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综 述

细胞穿膜肽介导生物大分子入胞机制研究进展*

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摘要 生物大分子药物与传统治疗方式相比作用靶点具有高度的专一性,成为21世纪药物研发中最具发展前景的领域之一,但由于细胞膜的天然屏障作用致使许多潜在的胞内药物靶标无法应用于新药研究。细胞穿膜肽(cell-penetrating peptides,CPP)是一类具有穿膜功能的小分子短肽,可高效携带核酸、蛋白质等生物大分子穿过细胞膜进入胞质发挥功能,在介导生物大分子药物入胞上有着高效、低毒等诸多优势,但仍存在效率低、靶向性差等问题。CPP携带货物分子入胞的方式可以根据是否依赖能量分为直接入胞和内吞。直接入胞依据孔隙形成的方式不同分为四种模型:桶板模型、超环面模型、地毯模型和反向胶团模型。内吞则根据受体的不同又分为巨胞饮、网格蛋白介导的内吞、小窝蛋白介导的内吞、硫酸乙酰肝素蛋白聚糖介导的内吞以及神经毡蛋白-1介导的内吞。CPP自身的类型、浓度、效应分子的物理化学性质以及分子大小都会影响CPP的入胞过程,进而决定CPP携带生物大分子入胞的途径。对CPP介导生物大分子的入胞机制进行综述,为研究更加高效、靶向性强的CPP提供依据,从而推动其在生物、医学领域的应用。

关键词 细胞穿膜肽 生物大分子 入胞机制 直接入胞 内吞

中图分类号 Q81

与小分子药物相比,生物大分子在药效、特异性以及靶分子类型识别方面更具优势[1]。生物大分子药物2012年占全球药物总收入的71%,美国FDA近年来批准的创新药物中近三分之一是生物大分子和生物制剂,且该比率在不断攀升[2-3]。因此,生物大分子药物已经成为目前新药研究的热门方向,无论在疾病治疗还是市场前景方面都具有巨大的潜力。然而,由于细胞膜的天然屏障作用,生物大分子无法直接进入细胞发挥作用,现有的生物大分子药物主要在胞外发挥作用。细胞大部分的物质位于胞内,由于缺少有效的递送手段,约80%的潜在胞内药物靶标无法用于生物大分子药物的开发。细胞穿膜肽(cell penetration peptide,CPP)介导的生物大分子的入胞因其相较于传统方式更加安全和高效引起了学者们的广泛关注,是目前最为成功的可在体内、外直接转运生物大分子的递送系统。但是,目前CPP介导的生物大分子的转运仍存在效率低、靶向性差等问题,限制了生物大分子药物的临床应用。CPP及其介导的生物大分子穿膜机制的研究将有助于研究更加高效的CPP。本文将对近年来CPP及其介导的生物大分子入胞的机制方面的进展进行综述,以期为研究更加高效的CPP提供依据,从而进一步推动生物大分子药物的研究和应用。

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1 细胞穿膜肽简介

CPP是一类由5~30个氨基酸残基组成,具有细胞膜穿透能力的多肽,可携带蛋白质、核酸等生物大分子进入细胞[4]。最早的CPP研究可以追溯到1988年由Frankel和Green两个团队独立开展的工作,他们发现

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HIV 的转录激活蛋白(TAT)可以有效穿过细胞膜并激活相应病毒启动子的转录。此后,TAT 的穿膜功能被精确定位于 11 个氨基酸的核心区段(aa47-aa57),该区段被命名为 TAT-蛋白转导域(protein transduction domain,TAT-PTD),并被成功应用于不同外源蛋白的胞内转运[5-6]。迄今,已有上百种来源于天然蛋白或人工合成的 CPP 被报道并用于生物大分子的胞内递送研究[5]。根据其化学性质的不同,可以分为三类:阳离子型、两亲型和疏水型[7]。(1)阳离子型,富含精氨酸和赖氨酸残基,在生理 pH 下具有强正电荷,主要包括 R9[8]、TAT[9]、hLF[10]等。此肽随着精氨酸个数的增加其穿透能力逐渐增强。比如核定位信号序列肽(nuclear localization sequences,NLSs)富含赖氨酸(K)、精氨酸(R)或脯氨酸(P)残基,可穿透细胞膜进入细胞核[11]。(2)两亲型肽类的正电荷由赖氨酸残基提供,这类 CPP 含疏水性和亲水性结构域,并且其两亲性的特征是由其一级结构和二级结构共同决定的,主要包括 MPG[12]、CADY[13]、pVEC[14]、SAP 等[15]。(3)疏水型 CPPs 具有较低的净电荷,主要由非极性氨基酸组成。相较于阳离子型穿膜肽和两亲型穿膜肽,目前仅发现非常少量的疏水型 CPP,比如卡波西成纤维细胞生长因子(K-FGF)肽[16](表 1)。CPP 自身的优势为生物大分子在胞内递送提供了新的手段,自 CellGate Inc 开展首个基于 CPP 药物递送的临床试验以来,陆续已有多家公司开展了基于 CPP 递送的局部或全身给药方案的临床试验[17]。

表 1　常见的几种细胞穿膜肽

Table 1　Examples of cell penetrating peptides

分类	CPP	特点	氨基酸序列	文献
Cationic	TAT	HIV-1 transcriptional activator	RKKRRQRRR	[18]
	R9	Synthetically created sequence of nine arginines	RRRRRRRRR	[8]
Amphipathic	Penetratin	Protein-derived form Drosophila antennapedia	RQIKIWFQNRRMKWKK	[19-21]
	MPG	Model amphipathic peptides	GLAFLGFLGAAGSTMGAWSQPKKKRKV	[22-23]
	pVEC	Murine vascular endothelial cadherin	LLIILRRRIRKQAHAHSK	[24]
Hydrophobic	VP22	A component of a capsid of HSV-1 virus	DAATATRGRSAASRPTE RPRAPARSASRPRRVD	[25]
	K-FGF	Artifcial peptide containing the penetrating motif and locating the cell nucleus sequence	AAVLLPVLLAAP	[16]

2　细胞穿膜肽的入胞机制

CPP 介导的生物大分子入胞机制根据过程中是否需要能量可分为两种:非能量依赖型直接入胞和能量依赖型的内吞途径[26-27]。CPP 入胞的方式不仅与 CPP 的类型和自身的浓度有关,也与效应分子的物理化学性质以及分子大小有关[28]。

2.1　直接入胞

直接入胞(又称膜转导),是指 CPP 通过自身携带的正电荷与细胞膜上磷脂双分子层表面的负电荷的相互作用穿过细胞膜实现入胞,该过程不依赖能量,在低温以及内吞抑制剂存在的情况下都可发生。根据孔隙形成方式不同将直接入胞分为四种模型:桶板模型、超环面模型、地毯模型和反向胶团模型。

桶板模型是指具有 α 螺旋结构的两亲性 CPP 成束排列平行插入细胞膜中形成桶样孔道,CPP α 螺旋的疏水氨基酸残基与细胞膜磷脂分子结合形成桶道的外表面,内部亲水性氨基酸残基与磷脂亲水头部结合形成桶道的内腔,促使 CPP 进行跨膜移位进入细胞质(图 1)[29-30]。聚精氨酸(Arg9)肽就是经此过程发生细胞内化[31]。

超环面模型是通过细胞膜磷脂和 CPP 的亲水性头部之间的吸引力和分子力,迫使脂质的尾部向上移动产生瞬间孔隙,从而使得 CPP 携带的效应分子进入胞内(图 1)[5,31]。Mastoparan X 肽通过此方式进入胞浆[32]。

地毯模型是 α 螺旋阳离子 CPP 像“地毯”一样平行覆盖在带负电荷的细胞膜表面,但并不插入细胞膜的磷脂双层[33]。随后 CPP 中碱性氨基酸残基朝向细胞膜表面,而疏水性氨基酸残基与细胞膜的疏水性区相互作用。当 CPP 达到一定浓度时,它们会自行旋转,增加细胞膜的流动性并在其中形成孔隙,促进 CPP 进

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入细胞(图1)[34-35]。

反向胶团模型是由CPP的阳离子与带负电荷的磷脂产生作用,将疏水性氨基酸如色氨酸插入细胞膜中,使得细胞膜的内侧或外侧开放,形成反向胶团使多肽进入磷脂双分子层区域,然后通过与细胞内侧层的磷脂相互作用使CPP进入细胞质(图1)[8,36]。此种模型需要CPP中疏水氨基酸残基参与,对于不含疏水性氨基酸残基的CPP并不适用。

直接入胞大多数情况下适用于单独的CPP或CPP偶联的小分子入胞,而CPP携带生物大分子则主要通过内吞方式入胞。

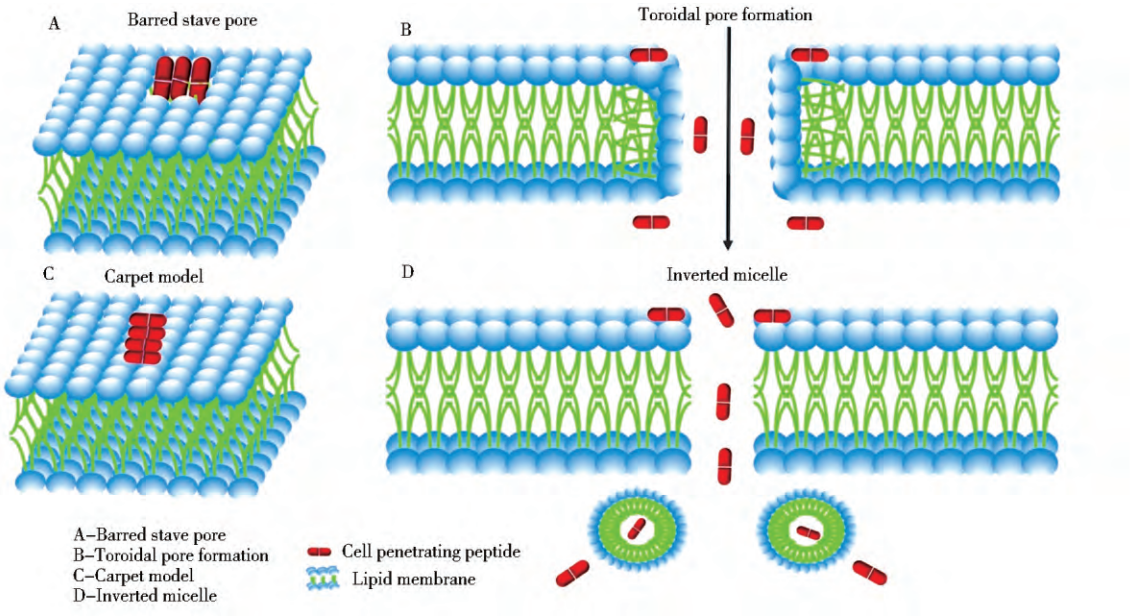

图1 细胞穿膜肽直接入胞示意图

Fig. 1 Schematic diagram of direct cell entry pathway of cell penetrating peptide

2.2 内 吞

内吞(endocytosis)又称胞吞作用,是指细胞通过细胞膜的变形运动将胞外物资转运入胞内,过程中依赖能量,是CPP携带生物大分子入胞的主要途径[37]。CPP及其携带的生物大分子通过内吞方式入胞后会生成内吞小泡(endosome),并由早期内吞小泡(early endosome)移向晚期内吞小泡(late endosome)单向成熟并伴随泡内pH的降低,最终与溶酶体(lysosome)融合[38-39]。生物大分子需要在进入溶酶体之前从内吞小泡逃逸(endosomal escape)进入细胞质,否则,将最终进入溶酶体被降解而不能发挥功能。

根据内吞过程中参与蛋白质种类的不同将内吞途径具体分为:巨胞饮(macropinocytosis)、网格蛋白(clathrin)介导的内吞、小窝蛋白(caveolae)介导的内吞、硫酸乙酰肝素蛋白聚糖(heparan sulfate Proteoglycans; HSPG)介导的内吞及神经毡蛋白-1(neuropilin-1; NRP1)介导的内吞[40](图2)。

2.2.1 巨胞饮 巨胞饮是受体依赖性的非特异性的内吞过程[17]。当细胞通过巨胞饮摄取外源物质时,局部细胞膜的肌动蛋白微丝进行收缩,使细胞膜凹陷变形并产突起行成内吞囊泡,囊泡包裹着CPP在驱动蛋白作用下将内吞小泡从细胞膜上分离进入细胞质(图2)[41-42]。

Wadia等[43-44]发现表达肌动蛋白的显性失活突变体Dyn^K44A不会阻断TAT融合蛋白的转导入胞,当加入阿米洛利(巨胞饮抑制剂)则会大大降低TAT融合蛋白的摄取效率,这表明TAT融合蛋白的入胞是通过脂质筏介导的巨胞饮作用发生的。上述发现与Lindsay小组结果一致,他们发现TAT及其融合蛋白与霍乱毒素会发生高度的共定位(霍乱毒素可对富含胆固醇的细胞膜区域进行标记),而当去除胆固醇后TAT融合蛋白的摄取会受抑制[45]。

2.2.2 网格蛋白介导的内吞 网格蛋白介导的内吞又称受体介导的细胞内吞,是细胞对外源物质的特异性摄取的过程。网格蛋白介导的内吞主要机制是:CPP和它偶联的生物大分子特异性的和细胞膜上的受体结

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合,导致局部细胞膜凹陷形成有被小窝,小窝与细胞膜脱离形成有被小泡。网格蛋白包被小泡进入细胞,随后脱去网格蛋白形成与细胞内早期内体融合的转运小泡。早期内体通过微管从细胞外周向核移动进入晚期内体,并与高尔基体囊泡融合形成内吞小泡,随后内容物从内吞小泡逃逸进入细胞质发挥作用(图2)[46-47]。

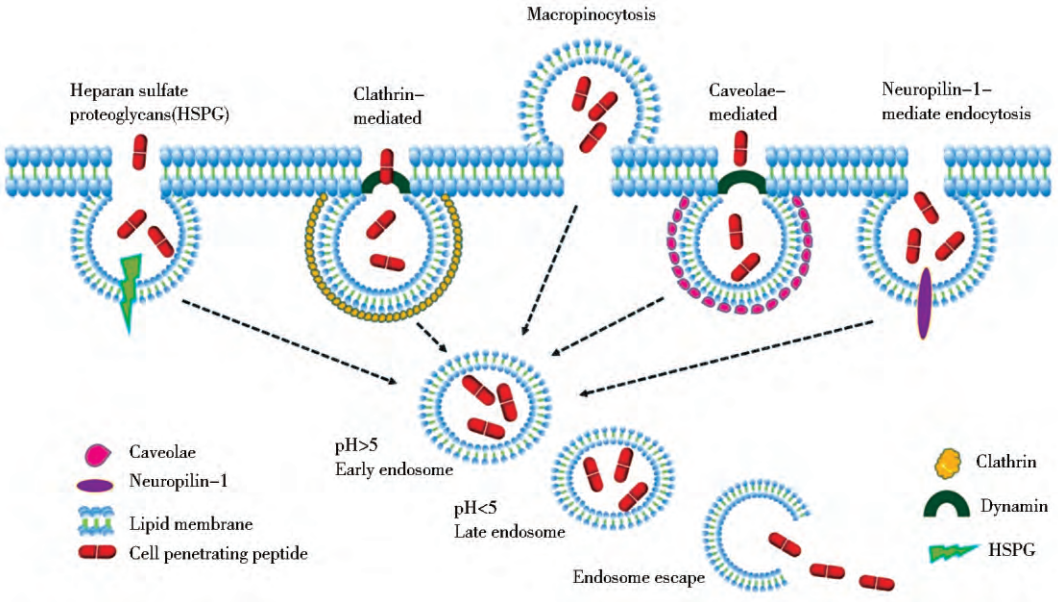

图2　细胞穿膜肽内吞途径示意图

Fig. 2　Schematic diagram of endocytic pathway of cell penetrating peptide

Richard 等[22]指出,TAT 的入胞可以通过网格蛋白介导的内吞作用。后续有研究证实,加入网格蛋白合成抑制剂(氯丙嗪盐酸盐),TAT 的入胞效率大大降低[21]。Doan 等[48]将不同的内吞抑制剂(网格蛋白介导的内吞抑制剂 CPZ、小窝蛋白介导的内吞抑制剂 MβCD、巨胞饮内吞抑制剂 EIPA)加入到[Cys-CPT^{2,9}]penetratin 穿膜肽中,观察细胞对[Cys-CPT^{2,9}]penetratin 摄取的影响,最终发现[Cys-CPT^{2,9}]penetratin 是通过网格蛋白介导的内吞途径入胞。

2.2.3　小窝蛋白介导的内吞　小窝蛋白是膜穴上的标志性蛋白。膜穴包含囊泡运输系统用于锚定和融合所需的全套分子机制。脂筏是细胞膜表面富含胆固醇和鞘磷脂的疏水性膜微区,脂筏内陷形成膜穴小泡,膜穴小泡的表面则附着小窝蛋白[49-51]。当细胞发生内化时,CPP 和它偶联的效应分子被募集到小窝,与膜穴小泡上的脂锚定蛋白进行特异性结合,此时酪氨酸激酶进行磷酸化招募发动蛋白-2 到达小窝的颈部,使小窝颈部缢缩,进而脱离细胞膜形成囊泡,囊泡在肌动蛋白的作用下从细胞膜上剥离将 CPP 释放(图2)[52]。

Jones 等[45]通过共聚焦显微镜发现 TAT-融合蛋白与霍乱毒素共定位,霍乱毒素已经被证明是通过小窝蛋白介导的内吞途径进入细胞[53]。

2.2.4　硫酸乙酰肝素蛋白聚糖　研究显示硫酸乙酰肝素蛋白聚糖(HSPG)可以作为 CPP 的主要结合位点介导其携带外源大分子的入胞[54-57]。HSPGs 由核心蛋白和多种硫酸乙酰肝素的糖胺聚糖链共同组成[58]。硫酸乙酰肝素与硫酸化的糖胺聚糖是自然界带负电荷最高的生物聚合物,它可以静电结合阳离子 CPP,作为初始附着位点提高货物的运输效率[59]。富含精氨酸的 CPP 与细胞表面 HSPGs 高亲和力相互作用,促使它们被细胞摄取(图2)[60-61]。

Jeffrey 等[62]发现与野生型 CHO 细胞相比,硫酸乙酰肝素缺陷型细胞系中聚精氨酸(4R-12R)、聚赖氨酸(4K-12K)和 TAT 的转导效率降低了 90 倍,而当把 HSPGs 加入到 HS 缺陷型细胞中时,转导效率恢复到野生型水平。这表明 HSPGs 参与到 CPP 介导生物大分子进入细胞的过程中[63]。

2.2.5　神经毡蛋白-1 介导的内吞　Morelli 等[64]证明神经毡蛋白-1(NRP1)参与细胞膜上 CPP 的摄取,而且 NRP1 只结合拥有 C 端精氨酸的 CPP,同时精氨酸的羧基没有取代基(图2)。Pang 等[65]发现 NRP1 和 HSPGs 也可以一起协同诱导巨胞饮途径,并能在电子显微镜

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下观察到 CPP 与 NRP1 或 HSPG 相互作用。

3 影响细胞穿膜肽的入胞方式的因素

CPP 自身的类型、浓度，效应分子的物理化学性质以及分子大小都会影响 CPP 的入胞过程，进而决定 CPP 携带生物大分子入胞的途径[27,66]。

3.1 CPP 的浓度

CPP 自身的浓度影响其介导的生物大分子的入胞效率。Duchardt 等[67] 研究表明浓度高于 10μmol/L 的 TAT 入胞途径是直接入胞和内吞方式同时进行，TAT 浓度小于 10μmol/L 是经小窝蛋白介导的内吞和巨胞饮方式进入胞质，而当浓度小于 40μmol/L 时，则通过巨胞饮，网格蛋白介导和小窝蛋白介导的内吞作用入胞。

3.2 细胞表面 CPP 的个数

Tseng 等[68] 发现细胞表面至少有 5 个 MAP 穿膜肽入胞过程才能发生。当 CPP 个数超过 100 个时，细胞的摄取效率才能达到最佳值[68-69]。低密度 CPP 一般通过小窝蛋白介导的内吞作用，高密度的 CPP 通过巨胞饮的方式入胞[70]。

3.3 CPP 的类型

同一类型的穿膜肽的入胞方式也未必是单一的，除了上述 TAT 可以通过多种方式入胞以外，Kim 等[71] 发现非离子型细胞穿膜肽 AA3H 介导的细胞内转运过程就是复杂混合式的能量依赖的过程，它的入胞存在多种机制，包括网格蛋白介导的内吞、小窝蛋白介导的内吞以及巨胞饮等过程。

3.4 效应分子的大小

某些 CPP 携带大分子蛋白或颗粒时，它的入胞机制是能量依赖的内吞途径，而单独的 CPP 或偶联小分子物质入胞时主要通过与细胞膜的磷脂类的相互静电作用进入细胞。Kaplan 等[72] 研究发现 TAT 偶联融合蛋白时，是通过小窝蛋白介导的内吞方式入胞，而当其与荧光基团偶联时，则通过网格蛋白介导的内吞方式入胞。Lim 等[73] 证明 dNP2 穿膜肽携带大分子物质的入胞机制是小窝蛋白介导的内吞，而当其偶联小分子入胞时则是多种内吞机制同时进行。

4 结语与展望

综上所述，单独的 CPP 可通过多种方式入胞，其入胞方式与 CPP 类型和浓度等有关，但其携带生物大分子时主要通过内吞方式入胞。内吞后，生物大分子成功的从内吞小泡释放才能发挥其功能。因此，内吞效率以及从内吞小泡释放的效率直接决定了生物大分子入胞的效率。CPP 介导的生物大分子入胞机制的探究为后续进一步研究更加高效的 CPP 提供了方向。随着 CPP 介导的生物大分子入胞机制的深入研究，将会开发出更多高效的 CPP，并通过与特定细胞表面受体联用，逐步解决 CPP 介导的生物大分子入胞的靶向性问题，从而进一步推动 CPP 介导的生物大分子药物在医学研究与疾病治疗中的应用。

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The Progress on The Mechanism of Cell Penetrating Peptides Mediated-Cellular Delivery of Biomolecules

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Abstract Compared to traditional small molecular drugs, biomacromolecular drugs have high specificity, and become one of the most promising areas in drug development in the 21st century. However, the natural barrier of cell membranes has prevented many potential intracellular drug targets from drug development. Cell-penetrating peptides (CPP) are a class of short peptides with membrane-permeating functions, could efficiently carry biomacromolecules such as nucleic acids and proteins into the cytoplasm through cell membranes and perform their functions. CPP have many advantages such as high efficiency and low toxicity on the transportation of biomacromolecular drugs. The mechanism of CPP mediated-cellular delivery of cargo can be divided into direct entry and endocytosis depending on whether energy is dependent. Direct entry could be divided into four models according to the way of pore formation: barrel model, toroid model, carpet model and inverted micelle model. endocytosis could be divided into micropinocytosis, clathrin-mediated endocytosis, caveolin-mediated endocytosis, heparan sulfate proteoglycans-mediated endocytosis, neuropilin-1-mediated endocytosis. The type, concentration of CPP, physicochemical properties and molecular size of cargo affected the process of CPP entry, and then determine its mechanism. To summarize the mechanism of CPP-mediated biomacromolecular entry, which provides a basis for the study of more efficient and targeted CPP and promote its application in biology and medicine research.

Key words Cell-penetrating peptide Biomacromolecule Cell-entry mechanism Direct penetration Endocytosis

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