ARTS, the unusual septin: structural and functional aspects

Yael Mandel-Gutfreund¹, Idit Kosti¹ and Sarit Larisch²,∗

¹Computational Molecular Biology Laboratory, Faculty of Biology, Technion-Israel Institute of Technology, Haifa 31096, Israel
²Cell Death Research Laboratory, Department of Biology, Faculty of Natural Sciences, University of Haifa, Mount Carmel, Haifa 31905, Israel

*Corresponding author
e-mail: saritlarisch@gmail.com

Abstract

The human Septin 4 gene (Sept4) encodes two major protein isoforms; Sept4_i1 (H5/PNUTL2) and Sept4_i2/ARTS. Septins have been traditionally studied for their role in cytokinesis and their filament-forming abilities, but subsequently have been implicated in diverse functions, including membrane dynamics, cytoskeletal reorganization, vesicle trafficking, and tumorigenesis. ARTS is localized at mitochondria and promotes programmed cell death (apoptosis). These features distinguish ARTS from any other known human septin family member. This review compares the structural and functional properties of ARTS with other septins. In addition, it describes how a combination of two distinct promoters of the Sept4 gene and differential splicing produces distinct mRNAs for Sept4_i1 and ARTS. Due to an intron retention event, ARTS acquired a novel, unique C-terminal domain that is important for its pro-apoptotic and tumor suppressor function.

Introduction

The human Septin 4 gene encodes two major protein isoforms, Sept4_i1 (H5/PNUTL2) and Sept4_i2/ARTS, which we will henceforth refer to as ARTS (apoptosis-related protein in TGF-β signaling pathway) (Larisch et al., 2000; Zieger et al., 2000). ARTS is localized at mitochondria and promotes programmed cell death (apoptosis) (Gottfried et al., 2004; Lotan et al., 2005). Septins have been traditionally studied for their role in cytokinesis and their filament-forming abilities, but subsequently have been implicated in diverse functions, including determination of cell polarity, cytoskeletal reorganization, membrane dynamics, vesicle trafficking, and oncogenesis (Longtime et al., 1996; Hall and Russell, 2004; Roeseler et al., 2009). ARTS is exceptional both in terms of its mitochondrial localization and its pro-apoptotic function, not shared by any other known human septin family member (Carp Nov and Larisch, 2008).

This review summarizes the structural and functional features that distinguish ARTS from other septins. In addition, we describe how a combination of two distinct promoters of the Sept4 gene and differential splicing produces distinct mRNAs for Sept4_i1 and ARTS. Due to an intron retention event, ARTS acquired a novel, unique C-terminal domain that is important for its pro-apoptotic and tumor suppressor function.

Programmed cell death by apoptosis

Apoptosis is a morphologically distinct form of programmed cell death that is important for regulating cell numbers and maintaining tissue homeostasis (Thompson, 1995; Jacobson et al., 1997; Meier et al., 2000). The main executioners of apoptosis are caspases, a family of cysteine proteases that preferentially cleave substrates after aspartate residue (Thornberry and Lazebnik, 1998; Yi and Yuan, 2009). Caspases are tightly regulated by both activators and inhibitors (Shi, 2002a,b; Steller, 2008). The best-studied family of caspase inhibitors is the inhibitors of apoptosis proteins (IAPs) (Crook et al., 1993; Deveraux and Reed, 1999; Salvesen and Duckett, 2002). There are two main pathways leading to caspase activation in mammalian cells: the mitochondrial pathway (‘intrinsic pathway’) and the death receptors pathway (‘extrinsic pathway’) mainly in immune cells (Degterev et al., 2003). All IAP proteins contain at least one baculoviral IAP repeat (BIR) domain. BIR domains can directly interact with caspases and inhibit their apoptotic activity (Shi, 2002a,b; Srinivasula and Ashwell, 2008). X-linked IAP (XIAP) is considered to be the most potent inhibitor of caspases in vitro, and elevated levels of this protein are found in human tumors (Tamm et al., 2000; Eckelman et al., 2006; Hunter et al., 2007). In cells that are doomed to die, inhibition of apoptosis has to be overcome to enable the cell death initiation process (Bangs and White, 2000; Ryoo et al., 2002; Kornbluth and White, 2005; Steller, 2008). A number of studies have suggested that the initiation of apoptosis can occur through the release of caspases from their binding to IAP proteins (Potts et al., 2003; Albeck et al., 2008; Hao and Mak, 2009; Jost et al., 2009). Several mammalian XIAP antagonists have been identified, including SMAC/DIABLO (Du et al., 2000; Verhagen et al., 2000), Omi/HtrA2 (Suzuki et al., 2001; van Loo et al., 2002), and ARTS (Larisch et al., 2000; Gottfried et al., 2004). SMAC and Omi, which are located in the mitochondrial intermembrane space, contain a short, conserved IAP binding motif (IBM) and are released to the cytosol upon apoptotic induction (Du et al., 2000;
ARTS induces apoptosis by inhibiting IAPs

ARTS was initially discovered in a screen for genes promoting apoptosis induced by TGF-β (Larisch et al., 2000; Larisch-Bloch et al., 2000). Later, ARTS was found to promote apoptosis induced by a variety of pro-apoptotic stimuli (Larisch et al., 2000; Elhasid et al., 2004; Gottfried et al., 2004; Lotan et al., 2005). ARTS was subsequently shown to activate caspases by binding and inhibiting XIAP (Gottfried et al., 2004; Bornstein et al., in press; Garrison et al., 2011). However, ARTS does not contain a canonical IBM. Instead, a stretch of 27 residues located at the C-terminus of ARTS was shown to be essential for binding of ARTS to XIAP (Gottfried et al., 2004). These 27 amino acids residing at the extreme C-terminus of ARTS show no detectable sequence similarity to any septin sequence nor to any other known protein (Figure 1). In particular, the composition of these 27 residues is entirely different from the IBM. Therefore, ARTS must bind IAPs by a mechanism that is distinct from the major known family of IAP antagonists. However, while the unique C-terminus of ARTS is necessary for the interaction of ARTS and XIAP, it appears that additional sequences are needed for efficient IAP binding. A somewhat similar situation is seen for the IBM. Although the pro-apoptotic function of SMAC/DIABLO requires a conserved four-residue IBM (AVPI), additional amino acids downstream make a second contact with the XIAP–BIR3 domain, and the Drosophila IBM-containing proteins, Reaper/Hid/Grim, use sequences beyond the fifth amino acid to bind Diap1 (Du et al., 2000; Liu et al., 2000; Verhagen et al., 2000; Wu et al., 2001; Yang and Du, 2004). However, the significance of additional amino acids contained within ARTS C-terminal sequence awaits further structural analysis studies using X-ray crystallography methods.

In living cells, ARTS localizes to the outer membrane of mitochondria (Edison et al., in press). Following induction of apoptosis, ARTS rapidly translocates to the cytosol in a caspase-independent manner where it binds XIAP. The translocation of ARTS from mitochondria precedes the release of both cytochrome c and SMAC and leads to degradation of XIAP before the exit of SMAC. Moreover, short hairpin RNA toward ARTS inhibits the release of both SMAC and cytochrome c, suggesting ARTS is required for proper ‘on time’ release of these two proteins (Edison et al., in press).

Structural features of ARTS

The hallmark of septin proteins is a conserved central G domain flanked by variable N- and C-termini, with the
C-terminal ends predicted as coiled coils (Sirajuddin et al., 2009). Similarly, the sequence of ARTS can be divided into three domains: an unstructured N-terminal domain, a central GTPase domain, and another disordered C-terminal region (Figure 2). However, these domains in ARTS are different from their counterparts in other septins. Sept4_i1 contains a central GTPase domain, which is catalytically active and can bind and hydrolyze GTP (Garcia et al., 2006). In contrast, although ARTS contains an identical P-loop GTPase domain, it lacks the G4 domain (Larisch et al., 2000). We therefore assume that unlike other septin GTPases, ARTS cannot hydrolyze GTP. Not all Septins are able to hydrolyze GTP, and this phenotype seems to be related to the phylogenetic division of human septins into four different subgroups (Kinoshita, 2003). Interestingly, mutating the P-loop GTPase domain in ARTS compromises its apoptotic activity (Larisch et al., 2000; Gottfried et al., 2004). We therefore conclude that the P-loop GTPase domain in ARTS may be important either for keeping its core structure and/or for binding to other apoptotic signaling proteins. Moreover, it appears that these interactions do not require hydrolysis of GTP. The interesting question whether ARTS can interact with Sept4_i1 or with other septins is still not resolved and awaits further exploration.

The N-terminal sequence of ARTS lacks the first 20 residues found in Sept4_i1 (Larisch et al., 2000). Amino acid analysis revealed that the N-terminus of ARTS is unstructured/disordered (Figure 2). Similar results were found for the N-terminal sequence of Sept4_i1 (residues 1–128) (Garcia et al., 2006). In addition, both ARTS and Sept4_i1 contain many proline-rich regions at their N-terminal part, suggesting a role in protein–protein interactions, as seen for example in SH3-binding domains (Kay et al., 2000; Garcia et al., 2006).

The lack of structural specificity, i.e., structural disorder, also has been recognized to be an important property of a significant fraction of protein domains in eukaryotes (Dyson and Wright, 2005). These unstructured regions have a lower density of internal contacts than compact globular structures, and their inherent disorder may confer the ability to interact differentially with several physiological partners (Tompa et al., 2005; Haynes et al., 2006). Indeed, Garrison et al. (2011) showed that ARTS binds to Siah-1 (seven in absentia homolog 1), an E3-ligase protein, through a Siah consensus motif found at amino acids 37–48 located within the N-terminal sequence of ARTS.

In addition, unlike other septins that are mainly localized to actin bundles or at the cytosol (Lindsey and Momany, 2006), we have found that ARTS is localized at the outer membrane of mitochondria (Edison et al., in press). Although septins in yeast appear to be tightly associated with the membrane of the mother–bud neck (Byers and Goetsch, 1976), the mechanism underlying the association of ARTS with the outer membrane of mitochondria is still obscure. ARTS, like other septins, lacks a recognizable transmembrane domain and other obvious membrane localization motifs (Zhang et al., 1999). However, the association of some septins with membranes has been attributed to a well-characterized polybasic sequence residing between the N-terminal domain and the GTPase, typically about nine residues before the P-loop. This polybasic sequence is known to bind directly to phosphatidylinositol bisphosphate (PtdInsP2) and phosphatidylinositol trisphosphate (PtdInsP3) and specifically confers membrane-binding capability to the mouse SEPT4_i1 protein (Byers and Goetsch, 1976; Xie et al., 1999; Zhang et al., 1999; Casamayor and Snyder, 2003; Bertin et al., 2010). Studies with Sept4-deficient mice have shown that during spermatogenesis, mouse Sept4 is essential for correct mitochondrial architecture and the formation of the annulus (Holstein and Roosen-Runge, 1981; Myles and Primakoff, 1984; Cesario and Bartles, 1994; Ibara et al., 2005; Kissel et al., 2005; Kwitny et al., 2010). The annulus is a ring structure composed of different septin proteins (Sept4, Sept12); it separates the midpiece and tail region of mammalian sperm and that acts as a diffusion barrier (Steels et al., 2007; Lin et al., 2009; Kwitny et al., 2010). Moreover, knockout of Septin 4 in mice prevents annulus formation, and induces flagellar bending and asthenozoospermia resulting in male sterility (Kissel et al., 2005). In addition, an asthenozoospermia patient was shown to exhibit an absence of the annulus structure and abnormal mitochondrial organization, confirming the role of the annulus septin ring for mitochondrial and sperm structure and function (Lhuillier et al., 2009). These observations are consistent with a role for Sept4 proteins in mediating protein–membrane interactions. Another mouse Septin 4 splice variant, M-septin, was found to localize to mitochondria (Takahashi et al., 2003). M-septin is suggested to play an important role in neuronal differentiation and axon guidance through the control of mitochondrial function (Takahashi et al., 2003). Interestingly, septin-like proteins of the ciliate Tetrahymena were also found to localize to mitochondria (Wloga et al., 2008). Yet, in contrast to ARTS, which has a pro-apoptotic activity, ciliate septins appear to protect against apoptosis-like damage of mitochondria and nuclear envelopes (Wloga et al., 2008). It has been proposed that the mitochondrial association of Tetrahymena and mammalian septins has resulted from the recruitment of septins to mitochondria, which has occurred independently in the lineages of protists and mammals (Wloga et al., 2008). Similarly, the adaptation of another type of membrane-associated GTPases, dynamins, to endocytosis has occurred independently in the lineages of ciliates and metazoans (Elde et al., 2005).

The C-terminal domains of septins generally include sequences characteristic of coiled coils (Kartmann and Roth, 2001; Garcia et al., 2006). Coiled-coil motifs exhibit periodic hydrophobic/polar sequence patterns for which core packing complementarity and optimized electrostatic interactions at the edges are expected to promote stability and specificity (Barth et al., 2008). The C-terminus of Sept4_i1 (residues 417–478) includes a second proline-rich sequence followed by a short stretch of uninterrupted leucine/isoleucine repeats at every seventh amino acid residue. This is expected to form part of a left-handed coiled coil, as suggested for most other septins (Garcia et al., 2006; Sirajuddin et al., 2007). In contrast, ARTS lacks the coiled coil domain at its C-terminus.
Figure 3  Evolutionary conservation of Septin 4 transcripts.
(A) Alignment of the translated amino acid sequence of the retained intron. Sequence alignment is shown for the eight fully sequenced mammalian species closest to human. On the left is the phylogenetic tree for the eight species, extracted from the University of California Santa Cruz (UCSC) human genome browser. The phylogenetic tree was calculated on the basis of the 16S rRNA. Amino acids are colored on the basis of their physicochemical properties: hydrophobic amino acids in pink, hydrophilic amino acids in green, aromatic amino acids in yellow, positively charged amino acids in blue, negatively charged amino acids in red, special amino acids in purple, and cysteine in bright yellow. As demonstrated, the human 27 amino acids are fully conserved among the apes and highly conserved in other mammals. Notably, the mouse sequence has an extension of 16 amino acids, resulting from a frameshift event. (B) A full representation of the two Sept4 isoforms, Sept4_V1 and Sept4_V2, as viewed from the UCSC human genome browser. Notably, the UCSC browser views all transcripts compared to the reference (+) strand, and thus the Sep4 transcript is shown from right (5') to left (3'). The top panel demonstrating that the transcript; CDS exonic regions are shown in thick blue bars, intronic regions in thin lines; arrow shows the direction of the transcript (from right to left); and UTRs are shown in thin blue bars. Below, the green bar represents the prediction of a CpG islands. The lower panel represents the evolutionary conservation of the Sept4 region. Conservation was calculated on the basis of an alignment of 17 fully sequenced mammalian species. The height of the bar represents the level of conservation, normalized from 0 to 1. (C) A zoom-in view into the region between exons 6 and 7. Lower panel representing the sequence conservation shows that the retained intron 6 is highly conserved among mammals.

The lack of a coiled-coil domain, which would have forced ARTS into a relatively ‘rigid’ structure, suggests that ARTS may be structurally more flexible than other septins to adopt distinct conformations upon interacting with other proteins. Indeed, the unique C-terminus of ARTS is predicted to be highly disordered and is required for its pro-apoptotic function (Larisch et al., 2000; Gottfried et al., 2004). Therefore, we propose that the unstructured nature of the unique C-terminus of ARTS provides the flexibility to bind other proteins, such as XIAP (Figure 2) (T.H. Reingewertz et al., unpublished data). The disordered structure of ARTS at its N- and C-termini provides it with another function, serving as a platform or adaptor protein that facilitates functional interactions between several proteins. An example for ARTS operating as a molecular bridge that targets Siah-1 onto XIAP was recently described (Garrison et al., 2011). In this study, it was shown that ARTS interacts with the E3 ligase Siah-1 to induce ubiquitination and degradation of XIAP. Cells lacking either Siah or ARTS contain higher steady-state levels of XIAP. Thus, ARTS serves as an adaptor to bridge Siah-1 to XIAP, targeting it for destruction (Garrison et al., 2011).
**Generation of the unique ARTS C-terminus through an intron retention event**

The *Sept4* gene contains two distinct promoters: a proximal promoter that generates *Sept4_V1* and a distal promoter that gives rise to ARTS pre-mRNA (V2). In the latter, the short (113 nt) intron 6 in the *Sept4_V1* pre-mRNA is retained in the ARTS spliced mRNA, resulting in a longer exon 6a with additional 27 amino acids that form the unique C-terminus of ARTS (Figure 1). A CpG island is predicted in the promoter region just upstream to the ARTS start site (shown as a green box under the transcript in Figure 3B). In contrast, no CpG island sequences are predicted for the *Sep4_V1* promoter, suggesting differential regulation of ARTS and V1.

The unique ARTS C-terminus is generated through an event termed ‘intron retention’. Intron retention is an alternative splicing event in which an intron is included in the mature RNA transcript (Galante et al., 2004). Intron retention is the rarest alternative splicing event among the four types of alternative splicing events that are prevalent in metazoans: exon skipping, alternative 3' splice sites, alternative 5' splice sites, and intron retention. Early studies suggested that at least one intron retention event occurs in approximately 15% of the human genes, the majority of them occurring at the untranslated regions (UTRs) (Galante et al., 2004). More recent studies suggest that intron retention events account for 10% of all alternative splicing events in the human transcriptome (Kim et al., 2007). For the retained introns interrupting the coding region, the GC content, codon usage, and the frequency of stop codons suggest that these sequences are under selection for coding potential (Galante et al., 2004). Furthermore, it has been suggested that other factors, such as the intronic length and the strength of the splice sites are associated with intron retention events in the human transcriptome (Sakabe and de Souza, 2007; Hu et al., 2010). While intron retention events are relatively rare events in the normal human transcriptome, impaired splicing events causing retention of intronic sequences are highly associated with human diseases, including genetic diseases and cancer (Baralle and Baralle, 2005). The relatively short length of the Septin 4 intron makes it a good candidate for an intron retention event. Moreover, conservation of nucleotides within the retained ARTS intron is much higher when compared with the upstream introns in the septin gene (shown in the bottom panels of Figures 3A and B). Thus, the intronic sequences found between exons 6 and 7 creating the unique C-terminus of ARTS were highly selected in evolution probably owing to their strong coding potential. Furthermore, the 5' splice site score calculated for the human sequence (data not shown) confirms that it has a weak 5' splice site. Similar results are seen in many other intron retention events (Sakabe and de Souza, 2007).

In the ARTS mRNA, the retention of the intronic sequence introduced a stop codon 27 amino acids after the last exon–intron junction. This creates a shorter open reading frame (ORF) of *Sep4_V2* and the unique 27 amino acids at the C-terminus of ARTS. As shown in Figure 3, the 27 amino acids are highly conserved among monkeys and close placental mammals. A deeper analysis of the intronic sequences among placental mammals and vertebrates suggests that the potential coding of the region is also conserved in other vertebrates. However, since in many mammals, intronic sequences and sufficient expressed sequence tags data are not available, it is impossible to exactly track the evolution of the intron retention event in the *Sept4* locus. Interestingly, as can be clearly seen from the amino acid sequence alignment (Figure 3), a frameshift event in the mouse intronic sequence resulted in an extension of 17 amino acids to the ARTS ORF. This extension has been confirmed at the protein level as well (Kissel et al., 2005). In summary, two separate promoters in the human *Septin 4* gene produce the two distinct pre-mRNAs for *Sept4_i1* and ARTS. This, in combination with differential splicing and an intron retention event occurring within the ARTS mRNA, allows the generation of an ‘unusual’ septin protein, ARTS, that contains a unique C-terminus that is critical for its pro-apoptotic and tumor suppressor function. The use of two distinct promoters also explains how it is possible to selectively silence ARTS, but not *Sept4_i1*, in human tumors (Elhasid et al., 2004).

**ARTS, IAPs, and cancer**

In recent years, IAP proteins have emerged as promising targets for cancer therapy (Elde et al., 2005; Hunter et al., 2007; Vucic and Fairbrother, 2007; LaCasse et al., 2008; Vaux, 2009). XIAP is considered to be the most potent inhibitor of caspases in vitro, and elevated levels of this protein are found in human tumors (Eckelman et al., 2006; Hunter et al., 2007; Tamm, 2008). ARTS expression is frequently lost in acute lymphoblastic leukemia and lymphoma patients, functioning as a tumor suppressor protein (Elhasid et al., 2004). Moreover, Sept4/ARTS-deficient mice exhibit increased tumor incidence, increased numbers of hematopoietic stem and progenitor cells, and increased resistance to cell death, demonstrating a physiological role of ARTS in regulating apoptosis and cancer in vivo (Garcia-Fernandez et al., 2010). Furthermore, the apoptosis, stem cell, and tumor phenotypes of *Sept4/ARTS*-null mice are suppressed by inactivation of XIAP, demonstrating that ARTS functions as a tumor suppressor by regulating stem cell apoptosis (Garcia-Fernandez et al., 2010). Because ARTS acts very early in the initiation of a caspase cascade and is lost in human cancers, ARTS-based drugs may offer new opportunities to manipulate apoptosis for therapeutic purposes.

**Acknowledgments**

We are very grateful to Hermann Steller for critical reading of the manuscript and to Juliana Kagan for technical assistance. Work from SL's laboratory described in this review was supported by funds from US Israel Binational Science Foundation grant 2003085 (to SL), Israel Science Foundation grant 1264/06 (to SL), and a grant from Israel Cancer Association (to SL).
References


Kwitny, S., Klaus, A.V. and Hunnicutt, G.R. (2010). The annulus


Received May 3, 2011; accepted July 2, 2011