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Research Article

2'-5' Oligoadenylate synthetase shares active site architecture with the archaeal CCA-adding enzyme

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Abstract. The 2'-5' oligoadenylate synthetases (OAS) are interferon-induced antiviral enzymes that recognise virally produced dsRNA and initiate an RNA destabilisation within the infected cell. We compared the structure of OAS to that of poly adenosine polymerase (PAP) and the class I CCA-adding enzyme from *Archeoglobus fulgidus* (AfCCA). This comparison revealed a strong structural homology between the three enzyme families. In particular, the active sites of OAS and CCA class I enzymes are highly conserved. We conducted an extensive mutagenesis of amino acid residues within the putative active site in OAS, thereby identifying enzymatically important residues and confirming the common active site architecture for OAS and the AfCCA. Our findings also have profound implications for our understanding of the evolutionary origin of the OAS enzymes and suggest that the OAS proteins diverged from a common 3'-specific ancestor at the beginning of metazoan evolution.

Keywords. Oligoadenylate synthetase, archaeal CCA-adding enzyme, poly adenosine polymerase, interferon, RNA metabolism.

Introduction

The 2'-5' oligoadenylate synthetases (OAS) are interferon-induced and double-stranded RNA (dsRNA) activated antiviral enzymes [1, 2]. They are produced as latent enzymes and binding of dsRNA to OAS result in their activation. The activated form of OAS produces 2'-5'-linked oligoadenylate (2–5A) in response to viral infections. The origins of the activating RNA are presumed to be viral but further investigations are required to establish the precise origin of *in vivo* activators of OAS. The formation of 2'-linked oligonucleotides is a unique property of the OAS proteins and they are currently the only enzymes known to catalyse the polymerisation of nucleotides into 2'-5'-linked oligomers [1]. This unusual nucleotide can bind to the inactive monomeric form of RNase L and lead to dimerisation and subsequently activation of this nuclease [3]. Thus collectively, the OAS-RNase L pathway constitutes a regulated RNA decay pathway, which is activated in response to viral infections.

It was initially presumed that the OAS proteins constituted a distinct family of proteins, which had emerged at a late stage during vertebrate evolution [4]. However, based upon conservation of short stretches of amino acid sequences in the active site, Holm and Sander suggested that the OAS proteins do belong to a larger superfamily of nucleotidyl-transferase enzymes, including the template-dependent polymerase β [5]. This familiarity was based upon the

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conservation of a short stretch of amino acids in the active site of the nucleotidyl-transferase enzymes, focused around the phosphate binding loop (also known as the P-loop or Walker A motif) and the first two of the metal-binding aspartic acids. The existence of an active site in OAS containing the three metal coordinating carboxylates was later confirmed by mutagenesis and molecular modelling [6] and ultimately by the crystal structure of OAS [7]. Site-directed mutagenesis identified S62 of the porcine OAS1 enzyme as part of the P-loop and K212 was suggested to interact with the phosphate of the incoming ATP molecule [7]. Interestingly, both S62 and K212 have structurally equivalent residues in both poly adenosine polymerase (PAP) and CCA, and the effect of mutating these residues in CCA and PAP is similar to that seen in OAS [8, 9]. The structure of OAS showed that the enzyme is characterised by a two-domain structure; a five-stranded β-sheet domain (also known as the palm domain in the hand analogy used for template-dependent polymerases) and a four-helix bundle domain [7]. The β-sheet domain harbours a set of three carboxyl side chains coordinating the catalytically active metal ions [6]. While the β-sheet domain and thus the basic catalytic mechanism is shared with all other known polymerases [7, 10], the four helix bundle is unique within the template-independent polymerase family replacing the thumb and finger domains found in template-specific enzymes.

In mammals the OAS family consist of four genes, the OAS1, OAS2, and OAS3, which all encode active synthetases [11, 12], and the OASL gene that encodes a protein with high similarity to the other OAS members but devoid of OAS activity [13, 14]. All the OAS genes are found on chromosome 12, with OAS1, OAS2 and OAS3 in a tightly coupled locus at 12q24.1 with the OASL gene situated further towards the telomeric end of chromosome 12. The OAS2 and OAS3 genes are made of a two- and threefold duplication of the basic OAS domain found in OAS1 and it has been shown that the OAS family evolved by duplication of a single ancestral OAS1 gene [15]. Throughout this paper we discuss the OAS1 protein exclusively since this is the only protein for which structural data is available.

We used the striking structural similarity between the OAS1 and the class I CCA-adding enzyme from *Archeoglobus fulgidus* (AfCCA) enzymes to produce a model of the active site of the OAS1 enzyme. Whereas the position and biochemical role of the metal co-ordinating aspartates found in the N-terminal domain of OAS1 are known, this study sheds light on the role played by the C-terminal domain of OAS1 in catalysis. Two highly conserved sequence motifs are found in the C-terminal domain of OAS1, often referred to as OAS signature motif one and two. In the 3-D structure, these sequence motifs correspond to the second helix in the C-terminal domain and a structure formed by three short β-strands and connecting loops. This region of the OAS1 structure is highly similar to the same region in the AfCCA enzyme and has formed the base of our investigations. We show that the residues Y230, E233 and D300, which are among the most conserved residues in the OAS family, play an important role in the catalysis of 2–5A. Furthermore, we have used a combination of structural comparison and site-directed mutagenesis to show that the OAS proteins shares a active site architecture with the class II CCA-adding enzymes and thus it is likely that OAS evolved from a ancestral 3'-specific enzyme.

Materials and methods

Structural superimposition and generation of molecular structure figures. The superimposition matrix was calculated by the LSQ-routine as implemented in O using active site residues [16]. For OAS, the superimposition was carried out separately for the two domains. For the N-terminal domain of OAS amino acids 73–78 (58–63) and 146–150 (109–113) were used, with the numbers for equivalent residues in AfCCA given in parentheses. This corresponds to the C-alpha atoms for the two β-strands where the catalytic carboxyl acids are situated. For the C-terminal domain of OAS, we used amino acids 206–215 (146–155) and 230–238 (173–181), corresponding to the first and second helix in the four-helix bundle. The superimposed OAS structure was written to a PDB file and read into the program PyMOL (Delano Scientific) used to generate the figures.

Generation of mutants. The wild-type porcine OAS1 genes were inserted into the *Nco*I and *Bam*HI sites of a modified pET 9d vector (Novagen), which contains a C-terminal 6×His-tag. Mutagenesis was carried out using the QuickChange Site-Directed Mutagenesis Kit (Stratagene) with appropriate oligonucleotide primers. The mutated plasmids were transformed into *E. coli* DH5α competent cells and plasmid DNA was purified using standard mini prep protocols (QIAprep Spin Miniprep Kit; Qiagen).

Expression and purification of wild-type and mutant proteins. Wild-type and mutant proteins were expressed in *E. coli* (BL21 DE3 Novagen) and grown to an OD_{600} of 0.5–0.7 in LB medium containing 30 μg/ml kanamycin before induction with 0.5 mM IPTG for 12 h at 22°C. Cells from 1-l culture were harvested by

centrifugation and washed with ice-cold PBS, then resuspended in 25 ml lysis buffer (300 mM NaCl, 50 mM NaH_2PO_4 pH 8.0, 20 mM Imidazole, 0.1 % (v/v) NP-40, 10 % glycerol, 5 mM β-mercaptoethanol) and lysed by sonication. The proteins were bound to Ni^{2+}-coupled agarose beads (Ni-NTA-agarose, Qiagen) in the lysis buffer for 1 h at 4°C with gentle shaking. The beads were cast onto a column and washed with wash buffer A (500 mM NaCl, 50 mM NaH_2PO_4, 20 mM Imidazole and 10 % glycerol at pH 8.0) and wash buffer B (as A but pH 6.0). The protein was eluted with the elution buffer (Wash buffer + 250 mM imidazole at pH 6.8) in fractions of 1 ml and the protein-containing fractions were identified by SDS-PAGE and Coomassie blue staining. The fractions containing the purified OAS protein were pooled and dialysed overnight against (200 mM NaCl, 25 mM HEPES, 1 mM EDTA and 5 % glycerol, pH 6.8).

Activity assays. Activity assays were performed essentially as described by [17]. In brief, protein was diluted in buffer E [20 mM Tris-HCl pH 7.8, 0.2 mM EDTA, 5 mM Mg(OAc)2 and 10 % (v/v) glycerol]. The activity of the wild-type and mutant proteins was determined with the following overall reaction mixture: 4 µl 5× OAS buffer [20 mM Tris-HCl pH 7.8, 20 mM Mg(OAc)2, 1 mM DTT, 0.2 mM EDTA, 0.5 mg/ml bovine serum albumin, 10 % (v/v) glycerol], 4 µl protein in buffer E, 4 µl 5 mM ATP including ATP pH 7.5 including α-^{32}P-labelled ATP, 4 µl 250 µg/ml poly(I)●poly(C) and 4 µl water. The different reaction mixtures were incubated in a thermocycler at 37°C for 1 h and the reaction was stopped by heating for 5 min at 95°C and than cooled to 4°C. Separation of the ATP and the 2'–5'-oligomers was performed by thin-layer chromatography (TLC) on PEI-cellulose plates (Merck) run in 0.4 M Tris pH 8.6 and 30 mM $MgCl_2$. The TLC plate was developed using a PhosphoImager™ and the Quantity One Software (Bio-Rad) to quantify the radioactivity within spots corresponding to the different-sized 2–5A. A control sample containing no poly(I)●poly(C) was used to estimate the background, which was then subtracted before calculating the specific activity.

Generation of alignments. We aligned the porcine (*Sus scrofa*) OAS1 sequence (AJ225090) to the sequence of OAS from chicken (*Gallus gallus*, BAB19016), sea sponges (*Geodia cydonium*, Y18497 and *Suberites domuncula*, AJ301652), starlet sea anemone, (*Nematostella vectensis,* XM_001635365), and sea squirt (*Ciona intestinalis* ci0100134101) using the Clustal W program. The *N. vectensis* sequence is a draft genomic sequence, which was modified prior to alignment. The first 70 residues at the N termini were removed since they have no similarity to any part of the OAS protein. Furthermore, four amino acids, included in the putative translated protein from the genomic sequence, were removed from the sequence, since they were not found in the EST sequences available. This is most likely due to inaccurate splice site prediction in the genomic draft sequence. The chicken sequence had the ubiquitin-like C-terminal domain removed. The *C. intestinalis* sequence is incomplete, as the start codon and possibly part of the N-terminal sequence is lacking. Accession number is given in parenthesis. The alignment was generated using the Clustal W program (http ://www.ch.embnet.org/software/ClustalW.html)

Results and discussion

Structural homology between OAS, PAP and AfCCA. The PAP adds a poly-A tail to mRNA and thus plays an important role in mRNA metabolism in eukaryotes. This enzyme is found in all eukaryotes and the crystal structure of the bovine and yeast PAPs are available [18–20]. Similarly, the class I CCA-adding enzyme adds the CCA tail to tRNAs without using a nucleic acid template. Recently, the crystal structure of the class I CCA-adding enzyme of the archaeal-bacteria *Archaeoglobus fulgidus* (AfCCA) was solved in complex with several different substrate analogues and with the mature $tRNA^{phe}$ [21, 22].

It was previously shown that OAS and PAP share a substantial structural homology [7]. We extended this comparison by superimposing the crystal structures of porcine OAS1 (1PX5) upon the crystal structure of AfCCA in complex with a substrate analogue mimicking the addition of the adenosine to the CCA tail of tRNA (1TFW). The best overall Root Mean Square Deviation (RMSD) values for the C-alpha atoms are achieved by superimposing the N- and C-terminal domains of OAS (equivalent to the head and neck in CCA) separately using all C-alpha atoms by the LSS algorithm as implemented in Coot [23]. This results in an RMSD value of 2.9 Å for the N-terminal domain and 2.1 Å for the C-terminal domain. The equivalent values for PAP are 3.1 Å and 2.5 Å, respectively, which was obtained using the recently published structure of PAP with bound substrate [20]. Thus, OAS, PAP, and class I CCA-adding enzymes share a substantial structural homology. However, since we were specifically interested in the conservation of the active sites of OAS and the AfCCA enzymes, we constructed a structural superposition of OAS on the AfCCA structure using short stretches of amino acids known to be involved in catalysis (see Materials and

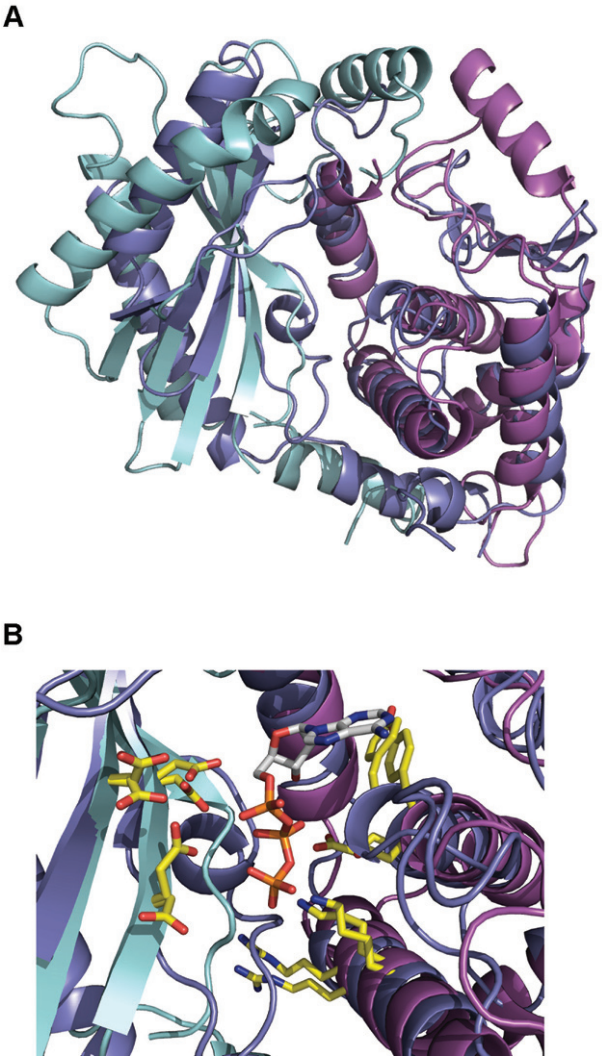

Figure 1. (*A*) Cartoon representation of 2'-5' oligoadenylate synthetase (OAS) (PDB: 1PX5) superimposed upon the CCA-adding enzyme from *Archaeoglobus fulgidus* AfCCA (PDB: 1TFW). The OAS N-terminal domain is shown in cyan, the OAS C-terminal domain is shown in purple, and the AfCCA is shown in blue. (*B*) A close up of the active site superimposition showing the three metal co-ordinating aspartic acid from the N-terminal domain and residues R209, K212, Y230 and D233 from the C-terminal domain of OAS in sticks. The ATP molecule originates from the AfCCA structure, and is positioned according to the superimposition of OAS on AfCCA.

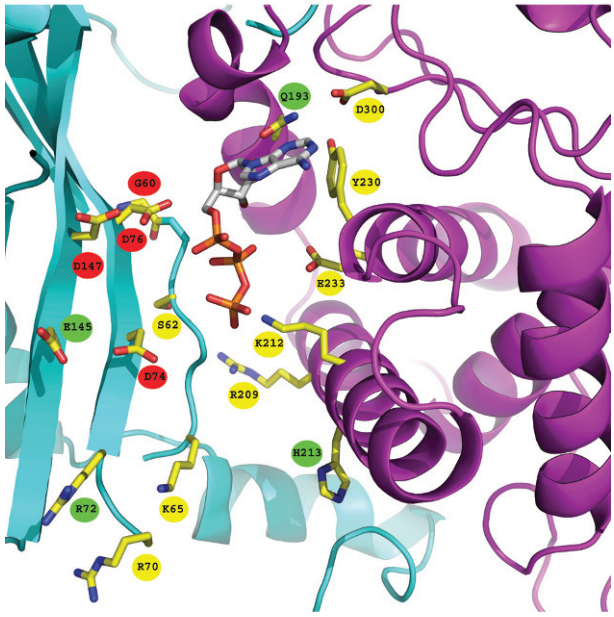

Figure 2. Model for ATP binding in OAS. The OAS molecule is shown in the same orientation as in Figure 1A (superimposed upon the AfCCA), the ATP molecule originates from the AfCCA structure, the amino acids targeted for mutation are shown in ball and stick, and the numbers of the relevant residues are shown inside coloured spheres. The colour indicates the effect of mutating the residues in question to alanine. Red indicates no detectable activity left upon mutation of the residue, yellow indicates a significant effect upon activity (less than 40% of wild type), and green indicates little or no significant effect (40% or more of wild type).

methods for details). Figure 1A shows the OAS structure superimposed upon the AfCCA structure, using the active site residues. Figure 1B shows a zoom in on the active site with OAS superimposed upon AfCCA and the active site residues used to guide the superimposition is shown in ball and stick. However, this way of constructing the superimposition, results in a slightly less accurate superimposition of the secondary structural element found distant from the active site (particularly the two helices found at the "back" of the β-sheet domain), but leads to a more accurate superimposition of the active site residues. Therefore, this superimposition is the best one for comparing the active site of OAS and AfCCA.

Identification of active site residues. We used the structural homology of OAS to AfCCA to identify putative active site residues in OAS. Residues in AfCCA, which has been implicated in catalysis by site-directed mutagenesis, or which makes obviously contact to the ATP in the AfCCA structure were identified and the corresponding residues in the OAS1 structure were target for mutagenesis (Fig. 1B). Subsequently, we produced 15 different mutants by an alanine scanning of the predicted active site residues and residues in the vicinity of the active site. We measured the production of 2–5A of the mutated proteins to identify residues critical for catalysis. The results of this mutagenesis are listed in Table 1.

The residue equivalent to R209 was mutated in the human OAS2 enzyme, where it has a substantial effect upon the activity of the enzyme [24]. We generated an R209A mutation in the porcine OAS1 and observed an effect similar to that seen in human OAS2. The structural equivalent residue to R209 has also been mutated in both PAP [9] and CCA [8], resulting in an enzymatically impaired enzyme. However, R209 is too

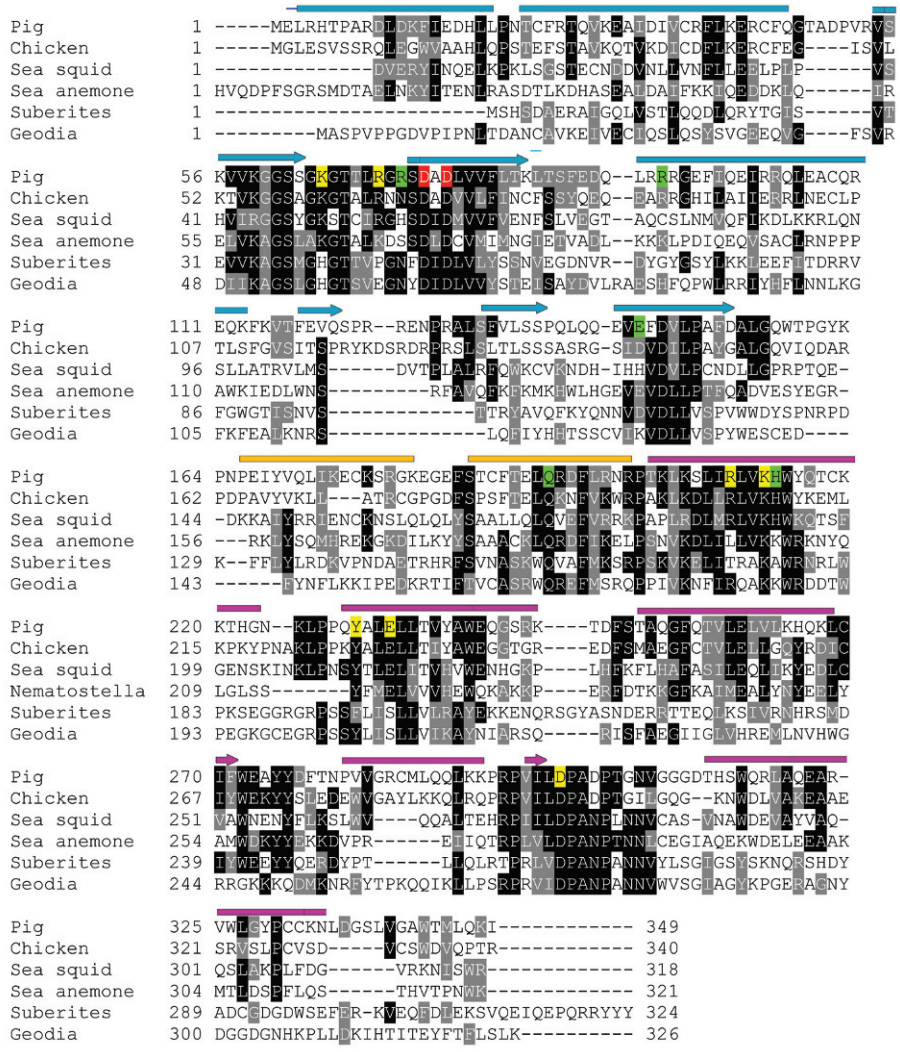

Figure 3. Alignment of distantly related OAS species. We aligned the porcine OAS1 sequence to the OAS from chicken, the two sea sponges *G. cydonium* and *S. domuncula* and to the putative OAS sequences from the starlet sea anemone *N. vectensis* and the sea squid *C. intestinalis* using the Clustal W program. The residues targeted for mutagenesis are highlighted using the same colour code as in Figure 2. The secondary structure elements (helices and sheets) are shown above the sequence. Cyan represents the N-terminal domain, orange represents the bridging helices found between the N- and C-terminal domains and purple represents the C-terminal domain.

far away from the active site to play any obviously role in catalysis (Fig. 2). It is possible that R209 interacts with the backbone of the flexible P-loop and thus indirectly stabilising the active site.

In the structure of AfCCA in complex with a substrate analogue mimicking the addition of the adenosine to the CCA tail of tRNA (1TFW), the adenosine base interacts with several residues conserved in OAS but not found in PAP. The residues Y230, E233 and D300 have direct counterparts in AfCCA (Table 1) and are highly conserved in different forms of OAS [1] (Fig. 3) but are not found in PAP. Mutating Y230A leads to a substantial loss of activity in OAS, whereas the more conservative mutation Y230F only has a marginal effect on activity. The aromatic side chain of Y230 is presumed to stack with base moiety of the incoming ATP molecule in our model (Fig. 2). Mutation of E233A leads to a substantial loss of activity, and this agrees with data from Yue *et al.* [25], who found that this mutation inhibited both CTP and ATP addition in the highly related *Sulfolobus shibatae* (AsCCA) enzyme, but disagrees with data from Okabe *et. al.* [26], who found no effect of mutating this residue on either CTP or ATP addition (in AfCCA). Our data suggest that Y230 and E233 of OAS interact with the incoming ATP in a manner similar to that seen in AfCCA [21]. The exact role played by E233 in catalysis is not clear. The carboxyl group of the equivalent residue in AfCCA, E176, is placed approximately 5 Å away from the β- and γ-phosphates of the ATP. Mutating D300A leads to a clear loss of activity, which is also seen in AsCCA but disagrees with data from AfCCA. Currently we do not have any explanation for the divergent results obtained by [26].

The mutational analysis was extended to residues in OAS that are not conserved in CCA-adding enzymes but are found in the vicinity of the active site. Mutating R72, R94, Q193, or H213 to alanine had little effect on the activity (Table 1), suggesting that these residues are not directly involved in catalysis. Similarly, the

Table 1. Activity of mutants[a].

			AfCCA		
Mutant	Activity (% of wt)	SD (% of activity)	Residue	Activity, (from [26])	Activity (from [24, 25])
Wt	100				
K65A	16.4	52.4	R50/K44	Active	Active
R70A	34.4	11.0	K55/R49		
R72A	64.4	11.0	S57/D51		
D74A/D76A	Inactive		E59/D61; D53/D55	Inactive	Inactive
R72N	103.0	61.2	S57/D51		
R94A	49	29.8	K72/K66		
E145A	45	2.8	E108/E104		Active
Q193A	49.1	3.0	H133/H129	Active	Active
R209A	1.6	34.6			
R209A/K212A	Inactive				
H213A	43.7	8.0	G153/R150		
Y230F	30.0	6.5	Y173/Y170		Active
Y230A	1.8	26.6	Y173/Y170	Active	Active
E233A	2.9	18.8	E176/E173	Active	Inactive
D300A	8.9	22.8	D218/D215	Active	Impaired

[a] The activity of the various mutants is listed as percentages of wild-type activity (normalised to 100 %). The SDs are listed as percentages of the actual activity for a given mutant. We have also listed the number of the equivalent residues in AfCCA and AsCCA for ease of comparison. The last two columns show a summary of the mutational studies performed previously on these enzymes [8, 25, 26]. As there is a substantial disagreement between the results obtained by [26] and [8, 25] we have cited the results from both groups.

conserved residue Glu145 (Glu104) has a limited effect on activity when mutated to alanine, which is in agreement with the data from [25]. Mutating R70 has some effect upon the activity (32 % of wild type) and mutating K65 leads to a substantial loss of activity (16 % of wild type). Both K65 and R70 are situated in a flexible loop between the first and second β-strand in the N-terminal domain of OAS. This part of the structure is not well ordered in OAS, possibly because it is stabilised by binding of the substrate and thus is disordered in the absence of substrate. Thus, it is difficult to predict the role played in catalysis based upon the current data.

The main characteristics differentiating the OAS proteins from the remaining template-independent polymerases are their 2' specificity and their ability to initiate synthesis without requiring a primer. The similarities identified between OAS and CCA are primarily concerned with the binding of the incoming nucleoside triphosphate and with the co-ordination of the metal ions required for catalysis. We believe that the 2' specificity is caused by the orientation of the sugar of the accepting AMP molecule, so that the 2'-hydroxyl is exposed the active site instead of the 3'-hydroxyl. However, as the binding site for the acceptor molecule has not been identified yet in OAS, we have little direct evidence for this.

Characteristics of the template-independent polymerases. Template-dependent polymerases grasp the nucleic acid in a hand-like structure simultaneously positioning the primer and template [27]. The nucleotidyl transferase reaction only occurs if the base of the incoming triphosphate nucleotide can form an appropriate Watson-Crick base pair with the template. There is very little contact between the base moiety and the protein during the catalysis step. In contrast, in both PAP and CCA, specific amino acids make direct contact with the base moiety of the incoming triphosphate nucleotide, thus accounting for selectivity in the absence of a template [21, 22, 28]. The similarity of OAS, PAP and class I CCA has been noted by others based on sequence analysis [5, 9, 29] and grouped together with DNA polymerase β. However, the template-independent polymerases share a common architecture that defines them as a separate subclass, where the catalytic site is found between the classic β-sheet domain and the α-helical bundle domain. The α-helical bundle domain is unique to this class of polymerases, whereas the β-sheet domain is both structurally and functionally homologous to the palm domain in template-dependent polymerases.

In addition to PAP, class I CCA, and OAS, there are other putative members of this class of template-independent polymerases. The structure of the RNA

editing terminal uridylyl transferase 2 (TUTase 2) from *Trypanosoma brucei* displays a clear structural homology to both OAS, AfCCA and PAP [30]. RDE-3 was identified by a genetic screen for RNA interference (RNAi) function in *Caenorhabditis elegans* and is required for siRNA accumulation and efficient RNAi function [31]. RDE-3 belongs to the same family of template-independent polymerases, as predicted by sequence comparison. This is further supported by the observation that motifs putatively identical to the active site of the nucleotidyl transferases are required for RDE-3 function [31]. Thus, RDE-3 can be added to this diverse family of template-independent polymerases involved in RNA metabolism. It appears that this class is not only structurally but also functionally related, as all enzymes identified so far are involved in some stage of RNA metabolism.

Another enzyme also exists that can polymerise nucleic acids without the use of a template. The terminal deoxynucleotidyltransferase (TdT) enzyme adds nucleotides to DNA ends without a template during generation of hypervariable loops in antibodies. This enzyme has a conserved hand-like structure [32] and is structurally more related to the template-dependent polymerases like polymerase-β, reflecting the fact that this enzyme still utilises dsDNA as the substrate and thus still binds to the dsDNA ends of DNA.

Evolutionary origin of the OAS proteins. The description of OAS as a member of this ancient class of template-independent polymerases has profound implication for our understanding of the evolutionary origin of the OAS gene family. Originally it was believed that the OAS system existed only in tetrapods [4, 33]. Based upon the evidence listed here, we find it highly likely that instead OAS originates from an ancestral template-independent polymerase; however, exactly when OAS diverged from the remainder of the family and became 2' specific remains unclear. Interestingly, an activity producing 2–5A independent of dsRNA stimulation has been identified in sea sponges and might be the first trace of OAS [34, 35]. Furthermore, genomic sequencing of *C. intestionalis* (sea squid, a chordate assumed to be a precursor to the vertebrate class) and *Callorhinchus milii* (elephant shark) revealed genes with a high degree of identity to the OAS gene family [36]. Bioinformatic searches in available databases revealed a putative OAS sequence in *N. vectensis* (starlet sea anemone), as well as several EST sequences from invertebrate species. Figure 3 shows an alignment of OAS1 from pigs, chicken, Sea Squid, starlet sea anemone and the two marine sponges. The existence of OAS in several invertebrate species as well as in sea sponges suggests that the OAS enzymes diverged from the PAP and CCA at the beginning of metazoan evolution. This is further supported by the fact that OAS has a much closer evolutionary link with the class I CCA-adding enzymes from archaea than with the class II CCA-adding enzymes found in eukaryotic species.

The only known function of OAS proteins is the production of 2–5A for the activation of RNase L. However, it is possible that the RNase L-OAS pathway is a late addition in evolution, possibly as late as the establishment of the tetrapods. This would suggest a function of OAS apart from activation of RNase L, probably involving RNA metabolism as is the case with the other members of this family. At the present this is speculative and needs further work, but we suggest that the OAS proteins had a role prior to the establishment of the RNase L pathway, and that this role might well be conserved in mammalian OAS proteins.

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1 Justesen, J., Hartmann, R. and Kjeldgaard, N. O. (2000) Gene structure and function of the 2'-5'-oligoadenylate synthetase family. Cell. Mol. Life Sci. 57, 1593–612.
2 Rebouillat, D. and Hovanessian, A. G. (1999) The human 2',5'-oligoadenylate synthetase family: Interferon-induced proteins with unique enzymatic properties. J. Interferon Cytokine Res. 19, 295–308.
3 Silverman, R. H. (2007) A scientific journey through the 2–5A/RNase L system. Cytokine Growth Factor Rev. 18, 381–8.
4 Cayley, P. J., White, R. F., Antoniw, J. F., Walesby, N. J. and Kerr, I. M. (1982) Distribution of the ppp(A2'p)nA-binding protein and interferon-related enzymes in animals, plants, and lower organisms. Biochem. Biophys. Res. Commun. 108, 1243–50.
5 Holm, L. and Sander, C. (1995) DNA polymerase beta belongs to an ancient nucleotidyltransferase superfamily. Trends Biochem. Sci. 20, 345–7.
6 Sarkar, S. N., Ghosh, A., Wang, H. W., Sung, S. S. and Sen, G. C. (1999) The nature of the catalytic domain of 2'-5'-oligoadenylate synthetases. J. Biol. Chem. 274, 25535–42.
7 Hartmann, R., Justesen, J., Sarkar, S. N., Sen, G. C. and Yee, V. C. (2003) Crystal structure of the 2'-specific and double-stranded RNA-activated interferon-induced antiviral protein 2'-5'-oligoadenylate synthetase. Mol. Cell 12, 1173–85.
8 Cho, H. D., Verlinde, C. L. and Weiner, A. M. (2005) Archaeal CCA-adding enzymes: Central role of a highly conserved beta-turn motif in RNA polymerization without translocation. J. Biol. Chem. 280, 9555–66.
9 Martin, G., Jeno, P. and Keller, W. (1999) Mapping of ATP binding regions in poly(A) polymerases by photoaffinity labeling and by mutational analysis identifies a domain conserved in many nucleotidyltransferases. Protein Sci. 8, 2380–91.

10 Steitz, T. A. (1998) A mechanism for all polymerases. Nature 391, 231–2.
11 Hovanessian, A. G. and Justesen, J. (2007) The human 2'-5'oligoadenylate synthetase family: Unique interferon-inducible enzymes catalyzing 2'-5' instead of 3'-5' phosphodiester bond formation. Biochimie 89, 779–88.
12 Hovanessian, A. G., Laurent, A. G., Chebath, J., Galabru, J., Robert, N. and Svab, J. (1987) Identification of 69-kd and 100-kd forms of 2–5A synthetase in interferon-treated human cells by specific monoclonal antibodies. EMBO J. 6, 1273–80.
13 Hartmann, R., Olsen, H. S., Widder, S., Jorgensen, R. and Justesen, J. (1998) p59OASL, a 2'-5' oligoadenylate synthetase like protein: A novel human gene related to the 2'-5' oligoadenylate synthetase family. Nucleic Acids Res. 26, 4121–8.
14 Rebouillat, D., Marie, I. and Hovanessian, A. G. (1998) Molecular cloning and characterization of two related and interferon-induced 56-kDa and 30-kDa proteins highly similar to 2'-5' oligoadenylate synthetase. Eur. J. Biochem. 257, 319–30.
15 Kumar, S., Mitnik, C., Valente, G. and Floyd-Smith, G. (2000) Expansion and molecular evolution of the interferon-induced 2'-5' oligoadenylate synthetase gene family. Mol. Biol. Evol. 17, 738–50.
16 Jones, T. A., Zou, J. Y., Cowan, S. W. and Kjeldgaard, M. (1991) Improved methods for building protein models in electron density maps and the location of errors in these models. Acta Crystallogr. A 47, 110–9.
17 Justesen, J., Ferbus, D. and Thang, M. N. (1980) 2'5' oligoadenylate synthetase, an interferon induced enzyme: Direct assay methods for the products, 2'5' oligoadenylates and 2'5' co-oligonucleotides. Nucleic Acids Res. 8, 3073–85.
18 Martin, G., Keller, W. and Doublie, S. (2000) Crystal structure of mammalian poly(A) polymerase in complex with an analog of ATP. EMBO J. 19, 4193–203.
19 Bard, J., Zhelkovsky, A. M., Helmling, S., Earnest, T. N., Moore, C. L. and Bohm, A. (2000) Structure of yeast poly(A) polymerase alone and in complex with 3'-dATP. Science 289, 1346–9.
20 Balbo, P. B. and Bohm, A. (2007) Mechanism of poly(A) polymerase: Structure of the enzyme-MgATP-RNA ternary complex and kinetic analysis. Structure 15, 1117–31.
21 Xiong, Y. and Steitz, T. A. (2004) Mechanism of transfer RNA maturation by CCA-adding enzyme without using an oligonucleotide template. Nature 430, 640–5.
22 Tomita, K., Fukai, S., Ishitani, R., Ueda, T., Takeuchi, N., Vassylyev, D. G. and Nureki, O. (2004) Structural basis for template-independent RNA polymerization. Nature 430, 700–4.
23 Emsley, P. and Cowtan, K. (2004) Coot: Model-building tools for molecular graphics. Acta Crystallogr. D Biol. Crystallogr. 60, 2126–32.
24 Sarkar, S. N., Miyagi, M., Crabb, J. W. and Sen, G. C. (2002) Identification of the substrate-binding sites of 2'-5'-oligoadenylate synthetase. J. Biol. Chem. 277, 24321–30.
25 Yue, D., Weiner, A. M. and Maizels, N. (1998) The CCA-adding enzyme has a single active site. J. Biol. Chem. 273, 29693–700.
26 Okabe, M., Tomita, K., Ishitani, R., Ishii, R., Takeuchi, N., Arisaka, F., Nureki, O. and Yokoyama, S. (2003) Divergent evolutions of trinucleotide polymerization revealed by an archaeal CCA-adding enzyme structure. EMBO J. 22, 5918–27.
27 Steitz, T. A. (1999) DNA polymerases: Structural diversity and common mechanisms. J. Biol. Chem. 274, 17395–8.
28 Tomita, K., Ishitani, R., Fukai, S. and Nureki, O. (2006) Complete crystallographic analysis of the dynamics of CCA sequence addition. Nature 443, 956–60.
29 Yue, D., Maizels, N. and Weiner, A. M. (1996) CCA-adding enzymes and poly(A) polymerases are all members of the same nucleotidyltransferase superfamily: Characterization of the CCA-adding enzyme from the archaeal hyperthermophile *Sulfolobus shibatae.* RNA 2, 895–908.
30 Deng, J., Ernst, N. L., Turley, S., Stuart, K. D. and Hol, W. G. (2005) Structural basis for UTP specificity of RNA editing TUTases from *Trypanosoma brucei.* EMBO J. 24, 4007–17.
31 Chen, C. C., Simard, M. J., Tabara, H., Brownell, D. R., McCollough, J. A. and Mello, C. C. (2005) A member of the polymerase beta nucleotidyltransferase superfamily is required for RNA interference in *C. elegans.* Curr. Biol. 15, 378–83.
32 Delarue, M., Boule, J. B., Lescar, J., Expert-Bezancon, N., Jourdan, N., Sukumar, N., Rougeon, F. and Papanicolaou, C. (2002) Crystal structures of a template-independent DNA polymerase: Murine terminal deoxynucleotidyltransferase. EMBO J. 21, 427–39.
33 Stark, G. R., Dower, W. J., Schimke, R. T., Brown, R. E. and Kerr, I. M. (1979) 2–5A synthetase: Assay, distribution and variation with growth or hormone status. Nature 278, 471–3.
34 Kuusksalu, A., Pihlak, A., Muller, W. E. and Kelve, M. (1995) The (2'-5')oligoadenylate synthetase is present in the lowest multicellular organisms, the marine sponges. Demonstration of the existence and identification of its reaction products. Eur. J. Biochem. 232, 351–7.
35 Wiens, M., Koziol, C., Hassanein, H. M., Muller, I. M. and Muller, W. E. (1999) A homolog of the putative tumor suppressor QM in the sponge *Suberites domuncula:* Down-regulation during the transition from immortal to mortal (apoptotic) cells. Tissue Cell 31, 163–9.
36 Venkatesh, B., Kirkness, E. F., Loh, Y. H., Halpern, A. L., Lee, A. P., Johnson, J., Dandona, N., Viswanathan, L. D., Tay, A., Venter, J. C., Strausberg, R. L. and Brenner, S. (2007) Survey sequencing and comparative analysis of the Elephant Shark (*Callorhinchus milii*) genome. PLoS Biol 5, e101.