The Role of ER Stress and Translation in Cell Death

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The Endoplasmic Reticulum:

Calcium storage and gated release

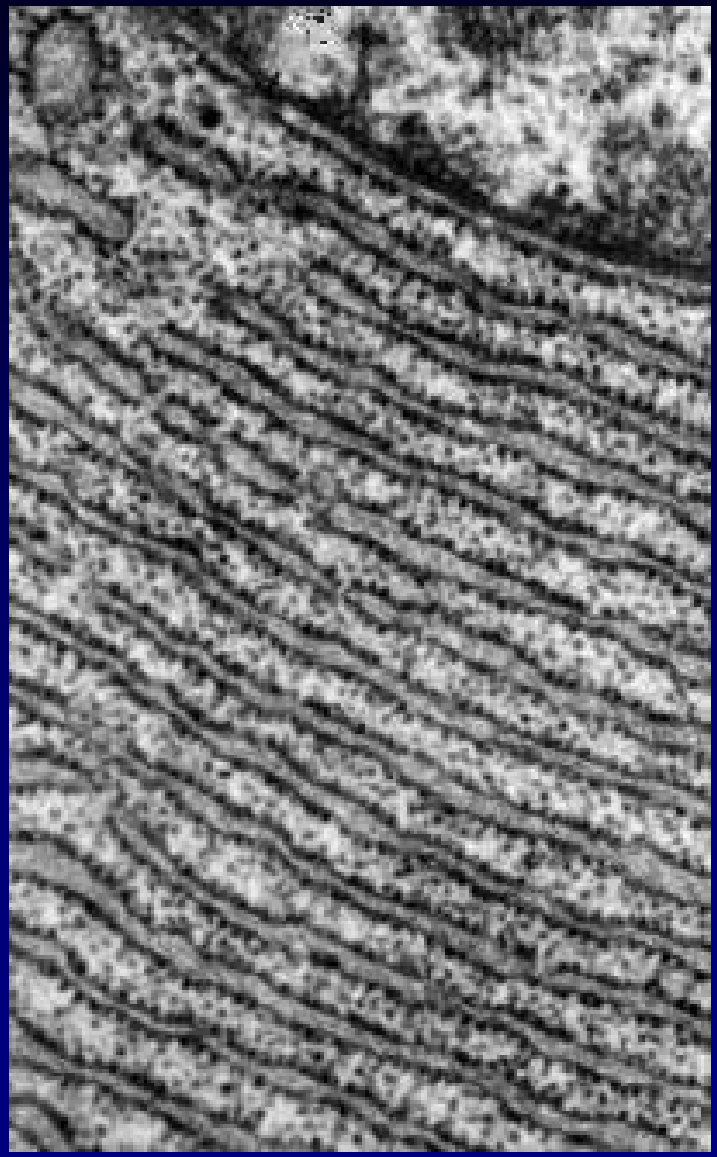
Oxidative protein folding

Quality control

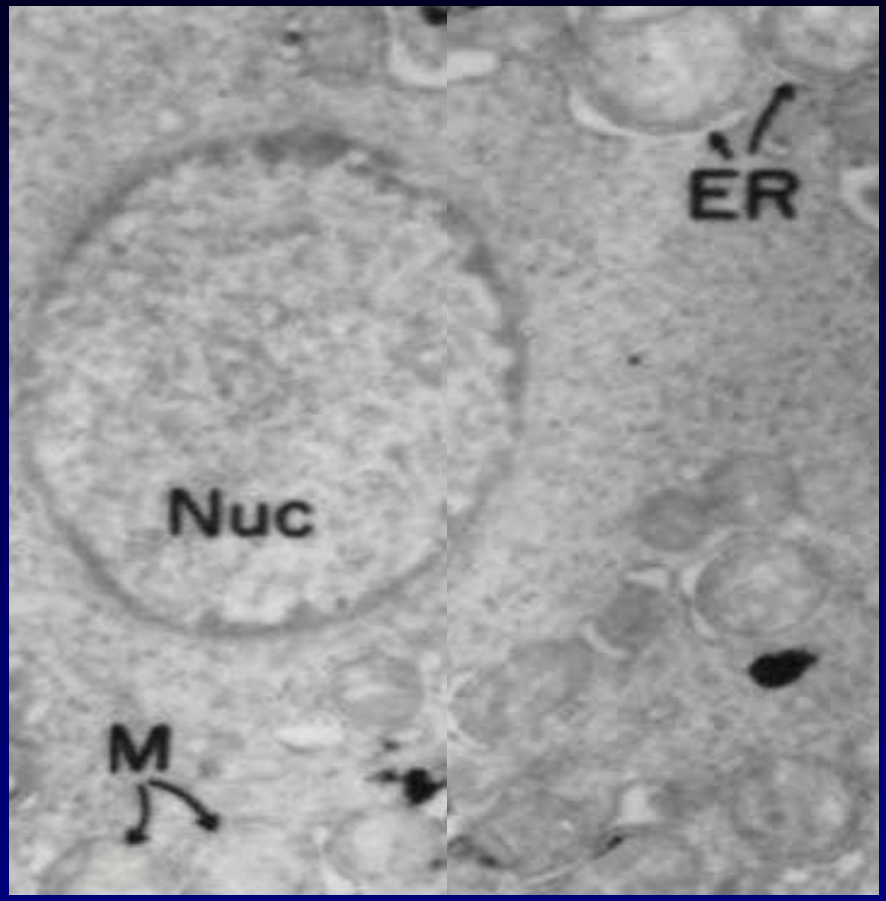
ER-associated protein degradation

Core oligosaccharide biosynthesis

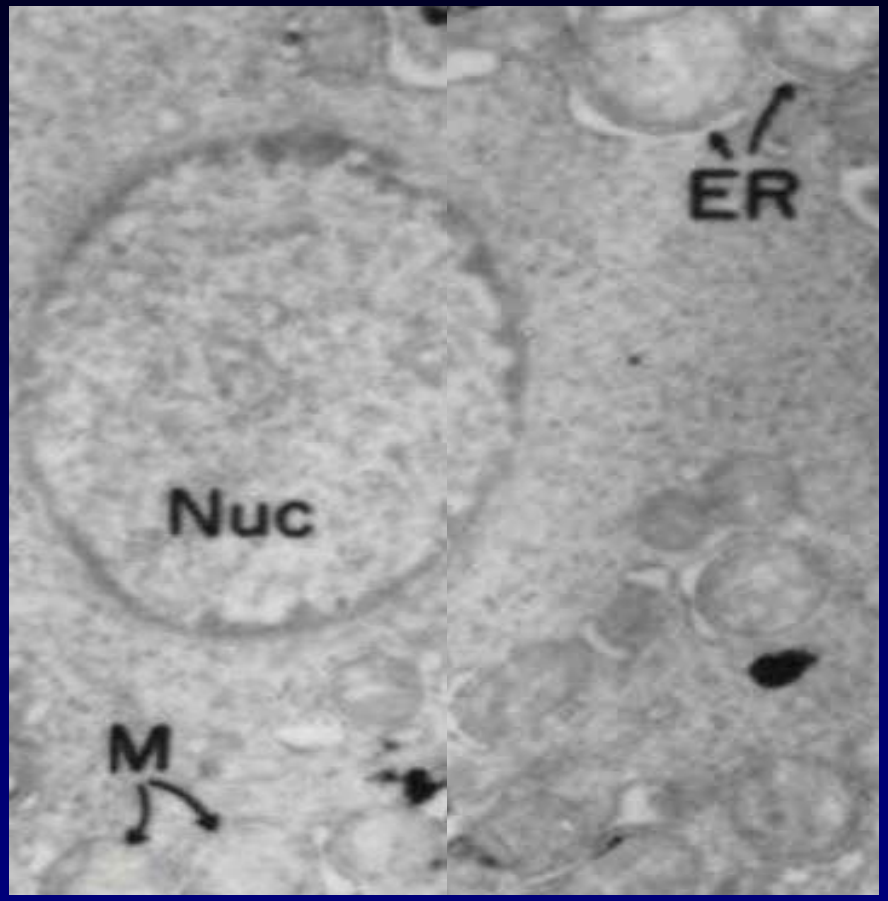
Lipid and sterol biosynthesis

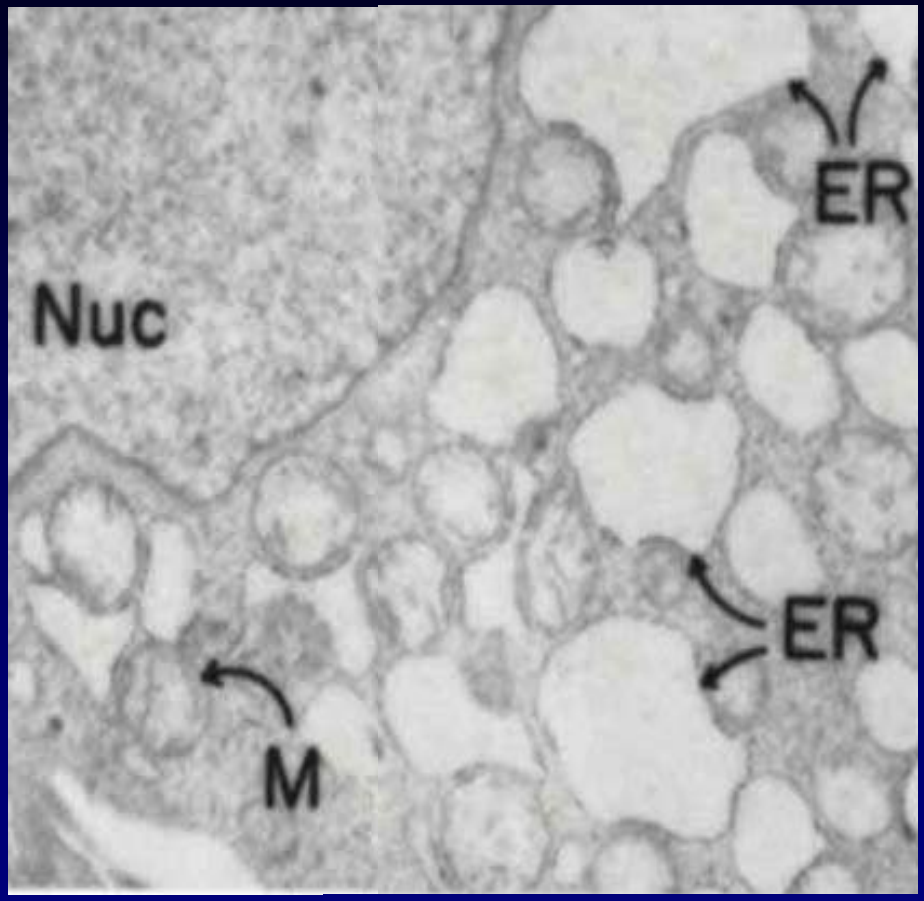


Chinese hamster ovary cell



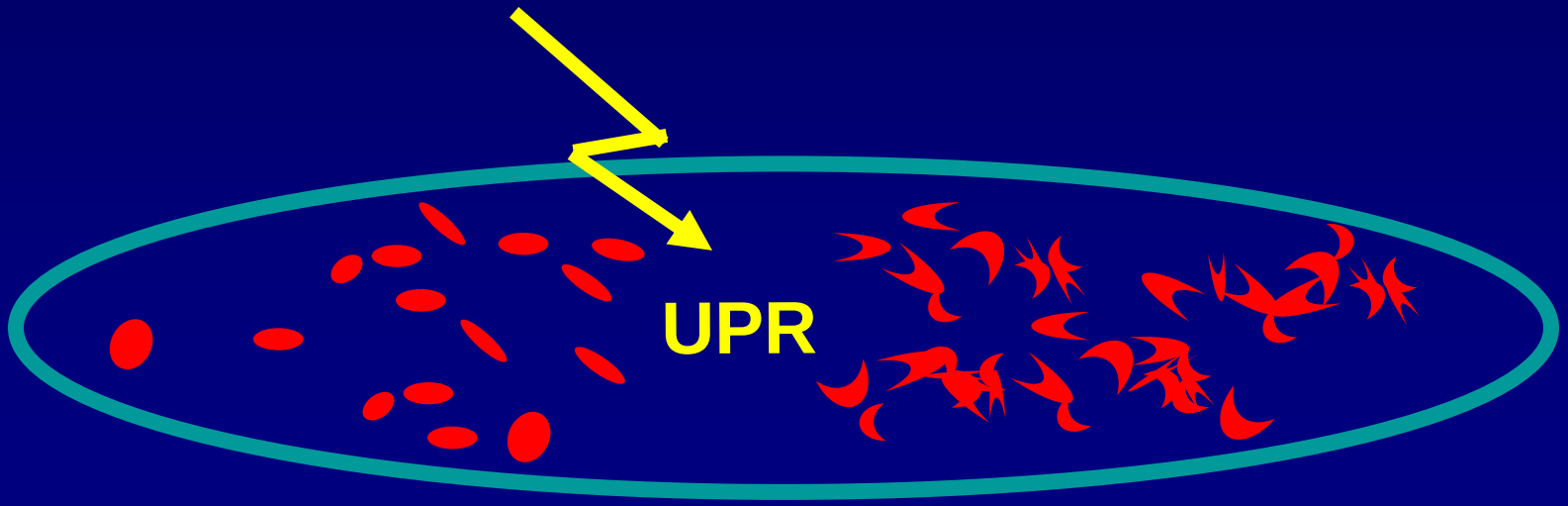
Factor VIII Expression Induces ER Stress





Activation of the Unfolded Protein Response

Calcium depletion	thapsigargin, ionophore
Altered glycosylation	tunicamycin, castanospermine
Nutrient deprivation (GRP)	glucose, hypoxia
Reductive/oxidative stress	DTT, homocysteine
Growth arrest / DNA damage (GADD)	etoposide, UV
Protein expression	wild-type / mutant / subunits



Activation of the Unfolded Protein Response

Pathological conditions:

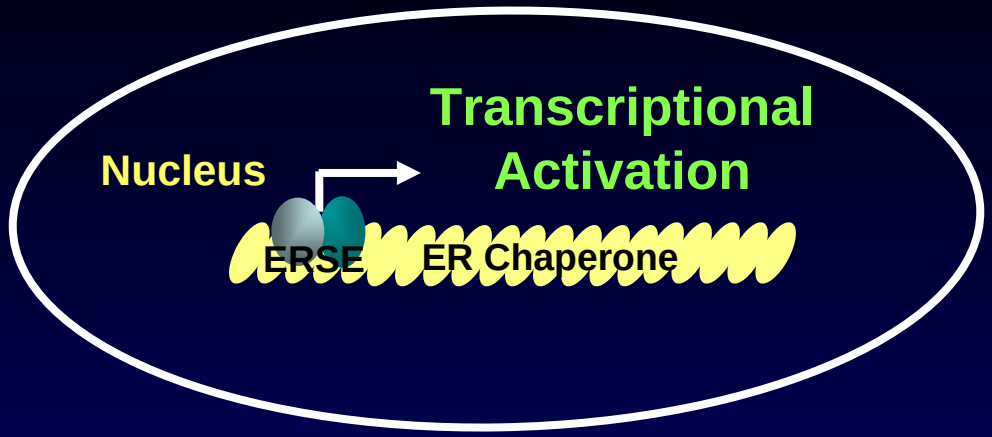
Viral infection, tumorigenesis, DNA damage, diabetes, atherosclerosis, ischemic injury, conformational diseases

Physiological responses:

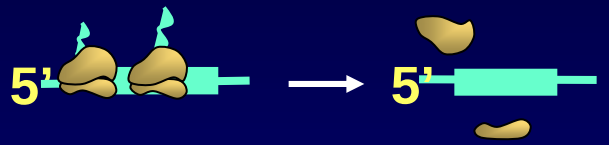
Glucose regulation of insulin production (β cells)

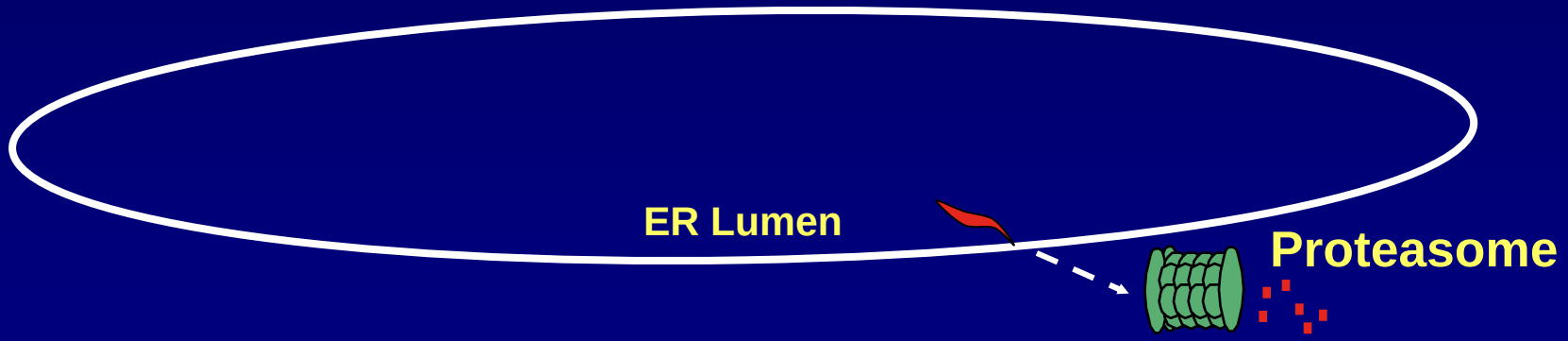
Response to a misfolded protein (hepatocytes)

The Unfolded Protein Response



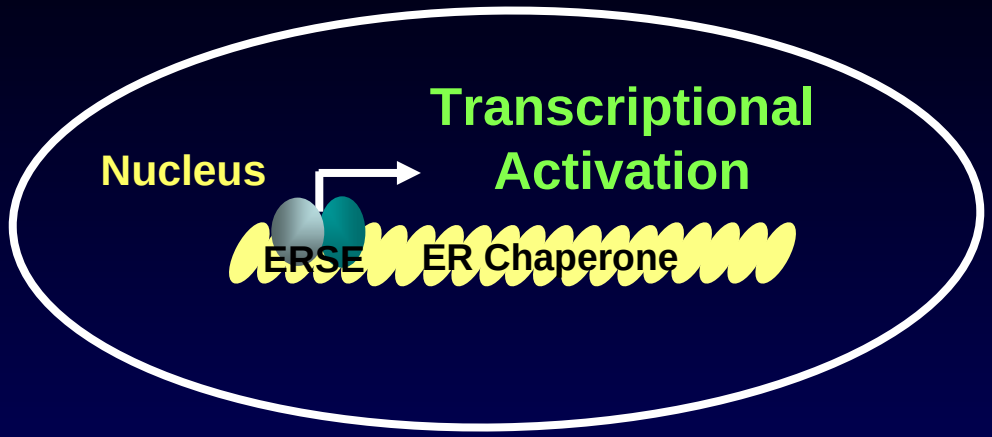
Translation Attenuation

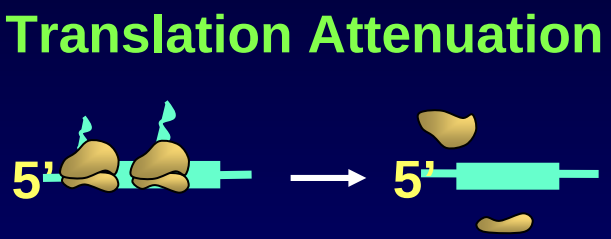


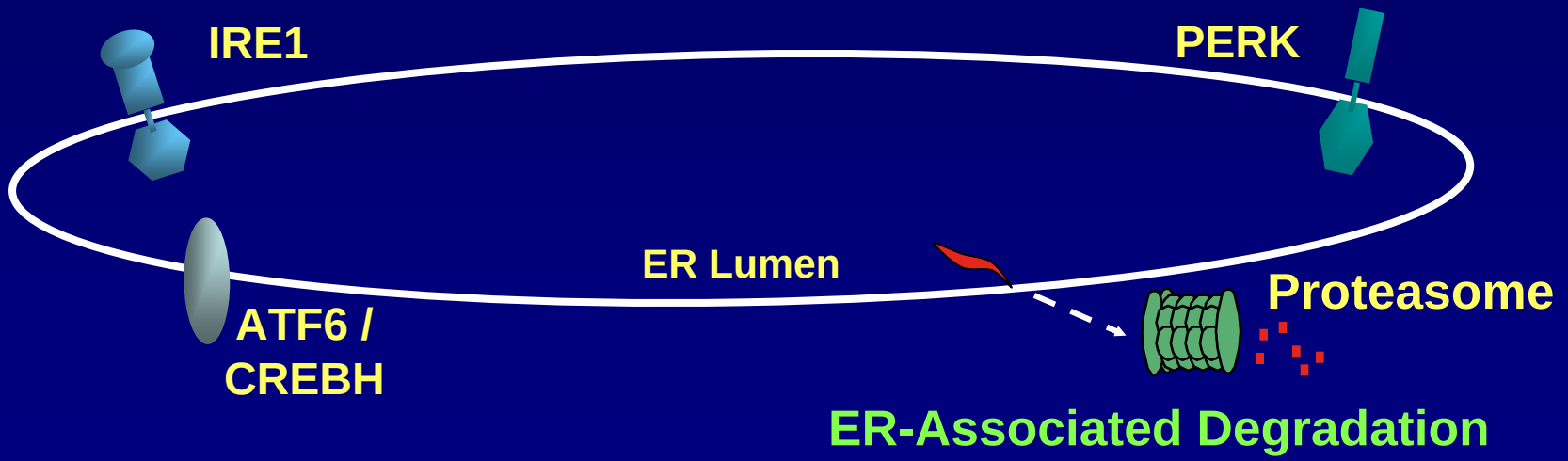


ER-Associated Degradation

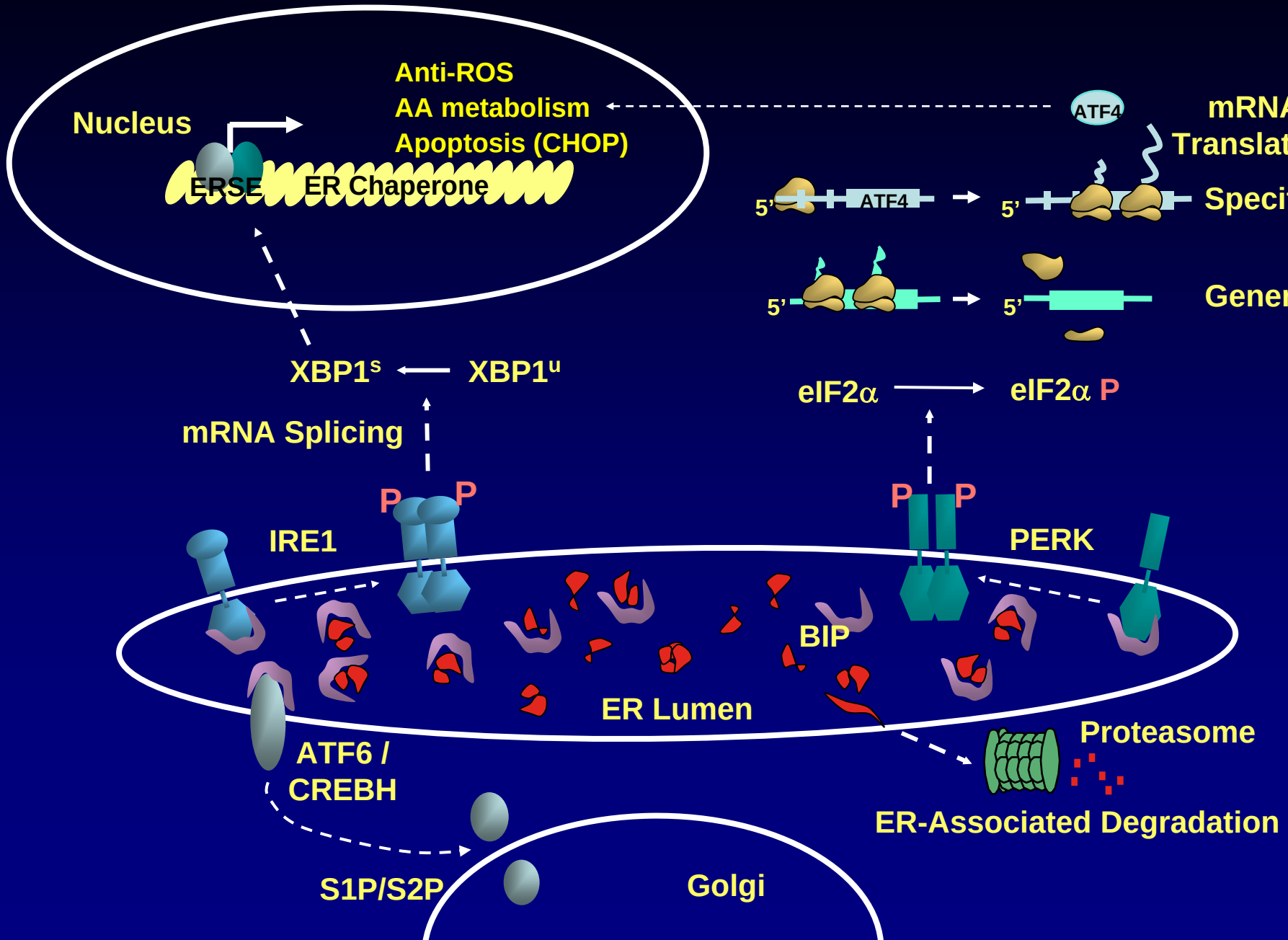
The Unfolded Protein Response



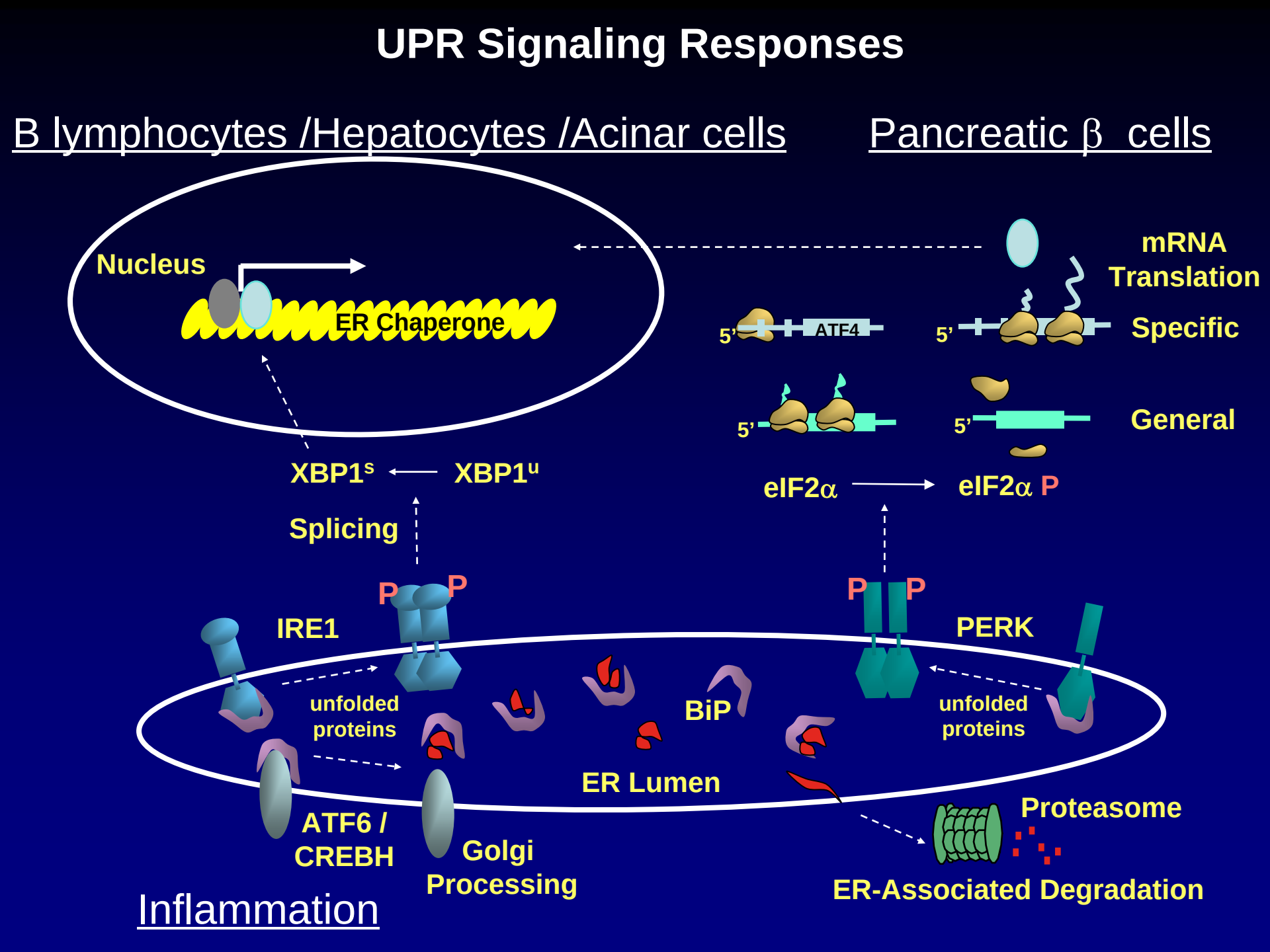




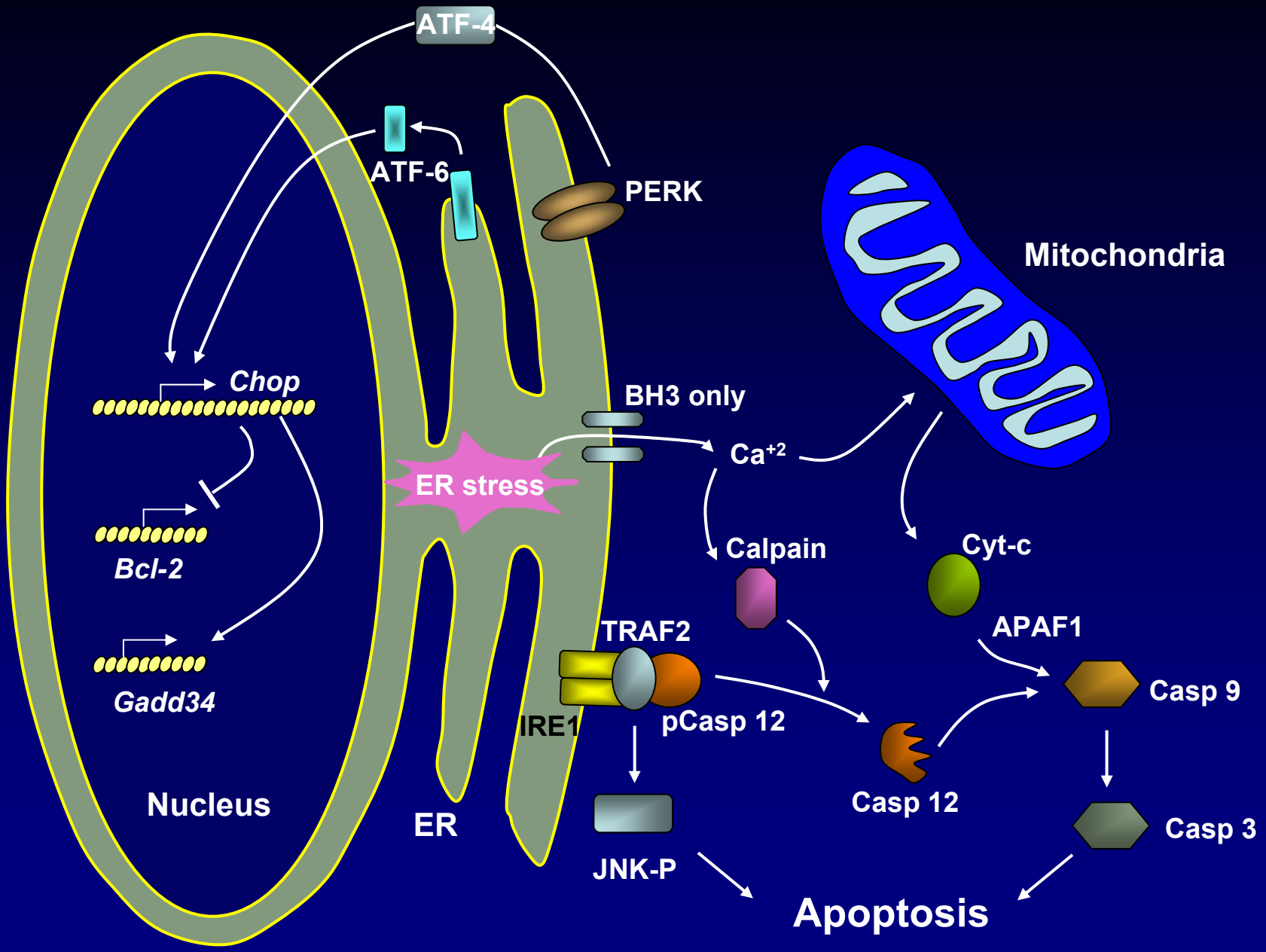
The Unfolded Protein Response



UPR Signaling Responses

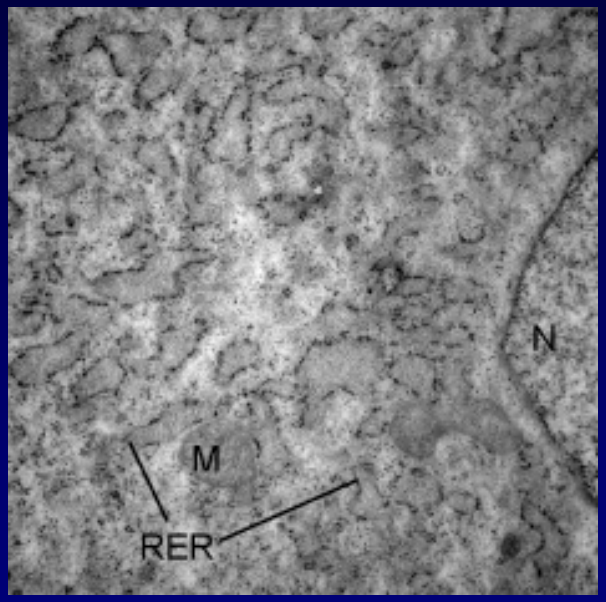


ER Stress-Induced Apoptosis

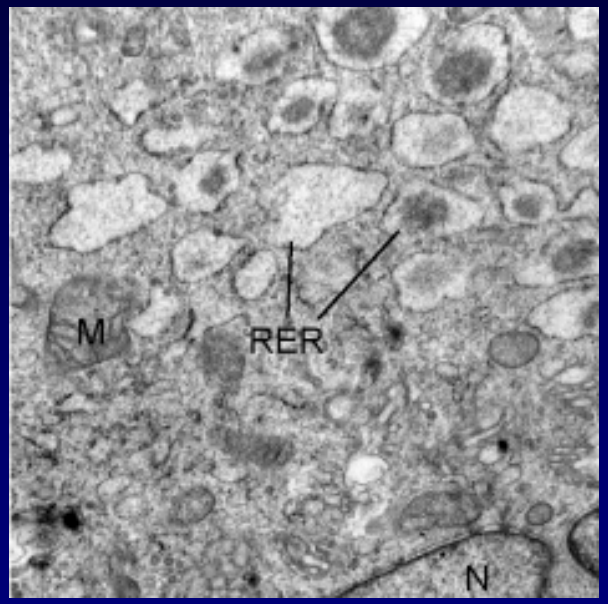


ER perturbation by mild stress

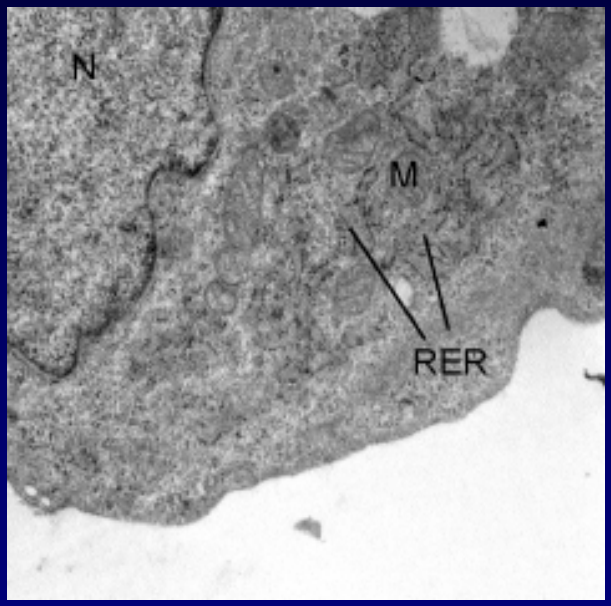
NT



25 ng/ml Tm overnight

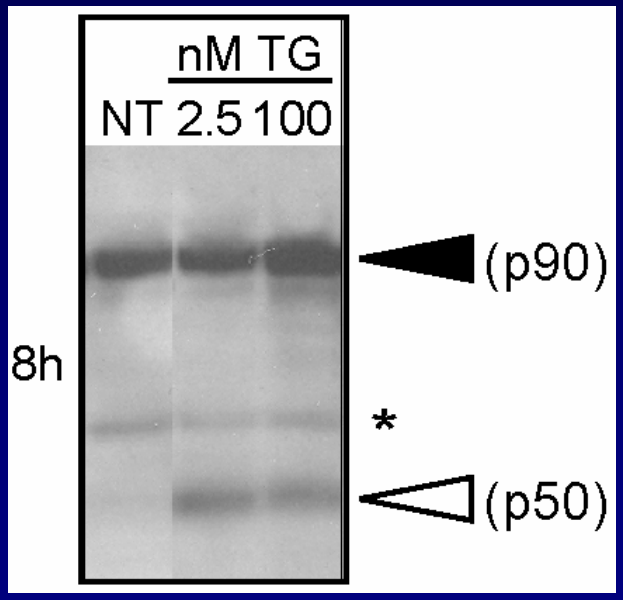


25 ng/ml Tm 2 weeks

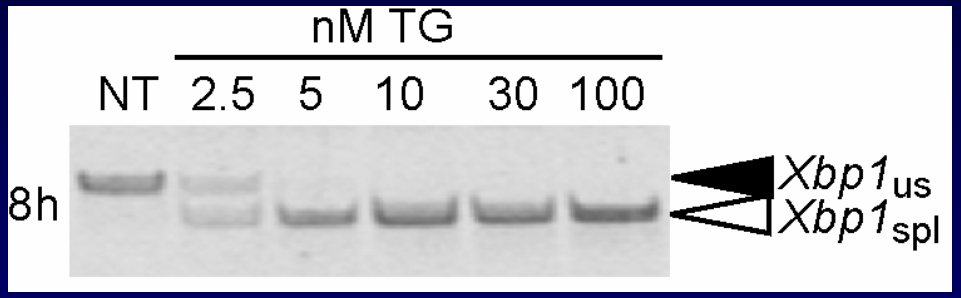


All stress pathways are activated during mild stress

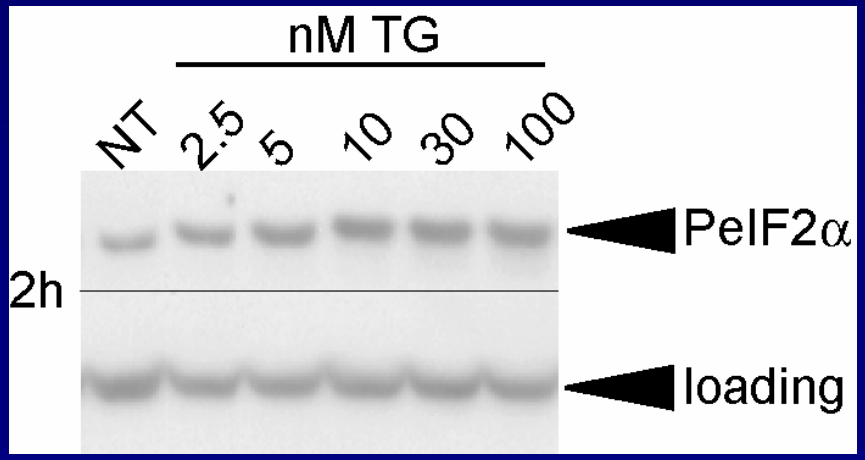
ATF6α



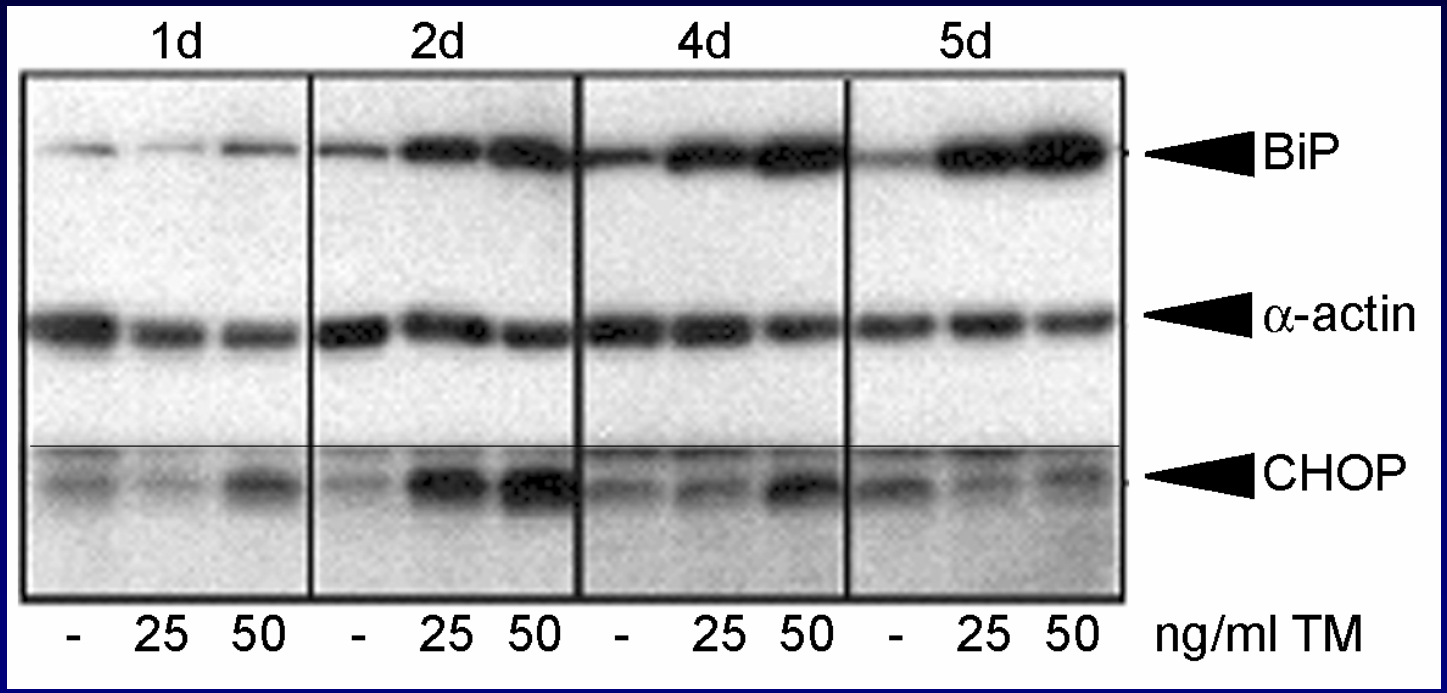
IRE1α



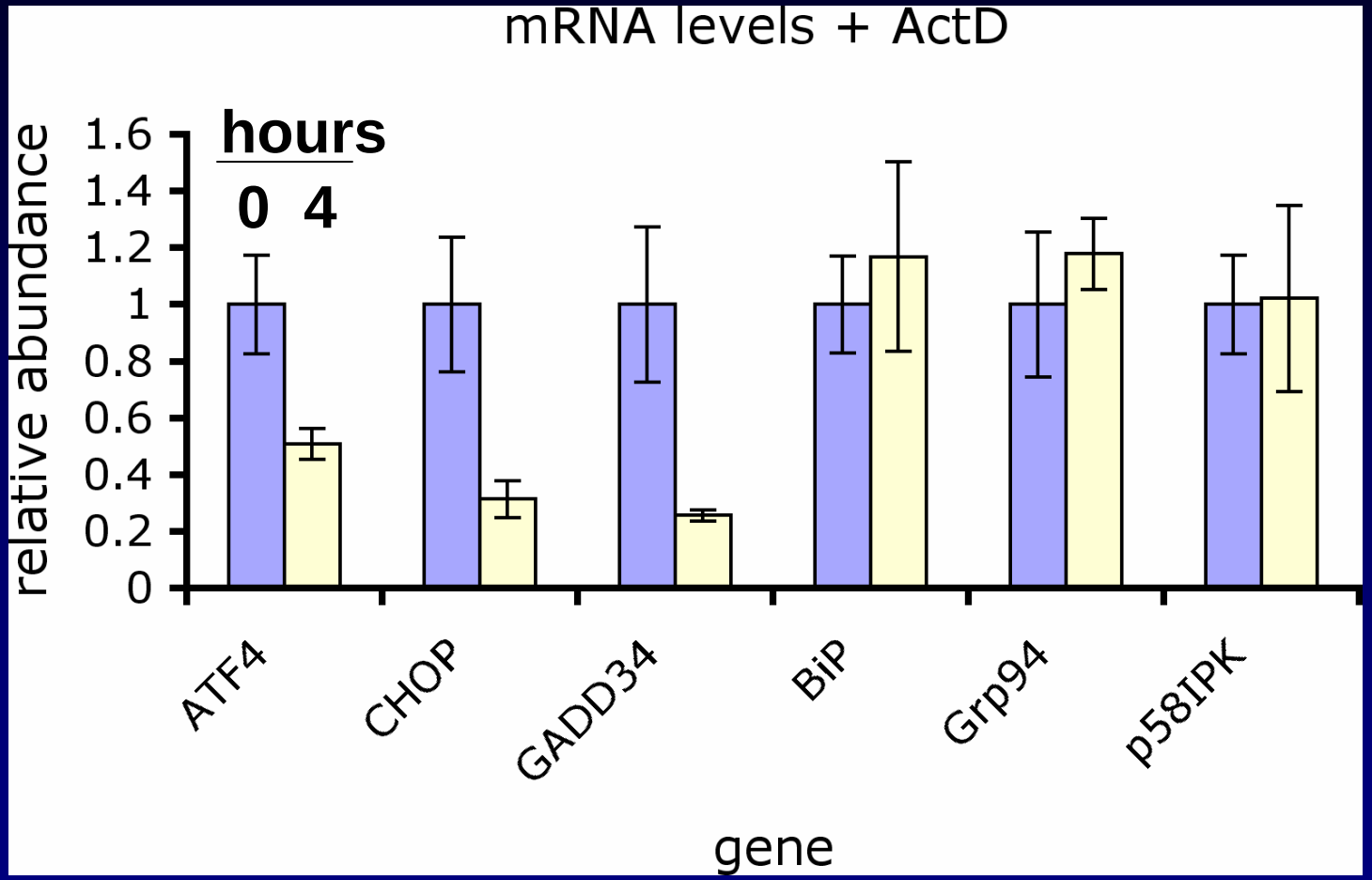
PERK



Differential outcomes for UPR pathways during persistent stress

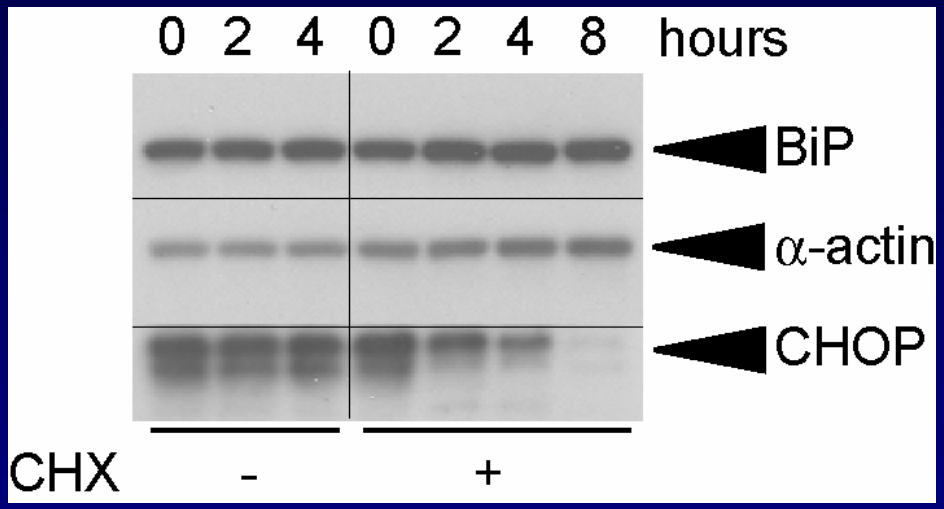


Selective instability of mRNAs in the PERK-CHOP axis



Cells pretreated to induce UPR and ActD added to block transcription

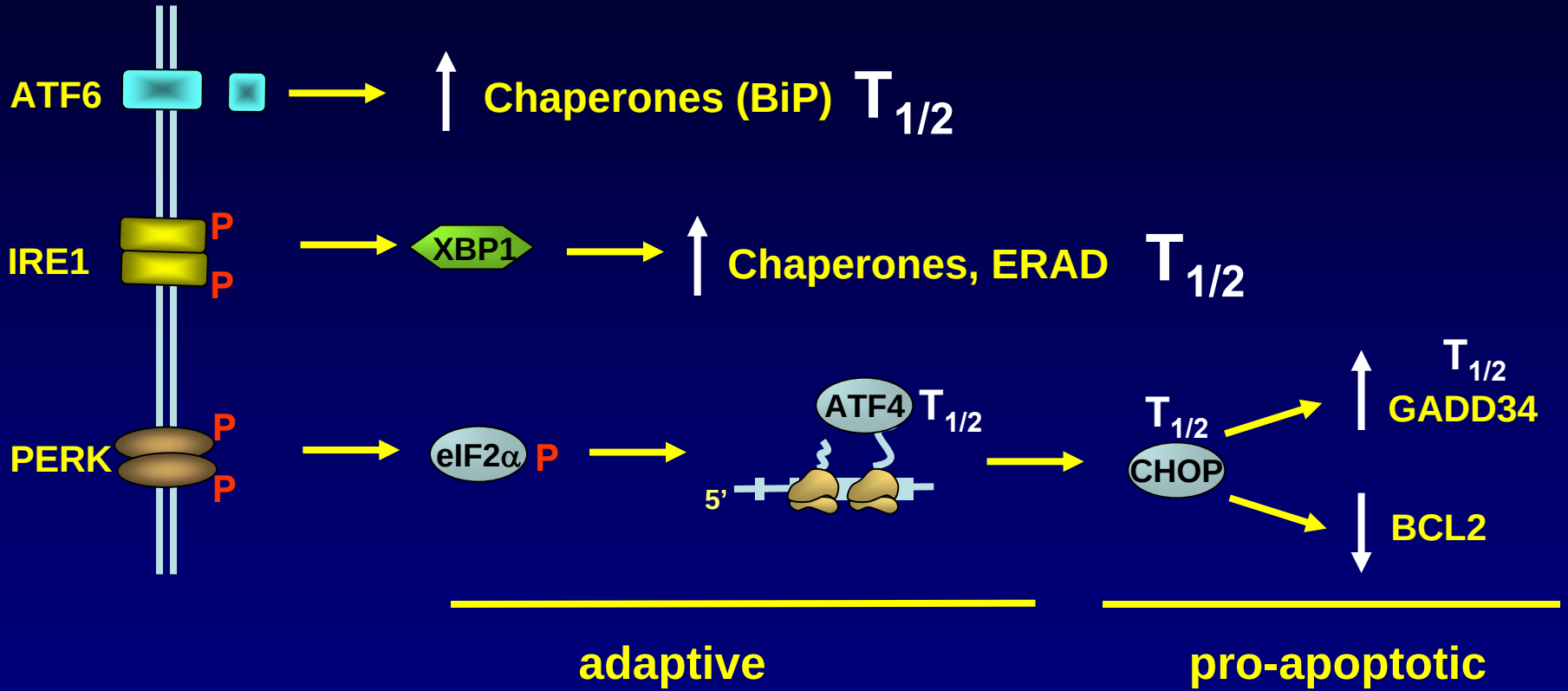
Selective instability of proteins in the PERK-CHOP axis



Short (<4h) half-life	Long (>8h) half-life
ATF4	BiP
CHOP	Grp94
GADD34	$p58^{IPK}$

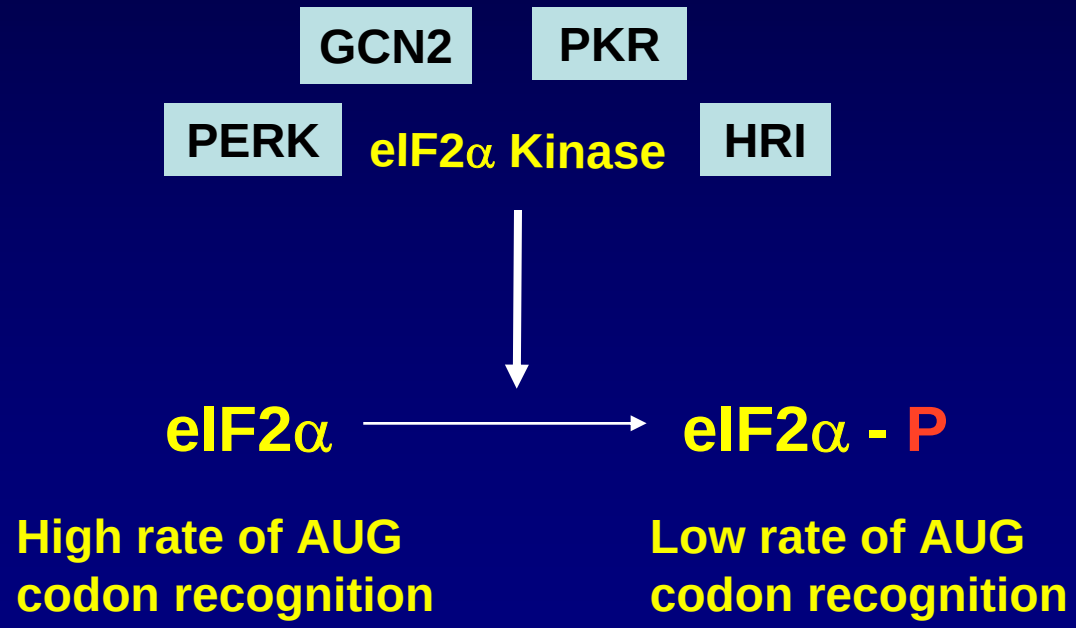
Cells pretreated to induce UPR and CHX added to block translation

UPR-induced Alterations in Gene Expression

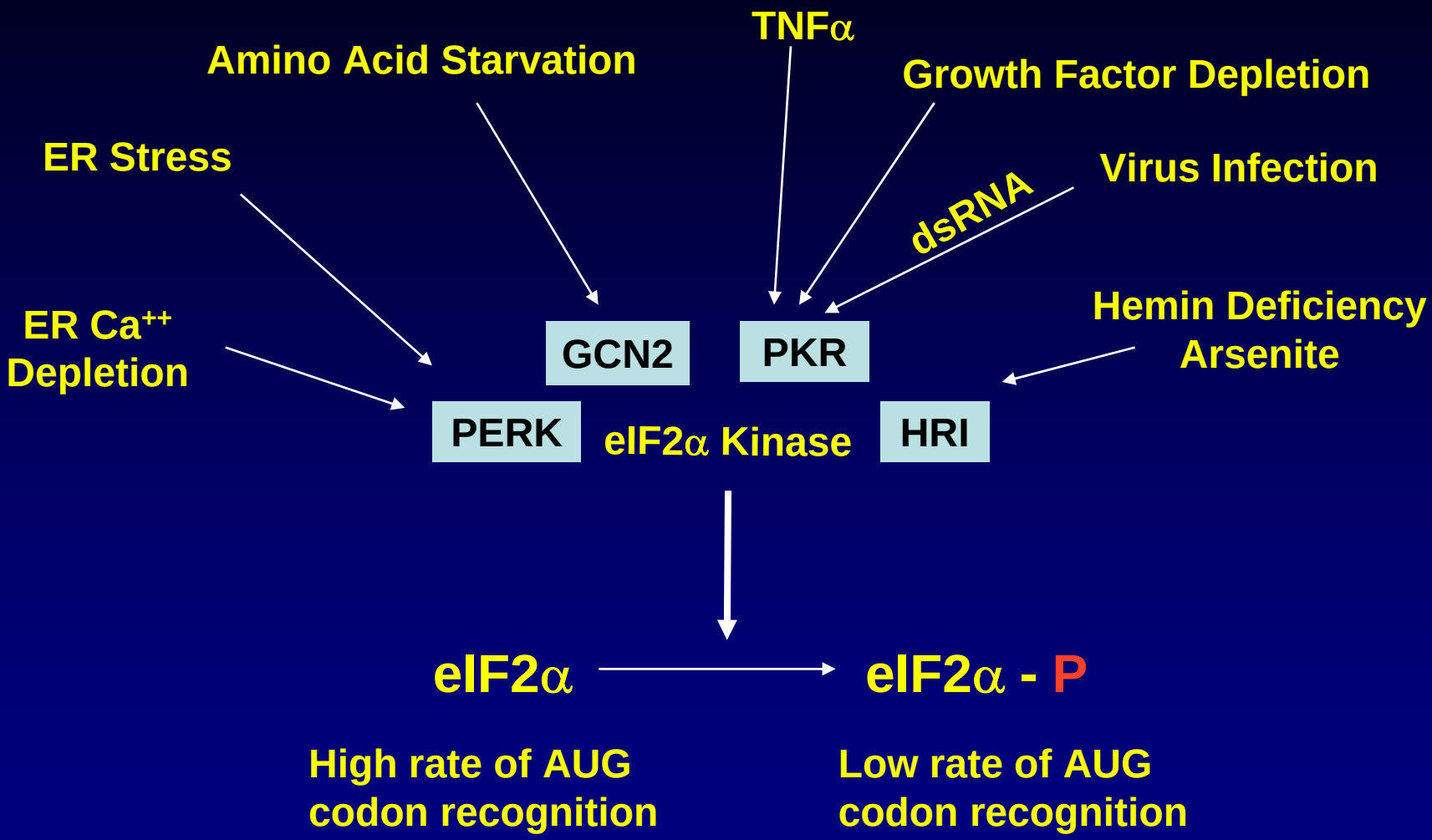


Rutkowski et al. PLOS Biol. (In Press)

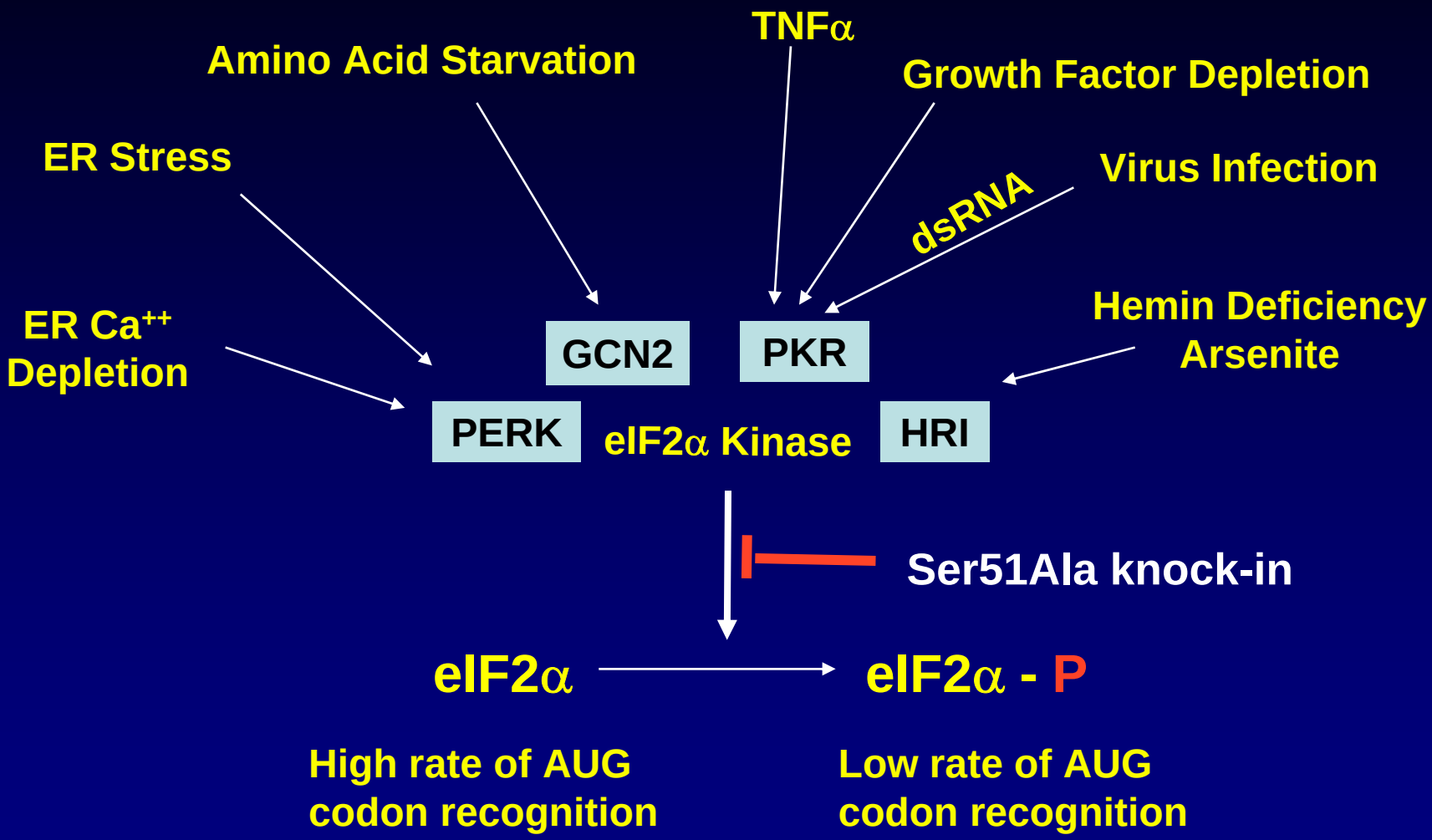
Translation Initiation Response to External Stimuli



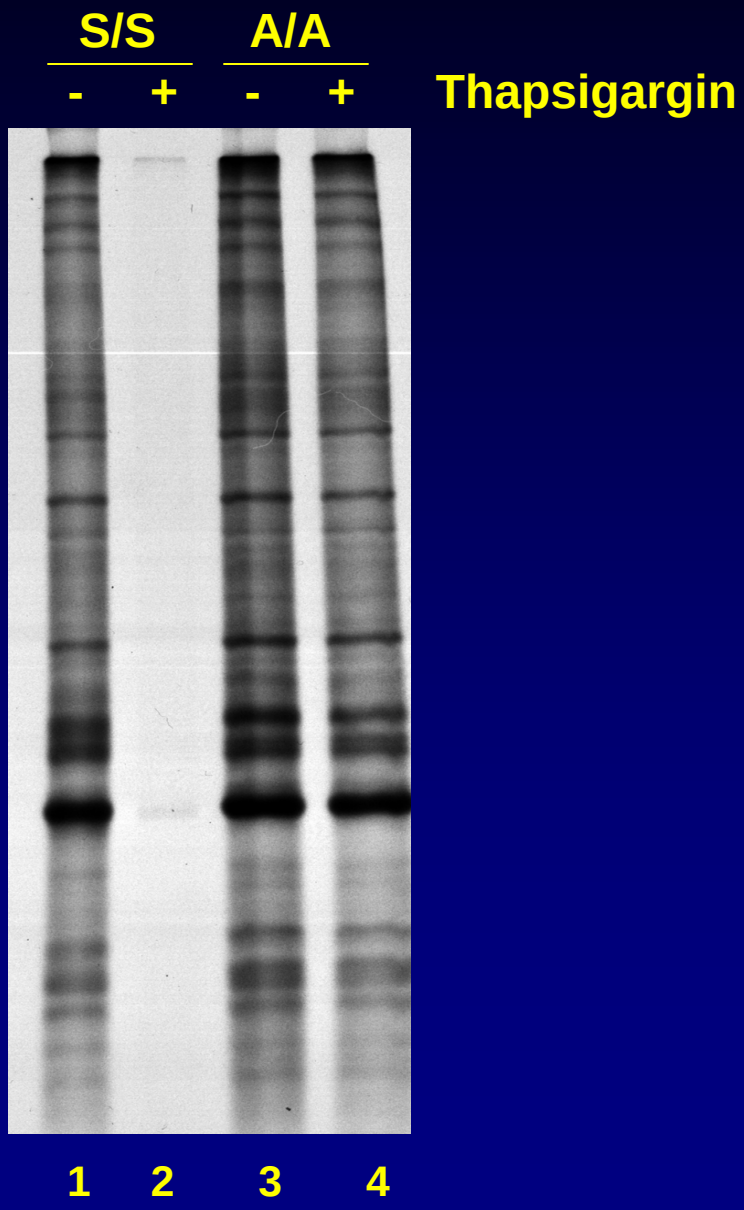
Translation Initiation Response to External Stimuli



Translation Initiation Response to External Stimuli



eIF2α phosphorylation is required for translation attenuation upon ER stress



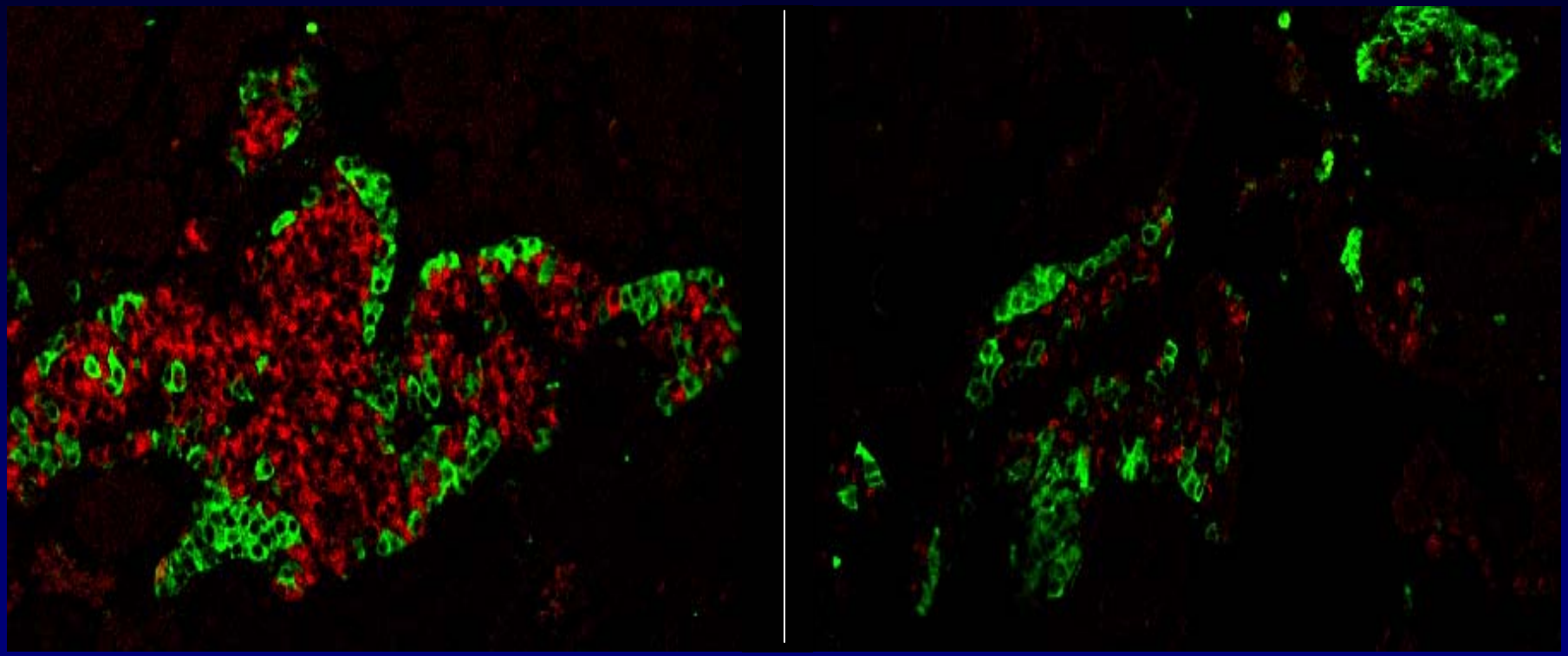
eIF2α A/A mice die within 24 hr after birth

Glucose Rescue of eIF2α A/A Mouse

A/A islets have reduced β-cells and insulin content

S/S

A/A



Insulin

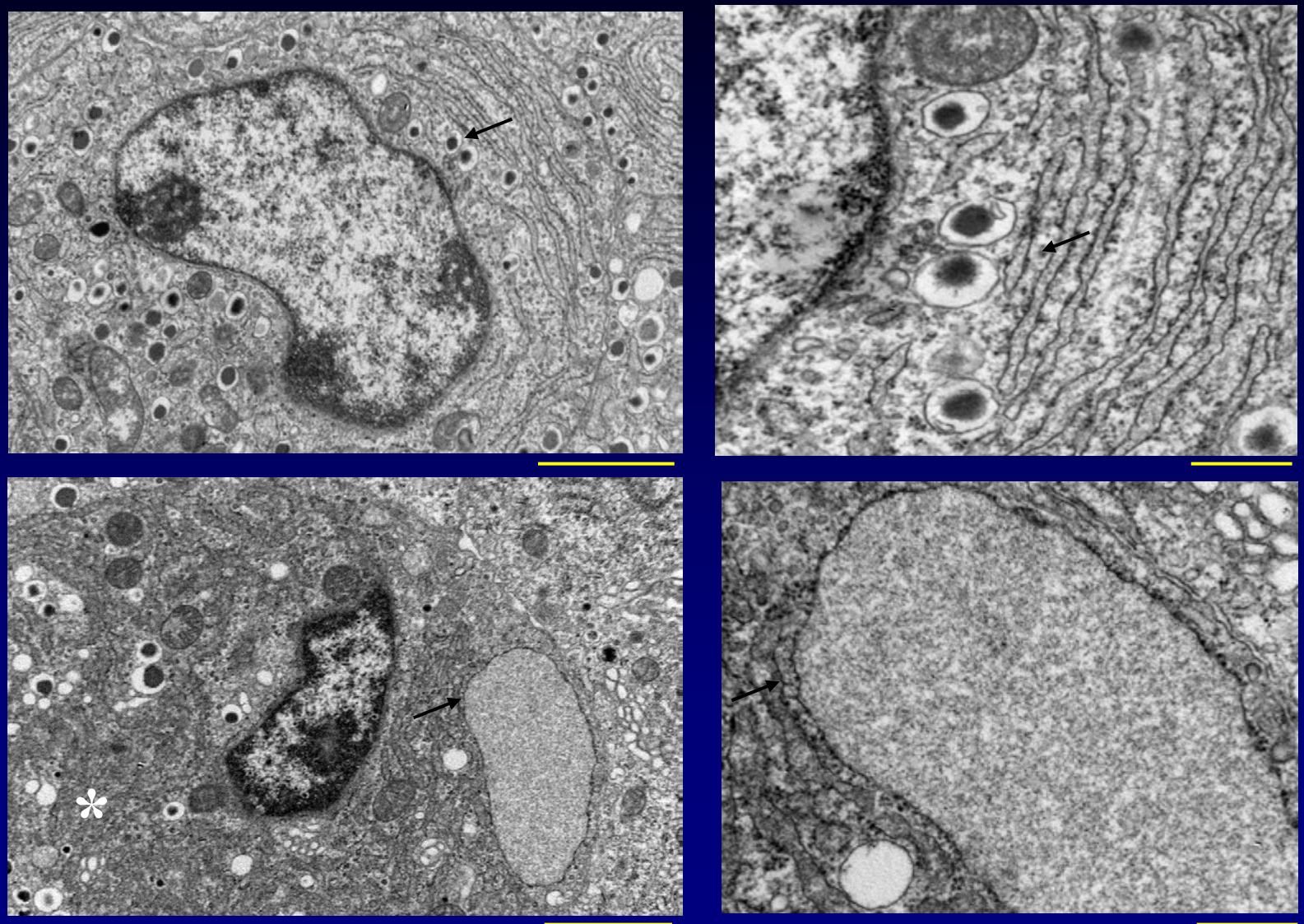
Glucagon

Scheuner et al. 2001 Mol. Cell 7: 1165

Translational control is required to prevent ER distension/stress in pancreatic β cells

S/S

A/A



2 μM

0.5 μM

Wolcott-Rallison Syndrome

- Rare autosomal recessive disorder
- Diabetes mellitus in early infancy
- Multiple epiphyseal dysplasia/osteoporosis
- Growth retardation
- Due to mutations in *PERK/PEK*

Delépine et al. 2000 Nat. Gen. 25: 406

Does the UPR play a role in the etiology of type II diabetes?

DIET **ENVIRONMENTAL STRESS** **GENETIC FACTORS**

+

Partial loss of UPR

↓ ?

Diabetes

Does the UPR play a role in the etiology of type II diabetes?

DIET **ENVIRONMENTAL STRESS** **GENETIC FACTORS**

High-Fat **or** ***db/db***

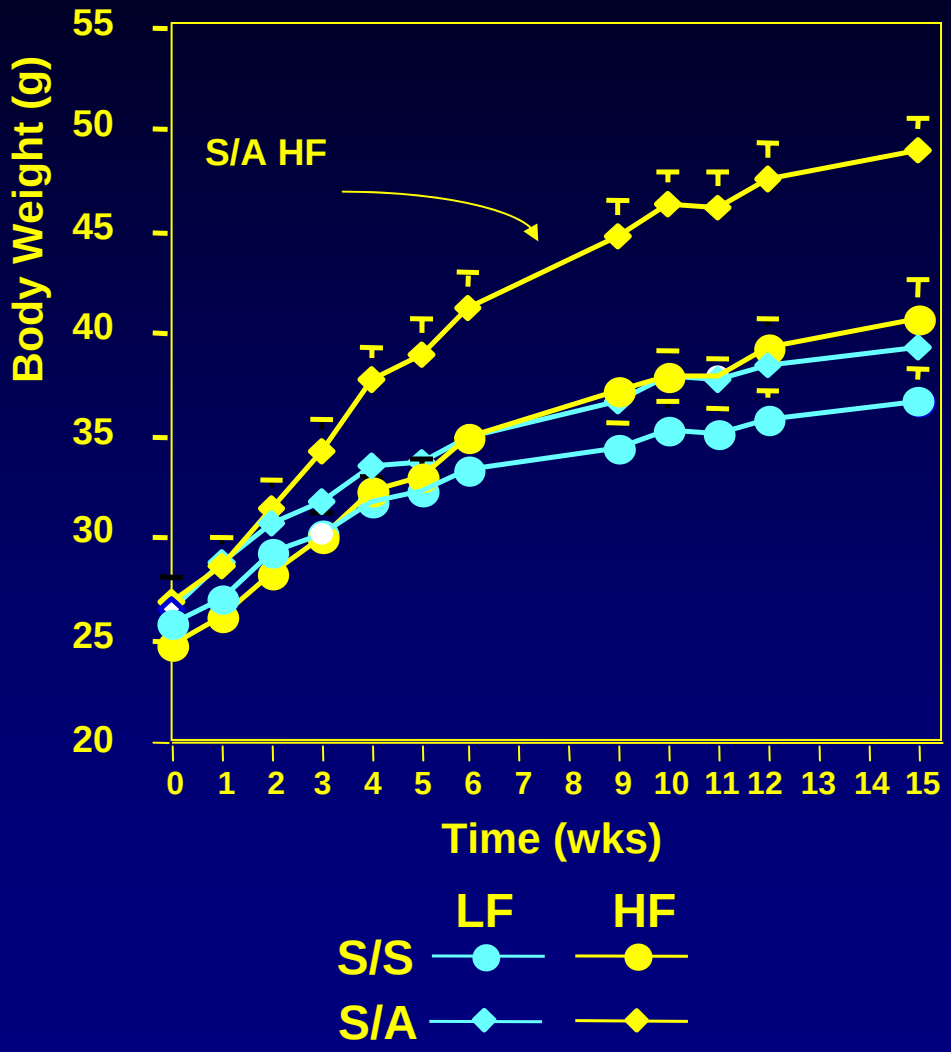
+

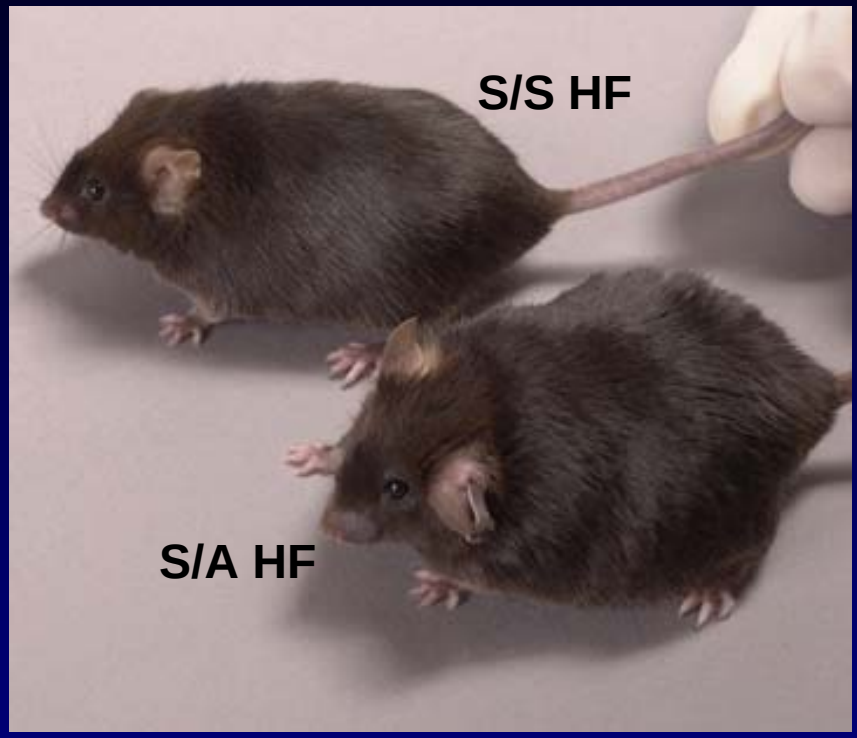
Partial loss of UPR (eIF2α S/A)

↓ **?**

Diabetes

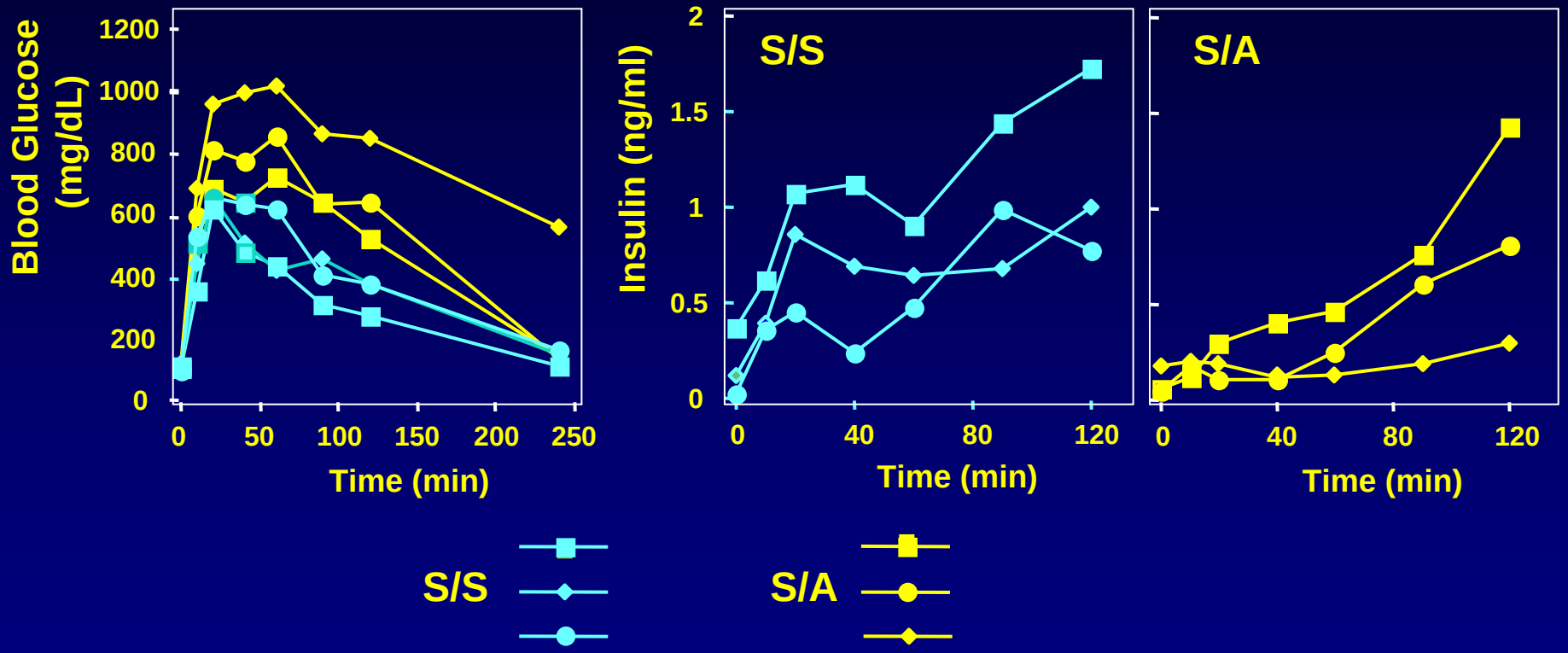
Heterozygous *eIF2α S/A* mice become obese upon high-fat diet





15 Wks

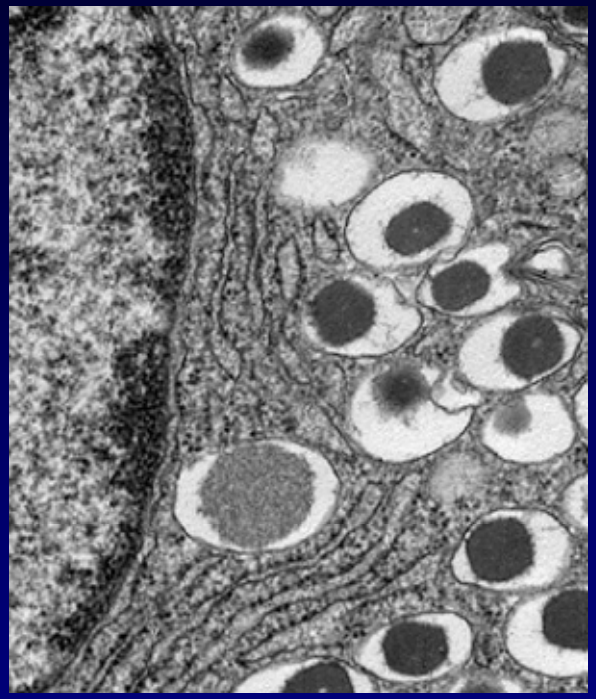
Heterozygous eIF2α S/A mice are glucose intolerant with impaired insulin secretion *in vivo*



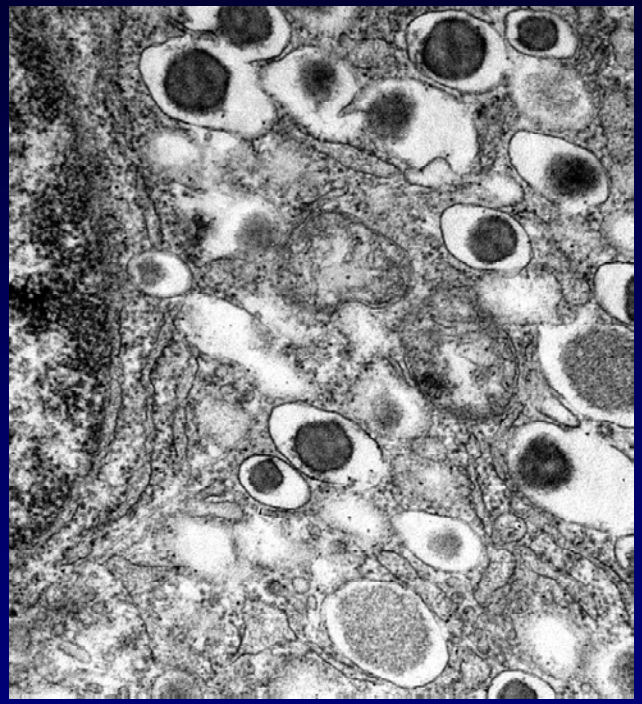
Scheuner et al. 2005 Nat. Med. 11: 757

High-fat diet induces ER stress in beta cells of eIF2α S/A mice

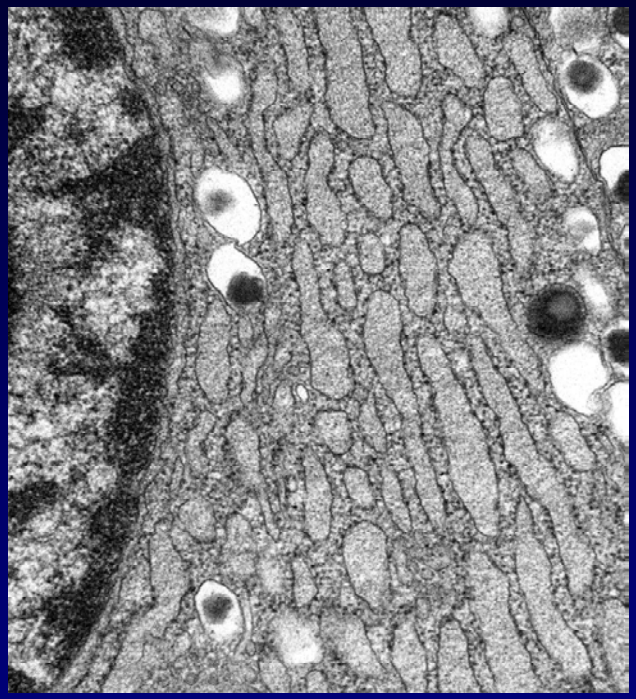
LF S/A



HF S/S

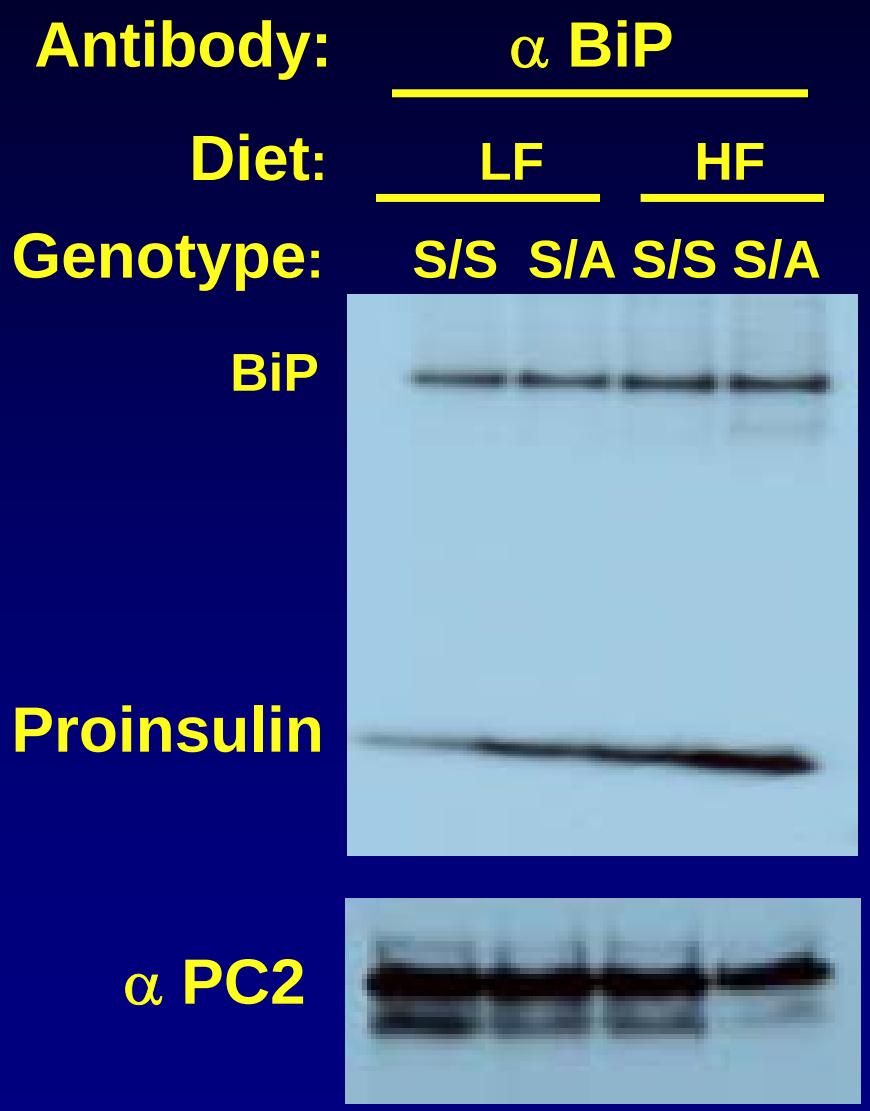


HF S/A



500 nm

Increased Association of Proinsulin with BiP in HF-Fed S/A Mice



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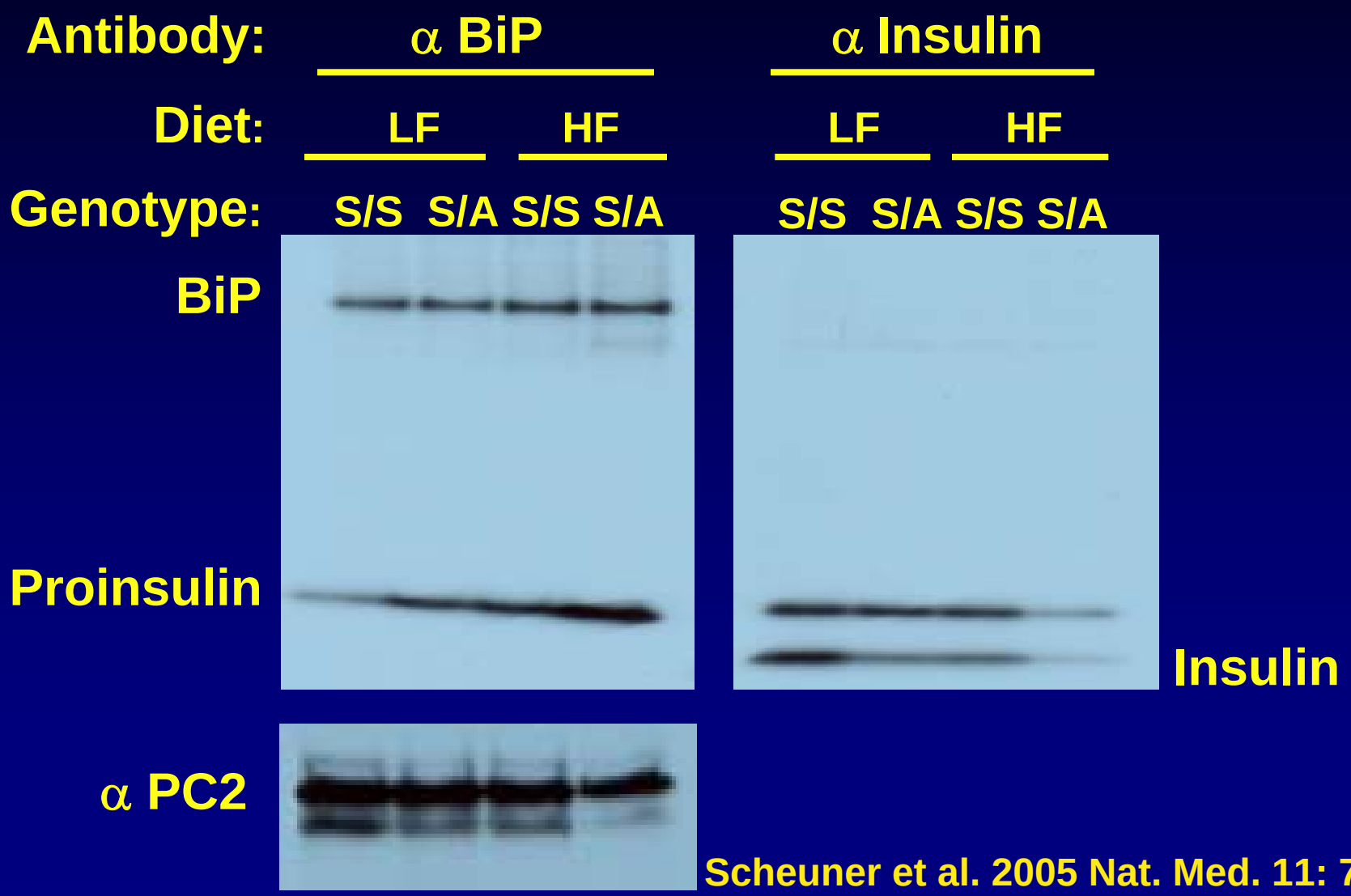


Scheuner et al. 2005 Nat. Med. 11: 757

The UPR is essential for beta cell compensation

Fatty acid

↓

Insulin resistance

↓

Insulin secretion / transcription / translation ↑

↓

Increased ER load

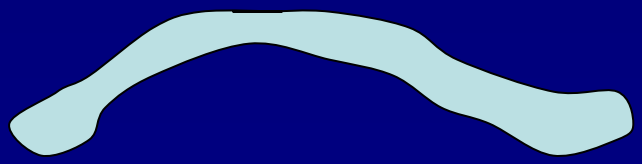
↓

UPR activation

↓

ER integrity preserved

(Beta cell compensation)



The UPR is essential for beta cell compensation

Fatty acid

↓

Insulin resistance

↓

Insulin secretion / transcription / translation ↑

↓

Increased ER load + eIF2α S/A (Partial loss of UPR)

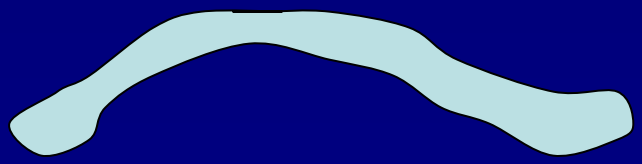
↓

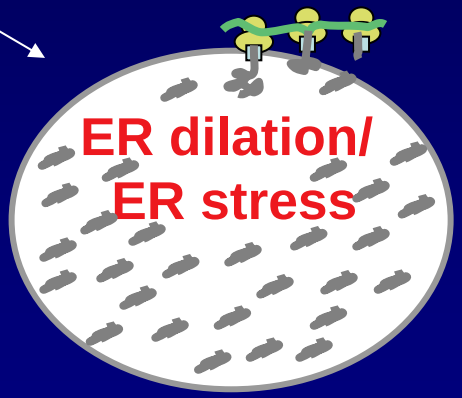
UPR activation

↓

ER integrity preserved

(Beta cell compensation)





Beta cell decompensation

Glucose intolerance

The UPR is essential for beta cell compensation

Fatty acid

↓

Insulin resistance

↓

Insulin secretion / transcription / translation ↑

↓

Increased ER load + eIF2α S/A (Partial loss of UPR)

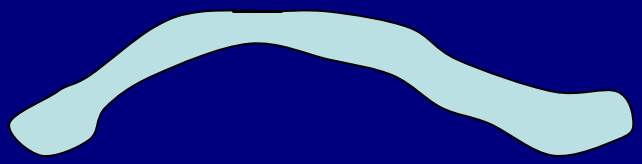
↓

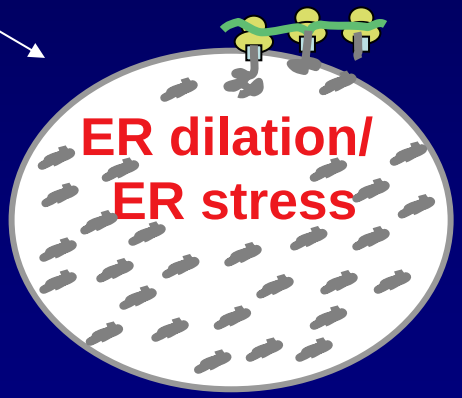
UPR activation

↓

ER integrity preserved

(Beta cell compensation)





CHOP

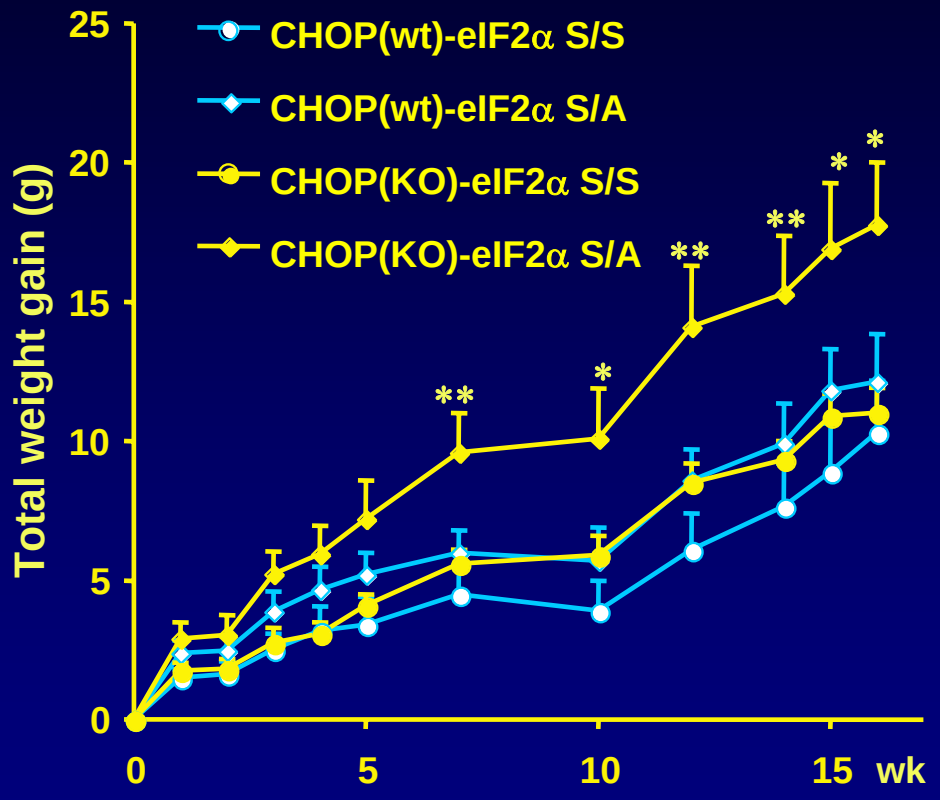
↓

?

Apoptosis

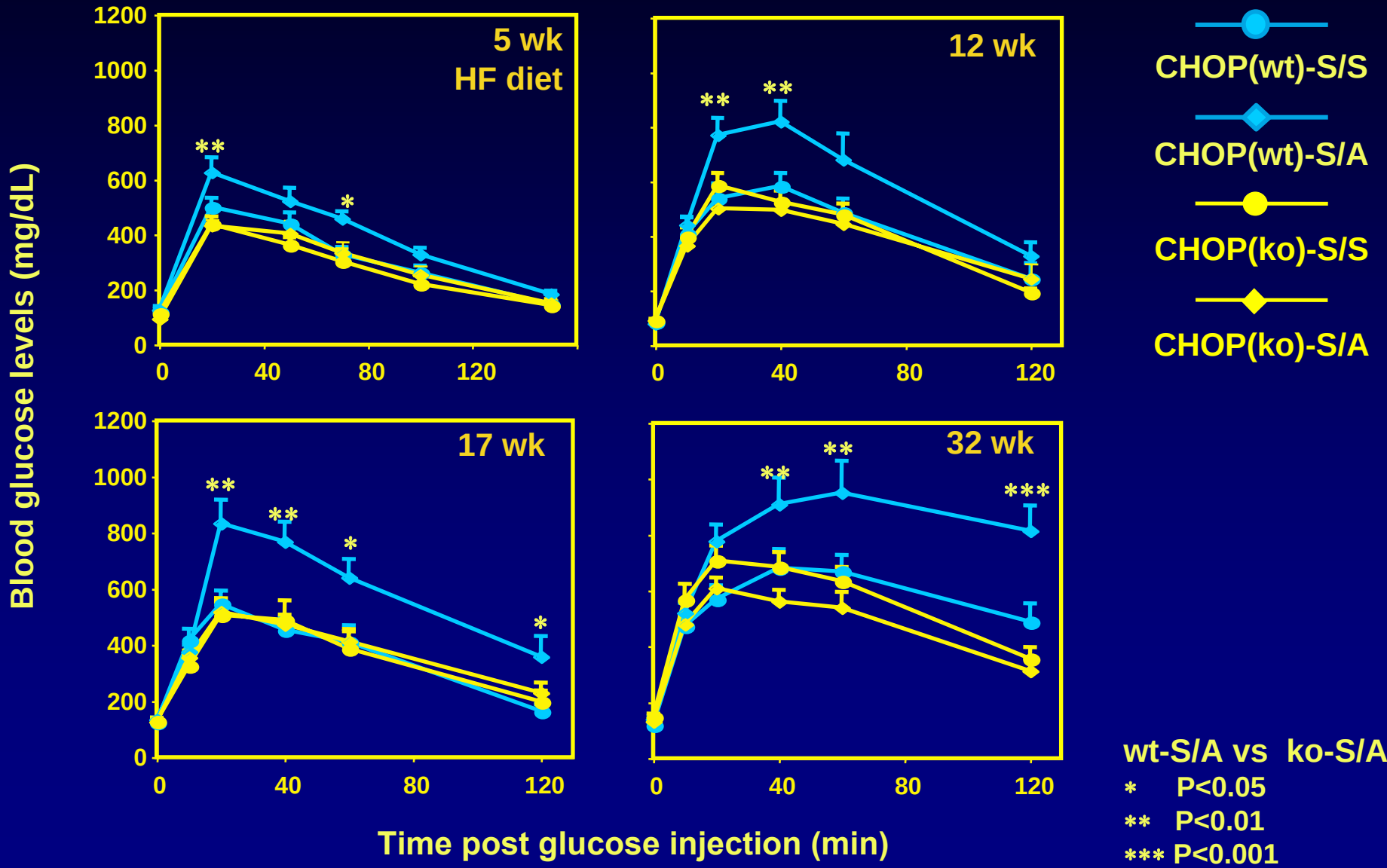
Beta cell
decompensation
Glucose intolerance

CHOP deletion increases HF-induced obesity in eIF2$\alpha^{S/A}$ mice

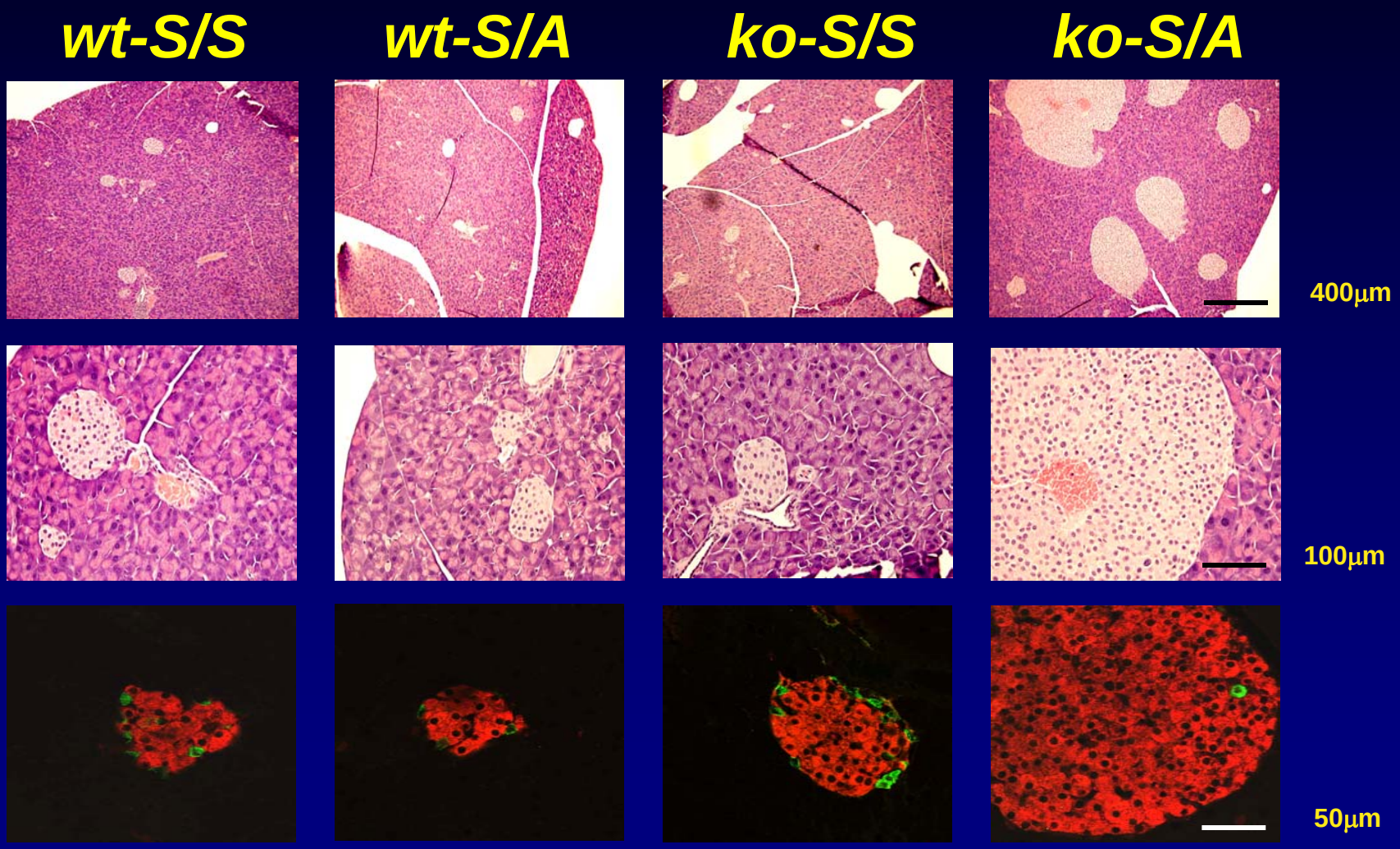


wt-S/A vs ko-S/A
* P<0.05; ** P<0.01

CHOP deletion prevents HF diet-induced glucose intolerance in eIF2$\alpha^{S/A}$ mice

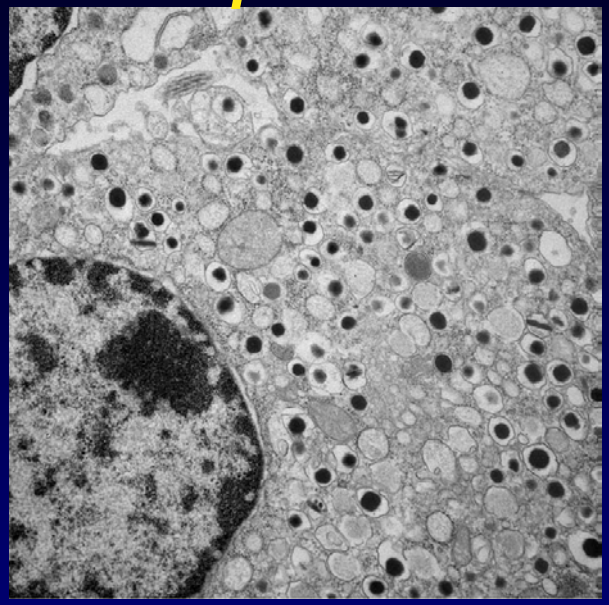


CHOP deletion promotes islet hyperplasia in high fat *eIF2α*$^{S/A}$ mice

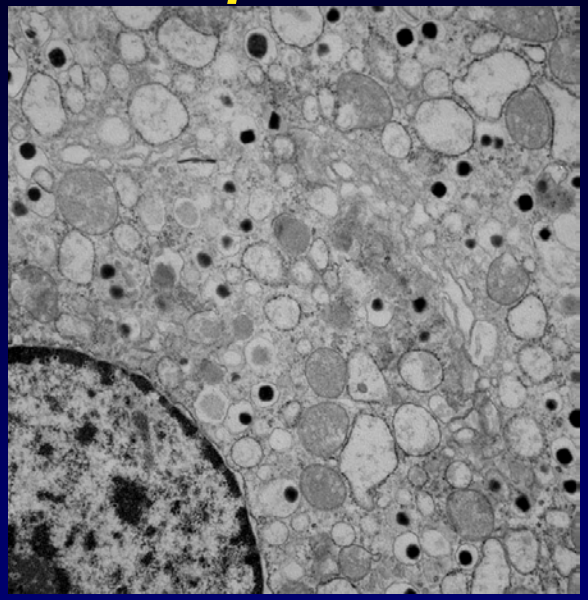


CHOP deletion restores insulin granules and secretion in HF-fed *eIF2*$\alpha^{S/A}$ mice

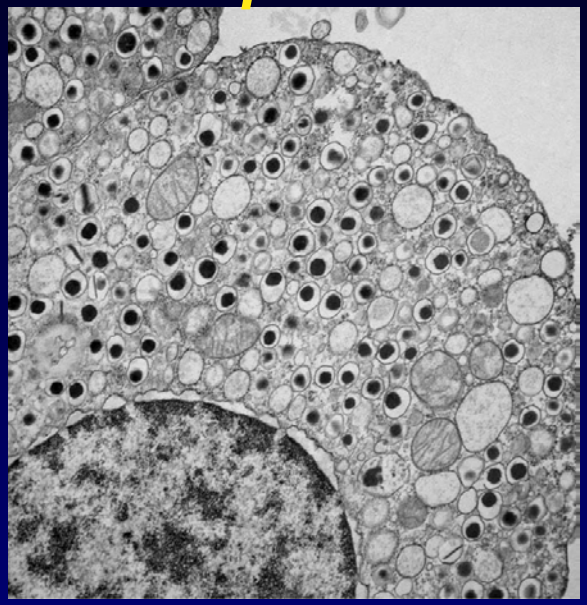
Chop+/+ S/S



Chop+/+ S/A



Chop-/- S/A



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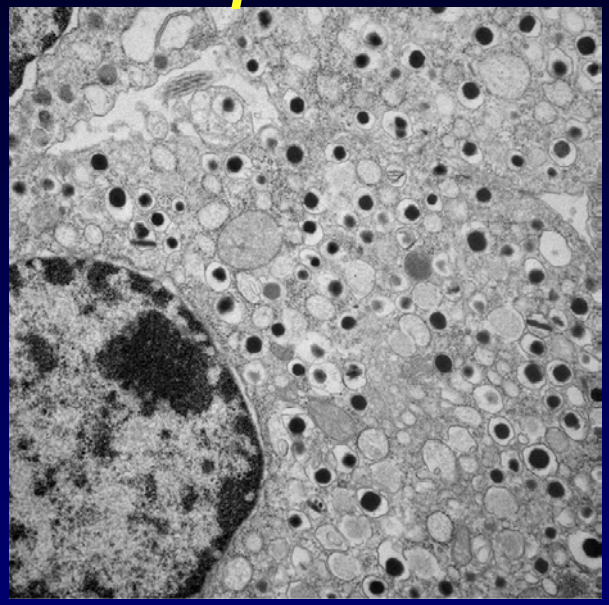
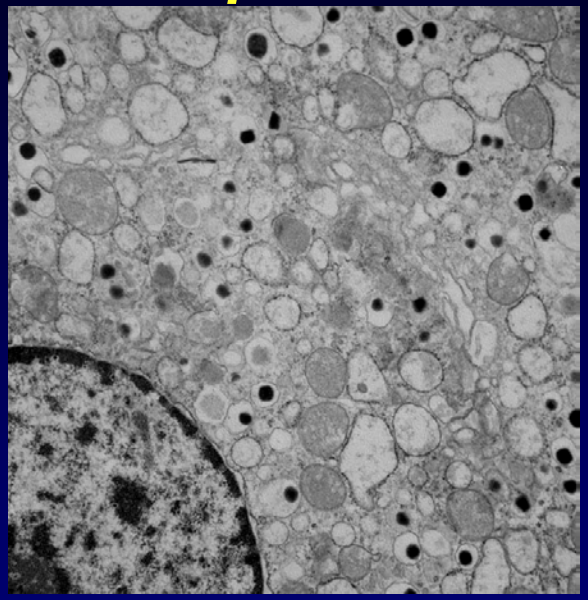
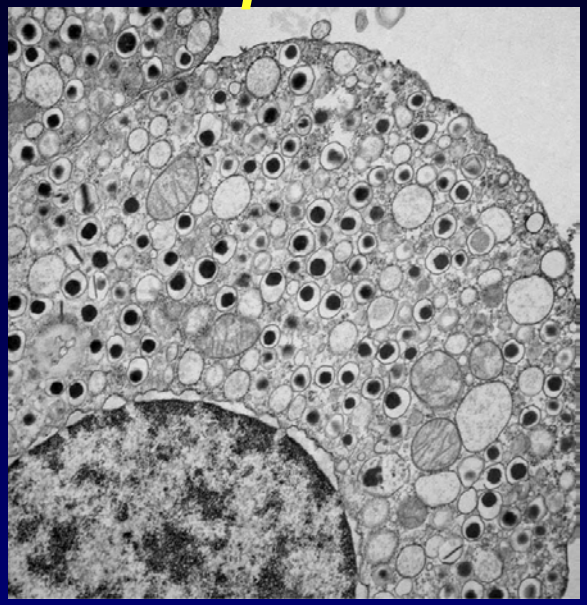


Chop+/+ S/A



Chop-/- S/A



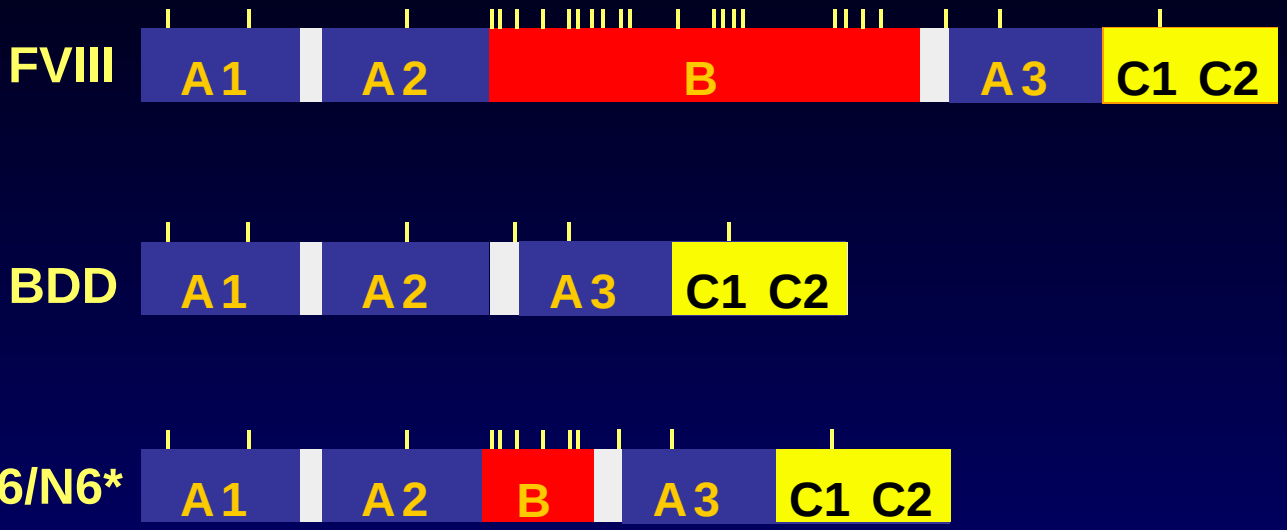
Does expression of a malfolded protein induce the UPR, oxidative stress, and apoptosis *in vivo*?

Does expression of a malfolded protein induce the UPR, oxidative stress, and apoptosis *in vivo*?

Clotting factor VIII inefficiently secreted due to misfolding.

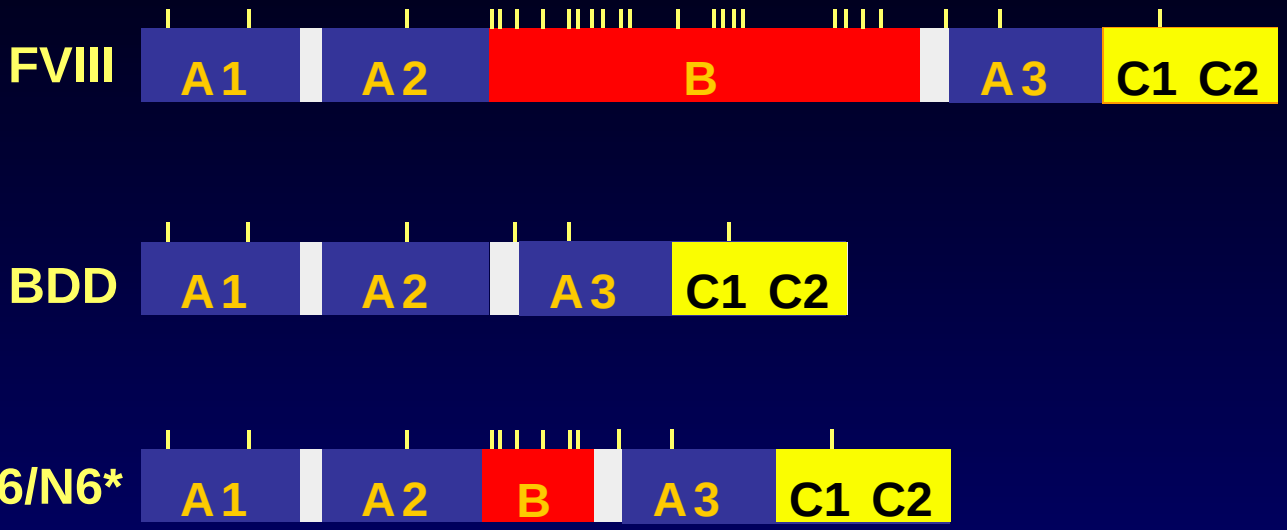
Does factor VIII expression activate the UPR and apoptosis *in vivo*?

FVIII expression after hydrodynamic DNA injection



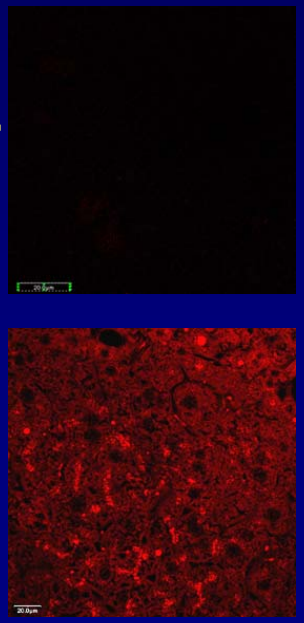
* Miao et al., 2004 Blood 103:3412

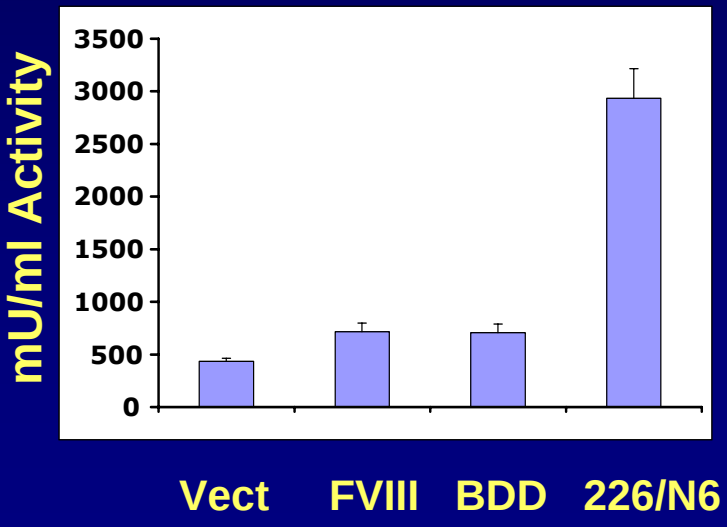
FVIII expression after hydrodynamic DNA injection



Vector

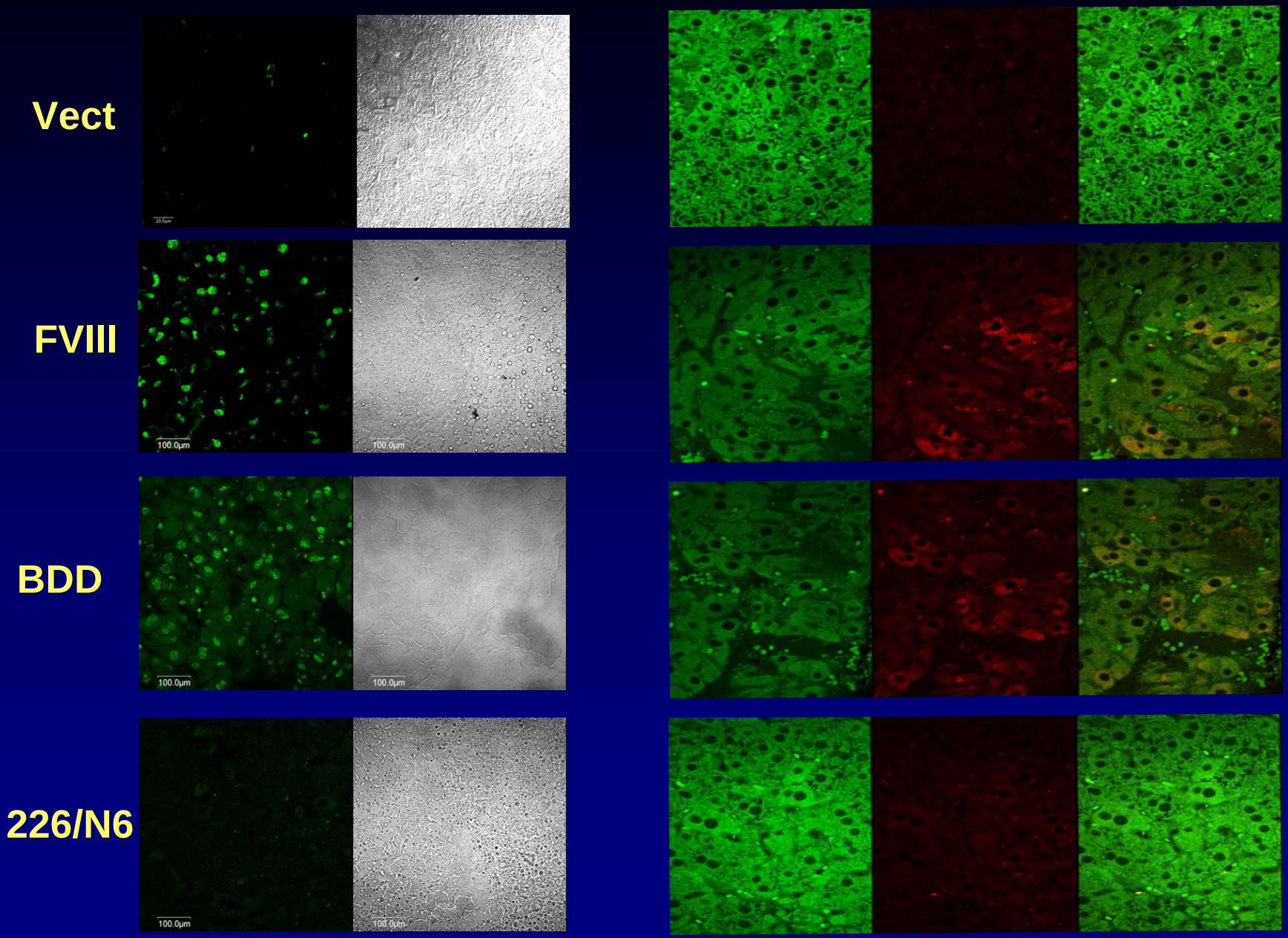
FVIII





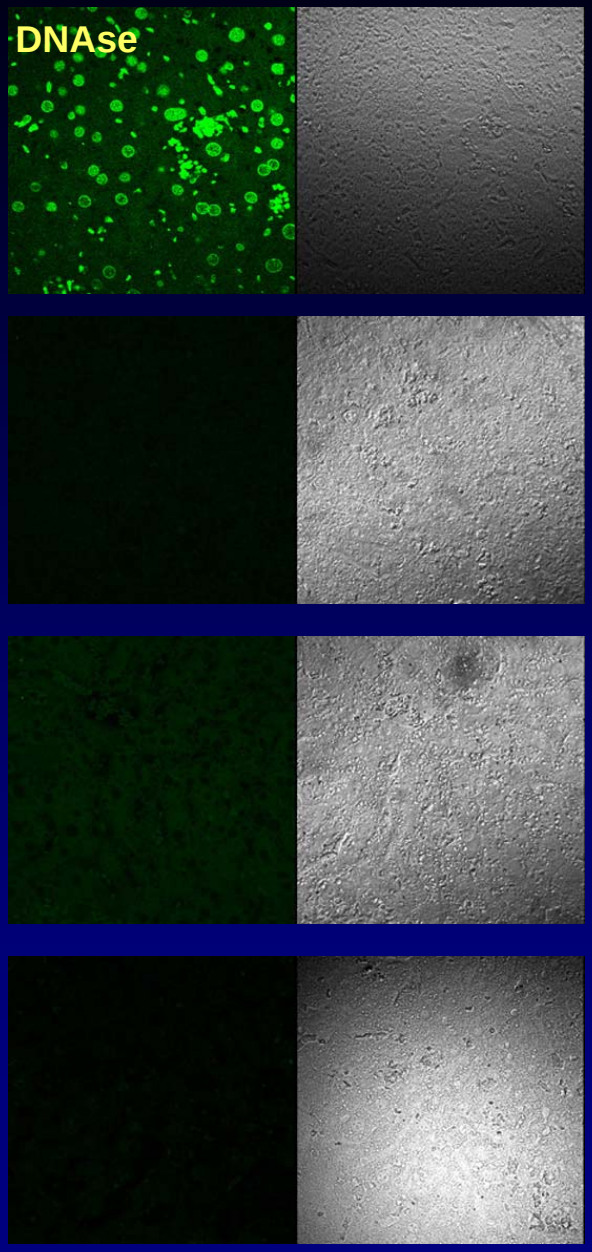
* Miao et al., 2004 Blood 103:3412

FVIII Expression Induces Apoptosis in Mouse Liver

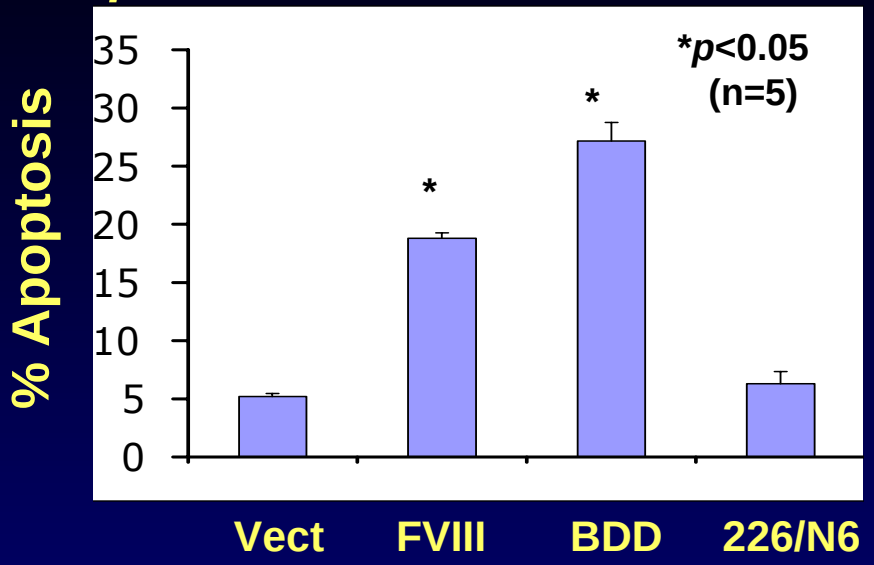


CHOP is required for FVIII induced apoptosis

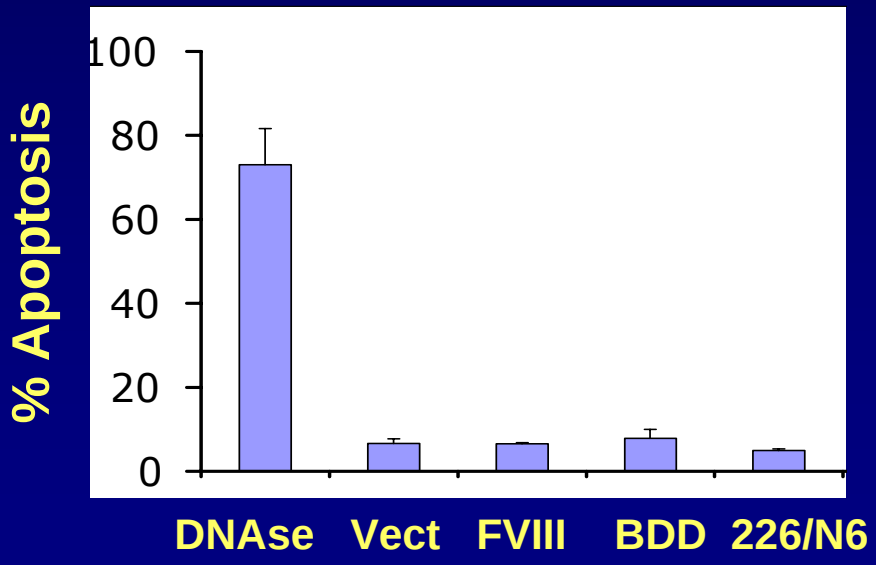
Chop$^{-/-}$



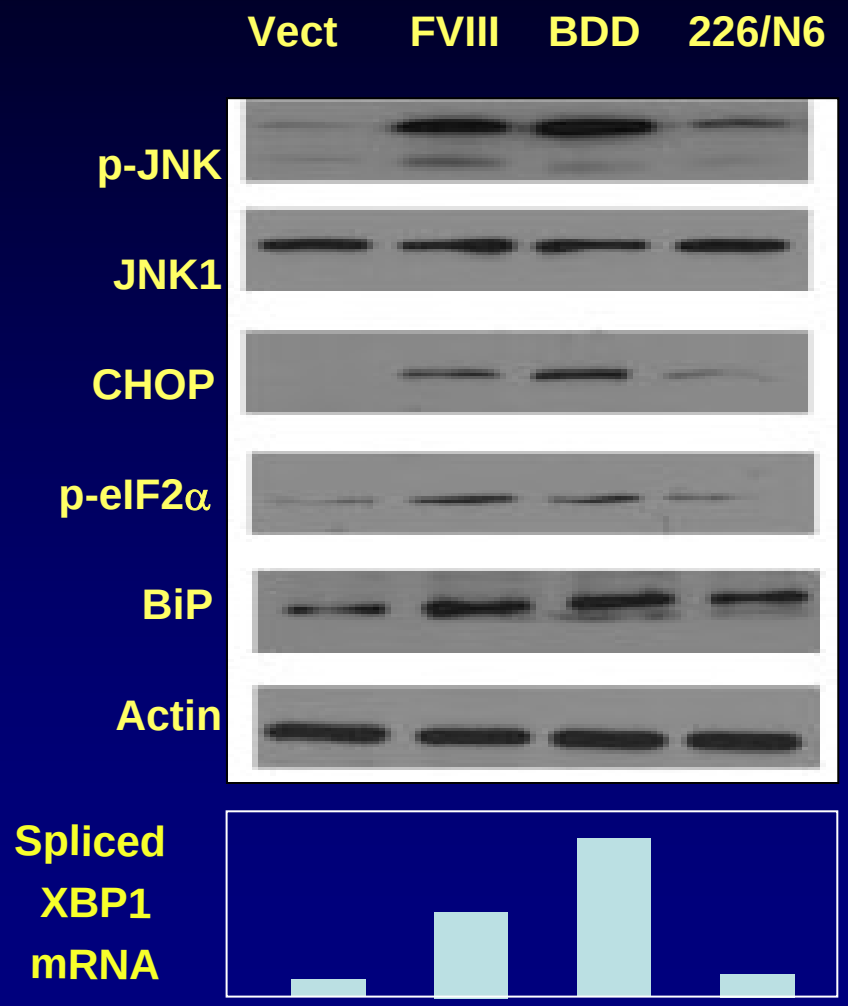
Chop$^{+/+}$



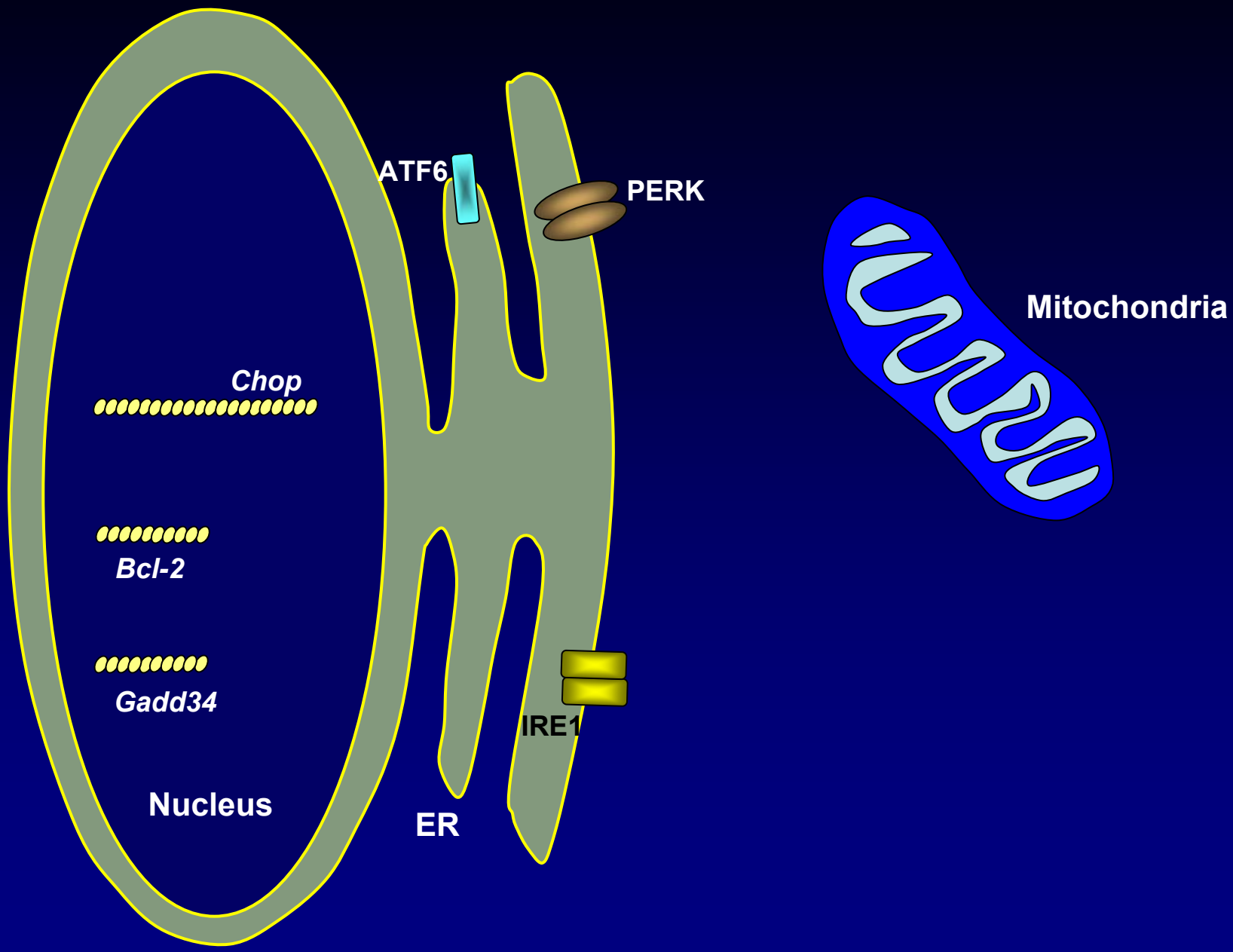
Chop$^{-/-}$



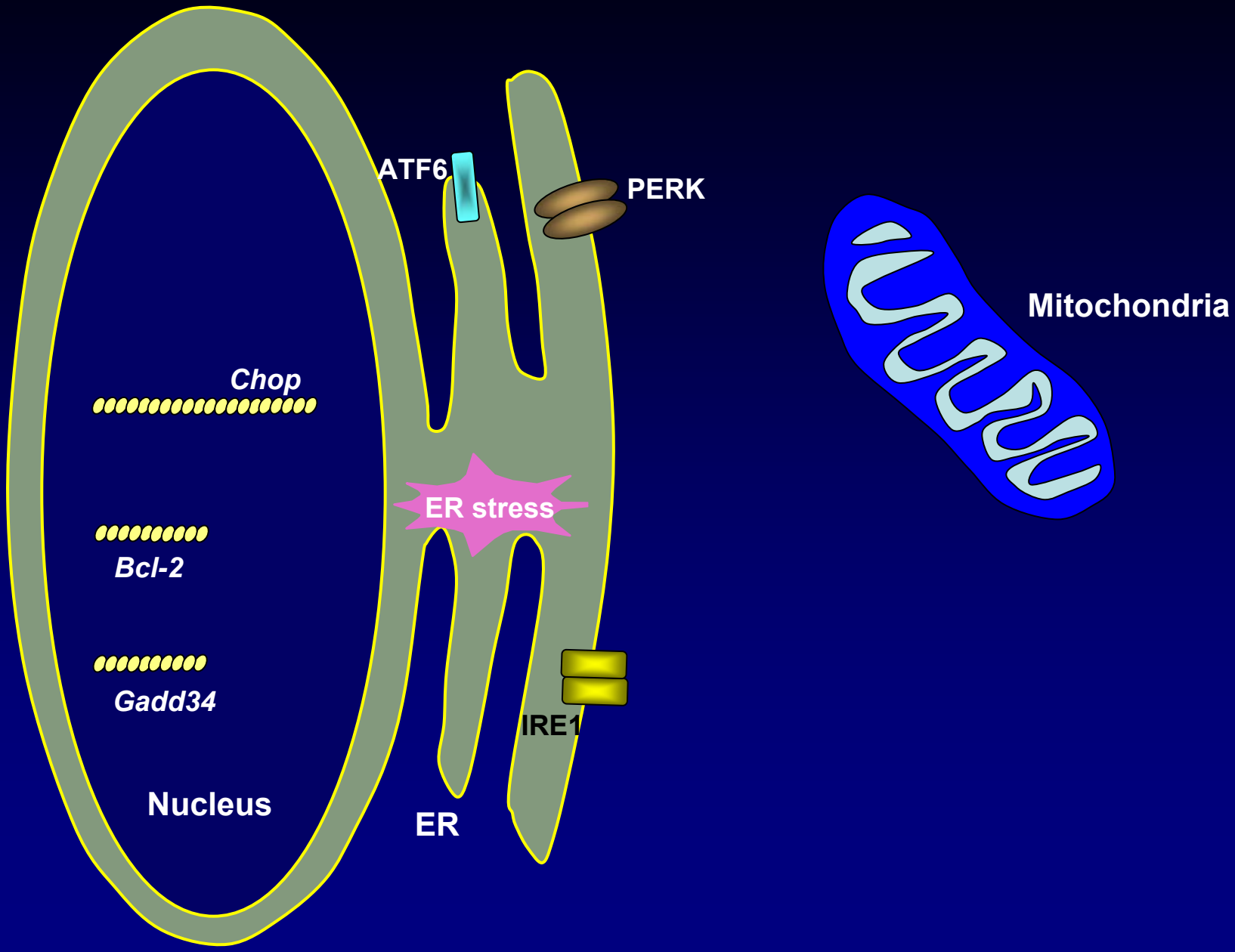
FVIII Expression Induces ER Stress in Liver



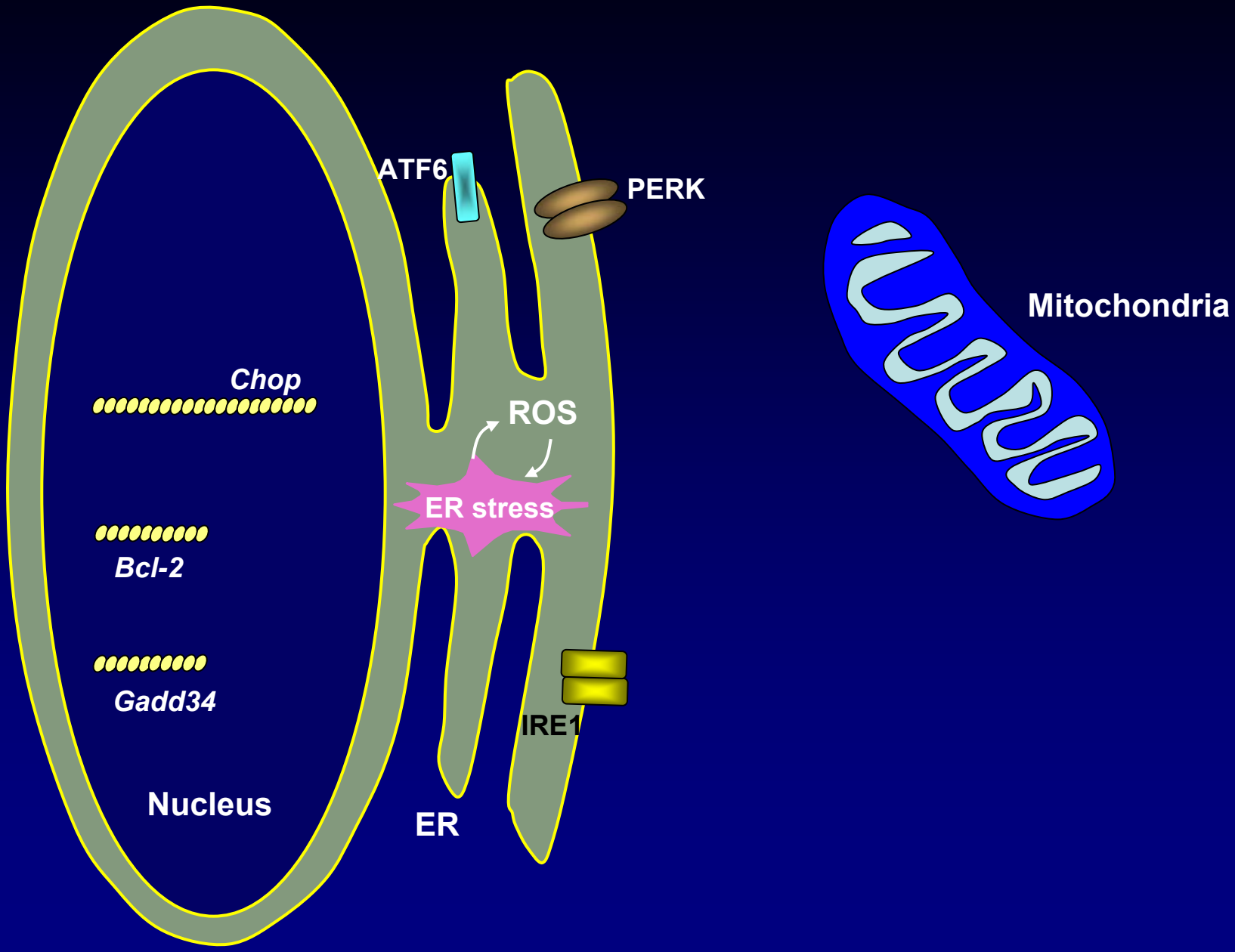
ER Stress-Induced Oxidative Stress



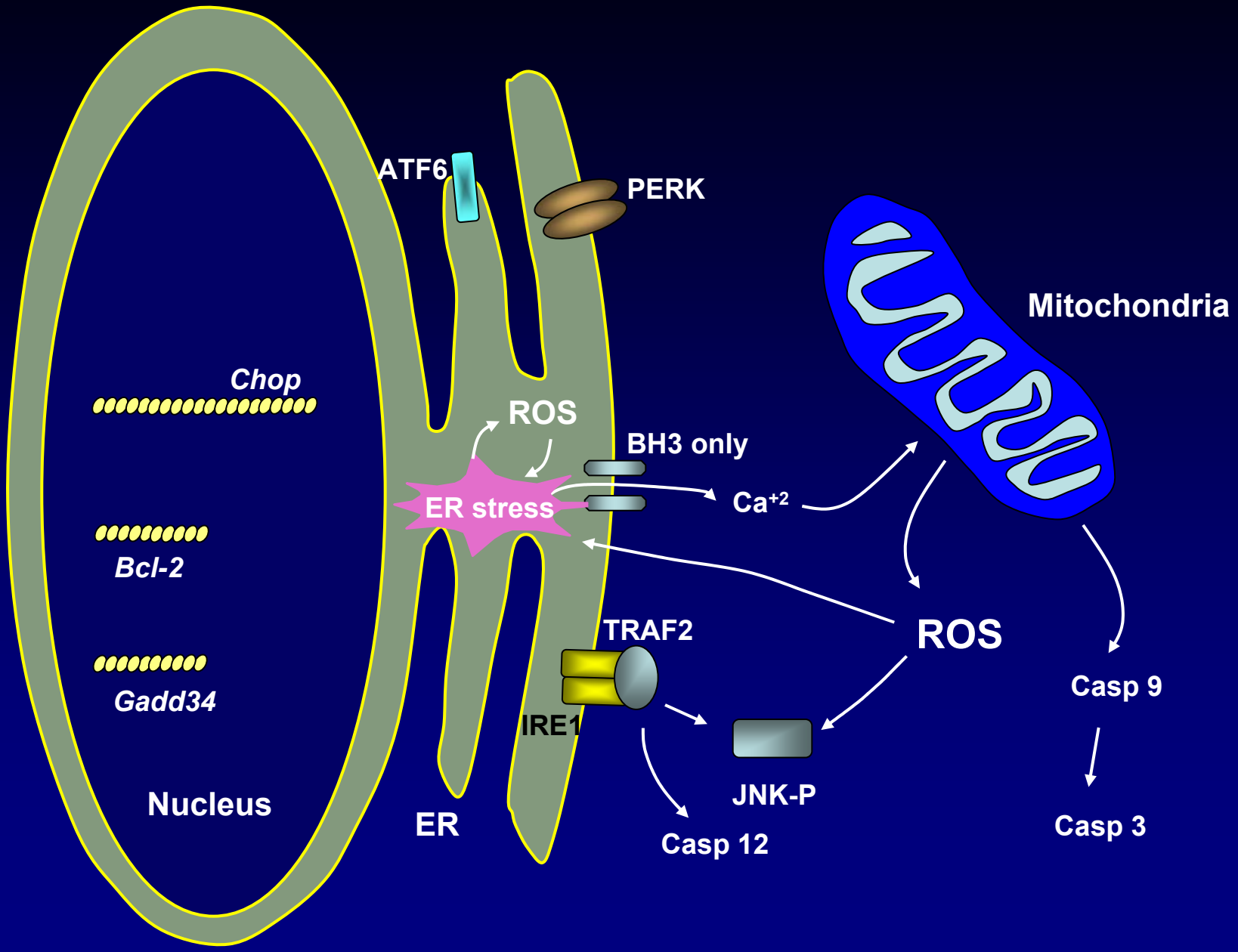
ER Stress-Induced Oxidative Stress



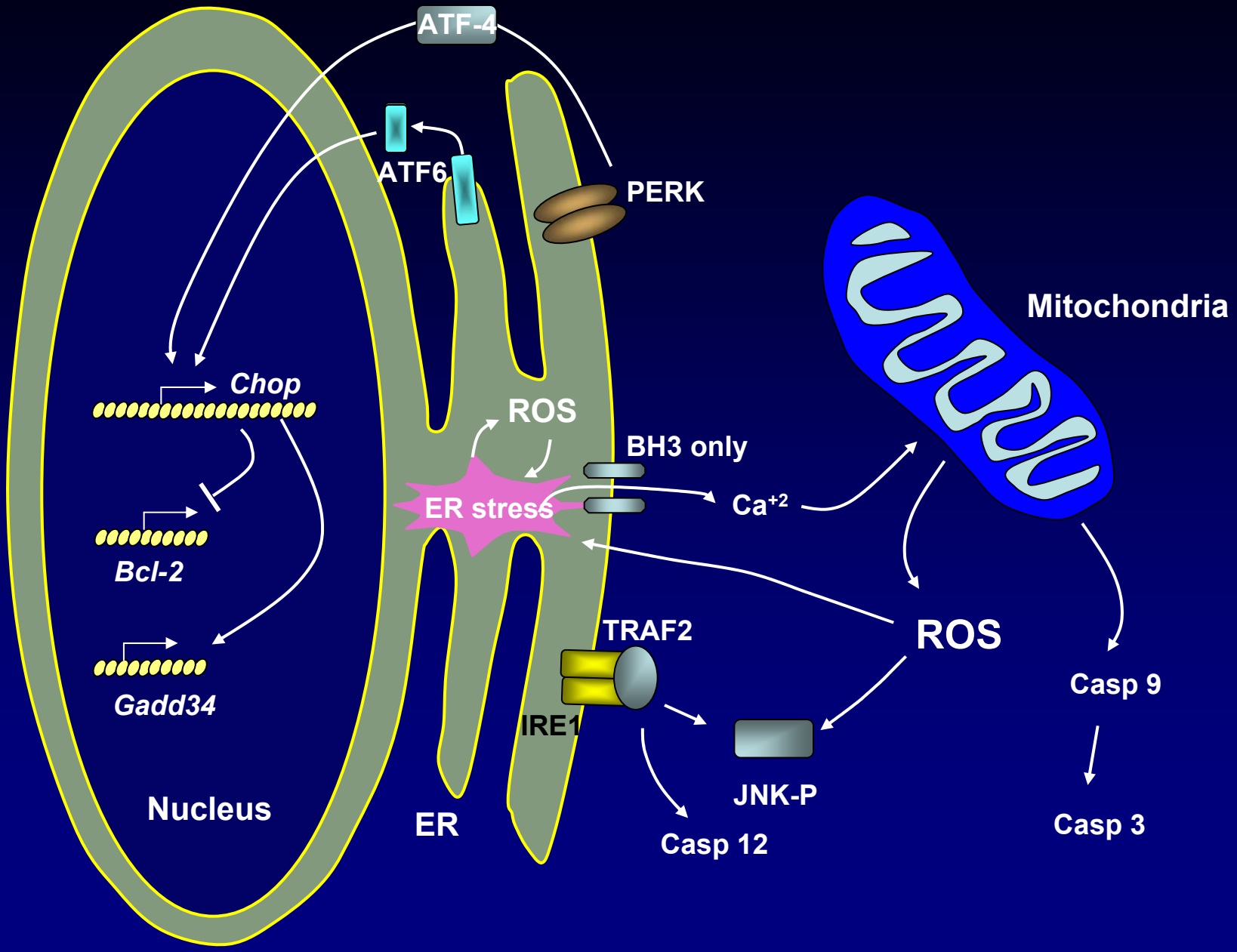
ER Stress-Induced Oxidative Stress



ER Stress-Induced Apoptosis



ER Stress-Induced Apoptosis



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