Faculty of science

University of Split

DNA condensation in vitro and in vivo

Mentor: prof.dr.sc. Rudolf Podgornik

Student: Lily Chowdhury

TABLE OF CONTENTS

1. INTRODUCTION

Deoxyribonucleic acid or simply known as DNA is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms and many viruses. Most DNA molecules are double strand helices that consist of two long biopolymers which are made of simpler units we call nucleotides. Each nucleotide is composed of a nucleobase guanine, adenine, thymine and cytosine (G, A, T and C respectively) as well as a backbone made of sugars deoxyribose and phosphate groups. DNA diameter is about 2 nm, while the length of a stretched single-molecule may be up to several dozens of centimeters depending on the organism. Genomic DNA is a very long molecule, which must fit into a very small space inside a cell or virus particle. DNA condenses when packed into bacteria, eukaryotic nuclei, and viruses. DNA condensation and decondensation are involved in gene expression, chromosomal changes during the cell cycle, and in the delivery of genes in gene therapy. DNA condensing into chromosomes is shown simplified on Figure 1.

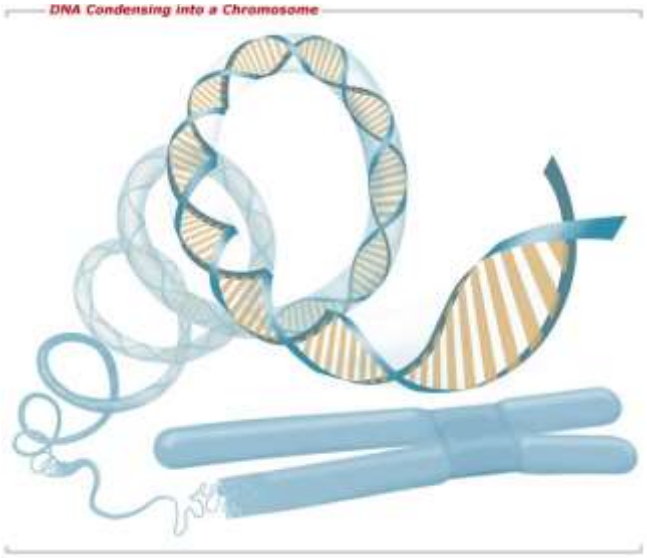

Figure 1. DNA condensing into a chromosome

If fully extended, the 160,000 base pairs of T4 phage DNA span 54 µm but condensed the T4 DNA molecule has to fit in a virus capsid about 100 nm in diameter, a 540-fold linear astounding compression. The space available for the DNA in vivo is much smaller than the space that it would occupy in the case of a free diffusion in the solution. In order to cope with the volume constraints, DNA has a striking property to pack itself in the appropriate solution conditions with the help of ions and other molecules. Just as file drawers help conserve space in an office, DNA condensation helps conserve space in cells. Packaging is the reason why the approximately two meters of human DNA can fit into a cell that is only a few micrometers wide. DNA condensation is defined as “the collapse of extended DNA chains into compact, orderly

particles that contain only a few molecules" (Bloomfield, 1997). The definition applies to many situations in vitro and is also close to the definition of DNA condensation in bacteria as "adoption of relatively concentrated, compact state occupying a fraction of the volume available" (Zimmerman and Murphy, 1996). In the condensed state, DNA helices may be separated by just one or two layers of water.

2. DNA CONDENSATION IN VITRO

2.1. Condensing agents

A condensing agent is a chemical compound which acts as a catalyst and also enters into the polycondensation reaction. DNA condensation can be induced in vitro by applying external force to bring the double helices together or by inducing attractive interactions between the DNA segments. Condensing agents generally work by decreasing repulsions between DNA segments, for example neutralizing phosphate charge, or by making DNA-solvent interactions less favorable, like adding ethanol, which is a poorer solvent than water for DNA. Multivalent cations may also cause localized bending or distortion of the DNA, which can also facilitate condensation. The forces that can also be used to condense DNA by pushing the double helices together are coming from entropic random collisions with the condensing polymers surrounding DNA condensates, and salt is required to neutralize DNA charges and decrease DNA-DNA repulsion. Most typical DNA condensing agents are either neutral polymers such as polyethylene glycol plus Na^+, or multivalent counterions (ligands). In water solutions, DNA condensation usually requires counterions with charge 3^+ or higher (Bloomfield, 1997). Among typical ligands used in experiments are trivalent metal ions and inorganic cations such as $Co(NH_3)_6^{3+}$, naturally occurring polyamines and their analogs protamines, natural and synthetic peptides, lipids and liposomes, bacterial nucleoid-associated proteins and eukaryotic chromatin proteins. Monovalent counterions such as Na^+ cannot induce DNA condensation unless under additional osmotic pressure exerted by neutral polymers such as polyethylene glycol (PEG). Divalent metal ions provide a boundary case. They cannot induce condensation of linear DNA molecules in water solutions, but they can do so in the presence of lipids that partition DNA molecules in lamellar sandwich-like structures (Mengistu et al., 2009).

Not all metal ions of the same charge are equally effective: transition metals such as Mn^{2+}, which can interact both with DNA phosphates (electrostatically) and bases (e.g. forming chelate complexes) are stronger condensing agents in comparison with alkali metals, which can interact only with DNA phosphates. Temperature elevation can help aggregate DNA in the presence of divalent ions. A recent study has shown a reversible condensation of T4 genomic DNA in solutions of poly(N-isopropylacrylamide) upon a temperature increase from 30 to 35 °C (Chen et al., 2010).

2.2. Morphology of condensed DNA

DNA molecules have been observed to condense into many different forms. When condensation is induced by a careful addition of polyamines or $Co(NH_3)_6^{3+}$ to very dilute aqueous DNA solutions, toroids and rods are the structures most commonly observed by electron microscopy (Arscott et al.1990), toroid structure is shown in Figure 2. This choice of rodlike over toroidal morphology may rest on the nonpolarity of the solvent or condensing agent. Flowerlike structures and globular structures are also a few of the condensed DNA structures commonly observed. Globular structure is shown in Figure 3. Both single and multiple strands may be involved in each aggregate.

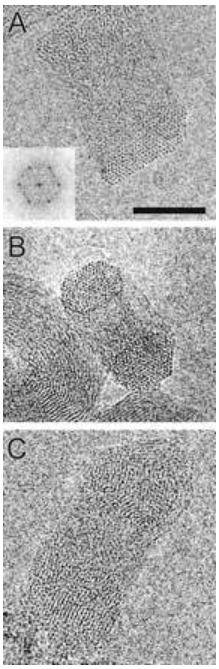

Figure 2. Cryoelectron micrographs of λ DNA toroids with the plane of the toroid oriented ≈90° with respect to the microscope image plane; edge-view toroid images.

(A) A toroid where the hexagonal packing of DNA helices is clearly apparent in the outer regions. (Inset) Fourier transform of image region containing the highly ordered DNA lattice.

(B) A toroid for which the outer regions are well-defined hexagons.

(C) A toroid in which the outer regions appear to have DNA packed in a nonhexagonal lattice. (Scale bar is 50 nm. All micrographs are shown at the same magnification.)

(Hud N V , and Downing K H PNAS 2001;98:14925-14930)

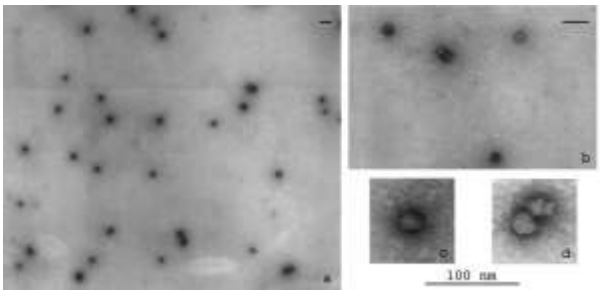

Figure 3. Transmission electron microscopy of an unfiltered solution of cationic lipid/DNA particles showing a very homogeneous population (*a*) of spheres (*b*) with a diameter of ≈23 nm (*c, d*). Bar = 100 nm. Similar results were obtained with complexes observed in 0.15 M NaCl.
(Dauty E, Remy JS, Blessing T, Behr JP. J Am Chem Soc. 2001 Sep 26; 123(38):9227-34)

DNA condensates are stable within a large interval of condensing ligand concentrations. If the ligand concentration continues increasing, a reverse transition in the DNA condensation occurs when the aggregates resolubilize. In the case of a long DNA, resolubilization is associated with decondensation of individual molecules. Reentrant condensation has been observed for short or long DNA molecules, single- or double stranded DNA, small or high DNA concentrations. The dimensions and morphology of condensed DNA particles depend greatly on the size of the DNA. Widom and Baldwin (Widom & Baldwin, 1980) discovered that DNA fragments that are shorter than about 400 base pairs will not condense into orderly, discrete particles. This shows that the net attractive interactions per base pair are very small and at least several hundred base pairs must interact.

3. PHYSICS IN DNA CONDENSATION

3.1. Polymer coil-globule transition

DNA condensation is an example of a polymer coil-globule transition. Different then protein folding, the general features of the DNA coil-globule transition like topology of the condensate are mostly determined by the average polymer and solution properties such as DNA length, concentration, solution temperature and content, etc, and not by the DNA sequence. DNA sequence determines the local interaction and recognition between the double helices in the condensed DNA.

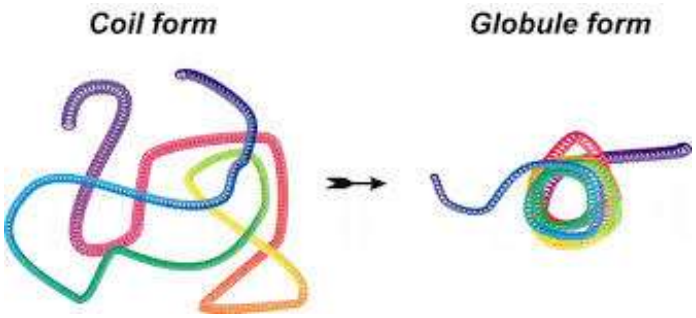

Figure 4. Simplified coil-globule transition

DNA is a very stiff polymer, and the coil-globule transition is abrupt for such polymers. High stiffness of the DNA double helix is due to both its internal properties (sugare-phosphate backbone with stacked base pairs) and its charge (like-charged phosphates repel each other and resist DNA bending). Post and Zimm who first discovered DNA condensation (Post and Zimm 1979) based their approach to the problem on the familiar Flory-Huggins lattice theory of polymer solutions, but they also included third virial coefficient effects so it would consider the high local concentration of polymer segments in the condensed phase. The Flory-Huggins

solution theory is a mathematical model of thermodynamics of polymer solutions which takes account of the great dissimilarity in molecular sizes in adapting the usual expression for the entropy of mixing. The result is an equation for the Gibbs free energy change ΔG_m for mixing a polymer with a solvent. Post and Zimm get their equation for the total mixing free energy ΔG of polymer and solvent:

$$\frac{\Delta G}{k_B T} = \frac{\Delta G_{ext}}{k_B T} + n_2 \frac{\Delta G_{int}}{k_B T} \quad (1)$$

Where k_B is the Boltzmann constant, ΔG_{ext} is the external free energy of mixing polymer and solvent molecules and T is the temperature.

$$\frac{\Delta G_{ext}}{k_B T} = n_1 \ln v_1 + n_2 \ln v_2 + \chi n_1 v_2 \quad (2)$$

ΔG_{int} is the internal free energy of dissolution of a single polymer chain.

$$\frac{\Delta G_{int}}{k_B T} = N\left[(\chi - 1) + \frac{B_2 \omega}{2^{3/2}\alpha^3} + \frac{B_3 \omega^2}{2 \cdot 3^{5/2}\alpha^6} + \frac{3}{2}(\alpha^2 - 1) - \ln \alpha^3\right] \quad (3)$$

In the equations (2) and (3) n_1 and n_2 are the numbers of solvent and polymer molecules, and v_1 and v_2 are the volume fractions of solvent and polymers. N is the ratio of molecular volume of polymer to solvent. The expansion parameter α is the ration of the polymer radius of gyration to that in the uncondensed state with no intramolecular excluded volume (α in the condensed state is «1). B_2 and B_3 are the second and third virial coefficients, which are defined in the terms of χ. The difference in interaction free energies of like and unlike species, meaning through the second and third virial coefficients are:

$$B_2 = \frac{1}{2} - \chi \quad (4)$$

$$B_3 = 1 + 12\frac{\chi^2}{q} - 16\frac{\chi^3}{q^2} \quad (5)$$

where q is the lattice coordiation number. The parameter ω is defined as

$$\omega = \left(\frac{9}{\pi \langle h_0^2 \rangle}\right)^{3/2} V_p \quad (6)$$

In which $\langle h_0^2 \rangle$ is the mean-square end-to-end distance in the unperturbed state and V_p is the molecular volume of the polymer. ω/α^3 is the volume fraction of the polymer in the solution. The key parameter χ is the difference in free energy between solvent-solvent, polymer segment-segment, and solvent-segment interactions. Equations for ΔG can be differentiated with respect to n_1 and n_2 in order to obtain the chemical potential μ_1 and μ_2 of solvent and polymer. If $\chi > 1/2$ the chemical potentials are no longer monotonic functions of polymer concetration, indicating coexisting phases. Boundaries of the phase diagram depend on DNA molecular weight, but three regions occur: extended random coils in solutions, collapsed DNA in dilute solution and concentrated precipitate ("aggregated"). The aggregated phase is assumed to be stable except in very dilute solutions. The extended-collapsed coil transition is predicted to be discontinuos due to the stiffnes of DNA, but the transition should appear macroscopically diffuse because individual molecules will collapse independently.

3.2. Toroid structure

A theory why DNA condensed formes a toroid structure was given by Grosberg and Zhestkov (Grosberg and Zhestkov 1986) explaining that the total free energy of DNA is the sum of compressive, repulsive and elastic contributions. Compressive free energy is either because of external osmotic pressure or poor solvent quality. The repulsive term comes from the excluded volume of the DNA, hightened by its high asymmetry. The elastic contribution comes from bending and other conformational entropy terms. Importance of these free energy terms also depend on polymer chain length. Phase diagram shows that short DNA molecules will form toroids, while very long molecules will form spherical globules. Bigger compressive forces prefer spherical globules without an inner hole, while greater stiffnes and excluded volume will favour toroids. Rodlike particles are sometimes also seen, but rarely in high proportion unless the solvent or the condensing agent is nonpolar. Seems like nonpolar enviroment lowers the free energy of exposed heterocyclic bases, favouring sharp local kinking.

3.3. Intermolecular free energy experimental estimates

The magnitude of the attractive free energy holding the molecule in the condensed state can be experimentally estimated. Simplest way is by remembering that DNA molecules smaller than 400 base pairs don't form ordered and compact particles. We assume that the total attractive energy must be at least an order of magnitude greater than the thermal energy so that it could maintain side-by-side bimolecular complex, then we could estimate that the attractive free energy per base pairs is:

$$10\ k_B T/400\text{bp} = 1/40\ k_B T/\text{bp} = 0.06\ \text{kJ/mole bp} = 0.015\text{kcal/mole bp}$$

The best way would be to measure it directly, which can be done using the osmotic stress technique with a thermodynamic analysis. DNA in an ordered fibre is equilibrated with a reservoir containing water and ions that can exchange, and a large inert polymer that can't exchange. Distance between DNA molecules is measured from Bragg spacing in x-ray diffraction, we assume hexagonal packing. Osmotic pressure is controlled with the polymer concentration. Because stress is a force per unit area, a measured osmotic stress can be converted to a force between surfaces, so we get osmotic stress vs distance curves.
Force per unit length on a DNA molecule at a distance *R* from its neighbours with π being the osmotoc stress or pressure is:

$$f = \frac{R\pi}{\sqrt{3}} \quad (7)$$

The $\sqrt{3}$ comes from the hexagonal packing of the double helices.
Thermodynamic analysis starts with the relation between free energy, temperature and pressure, but using the osmotic pressure π instead of external pressure *P*.

$$dG = -SdT + Vd\pi \quad (8)$$

We differentiate both sides with respect to T and π and get the Maxwell relation:

$$\left(\frac{\partial S}{\partial \pi}\right)_T = -\left(\frac{\partial V}{\partial T}\right)_\pi \quad (9)$$

This we can integrate and get entropy change which comes along with the change of osmotic stress:

$$\Delta S = -\int \left(\frac{\partial V(T,\pi)}{\partial T}\right)_\pi d\pi \quad (10)$$

In the region of abrubt change due to packing transition, we use a variant of the Clausius-Clapeyron equation.

$$\Delta S = -\left(\frac{d\pi_{trans}}{dT_{trans}}\right)\Delta V \quad (11)$$

The total entropy change as a function of helix spacing is a sum of the continuous and transition contributions. The total free energy change equals the work done on the system by changing the array spacing at constant osmotic pressure:

$$W = \Delta G = -\int \pi dV \quad (12)$$

The enthalpy change accompanying the approach of the DNA helices to their equilibrium distance is:

$$\Delta H = W + T\Delta S \quad (13)$$

Using this analysis we can be the free energy minimum at the equilibrium center-to-center separation of 28Å between DNA helices in 20mM $Co(NH_3)_6^{3+}$ and 0.25M NaCl is -0.14k_BT/bp, and also that a change of 0.20 ions bound per base pair accompanies a spontaneous condensation transition.

3.4. Hydration forces

Because double helices come very close to each other in the condensed phase, water molecules are restructured and we get so-called hydration forces. It can be attractive or repulsive. Repulsive component is short ranged, decaying, independent of ionic strenght and similar in behaviour for all types of molecular systems examined. Each water molecule represents a dipole that can be oriented in the electric field perturbed by the neighbouring surface of the DNA. That ordering decreases exponentially with the distance from DNA surface. Dipols in the solution orient themselves perpendicular to the charged surface. Leikin et al (Leikin et al 1991) state about hydration force magnitude that: "Hydration force magnitudes depend on the strenght of surface water ordering, while the decay length and sign, attraction or repulsion, depend on the mutual structuring of water on the two surfaces. Attraction results from a complementary ordering, while repulsion is due symmetrical structuring.". Hydration forces are very difficult to predict from first principles, since they depend on summation of hundreds or more of very weak intermolecular interactions. Equation to predict a repulsive hydration pressure between two similar, homogeneous planar surfaces separated by a distance *h* is given by:

$$P_{rep}^{homo} = \frac{R}{\sin h^2\,(h/2\lambda_w)} \approx 4Re^{-\frac{h}{\lambda_w}} \quad (14)$$

And the equation for an attractive pressure between two complementary surfaces:

$$P_{attr}^{homo} = -\frac{A}{\cos h^2\left(\frac{h}{2\lambda_w}\right)} \approx 4Ae^{-\frac{h}{\lambda_w}} \quad (15)$$

In the equations (7) and (8) *R* and *A* are coefficients and λ_w is the water correlation lenghts (approximately 4-5Å). This is the value observed for Na^+ as counterions.

In addition to water restructuring, the counterions dissolved in the water also rearrange in the vicinity of the charged DNA molecule, which gives rise to the counterion-correlated attraction.

3.5. Counterion condensation

DNA is a highly charged molecule, which cannot exist in solution without other ions. Although the abbreviation "DNA" means deoxyribonucleic acid, usually DNA comes as a salt of Na^+ or other alkali metals. Physiological solutions also contain large amounts of divalent metal ions, which are essential for functioning of enzymes and play structural roles in DNA, RNA and proteins. Finally, the DNA is surrounded by a variety of other charged molecules, from small polyamines to proteins.

Most electrostatic approaches treat DNA counterions as point-like, sphere-like or rod-like, but real multivalent ions and molecules possess an internal structure with spatially distributed charges. The simplest examples of such ions are spermidine3+ and spermine4+ which are naturally occurring polyamines and flexible linear molecules widely used as DNA condensing agents. The distribution of multivalent ions in aqueous solutions close to the macroion is mainly determined by the competition between electrostatic interactions within the system and the entropy of the constituents in the solution. At a thermodynamic equilibrium the counterions are attracted to the charged surface, while co-ions are depleted from this region, and a diffuse electric double layer is created. The Poisson-Boltzmann (PB) theory (Evans and Wennerstrom 1994) takes charges as point-like, interactions considered are the Coulombic interactions. Surfaces are uniformly charged. The aqueous solution is considered as a continuous medium with the dielectric constant of 78. The charge correlations and short-range interactions between charged particles are neglected. Distribution of ions is given by thr competition between the electrostatic interactions and the entropy of the ions in the solution which tends to scatter them. The electrostatics of the system is described by the Poisson equation and the ion number densities in the solution follow the Boltzmann distribution. From that we get the Poisson-Boltzmann equation for the electrostatic potential. The downside of the PB theory is that it does not predict attractive interactions between like-charged surfaces.

3.6. Counterion correlations

To understand DNA-DNA attraction we have to consider correlations between counterions. A very simple interpretation of the mechanism that leads to attraction can be given at zero temperature where counterions condense onto the charged surface. An alternation of positive and negative charges at the surface has been produced by Coulomb repulsion. Two of such opposing patterns adjust complementarily to each other and we have short range attractive forces.

3.7. Bending free energy

Consider a length *L* of DNA with persistence length *a* being bent in a path with curvature radius R_c, the bending free energy is given by:

$$\Delta G_{bend} = \frac{RTaL}{2R_c^2} \qquad (16)$$

Inside a condensed particle, R_c changes from point to point,being less inside of a toroid than at the edges, so a mean value is used. The equation provides an order of magnitude estimate of the energetic cost of bending. Usualy the radius of curvature of the DNA in a toroid is on the order of the persistence length $R_c \approx a$. If we consider a length of DNA equal to one persistence length L = a, then $\Delta G_{bend} \approx RT/2$. Due to the persistence length of DNA containing 150 base pairs, this corresponds to 1/300 k_BT per base pairs.

3.8. Mixing entropy

When DNA, DNA being a polymer, undergoes a coil-globule transition entropy is lost because of the demixing of polymer and solvent. This is given by the first two terms on the right within the equation:

$$\frac{\Delta G_{ext}}{k_B T} = n_1 \ln v_1 + n_2 \ln v_2 + \chi n_1 v_2 \qquad (17)$$

If we neglect the complications of the stiffnes and χ value, we get a simple approximation:

$$\Delta G_{mix} = -T\Delta S_{mix} = RT\frac{L}{a} \qquad (18)$$

This is equal to about 1/150 k_BT per base pair.

3.9. DNA charge reversal

In nucleoprotein complexes containing DNA and histones the negative DNA charge can be reversed by the positive charges of the bound proteins. It is also easy to reverse the DNA charge by basic lipids or proteins *in vitro*. DNA charge reversal by counterios can be understood through the concept of fractionalization. Fractionalization is based on an idea that long flexible counterions contacting DNA are not necessarily bound at a stoichiometric equlibrium, but may form brushes, etc and their total charge can be more than the bare DNA charge (shown on Figure 5.)

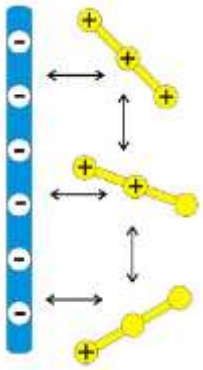

Figure 5. DNA charge reversal by bound multivalent counterions
(Teif V., Bohinc K.; Condensed DNA: Condensing the concepts (2010))

4. APPLICATIONS OF DNA CONDENSATION

Main potential application of DNA condensation in medicine is gene delivery in gene therapy. Gene therapy is the use of DNA as a drug to treat disease by delivering therapeutic DNA into a patient's cells. The most common form of gene therapy involves using DNA that encodes a functional, therapeutic gene to replace a mutated gene. Other forms involve directly correcting a mutation, or using DNA that encodes a therapeutic protein drug (rather than a natural human gene) to provide treatment. In gene therapy, DNA that encodes a therapeutic protein is packaged within a "vector", which is used to get the DNA inside cells within the body. Once inside, the DNA becomes expressed by the cell machinery, resulting in the production of therapeutic protein, which in turn treats the patient's disease.

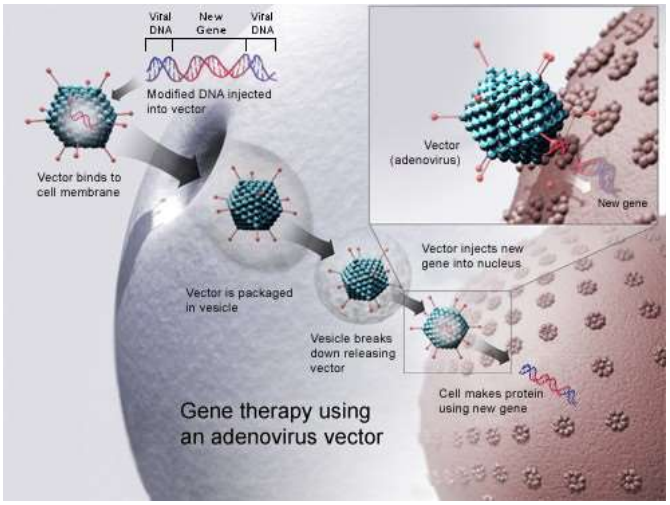

Figure 6. Gene therapy using an adenovirus vector. A new gene is inserted into a cell using an adenovirus. If the treatment is successful, the new gene will make functional protein to treat a disease

Biggest problem in gene therapy is target recognition, target modification and delivery DNA targeted drug to the cell. DNA condensation would be useful in drug delivery. Inserting genes into cells has been a goal of medical research for many years. The ways of doing this generally involve compacting the DNA and packaging it with something that will facilitate its uptake into cells. Receptor-mediated gene therapy is less popular at present than viral-mediated gene therapy, which has been more successful at productively introducing genes into cells. New questions about the safety of viral vectors provides a reason for continuing research on receptor-mediated DNA delivery, which can be used to introduce longer DNA sequences and can be targeted to specific cell types.

Other then gene therapy DNA condensation can also be used in the engineering of biosensors. We can now chemically contruct very sophisticated DNA-based nanostructures with almost any topology. Nanostructures combined with condensation can lead to construction of nano-tube based gene delivery vectors (Singh et al 2005).

5. LITERATURE

Teif, Vladimir B., and Klemen Bohinc. "Condensed DNA: condensing the concepts." Progress in biophysics and molecular biology 105.3 (2011): 208-222

Zimmerman, S.B., Murphy, L.D., 1996. “Macromolecular crowding and the mandatory condensation of DNA in bacteria”. FEBS Lett. 390, 245-248

Grosberg, A., Zhestkov, A.V., 1986. “On the compact form of linear duplex DNA: globular states of the uniform elastic (persistent) macromolecule”. J. Biomol.Struct. Dyn. 3, 859-872

Bloomfield, V.A., 1997. “DNA condensation by multivalent cations”. Biopolymers 44,269-282

Arscott, P. G., A.-Z. Li & V. A. Bloomfield. (1990). “Condensation of DNA by trivalent cations. 1. Effects of DNA length and topology on the size and shape of condensed particles”, Biopolymers. 30: 619-630

Leikin, S., D. C. Rau & V. A. Parsegian. (1991). “Measured entropy and enthalpy of hydration as a function of distance between DNA double helices”. Phys. Rev. A. 44: 5272-5278

Widom, J. & R. L. Baldwin. (1980). “Cation-induced toroidal condensation of DNA: Studies with $Co^{3+}(NH_3)_6$”. J. Mol. Biol. 144: 431-453

Post, C. B. & B. H. Zimm. (1979). “Internal condensation of a single DNA molecule”, Biopolymers. 18: 1487-1501

Chen, N., Zinchenko, A.A., Kidoaki, S., Murata, S., Yoshikawa, K., 2010. “Thermoswitching of the conformation of genomic DNA in solutions of poly(N-isopropylacrylamide)”, Langmuir 26, 2995-2998

Mengistu, D.H., Bohinc, K., May, S., 2009. “Binding of DNA to zwitterionic lipid layers mediated by divalent cations”. J. Phys. Chem. B 113, 12277-12282

Evans, D.F., Wennerström, H., 1994. “The Colloidal Domain, Where Physics, Chemistry, Biology and Technology Meet”. VCH Publishers, New York

Singh, Ravi, et al. "Binding and condensation of plasmid DNA onto functionalized carbon nanotubes: toward the construction of nanotube-based gene delivery vectors." Journal of the American Chemical Society 127.12 (2005): 4388-4396

6. LIST OF FIGURES