Cytokines, Chemokines and their Receptors

Louise Donnelly

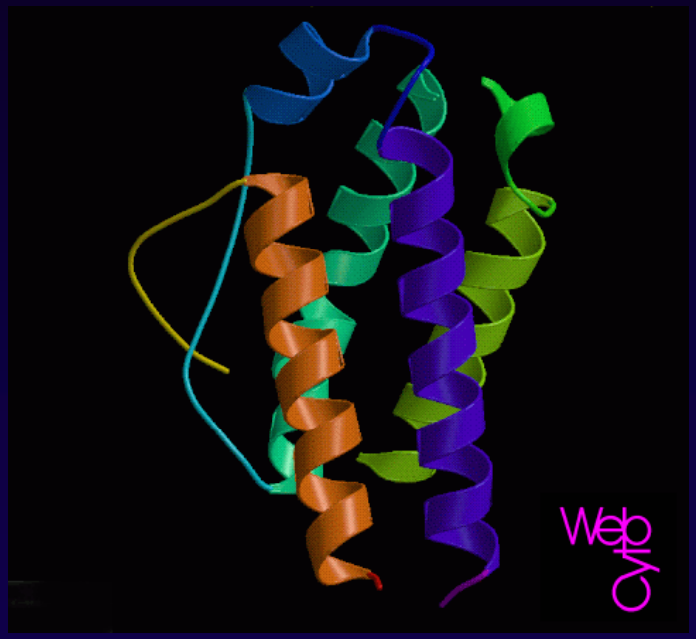
Definitions

Cytokines

Small secreted proteins which mediate and regulate immunity, inflammation, and haematopoiesis

Lymphokine – produced by lymphocytes
Monokine – produced by monocytes
Interleukin – made by one lymphocyte but acts on another
Chemokine – has chemotactic activity

Cytokines



Small proteins <30 kDa
Secreted by:
- T cells
- Macrophages
- BUT other cells too!

Four helix bundle
- most common eg IL-2

Cytokine Families

TNFα ↑ fever
Adhesion molecules
Mucus secretion
Neutrophil degranulation
Cytokine production

M-CSF – macrophages
G-CSF – neutrophils
GM-CSF – monocytes and granulocytes

Type I – virus infected cells
Type II – Th1, NK, MΦ

Differentiation, remodelling, wound healing

TNFα, IL-4 ↓ TGFβ1

Interleukins

IL-1 family

IL-1α, IL-1β, IL-18, IL-33 and IL-1ra

IL-4 and IL-13 → Th2

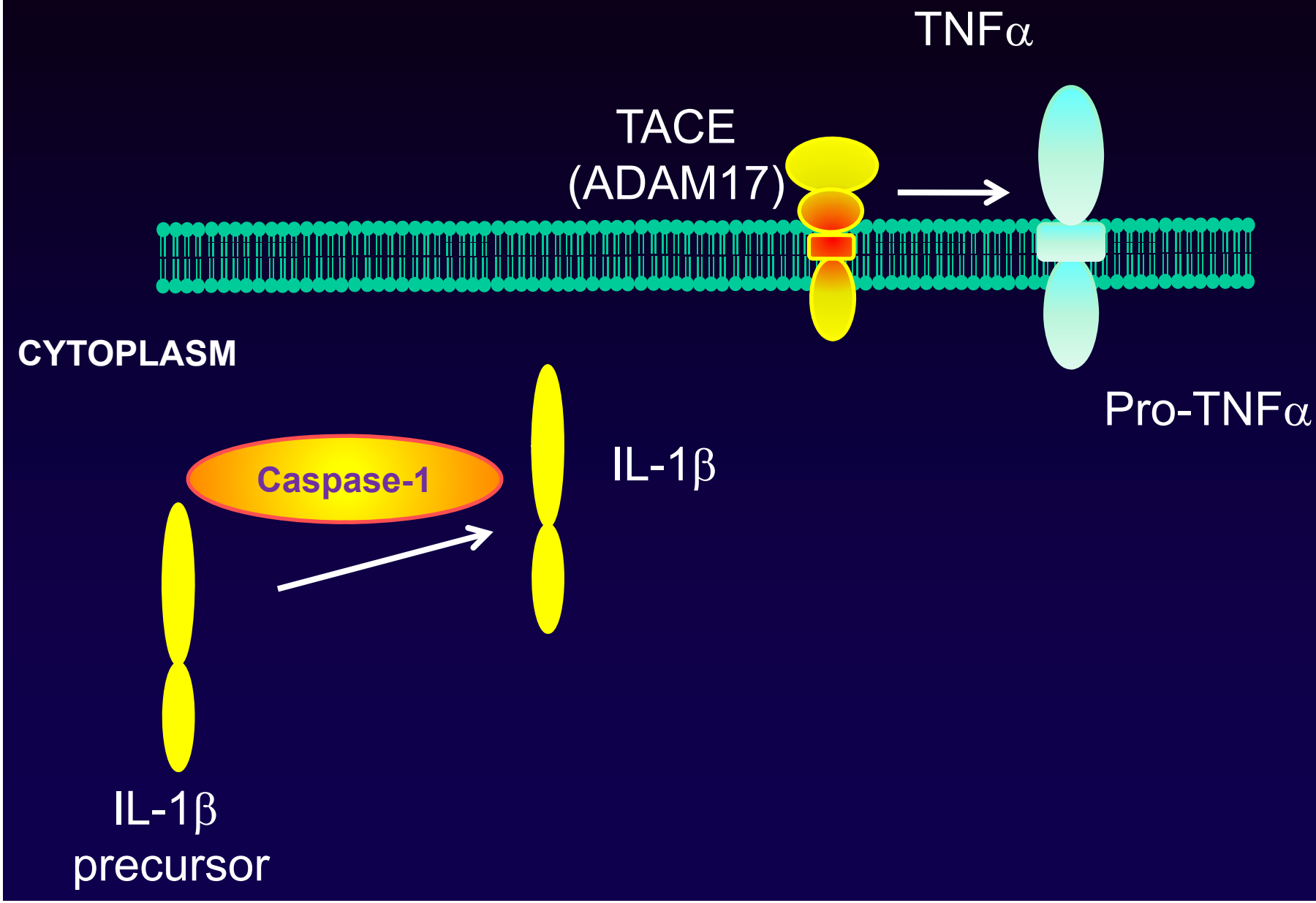
IL-10 family

IL-10, IL-19, IL-20, IL-22, IL-24, IL-26
IL-28, IL-29

IL-12 family → Th1

IL-12, IL-23, IL-27

Cytokine Processing



Cytokine Receptors

Cytokine receptor family I – recognise 4 helical cytokines

eg. IL-5, IL-6, IL-9

Haematopoeitin receptors

eg. GM-CSF, IL-2 receptors

Cytokine receptor family II

e.g. IFNα, IFNγ

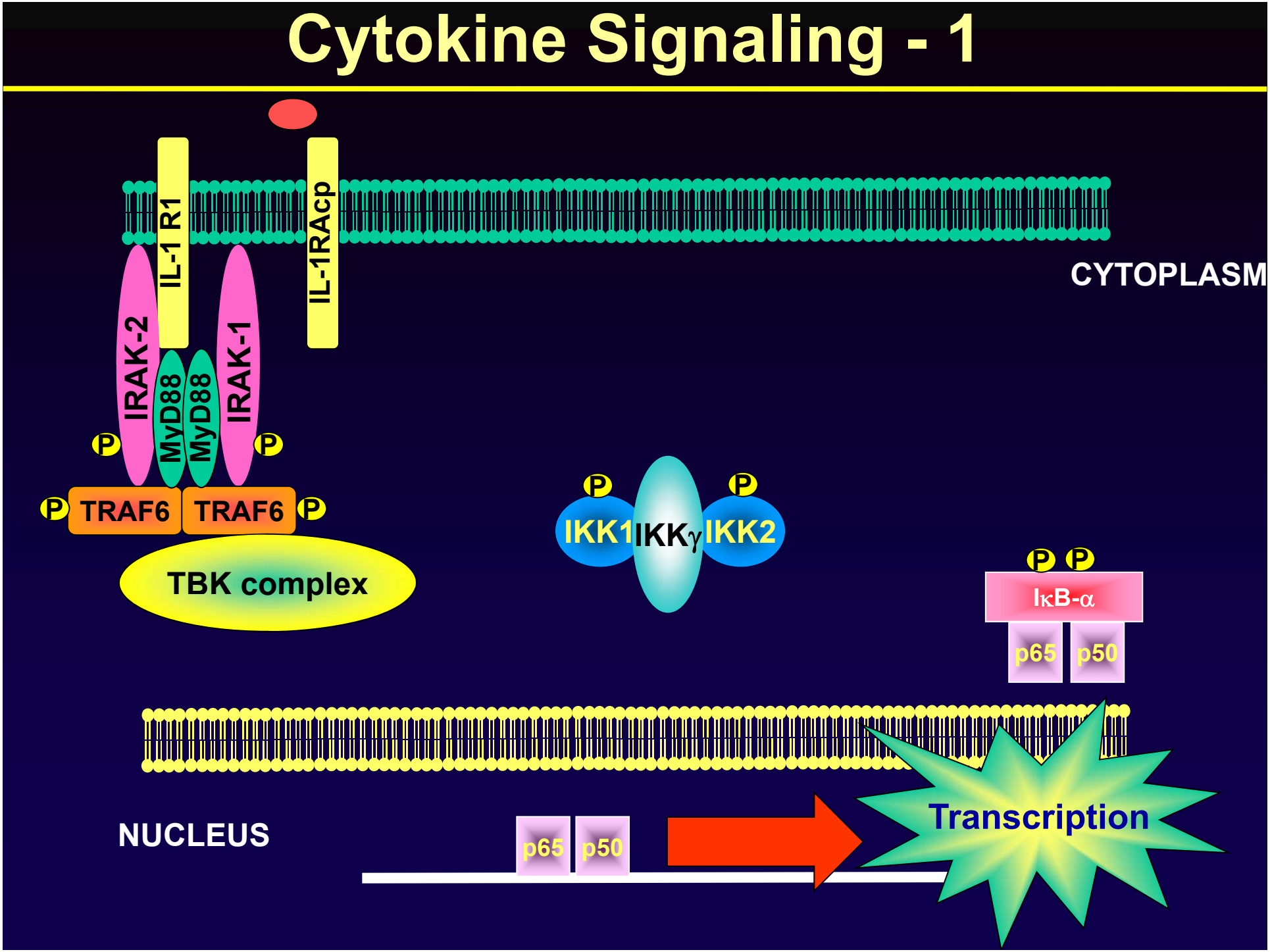
Ig superfamily

e.g IL-1, IL-18, CSF-1

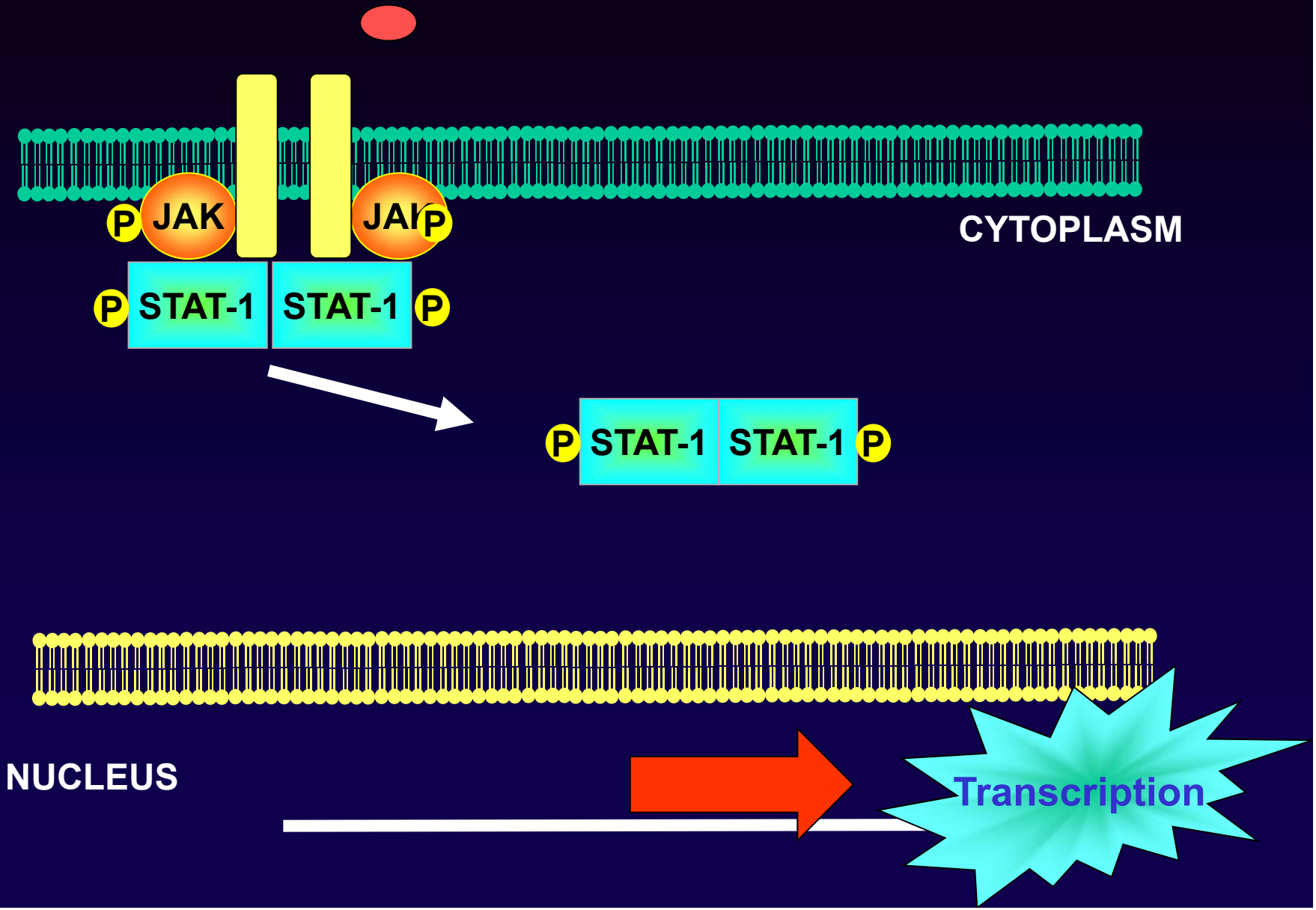
TNF receptor family

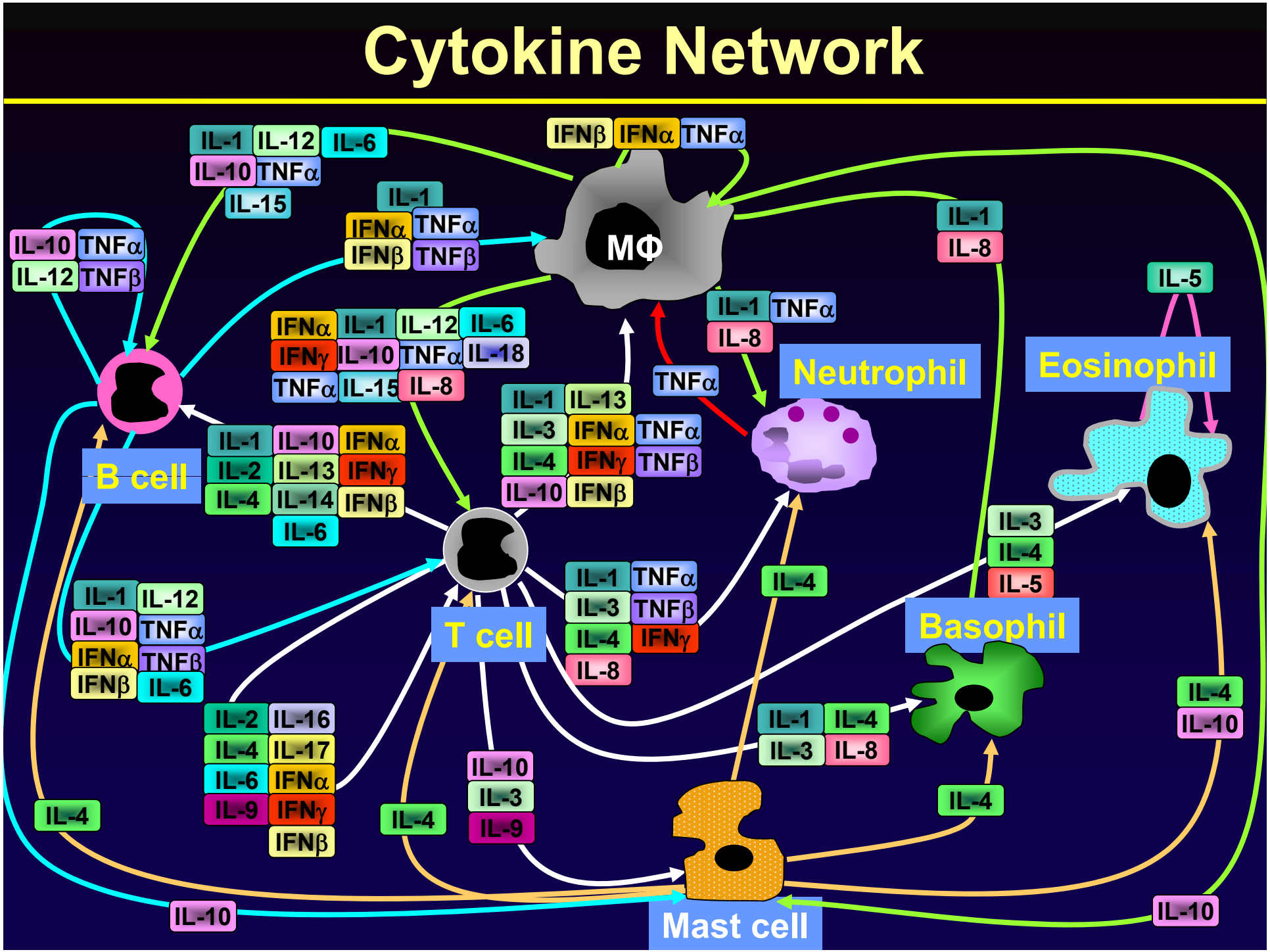
TNFα

Cytokine Signaling - 1



Cytokine Signaling - 2




Cytokine Network
IL-1 IL-12 IL-6
IL-10 TNFα
IL-15
IFNβ IFNα TNFα
IL-1
IFNα TNFα
IFNβ TNFβ
MΦ
IL-1
IL-8
IL-10 TNFα
IL-12 TNFβ
IL-5
IL-1 TNFα
IL-8
IFNα IL-1 IL-12 IL-6
IFNγ IL-10 TNFα IL-18
TNFα IL-15 IL-8
TNFα
Neutrophil
Eosinophil
IL-1 IL-13
IL-3 IFNα TNFα
IL-4 IFNγ TNFβ
IL-10 IFNβ
IL-1 IL-10 IFNα
IL-2 IL-13 IFNγ
IL-4 IL-14 IFNβ
IL-6
B cell
IL-3
IL-4
IL-5
IL-4
IL-1 TNFα
IL-3 TNFβ
IL-4 IFNγ
IL-8
T cell
Basophil
IL-1 IL-12
IL-10 TNFα
IFNα TNFβ
IFNβ IL-6
IL-2 IL-16
IL-4 IL-17
IL-6 IFNα
IL-9 IFNγ
IFNβ
IL-1 IL-4
IL-3 IL-8
IL-4
IL-10
IL-4
IL-10
IL-3
IL-9
IL-4
IL-4
IL-10
Mast cell
IL-10

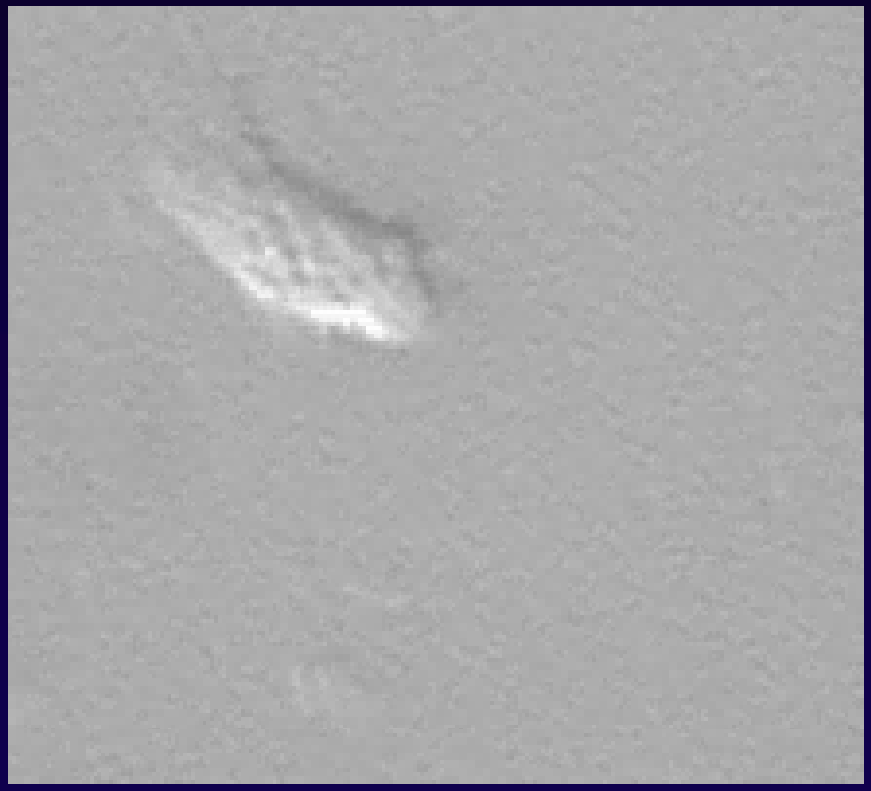
Cytokine Actions

Autocrine – act on self secreting cells
Paracrine - act on nearby cells
Endocrine – act on distant cells

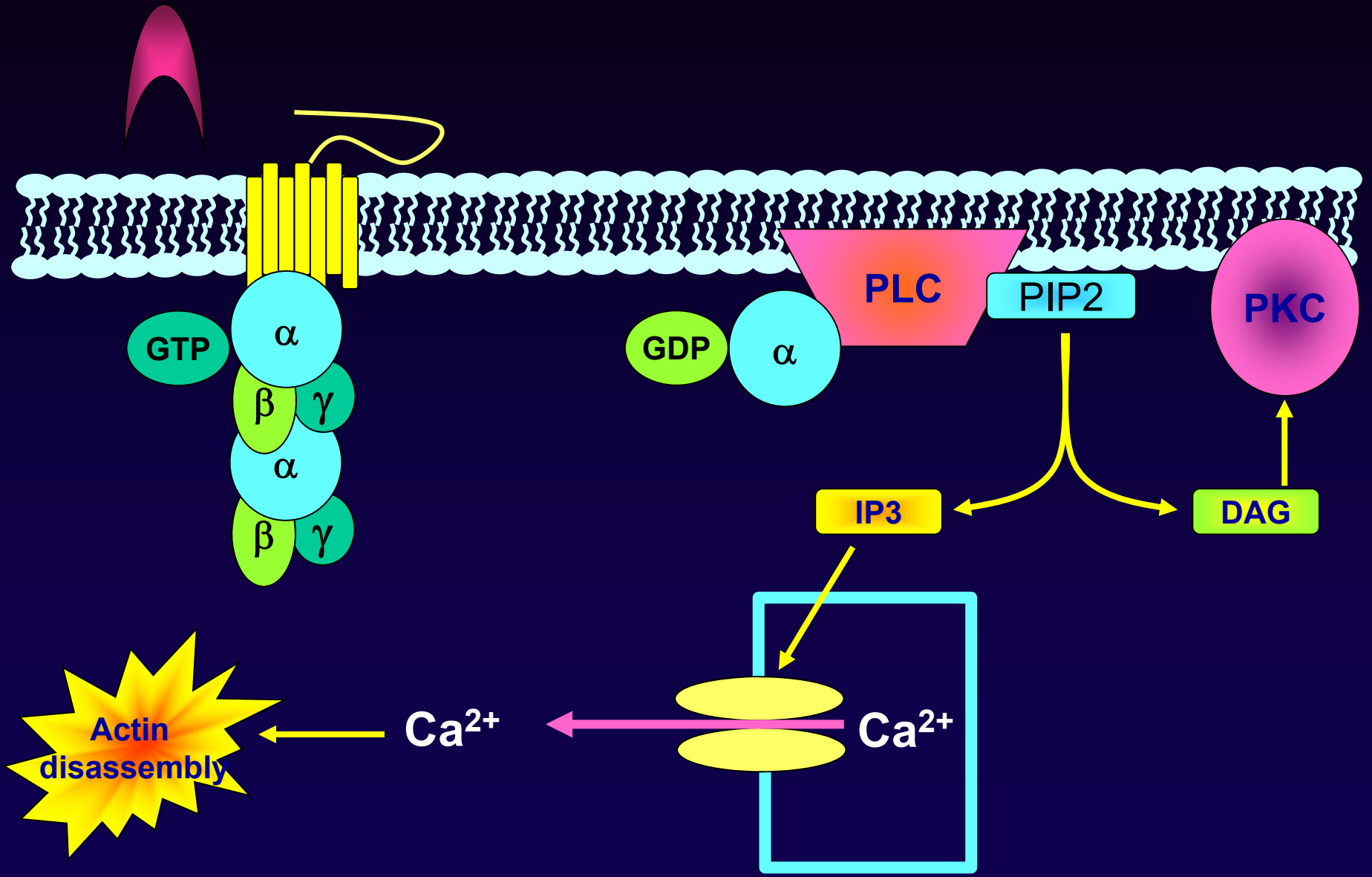
Pleiotropic – single cytokine can act on more than one cell type
Redundant – similar functions can be elicited by different cytokines

Chemokines

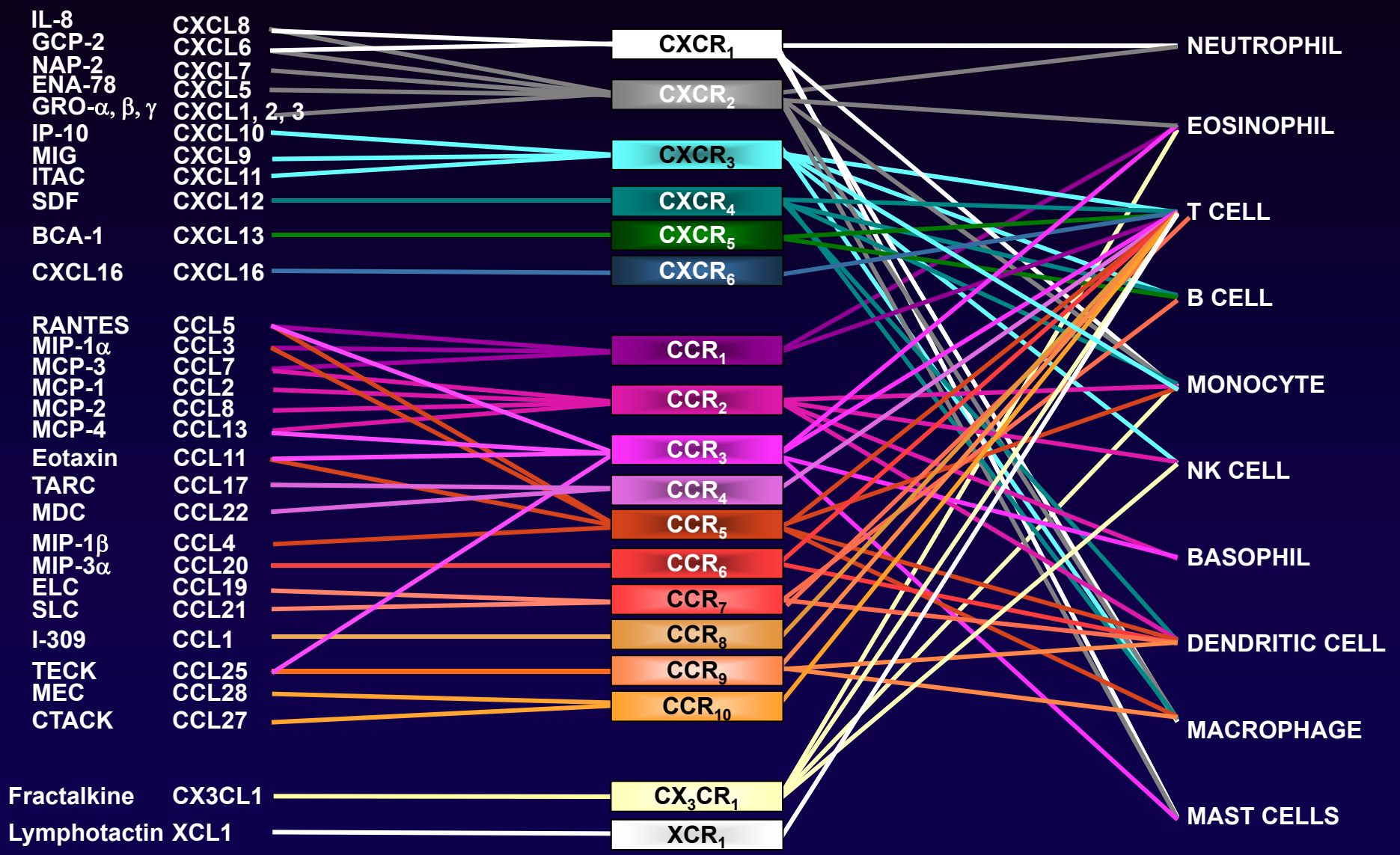
Cytokines that cause cell migration



Chemokine Receptor Signaling



Chemokine Receptor Redundancy

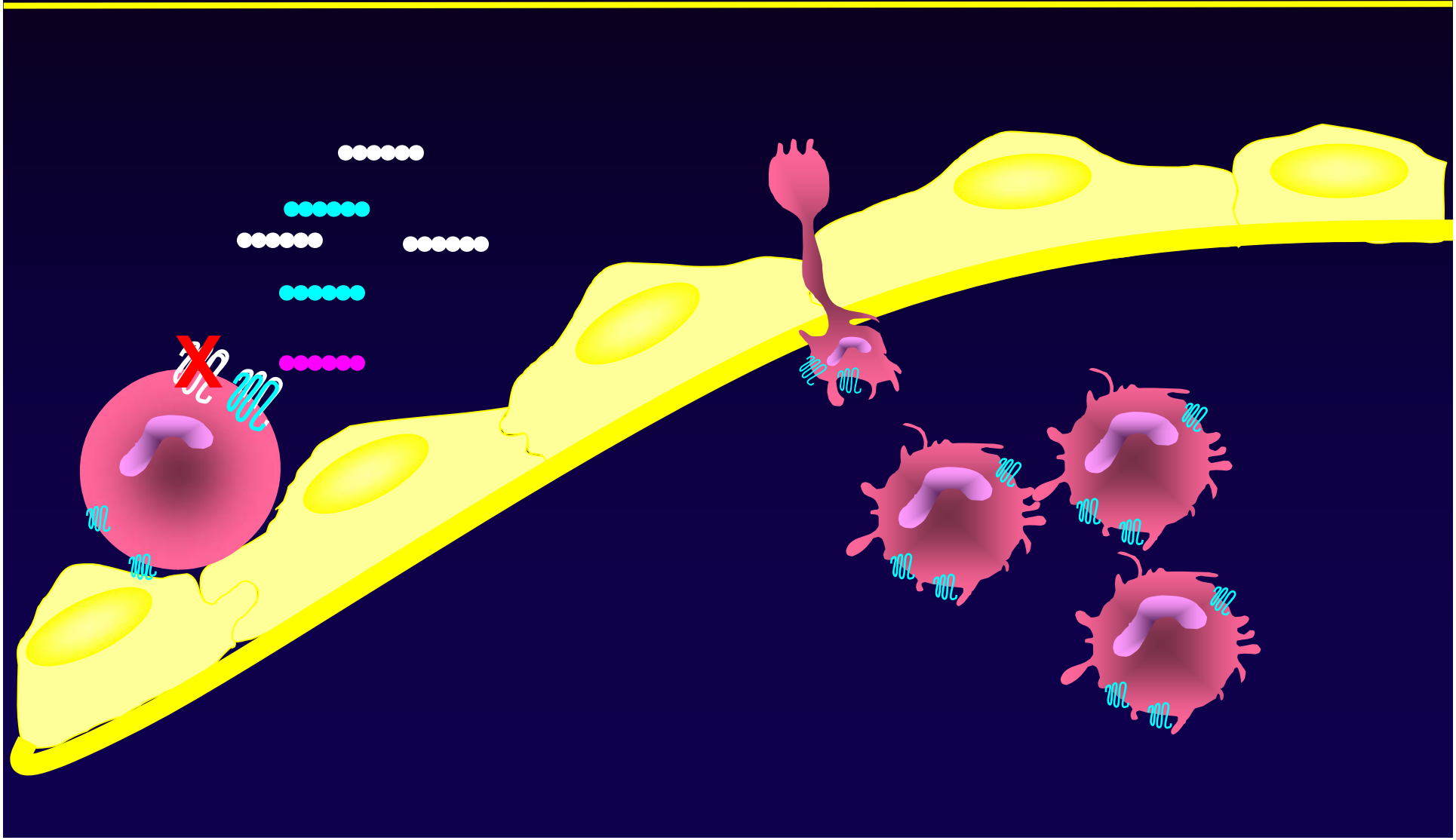


Redundancy

1. More than one chemokine/receptor

2. More than one receptor type/cell

Chemokine Receptor Redundancy



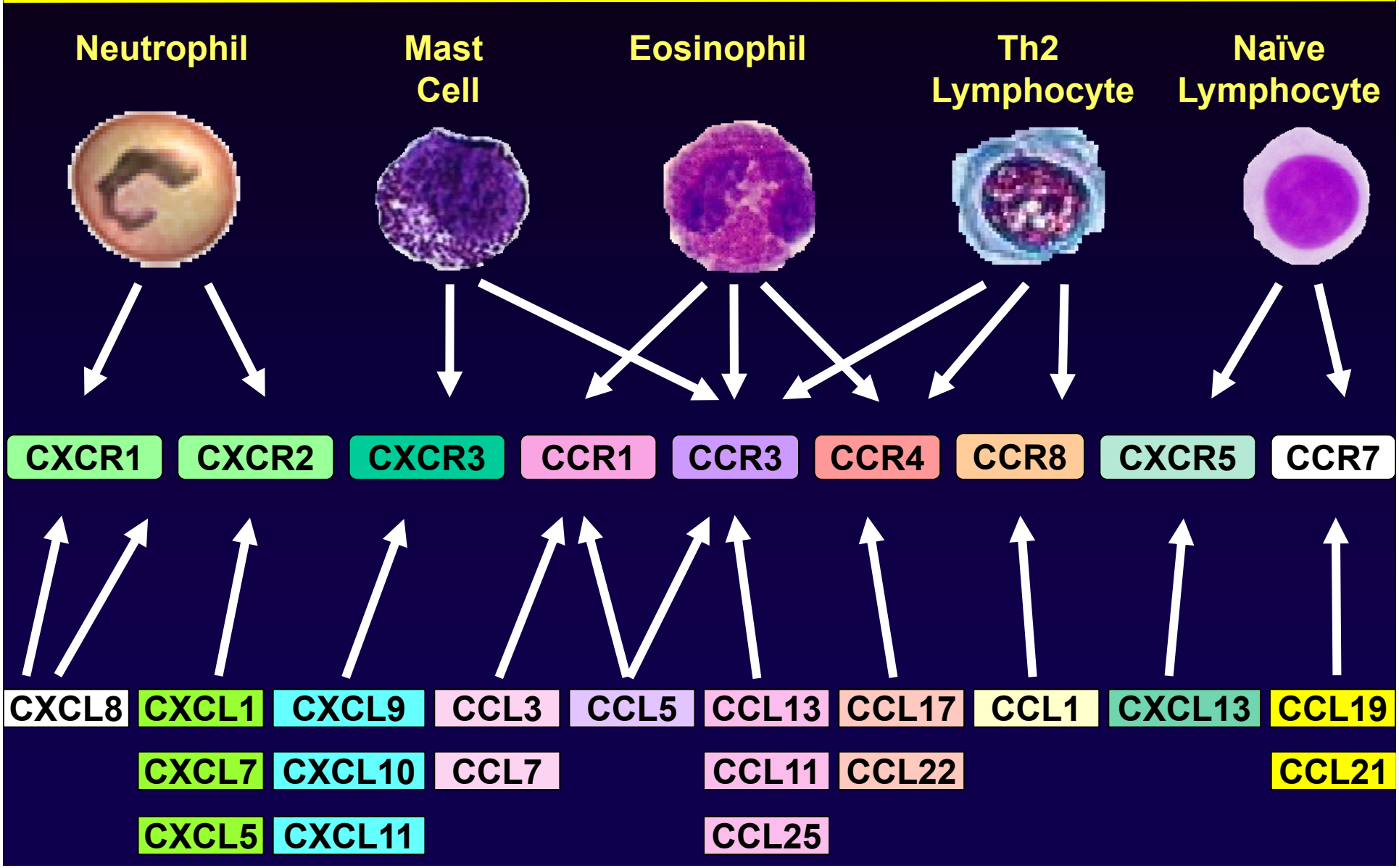
Redundancy - Assumptions

1. Different chemokines are equipotent at a single receptor

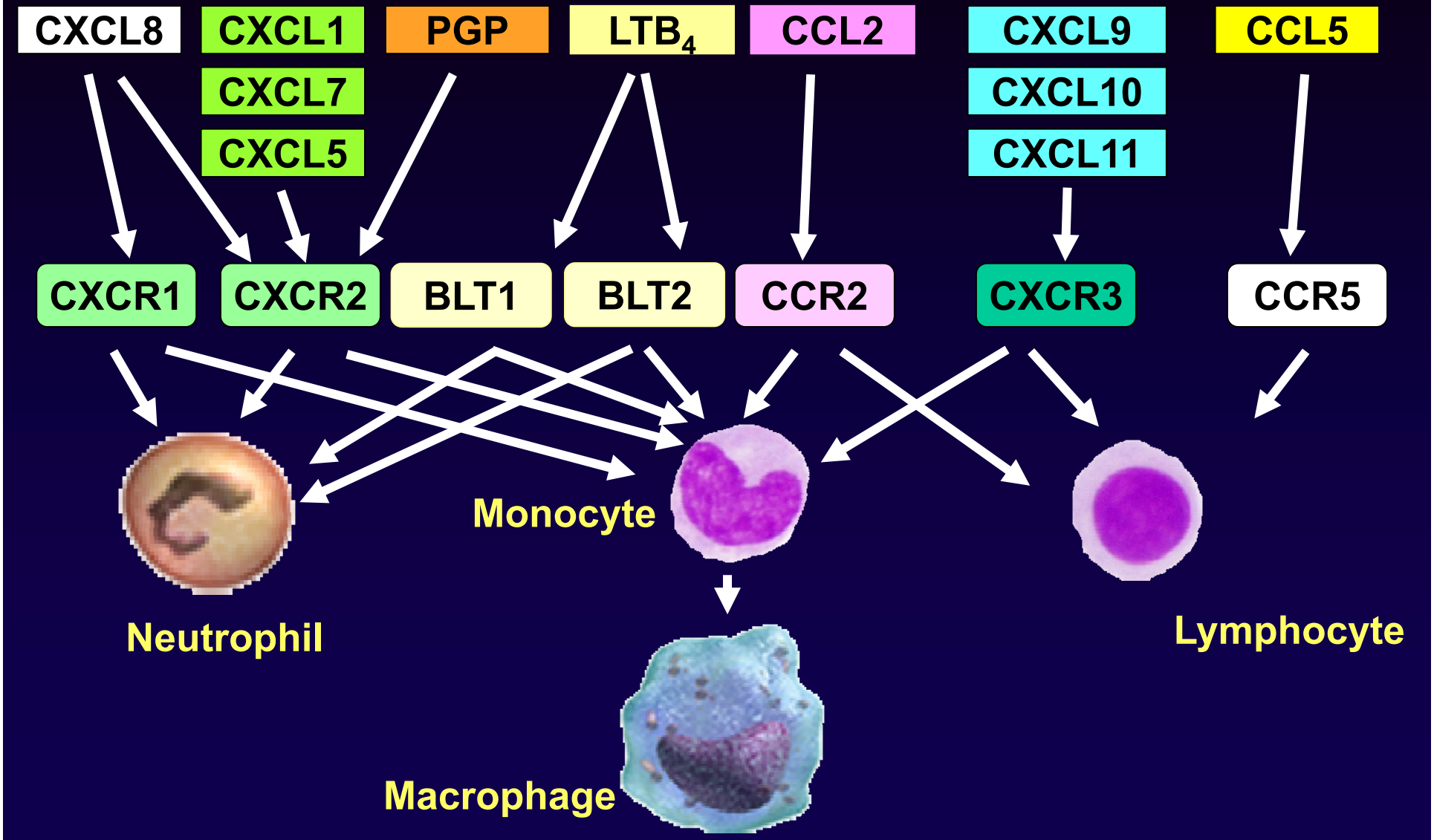
2. Different chemokines have the same function at a single receptor

3. Different chemokine receptors on a single cell have the same function

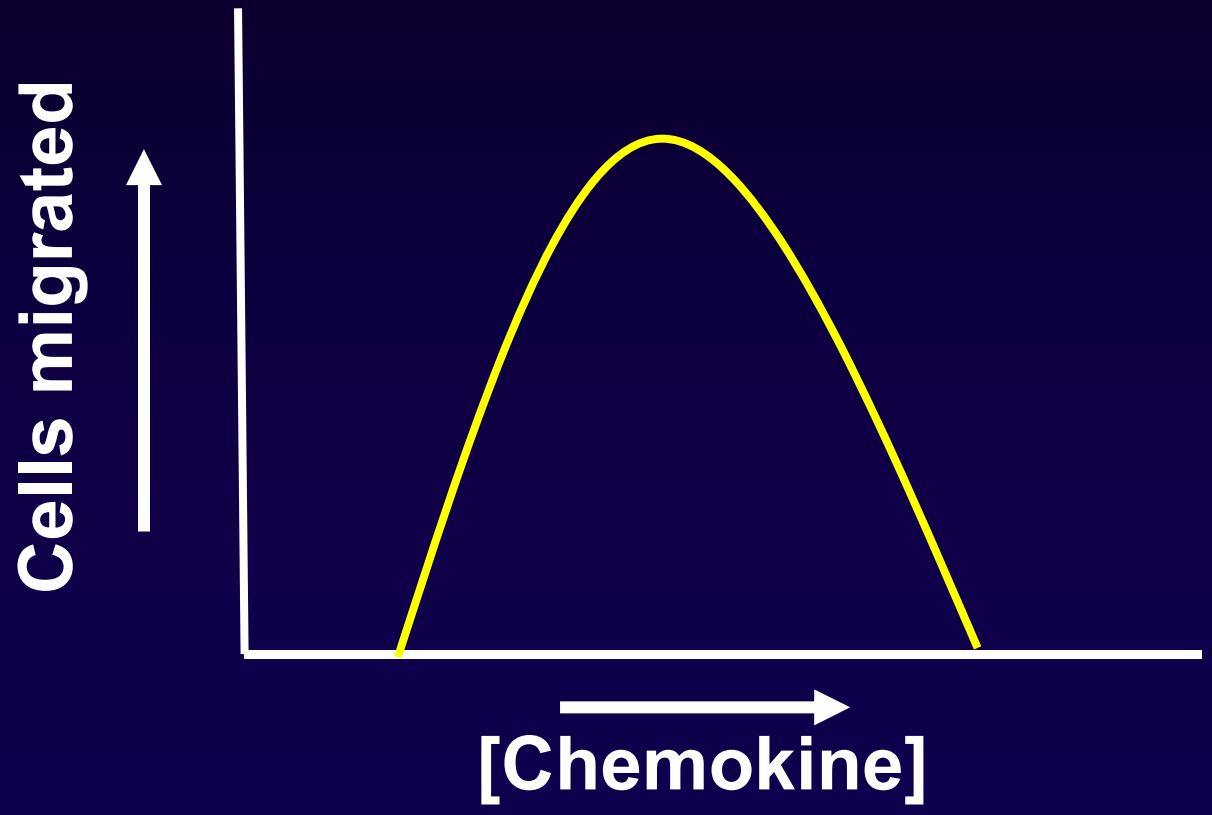
Which chemokine receptors to target? Asthma



Which chemokine receptors to target? COPD



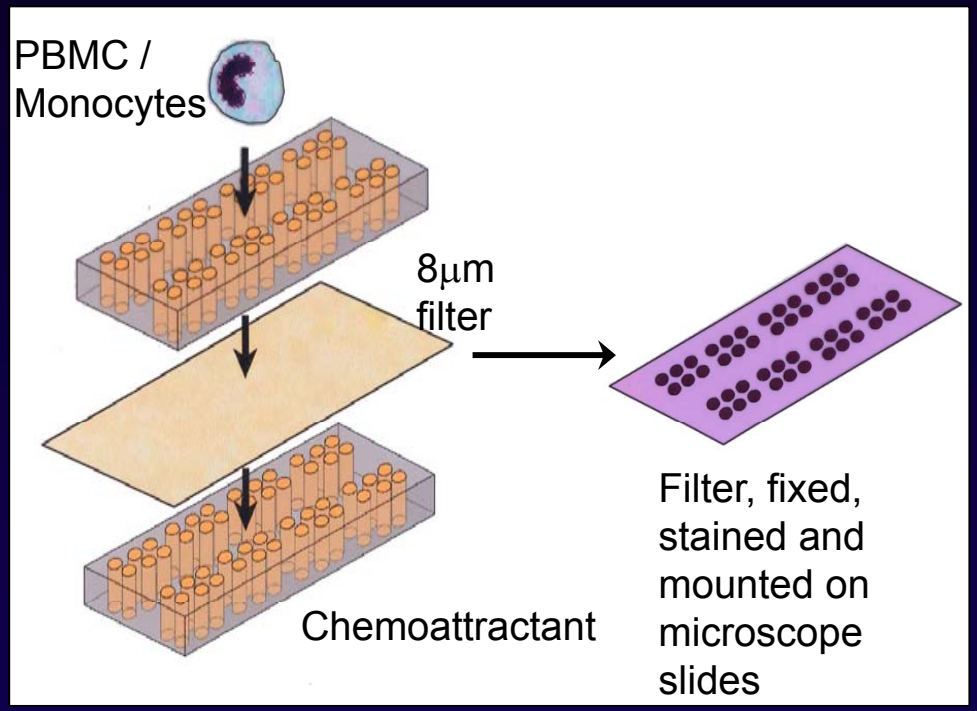
Bell-shaped Chemotactic Response



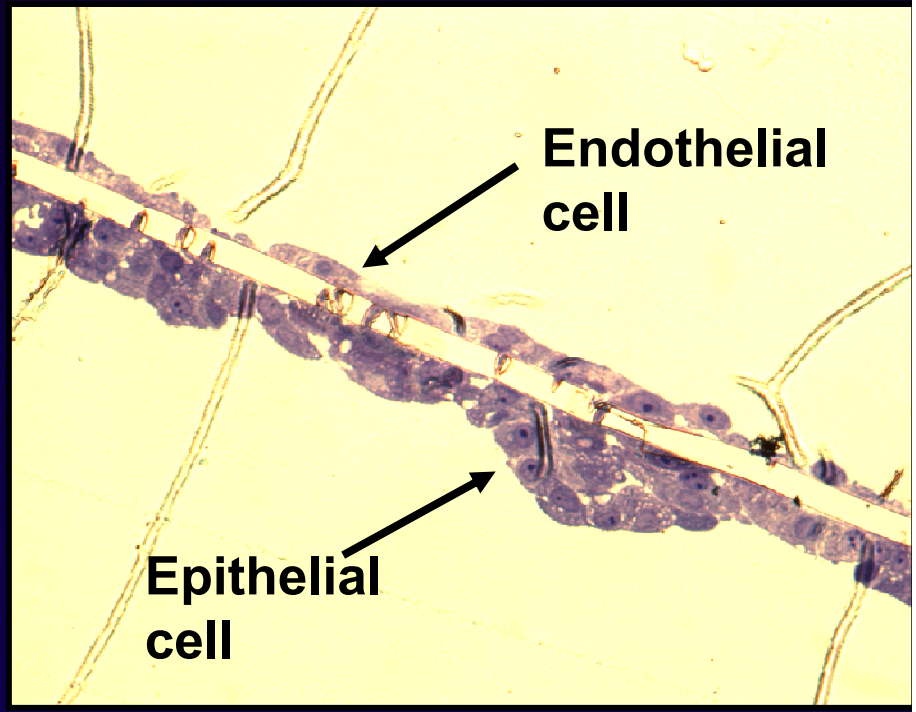
Assessing Antagonists

Chemotaxis assays

Boyden Chamber



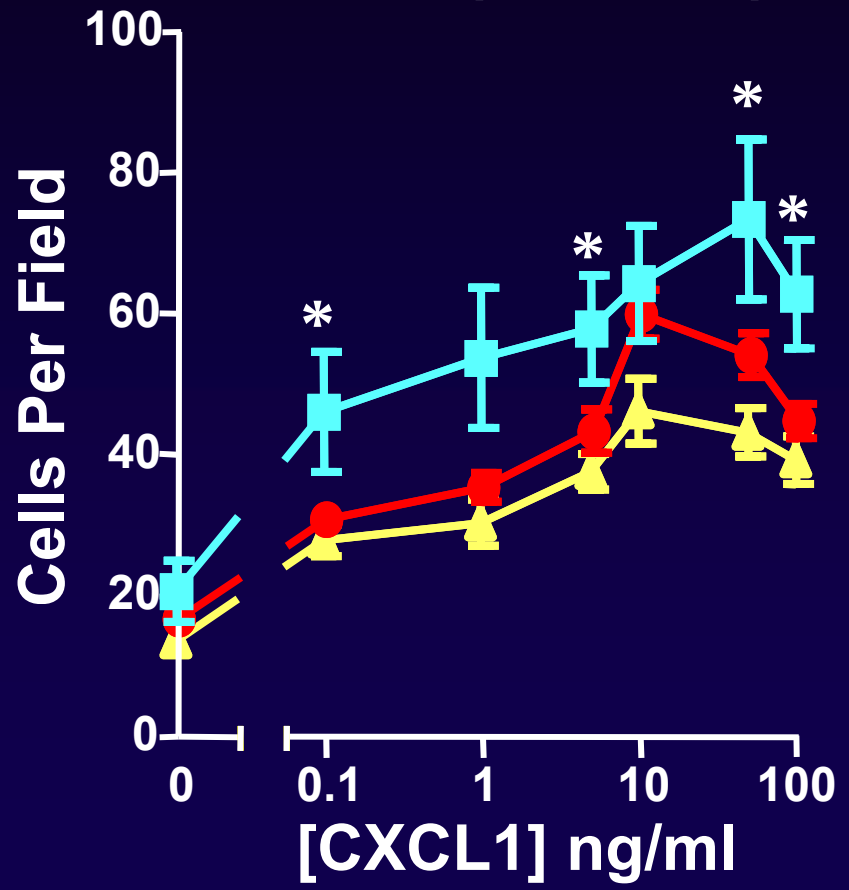
Transwell systems

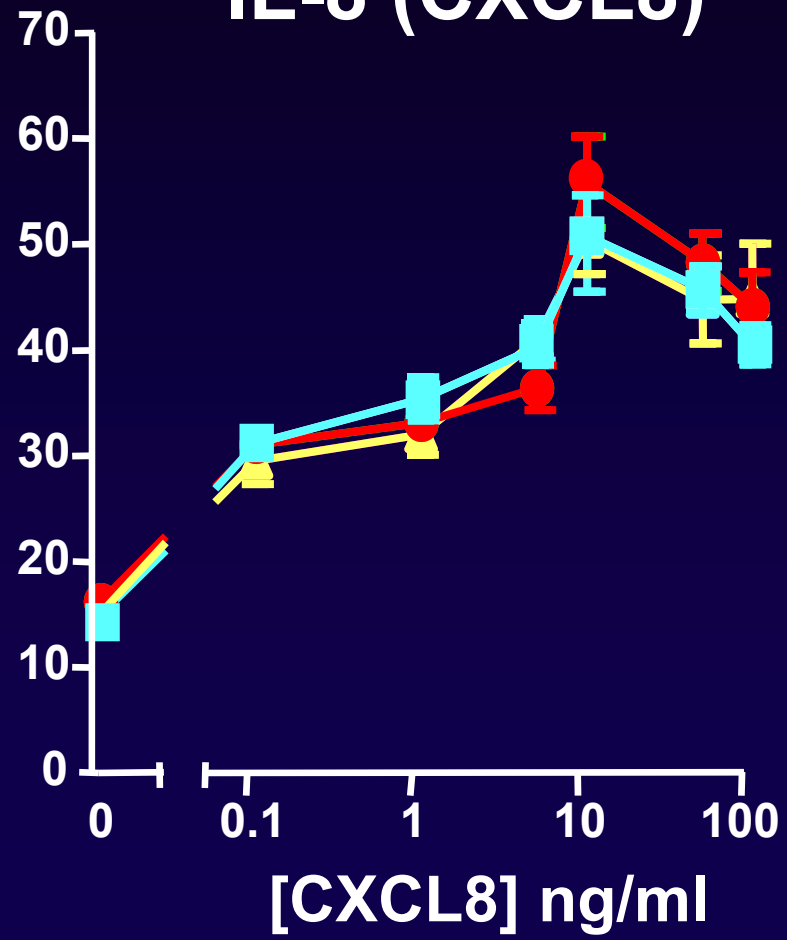


Surrogates – Shape change assays, Ca^{2+} mobilization

PBMC Migration

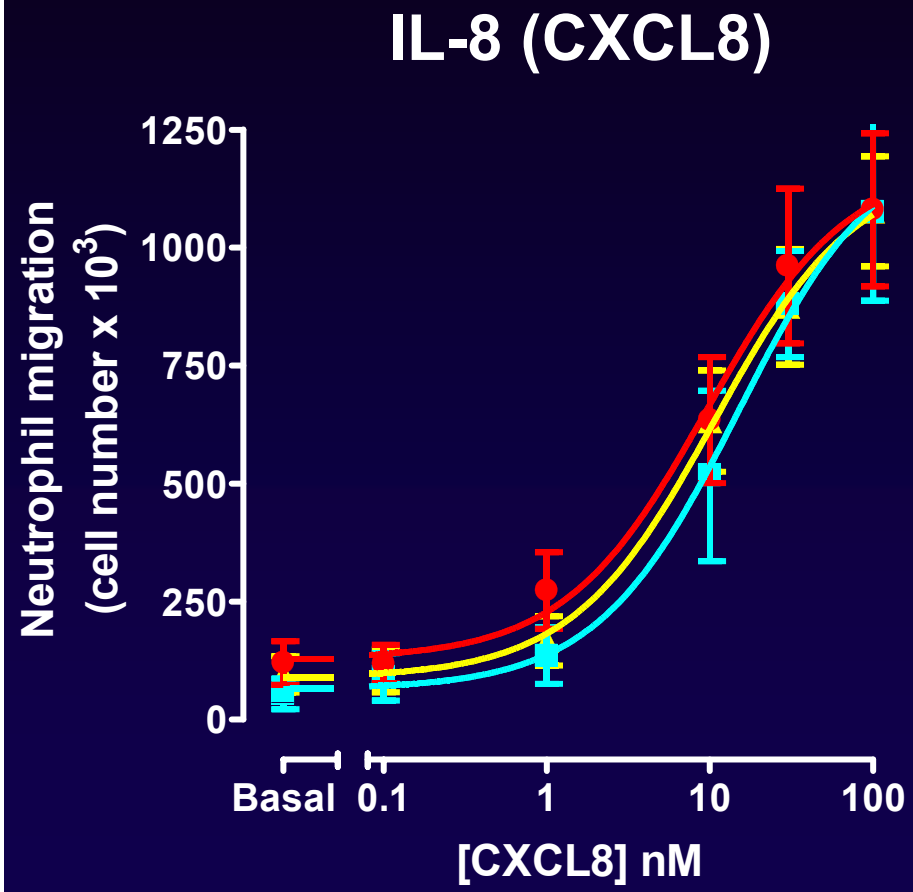
(Non-smokers (n=8), Smokers (n=8), COPD (n=10))

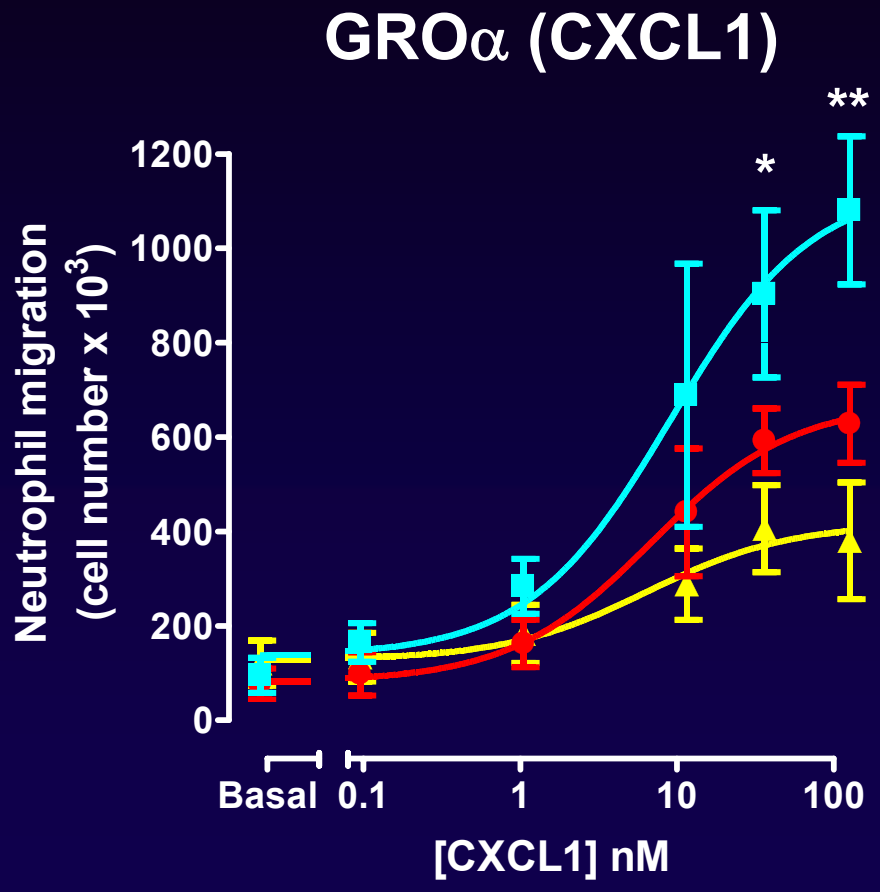




Traves *et al*., 2004 J. Leukoc. Biol

Neutrophil Migration to CXCL8 (IL-8) and CXCL1 (GROα)





▲ Non-smokers n=6 ● Smokers n=6 ■ COPD n=6

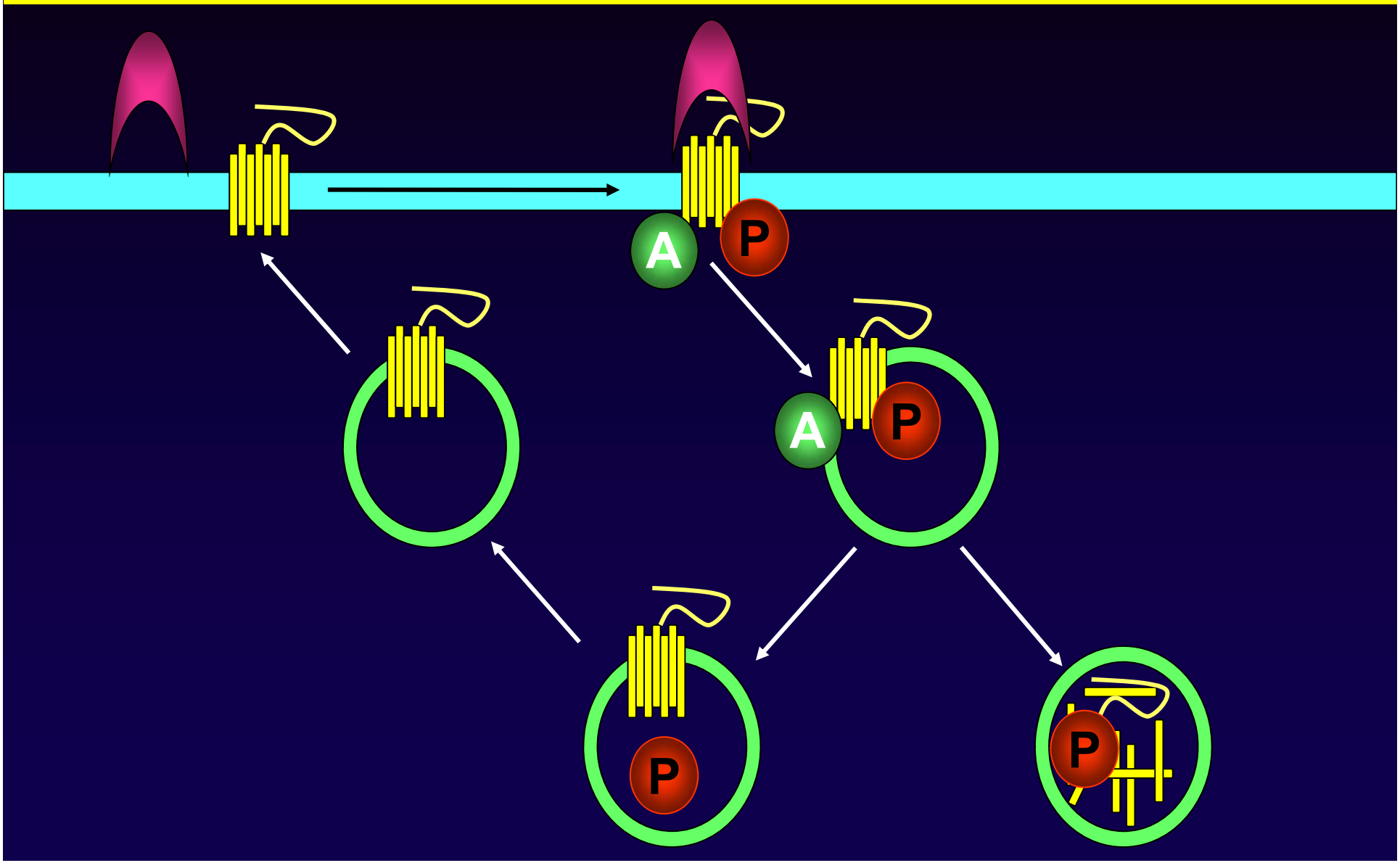
Receptor Expression by FACS

- $CXCR_1$ and $CXCR_2$
  - No difference in expression between the groups
  - Approx 30-40%

Are differences in migration due to differences in regulation of receptor expression?

Receptor recycling

Receptor Recycling



Summary

- Differential effects of GROα and IL-8 suggest non-redundancy of chemokine function

- This may be due to differences in receptor recycling

  Supporting evidence:

  IL-8, GROα and NAP-2 bind to different amino acids in the CXCR2 receptor

  Katancik *et al* 2000 Cytokine

  IL-8 and NAP-2 lead to differential phosphorylation of the CXCR2 receptor

  Ben-Baruch *et al* 1997 J. Immunol

Clinical Trials – CXCR1/2 Antagonist

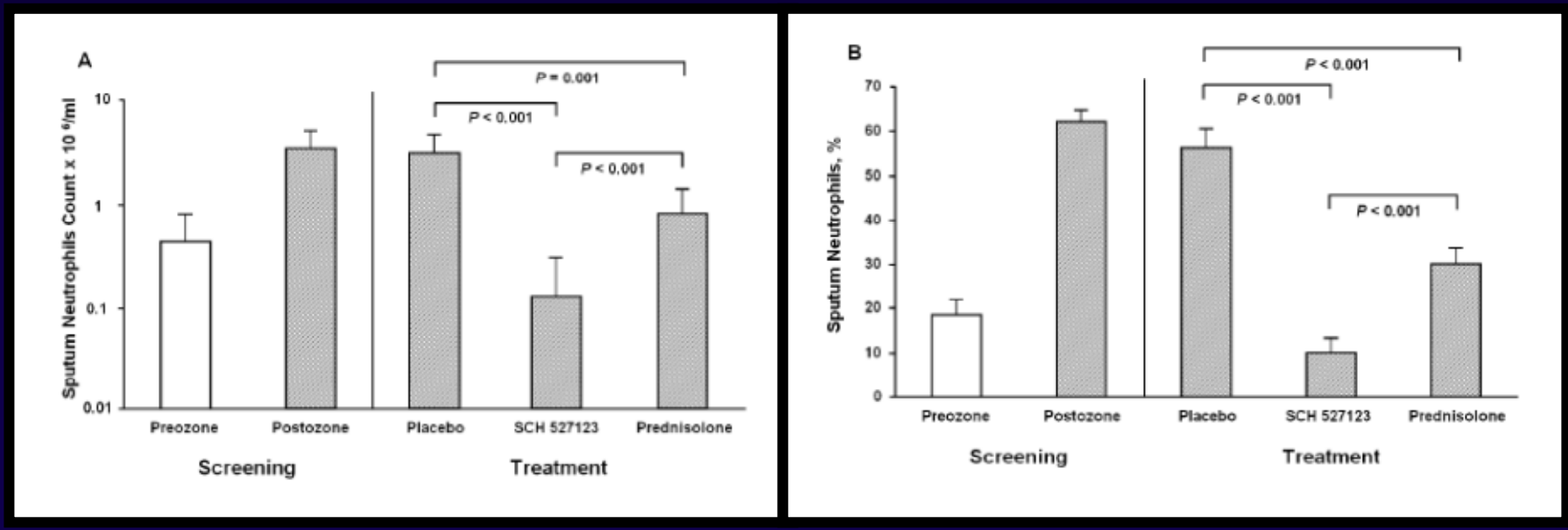
SCH 527123

Double blind, placebo controlled trial

SCH 527123 50mg for 4 days – healthy volunteers

Ozone challenge – induce sputum neutrophilia

Results



Holz *et al.*, ERJ, 2010

Clinical Trials – CXCR1/2 Antagonist

SCH 527123

Double blind, placebo controlled, escalating dose trial

SCH 527123 – 12 weeks 3,10, 30mg – COPD patients

Results

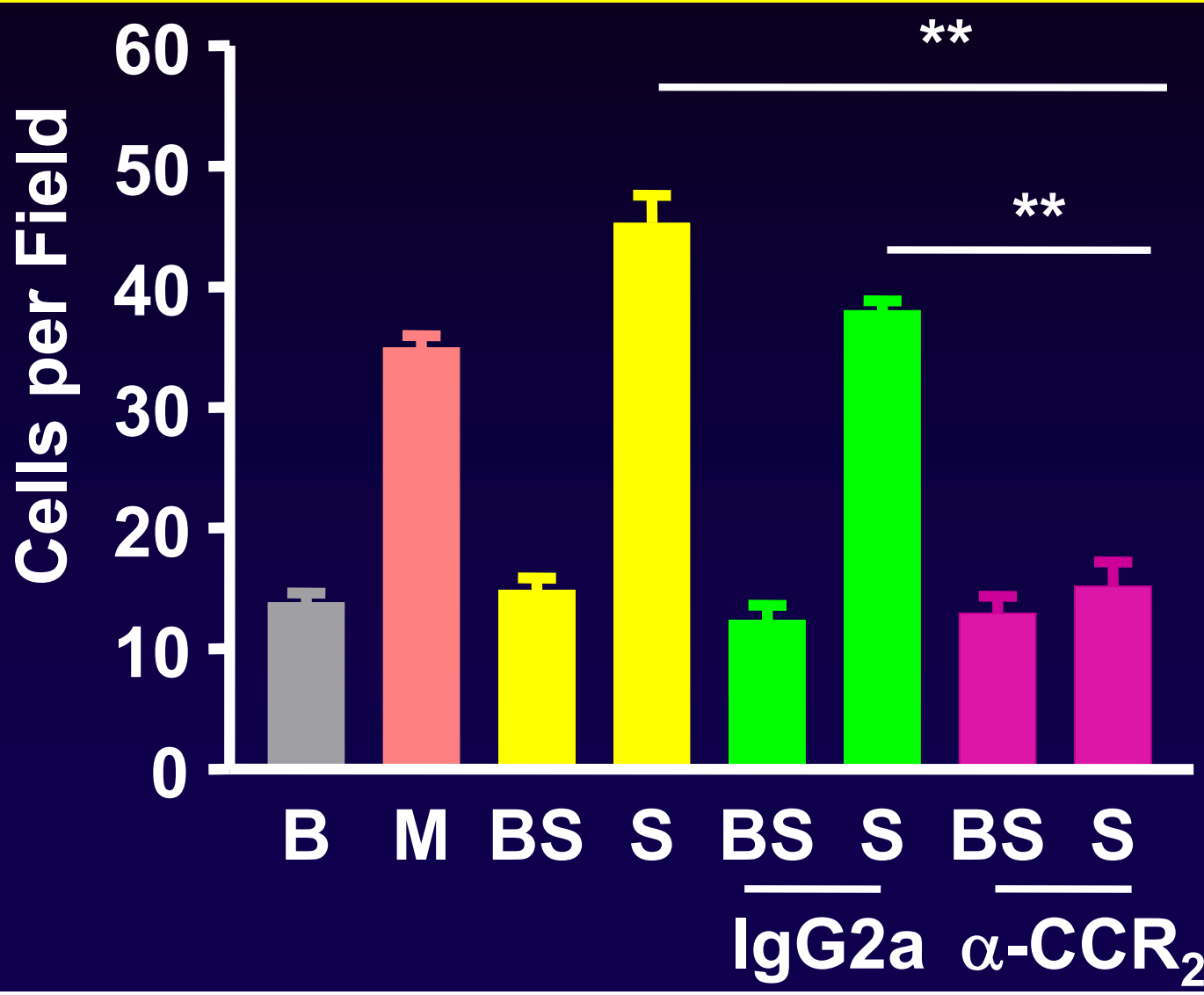
↓ Sputum neutrophils (47%)

↓ Sputum MMP-9 (59%)

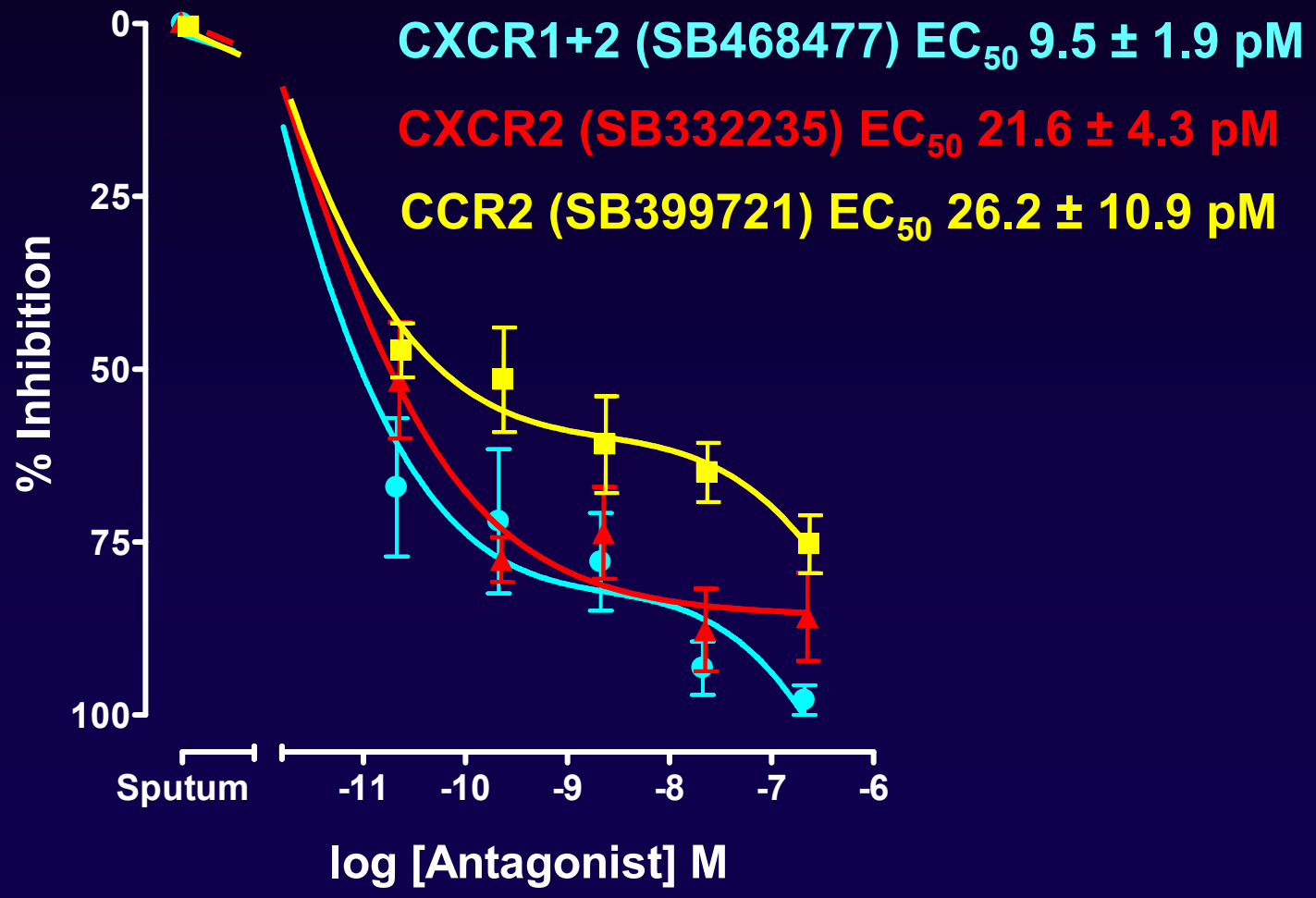
↑FEV_1 (91ml)

Magnussen *et al*., ERS, 2010

Effect of 10μg/ml Antibody on Monocyte Chemotaxis to Sputum



Effect of Receptor Antagonists on Monocyte Chemotaxis to Sputum



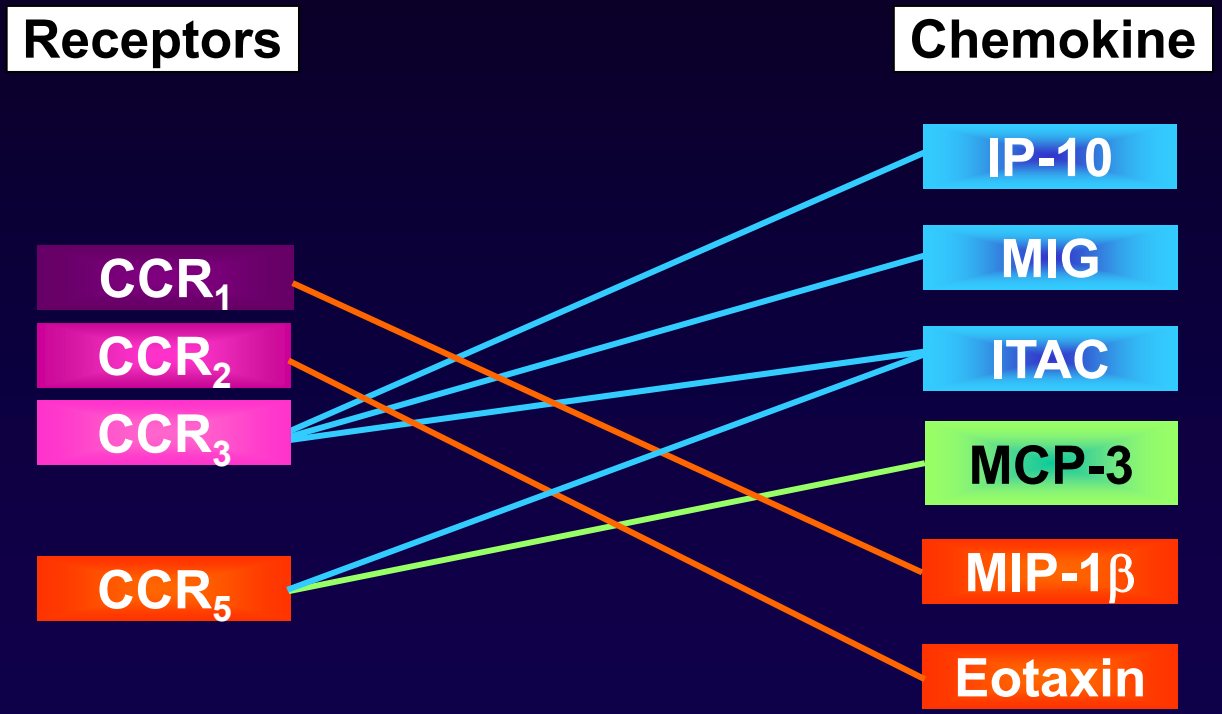
Chemokine Receptor Antagonism

Antagonists are more effective than IC_{50} values would suggest

SB468477:	**CXCR1 67 nM** **CXCR2 12 nM**
SB332235:	**CXCR2 19 nM**
SB399721:	**CCR2 219 nM**

More efficacious than would be predicted

Natural Chemokine Receptor Antagonists



Summary

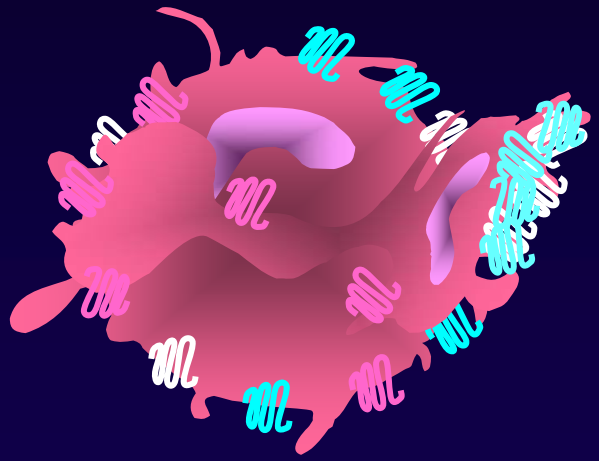
- Measuring effect of a chemokine receptor antagonist *vs* a single agonist does not predict efficacy *vs* a complex inflammatory infiltrate
- Chemokine receptor antagonists have increased efficacy *vs* complex inflammatory fluids (chemotaxis assay)

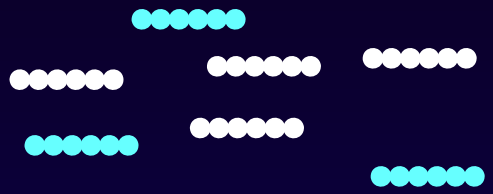
Why are antagonists more effective against mixtures?

Wang *et al* 2002 Nature Cell Biol

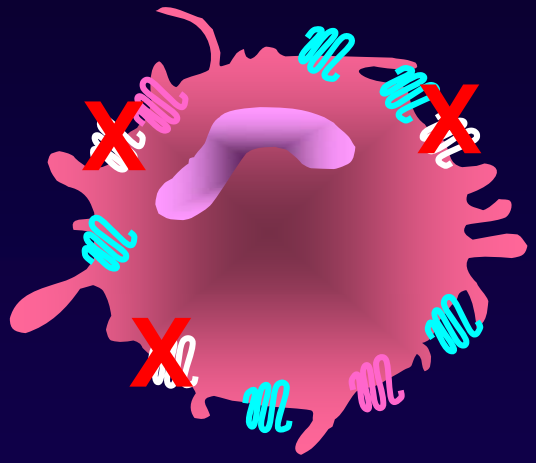
Chemotaxis to a single agonist

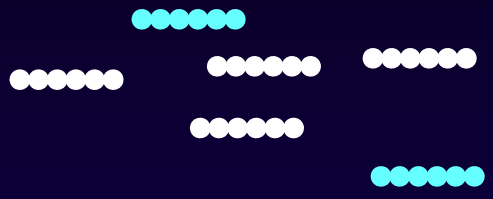
Chemotaxis to a mixed agonists





Chemotaxis to mixed agonists in the presence of an antagonist





Steric hindrance?

Other reasons

Receptor-Receptor interactions

- homodimerization
- heterodimerization
- oligomerization

Chemokine-Chemokine interactions

- heterodimerization

Receptor-Receptor Interactions

Homodimers

- CCR2, CCR5, CXCR2, CXCR4

Heterodimers

- CCR2-CCR5 →
- CCR2-CXCR4
- CCR5-CXCR4 →

CCR5 ligands inhibit CCR2 ligand binding

Modulate T cell function

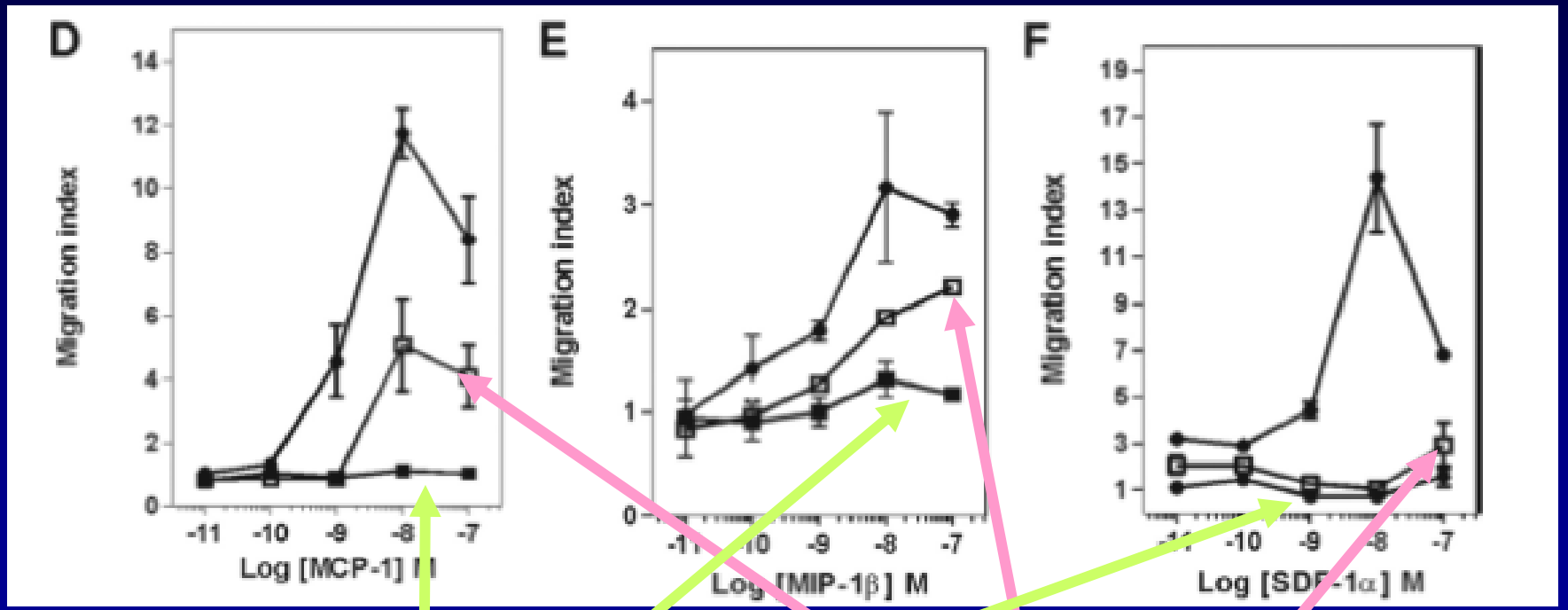
Contento *et al 2008* PNAS

Oligomers

- CCR2/CXCR4/CCR5/CXCR2

Human CD4+ Lymphocytes

CCR2 CCR5 CXCR4

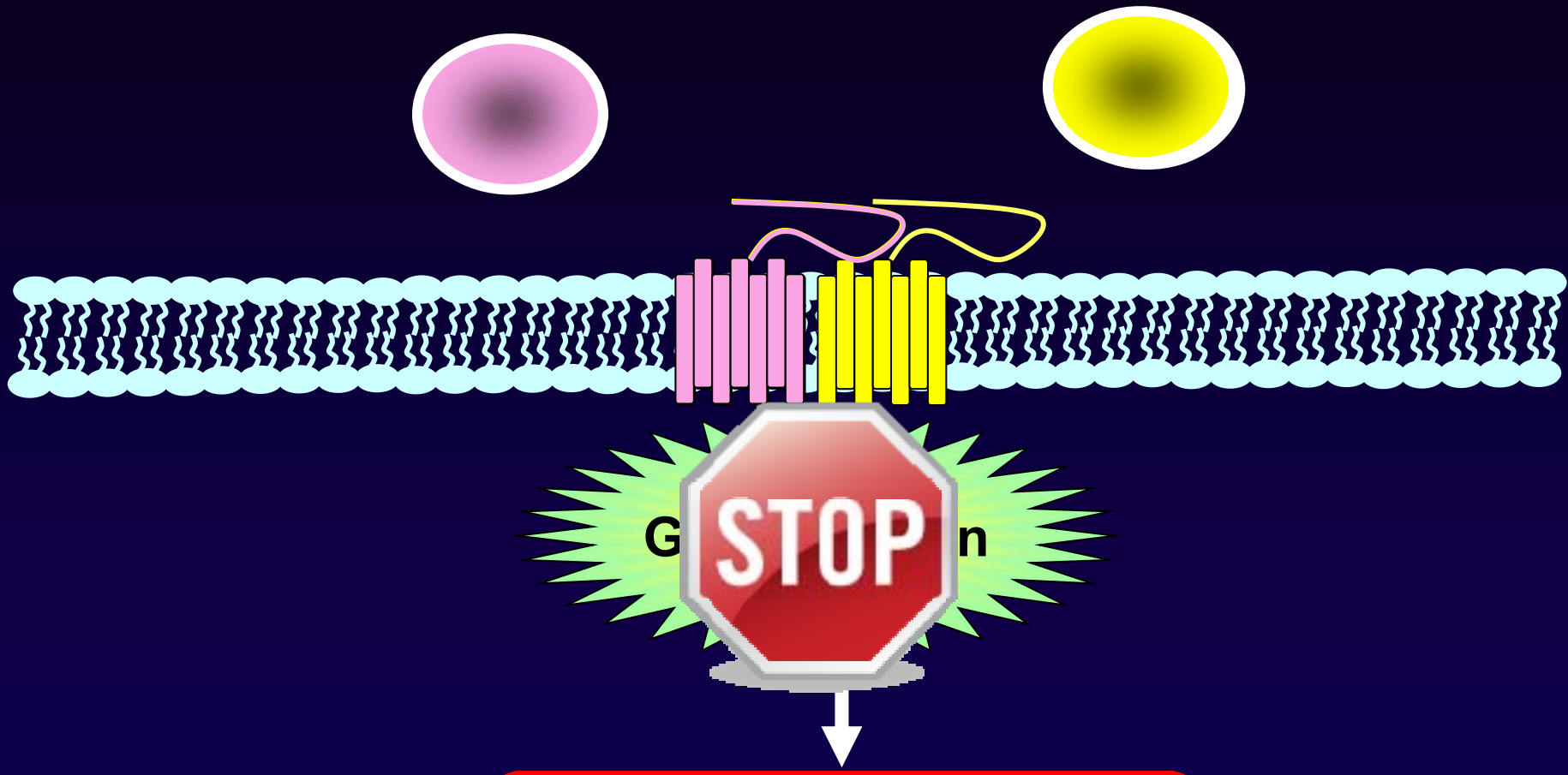


CCR2/5 antagonist

CXCR4 antagonist

Sohy *et al 2009* J Biol Chem

CXCR4 Opioid Receptor



Pello *et al 2008* Eur J Immunol

Chemokine Receptors – Dimers with other GPCR

CXCR2 - δ opioid receptor

+ CXCR2 Antagonist

Enhance δ opioid receptor signalling

Parenty *et al 2008* Biochem J

Chemokine-Chemokine Interactions

Homodimers

- IL-8, IP-10, MCP-1, RANTES, TARC, NAP-2 etc

→ **IL-8 dimers reduced CXCR1 binding**
Fernando *et al 2004* JBC

Heterodimers

- IL-8-PF-4
- MIP-1α-MIP-1β

→ **↑IL-8/CXCR2-dependent migration**
↑PF-4-dependent-anti-proliferative effect on EC
Nesmelova *et al 2005* JBC

Oligomers

TARC, PF-4, NAP-2 etc

Summary

Redundancy

- Little redundancy in the system
- Need to understand biology further

Target identification

- Need to understand receptor dimerization/oligomerization
- Cellular expression patterns

  eg. 40% monocytes express CXCR2
- Single antagonists *vs* dual antagonists(?)

Questions

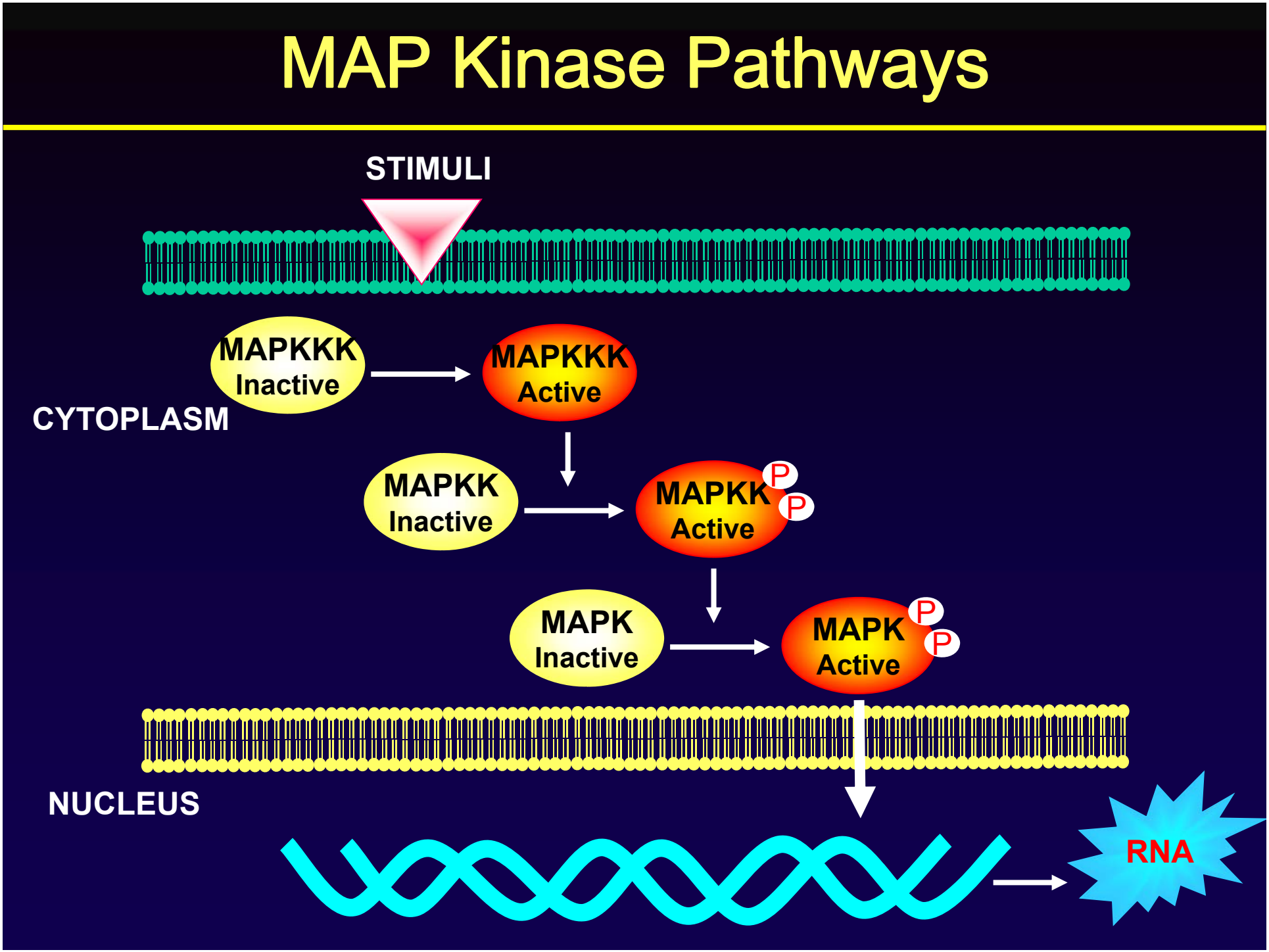
1. Is targeting a single cytokine a reasonable method for inhibiting inflammatory lung disease?

2. Would targeting specific signal transduction pathways produce more effective therapies?

3. How could you stop inflammatory cell migration?

4. Which chemokine/receptor would you select as a target for
   i) COPD?
   ii) Asthma?
   iii) CF?

MAP Kinase Pathways



MAP Kinase Pathways