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DNA Repair of Eukaryotes Associated with Non-coding Small RNAs

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Abstract In eukaryotes, most of the genome are transcribed, however only a small proportion of total transcripts encodes for protein, thus resulting in many of noncoding RNAs. In order to recover DNA damage including DNA double-strand breaks (DSBs) eukaryotes have evolved complex mechanisms and these are processed through coordinated mechanisms of protein sensors, transducers, and effectors including RNAs. During recent years, small RNAs have been increasingly studied and gradually considered as key regulators in various aspects of biology. Upon DNA damage, small RNAs including diRNAs (DSB induced RNA) are generated in both plant and human cell lines. Inhibition of their biogenesis has severe influence on DSB repair system.

Keywords DNA repair · small RNAs

Introduction

After the first description of miRNAs in *Caenorhabditis elegans* (Lee et al., 1993), small RNAs have been mainly discovered from *Arabidopsis thaliana, Oryza sativa, Zea mays, Populus trichocarpa*, *Saccharum officinarum, Sorghum bicolor, Medicago truncatula,* and *Glycine max* etc. as well as different animal cells. These include microRNAs (miRNAs), small nucleolar RNAs (snoRNAs), small interfering RNAs (siRNAs), small nuclear RNAs (snRNAs), piwi interacting RNAs (piRNAs), signal recognition particle (SRP) RNAs, some transcripts such as natural antisense 4 transcripts (NAT), and those derived from transposon or retrotransposon-rich regions (Rinn and Chang, 2012). Among these non coding RNAs, major classes of small RNAs are miRNAs and siRNAs, which differ each other in their biogenesis.

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MicroRNAs from plant or animal are evolutionarily small RNAs, 19–24 nucleotides in length, which are generated by cleavage from larger precursor molecules. These RNAs post-transcriptionally regulate gene expression by interacting with their target mRNAs. Despite the similarities in their biogenesis and length between plant and animal origines, the miRNAs exert their control in fundamentally different ways. In general, animal miRNAs repress gene expression by mediation of translational attenuation, which is processed by miRNA-binding sites that is located within the 3' untranslated region of target gene. On the other hand, many of miRNAs from plant regulate their target gene by cleavage of target mRNA at single sites in the coding regions. siRNAs show a similar structure, function, and biogenesis with miRNAs except that they are biosynthesized from long double-stranded RNAs, often resulting in DNA methylation at target sequences. Small RNAs in eukaryotes have been known to be involved in cell differentiation, development, apoptosis, stem cell self-renewal, removal of intronic sequences during splicing, site specific RNA modification, and telomere synthesis, etc. On the other hand, small RNAs can also play a role for DNA elimination by a developmental regulation, which was found in *Tetrahymera thermophila* and called scan RNAs (scnRNAs) (Mochizuki and Gorovsky, 2004). It is interesting that QDE-2 interacting small RNAs (qiRNAs) derived from rDNA repeats was observed in the cell of *Neurospora crassa*, a filamentous fungus, which was treated with DNA damaging agents (Lee et al., 2009).

Up to date, several small RNAs have been investigated for the roles in plants and animals.

The studies of small RNAs are being accelerated by the aid of high-throughput technique which can be helpful for identifying RNA population including promoter-associated short RNAs (PASRs) and termini-associated short RNAs (TASRs) in animals (Kapranov et al., 2010). In recent years, small RNAs were found to be also involved in the DNA repairs, even double strand breaks (Storici et al, 2007; Wei et al., 2012). On the analogies of diverse biological roles of small RNAs, many laboratories have interest in the concerns whether small RNAs could play a role in DNA repair including DNA double-strand breaks (DSBs) in plant or animals.

In this paper, some non-coding RNAs including small RNAs are reviewed for the better understanding of molecular and physiological mechanisms including DNA repair in eukaryotes.

Generation of Small RNAs

The biogenesis of miRNAs as well as other most small RNAs are synthesized from the transcription of pri-miRNA in the nucleus. The structure of pri-miRNA contains 60 to 80 nucleotide of hairpin stem-loop structure. For the biogenesis, the cleavage of this hairpin structure is necessary, which is processed by a protein complex consisting of Drosha and DGCR8. Drosha is an RNAse III and DGCR8 its binding protein. By this reaction, the pre-miRNA is produced, which includes a 22-bp stem, a loop, and a 2-nucleotide 3'-overhang (Denli et al., 2004; Gregory et al., 2004). Thus synthesized pre-miRNA is transported from the nucleus to the cytoplasm, which is facilitated by the protein, Exportin-5 (XPO5) (Yi et al., 2003). The pre-miRNA is further cleaved in the cytoplasm by the aid of another RNase III, Dicer. This removes the loop, resulting in about 22 nucleotide miRNA duplex (Hutvagner et al., 2001). At this step, the strand is unwound by helicase and one strand of miRNA destined for the mature miRNA, which is in general termed the guide strand. The another complementary strand, which is termed the passenger strand, is degraded. The mature miRNA is bind to Argonaute (AGO) family of proteins and then packed into a ribonucleo-protein complex, which is known as miRISC (miRNA-induced silencing complex). Small RNAs regulate various gene expression through transcriptional or post-transcriptional regulation by association with members of AGO protein family (Baulcombe, 2004; Carthew and Sontheimer, 2009). The endonuclease activity of Ago cleaves the double-strand complex consisting of mRNA and miRNA. But this can not cleave the single-strand mRNA. In contrast to the biosynthesis of most small RNAs, some small RNAs may be produced by different way. For instance, most miRNAs are synthesized in the cytoplasm but human miR-29b occurs in the nucleus (Hwang et al., 2007). AGO proteins contain some domains for its function such as a variable N-terminal domain and PAZ, MID, and PIWI domains, which are well conserved (Tolia and Joshua, 2007). The PAZ domain binds to the 3' ends of small RNAs and PIWI to 5' ends by the same way (Ma et al., 2004; 2005). PIWI domain shows endonuclease activity of which the catalysis is related with Asp-Asp-His triad (Rivas et al., 2005). In plants and animals, their specificity of miRNAs shows, in general, some differences. In plants, miRNAs may contain one single mRNA complementary sequence for the open reading frame of target gene and most corresponding miRNAs generally show a perfect complementarity to these sites and cleave the target mRNAs (Bartel, 2004). However, animal miRNAs often bind to their target mRNAs by imperfect complementarity at multiple sites of the 3' untranslated regions (UTR).

The most abundant small RNAs in plants are heterochromatic siRNAs (hc-siRNAs) which are derived from transposons and other repetitive sequences. Single-stranded RNA transcripts might be processed from DNA repeats by DNA-dependent RNA polymerase IV (Pol IV) and then converted into dsRNAs by RNA-dependent RNA polymerase II (RDR2), then transformed into 24 nucleotide hc-siRNAs by DCL3. Hc-siRNAs are associated with AGO4 subfamily for its function and involved in the DNA methylation by DNA methyl-transferase DRM2, which is known as RNA-directed DNA methylation (RdDM) (Law and Jacobsen, 2010). Similar to plant hc-siRNAs, piRNAs are specific to animals, which are often specifically generated in the germ line in order to inactivate transposons (for review, Malone and Hannon, 2009). When single stranded RNA transcripts are generated by the DNA-dependent RNA polymerase IV (Pol IV) in the heterochromatic siRNA system, the RNA transcripts are converted to dsRNAs by the mechanism of RNA-dependent RNA polymerase II (RDR II). The resulting dsRNAs are cleaved into hc-siRNAa by the aid of Dicer-like proteins, following by the formation of complex with agronaute protein AGO4. Thus hc-siRNAs can play a role for *de novo* DNA methylation.

General Aspects of DNA Repair

The biochemical and physiological complexity including DNA repairs of eukaryotes can not be explained merely by protein coding genes (Kasparek and Humphrey, 2011; Han et al., 2012; Khoba and Epe, 2012; Soria et al., 2012; Wei et al., 2012). Many DNA damages are frequently occurred even though any organisms maintain its normal metabolism and physiological state. Excision repair of nucleotides is important to repair large DNA lesions including those produced following UV damage or poly-aromatic hydrocarbons (Hoeijmakers, 2001). The repair of mismatched DNA is also important to remove misincorporated DNA bases occurring during DNA replication, which might otherwise result in mutation. The most common types of DNA lesions are small base lesions and DNA single-strand breaks (SSBs). Among DNA lesions, the most serious damages are interstrand crosslinks and DSBs (Lindahl, 1993) which can induce many types of mutation, genome instability and often lead to cell death. Table 1 summarizes transcription-associated genome instability. The repair pathways of DNA damage require well-regulated and coordinated enzymatic mechanisms of protein signals, transducers, and effectors in the DSBs or other damage signaling cascade (Ciccia and Elledge, 2010; Polo and Jackson, 2011). In order to overcome the high load of DNA damage, several DNA repair pathways have continually evolved throughout the microbial, plant and animal kingdoms (Hoeijmakers, 2001).

There is a variety of DNA glycosylases that specifically recognize different types of DNA base damage and then remove the site of DNA damage. AP endonuclease (APE1) recognizes the apurinic or apyrimidic (AP) site resulted from the DNA removal. The nick created by the endonuclease can be re-ligated by ligase 1 or 3 in a process controlled by additional proteins, after processing by PNPK. Similar to the repair of DSBs, similar proteins are involved

Table 1 Factors inducing transcription-associated genome instability. Transcription associated mutagenesis (TAM) generally always results from DNA damage (generated either by exogenous agents or spontaneously during transcription). Transcription-associated recombination (TAR) is initiated either due to DNA damage or by unusual DNA structural conformations (local single-strandedness, negative supercoiling, R-loop, at the backtracked replication forks. etc.). Such structures can be eventually converted into strand breaks, for instance by DNA repair enzymes

Initiating event	Type of genome instability	References
Mutations in transcription and RNA processing factors	TAR	Huertas et al., 2006
R-loop	TAR	Huertas and Aguilera, 2003
DNA damage (bulky lesions, strand breaks, AP sites)	TAR TAM	Hendriks et al., 2010
Topological constrain/topoisomerase defects	TAR	Hendriks et al., 2010
Collision of RNA polymerases with replication forks	TAR	Prado and Aguilera, 2005
Uracil and AP sites deamination	TAR	Tsai et al., 2008
Enzymatically induced deamination	TAR TAM	Chiu and Greene 2008

in the repair of SSBs, except that they are not recognized by glycosylases and is not required the APE1-mediated DNA incision. Instead of glycosylases, poly ADP-ribose polymerase (PARP1) is involved in the recognition of free SSBs and mediates the recruitement of the other factors needed for SSB endoprocessing and ligation (Caldecott, 2008). On the analogy that a SSB is present as an intermediate step occurring in the course of base excision, it has been speculated that PARP1 might be involved in this repair process. However, it was reported that PARP1 does not appear to influence the repair of base excision in vitro (Allinson et al., 2003) or in cells (Strom et al., 2011). There exist much common features between the nucleotide excision repair pathways and the interstrand crosslink repair pathway. Even though the repair of interstrand crosslink is fairly relevant for the response to anticancer drugs, many aspects of molecular pathway still remain unknown. Many miRNA promoters show characteristics that are similar to protein coding genes, implying that transcription of miRNAs can be controlled by common transcription factors. For example, p53 family members activate multiple target genes in response to DNA damage, thus leading growth arrest or programmed cell death. P53, one of transcription factor, can regulate expression of miRNA through direct binding to miRNA promoters and modulating their transcription. Su et al., (2010) elucidated that Tap63, one of the p53 family, binds to the Dicer promoter and activates the transcription.

DNA repair and damage tolerance mechanisms have been much studied in *E. coli*, *Saccharomyces cerevisiae* and human. But these mechanisms in plants are less studied. Recently Singh et al., (2010) identified genes potentially involved in DNA repair and recombination (DRR) in *Arabisdopsis* and rice. They also showed that many of DRR genes are very similar to those found in other eukaryotes. Among DRR proteins, the proteins belonging to the nucleotide excision repair pathway were relatively more conserved than proteins needed for the other DRR pathways.

Repair of Double-strand Breaks

Repair of DSBs has been known to be occurred either by nonhomologous end joining (NHEJ) or by homologous recombination (HR) (Helleday et al., 2007; Ciccia and Elledge, 2010; Lieber, 2010; Moynahan and Jasin, 2010; Sasaki et al., 2010). NHEJ repairs DSBs in an efficient mode but it can cause deletions or insertions at the break site, the reason of which is believed by the modification of DNA ends occurring before joining (Lieber, 2010). HR, in contrast to NHEJ, appears to occur in a mode of error-free, but it needs a resection of the DSBs and a sister chromatid for a template (San-Filippo et al., 2008; Moynahan and Jasin, 2010; Sasaki et al., 2010). There is another type of HR, single-strand annealing (SSA), which takes place when DSB resection is occurred at repetitive sequences, providing complementary strands which can be used for annealing (Hartlerode and Scully, 2009; Ciccia and Elledge, 2010). NHEJ ligates the two ends together, whereas the HR repair requires a DNA template, which is occurred after replication. A role of RNA in the DNA repair was observed in the non-homologous joining of DSB ends, following by reverse transcription and formation of a complementary DNA (Teng et al., 1996). In addition to the importance of DNA repair by HR, it mediates a restart of stalled replication forks (Petermann and Helledat, 2010). The finding of HR pathway is important in the sense that determines the responses to many anti-cancer agents and thus it can be helpful to identify inhibitors to HR would be very important to study resistances to many anti-cancer drugs (Helleday, 2010).

RNA can be served as a template for DNA synthesis, especially in the reverse transcription of retroviruses and retrotransposons or in the regulation of telomeres (Baltimore, 1985; Autexier and Lue, 2006). Despite the abundance of RNA in the nucleus, there has been insufficient evidence for a direct role of RNA as a template during the repair process of chromosomal DNA damage including DNA double-strand breaks. In the yeast *Saccharomyces cerevisiae*, in general, homologous recombination occurs readily (Paques and Haber, 1999). In this mechanism, RNA was shown to mediate recombination by the production of cDNA intermediate, which is generated by the reverse transcriptase function of Ty retrotransposon in Ty particles in the cytoplasm (Lesage and Todeschini, 2005). A pairing between duplex DNA and single-strand (ss) RNA can be occurred *in vitro* or also *in vivo* (Kasahara et al., 2000; Huertas and Aguilera, 2003). Direct homologous exchange between RNA and DNA has not been reported up to now. It was shown that RNA can be used as a template for DNA synthesis for repair of a chromosomal DSBs in yeast (Stori et al., 2007). In this research,

they showed that the repair was accomplished with RNA oligonucleotides complementary to the broken ends. It was shown that replicative DNA polymerases such as α and δ was able to copy short RNA template tracts *in vitro*, implying that RNA can transfer genetic information *in vivo* through direct homologous interaction with chromosomal DNA (Storici et al., 2007). Some investigations lead to conclude that DSBs induce a number of histone modifications around the DSB sites, which facilitates DSB repair (Lukas et al., 2011; Polo and Jackson, 2011). In response to DSBs, the phosphorylation of H2AX appears to be generated around the DSB sites, which may facilitate local recruitment and chromatin remodeling factors (Paull et al., 2000; Fillingham et al., 2006).

Research Trend in DNA Repair

RNAi technology has been adopted for investigation of DNA repair mechanism. The first use of siRNA using RNAi technique was intended to decrease the expression of ATR (Ataxia-Telangiectasia Rad 3)-interacting protein, which plays a role as a sense protein for DNA damage repair (Cortez et al., 2001). Transfection of RNA hairpins was used to knock down the 53BP1, a p53-binding protein, which relocalizes to discrete nuclear foci upon exposure of cells to ionizing radiation (Wang et al., 2002). When 53BP1 is inhibited in the cells, some defects occurred in intra-S-phase and G2-M checkpoints (Wang et al., 2002). Similarly, using 21-mer oligoribonucleotides, expression of *Prkdc* was shown to be knocked down, which codes for a protein involved in nonhomologous end joining of DNA double-strand breaks, resulting in the increased radiosensitivity of those cells (Peng et al., 2002). siRNAs, in tissue culture, can be introduced through transient transfection of oligoribonucleotides. A short hairpin RNA (shRNA) can be also introduced, which is subsequently processed to siRNAs by the aid of Dicer (Paddison et al., 2002). Virtually most method for expression of a heterologous transgene encoding the shRNAs can be used to induce RNAi.

Recently, Wei et al. (2012) observed some non-coding RNAs which are involved in the DSB repairs in plant and animal cells, using *Arabidopsis* and human cell lines, respectively. In this research, they found small RNAs which show a role in DNA double-strand break repair in the model plant *Arabidopsis thaliana* using an assay system which traces DSB repair by single-strand annealing (SSA). In this experiment system, a genetic cross causes a single DSB in an inactive reporter gene. When SSA repairs the DSB gene, the activity of the reporter gene is restored and allows a quantitative and visible repair of DSB. When this assay system was applied to the mutant for *atr* of *Arabidopsis*, which encodes a PI3 kinase known to be involved in DSB response, repair efficiency was markedly reduced (Wei et al., 2012). DSB repair system might be fairly related with Dicer or Dicer-like proteins (DCL). The efficiencies of DSB repair in three mutants for *dcl* genes, especially *dcl*3, were considerably reduced (Wei et al., 2012). They showed that a population of small RNAs approximately 21nt was observed when DSBs were induced in *A. thaliana*. Small RNAs are also produced in the vicinity of DSBs using a similar HR mediated DSB repair assay in a human cell line. The DSB-induced small RNAs were generated from the sequences flanking the DSB with both the sense and antisense strands. It is interesting that diRNAs were produced from sequences neighbouring the DSBs in plants, whereas in human cell lines they were generated from a broader vicinity around the break site. When Dicer or Ago2 were eliminated in human cells, DSB repair was not occurred (Wei et al., 2012). A small RNA system in plants can mediate heterochromatic silencing of repetitive sequences through DNA methylation. In order to dissect the diRNA system, they used this pathway. They found that diRNA population requires the activity of Pol IV, RDR2 and RDR6 and DCKs and DSB responsive kinase ATR is related with this repair system. They also showed that DSBs trigger the production of small RNAs from the sequences in the vicinity of DSB sites using a reporter assays for DSB repair in *A. thaliana* and human cells and the small RNAs are required for the DSB repair.

Table 2 The role of double-strand repair genes in genetic disease (Kasparek et al., 2011)

Gene	Chromosome location	Disease association
BRIP1	17q22	Fanconi anaemia
WRN	8p12	Werner's syndrome
Artemis	10p	Severe combined immunodeficiency
DNA ligase IV	13q22-24	Ligase IV (LIG4) syndrome
Cernunnos (XLF)	2q35	Severe combined immunodeficiency
BRCA1	17q21	Hereditary breast cancer
BRCA2(FANCD1)	13q12	Hereditary breast cancer
Mre 1	11q21	Ataxia telangiectasia-like disorder
PALB2	16p12	Fanconi anaemia
Nbs1	8q21	Nijmegen breakage syndrome
ATM	11q22	Ataxiatelangietasia
ATR	3q22	Seckel syndrome
FANCD2	15q	Fanconi anaemia
BLM	15q26	Bloom's syndrome

Conclusion and Perspectives

Small RNAs including miRNAs and siRNAs have opened a novel way for genetic researches as well as an application to various biotechnology including medical therapeutics. Table 2 summarizes the role of double strand repair genes in genetic disease. Different classes of small RNAs can mediate DNA repair, chromatin modification and other mechanism of DNA stabilization. In conclusion, small RNAs produced from the sequences flanking a DSB may play an important role for DSB pair. The abundance of small RNAs suggests that additional novel classes and subclasses of small RNAs might be continuously discovered. Development of miRNA chips and next gene sequencing technology also give

unprecedented chances to find these small RNAs. It is plausible that more discoveries of small RNAs can be much helpful for understanding molecular mechanisms including DNA repair.

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