rijksuniversiteit groningen

Bachelor Thesis Molecular Life Sciences

S-component interactions with group I and II ECF transport mechanisms and how they are mediated

Author: Moudon Thai (S3480844)

Supervisor: Professor Dirk-Jan Scheffers

Date: 10 July 2022

1. abstract

The ECF (energy-coupling factor) transporter family could be a new target for antibiotic targeting as antibiotic resistance becomes more and more prevalent. The ECF transporter consists of four subunits. The S-component is the scope of this thesis. The S-component is important as this subunit helps with the uptake of essential nutrients that cells cannot synthesize themselves. The mechanism of the S-component as part of the ECF transporter and how this mechanism is mediated is a question left unanswered. Findings show that the S-component in a group I ECF transporter, upon ATP binding, binds with its specific substrate. Upon ATP hydrolysis, the S-component will topple in the ECF complex due to conformational changes of the coupling helices of the T-component, consequently releasing the substrate into the cell, and then the process will be repeated. For group II ECF transporters the S-component will release from the ECF complex upon ATP binding and bind to the substrate in a free environment. Subsequently, the S-component will spontaneously topple over which is mediated by the membrane and then binds to the ECF complex due to complementary hydrophobic surfaces of the coupling helices of the T-component and the S-component. The S-component releases the substrate in the cell and the process will be repeated. Another mechanism is that due to lipid curvatures the ECF complex makes itself available for the S-component to bind to the complex and release its substrate. Questions remain about whether ATP hydrolysis can stabilize the toppled state of the S-component. Furthermore, techniques and new methods should be developed to create more understanding of the toppling mechanism. All of the previously mentioned information could provide more insight into the ECF complex and the S-component for antibiotic targeting.

Table of contents

1. abstract 2

2. introduction 4-7

3. Role of ATP on toppling mechanism group I ECF transporters 7-8

4. S-component mechanism in group II ECF transporters 8-13

4.1 toppling mechanism in group II ECF transporters 8-9

4.2 membrane-mediated toppling in group II ECF transporters 9-11

4.3 ATP mediated S-component mechanism for group II ECF transporters 11-13

5. discussion and conclusion 13-18

5.1 Testing toppling mechanism hypothesis 13-15

5.2 testing membrane-mediated toppling hypothesis 15-16

5.3 testing ATP-mediated hypothesis of S-component in group I ECF transporter mechanism 16-17

5.4 testing ATP-mediated hypothesis of S-component in group II ECF transporter mechanism 17-18

5.5 concluding remarks 18-19

6. references 19-21

2. Introduction:

At present-day, there is a rise of antibiotic resistance within bacterial pathogens. Antibiotic resistance causes current antibiotics to lose their effectiveness[1]. To solve this problem new antibiotics need to be found against the resistant pathogens. One way to deal with antibiotic resistance is to find new targets for antibiotics, to combat those pathogens. Possible targets which have been found recently are transporters. Transporters have the function of transporting essential nutrients inside the cell of the bacteria, which bacteria cannot synthesize on their own. ABC transporters (ATP binding cassette transporters) are a common example that bacteria uses to transport nutrients into their cell. The ABC transporter structure consists of two transmembrane proteins and two ATPases. The ATPases will hydrolyze ATP or use ATP to cause conformational changes in the transmembrane domain of an ABC transporter[2]. This conformational change causes the transmembrane domain to either be in an open state or a closed state. These states allow the ABC transporter to regulate the transport of those substrates specific to the transmembrane domain[2]. ABC transporters have multiple subgroups. Recently, a new ABC transporter subgroup had been discovered. These transporters are called energy-coupling factor transporter which is abbreviated as ECF transporters. The ECF transporters are mostly found in gram-positive bacteria, including many human pathogens[3]. This discovery makes the ECF transporter a great target for new antibiotics.

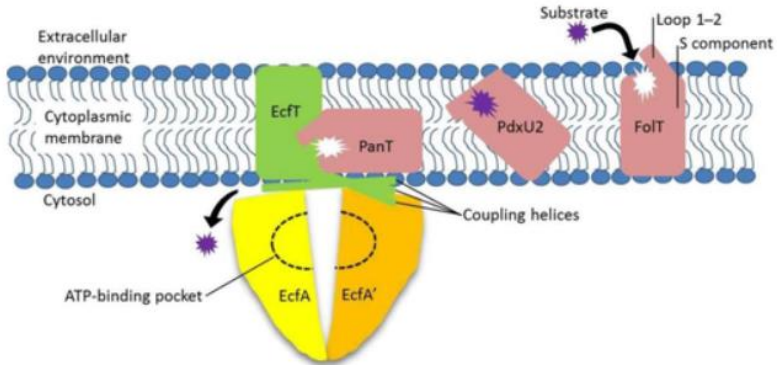

Figure 1. *Figure 1 shows the structure of an ECF transporter[3]. Figure is taken from Bousis et al. (2019)*

Figure 1 shows the structure of an ECF transporter. The ECF transporter has four subunits. It consists of an S component, an EcfT component, and an EcfA and EcfA' component. The EcfT and the S component are the transmembrane domains of the ECF transporter. The EcfA and EcfA' are the ATP-binding proteins of the ECF transporter[3]. The S component is responsible for taking substrates from the cell environment into the cell. The affinity of the S-component is specific for one substrate[3]. Group I ECF transporters each have an affinity for one S-component, whereas, group II ECF transporters individually have an affinity to multiple S-components[3]. The ATP-binding proteins of the ECF transporter help with the transport of substrates into the cell. Recently, many studies have been conducted on how the ECF transporter functions. Research has been done to discover the protein structure of the ECF transporter, however, of the four subunits, the mechanism of the S-component remains relatively unclear[4]. The protein structure of the S-component has been discovered, which is a significant step in researching how the S-component could interact with the ECF transport complex. Zhang et al (2010) have found that the S-component of the ECF transporter contains six transmembrane segments in which there are 5 loops[4].

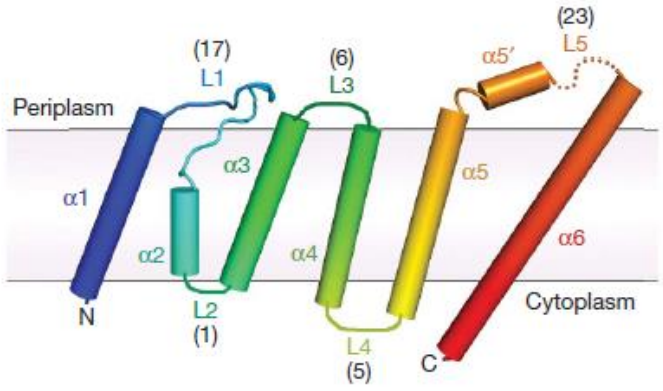

Figure 2. *Figure 2 shows the structure of the S-component specific for riboflavin[4]. Figure is taken from Zhang et al. (2010).*

Figure 2 shows the structure of the S-component. Zhang et al (2010) have found that the first loop of this specific S-component plays a big role in recognizing its substrate. The first loop (L1) is indicated in the transmembrane segments 1-3. The riboflavin is recognized by the L1 loop and transmembrane segments 4-6[4]. This recognition is due to Van der Waals interaction and hydrogen bonds of the riboflavin[4]. The L1 loop and transmembrane segments 4-6 will create a hydrophobic pocket to capture the riboflavin by binding to the non-polar side of the

riboflavin[4]. Zhang et al (2010) suggested a mechanism of the S-component where the L1 loop serves as a gate for the riboflavin[4]. Upon riboflavin binding, the L1 loop closes down[4]. As a result of ATP hydrolysis, transmembrane segments 1-3 move away from the transmembrane segments 4-6[4]. This allows the S-component to adopt an inward-open conformation, causing the riboflavin to be released into the cytoplasm of the cell[4]. The ADP-bound state of the riboflavin transporter may reset the transport system[4]. For the interaction between the S-component and the ECF transporter, it has been suggested that the mechanism of the S-component in groups I and II ECF transporter is done via a toppling mechanism of the S component[3].

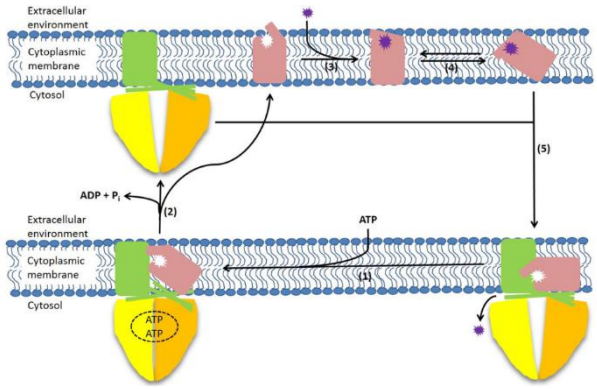

Figure 3. *figure 3 shows the suggested toppling mechanism of the S-component in group II ECF transporters[3]. Figure is taken from Bousis et al (2019)*

The suggested toppling mechanism can be seen in figure 3. Upon ATP binding, the empty S-component is released from the ECF module[3]. Then, the S-component will orientate into an outward-facing state, allowing substrate capture[3]. After substrate capture, it can be seen in figure 3 that the S-component is toppling over in the membrane and subsequently binds to the ECF module. Binding to the ECF module causes destabilization of the substrate-bound S-component leading to substrate release into the cytoplasm[3]

How this mechanism has been mediated is a question which is not entirely answered yet. In this thesis, the toppling mechanism will be explained. Furthermore, how the S-component interaction with the ECF group I and II transporter is mediated will be explained in the thesis. Two theories could explain the S-component interaction. The first theory suggests that the toppling mechanism of the S-component with group II ECF transporters is membrane-mediated and the other theory suggests that ATP binding mediates or stabilizes the S-component interaction with both groups I and II ECF transporters. The theories will be discussed, and a

conclusion is drawn whether these theories are supported or more research has to be conducted to have more clearance or insight into the theories.

3 Role of ATP on toppling mechanism group I ECF transporters

To see if this toppling mechanism applies to group I ECF transporters Bao et al (2017) used a cobalt group I ECF transporter to detect a toppling mechanism of the S-component[5]. Bao et al (2017) used mass spectroscopy and an assay system to analyse the cobalt group I ECF transporter structure and analyse the mechanism of the cobalt ECF transporter[5]. Bao et al (2017) made a working model of the cobalt transporter from the results which can be seen in figure 4.

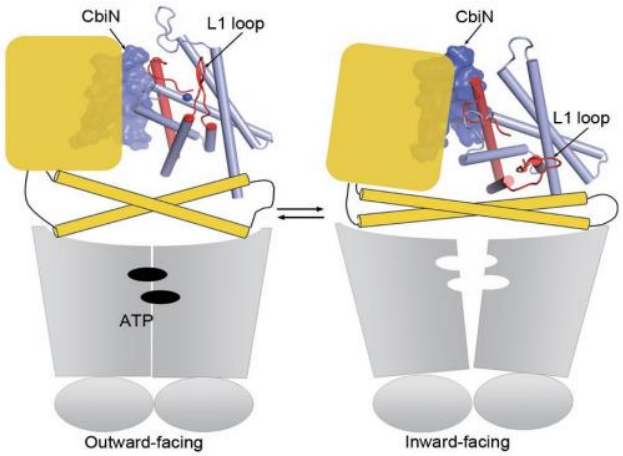

Figure 4. *figure 4 shows the working model of the cobalt group I ECF transporter. Figure is taken from Bao et al (2017).*

Looking at figure 4 Bao et al (2017) made a two-state working model of the cobalt ECF transporter. In the figure, the S-component is shown in blue and red cylinders[5]. The T-component is shown as a rectangle with two crossing cylinders, which depicts the coupling helices of the T-component. Bao et al (2017) suggested that in the outward-facing state ATP binds to the ATP-binding proteins of the ECF transporter, causing a closed conformation of the ATP-binding proteins[5]. While this happens, the T-component and the S-component adopt an upright conformation perpendicular to the membrane[5]. Subsequently, the L1 loop will interact with the substrate which has been described earlier by Zhang et al (2010) by guiding it into the cell[5]. This happens when ATP hydrolysis causes the ECF complex to go in an inward-facing conformation leading to an open conformation of the ATP-binding proteins[5]. The coupling

helices of the T-component move due to the change in the conformation of the ATP-binding proteins causing the S-component to topple over in the membrane, releasing the substrate into the cytoplasm[5].

4. S-component mechanism in group II ECF transporters

4.1 toppling mechanism in group II ECF transporters

This theory revolves around the group II ECF transporter. First, the theory suggested that the S-component topples over in the membrane to bind to the ECF transporter with their specific substrate[3]. The toppling mechanism of this S-component has been discovered by Xu et al (2013), when they made crystal structures of the group II ECF transporter specific for folate[6]. With the structures, they wanted to determine the crystal structures of the ECF transporter when no substrate is bound to the S-component of this ECF transporter[6.]

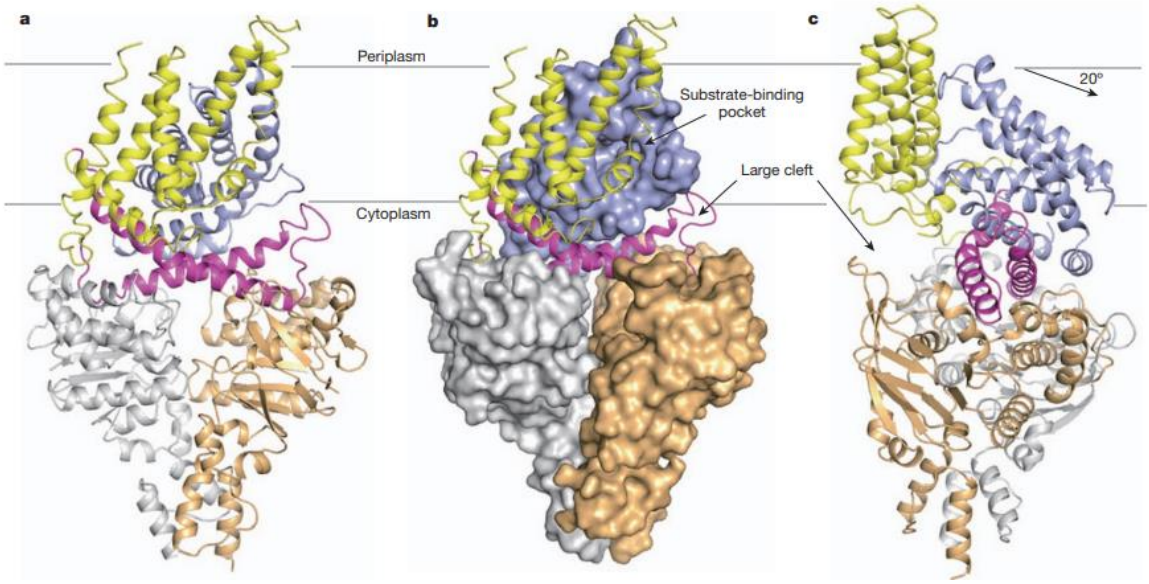

Figure 5. *figure 5 shows the crystal structure of the folate ECF transporter made by Xu et al (2013). The gold and grey structure shows the ATPases of the ECF transporter structure. The yellow structure shows the T-component of the ECF transporter and the light blue structure shows the S-component of the ECF transporter[6]. In c the structure has been rotated 90˚ clockwise from the structure of a[6]*

From the results, it could be seen that the S-component of the ECF transporter is parallel to the membrane in the crystal structure as seen in figure 5. This could indicate toppling of the S-

component however the crystal structures do not show if the toppling mechanism of the S-component occurs before or while the S-component is interacting with the ECF complex.

To answer this question, Xu et al (2013) tried to make crystal structures of the ECF transporter for folate by adding the folate to the non-substrate-bound ECF transporter. The results for that could not be obtained as no crystal structure of the ECF transporter in that state has been obtained[6]. Xu et al (2013) thus suggested that adding folate to the non-substrate-bound ECF transporter with the S-component causes the structure of the non-substrate-bound ECF transporter to be unstable[6]. This could indicate that substrate binding and toppling of the S-component occurs before the interaction of the S-component with the ECF complex[6]. The suggested mechanism of Xu et al (2013) is that in group II transporters the S-component releases from the ECF complex to bind to the substrate[6]. Then the substrate-bound S-component consequently topples over in the membrane and then binds to the ECF complex to release its substrate into the cytoplasm[6].

4.2 membrane-mediated toppling in group II ECF transporters

The earlier mentioned information said that the S-component is toppling over in the membrane. How this mechanism is mediated is still a question left to be answered. Faustino et al (2020) have researched this by creating this toppling mechanism and with the help of multi-scale molecular dynamics simulated how different lipids compositions could affect the toppling mechanism of the S-component in the membrane[7]. Faustino et al used an ECF transporter, which is categorized in ECF group II transporters, specific for folate, and created a simulated environment in which the folate ECF transporter is put into a mix of lipids consisting of POPE (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine), CL (cardiolipin) and POPG (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol) lipids[7]. The reasoning behind this is to replicate the conditions of the ECF transporter in the *Escherichia coli* membrane. Also, different forms of the S-component have been used for this experiment to see which form of the S-component is most suitable for this experiment[7]. In the *apo* form (when the S-component is not bound to its substrate folate), the S-component immediately reverts from the toppled state to the canonical state after toppling[7]. The canonical state is where the S-component is not parallel to the membrane, but rather perpendicular to the membrane[7]. In the *holo* form (when the S-component is bound to its substrate folate) the S-component stays toppled after being put into the membrane[7]. This is relevant because the structure of the *holo* S-component when toppled is stable enough to be put in the membrane so that experiments can be conducted if the

membrane can stabilize such state of the S-component[7]. Different lipid compositions have been used to see how stable the S-component in the toppled state can be under those conditions[7].

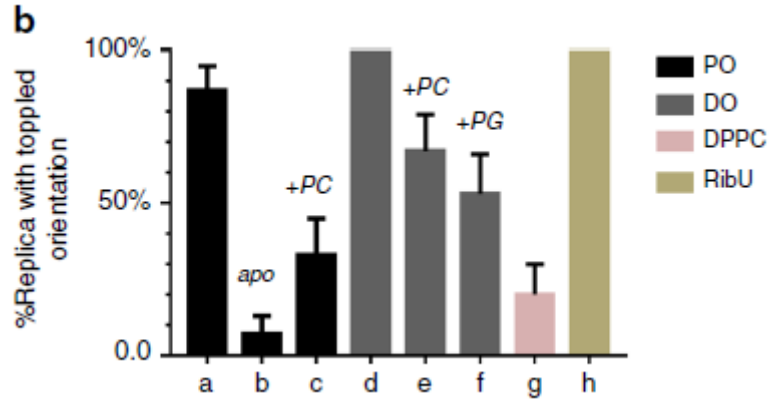

Figure 6. *Figure 6 shows how much of the S-component stayed toppled after changing the lipid composition of which the results can be seen in the table[7]. Figure taken from Faustino et al (2020)*

Figure 6 shows the result of how much of the S-components stay toppled when simulated in different lipid compositions. It can be seen that by replacing PO (palmitoyl oleoyl) lipids with DO (dioleoyl) lipids the S-component stays toppled. The difference between the two lipids is that DO lipids are more unsaturated and longer than PO lipids[7]. In figure 6 the bar above A and D represent how much the S-components stay toppled in lipid compositions with a PE headgroup. The bar above C and E represent how much the S-components stay toppled in lipid compositions with a PC headgroup[7]. From figure 6 it can be concluded that replacing the PC (phosphatidylcholine) headgroup with a smaller headgroup PE (Phosphatidylethanolamine), results in the S-component staying more in a toppled state[7]. This means that non-bilayer forming lipids with a small headgroup and longer tail help to stabilize the S-component in the toppled state[7]. Faustino et al (2020) suggested that binding and hydrolysis of ATP would cause the ECF complex to change its confirmation and help with flipping back the toppled S-component to the canonical state when the S-component is bound to the ECF complex[7].

Furthermore, Faustino et al (2020) have concluded another pathway in which the membrane can mediate S-component interaction with the ECF transporter. For this, the ECF transporter has been simulated into a non-bilayer membrane without an S-component[7]. With molecular dynamics, it can be seen how the membrane can interact with the ECF transporter[7]. Results show that the ECF transporter is curving the membrane in such a way that the ECF transporter

changes its configuration[7]. The configuration change of the membrane causes the ECF transporter to move down the membrane in which the ECF complex becomes available for the S-component to bind to it seen in figure 7 made by Faustino et al (2020).

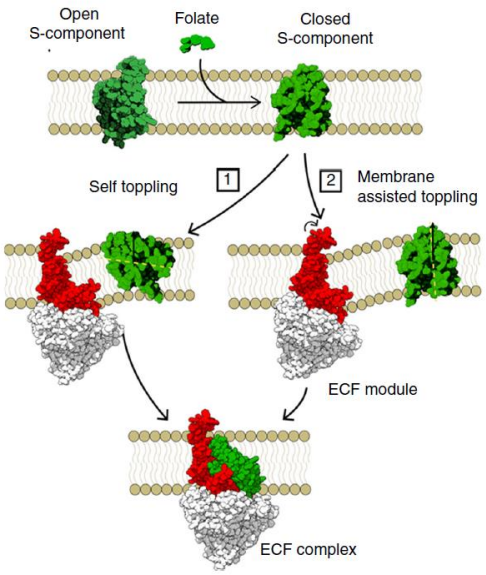

Figure 7. *Figure 7 shows the two possible pathways discussed in the research of Faustino et al (2020). The membrane stabilizes the toppled state of the S-component to enforce the binding of the S-component to the ECF complex. Another way is that the membrane enforces the ECF complex to move and change the configuration in such a way that it allows the S-component to bind to it*[7]*. Figure taken from Faustino et al (2020)*

4.3 ATP-mediated S-component mechanism for group II ECF transporters

Research on what effect ATP can have on the group II ECF transporters has also been conducted. Karpowich et al (2016) have researched how ATP affects the S-component and its influence on the ECF transport mechanism. As stated earlier by Bao et al (2017) et al, when ATP binds to the ECF complex the S-component will change its conformation so that the substrate binds to the S-component[5]. ATP hydrolysis will then follow and the substrate will then be released into the cell[8]. However, in group II ECF transporters, multiple S-components have an affinity for the same ECF complex[8]. Karpowich et al (2016) thus researched to see what effect ATP has on this group of transporters by using a group II ECF transporter specific for riboflavin for this research[8]. Karpowich et al. (2016) then trapped the riboflavin ECF transporter in an ATP-bound state[8]. This has been achieved by using a mutational strategy[8]. Previous research said that histidine in the H loop of ABC-type ATPases is essential for ATP hydrolysis[8]. Thus, mutations are introduced to the histidine in the H loop of the ATPases of the riboflavin ECF transporter

to prevent ATP hydrolysis and allow the transporter to be trapped in an ATP-bound state[8]. The reason for that is to see what effect the ATP-bound state has on the S-component of the group II ECF transporter. First wildtype riboflavin ECF transporter is used to test how ATP affects the wildtype riboflavin transporter.

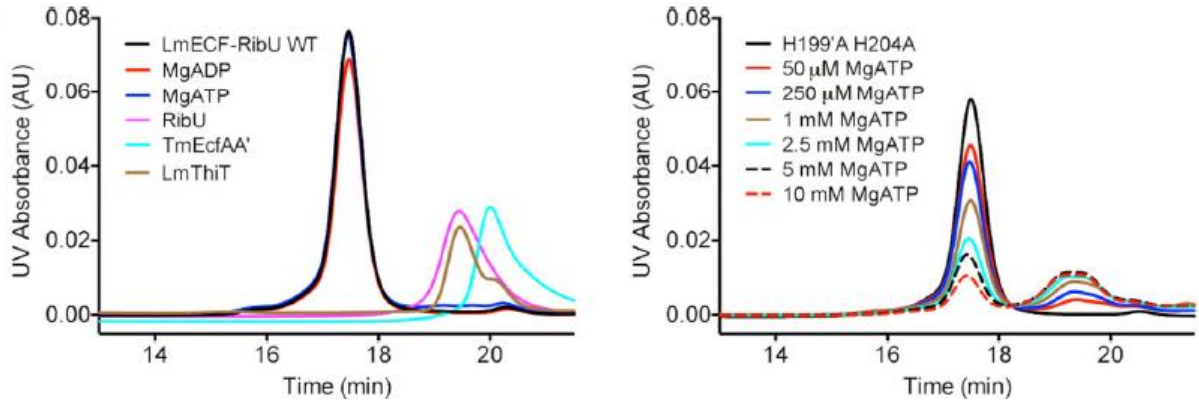

Figure 8. *Figure 8 shows two graphs. In the first graph absorbance of the wildtype ECF transporter for riboflavin and the subunits of it have been measured when ATP is added. In the second graph, absorbance is measured of the ECF transporter for riboflavin in ATP bound state when ATP is added[8]. Figure is taken from Karpowich et al (2016) and edited in Microsoft paint.*

Looking at figure 8 it can be seen in the left graph that when ATP is added to the wildtype ECF transporter specific for riboflavin, the peak of absorbance of the riboflavin ECF transporter (which is the black line) is declining and the peak of the ribU, which is the S-component of the riboflavin ECF transporter, is increasing[8]. This could mean that the addition and binding of ATP to the complex causes the S-component to disassociate from the ECF complex[8]. To further cement this theory ATP has also been added to the riboflavin ECF transporter mutant described earlier. The results show that the peak of the ECF transporter complex, which is labelled as H199'A H204A, is decreasing[8]. This could indicate that the S-component of the mutant ECF transporter is disassociated from the ECF transporter[8].

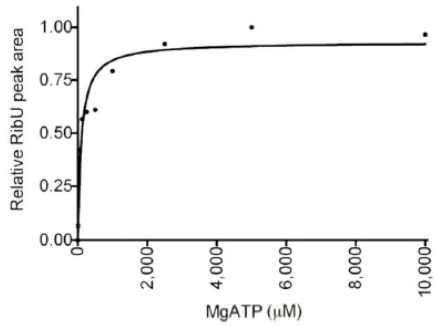

Figure 9. *Figure 9 shows the peak area of the ribU, which is the S-component of the riboflavin transporter[8]. By increasing the concentration of ATP it can be seen how ATP affects the height of the peak of the S-component. Figure is taken from Karpowich et al (2016) and edited in Microsoft paint.*

As seen in figure 9 increasing the concentration of the ATP also increases the peak area of the S-component of riboflavin. This also supports the theory of Karpowich et al (2016) that ATP binding causes disassociation of the S-component from a group II ECF transporter[8].

Showing this Karpowich et al (2016) have then suggested a release and catch model that ATP binding to the ECF complex leads to disassociation of the S-component in a group II ECF transporter[8]. This leads to the S-component binding to the substrate and then being captured by the ECF complex[8]. Then ATP hydrolysis will cause the substrate to be released into the cell in which the S-component topples over in the ECF complex to release the substrate. Then ATP will bind to the ECF complex and the process is repeated[8].

5. Discussion and conclusion

5.1 Testing toppling mechanism hypothesis

It has been earlier mentioned that the S-component interacts with the ECF complex via a toppling mechanism. Toppling of the S-component happens in the membrane in group II ECF transporters before the S-component interacts with the ECF complex, and toppling happens in group I transporters due to the change of the structure of the coupling helices of the T-component upon ATP hydrolysis. However, research has been done to test the hypothesis of the toppling mechanism[9]. The reasoning for that is that the toppling mechanism is still a very suggestive mechanism of the S-component in the ECF transporter[10]. Previous research has shown that the S-component is parallel with the membrane indicating a toppling mechanism[10].

Swier et al (2016) also researched this by making crystal structures of an ECF group II transporter specific for folate[10]. The crystal structures show that the S-component is parallel to the membrane in which the ECF complex is and that can indicate a toppling mechanism of the S-component. The crystal structures of the ECF transporters of Swier et al (2016) agree with the hypothesis of the mechanism of the S-component in group II ECF transporters studied by Xu et al (2013). However, the mechanism has not been studied in detail, meaning that the mechanism has not been captured yet. Stanek et al (2018) tested the hypothesis of the toppling mechanism to try and capture the mechanism[9]. To test the hypothesis, three methods have been used to try and capture the toppling mechanism of the S-component.

Crosslinking the proteins was the first method used to capture the toppling mechanism[9]. Crosslinking is when two proteins are bonded and then probed to research the interaction between the proteins[11]. In this case, cysteine mutants are introduced into the ECF complex which is specific for folate. The cysteine mutants are introduced by moving the cysteine from the interface between the S-component and T-component to the coupling helices of the T-component and to the part of the S-component which interacts with the coupling helices[9]. The crosslinking between these cysteine residues allows analysation of the S-component interaction with the coupling helices and the toppling mechanism resulting from it. First, protein activity of the ECF complex has been measured with the cysteine mutants[11]. The results showed that the activity of the ECF transporter declined due to the introduction of cysteine mutations into the ECF complex[9]. The crosslinking method has then been put to a halt before the probing step as no toppling mechanism can be studied on an impaired ECF transporter[9].

The second method was using a fluorescence-based study to detect and analyse the toppling mechanism of the folate ECF transporter[9]. Dyes have been used on the purified folate ECF transporter with the S-component and on the solitary S-component specific for folate, for emission fluorescence and results were seen on the spectrophotometer[9]. The goal was if emission is achieved by the solitary S-component and the ECF complex, a fluorescence assay can be made where movement and conformational changes of the S-component can be seen along with the ECF complex to test the toppling mechanism[9]. However, results did not show any stable signal when the fluorescence intensity has been measured by Stanek et al (2018) of the solitary S-component and the full ECF complex, meaning that fluorescence could not be used to test the toppling mechanism[9].

The last method is using FTIR (Fourier transform infrared spectroscopy) is used to test and analyse the toppling mechanism of an ECF transporter[9]. With this method, the chemical bonds between proteins can be identified and structural changes in the protein structure can be analysed[12]. Thus, this method could measure the structural changes of the S-component during the toppling mechanism. For this method, Stanek et al (2018) used a different S-component than used in the previous methods. The S-component used for this method is specific for thiamine[9]. The reasoning for the use of this specific S-component is that in comparison with other S-components thiamine does not contain carbonyl groups[9]. Carbonyl groups can influence infrared spectroscopy in a negative way[9]. Results of absorbance were used by Stanek et al (2018) to calculate the rotation of the S-component in the membrane to see if the S-component is indeed parallel to the membrane[9]. However, these results did not give more insight into the toppling mechanism of the S-component.

Hereby Stanek et al (2018) concluded that with the use of earlier mentioned methods the hypothesis of the S-component toppling mechanism could not be analysed[9]. To have more insight into the toppling mechanism, new techniques have to be developed to create the possibility to capture the toppling mechanism in detail[9].

5.2 testing membrane-mediated toppling hypothesis

Faustino et al (2020) have concluded that in membranes containing a small headgroup and increasing the length of the fatty acid chains the toppling mechanism is stabilised which could prove the hypothesis that the toppling mechanism is membrane mediated[7]. However, the results of Faustino et al (2020) are contradictory to the results shown in previous research done by Josts et al (2016)[13]. The research of Josts et al (2016) used a group I ECF transporter called YkoEDC[13]. The structure of the ECF transporter was then placed in a lipid bilayer to test the toppling mechanism using molecular dynamics. The simulated data showed when the S-component of the ECF transporter is placed horizontally in the membrane the lipid bilayer causes the S-component to move back vertically[13]. Josts et al (2016) used S-components from group II ECF transporters to test the toppling mechanism. The simulations with the S-components show that the S-components are unlikely to topple over by themselves[13]. This result contradicts Faustino et al (2020) in which the S-component topples over in the membrane by themselves with the help of the membrane[13]. The lipids used in Josts et al (2016) however were different from the lipids used in Faustino et al (2020)[7]. The lipids used in Josts et al (2016) were DDPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine)[7]. This could indicate that the DDPC

lipids are not favourable in membrane-mediated toppling instead of rejecting the hypothesis that toppling is membrane-mediated. To test this Faustino et al (2020) have used DDPC lipids to put in the holo form S-component for riboflavin[7]. The simulated data showed that the toppled orientation is unfavourable for the S-component, however when the S-component is put in the lipid composition used in Faustino et al (2020), the toppled orientation is favoured over the canonical, or vertical orientation called in Josts et al (2016)[7]. This concludes that the lipid composition is the deciding factor in stabilizing the toppled orientation of the S-component when the S-component is isolated in the membrane from the ECF complex of group II ECF transporters.

5.3 testing ATP-mediated hypothesis of S-component in group I ECF transporter mechanism

For group I ECF transporters it has been researched how ATP binding and hydrolysis affect the S-component and its interaction with the ECF complex during the transport cycle. In the thesis, the working model of the group I ECF transporter specific for cobalt has been described by Bao et al (2017). Previous research has been conducted on the group I ECF transporter by Finkenwirth et al (2015). A biotin transporter is purified and reconstituted in phospholipid nanodiscs to study the effect of ATP on the group I ECF transporter.

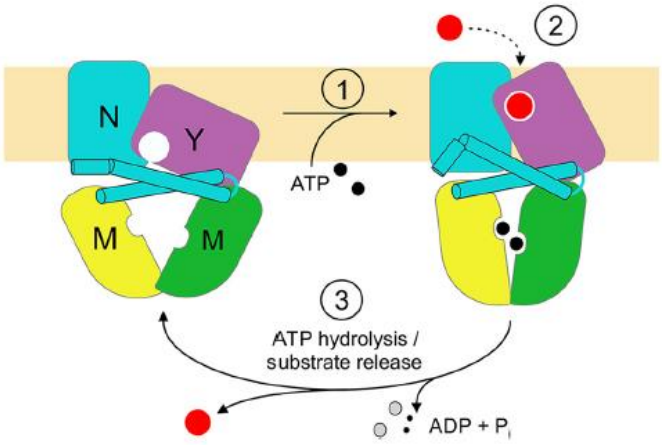

Figure 10. *Figure 10 shows a mechanism model of the biotin ECF transporter. ATPases help the S-component in the transport mechanism by releasing and taking up the substrate[14]. Figure taken from Finkenwirth et al (2015)*

From figure 10, Finkenwirth et al (2015) described that ATP binding to the ECF complex causes the S-component to change its conformation to an upright state from the ECF complex and bind to the specific substrate which is in this case biotin[14]. After capturing the substrate, ATP hydrolysis causes the S-component to topple over due to conformation changes of the coupling helices of the T-component. The substrate is then released into the cell due to the toppling of the S-component[14]. This model and description of it by Finkenwirth et al (2015) is similar to the model made by Bao et al (2017) which means that the role of ATP as described in Bao et al (2017) and Finkenwirth et al (2015) could apply to other group I ECF transporters. However, Bao et al (2017) stated that research has been minimal in comparison to group II ECF transporters, meaning that more research has to be done to see if the earlier described mechanism applies to all group I ECF transporters[5].

5.4 testing ATP-mediated hypothesis of S-component in group II ECF transporter mechanism

Karpowich et al (2016) have researched how ATP binding and hydrolysis affect the mechanism of the S-component in group II ECF transporters[8]. The conclusion was that upon ATP binding the S-component dissociates from the ECF transporter[8]. The S-component binds to its specific substrate and then binds back to the ECF complex and then releases the substrate into the cell by rotating over [8].

Finkenwirth et al (2017) have stated that there is still controversy about the ATP mechanism in group II ECF transporters[15]. The controversy stated by Finkenwirth et al (2017) is whether ATP hydrolysis help with stabilizing the S-component in the toppled state. Karpowich et al (2017) have concluded that ATP hydrolysis causes the S-component to rotate and release the substrate into the cell, which could indicate that ATP hydrolysis stabilizes and help the S-component to topple down in the ECF complex.

Swier et al (2016) have made a model in which the mechanism of the folate group II ECF transporter is described from the results of their research[10].

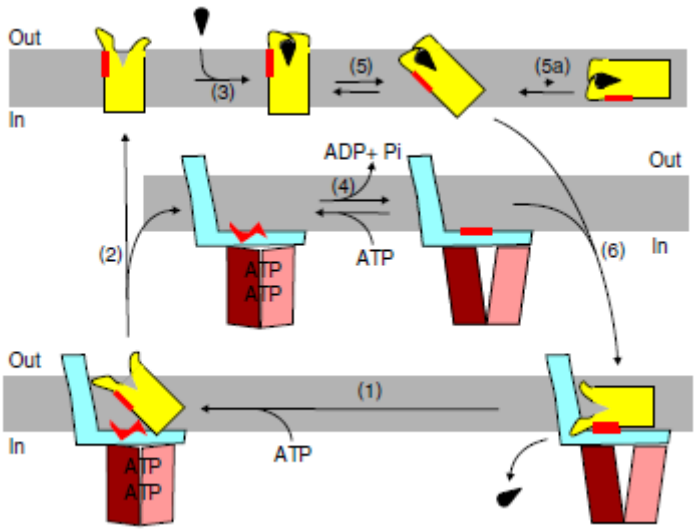

Figure 11. *Figure 11 shows the group II ECF transporter mechanism. Figure taken from Swier et al (2016)*

From figure 11, it can be seen that the S-component of the folate transporter upon ATP binding releases from the ECF complex[10]. This aligns with Karpowich et al (2016) model. The difference is that the S-component spontaneously topples over in the membrane, which has been a mechanism researched by Faustino et al (2020)[10,8]. ATP hydrolysis causes the ECF complex to become available to the substrate-bound S-component, for the substrate-bound S-component to bind to the ECF complex and then release the substrate in the cell. The binding to the ECF complex and the S-component is also explained by Swier et al (2016). Here the binding occurs between the complementary hydrophobic surfaces of the S-component and the T-component which are coloured in red[10]. The hydrophobic surfaces of the T-component are the coupling helices described by Swier et al (2016)[10]. Upon ATP binding the hydrophobic surface of the coupling helices is destroyed due to structural changes of the coupling helices. This could be the reason for the release of the S-component from the ECF complex to restart the transport process[10]. It is not said whether ATP hydrolysis is important in stabilizing the toppled state of the S-component meaning that this is still a question left to be answered. The model of Swier et al (2016) is a very plausible representation of the mechanism of the S-component as Faustino et al (2020) have further proved and analysed the mechanism in detail regarding the toppling mechanism and mediation of that mechanism.

5.5 concluding remarks

Most of the questions regarding the mechanism of the S-component and how that would be mediated in group I and group II ECF transporters have been answered, however, some

questions remain. First of all the toppling mechanism still needs to be analysed in detail as stated by Stanek et al (2018)[9]. New or improved techniques have to be developed to capture the toppling mechanism in detail. This could provide more information and insight into the toppling mechanism. For the membrane-mediated toppling mechanism, Faustino et al (2020) have made a lipid composition which could help stabilise the toppled state of the S-component. However, more lipid compositions could be created to have more variety and create maybe more insight into the toppling mechanism in different environments. This has also been stated by Rempel et al (2019) that more lipid compositions which could affect transporters should be tested on the ECF transporter[16]. How ATP binding and hydrolysis affect S-components in group I ECF transporters a consensus has been reached by researchers, however, more research has been conducted on group I ECF transporters regarding their structure and mechanism, which has also been stated by Bao et al (2017)[5]. More research on group I ECF transporters could provide more insight into the mechanism of this specific transporter and in turn, could create more distinctive and clear features for group II ECF transporters. For group II ECF transporters more research could be conducted to see whether ATP hydrolysis can stabilize the toppling mechanism of the S-component as stated by Finkenwirth et al (2017)[15]. As said earlier a lot of questions about the mechanism of the S-component with the ECF complex have been answered but some questions remain. Further research would provide more insight into the S-component interaction with the ECF complex and could in turn create more insight into the ECF complex transport cycle as a whole.

6. References

1. Ventola C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *P & T: a peer-reviewed journal for formulary management*, *40*(4), 277–283.

2. Rees, Douglas C., Eric Johnson, and Oded Lewinson. (2009). "ABC Transporters: ThePower to Change." *Nature Reviews Molecular Cell Biology*.

3. Bousis, Spyridon et al. (2019). "Energy-Coupling Factor Transporters as Novel Antimicrobial Targets." *Advanced Therapeutics*.

4. Zhang Peng, Jiawei Wang, and Yigong Shi. (2010). "Structure and Mechanism of the S Component of a Bacterial ECF Transporter." *Nature*.

5. Bao, Z., Qi, X., Hong, S., Xu, K., He, F., Zhang, M., … Zhang, P. (2017). Structure and mechanism of a group-I cobalt energy coupling factor transporter. *Cell Research*, *27*(5), 675–687. https://doi.org/10.1038/cr.2017.38

6. Xu, K., Zhang, M., Zhao, Q., Yu, F., Guo, H., Wang, C., … Zhang, P. (2013). Crystal structure of a folate energy-coupling factor transporter from Lactobacillus brevis. *Nature*, *497*(7448), 268–271. https://doi.org/10.1038/nature12046

7. Faustino, Ignacio et al. (2020). "Membrane Mediated Toppling Mechanism of the Folate Energy Coupling Factor Transporter." *Nature Communications* 11(1): 1–9. http://dx.doi.org/10.1038/s41467-020-15554-9.

8. Karpowich, Nathan K, Jin Mei Song, and Nicolette Cocco. (2016). "Release and Catch Mechanism." 22(7): 565–71.

9. Stanek, W. K. (2018). Insights into the transport mechanism of energy-coupling factor transporters. University of Groningen. "three attempts to test toppling hypothesis of S-components''. Chapter 7: 119-132

10. Swier, Lotteke J.Y.M., Albert Guskov, and Dirk J. Slotboom. (2016). "Structural Insight in the Toppling Mechanism of an Energy-Coupling Factor Transporter." *Nature Communications* 7

11. Yang, B., Tang, S., Ma, C., Li, S. T., Shao, G. C., Dang, B., … Wang, L. (2017). Spontaneous and specific chemical cross-linking in live cells to capture and identify protein interactions. *Nature Communications*, *8*(1), 1–10. https://doi.org/10.1038/s41467-017-02409-z

12. Berthomieu, C., & Hienerwadel, R. (2009). Fourier transform infrared (FTIR) spectroscopy. *Photosynthesis Research*, *101*(2–3), 157–170. https://doi.org/10.1007/s11120-009-9439-x

13. Josts, I., Almeida Hernandez, Y., Andreeva, A., & Tidow, H. (2016). Crystal Structure of a Group I Energy Coupling Factor Vitamin Transporter S Component in Complex with Its Cognate Substrate. *Cell Chemical Biology*, *23*(7), 827–836. https://doi.org/10.1016/j.chembiol.2016.06.008

14. Finkenwirth, Friedrich et al. (2015). "ATP-Dependent Conformational Changes Trigger Substrate Capture and Release by an ECF-Type Biotin Transporter." *Journal of Biological Chemistry* 290(27): 16929–42.

15. Finkenwirth, Friedrich, Franziska Kirsch, and Thomas Eitinger. (2017). "Complex Stability during the Transport Cycle of a Subclass i ECF Transporter." *Biochemistry* 56(34): 4578–83.

16. Rempel, S., Stanek, W. K., & Slotboom, D. J. (2019). ECF-Type ATP-binding cassette transporters. *Annual Review of Biochemistry*, *88*, 551–576. https://doi.org/10.1146/annurev-biochem-013118-111705