Chapter 3a – note that SLIDE REORGANIZATION IN PROGRESS

Week Day/Date	2021 Lecture Topics	Reading Assignment
1 Tue 1/19	NBOA Resources, Neurobio Primer	**Chap. 1 + Canvas Resource**
Fri 1/22	Senescence, Telomeres, ROS and DDR, Microglia	**Chapter 2a**
2 Tue 1/26	Astrocytes and IDPs/RNA dysregulation	**Chapter 2b**
Fri 1/29	Lipofuscin, Oxidation, Mitochondrial and Neuronal Aging	**Chapter 3**
3 Tue 2/2	Neocortical Nexus, Oscillators, WTA, Top-Down systems	**Chapter 4**
Fri 2/5	Structural Changes, Cognitive Slowing and Cognitive Decline	**Chapter 5**
	Mon Feb. 8 = last date to drop course without a W on transcript	
4 Tue 2/9	Neocortical Primer w/ PFC, ERC and DMN (see Glossary)	**Chapter 7**
Fri 2/12	Memory Systems Overview	**Chapter 8**
	MON is PREZ DAY – 2/15	
5 Tue 2/16	Working Memory: 5 Components w/ Vulnerabilities	**Chapter 9**
Fri 2/19	Competing Models and Executive Functions	**Chapter 9**
6 Tue 2/23	MTL, ERC/hippo and ACh Encoding	**Chapters 10/11**
Fri 2/26	Molecular Neurobiology of Dementia: Survey	**Chapter 12**
7 Tue 3/2	**MID-TERM** online, open book, notes, internet	*thru Chapter 11*
Fri 3/5	Molecular Neurobiology of Dementia: tau and A-beta	**Chapter 12**
	Mar 22nd = Fall Class Offerings POSTED	
8 Tue 3/9	Dementia Variants: Survey, LBD	**Chapter 13**
Fri 3/12	Dementia Variants: FTD, EOAD and AlzD	**Chapter 13**
9 Tue 3/16	Cognitive Reserve, Brain Reserve and other Stories	**Chapter 14**
Fri 3/19	GPUs, Dedifferentiation, Preclinical to Super-Agers	**Chapter 14**
10 Tue 3/23	Cell Assemblies, Gamma Oscillations and Hubs	**Chapter 15**
Fri 3/26	Language System Damage, bvFTD & Selective Vulnerability	**Chapter 15**
11 Tue 3/30	Functional Connectivity / fMRI in Aging and AlzD	**Chapter 17**
Fri 4/2	Biomarkers and PET Imaging of Dementia	**Chapter 18**

Syllabus SHOULD have been:

Chapter 2

Chapter 3a

Chapter 3b

Running a bit behind schedule but we do have some catch-up opportunities.

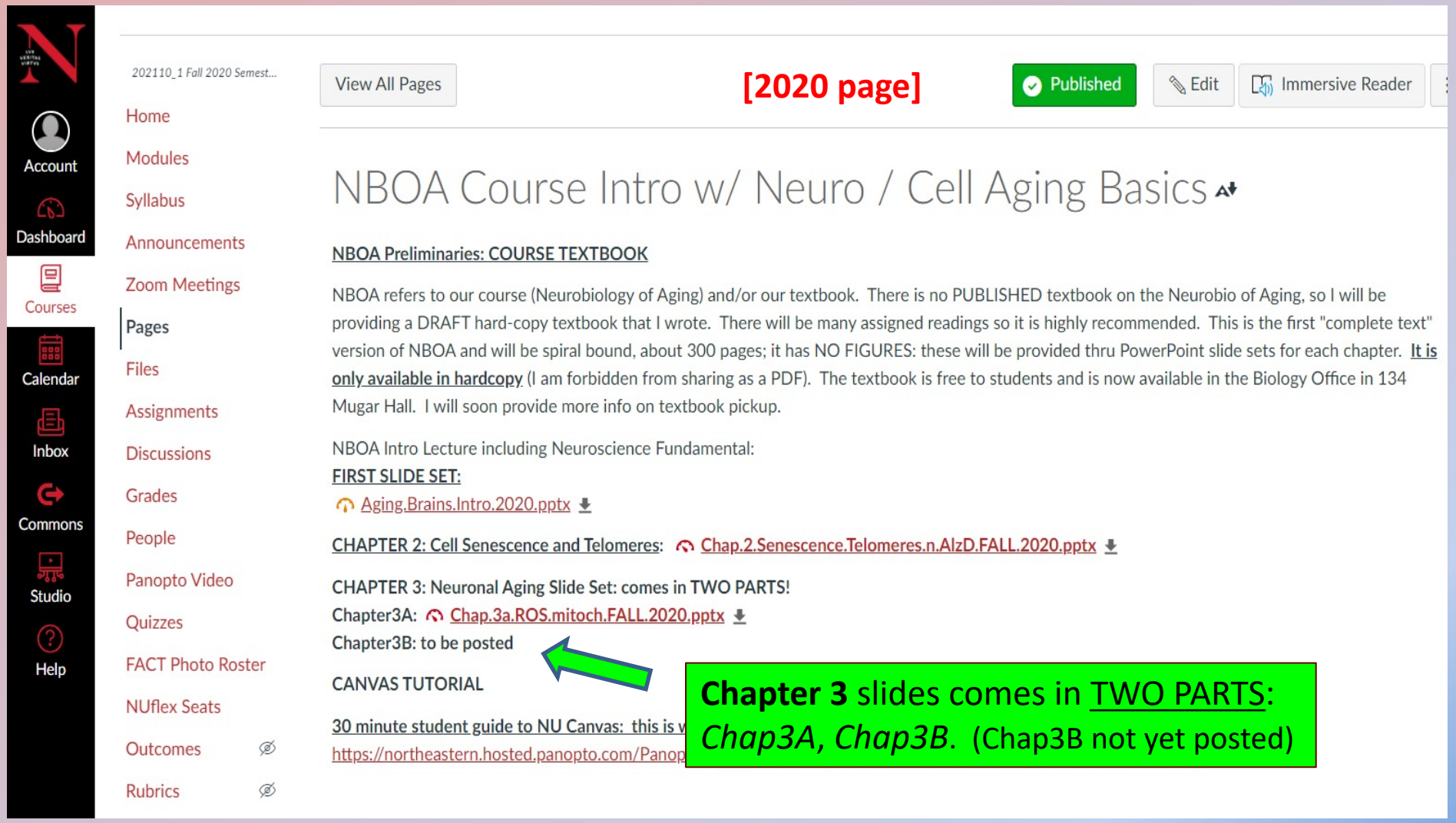
CHAT 3 TIMES on GLOSSARY ITEMS
- compose ONE chat in advance (you will paste this near the end of lecture AND AGAIN in the breakout room)
- once in the breakout rooms, during the discussion, you will comment on one of the glossary items posted by a classmate inside your room

DETAILS
Everyone please pick ONE glossary item and write a brief chat (2 or 3 sentences max) about why you picked that item. You can pick an item because it seems curious, or because you think it's of special prominence in the NBOA field or because it has some personal connection or interest for you.
ALSO: please try to pick a glossary item that is perhaps a little unique to you, so we don't all pick the same items.
BANNED ITEMS: AlzD, EOAD, LTP, neocortex, ROS, PFC. Many others to choose from!

IN-ROOM COMMENT: in addition to pasting your COMPOSED CHAT [once room comes to order], you should skim others' items, participate in conversation, and at some point paste a COMMENT about ONE of the other glossary items pasted (e.g. why you think its important or curious or of special interest to you). I know this is multi-tasking, but you should be able to talk, listen and post a very brief comment within the 15 minutes allotted to get your chats in.

CHAT LEADERS:
Please initiate some discussion by explaining your chat-post, and then encourage your chat mates to do the same. During discussion period everyone should post their IN-ROOM COMMENT on one of the others' glossary items. I will give a 6-minute warning for everyone to do this right away (if not pasted yet). I will also give a 3-minute warning for LEADER to collect all chats. In addition, the leader should nominate the TOP 3 ITEMS for their room like 1. xxx, 2. yyy 3.zzz and include that in their collected room chats. It is fine to paste this ranking at any time during the breakout room and also to have discussion and revision (time permitting). It is fine for leader to choose their own item, Leader's Prerogative!

Don's Chat:
I picked the AAN item because I think they are important for storing words, concepts and facts. Also, they may be important for preserving knowledge despite aging damage. Plus this is a research focus of mine.


202110_1 Fall 2020 Semest...
Account
Dashboard
Courses
Calendar
Inbox
Commons
Studio
Help
Home
Modules
Syllabus
Announcements
Zoom Meetings
Pages
Files
Assignments
Discussions
Grades
People
Panopto Video
Quizzes
FACT Photo Roster
NUflex Seats
Outcomes
Rubrics
View All Pages
[2020 page]
Published
Edit
Immersive Reader
NBOA Course Intro w/ Neuro / Cell Aging Basics
NBOA Preliminaries: COURSE TEXTBOOK
NBOA refers to our course (Neurobiology of Aging) and/or our textbook. There is no PUBLISHED textbook on the Neurobio of Aging, so I will be providing a DRAFT hard-copy textbook that I wrote. There will be many assigned readings so it is highly recommended. This is the first "complete text" version of NBOA and will be spiral bound, about 300 pages; it has NO FIGURES: these will be provided thru PowerPoint slide sets for each chapter. It is only available in hardcopy (I am forbidden from sharing as a PDF). The textbook is free to students and is now available in the Biology Office in 134 Mugar Hall. I will soon provide more info on textbook pickup.
NBOA Intro Lecture including Neuroscience Fundamental:
FIRST SLIDE SET:
Aging.Brains.Intro.2020.pptx
CHAPTER 2: Cell Senescence and Telomeres: Chap.2.Senescence.Telomeres.n.AlzD.FALL.2020.pptx
CHAPTER 3: Neuronal Aging Slide Set: comes in TWO PARTS!
Chapter3A: Chap.3a.ROS.mitoch.FALL.2020.pptx
Chapter3B: to be posted
CANVAS TUTORIAL
30 minute student guide to NU Canvas: this is w
https://northeastern.hosted.panopto.com/Panop
Chapter 3 slides comes in TWO PARTS:
Chap3A, Chap3B. (Chap3B not yet posted)

<u>Chapter 3 is covered in two parts,</u> expanded upon w/ PDFs, excerpts
3A = R0S, Mitochondrial dysfunction, and Lipofuscin
3B = IDPs (intrinsically disordered proteins) including
Alpha-Syn, plus Lewy Bodies and apoptosis

<u>Chapter 2:</u> Perfidious Processes: Cellular Preludes to Neurodegeneration
Briefly summarizes the major themes of general cellular/organismal aging. Includes mitochondrial damage, mutations, telomeres, SASP, IDPs, the DNA damage response, lipofuscin and ROS.

read chapters to preview & consolidate

<u>Chapter 3</u>. **Worms in our Brain**: The Aging of Neurons, Glia and Proteins
General mechanisms by which neurons and glia accumulate damage over time. Introduces neurogenesis, demyelination, apoptosis and necrosis. Includes brain-cell interactions, inflammation, glymphatic system and metabolic dysfunction, aka Type 3 Diabetes. Some details of neurodegeneration are briefly introduced, but Alzheimer's Disease (AlzD) and other dementias are covered in much more depth later (especially in Chapters 10, 11, 12, 15, 18 and 19).

<u>In our Chapter 3 topics</u>, we dig deeper into cell pathology and, incidentally, encounter some *neurodegenerative* items since reports on cell damage often concern diseases of the aged. The diseases *per se* (symptoms, imaging, etc.) are covered later, but it's fine to "look ahead" on these topics by reading **Chap. 12** which expands upon molecular/cellular details.

Mitochondrial Turnover and Aging of Long-Lived Postmitotic Cells: The Mitochondrial–Lysosomal Axis Theory of Aging

2010
Invited Review

BIG paper and NICE ABSTRACT: we will cover ONLY PARTS of this! ← major change!

It is now generally accepted that aging and eventual death of multicellular organisms is to a large extent related to macromolecular damage by mitochondrially produced reactive oxygen species, mostly affecting long-lived postmitotic cells, such as neurons and cardiac myocytes. These cells are rarely or not at all replaced during life and can be as old as the whole organism. The inherent inability of autophagy and other cellular-degradation mechanisms to remove damaged structures completely results in the progressive accumulation of garbage, including cytosolic protein aggregates, defective mitochondria, and lipofuscin, an intralysosomal indigestible material. In this review, we stress the importance of crosstalk between mitochondria and lysosomes in aging. The slow accumulation of lipofuscin within lysosomes seems to depress autophagy, resulting in reduced turnover of effective mitochondria. The latter not only are functionally deficient but also produce increased amounts of reactive oxygen species, prompting lipofuscinogenesis. Moreover, defective and enlarged mitochondria are poorly autophagocytosed and constitute a growing population of badly functioning organelles that do not fuse and exchange their contents with normal mitochondria. The progress of these changes seems to result in enhanced oxidative stress, decreased ATP production, and collapse of the cellular catabolic machinery, which eventually is incompatible with survival. *Antioxid. Redox Signal.* 12, 503–535.

Terman et al, in:
Antioxidants & Redox Signaling

aging mitochondria → more ROS, more cellular garbage
autophagy = garbage removal via eating of vesicles
proteasomes = removal of damaged cytosolic proteins

Mitochondrial Turnover and Aging, 2010:
The Garbage Theory of Aging/Degener.

ALSO NOTE:
ROS generation
SOD, hydrogen peroxide
hydroxyl radicals, lipofuscin, DA
aggresomes, autophagy fails
mitoch. fusion, size effects
apoptosis, necrosis
large chunks of PDF *ignored*!

How Many = # neurons that have substantial debris, dysfunction
How Badly = is the FUNCTION of debris-positive neurons *degraded*
What Consequences = in terms of failed computations, performance

↑↑ KEY QUESTIONS GOING FORWARD

can we model this?

Mitochondrial Turnover and Aging, 2010

As can be seen from the 5,000-year-old Sumerian Gilgamesh epos, the reasons for aging have been pondered, and the fountain of eternal youth sought after, ever since the beginnings of human reflection on life and death. During the short documented period of human history that is available to us, numerous theories on biologic aging, or senescence (and how it may be prevented) have been advanced, debated, and, in most cases, rejected (156, 187, 251, 255). Now, however, some agreement seems to exist that cellular oxidation and oxygen-derived radicals contribute to biologic aging (hereafter referred to as aging), which can be defined as a progressive decline in an organism's adaptability, followed by a consequent increase in morbidity and mortality (48, 221). The oxidative-stress theory of aging, although still far from proven, is presently one of the major aging hypotheses, even though its details are vaguely outlined, the conclusions are often obscure, and attempts to prevent aging by antioxidants are so far unsuccessful (10, 98, 213).

The amalgamation for metabolic symbiosis of anaerobic methane-producing bacteria and bacterial ancestors of present-day mitochondria into a prototype chimeric eukaryotic cell resulted in a capacity for much-enhanced energy production: oxidative phosphorylation (132). In many ways, a most successful unification of two different forms of bacteria, this amalgamation created organisms with substantially better access to energy than their ancestors. The transformation, however, had the inevitable side effect of exposing early eukaryotic cells to reactive oxygen species (ROS). These species, which have electrons that escape by accident from the mitochondrial electron-transporting system as their main cause of origin, may, in the presence of redox-active transition metals, damage a large variety of macromolecules by transforming them into dysfunctional and non-degradable garbage that accumulates intracellularly. In the long run, this accumulation results in cellular functional decay and, eventually, in cell death

All agree that oxidative damage of proteins and DNA must underlie much of the ravages of age, so why is this *not proven*?

- early research
- many theories
- *epic fail*: anti-oxidants?
  [remember to ask **Javier**]
- *symbiotic origins* led to vast amounts of ATP (and ROS)
- escaped e's + metals catalyze *this reaction*:
  macromolecules ➔ garbage

why anti-oxid story vanishes from my brain

Mitochondrial Turnover and Aging, 2010

However, such short-lived postmitotic cells may alter to some extent with organismal age, possibly reflecting changes in stem and progenitor cells, even though their continuous division considerably decreases their intracellular accumulation of waste products. [b/c dividing cells "grow in volume"?]

Recently it was shown that the proliferation potential of stem and progenitor cells decreases with age (218, 219). Because of this deterioration, the efficiency of biologic waste dilution by cell division also decreases in stem and progenitor cells with age, accompanied by the less-frequent replacement of mature short-lived postmitotic cells. It follows that stem cells, previously believed to escape aging, acquire over time some of the properties of aged long-lived postmitotic cells, in particular increased lipofuscin-related autofluorescence, elevated carbonyl content, and enhanced oxidative stress (218, 219). Conceivably, stem and progenitor cells, along with mature short-lived postmitotic cells, may then have to rely on a defective lysosomal compartment, the function of which is hampered by the presence of lipofuscin (see Section VI.B), which affects the turnover of essential structures and macromolecules.

It should be added that stem and progenitor cells are also prone to the accumulation of mutations that are reproduced during cell division and that may result in the development of neoplasms. The majority of tumors thus arise in actively proliferating cell populations that are characterized by relatively high numbers of stem and progenitor cells. Tumor bi-

Cells Decline with Age

- **focus on post-mitotic cells** e.g. cardiac and skeletal muscle cells, and neurons.
- **dividing (progenitor, stem) cells show** ↓ prolifer. capacity [can Parabiosis renew them?]
- **lipofuscin accumulates** & is autofluoresc.
- **lysosomes are** low pH vesicles loaded with digestive enzymes
- **w/ age lysosome function** declines ➔ due to accumulation of cellular debris?
- **endocrine cells** (hypothalamus & pituitary) are few in #, hence vulnerable AND control many organs so cell losses may compromise multiple organs

*

Stem and Progenitor cells
a. proliferate better with age
b. are immune from DDR and ROS
c. can accumulate lipofuscin
d. do not have lysosomes
e. ALL of the above are correct

challenging but faulty exam question

TABLE 1. RENEWAL AND AGE-RELATED CHANGES OF TERMINALLY DIFFERENTIATED (POSTMITOTIC) CELLS WITH DIFFERENT LIFE SPANS

REVISED, Note GreenBox

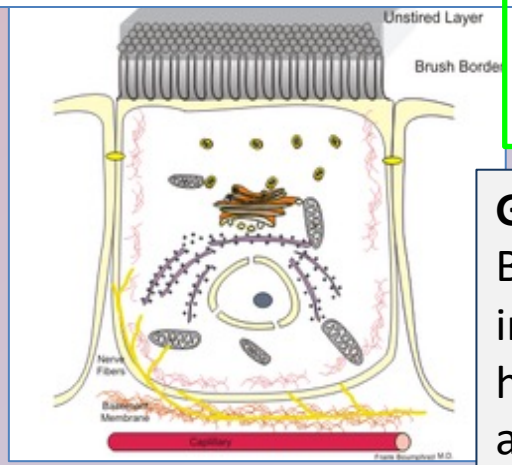
Characteristic	*Short-lived postmitotic cells*	**LLPMCs are** *Long-lived postmitotic cells*
Examples[a]	Mature enterocytes, peripheral blood cells	Neurons, cardiac myocytes, skeletal muscle fibers, RPE cells
Life span	Short, usually only days	Long, often comparable with that of the whole organism
Differentiation	Asymmetric division of stem cells gives rise to new stem cells and progenitor cells that divide sequentially and differentiate into mature cells	Similar to that for short-lived cells, although stem cells are scanty and differentiate rarely (more commonly in response to injury)
Regeneration capacity	High, usually associated with complete regeneration	Low, usually associated with incomplete regeneration, resulting in scarring
Malignant transformation[b]	Frequent, apparently due to high content of stem and progenitor cells	Rare, apparently due to low content of stem and progenitor cells
Senescent alterations	Minimal. Differentiated cells have a too-short life span to accumulate substantial amounts of damaged structures (waste materials). Stem and progenitor cells do not accumulate damaged structures either, because the latter are efficiently diluted by cell divisions	Pronounced. Differentiated cells have long life spans, resulting in progressive accumulation of waste materials [*e.g.*, lipofuscin, senescent (giant) mitochondria, and aberrant proteins]

mix of comments re: cells *OR* tissues

*

Mitochondrial Turnover and Aging, 2010

Enterocytes = cells lining GI system



GI epithelial cells, like neurons, are terminally differentiated. But they can be replaced as needed b/c THEY do not store information! The ***fate*** of LLPMCs (e.g. neurons) is at the very heart of the problem of ***Cognitive Decline***, although dividing and stem cells may have quite a lot to say about this.

It is known that aging is characterized by the increasing accumulation in long-lived postmitotic cells of dysfunctional, usually enlarged (sometimes called giant) mitochondria, lipofuscin-loaded lysosomes, and oxidatively modified cytosolic proteins and lipids. Damaged proteins often accumulate in the form of indigestible aggregates, termed aggresomes (92, 215). Since the emergence of the oxidative stress or free radical theory of aging, such alterations have been considered the result of a gradual accumulation of oxidatively injured macromolecules. Some other theories, such as the somatic mutation theory of aging (33, 54) and the error catastrophe theory (175), emphasized instead the role of the erroneous synthesis of macromolecules in aging. Later studies, however, did not show any substantial increase in the occurrence of synthetic errors with age (83, 97). Although somatic mutations do accumulate, they cannot explain the variety of changes associated with aging (120). Apparently, the role of somatic mutations is mostly restricted to the increased frequency of malignant neoplasms with age (see earlier).

Because damaged structures obviously would not accumulate if they were being perfectly removed, it can be reasoned that it is not the formation of dysfunctional and oxidized proteins and lipids that creates all the multifaceted problems that exist for aged long-lived postmitotic cells, but rather the malfunction of catabolic enzymes, such as the cytosolic proteasomes and calpains and the host of lysosomal enzymes that cannot completely degrade damaged structures. With this line of reasoning, aging, together with a number of neurodegenerative diseases, is starting to be considered a catabolic disorder.

Aging As:

Catabolic Disorder ~Catabolic History

- w/ age repair can't keep up w/ damage
- ➔ big mitoch. and clogged lysosomes
- aggresome = indigestible cytosolic clumps
  [failure of proteosomes leads to aggregates]
- "catastrophe theory" originally for DNA replication, biosynth, not relevant here
- "somatic mutations" not a factor either
  but errors do accum. in non germ-line cells
- to paraphrase *Butch Cassidy:*
  "it's not the damage that will kill you, it's the _____ __ _____"

PubMed Hits:

- aggresome = 492 (12,000 on scholar)
- inflammasome = 6,489
- "systemic inflammation" = 12,435

S.I. now 71,900

catabolic vs. metabolic vs. biosynthetic
cytosolic vs. cytoplasmic

broader term – "metabolic disorder" = 126k on GScholar

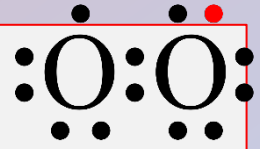
Soon after the important discovery that ROS, including the superoxide anion radical ($O_2^{\bullet-}$) and hydroxyl radical ($HO^{\bullet}$), both of which are short-lived with half-lives of 10^{-6} and 10^{-9} seconds, respectively, form within living cells as a consequence of normal respiration (94), Denham Harman (98) postulated that biologic aging (senescence) occurs because of the accumulation of oxidatively damaged macromolecules. This theory, called "the free radical theory of aging," although initially poorly accepted, has gathered an increasing number of followers over time as more supporting evidence has been presented. Today, the role of free radicals as important contributors to aging is considered most likely, and extensive studies on various biologic species ranging from yeast to humans are in support, although a final confirmation is still lacking (10, 87, 167, 200, 213). The free radical theory of aging, which points to an intrinsic mechanism underlying age-related molecular damage, does not in any way exclude that other factors may also be involved in the aging process (*e.g.*, evolution, somatic mutations, errors in protein synthesis, accumulation of waste products, neuroendocrine and immunologic disturbances). The possibility that many mechanisms may contribute to the aging process is reflected in the existing numerous theories of aging (some of them having only historic value), which are systematized in a number of reviews (156, 187, 255).

The process of cellular respiration is tightly associated with the electron-transport chain and the transfer of electrons from substrates (*e.g.*, NADPH from complex II) to the final acceptor (molecular oxygen) in complex IV. The electron transport is associated with translocation of protons from the mitochondrial matrix to the mitochondrial intermembrane space, which originates a membrane potential. This potential is coupled with phosphorylation of ADP to form ATP at complex V. Both free radicals and other ROS form continuously because of unavoidable electron leakage from mitochondrial complexes during electron transport and reductive one-

II. ROS, Mitochondrial Damage, and Aging

A. Biomolecular damage under normal conditions

TOP TWO RADICALS: superoxide, hydroxyl



Denham Harman, 1956

Free Radical Theory of Aging

- oxidation damages macromolecules
- vast amounts of data, but not "proven"
  but seems very likely to be a factor in aging

- other factors include "evolution", somatic mutations, biosynthetic errors, accumulation of wastes

- **role of mitochondria, ATP production**
  e's leak from electron transport train
  electron + 02 molecule = **superoxide**
  SOD (superoxide dismutase) ➔ H202
  H202 (hydrogen peroxide) also bad

- H202 is a signaling molecule *and*
  is removed by **catalase *but***
  can generate hydroxyl radicals OH•

Hydrogen peroxide is an important signaling molecule that regulates most cytosolic redox activity (220). However, if not eliminated, it can also react with Fe(II) during Fenton-type reactions, resulting in the formation of the very reactive hydroxyl radical. In addition, superoxide can directly reduce Fe(III) to Fe(II), which further contributes to the creation of $HO^{\bullet}$ or the likewise reactive ferryl or perferryl radicals. All of these radicals attack surrounding biomolecules (*i.e.*, nucleic acids, proteins, and lipids) at their very place of formation (*i.e.*, in direct relation to Fe(II) catalysis), resulting in damage to biomolecules with attached low-mass iron (94). Although most of the hydrogen peroxide is eliminated by glutathione peroxidase and catalase, some of it remains and may diffuse for some distance (*e.g.*, to the lysosomal compartment, which lacks hydrogen peroxide–degrading enzymes). Because lysosomes not only lack these enzymes, but also are rich in reactive iron as a consequence of the degradation of ferruginous materials (see Section VI.A), the formation of these radicals takes place mainly inside these organelles. This may result in lysosomal rupture, followed by damage to cytosolic structures as well as to nuclear and mitochondrial DNA as a result of the relocation of redox-active iron and hydrolytic enzymes (64, 127).

It may be assumed that the reason nature has found it necessary to speed up by 1,000 times the already rapid spontaneous dismutation of superoxide to hydrogen peroxide is that the capacity of superoxide to reduce Fe(III) to Fe(II) is a very dangerous one, allowing the formation of hydroxyl radicals if hydrogen peroxide is available (see earlier). Basic metabolic pathways involved in ROS production are schematically presented in Fig. 1.

Although ROS formation apparently is the main source of oxidative damage, it is not the only one. Another important damaging mechanism is glycation [*i.e.*, a reaction of glucose and other reducing sugars with protein amino groups, resulting in the formation of advanced glycation end

Mitochondrial Turnover and Aging, 2010

H_2O_2, OH• and Lysosomes

- SOD increases H_2O_2 production 1000x
  good b/c it removes O_2•
- H2O2 is degraded quickly BUT can undergo Fenton Reaction ➔ OH•
  which uses Fe, found in DA neurons
- occurs in lysosomes b/c H202 survives there
- finer details of reactions not testable

Free Radicals attack MANY biomolecules

- proteins, lipids and nucleic acids
- **lysosomes** lack H202 breakdown ability
  can damage contents, lead to rupture
  damage spreads thru cytosol, into nucleus

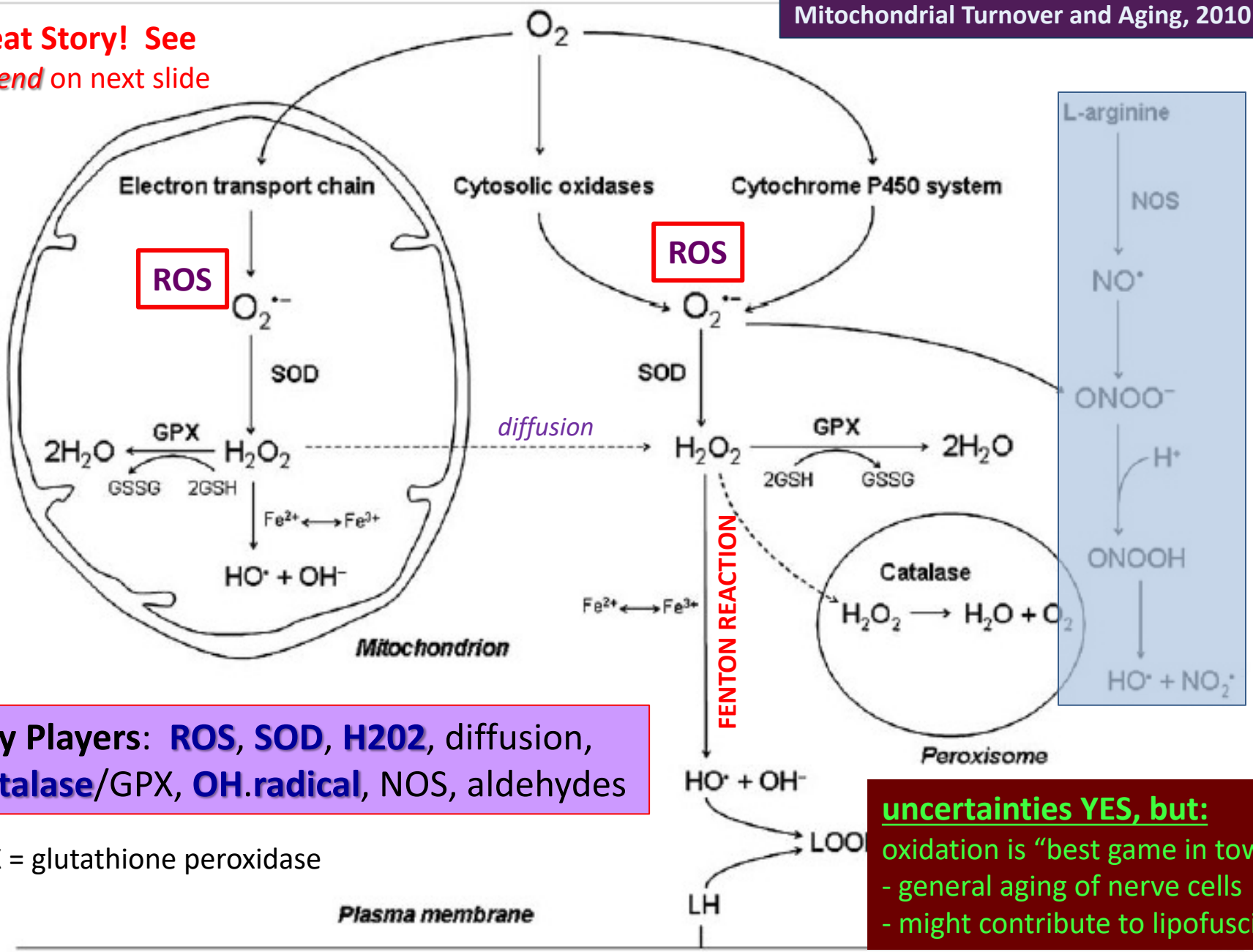
Other Kinds of Oxidative Damage

glucose / glycation end-products can cause DNA mutations, protein cross-linking (and stiffen extracellular matrix)

Fenton Reaction seems MOST destructive and are caused by Fe2 + H2O2 (hydrogen peroxide).

Great Story! See
Legend on next slide

Mitochondrial Turnover and Aging, 2010



Key Players: **ROS**, **SOD**, **H202**, diffusion, **Catalase**/GPX, **OH.radical**, NOS, aldehydes

GPX = glutathione peroxidase

uncertainties YES, but:
oxidation is "best game in town
- general aging of nerve cells
- might contribute to lipofuscin

FIG. 1. **Metabolic pathways involved in the production of cellular ROS.** Superoxide anion radicals ($O_2^{\bullet-}$) are produced

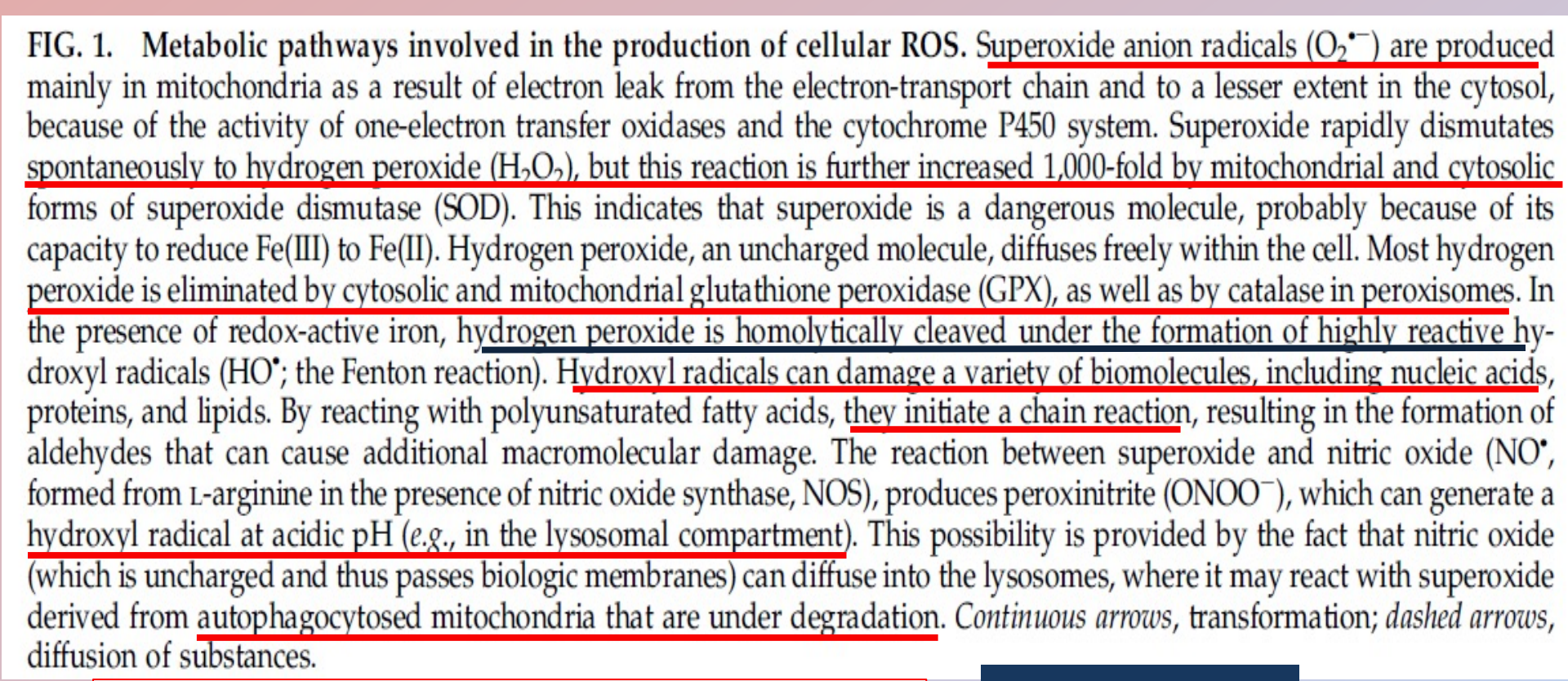


FIG. 1. **Metabolic pathways involved in the production of cellular ROS.** Superoxide anion radicals ($O_2^{\bullet -}$) are produced mainly in mitochondria as a result of electron leak from the electron-transport chain and to a lesser extent in the cytosol, because of the activity of one-electron transfer oxidases and the cytochrome P450 system. Superoxide rapidly dismutates spontaneously to hydrogen peroxide (H_2O_2), but this reaction is further increased 1,000-fold by mitochondrial and cytosolic forms of superoxide dismutase (SOD). This indicates that superoxide is a dangerous molecule, probably because of its capacity to reduce Fe(III) to Fe(II). Hydrogen peroxide, an uncharged molecule, diffuses freely within the cell. Most hydrogen peroxide is eliminated by cytosolic and mitochondrial glutathione peroxidase (GPX), as well as by catalase in peroxisomes. In the presence of redox-active iron, hydrogen peroxide is homolytically cleaved under the formation of highly reactive hydroxyl radicals ($HO^{\bullet}$; the Fenton reaction). Hydroxyl radicals can damage a variety of biomolecules, including nucleic acids, proteins, and lipids. By reacting with polyunsaturated fatty acids, they initiate a chain reaction, resulting in the formation of aldehydes that can cause additional macromolecular damage. The reaction between superoxide and nitric oxide ($NO^{\bullet}$, formed from L-arginine in the presence of nitric oxide synthase, NOS), produces peroxinitrite ($ONOO^-$), which can generate a hydroxyl radical at acidic pH (*e.g.*, in the lysosomal compartment). This possibility is provided by the fact that nitric oxide (which is uncharged and thus passes biologic membranes) can diffuse into the lysosomes, where it may react with superoxide derived from autophagocytosed mitochondria that are under degradation. *Continuous arrows*, transformation; *dashed arrows*, diffusion of substances.

note: **OH radicals**

ROS Summary

- **Fig. 1's Legend** summarizes key pathways
- mostly items we have covered

ARTICLE summary:

- massive article, slides cover the points that are MOST essential
- some portions overlap w/ other course materials
- details of PDF, figures and text excerpts that were never mentioned can be ignored

- **ROS are the lifeblood of cell death!**
- **this Figure Legend is legendary**

TABLE 2. CELLULAR DEGRADATION PROCESSES

Degradation process	*Location*	*Enzymes involved*	*Targets*
Cytosolic proteolysis	Cytosol	Calpains, proteasomes	Cytosolic proteins (mainly short-lived)
Mitochondrial proteolysis	Mitochondria	Lon, Clp-like, and AAA proteases	Mitochondrial proteins
Autophagy (macroautophagy, microautophagy, and chaperone-mediated autophagy)	Lysosomes	Acid hydrolases	All cytosolic macromolecules and organelles
Programmed cell death (PCD)	Whole cell	Effector caspases, lysosomal cathepsins, and endonucleases	All cellular components

many variations on this theme, stay tuned

in PCD-I (classic apoptos
acid hydrolases in PCD-
(autophagic cell death) a
PCD-III (programmed ne

Two Proteins Meet their Maker
cytosolic proteins tagged with ubiquitin
organelle proteins undergo autophagy

the "involved" enzymes are not-testable; necrosis & proteasomes are!

Mitochondrial Turnover and Aging. p. 507

B. Imperfect turnover of damaged biologic structures

Oxidatively or otherwise damaged biologic structures are either repaired (*e.g.*, single bases in DNA molecules are replaced) or degraded and completely replaced by newly synthesized structures, as is the case for proteins, organelles, and whole cells. Proteins, predominantly short-lived ones in the nucleus and cytosol, are degraded mainly by calpains and proteasomes, whereas most long-lived proteins and all organelles are digested in the lysosomal compartment in the process called autophagy, or autophagocytosis (49, 265). It has long been known that proteins intended for degradation by proteasomes have to be tagged by ubiquitin, but it is now recognized that some ubiquitinized proteins also are degraded by autophagy and that the proteasomal and lysosomal systems for degradation can compensate for each other (177, 242). Mitochondria possess their own proteolytic system, which includes Lon, Clp-like proteases, and AAA proteases (see Section IV). Irreversibly damaged cells are removed by self-killing programs, including apoptotic (caspase-dependent) programmed cell death (PCD-I), autophagic cell death (PCD-II), or, occasionally, necrosis (PCD-III) (71). Cellular catabolic pathways are summarized in Table 2.

structures. This will make the function of the cells less efficient and decrease their adaptability. The accumulation of biologic garbage also is associated with certain toxic effects, such as increased ROS production by senescent mitochondria, or enhancement of oxidative stress with release of lysosomal enzymes by lipofuscin-loaded lysosomes (see Section VI). These changes result in progressive functional decline of postmitotic cells, such as neurons, cardiac myocytes, and skeletal muscle fibers, making an aged organism fragile and unable to withstand stress. The lack of robustness that characterizes the aged individual is thus reflected on the cellular level.

Consistent with the idea that aging is largely dependent on the ultimate degeneration of long-lived postmitotic cells, a primitive cnidarian animal, *Hydra vulgaris*, has been shown to escape aging for 4 years in a controlled laboratory environment (150). The most plausible explanation for the absence of aging in hy is that th ani as well a ther cnidarians, totally lacks g-live ostmito cells. cells of cnidarian animals are co inu ly replace hro n the division and differentiation terstitial ste s. Interestingly, all higher animals, ch evolved later n cnidarians, contain postmitotic cell therefore ha ited life spans. It is possible that th app rance of lo live postmitotic cells, in particular, l g-live eurons ame a g evolutionarily because thi rait was soci d with c ain advantages, providing or better e ary fitness. Cnidarians are known to possess a primitive nervous system, consisting of a network of dissociated short-lived neurons. These animals can react only nonspecifically on external stimuli and do not develop conditioned responses. In contrast, higher animals have a more-developed nervous system, consisting of long-lived postmitotic neurons, allowing conditioned responses, and consequently, providing better adaptation to their environment. Apparently, the presence of long-lived neurons promoted the development of long-term memory, associated with conditioning. The price for this better evolutionary fitness was a limited lifespan…

← "Biological Garbage"

Theory: Frailty with Age is a function of accumulated cellular debris, affecting brain, heart and muscle. *Perhaps*. "cognitive slowing" tbd soon!

SKIP Cnidarian STORY

Claim: Cnidarians (simple invertebrates) do not have long-lived (post-mitotic) cells and so "escape aging" i.e. they do not suffer the fate of organisms that retain kno ledge nd th neur ns) over decad

But is it not b er to have **K wn and Lost** then t nev have *Kn wn* t all?

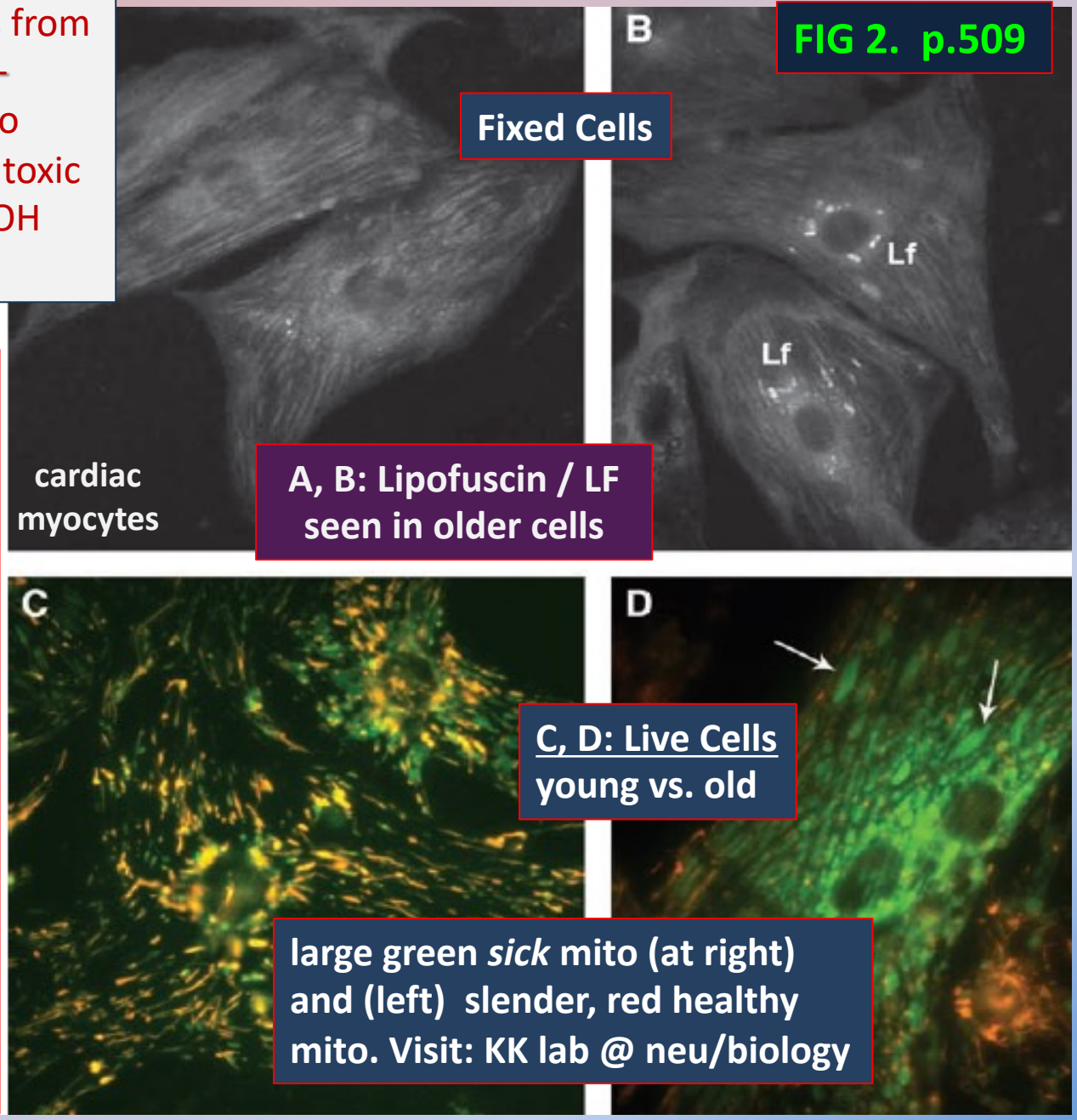
And one ore thin abo Cnidar ns: they've "escaped aging" but live 4 years- last I checked, 90 >> 4!

oh really?

Cellular Pathos: ensues from the linkages from aging-mitochondria damage to ROS, H2O2 production, toxic debris / lipofuscin and OH radicals. And apoptosis.

"The loading of lysosomes with lipofuscin is probably one of the most important contributors to the decline of autophagy in aged cells (see also Section VI).
As predicted by the mitochondrial–lysosomal axis theory of aging (30)". p. 524. [Brunk & Terman, 2002]

Each cell has many copies of the mitochondrial genome, which are being duplicated many times -- as new mitoch. are "born" old ones are broken down.



KK is Konstantin Khrapko

myocytes = *cardiac muscle cells* ➔ must last a lifetime

1 week in culture

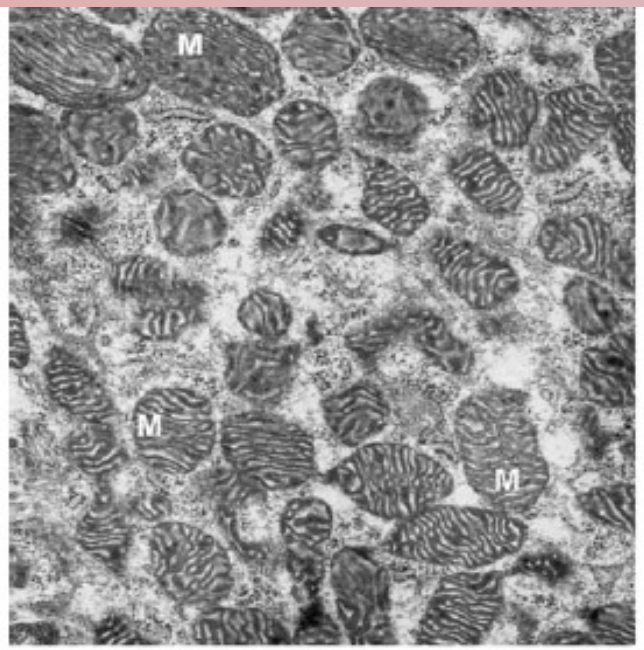
4 weeks in culture

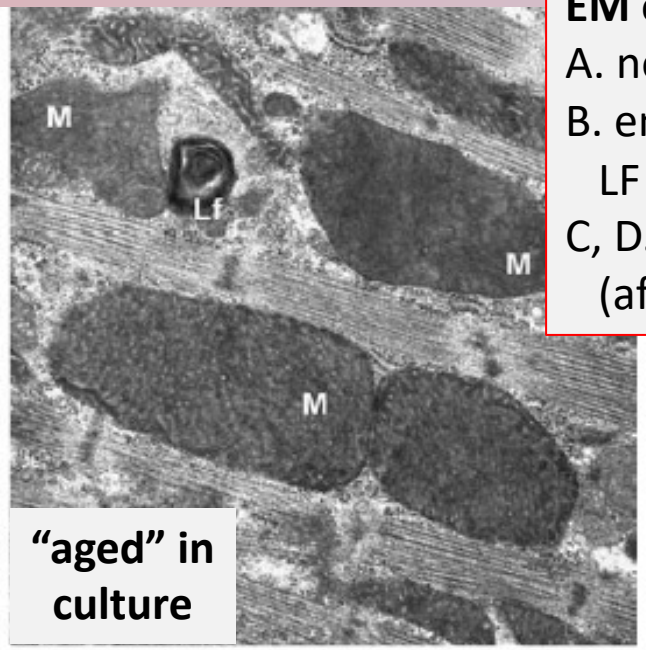
EM of Aged Cells in Culture:

A. normal mitochondria

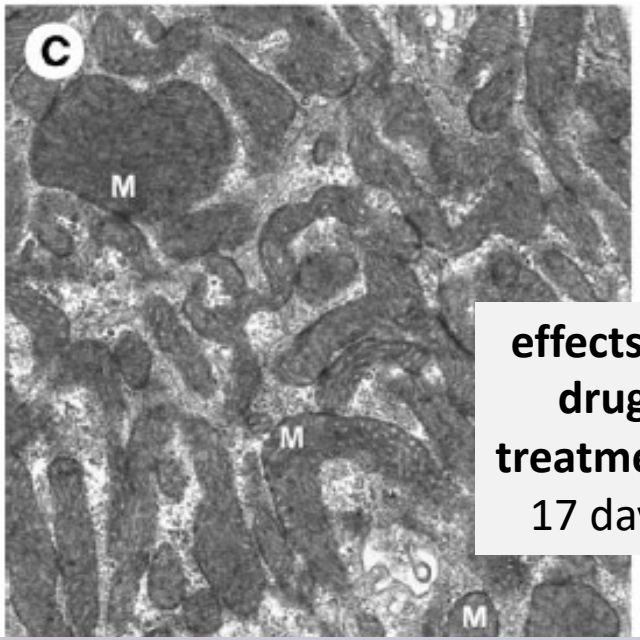
B. enlarged mitochondria with LF = lipofuscin granule

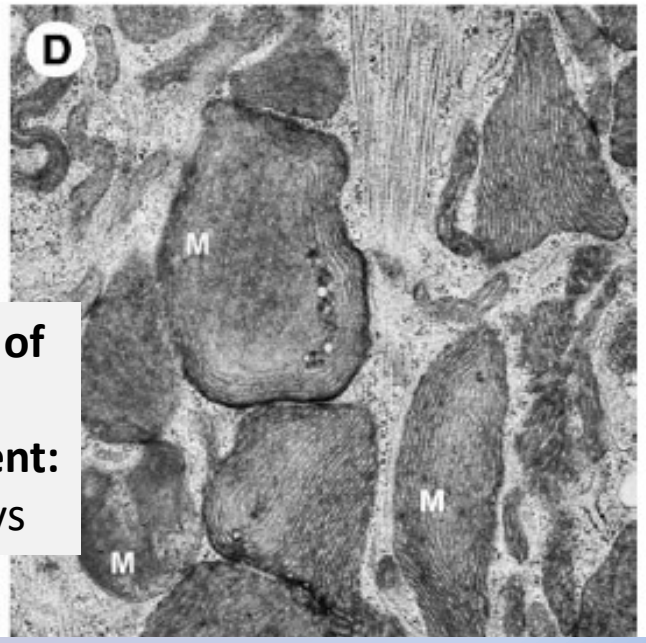
C, D. Senescent Mitochondria (after drug treatment)





“aged” in culture





effects of drug treatment: 17 days

FIG. 3. Ultrastructural mitochondrial changes associated with aging and inhibition of autophagy. (A, B) Electron microscopy images of neonatal rat cardiac myocytes cultured for 1 and 4 weeks, respectively. The aged cells contain enlarged (giant) mitochondria with irregular cristae and dense matrix. (C, D) The 17-day-old cardiac myocytes, exposed to 3-methyladenine for 12 days, accumulate numerous small, as well as some large senescent-like mitochondria (compare with Fig. 2). M, mitochondria; Lf, lipofuscin. Bar, 500 nm.

Oh Cristae, My Cristae

as two membranes become one
the oxide storm has seized the day
now brown-lipo blots the sun

by Walt Whitman

Mitochondrial Turnover, Calcium and Voltage are involved with Aging

compared with the generally accepted role of mitochondrial decay in aging, the roles of lysosomal malfunction and the cross-talk between lysosomes and mitochondria in aging remain less recognized.

The progress of age-related mitochondrial degeneration is, to a large extent, dependent on the failure of mitochondrial-turnover mechanisms, including (a) mitochondriogenesis or the generation of more mitochondrial mass, (b) mitochondrial fusion and fission, (c) the monitoring of protein folding and assembly by molecular chaperones and energy-dependent proteases, and (d) the removal of severely damaged mitochondria by autophagy (119). These mechanisms, as well as their age-associated malfunction, are described more in detail later.

…items (a)-(d) are all important; but our coverage of them will be limited

III. Mitochondrial Fusion, Fission, and Biogenesis

A. The role of mitochondrial dynamics

Mitochondria are dynamic organelles that are continuously fusing and dividing (Fig. 4). The harmonious balance of these two opposing processes is responsible for the prevailing morphologic features of mitochondria, their distribution, inheritance, and function (61). When the mitochondrial fusion is blocked, the normal tubular network of mitochondria transforms into fragmented mitochondria (40, 41, 90, 173), whereas the blocking of the opposing process, mitochondrial fission, results in elongated, interconnected mitochondrial tubules

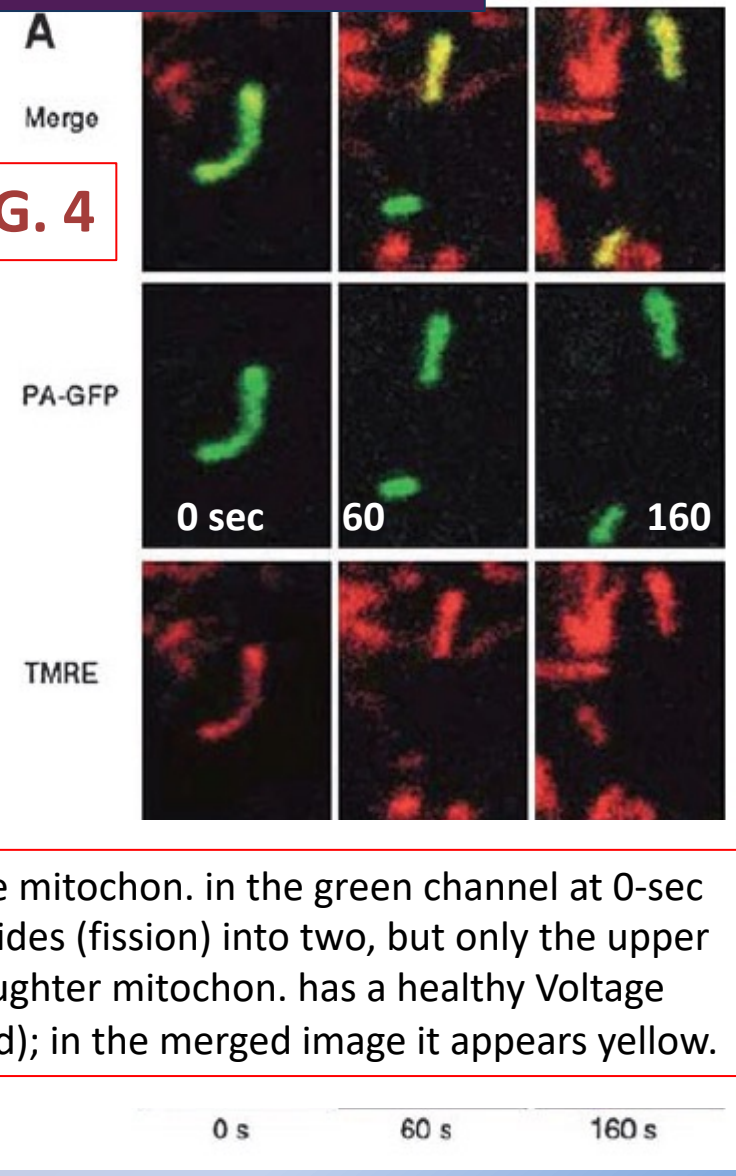


FIG. 4

The mitochon. in the green channel at 0-sec divides (fission) into two, but only the upper daughter mitochon. has a healthy Voltage (red); in the merged image it appears yellow.

Section III covers many nuances of Mitochondrial Biogenesis, Fusion and Fission

We are going to omit most details in this section. Fig. 4 shows that the membrane potential changes after fission as monitored with TMRE.

Apoptosis hits the WHOLE cell!

Without attending to all the molec. details, *the major theme* is that disruption of fission / fusion balance mucks up glucose oxidation and mitoch. membrane potential, calcium, which might ultimately lead to apoptosis!

In Progress Summary:

mitochondria turnover:

voltage-maintenance is key

calcium can be neurotoxic

apoptosis involves caspases

organelle preservation

lipofuscin is sign of damage?

or is it damaging?

lysosomes can be corrupted

possibly by toxic mitoch. fragments

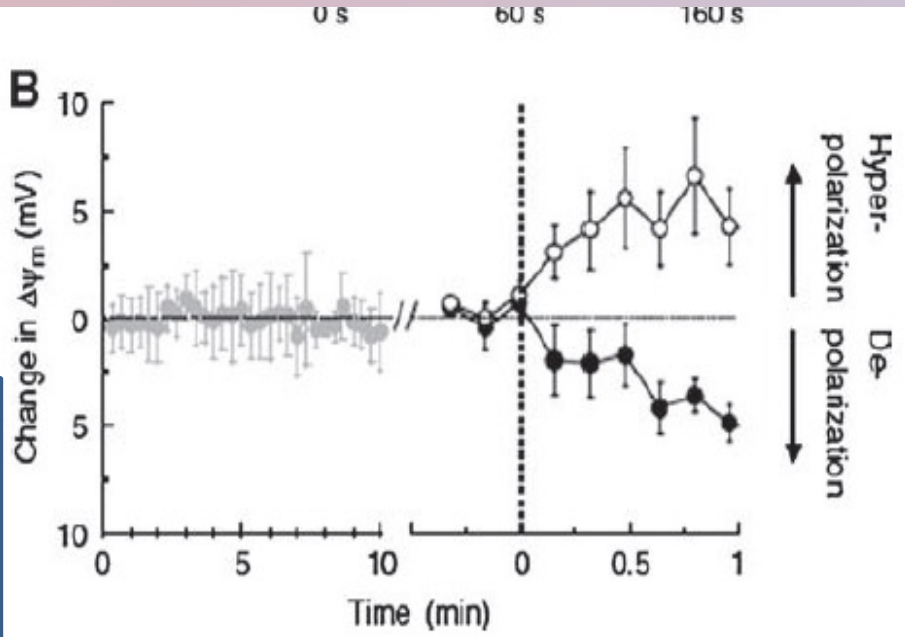
autophagy

led to Nobel Prize, new genes

lyso. might be corrupted by OH▪

next slide:

don't sweat confusing Figure



In Figure 4B, after fission, one mitoch. assumes a healthy voltage, whereas Vm collapses in the other daughter mitochondrion.

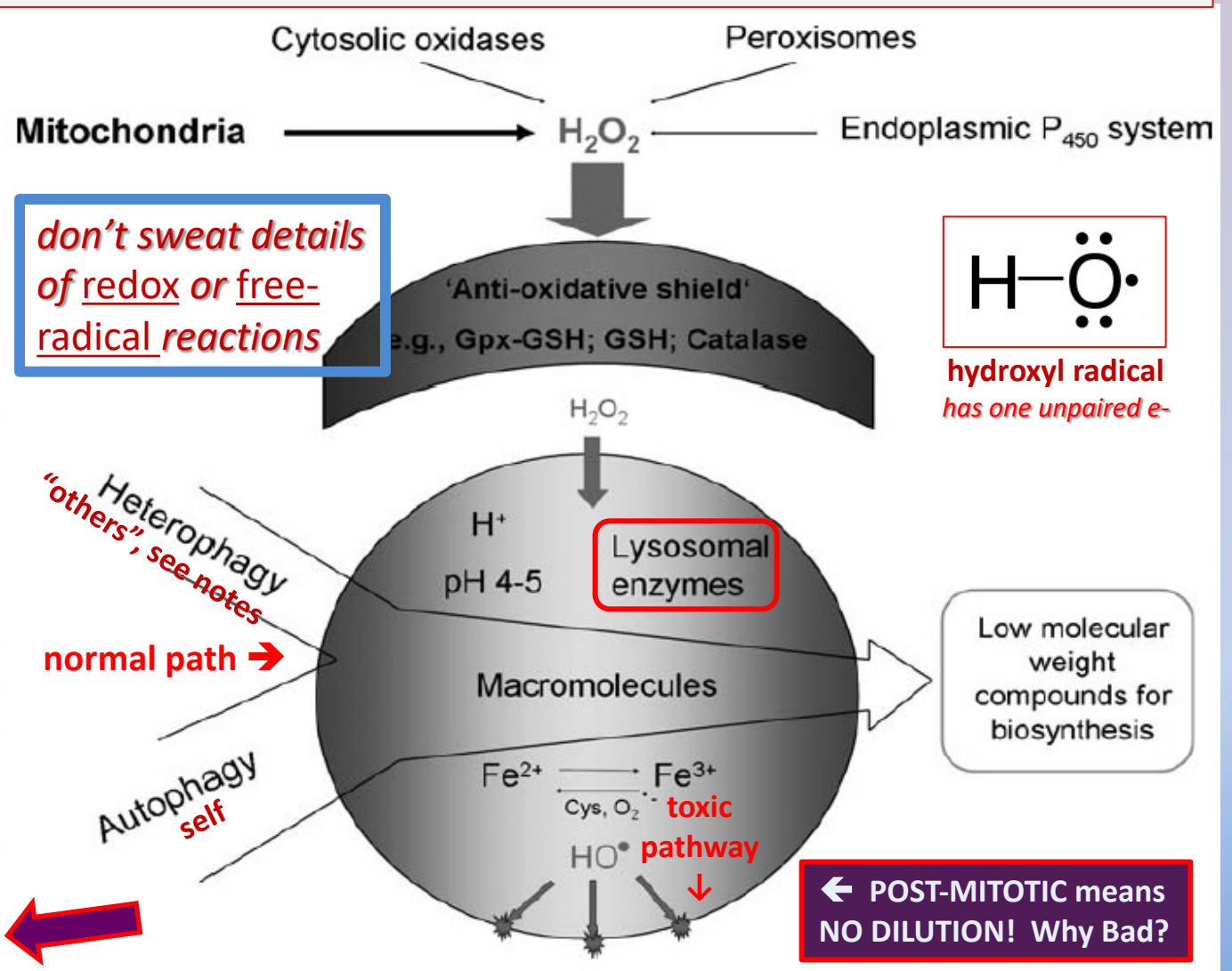
Select Details: healthy fission limits Ca++ waves and Ca++ uptake; this process also protects against ***apoptosis***. ***Excess*** Ca++ is widely thought to be neurotoxic, although its relation to neuronal damage in the course of aging remains a bit fuzzy. Only apoptosis protein to learn is: **CASPASE**

bonus material in notes

Mitochondrial Turnover and Aging, 2010

The flux of H2O2 into lysosomes generates hydroxyl free radicals aka HO•

FIG. 9. Results of intralysosomal formation of hydroxyl radicals. Hydrogen peroxide is formed normally, mainly from mitochondria. It is efficiently inactivated by the cell's antioxidative shield. Only a small portion of this oxidant manages to diffuse into lysosomes, a compartment rich in cystein and redox-active iron, the latter originating from the degradation of a variety of iron-containing proteins. Hydrogen peroxide and iron react in the Fenton reaction, yielding hydroxyl radicals. This process gives rise to intralysosomal oxidation/peroxidation with resulting damage to the lysosomal membrane and macromolecules undergoing autophagic degradation. Some oxidation products polymerize and become undegradable (lipofuscin) and accumulate in lysosomes of long-lived postmitotic cells, which do not dilute the pigment by division.



Lipofuscin Defined: plus OH (aka HO) radicals promote the formation of lipofuscin

Killer Cells: some cells are able to exocytose lysosomal contents OR actual lysosomes!

DQ: would dissolving all lipofuscin inside nerve/glial cells prevent cognitive aging?

VI. Lipofuscin Formation and Its Influence on Autophagy

p. 520

A. Influence of labile iron and ROS on lipofuscin formation

Lipofuscin (age pigment) is a nondegradable, yellowish-brown, autofluorescent, polymeric compound that slowly accumulates within aging postmitotic cells at a rate that is inversely correlated with species longevity (76, 123) and reviewed in refs.

It is now generally accepted that the aging of long-lived postmitotic cells is at least partly induced by endogenously formed ROS, affecting various cellular structures, but mainly mitochondria and lysosomes (10, 98, 124, 128, 132, 212, 239). Lipofuscin formation is one of the most important manifestations of ROS-induced damage that occurs within the lysosomal compartment (27).

-degradation of intracellular debris

Although rapid and effective, lysosomal (autophagic) degradation is not completely perfect. Even under normal conditions, some iron-catalyzed peroxidation occurs intralysosomally (as pointed out, lysosomes are rich in redox-active iron), resulting in oxidative modification of the autophagocytosed material, making it resistant to the hydrolytic activity of lysosomal enzymes. If cells do not divide, this material progressively accumulates in the form of lipofuscin inclusions. Lysosomes receive a wide variety of autophagocytosed subcellular structures, most importantly mitochondria, which are rich in lipidaceous membrane components and iron-containing proteins, such as cytochrome *c*. That lipofuscin/ceroid to a large extent originates from mitochondrial components is proven by the presence of the ATP-synthase subunit *c* in age pigment or ceroid granules (72). In Alzheimer disease (AD), large amounts of mitochondrial lipoic acid have been found associated with lipofuscin, which indicates pronounced mitochondrial autophagy (166). Thus mitochondria not only are the main generators of ROS, triggering lipofuscinogenesis, but also are a major source of the macromolecules from which lipofuscin forms.

What is Autophagy?

- "internal endocytosis" (more below)
- via "autophagosome" which fuses w/
- nascent or mature lysosome (methinks)
- is it the main cause of lipofuscin?

Lipofuscin: Autofluorescent Polymer

- lipofuscin is main damage occurring lysosomes
- ROS + iron ➔ *peroxidation* of lysosome contents
  i.e. it starts a chain-reaction of lipid radicals
- material becomes resistant to breakdown
  this includes material coming from mitochondria
  such as lipids, iron containing proteins
- lipoic acid seen in lysosomes (in AlzD) suggests failure to breakdown mitoch. (where lipoic acid is used; a sulfur cofactor in E-metab.)

Summary: Mitochondria are not only a major source of ROS but also contribute contents to lysosomes, via autophagy, which help trigger hydroxyl radical formation and the formation of lipofuscin.

see Exam Q. below

Mitochondrial Turnover and Aging, 2010

Time Line 2016
discovered this news the afternoon after my lecture on mito-lysosome autophagy

Press Release

2016-10-03

Swedish

Swedish (pdf)

The Nobel Assembly at Karolinska Institutet has today decided to award

the 2016 Nobel Prize in Physiology or Medicine

to

Yoshinori Ohsumi

for his discoveries of mechanisms for autophagy

see notes below on discovery

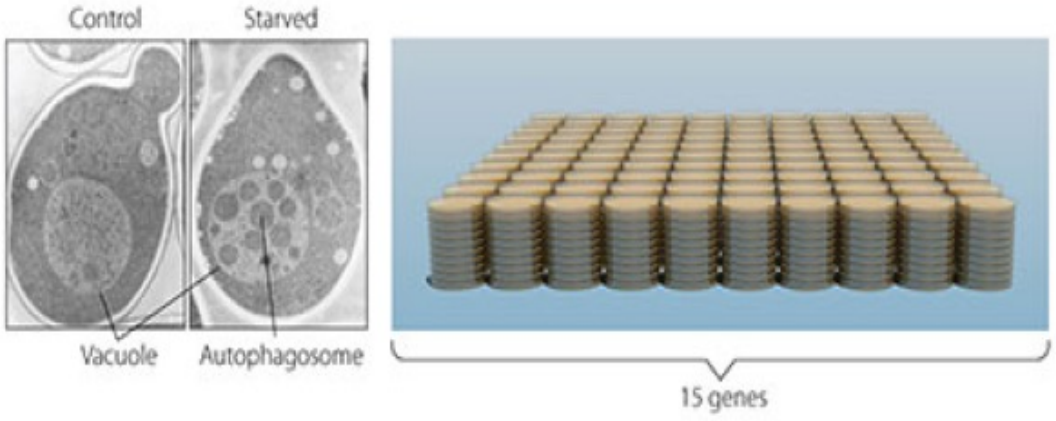
Summary

This year's Nobel Laureate discovered and elucidated mechanisms underlying *autophagy*, a fundamental process for degrading and recycling cellular components.

The word *autophagy* originates from the Greek words *auto-*, meaning "self", and *phagein*, meaning "to eat". Thus,autophagy denotes "self eating". This concept emerged during the 1960's, when researchers first observed that the cell could destroy its own contents by enclosing it in membranes, forming sack-like vesicles that were transported to a recycling compartment, called the *lysosome,* for degradation. Difficulties in studying the phenomenon meant that little was known until, in a series of brilliant experiments in the early 1990's, Yoshinori Ohsumi used baker's yeast to identify genes essential for autophagy. He then went on to elucidate the underlying mechanisms for autophagy in yeast and showed that similar sophisticated machinery is used in our cells.

Ohsumi's discoveries led to a new paradigm in our understanding of how the cell recycles its content. His discoveries opened the path to understanding the fundamental importance of autophagy in many physiological processes, such as in the adaptation to starvation or response to infection. Mutations in autophagy genes can cause disease, and the autophagic process is involved in several conditions including cancer and neurological disease.

Organelleathon: phagosomes and autophagosomes appear to be two different beasts. Use of Google Scholar advance search features revealed essentially separate literatures for the two terms, the first pertaining to phagocytosis and the second to autophagy. Delving ended.



see notes below on medical sequelae

Figure 2: In yeast (left panel) a large compartment called the *vacuole* corresponds to the lysosome in mammalian cells. Ohsumi generated yeast lacking vacuolar degradation enzymes. When these yeast cells were starved, autophagosomes rapidly accumulated in the vacuole (middle panel). His experiment demonstrated that autophagy exists in yeast. As a next step, Ohsumi studied thousands of yeast mutants (right panel) and identified 15 genes that are essential for autophagy.

Autophagy genes are discovered

Ohsumi now took advantage of his engineered yeast strains in which autophagosomes accumulated during starvation. This accumulation should not occur if genes important for autophagy were inactivated. Ohsumi exposed the yeast cells to a chemical that randomly introduced mutations in many genes, and then he induced autophagy. His strategy worked! Within a year of his discovery of autophagy in yeast, Ohsumi had identified the first genes essential for autophagy. In his subsequent series of elegant studies, the proteins encoded by these genes were functionally characterized. The results showed that autophagy is controlled by a cascade of proteins and protein complexes, each regulating a distinct stage of autophagosome initiation and formation (Figure 3).

Basically, lipofuscin may be regarded as a nondegradable plastic-like polymer that slowly matures by intramolecular reorganization. It has been found that lipofuscin contains oxidized proteins in which tyrosine residues have been replaced by DOPA (3,4-dihydroxy-L-phenylalanine, an oxidized form of tyrosine) (67, 117, 191, 201), secondary to an oxidation that most probably is mediated by redox-active iron. Because DOPA, being a hydroquinone-type structure, is capable of redox cycling (190), it is conceivable that lipofuscin, because of its oxidized protein residues, produces superoxide and hydrogen peroxide. This production might result in the labilization of the surrounding membrane, especially because lipofuscin is also rich in loosely bound iron. Consequently, lipofuscin-loaded lysosomes may be sites of pronounced Fenton-type reactions and be especially sensitive to oxidative stress (26, 29, 110).

Oxidized proteins are usually considered degraded by the proteasome system secondary to ubiquitination, but recent studies have shown that such proteins may also undergo autophagic degradation. Studies using a cell line with a thermolabile ubiquitin-conjugating enzyme showed that oxidized proteins were still being degraded at a normal rate, even in the absence of functioning ubiquitin-conjugating enzymes (209). Although it is not clear to what extent other pathways for ubiquitin conjugation could have been active, the latter finding suggests that the proteasomal pathway is not the only one involved in the degradation of oxidized proteins (109). *- see note below on ubiquitin -*

By using an approach in which oxidatively modified proteins were generated *in vitro* by allowing cells to incorporate DOPA into proteins, it was demonstrated that mildly modified proteins were efficiently degraded by proteasomes, because this process could be inhibited by the specific proteasome inhibitor, lactacystin (192). By increasing the amount of DOPA incorporated into proteins, it was, however, possible to generate proteins that were heavily modified and eventually to generate lysosomal autofluorescent lipofuscin

← a "plastic-like" polymer!

Lipofuscin: Autofluorescent Polymer

- **lipofuscin polymer** slowly matures inside lysosomes, perhaps aided by DOPA
- *beyond its role as debris* it may also help generate hydroxyl radicals via DOPA residues relevant to death of DA neurons in Parkinson's?
- **autophagy** may help proteasomes to breakdown oxidized proteins (in health)
- experiments show that increasing amt. of DOPA (oxidized tyrosine) ➔ lipofuscin .

Summary: This process may play a central role in the formation of very long-lived aggregates in post-mitotic cells like neurons. Possibly this is a slow, positive feedback loop where more ROS ➔ more lipofuscin and more lipofuscin ➔ more ROS.

article p. 520

Authors refer to "cross-talk" btw. proteasomes & lysosomes AND btw mitoch. & lysosomes: ***bonus*** *notes below*

Theory on How Lipofuscin Forms
degradation of aged mitochondria

Autophagy PRODUCES Lipofuscin

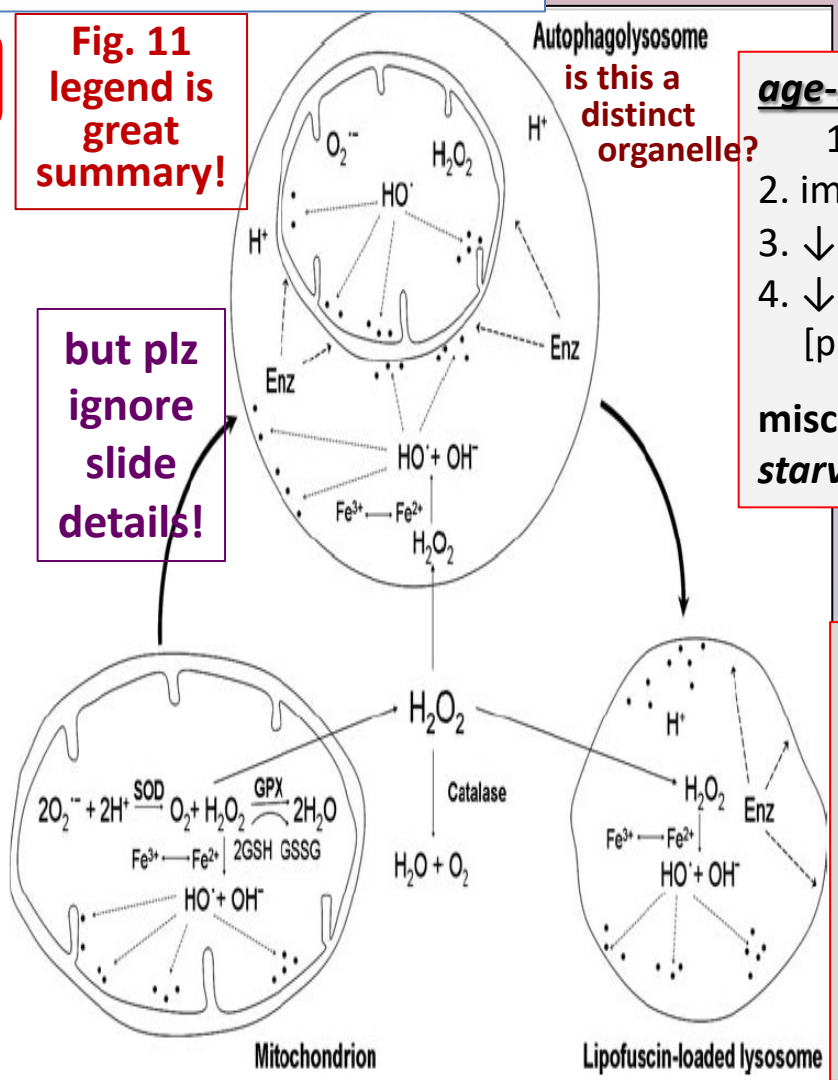


FIG. 11. Mechanisms of lipofuscin formation. Superoxide ($O_2^{\bullet-}$) forms mainly in mitochondria as a side product of biologic respiration. It is converted into hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). Hydrogen peroxide is further homolytically split, yielding the hydroxyl radical ($HO^\bullet$), in the presence of ferrous iron (the Fenton reaction). Hydroxyl radicals damage surrounding macromolecules, while H_2O_2 diffuses throughout the cell. Oxidatively damaged macromolecules (parts of mitochondria and other cellular structures) enter lysosomes through autophagy. In the autophagolysosomes, which are rich in iron, more hydroxyl radicals form, causing oxidative damage to autophagocytosed material, resulting in its polymerization and undegradability (*i.e.*, lipofuscin formation). Actions of lysosomal enzymes (Enz) and reactive oxygen species are indicated as dashed arrows. *Black dots*, oxidatively damaged macromolecules, including components of lipofuscin. *Bold curved arrows*, the sequence of events.

Fig. 11 legend is great summary!

but plz ignore slide details!

Autophagolysosome is this a distinct organelle?

age-related mito. degeneration **due to**:

1. ↓ mitochondriogenesis
2. impaired fusion and fission
3. ↓ chaperone & folding functions
4. ↓ removal of severely damaged mitoch
   [proper fusion & fission maintains vitality]

misc: liver cells regenerate & age less -- ***starve for a day*** to clean out lyso. dumps?

CODE RED:

Lysosome consumes old mitochondria
Enzymes break down old mitochondria
but old proteins and iron build up
- H202 in lysosomes becomes HO•
- oxidized proteins turn into polymers
- tyrosine/DOPA produces more ROS
AND oxidized lipids oxidize lipids
+ more reactions ➔ lipofuscin

Mitochondrial Turnover and Aging, 2010

more notes on next slide

Mitochondrial Turnover and Aging, 2010

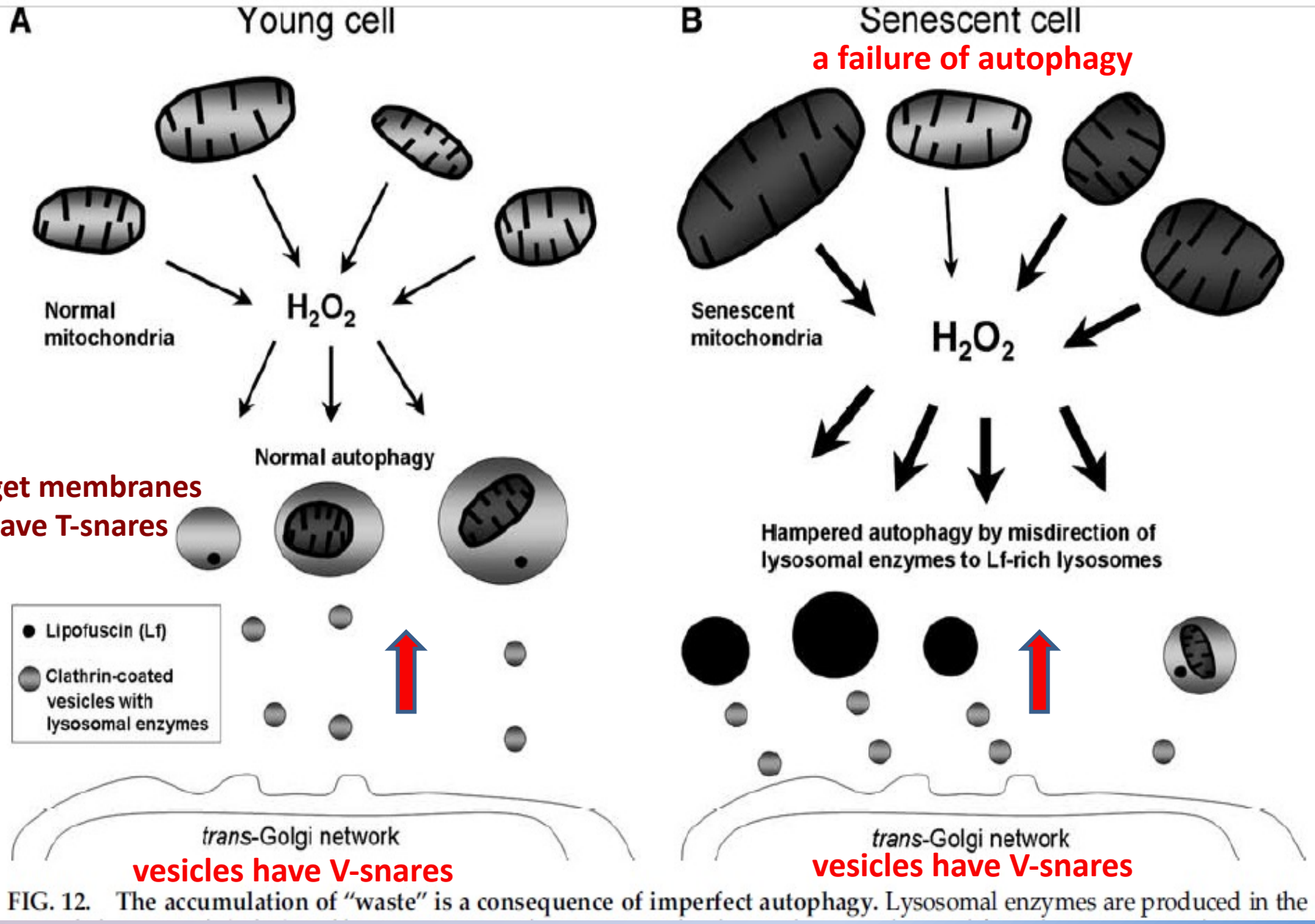


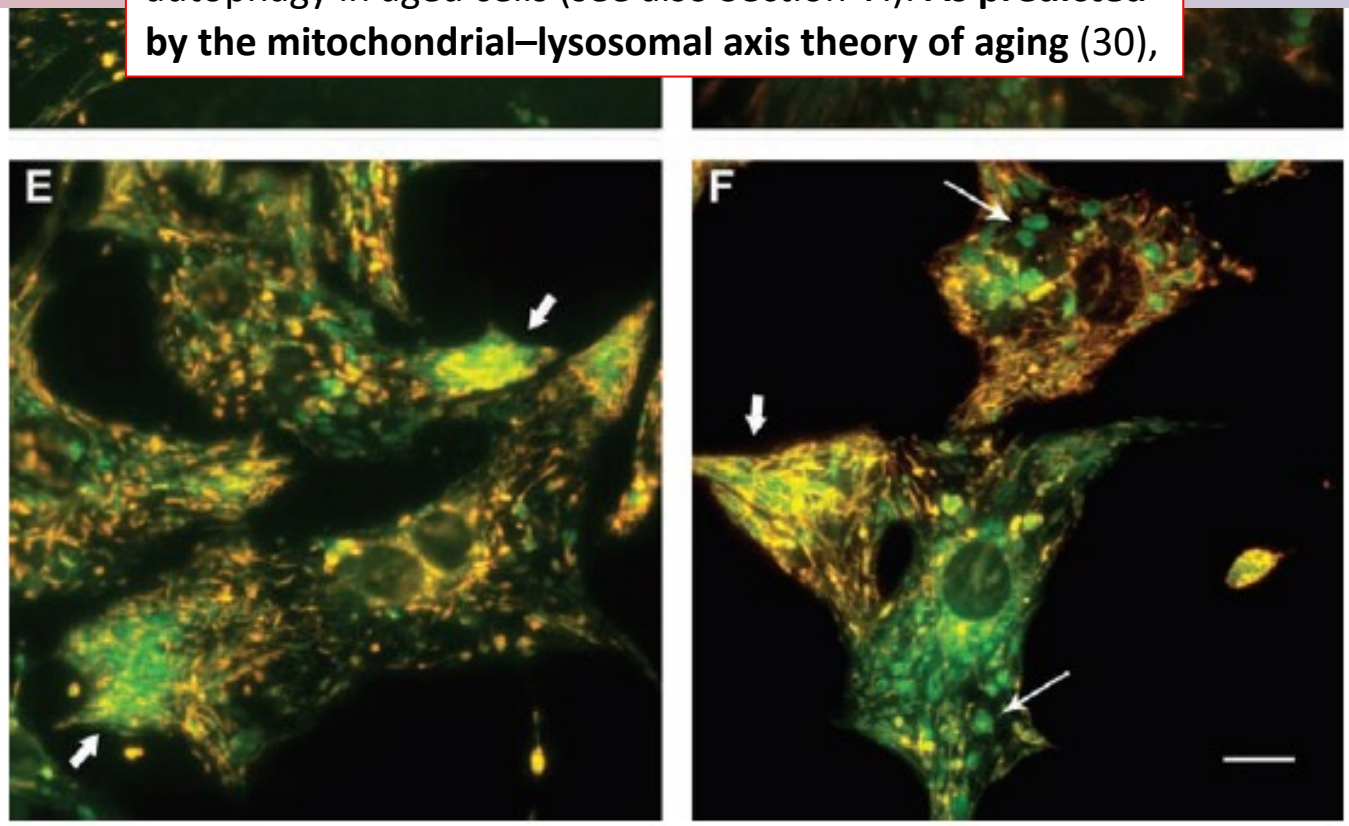
FIG. 12. The accumulation of "waste" is a consequence of imperfect autophagy. Lysosomal enzymes are produced in the

lysosomes, autophagosomes, have correct T-snares

ALLEGEDLY:

The loading of lysosomes with lipofuscin is probably one of the most important contributors to the decline of autophagy in aged cells (see also Section VI). **As predicted by the mitochondrial–lysosomal axis theory of aging** (30),

Mitochondrial Turnover and Aging, 2010



CAN IGNORE: The numerous small mitochondria are explained on a "bottleneck" slide further below. Their lowered membrane potential (after prevention of fusion and fission) might have to with inability to share good mito. proteins and genes, ala Prof. K's work on mitochondrial genomes. more in notes! KK: is Konstantin Khrapko in Biology Dept.