Slide 1. The common ancestor of Archaebacteria and Eukaryotes evolved molecules that served to compact its genome. These molecules are small basic proteins called histones. Histones are widespread among Archaea and belong to the genetic information of all Eukarya. We may therefore assume that the possession of at least one histone gene belongs to the synapomorphies of Archaea and Eukarya. In its most simple organization histones form a homo-tetramer around which DNA is wrapped in about one turn. The diversification of histone genes led to the evolution of the histone hetero-octamer, a derived, apomorphic character of the Eukarya. The histone octamer allowed even higher degrees of genome compaction.

Slide 6. For details see Luger et al. (1997)

Slide 9. Models for the 30 nm fiber are grouped into two classes. (i) The one-start helix with bent linker DNA connecting neighboring nucleosome cores, which are arranged along a helical path with 6-8 nucleosomes per turn. (ii) The two-start helix with straight linker DNA, derived from an “open” zig-zag arrangement of nucleosomes. Two two-start models are distinguished, (a) the helical ribbon model, and (b) the crossed linker model.

Slide 10. The histone H4 gene was expressed under control of the inducible GAL1 promoter, which is active in galactose-containing media and repressed in glucose-containing media. A shut down of H4 synthesis arrests the cells in G2, depleting nucleosomes about two-fold. The remaining nucleosomes are irregularly spaced as indicated by the near complete loss of nucleosomal ladders after partial Micrococcal nuclease digestion (left panel).

The PHO5 promoter, which becomes transcriptionally active in media lacking inorganic phosphate (406 (wt-H4)), was used to express the E. coli gene beta-galactosidase gene LacZ. Note that shutting down expression of H4 (switch from Gal to Glu) activates the PHO5 promoter under repressing conditions to a significant level. (401 (GAL-H4)). Deleting the UAS leads to loss of PHO5 promoter activity under activating
conditions in wt cells (407 (wt-H4), 424 (wt-H4), whereas activation by histone depletion does not require the upstream regulatory sequences (402 (GAL-H4), 423 (GAL-H4)).

**Slide 11.** Transcription perturbs chromatin structure in the absence of Spt6 function. FLO8 is transcribed under the experimental conditions, whereas GAL1 is not. The alleles spt6-1004 and rpb1-1 (encodes subunit of RNA pol II) are temperature sensitive. A shift from 30 to 39 degrees Celsius inactivates the mutant proteins.

**Slide 12.** Perturbation of chromatin structure over transcriptionally active loci in the absence of SPT6 function allows for transcription initiated at cryptic core promoters within the ORF.

**Slide 15.** The nucleosome changes geometric properties of the DNA double helix, namely its twist and writhe. As we shall see, the sum of twist and writhe is a topological property for circular DNA molecules. DNA circles, therefore, retain a “memory” of nucleosome structure even after the histones have been stripped off the DNA. This allows us to measure the extent of nucleosome formation on circular DNA molecules as a property of the DNA molecule itself.

**Slide 16.** Writhe provides a measure for how much a curve is contorted in space. Imagine a space curve $\gamma$ and its projection into a plane with normal vector $e$, the normal vector is perpendicular (orthogonal) to the plane. We now inspect self-crossings of the projection of $\gamma$ in the plane ($\gamma^e$). We can assign to each crossing $j$ a section that is the overcrossing and a section that is the undercrossing, and we assign a $+1$ or $-1$ to each crossing in the manner indicated at the bottom of the slide. The sum of these assignments is called the directional writhing number of $\gamma$ in the direction $e$. Obviously there are as many projection planes as there are points on the surface of the unit sphere. The integral of the directional writhing number over the unit sphere divided by $4 \pi$, the surface area of the unit sphere, gives us the average directional writhing number, where the average is taken over all possible directions of projection. This average is called the writhe of the space curve $\gamma$.

**Slide 17.** Conceptually related to the directional writhing number is the linking number. Imagine two oriented closed space curves (the orientation is indicated by an arrow) and
their normal projection into any plane, for example the plane of this slide. We call the pair of closed space curves a 'link'. Crossings of the two space curve projections can be distinguished according to their handedness. We use the same sign convention to assign +1 or -1 to each crossing that was introduced for defining the directional writhing number. Note however, that here we consider crossings between two distinct space curves rather than self-crossings of the same curve. We define the linking number, \( L_k \), as the sum of all crossing numbers divided by two. In contrast to the directional writhing number, the linking number does not depend on the choice of the projection vector! Indeed it can be shown that any continuous deformations of the link does not change the linking number. This result is intuitively amenable. Its prove, however, requires advanced mathematical instruments.

**Slide 18.** The two phosphodiester strands of the DNA double helix “twist” about the helix axis. To capture the essence of the twist idea we define the unit (length) tangential vector of the helix axis \( T \) and a vector field \( v \) on the curve of the axis gamma, where \( v \) is perpendicular (orthogonal) to \( T \) at each point of the axis pointing toward one of the two phosphodiester strands. As we follow gamma, \( v \) rotates about \( T \) defining the surface of a “twisted” ribbon. The ribbon serves as our geometric model for the DNA molecule.

**Slide 19.** The two boundaries of a closed ribbon represent two closed space curves. We define the linking number of the ribbon as the linking number of the ribbon boundaries, where the boundaries carry the same orientation as the ribbon axis. The picture shows a ribbon that had been wrapped around a cylinder in a left-handed helical path of complete 1 turn. The ribbon was closed such that the protruding loop is relaxed and the cylinder was removed. To determine the linking number we may transform the ribbon in any suitable conformation, for example the conformation shown on the right. Normal projection of this conformation into the plane of the slide allows to readily discern the linking number of the ribbon, which is -1.

**Slide 20.** The famous linking theorem states that the linking number of a ribbon is equal to the sum of its twist and writhe. The theorem is intuitively accessible, but its proof requires advanced mathematics. The linking number of the ribbon shown is -1. The twist of the conformation shown in the left panel is small, its writhe is therefore close to -1. Since the axis of the right panel conformation lies in a plane, its writhe must be zero.
Its twist is therefore -1. Transformation of the left panel conformation into the right panel conformation is accompanied by an exchange between writhe and twist, which is a reflection of the fact that their sum is a topological invariant, the linking number.

**Slide 21.** In the presence of topoisomerase activity, the linking number of a DNA molecule is changing constantly. Topoisomerase I, for example, introduces single strand breaks in the DNA molecule and reseals the breaks again. A phosphoester bond in the sugar phosphate backbone is intermittently exchanged for a phosphoester bond with a tyrosine residue of the enzyme which preserves the free energy of the phosphoester bond. Thermal fluctuation translate into rotations about the non broken phosphoester bond in the complementary strand. Resealing the bond after 1, 2 or more turns yields a DNA circle with altered linking number.

Clockwise and counterclockwise rotations occur with equal probability as indicated by the symmetrical linking number distribution of a population of circular DNA molecules treated with topoisomerase. We refer to DNA circles that differ only in their linking number as topoisomers. DNA molecules resist changes in twist and thus translate a change in linking number mostly into a change in writhe. Thus the more the linking number of a DNA molecule differs from the linking number of the relaxed circle, the more positively, or negatively it will be supercoiled and the more compact the molecule will be. Topoisomers with a linking difference to the fully relaxed topoisomer of either + n or - n exhibit the same degree of “compactness”. Nonetheless they can be separated from each other. The result of such a fractionation is shown on the right. Each peak represents a topoisomer, and the peak height indicates the abundance of the topoisomer. If the left most visible topoisomer peak represents topoisomers with linking number i than peaks to the right represent topoisomers with linking number i+1, i+2, etc.

(Imagine the nicked circle. In the state of least free energy, the two ends of the broken phosphoester bond may not oppose each other perfectly. We denote with $\theta$ the angle by which the two parts of the molecule on either side of the non-broken bond in the complementary strand can rotate relative to each other, where $\theta = 0$ for the least free energy conformation. Assuming, the free energy of rotation linearly depends on $\theta^2$, we expect a Gaussian probability density $P(\theta)$ for the continuous random variable $\theta$.

(Remember that $\Delta G^0 = -RT \ln \frac{c_i}{c_j}$, where $c_i$ and $c_j$ are the abundances of any two topoisomers i and j and $\Delta G^0$ is the Gibbs Free Energy change associated with the transition from j to i). I will refer to $P(\theta)$ as the “Gaussian envelop function” of the
topoisomer distribution. Since \( \theta \) can assume only discrete values if we require the phosphoester bond to be closed, we may imagine the topoisomer distribution as the result of sampling at discrete values of \( \theta \). Topoisomer distributions indeed obey \( \Delta G^0 = K\theta^2 \). See Depew and Wang (1975).

**Slide 22.** Hydrophobic planar compounds like ethidium are squeezed by the aqueous solvent between bases of the DNA helix. To accommodate the ethidium molecule the DNA has to unwind. This changes the DNA twist. Since the linking number remains constant, the change in twist is compensated by an equal change in writhe of opposite sign.

**Slide 23.** Imagine now a negatively supercoiled DNA circle. By adding ethidium to the solvent we can gradually increase the writhe, first titrating out negative supercoils and eventually adding positive supercoils. It is thus possible to electrophoretically resolve a population of topoisomers with positive and negative supercoils. Imagine three circles, one relaxed circle, and two additional circles, one with a negative supercoil and one with a positive supercoil. The positively and negatively circles are equally compact and cannot be separated from each other electrophoretically. However, by adding ethidium to the solvent we can add one positive supercoil to each topoisomer of the distribution. This will relax the negatively supercoiled circle, positively supercoil the previously relaxed circle and add an additional positive supercoil to the positively supercoiled circle, which now contains 2 positive supercoils. The three circles have now different conformation that are separable by gel electrophoresis.