Functions and Regulation of Artemis: A Goddess in the Maintenance of Genome Integrity

Aya KUROSAWA1* and Noritaka ADACHI1,2†

Artemis/DNA double-strand break/Non-homologous end-joining.

Artemis is a structure-specific endonuclease when associated with and phosphorylated by DNA-dependent protein kinase catalytic subunit. This structure-specific endonuclease is responsible for the resolution of hairpin coding ends in V(D)J recombination. In DNA double-strand break repair, Artemis is implicated in the end-processing step of the non-homologous end-joining (NHEJ) pathway. Recently, we have demonstrated that the involvement of Artemis in NHEJ depends on the type of DNA damage. Interestingly, recent evidence suggests that the end-processing activity is not the only function of Artemis. Indeed, Artemis is rapidly phosphorylated by ataxia telangiectasia mutated in response to DNA damage, and such phosphorylation of Artemis appears to be involved in the regulation of cell cycle checkpoints. These findings suggest that Artemis is a multifunctional protein participating in the maintenance of genome integrity at two distinct levels; one at the end processing step of NHEJ, and the other at the signaling pathway of cell cycle regulation. Therefore, understanding Artemis function may give us profound insights into the DNA repair network. In this review, we summarize the functions and regulation of Artemis.

DNA DOUBLE-STRAND BREAK REPAIR

DNA double-strand breaks (DSBs), which can be caused by a variety of exogenous and endogenous agents, pose a major threat to genome integrity and may cause cell death if left unrepaired.1,2 Eukaryotic cells have evolved two major pathways for repairing DSBs: homologous recombination (HR) and non-homologous DNA end-joining (NHEJ).2–5 DNA repair by the HR pathway is restricted to the late S and G2 phases of the cell cycle, while the NHEJ pathway is not restricted to a particular phase in the cell cycle and hence DSBs can be repaired via NHEJ throughout the cell cycle.5 Consistent with the roles of the HR and NHEJ pathways for DSB repair, cells deficient in HR or NHEJ proteins show increased sensitivity to DSB-causing agents.6–13 In higher eukaryotes, DSBs are mainly repaired by NHEJ,14 and thus NHEJ-deficient cells are in general more sensitive to ionizing radiation (IR) than are HR-deficient cells. Instead, as HR plays a major role at the replication fork, HR-deficient cells exhibit increased sensitivity to replication-associated, one-ended DSBs that arise from replication fork collapse. It should also be noted that NHEJ is also important for V(D)J recombination, which generates the diversity of antibody and T cell receptor molecules.14 The NHEJ reaction can be divided into three steps; (1) end binding, (2) end processing, and (3) ligation. In the end-binding step, the Ku complex (the heterodimer of Ku70 and Ku80) immediately binds to DSB ends. After the Ku complex binding to DSB ends, DNA-dependent protein kinase catalytic subunit (DNA-PKcs) is recruited to DSB ends. The Ku/DNA-PKcs complex is thought to participate in end bridging during NHEJ to protect the ends from nucleases as well as to facilitate end processing reactions.15,16 The end processing step is particularly important when DSBs contain unligatable ends, such as incompatible ends and chemically modified ends, because all DNA ligases, including DNA ligase IV, catalyze the formation of a phosphodiester bond between 5'-phosphate and 3'-hydroxyl termini.17 The end processing step relies on several enzymes, including nuclease and polynucleotide kinase, to generate DNA ends suitable for ligation reaction.18,19 Finally, the ligatable ends are rejoined by DNA ligase IV. Although higher eukaryotes have three different genes that code for DNA ligase (LIG1, LIG3, and LIG4), the LIG4 gene product DNA ligase IV is the only DNA ligase that can ligate DSB ends in the NHEJ reaction, and other
DNA ligases cannot substitute for the DNA ligase IV function.\(^7\)

**ARTEMIS** (named after the Hellenic goddess of the hunt who aids in childbirth) was identified as the gene responsible for radiosensitive-severe combined immunodeficiency (RS-SCID) or Athabascan SCID (SCIDA).\(^{20-23}\) *In vitro* and *in vivo* studies have revealed that Artemis is the nuclease required for the resolution of hairpin coding ends during V(D)J recombination.\(^{24,25}\) As mentioned above, Artemis-deficient cells display increased IR sensitivity, suggesting that Artemis is also required for the NHEJ pathway of DSB repair.\(^{20}\) Interestingly, recent work suggests that Artemis may be involved in the regulation of the cell cycle checkpoints as a downstream factor of ataxia telangiectasia mutated (ATM) and/or the ATM- and Rad3-related kinase (ATR).\(^{26-30}\) It is therefore possible that Artemis is a multifunctional protein in the maintenance of genome integrity and an important protein for understanding the mechanism of DSB repair. In this review, we summarize the recent progress on biological functions of Artemis in DSB repair and DNA damage response.

**BIOCHEMICAL AND STRUCTURAL PROPERTIES OF ARTEMIS**

Artemis has a ssDNA-specific 5' to 3' exonuclease activity and acquires an endonuclease activity when associated with and phosphorylated by DNA-PKcs.\(^{25}\) The Artemis/DNA-PKcs complex specifically cleaves boundary of ssDNA and dsDNA and hairpin DNA, generating blunt or 3' overhang DNA ends (Fig. 1).\(^{25}\) Although the three-dimensional structure has not been determined yet, two domains in the N-terminus of Artemis are shown to be important for enzymatic activity.\(^{31}\) One of the domains is called the metallo-β-lactamase domain, amino acids 1–155 of human Artemis, which is commonly observed in members of the metallo-β-lactamase superfamily (Fig. 2).\(^{32}\) Another domain, amino acids 156–385 of human Artemis, is called the β-CASP domain (metallo-β-lactamases-associated CPSF ARTEMIS SNM1 PSO2). This domain is highly conserved in other metallo-β-lactamases that specifically act on nucleic acids (Fig. 2).\(^{33}\) Recently, de Villartay et al. have reported that a histidine residue within the β-CASP domain (His254) is critical for full activation of Artemis (Fig. 2).\(^{32}\) Although the precise role of His254 is currently unclear, it is suggested that His254 is involved in zinc binding.\(^{32}\) Pannicke et al. have reported that aspartic acid residue 37 and histidine residues 33, 35, 38, 115, and 319 directly coordinate two proposed sites of metal (most likely Mg\(^{2+}\)) binding (Fig. 2).\(^{33}\)

While the N-terminus of Artemis is important for an enzymatic role, the C-terminus of Artemis appears to be a region involved in the interaction with DNA-PKcs.\(^{34,35}\) Indeed, Artemis is phosphorylated by DNA-PKcs only in the C-terminal domain.\(^{35}\) Because the C-terminal domain is dispensable for hairpin opening activity in V(D)J recombination *in vivo*,\(^{35,36}\) the phosphorylation of the C-terminus may cause a conformational change, resulting in an activated form of Artemis.\(^{35}\) On the other hand, Goodarzi et al. reported that autophosphorylated DNA-PKcs recruits Artemis to the sites of DSBs.\(^{37}\) As autophosphorylation of

---

**Fig. 1.** Endonucleolytic properties of the Artemis/DNA-PKcs complex. Shown are schematic structures of a hairpin end and DNA ends with a 3'- or 5'-overhang. Arrows mark the major cleavage sites.

**Fig. 2.** Schematic representation of human Artemis. Human Artemis consists of 692 amino acids. The metallo-β-lactamase/β-CASP domain contains the active site of the enzyme. The C-terminus of this protein is thought to be a regulatory domain. The positions of amino acid residues directly involved in metal ion-binding (Asp37, His33, His35, His38, His115, and His319), playing a key role in the Artemis activity (His254), and phosphorylated in response to DNA damage (Ser516 and Ser645) are indicated.
DNA-PKcs is also suggested to cause a conformational change in DNA-PKcs, the conformational change of DNA-PKcs rather than Artemis itself may be important for Artemis activity. Taken together, Artemis protein is divided into two functional domains, the N-terminal catalytic domain and the C-terminal regulatory domain, and the regulation of endonuclease activity of Artemis may contribute to preventing unnecessary DNA degradation (Fig. 3). 35)

ARTEMIS AND ITS RELATED PROTEINS

Artemis is a member of the SNM1 family, which is constituted by gene products homologous to yeast SNM1, and thus Artemis is often referred to as SNM1C. Yeast SNM1 possesses a 5’ to 3’ exonuclease activity, depending on its catalytic domain. 38) Genetic analysis has shown that SNM1 participates in the repair of interstrand cross-links (ICLs) and that exonuclease activity of SNM1 is important for the ICL repair. 38) Interestingly, however, Artemis exonuclease activity is independent of its catalytic domain, 33) implying that exonuclease activities of Artemis and SNM1 are functionally unrelated. Consistent with this, fibroblast cell lines established from RS-SCID/SCIDA patients do not exhibit increased sensitivity to ICL-causing agents, though these cells are hypersensitive to IR. 39,40) Similarly, in chicken DT40 cells, SNM1 is involved in ICL repair and not in DSB repair, while Smn1c-deficient cells are sensitive to IR but not to the ICL-causing agent cisplatin. 41) Recently, we performed targeted disruption of the ARTEMIS gene in the human pre-B cell line Nalm-6, and found that ARTEMIS−/− cells showed increased sensitivity to low-dose IR (Fig. 4), but not to cisplatin. 42) These findings are consistent with the notion that Artemis is not involved in ICL repair, but is involved in DSB repair by the NHEJ pathway. Indeed, human ARTEMIS−/− cells displayed increased sensitivity to etoposide, a potent topoisomerase II inhibitor that causes DSBs. Importantly, however, the extent of etoposide sensitivity of Artemis-deficient cells was much smaller than that of cells lacking DNA ligase IV, 42,43) suggesting a limited role for Artemis in DSB repair by NHEJ. Intriguingly, Riballo et al. have reported that Artemis is only required for the repair of a subset (15%) of IR-induced DSBs, specifically those repaired with slow kinetics and those located at regions of heterochromatin. 27,44) These findings provide important clues to Artemis function, as it is suggested that the Artemis-dependent DSB repair is ATM dependent (see below).

Another member of the SNM1 family, SNM1B, also possesses a 5’ to 3’ exonuclease activity. 45,46) SNM1B is referred to as Apollo, as SNM1B is closely related to Artemis; Apollo is the twin brother of Artemis in Hellenic mythology. 45,46) Despite structural similarities between Apollo and Artemis, it has been reported that Smn1b-deficient DT40 cells, unlike Smn1c-deficient cells, display increased sensitivity to ICL-generating agents, but not to IR, suggesting that Apollo is involved in ICL repair and not in DSB repair. 41) It is shown in HEK293 cells that shRNA-mediated knockdown of Apollo results in increased sensitivity to ICL-generating agents. 47) Interestingly, however, exonuclease activity of Apollo appears to be dispensable for repairing ICLs. Since
Apollo interacts with the human telomeric protein TRF2 via its N-terminal domain, Apollo may act to protect telomeres from unwanted DNA repair.\textsuperscript{45,46} Thus, unlike Artemis, SNM1 and Apollo are likely to be mainly involved in ICL repair.

**BIOCHEMICAL ROLE OF ARTEMIS IN NHEJ**

As mentioned above, genetic analysis indicates the involvement of Artemis in NHEJ.\textsuperscript{41,42} In vitro studies showed that the Artemis/DNA-PKcs complex can generate either blunt ends or 3’ overhangs of 2–4 bases.\textsuperscript{25} Therefore, Artemis is believed to be involved in the end processing step of NHEJ. As DNA ligase IV can ligate incompatible DNA ends with 3’ overhangs in the presence of Ku,\textsuperscript{43} Artemis may have a role in NHEJ only when end trimming is necessary prior to ligation. For example, the Artemis/DNA-PKcs complex may remove chemically modified termini. Consistent with this idea, biochemical analysis revealed that the Artemis/DNA-PKcs complex can convert such chemically modified ends to a form suitable for ligation with minimal loss of terminal sequence.\textsuperscript{49,50} Such chemically modified DSBs are often induced by IR and other radiomimetic agents.\textsuperscript{51} It is known that IR and other free radicals induce DSBs with either 3'-phosphate or 3'-phosphoglycolate termini,\textsuperscript{52–55} while radiomimetic enediyne antibiotics, such as neocarzinostatin, induce 5'-aldehyde termini.\textsuperscript{54,55} Thus, the end processing step mediated by an endonuclease activity of the Artemis/DNA-PKcs complex may be required for removing chemically modified termini. Recently, Ma et al. have constructed a biochemically defined system for mammalian NHEJ, and carefully examined the joining of incompatible DNA ends.\textsuperscript{56} In that system, the core NHEJ components, Ku70, Ku80, Artemis, DNA-PKcs, DNA ligase IV, and XRCC4, were able to join incompatible DNA ends.\textsuperscript{56} Interestingly, the length of the reaction products was affected by the presence of Artemis/DNA-PKcs, consistent with a nucleolytic role of this complex in end processing. Furthermore, recent studies have suggested that the C-terminus of Ku80 is important not only for repairing IR-induced DNA damage but also for Artemis-mediated processing of DNA ends.\textsuperscript{30} Although it is possible that other nucleases also participate in the processing reaction,\textsuperscript{57–59} the Artemis/DNA-PKcs complex is likely to be a central enzyme for the end processing step of NHEJ in higher eukaryotes.

**INVOLVEMENT OF ARTEMIS IN DNA DAMAGE RESPONSE**

It has been shown that ATM and ATR as well as DNA-PKcs phosphorylate Artemis in response to DNA damage,\textsuperscript{26–30,60} and the phosphorylated Artemis physically associates with the Mre11/Rad50/Nijmegen breakage syndrome 1 (Nbs1) complex in an ATM-dependent manner.\textsuperscript{28,29} Remarkably, Chen et al. revealed that serine residue 645 (Ser645) of Artemis is phosphorylated in response to IR irradiation (Fig. 2).\textsuperscript{29} Several groups have investigated the relationship between Artemis phosphorylation and cell cycle progression. Jeggo and coworkers reported that Artemis-deficient cells had normal G2/M checkpoint and thus exhibited a prolonged G2/M arrest after IR irradiation; by contrast, Leger and coworkers presented data showing that Artemis was required for normal G2/M arrest after IR irradiation.\textsuperscript{28,60,61} A possible explanation for this discrepancy could be that those studies employed different cell lines (primary fibroblasts derived from SCIDA patients versus transformed 293 kidney cells depleted for Artemis). Alternatively, the discrepancy may simply be due to the difference in cell cycle phases when cells were irradiated in those studies (asynchronous cells versus S phase-enriched cells).

The Legerski group also showed that Artemis is phospho-

---

**Fig. 5.** The Artemis/DNA-PKcs complex is required for generating ligatable DNA ends. IR and radiomimetic agents induce DSBs with unligatable ends, such as 3’ phosphoglycolate termini. After the Ku70/Ku80 complex immediately binds to DSB ends, the Artemis/DNA-PKcs complex generates ligatable DNA ends with minimal loss of nucleotides. Finally, the DNA ligase IV/XRCC4/XLF complex ligates the DSB ends. When NHEJ proteins leave the ligated DNA, NHEJ is completed.
rylated at Ser516 and Ser645 by ATR in response to UV light (Fig. 2), and this phosphorylation is involved in the recovery from S-phase arrest. Intriguingly, neither Chk1 nor Chk2 is involved in IR- and UV-induced hyperphosphorylation of Artemis. These observations suggest that Artemis is a direct downstream factor of the ATM (and presumably ATR) signaling pathway. Consistent with this notion, epistasis analysis using human fibroblasts derived from ataxia telangiectasia patients and from Artemis-deficient RS-SCID patients showed that ATM and Artemis function in a common DSB repair pathway. Additionally, this pathway requires H2AX, 53BP1 and DNA-PKcs, as well as Mre11 and Nbs1. Therefore, Ser645 phosphorylation may trigger a conformational change in Artemis that enables its physical interaction with the Mre11/Rad50/Nbs1 complex. A genetic interaction between 53BP1 and Artemis has been shown in mammalian cells, consistent with the observation that Artemis physically interacts with 53BP1, though in chicken DT40 cells 53BP1 reported to play a role in a pathway distinct from the Artemis-dependent ATM pathway.

In this review, we have described the biochemical properties of Artemis and its biological functions in light of the maintenance of genome integrity. It is conceivable that Artemis acts at two distinct levels; one at the end processing step of NHEJ and the other at the signaling pathway of cell cycle progression. In either case, such Artemis functions are likely to be regulated through phosphorylation. Since Artemis does not exist in yeast, it is reasonable to speculate that Artemis contributes to efficient DSB repair in higher eukaryotes. For instance, the Artemis/DNA-PKcs complex may serve to create DNA ends that are preferentially rejoined by DNA ligase IV, resulting in prompt DSB repair with minimal loss of nucleotides. Further analysis of Artemis function will provide detailed information about the mechanism that ensures the integrity of the genome in higher eukaryotes.

ACKNOWLEDGEMENTS

We thank the Editor-In-Chief of the Journal of Radiation Research, Dr. Yoshiya Furusawa, for giving us the opportunity to write this review article.

REFERENCES


