Poster C-7

In silico identification and analysis of new Artemis/Artemis-like sequences from fungal and metazoan species

Authors:
Diego Bonatto (*Universidade de Caxias do Sul*)
Martin Brendel (*Universidade Estadual de Santa Cruz*)
João Antonio Pêgas Henriques (*Universidade de Caxias do Sul*)

Short Abstract: The mammalian Artemis proteins have important functions in the repair of DNA double-strand breaks and in the V(D)J recombination. We have characterized new Artemis/Artemis-like sequences from the genomes of fungi and non-mammalian metazoan species using an in-depth phylogenetic analysis coupled to hydrophobic cluster analysis and three-dimensional modeling of selected sequences.

Long Abstract:

Eukaryotic chromatin is a relatively easy target for reactive chemical and physical agents, including cross-linking substances and ionizing radiation, respectively. Both DNA and the nucleoproteins that compose chromatin can be irreversibly modified by these agents, resulting in chromosomal rearrangements, deletions and other genetic alterations. As chromosomal DNA contains most of an organism's genetic information, modifications introduced in this molecule are potentially lethal if not repaired. Amongst all of these DNA lesions the double strand breaks (DSB) are the most dangerous lesions. Interestingly, the generation of DSB in genomic DNA is a common process in eukaryotic cells, occurring during certain stages of the life cycle, e.g. in meiosis or in DNA re-arrangements for antibody production in B cells. During evolution, eukaryotic cells have developed a complex network of proteins that, by sensing all types of DNA-damage and inducing the appropriate response, maintain the genome's integrity. This network can be sub-divided into different DNA repair pathways, each controlled by cell cycle, damage types and substrate requirements. DSBs are primarily repaired by homologous recombination (HR) and/or by non-homologous end joining recombination (NHEJ). In the case of HR, the presence of a DSB elicits a genomic search for similar (homologous) sequences and the repair involves base pairing of long stretches of matched base pairs. In contrast, NHEJ is a mechanism able to join DNA ends with no, or minimal, homology. In addition, NHEJ is also used to repair DSBs that arise during early lymphocyte development in the context of V(D)J recombination. The NHEJ pathway contains six protein members namely Ku70, Ku80, XRCC4, DNA ligase 4 (Lig4), DNA-dependent protein kinase catalytic subunit (DNA-PKcs), and Artemis. Many proteins that participate in NHEJ or V(D)J recombination share a high homology, from yeasts to plants and animals, indicating the essentiality of this mechanism for cellular well-being. Artemis is a group of proteins that belongs to the beta-CASP family, a member of the metallo-beta-lactamase superfamily. Artemis has 5' to 3' exonucleolytic activity with single-strand DNA specificity and, when associated with DNA-PKcs, forms a phosphorylated complex with endonucleolytic activity on both 5' and 3' DNA overhangs. Furthermore, it can cleave hairpins generated by the Rag-1/Rag-2 proteins in V(D)J recombination. Artemis cooperates with p53 to suppress chromosomal translocation and tumor development in mice

and, therefore, can be considered a tumor suppressor. Like other NHEJ/p53 doubly-deficient mice, most Artemis-deficient mice succumb to pro-B cell lymphomas at the age of 11–12 weeks. Moreover, Artemis interacts with the checkpoint kinase ataxia telangiectasia mutated protein (ATM) and ATM-/Rad3-related proteins (ATR) after exposure of cells to ionizing radiation (IR) or UV irradiation, respectively. These findings indicate that Artemis is required for the maintenance of a normal DNA damage-induced G2/M cell cycle arrest. However, despite the data obtained with mammalian cells on Artemis, little is known about how and when Artemis protein is recruited for DNA repair. Due to intrinsic difficulties in constructing mammalian cell lines with more than one knockout or knockdown gene, an alternative biological model allowing the study of Artemis in DNA repair would be welcome. Yeasts, especially the conventional species Saccharomyces cerevisiae and Schizosaccharomyces pombe, have many advantages as model organisms when compared to plants or metazoans. A large number of yeast mutant strains for many metabolic pathways and cellular components can be easily isolated, using a combination of sophisticated genetic and biochemical analyses. Also, yeast cells can grow rapidly in defined or complete culture media, their cell cycle can be synchronized, and many mutant strains can be tested for different phenotypes at the same time. An Artemis-like protein has not been discovered in conventional yeast species until now. But fungi, plants and metazoans contain an Artemis orthologue protein known as Pso2p/Snm1p. The family of Pso2p/Snm1p is divided in two groups: A and B, both associated with the recombinational repair of DSBs induced by chemical agents. Artemis and Pso2p/Snm1p have low aa sequence homology, indicating that both proteins possibly have different functions in DNA repair in metazoan cells. In this work, we have identified and characterized new members of the Artemis protein family, by searching in eukaryotic genomic databases using sensitive methods of phylogenetic analysis. Additional hydrophobic cluster analysis (HCA) allowed us to refine the results obtained from phylogeny and to map conserved domains in these new Artemis/Artemis-like proteins. HCA data was further confirmed by three-dimensional sequence modeling. The results indicates that Artemis probably belongs to an ancient DNA recombination mechanism that diversified with the evolution of multi-cellular eukaryotic lineage.