



昆虫血蓝蛋白功能研究进展*

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摘 要 血蓝蛋白是一种重要的昆虫呼吸蛋白, 参与昆虫的氧气转运、免疫防御和蛋白存储等多种生理过程, 并显著影响昆虫的生长发育及其对环境的适应性。近年来, 血蓝蛋白在不完全变态昆虫中被陆续报道; 血蓝蛋白的进化及功能已受到国内外学者的广泛关注。基于目前研究现状, 本文系统综述昆虫血蓝蛋白的结构和生物学功能, 并重点探讨血蓝蛋白对昆虫氧气转运和低氧适应的影响。

关键词 昆虫; 血蓝蛋白; 氧气转运; 低氧适应; 免疫防御

Progress in research on the function of insect hemocyanin

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Abstract Hemocyanin, an important respiratory protein, is involved in multiple physiological processes including oxygen transport, immune defense and protein storage, and plays an important role in the development and environmental adaptation of insects. In recent years, hemocyanin has been found in ametabolous insects and its evolution and function has become a popular research topic for both domestic and overseas scholars. This paper reviews the structure and function of insect hemocyanin, focusing on the effects of hemocyanin on oxygen transport and hypoxic adaptation in insects.

Key words insects; hemocyanin; oxygen transport; hypoxic adaptation; immune defense

血蓝蛋白 (Hemocyanin, Hc) 在不完全变态昆虫中广泛存在, 它与血红蛋白 (Hemoglobin, Hb) 和蚯蚓血红蛋白 (Hemerythrin, Hr) 共同组成动物的三大呼吸蛋白 (Terwilliger, 1998)。作为一种呼吸蛋白, 血蓝蛋白能够结合氧分子, 运输和储存氧气; 此外, 血蓝蛋白还参与生物的免疫防御和蛋白存储等多种生理过程 (Burmester, 2015)。目前, 血蓝蛋白的生物学功能已成为研究热点而广受关注。蛋白质的生物学功能取决于蛋白质的自身结构 (Berezovsky *et al.*, 2017), 所以, 血蓝蛋白的生物学功能也与其序列及空间

结构密切相关。例如: 血蓝蛋白活性部位螯合的双核铜离子能够与氧分子可逆结合, 这保证了血蓝蛋白的携氧功能 (Scherbaum *et al.*, 2018); 甲壳动物或螯肢动物血蓝蛋白 F3 位点苯丙氨酸 (位于 F1-F4 位点) 的变化 (被缬氨酸或苏氨酸取代) 导致血蓝蛋白酚氧化酶活性增强 (Decker and Terwilliger, 2000; Decker, 2004; Aguilera *et al.*, 2013; Masuda *et al.*, 2014)。为此, 本文详细介绍血蓝蛋白的结构和功能, 以期增强对血蓝蛋白的认识和促进对昆虫环境适应机制的理解。

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1 昆虫血蓝蛋白的进化和结构

1.1 昆虫血蓝蛋白的起源与进化

节肢动物血蓝蛋白是一个超家族,由血蓝蛋白、酪氨酸酶(含铜结合位点)、甲壳类拟血蓝蛋白(Pseudohemocyanins)/隐花青素(Cryptocyanins)、昆虫储存蛋白受体和昆虫六聚体储存蛋白组成;这些蛋白质关键元件的序列和结构高度保守(Burmester, 2004)。其中,血蓝蛋白起源于耗氧酚氧化酶,两者的分化节点为6亿年前的新元古代(Neoproterozoic)中期;直到4.3-4.4亿年前,昆虫血蓝蛋白和昆虫六聚体储存蛋白才从软甲纲Malacostraca血蓝蛋白分

化出来(Burmester, 2001)。随着时间的推移和生境变化,昆虫血蓝蛋白进化出2个亚基,即亚基1(Hc1)和亚基2(Hc2);并且,2个亚基在自然条件下独立进化(图1)。血蓝蛋白亚基的种类和数量在昆虫各目间存在一定差异。其中,石蛎目Archaeognatha昆虫仅含有Hc1,网蝽科Perlodidae的*Besdolus ravizzarum*、*Isoperla acicularis*、*Pachyleuctra benllochi*和*Arcynopteryx compacta*只含有Hc2,缨尾目Thysanura、革翅目Dermaptera、螳螂目Mantodea、蜚蠊目Blattaria、等翅目Isoptera和直翅目Orthoptera昆虫既含有Hc1也含有Hc2(Pick *et al.*, 2009; Amore *et al.*, 2011; Flachsbarth *et al.*, 2017)。

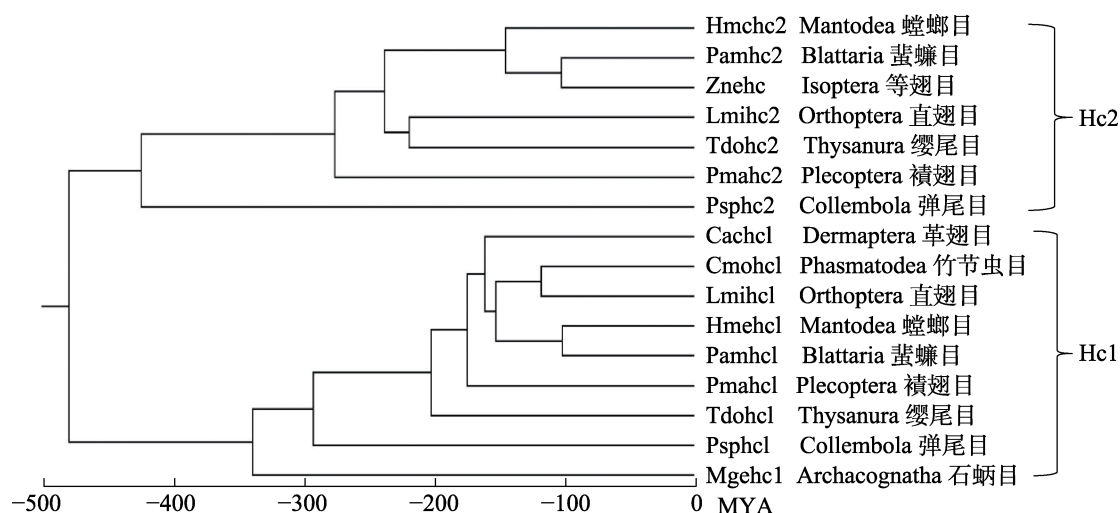


图1 六足动物血蓝蛋白亚基的进化树

Fig. 1 An evolutionary tree of hemocyanin subunits of Hexapoda

Lmihc1: 亚洲飞蝗血蓝蛋白亚基1, KJ713391.1; Lmihc2: 亚洲飞蝗血蓝蛋白亚基2, KJ713392.1; Pmahc1: 迁虫蜚蠊血蓝蛋白亚基1, AJ555403.1; Pmahc2: 迁虫蜚蠊血蓝蛋白亚基2, AJ555404.1; Psphc1: 须颚鳞翅目血蓝蛋白亚基1, QCC26464.1; Psphc2: 须颚鳞翅目血蓝蛋白亚基2, QCC26465.1; Cmohc1: 竹节虫血蓝蛋白亚基1, CAR85693.1; Tdohc1: 斑衣鱼血蓝蛋白亚基1, CAQ63321.1; Tdohc2: 斑衣鱼血蓝蛋白亚基2, CAQ63322.1; Mgehc1: 德国石蛎血蓝蛋白亚基1, CAR85692.1; Cachc1: 刺燕球螋血蓝蛋白亚基1, CAR85694.1; Hmehc1: 勇斧螳血蓝蛋白亚基1, CAR85695.1; Hmehc2: 勇斧螳血蓝蛋白亚基2, CAR85696.1; Pamhc1: 美洲大蠊血蓝蛋白亚基1, CAR85701.1; Pamhc2: 美洲大蠊血蓝蛋白亚基2, CAR85702.1; Znehc: 湿木白蚁血蓝蛋白, KDR21641.1。
Lmihc1: *Locusta migratoria* hemocyanin subunit 1, KJ713391.1; Lmihc2: *Locusta migratoria* hemocyanin subunit 2, KJ713392.1; Pmahc1: *Perla marginata* hemocyanin subunit 1, AJ555403.1; Pmahc2: *Perla marginata* hemocyanin subunit 2, AJ555404.1; Psphc1: *Pogonognathellus* sp. hemocyanin subunit 1, QCC26464.1; Psphc2: *Pogonognathellus* sp. hemocyanin subunit 2, QCC26465.1; Cmohc1: *Carausius morosus* hemocyanin subunit 1, CAR85693.1; Tdohc1: *Thermobia domestica* hemocyanin subunit 1, CAQ63321.1; Tdohc2: *Thermobia domestica* hemocyanin subunit 2, CAQ63322.1; Mgehc1: *Machilis germanica* hemocyanin subunit 1, CAR85692.1; Cachc1: *Chelidura acanthopygia* hemocyanin subunit 1, CAR85694.1; Hmehc1: *Hierodula membranacea* hemocyanin subunit 1, CAR85695.1; Hmehc2: *Hierodula membranacea* hemocyanin subunit 2, CAR85696.1; Pamhc1: *Periplaneta americana* hemocyanin subunit 1, CAR85701.1; Pamhc2: *Periplaneta americana* hemocyanin subunit 2, CAR85702.1; Znehc: *Zootermopsis nevadensis* hemocyanin, KDR21641.1。

1.2 昆虫血蓝蛋白的结构

血蓝蛋白是游离于节肢动物血淋巴中的六聚体或六聚体寡聚物(2×六聚体-8×六聚体),由分子量约为 75 ku 的单体组成,每个单体编码 600-660 个氨基酸(Markl, 1986);其中,弹尾目 Collembola 昆虫血蓝蛋白是由两个垂直排列的单体(分子量 73-83 ku)组成的 2×六聚体(Schmidt *et al.*, 2019)。血蓝蛋白结构复杂,主要包含 3 个结构域,即 α 螺旋组成的 N 端结构域、 α 螺旋和双核铜离子构成的活性部位和 7 股反向平行 β 桶组成的 C 端结构域(Linzen *et al.*, 1985; Markl and Decker, 1992)。血蓝蛋白的不同结构域拥有不同功能。例如,血蓝蛋白 N 端结构域 loop 区的保守占位残基 Phe49 的构象变化能够激活血蓝蛋白的酚氧化酶活性;位于活性部位 α 螺旋区的 6 个保守组氨酸残基能够螯合一对铜离子,结合一个氧分子;C 端结构域则能参与生物的免疫应答反应(Magnus *et al.*, 1994; Cong *et al.*, 2009; Zhao *et al.*, 2012; Qin *et al.*, 2018; Nangong *et al.*, 2020)。虽然不同昆虫血蓝蛋白的序列和结构存在多态性,但是血蓝蛋白活性部位的 6 个组氨酸残基(与铜离子配位)在昆虫中高度保守(图 2)。

1.3 昆虫血蓝蛋白与血红蛋白的异同

血蓝蛋白和血红蛋白在昆虫不同目中呈现互补性分布(表 1)。血蓝蛋白在弹尾目 Collembola、石蛎目 Archaeognatha、衣鱼目 Zygentoma、革翅目 Dermaptera、襀翅目 Plecoptera、直翅目 Orthoptera、螳螂竹节虫目 Mantophasmatodea、竹节虫目 Phasmatodea、螳螂目 Mantodea、蜚蠊目 Blattaria 和等翅目 Isoptera 等不完全变态昆虫中广泛存在,而血红蛋白主要分布在膜翅目 Hymenoptera、鳞翅目 Lepidoptera、鞘翅目 Coleoptera 和双翅目 Diptera 等全变态昆虫中。

除了物种分布外,血蓝蛋白和血红蛋白在分子结构和生物学功能等方面也存在一定差异。一方面,血红蛋白在细胞内和/或细胞外均有分布,是一种小型球蛋白;而血蓝蛋白仅分布在血淋巴中,通常以六聚体或六聚体寡聚物形式存在

(Coates and Decker, 2016)。另一方面,血红蛋白一般包含 8 个 α 螺旋片段和 1 个铁离子(Fe^{2+}),氧合状态下为红色(Wawrowski *et al.*, 2012; Burmester, 2015);然而,血蓝蛋白由 15 个 α 螺旋、7 个 β 折叠和 2 个铜离子(Cu^{2+})构成,氧合状态下为蓝色(Linzen *et al.*, 1985)。再者,血蓝蛋白比血红蛋白的生物学功能更广泛;血蓝蛋白除了参与昆虫免疫防御、解毒代谢以及转运和/或储存氧气外(与血红蛋白功能相似),还能加速木质素降解、储存蛋白和调节蜕皮(Qiu *et al.*, 2015; Zhang *et al.*, 2018; Hosseinzadeh *et al.*, 2019; Nangong *et al.*, 2020)。

2 昆虫血蓝蛋白的生物学功能

2.1 昆虫血蓝蛋白的携氧功能

大多数动物的有氧代谢需要消耗大量氧气。昆虫主要利用发达的气管系统(如不连续开闭气门)直接向组织或/和细胞中输送氧气(Talal *et al.*, 2016)。所以最初认为呼吸蛋白是昆虫非必需的,然而,研究发现并非如此。Hagner-Holler 等(2004)首次在石蝇 *Perla marginata* 若虫和成虫血淋巴中发现了能够结合氧气的血蓝蛋白($P_{50} = 8 \text{ torr}$);而且血蓝蛋白通过改变铜离子价位($\text{Cu}^{2+} \leftrightarrow \text{Cu}^{+}$)实现与氧分子的可逆结合(Bux *et al.*, 2018)。随后,具有氧结合特性的血蓝蛋白在美洲沙漠蝗 *Schistocerca americana*、斑衣鱼 *Thermobia domestica*、非洲马陆 *Archispirostreptus gigas*、飞蝗 *Locusta migratoria* 和白符跳 *Folsomia candida* 等昆虫中也被陆续报道(Sánchez *et al.*, 1998; Pick *et al.*, 2008; Damsgaard *et al.*, 2013; Chen *et al.*, 2015; Flachsbarth *et al.*, 2017; Liang *et al.*, 2019);另外,血蓝蛋白的表达缺失显著影响昆虫的正常发育和存活(Chen *et al.*, 2015, 2017);这些结果表明血蓝蛋白可能参与昆虫的氧气转运,并在昆虫有氧呼吸过程中发挥重要作用。

2.1.1 血蓝蛋白与昆虫胚胎呼吸 昆虫胚胎的气管系统未发育完全,而且被大量液体填充(Förster and Woods, 2012);这在一定程度上降低了气管系统运输氧气的效率。那么,昆虫胚

CuA									
Pspch1:	GTSR	NPE	QRVA	YF	GEDVGMNA	HH	SV	WH	KDHF
Cmohc1:	GTIR	NPE	QRVA	YL	GEDLGMNS	HH	AH	WH	MDF
Tdohc1:	GTIR	NPE	QRVA	YF	GEDIGMNS	HH	SH	WH	MDF
Mgehc1:	GTIR	NPE	QRVA	YF	GEDIGLNT	HH	HY	WH	MNF
Cachc1:	GTIR	NPE	QRVA	YL	GEDIGMNA	HH	AH	WH	MDF
Pamhc1:	GTIR	NPE	QRVA	YL	GEDVGMNA	HH	SH	WH	MDF
Hmehc1:	GTIR	NPE	QWVA	YL	GEDVGMNS	HH	SH	WH	MDF
Lmihc1:	GTIR	NPE	QRVA	YL	GEDLGMNS	HH	AR	WH	MDF
Pgrhc1:	GTIR	NPE	QRVA	YL	GEDVGLNS	HH	AH	WH	MDF
Csehc1:	GTVR	NPE	QRVA	YL	GEDVGIN	HH	SH	WH	MDF
Znehc:	GSVN	NPE	QRVA	YF	GEDVGMNS	HH	SH	WH	MDF
Lmihc2:	GSVN	NPE	QRVA	YF	GEDVGMNS	HH	QH	WH	MDF
Hmehc2:	GSVN	NPE	QRVA	YF	GEDIGMNS	HH	AH	WH	MDF
Pamhc2:	GSVN	NPE	QRVA	YF	GEDIGMNS	HH	SH	WH	MDF
Tdohc2:	GSIK	NPE	QRVA	YF	GEDIGMNS	HH	SH	WH	MDF
Pspch2:	GSVR	NPE	QRVS	YF	GEDIGMNA	HH	IS	WH	SLH
Csehc2:	GTIR	NPE	QRVA	YF	GEDIGMNS	HH	SH	WH	MDF
Pgrhc2:	GSVN	NPE	QRVA	YF	GEDIGINS	HH	SH	WH	MDF
CuB									
Pspch1:	KNPNY	YGS	HNLA	H	IMLSRVT	DPQ	GK	F	GMP
Cmohc1:	KHRDY	YGAL	HN	YG	HILLGKIT	DPK	GK	F	NMP
Tdohc1:	RHREY	YGAL	HN	YG	HILLGRIT	DPK	GK	F	NMP
Mgehc1:	PDIDY	YGS	HN	LG	HILLGEIM	DPD	HK	F	NLP
Cachc1:	KHPEY	YGAL	HN	YG	HILLGQIT	DPK	GK	F	NMP
Pmahc1:	PHEDY	YGS	HN	DA	HVLLGQIT	DPL	GK	F	NLP
Hmehc1:	KHPEY	YGAL	HN	YG	HIMLGQIV	DPK	GK	F	NMP
Lmihc1:	KHPEY	YGAL	HN	YG	HIMLGQIT	DPK	GK	F	NMP
Pgrhc1:	KHREY	YGS	HN	YA	HILLGKVT	DPL	GK	F	NLP
Csehc1:	PNPQY	YGAL	HN	YG	HILLGQIT	DPK	GK	F	NMP
Znehc:	VNPMF	YQQL	HNDG	H	VMLSKVT	DPQ	QRY	G	MP
Lmihc2:	VNPLY	YQQL	HNDG	H	VMLSKVT	DPD	QRY	G	MP
Hmehc2:	VNPAF	YQQL	HNDG	H	VMLSKVT	DPK	LY	GLP	
Pmahc2:	VNPFV	YQQL	HNDG	H	VMLSKVT	DPE	QK	F	GT
Tdohc2:	YNRHY	YQQL	HNDG	H	VMLSKVT	DYS	LK	Y	GLP
Pspch2:	QNKEL	YQQL	HNDG	H	VMLSKVT	DPK	GK	F	GLA
Csehc2:	VHPTL	YQQL	HNDG	H	VMLSKVT	DPL	QRY	G	VP
Pgrhc2:	VNPVY	YQQL	HNDG	H	VMLSKVT	DPE	QK	F	GT

图2 昆虫血蓝蛋白的双铜结合位点

Fig. 2 The bicopper binding sites of insect hemocyanin

Lmihc1: 亚洲飞蝗血蓝蛋白亚基 1, KJ713391.1; Lmihc2: 亚洲飞蝗血蓝蛋白亚基 2, KJ713392.1; Pmahc1: 迁虫血蓝蛋白亚基 1, AJ555403.1; Pmahc2: 迁虫血蓝蛋白亚基 2, AJ555404.1; Pspch1: 须鲸鳞蚱血蓝蛋白亚基 1, QCC26464.1; Pspch2: 须鲸鳞蚱血蓝蛋白亚基 2, QCC26465.1; Cmohc1: 竹节虫血蓝蛋白亚基 1, CAR85693.1; Tdohc1: 斑衣鱼血蓝蛋白亚基 1, CAQ63321.1; Tdohc2: 斑衣鱼血蓝蛋白亚基 2, CAQ63322.1; Mgehc1: 德国石蛾血蓝蛋白亚基 1, CAR85692.1; Cachc1: 刺燕球螋血蓝蛋白亚基 1, CAR85694.1; Hmehc1: 勇斧螳血蓝蛋白亚基 1, CAR85695.1; Hmehc2: 勇斧螳血蓝蛋白亚基 2, CAR85696.1; Pamhc1: 美洲大蠊血蓝蛋白亚基 1, CAR85701.1; Pamhc2: 美洲大蠊血蓝蛋白亚基 2, CAR85702.1; Znehc: 湿木白蚁血蓝蛋白, KDR21641.1; Csehc1: 堆砂白蚁血蓝蛋白亚基 1, CAR85697.1; Csehc2: 堆砂白蚁血蓝蛋白亚基 2, CAR85698.1。黑色标注区域代表与双铜离子结合的组氨酸, 灰色标注区域为血蓝蛋白的保守区域。

Lmihc1: *Locusta migratoria* hemocyanin subunit 1, KJ713391.1; Lmihc2: *Locusta migratoria* hemocyanin subunit 2, KJ713392.1; Pmahc1: *Perla marginata* hemocyanin subunit 1, AJ555403.1; Pmahc2: *Perla marginata* hemocyanin subunit 2, AJ555404.1; Pspch1: *Pogonognathellus* sp. hemocyanin subunit 1, QCC26464.1; Pspch2: *Pogonognathellus* sp. hemocyanin subunit 2, QCC26465.1; Cmohc1: *Carausius morosus* hemocyanin subunit 1, CAR85693.1; Tdohc1: *Thermobia domestica* hemocyanin subunit 1, CAQ63321.1; Tdohc2: *Thermobia domestica* hemocyanin subunit 2, CAQ63322.1; Mgehc1: *Machilis germanica* hemocyanin subunit 1, CAR85692.1; Cachc1: *Chelidura acanthopygia* hemocyanin subunit 1, CAR85694.1; Hmehc1: *Hierodula membranacea* hemocyanin subunit 1, CAR85695.1; Hmehc2: *Hierodula membranacea* hemocyanin subunit 2, CAR85696.1; Pamhc1: *Periplaneta americana* hemocyanin subunit 1, CAR85701.1; Pamhc2: *Periplaneta americana* hemocyanin subunit 2, CAR85702.1; Znehc: *Zootermopsis nevadensis* hemocyanin, KDR21641.1; Csehc1: *Cryptotermes secundus* hemocyanin subunit 1, CAR85697.1; Csehc2: *Cryptotermes secundus* hemocyanin subunit 2, CAR85698.1. The black area represents histidine which can bound to copper ions, and the gray area indicates conserved sites on hemocyanin.

表 1 血蓝蛋白与血红蛋白在昆虫中的分布
Table 1 Distribution of hemocyanin and hemoglobin for insects

目 Order	血蓝蛋白 Hc	血红蛋白 Hb	目 Order	血蓝蛋白 Hc	血红蛋白 Hb
原尾目 Protura	N	N	等翅目 Isoptera	Y	N
弹尾目 Collembola	Y	N	缨翅目 Thysanoptera	?	L
双尾目 Diplura	N	N	半翅目 Hemiptera	Y	Y
石蛎目 Archaeognatha	Y	N	虱目 Phthiraptera	Y	?
衣鱼目 Zygentoma	Y	N	啮虫目 Corrodentia	?	?
蜻蜓目 Odonata	N	N	膜翅目 Hymenoptera	Y	Y
蜉蝣目 Ephemeroptera	N	N	广翅目 Megaloptera	N	N
缺翅目 Zoraptera	?	N	蛇蛉目 Raphidioptera	N	N
革翅目 Dermaptera	Y	N	脉翅目 Neuroptera	N	N
襀翅目 Plecoptera	Y	N	捻翅目 Strepsiptera	N	N
直翅目 Orthoptera	Y	N	鞘翅目 Coleoptera	N	Y
螳螂竹节目 Mantophasmatodea	Y	N	毛翅目 Trichoptera	N	N
蛩蠊目 Grylloblattodea	?	N	鳞翅目 Lepidoptera	N	Y
纺足目 Embioptera	?	N	蚤目 Siphonaptera	N	N
竹节虫目 Phasmatodea	Y	N	长翅目 Mecoptera	N	N
螳螂目 Mantodea	Y	N	双翅目 Diptera	N	Y
蜚蠊目 Blattodea	Y	N			

N 代表对应类群中不存在; Y 代表对应类群中存在; ?代表尚未报道; L 代表对应类群中存在呼吸蛋白类似物。

N indicates the absence of respiratory proteins in the corresponding taxa; Y indicates the presence of respiratory proteins in the corresponding taxa; ? indicates respiratory proteins have not been found; L indicates the respiratory protein analogues.

胎如何维持高水平的氧气供给? Jaglarz 等 (2019) 研究发现,血蓝蛋白有助于螻蛄 *Arixenia esau* 1 龄若虫在雌性螻蛄生殖系统内进行有氧呼吸。血蓝蛋白在杜比亚螻蛄 *Blaptica dubia* 胚胎内特异性高表达,推测高表达的血蓝蛋白可能与杜比亚螻蛄胚胎的氧气运输有关 (Pick *et al.*, 2010); 类似的现象在美洲沙漠蝗、飞蝗和白符跳中也被报道 (Sánchez *et al.*, 1998; Chen *et al.*, 2015; Liang *et al.*, 2019)。另外,血蓝蛋白的表达缺失导致飞蝗胚胎发育迟缓和存活率降低 (Chen *et al.*, 2015)。由此可见,血蓝蛋白可能作为一种生理补充来弥补气管运输氧气的不足,进而辅助昆虫胚胎的有氧呼吸 (Pick *et al.*, 2010)。基于血蓝蛋白表达丰度和气管系统发育的关系,Chen 等 (2017) 提出了血蓝蛋白表达和气管系统发育的互补模型 (图 3)。该模型指出:没有成熟气管系统的昆虫胚胎在自然进化过

程中选择血蓝蛋白来辅助有氧呼吸;当昆虫进入胚胎后发育期,气管系统逐渐成熟,气管系统运输氧气的效率和实用性远高于血蓝蛋白,所以胚胎后发育期昆虫的氧气运输不需要血蓝蛋白参与。血蓝蛋白表达和气管系统发育的互补模型能够很好的解释昆虫的呼吸机制。

2.1.2 血蓝蛋白与昆虫低氧适应 生物低氧适应的一个重要方面是增强氧气供给和运输 (Förster and Woods, 2012; Harrison *et al.*, 2018)。为了应对大气氧分压降低,拥有成熟气管系统的昆虫成虫增加微气管密度和气管分支、缩小体型以及增加气门开闭的持续时间和频率 (Hetz and Bradley, 2005; Harrison *et al.*, 2006; Clapham and Karr, 2012)。没有成熟气管系统的昆虫胚胎如何适应低氧环境? Chen 等 (2017) 对西藏飞蝗和平原飞蝗胚胎血蓝蛋白进行了比较研究,发现西藏飞蝗胚胎血蓝蛋白的表达量显著高于平原

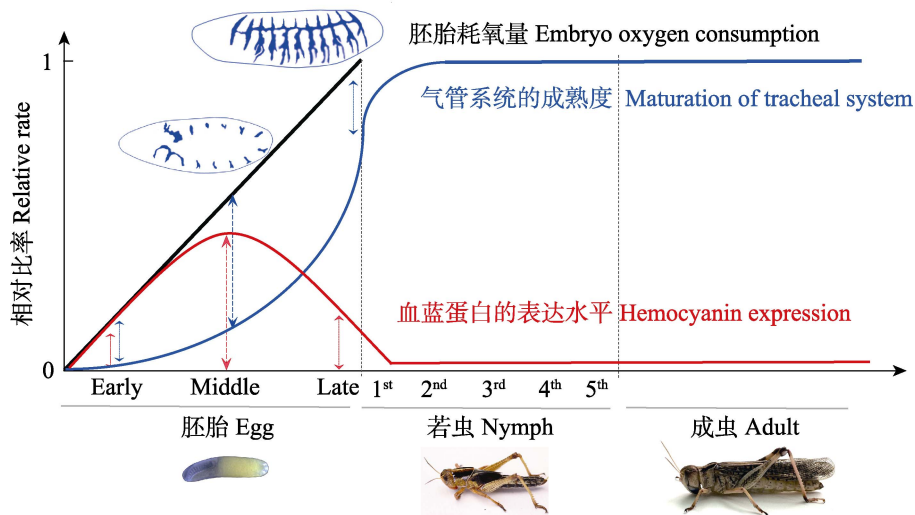


图3 飞蝗胚胎供氧机制的补充示意图 (Chen *et al.*, 2017)

Fig. 3 Schematic representation showing complementary mechanisms of oxygen supply for aerobic metabolism in migratory locust embryos (Chen *et al.*, 2017)

黑线表示昆虫胚胎的氧消耗速率, 蓝线为昆虫气管系统的发育水平, 红线为血蓝蛋白的表达趋势。

The black line shows the rate of oxygen consumption for embryos, the blue line presents the development rate of trachea system in insect, and the expression level of hemocyanin is denoted by red line.

飞蝗, 推测较高的血蓝蛋白含量可能有助于西藏飞蝗胚胎在低氧高海拔地区更好地获得充足和稳定的氧气。另外, 低氧诱导实验发现, 低氧条件下白符跳和飞蝗胚胎血蓝蛋白的表达量显著高于常氧条件 (Chen *et al.*, 2017; Flachsbarth *et al.*, 2017)。这些结果表明, 昆虫胚胎可能通过提高血蓝蛋白含量来增加氧气供给, 进而提高胚胎低氧适应性。另外, 血蓝蛋白调节动物低氧适应的现象在甲壳动物中也被报道。例如, 与常氧相比, 低氧条件下蓝蟹 *Callinectes sapidus* 血蓝蛋白的表达量显著增加 (McMahon, 2001; Lehtonen and Burnett, 2016); 长期缺氧诱导首长黄道蟹 *Cancer magister* 血蓝蛋白低聚物(如 $1\times$ 六聚体和 $2\times$ 六聚体)的表达显著上调 (Head, 2010)。

2.2 昆虫血蓝蛋白的免疫功能

血蓝蛋白是一种潜在的重要免疫分子, 在动物抵抗病原微生物(如细菌、真菌和病毒)入侵中发挥重要作用。一方面, 血蓝蛋白的酚氧化酶活性能够被高氯酸盐、尿素和 SDS 等激活, 进而参与宿主的体液免疫 (Coates and Nairn, 2014)。

例如, 经 SDS 处理的美洲蜃 *Limulus polyphemus* 或加利福尼亚庞蛛 *Eurypelma californicum* 血蓝蛋白表现出邻二酚氧化酶活性 (Decker, 2004)。另一方面, 血蓝蛋白能够自身降解产生抗菌肽, 参与宿主的免疫应答反应。例如, 凡纳滨对虾 *Litopenaeus vannamei* 血蓝蛋白 C 末端的降解片段 astacidin 1 可以显著抑制革兰氏细菌的增长 (Lee *et al.*, 2003); 凡纳滨对虾和克氏原螯虾 *Procambarus clarkii* 血蓝蛋白 N 末端的体外重组蛋白能够吞噬或凝集副溶血性弧菌 (Qin *et al.*, 2018; Fan *et al.*, 2019)。此外, 血蓝蛋白降解产生的抗菌肽还能诱导癌细胞凋亡以及抑制病毒增殖 (Destoumieux-Garzon *et al.*, 2001; Liu *et al.*, 2018; Zhan *et al.*, 2019)。

相比于甲壳动物和螯肢动物等节肢动物, 昆虫血蓝蛋白与免疫防御的研究相对薄弱。Ramsey 等 (2017) 研究发现, 柑橘黄龙病毒 (CLas) 显著诱导柑橘木虱 *Diaphorina citri* 血蓝蛋白的表达上调, 这表明血蓝蛋白可能在柑橘木虱抵抗 CLas 中发挥重要作用。血蓝蛋白含量和柑橘木虱抗病毒的相关性分析结果显示, 柑橘木虱抵抗 CLas 的能力随血蓝蛋白含量的升高而增强, 这

进一步说明血蓝蛋白可能参与柑橘木虱的免疫应答反应 (Hosseinzadeh *et al.*, 2019)。另外, 白粉虱 *Bemisia tabaci* 被黄叶卷曲病毒 (YLCV) 侵染后, 其体内血蓝蛋白的表达量显著升高, 推测血蓝蛋白在白粉虱抵抗黄叶卷曲病毒的过程中发挥重要作用 (Hasegawa *et al.*, 2018)。但是, 我们还未找到血蓝蛋白参与昆虫免疫防御的直接证据。

2.3 昆虫血蓝蛋白的其他功能

作为一种多功能蛋白, 昆虫血蓝蛋白除了参与氧气转运和免疫防御外, 还表现出其他功能。1) 加速木质素降解。台湾乳白蚁 *Coptotermes formosanus* 血蓝蛋白能够氧化木质素, 从而提高木质降解酶 (如纤维素酶和半纤维素酶) 降解木质素的效率 (Qiu *et al.*, 2015)。2) 调节蜕皮。在特定环境条件下, 加利福尼亚庞蛛通过血蓝蛋白 N 端结构域的疏水结构结合蜕皮激素来增强血淋巴对蜕皮激素的转运, 进而促进加利福尼亚庞蛛的蜕皮 (Jaenicke *et al.*, 1999)。3) 存储蛋白质。Zhang 等 (2018) 通过转录组学和蛋白组学分析发现, 滞育烟蚜茧蜂 *Aphidius gifuensis* 体内血蓝蛋白的含量约为非滞育烟蚜茧蜂的 8.45 倍, 推测血蓝蛋白可能参与烟蚜茧蜂的蛋白存储。此外, 甲壳纲动物的血蓝蛋白还能结合色素、调节渗透压和蛋白酶表达。例如, 凡纳滨对虾壳中的血蓝蛋白 LvPBP75 能够特异性结合虾青素 (Pan *et al.*, 2019); 远海梭子蟹 *Portunus pelagicus* 血蓝蛋白参与调控胰蛋白酶和胰凝乳蛋白酶的表达 (Kuballa *et al.*, 2011); 凡纳滨对虾血蓝蛋白还能改变胶体渗透压, 进而诱导脊椎动物红细胞裂解 (Zhang *et al.*, 2009)。

3 展望

综上所述, 昆虫血蓝蛋白不仅参与呼吸调节过程, 而且还表现其他功能, 对生物体的发育和生存都十分重要; 同时, 血蓝蛋白与生物的进化也有重要关联。但有关血蓝蛋白的研究还存在诸多有趣的问题有待解决。例如, 血蓝蛋白在生物体内是具有一种还是多种功能? 血蓝蛋白的功

能是否随生物的生长发育发生改变? 此外, 血蓝蛋白携氧功能及其他功能的具体调节机制尚不明确, 体现在诸多方面: 血蓝蛋白为什么在部分类群中的丢失或者仅在某一时期表达, 如飞蝗胚胎期? 血蓝蛋白在胚胎发育过程中的具体调节机制是什么? 血蓝蛋白表达的自然变异 (如高原和平原种群间的变异) 具有怎样的遗传及分子机制? 回答上述问题, 不仅有助于增强我们对血蓝蛋白功能的认识, 而且有利于促进对生物环境适应机制的理解。

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