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Brief report

# The Artemis:DNA-PKcs endonuclease cleaves DNA loops, flaps, and gaps

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**Abstract**

In eukaryotic cells, nonhomologous DNA end joining (NHEJ) is a major pathway for repair of double-strand DNA breaks (DSBs). Artemis and the 469 kDa DNA-dependent protein kinase (DNA-PKcs) together form a key nuclease for NHEJ in vertebrate organisms. The structure-specific endonucleolytic activity of Artemis is activated by binding to and phosphorylation by DNA-PKcs. We tested various DNA structures in order to understand the range of structural features that are recognized by the Artemis:DNA-PKcs complex. We find that all tested substrates that contain single-to-double-strand transitions can be cleaved by the Artemis:DNA-PKcs complex near the transition region. The cleaved substrates include heterologous loops, stem-loops, flaps, and gapped substrates. Such versatile activity on single-/double-strand transition regions is important in understanding how reconstituted NHEJ systems that lack DNA polymerases can join incompatible DNA ends and yet preserve 3′ overhangs. Additionally, the flexibility of the Artemis:DNA-PKcs nuclease may be important in removing secondary structures that hinder processing of DNA ends during NHEJ.


*Keywords:* Ionizing radiation; Nonhomologous DNA end joining; Double-strand break repair; DNA repair; DNA recombination; V(D)J recombination

## 1. Introduction

Nucleases can be grouped as exo- or endonucleases. Exonucleases are typically sequence-independent. Endonucleases can be further divided into those that are sequence-dependent, such as restriction enzymes, and those that are structure-specific. Among eukaryotes, the FEN-1 family was the first group of structure-specific endonucleases to be characterized and cloned [1–3]. FEN-1 stands for flap endonuclease 1, and it cleaves 5′ flap structures, which are thought to arise during the Okazaki fragment processing of lagging strand replicative synthesis [3,4]. XP-G has substantial homology to FEN-1, and XP-G is one of the two endonucleases required in nucleotide excision repair [5,6].

For most of the characterized structure-specific endonucleases, a region of double- to single-strand transition is important. FEN-1 and XP-G illustrate this, cleaving at the junction of the double-stranded region and the point where a single-stranded flap or overhang begins. In addition, structure-specific endonucleases are sensitive to the precise substrate structural features around the cleavage site. FEN-1, for example, is sensitive to the strand upstream of the 5′ flap [7,8]. FEN-1 cleavage is optimal when the strand upstream of the flap strand is directly adjacent to it (such that there is no gap). As this upstream strand is shortened to create a gap of 1–5 nt, FEN-1 recognition of the 5′ flap decreases markedly [7].

In vertebrates, the Artemis protein forms a complex with the DNA-dependent protein kinase, DNA-PKcs [9].

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DNA-PKcs is a serine/threonine protein kinase that phosphorylates Artemis, thereby activating it for endonucleolytic activity [9]. The Artemis:DNA-PKcs complex cleaves 5′ and 3′ overhangs, and it also nicks DNA hairpins near the tip [9]. The hairpin nicking is important in V(D)J recombination because without either of these nuclease components, the antigen receptor gene rearrangement process is arrested, resulting in severe combined immune deficiency (SCID) [10,11]. The 5′ and 3′ overhang cleavage is important in nonhomologous DNA end joining (NHEJ) for preparing double-strand DNA breaks for ligation by XRCC4:DNA ligase IV [12–16].

The precise manner in which the Artemis:DNA-PKcs complex cleaves its substrates is interesting. Hairpins are nicked mostly at the +2 position, in a system of numbering where the tip is defined as the zero position, and the plus direction is 3′ of the tip along the DNA strand [9]. Cleavage of 5′ overhangs preferentially results in a blunt configuration. Cleavage of 3′ overhangs is preferentially 4 nt into the single-stranded overhang from the double-/single-strand transition. These three endonucleolytic activities are similar in that all involve recognition of a double-/single-strand transition and all preferential cleavage sites are located immediately 3′ of a stretch of 4 nt of single-strandedness adjacent to the double-stranded region. Though the Artemis:DNA-PKcs complex has preferential cleavage sites, in all cases there are many sites of cleavage distributed on both sides of the preferred site [9]. This reflects the structural flexibility of this nuclease complex in processing its substrates.

We previously had reported testing of only single-stranded DNA, blunt duplex DNA, 5′ overhangs, 3′ overhangs, and DNA hairpins for action by Artemis [9]. Here we report the analysis of Artemis:DNA-PKcs action on a wider range of DNA structures including 3′ flaps, pseudo-Y structures, Y structures, stem-loop structures, symmetrical bubbles, heterologous loops, gapped DNA and nicked DNA. This analysis not only refines our view of the substrate range of the Artemis:DNA-PKcs complex as a structure-specific endonuclease, but also provides a much fuller insight into how Artemis:DNA-PKcs can contribute to junctional processing.

## 2. Materials and methods

### 2.1. Oligonucleotides

The sequences of the oligonucleotides that form the Artemis substrates are as follows. Flap structure: YM-220 (5′-CGATACTGAGCGTCACGGACTCTGCCTCAA-GACGGTAGTCAACGTGTTACAGACTTGATG-3′), YM-221 (5′-GATGTCA-AGCAGTCCTAACTTTGAGGCAGAGT-CCGTGACGCTCAGTATCG-3′), and YM-222 (5′-CATC-AAGTCTGTAACACGTTGACTACCGTC-3′). Pseudo-Y structure: YM-220 and YM-221. Y structure: YM-99 (5′-GTACGTAACCTGACTGCTATC-GACTGGACTTGA-TGCCGTC-3′), YM-100 (5′-GACGGCATCAAGTCCAG-TCGCAGATGGCGTATAGCAGTTA-3′), and YM-101 (5′-TAACTGCTATAC-GCCATCTGATAGCAGTCAGGTTA-CGTAC-3′). Stem-loop structure: YM-180 (5′-GCTGA-CTGAGTCCTACAGAAGGATCTTTTTTTTTTGATCCTT-CTGTAGGACTCAGTC AG-3′). Symmetrical bubble: YM-231 (5′-AGGCTGTGTTAAGTATCTGG-TTTTTTTTTTG-CTCGCCCTCAGGTCGACAA-3′) and YM-21 (5′-TTG-TCGACCT-GAGGGCGAGCCCGATGAATTCCAGATAC-TTAACACAGCCT-3′). Heterologous loop: YM-231 and YM-232 (5′-TTGTCGACCTGAGGGCGAGCCCAGATA-CTTAACACAG-CCT-3′). Gap: YM-49 (5′-CCTCTGAGG-GCGAGCCCGATTTTTTCCGCTT-GACCCAAGTAAGA-TTTTTTGCAGATACTTAACACAGCCT-3′), YM-233 (5′-AGCGGAAAAAATCGGGCTCGCCCTCAGAGG-3′), and YM-236 (5′-AGGCTGTGTTAAGTATCTGCAAAAAATC-TT-3′). Nick: YM-220, YM-235 (5′-TTGAGGCAGAG-TCCGTGACGCTCAGTATCG-3′), and YM-222.

The sequences of the oligonucleotides used as markers are as follows. YM-223: 5′-GATGTCAAGCAGTCC-3′; YM-224: 5′-GATGTCAAGCAGTCCTAACT-3′; YM-181: 5′-GCTGACTGAGTCCTACAGAAGGATCTTTTTT-TTTT-3′.

### 2.2. Protein purification

Artemis-myc-His (immunobeads) and DNA-PKcs were purified as described [9].

### 2.3. In vitro nuclease assay

One of the oligonucleotides in each substrate was labeled with T4 polynucleotide kinase (PNK) and [γ-$^{32}$P]ATP, and

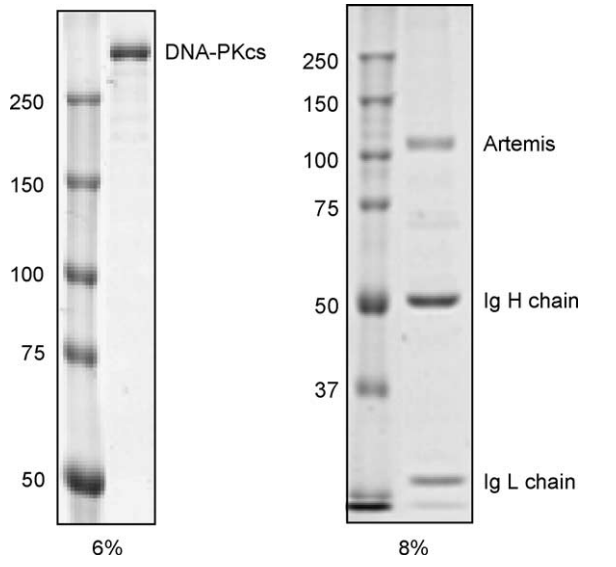


Fig. 1. Coomassie blue staining of purified proteins. Proteins were fractionated by SDS-PAGE (gel percentages for the resolving phase are noted at the bottom). The gels were stained with Coomassie Blue R250. Protein names are indicated on the right and the sizes of the protein standards (in kDa) are labeled on the left of each panel. The immunoglobulin (Ig) heavy (H) and light (L) chains in the Artemis preparation are also noted.