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LncRNA expression profiles of *Schizosaccharomyces pombe* in DNA damage inducing environments

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Abstract: LncRNAs are pervasively transcribed in eukaryotic cells and dynamically regulated in response to environmental changes. Compared to mRNAs, roles of lncRNAs in DNA damage responses are poorly understood. The lncRNA expression profiles of *Schizosaccharomyces pombe* under the treatment of four kinds of DNA damage generating drugs (camptothecin, hydroxyurea, methyl methanesulfonate and phleomycin) were systematically characterized by RNA-seq. Similar to mRNAs, lncRNA repertoires underwent drastic changes in response to DNA damage inducing environments. A Core DNA Damage Response lncRNA set of 161 commonly induced and 194 commonly repressed lncRNAs was identified. The differences between lncRNA and mRNA transcription profiles suggested that lncRNAs might conduct critical functions in DNA damage responses independent from their associated mRNAs. This profiling on lncRNA expressions provided data and resources for further functional studies of the fission yeast lncRNAs.

Keywords: long noncoding RNA; DNA damage; *Schizosaccharomyces pombe*
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1 Introduction

Pervasive genome transcription is a common feature of all eukaryotic organisms. In human cells, ~80% of the genome is transcribed, while only ~2% of these transcripts encode proteins[1,2]; in *Schizosaccharomyces pombe*, also known as fission yeast, at least 75% of the genome is transcribed as well[3]. A substantial portion of the noncoding transcriptome is constituted of long noncoding RNAs (lncRNAs). Distinguished from small RNA species, lncRNAs are more than 200 nucleotides in length[4]. Different from protein-coding mRNAs, lncRNAs are less conserved and expressed at lower levels[5]. It is suggested by quantitative RNA-seq that in proliferating fission yeast cells most lncRNAs are expressed below one copy per cell[6]. But it seems that lncRNAs are more sensitive to the environmental changes, that is, they have shown more variations in expression levels in different growth conditions and different tissues[6-8].

Although the precise annotation and classification of lncRNAs are still under development, in recent years, emerging evidence suggests that lncRNAs conduct critical functions in a variety of biological processes, including gene expressions, X chromosome inactivation, cell cycle regulations, chromatin remodeling, etc.[9]. In the past decade, lncRNAs have demonstrated their novel functions in DNA damage repair. The $ncRNA_{CCDN1}$, which is transcribed from the CCDN1 gene locus, is the first lncRNA that was found to participate in DNA damage responses[10]. After that, genome-wide screening has been conducted in several organisms with different DNA-damaging reagents or mutations to identify lncRNAs that are sensitive to DNA damages[11,12]. Correspondingly, in cancer cells, which have a relatively higher level of DNA damages, lncRNAs are found to be largely dysregulated[13]. Some of these DNA damage sensitive lncRNAs are not only deeply involved in the development and progression of cancers, but also associated with chemotherapeutic drug resistance of cancer cells[14,15]. Despite the large number of DNA damage sensitive lncRNAs, their roles in DNA damage response are just beginning to be elucidated.

Fission yeast (*S. pombe*) is a well-established model organism for studying DNA damage response. Its DNA damage response network is well characterized, and in some aspects, highly conserved with higher eukaryotes[16]. The RNA metabolism, including RNA interference, alternative polyadenylation features and

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RNA degradation process, of fission yeast is also quite similar to that of metazoan cells[17-19]. Therefore, it is also proposed as a potent model system for exploring the functions of lncRNAs. Currently, over 1500 lncRNAs has been annotated in *S. pombe* with continuous updates[20, 21]. But only a few of them are functionally characterized. The most famous one is meiRNA, which regulates homologous chromosome pairing during meiosis[22]. Early quantitative analyses suggested that most lncRNAs are expressed in low levels (< 1 copy per cell) in proliferating fission yeast cells[6]. Whereas, some of them showed the drastic increase in expression levels under the environmental stress. For example, the antisense transcript of *adh1* could be induced by zinc limitations[23]; *SPNGgCRNA. 1164* is activated under the oxidative stress to regulate *atf1* gene in *trans*[24]; and several lncRNAs that are transcribed from *fbp1* gene locus would all be activated by glucose starvation[25].

The transcriptome analysis under specific treatments has been proven useful for screening lncRNAs that conduct corresponding functions. Therefore, in order to define novel lncRNAs that participate in DNA damage responses and cytotoxic drug resistance, we analyzed lncRNA transcriptomes of fission yeast under four different kinds of DNA-damaging drugs – camptothecin (CPT), hydroxyurea (HU), methyl methanesulfonate (MMS) and Phleomycin (PLM). These drugs induce DNA damage through distinct mechanisms. By comparing their impacts on lncRNA expression profiles, we were able to identify 355 lncRNAs that are commonly induced (161) or repressed (194) by all four kinds DNA damage reagents, as well as those that are specifically responded to certain type of DNA damages. We verified the differentially expressed lncRNAs, and further analyzed them based on their genomic positions and degradation pathways. The difference and association between mRNA and lncRNA expression profiles are also discussed. This study provides general datasets and potent targets for further functional study of lncRNAs in DNA damage responses.

2 Material and methods

2.1 *S. pombe* strains and growth conditions

The wild type fission yeast strain PT286 (*h-ade*6-*M*216 *leu*1-32 *ura*4-*D*18) was used for RNA-seq and RT-qPCR verification. All the cell cultures were pre-grown overnight in YES liquid medium at 30 ℃. Cells were then span down and resuspended in new culture medium with corresponding drugs and grew for another 8 h. Cell concentrations were adjusted to ensure that all cell cultures were harvested at the mid-log phase. Final drug concentrations used for the treatments were 20 μmol · L^{-1} for camptothecin (APExBIO), 10 mmol · L^{-1} for hydroxyurea (Sigma), 0.02% for methyl methanesulfonate (Sigma), and 5 μg · mL^{-1} for phleomycin (APExBIO). Two biological replicates were collected for each treatment.

2.2 RNA-seq

Total RNA was extracted from harvested cells using TRIzol reagent (Invitrogen, USA) as previously described[26]. RNA quality was assessed on Agilent 2100 bioanalyzer prior to sequencing. RNA-seq libraries were prepared using NEBNext® Ultra™ Directional RNA Library ep Kit for Illumina®. Samples are depleted for rRNAs. Libraries were sequenced using PE150 (Pair End 150 bp) on an Illumina HiSeq 6000 instrument (Novogene, China). Low quality reads (one end low quality base > 50%), N-containing reads (unidentified reads ratio > 0.002) and reads with adapters are removed from the raw data to generate clean reads for further analyses. The sequencing data was submitted to GEO under the accession number GSE173677.

2.3 Analyses of RNA expression

Clean reads were mapped onto the fission yeast genome released on Pombase using HISAT2. Reads containing up to six mismatches (not clustered at read ends) were kept for further analysis. Around 30 million reads were obtained from each library, and the mapping rates were about 82% – 87%. The expressions of mRNA and lncRNA were evaluated with Stringtie followed by the annotation references[27]. LncRNAs and mRNAs with FPKM ≥ 0.5 in at least one sample were subjected to further RNA expression analysis. The differentially expressed mRNAs and lncRNAs were determined by edgeR package with the corresponding cutoff (p-value< 0.05, $|\log_2(\text{Fold Change})| \geq 1$ for mRNA and p-value<0.05, $|\log_2(\text{Fold Change})| \geq 1$ for lncRNA). For hierarchical clustering of expression data, $\log_2$(Fold Change) were clustered in R with the pheatmap package. KEGG pathways of differentially expressed mRNAs were analyzed using Metascape web server with default parameters (overlap ≥ 3, p-value < 0.01, enrichment ≥ 1.5) as previously described[28].

2.4 Classification of lncRNAs

LncRNAs are classified according to their degradation mechanisms and proximity to the nearby mRNAs as previously described. DUTs (Dicer-sensitive Unstable Transcripts), XUTs (Xrn1-sensitive Unstable Transcripts) and CUTs (Cryptic Unstable Transcripts) refer to lncRNAs that were significantly induced (expression ratio > 2 and adjusted p-value < 0.05) in *Δdcr1*, *Δexo2* and *Δrrp6* respectively[21].

The relative positions of lncRNAs to the nearest mRNAs were determined based on the annotation in Ensembl *S. pombe*, Assembly ASM294v2, release 33. LncRNAs shared ≥ 1 nt with mRNAs on the same and

opposite were categorized as Sense and Antisense respectively; those with no overlap with any nearby mRNAs were classified as Intergenic; lncRNAs are considered as Bidirectional if their transcription start sites (TSSs) were<300 nt up or downstream of a TSS of a mRNA on the opposite strand. LncRNAs meet the criteria for both Bidirectional and Intergenic or Bidirectional and Antisense were taken as Bidirectional. LncRNAs meet the criteria for both Sense and Antisense or Sense and Bidirectional were taken as Antisense or Bidirectional.

2.5 Pearson's correlation assay

The overall correlation between lncRNAs and paired mRNAs under each drug treatment was calculated with cor. test function in R software (v4. 0. 2) using $\log_2$(Fold Change) values. Pearson's correlation between each lncRNA-mRNA pairs was calculated with cor. test function in R software (v4. 0. 2) using $\log_2$(FPKM+0. 1) gene expression values of all 10 samples. Significance cutoff was set to p-value<0. 05.

2.6 RT-qPCR

Complementary DNA (cDNA) was prepared using ABScript II cDNA First-Strand Kit (ABclonal) according to the manufacturer's protocol. The quantitative real-time PCR was performed with Genious 2X SYBR Green Fast qPCR Mix (ABclonal) on an ABI QuantaStudio3 system followed by 40 amplification cycles according to standard procedures. All primers used are listed in Table S1.

3 Results

To identify lncRNAs that respond to DNA damage reagents in fission yeasts, we treated fission yeast cells with four kinds of DNA damage generating drugs. These drugs induce DNA damage through different molecular mechanisms. Camptothecin is a topoisomerase I (Top I) inhibitor, which generates single strand beaks (SSBs) during DNA replication by blocking Top I cleavage complex. The SSBs would develop further into double strand breaks (DSBs) if they are not repaired immediately[29]. CPT and its derivatives have long been used as chemotherapeutic drugs for treating colorectal cancer (CPC), ovarian cancer (OC), small cell lung cancer (SCLC), cervical carcinoma (CC), etc. The first FDA approval on its derivative Camptosar dates back to 1996[30]. Hydroxyurea is a ribonucleotide reductase inhibitor that has been widely used in both laboratories and clinics. Short-term presence of hydroxyurea would decrease dNTP levels and induce reversible cell cycle arrests, while longer treatment with hydroxyurea would lead to the collapse of replication forks, which will cause DSBs and cell death[31]. It has been listed on the model list of essential medicines by WHO for treating chronic myeloid leukemia (CML)[32]. Methyl methanesulfonate is an alkylating agent that modifies adenine and guanine, which leads to mispairing of DNA bases and stall of replication forks[33]. Phleomycin is a wide spectrum glycopeptide antibiotic in the bleomycin family. Based on their DNA cleavage activities, antibiotics in this family are often used in combination with other chemotherapeutic drugs to treat germ-cell tumors, Hodgkin's lymphoma, skin carcinoma, etc.[34]. Drug treatments continued for 8 h at 30 ℃ for all four kinds of drugs in order to test long-term effects on the transcriptome. Concentration of each drug used was pretested to ensure the viability of the cells was not largely affected. Transcriptome profiles under the drug treatments and physiological condition were acquired by using strand-specific RNA sequencing.

3.1 Expression profiles of mRNAs and lncRNAs

By comparing to the cells grown in physiological condition, we were able to visualize the effects of DNA-damaging drugs on both mRNA and lncRNA repertoires (Figure 1(a, b)). Among 5132 annotated mRNAs, 3849 (75. 00%), 3952 (77. 01%), 3948 (76. 93%) and 3893 (75. 86%) mRNAs are differentially expressed (> 2-fold change, $p<0.05$) under the treatments of camptothecin, hydroxyurea, methyl methanesulfonate and phleomycin respectively. The drastic changes in the mRNA transcription suggested that cells have altered their proteomes globally to deal with DNA damages and other stresses caused by these drugs.

Differentially expressed genes are classified into the corresponding pathways referring to the information provided in KEGG database (Figure 1(c), Table S2). Some pathways, including 'MAPK signaling pathway', 'nucleotide excision repair' and 'autophagy', etc., altered greatly in the presence of anyone of these four drugs. It indicates that there might be a common stress response pathway that assists the cells to combat the DNA damage reagents.

Fission yeast cells also showed specialized responses toward each drug. For example, all the genes (7 out of 7) in the mevalonate pathway are differentially expressed in the presence of CPT. Interestingly, activation of this pathway produces isopentenyl diphosphate (IPP), a necessary precursors for the natural production of CPT in plants[35, 36]. Uncovering the enigma how exogenous CPT activate the mevalonate pathway would be helpful to understand the processes and regulatory factors of CPT biosynthesis, and might be valuable for developing biotechnological interventions to improve CPT production[37]. On the other hand, it is in line with our expectations that inosine monophosphate biosynthesis and pyrimidine metabolism pathways are uniquely affected by HU and MMS respectively. Considering that hydroxyurea is a

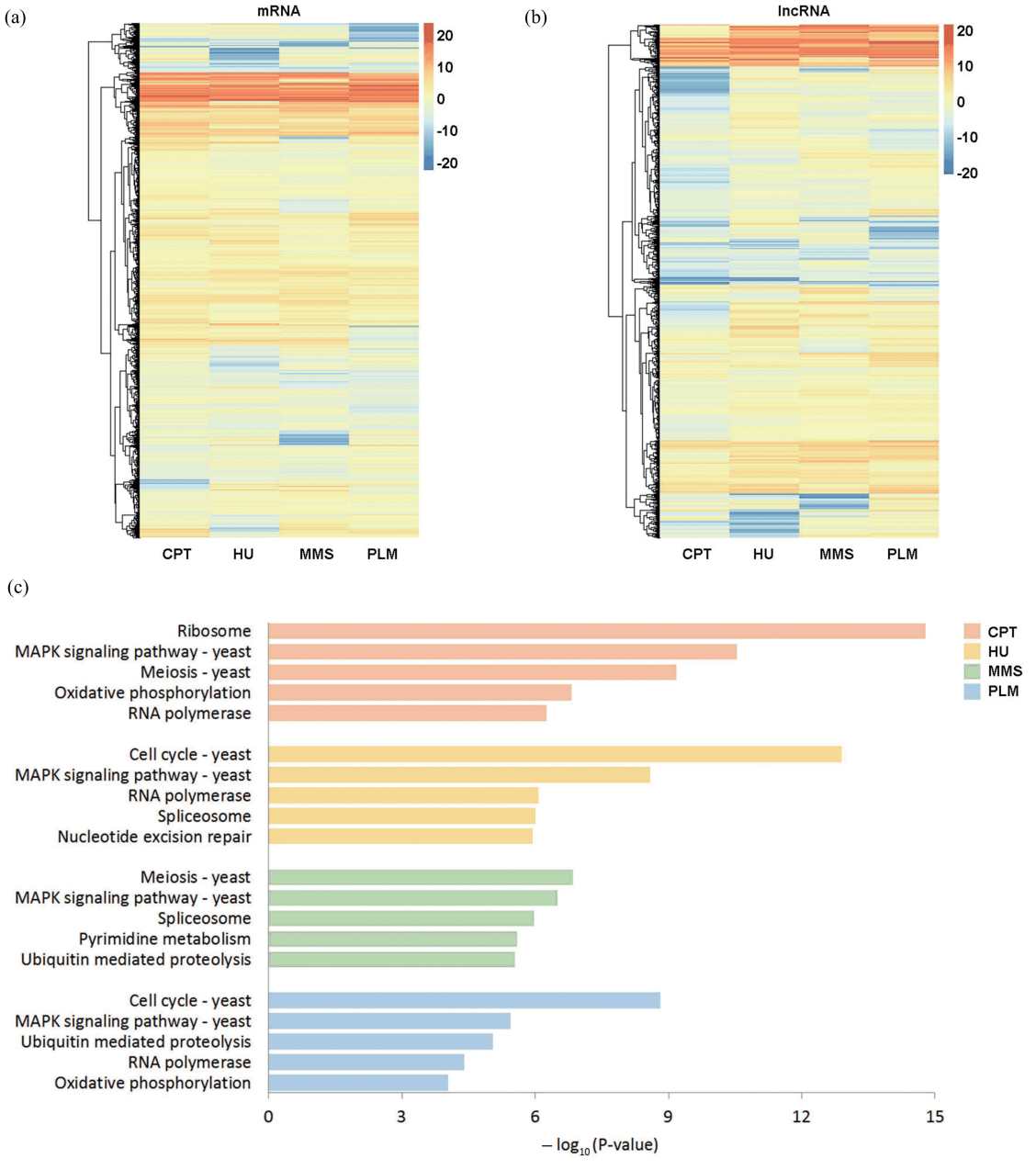

Figure 1. Expression profiles of mRNA and lncRNA under drug treatments. Expression profile of (a) mRNAs, (b) annotated lncRNAs, relative to control cells with no drug treatment. Color legends on top right represent $\log_2$(Fold Change) ratios relative to non-treated cells. (c) Top 5 enriched KEGG pathways of differentially expressed mRNAs under CPT, HU, MMS and PLM treatments.

ribonucleotide (RNR) reductase inhibitor, its presence would lead to the starvation of dNTPs (deoxyribonucleotide triphosphates)[31]. The activation of inosine monophosphate synthesis pathway, which generates the central intermediate IMP (inosine monophosphate) for guanine ribonucleotide, is likely a kind of feedback towards the shortage of dNTPs[38]. And the change in pyrimidine metabolism induced by MMS is probably associated with the biased translesion DNA synthesis occurred at damaged cytosines[39]. It has been reported that cytosines alkylated by MMS were more efficiently excised by the error-prone base excision repair than adenine and guanine. In accordance, a larger number of mutations were observed on cytosine though MMS induced more lesions on adenine and guanine[40].

Similar to mRNAs, lncRNA repertoires were also affected globally by the DNA damage reagents. LncRNA annotation in *S. pombe* is subject to constant updates. Here, we refer to the genome sequence and feature annotations in PomBase that were updated in September 2018, in which totally 1446 lncRNAs are annotated[27]. Among them, 1235 (85.41%), 1117 (77.25%), 1070 (74.00%) and 1111 (76.83%) lncRNAs are differentially expressed (> 2-fold change,

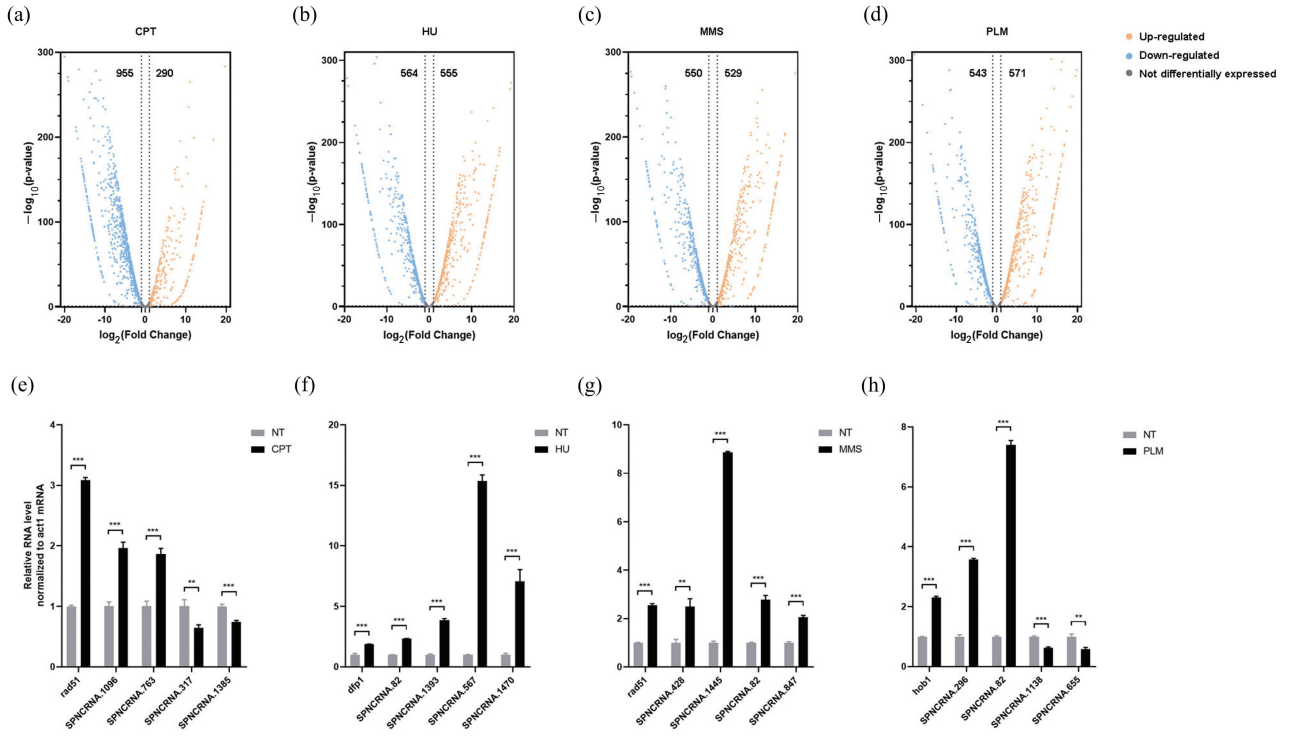

Figure 2. Differentially expressed lncRNAs in the presence of DNA damage reagents. Volcano plot of differentially expressed lncRNAs in the presence of (a) CPT, (b) HU, (c) MMS and (d) PLM. Numbers of more than 2-fold up-regulated and down-regulated lncRNAs are labeled in each plot. Quantitative PCR verification of lncRNAs differentially expressed in the presence of (e) CPT, (f) HU, (g) MMS and (h) PLM. Relative RNA levels were normalized to *act1* mRNA. NT, no treatment control. Expression level changes of *rad51*, *dfp1*, *rad51* and *hob1* were used as positive controls. Error bars depicted standard deviation of 3 biological repeats. **, $p<0.01$; ***, $p<0.001$. p, p-value of two-sided t-test.

$p<0.05$) in the presence of camptothecin, hydroxyurea, methyl methanesulfonate and phleomycin respectively (Figure 2(a)-(d)). RT-qPCR was conducted on some of the differentially expressed lncRNAs to verity the RNA-seq results (Figure 2(e)-(h)).

Noticeably, in CPT treated cells, 955 lncRNAs were down-regulated by more than 2 folds, while only 290 lncRNAs were up-regulated. This ratio between up- and down-regulated lncRNAs is distinct from the other three drugs (555 vs 564 for HU, 529 vs 550 for MMS and 571 vs 543 for PLM). We suspect this might due to the important functions of Top1, the target protein of CPT, in gene expression control[41]. Therefore, we checked the ratio of up-and down-regulated mRNAs as well. However, certain genome wide repression of transcription was not seen in the mRNA profile. In fact, more mRNAs were up-regulated and less mRNAs were down-regulated in CPT treated cells (2436 vs 1416 in CPT, 2072 vs 1888 in HU, 2050 vs 1909 in MMS and 2038 vs 1866 in PLM). It has been noticed previously that CPT favors the expression of short transcripts[42]. The different effects of CPT on mRNA and lncRNA repertoires might due to the difference in transcript length. On the other hand, it has also been reported that CPT may function through mechanisms that are independent of Top1. Taking lncRNAs into consideration might be helpful in exploring the unknown functional mechanisms of CPT and its analogues.

3.2 Classification of lncRNAs

To better understand the effects of DNA damage reagents on different types of lncRNAs, we classified lncRNAs into different groups. In both fission yeast and budding yeast, lncRNAs can be grouped according to their degradation pathways[21]. CUTs (Cryptic Unstable Transcripts) were defined as the lncRNAs that are degraded by nuclear exosome. They were accumulated in the strain lacking of Rrp6, which is the 3'-5' exonuclease of the nuclear RNA exosome; XUTs (Xrn1-sensitive Unstable Transcripts) are the lncRNAs that are targeted by the cytoplasmic exonuclease Exo2 (ortholog of Xrn1 in budding yeast); and DUTs (Dicer-sensitive Unstable Transcripts) referred to transcripts are degraded by Dcr1.

According to this standard, of totally 1446 annotated lncRNAs in fission yeast, 459 were CUTs, 179 were XUTs, 207 were DUTs and 601 remained unclassified (Figure 3(a)). Among 5132 mRNAs, ~80% were differentially expressed in the presence of these DNA damage reagents. Comparing to mRNAs, higher proportion of lncRNAs were repressed and lower

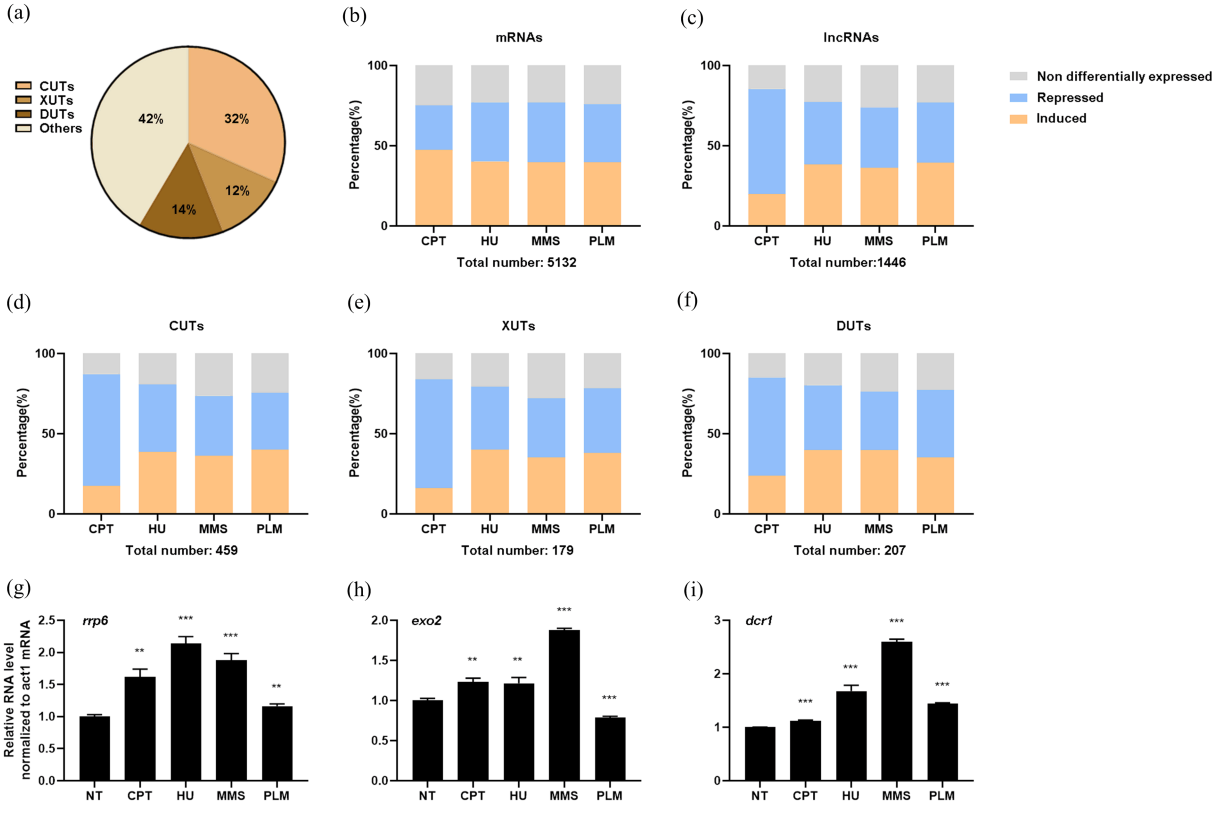

Figure 3. CUTs, XUTs and DUTs that differentially expressed under drug treatments. (a) Pie chart showed proportions of CUTs, XUTs, DUTs and other lncRNAs in the all annotated lncRNAs. Histograms showed proportions of induced (orange), repressed (blue) and not differentially expressed (gray) (b) mRNAs, (c) lncRNAs, (d) CUTs, (e) XUTs and (f) DUTs. Total number of each RNA set was labeled on the bottom. Expression level of (g) *rrp*6, (h) *exo*2 and (i) *dcr*1 under treatments of DNA damage generating drugs. NT, no treatment control. Error bars depicted standard deviation of 3 biological repeats. **, $p<0.01$; ***, $p<0.001$. p, p-value of two-sided t-test.

proportion of lncRNAs were induced under the treatments of all four kinds of drugs (Figure 3(b,c)). The changes in CPT treated cells were most drastic, which is consistent with our observation that CPT affects the expression of mRNAs and lncRNAs in the opposite ways (Figure 1(a,b)). However, we did not observe much specificity in lncRNA repression or induction when we classified lncRNAs into CUTs, XUTs and DUTs (Figure 3(d)-(f)). It seems that lncRNAs were generally more repressed and less induced under DNA damage treatments, but certain change was not due to the specific activation of any RNA degradation pathway. This observation was supported by the qPCR results of *rrp6*, *exo1* and *dcr1*, which encoded the enzymatic proteins of nuclear exosome, cytoplasmic exonuclease and dicer-dependent degradation pathways respectively. These three genes were generally up-regulated under the treatments of DNA damage reagents (expect for *exo2* with PLM), and the up-regulations were mostly less than 2 folds (except for *dcr1* with MMS) (Figure 3(g)-(i)).

Another common way to classify lncRNAs in different organisms is based on their templates' relative position to the nearest coding genes (Figure 4(a)). Antisense and sense lncRNAs completely or partially overlap with mRNAs, and are transcribed in the opposite or the same direction accordingly[9]. Many of them are known to participate in the regulation of local gene expressions. Long intervening/intergenic noncoding RNAs (lincRNAs), on the other hand, are transcribed from the intergenic regions that are outside of gene coding regions. This feature facilitates the explorations of their functions in many organisms[43-45]. And bidirectional lncRNAs are a special group of lncRNAs that are transcribed near the transcription start site but elongate in the opposite direction of the coding genes. Other than regulating gene expressions, they are also known as driving forces of the new gene origination[9].

Using these criteria, 1446 annotated lncRNAs were composed of 562 antisense lncRNAs, 86 sense lncRNAs, 290 intergenic lncRNAs and 508 bidirectional lncRNAs (Figure 4(b)). Among the lncRNAs induced by drug treatments, proportion of antisense lncRNAs

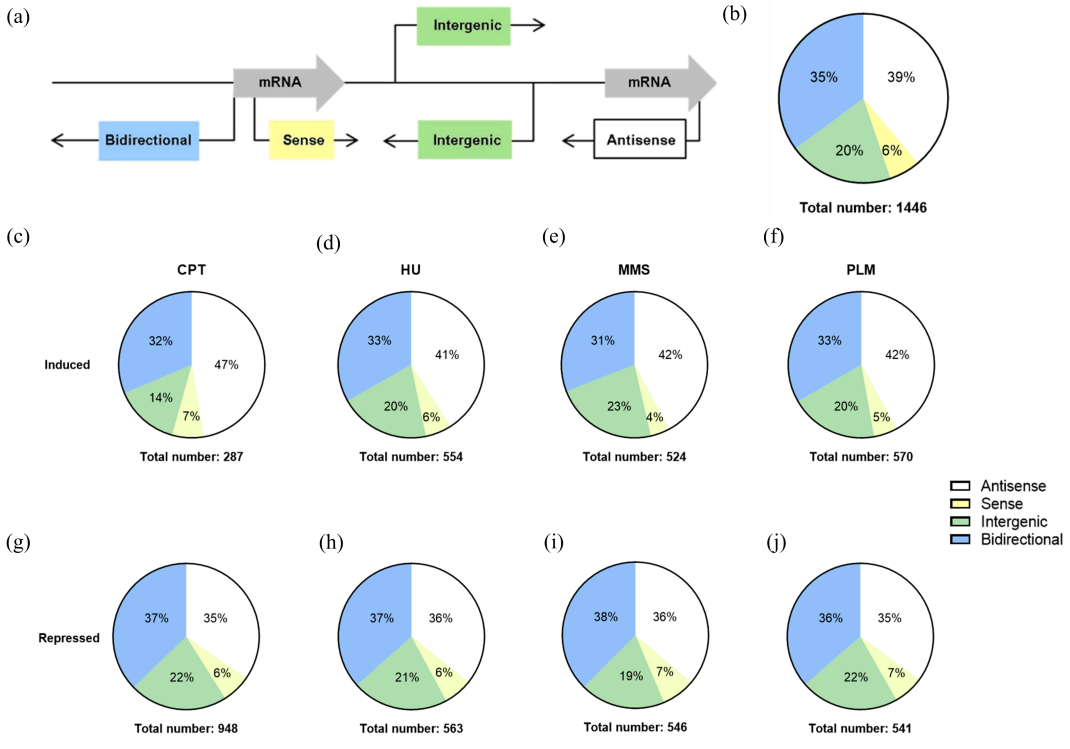

Figure 4. Analyses of lncRNA expression by relative positions to mRNAs. (a) Schematic representation of different types of lncRNAs' relative positions to mRNAs. (b) The proportions of antisense, sense, intergenic and bidirectional lncRNAs in all annotated lncRNAs. Pie charts showed the proportions of 4 types of lncRNAs that were induced by (c) CPT, (d) HU, (e) MMS and (f) PLM for more than 2 folds; and the proportions of 4 types of lncRNAs that were repressed by (g) CPT, (h) HU, (i) MMS and (j) PLM for more than 2 folds. The total number of each RNA set was labeled at the bottom of the corresponding pie chart.

increased and proportion of bidirectional lncRNAs decreased for all four kinds of drugs (Figure 4(c)–(f)). In contrast, among the lncRNAs repressed during drug treatments, proportion of antisense lncRNAs decreased and proportion of bidirectional lncRNAs increased (Figure 4(g)–(j)). Together, these indicated a general trend in which antisense lncRNAs are induced and bidirectional lncRNAs are repressed in the presence of DNA damage reagents. The expression levels of many antisense and bidirectional lncRNAs were known to be correlated with their neighboring mRNAs[9]. Usually, the expression of antisense lncRNAs were negatively correlated with the corresponding mRNAs, whereas the expression levels of bidirectional lncRNAs tended to be positively correlated with the surrounding mRNAs. It is highly possible that some of the differentially expressed antisense and bidirectional lncRNAs in this case were combating the DNA damage generating drugs through regulating the expression of their neighboring mRNAs.

3.3 Core DNA Damage Response lncRNAs

The existence of a group of Core Environmental Stress Response (CESR) genes, also known as Core Stress Response (CER) genes in *S. pombe* has long been proposed[46]. Since then, CERs, which referred to as a set of genes that is commonly induced or repressed in response to different types of stresses, have been discovered in many eukaryotic organisms[47]. Based on the lncRNA profiling in fission yeast cells treated with different kinds of DNA-damaging reagents, we were able to detect a set of 161 lncRNAs that were commonly induced in response to all 4 kinds of drugs, followed by a set of 194 lncRNAs that are commonly repressed (Figure 5(a), b), Tables S3 and S4). They were defined as Core DNA Damage Response (CDDR) lncRNAs. Their expression levels under drug treatments were verified by qPCR (Figure 5(c)–(e)). This indicated that similar to mRNAs, there was also a core group of lncRNAs that provide wide-spectrum protection against diverse kinds of stresses.

The CDDR lncRNAs were then categorized according to their relative positions to the mRNAs (Figure 6(a), b). Consistent with the observations in individual drug treatments, proportion of antisense lncRNAs increased in the commonly induced lncRNAs and decreased in the commonly repressed lncRNAs; whereas the proportion of bidirectional RNAs was less enriched in the commonly induced lncRNAs and more enriched in the commonly repressed lncRNAs.

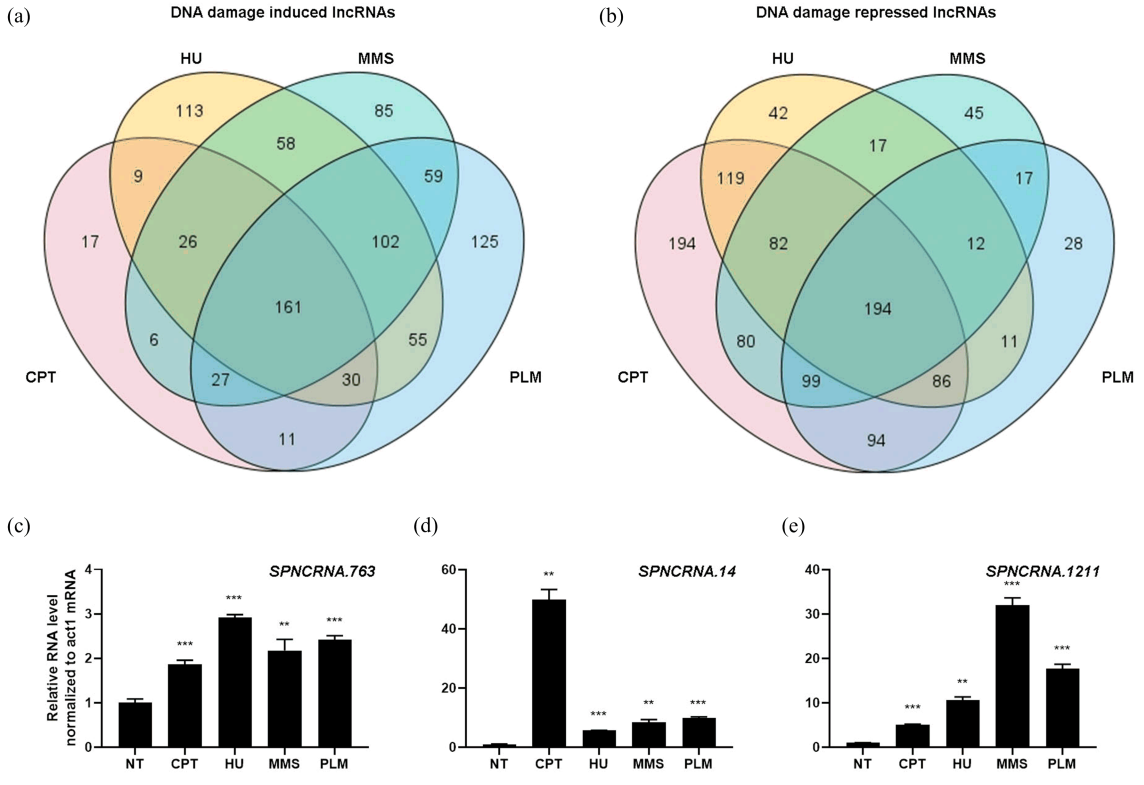

Figure 5. Detection and verification of CDDR lncRNAs. Venn diagrams illustrated numbers of lncRNAs that were commonly (a) induced and (b) repressed in the presence of DNA damage generating drugs. Relative expression levels of CDDR lncRNAs (c) *SPNCRNA. 763*, (d) *SPNCRNA. 14* and (e) *SPNCRNA. 1211* under drug treatments. NT, no treatment control. Error bars depicted standard deviation of 3 biological repeats. **, $p<0.01$; ***, $p<0.001$. p, p-value of two-sided t-test.

To explore the association between CDDR lncRNAs and coding genes, we define the paired mRNA of each lncRNA based on the relative position and compared changes of their expression levels in response to DNA damage inducing drugs. For intergenic lncRNAs, the nearest mRNA was taken as the paired mRNA. Overall, the expression level changes of CDDR lncRNAs are positively correlate with those of their associated mRNAs upon the treatment of each drug (Figure S1). Similar positive correlations with lower Pearson's correlation coefficients could also be observed between all the lncRNAs and their associated mRNAs (Figure S2). However, certain positive correlation might be largely attributed to the existence of some highly associated lncRNA-mRNA pairs. Therefore, we tested the correlation of expression levels of each CDDR lncRNA-mRNA pair under DNA damage conditions (Figure 6(c, d)). Among 355 CDDR lncRNAs, 97 (48 commonly induced lncRNAs and 49 commonly repressed lncRNAs) showed significant (Pearson $p < 0.05$) positive or negative correlation in expression levels with their paired mRNAs. Whereas, most CDDR lncRNAs did not behave in accordance with their paired mRNAs. These results indicated that correlated transcriptional regulation between lncRNAs and their neighboring mRNAs could only partially explain the genome-wide lncRNA expression profile changes in response to DNA damages. Many lncRNAs might act against environmental stresses in ways that were independent from their associated mRNAs. Understanding the functions and regulation mechanisms of these lncRNAs would be important for future studies on the DNA damage response.

4 Discussion

This study characterized the expression profiles of lncRNAs in fission yeast towards different DNA damage inducing reagents. About 80% of the annotated lncRNAs were differentially expressed in the presence of DNA damage reagents, suggesting that fission yeast cells made drastic changes not only on the coding genes but also on the noncoding RNAs in order to combat the DNA damage inducing environment. Differentially expressed lncRNAs were categorized based on their relative positions and degradation pathways. The identification and annotation of lncRNAs are still ongoing and evolving in the fission yeast. Nevertheless, this profiling of lncRNA repertoires under DNA damage inducing environments provides a useful reference for future research on the lncRNA function and regulations

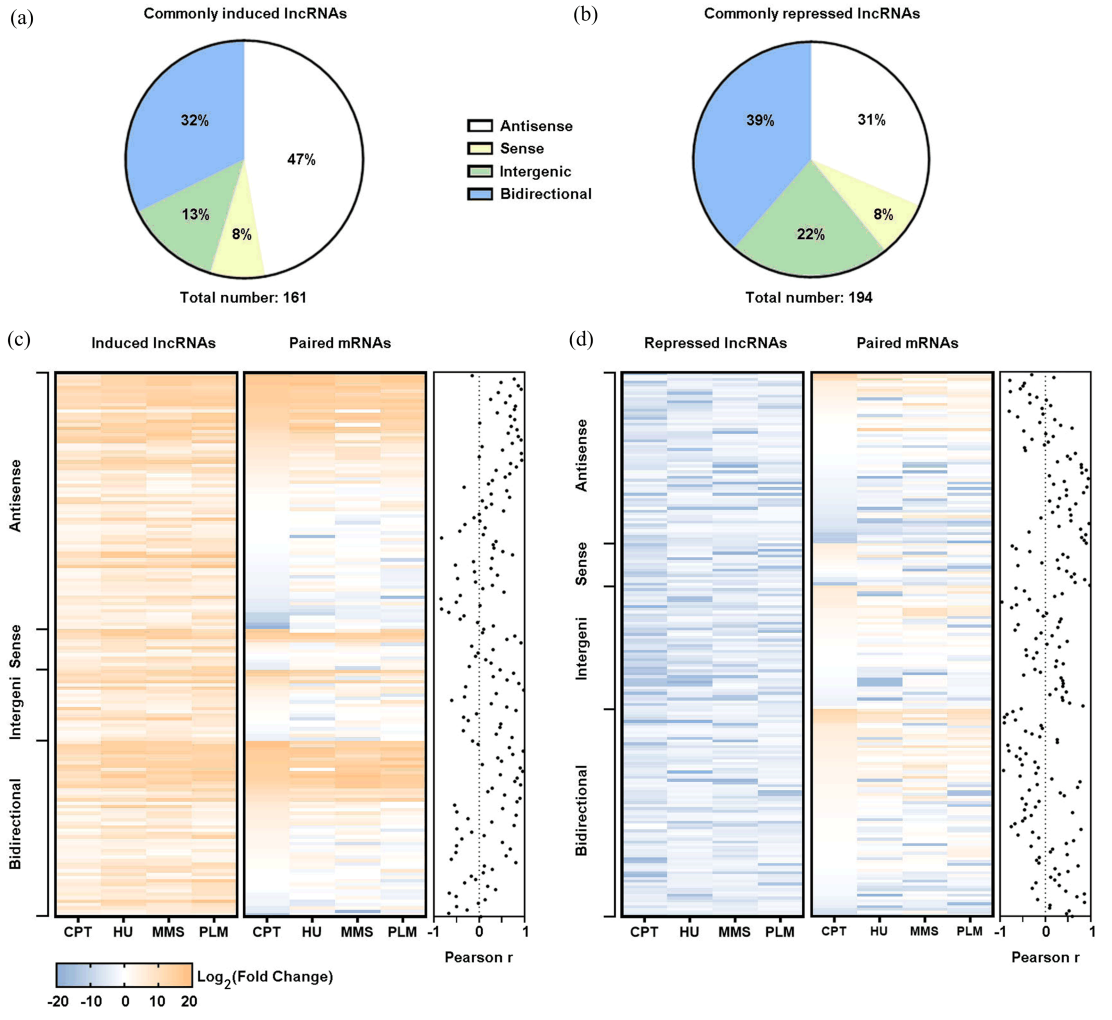

Figure 6. DNA damage responsive lncRNA-mRNA pairs. The proportions of antisense, sense, intergenic and bidirectional lncRNAs in (a) commonly induced lncRNAs and (b) commonly repressed lncRNAs. Total number of each RNA set was labeled at the bottom of the corresponding pie chart. Expression profiles of (c) commonly induced and (d) commonly repressed CDDR lncRNA-mRNA pairs in response to DNA damage reagents. Heatmaps represent the $\log_2$(Fold Change) relative to no treatment controls. Dot plots on the right side show the Pearson's correlation coefficients of lncRNA-mRNA pairs.

under diverse environmental conditions.

It has been proposed that microbes had little chance to live in the completely stress-free environment[48]. Therefore, microorganisms like yeasts need to make full use of their relatively small genome to efficiently fight against diverse kinds of environmental stresses. Not limited to single cell organisms, Core Environmental Stress Response genes have been discovered in a variety of organisms in recent years[47]. Compared to the protein-coding mRNAs, little is known about the roles of noncoding RNAs in stress responses.

In this study, we noticed that lncRNA profiles changed as much as mRNA profiles in DNA damage generating environments. The differential expression, especially the induction of many lncRNAs was quite unexpected. Since the pervasive transcription of lncRNAs is shown to be potentially harmful by affecting the expression of coding genes[49]. Even in the physiological condition, lncRNAs are surveilled and actively degraded to remain low expression levels[50]. It would seem quite "wasteful" for cells to generate "useless" lncRNAs under severe environmental stresses. Therefore, it is reasonable to believe that theses lncRNAs conduct important functions for fission yeasts to survive under DNA damage inducing environments.

Furthermore, 161 lncRNAs that were commonly induced and 194 lncRNAs that were commonly repressed by four kinds of drugs were defined as Core DNA Damage Response lncRNAs. Notably, the paired-mRNAs of Core DNA Damage Response lncRNAs defined in this study had little overlap with the CESR genes defined in the earlier study [46]. And only around 1/3 of the CDDR lncRNAs showed significant correlations in expression levels with their paired

mRNAs. These results suggested that most of CDDR lncRNAs may participate in DNA damage response independent from their associated mRNAs. A combination reaction of coding genes and noncoding RNAs is likely required for cells to combat or adapt to environmental stresses. The functions and regulations of lncRNAs in response to the DNA damage and other environmental stresses worth further explorations. This study provides a wide range of data and resources for further studies on DNA damage related lncRNAs in the fission yeast.

Supplementary data

Supplementary data are available at J. Univ. Sci. Tech. China online.

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Conflict of interest

The authors declare no conflict of interest.

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SHAN Ge (corresponding author) is currently a professor at University of Science and Technology of China, a recipient of the National Outstanding Youth Fund, and the chief scientist of the Ministry of Science and Technology Key Research and Development Program. His research interests include gene expression regulation and non-coding RNA function and functional mechanism. He was awarded the "New Century Talent" by the Ministry of Education, the Excellent Graduate Tutor of the Chinese Academy of Sciences, the "Young and Middle-aged Technological Innovation Leader" by the Ministry of Science and Technology, and the "National Outstanding Youth Fund" by the National Funding Committee.

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DNA 损伤环境下的裂殖酵母 lncRNA 表达谱分析

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摘要: LncRNA 在真核生物中广泛转录,且响应于细胞外环境的变化. 相比于 mRNA,人们对 lncRNA 在 DNA 损伤应答中的作用了解甚少. 基于高通量测序,本文系统地分析了裂殖酵母在 4 种 DNA 损伤药物(喜树碱、羟基脲、甲基磺酸甲酯和腐草霉素)处理下的 lncRNA 表达谱. 与 mRNA 相似,在 DNA 损伤环境下,lncRNA 的表达谱也发生了剧烈变化. 161 个受到 4 种药物共同诱导的 lncRNA 及 194 个受到共同抑制的 lncRNA 被定义为核心 DNA 损伤应答 lncRNA. LncRNA 表达谱和 mRNA 表达谱之间的差异表明,lncRNA 在 DNA 损伤应答中起着重要功能,且这些功能可能并不依赖于调控邻近的 mRNA. 这项研究为进一步研究裂殖酵母中的 lncRNA 在 DNA 损伤应答中的功能提供了基础和参考.

关键词: 长链非编码 RNA; DNA 损伤; 裂殖酵母