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The Mutation of a Novel *Saccharomyces cerevisiae* SRL4 Gene Rescues the Lethality of *rad53* and *lcd1* Mutations by Modulating dNTP Levels

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The SRL4 (YPL033C) gene was initially identified by the screening of *Saccharomyces cerevisiae* genes that play a role in DNA metabolism and/or genome stability using the SOS system of *Escherichia coli*. In this study, we found that the *srl4*Δ mutant cells were resistant to the chemicals that inhibit nucleotide metabolism and evidenced higher dNTP levels than were observed in the wild-type cells in the presence of hydroxyurea. The mutant cells also showed a significantly faster growth rate and higher dNTP levels at low temperature (16°C) than were observed in the wild-type cells, whereas we detected no differences in the growth rate at 30°C. Furthermore, *srl4*Δ was shown to suppress the lethality of mutations of the essential S phase checkpoint genes, RAD53 and LCD1. These results indicate that SRL4 may be involved in the regulation of dNTP production by its function as a negative regulator of ribonucleotide reductase.

***Keywords*:** SRL4, nucleotide metabolism, ribonucleotide reductase, SML1, checkpoint, *Saccharomyces cerevisiae*

Ribonucleotide reductase (RNRs) is the key enzyme in the maintenance and regulation of dNTP biosynthesis in all organisms, and catalyzes the rate-limiting step of the conversion of ribonucleoside diphosphates (NDPs) to their corresponding deoxy forms (dNDPs) (Nordlund and Reichard, 2006). Unlike multicelluar eukaryotes, the budding yeast *Saccharomyces cerevisiae* lacks deoxyribonucleoside kinase activity and, thus its dNTP synthesis is entirely dependent on RNR activity.

RNR is a primary target for the regulation of dNTP levels. The RNR activity is downregulated by dATP feedback inhibition. In addition to allosteric regulation, *S. cerevisiae* Sml1 (or Spd1 in *Schizosaccharomyces pombe*) inhibits RNR via direct interaction (Hakansson *et al*., 2006; Zhang *et al*., 2007). SML1 gene was initially identified as a suppressor of the lethality of *mec1* or *rad53* mutations, which encodes a small protein inhibitor which binds to RNR during G_1, G_2, and M phases (Zhao *et al*., 1998; Chabes *et al*., 1999). Sml1 protein is phosphorylated by the cascade of Mec1/Rad53 checkpoint kinases and degraded during S phase or following DNA damage in order to relieve RNR inhibition (Zhao *et al*., 2001; Zhao and Rothstein, 2002).

In budding yeast, Mec1 and Rad53 (ATR and Chk2 for mammalian homologues, respectively) are the essential checkpoint kinases that play central roles in cell cycle arrest at all checkpoints (Branzei and Foiani, 2006; Ben-Yehoyada *et al*., 2007). Lcd1 (also called Ddc2) associates stably with Mec1 and functions as a regulatory subunit for kinase (Rouse and Jackson, 2000). DNA damage, or incomplete DNA replication activates these signal transduction pathways, which results in a delay of cell cycle progression, DNA repair, stabilization of the replication fork, and inhibition of late origin firing in order to maintain genome integrity (Branzei and Foiani, 2006).

In addition to their involvement in the checkpoint pathways, Mec1 and Rad53 also function in transcriptional activation in response to DNA damage (Huang *et al*., 1998). They are required for the induction of DNA repair gene transcription. RNR is another target for transcriptional control by Mec1 and Rad53. The relevant mechanism involves Dun1, a downstream checkpoint kinase (Zhao and Rothstein, 2002; Chen *et al*., 2007). In response to DNA damage, Mec1 and Rad53 activate Dun1, which results in the transcriptional induction of the RNR genes. Such induction is achieved by the relief of transcriptional repression by Crt1 protein (Huang *et al*., 1998).

The lethality of the *mec1*Δ, *lcd1*Δ, and *rad53*Δ mutants is not likely to be associated with their checkpoint defects, considering that Rad3, the *S. pombe* homologue of Mec1, is not required for cell viability (Longhese *et al.*, 2003). Furthermore, the overexpression of RNR or the deletion of SML1 gene in *S. cerevisiae* has been shown to suppress the lethality of *mec1*Δ, *lcd1*Δ, or *rad53*Δ mutations by increasing *in vivo* dNTP pools (Huang *et al*., 1998; Zhao *et al*., 1998). As mentioned above, signal transduction through Mec1/Rad53/Dun1 kinase cascade induces RNR transcription and Sml1 degradation upon entry into S phase, thereby resulting in an increase in the levels of dNTPs. Therefore, it has been assumed that the essential function of Mec1 and Rad53 is the regulation and maintenance of the intracellular dNTP pools (Chabes and Stillman, 2007; Koc and Merrill, 2007).

In this study, we ascertained that a novel *S. cerevisiae*

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gene with the systematic name of YPL033C may be involved in the regulation of dNTP biosynthesis. The mutant cells proved resistant to a variety of dNTP production inhibitors and exhibited dNTP levels higher than those observed in wild-type cells in the presence of hydroxyurea. We refer to this gene as SRL4 (Suppressor of Rad53 null Lethality, or Suppressor of Rad53 and Lcd1), on the basis of our observation that the deletion of this gene suppresses the lethality of *rad53*Δ or *lcd1*Δ. Our results indicate that SRL4 may function as a negative regulator of RNR, which functions in a manner different from that associated with SML1.

Materials and Methods

Strains and media

All yeast strains used in this study were derived from YPH499 and YPH500 and listed in Table 1. The yeast media, YPD and synthetic drop-out (SD), were prepared as described by Chris *et al.* (1994).

Gene disruptions were conducted by one-step gene replacement methods as previously described by Baudin *et al.* (1993) and Sikorski and Heiter (1989). In order to disrupt the SRL4 and RAD53, selective markers (TRP1 or HIS3) were PCR amplified using the primers harboring 55 base-pairs (underlined) homologous to the 5' or 3' flanking region of each ORF. The primers utilized were as follows: Srl4-del1; 5'-GAA AAA CTG CGT ATG TCC GAA GCA CAG GTC CAC TAG TAA TAG AGC CCA AAA ATC TGA TTG TAC TGA GAG TGC ACC-3', Srl4-del2; 5'-TTG AAT TAT TTA TAG AAA TAT ATG TGC TTA GCA ATT TTA TCA CTG TTA CTT CGA TTG TGC GGT ATT TCA CAC CGC-3', Rad53-del1; 5'-GCA GGG TCA TTT GTA CCA CGG GTC AAA TTC CCA TCC GAG AGA CGT CTT ATT ATC ATG A-3', and Rad53-del2; 5'-GAA TGT ATT CTC TTC AAA ATG TTC CCC GTA TTC GCA TTA ACC TGA TGC GGT ATT TTC T-3'.

In order to construct the haploid yeast strains with double deletion mutations, heterozygous diploid strains were acquired by mating with the two haploid strains containing each deletion mutation to be combined. The diploid cells were sporulated, resulting in the formation of the tetrads. After the tetrads were dissected, the spores were recovered and germinated, followed by the selection of colonies harboring both of the deletion mutations by the marker test. For the induction of sporulation, 1 ml of diploid cells grown in YPD media were harvested and resuspened in the sporulation media (2% potassium acetate, pH 7.0), followed by 3~7 days of further cultivation at 25℃ with vigorous shaking. The tetrads were dissected with a micromanipulator (Singer MSM manual 100).

Measurement of chemical agent and UV sensitivity

The yeast cells were grown in liquid YPD media to mid-log phase and 10-fold serial dilutions from 10^7 cells/ml were spotted onto YPD or SD plates containing the selected chemicals. The plates were then incubated for 2~4 days at 30℃. Hydroxyurea (200 mM), mycophenolic acid (0.3 μg/ml), 6-azauracil (0.6 μg/ml), and methyl methanesulfonate (0.02%) were added to SD plates and 4-nitroquinoline 1-oxide (0.3 μg/ml) was added to YPD plates. For the UV sensitivity tests, YPD plates spotted with yeast cells were irradiated with various UV dosages (40~100 J/m^2) and incubated in darkness at 30℃.

Measurement of dNTP levels

Yeast cells were cultured to mid-log phase in liquid YPD media in the presence or absence of 100 mM hydroxyurea. Approximately 2×10^8 cells were harvested and extracted with 12% trichloroacetic acid as previously described (Muller *et al.*, 1993). Each of the four dNTPs in the extracts was measured by DNA polymerase-based enzymatic assay as previously described (North *et al.*, 1980). In brief, an alternating co-polymer template, poly(dA-dT) or poly(dI-dC) (GE healthcare), was extended by klenow DNA polymerase in the presence of the extract and an excess of ^{3}H-labeled complementary dNTP, where the amount of [^{3}H]dNTP incorporated into the template is dependent on the limiting amount of dNTP present in the extract.

Results and Discussion

The *srl4*Δ strain is resistant to nucleotide metabolism inhibitors but not to DNA-damaging agents

In an attempt to identify novel genes involved in DNA me-

Table 1. Yeast strains used in this study

Strains	Genotype	Reference/Source
YPH499	MATa *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*	Sikorski and Hieter (1989)
YPH500	MATα *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*	Sikorski and Hieter (1989)
DPD200-1a	YPH500 *srl4Δ::TRP1*	This study
YHM1	YPH499 *sml1Δ::URA3*	This study
YHM2	YPH499 *lcd1Δ::HIS3 sml1Δ::URA3*	This study
DPM300	YPH499 *lcd1Δ::HIS3 srl4Δ::TRP1*	This study
ARM531	YPH499 *rad53Δ::HIS3 sml1Δ::URA3*	This study
BRM532	YPH499 *rad53Δ::HIS3 srl4Δ::TRP1*	This study
DPM61	YPH499 *rad9Δ::HIS3 srl4Δ::TRP1*	This study
DPM62	YPH499 *rad17Δ::HIS3 srl4Δ::TRP1*	This study
DPM63	YPH499 *rad24Δ::HIS3 srl4Δ::TRP1*	This study

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Srl4   ---------MKKTIYKVLV-SFYQYVGLGK-KEHPSHDIVL-IIGGSSNELG-IELCEFFIEDYHIKV-INIDT----IDSINGKNAR-RSEKIYT-   77
Ag     MIEACLGTLM-KHAYRYTSLV-NDAVGPGKSL-ESGKDAVLIL-GGSSSTFGVE-LCKALLREGTIVVNLDTADLVKWRAPQQRR-ARARTPRARSA   91
Vp     --------MLIKSVYNTV-NRIYETFGSG-KTFEAGVDIV-LILGGSSNKF-GLEVCKTFVL-NYNSKVINIDTN-----DISYLLTM-QEAFVKN-   77

Srl4   -----FIS-CKDFSDIKCL---EESMLYLQNLEIIPT-------VLINN---------MQEGIESTLL-------KEDKFIRLDE-ESLNEFEKIV-R  141
Ag     GNVPRRAAA-GRYHFIHCRS-LTNKNDVLASLRMVLLGHFG-ISIFINNVNEGLFDFMQGSI-HAQAAQGPSDLMDRSELQGS----QELEQQLL-N  179
Vp     ------------YTFVECSSLSNRD-KLLESLKFIK-DNEINVTVFI--------NNLQEGLESLYS-------TDYEELLS-VEERIEHLER-CVT  143

Srl4   YNLQSVILITK-FCLSNIPP-KVQAEAQ-EKA-KGFYIVNISS-VLTLKPCKSGTHFITSKCGINSFHDGITSELKLKD-SNLNVKTLIAYLESF--  230
Ag     SNLINVMLSVK-FFLNQLVEQT-IELAQREHLTPGFYIVNVTN-TLSLHAPAFTGYYATSKAGLNQFHESLTSELSLYDKCRIK--TLLAFLEFLST  271
Vp     INLTNVM-IATKYYLTEI-----IPQIQQRLENPSFYIINMSCEQPDGSLLYA-SHYICSKMALNQFHDGLSSELSQRGKTSIE-KSQLPKIKML--  230

Srl4   --ESIAHWK----------------RLS-PSISKHLVHCLLEGRYGDTI-----------------LESKR---SIGDI-LLITGFKSSF-T-----  281
Ag     HTADETHWADSARILVRALKLGRTGDVSLAFPARRLSCA-RMLTFSYPVR-FWRAYEPWWWEKQLVASTNR-LPAPSAEHVV-RAQRPPLHIPDTGH  364
Vp     ----LIFLP----------------HIVDYNNWKNLCPAF-AKEEVKCIED-----------------GRRGFHVLPR-DVYTKRKR----------  278
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Fig. 1. An alignment of the amino acid sequences of Srl4-like proteins from *Ashbya gossypii* (Ag) and *Vanderwaltozyma polyspora* (Vp).

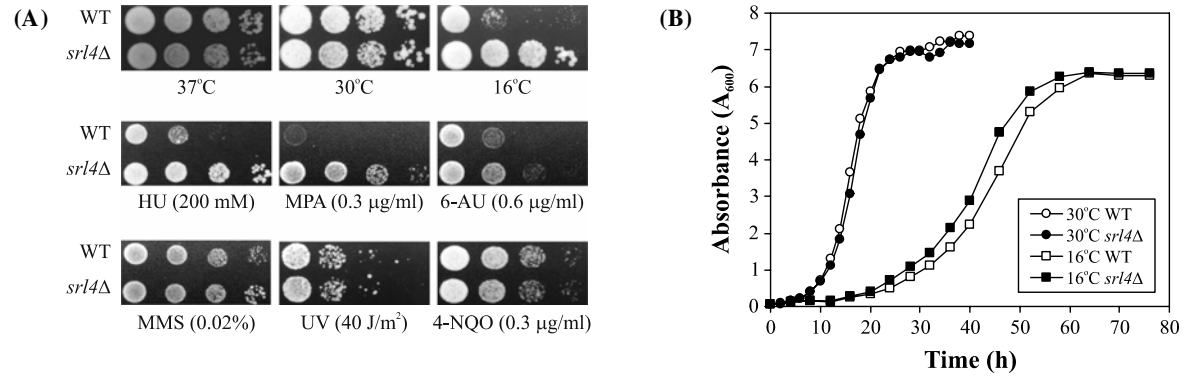

Fig. 2. Effects of *srl4*Δ mutation on the growth of yeast cells under a variety of growth conditions. (A) 10-fold serial dilutions (from 10^5 to 10^2 cells) of wild-type (WT) and *srl4*Δ cells grown in YPD were spotted onto YPD plates, followed by incubation at the indicated temperature (upper), or spotted onto plates containing the indicated chemicals and incubated at 30°C (middle and lower). HU, hydroxyurea; MPA, mycophenolic acid; 6-AU, 6-azauracil; MMS, methyl methanesulfonate; and 4-NQO, 4-nitroquinoline 1-oxide. (B) Temperature effects on the growth of wild-type and *srl4*Δ strains. Approximately 10^7 cells at mid-log phase were inoculated and grown in YPD media (200 ml) at 30°C or 16°C. Samples (1 ml) were withdrawn at a fixed time intervals and the cell densities were measured at 600 nm.

tabolism in *S. cerevisiae*, we searched uncharacterized or poorly characterized genes in the databases, whose annotations include the term 'DNA'. Among the candidate genes, we focused on the YPL033C gene (referred to hereafter as SRL4), as its function is predicted to be involved in nucleotide metabolism. This gene was initially identified by the screening *S. cerevisiae* genes that perform a role in DNA metabolism and/or genome stability using the SOS system of *E. coli* (Perkins *et al*., 1999). However its gene product had not yet been characterized.

SRL4 is a dispensable gene that encodes a protein composed of 281 amino acids with expected size of 32 kDa. Analyses of the amino acid sequence predict that Srl4 protein belongs to the short-chain dehydrogenases/reductases family. Although it is related to oxidoreductases, it misses the conserved Thr active site. Therefore it may not have any oxidoreductase activity (http://beta.uniprot.org). The homologues of Srl4 are found in the organisms closely related to *S. cerevisiae*, but not in multicellular organisms. The most homologous sequences are found in *Vanderwaltozyma polyspora* (GI:156845566) and *Ashbya gossypii* (GI:45188143) which are the members of Saccharomycetes group (Fig. 1). However, the homologies are not high even in these proteins. The *V. polyspora* protein shows 37% identity and 57% similarity to Srl4, and the *A. gossypii* protein shows 31% identity and 48% similarity.

We initially constructed a *srl4*Δ strain and assessed its susceptibility to a variety of agents. The deletion of SRL4 did not affect the growth rate at 30°C and 37°C (Fig. 2A and B). At 16°C, however, the mutant strain evidenced faster growth than the wild-type strain (Fig. 2A and B). We then assessed the susceptibility of the *srl4*Δ mutant to a variety of chemical agents. As a result, the mutant strain was determined to be significantly more resistant to hydroxyurea than was the wild-type strain (Fig. 2A). As hydroxyurea strongly inhibits ribonucleotide reductase (RNR), thereby preventing the *in vivo* reduction of NTPs to dNTPs (Nordlund and Reichard, 2006), this result compelled us to investigate the effects of other chemicals that reduce dNTP levels. Mycophenolic acid (MPA) and 6-azauracil (6-AU) exhaust the dGTP pool by inhibiting IMP dehydrogenase. It was determined that the *srl4*Δ mutant cells also exhibited a considerable level of resistance to these inhibitors (Fig. 2A), thereby suggesting that the SRL4 gene product may negatively influence the production of dNTPs.

As hydroxyurea is a DNA replication inhibitor and has

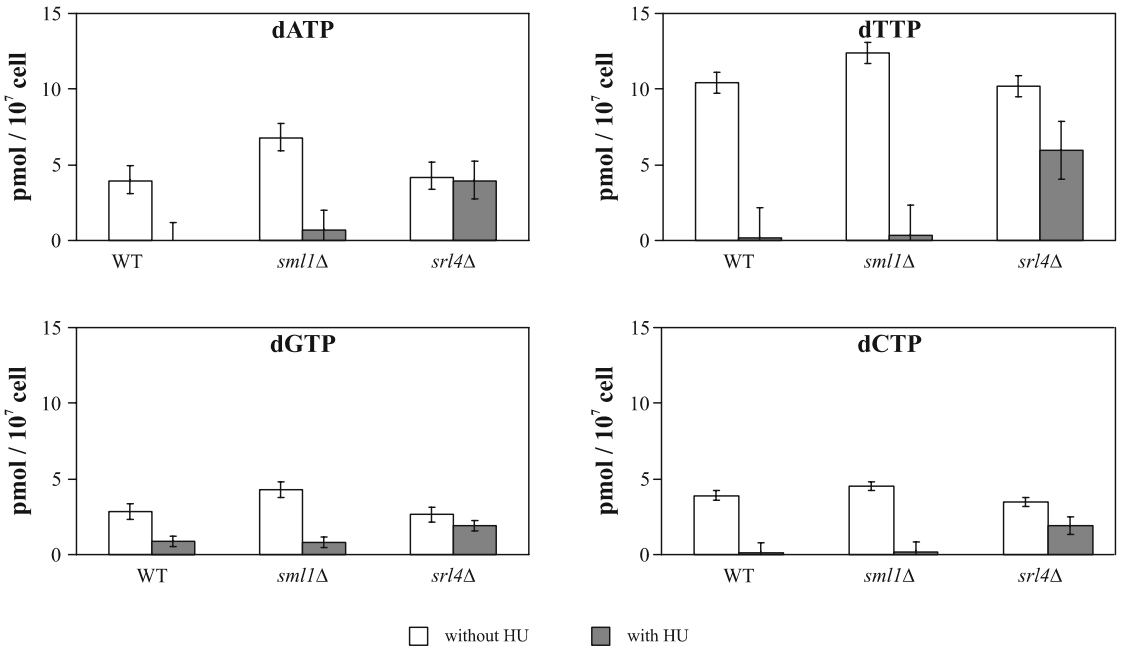

Fig. 3. dNTP levels in wild-type (WT), *sml1*Δ, and *srl4*Δ strains. Yeast cells were grown in YPD media in the presence (closed bars) or absence (open bars) of 100 mM hydroxyurea (HU). The dNTP levels were measured from 2×10^8 cells as described in the Materials and Methods section. The averages and standard deviations from three independent experiments are shown.

been shown to induce DNA damage (Alvino *et al*., 2007; Guarino *et al*., 2007), we also examined the effects of DNA damaging agents and other metabolic inhibitors. Neither treatment with the DNA damaging agents, methyl methanesulfonate (MMS) and 4-nitroquinoline 1-oxide (4-NQO), nor UV irradiation influenced the growth of the *srl4*Δ mutant strain (Fig. 2A). These results indicate that SRL4 may be involved in nucleotide metabolism but not in DNA repair processes.

When we transformed the *srl4*Δ strain with the plasmid containing wild-type SRL4 gene, we failed to observe the recovery of the mutant phenotypes (data not shown). Nevertheless, we believe that the presented mutant phenotype is derived from the absence of the SRL4 gene, not from any other mutation, since other *srl4*Δ strains independently constructed using a different genetic background strain, W303 (Thomas and Rothstein, 1989), also consistently showed the same mutant phenotypes (data not shown). The failure of complementation may be due to malfunction or no expression of our SRL4 clone in yeast. Another possibility is that the *srl4*Δ mutation may cause irreversible phenotypic changes that can not be recovered simply by re-introduction of wild-type SRL4 gene.

The *srl4*Δ strain evidences increased dNTP levels in the presence of hydroxyurea

Among the yeast genes involved in dNTP biosynthesis, the phenotypes of *sml1* mutations are similar to those of *srl4*Δ, in that *sml1* mutants also evidence increased resistance against hydroxyurea. Zhao *et al*. (1998) have demonstrated that the *sml1*Δ mutation induces an increase in dNTP levels and confers a hydroxyurea-resistant phenotype to yeast cells. Therefore, we attempted to determine whether the *srl4*Δ strain also evidences increased levels of dNTPs.

The level of each dNTP was determined in the extracts prepared from yeast cells grown in the presence or absence of hydroxyurea (Fig. 3). Without hydroxyurea, all four types of nucleotides are maintained at similar levels in the *srl4*Δ strain as compared with the wild-type cells, whereas they were slightly higher in the *sml1*Δ strain; this is consistent with the previous results (Zhao *et al*., 1998). In the presence of hydroxyurea, however, the dNTP pools were highest in the *srl4*Δ cells. In both the wild-type and *sml1*Δ strains, the nucelotide levels were greatly reduced as the result of hydroxyurea treatment. By way of contrast, a comparable level of dATP and more than 60% of the normal levels for dTTP, dGTP, and dCTP were detected in the *srl4*Δ strain treated with hydroxyurea (Fig. 3).

The dNTP levels are elevated in *srl4*Δ mutant at low temperature

As the *srl4*Δ strain grew faster than the wild-type strain at 16°C (Fig. 2 and 4A), we assumed that the deletion of SRL4 might also induce an increase in dNTP levels at low temperatures. When the dNTP levels in the wild-type and *srl4*Δ cells grown at 16°C were compared, it was determined that the levels increased by twofold for dGTP and more than fivefold for dATP, dTTP, and dCTP (Fig. 4B). This result indicates that the faster growth rate of the mutant strain is attributable to the increased dNTP pools.

The POL32 gene encodes the smallest subunit of DNA polymerase δ, which is not essential, but a deletion mutant evidences cold sensitivity for growth and is sensitive to hydroxyurea (Gerik *et al*., 1998). When we combined this mu-

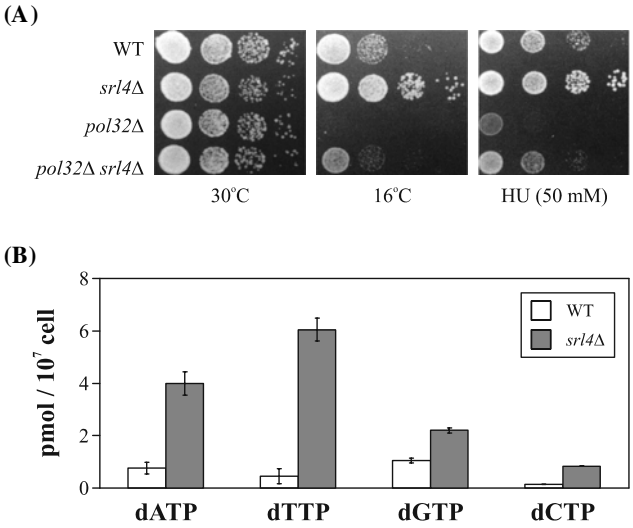

Fig. 4. Temperature effect on the dNTP pools in *srl4*Δ strain. (A) 10-fold serial dilutions (from 10^5 to 10^2 cells) of yeast cells were spotted onto YPD plates and incubated at 30°C or 16°C. For the hydroxyurea (HU) sensitivity test, the cells were spotted onto SD plates containing 50 mM HU and incubated at 30°C. (B) Wild-type cells (WT, open bars) and *srl4*Δ mutant cells (*srl4*Δ, closed bars) were grown in YPD at 16°C. The dNTP levels were measured from 2×10^8 cells as described in the Materials and Methods section. The averages and standard deviations from three independent experiments are provided.

tation with *srl4*Δ, we noted that both the cold and hydroxyurea sensitivity of the *pol32*Δ mutation was suppressed by *srl4*Δ (Fig. 4A). Thus, the enhanced growth rate observed at low temperature and increased resistance to hydroxyurea by the *srl4*Δ mutation are likely to be general effects.

The *srl4*Δ mutation rescues Lcd1 and Rad53 lethality

Since the deletion of SML1 gene rescues the lethality of the essential checkpoint genes, MEC1, LCD1, and RAD53 (Huang *et al*., 1998; Zhao *et al*., 1998), we attempted to determine whether *srl4*Δ can also suppress the lethality of these checkpoint genes. Tetrads were obtained following the sporulation of the heterozygous diploid strain (*lcd1*Δ/+ *sml1*Δ/+ *srl4*Δ/+), constructed from the two haploid strains, YHM2 (*lcd1*Δ::HIS3 *sml1*Δ::URA3) and DPD200-1a (*srl4*Δ::TRP1). After the dissection of the tetrads, we were able to recover the spores that evidenced the genotype *lcd1*Δ::HIS3 SML1$^+$ *srl4*Δ::TRP1 (Fig. 5A). This result shows that the *srl4*Δ mutation suppresses Lcd1 lethality, similar to the *sml1* mutation. However, no spores were obtained that were *mec1*Δ SML1$^+$ *srl4*Δ from the tetrad dissection (data not shown), indicating that *srl4*Δ mutation does not suppress the lethality of Mec1. In the case of Rad53, tetrad analysis proved impossible since RAD53 and SRL4 genes were located on the same chromosome (chromosome number 17). Instead, we directly deleted the RAD53 gene in the haploid strain lacking the SRL4 gene, and were able to acquire the double deletion mutant cells (Fig. 5B). These results indicate that the essential functions of Rad53 and Lcd1 can be bypassed by *srl4* mutation.

In an effort to determine whether the loss of checkpoint function in the *lcd1*Δ or *rad53*Δ background can also be restored by *srl4*Δ, we evaluated the sensitivities of the double mutant cells to 4-NQO, which functions as a UV mimetic DNA damaging agent, and determined that the *srl4*Δ or *sml1*Δ cells remained sensitive to 4-NQO (Fig. 5B). On the basis of these results, we assumed that the defect in the S-phase checkpoint in *rad53*Δ or *lcd1*Δ mutants cannot be suppressed by *srl4*Δ or *sml1*Δ, although they suppress the lethality of *rad53*Δ and *lcd1*Δ. To verify this conclusion, mu-

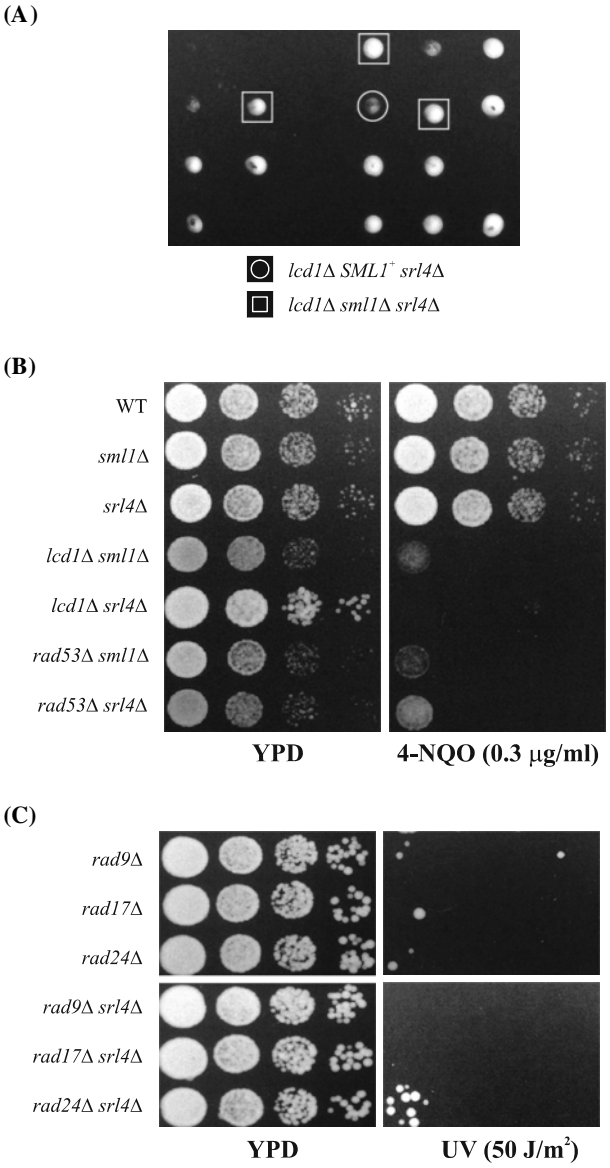

Fig. 5. Suppression of the lethality of *rad53*Δ and *lcd1*Δ by *srl4*Δ mutation. (A) Dissection of the tetrads obtained from the heterozygous diploid cells (*lcd1*Δ/+ *sml1*Δ/+ *srl4*Δ/+). The spore clones harboring the *lcd1*Δ mutation with SML1$^+$ *srl4*Δ (circle) or *sml1*Δ *srl4*Δ (squares) are indicated. (B) The growth of *rad53*Δ or *lcd1*Δ mutants combined with *sml1*Δ or *srl4*Δ mutations. Ten-fold serial dilutions (from 10^5 to 10^2 cells) of yeast cells were spotted onto YPD plates with or without 4-nitroquinoline 1-oxide (4-NQO) and incubated at 30°C. (C) Susceptibility of UV treatment in *srl4*Δ mutants. YPD plates spotted with the yeast cells were treated with UV and incubated at 30°C.

tations in the nonessential checkpoint genes (RAD9, RAD17, and RAD24) were combined with *srl4*Δ and assessed for UV sensitivity (Fig. 5C). Introduction to the *srl4*Δ mutation exerted no influence on the growth rate and UV sensitivity of these checkpoint mutants, indicating that *srl4*Δ does not affect S-phase checkpoint function.

Our results presented here indicate that SRL4, like SML1, may function as a negative regulator of RNR. However, the mechanism of inhibition is not likely to be the same, in that the dNTP levels in *srl4*Δ were almost identical to those in wild-type cells under normal conditions, whereas the dNTP pools were maintained at higher levels in the *sml1*Δ cells. Biochemical studies, such as investigation of the effect of Srl4 protein on the RNR activity and examination of the physical interaction between Srl4 and RNR, will provide useful clues to understand the precise role of SRL4.

S. cerevisiae contains two kinds of RNR complexes, which evidence unique $\alpha_2\beta\beta'$ structures (Nordlund and Reichard, 2006). The major RNR is composed of two identical large subunits (RNR1) and small subunits (RNR2 and RNR4). The minor RNR harbors the identical small subunits but differs with regard to its large subunit (RNR3), which is barely transcribed under normal conditions but is highly inducible by DNA damage. All RNR genes are inducible, and are regulated during the cell cycle and by DNA damage. The CRT1/CRT10 pathway is one of the most complicated regulatory circuits, in which CRT1 functions as an RNR repressor and CRT10 activates CRT1 transcription (Fu and Xiao, 2006). We are unable to dismiss the possibility that SRL4 may function as a transcriptional regulator which negatively affects the induction of RNR. Therefore, the levels of RNR gene transcription will, in future studies, be compared in wild-type and *srl4*Δ cells in order to evaluate this possibility.

Acknowledgements

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