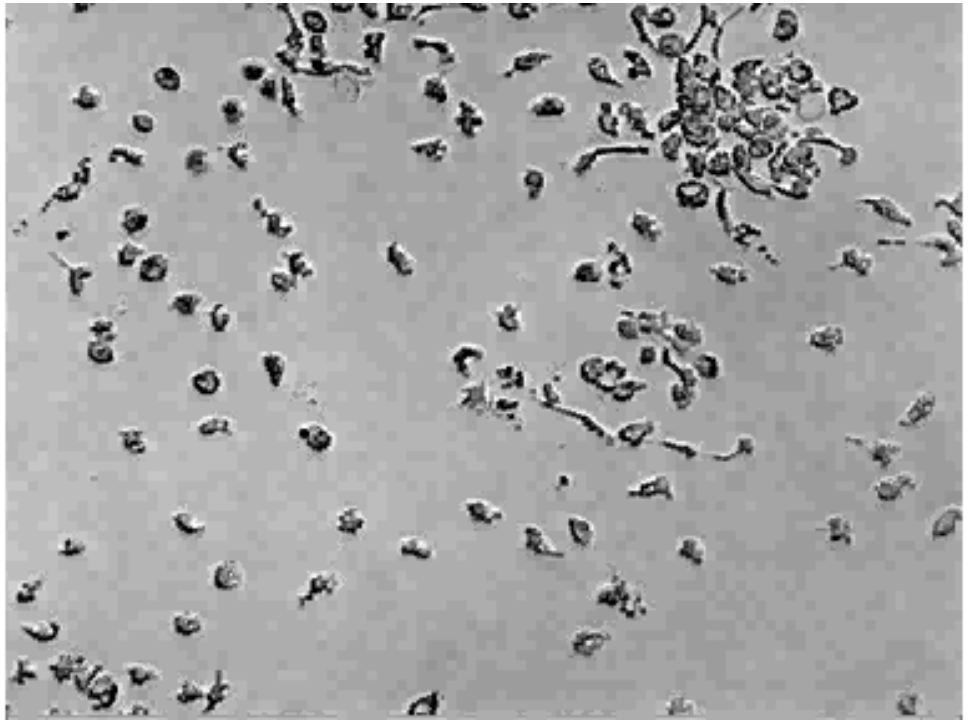
Chemotaxis
Chemotactic ligands



Inflammation Biology
Elective Course

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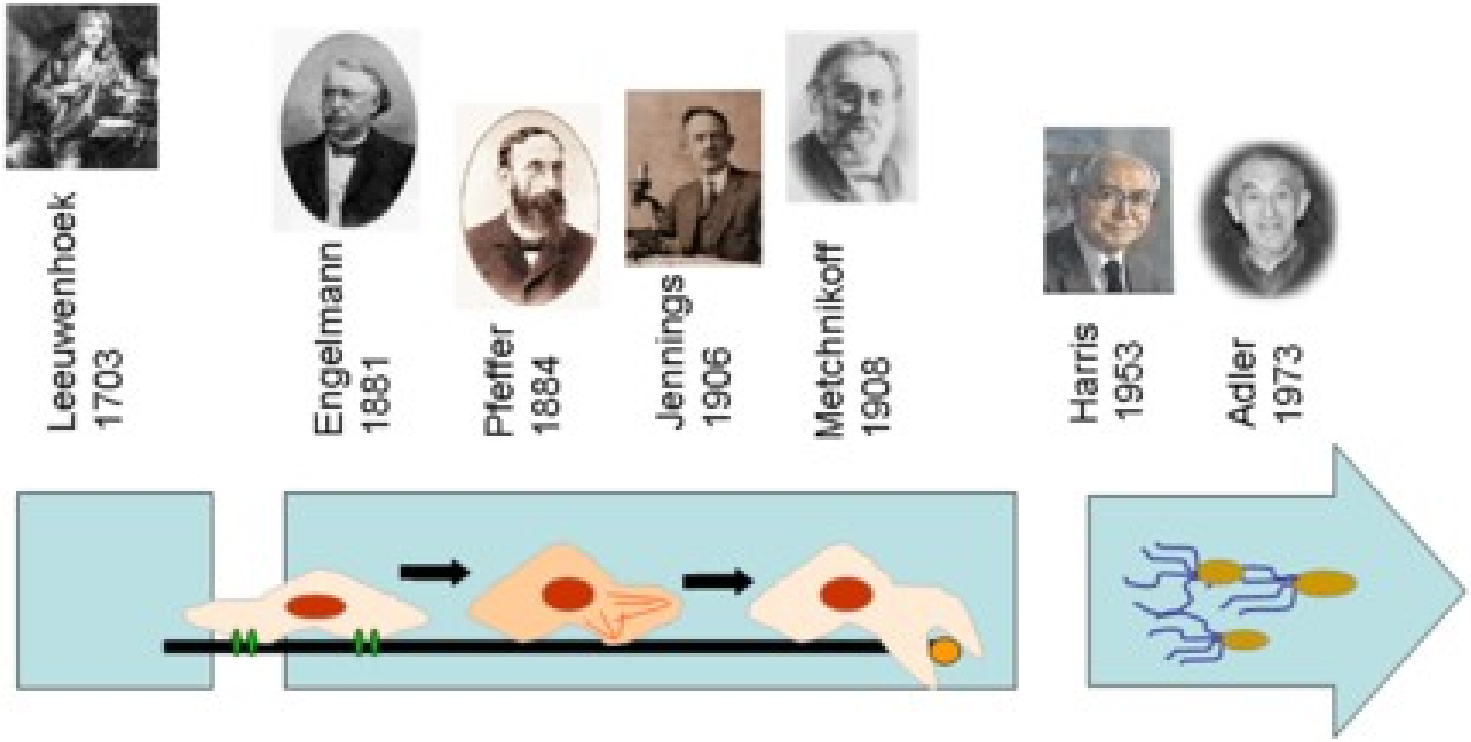
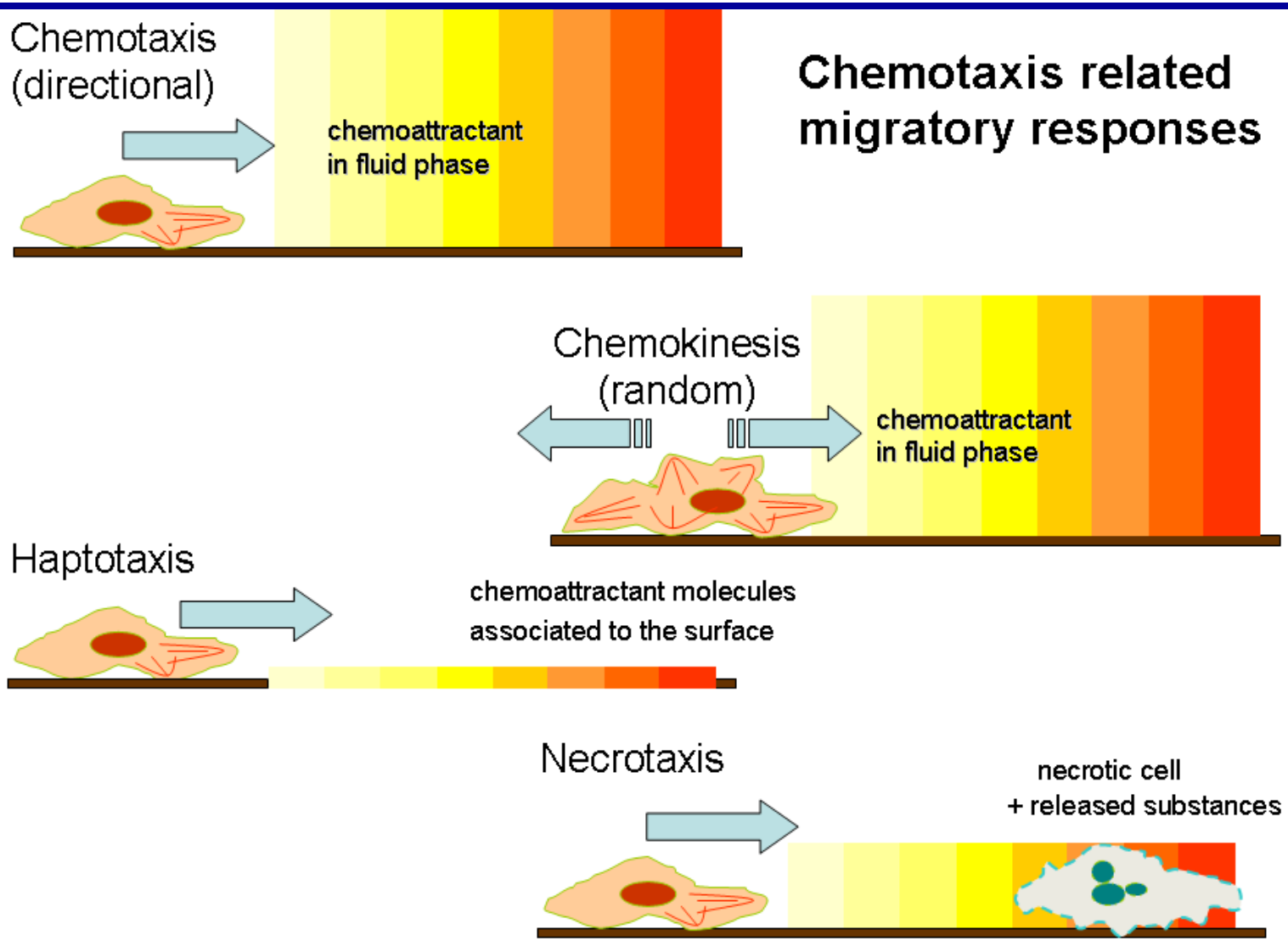
Chemotaxis Research Group, Dept. Genetics, Cell & Immunobiology, Semmelweis University
Budapest

2019

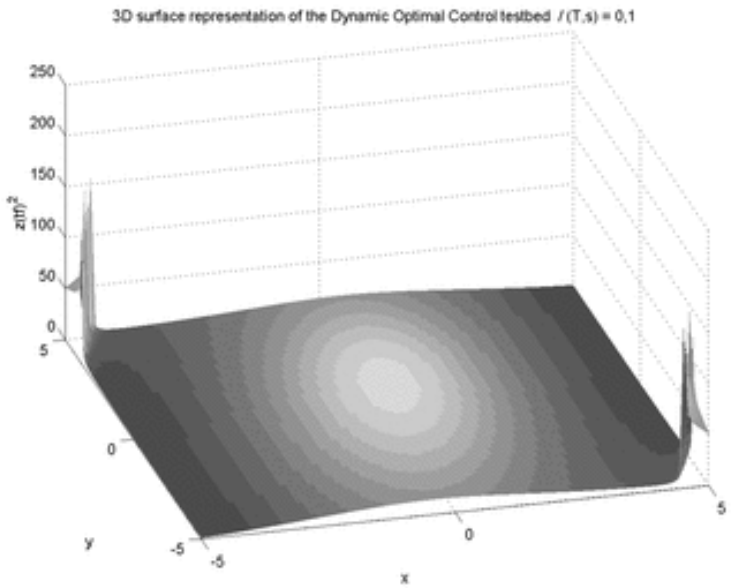
Milestones of Chemotaxis-research

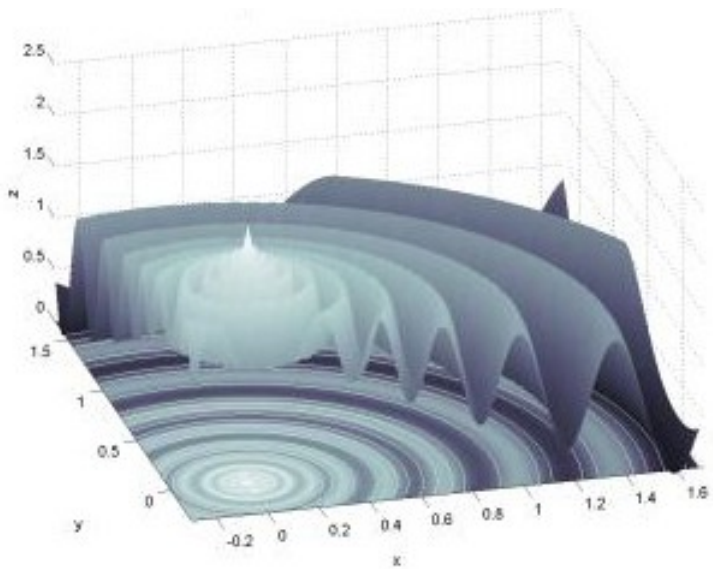


Main types of migratory responses

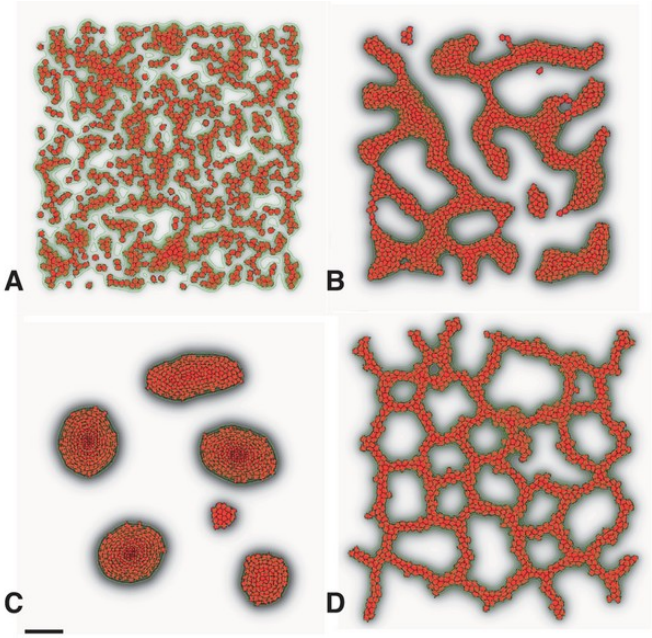


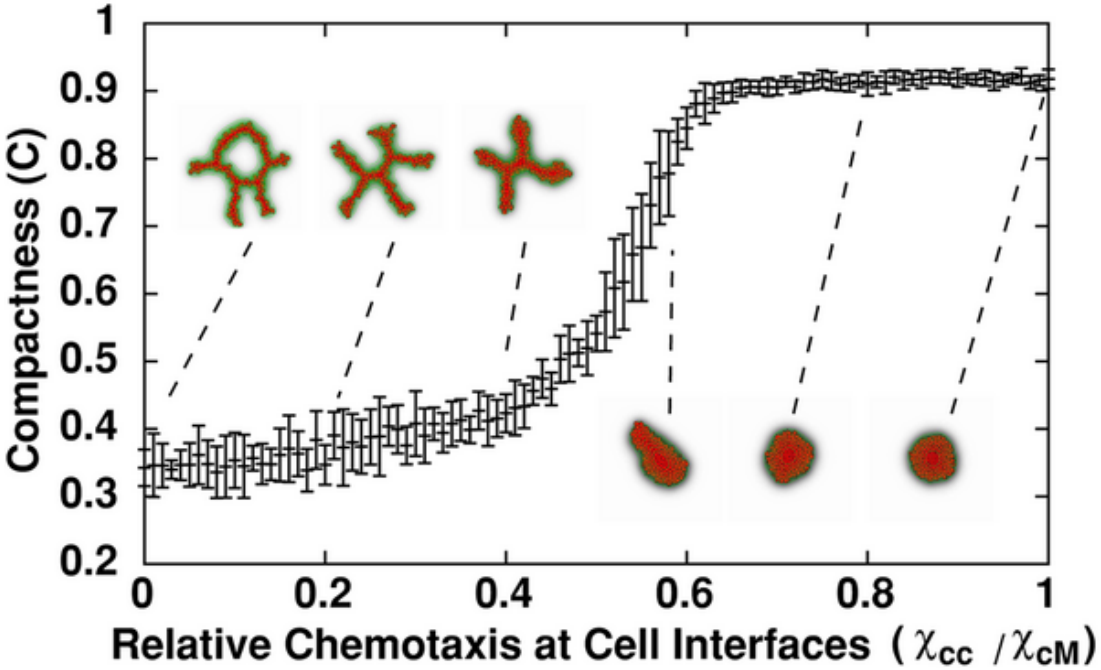
Concentration gradients and cell migration

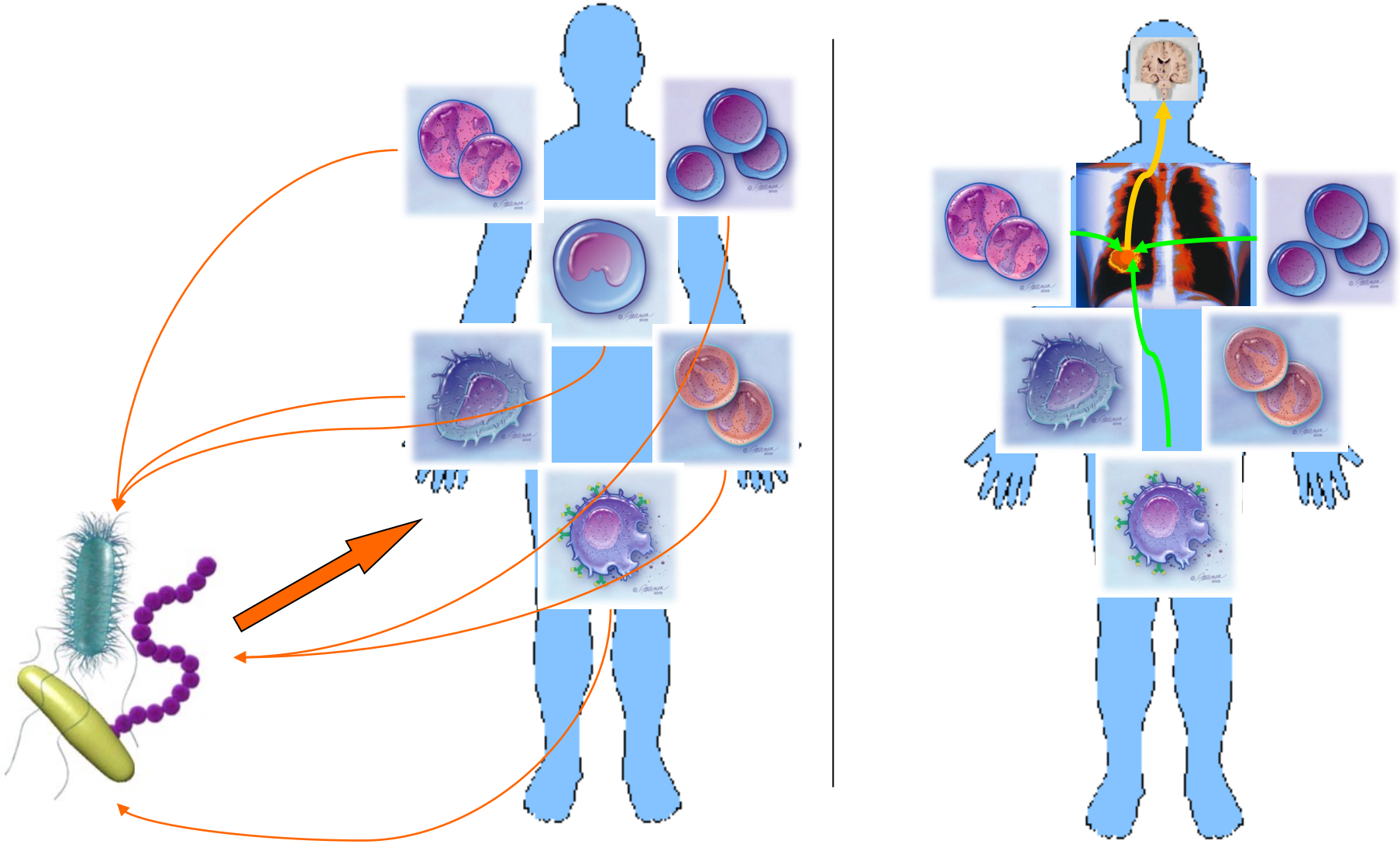




Tissue Differentiation – Chemotaxis



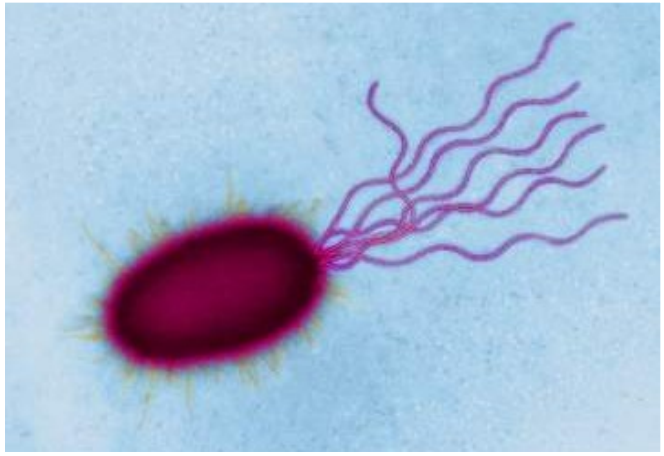




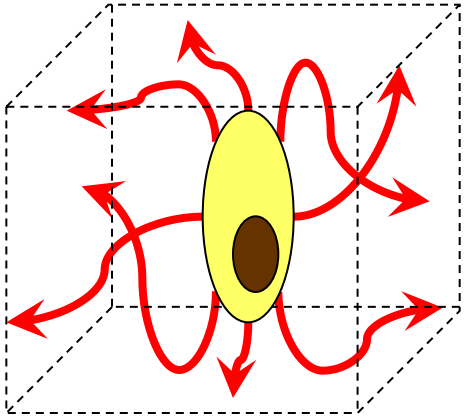
Significance of chemotaxis

- Helps to distinguish / to approach food molecules
- Helps to avoid biologically harmful/toxic substances
- Sperm to approach oocyte – fertilization
- Tissue transformation processes (e.g. CNS)
- Immune reaction of multicellular organisms
- Proliferation and growing of tumors, formation of metastasis

Cells migrating in 3D



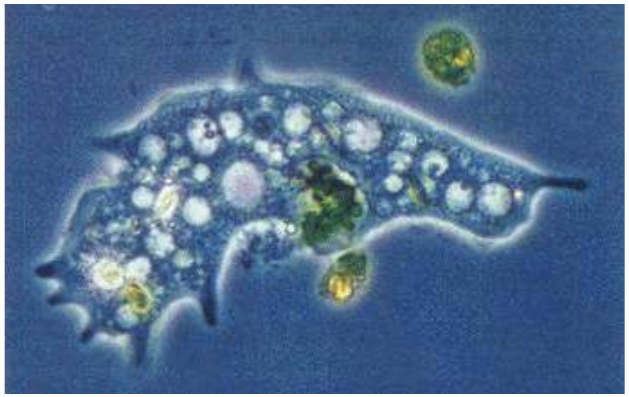
bacteria



ciliates

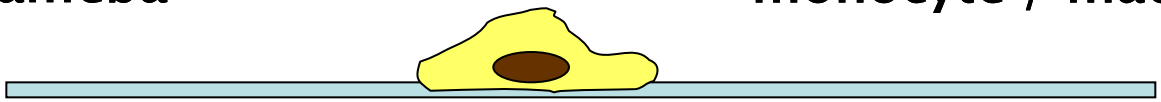
spermium

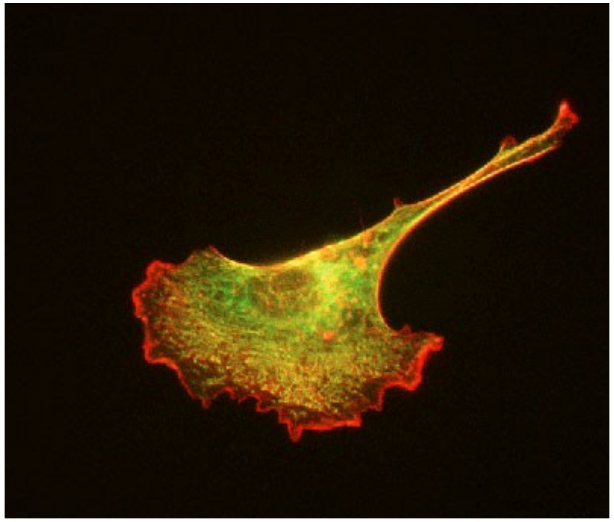
Cells migrating in 2D



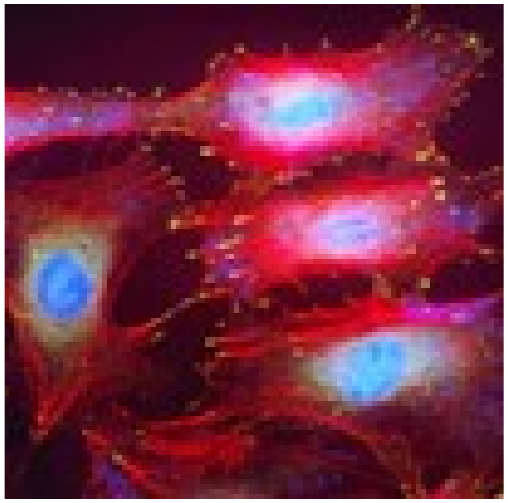
ameba

monocyte / macrophage





fibroblast



endothel

Molecules eliciting chemotaxis

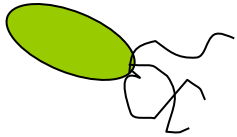
Professional chemoattractants

- N-formyl peptides (FPR)
- chemokines (CC and CXC subfamilies)
- arachidonic acid metabolism
- pheromones
- complement 5a, 3a

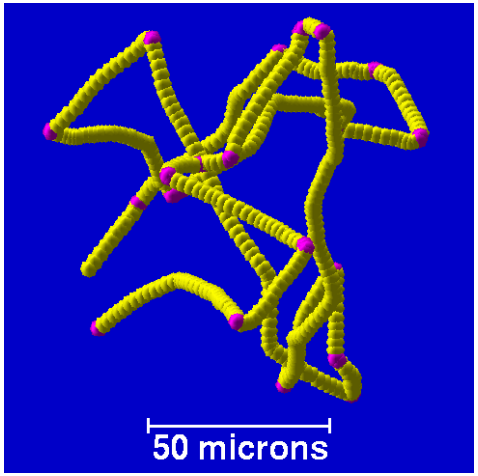
Ligands possessing chemoattractant activitv as a secondary effect

- amino acids
- oligopeptides
- polypeptide hormones
- steroids
- volatile oils
- synthetic substances

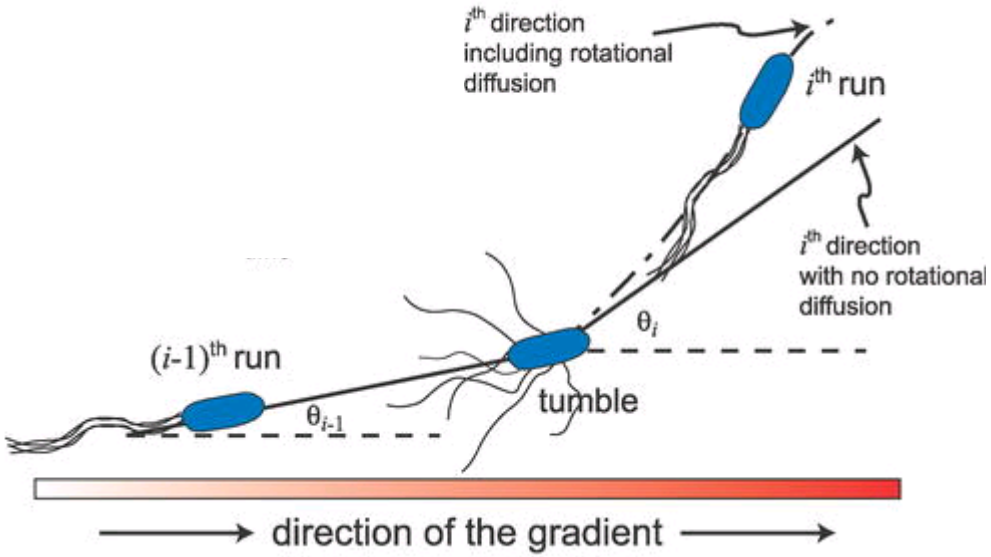
BACTERIAL CHEMOTAXIS

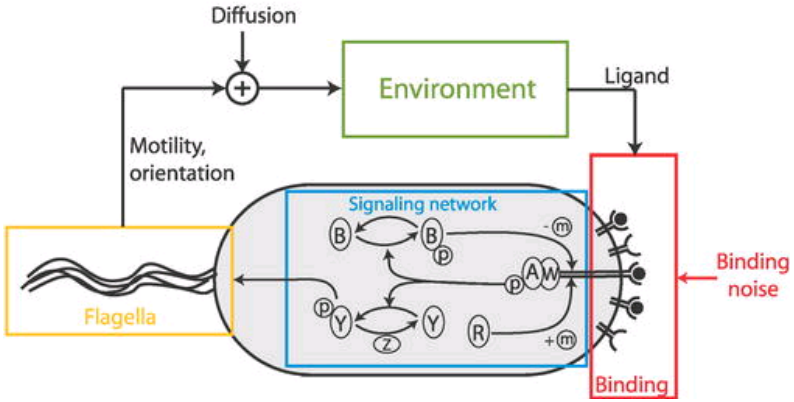


Mechanism of Adaptation



E.coli





Chemoattractant ligands – Bacteria

Attractant moiety:

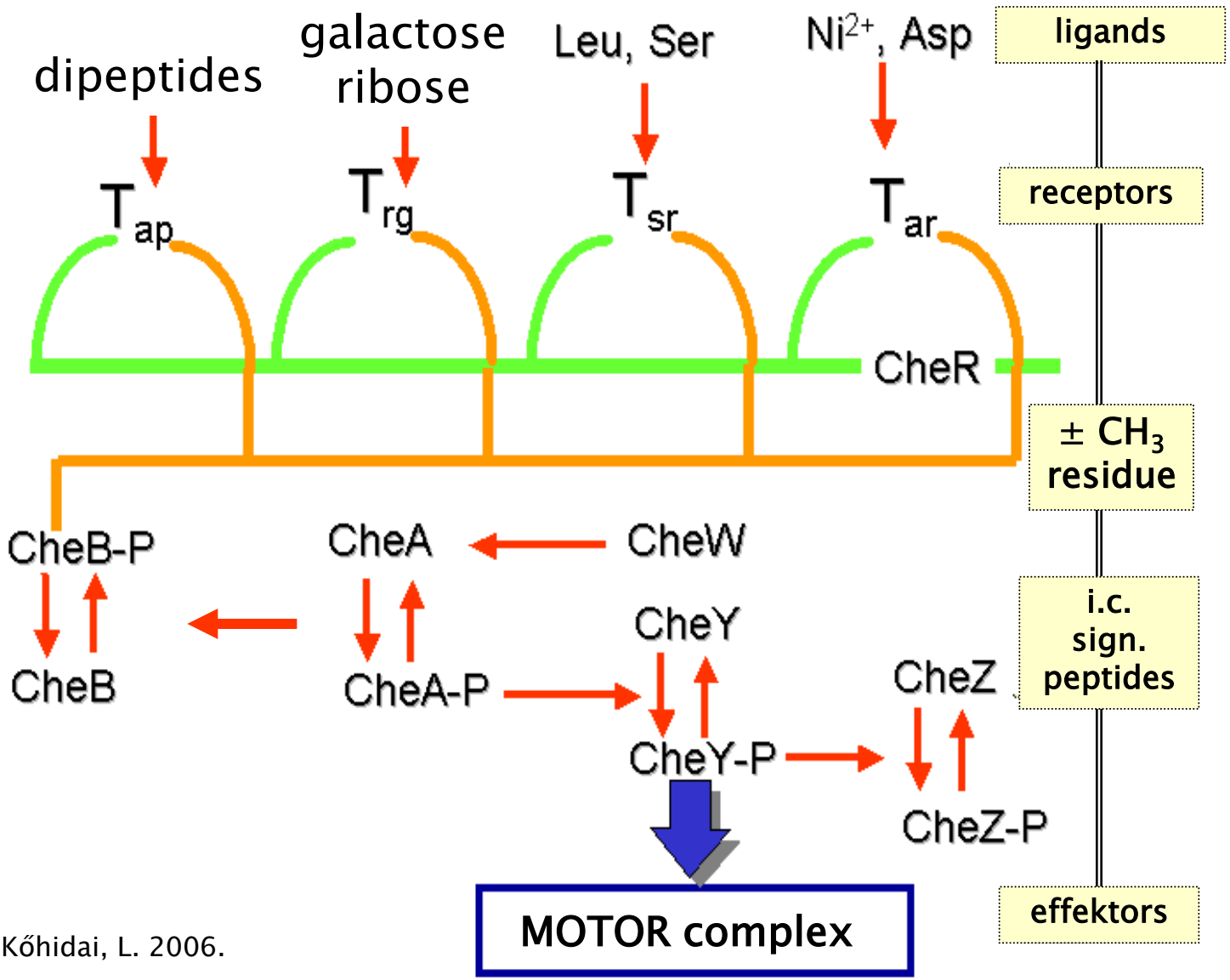
1. Does not require metabolic activity.

2. Does not require internalization of the ligand.

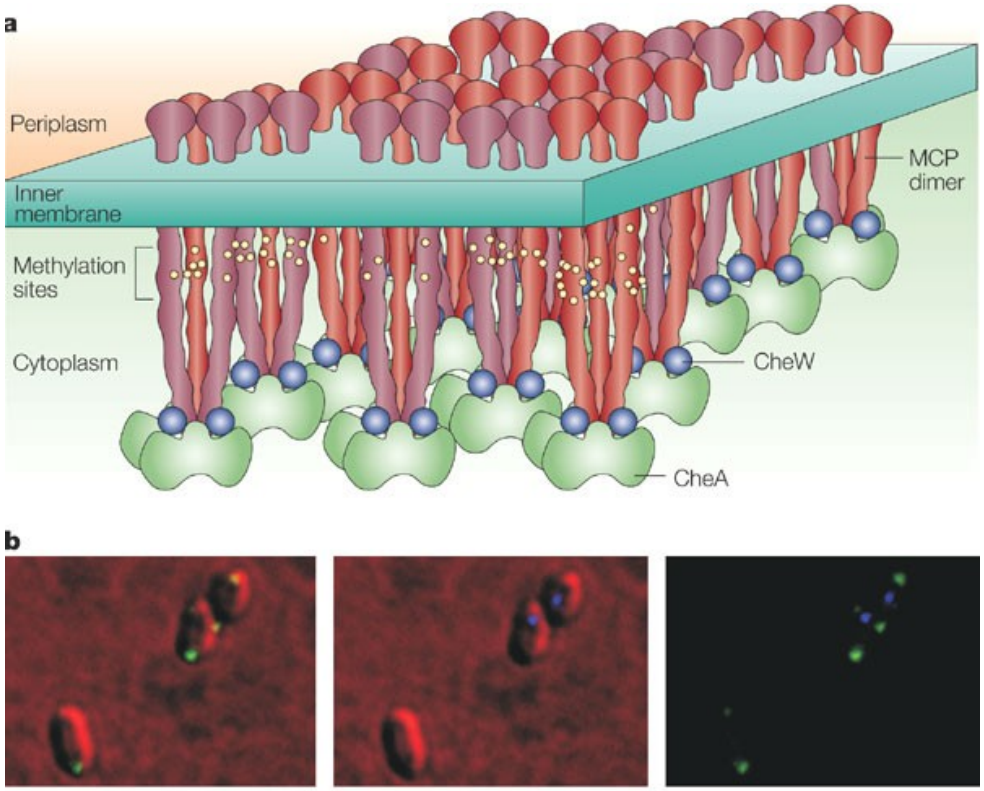
3. Determined by the interaction of ligand – receptor.

Gene	Receptor	Ligand
tsr	Tsr	Ser
tar	Tar	Asp
trg	Trg	ribose
tap	Tap	peptides
aer	Aer	oxygene

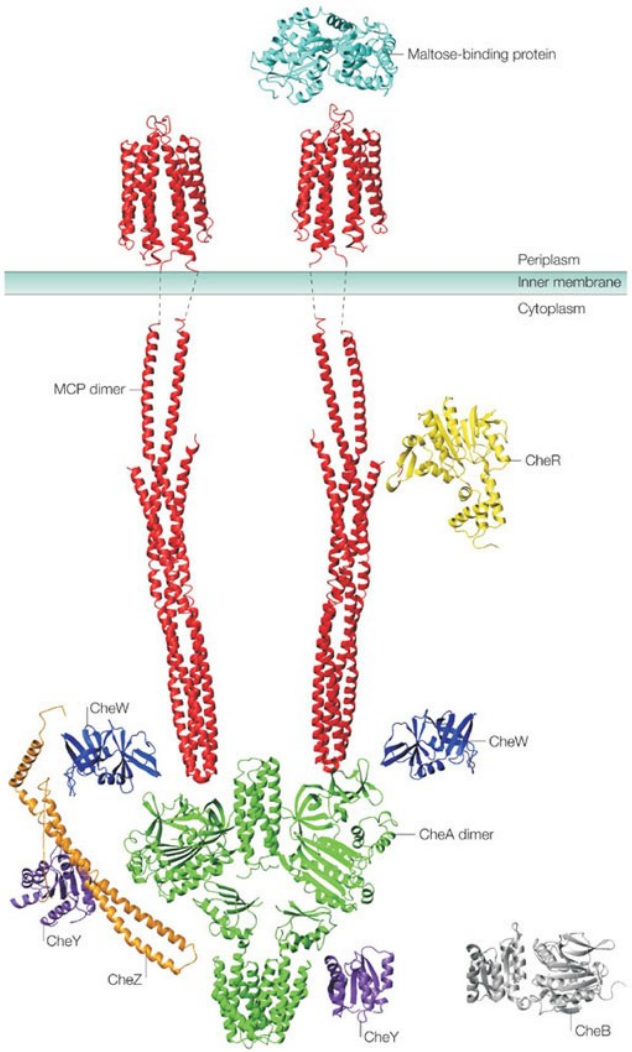
Bacterial chemotaxis



Bacterial chemotaxis



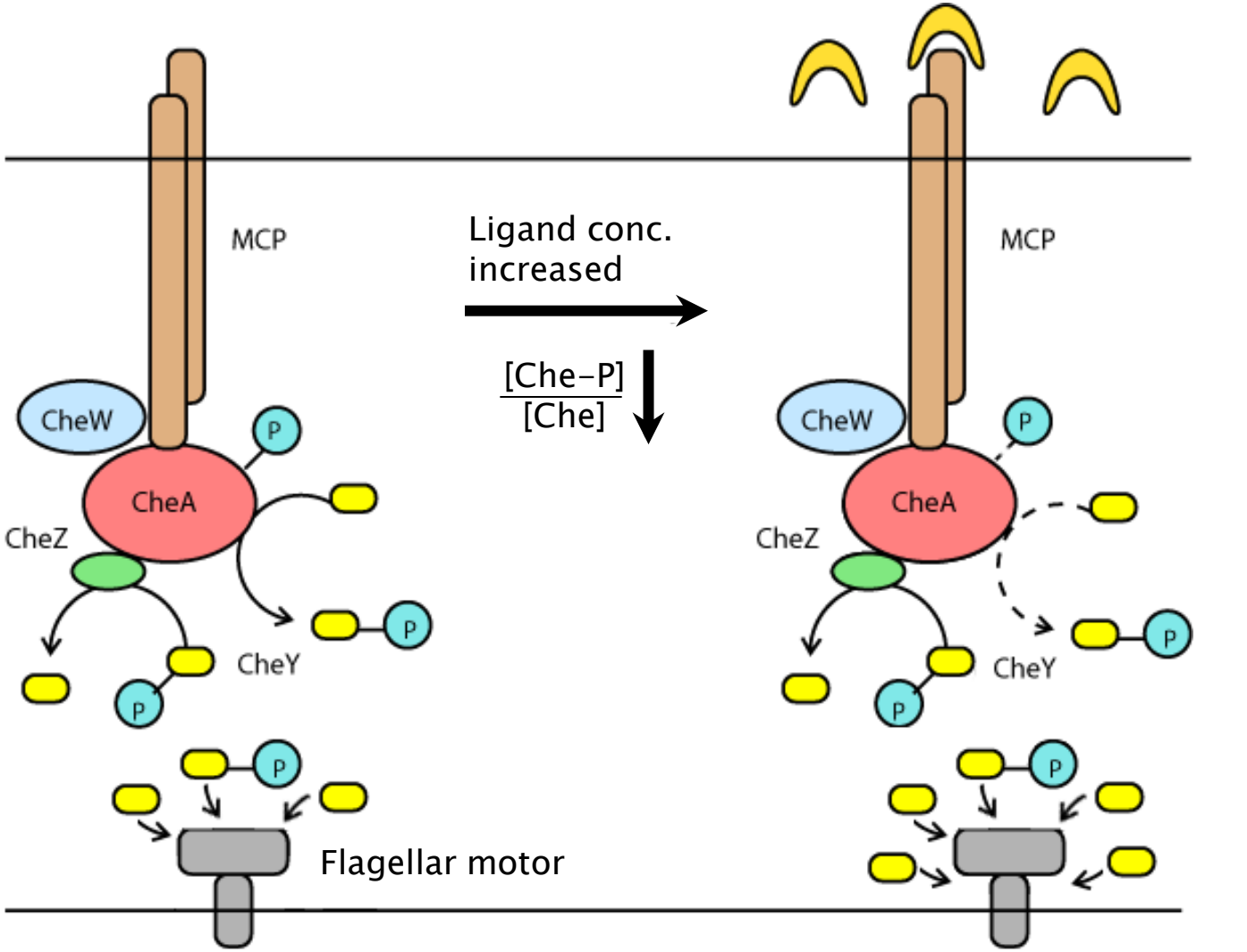
Nature Reviews | Molecular Cell Biology



Nature Reviews | Molecular Cell Biology

Chemotaxis – Rapid phase of intracellular signalling

– Bacteria –



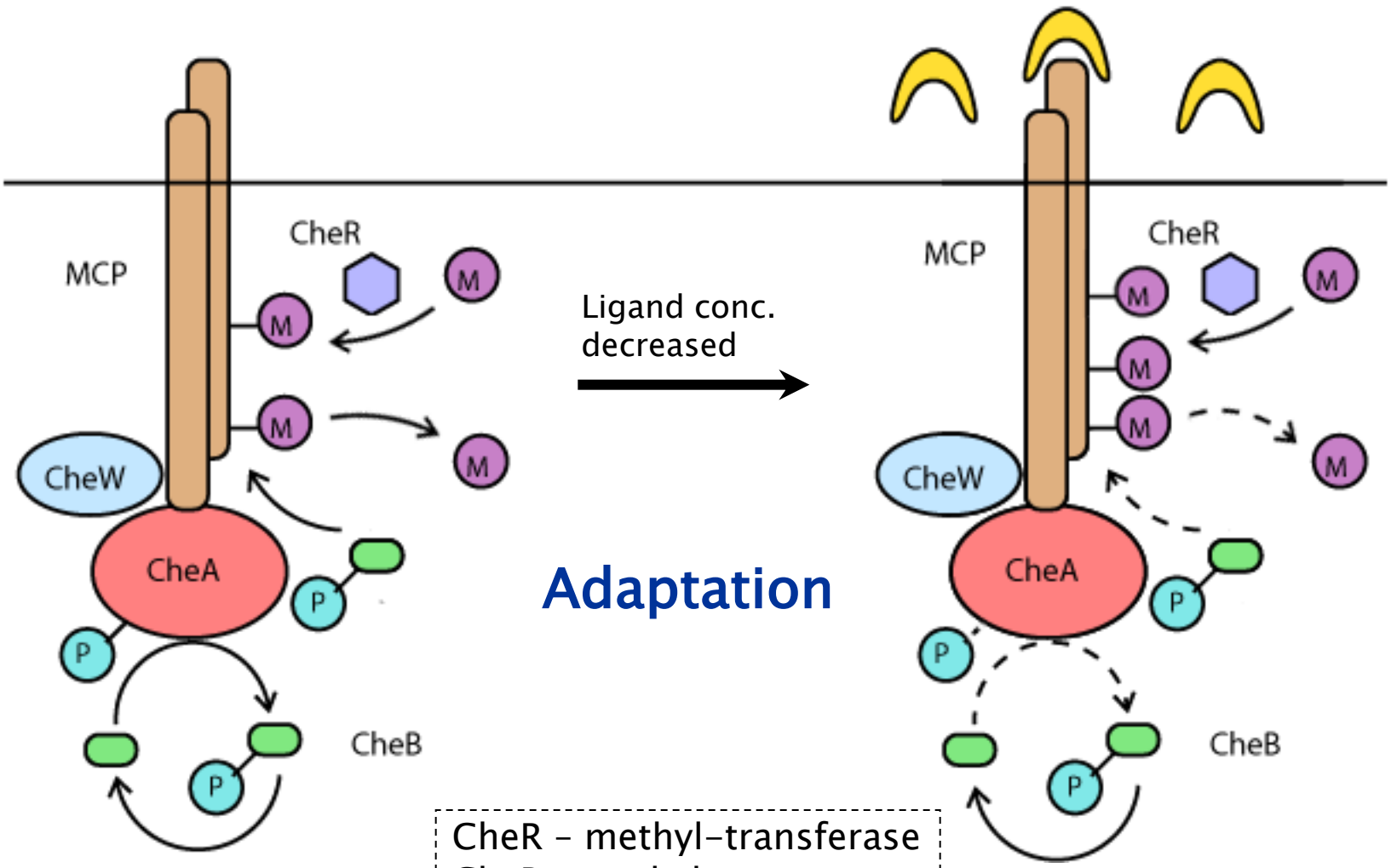
'Run' length – normal

'Run' length – expanded

Nature. 5, 1024-1037 (2004).

Chemotaxis – Slow phase of intracellular signalling

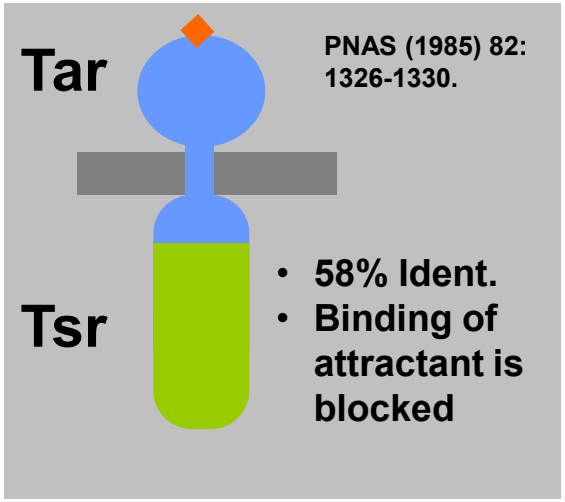
– Bacteria –



CheR – methyl-transferase
CheB – methyl-esterase

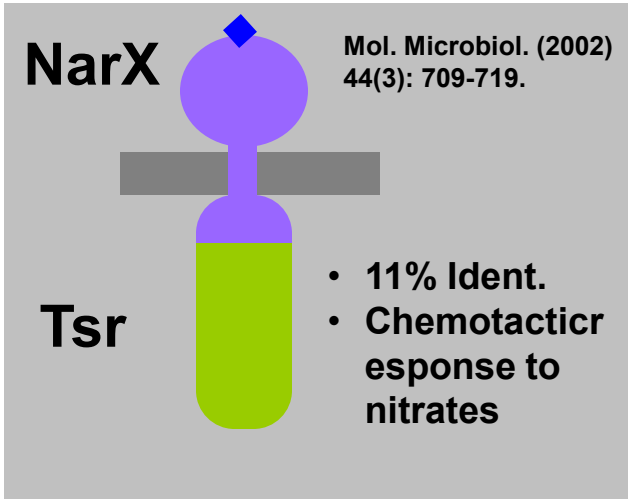
Nature. 5, 1024-1037 (2004).

Artificial new receptors – Recombination with MCP



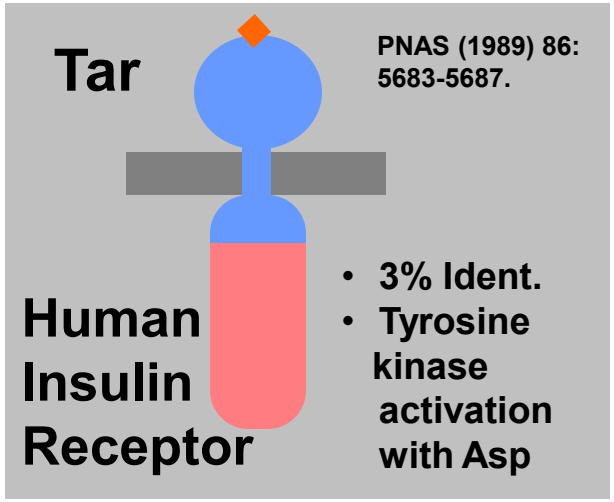
PNAS (1985) 82: 1326-1330.

- 58% Ident.
- Binding of attractant is blocked



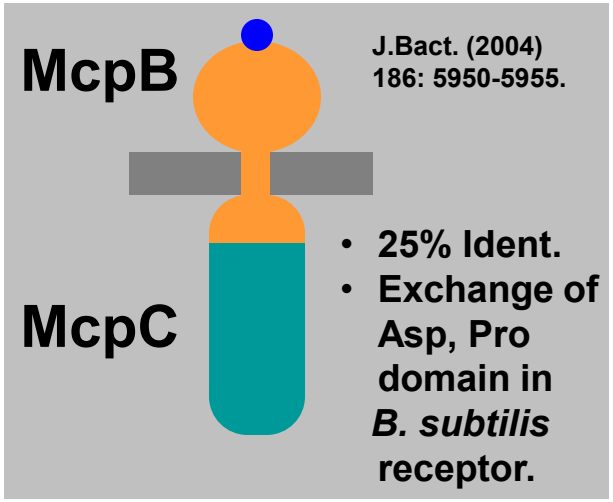
Mol. Microbiol. (2002) 44(3): 709-719.

- 11% Ident.
- Chemotacticr esponse to nitrates



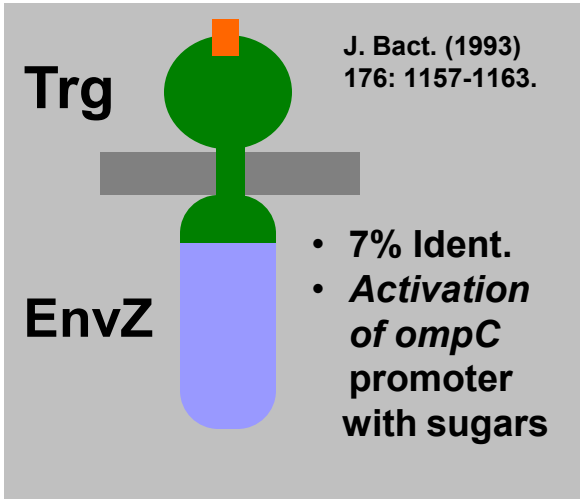
PNAS (1989) 86: 5683-5687.

- 3% Ident.
- Tyrosine kinase activation with Asp



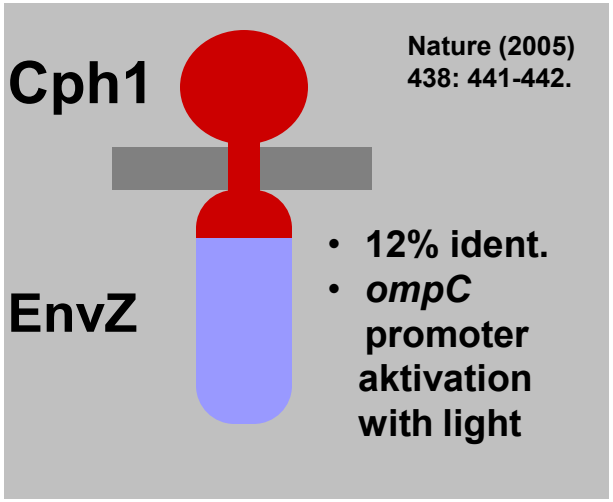
J.Bact. (2004) 186: 5950-5955.

- 25% Ident.
- Exchange of Asp, Pro domain in *B. subtilis* receptor.



J. Bact. (1993) 176: 1157-1163.

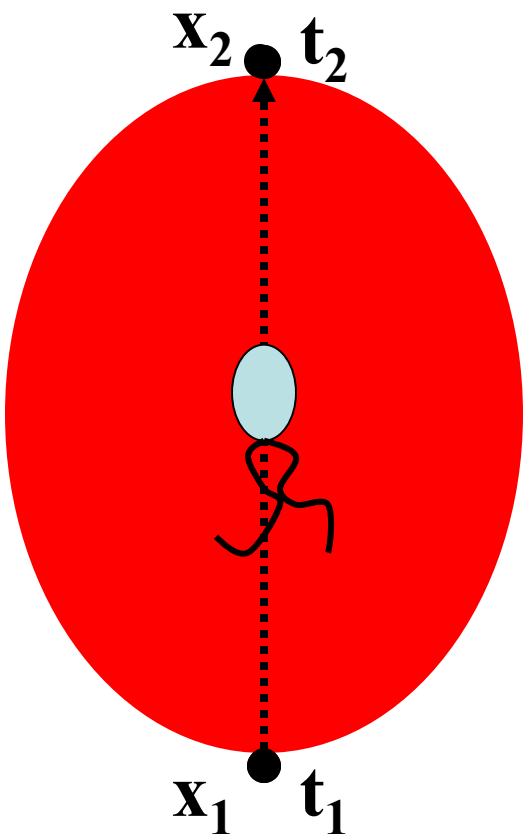
- 7% Ident.
- *Activation of ompC* promoter with sugars



Nature (2005) 438: 441-442.

- 12% ident.
- *ompC* promoter aktivation with light

Mathematical model of chemotaxis



Temporal Sensing (Differentiator)

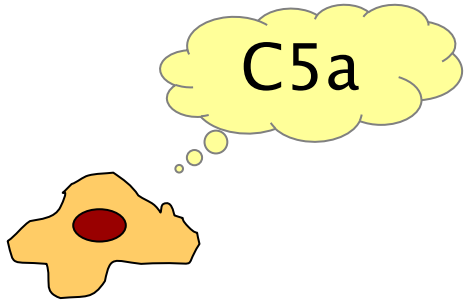
$$\frac{C(t_2)-C(t_1)}{t_2-t_1}=\frac{dC}{dt}$$

if $\frac{dC}{dt}>0$, "run" is frequent, "tumble" is rare

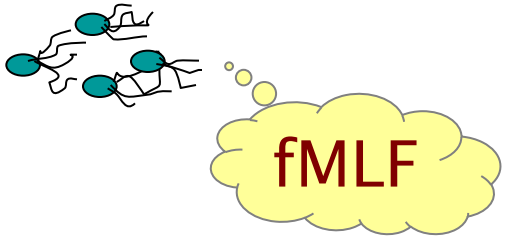
Spatial Sensing

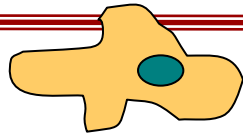
$$\frac{C(x_2)-C(x_1)}{x_2-x_1}=\frac{dC}{dx}$$

if $\frac{dC(x_2)}{dx}>0$, "shmoo" formation in x_2 direction

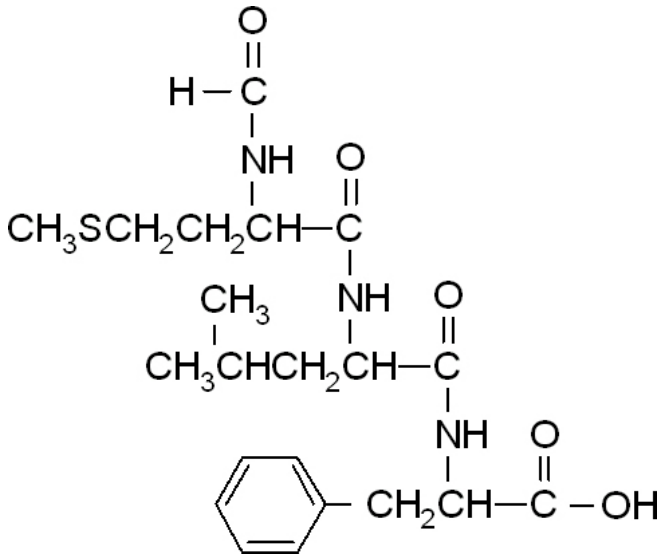


NON-CHEMOKINE TYPE SUBSTANCES

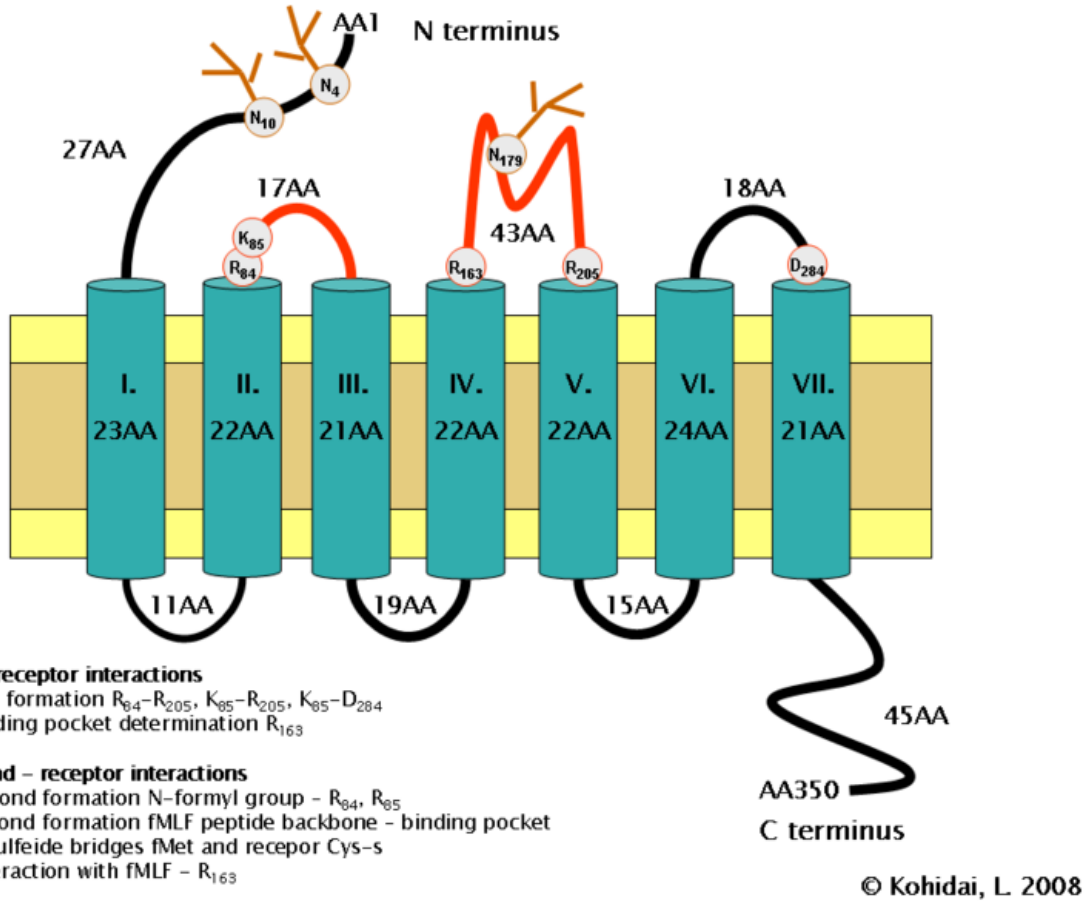




Formyl-Met-Leu-Phe (fMLF)



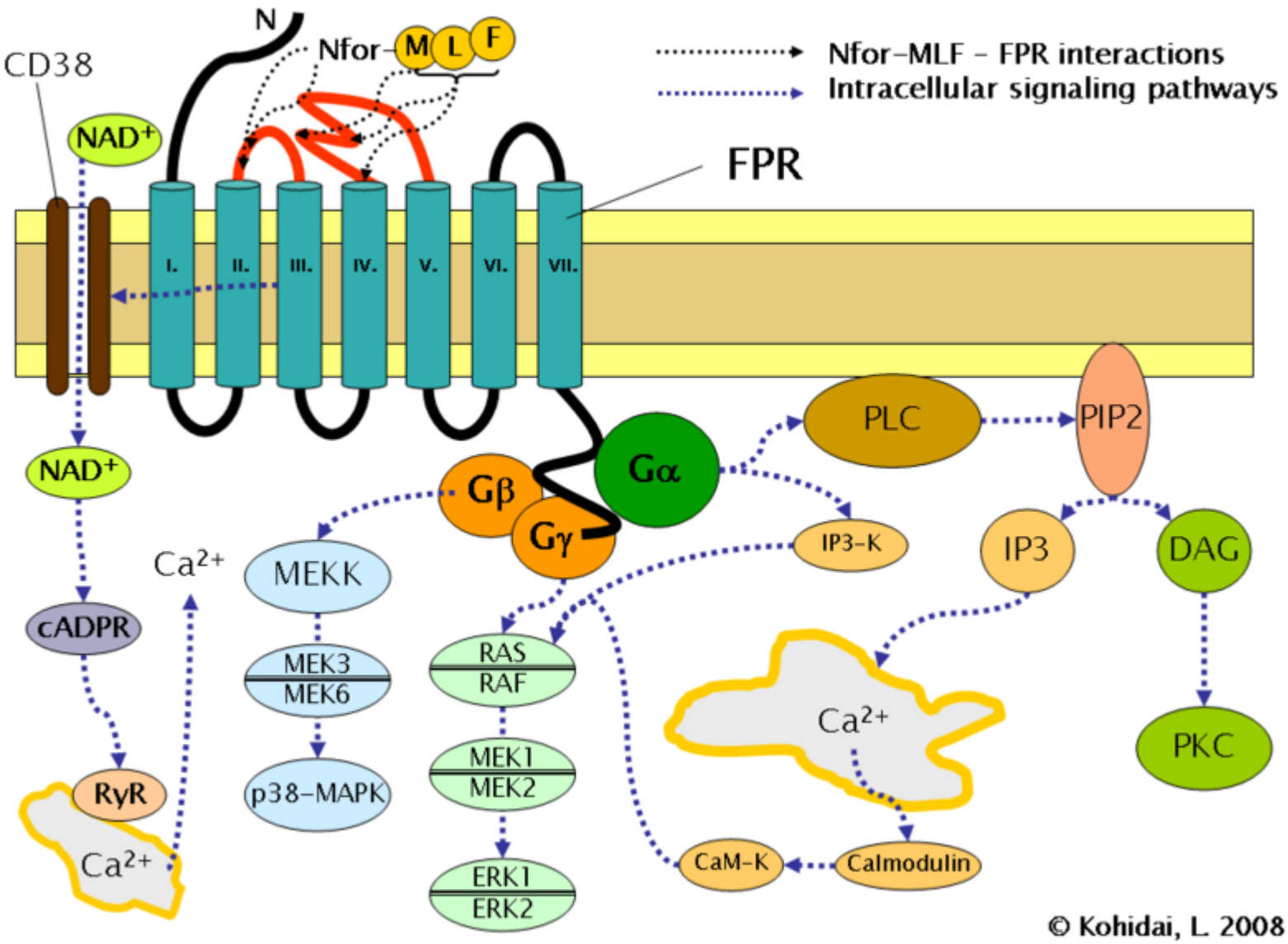
fMLF



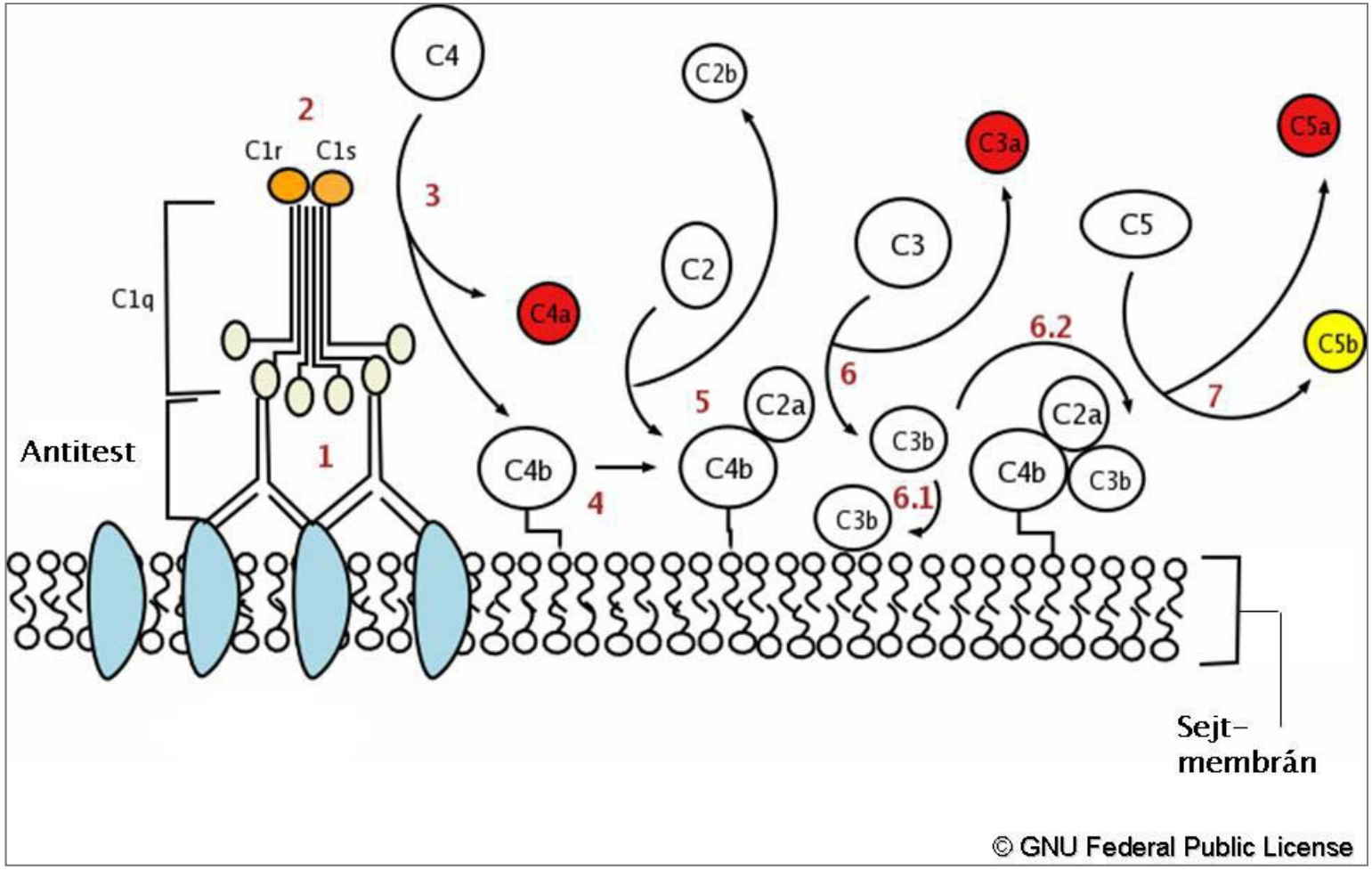
Intrareceptor interactions
- Salt formation R_{84}–R_{205}, K_{85}–R_{205}, K_{85}–D_{284}
- Binding pocket determination R_{163}

Ligand – receptor interactions
- H bond formation N-formyl group – R_{84}, R_{85}
- H bond formation fMLF peptide backbone – binding pocket
- Disulfeide bridges fMet and recepor Cys-s
- Interaction with fMLF – R_{163}

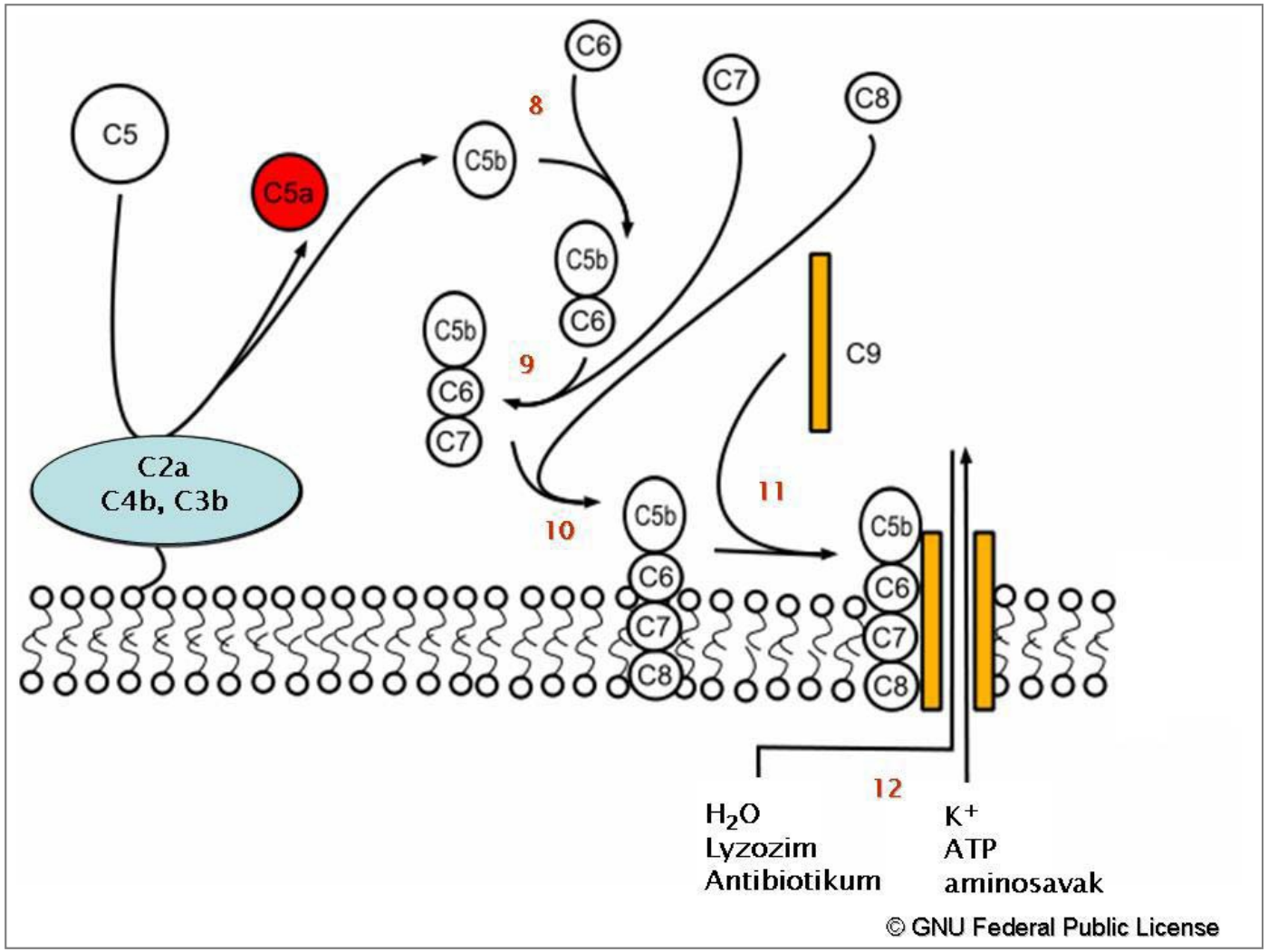
Formyl-Met-Leu-Phe (fMLF) signalling



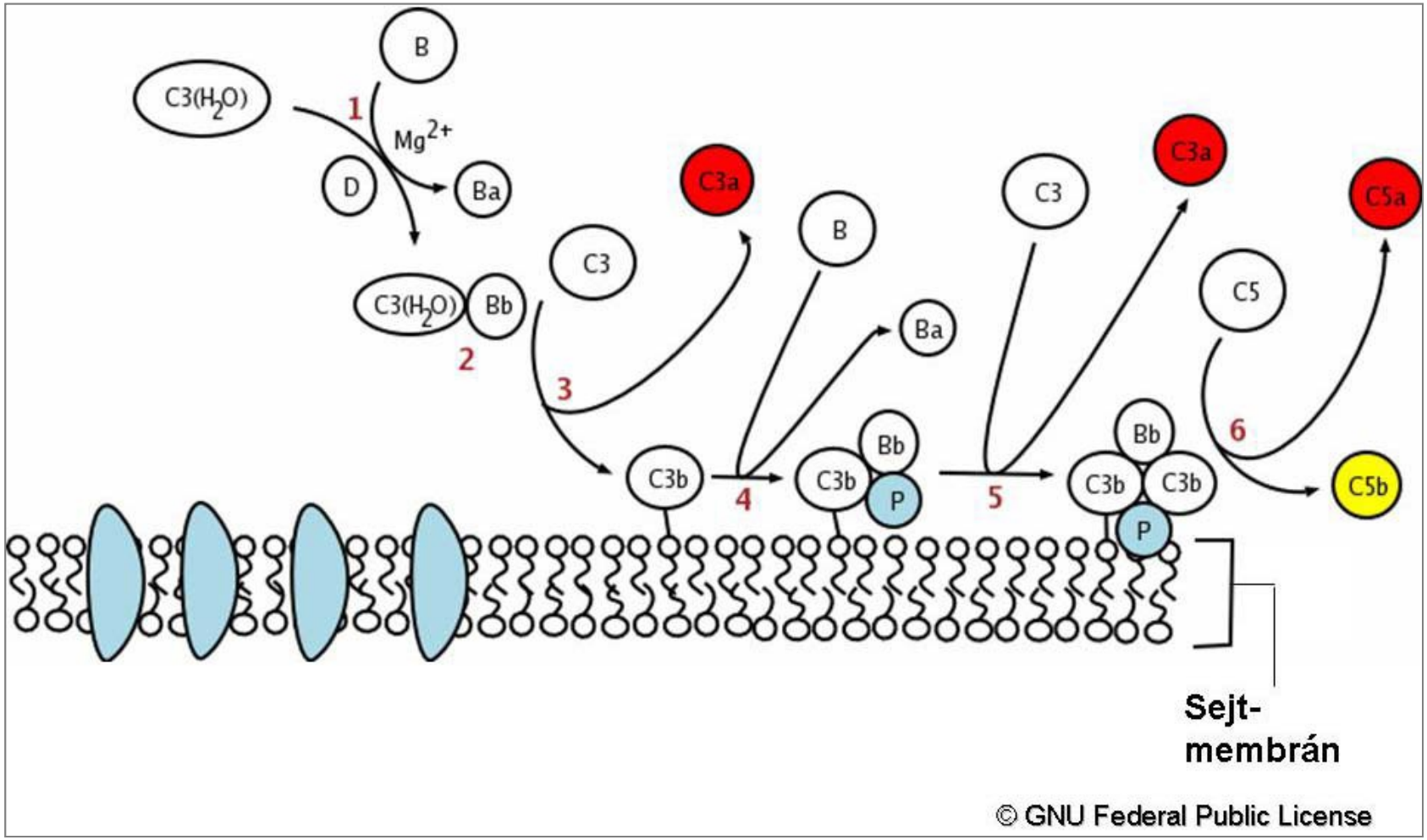
Complement – Classical pathway 1



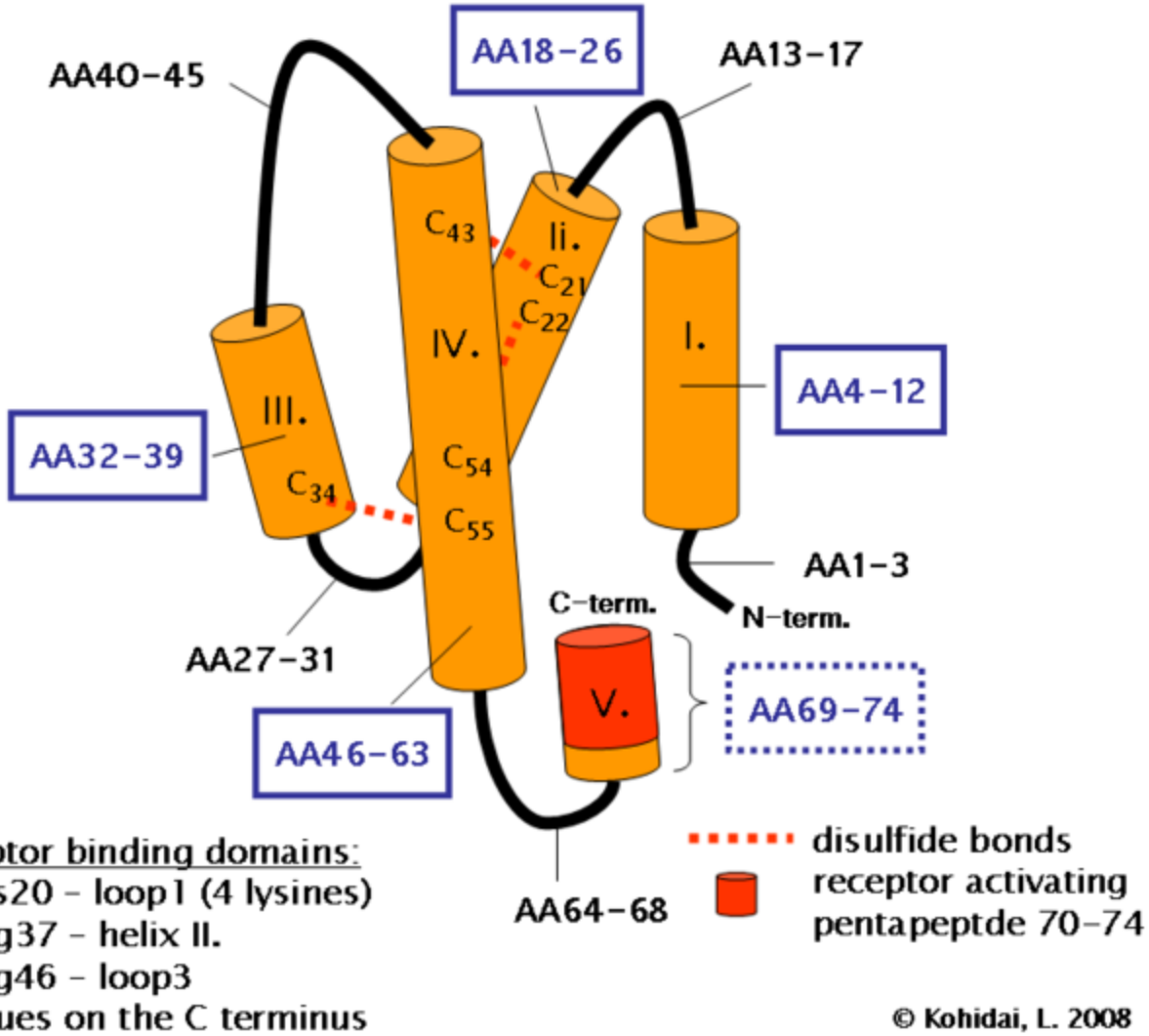
Complement – Classical pathway 2



Complement – Alternative pathway

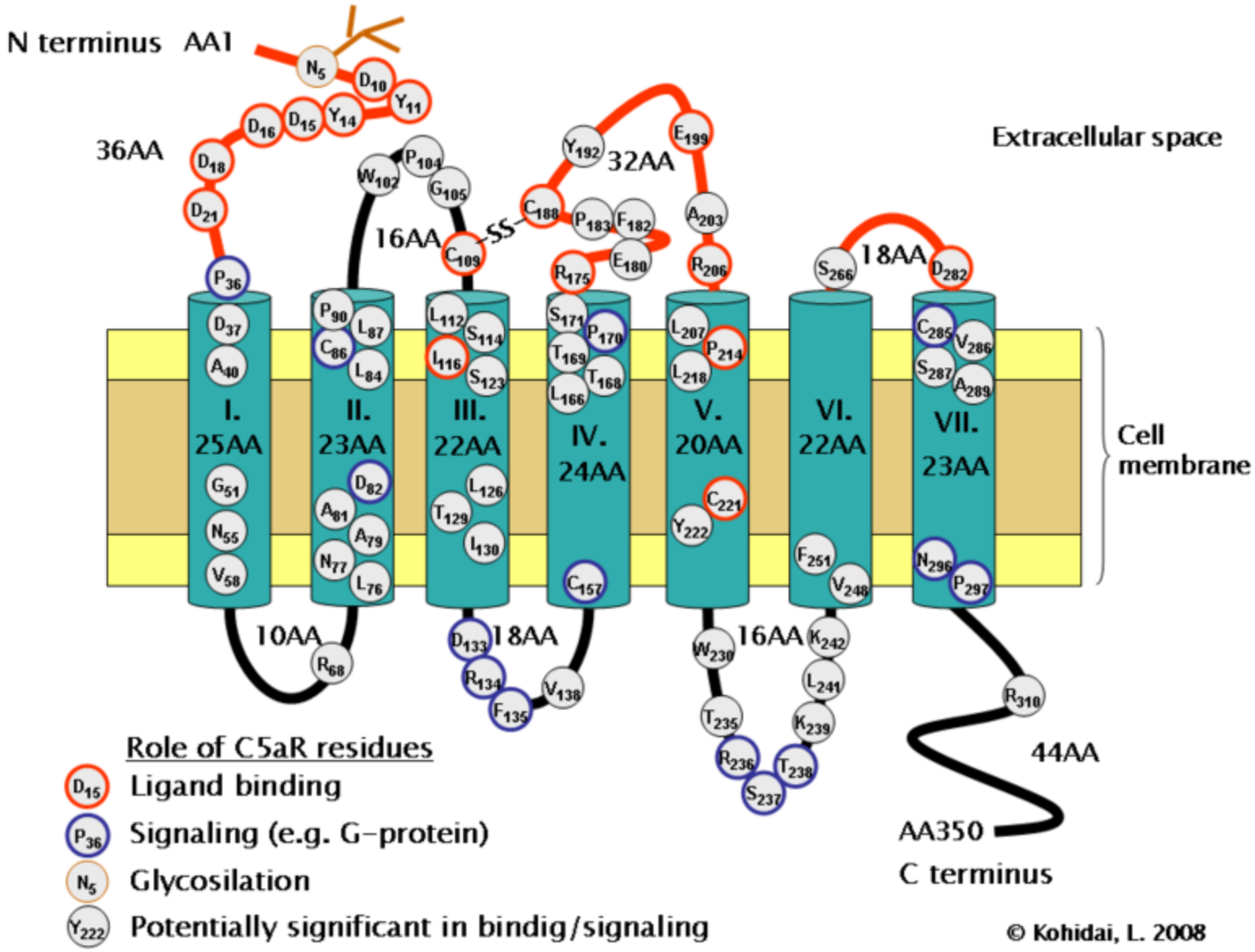


Complement 5a (C5a)



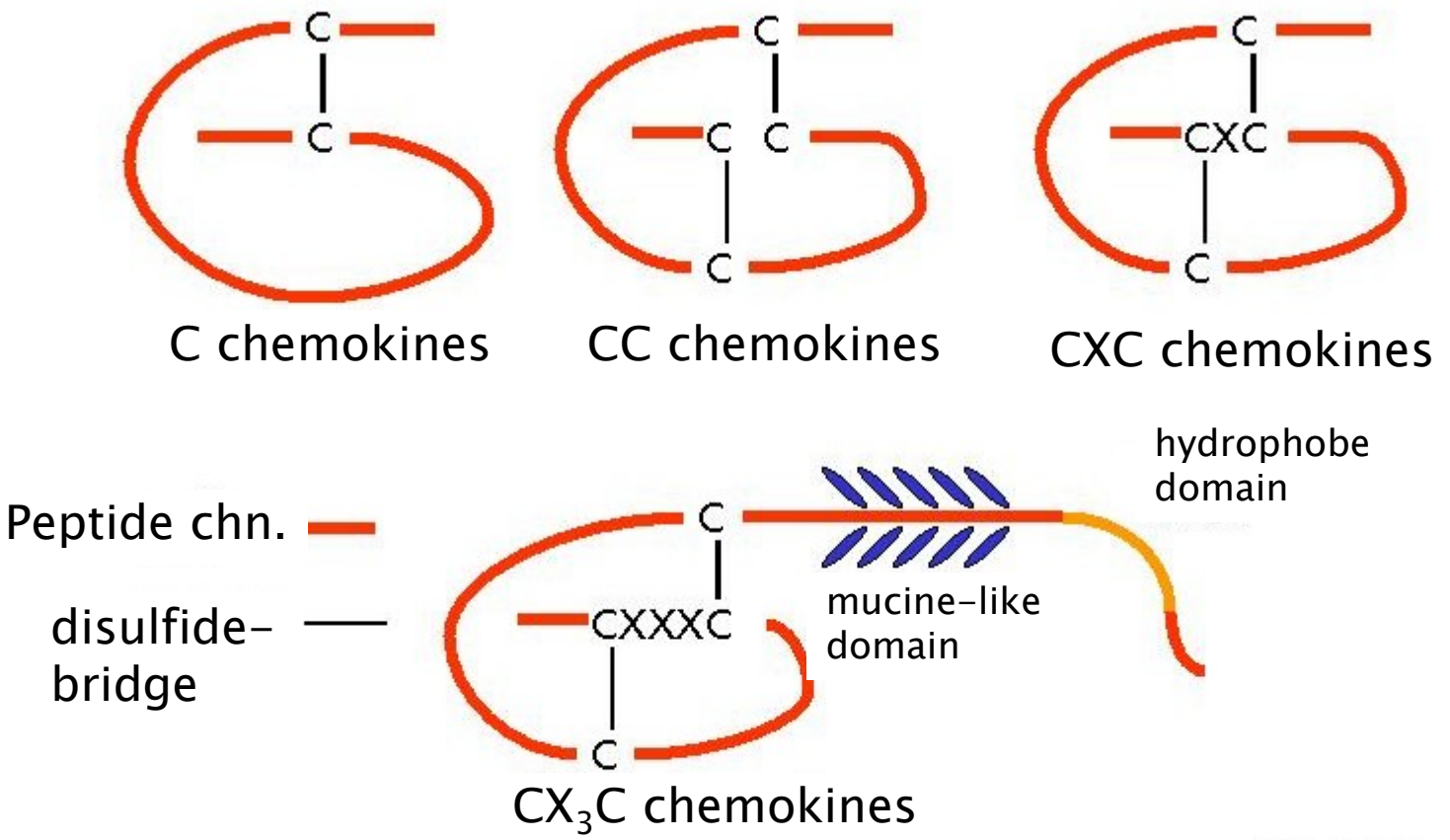
C5a receptor binding domains:
Lys12-Lys20 - loop1 (4 lysines)
Lys20-Arg37 - helix II.
Ala39-Arg46 - loop3
6-8 residues on the C terminus

C5a receptor

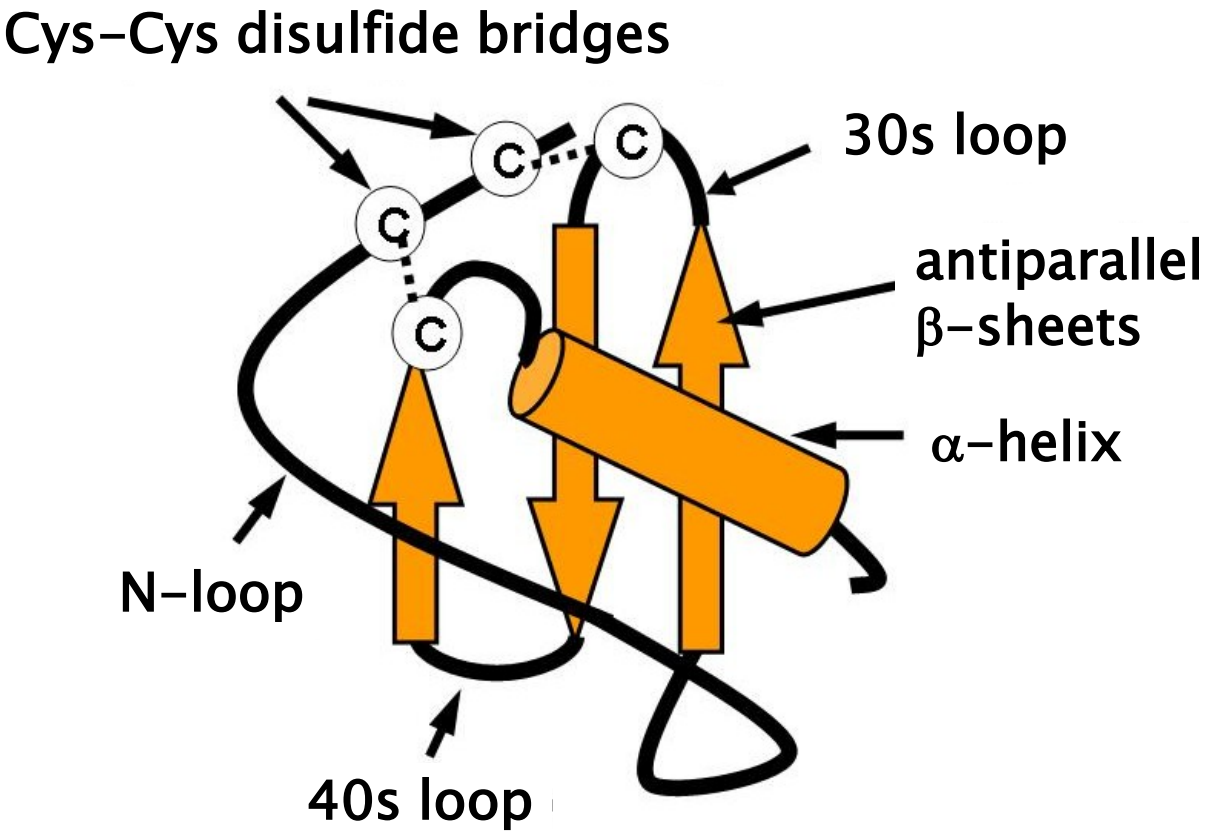


CHEMOKINES

Classification of Chemokines



3D structure of Chemokines



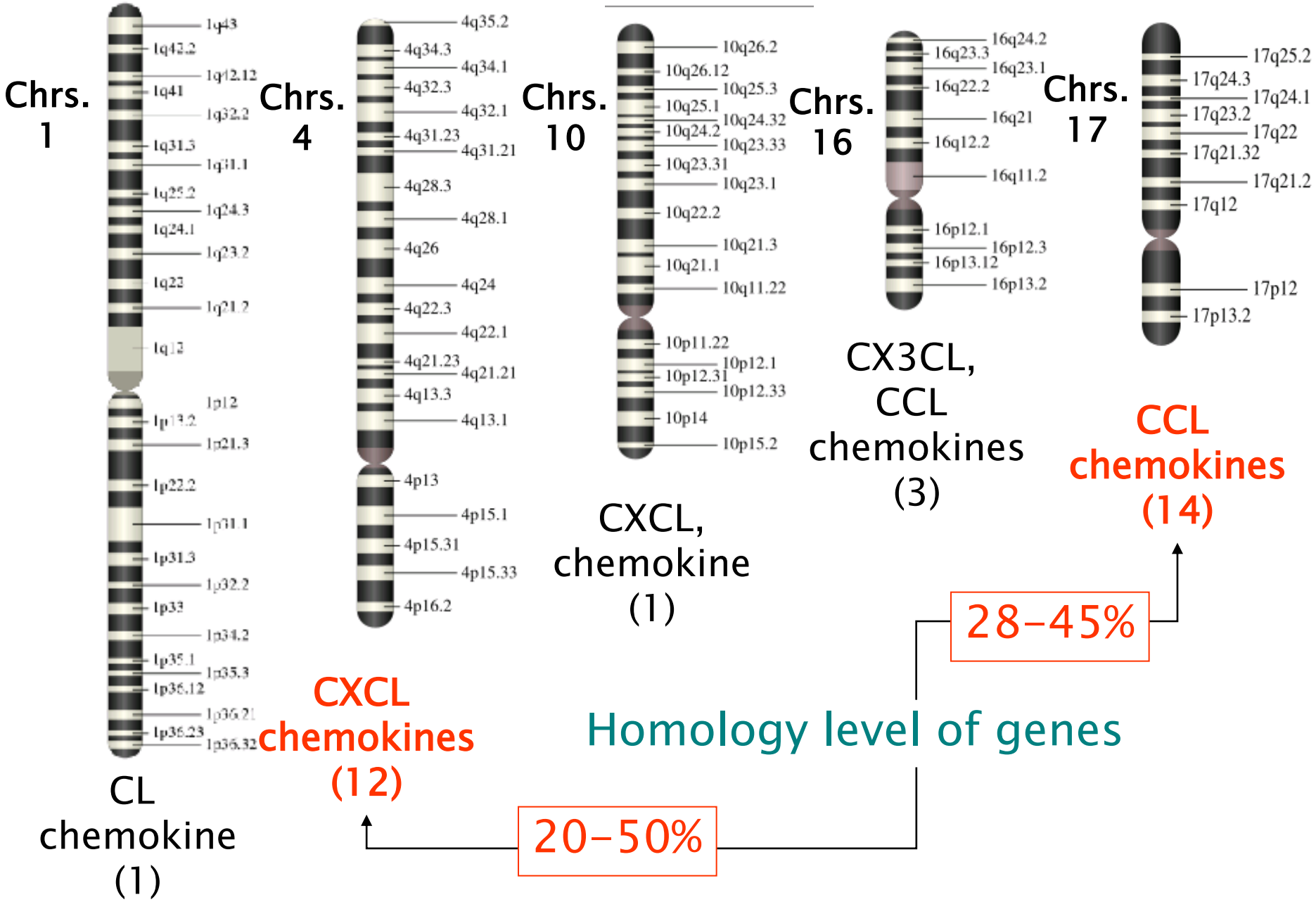
Chemokine-receptors and their ligands

CCR1	CCL3 (MIP-1α), CCL5 (RANTES), CCL7 (MCP-3), CCL14 (HCC1)
CCR2	CCL2 (MCP-1), CCL8 (MCP-2), CCL7 (MCP-3), CCL13 (MCP-4), CCL16 (HCC4)
CCR3	CCL11 (eotaxin), CCL13 (eotaxin-2), CCL7 (MCP-3), CCL5 (RANTES), CCL8 (MCP-2), CCL13 (MCP-4)
CCR4	CCL17 (TARC), CCL22 (MDC)
CCR5	CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES), CCL11 (eotaxin), CCL14 (HCC1), CCL16 (HCC4)
CCR6	CCL20 (MIP-3β, LARC)
CCR7	CCL19 (ELC), CCL21 (SLC)
CCR8	CCL1 (1309)
CCR9	CCL25 (TECK)
CCR10	CCL27 (CTACK), CCL28 (MEC)

CXCR1	CXCL8 (interleukin-8), CXCL6 (GCP2)
CXCR2	CXCL8, CXCL1 (GROα), CXCL2 (GROβ), CXCL3 (GROγ), CXCL5 (ENA-78), CXCL6
CXCR3-A	CXCL9 (MIG), CXCL10 (IP-10), CXCL11 (I-TAC)
CXCR3-B	CXCL4 (PF4), CXCL0 (MIG), CXCL10 (IP-10), CXCL11 (I-TAC)
CXCR4	CXCL12 (SDF-1)
CXCR5	CXCL13 (BCA-1)
CXCR6	CXCL16 (SR-PSOX)
CX_3CR1	CXCL1 (fractalkine)
XCR1	XCL1 (lymphotactin), XCL2

. Charo, IF and Ransohoff, RM NEJM 354:610, 2006

Genetics of Chemokines

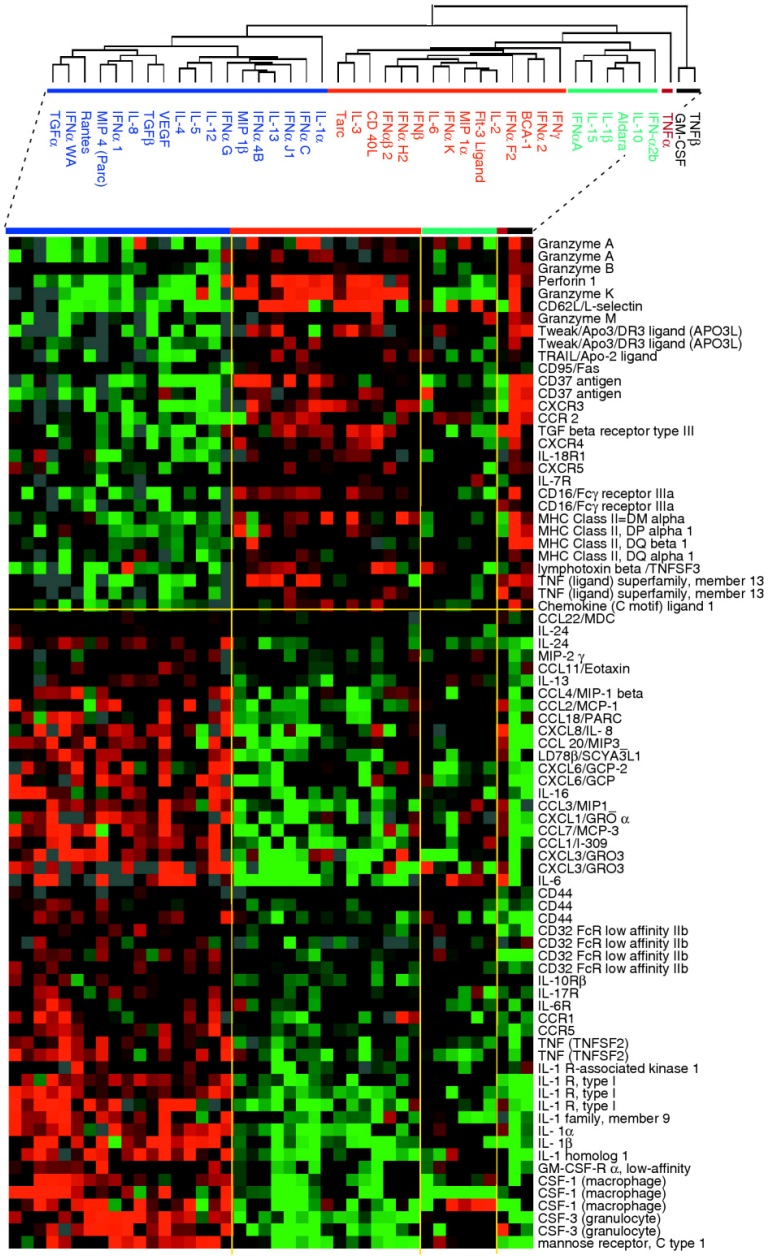


Genetics of Chemokines: SNP frequencies

Ligands	SNP/chemokin	Chemokines investigated
CCL	32,41	24
XCL	45	2
CX3CL	52	1
CXCL	36,76	13
Other	124,55	9
Receptors	**SNP/chemokin receptor**	**Chemokines investigated**
CCR	31,72	11
CX3CR	84	1
CXCR	34	5
Other	91,55	9

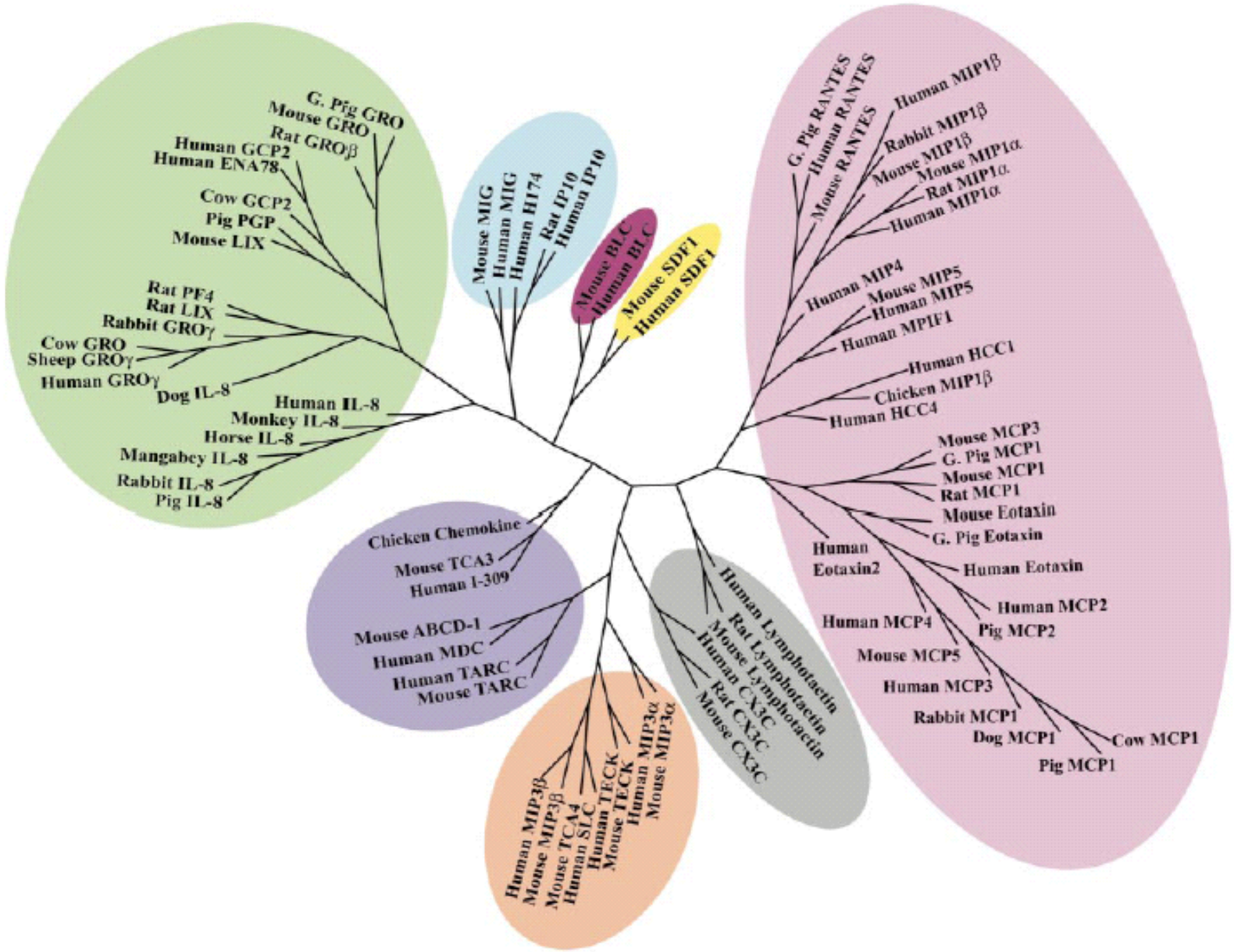
Gene Cards, Weizmann Inst. , Rehovot

Expression of ligand and its receptor(s)

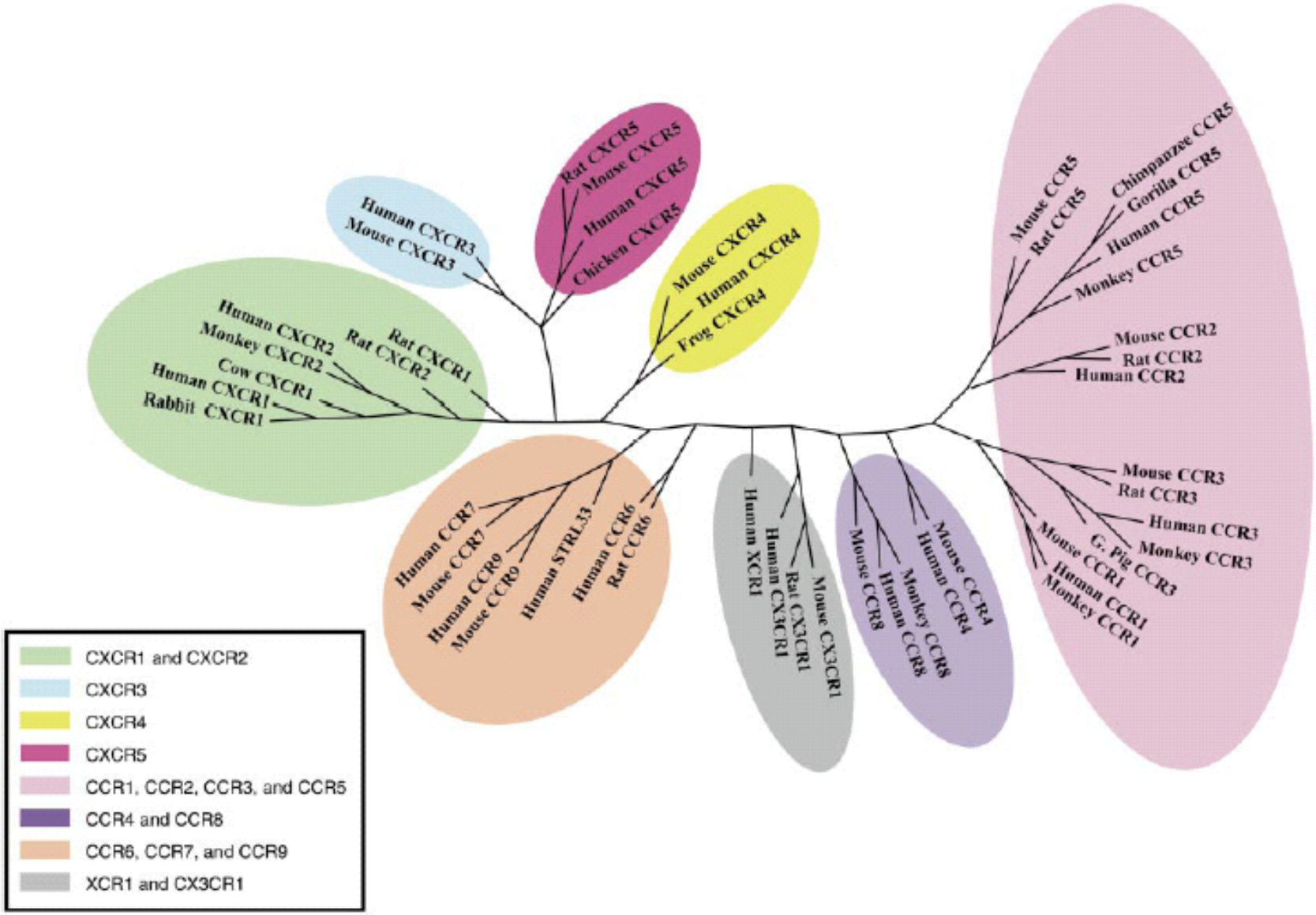


Gene expression in active human mononuclear phagocytes

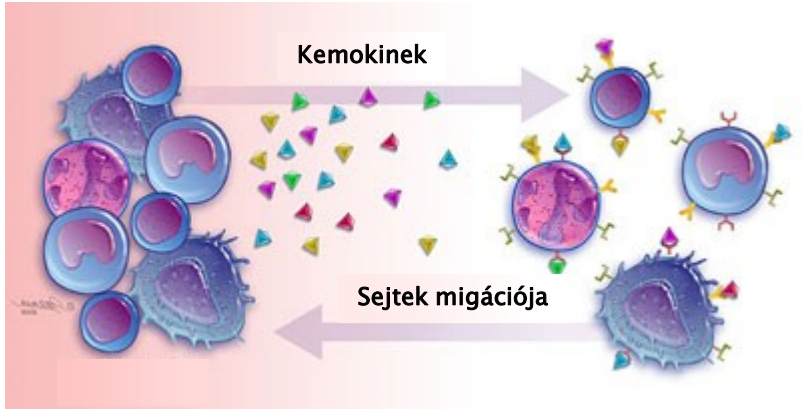
Phylogeny of Chemokines



Phylogeny of Chemokine receptors



Production of Chemokines and their Target cells



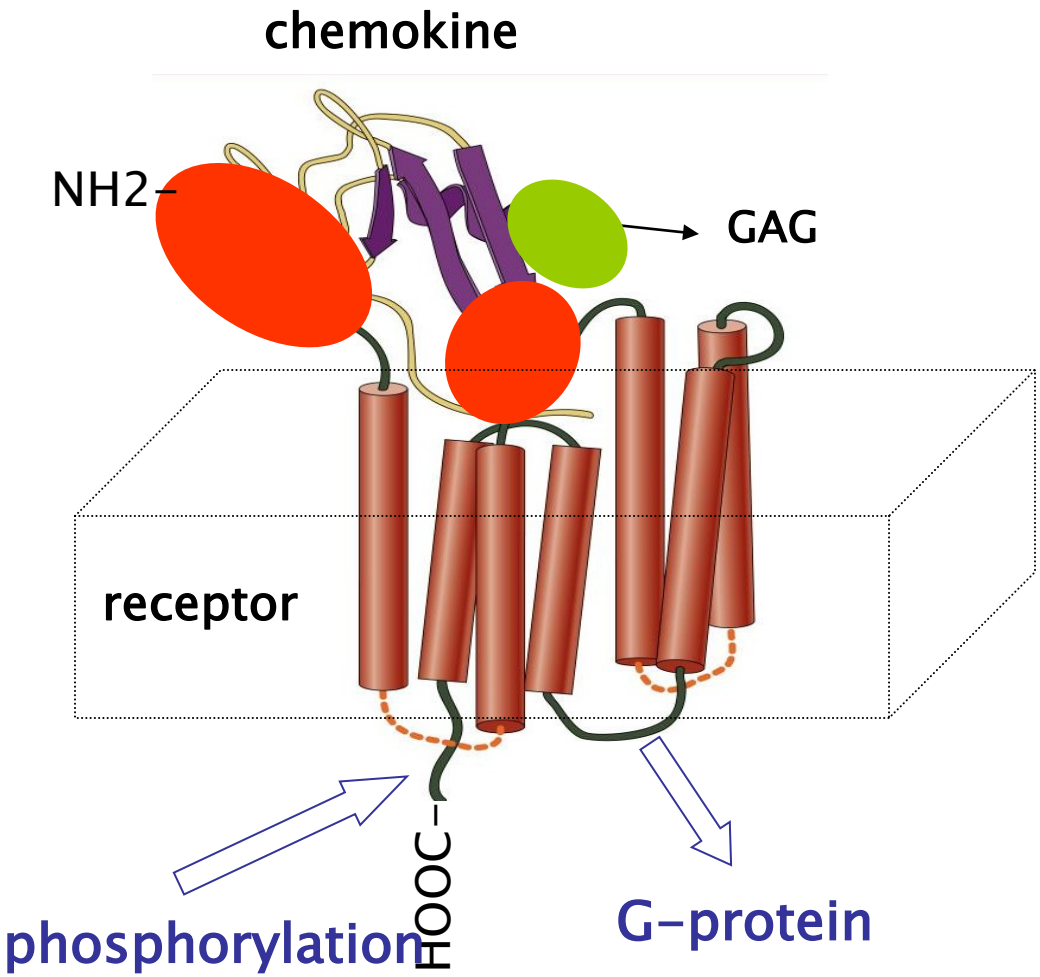
Chemokine producer cell

CXC chemokine	CC chemokine	CX_3C chemokine
monocyte	monocyte	endothel
lymphocyte	fibroblast	microglia
endothel	epithel	
	smooth muscle	
	melanoma	
	glioma	

Target cell of chemokine

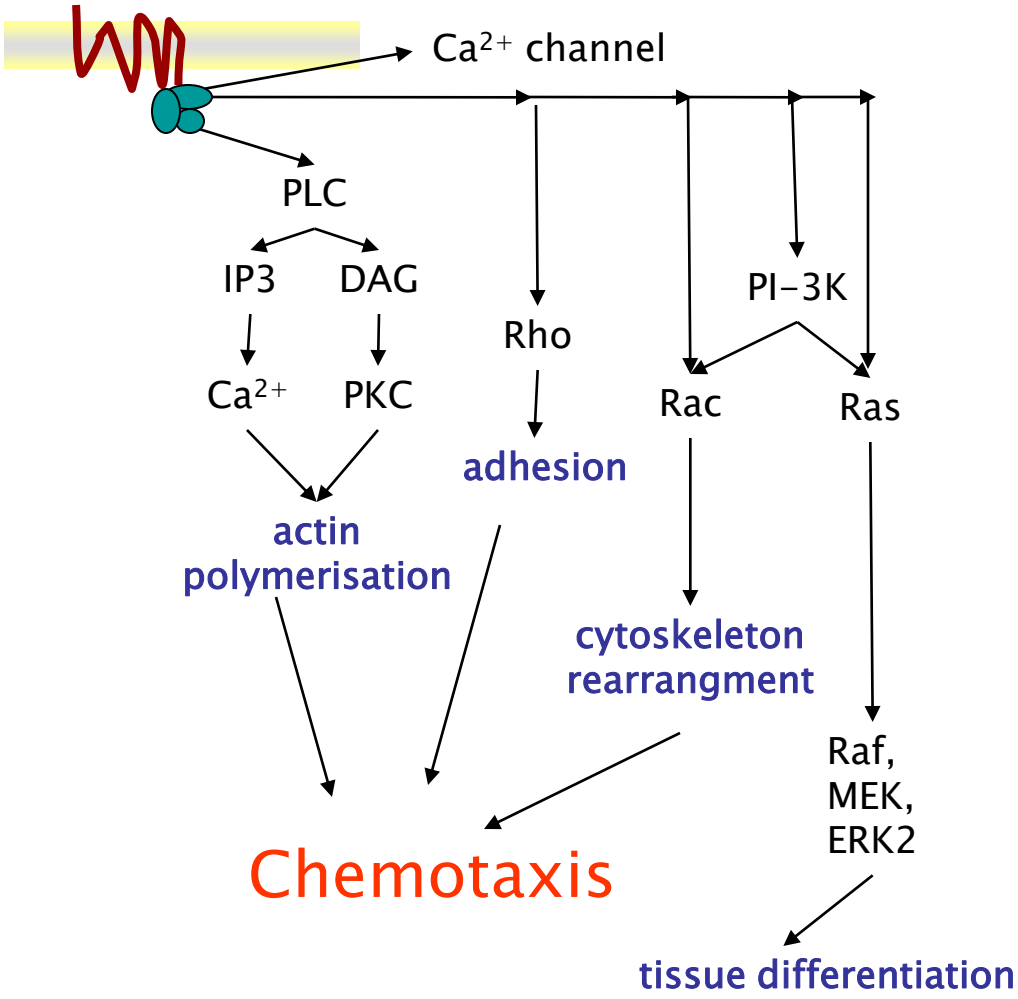
CXC chemokine	CC chemokine	CX_3C chemokine
neutrophil	monocyte	monocyte
Endothel	T-cell	T-cell
T-, NK-cell	dendritic cell	NK-cell
fibroblast	mastcell, basophil gr.cyte	
monocyte	eosinophil gr.cyte	

Chemokine receptors

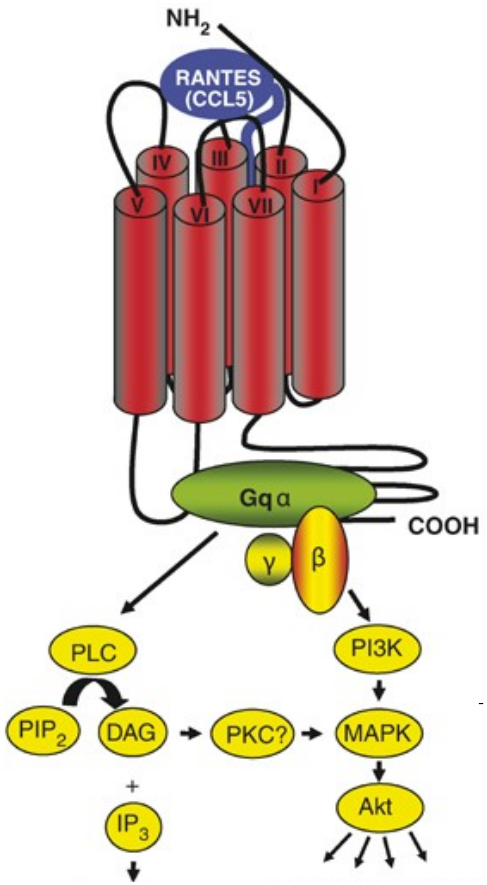


Cellular and Molecular Immunology 1999

Chemokines ic. signalling



Responses induced by chemokines



CELLULAR FUNCTIONS

Uropodium formation

Chemotaxis

Adh. mol. expr.

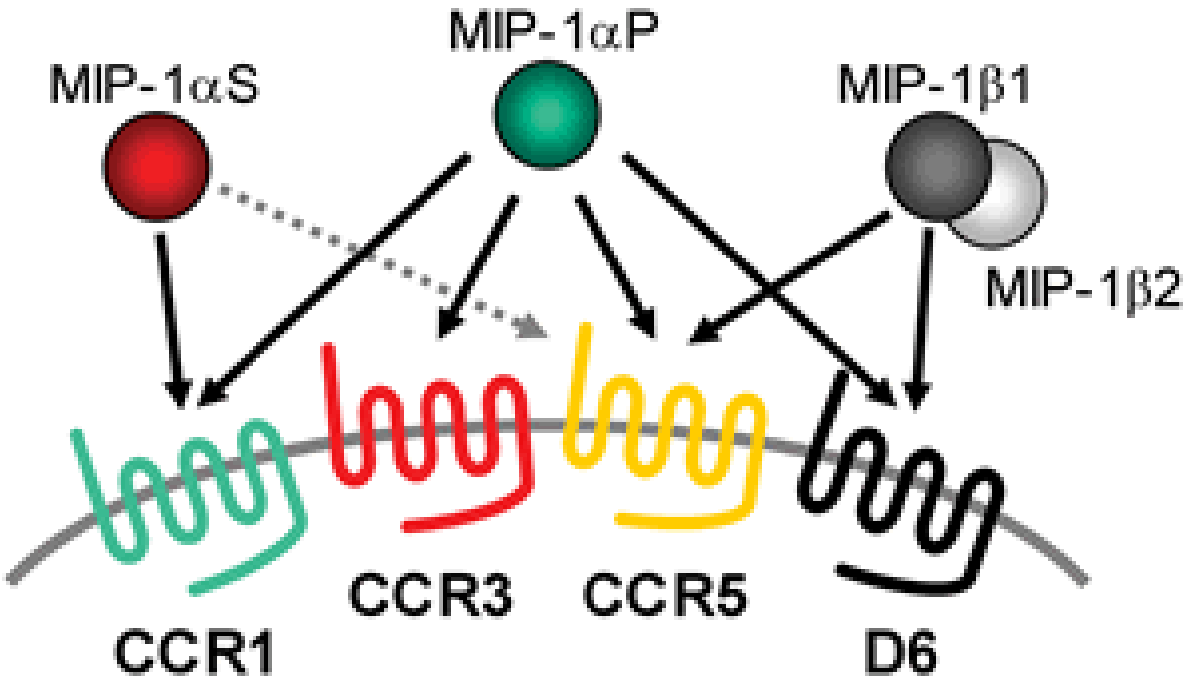
Proliferation

Prot. HIV infection

IL-2 rec. expr.

Cytokine release

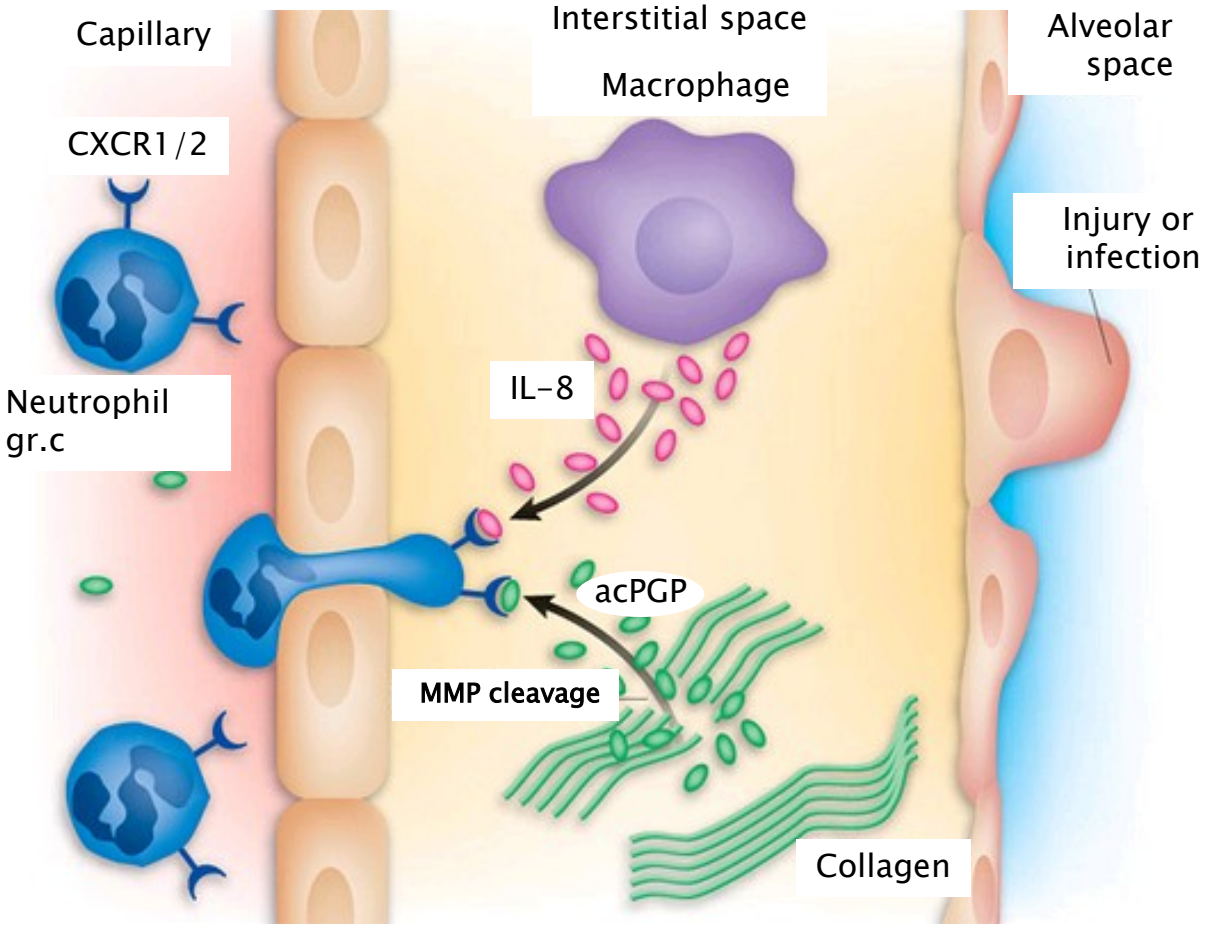
Receptor specificity



Other effects of chemokines

CCL19, CCL21	DC	increased expression of active markers increased production of cytokines elongation of dendrites blocked apoptosis increased rate of phagocytosis
CCL2	monocyte T-cell	decreased synthesis of IL-12 increased Th2 responsiveness
CCL3	DC	increased maturation of DC increasedTh1 responsiveness
CCL3, CCL4, CCL5	T-cell	DC-T cell interaction is increased decreased responsiveness to CCL21 increased synthesis of IL-12 in DC

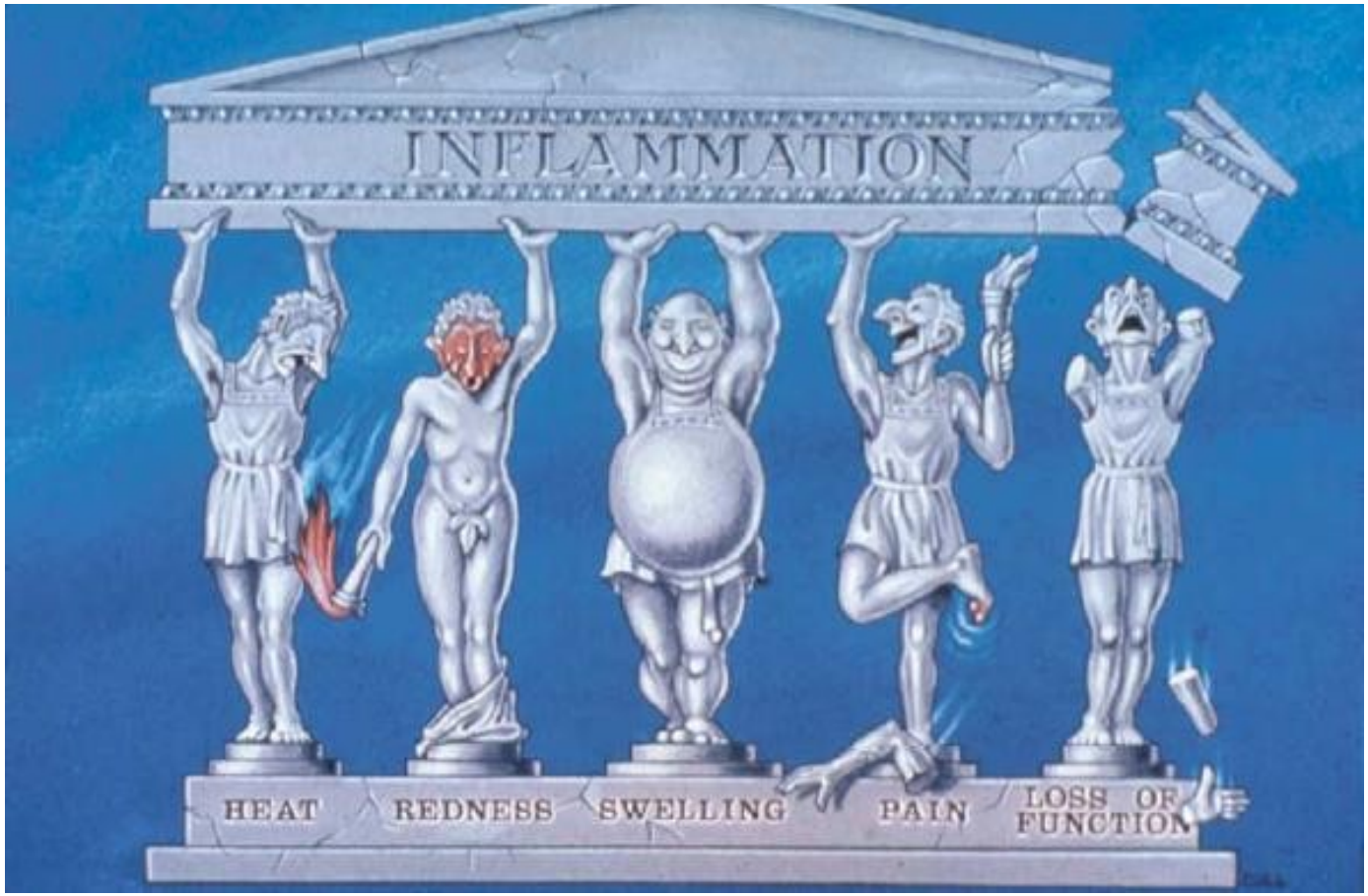
Fragment mimics chemokine



Nature Medicine 12, 317-323 (2006).

CLINICAL ASPECTS

Inflammation – Chemotaxis

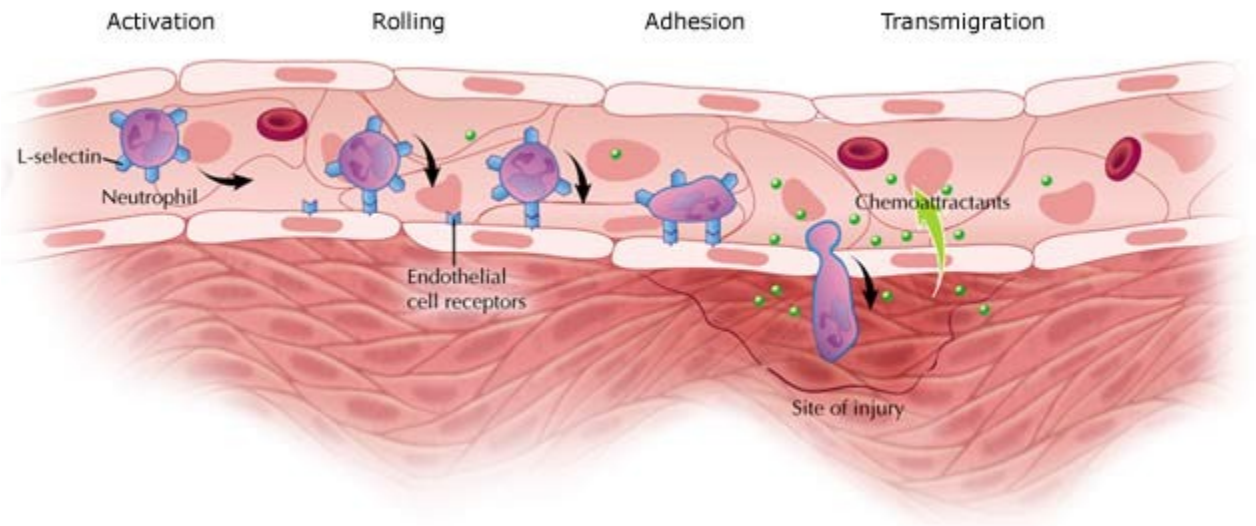


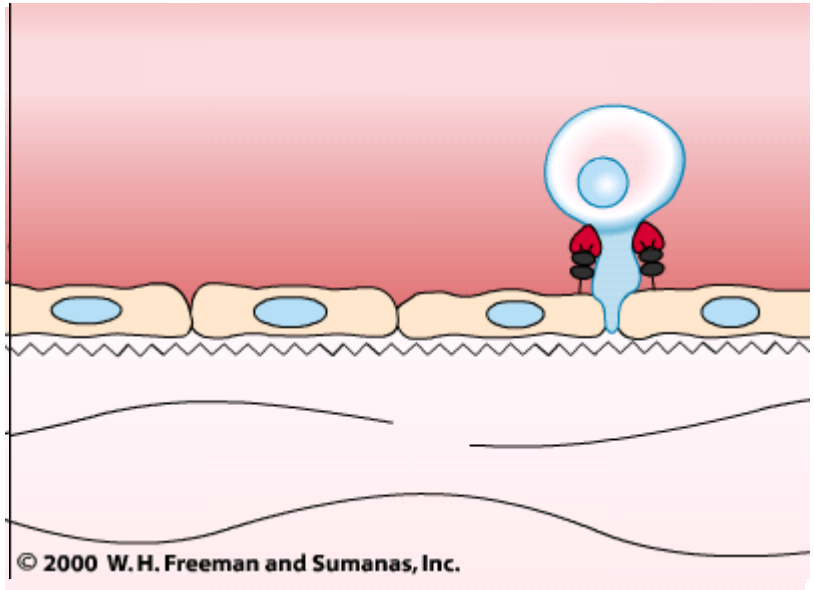
calor – rubor – tumor – dolor + functio laesa

Celsus
(Kr.e. 30～Kr. u 38)

Virchow
(1821–1902)

Inflammation – Migration



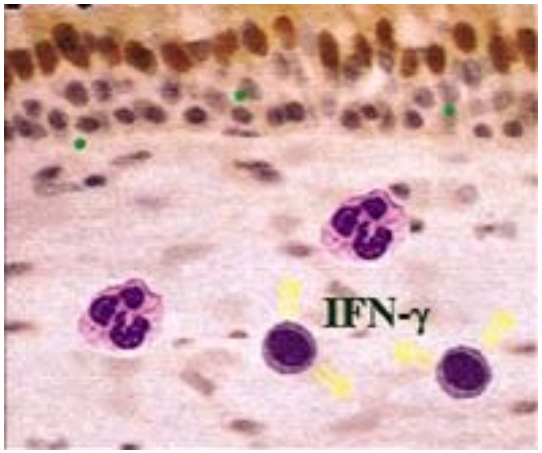


Inflammation – Migration 2

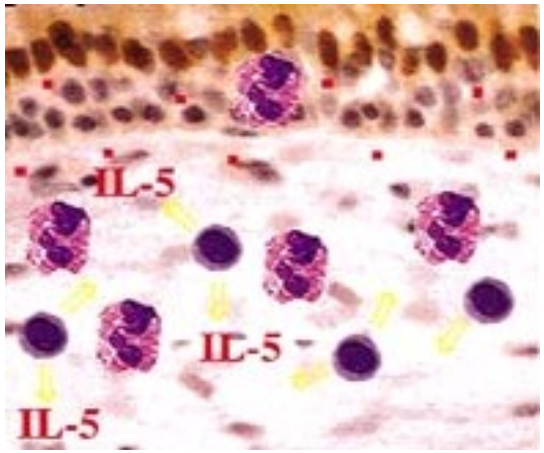
Infection
(e.g. bacteria)

„Sterile"
(e.g. allergen)

IL-8 release



RANTES, eotaxin release

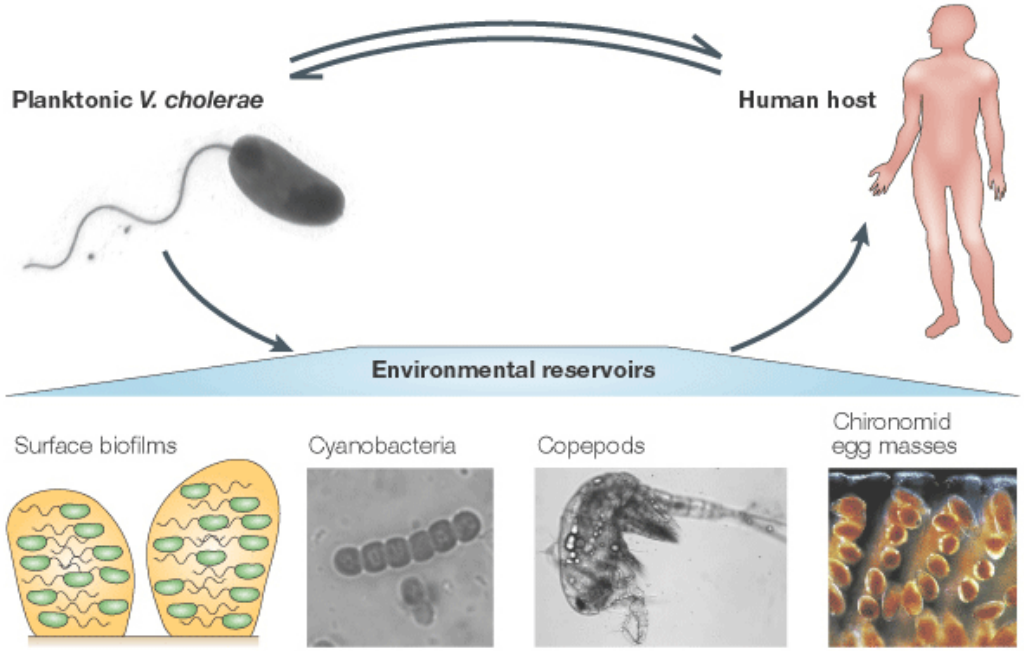


Clinical: Significance of Chemokines

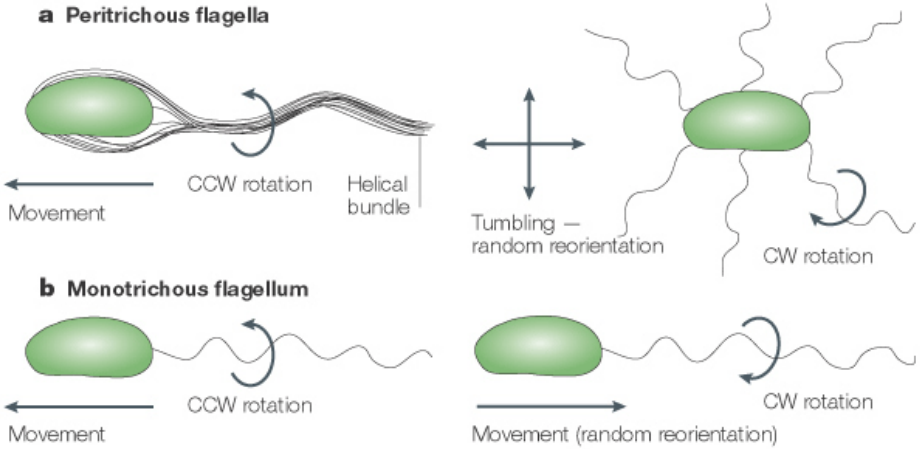
Acute respiratory distress syndrome	Neutrophil	Interleukin-8; GRO-α, -β, -γ; ENA-78
Asthma	Eosinophil, T cell, monocyte, basophil	MCP-1, -4; MIP-1α; eotaxin; RANTES
Bacterial pneumonia	Neutrophil	Interleukin-8; ENA-78
Sarcoidosis	T cell, monocyte	IP-10
Glomerulonephritis	Monocyte, T cell, neutrophil	MCP-1, RANTES, IP-10
Rheumatoid arthritis	Monocyte, neutrophil	MIP-1α, MCP-1, interleukin-8, ENA-78
Osteoarthritis		MIP-1β

Psoriasis	T cell, neutrophil	MCP-1, IP-10, MIG, GRO-β, interleukin-8
Bacterial meningitis	Neutrophil, monocyte	Interleukin-8; GRO-α; MCP-1; MIP-1α, -1β
Viral meningitis	T cell, monocyte	MCP-1, IP-10
Atherosclerosis	T cell, monocyte	MCP-1, -4; IP-10
Inflammatory bowel disease	Monocyte, neutrophil, T cell, eosinophil	MCP-1, MIP-1α, eotaxin, IP-10, interleukin-8

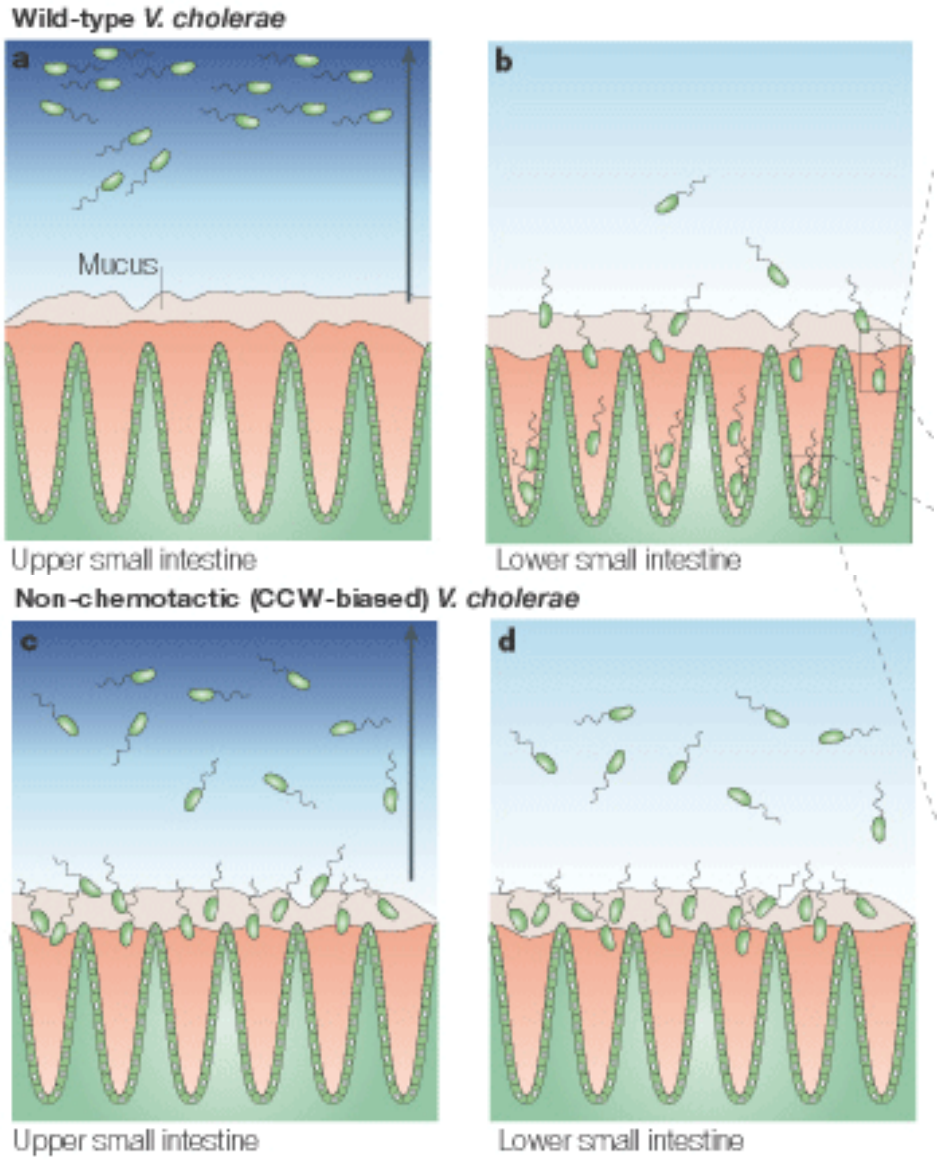
Clinical: Infcetions – Cholera



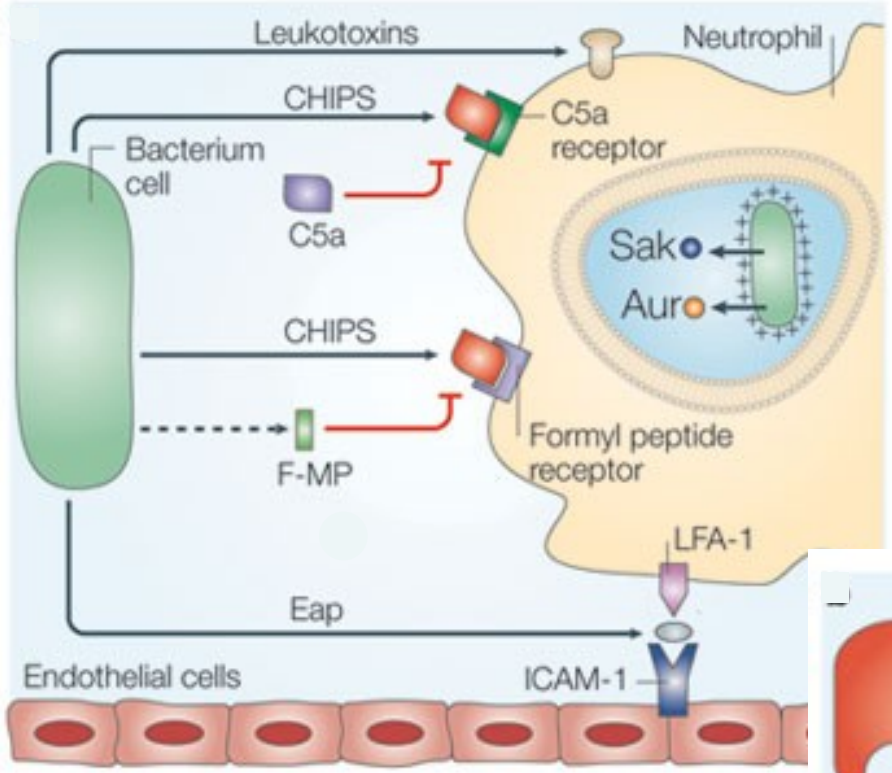
Nature Reviews | Microbiology



Clinical: Infections – Cholera 2

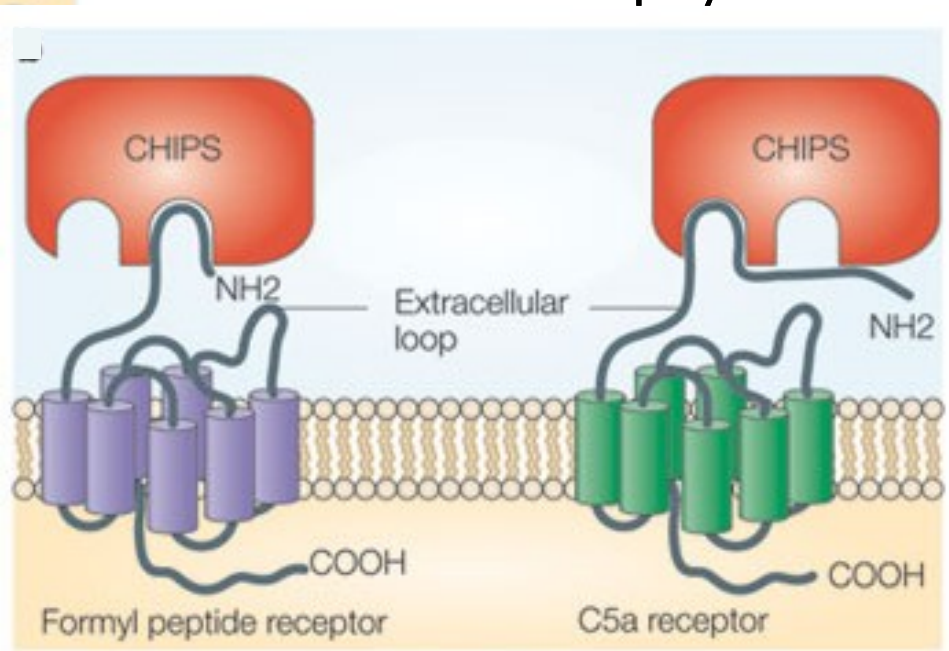


Clinical: Bacteria – Human



CHIPS ? =

CHIPS ! = Chemotaxis Inhibitory Protein of Staphylococci

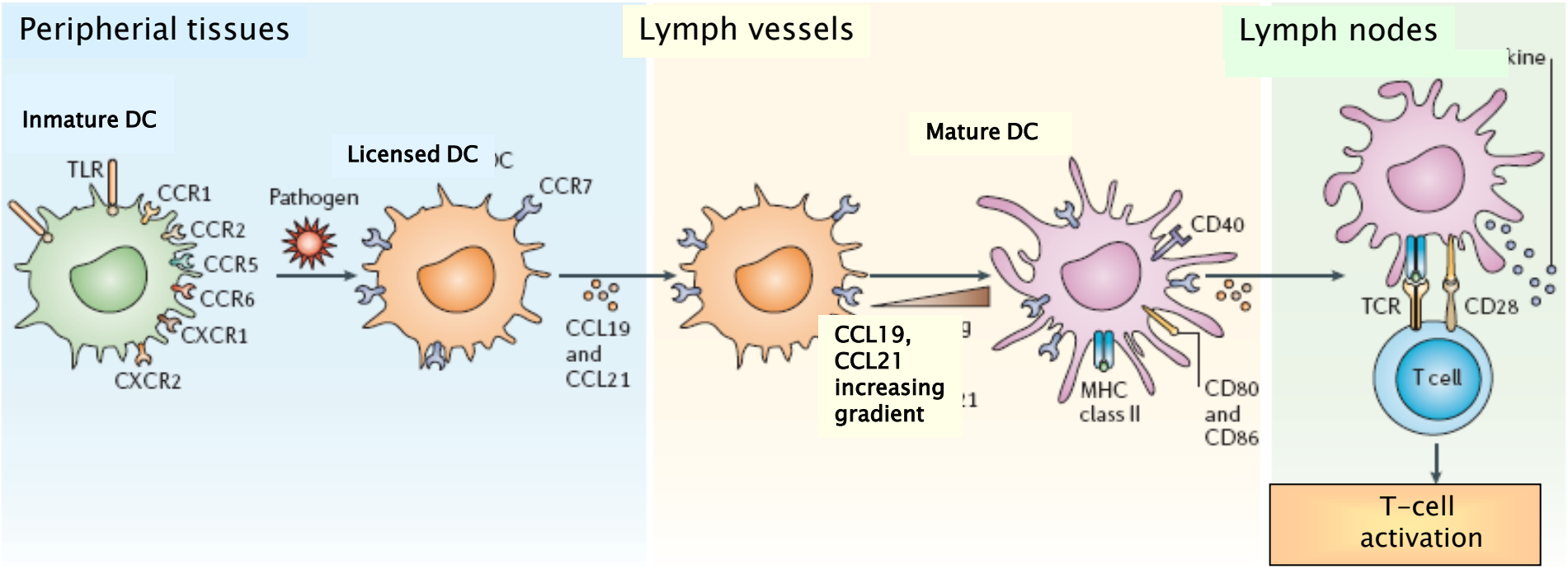


Eap = extracellular adherence protein

Sak = Staphylokinase

Aur = aureolysin

Chemokines and differentiation of DC



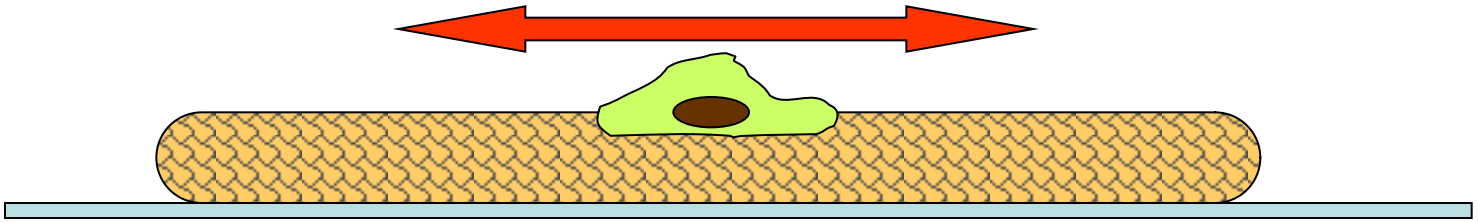
Cell type	CCR expressed	CXCR expressed	Representative surface markers
Immature DC	CCR1, CCR2, CCR5, CCR6, CCR7	CXCR1, CXCR2	$CD40^{low}$, $CD80^{low}$, $CD86^{low}$, MHC class II^{low}
Licensed DC	CCR7	None	$CD40^{low/int}$, $CD80^{low/int}$, $CD86^{low/int}$, MHC class $II^{int/hi}$
Mature DC	CCR7	None	$CD40^{hi}$, $CD80^{hi}$, $CD86^{hi}$, MHC class II^{hi}
Naive T cell	CCR7	CXCR4	$CD62L^{hi}$, $CD44^{low}$, $CD69^{low}$
Activated T cell	CCR2, CCR3, CCR4, CCR5, CCR6, CCR8	CXCR5	$CD62L^{low}$, $CD44^{hi}$, $CD69^{hi}$

CCR, CC-chemokine receptor; CXCR, CXC-chemokine receptor; DC, dendritic cell.

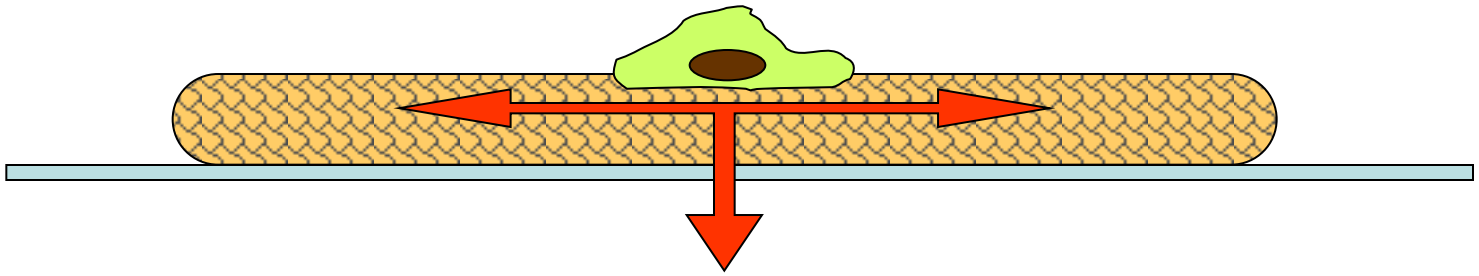
Nature Reviews Immunol. 6, 159–164 (2006)

Invasion

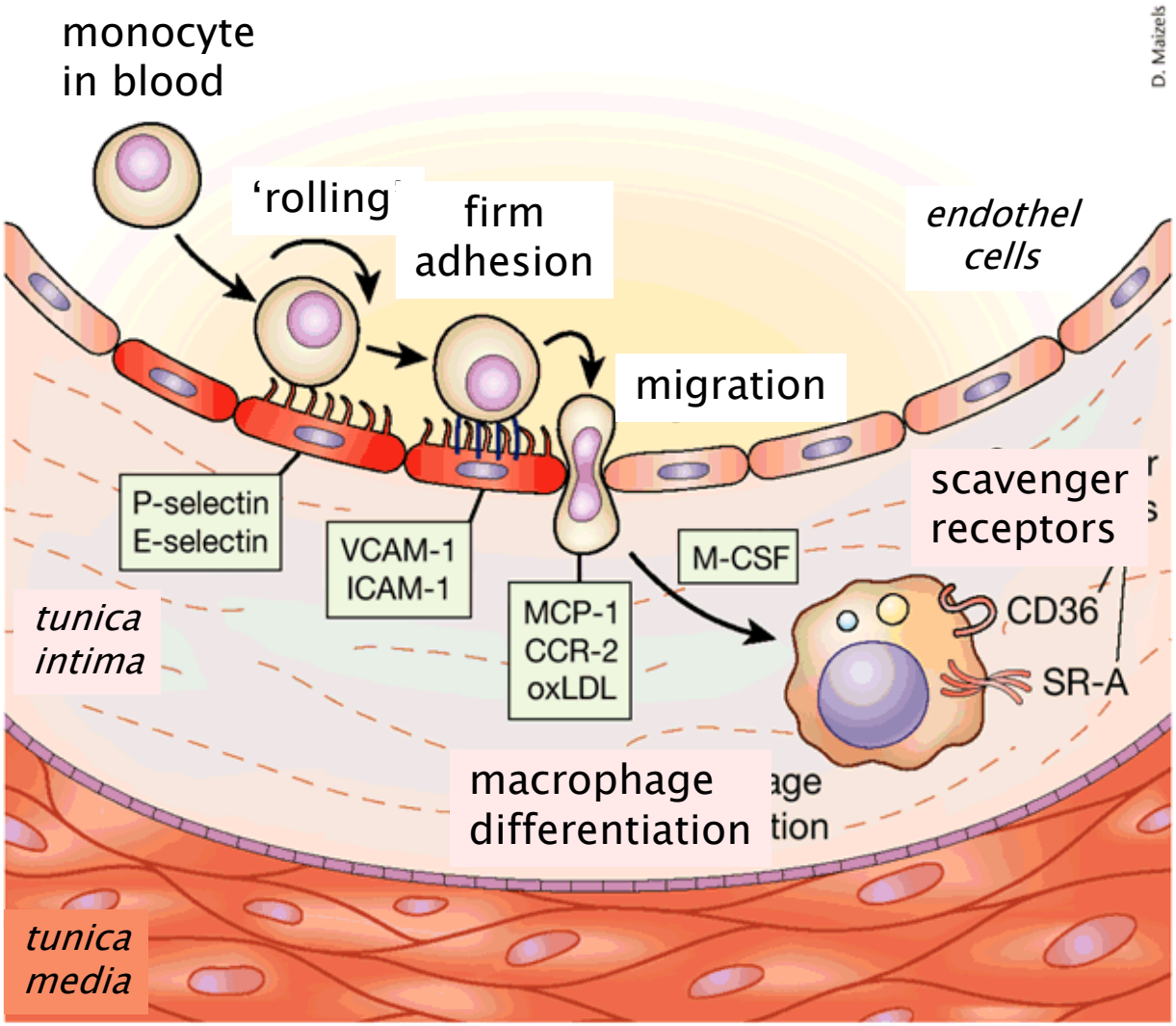
2D



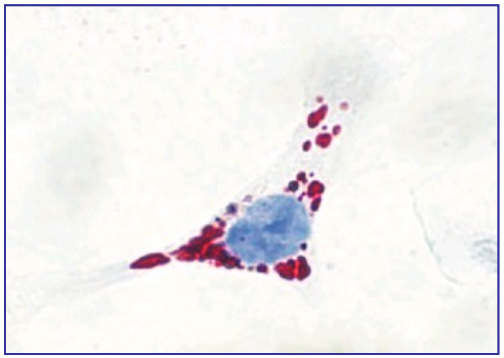
3D

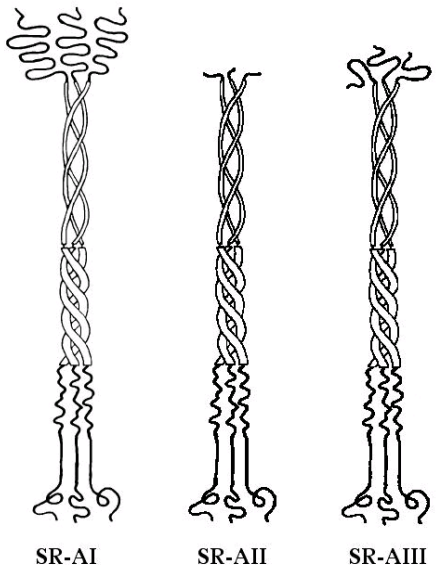


Clinical: Atherosclerosis



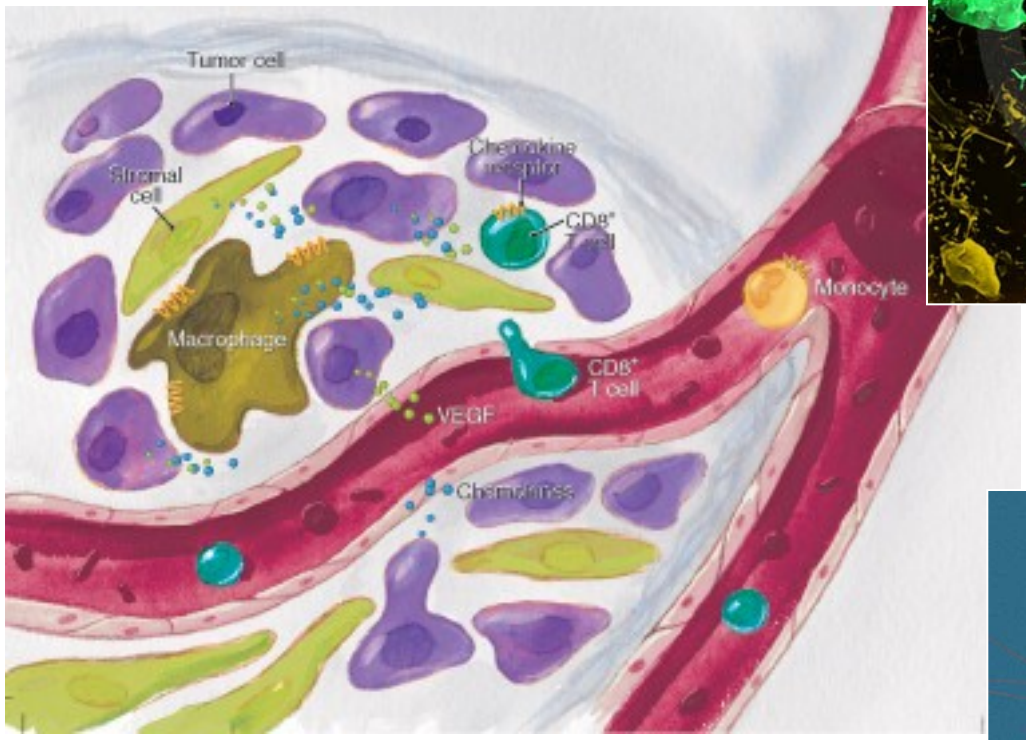
Nature Medicine 8, 1235 – 1242 (2002)

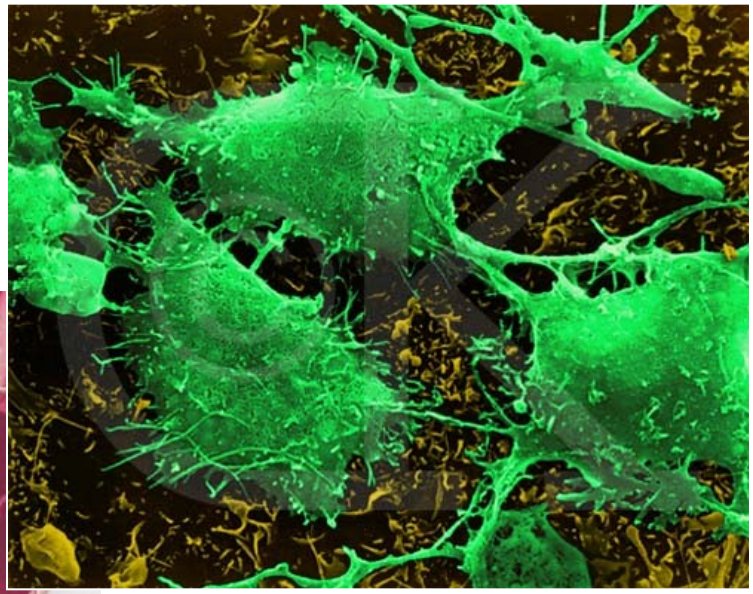


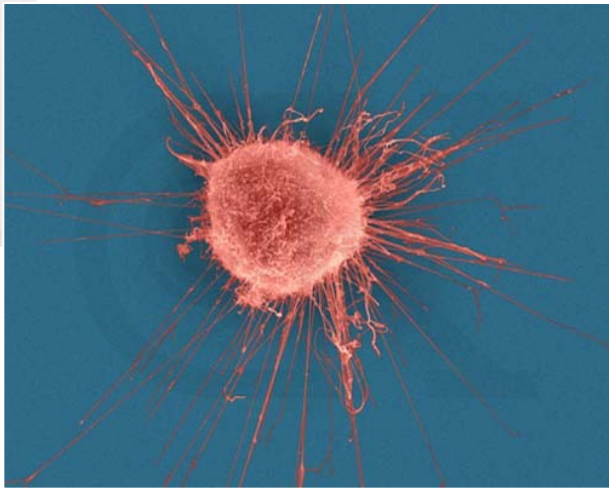


Scavenger receptors

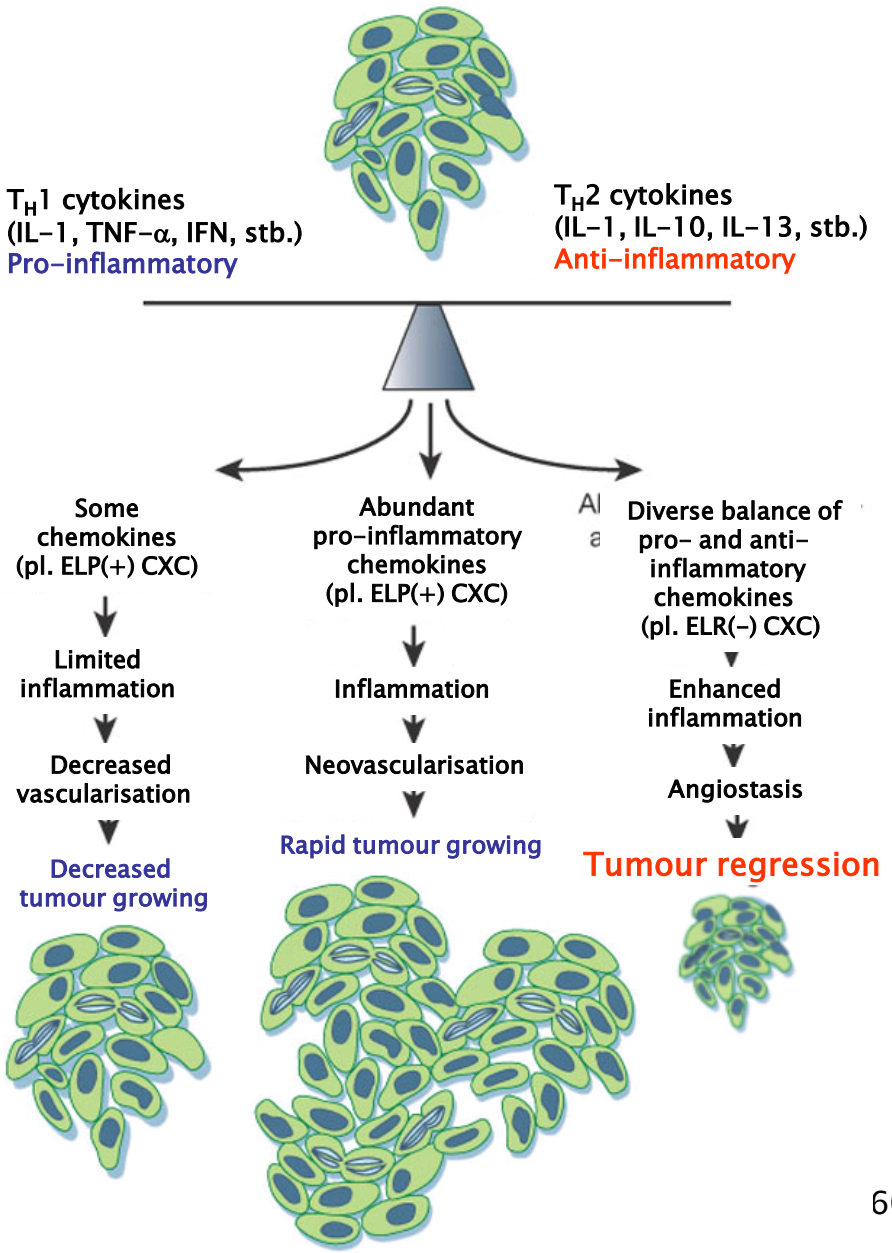
Pathway of tumour cells







Growing of tumours and cytokines/chemokines



60-867(19 December 2002)

Inflammation and Tumour

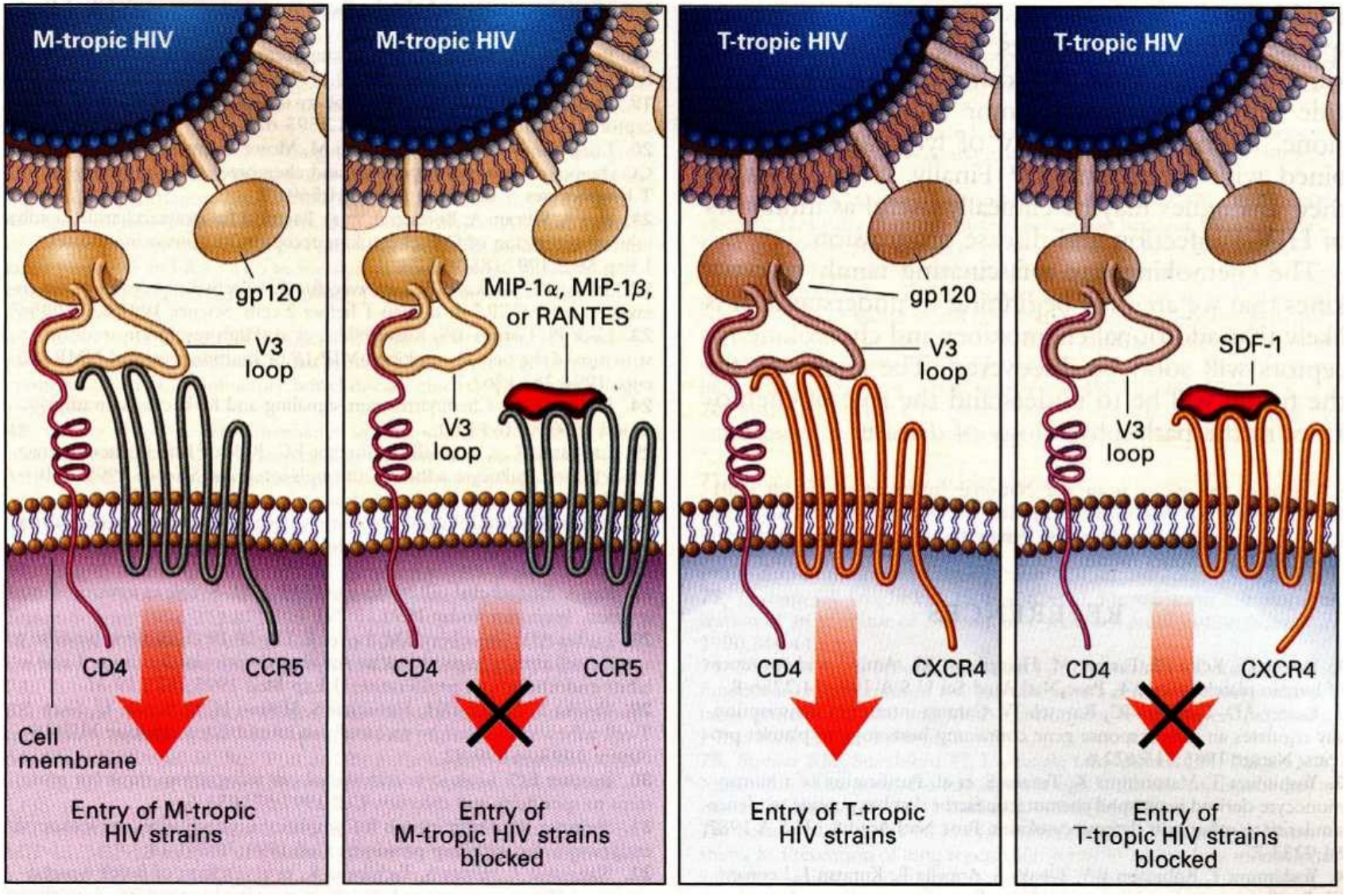
Table 1 **Chronic inflammatory conditions associated with neoplasms**

Pathologic condition	Associated neoplasm(s)	Aetiologic agent
Asbestosis, silicosis	Mesothelioma, lung carcinoma	Asbestos fibres, silica particles
Bronchitis	Lung carcinoma	Silica, asbestos, smoking (nitrosamines, peroxides)
Cystitis, bladder inflammation	Bladder carcinoma	Chronic indwelling, urinary catheters
Gingivitis, lichen planus	Oral squamous cell carcinoma	
Inflammatory bowel disease, Crohn's disease, chronic ulcerative colitis	Colorectal carcinoma	
Lichen sclerosus	Vulvar squamous cell carcinoma	
Chronic pancreatitis, hereditary pancreatitis	Pancreatic carcinoma	Alcholism, mutation in trypsinogen gene on Ch. 7
Reflux oesophagitis, Barrett's oesophagus	Oesophageal carcinoma	Gastric acids
Sialadenitis	Salivary gland carcinoma	
Sjögren syndrome, Hashimoto's thyroiditis	MALT lymphoma	
Skin inflammation	Melanoma	Ultraviolet light

Infection and Tumours

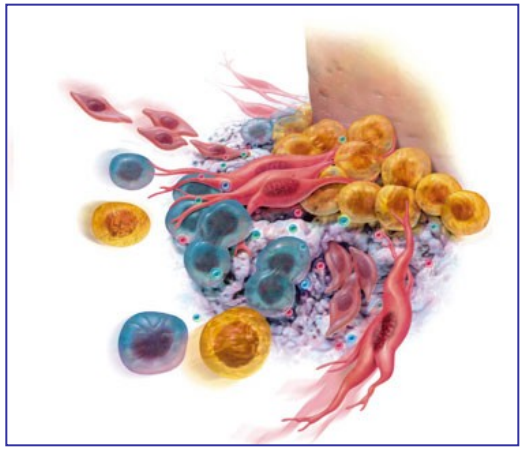
Cancers associated with infectious agents		
Opisthorchis, Cholangitis	Cholangiosarcoma, colon carcnoma	Liver flukes (*Opisthorchis viverrini*), bile acids
Chronic cholecystitis	Gall bladder cancer	Bacteria, gall bladder stones
Gastritis/ulcers	Gastric adenocarcinoma, MALT	*Helicobacter pylori*
Hepatitis	Hepatocellular carcinoma	Hepatitis B and/or C virus
Mononucleosis	B-cell non-Hodgkin's lymphoma, Burkitts lymphoma,	Epstein-Barr Virus
AIDS	Non-Hodgkin's lymphoma, squamous cell carcinomas, Kaposi's sarcoma	Human immunodeficiency virus, human herpesvirus type 8
Osteomyelitis	Skin carcinoma in draining sinuses	Bacterial infection
Pelvic inflammatory disease, chronic cervicitis	Ovarian carcinoma, cervical/anal carcinoma	Gonnorrhoea, chlamydia, human papillomavirus
Chronic cystitis	Bladder, liver, rectal carcinoma, follicular lymphoma of the spleen	Schistosomiasis

Clinical: HIV infection and chemokine receptoros

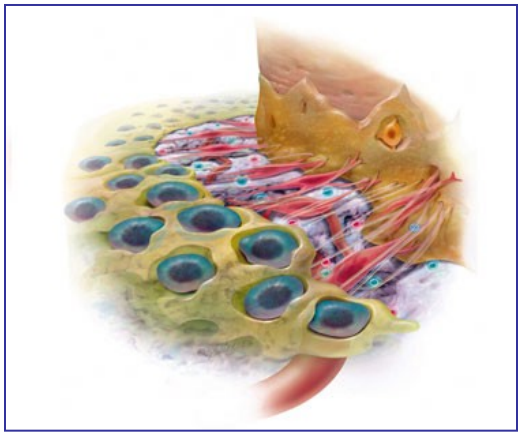
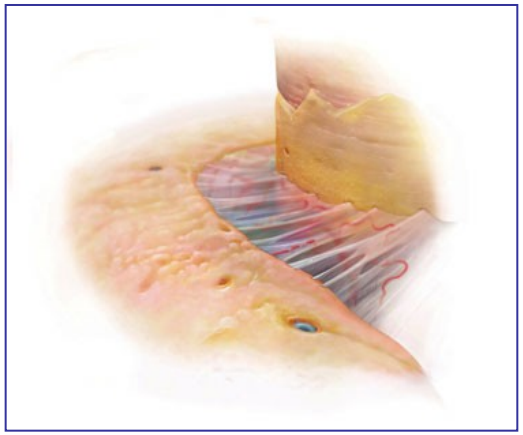


Clinical: Dentistry



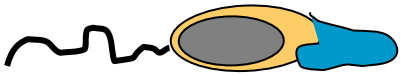


Maxillar/mandibular substitution with materials releasing chemoattractants (e.g. PDGF)

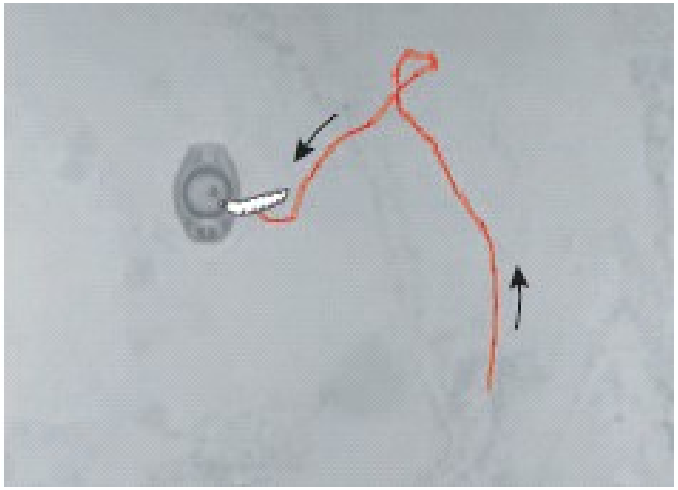




Increased migration and division of osteoblasts, fibroblasts and cementoblasts
– promotes reconstruction of bone and crevicular structure –

OTHER ASPECTS

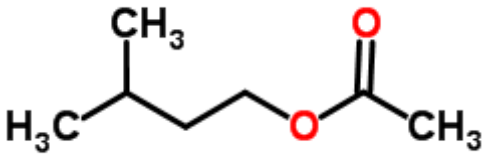


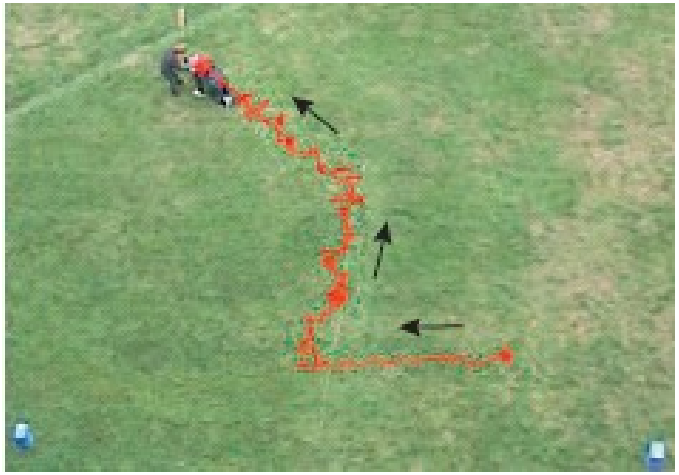
Olfactory receptors – Migration



Substance: isoamyl acetate
Path: 20.7 cm
Time: 3 min 50 sec

Test organism:
Drosophila larva

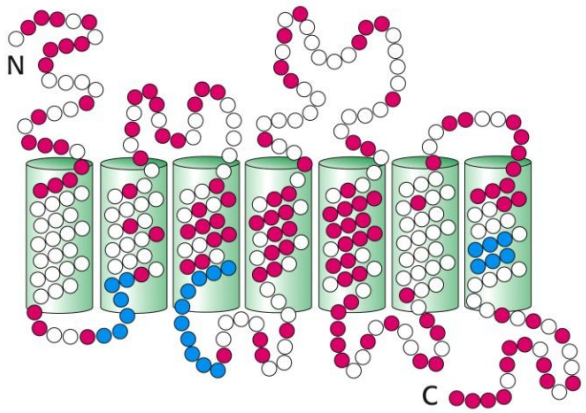


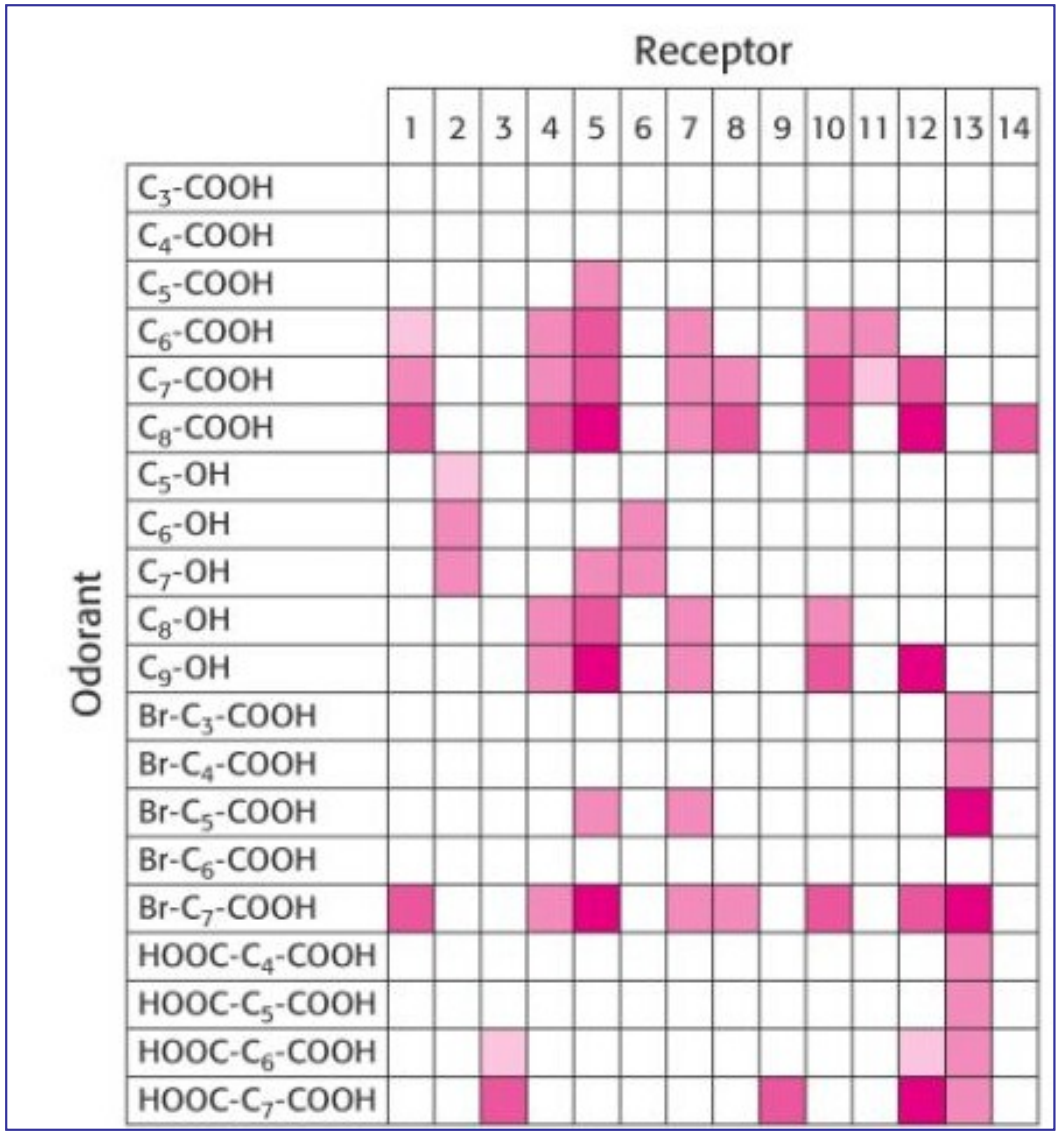


Substance: chocolate ess.oil
Area: 10x10m field
Time: 5 min 34 sec

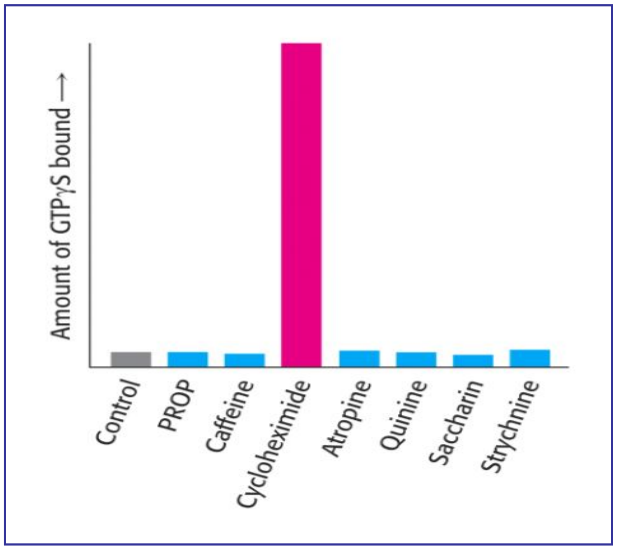
Test organism:
Human

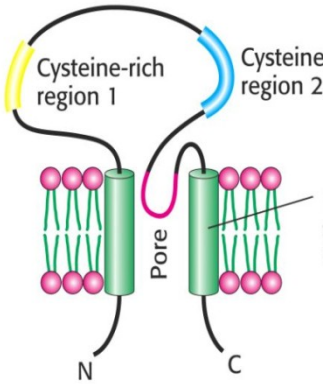
Ligand specificity of olphactory-receptors



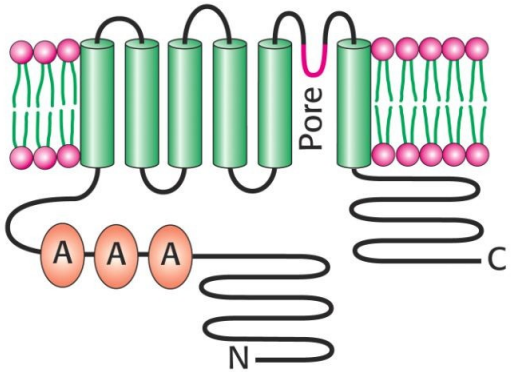


Structural diversity and sensibility of taste receptors

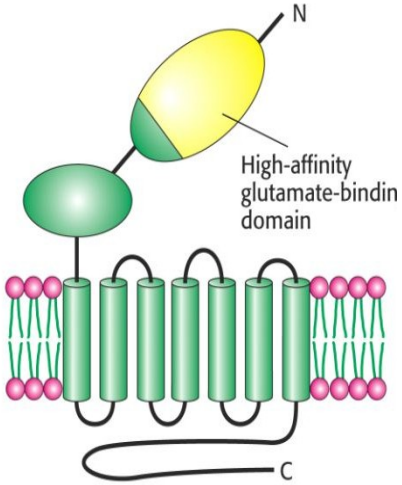




Na+

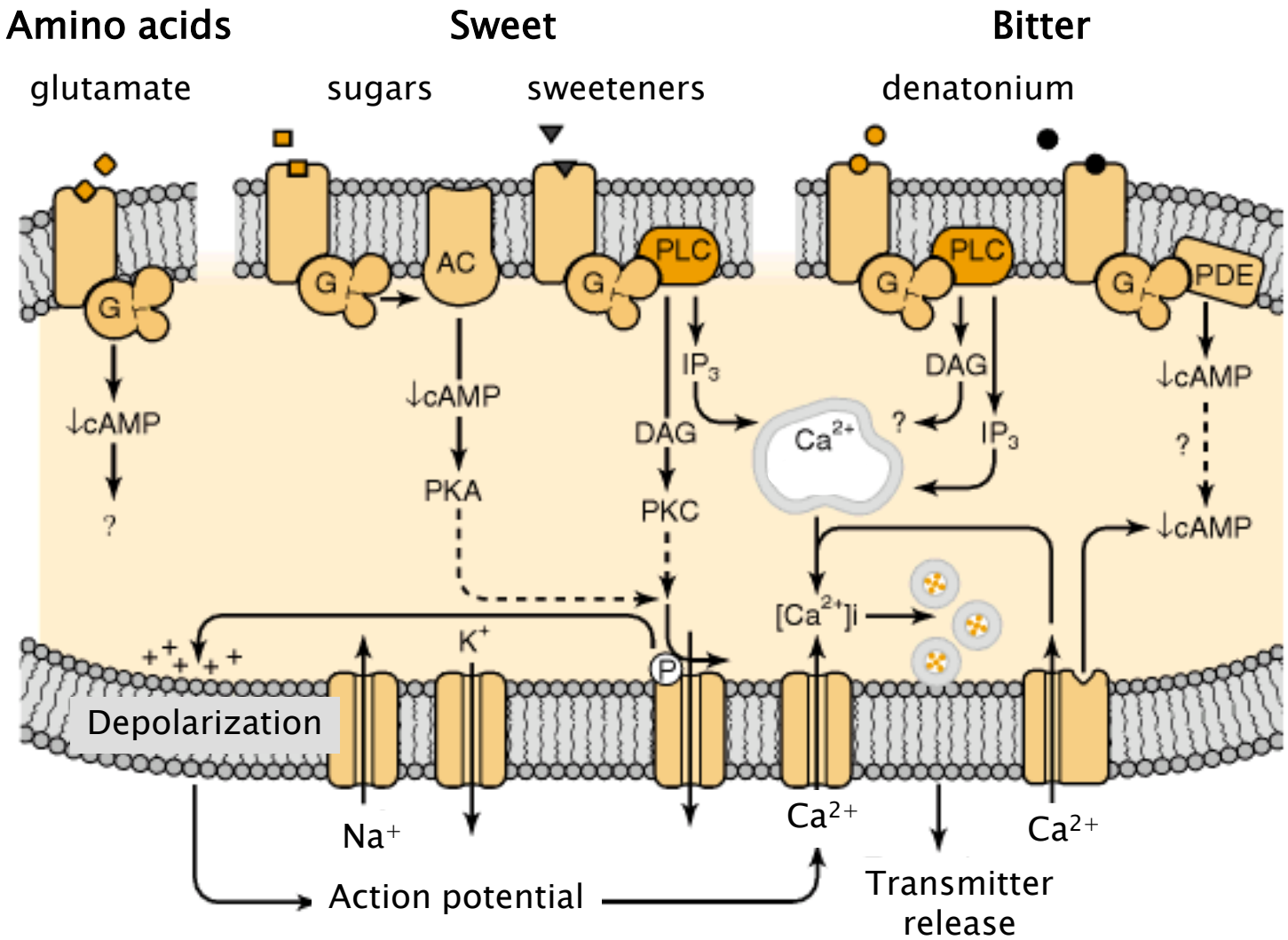


capsaicin



umami

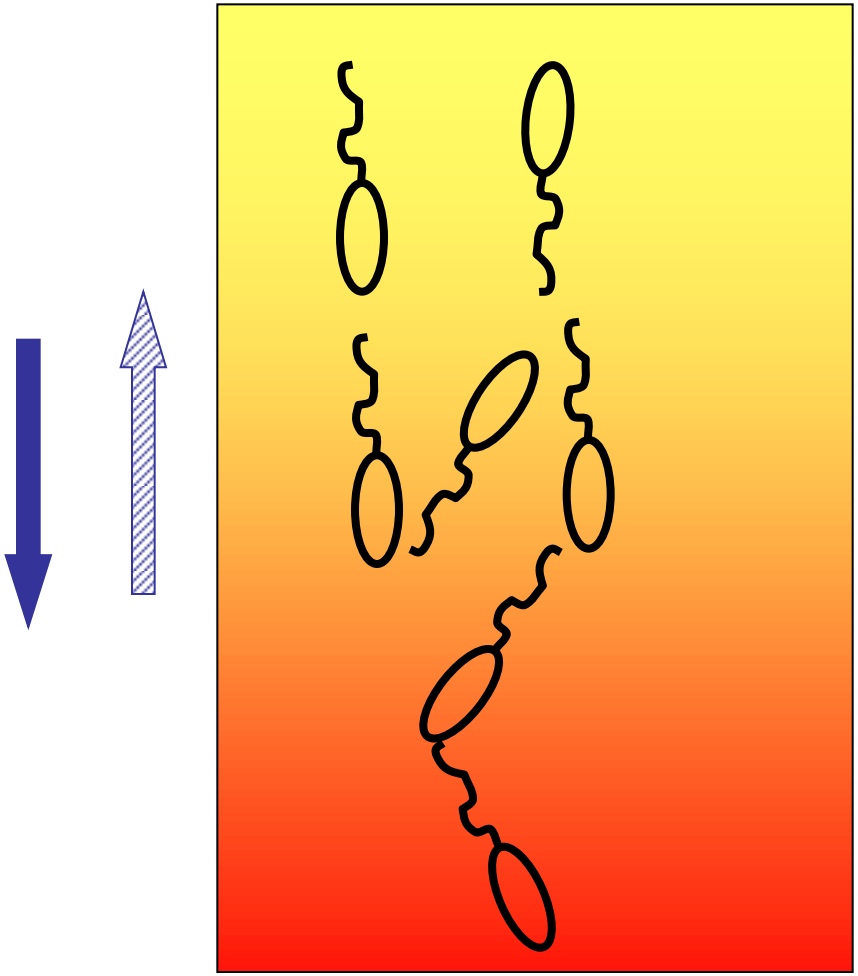
Intracellular signalling of taste receptors



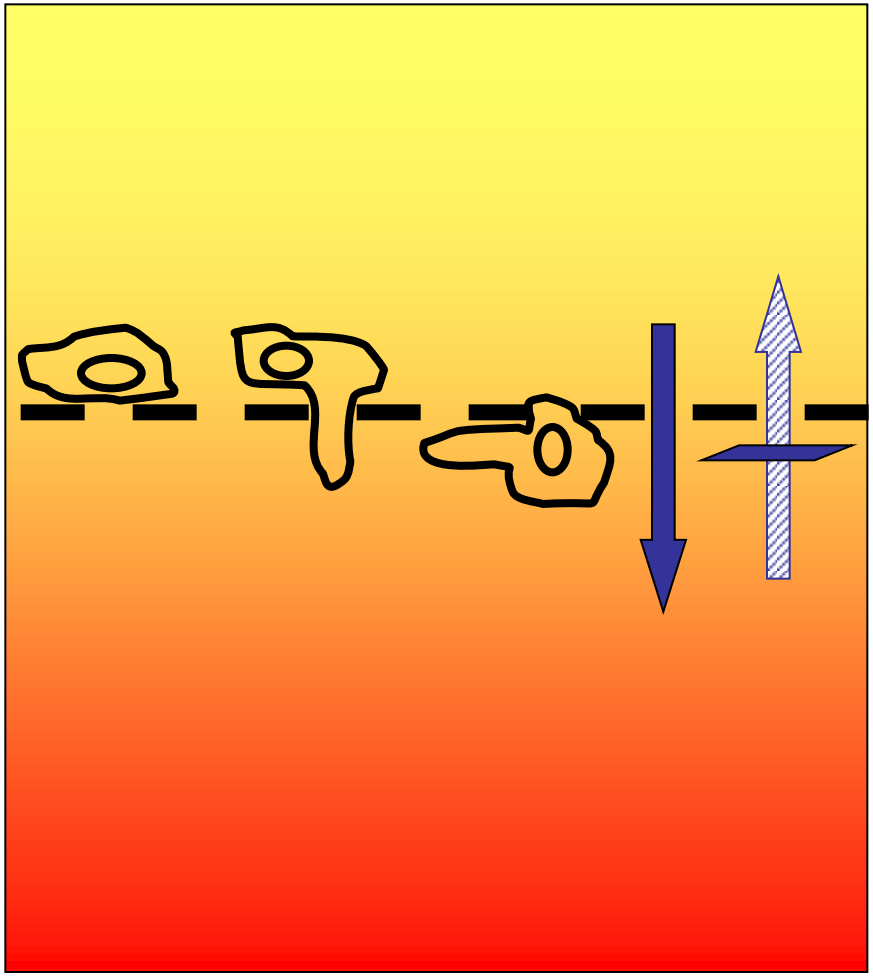
CHEMOTAXIS ASSAYS

Main types of chemotaxis assays

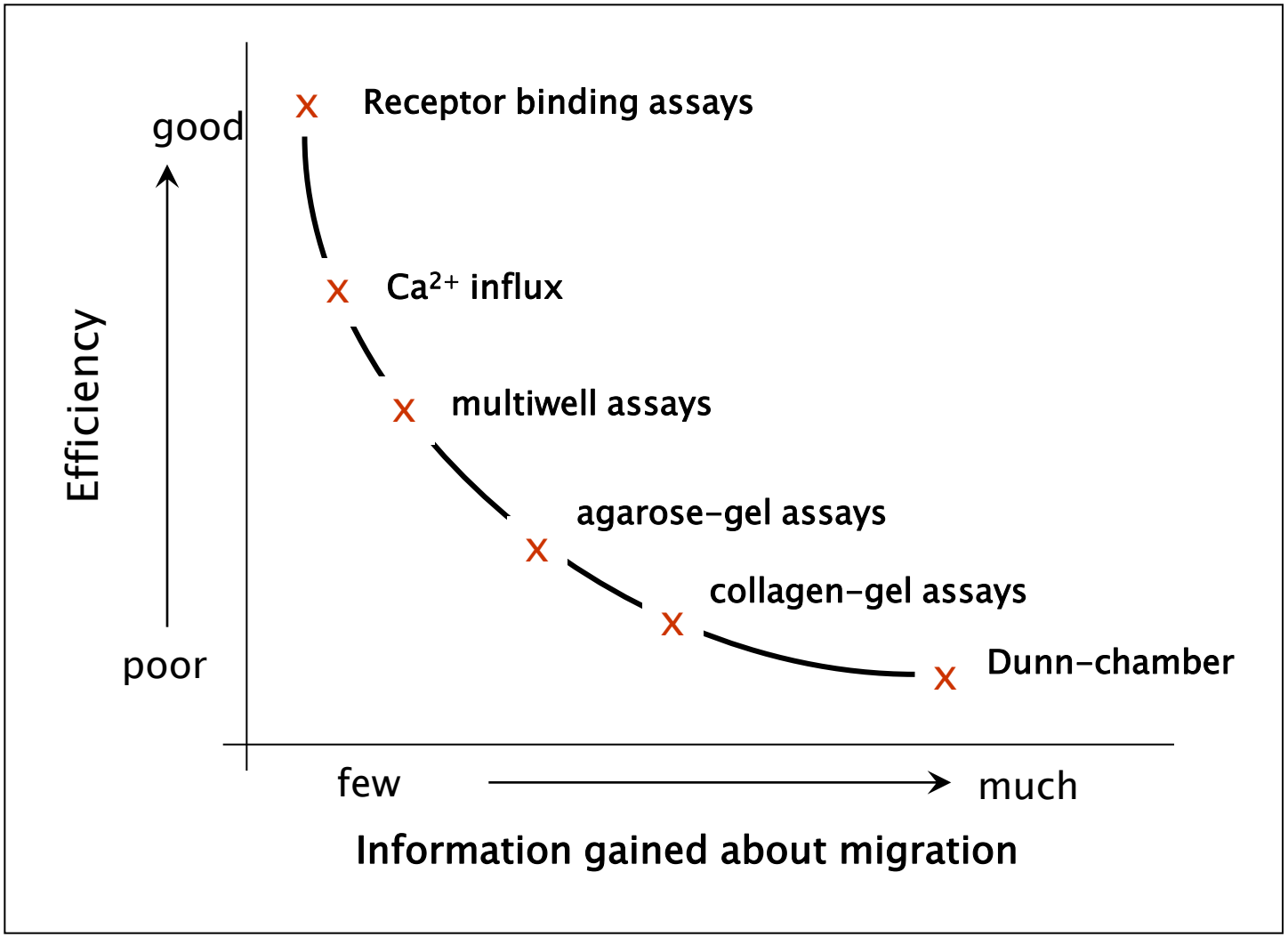
Reversible systems



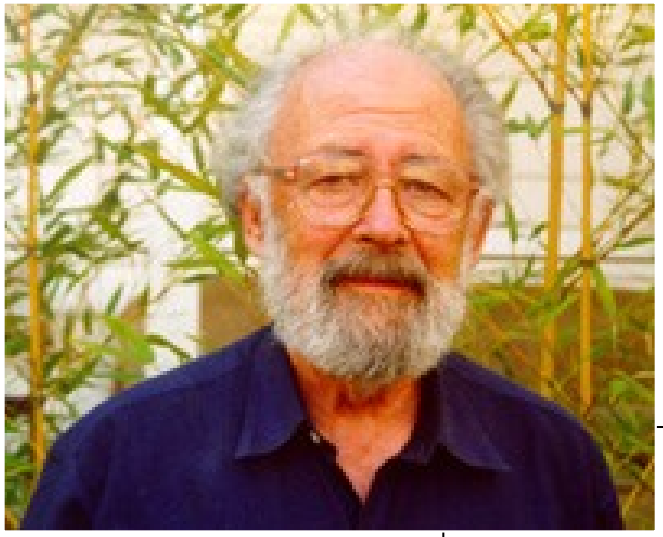
Irreversible systems



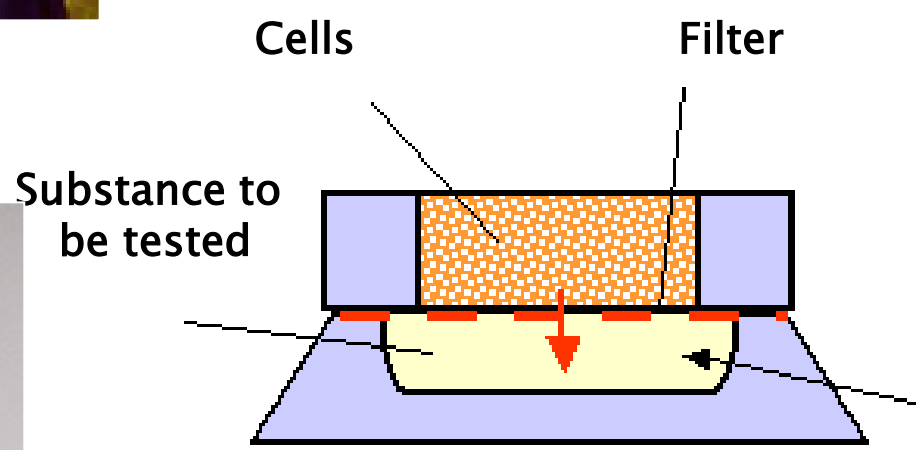
Comparative study of chemotaxis assays

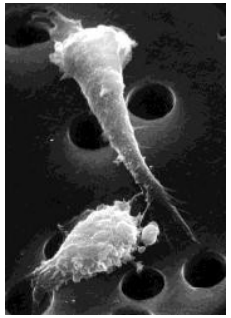


Chemotaxis assays 1



Boyden, Stephen
John Curtin School of Medical Research
Australian National University



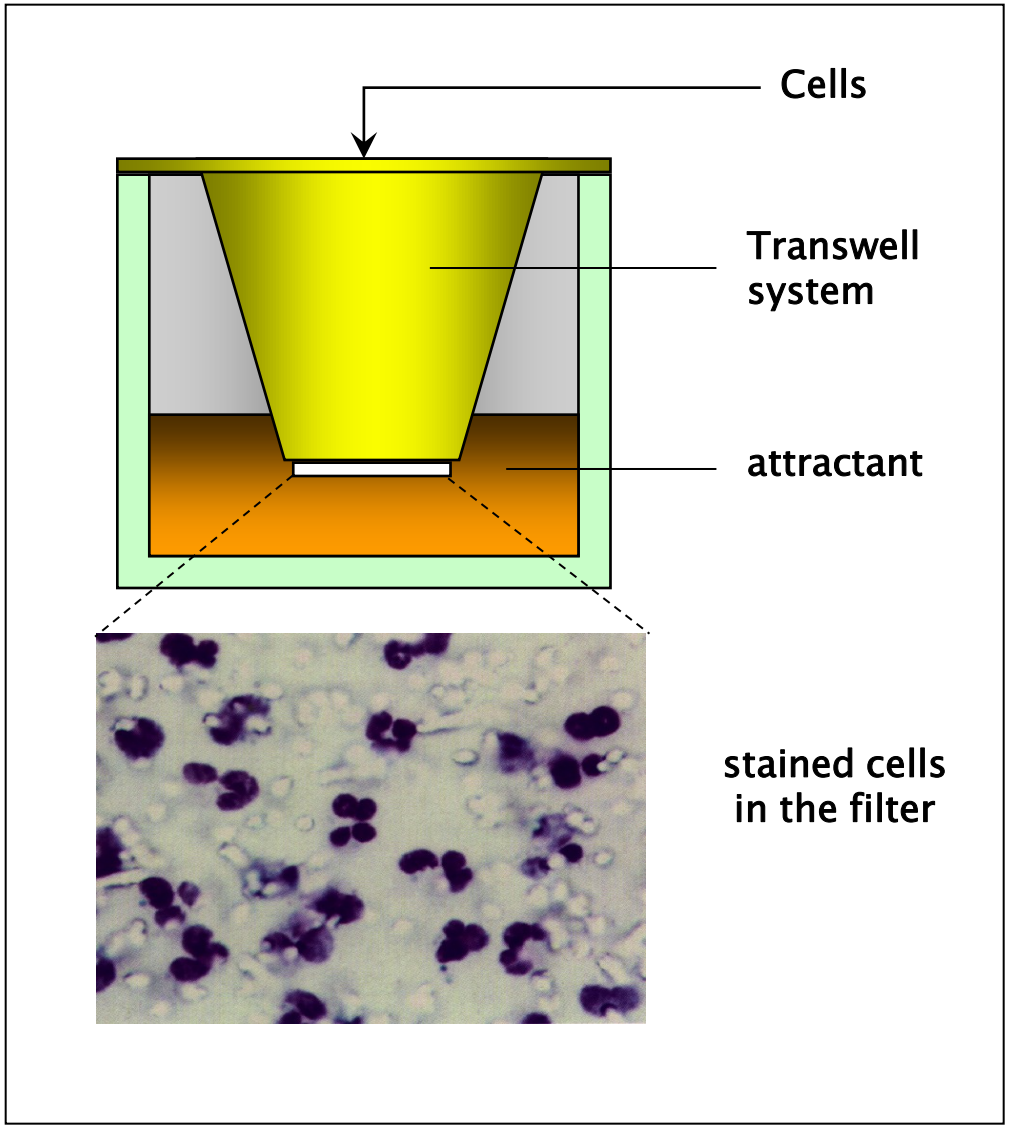


Counting of cells

Boyden-chamber

Chemotaxis assays 2

Repesh, Lilian, A.
Med.Sch. Duluth, Univ. Minnesota

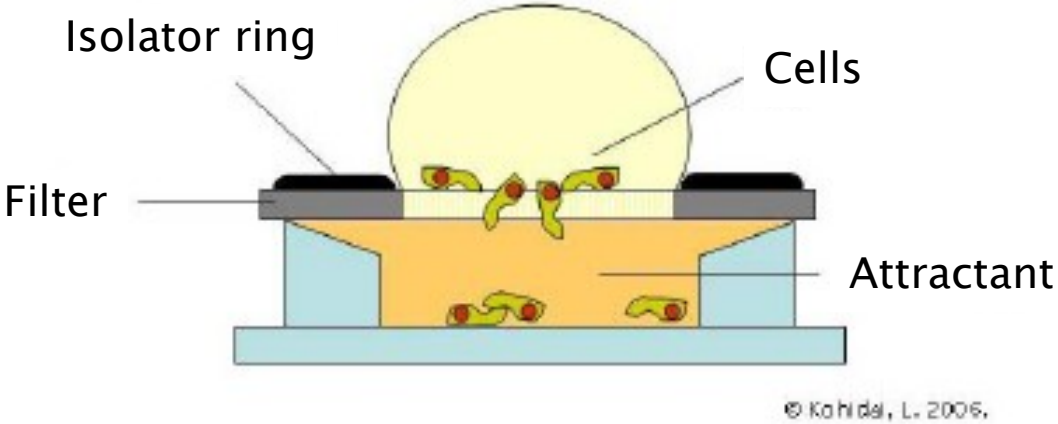


Transwell systems

Chemotaxis assays 3

Chemo Tx

Frevert, CW
Med.Res.Serv.
Univ. Washington



New chapter – Impedimetry

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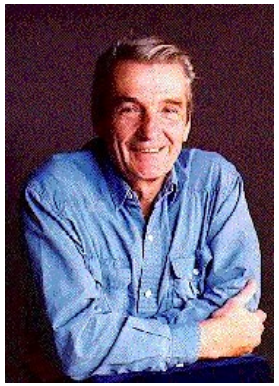
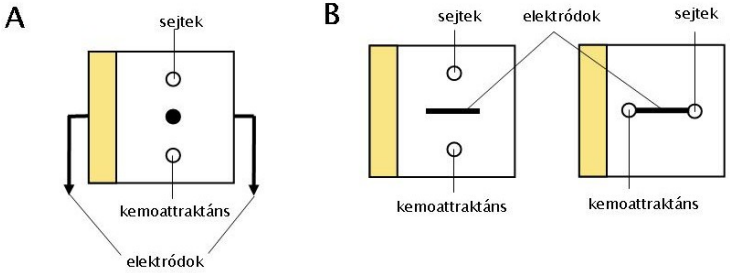


Giaver, I.

Applied BioPhysics

Electric Cell-substrate Inpedance Sensor (ECIS)



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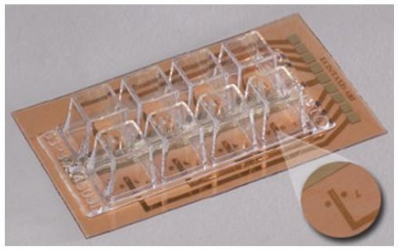
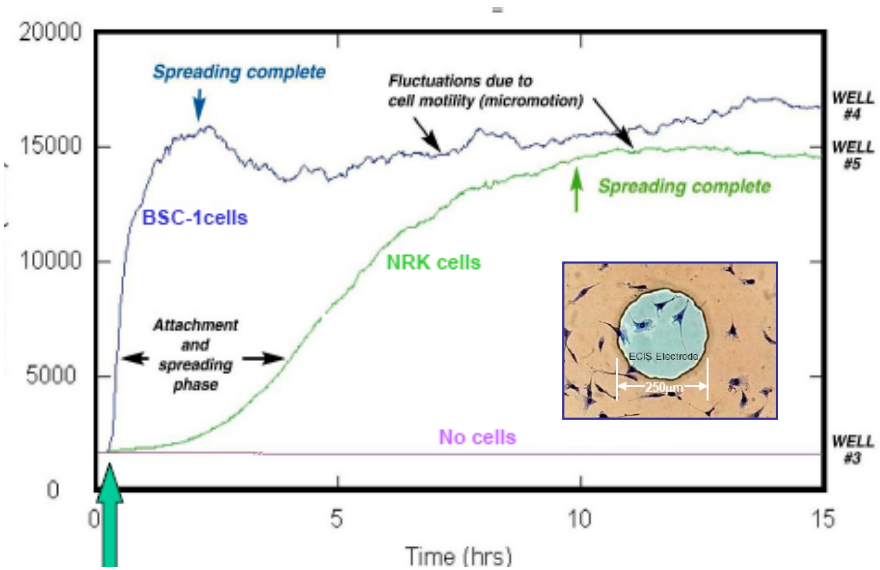
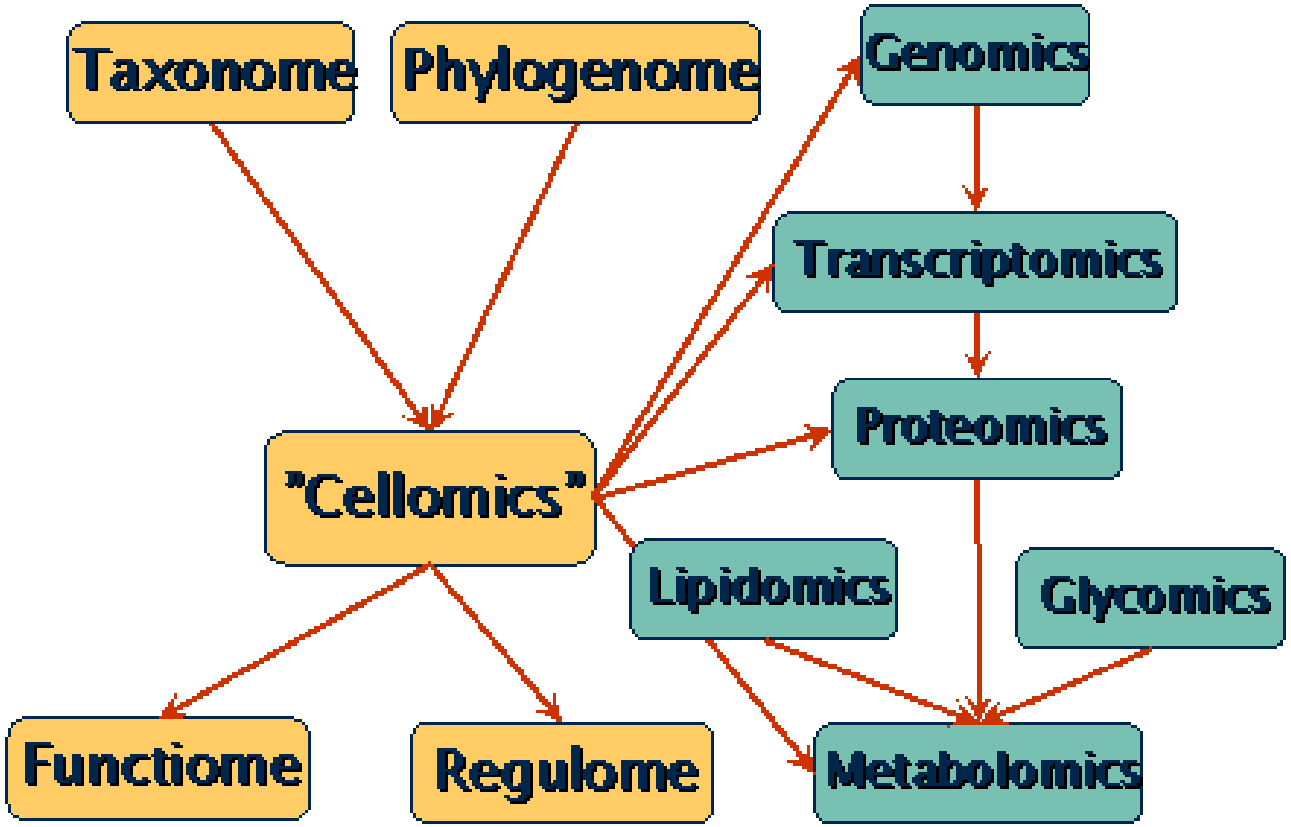


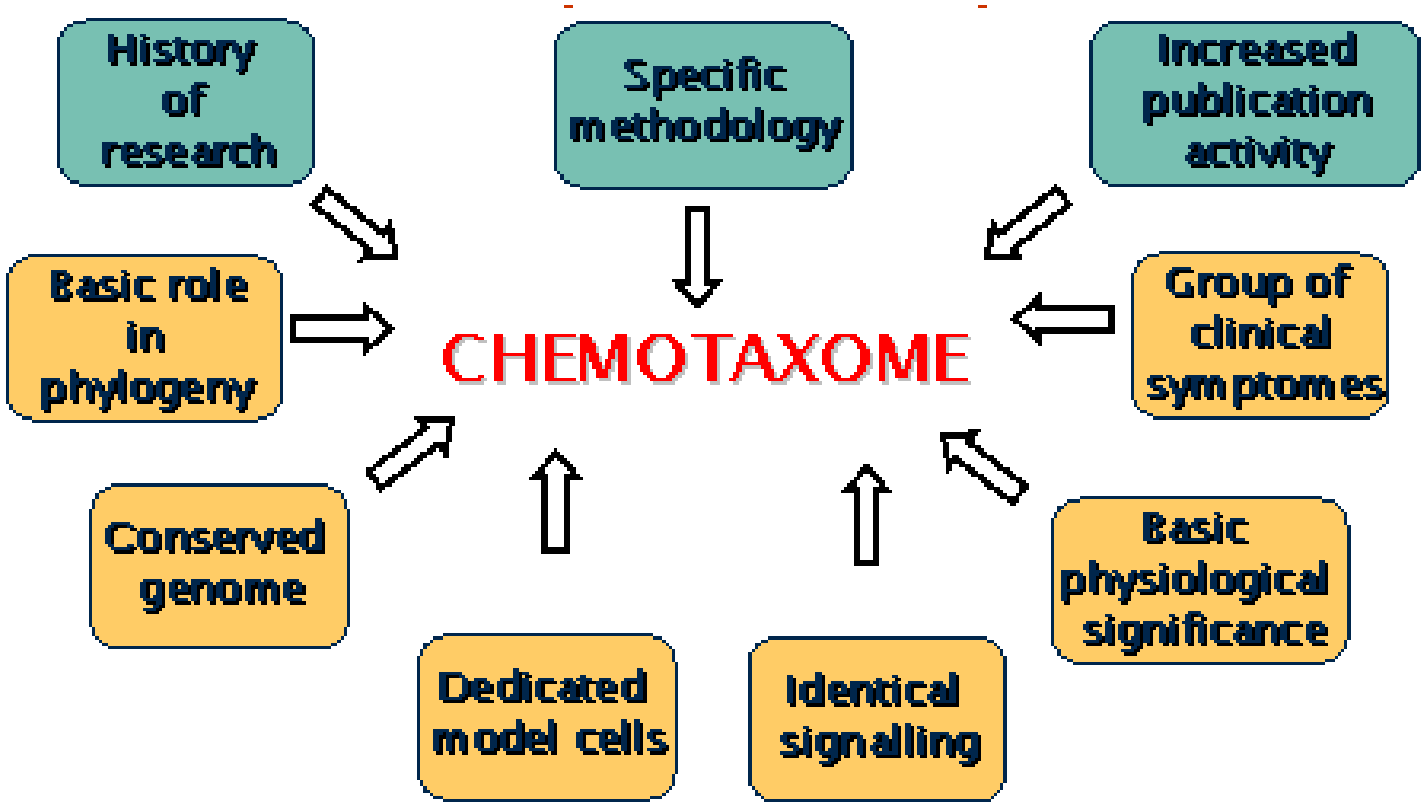


Systems biology and Chemotaxis

Network of significant 'Omics' as essential backgrounds of chemotaxis



Systems biology and chemotaxis: 'Chemotaxome'



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