Histone Structure
I. Current Concepts

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Transcript of narration

SLIDES 1-3
(No audio)
(Title page, acknowledgments, Table of Contents)

SLIDE 4
Title slide
(No audio)

Introduction

SLIDE 5

Hello. I'm Dr. Ken Biegeleisen. I'm going to show you the structure of the histone octamer as currently portrayed in all textbooks of molecular biology. Then I'm going to show you a modification of that structure, which adds order to the currently-disordered N-termini of its eight subunits.

This presentation is divided into three parts. Here, in Part I, I shall do no more than to review the current histone structure model. If you're interested in the eukaryotic cell nucleus, I think you'll find this to be quite enjoyable. I've gone to a lot of trouble to create 3D animations which should provide insight into this remarkable protein complex; an insight that cannot be had by simply staring at flat drawings.

Nor can the histone octamer be comprehended by simply spinning around a virtual model of the whole thing, using Jmol or comparable programs, especially if the virtual model is of the entire nucleosome, which also includes the DNA, further obscuring the intricate and highly-ordered state of the octamer core.

NOTE: THE FOLLOWING 4 PARAGRAPHS ARE ONLY HEARD ONLINE. IF YOU HAVE DOWNLOADED THE POWERPOINT PRESENTATION, YOUR NARRATION WILL JUMP DIRECTLY TO SLIDE 6:

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 6

Without further ado, then, let's get started. This first slide has the dramatic title "Heroes of the histone octamer". These are 3 people, or, more properly, 3 *groups* of people, whose work was of critical importance in our arriving at our current state of knowledge, and without whose contributions there is no way this current slide presentation could even exist. As Newton said, centuries ago, we are standing on the shoulders of giants, and I perceive that these are the giants of this field.

SLIDES 7-8

Our first hero is Roger Kornberg, Nobel laureate son of Nobel laureate Arthur Kornberg. The younger Kornberg, with co-worker Jean Thomas, first deduced the structure of the histone octamer core. A brief description of that exciting event was given by von Holde, of whom we shall also be making mention momentarily, on p. 23 of his book. In addition to the obvious fact that the elucidation of the core structure was a great step forward in science, it had a second, more subtle greatness, namely that it was a work of the human intellect, which I doubt is very much appreciated in this day and age of near-total dependence upon mechanical tools such as x-ray crystallography.

SLIDE 9

Our second hero, or heroes, are the team of Luger *et al*, whose 1997 crystallographic study of the histone octamer core structure both confirmed and precisely defined the Kornberg model. Pictures of this structure can now be found in essentially every textbook of molecular biology, and Luger *et al* remains, to the time of this writing, the definitive publication on the subject.

This paper had 5 authors, and I confess that I do not know much about any of them, so I have arbitrarily chosen a photograph of Karolin Luger, the first author, to represent the group. There she is, smiling coyly from behind what is either a drill press or an electron microscope; I can't tell which.

By the way, these pictures are from the researchers' own personal web pages, so I presume that this is how they wish to be seen .

SLIDE 10

My third hero is Kensal van Holde, a prominent histone researcher whose most durable contribution to histone science, however, was not, *per se*, his research itself, but rather his 1989 review book, entitled, appropriately enough, "Chromatin". Some might wonder why authoring a review book causes a man to be regarded by some as a "hero", but there are extenuating circumstances here. In the first place, this book represents one of the most thorough, all-inclusive and unbiased expositions of a subject which I can readily bring to mind. Secondly, it's now almost 25 years since the publication of van Holde, and, incredibly, it's *still* the leading text on the subject!

In science, anything more than a few years old is at risk of being regarded as hopelessly "obsolete", and in such a setting, for a reference work to still be current a quarter-of-a-century later is singular, to say the least.

It's far too early to say whether anything *I've* contributed to this field will stand up to scrutiny, but I can say this much: Whatever its merit, or lack thereof, my task would have been much more difficult, yea perhaps even impossible, had I not had this book, from which I was able to learn the history of this field in the few short months which were available to me at that difficult time.

SLIDE 11
Title slide
(No audio)

<u>Futility of working without ribbons</u>

SLIDE 12

Let's start to look at the current model of histone structure. We'll start with the nucleosome — the nucleosome core structure, that is — and we'll look at it in atomic view, without the help of ribbons. If you don't know what I mean by ribbons, don't worry, I'll be showing you that very shortly.

This is the current concept of the “nucleosome”, an 8-subunit protein called the “histone octamer”, with 146 base pairs of DNA wrapped around it. You can't tell where the protein starts or ends, but we can just about make out the outline of the DNA, and that only because of its recognizable helical twist and stacked bases. Looking into the core of this structure, however, is like looking into a forest!

Perhaps we'll be able to see the protein core more clearly if we remove the DNA.

SLIDE 13

Here's the same structure with the DNA removed. Well, that didn't help very much now, did it? Perhaps we'll comprehend the structure better if we move in closer?

SLIDE 14
(No audio)

SLIDE 15

Hmmm... that doesn't help either. It's still a forest. And please note, we haven't even included hydrogen atoms in our view. If we add all the hydrogen atoms...

SLIDE 16

...then we get an impenetrable thicket! Even an entire army of the world's greatest scientists would be hard-pressed to find law and order in a jungle of atoms of this complexity. Obviously something needs to be done to simplify this view.

And the thing scientists have done is to use cartoon representations, which replace logical structures composed of hundreds, or even thousands of atoms, with simplified graphic models that represent them.

There are various types of cartoon representations in use; in the current slide presentation, we shall be using ribbons.

SLIDE 17
Title slide
(No audio)

Introduction to octamer structure

-
- **Replacement of atoms by ribbons.**
- **Removal of N-terminal and C-terminal strands,**
- **leaving "truncated" octamer core.**

SLIDE 18

Here again is the atomic model of the nucleosome core structure that we have been looking at.

I should reiterate, in case it wasn't made perfectly clear in the previous slides, that this is the 1997 structure published in *Nature* by Luger *et al*, determined by x-ray crystallography. The material used for their study was a reconstituted octamer, made from purified histone subunits, which were then reassembled in the lab.

This virtual model is freely available at the Protein Data Bank, where it is filed under the requisition # 1aoi. If you'd like to obtain this file yourself, you can go directly to Luger *et al's* PDB page right now, by simply clicking the link at the top of *this* page. But you'll need virtual modeling software to look at it. Alternatively, you can just keep watching the current slide presentation, because we're going to examine this structure in minute detail, right here.

Let us now replace the atoms with ribbons, which will begin to reveal to us the rather extraordinary and highly-ordered structure of this very important biological substance.

SLIDE 19

Welcome to the ribbon representation of the histone octamer. It consists of 8 segmented structures, each segment having the form of a coil. If one didn't know better, one might think, at first glance, that this was a conglomeration of colored springs from the inside of some sort of children's toy.

Although it will be difficult to appreciate in this view, each of the 8 subunits has a fundamental structure consisting of 3 coils; a long one in the middle, and a shorter one at either end. This is easiest to see in the white-colored subunit at the 1:00 o'clock position [arrow], which is unfortunate, since this subunit is actually somewhat atypical in having not one, but *two* smaller coils at the N-terminus. More about that later.

SLIDE 20

If you take the trouble to count, you'll see that there are 8 colors, a different color for each of the 8 histone subunits. We shall adhere rigidly to this exact color scheme throughout this presentation, for if we do not, we shall go quite mad trying to keep track of the different subunits, as they move about, and as *we* move about *them*.

Now if we are to count the 'random coil' as a form of protein structure, then it can be said that, of the four fundamental forms of tertiary protein structure, all four, to a greater or lesser extent, are represented here. The many *ordered* coils are α-helices, which are clearly seen, as are the wavy strands projecting out from the core as *random* coils. Less clearly seen, mostly buried in the core in this view, are numerous short β-strands, connecting adjacent α-helical segments. Finally, the octamer as a whole is described by some as being a "globular protein", although most of the core structure would be better described as "the intersection of a pair of flat discs" than as "globular". We'll be looking at this all very closely in subsequent slides.

SLIDE 21

The histone octamer consists of two copies each of the four fundamental histone subunits, called H3, H4, H2A and H2B. To complicate matters, each subunit, in accordance with virtual modeling conventions, has a unique chain-identification name, which is generally *also* a letter, so we're going to have a lot of letters flying around, and I guarantee you it will be confusing. My job is to try to keep everything in focus, and all I can promise is that I'll do my best. Let's start by bringing in some labels.

As I said previously, the octamer core has *two* copies of each of the four histone subunits. Here are the pair of H3 subunits, which, in the Luger *et al* PDB file, are uniquely named with the chain letters "a" and "e". To minimize confusion, I shall give the subunit's formal name in upper

case letters, and the unique chain identifier in lower case. So the pair of H3 subunits shall henceforth be called H3[a], written as shown, which will always be colored white, and H3[e], which will always be yellow.

SLIDE 22

Although hardly evident from this picture, it's a known fact that these two H3 subunits, when carefully isolated, have identical amino acid sequences. Yet in the crystal structure shown here, the yellow H3[e] subunit has a much longer random coil segment at its end than its white H3[a] cousin. This, however, is merely an artifact of laboratory purification, which quite evidently degrades the free ends of the subunits in what appears to be a random and unpredictable fashion.

SLIDE 23

Next we have the pair of H4 subunits, whose chain identifiers are [b] and [f] respectively, hence we have H4[b], which is red, and H4[f] which we've colored brown. H4[f] is in the back of the drawing, and a bit more difficult to see.

SLIDE 24

Next let's bring in the pair of H2A subunits, whose chain names are [c] and [g]. The H2[c] subunit is colored green, but at this point we start to run into trouble coming up with distinctive primary sorts of colors for subunit coloring, wherefore we have chosen a turquoise color for H2A[g], a shade of color somewhere between blue and green.

Finally, we have the pair of H2B subunits, whose chain names are [d] and [h]. H2B[d] is a nice conservative blue color; H2B[h] is probably best-described as "pink", although the Crayola Crayon people might have a more exotic name. The main thing is to try to keep the memory of the color itself in your mind's eye, so you'll recognize it when you see it.

Eight chains, eight colors. OK, let's remove the name labels and look next at the N- and C-terminal ends of these eight chains.

SLIDE 25

We've already looked at the ends of the pair of H3 subunits, projecting out from the octamer core. These happen to be the ***N***-terminal ends. Now let's bring in labels for the N-termini of the H4 subunits. Next the N-termini of the H2A subunits, and finally, the N-termini of the H2B subunits.

The N-terminal ends of the eight histone subunits all project outward, like the arms of a starfish. You'll note, please, that the lengths are completely variable, and that they are without any evident structure. The *entire purpose* of the virtual modeling project I am reporting here is to provide a structure for these currently un-structured N-termini, but we shall not do that quite yet. That's for Part II of this histone slide show. For now, we're not going to do any modeling, we're just going to look.

The most important, and most singularly-defining characteristic of the N-termini is that they are the sites of the highest concentrations of positively-charged basic amino acid residues,

mostly lysine, and yet they are not, at least "not" according to the current point of view, considered to be places where DNA phosphate groups electrostatically bind. Again, we'll be dealing with that much more extensively in Part II.

Right now, let's take a brief look at the C-termini.

SLIDE 26

Unlike the N-termini, which are consistently basic, the C-termini of the 8 histone monomers have amino acid sequences that are markedly inconsistent from subunit-to-subunit. Thus, some of the C-termini are laden with basic amino acid residues, whereas others have very few, and still others are so *short* that there's almost no point in even making mention of their amino acid sequences.

Here are the C-termini of the H3 pair of subunits, the white-colored H3[a] and the yellow H3[e]. Recall how remarkably long the N-termini of these subunits were; now look how remarkably *short* are the C-termini. Re-stated in terms of amino acid count, the N-termini of fully-intact H3 chains are 63 residues in length, whereas the C-termini, shown here, are a mere 4 residues in length, barely long enough to even append a label to in this view!

Next are the H4 C-termini, namely the red-colored H4[b] and the brown-colored H4[f]. The H4 C-termini are also short, but not quite as short as those of H3. Unfortunately, the H4 C-termini are difficult to see from this angle. Not to worry, however, we shall be seeing them clearly later, when we look at the subunits individually.

SLIDE 27

Next is H2A, namely the green H2A[c], and the turquoise H2A[g]. Nothing short about these C-termini! Not only are they strikingly long, approximately 40 amino acid residues in length, but they project the full length of the octamer core, to extrude from it all the way on the other side; the side opposite their respective N-termini. Why so? At the present time, I have no answer to this question. They are also distinguished by having a small but significant number of the *acidic* amino acid residues, aspartic and glutamic acid, which species are generally rare in histones and other nuclear proteins.

Although I shall presently be talking at great length about the *N-termini* of all eight histone subunits, I confess, at the outset, that to this day, I have been unable to assign a logically-compelling function to the C-termini of either H2A, or any of the other histone subunit pairs.

SLIDE 28

Finally we have the C-termini of the H2B pair of subunits, namely H2B[d], the dark blue subunit, and H2B[h], which is colored pink. These C-termini are of middling length, 24 amino acids long, with a seemingly random array of different sorts of amino acid types. Oh, I should mention that they don't look very long in this view. That's because H2B has the distinguishing characteristic that 19 of its 24 C-terminal amino acids are, for some unknown reason, coiled up into an α-helix, which, especially in the case of H2B[d], the blue subunit, is clearly visible proximal to the tiny, unordered C-terminal strand.

Again, I have not, to this day, been able to assign any significance to the lengths, amino acid sequences, or conformations of any of the C-termini.

SLIDE 29

OK, we've removed all the labels; now let’s enlarge our octamer a bit. Next, we're going to remove all those *dis*orderly, and variably-lengthed N-terminal and C-terminal strands. This will leave only the octamer core, which, quite to the contrary, is not only ordered, but highly so, and also filled with fascinating symmetries.

SLIDE 30

So here we have the histone octamer core. I refer to this structure, with the N- and C-terminal strands removed, as the *truncated* octamer core. I think you can see, *without* even examining it very closely, that it is a logically-constructed object. Nevertheless, we *shall* examine it closely, starting with a consideration of its various component tetramers.

SLIDE 31

There are actually *two varieties* of tetramer contained within this octameric structure. First of all, there's [what one might call] a "thermodynamic" tetramer, by which I mean a tetramer that not only has important structural significance for the intact histone octamer, but which also forms *in vitro,* from association of purified histone subunits, because of their peculiar affinities for each other. This "thermodynamic" tetramer consists of the two H3 and the two H4 subunits. We're going to be looking at it later, but for now I want only to draw your attention to this "inverted V" shape which is formed by the pair of H3 subunits, the white H3[a] and the yellow H3[e]. This type of architectural relationship is found repeatedly in the histone octamer, but before examining this particular instance of it, I want to first look at the *other* variety of tetramer, which we might perhaps refer to as a "logical tetramer".

SLIDE 32

The expression "logical tetramer" refers to the grouping of one complete set of one each of the four unique histone subunit types, whose names, as we've previously seen, are H3, H4, H2A and H2B. Since each subunit type occurs twice in the octamer, the complete octamer therefore contains *two* such tetramers. I call them "logical", as opposed to "thermodynamic", because they don't necessarily form spontaneously *in vitro* from purified subunits, but rather, they require the complete octamer setting in order for them to be thermodynamically stable.

Let's remove the orange "V", and watch carefully now as I take away 4 of the 8 subunits, which will leave us with our first "logical" tetramer.

SLIDE 33

Here it is. Let's add the subunit labels. As you can see, there's a single occurrence of each of the subunit types, H3, H4, H2A and H2B. Now let's add the chain identification letters:

SLIDE 34

Here, now, are the complete names of the subunits which comprise this "logical" tetramer. If, perchance, the manner of assignment of lower-case chain-identifier letters seemed arbitrary in the previous slides, now you can see why they were so-assigned by Luger *et al* in their 1997 crystal structure. Note that the chain letters read: "a", "b", "c" and "d". Quite logical, and quite orderly.

Now let's bring back our orange "V" marker, which is no longer inverted, but almost an upright letter "V". If the V-shape seems a bit imperfect this time, it's because the structure we're looking at, at the moment, does not lie precisely in the plane of the computer screen.

SLIDE 35

This time, our V-shaped marker is marking the C-terminal ends of the red-colored H4, and the blue H2B, which form the heart of this tetramer. Although it's still too early in our discussion to demonstrate clearly, I'll tell you, by way of a preview, that the geometrical relationships between these 4 subunits will be repeated, almost exactly, in every instance in which we shall perceive a tetrameric sub-structure within the complete structure of the histone octamer.

Let's remove our "V" marker, and add back the 4 subunits which we removed, when we set out to demonstrate this first "logical" tetramer.

SLIDE 36

Here's the complete octamer again, with all 8 subunits in place. Now I'll remove the 4 subunits which comprised the first "logical tetramer", so that we can see the second one:

SLIDE 37

Here, then, is our second "logical tetramer". Once again, we see one instance each of the 4 subunit types, H3, H4, H2A and H2B. Now let's add the lower-case chain-identifier letters.

SLIDE 38

And here they are: "e", "f", "g" and "h". Once again, very logical, very orderly. Let's bring back our orange "V", which now marks the C-terminal ends of the brown-colored H4[f] and the pink H2B[h], which form the heart of this second 'logical' tetramer. As I've already implied previously, the geometrical relationships between these 4 subunits are the same as those of the first 'logical tetramer' we examined earlier.

SLIDE 39

Here's the complete structure again, with all 8 subunits restored.

Well, this completes our brief introduction to the truncated histone octamer core. Next we'll start to look at individual subunits, with the object in mind of eventually building back up,

in stages, to the complete octamer, only next time doing so with a good understanding of its component parts.

SLIDE 40
Title slide
(No audio)

Structure of the histone monomer

The “histone fold”

SLIDE 41

Here’s the octamer core again. Let’s remove everything except a single monomer; in this case one of the H3’s.

SLIDE 42

This is subunit H3[a]. I pointed out previously that each individual histone monomer consists of a long central helix, flanked by a pair of shorter helices. Now, with the H3 subunit isolated, that becomes much more clearly visible.

This arrangement, with the two smaller helices folded over the central helix, is referred to as the **"Histone Fold"**. It is found in all 8 histone monomers, and also in an ever-increasing number of other proteins involved in nuclear DNA metabolism.

Let's move it to the middle of the screen, where there's more room to manipulate it.

SLIDE 43

Now remember, this picture of subunit H3[a] was graphically-isolated from the "truncated" octamer, wherefore this is *not* a representation of the entire H3[a] subunit, but only of the central ‘histone fold’ region; that is, the region common to all histone subunits. Each subunit, however, has at least one additional α-helical region, these ranging in size from the tiny 3-residue helix found in the N-terminal region of subunit H4, to the 19-residue α-helix found in the C-terminal region of H2B, which we've already had occasion to look at in a previous slide. Remember also that the N-terminal and C-terminal unordered strands are excluded from the current view in this slide.

In this subunit, H3, the additional α-helix is located at the N-terminal end.

SLIDE 44

There it is. This additional α-helix, at the N-terminal end of the histone fold region, has a length of 12 amino acid residues, making it a rather substantial structure which can hardly be

ignored. But what is its role? We'll be considering that question in Part II of this series. Right now, all I want for us to do is to look at it, and to keep it in mind, as we proceed now to remove it from the picture again.

SLIDE 45

OK, we're back to our isolated histone fold. Let's look at its anatomy. First of all, let's label the N-terminal and C-terminal ends, since they otherwise look about the same in this truncated monomer. Here's the N-terminus; here's the C-terminus. All right, now that we're oriented, let's introduce some nomenclature for the three α-helices.

The lower helix, at the N-terminal end of this monomer, is generally referred to as "Helix I". Remember that the largest concentration of basic, potentially-DNA-binding residues, is in this N-terminal region, but most of these basic residues are *proximal* to Helix I, in the extreme N-terminus, whose un-ordered strands we've excluded from this view. That notwithstanding, Helix I is an important part of the so-called "superhelical ramp", a structure we'll be examining later. The superhelical ramp is a spiral path in the histone octamer upon which DNA is believed to bind, and some of the basic residues in Helix I, being part of this "ramp", are therefore presumed to be involved in DNA binding.

SLIDE 46

Next we have the long, central helix, called "Helix II". The distinguishing characteristic of Helix II is that it is strikingly hydrophobic. There are almost no basic residues to be found here – very little lysine, arginine or histidine. There is no doubt about the function of this region of the monomer – it is clearly a structural role, since, as we shall see, the histone octamer is held together primarily by hydrophobic interactions.

At the other end of Helix II, at the C-terminal end of the histone fold region, is Helix III. Some of the Helix III's have substantial numbers of basic residues, but others do not, therefore it would appear likely, at the present time at least, that its role, like that of Helix II, is largely structural.

SLIDE 47

Now there's one other pair of objects we've got to look at. These are the loops connecting the peripheral Helices I & III to the central Helix II. These are considered to be β-strands, wherefore the structure of the histone monomer core may be described as "helix-strand-helix", or "HSH" for short. Since I know of no "standard" names for these particular strands, I simply call them "β-Strand 1" and "β-Strand 2". As you can see, I have arbitrarily assigned the numbering "β-Strand 1" to the loop closest to *Helix* I. By default, then, the other β-strand shall be numbered "β-Strand 2" in the slides to come.

As we shall see later, these β-strands will interact with the β-strands of adjacent histone monomers, to form small β-sheet-like structures. These are referred to as "β-bridges". The β-bridges are rich in basic residues, and are considered to be important sites of DNA binding.

By the way, most of my terminology follows that of Ramakrishnan, whose excellent review was my own first introduction to histone science, wherefore I must now give the reference. His review is entitled "Histone Structure and the Organization of the Nucleosome".

This is a 1997 article, but, as we've pointed out earlier, with respect to the *structural* aspects of histone history, 1997 is still fairly current.

Well, we've been looking at a static, 2-dimensional picture of the histone monomer up to now. Let's see what this thing *really* looks like.

SLIDES 48-51

Here it is! This is the fundamental unit of the support matrix which has been about 3 Å from our own DNA, since the beginning of time. It's hard to know exactly what to say about it. Like life itself, it's hardly rectilinear, yet it does have a pleasing aesthetic quality to it.

SLIDE 52

On every level of structure in the histone octamer, starting here at the level of the monomer, a 2-fold axis of symmetry can be observed. The symmetries are not quite as exact as those you might observe in a crystal of a simple salt, but they're unmistakably present.

Here's a marker, showing the approximate location of the monomer 2-fold axis. To appreciate the symmetry, please take note of the positions and relative orientations of the three α-helices to each other, and try to keep a memory of them in your mind's eye. Now let's spin the molecule about its axis.

SLIDE 53

I think you can see that the appearance of the rotated monomer, insofar as the geometric relationships between the three α-helices, is pretty much the same as before. Now let's flip it back.

Next I want to show you a particular view of the monomer; a view that I have found helpful in understanding its geometry, and in assessing other molecules for their degree of histone-like structure.

SLIDE 54
(No audio)

SLIDE 55

Viewed from this angle, Helix II, the long central helix, appears as a small circle, Helix III is in the rear of the screen, pointing straight down, and Helix I is in the forefront, pointing upward at a characteristic angle. There are several descriptions of this angle that come to mind, all rather unflattering, such as, say, "Nazi salute", or worse, but rather than name the angle, we can measure it.

SLIDE 56

In this position, Helix I is at an angle of approximately 130º relative to Helix III. The reason I've taken the trouble to show this, is that there are many proteins known that are "histone-like", including a number of important transcription factors and other modulators of

gene expression. If you wish to assess their similarity to histone structure, I have found this position to be a good one from which to evaluate them in that regard.

SLIDE 57
Title slide
(No audio)

Other α-helices (in addition to Helices I, II and III)

SLIDE 58

Before going on to our next topic, which will be the joining together of histone monomers to form dimers, I wish to return to the "extra" helices I mentioned earlier. "Extra" here means in addition to the 3 central helices, upon which we have been concentrating *most* of our attention.

This I do for two reasons: First of all, to introduce some terminology for the extra helices, and secondly to show something of the geometry relative to the 3-helix core.

We'll look at two "extra" helices that we've looked at before, but now we'll look just a bit more closely. First, let's look at the extra helix in histone H3. We've seen it before, but the question I wish to raise now is, "What shall we call this?" We have Helices III, II and I, and this is *proximal,* or "N-terminal", or "N-ward" if you will, to Helix I, and the only number we know of, which is less than "1", is "zero". Therefore, with little available in the way of a logical alternative, I have decided to call this extra helix "Helix Zero"! It's admittedly a funny sort of name, but it gets even worse. It's highly-probable that there's yet a *fifth* small α-helix proximal to this one, and what shall we call that? Since I discovered it after getting quite accustomed to the terminology "Helix 0", I had no choice but to call the fifth helix, which is discussed in Part II of this series, "Helix *Sub-Zero!"* I know, it sounds more like a refrigerator brand than a chemical structure, but I got painted into a nomenclature corner, and I have resigned myself to my fate.

SLIDE 59

The second "extra" helix I'd like to show you is the one found in histone subunit H2B. Shown here is the blue H2B subunit, H2B[c]. Since *this* "extra" helix is *distal,* that is, C-terminal or "C-ward" to Helix III, I refer to it as "Helix IV". I'm going to rotate the subunit a bit now, to show you the close spatial relationship between Helices III and IV. Although we probably won't have time to discuss it in any detail, there is a strong hydrophobic alley between these helices, which means that the proximity of these two coils is certainly not mere coincidence. But, as I've said before, I don't know, at this point, what any of the histone C-terminal structures are actually for, so I present this view here strictly as an item of academic interest. So I hope it interests you as much as it interested me!

SLIDE 60
Title slide
(No audio)

Dimers

SLIDE 61

The next level of organization we shall look at is the dimer. Obviously, in an octamer there will be 4 of these, but we need only look at one, because the principles are the same in the other three. For our one dimer, let's look at H3[a] and H4[b]. We can begin, as we did with the monomer, by isolating them.

SLIDE 62

Now let's move the dimer to the center of the screen.

SLIDE 63

The first important thing to point out about the dimer is that there is an approximately-antiparallel orientation of the constituent monomers, which causes the N-terminal ends of the two monomers to be close together. This turns out to be a rather important relationship. In Part II of this presentation, where I'll show you how the currently-unordered N-terminal strands of the histone octamer can be made totally orderly, and optimally-arranged for DNA binding, we shall see that the precise spacing between the N-terminal α-helices of the dimer is critical to the structure we shall propose.

The C-terminal ends are also relatively close together in the dimer shown here, but not quite as close as the N-terminal ends.

The long central Helix II helices cross approximately in their midpoints, but not exactly so, which is accurately shown in this view. The anti-parallel orientation of the Helix II's is also not exact, but it's clear that they are a lot closer to being anti-parallel than parallel.

This structure is maintained by a combination of hydrophobic and ionic interactions, of which the hydrophobic are considerably more important. We'll look at both in a while. But first let's look at this structure in 3-D.

SLIDE 64

As the dimer rotates, you will notice that if the peripheral helices, that is, Helix I and Helix III of either of the monomers, are pointing *toward* you, then, at that moment, Helix I and Helix III of the *other* monomer will be pointing *away* from you. This is an indication of another approximate 2-fold axis of symmetry, which we shall demonstrate directly on the next slide.

This relative orientation of the 2 monomers is referred to by Ramakrishnan as a "handshake motif".

OK, I'm going to stop the rotation, and move the dimer slightly, to an angle which best displays the 2-fold axis:

SLIDE 65

Here's the axis of symmetry. In order to appreciate it, I draw your attention to the fact that the long central helix of subunit H3, the white subunit, is underneath its red partner, and its short helices, Helices I and III, are pointing slightly toward the rear of the computer monitor view. Now let's flip the dimer on its 2-fold axis.

SLIDE 66

Now the long central helix of the other subunit, H4, the red subunit, is underneath, and *its* short helices are now pointing slightly toward the rear. The approximate 2-fold axis is therefore clearly demonstrated.

SLIDE 67

Let's flip our dimer back to its former position. Next we'll begin to examine the forces which hold the histone octamer together. This is best done in the setting of the tetramer.

SLIDE 68
Title slide
(No audio)

Geometry of the $[H3\text{-}H4]_2$ tetramer

SLIDE 69

Just to make sure we remain fully-oriented, let's return momentarily all the way back to the beginning; the entire nucleosome, complete with its 146 base pairs of DNA wrapped 1-3/4 times around the histone octamer. Now let's again remove the DNA.

SLIDE 70

Here's the complete histone octamer, without DNA. Note again the wild and unruly state of the N- and C-terminal strands. The orderliness we have been examining in recent slides is only in the octamer core. So let's once again remove the unstructured N- and C-termini.

SLIDE 71

OK, we're back to the "truncated" octamer core, with its multitude of symmetries. Let's enlarge it a bit...

SLIDE 72

...and let's remove everything except the uppermost 4 monomer subunits.

SLIDE 73

This leaves us with a tetramer; one believed to be of critical importance in maintaining the stability of the histone octamer. Let's center it, and take a closer look.

SLIDE 74

This is not one of the tetramers I previously referred to as a "logical" tetramer, by which I meant a tetramer containing exactly 1 each of the 4 distinct subunit types found in the histone octamer, namely H3, H4, H2A and H2B. This tetramer contains only H3 and H4; two instances of each.

I referred to this previously as a "thermodynamic" tetramer, by which I meant a tetramer which forms spontaneously *in vitro* because of the favorable ΔG. I would like also to remind you of that 'inverted V' shape, made by the two H3 subunits, the white H3[a] and the yellow H3[e]. We'll be referring to that 'V' shape in some of the subsequent slides, and I'd just like you to keep it in mind.

Like the monomer and the dimer we just looked at, the H3-H4 tetramer has a two-fold axis of symmetry. Before getting into the energetics of formation of this structure, let's look at this symmetry. In order to appreciate it, please take careful note of the relative positions of the 4 monomer subunits, which, in this flat 2-dimensional view, seem to be stacked up like a small stack of playing cards (although the reality, as we shall see in a moment, is a bit more complex): On the right side of the slide, the long central helix of the red H4[b] is clearly sitting on top of the white H3[a]. In the middle of the slide, the C-terminal end of the white H3[a] is lying on top of the yellow C-terminal end of H3[e], and on the left side of the slide the long central helix of the yellow H3[e] is sitting on top of the brown H4[f].

So, from this viewing perspective at least, the tetramer seems to be *rising up* in a direction normal to the computer screen, that is, toward your eyes, as you go left-to-right, moving from subunit to subunit.

Now let's rotate it about its 2-fold axis:

SLIDE 75
(No audio)

SLIDE 76

I hope you can clearly see that the manner of stacking of the 4 subunits is the same in the rotated tetramer, with each successive subunit appearing to rise up in a direction normal to the screen, toward your eyes, as you move from subunit-to-subunit, in a left-to-right direction. This, I hope, illustrates the 2-fold axis. OK, let's rotate back to the starting position.

SLIDE 77
(No audio)

SLIDE 78

OK, we're back where we started from. Now let's repeat the rotation, but this time we'll stop halfway, which will allow us to more carefully assess the fascinating spatial relationships of these 4 subunits.

SLIDE 79
(No audio)

SLIDE 80

Now we get a nice picture of the 3-dimensional structure of this tetramer. It's not actually slanted at all; but rather a tri-laminar structure consisting of two major layers whose planes are almost perfectly parallel to one another, with a smaller third layer, a little less orderly-looking in this view, sandwiched in the middle.

The 1st layer consists mainly of the long Helix II's of H3[e] and H4[b]. It also includes Helix III of H3[a], which, in this view, is barely visible, but which I hope you recall from the frontal view, where we just recently saw it sitting prominently at the apex of the H3-H3 "inverted V" formation.

The 2nd layer consists of the long Helix II's of H3[a] and H4[f], also including Helix III of H3[e], which is barely visible in this view, but which, in the frontal view, forms the *other* side of the H3-H3 "inverted V" formation.

There's a small 3rd layer, consisting only of the Helix IIIs of H4[b] and H4[f], lying between the two main layers. It's seen better if we rotate a bit.

SLIDE 81

In this slightly rotated view, we can see that there is, in fact, good alignment between the Helix IIIs of H4[b] and H4[f] in this minor layer, although you can see that this slight rotation has knocked the two *major* layers somewhat *out* of alignment. Like everything in the histone octamer, yea, like everything in life generally, the symmetries and orderlinesses are all approximate.

Let's reverse the slight rotation, and return to our previous position.

SLIDE 82

Next, just for a moment, I'm going to add back the other 4 subunits of the octamer, to show where they sit relative to the H3-H4 tetramer.

SLIDE 83

The remaining subunits are H2A and H2B. The green and blue [c] and [d] chains project out to the right of the H3-H4 central tri-laminar structure, and the turquoise and pink [g] and [h] chains to the left.

It's quite impossible to appreciate it from this angle, but the pair of H2A and H2B subunits we've just added to the right of the central tri-laminar structure, combine with half of the central structure to form an additional tri-laminar structure just like it, and likewise for the pair of H2A and H2B subunits to the left. These two additional tri-laminar structures, which have all the parallelisms of the H3-H4 tetramer, correspond to the "logical" tetramers I defined earlier, consisting of one instance each of H3, H4, H2A and H2B.

We're not going to have time to look at these additional tri-laminar structures, and I mention them only to emphasize the fact that the octamer is rich in symmetries, and that any structural motif involving a fraction of the subunits in one part of it, is likely to be repeated in other parts.

SLIDE 84

OK – let's remove the extraneous subunits again, because there's one more thing I want to show you before we leave this view. I've been showing, or at least making mention of parallelisms; the parallel layers, or lamina, if you will, all involve Helix II and Helix III. There are four helices, however, which are not at all involved in these parallelisms: these are the 4 instances of Helix I. As you can see, none of the Helix I coils are in any of the parallel layers of the H3-H4 tetramer. This further emphasizes the fact that Helix II and Helix III are primarily involved in supporting the 3-dimensional conformation of the octamer, primarily through hydrophobic bonding, whereas Helix I, although it probably has some structural role as well, is *distinguished* by its involvement in DNA binding, as part of the superhelical ramp I mentioned earlier. Its different function is reflected here by its different geometrical relationship to the other α-helical coils, being absent from the parallel layers in which the other α-helical coils are found.

SLIDE 85

OK, let's unwind this view, and return to our frontal view of the H3-H4 tetramer.

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SLIDE 86
Title slide
(No audio)

What holds the octamer together?

1. H3-H3 interactions

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SLIDE 87

Here again is the structure I've referred to as the "thermodynamic tetramer", because it is believed to be a primary mediator of octamer stability. Let's look at some numbers.

SLIDE 88

This table is a combination of *two* tables from van Holde, showing the free energy changes associated with various types of dimer, tetramer and higher-order aggregate formation from histone monomers.

The 1st table, at the top of the page, shows the tendency of each subunit to self-associate to form homodimers and higher aggregates. The main thing to note here is that both H3 and H4 are significantly more likely to self-associate than the others.

The most important number on this page, however, located in the lower table, is the ΔG for association of individual H3 and H4 monomers, to form a tetramer. This tetramer is the self-same "thermodynamic tetramer" we have been looking at so closely, and this ΔG value is the reason I so-named it. At -28.1 kcal/mol, the tendency of H3 and H4 subunits, to form a tetramer, greatly exceeds the tendencies of any of the other monomer pairs listed in the table, to form a dimer. By comparison with the next line of data, just below the arrows, we may perhaps be justified in presuming that the lion's share of the free energy change for H3-H4 *tetramer* formation is to be found in the tendency of individual H3 and H4 subunits to first form dimers, because the next step, association of *pairs* of H3-H4 dimers to form tetramers, is no more favorable than the tendencies of the members of *any* of the other heterodimer pairs listed to associate into their respective dimers. I presume further, because of its conspicuous absence, that no tetramer will spontaneously form *in vitro* from the *other* pair of histone monomers, H2A and H2B.

The data in these tables led directly to our current concept of histone structure; a concept that was first suggested by Kornberg and Thomas in 1974.

SLIDE 89

Here's another page from the van Holde book, detailing the circumstances of the Kornberg-Thomas discovery, which came about through logical consideration of stoichiometric and energetic data, giving rise to a model which was confirmed by analytical ultracentrifugation and chemical crosslinking studies.

SLIDE 90

The passage is too long to read in its entirety, but the primary conclusion remains the central concept of histone science to this day: ***The stoichiometries of H2A, H2B, and DNA corresponded to a set of two each of all four histones and 200 base pairs of DNA: the globular nature of the H3-H4 tetramer called for wrapping the DNA around a histone core***.

Now, that DNA base-pair count of 200 is not carved in stone, and various estimates of the number of base-pairs per nucleosome exist, all, however, being somewhere between 100 and 200. The final conclusion, that the DNA must be wrapped around the histone core, is consistent

with all the known chemistry, *as long as the N-termini are ignored*, which has been the case up until now.

In Part II of this series, however, we shall look at *another* type of structure which *does* take the basic-residue-rich N-termini into account. But for now, we shall proceed with our examination of octamer core stability, and present the current nucleosome model in its entirety, which is generally considered to consist of 146 base-pairs of DNA wrapped around the octamer core.

SLIDE 91

With all these structural relationships in mind, we're now in a position to address the opening question of this section: "What holds the octamer together?". The answer is: Mainly hydrophobic interactions. For starters, there's the ubiquitous presence of Helix II-Helix II hydrophobic interactions in each of the four dimers that comprise the octamer. We'll be taking a close look at them later. But for now, since we now know about the probable importance of the H3-H4 tetramer in maintaining octamer stability, we want to look at the central joining point of these four particular subunits, where there is an interaction between the C-terminal ends of the white H3[a], and its 2-fold symmetrical "cousin", the yellow H3[e]. This interaction involves the C-terminal end of Helix II, parts of β-strand 2, and all of Helix III. It occurs at the apex of the "inverted V" that we have looked at several times.

SLIDE 92

When we examine the primary amino acid sequence in that area, we find a number of bulky hydrophobic residues. And note that I say "primary amino acid *sequence"*, as opposed to the plural *"sequences"*, because we must keep in mind that H3[a] and H3[e] have the same amino acid sequence.

The first amino acid residue we shall look at is Leucine 109. The pair of them are shown here. They are not particularly close together, but they are the beginning of a collection of such residues which will eventually fill the space between the Helix IIIs.

SLIDE 93

Next we have Leucine 126.

SLIDE 94

Then Isoleucine 130. Some of these residues are poorly-visualized from this angle, but in a moment we'll rotate.

SLIDE 95

Here are all 3, or I should say all *six* of these amino acid residues. I think you can start to see that there is a very hydrophobic pocket between these Helix IIIs. Let's rotate this structure to get a better look.

SLIDES 96-102
(No audio)

SLIDE 103
Close-up of final frame of the above sequence
0:18 (0:19)

Here's a closeup of the last frame. You can clearly see the Leu and Ile sidechains now. Note that the distances between them are correct for the formation of so-called "hydrophobic bonds", making this a very hydrophobic pocket.

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SLIDE 104
Title slide
(No audio)

What holds the octamer together?

2. H3-H4 interactions

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SLIDE 105

This completes our brief examination of the hydrophobic pocket at the H3-H3 junction, at the heart of the histone octamer. Now let’s turn our attention slightly, to both the right and the left, and look at the [H3-H4](*i.e.*, dimer) interactions. I should point out, at the outset of this, that the conclusions we shall reach for the H3-H4 dimer will be fully-applicable for all 4 dimer interactions in the octamer core, wherefore we shall dispense with the chore of looking at them all, but rather limit our inquiry to one example thereof.

H3 and H4 interact at three locations: Helix II, β-bridge 2, and β-bridge 1. Let's start with Helix II. I'll bring back, for the moment, our "X"-shaped Helix II marker, to focus our attention, and to remind us of the anti-parallel nature of the Helix II-Helix II relationship.

SLIDE 106

In order to properly emphasize the distinguishing characteristics of Helix II chemistry, we show now a table comparing and contrasting the properties of the eight histone monomer N-termini with their internal Helix II's. Our primary concern is with the percentage of basic and hydrophobic residues. If you glance at the middle of the table, you'll see that the total of residues classified as **"Other"** is about the same in both regions, but if you look to the left you'll see that they differ greatly in their **"Basic"** and **"Hydrophobic"** residue content.

The N-termini, which project outward into the surrounding aqueous environment, and which I believe to be the primary sites of DNA binding, because of their high concentration of lysine and arginine residues, are 34.4% basic, compared with only 9% in Helix II. The latter

helix, however, which is buried in a relatively-solvent-inaccessible position, is about 56% hydrophobic, which thereby establishes a predominantly-hydrophobic nature for the octamer core.

Let us now examine this directly.

SLIDE 107

Here again is the H3-H4 dimer, which we've already looked at in some detail previously. This is the right-hand half of the H3-H4 tetramer, which I presume I don't have to show again at this point.

Next I'm going to remove *everything* except the long central Helix II coils.

SLIDE 108

This, from the structural perspective, is the heart of the histone octamer. There are four such cross-shaped structures in the octamer core, and the forces that bind them are undoubtedly of major importance in the determination of octamer core conformation.

Next I'm going to selectively display the acidic, basic and large hydrophobic residues only. It'll look rather cluttered at first, but do not despair; when we rotate it you'll see the orderliness of it.

SLIDE 109

There we are. Please take note of our coloring scheme: The acidic residues, namely glutamic and aspartic acid, are entirely yellow; the basic residues, Arg and Lys, are blue. The atoms of the large hydrophobic residues, Leu/Ile/Val/Phe, are displayed but not specially-colored; that is, they are allowed to remain colored-by-atom, with hydrogen atoms white, carbon atoms gray, oxygen red, etc. Now let's rotate: As the structure rotates, you will clearly see the striking concentration of hydrophobic residues in the alley between H3 and H4:

SLIDES 110-115
(no audio)

SLIDE 116

In this view we see two things: We note that the alley between H3 and H4 is densely populated with Leu/Ile/Val/Phe residues, but *entirely* devoid of acidic or basic residues. Conversely, it's in the *periphery* of the Helix II/Helix II structure that we find all the acidic and basic residues. On the left, in H4, you can perhaps see that there are several high-quality intra-helix ionic bonds, between the R-groups of acidic and basic amino acid residues. Such ionic bonds support only the structure of this individual α-helix, but play no direct role in Helix II-Helix II association, since the latter association, as we can now see, is purely hydrophobic in nature.

The subunits shown here are the white H3[a] and the red H4[b]. Needless to say, an identical Helix II-Helix II hydrophobic alley is found in the space between the subunits of the 2-fold-rotated H3-H4 "cousin".

The alley between subunits H2A and H2B has a different amino acid sequence, but, with respect to its high content of hydrophobic residues, and virtual absence of basic and acidic residues, it is qualitatively the same as this one.

SLIDE 117

Next, let's look at the β-strand regions between the helices, starting at the top of the slide. In accordance with the naming convention I introduced earlier, the H3-H4 β-bridges at the top are each labeled "β-Bridge 2"; the pair of "β-Bridge 1"s, which we'll examine later, are at the bottom, and are not labelled in this view.

SLIDE 118

The first bulky hydrophobic residue we find in 'β-Bridge 2' is Ile 119, contributed by the H3 subunits.

SLIDE 119

The "hydrophobic bond" is completed by Ile 46, contributed by the H4 subunits.

SLIDE 120

Now let's look at the β-Bridges at the bottom of the slide, namely 'β-Bridge 1', so-named because it is adjacent to Helix I. The interactions, at this end of the structure, are a bit more interesting, because they include not just hydrophobic, but also ionic bonds. In order to see the latter, we're going to have to rotate the structure.

SLIDES 121-126
(No audio)

SLIDE 127

Now we can see, on the left, the salt bridge between Asp81 of histone subunit H3, and Lys 79 of histone H4. On the right, we find a "hydrophobic bond" between Phe 84 of H3 and Val 81 of H4. Let's take a closer look:

SLIDE 128

In this magnified view we can see, courtesy of Accelrys Discovery Studio, the ionic bond, which is actually a bit short at 2.6 Å. Above, the benzyl side chain of Phe 84 is seen, creating a mini-hydrophobic domain with the adjacent Val 81. Recall that ionic bonds are more powerful in hydrophobic environments than in aqueous ones, in the latter of which the charge interactions are attenuated by the surrounding layer of water molecules.

SLIDE 129
Title slide
(No audio)

Completion of the octamer

Addition of H2A-H2B dimers

SLIDE 130

To complete the octamer we must add back the pair of H2A-H2B dimers. I want to show you two things; first of all, their positions relative to the H3-H4 tetramer in this view, which I shall refer to as a "frontal" view. Then I want to emphasize their positions with respect to the the Z-axis, which, in the software used to create these images, is normal to the screen and points outward, towards you.

With respect to its position along the Z-axis, the H3-H4 tetramer shown here is at the center of the histone octamer. One of the H2A-H2B pairs, namely the [c] and [d] chains, will be seen momentarily to lie *in front* of the H3-H4 tetramer shown here; that is, closer to *you* along the Z-axis, and the other H2A-H2B pair, the [g] and [h] chains, will be in the rear.

The full octamer, as we shall shortly see, has a very definite helical twist, which is the basis of a so-called "superhelical ramp", believed to be the site of DNA binding.

Let's start with the [c] and [d] chains. We'll dim the H3-H4 tetramer, so we can focus on the location of these two subunits:

SLIDE 131

Here they are. The green subunit, H2A, chain [c], in this frontal view, appears to be the most anterior point of the histone octamer, and, as we shall shortly see, it is just that. Now let’s ‘un-dim’ the tetramer.

SLIDE 132

All right, we now have 6-out-of-8 subunits displayed. Let's dim these, and add in the 2nd instance of H2A and H2B, namely the [g] and [h] chains:

SLIDE 133

This shows the approximate location of H2A[g] and H2B[h]. I say "approximate" because they appear to be floating in front of the octamer at this moment, which is an artistic expedient, since they're actually all the way in the rear, like this:

SLIDE 134

This is all one can actually see of the H2A-H2B [g] and [h] chains in the completed octamer, in this view at least. Now we can un-dim everything:

SLIDE 135

Here, once again, is our complete, although truncated octamer core. We'll start now to look at some fine points of octamer geometry. I'm going to bring back our "inverted V" marker:

SLIDE 136

If we think of the yellow and white H3-H3 junction as being an "inverted V", or pyramid-like structure, then, to continue the architectural analogy, the brown and red pair of H4 subunits which, in this view, graphically bisect them, can be thought of as approximately upright pillars.

Note that the red pillar on the right is in *front* of the structure, and the brown one on the left is in the *rear*. This can, of course, be thought of as merely representing the 2-fold axis we've looked at previously, but I'm pointing this out also because I want you to start to get a sense of the helical nature of the octamer, which is not at all evident here, but which will become much clearer as we move along.

SLIDE 137

Let's restore the octamer, then move on to our first so-called "logical" tetramer, which, as you may recall, was so named because, first of all, it contains exactly one instance of each of the four distinct histone subunit types, namely H3-H4-H2A and H2B, and secondly because it does not form spontaneously *in vitro* from its individual subunits, but appears only in the presence of the central, and thermodynamically-stable H3-H4 tetramer:

SLIDE 138

We've seen this a few times before. Here's our "V" marker. Let's rotate this thing to the upright position.

SLIDE 139

We've rotated it upright, courtesy of the PowerPoint "Spin" function. I apologize for the inversion of the subunit labels, but if you're going to do molecular modeling, you often have to think upside-down anyway.

I'll show you the angle of rotation later. For now, I just want you to see that of the two upright "pillars", to continue our architectural analogy, the one on the right is anterior, exactly as we just saw in the H3-H4 tetramer. So that geometry is repeated here.

The green H2A[c] subunit, as I've mentioned previously, is the most anterior point of the the histone octamer in this frontal view.

Although I can't go through a complete consideration of the hydrophobic and ionic bonds which stabilize this structure, as we did for the H3-H4 tetramer, I just want to show you the pocket at the apex of the pyramid, to emphasize its similarity to the cognate part of the H3-H4 tetramer:

SLIDE 140

At the center of the slide is the apex of the pyramid, containing the Helix III's and the C-terminal ends of Helix II. As with the H3-H4 tetramer, we see a hydrophobic pocket. H4, the red subunit, contributes Thr 71 and Tyr 72. Although we don't think of Thr as having a "bulky hydrophobic R group", you will note that its methyl group is pointing advantageously into this hydrophobic realm. H2B, the blue subunit, contributes Leu 80, Thr 96 and Leu 100. Thr 96 also places its methyl group quite strategically between the two Leu residues. Thus, we see that this structure, like that of the H3-H4 tetramer, is maintained by strong hydrophobic forces, concentrated at its C-terminal end.

SLIDE 141

OK, we're back to the compete truncated octamer. Let's pare back down to the H3-H4 tetramer again:

SLIDE 142

I just want to emphasize something I've shown several times already, but haven't commented on. The H3-H4 subunit pairs, or dimers if you will, are each part of two "V"-shaped tetramers, which is, I suppose, self-evident, since we can discern 3 tetramers in the octamer, but there aren't 12 histone subunits, only 8. Let's look at the white and red subunits, on the right side of this slide, namely H3[a] and H4[b], in their two tetramer settings. Here we see them associated with their yellow-and-brown 2-fold-rotated H3-H4 cousins. *Here*...

SLIDE 143

...we see them associated with the green and blue H2A and H2B subunits. The joining geometry, as is always the case in the histone octamer, involves Helix III and the C-terminal ends of Helix II, in this case of the red H4 subunit and the blue H2B subunit. Let's look at that again.

SLIDES 144-145

I think you can see that the green and blue subunit pair, H2A[c] and H2B[d], are in the front of this picture, and ...

SLIDE 146

...the yellow and brown H3[e] and H4[f] are in the rear. The repetition of these spatial relationship motifs will ultimately give rise to a helical twist, but it's still to early to show that.

For now, let's look at the *other* H2A-H2B subunit pair, the turquoise- and pink-colored [g] and [h] subunits.

SLIDE 147

Now I'll flip back and forth, as I did previously:

SLIDES 148-149

I think you can see that, with H2A[g] and H2B[h], we've reached the rear of the octamer from the perspective of the current view. I think the helical aspect is still *not* well-demonstrated in this view, but we'll get a clearer view of that shortly.

SLIDE 150

OK, once again, we're back to our complete truncated octamer. Next we're going to really strip this structure down to its bare essentials, by looking exclusively at the Helix II's in the octamer core.

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SLIDE 151
Title slide
(No audio)

Helix II "backbone"

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SLIDE 152

Here's the most minimalist view of the octamer which is possible; a view showing nothing but the 8 Helix II's. In this view we will be able to ask a question which greatly concerned me at one time, perhaps unjustiafiably so. The question is: ***Can histone octamers combine in pairs, to form dimers of the octamer structure?*** Before addressing this question, let us run quickly through the structures we have already looked at, in this stripped-down form:

SLIDES 153-157

Here's the H3-H4 tetramer, in skeletal form...

SLIDE 154
[NOTE: Audio for this slide is on Slide 153, which plays over Slides 153-157]

...here's the 1st logical tetramer...

SLIDE 155
(No audio)

SLIDE 156
[NOTE: Audio for this slide is on Slide 153, which plays over Slides 153-157]

...and here's the 2nd logical tetamer.

SLIDE 157
(No audio)

SLIDE 158

In this stripped-down view, we can measure the hypothetical angles of rotation necessary to superimpose these tetramers on each other. To rotate the H3-H4 tetramer to the position of the H3-H4-H2A-H2B logical tetramer #1, we must rotate about +150º. You'll note that the “V” shape, after rotating, has to be moved a bit, because these structural relationships are all approximate, and because, as we've repeatedly seen, the octamer is far from being flat. In any event, it is quite evident that we are not dealing with a “perfect” equilateral triangular arrangement, where the corners of the rotations would be a perfect 120º apart.

SLIDE 159

Here's the comparable exercise for logical tetramer #2, which requires a rotation of approximately -145º. There's no special point to make here, other than to demonstrate this aspect of octamer geometry.

SLIDE 160
Title slide
(No audio)

Can the octamer structure be logically extended, to create a dimer from two adjacent octamers?

That is, can adjacent octamers join via H2A-H2A hydrophobic bonds?

SLIDE 161

We have now seen the recurrent nature of the hydrophobic interactions which stabilize our three tetrameric structures within the octamer core. These are centered at the C-terminal ends of Helix II and the adjacent Helix III. Because these interactions repeat, as we move

through the octamer, one might wonder, as I wondered: Is it possible that this sort of logical, "V"-shaped structure could be extended to an adjacent histone octamer ?

To re-phrase this question, is is possible that a *pair* of adjacent histone octamers could be joined together by hydrophobic inte ractions, based in the C-terminal end of Helix II and Helix III, similar to those seen within the octamer core?

The question is purely hypothetical, and I wouldn't bore you with it except for the fact that I investigated it, merely to satisfy my own curiosity, and found out, rather to my surprise, that the answer is **"Yes"!** A "dimer of octamers", so-to-speak, is unquestionably possible, although I'll say at the outset that that *doesn't* make it biologically-likely. That notwithstanding, the extent to which the octamer cooperates with this hypothetical exercise is somewhat startling, and more-or-less compels me to take the dimer-of-octamers seriously as a possibly-significant biological structure.

Let's see how this might come about. I'm afraid that if you're not already a histone scientist, the argument I'm about to make may be difficult to comprehend, but let's give it a try – I think it might be important.

SLIDE 162

First we rotate the core about 90º.

SLIDE 163

In this view, the structural elements we have been referring to as “pyramids” look more like track and field parallel bars! The central structural element of the histone octamer, the red, white, brown and yellow H3-H4 tetramer, still in the middle of the structure, now looks like 4 parallel upright posts. Let's bring in a little cameo picture of that same H3-H4 tetramer in frontal view, because the argument I'm going to make requires that we keep clearly in mind which structures we're looking at, since, in this side-view, they all look markedly different. In particular, keep in mind the "V" shaped interaction of the Helix II's, between which lie important hydrophobic bonds.

The structure I called the "H3-H4-H2A-H2B logical tetramer #1" is now displaced toward the "front" of the octamer, which, in this lateral view, is now on the left side of the slide.

SLIDE 164

Let's isolate it. The pyramidal shape we saw in the frontal view is replaced here by a chaotic-looking array of subunits, so let's bring in a little cameo of the frontal view, so we can be reminded what we're dealing with. Keep in mind also the downward-pointing "V" shape. If you look now at the middle of the slide, at the red and blue subunits, you'll see that even in this lateral view, the "V" shape can still be at least partially appreciated.

The point of this slide is that 3-out-of-4 of the subunits shown here are bound, by a "V"-shaped C-terminal hydrophobic interaction, to some other subunit in the histone octamer, but one subunit is *not* so bound. The C-terminal end of the white H3[a] subunit is firmly anchored, by hydrophobic bonds, to the yellow H3[e] subunit, by a "V"-shaped interaction which is best seen in the upper-right-hand cameo. The red and blue subunits are similarly anchored, which is best seen in the lower-right-hand cameo. But what about the C-terminal end of the green H2A

subunit? It's not hydrophobically-bonded to anything. The C-terminal end, indicated by the green arrow, is just projecting out into the surrounding medium, with no hydrophobic partner. Therefore, if a suitable partner happened to appear from without, a "V"-shaped hydrophobic bond could theoretically form.

SLIDE 165

An exactly parallel argument could be made for the turquoise H2A subunit, H2A[g], in the "rear" of the structure, the location of the 4 subunit complex we have previously referred to as "logical tetramer #2", which we now isolate...

SLIDE 166

The C-terminal end of the yellow H3 subunit, H3[e], is firmly anchored, by hydrophobic bonds, to the white H3[a] subunit, in a "V"-shaped interaction which is best seen in the cameo in the upper *left*-hand corner. The pink and brown subunits are similarly anchored, as best seen in the *lower*-left-hand cameo. But what about the C-terminal end of the turquoise H2A subunit in the rear of the structure? Like the H2A subunit in the front, it's not hydrophobically-bonded to anything. The C-terminal end, indicated by the arrow, is just projecting out into the surrounding medium, with no hydrophobic partner.

Therefore, this, histone subunit, H2A[g], in the rear of the octamer, *could* be the above-referenced "suitable partner" for H2A[c] in the front If two adjacent octamers linked together via such an H2A-H2A interaction, what might it look like?

SLIDE 167

Let's find out. Here's a skeletal view of one complete histone octamer, with its set of 8 Helix II's, and with the C-termini of the front and rear H2A subunits labeled.

SLIDE 168

Now let's bring in a second histone octamer. Look at the nice "V" shape we can arbitrarily draw. Ahhh, but alas, the joining geometry at the C-termini of Helix II is very particular, and this is ***not*** it.

SLIDE 169

Here's the correct geometry, in two views. This is the geometry that is found in all three of the tetramers we have been looking at. In the view we have called the "frontal" view, on the left, we do indeed see the "V" shape. But in the lateral, or "side" view, on the right, we need to see a perfect or nearly perfect alignment of Helix III of each subunit with Helix II of the other. I suspect that the view shown in the right-hand side of the slide is not particularly self-explanatory, so I'll show you again what I mean, because we have seen this before.

SLIDE 170

Here again is the octamer, stripped down to its component Helix II's. We're going to rotate it 90º. As it rotates, please keep your eyes on the H3-H4 tetramer; the yellow, brown, red and white subunits in the upper end of the octamer.

SLIDE 171

Note how, in lateral view, the "V" shape of the yellow-brown-red-white complex is replaced by this orderly array of parallel columns, which we've seen before. This is the sort of joining architecture we will need, if a dimer of octamers is to be seriously considered. It needs to be "V"-shaped in frontal view, with, moreover, the correct angle at the apex of the "V", *but* it also needs to have its Helix II and Helix III columns perfectly, or near-perfectly-aligned in the lateral view, as we see here.

SLIDE 172

And, believe it or not, here it is: the H2A—H2A joining point of two adjacent histone octamers. We'll show the entirety of these two octamers in a moment, but for now, please just note that the two subunits on the left are from the "rear" of one octamer, and the two subunits on the right are from the "front" of a second octamer. This *inter-octamer* bond results from a near-perfect, hydrophobic-bond-based C-terminal interaction, accurately mimicking the joining architecture seen within the octamer. In this view we have the "V" shape, and, if we rotate 90º...

SLIDE 173

...we see, to a fair degree of perfection, the parallel alignments of Helices II and III, which are required for these hydrophobic-bond-based junctions.

SLIDE 174

Let's rotate back to the frontal position, and look at the hydrophobic bonds.

SLIDE 175

Since we know the amino acid sequences of all the members of these hypothetically-interacting subunits, we therefore already know what the potential is for hydrophobic bonding. And that potential is vast, as we can see here. All the displayed residues have either bulky hydrophobic R-groups, or, at the very least, methyl groups pointing into a hydrophobic pocket. This hypothetical junctional structure is therefore energetically *very* plausible.

SLIDE 176

Speaking of energetics, I'm going to return, for just a moment, to our table of histone homodimer and heterodimer ΔG values. The only thing I want you to note here is the large

green arrow, which line of data indicates that H2A monomers can indeed form dimers, tetramers, and larger aggregates, further supporting the plausibility of this ***dimer-of-octamers*** concept.

SLIDE 177

And here's the final result. I was in a state of suspense during the entire prolonged period that I was working out the details of this octamer-octamer junction, and I had no idea what it would look like in the end. This slide shows what it would look like. I've applied a generally-uniform color to each of 2 histone octamers, except for the H2A subunits by which we shall join them, which are left with the original turquoise and green colors, respectively. Let's join them.

Look at the structure of the octamers after being joined by carefully-constructed and geometrically-correct H2A-H2A hydrophobic bonds. *Is that amazing, or what?*

It would appear to be possible, in theory, for DNA, superhelically coiling around one of these octamers, to just continue the coil onto the next octamer, and for such a structure to continue from one end of the cell nucleus to the other.

But is such a thing possible in "real life"? After all the time I've spent on this, I'd still have to answer "Probably not". For starters, electron micrographic studies consistently show the so-called "beads on a string" appearance for chromatin, which means that there are spaces between histone octamers, the octamers being connected by so-called "linker DNA". I have no reason to doubt that these EM studies correctly portray the structure of chromatin. In fact, in Part II of this series, I'm going to show you a new histone structure which has the observed lengths of linker DNA between octamer cores.

So why did I go to such lengths to explore this fascinating but totally hypothetical dimer-of-octamers structure? Because (a) it teaches a number of basic principles of histone science very nicely, and (b) a structure as astonishingly plausible as this one may yet have a role in cellular metabolism which we cannot foresee at this time.

As to what that role might be, I cannot even guess. I only know that if someone else had discovered this, I would have hoped that they would show it to me, so I could judge its relevance for myself. Likewise, therefore, I'm showing it to you, so that *you* can judge for *your*self.

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SLIDE 178
Title slide
(No audio)

X-axis, y-axis and z-axis rotation of histone octamer

(movies)

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SLIDE 179

Well, go get your microwave popcorn, because we're going to the movies. The time has come to rotate the histone octamer core about the x-, y- and z-axes, so we can finally see what this thing actually looks like in 3 dimensions.

The first two views we shall examine, the x-axis rotation and the y-axis rotation, are of general interest only, but the 3rd view we shall see is critical to our understanding of histone binding to DNA, according to current theory, and also according to the somewhat novel theory we're going to present in Part II of this series.

We'll start with the first two "general interest" views. Here's what you see if you rotate the histone octamer core about the x-axis:

SLIDE 180

Hopefully, as it rotates, you'll recognize some of the sub-structures we've been looking at. This slide is not really necessary if you have virtual visualization software, but if you don't, you'll never be able to satisfactorily comprehend the 3-D form of the histone octamer without movies such as this one.

So let's just watch, as it rotates a bit more.

SLIDE 181

OK, let's spin it again, this time about the y-axis.

SLIDE 182

This slide shows a rotational sense we have employed several times previously. Twice in each rotational cycle, for just a split-second each time, you'll be able to see the long Helix II helices of the central H3-H4 tetramer perfectly aligned, appearing momentarily as a pair of parallel columns.

SLIDE 183

For our final rotation, we shall rotate about the z-axis, but if we do so from the current perspective of this slide, we won't learn a thing from that, since it will be like looking at a spinning bicycle wheel. See what I mean?! You just get dizzy looking at it! So we'll first perform a preliminary rotation of -90º about the x-axis, then do what *would have been* a z-rotation from that new position.

SLIDE 184

Here's the preliminary rotation about the x-axis.

SLIDE 185

Now let's do what would have been a z-axis rotation, which has now been converted into a y-axis rotation. This is going to be the most functionally significant of these three rotation movies, because it shows the pathway along the octamer surface, upon which DNA is believed to bind.

SLIDE 186

Here we see, at long last, the helical twist in the histone octamer, that I've been harping on all along, up until now. Because this spiraling structure is not homogeneous, but rather composed of long, linear segments, the helical nature of it may not leap out at you. It's not like the Watson-Crick double helix, which spins smoothly like a barber pole; the spiral structure here is jointed and robotic-looking.

SLIDE 187

The spiral is composed of 4 rectilinear *domains*, which connect with one another at angular turning points. These domains each consist of a pair of histone subunits which are cross-shaped in the frontal view. To remind ourselves of that, let's bring in another cameo of the frontal view, so that we can keep in mind exactly what we're looking at, as we study the side view in the center of the slide. Each of the 4 subunit pairs provides a distinct domain of DNA binding. For now, let's take a look at those 4 domains from the point of view of the protein architecture only. We'll get to the DNA later.

SLIDE 188

We've added a clear plastic tube to mark the spiral path of the helical protein structure. The first DNA-binding domain is the turquoise-and-pink H2A/H2B dimer at the top of the slide.

Following the spiral path downward, we next arrive at the yellow-and-brown H3/H4 dimer in the middle...

...then the other H3/H4 dimer, colored white-and-red, to the right...

...then finally the other H2A/H2B dimer, colored green-and-blue, in the lower left-hand side, at the rear of the structure.

SLIDE 189

If, perchance, you didn't clearly see the spiral structure the first time, here's the movie again. Keep your eyes fixed on the center of the screen, and you'll surely see it.

SLIDE 190

We are now in a position to understand the current concept of DNA binding to histones, for this binding is considered to take place along the spiral pathway we are now looking at. By so binding, the DNA takes on a tertiary superhelical twist, in addition to its Watson-Crick

secondary twist, wherefore this spiral pathway is referred to in the literature as either the ***"superhelical groove"***, or the ***"superhelical ramp"***.

The single main purpose of this entire slide presentation is the understanding of this superhelical pathway for DNA, so, without further ado, let's look at it.

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SLIDE 191
Title slide
(No audio)

<u>Superhelical ramp</u>

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SLIDE 192

This is the picture of the complete nucleosome that is shown in all of todays textbooks of molecular biology. I believe this is actually the first time in this presentation that we've looked at the whole thing, other than in atomic view, which is incomprehensible to the naked eye.

What we see here is 146 base-pairs of DNA, superhelically wrapped around the octamer core, about 1-3/4 superhelical turns in all. The pathway followed by the DNA along the octamer surface is referred to as the "superhelical ramp". What constrains DNA to follow this pathway? It's not doubted, by any author, that this is a charge-charge interaction. The negative charges are in the phosphate groups of DNA, and the positive charges are in the basic amino acid residues of that portion of the histone octamer, those residues being mostly lysine.

We now wish to look at the details of these salt bridges. In order to do so effectively, we're going to have to remove all parts of the DNA and protein that are *not* involved. We'll start by temporarily removing the DNA, so that we can examine the protein structures that comprise the superhelical ramp beneath it.

SLIDE 193

Here's our truncated octamer core yet again, slightly enlarged and centered.

Now, there is an exact structural motif which repeats 4 times in the histone octamer, and which is the site of DNA binding. We only need to look at one example of it, because the other 3 are exactly the same. Well...which one shall we look at? We've been tending to neglect the turquoise and pink H2A and H2B [g] and [h] chains, because they're hidden away in the rear of the structure from our current perspective, so why don't we honor them now, by using *them* to illustrate the DNA-binding motif?

Let's isolate them.

SLIDE 194

And here they are. Now let's enlarge and center them, and add some subunit labels.

SLIDE 195

We've seen this H2A-H2B dimer before, but we need to review the anatomy, at least that portion of the anatomy which is involved in DNA binding.

SLIDE 196

Our DNA-binding motif starts with Helix I, proceeds to β-Strand 1, then to β-Strand 2. Note we have not put labels on the long Helix II helices, or on their C-terminal neighbors, Helix III. That's because Helix II, as we have seen, is 56% hydrophobic, and is poorly-endowed with DNA-binding basic residues. As for Helix III, it has *some* basic residues, but, on the whole, it has significantly fewer than Helix I, and, more importantly, its interior location and unfavorable 3-D spatial orientation make it relatively unavailable for binding to DNA.

SLIDE 197

Here's a table I hope you won't find *too* boring, showing the *percentage* of basic residues in each of the above-referenced anatomical parts of the superhelical ramp, namely Helix I, β-Strand 1 and β-Strand 2.

We certainly don't need to discuss all these numbers, but I *would* direct your attention to the figures in the bottom row, which show that Helix I and β-Strand 2, at 35- and-36% respectively, are substantially-enriched in basic residues, although β-Strand 1, at 19%, is curiously lacking.

Overall, however, as shown in the very-last entry in the lower right-hand corner of the table, there are a grand total of 106 amino acid residues in the superhelical ramp, of which 32 are basic, giving an overall figure of 30% basic residues for the entire DNA-binding superhelical ramp.

Just to put this in perspective, the 8 histone N-termini, which, in Part II, I'm going to propose to be major DNA-binding domains, are *34%* basic. Protamine, as shown in our slide presentation on that subject, is considerably more, namely 50% basic, and moreover, unlike histone, where the predominant basic residue is lysine, most of the basic residues in protamine are the powerfully-basic amino acid *arginine.*

This establishes, in my opinion at least, a *hierarchy* of metabolic states as a function of amino acid sequence basicity. I would presume, for example, that a hypothetical protein-DNA complex whose protein component was ***100%*** basic residues would be an inert, lifeless crystal; at 50% basic residues, such as is found in protamine, we see a fluid-but-metabolically-inert storage environment, useful mainly for passing DNA from one generation to the next; and at about 1/3 basic, which we see in histones, you get an environment which also allows for long-term storage of DNA, but also for metabolic interaction with the myriad enzymes which give rise to the processes of somatic life.

In other words, the amino acid composition of the superhelical ramp is consistent with what would be expected for DNA binding, but slightly-less-so than that of the N-termini of the same histone subunits.

Moreover, as we shall see later, there are alignment issues in the superhelical ramp.

SLIDE 198

Let us return to our anatomical chart of the turquoise and pink H2A-H2B dimer. The labeled portions are going to be our binding motif, which will repeat for each of the four subunit dimers in the histone octamer. But...the angle at which we will be looking at these pairs of histone subunits, as we examine the surface of the superhelical ramp, is rather different from the angle shown here. Let's rotate it to *that* angle, so that we will be able to recognize the binding motif when we see it.

SLIDE 199

First we must rotate the structure 90º about its Y-axis.

SLIDE 200

Then we'll rotate it -120º about its X-axis.

This, now, is the position of each of the 4 histone subunit dimers, as you will see them in the upcoming view of the superhelical ramp. Before we look at that, let's do one more thing, to make this task as easy as possible. Let's remove the parts of this motif that don't actually bind DNA. Since we don't need to see Helix II, let's highlight it...

SLIDE 201

...and delete it.

SLIDE 202

Likewise, we really don't need to see Helix III either, so let's highlight that…

SLIDE 203

...and delete that too.

SLIDE 204

This, then, is the shape we're going to see for each dimer. That is, as we travel around the superhelical ramp, the spiral path that binds the nucleosome's 146 base pairs of DNA, we'll encounter this motif four times, once for each of the 4 dimers that comprise the histone octamer.

SLIDE 205

Just for a moment, let's review our labels, as they apply to this maximally-stripped-down view of the protein structure. Proceeding from bottom-to-top, the DNA will encounter the binding portions of the motif in the following exact sequence, starting with β-Strand 2 of the lower-numbered histone subunit; in this case, the turquoise-colored H2A:

First, H2A β-Strand 2...
then H2B β-Strand 1...
then H2B Helix I...

then H2A Helix I...
then H2A β-Strand 1...
then H2B β-Strand 2.

You may perhaps have noticed that the geometry at the top of the slide is a mirror-image of that at the bottom. There is a great deal of orderliness to this structure.

SLIDE 206

Let's return to the entire nucleosome, and get started. Once again, let's center it, and remove the random-coil N- and C-termini.

SLIDE 207

Now, as I've been saying, we don't need to include all elements of the structure to illustrate the salt bridges between DNA and histone, and in fact, most of it just gets in the way. So, for starters, let's remove all the protein components which are NOT involved in DNA binding, leaving only the maximally-stripped-down motif developed above.

SLIDE 208

All right, this is almost manageable. We need to do one more thing, which is to remove the DNA bases, which merely clutter the picture up, but have no role in protein binding, since the protein-binding function is entirely contained in the sugar-phosphate backbone.

SLIDE 209

There, that's better. Now we'll be able to see something. But we need to change the viewing angle. First we rotate the entire structure +90º around its Y-axis.

SLIDE 210

Next I'm going to rotate it upwards, like a water wheel on a Mississippi River steamboat, until we see the end of the DNA strand, so that we can have a reference point, from which to start looking at this structure.

SLIDE 211
(No audio)

SLIDE 212

OK, now we can see the end of the DNA duplex, at the left-hand side of the structure. Before looking at details, let's just first spin it about, so we can get a feel for what it looks like.

SLIDE 213

This movie is only to demonstrate the spiral arrangement of the DNA, as it superhelically wraps around the histone core. See if you can discern the spiral. I see it best when I focus my attention on the top of the structure as it whirls about. You won't be able to see much of the histone geometry beneath the DNA while it's moving, but we're going to slow it down in a moment.

SLIDE 214

It is essential that we keep in mind that what we are looking at here is exactly and precisely the same thing we looked at previously, when we used a clear plastic tube to demonstrate the octamer's spiral structure.

SLIDE 215

Here's that previous view. It looks very different from what we're looking at now, but it isn't really different at all.

SLIDE 216

The reason it looks different is that we've stripped down the protein to a minimum skeleton, rotated this structure 90º about the y-axis, and added back the DNA.

Let's return now to our current view.

SLIDE 217

Now we come to the crux of the matter. We're going to repeat the rotation of the nucleosome, but this time we're going to stop at each DNA-binding domain, to see the structure and placement of the underlying histone subunits. We'll start at the end of the DNA duplex, and wind positively around the X-axis.

SLIDE 218
(No audio)

SLIDE 219

Here we are at our first stop, H2A and H2B; the turquoise and pink [g] and [h] chains. They'll be much easier to see if we temporarily remove the DNA.

SLIDE 220

Remember this shape? This is the DNA-binding motif we spent so much time developing in previous slides. Perhaps now you can see why. The four occurrences of this motif are what fastens the DNA to the octamer core. We're going to look into even more detail later, at the individual basic residues which reside in these motifs, but for now we're only going to focus on the basic ribbon architecture.

OK, let's add back the DNA.

SLIDE 221

From this viewing angle, the turquoise subunit, H2A chain [g], looks apparently-well-situated to bind DNA, *but look at the pink subunit, H2B chain [h]*. Most of Helix I is nowhere near the DNA, and whatever basic amino acid residues are present within it, they are not likely to be able to form strong salt bridges with DNA phosphate oxygen atoms.

We shall now continue rotating the nucleosome, to look at the other 3 histone dimers that reside inside this DNA spiral. To facilitate the next steps in this examination, we shall now remove the turquoise and pink subunits, since we've already seen all we need to see of them, and in the subsequent steps they'll only get in the way.

SLIDE 222

Okay, that clears the field a bit. Now let's continue our rotation.

SLIDE 223
(No audio)

SLIDE 224

Here's our second stop, H3 and H4; the yellow and brown [e] and [f] chains. As previously, we'll temporarily remove the DNA.

SLIDE 225

And here we see the second occurrence of our DNA-binding motif, identical to the first except for the subunit color scheme. Let's add back the DNA.

SLIDE 226

At this viewing angle, the alignment with the DNA sugar-phosphate backbone is not as easily assessed for this subunit pair, but if you look closely, you'll see that here too the alignment is surely less than perfect. We'll be re-examining this whole question in atomic view shortly. For now let's remove these subunits, and continue our journey.

SLIDE 227

OK, the H3-H4 [e] and [f] chains are removed. Let's continue.

SLIDE 228
(No audio)

SLIDE 229

This is the third stop, the other instance of H3 and H4; the white and red [a] and [b] chains. Let's again remove the DNA.

SLIDE 230

This is the third occurrence of our DNA-binding motif, once again looking very much like the first two, except for the colors. Let's add back the DNA.

SLIDE 231

Again, the alignment with the DNA sugar-phosphate backbone is not entirely clear for this subunit pair, at this viewing angle, but if you look closely, you'll surely see that it's, at the very least, a bit off. Let's remove these subunits, and continue to the last leg of our journey.

SLIDE 232

The H3 and H4 [a] and [b] chains are now removed. Here's the final rotation.

SLIDE 233
(No audio)

SLIDE 234

This is the final stop, the other instance of H2A and H2B; the green and blue [c] and [d] chains. We'll remove the DNA...

SLIDE 235

...to reveal the 4^{th} and final occurrence of our DNA-binding motif., once again looking very much like the others, except for the coloring scheme. Let's add back the DNA.

SLIDE 236

As was the case with the other instance of H2A-H2B, we see again that the alignment with the DNA sugar-phosphate backbone is rather poor here. Let's remove these subunits, and, just for orientation's sake, let's fast-forward to the end of the DNA duplex.

SLIDE 237
(No audio)

SLIDE 238
(No audio)

SLIDE 239

This is the end of the 146-base-pair strand of DNA, which wraps around the 8-subunit histone octamer core to give us the complete nucleosome.

Next, as I've been saying for a while, we need to look a bit more closely at the contacts between the positively-charged basic amino acid Lysine and Arginine residues in histone, and the negatively-charged phosphate groups in DNA.

SLIDE 240
Title slide
(No audio)

Atomic view of histone-DNA contacts in the Superhelical Ramp

SLIDE 241

The time has come to look at the atomic details of superhelical ramp DNA-protein binding. We won't have time to look into it in too much detail, but just enough to illustrate the basic principles involved.

As usual, we'll start with the complete nucleosome.

SLIDE 242

We'll remove all the protein, except the 4 DNA-binding motifs.

SLIDE 243

For the moment, let's remove the DNA. We'll bring it back after we look at the atomic view of the underlying protein structure.

SLIDE 244

Let us now display, and highlight, all the lysine and arginine residues in these 4 DNA binding sites. There are exactly 60 such residues, distributed among the 8 histone subunits.

SLIDE 245

Now we're going to remove *everything* except the lysine and arginine R-groups; we'll even remove the peptide backbone, so that we can better-comprehend the positions of the positive charges that cause the DNA to be bound to the superhelical ramp.

SLIDE 246

And now let us remove the highlights, and color these Lys and Arg side-chains according to atom type. The positively-charged guanidinium group of a representative arginine residue, and the ε-amino group of a lysine residue, are each illustratively marked with a "plus sign".

If you now look carefully around the circular structure, you'll see that these positively-charged R-groups are everywhere, pointing every which way, with little order to be seen, other than that they very generally follow the circular shape.

Now let's add back the DNA, to see how it interacts with these basic R-groups.

SLIDE 247

From this perspective and viewing angle, you can readily see two things:

First of all, whereas the DNA is relatively neatly wrapped around the histone core, and has a relatively orderly appearance, the R-groups of the Lys and Arg residues to which it needs to bind are visibly haphazard, and distinctly devoid of any obvious order, pointing, as they are, in seemingly-random directions.

Secondly, we can now see that there is a concentric quality to the distributions of Lys and Arg residues. An outer circle appears, in this view, to be close to the DNA, but there is clearly what may be thought of as an inner circle of Lys-Arg residues which are quite obviously not very close to DNA.

In college chemistry, I was taught that an ideal salt bridge is about 3 Å in length; about the same size as a good hydrogen bond. In the structure before us right now, there are, in fact, a small number of 3 Å salt bridges between DNA and the basic residues of the outermost circle of the histone core structure shown. But most of the distances from basic R-groups to DNA phosphates are far larger than 3 Å, many being in the 5-10 Å range, which would afford only weak, relatively ineffectual ionic bonding of protein to DNA.

SLIDE 248

Ladies and gentlemen, the entire purpose of our slide show is to present this current view. The Second Law of Thermodynamics teaches that nothing happens by mistake, but rather in accordance with a relentless force of nature, which causes an attribute of the universe, called the Free Energy, to *decrease.* What we see here is a structure maintained by exactly 60 salt bridges, most of which are rather poor, being 2-3 times the optimal length. This means that the attractive forces between the members will only be about 10-25% the attractive force of a properly-constructed salt bridge.

The question we are going to raise next is whether we can reasonably expect to see the structure shown in this slide, if *another* structure; one which is thermodynamically *more favorable,* exists and can readily form.

SLIDES 249-260 (END)

NOTE: THE FOLLOWING NARRATION IS ENTIRELY LOCATED IN SLIDE 249, BUT PLAYS OVER MULTIPLE SLIDES, FROM 249-260 (THE END). IF YOU REPLAY ANY OF THOSE SLIDES IN ISOLATION, IT WILL BE SILENT. ITS AUDIO CAN ONLY BE HEARD BY RETURNING TO SLIDE 249, AND PLAYING THE FINAL SEQUENCE OF SLIDES, 249-260, OVER AGAIN.

The next part of this series is entitled "Histone Structure, Part II: A model which places DNA in the N-terminal region of the octamer". In it, I'll show you a new model in which there are more than 100 salt bridges between protein and DNA, each salt bridge being of perfect design and length.

The question then before you will be: ***"Why should DNA bind superhelically to a structure that has 60 poor salt bridges; bridges of random shape and size, mostly too long to be effective; when DNA can, instead, bind linearly to a structure which has over 100 salt bridges, each of which is perfect in size and shape?"***

The structure we present in Part II is based upon the structure of protamine. I regard the protamine structure, which is reviewed extensively in another slide show on this web site, to be incontrovertible. It is, moreover, the only detailed model for protamine that has ever even been suggested, and not a single word of protest has been publicly posted since it was published in 2006.

The new histone structure, however, is *not* incontrovertible. For one thing, like the protamine structure, it is not so much a single structure as a *type* of closely-related structures, and, in our current state of knowledge, it's impossible to say which one is best. But, in the case of protamine, we can say with certainty that the *type* of structure is correct, even though we may not be able to precisely adjudicate issues such as small variations of phi and psi angles.

In the case of histone, however, the correctness of our new structure depends entirely upon the correctness of the octamer core structure upon which it is built. The Kornberg-Luger histone core is found in essentially all textbooks on earth at this point: If it is correct, then our modified *type* of new histone structure is almost certainly also correct. But if, G-d forbid, the Kornberg structure turns out to be a grand laboratory artifact, then all bets are off. In that case, G-d only knows what the truth is.

SLIDE 250

[NOTE: Audio for this slide is on Slide 249, which plays over Slides 249-260]

... "Histone Structure, Part II: A model which places DNA in the N-terminal region of the octamer".

SLIDE 251

[NOTE: Audio for this slide is on Slide 249, which plays over Slides 249-260]

In it, I'll show you a new model in which there are more than 100 salt bridges between protein and DNA, each salt bridge being of perfect design and length.

The question then before you will be...

SLIDE 252

[NOTE: Audio for this slide is on Slide 249, which plays over Slides 249-260]

..."Why should DNA bind superhelically to a structure that has 60 poor salt bridges; bridges of random shape and size, mostly too long to be effective; when DNA can, instead, bind linearly to a structure which has over 100 salt bridges, each of which is perfect in size and shape?"

SLIDE 253-254

[NOTE: Audio for this slide is on Slide 249, which plays over Slides 249-260]

The structure we present in Part II is based upon the structure of protamine. I regard the protamine structure, which is reviewed extensively in another slide show on this web site, to be incontrovertible. It is, moreover, the only detailed model for protamine that has ever even been suggested, and not a single word of protest has been publicly posted since it was published in 2006.

SLIDE 255

[NOTE: Audio for this slide is on Slide 249, which plays over Slides 249-260]

The new histone structure, however, is *not* incontrovertible. For one thing, like the protamine structure, it is not so much a single structure as a *type* of closely-related structures, and, in our current state of knowledge, it's impossible to say which one is best.

SLIDE 256

[NOTE: Audio for this slide is on Slide 249, which plays over Slides 249-260]

But, in the case of protamine, we can say with certainty that the *type* of structure is correct, even though we may not be able to precisely adjudicate issues such as small variations of ϕ and ψ angles.

SLIDE 257

[NOTE: Audio for this slide is on Slide 249, which plays over Slides 249-260]

In the case of histone, however, the correctness of our new structure depends entirely upon the correctness of the octamer core structure upon which it is built.

SLIDE 258

[NOTE: Audio for this slide is on Slide 249, which plays over Slides 249-260]

The Kornberg-Luger histone core is found in essentially all textbooks on earth at this point: If it is correct, then our modified *type* of new histone structure is almost certainly also correct.

SLIDE 259

[NOTE: Audio for this slide is on Slide 249, which plays over Slides 249-260]

But if, G-d forbid, the Kornberg structure turns out to be a grand laboratory artifact, then all bets are off. In that case, G-d only knows what the truth is.

SLIDE 260

(No audio)

(The End)