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裂殖酵母中 Dnt1 参与纺锤体组装检验点沉默机制的研究

A study on mechanism of fission yeast Dnt1 participating in spindle assembly checkpoint silencing

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缩写词表

APC	anaphase promoting complex 后期促进复合物
APS	ammonium persulfate 过硫酸铵
BSA	bovine serum albumin 牛血清白蛋白
CDK	cyclin-dependent kinase 周期蛋白依赖性蛋白激酶
CIAP	calf-intestinal alkaline phosphatase 小牛小肠碱性磷酸酶
co-IP	co-immunoprecipitation 免疫共沉淀
DAPI	4',6-diamidino-2-phenylindole 4,6-二脒基-2-苯基吲哚
DMSO	dimethyl sulfoxide 二甲亚砜
dNTP	deoxyribonucleoside triphosphate 脱氧核苷三磷酸
DTT	dithiothreitol 二硫苏糖醇
<i>E.coli</i>	<i>Escherichia coli</i> 大肠杆菌
EB	ethidium bromide 溴化乙啶
EDTA	ethylene diamine tetraacetic acid 乙二胺四乙酸
GBP	GFP binding protein GFP 结合蛋白
GFP	green fluorescent protein 绿色荧光蛋白
HU	Hydroxyurea 羟基脲
IP	immunoprecipitation 免疫沉淀
LB	luria broth medium 肉汤培养基
MCC	Mitotic checkpoint complex 有丝分裂检验点复合物
mCherry	红色荧光蛋白
1NM-PP1	1-(tert-butyl)-3-(naphthalen-1-ylmethyl)- 1H-pyrazolo[3,4-d]pyrimidin-4-amine
OD	optical density 光密度
PAGE	polyacrylamide gel electrophoresis 聚丙烯酰胺凝胶电泳
PCR	polymerase chain reaction 聚合酶链式反应

PMSF	phenylmethylsulfonyl fluoride 苯甲基磺酰氟
SAC	spindle assembly checkpoint 纺锤体组装检验点
SDS	sodium dodecyl sulfate 十二烷基磺酸钠
TAP	tandem affinity purification 串联亲和纯化
TBZ	Thiabendazole 2-(4'-噻唑)苯丙咪唑
TE(Tris/EDTA)	Tris/EDTA 缓冲盐溶液
TEMED	N N N' N'-tetramethylethylenediamine N N N' N'-四 甲基二乙胺
Tris	tris(hydroxymethyl)aminomethane 三羟基甲基氨基甲烷

摘要

在细胞进行有丝分裂的过程中，染色体需要精确地被分离到两个子细胞内以维持基因组的稳定性。一旦动粒和微管发生错误连接或者动粒受到两端微管的拉力不均衡时，细胞就会激活 SAC 并阻止细胞进入后期，直至所有的染色体都被来自两极的微管正确连接，并发生双极定向之后，SAC 才能失活，进而解除对细胞中后期转换的抑制，促使姐妹染色单体的分离和有丝分裂的退出。

在裂殖酵母中，Dnt1主要定位在核仁里，在有丝分裂后期还定位在纺锤体及纺锤体极体上。我们实验室已有的研究发现Dnt1是一个具有多重功能的蛋白。首先，Dnt1通过与检验点蛋白Dma1相互作用并抑制其E3泛素连接酶功能，从而抑制胞质分裂。其次，Dnt1还通过下调Wee1蛋白水平，调控细胞G2/M转换。另外，我们最近还发现，Dnt1在纺锤体组装检验点沉默过程中发挥一定的功能。本研究旨在进一步研究Dnt1调控纺锤体组装检验点沉默的可能机制。

我们利用 $nda3-KM311\ cut2-GFP$ 和 $nda3-KM311\ cdc13-GFP/mCherry\ ark1-as3$ 纺锤体检验点沉默筛选系统，将酵母细胞在低温18°C阻断6小时后，释放到允许温度30°C，每隔10分钟采样计数Cut2蛋白（Securin）和Cdc13蛋白（Cyclin B）分别在细胞核内和SPB上的定位比例。通过这种方法我们发现：Dnt1对于纺锤体组装检验点沉默是必需的；人为定位在SPB上的Dnt1参与调控纺锤体组装检验点的沉默；Dnt1与Dis2在平行通路上调控纺锤体组装检验点沉默；Dnt1调控的纺锤体组装检验点沉默部分依赖于Dma1和 $slp1-mr63$ 。结合遗传学实验我们发现Dnt1和Apc15有可能在一条通路上调控检验点沉默。另外我们还发现过表达Slp1导致检验点沉默缺陷但不依赖于SAC的关键组分Mad2。

通过细胞生物学定位研究以及生化分析，我们发现了 $dnt1$ 缺失引起纺锤体组装检验点沉默缺陷的潜在机制。一方面，我们发现Dnt1有可能通过调控Apc15而影响APC/C的活性，另一方面，Dnt1通过下调Slp1蛋白水平而促进APC/C的激活进而调控检验点沉默。但是目前的研究并不能确定这两条途径之间的关系，具体的机制还不是很清楚。

关键词：Dnt1；纺锤体组装检验点；Dis2；有丝分裂检验点复合物；Apc15

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Abstract

During mitosis, the spindle assembly checkpoint acts to maintain genome stability by delaying cell division until all chromosomes have been segregated into two poles. When kinetochores are not correctly attached to the spindle or tension between sister kinetochores is not established, cells will activate the spindle assembly checkpoint network, which in turn blocks cell cycle progression. Only after all kinetochores are stably attached to the spindle and have established a bipolar orientation, the checkpoint is inactivated, which alleviates the cell cycle block and thus allows chromosome segregation and cell division to proceed.

In the fission yeast, Dnt1 mainly localizes in the nucleolus, and it also appears on the spindle and spindle pole body at anaphase. Our previous studies found that Dnt1 has multiple functions. Dnt1 inhibits cytokinesis through interacting with checkpoint protein Dma1 and suppressing its E3 ubiquitin ligase function. Dnt1 reduces Wee1 protein level to regulate G2/M transition. Recently we have found Dnt1 plays important roles in regulating spindle checkpoint silencing. This study aimed at finding the possible mechanism of spindle checkpoint silencing regulated by Dnt1.

In this study, we used the *nda3-KM311 cut2-GFP* and *nda3-KM311 cdc13-GFP/mCherry ark1-as3* silencing assay systems. Cells were arrested at 18°C for 6hr and shifted to permissive temperature of 30°C. By counting the percentage of nuclear Cut2-GFP or Cdc13-GFP/mCherry on SPB, we found that Dnt1 is required for spindle checkpoint silencing. Artificial targeting of Dnt1 at SPB affects SAC silencing. Dnt1 and Dis2 regulate SAC silencing through parallel signaling pathways. Dnt1-mediated SAC silencing partly depends on Dma1 and *slp1-mr63*. Besides, Dnt1 and Apc15 may regulate SAC silencing in the same pathway. Slp1 overexpression leads to SAC silencing defects independent of SAC protein Mad2.

Through the cell biology and biochemical analyses, we found that Dnt1 participates in the spindle checkpoint silencing by two ways. On one hand, Dnt1 may cooperate with Apc15 to regulate SAC silencing by influencing the activity of APC/C. On

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