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Richard McIntosh's Lecture Part 1:
Cell Division in Eukaryotes

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Contents

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1. Keywords and Terms

Cell division cycle; chromosome; chromatin; DNA; replication; condensation; mitosis; microtubule; centrosome; kinetochore; metaphase; anaphase; nucleus; complexity; assembly

2. Lecture Notes

Introduction

The process of cell growth and division is introduced as a fundamental aspect of cell behavior. The difficulty of the process is expressed in terms of the complexity of any one cell, which makes it hard to synthesize and assemble all the parts necessary to make a second cell. Many macromolecules must be made and set in their correct

places before a cell is ready to divide. All this synthetic activity occurs during "interphase", the time between successive cell division.

Events of the cell growth and division cycle

One key event of interphase is the replication of the cell's DNA, which occurs during the "S-phase".

Another is the duplication of the centrosome, which usually occurs at about the beginning of S-phase. There is, however, a gap in time after the previous cell division is over and before S-phase begins (G1).

During G1, cells synthesize many macromolecules, like proteins that are essential for cell growth. It is also during this phase that most cells decide either to enter S-phase and divide again or to leave the cell division cycle and synthesize more specialized proteins, so they can "differentiate" into a form that will allow them to do a particular job, for example, become a neuron. This time of decision is important for cell behavior, because cells in a multicellular organism should divide only often enough to make new cells as needed.

Once S-phase has started, the cell is committed to divide again, but after S-phase is finished, there is usually yet another gap (G-2), allowing time for more RNA and protein synthesis before the cell is ready to go through the mechanical process of separating the now-double cell into two distinct objects, the "daughter cells".

The logic of dividing the parts of a biochemically doubled cell

Most of the cell's parts (organelles like mitochondria and macromolecular machines like ribosomes, etc.) are so numerous that they get no special attention at the time of cell division; roughly half will wind up in one cell and roughly half in the other, simply by chance.

Some cell parts are present in only one or a few copies, for example, each chromosome and the centrosome, a structure that helps to organize the microtubules of the cell's cytoskeleton. Cells take special pains to make sure that each daughter cells gets one of each of these objects, so it will have all that it needs to grow and divide again. To achieve reliable separation of such structures, the cell builds a special machine that is

designed to deliver one copy of each structure to each of the two daughter cells. This machine is called the “mitotic spindle”.

The mitotic spindle is built largely from microtubules and the many proteins that associate with them. In most cells the spindle microtubules are initiated by the already duplicated centrosomes, so the structure that forms has two, essentially identical parts. These microtubules grow into the space where the chromosomes reside, and some of them encounter a specialization found on each chromosome called a “kinetochore”, to which they bind, allowing the spindle to affect chromosome orientation and position. The molecular mechanisms by which this microtubule-based machine can accomplish chromosome organization and segregation are the subjects of my next two lectures.

The problems of segregating DNA

In spite of this special, chromosome-moving machine, the problem of chromosome segregation is difficult because each chromosome is made of one long piece of DNA. The chromosomes in our own cells are millimeters in length, i.e., thousands of times longer than the diameter of a human cell nucleus. Thus, the DNA of each chromosome must be condensed many fold to make an object that is small enough for the cell to move it around in an organized fashion. The “condensation” of each chromosome is achieved in part by wrapping the DNA around small clusters of proteins called “histones” to make “nucleosomes”, in part by packing the nucleosomes into helical fibers, in part by folding those fibers into loops, and in part by wrapping those looped fibers into yet another set of helices. This hierarchy of condensation shortens the DNA of each chromosome the necessary thousands of fold, making an object that is short enough to fit easily into a nucleus and thick enough to be seen in the light microscope.

DNA must be extended during interphase, so it can serve as a template for both RNA synthesis and DNA replication. As DNA replicates, the two identical double-stranded molecules that are produced become linked together by “cohesins”, so the two copies will be connected as they go into mitosis. These identical pieces of DNA are called “sisters”, and each one is commonly called a “chromatid”, rather than a chromosome. Every chromosome condenses in preparation for cell division during the stage of the cell cycle called “prophase”. Because S-phase precedes prophase, and because cohesins bind sister chromatids together, each chromosome is a double structure as it condenses for cell division.

Once condensation is far enough along, the mitotic spindle forms. It interacts with the now almost fully condensed chromosomes and organizes them into the 2-fold symmetric structure seen in “metaphase”, the stage just before the separation of the duplicated chromosomes.

The problems of segregating already duplicated chromosomes

The essential problem of mitosis is to get the sister chromatids of each duplicated chromosome attached to the two opposite ends of the spindle. This is accomplished in part because each chromatid contains one and only one site to which the fibers of the mitotic spindle can attach. This site, the “kinetochore”, is able to bind either the side or the tip of spindle microtubules. Stable connections between kinetochores and spindle fibers are made only when these links are under tension. This tension is produced largely at the kinetochore. It probably comes from both motor enzymes, like dynein, and from non-motor protein links that couple the kinetochore to spindle microtubules in such a way that microtubule depolymerization can exert force on the kinetochore. Once each chromosome is being pulled toward both of the spindle poles, the cell needs only separate the sister chromatids and allow them to respond to the forces acting on them. Then accurate separation of the duplicated chromosomes is achieved.

There are many different ways to look at a mitotic spindle

The process of mitosis can be followed with “phase” optics, in which chromosomes appear dark, but the spindle is essentially invisible. It can also be seen with “polarization optics”, in which the chromosomes are only ghosts, but the fibers of the spindle appear bright or dark. Fluorescence microscopy can be used in at least two ways to reveal aspects of spindle structure and function: by fixing cells to stabilize their structure, then staining particular spindle components with protein-specific antibodies tagged with a fluorescent dye (immunofluorescence), or by staining part(s) of the spindle with a fluorescent tag in vivo. The latter method can use both fluorescent dyes that bind to cell parts, like chromosomes, and mutant proteins that are made by coupling the gene that encodes a particular spindle component directly to the gene for a fluorescent protein, e.g., the green-fluorescent protein (GFP). If GFP is coupled to tubulin, the protein subunit of microtubules, the whole spindle is stained.

Spindles can be seen at higher resolution with the electron microscope, in which the fibers that attach to the chromosomes appear as bundles of microtubules. With this

same instrument, the kinetochore often appears as a dark-staining layer on the underlying material of the condensed chromosome, the “chromatin”.

Protein localization by immunofluorescence and GFP-tagging has shown that kinetochores are complex assemblies of multiple proteins, probably around 100 different molecules. These many components give each kinetochore lots of useful properties in addition to microtubule binding: motor activity in two directions, enzyme activities that can help to depolymerize microtubules and protein kinase activities that can help to regulate the function of other proteins.

Each of these ways of looking at the mitotic spindle shows us a part of the full complexity of the machine that segregates the chromosomes.

The process of mitosis is somewhat different in different organisms

In yeast cells, the spindle is small; it forms inside the nucleus, whose membrane remains intact throughout mitosis. In mammals, vertebrates in general, and many higher plants, the spindle forms in the cytoplasm, but the nuclear envelope disassembles as the spindle forms, so the cytoplasmic microtubules gain access to the chromosomes as they condense in the nucleus. In some cells, like the flagellate, *Barbulanympha*, the nuclear envelope stays intact, the chromosomes condense in the nucleus, the spindle forms in the cytoplasm, and the two interact right through the nuclear envelope. Thus, there are numerous ways to solve the essential problems of mitosis. This kind of “biological variability” can make the study of mitosis complex, but it also give informative variation that can help the experimenter find a useful system for answering a particular question.

Some features of mitosis are almost universal

All spindles form with microtubules as their principal fibrous component. All spindles contain some microtubules that interact with kinetochores to organize the chromosomes and exert forces on them. All spindles form into a structure that is roughly 2-fold symmetric, and as the spindle acts on the duplicated chromosomes during anaphase, the structural symmetry plays out in the motion of the chromosomes to opposite ends of the cell, making it comparatively easy for a simple cleavage event to separate the mother cell into two similar and fully viable daughter cells.

Vocabulary: Words whose meaning you should know.

DNA; chromosome; chromatin; replication; nucleosome; histone; condensation; cohesin; sister chromatids; interphase; S-phase; G1 and G2; differentiate; mitosis; microtubule; centrosome; kinetochore; prophase; metaphase; anaphase; nucleus; cytoplasm; nuclear envelope; phase optics; polarization optics; fluorescence microscopy; green fluorescent protein; electron microscopy; biological variability; 2-fold symmetry.

3. Recommended Reading

Alberts, et al., Molecular Biology of the Cell or Essential Cell Biology: chapters on the cell cycle and mitosis.

Rieder, C. L. and E. D. Salmon (1998). "The vertebrate cell kinetochore and its roles during mitosis." Trends Cell Biol 8(8): 310-8.

More advanced basic reading

Nicklas, R. B. (1997). "How cells get the right chromosomes." Science 275(5300): 632-7. *A readable paper that summarizes the life's work of a great scholar of mitosis. Nicklas develops the theme that stability of the chromosome attachment is a result of tension and shows how this provides a logical solution to one of mitosis' deepest problems.*

Nurse, P. M. (2002). "Nobel Lecture. Cyclin dependent kinases and cell cycle control." Biosci Rep 22(5-6): 487-99. *A review of some great genetic studies that allowed Nurse and his colleagues to help open up a molecular understanding of cell cycle control.*

McIntosh, J. R., E. L. Grishchuk, R.R. West (2002). "Chromosome-microtubule interactions during mitosis." Annu. Rev. Cell Dev Biol 18: 193-219. *A review that*

focuses on the processes associated with getting chromosomes onto the spindle and segregated, rather than the identification of components.

4. Review Questions

1. Why do cells complete S-phase before starting mitosis?
2. Why do cells have periods of biosynthesis and growth before starting mitosis?
3. What are the important sections of the interphase period of the cell cycle, and what happens in each of them?
4. What are the commonly discussed stages in the mitotic period of the cell cycle, and what happens in each of them?
5. Why is chromosome condensation essential for a successful mitosis?
6. What strategies do cells use in addition to chromosome condensation in order to deal with the problem of segregating millimeters of DNA into two daughter cells?
7. What is presented as the “essential problem of mitosis”?
8. What are the differences between the aspects of mitosis that you can see by phase, polarization, fluorescence, and electron microscopy?
9. Give three examples of differences found in mitotic spindles from different organisms.
10. What aspects of mitosis appear to be essentially universal among all eukaryotic organisms?

5. Answers to Review Questions

1. DNA must be duplicated before cell division, or there is not a copy of each chromosome available to go into each daughter cell.

2. A cell must make enough of all its parts to provide the materials, meaning enzymes, membrane, organelles, and everything else that will allow each “daughter cell” to be viable; otherwise cell division would be a prelude to cell death.

3. S-phase for DNA synthesis, G1 for cell growth or differentiation before DNA duplication, and G2 for further growth after DNA duplication, allowing the cell to make all the components necessary for the cell division process.

4. Prophase: chromosomes condense. Prometaphase: the now almost-condensed chromosomes interact with the forming mitotic spindle and begin to become organized. For example, sister kinetochores develop attachments to opposite ends of the spindle. Metaphase: the chromosome attachment process goes to completion, and each chromosome completes is motion to the midplane, or “equator” of the spindle. Anaphase: the duplicate chromosomes separate into two distinct parts and move in opposite directions to the end of the spindle they face. Telophase: Chromosomes decondense and go back into the interphase state. This is accompanied by the reformation of the nuclear envelope in those cells that disassembled the envelope for mitosis.

5. Chromosomes are usually much longer than the diameter of the cell in which they reside. It would be impossible to separate the duplicated chromosomes into two distinct groups unless they become short enough to move around as objects in the cell.

6.
    a. package DNA in pieces, so no one is too big
    b. replicating all the DNA before starting to segregate it
    c. coupling sister chromatids with cohesin as they are made by DNA replication, so sister chromatids are in a defined position relative to one another.
    d. develop a special machine that can do the segregation job right

7. The problem of attaching the kinetochores on sister chromatids to opposite ends of the spindle.

8. Phase microscopy shows the chromosomes as dark on a lighter background, so their motions are easy to see, but the spindle is almost invisible. Polarization microscopy shows up the spindle very nicely, but now the chromosomes are pale. Fluorescence microscopy reveals the position of only those molecules that have

been stained. These, however, are very clearly seen against a black background. In live-cell fluorescence microscopy, the behavior of those components during mitosis can be followed over time. Electron microscopy shows most chromosomes as dark-staining masses of fibers and the spindle as containing many microtubules. Kinetochores and structures at the spindle poles are also commonly seen, but electron microscopy requires cells to be fixed, so events that happen in a living cell cannot be watched in real time.

9. Size (number of microtubules, length of microtubules, number and size of chromosomes); mitosis occurs inside a nuclear envelope (as in yeasts and many algae) or the nuclear envelope disperses, so the chromosomes are now accessible to fibers, like microtubules, that form in the cytoplasm; mitosis occurs with the chromosomes inside the nucleus and the spindle outside in the cytoplasm, but the two interact through the nuclear envelope.

10. All spindles use microtubules as their principal fibrous component. All spindles contain some microtubules that interact with kinetochores to organize the chromosomes and exert forces on them. All spindles form into a structure that is roughly 2-fold symmetric, and as the spindle acts on the duplicated chromosomes during anaphase, the structural symmetry plays out in the motion of the chromosomes to opposite ends of the cell.

6. Discussion Questions

1. Everyone knows that cells are complex, but how could you measure how complex they really are? How could you compare the complexity of a cell with that of some other structure?
2. Why are cells so small?
3. Why do unicellular organisms grow and divide whenever they can? Wouldn't it be simpler just to differentiate into a stable form as stay in one place?
4. Why is the cell growth and division cycle in multicellular orgnanisms regulated?
5. What properties would you want a microtubule to have to accomplish the jobs it performs during mitosis?

6. Do you see advantages for a cell in having its spindle form inside the nucleus, rather than in the cytoplasm? How about having the spindle in the cytoplasm and the chromosome in the nucleus?

7. How does a cell know when to start anaphase? If you were designing such a system, what properties would you give it, and how would you make it work?

8. What time in the cell cycle is most suitable for the decision to start the division process?

7. Answers to Discussion Questions

1. One measure of a system's complexity is the number of parts it contains, but for a cell this is hard to know. Is each water molecule a part? No, but each protein molecule is a part in a way. But big things with lots of identical parts are not necessarily complex, so it isn't just the number of parts, it is the numbers of different parts. But not all parts are equivalently complicated, and clearly component complexity has to be taken into account. Thus, the number of instructions necessary to build a structure sounds like a pretty good method for assessing complexity. But even this is not easy to measure for a cell: genes, yes, but what do you do about alternative splice forms? about post-translational modifications? It's an interesting problem to think about.

2. Part of the answer here is that the macromolecules of which cells are made are held together by rather weak forces. You can't build very big objects with things that are held together by weak bonds, or they just fall apart. If you made the bonds between macromolecules stronger, the cell would not have the flexibility that is characteristic of living things: growth and fluidity of motion, etc. Another part of the answer is that small size means the molecules can diffuse around in the aqueous solutions of each cellular compartment with relative ease and speed. As sizes increase, the time required for a given level of diffusion goes up with the square of the size, so diffusion of a nutrient from the place it enters the cell to the opposite end of a 10 mm cell takes 100 times longer than it does in a 1 mm cell. In a 10 mm cell, it would take a million times longer than in a 10 mm cell, and now things would really slow down. The importance of this issue is seen in the fact that when cells are really big (giant algal cells that are centimeters long, or neurons whose axons are even a meter or so in length), they have to build special machinery to supplement diffusion as the

mechanism for getting molecules around inside them. This machinery causes the processes called “cytoplasmic streaming”.

3. Most unicellular organisms “succeed” by growing and dividing, so their numbers increase relative to the competition. This is really one definition of “success” at a biological level. By growing and dividing whenever food supplies permit, a given species of microorganism is maximizing its likelihood of persisting over time and being around, even when times are bad.

4. Cells in a multicellular context must contribute to the well-being of the organism as a whole. If individual cells grow and divide when additional copies of their kind are not needed, imbalance occurs, and one gets a disease like cancer.

5. Its polymerization should be controlled so it occurs at the right time and in the right place. A microtubule should be strong enough to exert useful forces on chromosomes. This strength should include both tensile strength (the ability to bear the tension that acts at a kinetochore) and rigidity (so the fibers will not be flaccid and bend when compressed). If kinetochores and/or the ends of the spindle have motor enzymes that interact with microtubules, these polymers should be compatible with those motors. They also must have intrinsic directionality (if a fiber is not polar, a motor won’t know which way to move along it).

6. This question inspires very speculative answers. The nucleus is always smaller than the cell as a whole, so this behavior might allow the cell to use only a smaller spindle, saving the biosynthetic work of making all the proteins necessary for a big spindle. There may be many more factors, so think about it.

7. A cell should not start anaphase until all the chromosomes are properly attached to the spindle. One could measure the net tension acting on all chromosomes and in this way count the number of chromosomes that had become properly attached to the spindle, but for cells with lots of chromosomes (our cells have 46), this means having a system that is so accurate that it can tell the difference between 45 and 46 without a mistake. It is probably easier to count the number of UNATTACHED chromosomes, because then you are looking for the difference between 1 and 0, when the last chromosome does become attached, and that is a bigger fractional change. This has led to the hypothesis that chromosomes that are not attached to the spindle emit a signal that says, “Don’t start anaphase!” This is currently the

favored idea about a control that is called the “Spindle assembly checkpoint.” Just how it works is still unknown, but it is fun to think about.

8. Before DNA has been replicated would be a good time, because the cell has not yet invested all the energy required to replicate its DNA. In late G1, just before S, there is a control point called “Start” in yeasts and the “Restriction point” in vertebrate cells. This is the decision point at which the cell sums the information it has received: nutrients in the environment, its own size, signals from neighboring cells, etc., and makes the decision whether to go on and divide or pursue some other fate.

8. Explain or Teach These Concepts to a Friend

1. Explain why a cell’s DNA is long, why it is only slightly compacted during interphase, but must become significantly condensed for the cell to be able to segregate its chromosome successfully.
2. Explain why the metaphase spindle is 2-fold symmetric.
3. Explain why microtubules are polar structures, not themselves 2-fold symmetric.
4. Explain why the system of using tension at the kinetochore-microtubule junction is an effective one for assuring that chromosomes are properly segregated during anaphase.
5. Explain why anaphase generally includes both Anaphase-A (the chromosomes approach the spindle poles) and Anaphase-B (the spindle poles move apart).

9. Research the Literature on Your Own

1. What controls spindle microtubule polymerization? (Hints: check the literature on centrosomes with special attention to an isoform of tubulin called “gamma-tubulin.” Look into the idea that chromosomes too are able to increase the probability of microtubule polymerization by looking up “ran-GTP” and its ability to alter the tubulin-microtubule equilibrium. Look also at recent evidence for kinetochore-mediated initiation of microtubule growth.) When all these facts are in hand, consider the implications of your knowledge for a basic understanding of how the spindle forms. Is the diversity you have now seen the source of fundamental

variation in spindle function, or is it just another example of biological diversity on a fundamental process?

2. How are sister chromatids held together, and how is this connection severed to allow anaphase onset? (Hints: check out the literature on cohesin, but be sure to get more than one point of view, e.g., work from both the Nasmyth and the Koshland labs. Find out how cohesin is removed from the chromosomes at anaphase onset. See if you can link this process with the issues raised above concerning our understanding how a cell decides when to begin anaphase.)

3. What is the evidence that motor enzymes contribute to the successful formation of the mitotic spindle? (Hints: check out the literature on loss-of-function alleles of motor enzymes in yeasts. Look as studies that use the injection of function-blocking antibodies into living cells. Look at studies carried out in extracts of frog oocytes, where antibodies can be use to deplete a specific spindle component. Look at more recent literature that uses RNA interference to knock down expression of motor genes.) Now compare your results and ask yourself, which kind of data do you think is the most reliable and why. Are the differences you find a result of biological variability or experimental method?

4. What aspects of the mitotic spindle contribute to the accuracy of chromosome segregation? (Hints: think first about how you would measure mitotic accuracy, then go to the yeast literature (both budding and fission yeasts) to see how it has actually been done. See what you can find about measurements of segregation accuracy in different genetic backgrounds, i.e., when particular spindle components are compromised by mutation. Now think of other ways in which you could compromise spindle action and see if you can find data on the fidelity of chromosome segregation in these different experimental situations.