THE DEVELOPMENT OF AN INFORMATIONAL VIDEO USING THREE-DIMENTIONAL ANIMATION TO TEACH THE FUNDAMENTALS OF THE CELLULAR PROCESS OF APOPTOSIS

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For my father, and for all cancer patients.

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by

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THESIS

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Thank you to my committee members, Lew Calver, Kim Krumwiede and John Abrams, and to Tony Frisbie, Perry Sparks, Susan Douglas and Jean Ann Haag. Thank you also to my husband, Chance, for his endless love, support and inspiration, my mother for nurturing the artist and the scientist in me, and my brother, who keeps me thinking, keeps me curious and makes me wonder how anyone gets through life without a twin.

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The goal of this thesis was to create an animated video, with narration, that explains the fundamentals of the process of apoptosis. The objectives were to produce a narrated 3D animation of apoptosis presented in an accurate efficient way, and format it for distribution on CD or DVD. Topics discussed in the video include: the difference between necrosis and apoptosis, the physical changes occurring in the cell during apoptosis, triggers of apoptosis and the effect apoptosis has on disease processes. The creation process began by determining subject, scope and audience. After these initial decisions were made a script was written and storyboards were produced. Narration was then recorded and combined with stills of the storyboards and preliminary animation to create an animatic. All animation was created in 3D Studio Max®. Editing was accomplished using Adobe Premiere®. The final product was then copied to CD and to DVD.

This document discusses the process of creating this video from formation of the idea to DVD creation. Results of an informal test of the video are also discussed as well as ideas for further research.

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CHAPTER ONE
Introduction

Goal

Great effort is spent teaching students the details and the importance of the cell cycle. While knowledge of mitosis is essential to understanding the complexities of life, the same can be said of its antithesis, cell death. In the human body, as in all living things, equilibrium is achieved between multiplying cells and dying cells. In the past decade much has been uncovered about programmed cell death. It was the goal of this project to create an informational video using 3D animation and narration to introduce the fundamentals of this important process to students of a post-graduate level. The objectives were to produce a narrated 3D animation of apoptosis presented in an accurate efficient way, and format it for distribution on CD or DVD.

Background Information

Cell death occurs by two different means. The first way is injury, or necrosis. This process occurs when cells are damaged during trauma or disease processes. During this process, the whole cell and organelles inside the cell swell due to interruption of normal ion transport across membranes. The cell contents then leak out which leads to inflammation of surrounding tissue.

The second type is a programmed type of cell death called apoptosis. Apoptosis is important in development, immune system function, and maintaining homeostasis.

Unlike cell injury, cells undergoing apoptosis first shrink, then bubble-like blebs form on the surface of the cell in a process called blebbing. Next, all chromatin in the nucleus is degraded, and the cell breaks into small membrane wrapped fragments. (fig. 1)

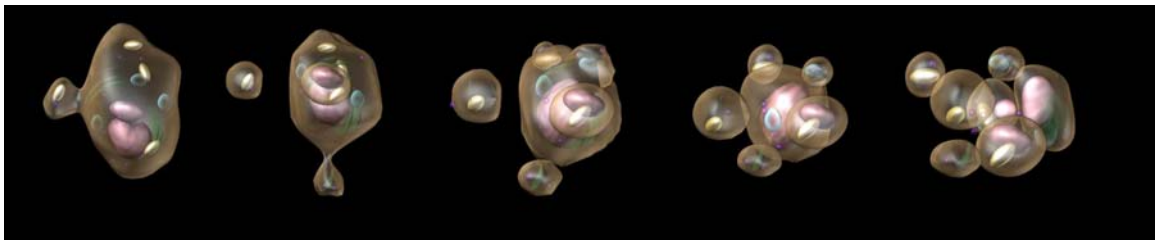


Fig. 1 – This is a series of stills taken of the animated blebbing sequence.

Apoptosis is an important mechanism in both development and homeostasis in adult tissues for the removal of unnecessary, infected, transformed or damaged cells by activation of an intrinsic suicide program. Apoptosis is a form of programmed cell death which is characterized by maintenance of intact cell membranes during the suicide process. This maintenance allows adjacent cells to engulf the dying cell so that the cell contents are not released and an inflammatory response is therefore not elicited. Cells undergoing apoptosis usually go through a process including fragmentation of the cell into membrane-bound apoptotic bodies, nuclear and cytoplasmic condensation and endolytic cleavage of the DNA. The cells, or cell fragments, are then phagocytized by macrophages or neighboring cells. Phosphatidylserine is exposed on the surface of the fragments, which causes phagocytic cells, like macrophages, to engulf them. (fig. 2)

These phagocytic cells then secrete cytokines that inhibit inflammation of the surrounding tissue.

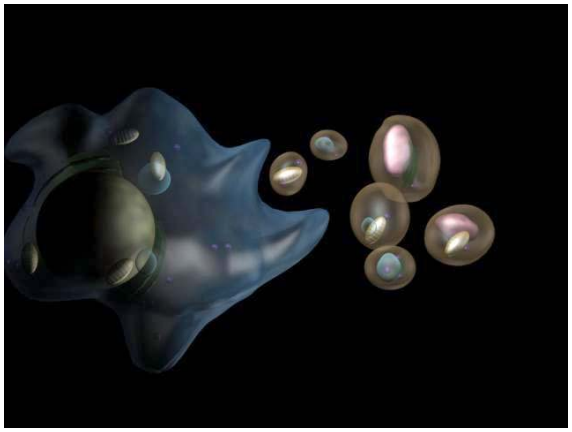


Fig. 2 – This is a still from the animation of a macrophage engulfing a cell fragment.

Apoptosis is as important to viability as mitosis, and yet only now are researchers beginning to learn the intricacies of this process. Understanding programmed cell death is a key step in the search for cures for diseases such as cancer, Alzheimer's disease, AIDS and many more. It is therefore important to teach the basics of this process to students in an accurate and efficient way.

Significance of Project

Diagrams, charts and two-dimensional representations of cellular processes such as apoptosis are important in the learning process. Additionally, an animated representation of the same process can prove invaluable to students by teaching them an important concept in an efficient way that will keep them interested. Since most

knowledge of apoptosis has been acquired in the last few years there are relatively few effective illustrations and little or no animation designed to teach the important points.

This video is fully narrated and provides an introduction to the fundamentals of apoptosis, including sequence of important cellular events, possible triggers and a discussion of the importance of further research.

Limitations

Several factors placed limitations on the project. Budget concerns made it impossible to hire a professional narrator. Time and scope constraints limited the video to a basic introduction to apoptosis. And, since the entire video was produced on a personal computer rendering time and processor speed limited the complexity of the animation.

This video only covers an introduction to this process of cell death. Biochemical and genetic details are not included. Also, because of the amount of current research on this topic, and the new discoveries that are being made every year this video may need to be updated as new facts are uncovered.

Creation of this video, including recording of all narration and all animation and editing, was completed using a personal computer. Because of this, some animation sequences were simplified to accommodate the memory and processor speed of the computer.

Production Methods

The original idea was storyboarded and a script was written. Narration was recorded and animation sequences were created. The various components were then edited together, and the final video was created in QuickTime format. The video was then recorded on to CD-ROM and DVD-ROM for use on most computers and DVD players. It can be played in classrooms or be made available to students to view on personal computers or DVD players.

Evaluation

An informal survey was taken by a sample of the target audience. Participants viewed the final video then completed a questionnaire designed to provide some informal feedback concerning the effectiveness of the project.

CHAPTER TWO
Review of Existing Literature

HISTORIC OVERVIEW

Introduction

Cells die in one of two ways, necrosis or apoptosis. Necrosis is caused by injury to the cell and is always a pathologic process. Until a few decades ago, necrosis was thought to be the only way in which cells die (5). In 1972 Kerr, Whyllie and Currie published *Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics.* This was the first group to propose a type of cell death distinct from necrosis (12). Apoptosis is a programmed, managed form of cell death, while necrosis is an unordered and accidental form of cell death (11).

Characteristics

A cell undergoing necrosis will first swell due to the lack of proper ion and water transport through the plasma membrane. Cell contents will then leak from the cell and inflammation of surrounding tissue will ensue (21).

A cell undergoing apoptosis will shrink and lose contact with surrounding cells. Then, the cell membrane will bleb, producing bubble-like formations on the surface of the cell. Chromatin will condense and become visible near the inside of the nuclear envelope, and blebbing will produce membrane bound fragments. Finally, phagocytic cells react to phosphatidylserine which becomes exposed on the fragments' surfaces. The

fragments are engulfed and degraded by the phagocytic cell. In some cases, such as the liver, neighboring cells will engulf and degrade the fragments (13,10)

Caspase

The major enzymes in the apoptotic process are proteases called caspases (cysteine-dependent, aspartate-specific proteases). These enzymes directly and indirectly cause the morphologic changes of the cell during apoptosis (5). Caspases are the mammalian homologue of the cell death protease, CED-3, in the nematode (16). Caspase enzymes are specialized proteases which are dormant in healthy cells. When activated, they initiate a complicated series of biochemical interactions called the caspase cascade. When cells are specified to die, caspases are converted to an active state and can cleave other proteins in specialized ways. Cytoskeletal and nuclear material is cleaved in specific regions causing the breakdown of the cell. (16, 5)

Examples of Triggers

Apoptosis can be caused by many factors. Three examples include: withdrawal of hormones and survival factors, receptor ligand interactions and exposure to toxins or radiation (21).

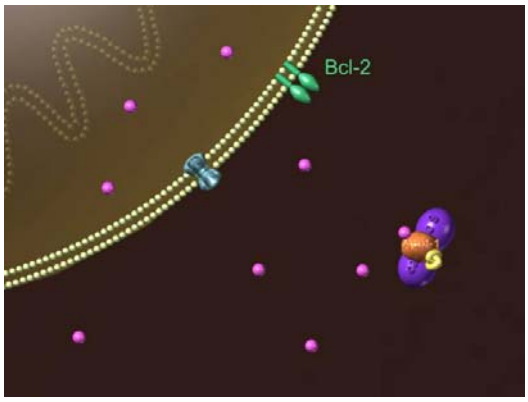


Fig. 3 – Bcl-2 causes cytochrome c to be released from the mitochondria. Cytochrome c, Apaf-1, ATP and caspase then bind creating the apoptosome.

Withdrawal of hormones and survival factors is a theory currently being researched. Cell death elicited by this method is believed to involve the Bcl-2 family of proteins and Caspase 9. Bcl-2 proteins are often located on the outer mitochondrial membrane and function to promote the release of cytochrome c from the mitochondria. (21). Cytochrome c, Apaf-1 and ATP bind together and then subsequently bind to caspase 9, activating it. The activated caspase then initiates the apoptosis process (19). (Fig. 3)

Apoptosis can also be triggered by specific receptor/ligand reactions. For example, the FAS ligand, produced by cytotoxic T cells of the immune system, binds to the FAS receptor on the surface of a virus infected cell. (Fig. 4) With the help of FADD, an adapter protein, the FAS ligand activates caspase 8, which then initiates the caspase cascade causing apoptosis. Another example of receptor-ligand mediated apoptosis involves the tumor necrosis factor (TNF) receptor also produced by cells of the immune system (21).

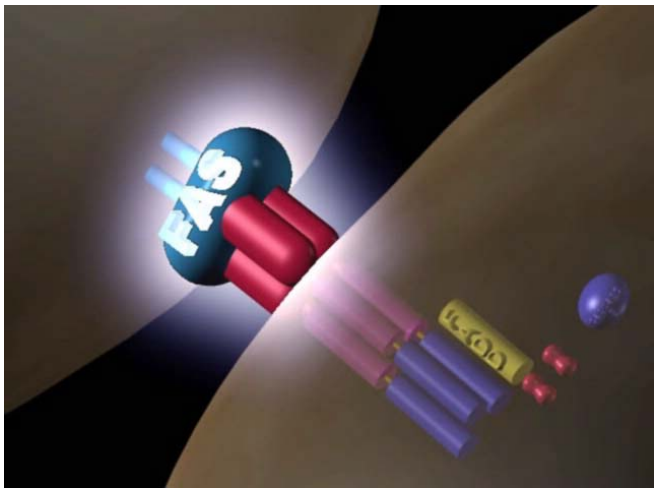


Fig. 4 – The FAS ligand binds with the FAS receptor on a virus infected cell.

Cells exposed to factors such as radiation, free radicals, toxins and chemotherapeutic agents produce p53, which accumulates in the cells, arresting the cell cycle or triggering apoptosis of the damaged cell. (Fig. 5) It has been found that the gene encoding this protein is mutated in more than 50% of human cancers (21,1).

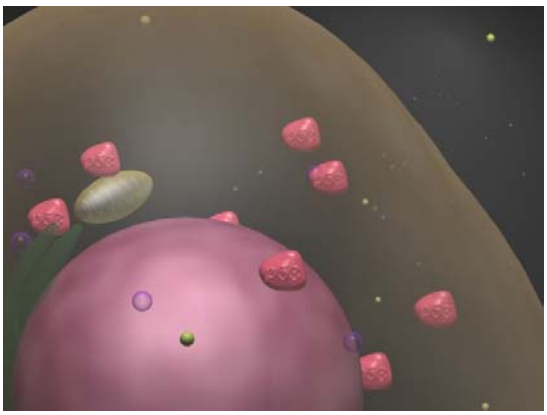


Fig. 5 – P53 is present in a damaged cell.

Research Significance

Apoptotic cell death represents an important mechanism for the regulation of the number of cells in normal tissue (6). Survival of all somatic cells requires the continuous input of survival signals to suppress apoptosis. Deregulation of these signals can cause inhibition of apoptosis, and is a key factor in tumor development (4). A great deal of research is now being done to identify methods of cancer treatment related to apoptosis.

It has been found that in chronic neurodegenerative diseases, caspase-mediated apoptotic pathways have the dominant role in mediating cell death. Also, in acute neurologic diseases, both necrosis and caspase-mediated apoptotic cell death occur (5). Apoptosis plays a pivotal role in the progression of a variety of neurologic disorders such as Alzheimer's, Parkinson's, Huntington's, Amyotrophic Lateral Sclerosis and Multiple Sclerosis.(5)

EXISTING LITERATURE

Since apoptosis is relatively a recently discovered process, there are few illustrations of it and even fewer animations. Most of the illustrations, which accompany texts or journal articles, are diagrammatic and sometimes difficult to understand. Recently, better and more detailed illustrations have been produced, but they can only explain one or two details of the process per illustration whereas a video can focus on several concepts. Video of an actual cell, shot through a microscope, is available, but does little to enlighten the viewer about the details of the apoptotic process. Some

diagrammatic animation of apoptosis has been created, such as in the video, "Programmed Death of a Cell," produced by Films for the Humanities (8), but no examples of 3D animation on this subject are available.

CHAPTER THREE
Methodology

Concept and Planning

The original idea to create a three-dimensional representation of a cell dieing came about after several medical and graduate students expressed a need for better, more interesting visual teaching tools in their cell biology class. After discussing the idea with committee members it was decided that the scope of the project only allowed for the basics of the process of apoptosis to be explained, and that it was best to limit the video to less than ten minutes long. Since the audience consists of post-graduate students a certain level of knowledge of biology and chemistry could be expected.

After these initial decisions were made, it was determined that narration was necessary. Topics to discuss in the video were limited to: a description of the basic characteristics of the process, the importance of caspase enzymes, examples of cell death triggers and the relationship of apoptosis to diseases such as cancer and Parkinson's. The animation would be completed with 3D Studio Max®, and the editing would be done with Adobe Premiere®.

Because of students' ready access to DVD and CD-ROM drives it was decided that the video would remain digital and be available as both a DVD and as a CD-ROM in QuickTime and .avi format. The video would be available for use by students in campus computer labs, or would be shown during lecture.

Hardware

All aspects of this video were produced on a Dell Demension PC with an Intel Pentium® 4 1.70 GHz processor, 1.00 GB of RAM and running Microsoft Window XP.

Software

Narration was recorded using Advanced Sound Recorder®. Storyboard stills and production stills were edited using Adobe Photoshop® 7.0. Animation was produced with 3D Studio Max® 5.0. Editing was accomplished with Adobe Premiere® 6.0. CD-ROMs were burnt using Roxio Easy CD Creator® 5.0.

Research

Research was done on several topics. Available illustration, video and literature on apoptosis, visual learning modalities and methods of 3D animation were all researched prior to production of the video.

To assure the accuracy of the video and to assure that it would be up to date with the current research, journal articles, books and websites that dealt with the topic were used. John Abrams, PhD, Associate Professor Department of Cell Biology at UT Southwestern, was able to answer questions and to give feedback. Xiaodong Wang ,PhD, from the department of Biochemistry, was additionally contacted with specific questions about the binding of the elements of the apoptosome.

Research on teaching using audiovisual-based systems was also done. Representational images, such as the cell itself and the various molecules, were kept as simple as possible so as not to detract from the objective of the video. A dramatic opening scene was placed in the video to gain the audiences' attention. Similar images and colors were used throughout the video in order to establish a feeling of continuity. Narration was added to aid in the explanation of the process, and was kept clear and concise. And attention-focusing devices such as zooming in on important features and use of labels were also used.

The best way to build and animate the models necessary for the video also required some research. Several online sources and bulletin boards designed for 3D Studio Max® users were used. The help file that accompanies the software was invaluable for solving quick problems, and texts that outlined in detail some of the animation processes help to determine the most efficient method of animation.

In order to complete the editing Adobe Premiere® was used. To become more comfortable with this program Adobe Press's Adobe Premiere Classroom in a Book was utilized. This book included lessons on all of the important features and a CD of examples to work on. This was a quick and easy way to become familiarized with this program.

The Script

The script began as an outline of the topics to be discussed in the video. A quick introduction was followed by an explanation of the process, possible triggers,

relationship of cell death to disease and a simple conclusion. The outline was then fleshed out and each topic was given a few sentences or a short paragraph.

Each committee member reviewed and suggested revisions for the script. Language was chosen that would convey the concept in a accurate, efficient way, and still take into consideration the education level of the audience. Many changes were made before the final script was ready for narration, and when read aloud the script was approximately five minutes long. (Appendix A.)

The Narration

The narration was recorded using Advanced Sound Recorder 3.1 and the M-560 Super-Directional USB Digital Microphone from Telex®. Original problems with background noise were solved by upgrading the Windows Sound Recorder to Advanced Sound Recorder 3.1, and by investing in a higher quality microphone. The narration was recorded in one or two sentence segments and saved as .wav files. Chance Litton was chosen as the narrator because, as the author's husband, he was readily available to make changes or corrections in the narration.

After recording the segments of narration, it was put together in Adobe Premiere®. Long pauses were edited out and the final length of narration was six minutes and three seconds.

The Storyboard

The script was used to create a storyboard, which is a series of simple illustrations depicting key events or action in the video. (Fig. 6) One or two sentences of script were written under each illustration as well as direction for transitions or effects. These storyboards were discussed during committee meetings and visual changes were made accordingly. (Appendix B)

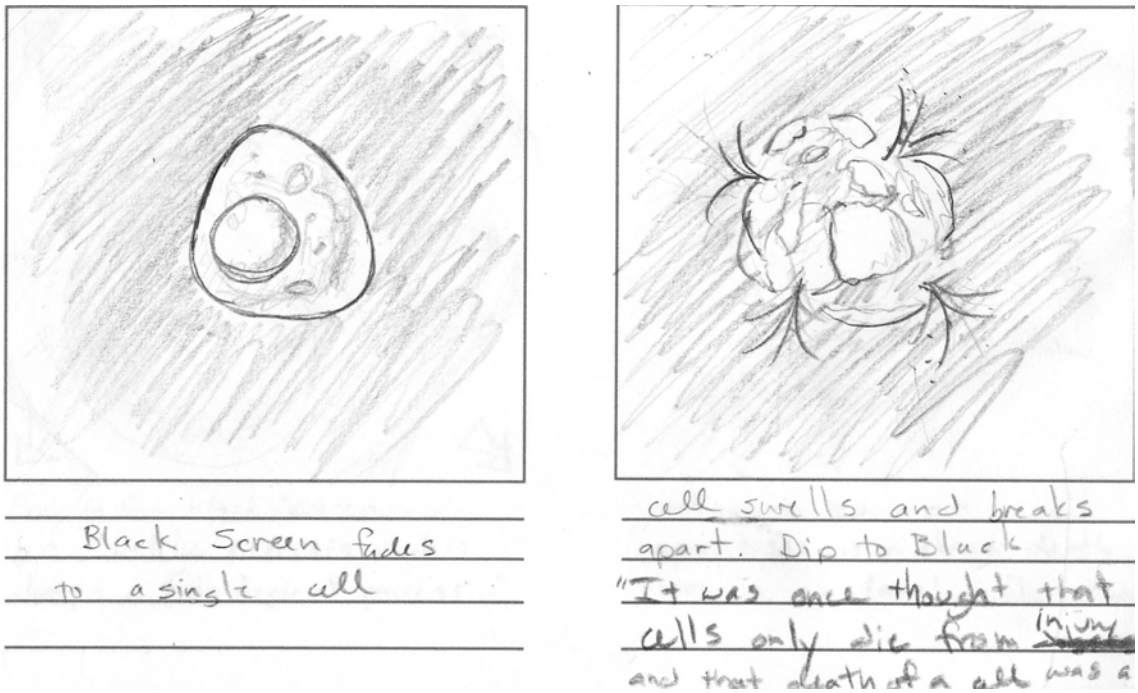


Fig. 6 – Storyboards were created using sketches of key events.

The Anamatic

Anamatics have been used by Hollywood directors to better visualize the movies they are creating. Stills of the storyboards drawn by artists and rough low polygon count animation is edited together with sound. By doing this, changes can be made early in the production process, saving time and money.

The anamatic became an invaluable tool during the production process of the video. By scanning the storyboard stills and placing them as still frame images into the editing software along with the narration, a narrated storyboard was created. Scene length was simple to determine, and an overall picture of the final product was taking shape. As preliminary model building and rough animation was begun, storyboard stills were replaced by early clips. And, as final animation was completed it simply replaced the stills or the earlier clips. (Fig. 7) This cut down on editing time, and kept everything organized and in place.

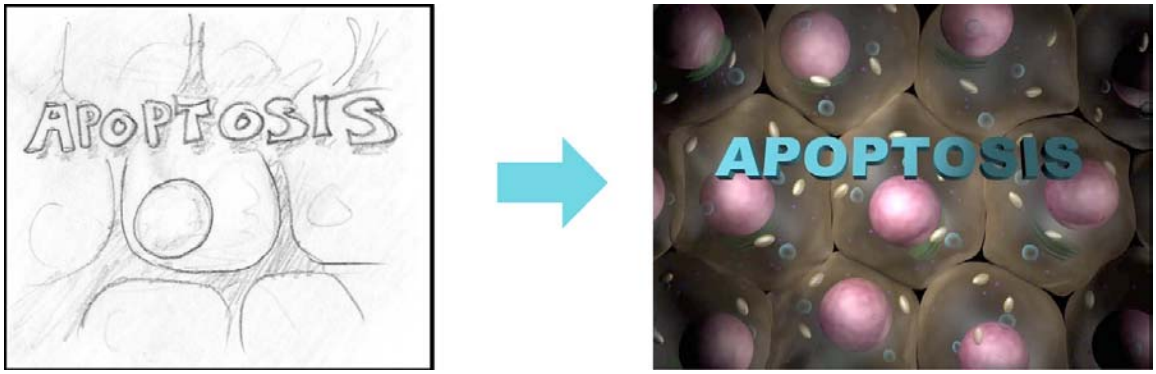


Fig. 7 – The storyboard contained the ideas that would soon be animated.

The Animation

All animation was created using 3D Studio Max®. Models were created as well as some maps for use as materials. Animation was accomplished by several methods, and some still images were taken. All animated sequences were rendered as .avi files for use in the editing software.

Model building

The main model to be built was the cell. (Fig. 8) A sphere manipulated by editing the mesh into an irregular shape was used to create the cell membrane, then another was used as the nucleus. Various other shapes were used to represent organelles within the cell. Manipulated spheres were used for the vacuoles, ribosomes and outer mitochondrial membrane. Splines were lathed to make the inner mitochondrial membrane, and a mesh-smoothed rectangle was copied several times to make the Endoplasmic Reticulum.

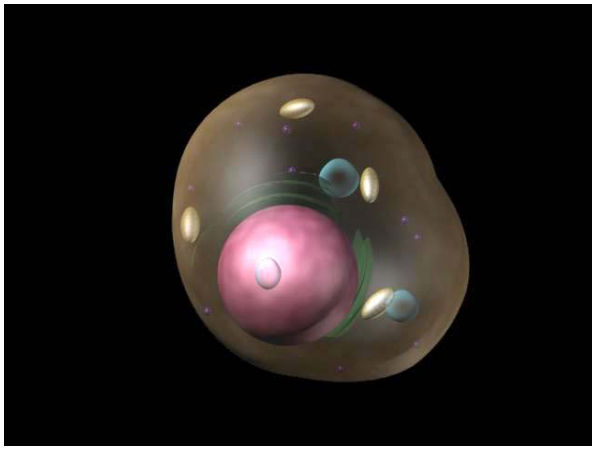


Fig. 8 – The cell was the most complex model to be animated.

Molecules such as the caspase enzymes, Bcl-2 and Apaf-1 were also manipulated from basic shapes such as spheres and cylinders. (Fig. 9) This uncomplicated method of model building resulted in lower polygon count models which made the files smaller and quicker to render.

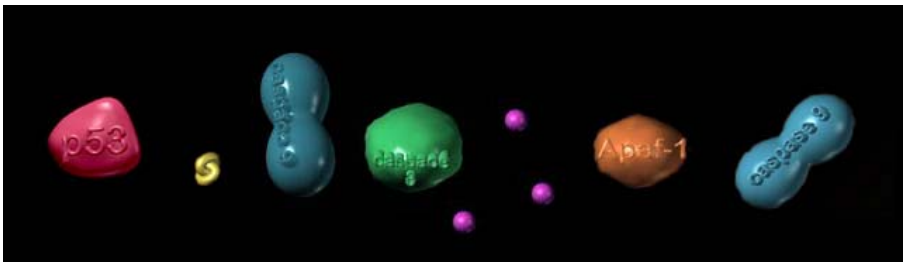


Fig. 9 – Molecules were built using simple shapes.

The leaf was made by drawing the shape as a spline and then using the face extrude command. The edit mesh command was then used to make the sides curl up in a more realistic way. (Fig. 10)

Fig. 10 – The leaf was created by using a spline to draw the shape.

Materials and Maps

For the cell, the membrane was given a material with 100% fallout. This accounts for the transparent appearance. A smoke map was also used to make the membrane more organic. The nucleus was also given a smoke map, but remained completely opaque. All other organelles were set at 100% fallout as well, so they didn't appear completely solid.

All molecules were made with bright colors with high glossy and specular highlight levels. A drawing created in Adobe Photoshop® of a brown leaf was used as a map in the diffuse color channel of each leaf model to make a more realistic looking leaf.

Manipulating the Models

Most animation in the video was simple movement. This was simple to accomplish using the auto set animation button, the timeline and the movement or rotation tool.

The most complicated animation was used for the blebbing sequence and for the phagocytosis sequence. The cell membranes were manipulated using free-form deformations or FFDs. This simple type of space warp provides a method of deforming an object by adjusting the control points of a lattice. By binding a cylindrical FFD to the cell membrane small areas of the object surface were then manipulated to obtain the desired effect. (Fig. 11)

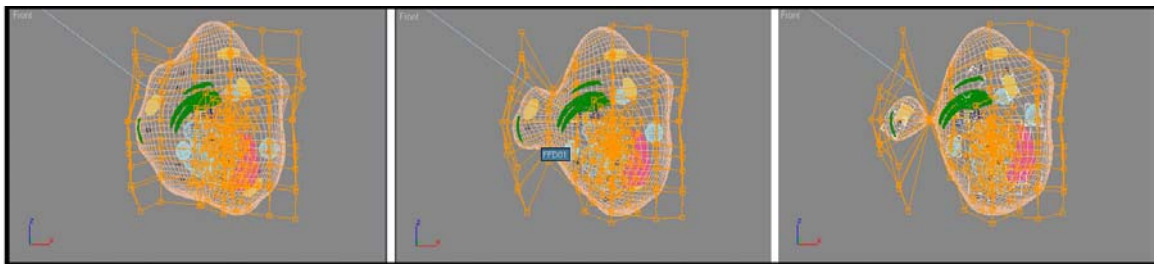


Fig. 11 – FFDs were used to animate the cell membrane.

Some animation was also done in the material editor. To create the illusion of condensing chromatin in the nucleus, the first color in the smoke map was faded from dark pink to black, and the size was decreased. The color in the diffuse color channel was changed

abruptly to signify enzyme activation, and color saturation was decreased during the opening necrosis scene.

Super-spray particle systems were used also used as animated effects. (Fig. 12) Seven super-sprays were used in the necrosis scene, with particle size set low. One super-spray was used to create the radiation in the scene explaining p53, with the particle size set higher.

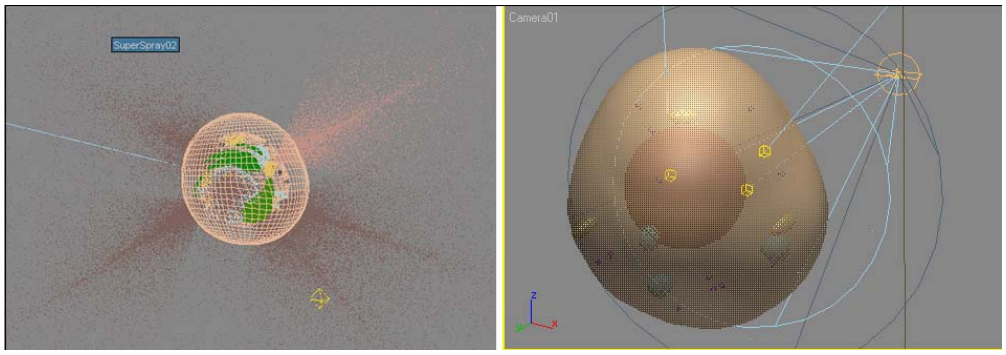


Fig. 12- Super Sprays were used for the necrosis and radiation scenes.

Still Images

Several still images were also used in the production of the video. Jpeg images of a CT scan were placed to illustrate disease associated with apoptosis, and stills taken from earlier animation were used during the conclusion of the video.

Rendering

Since hard drive space was not limited, and quality was important, all animation sequences were rendered from 3D Studio Max® as full-frames uncompressed .avi files. While this method produced higher quality clips, it is not recommended for those working with limited storage space. After rendering, the clips were ready to be placed in the editing software with the narration.

Editing

Editing for the video was done using Adobe Premiere® software. Narration was brought in as .wav files, animation was brought in as .avi files and stills were placed as .jpg files.

Editing progressed as the video process progressed. The first files to be placed were the narration files. These were put in the proper order, and dead space and errors were edited out. Then storyboard stills were brought in and synchronized with the narration. Rough animation and stills of built, but not animated models were placed next in some scenes. Finally, as each scene was completed it was brought in and dropped in place. Each completed scene replaced either a storyboard still or an earlier, rough version of the same scene. Transitions such as cross-dissolves were added between scenes as needed.

Finally, the timeline was exported as a QuickTime movie and an .avi file. For producing the QuickTime movie the frame size was set at 640x480 at 15 frames per second. Sorenson Video 3 was used at 90% quality for compression. For production of the .avi file frame size and frames per second remained the same, but Intel Indeo® Video 4.5 was used for compression, also at 90% quality.

CD-ROM Creation

Animation was recorded to a compact disc using a high speed rewritable drive. Roxio Easy CD Creator® 5 software was also used.

DVD Creation

Animation was also recorded to a DVD using a DVD writer and iDVD software.

CHAPTER FOUR
Analysis

It was the goal of this project to create an informational video using 3D animation and including narration to introduce the fundamentals of this important process to students of a post-graduate level.

After all animation and editing were completed, the final video was presented to a group of sixteen first-year medical and graduate students at the Medical University of South Carolina. They were then given an informal survey to complete. (Appendix C) All of the students claimed to have little knowledge of apoptosis before viewing the video. The questionnaire asked two questions pertaining to how helpful or effective they thought the video was, one question about the student's access to a CD-ROM or a DVD player and two final knowledge questions to test the students' understanding of the information presented.

The first question presented to the students was*, On a scale of five, one being the least effective, how would you rate the effectiveness of this video to introduce the basic concept of apoptosis?* Of the sixteen students who participated, thirteen gave the video a 5 and three students gave the video a 4.

The second question was, *On a scale of five, one being the least helpful, how helpful do you believe this video would be as supplementary material for a lecture on apoptosis?* Fourteen of the sixteen students gave the video a 5 and two gave the video a 4.

Next the students were asked if they had access to a CD-ROM or a DVD player. One hundred percent of the students answered yes to this question.

The next two questions were designed as an informal test of the student's knowledge. The first of these questions asked, *Name the two ways in which cells die?* Two blanks were provided for the student to write his or her answer. One hundred percent of the students wrote *apoptosis* as one of the answers. For the second method ten of the students wrote *necrosis* and six wrote *injury*. Both of these answers were acceptable.

Finally the students were asked, *What family of proteins is thought to cause the break down of the cell in apoptosis?* All students answered the question correctly, writing *caspase* in the blank.

Overall the students gave the video an average score of 4.81 out of 5 in effectiveness, and a 4.87 out of 5 for helpfulness. All of the students had access to a CD ROM or a DVD player, and all of the knowledge questions were answered satisfactorily.

While the questionnaire was informative, it is important to remember that this was a very informal study done on a very small group. The goal of creating an informational video using 3D animation to explain the basics of apoptosis was met. The video was produced, but formal evaluation of the video would require a much larger audience, and a more specific questionnaire.

CHAPTER FIVE
Conclusion

Conclusion

In 2002 the Noble Prize for medicine was awarded jointly to: Sydney Brenner**,** H. Robert Horvitz and John E. Sulston for their discoveries concerning genetic regulation of organ development and programmed cell death. Research in this field has been growing rapidly in the last decade and new discoveries are being made everyday. The goal of this thesis was to produce a video that included narration and 3D animation to explain the process of apoptotic cell death to students beginning their medical or graduate studies.

The final product was a video, available on a CD or DVD, with 3D animation and narration. (Fig. 13) Animation was created on 3D Studio Max® and editing was completed using Adobe Premiere®. Topics discussed included a description of the basic characteristics of the process, the importance of caspase enzymes, examples of cell death triggers and the relationship of apoptosis to diseases such as cancer and Parkinson's.

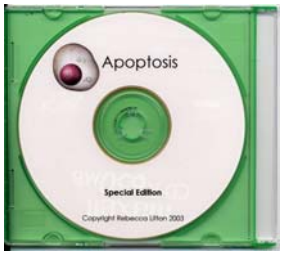


Fig.13 – The CD and DVDs were given labels and cases.

An informal questionnaire was given to a small group of students who agreed that the video was both effective as a teaching tool and would be helpful as supplementary

material for a lecture. The students were also able to answer two knowledge questions correctly after viewing the video and all claimed to have access to a CD-ROM or a DVD player.

Further Study

This video covers only the basic concepts of one cellular process. Each concept presented on this video could be expanded and explained in depth. Videos could also be produced to explain other cellular processes. An entire series could be completed with a video explaining the basics of processes such as mitosis, necrosis, DNA replication and membrane transport.

A video is not always the best way to introduce material. The information presented in this video could easily be presented in print media or as web pages. These methods combined with the video could reach more students.

The animation used in this video could also be used as small segments without narration. These segments could be used in a lecture, and designed to aid the instructor as he or she speaks.

Actual video and still shots of cells could be added to give the students an idea of what the process really looks like.

Further study could also be done on the effectiveness of this or other videos to teach the concepts they are designed to teach.

APENDIX A

THE FINAL SCRIPT

It was once thought cells only die from injury and the death of a cell was always detrimental to the organism. We now know a natural and controlled process of cell death is necessary for a healthy organism to rid itself of damaged or unnecessary cells. This process is called programmed cell death, or apoptosis, from the Greek meaning, “to fall off”, referring to leaves falling off trees.

Unlike necrosis, which is always a pathologic process, apoptosis is a clean death. There is no local inflammation or harm to the organism.

In the early stages of apoptosis, the cell will shrink and lose contact with surrounding cells.

Within the nucleus, chromatin condenses near the inside of the nuclear envelope. DNA strands are cleaved in the intervening segments between the nucleosomes called the spacer region, creating fragments of DNA characteristic of apoptosis.

Next, blebbing begins as the cell breaks into membrane bound fragments, with organelles generally remaining intact. These fragments, called apoptosomal bodies, remain membrane bound and no intracellular material is ever in contact with the extracellular space.

Phosphatidylserine, a protein found in the cell membrane, then changes orientation and becomes exposed on the fragment surface. This is one of many known flags that will attract a macrophage, or in some cases such as the liver, it will cause neighboring cells to engulf and degrade the fragments. Finally, all material is broken down in the phagocytotic cell.

The latest research has found that activating and inhibiting a family of proteins known as caspase enzymes affects the process of apoptosis. Caspase enzymes are specialized proteases which are dormant in healthy cells but when activated have been found to cause the activation of a caspase cascade. When cells are specified to die, caspases are converted to an active state and can cleave other proteins in specialized ways. This is a complicated series of biochemical interactions, ultimately causing the breakdown of the cell by cleavage of cytoskeletal and nuclear material.

There are many known triggers of this caspase cascade which lead ultimately to apoptosis. Withdrawal of hormones and survival factors is a current theory. Cell death elicited by these triggers is believed to involve the Bcl-2 family of proteins and Caspase 9. Bcl-2 proteins are often located on the outer mitochondrial membrane and function to promote the release of cytochrome c from the mitochondria. The result is a complex of cytochrome c, Apaf-1, caspase 9 and ATP called an apoptosome. Caspase 9 is now active and begins a caspase cascade, which leads to apoptosis.

Apoptosis can also be triggered by specific receptor/ligand reactions. For example, the FAS ligand, produced by cytotoxic T cells of the immune system, binds to the FAS receptor on the surface of a virus infected cell and with the help of FADD, an adapter protein, activates caspase 8, which then activates the caspase cascade causing apoptosis. Another example of receptor-ligand mediated apoptosis involves the tumor necrosis factor (or TNF) receptor also produced by cells of the immune system.

Cells exposed to factors such as radiation, free radicals, toxins and chemotherapeutic agents produce p53. It has been found that the gene encoding this preotin is mutated in more than 50% of human cancers. p53 accumulates in the cells, arresting the cell cycle and or triggering apoptosis of the damaged cell.

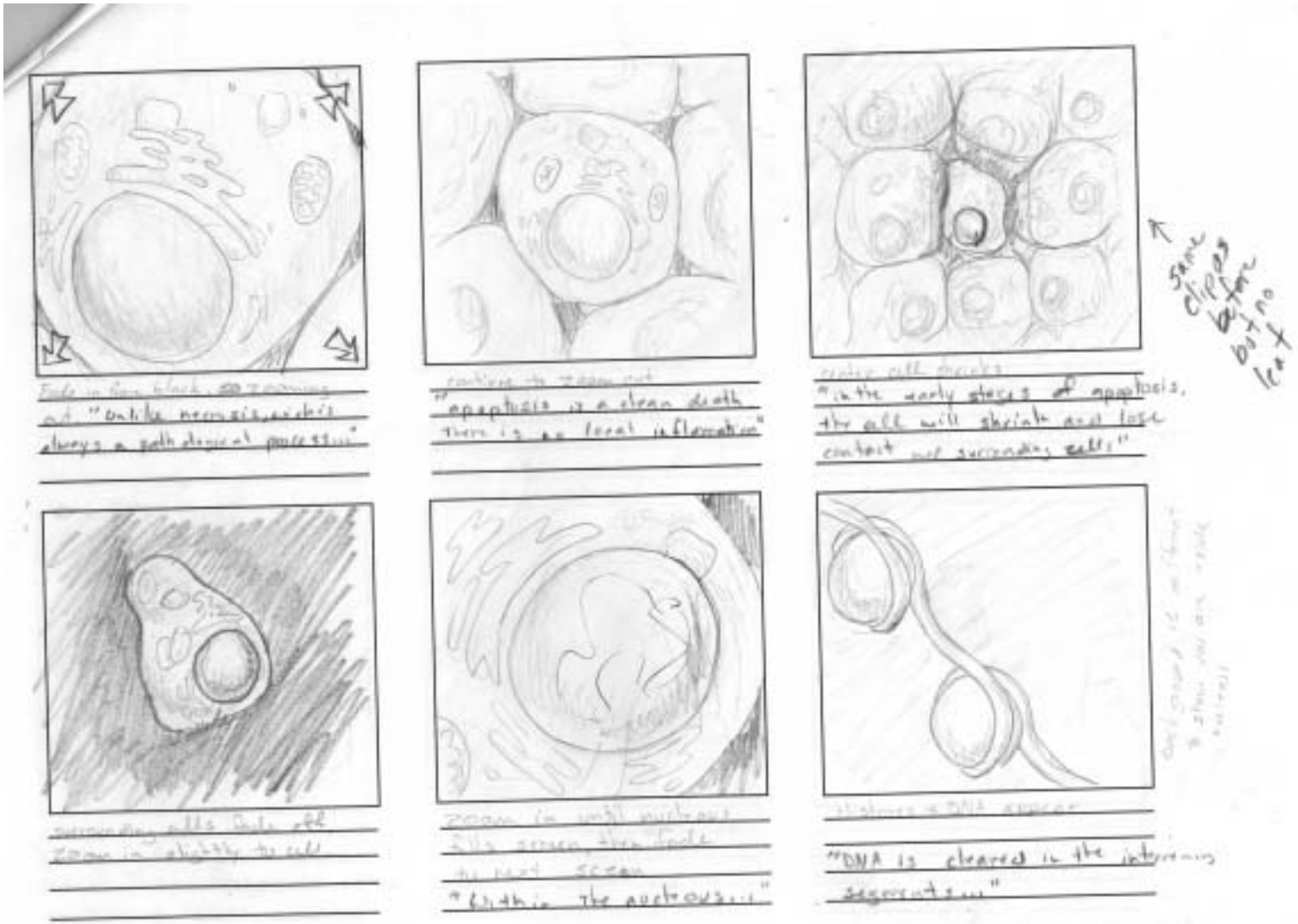
When certain factors alter the apoptotic processes, diseases can result. For example, cells can lose the ability to initiate apoptosis- a condition that often leads to cancer. Alternatively, brain cells in neurodegenerative diseases such as Parkinson's and Alzheimer's are victims of premature cell loss due to the same apoptotic process.

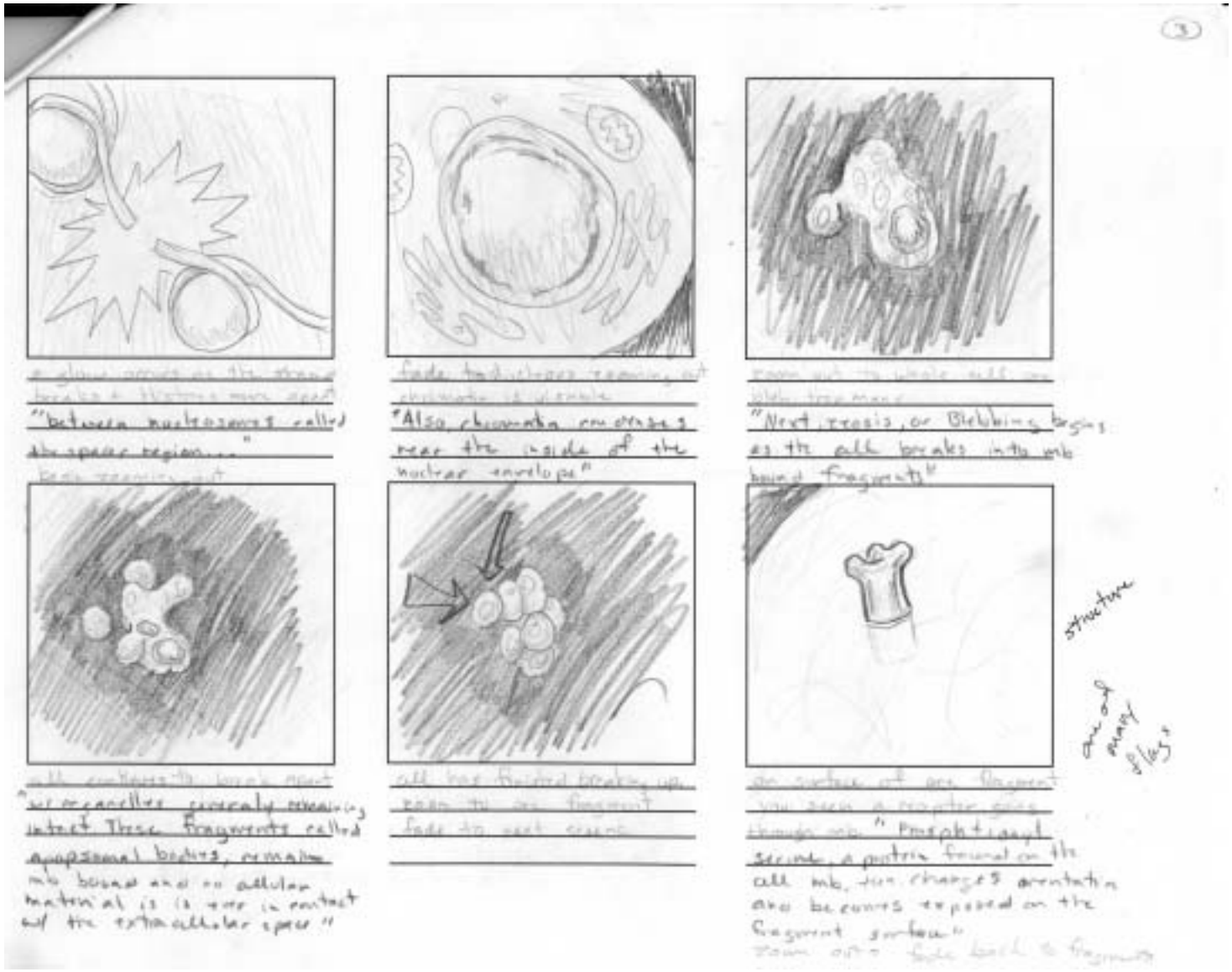
Apoptosis is an integral cellular process for a healthy organism. It allows the organism to rid itself of damaged or unnecessary cells. It has been discovered when the normal apoptotic process is altered the organism can become diseased. The importance of this process is becoming more and more apparent as research continues.

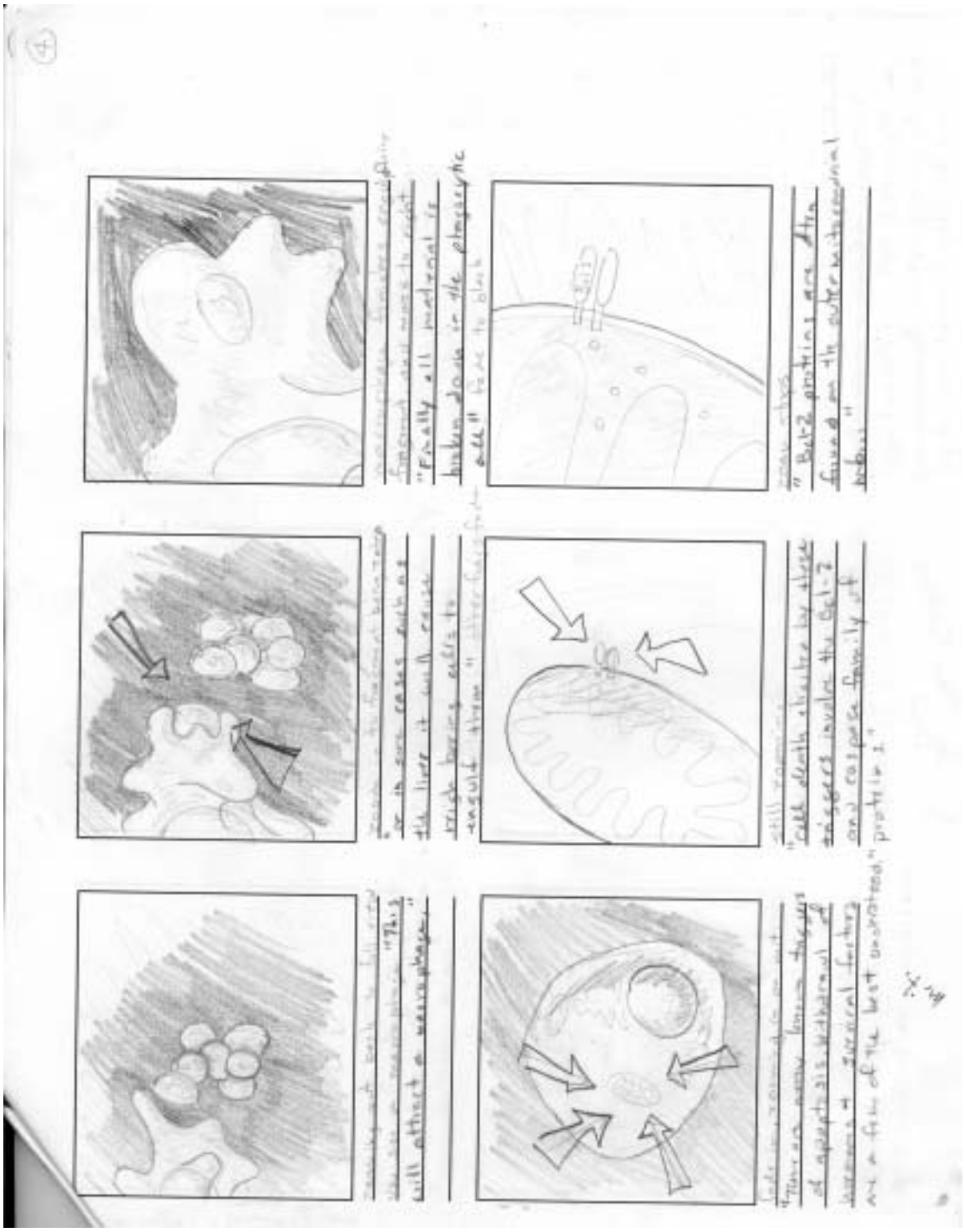
APENDIX B

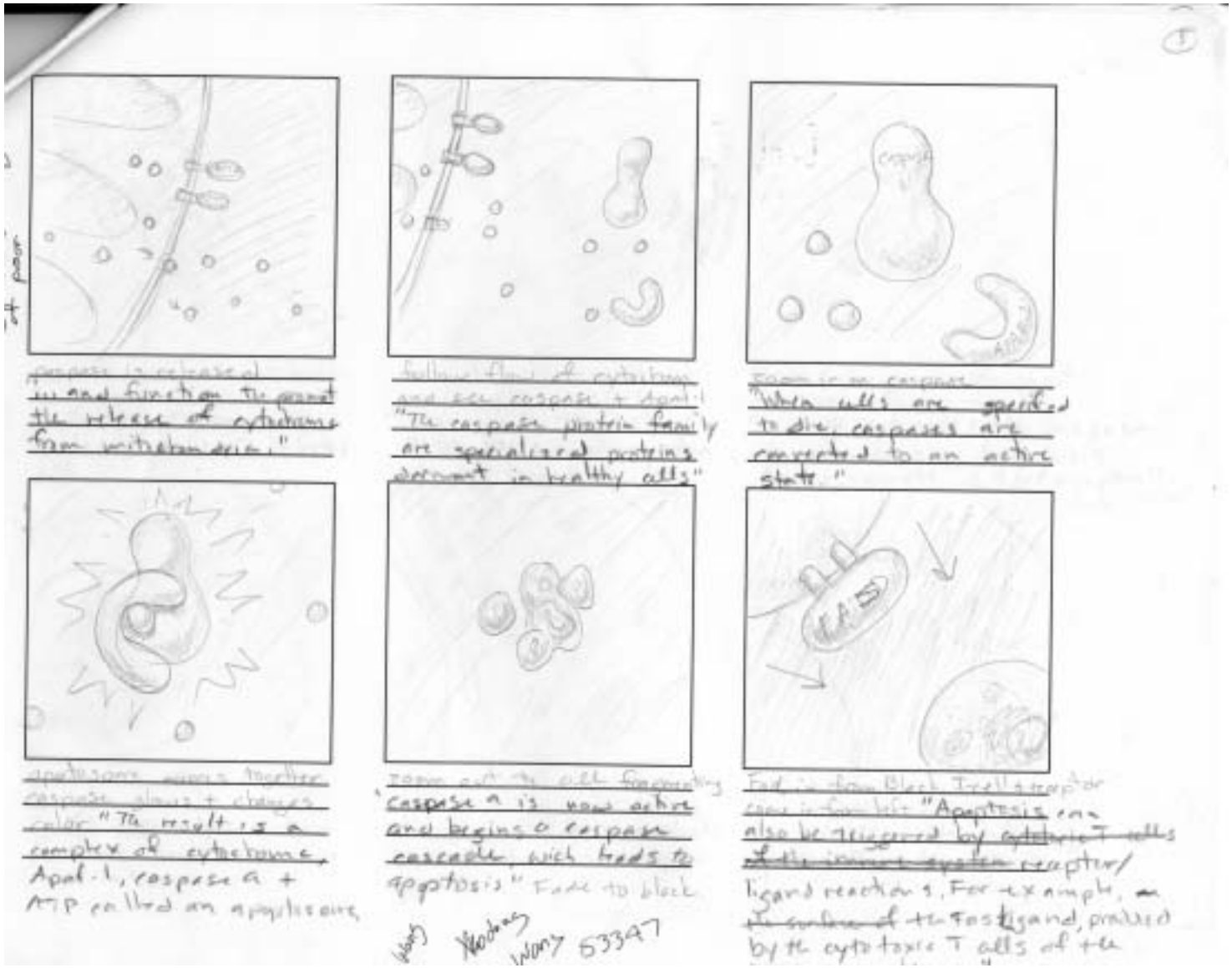
STORYBOARDS

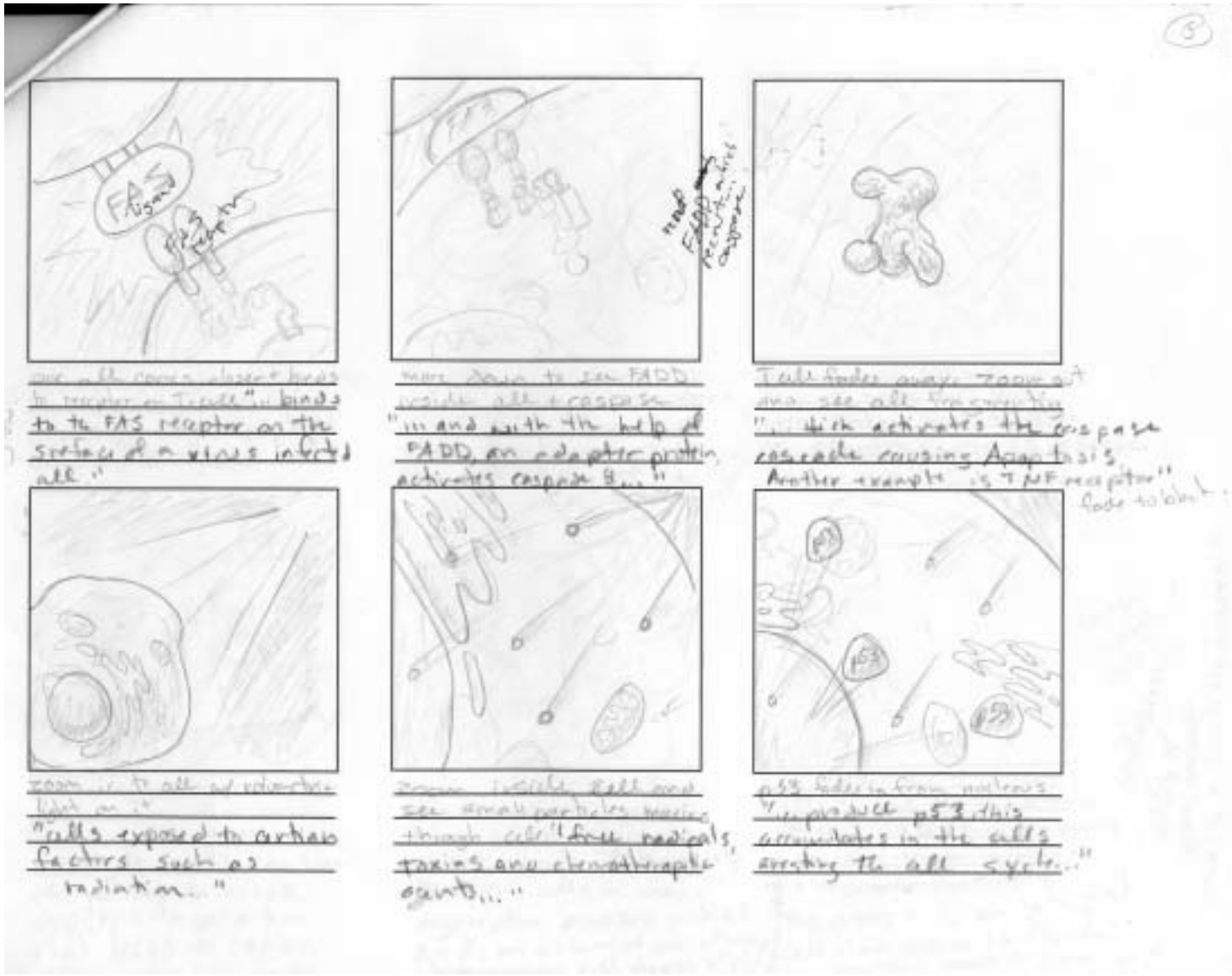
APOPTOSIS


Fade in from black, so zooming out "Unlike necrosis, which is always a pathological process..."
continue to zoom out
"apoptosis is a clean death there is no local inflammation"
center cell shrinks
"in the early stages of apoptosis, the cell will shrink and lose contact w/ surrounding cells"
same clips before but no leaf
surrounding cells fade off zoom in slightly to cell
zoom in until nucleus fills screen, then fade to next scene
"within the nucleous..."
histones & DNA appear
"DNA is cleared in the intervening segments..."

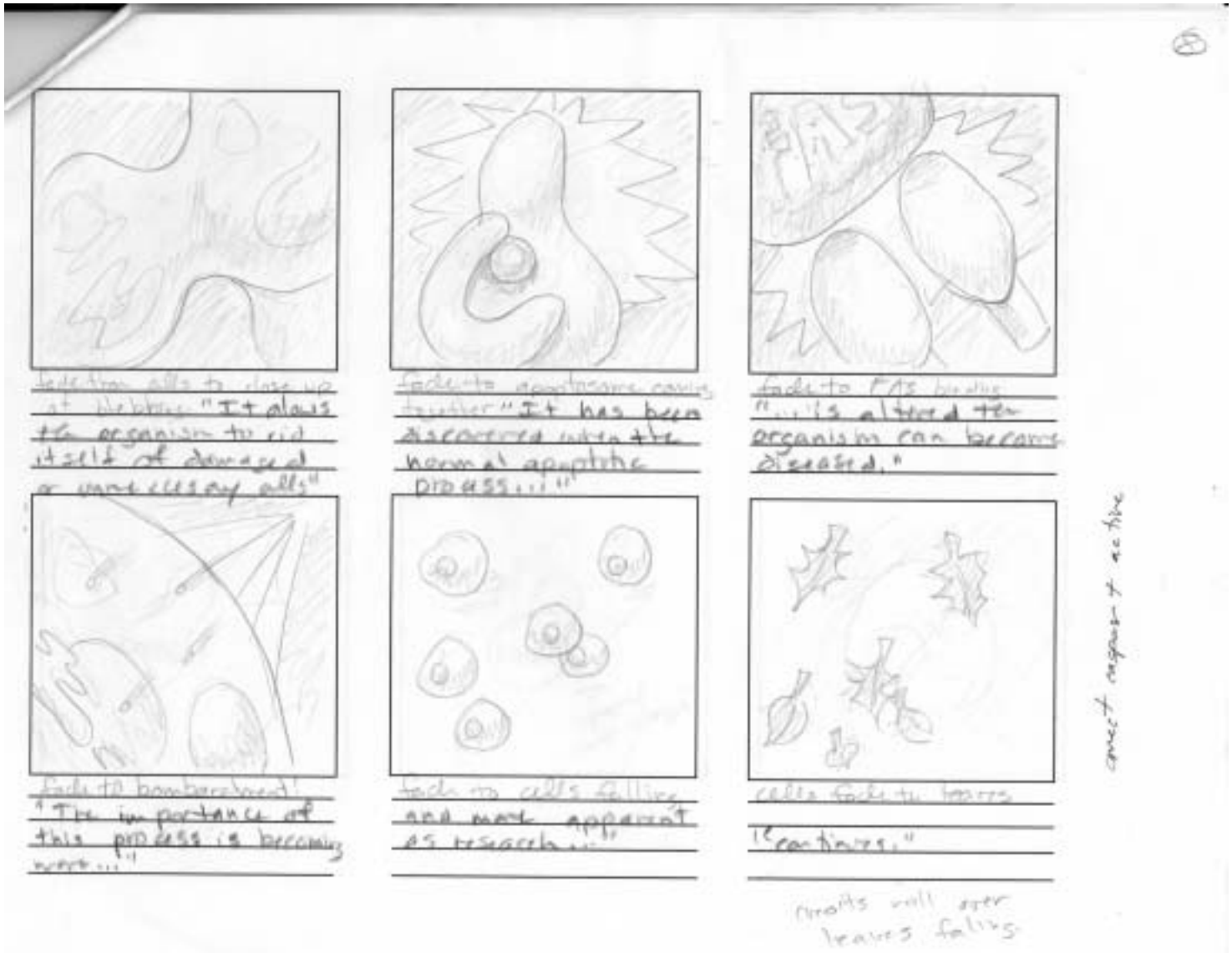

3
a glow appears as the strand breaks & Histone mon appear
"between nucleosomes called the spacer region..."
fade, last stages removing all chromatin is visible
"Also, chromatin concentrates near the inside of the nuclear envelope"
from out to whole cell see with two more
"Next, crasis, or blebbing begins as the cell breaks into membrane bound fragments"
with contents to break apart
"nucleolus viscerally remaining intact These fragments called apoptosomal bodies, remain membrane bound and no cellular material is in close contact w/ the extracellular space"
cell has finished breaking up, zoom in on fragment fade to next scene
on surface of one fragment you see a receptor going through one. "Phosphatidyl serine, a protein found on the cell mb, then changes orientation and becomes exposed on the fragment surface"
structure
one of many flags
from out - fade back to fragments






FAS
FAS receptor
more down to see FADD inside all + caspase
"... and with the help of FADD, an adapter protein, activates caspase 8..."
"... tick activates the caspase cascade causing Apoptosis. Another example is TNF receptor"
"cells exposed to certain factors such as radiation..."
"free radicals, toxins and chemotherapic agents..."
p53 fades in from nucleus
"... produce p53, this accumulates in the cells arresting the cell cycle..."

MRI
CSCAN
MRI
CSCAN


8
fade from cells to close up of blebbing "It allows the organism to rid itself of damaged or unnecessary cells"
fade to apoptosome coming together "It has been discovered when the normal apoptotic process..."
fade to FAS binding "...is altered the organism can become diseased."
fade to bombardment! "The importance of this process is becoming more..."
fade to cells falling and more apparent as research..."
cells fade to leaves "continues."
credits roll over leaves falling
correct response + active

APENDIX C

QUESIONNAIRE

Apoptosis Video Questionnaire

1. On a scale of five, one being the least effective, how would you rate the effectiveness of this video to introduce the basic concept of apoptosis?

1 2 3 4 5

2. On a scale of five, one being the least helpful, how helpful do you believe this video would be as supplementary material for a lecture on apoptosis?

1 2 3 4 5

3. Do you have access to a C-ROM or a DVD player?

yes no

4. Name the two ways in which cells die?

_____ _____

5. What family of proteins is thought to cause the break down of the cell in apoptosis?

_____

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