Evolution of Mitochondrial Power in Vertebrate Metazoans

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Abstract

**Background:** Basal metabolic rate (BMR) has a very strong body-mass (M) dependence in an individual animal group, and BMR per unit mass (msBMR) converges on a markedly narrow range even across major taxonomic groups. However, it is here a basic question in metazoan biology how much BMR per unit mitochondrion (mtBMR) changes, and then whether mtBMR can be related to the original molecular mechanism of action of mt-encoded membrane proteins (MMPs) playing a central role in cellular energy production.

**Methodology/Principal Findings:** Analyzing variations of amino-acid compositions of MMPs across 13 metazoan animal groups, incorporating 2022 sequences, we found a strong inverse correlation between Ser/Thr composition (STC) and hydrophobicity (HYD). A majority of animal groups showed an evolutionary pathway of a gradual increase in HYD and decrease in STC, whereas only the deuterostome lineage revealed a rapid decrease in HYD and increase in STC. The strongest correlations appeared in 5 large subunits (ND4, ND5, ND2, CO1, and CO3) undergoing dynamic conformational changes for the proton-pumping function. The pathway of the majority groups is well understood as reflecting natural selection to reduce mtBMR, since simply raising HYD in MMPs (surrounded by the lipid bilayer) weakens their mobility and strengthens their stability. On the other hand, the marked decrease in HYD of the deuterostome elevates mtBMR, but is accompanied with their instability heightening a turnover rate of mitochondria and then cells. Interestingly, cooperative networks of interhelical hydrogen-bonds between motifs involving Ser and Thr residues can enhance MMP stability.

**Conclusion/Significance:** This stability enhancement lowers turnover rates of mitochondria/cells and may prolong even longevity, and was indeed founded by strong positive correlations of STC with both mtBMR and longevity. The lowest HYD and highest STC in Aves and Mammals are congruent with their very high mtBMR and long longevity.

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Introduction

Because the basal metabolic rate (BMR) is a fundamental currency to sustain metazoan life, it must be profoundly relevant to the mt power in energy production. However, its strong mass (M)-dependence makes unclear the existence of a relationship between BMR and this mitochondrial (mt) energy power across major taxonomic groups. A recent allometric study reports that the mass specific BMR (msBMR) converges on a markedly narrow range in these groups [1]. This viewpoint of the normalized energy inclines us to convert msBMR into the mt BMR (mtBMR) per unit mitochondrion which stands for the mt energy power, since the conversion can be done when msBMR includes the falling effect of the mt density (the mean number of mitochondria per unit cell) with increasing M [2], [3]. It is intriguing to estimate how much mtBMR changes across taxonomic groups, because recent structural studies report a high degree of sequence conservation of the membrane integral central subunits [4], [5], the mechanism of which is therefore likely to be similar throughout species [6].

The first step to relate mtBMR to the mt energy production power is to investigate the molecular structure of mt-encoded membrane proteins (MMPs) by using a number of amino acid sequences which are available in the NCBI database [7] (the accession numbers of these sequences are listed up in Table S1). The great majority of MMPs belongs to the 3 proton-pumping complexes of I, III and IV. Recent structural studies suggest that proton translocation in complex I requires large dynamic conformational changes across several subunits [6], [8], [9]. Likewise, the two large subunits of complex IV, i.e., CO1 and CO3, transfer protons across the membrane via conformational changes induced by electron transport [10–12].

MMPs are mostly embedded in the hydrophobic environment of the lipid bilayer, and their amino acid composition is primarily hydrophobic, with approximately 90–95% of these amino acids...
being non-polar. Therefore, the degree (mobility) of their conformational changes much depends on hydrophobicity (HYD). Raising HYD weakens their mobility and strengthens their stability according to the trade-off relation between mobility and stability [13]. Interestingly, a recent study of membrane proteins reports that the dynamic conformational stability of membrane helices can be typically enhanced by cooperative networks of interhelical hydrogen bonds between moderately polar residues, notably Ser and Thr [13–15]. The above-mentioned two features of HYD and Ser/Thr composition (STC) allow us to conceive a basic scenario of the metazoan evolution that lowering mtBMR (on the basis of the multicellular effect) requires less dynamic conformational changes of MMPs which induce an increase in HYD and a decrease in STC.

Here we report that most members of major animal groups follow this evolutionary scenario. However, the deuterostome lineage reveals the converse, i.e., rapid increases in STC and mtBMR, and a rapid decrease in HYD toward the endpoints (Aves and Mammals) of this lineage. Aves and Mammals seem ready to power up the mt energy by activating dynamical conformational changes of MMPs and still then enhance stability (durability) of them by increasing helix-helix interactions. This durability lowers turnover rates of mitochondria and cells, and may prolong longevity of organisms. Indeed, a strong correlation between STC and maximum lifespan (MLS) (observed in a previous vertebrate analysis [16]) was found to extend as a global rule beyond vertebrates across metazoans.

Materials and Methods

Derivation of mtBMR from msBMR

The allometric scaling law provides a very strong correlation between BMR and M in each animal group, and is expressed as BMR = \(C M^\alpha\) with an allometric exponent \(\alpha\) and constant \(C\). Makarjeva et al. [1] well described a variation of BMR data across different animal groups by using BMR per unit mass (msBMR), i.e.,

\[
msBMR = \frac{BMR}{C M^\alpha} = C \frac{M^\alpha}{M} = C M^{\alpha-1}
\]

They reported that msBMR data across dramatically different life forms converge on a markedly narrow range. This unit-mass representation of msBMR implicitly means that an organism is approximately regarded as a homogeneous matter of standard (representative) cells; the number of cells in unit mass and also that of mitochondria (the mt density) in unit cell are invariant, respectively, although, in practice, metabolically active cells, such as those of the liver, kidneys, muscles, and brain, have hundreds or thousands of mitochondria [17]. Therefore, msBMR is proportional to BMR per unit cell (we put this proportional constant equal to 1.0). Next, to get BMR per unit mitochondrion, we divide msBMR by the factor \(M^{\beta}\) which takes into account the decreasing effect of the mt density with increasing \(M\) [2], [3]. Then we have

\[
mtBMR = \frac{msBMR}{M^\beta} = C \frac{M^\alpha}{M^{\alpha+\beta}} = C M^{\alpha-\beta}
\]

where \(C\) is a constant. The above equation shows appreciable correlation. For simplicity, we put the proportional constant \(D\) equal to 1.0, since the value of \(D\) is 1.0. Here, \(F\) is a new parameter to adjust the allometric scaling effect of the mt-dependence. In the previous allometric analysis, the value of \(F\) was selected as providing the strongest correlation between msBMR and MLS [16].

In the present analysis, we redefine \(M\) as the mean value of the individual body masses in each animal group, to examine a relationship between mtBMR and amino acid compositions of MMPs in major taxonomic groups.

Data retrieval

To select a hydrophobic domain in MMPs, we applied the primary structure analysis (ExPaSy Proteomics Server; http://www.expasy.org/), using a standard model for the hydrophobic score (HIDSC) given by Cowan and Whittaker [18]. We calculated the moving average, \(S_n\), of HIDSC(m; m takes n-1, n, and n+1) around the n-th amino acid site in a protein and obtained a smooth function \(\bar{S}(n)\) of \(n\) by repeating this procedure. As a result, \(\bar{S}(n)\) was defined as the average value of \(S(n)\) with \(S(n) > 0.0\) in all or selected proteins of a given species. The obtained \(\bar{S}(n)\) is suitable for examining correlations with other quantities of present interest, such as amino acid compositions and lifespan. We predicted the helix domain of MMPs by using SOSUI and TMHMM servers [19], [20].

Results

A) HYD-TC correlation within respective MMPs

By including 13 metazoan animal groups with many amino acid sequences (more than 20) in the NCBI database [7], we analyzed 13 MMPs with a score S>0 for their hydrophobic domain (Materials and Methods). As a result, we selected 4 MMP variables (HYD, STC, TC and CC) of amino acid compositions as having significant correlations with one another, and found that HYD-TC provided an especially strong correlation. Here, TC and CC denote the Thr and Cys compositions, respectively. Table 1 shows a list of MMPs in the order of strong correlations. The 3 large subunits of ND4, ND3, and ND2 in complex I appeared as the first group with the largest \(R^2\)-values (\(R^2>0.86\)). Likewise, 2 large subunits of CO1 and CO3 in complex IV appeared as the second group (with \(R^2>0.78\)). These subunits just correspond to the proteins which require dynamic conformational changes for proton translocation [6], [8], [9]. The TC-CC correlation was appreciable in only these 2 proton-pumping complexes (with \(R^2>0.40\)) undergoing dynamic conformational changes in their helices.

B) Correlations between the MMP variables (HYD, STC, TC and CC)

We investigated the intra-correlations between the MMP variables, by using the following 5 sets of proteins according to the order of strong correlations shown in Table 1: 1) 3-protein set (ND4, ND5, ND2); 2) 4-protein set (ND4, ND5, ND2, ND1); 3) 5-protein set (ND4, ND5, ND2, CO1, CO3); 4) 6-protein set (ND4, ND5, ND2, CO1, CO3, ND3); 5) 7-protein set (ND4, ND5, ND2, CO1, CO3, ND1, CYTB). Here, the 3-protein set included 39% of the total site number of the complete amino acid sequence in humans, and the 7-protein set, 76% of it. The 7-protein set did not include ND3 and ATP8 with small numbers of helices (3 or less in humans). As seen in Table 2, TC provided predominantly strong correlations with HYD in all protein sets, and the strongest correlation (\(R^2=0.9\)) in the 5-protein set (Figure 1). In addition to this, TC-CC, HYD-CC and HYD-STS showed appreciable correlations. Here, we used the average values of TC and HYD in each animal group, in order to describe the correlation pattern lucidly (the raw data without the averaging procedure also showed a strong correlation of \(R^2=0.9\) (Figure S1). As a result, the TC-values in Aves and Eutheria with very high BMR were 2.5 fold larger than those of Nemotoda and Platyhelminthes with very low BMR, and the HYD values of the former were decreased by about 22% compared with those of the latter. The validity of these estimations of TC and HYD was supported by speculating the TC and HYD distributions in the helix domain of the above-mentioned 4 animal groups (Figure 2).
Table 1. HYD-TC and TC-CC correlations (R²) within respective proteins.

|          | HYD-TC  | TC-CC  |
|----------|---------|--------|
| ND6²     | 0.89    | 0.68   |
| ND5      | 0.89    | 0.60   |
| ND4      | 0.89    | 0.60   |
| ND3      | 0.81    | 0.61   |
| ND2      | 0.87    | 0.64   |
| ND1      | 0.87    | 0.49   |
| CO3      | 0.78    | 0.41   |
| CO2      | 0.71    | 0.38   |
| CO1      | 0.63    | 0.32   |
| CO3      | 0.68    | 0.32   |
| CO1      | 0.60    | 0.30   |
| CO3      | 0.60    | 0.25   |
| CO1      | 0.60    | 0.26   |
| CO3      | 0.60    | 0.26   |
| CO1      | 0.60    | 0.25   |
| CO3      | 0.60    | 0.25   |
| CO1      | 0.60    | 0.26   |
| CO3      | 0.60    | 0.25   |
| CO1      | 0.60    | 0.26   |
| CO3      | 0.60    | 0.25   |
| CO1      | 0.60    | 0.26   |
| CO3      | 0.60    | 0.25   |
| CO1      | 0.60    | 0.26   |
| CO3      | 0.60    | 0.25   |
| CO1      | 0.60    | 0.26   |
| CO3      | 0.60    | 0.25   |

C) Correlations between MMP variables and total site number of amino acids

We found that the total site number (TSN) of amino acids in a protein set steadily changes across the 13 animal groups (by 20% as a whole) and is a good index to describe the mutually contrasting evolutionary pathways of TC and HYD in metazoans (Figure 3). The TSN order of the animal groups (their relative TSN-dependence) was invariant in all protein sets (Figure S2). As a result, TSN strongly correlated with TC and HYD, when the deuterostomes were excluded (Table 2). Indeed, TC gradually decreased with decrease in TSN of many animal groups except for the deuterostomes (Figure 3, blue regression line), whereas HYD increased with a decrease in TSN (Figure 3, red regression line). On the other hand, TC and HYD in the deuterostome lineage presented rapidly increasing and decreasing trends with a decrease in TSN towards the terminal branch of Aves, clearly splitting from the 2 regression lines. This splitting pattern could be identified by looking at the TC-HYD relationship of Figure 1, because the non-linear regression curve $A (TC = 0.429 \cdot HYD^{1.241})$ with $R^2 = 0.90$ as a whole was decomposed into a steep slow slope dotted-line $B (TC = -65.66 \cdot HYD+41.75$ with $R^2 = 0.92$) for the deuterostomes and a slow slope dotted-line $C (TC = -18.32 \cdot HYD+14.92$ with $R^2 = 0.89$) for the other animal groups. The splitting pattern of Figure 3 became compatible with a molecular (rRNA)-based phylogeny [21], in the point that the tree starts with the root of Porifera and splits into the two lineages of Deuterostomia and Protostomia via Cnidaria. In this way, the TC-HYD relationship globally reflected the evolutionary pathway of metazoans.

D) Correlations between MMP variables and mtBMR

We examined correlations between mtBMR and MMP variables (TC, STC, and HYD) at the same mt function level, by increasing the F-value from 1.0 (corresponding to mtBMR) to infinity. The mtBMR values were estimated by extending mtBMR in the respective animal groups (Materials and Methods). Since the data on metazoans with low BMR were very limited, we here applied the recent data reported by Makarieva et al. [1] and also the AnAge database for vertebrates (Table S2). We investigated the correlation between STC and mtBMR by changing the F-value included in this quantity from 1.0 to infinity (Materials and Methods). Then we found that excluding the M-dependence of mtBMR with $F = \infty$ provides the strongest correlation (Figure S3). As a result, mtBMR correlated significantly with all MMP variables (STC, TC, HYD, and CC) in almost all of the protein sets, whereas mtBMR weakly correlated with STC in only the 3-, 6-, and 7-protein sets. Here, mtBMR showed an especially strong correlation with STC in all protein sets (Table 2), since STC well describes vertebrates [16] and this analysis includes relatively many vertebrates. Figure 4A demonstrates a typical case of the 3-protein set, which shows a significant STC-mtBMR correlation with markedly high mtBMR-values in Aves and Eutheria. We here note that the STC-mtBMR correlation was not strong with $R^2 = 0.28$.

E) Correlations of STC with MLS

The significant correlation between STC and mtBMR prompted us to examine the relationship between STC and mtBMR-MLS, because mtBMR-MLS corresponds to the total consumption energy per mitochondrion during the time (MLS) and may therefore be interpreted as a performance of the mt function. Here, MLS is redefined as the mean value of the individual MLSs in an animal group. By taking account of this time effect, the STC-mtBMR-MLS correlation ($R^2 = 0.81$) became much stronger than the STC-
mtBMR correlation ($R^2 = 0.64$) (the 3-protein set in Table 2), and separated vertebrates from other animal groups (Figure 4B). Figure 4C demonstrates a strong STC-MLS positive correlation ($R^2 = 0.71$) in the 3-protein set, and we found significant correlations between the MMP variables and MLS (Table 2). The CC-MLS correlation is likely to be related to oxidative damage to mitochondrial proteins or mtDNA [22–24], but was always weaker than the STC-MLS correlation in all protein sets (Table 2).

**Discussion**

Gradual increase in HYD for natural selection in many animal groups

A recent structural elucidation of ion channels in transmembrane proteins has provided evidence that these proteins undergo conformational changes during their function [13]. An easily understandable strategy of ecological and natural selection in metazoans is a reduction in their BMR by utilizing the multicellular effects of allometric scaling. Indeed, in many animal groups except for the deuterostomes, HYD and TC gradually
increase in decrease in underwent the evolutionary pathway of an increase in returned from the land to water, because these animal groups are therefore a strong evidence of adaptive evolution at the mt genome level. Another reverse process was previously observed in pathways of many other animal groups. These mutually reverse pathways of HYD and TC/STC may be consistent with their phylogenetic tree. Indeed, the order of animal groups along the TSN-HYD regression line in Figure 3 became globally compatible with the branching pattern projected on the evolutionary pathway from Porifera toward Platyhelminthes in the molecular (rRNA)-based phylogeny reported by Adoutte et al. [21]. This compatibility was supported by the neighbor-joining tree [25] in terms of TSN and TC (Figure S4) and also by a multidimensional vector space method of tree building (Figure S5) [26], [27]. However, the 2 lowest values of TSN were occupied by Platyhelminthes (Acoelomata) and Nematoda (Pseudocoelomata). The life style of these two groups seems to be closely related to each other, since they live mostly in anaerobic environments. On the other hand, the molecular-based phylogeny coupled Platyhelminthes with Mollusca (Coelomata) as Lophotrochozoa [26], [27]. However, the 2 lowest values of TSN were occupied by Platyhelminthes (Acoelomata) and Nematoda (Pseudocoelomata). The life style of these two groups seems to be closely related to each other, since they live mostly in anaerobic environments. On the other hand, the molecular-based phylogeny coupled Platyhelminthes with Mollusca (Coelomata) as Lophotrochozoa. Apart from this problem, the gradual increase in HYD of MMPs well explains the evolutionary pathway of ecological selection to strengthen their stability in many animal groups except for the deuterostomes.

Increase in STC and decrease in HYD for adaptive evolution in Deuterostomes

The marked decrease in HYD and increase in STC of the deuterostome lineage are quite interesting (Figure 3), because they are likely to break the ordinary trade-off relationship rule between mobility and stability as being well understood in the evolutionary pathway of many other animal groups. These mutually reverse pathways of HYD and STC in the 2 large animal groups are far from regarding mtDNA as the neutral marker long held to be [28], cannot be explained by the nucleotide mutation pressure [29–32], and are therefore a strong evidence of adaptive evolution at the mt genome level. Another reverse process was previously observed in vertebrate marine animals such as cetaceans and alligators which returned from the land to water, because these animal groups underwent the evolutionary pathway of an increase in HYD and a decrease in STC toward Fishes in contrast to that of the decrease in HYD and increase in STC [16].

Marked decrease in HYD and increase in STC heighten mt function in vertebrates

To pursue the biological meaning of the decrease in HYD and increase in STC in the deuterostome lineage, we introduced the quantity, mtBMR, being an energetic function at the same mt level as HYD and STC. Indeed, mtBMR was correlated negatively with HYD and positively with STC (Table 2). Figure 4A demonstrates a typical STC-mtBMR correlation (R² = 0.64) in the 3-protein set (a linear combination of STC and H1D provided a stronger correlation (R² = 0.77) with mtBMR). The marked decrease in HYD supports the appearance of a very large mtBMR in Aves and Eutheria, since a highly active mt-function can be attained by realizing MMPs with greater conformational freedom. However, on the other hand, higher MMP instability induced by this greater freedom heightens turnover rates of mitochondria, which requires a higher cost to reproduce a large number of them within cells. Furthermore, spatial constraints in metazoan tissues make it difficult for organisms to develop much higher power by simply accumulating more mitochondria, because mitochondria in metabolically active cells (such as those of the liver and brain) of, for example, humans make up 40 percent of the cytoplasm [17]. In this situation, the marked increase in STC in MMPs of Aves and Eutheria must be a critical condition to compensate or overcome their instability.

The reason why Ser and Thr residues can enhance dynamic stability of MMPs

Interestingly, membrane proteins have an outstanding feature of being able to strengthen their dynamic stability by interhelical interactions between motifs involving moderately polar residues such as Ser and Thr [13–15]. Indeed, the decrease in HYD and increase in STC in Aves and Eutheria were markedly large within the membrane itself, as is well understood by comparing their differences between Aves/Eutherians and Platyhelminthes/Nematoda (Figure 2). It is therefore likely that the increase in STC corresponds to increased hydrogen bonding between helices, within and between subunits, as pointed out by Dawson et al. [15] and Hildebrand et al. [13]. Because Ser and Thr residues are small and only moderately polar, helical structures tend to be stabilized by cooperative networks of interhelical hydrogen bonds. In a
Table 2. Correlations ($R^2$) between the pairs of variables (HYD, TC, STC, CC, TSN, mtBMR, msBMR and MLS).

|                  | 3 proteins | 4 proteins | 5 proteins | 6 proteins | 7 proteins | mean |
|------------------|------------|------------|------------|------------|------------|------|
|                  | <39%>      | <47%>      | <59%>      | <66%>      | <76%>      |      |
| TC-HYD           | N 0.8898   | 0.8939     | 0.8997     | 0.8958     | 0.8867     | 0.89318 |
| TC-CC            | N 0.6554   | 0.6916     | 0.6994     | 0.7264     | 0.6691     | 0.68838 |
| HYD-CC           | P 0.5295   | 0.6081     | 0.6367     | 0.5449     | 0.5337     | 0.57058 |
| HYD-STC          | N 0.5052   | 0.4803     | 0.2992     | 0.3503     | 0.3151     | 0.3901 |
| STC-CC           | N 0.2387   | 0.2298     | 0.209      | 0.1887     | 0.1551     | 0.20426 |
| TSN-TC           | P 0.9232   | 0.9014     | 0.8907     | 0.8019     | 0.6699     | 0.83742 |
| TSN-HYD          | N 0.7591   | 0.7364     | 0.7484     | 0.6491     | 0.7171     | 0.7221 |
| STC-ln(mtBMR)    | P 0.6434   | 0.6483     | 0.7048     | 0.6762     | 0.6751     | 0.66956 |
| TC-ln(mtBMR)     | P 0.4999   | 0.4995     | 0.4948     | 0.5159     | 0.5316     | 0.5083 |
| HYD-ln(mtBMR)    | N 0.3403   | 0.3206     | 0.3547     | 0.3379     | 0.3574     | 0.34218 |
| CC-ln(mtBMR)     | N 0.4041   | 0.3441     | 0.3401     | 0.3184     | 0.2851     | 0.33836 |
| STC-ln(msBMR)    | P 0.1937   | 0.1979     | 0.2804     | 0.2514     | 0.2428     | 0.23324 |
| STC-ln(MLS)      | P 0.7065   | 0.6861     | 0.5691     | 0.5691     | 0.5687     | 0.6944 |
| CC-ln(MLS)       | N 0.3732   | 0.3962     | 0.3447     | 0.3667     | 0.3441     | 0.3654 |
| STC-ln(mtBMR-MLS)| P 0.8133   | 0.8052     | 0.7737     | 0.7561     | 0.7551     | 0.78068 |

<%n> denotes that TSN of each protein set occupies the n % of that of the complete amino acid sequence in Human. P and N stand for the positive and negative correlations, respectively. The best results in the respective correlation croups are denoted by italics.

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previous paper [16], we showed that the short-range force of hydrogen bonds (1–2 Å) can be extended 2 to 3-fold (on average) by dynamic conformational changes in MMPs, because the relative distance of hydrogen bonding oscillates with the average amplitude R around R. Such a long-range potential amplifies the probability of interhelical interactions (in three-dimensional space) between cooperative networks of hydrogen bonds between motifs involving Thr or Ser residues. We envisage that such dynamic interactions could enable rapid resonance between metastable conformational states in MMPs, which have individual enzyme turnover rates of tens to hundreds of electrons per second [33]. In contrast, other types of hydrogen bonding, such as Cα-H—O

Figure 4. Relationships of STC with mtBMR (A), mtBMR MLS (B), and MLS (C).
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hydrogen bonding between Gly and Ala residues and the helical backbone, produce more rigid structures [13].

Proton-pumping machinery and dynamic conformational changes in MMPs

Three large subunits (ND2, ND4 and ND5) in complex I provided the strongest correlations between TC and HYD (Table 1), which were further correlated with mtBMR (Table 2). These subunits exhibit homology with sodium-proton antiporters and are known to be part of the proton pumping machinery of complex I [34]. Structural models of complex I suggest that electron transfers known to be part of the proton pumping machinery of complex I subunits exhibit homology with sodium-proton antiporters and are provided the strongest correlations between HYD and STC (Table 2). These overall comments about the results

Thus, overall, our findings are consistent with the hypothesis that cooperative networks of hydrogen bonds involving Thr and Ser residues stabilize dynamic conformational changes in MMPs, presumably increasing aerobic capacity, although we have not measured that directly. Direct measurements of the effect of increased TC or STC on MMP catalytic efficiency (Kcat), either in vitro or in vivo, are very difficult, as each substitution is likely to be highly dependent on the context. Cryptic epistasis is common in molecular evolution [40], and the requirement for multiple interactions with nuclear as well as mitochondrial genes [41], [42] only makes the problem more extreme in the case of respiratory proteins. Moreover, respiratory flux can be increased by adaptations throughout the entire supply network, including lung structure, hemoglobin kinetics, and capillary density [43], [44], as well as substrate channeling via respirasome assembly [45]. Given this complexity, the pervasive correlation between TC (STC) and HYD in MMPs right across metazoans stands as strong evidence that selection for aerobic capacity at the level of mitochondrial-encoded subunits has indeed taken place. This view is consistent with a number of studies indicating regular selective sweeps on mitochondrial genes: mtDNA is far from the neutral marker it was long held to be [46].

Remaining problems

It was difficult to detect the species-to-species coincidence between the sequence data and the observed data on BMR and/or MLS. Therefore, we used the average values of these data in respective animal groups without taking account of this coincidence. More available data in future will provide a clearer relationship between the MMP variables and mtBMR/MLS in more animal groups. We did not perform temperature adjustments of metabolic rates. One reason is that a common measurement temperature does not exist because endothermic groups do not live at body temperatures of 25°C as in many other animal groups.
Another reason is that metabolic rate and temperature are not independent variables with each other, but may be rather correlated.

Conclusion

The deuterostome lineage presented a quite unique evolutionary pathway of a marked decrease in HYD and increase in STC, in sharp contrast with the pathway of many other animal groups showing a gradual increase in HYD and decrease in STC, reflecting the natural selection to utilize the multicellular effect. These decreases in HYD and increases in STC were remarkable in the 5 large subunits (ND4, ND5, ND2, CO1 and CO3) in complexes I and IV, which require their dynamic conformational changes to exert a high degree of proton-pumping function. The low HYD values for these subunits are congruent with the large mtBMR values associated with their dynamic mobility. Furthermore, the marked increase in STC can strengthen dynamic stability of them via helix-helix interactions. As a result, this dynamic stability can lower the turnover rate of mitochondria and cells, and ultimately prolong the lifespan of organisms. In this way, vertebrates (especially Aves and Mammals) are considered to have equipped an excellent mechanism of action in MMPs to attain both very high metabolic rate and long longevity.

Supporting Information

Figure S1 Global relationship between TC and HYD in MMPs throughout metazoans. Strong correlations with (R²>0.9) were obtained by analyzing all 13 proteins with S>0 (see Materials and Methods).

Figure S2 The TSN-dependence of the animal groups in various protein sets. This figure shows that the relative positions of the 13 animal groups are invariant in any protein sets of 1) 3-protein set (ND4, ND5, ND2), 2) 4-protein set (ND4, ND5, ND2, ND1), 3) 5-protein set (ND4, ND5, ND2, CO1, CO3), 4) 6-protein set (ND4, ND5, ND2, CO1, CO3, ND1), and 5) 7-protein set (ND4, ND5, ND2, CO1, CO3, ND1, CYTB).

Figure S3 The F-value dependence of correlation (R²) between STC and mtBMR. The correlation (R²) between STC and mtBMR is estimated by changing the F-value in this quantity from 1.0 to infinity (Materials and Methods). Here, F=∞ excludes the M-dependence of mtBMR completely, and mtBMR depends only on the constant C in each animal group.

Figure S4 Neighbor-joining tree in terms of TC and TSN. We defined the pairwise distance between the i-th and j-th animal groups by Di,j = {(TCi−TSNj)² + (TSNi−TSCj)²} / σTSN². TCi and TSNj denote the average values of TC and TSN in the i-th animal group, respectively, by using the 5-protein set (ND4, ND5, ND2, CO1, and CO3). σTC and σTSN denote the standard deviations of TC and TSN, respectively.

(TIF)

Figure S5 Two dimensional display of a tree prepared by a multidimensional vector space method. According to the multidimensional vector space (MVS) method for preparation of a phylogenetic tree [19], the molecular evolution of a tree branch is described as going into a new dimensional space, the direction of which is therefore perpendicular to that of the original pathway. For simplicity, let us consider a tree structure in 2-dimensional (X-Y) space, as illustrated in this figure. Here, the lineage A represents the main pathway from the tree root to species α, and the lineage B represents a branch pattern from the lineage A toward species β. When X and Y are variables independent from each other without any attractions (convergent evolution), the angle between the 2 lineages is 90° (when they are closely correlated, the angle may be much deviated from 90°, as seen in the case of lines B and C of Figure 1 for the HYD-TC/STC strong correlation). The other species except for A, B and tree root must lie on the line A or B because they evolve into new dimensional spaces. If there are long-branch attractions, they fluctuate around these lines, as seen in this figure. We note that the lineage C may include many species but degenerates into one point in the X-Y plane and that the variables (to be used) must identify as many species as possible so as to explicitly describe a tree structure within these variables.

(TIF)

Figure S6 The M-dependence of BMR in Aves and Mammals.

(TIF)

Table S1 The accession numbers of amino acid sequences of 13 animal groups.

(XLSX)

Table S2 A list of msBMR in the 7 metazoan animal groups. The 4 quantities of mtBMR, scaling exponent α and constant C is defined in Materials and Methods.

(XLSX)

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Author Contributions

Analyzed the data: YK MT. Wrote the paper: YK. Conceived the direction of research: YK MT. Read and approved the final manuscript: YK MT.

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