Lecture #4
The Cell as a Machine

Nuclear Functional Organization

Background readings from Chapters 4 of Alberts et al. Molecular Biology of the Cell (4th Edition)

Description of Functions by Biosystems Engineering

Biosystems engineering is a term used to describe the application of the thinking in systems engineering to the understanding of biological functions. Systems engineering in chemical engineering is involved with the optimization of processes for the commercial production of chemicals. For example, if you can increase the efficiency of a reaction in the synthesis of a major raw material and produce it for less money, then you can take over a market. I present this in terms of competition since the biological systems were selected by the criterion of competing in an open market. Unlike the systems engineering case where it is up to the engineer to design the facility that will produce the desired product, cells have functioning facilities and it is our job to understand how the facilities work. To carry this production facility analogy further, biosystems engineering is reverse systems engineering where you try to understand how an operational facility functions. We can now alter components and see how those changes alter output. Cells are much too complicated to be explained by one complex diagram such as the metabolic charts that are fading on the walls in most biochemistry departments. We can only hope to describe aspects of different functions and it is essential in doing so to break complex functions such as DNA replication or RNA transcription into a series of much smaller steps that depend upon a few proteins or components.

Basic functions of the nucleus during one cell cycle

1. Information Storage and Retrieval
    A. Storage (the packing problem)
    B. Retrieval
    i. mRNA for each protein required by the cell needs to be synthesized and processed selectively with the right number of copies, while other mRNAs are not made
    ii. rRNA must be made and assembled into ribosomes
    iii. tRNA must be made and processed for transport to cytoplasm
2. Replication (total DNA needs to be copied once and only once, ends-Telomers)
3. Division and reformation (one copy needs to be transferred to each daughter)

Physical Constraints on DNA functions

1. DNA and nuclear size: 6×10^9 base pairs at 0.3 nm/bp or 2 m of DNA in 46 chromosomes is packaged in a 5-6 μm diameter nucleus
2. DNA is a helical polymer with complimentary strands that is unwound in reading (10 bp/360°). Also, it has 2 neg. charges per bp or 0.3/nm of length.
3. Number of mRNAs encoded (about 50,000)

4. Number of different mRNAs translated (6-10,000) -

5. Total number of mRNAs produced per day is

6. 46 chromosomes must be separately packaged with daughter chromosomes and separated.

System Properties

1. Histones bind to DNA to neutralize charge and organize DNA into nucleosomes which have 140 bp or 47 nm of DNA and are spaced on average every 200 bp of DNA.

2. Nucleosomes are organized into solenoids (30 nm in diameter).

3. Solenoids are further organized into loops that are condensed into chromosomes

4. Replication has an error rate of 1 in 10^9 bp whereas the polymerases have an error rate of 1 in 10^6 bp

Summary:

The nucleus of eukaryotic cells serves to compartmentalize the DNA which is essential for the propagation of the organism but mechanically fragile (a covalent bond will withstand on the order of 1000 pN of force) and very long (6×10^9 base pairs at 0.3 nm/bp or 2 m of DNA that is about 1.5 nm in diameter, equivalent of 1.5 mm fish line that is 2000 km long and packaged in a 5 m diameter sphere). Critical functions are to replicate the DNA code for daughter cells and to encode RNA for proteins (mRNA), ribosomes (ribosomal RNA) and transfer RNA (tRNA). Throughout the cell cycle (G1-S-G2-M, which stands for Growth 1, Synthesis of DNA, Growth 2, and Mitosis) there are changes in the role of the nucleus (S is for DNA synthesis, M is for mitosis and the separation of the chromosomes to daughter cells, which typically involves nuclear breakdown) and we will discuss nucleus function in terms of the cell cycle. Big problems for the nucleus include helicity (360° per 10 bp and solution is Topoisomerase 1), tangling of DNA (fishing line problem and solution is Topoisomerase II), replication (50 bp/sec in eucaryotes vs. 500 bp/sec in bacteria, whole genome in 8 hr.) proofreading (typical error rates are 1 in 10^9 but should be in 10^6 or so in other views), DNA breaks and damage repair. DNA is a polyanion and much of the charge is neutralized by histones in the packaging of the nucleosome ($2 \times 6 \times 10^9$ P^- or 2×10^{-14} moles of P^- in $4/3\pi r^3$ = 4-10 $\times 10^{-14}$ l or 0.2-0.5 M of negative charge).

Why Make a Nucleus?

Since prokaryotic cells don't have a nucleus, there is some question about why a nucleus should be created. The presence of the nucleus creates many problems for the cell such as how to move proteins in and mRNA out of the nucleus, how to divide the DNA to two daughter cells. In cells with a cytoskeleton, the problems of handling meters of DNA in cytoplasm are enormous. Tangling of fibers and the need to move the cell with internal fibers make it much easier for the cell to compartmentalize the DNA in the nucleus. Specialization of function can occur in the nucleus without affecting events in the cytoplasm and vice versa. Compartmentalization and specialization are hallmarks of a highly engineered system. The cost in construction of the compartment and transport is easily offset by the ability to develop highly specialized functions in a controlled environment.

The nucleus is covered with the nuclear membrane. It is a double membrane that is contiguous with the endoplasmic reticulum. Sperm DNA will assemble a nuclear membrane in a complex frog egg cytoplasmic extract presumably through interaction of the lamin intermediate filament proteins with the DNA followed by membrane fusion. To communicate with cytoplasm, there are nuclear pores composed of over 100 proteins that aid in the transport of RNA from the nucleus and import of proteins into the nucleus. In addition, ribosomes are assembled in the nucleoli and moved out through the pores. Filaments extend from the cytoplasmic surface of the pores and potentially aid in the capture and transport of proteins with a nuclear import signal. Although we don't have time here to consider nuclear transport, it is an important area that has a number of interesting specializations that don't apply to other cellular transport systems. Most notable is the fact that pores in the nuclear membrane are relatively large holes that allow molecules up to 9 nm in diameter to readily diffuse through them. However, larger objects such as mRNAs (up to 26 nm in diameter) can be transported through pores.

How is 2 Meters of DNA Packed into a 5 Micron Diameter Nucleus?

The problem is how to compress 2 m of DNA in 46 pieces into a spherical nucleus of only 4-6 micrometers. Many different mechanisms could be used to compact the DNA; however, several other necessary DNA functions limit the possible mechanisms. We will first describe the system used by cells to compact DNA and then consider how this method of compaction might participate in other functions.

Nucleosomes are complexes of 8 histone molecules (2-H3-H4 dimers and 2-H2A-H2B dimers) and 140 base pairs of DNA. In the electron microscope, the complex is 11 nm in diameter and the crystal structure is known (p. 209 of Mol. Biol. Cell). The DNA strand goes 1.65 times around the nucleosome and puts a strain on the DNA that is best accommodated by A-T pairs in the portions nearest the histones. On average, the nucleosomes are present every 200 bp and the overall compression of the DNA is about 4 fold.

In the electron microscope, a common form of chromatin is the 30 nm solenoid which is formed from the nucleosomes by further aggregation aided by histone H1. The solenoids are remarkably regular in size and can extend for many microns. Since the length compression is 35-40 fold, the average chromosome (1.4×10^8 bp or 45 cm of DNA) forms about a cm of solenoid. Although to this point there has been considerable condensation, much more condensation is needed to reach the size of an average condensed mitotic chromosome, which is about 6 by 0.7 microns. To reach this highly condensed state, the solenoid fibers are first folded to form loops of 20,000-100,000 nucleotide pairs (a loop of 0.1 to 0.5 microns in length). Interphase chromatin is also believed to be organized in loops and those loops are further condensed to form a mitotic chromosome. The SMC proteins have ATPase activity and are linked to the condensation process (Ball Jr and Yokomori, 2001). Possibly, they bridge between loops and move the solenoids in a manner similar to the other motors (Wanner and Formanek, 2000) .

DNA and the Cell Cycle

An important aspect of DNA packaging in cells is the need to move through the cell cycle. The implication is that the DNA must be dynamic and change dramatically in

the transition to each step. The cell cycle is [(G1-S-G2-M-G1) Growth-phase 1-DNA Synthesis (replication)-Growth-phase 2-Mitosis (Metaphase-Anaphase-telophase-cytokinesis)-Growth -phase 1]. In the two growth phases, there is only transcription of DNA to RNA. However, in S the DNA must be replicated. At early M, the DNA must be highly condensed (nucleus is broken down) and then must be separated equally into the daughter cells.

Mitosis and DNA Packaging

During mitosis, it is critical to move one copy of the genome to each of the daughter cells. Mitosis involves a well-choreographed series of events. Metaphase is the first step during which time the DNA is condensed, the nuclear membrane is removed (by absorption into the ER and vesiculation), and the mitotic spindle is formed. After chromosomes are aligned at the metaphase plate, anaphase begins with the elongation of the spindle and the separation of the chromosome pairs with movement toward the spindle poles. In telophase the remaining microtubules in the overlap region are depolymerized, cytokinesis is completed and the chromosomes decondense with the reformation of the nucleus.

In terms of DNA manipulations during mitosis, the critical aspects are the initial condensation, the binding of the two kinetochores in a chromosome pair to microtubules from opposite spindle poles, separation with transport to the poles, cell division, and the decondensation of the DNA with the reformation of the nucleus.

Condensation involves the SMC proteins and discuss the model in the paper with anti-parallel filaments. Although the process can be recapitulated in vitro, there is not an extensive understanding of the important players (particularly the filaments).

Alignment at the metaphase plate has been studied extensively because it has been observed for over a hundred years. Spindle assembly is initially critical and that process can be broken down into many steps (separation of the duplicated centrosomes and movement to the opposite ends of the cell followed by increased microtubule dynamics). Dynamic microtubules are presumably captured by the kinetochores (possibly a role for cytoplasmic dynein in capture and creation of tension). Alignment is a push-pull exercise that involves force sensing at the kinetochores. Chromosome alignment signals are a major mystery in cell biology in the sense that it is hard to quantitatively model how the alignment is sensed with high fidelity. Force sensing is critical in process because of repulsive wind.

Once chromosomes are aligned, a signal is given to separate daughter chromosomes and move them to the poles (anaphase A). Separation requires the depolymerization of the kinetochore microtubules as well as their physical

How are Daughter Chromosomes Separated?

How are the Solenoids unfolded for Transcription?

nucleosome (histone-DNA 140 bp, and ~200 bp on average) (length compression is about 4 fold)

solenoids (30 nm in diameter and held together by histone H1) (length compression is 35-40 fold)

Solenoids are thought to be modified by methylation and acetylation to increase or decrease dynamics (also phosphorylation)

Chromosomes unfold and refold the solenoids in a process like reshuffling the deck of cards.

euchromatin and heterochromatin

Loops (to further consolidate the DNA, the solenoids are arranged in loops that

Arms of chromosomes and kinetochore (46 chromosomes in humans and a 10,000x compression from 1.5 cm to 2.2 microns for chromosome 22) Role of SMC proteins as part of condensins have a role in the 10X condensation occurring during prometaphase.

Cell cycle (G1-S-G2-M-G1) Growth phase 1-DNA Synthesis (replication)-Growth phase 2-Mitosis (Metaphase-Anaphase-telophase-cytokinesis)-Growth phase 1

Replication of DNA

polymerases (always 5' to 3' and hence Okazaki fragments ~200 bp in eucaryotes)

Helicases, Topoisomerases

Problems:

1. Helicases put torsional energy into DNA that is relieved by topoisomerase 1. If we assume that the helicases create a torsional force, then how would you convert that force into a signal recognized by the topoisomerase.
2. Assuming that histone complexes neutralize 140 bp and are spaced on average every 200 bp and that the whole human genome is replicated in eight hours, what is the average rate of histone synthesis? What is the concentration of negative charges in the replicated nucleus (7 microns in diameter)?
3. Describe a way to check for unpaired bases that involves interaction with the double stranded DNA and diffusion along it. Assuming a diffusion coefficient of 10^{-10} cm^2/sec, how long a strand of DNA can be scanned in one hour (to get a reliable scan we suggest that you should only consider half the normal diffusion distance, i.e. 2X sampling).

Ball Jr, A.R., and K. Yokomori. 2001. The structural maintenance of chromosomes (SMC) family of proteins in mammals. *Chromosome Res*. 9:85-96.

Wanner, G., and H. Formanek. 2000. A new chromosome model. *J Struct Biol.* 132:147-61.