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Phylogenetic analysis of Na^+/H^+ (NuoL/MrpA) antiporters

Análisis filogenético de los antiportadores Na^+/H^+ (NuoL/MrpA)

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Abstract

Objectives: Sodium/proton (Na+/H+) antiporters NuoL/MrpA-like proteins are important in monovalent cations homeostasis, ATP synthesis, are involved in growth using low concentrations of acetate, and in management of protons during methane production. To learn more about the evolutive origin and biological relevance of this protein, in this work a phylogenetic analysis of the NuoL/MrpA superfamily of proteins was done. Methodology: Phylogeny reconstruction was done with 596 NuoL/MrpA proteins and 39 MrpD-NuoM/N proteins. The algorithms used were minimum evolution and maximum likelihood, using MEGA program. Additionally, a conserved domain analysis was done. Contribution: NuoL/MrpA superfamily and their homologous proteins, MrpD-NuoM/N, form two paralogous groups. The NuoL/MrpA superfamily consists of two families. Family NuoL consist of arqueal, bacterial and eukaryotic proteins of around 600 aa in size. Family MrpA are formed by proteins from bacteria and archaea, with a 600 to 850 aa in size. Using the phylogenetic analysis and conserved domain analysis, a superfamily NuoL/MrpA evolution model was proposed.

Phylogeny, antiporters Na^+/H^+, NuoL/MrpA

Resumen

Objetivos: Las proteínas antiportadores de Na^+/H^+ similares a NuoL/MrpA son importantes para la homeostasis de cationes monovalentes, la correcta síntesis de ATP, el crecimiento a bajas concentraciones de acetato, y se piensa que es importante el manejo de los protones durante la producción de metano. Para conocer más sobre origen evolutivo y relevancia biológica de estas proteínas, en este trabajo se llevó a cabo un análisis filogenético de la superfamilia de proteínas NuoL/MrpA. Metodología: La filogenia se efectuó usando 596 proteínas NuoL/MrpA y 39 MrpD-NuoM/N. Los algoritmos de reconstrucción usados fueron el de mínima evolución y máxima verosimilitud, en el programa MEGA. Además, se llevó a cabo un análisis de dominios conservados (CDD). Contribución: Las proteínas NuoL/MrpA y sus homólogos, MrpD-NuoM/N, forman dos grupos parálogos. La superfamilia NuoL/MrpA consta de dos familias. La familia NuoL que está formada por proteínas de arqueas, bacterias y eucarióticas de alrededor de 600. La familia MrpD, formada por proteínas de 600 a 850 aa de bacterias y arqueas. Mediante el análisis filogenético y de dominios conservados, se propone el modelo evolutivo que dio origen a la superfamilia antiportadores NuoL/MrpA.

Filogenia, antiportadores Na^+/H^+, NuoL/MrpA

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Introduction

The Na + / H + antiporters similar to NuoL / MrpA are a group of secondary transporters that use the motive force of the proton gradient to displace positive cations such as sodium, which are widely distributed in organisms in the three phylogenetic domains of life, Bacteria, Archaea and Eukarya (Masahiro Ito, Morino, & Krulwich, 2017; Jasso-Chávez, Diaz-Perez, Rodríguez-Zavala, & Ferry, 2017).

The main physiological role of these transporters is the maintenance of intracellular pH homeostasis and the flow of cations such as Na +, K + and Li +, in addition these proteins play an important physiological role in bacteria regulating cation homeostasis by means of Na + / H + (Morino, Suzuki, Ito, & Krulwich, 2014), of K + / H + anti-support (Putnoky et al., 1998), pH homeostasis (Blanco-Rivero, Leganes, Fernandez-Valiente, Calle, & Fernandez-Pinas, 2005), resistance to bile salts (J. Dzioba-Winogrodzki et al., 2008), arsenite oxidation (Kashyap, Botero, Lehr, Hassett, & McDermott, 2006), pathogenesis (J. Dzioba-Winogrodzki et al., 2008) and energy conservation (Blanco-Rivero, Leganes, Fernandez-Valiente, & Fernandez-Pinas, 2009). The majority of cation / proton anti-carriers are monomers, however, NuoL / MrpA anti-carriers are complexes of several subunits and have been classified as an independent family in the carrier classification system called cation: proton antiporter-3, or CPA3 (Masahiro Ito et al., 2017).

NuoL is part of the respiratory complex I, which is the main proton entrance into the respiratory chains of mitochondria of most eukaryotes and some bacteria (Steimle et al., 2011). The resolved crystal structure of complex I shows that NuoL helps in the translocation of protons (Baradaran, Berrisford, Minhas, & Sazanov, 2013; Efremov & Sazanov, 2011), interacting with the NuoM, NuoN and NuoJ subunits through one arm formed by its C-terminal (Figure 1A). In the genomes of Bacillus and methanogenic archaea Methanosarcina acetivorans, Mrp complexes have been found, formed by the MrpABCDEFG subunits (Jasso-Chávez et al., 2017; TH Swartz, S. Ikewada, O. Ishikawa, M. Ito, & TA Krulwich , 2005), while in Vibrio cholerae the Mrp system is formed by the MrpABCDEFG subunits, since the MrpA and MrpB subunits are merged (Judith Dzioba-Winogrodzki, Winogrodzki, Krulwich, Boin, & Dibrov, 2009). The MrpA protein is the A subunit of the Mrp complex, which has the function of translocating Na + / H +, is involved in the synthesis of ATP and cell growth by converting acetate into methane and carbon dioxide (Jasso-Chavez, Apolinario, Sowers, & Ferry, 2013), it has also been seen that this protein is functional independently of the MrpABCDEFG complex in M. acetivorans (Jasso-Chavez et al., 2017).

MrpA is a membrane protein formed by 20 transmembrane segments (STM), which has a conserved structure with the anti-carrier subunit, NuoL. The N-terminal end of MrpA contains the anti-support domain, in addition to a protruding alpha helix that forms an arm that interacts with the MrpA subunit. The structure model of MrpA predicts that the mechanism of proton translocation is through STM 5, 7, 8 and 12, in addition to the fact that transport-related amino acids are conserved (Jasso-Chavez et al., 2017). The C-terminal end of MrpA contains another domain that is similar to the NuoJ subunit (Figure 1B), and possibly is part of the proton transport channel (Baradaran et al., 2013; Kao et al., 2005).

It is widely known that the subunits of the I NuoL, NuoM and NuoN complex are homologous to each other, as are the MrpA and MrpD subunits, suggesting a common origin for these proteins, in addition, there is a greater similarity between MrpA and NuoL, and between MrpD and NuoM / N, which between the subunits of its same complex, suggesting that a functional difference between them, which has been tested experimentally (Sperling, Górecki, Drakenberg, & Hägerhäll, 2016). It has also been proposed that the subunits of the Mrp complex arose from an ancestor similar to Complex I (Mathiesen & Hagerhall, 2003), however, an exhaustive analysis has not been carried out in this superfamily of transporters to know the evolutionary origin of these proteins. This is why in this work the reconstruction of the phylogeny of the Na + / H + anti-carrier superfamily similar to NuoL / MrpA was carried out and an analysis of its members to elucidate its evolutionary history.

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Methodology to be developed

Obtaining the working group

First, a sequence search was carried out using the NuoL proteins of Escherichia coli (accession number: P33607) and MrpA of M. acetivorans (accession number: AAM07911) on the GenBank platform using the Blastp program (Altschul et al., 1997) against the nr and swiss-prot protein bases, the BLOSUM 62 substitution matrix was used, and an expectation cut-off value (e) of 5x10-10 was taken. To narrow down the search, only nr database proteins that had the complete NADH-Ubiquinone / plastoquinone (pfam00361) domains were taken into account. Redundant proteins and fragments were removed using the guide trees generated by Clustalw (Thompson, Gibson, & Higgins, 2002). After five debug iterations, a working group of 635 sequences was obtained (39 MrpD and 596 MrpA).

Reconstruction of phylogeny

The 635 work group sequences were aligned using the Clustalw program. The alignment obtained was corrected by hand using the alignments generated with Blastp of each sequence as a reference. The correction was carried out using the Seaview program (Gouy, Guindon, & Gascuel, 2009). The MEGA version 6 program (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) was used for the reconstruction of phylogeny. Phylogenetic trees were reconstructed with the Minimum Evolution (ME) and Maximum Likelihood (ML) algorithms using the JTT matrix and the LG model plus 2.0 range as methods for estimating evolutionary distance. As a statistical test, a Bootstrap analysis of 1000 repetitions was performed for each tree. The taxonomy of the organism from which each sequence came from to verify the phyllogenetic data was verified. To place the root of the tree was used as an external group to MrpD proteins.

Domain Analysis

The analysis of the domains that make up the 635 sequences was carried out using the CDD conserved domain search platform (Marchler-Bauer et al., 2014), using the standard values.

To facilitate the analysis, scripts were generated in Perl 5.0 to generate images of the location of the domains from the results table of the CDD platform.

Results

Reconstruction of the phylogeny of the MrpA family

To know the evolutionary relationships and the origin of MrpA proteins, a phylogenetic analysis was carried out using the NuoL protein of Escherichia coli and MrpA of M. acetivorans as model proteins.

After the search and purification process, a working group of 635 sequences similar to NuoL / MrpA was obtained, within this group it was observed that there was a small subgroup of 39 sequences that were 300 amino acids smaller than the model protein, in In this subgroup, MrpD-NuoM / N proteins were identified, as already mentioned, MrpD-NuoM / N proteins are homologous to NuoL / MrpA proteins, which do not have a domain at the C-terminal end, so these proteins were not excluded from the analysis, since they can be used as an external group to root the phylogenetic tree. Excluding MrpD-NuoM / N proteins, a group of 596 proteins belonging to the Na + / H + (NuoL / MrpA) anti-carrier superfamily were found, of which 47 come from archaea, 376 from bacteria and 173 from eukaryotes, confirming that this is an ancestral family.

The phylogenetic tree shows three main groups well supported by high Bootstrap values (> 96). One of the groups consists exclusively of the proteins identified as MrpD-NuoM / N, none of these is mixed in any group of NuoL / MrpA proteins, which leads us to conclude that, although they are homologous proteins, they form a paralogue group to the NuoL / MrpA superfamily, as previously suggested (Mathiesen & Hagerhall, 2002; Moparthi et al., 2014). The other two groups, which form the family of cation / proton anti-carriers (Figure 2), were named group I (NuoL Family) and group II (MrpD Family).

The NuoL family consists of 196 members, mainly eukaryotic; It is divided into two subgroups, called IA and IB.

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In subgroup IA are eukaryotic proteins encoded in mitochondria of NuoL metazoans (ND5, NADH dehydrogenase 5), including human NuoL (P03915). It has been found that certain mutations in the NuoL protein have been related to various diseases, since they affect oxidative metabolism (Lodi et al., 2000; Pulkes et al., 1999; Talia H Swartz, Sayuri Ikewada, Osamu Ishikawa, Masahiro Ito, & Terry Ann Krulwich, 2005). Subgroup IB contains mainly eukaryotic proteins from chloroplasts, and from fungal mitochondria, although there is also a small group of 16 bacterial proteins and one archaea protein.

Within the bacterial proteins, the Escherichia coli NuoL protein (P33607) is located, whose three-dimensional structure has been resolved (PDB: 3RKO, subunit L), from which the proton expulsion mechanism has been better understood in the respiratory complex I (Efremov & Sazanov, 2011). The archaeological protein in this group is about the FpoL subunit of the Methanosarcina mazei Gö1 oxido-reductase F420 (Baumer et al., 2000), this result shows that the F420H2 oxido-reductases proteins are orthologs of the NADH dehydrogenases and paralogs of the MrpD proteins.

The MrpA family, consisting of 400 members, is divided into four subgroups, named IIA-D (Figure 2). Subgroups IIA, IIB and IIC are formed exclusively by bacterial proteins, while the IID group only contains archaea proteins (Figure 2). The Vibrio cholera MrpA protein (WP_001911623) is located in subgroup IIB, the Bacillus subtilis MrpA protein (Q9K2S2) is in the IIC group, and the M. acetivorans archaeus MrpA protein is in the IID group, these three proteins they have been associated with the resistance of these organisms to salts (J. Dzioba-Winogrodzki et al., 2009; M. Ito, Guffanti, Oudega, & Krulwich, 1999; Jasso-Chavez et al., 2013).

The fact that these proteins are in different groups suggests that members of this family have these same physiological functionalities. The M. acetivorans MrpA protein has also been associated with methanol metabolism and ATP synthesis (Jasso-Chavez et al., 2013; Rohlin & Gunsalus, 2010), so it is feasible that more members of the archaea MrpD family also have this functionality.

Analysis of the domains of MrpA proteins

It was observed that the distribution of the MrpA family tree shows a metabolic distribution, suggesting duplication events and subsequent divergence of the family. The MrpD-NuoM / N and NuoL / MrpA proteins vary in size due to an extension of the carboxyl end in the NuoL / MrpA family. To obtain more information about the evolutionary events in this superfamily, an analysis of the domains of the 635 sequences obtained in the search for homologues was carried out.

When analyzing the domains that make up this protein superfamily, it was observed that all members, both MrpD-NuoM / N and NuoL / MrpA, share the NADH-Ubiquinone / plastoquinone domain (pfam00361), and that the extension in the carboxyl terminal of NuoL / MrpA proteins range from 50 to 350 amino acids (Figure 3). It was observed that NuoL / MrpA proteins could be divided into two groups according to their size, which coincide with the families found in the phylogenetic tree. Group I (NuoL Family) contains proteins from 550 to 750 amino acids, Group II (MrpD Family), encompasses members that vary from 700 to 850 amino acids.

Group I contains the NuoL-1 subgroups, which is made up of proteins that have the pfam06455 domain (called C-terminal of the NADH dehydrogenase subunit 5), the NuoL-2 subgroup, which contains a F subunit domain of the NADH dehydrogenase (pfam01010), and finally subgroup I-3, whose carboxyl terminus contains another uncharacterized domain of about 100 to 200 amino acids (Figure 3). Group II contains only one type of protein whose terminal carboxyl end is similar to the proteins of the NuoL-3 subgroup, but also contains an mnhB domain (COG2111) (Figure 2).

Evolutionary model of the Na + / H + family of anti-carriers

The phylogenetic tree topology suggests that the MrpD-NuoM / N and NuoL / MrpA proteins arose from a duplication event from a single, small ancestral protein, with a NADH-Ubiquinone / plastoquinone domain (pfam00361).

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A primitive NuoL / MrpA protein lineage was generated that gave rise, after a fusion event with the mnhB domain (COG2111) to the larger archaea and bacteria MrpA-1 proteins. In the same way, the original lineage in a first diversification gave rise to the NuoL-3 group proteins, which are found in archaea and bacteria. Subsequently, this lineage, through a specialization event, gave rise to the NuoJ proteins present in eukaryotic organelles, suggesting that they arose before the symbiosis phenomena that gave rise to the mitochondria and chloroplasts of eukaryotic cells (Figure 4).

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Conclusions

The phylogenetic analysis of the Na + / H + (NuoL / MrpA) anti-carrier superfamily showed us that this is an ancestral superfamily, composed of at least 596 members, forming a group of paralogs with the MrpD-NuoM / N family. The phylogenetic tree shows a clear division of the NuoJ and MrpA families, which probably arose from a diversification event. NuoL proteins have formed specialized groups, highlighting the NuoL proteins of mitochondria and chloroplasts, which have a bacterial origin. MrpA proteins were also fused with an mnhB protein, to give rise to current MrpA.

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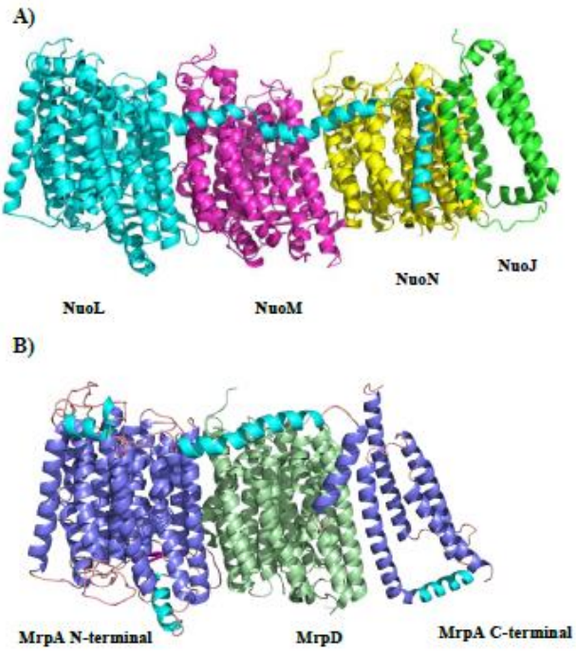

Figure 1 Three-dimensional structure of MrpAD and NuoLMNJ proteins. A) NuoLMNJ subunits of E. coli respiratory complex I (PDB: 3RKO). The anti-carrier proteins are linked by the arm that is part of the NuoL subunit. You can see the similarity between the LMN subunits. B) Molecular model of Mrp proteins of M. acetilovorans. The anti-carrier proteins, MrpA and MrpD, are similar to NuoL and NuoMN proteins respectively. MrpA contains an extra domain, which is similar to the NuoJ subunit

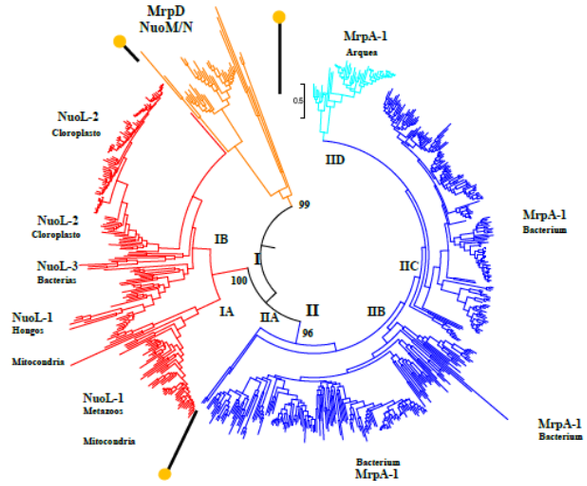

Figure 2 Phylogeny of the anti-carrier superfamily Na + / H + NuoL / MrpD. The NuoL / MrpD superfamily consists of two main groups. The NuoL family is marked as group I and the MrpD family is indicated as group II. Bootstrap values are shown in the main groups. The scale represents 0.5 amino acid substitutions per site. The tree was rebuilt using the ML method with a 1000-repetition Bootstrap

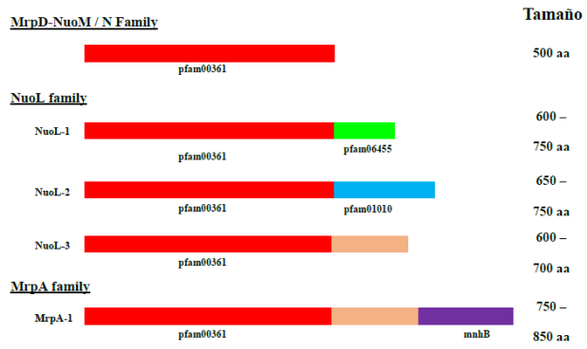

Figure 3 Arrangement of domains in the superfamily of anti-carriers Na + / H + NuoL / MrpD. The distribution of superfamily domains reflects the phylogenetic distribution. The domain of NADH-Ubiquinone / plastoquinone (pfam00361, red) is observed, conserved in the superfamily, as well as the different domains that form the extension of the C-terminal, and that generate a difference in the size of the family proteins

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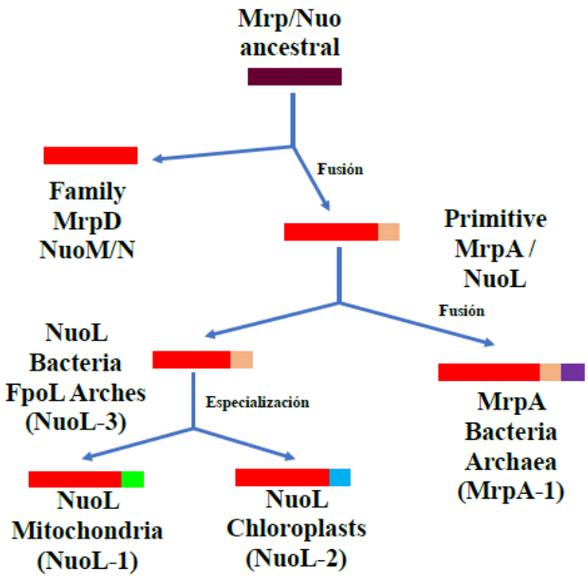

Figure 4 Evolutionary model of the origin of the superfamily of the anti-carriers Na + / H + NuoL / MrpD. The events of diversification, fusion and specialization that possibly gave this superfamily and that are proposed from the phylogenetic tree topology and the distribution of conserved domains are shown

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