行政院國家科學委員會專題研究計畫　成果報告

酵母菌減數分裂中粗絲期檢控機制之研究

計畫類別：個別型計畫
計畫編號：NSC92-2311-B-002-102-
執行期間：92 年 08 月 01 日至 94 年 01 月 31 日
執行單位：國立臺灣大學分子與細胞生物學研究所

計畫主持人：董桂書

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 94 年 4 月 30 日

中文摘要

關鍵詞：減數分裂、細胞週期、粗絲期檢控點、酵母菌

減數分裂在高等生物有性繁殖中扮演關鍵性的角色，一次完整的減數分裂包含了一次 DNA 複製，卻有二次染色體分離，因此而能平衡子代染色體的數目。減數分裂過程中，藉由同源染色體的配對、互換，而使染色體得以正常分離。為確認配對和互換在第一次染色體分離前完成，減數分裂細胞週期中從粗絲期進入到第一次核分裂期的轉換受到粗絲期檢控點的嚴密監控。此檢控點的分子作用機制是我們研究的主要目標。

酵母菌在減數分裂時特定表現的轉錄活化因子 Ndt80 是驅動細胞進入第一次染色體分離期的必需蛋白。而 Ndt80 的多重磷酸化與減數分裂週期的正常進行又有直接的關聯，因此 Ndt80 蛋白質本身或是其磷酸化可能正是粗絲期檢控機制的直接作用點。但亦有研究報告認為粗絲期檢控點對 Ndt80 活性的檢控是透過 Sum1 轉錄抑制因子對於 *NDT80* 基因表現的調控而間接作用。我們在界定 Ndt80 功能區的研究中發現了一個顯性的片段缺失突變型，*NDT80-bc*。此一片段缺失使得 Ndt80-bc 蛋白似乎不再受粗絲期檢控點的控制而維持恆常活性。Ndt80-bc 提供了研究粗絲期檢控點的重要方向。

本研究計畫即針對 Ndt80-bc 與粗絲期檢控點間的作用關係，做進一步的分析，以確認 Ndt80-bc 的作用確實是一般性的不受粗絲期檢控點控制，而非只針對特殊突變體（如 *zip1*）。我們的實驗結果也充分支持 Ndt80 蛋白本身即是粗絲期檢控點的作用標的。但磷酸化與 Ndt80 功能之間的關係，目前尚未有明確的結論。此外，利用此缺失片段篩選與 Ndt80 蛋白質直接作用的檢控點蛋白質也已開始進行。這些實驗結果除了可以增進我們對減數分裂的了解，同時也能幫助我們進一步探討細胞週期，更可以應用在許多人類遺傳疾病和癌症的研究上。

Abstract

Keywords：meiosis, cell cycle, pachytene checkpoint, budding yeast

Meiosis is essential for sexual reproduction. To ensure the success of meiosis, meiotic checkpoints operate to coordinate the proper order of meiotic events. In particular, the pachytene checkpoint prevents exit from the pachytene stage of meiotic prophase when meiotic recombination and chromosome synapsis are incomplete. Our research is planned to study the molecular mechanism of the pachytene checkpoint in detail.

In budding yeast *Saccharomyces cerevisiae*, the *NDT80* gene encodes a meiosis-specific transcription activator that is required for progression from pachytene into meiosis I. Multiple phosphorylation of Ndt80 occur in wild-type cells, but it is inhibited in cells arrested at the pachytene stage by the pachytene checkpoint. Based on these results, we suggested that Ndt80 is a direct target of the pachytene checkpoint. On the other hand, other reports proposed that Ndt80 activity is regulated indirectly at a transcriptional level by the pachytene checkpoint through the Sum1 transcription repressor.

In our studies on defining Ndt80 functional domains, we have isolated a dominant deletion mutation, *NDT80-bc*. In this study, we have confirmed that the Ndt80-bc protein is resistant to the control of the pachytene checkpoint. It bypassed all the pachytene-arrested mutants tested, and it did not restore spore viability and crossover frequency. We proposed that the Ndt80 protein itself is a direct target of the pachytene checkpoint. The deleted region from Ndt80-bc might be the interacting site with an unidentified checkpoint protein(s). The Ndt80-bc provides a good evidence for our hypothesis and points out an important direction for the studies on pachytene checkpoint.

The information learned from this project will provide valuable information for the understanding of meiotic cell-cycle control. It is also useful and applicable to researches on mitotic cell cycle, human genetic diseases, and cancers.

I. Introduction

In eukaryotic organisms, the integrity of genetic information is maintained through the operation of cell-cycle checkpoints. Checkpoints ensure the proper order of events in mitotic cell cycle by arresting or delaying in response to defects in cellular process. As in the mitotic cell cycle, checkpoint machinery also operates in meiosis to ensure that one event does not occur until the preceding event has been completed. In particular, there is a checkpoint prevents exit from the pachytene stage of meiotic prophase when meiotic recombination and chromosome synapsis are incomplete. This “pachytene checkpoint” prevents chromosome damage or chromosome missegregation that would lead to the production of inviable or aneuploid gametes (Roeder 1997, Roeder and Bailis 2000). In the budding yeast *Saccharomyces cerevisiae*, mutants that confer defects in recombination (e.g., *zip1*, *dmc1*) cause cells to arrest at the pachytene stage of prophase (Bishop et al. 1992, Sym et al. 1993, Storlazzi et al. 1996, Tung and Roeder 1998). This arrest is triggered by defective meiotic recombination intermediates and is dependent on checkpoint genes, e.g., *RAD17*, *MEK1*, and *PCH2* (Lydall et al. 1996, Xu et al. 1997, San-Segundo and Roeder 1999). The *MEK1* and *PCH2* are meiosis-specific genes. Mutations in these checkpoint genes allow *zip1* and *dmc1* mutants to exit pachytene and to complete meiosis and to produce defective spores. However, the molecular mechanism for the pachytene checkpoint control is not clear.

The progression from pachytene into meiosis I is a critical step for meiosis. *NDT80* is a meiosis-specific gene. It was independently identified from screens for mutants defective in sporulation (Xu et al. 1995, Hepworth et al. 1998), and from a screen for multicopy suppressors of *zip1* sporulation defects (Tung et al. 2000). Further studies have shown that Ndt80 is a meiosis-specific transcription factor, it binds to middle sporulation element (MSE) and activates transcription of genes required for both spore formation and meiotic nuclear divisions, including *NDT80* itself and most B-type cyclins (Chu and Herskowitz 1998, Hepworth et al. 1998). The essential role of *NDT80* suggests that Ndt80 would be a central target of meiotic checkpoint machinery. It was proposed that the pachytene checkpoint controls the activation of Ndt80 is indirectly through its expression from the MSE-containing promoter (Lindgren et al. 2000, Pak and Segall 2002a, 2002b). The activated expression of *NDT80* is repressed by Sum1 protein, and the stability of the Sum1 protein is regulated

by the pachytene checkpoint. The Sum1 binds to MSEs and represses middle sporulation gene expression during vegetative growth (Xie et.al. 1999). The level of Sum1 decreases when middle genes are induced, and on activation of the pachytene checkpoint, Sum1 is stabilized and MSE containing genes, including *NDT80*, is repressed (Lindgren et al. 2000, Pak and Segall 2002a).

Recently, among a set of in-frame *ndt80* deletion mutations, we have isolated a very interesting dominant allele, *NDT80-bc* (*NDT80*-bypass checkpoint). This allele is insensitive to the control of pachytene checkpoint. In the presence of this allele, *zip1* mutants complete meiosis and spore formation to a wild-type level. This exciting result suggest that Ndt80 is a direct target to the pachytene checkpoint. In addition, it points out a new direction in studying the molecular mechanism of the pachytene checkpoint control.

II. Aims

The critical differences in the behavior of chromosome segregation and the control of cell cycle progression distinguish meiosis from mitosis. The detail mechanisms that control cell cycle progression and coordinates chromosomes segregation are not clear. However, as described above, Ndt80 plays a central role in coordinating these procedures. Exploring the detail of Ndt80 function and its regulation will be essential for understanding meiotic cell cycle control. The Ndt80-bc provides an excellent tool to study the regulation of Ndt80 by the pachytene checkpoint machinery. In this study, we were focused on characterizing Ndt80-bc suppression in detail and establishing the model that Ndt80 protein is the direct target to the pachytene checkpoint machinery

III. Research Design

A). Detail characterization of Ndt80-bc suppression

The *NDT80-bc* allele suppresses *zip1* mutants in sporulation to a wild-type level. This interesting result raises lots of questions need to be answered. Is this suppression a general effect to the pachytene checkpoint, or only to the defects caused by *zip1*? Does the *NDT80-bc* also suppress the defects of *zip1* in spore viability, crossover, and crossover interference, or not? All of these information are necessary for the understanding of Ndt80-bc.

1). Besides the original tested *zip1* mutants, there are mutations in other meiosis-specific genes also cause pachytene arrest due to different defects, e.g., *dmc1* and *hop2* (Bishop et al. 1992, Leu et al. 1998). Previous studies showed that suppression to these arrested mutants by other known suppressors are variable in extent, and hardly to the wild-type levels (San-Segundo and Roeder 1999, Leu and Roeder 1999, Pak and Segall 2002b). To test the suppression, we will perform gene replacement experiments using the pop-in/pop-out methods to test if Ndt80-bc bypasses pachytene checkpoint in general.

2). The *zip1* mutants cause pachytene arrest in some strain backgrounds (e.g., BR2495, W303), but *zip1* mutants do not arrest in all strains. In the SK1 strain background, *zip1* cells sporulate, however, crossover frequency is reduced and crossover interference is completely abolished. These defects result in a decrease in spore viability, mainly due to homolog nondisjunction (Sym and Roeder 1994). The *NDT80-bc* allele will be put into SK1 strains and test for spore viability, crossover and interference. This experiment will demonstrate that whether Ndt80-bc is only a checkpoint suppressor, or it may confer other unexpected effects in crossover or crossover interference.

B). The functional relationship between Ndt80 and Sum1

It was suggested that the MSE-repressor Sum1 is required for the pachytene checkpoint, based on the observation that mutation of *SUM1* allows *dmc1* strains to complete nuclear divisions (Lindgren et al. 2000). More recently, it was further suggested that the pachytene checkpoint directly controls the stability of Sum1, which in turn regulates the activated-level transcription of *NDT80*, i.e., the control of Ndt80 activity is mainly at the transcription level (Pak and Segall 2002a, 2002b). However in those studies, nuclear divisions in the *dmc1 sum1* cells only reached 30 to 60% of the wild-type level, and no normal spores formed (Lindgren et al. 2000, Pak and Segall 2002b). We think this data indicate that Sum1 does not play the central role in pachytene checkpoint control. On the contrary, in our *zip1 NDT80-bc* cells, both nuclear divisions and spore formation are equivalent to that of the wild type. We propose that post-translational regulation of Ndt80, but not the transcriptional regulation through Sum1, is the real major target of pachytene checkpoint,. More analyses are necessary to distinguish between the two models.

1). One possible reason for the difference between our results and that of the others may be the different strain backgrounds and the pachytene-arrest mutants used in the studies. We will clarify this difference by testing the suppression by *sum1* mutants in our strains. These reconfirmation experiments shall provide a standard point to discuss the control target of pachytene checkpoint.

2). If the Sum1/MSE model (Lindgren et al. 2000, Pak and Segall 2002a, 2002b) were correct, then the interpretation for our result would be that the basal amount of Ndt80-bc were more competitive or active than the wild-type Ndt80 protein in binding to MSEs. Accordingly, it is possible that an overproduction of the Sum1 protein shall overcome the Ndt80-bc effect and cause a significant decrease in sporulation of *zip1 NDT80-bc* or *dmc1 NDT80-bc* cells. On the other hand, based on our model, the Sum1 does not play a critical role. Overproduction of Sum1 will not confer pronounced effect on the Ndt80-bc suppression. Overproduction of Sum1 in an isogenic wild-type strain will be used as a control.

IV. Results and Discussion

1.). *NDT80-bc* is a general suppressor to pachytene-arrest mutants

We have tested the suppression ability of endogenous *NDT80-bc* in another two pachytene-arrest mutants, *dmc1* and *hop2* in the BR2495 strain background. The *NDT80-bc* restored sporulation of *dmc1* and *hop2* cells to the wild-type level, as in the *zip1* cells. This result indicates that *NDT80-bc* suppression is not specific to *zip1* defects, but is general to pachytene-arrest mutants, suggesting that Ndt80-bc is really insensitive or resistant to the pachytene checkpoint machinery.

2). *NDT80-bc* has no effect on crossover

We have performed tetrad analysis in WT, *NDT80-bc*, *zip1*, and *zip1 NDT80-bc* to assess the effect of NDT80-bc on crossover in two intervals, *MAT-CEN3* and *CEN3-HIS4*. There was no obvious difference in spore viability and crossover frequency between *NDT80* and *NDT80-bc* in both WT and *zip1* cells. This indicates that Ndt80-bc suppression is simply a due to bypassing pachytene checkpoint.

3). The *sum1* mutation does not bypass *zip1* in the BR2495 background

To compare the suppression ability of *sum1* to *NDT80-bc* in the same strain background, we put *sum1* into the *zip1* strain used to test *NDT80-bc* suppression and checked sporulation. However, sum1 did not suppress *zip1* at all. This result suggests that Sum1 is probably not a general regulation target to the pachytene checkpoint.

4). Overproduction of Sum1 does not affect *NDT80-bc* suppression

To further test our hypothesis of Ndt80 regulation, we overproduced Sum1 in the *zip1 NDT80-bc* cells. We found that the sporulation was not affected by Sum1 overproduction at all. This result supports our model that Ndt80 protein itself, but not Sum1, is the major target of the pachytene checkpoint.

V. Significance and Evaluation

Meiosis plays a central role in the sexual reproduction of eukaryotes. Regulation of cell cycle progression and control of chromosome segregation is critical for producing normal chromosome set in gametes. Defects in meiotic chromosome segregation are the primary causes of aneuploidy, which contributes significantly to infertility, reproductive failure, birth defects, and has been associated with various cancers. Dawn's syndrome (trisomy 21) is one of the well-known diseases that are caused by aneuploidy. Studies of meiotic cell cycle control and chromosome segregation can provide insight into the molecular basis of human disease due to chromosome abnormality.

In this study, we have isolated a very interesting mutation, the *NDT80-bc*. This deletion causes Ndt80 to become resistant to the control of the pachytene checkpoint, i.e., this dominant *NDT80-bc* allele can suppress the pachytene-arrested mutants in both nuclear divisions and spore formation to the wild-type level. This exciting result provides strong evidence for the idea that Ndt80 itself is a direct target of the pachytene checkpoint. This is different from the model proposed by several research groups. Our finding opens a new direction in studying the molecular mechanism of meiotic cell cycle control. We are currently writing a manuscript of these results for publication.

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