國立臺灣大學生命科學院生化科學研究所

碩士論文

Graduate Institute of Biochemical Sciences
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Master Thesis

闡釋 Mer2 與 DNA 結合之生化特性在減數分裂所扮演的角色

Deciphering the role of DNA binding activity of Mer2 in meiotic DNA double-strand breaks

柯曉涵

Hsiao-Han Ko

指導教授：冀宏源 博士

Advisor：Hung-Yuan Chi, Ph.D.

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摘要

減數分裂中，計畫性的雙股脫氧核糖核酸斷裂 (DNA double-strand breaks) 是必要的條件，缺乏雙股脫氧核糖核酸斷裂的染色體無法形成交叉 (chiasmata)，最終染色體不能正常分配至配子中，這會導致配子中染色體數目異常，造成配子死亡。在出芽酵母菌 (budding yeast) 中，至少有十個基因參與雙股脫氧核糖核酸斷裂的形成，而 Mer2 (meiotic recombination protein 2) 是其中之一。酵母菌遺傳研究發現，剔除 Mer2 的酵母菌其表現型為無法產生雙股脫氧核糖核酸斷裂，使減數分裂完的孢子無法存活。在 Panizza (2011) 等人的文獻研究指出，在減數分裂時，Mer2 會在染色體上聚集，並召集 Mei4 與 Rec114 形成複合體，最後促成雙股脫氧核糖核酸斷裂。作者更進一步推測 Mer2 可能具有結合雙股脫氧核糖核酸能力，但體內 (*in vivo*) 實驗無法證明 Mer2 是否能直接結合雙股脫氧核糖核酸。因此我們的實驗首先想要證實，Mer2 是否具有雙股脫氧核糖核酸結合能力；其次 Mer2 結合雙股脫氧核糖核酸的能力，是否在減數分裂中造成雙股脫氧核糖核酸斷裂扮演重要角色。我的初步實驗結果發現：(1) 利用純化出的 Mer2 蛋白運用膠體電泳位移分析 (DNA mobility shift assay)，證實 Mer2 的確能直接結合雙股脫氧核糖核酸。(2) 發現 Mer2 至少有兩個區域能結合雙股脫氧核糖核酸。我們進一步利用序列分析這兩段區域的鹼性胺基酸，並藉由 Mer2 鹼性胺基酸突變蛋白，希望在體外 (*in vitro*) 能找出 Mer2 結合雙股脫氧核糖核酸的主要鹼性胺基酸。另一方面，在體內實驗我們可以將雙股脫氧核糖核酸結合突變的 Mer2 基因送回缺乏 Mer2 的酵母菌中，觀察是否能回復孢子存活率，來證實 Mer2 結合雙股脫氧核糖核酸的能力，對於減數分裂產生雙股脫氧核糖核酸斷裂的重要性。

關鍵字：Mer2、減數分裂、雙股脫氧核糖核酸斷裂、同源重組、交叉

ABSTRACT

Programmed double-strand breaks (DSBs) are essential for a proper chromosome segregation during meiosis, because DSBs initiate physical connection between two homologous chromosomes through homologous recombination (HR). Dysegulation of DSBs formation can lead to the aneuploid of inviable gamete caused by abnormal chromosome disjunction. In the budding yeast, *Saccharomyces cerevisiae*, there are at least ten genes mediated the formation of DSBs including Mer2. The deletion of *mer2* in yeast exhibits no DSB formation and poor meiotic spore. Recent study by Panizza et al. (2011) further infers that Mer2 protein accumulates on chromosome and recruits it's interacting partners ; Mei4 and Rec114, to promote the formation of DSBs. However, it still remains largely unknown whether Mer2 itself possesses physical interaction with double-strand DNA and whether the DNA binding activity of Mer2 is prerequisite for generating DSBs. We aim to address this question by employing highly purified recombinant Mer2 protein for *in vitro* DNA binding analysis. Our results indicate that Mer2 is a DNA binding protein and possesses at least two DNA binding motifs. We further narrowed down the clusters of positive charge amino acids within Mer2 that contributes to its DNA binding ability, and currently in the progression of identifying those key residues. Besides identifying DNA binding defective mutant variants of Mer2, we will also examine whether those Mer2 mutants can rescue the phenotype of poor spore viability in the deletion of *mer2* strain. We have successfully generated *mer2* deletion in SK1 strain, and as reported, *mer2* null showed poor spore viability. In the future, we will delineate whether Mer2 DNA-binding defective mutant variants can rescue the *mer2* null phenotype by complementation experiments. Our findings will shield light on the mechanism of DNA binding property of Mer2 in contributing to make DNA double-strand breaks during meiosis.

keyword：Mer2、meiosis、DNA double strand breaks、homologous recombination、chiasmata

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CHAPTER 1：Introduction

1-1 The Biology of Meiosis

Most organisms propagate themselves by sexual reproduction, which increase the genetic diversity of offspring to adapt varied environmental challenges. Germ cells utilize meiotic processes to generate haploid gamete (Wilkins and Holliday, 2009) **(Figure 1)**. The molecular mechanism of meiosis is a sophisticated process involving DNA replication, homologous recombination, and chromosome dynamics. In meiosis, meiotic cells follow one round of DNA replication and two rounds of chromosome segregation during meiosis I and II. Meiosis I is divided into five phases as interphase I, prophase I, metaphase I anaphase I and telophase I **(Figure 2)**. Most importantly the meiotic recombination-mediated homologous chromosome exchange, which increases genetic diversity, and a proper chromosome segregation are performed during prophase I of meiosis I. The prophase I consists of (1) leptotene phase : including chromatin condensation and the formation of DSBs ; (2) zygotene phase : the formation of synaptonemal complex and homologous chromosome paring ; (3) pachytene phase : chromosome exchange by meiotic recombination ; (4) diplotene phase : completion of chromosome crossover and the formation of chiasmata ; (5) diakinesis phase : including nuclear envelope disappearance, the spindle formation and chromosome separation. Following completion of meiosis I, meiosis II is to

ensure sister chromosome can segregate properly. Eventually, meiosis produces four haploid gametes.

1-2 The Importance of Meiotic Chiasmata

Chiasmata are the cross-shapes of chromosomal structure, discovered by Frans Alfons Janssens in 1909 (Janssens, 1909), with two homologous chromosomes exchanging genetic materials between maternal and paternal chromosomes during chromosome crossover during meiosis I **(Figure 4)**. To successfully establish chiasmata, both synaptonemal complex (SC) and homologous recombination (HR) processes need to be coordinated properly.

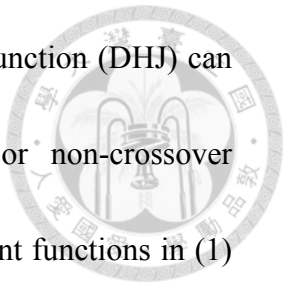
Before chiasmata formation, the synaptonemal complex (SC) mediated the pairing of homologous chromosomes plays an important role to assure the formation of chiasmata. SC complex is a protein complex that links two homologous chromosomes together and is divided by two parallel lateral regions (axial element) and a central element (Moses, 1958) **(Figure 3)**. The axial element consists of Scp2, Scp3, and forms chromosome axes with cohesin encircling sister chromatids (Schalk et al., 1998); and the central element consists of Scp1 to connect two parallel axial elements (Meuwissen et al., 1992; Schmekel et al., 1996) **(Figure 3)**. In addition to the formation of SC, homologous recombination is also required to establish the physical

connection between two homologous chromosomes. We will discuss the detailed mechanism of homologous recombination in the next section.

There are many functions of chiasmata during meiosis including : (1) physical connection between two homologous chromosomes ; (2) the proper chromosome orientation for spindle attachment ; (3) ensuring proper chromosome segregation ; and (4) enhancement of genetic diversity. Importantly, as expected, dysregulation of chiamata formation exhibits severe phenotypes including aberrant chromosome segregation, aneuploid, and spore inviability (Petronczki et al., 2003).

1-3 The Mechanism of Homologous Recombination (HR) in Meiosis

As mentioned above, the homologous recombination is required to generate chromosomal crossover structure, chiasma. In meiosis, homologous recombination initiated by programmed DNA double-strand breaks (DSBs) mechanisms. After the formation of DSBs, Rad50, Mer11, Xrs2, and Com1/Sae2 complex will process the breaks with 5’ to 3’ end resection to generate 3’ overhang single-stranded DNA (ssDNA). Then both Rad51 and Dmc1 recombinases polymerize on ssDNA to form a presynaptic filament. The presynaptic filament then invades into homologous duplex DNA to form a D-loop structure. Following by DNA synthesis and ligation the double holiday junction (DHJ) structure is formed. Holiday junction is a special chromosome



crossover structure, as chiasmata, in meiosis. The double holiday junction (DHJ) can then be resolved by resolvase to generate either crossover or non-crossover recombinant products **(Figure 5)**. Therefore, HR possesses important functions in (1) generating homologous chromosomal crossover, and (2) increasing genetic diversity (3) producing viable gametes (Kleckner, 1996; Lichten and Goldman, 1995; Roeder, 1995).

1-4 Formation of Double-Strand Breaks (DSBs) during Meiosis

Double-strand breaks (DSBs) appear at the initiation sites of meiotic recombination (Cao et al., 1990; Game et al., 1989; Sun et al., 1989). In general, DSB is generated in the loops which anchor along axis of chromosome (Blat et al., 2002) **(Figure 6.)**. There are at least ten genes is required for making DBSs formation including : Spo11 (Cao et al., 1990), Mei4 (Menees et al., 1992), Mer2 (Rockmill et al., 1995), Rec102, Rec104, Rec114 (Bullard et al., 1996), Ski8 (Ridley et al., 1984), Mre11 (Ajimura et al., 1993), Red50 (Alani et al., 1990) and Xrs2 (Ivanov et al., 1992). Each ten gene is essential for DSBs formation **(Figure 7) (Table 1)**. The possible roles of those DSB-forming components were summarized in Table 1. As mentioned previously the chiasmata established by DSBs-mediated homologous recombination, ensure chromosomes segregation properly. As expected without DSB formation leads to the

aneuploid and inviable gamete due to the consequence of abnormal chromosome disjunction.

Table 1. Genes are essential for DSB formation

S. cerevisiae gene	Function	Phenotype			Protein expression	Tag	M. musculus	H. sapiens
		DSB	Sporulation	Spore viability				
Spo11	Catalyzing DSB formation ;Transesterase, ortholog of TopoVI A	No	Decrease	Inviable	Meiosis	C term	Spo11	SPO11
Ski8	Required for DSB formation; involved in stabilizing chromatin localization of Spo11	No	Decrease	Inviable	Mitosis (interact with Ski2 and Ski3, result in RNA degradation) Meiosis	C term	WDR61	WDR61
Mer2	Required for DSB formation ; Axis associated, phosphorylated by Cdc28, required for Mei4 and Rec114 loading.	No	Decrease	Inviable	Meiosis	C term	?	?
Mei4	Required for DSB formation	No	Efficiently	Inviable	Meiosis	C term	Mei4	MER4
Rec114	Required for DSB formation ; Spo11 association to chromatin, axis associated	No	Decrease	Inviable	Meiosis	C term	Rec114	REC114
Rec102	Required for DSB formation ; Spo11 dimerization and binding to chromatin	No	Decrease	Inviable	Meiosis	C term	?	?
Rec104	Required for DSB formation; Spo11 dimerization and binding to chromatin	No	Decrease	inviable	Meiosis	N term	?	?
Mre11	Required for DSB formation, Spo11 removal, end processing and checkpoint activation ; endonuclease and exonuclease activity	No	Decrease	low spore viability	Meiosis/ Mitosis		Mre11	MRE11
Xrs2	Required for DSB formation and processing, checkpoint activation	No	Decrease	low spore viability	Meiosis/ Mitosis		Nbs1	NBS1
Rad50	Required for DSB formation and processing, checkpoint activation; DNA binding, ATP binding	No	Decrease	low spore viability	Meiosis/ Mitosis		Rad50	RAD50

This table is cooperated with Chia-Chia Hsu.

1-5 Spo11-mediated DSBs Machinery

Among DSB-forming machinery, Spo11 is believed in directly participating DNA cutting due to (1) Spo11 shares homology with archaea topoisomerase type VI subunit Top6A (Diaz et al., 2002) and (2) the catalytic Tyrosine135 of Spo11 is critical for the formation of DBSs (Keeney et al., 1997). Moreover, it has been documented that Spo11 covalently links to DNA forming Spo11-oligonucleotide complex in budding

yeast **(Figure 8)**. Consistent with the finding in yeast, the mSpo11-associated oligonucleotide complexes with length between 12-26 and 28-34 nucleotides were also identified in mouse testis (Neale et al., 2005).

Spo11 is evolutionally conserved from yeast, mouse to human. In contrast to have only one Spo11 isoform in yeast, mouse and human harbor two spliced Spo11 isoforms. Both Spo11α and β forms contain catalytic tyrosine residue; however, the mouse genetic data indicated that Spo11β is the one making DSBs due to Spo11 β transcripts reaching maximum levels in early stages (Bellani et al., 2010).

1-6 The Functional Role of Mer2 the Formation of Meiotic DSBs

In the budding yeast, at least ten genes are prerequisite generating DSBs including Mer2. Meiotic Recombination protein 2 (Mer2), also known as Rec107 is located on the right arm of chromosome X. Mer2 is initially identified as a suppressor of *mer1Δ*, which defected in intragenic recombination, by overexpression of Mer2 cDNA (Engebrecht et al., 1990). It turns out that Mer1 and Nam8/Mre2 are required for efficient Mer2 mRNA splicing to form a mature mRNA for its function (Engebrecht et al., 1991; Nakagawa and Ogawa, 1997; Nandabalan and Roeder, 1995; Ogawa et al., 1995). *mer2Δ* exhibits：(1) reduction of synaptonemal complex formation and homologous DNA pairing ; (2) failure to form DNA double strand breaks ; (3) no

meiotic interchromosomal recombination ; (4) poor meiotic spore viability (Rockmill et al., 1995). Although yeast phenotypes indicated that Mer2 is essential for meiosis, up till now no Mer2 ortholog have been identified in mouse and human. Moreover, Mer2 is directly phosphorylated at Serine30 (S30) by Cdc28-Clb5/Clb6 and mutation Mer2 at Serine 30 to Alanine showed no DSB formation and a reduced spore viability (Henderson et al., 2006). Further studies demonstrated that phosphorylation of Mer2 is required to recruit Mei4 and Rec114 to form a complex and then to generate DSBs **(Figure 9)** (Panizza et al., 2011).

Recent studies have demonstrated that Mer2 prefers to bind axis sites of chromosome by ChIP (chromatin immunoprecipitation)-sequencing with myc antibody for Mer2-myc (Panizza et al., 2011). Moreover, Mer2 interacts with Spp1 which binds H3K4me3 on chromatin loop and tethers chromatin loop to axis sites resulting in Spo11-mediated cleavage at near nucleosome-depleted region (NDR) (Acquaviva et al., 2013; Sommermeyer et al., 2013).

1-7 Motivation of My Thesis Studies

Genetic studies clearly documented that Mer2 is essential for induction of DSBs and required for spore viability in SK1 strain. Furthermore, biochemical fractionation experiments and ChIP analyses also indicated that Mer2 is enriched in axis sites of chromosome during meiosis. However, it remains unknown whether Mer2 directly binds DNA and what is the role of DNA binding property of Mer2 in making DSBs. To address this question, we established the expression and purification system to obtain a recombinant Mer2 protein. Our initial findings suggest that Mer2 physical binds double-stranded DNA and harbors at least two DNA binding domains. We are in the progression to identify DNA-binding defective mutants. Our final goal is to use these DNA binding mutants to further address whether DNA binding property plays an essential role for Mer2 to make DSBs *in vivo* **(Figure 10)**.

CHAPTER 2：Materials and Methods

2-1 DNA substrates

To prepare linear double strand DNA, the 5386 bases pair of ΦX174 RF I supercoiled dsDNA (New England Biolabs) was treated with PstI restriction enzyme (NEB) at 37℃ overnight. Then the linear dsDNA was purified by the QIAquick PCR purification kit (Qiagen).

2-2 *E. coli* strain

(1) ECOS101/DH5α (YE607-J) is used to amplify and stock plasmids (Yeastern Biotech Co., Ltd).

(2) Rosetta/DE3 (YE437-J) with pPARE (CamR) plasmid that supply tRNA for mammal codons is used for protein expression (Yeastern Biotech Co., Ltd).

2-3 Plasmids

We note that there is one amino acid polymorphism in Mer2 at 43th position between S288C (A) and SK1 (T). S288C Mer2 full-length cDNA was cloned into pGEX6P-1 expression vector with GST tag in N terminal and six Histindins tags in C terminal. The truncated Mer2 variants were cloned into pET-Dutet vector (Novagen) by NcoI and EcoRI restriction sites. To make site-specific mutations in Mer2, we used

site-directed mutagenesis method to change either Lysine (K) or Arginine (R) codon to Alanine (A). All plasmids were sequenced to make sure that sequences are correct, in frame, and no other unwanted mutations.

2-4 Protein expression and purification

2-4.1 GST-Mer2-His expression and purification

GST-Mer2-His expression : 65.1kD

GST-Mer2-His plasmid was transformed into Rosetta cells. The cells were grown on Ampicilin (Amp, 100μg/ml) and Chloramphenicol (Cam, 34μg/ml) Luria broth (LB) plate at 37°C incubator. Single colony was then cultured Amp/Cam LB liquid overnight. Following by diluting 25-50 folds of overnight culture in Amp/Cam LB liquid to let cell continue to grow until the A600 reached 0.6-0.8, at which time IPTG was added to final 1mM to induce protein expression. Cells were collected by centrifugation after a 3hr incubation.

GST-Mer2-His purification

10g GST-Mer2-His pellets were dissolved in cell breakage buffer (25 mM Tris-HCl, pH 7.5, 10% glycerol, 500 mM KCl, 0.01% Igepal, 2 mM β-mercaptoethanol) with 0.5 mM EDTA, 1 mM Benzamidine, 0.1 M PMSF, and 3 μg/ml following protease inhibitors: Aprotinin, Chymostatin, Leupeptin, Pepstatin A,

and Bestatin. Resuspended cells were then broken by sonication. After ultracentrifugation (100,000 X g for 60 min), the clarified lysate was incubated with Glutathione Sepharose 4 (GST) resins (GE) for overnight. All the purification steps were carried out at 4°C. After overnight incubation, the GST resins were wash by buffer A (25 mM Tris-HCl, pH 7.5, 10% glycerol, 0.01% Igepal, 2 mM β-mercaptoethanol) containing 500 mM KCl, and then the bound proteins were eluted with buffer A containing 150 mM KCl and 15mM glutathione. Then, the eluted fractions were incubated with Talon resins (Clontech) for 3hr. After 3hr incubation, the Talon resins were wash by buffer A containing 150 mM KCl, and then the bound proteins were eluted with buffer buffer A containing 150 mM KCl and 200 mM imidazole. The Mer2-containing fractions were collected and diluted with 1:1 volume ratio of buffer A. Followed by chromatographic fractionation in Source Q column (GE). The Mer2-containing fractions were pooled and concentrated to 5-15 mg/ml in buffer A containing 300 mM KCl in a Centricon-30 concentrator (Millipore). The concentrated preparation was divided into small aliquots and stored at -80°C.

2-4.2 Mer2 1-202-His expression and purification

Mer2 1-202-His expression : 23.5kD

Expression of Mer2 1-202-His fragment was followed the same transformation and

expression protocols as full-length Mer2, except induction with 0.2 mM IPTG at 16°C for 14-16 hr, as described above.

Mer2 1-202-His purification

16g Mer2 1-202-His pellets were disrupted and the clarified lysate was incubated with Talon resins as described above as full-length Mer2. The Mer2 1-202-His-containing fractions were collected and then followed by chromatographic fractionations in Source Q and hydroxyaptite columns (GE). The Mer2 1-202-containing fractions were pooled and concentrated to 5-15 mg/ml in buffer A containing 300 mM KCl in a Centricon-30 concentrator. The concentrated preparation was divided into small aliquots and stored at -80°C.

2-4.3 Mer2 1-202-His AATA expression and purification

Mer2 1-202-His AATA expression : 23.4kD

Expression of Mer2 1-202-His AATA variant was followed the same transformation and expression protocols as Mer2 1-202 His fragment as described above.

Mer2(1-202a.a)-His AATA purification

Purification procedures of Mer2 1-202-His AATA variant was followed the same purification protocols as Mer2 1-202-His fragment as described above.

2-4.4 Mer2 203-314-His expression and purification

Mer2 203-314-His expression : 14kD

Expression of Mer2 203-314-His fragment was followed the same transformation and expression protocols as full-length Mer2 as described above.

Mer2 203-314-His purification

Purification procedures of Mer2 202-313-His fragment was followed the same purification protocols as Mer2 1-202-His fragment as described above.

2-4.5 Mer2 203-314-His AQAAA expression and purification

Mer2 203-314-His AQAAA expression : 13.7kD

Expression of Mer2 203-314-His AQAAA variant was followed the same transformation and expression protocols as Mer2 203-314-His as described above.

Mer2 203-314-His AQAAA purification

Purification procedures of Mer2 203-314-His AQAAA variant was followed the same purification protocols as Mer2 203-314-His fragment as described above.

2-4.6 Mer2 203-314-His AAAA expression and purification

Mer2 203-314-His AAAA expression : 13.7kD

Expression of Mer2 203-314-His AAAA variant was followed the same

transformation and expression protocols as Mer2 203-314-His as described above.

Mer2 203-314-His AAAA purification

12g Mer2 203-314-His AAAA pellets were disrupted and the clarified lysate was incubated with Talon resins as described above as full-length Mer2. The Mer2 203-314-His-AAAA-containing fractions were collected and then followed by chromatographic fractionations in Source S (GE) and hydroxyaptite columns. The Mer2 203-314-His-AAAA-containing fractions were pooled and concentrated to 5-15 mg/ml in buffer A containing 300 mM KCl in a Centricon-30 concentrator. The concentrated preparation was divided into small aliquots and stored at -80°C.

2-4.7 Mer2 1-136-His expression and purification

Mer2 1-136-His expression : 15.8kD

Expression of Mer2 1-136-His fragment was followed the same transformation and expression protocols as full-length Mer2 as described above.

Mer2 1-136-His purification

Purification procedures of Mer2 1-136-His fragment was followed the same purification protocols as Mer2 1-202-His fragment as described above.

2-5 DNA mobility shift assay

The indicated amounts of full-length, truncated, and specific mutant variants of Mer2 proteins were incubated with the 5kb of ΦX174 linear DNA in 10 μl of reaction buffer (35 mM Tris-HCl, pH 7.5, 1 mM DTT) at 37°C for 10 min. The reaction mixtures were run in 1% agarose gel in TBE buffer at 4°C. The DNA species were stained with ethidium bromide and detected by gel documentation station (Bio-Rad).

2-6 Yeast strains

SK1 strains were obtained from Dr. Kuei-Shu Tung (Institute of Molecular and Cellular Biology, National Taiwan university).

(1) SK1a

The other name : YBY3, NKY2997, TEF411

Genotype : ho::hisG, lys2, leu2::hisG, arg4-nsp, ura3

(2) SK1α

The other name : YBY3, NKY2997, TEF412

Genotype : ho::hisG, lys2, leu2::hisG, arg4-nsp, ura3

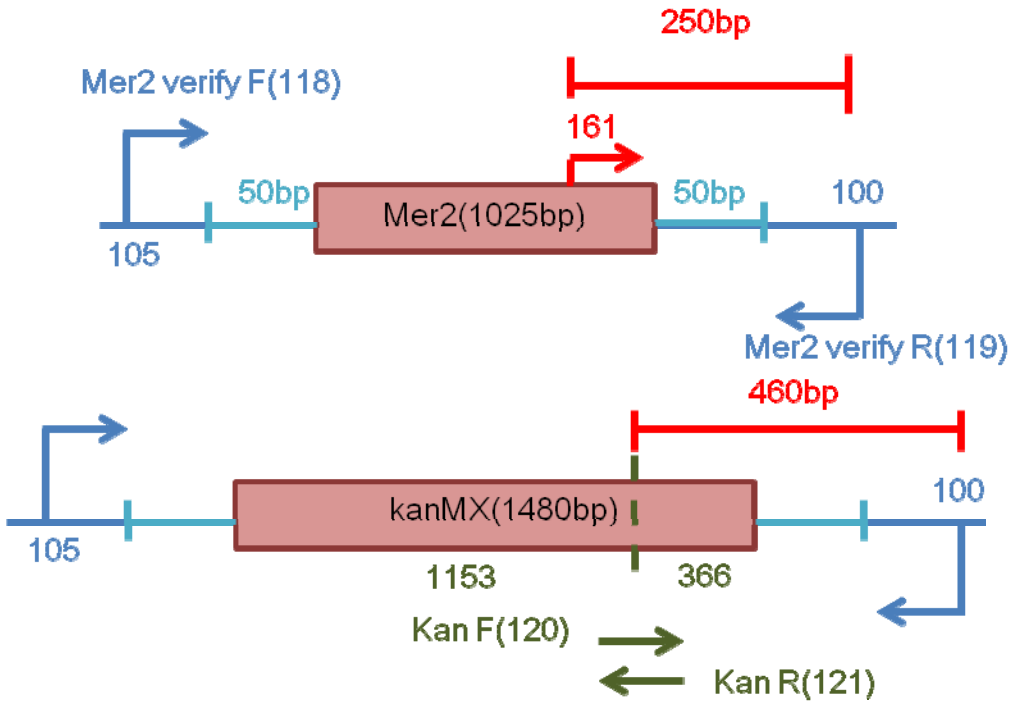
2-7 Yeast *MER2* gene deletion

2-7.1 Lithium transformation

Reference : Transformation of intact yeast cells treated with alkali cations (Ito et al.,

1983). We followed the standard yeast transformation protocol to perform *MER2* gene deletion.

2-7.2 Verify knockout primer design Primer designs to verify *MER2* gene deletion status in SK1



PCR wild-type：primer161+119=250bp

PCR knockout：primer120+119=460bp

2-7.3 Colony polymerase chain reaction

A single colony was dissolved into 10 µl filtered water. Followed by adding 2µl Lyticase (5U/µl) (SIGMA), the reaction mixtures were incubated digest cell wall at 30°C for 30 min. Cells were then disrupted through freeze and thaw cycles. A 2.5 µl

cell lysate was used for PCR reaction with a standard PCR protocol. The PCR products were run in 1% agarose gel in TBE buffer, and then the DNA species were stained with ethidium bromide and detected by gel documentation station (Bio-Rad).

2-7.4 Tetrad dissection

5 single colonies on G418 YPAD plate were replicated to SPO plate (2% lithium acetate, 0.2% yeast extract, 0.1% glucose, 0.1% amino acid mix and 2% agar) at 30 °C for 2～3 days. Usually it takes 3 days for ascus formation. After make sure ascus formation under the light microscopy, we will then use a toothpick to pick up a tip of sporulating cells into digestive buffer containing glusulase (SINGER) to remove ascus wall at 25°C for 15 min. The cell mixtures were gently mixed and then put few drops onto a YPAD plate. Spread out the cells in the centerline of plate with the flat end of a toothpick and then the cells were ready for tetra dissection under the microscope by Dr. Kuei-Shu Tung.

CHAPTER 3：Results

3-1 Expression and purification of recombinant Mer2 protein

There was one amino acid polymorphism at A43T between S288C and SK1 strains **(Figure 11A) (A-5)**. First, we expressed yeast S288C Mer2 full-length protein which had GST tag in amino-terminus and 6 Histidine tags in carbon-terminus and induced in *E. coli* with 1 mM IPTG and in 37 degree for 3hrs inductions. This Mer2 full-length protein purified by affinity column and ionic exchange column **(Figure 11B, panel I)**. First step, GST beads eluted by 15mM glutathione. Second step, Talon beads eluted by 200mM imidazole. Third step, Source Q column eluted by 0-50% of 1000mM T buffer. The purity of Mer2 full-length protein was 50% but no contamination with nuclease **(Figure 11B, panel II)**.

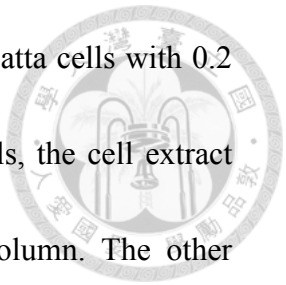
3-2 Mer2 harbors DNA binding property

It has been shown that Mer2 associates with chromatin and enriched in DSBs hotspot by biochemical fractionation and ChIP experiments (Henderson et al., 2006), suggesting that Mer2 may potentially interact with DNA. Interestingly, Mer2 has no consensus DNA binding motifs in their primary amino acid sequence. It raises a question whether Mer2 can bind DNA directly or associate with chromatin through a third protein such as histone. For this reason, we want to examine whether our

purified recombinant Mer2 protein can physically interact with duplex DNA by *in vitro* DNA mobility shift assay. To do so, we use a linear 5 kb duplex DNA to measure the DNA binding ability of Mer2 protein. As shown in Figure 11, recombinant Mer2 protein exhibits a dosage-dependent DNA binding activity **(Figure 11C, panel II, lanes 2-5)**. Moreover, we also incubated the protein-DNA complex with SDS and protease K **(Figure 11C, panel II, lane 6)** to digest the protein and demonstrated that the DNA band shifting was due to the Mer2 binding. Taken all together, our results conclude that Mer2 possesses the ability to bind duplex DNA.

3-3 Mer2 possesses at least two DNA binding domains

Next, we wish to determine the DNA binding domain of Mer2 protein. To do so, we made different truncation variants of recombinant Mer2 protein to examine their DNA binding affinity by DNA mobility shifting assay. Mer2 protein consists of 314 amino acids and several α helix and β sheet through on-line secondary structure prediction **(A-1)**. Based on the secondary structure prediction, we simply divided Mer2 into two fragments without disrupting their secondary structures: Mer2 1-202 and Mer2 203-314 variants **(Figure 12A)**. We have successfully made those expression constructs with 6 Histidine tags on the carbon-terminus and expressed them in the *E. coli* expression system. Both Mer2 truncation variants have a different expression and



purification pattern. Briefly, Mer2 1-202 protein is induced in Rossatta cells with 0.2 mM IPTG and in 16 degree for 16 hrs inductions. After lyses cells, the cell extract purified by Talon beads, Source Q column and hydroxyaptite column. The other truncation Mer2 203-314 protein is induced in Rossatta cells with 1 mM IPTG and in 37 degree for 3 hrs inductions then purified by Talon beads, Source Q column and hydroxyaptite column. We can obtain a near 95% homogeneity of Mer2 truncation proteins with the expected protein size **(Figure 12B)**. We also confirmed those Mer2 truncation variants by Western blot with anti-Histidine antibody. Importantly, there is no nuclease contamination in our protein preparation.

To test the DNA binding affinity of those Mer2 truncation variants. As shown in Figure 12C panel I and II, both Mer2 1-202 and Mer2 203-314 variants exhibit a dosage-dependent DNA binding activity. We note that in the same protein concentration, Mer2 203-314 has a higher DNA binding affinity than Mer2 1-202 **(Figure 12C, panel II, comparing lanes 3 with 7)**. In summary, our results demonstrated that Mer2 possesses at least two DNA binding domains.

3-4 Mer2 1-136 protein fragment is devoid of DNA binding activity

In order to isolate the DNA binding defective variant of Mer2, we wish to further narrow down the DNA binding residues of Mer2 protein. As we know that

protein-DNA interaction mainly through (1) the ionic interaction with negative phosphate backbone of DNA and/or (2) stacking interaction through hydrophobic residues of protein with the DNA bases **(A-2)**. Interestingly, we found that the primary amino acid sequence of Mer2 protein exhibits three significant clusters of basic amino acid residues including Lysine (K) and Arginine (R) as shown in Figure 13A. One basic cluster is located in Mer2 1-202 and the others are located in Mer2 203-314 **(A-2, part III)**. We suspect that those basic amino acid clusters may contribute the DNA binding activity of Mer2 protein. To examine this idea we first examine whether Mer2 1-136, which has no basic amino acid cluster, is capable of binding DNA. We constructed Mer2 1-136 expression plasmid and expressed it in *E. coli* expression system as described for Mer2 truncation variants in above section. Through a series of ionic chromatography and affinity column we can obtain a more than 95% purity of Mer2 1-136 fragment **(Figure 13B)**. Importantly, Mer2 1-136 lacks significant DNA binding activity **(Figure 13C, comparing lanes 2 with 6)**.

3-5 Basic amino acid clusters of Mer2 contribute the DNA binding ability

As discussed above, we identified Mer2 protein harboring three significant basic amino acid clusters and Mer2 1-136 is devoid of binding DNA. It raises a possibility

that Mer2 could potentially bind dsDNA through those basic clusters. To explore this possibility, we aim to firstly identify which basic cluster has a higher DNA binding affinity. To examine this idea, we systematically generated three mutant variants that are specifically defective in each basic amino acid cluster: Mer2 1-202 AATA, Mer2 203-314 AQAAA and Mer2 203-314 AAAA. We utilized a site-direct mutagenesis approach to mutate basic amino acid Lysine (K) and Arginine (R) to Alanine (A) on individual Mer2 fragments **(Figure 14A-B and Figure 15A-B)**. We have successfully obtained those mutant variants and expressed them in *E. coli* system. Moreover, we can purify those mutant variants to near 95% homogeneity as their wild-type counterpart **(Figure 14C and Figure 15C)**. Again, there is no nuclease contamination during protein preparation.

Next, we wish to determine the DNA binding affinity of those mutant variants. As shown in Figure 14D, Mer2 1-202 AATA mutant is significant defective in DNA binding ability compared to their wild-type counterpart in the same protein concentration **(Figure 14D, comparing lanes 4 and 7)**. However, in the higher protein concentration, Mer2 1-202 AATA mutant is still capable of interacting with DNA **(Figure 14D, lane 5)**. Like Mer2 1-202 AATA mutant, Mer2 203-314 AQAAA mutant variant also significantly reduces their DNA binding activity in comparing to their wild-type counterpart in the same protein concentration **(Figure 15D, panel I,**

comparing lanes 2 and 7). Again, Mer2 203-314 AQAAA variant possesses a residue DNA binding capability in higher protein concentrations **(Figure 15D, panel I, lane 5)**. In contrast, Mer2 203-314 AAAA variant is devoid of any detectable DNA binding in the condition we have tried **(Figure 15D, panel II)**. In conclusion, defective in either of three basic amino acid cluster of Mer2 cause a significant attenuation of DNA binding ability.

3-6 Establish the Mer2 deletion in *Saccharomyces cerevisiae* SK1 strain

Our long-term goal is to address whether the DNA binding activity of Mer2 plays a role in making double-strand break during meiosis. As mentioned above, we will firstly identify the DNA binding mutant of Mer2 and then perform genetic complementation experiments to address whether DNA binding defective Mer2 is still capable of rescue the meiosis phenotype as efficiently as their wild-type counterpart. To do so, we generated a Mer2 deletion in *Saccharomyces cerevisiae* SK1 strain, which is used in meiosis study. We deleted *MER2* gene in yeast by kanMX with *MER2* gene up and down homologous sequence (50 nucleotides each side). That used kanMX fragment to displace *MER2* gene in yeast genome **(Figure 16A)**. The deletion of *MER2* gene was verified by colony PCR with three primers **(Figure 16B)**. The size

of DNA band for wild-type is 250bp and deletion is 460bp **(Figure 16C)**. It has been well documented that Mer2 deletion has no DSBs, defective in sporulation and spore inviability (Henderson et al., 2006). To verify the phenotype of our *mer2* null strain, we made a *mer2* null in a diploid zygote and then transferred to SPO plate with low nutrient to induce meiotic sporulation **(Figure 16D)**. The spore viability are scored colony in 48 spores of 12 ascus. As shown in Figure 16E, the spore viability was poor which only had 0% spore viability compare to wild-type spore had 92% spore viability **(Figure 16E)**.

CHAPTER 4：Conclusion and Discussion

4-1 Summary of Key Findings

DSB is required to initiate meiotic recombination which is essential for the formation of chiasmata and a proper chromosome segregation during meiosis. It is known that Mer2 participates generation of DSBs and accumulates at chromosome axis sites, indicating that the possibility of Mer2 possesses a DNA binding ability. However, due to the limitation of obtaining the Mer2 protein, it remains largely unknown whether Mer2 harbors the ability to bind DNA and the role of this DNA binding activity in making DSBs. To address this question, we have purified the recombinant Mer2 protein and examined its DNA binding activity. We found that (1) Mer2 belongs a DNA binding protein and directly binds duplex DNA, and (2) Mer2 possesses at least two DNA binding domains **(Figure 17)**.

Both truncated Mer2 variants: Mer2 1-202 and 203-314 harbor the DNA binding ability, and Mer2 203-314 has a better DNA binding affinity than 1-202. Moreover, the DNA binding domain of Mer2 is further narrow down to 137-314 fragment based on that Mer2 1-136 lacks the DNA binding ability. We further identify three basic amino acid clusters within Mer2 contributing to the DNA binding activity **(Figure 17)**. Because mutations in individual cluster exhibit a different degree of DNA binding deficiency compared with their wild-type counterpart. We are currently introducing

individual cluster mutation into the full-length of Mer2 to see which mutant variant has a dramatically effect in abolishing Mer2 DNA binding activity. Our long-term goal is to identify a Mer2 mutant that is devoid of DNA binding activity, and then address whether the DNA binding defective mutants can rescue the inviable spore phenotype in *mer2* null SK1 strains.

4-2 Discussion and Future Direction

4-2.1 The DNA binding property of Mer2: sequence or/and structure specificity

In general, the DNA binding protein interacts with DNA either by sequence-specific interaction such as transcriptional factor, or by no sequence-specific interaction such as histone. ChIP (chromatin immunoprecipitation)-sequencing with myc antibody indicates that Mer2-myc is enriched in axis sites of meiotic chromosomes (Panizza et al., 2011). The properties of axis sites are locally AT-rich and association with protein complexes including cohesin (Blat and Kleckner, 1999; Blat et al., 2002; Glynn et al., 2004). There are two possibilities for Mer2 localization at axis sites. One is Mer2 binding to axis site thorough interacting with axis sites' protein components, such as cohesin or synaptonemal complex (SC). The other possibility is Mer2 preferentially to bind AT-rich DNA sequence. In our DNA mobility shift experiments, we used a random sequence of duplex DNA to monitor the DNA

binding activity of Mer2. Even though we find Mer2 binds DNA, we can't rule out the possibility that Mer2 possesses a higher DNA binding affinity with AT-rich DNA sequence. In the future experiments we will amplify the same lengths of DNA with sequences specific to axis sites, to DSB sites or to chromatin loops by PCR, and then examine the DNA binding affinity of Mer2 to those DNAs. If Mer2 binds DNA without sequence-specificity and is enrich in axis sites, it suggests that the preferential axis sites binding of Mer2 stems from the local chromosome structures or through interacting with unknown axis sites' protein components **(A-3)**.

4-2.2 Biochemical property of Mer2 phosphorylation

During meiosis, Mer2 is phosphorylated at Serine 30 (S30) by Cdc28-Clb5/Clb6 (Henderson et al., 2006). The phosphorylation is essential for Mer2 to recruit Rec114 and Mei4 to form a complex on axis site of meiotic chromosome. Further, the RMM complex contributes to the formation of DSBs. Moreover, although Mer2 phosphorylation is required for the RMM formation it does not affect the accumulation of Mer2 in the axis sites (Henderson et al., 2006). These findings indicate that Mer2 can recruit to axis site without phosphorylation. Consistent with the *in vivo* observations, we purified recombinant Mer2 from *E. coli* expression system without any phosphorylation and it still binds dsDNA avidly. Our results

further emphasizing that the phosphorylation of Mer2 is mainly to recruit its interacting partners rather than alters Mer2 DNA binding affinity.

4-2.3 The stoichiometry of Mer2 protein

It has been documented that Mer2 can interact with itself by yeast two hybrid analyses (Arora et al., 2004; Henderson et al., 2006). It raises an interesting question whether Mer2 forms a dimer or oligomer complex. Since our purified recombinant Mer2 protein has a GST tag, which forms a dimer in solution, it prohibits us to further address the stoichiomertric ratios of Mer2 by gel filtration experiment. We are currently in the progression of expressing and purifying recombinant Mer2 protein without any tags. In this way we can solve the stoichiometry of Mer2 protein in the near future. Obtaining the stoichiometry ratio will assist us to delineate the DNA binding property of Mer2. Moreover, it will be interesting to investigate whether interaction with Mei4 and Rec114 will inhibit the oligomerization of Mer2.

4-2.4 Complementation experiments with DNA-binding defective mutants of Mer2

To address the function of DNA binding ability of Mer2 in contributing to make DSBs, we aim to perform the rescue experiments with DNA-binding defective

mutants of Mer2 that we identify from *in vitro* study. It has been well documented that deletion of Mer2 in SK1 strains exhibit no DSBs and lead to inviable spores. We will perform the genetic complementation experiments with spore viability as a readout to indicate the formation of DSBs. If Mer2 mutants fail to rescue the inviable spores, it reveals that the DNA binding ability of Mer2 plays an important role to make DSBs formation. We will also perform ChIP analyses to confirm that our Mer2 mutants are not in the axis site.

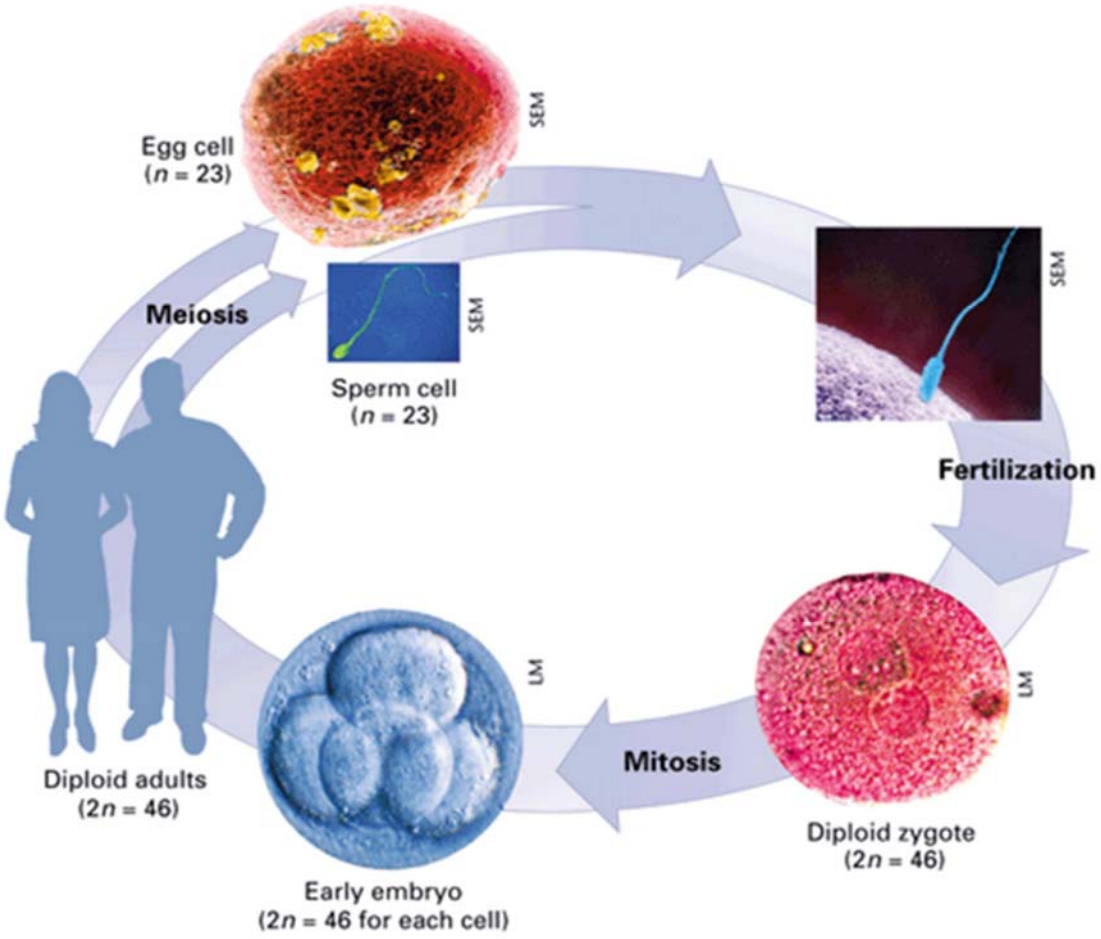
We also notice that one of positive charge cluster, KRRR at the positions 265-268 of Mer2, is also predicted as a nuclear localization signal (NLS) **(A-4)**. If later we decide to use this particular mutant for our complementation experiment, we will also examine whether this mutant variant can shuttle to nucleus by immunostaining.

4-2.5 The biochemical properties of Mer2 variants

As described above, Mer2 (1) interacts with Rec114 and Mei4 to form RMM complex, (2) binds double-stranded DNA, and (3) can be phosphorylated by Cdc28-Clb5/Clb6 during meiosis. We wonder whether our Mer2 mutants, that defective in DNA binding activity, are simply due to a protein misfolding or unstructured conformation. To answer this concern, it is important to prove that DNA binding mutants of Mer2 are only defective in DNA binding ability rather than

defective in other functions such as forming RMM complex. Thus, in the future we will examine our Mer2 mutant variants regarding their ability to (1) form RMM complex by yeast two hybrid and affinity pull-down analyses, and (2) be phosphorylated by Cdc28-Clb5/Clb6 by western blot with Mer2 S30 specific antibody. We wish to identify a Mer2 mutant that is specifically defective in DNA binding, but not other Mer2 function, for our complementation experiments.

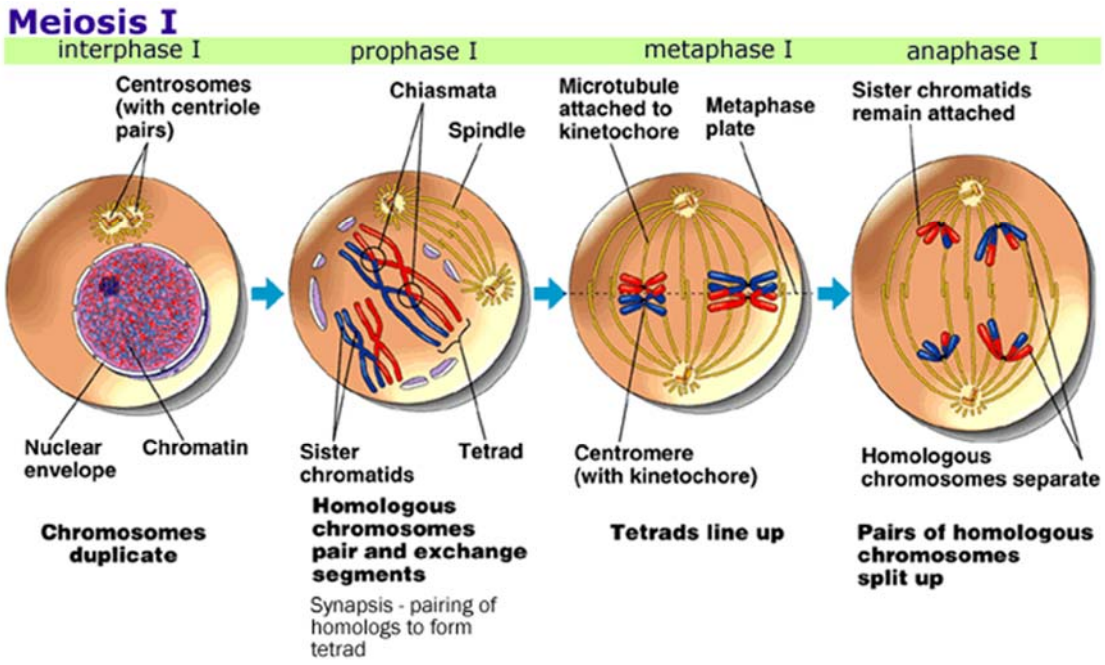
FIGURES



http://www.docstoc.com/docs/14940547/Cell-Division-Life-Cycle

Figure 1. The life cycle of meiosis and mitosis.

Diploid adults produce haploid gamete such as egg and sperm cell. Fertilization of egg and sperm become diploid zygote. Diploid zygote uses mitosis to increase cell numbers and growth to adult.



https://sites.google.com/site/accessrevision/biology/cell-form-and-function/meiotic-division

Figure 2. The processing stages in meiosis I.

In interphase I, the DNA replication results in chromosomes duplication. In prophase I, homologous chromosomes are engaged to form chiasmata. In metaphase I, spindles capture centromeres of chromosome and bivalent chromosomes align at metaphase. In anaphase I, homologous chromosomes segregate into opposite directions.

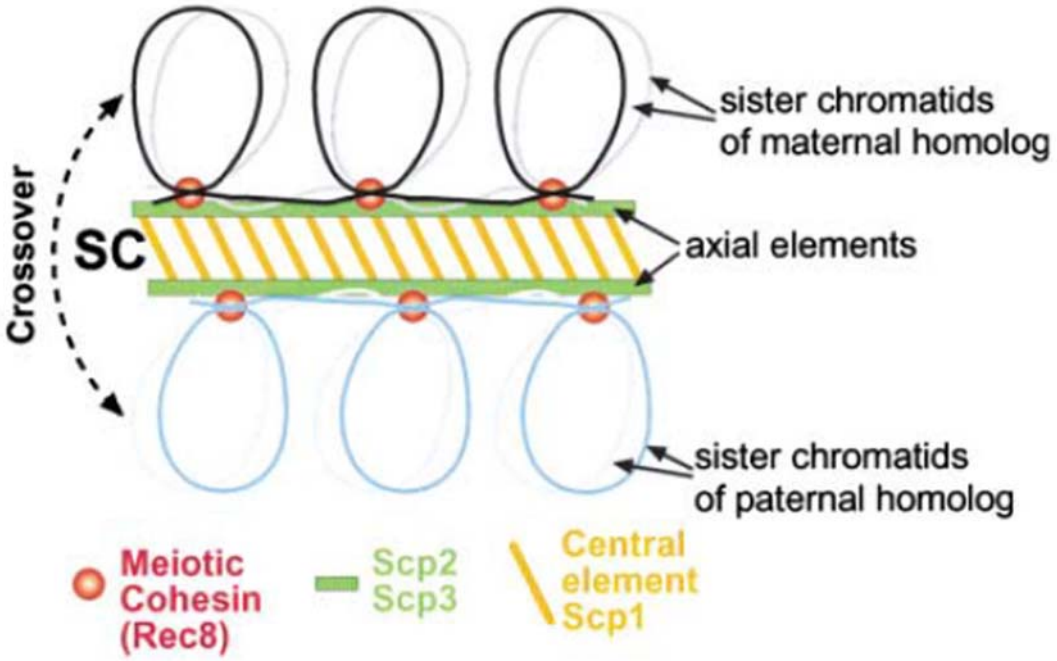


Figure 3. The structures of parental chromosomes and synaptonemal complex.

The synaptonemal complex (SC) contributes to homologous chromosome pairing between maternal and paternal. Meiotic cohesin Rec8 connects sister chromatids together. Homologous chromosomes paring is connected by SC components including axial elements (Scp2 and Scp3) and central elements (Scp1) (Adopted from Petronczki et al., 2003).

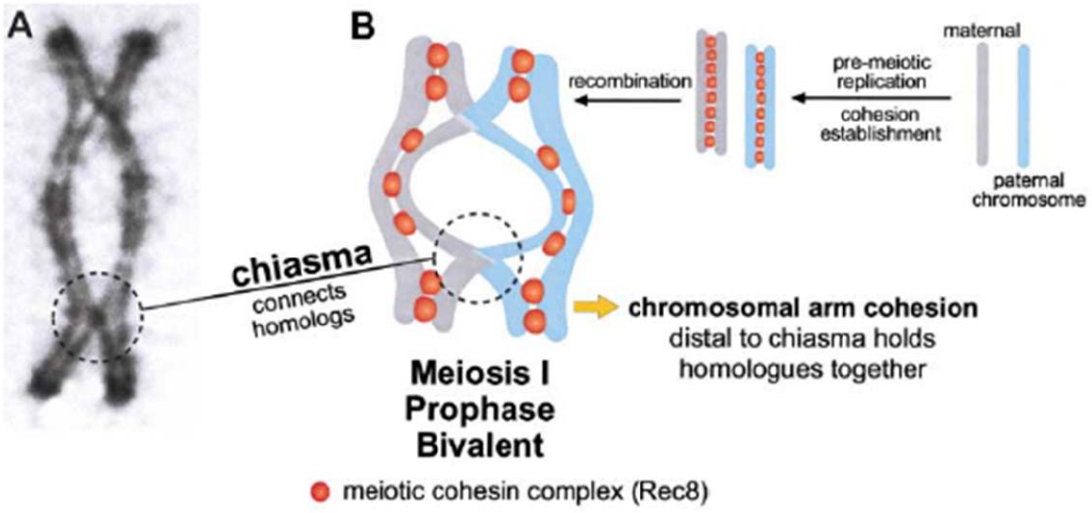


Figure 4. The formation of chiasma.

In meiosis, maternal and paternal chromosomes are connected by chiasmata and cohesin. During meiosis, chromosome go through pre-meiotic replication and cohesin establishment, then meiotic recombination engages homologous chromosomes to form chiasmata (Adopted from Petronczki et al., 2003).

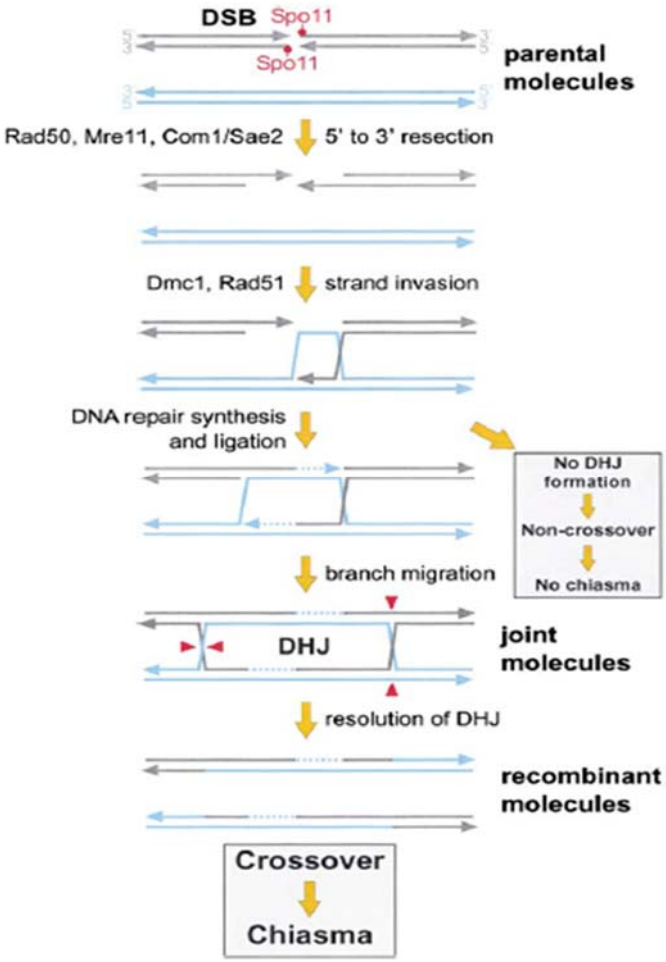


Figure 5. The establishment of chiasmata by meiotic recombination.
Meiotic recombination is initiated by double-strand breaks (DSBs). Spo11 generates DNA breaks at DNA and then the DNA end is resected by Rad50, Mre11, Xrs2 and Com1/Sae2 to produce 3' overhang single-strand DNA (ssDNA). The ssDNA invades to the homologous chromosome by Rad51 and Dmc1 recombinases to form a D-loop. Following by DNA synthesis and ligation, the double holiday junction (DHJ) is formed. Then the double holiday junction can be resolved by resolvase to produce recombinant molecules (Adopted from Petronczki et al., 2003).

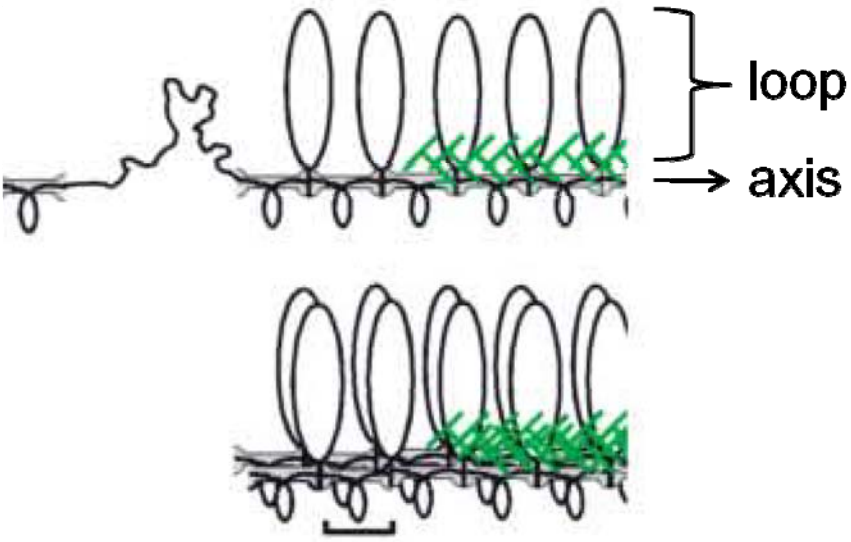


Figure 6. The definition of chromatin loops and axis.

During meiosis chromosomes are organized into a linear array of chromatin loops, and the bases of these chromatin loops is defined as an axis, which developed by protein complexes. Along the axis, gray units represent basic loop modules (containing cohesins); green units represent other axis-associated proteins (such as Red1). The homolog axis includes a linear array of "dual loop modules" (Adopted from Blat et al., 2002).

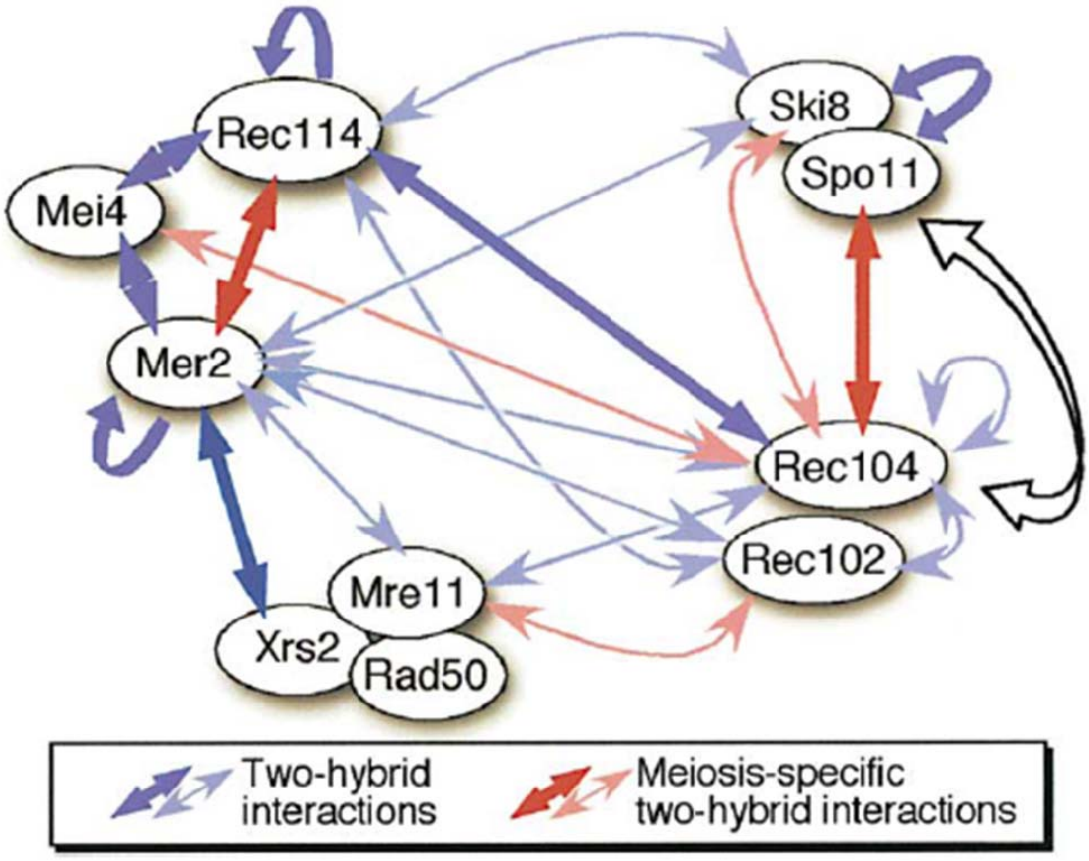


Figure 7. A network of interactions connects the DSB proteins to one another.

In budding yeast at least ten genes participate in DSB formation. The blue lines indicated mitotic interaction and red lines indicated meiosis specific interaction by yeast two hybrid. The thick lines indicated strong interaction. Deletion of any gene of ten is no DSB formation (Adopted from Arora et al., 2004).

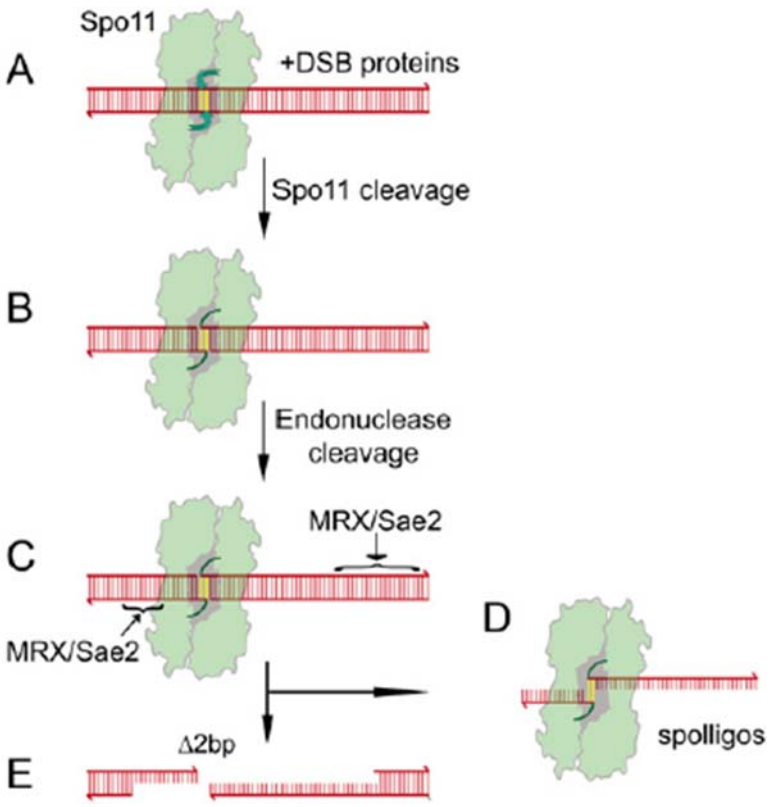


Figure 8. Spo11-mediated DNA double-strand break formation.

Spo11 forms a dimer and utilizes catalytic tyrosine to attack phosphodiester backbone of DNA to create DSBs. Spo11 covalently links to the DNA and then the endonuclease machinery of Mre11-Rad50-Xrs2 (MRX) and Sae2 cleaves DNA to release Spo11-oligonuclotide complex (Adopted from Cole et al., 2010).

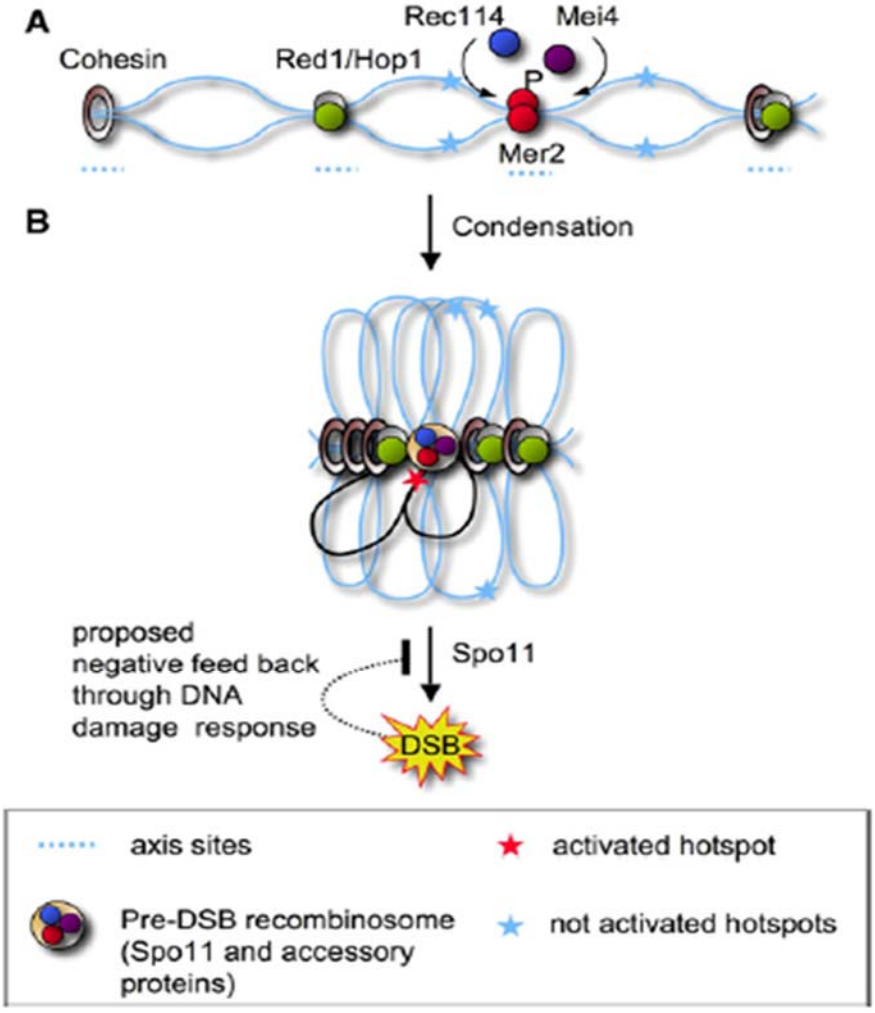


Figure 9. Model depicting the role of Mer2 in DSB-forming machinery.

(A) After pre-meiotic DNA replication, cohesins encircle the sister chromatins and Mer2 binds to axis sites. Moreover, phosphorylated Mer2 recruits Rec114 and Mei4 to form a complex at axis sites.

(B) Then, the linear chromatin loops is condensed and recruits Spo11-medated DSB machinery to cleave DNA(Adopted from Panizza et al., 2011).

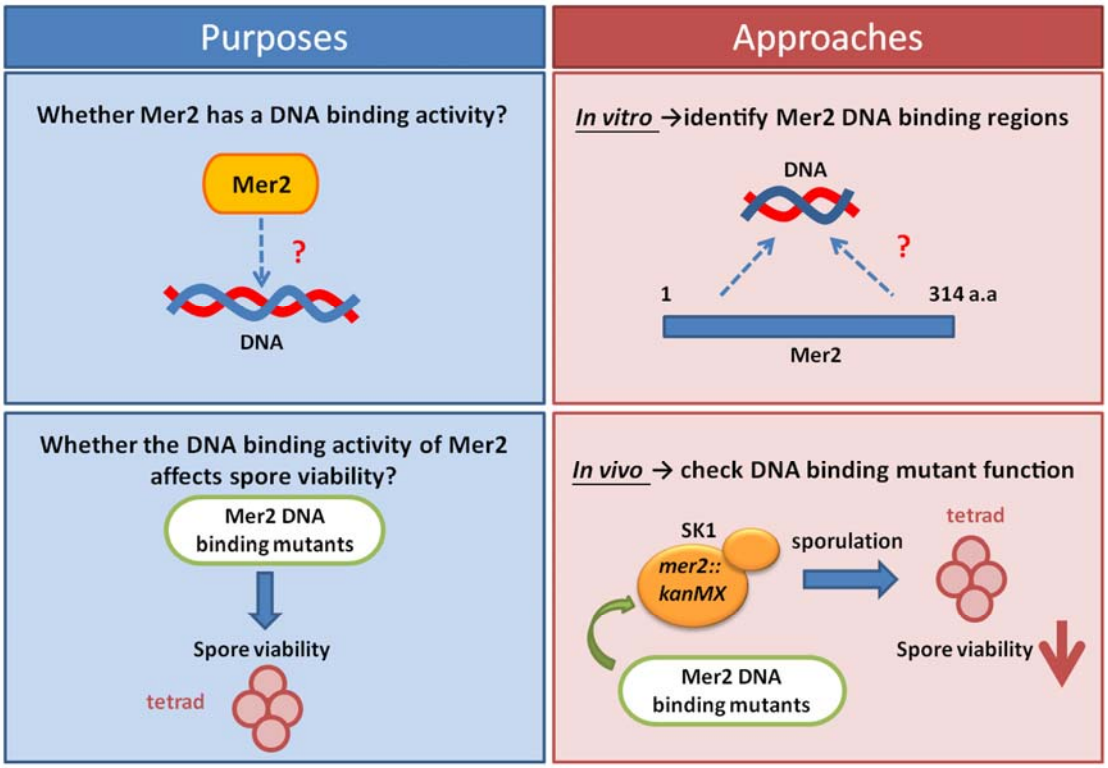


Figure 10. Schematics of my thesis study.

We aim to examine (1) whether Mer2 can directly bind double-stranded DNA *in vitro*, and to find out the DNA binding sites of Mer2, and (2) whether DNA binding activity of Mer2 plays an important role in making meiotic DNA double-strand breaks *in vivo*, by complementation experiments with spore viability as a readout.

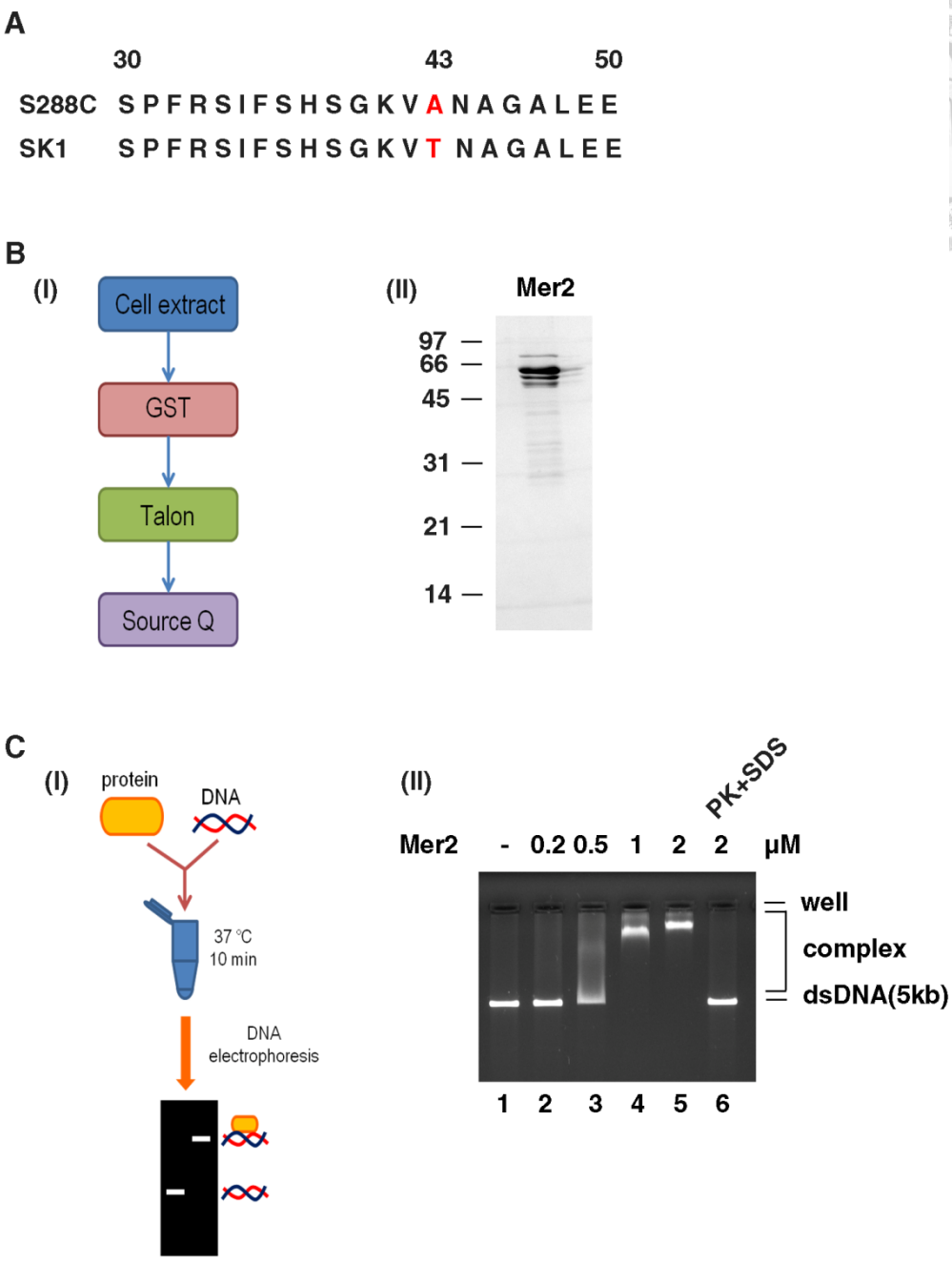


Figure 11. Mer2 possesses a DNA binding ability.

(A) The amino acid polymorphism between S288C and SK1 strains. At 43 amino acid S288C is Alanine (A) and SK1 is Threonine (T). (B) The purification strategy of Mer2 protein (panel I). Purified 2μg GST-Mer2-His was run in 15% SDS-PAGE and stained with Coomassie Blue (panel II). (C) A DNA binding assay to examine Mer2 DNA binding activity. Scheme of DNA mobility shift assay (panel I). Mer2 exhibits a dosage-dependent DNA binding ability. In lane 6, proteins were digested by SDS and protease K to demonstrate that the DNA band shifting was due to the Mer2 binding (panel II).

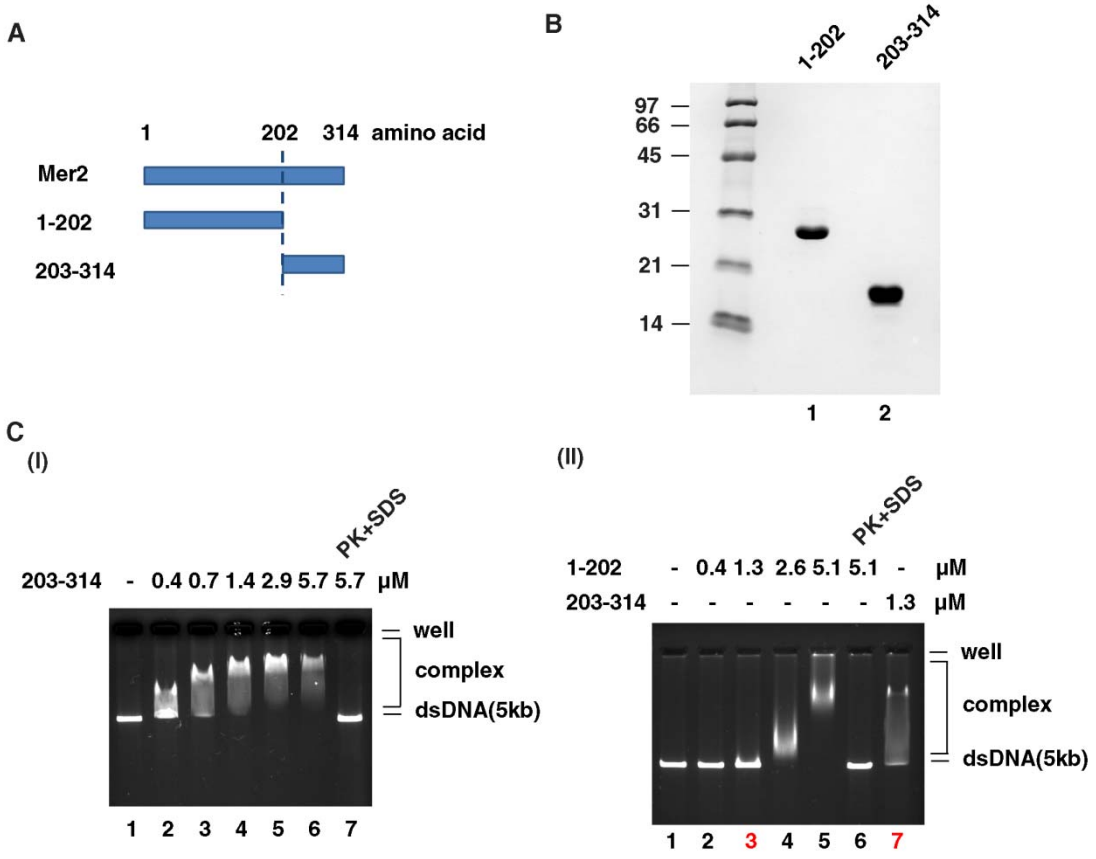


Figure 12. DNA binding properties of truncated recombinant Mer2.

(A) Design of truncated Mer2 with either N terminal or C terminal deletion. (B) Purified Mer2 1-202 (lane 1) and Mer2 203-314 (lane 2), 2μg each, were run in 15% SDS-PAGE and stained with Coomassie Blue. (C) DNA binding analyses. Mer2 203-314 (panel I) and 1-202 (panel II) exhibit a dosage-dependent DNA binding ability. The DNA binding affinity of Mer2 203-314 is better than Mer2 1-202 (panel II, comparing lanes 3 and 7 in the same molar ratio).

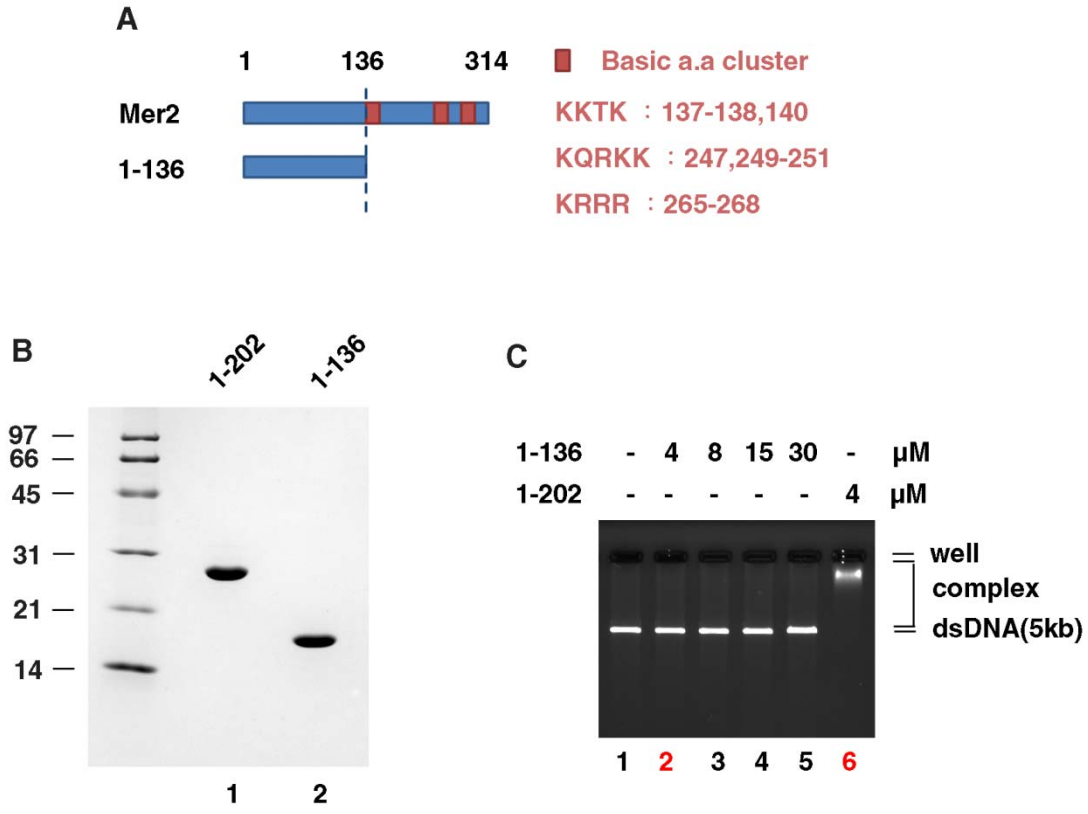


Figure 13. Recombinant Mer2 1-136 protein lacks the DNA binding activity.

(A) Design of truncated Mer2 1-136 without basic amino acids cluster. (B) Purified Mer2 1-202 (lane 1) and Mer2 1-136 (lane 2), 2μg each, were run in 15% SDS-PAGE and stained with Coomassie Blue. (C) Mer2 1-136 is devoid of binding duplex DNA. Mer2 1-202 was included in the reaction as a positive control (lane 6).

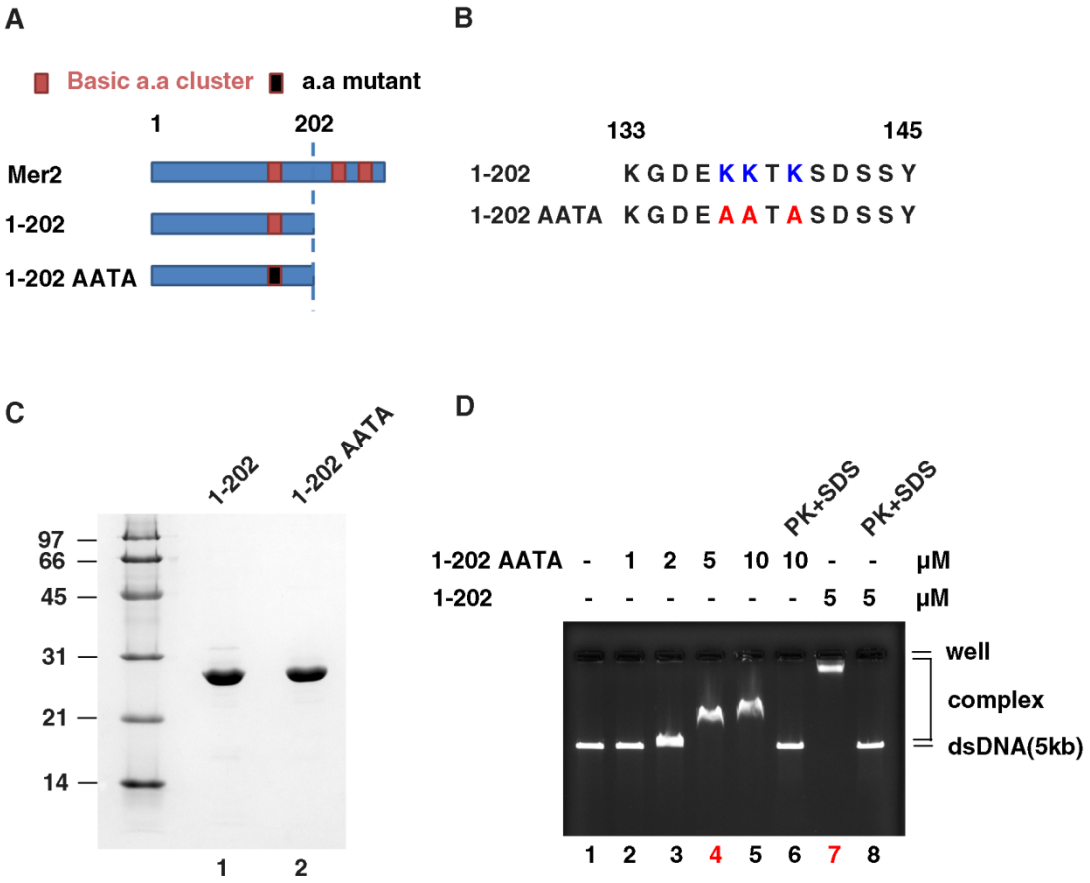


Figure 14. The DNA binding property of Mer2 1-202 AATA mutant variant.

(A) Design of truncated Mer2 1-202 with Lysine (K) or Arginine (R) mutated to Alanine (A) within the basic amino acid cluster. (B) The Lysine (K137, K138 and K140) of Mer2 1-202 were changed to Alanine (A). (C) Purified Mer2 1-202 (lane 1) and Mer2 1-202 AATA (lane 2), 2μg each, were run in 15% SDS-PAGE and stained with Coomassie Blue. (D) The DNA binding affinity of Mer2 1-202 AATA mutant variant is attenuated in comparing with the same molar ratio of wild-type 1-202 (comparing lanes 4 and 7).

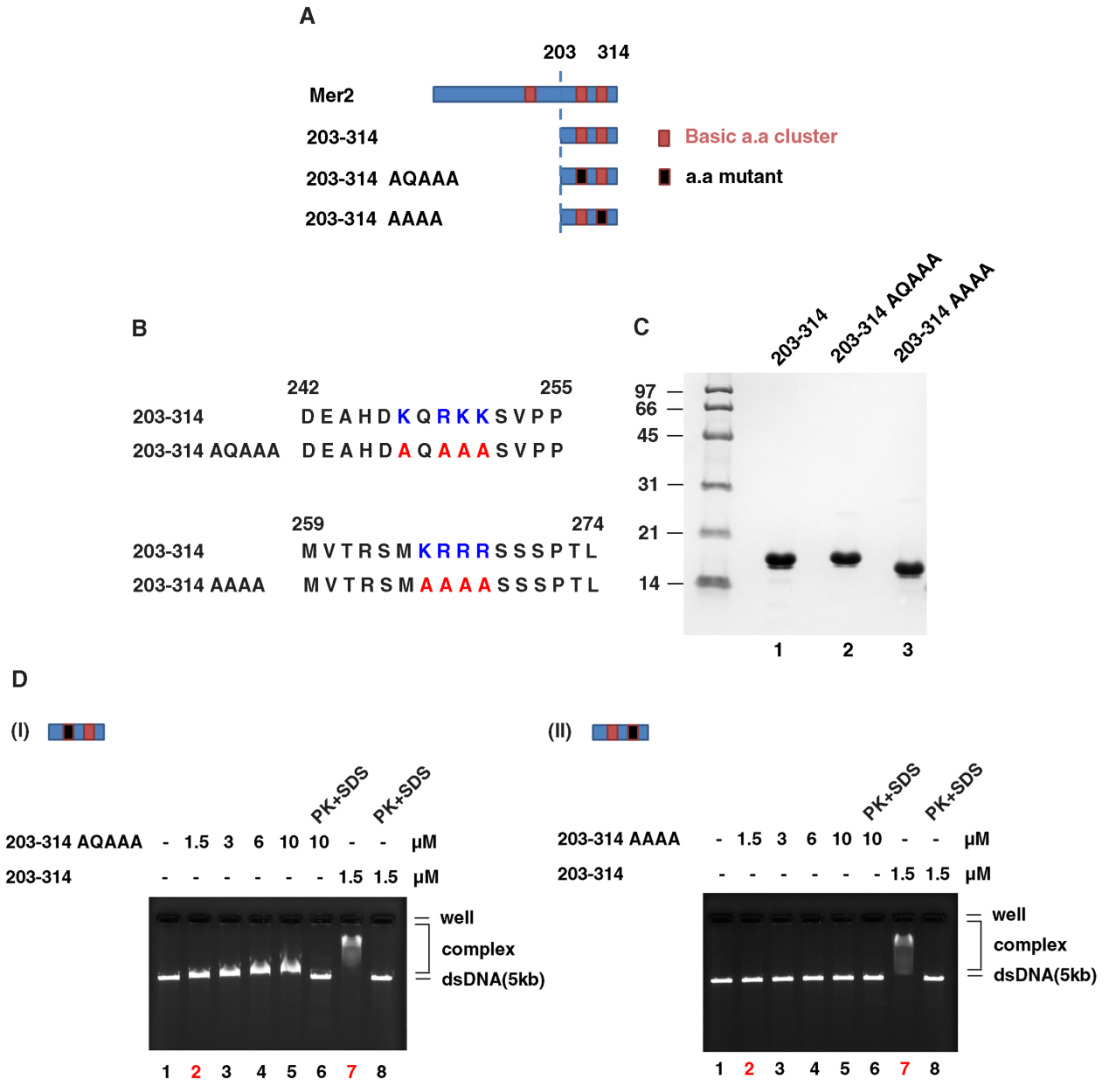


Figure 15. The DNA binding property of Mer2 203-314 AQAAA and AAAA mutant variants.

(A) Design of truncated Mer2 203-314 with Lysine (K) or Arginine (R) mutated to Alanine (A). (B) The Lysine (K247, K250, K251 and K265) and Arginine (R249, R266, R267 and R268) of Mer2 203-314 were changed to Alanine (A). (C) Purified Mer2 203-314 (lane 1), Mer2 203-314 AQAAA (lane 2) and Mer2 203-314 AAAA (lane 3), 2μg each, were run in 15% SDS-PAGE and stained with Coomassie Blue. (D) DNA binding analyses. Both Mer2 203-314 AQAAA (panel I) and AAAA (panel II) mutant variants show a significant defective in DNA binding ability. Wild-type Mer2 203-314 was included in the reaction as a positive control (lane 7).

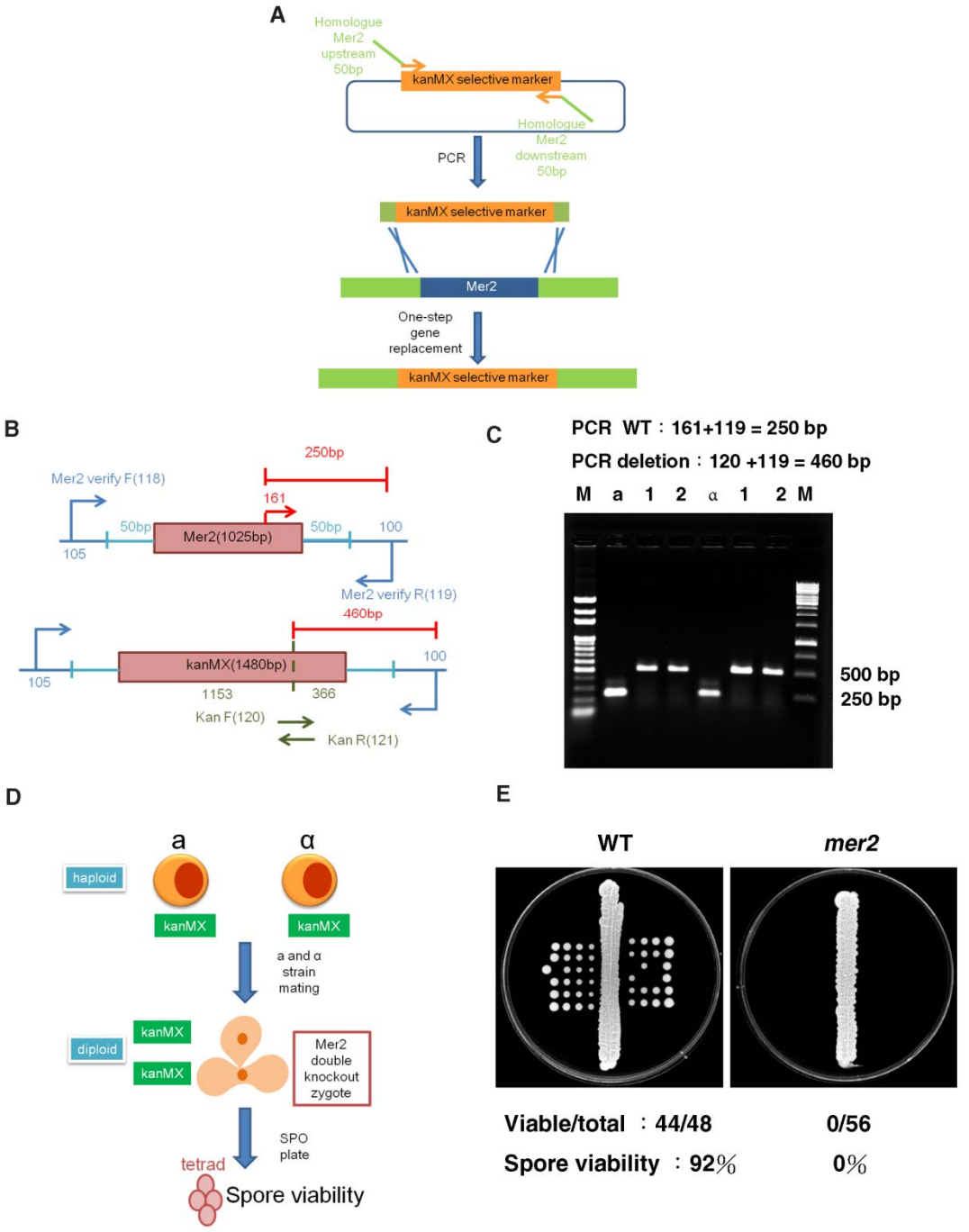


Figure 16. Deletion of *MER2* in SK1 strain exhibits a poor spore viability.

(A) Scheme of depletion of Mer2 in yeast SK1 strain by recombination-based gene replacement. (B) The diagram of primers design for colony PCR to verify Mer2 replacement. Wild-type haploid exhibits a 250bp PCR product by primer 161 and 119; in contrast, Mer2 deleted haploid shows a 460bp product by primer 120 and 119. (C) We confirmed deletion of *MER2* gene in haploid yeast (both a and α strains) by colony PCR. (D) Scheme of yeast sporulation experiment. (E) Tetrad dissection for measuring spore viability in *mer2* deletion strain by Dr. Kuei-Shu Tung.

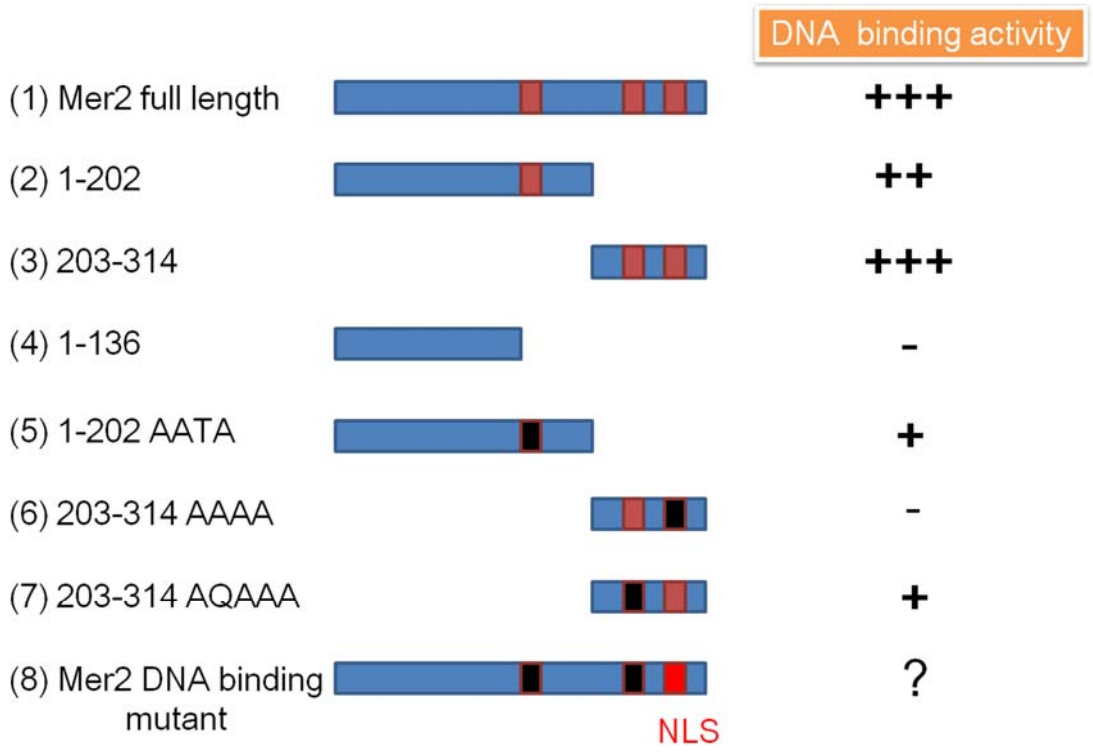


Figure 17. The summary of DNA binding activity of Mer2 variants.

We found that (1) Mer2 belongs a DNA binding protein and directly binds duplex DNA, and (2) there are at least three basic amino acid clusters within Mer2 contributing to the DNA binding activity. Symbol : NLS, nuclear localization signal.

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APPENDIX

A-1 On-line prediction of Mer2 secondary structure prediction

(I)CFSSP - Chou & Fasman Secondary Structure Prediction Server

http://www.biogem.org/tool/chou-fasman/

Name of the sequence is *gi|1019811|emb|CAA89546.1| REC107 [Saccharomyces cerevisiae].*

Sequence consists of 314 amino acids.

Target Sequence:

MVARGRTDEI STDVSEANSE HSLMITETSS PFRSIFSHSG KVANAGALEE SDKQILEWAG KLELESMELR
ENSDKLIKVL NENSKTLCKS LNKFNQLLEQ DAATNGNVKT LIKDLASQIE NQLDKVSTAM LSKGDEKKTK
SDSSYRQVLV EEISRYNSKI TRHVTNKQHE TEKSMRCTQE MLFNVGSQLE DVHKVLLSLS KDMHSLQTRQ
TALEMAFREK ADHAYDRPDV SLNGTTLLHD MDEAHDKQRK KSVPPPRMMV TRSMKRRRSS SPTLSTSQNH
NSEDNDDASH RLKRAARTII PWEELRPDTL ESEL

Secondary Structure:

```
                 *         *         *         *         *
Query 1    MVARGRTDEISTDVSEANSEHSLMITETSSPFRSIFSHSGKVANAGALEESDKQILEWAG 60
Helix 1           <---------------->         <------------------------ 60
Sheet 1        EEEEEEEEE          EEEEE     EEEE                  EEEE     60
Turns 1        T  T    T       T          T         T         T  T      T 60

                 *         *         *         *         *
Query 61   KLELESMELRENSDKLIKVLNENSKTLCKSLNKFNQLLEQDAATNGNVKTLIKDLASQIE 120
Helix 61   ---------------------->     <------------>     <------------ 120
Sheet 61    EEEEE          EEEE       EEEEEEEEEEEEEE         EEEEEEEEEEEEE 120
Turns 61             T  T       T TT              T     T          T    120

                 *         *         *         *         *
Query 121  NQLDKVSTAMLSKGDEKKTKSDSSYRQVLVEEISRYNSKITRHVTNKQHETEKSMRCTQE 180
Helix 121  ------------------->      <---->        <------------------- 180
Sheet 121  EEEEEEEEEE                EEEEEEEEEEEEEEEEEEEEEEE        EEEEEE 180
Turns 121  T           T    T    T T              T        T     T     T  180

                 *         *truncate *         *         *
Query 181  MLFNVGSQLEDVHKVLLSLSKDMHSLQTRQTALEMAFREKADHAYDRPDVSLNGTTLLHD 240
Helix 181  ----------------------------------------->            <--- 240
Sheet 181  EEEEEEEEEEEEEEEEEE    EEEEEEEEEEEEEE             EEEEEEEEEEE 240
```

```
Turns 181       T             T                T       TT     T       240

                    *         *         *         *         *

Query 241 MDEAHDKQRKKSVPPPRMMVTRSMKRRRSSSPTLSTSQNHNSEDNDDASHRLKRAARTII 300
Helix 241 ----------->                                        <---------- 300
Sheet 241                   EEEEEE          EEEEEE                   EEE 300
Turns 241       T   T   TT      T   T    TT      T   T   T       T       300

                    *
Query 301 PWEELRPDTLESEL 314
Helix 301 --->     <>    314
Sheet 301 E   EEEEE      314
Turns 301       TT   T   314


Total Residues: H: 217   E: 161   T: 47
       Percent: H: 69.1  E: 51.3  T: 15.0
```

(II)GOR - Garnier et al, 1996

http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_gor4.html

GOR4 result for : mer2xx0

Abstract GOR secondary structure prediction method version IV, J. Garnier, J.-F. Gibrat, B. Robson, Methods in Enzymology,R.F. Doolittle Ed., vol 266, 540-553, (1996)

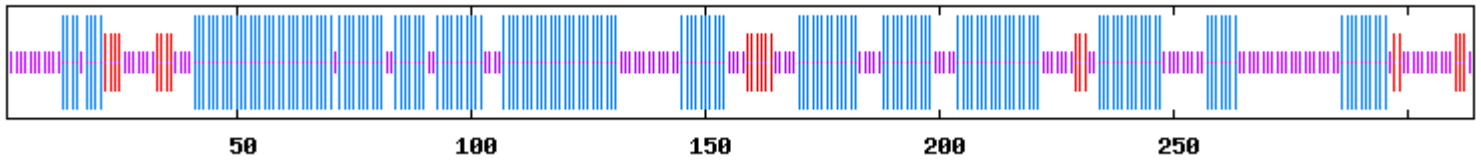
View GOR4 in: [AnTheProt (PC) , Download...] [HELP]

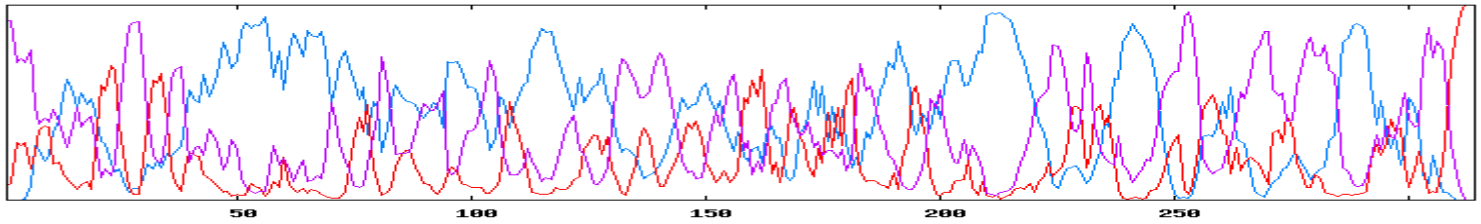
```
         10        20        30        40        50        60        70
          |         |         |         |         |         |         |
MVARGRTDEISTDVSEANSEHSLMITETSSPFRSIFSHSGKVANAGALEESDKQILEWAGKLELESMELR
ccccccccccccchhhhchhhheeeecccccccceeeecccchhhhhhhhhhhhhhhhhhhhhhhhhhhh
ENSDKLIKVLNENSKTLCKSLNKFNQLLEQDAATNGNVKTLIKDLASQIENQLDKVSTAMLSKGDEKKTK
hchhhhhhhhhhcchhhhhhhhcchhhhhhhhhhcccchhhhhhhhhhhhhhhhhhhhhhhhhhcccccccc
                                                          truncate
SDSSYRQVLVEEISRYNSKITRHVTNKQHETEKSMRCTQEMLFNVGSQLEDVHKVLLSLSKDMHSLQTRQ
ccccchhhhhhhhhhcccceeeeeecccccchhhhhhhhhhhhhhcccccchhhhhhhhhhhcccccchhhhhh
TALEMAFREKADHAYDRPDVSLNGTTLLHDMDEAHDKQRKKSVPPPRMMVTRSMKRRRSSSPTLSTSQNH
hhhhhhhhhhhhcccccccceeeccchhhhhhhhhhhhhhhcccccccccchhhhhhhccccccccccccccc
NSEDNDDASHRLKRAARTIIPWEELRPDTLESEL
cccccchhhhhhhhhhceecccccccccccceeec
```

Sequence length : 314

GOR4 :

Alpha helix	(Hh) :	173 is	55.10%
3_{10} helix	(Gg) :	0 is	0.00%
Pi helix	(Ii) :	0 is	0.00%
Beta bridge	(Bb) :	0 is	0.00%
Extended strand	(Ee) :	22 is	7.01%
Beta turn	(Tt) :	0 is	0.00%
Bend region	(Ss) :	0 is	0.00%
Random coil	(Cc) :	119 is	37.90%
Ambigous states	(?) :	0 is	0.00%
Other states	:	0 is	0.00%





A-2 On-line prediction of potential DNA binding amino acids in Mer2 protein

(I)BindN : prediction of DNA-binding residues

Website: http://bioinfo.ggc.org/bindn/

Summary

Your sequence: gi|854595|emb|CAA60944.1| MER2 [Saccharomyces cerevisiae]

Input sequence length: 314 amino acids

Predicted binding sites: 94 residues

User-defined specificity: 80.00%

Estimated sensitivity: 56.96%

Overview

Sequence:

MVARGRTDEISTDVSEANSEHSLMITETSSPFRSIFSHSGKVANAGALEESDKQILEWAG

Prediction:

---+-++-+----------------+-+++--++--+-+-+------------------

Confidence:

55695652462259237426427575244425965454565673988775373697779 9

Sequence:

KLELESMELRENSDKLIKVLNENSKTLCKSLNKFNQLLEQDAATNGNVKTLIKDLASQIE

Prediction:

---------+--------------++--++--+----------+-+-++--+-------

Confidence:

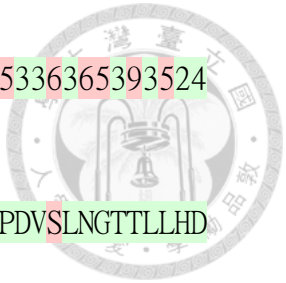
29686276764426299397353276438552583599858882544744594689549 7

Sequence:

NQLDKVSTAMLSKGDEKKTKSDSSYRQVLVEEISRYNSKITRHVTNKQHETEKSMRCTQE

Prediction:

----+-++----+---++++++++++--------++-++-+++-++++--+-++-+-+--



```
Confidence:
        75886746425264434849869748279856828527938964545533636539352 4

Sequence:
        MLFNVGSQLEDVHKVLLSLSKDMHSLQTRQTALEMAFREKADHAYDRPDVSLNGTTLLHD
Prediction:
        -----------------+-+----+--++++------+--------+---+---------
Confidence:
        79648755878984877464245354278546588866627747276447452522798 9

Sequence:
        MDEAHDKQRKKSVPPPRMMVTRSMKRRRSSSPTLSTSQNHNSEDNDDASHRLKRAARTII
Prediction:
        ------+-++++----+---+++-+++++++++-+++++---------+-+-++--+---
Confidence:
        99985452869842259325797379998898938856623269478742867946938 8

Sequence:       PWEELRPDTLESEL
Prediction:     -----+--+-----
Confidence:     86979545576278


*** Prediction:  binding residues are labeled with '+' and in red;
                 non-binding residues labeled with '-' and in green.
*** Confidence:  from level 0 (lowest) to level 9 (highest).
```

(II)DNABindR : A Server for Prediction of Protein-DNA interaction sites

Website: http://turing.cs.iastate.edu/PredDNA/predict.html

Protein-DNA interaction site Predictions:

Mon Sep 10 00:41:48 CDT 2012

Prediction Model: Naive Bayes
Feature Representation AA Identity

MVARGRTDEISTDVSEANSEHSLMITETSSPFRSIFSHSGKVANAGALEESDKQILEWAGKLELESMEL
RENSDKLIKVL
---#-##-----------------#-###############-#-----------------------
----

NENSKTLCKSLNKFNQLLEQDAATNGNVKTLIKDLASQIENQLDKVSTAMLSKGDEKKTKSDSSYRQVL
VEEISRYNSKI
##-###-###-#-----------###-##-------------#------##---##########---
-----######

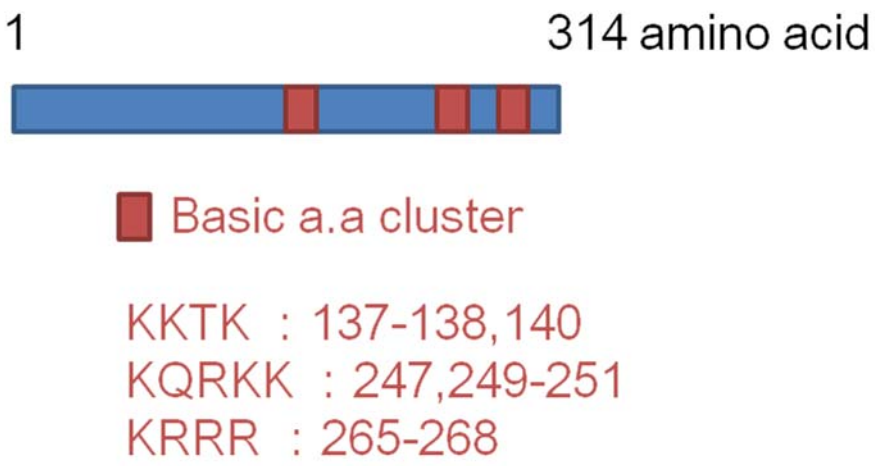
TRHVTNKQHETEKSMRCTQEMLFNVGSQLEDVHKVLLSLSKDMHSLQTRQTALEMAFREKADHAYDRPD
VSLNGTTLLHD
###########----#----------------------------#####----------------
----

MDEAHDKQRKKSVPPPRMMVTRSMKRRRSSSPTLSTSQNHNSEDNDDASHRLKRAARTIIPWEELRPDT
LESEL
---------###-##########################----------##-##--#----------
-----

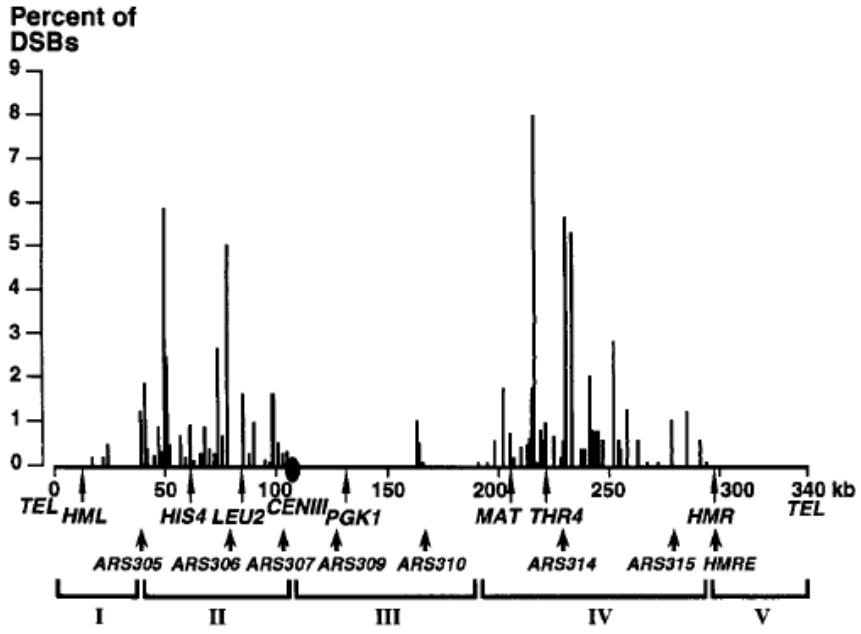
(III) The prediction of three potential DNA binding clusters in Mer2 protein

Basic amino acids : Lysine (K)；Argnine (R)
Aromatic amino acids : Phenylalanine (F)；Tyrosine (Y)；Tryptophan (W)

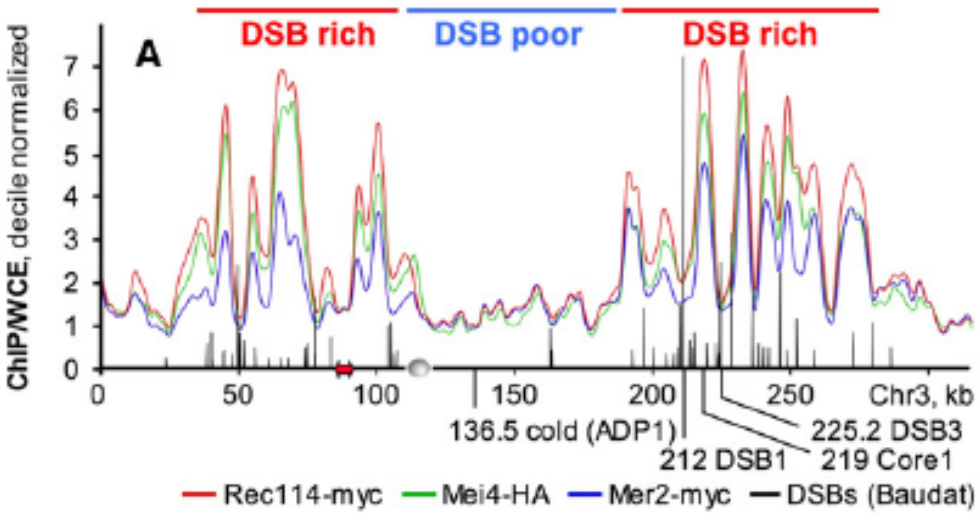
MVARGRTDEISTDVSEANSEHSLMITETSSPFRSIFSHSGKVANAGALEESD
KQILEWAGKLELESMELRENSDKLIKVLNENSKTLCKSLNKFNQLLEQDAAT
NGNVKTLIKDLASQIENQLDKVSTAMLSKGDEKKTKSDSSYRQVLVEEISRYN
SKITRHVTNKQHETEKSMRCTQEMLFNVGSQLEDVHKVLLSLSKDMHSLQT
RQTALEMAFREKADHAYDRPDVSLNGTTLLHDMDEAHDKQRKKSVPPPRM
MVTRSMKRRRSSSPTLSTSQNHNSEDNDDASHRLKRAARTIIPWEELRPDT
LESEL.



A-3 Design sequences of DSB-hot, DSB-cold and axis sites



(Adopted from (Baudat and Nicolas, 1997))



(Adopted from(Panizza et al., 2011))

Core 1 is a Rec8-binding site at 219.5 kb, ADP1 lies in a DSB-cold region (136.5 kb), and DSB1 and DSB3 are hotspots at positions 212 and 225.2 kb.

DSB1:211001-213000bp, length=2000bp
DSB3:224001-226000bp, length=2000bp
Core 1:218001-22000bp, length=2000bp
ADP1:135001-137000bp, length=2000bp

A-4 The prediction of nuclear localization signal (NLS) in Mer2

(I) NucPred: A server for prediction of nuclear localization signal in Mer2

Website: http://www.sbc.su.se/~maccallr/nucpred/cgi-bin/single.cgi

SBC STOCKHOLM BIOINFORMATICS CENTER

Authors: Amine Heddad, Andrea Krings, Markus Brameie

NucPred

The NucPred score for your sequence is 0.86 (see score help below)

```
  1  MVARGRTDEISTDVSEANSEHSLMITETSSPFRSIFSHSGKVANAGALEE   50
 51  SDKQILEWAGKLELESMELRENSDKLIKVLNENSKTLCKSLNKFNQLLEQ  100
101  DAATNGNVKTLIKDLASQIENQLDKVSTAMLSKGDEKKTKSDSSYRQVLV  150
151  EEISRYNSKITRHVTNKQHETEKSMRCTQEMLFNVGSQLEDVHKVLLSLS  200
201  KDMHSLQTRQTALEMAFREKADHAYDRPDVSLNGTTLLHDMDEAHDKQRK  250
251  KSVPPPRMMVTRSMKRRRSSSPTLSTSQNHNSEDNDDASHRLKRAARTII  300
301  PWEELRPDTLESEL                                      314
```

Positively and negatively influencing subsequences are coloured according to the following scale:

(non-nuclear) negative positive (nuclear)

(II) cNLS Mapper: A server for prediction of importinα-dependent nuclear localization signals in Mer2

Website: http://nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi

Predicted NLSs in query sequence

```
MVARGRTDEISTDVSEANSEHSLMITETSSPFRSIFSHSGKVANAGALEE 50
SDKQILEWAGKLELESMELRENSDKLIKVLNENSKTLCKSLNKFNQLLEQ 100
DAATNGNVKTLIKDLASQIENQLDKVSTAMLSKGDEKKTKSDSSYRQVLV 150
EEISRYNSKITRHVTNKQHETEKSMRCTQEMLFNVGSQLEDVHKVLLSLS 200
KDMHSLQTRQTALEMAFREKADHAYDRPDVSLNGTTLLHDMDEAHDKQRK 250
KSVPPPRMMVTRSMKRRRSSSPTLSTSQNHNSEDNDDASHRLKRAARTII 300
PWEELRPDTLESEL                                      314
```

Predicted monopartite NLS

Pos.	Sequence	Score
260	VTRSMKRRRSS	11
262	RSMKRRRSSS	13

Predicted bipartite NLS

Pos.	Sequence	Score

[Note] : The basic amino acids KRRR could be the nuclear localization signal (NLS) in Mer2.

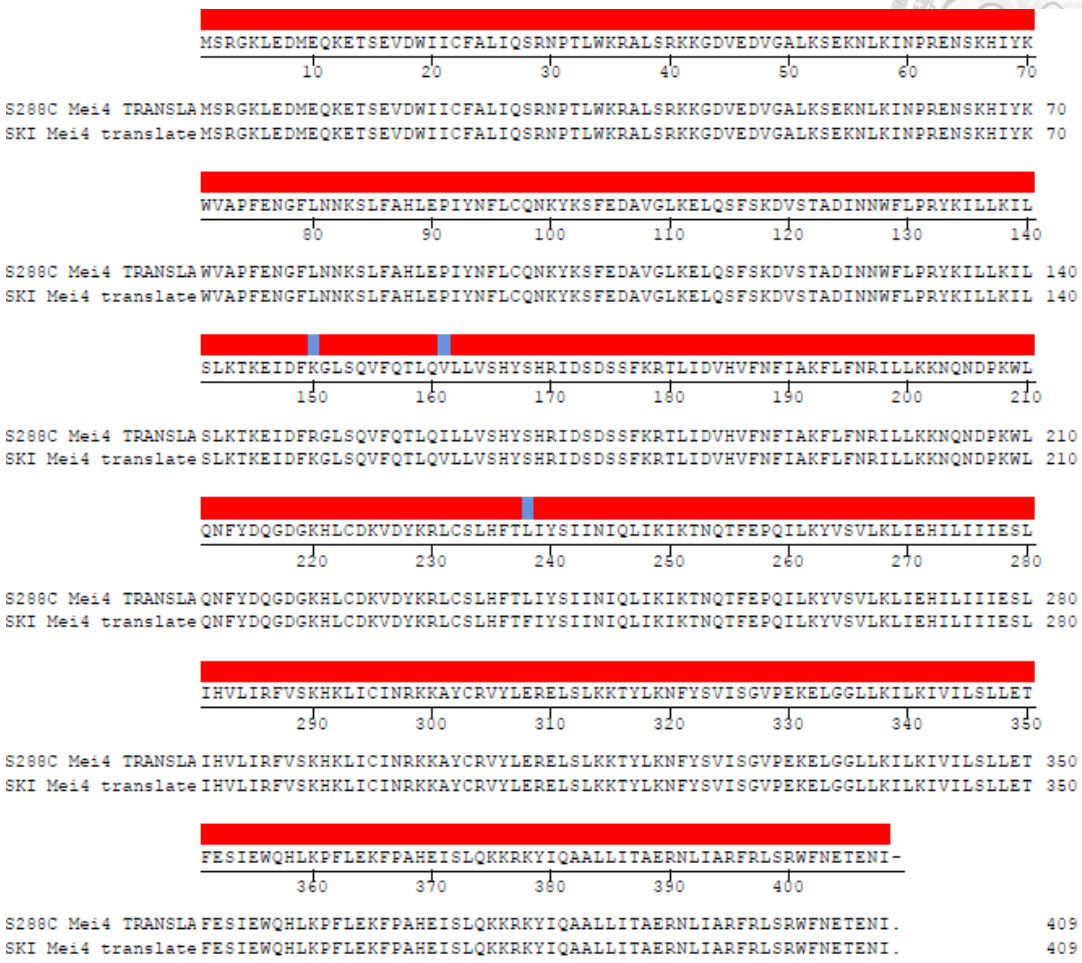
A-5 The amino acid polymorphism between S288C and SK1

(I) The amino acid polymorphism between S288C and SK1 strains in Mer2.

At 43th amino acid：S288C is Alanine (A) and SK1 is Threonine (T).

Our sequence of Mer2 is S288C.

(II) The amino acid polymorphism between S288C and SK1 strains in Mei4.



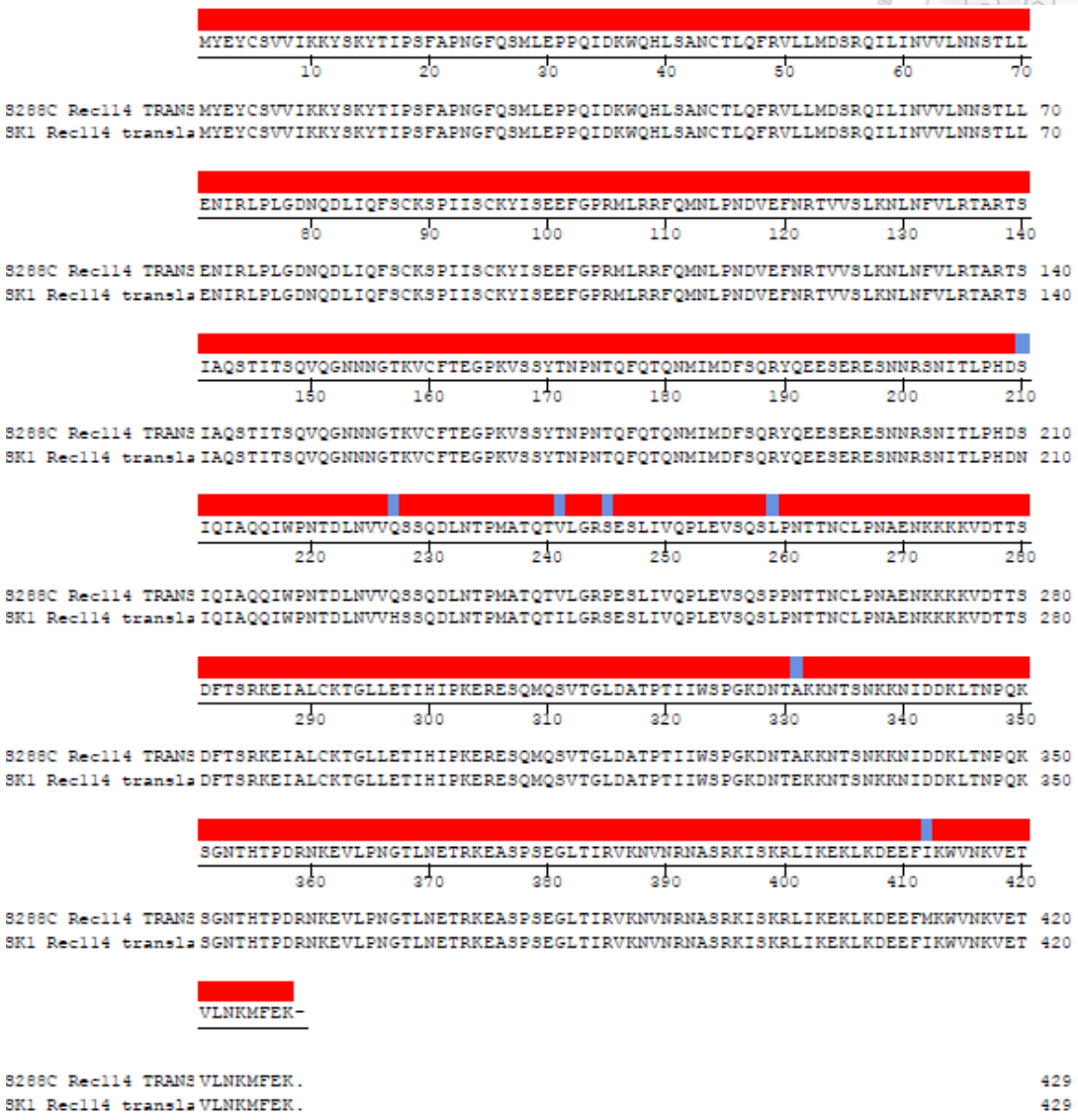
At 150th amino acid：S288C is Arginine (R) and SK1 is Lysine (K).

At 161th amino acid：S288C is Isoleucine (I) and SK1 is Valine (V).

At 238th amino acid：S288C is Leucine (L) and SKI is Phenylalanine (F).

Our sequence of Mei4 is S288C.

(III) The amino acid polymorphism between S288C and SK1 strains in Rec114.



At 210th amino acid：S288C is Serine (S) and SK1 is Asparagine (N).

At 227th amino acid：S288C is Glutamine (Q) and SK1 is Histidine (H).

At 241th amino acid：S288C is Valine (V) and SK1 is Isoleucine (I).

At 245th amino acid：S288C is Proline (P) and SK1 is Serine (S).

At 259th amino acid：S288C is Proline (P) and SK1 is Leucine (L).

At 331th amino acid：S288C is Alanine (A) and SK1 is Glutamate (E).

At 412th amino acid：S288C is Methionine (M) and SK1 is Isoleucine (I).

Our sequence of Rec114 is SK1.