular spatial arrangement, and the corresponding histone octamers, of each of the 25 stories in the structure, are vertically-aligned, with a single DNA duplex strand passing through, alternatively from bottom-to-top, then top-to-bottom.

Each histone octamer is associated with 8 strands of DNA, so, for each vertical histone-DNA complex, there are eight times our previous bp total, or (441x8)=3,528 bp's of DNA. There are 6 histone octamers on each of the 25 stories of the tower, therefore the grand total number of bp's in the tower is 3,528x6 = 21,168 bp.

SLIDE 63

To comprehend the compaction of DNA by virtue of the 300-nm fold, we should first consider what the length of the average human chromosome would be if the 30-nm fiber was *not* folded at 300-nm intervals, but was merely stretched out to its full length. Let's start with the whole cell. As you probably know, the human genome, if all of its 46 chromosomes were laid out end-to-end as a single linear W-C double-helix, would be 2 m long. Our Wu straight-ladder structure, however, has 6.8 Å base-pair-spacing, so it would be twice as long, *i.e.*, 4 m. The average human chromosome would be $1/46^{th}$ of this length, which, for the W-C structure, would be approximately 44 mm. For the Wu straight-ladder structure, it would be twice as long, or about 88 mm.

If, instead of folding the DNA into 300-nm segments, such as the one shown here, we instead laid all the DNA from a single chromosome out in the form of a 30-nm fiber, according to this model, how long would it be? Well, as I showed previously, there are 48 Wu duplexes in this tower-like structure, so the total length of an entire chromosome, packaged in the form of this 30-nm fiber model, would be $1/48^{th}$ of its former length, or about 1.8 mm.

Now let's consider our Newton-sized macroscopic model of the same structure, which is 10,000,000-fold enlarged. 1.8 mm then becomes 1.8×10^7 mm = 18,000 m = 11.1 miles. In New York City, this would be a 1-ft wide tennis-ball-laden fiber stretching approximately from 1st Street, just about to the end of Manhattan Island, at 220th Street and Broadway.

SLIDE 64

On the left we see Sir Isaac with his 30-nm fiber. He's given it a single 300-nm fold, only to aim it north, sending it through the famous Washington Square Arch, which is just above 1st Street in New York City. It travels the 11.1 miles and comes to rest at the entrance of the Broadway Bridge, the northern tip of Manhattan Island, and the last stop before the Bronx. If you've ever been to New York, this should give you a pretty good picture of the relative proportions of our 10,000,000-fold enlarged model. It's 1 foot wide and 11.1 miles long, about the length of Manhattan Island.

SLIDE 65

Now we're going to take our 30-nm fiber and fold it every 300-nm. Here's a view of a number of 300-nm segments connected at the bottom by strands of duplex DNA, and arranged in a very approximately parallel manner, but more haphazard than parallel. Next we'll compact them.

SLIDE 66

Here's a compacted structure containing 4 adjacent 30-nm fibers, which now looks like a single un-segmented, homogeneous structure, because of the close interactions at the borders thereof. I'm bringing back Newton so that we don't lose sight of our scale. The actual structure is a lot longer than this, of course. If you count the segments in the finished product, you'll see that the slide will contain 44 adjacent 300-nm folds, after which we run out of room on the screen.

Again, if you look closely at the bottom of the structure, which is starting to look like a long picket fence, or perhaps a big green curtain, you'll still see the connecting loops, because in reality, it's still one long duplex strand of DNA.

If the entire chromosome is folded in this manner, how long will it be? It'll be the width of a 300-nm fold, multiplied by the number of 300-nm folds in the average human chromosome. That latter number is no mystery: it's simply the average chromosome length divided by 300, which is just over 6,000.

As for the width of a 30-nm fiber, folded every 300 nm, well, that width is still 30 nm. I'm going to ignore the spaces between the folds, because adjacent 300-nm towers will interact by the peptide backbone H-bonds and protein-DNA salt bridges we have looked at previously, and would likely fit together very closely. I have therefore taken the liberty of presuming the width of each 300 nm fold to be exactly 30 nm, ignoring the small spaces which must exist between them, which are probably somewhere in the neighborhood of 3 angstroms. My fold-count is therefore probably off a bit, and the actual number of folds is probably a few percent smaller than is reported in this table, within which we see that the new length of a 300-nm-folded chromosome is approximately 2 tenths of a mm.

In our 10,000,000-fold enlarged macroscopic model, the length of the 300-nm-folded structure will be blown up to a little under 2000 m, which is a little *over* 1 mile. If we were to go through the math more precisely, the length would prove to be exactly 1/10 of the previous length, which makes perfect sense when you think about it: each 300-nm segment has been rotated upright, so that its 300-nm length has been replaced by its 30-nm width.

So if Sir Isaac still starts his chromosome in Washington Square Park, it won't reach anywhere near the northern tip of Manhattan anymore, but only about a mile up, to Madison Square Park on 23rd Street. Let's see how he's doing!

SLIDE 67

Here's our previous view of Sir Isaac's un-folded 30-nm fiber, looking like some sort of weird green electrical trunk line, and extending 11.1 miles, all the way to the border of the Bronx. After 300-nm folding, it now looks more like a green picket fence, or a curtain of some sort. It's been compacted to a new structure only about a mile in length, a fraction of its former self, barely extending beyond downtown Manhattan. On the right is Sir Isaac, keeping an eye on the northern end of his chromosome, which is now terminating at the entrance to Madison Square Park, where Manhattan's famed 5th Avenue and Broadway intersect, just about a mile north of Washington Square Park.

So the 300-nm fold reduces the length of our magnified structure from about 11 miles to 1 mile, about a 90% compaction.

SLIDE 68

Here's our 300-nm-folded fence-like structure.

To complete our chromosome, we're going to have to fold it into 700-nm folds at right angles to the direction of the 300-nm fold, in the manner of a curtain. But our folds will have to be much larger than those of a real fabric curtain. Let us review our structure up to this point before proceeding.

SLIDE 69

We started with our proposed model for a 30-nm fiber, based upon this grouping of 6 histone octamers. A single strand of DNA is threaded through the complex. Again I mention that the DNA columns are drawn vertically, but I've unrealistically left the histone structure tipped forward so that at least a part of the path of the DNA can be seen. I don't think I've mentioned it before, but, in case it's not completely obvious, the pathway given here for the threading of DNA through the 6-histone group is arbitrary; any number of alternative pathways could be proposed. The only requirement of our model is that any proposed pathway have a single entry point and a single exit point for the single DNA duplex, and that the DNA acquire no net twists during its journey.

Remember too that the loops of DNA are much longer than shown here.

Next we stack up 25 of these 6-histone-octamer planar units to make our 300-nm-tall tower.

Then we take a large number of these unit towers, and compress them together to make our fence-like, or wall-like structure. This wall, containing a single DNA duplex with no twists,

extends the entire length of the chromosome, and has a width of about 30 nm everywhere, and a height of 300 nm. Now we'll fold it like a curtain. To appreciate the geometry of the fold, we shall look down upon the wall from above, in the viewing direction indicated by the arrows.

SLIDE 70

At the risk of boring you to death, I'm going to reconstruct the axial view from its building blocks, so that we never lose sight of the fundamental building blocks of our structure. Here is our model of the 30-nm fiber, showing a single 6-histone-octamer complex in cross-section. This time there's no tilt, because we're not going to be tracing the path of the DNA, so this is a perfectly axial view. Let's put 3 cross-sections together. The green lines are not biological structures, but merely an artistic contrivance so that, as we lengthen the axial view, the details aren't totally obscured. OK, let's double the length. Now let's double it again.

This axial view is now 24 cross-sectional units long, which is very close to 700 nm, the length of the highest known unit of chromatin folding before the complete metaphase chromosome. So this will be the motif for our 700-nm fold. But before we look at that, I wish to bring back the "front view", or longitudinal view, so I'm sure both I and you remain oriented, and continuously cognizant of the view at which we are looking.

SLIDE 71

First, let's move the axial view up a bit to make room...

SLIDE 72

Now let's momentarily bring back the longitudinal view, which we just recently dismissed. At the bottom of the slide is our 300-nm-high "wall", or "fence", that we've looked at several times previously. I just want it to be clear that our axial view is merely the top view of the wall below. We've intentionally limited our view to a segment of length 700 nm, for curtain-like folding purposes, to be shown shortly. So the dimensions of our chromatin "wall" segment are W=700 nm, H=300 nm, and the thickness of our "wall", as hopefully made clear by the upper drawing, is in the neighborhood of 30 nm. Now we're ready to concentrate on the axial view.

SLIDE 73

So here we have our axial view of a 24-unit-lengthed segment of 300-nm-folded chromatin. Each of the 24 units shown contains a column of sextets of histone octamers, which you can still see if you look closely. The length of the structure shown, as we have already said, is about 700 nm. Now let's imagine it extends the full length of the chromosome. Now let's fold it every 700 nm.

SLIDE 74

Here we now see the beginning of 700-nm folding. This is the "curtain-like" fold we mentioned previously. Remember, we're looking down from the top; as if we were looking

down at the top of a curtain from the ceiling of a room. Of course our molecule is more angular than a free-flowing fabric curtain would be, but it's the same principle of back-and-forth folding. If we looked at the front of the structure, we'd see a more literally-curtain-like appearance, which we'll get to in a moment. First we want to complete the process of compaction by squeezing these 700-nm segments together.

SLIDE 75

When all the 700-nm folds are fully-compressed, one will have a rather amorphous axial appearance, just as one gets in the longitudinal green "wall" we have been looking at. In other words, we're now looking at the top of a solid cube of DNA. Again, if you look closely, you'll see lots of histone octamers in this structure.

The reason the segments can be packed together so closely, as we have explained previously, is that the sides of our proposed 30-nm structure – both the long *and* the short sides – are, so-to-speak, "sticky"; that is, they will interact very well by either hydrogen-bonding of adjacent peptide backbones, or else by that peculiar square-array of salt bridges we learned of from the protamine-DNA complex. Let's look at the front, or longitudinal view of our 700-nm-folded structure.

SLIDE 76

Here are 7 stages in the curtain-like 700-nm folding of the 300-nm "wall".

SLIDE 77

Our chromosome is now complete! How big is this structure now? Does it have the proportions known for chromosomes? It does indeed. Let's check the new length. The number of 700-nm folds, like the number of 300-nm folds before it, is readily calculated as the previous length divided by 700, so there are something like *263* 700-nm folds.

By the way, although the structure depicted here is hardly what one would call a "molecular model", but merely a picture created using graphics software, it's interesting to note that the graphic method I used resulted in a 256-segment structure, so this is actually a reasonable facsimile of a 700-nm-folded structure.

As for the length of the new structure, that's simply the number of 700-nm folds multiplied by their width. Switching back to the previous axial view...

SLIDE 78

...you'll recall that the thickness of the wall to which we're applying the curtain-like fold is still only 30 nm.

SLIDE 79

Therefore the length of the new structure is 263 x 30 = 7890 nm, which we can surely round off to 8000 nm.

SLIDE 80

Here's the shape of our new chromosome. Its length, as we've just seen, is about 8000 nm. The height of its front edge is 300 nm, corresponding to the roughly *6000* 300-nm folds we've introduced. The 700 nm front-to-back dimension corresponds to the 263 curtain-like folds we introduced at the end of the compaction process. Let's rotate the structure longitudinally, so the 700 nm face is in front.

SLIDE 81

The reason I've rotated it is to compare it to the original artist's conception of the chromosome, according to a slide we looked at recently. Here's the isolated lower panel of that slide, showing the 700-nm fold giving rise to one of a pair of replicating metaphase chromosomes. If we further isolate the top chromatid, we can see that the proportions of our chromosome are quite compatible with the picture.

Now the picture is surely just an artist's representation of a chromosome, but the measured lengths of chromosomes range from under 1000 nm up to 20,000 nm, so our 8000 nm artificially-rectangular model with a height of 700 nm is clearly in the range of actual chromosome sizes.

SLIDE 82

Now let's continue the examination of our fully-compacted chromosome by considering our 10,000,000-fold enlarged macroscopic model. We've now moved across town in Manhattan, to the East Side at 75th Street and 3rd Avenue, New York. Shown here is the street where I lived when, as a young man, I vowed to never become a medical doctor. As you can see from my titles, I failed to keep that vow. Medicine was the "family business" and it got the best of me. So all my chemistry endeavors have been carried out in back rooms, at odd times like 3 in the morning, and sometimes in secret. So it's a fitting end to those endeavors that I should complete the folding of the human chromosome on my home block, with Newton staring quizzically from across the street. As we have seen, the 700-nm folding reduces the length of the chromosome to about 8000 nm, which, in this 10,000,000-fold enlarged macroscopic model, is about 80 m, or 262 feet, which is almost exactly 1 New York City block, or 1/20th of a mile.

SLIDE 83

Look how far we've come! We started out with a 30-nm fiber which stretched nearly the entire length of Manhattan Island, requiring a map to show the complete extent of it.

SLIDE 84

By introducing a simple folding at 300-nm intervals, taking advantages of the "sticky corners" of our 30-nm structure, we converted the 30-nm strand into a fence-like structure, which, in the macroscopic model, only extended for a mile, a 90% reduction in length.

SLIDE 85

Finally, by adding a 700-nm fold on top of the 300-nm fold, we reduced the structure to one which, in the macroscopic model, is a mere city block in length, effortlessly achieving an 11,000-fold compaction over the original duplex, without the introduction of a single twist.

SLIDE 86

Perhaps the most important thing to emphasize about this structure is that it consists of a single strand of DNA, with *no twists.* If there was actually a way to pull on the two ends of this long, enormous strand, in principle, all the DNA would come out effortlessly, with no tangles, no twists, no knots and no strand breakage.

In stark and terrible contrast, the current widely-accepted, double-helix-based model of chromosome structure, calls for an average of *13,000,000* Watson-Crick twists per chromosome, for each of the 46 chromosomes of the human cell. All this twisted DNA is then proposed to be further twisted, about 1,500,000 times per chromosome, around a vast array of histone octamers — a twist upon a twist, as it were. Many authors assume further that this frightfully-twisted nightmare structure is then twisted yet a *third* time to give not a 700-nm fold, but a 700-nm *additional helical twist!* Even if it was remotely possible that such an impenetrable jungle of topological horrors could ever unwind, it is a known but inexplicably-ignored fact that the amount of topoisomerase in a cell is woefully insufficient to get the job done in the time available for cell division — even if the job *was* possible; which it isn't!

What we are proposing, therefore, is a structure with many desirable characteristics: it fits the chemistry, it accords well with the known size of metaphase chromosomes, and it allows for topologically-trouble-free DNA replication.

SLIDE 87

THE END