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硕士学位论文

秀丽隐杆线虫 AMPK 蛋白 AAK-2 及线粒体腺苷酸转运蛋白 ANT-1.1 在热应激中的功能研究

The study of *C. elegans* AMPK protein AAK-2 and Mitochondrial Adenine Nucleotide Translocator ANT-1.1 function in heat stress response

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缩略词表

英文缩写	英文名称	中文名称
Amp	Ampicillin	氨苄青霉素
cm	centimeter	厘米, 长度单位
DNA	Deoxyribonucleic acid	脱氧核糖核酸
FUDR	Floxuridine	氟脲嘧啶脱氧核苷
GFP	Green Fluorescent Protein	绿色荧光蛋白
HS	Heat Shock	35℃热激
h	hour	小时, 时间单位
L	liter	升, 体积单位
L4	Larva 4	第四期幼虫
LB	Luria-Bertani culture medium	溶菌肉汤培养基
mCherry	Red Fluorescent Protein	红色荧光蛋白
Min	minute	分, 时间单位
mL	milliliter	毫升, 体积单位
mm	millimeter	毫米, 长度单位
NGM	Nematode Growth Medium	线虫生长培养基
RNA	RiboNucleic Acid	核糖核酸
RNAi	RNA interference	RNA 干扰
RT-qPCR	Real-time PCR	实时定量 PCR
S	second	秒, 时间单位

摘要

随着对 AMPK 蛋白研究的深入，人们发现其在能量代谢、压力应激和寿命等方面都有重要的调控作用，对其功能的进一步研究也受到越来越广泛的关注。秀丽隐杆线虫 AMPK α 催化亚基 AAK-2 功能缺失后会导致寿命缩短，热敏感等表型，揭示了 AAK-2 在热应激中的重要功能。为了进一步探究线虫中 AAK-2 在压力应激过程中的调控机制，本文检测了热激处理前后野生型虫株中 *aak-2* mRNA 水平，并比较了几种热激蛋白的基因表达水平在野生型及 *aak-2* 缺失突变株中的差异。RT-qPCR 实验显示 *aak-2* mRNA 水平在经过 20 小时非致死温度热处理后显著上调。热激处理都可以显著上调野生型及 *aak-2* 缺失突变株中几种热激蛋白的基因表达水平，暗示 AAK-2 在热应激中的功能可能不是通过调控热激蛋白的基因表达水平来实现的。同时，本论文通过免疫沉淀技术分离出在压力条件下可能与 AAK-2 特异结合的相互作用蛋白，并进一步确认 AAK-2 与它们之间的相互作用，试图通过对 AAK-2 相互作用蛋白功能的分析，来进一步揭示 AAK-2 在压力应激中的作用。我们对分离到的一个 AAK-2 的可能作用蛋白 ANT-1.1 进行了研究，发现 *ant-1.1* RNAi 敲降可增强热抗性，并依赖于热激蛋白因子 HSF-1。RT-qPCR 结果显示 HSP16.2 mRNA 基底表达水平的上调可能是 *ant-1.1* RNAi 敲降热抗性增强的原因之一。本研究虽然多次尝试在体外或体内来验证 AAK-2 与 ANT-1.1 间的相互作用，但都由于各种已知或未知的原因未能证明它们之间存在特异结合，还有待今后优化实验条件来得出确切的结论。本研究揭示了 AAK-2 和 ANT-1.1 参与热抗性调控过程的部分机制，为秀丽隐杆线虫压力应激反应调控提供了新的启示。鉴于 AMPK 和 ANT 蛋白在进化上的保守性，本文的研究工作将有助于人们认识其他物种中应激调控的可能作用机制。

关键词：秀丽隐杆线虫；AAK-2 蛋白；ANT-1.1 蛋白；应激反应

Abstract

Recently, people paid more and more attention to AMP-activated protein kinase (AMPK) due to their important roles on energy metabolism, stress response and life span regulation. In *C. elegans*, the absence of AMPK catalytic subunit AAK-2 leads to shortened lifespan and heat sensitivity, indicating that AAK-2 as an energy sensor plays an important role in lifespan and stress responses. To further explore the regulatory mechanism of AMPK protein AAK-2 on stress response, we examined *aak-2* mRNA level in wild-type worms with or without heat shock treatment, and compared the gene expression level of several heat shock proteins in wild-type and *aak-2* mutants. Our results showed that *aak-2* mRNA level was significantly increased in wild-type worms after 20 hours of 30°C non-lethal heat shock treatment. The heat shock treatment could significantly up-regulate several heat shock protein gene expression level in both wild-type and *aak-2* mutants, suggesting AAK-2 function in heat stress response was not through regulating the heat shock protein gene expression. In order to further elucidate AAK-2 function in stress response, we isolated the possible AAK-2 interacting proteins under stress conditions by immunoprecipitation and mass spectrometry, and tried to analyze the function of these AAK-2 interacting proteins. We further studied ANT-1.1, one of possible AAK-2 interacting proteins identified from mass spectrometry and found that *ant-1.1* RNAi knock-down could lead to increased thermotolerance, which was dependent on heat shock factor HSF-1. RT-qPCR results showed that higher HSP16.2 mRNA basal level might be responsible for the increased heat resistance caused by *ant-1.1* RNAi knock-down. Although we have tried very hard to determine the interaction between AAK-2 and ANT-1.1 in vitro or in vivo, we could not get the final conclusion due to some known or unknown reasons. It still requires further experiments to confirm their interaction. Our results in this thesis have partially revealed the regulatory mechanism of AAK-2 and ANT-1.1 on heat stress response and may provide new insight for stress response in *C. elegans*.

Abstract

Given AMPK and ANT proteins are highly conserved in evolution, the research work in this paper will help to understand the regulation mechanism of AMPK and ANT proteins in stress response in other species.

Key words: *C. elegans*; AAK-2; ANT-1.1; stress response

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