

蓝藻伪空胞的特性及浮力调节机制

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摘要: 伪空胞为蓝藻在水体中提供浮力, 使其获得适宜的生长条件, 最终导致蓝藻水华暴发, 了解伪空胞的特征对控制蓝藻水华暴发有重要意义。文章简要回顾了蓝藻伪空胞自 1865 年被 Klebahn 发现到 1965 年被正式命名的研究历程, 目前已发现 150 多种原核生物中含有伪空胞; 伪空胞是两末端呈圆锥状的中空圆柱体, 伪空胞半径与临界压强遵循方程: $P_c = 275 (r/\text{nm})^{-1.67}$ MPa; 伪空胞气体含量可根据不同原理, 利用 Walsby 伪空胞测定装置、压力浊度计和细胞流式仪测得。总结了伪空胞组成的化学特性, 评述了伪空胞 *gvp* 基因丛结构功能和 GvpA、GvpC 的蛋白空间结构。GvpA 是伪空胞合成的主要成分, *gvpA* 在伪空胞内存在多个拷贝, 其功能仍不清楚; GvpC 由 33 个氨基酸重复单位组成, 重复单位越多, 伪空胞越不易破裂; 概述了伪空胞 3 种浮力调节机制: 镇重物的改变、伪空胞的合成、伪空胞的破裂; 归纳了环境因子(光照、温度、氮、磷、钾)参与伪空胞浮力网络调控的途径。提出了目前伪空胞研究面临的困难和问题, 对伪空胞的未来研究方向提出探索性的建议。

关键词: 伪空胞; 理化特性; 遗传特性; 网络调控; 浮力调节

The characteristics and buoyancy regulations of cyanobacterial gas vesicles

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Abstract: Gas vesicles are important factor for the occurrence of cyanobacterial water-bloom, which provide varying buoyancy for cyanobacteria to obtain favorable growth conditions. Therefore, it is important to understand the characteristics of gas vesicle for cyanobacteria water-bloom control. In this article, we introduced the research history of gas vesicle, and then conducted a systematic review about gas vesicle morphology, the relationship between the cylinder radius and critical pressure, the measurement approach of gas vesicle volume and its chemical features. Gas vesicle was discovered by Klebahn in 1895 and this name was officially accepted in 1965. So far, gas vesicles have been found in more than 150 prokaryotes. Gas vesicle shape is described as a cylinder with two cones on two ends and its cylinder radius is closely related to critical pressure following the equation: $P_c = 275 (r/\text{nm})^{-1.67}$ MPa. Gas vesicle volume could be measured by capillary apparatus, pressure nephelometry and flow cytometry. Furthermore, we reviewed the genetic features of gas vesicle including *gvp* gene cluster structure, the function and the spatial structure of GvpA and GvpC. Until now, 14 *gvp* genes (*gvpA*, *gvpC*, *gvpN*, *gvpO*, *gvpD*, *gvpE*, *gvpF*, *gvpG*, *gvpH*, *gvpI*, *gvpJ*, *gvpK*, *gvpL*, *gvpM*,) have been identified in *gvp* gene cluster, but it is still unclear that which one is imperative during the process of gas vesicle synthesis. In this paper, we summarized the function of all the 14 Gvp proteins, especially, *gvpA* copy number and the 33 amino acid repeat number of GvpC. GvpA is present in the gas vesicles of most cyanobacterium, but its copy number is different in various taxonomic groups. GvpC is mainly composed of 33 amino acid repeat sequences, and the increased number of which could strengthen the structure of cyanobacterial gas vesicles against collapse. The spatial structure of GvpA and GvpC protein were also described in this paper. In addition, three buoyancy regulation mechanisms and the ways of environmental factors involving in buoyancy network regulation were summarized. Gas vesicles could adjust buoyancy through change in cell ballasts, production and dilution of gas vesicles and irreversible collapse of gas vesicles. Environmental factors, such as light and nutrients are

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important for gas vesicles synthesis. Nitrogen and phosphorus limitation lead to gas vesicle content decrease. Continuous carbon limitation could restrain gas vesicle synthesis due to energy shortage. Additionally, we addressed the current difficulties and problems in gas vesicle researches, such as the uncertainty of whether gas vesicles exclusively determine cyanobacterial buoyancy and the difficulties to answer ecological questions based on the results of molecular biology of gas vesicles. Eventually, we proposed the future research directions in the physiological ecology and molecular biology of gas vesicles, e. g. the relative contribution of gas vesicles to regulate cyanobacterial buoyancy compared with that of gap junction, the influence of environmental factors on gas vesicles, the genetic features of gas vesicles, as well as the function and the spatial structure of individual *gvp* gene.

Key Words: gas vesicle; physic-chemical characteristics; genetic characteristics; network regulation

我国是世界上湖泊富营养化最严重的国家之一。湖泊富营养化导致浮游藻类过度生长形成水华,从而加剧水质的恶化。在形成蓝藻水华的四个阶段,即休眠、复苏、生物量增加(生长)、上浮及聚集过程中,蓝藻通过伪空胞调节自身浮力进行垂直迁移,以获取适宜的光能,得到充足的营养盐,使其成为优势藻种^[1]。因此,研究伪空胞的特性对弄清蓝藻水华形成机制有十分重要的意义。蓝藻伪空胞研究自 20 世纪 90 年代后以来进展缓慢,目前可以参考的文献大部分是 20 世纪 90 年代以前的研究成果,近几年的综述大多数就某一方面进行简单阐述,没有对伪空胞的最近研究成果进行系统深入的总结归纳,且其研究综述大部分局限在理化性质、编码基因、蛋白空间结构方面,很少提及环境因子对伪空胞的浮力网络调控和研究瓶颈,更没有对伪空胞的未来研究方向提出相应的对策。本文简要概述了伪空胞的研究历史、理化特性,对伪空胞分子生物学特性进行了详细评述,总结了伪空胞的 3 种浮力调节机制,对环境因子如何影响浮力调节的途径进行归纳,并提出伪空胞研究目前存在的问题和困难,探讨了伪空胞未来研究方向,以期从不同角度研究伪空胞提供参考。

1 伪空胞的研究

1895 德国微生物学家 Klebahn 在利用光学显微镜观察到蓝藻细胞内存有含有气体的气囊^[2],可以为细胞提供浮力。气囊具有明显的折光特征,在光学显微镜下略带红色。1915 年 Lauterborn 在古细菌(嗜盐菌和产甲烷菌)和革兰氏阴性菌中也发现有类似结构的气囊^[3]。1965 年, Bowen 和 Jensen 运用超薄切片技术证实这些气囊由众多圆柱形的气泡叠加而成,于是将这些气泡命名为伪空泡或伪空胞^[4]。但伪空胞与水中简单气泡在结构上又存在差异:首先,伪空胞形状大部分呈圆柱型;其次,伪空胞中气体的压力比同样大小的简单气泡要小;最后,受到一定外部压力时,伪空胞的大小几乎不发生变化。因此, Klebahn 认为伪空胞中的气体可能是被一种有一定强度的膜包裹着的,它不受表面张力的影响,并能承受一定的外部压力^[2]。Walsby 等实验结果也证实,当受到较大的压力时,细胞中的伪空胞结构会消失^[5]。

迄今已在 150 多种的原核生物中发现伪空胞^[6-8],至少涉及了细菌的 5 个门:绿色硫细菌、紫色细菌(变形菌)的 α 和 γ 分支、蓝藻(包括原绿藻)、浮霉菌和革兰氏阳性菌。细菌的其它门中也发现了类似伪空胞的结构,例如:极端嗜盐生物门(盐杆菌属)和产烷生物门(甲烷八叠球菌属)^[9]。

2 伪空胞形态及气体测量方法

大部分伪空胞是两末端呈圆锥状的中空圆柱体(图 1)^[10]。冷冻断裂细胞图片(图 2)^[11]和负染色电镜图片也证实了这样的结构特点^[12]。不同种类的蓝藻中,伪空胞的平均直径不尽相同;而在每一种蓝藻中,伪空胞圆柱体的直径比较统一^[9]。伪空胞圆柱体的直径(d)是通过测量电镜图上坍塌伪空胞的宽度(w)计算得到: $d = 2w/\pi$ ^[12]。但是细胞内伪空胞的长度变化就很大,可以从 100 nm 到 800 nm 甚至更长^[9]。

水中游离的伪空胞受到压力有:上方水柱压强(P_h)、大气压强(P_i)和伪空胞内部气体压强(P_g)。细胞内的伪空胞主要承受 4 种来源的压强,即细胞膨压(P_i)和上方水柱压强(P_h)、大气压强(P_i)和伪空胞内部气体

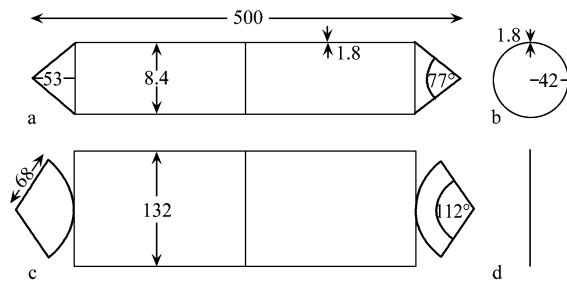


图1 *Anabaena* 伪空泡的几何特征/nm^[10]

Fig.1 Geometry of the *Anabaena* gas vesicle drawn to scale, with all measurements in nanometers^[10]

a:完整伪空泡的纵切面;b:a图的横切面;c:破裂伪空泡的纵切面;d:c图的横切面

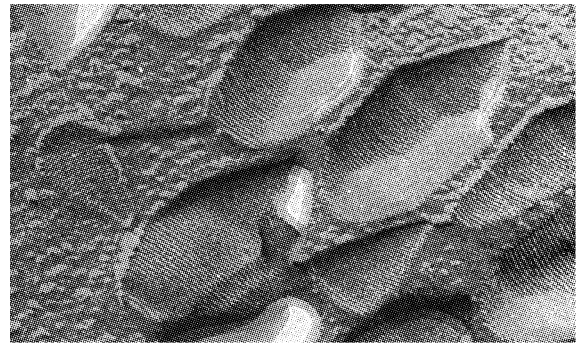


图2 *Prosthecomicrobium pneumaticum* 伪空泡在冷冻细胞的剖面图^[11]

Fig.2 Gas vesicles in a freeze-fractured cell of *Prosthecomicrobium pneumaticum*^[11]

压强(P_g)(图3)^[9],两种状态下,伪空泡能够承受的净压强可用以下公式计算^[9]:

$$P_n = P_h + P_f - P_g \text{ (游离状态)}$$

$$P'_n = P_h + P_t + P_f - P_g \text{ (完整细胞状态)}$$

伪空泡受到较小的压强时,体积变化很小。当压强在 $6 \times 10^5 \text{ Pa}$ 以下时,水华鱼腥藻的伪空泡上受到的压强每增加 10^5 Pa ,它的体积仅改变 $1/650$ ^[13]。但是一旦受到的压力达到某个阈值,伪空泡就会突然坍塌破裂^[14]。能引发坍塌的伪空泡内外压力之差的最小值被称为临界压力(P_c)。对于不同的生物体来说,临界压力有所不同,Hayes 和 Walsby 等人认为这主要是因为伪空泡圆柱体的直径不同,他们比较了从水华鱼腥藻、水华束丝藻、微囊藻、阿氏颤藻、蓝纤维藻属的 *Dactylococcopsis salina* 和一种海洋蓝藻铁氏束毛藻中分离得到的伪空泡的圆柱体平均直径以及它们的临界压力,提出蓝藻伪空泡的临界压力(P_c)与平均半径(r)之间呈反相关的关系(图4)^[15-16]:

$$P_c = 275(r/\text{nm})^{-1.67} \text{ MPa} = 275(d/2\text{nm})^{-1.67} \text{ MPa} \approx 275(d/2\text{nm})^{-5/3} \text{ MPa}$$

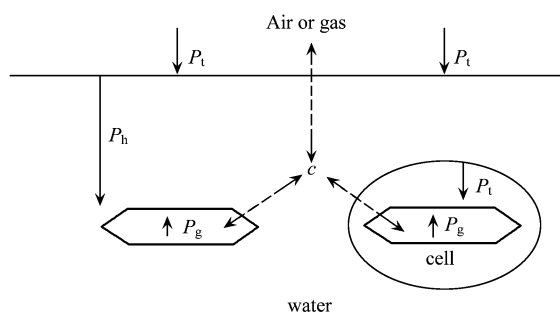


图3 水中伪空泡承受的压力^[9]

Fig.3 Pressures acting on a gas vesicle suspended in water^[9]

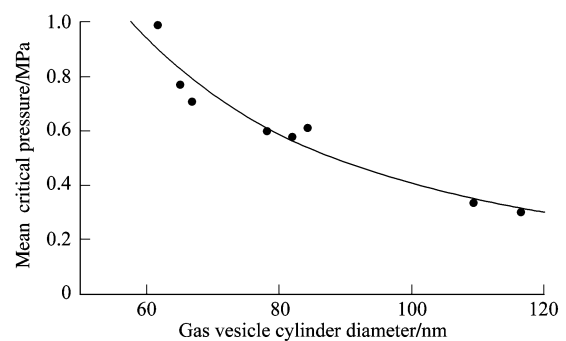


图4 临界压力(P_c)与伪空泡半径(r)的关系^[16]

Fig.4 Relationship between critical pressure (P_c) and cylinder radius (r) of gas vesicles^[16]

而临界压力与伪空泡的长度之间没有发现类似的相关关系,这一结果也与力学的理论相吻合。在细胞内,伪空泡的临界压力还会受其他一些因素的影响,如:温度、pH、盐度、酶等^[17],其中最为重要的是细胞膨压,当膨压升高时伪空泡会发生坍塌^[18-19]。

伪空泡和光存在着相当复杂的关系。完整的伪空泡不吸收可见光,但对光具有强烈的散射作用,在前后

2 个方向散射光最强烈;当伪空胞破裂后,其明显的光学特征就会消失,散射的量可能减少 98% 甚至更多,悬液也随之变澄清^[20]。因此可以利用改进的 Walsby 伪空胞测定装置(图 5)^[21]和压力浊度计(presure nephelometry)构建破裂-压力曲线(图 6)^[22],从而测量细胞中伪空胞的体积、临界压强和相对含量等参数^[13,22-23]。

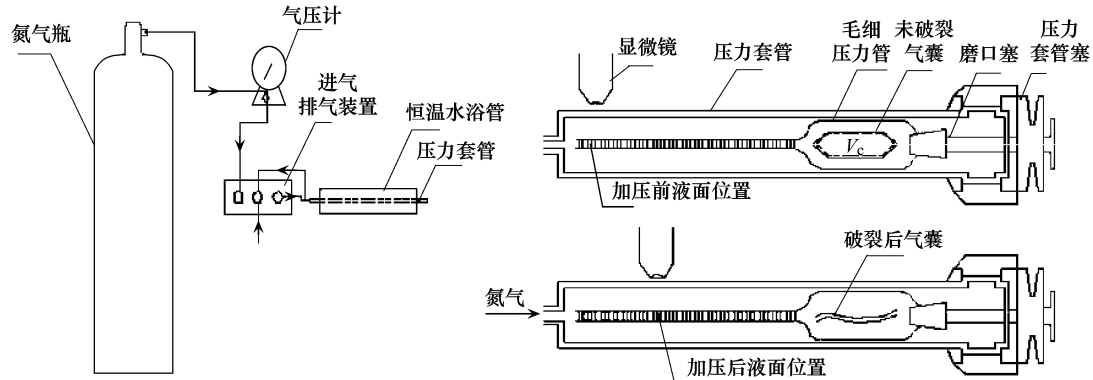


图 5 伪空胞体积测定装置示意^[21]

Fig. 5 Schematic diagram of gas vesicles measurement apparatus^[21]

通过测定加压前后的伪空胞散射光值变化,便可以估算出细胞中伪空胞的相对含量(RGV)和一定压强下破裂伪空胞所占有的比率($C\%$)^[23]:

$$RGV = \Delta T_a / T_c$$

$$\Delta T_a = T - T_c$$

$$C\% = (T - T_b) / (T - T_c) \times 100$$

其中, ΔT_a 为所有伪空胞破裂前后比浊度的变化值; T_b 是加压后部分伪空胞破裂时细胞悬液的比浊度; T_c 为所有伪空胞破裂后细胞内其它物质的比浊度; T 为初始细胞悬液的比浊度。

细胞中伪空胞的体积、临界压强和相对含量等参数还可以利用细胞流式仪和高斯-牛顿方程计算得到^[24]:

$$y\% = (S_p - S_f) / (S_a - S_f) \times 100$$

$$y = M \frac{1}{1 + e^{a-bx}}$$

其中, y 是某一压强下剩余伪空胞的百分含量; S_p 是某一压强下部分伪空胞破裂后悬液的 SSC (side scatter) 值; S_f 是伪空胞全部破裂后悬液的 SSC 值; S_a 是伪空胞全部完整时悬液的 SSC 值; M 是没有加压时的伪空胞百分含量(100%); x 是悬液受到的压强。表观压强 P_a (悬液中还剩余 50% 完整伪空胞时的压强) 和临界压强 P_c (悬液中加入 $0.5 \text{ mol} \cdot \text{L}^{-1}$ 蔗糖后剩余 50% 完整伪空胞时的压强) 可以利用各自的破裂-压力曲线和高斯-牛顿方程计算可得, 即 $y = 50\%$, 则 $x = a/b$ 。细胞膨压 $P_t = P_c - P_a$ 。

对于伪空胞的绝对含量通常是按照 Walsby 等人对纯化的水华鱼腥藻伪空胞的测量值来进行估算的, 1 mg 伪空胞在 500 nm 处的压力敏感衰减值为 20.84 cm^{-1} ^[25]。

3 伪空胞组成成分

研究证明, 蛋白质是嗜盐菌伪空胞的主要组成成分^[8]。对鱼腥藻^[26-27]、微囊藻^[12] 以及异养菌水生微环菌^[28] 研究表明, 蛋白质是伪空胞的唯一成分。利用 ^{32}P 标记技术检测伪空胞中磷酸盐含量, 发现磷酸盐含量

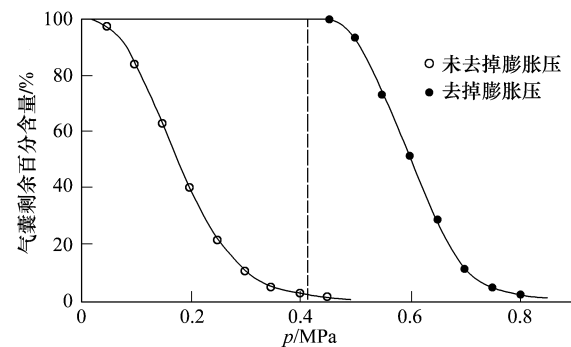


图 6 蓝藻 *Cyanobacteria* 的临界破裂压力分配^[22]

Fig. 6 Critical pressure distribution of gas vesicles in *Cyanobacteria*^[22]

甚少,甚至可以忽略不计^[29],由此可以证明伪空胞与一般的生物膜组分不尽相同。伪空胞在去污剂中很难溶解,但可以在 80% 的甲酸和苯酚-乙酸-水(2:1:1)中部分溶解,对溶解液进行聚丙烯酰胺凝胶电泳,只出现一条带,并在点样孔中留有不溶的物质,将该不溶物质用 90% 的甲酸再次溶解、电泳,出现同样的条带, Jones 和 Jost 认为点样孔和凝胶中物质是处于不同形态的同一种蛋白质,分子量约为 14kDa 左右^[30]。Smith 和 Walker 等人分别对鱼腥藻细胞粗提液进行电泳,同样在点样孔中发现不溶物质,并且凝胶上均显示一条明显的条带,分子量约为 22 kDa^[31-32]。但是,X-射线衍射的研究结果表明晶体结构上的蛋白质可能为 7.5 kDa^[33]。造成这种现象的原因可能是:(1)伪空胞很难溶解,溶解部分以多聚体的状态存在;(2)以前学者认为伪空胞只有一种蛋白组成,即伪空胞蛋白(GVP)。但现在研究结果表明,留在点样孔里的蛋白疏水性强,称为 GvpA,分子量约 7.5 kDa,是伪空胞组成的主要蛋白;而进入凝胶的是另外一种蛋白——GvpC,分子量约为 22 kDa,是伪空胞组成的次要组分。伪空胞组成还包含其他微量结构蛋白,具体参见表 1。

表 1 *H. halobium*, *H. salinarium*, 和 *H. mediterranea* 中 *gvp* 基因编码产物^[34-38]

Table 1 Predicted products of *gvp* genes from *halobium*, *H. salinarium* and *H. mediterranea*^[34-38]

基因产物 Protein	分子量 Approx mol mass (kDa)	氨基酸的数目 No. of amino acids				注释 Comments
		<i>H. halobium</i> 质粒基因 plasmid gene	<i>H. salinarium</i> 质粒基因 plasmidgene	<i>H. salinarium</i> 染色体基因 chromosomal gene	<i>H. mediterranei</i> 染色体基因 chromosomal gene	
GvpA	8.1	75	75	78	77	伪空胞骨架主要组分
GvpC	42.4	382	382	385	381	伪空胞外部蛋白;稳定结构
GvpN	39.2	347	346	345	347	核苷结合位点
GvpO	—	—	120	111	140	
GvpD	59.3	536	536	492	545	核苷结合位点;表达调控
GvpE	21.0	191	191	192	192	
GvpF	24.0	213	214	217	213	
GvpG	10.0	83	83	83	83	
GvpH	19.9	182	182	163	216	
GvpI	16.3	144	140	114	162	伪空胞基本蛋白
GvpJ	12.0	114	114	98	114	与 GvpA 和 GvpM 部分同源
GvpK	12.7	113	113	119	117	
GvpL	32.0	281	281	273	322	
GvpM	9.2	84	84	73	86	与 GvpA 和 GvpJ 部分同源

4 伪空胞的遗传学特性

迄今为止,文献共报道 14 个不同的基因与伪空胞的形成有关(表 1)^[34-38],它们成簇排列,基因组两端分别是两个插入序列 ISH27 和 ISH2,基因组共分成 4 个转录子,转录方向不同(图 7)^[38-40]。但是哪些是伪空胞合成所必需的基因并不清楚^[41-45]。*gvpA*、*gvpF*、*gvpG*、*gvpJ*、*gvpL* 和 *gvpM* 几乎在所有产伪空胞的微生物中出现^[42,46-49],因此推测它们是合成伪空胞所必需的。在这些基因中,*gvpA* 和 *gvpC* 研究最为透彻。

4.1 *gvpA* 基因与 GvpA 蛋白

Walker 等人对水华鱼腥藻的伪空胞进行氨基酸测序,得到一个具有 70 个残基序列^[50-51]。该序列就是 GvpA 蛋白,是伪空胞的主要组分,具有很强的疏水特性^[52]。GvpA 是一个保守性很强的蛋白,在不同蓝藻之间具有很高的相似性(图 8)^[53]。

7—8 个 GvpA 蛋白分子利用肽键连接成 β -链,相邻的 β -链以氢键连接,相距 0.994nm,多条 GvpA β -链以 β -转角的方式连接形成反平行 β -折叠,从而构成 GvpA 骨架(图 9)^[11]。

编码 GvpA 蛋白的基因序列最早是通过 DNA 杂交的方法获得的^[54]。在 *gvpA* 基因的下游,有两个开放阅

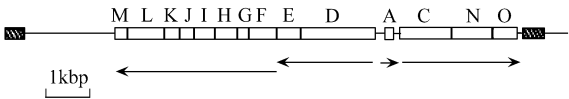
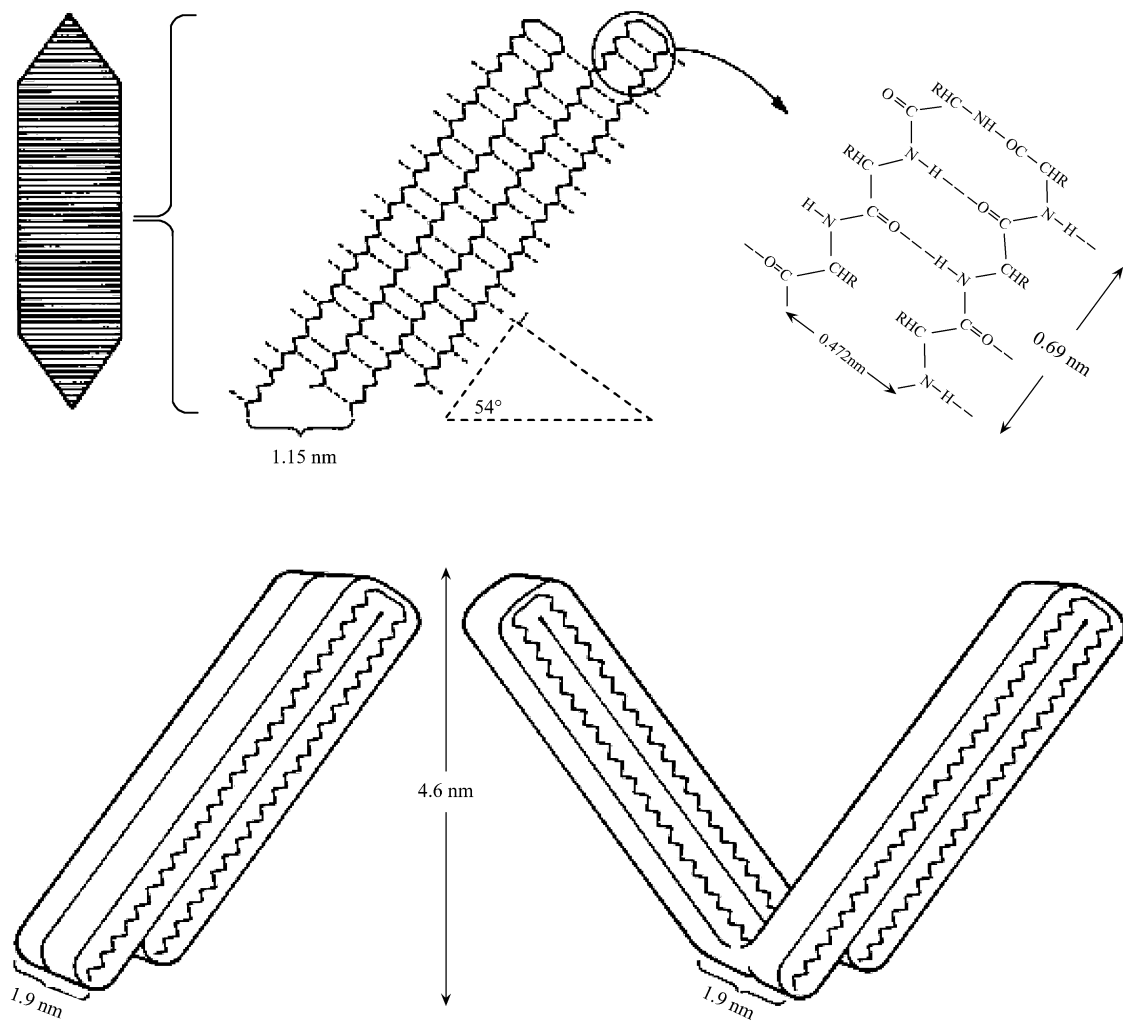


图 7 *gvp* 基因簇结构图^[38]

Fig.7 Organization of the *p-gvp* gene cluster in *Halobacterium halobium*^[38]

图9 GvpA 分子在伪空胞骨架中的排列^[11]Fig. 9 Possible arrangement of GvpA molecules in the rib of the gas vesicle^[11]

宽度 4.57nm 稍宽;GvpA 骨架中每个 GvpA 分子的宽度为 1.15nm,相邻 GvpA 骨架中位置对等的 GvpA 分子连线与 GvpA 骨架呈 86°,且位移 0.72nm;每 33 个氨基酸残基重复序列与 GvpA 骨架(5 个 GvpA 蛋白分子)呈 24°结合,因此 5 个 33 氨基酸残基重复序列将结合 25 个 GvpA 蛋白分子,即 GvpC:GvpA = 1:25(图 11)^[33,58]。最新的 MALDI-TOF 质谱分析结果证明,GvpC 蛋白与 GvpA 蛋白骨架的结合位点位于保守重复区的两个末端^[65-66]。

4.3 gvp 基因簇结构

在 *Anabaena flos-aquae* 的 gvpC 基因的上游发现 4 个 gvpA 拷贝,在其下游发现了 4 个与盐生盐杆菌 gvp 基因同源的开放阅读框:ORF1 的产物与 GvpN 有 40% 同源,ORF2 的产物与 GvpJ 和 GvpA 同源,ORF3 的产物与 GvpK 和 GvpJ 同源,ORF4 的产物与 GvpF 和 GvpL 同源(图 12)^[42]。

大多数藻株的伪空胞基因簇只有一个 gvpC 基因,位于串联的多拷贝 gvpA 基因下游,但也有 gvpA 和 gvpC 交替排列的(红色颤藻)。Dunton 等人认为,伪空胞基因簇中的 gvpC 越长,产生的伪空胞就越宽,临界压力也就越小^[57]。铜绿微囊藻 PCC7806、PCC9354 和微囊藻 BC8401 的伪空胞基因簇都由两个操纵子(gvpAIIAIIHCNIX 或 gvpAIIAIIHCNIX 和 gvpKFG)、gvpV 和 gvpW 组成^[45,57](表 2)。在 gvpN、gvpV 和 gvpW 突变

Anabaena flos-aquae GvpC

N-terminal sequence

I	V	A	E	L	S	L	E	T	R	E	F	L	S	V	T	T	A	K	R	Q	E	Q	A	E	K	Q	A	Q	E	L	Q	A	F
II	Y	K	D	L	Q	E	T	S	Q	Q	F	L	S	E	T	A	Q	A	R	I	A	Q	A	E	K	Q	A	Q	E	L	L	A	F
III	H	K	E	L	Q	E	T	S	Q	Q	F	L	S	A	T	A	Q	A	R	I	A	Q	A	E	K	Q	A	Q	E	L	L	A	F
IV	Y	Q	E	L	Q	E	T	S	Q	Q	F	L	S	A	T	A	Q	A	R	I	A	Q	A	E	K	Q	A	Q	E	L	L	A	F
V	H	K	E	L	Q	E	T	S	Q	Q	F	L	S	A	T	A	D	A	R	T	A	Q	A	K	E	Q	K	E	S	L	L	K	F
C-terminal sequence	R	Q	D	L	F	V	S	I	F	G																							

Calothrix sp. GvpC

N-terminal sequence

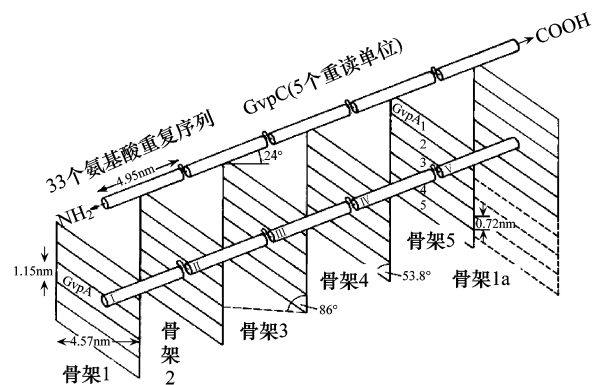
I	V	T	Q	L	F	K	D	T	Q	E	F	L	S	V	T	T	A	Q	R	Q	A	Q	A	K	E	Q	A	E	N	L	H	Q	F
II	H	K	D	L	E	K	D	T	E	E	F	L	T	D	T	A	K	E	R	M	A	K	A	K	Q	Q	A	E	D	L	F	Q	F
III	H	K	E	M	A	E	N	T	Q	E	F	L	S	E	T	A	K	E	R	M	A	Q	A	Q	E	Q	A	R	Q	L	R	E	F
IV	H	Q	N	L	E	Q	T	T	N	E	F	L	A	D	T	A	K	E	R	M	A	Q	A	Q	E	Q	K	Q	Q	L	H	Q	F
C-terminal sequence	R	Q	D	L	F	A	S	I	F	G	T	F																					

Anabaena consensus*Calothrix* consensus

H	K	E	L	Q	E	T	S	Q	Q	F	L	S	A	T	A	Q	A	R	I	A	Q	A	E	K	Q	A	Q	E	L	L	A	F
H	K	D	L	E	K	D	T	Q	E	F	L	S	D	T	A	K	E	R	M	A	Q	A	K	E	Q	A	E	Q	L	H	Q	F

图 10 *Anabaena flos-aquae* 和 *Calothrix* sp 中 GvpC 蛋白 33 个氨基酸残基重复单位^[41]Fig. 10 Amino acid sequences of GvpC from *Anabaena flos-aquae* and *Calothrix* sp^[41]表 2 部分蓝藻伪空胞基因簇结构^[71]Table 2 The structure of gas vesicle gene clusters in cyanobacteria^[71]

蓝藻名称 Name of cyanobacteria	伪空胞基因簇 Gas vesicle gene clusters
眉藻 PCC7601	(A) ₂ -C
水华鱼腥藻	(A) ₇ -C-N-J-K-F
拟鱼腥藻 PCC6901	A-C-N-J
红色颤藻	A-C-A-C
鱼腥藻 PCC7120	(A) ₂ -C-N-J-K-F-G
微囊藻 PCC7806、PCC9354	(A) ₃ -C-N-J-X-K-F-G-V-W
微囊藻 BC8401	(A) ₂ -C-N-J-X-K-F-G-V-W

图 11 GvpC 蛋白与 GvpA 骨架结合的模式^[58]Fig. 11 Speculations on how GvpC might interact with GvpA in the ribs^[58]图 12 *Anabaena flos-aquae* 中的 *gvp* 基因簇^[42]Fig. 12 The cluster of *gvp* genes in *Anabaena flos-aquae*^[42]

株的研究中, *gvpA* 虽然能够表达, 但没有检测到 GvpA 蛋白, 也没有观察到伪空胞结构, 说明这 3 个基因是伪空胞合成必需的^[45]。

目前, 关于蓝藻 *gvp* 基因表达调控方面的研究鲜有报道。拟鱼腥藻 PCC6901 从低光强 ($5 \mu\text{E m}^{-2} \text{s}^{-1}$) 转移到高光强 ($50 \mu\text{E m}^{-2} \text{s}^{-1}$) 时, *gvpA* 的转录量逐渐减少, 重新放置低光强后, *gvpA* 的转录子在 12h 后再次达到最初的表达量^[55]。眉藻 PCC7601 的 *gvpABC* 操纵子上游只有一个转录起始位点, 位于 *gvpA* 基因上游的 -56 核苷酸处; 另外一个转录子是反义 RNA, 起始于 *gvpA2* 的 3' 末端, 并延伸至 *gvpA* 基因中, 该转录子可能会削弱 *gvp* 基因的转录^[67]。水华束丝藻的伪空胞含量随着光强的增加有所下降^[68], 但没有研究表明这种变化是否

与 *gvp* 基因表达调控有关,因为该藻种的伪空胞含量还受到磷浓度变化的影响^[69-70],但机制不清楚。许敏和徐旭东等人^[71]利用微囊藻 FACHB930 为材料,在不同温度、光照强度、氮源、碳源以及 pH 值条件下研究了蓝藻漂浮与 *gvp* 基因转录调控的关系:在不同初始 pH 条件下,3 个 *gvpA* 拷贝和 1 个 *gvpC* 基因的转录水平存在差异;实时荧光定量 PCR 的方法证实了微囊藻 FACHB930 中 3 个 *gvpA* 拷贝与 1 个 *gvpC* 基因的转录调节是相互协调的。

5 伪空胞浮力调节机制

伪空胞的主要功能是为细胞提供浮力并参与浮力调节,蓝藻在不同水层中的定位以及昼夜垂直迁移可能都与此相关。伪空胞浮力的调节涉及到细胞镇重物的含量改变、伪空胞合成和伪空胞的破裂等 3 种调节机制^[72-73]。

(1) 镇重物含量改变 细胞的镇重物主要包括糖类(约为 $1550 \text{ kg} \cdot \text{m}^{-3}$)、蛋白质(约为 $1330 \text{ kg} \cdot \text{m}^{-3}$)、糖脂(约为 $1050 \text{ kg} \cdot \text{m}^{-3}$)、核酸($>1660 \text{ kg} \cdot \text{m}^{-3}$),它们密度均大于水($998 \text{ kg} \cdot \text{m}^{-3}$, 20°C)^[74]。镇重物的积累可以抵消伪空胞产生的浮力,这是浮游蓝藻中普遍存在的一种浮力调节方式。高光强下,多余的光合产物会以淀粉的形式积累,糖类占细胞干重的比例可由 30% 升至 60%^[75],如果没有新的伪空胞合成,或新合成的伪空胞所提供的浮力不足以抵消镇重物的积累,那么细胞将会下沉。而当光强降低、糖类物质逐渐被呼吸消耗或转化为密度较低的蛋白质时,细胞密度会降低,从而引起细胞上浮。Bright 和 Walsby 利用束丝藻(*Aphanizomenon ovalisporum*)研究蓝藻在夏季分层的湖泊中的垂直迁移和定位中发现^[76]:在昼夜交替过程中,蓝藻的镇重物白天增加而夜间下降。进一步的研究表明,当培养光强为 $3.9\text{--}8.0 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ 时,丝状体在光下达到浮力平衡状态,即 50% 的丝状体呈漂浮状态^[77]。这种与光相关的浮力调节机制可能是:白天光合作用合成的糖类以淀粉的形式积累而使丝状体密度增加,晚上呼吸作用消耗淀粉使细胞密度下降。蓝藻的这种昼夜垂直迁移有利于藻细胞获取更多的光能和营养物质,避免中午强光引起的光抑制和光氧化的破坏作用,从而有利于自身的生长发育并最终成为优势种群^[73]。

(2) 伪空胞合成 伪空胞合成的调节主要在分子和生理 2 个水平上进行^[73],前者主要是通过基因调控进行调节,后者主要是通过控制伪空胞合成所需要的能量和结构物质进行调节。

在生理水平上,主要通过光能和营养物(如:碳、氮和磷等)的供给对伪空胞合成影响进行调节(图 13)^[72],最近也有文献报道伪空胞的合成与蓝藻培养物的初始 pH 值有关^[71]。光照通过影响细胞光合作用捕获能量的多少和所固定碳的去向,从而影响浮力调节。当氮和磷受到限制时,细胞捕获的能量超过生长用量从而引起镇重物的积累和细胞膨压升高,导致了细胞浮力下降。持续的氮限制还会阻碍蛋白质合成,进而抑制伪空胞的合成,使细胞浮力下降^[78-79]。当细胞已有足量的伪空胞提供浮力或有足够的能量用于合成新的伪空胞时,短期的间歇性碳限制会使糖类含量降低,从而导致细胞密度降低和细胞浮力升高,但是持续的碳限制则抑制伪空胞的合成,从而导致细胞浮力下降^[78-79]。碳源和氮源的相对比值也影响伪空胞合成,高 C/N 下伪空胞的合成滞后于细胞生长,因而细胞伪空胞含量下降,低 C/N 下伪空胞则相对增加^[9,78-79]。

(3) 伪空胞破裂 淡水蓝藻的细胞膨压为 $0.1\text{--}0.6 \text{ MPa}$,通常在 $0.2\text{--}0.4 \text{ MPa}$ 之间^[75]。光限制条件下细胞膨压随着光强增大而升高,其原因可能是:(1)随着光强的增大细胞光合速率升高,因而可溶性有机产物(如:蔗糖、有机酸等)增加;(2)随着光强的增大对光依赖的 K^+ 吸收增强。细胞膨压升高会导致一些脆弱的伪空胞破裂,从而使细胞浮力下降。对于生活在深水中的蓝藻来说,其伪空胞较为坚固,因此很难通过这种方式进行浮力调节^[73]。

浮力调节与蓝藻生存的生理条件密切相关,而且这 3 种浮力调节机制有重叠作用。细胞镇重物的调节和伪空胞的破裂机制之间谁占优势取决于碳分配、膨压变化以及伪空胞的强度。不同浮力调节机制的响应时间也存在差异,虽然伪空胞破裂导致浮力迅速丧失($20\text{--}180 \text{ min}$),但浮力恢复所需的伪空胞合成过程较慢($>24 \text{ h}$)^[80]。细胞增殖对伪空胞的稀释作用需以细胞的世代时间来度量,相反,影响细胞镇重物的调节机制反应迅速,可以响应短时间内的环境变化。

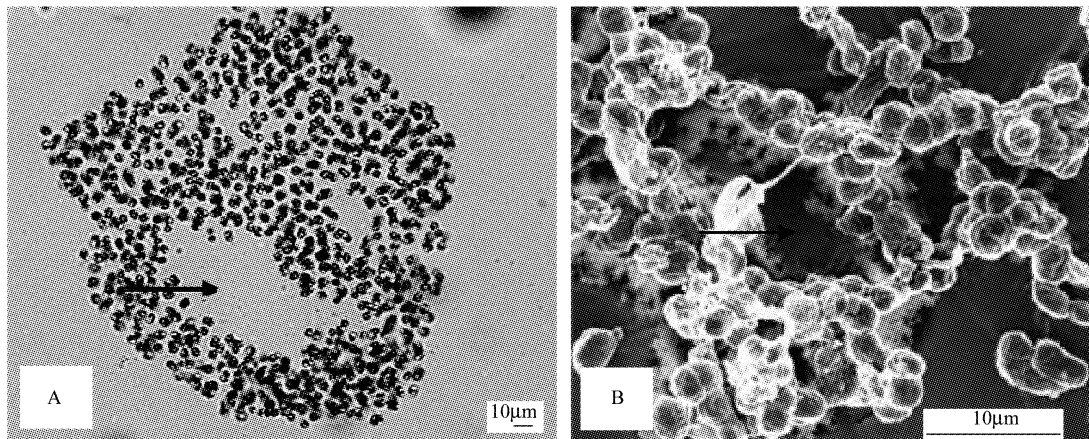


图 14 蓝藻群体细胞间隙 (箭头所指)^[81]

Fig. 14 Intercellular space (arrow) in aggregates of *Cyanobacteria*^[81]

空胞 *gvp* 基因的转录调控机制研究较少,特别是在自然水体中 *gvp* 基因的转录水平的调控更是未见报道,是否可以通过比较 *gvp* 基因在自然水体中的转录水平的差异预报蓝藻水华暴发的时间?(5)伪空胞基因网络调控研究在国内外研究中尚属空白,而该研究内容是全面理清环境因子与伪空胞表型、基因型之间关系的核心,理应成为今后研究的重中之重。(6)既然 GvpC 蛋白 33 个残基重复序列单位的数目与伪空胞的临界压力有关,那么 GvpC 蛋白的二级或三级结构如何?它们与伪空胞的临界压力关系如何?

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