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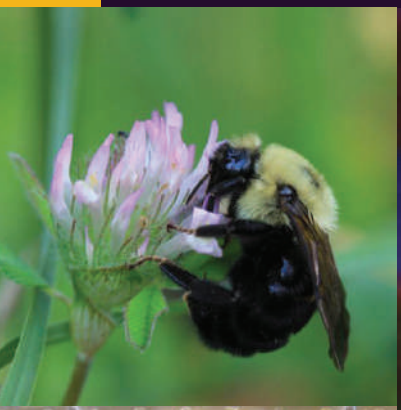

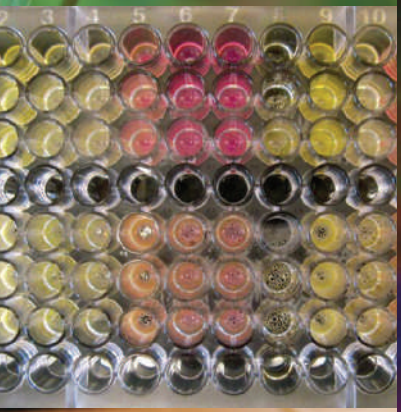

Animal Physiology

A. Malcolm Campbell
Christopher J. Paradise

MOMENTUM PRESS
APPLIED SCIENCES

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Abstract

This book examines four examples of animal physiology that illustrate emergent properties in whole organisms. The first example shows how mammals coordinate the activity of all their cells using a daily rhythm. The second case explains an apparent contradiction that happens every time a woman gets pregnant and delivers a healthy baby—how the immune system tolerates a foreign tissue such as the fetus. The next case study in this book shows how bodies regulate the amount of fat using a complex interaction of proteins that function as a lipostat, a self-regulating fat maintenance system. Finally, the book provides an understanding of why some species live long lives while others die after very short lives, and under what conditions each situation is favored. What is evolutionarily adaptive about death? These four case studies provide sufficient evidence to understand how animals regulate many of their own metabolic functions.

Keywords

allografts, antagonistic pleiotropy, autograft, cerebrospinal fluid, choroid plexus, circadian, disposable soma hypothesis, dopamine, eclampsia, emergent property, endometrium, eusocial, first-set rejection, graft, homeostasis, leptin, lipostat, melatonin, MHC I, natural killer, negative feedback loop, orthologs, pair-fed, parabiotic, placenta, positive feedback loop, preeclampsia, second-set rejection, senescence, suprachiasmatic nucleus, trophoblast

Contents

Preface

This book about animal physiology is part of a thirty book series that collectively surveys all of the major themes in biology. Rather than just present information as a collection of facts, the reader is treated more like a scientist, which means the data behind the major themes are presented. Reading any of the thirty books by Campbell and Paradise provides readers with biological context and comprehensive perspective so that readers can learn important information from a single book with the potential to see how the major themes span all size scales: molecular, cellular, organismal, population and ecologic systems. The major themes of biology encapsulate the entire discipline: information, evolution, cells, homeostasis and emergent properties.

In the twentieth century, biology was taught with a heavy emphasis on long lists of terms and many specific details. All of these details were presented in a way that obscured a more comprehensive understanding. In this book, readers will learn about four case studies (the mammalian circadian clock, immune tolerance of fetuses, obesity and the lipostat, and why all organisms age) and some of the supporting evidence behind our understanding. The historic and more recent experiments and data will be explored. Instead of believing or simply accepting information, readers of this book will learn about the science behind animal physiology the same way professional scientists do—with experimentation and data analysis. In short, data are put back into the teaching of biological sciences.

Readers of this book who wish to see the textbook version of this content can go to www.bio.davidson.edu/icb where they will find pedagogically-designed and interactive *Integrating Concepts in Biology* for introductory biology college courses or a high school AP Biology course.

Acknowledgments

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Kristen Eshleman, Paul Brantley, Bill Hatfield and Olivia Booker helped us with technology at Davidson College. We are grateful to administrators Tom Ross, Clark Ross, Carol Quillen, Wendy Raymond, Verna Case, and Barbara Lom who had confidence in us and encouraged us to persist despite setbacks along the way.

These books were the product of the shared labor of my two visionary coauthors Laurie Heyer and Chris Paradise. We shared the dream and the hardships and developed this book from scratch. My family has been very supportive and I thank Susan, Celeste and Paulina for their support and patience. I also want to thank Jan Serie, my pedagogical mentor, who taught me so much about the art and science of helping students learn. I benefited from the support of the Howard Hughes Medical Institute grant 52006292, the James G. Martin Genomics Program, and Davidson College. This book would not have survived its first draft without my students who endured the typos and the early versions of this book. These undergraduates participated in a bold experiment to see if beginners could construct their own knowledge, retain what they learned, and transform the way they see themselves and the discipline of biology. While many people said that beginning students were not up to the task, my students proved them wrong.

Introduction

René Descartes's famous quote, "I think, therefore I am" is a powerful statement on emergent properties. A person exists because he or she has consciousness and can think. A human brain can be described in terms of cellular functions and biochemical reactions. Yet describing a brain does not explain the emergent property of human consciousness. Brains perform at a level that exceeds the functions of individual neurons. Truly, it would not be possible to predict that consciousness would arise from all the components that make up the human brain.

This book explores four case studies and three ethical, legal, social implications. The first case considers evidence that contributed to our understanding of the mammalian circadian rhythm. The second case confronts a logical contradiction that happens every day. How can a mother's immune system tolerate a fetus that 50% different from the cells of her own body? In line with Descartes, the reader will contemplate how to define individual organisms (mother and fetus) when the lines are blurred. The third case study addresses a topic very familiar but rarely studied in biology classes. The chapter will examine data that illuminate the emergent properties inherent in body weight homeostasis. The final case study takes on another topic debated by philosophers. Why do we age and die when these processes seem counter to the principle of evolution?

CHAPTER 1

Mammals Have Cellular Clock Mechanism

Diverse multicellular organisms (such as, insects, plants, and mammals) have evolved three distinct molecular circadian clock pathways that share some overall features. This chapter focuses on the mammalian molecular clock in part because it allows the reader to consider genetic variations in the human population and the effects different alleles could have on personal sleep patterns. The mammalian clock mechanism is much more complicated than the KaiABC clock in cyanobacteria. The best way to understand the mammalian mechanism is to walk through it slowly and don't let the names of the proteins become distracting (Figure 1).

Analysis of the mammalian **circadian** mechanism begins with two genes called *Clock* and *BMAL1* that encode proteins by the same names. Like all proteins, Clock and BMAL1 are translated in the cytoplasm, but they return to the nucleus and form a heterodimer transcription factor that initiates transcription of many genes. Clock/BMAL1 heterodimers bind to specific promoters and induce the production of two families of proteins called Cry1 and Cry2 as well as Per1, Per2, and Per3. *Period* (*Per*) genes are **orthologs** of the fly proteins identified by Benzer and Konopka in 1971. The first **negative feedback loop** in the mammalian system occurs because the Cry/Per protein complex binds to and inactivates the Clock/BMAL1 transcription factor. By inactivating Clock/BMAL1, the mammalian cells produce fewer Cry and Per proteins. This form of self-regulated Cry and Per protein production via negative feedback is an **emergent property** seen in all four chapters of this book. Clock/BMAL1 also stimulates the production of Aanat, which will be addressed later. Clock/BMAL1 also leads to the production of two opposing proteins called Rev-Erbα and Rora. Rora is part of a **positive feedback loop**

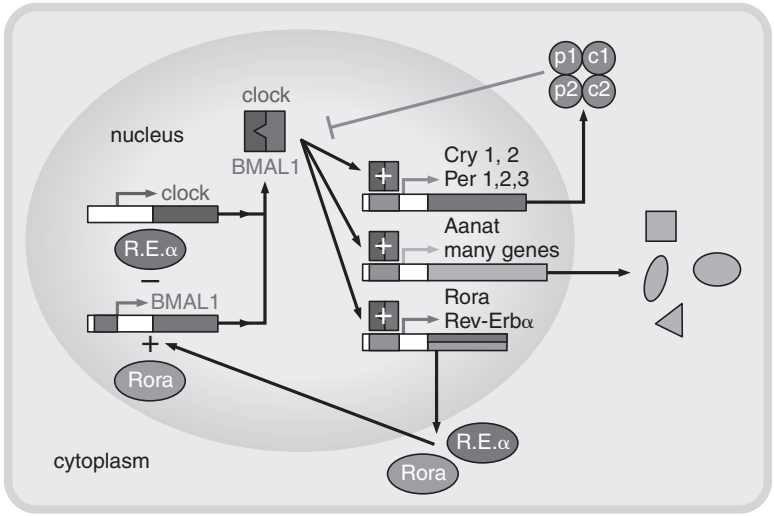

Figure 1 Mammalian circadian time keeper proteins. Clock and BMAL1 proteins increase at sunrise, activate* Cry, Per, Aanat, Rora, *and* Rev-Erbα *genes. Several positive and negative feedback loops compete and counteract each other to produce a daily cycle.

Source: Modified from Tosini *et al.*, 2008, figure 1.

because it stimulates the production of BMAL1, which stimulates the production of Rora. Rev-Erbα is part of a competing negative feedback loop, because Rev-Erbα blocks the production of Clock/BMAL1 and thus Rev-Erbα's own production. The positive feedback loop and the competition between Rora and Rev-Erbα are two more examples of emergent properties. In the mammalian system, Rev-Erbα and Rora compete for the same binding spot on the BMAL1 promoter. The combination of Rev-Erbα and Cry/Per eventually leads to reduced amounts of Clock/BMAL1 protein until the next morning when the heterodimer accumulates again.

Based on Figure 1, the mammalian circadian clock contains the emergent properties of positive and negative feedback loops and molecular competition. Nearly every cell in the human body maintains its own Clock/BMAL1 molecular mechanism, but circadian systems are not precise and require environmental cues to be maintained. Humans need a way to coordinate 50 trillion cellular clocks and synchronize them with the sun (Figure 2). Mammals use their eyes to see, but also to collect

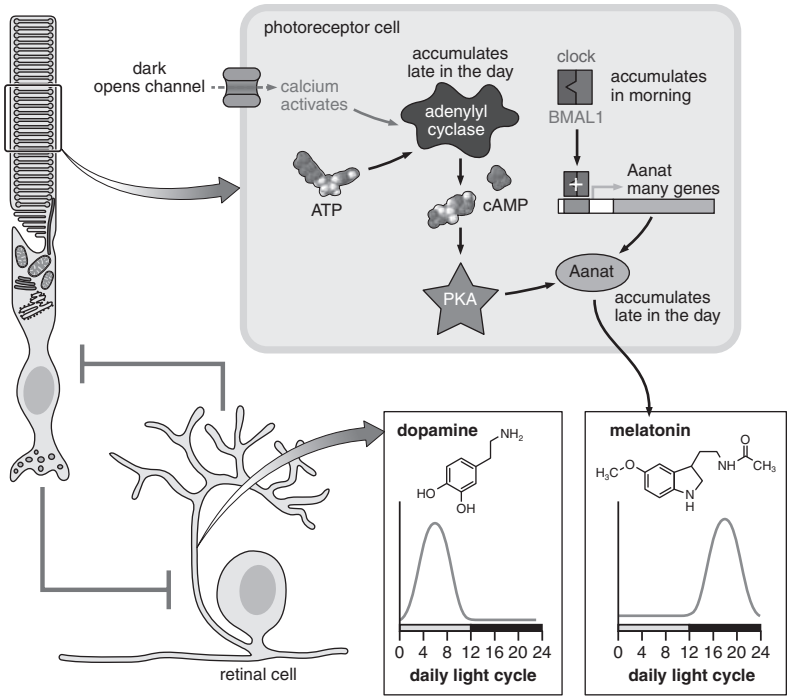

Figure 2 Light keeps your circadian clock synchronized. Darkness triggers the production of melatonin in photoreceptor cells and suppresses (T-end line) other retinal cells from producing the daytime molecule dopamine.

Source: Panel compiled and modified from Tosini *et al.*, their figures 2 and 3. Tosini, Gianluca, Nikita Pozdeyev *et al.* 2008. The circadian clock system in the mammalian retina. BioEssays Vol. 30(7): 624–633. Reprinted with permission from John Wiley and Sons, copyright 2008.

critical information for behaviors with daily and seasonal solar cycles. One of the many genes regulated by circadian rhythm in retinal rod cells encodes **adenylyl cyclase**. Adenylyl cyclase converts adenosine triphosphate (ATP) into **cyclic adenosine monophosphate (cAMP)** with two phosphates as waste products. Adenylyl cyclase requires allosteric modulation by Ca^{2+} ions to become activated. Retinal rod cells contain darkness-gated Ca^{2+} channels that open when human eyes are not exposed to light, which means at night. Darkness triggers an influx of Ca^{2+} ions, which leads to the production of cAMP by adenylyl cyclase in rod cells. cAMP **allosterically** activates protein kinase A (PKA), which can phosphorylate multiple substrates. In rod cells, PKA covalently modulates the enzyme

called Aanat, which was one of the proteins produced in response to Clock/BMAL1 (Figure 1). Aanat leads to the accumulation of a very important circadian rhythm nonprotein molecule called **melatonin**. Melatonin is produced in rod cells at night and initiates many processes related to circadian rhythm. One of melatonin's effects is to block the production of **dopamine** in a different population of retinal cells. Dopamine is produced during the day, and it inhibits the production of melatonin. Dopamine and melatonin are antagonistic in their functions.

Every cell contains the Clock/BMAL1 circadian rhythm time keeper. In addition, cells in human eyes generate interconnected biochemical signals for night (melatonin) and day (dopamine) signaling. The integrated daily signal needs to reach every part of the body quickly. From the eyes, the message of light or dark is transmitted to the master circadian rhythm control region of the brain, the **suprachiasmatic nucleus**. "Supra" means above, and the chiasma is the location where the two optic nerves cross at the base of the brain. A functional region of the brain is often referred to as a nucleus, but it refers to a collection of cells, not a subcellular organelle. The suprachiasmatic nucleus contains about 10,000 neurons, and these neurons communicate chemically with many other regions of the brain, including the pineal gland. The pineal gland has been referred to as the "third eye" because of its critical role in circadian rhythm and its massive production of melatonin, the nighttime biochemical signal.

The mammalian circadian clock molecules produce two negative feedback loops. Clock/BMAL1 activates the transcription of *Cry* and *Per* genes, which inhibit Clock/BML1. Per and Cry proteins are most abundant at dusk. The Clock/BMAL1 transcription factor is most abundant at dawn. If the production of Clock/BMAL1 and Cry/Per were altered due to environmental stimulus or a genetic mutation, then the timing of the clock would be phase-shifted. Rev-Erbα also participates in a negative feedback loop that brings to an end the accumulation of Clock/BMAL1 transcription factor, as did Cry/Per. Rora is part of the only positive feedback loop, which may play a role in reestablishing the accumulation of Clock/BMAL1 heterodimers when the time is right. The mammalian molecular clock produces an oscillation of protein accumulation and depletion that is regulated by a small number of interacting proteins. The mammalian system does not use phosphorylation the way cyanobacteria

do. In the human retina, darkness regulates calcium levels, which affects cAMP levels. Light's role in human circadian rhythm may help explain why it is easier to sleep in the dark than in bright light. The signals of dark and light allow a person to process seasonal changes in sunrise and help reset the biological clock each day in every month.

The circadian clock in the retina ensures that the information of darkness at night is processed differently than darkness produced when a person briefly blinks during the day. Adenylyl cyclase accumulates late in the day, so only darkness at night signals the body to progress toward fatigue and sleep. The connection between the Clock/BMAL1 circadian periodicity and the light-regulated periodicity in the retina are connected through the transcriptional control of Aanat and adenylyl cyclase. Another instance of emergent properties is that melatonin in rod cells blocks the production of dopamine in non-rod cells and vice versa, which is a form of positive feedback. The molecular outcome is the production of melatonin at night but not during the day. If someone lacked functional PKA or adenylyl cyclase, they would not be able to produce melatonin and thus the suprachiasmatic nucleus would not be able to coordinate nighttime physiology. It seems reasonable to hypothesize that the location of the suprachiasmatic nucleus adjacent to the optic nerves facilitates a rapid communication of nighttime darkness via melatonin or daylight via dopamine.

One other feature that cyanobacteria and mammals have in common is that temperature can affect the circadian rhythm. When investigators exposed mammalian cells to a temporary heat increase, the cells altered their *Per2* transcription (Figure 3). Most people think of humans as maintaining a constant temperature, but this is a common misconception. Mammals have an average body temperature, but that temperature varies over the course of a normal day. At night, the body temperature goes down and rises just prior to waking. Per2 protein normally accumulates at dusk, so an increase in body temperature at dawn would help reduce Per2 protein levels, which is consistent with the normal circadian rhythm of this protein.

Many questions remain unanswered about how the circadian rhythm works in mammals as well as insects and plants. Contrary to the belief of many science students, we understand only small fraction of the biological world, and our understanding will progress slowly through the efforts

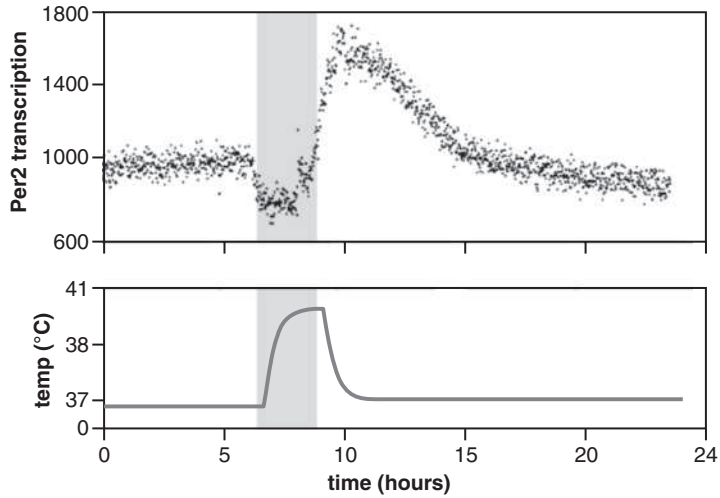

Figure 3 Experimental manipulations of circadian clock. An increase in temperature in mammalian cells altered* Per2 *transcription.

Source: Kornmann B, Schaad O, Bujard H, Takahashi JS, Schibler U. (2007). System-Driven and Oscillator-Dependent Circadian Transcription in Mice with a Conditionally Active Liver Clock. PLoS Biol 5(2): e34. doi:10.1371/journal.pbio.0050034. © 2007 Kornmann *et al.* This is an open-access article distributed under the terms of the Creative Commons Attribution License.

of biology students who ask questions and perform experiments to discover the answers.

Biologists from Switzerland and the USA wanted to determine whether healthy humans exhibit measurable variation in their genetically encoded circadian rhythms (Figure 4A). The biologists isolated skin cells from healthy volunteers and grew the cells in petri dishes to measure the circadian rhythm by quantifying BMAL1 mRNA. As can be seen in the data, the periodicity in the 19 people ranges from 22.75 hours to 26.25 hours. If individuals A and S were astronauts living on the space station, they would find their daily patterns increasingly out of synch because they would not receive regular information from the sun; sunrise to sunset is 45 minutes on the space station. The investigators also removed skin from multiple locations from four volunteers (arrows) and compared the length of circadian periods for each sample (Figure 4B). The variation between people is greater than the variation within a person, which further supports a genetic cause for human variation in circadian rhythms.

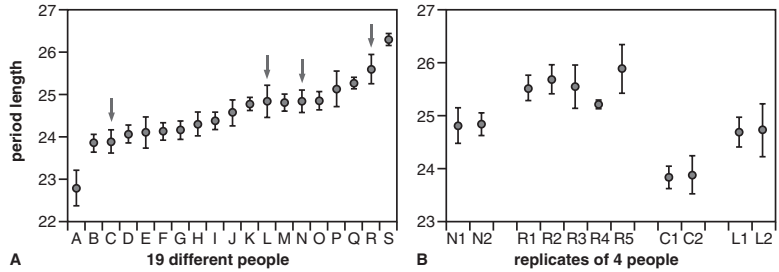

Figure 4 Human variation of circadian cycle lengths. A, BMAL1 messenger RNA (mRNA) periodicity from skin cells isolated from 19 healthy volunteers (error bars ± standard deviation [SD]). Arrows show which individuals are studied further in panel B. B, BMAL1 mRNA periodicity from skin biopsies taken from multiple places on four individuals (error bars are ± SD).

Source: Brown SA, Fleury-Olela F, Nagoshi E, Hauser C, Juge C, et al. (2005). The Period Length of Fibroblast Circadian Gene Expression Varies Widely among Human Individuals. PLoS Biol 3(10): e338. doi:10.1371/journal.pbio.0030338. © 2005 Brown et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License.

Despite being endothermic organisms that maintain a relatively stable body temperature, humans do experience temperature fluctuations over the course of a day. As shown in Figure 3, an elevated temperature can decrease transcription of the *Per2* gene. This transcriptional response is well suited to synchronizing our circadian rhythm because Per2 protein should go down at dawn when the air begins to warm. Therefore, the normal bump in body temperature before waking ensures that all of the cells in the body produce less Per2 protein when waking up. However, Figure 4A shows that people can vary by more than 3 hours in their daily cycle. The data in Figure 4B shows that even when skin cells are taken from different body parts and grown in identical conditions of tissue culture, the *Per2* mRNA variation within a person is much less than the variation between people. This supports the conclusion that variation in circadian rhythm is genetically encoded. This conclusion is consistent with common experience because everyone knows some people prefer to wake early in the day and others tend to stay up late and sleep in.

Variation in the population is visible every day among any gathering of people. Perhaps the reason famous biologist Seymour Benzer wanted to study the genetics of time-dependent behavior is because he was a night

owl who preferred to work through the night and into the early morning. Many high school and college students like to stay up late too. However, sleep patterns may be manageable today, but as people mature and their obligations to others change, they may realize that they have a sleep disorder they would like to modify. The following Ethical, Legal, Social Implications will consider sleep habits and irregularities.

Ethical, Legal, Social Implications: Sleep is Connected to Daily Rhythms

Everyone knows that people sleep different amounts through their lifetimes. Infants obviously sleep the most, whereas some senior citizens have a difficult time sleeping. Sleep affects people in many ways. The immune system and ability to digest food are tied to rest and circadian rhythms. Sleep is known to be essential for consolidating memories of what people have learned the day before. If someone pulls an all-nighter, they are more likely to forget the material recently crammed in for the test. Furthermore, the person will accumulate a deficit of sleep that must be compensated, which is why people often sleep very long when they are exhausted. However, sleep cannot be stored in advance of a time in the future when needed.

Due to hormonal changes during development, circadian rhythm shifts as people age. For teens, the preferred times for falling asleep and waking are shifted later into the night and morning, respectively. Teens and their parents probably experienced this circadian shift during high school as teens wanted to go to bed later because they did not feel sleepy until later. Unfortunately, school schedules are established by adults, many of whom do not know much biology. Schools often begin much earlier in the day than a typical teenager wants to wake up. As a result, students have to wake up contrary to their circadian rhythms and often after getting too little sleep. One student tried to use science to convince his school to shift the start of his school day to be more in line with the biology of teenagers. Exposure to artificial indoor light after the sun goes down disrupts the normal retinal mechanism to adjust wake/sleep cycles. A prolonged exposure to light makes the body think that it is earlier in the day than it actually is. Most students try to catch up on sleep by sleeping

in during weekends, but this habit further shifts their circadian rhythm out of phase with their school schedule. As people age, the window for optimal sleeping narrows as the circadian and retinal time keepers continue to shift. The loss of coordination between the eye and the molecular clock may be an important reason that the elderly tend to wake up early in the morning and fall asleep earlier in the evening.

No one is exactly sure why animals sleep. As stated earlier, sleeping is necessary for a good immune system and for retaining newly learned information. From what we can tell, all animals sleep in one way or another. Some animals "sleep" even when they are active. For example, aquatic mammals such dolphins and whales rest half of their brains at a time. It is easy to imagine why it would be maladaptive to fully sleep in the water for an air breathing marine mammal. Even invertebrates show signs of sleeping based on their gene transcription patterns that resemble mammalian transcription patterns. In 2013, a group of biologists from the University of Rochester reported that mouse brain cells shrink in size during sleep. They reported that the cerebrospinal fluid bathing brain cells was able to flow 60% faster and thus the brain is "cleansed" better during sleep than when we are awake. More research will be required to verify or disprove the recent findings, but that is the nature of science.

Like all other species, mammals coordinate their physiology with the daily and seasonal cycle of the sun. The periodicity of a circadian clock varies depending on the combination of alleles given to a person by their biological parents. Light and temperature can help coordinate a body's circadian rhythm, which allows each person to adjust to seasonal changes, east-west long distance travel, different work shifts, and sleep disorders. Regardless of how sleep patterns changes with age, everyone must sleep to stay healthy and to maximize their learning.

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CHAPTER 2

Maternal Immune System Tolerates Non-self Fetus

There are two facts from everyday life that seem normal, and yet when considered carefully, they contradict each other. First, a pregnant woman carries a fetus inside her body for 9 months to nurture and protect it from harm. Second, successful organ transplantation requires the donor and the recipient to be genetically matched or else the donated organ will be rejected by the recipient's immune system. However, a fetus is tolerated by the mother despite the fetal tissue being genetically 50% foreign. How can a pregnant woman tolerate a fetus for 9 months? The answer to this question is an emergent property at the organismal level and the focus of this chapter.

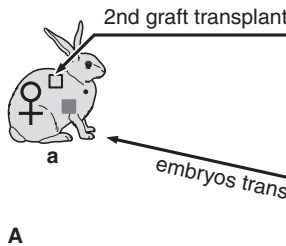
To understand how mothers can tolerate their fetuses, this case starts at a very unlikely place—a British military field hospital during World War II. In particular, the immunology observations of Peter Medawar ultimately resulted in him winning a Nobel Prize in 1960. Medawar treated many soldiers whose skin had been badly burned. Burned skin needs to be replaced by healthy skin, a **graft**, which must be sewn over the burned area. If the patient has only small areas of burned skin, the replacement skin graft can be taken from another part of the patient's body. Tissue transferred from one part of an individual's body to another part of the same person is called an **autograft**. Autografts are always successful because the transplanted skin cells are genetically equivalent to all of the other cells in the patient's body. However, when a patient is burned over a large portion of his or her body, there is not enough healthy skin to graft onto all of the burns and another source of skin is needed. **Allografts** are taken from another person and transplanted to the patient in hopes that the skin graft will grow and fill in the damaged areas. Many surgeons

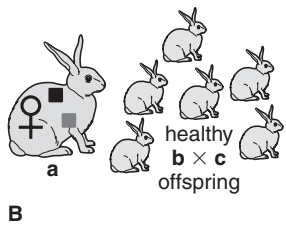
before Medawar had discovered that autografts were successful, but allografts often turned black and died. Medawar's big insight was recognizing a pattern and deducing a mechanism to explain why allografts were rarely successful.

Medawar's observations stimulated him to ask some questions that he could answer through careful experimentation. Medawar took small patches of skin from healthy volunteers and transplanted them to different locations. After the war, he performed many grafts similar to the kind he performed during the war. When an allograft was rejected by the host recipient, Medawar called this **first-set rejection**. In some cases, Medawar transplanted a second skin graft from the same donor onto the same recipient. If the second graft was also rejected by the recipient, Medawar called this **second-set rejection**. After World War II, Medawar published a series of important papers describing a wide range of skin graft experiments he performed using rabbits instead of humans. Medawar described in detail the stages of allograft rejection, which included redness and swelling of the area prior to the tissue turning black and dying. In some experiments, he compared the rejection rates depending on whether the second allograft was surgically placed at the same site as the first one or somewhere else, and the relative sizes of the grafts.

Medawar performed hundreds of skin graft experiments to quantify the rate of rejection and found that large skin patches were rejected faster than small patches as long as both were first-set rejections. Second-set rejections always occurred faster, and the time course of the rejection was not significantly influenced by whether the graft was transplanted to the same site or a different site. Based on the redness and swelling, Medawar correctly hypothesized that allograft rejection was caused by an immune response. Autografts are "self" tissue meaning the tissue is genetically identical to the recipient, whereas allografts are "non-self" tissues and rejected the same way viral infections are destroyed. Immune systems attack and reject non-self tissue, which makes the contradiction of a pregnant mammal more apparent. By definition, a fetus is 50% non-self and therefore should be rejected like an allograft, but fetuses are not rejected. This contradiction stimulated a very compelling set of experiments to understand why pregnant women fail to reject the non-self fetal tissue inside them.

By the 1960s, biologists agreed that the immune system rejected allografts but not fetal tissue. Two possible explanations were proposed: 1) pregnant women have a reduced immune function, or 2) fetal tissue benefits from a protected status through some unknown mechanism. A group of immunologists tested these two hypotheses directly in a beautifully designed experiment (Figure 5). Female rabbit "a" received a skin allograft from female rabbit "b", and the allograft was rejected (gray square on rabbit a). After the allograft was first-set rejected, eggs from rabbit b were mixed with sperm from rabbit c; and the fertilized embryos were transferred to the uterus of rabbit a. Rabbit a received a second skin allograft from rabbit b either at the time of the embryo transfer, or halfway





C

results	second graft transplanted	
	at transferral	mid-pregnancy
healthy litter	3	7
no litter	1	2

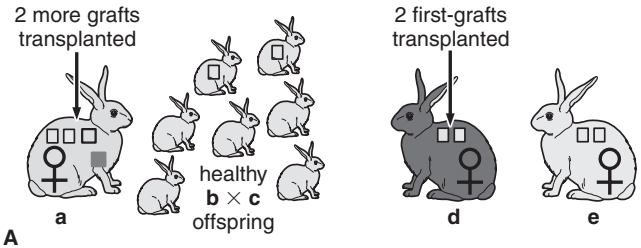
Figure 5 Allografts are rejected but "allo-embryos" are not rejected. A, Foster mother a was implanted with embryos from the mating of rabbits b and c. Rabbit a rejected one allograft from rabbit b prior to embryo transfer and a second allograft transplantation was also rejected. B, Healthy bunnies from b × c embryo transfers to rabbit a. Black square on rabbit a shows 100% second-set skin rejection; gray square was previous skin rejection. C, The number of pregnancies that produced offspring as a function of the timing for second allograft transplantation.

Source: A-B. Original Art. Panel C modified from Lanman *et al.*, 1962, their table 1. Lanman, Jonathan T., Jenny Dinerstein and Senih Fikrig. 1962. Homograft immunity in pregnancy: lack of harm to the fetus from sensitization of the mother. Annals of the New York Academy of Sciences. Vol. 99: 706–716.

through the pregnancy of rabbit a. The experiment in Figure 5 was technically challenging and not every transfer of embryos was successful, but a general trend was evident (Figures 5C).

After analyzing the data in Figure 5, the investigators designed a more rigorous experiment to test directly the ability of pregnant rabbit a to reject non-self tissue (Figure 6). After giving birth to a healthy litter, rabbit a received two more skin allografts. One allograft was from one foster b × c offspring, and the other allograft was from a new, unrelated rabbit e. As a control, the immunologists transplanted equivalent allografts from b × c foster offspring as well as unrelated rabbit e onto a third, unrelated rabbit d. All of the rabbits in this experiment were female and the data are summarized in Figure 6B.

The experiment in Figure 5 directly tested whether pregnant females had weakened immune systems. If pregnant females had reduced immune function, they would not be expect to reject skin allografts, but



A

experimental conditions	baby skin transplanted to:		rabbit E skin transplanted to:	
	foster mother A	unrelated rabbit D	foster mother A	unrelated rabbit D
average days graft survived	4.0*	6.5	6.0*	7.0

*indicates $p < 0.01$; experiment replicated 5 times

B

Figure 6 Follow-up experiment from Figure 5. A, After raising her offspring, mother a receives two more skin grafts, one from her b × c offspring and one from unrelated female e. Unrelated rabbit d receives two equivalent allografts. B, Quantified results showing the average number of days skin grafts survived on recipient rabbits. * indicates significant difference between 4.0 and 6.0 for foster mother a.

Source: A. Original Art. Panel B modified from data from Lanman *et al.*, 1962, their table 4.

they did. About 75% of the foster mothers were able to carry the implanted b × c embryos to term and deliver healthy offspring even though these transplanted offspring are 100% non-self. The immune systems of both rabbits a and d in Figure 6 rejected the allografts from the b × c offspring as well as the new rabbit e graft. Rabbit d rejected both allografts at about the same rate (6.5 and 7 days), but rabbit a responded differently. Rabbit a rejected the allograft from foster b × c offspring significantly faster ($p < 0.01$) than it rejected the allograft from rabbit e, even though both patches of skin were transplanted at the same time. The b × c allografts were rejected faster because the skin patches were second-set transplants, whereas the allograft from rabbit e was a first-set rejection. Rabbit a rejected allograft from her b × c foster offspring faster because rabbit a had previously been exposed to skin from rabbit b. Rabbit a rejected allograft e slowly because rabbit a had not been exposed to tissue from rabbit e before. Immune responses are specific for the source of non-self tissue, and immunity does not uniformly affect all allografts equally.

A lot of progress was made between Medawar's initial observations and the experiments in the 1960s with the pregnant rabbits. The fetus must possess some mechanism to protect itself from the mother's functional immune system. However, the immune system is a difficult area of physiology to study, and many fundamental questions remained unanswered about how the normal immune system works. How does the immune system treat second-set and first-set rejection differently? What cells are responsible for "remembering" a previous exposure to a particular source of non-self tissue? Although rabbits were the model system of choice early on, many immunologists prefer to work with mice, because they are smaller, less expensive to maintain, reproduce faster, and investigators have many more mutant strains of mice to help dissect the role of particular genes.

As with rabbits, mice respond differently to first-set and second-set allografts (Figure 7). In this experiment, the investigators isolated white blood cells of the immune system and separated them into different subtypes, such as B cells, T cells, and **natural killer** (**NK**) cells. The immunologists injected **naïve** mice with different subtypes of white blood cells from a mouse that had already exhibited first-set rejection. Shortly after injecting a naïve mouse with white blood cells, the investigators

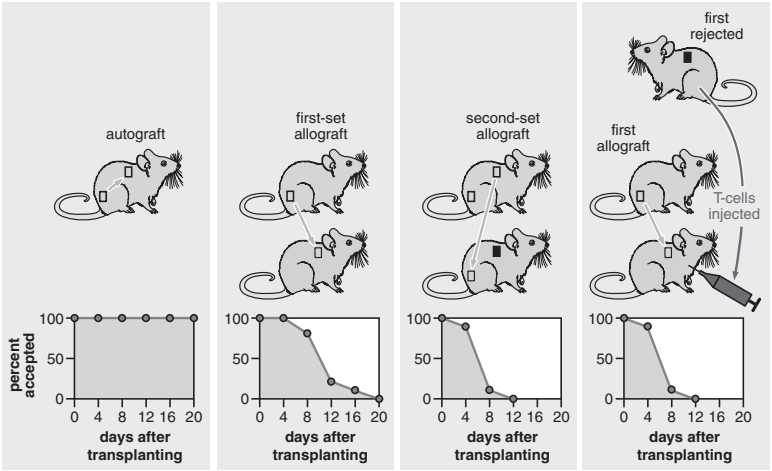


Figure 7 Rate of graft rejection is affected by the state of the recipient mouse. Mice were given grafts as diagramed, and the outcome of each grafts is plotted as percent of grafts accepted (shaded areas) over 20 days. The injected mouse was immunologically naïve prior to the injection and first-set transplant.

Source: Common knowledge. Original art.

transplanted skin allografts that had been rejected previously by the white blood cell donor.

Second-set rejection is caused by T cells, a subtype of white blood cells. First-set rejection in half of the mice takes about 10 days, whereas second-set rejection in half the mice only takes about 6 days. The experiment in Figure 7 was a critical breakthrough in understanding the normal immune system. Immunologists would need to understand a normal immune system before they could determine how a fetus avoids being rejected. Another critical component of the immune system was the discovery shown in Figure 8. With very few exceptions, every cell in a mammal's body displays many copies of the **major histocompatibility complex type one** (**MHC I**) molecule. MHC I molecules are integral membrane proteins as illustrated by the thin line passing from outside the cell into the cell's cytoplasm. The vast majority of MHC I protrudes into the extracellular world. MHC I proteins look like moose heads and between their antlers is an empty space. However, the thousands of MHC I molecules found on the surface of each cell never have empty spaces;

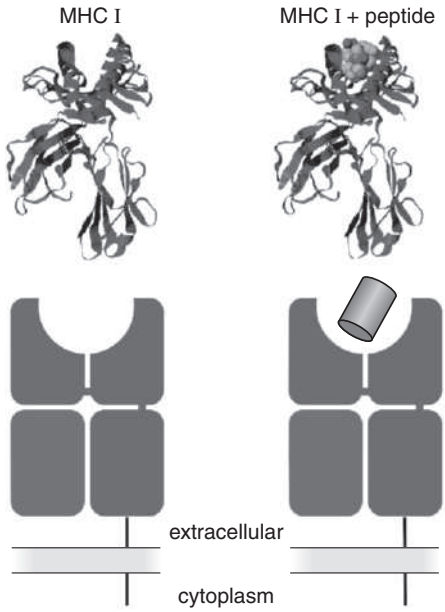


Figure 8 Structure of MHC I protein. MHC I protein is composed of two subunits and contains a binding groove for protein fragments made inside of the cell displaying the fragment. Three-dimensional (3D) structures are on top, stylized diagrams below; shaded barrel represents a self peptide.

Source: Jsmol images taken from PDB ID# 1OGA.

the space is always occupied by a protein fragment, or **peptide**, that was translated inside the cell displaying the MHC I molecule. MHC I plus peptide is a cell's way of defining "self." In other words, MHC I molecules display fragments of every protein made inside that cell like a proud grandparent showing photos of grandchildren. All of the cells of an organism define "self" by displaying peptide fragments from every protein produced inside each cell of an individual.

With the discovery of MHC I molecules, the rejection of allografts and acceptance of autografts made sense. MHC I presents self peptides on the surface of every grafted skin cell (Figure 9). Autograft cells display MHC I molecules on their surfaces with peptide fragments to inform the T cells that the skin graft is self, so the cells are never rejected. Allografts display MHC I plus peptides from proteins made inside genetically non-self cells and thus are recognized as foreign by the recipient's T cells.

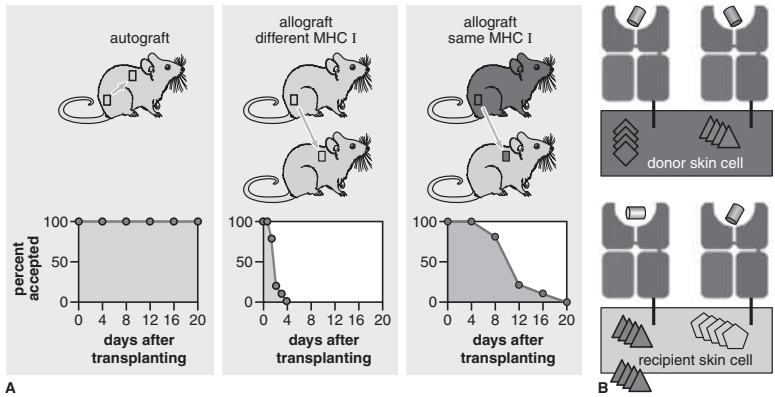


Figure 9 Cells display self peptides in MHC I. A, Mice were given grafts as diagramed, and the outcome of the grafts are plotted as percent of grafts accepted (shaded areas) over 120 days. B, Adjacent skin cells with identical MHC I proteins, and each cell is displaying fragments of proteins made inside the indicated cell.

Source: Panel A modified from Janeway *et al.*, figure 13.36. B. Panel B modified from Janeway *et al.*, figure 13.37. 2005.

However, the data in Figure 9A uncovered an important characteristic of the MHC I molecules. Through careful breeding of mice, immunologists produced different strains of mice that were genetically identical at every locus except MHC I. Conversely, immunologists bred mice that were genetically identical only at the MHC I locus and varied at all other loci. Therefore, immunologists could distinguish the impact of MHC I differences versus the impact of the peptide differences on allograft rejection (Figure 9B).

Because MHC I molecules display self protein fragments, these peptides must bind tightly to prevent peptides produced in other cells from binding to an empty space and incorrectly appearing as a self peptide. T cells use receptor proteins in their plasma membranes to "feel" MHC I + peptide on the surfaces of all cells to distinguish self from non-self. In their efforts to recognize cells, T cell receptors physically interact with the peptide and MHC I molecules, as depicted in Figure 10A. The peptide and the "antlers" of MHC I molecules are about the same height, which gives a T cell receptor equal access to both at the same time. Unlike autografts, which are never rejected, allografts containing different alleles of MHC I are first-set rejected in 4 days. However, the immune

system takes 20 days to first-set reject allografts with identical MHC I alleles but displaying non-self peptides as defined by the recipient's T cells (Figure 9B). Therefore, T cell receptors use MHC I as the primary means for rejecting non-self tissue, which is how the name major histocompatibility was chosen for the protein. As with mice, human organs and tissue with different MHC I alleles are not compatible for transplantation and are rapidly rejected as non-self. T cells more slowly reject a graft if the peptides are non-self and the MHC I is self. Identifying non-self peptides within MHC I is how viruses are detected and destroyed by T cells when human cells become infected. These data highlight the fact that T cells must be "educated" to distinguish self peptides from non-self peptides. The education of T cells is beyond the scope of this book but is a key element of an immunology course.

T cells are essential to the vertebrate immune system (Figure 10B). T cells interact with every cell in the human body and use their receptors to determine the identity of each cell as self or non-self. If the MHC I molecule is self, the T cell perceives the cell as self. If the peptide bound to MHC I is self, then the T cell recognizes the self cell as containing only self proteins, and the T cell moves on. However, if the T cell does not recognize the MHC I protein or does not recognize the bound peptide as self within a self MHC I, then the T cell has the capacity to kill the offending cell. T cells kill virally-infected cells displaying viral proteins within self MHC I molecules. Some viruses try to avoid being displayed by their host cells and block the movement of MHC I molecules to the surface of the infected cell. Another type of white blood cell called natural killer (NK) cells destroy cells that lack MHC I on their surfaces. Therefore, every cell must display MHC I plus peptide to avoid being killed by NK cells. If a cell displays non-self peptides, the offending cell will be killed by T cells. T cells and NK cells of a pregnant mother maintain their immune function and yet these two white blood cells do not kill fetal cells that are, by definition, non-self. White blood cells such as NK cells can engulf extracellular material and digest it in their stomach-like organelle called the lysosome.

The immune system rejects all allograft cells as non-self because they contain different MHC I proteins, because the peptides are non-self, or both. Contemporaries of Medawar wanted to know if the immune system

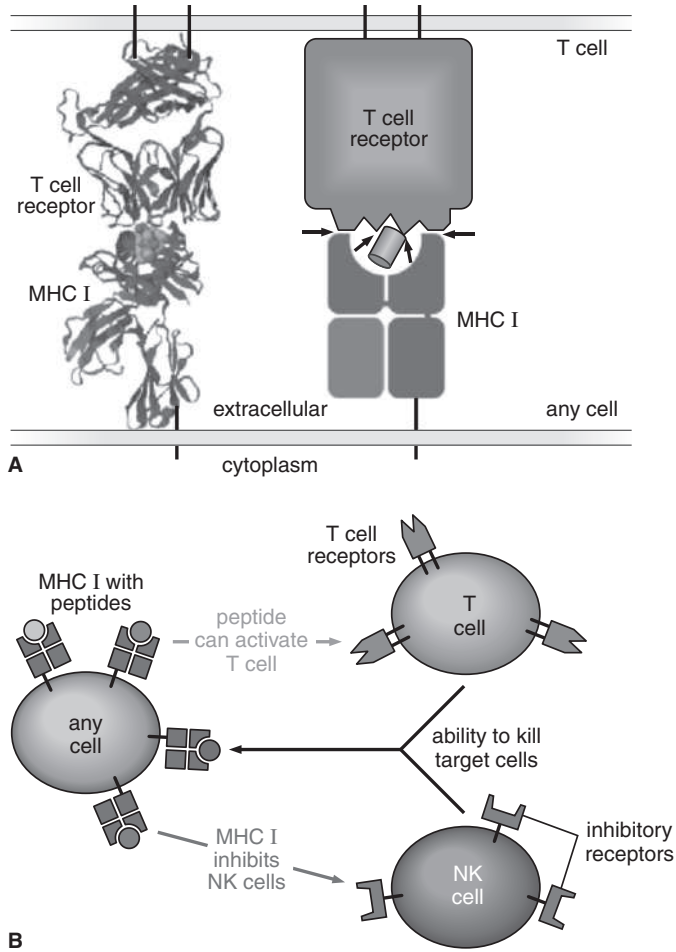


Figure 10 Immune cells kill non-self cells. A, T cells recognize self cells through their receptors that touch both MHC I molecules and their presented peptides (arrows). The 3D structure is on left; line diagram is on the right. B, All cells use MHC I + peptide to identify themselves to T cells and NK cells.

Source: Panel A images taken from PDB ID# 1OGA. Panel B modified from Bainbridge, 2000; his figure 1.

could distinguish male from female cells (Table 1). The investigators had bred mice carefully so that all offspring carried identical alleles on every chromosome and the only differences were the 53 genes unique to the Y chromosome present only in males. The immunologists performed a series of skin allografts with these highly inbred mice and determined the

Table 1 Gender effects on graft rejection rates for genetically identical, inbred mice.

donor→ recipient	number of animals	% rejected	average days to rejection ± stdev
male→ male	16	0	n.a.
female→ female	15	0	n.a.
female→ male	15	0	n.a.
male→ female	15	100	28 ± 3
male→ primed female*	10	100	14 ± 2

*primed female injected with sperm two weeks prior to skin graft.
Modified from *Katsh etal.*, 1946; their Table 1.

Source: Modified from Katsh *et al.*, 1946; Table 1. Katsh, Grace F., David W. Talmage and Seymour Katsh. 1964. Acceptance or rejection of male skin by isologous female mice: Effect of injection of sperm. Science. Vol. 143: 41–42. Reprinted with permission from AAAS.

rate of graft rejection. In one set of allografts, they first injected (primed) the female recipients with mouse sperm cells and transplanted the skin graft 14 days later to determine the rate of allograft rejection with or without previous exposure to male sperm cells.

Many human diseases are understood in part because of mutant mice with similar causes and symptoms. Nude mice and humans with the mutation described by OMIM code 242700 lack T cells due to a nonfunctional thymus. T cells are called "T" because they mature and become "educated" to recognize self in the thymus. Without a thymus, animals are doomed to die from viral infections because T cells are primarily responsible for protecting us from viruses. T cell receptors interact with the MHC I molecule more than the peptide, but the exact number of peptide interactions varies with different T cell receptors and different peptides. Both MHC I and the peptide are in direct physical contact with the T cell receptor. Fetal cells must have MHC I molecules or the NK cells would attack and destroy the fetus.

Before examining more data, reflect upon some life experience. Most people have heard about bone marrow donation and other organ donor programs, but most do not realize that these programs are trying to find MHC I allele matches between donors and recipients. Every day, 18 people die in the United States due to a lack of suitable donor tissue. The total human gene pool consists of about 2,000 MHC I alleles. (Humans have 3 MHC genes, but for simplicity sake, let's assume there is only one

gene.) Given that humans are diploid, there are approximately 4 million ($2{,}000^2$) different MHC I genotypes. Therefore, the probability of two unrelated people matching two MHC I alleles is greater than 1 in 4 million, which explains why it is so hard to find a good tissue match for human organ donation. It is possible to learn more about human organ donation and tissue typing from the United Network for Organ Sharing and the National Marrow Donor Program. With regards to pregnancy, the odds are at least one in four million that a couple would have identical MHC I alleles. Therefore it might be expected that a pregnant woman to reject her fetus because it is 50% non-self MHC I. Furthermore, the 53 male-specific genes on the Y chromosome were sufficiently non-self to be rejected by female mice as summarized in Table 1. It would be expected that male fetuses are rejected faster than female fetuses, but they are not.

In order to understand how all fetuses are protected, it is important to learn the physical interaction between the mother and fetus (Figure 11A). The fetus is 50% identical to the mother, but half of all its MHC I molecules differ from the mother's. The amniotic sac that surrounds the fetus and the umbilical cord that brings nutrients and oxygen to the fetus are both fetal tissue. These two tissues do not directly interact with the mother's cells, so it might be predicted that they would be safe from T cell attack. However, notice that the finger-like projections of the **trophoblast** fetal tissue interacts with the mother's blood-rich **endometrium**. Cells in the fetal trophoblast area are called **cytotrophoblasts**. Collectively, the trophoblast and the endometrium form the **placenta**, which is a defining characteristic of all mammals that nurture their young internally until birth. Given its high blood content, the placenta is where the maternal immune system attacks fetal cells and thus kills the entire embryo. The key to an embryo's survival is in the MHC I alleles that it presents on every cell derived from the fertilized egg.

When immunologists were able to sequence DNA, they very quickly wanted to know which genes were encoded in the MHC locus of the mammalian genome. The human MHC locus contains about 200 genes and spans 4 million base pairs on chromosome 6. Immunologists discovered a new MHC I gene called G, or MHC IG, within the MHC locus (Figure 11C). Once immunologists knew to look for MHC IG, they discovered only fetal cells produce MHC IG proteins. MHC IG

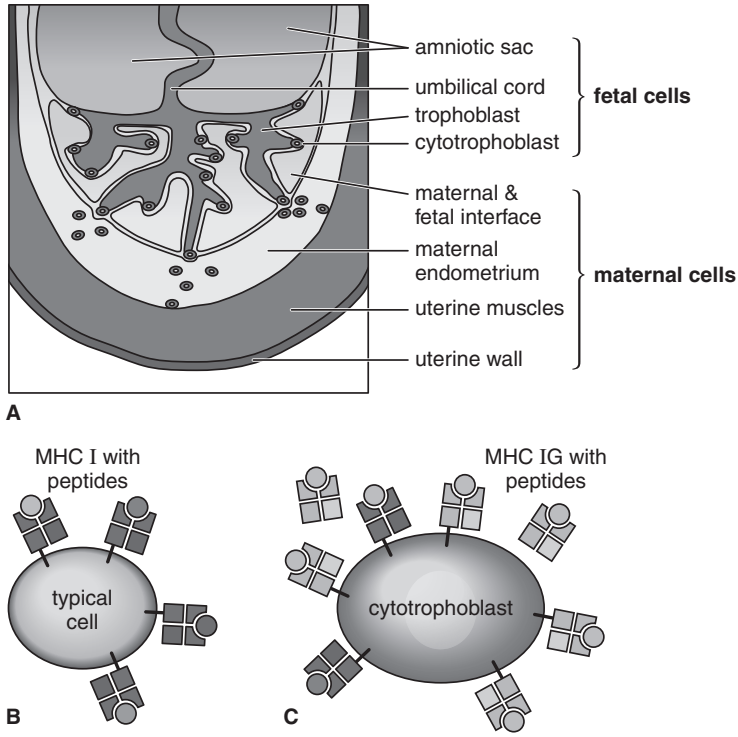


Figure 11 Maternal and fetal cells intermix during mammalian pregnancy. A, Diagram of developing placenta below a fetus (not shown). B, Cells use MHC I + peptide to identify themselves to immune cells. C, Fetal cells also secrete and display peptides bound to unique MHC IG molecules.

Source: Panel A modified from Le Bouteiller et al., 1999; figure 2. Panel B modified from Bainbridge, 2000; his figure 3.

molecules are very similar in shape to the regular MHC I proteins. MHC IG presents peptides from proteins translated inside fetal cells. Through alternative mRNA splicing, some of the MHC IG proteins are not anchored to the membranes of fetal cells, and they float freely in the area surrounding fetal cells.

With the discovery of MHC IG, immunologists had a very good candidate mechanism for fetal protection from the maternal immune system. Investigators used the very sensitive method of reverse transcriptase polymerase chain reaction (RT-PCR) to detect MHC IG mRNA produced inside recently fertilized human embryos. Manipulating mRNA and tiny

embryos is technically difficult, but the investigators were able to detect MHC IG mRNA in the fertilized human embryos prior to implantation. A different group of immunologists wanted to detect MHC IG protein in slightly older embryos. The investigators produced antibodies that bind to human MHC IG and covalently linked a green dye to the antibodies. When embryos and the antibodies were mixed together, the investigators could detect MHC IG protein by the appearance of green tissue when viewed using fluorescence microscopy.

The MHC IG is within the MHC locus at 6p.21.3 (6p = short arm of chromosome six; band 21.3), and the gene is composed of eight exons. Only the first six exons encode amino acids and the last two exons are non-coding. The investigators learned trophoblast cells produce more MHC IG than the small circular embryo, as indicated by the brighter green cytotrophoblasts. Given that cytotrophoblasts directly interact with maternal NK and T cells, it makes sense that the trophoblast would benefit from a higher density of MHC IG than the fetus, which is physically separated from the mother's immune system

Detecting a protein by antibody binding does not definitively demonstrate MHC IG protects embryos from the mother's immune system. What was needed was a functional test to determine if MHC IG prevents destruction of non-self cells (Figure 12). The investigators isolated trophoblast cells from placenta and tested their ability to withstand lysis when challenged by NK cells. The trophoblast sample was divided into three aliquots and a different set of antibodies was added to each aliquot. One portion of trophoblast was incubated with an arbitrary antibody that did not bind to any human protein. Another portion was incubated with antibody that bound specifically to human MHC IG, and the final portion was incubated with an antibody that bound to MHC I but not MHC IG. If an antibody blocked a trophoblast's ability to prevent destruction by NK cells, then the percentage of lysis would increase. Three sources of NK cells were incubated separately with the three aliquots of trophoblasts pretreated with antibodies for a total of nine tubes of cells and antibodies.

MHC IG protects trophoblast cells from lysis by NK cells in Figure 12, as indicated by the increased cell lysis when incubated with the anti-MHC IG antibody. The two irrelevant antibodies in Figure 12 did not

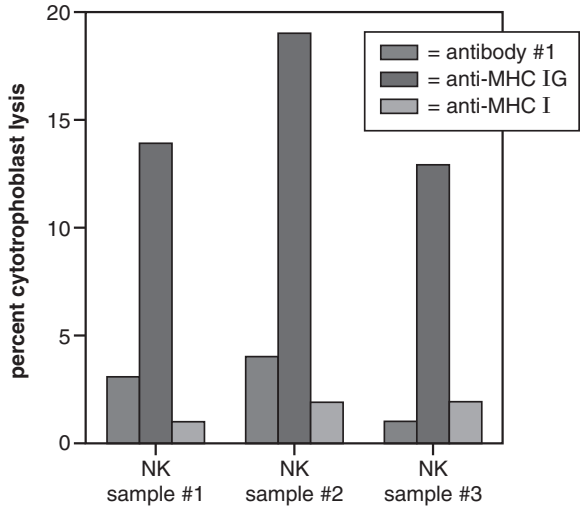


Figure 12 Exposing trophoblast cells to NK cells from unrelated individuals. NK cells from three different people were added to three samples from one population of cytotrophoblasts. Three different antibodies (see text) were added to cytotrophoblasts prior to adding NK cells and measuring lysis. SD was less than 5% in triplicate experiments.

Source: Modified from Rouas-Freiss *et al.*, 1997; their figure 4A.

prevent NK cells from ignoring non-self cytotrophoblasts. However, the anti-MHC IG antibody masked the protective MHC IG proteins and allowed the NK cells to attack the non-self cells. Only about 20% of the cytotrophoblasts were lysed when incubated with anti-MHC IG because *in vitro* experiments are never 100% efficient.

In 1944, Medawar published his ground-breaking paper in which he proposed that a healthy immune system was the cause of allograft rejection. Fifty years later, immunologists had a very solid working model to explain how the immune system distinguished self from non-self. Every cell uses MHC I molecules to display self peptides. T cells scan all cells to find and destroy any cells that appears to be non-self or virally infected cells. One benefit of conducting research on mice rather than humans is that it is possible to make predictions and test them in whole animals. For example, they predicted that mutations causing the loss of MHC IG would cause problems during pregnancy, such as spontaneous abortions and sterility.

Based on maternal tolerance of a non-self fetus, they predicted that genetic deficiencies in MHC IG should lead to immune rejection and spontaneous abortion. The term **preeclampsia** describes a serious medical complication for a pregnant woman. The expectant mother experiences high blood pressure and protein appears in her urine after the twentieth week of the pregnancy. **Eclampsia** is a life-threatening condition when the mother experiences convulsions, or seizures, due to high blood pressure brought about by the pregnancy. Do these conditions have anything to do with maternal tolerance of non-self fetal cells?

Hylenius and colleagues documented a correlation between particular MHC IG alleles and preeclampsia (Figure 13). The human MHC IG gene is composed of eight exons with the last two exons being part of the 3′ untranslated region of the mRNA. The paper by Hylenius and colleagues discovered that preeclampsia fetuses often contain a 14 bp insertion in exon 8.

The 14 bp insertion into exon 8 of MHC IG mRNA does not affect the primary amino acid structure of the MHC IG protein. Because the protein is not altered, there must be another mechanism for reduced MHC IG function. MHC IG mRNA that contains the 14 bp insertion was degraded faster than mRNA lacking the insertion. Therefore, fetuses that produce the mutant mRNA produce less MHC IG protein and are more vulnerable to maternal immune system attack.

This chapter began with an apparent contradiction that was the focus of the emergent property at the organismal level—tolerance of a

Figure 13 Structure of human MHC IG gene. The gene is about 4 kilobases long and composed of 8 exons. Fourteen bases are inserted in mutant alleles of the gene.

Source: Modified from Hylenius *et al.*, 2004; figure 1.

mammalian fetus. If biologists studied only the immune system, they would not have predicted that a non-self fetus could be tolerated. Conversely, if biologists only studied reproduction, they would not have predicted allograft rejection and how the immune system identifies and rejects non-self tissue. The emergent property of a mother tolerating her fetus exhibits competition between tolerance and immune rejection as well as cooperation within the placenta. MHC I proteins display peptide fragments produced inside every cell in the body, which helps the immune system survey cells for the potential of viral infection. MHC IG inhibits the rejection of non-self paternal MHC I proteins as well as the non-self peptide fragments the fetus displays. Of course, MHC I is just one of many genes that play critical roles in embryo development. Once the child is born, it begins to gain weight and the next chapter examines how adult mammals maintain a fairly consistent weight.

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CHAPTER 3

Maintaining Body Weight and Fat Levels

Obesity is a growing problem worldwide, especially in the United States. Over the past 35 years, the percentage of teens categorized as obese has tripled (Figure 14). As illustrated in the 2010 White House report, gender and ethnicity correlate with the rate of obesity in teens between the ages of 12 and 19. What has changed in the last 35 years to cause a substantial shift in the body weight of Americans? Isn't body weight constrained by organismal **homeostasis**? For many years, physiologists demonstrated the importance of exercise in regulating weight. From Figure 14C, it is apparent that about half of all high school boys exercise less than 1 hour a week, whereas 75% of high school girls exercise less than 1 hour a week. Is exercise the only factor that regulates body weight? Obesity is defined by the Centers for Disease Control and Prevention (CDC) as a body mass index (BMI) of 30 or greater. The physiological trait of body weight may not seem like an emergent property, but fat regulation is connected to many other physiological processes. It is important to consider the experimental results that explain how mammals regulate their body fat.

The percentage of obese American teens is increasing, but Figure 14 does not provide enough information to determine if the yearly differences are significant or not because the data lack error bars. The data lack any indication of variance, so it is not possible to evaluate whether changes are significantly different. Variance was probably omitted from the data to keep it simple for the typical American who does not understand the use of error bars. Non-Hispanic black girls have the highest incidence of obesity, followed by Hispanic boys. Non-Hispanic boys and girls have the lowest levels of obesity nationally, but regionally this trend may not hold true everywhere. For example, the state with the

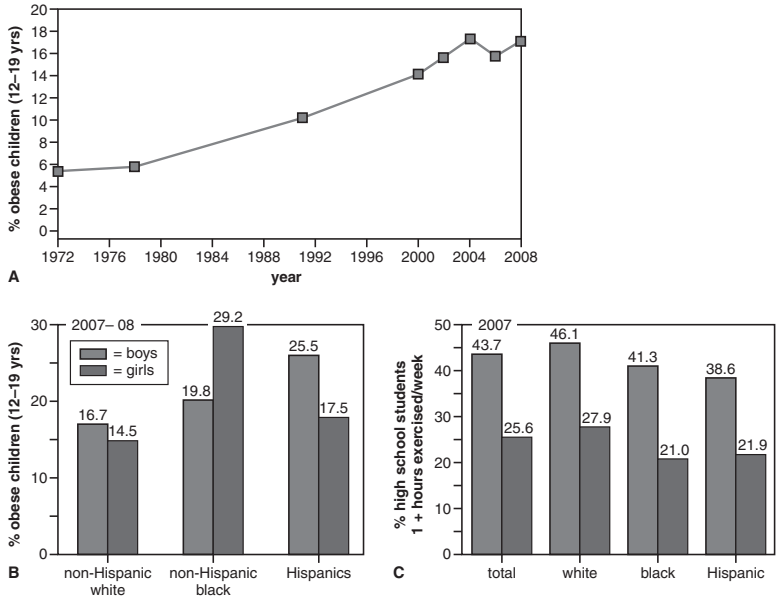

Figure 14 Obesity is an epidemic in America. A, Percentage of American teens classified as obese. B, Percentage of obese teens sorted by gender and ethnicity. C, Percentage of high school students who get 1 hour or more of exercise per week, sorted by gender and ethnicity.

Source: White House Task Force on Childhood obesity Report to the President. Solving the problem of childhood obesity within a generation. Public domain.

highest percentage of obesity in 2013 was Mississippi with 35.4%, whereas Montana had the lowest percentage with 19.6%.

Figure 15 contains the data that implicated the hypothalamus as an important component in body weight regulation. In 1959, physiologist G. R. Hervey at the University of Cambridge published his results describing a clever set of experiments on weight regulation in rats. During the 1950s, physiologists developed a method where they surgically integrated the circulatory systems of two individuals, similar to conjoined or Siamese twins, which are termed **parabiotic**. Hervey produced several pairs of 1-month-old parabiotic rats and compared their weight gain to age-matched single rats that lived independently. Four months after joining the parabiotic rats, he segregated the conjoined rats into two populations. For half of the parabiotic rats, Hervey surgically damaged a small portion of the hypothalamus in the individual on the right side of the paired rats (Figures 14A and 14B).

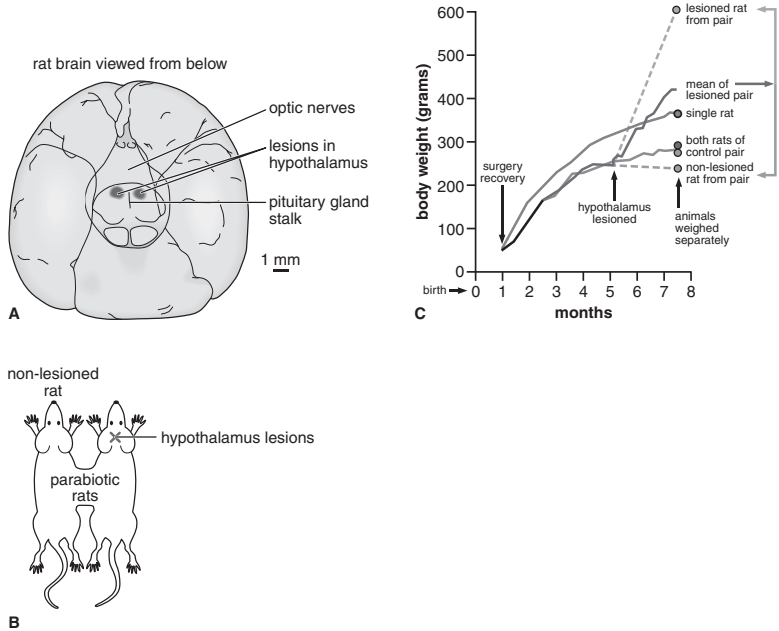

Figure 15 Rat brain regulates body mass. A, Dissected rat brain viewed from below (ventral) at the conclusion of the experiment. Surgical lesions in hypothalamus indicated by two shaded areas. B, Parabiotic rats share circulatory systems. Rats on the right side of a pair received surgical hypothalamus lesions but ones on the left remained unaltered. C, Body weight data on individual rats or paired rats after parabiotic surgery and hypothalamic lesion surgery. At the end of the experiment, parabiotic rats were separated and measured individually. Dashed lines are predicted weight changes before separation.

Source: Panel A modified from Hervey, 1959, figure 1. Hervery, G. R. The effects of lesions in the hypothalamus in parabiotic rats. Journal of Physiology. Vol. 145: 336–352. Copyright 1959, Wiley and Sons. Panel B original art. Panel C modified from Coleman, 1978, figure 1. Springer and Diabetologia. Coleman, D. L. 1978. *obese* and *diabetes*: Two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia. Vol. 14: 141–148. Modified with kind permission from Springer Science and Business Media.

He allowed all rats to eat freely as much as they wanted for 3 months when the experiment was terminated, and each rat was weighed individually. Periodically during the 3 months, Hervey weighed the parabiotic rat pairs and calculated the average weight of each member of the pair as shown in the graph. The dashed lines are estimations of the gradual change in weight based on the final weights of the paired experimental rats.

The data in Figure 15 were among the first to firmly establish the critical role the hypothalamus plays in body fat regulation. Parabiotic control rats gained less weight than the single rat, but it is not possible to determine if this difference is significant because the figure does not display any variance. The experimental parabiotic rats collectively gained more weight than the single rat, and the rat from the conjoined pair that had its hypothalamus surgically lesioned gained the most weight of all rats. However, the parabiotic rat with a functional hypothalamus gained the least weight. Perhaps the obese condition of the conjoined individual with a hypothalamus lesion was able to biochemically communicate its physiological status to the conjoined control rat, but more data would be needed to confirm this hypothesis. Preliminary data are good for generating hypotheses, not reaching firm conclusions.

In 1949, one of the world's leading mouse genetics facilities, Jackson Labs, isolated a spontaneous mutation in a single gene called *obese*, abbreviated *ob*. The *ob* mice were very fat, had impaired immune systems, abnormal regulation of glucose and insulin, and were less fertile than normal mice. From this genetic starting place, it appeared that weight regulation was intimately connected to several other physiological processes. The multifaceted phenotypes from one mutated gene are why weight regulation is an emergent property at the organismal level. Years later, rat geneticists isolated the *ob* ortholog that caused the same complex phenotypes as *ob* mice. A pair of physiologists at Pennsylvania State University wanted to quantify and characterize the weight gain in *ob* and *wt* rats with and without exercise (Table 2). Five-week-old, *ob* and *wt* male rats were randomly assigned to either an exercise or non-exercise group of six individuals.

Table 2 Effects of exercise on rat body weight and body composition.

	initial body weight	final body weight (+ 11 weeks)	percent protein	percent lipid	percent dry matter
lean rats					
no exercise	224.8 ± 7.1*	301.4 ± 9.8	22.8 ± 0.34[a]	6.4 ± 0.49[a]	32.7 ± 0.51[a]
exercise	214.0 ± 10.1	297.0 ± 12.0	23.5 ± 0.23[a]	4.9 ± 0.41[a]	31.8 ± 0.61[a]
obese rats					
no exercise	210.7 ± 11.1	327.6 ± 15.2	14.8 ± 0.28[b]	39.0 ± 0.81[b]	56.6 ± 0.66[b]
exercise	218.0 ± 8.8	317.8 ± 13.3	17.3 ± 0.62[c]	27.7 ± 1.43[c]	48.2 ± 1.71[c]

*mean values ± standard error of the mean with 6 rats in each group. Different superscript letters within the same column indicate significant differences $p < 0.05$.

Source: From Deb and Martin, 1972, their tables 1 and 2.

Each rat was allowed to feed freely over the course of the 11-week experiment. At the end of the experiment, the investigators weighed each rat and determined the percentage of body mass for protein, lipid, and total dry matter (all water evaporated from the tissue). The average weight of each group of six rats increased, but there were no significant differences in their total weights. However, the physiologists did find significant differences in the percentage of protein, lipid, and total dry matter among the different groups of rats. In Table 2, a significant difference of $p < 0.05$ is denoted by different superscript letters when comparing values within a column. For example, there are three significantly different groups in the percent protein as indicated by superscripts a, b, and c. The lean rats, with or without exercise, were indistinguishable from each other (superscript a), but different from the *ob* rats lacking exercise (superscript b), which were also different from the *ob* rats with exercise (superscript c).

Table 2 revealed that exercise has a profound impact on the body composition of *ob* rats. *ob* rats that exercised had significantly less fat than *ob* rats that did not exercise. Similarly, the amount of protein was significantly reduced in *ob* rats compared to *wt* rats, but *ob* rats that exercised had significantly more protein, presumably muscle protein, than *ob* rats lacking exercise. Although the *ob* rats were heavier than their *wt* counterparts, the total body weight differences were not significant. Compare only their dry matter and notice that *ob* rats were heavier, which implies water was more abundant in *wt* rats than *ob* rats. Based on these findings, it would be possible to predict that American teens who exercise less have less muscle protein and more fat than their peers of the same weight who exercise more. These data and many others clarify the link between lack of exercise and obesity.

Building on the data in Table 2, physiologists wanted to determine if the amount of food consumed was the cause of weight differences between *ob* and *wt* mice (Table 3). In 1966, a second spontaneous mutant strain of mice called *db* was isolated at Jackson Labs that also exhibited the overweight phenotype. In particular, *db* mice display signs of diabetes, from which the locus name *db* was derived. The connection between obesity and diabetes is another emergent property at the organismal level. In this experiment, *wt*, *ob*, and *db* mice were randomly assigned to one of two groups composed of four males. One group was allowed to eat freely with no restrictions, whereas the other group was restricted to the

Table 3 Body weight and percent body fat in free-feeding and pair-fed mutant and wild-type mice.

genotype	age (days)	free-feeding mice			pair-fed mice		
		body weight (g)	weight change (g)	percent fat	body weight (g)	weight change (g)	percent fat
wt	20	14.6 ± 0.5*	—	9.5 ± 0.4	15.1 ± 0.6	—	n.d.
wt	48	26.1 ± 0.9	+ 11.5	9.1 ± 0.8	21.4 ± 0.8	+ 6.3	14.9 ± 0.8
ob/ob	20	17.0 ± 0.5	—	23.8 ± 1.1	17.1 ± 0.4	—	n.d.
ob/ob	48	38.6 ± 0.4	+ 21.6	42.3 ± 1.4	25.6 ± 1.0	+ 8.5	43.7 ± 1.0
db/db	20	16.8 ± 0.4	—	24.3 ± 0.9	16.6 ± 0.5	—	n.d.
db/db	48	38.2 ± 0.5	+ 21.4	36.8 ± 0.8	24.3 ± 1.2	+ 7.7	41.7 ± 0.7

*mean values ± standard error of the mean with 4 mice in each group.

Source: Modified from Coleman, 1978, his table 3.

smaller amount of food eaten by *wt* mice, which were referred to as **pair-fed** amounts of food. The experiment was terminated after 4 weeks, and each animal weighed individually. Table 3 presents the averaged weights and percent body fat for each of the six groups of mice. As expected, every group showed an increase in weight as the young mice grew. The investigators presented the variance in their data, which is helpful, but they did not indicate whether any of the differences were significant or not. The reader is left to estimate significant differences based on prior biology experience.

As expected, the *wt* mice in Table 3 gained weight over time and their percent body fat was similar regardless of feeding regime. By comparison, free-feeding *ob* and *db* mice gained about twice as much weight as *wt*, but pair-fed *ob* and *db* mice gained no more weight than the control *wt* mice. However, the *ob* and *db* pair-fed mice had much larger percentages of fat than the *wt* mice despite their equivalent overall weights. Although *ob* and *db* mice would eat more if given the option, the amount of food is not the reason they accumulated more fat. Something about their mutations caused the pair-fed mutant mice to accumulate fat even when their diets were restricted to *wt* amounts of food and their overall weight was no greater than *wt* mice. The rat data suggest that human obesity might be influenced by diet as well as genetics.

Genetically, biologists knew that *ob* and *db* were different genes and not alleles of the same gene, but no one knew their functional relationship to one another. To test how *db* and *ob* genes function and interact with each other, investigators produced parabiotic mice with all pair-wise combinations of *wt*, *ob*, and *db* mice (Figure 16). Both *ob* and *db* are autosomal recessive mutations and their phenotypes are only present in

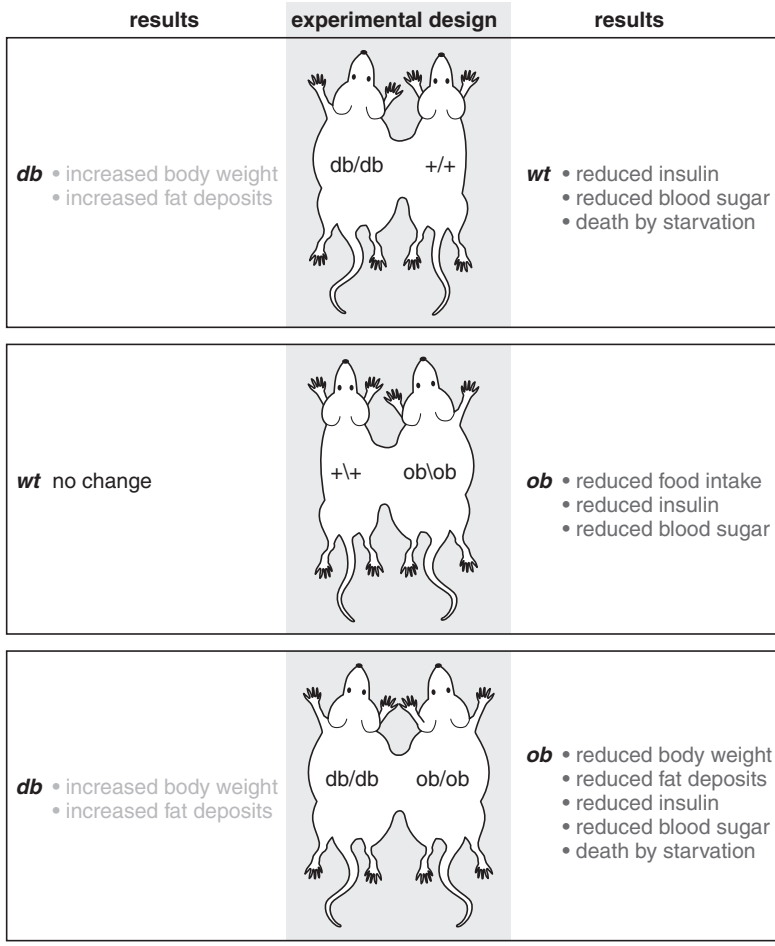

Figure 16 Summary results from* wt *and mutant parabiotic mice. Parabiotic mice in all pair-wise combinations of* wt, ob, *and* db *mice. Parabiotic mice lived for several months under free-feeding conditions before the end of the experiment when investigators collected measurements.

Source: Modified from Coleman, 1978, figure 1. Springer and Diabetologia. Coleman, D. L. 1978. Obese and diabetes: Two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia. Vol. 14: 141–148. Modified with kind permission from Springer Science and Business Media.

homozygous individuals. Parabiotic mice co-circulate blood due to the surgery used to connect two individuals.

The experiments outlined in Figure 16 produced some unexpected results. A parabiotic *db* mouse caused its conjoined partner (*ob* and *wt*) to

stop eating and eventually die from starvation. Therefore, it appears that something produced by the *db* mice was capable of overriding appetite control in both types of mice. When *ob* was conjoined to *wt* mice, the *wt* mice were unchanged, but the *ob* mice reduced their weight and other phenotypes associated with the *ob* phenotype. It appears the *wt* mouse produced something that was dominant to the *ob* phenotype. The dominant aspects of *db* and *wt* mice indicate a factor is present in the blood, because they co-circulate blood in parabiotic experiments. Because *ob* mice were homozygous recessive, they must lack the *ob*-encoded protein. Loss of functional in *ob* mice explains why *wt* mice **complemented** the *ob* deficiency and restored normal eating and fat deposition in conjoined *ob* mice. What is unclear at this time is why *db*, a recessive mutation, overwhelmed both *ob* and *wt* mice to the point of starvation.

It was not until 1994 that Jeffrey Friedman and his collaborators at Rockefeller University were able to clone and sequence the mouse and human *ob* genes. It is customary to name a protein in a descriptive way and add the suffix "-in" similar to dystrophin, which is the protein causing muscular dystrophy. Friedman's team named the protein **leptin** and the *ob* gene has been renamed *leptin* as well, although the strain of mice is still referred to as *ob*. To determine which tissues produce leptin protein, the investigators used reverse transcriptase PCR (RT-PCR). RT-PCR is more sensitive than an RNA Northern blot, so even rare mRNAs are detectable. The investigators predicted that because leptin regulates a fundamental aspect of physiology, many animals would have orthologs of leptin too. The team of biologists tested genomic DNA from many species using the mouse leptin gene as a probe.

Mammals share very similar leptin gene and protein sequences and all animals tested appear to have orthologs of leptin genes as measured by genomic DNA Southern blotting. In fact, mice can use human leptin protein when injected experimentally, and mouse leptin protein functions in human cells grown in tissue culture. Perhaps the biggest insight gained by the cloning and sequencing of the *leptin* gene was that its mRNA is detected only in fat cells and not any other tissue type, including the brain. The positive control of actin (produced in all cells) in the RT-PCR experiment indicated the mRNA was of high quality for all tissues and

thus a negative result in the top gel indicates *leptin* mRNA was not detectable in these other tissues.

The mid-1990s was an exciting time for research into body weight and fat regulation. Soon after the cloning of the *leptin* gene, investigators wanted to understand what type of mutation led to the *ob* phenotype. To help understand how leptin works, investigators used two different strains of *ob* mice, the original *ob* allele (*ob*) and a more recently isolated strain called ob^{2J}. *ob* and ob^{2J} are two different alleles of the *leptin* gene. Using the less sensitive method of Northern RNA blotting, the investigators measured the amount of *leptin* mRNA isolated from *wt* brain as well as fat tissues from *wt*, *ob*, and ob^{2J} mice. Actin was the positive control again and was detected to varying degrees in all RNA samples. The investigators detected *leptin* mRNA in fat cells from both *wt* mice as expected. In addition, they cloned and sequenced the original *ob* mRNA and compared its **cDNA** sequence (converted from mRNA) to that of *wt* leptin sequence.

It might be difficult to understand how it is possible to have two different mutant alleles of *leptin*. From their Northern blot data, the investigators found that the recessive *ob* allele produced much more *leptin* mRNA than the *wt* allele, which is counterintuitive. Given that *ob* is a recessive mutation, the *ob* leptin mRNA cannot be functional. The mRNA over abundance appears to be the body's homeostatic mechanism attempting to balance food intake and energy storage in a broken system. Leptin protein is produced in fat cells of *wt* mice, and an *ob* mouse has excessive fat deposits. As *ob* fat cells accumulated, the fat cells transcribed more and more *leptin* mRNA. When it was sequenced, investigators learned that the *ob* allele contained a nonsense mutation, a premature stop codon. Producing a truncated, nonfunctional leptin protein means *ob* mice lack a negative feedback loop that would normally slow eating and fat storage. The *ob* mouse continued to produce more fat cells, which transcribed more defective leptin mRNA and more nonfunctional truncated leptin protein in a futile feedback loop of failed regulation. However, the more recently discovered ob^{2J} allele did not produce any detectable levels of leptin mRNA, which indicated that the mutation may be in the ob^{2J} leptin promoter. Therefore, the two *leptin* alleles contain two different

mutations in the same gene, and the alleles produce two different errors that result in identical phenotypes caused by a lack of leptin protein.

Although the Northern RNA blot data were compelling, investigators could not be sure that leptin was the protein that caused the *ob* phenotype until they tested it functionally (Figure 17). The best way to determine whether a gene is the cause of a phenotype is to complement the mutation by adding back the *wt* protein and rescuing a normal phenotype. One year

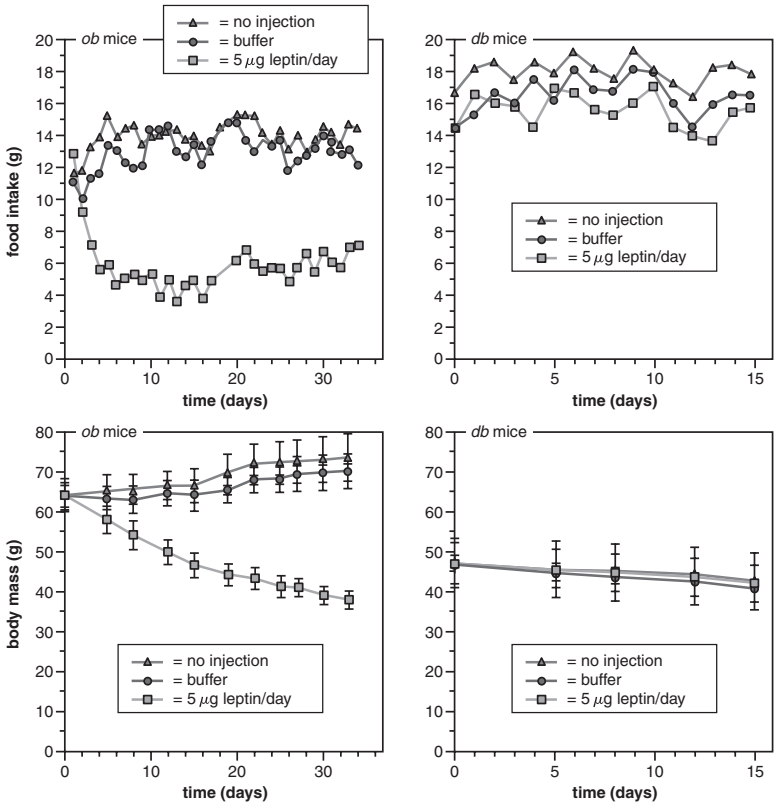

***Figure 17 Functional tests of leptin protein. Investigators produced mouse leptin protein in bacteria and injected the protein into* ob *and* db *mice as indicated. The amount of food mice ate and their body weight was measured periodically for a month. Each point represents the average of ten animals ± SD. The differences between leptin treated* ob *mice and control mice were significant* p < 0.0001.**

Source: Modified from Halaas *et al.*, figure 2. Halaas, Jeffrey, Ketan S. Gajiwala, *et al.*, 1995. Weight-reducing effects of the plasma protein encoded by the *obese* gene. Science. Vol. 269: 543–546. Reprinted with permission from AAAS.

after cloning *leptin*, Friedman's research team was testing its function by injecting leptin protein into *ob* and *db* mice. When performing this type of experiment, it is very important to make sure the simple act of injecting test subjects with an inactive agent does not produce the same effect. The investigators tested a phosphate buffered salt solution, as well as simply sticking the mice with a needle but not injecting anything at all. Over the month, Friedman's group measured food intake and weight changes.

Figure 17 demonstrated for the first time that the leptin protein could functionally complement the *ob* phenotype. When human or mouse leptin protein was produced in *E. coli* and injected into *ob* mice, the mice ate significantly less food and lost significantly more weight, predominantly in the form of less fat. The two negative controls of no injection or injecting buffer did not affect the mice. The *db* mice, however, did not change their eating behavior, nor did they lose any weight when injected with leptin. How can a functional leptin protein have no effect on *db* mice? Leptin is a protein ligand secreted by fat cells that circulates in the blood. Circulating leptin explains the parabiotic results of Figure 16 because functional leptin protein from *wt* mice was able to complement *ob* mice and cause the *obese* mice to lose weight. To affect change, a ligand must bind to a functional receptor to initiate signal transduction. Understanding signal transduction in general led to the prediction that the *db* mutation might produce a nonfunctional leptin receptor. This hypothesis is consistent with the data, including the inability of leptin injections to rescue *db* mice in Figure 17 and the inability of *wt* mice to rescue conjoined *db* mice in Figure 16. The parabiotic data indicated the leptin receptor did not circulate in the blood. The receptor is likely to be embedded in the plasma membrane of the appropriate cells, whatever those are. The receptor hypothesis for *db* led many investigators to search for the leptin receptor gene and determine the cause of the *db* phenotype.

A multinational group of molecular biologists working for a pharmaceutical company were the first to clone and sequence the *leptin receptor* gene (*db*) despite the fact that Friedman and many other academics were trying too. As soon as the pharmaceutical team cloned and sequenced the *leptin receptor* gene, they wanted to know which tissues were capable of receiving the leptin signal, so they used a RNA Northern blot to detect *leptin receptor* mRNA. Instead of relying solely on mRNA to reveal the

location of the leptin receptor transcription, the investigators also used radioactive leptin protein to incubate with brain tissue and detect functional leptin receptor protein. They obtained similar results with both mouse and rat brain tissues. In this type of ligand-binding experiment, white on a black background indicates where the radioactive leptin is bound to its receptor in thin brain tissue slices.

From the RNA Northern blot data, it appeared every tissue produced some *leptin receptor* mRNA, but some tissues produced more than others. However, the investigators did not include an actin control that would allow them to evaluate the amount and quality of mRNA loaded in each lane. Although lung cells appeared to produce the most *leptin receptor* mRNA, it is not possible to be sure because perhaps more mRNA was loaded in the lung lane than any of the other lanes. The NCBI database can be used now to determine how much leptin receptor mRNA each human tissue transcribes as well as the health status and developmental stages that affect leptin receptor mRNA production. From the NCBI database and the legend at the bottom of the web page, it is possible to see that human lung cells do produce leptin receptor mRNA but no more than the human brain and only 10% as much as human liver. The investigators did not include fat cells in their RNA Northern blot. The NCBI database indicates that adipose, or fat cells, produces more *leptin receptor* mRNA than lung and liver combined. Oddly, the tissue with the highest levels of *leptin receptor* mRNA is the trachea, which means more research needs to be conducted to understand all of leptin's emergent properties. The NCBI data reveal the importance of a loading control for Northern blots that was absent in the paper describing the cloning of the *leptin receptor* gene.

Soon after determining the DNA sequence of genes encoding leptin and its receptor, physiologists formulated a working model to explain how organisms maintain about the same body weight and fat deposits through homeostasis (Figure 18). When a *wt* individual accumulates more fat, more leptin protein is produced and the individual eats less, burns more energy, and accumulates less fat. Conversely, when a *wt* individual has less fat, less leptin is produced, which triggers more eating and more fat storage. Because fat regulation is similar to temperature regulation in a building, biologists coined the term **lipostat** to indicate that fat deposition is regulated like a thermostat regulates a building's temperature.

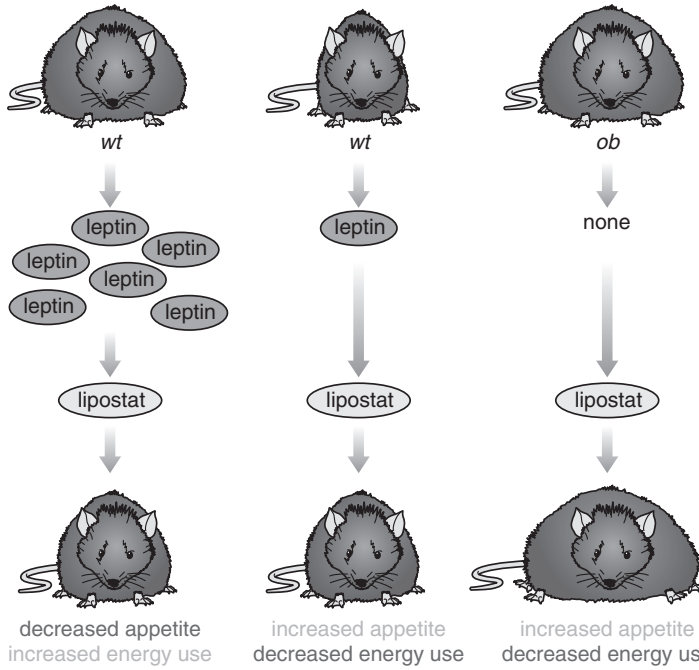

Figure 18 Proposed lipostat mechanism for fat regulation. Fat* wt *mice produce more leptin, which causes them to lose fat. Skinny mice produce less leptin, which causes them to store more fat.* ob *mice produce no functional leptin, which causes them to store more fat.

Source: Modified from Barinaga, 1995, figure 1.

The brain tissue of the hypothalamus did not labeled brightly with radioactive leptin, but instead the **choroid plexus** contained the highest concentration of leptin receptor protein. The choroid plexus is composed of capillary blood vessels within the spaces, called **ventricles**, of the brain. The **cerebrospinal fluid** is produced by the choroid plexus and provides a physical barrier to exclude many blood-borne molecules from the brain and the cerebrospinal fluid. Leptin protein is present in cerebrospinal fluid, which indicates some cells of the brain and spinal cord probably express leptin receptors on their plasma membranes too. The current understanding of why the choroid plexus has the highest concentration of leptin receptors is that this capillary bed transports leptin from the blood into the cerebrospinal fluid. Leptin is produced in fat cells, circulates through the blood, and is transported to the cerebrospinal fluid,

which supplies the leptin ligand to cells of the hypothalamus. It would be reasonable to predict at least some hypothalamus neurons produce leptin receptors on their plasma membranes, but the data were not provided in the research paper.

Whenever scientists collect data about a particular system, they try to develop a model that can explain the existing data and allow them to formulate testable predictions. The lipostat predicted that leptin initiates a negative feedback loop, which signals the organism to become lean. More fat produces more leptin, which results in less fat and less leptin. *ob* mice fail to produce functional leptin, so they cannot benefit from homeostasis and therefore continue to accumulate more fat. In parabiotic mice, *ob* mice benefited from the *wt* leptin protein that circulated in the shared blood. However, *db* mice lack the leptin receptor, so their cells cannot bind leptin protein and thus continue to accumulate more fat, which results in more leptin production (a positive feedback loop). When conjoined to other mice, the *db* mouse overwhelms its parabiotic partner with excess leptin protein, which causes the partner to become fatally thin. Unfortunately, the lipostat model does not explain the emergent properties in *ob* and *db* mice that develop diabetes and are less fertile than their *wt* counterparts. With the available data, one can conclude that fat and energy regulation is an ancient trait, shared by all animals, and one that is connected to sugar homeostasis and reproduction.

Once the regulation of a particular trait is understood, investigators often want to understand the trait's natural variation within a population. In Figure 19A, investigators wanted to know whether the amount of leptin circulating in the blood was consistent throughout the day. Periodically over the course of 24 hours, physiologists from Ohio State University measured the leptin levels in the serum of 25 13-year-old girls. The box plot shows the mean values (horizontal line), the middle 50% (box), and the variability (error bars are +/− 2 SE). A statistical test confirmed that leptin levels are not the same all day long ($p < 0.0001$). For Figure 19B, the investigators calculated how much leptin levels increased during the night for each girl, and graphed her percent nocturnal increase in leptin against her change in body fat over a six-month period. The *p*-value of < 0.024 indicates that the slope of the best fit line is significantly

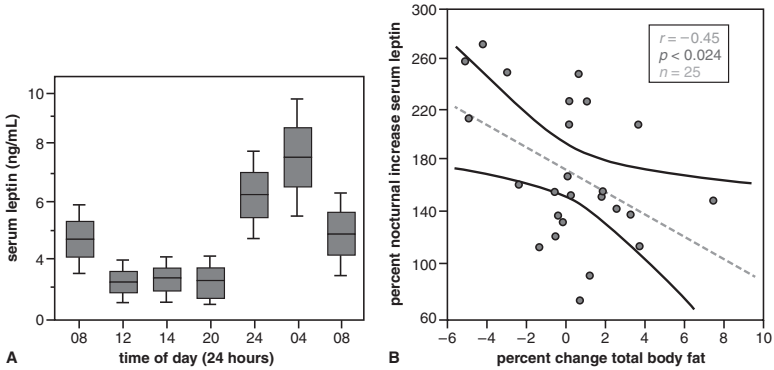

Figure 19 Variation in leptin levels. A, Box plot showing variation in daily leptin levels in 25 13-year-old girls; midnight and 4 AM were significantly higher, p < 0.0001. B, The same 25 girls were weighed six months later and their percent change in total body fat was negatively correlated with their percent nocturnal increase in serum leptin. Dotted line shows the line of best fit, and black arcs show the 95% confidence curves.

Source: Modified with permission from Matkovic *et al*., 1997.

different from a horizontal line with slope of 0. The correlation coefficient (r) of −0.45 indicates that girls with the largest percent increase in nocturnal leptin lost the most body fat. The confidence curves reflect the variability in the percent change in nocturnal leptin levels (y-axis) for different values of the percent change in total body fat (x-axis). Despite variance in the data, the lipostat model was supported by the data in Figure 19. The box plot revealed that leptin levels are not the same all day long which indicates more emergent properties probably exist in leptin gene regulation.

Figure 19A showed that leptin increases at night when most people sleep and therefore people can go longer without feeling hungry at night than during the day when leptin levels are lower. These data also illustrate why it is a bad idea to have a midnight snack when trying to reduce fat accumulation. By snacking at night, the eater is overriding the natural tendency not to eat at night, which could hasten the onset of obesity. For the girls in Figure 19B with lower levels of nocturnal leptin, the lipostat predicted an increase in fat accumulation. As predicted, the

girls with lower nocturnal leptin levels gained more body fat over the 6 months, whereas those with higher leptin at night lost body fat.

According to the CDC, obese people have a higher probability of suffering from heart attacks, strokes and diabetes. No one fully understands the biochemical connection between leptin and these disease-based emergent properties associated with obesity. Physiologists have been trying for many years to understand the mechanisms linking leptin to a variety of phenotypes. In 2004, a group from Lublin, Poland, studied the effects of leptin on the heart rate and blood pressure in adult male rats (Figure 20A). Rats were randomly assigned to one of three groups of eight individuals—free-feeding control rats, leptin-injected rats, and pair-fed rats matching the amount of food eaten by the leptin-injected group. These data show a connection between leptin and circulatory problems, but the mechanism remains unknown. A team of French cell biologists wanted to test the effects of elevated insulin on

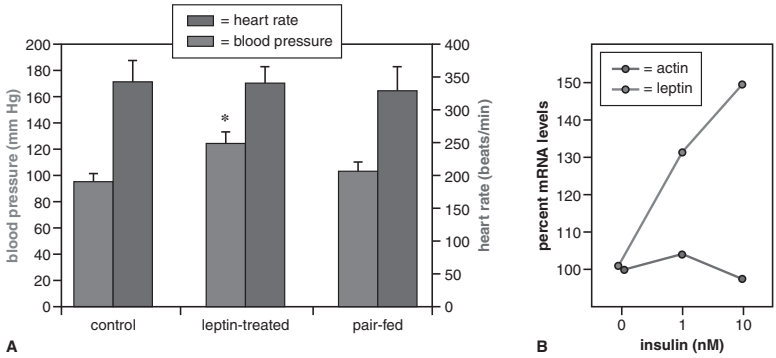

Figure 20 Leptin affects many aspects of homeostasis. A, Three groups of eight wt male rats were treated as indicated and measured for blood pressure and heart rate; * indicates significant difference in blood pressure ($p < 0.05$). B, Change in mRNA levels of fat cells isolated from rats cells two hours after insulin exposure.

Source: Panel A modified from Beltowski *et al.*, 2004, their figure 1. Beltowski, Jerzy, Anna Jamroz-Wisniewska, *et al.* 2004. Up-regulation of renal Na+, K+-ATPase: the possible novel mechanism of leptin-induced hypertension. Polish Journal of Pharmacology. Vol. 56: 213–222. Reprinted with permission by Institute of Pharmacology, Polish Academy of Sciences. Panel B modified from Saladin *et al.*, 1995, figure 4c. Reprinted by permission from Macmillan Publishers Ltd: Saladin, Régis, Piet De Vos, *et al.* 1995. Transient increase in obese gene expression after food intake or insulin administration. Nature. Vol. 377: 527–529.

leptin production because obese individuals typically have elevated levels of insulin. When investigators exposed adult male rat fat cells grown in tissue culture to two different concentrations of insulin, they detected substantial differences in leptin mRNA (Figure 20B).

The emergent properties of weight regulation and the many functions of leptin are apparent in Figure 20. Elevated leptin in *wt* rats led to increased blood pressure, which is a contributing factor in heart attacks and strokes. Added insulin produced elevated leptin mRNA in isolated rat cells, which indicates glucose regulation influences leptin production and fat accumulation. It makes sense that glucose and fat would have overlapping cell signaling mechanisms, but their overlap makes it very difficult for investigators to distinguish cause and effect for obesity and diabetes. Regardless of the mechanisms, obesity and leptin are linked to high blood pressure and diabetes. Because obesity results in elevated insulin levels and more leptin, it appears the two protein ligands may spiral upwards in a connected positive feedback loop—more insulin produces more leptin and more leptin stimulates more insulin.

The last emergent property considered in this chapter is the connection between leptin and fertility. Perhaps no phenotype plays a bigger role in evolution and natural selection than fertility. Three investigators from the University of California at San Francisco examined sperm production in *ob* mice. They microscopically examined testicular tissue isolated from untreated *wt* mice, untreated *ob* mice, pair-fed *ob* mice, and leptin-treated *ob* mice. For each sample, they focused on the presence or absence of sperm cells. Obviously, males that fail to produce sperm cells are sterile, so they were able to determine the cellular cause of *ob* sterility in males. If *ob* mice are sterile, how can they exist? Mouse geneticists maintain their collection of *ob* mice by mating heterozygotes so that 25% of the offspring will be *ob*.

The connection between *ob* and male sterility was clear. *ob* males who received leptin injections regained their fertility because they produced sperm for as long as the injections continued. Pair-fed mice did not produce sperm, which indicates the amount of food was not the important factor. Unfortunately, few studies have been published trying to connect leptin and female fertility.

This chapter explained how animals maintain body fat with their lipostats. Leptin signals many different physiological processes including sugar regulation, blood pressure, and sperm production, but no one understands how these different pathways intersect. With the lipostat, temporary weight gain, such as what happens with overeating during the holidays, is not a problem. Slight increases in fat produce more leptin, which reduces appetite and fat storage. However, obese individuals tolerate elevated leptin levels, which means their lipostats have adjusted to a new set point. When the lipostat adjusts to a higher level of leptin, it is as if the leptin receptors have desensitized to leptin just as the sense of smell can desensitize to a persistent smell that becomes unnoticeable with time. Exercise is the only way one can move the lipostat to a lower set point. To confirm this prediction, look back at Table 2 to remember the positive effects exercise had on fat regulation in *ob* mice. Exercise reduces body fat content and increases protein content. Weight loss can be sustained through persistent exercise but not through fad diets. Short-term fat loss through extreme dieting results in regaining the fat later. Changing behavior to eat less and cutting out nighttime snacks can help restore the lipostat to its original function. Among these options, the most important behavior change is exercise. Exercise is the most reliable way to reduce fat and keep it off.

Animals have evolved the emergent property of negative feedback loops to maintain a consistent weight and percent body fat. Short-term overproduction of leptin after temporary fat gain slows eating, increases energy use and maintains the lipostat set point for the long-term amount of body fat. However, long-term exposure to elevated leptin makes individuals insensitive to leptin and their lipostats become reset for increased fat storage. A lipostat broken by too much leptin is one of many emergent properties for fat regulation. All animals require resources, which results in feedback loops associated with leptin and fat regulation. People produce different amounts of leptin over 24 hours, which provides a flexible response to fat regulation. Finally, leptin is connected to many important physiological processes, which shows that fat regulation is more than the sum of leptin and its receptor. The following Ethical, Legal, Social Implications integrates fat regulation at the organismal level to a national health policy. The

last chapter in this book considers the ultimate emergent property—senescence and death.

Ethical, Legal, Social Implications: Challenges of the Obesity Epidemic

Chapter 3 explained that individuals have different set points for their lipostats. Is the set point simply a matter of behavior and choices, or do other factors contribute to obesity? Two facts are indisputable—exercise and consuming fewer calories can maintain lower fat and total body weight. But a lot remains to be discovered about obesity. Several research groups have found that the bacterial ecosystem living in the intestines of obese mammals is significantly different than the microbes living in lean individuals. In 2010, a group of immunologists found that a person's immune response to the gut bacteria plays a role in the development of obesity. An international team from the USA, Canada, and Australia published a study of 142 inbred *Drosophila* fruit fly strains and found significant differences in weight regulation when fed identical diets. The implication of their research is consistent with Dr. Friedman's contention that the alleles we inherit from our parents play a role in our likelihood to become obese. Three investigators from Louisiana State University found that obese mother rats produced obese offspring at a higher rate than non-obese mothers, even when diet was consistent between the two populations. Despite the ability to affect one's lipostat by exercise and food consumption, weight and fat regulation contain emergent properties that are influenced by gender, genetics, epigenetics, immune responses, and bacterial symbiosis. Therefore, can a nation formulate a national public health strategy to obtain the desired goal of reduced obesity?

As with most ELSI topics, developing a public health strategy involves personal values, opinions and emotions. Except for extreme athletes (such as sumo wrestlers), no one wants to be obese and risk diabetes and heart failure. Furthermore, being obese is more expensive given that airlines have started charging obese people for two seats, extremely large clothes are more expensive, and of course medical care is much more expensive. Therefore, it would seem that a national policy on good health would be

easy. If it were easy, we would already have a public health strategy, so producing good health policy must not be easy.

Some people resent government intrusion even if the advice is for their own good and consistent with their own wishes. "No one can tell me what to do." For example, some motorcycle riders resent helmet laws despite their obvious benefits. The US government already requires nutrition labels on all packaged food. Fast food restaurants are required to inform customers how much fat is in the food they consume. For example, go to the Fast Food Nutrition Calculator and search the database for the number of calories in chicken products. In 2010, Dairy Queen served a chicken meal for one person that contained 1,340 calories, 870 from fat, whereas KFC sold a chicken breast without skin or breading that contained only 140 calories. Should people be rewarded when they make healthier choices and punished for unhealthy choices? Most governments tax tobacco products in part because they want to discourage people from smoking. Smoking costs everyone money through lost productivity at work and increased healthcare costs. Similarly, obesity increases healthcare costs. The CDC calculated that healthcare in America due to obesity accounted for 9.1% of all money spent on healthcare in 2006, for a total of 146 billion dollars annually, or $1,429 per person. Should there be a fat tax on foods that exceed a maximum calorie limit just as we tax tobacco?

Rather than punishing bad choices, does it make more sense to reward good behavior? Would the country support a 9% discount on health insurance for being lean? Should life insurance be adjusted for people whose BMI is below 30? Should airline tickets cost less for people who are in the lightest 10% of the population for their height? Economic incentives can change people's behavior, and perhaps behavioral modification could accomplish the desired outcome for weight and fat maintenance. Would such policies discriminate against people based on their genetics or microbiome? Given that obesity varies by state, this may not be a feasible policy since being in the lightest 10% of the population might depend on where the ticket was bought. We cannot alter people's genetics, nor their mothers' eating habits, but it is possible to influence their current behavior. Should the government or companies who offer their workers employee-based health insurance develop strategies to help people achieve their weight goals?

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CHAPTER 4

Animals Age and Die at Different Rates

Chapter 4 is based on an emergent property of which everyone is very aware—all individuals age with time and eventually die. Aging, from conception to death, could not be predicted by studying an individual for a short amount of time. Death and aging are two certainties that come with life. By studying individuals for short periods of time, it would not seem obvious that they would become old and die. Tom Kirkwood, a leading researcher in senescence said, "Aging is a process where the end result is obvious but the mechanism remains obstinately obscure." Getting older and becoming less fit, **senescence**, seems counter to natural selection and evolution. Why don't individuals stay young and reproduce forever? Why has natural selection favored senescence over eternal youth?

Research in senescence focuses on two major categories of questions—why and how? Why do all individuals decline in their functions as they age? How do individuals become less fit as they age? How do cellular and molecular processes lead to deteriorating health and performance? These questions are not new and were asked in 350 BCE by Aristotle who wrote, "The reason for some animals being long-lived and others short-lived calls for investigation." Although senescence has been studied for over 2,000 years, the first modern hypothesis was published in 1891 by August Weismann. Weismann proposed that aging makes room for the young. Although Weismann's hypothesis tried to accommodate natural selection, he was not right because most organisms die from predation, disease, or starvation before senescence begins. Furthermore, Weismann implied that older individuals would essentially sacrifice themselves for the sake of younger individuals, which is a claim not supported by data.

When searching the AnAge database, the longevity winner is the hexactinellid sponge with an estimated lifespan of 15,000 years, which is about 100 times older than the oldest human! Sponges are colonial animals that feed on small plankton in the water. It is also surprising to realize that 14 of the oldest species live in the water and that 14 species are known to live longer than humans. The oldest silver-haired gorilla was 55.4 years old; lived in the Dallas, Texas zoo; died of cancer; and was diagnosed with cataracts in her eyes.

Biologists made little headway on aging research until the 1950s with the publication of two new hypotheses for senescence. During the twentieth century, biologists had documented many life histories with one of two typical mortality and reproduction rates (Figure 21A). The constant mortality rate for many species demonstrated that Weismann's hypothesis could not be correct because most individuals die early, and their reproduction is constant once they mature. Constant mortality and reproduction rates contrast with the human life history where mortality is high for the first 5 years, slows down, and later accelerates again. During the time of lowest mortality, humans and other species reach sexual maturity but experience reduced fertility later in life as senescence progresses. In 1952, Peter Medawar proposed a new hypothesis that tried to account for the two major types of mortality and reproduction. According to Medawar's **mutation accumulation hypothesis**, genes that cause aging and death evolved because they were not selected against due to the lateness of their activity, after reproduction. Aging alleles that are transcribed after reproduction would be passed on to subsequent generations. Mutation accumulation predicts that all cells experience a steady number of mutations (Figure 21B). If a cell experienced a small burst of errors in replication, transcription, or translation, then the cell would pass a threshold of quality control and produce ever increasing errors (mutation accumulation) that would ultimately lead to that cell's death.

The hypothesis that aging and death made room for the young was discredited because of data similar to the "constant" graph in Figure 21A. Most organisms die prior to senescence, so aging to make room for the next generation was inconsistent with the data. Mutation accumulation required that different species accumulate mutations at two different rates: one mutation rate for the "constant" and another mutation rate

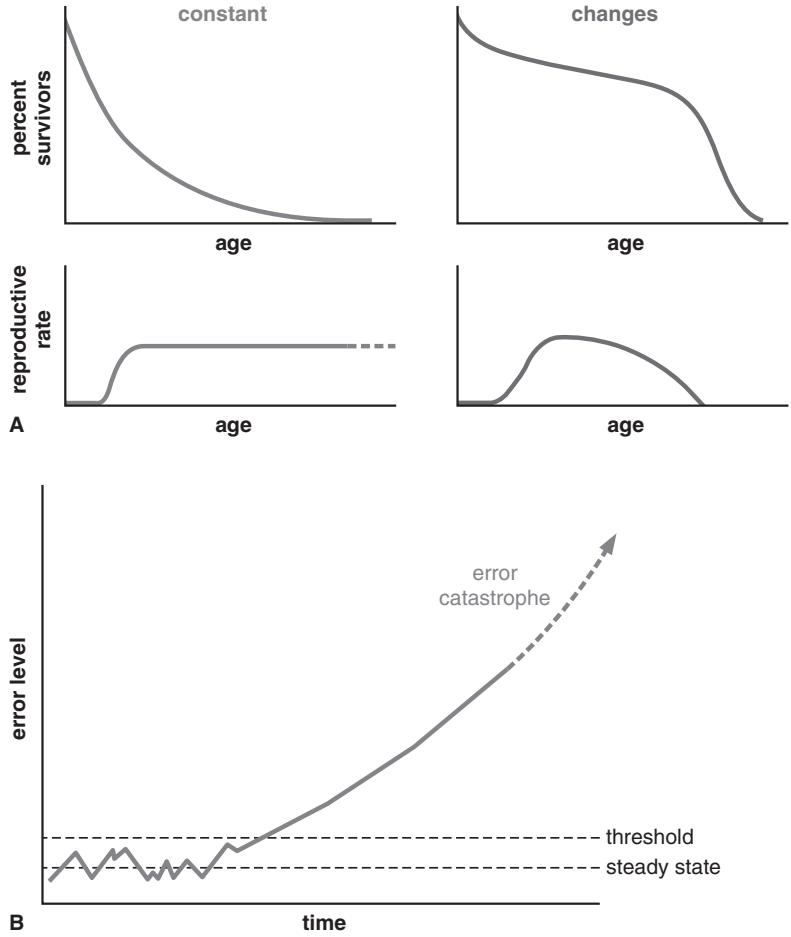

Figure 21 Aging, reproduction, and information errors are affected by time. A, On the left, mortality is constant for an individual's entire life, and reproduction is constant once it matures. On the right, mortality varies during an individual's lifetime, as does its reproductive success. B, DNA and protein information processing varies around a steady state level of errors unless errors accumulate by chance above a point of no return.

Source: Panel A modified from Kirkwood and Holliday, 1979; their figure 2. Panel B modified from Kirkwood and Holliday, 1979; their figure 4. Kirkwood, T. B. L., and R. Holliday. 1979. The Evolution of Ageing and Longevity. Proceedings of the Royal Society of London. Vol. 205: 531–546. By permission of the Royal Society.

for the "changes" life histories. Furthermore, the error catastrophe shown in Figure 21B would require two different slopes to account for the two different mortality rates in Figure 21A. Perhaps the greatest inconsistency in mutation accumulation is that natural selection should result in extremely long-lived individuals that accumulated particular mutations that made them resistant to subsequent mutations. If an individual could postpone the error catastrophe, then it would produce more offspring, and the entire population would evolve to live longer and longer, which is contradictory to all the available data.

Mutation accumulation was revised in 1957 with an explanation called **antagonistic pleiotropy**. Antagonistic pleiotropy states that genes responsible for senescence evolved with tradeoffs; they were beneficial when an individual was young but became detrimental late in life. Antagonistic pleiotropy was supported in a 1961 study conducted by Hayflick and Moorhead who cultured human cells isolated from tissue samples. The biologists worked with 25 different sources of cells, and all of them grew exponentially. The original cells grew slowly at first and then the populations grew very large quickly. After about six months in culture, most cells began to slow their growth and reached a maximum number by about the 50th subculturing. After about 12 months of growing in culture, the human cells quickly senesced and died. However, a small number of cells experienced DNA mutations and become cancerous and immortal. The cancerous cells never stopped growing as long as they were fed.

The data from human cells grown in culture supported antagonistic pleiotropy in two ways. First, alleles that led to rapid growth early in the cells' existence in culture would eventually lead to a coordinated senescence. Antagonistic pleiotropy allows the transmission of alleles that lead to senescence to be passed on to the next generation, because the alleles are beneficial early in life and detrimental only after an organism has reproduced, as shown in the "changes" graph in Figure 21A. Second, antagonistic pleiotropy was altered by mutation as indicated by chromosomal changes that lead to cancer formation and cellular immortality. Cells normally die after a set number of cell divisions, except when mutations alter the normal pathway of senescence.

Antagonistic pleiotropy was accepted as a good explanation for the evolution of senescence until 1979 when Tom Kirkwood offered a

different explanation he called the **disposable soma hypothesis**. Kirkwood proposed each species exhibits one of two possible evolutionarily fates. Under the disposable soma hypothesis, one evolutionary fate was that an individual could expend a substantial amount of energy to maintain its body, or soma, and reproduce over a longer period of time because it lived longer. The alternative fate was for an individual to devote very little energy to maintaining its body and reproduce early. These individuals devoted the majority of their energy to mating early, and often, due to the high mortality rate experienced by individuals of the species. The significant innovation in the disposable soma hypothesis was that it did not invoke the use of genes to directly influence senescence as both mutation accumulation and antagonistic pleiotropy had.

The disposable soma hypothesis matched the existing data but had not been rigorously tested until 1993 when Steven Austad went to Sapelo Island, Georgia, in search of American opossums, *Didelphis virginiana*. To critically test the disposable soma hypothesis, investigators would need to compare two genetically isolated populations of a species that had evolved under different environmental conditions. One population would need to experience relatively high mortality due to the local conditions (from predators), whereas the other population would experience a much lower mortality rate due to a lack of predators. Disposable soma hypothesis predicted that senescence and reproduction would be postponed in the individuals experiencing lower predation pressure because of their safer environment. To find the ideal natural populations, Austad needed two field research stations in close proximity that both contained opossum populations. Savannah River Environmental Research Park on the coast of South Carolina was very near the University of Georgia's Marine Research Station on Sapelo Island, and both sites had genetically related populations of opossums. Austad put radio beacons on 34 female opossums on Sapelo Island, 37 radio beacons at Savannah River, and tracked them until their natural deaths. Opossums normally reproduce for 2 years before senescence. Previous research showed that more than half of all opossum deaths are due to predation. Austad captured the females and took physical measurements of the mothers as well as their offspring, which they carry in pouches because they are marsupials.

After tracking opossums for over 2 years, Austad was able to determine the mortality rates and whether the opossums experienced constant or changing types of mortality, as in Figure 21A. Austad compared the mortality rates of the two populations of opossums and graphed them after transforming the mortality rates to their natural log (*ln*) values to convert the graphs to straight lines. As predicted by the disposable soma hypothesis, island opossums lived longer and had significantly lower mortality rates. Furthermore, island opossums had lower fertility in their first year of reproduction than mainland opossums, as predicted by the disposable soma hypothesis. Austad measured physiological traits from the two populations to see if something other than mortality could explain the differences in reproduction (Table 4). He also measure temperature and precipitation, which showed no weather differences for the two field sites.

AnAge database contains information about *Didelphis virginiana*, including Austad's data from Sapelo Island as described in this chapter. Male opossums mature later than females, and it would be expected that island males would mature later and reproduce longer than mainland opossums if the disposable soma hypothesis were true. Based on the data, island opossums do not vary in total mass of their offspring between year one and two, whereas mainland opossums produce a greater mass in year one. The mass of mainland opossums' second year litter is approximately the same as the mass for both litters for island opossums. If island opossums reproduce during year three and into year four, the island opossums eventually to produce more offspring than their mainland counterparts,

Table 4 Comparison of island and mainland opossum traits. The p values quantify the statistical significance of the differences between the two populations. n.a., Not applicable.

trait	island	mainland	p value
mean longevity	24.6 months	20.0 months	0.002
maximum longevity	45 months	31 months	n.a.
age at first litter	11.56 months	10.55 months	0.297
average size of litter	5.66 born	7.61 born	< 0.001
tick parasite load (second year)	22.7 on ears	10.6 on ears	0.036
blood glucose level (mg/dL)	102.3	91.0	0.126

Source: From Austad, 1993 Tables 1 and 2. Austad, Steven N. 1993. Retarded senescence in an insular population of Virginia opossums (*Didelphis virginiana*). Journal of Zoology, London. Vol. 229: 695–708.

but the data do not extend beyond two years. Disposable soma hypothesis correctly predicted that short-lived individuals would produce more offspring sooner because these mainland opossums allocated less energy to longevity than island opossums. Island opossums allocated more energy to long-term survival because the reduced island predation means they enjoy the possibility of living four years instead of only two.

Island opossums have much smaller litters and carry many more ticks than their mainland counterparts. Despite the higher load of ticks, the island opossums have comparable glucose levels to mainland individuals. In short, the disposable soma hypothesis predictions held true, and Austad's data supported the explanation for the evolution of senescence, which is an emergent property at the organismal level. With experimental support from Austad and many others, the disposable soma hypothesis is now referred to as a theory. For *Homo sapiens*, our life history in Figure 21A was initially determined by survival in the wild, but now humans have altered the selection pressures and we tend to live longer and reproduce later. People allocate many of their resources to live long lives, but Kirkwood comically summarized his opinion when he wrote, "The best recipe for a long life is to choose your parents well."

The disposable soma theory does not focus on why evolution might have resulted in aging at all. Instead, senescence evolved because natural selection is unable to affect late-life traits that are the consequences of resource allocation based on mortality rates in natural conditions. Other biologists reasoned that if the disposable soma theory is true, then it should apply in laboratory as well as field settings. Biologists who study evolution are aware of the tradeoffs between field research (realistic but uncontrolled) and laboratory research (highly controlled but not realistic). Austad had tested disposable soma theory predictions in the field; and in 2000, Stephen Stearns and his collaborators tested this theory in a laboratory setting using the fruit fly *Drosophila melanogaster* (Table 5). Stearns and his colleagues let evolving populations determine which traits would change, such as longer lives with fewer offspring early in life. In this case, the biologists subjected two equivalent fly populations to different treatments. Both populations initially consisted of 100 males, 100 females, and 250 larvae. Twice each week, only a subset of the adults was moved to a new vial and allowed to reproduce. In the high adult

Table 5 Comparison of physical traits for fruit flies subjected to high or low adult mortality.

trait	high mortality rate		low mortality rate		p values
	# of flies	averages	# of flies	averages	
female development (hours)	389	254	345	272	0.0041
female dry weight (μg)	90	242	90	261	0.0156
fecundity (average # offspring)	340	40.8	322	27.0	0.0035
male development (hours)	389	260	334	276	0.0061
male dry weight (μg)	388	197	332	217	0.0182

Source: From Stearns *et al.*, 2000 Table 1. Stearns, S. C., M. Ackermann, *et al.* 2000. Experimental evolution of aging, growth, and reproduction in fruitflies. PNAS. Vol. 97(7): 3309–3313. Copyright 2000 National Academy of Sciences, U.S.A.

mortality treatment, 90% of the adults were killed so that the probability of an adult surviving one week was $0.1 \times 0.1 = 0.01$ (two weekly exterminations in which only 10% survived). The low adult mortality treatment killed only 10% of the adults twice a week, and the probability of an adult surviving one week was $0.9 \times 0.9 = 0.81$. These experimental selection pressures continued uninterrupted for three years. At the end of the experiment, they measured many traits, some of which are in Table 4.

In Table 4, all five fly traits showed significant differences between the two treatments. The male and female flies of the high mortality group developed faster, weighed less, and produced about 50% more offspring. All of these physical traits responded as predicted from the disposable soma theory as a result of the strong selection pressure applied to the adults.

Combining laboratory and field-based methods in 2008, Salma Balazadeh and her colleagues tested the disposable soma theory using several natural variants of the model flowering plant *Arabidopsis thaliana*. The biologists monitored laboratory growth, reproduction, and senescence rates of eight strains of plants taken from natural populations. All of the plants were grown in identical laboratory conditions of light, temperature, and moisture. Each plants bolted (sprouted their flowering stalks) at different times with four bolting sooner than the other four. Bolting was defined as the time when a plant sent up its stalk 1 cm or higher. Flowers and the subsequent seeds were produced from the top portion of the stalk, as shown for the four plants on the right side of the photograph. The extent of plant senescence was calculated by the percentage leaf surface area with reduced green chlorophyll pigmentation from maximum chlorophyll levels. Balazadeh and her colleagues measured many physiological

parameters, including the timing of bolting and the total number of leaves on the plant. They measured the correlation between the extent of whole-plant senescence and either the age of the plant at bolting, or the total number of leaves at the time when the slowest aging isolate began to senesce. The fast-bolting plants put more energy into reproduction by bolting sooner, and as a consequence, produced fewer leaves and senesced sooner than their late blooming counterparts. Once again, the disposable soma hypothesis was supported but this time by data collected on plants.

Before considering mechanistic causes of aging, it is important to confront a widely held misconception that bacteria do not age. Most textbooks or websites addressing aging and senescence state that not all organisms age. These sources indicate that microbes do not age because they divide evenly and do not have sex cells. Rather than accepting these statements on faith, a scientist asks for the data to support these claims. It is well known that microbes, including *E. coli*, divide evenly and do not have sex cells but is that the same as not aging?

In 2005, Eric Stewart and his colleagues documented aging and senescence of individual *E. coli* cells. Stewart's lab isolated single cells and used microscopes to film the binary fission of genetically equivalent, clonal populations of cells. Using movies and software, Stewart was able to color code both ends of a cell and count the number of times each end had participated in a cell division. For example, an older cell end might have participated in three cell divisions, whereas the younger end might have only participated in one cell division. Using computer image analysis, Stewart was able to determine the age of individuals within a clonal population. Furthermore, he could quantify the growth rate of each cell and correlate age with growth rate. By showing the data as a branching tree, the scientists did not have to distinguish individual lines to understand the trend. Each cell division would produce one cell that was older than its sibling cell. The older cell was placed on the right side of the branch point and colored red, whereas the younger cell was colored blue and placed on the left side of the branch point. The length of any particular branch was proportional to its growth rate so that cells that divided quickly were displayed as longer lines. After four cell divisions, each generation was separated by a horizontal line placed at the end of the shortest branch from the previous generation. By systematically placing the oldest cells

to the right of each branch, it was possible to see a general trend of short branches being on the right of the figure and longer branches on the left. The slowest growing cell in the branching tree was the one on the far right, and it was also the oldest cell in the entire population. The youngest cells are on the left side, and they have the longest branches. Bacteria such as *E. coli* do age and show signs of senescence in their growth rates.

Younger *E. coli* cells grow faster than the population average and older cells grow slower than the population average. The older cells decrease their growth rate faster than the young cells increase their growth rate as indicated a graph showing cell growth as a steeper negative slope for older cells than the positive slope for younger cells. Because the young cells grow faster, they will become a larger percentage of the total population. Over time, the oldest cells become a smaller proportion of the total population, especially because young cells are produced fresh each generation but the oldest cells only get older. It would be interesting to know if the oldest cells eventually die, but unfortunately the data are not available to determine if they die or simply divide slower with each generation and thus become less abundant in the population. Now that it was known that individual bacteria senesce, they wondered how could aging happen in a single-celled organism? Does the disposable soma theory still apply to prokaryotes? With the available data, it is not possible to determine whether disposable soma theory holds true for *E. coli* and other prokaryotes.

For plants and animals, the disposable soma theory seems to be the best explanation why organisms age and senesce. Pacific salmon provide an extreme example of the disposable soma theory. They expend all their energy to migrate up a stream, jumping up waterfalls to spawn one season and then die minutes later. All of their resources are allocated to a single mating period because it would be impossible for one individual to survive a return trip to the ocean and another migration upstream to spawn again. On the other extreme is the ugly, but long lived, naked mole rat *(Heterocephalus glaber*), which can live 30 years, much longer than a typical mouse (*Mus musculus*), which lives about 2 to 3 years. Mice and naked mole rats seem similar enough in size that it would be logical to predict that they would both live about the same amount of time, but they do not.

Oxidation in one way to damage cells that can lead to senescence and death. Commercial websites claim their products can prevent oxidation of molecules in cells. If antioxidants really are the key to a long life, then it would seem logical that naked mole rats would have high levels of antioxidants and their cells would exhibit very little evidence of oxidative damage. Blazej Andziak and his colleagues wanted to determine if naked mole rats had lower levels of oxidative damage than laboratory mice. The biologists measured the amount of oxidative protein damage in the kidneys of mice and naked mole rats of functionally equivalent age (4-month-old mice and 2-year-old naked mole rats). The investigators also tested the amount of oxidized lipid isoprostanes in the urine of age-equivalent rodents as well as rodents that were the exact same age.

The experiment revealed that mice have lower levels of oxidized kidney protein as well as oxidized urinary lipids. These differences are significant for all comparisons as indicated by the p values of less than 0.001. Therefore, the prediction that mice would have higher concentrations of oxidized products was incorrect and contrary to what people say who sell antioxidants to prevent aging. However, it would be over-interpreting the data to say the naked mole rat data demonstrate that oxidation plays no role in aging. Oxidation may well play a role, but it is not the only player in the aging process, as shown in Table 6. There appear to be at least five pathways that contribute toward aging, but their relative importance may differ between species. Mice may experience the other four molecular events much more than naked mole rats. There is not enough

Table 6 Proposed molecular events that can contribute to physical aging and senescence.

molecular events	description
oxidation	DNA, lipids, proteins and mitochondria can be damaged by reactive oxygen ions produced as byproducts of biochemical reactions in the cell. Anti-oxidants can neutralize these damaging ions.
cross linking	proteins form inappropriate covalent bonds with DNA, blocking their own function and the cellular recycling processes.
DNA errors	nuclear and mitochondrial DNA mutations can accumulate and lead to malfunctions for the cell – mutation accumulation leading to error catastrophe.
telomeres shorten	telomeres, the ends of chromosomes, shorten with each round of replication and eventually chromosomes can "unravel" and stop working properly. See population crash in Figure 15.3.
hormonal changes	hormonal levels change as individuals age but it is not clear if these are the cause or consequence of senescence.

Source: General knowledge from various sources.

information to know yet which is what keeps biologist asking questions and designing experiments.

Because mice live aboveground, they are more likely to experience predation pressure than the subterranean naked mole rats. As with opossums on the mainland, mice have shorter lives, produce more offspring quickly, and allocate fewer resources to maintaining their body for many years. One pair of mice can produce an average litter of seven pups ten times a year. In contrast to mice, naked mole rats are one of only two **eusocial** mammals. Only the queen naked mole rat mates with males, which means most females are effectively sterile. In their native habitat, a queen produces only one litter a year for an average of eleven offspring per year. These reproductive outcomes are consistent with predictions based on the disposable soma theory. Because the mice die primarily by predation, they have evolved to put their resources into quick reproduction rather than long term maintenance of their bodies.

If senescence is considered a failure to maintain the body, then aging could be a failure of any one of the five different maintenance systems in Table 6. Aging and senescence are organismal emergent properties that evolved in response to events during individuals' lives, such as predation and natural selection when competing for limited resources. Everyone is aware of aging and senescence. Parents and grandparents age and decline physically no matter how hard they try to stop this natural progression. Organisms are the end product of many emergent properties, but the only certainties in life are aging and death.

Ethical, Legal, Social Implications: End of Life Issues

Aging, senescence, and death are natural components of life. But if life could prolonged, should it be? Eating healthy food and exercising will keep people healthier and prolong life. Caloric restriction, eating 40% less food than normal, has prolonged the life of every organism tested so far including yeast, flies, worms, mice, and monkeys? Caloric restriction not only postponed senescence and death, it also postponed or prevented reproduction in many species. Should we reduce senescence in humans?

What if a person could take a pill that allowed him or her to live to be 150 years old? Aging researcher Steve Austad wrote, "All the life-extending

genes…investigated in detail have turned out to [show] side effects. As we move towards the development of interventions in the aging process in the not-too-distant future, we must not forget [these side effects]." When contemplating prolonged life and the possible side effects (such as, new diseases), many investigators have described this outcome as a Pyrrhic victory against aging. Pyrrhus was a Greek general who fought the Romans and won a battle but lost a very large number of his soldiers in the process. Now any victory won with excessive costs that outweigh the benefits are described as Pyrrhic victories. Pyrrhus declared after his conquest, "One more such victory will undo me!" What would be the biological consequence if someone lived to be 200 years old? Would this be a Pyrrhic extension of life?

A related question is whether humans should be allowed to voluntarily end their lives and "die with dignity." In 1997, the state of Oregon enacted the Death with Dignity Act, which has two stipulations:

1. An adult who is capable, is a resident of Oregon, has been determined by an attending physician plus a consulting physician to be suffering from a terminal disease, and who has voluntarily expressed his or her wish to die may make a written request for medication for the purpose of ending his or her life in a humane and dignified manner.
2. No person shall qualify solely because of age or disability.

Physician-assisted suicide is legal in several countries, but in the United States, only citizens in Oregon, Vermont, Washington, California, and Montana can exercise this option (as of 2015). The major opposition to physician-assisted suicide is one called a "slippery slope." As the name implies, opponents fear that if one person is allowed to die with the assistance of a physician, then what would stop physicians from killing quadriplegics, people with Down syndrome, or anyone else? The concern is that momentum will grow once the procedure is allowed at all. The number of assisted suicides in Oregon has risen over time. Does this increase in number support a slippery slope, or merely gradual awareness of the practice? Once again, one could invoke Pyrrhus and ask whether medical intervention to prolong life is better than natural or medically hastened death. Is it ever acceptable to hasten a person's death if they are suffering? If it is

morally acceptable to euthanize pets to stop their suffering, is it unethical to offer the same consideration to family members?

Death is a natural outcome of life. Opponents of Oregon's Death with Dignity Act say, "Let nature takes its course and die naturally—no sooner or later." Letting nature take its course could be interpreted to mean no medical interventions should be employed to prolong someone's life either. Dying naturally might be a very painful process that would tempt many family members to intervene with pain relief. Letting nature take its course offers its own slippery slope.

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Conclusion

This book presented four remarkable cases of emergent properties at the organismal level. The first case examined how multicellular animals coordinate the circadian rhythms in all their cells. Chapter 2 explained how female mammals do not reject offspring *in utero* and in the process how the immune system identifies virally-infected cells. There are emergent properties associated with fat regulation that also affect the immune system and reproduction. The final emergent property of aging and death is shared by all species. The disposable soma theory explains how senescence evolved, but cannot address the ethical questions of whether it is right for a person hasten his or her own death. This book gave examples of competition or cooperation as a result of an organism's need for resources. There were multiple examples of organisms using random processes to provide flexible responses to environmental factors and physiological processes. In short, organisms rely upon their emergent properties to produce more than just the sum total of their molecules and cells. Emergent properties are fascinating and surprising, which is why studying biology is so much fun. Biologists do not memorize trivial facts. Scientists ask questions, design experiments, collect data, and interpret our data to test hypotheses or to make discoveries unimagined before. The first half of the twenty-first century is a wonderful time to be a biologist and shed light on new emergent properties.

Glossary

adenosine monophosphate. AMP, RNA nucleotide precursor to ATP.

adenylyl cyclase. it converts ATP into cAMP when liver cells detect epinephrine.

allografts. transplanted tissue from one individual to another.

allosterically. allosteric modulation is non-covalent change of protein shape and function.

antagonistic pleiotropy. it comes from the terms antagonist, which means a substance that interferes with the function of another, and pleiotropy, which means one gene produces more than one phenotypic effect.

autograft. transplanted tissue from one place to another on the same individual.

cDNA. complementary DNA is generated experimentally using reverse transcriptase to produce DNA from mRNA template

cerebrospinal fluid. fluid made by choroid plexus that bathes the brain and spinal chord

choroid plexus. capillary blood vessels that produce cerebrospinal fluid within the spaces, called ventricles, of the brain.

circadian. daily biological cycle roughly in synch with 24 hour day and uses environmental cues to recalibrate.

complemented. the wt individual provided what was missing from the recessive individual.

cyclic adenosine monophosphate (cAMP). signaling molecule made from ATP by the enzyme adenylyl cyclase; can activate protein kinase A.

cytotrophoblasts. cells of the trophoblast tissue.

disposable soma hypothesis. it states that species either live long and reproduce later in life, or die young and mate when younger.

dopamine. an organic molecule produced in retinal neurons during daytime.

eclampsia. a life-threatening medical condition brought on by pregnancy-associated high blood pressure and no other cause.

emergent property. an emergent property is an unexpected consequence apparent only when examining combined systems.

endometrium. the blood-rich female tissue in the uterus that provides nutrients to the embryo and is discarded every month during menstruation.

eusocial. eusocial organisms live in colonies and restrict reproduction to a subset of specialized breeders, such as bees, ants, and termites.

first-set rejection. it happens when an allograft is transplanted onto a recipient for the first time.

graft. the pieces of skin surgically attached to a different location.

homeostasis. it maintains internal conditions within a range of acceptable extremes.

leptin. the name of the gene and protein that when mutated can cause obesity in ob mice.

lipostat. the homeostatic mechanism that uses leptin to maintain a consistent amount of fat.

major histocompatibility complex type one (MHC I). a region of the genome that encodes the type one proteins.

melatonin. an organic molecule produced in retinal rod cells at nighttime.

MHC I. see major histocompatibility complex type one.

mutation accumulation hypothesis. no longer accepted as accurate explanation of aging, stated that as cells age, they accumulate mutations until they reach a lethal level.

naïve. one that has not been presented with non-self tissue.

natural killer. destroy any cell that lacks MHC I molecules on its surface.

negative feedback loop. it occurs when the product of a process results in a reduction of the same product being produced.

NK. natural killer cells; immune white blood cells that kill cells which lack MHC I proteins on their surface.

orthologs. two genes with very similar sequences found in two different genomes.

pair-fed. it is permitted to eat only as much food as control animals to eliminate the amount of food as a variable.

parabiotic. organisms share circulatory systems and many other physiological processes.

peptide. smaller protein pieces derived from a larger protein.

placenta. a mixture of fetal and maternal tissue composed of the trophoblast and the endometrium.

positive feedback loop. it occurs when the product of a process results in more of the same product being produced.

preeclampsia. a pregnancy-induced medical condition that results in protein in the urine due to high blood pressure.

second-set rejection. it happen after the same donor tissue is transplanted onto the same recipient as the first-set rejection.

senescence. aging with decreased physiological functions that come with getting older.

suprachiasmatic nucleus. it interprets day and night signals from your eyes and informs the rest of your brain.

trophoblast. the fetal tissue that physically interacts with the mother to transport nutrients and oxygen to the fetus.

ventricles. fluid filled spaces of the brain where the choroid plexus is located.

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Animal Physiology

A. Malcolm Campbell • Christopher J. Paradise

This book examines four examples of animal physiology that illustrate emergent properties in whole organisms. The first example shows how mammals coordinate the activity of all their cells using a daily rhythm. The second case explains an apparent contradiction that happens every time a woman gets pregnant and delivers a healthy baby—how the immune system tolerates a foreign tissue such as the fetus. The next case study in this book shows how bodies regulate the amount of fat using a complex interaction of proteins that function as a lipostat, a self-regulating fat maintenance system. Finally, the book provides an understanding of why some species live long lives while others die after very short lives, and under what conditions each situation is favored. What is evolutionarily adaptive about death? These four case studies provide sufficient evidence to understand how animals regulate many of their own metabolic functions.

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