Supplementary Information Results and Discussion

The mechanism of transport by mitochondrial carriers based on analysis of symmetry

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Contents

Supplementary text

1. Assembly of symmetry-related triplets. 4
2. Conserved triplets of symmetric residues 4
3. Mitochondrial dicarboxylate carriers 6
4. Mitochondrial oxoglutarate transporters 7
5. Mitochondrial carnitine/acylcarnitine carriers 8
6. Mitochondrial ADP/ATP carriers 9
7. Mitochondrial GTP/GDP carrier 10
8. Mitochondrial phosphate transporters 12
9. Mitochondrial oxaloacetate carriers 13
10. Mitochondrial aspartate/glutamate carriers 14
11. Mitochondrial oxodicarboxylate carriers 16
12. Mitochondrial Mg^{2+}-ATP/Pi transporters 17
13. Mitochondrial ornithine transporters 18
14. Mitochondrial succinate/fumarate carriers 19
15. Mitochondrial citrate transporters 20
16. Mitochondrial ScMrs3p-like transporters 21
17. Mitochondrial ScYFR045w-like transporters 22
18. Peroxisomal adenine nucleotide transporters 23
19. Mitochondrial UCP4 orthologs 24
20. Mitochondrial UCP5 orthologs 25

21. Mitochondrial CoA transporters 26
22. Mitochondrial pyrimidine nucleotides transporters 27
23. Mitochondrial NAD^+ transporters 28
25. Mitochondrial *S*-adenosyl-methionine transporters 30
26. Mitochondrial thiamine pyrophosphate transporters 32
27. Mitochondrial glutamate transporters 33
28. Mitochondrial MCART1 and MCART2 transporters 34
29. Mitochondrial MCART6 transporters 35
30. Mitochondrial ScYhm2p-like transporters 36
31. Mitochondrial ScYmc1p and ScYmc2p transporters 37
32. Mitochondrial YMR166c transporters 38
33. Mitochondrial YPR011c transporters 39
34. Mitochondrial ScMtm1p and HsSLC25A39/HsSLC25A40 transporters 39
35. Mitochondrial ScYDL119c and SLC25A38 transporters 40
36. Mitochondrial HsSLC25A29 transporters 42
37. Mitochondrial HsSLC25A42 transporters 42
38. Mitochondrial HsSLC25A43 transporters 43
39. Mitochondrial HsSLC25A44 transporters 44
40. Supplementary references 46
41. Supplementary figures 57

Supplementary figures

Fig. S1. Alignment of symmetry-related triplets from the odd-numbered α-helices. 57
Fig. S2. Alignment of symmetry-related triplets from the matrix and linker α-helices. 58
Fig. S3. Alignment of symmetry-related triplets from the even-numbered α-helices. 59
Fig. S4. Alignment of symmetry-related triplets of the cavity residues. 60
Fig. S5. Asymmetry and symmetry in the fungal and metazoan dicarboxylate carriers. 61
Fig. S6. Asymmetry and symmetry in the metazoan oxoglutarate transporters (OGC). 62
Fig. S7. Asymmetry and symmetry in the fungal and metazoan mitochondrial carnitine-acylcarnitine carriers. 63
Fig. S8. Asymmetry and symmetry in the fungal and metazoan ADP/ATP carriers. 64
Fig. S9. Asymmetry and symmetry in the fungal mitochondrial GTP/GDP carrier (ScGgc1p). 65

Fig. S10. Asymmetry and symmetry in the fungal and metazoan mitochondrial phosphate transporters. 66
Fig. S11. Asymmetry and symmetry in the fungal and metazoan oxaloacetate carriers. 67
Fig. S12. Asymmetry and symmetry in the fungal and metazoan mitochondrial aspartate/glutamate carriers. 68
Fig. S13. Asymmetry and symmetry in the fungal and metazoan oxodicarboxylate carriers. 69
Fig. S14. Asymmetry and symmetry in the fungal and metazoan Mg^{2+}-ATP/Pi transporters. 70
Fig. S15. Asymmetry and asymmetry in the fungal and metazoan mitochondrial ornithine transporters. 71
Fig. S16. Asymmetry and symmetry in the fungal succinate/fumarate carriers (ScSfc1p). 72
Fig. S17. Asymmetry and symmetry in the fungal and metazoan citrate transporters. 73
Fig. S18. Asymmetry and symmetry in the fungal and metazoan mitochondrial ScMrs3p-like transporters. 74
Fig. S19. Asymmetry and symmetry in the fungal ScYFR045w transporters. 75
Fig. S20. Asymmetry and symmetry in the fungal and metazoan peroxisomal adenine nucleotide transporters. 76
Fig. S21. Asymmetry and symmetry in the metazoan HsUCP4. 77
Fig. S22. Symmetry and asymmetry in the metazoan HsUCP5. 77
Fig. S23. Asymmetry and symmetry in the fungal and metazoan mitochondrial CoA transporters. 78
Fig. S24. Asymmetry and symmetry in the fungal and metazoan pyrimidine nucleotides transporters. 79
Fig. S25. Asymmetry and symmetry in the fungal NAD^+ transporters (ScNdt1p and ScNdt2p). 80
Fig. S26. Asymmetry and symmetry in the fungal and metazoan FAD transporters. 81
Fig. S27. Asymmetry and symmetry in the fungal and metazoan *S*-adenosyl-methionine transporters. 82
Fig. S28. Asymmetry and symmetry in the fungal and metazoan thiamine pyrophosphate transporters. 83
Fig. S29. Asymmetry and symmetry in the metazoan glutamate transporters GC1 and GC2. 84
Fig. S30. Asymmetry and symmetry in the metazoan MCART1 and MCART2. 84
Fig. S31. Asymmetry and symmetry in the metazoan MCART6. 85
Fig. S32. Asymmetry and symmetry in the fungal ScYhm2p transporters. 85

Fig. S36. Asymmetry and symmetry in the fungal ScMtm1p and metazoan HsSLC25A39/HsSLC25A40 transporters. 88
Fig. S37. Asymmetry and symmetry in the fungal ScYDL119c and metazoan HsSLC25A38 transporters. 89
Fig. S38. Asymmetry and symmetry in the metazoan HsSLC25A29 (CACTL). 90
Fig. S39. Asymmetry and symmetry in the metazoan HsSLC25A42. 90
Fig. S40. Asymmetry and symmetry in the metazoan HsSLC25A43. 91
Fig. S41. Asymmetry and symmetry in the metazoan HsSLC25A44. 91

Supplementary text

1. Assembly of symmetry-related triplets.

The mitochondrial transport proteins are called also carriers or transporters. The residues of all redundant *S. cerevisiae* and *H. sapiens* transporters were grouped in symmetry-related triplets of the odd-numbered α-helices (Fig. S1), the matrix and linker α-helices (Fig. S2), and the even-numbered α-helices (Fig. S3). In two cases, the human proteins were truncated compared to all other metazoans and we used the mouse sequences instead (HsMmSLC25A45 and HsMmAu042651). *S. cerevisiae* does not contain an ortholog of the oxoglutarate carrier, but other fungi do and the *Aspergillus oryzae* version was used (AoOgc1p). Separately, all of the symmetry-related triplets that are present in the water-filled cavity were grouped to analyze residues that are most likely to be involved in substrate binding and ion coupling (Fig. S4). The order of the sequences reflects the phylogenetic relationship between the transporters.

2. Conserved triplets of symmetric residues

Triplets of symmetry-related residues with a high level of symmetry and conservation define members of the mitochondrial transporter family, and they are likely to be important for mechanism (Fig. S1-S3) (1). The triplets are numbered according to the equivalent residue of the first repeat in the bovine

ADP/ATP carrier. The most obvious are the residues of the signature motif containing the matrix network in triplets 27, 29, and 32 (1-3) (Fig. S1 and Fig. 3). The residues of triplet 36 (Gln) interact with the charged residues of the matrix salt bridges. The cytoplasmic network consists of the charged residues in triplet 92 (Asp or Glu) and 95 (Lys or Arg), which have been noted to be symmetrical (1), but had no assigned function (Fig. S3 and Fig. 3). Two triplets of aromatic residues (triplet 88 and 91) (4) precede the residues of the cytoplasmic network, which may form transmembrane helix-helix interaction motifs (5). The matrix α-helices are the least conserved structural elements, even among orthologs, but there are notable symmetry-related triplets. Gly in triplet 65 (1) causes a break between the matrix and linker α-helices, whereas Arg and Lys in triplet 62 (1) do not have an assigned role. On the basis of an interaction between residue E264 on the matrix α-helix h56 and residue R236 on the odd-numbered α-helix H5 (3), conserved acidic residues of triplet 64 and basic residues of triplet 34 (4) are predicted to interact. There are hydrogen bonds between the side chains of residues of triplet 38 and the backbones of the loops between the odd-numbered and matrix α-helices (3). Between the short linker α-helix and the even-numbered α-helices is a motif [FWY][KR][G] formed by triplets 70-72 (3), which are important for function (6). In addition, there are conserved glycine residues (triplets 14 and 18) in a GXXXG motif (7), which are at the level of the substrate binding site and may promote helix-helix interactions. Often, proline residues are found in triplet 75, which are at the N-terminal end of the even-numbered α-helices, and in triplet 82, which may, like P82 in the bovine ADP/ATP carrier (3), cause kinks in the even-numbered α-helices at the level of the substrate binding site.

The uncharacterized human HsMCART1, HsMCART2, HsMCART6 , HsMTCH1, HsMTCH2 (8), HsSLC25A44 and HsTB1 (9) as well as the yeast ScYhm2p (10) are

characterized by a lack of symmetry in most but not all symmetric triplets (Fig. S4).

3. Mitochondrial dicarboxylate carriers

The mitochondrial dicarboxylate carrier of *S. cerevisiae* (Dic1p) translocates malonate, malate, succinate, and phosphate (11, 12). The main function is to transport cytosolic dicarboxylates into the mitochondrial matrix rather than to direct carbon flux to gluconeogenesis by exporting malate (13). The rat dicarboxylate carrier (14) and the human HsSLC25A10 are orthologs. Phylogenetically, the dicarboxylate transporters belong to the keto acid transporters in agreement with their substrate specificity (15).

Phosphate and dicarboxylates are small substrates and in accordance relatively few asymmetric residues are present in the cavity. Hence they do not have many defining triplets either (Fig. S4). They share with other dicarboxylate transporters, uncoupling proteins and ScYDL119c a fully symmetric Arg triplet 79, which forms the contact points of the common substrate binding sites (16, 17). In ScDic1p these residues are R77, R177 and R271, which could be involved in phosphate and dicarboxylate binding (Fig. S5 *B*). Around the substrate binding site, there are several asymmetric hydrophobic residues such as M181 and L272, which may guide the substrate to the contact points of the binding site by repulsion (Fig S5 *A*). The equivalent residues in HsSLC25A10 are R70, R170, R262, V174, and L263 (Fig. S5 *D* and *E*). There are no negatively charged residues in the vicinity of the substrate binding site, suggesting that transport is not proton-driven.

The fungal matrix networks have Asn (85%) or Leu (15%) instead of a positively charged residue on H3, whereas the metazoan ones have Asn (85%) or Thr (15%) instead, meaning that the networks have one polar interaction (Fig. S5 *C* and *F*,

bottom panels). The cytoplasmic networks are complete (Fig. S5 *C* and *F*, top panel), indicating that the transporters function as exchangers. The binding of phosphate would be just sufficient to overcome the energy barrier posed by the matrix network, but not of the cytoplasmic network, suggesting that phosphate can be imported only.

4. Mitochondrial oxoglutarate transporters

The mitochondrial oxoglutarate transporters (OGC) exchange cytosolic malate for 2-oxoglutarate from the mitochondrial matrix and play an important role in the malate-aspartate shuttle, the oxoglutarate-isocitrate shuttle and gluconeogenesis (18-22).

Phylogenetically, the oxoglutarate transporters cluster with the keto-acid transporters in agreement with their substrate specificity. They share with other dicarboxylate transporters, uncoupling proteins and Ydl119c a fully symmetric Arg triplet 79, which forms the contact points of the common substrate binding sites (16, 17). They differ from other keto acid transporters in triplets 83 in the cavity, and in triplet 95 of the cytoplasmic network and in triplet 32 of the matrix network (Fig. S4).

In agreement with the small size of the substrates, the cluster of asymmetric residues is small. Among the asymmetric residues are Q91 and H292 on contact point I and III, respectively (Fig. S6 *A*). In addition, the symmetry-related R90, R190 and R288 are in the same area, coupling substrate binding to a three-fold mechanism of transport (Fig. S6 *B*). H292 may be involved in proton-coupling. When mutated to cysteine all of these residues have a transport activity that is less than 6% of the wild-type (6). There are no negatively charged residues in the vicinity of the substrate binding site, suggesting that transport is not proton-coupled.

The matrix network of the oxoglutarate transporter is incomplete, as a Leu is present on H3, where a positively charged residue is expected (Fig. S6 *C*, bottom). The cytoplasmic network has several deviations (Fig. S6 *C*, top). Some OGC have a Thr (30%) or Ser (20%) on H2, where a positively charged residue is expected. All OGC have a Ser on H4, where a negatively charged residue is expected, and an Asn on H4, where either Arg or Lys is expected. The strengths of the networks may relate to the relative weak binding energy of oxoglutarate or malate to the transporter, as we have argued previously (23), as substrate binding needs to overcome the energy barrier posed by the two networks. Given the strength of the two networks the transporter would operate as an exchanger in agreement with experimental data (24). All of the residues of the matrix network and the three tested residues S203, E301 and N304 of the cytoplasmic network were found to be important for function when they were replaced by Cys (6, 23, 25).

5. Mitochondrial carnitine/acylcarnitine carriers

Mitochondrial carnitine/acylcarnitine carriers exchange cytosolic acyl-carnitine for carnitine in the matrix. The mammalian version allows the alkyl moieties of acylcarnitine to be oxidized by the enzymes of the β-oxidation pathway, which are located in the mitochondrial matrix (26). In yeast, fatty acid β-oxidation takes place in peroxisomes and the carrier most likely transports acetylcarnitine instead (27). The rat carnitine/acylcarnitine carrier operates according to a ping-pong mechanism (28). The carriers belong to the amino acid carriers, because of the [KR][DE] motif on H4. They are related to the ornithine carriers (29, 30), but for triplets 83 and 87, which are in the substrate binding site (Fig. S4).

Among the conserved and asymmetric residues in the cavity are G108, V109, I112 (or Met in most) and F113 of the yeast ScCrc1p and G81,V82, M85 and F86 in the human version HsCACT, which form a hydrophobic patch on H2, potentially involved in van der Waals interactions with the hydrophobic acetyl or alkyl moieties (Fig. S7 *A* and *D*). D204 in ScCrc1p and D179 in HsCACT on H4 could be involved in the binding of the positively charged trimethyl ammonium group and the symmetry-related R203/R303 in ScCrc1p and R178/R275 in HsCACT could bind the carboxylate group of acetyl-carnitine.

In the fungal carriers the matrix network is complete, but the cytoplasmic network has a His on H6 rather than the expected Arg or Lys (Fig S7 *C*). The $N\delta_1$ and $N\varepsilon_2$ atoms of His can form ion pairs with negatively charged Asp, but they are statistically less frequent than ion pairs with Arg or Lys (31). As the salt bridge networks are complete we postulate that the fungal carriers operate via a strict exchange mechanism. In the metazoan carriers, the cytoplasmic network is incomplete (Fig S7 *F*), as the acidic residue on H2 is a Gly and the expected basic residue on H6 a Met, suggesting a weaker network and a higher degree of uniport activity. There may be a correlation between the different strengths of the cytoplasmic networks and the different physiological roles of the fungal and metazoan carriers, as the transport via HsCACT is coupled to the β-oxidation reactions inside mitochondria in agreement with experimental observations (32).

6. Mitochondrial ADP/ATP carriers

Mitochondrial ADP/ATP carriers exchange cytosolic ADP for ATP, which is synthesized in the mitochondrial matrix by ATP synthase and they thereby replenish the cell with metabolic energy (33-36). Nucleotide exchange is driven by the chemical gradients of the substrates and the membrane potential, as the mechanism is electrogenic (37-39). The ADP/ATP carriers belong to the class of

mono-nucleotide carriers and they differ most significantly from related carriers in triplets 18, 80, 83, 84, and 87, which are in the vicinity of the substrate binding site, and 91, which is close to the cytoplasmic network (Fig. S4).

The cluster of conserved and asymmetric residues contains G199/Y203 in ScAac2p (Fig. S8*A*) and G183/Y187 in HsAAC1 (Fig. S8 *D*), which are part of the adenine nucleotide binding site together with I184 and I200, respectively (17). In addition, the asymmetric K38 in Aac2p and K23 in HsAAC1 could be involved in the binding of the phosphate moieties of adenine nucleotides together with the symmetry-related R96/R294 and R80/R280, respectively (Fig. S8 *B* and *E*).

In alignments the transmembrane α-helix H6 is one residue shorter than the H2 and H4 (Fig. S3 and for the bovine version in Fig. 1), which could be due to the multiple glycines that precede the deletion. The networks of the yeast (Fig S8 *C*) and human ADP/ATP carrier (Fig S8 *F*) are complete, but the cytoplasmic networks of some fungal carriers, such as Aac2p (Fig S8 *C*, top panel), are marginally weaker, as they contain a polar residue rather than the expected positively charged residue on H6. The networks would prevent a change of conformation in the absence of the substrate and thus adenine nucleotide exchange is expected to be equimolar in agreement with experimental observations (40, 41). The binding of ADP or ATP would provide sufficient energy to overcome the energy barrier posed by the networks, as its phosphate moieties form three salt bridges with the positively charged residues in the substrate binding pocket in addition to other interactions.

7. Mitochondrial GTP/GDP carrier

The mitochondrial GTP/GDP carrier (ScGgc1p) transports (deoxy)GTP and (deoxy)GDP, and the structurally related ITP and IDP for nucleic acid and protein synthesis (42). The GTP/GDP carrier is present in fungi, but they may not

have orthologs in metazoans, even though HsMCART1 and HsMCART2 have triplets in common with ScGgc1p, most notably triplet 22, 79, 80 and 95 (Fig. S4). The triplet analysis shows also that the ScGgc1p-like carriers most likely form a separate class, as the vast majority of triplets are unique. Triplets that deviate significantly from other subfamilies are 21, 22, 76, and 83(Fig. S4), which are in the predicted substrate binding site. Deviating triplet 33 is located below the matrix network and triplet 88 is two α-helical turns above the common substrate binding site.

Guanine could be bound to asymmetric E133, which could bind the amine group, together with R180 and asymmetric N181, which are in contact point II of the common substrate binding site (Fig. S9 *A*). The asymmetric E24, which is close to K279, could be the site for proton binding, as GTP/GDP hetero-exchange is proton-compensated (42). Asymmetric R86 together with the symmetry-related R279 on H6 and K89 or K82 on H2 could be involved in binding of the phosphate moieties of GTP (Fig. S9 *B*).

The only fully symmetric triplets are Pro and Asp of the signature motifs (Fig. S9 *C*). The matrix network of fungal GTP/GDP carriers is incomplete, as Ser (33%) or Ala (67%) are found instead of the expected positively charged residue on H1, meaning that this network is marginally weaker than a complete network (Fig. S9 *C*, bottom). The cytoplasmic network has Thr (46%), Asn (36%) or Ala (18%) present instead of a positively charged residue on H2, and an Ala instead of a negatively charged residue on H4. 45% of the orthologs have Gly instead of the expected negatively charged residue and a hydrophobic substitution instead of a positively charged residue on H6 (Fig. S9 *C*, top). Thus, the cytoplasmic network is very weak, indicating that the carrier is likely to be an exchanger with a large net import activity, which is fully compatible with its biological function, as

nucleotides are required for mitochondrial DNA and RNA synthesis, but no significant efflux activity has been observed experimentally (42).

8. Mitochondrial phosphate transporters

The fungal ScMir1p (43) (Fig S10 *A-C*) and ScPic2p (44) (Fig 2 *A-C*) and the metazoan HsPTP (45) (Fig. S10 *D-F*) are mitochondrial phosphate transporters. Phylogenetically, ScPic2p is more closely related to the metazoan orthologs than Mir1p. The main function of the phosphate carriers is to supply the mitochondrion with inorganic phosphate for the synthesis of the ATP from ADP. The phosphate transporters are unrelated to other classes, but they share triplet 22 and 76 with ScMrs3p and orthologs. The phosphate transporters are typified by triplets 18, 84, and 87, which are in the common substrate binding site (Fig. S4).

In the phosphate transporters, a cluster of conserved and asymmetric amino acid residues can be found at the midpoint of the membrane, consisting of R276, K179 and Q180 in Mir1p (Fig S10 *A*) and R317, R225, and Q226 in HsPTP (Fig S10 *D*), which are contact points II and III of the common substrate binding site (17). Close to the putative phosphate binding site is the negatively charged E126 in ScMir1p and E172 in HsPTP, which could be the site for proton binding. Other possible sites for proton coupling could be the asymmetric and conserved H79 and D176 in HsPTP and H32 and D130 in ScMir1p. These arrangements mean that phosphate transport is most likely to be driven by proton symport rather than hydroxyl ion exchange, as suggested previously (46). R276A, K179A, E126Q, and H32A mutations in mutant ScMir1p transporters render the carrier devoid of transport activity, and D130N had 30% of the wild-type activity (47), supporting the importance of these residues in transport.

The matrix network is incomplete and has on H5 a hydrophobic Leu in ScMir1p (Fig. S10*C* bottom) and Val in HsPTP (Fig. S10 *F* bottom) rather than the expected positively charged residue. The cytoplasmic network is also incomplete; (i) the expected positively charged residue on H4 is a Val in HsPTP (Fig. S10 *F* top) and Ala (40%), Ser (27%), Val (20%), or Thr (13%) in ScMir1p (Fig. S10 *C* top), and (ii) the negatively charged residue on H6 is replaced by Gly (83%) or Ala (17%) in ScMir1p (Fig. S10 *C* top). Thus, despite different compositions all of the networks, including that of Pic2p (Fig. 2 *C*), have only two salt bridges rather than the expected three. We have argued previously that a weaker network is correlated with a lower binding energy of the substrate (23). The expected interactions with the substrate binding site would be sufficient to overcome two, but not three salt bridges of the networks. The weaker networks may indicate also that the transporter has some uniport activity in agreement with experimental observations (46, 48). The mutation of E95Q, K98A, E192Q, S195A and K295A in ScMir1p reduced the transport activity to 72%, 3%, 39%, 3%, and 33% of the wild-type activity, showing that apolar substitutions of the cytoplasmic network residues render the transporter dysfunctional, whereas polar substitutions are less severe.

9. Mitochondrial oxaloacetate carriers

The mitochondrial oxaloacetate carriers translocate oxaloacetate, sulfate, thiosulfate, and malonate. Their main physiological role may be to take up oxaloacetate, produced from pyruvate by the cytoplasmic pyruvate carboxylase into mitochondria (49). The human orthologs may be SLC25A34 and SLC25A35, but their function has not been confirmed experimentally. The carriers belong to the keto acid carrier group, but they differ from the other carriers in triplets 79

and 83, which are in the substrate binding site, and in triplet 29 and 32, which are in the matrix network (Fig. S4).

The cluster of asymmetric residues is relatively small in agreement with the substrate being small. Among the most remarkable conserved and asymmetric residues in ScOac1p are H298 and Q94 and in HsSLC25A34 H281 and Q80, which are in contact point III and I, respectively (Fig S11*A* and *D*). In the same region are the symmetry-related R200 and R294 in ScOac1p and R176 and R277 in HsSLC25A34, which are contact points II and III, respectively (Fig S11 *B* and *E*) and could be involved in the binding of the carboxylic moieties. In Sc Oac1p Y93 and L97 in contact point I together with the asymmetric G142, A143 form a hydrophobic pocket. The equivalent residues are Y79, M83, G117, and A20 in HsSLC25A34. Recently, it has been shown that the oxaloacetate carriers also transport α-isopropylmalate, which has a hydrophobic functional group that could bind to this site (50).

The cytoplasmic as well as the matrix network are relatively weak, which is expected as the substrates provide a small amount of binding energy to overcome the energy barrier posed by the network (Fig. S11 *C* and *F*). Residues that do not form hydrogen bonds or salt bridges in the networks are poorly conserved. In addition to exchange, it is expected that the carrier is able to change conformation without substrate, meaning that its task would be to equilibrate substrate pools on either side of the membrane. Exchange and uniport activity of the oxaloacetate carrier have been shown experimentally (49).

10. Mitochondrial aspartate/glutamate carriers

Mitochondrial aspartate/glutamate carriers exchange aspartate from the matrix for cytosolic glutamate plus a proton (51, 52). In mammalian mitochondria, aspartate/glutamate carriers play a key role in the malate-aspartate NADH

shuttle that transfers electrons from cytosolic NADH via inter-conversions and transport steps to matrix NADH, and finally to the mitochondrial electron transport chain. In *Saccharomyces cerevisiae* a malate-aspartate NADH shuttle may exist (53) despite the presence of external NADH dehydrogenases that oxidize NADH on the external face of the inner mitochondrial membrane (54). The aspartate/glutamate carriers have an extra soluble domain in the intermembrane space that is absent in other mitochondrial carrier subfamilies (51, 52). The aspartate/glutamate carriers belong clearly to the amino acid carriers, even though they share properties with the keto acid carriers too. Among the distinctive triplets are 21, 76, 83, 87, and 91, which are in the substrate binding site (Fig. S4).

Among the conserved and asymmetric residues in the cavity of the fungal ScAgc1p is D693, which forms the amino group binding motif together with the symmetric R692 (16, 17) (Fig. S12 *A* and *B*). The asymmetric residues K601 and K604 could be receptors for the carboxylate side chain of glutamate and aspartate, respectively. E600 is close to these two lysines and may be the binding site for a proton to drive the import of these amino acids. The equivalent residues in HsAGC1 are D491, R490, K403, K406 and E402, respectively (Fig. S12 *D* and *E*). The cavities contain aromatic and hydrophobic residues that may help to guide the substrate to the binding site.

The fungal matrix network is complete, but the cytoplasmic network has Ala (79%), Ser (14%) and Asn (7%) instead of a negatively charged residue on H4 (Fig. S12 *C*). Only the *S. cerevisiae* carrier has a Lys on H6, but most fungal carriers have either Gln (57%) or His (36%). In the metazoan orthologs, the matrix network is also fully conserved, but the cytoplasmic network has an Ala on H4 instead of Asp or Glu and a Gln on H6, where a Lys or Arg is expected (Fig. S12 *F*). The difference in strength of the two networks suggests that the carrier is an

exchanger with net import activity, which is supported by efflux experiments (51). This observation would agree also with its biological function as the amino acids would be incorporated into the proteins that are synthesized in the mitochondrial matrix.

11. Mitochondrial oxodicarboxylate carriers

Oxodicarboxylate carriers transport 2-oxoadipate, 2-oxoglutarate, adipate, glutarate and to a lesser extent malate and citrate (55, 56). The main differences between the human and yeast carriers are that 2-aminoadipate is transported by the former but not by the latter, whereas the reverse is the case for malate. The main physiological role is probably to supply 2-oxoadipate and 2-oxoglutarate from the mitochondrial matrix to the cytosol where they are used in the biosynthesis of lysine and glutamate, respectively. The carriers differ from other keto acid carriers in triplets 25, 76, 80, 83, 84, and 87, which are in the region of substrate binding site, and in triplet 92, which belongs to the cytoplasmic network (Fig S4).

The carriers have relatively few asymmetric residues at the midpoint, indicating that adaptations are relatively small in order for the substrate to bind. Among the conserved asymmetric residues in ScOdc1p are K94, R95, and K98 in contact point 1 on H2, H187 in contact point II on H4 and K279 in contact point III on H6 (Fig. S13 *A*). The asymmetric E91 is close to K94 and R95 and could be the site for proton coupling. In addition, triplet 21 has two glutamate residues E25 and E136 that could be involved in proton transport also. The equivalent residues in HsODC are K86, R87, K90, H179, K273, E83, E27 and E123, showing that the analysis is consistent between taxa (Fig. S13 *A* and *D*).

The matrix network is complete in the fungal and metazoan carriers (Fig. S13 *C* and *F*, bottom), whereas the cytoplasmic one is incomplete. The fungal

cytoplasmic network has Gly (60%), Gln (20%), Thr (13%) or Ser (7%) on H2 instead of a positively charged residue, and a Phe on H4 instead of a negatively charged residue (Fig. S13*C*, top). The metazoan cytoplasmic network has on H4 His (30%), Tyr (30%), Phe (20%), Gln (10%) and Asn (10%) instead of a negatively charged residue, meaning either cation-π interactions or hydrogen bonds, which are equivalent in strength. On H6, predominantly Tyr is found where a positively charged residue is expected (Fig. S13 *F*). The carriers are exchangers, but the metazoan orthologs may have a weak net import activity, not observed experimentally (55, 56).

12. Mitochondrial Mg^{2+}-ATP/Pi transporters

ScSal1p is the Mg^{2+}-ATP/Pi transporter in *S. cerevisiae* and its transport activity is regulated by cytosolic calcium (57). In man, there are three isoforms with 66–75% similarity that transport ATP-Mg^{2+} in exchange for phosphate (58, 59), called HsAPC1, HsAPC2 and HsAPC3. The proteins have three EF-hand Ca^{2+}-binding motifs in their N-terminal domains. As mitochondrial ADP/ATP transporters carry out equimolar exchange (40, 41), ATP-Mg^{2+}/Pi transporters regulate the adenine nucleotide pools in the mitochondrial matrix. The transporters belong to the mono-nucleotide transporters and are classified by triplets 33, 83, and 87, which are in the substrate binding site, and triplet 29, which is in the matrix network (Fig. S4).

In ScSal1p the asymmetric G416 and Y420 form the putative adenine binding site together with the hydrophobic I417 in contact point II (Fig. S14 *A* and *D*). In contact point I are the asymmetric E318 and K322, which could be the site for Mg^{2+} binding and phosphate binding, respectively. The symmetry-related K314 and K523 are in the same area and might also be involved in phosphate binding and thus they would couple the binding of the substrate to a symmetric

mechanism (Fig. S14 *B*). The equivalent residues in HsAPC1 are G353, Y357, I354, E264, and K268 (Fig. S14 *E*).

The matrix network in the fungal network has an Asn instead of a negatively charged residue on H5 (Fig. S14 *C*, bottom), whereas the metazoan transporter has an Ala in that position (Fig. S14 *F*, bottom). The cytoplasmic network of metazoan transporters is complete, whereas half of the fungal transporters have Ser instead of a negatively charged residue on H4 (Fig. S14 *C* and *F*, top). The transporters are predicted to be strict exchangers, which has been confirmed experimentally (58).

13. Mitochondrial ornithine transporters

The fungal mitochondrial ornithine transporter transports ornithine, arginine and lysine (29, 60), whereas the mammalian ornithine transporter translocates citrulline and ornithine (61), which form the start and end products of the urea cycle. A defective human ORNT1 transporter causes hyperammonaemia-hyperornithinaemia-homocitrullinuria (HHH) syndrome, which is characterized by the accumulation of ammonia and ornithine (62). The ornithine transporters belong to the amino acid transporters phylogenetically. They differ significantly from other amino acid transporters by a glutamate in the first repeat of the triplet 83 (Fig. S4). The fungal transporter differs from the metazoan transporter by Lys in triplet 22 and Glu in triplet 25.

The asymmetric residues cluster in a small area in agreement with the fact that the substrates are relatively small. The fungal and metazoan ornithine transporters have two asymmetric and conserved residues in common, i.e. E83 and E179 in Ort1p (Fig. S15 *A*) and E77 and E180 in ORNT1 (Fig. S15 *D*). They are likely to be interacting with the two amine groups of the substrates. The carboxylate group of the substrate may bind to the symmetry-related R178/R274

and R179/R275 in the fungal and human versions, respectively (Fig. S15 *B* and *E)*). Indeed, mutations in E180 and R275 cause the HHH syndrome (62).

In addition to the charged residues involved in substrate binding, the fungal ornithine transporter ScOrt1p has E31 and K28, which are close to each other and could be involved in proton coupling (Fig. S4). K28 would prevent the binding of the amine group of the side chain of ornitine or arginine to the binding by charge repulsion, but if the amine group is protonated it would interact with E31 and allow the binding of the substrate. The efflux of ornithine was found to be pH dependent in agreement with this notion (29). The metazoan ornithine transporter does not have an arrangement like this and is thus expected to exchange ornithine and citrulline without proton coupling.

The matrix network is complete in both fungal and metazoan ornithine transporters (Fig. S15 *C* and *F* bottom), whereas the cytoplasmic network is not (Fig. S15 *C* and *F* top). As the cytoplasmic network is relatively weak some net import is expected. Indeed, a protein-mediated uniport activity has been observed in the case of the fungal ScOrt1p (29) and for the rat ornithine transporter (63).

14. Mitochondrial succinate/fumarate carriers

The succinate/fumarate carrier ScSfc1p transports cytoplasmic succinate, derived from isocitrate in the cytosol, into the mitochondrial matrix in exchange for fumarate (64). This exchange activity and the subsequent conversion of fumarate to oxaloacetate in the cytosol are essential for the growth of yeast on ethanol or acetate as the sole carbon source. Phylogenetically, ScSfc1p belongs to the keto acid transporters and the triplet analysis suggests that ScSfc1p has many features in common with the citrate carriers (65, 66), but they differ in triplets 22, 84, 87, which are in the substrate binding site (Fig. S4). Among the conserved

asymmetric residues in the cavity are K85/R89, Q185 and R282 in contact points I, II and III, respectively (Fig. S16 *A*). Another asymmetric residue is E24 is in the vicinity of the substrate binding site and could be the site of proton binding for co-transport.

In the fungal succinate/fumarate carriers the matrix network is complete, but the cytoplasmic network has a polar residue instead of the expected negatively charged residue on H4 (Ser 68%, Gln 19% and Thr 13%). Thus, the carrier is expected to be an exchanger in agreement with experimental observations (64) and its biological function.

15. Mitochondrial citrate transporters

The fungal mitochondrial citrate transporters translocate citrate and isocitrate, but no dicarboxylates (66) and their rat (67) and human orthologue is HsSLC25A1 (68). The citrate transporters belong to the keto acid transporters in agreement with their substrate specificity. Characteristic triplets for citrate transporters are 80, 84, and 87, which are in the substrate binding site (Fig. S4).

In ScCtp1p the conserved and asymmetric R276, K83 and possibly Q182 together with the symmetric R279 and R181 could form a substrate binding site. The equivalent residues in the human CTP are R282, K97, Q191, R285 and K190, respectively. E26 and E122 in ScCtp1p and E39 and E135 in HsCTP are in the vicinity of the substrate binding site and could be involved in proton coupling.

Recently, two alternative binding sites of citrate have been proposed based on a comparative model of ScCtp1p and interpretations of kinetic data (69). One citrate binding site consisted of K83, R87 and R189 and another of K37, R181, R239, R276 and R279. These two binding sites contain residues of the common substrate binding site (16, 17) with the following four exceptions. The proposed residues K37 and R239 are part of the matrix network, explaining fully their

critical role in mechanism without the need to invoke a role in substrate binding. The symmetry-related R87 and R189 are in the contact points I and II of the common substrate binding site, respectively, although they are two turns of an α-helix away from the most common interaction sites. The equivalent residues in the position of R87 in the oxodicarboxylate and aspartate/glutamate carriers allow different carbon lengths of the substrates and thus a broader specificity, but to our knowledge longer chain carboxylates have not been tested.

The matrix network is conserved fully in fungi and metazoans, but the cytoplasmic network deviates from the norm. In ScCtp1p a polar residue (Ser 62%, Gln 28% and Thr 10%) replaces the negatively charged residue on H4, whereas eight different, mostly apolar, residues replace the positively charged residue on H6. In the human HsCTP, Ser (67%) can be found on H2 instead of a positively charged residue, Thr (56%), Glu (33%) and Asn (11%) on H4 where a negatively charged residue is expected, and on H6 a hydrophobic residue is found (Val 67%, Met 22%, Ile 11%) instead of a positively charged residue. Thus, the cytoplasmic network is significantly weaker than the matrix one and thus a net import activity would be expected. In agreement, uniport activity from loaded proteoliposomes containing the rat citrate transporter has been observed experimentally (70).

16. Mitochondrial ScMrs3p-like transporters

The fungal ScMrs3p and ScMrs4p are related to the metazoan orthologs of the human HsSLC25A28 (HsMF2) and HsSLC25A37. The transporters might have roles in the utilization and/or transport of iron *in vivo* (71) for heme (72) or iron-sulfur cluster (73) assembly, but the substrate has not been confirmed in transport assays with purified reconstituted transporters. These transporters do not belong to any of the known classes, such as keto acid, amino acid, phosphate

or nucleotide transporters, indicating that their substrates are likely to be very different structurally. This class of transporters is defined by triplets 18, 21, 79, 80, and 84, which are in the region of the substrate binding site. They share triplet 22 with the phosphate transporters.

In ScMrs3p or HsMF2, there are two asymmetric and conserved histidines in the substrate binding site; one on H1 (H48 or H87, respectively) and one in contact point I on H2 (H105 or H145) (Fig. S18 *A* and *D*). In the vicinity of the His on H1 is a Glu (E47 and E86), which could be involved in proton coupling (Fig. S18 *A* and *D*). Among the conserved asymmetric residues is also an Arg (R289 and R331) in contact point III (17). Surprisingly, there are no strictly conserved residues in contact point II, but in most cases a Met and Asn can be found as adjacent residues. The substrate is unlikely to be a keto acid, phosphate, amino acid or nucleotide, and it may have one negative charge based on the observation that the substrate binding site has one positive counter charge. Although iron could bind to The cytoplasmic networks of the fungal and metazoan transporters are complete (Fig. S18 *C* and *F*). The matrix network on H4 has a Gln in metazoan transporters and a Ser (52 %), Thr (26%), Lys (11%), Arg (11%) in fungal transporters, where a positively charged residue is expected (Fig. S18 *C* and *F* top). Therefore, the transporter is expected to be an exchanger.

17. Mitochondrial ScYFR045w-like transporters

The function of the fungal orthologs of *S. cerevisiae* ScYFR045w is unknown, but they belong to the class of keto acid transporters. Triplet analysis shows that ScYFR045w orthologs share many properties with citrate (65, 66) and the succinate/fumarate transporters (64), but they differ from these in triplets 21, 84, and 87, which are in the substrate binding site, and in triplet 95 in cytoplasmic network.

The cluster of asymmetric residues is rather small, indicating that the substrate is relatively small, as observed in other known transporters of small keto acids. Among the conserved asymmetric residues in ScYFR045w are K69, Q194 and R281, which are in the contact points I, II and III of the common substrate binding site (16, 17), respectively. Also present is E113, which could be involved in proton coupling.

The matrix networks of the fungal ScYFR045w transporters are fully conserved, but the cytoplasmic network is largely incomplete and not well conserved, suggesting that it is a net importer of its cytosolic substrate.

18. Peroxisomal adenine nucleotide transporters

The fungal peroxisomal adenine nucleotide transporter ScAnt1p (74) and the metazoan homologue HsPMP31 are members of the mitochondrial transporter family, but they are found in peroxisomal membranes. Their expression is oleic acid inducible and the transporter is essential for growth on medium-chain fatty acids as the sole carbon source. The physiological role is probably the transport of cytoplasmic ATP into the peroxisomal lumen in exchange for AMP generated in the activation of fatty acids (74). The peroxisomal adenine nucleotide transporters belong to the class of di-nucleotide transporters, even though the substrates are mono-nucleotides (Fig. S4). They differ significantly from other transporters in triplet 79, 83, and 84, which are in the predicted substrate binding site, and in triplets 29 and 32, which are in the matrix network.

The substrate and inhibitor specificity differ from those of the mitochondrial ADP/ATP transporters. In agreement with this notion, the substrate binding site for adenine, typically G[ILMV] (17), is not conserved in the peroxisomal transporters. Alternatively, the asymmetric L191 in ScAnt1p may form part of the adenine binding pocket (Fig. S20 *A*). The asymmetric conserved K233, Q283, and

K286 could be involved in the binding of the phosphates (Fig. S20 *A* and *B*). K233 is also present in the adenine dinucleotide transporters ScFlx1p (75), ScPyt1p (76), ScNdt1p and ScNdt2p (77), suggesting a closer phylogenetic relationship with this class of transporters than with the adenine mononucleotide transporters. The equivalent residues in the HsPMP34 are L175, K213, K273, and Q276 (Fig S20 *D* and *E*).

Notably, the matrix network is virtually absent in the fungal transporters with the exception of Asp and Lys on H1 and Lys on H5, whereas the cytoplasmic network is complete. The cytoplasmic network has a polar residue (Ser 47%, Thr 53 %) instead of a negatively charged residue on H2, a Gln (29%) instead of the more common Glu on H4, and Glu (52%), Asp (18%), Lys (18%), Arg (6%) and Thr (6%) on H6 where a negatively charged residue is expected. The matrix networks of the metazoan transporters have a Trp and an Asn on H3 and two Gln on H5, where negatively and positively charged residues are expected, respectively. The cytoplasmic network has on H2 Asn (50%), His (33%) and Ser (17%), where a negatively charged residue is expected, and on H6 Thr (66%), Val (17%) and Ala (17%), where a positively charged residue is expected. Unusually, the cytoplasmic networks are stronger than the matrix networks, and thus the transporter could have a significant uniport activity.

19. Mitochondrial UCP4 orthologs

HsUCP4 are related to the uncoupling proteins (78) and more broadly to the dicarboxylate and oxoglutarate transporters. They differ from the uncoupling proteins by having Glu rather than Asp in triplet 22 and Asp rather than Glu in triplet 87, which are both absent in keto acid transporters.

HsUCP4 orthologs are highly symmetric and have a very small cluster of asymmetric residues, suggesting that the intended substrates are small (Fig. S21

A and *B*). They have a fully symmetric set consisting of R97, R199 and R299 (Fig. S21 *B*), which are found in oxoglutarate transporters, dicarboxylate transporters, ScYDL119c-like transporters and uncoupling proteins HsUCP1, HsUCP2 and HsUCP3. The asymmetric residues R105 and F307 belong to the triplet that includes D207. Like other uncoupling proteins, HsUCP4 orthologs have the two aforementioned negatively charged residues E34 and D207, which could be the sites for proton binding. Thus, there are remarkably few asymmetric adaptations in UCP4 orthologs, indicating that the intended substrate is possibly a small keto acid.

The matrix and cytoplasmic networks of UCP4 orthologs are complete, indicating that the transporter is likely to operate as a strict exchanger of keto acids coupled to proton import.

20. Mitochondrial UCP5 orthologs

The UCP5 orthologs are related to the uncoupling proteins (78) and more broadly to the dicarboxylate and oxoglutarate transporters. They differ from the uncoupling proteins UCP1-3 by having a Glu rather than an Asp in triplet 22 and by having a Lys rather than Arg in triplet 87, which differ from triplets found in keto acid transporters (Fig. S4).

The UCP5 proteins are very symmetric and have a very small cluster of asymmetric residues in the cavity, indicating that the substrate is relatively small (Fig S22 *A* and *B*). A fully symmetric set of R113, R206 and R305 is found in HsUCP5, which are present also in oxoglutarate transporters, dicarboxylate transporters, ScYDL119c-like transporters and uncoupling proteins. Like uncoupling proteins, UCP5 orthologs have two negatively charged and asymmetric residues E55 and E214, which could be the sites for proton binding.

Apart from Q114, there are remarkably few asymmetric adaptations in UCP5 orthologs.

The matrix network of UCP5 orthologs is complete, but the cytoplasmic network has Gln (40%), Tyr (40%) and Asn (20%) on H2 instead of a negatively charged residue and thus is slightly weaker (Fig S22 *C*). Thus, UCP5 orthologs are likely to be exchangers of small keto acids.

21. Mitochondrial CoA transporters

The yeast mitochondrial transporter ScLeu5p and human homologues Graves' disease carrier protein HsGDC (SLC25A16) and putatively HsSLC25A42 could be involved in the uptake of coenzyme A (CoA) or its precursor into mitochondria. Deletion of the gene for Leu5p caused a 15-fold reduction of mitochondrial CoA levels, but did not affect the cytosolic CoA content (79). CoA is required for the conversion of pyuvate to acetyl-CoA, tricarboxylic acid cycle, fatty acid β-oxidation and the metabolism of amino acids, such as leucine. To our knowledge, the CoA transport activities have not been confirmed in proteoliposomes with purified transporters. ScLeu5p, HsGDC and HsSLC25A42 belong to the subgroup of mono-nucleotide transporters (Fig. S1-4), but they differ from other transporters in this group in triplet 33. ScLeu5p has a lysine in triplet 87 and histidine in triplet 91, which are not present in the human ortholog HsGDC (Fig. S4).

In ScLeu5p, the conserved symmetric G213 and the adjacent M214 and Y217 could form the adenine binding pocket, as observed for other transporters in this group (Fig. S23 *A* and *B*). The asymmetric Q279 could be binding the phosphate group on the ribose. The asymmetric K48 together with K336 could be involved in the binding of the two phosphate moieties. K112 could bind the terminal sulfur atom of pantothenate moiety of CoA (Fig. S23 *A* and *B*). The equivalent

residues in the HsGDC are G198, M199, Y202, Q255, K51, R310 and Q110 (Fig. S23 *D* and *E*).

In fungal CoA transporters, the matrix and cytoplasmic networks are complete (Fig. S23 *C*). (Fig. S23 *F*)

22. Mitochondrial pyrimidine nucleotides transporters

The *S. cerevisiae* pyrimidine nucleotide transporter ScPyt1p translocates pyrimidine (deoxy)nucleoside tri- and di-phosphates and, to a lesser extent, pyrimidine (deoxy)nucleoside monophosphates (76). The main physiological role of the transporter is to transport (deoxy)pyrimidine nucleoside triphosphates into mitochondria for the synthesis of mitochondrial DNA and RNA in exchange for the generated (deoxy)pyrimidine nucleoside monophosphates in the matrix. HsSLC25A33 (PNC1) and HsSLC25A36 are the human orthologs (80), but these transport UTP primarily.

Even though the substrates are mononucleotides, the transporters belong to the class of dinucleotide transporters, as they contain the characteristic triplet 18 and 29, the latter containing the defining Trp in the matrix network. The transporters distinguish themselves from others in the group by triplets 22, 76, 83, and 84, which are in the substrate binding site, and 95, which is in the cytoplasmic network (Fig. S4).

Asymmetric residues R150 on H2 (contact point I), K299 on H5, and R357 on H6 (contact point III) could be interacting with the phosphates of pyrimidine nucleotides (Fig. S24 *A*). The asymmetric E248 could be involved in proton coupling, as TTP/TMP and TTP/TDP heteroexchange are electroneutral and most likely proton-compensated. The binding site for the bases is not apparent from the analysis, but the whole orientation seems to be very different from that found

in mononucleotide transporters. The equivalent residues in the human ortholog HsSLC25A36 are R95, K237, R290, and E189 (Fig. S24 *D* and *E*).

The matrix network is complete but for Trp on H4 rather than the expected negatively charged residue, which can form a cation-π interaction (16) (Fig. S24 *C* and *F*). The cytoplasmic network is lacking one salt bridge between H2 and H6, as H6 contains a hydrophobic residue instead of a positively charged residue. The transporter is predicted to be a strict exchanger in agreement with experimental observations (76).

23. Mitochondrial NAD^+ transporters

ScNdt1p and ScNdt2p transport NAD^+ and to a lesser extent (d)AMP and (d)GMP, but not α-NAD^+, NADH, $NADP^+$, or NADPH (77). Their main role is to import NAD^+ into mitochondria by exchange with matrix (d)AMP or (d)GMP. The alignments of symmetry-related triplets suggest that there may not be a human ortholog, but in agreement with their substrate specificity the transporters cluster with di-nucleotide transporters, which share similar triplets 18 and 29 (Fig. S4). The two most similar proteins, HsSLC25A33 (PNC1) and HsSLC25A36 (80), are related to the fungal pyrimidine nucleotide transporter (ScPyt1p) (76), with which they share triplets 26, 29 and 33. The ScNdt1p transporters also share triplets with HsMFTC (81), such as 79 and 84. Unique triplets for members of the ScNdt1p subfamily are 22 and 83, which are in the region of the substrate binding site.

The conserved asymmetric residues Y149, W153 and Y156 in contact point I on H2 of ScNdt1p are possibly involved in binding the nicotinamide moiety of NAD^+ (Fig. S25 *A*). The asymmetric R346 in contact point III and/or K289 could be involved in binding the phosphate moieties of NAD^+. The carrier may have an

adenine binding site, but this is not apparent from the symmetry analysis, as the triplets are G-G-R and Y-L-T

The matrix network has a conserved Trp rather than the expected negatively charged residue on H3, which can form a cation-pi interaction (16) with a positively charged residue of the neighboring repeat (Fig. S25 *C*). The cytoplasmic network has in 15% of the fungal NAD^+ transporters an Asn instead of the negatively charged residue on H2 and in 25% a Met rather than a positively charged residue on H6. Thus, most fungal NAD^+ transporters are expected to be strict exchangers, but some may have a net import activity. For the ScNdt1p and ScNdt2p transporters a protein-dependent efflux activity was observed (77), which is not expected on the basis of its networks or its biological function. NAD^+ is recycled in mitochondria by oxidation/reduction reactions and thus the only requirement for net import is to replenish the pools upon mitochondrial division, which could be achieved by exchange with mitochondrial AMP or GMP24. Mitochondrial flavin nucleotide transporters

The yeast mitochondrial ScFlx1p is important for flavin nucleotide homeostasis in mitochondria, but there is disagreement on whether the transporter is involved in import (75) or export (82) of FAD. To date the activity has not been studied by transport assays with proteoliposomes using a purified transporter. The ScFlx1p transporter is phylogenetically closely related to HsMFTC, which has been implicated in folate transport (81), but was found to complement a *flx1* deletion in yeast (83). The transporters belong to the class of di-nucleotide transporters, which are characterized by triplet 18 and 29, which contain a Lys and Trp, respectively. They differ from other transporters in this class by triplet 84, which is in the predicted substrate binding site (Fig. S4).

In ScFlx1p the asymmetric Y91 and W88 in contact point I on H2 could be involved in the binding of the riboflavin moiety, whereas K237 on H5 and R292

on H6 (contact point III) could interact with the two phosphate groups of FAD (Fig. S26 *A*). In addition, the transporter has G193 and V194 on H4, which could form the adenine nucleotide binding pocket in contact point II (17). Together these residues would form a plausible binding site for FAD. The equivalent residues in HsMFTC are Y99, W96, K235, R288, G189, and L190.

The fungal and metazoan matrix networks have two conserved anomalies; (i) a Trp on H3 where a negatively charged residue is expected, but this residue could form a cation-π interaction (16, 17), and (ii) a Gln on H5 instead of a negatively charged residue, which could form a hydrogen bond (Fig. S26 *C*). On H2 the matrix network of fungi has eight different substitutions, whereas the metazoan has an Asn instead of the expected negatively charged residue. On H6 the metazoan cytoplasmic network has a Ser instead of the expected positively charged residue. Thus, the cytoplasmic network is equivalent in strength to the matrix network and they agree with two salt bridges being involved in substrate binding. Although they would function mainly as exchangers, a small amount of uniport activity would be expected, as FAD is incorporated into complex II and acyl-CoA dehydrogenase, for instance.

25. Mitochondrial *S*-adenosyl-methionine transporters

The main function of the mitochondrial ScSam5p or HsSAMT is to exchange cytosolic *S*-adenosylmethionine (SAM) for matrix *S*-adenosylhomocysteine, but it also transports adenosylornithine and *S*-adenosylcysteine (84, 85). The transport of *S*-adenosylmethionine was previously characterized in rat mitochondria (86). *S*-adenosylmethionine is required for the methylation of DNA, RNA and proteins, and the biosynthesis of lipoic acid (87, 88), ubiquinone (89) and in yeast of biotin (90). The transporters belong to the class of amino acid transporters, even though the substrates contain an adenine moiety. They are most closely

related to ScYmr166c, as triplets 18, 22, 79, and 87 are very similar, but they differ in triplet 25, where the former has introduced a symmetry-breaking Arg and also in the cytoplasmic network (Fig. S4). The transporter distinguishes itself from other amino acid transporters by triplets 25, 83, and 84.

The putative binding site is at the midpoint of the membrane, and the spatial arrangement of the asymmetric and conserved residues is relatively small. Among them are R160 and E161 in ScSam5p or R150 and E151 in HsSAMT in contact point II, which could be involved in binding the amino group of the methionine moiety in analogy of the other amino acid transporters (16, 17) (Fig. S27 *A-B* and *D-E*). R250 in ScSam5p or R247 in HsSAMT are in contact point III and could be involved in binding the carboxylate group. The asymmetric S58 in ScSam5p or S60 in HsSAMT could bind of the N3 of the adenine group, whereas D19 or the equivalent D21 in HsSAMT could bind the N6. The asymmetric R112/R106 could be interacting with the OH groups of the ribose, and the oxygen atom in E105/E99 with the sulphur atom in ScSam5p and HsSAMT, respectively.

Both networks in the fungal and metazoan SAM transporters are fully conserved, indicating that the transporter functions according to a strict exchange mechanism (Fig. S27 *C*). In some fungal transporters the negatively charged residue on H6 is a Gln, whereas the positively charged residue is a Leu, which indicates that some transporters may be net importers. This notion is in agreement with the biological function of *S*-adenosylmethionine and *S*-adenosylhomocysteine exchange and with the observations that the human HsSAMT has no efflux activity (84). Yet, a significant protein-dependent uniport activity was measured for the *S. cerevisiae* transporter (85), which is in disagreement.

26. Mitochondrial thiamine pyrophosphate transporters

The yeast thiamine pyrophosphate transporter ScTpc1p translocates the essential cofactor thiamine pyrophosphate for several enzymes in mitochondria, such as pyruvate dehydrogenase and oxoglutarate dehydrogenase (91). Initially, the human ortholog had been identified as the deoxynucleotide transporter (HsDNC) (92), but its main function is likely to be the transport of thiamine pyrophosphate (93). The transporter belongs to the mononucleotide transporters, but differs from others in the same class by triplets 22, 79, and 84, which are in the substrate binding site (Fig. S4).

There are relatively few highly conserved and asymmetric residues in the cavity, but there are many more subtle adaptations. In ScTpc1p the asymmetric R31 on H1 and K292 on H6 (contact point III) could be involved in the binding of the pyrophosphate possibly with K227 (Fig. S28 *A* and *B*). In addition, there is the asymmetric Glu in the vicinity of R31, which could be a site for proton coupling, but is not conserved ScTpc1p (S83) and in metazoans. The analysis does not reveal the position of the thiamine binding site, which could fall outside the significance level. There are three asymmetric residues K238, T138, and I42, which are predicted to be at the bottom of the cavity when the transporter is in the cytoplasmic state. The equivalent residues in the human ortholog HsDNC are R30, K291, K231, H82, K242, T144, and I41.

The matrix network is fully conserved in the fungal and metazoan thiamine pyrophosphate transporters. In the metazoan and yeast transporters non-conserved polar residues are found where positively charged residue on H2 and the negatively charge residue on H4 are expected, whereas on H6 there is an apolar substitution of the positively charged residue (Fig. S28 *C*). The cytoplasmic network has approximately half the strength of the matrix network

and thus a significant net import of thiamine pyrophosphate is expected. In agreement, a transporter-dependent efflux activity has been established experimentally for the yeast orthologue (91).

27. Mitochondrial glutamate transporters

The main physiological role of GC1 and GC2 is to import L-glutamate, produced by transamination in the cytosol, into the mitochondrial matrix, where glutamate dehydrogenase is located (94). Phylogenetically, the glutamate transporters belong to the amino acid transporters and they are most closely related to the aspatate/glutamate exchangers (51, 52), but they lack the extra cytoplasmic domain. The two types of transporters share the similar triplets 21, 22, 80, 83, 84, and 87, which are in the region of the substrate binding site, but they differ in triplets 79 and in 92 and 95, which are in the cytoplasmic network (Fig. S4).

Among the asymmetric residues in HsGC1 are R196 and D197, which could form the amino group binding site in contact point II on H4 (Fig. S29 *A*). The asymmetric K80 and K83 are in contact point I and R291 in contact point III (Fig. S29 *A* and *B*). E79 could be the site for proton binding, which is required for the import of glutamate against the proton electrochemical gradient. The pH dependency of transport has been confirmed experimentally (94). The arrangement of the charged residues resembles that of aspartate/glutamate transporters very well and at first sight does not explain why HsGC1 will only transport glutamate and not aspartate (94). A plausible explanation is that the hydrophobic Val in triplet 79, which is the greatest deviation in the binding site, affects the binding of the smaller Asp to the contact points. The binding site also resembles that of oxodicarboxlate transporters apart from contact point II, which makes HsGC1 and HsGC2 amino acid rather than keto acid transporters.

The matrix network is fully conserved, but the cytoplasmic network is not functional (Fig. S29 *C*). On H2 the negatively and positively charged residue are Ala and Asn instead, whereas on H6 they are non-conserved aromatic or hydrophobic residues, respectively. Thus, this transporter is expected to be an importer of glutamate, consistent with its role in protein synthesis, amino acid degradation and inter-conversion. The uniport activity has been confirmed experimentally (94).

28. Mitochondrial MCART1 and MCART2 transporters

The MCART1 and MCART2 orthologs do not belong to a known class and they have a very low degree of conserved symmetry. They differ from all other subfamilies by triplets 22, 33, 76, 80, 83, 84, and 87 in the substrate binding site, as well as in triplets 29 and 32 of the matrix network and 95 of the cytoplasmic network. They bare some resemblance to the GTP/GDP transporters in triplets 22, 79, and 80, which are in the substrate binding site (Fig. S4 and S9).

Among the asymmetric residues in MCART1 are N44 and V45 on H1, K91 on H2 (contact point I), E132 on H3, W283 (contact point III) on H6 (Fig. S30 *A*). It could be that E132 is involved in proton coupling. The transporter also has the symmetry-related R182 (in contact point II) and R278 (in contact point III) (Fig. S30 *B*).

Both networks in the MCART1-like transporters are incomplete and poorly conserved (Fig. S30 *C*). The matrix network lacks the negatively and positively charged residue on H1, which has been replaced by Gln (55%), Tyr (12%), His (9%) or Asn (9%), and by Leu (64%), Ile (27%) or Met (9%), respectively. On H3 it has a Gln instead of a positively charged residue and on H5 an Asn instead of a negatively charged residue. Most MCART1-like transporters have a Ser instead of a Lys or Arg on H2 and a Gly instead of a Glu or Asp on H4. On H6 the

positively charged residue is mostly a hydrophobic Leu instead. Both networks are extremely weak and the transporter may cycle between states in the absence of substrate, equilibrating the substrate pools on either side of the membrane. It is also possible that the substrate has a very low binding energy.

29. Mitochondrial MCART6 transporters

The MCART6 transporters do not cluster with known classes. On the whole very little conserved symmetry is present and they differ from other transporters in triplets 21, 33, 76, 83 and 91, as well as triplets 29 and 32 of the matrix network and 95 of the cytoplasmic network (Fig. S4). Very few conserved and asymmetric residues are present in the cavity and most are unremarkable with the exception of P84 (and neighbouring P83) on H1, E128 on H3, and G188 on . There is also a very low degree of conserved symmetric residues, indicating that the transporter is highly divergent. Two symmetry-related R184 and R279 are in contact points II and III of the common substrate binding sites, respectively (Fig. S31 *B*). In contact point I is K88, which is an Arg in some related transporters and E128 could be involved in proton coupling.

There are only three conserved and symmetric triplets 14, 27, 36, and 92 (Fig. S1-3). The matrix network is non-existent and also poorly conserved, meaning that there is no energy barrier to prevent the conversion to the matrix state (Fig. S31 *C*). On H1 a Tyr is present instead of a negatively charged residue, whereas the expected negatively charged residue is either a Val (66%) or Ile (33%). On H3 the positively charged residue is Gln instead, whereas on H6 a Ser (66%) or Ile (33%) replace Asp/Glu and a Val the positively charged residue. The cytoplasmic network contains two hydrogen bonds and is thus marginally stronger than the matrix network. The expected positively charged residue on H2 is a Leu (57%), Gln (21%) or His (21%). Some transporters in the subfamily have on H4 Gly

(29%) instead of a negatively charged residue and a Tyr (29%) or Gln (29%) for the positively charged one. The positively charged residue on H6 is not conserved at all. On the basis of the strengths of the two networks alone the transporter is expected to have a major uniport activity, and would equilibrate the substrate pools on either side of the membrane. Overall, this transporter is highly divergent.

30. Mitochondrial ScYhm2p-like transporters

The transporter ScYhmp2 and orthologs in other fungi have not been characterized by transport assays, but it has been suggested that they are involved in mitochondrial DNA maintenance (10). The transporters do not belong to known classes, such as keto acid, amino acid, nucleotide, or phosphate transporters. Apart from the matrix network, the transporter shows remarkably little symmetry conservation and does not have many of the typical sequence and structural features that epitomize mitochondrial transporters. The asymmetric Q182 together with R181 may form a keto acid binding site in contact point II (16, 17) (Fig. S32 *A*). The conserved and asymmetric E33 could be involved in proton coupling. Asymmetric R280 in triplet 76 is commonly found in keto acid, amino acid and phosphate transporters, K87 and N185 in triplet 83 and R189 in triplet 87 occur in citrate transporters specifically, but all of them are in the contact points of the common substrate binding site (16, 17) (Fig. S4). The asymmetric E83 is unique among all known yeast and human transporters and might be a second site for proton coupling or for positively charged side chains of the substrate (Fig. S32 *A*).

There are very few conserved and symmetric residues apart from triplets 65, 72 and the residues of the signature motif (Fig. S32 *B*). The matrix network of the fungal ScYhm2p is complete and well-conserved in contrast to the cytoplasmic

network that deviates considerably from the norm (Fig. S32 *C*). On H2 the usual negatively charged residue is either an Ala (50%) or a Ser (50%), whereas the common positively charged residue is an Ala (69%), Val (13%), Ile (6%), Leu (6%) or a Phe (6%). Thus, the analysis shows that the highly divergent ScYhm2p transporter might be a net importer of keto acids, most likely citrate, driven by the proton electrochemical gradient.

31. Mitochondrial ScYmc1p and ScYmc2p transporters

The unknown ScYmc1p and ScYmc2p transporters (95) and their orthologs belong to the amino acid transporters and they are most closely related to the carnitine/acylcarnitine transporters (27, 96). They differ in triplet 21, 26, 84, 87, which are in the common substrate binding site, and in triplet 95 from the cytoplasmic network.

In Ymc1p asymmetric E187 together with symmetric R186 form the amino acid binding motif on H4 (16, 17) (Fig. S33 *A* and *B*), indicating that the carrier transport amino acids or amino acid-containing substrates. The related Ymc2p lacks the negatively charged residue and has Ala209 instead, indicating that it is not a transporter of amino acids. The asymmetric G88, V89, and V93 in Ymc1p form a hydrophobic patch in contact point I on H2 similar to carnitine/acylcarnitine transporters, which could interact with a hydrophobic side chain. Another interesting residue is W234 in triplet 22, which are also present in the ornithine transporters (61, 97) and the uncharacterized HsMmAU042651, HsSLC25A29 (CACTL), HsSLC25A45 and HsC14ORF68 (Fig. S4).

The matrix network is fully conserved, but the cytoplasmic network is relatively weak (Fig. S33 *C*). Among the deviations of the cytoplasmic network are His instead of Asp or Gly on H2 in half of the fungal transporters, a hydrophobic

residue on H4, and a Met or Leu instead of a negative charged residue in virtually all transporters. As the cytoplasmic network is relatively weak, it is expected that the transporter can change to the cytoplasmic state without binding of substrate and thus they are expected to be net importers. This notion would be consistent with amino acids being the substrates, as net import would be required for protein synthesis in mitochondria.

32. Mitochondrial YMR166c transporters

The unidentified *S. cerevisiae* ScYMR166c and related transporters belong to the amino acid transporters, as they contains the [RK][DE] motif on H4, which could be involved in the binding of an amino group (16, 17). They are most closely related to the *S*-adenosyl-methionine transporters (85) (Fig. S27), but differ significantly in triplets 25, 26, and 83, which are in the substrate binding site, and in triplets 92 and 95, which are in the cytoplasmic network (Fig. S4).

The asymmetric R222 together with the D223 could be involved in binding the amino group of the substrate on contact point II (Fig. S34 *A*). Asymmetric R339, W342 and Q346 in contact point III on H6 could also be involved in substrate binding, as well as M71 on H1. Triplet analysis shows that D68 and D159, which also present in *S*-adenosyl methionine transporters, are also typical of this subfamily (Fig. S4 and S27).

The matrix network is complete, but the cytoplasmic network has Gln and Leu on H6, where a negatively and positive charged residue are expected, respectively (Fig. S34 *C*). Thus, the transporter is expected to be a net importer, as the transporter may return from the matrix state to the cytoplasmic state without substrate.

33. Mitochondrial YPR011c transporters

The uncharacterised *S. cerevisiae* ScYPR011c transporter and orthologs belong phylogenetically to the mono-nucleotide transporters, and they are most closely related to the putative CoA transporters (79), but for triplet 22 in the substrate binding site and 95 in the cytoplasmic network (Fig. S4). They are closely related to HsSLC25A42 also (Fig. S39).

The ScYPR011c transporter is largely symmetric, but it has a cluster of asymmetric residues in the putative substrate binding site (Fig. S35 *A*). The substrate binding site is relatively large suggesting that this transporter may be a nucleotide or cofactor transporter, like CoA. In analogy to the ADP/ATP transporter, the asymmetric G201 together with V202 and Y205 may form the binding pocket for adenine or another hydrophobic moiety (Fig. S35 *A*). The asymmetric R37 together with the symmetry related R89 and K304 could be interacting with phosphates or other negatively charged chemical groups (Fig. S35 *A* and *B*). The putative substrate may have three negative charges overall, which could be compensated by the interactions with the positively charged side chains. A cluster of asymmetric residues consisting of I48, T148, R258 and R259 can be found at the matrix side of the matrix network.

The matrix network is complete, whereas the cytoplasmic network lacks one salt bridge (Fig. S35 *C*). Thus, the transporter of (deoxy)-nucleotides or di-phosphate co-factors is likely to be an exchanger with a minor net import activity.

34. Mitochondrial ScMtm1p and HsSLC25A39/HsSLC25A40 transporters

The metazoan orthologs of the human HsSLC25A39 (HsCGI-69) and HsSLC25A40 (HsMCFP) are related to the *S. cerevisiae* ScMtm1p (previously ScYGR257c) (98) and its fungal orthologs. ScMtm1p may have a role in manganese trafficking for mitochondrial superoxide dismutase, which plays a

critical role in guarding against mitochondrial oxidative stress, but the activity has not been confirmed by transport assays. Phylogenetically, these transporters cluster with translocators of amino acids or amino acid-containing substrates, as they contain the amino group binding motif on H4 (16, 17). The transporters are similar to HsSLC25A38 and ScYDL119c, but differ in triplets 79 and 80 (Fig. S4). They differ from other amino acid transporters in triplets 18, 83, 84, and 87, which are in the predicted substrate binding site.

Among the conserved and asymmetric residues are D233 in ScMtm1p or D226 in HsSLC25A39, which are in contact point II and could be involved in the binding of the amino group together with the symmetric R232 and R225, respectively (16, 17) (Fig. S36 *A* and *B*). Very close to this site there is a positively charged residue R169 in Mtmt1p and R172 in HsSLC25A39, similarly to YDL119c and SLC25A38. In addition, there are M131 and R338 in ScMtm1p and M133 and R326 in HsSLC25A39, which are in contact point I and III on H6, respectively.

Both the matrix and the cytoplasmic network are fully conserved in the metazoan and fungal transporters, except for a few cases in which the expected positively charged residue on H2 is replaced by Ser, capable of forming a hydrogen bond (Fig. S36 *C*). Thus, the transporter is expected to operate according to a strict exchange mechanism. The binding of the substrate, likely to be an amino acid, must be sufficient to break three salt bridges, in agreement with the predicted ionic interactions.

35. Mitochondrial ScYDL119c and SLC25A38 transporters

The uncharacterized fungal ScYDL119c transporters and metazoan HsSLC25A38 are related and they cluster with the transporters of amino acids or amino acid-containing substates, such as the *S*-adenosyl-methionine, ornithine, carnitine transporters. They are most closely related to ScMtm1p-like carriers because of

triplets 18, 21, and 76 and they have also a fully symmetric triplet 79 of Arg similar to dicarboxylate transporters and uncoupling proteins (Fig. S4). Defining triplets for these transporters are 79, 80, 83 and 87, which are all in the substrate binding site, and 29, which is in the matrix network.

The cluster of asymmetric residues at the midpoint of the membrane is relatively small indicating that the substrate might be relatively small also. Noticeably, among the conserved residues in the substrate binding site there are three asymmetric and three symmetric positively charged residues, i.e. the asymmetric R128, R284 and K288, and symmetric R69, R181 and R287 in ScYDL119c (Fig. S37 *A* and *B*), and the asymmetric R134, R278, and R282 and symmetric R96, R187 and R281 in HsSLC25A38 (Fig. S37 *D* and *E*). R187 and D188 in HsSLC25A38 and R181 and D182 in ScYDL119c might be involved in the binding of an amino group (16, 17). In the area of the common binding site are two other conserved asymmetric residues G100 and T184 in SLC25A38, and G73 and T178 in ScYDL119c.

In the fungal and metazoan transporters the matrix network has a Thr instead of a negatively charged residue on H3 (Fig. S37 *C* and *F)*. The cytoplasmic networks of the fungal transporters are incomplete with an Asn (78%) or Ser (22%) instead of a negatively charged residue on H2 and a hydrophobic substitution (Ile 61%, Leu 22% or Val 17%) on H6 instead of the expected positive charged residue. In the case of the metazoan transporters, Tyr substitutes the expected negatively charged residue on H2, the polar Asn (33%), Ser (33%) or Thr (33%) the negatively charged residue on H4 and Met the positively charged residue. Thus, in addition to exchange a significant net import is expected especially in the case of the metazoan transporters. This activity would be compatible with amino acid transport as net import is required for the mitochondrial protein synthesis.

36. Mitochondrial HsSLC25A29 transporters

Phylogenetically, the metazoan HsSLC25A29 transporters cluster with the amino acid transporters, and they are related to the ornithine (29, 30, 60) (Fig. S15) and carnitine/acylcarnitine transporters (26, 27) (Fig. S7) and the uncharacterized HsMmAU042651, HsMmSLC25A45 and HsC14ORF68. Defining triplets are 83, in the binding site, and 92 and 95, which are in the cytoplasmic network (Fig. S4). Among the highly conserved asymmetric residues are E161, which could form with the symmetric R160 in contact point II an amino group binding motif (17) (Fig. S38 *A* and *B*). They may transport an amino acid or amino acid containing substrate, which would be in agreement with the relatively small cluster of asymmetric residues. The conserved G68 and L69 in contact point I may interact with the side chain of the amino acid. The putative binding site may contain also R257 in contact point III, which generally interacts with a carboxylate or phosphate group.

The matrix network is complete, but the cytoplasmic network is not, as on H2 the expected negatively charged residue is a Gly (75%) or Ala (25%) and on H6 the expected positively charged is a hydrophobic one, such as Leu (50%), Met (33%), Ile (8%) or a polar Asn (17%). The transporters are exchangers possibly of amino acids with net import activities.

37. Mitochondrial HsSLC25A42 transporters

The HsSLC25A42 transporters belong to the class of mono-nucleotide transporters and are phylogenetically closely related to the CoA transporters (ScLeu5p and HsGDC) (79, 99) and to the uncharacterized ScYPR011c-like transporters (Fig. S4).

Among the asymmetric residues are Q107 on H2 in contact point I and G196 that with V197 and Y200 could form an adenine binding site in contact point II (Fig. S39 *A*). In addition, there are K48, R99 and K294 nearby (Fig. S39 *B*).

At the bottom of the predicted cavity in the cytoplasmic state is a cluster of asymmetric residues consisting of R56, I59, A157, and R252, which may flank the passage to the matrix (Fig. S39 *C*). The matrix network is complete and the cytoplasmic network is marginally weaker as there is a Gln on H6, where a positively charged residue is expected. Thus, the transporter is likely to operate as an exchanger of adenine nucleotides, possibly CoA.

38. Mitochondrial HsSLC25A43 transporters

The uncharacterized HsSLC25A43 and orthologs have many features in common with mono-nucleotide transporters (triplet 18, 26, 29, 33, 79, 83), but they are lacking the basic residue in triplet 22. They differ significantly from other transporters in triplets 83 and 84, which could be involved in substrate binding, and 32 in the matrix network and 92 and 95 in the cytoplasmic network (Fig. S4).

There are remarkably few asymmetric residues in the cavity and most can be found in the cytoplasmic network. Among the asymmetric residues are G173 and A174, which are in contact point II and they may form an adenine binding pocket (Fig. S40 *A*). Q216 on H5 and F280 on H6 are the only other remarkable residues. The symmetry-related R78 and K275 might be binding phosphate of nucleotides or cofactors (Fig. S40 *B*). Quite a large number of asymmetric residues cluster at the bottom of the cavity in the predicted structure of the cytoplasmic state: V38, L39, T132, I135, and R227 (Fig. S40 *A*).

The matrix network deviates from the norm by having a polar Thr instead of a positively charged residue on H1 in 75% of the HsSLC25A43 transporters (Fig. S40 *C*). The cytoplasmic network has on H2 a positively charged residue instead

of a negatively charged residue and a hydrophobic Val (71%) or Leu (29%) instead of a positively charged residue. On H4 the network has a hydrophobic Met (43%), Ile (43%) or Val (14%) instead of Asp/Glu, whereas the expected positively charged residue is a negatively charge residue. The network is extremely weak or not functional and, thus, the transporter is likely to be an importer.

39. Mitochondrial HsSLC25A44 transporters

The HsSLC25A44 and orthologs do not belong to any known class of transporters and they have a low degree of conserved symmetry. Only triplets 14, 29, 32, 33, 91, 92 and 95, most of which belong to the networks, have features of other carriers, but most others are unique (Fig. S4). Very few asymmetric residues are present in the cavity (Fig. S41 *A*). In contact point II is the asymmetric and conserved Y193, which is unique in that position, and in contact point III is R281, which is commonly found in phosphate, keto and amino acid transporters. Thus, the substrate may have a carboxylate group and an uncharged polar or hydrophobic side chain, as the interacting residues will not deviate significantly from other residues in the cavity to show in the analysis.

The matrix network is in most cases slightly weaker with Thr (67%), Ser (17%), Ala (8%) or Asn (8%) instead of a negatively charged residue on H1, and Ser (83%) or Ala (17%) on H3 rather than a positively charged residue (Fig. S41 *C*). The cytoplasmic network is also incomplete, as on H4 the expected negatively charged residue is either a His (75%) or or Tyr (25%), and the positively charged residue is an Ala (50%), Gln (17%), Leu (17%), Ser (8%) and Gly (8%). Thus, the cytoplasmic network has about half the strength of a complete network. The relative strength of the networks indicates that the carrier has a significant net import activity in addition to an exchange activity.

The residues of the triplets of mitochondrial transporters of *S. cerevisiae* (Sc), *H. sapiens* (Hs), *M. musculus* (Mm), and *Aspergillus oryzae* (Ao) are shown adjacent to emphasize the symmetry in the three-fold repeat. The triplets are numbered according to the residue number of the first repeat in the bovine ADP/ATP carrier.

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41. Supplementary figures

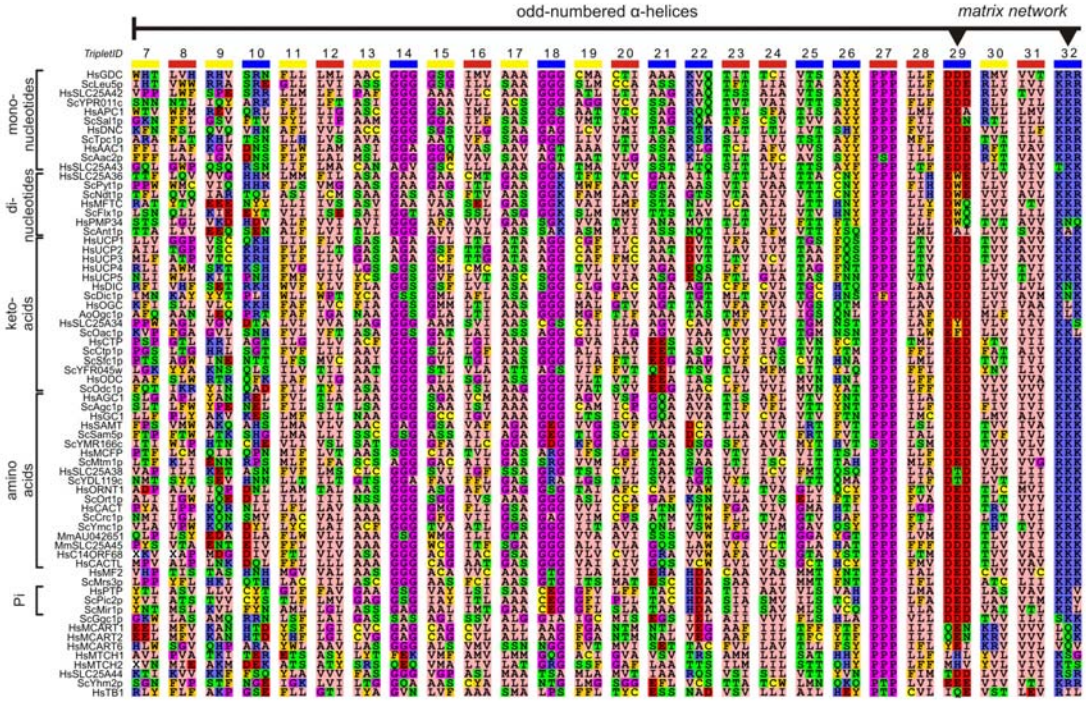

Fig. S1. Alignment of symmetry-related triplets from the odd-numbered *α*-helices. The residues of the triplets of mitochondrial transporters of *S. cerevisiae* (Sc), *H. sapiens* (Hs), *M. musculus* (Mm), and *Aspergillus oryzae* (Ao) are shown adjacent to emphasize the symmetry in the three-fold repeat. The triplets are numbered according to the residue number of the first repeat in the bovine ADP/ATP carrier. The order of the sequences is based on the sequence similarity calculated by using CLUSTALW (100) and is consistent with their classification

based upon the chemical similarity of their substrates (16, 17). The dark blue, red and yellow bars indicate residues of the transmembrane α-helices that are facing the cavity, are interacting with the lipid bilayer or are inter-helical, respectively. The arrow heads indicate the charged residues of the salt bridge networks. Amino acids are colored according to their properties: basic Lys, Arg and His are blue, acidic Asp and Glu red, polar Asn, Gln, Ser and Thr green, aliphatic Ala, Ile, Leu, Met and Val pink, aromatic Phe, Tyr and Trp orange, structural Gly and Pro magenta, and Cys yellow.

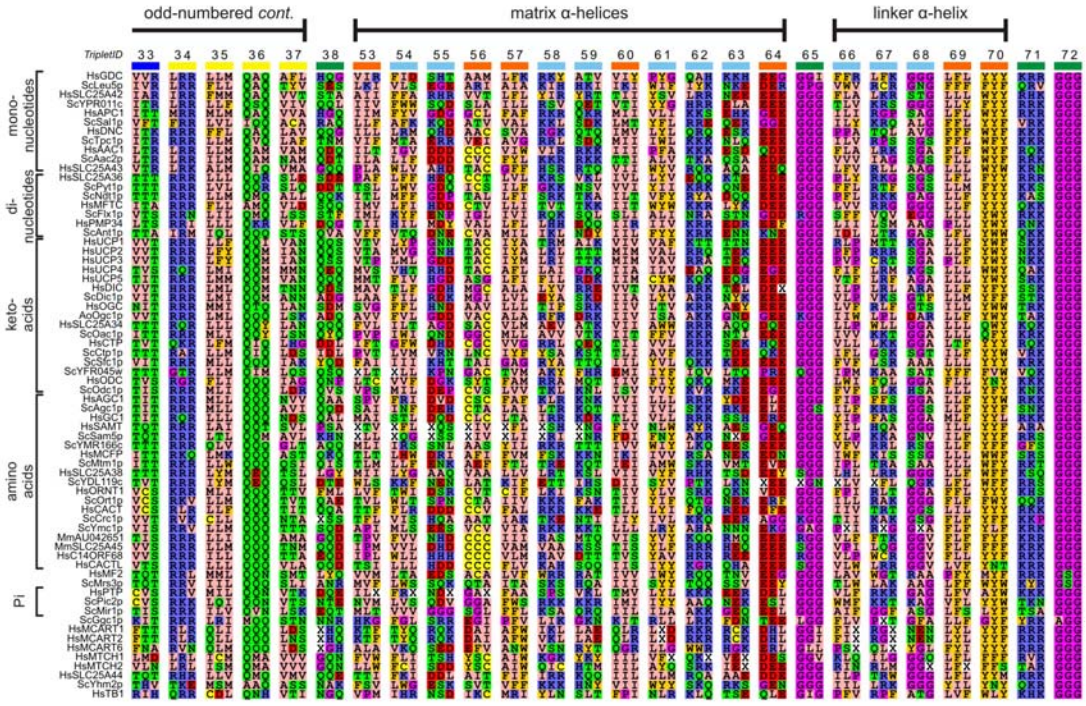

Fig. S2. Alignment of symmetry-related triplets from the matrix and linker α-helices. For details, see the legend to Fig. S1, except for the light blue or orange bars, which indicate residues of the matrix and linker α-helices that interact with the water phase of the mitochondrial matrix or with the transmembrane α-helical bundle, respectively.

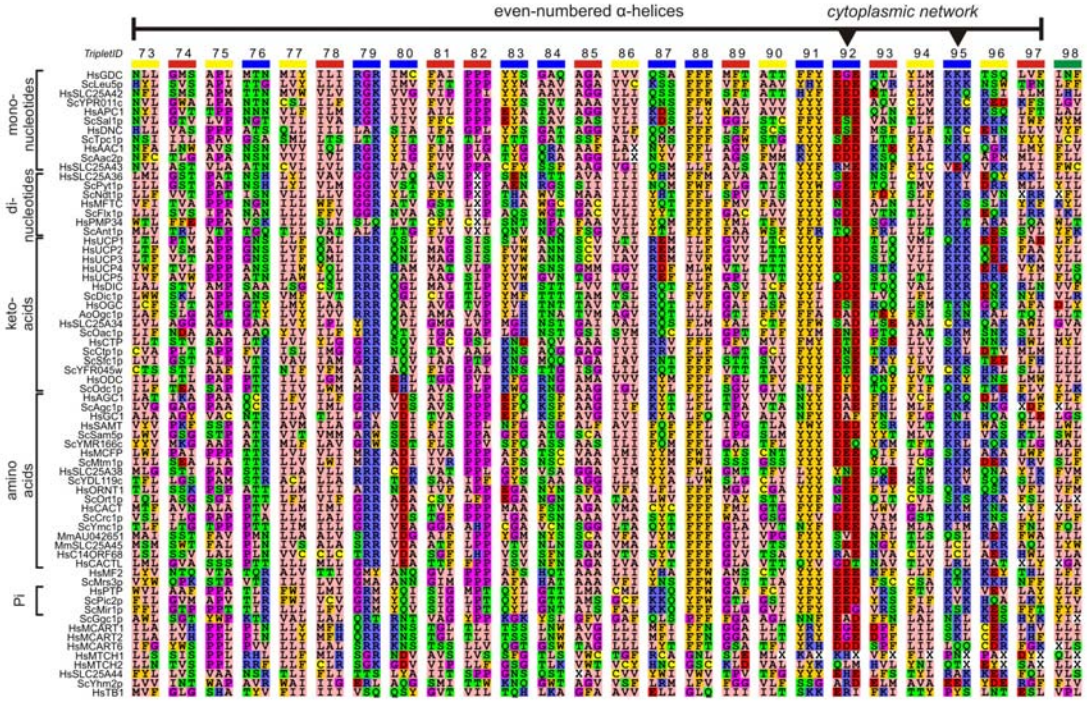

Fig. S3. Alignment of symmetry-related triplets from the even-numbered α-helices. **F**or details, see the legend to Fig. S1, except for the green bar, which indicates residues that are in a loop region.

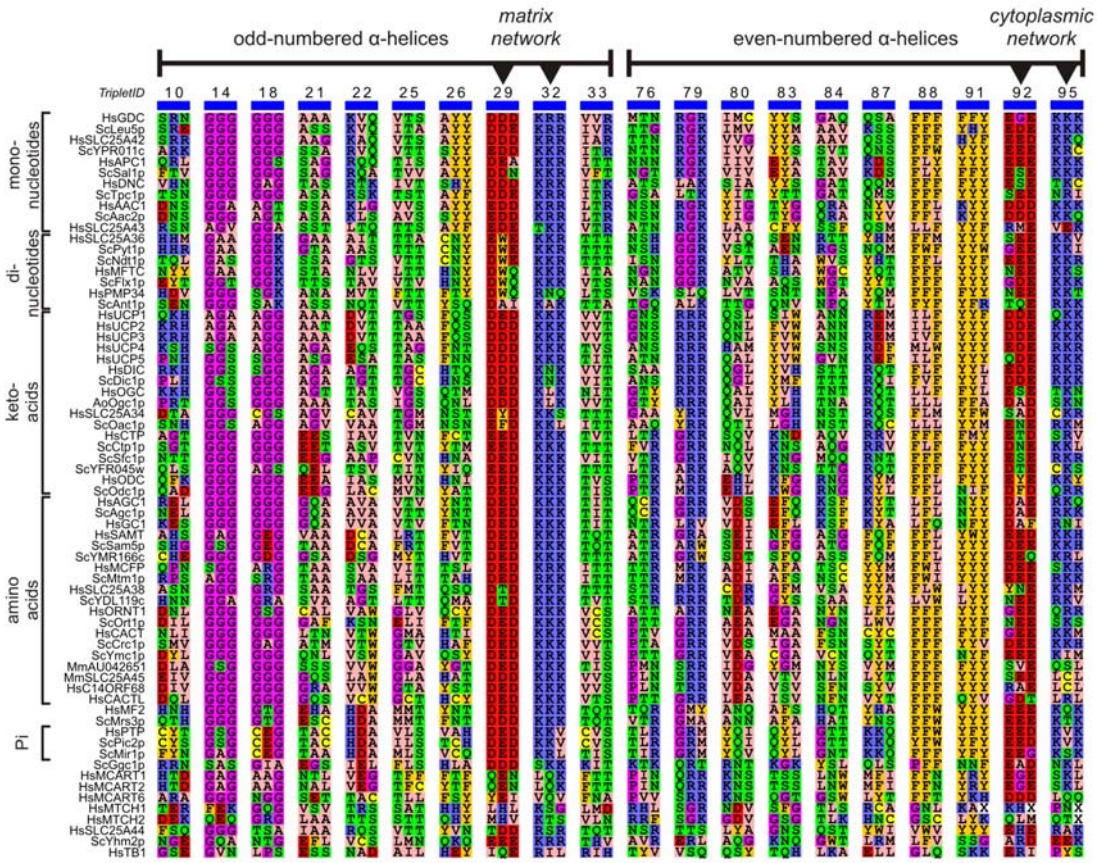

Fig. S4. Alignment of symmetry-related triplets of the cavity residues. For details see the legend to Fig. S1.

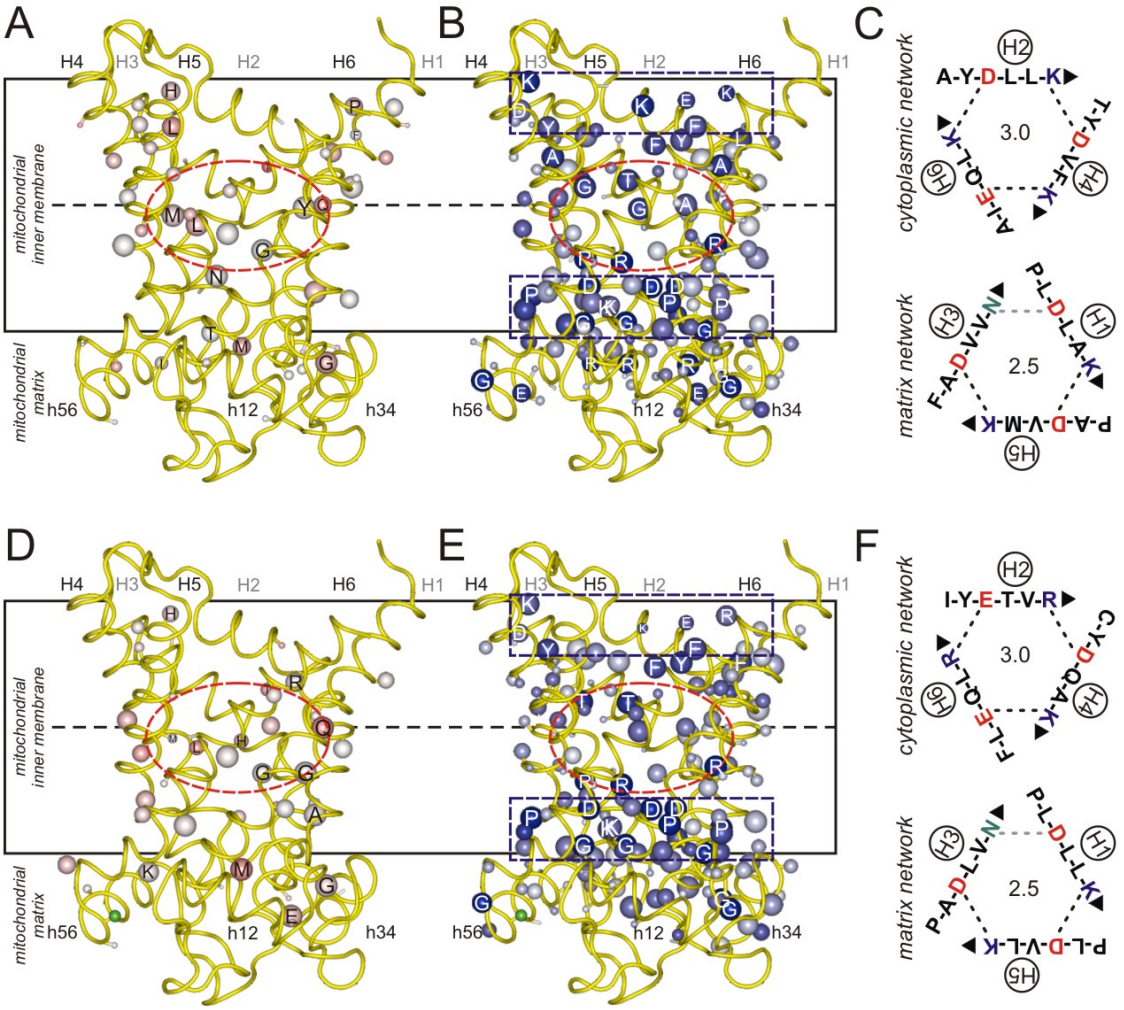

Fig. S5. Asymmetry and symmetry in the fungal and metazoan dicarboxylate carriers. For details, see the legend to Fig. 2, except for (*A-C*) fungal (ScDic1p) and (*D-F*) metazoan (HsDIC) dicarboxylate transporters.

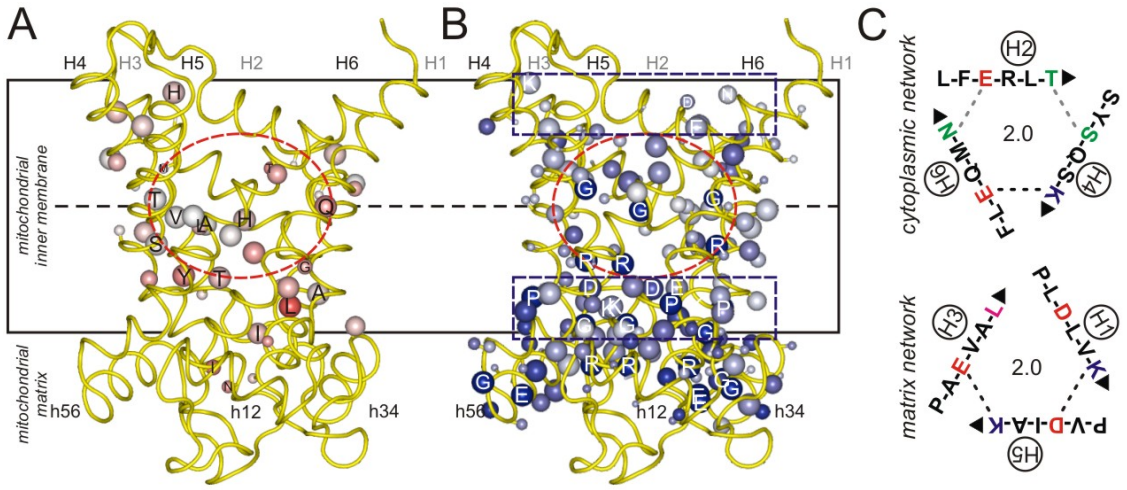

Fig. S6. Asymmetry and symmetry in the metazoan oxoglutarate transporters (OGC). **F**or details, see the legend to Fig. 2.

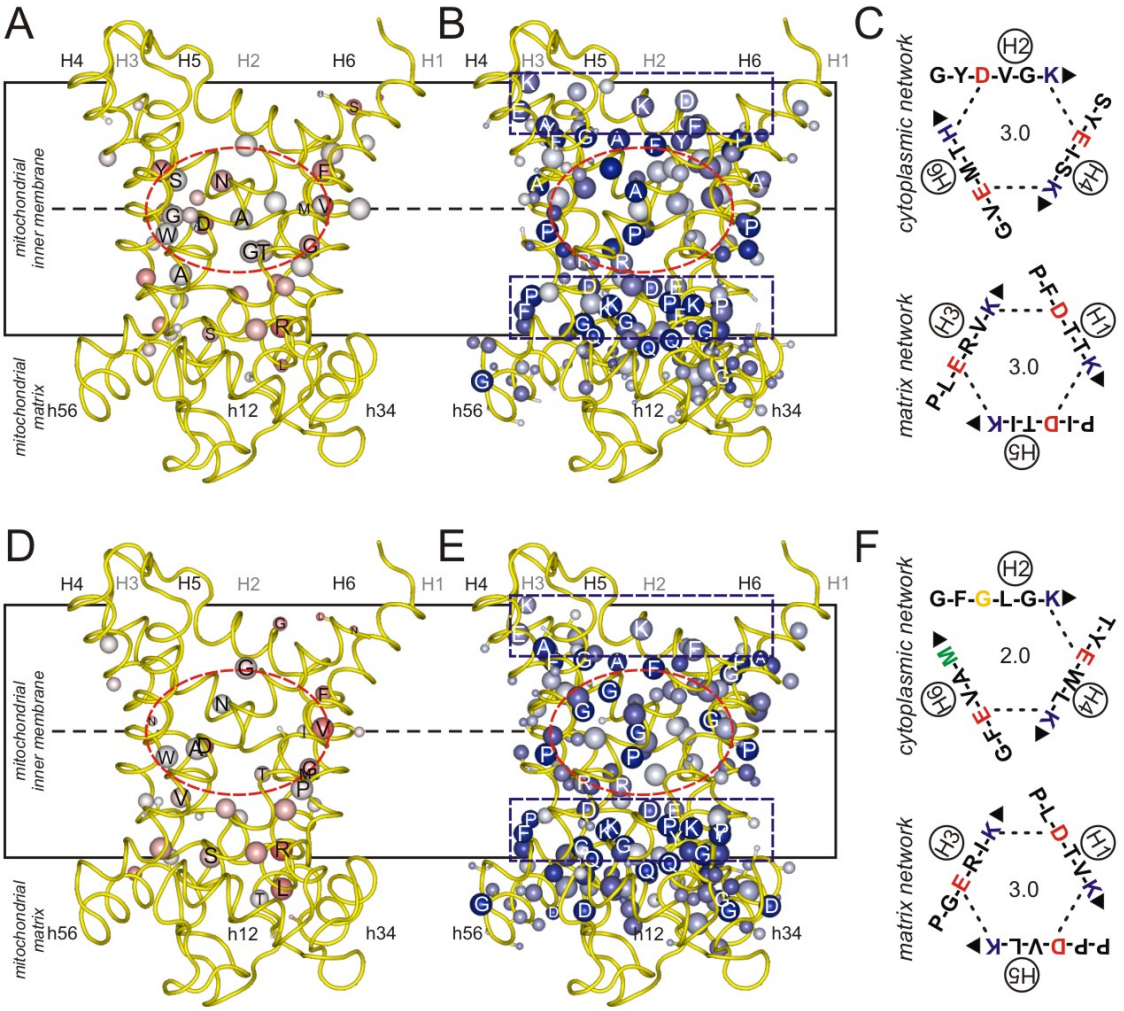

Fig. S7. Asymmetry and symmetry in the fungal and metazoan mitochondrial carnitine-acylcarnitine carriers. For details, see the legend to Fig. 2, except for (*A-C*) fungal (ScCrc1p) and (*D-F*) metazoan (CACT) carnitine-acylcarnitine transporters.

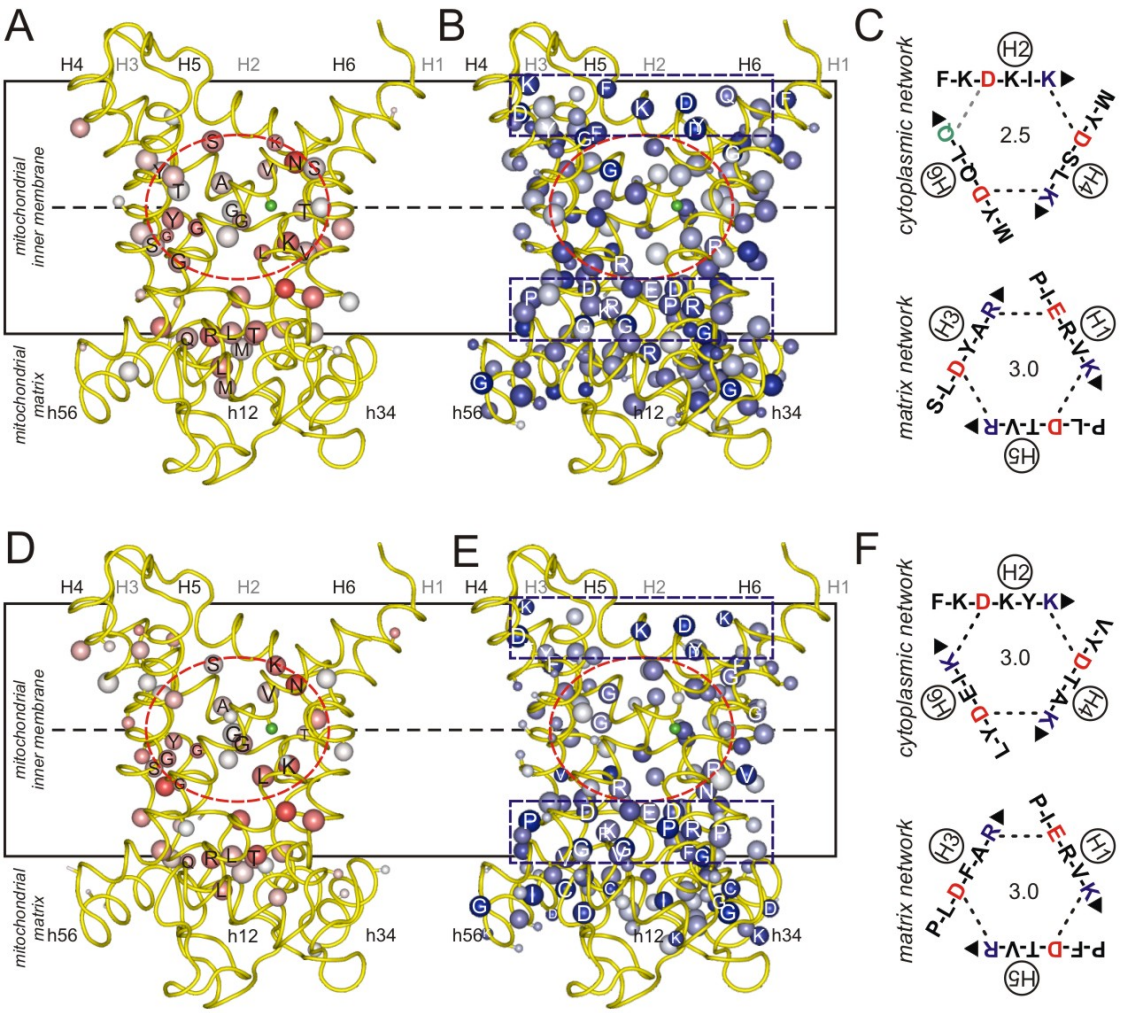

Fig. S8. Asymmetry and symmetry in the fungal and metazoan ADP/ATP carriers. **L**egend as that of Fig 2, except for (*A-C*) fungal (ScAac2p) and (*D-F*) metazoan (HsAAC1) ADP/ATP transporters.

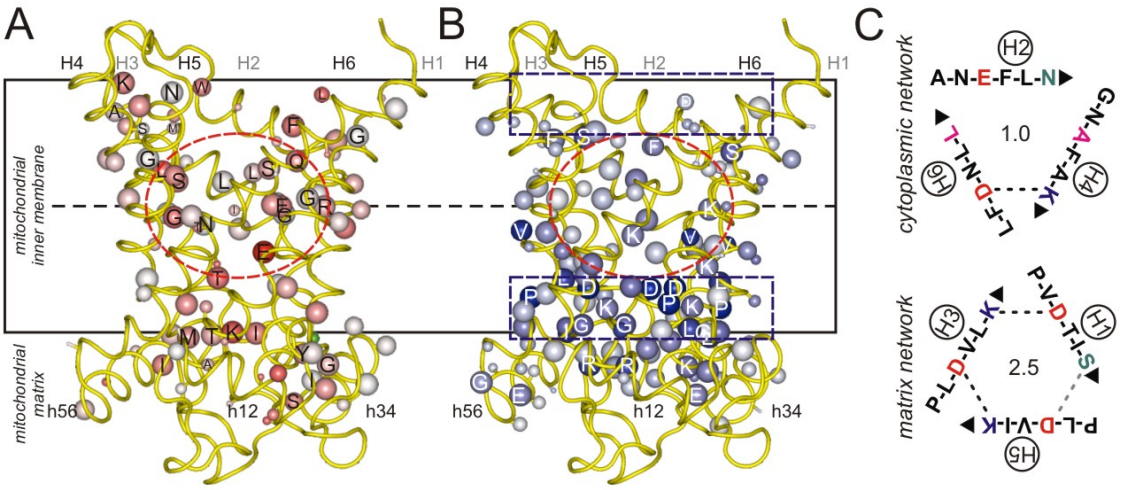

Fig. S9. Asymmetry and symmetry in the fungal mitochondrial GTP/GDP carrier (ScGgc1p). For details, see the legend to Fig. 2.

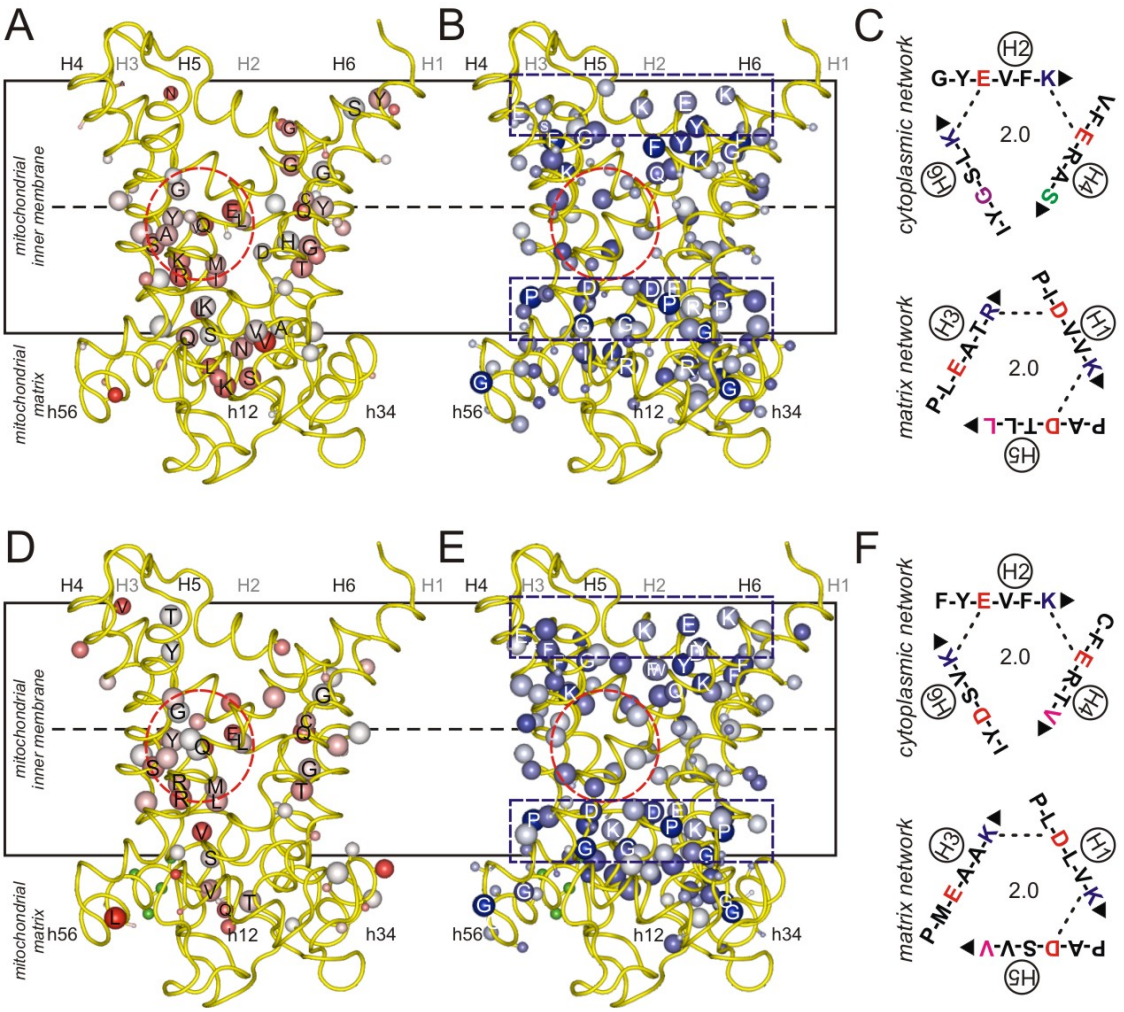

Fig. S10. Asymmetry and symmetry in the fungal and metazoan mitochondrial phosphate transporters. For details, see the legend to Fig. 2, except for (*A-C*) fungal (ScMir1p) and (*D-F*) metazoan (HsPTP) phosphate transporters.

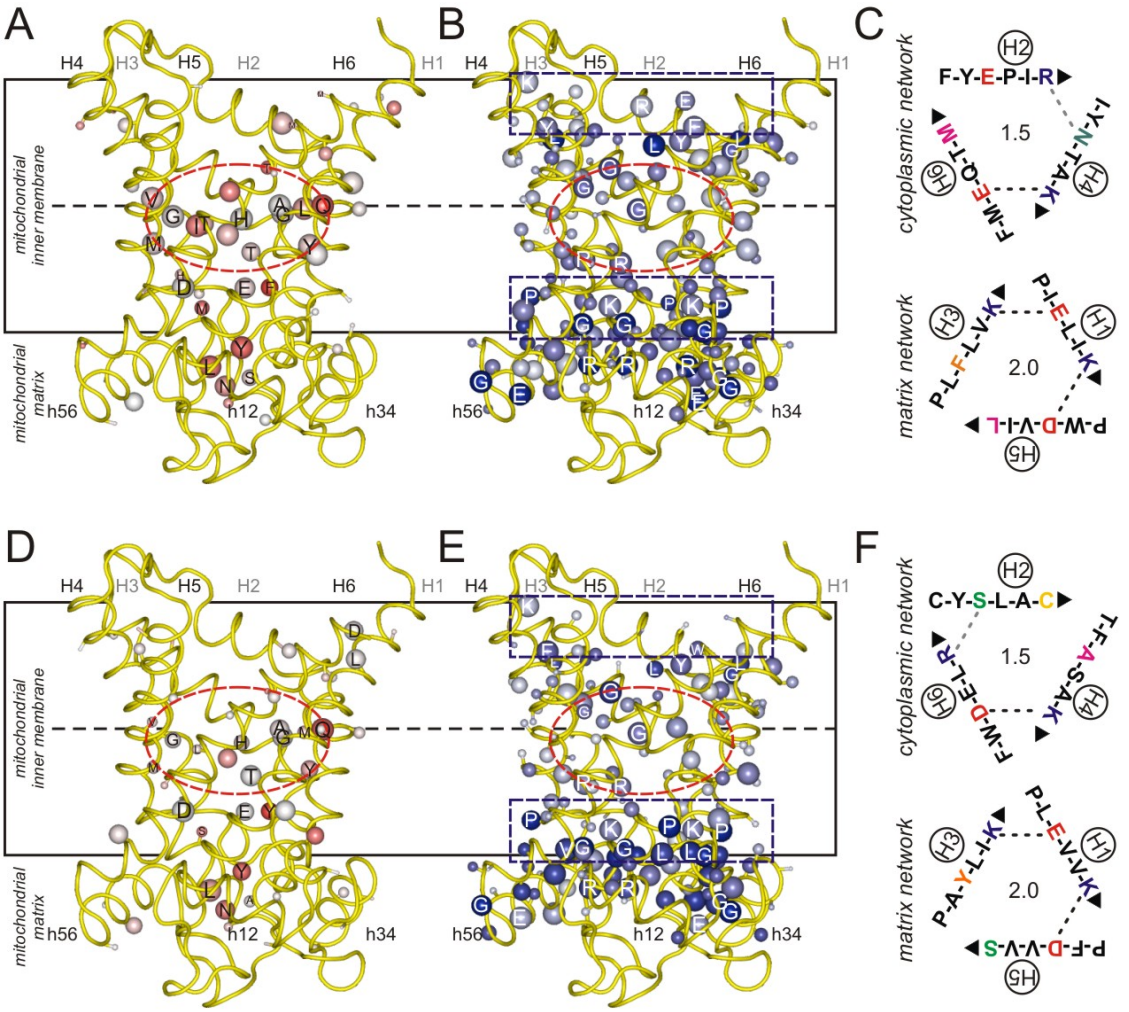

Fig. S11. Asymmetry and symmetry in the fungal and metazoan oxaloacetate carriers. For details, see the legend to Fig. 2, except for (*A-C*) fungal (ScOac1p) and (*D-F*) putative metazoan (HsSLC25A34, HsSLC25A35) oxaloacetate carriers.

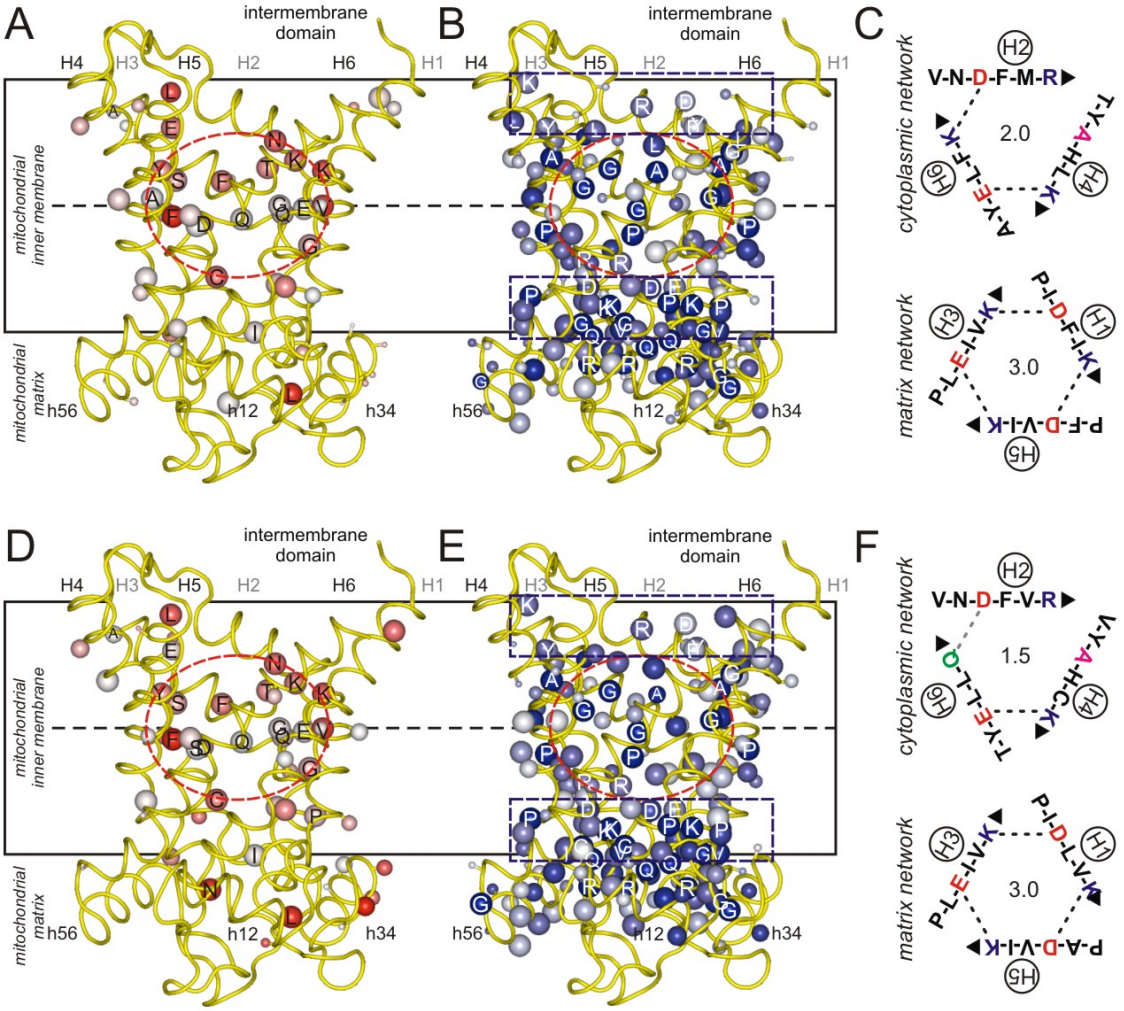

Fig. S12. Asymmetry and symmetry in the fungal and metazoan mitochondrial aspartate/glutamate carriers. For details, see the legend to Fig. 2, except for (*A-C*) fungal (ScAgc1p) and (*D-F*) metazoan (HsAGC1) aspartate/glutamate carriers.

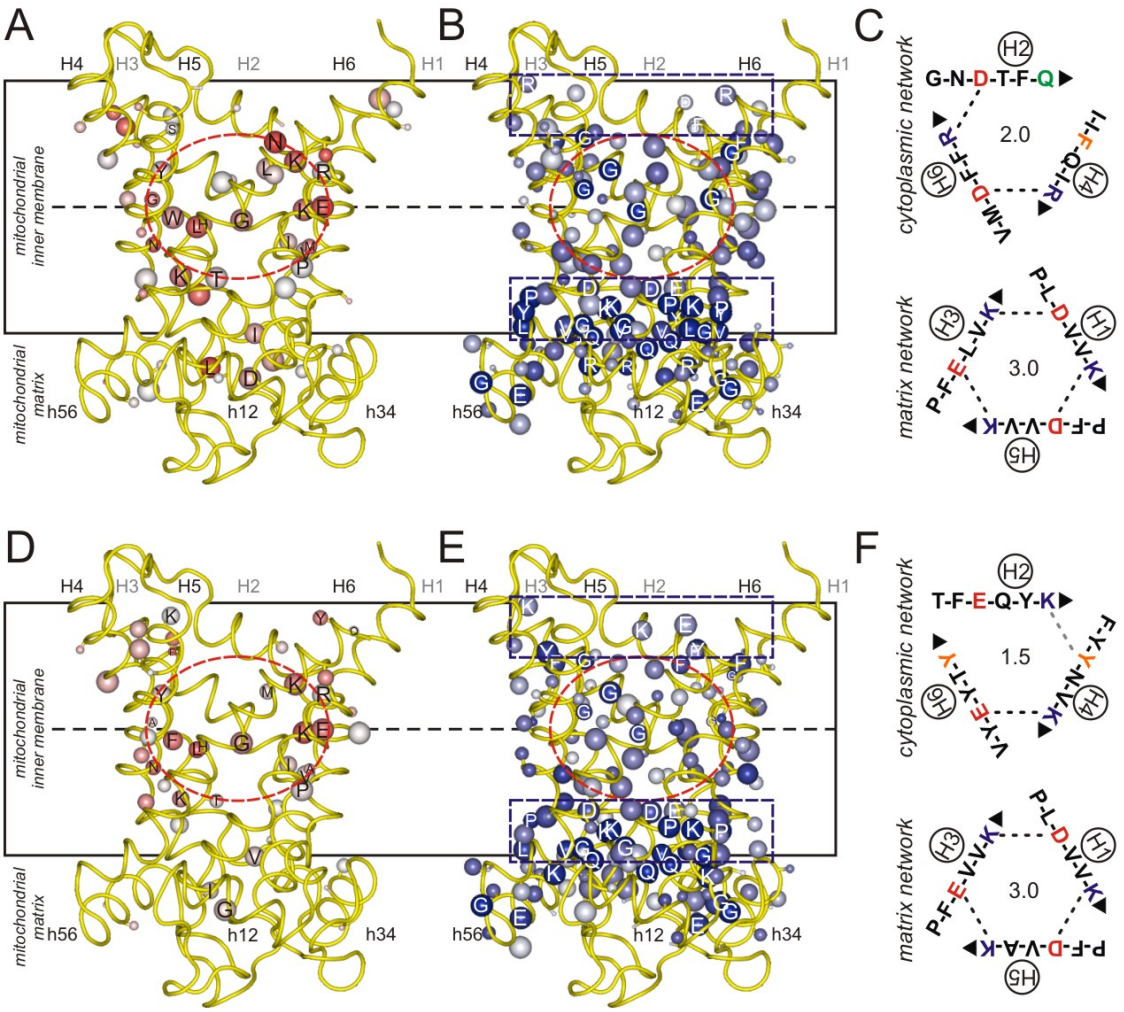

Fig. S13. Asymmetry and symmetry in the fungal and metazoan oxodicarboxylate carriers. For details, see the legend to Fig. 2, except for (*A-C*) fungal (ScOdc1p) and (*D-F*) metazoan (HsODC1) oxodicarboxylate carriers.

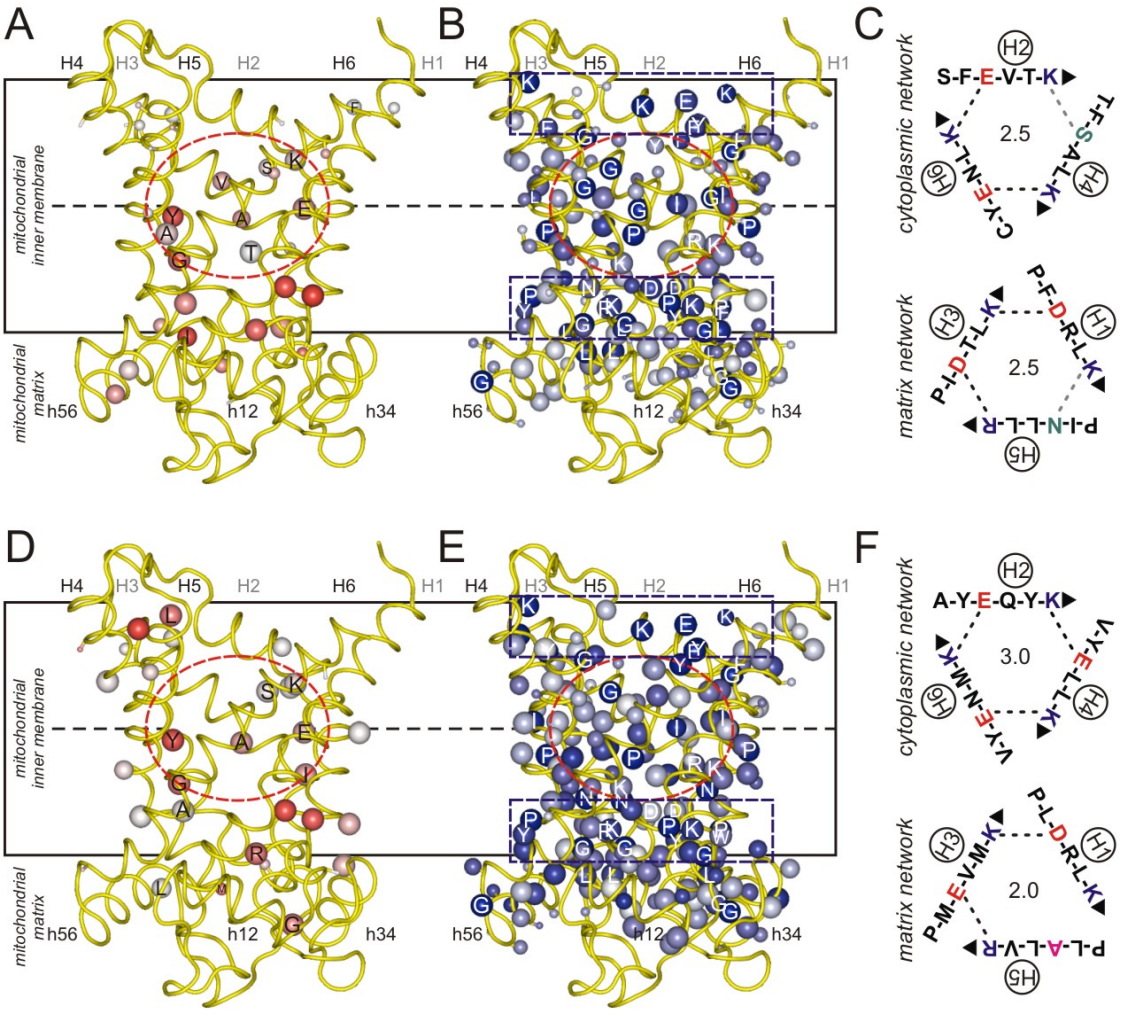

Fig. S14. Asymmetry and symmetry in the fungal and metazoan Mg^{2+}-ATP/Pi transporters. For details, see the legend to Fig. 2, except for (*A-C*) fungal (ScSal1p) and (*D-F*) metazoan (HsAPC1) Mg^{2+}-ATP/Pi transporters.

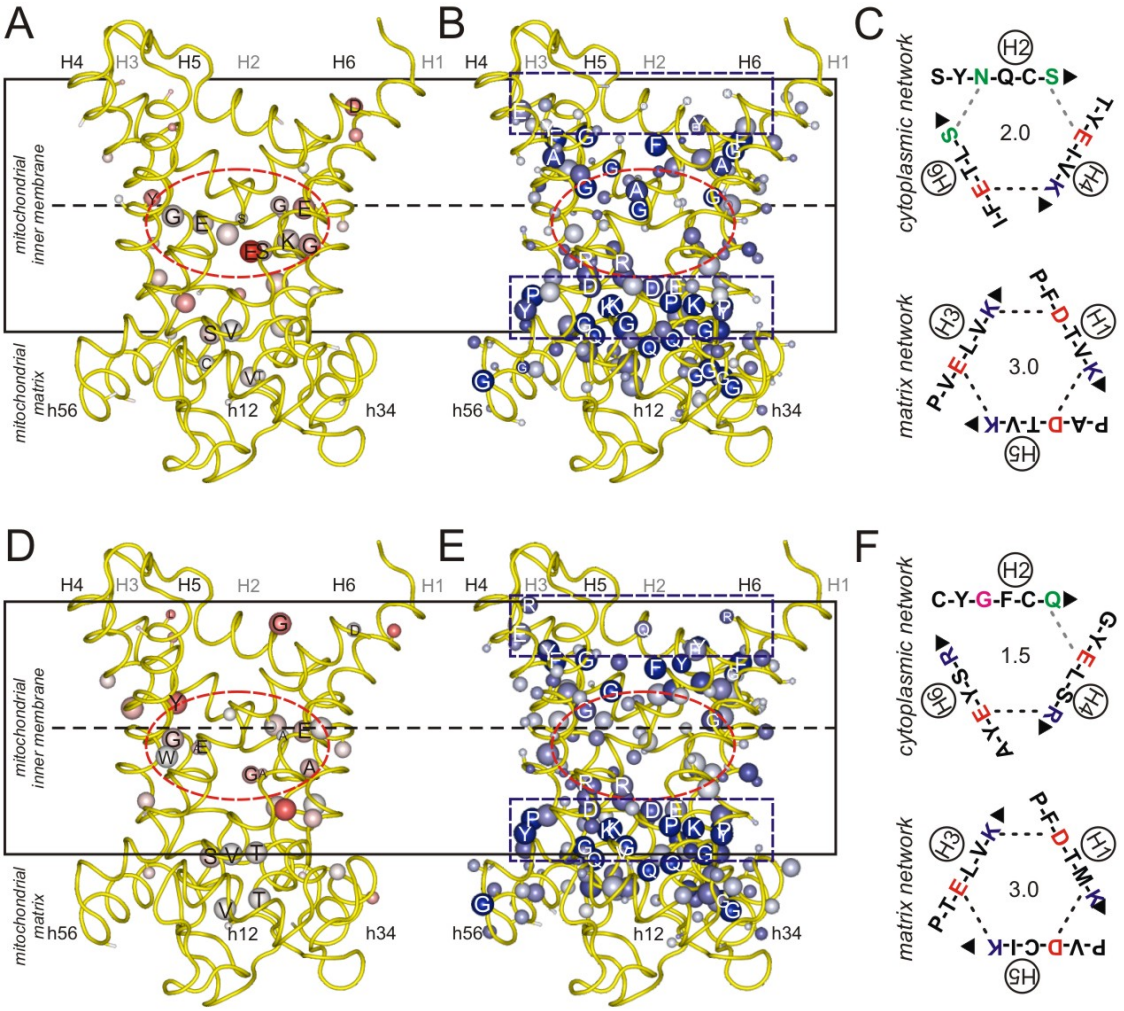

Fig. S15. Asymmetry and asymmetry in the fungal and metazoan mitochondrial ornithine transporters. For details, see the legend to Fig. 2, except for (*A-C*) fungal (ScOrt1p) and (*D-F*) metazoan (HsORNT1) ornithine transporters.

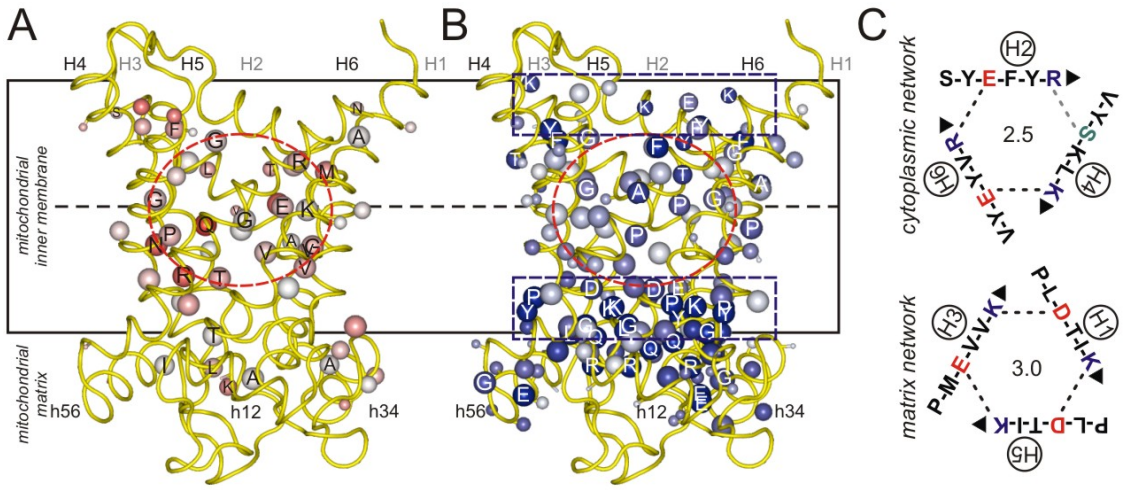

Fig. S16. Asymmetry and symmetry in the fungal succinate/fumarate carriers (ScSfc1p). **F**or details, see the legend to Fig. 2.

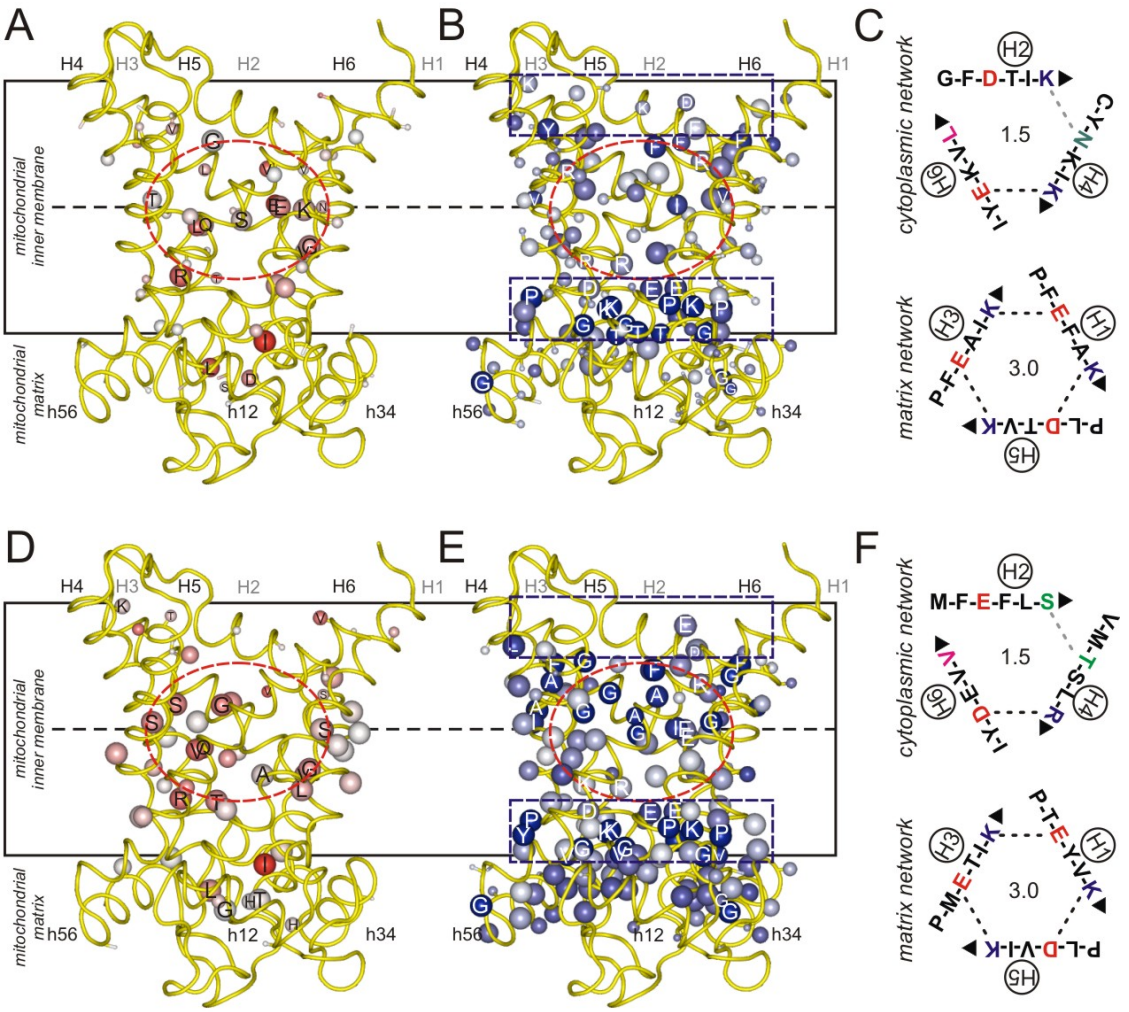

Fig. S17. Asymmetry and symmetry in the fungal and metazoan citrate transporters. **F**or details, see the legend to Fig. 2, except for (*A-C*) fungal (ScCtp1p) and (*D-F*) metazoan (HsCTP, HsSLC25A1) citrate transporters.

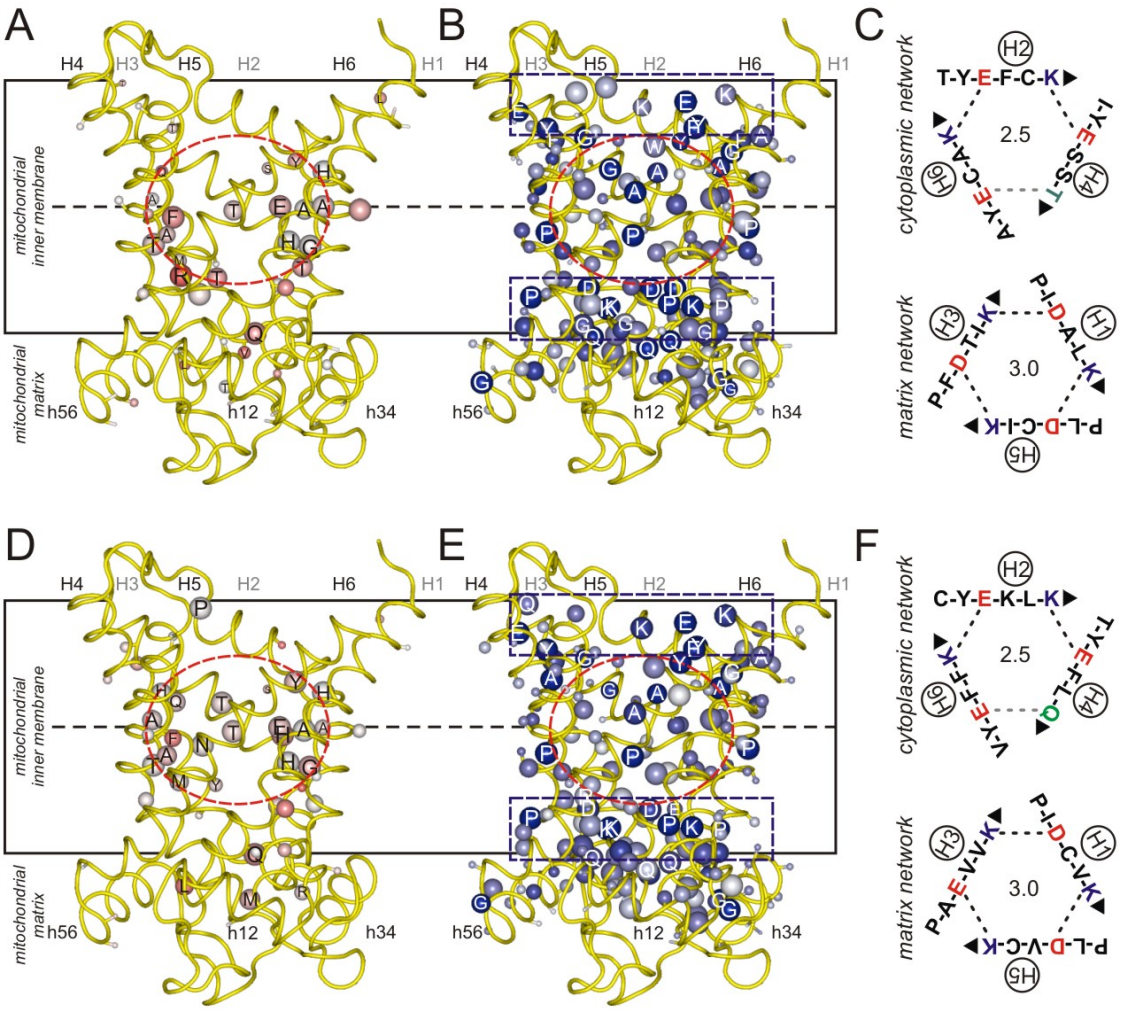

Fig. S18. Asymmetry and symmetry in the fungal and metazoan mitochondrial ScMrs3p-like transporters. For details, see the legend to Fig. 2, except for (*A-C*) fungal (ScMrs3p) and (*D-F*) metazoan (HsMF2, SLC25A28) ScMrs3p-like transporters.

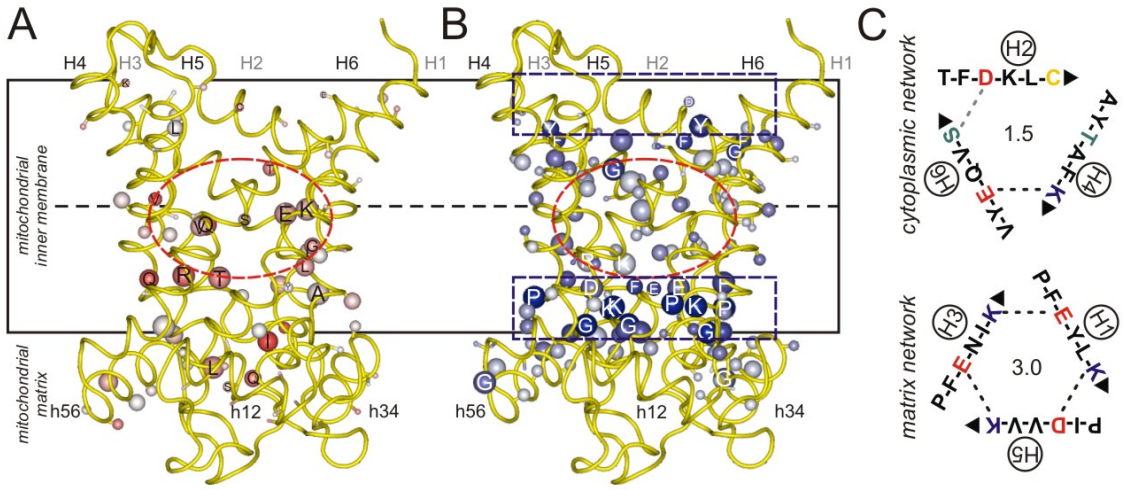

Fig. S19. Asymmetry and symmetry in the fungal ScYFR045w transporters. For details, see the legend to Fig. 2.

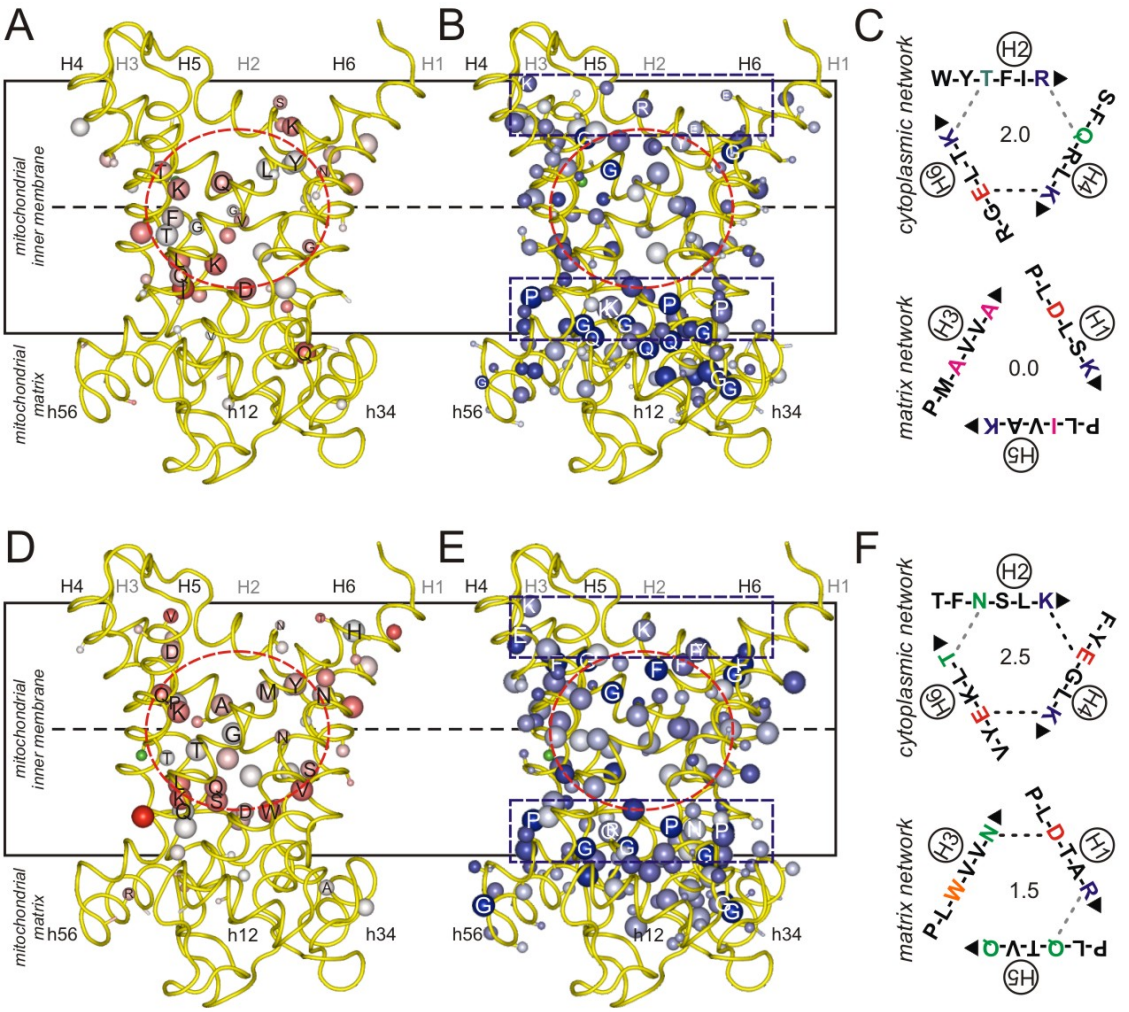

Fig. S20. Asymmetry and symmetry in the fungal and metazoan peroxisomal adenine nucleotide transporters. For details, see the legend to Fig. 2, except for (*A-C*) fungal (ScAnt1p) and (*D-F*) metazoan (HsPMP34) peroxisomal adenine nucleotide transporters.

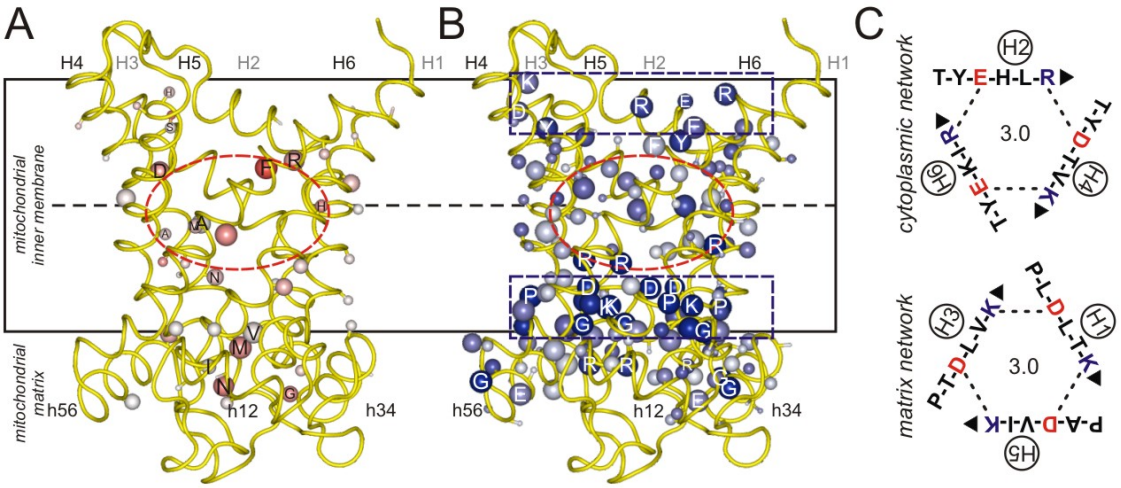

Fig. S21. Asymmetry and symmetry in the metazoan HsUCP4. For details, see the legend to Fig. 2.

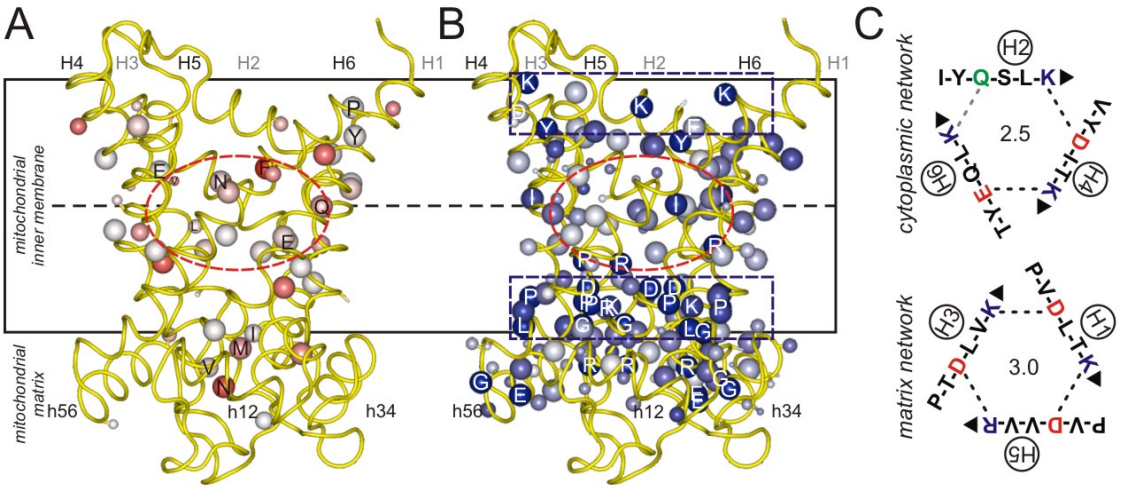

Fig. S22. Symmetry and asymmetry in the metazoan HsUCP5. For details, see the legend to Fig. 2.

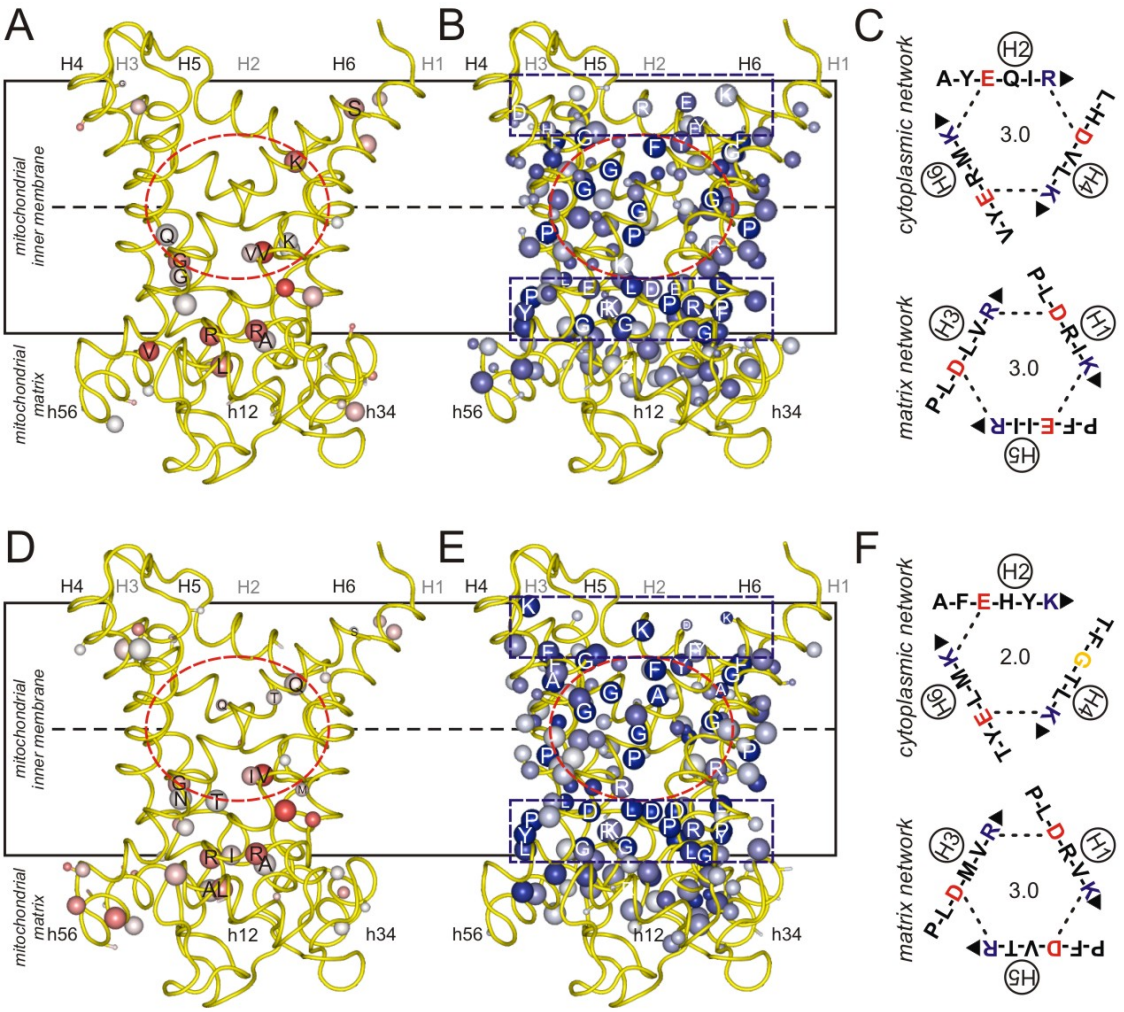

Fig. S23. Asymmetry and symmetry in the fungal and metazoan mitochondrial CoA transporters. For details, see the legend to Fig. 2, except for (*A-C*) fungal (ScLeu5p) and (*D-F*) metazoan (HsGDC/SLC25A16) CoA transporters.

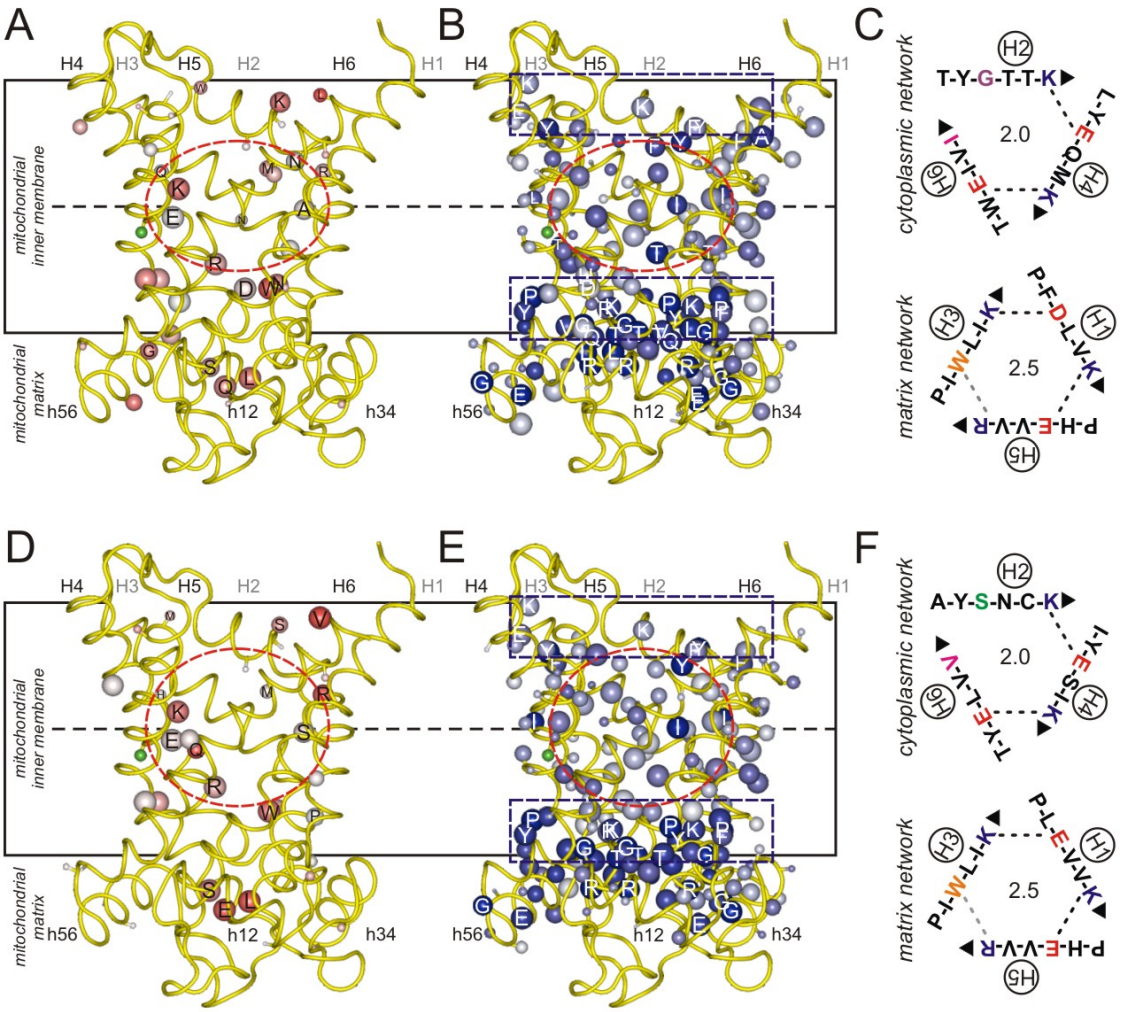

Fig. S24. Asymmetry and symmetry in the fungal and metazoan pyrimidine nucleotides transporters. For details, see the legend to Fig. 2, except for (*A-C*) fungal (ScPyt1p) and (*D-F*) metazoan (HsSLC25A33 and HsSLC25A36) pyrimidine nucleotide transporters.

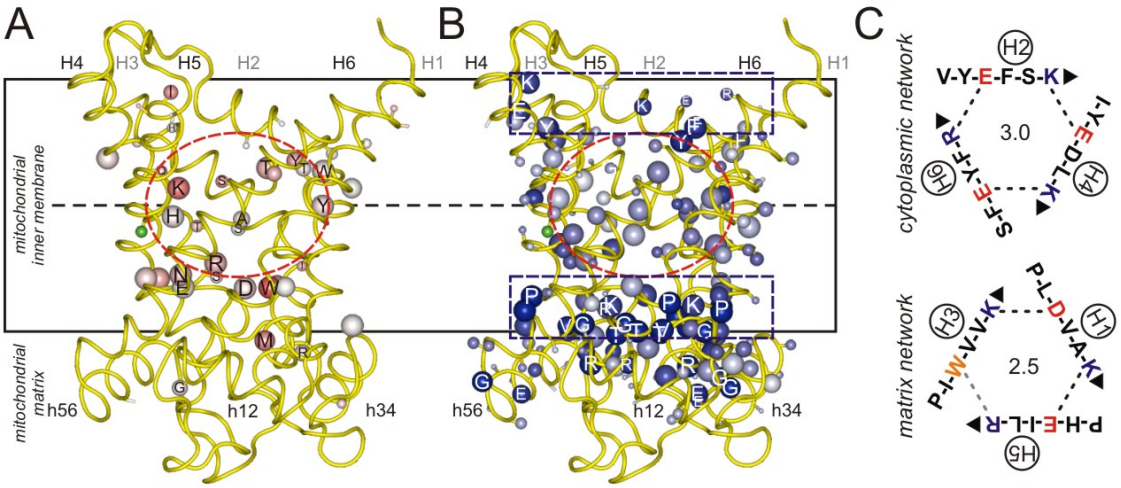

Fig. S25. Asymmetry and symmetry in the fungal NAD⁺ transporters (ScNdt1p and ScNdt2p). For details, see the legend to Fig. 2.

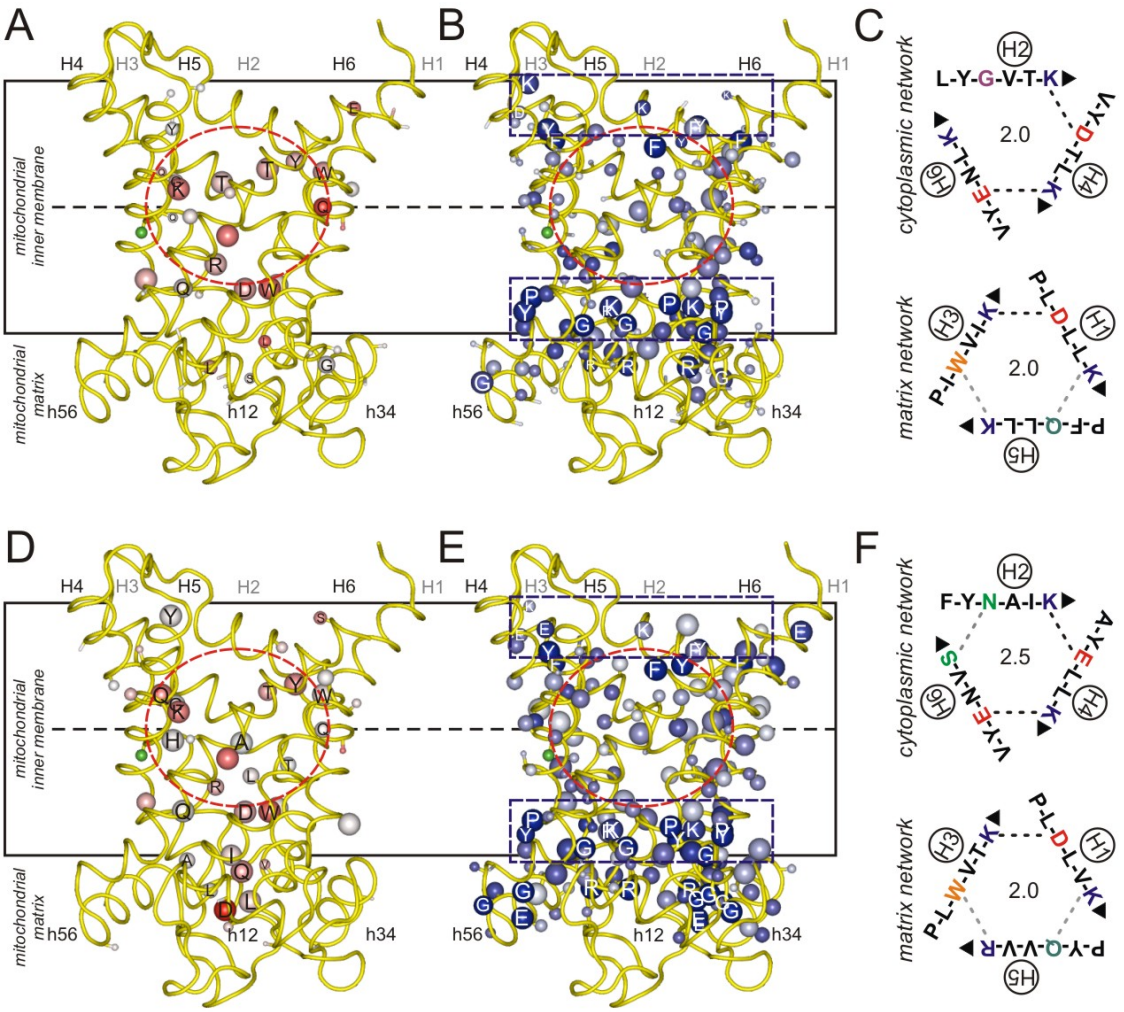

Fig. S26. Asymmetry and symmetry in the fungal and metazoan FAD transporters. For details, see the legend to Fig. 2, except for (*A-C*) fungal (ScFlx1p) and (*D-F*) metazoan (HsMFTC) flavin nucleotide transporters.

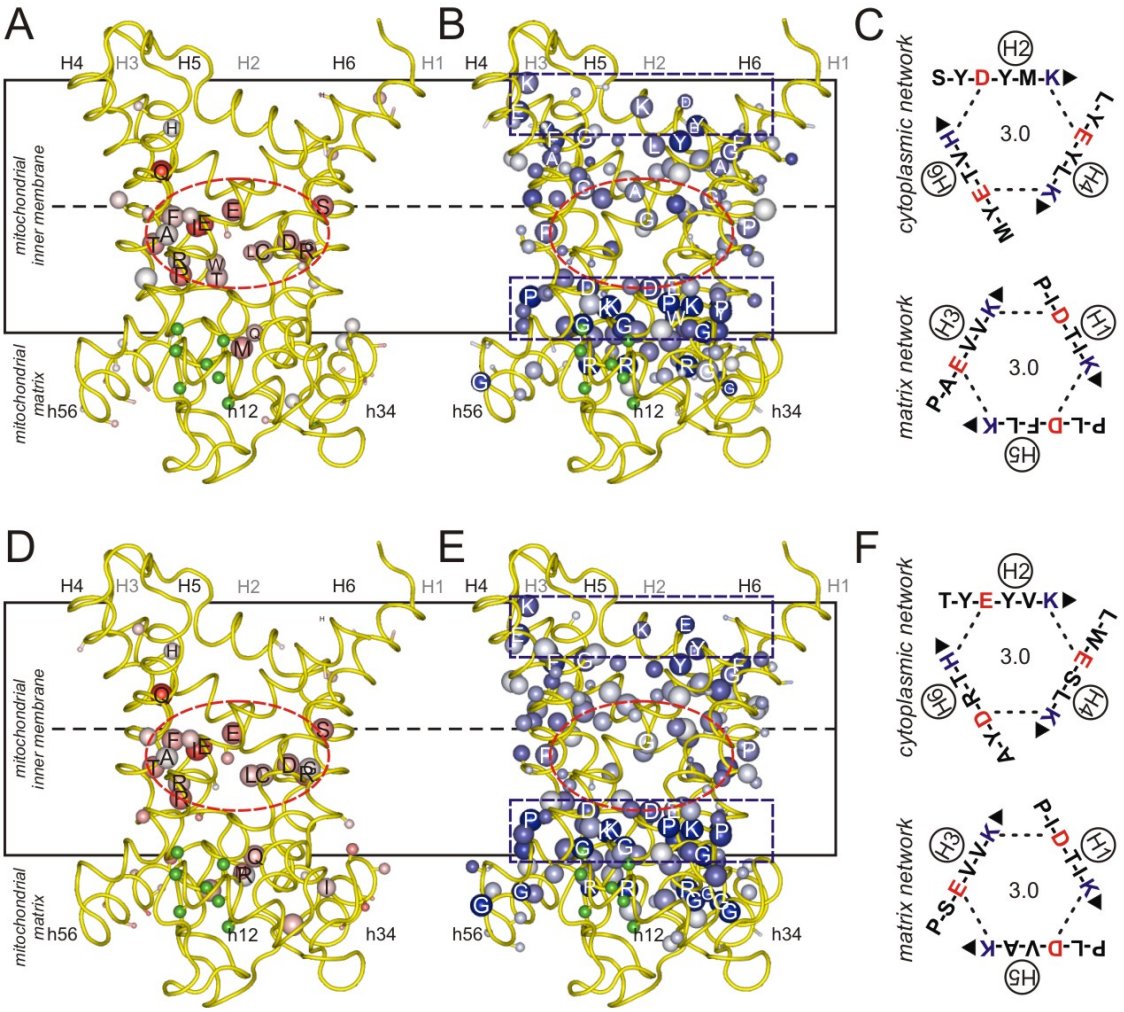

Fig. S27. Asymmetry and symmetry in the fungal and metazoan *S*-adenosyl-methionine transporters. For details, see the legend to Fig. 2, except for (*A-C*) fungal (ScSam5p) and (*D-F*) metazoan (HsSAMT) *S*-adenosyl-methionine transporters.

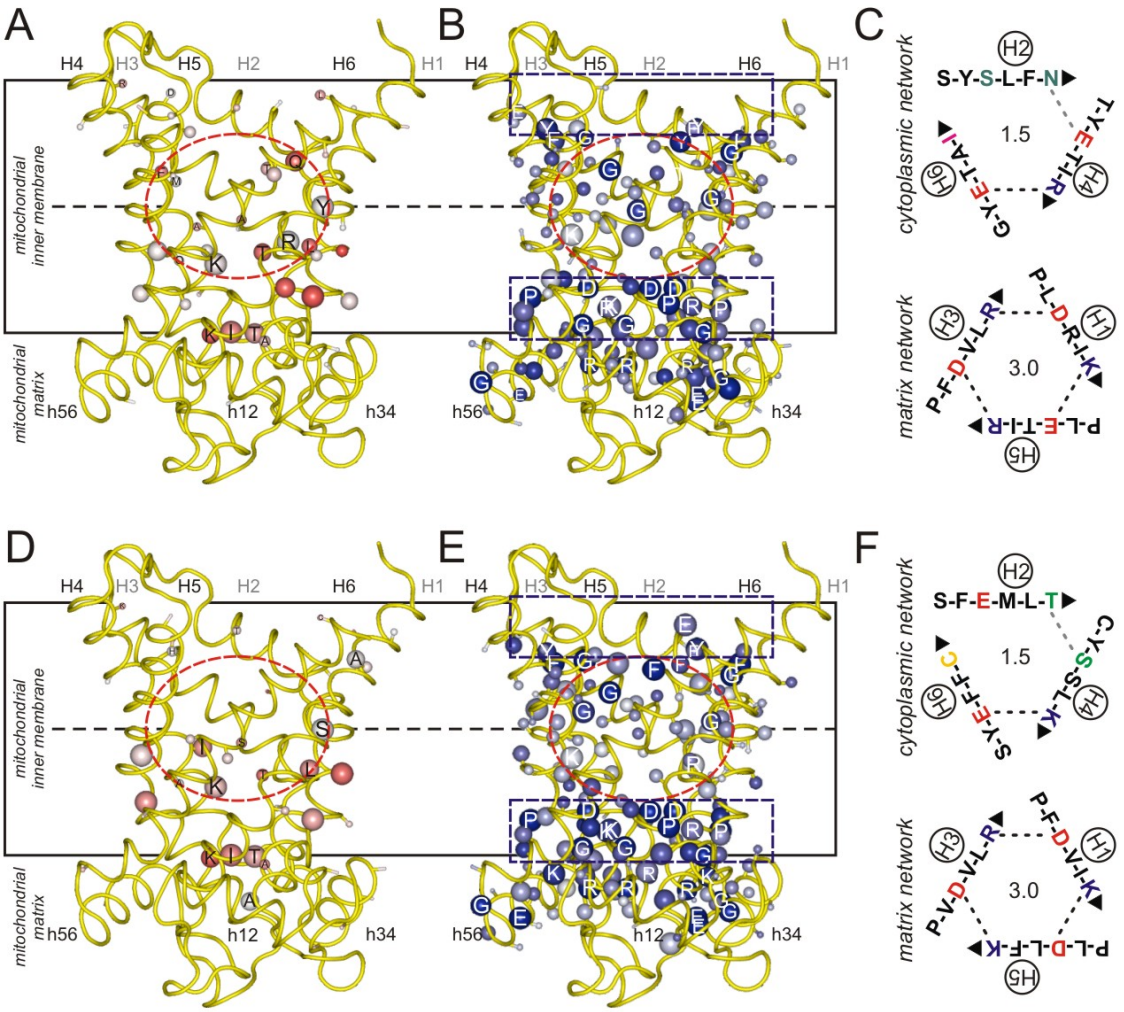

Fig. S28. Asymmetry and symmetry in the fungal and metazoan thiamine pyrophosphate transporters. For details, see the legend to Fig. 2, except for (*A-C*) fungal (ScTpc1p) and (*D-F*) metazoan (HsDNC) *S*-adenosyl-methionine transporters.

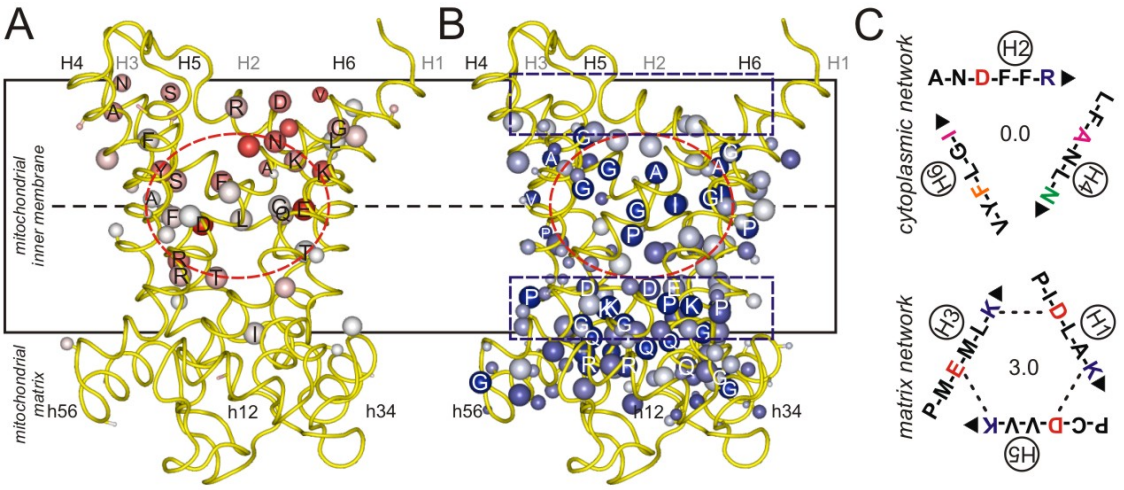

Fig. S29. Asymmetry and symmetry in the metazoan glutamate transporters GC1 and GC2. For details, see the legend to Fig. 2.

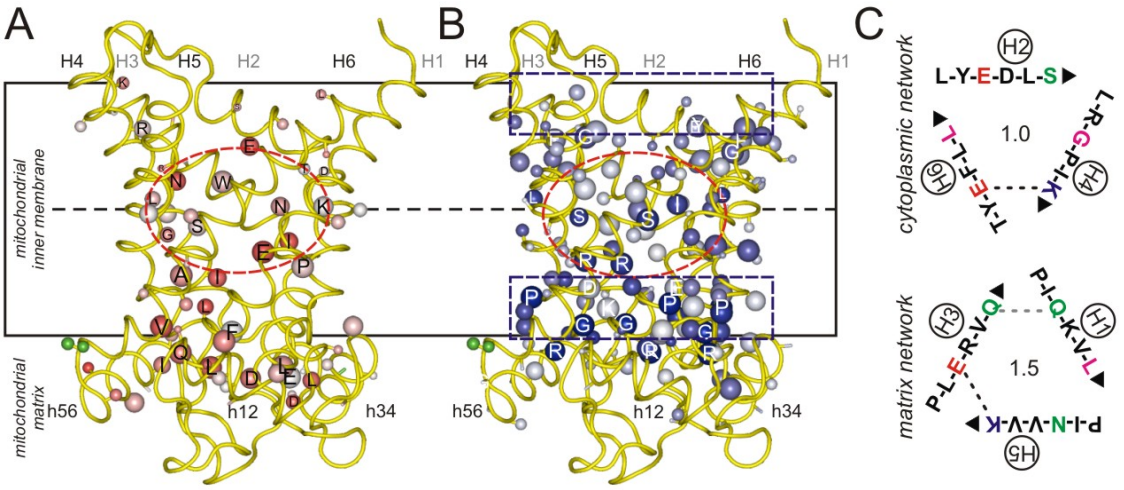

Fig. S30. Asymmetry and symmetry in the metazoan MCART1 and MCART2. For details, see the legend to Fig. 2.

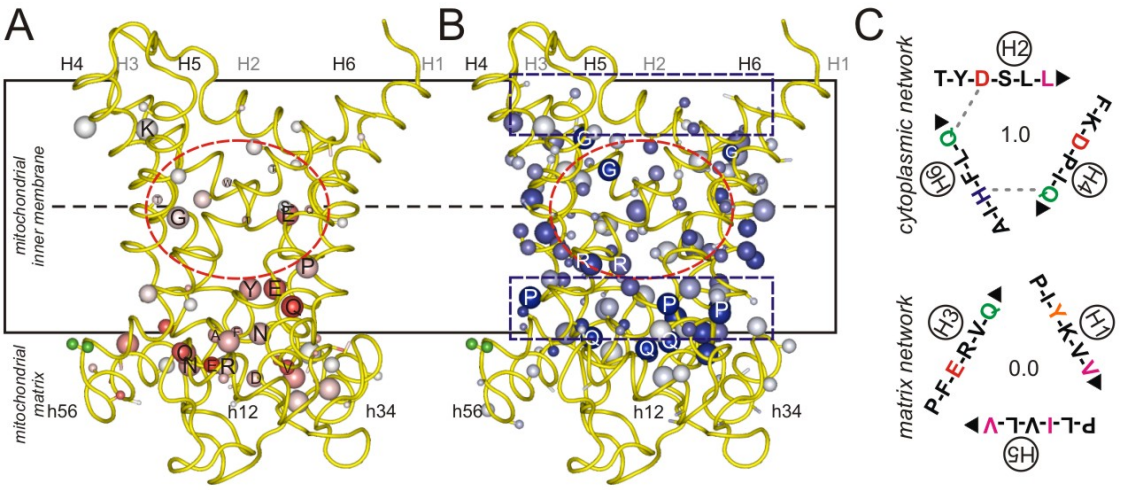

Fig. S31. Asymmetry and symmetry in the metazoan MCART6. For details, see the legend to Fig. 2.

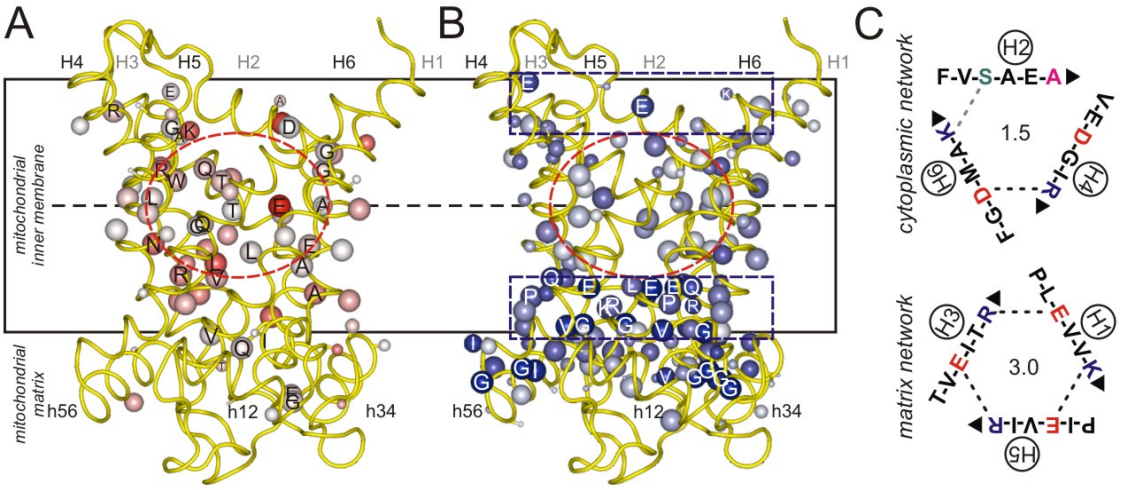

Fig. S32. Asymmetry and symmetry in the fungal ScYhm2p transporters. For details, see the legend to Fig. 2.

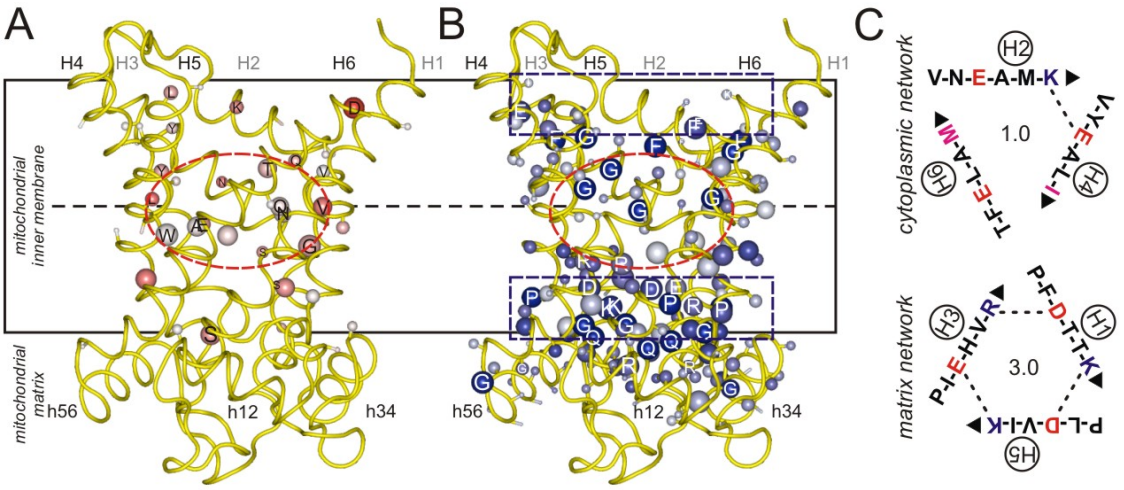

Fig. S33. **Asymmetry and asymmetry in the fungal ScYmc1p and ScYmc2p transporters.** For details, see the legend to Fig. 2.

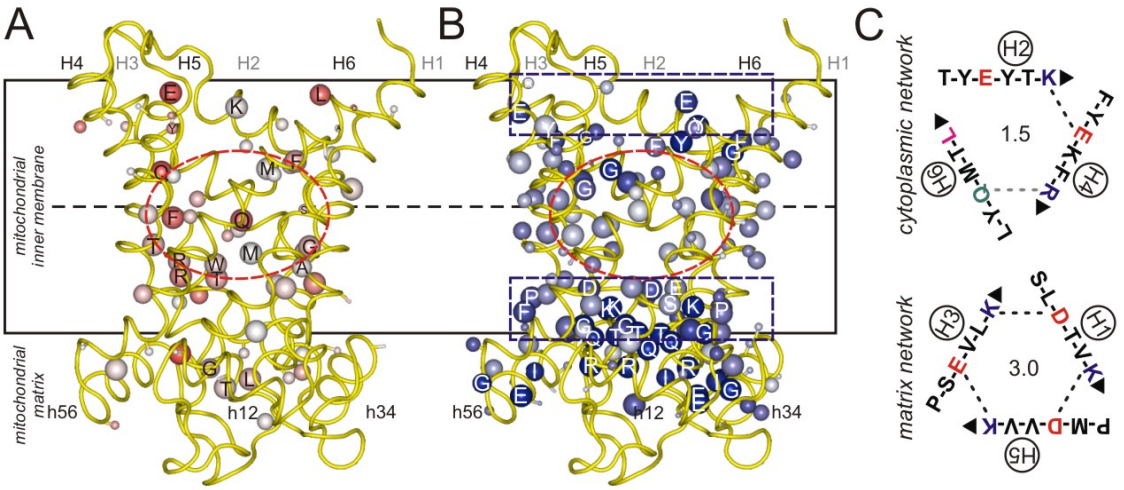

Fig. S34. **Asymmetry and symmetry in the unidentified fungal ScYmr166c transporter.** For details, see the legend to Fig. 2.

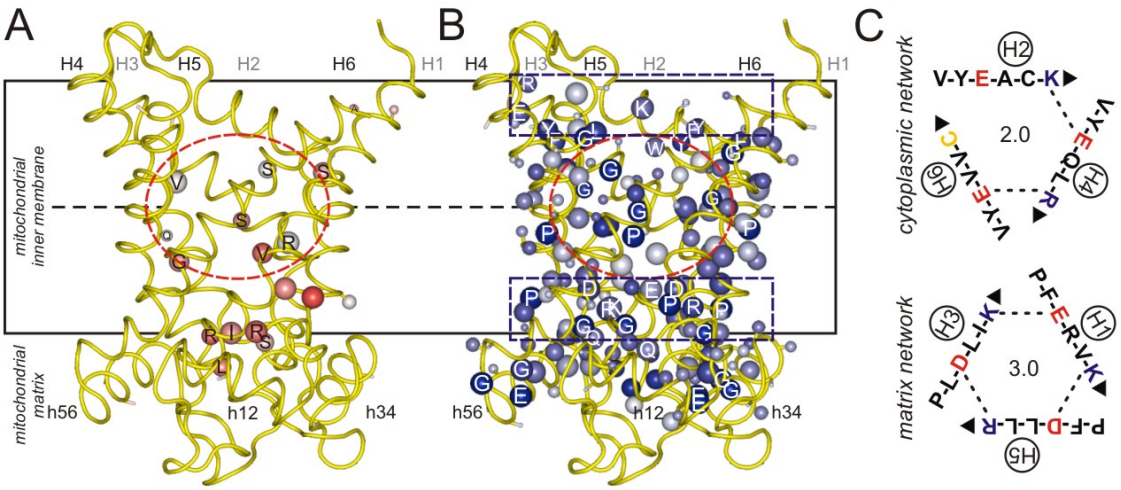

Fig. S35. **Asymmetry and symmetry in the unidentified fungal ScYPR011c transporter.** For details, see the legend to Fig. 2.

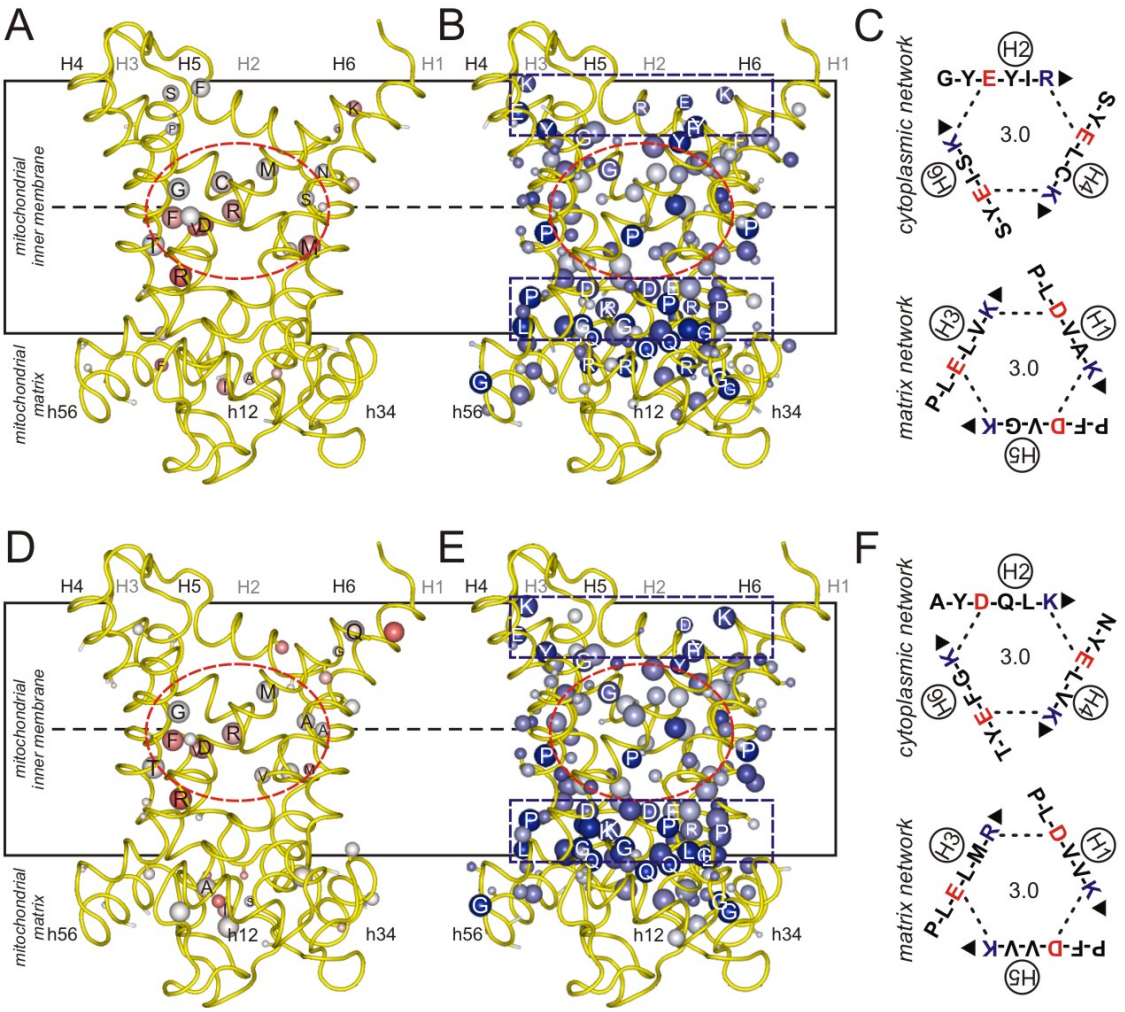

Fig. S36. Asymmetry and symmetry in the fungal ScMtm1p and metazoan HsSLC25A39/HsSLC25A40 transporters. For details, see the legend to Fig. 2, except for (*A-C*) fungal (ScMtm1p) and (*D-F*) metazoan HsSLC25A39 (HsCGI-69) and HsSLC25A40 (HsMCFP) transporters.

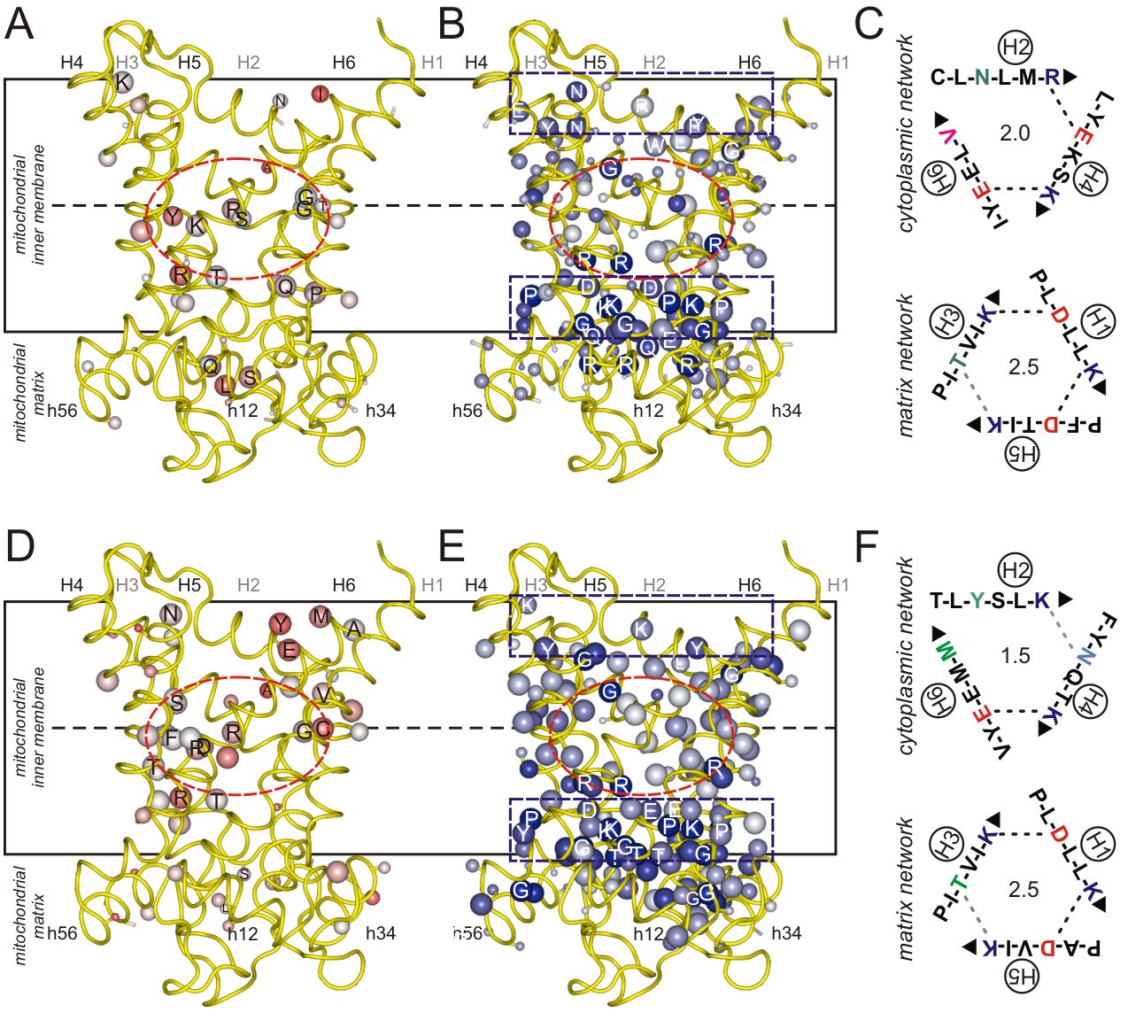

Fig. S37. Asymmetry and symmetry in the fungal ScYDL119c and metazoan HsSLC25A38 transporters. For details, see the legend to Fig. 2, except for (*A-C*) fungal (ScYDL119c) and (*D-F*) metazoan HsSLC25A38 transporters.

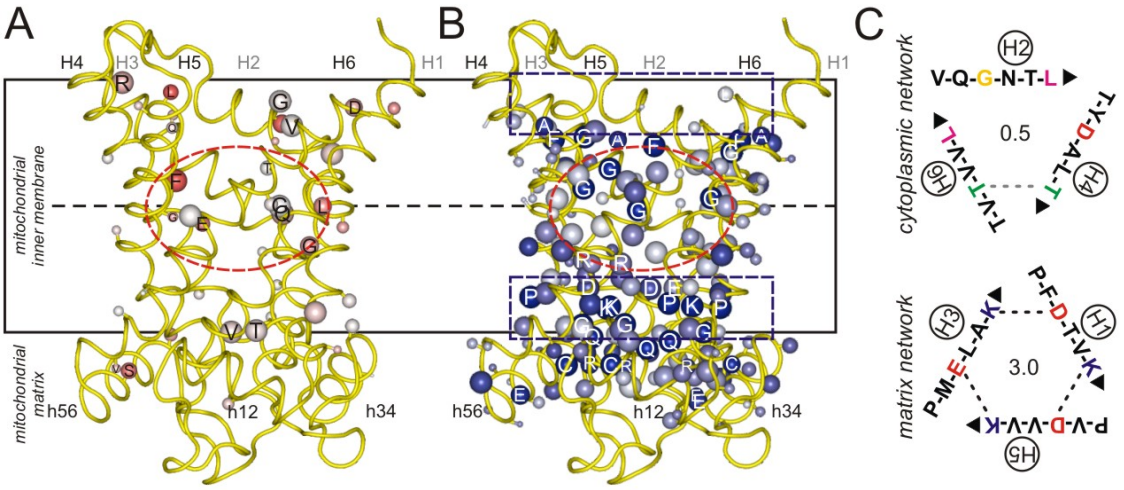

Fig. S38. Asymmetry and symmetry in the metazoan HsSLC25A29 (CACTL). For details, see the legend to Fig. 2.

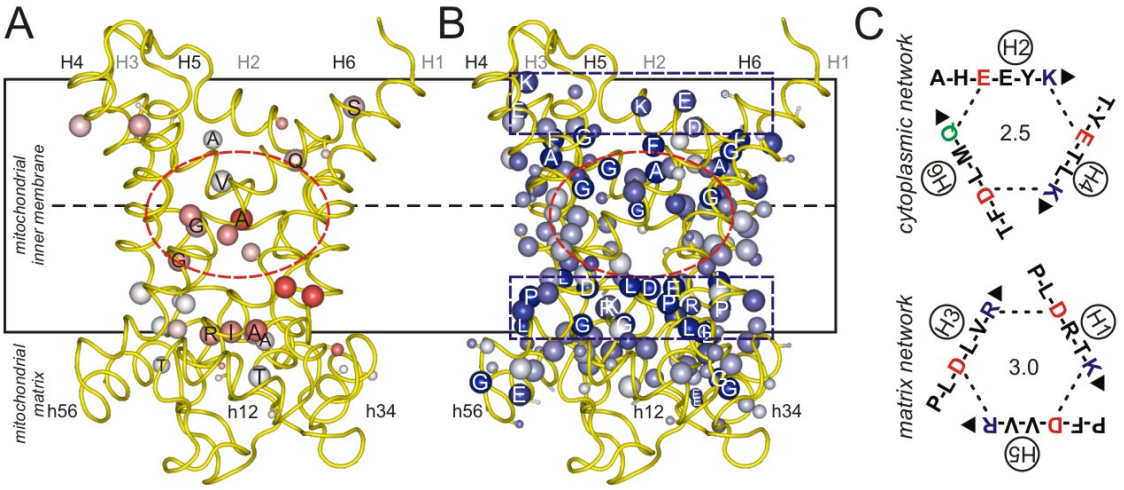

Fig. S39. Asymmetry and symmetry in the metazoan HsSLC25A42. For details, see the legend to Fig. 2.

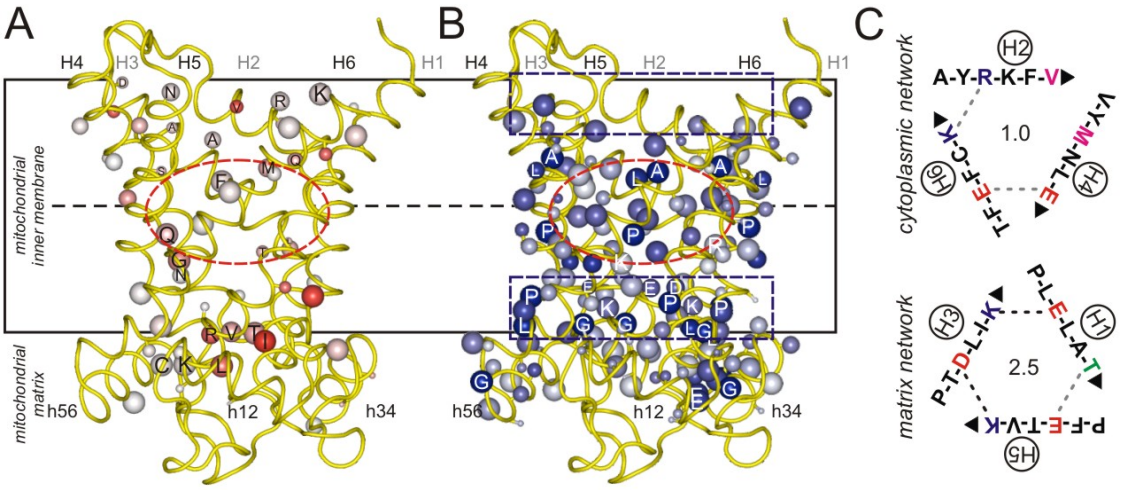

Fig. S40. Asymmetry and symmetry in the metazoan HsSLC25A43. For details, see the legend to Fig. 2.

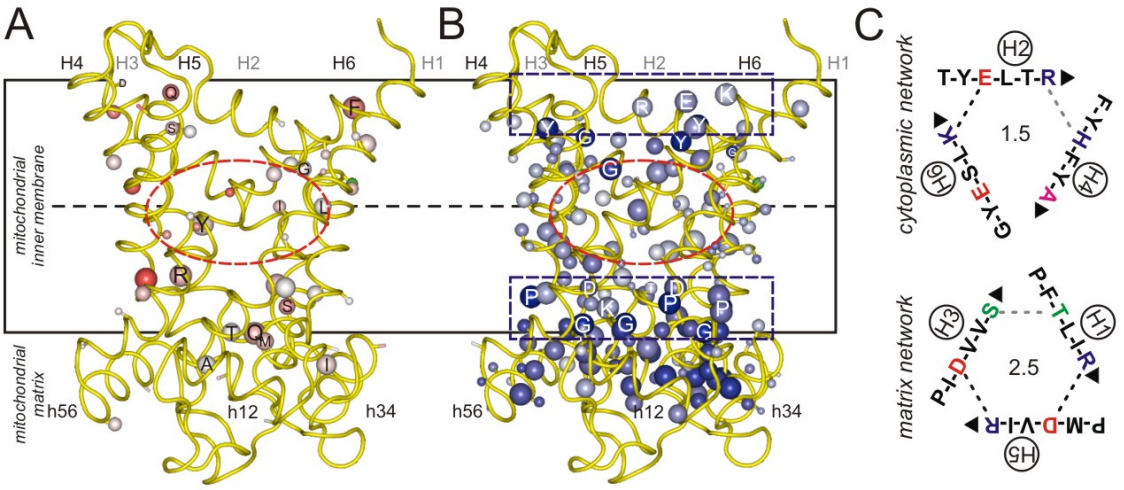

Fig. S41. Asymmetry and symmetry in the metazoan HsSLC25A44. For details, see the legend to Fig. 2.