A COMPARATIVE ANALYSIS OF THREE CLASSES OF BACTERIAL NON-SPECIFIC ACID PHOSPHATASES AND ARCHAEOAL PHOSPHOESTERASES: EVOLUTIONARY PERSPECTIVE

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ABSTRACT

Introduction: Bacterial nonspecific acid phosphohydrolases (NSAPs) or phosphatases are group of enzymes secreted as soluble periplasmic proteins or retained as membrane bound lipoproteins that are usually able to dephosphorylate a broad array of structurally unrelated organic phosphoesters (nucleotides, sugar phosphates, phytic acid etc.) to acquire inorganic phosphate (Pi) and organic byproducts. They exhibit optimal catalytic activity at acidic to neutral pH values. On the basis of amino acid sequence relatedness, phosphatase are grouped into different molecular families namely Class A, Class B and Class C acid phosphatase respectively. Results and discussion: In this article out of thirty three sequences, twenty six belonging to each of the three classes of bacterial acid phosphatase and seven belonging to archaean phophoesterases were analyzed using various tools of bioinformatics. Phylogenetic analysis, dot plot comparisons and motif analysis were done to identify a number of similarities and differences between three classes of bacterial acid phosphatases and archaean phosphoesterases. In this research we have attempted to decipher evolutionary relationship between three classes of bacterial acid phosphatase and archaean phosphoesterases using bioinformatics approach.

Key words: Non-specific acid phosphatase, archaean phosphoesterase, NSAP, evolution.

1. INTRODUCTION

Phosphatases constitute a diverse group of enzymes that hydrolyze phosphoesters in various kinds of substrates under different conditions (1). Based on the criteria such as specificity and optimum pH, phosphatase can be classified into several families, one of which is the family of bacterial non-specific acid phosphohydrolases or phosphatases (NSAPs). In general, acid phosphatases hydrolyze phosphoester esters via the two-step mechanism shown in Scheme 1 (1).

\[
E + ROPO_2H \rightarrow E \cdot ROPO_2 \rightarrow E \cdot PO_2H \rightarrow E + Pi
\]

NSAPs are found to be widely distributed among many Gram-positive, Gram-negative bacterial species and eukaryotes. NSAPs are considered physiologically important because they either help the cell to utilize the organic phosphoesters that cannot cross the cytoplasmic membrane thus providing the cell with essential nutrients (2) or have functional importance in gene expression (3). Some of these enzymes can be secreted outside the cell, where they are either released in soluble form or retained as membrane-bound proteins where they can dephosphorylate a broad array of structurally unrelated phosphoester substrates and exhibit optimal catalytic activity at acidic to neutral pH values (Rossolini et al., 1998) (4). In addition to their role in P acquisition, phosphohydrolases regulate cellular metabolism, are involved in signal transduction and may also be critical to bacterial pathogenicity (5, 6, 7).

NSAPs are usually grouped into three types (Classes A, B and C) on the basis of amino acid sequence relatedness (8, 9, 10, 11). The structural criterion has led to the definition of various molecular families of phosphohydrolases for which the signature sequence motifs have been identified (12). NSAPs have been detected in several microbial taxa, and the enzymes of different classes can be produced by the same bacterial species (4). Class A NSAPs are secreted monomeric to oligomeric proteins containing a polypeptide component of approximately 25-27 kDa (8, 13, 14, 15, 16). This group of enzymes has recently been demonstrated to share some conserved sequence motifs with other bacterial and eukaryotic phosphatases, suggesting that the conserved residues could be essential for catalytic activity and possibly part of the active site.
of these enzymes (17). The *Zymomonas mobilis* PhoC-Zm enzyme represents the major Pi-irrepressible acid phosphohydrolases and was the first sequenced class A enzyme (13).

Class B NSAPs are secreted homotrimeric metallo-proteins containing a 25-kDa polypeptide component (10, 11, 18,) that are completely unrelated to class A enzymes at the sequence level. The *Salmonella enterica* Apha-Se enzyme was the first class B NSAP purified and characterized in detail (18, 19). Class C NSAPs are secreted bacterial lipoproteins that contain a polypeptide component with a molecular mass of approximately 30kDa and share conserved sequence motifs (11, 20). At the sequence level Class C appear to be related, although distantly, to Class B NSAPs and also to some plant acid phosphohydrolases (4).

This represents the first example of family bacterial NSAPs that has a relatively close eukaryotic counterpart. The first identified Class C enzyme was OlpA enzyme of *Chryseobacterium meningosepticum* (21). All the three class contain conserved signature sequence motifs (22).

These structural similarities together with dephosphorylating activity exhibited by bacterial, eukaryotic and archaeal phosphatases support the definition of this phosphatase motif and the inclusion of all of these enzymes into a molecular superfamily of phosphohydrolases which is indicated as “DDDD” due to the couple of invariant aspartate residues present in each domain. (4). The invariant residues could be essential for the phosphohydrolase catalytic activity of these enzymes and part of active site (22). Thus, the similar catalytic mechanism of all the bacterial acid phosphatase, archaeal phosphatase and archaeal membrane bound phosphoesterases belonging to phosphohydrolase family would seem to suggest that they evolved from a common ancestor. In this article, we analyzed the sequences of the three classes of bacterial acid phosphatase and archaeal phosphoesterase to determine their evolution using bioinformatics tools.

**2. METHODS**

The sequences for bacterial acid phosphatases and archaeal phosphoesters were obtained from the NCBI website (http://www.ncbi.nlm.nih.gov/). From the available several acid phosphatase sequences, only the bacterial and archaeal acid phosphatases were selected whereas the viral and eukaryotic sequences were excluded. A total of 26 sequences belonging to bacterial acid phosphatase and 7 sequences belonging to archaeal phosphoesterases were meticulously chosen based on the size of sequences, substrate specificity, structural criterion, functional differences, biophysical features, distribution of NSAPs/ organism origin, signature sequence motifs, conserved structural motifs. We obtained 10 sequences belonging to bacterial Class A acid phosphatases, 7 sequences belonging to bacterial Class B acid phosphatases, 9 sequences belonging to bacterial Class C acid phosphatases, and 7 sequences belonging to archaeal phosphoesterases. Structural and phylogenetic relationships have existed among various bacterial NSAPs and some other bacterial and eukaryotic phosphohydrolases. Here for the first time we tried to analyze the evolution of bacterial acid phosphatases and archaeal phosphoesterases using bioinformatics tools.

Similarity motifs were identified in all the sequences using MEME Motif discovery tool. All the settings were set to default, except for the maximum number of Motifs which was increased from three to thirteen (23). The phylogenetic tree and motif analysis were then used to construct dot plots. The position of a specific amino acid motif in the selected protein sequence was found by dot plots.

In order to compare the similarity and differences in the sequences of each class of bacterial acid phosphatases and archaeal phosphoesterases the dot matcher program was used to construct dot plots. The similarity in the protein sequences can be easily accessed from dot plots simply by seeing the diagonal fragment in between the X and Y axis of a graph, which is constructed by using data matrix, distance matrix and chi-squared analysis (24). Here similar sequences show a diagonal line
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**MOTIF 2**

15, 80  DDTVLFSSPG  FWVRGKTFTSFESEDLYLKNVFWEKMNNGWDEFSPKEWARGDGRMVKRMVRG  DAIFVFTGRS
17, 79  DDTVLFSSPG  FWVRGKTTSDFSDYLKNNDFWPKMNNNGWEFSPEKEWARGDGRMVKRMVRG  DSIFVFTGRS
14, 80  DDTVLFSSPG  FWVRKKNFTSFESEDLYLKNVFWEKMNNGWDEFSPKEWARGDGRMVKRMVRG  DAIFVFTGRS
16, 80  DDTVLFSSPG  FWVRKKNFTSFESEDLYLKNVFWEKMNNGWDEFSPKEWARGDGRMVKRMVRG  DSIFVFTGRS
13, 80  DDTVLFSSPG  FSIRGQKTFSDFSDYLKNDFWPKMNNNGWEFSPEKEWARGDGRMVKRMVRG  DSIFVFTGRS
12, 79  DDTVLFSSPG  FSIRGQKTFSDFSDYLKNDFWPKMNNNGWEFSPEKEWARGDGRMVKRMVRG  DTIVYFTGRS
11, 78  DDTVLFSSPG  FSIRGQKTFSDFSDYLKNDFWPKMNNNGWEFSPEKEWARGDGRMVKRMVRG  DTIVYFTGRS

**MOTIF 3**

8, 102  VANAFSSAFG  SITSGKDAQOLKLLTINMEDALINSLATRS  AKEKYMIRP
5, 102  VANAFSEAFG  SITSGKDAQOLKLLTINMEDALINSLATRS  AKEKYMIRP
19, 155  OQADDNGVOI  YYSGRTSTODATMENLOLDGEVQGRD  HLLFLEEGKV
7, 102  VANAFSFAFG  SITSGKDAQALKLLTINMEDALINSLATRS  AKDYHYMIRP
17, 132  DMMVRGODSI  YFTFRSQTKEITVSCLKALNFIATANMM  NVPVIAGDFK
16, 133  DMMVRGODSI  YFTFRSQTKEITVSCLKALNFIATANMM  NVPVIAGDFK
15, 133  DMMVRGODSI  YFTFRSQTKEITVSCLKALNFIATANMM  NVPVIAGDFK
14, 133  DMMVRGODSI  YFTFRSQTKEITVSCLKALNFIATANMM  NVPVIAGDFK
6, 102  VANAFSFAFG  SITSGKDAQOLKLLTINMEDALINSLATRS  AKDYHYMIRP
24, 143  KYTESKGVDI  YYSNRFKNOQATNKLERNVQATKE  HILDFPKEK
22, 136  KYTESKGVDI  YYSNRFKNOQATNKLERNVQATKE  HILDFPKEK
23, 143  KYTESKGVDI  YYSNRFKNOQATNKLERNVQATKE  HILDFPKEK
9, 102  VANAFSSAFG  SITSGKDAQALKLLTINMEDALINSLATRS  AKEKYMIRP
2, 155  OFADDNGVOI  YYSSRASDAODATMENLOLDGEVQGRD  HLLFLEEGKV
20, 155  OFADDNGVOI  YYSSRASDAODATMENLOLDGEVQGRD  HLLFLEEGKV
13, 133  AMVHRGODSI  WFTVRQSTKKEITVSCLKLDLFIATANMM  NVPVIAGDFK
18, 160  KYANEKGKIKI  YYSQRTSTODATMENLOLDGEVQGRDM  HLLFLEXGKM
12, 132  DQHMLRGODSI  YFTFRSQTKEITVSCLKALNFIATANMM  NVPVIAGDFK
4, 92  IARIFSPWVG  AKINQIOTETQNMKLNLGMYATAS  AKEKYMIRP
3, 92  IARIFSPWVG  AKINQIOTETQNMKLNLGMYATAS  AKEKYMIRP
11, 131  NH4HARGDQGV  YFTFTTDAKQDAGTTLKETKNIKNMSPF  VEMGSRERT

**MOTIF 5**

15, 59  SVADIENSLA  GRTYMAVMFGFDITDLVFSSPG  FWVRGKTFTSF
17, 58  SVADIENSLT  GRTYMAVMFGFDITDLVFSSPG  FWVRGKTFTSF
16, 59  SVADIENSLT  GRTYMAVMFGFDITDLVFSSPG  FWVRGKTFTSF
14, 59  SVADIENSLA  GRTYMAVMFGFDITDLVFSSPG  FWVRGKTFTSF
21, 89  DRLKDNLDQQA  TIKYVSVLDITTVLNSY  QAKNVLEGT
19, 89  DRLKDELDNKP  TIKYVSVLDITTVLNSY  QAKNVLEGT
20, 89  DRLKDNLDQQA  TIKYVSVLDITTVLNSY  QAKNVLEGT
13, 59  SVADIENSSL  GRTYMAVMFGFDITDLVFSSPG  FSIRGQKTFSF
24, 78  LKLDAILAKG  TEKKAILVLKTVINSFHP  QAMSVKSGK
22, 71  LKLDAILAKG  TEKKAILVLKTVINSFHP  QAMSVKSGK
23, 78  LKLDAILAKG  TEKKAILVLKTVINSFHP  QAMSVKSGK
12, 58  ISVEDEEESL  KQGGTMAVMFGFDITDLVFSSPG  FYRGRKLYFSP
18, 94  MKLIDWLOKPK  SEKYSSISDDITTVLNSY  QAKNVEQSSS
25, 90  RVVDLQVEEP  TAKVTVLUTQITDQNSP  AGYVXHNNEL
11, 57  SVDDIKNSLE  SKAAANVMFMFGFDITDLVFSSPG  FHYQGQDFSPG
26, 72  KMAFDHAKAK  KSKKAKAVVOLQITDQNSAP  AGWVDQGSGG

**MOTIF 7**

24, 43  EEEKVKLQDQQ  LMAOLOKITATGEMKALYYGTGNTGQTDQLKLD  AALAKGEKKEK
23, 43  EEEKVKLQDQQ  LMAOLOKITATGEMKALYYGTGNTGQTDQLKLD  AALAKGEKKEK
22, 36  EEEKVKLQDQQ  LMAOLOKITATGEMKALYYGTGNTGQTDQLKLD  AALAKGEKKEK
18, 59  SSIIKLLAKEN  TMSVLFVONSAAEAKAILQLGWNRMLDK  DMLDPKSEPK
21, 54  SDEQURTJNEN  TMSVLFVOTMAAEAKAILQLGWNRMLDK  NLDQIATDKP
20, 54  SDEQURTJNEN  TMSVLFVOTMAAEAKAILQLGWNRMLDK  NLDQIATDKP
19, 54  SDEQURTJNEN  TMSVLFVOTMAAEAKAILQLGWNRMLDK  NLDQIATDKP
25, 55  NAVEEVNKG  VATLWLVONSSEVKALREAKNSKRVWD  ELVKEKPARK
26, 38  ANQAKLQDDQV  AGMLVWTQOGTETALQAQFNSEQAKKFMD  HAKAKKHKKKK

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whereas in dissimilar sequences this line is absent or highly fragmented. We first constructed the dot plots by using sequences belonging to same class bacterial acid phosphatase and archaeal phosphoesterases and then used each sequences from different class using different combinations of different class of bacterial and archaeal phosphoesterases each time. The parameters of the program were mostly set at default except for the window size of 10 and threshold of 23 (25).

The selected sequences were obtained in FASTA format and then aligned by using Clustal X (Thompson et al., 1997) (26). Neighbour joining method was used to construct the phylogenetic tree from the sequences which were aligned using PHYLIP (27). The phylogenetic tree was then bootstrapped in order to see how well the sequences related to each other. Finally, treview was used to see their position in each clade and the study of bacterial acid phosphatase and archaeal phosphoesterases were related by evolution (28).

3. RESULTS

Analysis of motifs of bacterial acid phosphatases and archaeal phosphoesterases reveals distinct features in all three classes of bacterial acid phosphatases and archaeal acid phosphatases. As seen in Figure 1a, Class A acid phosphatase consisting of sequences 1 (Rahnella sp. Y9602) through 10 (Enterobacter aerogenes) share a common Motif 1 and Motif 4. Class B acid phosphatase consisting of sequences 11 (Haemophilus influenzae) through 17 (Salmonella enterica) share a common Motif 2 and Motif 9. Class C acid phosphatase consisting of sequences 18 (Streptococcus agalactiae 2603V/R) through 26 (Pasteurella multocida) share common Motif 7 and Motif 12. Sequences 27 (Thermococcus gammatolerans EJ3) through 33 (Methanocococcus vulcanus M7) belonging Archaeal phosphoesterases have only one common Motif 13. Motif 5 and Motif 10 is common to Class B and Class C acid phosphatase. Motif 6 is common to Class A acid phosphatase from sequences 2 through 10 and Class B acid phosphatase from sequences 11 through 17. Motif 3 is common to sequences 3 through 10 of Class A acid phosphatase, sequences 11 through 17 of Class B acid phosphatase and sequences 18 through 25 of Class C acid phosphatase. The degree of similarity between the protein sequences of the three classes of bacterial acid phosphatase and archaeal phosphoesterase can be seen by comparing the dot plots in Figure 2a. The dot plots constructed with sequences belonging to same class shows linear graph whereas the dot plots constructed between two different bacterial acid phosphatase classes and archaeal phosphoesterase class show a high degree of dissimilarity or fragmentation as seen in Figure 2b. These findings correlate with the motifs discovered which are shared only by that particular class.

As seen in Figure 3, the phylogenetic analysis of the three classes of bacterial acid phosphatase and archaeal phosphoesterase resulted in the formation of a tree with three distinct clades for Class A, Class B-Class C and archaeal phosphoesterase, as we can see from the tree that the bootstrap values of three classes of bacterial acid phosphatase and archaeal phosphoesterase is mostly above ninety, we can infer that bacterial acid phosphatase and archaeal phosphoesterase share a common ancestor.

4. DISCUSSION

Multiple sequence alignment was performed on 26 bacterial acid phosphatase and 7 archaeal phosphoesterase giving sufficient evidence to conclude that they have a common evolutionary origin. The phylogenetic tree shows three distinct clades;
one clade belonging to Class A bacterial acid phosphatase, second clade belonging to Class B and Class C being closely related (4); third clade belonging to archaeal phosphoesterases providing a definitive evidence of common ancestral origin of bacterial acid phosphatase and archaeal phosphoesterase with divergent evolution. Our analysis found sequence 4 (Salmonella enterica subsp. enterica serovar Typhimurium) is a Class A acid phosphatase and also Class B acid phosphatase as seen in sequence 16. This shows that enzymes of different classes is produced by same bacterial species (8, 10, 14, 29).

Membrane bound type 2 phosphatidic acid phosphatase (PAP2) shares sequence motifs with a soluble vanadium-dependent chloroperoxidase of known structure. These regions are also conserved in other soluble and membrane bound proteins including bacterial acid phosphatases, mammalian glucose-6-phosphatases and the Drosophila developmental protein Wunen. This shows that similar arrangement of the catalytic residues specifies the active site within the soluble and membrane spanning domains (30). Vanadate and vanadate derivatives have been employed to interrogate a range of enzymes that interact with phosphorylated substrates (31). Acid phosphatase enzymes have evolved to accommodate vanadate as a redox cofactor (32, 33).

In this article, archaeal phosphatase belonging to vanadium-dependent haloperoxidase superfamily (Thermococcus gammatolerans EJ3) representing sequence 27 has been included as well as membrane-bound phosphoesterase, belonging to PAP2 superfamily (Thermococcus gammatolerans EJ3) representing sequence 31 is also studied. Our analysis found conserved sequence motif 13 in the soluble and membrane bound phosphatase enzyme. Also, sequence 32 (Methanocaldococcus sp. FS406-22) phosphoesterase PA-phosphatase related protein and sequence 33 (Methanocaldococcus vulcanius M7) a phosphoesterase PA-phosphatase related protein sharing conserved sequence motif 13 with all of the other archaeal membrane bound phosphoesterases and soluble phosphatase. This analysis suggests that membrane-associated PAP2 like proteins share conserved structural elements and similar catalytic mechanism with related soluble globular enzymes (haloperoxidases, bacterial acid phosphatases, ATP diphosphohydrolase). Assuming divergent evolution, this implies that catalytic ac-

**Figure 2a.** The dot plot comparison of sequences belonging to same classes resulted in collinear diagonal fragment. The dot plots shown are within the same class of bacterial acid phosphatase ClassA/ClassA, ClassB/ClassB, ClassC/ClassC and archaeal phosphoesterases, Archaea/Archaea.

**Figure 2b.** The dot plot comparison of sequences belonging to different classes such as ClassA/ClassB, ClassB/ClassC, ClassC/ClassA, Archaea/ClassB resulted in a plot with non-collinear fragments.
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Figure 3: A Phylogenetic tree constructed by using amino acid sequences belonging to all the tree classes of bacterial acid phosphatase and archaeal phosphoesterase. The name of the bacteria with the class it belongs to makes the first and second clade and archaea that makes the third clade is shown on the right hand side of the cladogram. The scores seen on the tree do degree of sequence similarity between the sequences of each class. The names of bacteria and archaea along with the accession numbers in parentheses are as follows: The numbers 1 to 10 represent Class A bacterial acid phosphatase. Numbers 11 to 17 represent Class B acid phosphatase, numbers 18 to 26 represent Class C acid phosphatase and number 27-33 represent Archaeal phosphoesterase. 1. Rathnella sp. Y9602 (ADW727173.1), 2. Zymomonas mobilis (AAY27700.1), 3. Salmonella enterica subspp. enterica serovar Typhi str. CT18 (NP_458615.1), 4. Salmonella enterica subspp. enterica serovar Typhimurium (AAI47560.1), 5. Providencia retiarum (PA698298.1), 6. Enterobacter cloacae SCF1 (YP003942142.1), 7. Escherichia coli blattae (BA484942.1), 8. Klebsiella pneumoniae subspp. rhinoscleromatis ATCC 13884 (YP.060162171.1), 9. Raoultella planticola (BAB18198.1), 10. Enterobacter aerogenes (ABW37174.1), 11. Haemophilus influenzae (CA68889.1), 12. Morganella morganii (CAAS131.1), 13. Klebsiella pneumoniae (AAL59317.1), 14. Shigella boydii CDC 3083-94 (ACD06580.1), 15. Escherichia coli str. K-12 substr. MG1655 (CAAC5534.1), 16. Salmonella enterica subspp. enterica serovar Typhimurium (AAW28968.1), 17. Salmonella enterica (CA40974.1), 18. Streptococcus agalactiae 2603V8/9 (NP_688757.1), 19. Streptococcus pyogenes (MGAS5463.1) (YP.597336.1), 20. Streptococcus dysgalactiae subspp. equisimilis G55 124 (YP. 002997631.1), 21. Streptococcus dysgalactiae subspp. equisimilis (CAAC3715.1), 22. Bacillus sp. FRC Y9-2 (AB060628.1), 24. Bacillus anthracis str. Ames Ancestor (YP.921394.1), 25. Clostridium perfringens (ACB11490.1), 26. Pasteurella multocida (ACMB930.1), 27. Thermococcus gammatolerans EJ3 (YP.002960201.1), 28. Thermococcus kodakarenensis KO01 (BAB04854.1), 29. Thermococcus sibiricus MM 739 (YP.002993470.1), 30. Thermococcus sp. AM4 (YP.08400406.1), 31. Thermococcus gammatolerans EJ3 (YP.002959503.1), 32. Methanocaldococcus sp. F5406-22 (YP.003458929.1), 33. Methanocaldococcus vulcanus M7 (YP.003427626.1).

The analyses of the different motifs show that bacterial Class A has conserved sequence motifs 1 and 4. Bacterial Class B has conserved sequence motifs 2 and 9; bacterial Class C has unique motifs 7 and 12; archaeal phosphoesterase has unique motif 13. Class A, B, and C share common Motif 3 and Class A and B share common Motif 5 and Class B and C share a common Motif 5 and 10. Archaeal phosphoesterase do not share any common Motifs with any of the bacterial Class A, B and C acid phosphatases except sequence 28 (Thermococcus kodakarenensis KOD1) share Motif 12 with Class C bacterial acid phosphatase. It suggests that it must have evolved and adapted separately but seem to have a common ancestral origin. The high bootstrap scores also reveal the common ancestral origin of bacterial acid phosphatase and archaeal phosphoesterase.

The dot-plots which were used as a comparative tool between the two sequences showed a high degree of similarity within members of the same class. However, dot plots between members of different classes resulted in multiple fragments without solid collinear lines suggesting no similarity between sequences of different classes. These results are consistent with the motif analysis where each class of bacterial acid phosphatase and archaeal phosphoesterase has unique motifs and share only one motif between all the three classes of acid phosphatase. Thus by comparing the dot plots, motif analysis, protein sequences, phylogenetic analysis we could conclude that the three classes of bacterial acid phosphatase and archaeal phosphoesterases have evolved separately but have a common ancestry.

In this research we considered only the bacterial and archaeal se sequences. Further research may be done by comparing all other soluble globular and membrane associated proteins to get a more comprehensive picture of evolution of phosphoesterases. This knowledge will be useful to correlate bacterial, eukaryotic and archaeal world. This study may also offer insight into the study of immobilization of toxic uranium (U VI) mediated by the intrinsically phosphate activity of naturally occurring bacteria isolated from contaminated subsurface soils (Martinez et.al., 2007) (34). It also may be promising towards bio remediation of contaminated soils, ground water and waste streams using bacteria and archaea.

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