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厦门大学

硕 士 学 位 论 文

裂殖酵母中 Dnt1 参与 DNA 损伤及修复的初步研究

A preliminary study on fission yeast Dnt1 participating in DNA damage and repair

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缩写词汇

APC	anaphase promoting complex　后期起始复合物
ATM	Ataxia Telangiectasia Mutated　毛细血管扩张性运动失调突变蛋白
ATR	Ataxia Telangiectasia Related　毛细血管扩张性运动失调相关蛋白
BIR	bread-induced replication　断裂引发的复制
CDK	cyclin-dependent kinase　周期蛋白依赖性蛋白激酶
CO	crossover　交叉互换
DDK	DBF4-dependent kinase　DBF4依赖的激酶
DDR	DNA damage response　DNA 损伤反应
D-loop	Displacement-loop　置换环
DSBR	double-strand break repair　双链断裂修复
DSB	DNA double-strand breaks　DNA 双链断裂
FPC	Replication fork protection complex　复制叉保护复合物
HJ	Holliday Junction　Holliday 连接体
HR	homologous recombination　同源重组
HU	hydroxyurea　羟基脲
IR	ionizing radiation　电离辐射
LOH	loss of heterozygosity　杂合性丢失
MCM	minichromosome maintenance proteins　微小染色体维持蛋白
NER	Nucleotide Excision Repair　核苷酸切除修复
NHEJ	non-homologous end-joining　非同源末端连接
ORC	Origin recognition complex　起始识别复合体
PIKK	phosphatidylinositol 3-kinase-like protein kinase　磷脂酰肌醇-3-激酶样蛋白激酶
pre-RC	prereplication complex　前复制复合物
RENT	regulator of nucleolar silencing and telophase exit　核仁沉默与末期退出调控
RFB	replication fork barriers　复制叉障碍区
RPA	Replication protein A　复制蛋白 A
SDSA	synthesis-dependent strand-annealing　合成依赖的链互补配对

SPB	spindle pole body　纺锤体极体
SSA	single-strand annealing　单链互补配对
SSB	single-strand breaks　单链断裂
Ter site	site-specific replication terminators　位点特异性复制终止子

摘　要

双链 DNA 断裂是 DNA 损伤的主要形式。DNA 损伤不仅发生在常染色质区，还发生在异染色质区，如 rDNA 区。

本实验室前期的结果发现，粟酒裂殖酵母(fission yeast，*Schizosaccharomyces pombe*)中核仁蛋白 Dnt1 在 rDNA 区有功能。在 *dnt1*Δ菌株中，以 *ura4*$^{+}$作为 rDNA 的报道基因，呈现 rDNA 沉默的解除和 rDNA 重组的增加。理论上，rDNA 作为一种重复序列，相当不稳定，彼此之间很容易发生同源重组。然而在细胞的长期进化过程中，产生了保护 rDNA 重复序列稳定性和完整性的内在机制。

本论文中，我们主要用 DNA 损伤试剂使 DNA 产生一定损伤，然后观察 Dnt1 与 DNA 损伤和修复相关蛋白之间的遗传关系。研究发现 Dnt1 在裂殖酵母 S 期是一个重要的蛋白。DNA 发生损伤，细胞会作出一系列的反应，这些反应需要复制叉相关蛋白，DNA 维持检验点蛋白，HR 修复蛋白，G2/M 调控蛋白等。

DNA 复制叉相关蛋白主要指复制叉保护复合物，包括 Swi1，Swi3。结果显示，Dnt1 与 Swi1-Swi3 在不同的通路发挥功能。DNA 维持检验点蛋白首先对 DNA 损伤作出反应，主要通过 DNA 复制检验点蛋白和 DNA 损伤检验点蛋白发挥功能。我们用 *dnt1*Δ与有关 DNA 复制检验点和 DNA 损伤检验点基因组成双突变菌株，结果发现，Dnt1 与 Rad1、Rad3、Crb2、Chk1、Cds1 都存在不确定的遗传关系，而与 Rad26 呈负的遗传相互作用。而且，我们发现 *dnt1*Δ完全逆转 *rad17*Δ菌株对 DNA 损伤试剂的敏感性，这就暗示 Dnt1 负调控 Rad17。机体主要通过同源重组(homologous recombination，HR)、非同源末端连接(non-homologous end-joining，NHEJ)对 DNA 损伤进行修复，而我们的结果显示 Dnt1 可能独立于 HR，NHEJ 修复发挥功能。另外，我们还探索了 Dnt1 与 Smc5-Smc6 复合物，DNA 聚合酶δ和ε等蛋白之间的遗传关系，都没有发现 Dnt1 与其有明显的作用。

另外，我们还发现无论 *ura4*$^{+}$正向还是反向整合到 rDNA 的不同区域，沉默结果都不同。并且还发现，结合于 RFB 的蛋白 Reb1 缺失之后，对 Dnt1 的定位没有影响，但是能够逆转 *dnt1*Δ菌株对 DNA 损伤试剂的敏感性。另外，与 RFB 有关的蛋白 Sap1，与 Dnt1 组成的双缺失菌株会严重影响细胞的生存能力，而且 *dnt1*Δ会影响 Sap1 的定位，而 *sap1-48*^{ts} 对 Dnt1 的定位并没有影响。

关键词：Dnt1；DNA 损伤；DNA 修复；

Abstract

DNA double-strand breaks is a main formation of DNA damage. DNA damage not only are generated in the euchromatic region, but also in the heterochromatin region, such as telomere domain and rDNA regions.

The early results of our lab demonstrated the nucleolar protein Dnt1 in fission yeast had relation to rDNA regions.The *dnt1*Δ mutant reduced the rDNA silencing and increased the rDNA recombination rates using the *ura4*$^{+}$ regarded as the reportor gene of rDNA repeat units. As such a large and repetitive locus, it's thought to be unstable, unequal recombination events occurring. However, cells have evolved mechanisms to protect the stability and integrity of rDNA.

In this paper, We wonder that how does Dnt1 participate in the DNA damage that were caused under the stress of DNA damage reagents using the genetic methods. Our results showed that Dnt1 is important during a perturbed S-phase in *S.pombe*. As is known to all, as soon as the damaged DNA were produced, the cells induced DNA damage response to cope with these assaults, but the reactions required lots of proteins, which including replication fork-associated proteins, DNA maintenance checkpoint proteins, homologous recombination proteins and G2/M regulation proteins.

Swi1 and Swi3 form the replication fork protection complex, which were the most important proteins of replication fork-associated proteins. As shown in our drop test, the Dnt1 and FPC do not function exclusively together. DNA damages were firstly responded with the DNA maintenance checkpoint proteins，which are made of the DNA replication checkpoints and DNA damage checkpoints. We constructed the double mutant strains between *dnt1*Δ and components of the DNA replication checkpoints and DNA damage checkpoints proteins. In our genetic analysis, there are some functions between Dnt1 and Rad1, Rad3, Crb2, Chk1, Cds1, but no genetic interactions with Rad26. Fortunately, The genetic results that *dnt1*Δ could rescue the *rad17*Δ sensitivity in response to different DNA-damaging agents suggesting that Dnt1 negative regulation Rad17. The damaged DNA were mainly repaired by

homologous recombination and non-homologous end joining. We found that Dnt1's functions are independent to HR and NHEJ repair pathway at least in response to these DNA-damaging agents. Besides, we had explored the genetic relationships between Dnt1 and Smc5-Smc6 complex, and DNA polymerase δ and ε et.cl, and observed that they could no genetic connections.

In addition to, we discovered that the silencing represent diverse levels however different regions of rDNA is integrated with the *ura4*$^{+}$ in forward or reverse directions. We also found that the locus of Dnt1 were not influenced by the *reb1*Δ, which associated with the RFB sites of rDNA regions. But, *the reb1*Δ could rescue the *dnt1*Δ sensitivity on these DNA-damaging agents. Sap1, which also was relative to rDNA's RFB. The *sap1-48*^{ts} *dnt1*Δ mutants showed a severe growth defect compare to the parent's mutant. Besides, the Dnt1 locations were not affected by the *sap1-48*^{ts} mutant, in contrast, the locus of Sap1 were influenced by the Dnt1.

Key word: Dnt1; DNA damage; DNA repair;