

纤毛虫与藻类共生关系的研究现状与展望

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周庆兰, 饶小珍, 邱大俊. 纤毛虫与藻类共生关系的研究现状与展望[J]. 微生物学通报, 2023, 50(6): 2753-2764.

ZHOU Qinglan, RAO Xiaozhen, QIU Dajun. The study of symbiosis and interactions between ciliates and algae: current status and future directions[J]. Microbiology China, 2023, 50(6): 2753-2764.

摘要: 纤毛虫与藻类的共生关系在水体环境中广泛存在并有着重要的生态功能。文章回顾了国内外纤毛虫与藻类共生研究的发展历程, 主要介绍了纤毛虫与藻类共生的生态功能, 以及显微观察与分子生物学技术在纤毛虫与藻类共生研究中的应用; 阐述了包括草履虫与小球藻共生关系建立的 4 个过程及其互作机制、红色中缢虫与隐藻的共生关系、宿主与共生体之间的互作等内容; 提出了纤毛虫与藻类共生研究中亟待解决的科学问题, 包括草履虫食物泡膜(digestive vacuole, DV)与围藻膜(perialgal vacuole, PV)发挥作用的分子机制、红色中缢虫与隐藻共生关系的建立过程、红色中缢虫在共生过程中的功能作用等, 并展望未来的研究方向。

关键词: 共生机制; 绿草履虫; 小球藻; 红色中缢虫; 隐藻

The study of symbiosis and interactions between ciliates and algae: current status and future directions

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Abstract: Ubiquitous ciliate-algae symbiosis plays a key role in the water environment. In this review, we summarized the current knowledge on ecological functions of ciliate-algae symbiosis. Moreover, the steps of symbiosis establishment and the interactions between *Paramecium bursaria* and *Chlorella* sp., the symbiotic relationship and interaction between *Mesodinium rubrum* and cryptophytes were summarized. Furthermore, several key biological

资助项目: 国家自然科学基金面上项目(42276165, 41776154)

This work was supported by the National Natural Science Foundation of China (42276165, 41776154).

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Received: 2022-09-19; Accepted: 2022-11-26; Published online: 2023-01-11

questions on ciliate-algae symbiosis were raised, including the molecular mechanisms of the functions of *Paramecium* digestive vacuole (DV) and perialgal vacuole (PV) membrane, the establishment process of the symbiosis between *M. rubrum* and cryptophytes, and the roles of *M. rubrum* in the symbiotic process. Finally, we prospected the future directions on ciliate-algae symbiosis study.

Keywords: symbiosis mechanism; *Paramecium bursaria*; *Chlorella* sp.; *Mesodinium rubrum*; cryptophyte

1 共生的概念及其多样性

共生(symbiosis)一词源自希腊语 sym (在一起)和 biosis (生活), 1879年由德国植物学家 Bary 研究地衣时首次提出并定义其概念: 不同种类的生物在一起生活^[1]。由于共生关系的复杂性, 出现了概念上的混乱与争议; 1960–1990年, 共生的原始含义曾被狭义共生取代, 即共生为互利共生。但目前普遍认同广义的共生包含互利共生(mutualism)和偏利共生(commensalism)^[2]。

自然界中生物体之间的共生关系无处不在, 从细胞、个体至种群, 从陆地到水域生态系统, 如豆科植物与根瘤菌共生^[3]、海洋热带珊瑚礁中腔肠动物与虫黄藻共生^[4]、人类与体内的微生物群落共生^[5]等。在海洋生态系统中, 藻类贡献了约 50% 的全球初级生产力, 同时与多种生物之间营互利共生生活。藻共生体与宿主的共生类型十分多样, 包括蓝藻与定鞭藻共生、固氮菌与硅藻共生、隐藻与纤毛虫共生、虫黄藻与热带珊瑚共生等^[6–11]。已报道的共生藻类有小球藻、蓝藻、棕囊藻和隐藻等; 海洋生物中的宿主有纤毛虫、海葵、巨蛤、海绵和热带珊瑚等^[12–16]。例如, 底栖纤毛虫(*Euplotes uncinatus*)与珊瑚礁中的甲藻内共生^[17]; 三亚湾鹿角珊瑚体内发现一种纤毛虫吞食虫黄藻, 虫黄藻在纤毛虫体内进行临时性内共生^[18]。共生互作很少为专性共生, 多为非专一性共生, 例如, 温带海葵(*Anthopleura xanthogrammica*)可同时与虫黄藻和绿藻共生^[19], 蛞蝓中缢虫

(*Mesodinium chamaeleon*)可与 6 种隐藻共生^[20–22]。

宿主不仅为共生体藻类提供相对安全稳定的生长环境, 还为其提供 CO₂、无机盐和有机物等营养物质, 共生藻类则为宿主提供光合作用产物^[23]。大多数进行光合共生的原生动物的兼性营养, 它们既能利用共生体进行光合作用合成有机物, 又能通过摄食细菌或其他微生物来满足其生长和繁殖^[24], 这样的生活方式提高了纤毛虫等原生动物对环境的适应能力^[25–26]。纤毛虫等原生动物通过光合共生结合异养的营养方式缩短了传统的微生物食物网, 在海洋生态系统中对初级生产力有巨大贡献; 尤其在寡营养海域中, 混合营养型生物可以构成总生物量和生产力的大部分^[25,27]。广泛分布的光合共生生物对海洋生态系统的初级生产力与碳固定都做出了重要贡献^[28–29]。本文对藻类与纤毛虫的共生研究进行回顾、总结和展望, 主要针对草履虫与小球藻、红色中缢虫与隐藻的共生关系研究, 并提出纤毛虫与藻类共生研究中亟待解决科学问题, 以期为后续的相关研究工作提供参考。

2 纤毛虫与藻类共生的生态功能

纤毛虫是原生动物中分化最复杂的一个类群, 其作为超微型与小型浮游生物之间的连接, 是海洋微食物网物质循环和能量流动的重要一环, 对海洋生态系统初级生产力有重要贡献^[30–31]。纤毛虫与藻类共生现象广泛存在于各种生态系统, 包括河口^[6]与海洋中沿岸海域、底栖生物栖息

地和贫营养海区^[32-34]。在淡水生态系统中, 绿草履虫(*Paramecium bursaria*)常与绿藻(如 *Chlorella variabilis*)营共生生活^[35], 四膜虫(*Tetrahymena utriculariae*)与微星藻(*Micractinium* sp.)也存在共生关系^[36]; 在海洋生态系统中, 纤毛虫宿主常与蓝藻或者真核微藻(如隐藻)形成共生关系^[37]。

纤毛虫与藻类建立的共生关系使两者均受益。一方面, 极大地提高了宿主对环境的适应性^[38], 有利于纤毛虫在食物匮乏、氧气稀缺和强光照的环境条件下生存, 不仅能延长饥饿条件下宿主的存活时间^[39-40], 还能提高其在厌氧环境中的适应能力^[41]。如草履虫体内共生藻可向宿主提供的碳源量最高可达 85%, 在饥饿时草履虫还能通过消化体内的小球藻生存更长时间^[42]; 在强紫外线环境中, 共生藻可为宿主纤毛虫的大分子比如 DNA 提供遮蔽^[43]; 另一方面, 纤毛虫则为共生藻类提供较安全稳定的环境, 其出色的运动能力可使体内共生藻细胞在合适的光照下进行光合作用^[44]; 在草履虫体内生活的共生小球藻还可以避免遭受小球藻病毒的侵害^[45]。

纤毛虫与藻类共生群体产生的藻华与环境关系十分密切, 纤毛虫纲中缢虫属的红色中缢虫(*Mesodinium rubrum*)常在河口、峡湾和沿海上升流区形成大面积的赤潮^[46]。如红色中缢虫暴发赤潮时, 隐藻(*Teleaulax amphioxeia*)在红色中缢虫体内进行共生生活(图 1)^[47-48], 红色中缢虫引发的赤潮造成巨大的经济损失和严重的环境问题, 但其赤潮暴发机理仍然不明^[49]。因此, 红色中缢虫与隐藻共生研究一直是海洋生态学研究热点之一。

3 纤毛虫与藻类共生机制的研究

3.1 显微观察确定纤毛虫与藻类的共生

显微观察技术伴随着共生研究的不同历史

阶段推动着共生研究不断地向前发展。20 世纪 60 年代, 光学显微镜与电子显微镜的应用有助于对纤毛虫与共生藻及其细胞结构的观察, 促进了对纤毛虫与藻类共生的研究(表 1)。

光学显微镜出现后, 应用到纤毛虫与藻共生体系的研究中^[51-52]。通过显微观察确定绿草履虫可与小球藻属的不同种类形成共生关系^[55-56], 研究观察到一个绿草履虫细胞内共生着数百个完整的小球藻细胞, 而且在分裂过程中, 藻共生体可从亲本细胞直接传递到子细胞并分隔于各个子细胞中^[50]。

Powers^[65]使用光学显微镜观察赤潮水样中红色中缢虫的细胞结构。Taylor 等首次用电子显微镜对红色中缢虫进行超微结构观察, 确定了其细胞内保留着吞食藻类的叶绿体和线粒体等细胞器^[53,66]。1973 年, Blackbourn 等对温哥华附近海域的纤毛虫样品进行切片和显微观察, 发现其细胞质中含有完整的藻叶绿体, 首次提出纤毛虫可能保留了吞噬藻类叶绿体的观点^[67]。1988 年, Stoecker 等通过显微镜观察了 4 种纤毛虫, 观察到纤毛虫体内存在完整的藻共生体或藻叶绿体^[68], 提出纤毛虫可能利用藻类叶绿体的观点。1990 年通过荧光显微镜观察实验室培养的急游虫(*Strombidium capitatum*), 发现纤毛虫体内有 3 种藻类叶绿体即隐藻(*Pyrenomonas salina*)、等鞭金藻(*Isochrysis galbana*)和塔胞藻(*Pyramimonas* sp.)的存在, 并能通过不断吞噬藻细胞来获得新的叶绿体; 发现共生藻叶绿体缺乏时, 纤毛虫的增殖会受到影响^[69]。2000 年, Gustafson 等在实验室以隐藻(*Geminigera cryophila*)喂养红色中缢虫, 通过流式细胞术和荧光显微镜检测其细胞内的叶绿素含量变化, 结果表明红色中缢虫体内叶绿素 a 的含量增加; 他们推测共生体隐藻的叶绿体等细胞器在宿主红色中缢虫体内停

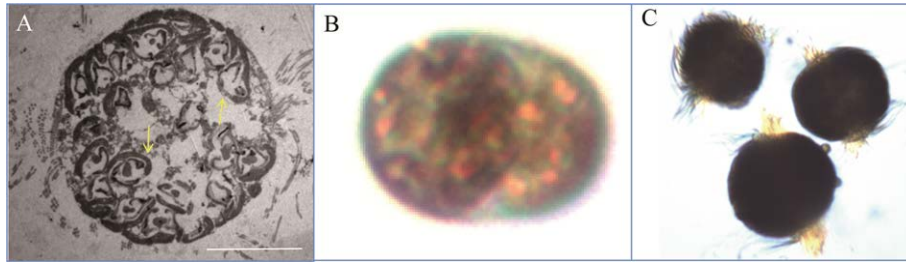


图 1 红色中缢虫与隐藻显微图 A: 红色中缢虫及其体内共生隐藻细胞亚显微结构图, 隐藻细胞(黄色箭头), 标尺=10 μm . B: 红色中缢虫光学显微图(未固定) (10 \times). C: 红色中缢虫光学显微图(鲁格氏固定) (20 \times)

Figure 1 Micrographs of *Mesodinium rubrum* and *Teleaulax amphioxeia*. A: TEM image showing *Teleaulax amphioxeia* cells within *Mesodinium rubrum*. Yellow arrowheads indicate *Teleaulax amphioxeia* cells, Scale bar=10 μm . B: Light microscopic image of a free living *Mesodinium rubrum* cell (10 \times). C: Light microscopic image of fixed *Mesodinium rubrum* cells by lugol's solution (20 \times).

留, 红色中缢虫可周期性摄食隐藻并利用其叶绿体进行光合作用, 从而提出了“红色中缢虫窃取隐藻细胞器”的观点^[63]。该研究确定了红色中缢虫细胞内叶绿体的存在, 但无直接证据表明只有叶绿体被保留。最近, 通过透射电镜观察到了红色中缢虫内共生隐藻具有完整的细胞结构(图 1A), 包括细胞膜、细胞核和叶绿体等^[54]。

由于样品采集仅是纤毛虫与藻类共生状态的一瞬间, 不同共生阶段纤毛虫与其共生体藻细胞结构的观察结果存在差异, 一些研究只观察到共生藻的叶绿体和线粒体等细胞结构^[70-71], 一些研究观察到纤毛虫细胞质中存在着完整藻细胞^[72-73]。同时, 藻共生体的细胞状态相较于自由生活状态下有巨大差异, 这些差异可能是隐藻在纤毛虫体内营共生生活的适应性变化。

草履虫易于采集与培养且便于显微观察, 用草履虫与小球藻作为研究对象在共生机制方面获得了较多成果; 而红色中缢虫纤毛过多且隐藻的细胞很小, 通过光学显微镜较难直接观察其细胞质内隐藻细胞结构的变化(图 1B、1C), 使用电子显微镜观察切片均为固定的样品, 其结果存在着一定局限性。因此, 红色中缢虫与

隐藻共生关系的建立过程还需更加细致与深入的研究。

3.2 纤毛虫与藻类共生关系的建立过程及其调控

对于纤毛虫与藻类共生关系的研究, 绿草履虫与小球藻共生关系建立过程的研究已经比较深入。

Karakashian 等^[74-75]利用电子显微镜和钙钛矿荧光标记, 观察到绿草履虫的次级溶酶体与食物液泡融合, 但不与含有共生藻类的液泡融合, 提出共生藻会改变其周围液泡的膜从而抑制与次生溶酶体的融合; Reisser 等^[59]研究最早发现, 绿草履虫对小球藻的识别吞噬可能是通过细胞表面的糖结合物介导, 通过荧光显微镜分别观察染色后自由生活的小球藻与绿草履虫中共生小球藻的细胞壁成分, 研究结果表明: 自由生活的小球藻细胞壁中包含葡萄糖-甘露糖成分, 共生小球藻细胞壁则不含该成分。后续的相关研究表明小球藻细胞壁中的葡糖胺可能是与绿草履虫建立共生关系的决定因素^[60]。Wootton 等^[76]对浮游鞭毛虫和海洋纤毛虫摄食的研究结果进一步证实了这一推论, 该实验鉴定了用于识别与吞噬的 c 型凝集素。

表 1 纤毛虫与藻类共生研究的历程

Table 1 The studies of ciliates-algae symbiosis: history, methods, and knowledge

研究内容	绿草履虫与小球藻共生体	红色中缢虫与隐藻共生体	参考文献
Contents	<i>Paramecium bursaria</i> and endosymbiotic <i>Chlorella</i> sp.	<i>Mesodinium rubrum</i> and endosymbiotic cryptophyte	Reference
显微观察	实验室培养观察较易	实验室培养较难	[50]
Microscopy	Easy to cultivate in the laboratory	Difficult to cultivate in the laboratory	
Observation	确定绿草履虫与完整小球藻细胞内共生	红色中缢虫体内观察到完整隐藻细胞内共生或细胞器等结构	[51-52] [53-54]
	Intact endosymbiotic <i>Chlorella</i> sp. cells within <i>Paramecium bursaria</i> were observed	Intact endosymbiotic <i>Teleaulax amphioxeia</i> cells and its organelles were observed	
	绿草履虫可与多种绿藻共生	红色中缢虫可与多种隐藻共生	[55-56] [57-58]
	Several <i>Chlorella</i> sp. species symbiosis with <i>Paramecium bursaria</i> , respectively	Several cryptophyte species symbiosis with <i>Mesodinium rubrum</i> , respectively	
共生机制	绿草履虫对小球藻的识别可能通过糖结合物介导; 发现小球藻与绿草履虫建立共生关系的 4 个阶段	红色中缢虫与隐藻共生机制建立过程暂不清楚	[59-60] [61-62]
Symbiosis mechanism	The establishment of symbiotic relationship between <i>Chlorella</i> sp. could be recognized by <i>Paramecium bursaria</i> by glycoconjugates. Four steps of symbiosis establishment between host <i>Paramecium bursaria</i> and endosymbiotic <i>Chlorella</i> sp. were described	The establishment of symbiotic relationship between <i>Mesodinium rubrum</i> and cryptophytes is unclear	
纤毛虫与藻共生体的互动	绿草履虫与小球藻建立共生关系后某些基因表达发生变化	共生隐藻蛋白质丰度和基因表达均产生变化; 红色中缢虫窃取隐藻核与叶绿体	[35,63] [57-58]
The interaction between host and symbiont	The expression of several genes was changed after the establishment of the <i>Paramecium-Chlorella</i> symbiosis	Differential cryptophytes protein abundance and gene expression were observed during the ingestion by <i>Mesodinium rubrum</i> . <i>Mesodinium rubrum</i> ingest cryptophytes and steal their organelles	
	绿草履虫不同品系的大核基因组测序分析, 在蛋白家族和基因谱系中发现了几个可能与内共生相关的特异性基因	红色中缢虫体内发现完整共生隐藻细胞结构且功能活跃, 提出了“红色中缢虫培育隐藻”的共生新模式	[54,64]
	Several specific genes in protein families and lineages were identified, which were potentially associated with endosymbiosis in different strains of <i>Paramecium bursaria</i>	Endosymbiotic <i>Teleaulax amphioxeia</i> cells were intact and active. “ <i>Mesodinium</i> -farming- <i>Teleaulax</i> ” relationship was posited	

Kodama 等^[61]对绿草履虫与小球藻共生系统进行了一系列显微观察, 为两者共生关系的建立提供直接证据: 他们首先从绿草履虫内分离出共生的藻细胞, 再将其与无藻的绿草履虫进行共同培养, 并对藻细胞进行脉冲标记和追踪, 结合染色技术, 观察到二者共生关系建立过程中草履虫内部生理变化与小球藻的动态迁移, 提出了绿草履虫与小球藻共生关系的建立过程可分为 4 个阶段(图 2): (1) 小球藻通过胞口进入绿草履虫

胞质内形成食物泡(digestive vacuole, DV); (2) 绿草履虫溶酶体与食物泡结合, 部分藻细胞获得了对溶酶体酶一段时间的抗性; (3) 食物泡内的小球藻随着 DV 膜出芽进入胞质, 包裹小球藻的 DV 膜分化为围藻膜(perialgal vacuole, PV), 围藻膜可阻断宿主溶酶体融合从而为共生藻提供保护; (4) 小球藻通过 PV 膜定位于绿草履虫细胞皮层下方, 两者建立内共生关系。最新的研究通过荧光标记颗粒探索草履虫的 DV 膜与 PV

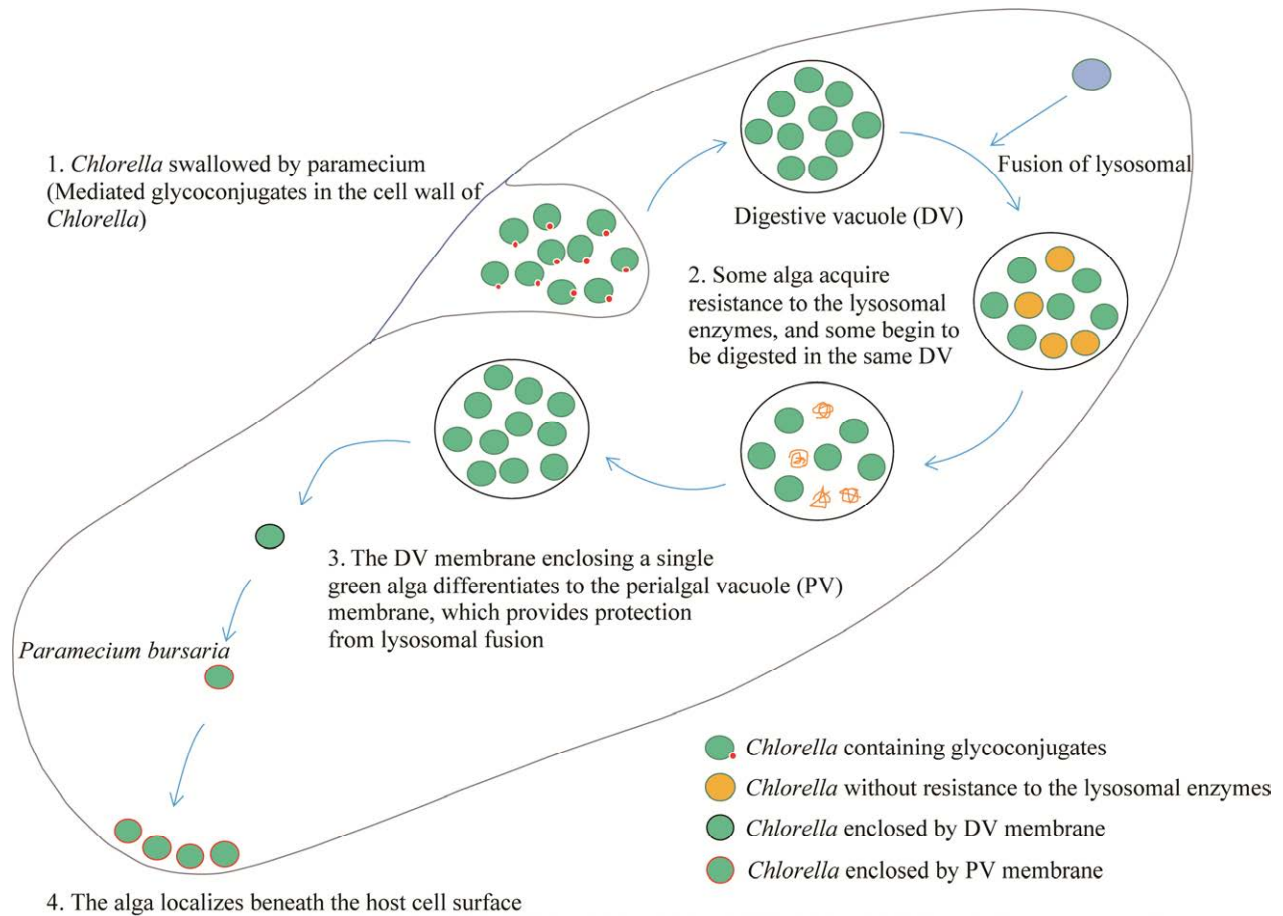


图2 绿草履虫与小球藻共生机制的建立过程

Figure 2 Establishment process of symbiotic mechanism between *Paramecium bursaria* and *Chlorella* sp..

膜对小球藻的接触与识别机制^[62]。近年来，有研究通过透射电镜和3D结构重建分析，发现绿草履虫宿主的线粒体与PV膜和共生体小球藻之间有明显的结构联系，70%以上的线粒体与PV膜直接接触^[77]，而且含共生藻的绿草履虫细胞内线粒体数量显著减少，宿主线粒体的变化与共生藻之间的关联可能是两者共生关系中重要的组成部分^[78]。

受环境和细胞大小等因素的影响，不同的纤毛虫宿主内所含共生藻的数量不同，一个大型海洋喇叭虫(*Maristentor dinoferus*)的细胞质内含有500–800个共生甲藻细胞^[79]，一个绿色的喙纤虫

(*Loxodida karyorelictea*)细胞内仅含有1–20个绿藻细胞^[80]。在一定的光照和营养条件下，纤毛虫宿主细胞中共生藻类会维持在一个稳定的丰度^[81]。宿主细胞内的藻细胞数量大量增加可能会导致光合作用衍生的活性氧(reactive oxygen species, ROS)积累，而活性氧含量过高会影响宿主细胞，可能导致共生体藻类被排出宿主细胞^[82]。纤毛虫内共生藻的数量进行一定的调节以适应多变的环境，如纤毛虫液泡的pH对共生藻数量调节具有明显的影响^[32]，在酸性pH条件下，小球藻细胞能合成和释放更多的碳水化合物，同时也降低了对铵的吸收，从而减缓藻细胞

的分裂^[83-85]。纤毛虫对共生藻数量的调控机制还需要进一步的研究来确定和量化。

相比绿草履虫与小球藻共生关系的研究,有关红色中缢虫与隐藻共生关系建立过程的研究十分稀少。已有的研究包括通过荧光原位杂交技术,观察到红色中缢虫每次摄取隐藻后其体内隐藻细胞核可保持数周的转录活性,当隐藻核消失后,与隐藻细胞器相关功能的基因表达下降^[86]。有研究认为红色中缢虫需不断摄取隐藻窃取细胞核,以此来控制隐藻叶绿体的功能与分裂,提出红色中缢虫可能处于永久获取叶绿体的过渡阶段^[87]。但近 10 年转录组数据的分析结果并不支持该理论^[65]。

3.3 分子生物学技术在纤毛虫与藻类共生机制研究中的应用

过去 20 年,通过分子生物学技术对纤毛虫与藻类的共生机制开展了一系列的研究(表 1),使纤毛虫与藻类间的共生关系研究进入到分子水平。利用 28S rDNA 作为标记物观察纤毛虫体内共生体的替换^[88];通过转录组分析技术对有共生藻和无共生藻的草履虫宿主进行深度比较分析,可确定二者差异表达的基因^[35];Hoshina 等利用藻类靶向 PCR 技术获得了 4 种纤毛虫体内的共生藻 rDNA 片段序列,发现同一湖泊内不同时间采集的不同种类纤毛虫体内的藻类序列差异极小(<1%)^[89]。同时,对不同品系的绿草履虫的大核基因组进行了测序和注释,其蛋白质和基因谱系中发现了几个可能与内共生相关的物种特异性基因^[64]。这表明内共生可能是真核生物的一种协同进化策略^[7]。

2015 年, Lasek-Nesselquist 等对自由生活状态下的隐藻(*Geminigera cryophila*)与红色中缢虫体内共生隐藻的基因表达进行了比较分析,结

果显示共生隐藻许多功能基因的表达出现减少^[57]。来自韩国的研究团队在 2016 年通过焦磷酸测序方法分析了红色中缢虫和隐藻(*Teleaulax amphioxeia*)的基因表达,并对宿主红色中缢虫进行了时间序列基因芯片分析,研究结果表明红色中缢虫内的隐藻核保留了 13 周以上的转录活性,可大量表达隐藻基因并存在大量的表观遗传修饰^[58]。目前大量研究证明红色中缢虫与隐藻共生会影响二者的基因表达,但仍无直接证据证实红色中缢虫能窃取利用隐藻的细胞器。近年来,在赤潮样品中观察到红色中缢虫细胞内的隐藻具有完整的细胞结构,包括细胞膜、细胞核和叶绿体等细胞器^[65]。有研究通过宏转录组数据甄别出共生隐藻表达的基因,描绘出红色中缢虫内隐藻较完整的代谢通路图,揭示了共生隐藻的光合作用、代谢通路、物质输送和细胞增殖等分子机制,确定宿主细胞内共生着细胞结构完整及功能活跃的隐藻,提出了“红色中缢虫培育隐藻”的共生新模式,即宿主从环境中运输无机物等营养物质供共生藻细胞增殖,共生藻的光合作用产物为宿主所用^[65,90]。大量研究表明红色中缢虫与隐藻的确存在着共生关系,但二者共生关系的建立过程仍有待进一步深入的研究。

4 总结与展望

纤毛虫与藻类的共生体系在海洋生态系统中有重要的生态学意义,对藻类共生的研究有助于对真核生物进化机制的认识。目前国内外的研究已经对两者的共生机制有了一定的认知,但仍有很多科学问题需要进一步探究。结合目前国外学者研究的热点,并针对在纤毛虫与藻类共生机制研究上的不足,我们对未来需开展的工作进行了展望(图 3)。

(1) 加强草履虫与小球藻基因组水平以及转录组水平上的研究

草履虫与小球藻之间稳定的内共生系统为共生研究提供了很好的模式种, 以往的研究大多基于形态和理化指标水平上的研究, 还有许多问题需要通过分子水平上进一步研究进行解释。比如: 影响草履虫与共生小球藻生长速率的机制, 草履虫细胞器在共生过程中发挥的作用与其机制, DV膜和PV膜在共生过程中变化的分子机制, 内共生的建立过程及其维持机制, 以及共生互动与协同进化机制等(图3)。

(2) 红色中缢虫在高氮氮养殖环境与水环境处理中的应用前景有待进一步深入研究

红色中缢虫具有较强的氮吸收能力^[54]。加强红色中缢虫与隐藻共生机制的研究, 可为

红色中缢虫引发的赤潮的预测与防控提供理论依据。由于红色中缢虫与隐藻共生关系的建立过程暂不清楚, 需要进一步研究解答红色中缢虫与隐藻是暂时性内共生还是长期内共生等问题。包括: 红色中缢虫如何识别隐藻, 宿主红色中缢虫在共生建立过程中发挥了什么作用, 以及红色中缢虫与隐藻共生可维持多长时间(图3)。应采用共聚焦显微镜观察、三维重建等方法, 结合多组学技术深入探讨, 进一步确定红色中缢虫与隐藻的共生模式与互作机制。

这些问题都需要在下一步的研究中加以关注, 同时结合基因组和转录组分析、共聚焦显微镜观察等研究方法, 进一步深入开展纤毛虫与藻类共生关系的研究。

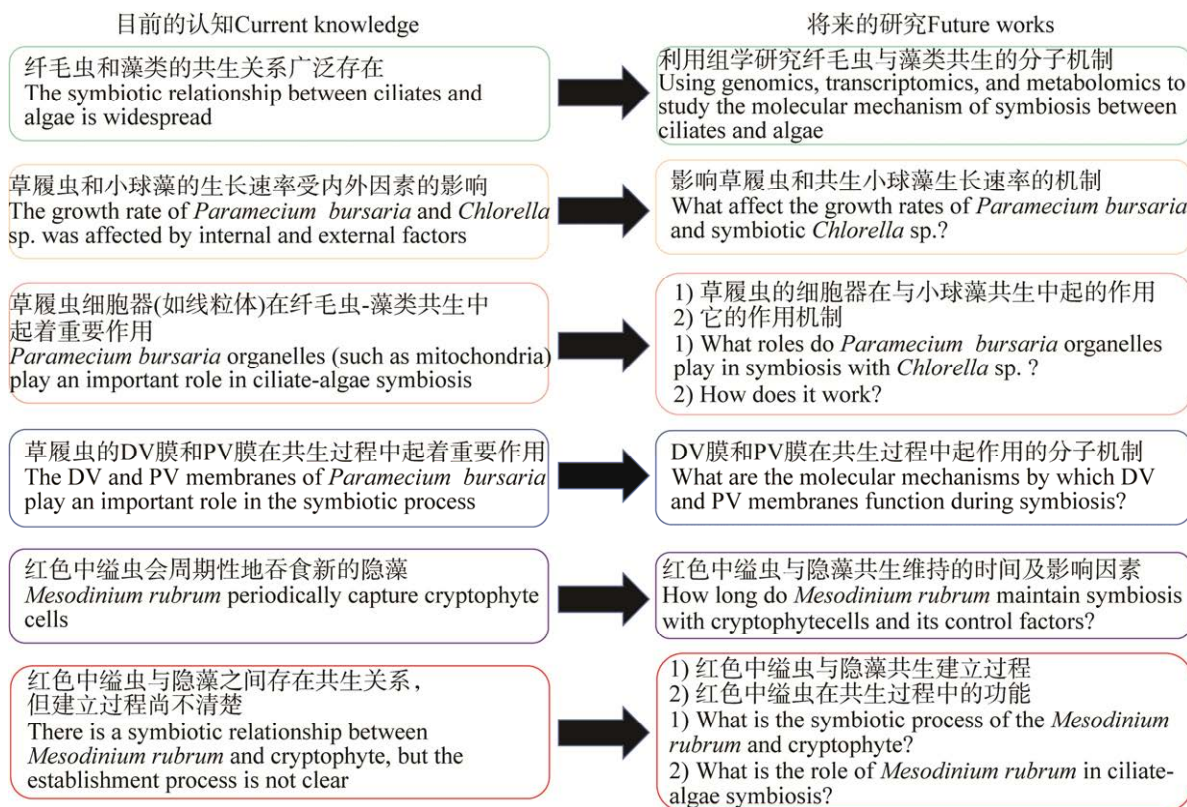


图3 当前取得的关键性研究成果与将来的研究方向

Figure 3 Current status of research achievements in key areas and future research directions.

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