水产学报
JOURNAL OF FISHERIES OF CHINA

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Research progress on the effects of hypoxia stress on crustacean and its molecular regulation

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在线阅读 View online: https://doi.org/10.11964/jfc.20191112052

文章编号：1000-0615(2020)04-0690-15　　水产学报, 2020, 44(4): 690−704

JOURNAL OF FISHERIES OF CHINA

DOI: 10.11964/jfc.20191112052

Science Press

·综述·

低氧对甲壳动物的影响及其分子调控研究进展

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摘要：氧是众多生物赖以生存的首要条件，动物处于低氧环境时，机体的生化反应和生理功能也会相应发生改变，严重时可引起一系列机体损伤甚至死亡。低氧胁迫下的应激机制是涉及多基因参与的复杂的生理调控过程，与哺乳动物相比，甲壳动物的低氧应激与分子适应机制尚不明晰。本文分析了低氧胁迫产生原因，并从生理层面阐述了甲壳动物对低氧胁迫的应激反应和生理适应策略、以及低氧对甲壳动物的行为、存活、抗氧化能力和代谢的影响，又从HIF-1信号通路、AMPK信号通路以及细胞凋亡通路阐述了甲壳动物对低氧环境胁迫的分子响应机制。本文在总结低氧信号传导及其调控通路研究进展的基础上，提出了低氧胁迫对甲壳动物影响的预防和调控手段，包括选育耐低氧新品种和营养调控手段，以期为人们更深入地理解甲壳动物低氧应激和分子适应机制提供理论参考。

关键词：甲壳动物；低氧；缺氧诱导因子；分子调控

中图分类号：Q 785；S 966.1　　**文献标志码：**A

低氧(hypoxia)一般情况下是指水中的溶解氧(dissolved oxygen, DO)浓度低于2 mg/L[1]，鉴于水生生物氧需求量各有不同，因此低氧浓度的具体数值并不固定，但有一种较为合理的定义：当水体DO值高于某个临界值(critical oxygen level, COL)时，水生生物耗氧量不再受水体DO降低的影响，这一临界数值就可以用来判断该生物是否缺氧[2]。

迄今，关于哺乳动物内部的氧气调节机制从细胞及分子水平进行了大量研究工作，证实低氧诱导因子- 1(hypoxia-inducible factors-1, HIF-1)、脯氨酸羟化酶(prolyl hydroylase, PHD)、促红细胞生成素(erythropoietin, EPO)、血管内皮生长因子(vascular endothelial growth factor, VEGF)等相关基因参与其调控[3-7]，而细胞如何感知和适应不同氧气环境，这一杰出发现获得2019年诺贝尔生理学或医学奖。相比之下，有关甲壳动物低氧应激的研究相对有限，主要集中在对行为反应、能量代谢和免疫机能等生理生化层面的探讨[8-13]，对甲壳动物低氧胁迫过程中体内基因表达机制和信号传导途径的研究极其缺乏，甲壳动物低氧应激的分子机制尚不清楚[14]，低氧诱导因子表达增强只是表象，具体原因有待查明。

相较于陆生哺乳动物和水生动物鱼类，生

收稿日期：2019-08-31　　修回日期：2019-12-26

资助项目：国家自然科学基金(31402280, 31672633)；江苏省“六大人才高峰”资助项目(2017-NY-112)；现代农业产业技术体系专项(CARS-48)

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活在不同水域的甲壳动物由于在长期演化进程中受到不同溶解氧浓度水体的自然选择，首先甲壳动物尽管没有下丘脑-垂体-肾间组织轴和下丘脑-垂体-甲状腺轴等神经传导系统，但已存在传统意义上的X-器官窦腺复合体、Y-器官和大颚器等内分泌器官，这些器官分泌的神经激素在机体应激发生中扮演着重要角色[15-16]。其次，相比哺乳动物具有复杂的闭管式循环系统并以肺为呼吸器官，甲壳动物依靠较为原始的开管式循环系统完成营养物质和代谢产物的运输[17]，以鳃小片作为水与血淋巴实现气体交换的场所，低氧应激下关键代谢物质、适应性反应进程、以及氧化应激的联系还不是很清楚。再次，甲壳动物呼吸蛋白(血蓝蛋白)在携氧效率上只有哺乳动物呼吸蛋白(血红蛋白)的1/4，低氧应激下甲壳动物通过减少能量的消耗来维持机体的正常代谢水平[18]。因此，深入阐明甲壳动物的低氧应激与分子适应机制，不仅对培育耐低氧虾蟹新品种具有重要意义，而且对促进甲壳动物养殖业健康发展也有重要意义。

1　甲壳动物对低氧应激的适应策略

1.1　水产养殖低氧胁迫产生原因

农业化学肥料污染　农业化肥的过量施用不仅会导致地表水的富营养化而且容易造成地下水硝酸盐污染等一系列水环境问题[19]。水体富营养化导致水产养殖池塘中营养盐和有机物不断增多，引起藻类及其他浮游生物的疯狂生长，水体透明度降低，水中植物光合作用减弱，产氧量下降，同时水中浮游植物的大量繁殖又不断消耗水体中的氧气促使池塘养殖水体中溶解氧严重不足[20]，导致甲壳动物“浮头泛塘”甚至出现大规模死亡。

高密度集约化养殖　20世纪80年代以来，世界水产养殖产量持续增高，甲壳动物作为我国重要的水产养殖经济动物[21]，为满足包括中国在内的许多国家的蛋白质供应作出了重要贡献。然而，伴随着淡水养殖的发展，因受温度、昼夜节律和季节变化以及富营养化作用的影响，再加上人为养殖管理不完善，高密度养殖水体时常处于低氧或缺氧状态；尤其在高温时节，养殖场所底层大量累积物(未消化饲料和排泄物)腐败分解，造成水体底层缺氧[22]，致使具有底栖习性的甲壳动物长期处于低氧胁迫状态。

1.2　甲壳动物对低氧应激的生理适应策略

甲壳动物在长期的演化过程中表现出对低氧耐受能力的差异性，例如中国明对虾(*Fenneropenaeus chinensis*)幼虾对低溶解氧的耐受能力在2~2.5 mg/L，而克氏原螯虾(*Procambarus clarkii*)成虾氧窒息点可低至0.061 mg/L[23]，其原因是甲壳动物逐渐进化出多种无氧代谢途径来应对低氧环境[24]。甲壳动物缺氧时会动员糖原进行无氧代谢产生最终产物乳酸，低氧下乳酸含量在斑节对虾(*Penaeus monodon*)[25]、凡纳滨对虾(*Litopenaeus vannamei*)[8]、淡水螯虾(*Orconects limosus*)[26]、长臂虾(*Palaemonetes pugio*)[27]和中华绒螯蟹(*Eriocheir sinensis*)[28]等不同组织中相继报道，而乳酸积累速率可能与甲壳动物耐低氧能力密切相关。

甲壳动物具有依靠自身平衡机制(代偿性反应)以维持机体保持平衡的功能，即使遭受某些胁迫因子的刺激，甲壳动物仍然能够在生命活动中保持机体内环境的稳定[29]。初步研究表明，甲壳动物应答低氧胁迫主要采用以下3种生理适应策略：①增加通气量和脉搏输入量，通过提高氧结合蛋白的浓度和亲和力以维持需氧量[18, 30]，甲壳动物对低氧环境的短期生理适应策略包括血蓝蛋白的迅速合成以满足机体对氧的需求，而长期的生理适应策略则是通过增大鳃的滤水量等方式提高氧的运输效率；②减少运动量，通过降低蛋白合成速率来减少自身代谢需求[31]，不过这种限制行为会增加其被捕食的风险；③诱导厌氧途径以产生能量来临时补充ATP合成所消耗的能量，但其能量消耗很大且这种途径的终产物乳酸的过量积累往往是有害的[32]，然而适量的乳酸积累又能够增加血蓝蛋白与氧气的亲和能力，由此形成了反馈机制[33]。

2　低氧胁迫对甲壳动物的影响

2.1　低氧胁迫对甲壳动物行为的影响

当水生生物处于低氧环境时，水生生物自身会通过调整一系列的个体行为来适应低氧环境并最大限度地减轻低氧胁迫对自身的危害，如通过逃离低氧区域、跳跃及降低摄食频率等行为回避低氧水环境[34]。有趣的现象是，水体缺

氧时虾类会趋向浅水区及水草上以便接触空气来增加自身摄氧量[35]，例如日本囊对虾(*Marsupenaeus japonicus*)[36]、褐虾(*Cragon cragon*)[37]、大西洋蓝蟹(*Callinectes sapidus*)[38]和日本沼虾(*Macrobrachium nipponense*)[39]等具有躲避低氧区域行为。Hagerman等[40]发现挪威海螯虾(*Nephrops norvegicus*)能通过加快游泳足的摆动速度增加进入洞穴的水流来应对暂时的低氧环境。在实际养殖生产中发现，在水体溶解氧含量逐渐下降的过程中，虾类的行为反应从低氧初期活动性增加到持续低氧时停止活动并伏底，若溶解氧继续降低甚至导致其大规模死亡[41-42]。

2.2 低氧胁迫对甲壳动物存活的影响

不同种类水生生物所能承受的窒息点(lethal dissolved oxygen level, LDOL)和半致死浓度(lethal Concentration 50, LC_{50})不尽相同，相比于蟹类，甲壳动物中虾类对低氧胁迫较为敏感(表1)。

2.3 低氧胁迫对甲壳动物抗氧化能力的影响

抗氧化相关指标可以在一定程度上反映无脊椎动物对环境胁迫的适应能力[57-60]。在机体正常生命活动中，其体内不断产生自由基和活性氧(reactive oxygen species, ROS)，随即又不断地被机体内抗氧化酶防御体系清除，包括超氧化物岐化酶(superoxide dismutase, SOD)、过氧化氢酶(catalase, CAT)和谷胱甘肽过氧化物酶(glutathione peroxidas, GPX)等，从而避免了机体的氧化损伤[3]。低氧胁迫对甲壳动物抗氧化能力的影响一直是研究热点，如Arun等[61]报道了罗氏沼虾不同组织CAT活力：鳃<肌肉<肝胰腺；不同组织SOD活力：肌肉<鳃<肝胰腺，说明肝胰腺与肌肉、鳃组织相比，是机体对外界刺激反应最早、最敏感、也最容易出现损伤的组织。凡纳

表 1 甲壳动物低氧窒息点

Tab. 1 Hypoxia asphyxia points of crustaceans

物种 species	发育阶段 developmental stage	耐受值/(mg/L) tolerance value	低氧耐受能力指标 hypoxia tolerance index	参考文献 reference
斑节对虾 *Penaeus monodon*	稚虾	0.90	96 h-LC_{50}	[43]
斑节对虾 *P. monodon*	稚虾	0.38	LDOL	[44]
刀额新对虾 *Metapenaeus ensis*	稚虾	0.30	LDOL	[44]
刀额新对虾 *M. ensis*	稚虾	0.77	8 h-LC_{50}	[42]
中国明对虾 *Fenneropenaeus chinensis*	稚虾	0.74	LDOL	[45]
日本囊对虾 *Marsupenaeus japonicus*	稚虾	0.48	LDOL	[44]
白滨对虾 *Litopenaeus setiferus*	仔虾	1.27	48 h-LC_{50}	[46]
白滨对虾 *L. setiferus*	稚虾	1.16	72 h-LC_{50}	[46]
凡纳滨对虾 *Litopenaeus vannamei*	稚虾	0.20	1 h-LC_{50}	[8]
日本沼虾 *Macrobranchium nipponense*	稚虾	0.85	8 h-LC_{50}	[47]
波纹龙虾 *Panulirus homarus*	成虾	0.23		[48]
中国龙虾 *Panulirus stimpsoni*	成虾	0.16		[49]
秀丽白虾 *Exopalaemon modestus*	幼虾	2.36	LDOL	[50]
罗氏沼虾 *Macrobrachium rosenbergii*	仔虾	0.98	LDOL	[51]
脊尾白虾 *Exopalaemon carinicauda*	仔虾	0.55	LC_{50}	[52]
锯缘青蟹 *Scylla serrata*	幼蟹	0.17	LC_{50}	[53]
拟穴青蟹 *Scylla paramamosain*	幼蟹	0.67		[54]
合浦绒螯蟹 *Eriocheir hepuensis* Dai	豆蟹	0.24		[55]
中华绒螯蟹 *Eriocheir sinensis*	幼蟹	0.21		[36]
三疣梭子蟹 *Portunus trituberculatus*	幼蟹	2.90		[56]

滨对虾在短期循环低氧下，其肝胰腺中抗氧化酶活性增强，这表明凡纳滨对虾具有耐受低氧和避免氧化损伤的早期适应机制[62]。颗粒张口蟹和日本沼虾暴露于低氧后，其鳃和肝胰腺组织中CAT和GPX活性升高，而在复氧阶段均能恢复到对照水平[63, 48]。以上研究表明，低氧胁迫能够均速激活甲壳动物的抗氧化系统(表2)。

2.4　低氧胁迫对甲壳动物呼吸代谢和线粒体功能的影响

能量是维持机体正常生命活动的必要条件，有氧代谢是机体获取能量的根本途径，主要分为3个阶段：糖酵解、三羧酸循环以及电子传递链。本实验室通过分析慢性低氧胁迫下日本沼虾不同组织转录组数据，发现低氧诱导日本沼虾糖酵解途径[70]，其最终产物(如乳酸)很快会积累到具有毒性的水平，从而最终在细胞内产生的氧代谢失衡。基于RNA干扰、重组蛋白和启动子序列分析方法，探明日本沼虾和凡纳滨对虾糖酵解关键基因已糖激酶(hexokinase, HK)和乳酸脱氢酶(lactic dehydrogenase, LDH)基因在低氧应激中的作用[71-74]。由于蛋白质是生命体功能的执行者和体现者，进一步采用双向凝胶电泳技术证实糖酵解关键酶，6-磷酸果糖激酶(6-phosphofructokinase, PFK)、丙酮酸激酶(pyruvate kinase, PK)在日本沼虾低氧应激过程中高丰度表达[75]。线粒体是细胞呼吸代谢的主要场所，电子传递链是线粒体内膜上由呼吸传递体组成的电子传递总轨道[76]，电子传递链能把代谢物脱下的电子有序地传递给氧，其电子传递顺序是：代谢物-烟酰胺腺嘌呤二核苷酸(nicotinamide adenine dinucleotide, NAD)- 黄素腺嘌呤二核苷酸(flavin adenine dinucleotide, FAD)-辅酶Q-细胞色素系统-O_2，其中琥珀酸脱氢酶(succinate dehydrogenase, SDH)是电子传递链前端的酶，在氧分子的参与下将琥珀酸氧化为延胡索酸并产生ATP，而延胡索酸还原酶(fumarate reductase, FRD)能够在无氧条件下催化延胡索酸生成乳酸，并产生少量的ATP。克氏原螯虾在低氧情况下能通过FRD催化延胡索酸生成琥珀酸这一途径为机体提供能量[77]；LDH可催化乳酸和丙酮酸之间的相互转化，FRD和LDH是无氧代谢过程的重要酶。管越强等[64]研究发现日本沼虾SDH活力随着低氧暴露时间延长而显著下降，而FRD和 LDH 活力显著增加；细胞色素氧化酶(cytochrome oxidase, CCO)是电子传递链末端的酶,在它的作用下,电子和还原态的氧结合生成水,是限制有氧代谢速率的酶，孙盛明等[78]研究发现日本沼虾在低氧胁迫下，细胞色素氧化酶相关基因表达量显著变化。Jimenez-Gutierrez等[79]通过凡纳滨对虾血浆中乳酸浓度的增加证实了缺氧的影响，低氧条件下凡纳滨对虾细胞色素c氧化酶活性下降，这表明了线粒体氧摄取量降低，然而由于甲壳动物细胞生物学研究还相对较少，低氧下甲壳动物细胞中线粒体的功能研究往往被忽略了。

表 2　低氧胁迫对甲壳动物抗氧化酶活性的影响

Tab. 2　Effect of hypoxia on antioxidant enzyme activities of crustaceans

物种 species	抗氧化酶活性变化 changes in antioxidant enzyme activities	参考文献 reference
颗粒张口蟹　*Chasmagnathus gramulata*	过氧化氢酶(catalase, CAT)、谷胱甘肽S转移酶(glutathione S-transferase，GST)活性上升，超氧化物岐化酶(superoxide dismutase, SOD)活性下降	[63]
日本沼虾　*Macrobranchium nipponense*	总抗氧化能力(Total antioxidant capacity，T-AOC)、CAT活性上升，SOD活性下降	[64]
日本沼虾　*M. nipponense*	CAT、谷胱甘肽过氧化物酶(glutathione Peroxidases，GPX)、SOD活性显著上升	[48]
凡纳滨对虾　*Litopenaeus vannamei*	SOD、GPX、丙二醛(malondialdehyde，MDA)活性显著上升	[65]
凡纳滨对虾　*L. vannamei*	GST、金属硫蛋白(metallothionein，MT)活性显著上升，GPX、SOD活性显著下降	[64]
中华绒螯蟹　*Eriocheir sinensis*	CAT、GST活性显著上升，SOD活性显著下降	[3]
大西洋蓝蟹　*Callinectes sapidus*	酚氧化酶(phenoloxidase，PO)活性显著下降	[66]
细角滨对虾　*Penaeus stylirostris*	PO活性显著上升，血细胞总数(total haemocyte count，THC)显著下降	[67]
中国明对虾　*Fenneropenaeus chinensis*	SOD、过氧化物酶(peroxidase，POD)活性显著上升	[68]
北方长额虾　*Pandalu sborealis*	GPX活性显著上升	[69]
罗氏沼虾　*M. rosenbergii*	PO活性显著下降	[13]

3 甲壳动物对低氧胁迫的相关信号通路研究

3.1 低氧诱导甲壳动物 HIF-1信号通路

为了应对低氧环境，生物机体形成了一系列的调节机制，作为一种氧敏感的转录激活因子，HIF-1是参与缺氧反应的主要因子[80]。HIF-1信号途径是从无脊椎动物到脊椎动物都十分保守的一个细胞信号传导途径系统，它对于维持动物的氧稳态至关重要[81]。HIF-1是由HIF-1α和HIF-1β两个亚基组成，常氧时，HIF-1α的Pro402和Pro564被PDHs羟基化，然后被VHL识别，随后传递给E3连接酶的其他成分(elonginB, ElonginC, Rbx1和Cul2)，最后被蛋白酶体降解；但在低氧状态下，HIF-1α进入细胞核与HIF-1β亚基组成的异源二聚体，形成具有转录功能的复合体参与维持体内氧气和能量平衡(图1)。国内外学者相继克隆分析了凡纳滨对虾、大西洋蓝蟹、虾蛄(*Oratosquilla oratoria*)、日本沼虾等甲壳动物的HIF-1α基因cDNA全序列[82-85]，越来越多研究证实，虾蟹HIF-1α基因均具有与哺乳动物相一致感受缺氧信号的活性调控区域(氧依赖降解结构域和反式激活结构域)。值得注意的是，大多数甲壳动物在低氧胁迫下HIF-1α的表达丰度显著增加，这与先前观察到的脊椎动物缺氧反应类似，然而低氧下甲壳动物体内HIF-1如何调控下游效应靶基因从而产生缺氧适应性反应的研究尚十分薄弱。依据研究报道，甲壳动物HIF-1下游基因主要涉及氧运输、糖酵解及代谢等相关基因，如血蓝蛋白、葡萄糖转运蛋白、已糖激酶、乳酸脱氢酶等(表3)。

动物的呼吸蛋白通常划分为3大类：血红蛋白、血蓝蛋白和蚯蚓血红蛋白。其中，血红蛋白分布最为广泛，不仅存在于脊椎动物，还存

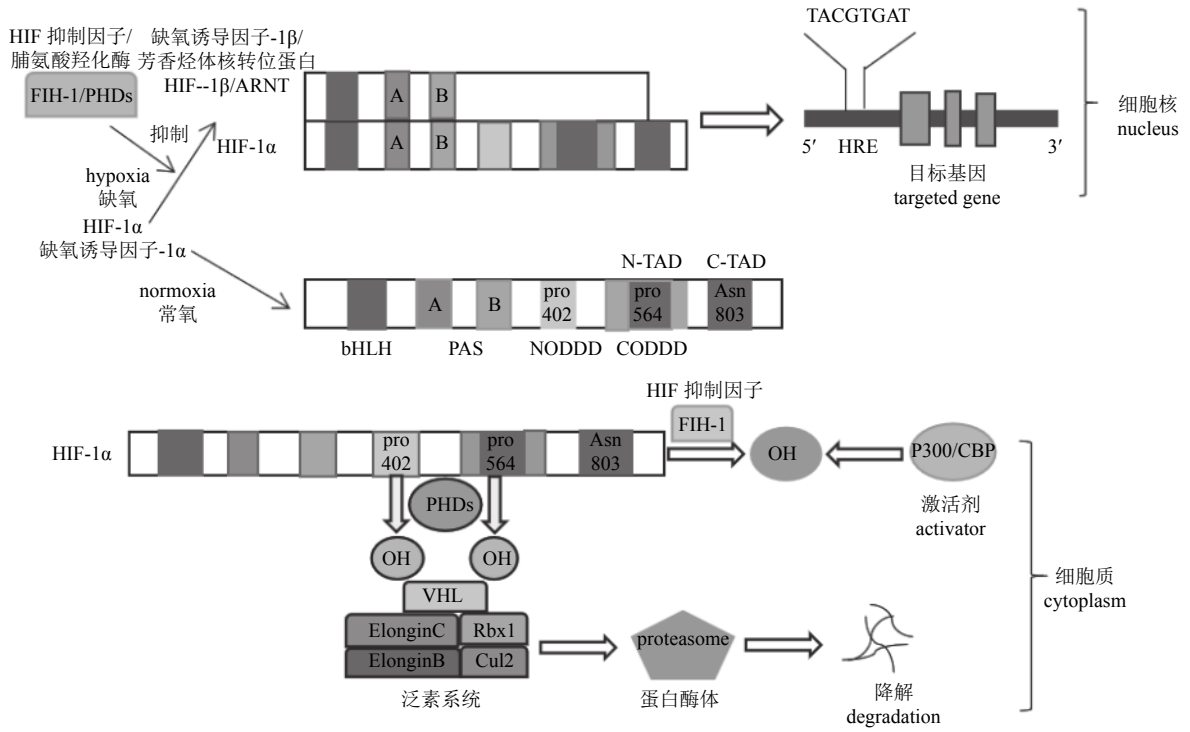

图 1 HIF-1的结构及转录调控[86]

HIF-1α. 缺氧诱导因子α亚基, FIH-1. HIF-1α的抑制因子, PHDs. 脯氨酰羟化酶, ARNT. HIF-1β芳香烃受体核转运蛋白, HRE. 缺氧反应元件, bHLH. 碱性螺旋-环-螺旋, PAS. 蛋白结构域, NODDD. N端氧依赖降解结构域, CODDD. C端氧依赖降解结构域, N-TAD. N端转录激活结构域, C-TAD. C端转录激活结构域, VHL.希佩尔林道.

Fig. 1 Structure and transcript regulation of HIF-1

HIF-1α. hypoxia-inducible factors-1α, FIH-1. factor- inhibiting HIF- 1α, PHDs.prolyl hydroxylase domain enzymes, ARNT. arylhydrocarbon receptor nuclear translocator, HRE. hypoxia response element, bHLH. basic-helix-loop-helix, PAS. PER-ARNT-SIM, NODDD.N-oxygen- dependent degradation domain, CODDD. C-oxygen- dependent degradation domain, N-TAD.N-terminal activation domain, C-TAD. C-terminal activation domain, VHL.Von Hippel-lindau

表 3　甲壳动物HIF-1α的靶基因

Tab. 3　Target gene of HIF-1α in crustaceans

基因 gene	物种 species	生物学效应 biological effect	参考文献 reference
血蓝蛋白　hemocyanin	日本沼虾　*Macrobrachium nipponense*	氧的运输　oxygen transport	[87]
血红蛋白　haemoglobin	大型蚤　*Daphnia magna*	氧的运输　oxygen transport	[88]
血蓝蛋白　hemocyanin	黄道蟹　*Cancer magister*	氧的运输　oxygen transport	[89]
膜结合血红蛋白　membrane-bound hemoglobin	日本沼虾　*M. nipponense*	抗氧化　antioxidant ability	[90]
磷酸果糖激酶　PFK	凡纳滨对虾　*Litopenaeus.vannamei*	糖酵解　glycolysis	[91]
果糖1,6-二磷酸酶 FBP	凡纳滨对虾　*L. vannamei*	糖异生　gluconeogenesis	[91]
甘油醛-3-磷酸脱氢酶　GAPDH	凡纳滨对虾　*L. vannamei*	糖酵解　glycolysis	[92]
乳酸脱氢酶　LDH	凡纳滨对虾　*L. vannamei*	糖酵解　glycolysis	[72]
己糖激酶　HK	凡纳滨对虾　*L. vannamei*	糖酵解　glycolysis	[73]
乳酸脱氢酶　LDH	日本沼虾　*M. nipponense*	糖酵解　Glycolysis	[74]
葡萄糖-6-磷酸脱氢酶　G6PDH	凡纳滨对虾　*L. vannamei*	葡萄糖代谢　glucose metabolism	[72]
葡萄糖转运蛋白　glut1	凡纳滨对虾　*L. vannamei*	葡萄糖代谢　glucose metabolism	[72]

在于无脊椎动物甲壳纲[86]。文献报道，甲壳动物大型蚤(*Daphnia magna*)血红蛋白启动子中含有低氧应激元件(hypoxia response element, HRE, 可与HIF-1结合)，其核心序列为5′-RCGTG-3′[88]。血蓝蛋白是虾蟹血淋巴中一种含铜呼吸蛋白，机体可以通过调节血蓝蛋白合成来适应溶解氧的变化，例如Hagerman[93]研究表明褐虾在低氧1~2 w内，其血蓝蛋白含量显著升高；Mangum等[94]和Brouwer等[95]报道低氧环境会引起大西洋蓝蟹血蓝蛋白的表达及合成，血蓝蛋白基因增加能够作为虾蟹低氧应答的分子指标。本课题组也克隆了日本沼虾血蓝蛋白启动子序列，发现启动子序列存在潜在的HRE 元件，双荧光素酶报告基因系统分析显示，克隆得到的5'调控区序列中转录因子与目的基因启动子区DNA相互作用，由此证实日本沼虾血蓝蛋白基因是HIF-1潜在靶基因。尽管近年来HIF-1信号通路在甲壳动物低氧应激中的作用研究取得了一定进展，但是仍然有甲壳动物HIF-1靶基因的生物学功能有待阐明。

3.2　低氧诱导甲壳动物AMPK (adenosine 5'-monophosphate (AMP)-activated protein kinase)信号通路

AMPK是真核生物细胞中广泛存在的一种重要的蛋白激酶，AMPK通过调节一系列底物酶而改变脂类和碳水化合物代谢，使其朝着抑制ATP消耗、促进ATP生成的方向进行，从而广泛参与糖脂代谢相关的信号通路。在影响低氧应激一系列复杂分子事件中，作为能量感受器的AMPK被证明是最关键因子之一，它在调控高等动物能量代谢和激活HIF-1转录活性具有重要作用[96]。那么甲壳动物AMPK的氨基酸序列是否相对保守，思考这个疑问过程中，我们从甲壳动物中AMPK基因克隆与序列分析的研究进展里找到了证据，将斑纹黄道蟹(*Cancer irroratus*)[97]、锯缘青蟹(*Scylla paramamosain*)[98]，凡纳滨对虾[99]、卤虫(*Artemia*)[100]和日本沼虾[101]的AMPK基因氨基酸序列进行比较分析，发现虾蟹AMPKα结构域均含有典型的丝、苏氨酸蛋白激酶催化区域，可通过AMPKα亚基172位苏氨酸磷酸化而激活AMPK来调节细胞能量平衡。本课题组克隆了日本沼虾AMPKα/β/γ等AMPK通路相关基因，并证明低氧诱导AMPK基因表达量并激活AMPK信号通路[101]，这一发现也在水蚤(*Daphnia pulex*)和岸蟹(*Carcinus maenas*)中证实[102-103]。那么，低氧下AMPK信号通路在甲壳动物糖脂代谢中调控机制还值得深入的探究。

3.3　低氧诱导甲壳动物细胞凋亡信号通路

细胞凋亡是一种不同于细胞坏死的多基因严格控制的过程，如Bcl-2家族、caspase家族、p53等[104]。p53是肿瘤抑制基因，可作为一个引发细胞凋亡通路的转录因子。研究发现，细胞

核外p53在线粒体中与Bcl-2家族中含有BH3结构域的蛋白结合后，使促凋亡蛋白Bak和Bax游离下来并活化，由于Blc-2家族蛋白能控制线粒体膜的通透性，从而将细胞色素C从线粒体中释放出来诱导细胞凋亡[105]。Caspase是重要的半胱氨酸依赖性的特异蛋白酶，它能够切割底物天冬氨酸残基后的肽键，使底物激活或者失活，其中capase-3是细胞凋亡通路下游的执行者。Nuñez-Hernandez等[106]以caspase-3表达和caspase活性作为细胞凋亡的指标，低氧处理48 h后凡纳滨对虾肝胰腺中p53水平和caspase-3表达显著升高，表明p53和caspase-3参与低氧诱导凡纳滨对虾细胞凋亡的分子过程。Sun等[107]从日本沼虾鳃中克隆了caspase-3基因，并证实急性低氧刺激后caspase-3在mRNA和蛋白质水平都显著升高，说明低氧也启动了细胞凋亡途径。Felix-Portillo等[108]研究表明低氧诱导凡纳滨对虾细胞凋亡基因p53的表达，RNA干扰p53后检测出凡纳滨对虾血细胞中的caspase-3活性升高，这些结果都证实低氧会使p53和caspase-3诱导甲壳动物细胞凋亡信号通路，Nuñez-Hernandez等[106]通过RNAi干扰技术，也发现凡纳滨对虾肝胰腺中p53参与急性低氧诱导细胞凋亡分子过程。微小RNA(microRNA，miRNA)是一类广泛存在于动植物中，大小约22 nt的单链非编码小分子RNA，它通过与靶mRNA 3′末端非翻译区结合，使mRNA降解或翻译抑制，研究表明，通过miRNA芯片和荧光定量PCR技术验证了显著差异表达miRNA在低氧诱导甲壳动物的细胞增殖与凋亡环节发挥着重要的调节作用[109-110]，例如miR-210, let-7, miR-143和miR-101等，它们潜在靶向调控p53和HIF-1。在脊椎动物中，凋亡相关基因在低氧应激与分子适应过程中的作用已经研究较为透彻，而在甲壳动物中细胞凋亡途径的研究尚处于起步阶段，究竟甲壳动物中哪个细胞凋亡途径在低氧应答分子过程中起主导作用? 细胞凋亡通路成员是否通过表观修饰而影响低氧适应机制？这些都有待今后的研究来回答。

4 低氧胁迫对甲壳动物影响的预防和调控

4.1 培育耐氧品种

为了培育耐低氧甲壳动物新品种，亟需阐明甲壳动物低氧应激与适应分子机制。本文认为基本策略是：①通过蛋白组、代谢组、表观组和基因组等多组学技术，对不同耐低氧能力甲壳动物进行比较分析，筛选和解析甲壳动物低氧适应关键基因或蛋白；②选取具有代表性的低氧敏感或低氧耐受的甲壳动物物种，利用生物信息学方法和CRISP/Cas9 技术，以低氧应激为导向进行低氧适应相关基因的功能解析；③筛选出有效分子标记，建立青虾低氧适应/耐受品系，进行甲壳动物耐低氧群体选育；④在此基础上，利用基因工程手段改造甲壳动物的遗传特性，培育耐低氧甲壳动物新品种。

4.2 营养调控

愈来愈多的研究表明，营养与水生生物的健康之间有着密切的联系。营养不良将导致机体抗应激能力和免疫功能性障碍，提升传染病易感性风险。在养殖生产中，营养素是影响水生动物抗应激能力最重要且最易于调控的因子之一。各种营养素如肌醇、硫辛酸和纳米硒等均以不同的方式影响和缓解甲壳动物的低氧应激。与对照组相比，投喂肌醇的凡纳滨对虾在低氧胁迫的条件下抗氧化能力和存活率显著增加，表明饲料中补充肌醇能够提高凡纳滨对虾耐低氧应激能力[111]。硫辛酸是一种已知的强效抗氧化剂，de Oliveira等[112]研究发现摄食硫辛酸对低氧/复氧诱导的凡纳滨对虾脂质过氧化的具有保护作用。Qin等[113]研究发现，低氧条件下纳米硒能够增强中华绒螯蟹幼蟹免疫反应和抗病力。邱仁杰[114]研究发现饲料中DHA(二十二碳六烯酸)/EPA(二十碳五烯酸)比例为1和2时对中华绒螯蟹幼蟹的免疫和生理更有益，对其抗低氧胁迫也更有帮助。总之，营养调控手段是缓解低氧胁迫对甲壳动物产生的不利影响有效途径。

5 展望

甲壳动物处在由无脊椎动物向脊椎动物进化的特殊阶段，其独特的进化地位也决定了发育过程和组织构造的特殊性。在未来甲壳动物低氧应激与分子适应研究过程中，有3点值得关注：①有关甲壳动物低氧应激的研究已从生长繁殖、呼吸代谢和免疫机能等生化层面延伸至信号传导、基因转录调控和关键基因功能等分子层面，但对于细胞能量代谢机制的研究还极为匮乏，无法全面深入地揭示甲壳动物“特有”的低氧应激分子机制；②随着三代测序技术的快速发展，甲壳动物基因组的研究必将有助于全

面阐释甲壳动物低氧适应相关基因功能及其调控网络；③低氧胁迫对甲壳动物的生理生化影响外，对行为学、神经内分泌等方面的研究也亟待加强。

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Research progress on the effects of hypoxia stress on crustacean and its molecular regulation

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Abstract: Oxygen is the most critical condition for animal's life activities. When animals are exposed to hypoxia environment, the biochemical reaction and physiological function of the animals will correspondingly change, which can finally cause a series of metabolic disorders and damage to organisms or even death. Hypoxia stress mechanism is complex physiological regulation process involving multiple genes and regulated by fine-tuning. Hypoxia stress and molecular adaptation mechanism of crustaceans are unclear compared with mammals. In this paper, the causes of hypoxia stress were analyzed, and stress reacting process and physiological adaptation strategies of crustaceans in response to hypoxia, as well as the effects of hypoxia on behavior, survival, antioxidant ability, metabolic reaction, and molecular mechanisms of hypoxia stress of crustacean were described and reviewed. In addition, the molecular mechanism in response to hypoxia was discussed from HIF-1 (hypoxia-inducible factor 1) signaling pathway, AMPK (adenosine 5′-monophosphate (AMP)-activated protein kinase) signaling pathway and apoptosis pathway. On this basis, the multiple measures are necessary to prevent and regulate negative effects of hypoxia stress, including the breeding hypoxia-resistant variety, and nutritional regulation. This paper may provide a theoretical reference for people to deeper understand the mechanism of hypoxia stress and molecular adaptation in crustaceans.

Key words: crustacean; hypoxia; HIF-1; molecular regulation

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Funding projects: National Natural Science Foundation of China (31402280, 31672633); Jiangsu Province "Six Talent Peak" Funded Project (2017 - NY - 112)