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The multifunctional *SNM1* gene family: not just nucleases

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Abstract

The archetypical member of the *SNM1* gene family was discovered 30 years ago in the budding yeast *Saccharomyces cerevisiae*. This small but ubiquitous gene family is characterized by metallo-β-lactamase and β-CASP domains, which together have been demonstrated to comprise a nuclease activity. Three mammalian members of this family, *SNM1A*, *SNM1B*/Apollo and Artemis, have been demonstrated to play surprisingly divergent roles in cellular metabolism. These pathways include variable (diversity) joining recombination, nonhomologous end-joining of double-strand breaks, DNA damage and mitotic cell cycle checkpoints, telomere maintenance and protein ubiquitination. Not all of these functions are consistent with a model in which these proteins act only as nucleases, and indicate that the *SNM1* gene family encodes multifunctional products that can act in diverse biochemical pathways. In this article we discuss the various functions of *SNM1A*, *SNM1B*/Apollo and Artemis.

Keywords

Apollo; Artemis; cell cycle; phosphorylation; SNM1; telomere

Approximately 30 years ago, independent genetic screens conducted in *Saccharomyces cerevisiae* for hypersensitivity to the DNA interstrand cross-linking (ICL) agents nitrogen mustard and psoralen identified the founding member of a small but ubiquitous gene family referred to as sensitivity to nitrogen mustard (*SNM1*) or sensitivity to psoralen (*PSO2*) [1–7]. An intriguing aspect of the yeast *SNM1/PSO2* mutants is that they were singularly hypersensitive to bi- and polyfunctional alkylating compounds, while exhibiting an essentially wild-type level of sensitivity to monofunctional alkylating agents, ionizing radiation (IR) or UV. This unique phenotype led a number of laboratories to search for mammalian homologs of *SNM1/PSO2*, and five such genes were identified, including *SNM1A*, *SNM1B/*Apollo, Artemis, *CPSF73* and *ELAC2*. Both *CPSF73* and *ELAC2* have roles in RNA processing [8–10], while the other three members have various roles in DNA metabolism and cellular stress responses. It is the functions of *SNM1A*, *SNM1B/*Apollo and Artemis that are the subject of this article.

Molecular cloning of the yeast *SNM1/PSO2* gene and its mammalian homologs demonstrated that the common features of this family are a metallo-β-lactamase (MBL) fold and an appended

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β-*CPSF*–Artemis–*SNM1*–*PSO2* (CASP) domain [11,12], which together are referred to as the *SNM1* domain. The β-CASP domain is unique to the *SNM1* gene family, and is predicted to be a nucleic acid binding domain, which, together with the MBL hydrolase has been demonstrated to possess a nuclease activity in the SNM1 proteins [13–21]. More detailed discussions of the structure of the SNM1 domain and its function as a nuclease can be found in recent reviews [5,22]. Outside of the *SNM1* domain the sequence of each of the proteins is distinct (Figure 1), suggesting that each of the mammalian homologs may have a unique function. The yeast *SNM1/PSO2* gene is, under normal conditions, poorly transcribed, however, it can be induced approximately fourfold upon treatment with agents that induce ICLs, but not by monofunctional alkylating agents or the UV mimetic agent 4-nitroquinoline 1-oxide [23]. Interestingly, induction of *SNM1/PSO2* is dependent upon the *DUN1* gene, which is known to be required for transcription of genes induced by yeast cell cycle checkpoint pathways activated by DNA damage or replication blocks [24]. Molecular analysis of *SNM1* mutants treated with either psoralen or nitrogen mustard demonstrated that incision near crosslinks, and also possibly excision, proceeded normally in these mutants. However, a late step in crosslink repair that was required for restoration of high molecular weight DNA after the initial repair processes appeared to be defective [25–28]. When replication forks encounter a barrier such as an ICL, a common observation is that the fork will collapse resulting in the formation of a one-sided double strand break (DSB) [29]. It is apparently the resolution of this unique substrate that contains a DSB and a closely associated ICL that is defective in *SNM1/PSO2* mutants. Repair of DSBs created by IR or that occur in mating type switching are not defective in *SNM1/PSO2* mutants.

Interestingly, an epistasis analysis has demonstrated that there are only three extant pathways of ICL repair in *S. cerevisiae*. These pathways are defined by *RAD52, REV3* and *SNM1/PSO2*, indicating that they represent homologous recombination, translesion bypass synthesis and an unknown mechanism presumably involved in restoring ICL-induced DSBs [25,30]. Break-induced replication is a well-recognized pathway in *S. cerevisiae* known to repair one-sided DSBs via homologous recombination. In fact, there are two demonstrated subpathways of yeast break-induced replication, one dependent on *RAD51* and one independent of *RAD51* [31–33]. It is possible that *SNM1/PSO2* participates in the latter of these pathways, which might account for the results of the epistasis analysis. Snm1/Pso2 has also been demonstrated to have an overlapping role with the 5′–3′ mismatch repair exonuclease Exo1 during processing of collapsed replication forks via homologous recombination [27]. It was also demonstrated that Snm1/Pso2 has a separate role in G1 phase repair of ICLs that requires the nucleotide excision repair pathway, but not homologous recombination. Thus, Snm1/Pso2 appears to play a role in both homology-dependent and homology-independent pathways of ICL repair in *S. cerevisiae*, although its precise function in either of these pathways has remained elusive.

SNM1A

The first reported study of mammalian *SNM1A* was by Dronkert *et al.* who disrupted this gene in mice and demonstrated that homozygous −/− mice developed normally, but exhibited an increased mortality compared with wild-type littermates after injection of the crosslinking agent mitomycin C (MMC) [34]. Mouse embryonic stem cells that were homozygous −/− for *SNM1A* exhibited a twofold sensitivity to this drug compared with wild-type cells, but an increased sensitivity to other ICL agents or to IR was not observed. Two other knockouts of *SNM1A* in mice have also been reported. In a study conducted by our laboratory we observed a very similar phenotype in terms of normal development and sensitivity to DNA damaging agents as reported by Dronkert *et al.* [34]. However, we also observed a decreased lifespan, owing primarily to accelerated tumorigenesis, primarily lymphomas, adenomas and a curious propensity for bacterial glandular infections, particularly in male animals [35]. A cross of the *SNM1* mutant mice with *Trp53* nullizygous animals caused a decrease in survivability owing

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to tumorigenesis compared with that observed in either single mutant. Taken together, these findings suggested that *SNM1A* acts as a tumor suppressor. Along these lines, polymorphisms in human *SNM1A* have been associated with small-cell lung carcinoma risk [36]. However, a third reported knockout of *SNM1A* indicated a normal lifespan with no observed accelerated tumorigenesis [37]. These conflicting results in tumor incidence may have been owing to strain differences, or in the nature of the disruption in the *SNM1A* gene. Interestingly, a cross with *FancD2* null animals caused a perinatal lethality suggesting that *SNM1A* and *FANCD2* function in parallel pathways that are essential for normal development in the other's absence [37]. Finally, while these studies indicated that *SNM1A* nullizygous mice exhibited only a minor sensitivity to MMC, homozygous deletion of *SNM1A* in chicken DT40 cells demonstrated that the mutants were sensitive to both MMC and cisplatin [38,39]. In addition, of the three mammalian *SNM1* genes involved in cellular stress responses, only *SNM1A* was able to rescue the cellular hypersensitivity of the yeast *SNM1* mutant to crosslinking agents [17]. In total, these studies clearly suggest that *SNM1A* has a role in mediating resistance to ICLs, although this role may be masked to some extent in mammalian cells by a possible partially redundant function with *SNM1B*/Apollo, as was observed in chicken DT40 cells [38].

Cellular studies of SNM1A have demonstrated that it is a poorly expressed nuclear protein (it possesses a nuclear localization signal) that in unstressed cells is largely confined to one or two regions or bodies (SNM1 bodies) in the mammalian nucleus [40]. SNM1 bodies vary somewhat in size but are typically approximately 2 µm in diameter and irregular in shape. These bodies are regulated as a function of the cell cycle, appear largely in the G1 phase, and are observed far less frequently in S and G2 phases. They do not appear to coincide with other known structures, such as promyelocytic leukemia protein or Cajal bodies. SNM1 bodies may be sites of sequestration of SNM1A to protect cells from the inappropriate activity of the protein since, as discussed below, overexpression of SNM1 is highly toxic to mammalian cells. Interestingly, 53BP1 is also localized to these bodies in unstressed cells, and co-immuno-precipitation (co-IP) experiments demonstrated that SNM1A and 53BP1 interact *in vivo*. Upon exposure to DNA damaging agents such as IR or crosslinking agents, both SNM1A and 53BP1 relocate to the sites of DNA damage, where they colocalize in foci with other DNA damage response (DDR) proteins such as MRE11 and BRCA1. Interestingly, there is little colocalization between SNM1A and the homologous recombination protein RAD51 after DNA damage, suggesting that these proteins function in distinct pathways as suggested by findings in budding yeast described above. In a yeast two-hybrid screen, SNM1A was demonstrated to interact with the sumo E3 ligase, PIAS1, and the proteins exhibited colocalization in IR-induced foci (IRIF) [38]. Recently, PIAS1 and PIAS4 have been demonstrated to sumoylate BRCA1 and 53PB1, respectively, at sites of DNA damage, and to be required for efficient repair of DSBs [41,42]. These findings suggest the possibilities that SNM1A is either a substrate of the PIAS1 ligase or possibly facilitates the sumoylation of BRCA1 and 53BP1.

Overexpression of SNM1A results in the formation of numerous nuclear foci in untreated mammalian cells. However, these foci are unlikely to be sites of DNA damage since they do not stain positive for 53BP1 [40], although, they do exhibit colocalization of PIAS1 [38]. Rather, these foci may be sites of sequestration of SNM1A akin to the function of the SNM1 bodies. Even low levels of overexpression of SNM1A are toxic to mammalian cells, and lead to a potent G1 phase cell cycle arrest followed by apoptosis [40]. This phenomena has greatly limited studies of SNM1A in regard to its role in the cell cycle, and the effects of mutations on its functions *in vivo*. Interestingly, over expression of human SNM1A is apparently not toxic to yeast cells [17].

Although endogenous SNM1A readily locates to sites of DSBs, its function at these sites remains unclear. Disruption of *SNM1A* in mouse cells indicated that it has little or no role in mitigating sensitivity to IR, suggesting that it is either not involved in the repair of DSBs, or

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that it plays a redundant role in this process, possibly with *SNM1B/*Apollo. Localization of SNM1A to IRIF is dependent upon the checkpoint kinase ATM, and this kinase can phosphorylate SNM1A *in vitro* [43]. Furthermore, loss of SNM1A results in a defective G1 checkpoint after exposure to IR. These findings suggest that SNM1A may play a role in cellular signaling in response to DNA damage, possibly through processing of damaged DNA mediated by its nuclease activity [17,18].

The human *SNM1A* gene has an unusual genomic structure with a long 5′ untranslated region that contains numerous open reading frames, and which is predicted to fold into a complex secondary structure. Using a reporter gene assay, this structure was demonstrated to act as an internal ribosome entry site (IRES) [44]. IRESs are structures that allow cap-independent translation of mRNAs, and many mammalian genes that contain an IRES are upregulated during mitosis [45,46]. This also proved to be true for the *SNM1A* IRES. During interphase the *SNM1A* IRES reduced expression of a green fluorescent protein reporter gene by approximately tenfold, while a two- to four-fold increase in expression was observed during mitosis. These observations led us to examine whether *SNM1A* has a function during mitosis, which resulted in the discovery that *SNM1A* participates in an early mitotic checkpoint in response to spindle poisons such as nocodazole [47]. This early mitotic checkpoint referred to variously as the prophase or antephase checkpoint was first discovered in mammalian cells through the characterization of the checkpoint with forkhead-associated (FHA) and really interesting new gene (RING) domains (*CHFR*) [48]. CHFR-deficient cells exhibit a failure to arrest the cell cycle in prophase prior to chromosome condensation upon exposure to spindle poisons, which is distinct from the more extensively studied spindle checkpoint that regulates the metaphase to anaphase transition [49–52]. Similar to *CHFR*-deficient cells, $SNM1A^{-/-}$ mouse embryonic fibroblasts (MEFs), when exposed to spindle poisons, exhibited a failure to arrest in mitosis prior to chromosome condensation, elevated levels of micronuclei formation, a decreased mitotic delay, supernumerary centrosomes and decreased viability [47]. In addition, both SNM1A and 53BP1, which as discussed above are interacting partners, were demonstrated to co-IP with components of the anaphase-promoting complex (APC)/ cyclosome, further indicating a mitotic function for SNM1A. It should be noted, however, that 53BP1-deficient cells do not exhibit a significant defect in the prophase checkpoint [Akhter *et al.*, Unpublished Data]. The exact biochemical function of SNM1A in the prophase checkpoint remains unclear. In fact, the molecular details of the prophase checkpoint pathway in general have yet to be resolved, although, CDK1-cyclin B appears to be the ultimate target. Recently, a novel poly(ADP-ribose)-binding zinc finger motif was found in a small number of eukaryotic proteins involved in the DDR and checkpoint regulation [53]. These proteins include CHFR, aprataxin PNK-like factor, and SNM1A. Mutations in the poly(ADP-ribose)-binding zinc finger domain of CHFR abrogates its function at the prophase/antephase checkpoint. The role of this domain in SNM1A function has yet to be characterized primarily owing to the problems with its overexpression discussed above. Finally, whether or not SNM1A and CHFR function in the same checkpoint pathway is unclear. The phenotypes of the respective deficient cells are highly similar, however, despite repeated attempts, researchers have not been able to demonstrate a physical association between these proteins.

In summary, SNM1A appears to function in multiple pathways in response to various forms of cellular stress such as DNA damage and spindle poisons. However, its fundamental activity in these pathways remains unresolved, particularly since its role as a nuclease does not appear to explain all its functions.

SNM1B/Apollo

Only a handful of studies have been conducted on the function of *SNM1B/*Apollo in vertebrate cells. Concannon and colleagues were the first to examine its role in the DDR in human cells

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[54]. Using siRNA-mediated knockdown, they demonstrated that cells depleted of *SNM1B*/Apollo were moderately sensitized to both ICL drugs and to IR, suggesting that *SNM1B*/Apollo may have a direct role in the repair of DSBs. Subsequently, this group also demonstrated that *SNM1B*/Apollo localized to IRIF, and that the autophosphorylation of ATM, which is required for its activation as a kinase, and phosphorylation of downstream ATM targets, was attenuated in *SNM1B/*Apollo-deficient cells [55]. Studies in DT40 chicken cells have also demonstrated that *SNM1B/*Apollo-deficient cells are hypersensitive to crosslinking drugs, such as MMC and cisplatin, but hypersensitivity to IR was not observed [38,39]. Both *SNM1A*–deficient and *SNM1B/*Apollo-deficient cells have exhibited sensitivity to ICL-inducing agents, however, analysis of a double mutant demonstrated that it was more sensitive than either single mutant, suggesting that there may be a partially redundant DNA repair function for these two genes [38,39]. Alternatively, these findings are consistent with *SNM1A* and *SNM1B/*Apollo having functions in distinct ICL response pathways.

Legerski and colleagues used shRNA technology to deplete expression of *SNM1B/*Apollo in stable clones of HEK293 cells [56]. Consistent with previous reports, these *SNM1B/*Apollo-deficient cells were hypersensitive to the cross-linking agents MMC, cisplatin and psoralen plus UV-A, however an increased sensitivity to IR or UV was not observed. It was also demonstrated that *SNM1B/*Apollo-deficient cells exhibit a defective S phase checkpoint in response to MMC, but not to IR, and this finding may account for the specific sensitivity to crosslinking compounds. Interestingly, although previous studies have largely implicated ATR as the major kinase activated in response to ICLs, we demonstrated that it is activation of the ATM-mediated checkpoint that is defective in *SNM1B/*Apollo-deficient cells. The requirement for *SNM1B*/Apollo in ATM checkpoint activation following ICL damage was correlated with its role in promoting DSB formation and thus DNA replication fork collapse. Others have demonstrated previously that when the replication fork encounters an ICL, the ATR-mediated S phase checkpoint is activated, leading to a transient arrest [57]. Over a period of hours this checkpoint is attenuated resulting in the collapse of the replication fork and the formation of a double-strand break by incision of a template strand. Current evidence indicates that the incision is performed by the MUS81-EME1 endonuclease, since cells deficient in this enzyme are defective in efficient formation of ICL-induced DSBs [29,58,59]. Most current models of ICL repair predict that formation of DSBs is an obligatory step that is required for uncoupling of the ICL prior to the ultimate repair by homologous recombination. The HEK293 *SNM1B/*Apollo-deficient cells have a similar phenotype, in that formation of DSBs is greatly reduced upon exposure to ICL-inducing agents. A possible function for SNM1B/Apollo in the formation of DSBs is to resect the lagging strand of a regressed fork in order to produce a substrate that is highly amenable to cleavage by MUS81-EME1 [60]. Consistent with this result was our finding that SNM1B/Apollo directly interacts with MUS81-EME1 via its MBL domain, suggesting that these proteins may act cooperatively to process stalled forks into DSBs [56]. These findings provide an explanation as to why no checkpoint defect was observed after IR, since SNM1B/Apollo produces DSBs in response to ICLs, which are the activating signal for the ATM-mediated checkpoint. In addition, SNM1B/Apollo interacts with the MRE11-RAD50-NBS1 (MRN) complex via its β-CASP domain and with FANCD2, suggesting that it may have additional roles in the repair processing of ICLs and/or in checkpoint signaling in response to these lesions. It should be noted that the interaction with the MRN complex was direct, while the interaction with FANCD2 was indirect and likely mediated by MRN. Also, the activation of ATR as assessed by CHK1 phosphorylation was not affected by SNM1B/Apollo depletion upon exposure to MMC, however, a recent study demonstrated that in response to UV, ATR activation is impaired in *SNM1B/*Apollo-deficient cells [61]. The reason for this discrepancy is unclear, however *SNM1B/*Apollo-deficient cells do not appear to be sensitive to UV, which would be expected if ATR-mediated checkpoint signaling was disrupted. This study also reported that SNM1B/Apollo interacts with members of the heat shock protein 70 family, and that HSP72 is required for the localization of SNM1B/Apollo to

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nuclear foci. HSP70 chaperones have been implicated in genome stability, and this interesting connection to SNM1B/Apollo might at least partially explain their involvement, since, as discussed below, SNM1B/Apollo is required for protection of telomeres.

A number of laboratories have reported that SNM1B/Apollo interacts with the telomere binding protein TRF2 and has a role in telomere maintenance [13,55,62–64]. Proper telomere length maintenance is critically important during development of multicellular organisms, since telomere length is the determining factor for the number of cell divisions a cell can undergo in the absence of telomerase. Telomeres can fold into a structure termed the t-loop, in which the 3′ single-stranded overhang invades a duplex region of the telomere to sequester the overhang [65,66]. Telomeres are protected from inappropriate activation of the DDR by a complex of proteins referred to as the shelterin complex [67]. Depletion or mutation of members of the shelterin complex renders the telomeres unprotected and can lead to chromosomal fusion and hence genomic instability. In addition to the shelterin complex, other telomere-associated proteins have also been demonstrated to be required for telomere protection. One of these accessory proteins is SNM1B/Apollo, and its depletion by siRNA experiments was demonstrated to lead to activation of the DDR and increased levels of chromosomal end-to-end fusions. SNM1B/Apollo forms nuclear foci in untreated cells, and these foci were demonstrated to coincide with telomeres and to require TRF2 for their formation. The role of SNM1B/Apollo at telomeres has yet to be resolved, however, like other members of the SNM1 family, SNM1B/Apollo possesses a 5′–3′ exonuclease activity [13]. Thus, one postulated role for SNM1B/Apollo is that it is required to resect the leading strand upon completion of DNA replication in order to create the single-stranded overhang necessary for the formation of t-loops. Recently, we generated a knockout of *SNM1B/*Apollo in mice and observed that null animals exhibited multiorgan developmental failure, which led to a perinatal mortality [Akhter *et al.*, Unpublished Data]. MEFs derived from the null embryos demonstrated an increased incidence of chromosomal fusions, which were the likely cause of the impaired organ development [68]. This conclusion is supported by the finding that a cross of the *Snm1B*/Apollo null mice with *Ku* null mice rescued the lethal phenotype, suggesting that the nonhomologous end-joining (NHEJ) pathway is responsible for the observed chromosomal end-to-end joining. Taken together, these data implicate SNM1B/Apollo as a key 5′–3′ exonuclease required for generation of 3′ single-stranded overhangs at newly replicated leading-strand telomeres to protect them from engaging the NHEJ pathway. Of the three *SNM1* mammalian homologs discussed here, only the knockout of *SNM1B/*Apollo leads to an embryonic lethal phenotype, which appears to be owing to the unique role of this protein in telomere maintenance.

As discussed earlier, we have demonstrated that *SNM1A* is a multifunctional gene involved in both the DDR and in an early mitotic or prophase checkpoint in response to spindle stress. To find interaction partners for SNM1A and SNM1B/Apollo, we conducted yeast two-hybrid screens, and among the positives found in both screens was a gene with a known mitotic function referred to as *Astrin* (also termed *DEEPEST*) [69–71]. Interestingly, an interaction between Astrin and Artemis was not observed, suggesting that it was specific to SNM1A and SNM1B/Apollo. The function of Astrin is not well understood, but it is an essential protein that localizes both to the mitotic spindle and to the centrosome and has been found to be involved in the regulation of the mitotic enzyme separase [71].

Previous findings with SNM1A and the two-hybrid results with Astrin led our laboratory to investigate whether SNM1B/Apollo might also play a role in early mitosis in response to spindle poisons. Using the SNM1B/Apollo-depleted HEK293 clones described above, we demonstrated that these cells were defective in the prophase checkpoint as indicated by a failure to arrest prior to chromosome condensation upon exposure to nocodazole [72]. Abrogation of the prophase checkpoint resulted in an extended spindle checkpoint causing a prolonged duration of mitosis. Similar to CHFR the target of the SNM1B/Apollo pathway appeared to

be CDK1-cyclin B since the inhibitory phosphorylation of CDK1 promoted by the WEE1 kinase and regulated by the CDC25 phosphatase was not maintained in the presence of nocodazole in *SNM1B/*Apollo-deficient cells in comparison with control cells. This result was also confirmed by an excessive and prolonged CDK1-cyclin B-mediated phosphorylation of the APC subunit CDC27. In addition, SNM1B/Apollo was demonstrated to interact with Astrin *in vivo* by co-IP experiments through its MBL domain, and disruption of this interaction by independent point mutations (D14N and H276A) resulted in a deficient prophase checkpoint. Interestingly, live cell imaging studies indicate that the levels of SNM1B/Apollo are rapidly and dramatically reduced at the onset of mitosis, and that the residual protein is confined to the centrosome where it colocalizes with Astrin. Consistent with this finding is the fact that the initial activation of CDK1-cyclin B is known to occur in the centrosome [73]. Furthermore, treatment with nocodazole appeared to prevent the reduction in SNM1B/Apollo levels during mitosis, and the point mutants mentioned above were found not to undergo extensive reduction in levels in untreated cells. The mitotic reduction of SNM1B/Apollo appears to be owing to degradation by the ubiquitin/proteosome system, since the inhibitor MG132 stabilizes the protein. However, the E3 ubiquitin ligase responsible has yet to be identified. Taken together, these findings indicate that SNM1B/Apollo and Astrin function together to enforce the prophase checkpoint in response to spindle stress and that the target of this checkpoint is CDK1-cyclin B. Whether SNM1B/Apollo and SNM1A function in the same prophase checkpoint pathway is unclear at present, but it is certain that these proteins do not act redundantly.

Artemis

Artemis is by far the most intensively studied member of the mammalian *SNM1* gene family, and was initially genetically characterized as defective in a form of human severe combined immunodeficiency (SCID) [12]. This syndrome is characterized by a defect in variable (diversity) joining (V[D]J) recombination resulting in a virtually complete premature arrest of both B- and T-cell maturation. In addition, patient cell lines exhibited an increase in sensitivity to IR compared with normal cells [74–76]. This form of SCID is usually lethal within the first year of life owing to protracted infections caused by opportunistic microorganisms. This SCID syndrome resembles murine SCID caused by defects in DNA-dependent protein kinase (DNA-PK), a protein complex involved in both V(D)J recombination and repair of DNA double-strand breaks via the NHEJ pathway. Disruption of Artemis in a mouse model and the characterization of these mice indicated that they exhibit a SCID with sensitivity to ionizing radiation phenotype congruent to the human syndrome [77]. In addition, MEFs derived from these mice demonstrated an elevated frequency of chromosomal abnormalities, indicating a clear role for Artemis in maintenance of genomic stability. Interestingly however, Artemis null mice did not exhibit accelerated tumorigenesis compared with wild-type littermates. Yet when Artemis/*p53* double null mice were examined, an accelerated tumorigenesis was observed compared with *p53* null mice, indicating that Artemis is a tumor suppressor in the absence of *p53* [78]. Interestingly, the observed tumorigenesis mostly affected the B-cell lineage, with the development of aggressive pre-B-cell lymphomas. Hypersensitivity to MMC was not noted in the Artemis null MEFs, in agreement with studies of human mutants [79].

Lieber and his colleagues defined the role of Artemis in V(D)J recombination by demonstrating that, when complexed with DNA-PKs it acquires an endonucleolytic activity on 5′ and 3′ overhangs and the ability to open hairpins in duplex DNA [15,16,80,81]. The latter activity is consistent with the observed defect in coding joint formation in Artemis-deficient cells, since coding joints terminate in hairpins upon processing by the RAG1/RAG2 complex. It was also demonstrated that Artemis is a substrate of the kinase activity of the DNA PK catalytic subunit (DNA-PKcs) *in vitro*, which was the first evidence suggesting that Artemis was a phosphoprotein. Artemis was also demonstrated, independent of DNA-PKcs, to possess a 5′–3′ exonucleolytic activity on single-stranded DNA. However, when an extensive point mutation

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analysis of conserved residues within the MBL and β-CASP domains of Artemis was performed, almost all the mutants were defective in the DNA-PKcs-dependent endonucleolytic activity, but none were defective in the Artemis-associated exonuclease activity [16]. This raised the specter that the observed exonucleolytic activity was owing to a contaminant; otherwise, the putative exonuclease activity would presumably have to lie outside of the conserved MBL and β-CASP domains. More recently, Artemis endonuclease activity against single-stranded DNA, which is stimulated by DNA-PKcs, has also been reported [82]. There are a number of additional reports of the characterization of the DNA-PKcs-dependent endonuclease activity of Artemis [19–21,83–85]. These will not be discussed here, as an excellent recent review has summarized the nuclease activities of the MBL protein family in depth [5]. However, it should be noted that one study has demonstrated that an Artemis mutant that is unable to interact with DNA-PKcs either *in vitro* or *in vivo* is able to rescue the V(D)J defect in Artemis-deficient cells [86]. This finding appears to conflict with the *in vitro* results, however, it is possible that overexpression of the mutant protein results in the observed complementation owing to weak interactions of Artemis with components of the V(D)J machinery. This consideration aside, these findings have defined the mechanism by which Artemis acts in V(D)J recombination and, combined with the hypersensitivity of Artemis-deficient cells to IR, led to the proposal that the protein also plays a role in the NHEJ pathway of DSB repair.

A role for Artemis in the NHEJ pathway was further strengthened by work from the Jeggo and Lobrich laboratories, which demonstrated that Artemis was required for the repair of approximately 10–15% of all DSBs caused by IR [87]. This subfraction of DSBs was proposed to be structurally complex in some way and require processing by the nuclease activity of Artemis prior to rejoining by NHEJ. In addition, this report demonstrated that Artemis was a substrate of the checkpoint/DNA repair kinase ATM and that ATM's direct role in NHEJ was mediated by Artemis. These conclusions are also supported by work from other laboratories [79,83,88]. Thus, the concept that Artemis, largely regulated by ATM and to some extent by DNA-PK, is involved in the nucleolytic processing of a subset of IR-induced DSBs ,became the dominant paradigm of Artemis function. However, subsequent papers from the Jeggo laboratory and results from our laboratory (discussed below) have raised doubts about this simple model [89,90]. In these studies it was proposed that ATM, and presumably Artemis, is required for the repair of DSBs that occur only in highly compacted heterochromatin and thus, ATM's function is in chromatin relaxation rather than directly in DNA repair processing. In support of this model it was demonstrated that depletion of the hetero chromatin binding factor KAP1 dispensed with the requirement for ATM, although whether this finding also applies to Artemis was not addressed. KAP1 has been demonstrated to be phosphorylated by ATM in response to IR, which promotes relaxation of compacted chromatin and access by the DNA repair machinery [91]. Thus, these findings refocused ATM's role in DSB repair from direct DNA processing to remodeling of highly compacted chromatin to allow access by DNA repair enzymes. With regard to Artemis, this model raises a number of issues. If, in fact, ATM is primarily required for chromatin relaxation in heterochromatin, what is the role of the Artemis nuclease function in this process, or on the other hand, why would the nuclease activity of Artemis be specifically required for DNA repair in heterochromatin. Possibly, as discussed below, ATM, and also ATR, regulate Artemis with respect to functions such as chromatin remodeling, cell cycle control and so on, while DNA-PKcs regulates Artemis specifically in the processing of complex broken ends throughout the genome.

Artemis has been demonstrated to be extensively phosphorylated both *in vitro* and *in vivo* by the three phosphatidylinositol-3-OH kinase-like (PIK) kinases ATM, DNA-PKcs and ATR depending on the type of DNA damage or cellular stress [86–88,92–98]. Initially, it was assumed that DNA-PK was the major kinase that regulated Artemis in response to IR, since this enzyme robustly phosphorylates Artemis *in vitro* and strongly co-IPs with it *in vivo* [39,

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95]. In fact, using mass spectroscopy analysis the Lieber group identified 24 sites of phosphorylation in Artemis, 21 of which were phosphorylated by DNA-PKcs *in vitro* [96]. Sites that have been confirmed to be phosphorylated *in vivo* are shown in Figure 2. However, a number of reports have indicated that, in fact, ATM is the more important regulator of Artemis *in vivo* after IR exposure [87,88,92–95]. For instance, using phospho-specific antibodies, it was demonstrated that serines S516, S534, S538 and S645, all located in the (serine/threonine)–glutamine cluster domain of Artemis, were phosphorylated in response to IR *in vivo* [93]. At low-to-moderate doses of IR (2–3 Gy) ATM was almost solely responsible for these phosphorylations, however, at a substantially higher dose (10 Gy) DNA-PKcs also contributed to these modifications. Thus, the evidence from these studies indicates that it is ATM that is the more relevant PIK kinase with regard to Artemis phosphorylation following exposure of cells to IR. Interestingly, Artemis was also demonstrated to be phosphorylated at the same four sites by the ATR kinase *in vivo* in response to other forms of DNA damage and cellular stress such as UV irradiation, MMC and hydroxyurea [88,93–95]. In fact, it was these findings that led us to the concept that Artemis has a wider role in the DDR than acting as a nuclease in the processing of DSBs during NHEJ or homologous recombination.

The role of phosphorylation in Artemis function remains undefined to a large extent, which is not surprising given the large number of potential sites identified in this protein. Current findings indicate that phosphorylations by PIK kinases, at least at serine–glutamine or serine–proline sites, are not involved in regulating the role of Artemis in V(D)J recombination or NHEJ. A construct of Artemis containing only the catalytic core (MBL plus β-CASP) is able to fully rescue the defects in these pathways *in vivo* [85,94]. This result is compatible with a model in which phosphorylation of the C-terminal region of Artemis is required to relieve negative regulation of the endonuclease activity. However, several reports have demonstrated that multisite mutation of Artemis phosphorylation sites does not affect Artemis endonuclease activity *in vitro*, or the ability of Artemis to rescue defects in V(D)J or NHEJ *in vivo* [85,86, 94,98]. Interestingly, it was demonstrated that autophosphorylation of DNA-PKcs was required for the activation of the Artemis nuclease, which suggests that a conformational change in the kinase is required to engage Artemis nucleolytic activity [20,98].

As discussed above, Artemis is phosphorylated by the checkpoint kinases ATM or ATR in response to DSBs or lesions that form replication barriers, respectively; the sites of these phosphorylations appear to be the same for each kinase. Artemis-deficient cells are not hyper sensitive to UV or MMC, suggesting that Artemis does not have a direct role in the repair of these lesions [77]. In fact, the repair of UV-induced photo-lesions by nucleotide excision repair has been reconstituted *in vitro* [99], and it is highly unlikely that there is a role for the Artemis nuclease in this pathway. These considerations led our laboratory to examine a role for Artemis in cell cycle regulation in response to DNA damage. An analysis of Artemis phosphorylation demonstrated that the S516, S534, S538 and S645 sites were all rapidly phosphorylated within minutes by either IR or UV exposure [93,95]. However, the S534 and S538 sites were rapidly dephosphorylated within 1 h, while the other two sites remained phosphorylated for at least 24 h. These results suggest that S516 and S645 may have a common function in Artemis regulation and that S534 and S538 have a separate function. This prediction was borne out by studies demonstrating that an allele of Artemis in which both the S516 and S645 residues were converted to alanine was defective in recovery from the G2/M cell cycle checkpoint [93,95]. An allele in which the serine residues were replaced with aspartic acid residues to mimic phosphorylation displayed a phenotype equivalent to that of the wild-type allele. This result indicates that phosphorylation at these sites was causative of the phenotype. The initial imposition of the G2/M checkpoint was normal, however the activation of CDK1-cyclin B in the centrosome was delayed when this Artemis alanine mutant was overexpressed. It is conceivable that this phenotype is owing to incomplete repair of DSBs, which could lead to an extended checkpoint, as has been reported for Artemis null mutants [100,101]. However,

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as mentioned above, mutation of these particular residues in Artemis is not detrimental to NHEJ, and we did not observe altered levels of phosphorylated H2AX (γH2AX) when this allele was overexpressed compared with wild-type Artemis. These findings suggested that Artemis has a role in regulating checkpoint recovery from the G2/M checkpoint by impacting the activation of CDK1-cyclin B, albeit through an unknown mechanism.

In a separate study the role of Artemis phosphorylation by ATR after UV or MMC exposure was investigated [102]. The major checkpoint in response to these agents occurs in S phase as a result of the DNA replication fork encountering lesions that block progression. Similar to the studies mentioned above, mutation of the S516 and S645 residues resulted in a delayed recovery from the S phase checkpoint, which was owing to impaired degradation of cyclin E. Cyclin E is regulated by the proteosome system via ubiquitination by the SCF^{Fbw7} E3 ligase complex [103–105]. Phosphorylation of Artemis at the S516 and S645 sites strongly enhances its binding to the F-box protein Fbw7 and thereby promotes ubiquitination of cyclin E. These findings provide an explanation for the ATR-mediated phosphorylation of Artemis and define a dramatically novel role for this protein as a component of the ubiquitin–proteosome system. In support of this finding, our unpublished studies have demonstrated that Artemis is also involved in protein ubiquitination as part of the Cul4A–DDB1 E3 ligase complex [Yan *et al.*, Unpublished Data].

As a further testament to the wide-ranging functions of Artemis, we have also demonstrated that it is a negative regulator of p53 in response to oxidative stress derived from mitochondrial respiration [106]. Oxidative stress was demonstrated to activate DNA-PKcs to phosphorylate p53 and induce its transcriptional program, such as expression of *p21*, *BAX, SESTRIN2* and *TIGAR*. These latter two p53 target genes are involved in mitigating the levels of reactive oxygen species in cells. Artemis, which interacts with both p53 and DNA-PKcs, inhibits the phosphorylation of p53 by DNA-PKcs. Markers of DNA damage such as H2AX, CHK1 or CHK2 were not activated, indicating that this pathway was not a component of the DDR, but rather a response to reactive oxygen species created during oxidative phosphorylation. These findings also suggest that Artemis is a regulator of DNA-PKcs kinase activity, although the mechanism by which Artemis inhibits the phosphorylation of p53 is unclear.

In consideration of the foregoing discussion, we propose a working model for the role of phosphorylation in Artemis function (Figure 3). In response to DNA damage or replication fork barriers, Artemis is phosphorylated at multiple sites, principally by the ATM or ATR kinases. These phosphorylations do not affect the function of Artemis with respect to its nuclease activities in NHEJ or V(D)J recombination. In fact, the entire C-terminal region of Artemis is dispensable for these functions [85]. Rather, phosphorylation regulates separate functions of Artemis in cell cycle checkpoints, chromatin remodeling and possibly other responses to cellular stress. This model suggests that Artemis is a multifunctional protein that acts as a simple nuclease in certain aspects of DNA metabolism on one hand, and as a phosphoprotein that is a regulator of cellular stress responses on the other.

Functions of the conserved SNM1 domain

As discussed earlier, there is extensive evidence that the SNM1 domain, composed of the MBL and β-CASP folds, contains a nucleolytic activity. SNM1A and SNM1B/Apollo have both been reported to possess a 5′–3′ exonuclease that acts on single-stranded DNA and Artemis, in association with DNA-PKcs, possesses an endonuclease activity [5,13–21]. Mutational analysis confirms that this nuclease activity resides within the SNM1 domain. However, surprisingly we and others have noted that the SNM1 domain also constitutes an interface for protein–protein interactions. SNM1A interacts with the SUMO ligase PIAS1 and the mitotic factor Astrin [38,72]; SNM1B/Apollo interacts with the MRN complex, the nuclease

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heterodimer MUS81-EME1, and Astrin [56,72]; and Artemis interacts with the Cul4A–DDB1 ubiquitin ligase complex [Yan *et al.*, Unpublished Data]. All of these inter actions have been mapped to the SNM1 domain of the respective proteins and indicate that this domain has biochemical functions in addition to its nuclease activities (Figure 1).

Cancer relevance

The *SNM1* gene family has a clear role in the maintenance of genomic integrity and in the suppression of tumorigenesis. All three genes have been demonstrated to have various roles in the direct repair of DNA damage and have been implicated in cell cycle checkpoint responses to different forms of cellular stress. Both Artemis and SNM1A are substrates of checkpoint kinases and both have been demonstrated to act as tumor suppressors in mouse models. The *CHFR* gene is mutated in a high proportion of tumors and cancer cell lines, indicating the importance of the prophase checkpoint in suppressing oncogenesis [48,107–113]. Both SNM1A and SNM1B/Apollo have roles in this pathway and polymorphisms in *SNM1A* have been linked to small-cell lung cancer [36]. SNM1B/Apollo plays a role in telomere protection, and a knockout of this gene in the mouse causes highly increased levels of chromosome end-to-end fusions, resulting in chromosome instability. While the null genotype results in embryonic lethality in mice, more subtle mutations could lead to cancer in humans. Artemis has been demonstrated to be a negative regulator of p53 and, along with its role in protein ubiquitination, suggest that it might, paradoxically, be a target for cancer chemotherapy. In fact, Artemis may act as both a tumor suppressor and an oncogene, since, in its absence, genomic instability will occur, while its overexpression may inhibit the activity of p53 and deregulate other cell cycle regulators.

Future perspective

The mammalian SNM1 family of proteins have proven quite surprising in the range of their identified activities and functions. Initial observations indicated that they were seemingly run-of-the-mill nucleases involved in various aspects of DNA metabolism. However, as investigations have continued, their range of functions have come to include checkpoint recovery in response to DNA damage, imposition of mitotic checkpoints in response to spindle poisons, telomere maintenance, possibly chromatin remodeling and as cofactors in the ubiquitination of other proteins. It is the functions of SNM1 proteins in these latter pathways that require investigation in the coming years. The fundamental role of SNM1A in cell cycle regulation remains unresolved, and is particularly puzzling since it is involved in pathways that respond to both DNA damage and mitotic spindle disruption. Possibly, as has been demonstrated for Artemis, SNM1A may be involved in protein ubiquitination since it interacts with the APC/cyclosome. Recently, the APC/cyclosome has also been demonstrated to be a component of the DDR in that it regulates the implementation of the G2/M cell cycle checkpoint [114]. Thus, SNM1A could participate in these various checkpoint pathways via its association with the APC/cyclosome. SNM1B/Apollo has a clearly defined role in telomere maintenance mediated through its nuclease activity, and future studies should further refine its function at telomeres. In addition, similar to SNM1A, SNM1B/Apollo has a role in mediating the prophase checkpoint in response to spindle poisons. The target of this pathway appears to be CDK1-cyclin B, however, the biochemical role of SNM1B/Apollo requires explication and should be the subject of future studies. Artemis has multiple cellular functions, but perhaps the most interesting new development is its role in protein ubiquitination. It interacts with both the Cul1 and Cul4A E3 ligase complexes, and a future direction will be to identify proteins that require Artemis for their regulation via ubiquitination and how this activity of Artemis manifests itself in cell cycle control. Finally, an analysis of the expression and mutational spectrum of *SNM1* genes in human cancers is warranted given the demonstrated role of these genes in maintenance of genomic stability and cell cycle regulation.

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The SNM1 proteins have proven to be more interesting than originally anticipated, and additional surprises should be in store as investigations continue on this intriguing protein family.

Executive summary

- The first member of the sensitivity to nitrogen mustard (*SNM1*) gene family was discovered in budding yeast and has a unique role in the repair of DNA interstrand crosslinks.
- The *SNM1* gene family is characterized by metallo-β-lactamase and CPSF–Artemis–SNM1–PSO2 domains that together possess a DNA nuclease activity in the proteins.
- There are five mammalian homologs of SNM1 and three of these; SNM1A, SNM1B/Apollo and Artemis, are nuclear proteins involved in DNA metabolism and cell cycle regulation.

SNM1A

- Knockout studies of *SNM1A* have demonstrated that it has only a minor role in mitigating sensitivity to interstrand crosslinking agents, and that it has tumor suppressor activity in a mouse model.
- SNM1A localizes to sites of double-strand breaks and has a role in mediating the G1 cell cycle checkpoint. ATM is required for this localization and SNM1A is a substrate of ATM *in vitro*.
- SNM1A interacts with the SUMO ligase PIAS1 but the functional significance of this interaction is unclear.
- Translation of *SNM1A* is regulated by an internal ribosome entry site.
- SNM1A has a role in mediating the prophase/anaphase cell cycle checkpoint in response to spindle poisons. It interacts with the anaphase-promoting complex/cyclosome but the significance of this interaction is unclear.
- The biochemical function of SNM1 in mediating cell cycle checkpoints is unknown.

SNM1B/Apollo

- SNM1B/Apollo-deficient cells generally exhibit sensitivity to interstrand crosslinking agents.
- SNM1B/Apollo has been demonstrated to participate in mediating replication fork collapse in response to interstrand crosslinks.
- SNM1B/Apollo interacts with proteins involved in DNA repair and checkpoint signaling, such as MUS81-EME1, the MRE11-RAD50-NBS1 complex and FANCD2.
- SNM1B/Apollo is required for telomere maintenance during S phase and interacts with the telomere protein TRF2.
- A knockout of SNM1B/Apollo in mice results in embryonic lethality owing to chromosome end-to-end fusions.
- SNM1B/Apollo is required for the resection of newly replicated leading strands at telomeres.

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- Similar to SNM1A, SNM1B/Apollo has a role in mediating the prophase/anaphase checkpoint in response to spindle poisons. The identified target of SNM1B/Apollo is cyclin B-CDK1, although its biochemical role in this pathway is unknown.

Artemis

- Defects in Artemis result in a severe-combined immunodeficiency syndrome owing to impaired variable (diversity) joining (V[D]J) recombination.
- The nuclease activity of Artemis is required for V(D)J recombination, the repair of a subset of DNA double-strand breaks mediated by nonhomologous end joining and for resection of ends during homologous recombination.
- Artemis is a phosphoprotein and in response to DNA damage Artemis is primarily phosphorylated by ATM and ATR at multiple sites.
- Phosphorylation of Artemis is not required for its roles in V(D)J recombination and nonhomologous end joining.
- Artemis is involved in the recovery from the G2/M cell cycle checkpoint by regulating the activation of cyclin B-CDK1 in the centrosome.
- Artemis is involved in regulating recovery from the S phase checkpoint through the ubiquitination and degradation of cyclin E via the SCF^{Fbw7} E3 ligase complex.
- Artemis interacts with both the Cul1 and Cul4A E3 ubiquitin ligase complexes.
- Artemis is a negative regulator of p53 in response to oxidative stress induced by mitochondrial respiration.
- Artemis is a multifunctional protein whose nuclease activity is regulated by DNA-dependent protein kinase catalytic subunits during V(D)J recombination and nonhomologous end joining. This regulation does not involve phosphorylation of Artemis.

Functions of the conserved SNM1 domain

- The SNM1 domain also mediates protein–protein interactions indicating it has biochemical functions in addition to its nuclease activity.

Cancer relevance

- *SNM1* genes have been demonstrated to act as tumor suppressors and participate in the maintenance of genomic stability through multiple pathways.

Future perspective

- Mammalian SNM1 proteins are involved in a wide range of functions, including checkpoint recovery in response to DNA damage, imposition of mitotic checkpoints in response to spindle poisons, telomere maintenance, chromatin remodeling and as cofactors in the ubiquitination of other proteins.
- Resolving the detailed biochemical functions of SNM1 proteins in these various pathways is an area for future investigation.

Bibliography

Papers of special note have been highlighted as:

▪ of interest

▪▪ of considerable interest

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1. Haase E, Riehl D, Mack M, Brendel M. Molecular cloning of *SNM1*, a yeast gene responsible for a specific step in the repair of cross-linked DNA. Mol. Gen. Genet 1989;218(1):64–71. [PubMed: 2550766] ▪ Cloning of the founding member of the sensitivity to nitrogen mustard (*SNM1*) gene family in *Saccharomyces cerevisiae*
2. Henriques JA, Moustacchi E. Isolation and characterization of PSO mutants sensitive to photo-addition of psoralen derivatives in *Saccharomyces cerevisiae*. Genetics 1980;95(2):273–288. [PubMed: 7009316]
3. Ruhland A, Kircher M, Wilborn F, Brendel M. A yeast mutant specifically sensitive to bifunctional alkylation. Mutat. Res 1981;91(6):457–462. [PubMed: 7027037]
4. Bonatto D, Revers LF, Brendel M, Henriques JA. The eukaryotic Pso2/Snm1/Artemis proteins and their function as genomic and cellular caretakers. Braz. J. Med. Biol. Res 2005;38(3):321–334. [PubMed: 15761611]
5. Dominski Z. Nucleases of the metallo-β-lactamase family and their role in DNA and RNA metabolism. Crit. Rev. Biochem. Mol. Biol 2007;42(2):67–93. [PubMed: 17453916]
6. Bonatto D, Brendel M, Henriques JA. *In silico* identification and analysis of new Artemis/Artemis-like sequences from fungal and metazoan species. Protein J 2005;24(6):399–411. [PubMed: 16323046]
7. Bonatto D, Brendel M, Henriques JA. The eukaryotic Pso2p/Snm1p family revisited *in silico* analyses of Pso2p A , B and *Plasmodium* groups. Comput. Biol. Chem 2005;29(6):420–433. [PubMed: 16290064]
8. Sullivan KD, Steiniger M, Marzluff WF. A core complex of CPSF73, CPSF100, and Symplekin may form two different cleavage factors for processing of poly(A) and histone mRNAs. Mol. Cell 2009;34 (3):322–332. [PubMed: 19450530]
9. Takaku H, Minagawa A, Takagi M, Nashimoto M. A candidate prostate cancer susceptibility gene encodes tRNA 3′ processing endoribonuclease. Nucleic Acids Res 2003;31(9):2272–2278. [PubMed: 12711671]
10. Jenny A, Minvielle-Sebastia L, Preker PJ, Keller W. Sequence similarity between the 73-kilodalton protein of mammalian CPSF and a subunit of yeast polyadenylation factor I. Science 1996;274(5292): 1514–1517. [PubMed: 8929409]
11. Callebaut I, Moshous D, Mornon JP, de Villartay JP. Metallo-β-lactamase fold within nucleic acids processing enzymes. the β-CASP family. Nucleic Acids Res 2002;30(16):3592–3601. [PubMed: 12177301] ▪ Identification of the β-CPSF–Artemis–SNM1–PSO2 subfamily of metallo-β-lactamase, which act as nucleases
12. Moshous D, Callebaut I, de Chasseval R, et al. Artemis, a novel DNA double-strand break repair/V (D)J recombination protein, is mutated in human severe combined immune deficiency. Cell 2001;105 (2):177–186. [PubMed: 11336668] ▪▪ Discovery of the role of Artemis in severe combined immunodeficiency
13. Lenain C, Bauwens S, Amiard S, Brunori M, Giraud-Panis MJ, Gilson E. The Apollo 5′ exonuclease functions together with TRF2 to protect telomeres from DNA repair. Curr. Biol 2006;16(13):1303–1310. [PubMed: 16730175] ▪ Demonstrates that SNM1B/Apollo interacts with TRF2 and possesses a nuclease that is required to prevent chromosome end-to-end fusions during S phase
14. Li X, Hejna J, Moses RE. The yeast Snm1 protein is a DNA 5′-exonuclease. DNA Repair (Amst.) 2005;4(2):163–170. [PubMed: 15590324]
15. Ma Y, Pannicke U, Schwarz K, Lieber MR. Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. Cell 2002;108(6):781–794. [PubMed: 11955432] ▪▪ Describes the requirement for Artemis and DNA-dependent protein kinase catalytic subunits (DNA-PKcs) for variable (diversity) joining (V[D]J) recombination
16. Pannicke U, Ma Y, Hopfner KP, Niewolik D, Lieber MR, Schwarz K. Functional and biochemical dissection of the structure-specific nuclease ARTEMIS. EMBO J 2004;23(9):1987–1997. [PubMed: 15071507]
17. Hazrati A, Ramis-Castelltort M, Sarkar S, et al. Human SNM1A suppresses the DNA repair defects of yeast pso2 mutants. DNA Repair (Amst.) 2008;7(2):230–238. [PubMed: 18006388]
18. Hejna J, Philip S, Ott J, Faulkner C, Moses R. The hSNM1 protein is a DNA 5′-exonuclease. Nucleic Acids Res 2007;35(18):6115–6123. [PubMed: 17804464]

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19. Povirk LF, Zhou T, Zhou R, Cowan MJ, Yannone SM. Processing of 3′-phosphoglycolate-terminated DNA double strand breaks by Artemis nuclease. J. Biol. Chem 2007;282(6):3547–3558. [PubMed: 17121861]
20. Weterings E, Verkaik NS, Keijzers G, et al. The Ku80 carboxy terminus stimulates joining and artemis-mediated processing of DNA ends. Mol. Cell. Biol 2009;29(5):1134–1142. [PubMed: 19103741]
21. Yannone SM, Khan IS, Zhou RZ, Zhou T, Valerie K, Povirk LF. Coordinate 5′ and 3′ endonucleolytic trimming of terminally blocked blunt DNA double-strand break ends by Artemis nuclease and DNA-dependent protein kinase. Nucleic Acids Res 2008;36(10):3354–3365. [PubMed: 18440975]
22. Cattell E, Sengerova B, McHugh PJ. The SNM1/Pso2 family of ICL repair nucleases: from yeast to man. Environ. Mol. Mutagen. 2010 (Epub ahead of print).
23. Wolter R, Siede W, Brendel M. Regulation of SNM1, an inducible *Saccharomyces cerevisiae* gene required for repair of DNA cross-links. Mol. Gen. Genet 1996;250(2):162–168. [PubMed: 8628215]
24. Elledge SJ. Cell cycle checkpoints: preventing an identity crisis. Science 1996;274(5293):1664–1672. [PubMed: 8939848]
25. Li X, Moses RE. The β-lactamase motif in Snm1 is required for repair of DNA double-strand breaks caused by interstrand crosslinks in *S. cerevisiae*. DNA Repair (Amst.) 2003;2(1):121–129. [PubMed: 12509272]
26. Magana-Schwencke N, Henriques JA, Chanet R, Moustacchi E. The fate of 8-methoxypsoralen photoinduced crosslinks in nuclear and mitochondrial yeast DNA: comparison of wild-type and repair-deficient strains. Proc. Natl Acad Sci. USA 1982;79(6):1722–1726. [PubMed: 6281782]
27. Barber LJ, Ward TA, Hartley JA, McHugh PJ. DNA interstrand cross-link repair in the *Saccharomyces cerevisiae* cell cycle: overlapping roles for PSO2 (SNM1) with MutS factors and EXO1 during S phase. Mol. Cell. Biol 2005;25(6):2297–2309. [PubMed: 15743825] ▪ Describes a role for SNM1 in interstrand crosslinking (ICL) repair in both G1 and S phase
28. Wilborn F, Brendel M. Formation and stability of interstrand cross-links induced by *cis*- and *trans*-diamminedichloroplatinum (II) in the DNA of *Saccharomyces cerevisiae* strains differing in repair capacity. Curr. Genet 1989;16(5–6):331–338. [PubMed: 2692851]
29. Hanada K, Budzowska M, Modesti M, et al. The structure-specific endonuclease Mus81-Eme1 promotes conversion of interstrand DNA crosslinks into double-strands breaks. EMBO J 2006;25 (20):4921–4932. [PubMed: 17036055]
30. Grossmann KF, Ward AM, Moses RE. *Saccharomyces cerevisiae* lacking Snm1, Rev3 or Rad51 have a normal S-phase but arrest permanently in G2 after cisplatin treatment. Mutat. Res 2000;461(1):1–13. [PubMed: 10980408] ▪ Demonstrated that there are three and only three pathways of ICL repair in budding yeast
31. Kraus E, Leung WY, Haber JE. Break-induced replication: a review and an example in budding yeast. Proc. Natl. Acad. Sci. USA 2001;98(15):8255–8262. [PubMed: 11459961]
32. Malkova A, Naylor ML, Yamaguchi M, Ira G, Haber JE. RAD51-dependent break-induced replication differs in kinetics and checkpoint responses from RAD51-mediated gene conversion. Mol. Cell. Biol 2005;25(3):933–944. [PubMed: 15657422]
33. Malkova A, Signon L, Schaefer CB, et al. RAD51-independent break-induced replication to repair a broken chromosome depends on a distant enhancer site. Genes Dev 2001;15(9):1055–1060. [PubMed: 11331601]
34. Dronkert ML, de Wit J, Boeve M, et al. Disruption of mouse SNM1 causes increased sensitivity to the DNA interstrand cross-linking agent mitomycin C. Mol. Cell. Biol 2000;20(13):4553–4561. [PubMed: 10848582] ▪▪ Knockout of *SNM1A* in a mouse and the first description of the mammalian *SNM1* family of genes
35. Ahkter S, Richie CT, Zhang N, Behringer RR, Zhu C, Legerski RJ. Snm1-deficient mice exhibit accelerated tumorigenesis and susceptibility to infection. Mol. Cell. Biol 2005;25(22):10071–10078. [PubMed: 16260620]
36. Kohno T, Sakiyama T, Kunitoh H, et al. Association of polymorphisms in the *MTH1* gene with small cell lung carcinoma risk. Carcinogenesis 2006;27(12):2448–2454. [PubMed: 16774934]
37. Hemphill AW, Bruun D, Thrun L, et al. Mammalian SNM1 is required for genome stability. Mol. Genet. Metab 2008;94(1):38–45. [PubMed: 18180189]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

38. Ishiai M, Kimura M, Namikoshi K, et al. DNA cross-link repair protein SNM1A interacts with PIAS1 in nuclear focus formation. Mol. Cell. Biol 2004;24(24):10733–10741. [PubMed: 15572677] ▪ Demonstrates a connection between the PIAS1 sumoylation pathway and SNM1A
39. Nojima K, Hochegger H, Saberi A, et al. Multiple repair pathways mediate tolerance to chemotherapeutic cross-linking agents in vertebrate cells. Cancer Res 2005;65(24):11704–11711. [PubMed: 16357182]
40. Richie CT, Peterson C, Lu T, Hittelman WN, Carpenter PB, Legerski RJ. hSnm1 colocalizes and physically associates with 53BP1 before and after DNA damage. Mol. Cell. Biol 2002;22(24):8635–8647. [PubMed: 12446782]
41. Galanty Y, Belotserkovskaya R, Coates J, Polo S, Miller KM, Jackson SP. Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks. Nature 2009;462(7275):935–939. [PubMed: 20016603]
42. Morris JR, Boutell C, Keppler M, et al. The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress. Nature 2009;462(7275):886–890. [PubMed: 20016594]
43. Akhter S, Legerski RJ. SNM1A acts downstream of ATM to promote the G1 cell cycle checkpoint. Biochem. Biophys. Res. Commun 2008;377(1):236–241. [PubMed: 18848520]
44. Zhang X, Richie C, Legerski RJ. Translation of hSNM1 is mediated by an internal ribosome entry site that upregulates expression during mitosis. DNA Repair (Amst.) 2002;1(5):379–390. [PubMed: 12509242]
45. Qin X, Sarnow P. Preferential translation of internal ribosome entry site-containing mRNAs during the mitotic cycle in mammalian cells. J. Biol. Chem 2004;279(14):13721–13728. [PubMed: 14739278]
46. Spriggs KA, Stoneley M, Bushell M, Willis AE. Re-programming of translation following cell stress allows IRES-mediated translation to predominate. Biol. Cell 2008;100(1):27–38. [PubMed: 18072942]
47. Akhter S, Richie CT, Deng JM, et al. Deficiency in SNM1 abolishes an early mitotic checkpoint induced by spindle stress. Mol. Cell. Biol 2004;24(23):10448–10455. [PubMed: 15542852] ▪▪ Defined a role for SNM1A in the mitotic prophase checkpoint pathway
48. Scolnick DM, Halazonetis TD. Chfr defines a mitotic stress checkpoint that delays entry into metaphase. Nature 2000;406(6794):430–435. [PubMed: 10935642] ▪▪ Defined the existence of the prophase checkpoint pathway, which responds to spindle stress in mammalian cells
49. Cleveland DW, Mao Y, Sullivan KF. Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling. Cell 2003;112(4):407–421. [PubMed: 12600307]
50. Shah JV, Cleveland DW. Waiting for anaphase: Mad2 and the spindle assembly checkpoint. Cell 2000;103(7):997–1000. [PubMed: 11163175]
51. Smith AP, Gimenez-Abian JF, Clarke DJ. DNA-damage-independent checkpoints: yeast and higher eukaryotes. Cell Cycle 2002;1(1):16–33. [PubMed: 12429904]
52. May KM, Hardwick KG. The spindle checkpoint. J. Cell Sci 2006;119(Pt 20):4139–4142. [PubMed: 17038540]
53. Ahel I, Ahel D, Matsusaka T, et al. Poly(ADP-ribose)-binding zinc finger motifs in DNA repair/checkpoint proteins. Nature 2008;451(7174):81–85. [PubMed: 18172500]
54. Demuth I, Digweed M, Concannon P. Human SNM1B is required for normal cellular response to both DNA interstrand crosslink-inducing agents and ionizing radiation. Oncogene 2004;23(53):8611–8618. [PubMed: 15467758] ▪ First report of the involvement of SNM1B/Apollo in the response to DNA damage
55. Demuth I, Bradshaw PS, Lindner A, et al. Endogenous hSNM1B/Apollo interacts with TRF2 and stimulates ATM in response to ionizing radiation. DNA Repair (Amst.) 2008;7(8):1192–1201. [PubMed: 18468965]
56. Bae JB, Mukhopadhyay SS, Liu L, et al. Snm1B/Apollo mediates replication fork collapse and S Phase checkpoint activation in response to DNA interstrand cross-links. Oncogene 2008;27(37):5045–5056. [PubMed: 18469862] ▪ Demonstrates a mechanism by which SNM1B/Apollo participates in the removal of ICLs
57. Pichierri P, Rosselli F. The DNA crosslink-induced S-phase checkpoint depends on ATR-CHK1 and ATR-NBS1-FANCD2 pathways. EMBO J 2004;23(5):1178–1187. [PubMed: 14988723]

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58. Hanada K, Budzowska M, Davies SL, et al. The structure-specific endonuclease Mus81 contributes to replication restart by generating double-strand DNA breaks. Nat. Struct. Mol. Biol 2007;14(11): 1096–1104. [PubMed: 17934473]

59. McPherson JP, Lemmers B, Chahwan R, et al. Involvement of mammalian Mus81 in genome integrity and tumor suppression. Science 2004;304(5678):1822–1826. [PubMed: 15205536]

60. Whitby MC, Osman F, Dixon J. Cleavage of model replication forks by fission yeast Mus81-Eme1 and budding yeast Mus81-Mms4. J. Biol. Chem 2003;278(9):6928–6935. [PubMed: 12473680]

61. Anders M, Mattow J, Digweed M, Demuth I. Evidence for hSNM1B/Apollo functioning in the HSP70 mediated DNA damage response. Cell Cycle 2009;8(11):1725–1732. [PubMed: 19411856]

62. Freibaum BD, Counter CM. hSnm1B is a novel telomere-associated protein. J. Biol. Chem 2006;281 (22):15033–15036. [PubMed: 16606622] ▪ Demonstrates that SNM1B/Apollo interacts with TRF2 and is required to prevent chromosome end-to-end fusions during S phase

63. van Overbeek M, de Lange T. Apollo, an Artemis-related nuclease, interacts with TRF2 and protects human telomeres in S phase. Curr. Biol 2006;16(13):1295–1302. [PubMed: 16730176] ▪ Demonstrates that SNM1B/Apollo interacts with TRF2 and is required to prevent chromosome end-to-end fusions during S phase

64. Chen Y, Yang Y, van Overbeek M, et al. A shared docking motif in TRF1 and TRF2 used for differential recruitment of telomeric proteins. Science 2008;319(5866):1092–1096. [PubMed: 18202258]

65. Griffith JD, Comeau L, Rosenfield S, et al. Mammalian telomeres end in a large duplex loop. Cell 1999;97(4):503–514. [PubMed: 10338214]

66. Murti KG, Prescott DM. Telomeres of polytene chromosomes in a ciliated protozoan terminate in duplex DNA loops. Proc. Natl. Acad. Sci. USA 1999;96(25):14436–14439. [PubMed: 10588723]

67. de Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. Genes Dev 2005;19(18):2100–2110. [PubMed: 16166375]

68. Lam YC, Akhter S, Gu P, et al. SNM1B/Apollo protects leading-strand telomeres against NHEJ-mediated repair. EMBO J. 2010 (In press).

69. Mack GJ, Compton DA. Analysis of mitotic microtubule-associated proteins using mass spectrometry identifies astrin, a spindle-associated protein. Proc. Natl. Acad. Sci. USA 2001;98(25):14434–14439. [PubMed: 11724960]

70. Gruber J, Harborth J, Schnabel J, Weber K, Hatzfeld M. The mitotic-spindle-associated protein astrin is essential for progression through mitosis. J. Cell Sci 2002;115(Pt 21):4053–4059. [PubMed: 12356910]

71. Thein KH, Kleylein-Sohn J, Nigg EA, Gruneberg U. Astrin is required for the maintenance of sister chromatid cohesion and centrosome integrity. J. Cell Biol 2007;178(3):345–354. [PubMed: 17664331]

72. Liu L, Akhter S, Bae JB, et al. SNM1B/Apollo interacts with astrin and is required for the prophase cell cycle checkpoint. Cell Cycle 2009;8(4):628–638. [PubMed: 19197158] ▪ SNM1B/Apollo regulates CDK1-cyclin B as a target of the prophase checkpoint

73. Jackman M, Lindon C, Nigg EA, Pines J. Active cyclin B1-Cdk1 first appears on centrosomes in prophase. Nat. Cell Biol 2003;5(2):143–148. [PubMed: 12524548]

74. Cavazzana-Calvo M, Le Deist F, De Saint Basile G, Papadopoulo D, De Villartay JP, Fischer A. Increased radiosensitivity of granulocyte macrophage colony-forming units and skin fibroblasts in human autosomal recessive severe combined immunodeficiency. J. Clin. Invest 1993;91(3):1214–1218. [PubMed: 8450050]

75. Moshous D, Li L, Chasseval R, et al. A new gene involved in DNA double-strand break repair and V(D)J recombination is located on human chromosome 10p. Hum. Mol. Genet 2000;9(4):583–588. [PubMed: 10699181]

76. Nicolas N, Moshous D, Cavazzana-Calvo M, et al. A human severe combined immunodeficiency (SCID) condition with increased sensitivity to ionizing radiations and impaired V(D)J rearrangements defines a new DNA recombination/repair deficiency. J. Exp. Med 1998;188(4):627–634. [PubMed: 9705945]

77. Rooney S, Sekiguchi J, Zhu C, et al. Leaky Scid phenotype associated with defective V(D)J coding end processing in Artemis-deficient mice. Mol. Cell 2002;10(6):1379–1390. [PubMed: 12504013] ▪▪ A knockout of Artemis in the mouse confirms its role in V(D)J recombination

78. Rooney S, Sekiguchi J, Whitlow S, et al. Artemis and p53 cooperate to suppress oncogenic N-myc amplification in progenitor B cells. Proc. Natl Acad. Sci. USA 2004;101(8):2410–2415. [PubMed: 14983023] ▪ Demonstrates that Artemis is a tumor suppressor when combined with a p53 knockout

79. Darroudi F, Wiegant W, Meijers M, et al. Role of Artemis in DSB repair and guarding chromosomal stability following exposure to ionizing radiation at different stages of cell cycle. Mutat. Res 2007;615 (1–2):111–124. [PubMed: 17169382]

80. Ma Y, Schwarz K, Lieber MR. The Artemis:DNA-PKcs endonuclease cleaves DNA loops, flaps, and gaps. DNA Repair (Amst.) 2005;4(7):845–851. [PubMed: 15936993]

81. Niewolik D, Pannicke U, Lu H, et al. DNA-PKcs dependence of Artemis endonucleolytic activity, differences between hairpins and 5′ or 3′ overhangs. J. Biol. Chem 2006;281(45):33900–33909. [PubMed: 16914548]

82. Gu J, Li S, Zhang X, et al. DNA-PKcs regulates a single-stranded DNA endonuclease activity of Artemis. DNA Repair (Amst.) 2010;9(4):429–437. [PubMed: 20117966]

83. Kurosawa A, Koyama H, Takayama S, et al. The requirement of Artemis in double-strand break repair depends on the type of DNA damage. DNA Cell Biol 2008;27(1):55–61. [PubMed: 17941805]

84. Inagaki K, Ma C, Storm TA, Kay MA, Nakai H. The role of DNA-PKcs and artemis in opening viral DNA hairpin termini in various tissues in mice. J. Virol 2007;81(20):11304–11321. [PubMed: 17686847]

85. Poinsignon C, Moshous D, Callebaut I, de Chasseval R, Villey I, de Villartay JP. The metallo-β-lactamase/β-CASP domain of Artemis constitutes the catalytic core for V(D)J recombination. J. Exp. Med 2004;199(3):315–321. [PubMed: 14744996] ▪ Only the the SNM1 domain of Artemis is required to rescue the hairpin cleavage defect in V(D)J recombination *in vivo*

86. Soubeyrand S, Pope L, De Chasseval R, et al. Artemis phosphorylated by DNA-dependent protein kinase associates preferentially with discrete regions of chromatin. J. Mol. Biol 2006;358(5):1200–1211. [PubMed: 16600297]

87. Riballo E, Kuhne M, Rief N, et al. A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to γ-H2AX foci. Mol. Cell 2004;16(5):715–724. [PubMed: 15574327] ▪ Demonstrates that ATM and Artemis act together to repair double-strand breaks

88. Wang J, Pluth JM, Cooper PK, Cowan MJ, Chen DJ, Yannone SM. Artemis deficiency confers a DNA double-strand break repair defect and Artemis phosphorylation status is altered by DNA damage and cell cycle progression. DNA Repair (Amst.) 2005;4(5):556–570. [PubMed: 15811628]

89. Goodarzi AA, Noon AT, Deckbar D, et al. ATM signaling facilitates repair of DNA double-strand breaks associated with heterochromatin. Mol. Cell 2008;31(2):167–177. [PubMed: 18657500]

90. Goodarzi AA, Noon AT, Jeggo PA. The impact of heterochromatin on DSB repair. Biochem. Soc. Trans 2009;37(Pt 3):569–576. [PubMed: 19442252]

91. Ziv Y, Bielopolski D, Galanty Y, et al. Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway. Nat. Cell Biol 2006;8(8):870–876. [PubMed: 16862143]

92. Chen L, Morio T, Minegishi Y, et al. Ataxia-telangiectasia-mutated dependent phosphorylation of Artemis in response to DNA damage. Cancer Sci 2005;96(2):134–141. [PubMed: 15723659]

93. Geng L, Zhang X, Zheng S, Legerski RJ. Artemis links ATM to G2/M checkpoint recovery via regulation of Cdk1-cyclin B. Mol. Cell. Biol 2007;27(7):2625–2635. [PubMed: 17242184] ▪ Demonstrates that Artemis acts downstream of ATM to effect recovery from the G2/M checkpoint via regulation of CDK1-cyclin B

94. Poinsignon C, de Chasseval R, Soubeyrand S, et al. Phosphorylation of Artemis following irradiation-induced DNA damage. Eur. J. Immunol 2004;34(11):3146–3155. [PubMed: 15468306]

95. Zhang X, Succi J, Feng Z, Prithivirajsingh S, Story MD, Legerski RJ. Artemis is a phosphorylation target of ATM and ATR and is involved in the G2/M DNA damage checkpoint response. Mol. Cell. Biol 2004;24(20):9207–9220. [PubMed: 15456891] ▪▪ First report demonstrating that Artemis is a substrate of ATM and ATR and is involved in checkpoint responses

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96. Ma Y, Pannicke U, Lu H, Niewolik D, Schwarz K, Lieber MR. The DNA-dependent protein kinase catalytic subunit phosphorylation sites in human Artemis. J. Biol. Chem 2005;280(40):33839–33846. [PubMed: 16093244]

97. Drouet J, Frit P, Delteil C, de Villartay JP, Salles B, Calsou P. Interplay between Ku, Artemis, and the DNA-dependent protein kinase catalytic subunit at DNA ends. J. Biol. Chem 2006;281(38): 27784–27793. [PubMed: 16857680]

98. Goodarzi AA, Yu Y, Riballo E, et al. DNA-PK autophosphorylation facilitates Artemis endonuclease activity. EMBO J 2006;25(16):3880–3889. [PubMed: 16874298] ▪ Phosphorylation of Artemis is not required for either double-stand break repair or V(D)J recombination, but DNA-PKcs autophosphorylation is required

99. Aboussekhra A, Biggerstaff M, Shivji MK, et al. Mammalian DNA nucleotide excision repair reconstituted with purified protein components. Cell 1995;80(6):859–868. [PubMed: 7697716]

100. Deckbar D, Birraux J, Krempler A, et al. Chromosome breakage after G2 checkpoint release. J. Cell Biol 2007;176(6):749–755. [PubMed: 17353355]

101. Krempler A, Deckbar D, Jeggo PA, Lobrich M. An imperfect G2M checkpoint contributes to chromosome instability following irradiation of S and G2 phase cells. Cell Cycle 2007;6(14):1682–1686. [PubMed: 17637566]

102. Wang H, Zhang X, Geng L, Teng L, Legerski RJ. Artemis regulates cell cycle recovery from the S phase checkpoint by promoting degradation of cyclin E. J. Biol. Chem 2009;284(27):18236–18243. [PubMed: 19423708] ▪▪ First report demonstrating that Artemis has a role in protein ubiquitination

103. Koepp DM, Schaefer LK, Ye X, et al. Phosphorylation-dependent ubiquitination of cyclin E by the SCFFbw7 ubiquitin ligase. Science 2001;294(5540):173–177. [PubMed: 11533444]

104. Strohmaier H, Spruck CH, Kaiser P, Won KA, Sangfelt O, Reed SI. Human F-box protein hCdc4 targets cyclin E for proteolysis and is mutated in a breast cancer cell line. Nature 2001;413(6853): 316–322. [PubMed: 11565034]

105. van Drogen F, Sangfelt O, Malyukova A, et al. Ubiquitylation of cyclin E requires the sequential function of SCF complexes containing distinct hCdc4 isoforms. Mol. Cell 2006;23(1):37–48. [PubMed: 16818231]

106. Zhang X, Zhu Y, Geng L, Wang H, Legerski RJ. Artemis is a negative regulator of p53 in response to oxidative stress. Oncogene 2009;28(22):2196–2204. [PubMed: 19398950] ▪ Demonstrates that Artemis regulates p53 via DNA-PKcs in response to oxidative stress generated by mitochondrial respiration

107. Brandes JC, van Engeland M, Wouters KA, Weijenberg MP, Herman JG. CHFR promoter hypermethylation in colon cancer correlates with the microsatellite instability phenotype. Carcinogenesis 2005;26(6):1152–1156. [PubMed: 15760919]

108. Cheung HW, Ching YP, Nicholls JM, et al. Epigenetic inactivation of CHFR in nasopharyngeal carcinoma through promoter methylation. Mol. Carcinog 2005;43(4):237–245. [PubMed: 15937956]

109. Corn PG, Summers MK, Fogt F, et al. Frequent hypermethylation of the 5′ CpG island of the mitotic stress checkpoint gene Chfr in colorectal and non-small cell lung cancer. Carcinogenesis 2003;24 (1):47–51. [PubMed: 12538348]

110. Homma N, Tamura G, Honda T, et al. Hypermethylation of Chfr and hMLH1 in gastric noninvasive and early invasive neoplasias. Virchows Arch 2005;446(2):120–126. [PubMed: 15735977]

111. Kobayashi C, Oda Y, Takahira T, et al. Aberrant expression of CHFR in malignant peripheral nerve sheath tumors. Mod. Pathol 2006;19(4):524–532. [PubMed: 16554732]

112. Mariatos G, Bothos J, Zacharatos P, et al. Inactivating mutations targeting the chfr mitotic checkpoint gene in human lung cancer. Cancer Res 2003;63(21):7185–7189. [PubMed: 14612512]

113. Privette LM, Gonzalez ME, Ding L, Kleer CG, Petty EM. Altered expression of the early mitotic checkpoint protein, CHFR, in breast cancers: implications for tumor suppression. Cancer Res 2007;67(13):6064–6074. [PubMed: 17596595]

114. Bassermann F, Frescas D, Guardavaccaro D, Busino L, Peschiaroli A, Pagano M. The Cdc14B-Cdh1-Plk1 axis controls the G2 DNA-damage-response checkpoint. Cell 2008;134(2):256–267. [PubMed: 18662541]

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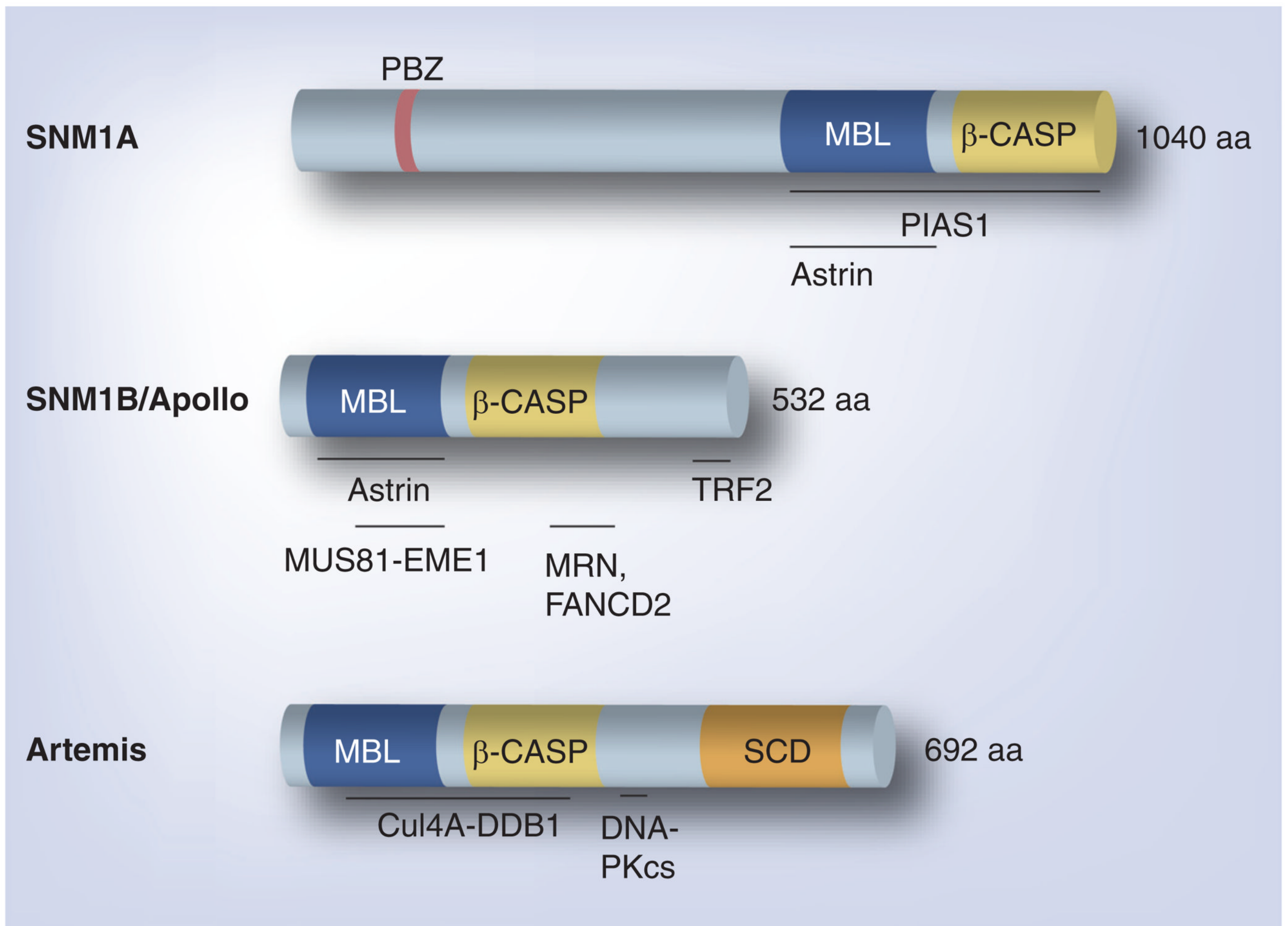

Figure 1. Structural domains of mammalian SNM1 proteins and identified interacting partners
Domains are described in the text. Horizontal lines indicate identified regions of interaction with the proteins.
β-CASP: β-CPSF–Artemis–SNM1–PSO2; DNA-PKcs: DNA-dependent protein kinase catalytic subunit; MBL: Metallo-β-lactamase; MRN: MRE11-RAD50-NBS1; PBZ: Poly (ADP-ribose)-binding zinc finger; SCD: (Serine/threonine)–glutamine cluster domain; SNM1: Sensitivity to nitrogen mustard.

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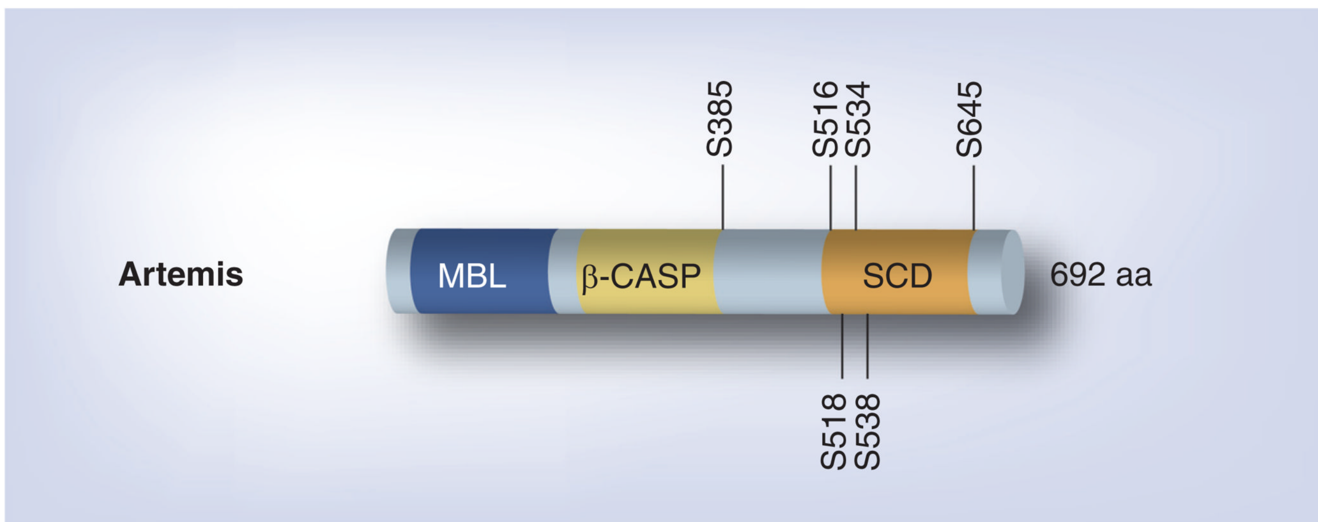

Figure 2. Sites of Artemis phosphorylation that have been confirmed *in vivo*

S516, S534, S538 and S645 are all serine–glutamine sites and have been confirmed to be phosphorylated *in vivo* through the use of phospho-specific antibodies [86,93]. The S385 and S518 sites have been confirmed by mass spectroscopy and phospho-specific antibodies [39]. The S385 site represents a consensus sequence for phosphorylation by casein kinase II. The S518 site is a serine–proline motif.

β-CASP: β-CPSF–Artemis–SNM1–PSO2; MBL: Metallo-β-lactamase;
SCD: (Serine/threonine)–glutamine cluster domain.

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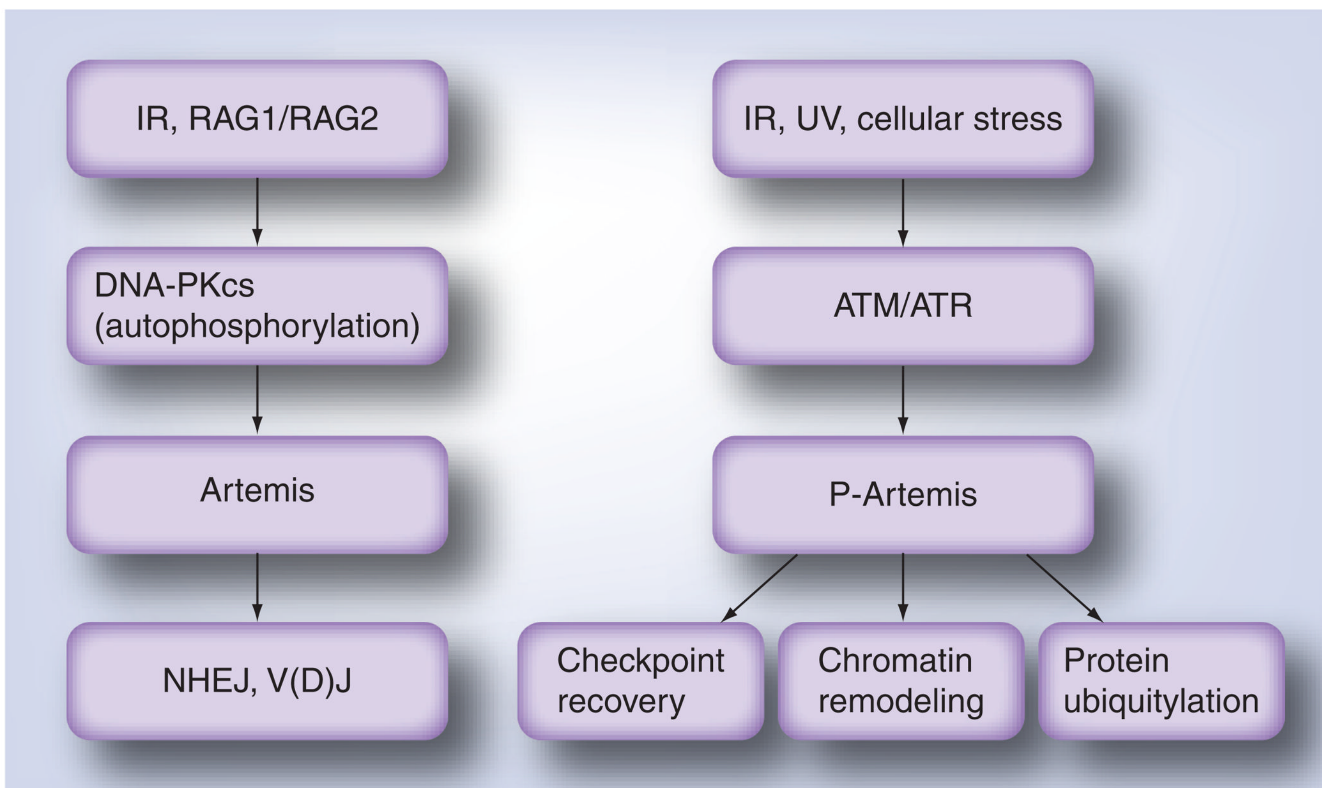

Figure 3. Model for the functions of Artemis in mammalian cells

In response to double-strand breaks created by IR or the RAG1/RAG2 complex, Artemis interacts with autophosphorylated DNA-PKcs to facilitate repair. These processes are independent of Artemis phosphorylation and require only the SNM1 domain (left side of schematic). In response to various forms of cellular stress, Artemis is phosphorylated at multiple sites and acts in various response pathways (right side of schematic).

DNA-PKcs: DNA-dependent protein kinase catalytic subunit; IR: Ionizing radiation; NHEJ: Nonhomologous end joining; P-Artemis: Phosphorylated Artemis; SNM1: Sensitivity to nitrogen mustard; V(D)J: Variable (diversity) joining.