SYNTHETIC LETHALITY AND SYNTHETIC CYTOTOXICITY STRATEGIES FOR SELECTIVE KILLING OF ATM DEFICIENT CELLS

by

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Abstract

Chromosome instability (CIN) is a hallmark of cancer cells and could, in theory, be exploited in the design of cancer therapeutics. Tumor cells harboring CIN mutations may be dependent on certain DNA repair pathways for viability. Thus, inhibition of specific DNA repair enzymes may enhance the CIN phenotype to an intolerable level, or may sensitize cells to DNA damage stress. To test this hypothesis, I focused on the CIN gene ATM, which is often mutated in human tumors. I hypothesized that knockdown of certain second site DNA repair genes would selectively kill ATM-deficient cells resulting in synthetic lethality (SL), or sensitize ATM-deficient cells to a sub-lethal dose of DNA damaging agent resulting in synthetic cytotoxicity (SC). The goal of this research is to use budding yeast as a model system to identify candidate SL or SC interaction partner genes for ATM with/without sub-lethal doses of DNA damaging agents, using mutations in the yeast ATM homologues, TEL1 and MEC1. I tested for interactions with TEL1 and MEC1 in a small matrix of three DNA repair genes (RAD27, TDP1 and TPP1) and four DNA damaging agents (hydroxyurea, 5-fluorouracil, bleomycin, and camptothecin). I also performed a genome-wide screen for interactions between TEL1 and ~5000 non-essential genes, both in the presence and absence of low doses of camptothecin. I discovered one SL interaction with MEC1 and fourteen SC interactions with TEL1. Most of the SC interaction partner genes are involved in DNA repair and show sensitivity specifically to camptothecin. These data provide a rationale for testing specific combination therapies for selective killing of cancer cells bearing ATM mutations. Specifically, the Shu complex, Ku complex, Rrm3, Rad27 and CK2β subunits can be further tested as potential combination therapeutic targets, together with a sub-lethal dose of camptothecin, to kill ATM-deficient cancer cells.

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List of Abbreviations

AT: ataxia telangiectasia

ATM: Ataxia Telangiectasia Mutated

ATR: ATM- and Rad3-related

AUC: area under the curve

BER: base excision repair

CIN: chromosome instability

DDR: DNA damage response

DMA: deletion mutant array

DSB: double strand break

dSLAM: diploid-based synthetic lethality analysis with microarrays

GCR: gross chromosomal rearrangements

IR: ionizing radiation

HR: homologous recombination

MIN: microsatellite instability

MMR: mismatch repair

MMS: methyl methanesulfonate

NBD: nuclear binding domain

OD: optical density

OFP: Okazaki fragment processing

PARP: poly (ADP ribose) polymerase

PI3K-related: phosphoinositide 3-kinase-related

RNAi: RNA interference

SC: Synthetic Cytotoxicity

SGA: Synthetic Genetic Array

SL: Synthetic Lethality

WT: Wild Type

YKO: Yeast Knock-out deletion collection

YPD: yeast peptone dextrose

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Chapter 1 : Introduction

1.1 Accumulation of mutations underlies cancer

Although most spontaneous mutations are neutral, they can often be harmful rather than beneficial (Taddei et al., 1997). The survival of a species requires the protection of genetic material and the passage of intact accurately copied genetic information. This can be quite a challenge as DNA is constantly under attack by both endogenous and exogenous DNA damaging factors (Hoeijmakers, 2009). DNA damage can result from endogenous processes such as cellular metabolism or spontaneous errors in DNA replication and recombination (De Bont and van Larebeke, 2004). In addition, exogenous genotoxic agents, such as ultraviolet light, ionizing radiation (IR) and chemical mutagens, can cause nucleotide alterations and DNA breaks (Wogan et al., 2004, Friedberg et al., 2006). Unlike damaged protein, which can be replaced by synthesis, any modification of DNA has to be accurately repaired. Unrepaired DNA damage can lead to cell cycle arrest, cell death or mutations that may contribute to oncogenesis or other disease phenotypes (Vogelstein and Kinzler, 2001). Cells have evolved a response system to maintain and repair the organized state of DNA structure and the sequence of the nucleotide code. The DNA damage response (DDR) system activates the appropriate DNA repair pathway and arrests or slows the cell cycle to allow time for repair, or, in the case of irreparable damage, induces apoptosis (Jackson and Bartek, 2009). There are multiple pathways that must collaborate for the efficient repair of DNA damage and because of the critical role of genome maintenance mechanisms in all cells, DDR genes tend to be highly conserved throughout eukaryotes (Friedberg et al., 1995). Approximately 230 human DNA repair genes have been identified to date and most have homologues in budding yeast (Wood et al., 2005, Milanowska et al., 2011).

The integrity of the genome is crucial for tumor suppression in somatically proliferating cells (Hoeijmakers, 2001). To ensure faithful genome transmission eukaryotic cells have a remarkable DNA repair capacity (Vilenchik and Knudson, 2000). However, errors do happen, some of which escape the repair mechanisms and apoptosis, thus becoming permanent mutations. These mutations can be propagated to the next cellular generation and will accumulate with time. Accumulation of multiple mutations underlies many genetic diseases including cancer. During early cancer development, it is hypothesized that tumor cell precursors undergo a multistep mutational process to acquire a set of genetic changes that leads to oncogenesis. These oncogenic mutations in tumor cells result in a set of specific phenotypes or "hallmarks" that define tumor cells. These traits include uncontrolled proliferation, escape from apoptotic signals, and metastasis (Hanahan and Weinberg, 2000, Michor et al., 2004). Therefore, anticancer therapeutics are designed to specifically exploit one or more of these hallmarks to kill tumor cells.

The effectiveness of the most common chemotherapy agents is based on increased proliferation of cancer cells. Cytotoxic drugs, which are more toxic to proliferating cells, can kill the highly proliferative tumor cells. However, they also harm normal proliferating cells, such as cells in the bone marrow, digestive tract, and hair follicles. This results in many side effects, such as myelosuppression, mucositis and alopecia (Chabner and Roberts, 2005, Weinberg, 2007). Therapeutic effects could be improved by developing therapies that are highly selective for cancer cells, and do not rely on cell proliferation alone for their toxicity.

1.2 Chromosomal instability (CIN) as a hallmark of cancer

The phenotypic hallmarks of cancer cells are caused by an accumulation of two main kinds of mutations: activating mutations in oncogenes and inactivating mutations in tumor suppressor genes (Weinberg, 2007). Usually, when cancer is detected, a large number of mutations have accumulated in tumor cells (Tomlinson et al., 2002). However, given the extremely high fidelity of replication, repair, and segregation of chromosomal DNA during normal cell division, the multiple cancer mutations are accumulating at a much higher rate than the spontaneous mutation rate in normal cells. So there is a third class of mutations that lead to genome instability that occur in the early development stage of tumorigenesis (Gatenby and Vincent, 2003, Hahn, 2004). Many tumor cells acquire a mutator or chromosome mis-segregation phenotype early in their development, which increases the frequencies of errors that occur during replication, repair, and segregation of chromosomes in dividing cells, and therefore increases the likelihood of accumulating the other two categories of cancer gene mutations (Lengauer et al., 1997, Negrini et al., 2010).

Multiple pathways function to maintain genetic integrity in normal cells. Accordingly, genetic instability in cancer can be caused by different classes of mutations. There are at least two general types that are observed in tumor cells: 1) chromosome instability (CIN) involving changes of chromosome structure and number as a result of an abnormal mitosis; 2) microsatellite instability (MIN) involving deletions or amplifications at the nucleotide level, especially at short tandem repeats (microsatellites) due to loss of mismatch repair (MMR) (Storchova and Pellman, 2004). The majority of solid colorectal tumors (>80%) exhibit CIN (Rajagopalan et al., 2003), and it is believed that the emergence of CIN provides

a fundamental source of mutation to drive tumorigenic progression rather than being simply a passenger phenotype (Jallepalli and Lengauer, 2001, Vogelstein and Kinzler, 2004). A large amount of data from human tumors suggests that CIN correlates with tumor grade and prognosis (Kronenwett et al., 2004, Carter et al., 2006). In addition, the high mutation rates and heterogeneity of tumor cells caused by CIN can provide growth advantages particularly in cells challenged by chemotherapeutic agents. So CIN contributes to both tumor evolution and therapeutic resistance (Rajagopalan and Lengauer, 2004). However, because CIN is specifically present in most types of cancer cells and not normal cells, it could be exploited for selective killing of tumor cells based on that phenotypic difference.

1.3 Synthetic Lethality (SL) and Synthetic Cytotoxicity (SC) strategies to target CIN

One strategy to target CIN is to identify gene mutations that are synthetic lethal with mutations that cause CIN. Synthetic lethality (SL) is a phenomenon that is well-characterized and studied in yeast and the fruit fly: two gene mutations that have little or no effect on cell viability individually but cause lethality when combined in a double mutant are said to be synthetic lethal (Kaelin, 2005). Early in 1997, Hartwell and colleagues hypothesized that cancer cells harboring known mutations or deletions might represent genetically sensitized cells relative to the normal surrounding cells that may be susceptible to drug therapies selectively targeting a second gene product (Hartwell et al., 1997). Recently this approach has proven effective in targeting cancer cells with loss of function of the homologous recombination (HR) pathway for DNA double-strand break repair (BRCA1/BRCA2 mutation), using small molecule inhibitors of PARP, a protein involved in DNA single-strand break repair (Bryant et al., 2005, Farmer et al., 2005). The CIN as a “driver” mutation in

cancer cells may therefore create opportunities for selective killing of cancer cells by mimicking the effect of the second genetic mutation with a targeted therapy (McManus et al., 2009, McLellan et al., 2009, McLellan et al., 2012) (Figure1.1).

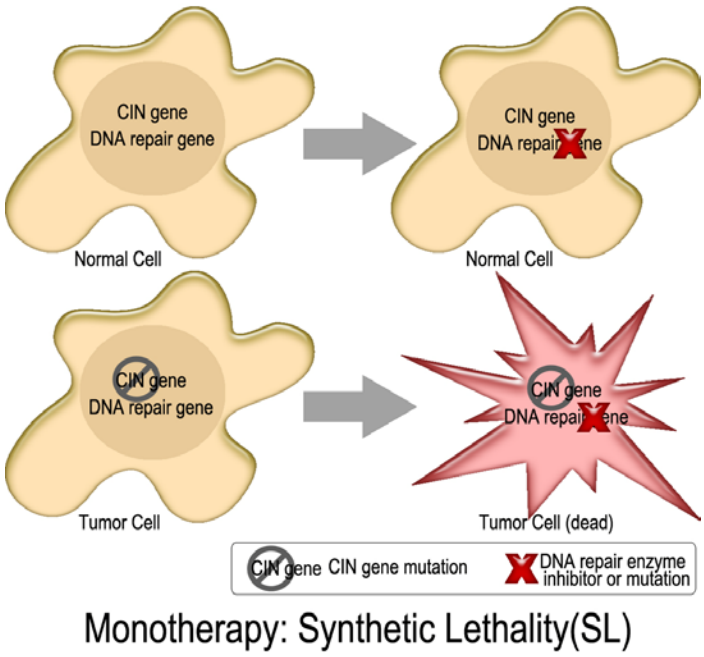

Figure 1.1 SL strategy to target tumor cells with a CIN mutation

Tumor cells harboring a CIN mutation may require certain DNA repair pathways for viability. The inhibition or mutation of the DNA repair enzyme in this pathway may selectively kill the tumor cell as a monotherapy.

DNA repair enzymes might be good candidates for targeting tumors for SL (Shaheen et al., 2011). In normal proliferating cells, DNA lesions are detected and repaired by the DNA damage response machinery. Many CIN proteins are involved in damage-induced signaling cascades and various DNA repair pathways (Paulsen et al., 2009, Schvartzman et al., 2010). The tumor cells harboring CIN mutations may exhibit a high level of endogenous DNA lesions that result in genetic instability. While being the origin of DNA mutation, CIN may also be a weakness: for example, causing reduced ability to replicate DNA and proliferate

(Negrini et al., 2010). Successful CIN cells have acquired additional mutations that adapt its genome maintenance system to cope with these genome imbalances. It could be said that some tumor cells become addicted to certain DNA repair pathways and would be sensitive to the inhibition of these pathways. Hence, in tumors with defined somatic CIN gene mutations, certain DNA repair enzymes may be excellent drug targets as monotherapies with high efficacy.

The level of DNA damage occurring spontaneously in cancer cells might be not enough to convert to fatal lesions and trigger cell death in the absence of certain DNA repair functions. Therefore, not all tumors will be susceptible to DNA repair inhibitor monotherapies. It is possible that these tumors will be sensitive to a combination therapy of DNA repair enzyme inhibitor and DNA damaging agent. Inhibiting the function of DNA repair enzymes could synergize with somatic mutations in the tumor cell to sensitize the tumors to DNA-damaging therapeutic agents resulting in Synthetic Cytotoxicity (SC) (Figure 1.2). In addition to the potential to improve efficacy and selectivity of treatment, another benefit of using the SC strategy to target cancer cells is to minimize the cytotoxic side effects because SC would permit significant reductions in the dose of cytotoxic agents from those used in standard chemotherapy. Furthermore, the toxicity of chemotherapy can be reduced by the increased activities of several DNA repair pathways (Helleday et al., 2008). So by identifying and targeting DNA repair enzymes as damage agent-enhancers, it may be possible to overcome natural and acquired tumor cell chemoresistance. Currently, clinical trials are assessing the efficacy of combination therapy with PARP inhibitors coupled with DNA damaging agents such as carboplatin, gemcitabine, and temozolomide (Chan and Giaccia, 2011). Although the

molecular mechanism behind the increased sensitivity to these therapeutics remains to be elucidated, these findings demonstrate the power of DNA repair enzyme inhibitors as sensitizers of tumor cells to chemotherapy. The same SC strategy could be used to target tumors with a CIN gene mutation.

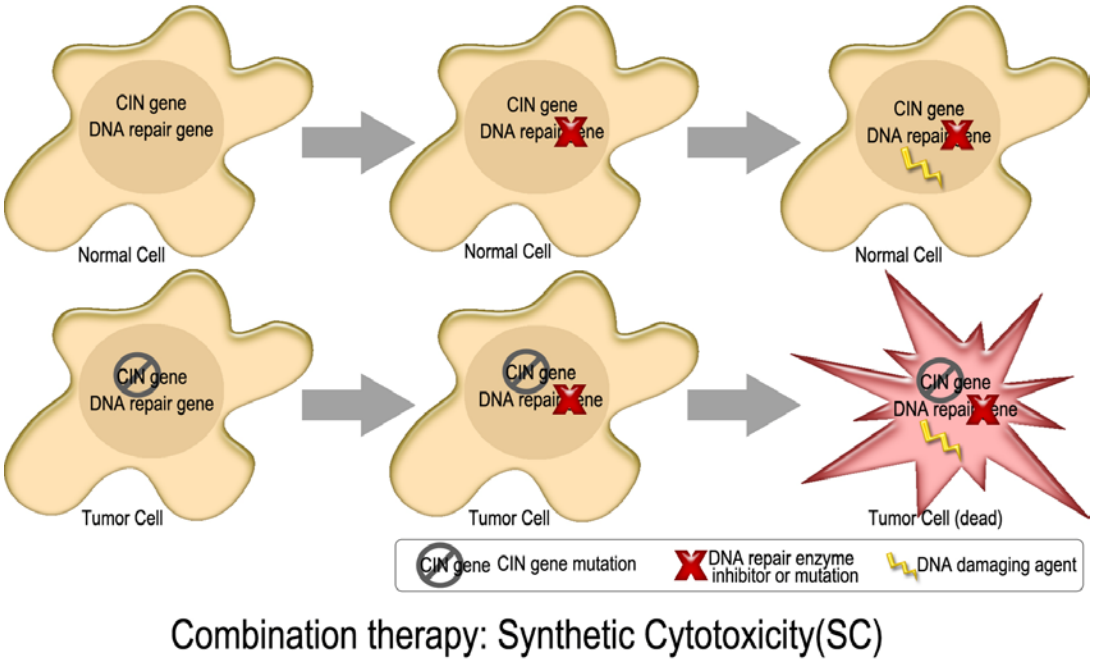

Figure 1.2 SC strategy to target tumor cells with a CIN mutation

The inhibition or mutation of certain DNA repair enzymes may sensitize the tumor cell harboring a CIN mutation to low doses of a DNA damaging agent resulting in Synthetic Cytotoxicity (SC).

1.4 CIN gene ATM mutations in cancer cell

To test SL and SC strategies to target tumor cells with CIN mutations, I chose the ATM (Ataxia Telangiectasia Mutated) gene, a CIN gene frequently mutated in many types of cancer and which represents the major CIN mutational spectrum of a tumor cell.

The ATM gene is a large gene encoding a 13 kb transcript. It has 66 exons extending over 160kb of genomic DNA (Shiloh, 2003). This gene was first discovered in the hereditary cancer-prone human disease ataxia telangiectasia (AT) (Savitsky et al., 1995). People with this disorder have mutations in both copies of the ATM gene in each cell. It is an autosomal recessive disorder which is caused by absent or inactivated ATM protein (Broeks et al., 2000). Researchers have identified several hundred mutations in the ATM gene in AT patients. The majority of these mutations are nonsense changes that lead to the generation of a premature stop codons and give rise to truncated proteins (Stankovic et al., 1998). People with AT have a 40% risk of developing cancer. The most common types of cancer seen are leukemia and lymphoma and often appear in childhood (Boultwood, 2001). As AT patients live longer, there appears to be an increased risk of other cancer types, including breast cancer, ovarian cancer, stomach cancer, melanoma, and sarcoma (Ahmed and Rahman, 2006, Kastan and Bartek, 2004, Renwick et al., 2006). AT cells exhibit radiosensitivity, altered DNA repair, cell cycle checkpoint defects and chromosomal instability (Lavin, 2008).

AT is rare disease with a frequency of ~1/40,000 (Savitsky et al., 1995). However, about 1% of the western populations are ATM heterozygotes (Renwick et al., 2006). It has been estimated that ATM gene mutation carriers have an increased hereditary pancreatic cancer risk (Fillon, 2012). They also have a 2.2-fold higher risk of developing breast cancer, and this number increases to nearly 5-fold for women under 50 (Thompson et al., 2005). The genomic instability present in the primary cells from AT patients and the high propensity of ATM mutation carriers to develop cancer strongly support the idea that CIN gene mutations in ATM drive tumor development by increasing the spontaneous mutation rate.

In addition to hereditary cancer, CIN gene ATM mutations were often detected in diverse sporadic cancers as well. Several studies have reported a high prevalence of ATM gene mutations in sporadic lymphoid cancer (Stankovic et al., 2002, Gumy-Pause et al., 2004). Recently, research showed that ATM is the 7th most frequently mutated gene in lung adenocarcinoma, detected in nearly 10% of cases (Kang et al., 2008). Mutation or reduced expression of ATM protein was found in 15% of gastric cancers. Low ATM protein levels were also detected in 10-20% of breast cancer cases and a strong correlation between ATM disruption and breast cancer was suggested (Hall, 2005).

The phenotype of AT patients and of cells carrying ATM mutations provided many clues to understand the function of ATM protein. ATM is a member of the phosphoinositide 3-kinase-related (PI3K-related) kinase family and is mainly involved in initial response to double-stranded DNA breaks (DSB) as a principal sensor. The activation of ATM in response to DSBs leads to phosphorylation and activation of multiple downstream protein effectors, which in turn results in the delay of cell cycle progression to facilitate DNA repair or the induction of apoptosis to eliminate the damaged cells. Many of these integrated cellular response processes are also required for subsequent activation of another master checkpoint kinase, the ATM- and Rad3-related (ATR) protein which also belongs to the PI3K-related kinase family (Shiloh, 2003).

Because of its central role in the DNA damage response, ATM is an important protein for preventing malignant transformation through induction of cell cycle checkpoints, senescence and apoptosis. However, in cancer cells, the response to genome alterations is commonly

impaired owing to accumulation of mutations in the ATM checkpoint regulator. Such alterations disrupt the ability of the cell to maintain genomic stability, generating the CIN and mutator phenotype, and making the acquisition of additional genomic alterations in the cancer cell more likely. Due to well-established problems in the means of gene delivery, therapeutic restoration of ATM is currently not a viable option for cancer therapy. However, an ATM mutation inducing CIN may represent a weakness relative to normal cells that can be exploited for tumor therapy. Therefore, the study to exploit SL and SC strategies-based target therapy through investigating the synthetic genetic interactions with ATM mutation is of interest in cancer research.

1.5 Conserved SL interactions and the ATM homologue gene in yeast

Given the large number of DNA repair enzymes and various types of DNA damaging agents, it is necessary to develop a robust method to screen for combinations of gene pair and DNA damaging agents for synthetic lethal and synthetic cytotoxic interactions with ATM mutations. Since genome stability and DNA repair are basic and fundamental cellular processes, the mechanisms and genes involved are highly conserved among eukaryotes (Kitagawa and Hieter, 2001). Many insights into genetic interactions have been obtained from the model organism *Saccharomyces cerevisiae*, in which a genome-wide deletion collection has been generated that can be used with high-throughput screening techniques (Scherens and Goffeau, 2004). Instead of searching for SL and SC genetic interactions with ATM mutation directly in human cells, I use a budding yeast with a dysfunctional ATM homologue gene to mimic ATM mutations in cancer cells. I then knocked out second site DNA repair genes to mimic the inhibition of the DNA repair enzyme. Yeast is a genetically

amenable model organism with a short life cycle that greatly facilitates my research and allows the rapid translation of data from the genetic networks with ATM to Human. In this way, I can narrow down the number of synthetic lethal and cytotoxic candidate gene pairs and then directly test these candidates in human cells. This approach is based upon the observation that many synthetic interactions are conserved across species (McManus et al., 2009, McLellan et al., 2009).

The yeast homologues of ATM and the similar PI3K-related kinase ATR are TEL1 and MEC1, respectively. TEL1 and MEC1 have overlapping functions in mediating the cellular response to DNA damage. While ATM/TEL1 is not essential for growth in mammals and yeast, loss of function of the related kinase ATR/MEC1 is lethal in both human and budding yeast (Brown and Baltimore, 2000, de Klein et al., 2000). However, MEC1 deletion mutants are viable when an inhibitor of ribonucleotide reductase, SML1, is also deleted (Zhao et al., 1998). Therefore, I can create a set of clean deletion strains for my assays bearing *tel1* or *mec1* deletions or *tel1* and *mec1* double deletions. *sml1* deletion strains will serve as an additional "wild type" control for any strain deleted in MEC1 to ensure that SML1 is not having an effect.

1.6 Overview of thesis

I propose to use the model organism *Saccharomyces cerevisiae* to screen for synthetic lethal interactions between DNA repair genes and the CIN gene ATM, and for synthetic cytotoxic interactions in combination with sub-lethal doses of DNA damaging agents. I hypothesize that knockdown of certain second site DNA repair genes will selectively kill TEL1 deficient

cells resulting in synthetic lethality, or sensitize TEL1 deficient cells to DNA damaging agents resulting in synthetic cytotoxicity.

As proof of principle for this hypothesis, I first directly tested genetic interactions in a small matrix of three DNA repair enzyme genes and then used high throughput genetic methods to screen a large matrix of the complete non-essential gene deletion collection in budding yeast to discover synthetic lethal and synthetic cytotoxic relationships between TEL1, DNA repair enzymes and sub-lethal doses of DNA damaging therapeutic agents. SL and SC interactions data derived in model organisms may or may not be conserved in humans and will need to be further validated.

This study would provide the basis for monotherapies and combination therapies for selective killing of ATM deficient cancer cells. The identification of negative genetic interactions (the extreme example: SL) with ATM mutation can be exploited directly as drug targets for monotherapies for ATM deficient cancer. Furthermore, the genes that upon knockdown sensitize cells to a specific DNA damaging agent can be used as targets in combination therapy.

Chapter 2 : Direct testing for SL and SC with ATM mutation in combination with three DNA repair enzymes

2.1 Introduction

Studies show that the *Saccharomyces cerevisiae* homologue of ATM, TEL1 plays a similar role in maintaining genome stability as a central mediator of the DNA damage and replication checkpoints (Ritchie et al., 1999, Zhou and Elledge, 2000). Also, based on the observation of molecular structures, the homology between ATM and TEL1 is not restricted to the catalytic domain at the C terminus of the protein, but rather extends over the whole length (Mallory and Petes, 2000). Unlike its homologue ATM, TEL1 is also involved in telomere maintenance. TEL1 was originally isolated as a mutant that had stable shorter (about 50 bp) telomeres (Greenwell et al., 1995, Lustig and Petes, 1986). Although the phenotype of TEL1 mutated yeast cells are not identical to ATM mutated mammalian cells, it has been observed that the TEL1 gene, when transformed into an AT cell line, could complement many of the mutant phenotypes associated with the ATM mutation (Fritz et al., 2000).

In human cells, many proteins involved in cell cycle checkpoints and DNA repair are phosphorylated in an ATM-dependent manner in response to DNA damage, including many proteins involved in the homologous recombination (HR) repair pathway (Mre11, Nbs1, Rad50, Rad51, Rad52, Rad54, RPA, Exo1, CtIP and BRCA1/2) and several non-homologous end joining (NHEJ) repair pathway core proteins (DNA-PKcs, XLF and Artemis) (Bensimon et al., 2010). HR and NHEJ are the two main pathways for DNA DSB repair in human cells (Mao et al., 2008). Although ATM has many substrates, its contribution to the DNA damage

response is partially masked by ATR. Many IR-induced phosphorylation sites targeted by ATM can also be phosphorylated by ATR (Matsuoka et al., 2007). In yeast, TEL1 has been shown to partially restore the DNA damage checkpoint in cells with a MEC1 (ATR in humans) deficiency (Clerici et al., 2004). And cells lacking both TEL1 and MEC1 are more sensitive to DNA damaging agents than either single mutant. Also, because of this redundancy in function, the TEL1 or MEC1 mutation alone demonstrates low or moderate effects on genome stability, while loss of both genes leads to synergistically elevated levels of genome instability in budding yeast.

Genetic interactions of the CIN genes TEL1 and MEC1 have been widely explored in the model organism budding yeast. Only a few SL interactions with TEL1 have been found and most of the SL partners are essential genes, such as DNA2 and MEC1 (Budd et al., 2005, Chakhparonian et al., 2005). In *S. cerevisiae*, *mec1* null cells are inviable, so most genetic interaction data are based on hypomorphic *mec1* mutations or in combination with the *sml1* deletion background. In my primary screen in budding yeast for SL and SC genetic interactions with TEL1 or MEC1 mutations, I focused on three DNA repair enzymes, tyrosyl-DNA-phosphodiesterase 1 (TDP1), DNA 3'phosphatase (TPP1/PNKP in humans) and RAD27 5'flap endonuclease (FEN1 in humans), for which small molecule inhibitors have been identified or are being developed (Huang et al., 2011, Freschauf et al., 2009).

In my study, the inhibition of the DNA repair enzyme is modeled through knockout of the corresponding yeast homologue. The combination of mutations in three repair enzyme genes (TDP1, TPP1, RAD27) and two CIN genes (TEL1, MEC1) results in a 2x3 matrix of double

gene deletion strains. Any inviable double mutant combinations of CIN gene and DNA repair gene will be considered SL and can be prioritized for validation in human cells as a potential single-agent target. On the other hand, viable double mutants will be screened for synthetic cytotoxicity by exposing them to sub-lethal doses of various classes of DNA-damaging agents.

In order to test whether SC occurs with general DNA damage or a specific lesion type, four DNA damaging agents that cause different types of lesions, were chosen: bleomycin (BLEO), 5-fluorouracil (5FU), camptothecin (CPT) and hydroxyurea (HU) (Helleday et al., 2008) (Table 2.1). After the construction of all double gene deletion strains between CIN genes and DNA repair genes, their sensitivities to 4 types of DNA damage were tested by plate assays on YPD media with/without corresponding DNA damaging agents, resulting in 2X3X5 matrix screen (Figure 2.1). The SC interactions were quantified by growth curve analyses.

DNA damaging agent	Compound Description	Toxic Lesions
5-Fluorouracil (5FU)	Antimetabolite	Base damage; Replication lesions
Bleomycin (BLEO)	Radiomimetic DNA-cleaving agents	DNA strand breaks; Base damage
Camptothecin (CPT)	Topoisomerase I inhibitor	DNA strand breaks; Replication lesions
Hydroxyurea (HU)	Ribonucleotide reductase inhibitor	Stalled/collapsed replication forks

Table 2.1 DNA damaging agents

Four DNA damaging agents that cause different types of lesions were chosen for DNA damage sensitivity test: bleomycin (BLEO), 5-fluorouracil (5FU), camptothecin (CPT) and hydroxyurea (HU) (Helleday et al., 2008).

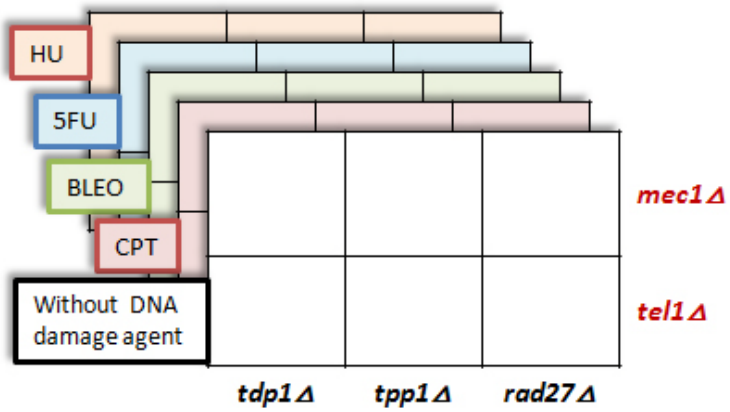

Figure 2.1 Screen in 2X3X5 matrix

The combination of double mutations of three repair enzyme genes (TDP1, TPP1 and RAD27) and two CIN genes (TEL1 and MEC1) results in a 2x3 matrix of double gene deletion strains. All double mutants were first tested for synthetic lethality by growing in media without DNA damaging agent and then screened for cytotoxicity by exposing to sub-lethal doses of four DNA-damaging agents: bleomycin (BLEO), 5-fluorouracil (5FU), camptothecin (CPT) and hydroxyurea (HU).

2.2 Material and methods

2.2.1 Yeast strains and media

All strains are BY4743 background (Brachmann et al., 1998). The heterozygous double mutant strains were constructed by mating each of the corresponding single mutants. The haploid double mutant strains were generated by sporulation of diploid heterozygous double mutant strains followed by tetrad dissection. The genotypes of all the strains used in the experiment were checked by PCR for confirmation of the gene deletions (Table 2.2).

Yeast was grown in rich media at 30°C (Amberg et al., 2005). Plasmid bearing strains were grown in synthetic complete media lacking the appropriate nutrient.

Strain	Genotype
BY4741	*MAT*a *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*
BY4742	*MAT*α *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*
PSY437	*MAT*a *his3 leu2 lys2 ura3 tel1::KANMX*
PSY438	*MAT*α *his3 leu2 lys2 ura3 tel1::KANMX*
PSY765	*MAT*α *his3 leu2 lys2 ura3 mec1::HYG sml1::KANMX*
PSY766	*MAT*a *his3 leu2 lys2 ura3 mec1::HYG sml1::KANMX*
YDV103	*MAT*a *his3 leu2 lys2 ura3 rad27::HYG*
YDV104	*MAT*α *his3 leu2 LYS2 ura3 rad27::HYG*
YNM1	*MAT*a/*MAT*α *his3/his3 leu2/leu2 LYS2/lys2 MET15/ met15 TRP1/TRP1 ura3/ura3 CAN1/can1::LEU2-MFA1pr-HIS3 TDP/tdp1::KANMX*
YNM10	*MAT*α *his3 leu2 lys2 ura3 tel1::NAT*
YNM11	*MAT*α *his3 leu2 lys2 ura3 mec1::NAT sml1::KANMX*
YNM12	*MAT*α *his3 leu2 lys2 ura3 mec1::HYG sml1::NAT*
YNM14	*MAT*a *his3 leu2 LYS2 TRP1 ura3 tpp1::KANMX*
YNM15	*MAT*a *his3 leu2 lys2 ura3 rad27::HYG sml1::KANMX*
YNM16	*MAT*a *his3 leu2 lys2 ura3 rad27::HYG sml1::KANMX*
YNM17	*MAT*a *his3 leu2 lys2 ura3 tdp1::KANMX tel1::NAT*
YNM18	*MAT*a *his3 leu2 lys2 ura3 tdp1::KANMX tel1::NAT*
YNM2	*MAT*a/*MAT*α *his3/his3 leu2/leu2 LYS2/lys2 MET15/ met15 TRP1/TRP1 ura3/ura3 CAN1/can1::LEU2-MFA1pr-HIS3 TPP/tpp1::KANMX*
YNM20	*MAT*α *his3 leu2 lys2 ura3 tdp1::KANMX tel1::NAT*
YNM22	*MAT*α *his3 leu2 lys2 ura3 tdp1::KANMX mec1::HYG sml1::NAT*
YNM23	*MAT*a *his3 leu2 lys2 ura3 tpp1::KANMX tel1::NAT*
YNM24	*MAT*α *his3 leu2 lys2 ura3 tpp1::KANMX tel1::NAT*
YNM25	*MAT*a *his3 leu2 LYS2 ura3 tpp1::KANMX mec1::HYG sml1::NAT*
YNM26	*MAT*α *his3 leu2 lys2 ura3 tpp1::KANMX mec1::HYG sml1::NAT*
YNM27	*MAT*a *his3 leu2 lys2 ura3 rad27::HYG tel1::NAT*
YNM28	*MAT*α *his3 leu2 lys2 ura3 rad27::HYG tel1::NAT*
YNM3	*MAT*a *his3 leu2 LYS2 TRP1 ura3 tdp1::KANMX*
YNM30	*MAT*α *his3 leu2 LYS2 ura3 rad27::HYG sml1::KANMX*
YNM31	*MAT*a *his3 leu2 lys2 ura3 tel1::KANMX mec1::HYG sml1::NAT*
YNM32	*MAT*α *his3 leu2 lys2 ura3 tel1::KANMX mec1::HYG sml1::NAT*
YNM4	*MAT*α *his3 leu2 lys2 TRP1 ura3 tdp1::KANMX*
YNM6	*MAT*α *his3 leu2 lys2 TRP1 ura3 tpp1::KANMX*
YNM7	*MAT*a *his3 leu2 lys2 ura3 rad27::NAT*
YNM8	*MAT*α *his3 leu2 LYS2 ura3 rad27::NAT*
YNM9	*MAT*a *his3 leu2 lys2 ura3 tel1::NAT*

Table 2.2 Strains used in the study in Chapter 2

2.2.2 DNA damaging agent sensitivity assay

Both plate assays and growth curve analyses were utilized to identify SC interactions by comparing the agent sensitivity of double mutant strains with the two single mutant parental strains.

All cells were grown in YPD media without DNA damaging agent until log phase before spotting on YPD plates or addition to YPD liquid media containing sub-lethal doses of the DNA damaging agents. OD_{600} values were checked in order to transfer the same number of single and double mutant cells. The sub-lethal doses of the DNA damaging agents were decided based on previous tests of the fitness of single mutants in serially diluted concentration of agents on plates or in liquid media. The concentration at which the growth of one parental single mutant was mildly affected was used as the inhibitory concentration. The double mutants and parental strains being tested were exposed to three concentrations (inhibitory concentration diluted 2X, 8X, and 16X) of each DNA-damaging agent during on plate assay and growth curve analyses (Table 2.3).

DNA damaging agents	**Concentrations for on plate assay**			**Concentrations for growth curve assay**		
Camptothecin (CPT)	1μg/ml	2 μg/ml	8 μg/ml	0.5μg/ml	1 μg/ml	4 μg/ml
Bleomycin (BLEO)	0.5μg/ml	1 μg/ml	4 μg/ml	0.1μg/ml	0.2μg/ml	1 μg/ml
5Fluorouracil (5FU)	2.5μg/ml	5 μg/ml	20 μg/ml	1 μg/ml	2 μg/ml	8 μg/ml
Hydroxyurea (HU)	10 mM	20 mM	80 mM	2 mM	4 mM	16 mM

Table 2.3 Concentrations of DNA damaging agents for the sensitivity test

For on plate spot assays, an identical optical density (OD_{600}) of cells was serially diluted 5-fold and spotted on the indicated plate at the indicated temperature for 72 hours. For growth curve analysis logarithmic phase cultures were diluted to an OD_{600} value of 0.15±0.02 in 96-well plates and grew for 24 hours in a TECAN M200 plate reader at 30°C. Each strain was tested in three replicates.

2.2.3 Quantitative growth curve analysis

The fitness of strains in growth curve analysis is often calculated by two ways: doubling time (the time required for a population to double in size during log phase); and specific growth rate (the maximum growth rate estimated through the slope) (Shah et al., 2007). However, both calculations only focus on the exponential growth region, a small area of the growth curve. They work well with most of the healthy strains, but when estimating very sick strains with slow growth, it is hard to define the exponential growth regions. In addition, the ability to adapt to growth conditions is different between mutants. And as population increases, the limited pool of resource and toxic waste buildup prevent growing beyond certain threshold. So the resulting lag and plateau phases are also very important factors when estimating fitness, especially for the mutants with growth defects. To avoid these issues, I used the area-base calculation method developed by Kevin Ushey (UBC Statistics MSc student). Strain fitness F was defined as the presentation of the logarithm of the area under the curve (AUC) of mutants relative to wild type (Ushey, 2011, McLellan et al., 2012).

The genetic interactions are quantified through the comparison of fitness of double mutants and expected phenotype base on two single mutants. Mutations in independent genes (two

genes with a neutral interaction) often combine in a multiplicative manner. The expected fitness of resulting double mutant is assumed to be $F_{ab}^* = F_a \times F_b$. The interaction can be quantitatively measured by comparing double mutant F_{ab} against expected F_{ab}^* (Mani et al., 2008).

2.3 Results

2.3.1 Most double mutant strains are viable without growth defects

All double heterozygous deletion strains for genetic analysis were generated by mating two haploid single deletion strains bearing either the CIN or DNA repair gene deletion. After sporulation, the viability of double mutants was first checked by the growth of double mutant offspring after tetrad dissection. Most spores from *tel1* and DNA repair gene double heterozygous deletion diploids were viable. When selected for the antibiotic resistance maker, most tetrads exhibited 2:2 segregation patterns. Because MEC1 is an essential gene, we used a *mec1 sml1* double mutant, which is viable. As expected, only half of the offspring spores generated from triple heterozygous deletion strains (*mec1Δ sml1Δ tdp1Δ* or *mec1Δ sml1Δ tpp1Δ*) were viable.

All 5 combination double mutants grew similar to wild type, and did not show sick/slow growth on plates. The viable double deletion strains were further tested for synthetic sickness by comparing to parental single deletion strains. The fitness relative to wild type was quantified using liquid growth curve analysis (Ushey, 2011, McLellan et al., 2012), which show no synthetic growth defects.

2.3.2 SL interactions between *rad27* and *mec1* mutations

For the *mec1 rad27* double heterozygote, only 13 in total 55 spores derived from asci separated on YPD plates were viable suggesting other reasons causing death beside the separation of mec1 deletion and sml1 deletion. *mec1 sml1 rad27* triple deletion haploid strains could not be isolated after replication to selection media plates. The genotype of some inviable spores deduct from the rest spore on selection plate should be triple deletion (representative tetratype tetrad is shown in Figure 2.2). A *rad27 mec1* double heterozygous deletion and *sml1* homozygous diploid was constructed to eliminate the effect of SML1 and confirm the *rad27 mec1* synthetic lethal interaction. Inviability of the *rad27 mec1* double mutant synthetic lethality was confirmed in 22 tetrads, and no viable double deletion haploid was found.

Plates	Tetrad spores		phonotype	genotype
YPD	1		viable	RAD27 MEC1
	2			*rad27Δ mec1Δ*
	3		viable	RAD27 *mec1Δ*
	4		viable	*rad27Δ* MEC1
YPD HYG	1		inviable	RAD27
	2			*rad27Δ*
	3		inviable	RAD27
	4		viable	*rad27Δ*
YPD NAT	1		inviable	MEC1
	2			*mec1Δ*
	3		viable	*mec1Δ*
	4		inviable	MEC1

Four haploid spores from one *mec1 sml1 rad27* triple heterozygous deletion diploid cell were dissected onto an YPD plate and replica-plated onto marker selection plates with different antibiotics: YPD HYG and YPD NAT. Only *rad27Δ*::Hyg mutants can survive on YPD HYG plates while *mec1Δ*::NAT mutants can grow on YPD NAT plates. There were three viable spores that grew to colonies on the YPD plate. Marker segregation patterns were 2:2, as expected. The genotype of the inviable spores could be deducted from the growth of viable spores on selection plates and corresponded to *rad27Δ*::Hyg *mec1Δ*::NAT deletion mutants.

Figure 2.2 Inviable spores due to SL after tetrad dissection

2.3.3 Most double mutants show the same DNA damaging agent sensitivity as single mutants

Comparing the concentrations of the DNA damaging agents used in the on plate spot assay and in liquid growth curve assay, it was clear that yeast cells are much more sensitive to DNA damaging agents in liquid than on the surface of solid media. Among all single deletion strains, *mec1Δ* and *rad27Δ* are more sensitive to DNA damaging agents especially camptothecin and bleomycin, *tel1* null mutants are mildly sensitive to CPT. *tpp1* and *tdp1* single deletion strains grow similar to wild type and double mutants show similar sensitivity with their parental *mec1* or *tel1* single deletion strains. In addition, growth curve assays to quantify the interaction also show the same results.

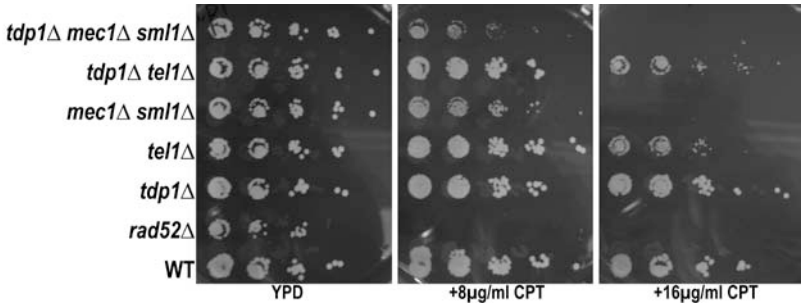

Figure 2.3 Example of no SC interaction (TDP1, TEL1/MEC1 and CPT) by spot assay

A serial 5x dilution of cells was spotted on YPD plates with/without CPT. On YPD plates all mutant strains grow similarly as compared to wild type. *rad52Δ* mutant which is hypersensitive to CPT, was used as positive control. *tdp1Δ* strain was not sensitive to CPT even at 16 µg/ml CPT. *tdp1Δ mec1Δ sml1Δ* mutants show slow growth on YPD plate with 8 µg/ml CPT, similar to *mec1Δ sml1Δ* mutants. *tdp1Δ tel1Δ* double mutants grow sick similarly with *tel1Δ* mutant on 16 µg/ml CPT plate.

Synthetic cytotoxicity of viable double mutants was directly tested by plating onto YPD plates with sub-lethal doses of DNA damaging agents and comparing sensitivity to the two single mutant parental strains. The double mutants and parental strains were tested along with the wild type as native control and *rad52* single deletion as positive control (one example of spot assay for *tdp1Δ* CPT sensitivity test is shown in Figure 2.3). Tdp1 participates in the

repair of topoisomerase I inhibitor-induced DNA damage, but knock out of TDP1 in budding yeast is not sensitive to CPT (Pouliot et al., 2001). This is consistent with the observation on spot assays that the *tdp1Δ* mutant did not show a growth defect on CPT plates, suggesting the existence of other repair mechanisms in addition to Tdp1 to remove Top1-DNA cleavage complexes trapped by CPT (Liu et al., 2002).

2.3.4 SC interactions between *rad27* and *tel1* mutations

The spot assays suggested *rad27* and *tel1* exhibited a clear SC interaction on CPT plates and weak negative interaction on HU plates (Figure 2.4). However, on BLEO and 5FU plates, double mutants grew similar to *rad27Δ* single mutants indicating the sensitivity of double mutants is specific to CPT and HU induced DNA lesions. The growth curve assays show the similar results (Figure 2.5). In 0.5 µg/ml CPT, the double mutant growth curve was much slower than either single mutant growth curve, about 7% worse than the prediction, indicating a SC interaction (Figure 2.6).

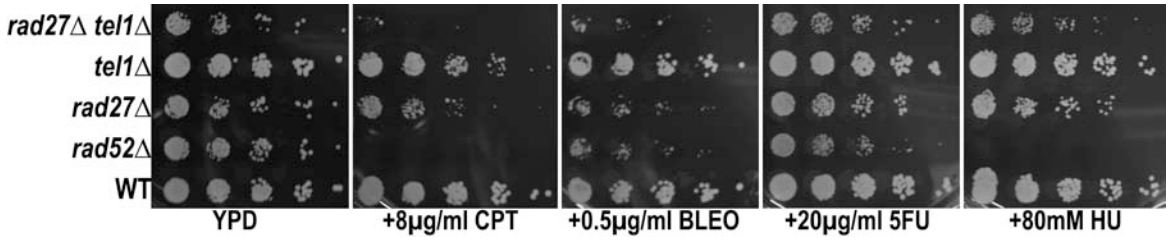

Figure 2.4 SC interaction between RAD27 and TEL1 by spot assay

A serial 5x dilution of cells was spotted on YPD plates containing different DNA damaging agents. On YPD control plates, *tel1Δ* grows as well as wild type. Both *rad27Δ* single mutant and *rad27Δ tel1Δ* mutant grow a little slower than wild type. On CPT plate, *rad52Δ* (positive control) and *rad27Δ tel1Δ* mutants are hypersensitive to CPT. *tel1Δ* is slightly sick compared to wild type, while *rad27Δ* shows a mild growth defect. On HU plates, the double mutant is a little sicker than *rad27Δ* and relatively more sensitive compared to *tel1Δ*. On BLEO and 5FU plates growth of the double mutant is similar to *rad27Δ* single mutant.

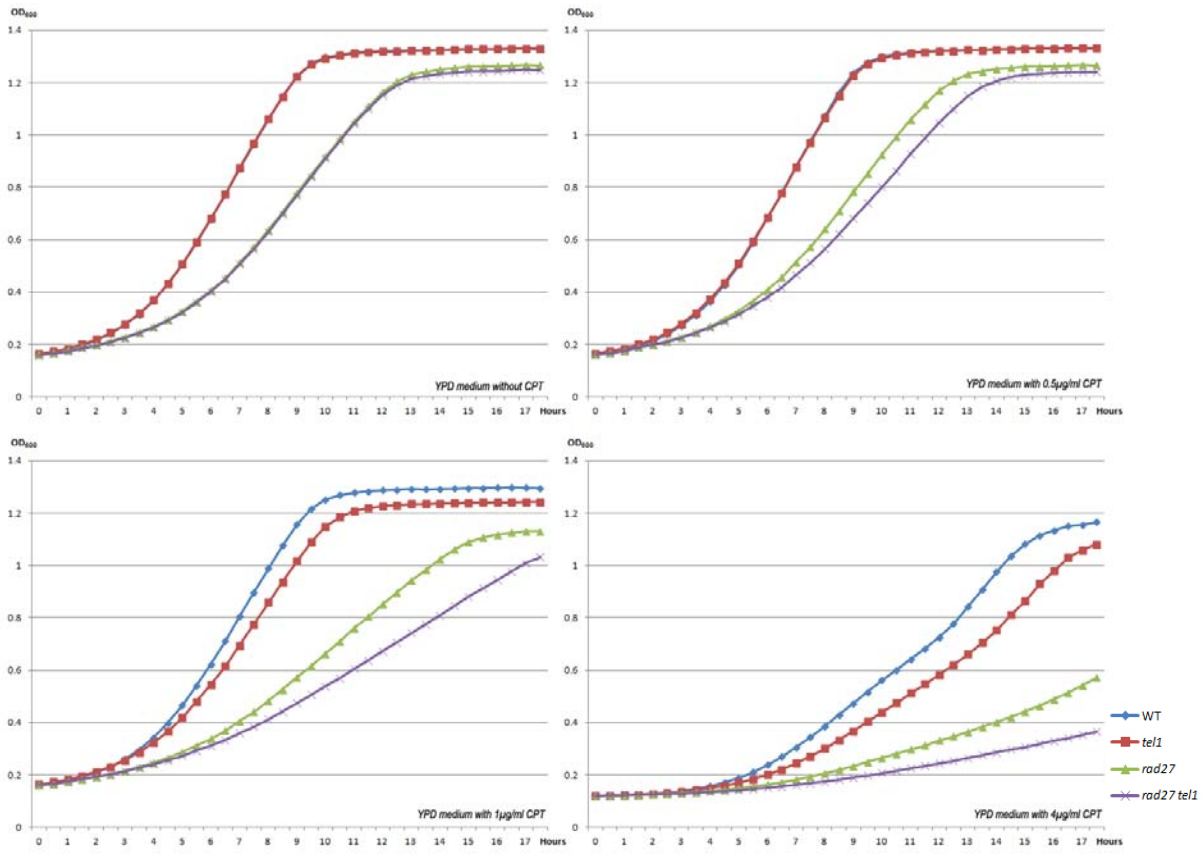

Figure 2.5 SC interaction between RAD27 and TEL1 by growth curve assay

In YPD control media, *tel1Δ* grows as same as wild type, while *rad27Δ* and *rad27Δ tel1Δ* mutants grow a little slower. A low dose of CPT (0.5µg/ml) shows no/little effect on all strains except double mutants. In YPD media with 1 µg/ml CPT, the growth of double mutants is much slower than single mutants. The growth curves shown are using average reads from 3 replicates data.

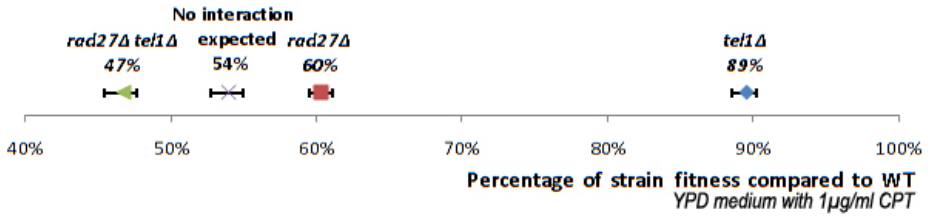

Figure 2.6 Quantitative SC analyses of fitness data from growth curve assay

The fitness compared to wild type during the growth curve assay is calculated using curve area based methods (Ushey, 2011, McLellan et al., 2012). The percentages of each strain relative to wild type are shown above the line and average numbers are noted. The expected fitness values of double mutants with no interaction between *rad27* and *tel1* are obtained by multiplying two single mutant fitness values together. The actual fitness of *rad27Δ tel1Δ* double mutants in the presence of 1 µg/ml CPT is about 7% lower than expected, indicating a SC interaction between *rad27* and *tel1*.

2.4 Discussion

Similar to their homologues ATM and ATR in human cells, MEC1 and TEL1 initiate the DNA damage response in response to single-strand and double-strand DNA breaks. A cascade of protein kinases are activated to arrest or slow the cell cycle, allowing time to repair the damage (Clerici et al., 2004). FEN1/RAD27 is highly conserved through evolution. RAD27 encodes a nuclease involved in many aspects of DNA metabolism including processing Okazaki fragments during DNA replication, base excision repair (BER), and other pathways maintaining genome stability (Liu et al., 2004). Inactivation of Rad27 in budding yeast leads to the accumulation of double-strand breaks, chromosome instability, and sensitivity to methylmethane sulfonate (MMS) (Debrauwere et al., 2001). I have found that *rad27* null mutants are also sensitive to CPT. Because of its multifunctional role, more than 30 SL interactions with RAD27 have been reported that include many DNA damage checkpoint genes including RAD9, RAD24, RAD17 and MEC1 (Pan et al., 2006), which is consistent with my experimental results. It suggests that double-strand breaks created in *rad27* mutants may require the TEL1/MEC1 checkpoint to delay cell division to allow DNA damage repair to maintain viability. The three DNA enzymes tested during the first screening did not provide any hint of the mechanism behind the only SC interaction identified. Also because of the multiple functions of RAD27 and the indirect DNA damage caused by camptothecin, there are still many questions about this SC interaction between RAD27 and TEL1 in the presence of CPT. But the discovery of SC interaction in yeast proved my hypothesis and suggested SC interaction could be specific to certain types of DNA lesions.

Furthermore, the direct screening of the matrix (2 CIN genes X 3 DNA repair enzyme genes X with/without 4 damage agents) revealed FEN1/RAD27 SL with ATR/MEC1 and SC to camptothecin with ATM/TEL1 in budding yeast. This work suggests a new target for combination therapy: FEN1 inhibitor combined with low non-toxic dose of camptothecin may enhance cytotoxicity to ATM deficiency cancer cell.

Chapter 3 : Genome wide screen for synthetic interactions with TEL1

3.1 Introduction

From directly testing a 2 gene X 3 gene X 4 DNA damaging agent matrix, I found that the *rad27 tel1* double mutant shows a strong Synthetic Cytotoxicity (SC) to CPT treatment in yeast. Because Rad27 is a multifunctional protein with roles in DNA replication, base excision repair (BER) and double-strand break repair (DSBR) pathways (Liu et al., 2004), the mechanism behind the SC with TEL1 is not clear. Testing of more synthetic interactions with TEL1 (i.e. with more than one DNA repair gene in certain repair pathway) would provide a more comprehensive view and may lead to a better insight into the mechanism. In addition to DNA repair enzymes, genes involved in other process may lead to SC as well, such as other CIN genes or proteins that exclude DNA damaging agents from the cell. To get a better view of the TEL1 SC network and to identify more candidate genes that target *tel1* deficient cells, I expanded my search to the collection of non-essential gene deletions in yeast using high throughput Synthetic Genetic Array (SGA) technology.

With the SGA technique, it is possible to systematically construct double mutants by introducing the tel1 deletion allele in a query strain and mating to an ordered array of ~5000 non-essential gene deletion mutants (representing ~80% of all yeast genes) (Tong and Boone, 2006). (Figure 3.1) The generated single and double deletion haploid strains were transferred and grown on selective plates separately. Because the amount of cells of each strain transferred by robotic pinning are relatively similar, the fitness of haploid mutants can be estimated based on strain spot size on the final plate. By comparing the fitness of single mutants and their corresponding double mutants, a quantitative measurement of genetic

interactions can be determined. In this way, I could identify all non-essential SL partner genes with *tel1*. In parallel, each strain was replicated onto media containing sub-lethal doses of DNA damaging agent, and comparison of the growth on plates with and without DNA damaging agent demonstrates the differential sensitivity of every single and double deletion strain. The SC partner genes were identified when single gene deletion strains exhibited low sensitivity to DNA damaging agent and the corresponding double mutants exhibited hypersensitivity. All hits identified in high throughput fashion could be further validated by tetrad dissection or random spore assay.

Because the only SC interaction identified in the previous experiment in the 2X3X5 matrix shows specificity to CPT, I chose CPT as the DNA damaging agent in the SGA screen. All SC hits identified during SGA screening could be tested with other DNA damaging agents to test whether the SC was specific for a type of lesion or was for DNA damage in general.

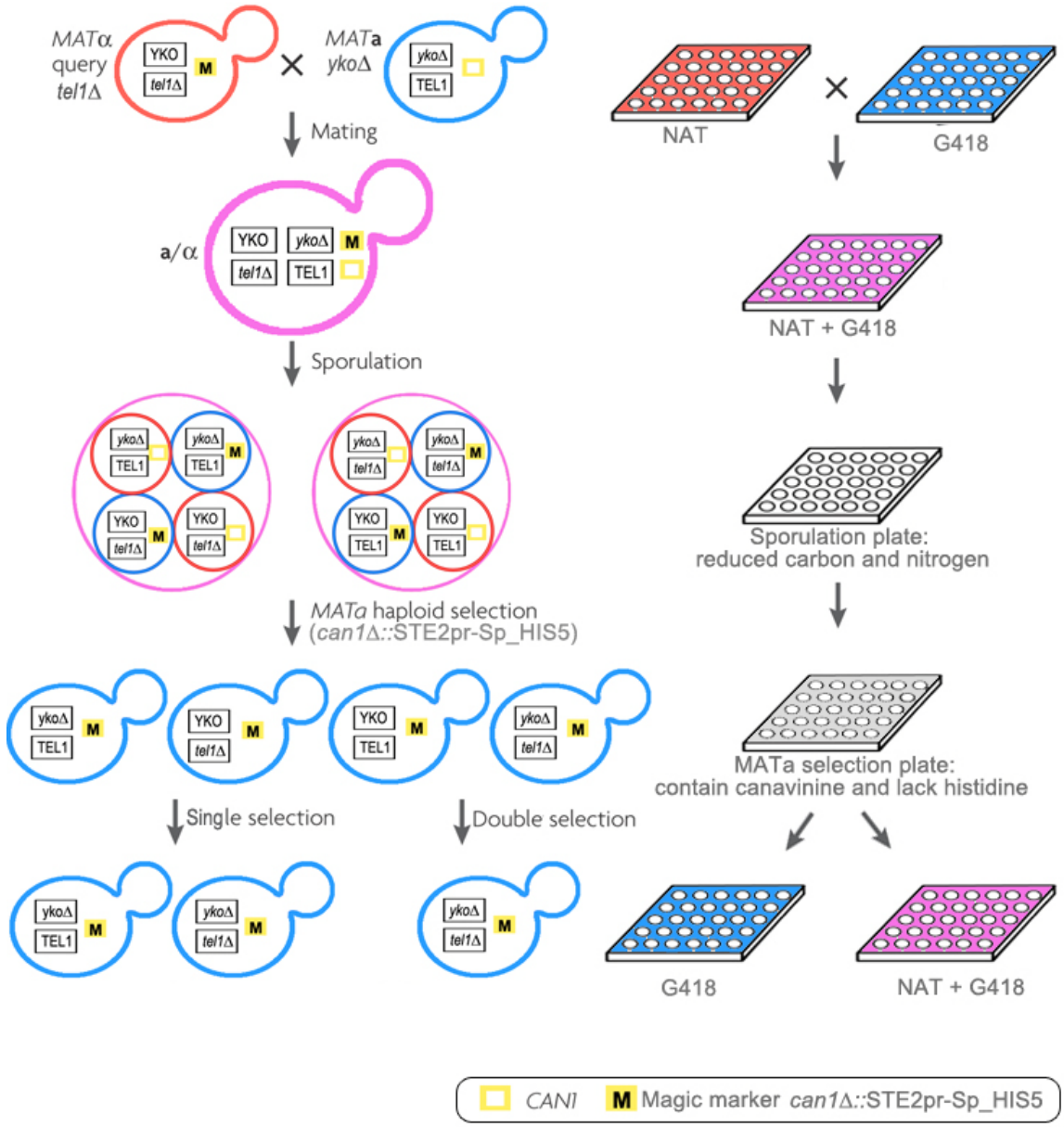

Figure 3.1 Synthetic Genetic Array (SGA)

MATα strain carrying the magic marker (*can1Δ*::STE2pr-Sp_HIS5) and a query gene TEL1 replaced by selectable marker (*tel1Δ*::NAT) is crossed to an ordered array of MATa deletion mutants (*ykoΔ*::kanMX). The resulting heterozygous diploids are induced to sporulate on medium with reduced carbon and nitrogen. The generated haploid meiotic spores are selected on medium lacking histidine and containing canavinine (a toxic analogue of arginine) for MATa cells which express *S.pombe* His5 protein and are deleted of CAN1 gene (encoding arginine permease). The selected MATa progeny are transferred to a medium that contains G418 / G418+NAT, which selects for single/double mutants.

3.2 Materials and methods

3.2.1 Yeast strains and media

All strains are BY4743 background (Brachmann et al., 1998). The heterozygous double mutants were constructed by mating each of the corresponding single mutants. The haploid double mutants were generated from the sporulation of diploid heterozygous double mutants followed by tetrad dissection. The genotypes of all the strains used in the experiment were checked by PCR confirmation of the genomic DNA (Table 3.1).

MATa deletion mutant array (DMA) for SGA was obtained from Research Genetics Company. The MATa deletion collection was originally derived from genome-wide "bar-coded" yeast knockout (YKO) mutants constructed by the *Saccharomyce* Genome Deletion Project (http://www.sequence.stanford.edu/group/yeast_deletion_project/deletions3.html). The overall genotype of the MATa deletion mutants from the DMA collection is: MATa *ura3Δ leu2Δ his3Δ lys2Δ met15Δ ykoΔ*::kanMX.

Heterozygous gene deletion collection provided by Jef Boeke was used to obtain single gene knockout alleles through tetrad dissection. It was originally constructed for the dSLAM technology (heterozygous diploid-based Synthetic Lethality Analysis on Microarrays) (Pan X et al., 2004). The overall genotype of strains from this Boeke collection is: MATa/α *ura3Δ leu2Δ his3Δ lys2Δ*/LYS2 *met15Δ*/MET15 *can1Δ*::LEU2-MFA1pr-HIS3/CAN1 *xxx*::kanMX/XXX

The media for SGA were the same as described in (Tong et al., 2004).

Strain	Genotype
DHL1	*MATα his3 leu2 LYS2 TRP1 ura3 lyp1Δ can1Δ::STE2pr-Sp _HIS5*
DHL10	*MAT*a *his3 leu2 LYS2 TRP1 ura3 yku70::KANMX*
DHL11	*MATα his3 leu2 lys2 TRP1 ura3 yku70::KANMX*
DHL12	*MATα his3 leu2 lys2 TRP1 ura3 yku70::KANMX tel1::NAT*
DHL13	*MAT*a *his3 leu2 lys2 TRP1 ura3 yku80::KANMX tel1::NAT*
DHL14	*MAT*a *his3 leu2 lys2 TRP1 ura3 yku80::KANMX*
DHL15	*MATα his3 leu2 lys2 TRP1 ura3 yku80::KANMX*
DHL16	*MATα his3 leu2 lys2 TRP1 ura3 yku80::KANMX tel1::NAT*
DHL17	*MAT*a *his3 leu2 lys2 TRP1 ura3 dnl4::KANMX tel1::NAT*
DHL18	*MAT*a *his3 leu2 lys2 TRP1 ura3 dnl4::KANMX*
DHL19	*MATα his3 leu2 lys2 TRP1 ura3 dnl4::KANMX*
DHL20	*MAT*a *his3 leu2 LYS2 TRP1 ura3 psy3::KANMX*
DHL21	*MATα his3 leu2 lys2 TRP1 ura3 psy3::KANMX*
DHL22	*MATα his3 leu2 lys2 TRP1 ura3 dnl4::KANMX tel1::NAT*
DHL23	*MAT*a *his3 leu2 lys2 TRP1 ura3 shu1::KANMX tel1::NAT*
DHL24	*MAT*a *his3 leu2 lys2 TRP1 ura3 csm2::KANMX*
DHL25	*MATα his3 leu2 lys2 TRP1 ura3 csm2::KANMX*
DHL26	*MATα his3 leu2 lys2 TRP1 ura3 shu1::KANMX tel1::NAT*
DHL27	*MAT*a *his3 leu2 lys2 TRP1 ura3 shu2::KANMX tel1::NAT*
DHL28	*MAT*a *his3 leu2 lys2 TRP1 ura3 erg5::KANMX*
DHL29	*MATα his3 leu2 lys2 TRP1 ura3 erg5::KANMX*
DHL30	*MAT*a *his3 leu2 LYS2 TRP1 ura3 pif1::KANMX*
DHL31	*MATα his3 leu2 lys2 TRP1 ura3 pif1::KANMX*
DHL32	*MATα his3 leu2 lys2 TRP1 ura3 shu2::KANMX tel1::NAT*
DHL33	*MAT*a *his3 leu2 LYS2 TRP1 ura3 psy3::KANMX tel1::NAT*
DHL34	*MATα his3 leu2 lys2 TRP1 ura3 erg5::KANMX tel1::NAT*
DHL35	*MATα his3 leu2 lys2 TRP1 ura3 rrm3::KANMX*
DHL36	*MATα his3 leu2 lys2 TRP1 ura3 psy3::KANMX tel1::NAT*
DHL37	*MAT*a *his3 leu2 lys2 TRP1 ura3 csm2::KANMX tel1::NAT*
DHL38	*MAT*a *his3 leu2 LYS2 TRP1 ura3 yku70::KANMX tel1::NAT*
DHL4	*MAT*a *his3 leu2 lys2 TRP1 ura3 tel1::NAT*
DHL40	*MATα his3 leu2 lys2 TRP1 ura3 lte1::KANMX*
DHL41	*MAT*a *his3 leu2 lys2 TRP1 ura3 lte1::KANMX*
DHL42	*MATα his3 leu2 lys2 TRP1 ura3 csm2::KANMX tel1::NAT*
DHL43	*MAT*a *his3 leu2 lys2 TRP1 ura3 erg5::KANMX tel1::NAT*
DHL44	*MATα his3 leu2 lys2 TRP1 ura3 bfa1::KANMX*

Strain	Genotype
DHL45	*MAT*a *his3 leu2 lys2 TRP1 ura3 ckb2::KANMX*
DHL46	*MAT*α *his3 leu2 lys2 TRP1 ura3 cka2::KANMX*
DHL47	*MAT*a *his3 leu2 LYS2 TRP1 ura3 pif1::KANMX tel1::NAT*
DHL48	*MAT*α *his3 leu2 lys2 TRP1 ura3 ckb2::KANMX*
DHL49	*MAT*a *his3 leu2 lys2 TRP1 ura3 cka2::KANMX*
DHL5	*MAT*α *his3 leu2 lys2 TRP1 ura3 tel1::NAT*
DHL50	*MAT*α *his3 leu2 lys2 TRP1 ura3 rrm3::KANMX tel1::NAT*
DHL51	*MAT*a *his3 leu2 lys2 TRP1 ura3 bfa1::KANMX*
DHL54	*MAT*α *his3 leu2 lys2 TRP1 ura3 lte1::KANMX tel1::NAT*
DHL55	*MAT*a *his3 leu2 lys2 TRP1 ura3 bfa1::KANMX tel1::NAT*
DHL56	*MAT*a *his3 leu2 lys2 TRP1 ura3 srs2::KANMX*
DHL58	*MAT*α *his3 leu2 lys2 TRP1 ura3 srs2::KANMX*
DHL59	*MAT*a *his3 leu2 LYS2 TRP1 ura3 rrm3::KANMX*
DHL6	*MAT*a *his3 leu2 lys2 TRP1 ura3 shu1::KANMX*
DHL60	*MAT*α *his3 leu2 lys2 TRP1 ura3 ckb2::KANMX tel1::NAT*
DHL61	*MAT*a *his3 leu2 lys2 TRP1 ura3 lte1::KANMX tel1::NAT*
DHL62	*MAT*α *his3 leu2 lys2 TRP1 ura3 srs2::KANMX tel1::NAT*
DHL63	*MAT*a *his3 leu2 LYS2 TRP1 ura3 srs2::KANMX tel1::NAT*
DHL64	*MATa his3 leu2 LYS2 TRP1 ura3 psy3::KANMX srs2::KANMX*
DHL65	*MAT*α *his3 leu2 lys2 TRP1 ura3 psy3::KANMX srs2::KANMX*
DHL66	*MAT*α *his3 leu2 lys2 TRP1 ura3 shu1::KANMX srs2::KANMX*
DHL67	*MAT*a *his3 leu2 lys2 TRP1 ura3 shu1::KANMX srs2::KANMX*
DHL68	*MAT*α *his3 leu2 lys2 TRP1 ura3 bfa1::KANMX tel1::NAT*
DHL69	*MAT*a *his3 leu2 lys2 TRP1 ura3 ckb2::KANMX tel1::NAT*
DHL7	*MAT*α *his3 leu2 lys2 TRP1 ura3 shu1::KANMX*
DHL71	*MAT*a *his3 leu2 lys2 TRP1 ura3 cka2::KANMX tel1::NAT*
DHL72	*MATa his3 leu2 LYS2 TRP1 ura3 psy3::KANMX srs2::KANMX tel1::NAT*
DHL73	*MAT*α *his3 leu2 lys2 TRP1 ura3 psy3::KANMX srs2::KANMX tel1::NAT*
DHL76	*MAT*α *his3 leu2 lys2 TRP1 ura3 shu1::KANMX srs2::KANMX tel1::NAT*
DHL77	*MAT*a *his3 leu2 lys2 TRP1 ura3 shu1::KANMX srs2::KANMX tel1::NAT*
DHL8	*MAT*a *his3 leu2 lys2 TRP1 ura3 shu2::KANMX*
DHL9	*MAT*α *his3 leu2 lys2 TRP1 ura3 shu2::KANMX*
YJM164	*MAT*α *his3 leu2 lys2 TRP1 ura3 cka2::KANMX*
YNM9	*MATa his3 leu2 lys2 ura3 tel1::NAT*

Table 3.1 Yeast strains used in the study in Chapter 3

3.2.2 Genome-wide screens

3.2.2.1 *tel1* deletion query strain construction

The SGA selection scheme was used to introduce the *tel1* deletion into MATa deletion mutants. The MATα *tel1* deletion query strain, DHL1, was created by a non-essential gene switching method (Tong et al., 2004). I crossed YNM9 bearing *tel1Δ*::NAT with YJM164 carrying the magic marker (LYS2 *lyp1Δ can1Δ*::STE2pr-Sp _HIS5) (Tong and Boone, 2006). The resulting heterozygous diploid was sporulated and followed by tetrad dissection to get the MATα *tel1Δ* strain containing the magic maker. The genotype of DHL1 (MATα *tel1Δ*::NAT *his3Δ* LYS2 *lyp1Δ can1Δ*::STE2pr-Sp_HIS5) was confirmed by PCR.

The introduction of the magic maker was to allow selection of haploids during SGA. HIS3, a *S.cerevisiae* gene required for histidine biosynthesis, was deleted from both the MATα query strain DHL1 and MATa deletion mutant array strains thus they cannot grow without the addition of histidine. *S.pombe* HIS5 gene (Sp_HIS5) can complement a *S. cerevisiae* HIS3 mutation, so *his3Δ* Sp_HIS5 cells can grow on minimal medium without histidine. STE2 encodes the α-factor pheromone receptor which is only expressed in MATa cells. The STE2 promoter linked with the *S.pombe* HIS5 gene (STE2pr-Sp_HIS5) allows specific selection of MATa *his3Δ* haploids in media lacking histidine, because only MATa cells express the *S.pombe* HIS5 gene (Figure 3.1). The CAN1 gene and LYP1 gene encode an arginine permease and a lysine permease, respectively, which allows respective antibiotics, canavanine (a toxic analogue of arginine) and thialysine (a toxic analogue of lysine), to enter and kill cells. So only *can1Δ lyp1Δ* double mutants can survive in the presence of these toxic analogues. Sometimes mitotic crossover events between homologous chromosomes in

MATa/α diploids can result in MATa/a or MATα/α diploids. The STE2pr-Sp_HIS5 in MATa/a diploid can be also expressed like MATa haploid and live in the media without histidine. Introduction of *can1Δ* and *lyp1Δ* markers into the query strain enable MATa/a diploid cells to be killed by canavanine and thialysine because they carry a wild-type copy of the CAN1 and LYP1 genes (Tong and Boone, 2006). The magic maker (LYS2 *lyp1Δ can1Δ*::STE2pr-Sp_HIS5) in query strain DHL1 ensured the specific selection of MATa haploid progeny and substantially reduced the potential for false positives.

3.2.2.2 SC screen by synthetic genetic array (SGA)

SGA analyses were performed using a Singer RoToR as described in (Tong et al., 2004) with the following modifications (Figure 3.2). The MATa yeast deletion mutant set (*ykoΔ*::kanMX) was arrayed at a density of 1536 colonies/plate by robotic pining. The query strain DHL1 was mated with yeast deletion mutants on YPD medium for 1 day. This mating step was done in triplicate to increase confidence. All plates were incubated at 30 degrees. Then diploids were selected twice on YPD plates contain 200µg/ml G418 and 200µg/ml clonNAT. Diploids were then pinned onto sporulation medium for 7 days at 25 degrees to induce sporulation. The resulting haploid spores were a combination of wild type, single and double mutants due to the independent assortment of chromosomes and recombination within the chromosomes. MATa spore progenies were selected twice on haploid selection medium (synthetic complete medium lacking histidine, lysine and arginine but containing 50µg/ml thialysine and 50µg/ml canavanine). Then MATa deletion mutants were selected twice on single deletion selection plate containing 200µg/ml G418 and double deletion selection plate containing 200µg/ml G418 and 200µg/ml clonNAT. The single deletion selection medium

allowed the germination of the MATa meiotic progeny carrying a *yko⊿*::kanMX single gene deletion and carrying a *yko⊿*::kanMX *tel1⊿*::NAT double gene deletion. The double deletion selection medium only allowed the *yko⊿*::kanMX *tel1⊿*::NAT double gene deletion MATa progeny to grow. The resulting single/double gene deletion strains were also pinned onto single/double selection plate with a sub-lethal dose of camptothecin to test sensitivity. The final selection plates with/without camptothecin were replicated to three identical selection plates to increase the statistical power. All SGA plates were incubated at 30 degrees for 24 hours before scanning.

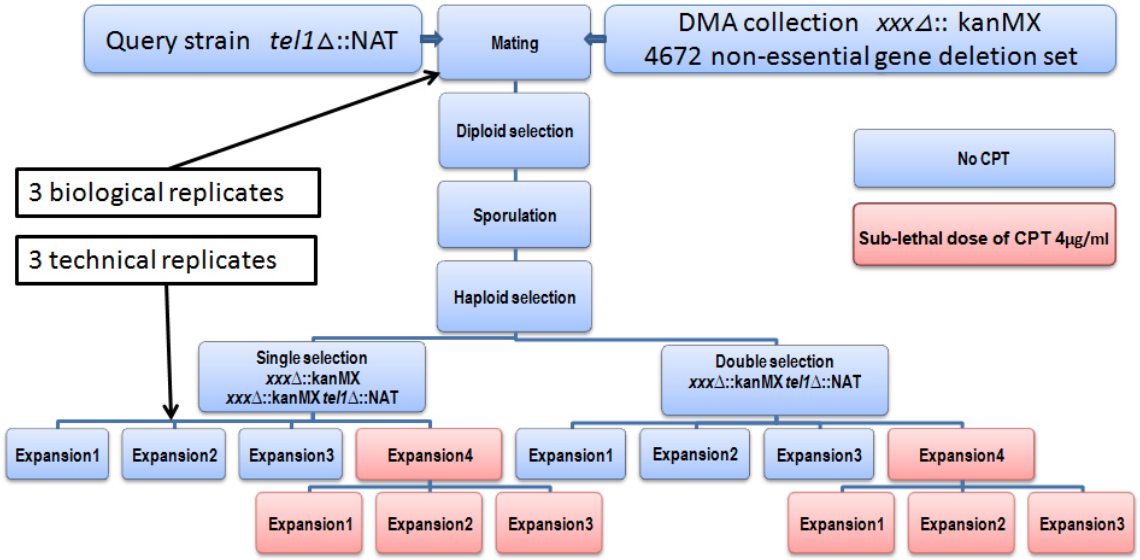

Figure 3.2 SC screen using synthetic genetic array (SGA)

During SGA, tel1 deletion was introduced into non-essential gene mutants by mating MATα query strain (*tel1⊿*::NAT) to the MATa yeast deletion mutant set (*yko⊿*::kanMX). Mating step was done in three biological replicates. The resultant double heterozygous diploid mutants were selected and induced to sporulate. The MATa haploid spores were selected by magic marker and then selected by single deletion (*yko⊿*::kanMX) and double deletion (*yko⊿*::kanMX *tel1⊿*::NAT) in parallel. Finally, the single/double mutants were also copied onto single/double selection plate with a sub-lethal dose of camptothecin to test sensitivity. The final selection plates with/without camptothecin were replicated to three identical selection plates before scanning.

3.2.3 Bioinformatic analysis

3.2.3.1 Fitness score and normalization

The fitness of each strain was determined by Balony, a yeast colony plate scanning image analysis program developed by Barry Young in the Loewen Lab (http://code.google.com/p/balony/). The area of each spot was first measured by pixel counts on the SGA plate scanning pictures. The program averaged the area values of all spots on the entire plate and normalized to 1. The fitness score resulted from comparing the area of every spot with average value. Edge effects, in which spots on the plate edge grow better than at plate centre due to more nutrition distribution, was also buffered and eliminated during normalization. So the fitness score of most strains was around 1. A score below 1 means this strain grew worse than others and 0 means an empty spot or lack of growth. Because three biological replicates at the mating step and three technique replicates at the selection step were done during SGA, every strain had nine fitness scores. Also, four sets of fitness scores of each non-essential gene from both single/double selection media with or without CPT were collected.

3.2.3.2 SGA fitness data comparison by R

R program was used to compare the fitness score between four sets of data for each gene. The difference of the average fitness score on single selection media and double selection media gives the EC score (experiment versus control) of certain gene. The more negative EC score that a certain gene was, the worse the double mutant grew compared with single mutants. Also because there were nine fitness scores for each data set, R program also gave the P value of this EC score.

3.2.4 Hit validation

3.2.4.1 Random spore assay (RSA)

Double heterozygous deletion diploids were created by the same method in SGA by mating query strain DHL1 to single gene deletion strains derived from the DMA collection which was the same collection used in the SGA screen. After sporulation, a small amount of germinated spores was resuspended in 1ml sterile H_2O and plated out onto different medium:
1) 20 µL on SC-His/Arg/Lys + canavanine/thialysine select for the MATa spore progeny;
2) 40 µL on SC-His/Arg/Lys + canavanine/ thialysine/clonNAT select for MATa tel1 mutants;
3) 40 µL on SC-His/Arg/Lys + canavanine/ thialysine/G418 select for MATa candidate gene mutants;
4) 80 µL on SC-His/Arg/Lys + canavanine/ thialysine/clonNAT/G418 select for MATa tel1 candidate gene double mutants.

In parallel, the same amount of spores was plated out onto corresponding selection media with 4µg/ml CPT to test the sensitivity. All plates were incubated at 30 degrees for 2 days. Colony growth under the 8 conditions was compared by eye and the double mutants were scored as SL, SC or no interaction.

The SC interaction between CSM2 and TEL1 had been validated by tetrad dissection followed by spot assay and growth curve assay. No SC interaction between TDP1 and TEL1 had been tested in the small matrix. CSM2 and TDP1 were used as positive control and negative control, respectively, to test RSA sensitivity.

3.2.4.2 Validation by tetrad dissection followed by spot assay and growth curve analysis

Methods of spot assay and growth curve assay were the same as described in Chapter 2.

To increase confidence as well as strains with a clean genetic background, all single deletion mutants for construction of double deletion strains were derived by tetrad dissection from the Boeke collection (Pan et al., 2004), which was different from the DMA collection used in SGA screen. It was a heterozygous gene deletion collection with less contamination and more stable deletion alleles. Gene deletions in strains for spot assay and growth curve analysis were confirmed by PCR.

3.3 Results and discussion

3.3.1 Camptothecin (CPT) concentration for SGA

From the previous small matrix screen, single deletion mutants themselves show different sensitivities to camptothecin (CPT). It was inevitable that some hypersensitive single deletion mutants were killed in the presence of a sub-lethal dose of CPT during the screen. In contrast, if the concentration was too low it would not elicit an effect on double deletion mutants. Unfortunately, there was little information from the literature about using SGA in the presence of CPT. It was therefore important to define an appropriate CPT concentration for the genome-wide screen that slightly inhibited *tel1* mutants and allowed most of the other single mutants to grow without killing them.

In previous on plate spot experiments, the *tel1* strain exhibited a mild sensitivity on YPD plates containing 16µg/ml CPT. But the SGA plates used synthetic complete media (SC-His/Arg/Lys + canavanine/thialysine) to select the MATa spore progeny and I found that the same strains grown on synthetic complete medium show higher sensitivities to CPT than on YPD rich medium with the same concentration of CPT (data not show). To mimic the final DNA damaging agent sensitivity assay during SGA, I ran CPT sensitivity tests on the third plate of 1536 density single deletion strains array from the MATa single selection step of the other SGA experiment ("G418 plate" step in Figure 3.1) because the genotype of strains on this plate was roughly the same as the SGA final single deletion array plate ("Expansion 4" plate in Figure 3.2) and it contains *tel1* deletion strain. The strains were transferred by robot onto single haploid selection medium (SC-His/Arg/Lys + canavanine/thialysine + G418) with serial diluted concentrations of CPT (diluted from 16µg/ml to 1µg/ml) and grown at 30 degrees C for 24 hours. The severity of the phenotype was assessed qualitatively by eye. Because *rad52* and *rad54* are hypersensitive and *tel1* is mildly sensitive to CPT, *rad52Δ* and *rad54Δ* spots exhibited no growth on plates with 4µg/ml CPT and there was ~20% reduction in growth in comparison to the plate with no CPT present at *tel1Δ* spot. On the plate with 8µg/ml CPT, most strains grew slower than on 4µg/ml CPT plate and *tel1Δ* was very sick. Comparing the growth on all plates, 4µg/ml was chosen as the concentration of CPT for SGA.

After SGA was completed and fitness data of each strain obtained, to determine if an appropriate concentration of CPT was chosen for SGA plates, I compared my data of single mutant sensitivity to CPT with the published data of the chemical-genetic interaction profiles generated by screening CPT against the *S. cerevisiae* viable deletion set from two groups

(Deng et al., 2005, Parsons et al., 2003). The fitness of single mutants in the presence of CPT was compared to the fitness in the absence of CPT. The larger the difference of fitness value (EC value) there was between the CPT plate and the no CPT plate, the more sensitive the single mutant was. The comparison provided a genome-wide view of the CPT effect on each non-essential gene single deletion strain during my SGA screen. The Deng *et al* study used a barcode pool based assay by growing homozygous diploid deletion strains in liquid YPD medium containing 50 μM (~17μg/ml) CPT. The Parsons *et al* study used colony size on plate based assay by growing MATa hypoid deletion strains onto YPD solid YPD medium containing 15 μg/ml CPT. I compared my top 30 most sensitive genes (P value<0.05) with the top 30 genes from the Deng paper and the 27 genes that show strong sensitivity from Parsons paper (Table 3.2). I found 15 and 17 overlapping genes respectively. They included 13 of top 15 CPT sensitivity genes from Deng paper. These suggested that 4μg/ml CPT works well on SGA plates and was an appropriate concentration for the screen.

	Top 30 sensitive mutant strains in SGA screen				(Deng et al., 2005)	(Parsons et al., 2003)
Rank	ORF	GENE (a)	EC value	P value	GENE (b)	GENE (c)
1	YOR368W	RAD17	-0.9285	6.09E-07	MMS4	CTF4
2	YGL175C	SAE2	-0.89549	1.72E-05	(*mms4*)	DDC1
3	YDR386W	MUS81	-0.87715	8.61E-07	RAD59	MMS1
4	YBR094W	PBY1	-0.83529	4.74E-05	SRS2	MMS22
5	YBR098W	MMS4	-0.82795	2.69E-07	MUS81	MMS4
6	YGL163C	RAD54	-0.82453	1.87E-05	SAE2	MRE11
7	YDR388W	RVS167	-0.80862	0.000753	RAD54	MUS81
8	YLR288C	MEC3	-0.80441	1.02E-05	RAD55	PAT1
9	YJL047C	RTT101	-0.80415	9.69E-06	RAD51	RAD24
10	YDR076W	RAD55	-0.8006	3.51E-06	RTT101	RAD50
11	YDL059C	RAD59	-0.79696	5.72E-05	MMS1	RAD51
12	YJL092W	SRS2	-0.77672	8.71E-05	RAD57	RAD52
13	YBR099C	YBR099C	-0.76826	1.09E-06	RAD50	RAD54
14	YJL124C	LSM1	-0.75732	1.18E-05	VAC14	RAD55
15	YDR369C	XRS2	-0.74981	1.27E-05	TOF1	RAD57
16	YCL016C	DCC1	-0.74375	1.17E-05	CLB5	RAD59
17	YJL115W	ASF1	-0.69261	3.50E-05	HMO1	RTT101
18	YML032C	RAD52	-0.68893	1.32E-05	(*mms4*)	RTT109
19	YOR233W	KIN4	-0.67891	0.001929	PPH3	RVS161
20	YNL250W	RAD50	-0.67503	3.37E-07	HTZ1	SPT10
21	YHR191C	CTF8	-0.67347	6.84E-05	PAT1	SPT20
22	YHR154W	RTT107	-0.66587	4.17E-05	PSY2	XRS2
23	YDR004W	RAD57	-0.64499	0.000154	FUN30	PBY1
24	YMR258C	ROY1	-0.63599	0.028126	LSM1	YBR099C
25	YER095W	RAD51	-0.63033	0.000107	CSM3	YBR100W
26	YBR260C	RGD1	-0.61873	0.0137	NUP60	YLR235C
27	YMR190C	SGS1	-0.61561	0.004059	SPT21	ZUO1
28	YOL106W	YOL106W	-0.60491	0.005285	ILM1	
29	YMR048W	CSM3	-0.60411	0.000455	ULA1	
30	YLL002W	RTT109	-0.58245	5.80E-06	UBC4	

Table 3.2 Comparison of most sensitive genes to CPT

GENE (a) list shows the top 30 sensitive single mutant strains in my SGA screen ordered by EC value (P value < 0.05). More sensitive mutant has more negative EC value. GENE (b) list shows top 30 gene list from Deng paper ordered by rank of sensitivity. (*mms4*) indicates that deletion of the ORF deletes MMS4 rather than a new gene. GENE (c) list shows 27 genes with strong sensitivity from Parsons paper ordered by alphabetical. Red box indicates mutations common to my top 30 CPT sensitivity gene list and the Parsons et al 2003 and Deng et al 2005 studies.

3.3.2 SC/SL candidate genes list obtained through two-step analysis

To ensure I did not miss any potential SL and SC interactions, I used a two-step strategy to analyze the SGA data. (Figure 3.3)

First, I compared the data from single selection plates without CPT against double selection plates with CPT to identify all double mutants that were sensitive to CPT which had largest fitness difference compared with single mutant without CPT. 173 candidates genes were deemed significant (EC<-0.4 P value<0.05). These hits included all potential SC/SL interaction as well as the ones in which both single and double mutants were sensitive to CPT.

Second, I sorted out all the single mutants sensitive to CPT by comparing the fitness of single and double mutants in the presence of CPT (EC<-0.4 P value<0.05). There were 27 candidates on the final gene list (Table 3.3).

After checking each gene profile and phenotype on SGA plates, I identified 2 SL candidate genes, 20 SC candidate genes and 5 linkage genes (including TEL1) whose ORF is “linked” to the query TEL1 gene. Because double mutants were created by meiotic recombination, the linkage genes tended to form double mutants at a reduced frequency, appearing as false positive synthetic lethal/sick with the query gene mutation.

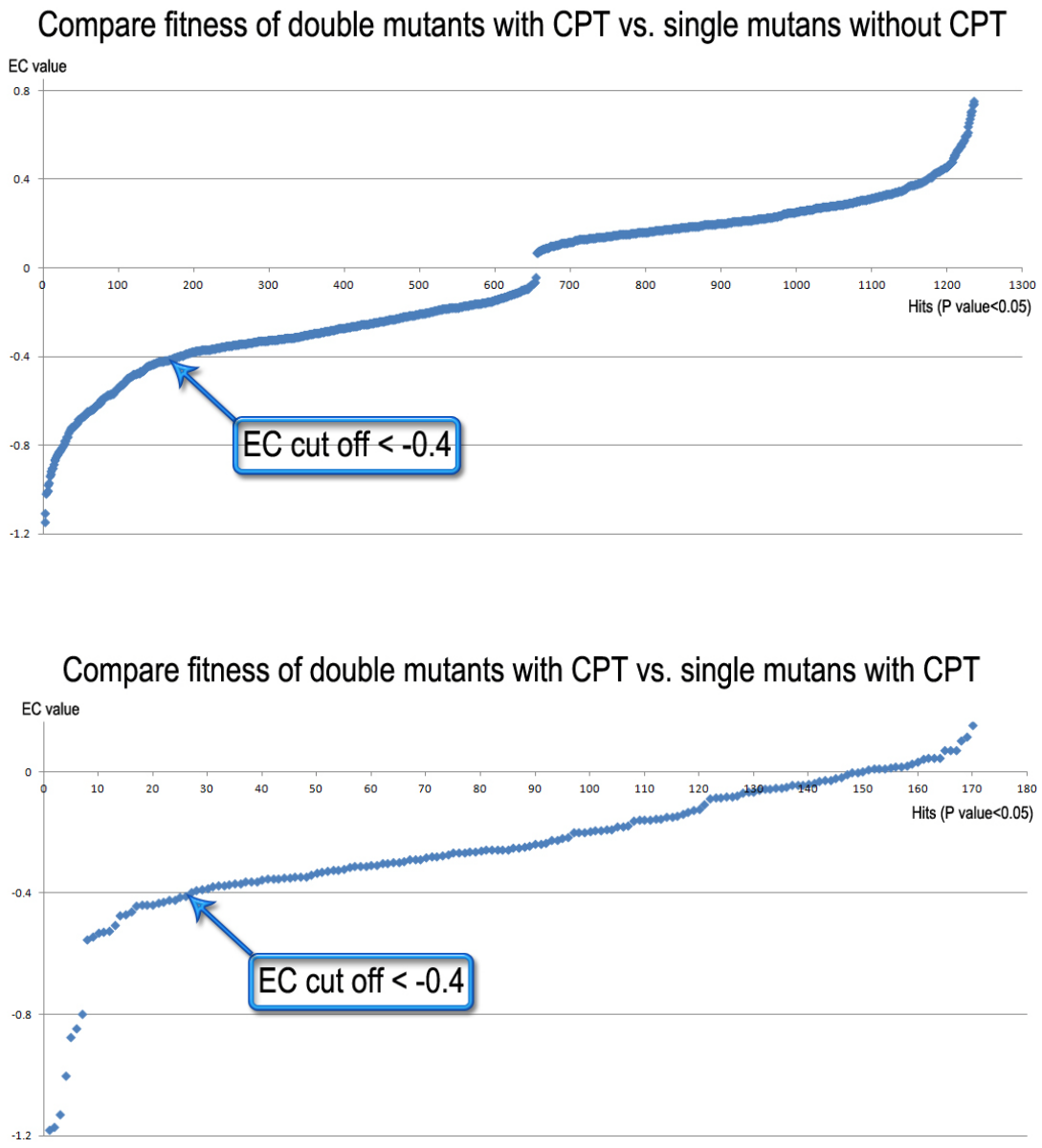

Figure 3.3 Two steps to filter for SC and SL interaction hits

All hits (value<0.05) shown on scatter chart are ordered by EC value. The comparison of the data from single selection plates without CPT against double selection plate with CPT filtered 173 candidates (EC<-0.4). The comparison of the data from single selection plates with CPT against double selection plate with CPT identified 27 candidates (EC<-0.4).

GENE	Linkage	EC value	P value	Function
HBT1		-1.18115	1.31E-06	Cell morphogenesis
SCS22	linked	-1.1702	8.33E-06	Phospholipids metabolism
RAS1		-1.1282	3.87E-06	Adenylate cyclase activating pathway
MRP21	linked	-1.00322	8.94E-05	Mitochondrial ribosomal protein
YBL071C	linked	-0.87391	5.27E-06	Putative protein of unknown function
CKI1		-0.84728	0.000266	Phosphatidylcholine synthesis
YJR128W		-0.79801	0.03098	Dubious open reading frame
SRO77	linked	-0.7667	0.013652	Exocytosis and cation homeostasis
RRM3		-0.55443	0.000345	DNA helicase
YPR063C		-0.54455	0.013964	Protein of unknown function
MVB12		-0.53027	0.006265	ESCRT-I subunit
YPR1		-0.52772	0.047448	NADPH-dependent aldoketo reductase
TEL1	linked	-0.52577	0.000147	DNA damage cell cycle checkpoint
RRT6		-0.50463	0.03739	Putative protein of unknown function
YCL046W		-0.47278	0.008209	Dubious open reading frame
RUB1		-0.47079	0.005797	Ubiquitin-like protein
FPK1		-0.45977	0.005096	Ser/Thr protein kinase
CSM2		-0.43987	0.003578	Subunit of SHU complex
CKB2		-0.4374	0.002063	B Subunit of casein kinase 2 (CK2)
TRE1		-0.43697	0.002569	Regulation of metal transporter
YKU80		-0.43685	0.012981	Subunit of the telomeric Ku complex
LTE1		-0.43321	0.028525	spindle orientation checkpoint
YOR223W		-0.42895	0.044181	Protein of unknown function
AGX1		-0.42142	0.000498	Glycine synthesis
ERG5		-0.42098	0.013983	Ergosterol synthesis
YBR134W		-0.41134	0.032558	Dubious open reading frame
PSY3		-0.41089	0.000613	Subunit of SHU complex

Table 3.3 Twenty-seven candidate genes (include linkage genes) list after SGA data analysis

3.3.3 No SL and 13 SC interactions with TEL1 were uncovered

The random spore assay test demonstrated that the two SL candidate genes interactions were false positives. Double mutants grew normally and didn't show sensitivity to CPT. Only two genes, MEC1 and DNA2, are reported synthetic lethal with TEL1 in yeast and both of them are essential genes and were not tested in this experiment (Budd et al., 2005, Chakhparonian et al., 2005).

Among 20 SC candidates, 4 genes are related to DNA repair and 2 of them are in the same complex. So I decided to validate these 4 hits directly by tetrad dissection, while the remaining 16 were confirmed by random spore assay. After removing false positive SC candidates through random spore assay and tetrad dissection, I identified 7 candidates that appear to have SC interactions with TEL1. Among them, CSM2 and PSY3 are in the same SHU complex which contains the other two subunits SHU1 and SHU2; YKU70 is a component of the yKu70-yKu80 heterodimer; CKB2 is the subunit of casein kinase 2 (CK2) which is also comprised of CKA1, CKA2 and CKB1; LTE1 is involved in spindle checkpoint and interacts with BFA1-BUB2 complex. All function related non-hit genes mentioned have negative EC value >-0.4 or P value > 0.05 (Table 3.4). So I also included them to test the SC interactions with TEL1 by random spore assay and tetrad dissection. 6 of 9 were also found to be SC to CPT with *tel1*. In total 13 SC interactions were uncovered.

Function	Gene	EC value	P value	Random spore assay	Tetrad dissection	Interaction
Shu complex	CSM2	-0.43987	0.003578	√	√	SC
	PSY3	-0.41089	0.000613		√	SC
	SHU1	-0.29365	0.135906		√	SC
	SHU2	-0.24395	0.054303		√	SC
Ku complex	YKU80	-0.43685	0.012981		√	SC
	YKU70	-0.22265	0.01981		√	SC
DNA helicase	RRM3	-0.55443	0.000345		√	SC
	PIF1	0.321353	0.65881	X	X	
Bfa1-Bub2 complex	LTE1	-0.43321	0.028525	√	√	SC
	BFA1	-0.37092	0.059806	√	√	SC
	BUB2	-0.30669	0.018664	√	√	SC
Casein kinase 2	CKB2	-0.4374	0.002063	√	√	SC
	CKA2	-0.31277	0.117162	X	X	
	CKB1	-0.27383	0.051928	√	√	SC
	CKA1	-0.06364	0.77864	X		
Other function	YPR1	-0.52772	0.047448	X		
	CKI1	-0.84728	0.000266	X		
	MVB12	-0.53027	0.006265	X		
	RUB1	-0.47079	0.005797	X		
	FPK1	-0.45977	0.005096	X		
	ERG5	-0.42098	0.013983	√	√	SC
	TRE1	-0.43697	0.002569	X		
	AGX1	-0.42142	0.000498	X		
	RAS1	-1.1282	3.87E-06	X		
	HBT1	-1.18115	1.31E-06	X		
Unknown function	YJR128W	-0.79801	0.03098	X		
	YBR134W	-0.41134	0.032558	X		
	YCL046W	-0.47278	0.008209	X		
	YPR063C	-0.54455	0.013964	X		
	YOR223W	-0.42895	0.044181	X		
	RRT6	-0.50463	0.03739	X		

Table 3.4 SL/SC candidate validation

The red box indicates P value >0.05 or EC value >-0.4. "√" and "X" indicates confirmed true and false positive respectively.

3.3.3.1 SHU genes

SHU genes (CSM2, PSY3, SHU1, and SHU2) were first identified in the same epistasis group that suppresses severe sensitivity to HU and MMS in *sgs1* and *top3* mutants. All four SHU gene products interact in two-hybrid assays suggesting they are stably associated as a multi-protein complex in the cell. The single SHU gene deletion mutants all demonstrate similar phenotypes: a mutator phenotype, increased gross chromosomal rearrangement (GCR) and moderate sensitivity to methyl methane sulfonate (MMS). Mutation of all four genes does not cause any additive effects. It has been proposed that these proteins exist in a multimeric complex that fails to function when any one member is missing (Shor et al., 2005). In spot assays, compared with YPD control plates, both TEL1 and SHU gene single mutants exhibited weak sensitivity to CPT, while all 4 double mutants were almost completely inviable on CPT plate (Figure 3.4). The SC interactions between SHU genes and TEL1 were clearly demonstrated.

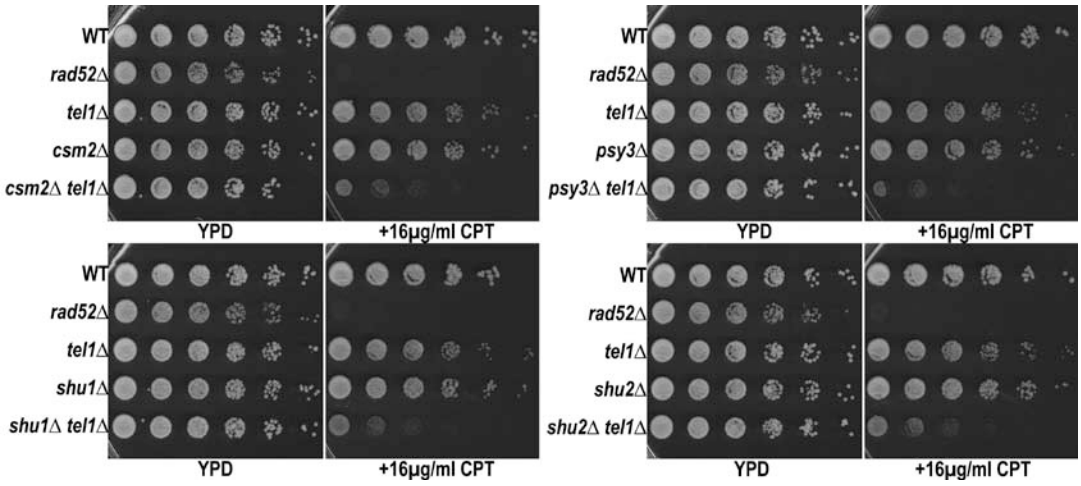

Figure 3.4 SC interactions between SHU genes and TEL1 by spot assay

In spot assays, single and double mutants were grown on the same plate, wild type and *rad52Δ* mutants were used as negative and positive controls, respectively. All strains grew normally on YPD plates except the *rad52Δ* mutant grows a little slower. On CPT plates, SHU gene single mutants show a minor growth defect and tel1 null cells grew slightly less, while the double mutants were almost dead.

To quantify the PSY3, SHU1, SHU2 SC interactions with TEL1, I used growth curve assays in presence of CPT and compared the fitness ratio of wild type and double mutants to the expected ratio based on each single mutant. The calculation methods of the fitness ratio and expected ratio are described in Chapter 2. An example of a PSY3 growth curve is shown in (Figure 3.5). The growth curve of *psy3Δ tel1Δ* double deletion strain in media with 2µg/ml CPT is much slower than both single mutants. The quantitative analysis results indicate *psy3Δ tel1Δ*, *shu1Δ tel1Δ* and *shu2Δ tel1Δ* double mutants are 15%, 10% and 10% respectively lower than expected when exposed to 2µg/ml CPT.

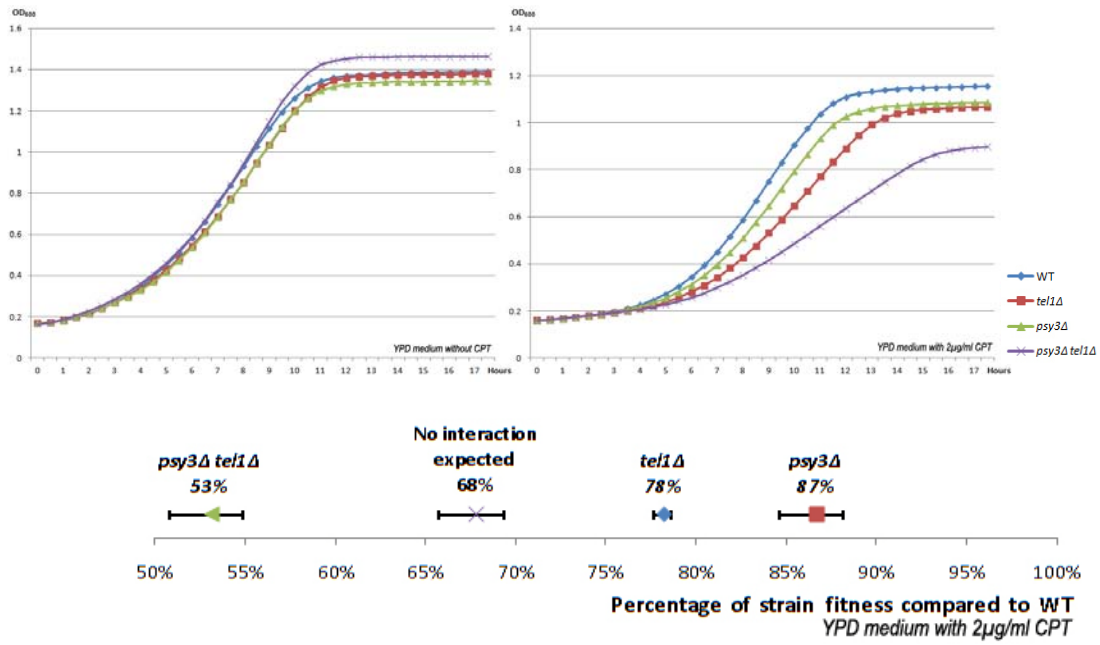

Figure 3.5 SC interaction between PSY3 and TEL1 by growth curve assay

Growth curve assays were done in YPD media containing different concentrations of CPT. All single and double mutants grew similarly to wild type in YPD media without CPT. When treated with 2µg/ml CPT, the growth of *psy3Δ tel1Δ* double deletion is much slower than either single mutants. The quantitative analysis shows the fitness of double mutants is around 15% lower than expected when exposed to 2µg/ml CPT.

Next I asked if these SC interactions with TEL1 are specific to CPT-induced DNA damage. I tested sensitivity to high concentrations of hydroxyurea (HU, blocks DNA replication), and cycloheximide (CHX, inhibits protein synthesis). The results show that the SHU gene SC interactions with TEL1 are specific to CPT and do not extend to HU or CHX (Figure 3.6).

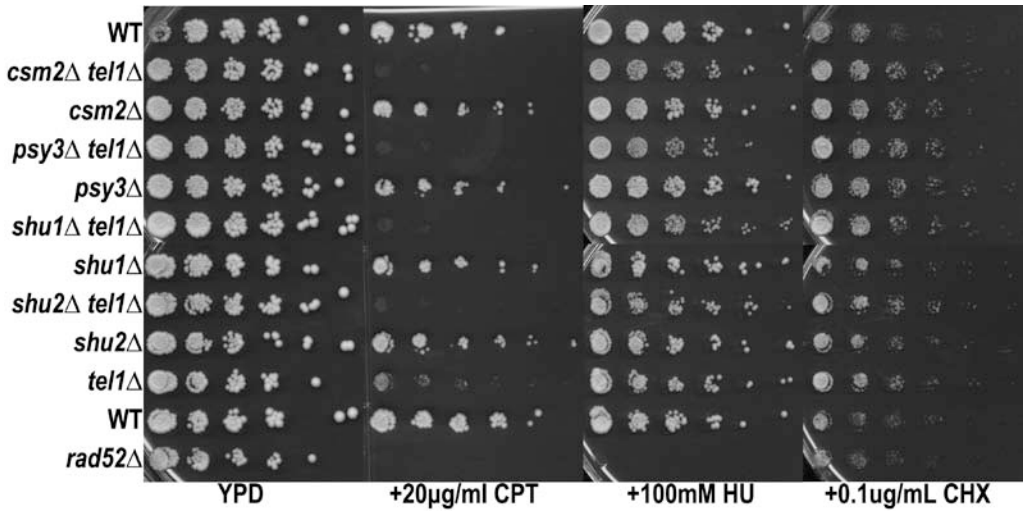

Figure 3.6 SC interactions between SHU genes and TEL1 are specific to CPT

Spot assays were done on YPD plates with different chemicals: 20 µg/ml CPT, 100 mM HU (DNA replication inhibitor) and 0.1 µg/ml cycloheximide (CHX; protein synthesis inhibitor). The double mutants are completely inviable on CPT plate, but exhibit no/little growth defect on HU and CHX plates compared with single mutants.

As an inhibitor of protein synthesis, CHX induces defects in cell growth which is more general compared to the two DNA damaging agents: CPT and HU (del Pozo et al., 1991). The topoisomerase I inhibitor CPT induces formation of DNA Topoisomerase I (Top1) cleavage complexes (Top1cs) that block the leading strand during DNA synthesis, which often leads to replication fork collapse. Also, the accumulation of single-stranded nicks after CPT treatment can result in double-strand breaks (DSBs) upon passage of the fork (Redon et al., 2003). In contrast, HU is an inhibitor of ribonucleotide reductase that slows down fork progression and induces stalling of the replication fork by reducing dNTP pools (Lopes et al.,

2001, Alvino et al., 2007). So both HU and CPT can increase the DNA replication stress during S phase. Compared to HU, CPT induces more DSBs and DNA replication fork collapse rather than arresting or slowing down the replication process. On spot assays, the resistance to HU of double mutants indicates cells are able to resume DNA replication after HU-dependent fork arrest. The sensitivity to CPT suggests double mutants are defective either in recovery after CPT-dependent fork breakage or in DSB repair pathways.

While the formation of DSBs triggers activation of the DNA damage checkpoint TEL1 and MEC1, DNA repair enzymes in the cell also are activated to repair DSBs by either non-homologous end joining (NHEJ) or homologous recombination (HR) (Mao et al., 2008). Recently, it has been proposed that the Shu complex stabilizes Rad51 filaments to promote HR by inhibiting the disassembly reaction of Srs2 (Bernstein et al., 2011). To investigate if SRS2 is involved in the SC interaction with TEL1, I knocked out the SRS2 gene in double deletion mutants and tested the sensitivity to CPT by spot assay (Figure 3.7). The deletion of SRS2 gene doesn't alleviate the CPT sensitivity of *psy3Δ tel1Δ* or *shu1Δ tel1Δ* double mutant. Hypersensitivities to CPT of triple mutants suggest that the SC interactions are SRS2 independent. The SHU complex is also suggested to be involved in the error-free repair pathway in DNA post-replication repair (PRR) (Ball et al., 2009). It functions to bypass replication-blocking lesions and prevent damage-induced cell death, but little is known about the detailed mechanism. A recent study shows the Shu complex has DNA-binding activity *in vivo* (Tao et al., 2012). It is possible that SHU facilitates efficient repair of broken replication forks and the activation of this repair is independent of TEL1 checkpoint, further investigation is needed to address this possibility.

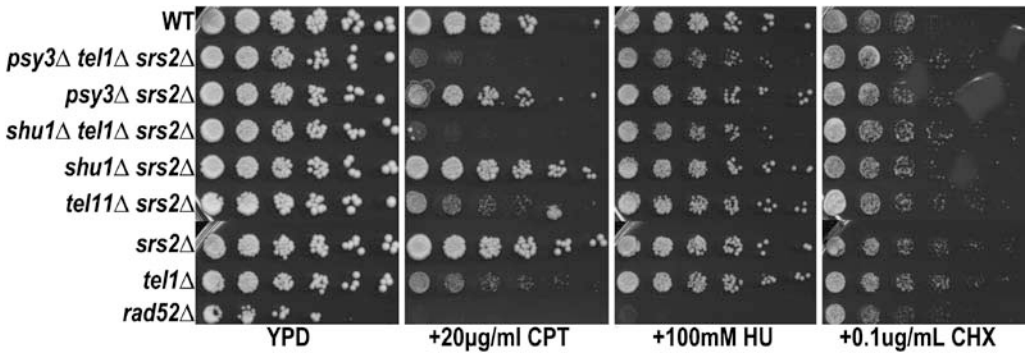

Figure 3.7 SC interactions between SHU genes and TEL1 are SRS2 independent

SHU gene, TEL1 and SRS2 triple deletion strains are constructed and test by spot assay. The triple deletion was still much more sensitive to CPT than tel1Δ single mutant and *srs2Δ tel1Δ*, *srs2Δ psy3Δ*, *srs2Δ shu1Δ* double mutants.

3.3.3.2 YKU genes

The highly conserved Ku genes are involved in double strand break repair by NHEJ and telomere maintenance. In *S. cerevisiae*, the core components of the NHEJ machinery include the Yku (Yku70–Yku80), MRX (Mre11–Rad50–Xrs2) and Dnl4–Lif1 complexes, which are recruited rapidly to DSBs, where the two Yku subunits form a ring-like structure that binds to DNA ends and initiates NHEJ (Daley et al., 2005). In humans, the essential NHEJ components also include the catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs), Artemis, and XRCC4. It has been reported that siRNA targeting the Ku70 protein enhances the response to topoisomerase II inhibition in human cancer cells (Ayene et al., 2005). Upon both the spot assay and growth curve assay, cells with mutations in a YKU gene alone show no growth defect compared with wild type. *yku70Δ tel1Δ* double mutants are very sensitive to CPT, similar to the SHU complex. The fitness of *yku70Δ tel1Δ* double mutant is around 13% lower than the expected fitness if there was no interaction (Figure3.8). Both subunits of the yKu70-yKu80 heterodimer are confirmed as SC partner genes of TEL1, and

these SC interactions are also specific to CPT-induced DNA lesions and do not extend to HU or CHX (Figure3.9).

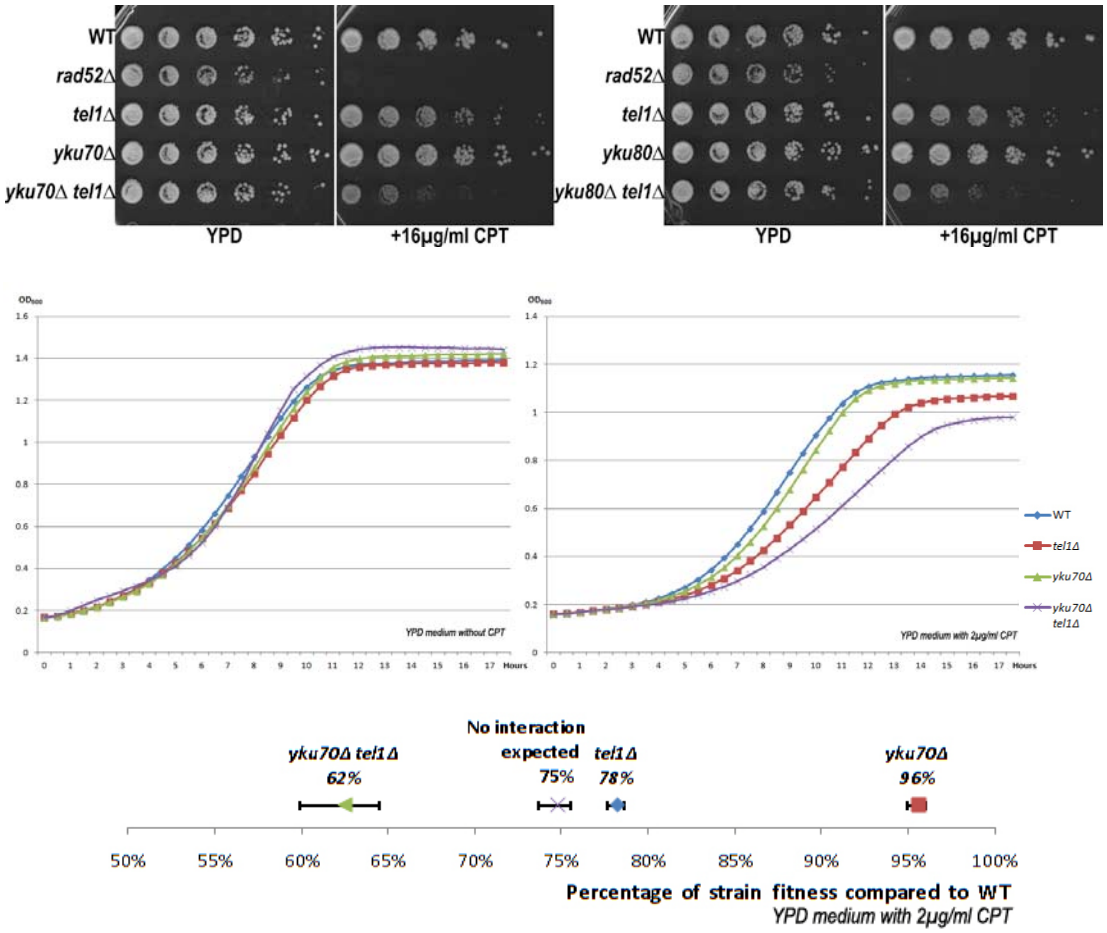

Figure 3.8 SC interactions between YKU genes and TEL1

The SC interactions between YKU70/YKU80 and TEL1 are demonstrated by spot assays and growth curve assays. On the plate spot assay, *yku70Δ* and *yku80Δ* exhibit no sensitivity to CPT as compared with wild type, while double mutants of TEL1 and either YKU gene are more sensitive than *tel1Δ* single mutant, which is mildly sensitive to CPT. The quantitative analysis from growth curve data shows the fitness of *yku70Δ* tel1Δ double mutant is around 13% lower than the expected fitness if there was no interaction.

In *S. cerevisiae*, the deletion of YKU70 or YKU80 causes defective NHEJ and can suppress the lethality of *mec1* mutants, and this suppression is dependent on wild type TEL1 (Corda et al., 2005). It has been reported that chemosensitization of ATM-deficient tumor cells occurs when DNA-PK, which is also involved in NHEJ but has no yeast homologue, is knocked down by RNAi or small molecule inhibitors *in vitro* and *in vivo* (Jiang et al., 2009). These findings suggest that there is a connection between the DNA damage checkpoint ATM/TEL1 and NHEJ pathway. However, another study reported that CPT shows a stronger inhibition of DNA replication in $Ku80^{-/-}$ cells than $Ku80^{+/+}$ cells. The inhibition was correlated with the activities of ATR and CHK1 but not DNA-PK which suggests that Ku may function in the DNA damage checkpoint in a DNA-PK independent fashion (Wang et al., 2002). In budding yeast, *yku* null mutants exhibit increased gross chromosomal rearrangements (GCR), and overexpression of the YKU complex can suppress GCRs after MMS treatment. Furthermore, this suppression cannot be attenuated by the deletion of DNL4 gene (DNA ligase involved in NHEJ, Lig4 in humans), suggesting another function of the Yku complex in maintaining genome stability besides NHEJ (Banerjee et al., 2006). DNL4/Lig4 is exclusively required for the Ku-dependent NHEJ pathway of DSB repair and no other DNA ligases have been found that can substitute for this function both in human cells and budding yeast (Adachi et al., 2001). To test whether the SC interaction with TEL1 is with NHEJ in general or just the Yku complex, I knocked out the DNL4 gene in a *tel1Δ* mutant to check if it too was SC to CPT (Figure 3.9). The *dnl4Δ tel1Δ* double mutant shows a similar sensitivity with *tel1Δ* single mutants suggesting that the SC interactions between YKU70/YKU80 and TEL1 is due to an NHEJ independent mechanism.

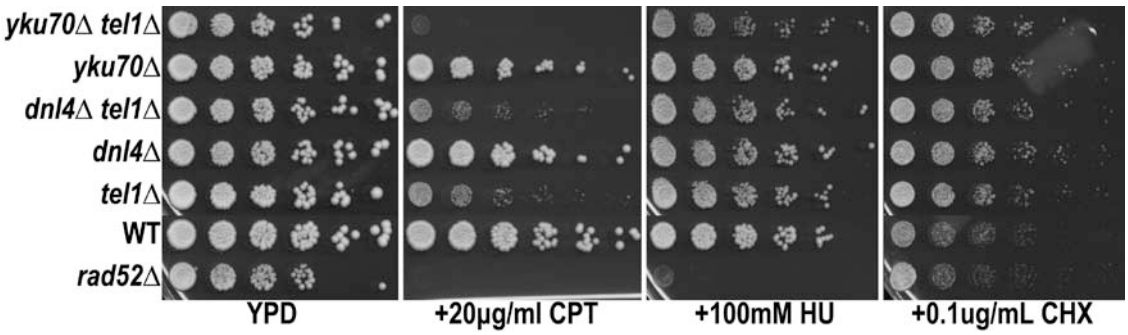

Figure 3.9 Interactions between YKU genes and TEL1 are CPT specific and due to an NHEJ independent mechanism

On spot assays, YKU70 doesn't exhibit SC interaction with TEL1 on HU or CHX plate. *dnl4Δ tel1Δ* double mutant shows a mild sensitivity similar to *tel1Δ* single mutants suggesting no SC interaction between DNL4 and TEL1.

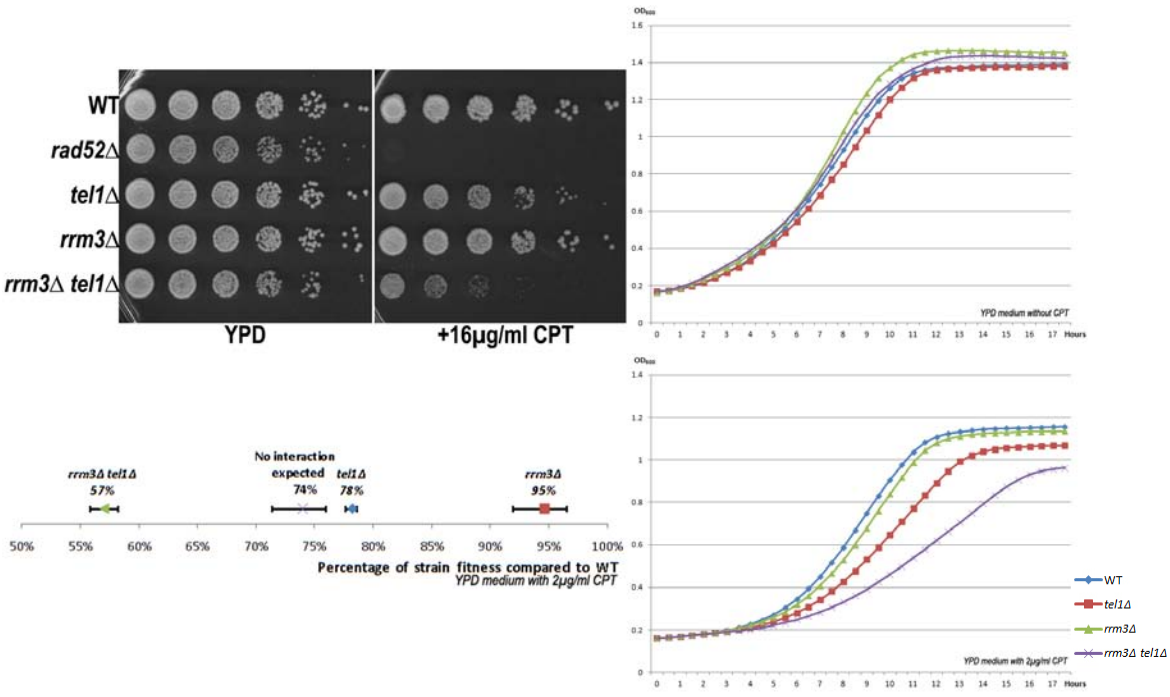

Figure 3.10 SC interactions between RRM3 and TEL1

The SC interactions between RRM3 and TEL1 are demonstrated by spot assays and growth curve assays. The quantitative analysis from growth curve data shows the fitness of *rrm3Δ tel1Δ* double mutant is around 17% lower than expected.

3.3.3.3 RRM3 gene

RRM3 shows the strongest SC interaction with TEL1. Like YKU genes, in medium containing 2µg/ml CPT, the *rrm3Δ* single mutant is not sensitive and *rrm3Δ tel1Δ* double mutant shows slow growth, about 17% slower than expected (Figure 3.10). In addition, on spot assays compared with single mutants, the slower growth of the *rrm3Δ tel1Δ* double mutant on all CPT, HU and CHX plates suggests the SC interaction between RRM3 and TEL1 is not specific to CPT (Figure 3.11).

Rrm3 protein in yeast is a 5' to 3' DNA helicase involved in rDNA replication and relieves replication fork pauses at telomeric regions (Ivessa et al., 2000, Makovets et al., 2004). It has a structurally and functionally related protein Pif1. Both of them belong to the conserved Pif1 subfamily, of which humans have only one identified Pif1 family member, Pif1. Cells mutated in either helicase are repair DNA proficient (Bochman et al., 2010). Therefore, I also tested PIF1 gene for SC interaction with TEL1. PIF1 also functions in mitochondrial DNA recombination and genome maintenance. The observation that the *pif1* deletion strain loses functional mitochondria rapidly could explain the slow growth phenotype of *pif1Δ* mutants on spot assays (Foury and Kolodynski, 1983)(Figure 3.11). Interestingly, the phenotypes of *rrm3Δ* and *pif1Δ* mutants on CPT plates are opposite: RRM3 show a negative interaction with TEL1 while PIF1 acts as a phenotypic suppressor. RRM3 and PIF1 appear to have different roles in several areas other than mitochondria in yeast. Rrm3 is required for telomere-proximal replication forks, while Pif1 is thought to be an inhibitor of telomerase (Schulz and Zakian, 1994). RRM3 and PIF1 also have different functions during Okazaki fragment maturation and HR DNA repair. The *rrm3Δ* mutant is synthetically lethal with *dna2*

mutants, a helicase/nuclease required for Okazaki fragment processing (OFP) and HR repair. In contrast, *pif1Δ* suppresses both the DNA replication and repair defects of *dna2* mutants and even the lethality of deletion of DNA2 (Ivessa et al., 2000).

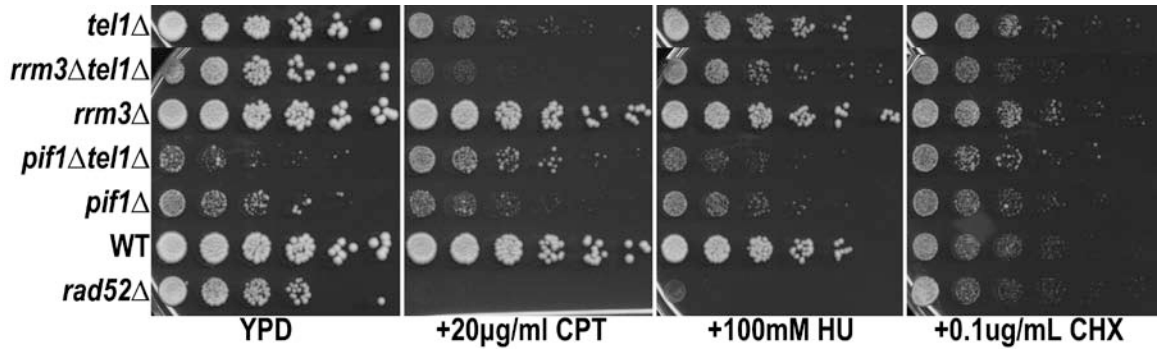

Figure 3.11 SC interactions between RRM3 and TEL1 are not specific to CPT

Both the *pif1Δ* single mutant and *pif1Δ tel1Δ* double mutant show growth defects on YPD plates and HU plates. *pif1Δ tel1Δ* double mutant is even sicker. On CPT plate, the growth of *rrm3Δ tel1Δ* double mutant was entirely inhibited by CPT, whereas *pif1* appears to partially suppress the *tel1* growth defect. This positive interaction between PIF1 and TEL1 only occurs on CPT plate but not on HU or CHX plate.

3.3.3.4 Casein kinase 2 beta subunit genes

Casein kinase 2 is a serine/threonine protein kinase, which has been implicated in cell cycle control, DNA repair, regulation of the circadian rhythm and other cellular processes (Guerra et al., 1999). In budding yeast, it is a tetramer of two alpha subunits (CKA1 and CKA2) and two beta subunits (CKB1 and CKB2). The alpha subunits have the catalytic kinase domain. The beta subunits function as the regulator of the two catalytic subunits, enhancing their stability, activity and specificity (Pinna, 1990). Random spore assay (RSA) of all 4 subunits shows only CKB1 and CKB2, the beta subunits of CK2 kinase are SC with TEL1. One example of RSA plates with CKB1 is shown (Figure 3.12)

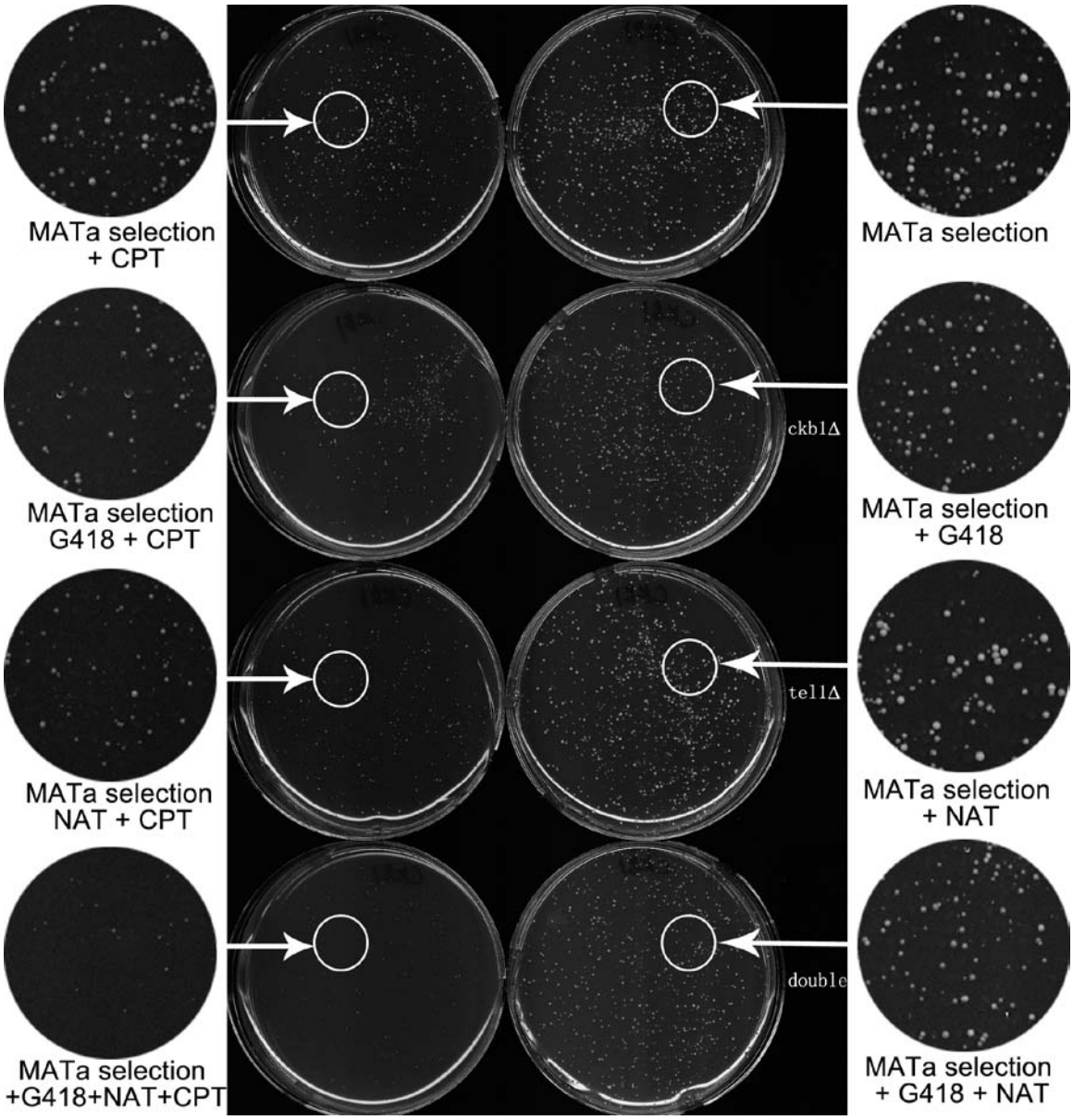

Figure 3.12 SC interactions between CKB1 and TEL1 by RSA

On the control plate (right), both the amount and size of colonies grown on double selection plate are similar with single selection plates. On experimental plate with 4μg/ml CPT (left), only a few small colonies can be found on the double selection plate.

The spot assay shows that knockout of CKA2 in a *tel1Δ* mutant doesn't cause a more severe phenotype in the presence of CPT, however CKB2 displays a clear SC interaction with TEL1 (Figure 3.13). These observations suggest only CK2 beta subunits exhibit SC interaction with TEL1. The same results from both random spore analysis and tetrad dissection followed

by spot assay indicate a CKA-independent role of CKB1/CKB2 in addition to regulating the CK2 tetramers leads to the SC interaction with TEL1. In *S. pombe*, only CK2β mutants show adaptation defectives in response to DNA damage and this defect is independent of the catalytic CK2 subunits (Toczyski et al., 1997). Study in mammalian cells demonstrates that CK2β interacts with Chk1 (G2 checkpoint) and this interaction does not extend to the CK2α subunit (Guerra et al., 2003). It has been reported that high level expression of CK2β relative to CK2α was observed in a variety of tumors (Vilk et al., 2001). The distinct cellular functions of CK2β from CK2 including the SC interaction with TEL1, may offer insights into the multiple roles of the regulatory subunit of CK2 kinase.

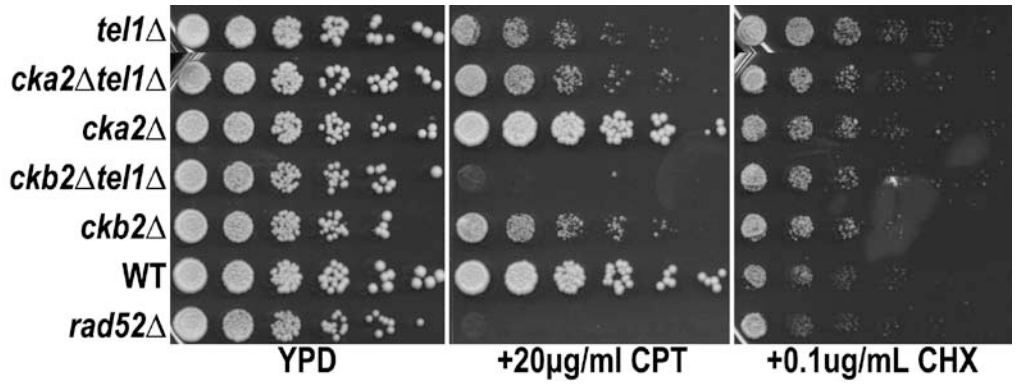

Figure 3.13 Only CK2 beta subunits show SC interactions with TEL1

On spot assays, *cka2Δ*, *ckb2Δ* and *tel1Δ* single mutation strains show a mild sensitivity to CPT. *ckb2Δ tel1Δ* double mutant is dead on CPT plate, while *cka2Δ tel1Δ* grows similar to *cka2Δ* single mutant.

3.3.3.5 Spindle checkpoint genes and ERG5 gene

The other SC partner genes confirmed by random spore analysis and further validated by tetrad dissection include three spindle checkpoint genes (BFA1, BUB2 and LTE1) and the ERG5 gene involved in ergosterol biosynthesis.

On spot assay, *lte1* null mutants are hypersensitive to CPT; both *lte1Δ* and *lte1Δ tel1Δ*

mutants are inviable on CPT plates. The double mutants also show strong sensitivity on HU plates but not on CHX plate, as compared with single mutants, suggesting that the SC interaction with the spindle checkpoint genes is specific to CPT and HU induced DNA damage (Figure 3.14).

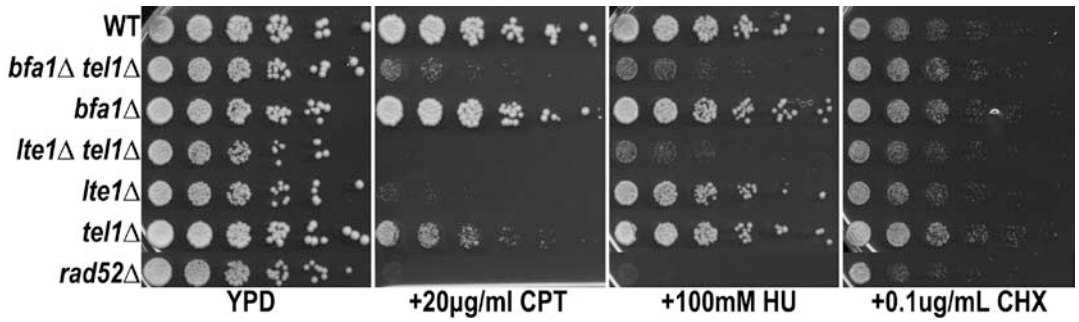

Figure 3.14 SC interactions between spindle checkpoint genes and TEL1

On CPT plates, the *bfa1Δ* single mutant is not sensitive while the *bfa1Δ tel1Δ* mutant is very sick. Both *lte1Δ* and *lte1Δ tel1Δ* mutants are inviable in the spot assay to CPT. Both TEL1 SC interactions with LTE1 and BFA1 are clearly shown on HU plate but not CHX plate.

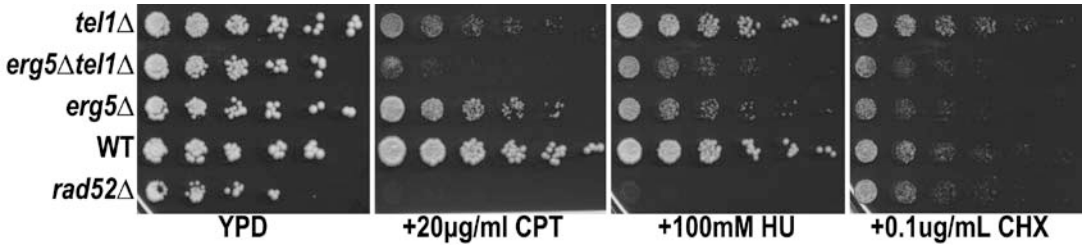

Figure 3.15 SC interactions between ERG5 genes and TEL1

Erg5 shows SC interaction with TEL1 on the CPT plate. Whereas both the single and double mutants are sensitive to HU and CHX.

Erg5 shows a clear SC interaction with TEL1 on CPT plates by spot assay. However, both *erg5Δ* and *erg5Δ tel1Δ* mutants display a similar sensitivity to HU. (Figure 3.15) One possible explanation is that membrane lipid composition affects CPT susceptibility in yeast. It has been observed that cells deleted for genes encoding ergosterol biosynthetic enzymes

such as ERG3, ERG4 and ERG6 were hypersensitive to various DNA damaging agents include CPT (Parsons et al., 2003). The defective membrane in *erg5Δ* cells may allow more CPT to enter cell and cause more DNA damage and eventually lead to cell death.

All four candidate genes have no detectable homologues in human.

Chapter 4 : Conclusion, implications and future directions

4.1 Summary

Chromosome instability (CIN) is characterized by aberrant chromosomal states including changes in ploidy, alterations in the number of chromosomes (aneuploidy), or gross chromosomal rearrangements (GCR), all of which are consistently observed in most cancers in contrast to normal cells (Storchova and Pellman, 2004). As a hallmark of cancer cells, CIN can be exploited as a tumor-specific target for selective killing of cancer cells. The emerging data from research of using PARP inhibitors to target tumor cells with BRCA1/BRCA2 mutation with/without DNA damaging agents suggests that synthetic lethality (SL) and synthetic cytotoxicity (SC) can be exploited to preferentially kill tumor cells with specific mutations relative to adjacent normal cells (Bryant et al., 2005, Farmer et al., 2005, Chan and Giaccia, 2011). Using the same SL/SC strategies, cancer cells with CIN gene mutations may be inviable with/without the presence of a sub-lethal dose of DNA damaging agents when combined with another non-essential gene mutation. While CIN mutations may contribute to cancer development, they may also cause cells to have difficulty in replicating their DNA and proliferating. In such cases, it is possible that tumor cells can proliferate by overcoming the negative consequence of CIN through adaptive DNA repair mechanisms. In other words, tumor cells harboring CIN mutations may become heavily dependent on certain DNA repair pathways for viability. Thus use of SL/SC strategies by inhibition of these DNA repair enzymes may enhance CIN to an intolerable level or sensitize cells to DNA damage stress and eventually lead to cell death. To test this theory, I focused on the CIN gene ATM, which is often mutated in human lymphoid and epithelial tumors (Ahmed and Rahman, 2006). I hypothesized that knockdown of certain second site DNA repair genes would selectively kill

ATM deficient cells resulting in SL, or sensitize ATM deficient cells to a specific DNA damaging agent resulting in SC. The goal of this thesis is to use *Saccharomyces cerevisiae* as a model to identify SL interaction partner genes with ATM, and SC interaction partner genes together with a sub-lethal dose of DNA damaging agents. After directly testing a small matrix of the ATM and ATR homologues, TEL1 and MEC1 with three DNA repair enzyme genes and four DNA damaging agents, I focused on TEL1 and camptothecin (CPT) and screened a large matrix of the non-essential gene collection using high throughput genetic methods. In total, fourteen SC interactions with TEL1 (ATM in humans) were uncovered in budding yeast. Most of the SC genes found are involved in DNA repair and show a specificity of sensitivity to CPT which proved my hypothesis.

4.2 Improvement of methodology

To determine which combinations of the DNA repair enzymes inhibition and sub-lethal doses of various types of DNA damaging agent might result in effective killing of tumor cells compared to healthy cells, I adapted the SGA methods in yeast, which enabled rapid screening of the thousands of possible combinations on one plate and determining SL/SC interactions through bioinformatic analysis. Compared with the direct test in the small matrix with of three DNA repair enzymes, the SGA approach was much faster and more scalable. However, the high throughput approach yielded a high proportion of false positives in the initial screen data that need to be resolved by retesting.

After validation by random spore analysis and tetrad dissection, only 7 out of 22 candidate genes from SGA screen turned out to be true positive hits. The biological processes involved

and the single mutant phenotype of the 15 false positive hit genes are various. Also there were 6 false negative hits confirmed by tetrad dissection and random spore assay. The similarity of principles between random spore assay and SGA implied technical problems during SGA, such as the contamination or mis-location of single mutants in the DMA collection or growth variation caused by differences in cell transfer by pinning. Also, many DNA repair gene mutants are very sensitive to DNA damaging agents because of DNA damage response defects. These genes were filtered out during the second comparison analysis because the single mutant was itself inviable or sick when exposed to the sub-lethal dose of CPT. In addition, there was a maximum number of times that replicate plates could be performed which limited the number of DNA damaging agents that could be tested during SGA. Because the pins controlled by the robot picked cells always at the same position, one mother plate of 1536 density array can be used to replicate to ~5-6 daughter plates, at most.

One way to fix these problems is to make customized array plates with lower density (for example, 384 colonies/plate). The contamination can be minimized by barcode sequence checking. Low density would also prevent the occurrence of contamination and mis-location during SGA. The larger pin head allows robot transfer of more cells when replicate-plating and reduces the noise inherent in colony growth. In addition, the larger colonies area would show greater differences of the fitness when compared to different plates and result in a more confident EC value. Finally, greater replicate-plating enables multiple chemical sensitivity tests needed to provide the information about the specificity of the SC interaction during SGA.

Other model organisms and high throughput methods also can be employed for the SC screen. For example, using barcode analysis by deep sequencing in yeast could allow the growth of mutants pooled in liquid media with different DNA damaging agents and quantitatively assessing the complex pools following outgrowth (Smith et al., 2009). An RNAi based screen in *Caenorhabditis elegans* would allow testing of the human genes which are not conserved in yeast but are conserved in worm, such as BRCA1 and BRCA2 (Kirienko et al., 2010).

4.3 Clinical implications

Fourteen SC interactions with TEL1 provide the basis to investigate the mechanism behind the SC interaction and can be used to propose potential drug targets for ATM deficient cancer cell-specific therapy. Among them, at least 8 proteins have known homologues or functional orthologues in humans.

Human Fen1 and yeast RAD27 show a high degree of sequence similarity (73%) with a BLAST value of e^{-104}. Similar to RAD27 in yeast, Fen1 is also a flap endonuclease and exhibits nick exonuclease activities which are required for DNA synthesis and DNA repair including BER and HR. However, Fen1 is an essential gene required for DNA replication in human cells (Liu et al., 2004). FEN1 homozygous knockouts lead to embryonic lethality in mice (Larsen et al., 2003). Together, these findings suggest its critical role in DNA replication and repair. It has been reported that FEN1 silencing can specifically kill RAD54B-deficient tumor cell (McManus et al., 2009). FEN1 inhibitors are currently in pre-

clinical development. It will be of great interest to treat ATM deficient cells with a combination of FEN1 inhibitors and DNA damaging agents such as CPT.

The Shu complex subunits, PSY3 and SHU2, appear to have homologues in human cells (RAD51D and SWI1 respectively) (Martin et al., 2006) and a human Shu-like complex was been found recently, which consists of SWS1 and SWSAP1 proteins (Liu et al., 2011). Their functions are reminiscent of SHU with respect to promoting HR and suppressing RecQ family mutant phenotypes. Like the SHU complex in budding yeast, the depletion of SWS1 and/or SWSAP1 leads to increased MMS sensitivity but not appreciable sensitivity to CPT. The function in HR of SHU genes in both organisms is not yet clarified and additional studies are needed.

As an essential component in NHEJ pathway, the Ku heterodimer is composed of Ku70 and Ku80 homologues in all eukaryotes (Daley et al., 2005). There are some variations between yeast and human. In humans, Ku interacts with DNA-PK, recruiting DNA-PKcs to DNA ends (Gottlieb and Jackson, 1993); while in *S. cerevisiae*, direct homologues of DNA-PKcs do not exist, and Ku lacks the DNA-PKcs-recruitment motif. The lack of DNA-PKcs suggests a more fundamental role of Ku genes in budding yeast. Strikingly, the study from Weller et al. shows Ku is also conserved in prokaryotes, which reinforces the idea of conservation of DNA repair throughout evolution (Weller et al., 2002). On the other hand, unlike NHEJ as a dominant repair pathway for DSB in higher organisms with larger genomes, prokaryotes might not need for NHEJ. This could be explained by their small genomes size making homology searches during HR quite easy. Alternatively, the prevalence

of Ku might reflect its action in other processes beside NHEJ. My study shows that the YKU gene SC interaction with *tel1* is NHEJ-independent. It will be of great interest to determine whether this interaction is conserved in human.

While *S. cerevisiae* has two Pif1 family helicases: Rrm3 and Pif1, higher eukaryotes contain only one: Pif1. Human Pif1 shows 24% identity with ScRrm3 and ScPif1 over the helicase domain (Mateyak and Zakian, 2006). Only RRM3 shows SC interaction with TEL1 in my study, which could be explained by the very different roles PIF1 and RRM3 play in yeast in several cellular processes including DNA replication, DNA repair and telomere maintenance (Ivessa et al., 2000). The reason that such similar proteins have very divergent roles is unknown. Also the functions and mechanisms of human Pif1 are still not very clear due to the difficulties in expressing and purifying full-length protein. A recent study shows human Pif1 helicase has activity akin to Rrm3 in the nucleus and Pif1 in the mitochondria (Bochman et al., 2010). It is therefore possible that the SC interaction between RRM3 and TEL1 is conserved in human cells.

CK2 beta subunit genes were unexpected hits from my screen. Protein kinase CK2 is composed of a catalytic and regulatory subunit and is ubiquitously present in eukaryotic organisms (Pinna, 1990). It is involved in a myriad of cellular processes including cell growth and proliferation with more than 300 substrates (Meggio and Pinna, 2003). In *S. cerevisiae*, there are two regulatory isoforms: CKB1 and CKB2; while there is only one regulatory subunit, CK2 β, in humans. It has been shown that CK2 protein levels and activity are increased in many cancers (Ahmad et al., 2005). Several CK2 inhibitors have been

developed, but most of them target the ATP-binding pocket on the catalytic subunit (Mazzorana et al., 2008). A two-hybrid screening of a combinatorial library of peptide aptamers to target CK2 beta subunit was carried out (Martel et al., 2006). This group found one potent CK2β-interacting peptide (KD=0.4 μM) and it can induce apoptosis in cells with wild-type p53. The CK2β subunit will be particular interesting to test in combination therapy.

4.4 Conclusion

In conclusion, I used *Saccharomyces cerevisiae* as a model to discover SL and SC relationships between DNA repair enzyme inhibitors, TEL1/ATM mutations and sub-lethal doses of DNA damaging therapeutic agents. After directly testing in a small matrix with three DNA repair genes and genome-wide screening in a large matrix with ~5000 non-essential genes, I identified 1 SL interaction with MEC1 and 14 SC interactions with TEL1 and CPT. SL and SC interaction data derived in model organisms may or may not be conserved in humans. The next step will be to validate these results in ATM deficient cultured mammalian cells. The data derived in model organism budding yeast provided the basis for testing specific combination therapies for selective killing of cultured cancer cells bearing ATM mutations. Specifically, the Shu complex, Ku complex, Rrm3, Rad27 and CK2 β subunits can be further tested as potential combination therapy targets with sub-lethal doses of camptothecin to kill ATM-deficient cells.

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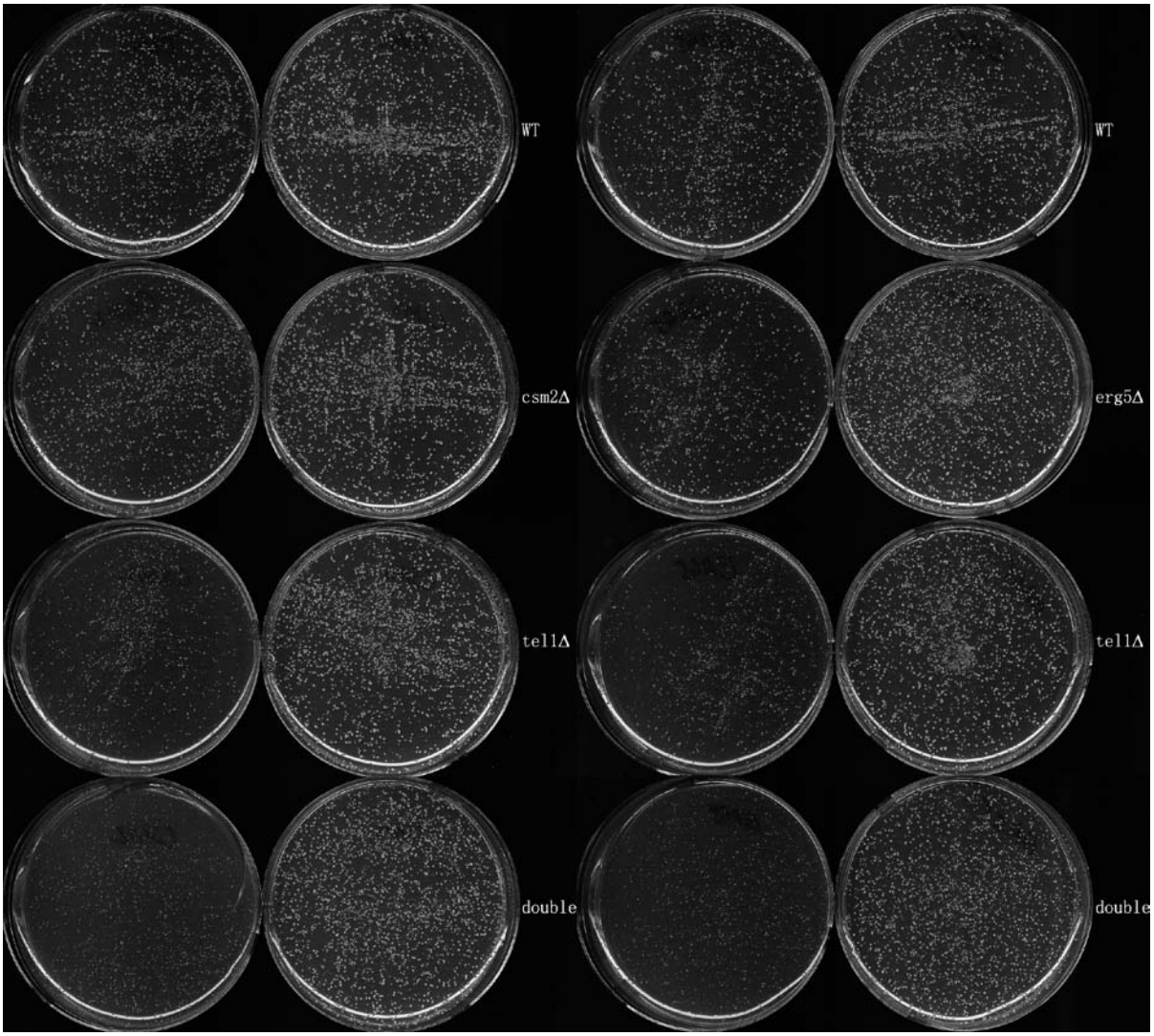
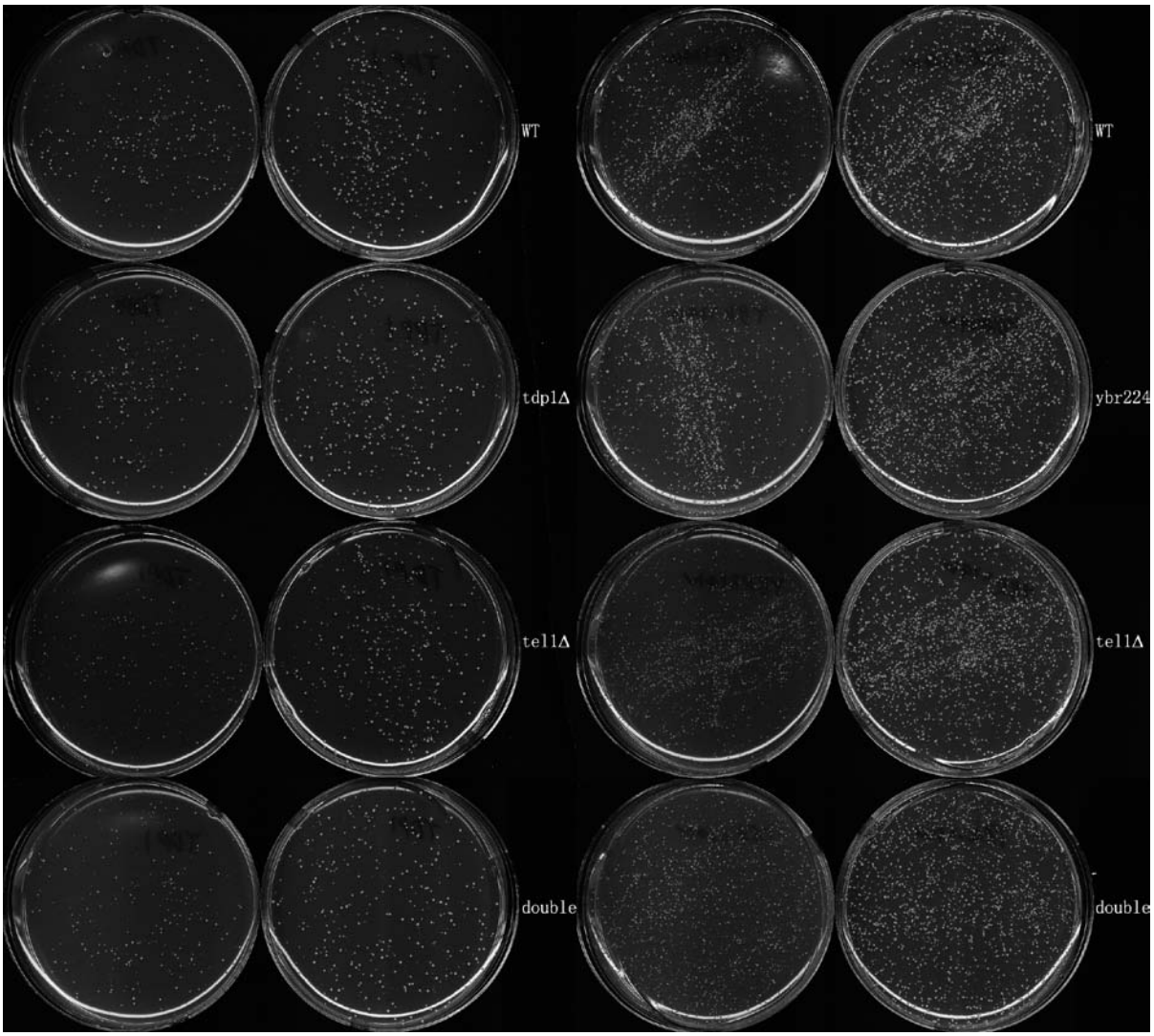
Appendices

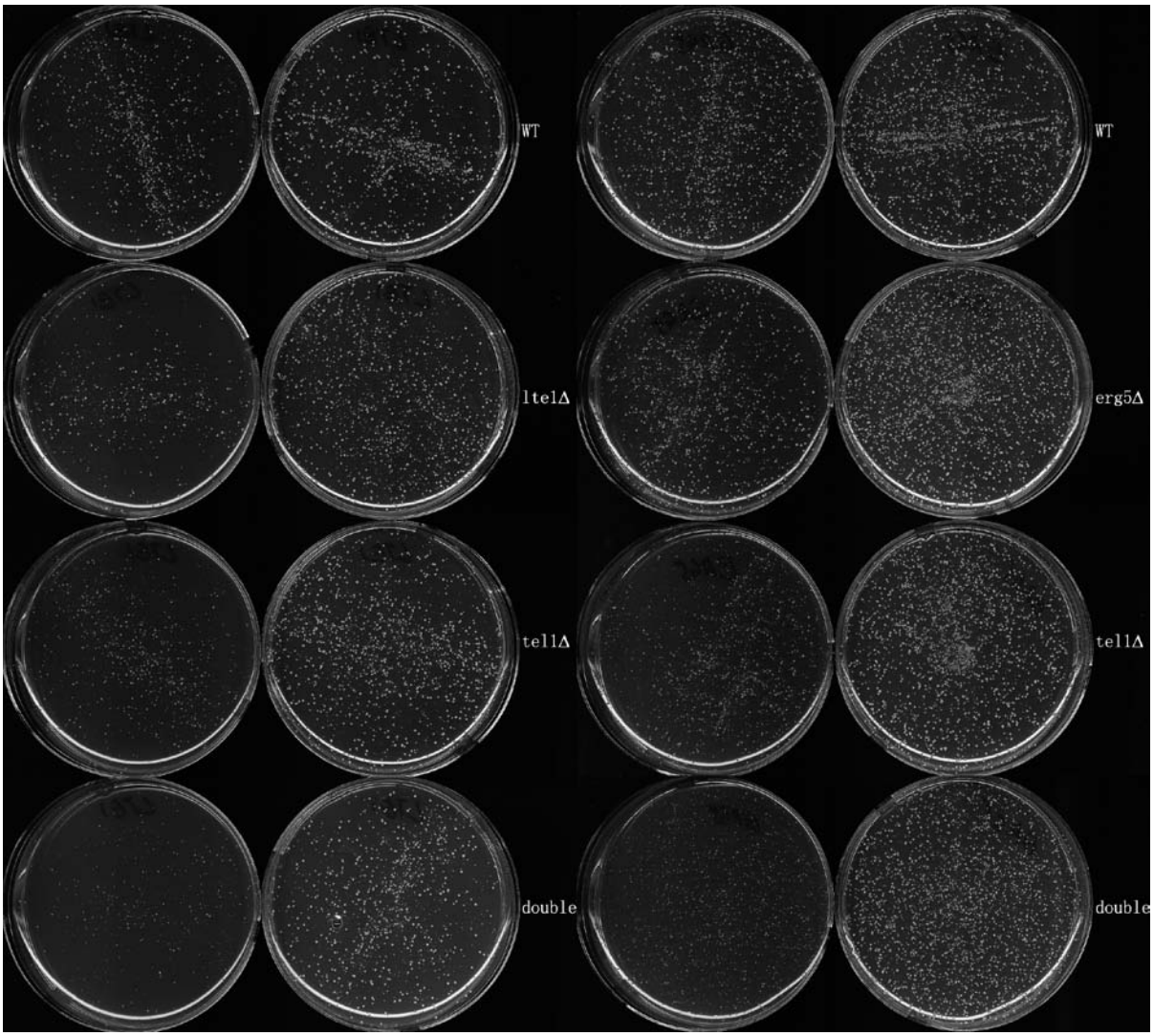
Appendix A Other positive SC interaction hits validated by RSA



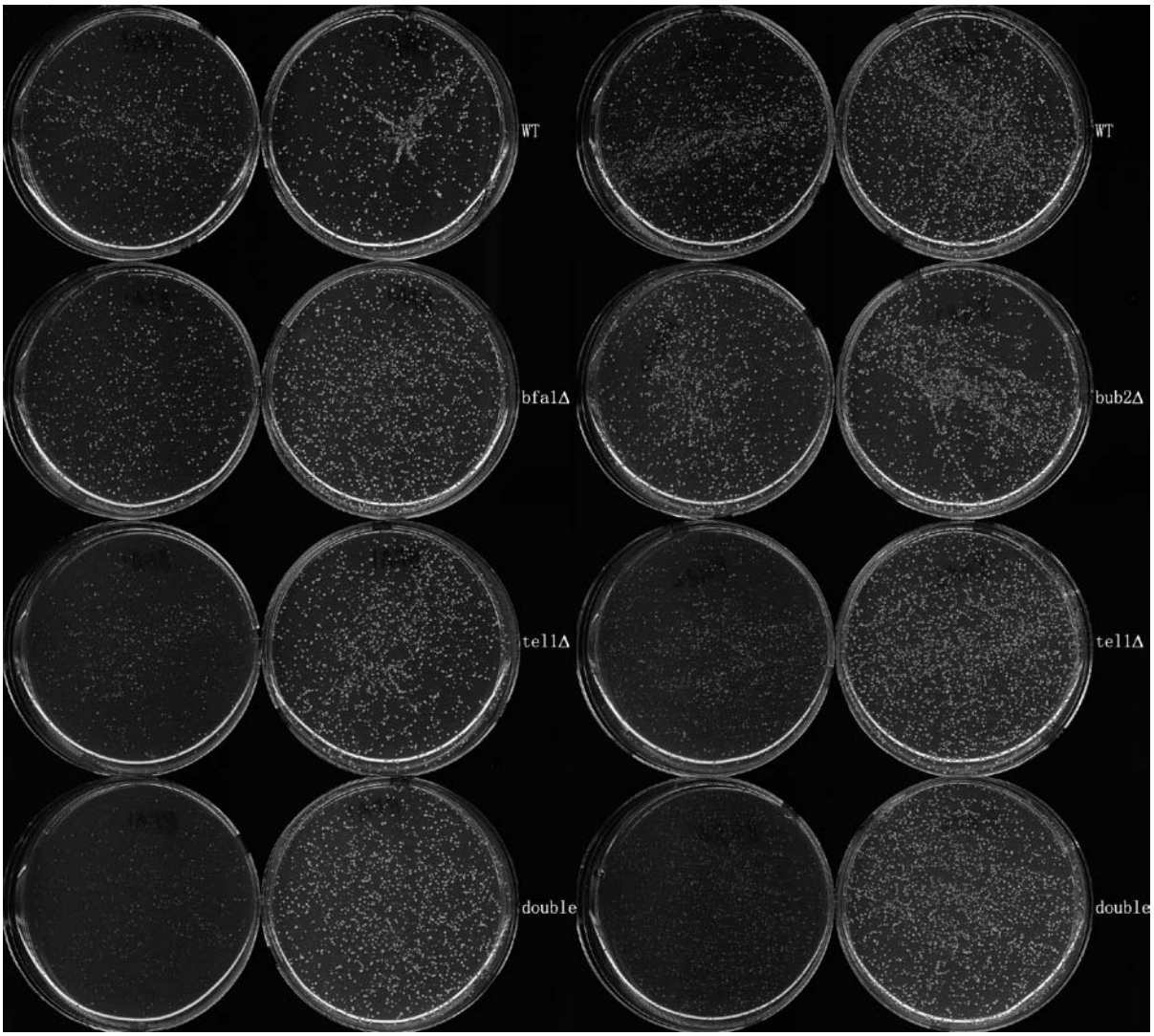
a CSM2 ERG5



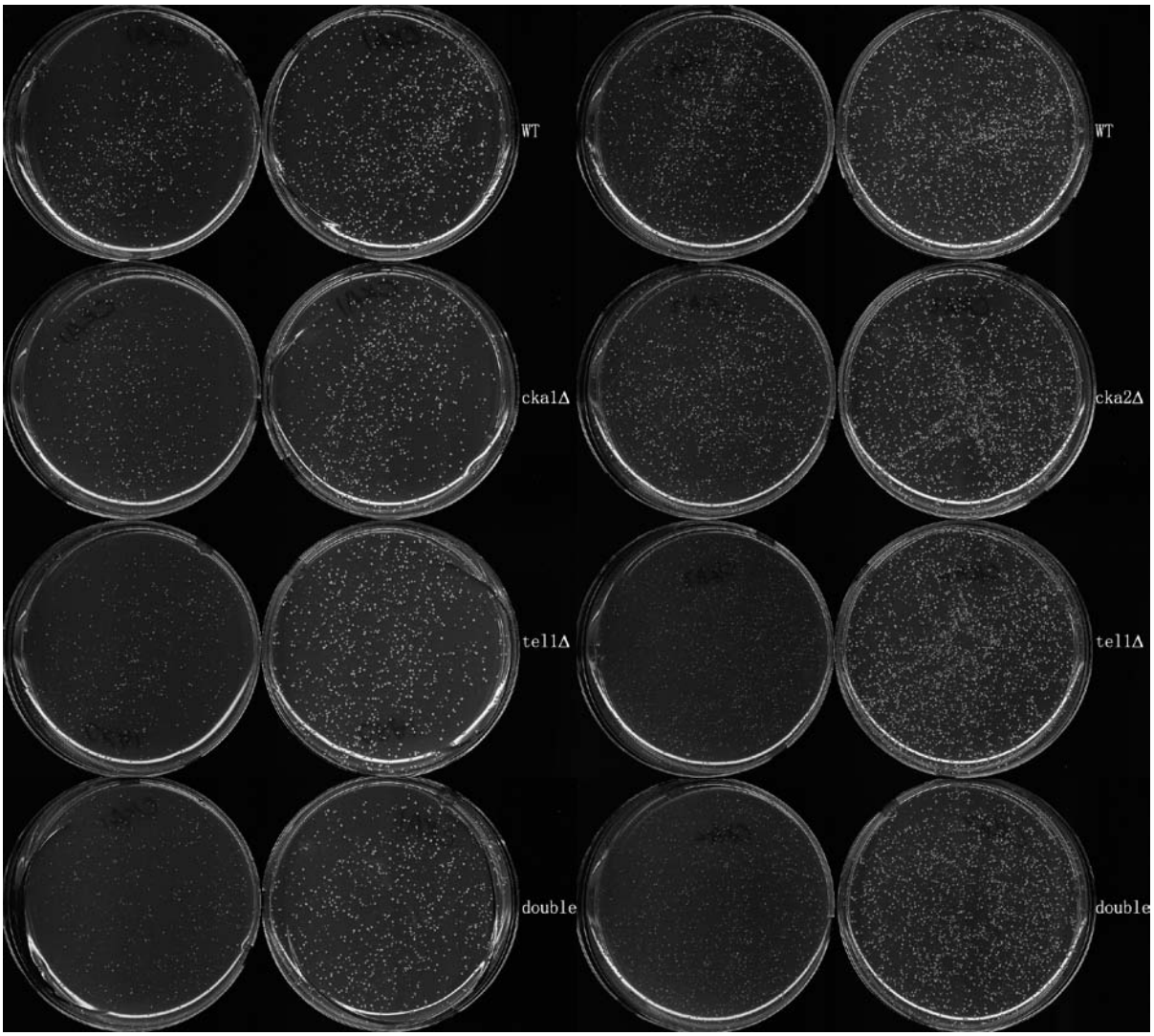
b TDP1 YBR224W



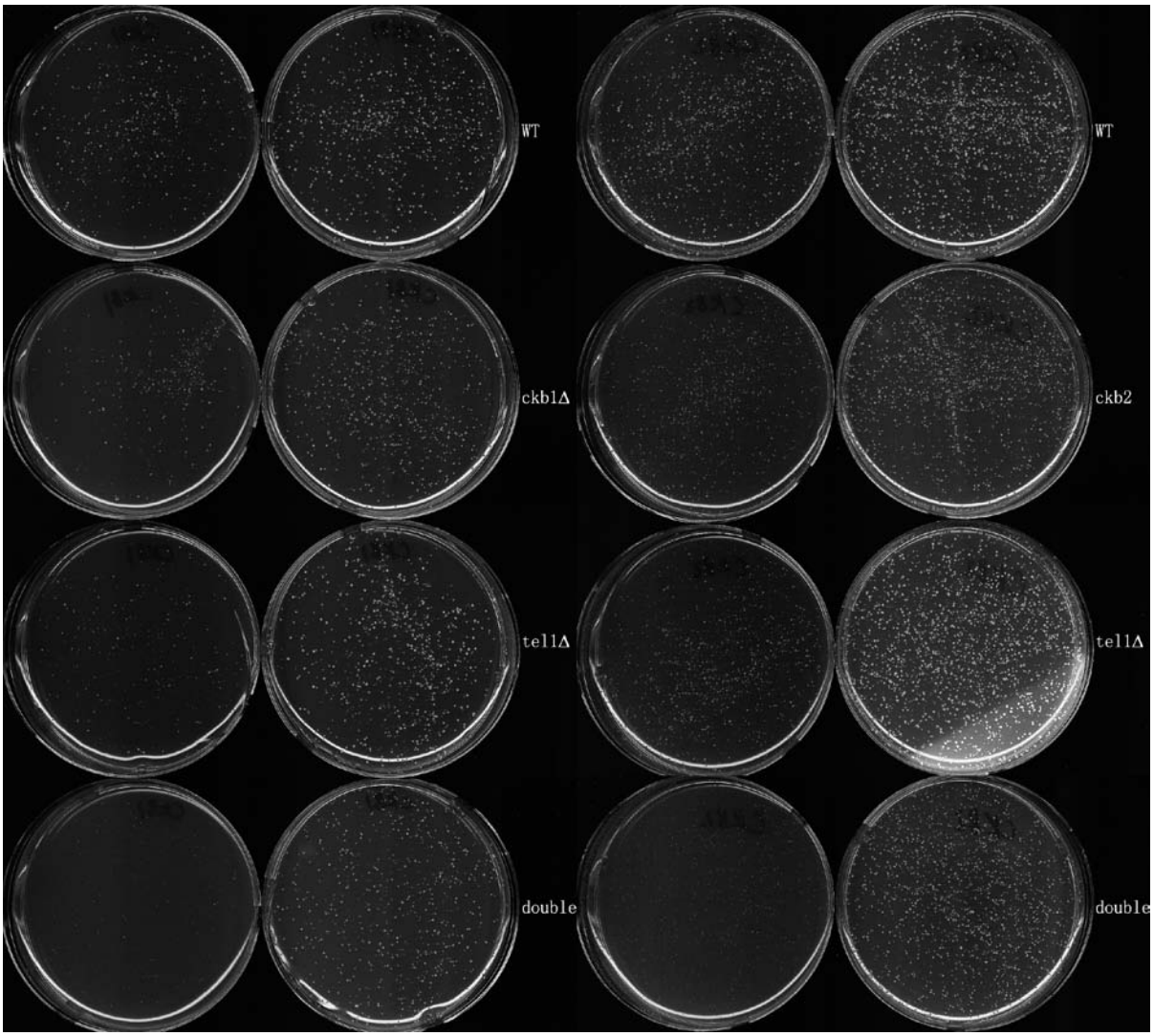
c LTE1 ERG5



d BFA1 BUB2



e CKA1 CKA2



f CKB1 CKB2

The SC interaction between CSM2 and TEL1 had been validated by tetrad dissection followed by spot assay and growth curve assay. No SC interaction between TDP1 and TEL1 had been tested in small matrix. So CSM2 and TDP1 were used as positive control and negative control respectively during RSA to test other screen hits. Other uncovered real SC interaction hits shown here include BFA1, BUB2, LTE1, CKB1, CKB2 and ERG5. One example of false positive hits excluded by RSA (YBR224W) is also shown here.

Appendix B SHU1 SHU2 SC interactions with TEL1 by growth curve assay