BSc in Pharmacology – Nov 2011

Airway mucus production and pharmacotherapy of overproduction

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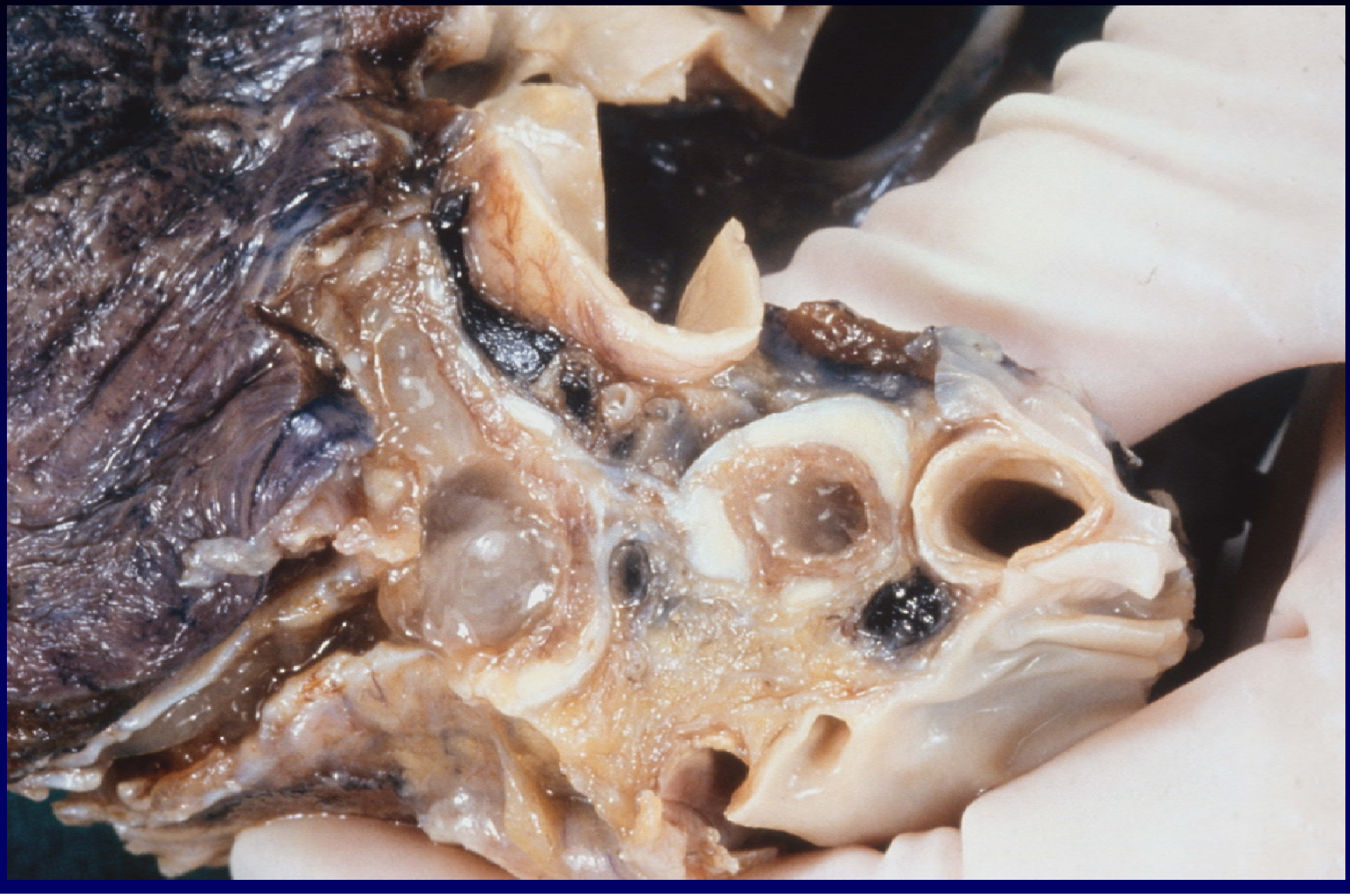
Learning objectives

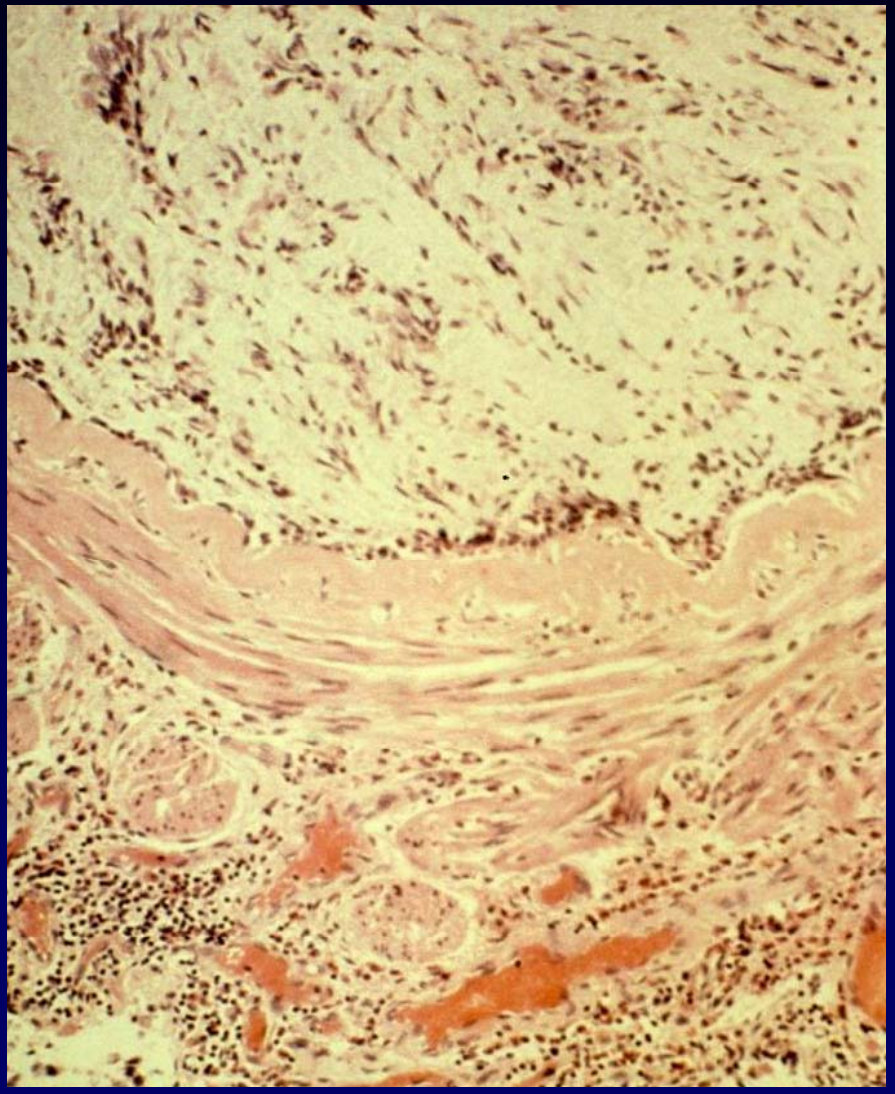
After the lecture (and appropriate revision) you will be able to:

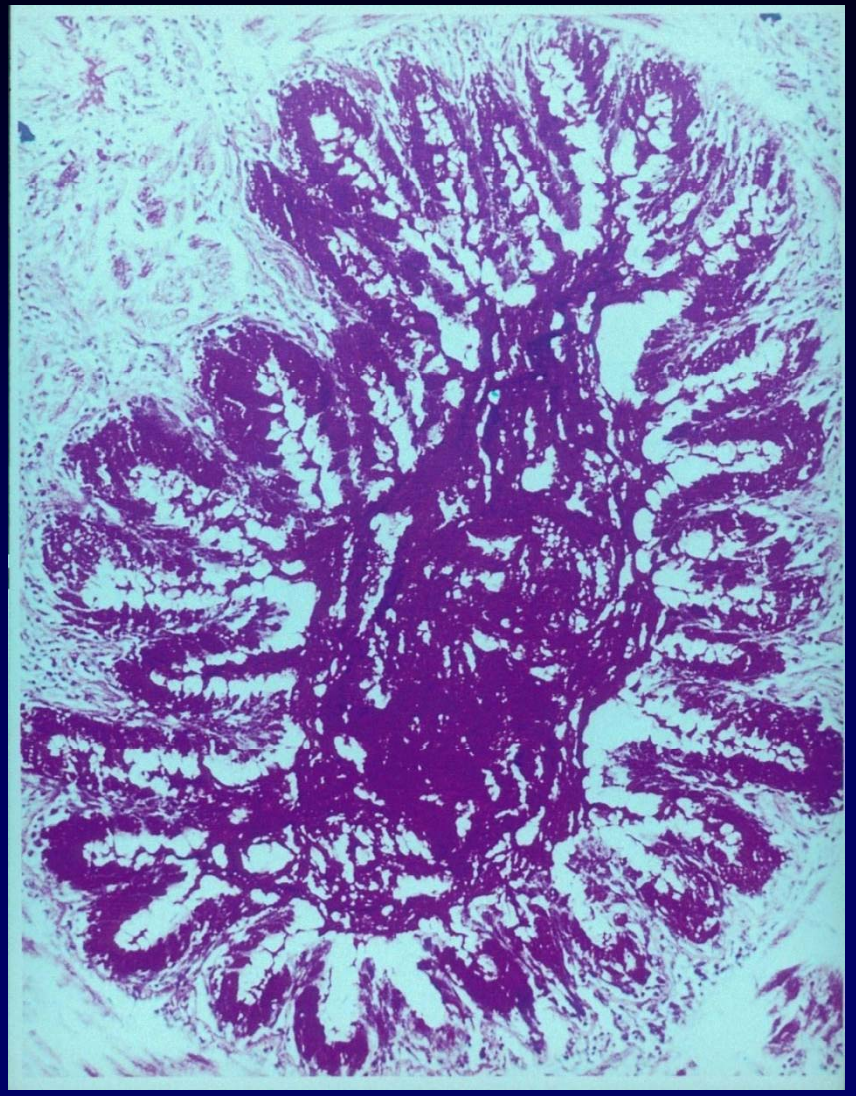
- Describe the physiology of airway mucus secretion (exocytosis) and how this might be inhibited
- Describe the pathophysiology and clinical impact of airway mucus hypersecretion in asthma and chronic obstructive pulmonary disease (COPD)
- Understand how potential treatments for airway mucus hypersecretion can be elucidated rationally by study of the inflammatory 'cascade'
- Describe a variety of potential treatments for airway mucus hyper-secretion (including epidermal growth factor receptor antagonists and different 'natural' products)

Airway obstruction by mucus in asthma

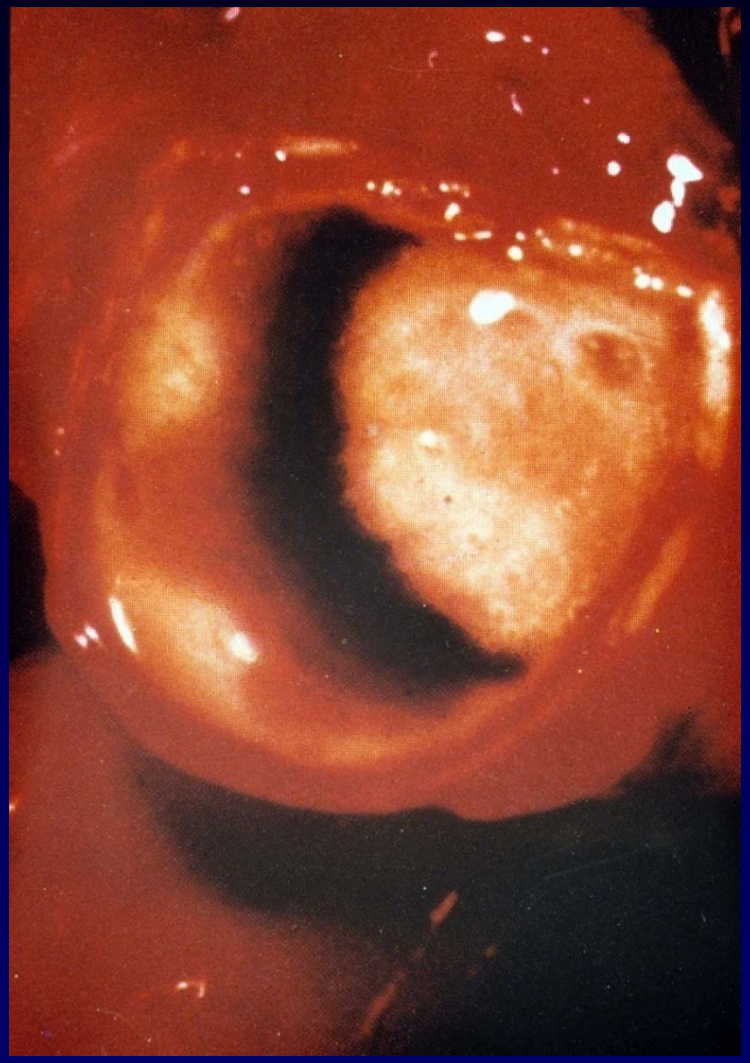


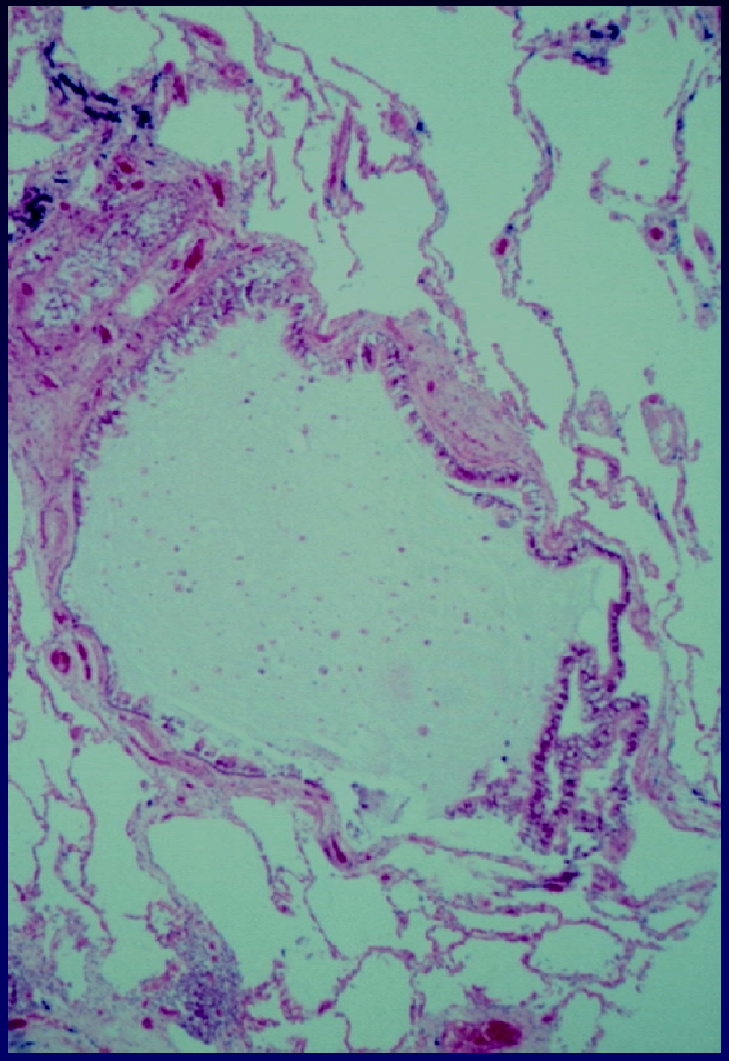
Asthma histopathology





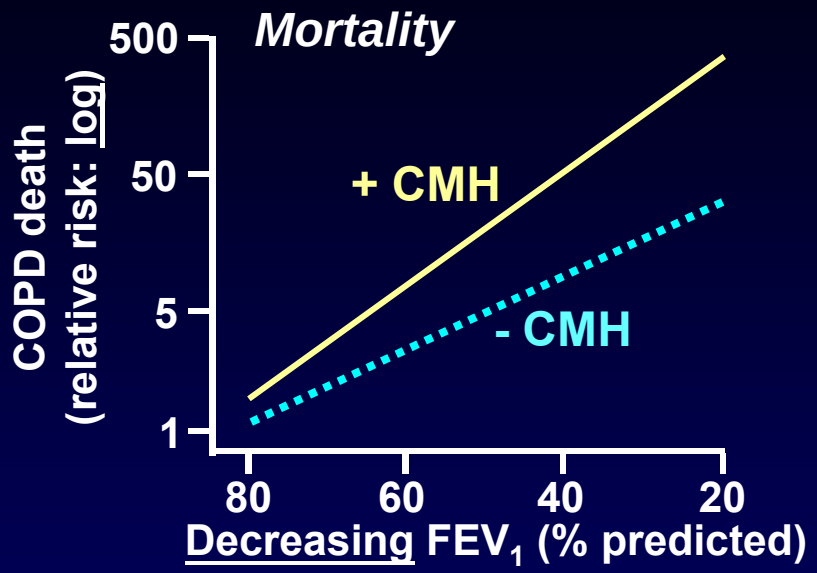
Airway obstruction by mucus in chronic obstructive pulmonary disease (COPD)

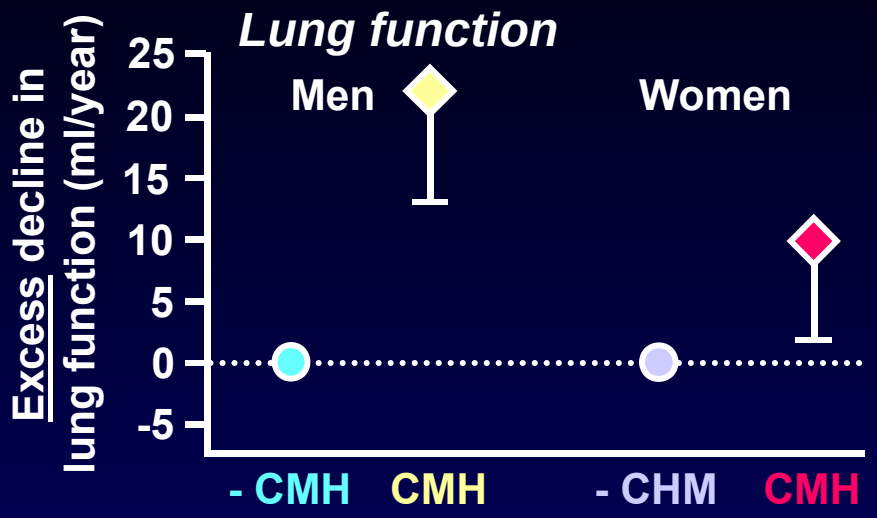


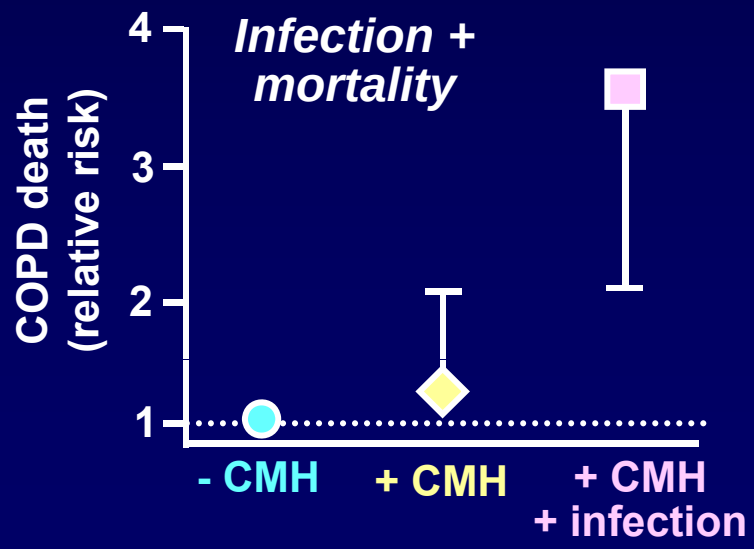


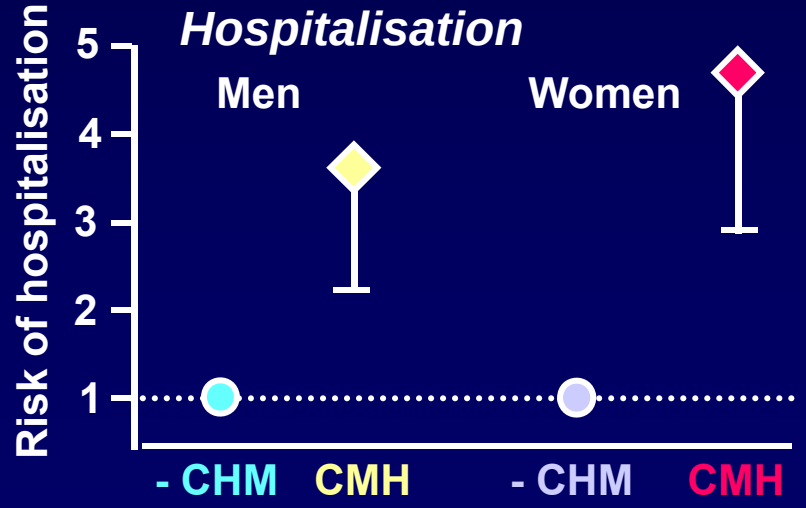
Clinical impact of airway mucus hypersecretion in COPD

(Vestbo *et al*, Copenhagen Heart Study: CMH = chronic mucus hypersecretion)

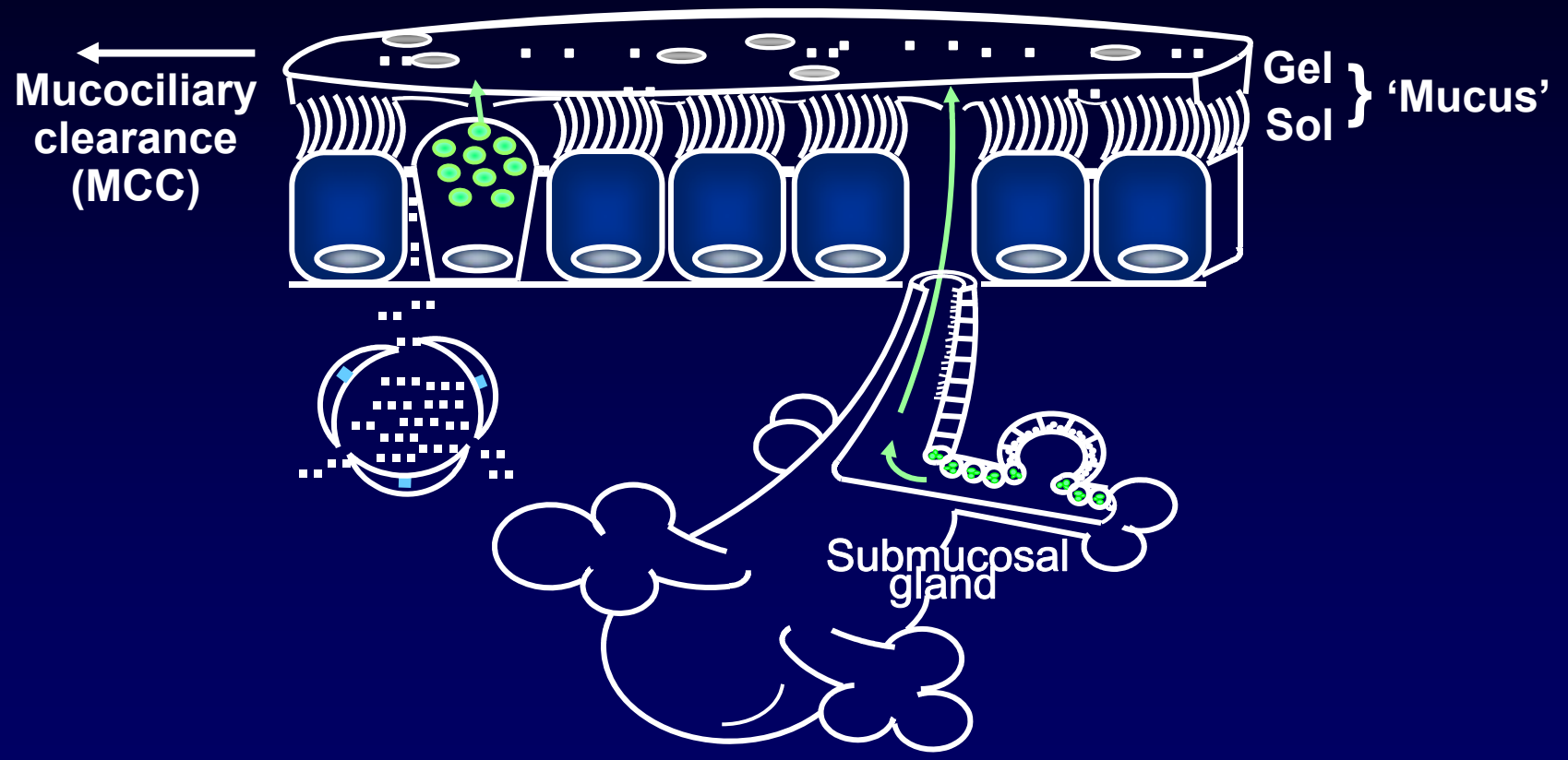




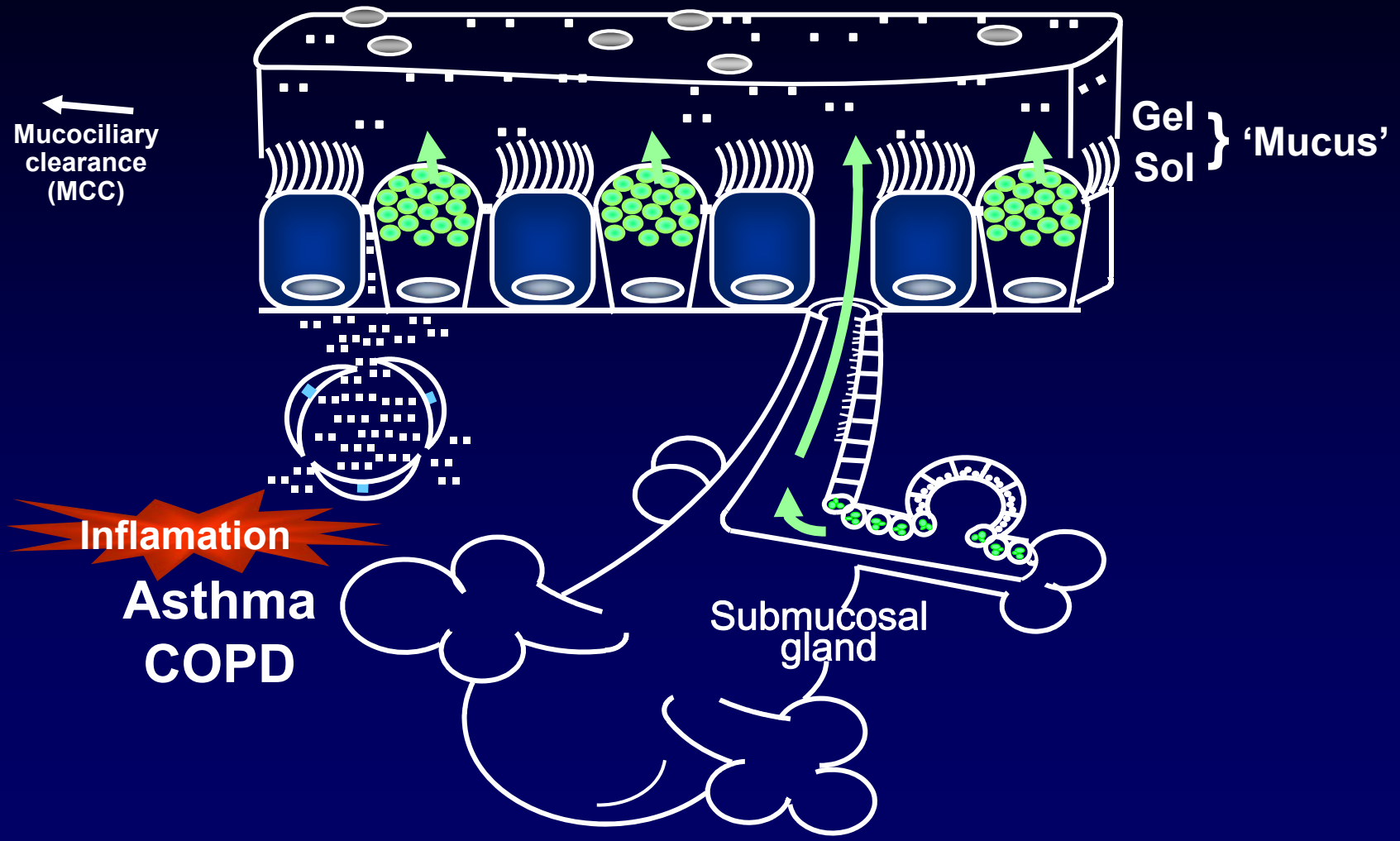




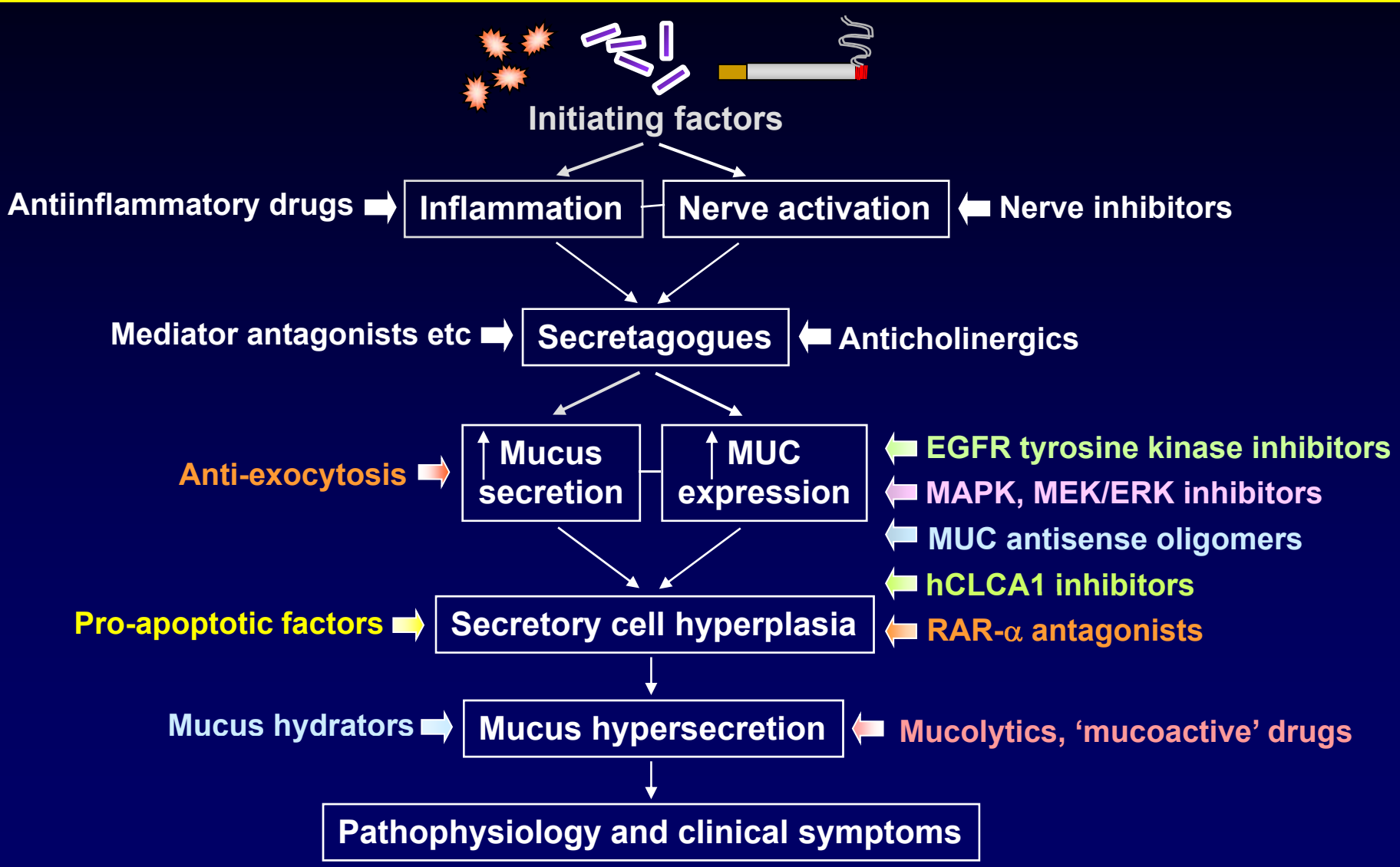
Airway mucus and hypersecretion



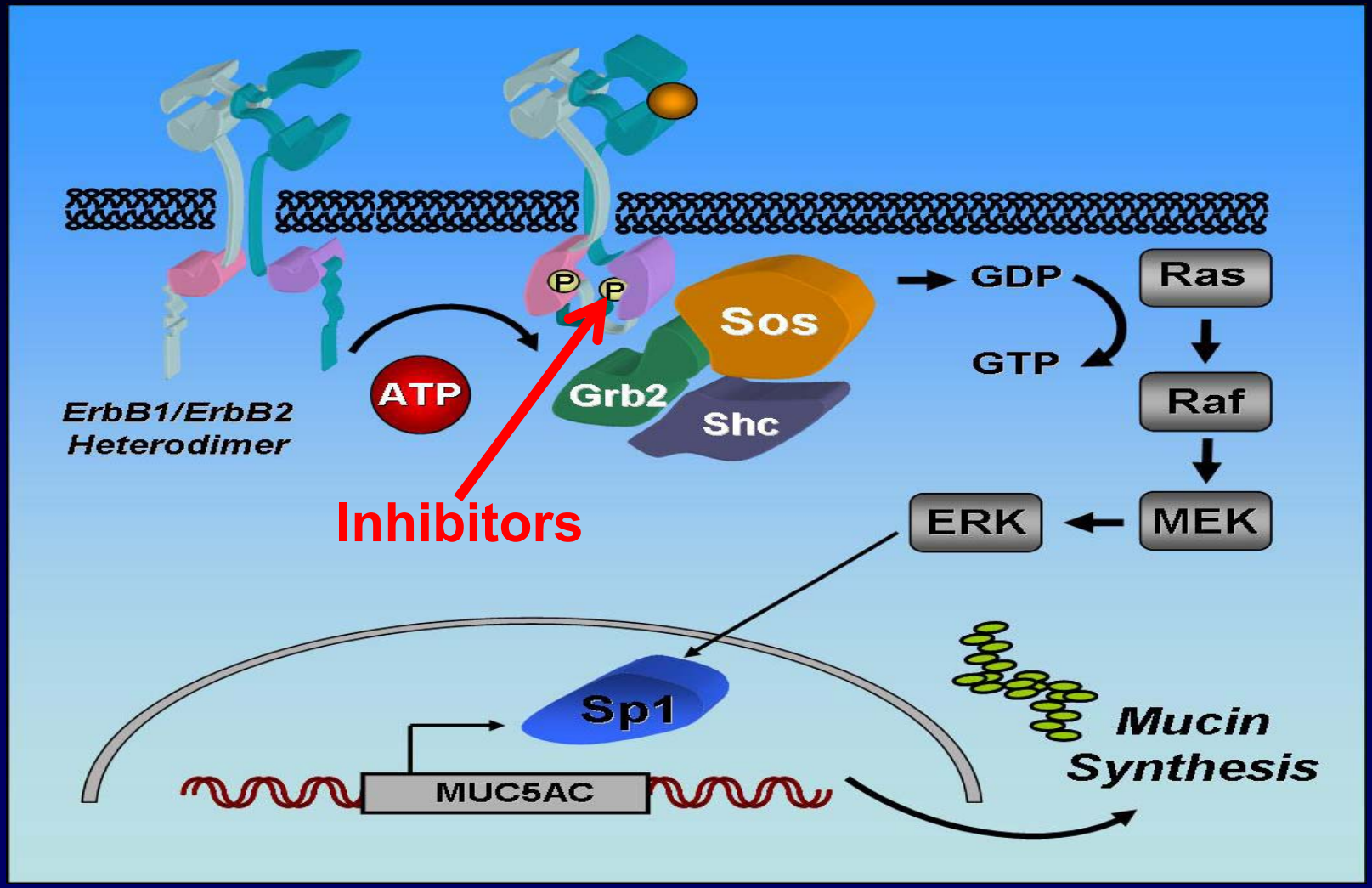
Airway mucus and hypersecretion



Pharmacotherapy of airway mucus hypersecretion: possibilities



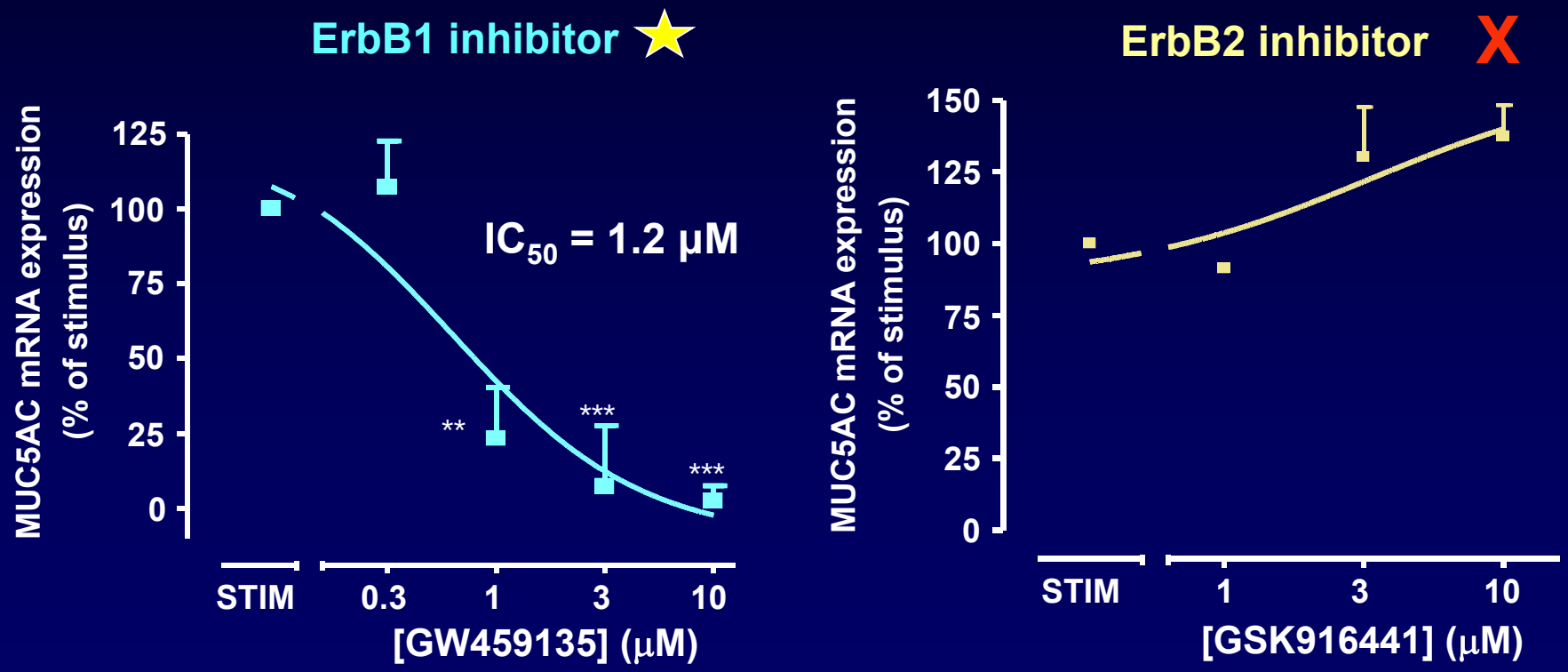
EGF (ErbB) receptor signalling-induced mucin gene expression



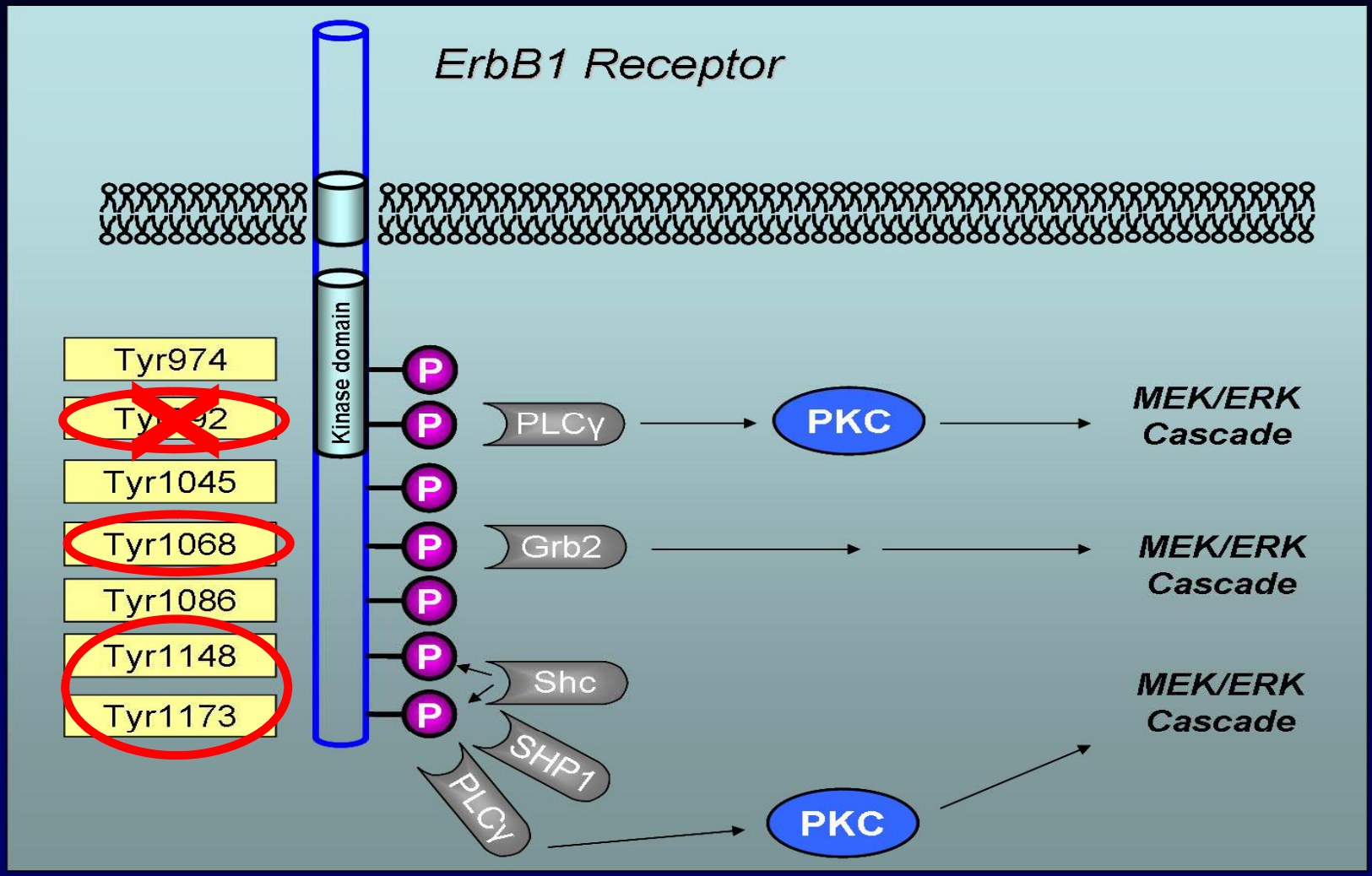
Effect of Glaxo (Pharma) ErbB kinase inhibitors on EGF-induced mucin (MUC)-5AC mRNA expression

(test compounds from anti-cancer programme)

H292 cells pre-treated with inhibitor for 45 min and stimulated with EGF 20 ng/ml for 4 h ± inhibitor



ErbB1 (EGFR) tyrosine autophosphorylation sites initiating the ERK1/2 cascade

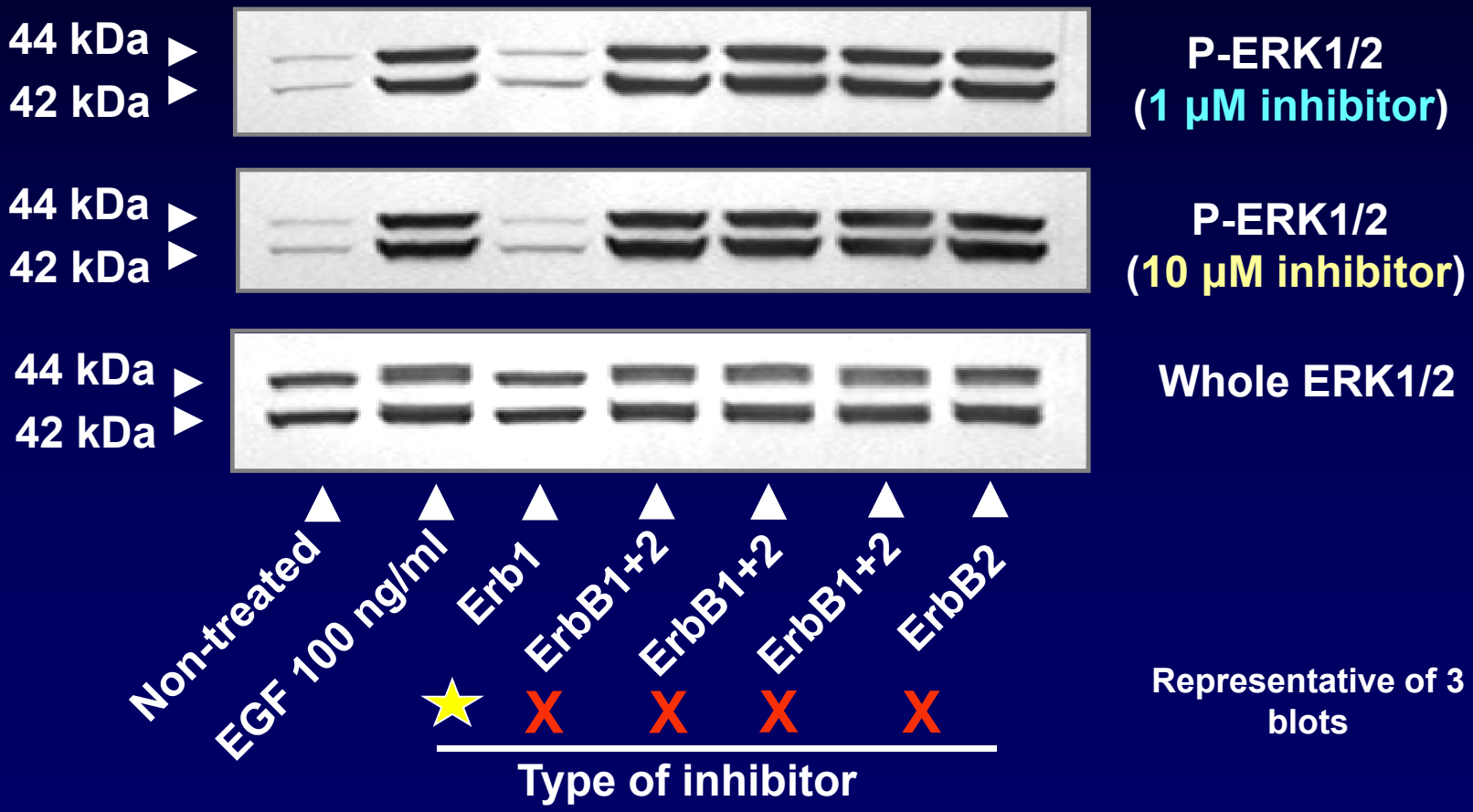


Cell-based ELISA

Western Blotting

Effect of ErbB inhibitors on downstream signalling: ERK1/2 phosphorylation

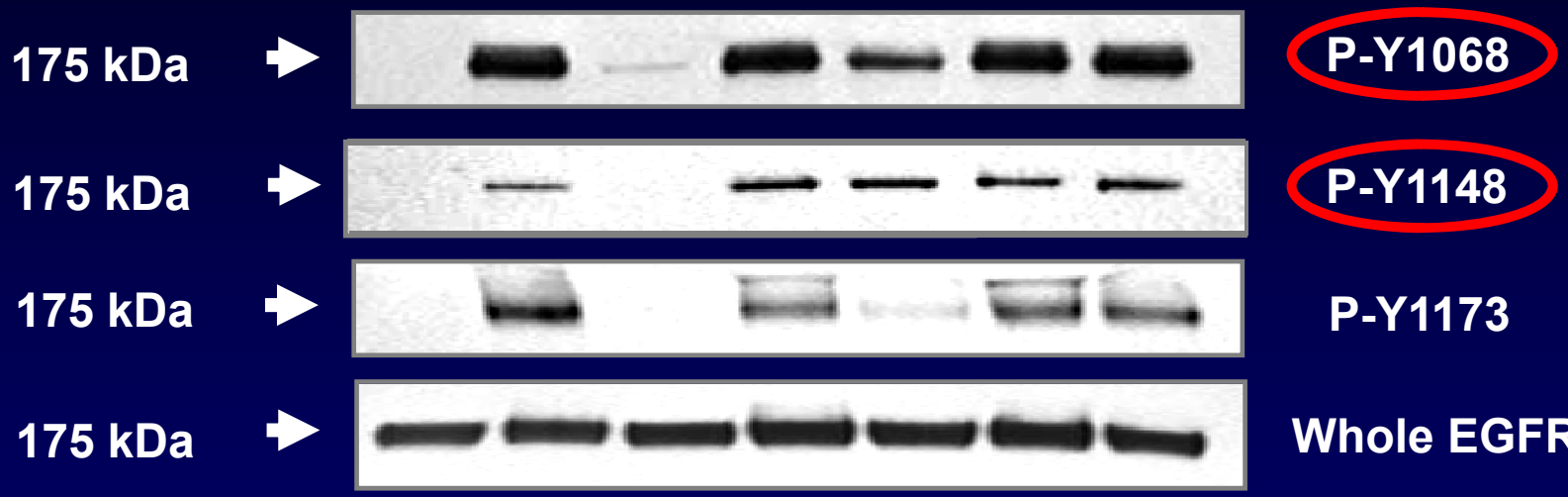
H292 Cells pre-treated for 45 minutes with compounds then stimulated (± compound) with EGF for 2.5 minutes



Representative of 3 blots

Effect of ErbB inhibitors on ErbB1 Y1068, Y1148 and Y1173 autophosphorylation

H292 cells pre-treated for 45 min with compounds (1 μM) then stimulated (± compound) with EGF for 2.5 min



	Non-treated	EGF 100 ng/ml	Erb1	ErbB1+2	ErbB1+2	ErbB1+2	ErbB2
	★		X	X	X		X

Type of inhibitor

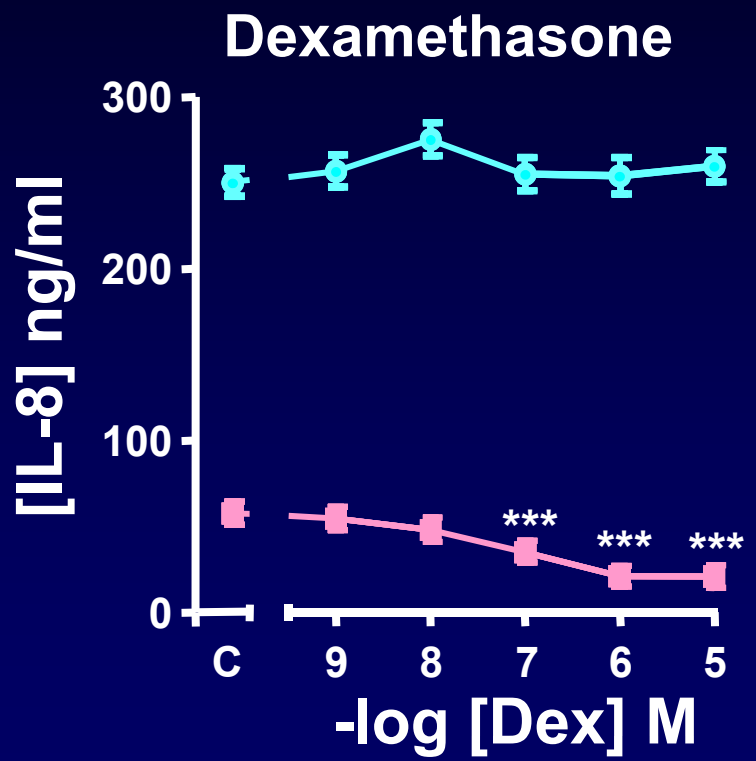
(Representative of 3 blots)

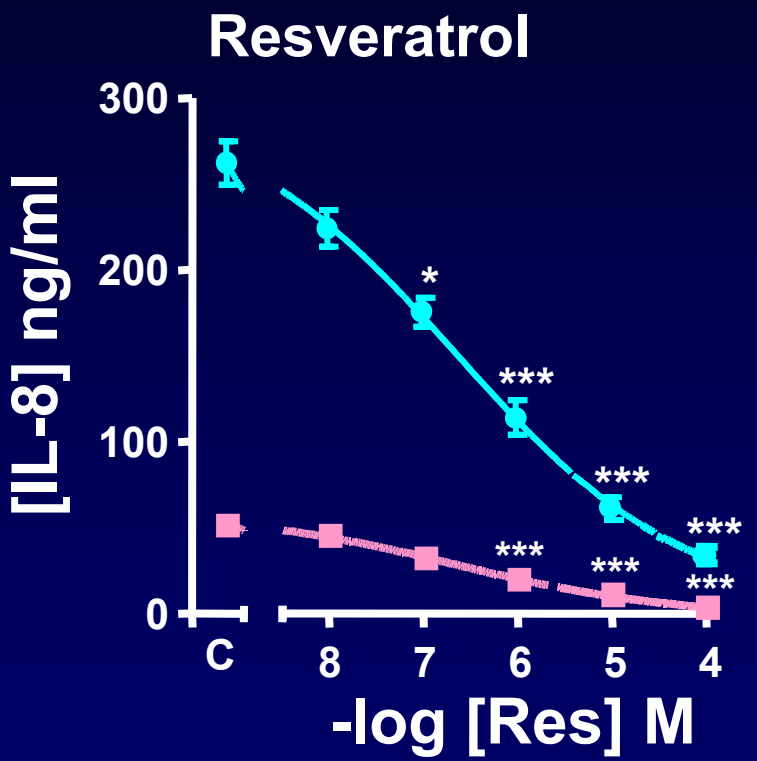
Health benefits of red wine

- 'French paradox'
- ? Reduce heart disease
- **Resveratrol**
  - found in skin of red fruits → red wine
  - antioxidant, antiproliferative
  - antiinflammatory?
  - ? use in ischaemic heart disease, atherosclerosis, cancer

3,5,4'-trihydroxystilbene

Effect of a corticosteroid or resveratrol on IL-8 release by BAL alveolar macrophages: Smokers *vs* COPD patients

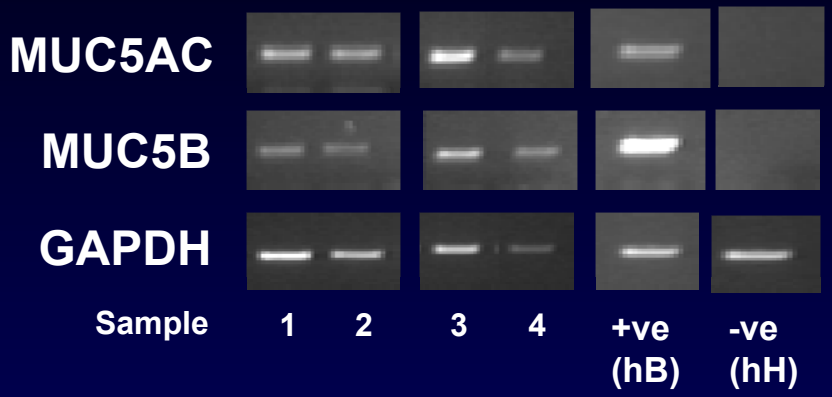




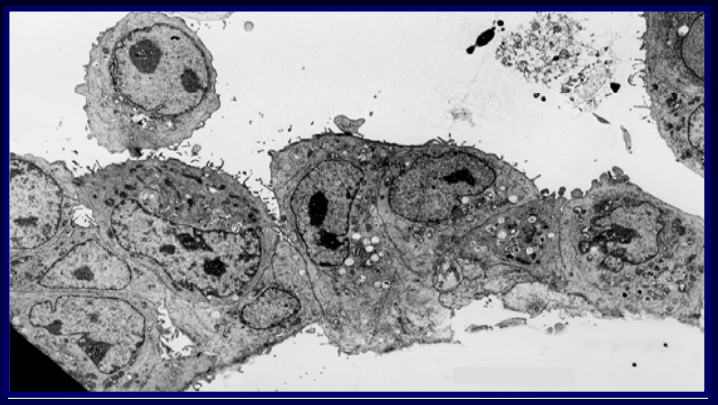
(Culpitt *et al* Thorax 2003, 58: 942-946)

A549 cells and mucus measurements

Mucin gene expression



Appearance

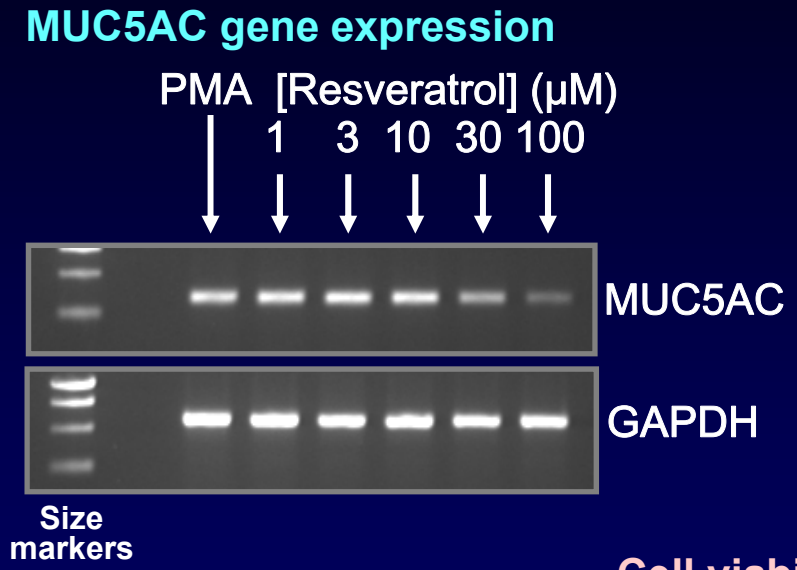


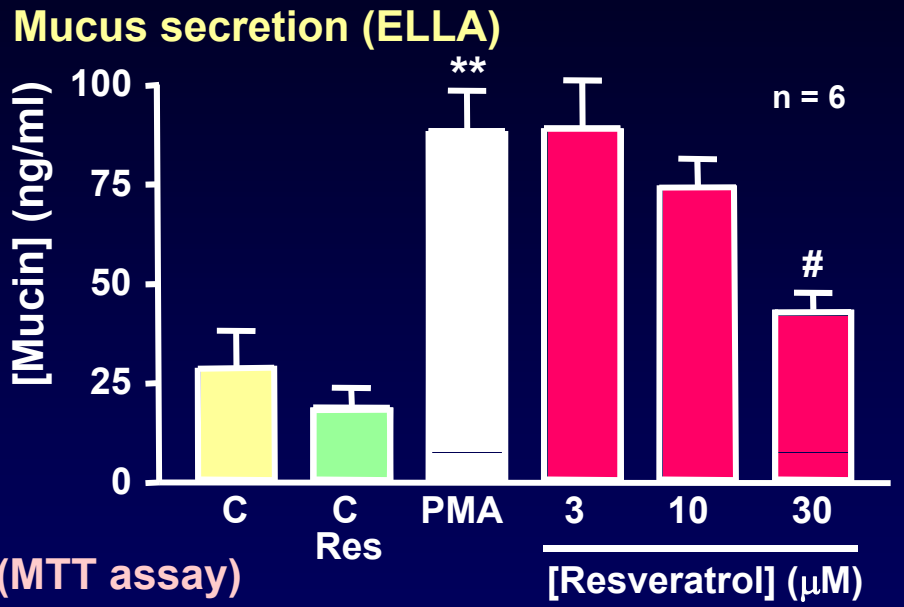
Mucin quantification

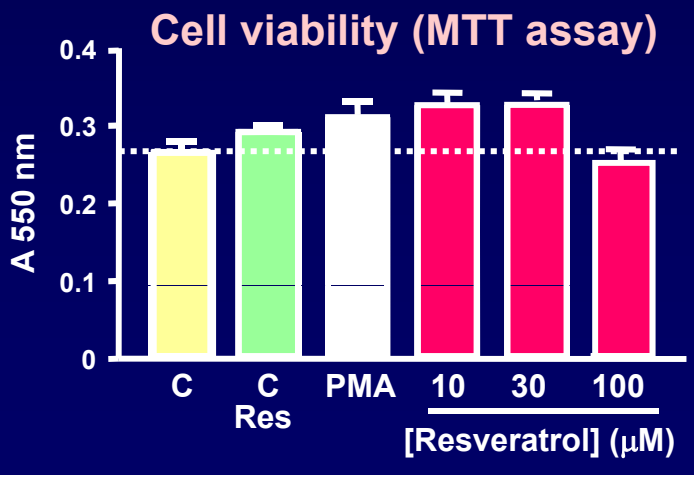
- Enzyme Linked Lectin-Binding Assay (ELLA):
  lectin binds to specific terminal sugars on mucin molecule
- Lectin: *Helix pomatia* (edible snail) agglutinin, conjugated to HRP
- Samples compared with human airway mucin standard

Effect of resveratrol on mucus expression in A549 cells

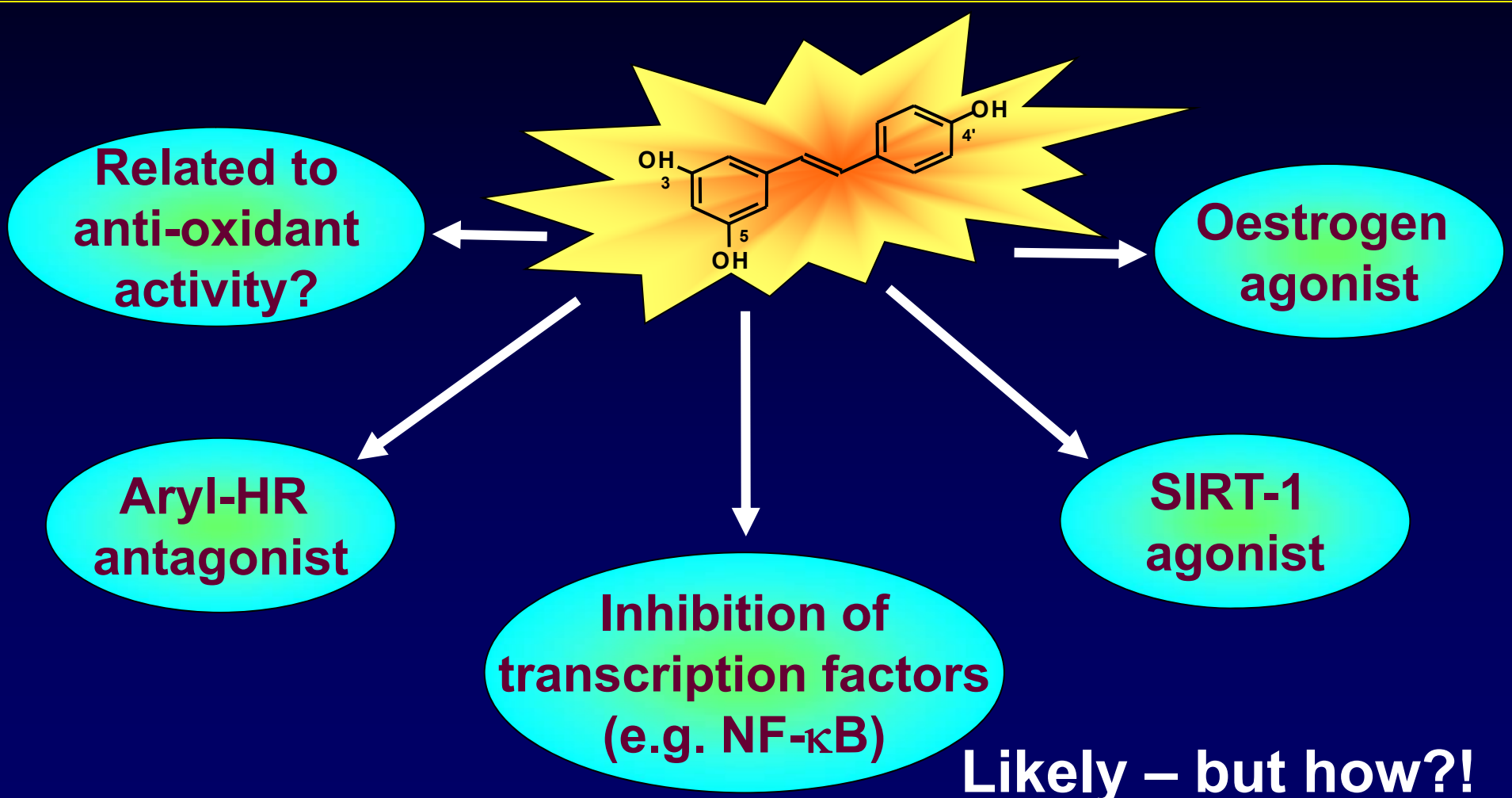
(24 h pre-incubation with resveratrol before stimulation with 1 μM PMA for 4 h)





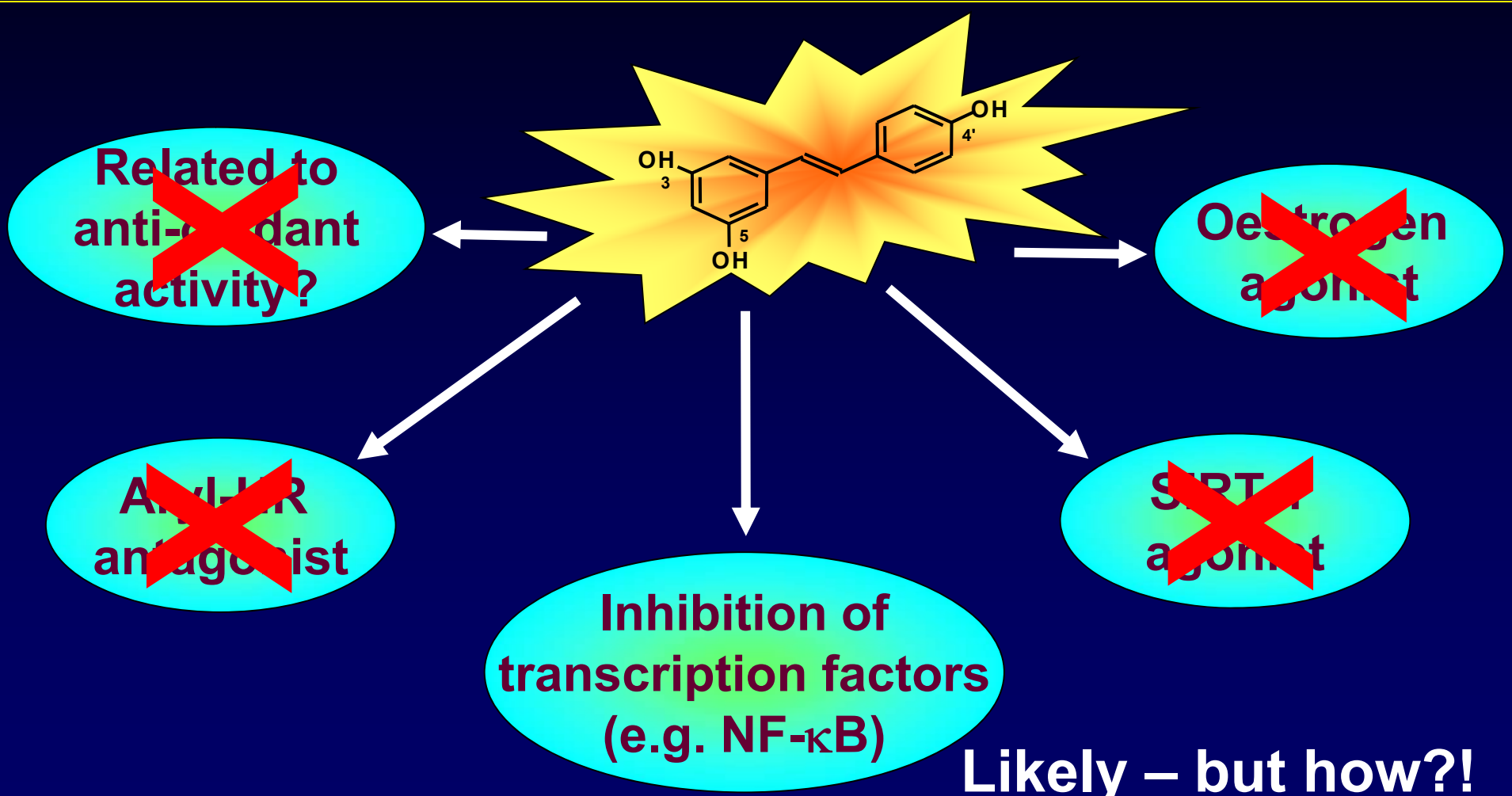


Mechanism(s) of anti-mucus effects of resveratrol?

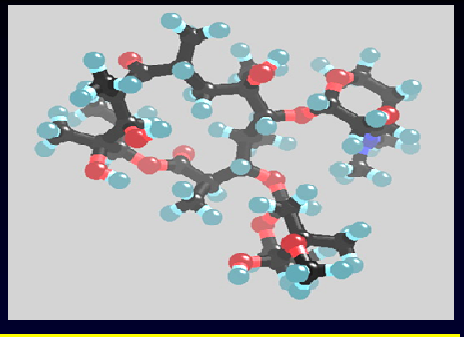


Likely – but how?!

Mechanism(s) of anti-mucus effects of resveratrol?



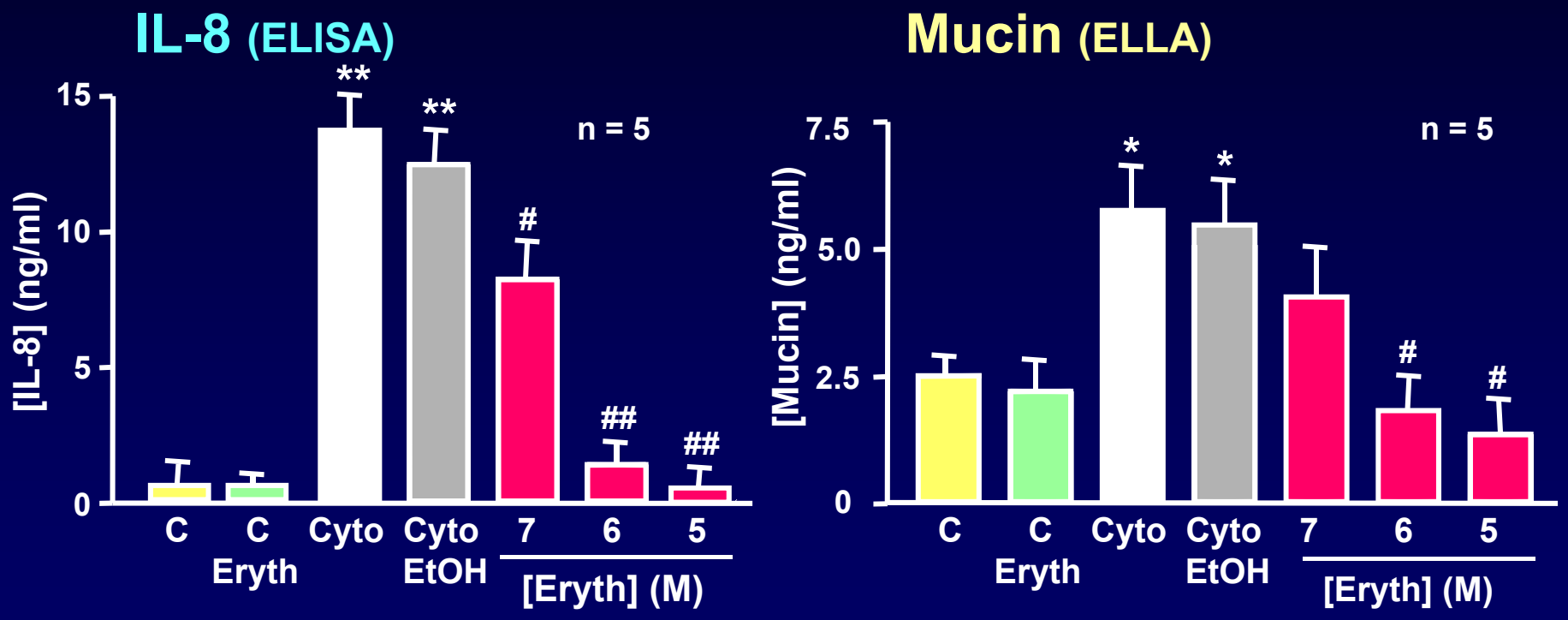
Erythromycin



- 14-member ring macrolide antibiotic
- Widely used in treatment of upper and lower respiratory tract infections
- Improves chronic inflammatory processes including diffuse panbronchiolitis, asthma and chronic sinusitis
- Relieves ‘bronchorrhea’ in patients

Effect of erythromycin on IL-8 release and mucin secretion in A549 cells

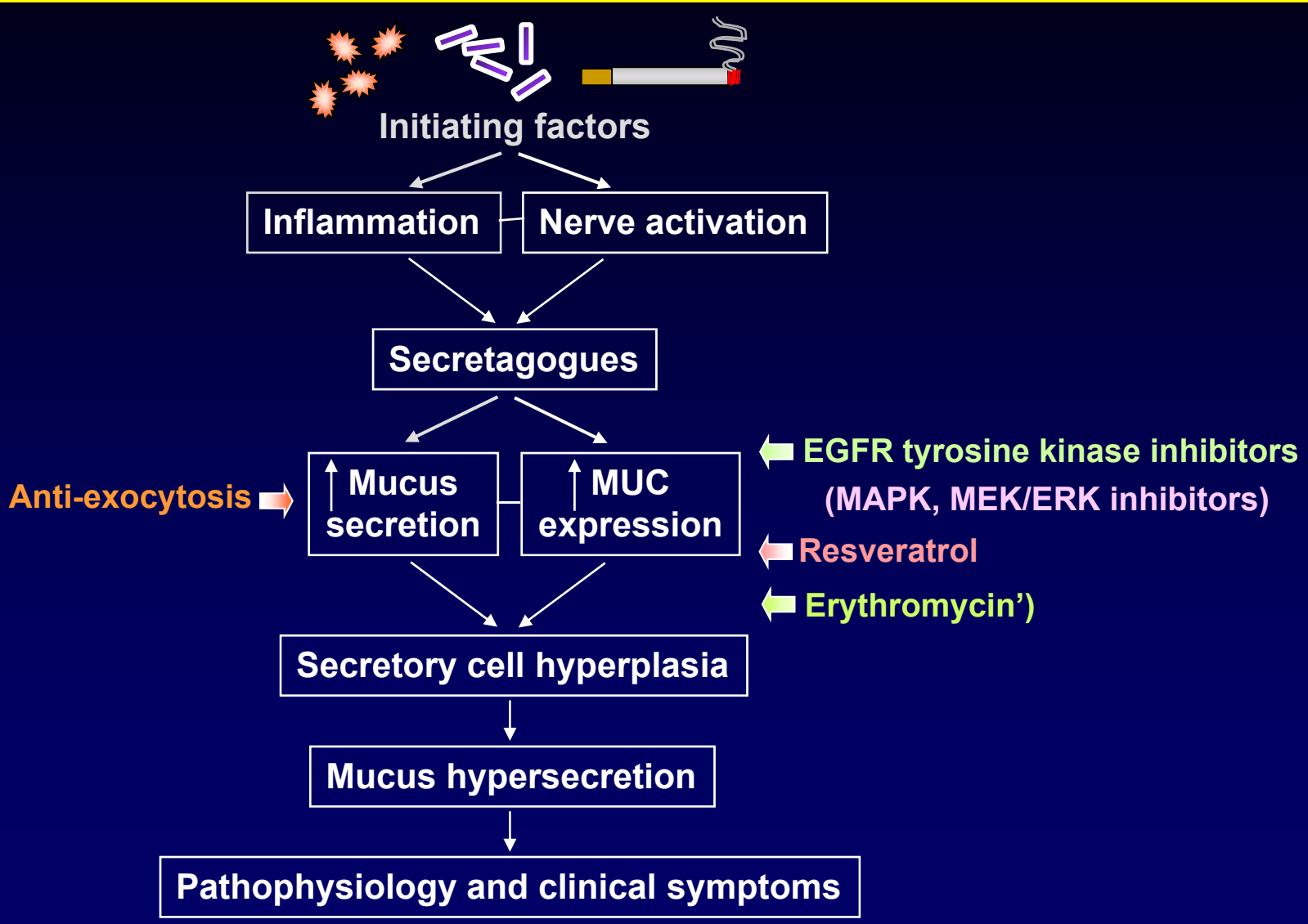
(24 h pre-incubation with erythromycin before stimulation with cytomix, 1 ng/ml)



‘Antiinflammatory’

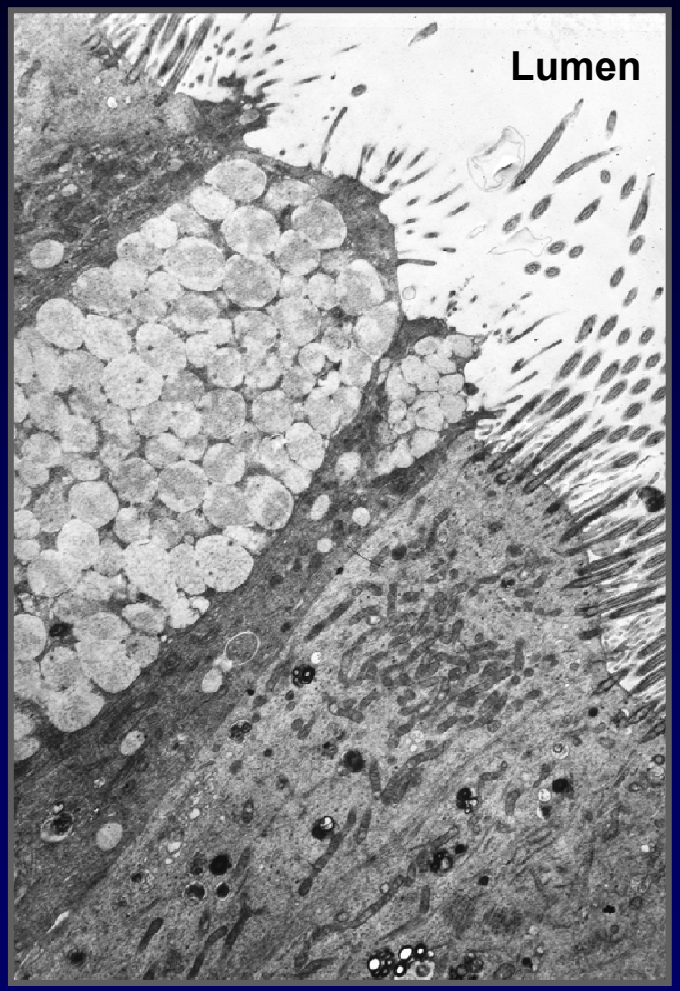
‘Anti-mucus’

Pharmacotherapy of airway mucus hypersecretion: possibilities

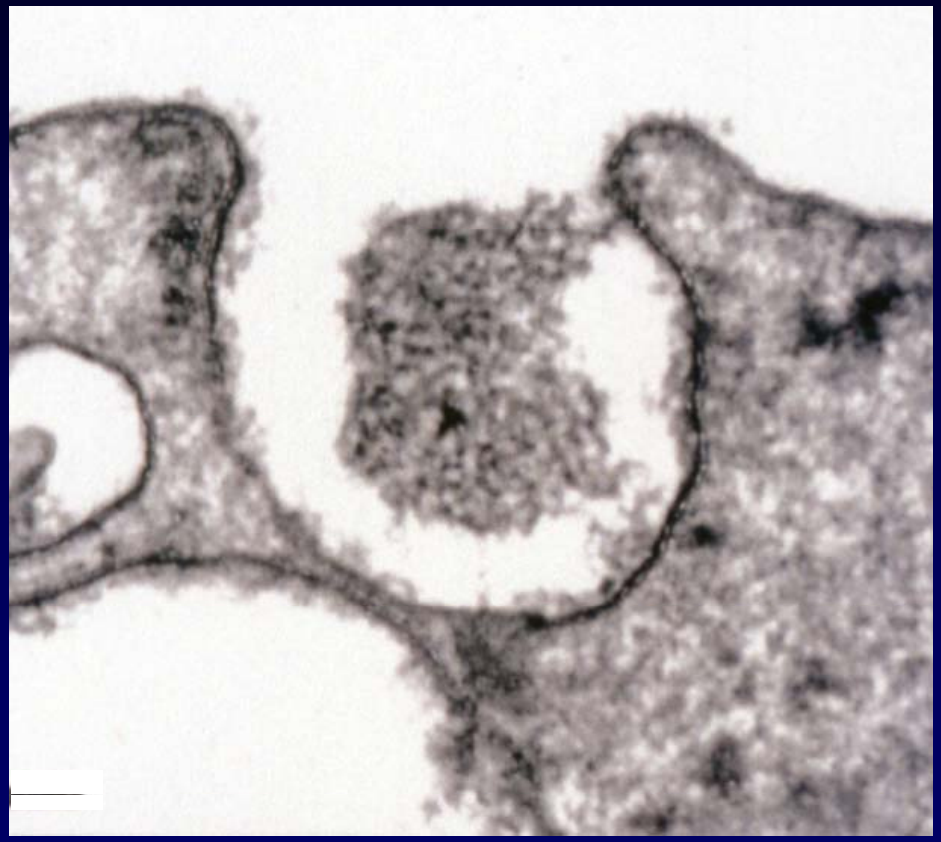


Airway secretion: goblet cell and Ω profile

Human epithelium

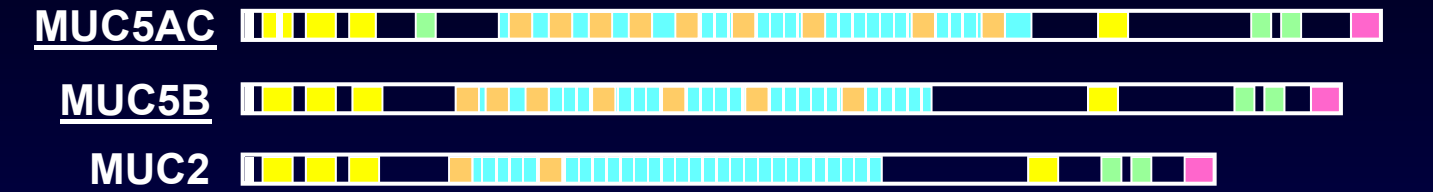


Guinea pig tracheal goblet cell



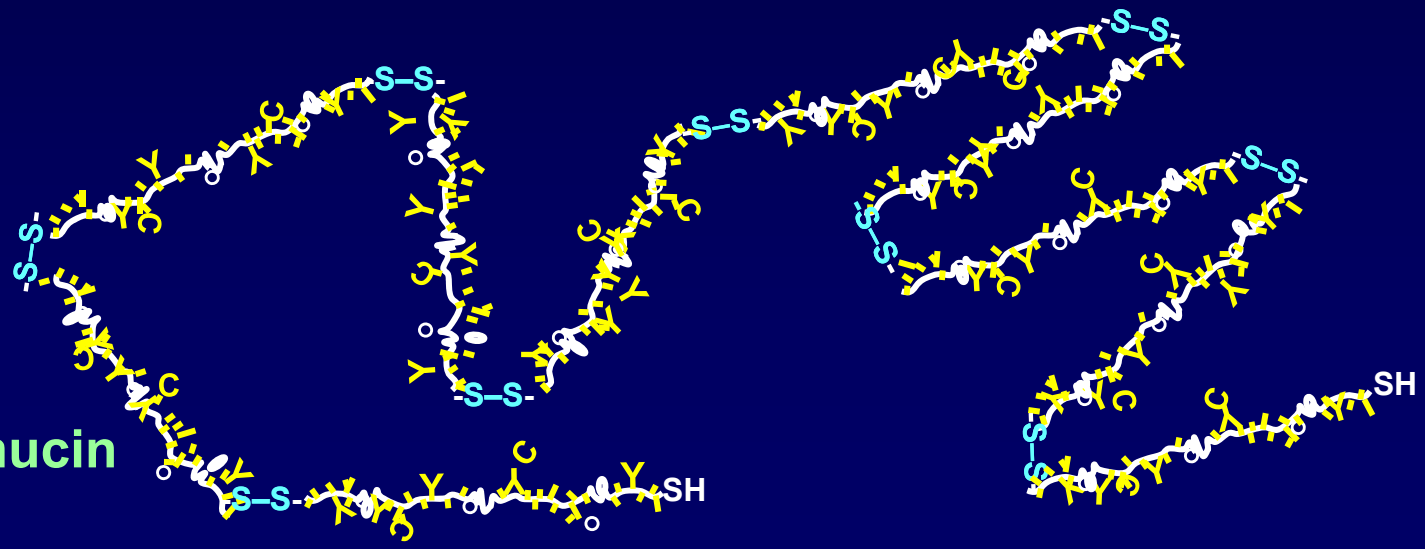
Respiratory tract mucins

Respiratory tract MUC gene products (out of >20 human MUC genes)

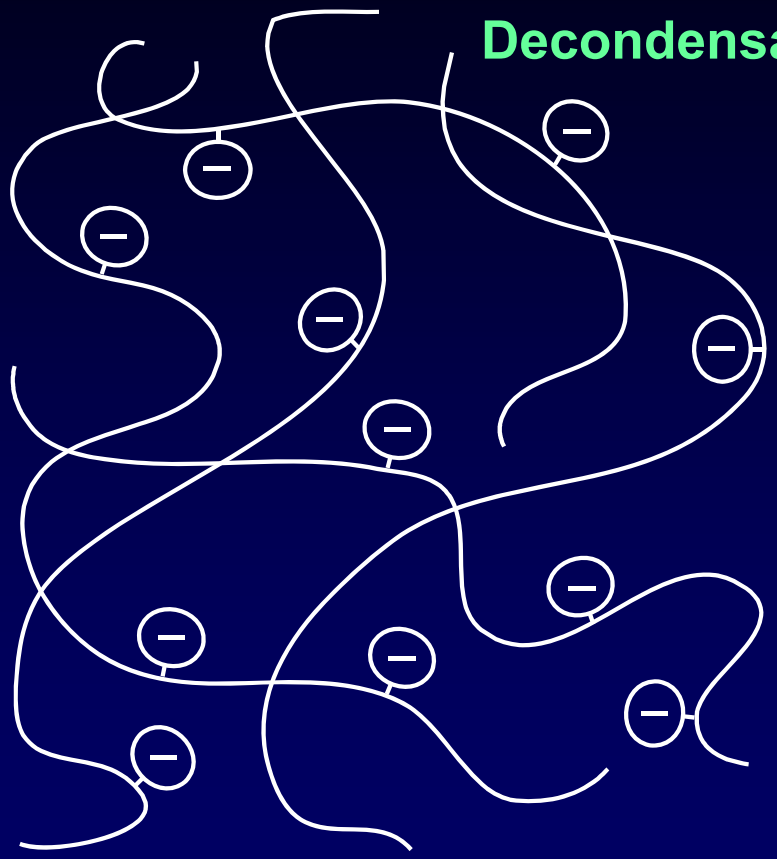


Mucin monomer

Mature mucin



Mucin exocytosis



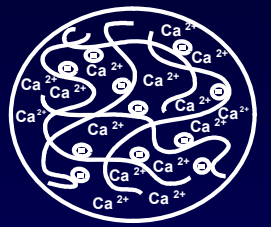
Decondensation

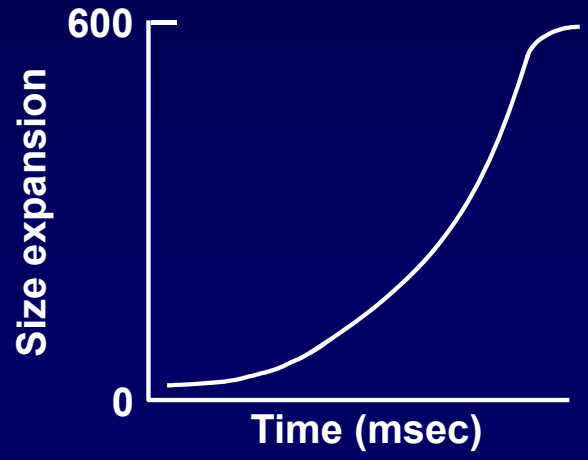
\+ Ca^{2+}

Packaging

Secretion

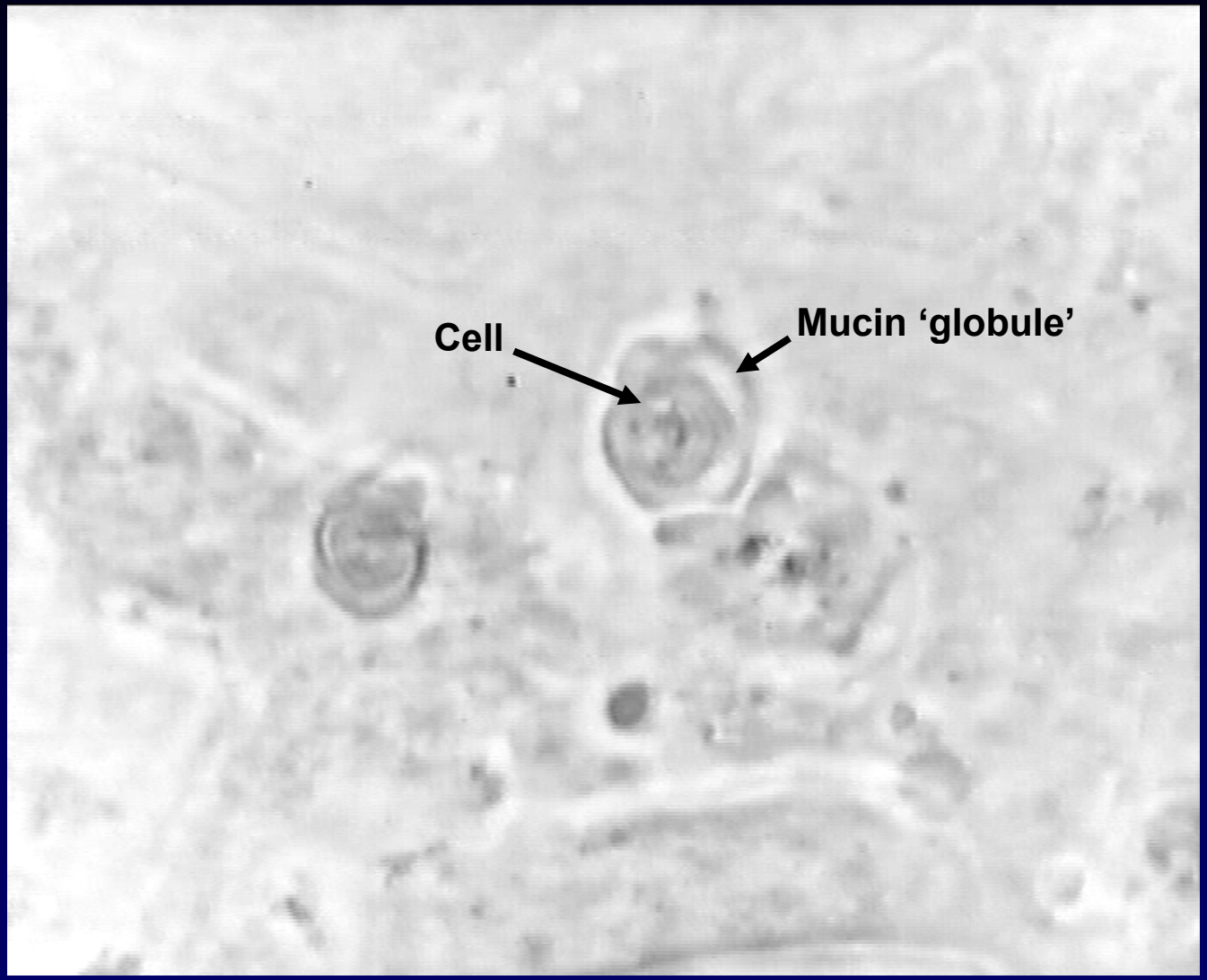
Condensation



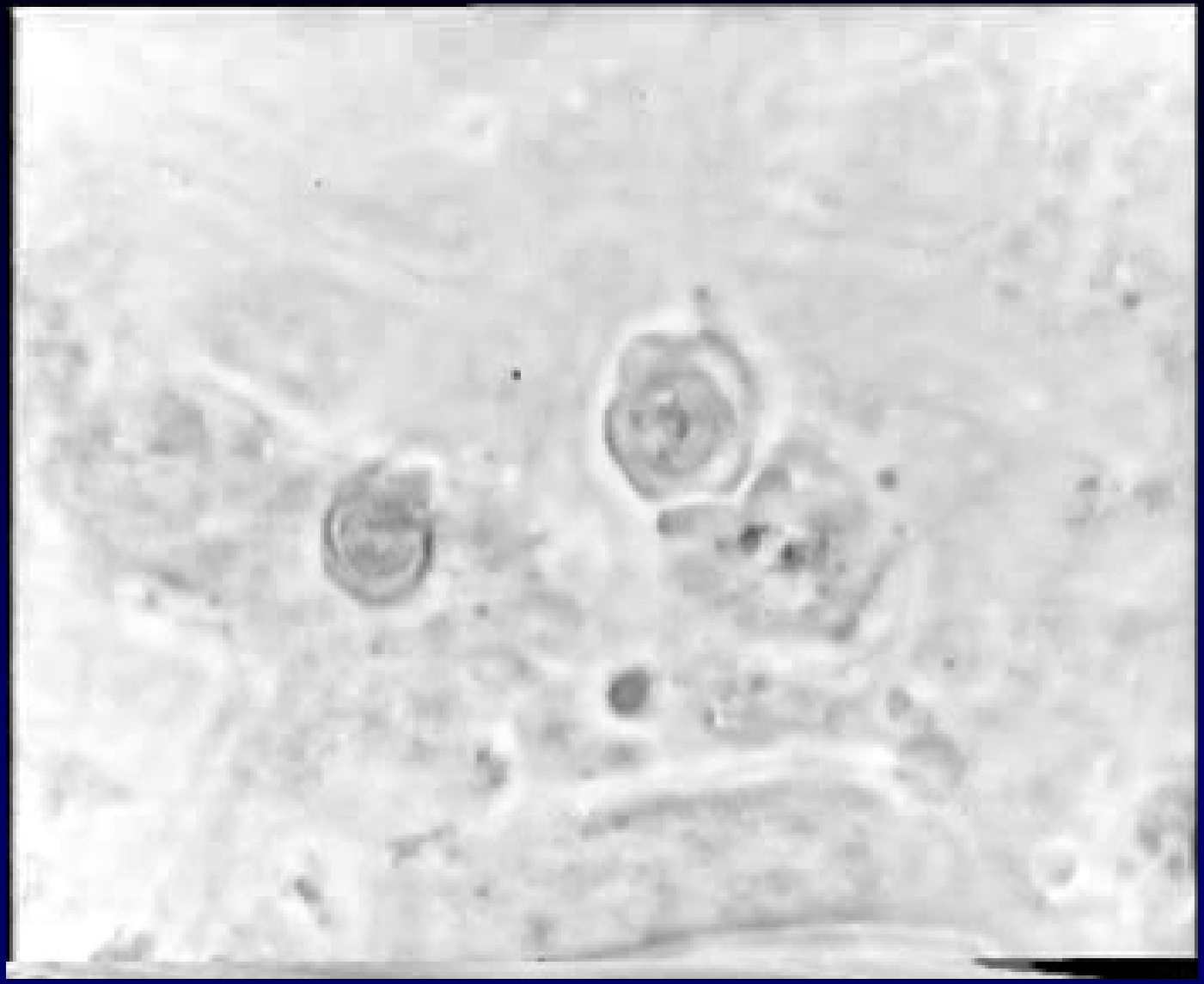


(After Pedro Verdugo)

Guinea pig tracheal goblet cells



Goblet cell mucin exocytosis (to ATP: real time)



Clostridium botulinum

- Gram +ve bacteria

  - soil, badly preserved food (canned raw meat)

- Produce botulinum neurotoxins (BoNT)

  - 7 serotypes: A – G

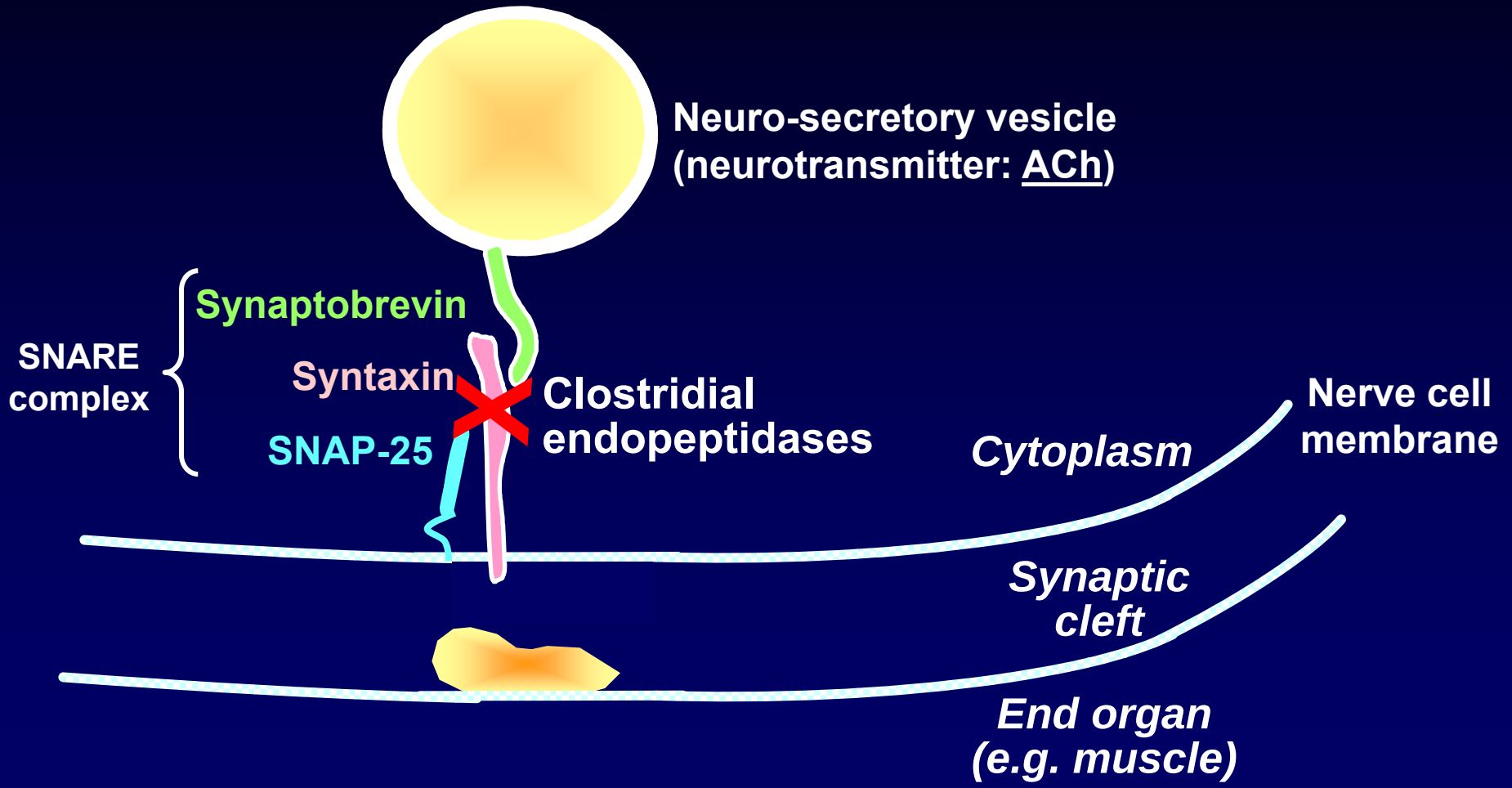
    (serotype C degraded in gut)

  - target neuromuscular junction

- Block secretion of neurotransmitters by cleaving

  SNARE proteins (*via* endopeptidase enzymes)

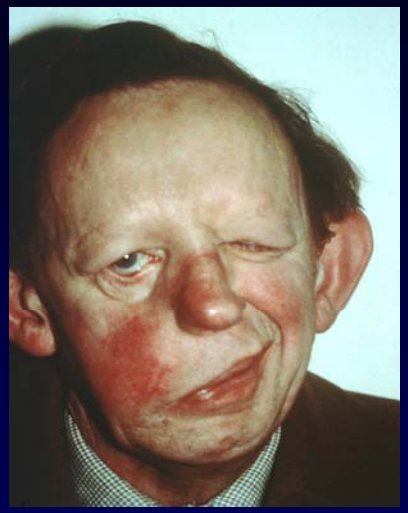
BoNTs and inhibition of neurosecretion

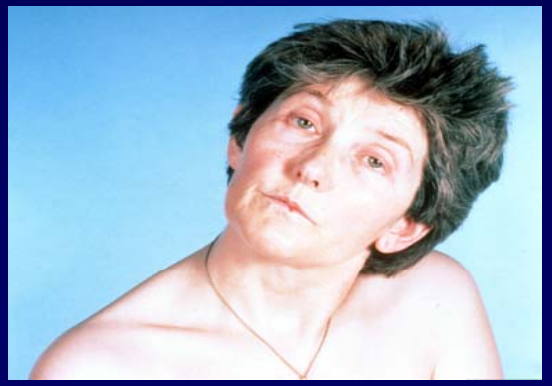


BoNT: Therapeutic products

(for nerve-related problems, very low doses)

- Serotype A product:
  - 1980 Alan Scott - squint
  - USA licence 1989
  - UK licence 1991
  - Now licenced in over 70 countries
- Serotype B product approved in 2004
- Over 180 applications:
  - Botox (wrinkles)
  - dystonia
  - overactive bladder
  - hyperhidrosis (sweating)
  - cerebral palsy





Hypothesis and target for mucus cells

"BoNT, targeted appropriately, will inhibit mucin secretion from respiratory epithelial cells"

- Which target?
  - what about epidermal growth factor (EGF) receptors?
- EGF receptors on respiratory epithelial cells
- EGF receptors upregulated in airway epithelium of asthmatics, smokers and patients with COPD

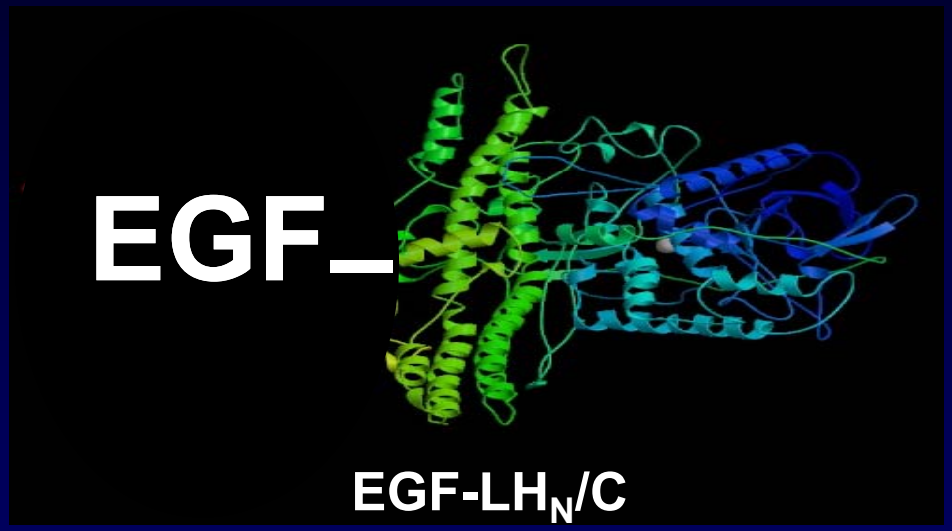
Re-targeting BoNT/C to EGF receptors

- BoNT/C domains: neuronal binding, translocation, catalytic

- Neuronal binding domain removed
  - leaves LH_N/C (translocates and catalytically active)
- Replace neuronal binding domain with EGF = EGF-LH_N/C

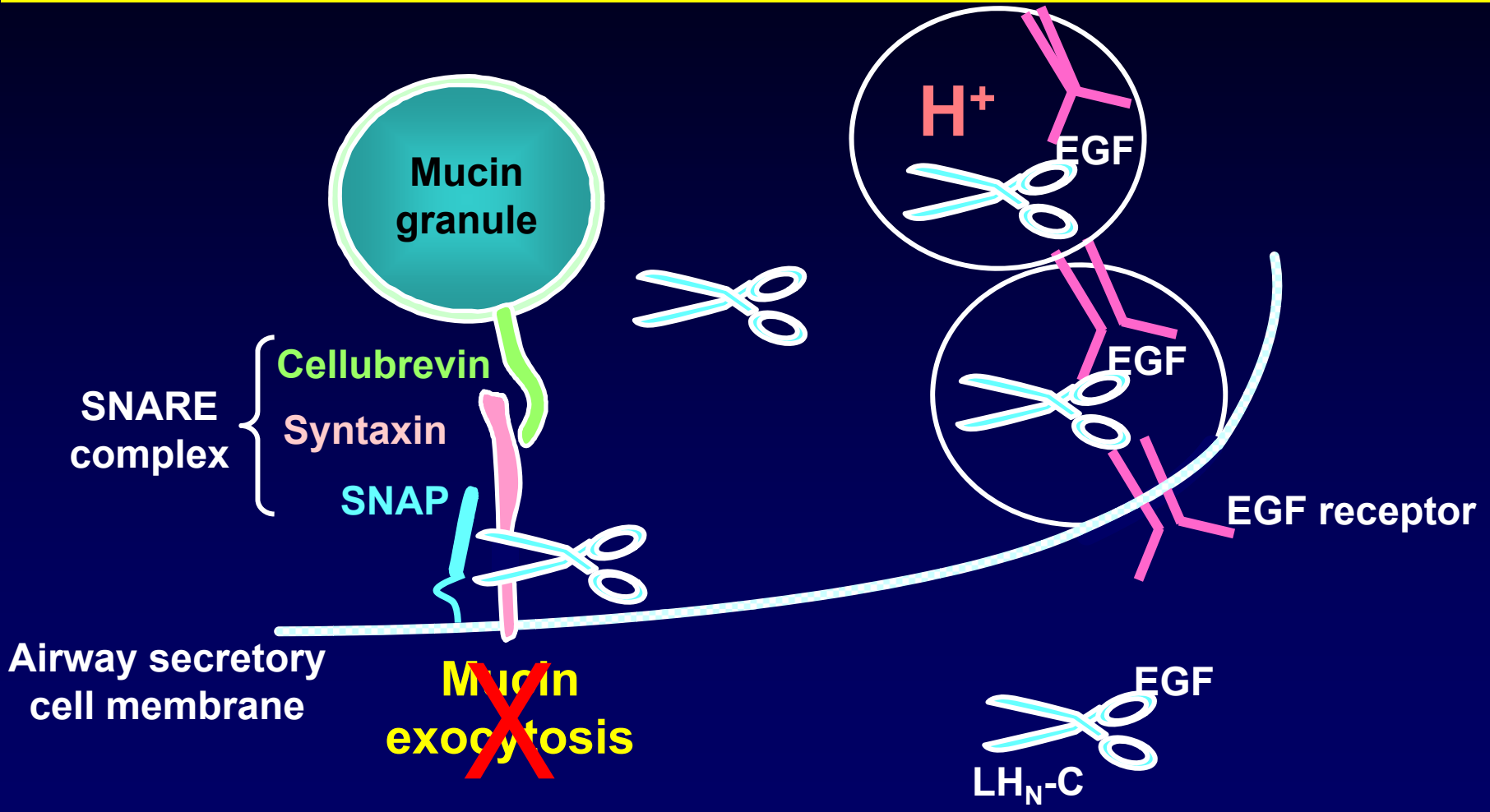
Re-targeting BoNT/C to EGF receptors

- BoNT/C domains: neuronal binding, translocation, catalytic



- Neuronal binding domain removed
  - leaves LH$_N$/C (translocates and catalytically active)
- Replace neuronal binding domain with EGF = EGF-LH$_N$/C

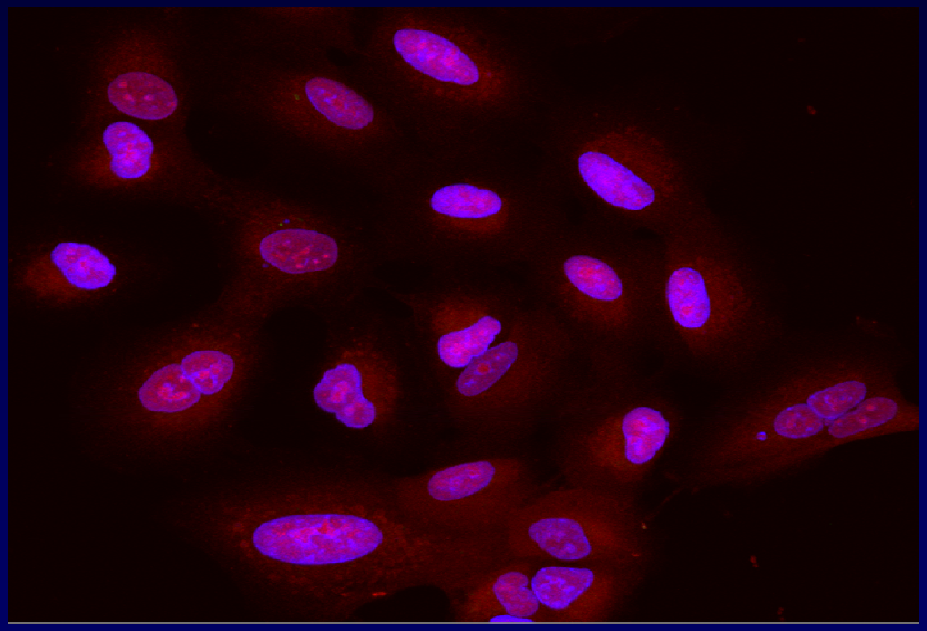
EGF-LH_N-C and inhibition of airway mucin secretion: theory



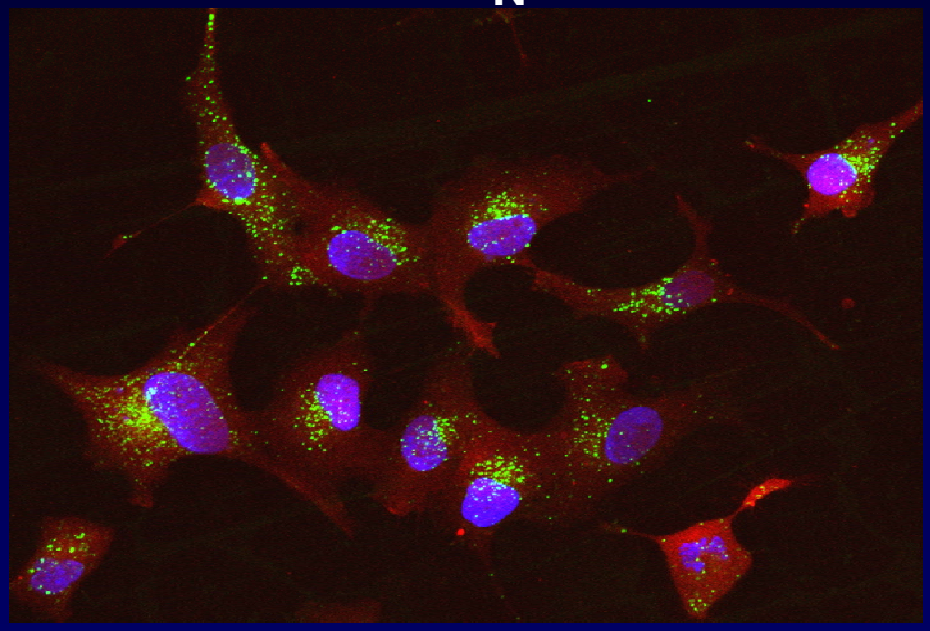
EGF-LH_N/C enters human respiratory epithelial (A549) cells

(Confocal microscopy)

Vehicle



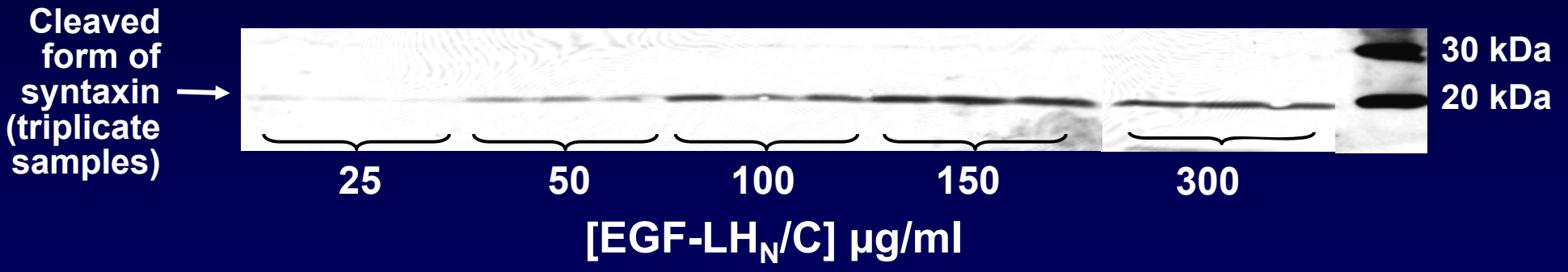
EGF-LH_N/C



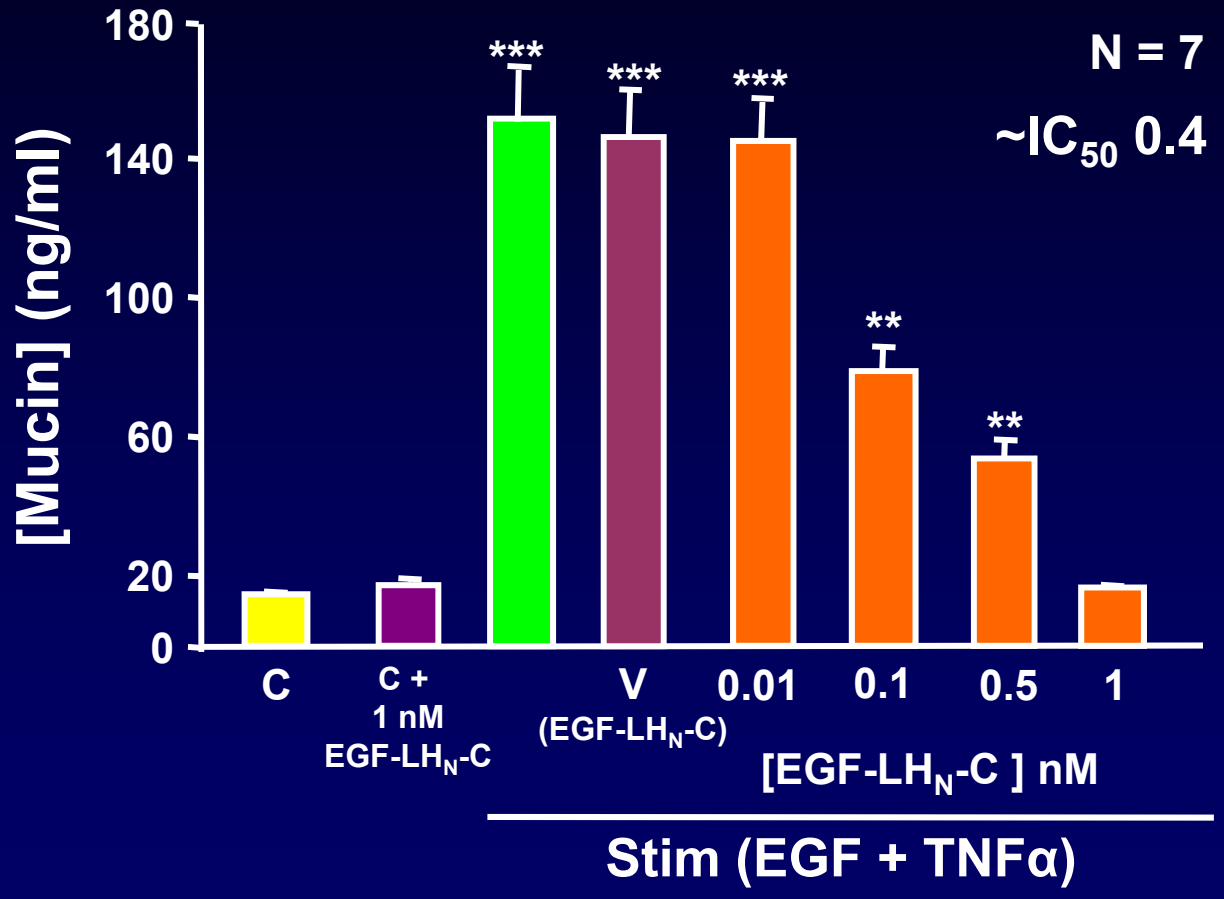
Blue/pink = nucleus, Red = cytoplasm, Green = EGF-LH_N/C

EGF-LH_N/C cleaves syntaxin in human respiratory epithelial cells

(AVKY antibody for C-terminus cleavage product)

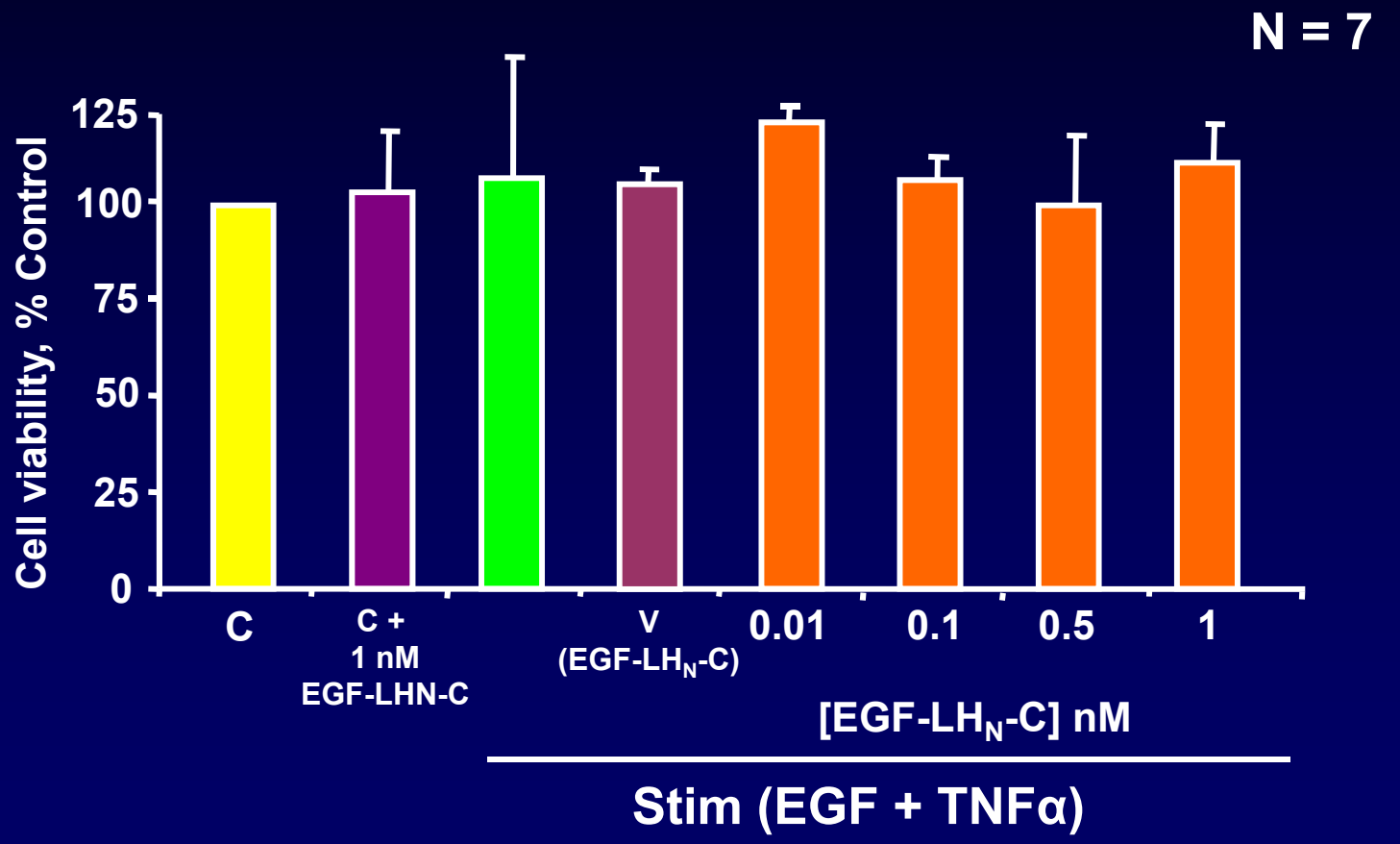


EGF-LH_N/C inhibits mucus secretion

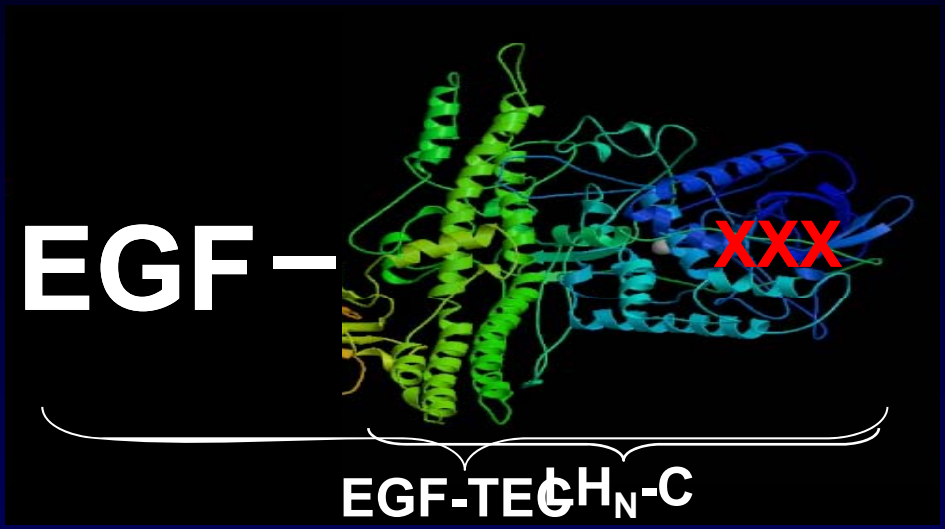


EGF-LH_N/C does not affect cell viability

(MTT assay)



Controls: LH_N/C and EGF-TEC



LH_N/C: non cell-binding (no EGF fused to it)

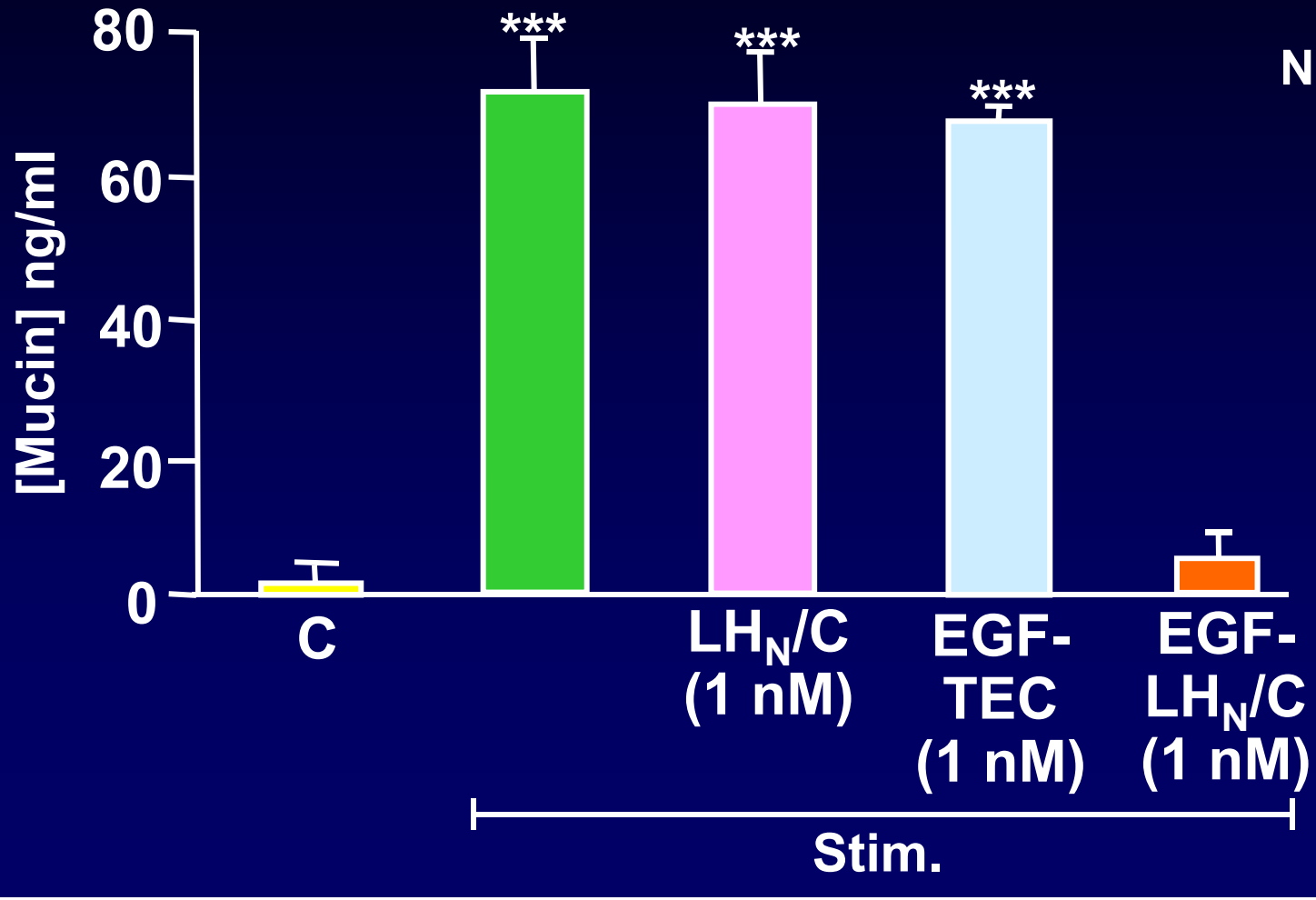
- should not enter cells (tests for 'targeting')

Triple endopeptidase negative (EGF-TEC):

- enters cells but not active

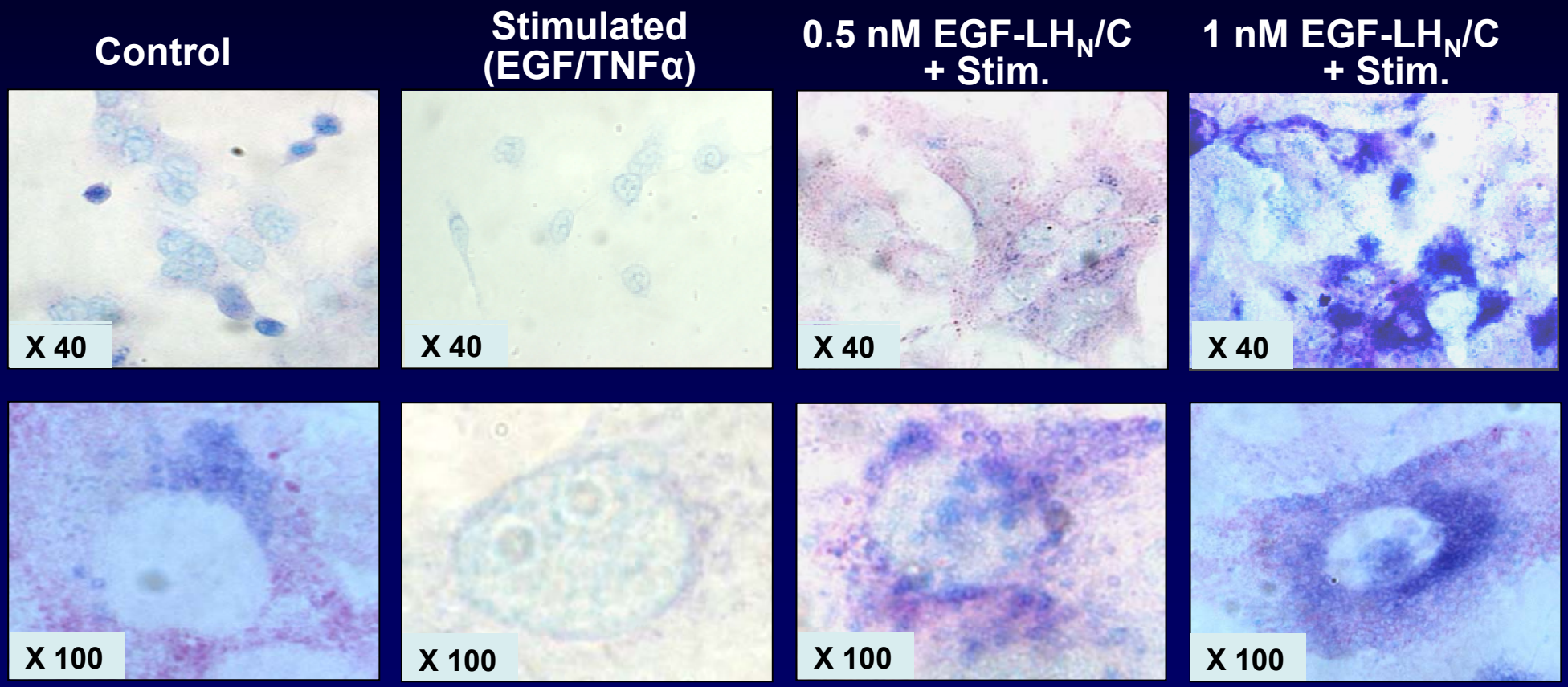
(tests for non-selective inhibition)

LH_N/C and EGF-TEC do <u>not</u> inhibit secretion
(∴ needs to be targeted and enzyme active)



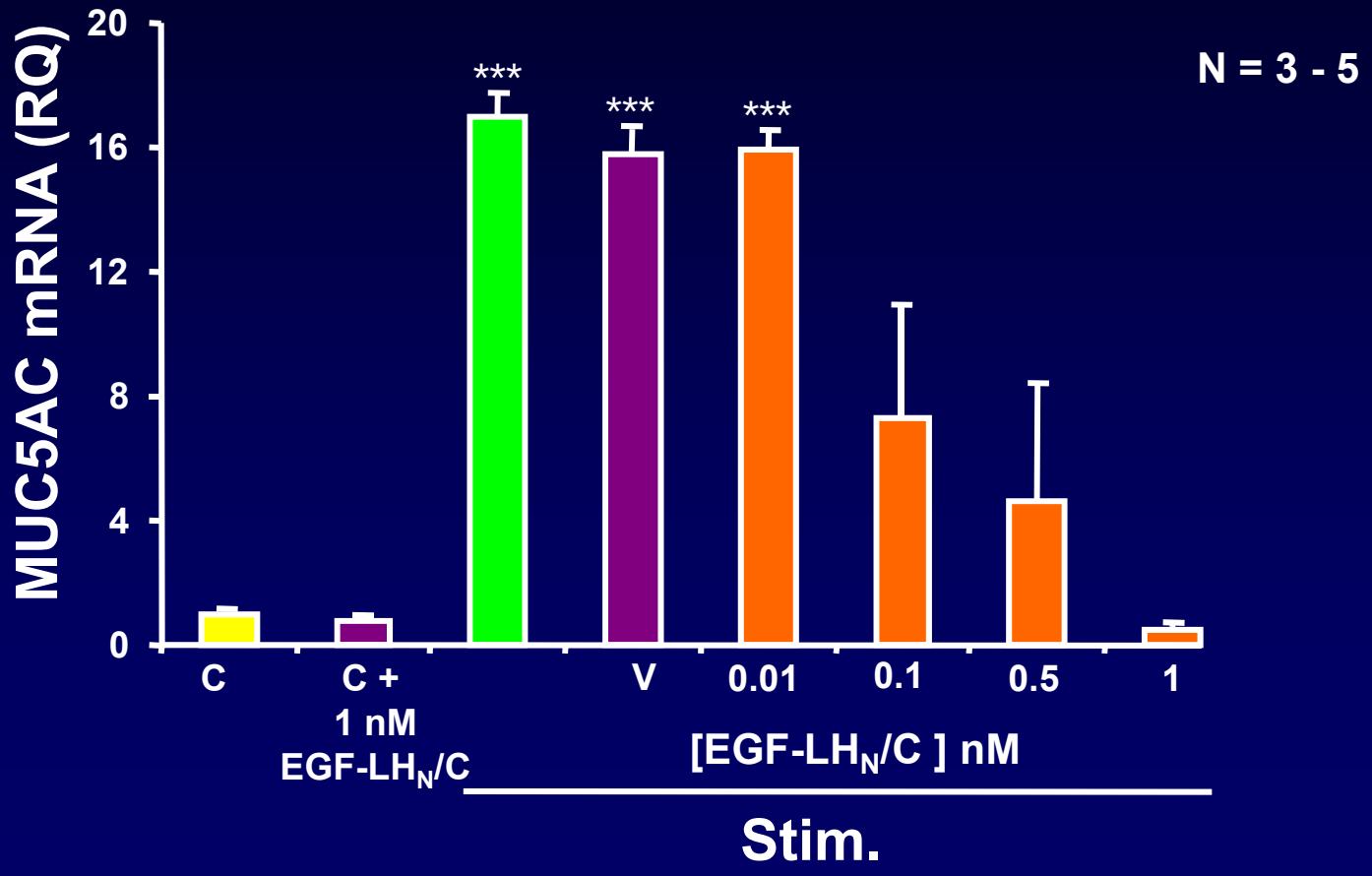
Effect of EGF-LH_N/C on intracellular mucin

(A549 cells, Alcian blue/PAS staining)

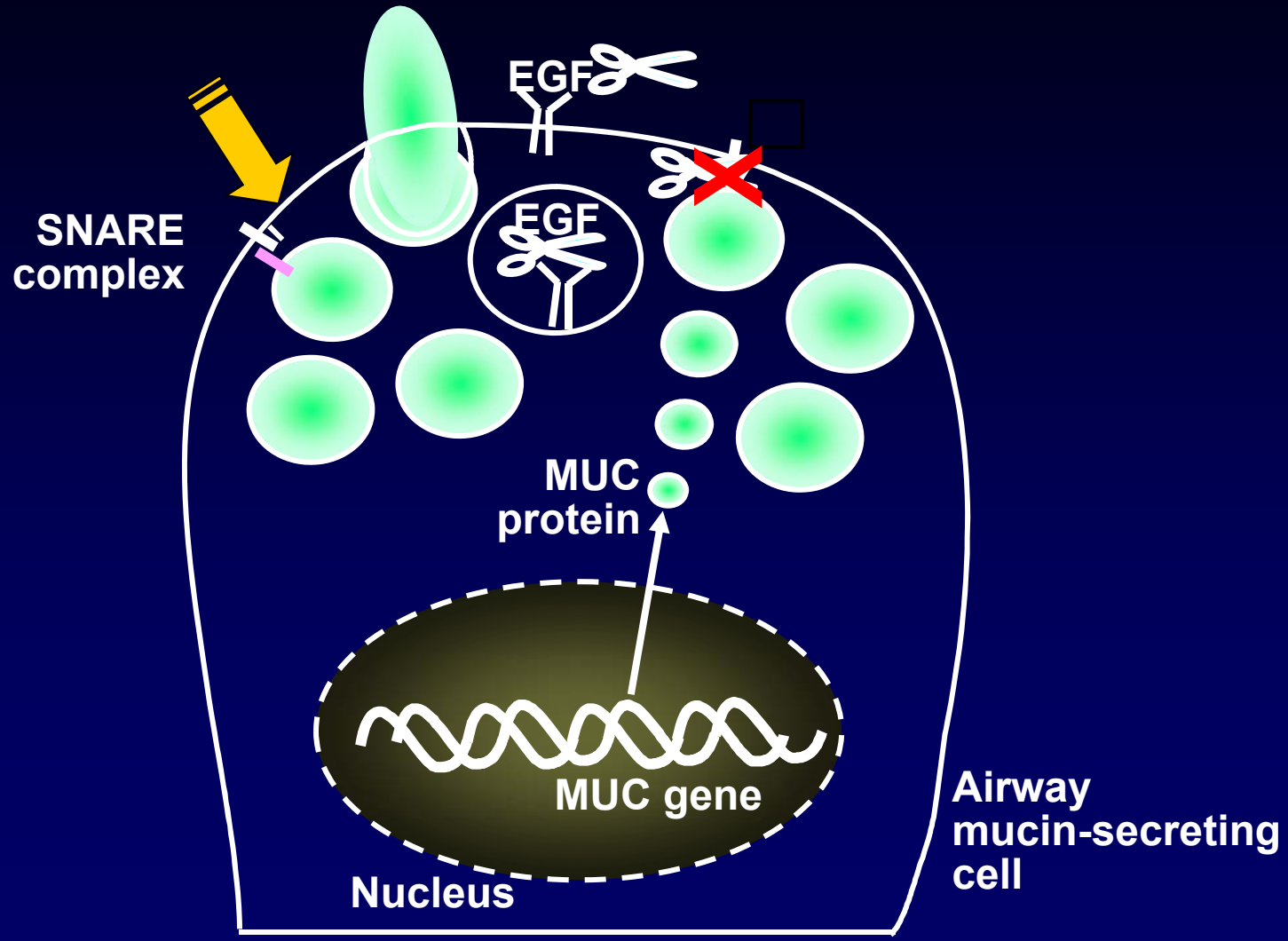


Effect of EGF-LH_N/C on EGF-TNFα-induced MUC5AC gene expression

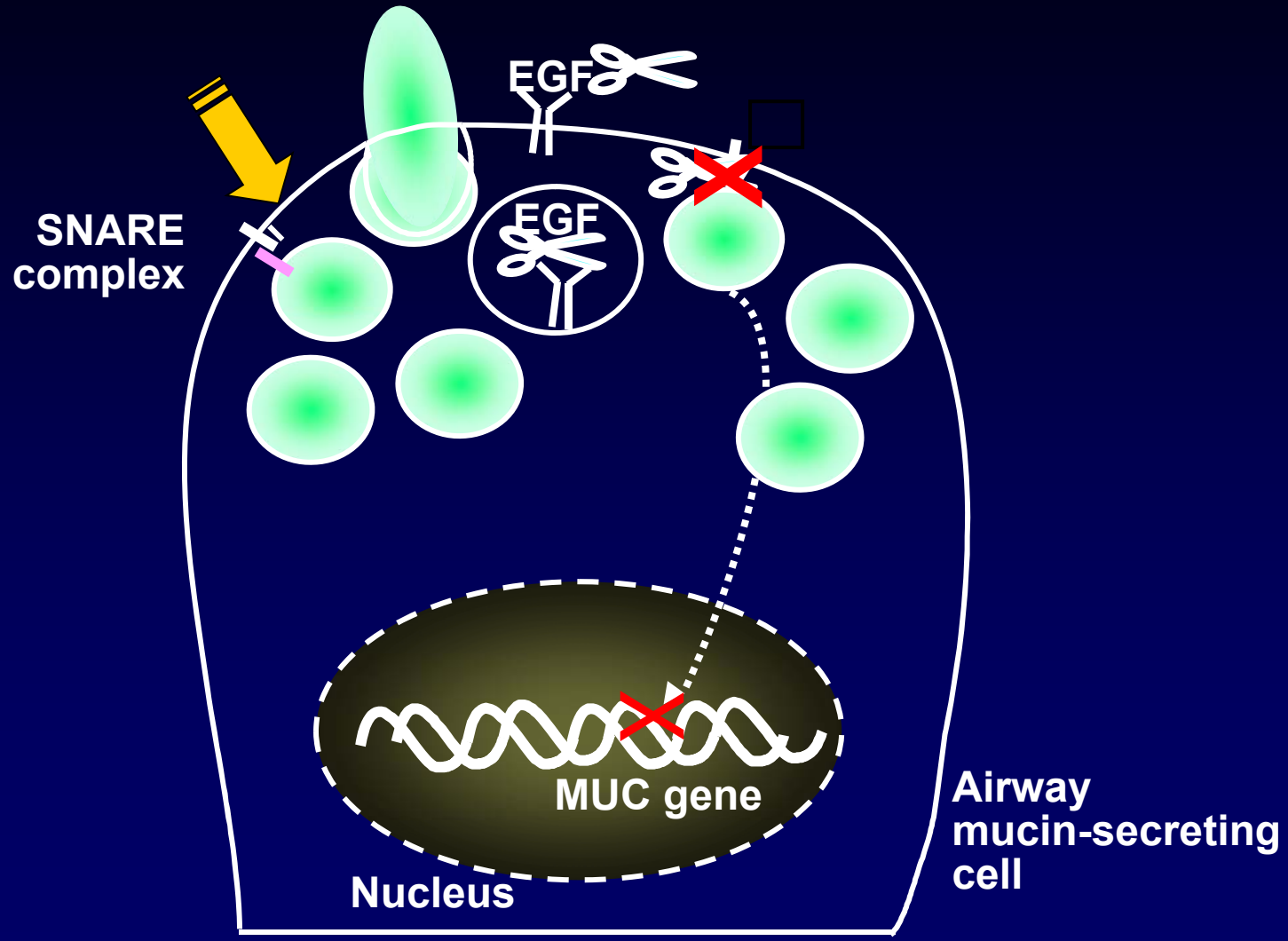
(A549 cells, Taqman RT-PCR)



Feedback mechanism (+/-?)



Feedback mechanism (+/-?)



Summary
Airway mucus hypersecretion and pharmacotherapy

- Increased airway mucus production (synthesis and secretion) in asthma and COPD → pathophysiology and clinical symptoms
- Goblet cell hyperplasia and submucosal gland hypertrophy
- Numerous possible intervention sites in mucus hypersecretory 'cascade'
- EGFR antagonists look promising – but have to be specific
- Promising resveratrol effects indicate uses for phytoceuticals
- Promising eythromycin effects indicate additional uses for macrolides
- Inhibition of mucus exocytosis associated with inhibition of synthesis
- Pharmacotherapy for airway mucus at an early stage, but promising!