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Analyzing Flagellum Structure of Trypanosoma Brucei Using Electron Tomography Datasets from Electron Microscopy Data Bank

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Abstract. African trypanosomiasis is a vector-borne parasitic disease. The main parasite that causes the disease is Trypanosoma brucei, also known as T. brucei, which infects its hosts through the tsetse fly. Trypanosoma brucei has an important structure, the flagella. The parasite relies on the flagella for survival and movement, so studying the flagella structure in detail is essential to understanding its function. Therefore, we use electron microscopes for multi-angle observation and photography. We identified some important structures of flagella which aligned with previous literature including the 92+2 axoneme, and the paraflagellar rod (PFR), and we even captured some novel features including densities connecting the axoneme and PFR, densities that interconnect the microtubule, and densities that bridges the different zones of PFR. We also did literature reviews and proposed hypotheses about the functions of these structures. Our structural study could shed light on the inhibition of the flagella movement, thereby treating trypanosomiasis.

Keywords: African trypanosomiasis, Trypanosoma brucei, flagella

1. Introduction

African trypanosomiasis, also known as African sleep disease or lethargic encephalitis, is a zoonotic parasitic disease transmitted by T. brucei through the bite of the tsetse fly. It is rampant in sub-Saharan Africa, with prevalence rates as high as 80% in some endemic areas [1]. Rural populations living in areas where transmission occurs and dependent on agriculture, fishing, livestock or hunting are most likely to be exposed to tsetse flies and therefore to the disease. The disease can occur in a single village or an entire region [1]. The disease can cause fever, rash, edema, and swollen lymph nodes in the early stages [1,2], followed by inflammation of the brain and meninges. Sleeping sickness poses a threat to an estimated 60 million people [3]. There is currently no vaccine available, and treatments are antiquated, toxic, and increasingly ineffective [1].

Infection of a mammalian host initiates when a tsetse fly bite delivers growth-arrested metacyclic trypomastigotes to the mammalian bloodstream. Metacyclic trypomastigotes then differentiate into proliferating long slender forms that establish and maintain a bloodstream infection. Parasites eventually penetrate the blood vessel endothelium and invade extravascular tissues, including the central nervous system (CNS). In the bloodstream, T. brucei changes from a long, slender form to a short, pudgy one. When parasites are taken up with the blood meal into the midgut, short stumpy forms differentiate into procyclic trypomastigotes that resume cell division and establish a midgut infection. Then the

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trypanosomes migrate and eventually attach themselves to the salivary glands. And they can keep moving because they have a flagellum. T. brucei has only one flagellum, which is present throughout the cell cycle and at all stages of development. Flagella are necessary for survival, the only means of movement, and play a key role in many aspects of development, development, and pathogenesis[3].

The parasite relies on its flagella for movement. Early work showed that motility is driven by a flagellar wave that initiates at the tip of the flagellum and moves towards the base of the flagellum. Flagella beating is a feature of trypanosomes, because when the flagella wiggles, it causes recoil in the surrounding fluid to move the trypanosome in the direction of the flagella. Unlike other flagellated eukaryotes, this movement is in the opposite direction [3]. Because the flagellum is closely combined with the cell body, the flagellum wave will make the whole cell body begin to rotate and other movements when the flagellum swings.

Electron microscopy is a very important instrument in the study and observation of T. brucei. Although electron microscopes are more expensive and are very complex to operate, the key advantage Electron microscopy has is that it can achieve far higher resolution than light microscopes. And in order to capture high-resolution information of the T. brucei in detail from collected image series, sample preparation is an essential procedure. The three scientists, George Palade, Albert Claude, and Christian de Duve, have developed a method for grid sample preparation. This method effectively makes the sample stable and easy to observe.

2. Materials and methods

The data source for the analysis in this paper is the CryoET data related to the flagellar structure of T brucei from the Electron Microscopy Data Bank (EMDB) website. I downloaded several CryoET data for observation and analysis, including EMD-23621, EMD-11305, EMD-11307, EMD-11308, EMD-11303, EMD-20012, EMD-20013, EMD-20014, EMD-6748 [8-11].

CryoET is very important for observing and photographing experimental samples. The workflow for CryoET starts with making a sample and the detailed steps are described below.

The sample was first stained, and then the fixed mass sample was transferred with a pipette gun to the tweezers, which transferred the sample to the grid (this typical EM grid is covered with a layer of plastic and carbon on top of it). The samples in the grid were frozen with liquid nitrogen.

Then we need to use the CryoET. A tank was prepared with liquid nitrogen on the outside and ethane on the inside. Once this was ready, we need to pick up the mesh with tweezers and place it in the CryoET to secure it. And later we need to also place the frozen ethane into the CryoET. The sample of fixed mass was then sucked out of the tube with a pipette gun and inserted through a small hole at the side of the CryoET so that the sample comes into contact with the grid on the tweezers. Two mechanical arms with filter paper control the filter paper to fit the mesh at a very small Angle, absorbing excess water from the sample. After these treatments, the sample was then quickly placed into the frozen ethane, which is then placed in a box.

The third step was to place the sample into an electron microscope, which will photograph the sample from multiple angles and create a 3D model.

3. Result

3.1. Part of the flagella with a structure of 92+2 under the electron microscope

To observe the flagella structure of the T. brucei in detail, I have reviewed the uploaded cryoEM datasets and cryoET datasets in the EMDB databank and identified some high-quality cryoET images as shown in Figure 1. From the reconstructed tomographic image (Figure 1), the 92+2 axoneme and the adjacent PFR structures can be readily observed. In the cross-section shown in Figure 1, nine pairs of outer doublet microtubules can be seen, with a pair of central pair apparatus in the center. As shown in Figure 1, the structure of 92+2 is very complete which means no structure loss during the sample preparation, and you can observe all the key components such as the axoneme and PFR. The diameter of the cross-section is measured at about 215nm, and the diameter of the central pair apparatus is measured at about

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23.1nm. The outer doublet microtubules are a special pair of microtubules with two different diameters, one large and one small. The larger microtubules are about 26.9nm in diameter, and the smaller ones are 23.1nm in diameter, which is about the same as the central pair apparatus. Because the resolution of this tomography image is limited due to the issue of missing wedge compensation, the more detailed structure of flagella is hard to get from it without further image processes such as sub-tomographic averaging of hundreds of tomographic images.

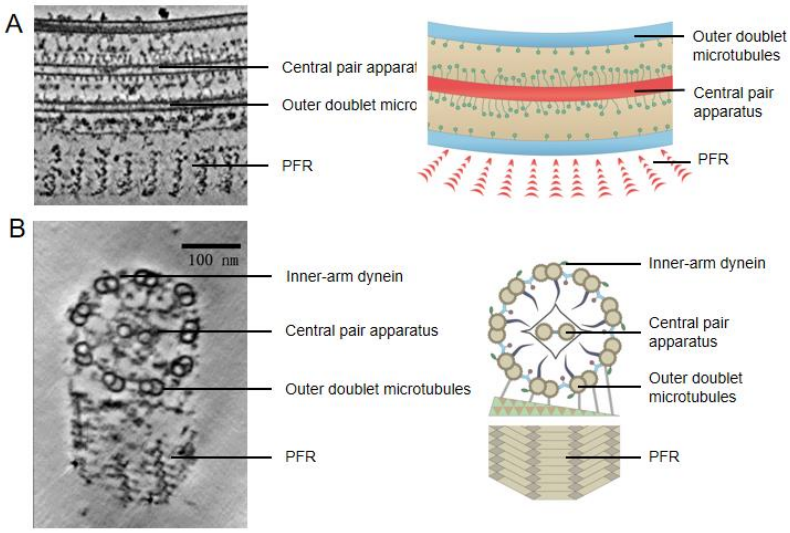

Figure 1. The structure of a part of 9^2+2 flagellum.

(A)Lateral view of the flagellar structure observed by Gygwin64 Terminal. the rotation of X is -2.0, the rotation of Y is -1.0, and the rotation of Z is -90.0. The view axis position is 86. From this Angle, we can observe the overall changes in flagella structure, and Some tissues or structures attached to the walls of the central pair apparatus and the outer doublet Microtubules were observed.

(B)This perspective is viewed from a cross-sectional perspective and also observed by Gygwin64 Terminal. The rotation of X is 0.4, the rotation of Y is -75.6, and the rotation of Z is -94.4. The view axis position is 268. This image has the outer doublet microtubules, a central pair of devices, internal-arm dynamin, and PFR.

We have utilized the eTomo software package to observe this tomogram (Figure 1) in different rotation angles along the X, Y, and Z-axis using the command 3dmod. We observed that there were some connection-like densities between each pair of inner-doublet microtubules, and we speculated that this density structure was the nexin dynein regulatory complex (NDRC), present in almost all motile axonemes. But the NDRC in T. brucei has not been determined. According to image A, the blue arrow shows one of the detailed structures. Based on the shape, we can readily recognize this tiny structure to be the outer-arm dynein. The inner-arm dynein structure in the essay [3] is shaped like a boomerang, long at the top and short at the bottom. The shape is roughly consistent with that in the book. But these are all extrapolations, and determining the exact structure requires photographing with higher-resolution instruments. A simple diagram makes structure clearer, simple diagram size, and real picture size, shape similar, more conducive to understanding.

Through the reconstructed tomographic images of these two flagella, we can observe the basic structure of the flagella from different angles and have a general understanding of the flagella components, which is conducive to the discussion and speculation of the flagella later.

3.2. Comparing flagella size to other common species

Haven seen the detailed structure of the flagella as shown in figure 1, further comparison analyses have been performed. In this section, I first rotated the 3D images of the flagella structure at different angles. The flagella are divided into 9 parts in total (Figure 2). There are different components in the flagella,

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and each part has a different size, and their specific sizes are marked in image A. In the cross-section, nine pairs of outer doublet microtubules can be seen, with a pair of central pair apparatus in the center. The structure of 92+2 is very complete. The length of this structure is 355nm, the diameter of the flagella is about 215nm, and the central pair apparatus is about 70nm. The distance between the two outer doublet microtubules is about 110nm. On the far left side of the structure is the PFR structure, which is very clearly divided by observation. The length of the upper part on the left is about 65nm, which may be shorter due to the shooting angle. The length of the second upper left segment is about 135nm, the third lower left segment is about 125nm, and the fourth lower left segment is about 40nm. The short length of the fourth paragraph is also related to the shooting angle. The body of T. brucei is streamlined, and its curved body length varies from 12 to 35 microns. T. brucei also has structures such as the nucleus and dynamic matrix. The flagella are one of its most important structures, extending from the top of the body, clinging to the body, and finally extending outward. These are shown in image B.

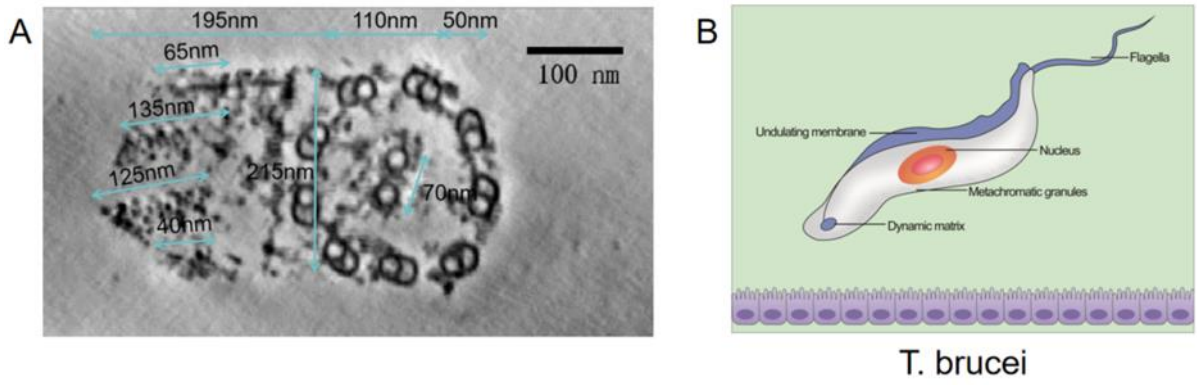

Figure 2. The size and proportion of the complete T. brucei and its flagella.

(A)Image A shows part of the structure of T. brucei flagella and its size and proportion. The degree of x rotation is -71.2, the degree of y rotation is 0, the degree of z rotation is -180. And view axis position is 267.

(B)Image B is a simplified view of the complete T. brucei and its size and proportions.

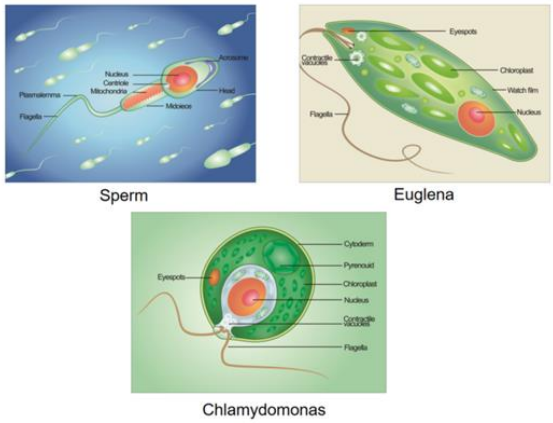

Figure 3. Comparison of T. brucei with sperm, Euglena and chlamydomonas.

Next, I have also compared the flagella size to other common species such as human sperm Euglena, and Chlamydomonas. As we all know that flagella are not only an important structure of T. brucei but many species in nature and humans rely on flagella for movement and survival [3]. Therefore, three species with flagella are commonly found in life to emphasize the important role of flagella, and the structure in flagella is hypothesized below. The three species I have analyzed and compared with T. brucei are spermatozoa, Euglena and Chlamydomonas. These are the "resident guests" of biology

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textbooks, which are more conducive to understanding the role of flagella. The average volume of sperm is 111 cubic micrometers, and each sperm has an average of 100 flagella. The image of the sperm is presented in panel G. Euglena has an average volume of 100 cubic microns and has an average of two flagella. Euglena is represented in Figure 3. Chlamydomonas is also about 100 cubic microns in size and has two flagella. The basic structure of Chlamydomonas is shown in Figure 3 too. The description of the structure in the image is still relatively clear and detailed.

Comparing flagella with viruses, fungi, bacteria, etc. is also a point worth analyzing. All three species choose examples that are widespread and close to life. It is more conducive to having a better understanding of the size of flagella and T. brucei. specific concepts. The virus selected is HIV. The structure of HIV includes cytoplasm, cell membrane, membrane proteins, etc. The simple structure of HIV is presented in image C. The diameter of the HIV is about 100 nm. The bacteria chose Escherichia coli as an example, because Escherichia coli exists everywhere in our life, including in our bodies. Escherichia coli are round rods with a length of about 2000 nm. Image D shows the simplified structure of E. coli: cytoplasm and membrane. The fungi selected are yeasts, which are involved in common foods, such as yogurt and rice wine. Yeasts are oval and about 5000nm in length. Image E also shows the simplified structure of yeast, including the vacuole, nucleus, cytoplasm, and membrane. Comparing the sizes of flagella, viruses, bacteria, and fungi, the graph shows very clear differences (Figure 4). With a basic understanding of the size of flagella and T. brucei, it is possible to further analyze what kind of damage their size may cause to the human body. For example, how much impact the breakthrough of the blood-brain barrier may have [12].

This group of pictures makes the structure of flagella more clear and detailed, and the size of each place is marked. Comparing the size, volume, and flagellum number of T. brucei with other common species or species with the same structure can highlight some characteristics of T. brucei and make it easier to understand.

3.3. Some special structures in the flagella

Beside the comparison studies, we also performed in-depth analyses and found some special features inside the flagella. As you can see in image A of figure 5, three special parts have been indicated by 3 boxes in different colors. Image B in figure 5 shows a pair of outer doublet microtubules with a protein-like protruding between two tubular structures revealed by careful observation. The protuberance is an unknown and special structure, which is worth studying. There is only one protrusion in this figure, and it is triangular. This bulge is not a very unusual structure. It can be seen from image A that these processes are present in almost every pair of outer doublet microtubules and that some of them are very obvious, while others are numerous and dense. There are four more examples at the bottom of the big picture where the bumps can be seen very clearly. The protrusion in the image on the upper left is very obvious and larger than normal. The other three images all have two or three projections and are relatively dense. High density suggests that protruding structures are common, but may be too small to understand in detail. That's why we're doing research here.

Image B of figure 5 also shows some parallel spring-like structures that can be inferred as PFR based on the basic understanding of the flagellar structure [3]. By reading the literature, we speculate that the PFR is elastic and not only supports the flagellar structure but also effectively mitigates the effects of flagellar oscillations. The spring structure in the figure can verify our conjecture very well. The spring is elastic and can be stretched and shortened to some extent. In addition to the protrusions in the PFR and microtubules, I also observed a structure coming across between the microtubules and the PFR. Based on the understanding of the whole 92+2 structure, it is reasonable to guess that this structure is a transition structure that connects the upper microtubule structure to the lower PFR structure to make the flagellum more stable as a whole.

Furthermore, we also observed some special tubular structure in the PFR region as shown in Figure 6. Basically, two parallel tubular densities have been captured which seem interconnected. This interesting observation imply that the flagella is a complicated system containing several components

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that work together to function well. Energy and signal could transmit through these interconnected wires and to further control the movements of the flagella (Figure 6).

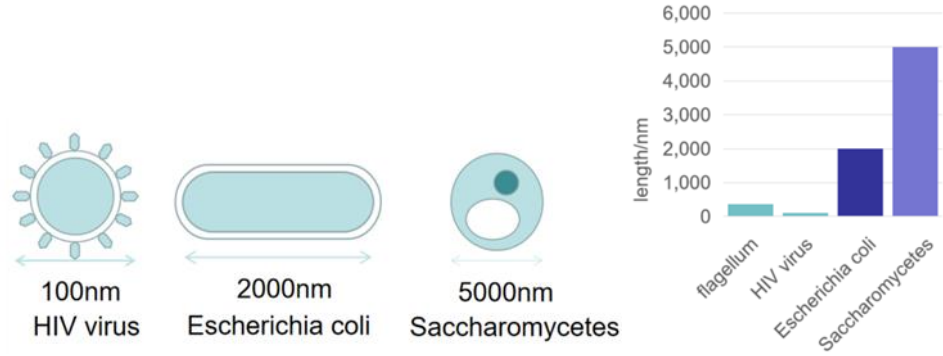

Figure 4. Comparison of T. brucei with viruses, fungi, bacteria.

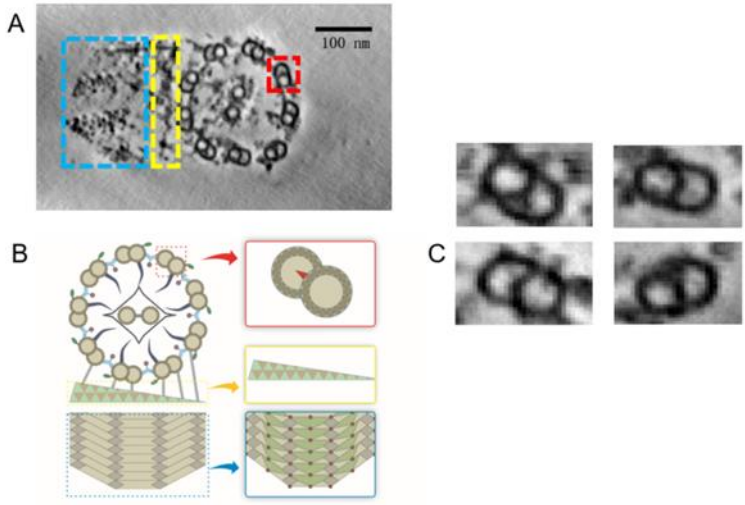

Figure 5. Some special structures in the flagella.

(A) Three parts are marked in the 92+2 structure in (A). There are some spring-like structures in the blue box, the PFR. A very straight structure in the yellow box, is an unknown feature. And a pair of outer double-line microtubules in the red box. The odd part, however, is the protein protrusions between the two tubes.

(B) Image B enlarges each section to show it clearly. Using simple, clear images makes the structure easier to understand.

(C) Image C presented are the outer doublet microtubules with a structure similar to the red box in image A. There are distinct protein protrusions between the two tubes.

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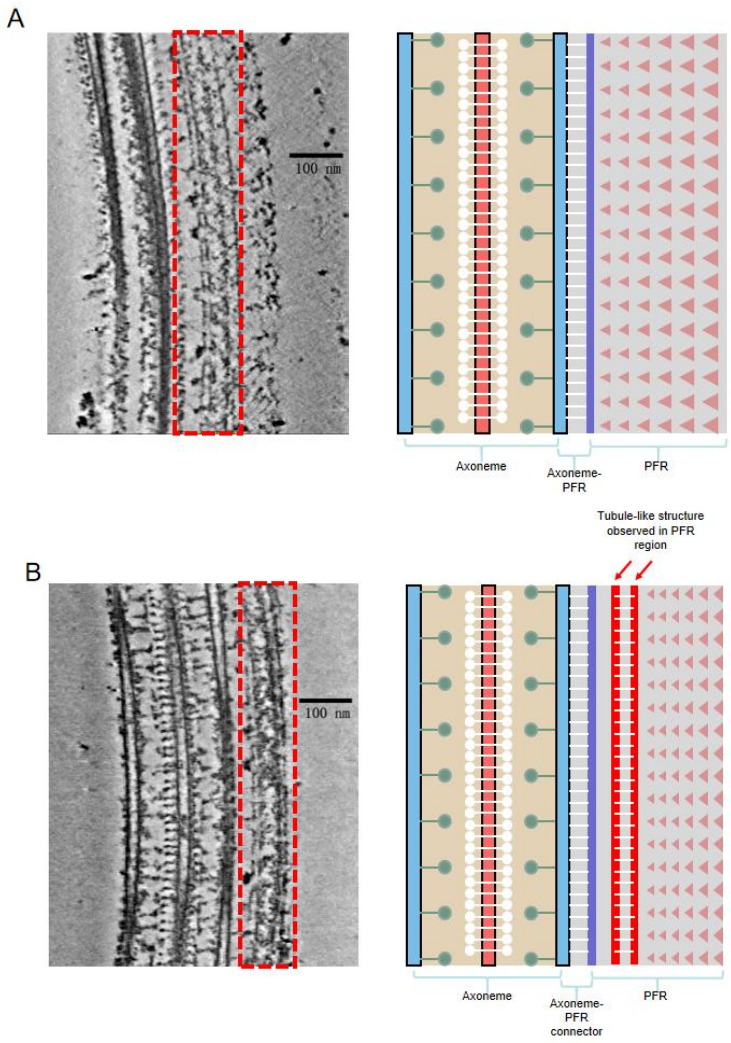

Figure 6. Special tubular structure.

(A)Image A shows a cross-section view observed by the Gygwin64 Terminal, the rotation of X is 0.0, the rotation of Y is 0.1, and the rotation of Z is 0.0. The view axis position is 127. other tubular structures will be found in the cross-section. The two tubular structures are framed in red, and the detailed structure is shown in a clearer cartoon. Known structures are drawn and marked in the cartoon: Axoneme, Axoneme-PFR connector, and PFR. Particular tubes observed are marked in purple.

(B) Image B shows a cross-section view observed by Gygwin64 Terminal too, the rotation of X is 0.0, the rotation of Y is 27, and the rotation of Z is -2.2. The view axis position is 108. Two other tubular structures can also be observed in the section Figure B, which proves that these two tubular structures do exist, and their structures are described in detail with cartoon drawings. Known structures are drawn and marked in the cartoon: Axoneme, Axoneme-PFR connector, and PFR. The two observed special tubular structures are also drawn in red and marked with the Tubule-like structure observed in the PFR region', indicating that this structure is unknown.

4. Discussion

I have conducted a literature review of more than ten papers on the T. brucei flagella topic, and based on that, I have made a couple of assumptions in this discussion section. The first point I want to discuss here is related to the role of PFR structures in flagella. I have made three hypotheses during my initial research. In the accompanying drawings of many pieces of literature, I have found that whether it is a

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simple drawing or a complex drawing, the part of the flagella parallel to the body is not closely attached to the cell body, except for a small part connected in the flagellar pocket, the rest is most have gaps [1,3,4]. So I started thinking about how this void is formed and whether it has something to do with the role of the PFR structure. This gap is very small. What properties of PFR can maintain this gap? This can't help but remind me of magnets. Magnets are very common objects in life. They are attractive and repulsive, so they can maintain a steady distance. Therefore, we think that the gap between the PFR and the cell body is maintained like two magnets. So my first initial hypothesis about the function of PFR structure was that PFR is functioning as a magnet, not double-sided tape. It can be assumed that the PFR and the cell body are directly maintained by suction and repulsion forces, rather than being tightly held together like double-sided tape.

I know that flagella are the only mode of movement in T. brucei and play a very important role in their development and infection [4,6,7]. What makes T. brucei truly pathogenic is that it penetrates the blood-brain barrier, which requires adaptive structures. Many parasites have a subpellicular array of microtubules that shape their cells into shapes that can efficiently infect their hosts. The tip shape of T. brucei is produced by the subpellicular array. The beating of the flagellum deforms the subpellicular array and invades the blood-brain barrier in a unique movement pattern [5], so the beating of the flagella must be very powerful to drive whole-body motion. Through cell observation [3], I found that the main structures of flagella are microtubules and PFR. These microtubules are made of tubulin, and they are not only incapable of powering themselves, but they are also fragile. Only PFR helps flagella wiggle. So my second thought is that the PFR acts like a muscle and provides a very large amount of power for the flagella to wiggle. If you look at the shape of the PFR, it looks like a bicep on a human arm [3]. That's the rationale why I hypothesize that PFR acts like a muscle.

The discovery process is the same as the second one, but I have a different idea about PFR as a muscle to provide power. It may be other structures in some flagella to provide power, but the structure of microtubules is still fragile, so I think that PFR can be used as a protective structure of microtubules, which is the third idea. The PFR acts as a spongy structure to cushion the impact of flagellar oscillation. Since the movement of trypanosomes is entirely dependent on flagellum oscillations, the flagellum oscillations should have a lot of force, so it is assumed that PFR acts as a buffer to protect the structural integrity of axons.

Besides the function of the PFR, the second point I want to make in this discussion section is whether T. brucei can cause other pathogens to infect humans. Because T. brucei lives in its host, it causes African trypanosomiasis. Breaking the blood-brain barrier is the key to causing disease. But because of T. brucei's size, the only way it can penetrate the blood-brain barrier is to burrow into a blood vessel and make a hole in its wall. This can cause the blood vessel to rupture, likely invaded by other pathogens, or more severely, causing surrounding cells to be deprived of nutrients and die. The blood-brain barrier is a very important line of defense in the human body. It is an efficient barrier that protects the brain and limits the delivery of drugs to the central nervous system [13]. So when this line of defense is broken, it may cause other more serious consequences. For example, in 2010, Sun Rui and Wu Zhongdao studied brain cysticercosis infection and used mice as research objects to observe. It was found that disruption of the blood-brain barrier and migratory exudation of leukocytes from the pia mater occurred within days of infection. And the overflow of leukocytes in the blood vessels can cause aggravation of local inflammation, and finally cause most of the infections of the brain. The two experiments proved that the harm caused by the parasites penetrating the blood-brain barrier is great [12].

And the third point I want to hypothesize involves a strange structure that I have observed. This structure is between the microtubule and PFR structure, and I have two ideas about this structure. By looking at the shape of the structure (Figure 1B), I found that it resembled a bridge, so I speculated by analogy whether it would function as a bridge. This led to my first thought that it acts as a bridge, transmitting signals that align microtubules with PFR action. In the literature, I read I found similar conclusions - the physical link between PFR and outer arm dynein provides a mechanism by which chemical or mechanical regulatory signals from PFR can be transmitted directly outward Arm dynein [6]. The published paper by Louise C Hughes et al. came to a similar conclusion. They used biaxial

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electron tomography to determine the 3D structure of the T. brucei flagella. observed a direct link between PER and dynein in axons and concluded that mechanochemical signals could be transmitted from PFR to axonal dynein [6].

The second idea is also based on literature reading [3,6] and the electron micrographs of the cells I observed (Figure 6). In both different views, I found unusual tubular structures, which make the connection between PFR and microtubules more numerous and stable. There is a connection between the microtubule and the PFR, and this structure allows the PFR to connect to the entire microtubule, making the structure more stable and the flagellar wiggling stronger. For example, in the process of breaking the blood-brain barrier, the structure of T. brucei can be more stable and powerful.

5. Conclusion

African trypanosomiasis disease also called sleeping sickness, is caused by the parasite, namely, Trypanosoma brucei, also known as T. brucei. The human can get infected when the Tsetse fly bites them. One of the most important structures for T. brucei is the flagella which not only account for its survival but also play an essential role in its movement. Therefore, studying the detailed structure of the flagellar is needed to understand its assembly and its function. Here in this study, we have leveraged the Electron Microscopy Data Bank (EMDB) database, especially the cryo-electron tomography data, to conduct a series of analyses. We also utilized the software of eTomo to perform the multi-angle observation of the three-dimensional tomography images. We identified some important structures of flagella which aligned with previous pieces of literature, for example, the 92+2 axoneme structure, and the PFR structure which attached to the Axoneme. More importantly, we even captured some novel features from the cryoET datasets, including electron densities that bridge the axoneme and PFR, densities that interconnect the microtubule, and densities that bridge the different zones of PFR. We also conducted literature reviews of published papers in this field and proposed hypotheses about the functions of these novel structures. Our structural study could shed light on the inhibition of the flagella movement, thereby it might represent a great opportunity for us to treat the trypanosomiasis disease.

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