第四章 跨膜运输

MEMBRANE TRANSPORT

内容提要

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一、简单扩散

二、协助扩散

第二节、主动运输

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二、钙离子泵

三、质子泵

四、ABC 转运器

五、协同运输

第三节、膜泡运输的基本概念

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二、胞饮作用

三、外排作用

四、穿胞运输

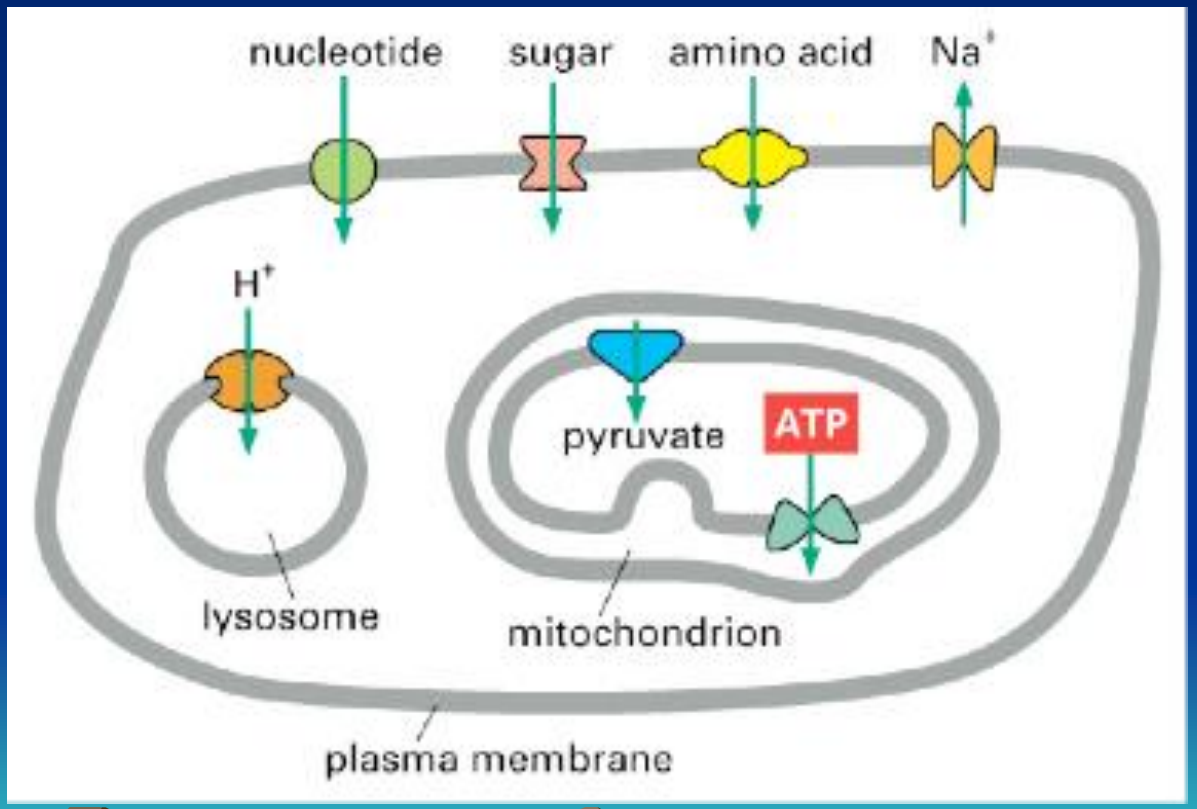
五、胞内膜泡运输

Table 12–1 A Comparison of Ion Concentrations Inside and Outside a Typical Mammalian Cell

COMPONENT	INTRACELLULAR CONCENTRATION (mM)	EXTRACELLULAR CONCENTRATION (mM)
Cations		
Na^+	5–15	145
K^+	140	5
Mg^{2+}	0.5	1–2
Ca^{2+}	10^{-4}	1–2
H^+	7×10^{-5} ($10^{-7.2}$ M or pH 7.2)	4×10^{-5} ($10^{-7.4}$ M or pH 7.4)
Anions*		
Cl^-	5–15	110

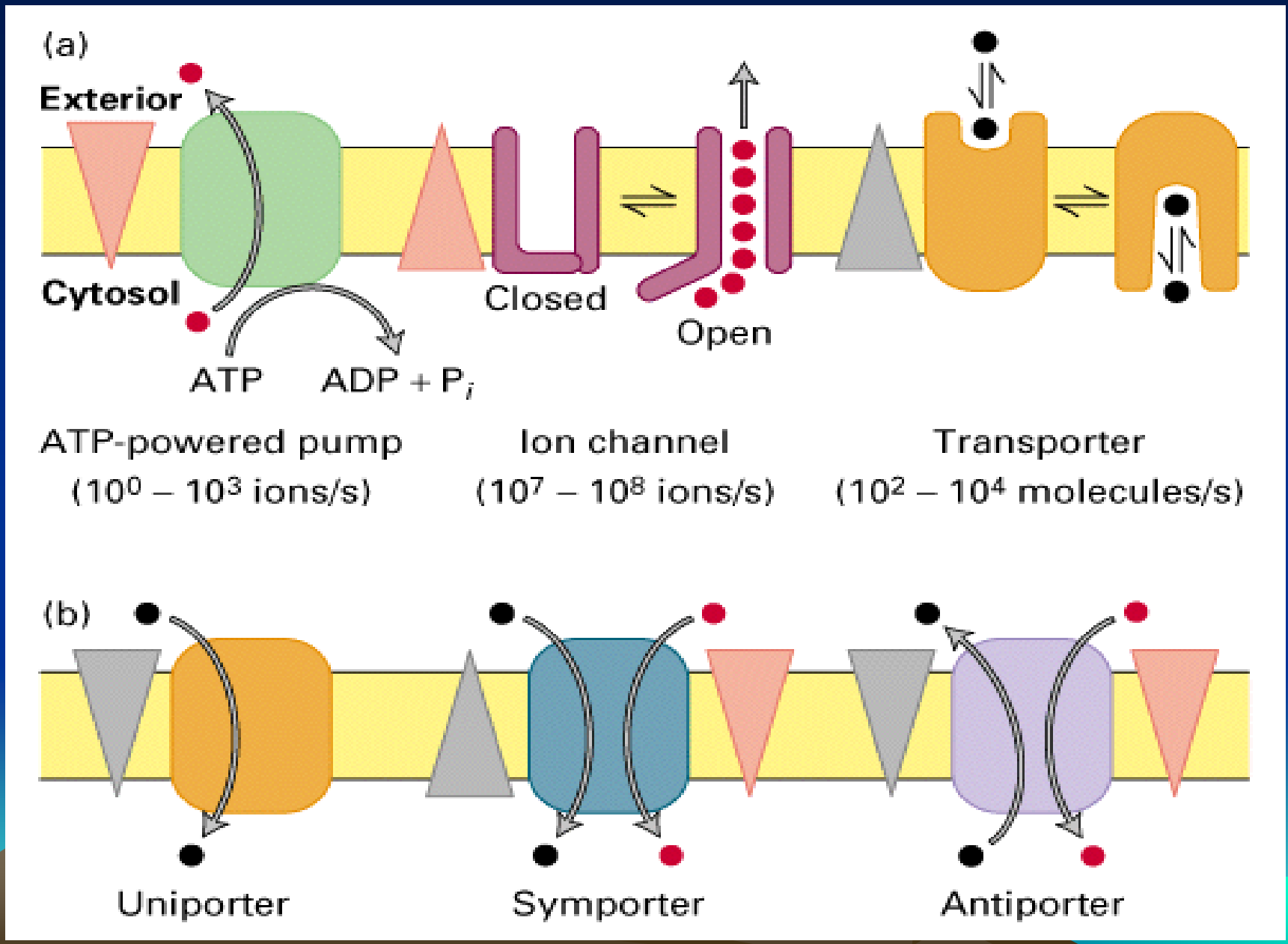
* The cell must contain equal quantities of positive and negative charges (that is, be electrically neutral). Thus, in addition to Cl^-, the cell contains many other anions not listed in this table; in fact, most cellular constituents are negatively charged (HCO_3^-, PO_4^{3-}, proteins, nucleic acids, metabolites carrying phosphate and carboxyl groups, etc.). The concentrations of Ca^{2+} and Mg^{2+} given are for the free ions. There is a total of about 20 mM Mg^{2+} and 1–2 mM Ca^{2+} in cells, but this is mostly bound to proteins and other substances and, for Ca^{2+}, stored within various organelles.

Each cell membrane transports specific molecules

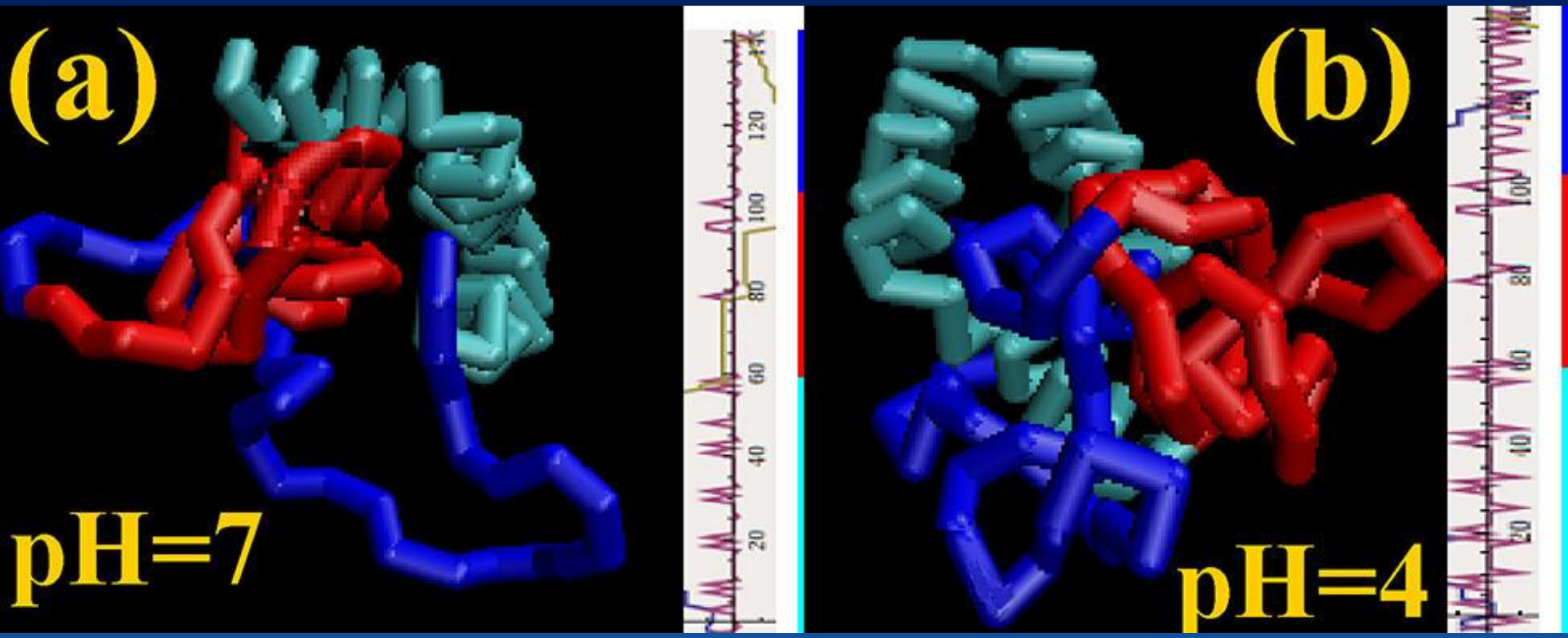


- 估计细胞膜上与物质转运有关的蛋白占核基因编码蛋白的15~30%，细胞用在物质转运方面的能量达细胞总消耗能量的2/3。
- 两类主要转运蛋白：
  - 载体蛋白（Carriers)：又称做载体、通透酶和转运器。
  - 通道蛋白(Channels)：能形成亲水的通道，允许特定的溶质通过。

Membrane Transport Proteins



构象 Conformations



第一节 被动运输

一、简单扩散

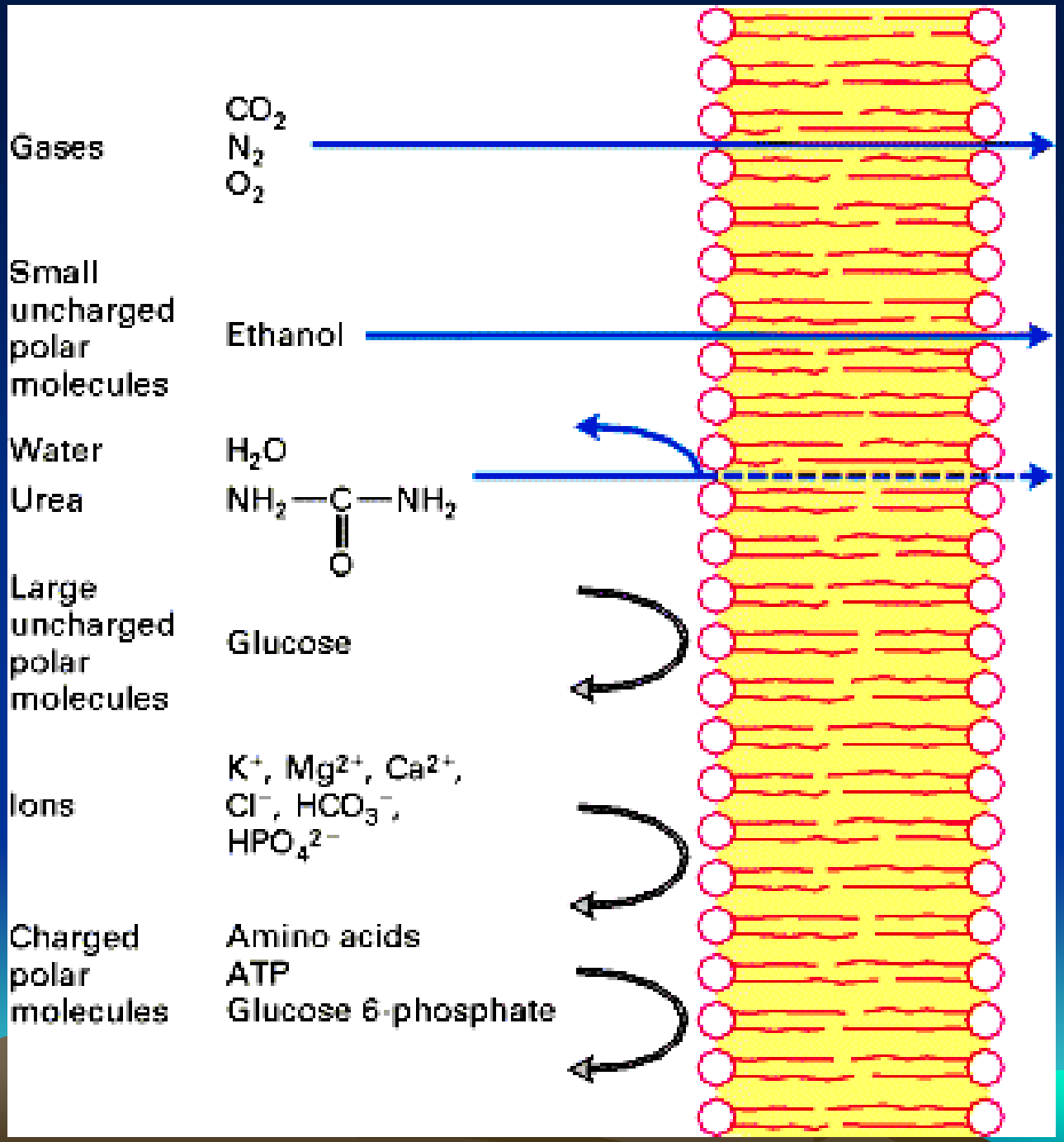
- 也叫自由扩散（free diffusion）：

  ①沿浓度梯度（或电化学梯度）扩散；

  ②不需要提供能量；

  ③没有膜蛋白协助。

- 通透性P=KD/t， K为分配系数， D为扩散系数，t为膜的厚度。

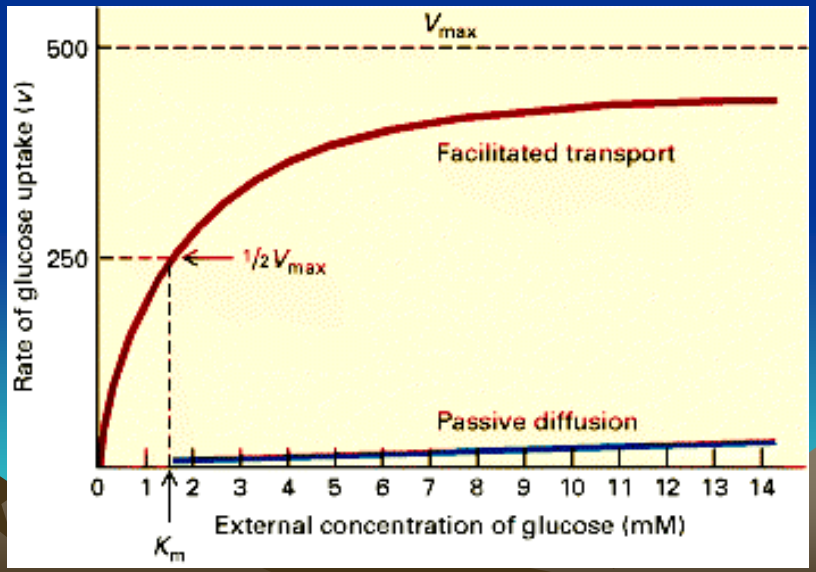

Gases
CO_2
N_2
O_2
Small uncharged polar molecules
Ethanol
Water
H_2O
Urea
$NH_2—C(=O)—NH_2$
Large uncharged polar molecules
Glucose
Ions
K^+, Mg^{2+}, Ca^{2+}, Cl^-, HCO_3^-, HPO_4^{2-}
Charged polar molecules
Amino acids
ATP
Glucose 6-phosphate

人工膜对各类物质的通透率：

- 脂溶性越高通透性越大；
- 小分子比大分子易透过；
- 非极性分子比极性容易透过；
- 极性不带电荷的小分子可透过人工脂双层；
- 人工膜对带电荷的物质，如离子是高度不通透的。

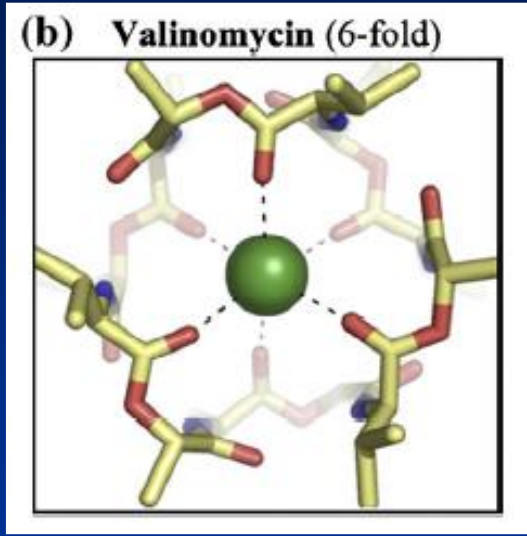
二、协助扩散

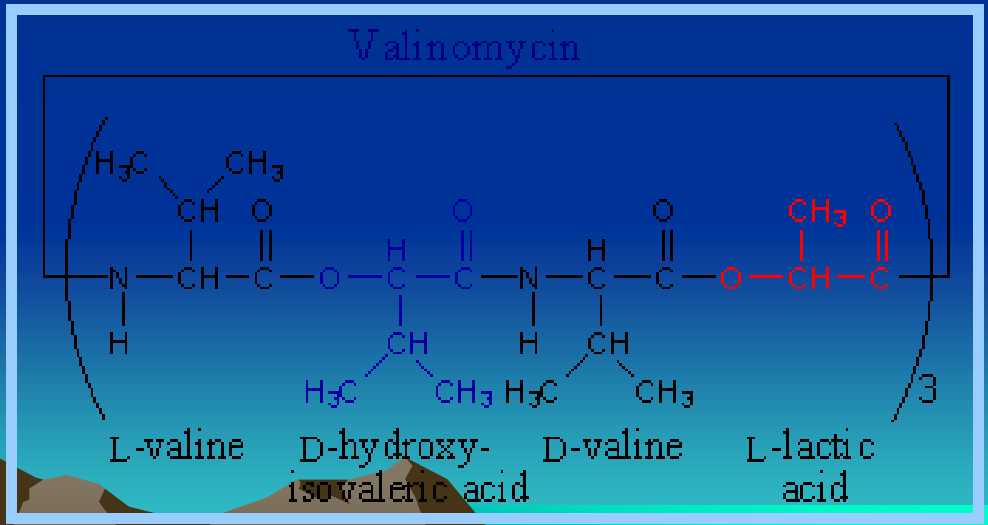
- 也称促进扩散（**facilitated diffusion**）。
- 特点： ①转运速率高； ②运输速率同物质浓度成非线性关系； ③特异性；④饱和性。
- 载体：离子载体、通道蛋白。



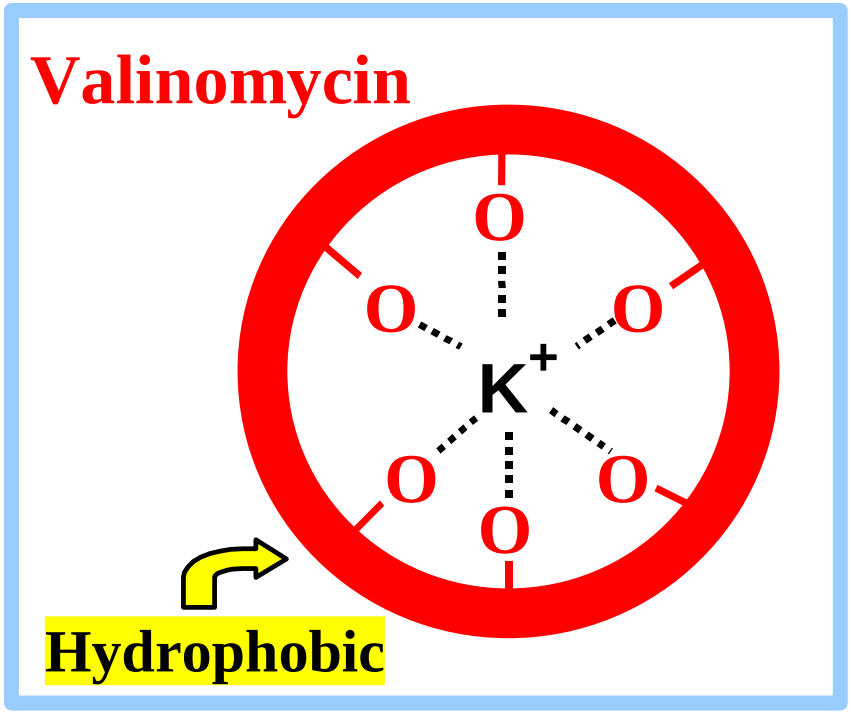
（一）离子载体（ionophore）

- 疏水性小分子，可溶于双脂层。分为：可动离子载体(Valinomycin)和通道离子载体(Gramicidin)。



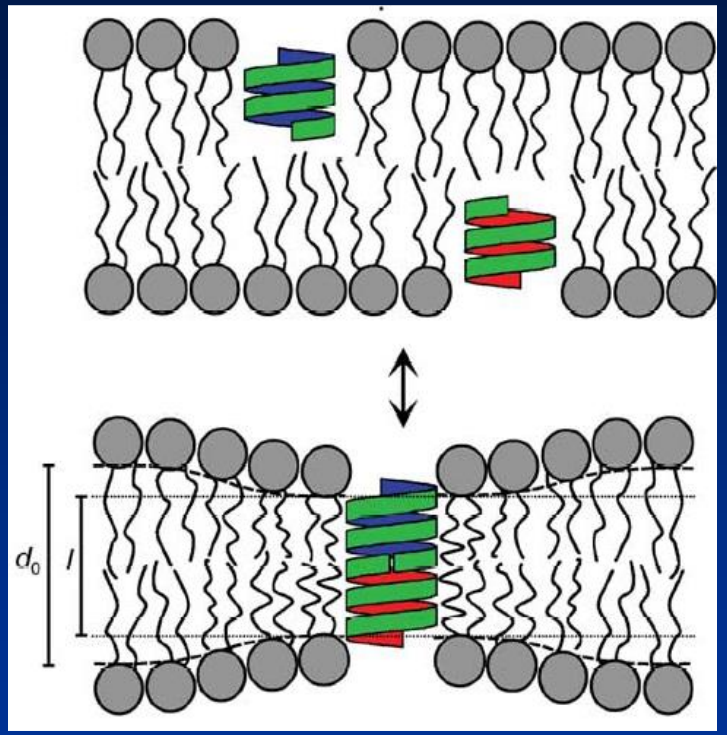


•缬氨霉素能转运K+； DNP和FCCP可转运H+；离子霉素、A23187可转运钙离子。



Whereas the interior of the valinomycin-K^+ complex is polar, the surface of the complex is hydrophobic. This allows valinomycin to enter the lipid core of the bilayer, to solubilize K^+ within this hydrophobic milieu. Crystal structure (at Virtual Museum of Minerals & Molecules).

•短杆菌肽A，15个疏水氨基酸构成，2分子形成一跨膜通道，有选择的使单价阳离子如H+、Na+、K+按化学梯度通过。



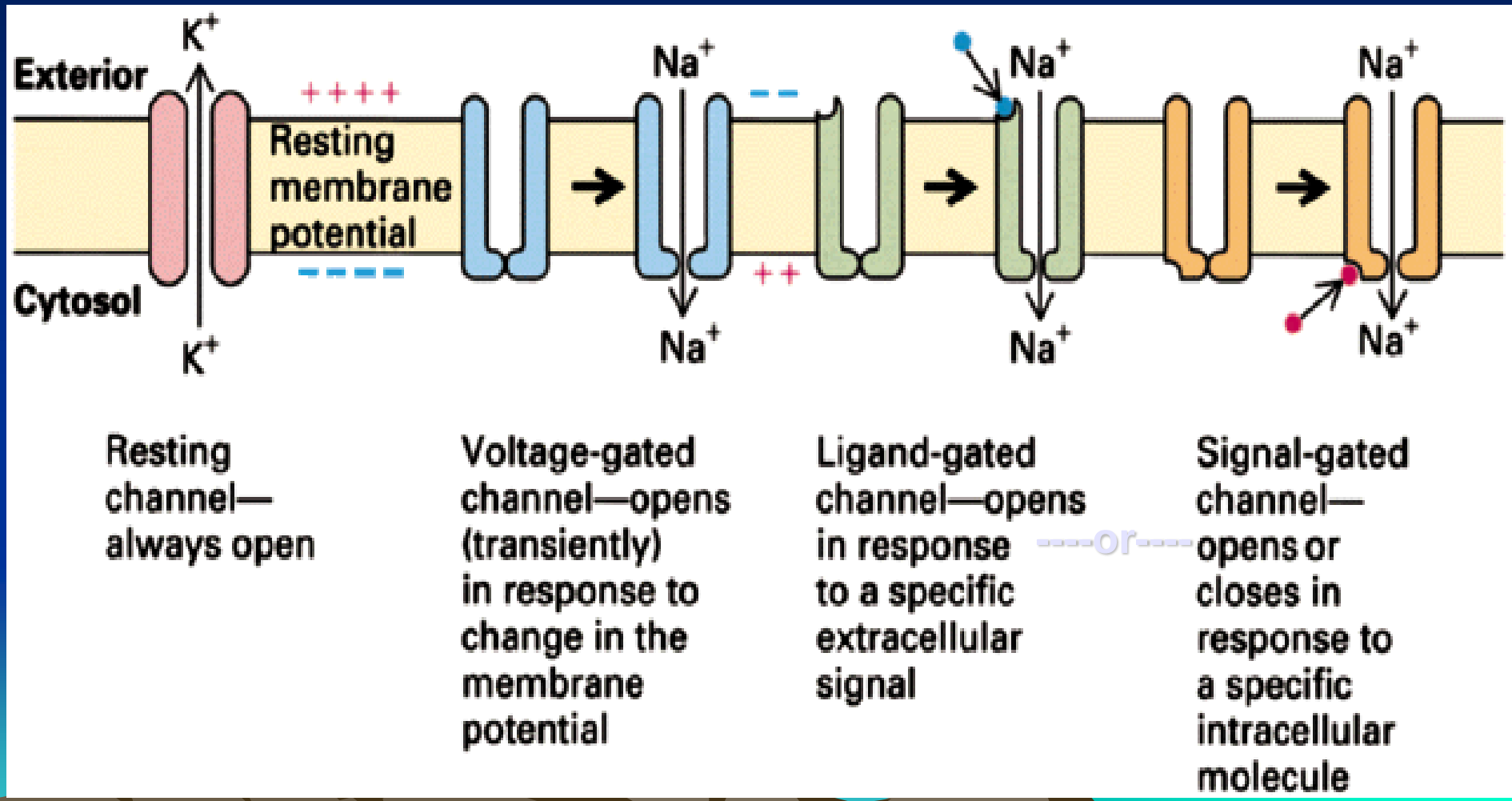
Formyl–L–Val–––Gly–L–Ala–D–Leu–L–Ala–
–D–Val–L–Val–D–Val–L–Trp–D–Leu–L–Trp–
–D–Leu–L–Trp–D–Leu–L–Trp–ethanolamine

Gramicidin A　an antibiotic that acts as an ion pore.

（二）通道蛋白（channel protein）

- 跨膜亲水性通道，允许特定离子顺浓度梯度通过，又称离子通道。
- 有些通道长期开放，如钾泄漏通道；
- 有些通道平时处于关闭状态，仅在特定刺激下才打开，称为门通道（电位门通道、配体门通道、环核苷酸门通道、机械门通道）。

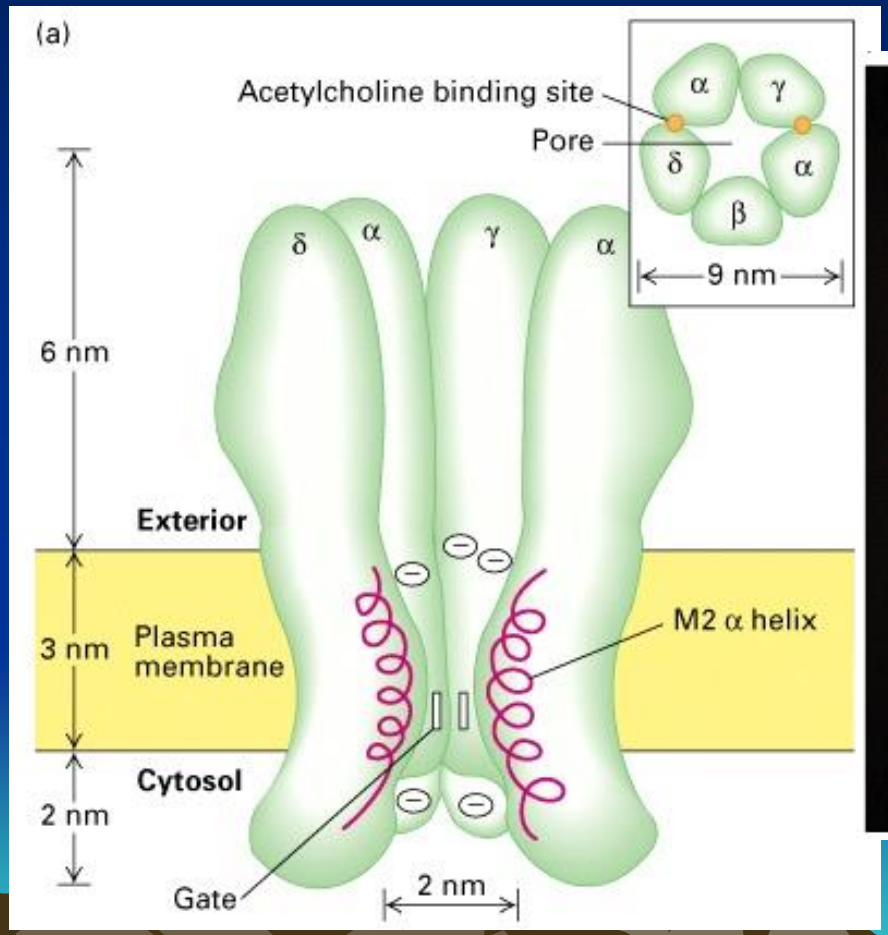
Ion Channels

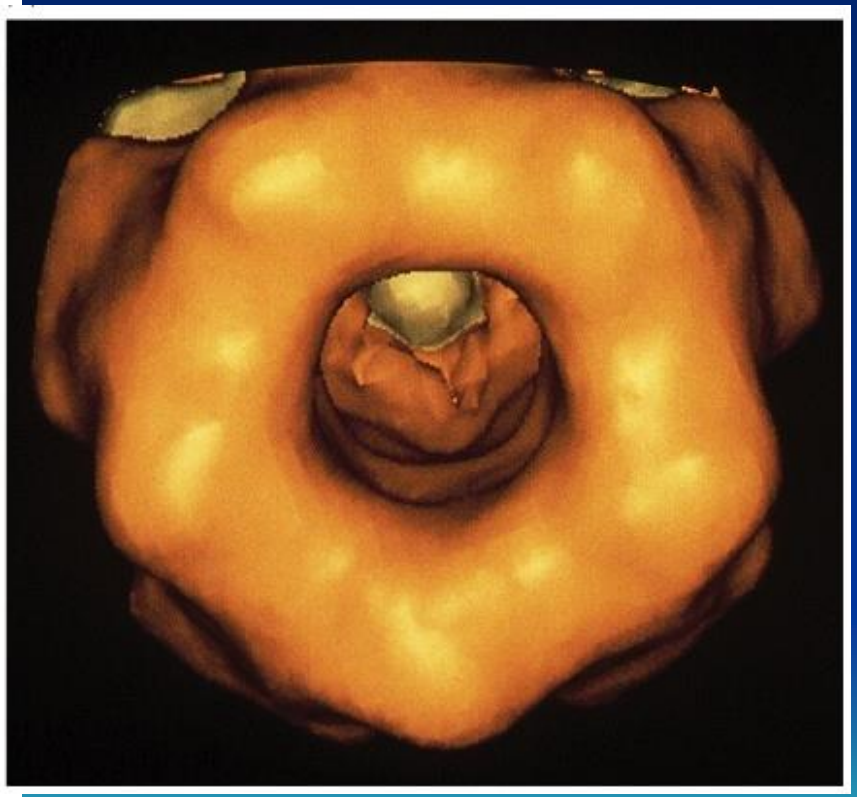


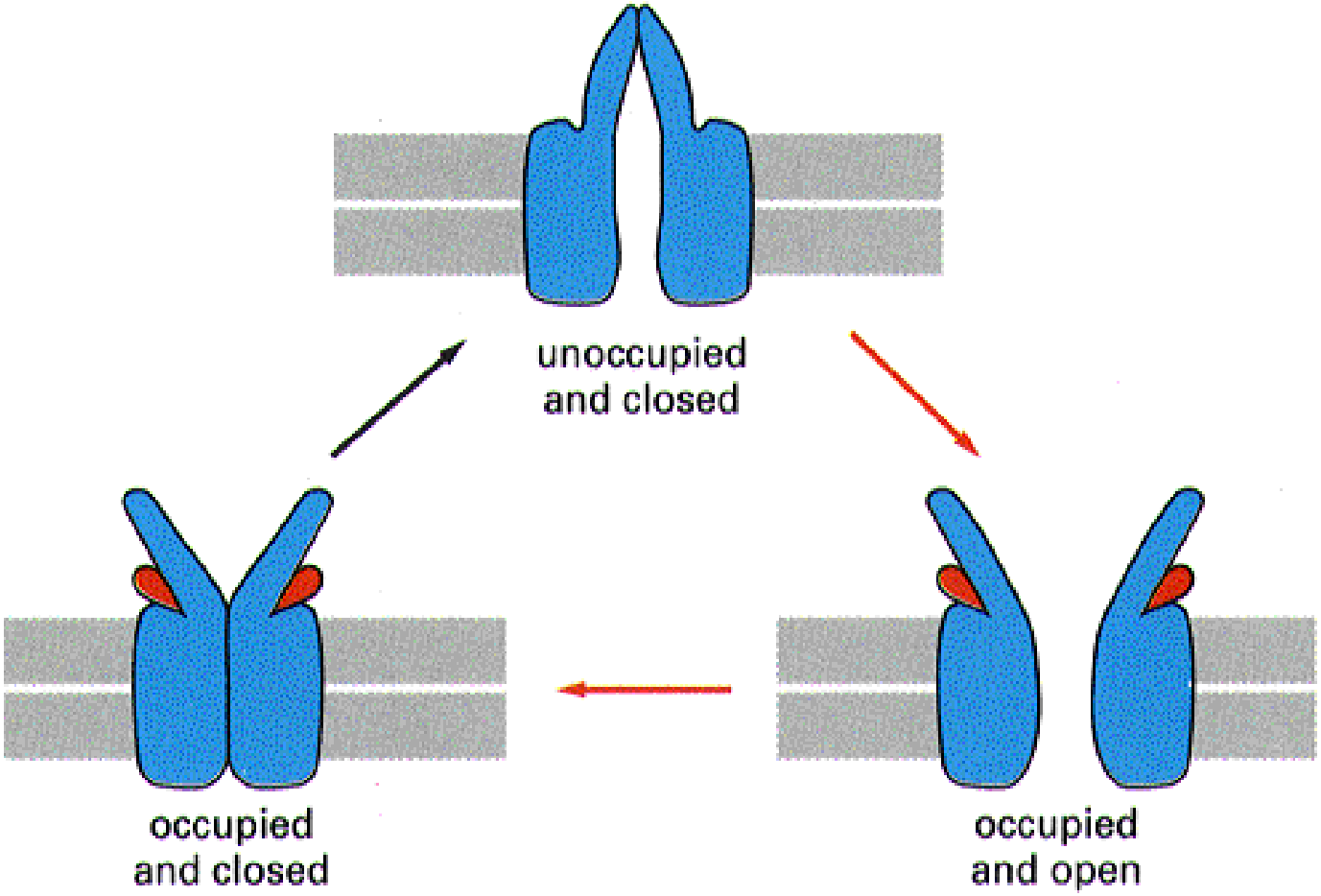
1、配体门通道(ligand gated channel)

- 特点：受体与细胞外的配体结合，引起通道构象改变，“门”打开，又称离子通道型受体。
- 分为阳离子通道，如乙酰胆碱受体；和阴离子通道，如γ一氨基丁酸受体。
- Ach受体由4种亚单位（$\alpha_2\beta\gamma\delta$）组成。

Nicotinic acetylcholine receptor





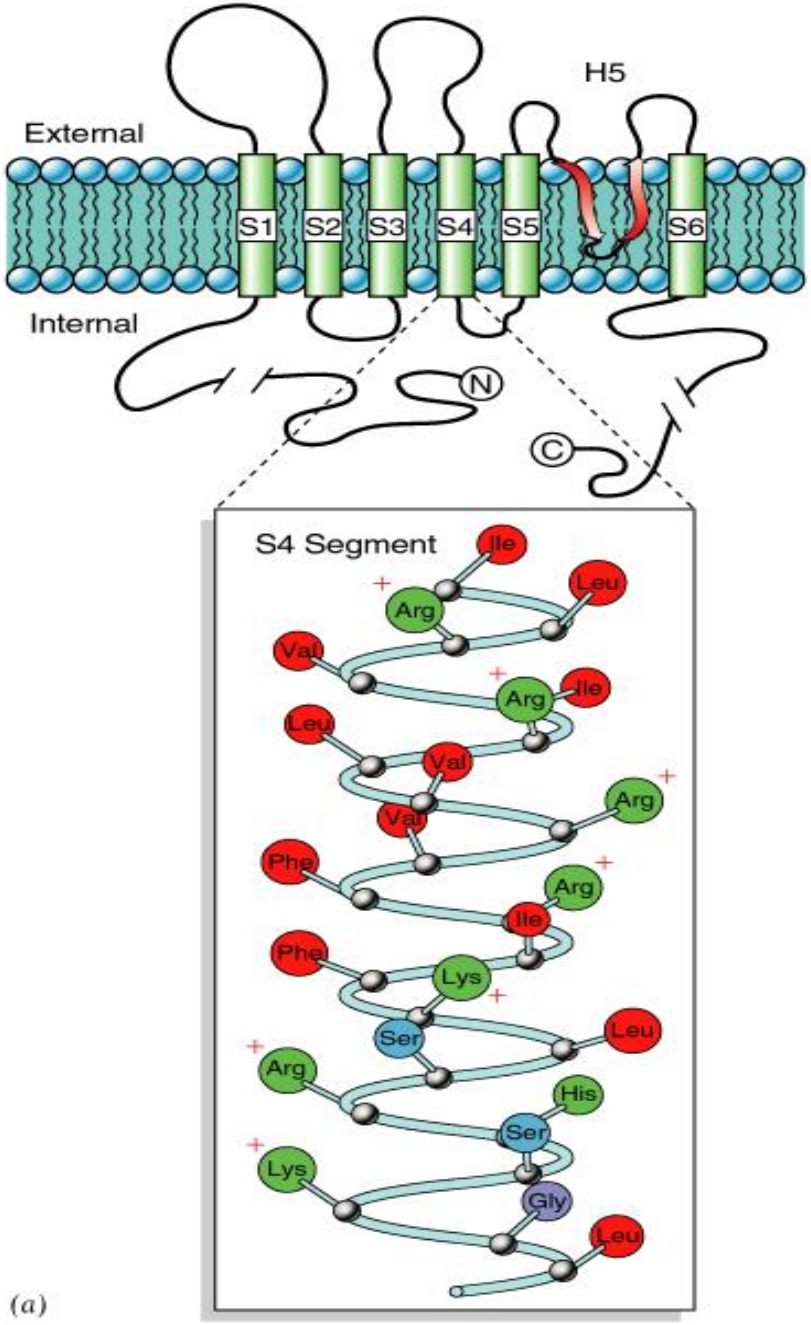


2、电位门通道(voltage gated channel)

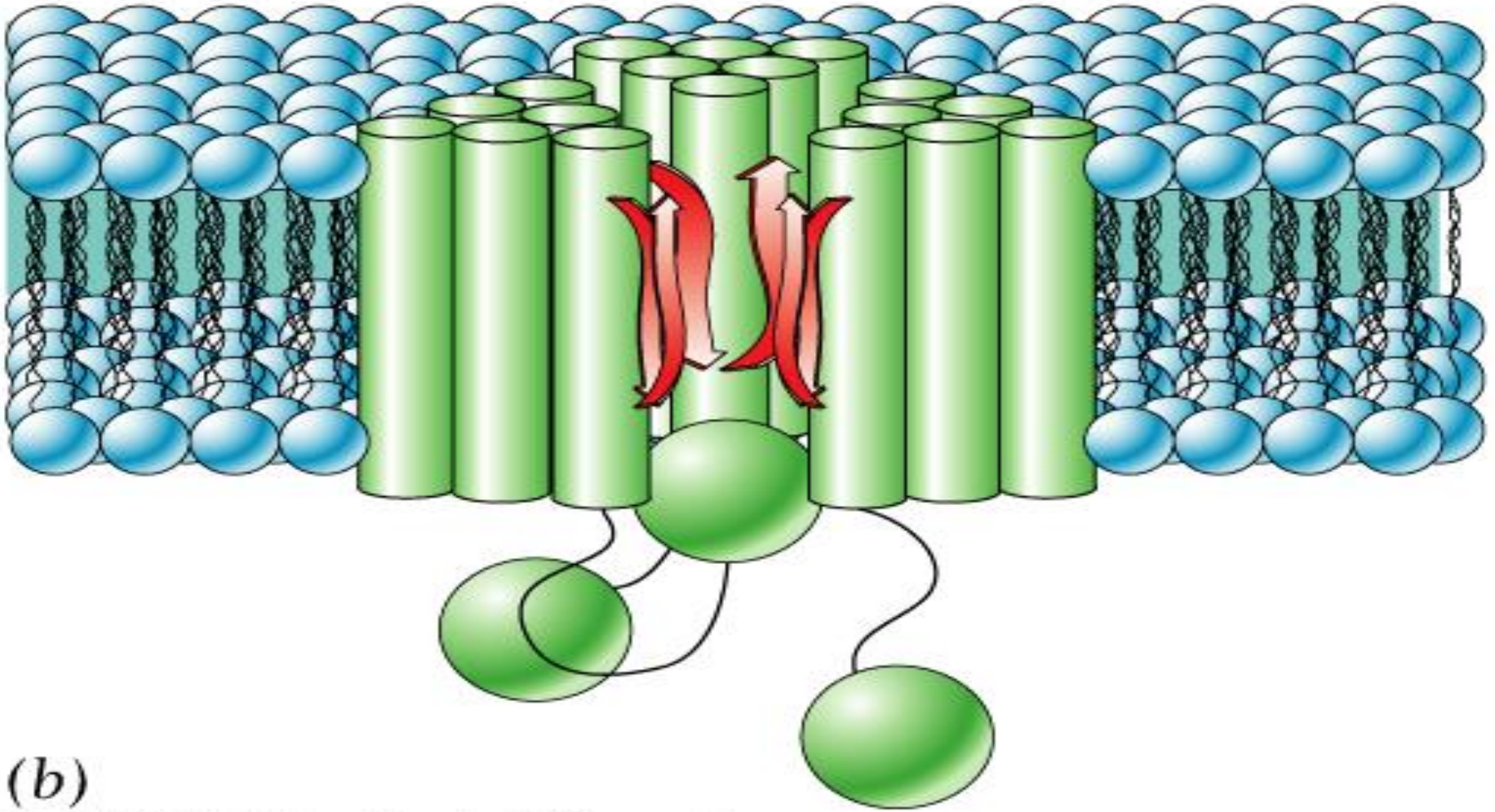
- 特点：膜电位变化可引起构象变化，“门”打开。
- 结构：四聚体，每个单体跨膜6次。
- Na^+、K^+、Ca^{2+}电压门通道结构相似，由同一个远祖基因演化而来。

Voltage gated K^+ channel

K+电位门有四个亚单位，每个亚基有6个跨膜α螺旋(S1-S6)，N和C端均位于胞质面。连接S5-S6段的发夹样β折叠(P区或H5区)，构成通道内衬，大小允许K+通过。目前认为S4段是电压感受器

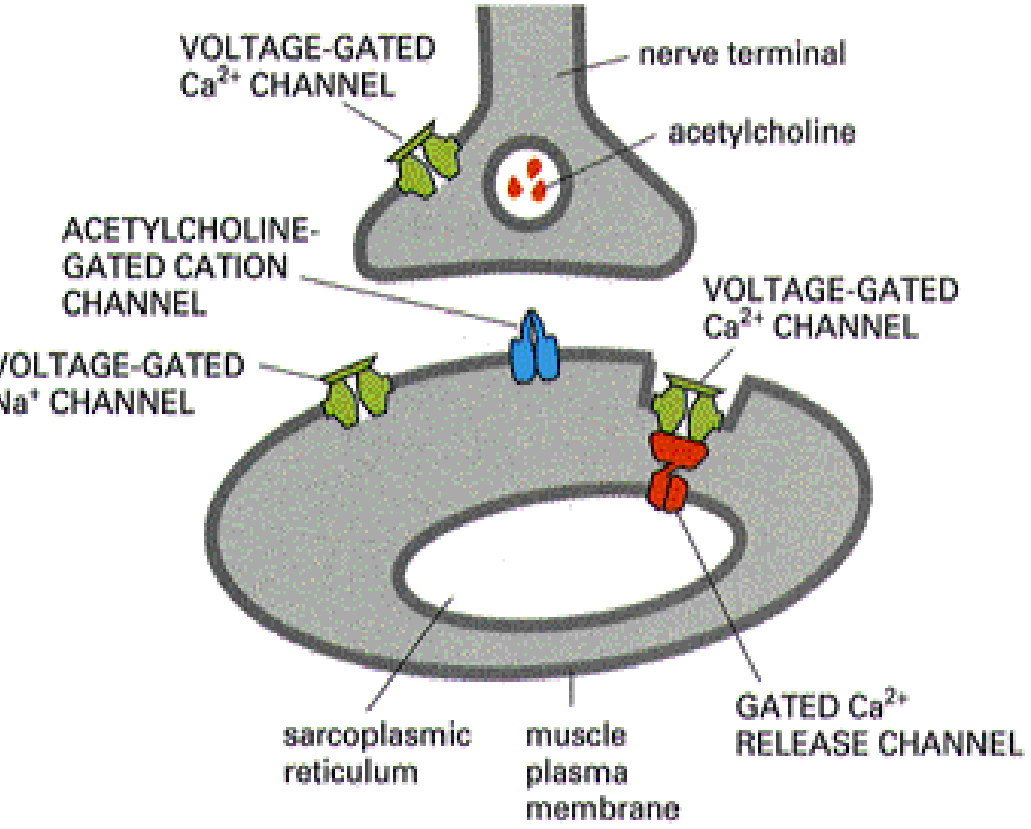


K^+ channel

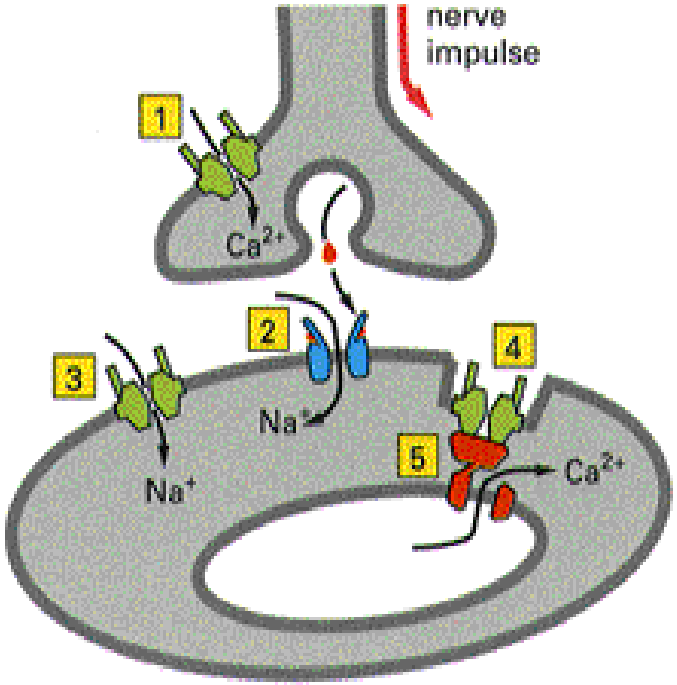


(b)

RESTING NEUROMUSCULAR JUNCTION



ACTIVATED NEUROMUSCULAR JUNCTION



3、环核苷酸门通道

- CNG结构与钠电位门通道相似。细胞内的C末端较长，有环核苷酸的结合位点。
- 分布于化学和光感受器中。
  - 如气味分子与化学感受器中的G蛋白偶联型受体结合，激活腺苷酸环化酶，产生cAMP，开启cAMP门控阳离子通道，引起钠离子内流，膜去极化，产生神经冲动，最终形成嗅觉或味觉。

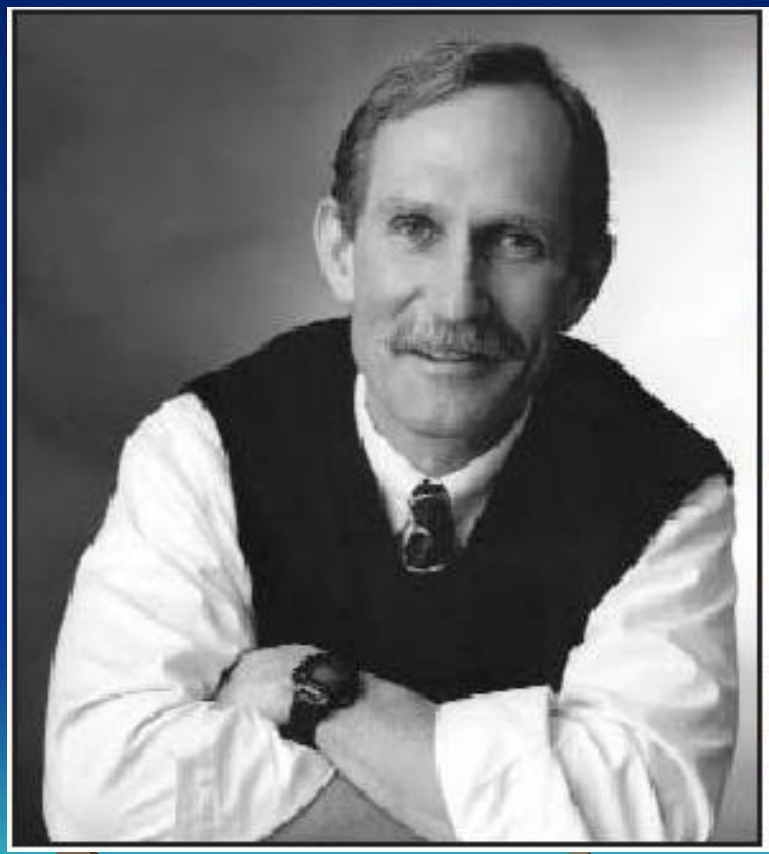
4、机械门通道

- 感受摩擦力、压力、牵拉力、重力、剪切力等。
- 目前比较明确的有两类机械门通道，一类对牵拉敏感，为2价或1价的阳离子通道，有Na^+、K^+、Ca^{2+}，以Ca^{2+}为主，几乎存在于所有的细胞膜。另一类对剪切力敏感，仅发现于内皮细胞和心肌细胞。

5、水通道

- 1991年Agre发现第一个水通道蛋白CHIP28 （28 KD ），CHIP28的mRNA能引起非洲爪蟾卵母细胞吸水破裂，已知这种吸水膨胀现象会被Hg^{2+}抑制。
- 目前在人类细胞中已发现至少11种此类蛋白，被命名为水通道蛋白（Aquaporin，AQP）。

2003年，美国科学家彼得·阿格雷和罗德里克·麦金农，分别因对细胞膜水通道，离子通道结构和机理研究而获诺贝尔化学奖。



Peter Agre

Roderick MacKinnon

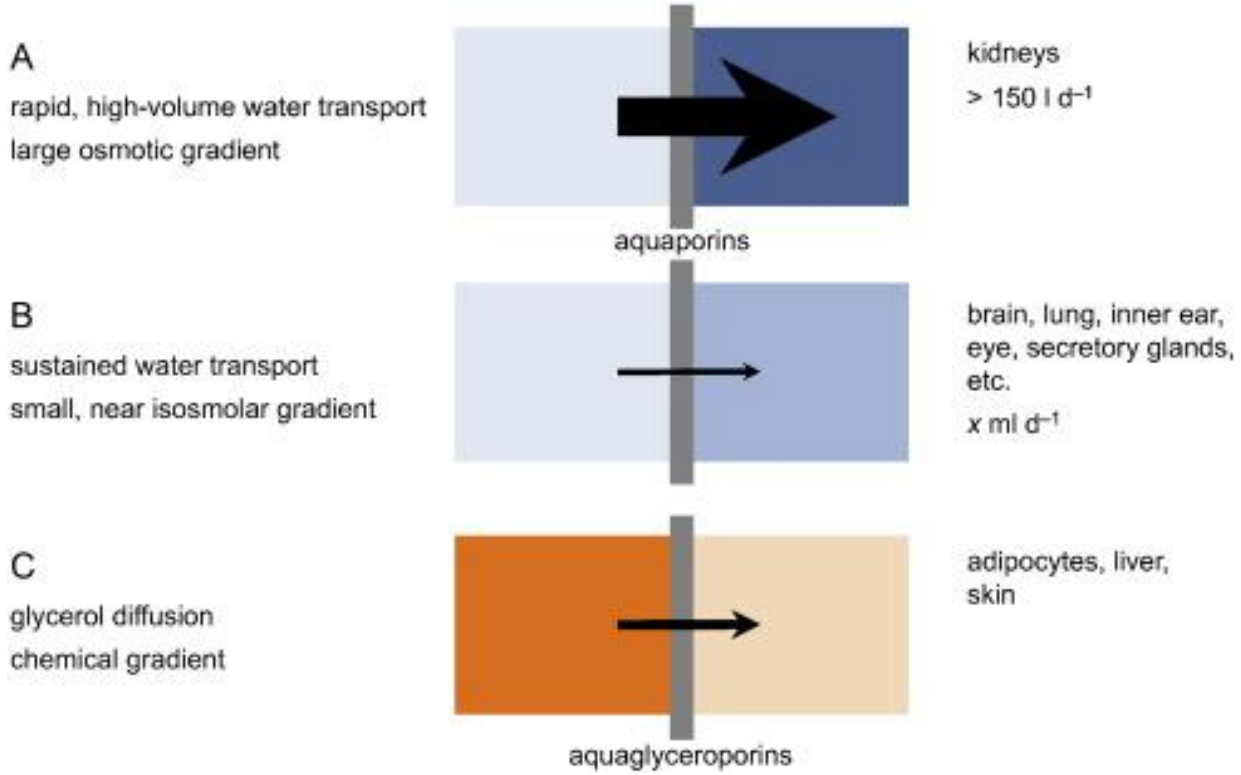


Fig. 1. Physiological situations that require AQP water or glycerol facilitation across cell membranes.

Eric Beitz, André Golldack, Monja Rothert, Julia von Bülow

Challenges and achievements in the therapeutic modulation of aquaporin functionality

Pharmacology & Therapeutics, Volume 155, 2015, 22–35

http://dx.doi.org/10.1016/j.pharmthera.2015.08.002

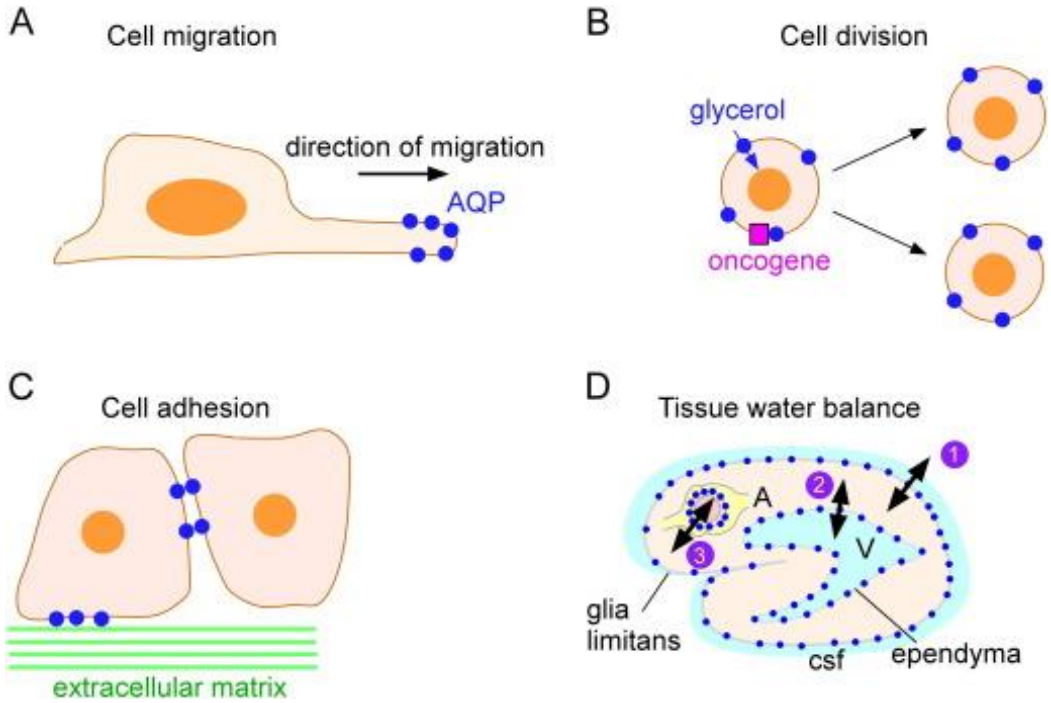


Fig. 1. Roles of AQPs in cancer. A. Cell migration. AQP polarizes to the leading end of the cell and facilitates formation of the lamellipodium. B. Cell proliferation. Aquaglyceroporin facilitates glycerol entry into the cell, which is essential for biosynthes...

Marios C. Papadopoulos, Samira Saadoun

Key roles of aquaporins in tumor biology ☆

Biochimica et Biophysica Acta (BBA) - Biomembranes, Volume 1848, Issue 10, Part B, 2015, 2576–2583

http://dx.doi.org/10.1016/j.bbamem.2014.09.001

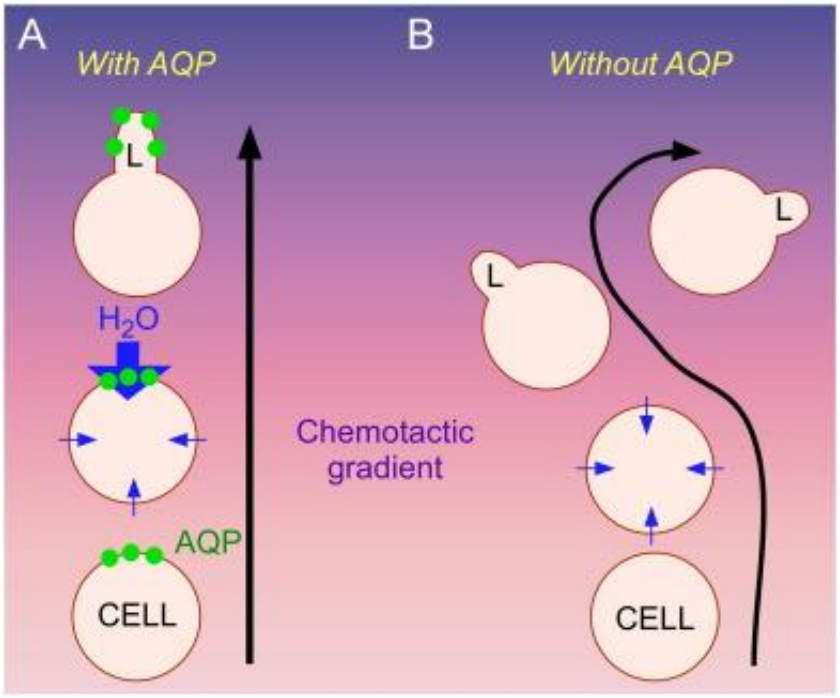


Fig. 2. Proposed role of AQP in increasing directionality of migrating cell. A. AQP polarizes to the leading end of the cell thus ensuring that the lamellipodium forms in the direction of the chemotactic gradient. The cell migrates toward the chemotactic gradi...

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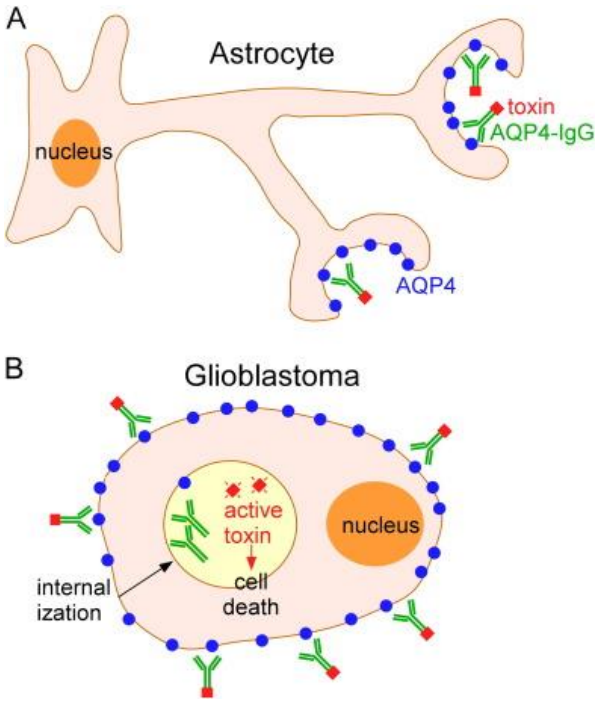

Fig. 3. AQP4-IgG linked to toxin as a potential treatment for glioblastoma (恶性胶质瘤). A. AQP4-IgG binds AQP4 on astrocyte foot processes and is not internalized. Toxin remains linked to AQP4-IgG. B. AQP4-IgG binds AQP4 on glioblastoma cell and becomes internalized. Toxi...

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http://dx.doi.org/10.1016/j.bbamem.2014.09.001

第二节 主动运输

- 特点：

  ①逆浓度梯度（逆化学梯度）运输；

  ②需要能量；

  ③都有载体蛋白。

- 能量来源：

  ①协同运输中的离子梯度动力；

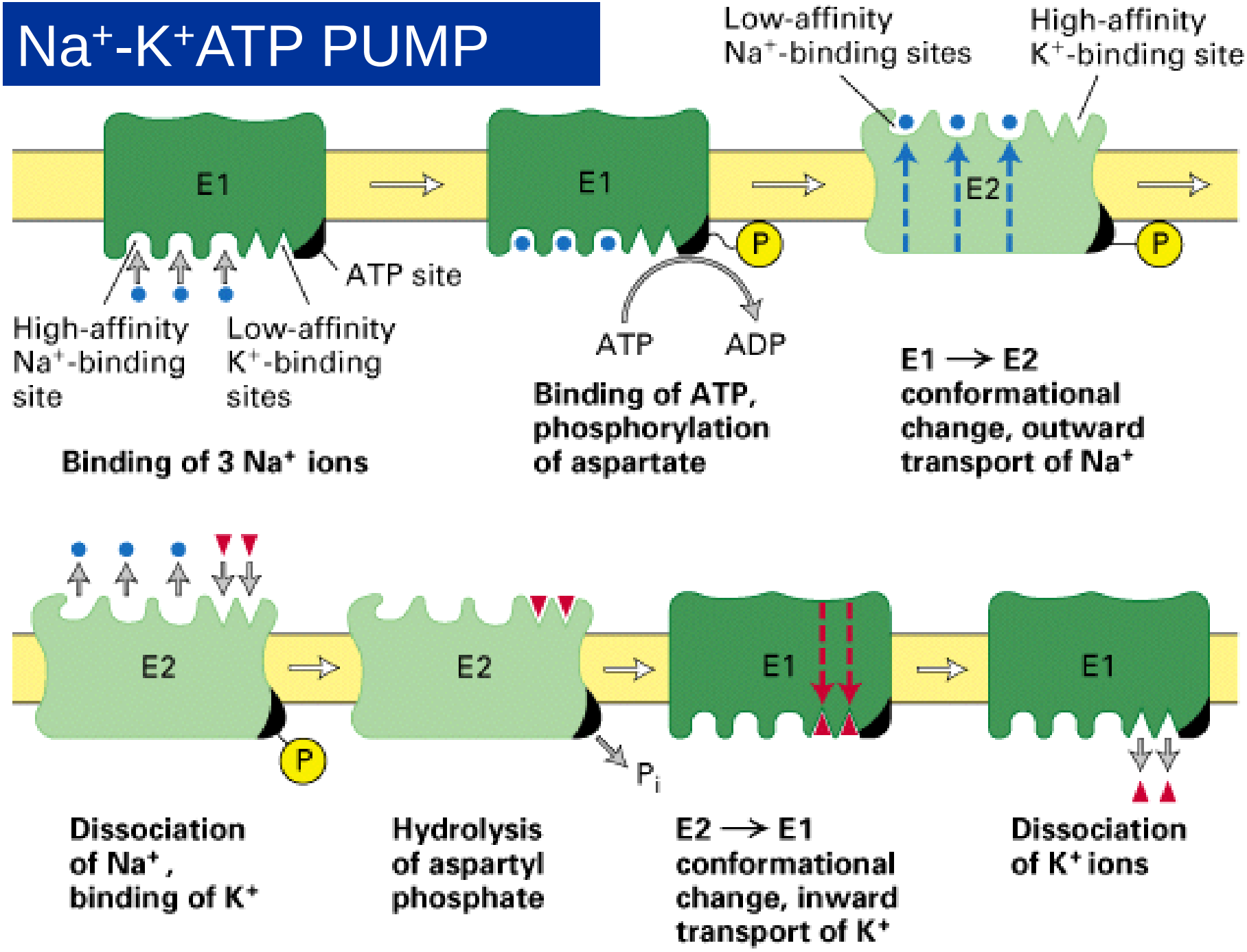
  ② ATP驱动的泵通过水解ATP获得能量；

  ③光驱动的泵利用光能运输物质，见于细菌。

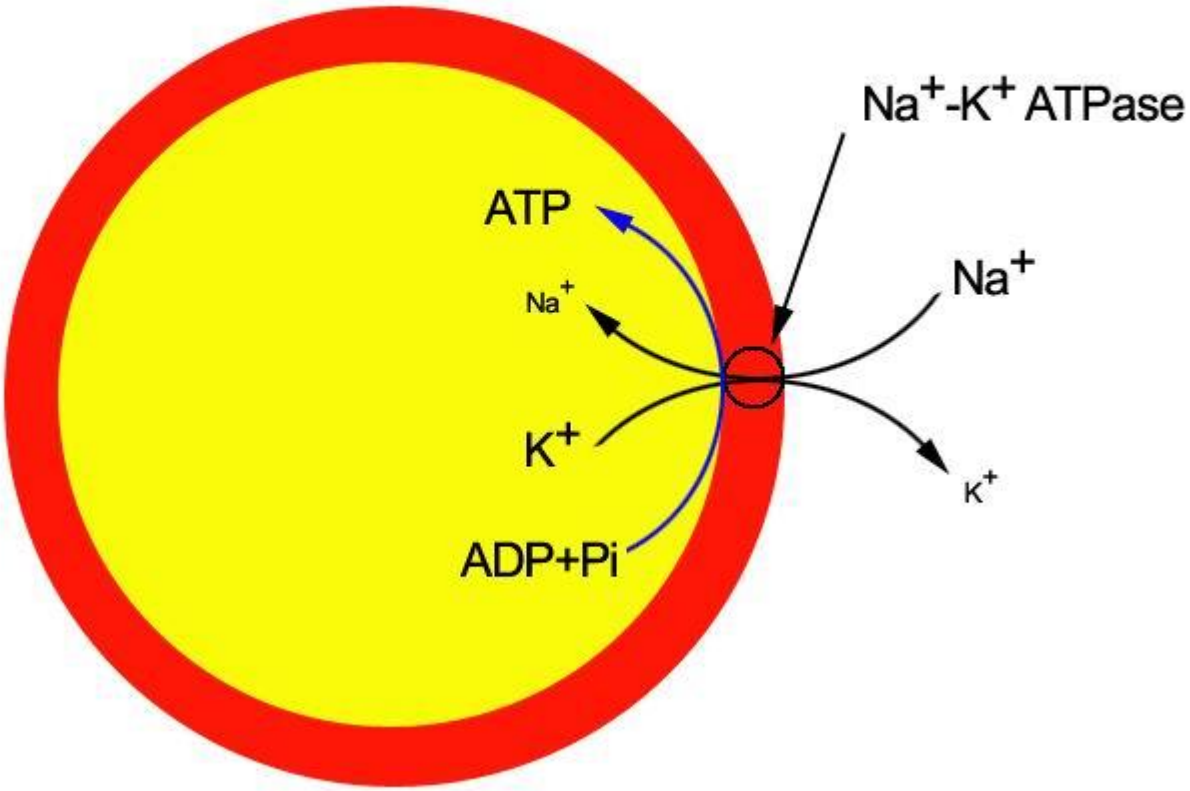
一、钠钾泵

- 构成：由2个大亚基、2个小亚基组成的4聚体，也叫Na^+-K^+ATP酶，分布于动物细胞的质膜。
- 工作原理：
  - 对离子的转运循环依赖自磷酸化过程，所以叫做P-type离子泵。每个周期转出 3 个钠离子， 2 个钾离子。

Na^+-K^+ATP PUMP


Low-affinity
Na+-binding sites
High-affinity
K+-binding site
E1
E1
E2
P
P
ATP site
High-affinity
Na+-binding
site
Low-affinity
K+-binding
sites
ATP
ADP
Binding of 3 Na+ ions
Binding of ATP,
phosphorylation
of aspartate
E1 → E2
conformational
change, outward
transport of Na+
E2
E2
E1
E1
P
Pi
Dissociation
of Na+,
binding of K+
Hydrolysis
of aspartyl
phosphate
E2 → E1
conformational
change, inward
transport of K+
Dissociation
of K+ ions

Na^+-K^+ATP pump can catalyze the formation of ATP under laboratory condition



- 钠钾泵的作用：

  ①维持细胞的渗透性，保持细胞体积；

  ②维持低Na^+高K^+的细胞内环境；

  ③维持细胞的静息电位。

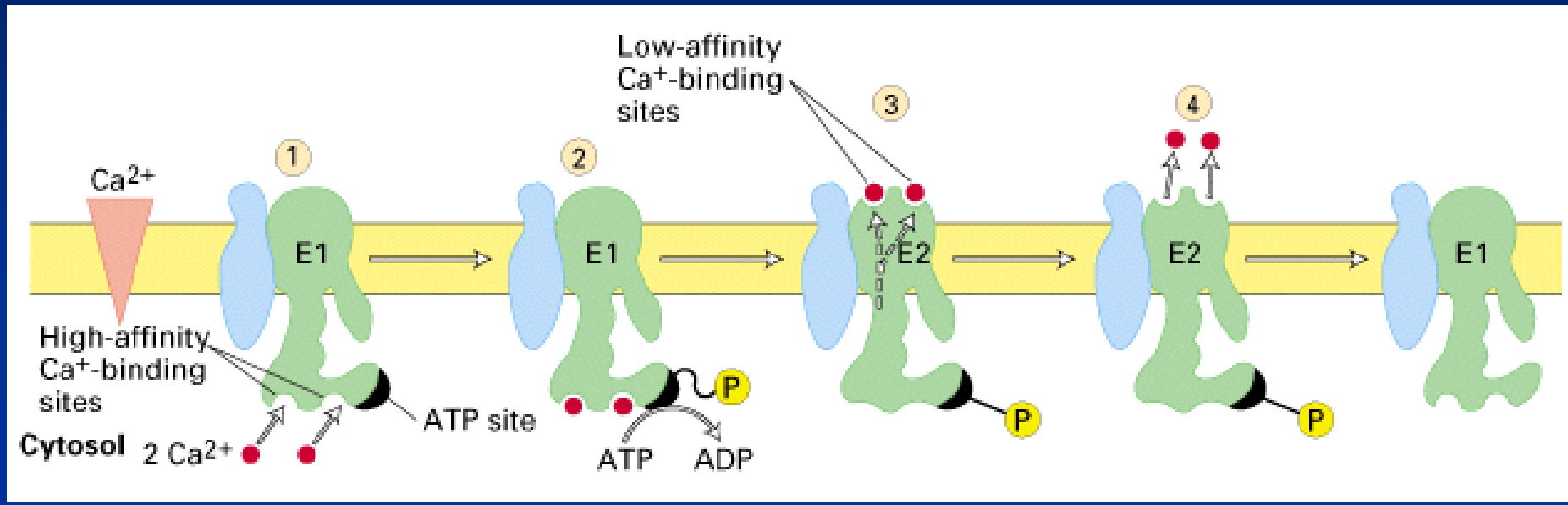
- 地高辛、乌本苷等强心剂抑制其活性；Mg^{2+}和少量膜脂有助提高于其活性。

二、钙离子泵

- 作用：维持细胞内较低的钙离子浓度（胞内钙浓度$10^{-7}M$，胞外$10^{-3}M$）。
- 位置：质膜、内质网膜。
- 类型：
  - P型离子泵，每分解一个ATP分子，泵出2个Ca^{2+}。位于肌质网上的钙离子泵占肌质网膜蛋白质的90%。
  - 钠钙交换器（Na^{+}-Ca^{2+} exchanger），属于反向协同运输体系，通过钠钙交换来转运钙离子。

Ca^{++} ATPase

Maintains low cytosolic [Ca^{++}]
Present In Plasma and ER membranes



Model for mode of action for Ca^{++} ATPase
Conformation change

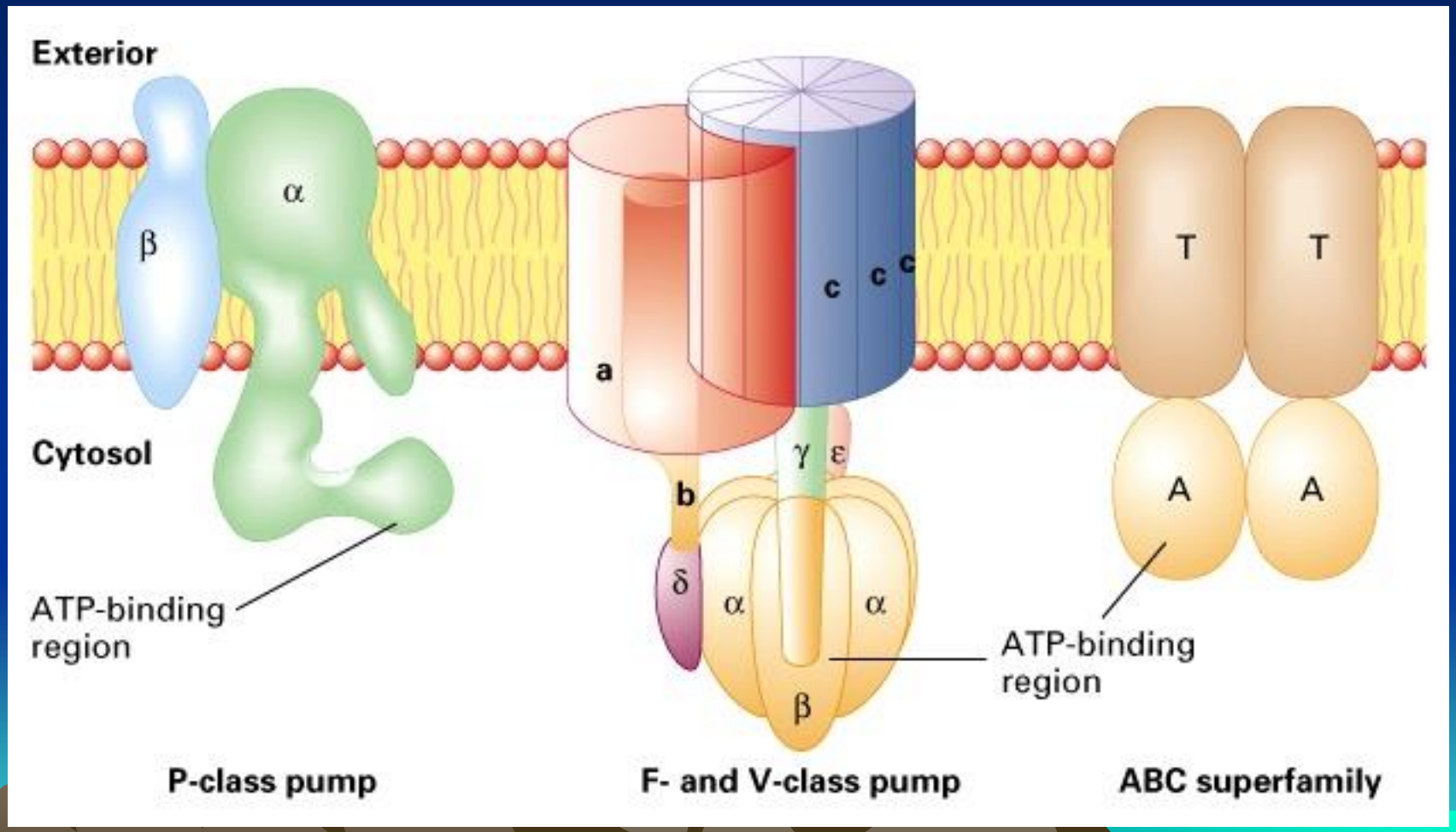
三、质子泵

1、**P-type**：如植物细胞膜上的H^+泵、动物胃表皮细胞的H^+-K^+泵（分泌胃酸）。

2、**V-type**：存在于各类小泡膜上，水解ATP产生能量，但不发生自磷酸化，位于溶酶体膜、内体、植物液泡膜上。

3、**F-type**：利用质子动力势合成ATP，即ATP合酶，位于细菌质膜、线粒体内膜、类囊体膜上。

Four types of ATP-powered pumps



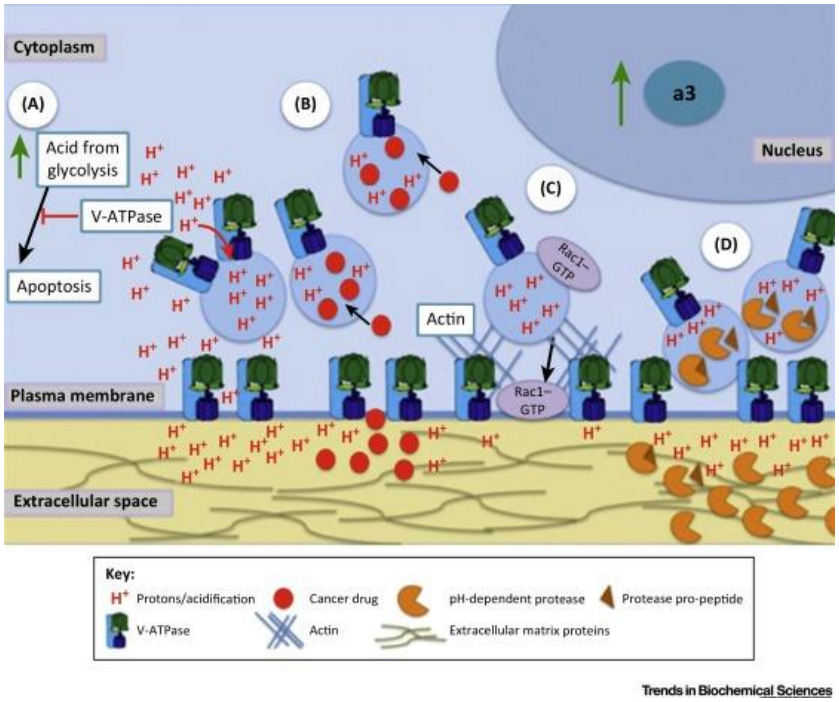


Figure 4. Functions of V-ATPase in Cancer. (A) The V-ATPase aids cancer cell survival, likely by its role in regulating cytoplasmic pH. Cancer cells experience an increased acid load due to enhanced glycolysis that can induce apoptosis. By transporting protons...

Kristina Cotter, Laura Stransky, Christina McGuire, Michael Forgac

Recent Insights into the Structure, Regulation, and Function of the V-ATPases

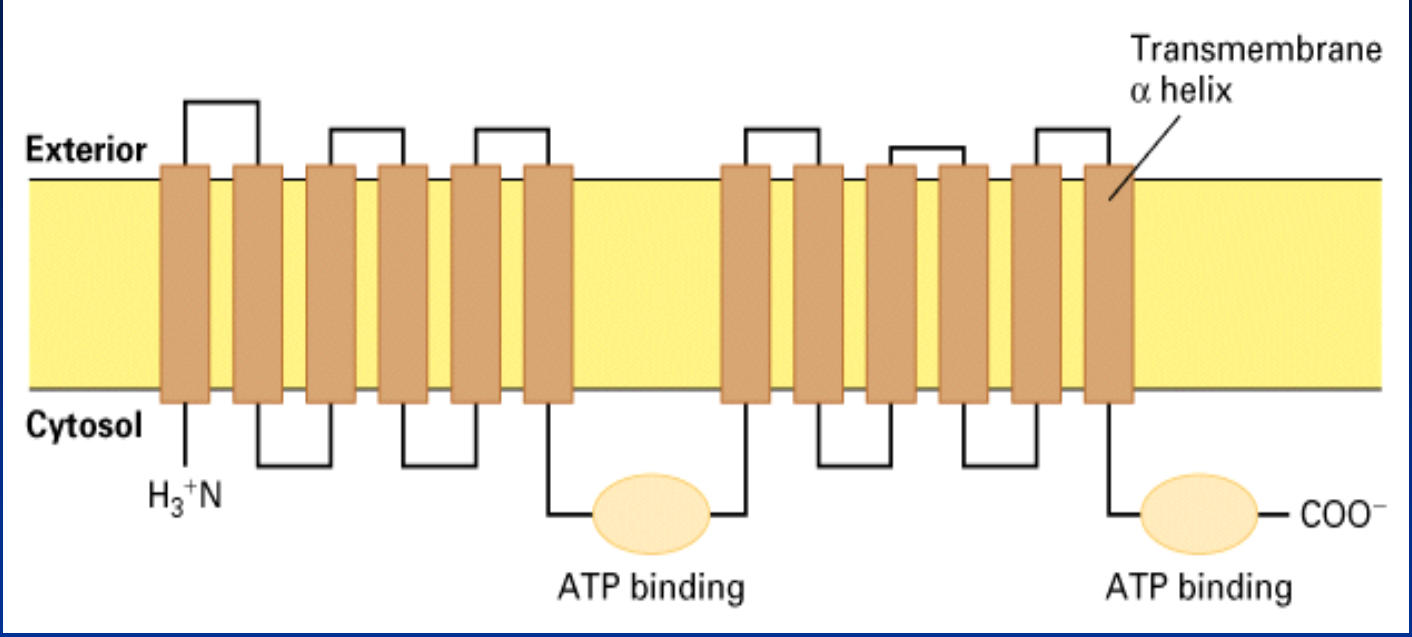
null, Volume 40, Issue 10, 2015, 611–622

http://dx.doi.org/10.1016/j.tibs.2015.08.005

四、ABC 转运器（ABC transporter）

- 最早发现于细菌，是一庞大的蛋白家族，都有两个高度保守的ATP结合区（ATP binding cassette），故名。
- 一种ABC转运器只转运一种或一类底物，不同成员可转运离子、氨基酸、核苷酸、多糖、多肽、蛋白质；可催化脂双层的脂类在两层之间翻转。

Mammalian MDR1 protein



ABC转运器与病原体对药物的抗性有关。MDR（multidrug resistance protein ）是第一个被发现的真核细胞ABC转运器，是多药抗性蛋白，约40%患者的癌细胞内该基因过度表达。

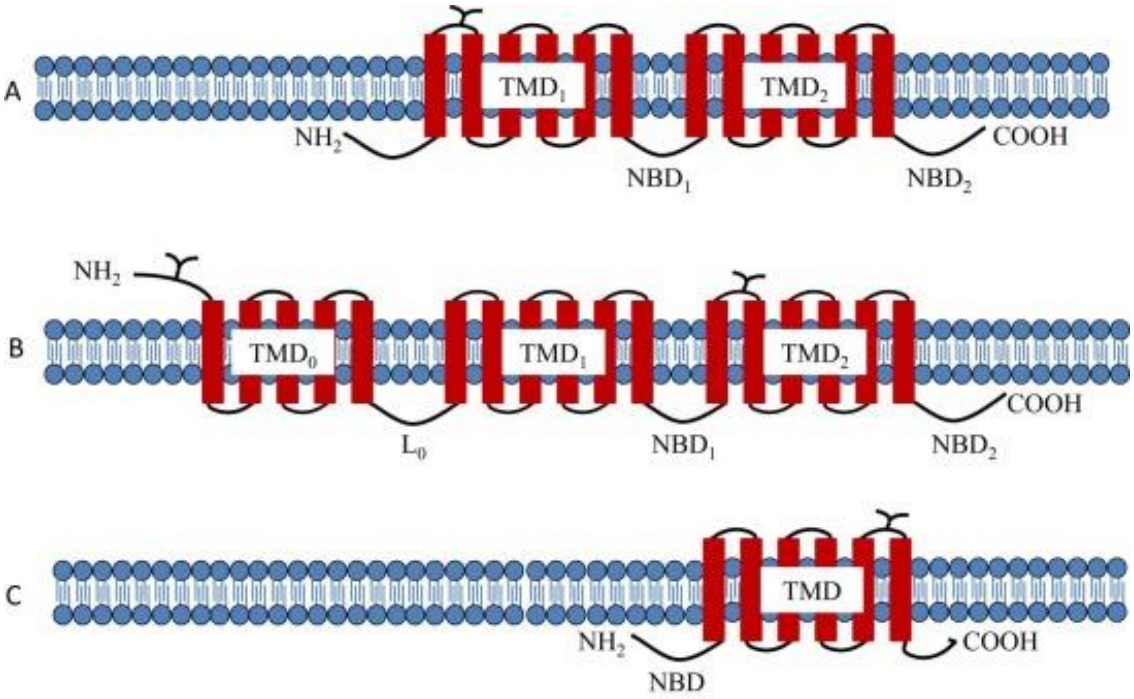


Fig. 1. Secondary structure models of drug efflux transporters of the ATP-binding cassette family. (A) P-gp/ABCB1, (B) MRP2/ABCC2, (C) BCRP/ABCG2. TMD – transmembrane domain; NBD – nucleotide-binding domain; L0 – loop 0.

Zhaolin Chen, Tianlu Shi, Lei Zhang, Pengli Zhu, Mingying Deng, Cheng Huang, Tingting Hu, Ling Jiang, Jun Li

Mammalian drug efflux transporters of the ATP binding cassette (ABC) family in multidrug resistance: A review of the past decade

Cancer Letters, Volume 370, Issue 1, 2016, 153–164

http://dx.doi.org/10.1016/j.canlet.2015.10.010

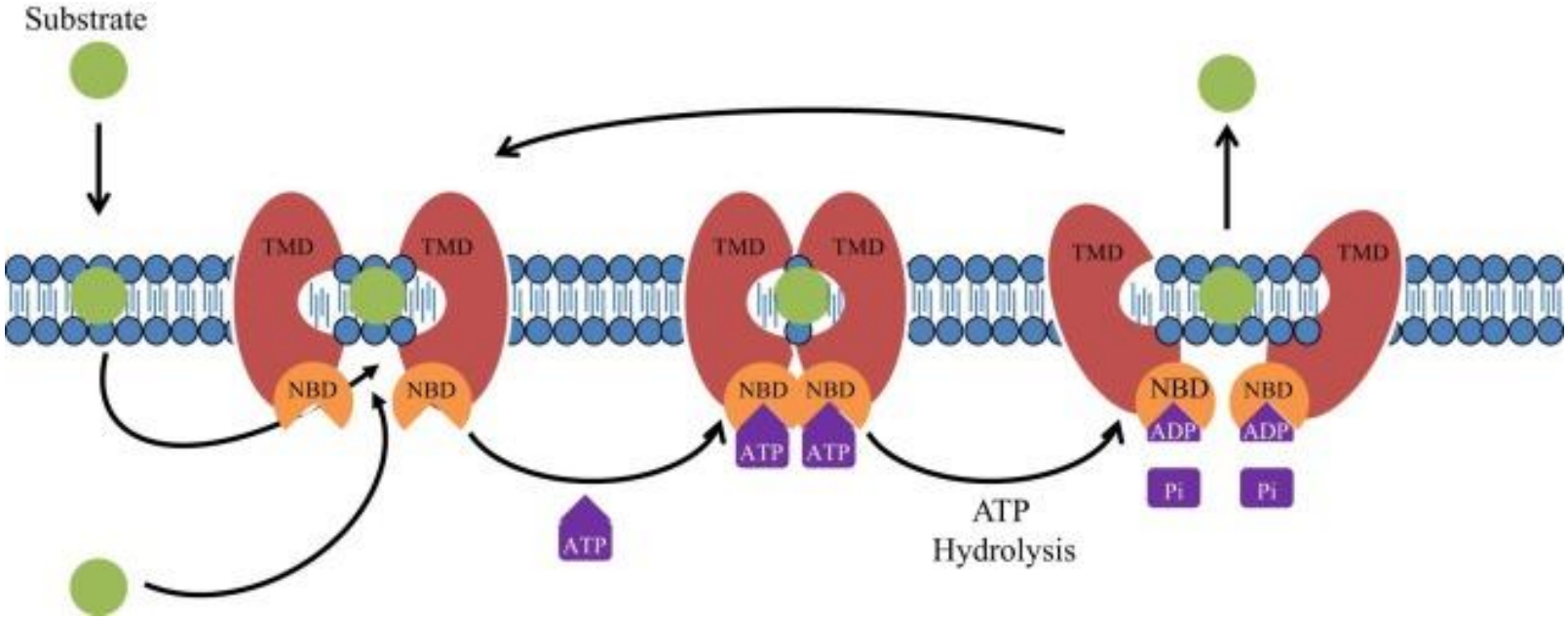


Fig. 2. Function of ABC transporters. ABC transporters are energy-dependent transporters; they exhibit a conformational change upon substrate binding and ATP hydrolysis which drives the transport process of the substrate.

Zhaolin Chen, Tianlu Shi, Lei Zhang, Pengli Zhu, Mingying Deng, Cheng Huang, Tingting Hu, Ling Jiang, Jun Li

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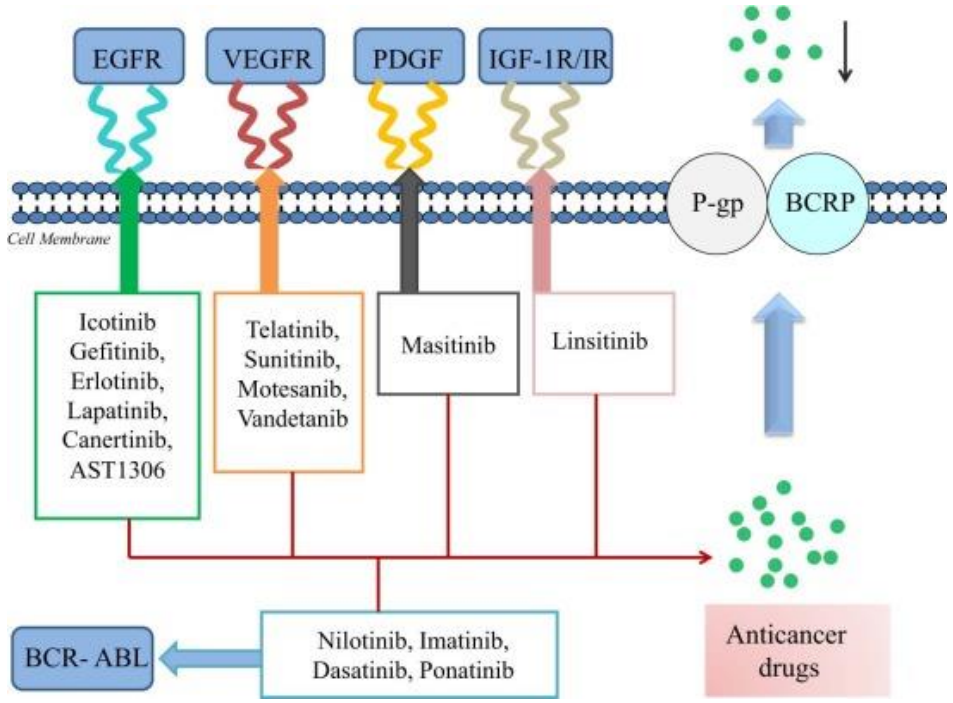

Fig. 3. Overview of TKIs as antagonists in the regulation of ABC transporters. BRAF TKI, breakpoint cluster region-Abelson (BCR-ABL) TKIs, epidermal growth factor receptor (EGFR) TKIs, vascular endothelial growth factor receptor (VEGFR) TKIs, platelet-derived ...

Zhaolin Chen, Tianlu Shi, Lei Zhang, Pengli Zhu, Mingying Deng, Cheng Huang, Tingting Hu, Ling Jiang, Jun Li

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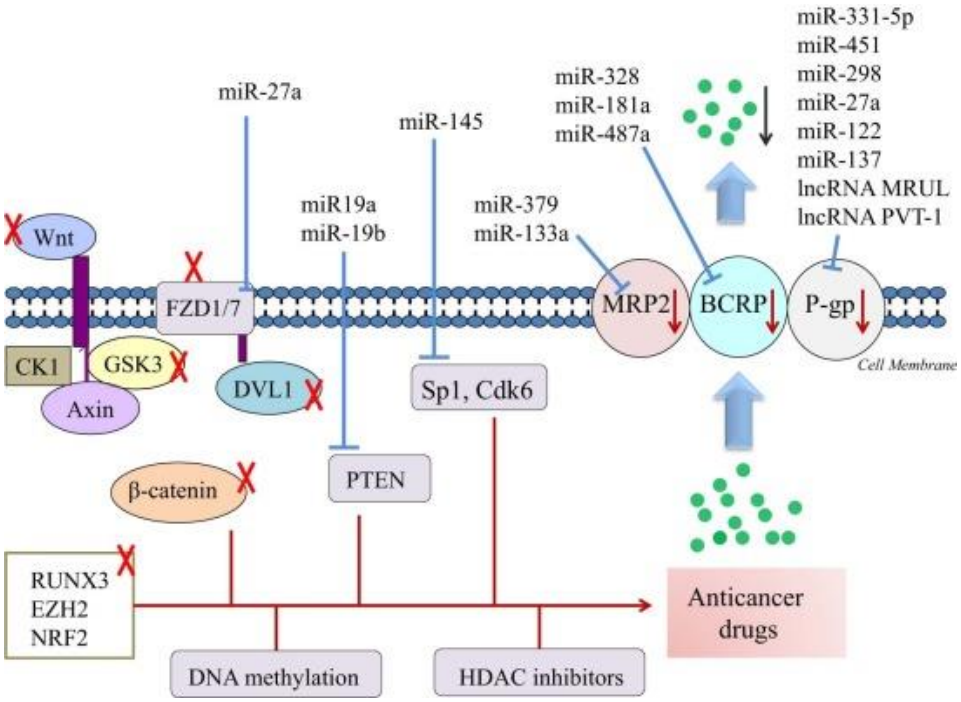

Fig. 5. Selected natural drugs as potential reversing agents of MDR. Black solid arrow indicates a reduction on the efflux of various anticancer drugs. Red solid arrow indicates a reduction on the expression of P-gp/ABCB1. Orange solid arrows indicate a reduct...

Zhaolin Chen, Tianlu Shi, Lei Zhang, Pengli Zhu, Mingying Deng, Cheng Huang, Tingting Hu, Ling Jiang, Jun Li

Mammalian drug efflux transporters of the ATP binding cassette (ABC) family in multidrug resistance: A review of the past decade

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五、协同运输cotransport

- 靠间接提供能量完成主动运输。所需能量来自膜两侧离子的浓度梯度。
  - 动物细胞中常常利用膜两侧Na^+浓度梯度来驱动。
  - 植物细胞和细菌常利用H^+浓度梯度来驱动。
- 分为：同向协同（symport）和反向协同（antiport）。

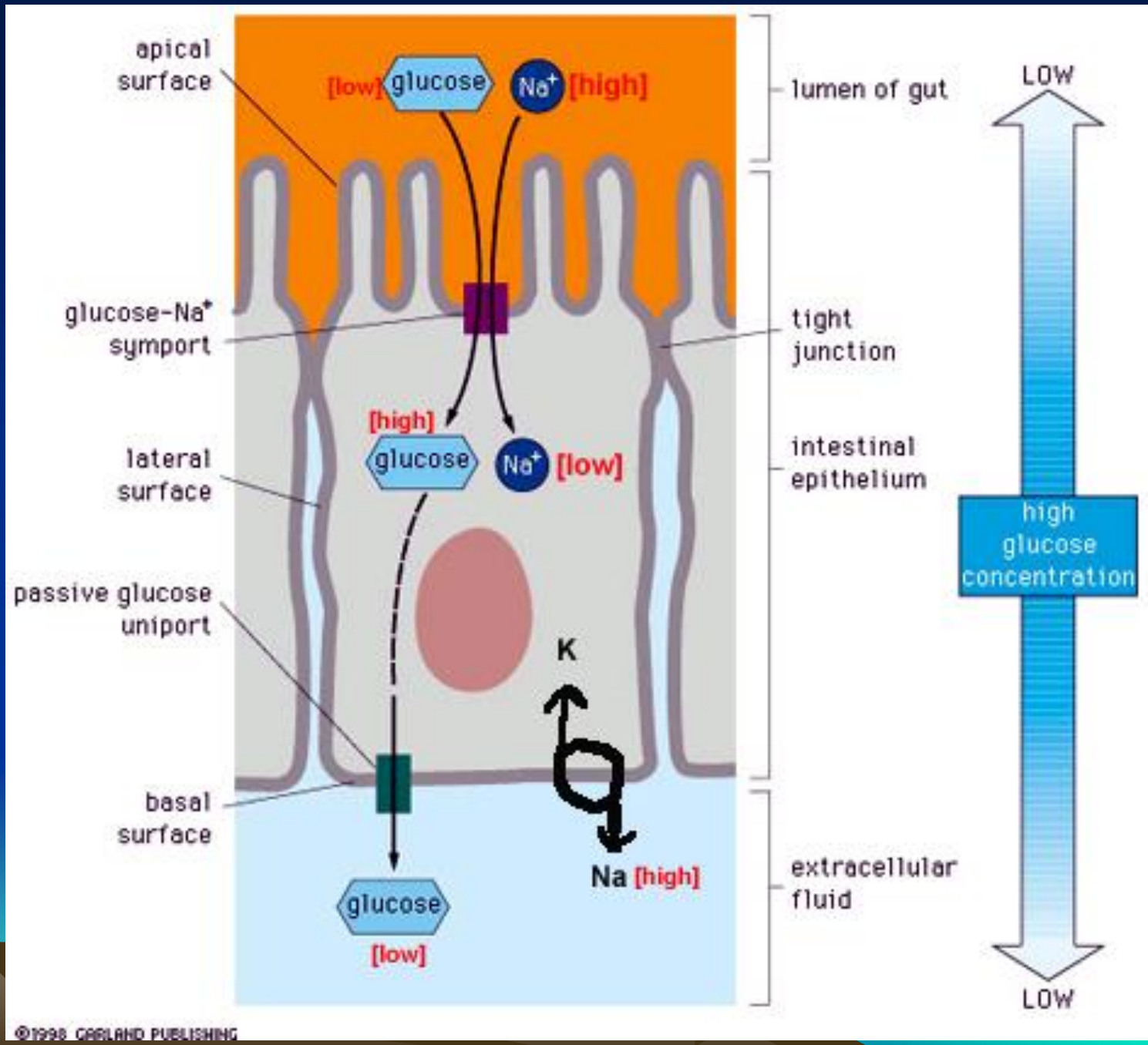
1、同向协同（symport）

- 如小肠细胞对葡萄糖的吸收伴随着Na^+的进入。某些细菌对乳糖的吸收伴随着H^+的进入。

2、反向协同（antiport）

- 如Na^+驱动的Cl^--HCO_3^-交换，即Na^+与HCO_3^-的进入伴随着Cl^-和H^+的外流，如存在于红细胞膜上的带3蛋白。

Glucose is absorbed by symport

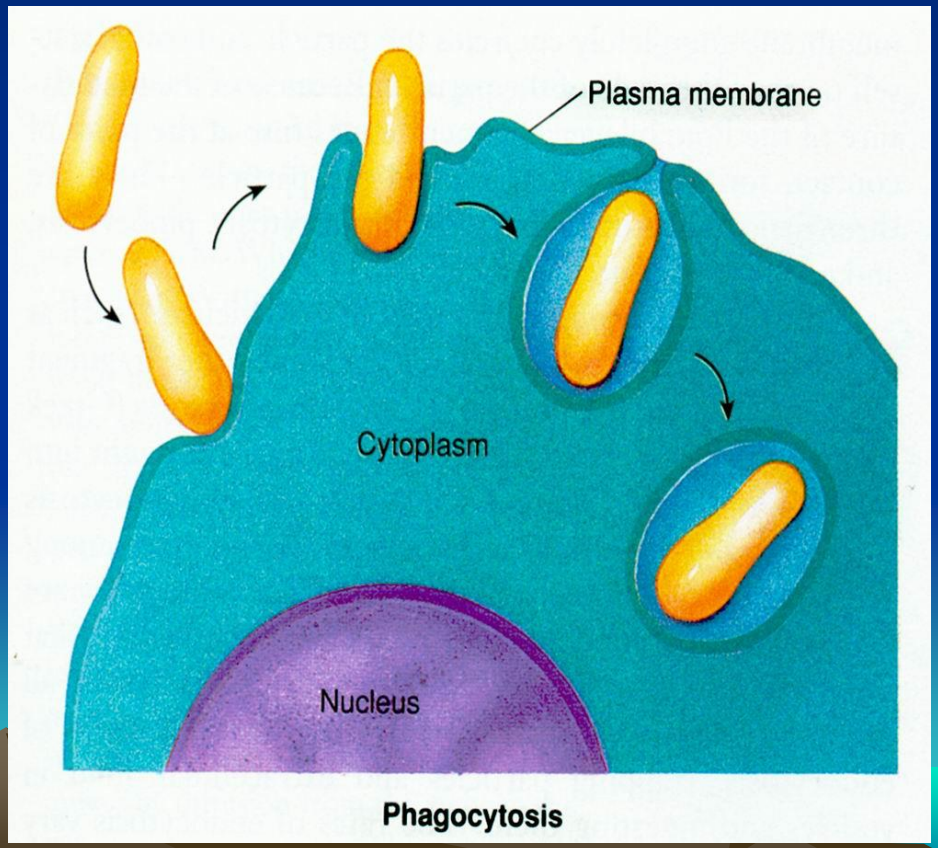


第三节 膜泡运输的基本概念

- 真核细胞通过内吞作用（endocytosis）和外排作用（exocytosis）完成大分子与颗粒性物质的跨膜运输。
- 因货物包被在囊泡中，又称膜泡运输。

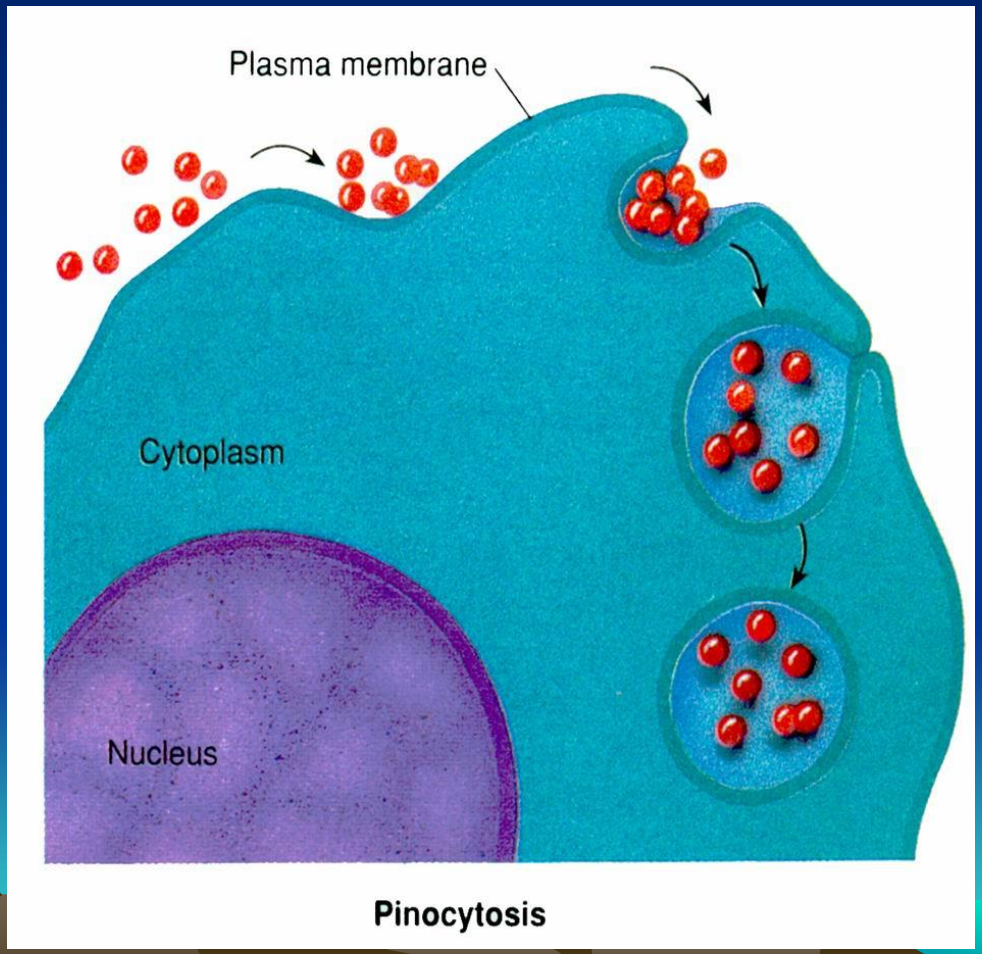
一、吞噬作用

- 细胞内吞较大的固体颗粒物质，如细菌、细胞碎片等。



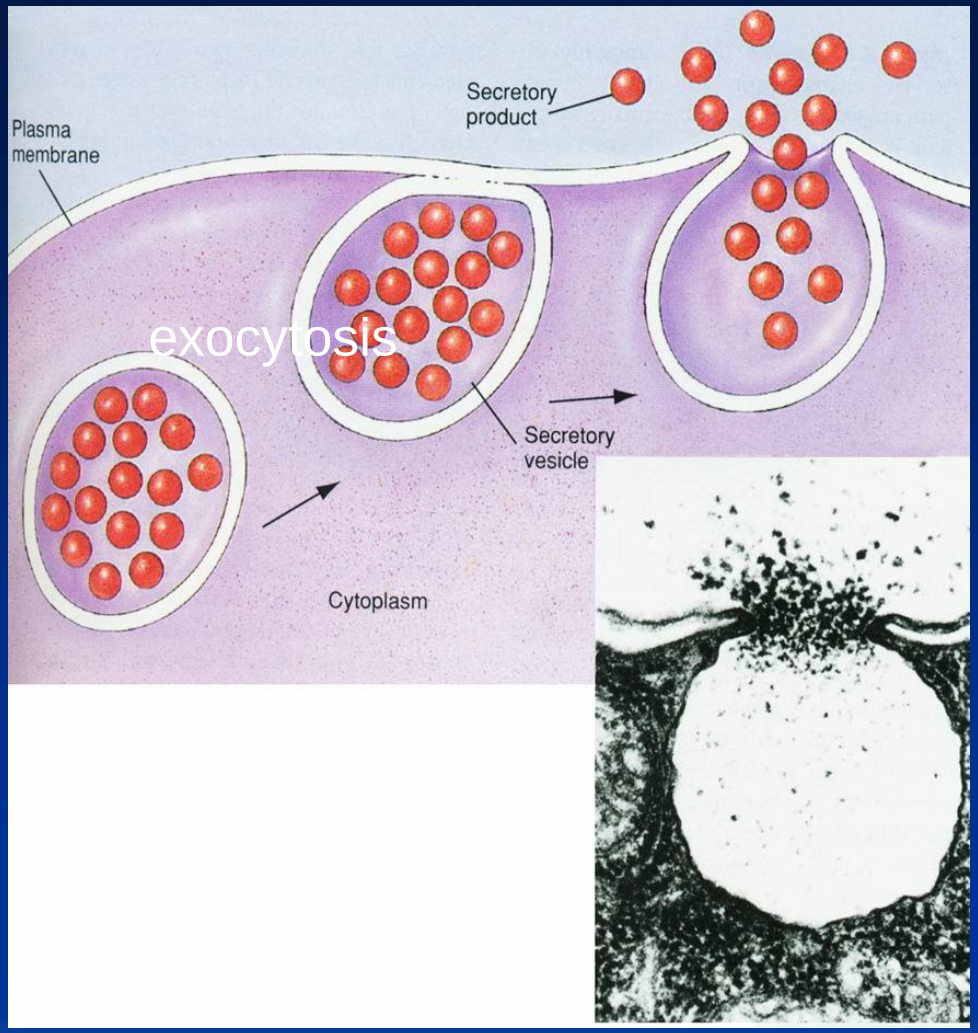
二、胞饮作用

- 细胞吞入液体或极小的颗粒物质。



Pinocytosis

三、外排作用



- 包含内容物的囊泡移至细胞表面，与质膜融合，将物质排出细胞之外。

四、穿胞运输

- 在细胞的一侧形成胞饮小泡穿越细胞质，另一侧使小泡中的物质释放出去。如：母鼠血液中的抗体经穿胞运输进入乳汁。

五、胞内膜泡运输

- 细胞内膜系统各个部分之间的物质传递也通过膜泡运输方式进行。如从内质网到高尔基体；高尔基体到溶酶体等。

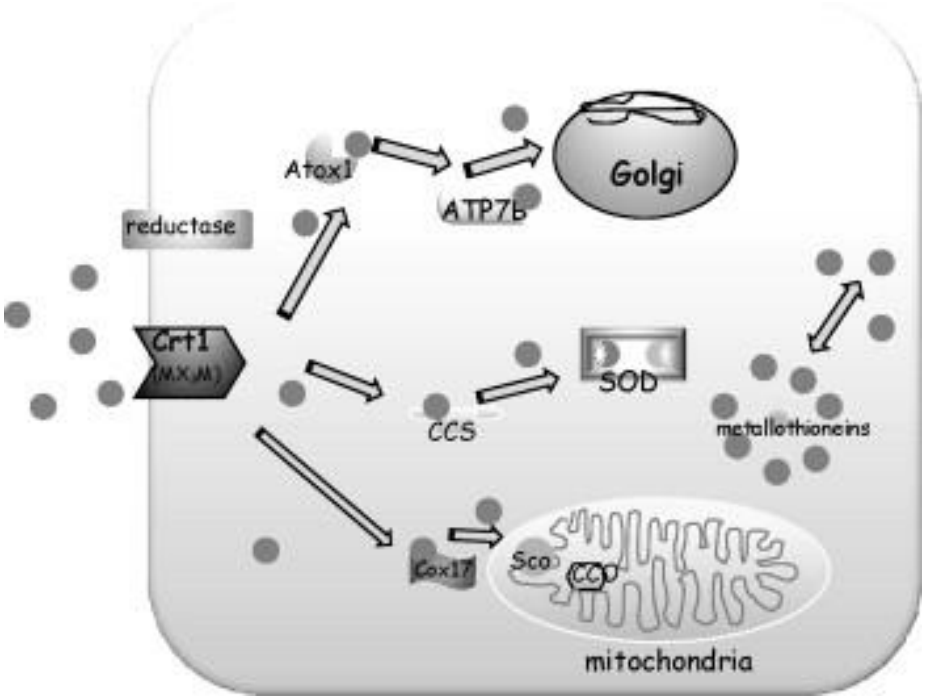


Fig. 1. Scheme of copper(I) trafficking pathways in mammals.

Henryk Kozlowski, Anna Janicka-Klos, Justyna Brasun, Elena Gaggelli, Daniela Valensin, Gianni Valensin

Copper, iron, and zinc ions homeostasis and their role in neurodegenerative disorders (metal uptake, transport, distribution and regulation)

Coordination Chemistry Reviews, Volume 253, Issues 21–22, 2009, 2665–2685

http://dx.doi.org/10.1016/j.ccr.2009.05.011

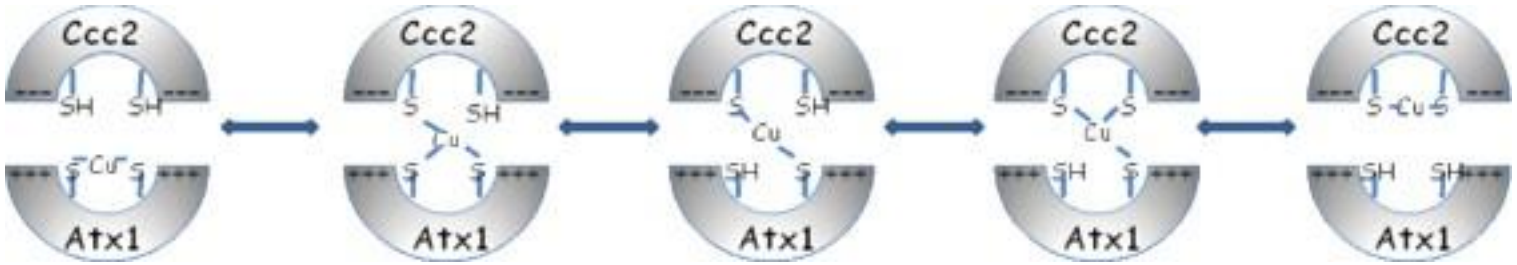


Fig. 2. Proposed mechanism for yeast model of copper transfer from Atx1 to Ccc2.

Henryk Kozlowski, Anna Janicka-Klos, Justyna Brasun, Elena Gaggelli, Daniela Valensin, Gianni Valensin

Copper, iron, and zinc ions homeostasis and their role in neurodegenerative disorders (metal uptake, transport, distribution and regulation)

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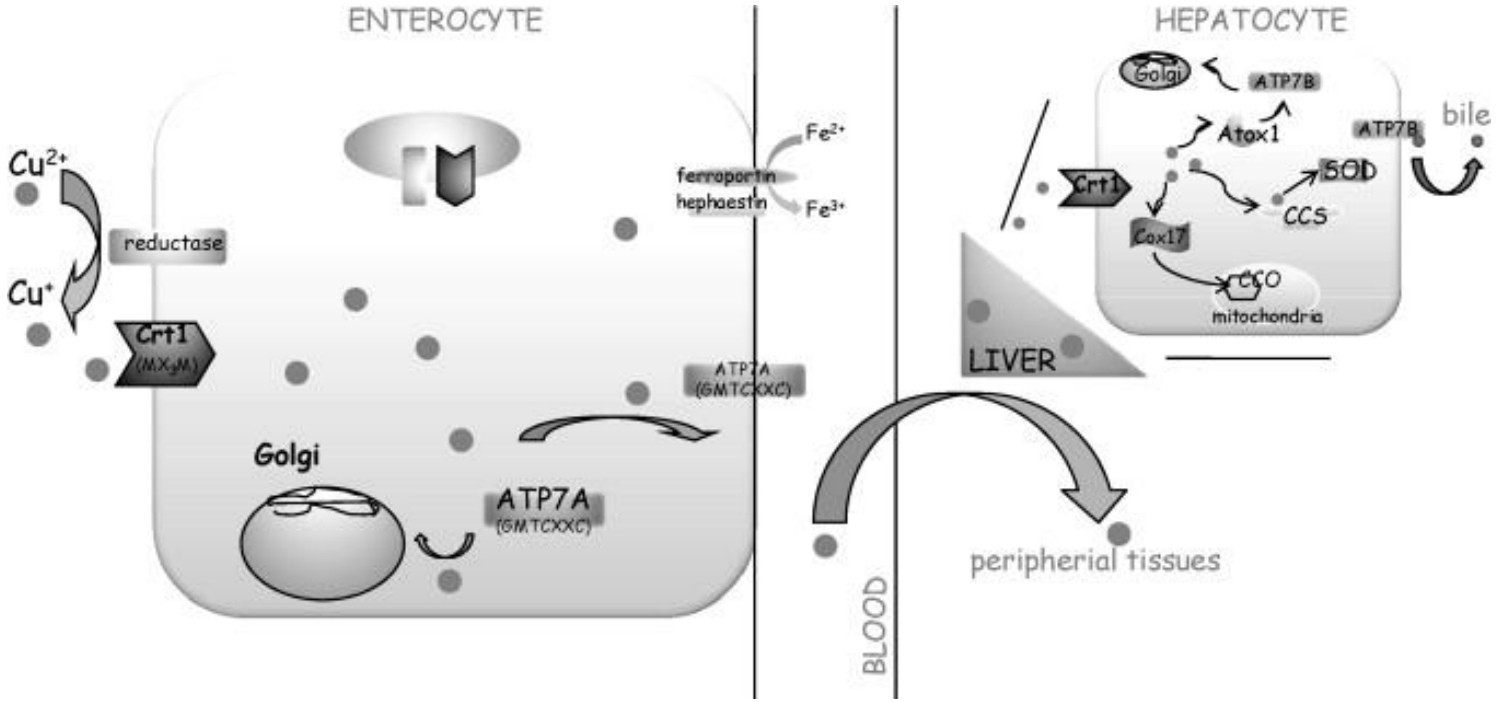

Fig. 4. Schematic model of intestinal copper absorption and its peripheral distribution.

Henryk Kozlowski, Anna Janicka-Klos, Justyna Brasun, Elena Gaggelli, Daniela Valensin, Gianni Valensin

Copper, iron, and zinc ions homeostasis and their role in neurodegenerative disorders (metal uptake, transport, distribution and regulation)

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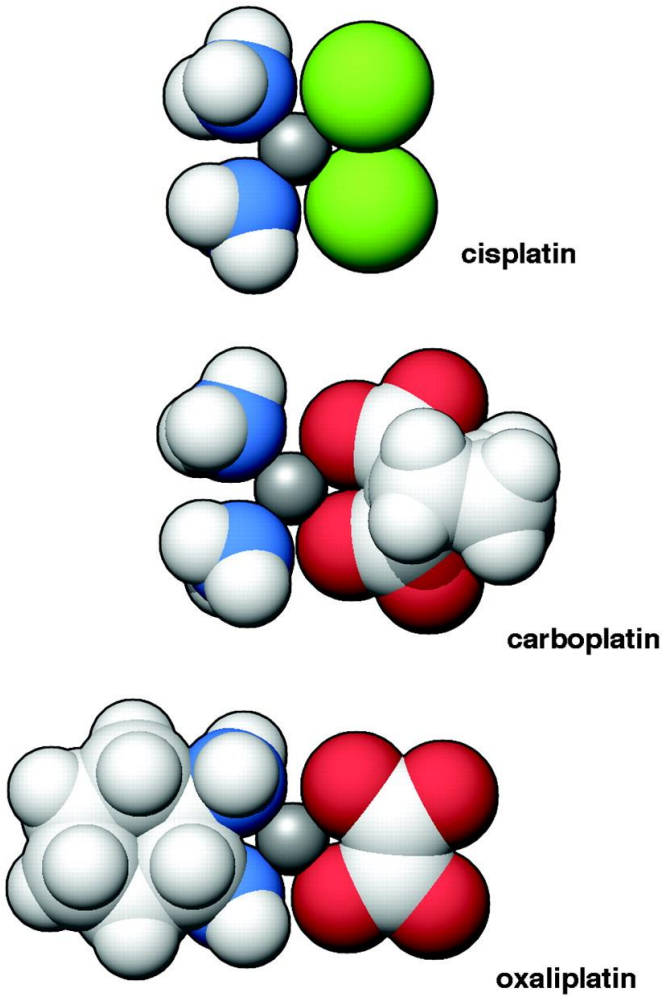
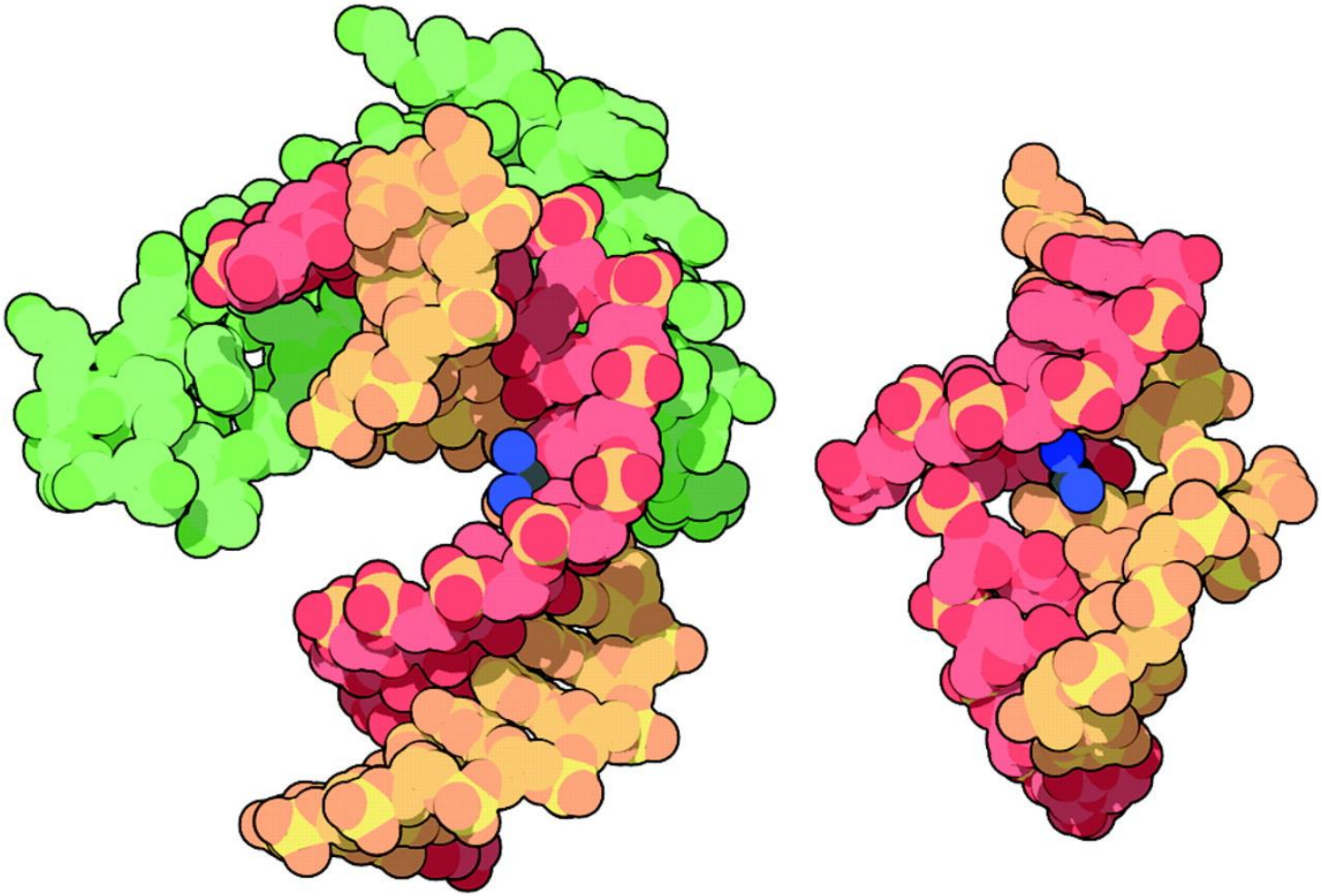
Cisplatin and its relatives are composed of a doubly charged platinum ion (in grey) surrounded by four ligands.



David S. Goodsell The Oncologist 2006;11:316-317

The Oncologist

Cisplatin forms crosslinks in DNA. On the left, cisplatin has formed a crosslink between two neighboring guanine bases, causing the DNA double helix to bend around the site of damage.



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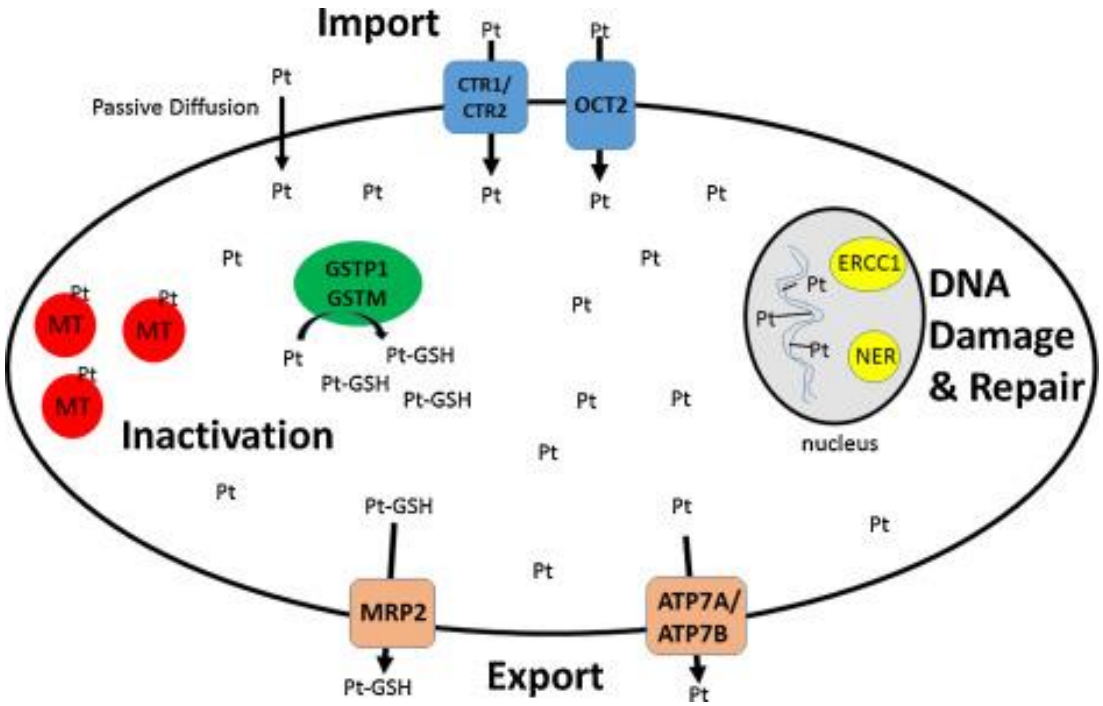


Fig. 2. Cellular fate of cisplatin (Pt,顺氯氨铂). Cisplatin crosses the cell membrane by passive diffusion or by transmembrane transporters. CTR1, CTR2, and OCT2 have been identified as transporters that import cisplatin into the cell. Once inside the cell, cisplatin b...

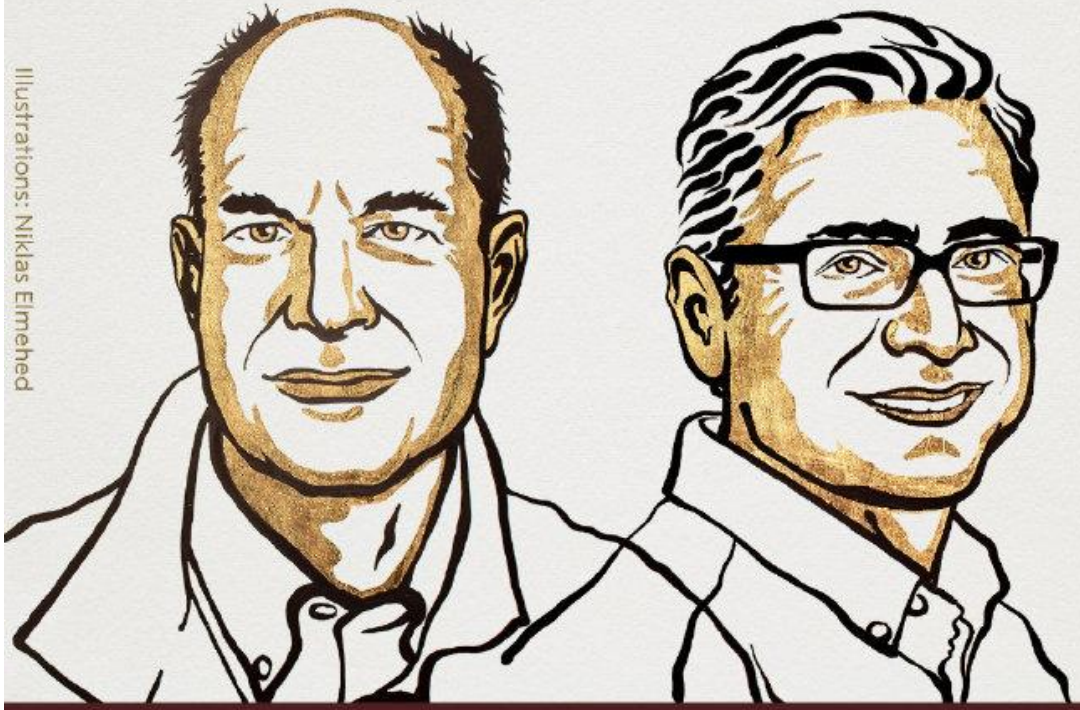
Lauren Amable

Cisplatin resistance and opportunities for precision medicine

Pharmacological Research, Volume 106, 2016, 27–36

http://dx.doi.org/10.1016/j.phrs.2016.01.001

THE NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE 2021



Illustrations: Niklas Elmehed

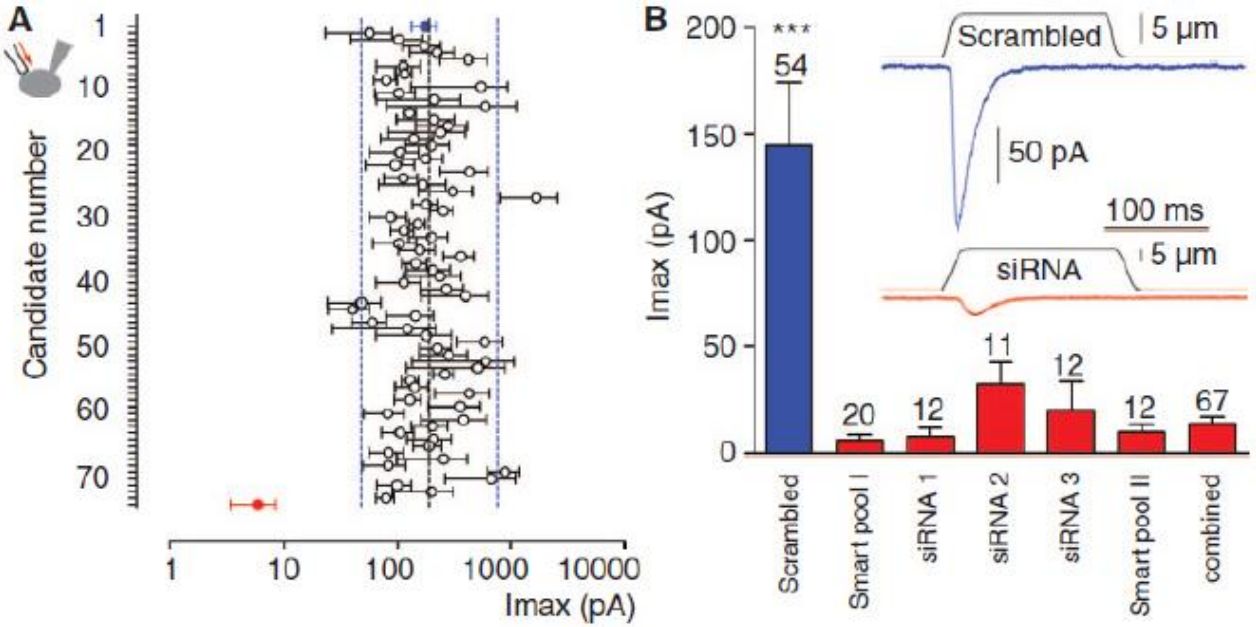
David Julius Ardem Patapoutian

"for their discoveries of receptors for temperature and touch"

THE NOBEL ASSEMBLY AT KAROLINSKA INSTITUTET

Piezo1 and Piezo2 Are Essential Components of Distinct Mechanically Activated Cation Channels

Bertrand Coste,[1] Jayanti Mathur,[2] Manuela Schmidt,[1] Taryn J. Earley,[1] Sanjeev Ranade,[1] Matt J. Petrus,[2] Adrienne E. Dubin,[1] Ardem Patapoutian[1,2]*

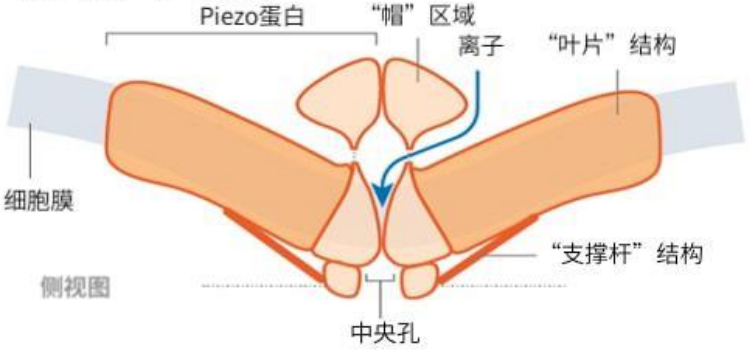


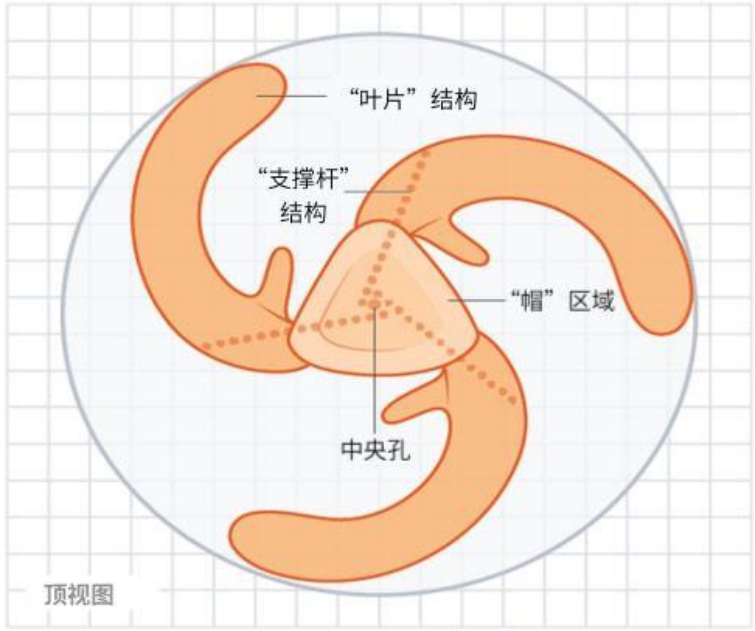
压力感受器

细胞有特殊的蛋白质帮助其感知机械力。哺乳动物中表征最好的包括Piezo蛋白——形成离子通道，帮助离子进出细胞。研究人员正在探究这些通道如何打开。

关闭状态

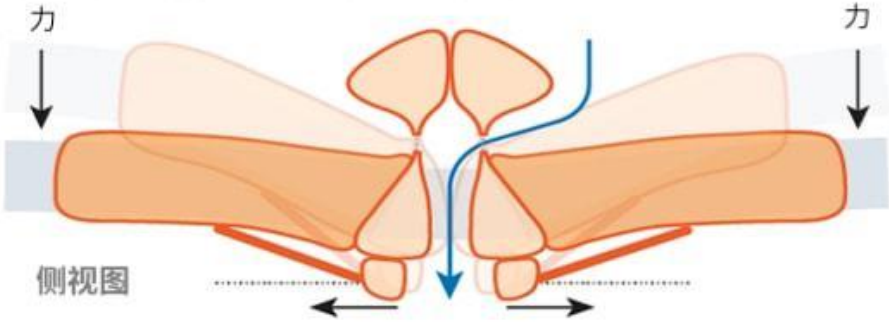
通道关闭时的图像显示，通道由三个相同蛋白质围绕中央孔构成，每个蛋白质包含一个“叶片”结构和一个“支撑杆”机构。细胞膜在通道处起了个褶，形成一个凹陷结构。



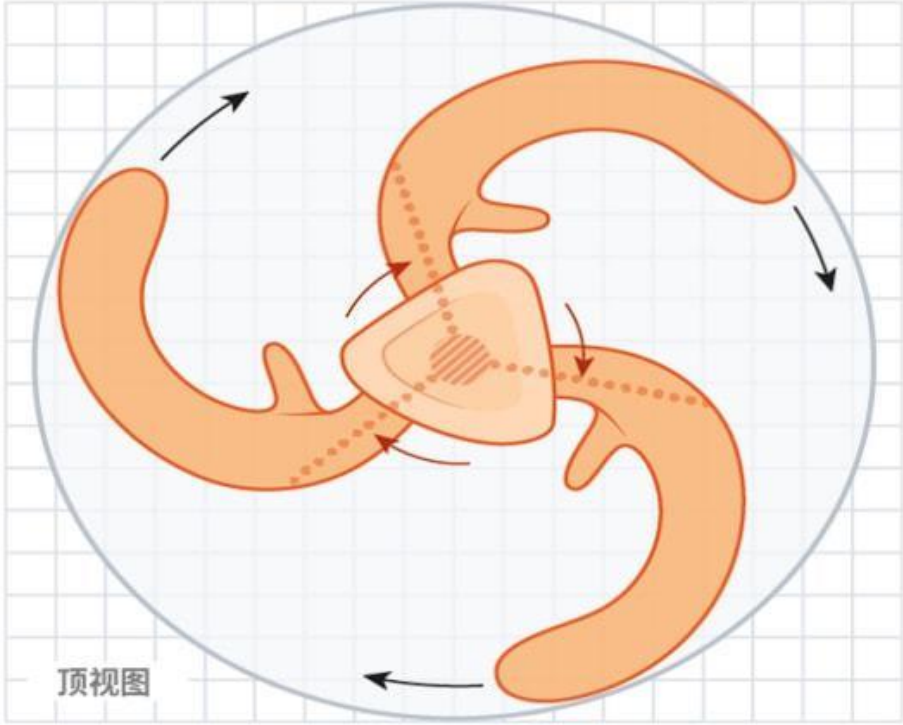


通道开启

目前尚没有Piezo通道开启状态的图片，但关于通道如何打开，研究人员提出了诸多假设。弯曲的“叶片”结构可能被拉平从而开启通道；或者某种力作用于“叶片”结构，带动“支撑杆”结构打开通道。



“叶片”结构可能通过旋转，使中央孔打开。部分研究提示，三个“叶片”结构可独立运作。



THANK YOU!