Some Tensions are Good for Life

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Like every animal has its characteristic size and shape, each cell type in our body has a size and shape. Neuron (nerve cell) is very different from muscle cells that are dramatically different from the cells in the bone. All these cells look different, perform diverse activities, share things with each other and multiply, albeit somehow maintaining their individual size and form. The plasma membrane of a cell defines the boundary of a cell and thus its size. However, it is not a static wall. The membrane is under constant fluxevery moment because a cell talks to its surroundings via this membrane. Cells uptake enutrients and other material from surrounding via endocytosisby bending the membrane inwardand forming a vesicle containing these(*Endon- within, kytos- cell: uptake of material by a cell from the environment by invagination of its plasma membrane*).Exocytosis, on the other hand, helps add material to the surface through vesicles. However, taking material in each time also removes a bit of plasma membrane from surface. Imagine each time you enter a room, you take in the door and a portion of the wall with you. If membrane is not carefully put back each time through exocytosis, the cell would shrink or enlarge. To make it even more

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complex, cell makes multiple doors every moment to take in or remove stuff from the cell. So how does the cell maintain the endo-exocytic flux to help it maintain a size?

"Don't underestimate The Force"

- Darth Vader. (Star Wars)

We find that like 'Jedis', cells also maintain the balance in their lives and processes by utilizing the Force. Force, in this cellular context is called the membrane tension. I found that endo-exocytic processes are intimately connected to membrane tension. Though there are myriad of endocytic pathways operating in parallel at a micron scale,only one of them is able to quickly sense and respond to changes in forcei.e. membrane tension.This pathway is called CLIC/GEEC pathway. Increasing membrane tension decreases this endocytic pathway while decreasing membrane tension increases the endocytosis. I find that the CLIC/GEEC pathway can sense and respond to forces. This pathway, if you will, is the 'Yoda'among the many endocytic mechanisms.

How did we change tension of a cell? For this we collaborated with physicists in Barcelona, Spain who were interested in understanding how forces influence life. A cell stretcher was made using a silicon based membranemuch similar to a contact lens but with a diameter of 6 inches. Cells are attached to the membrane and stretching them causes cells to stretch and thus change the membrane tension. This silicon membrane is divided into two concentric circles and cells are added to the inner circle. To stretch it, we apply precisely calibrated vacuum to the outer concentric circle, sucking the membrane in and thus causing the membrane in the inner circle to stretch. To look at endocytosis, we add fluorescent material from outside (that is not permeable through plasma membrane) and ask how much the cell takes in for a given amount of time. We image the fluorescent material in a cell to study endocytosis with changes in tension of a cell.

We then used a special microscope called 'optical tweezer' which as the name suggest acts as a light based tweezer. Using a focused laser, you can hold objects (in our case, a micron size transparent bead) with high precision.We used this tweezer to carry out a tug-of-war between the cell membrane and the bead. We held a bead in this tweezer and pull a thin membrane from the cell. If cell applies higher force, it will pull the bead closer to it while if the force is less, the bead would move less. Using this we asked what happens to the forces applied by the membrane tethers (called here as 'tether forces'). We use this tether forceas proxy for membrane tension.We find that this CLIC/GEEC pathway is essential for the maintenance of the membrane tension. Removal of plasma membrane on increasing the pathway increases the membrane tension and inhibiting the pathway, decreases the tension. Thus, addition and removal of membrane directly influences the tension of membrane.

So, every time a door and wall is removed when you enter a room (endocytosis) there is a change in the physical property of the rest of the wall (membrane tension) that indicates how much is removed. This provides a clever way for the cell to maintain homeostasis. So, each time membrane is removed by endocytosis, membrane tension increases and if a cell could sense and respond to this change in tension by adding membrane, it could maintain the homeostasis of membrane turn over.

So how would a cell sense the change in force and control an endocytic pathway? In other words, how does the cell convert physical information to a bio-chemical one to control a biological process? By using mutant cell lines, we found that a molecule called vinculin is responsible for this. Vinculin remains closed under low forces while on higher forces, it changes its conformation and opens up like a hair pin that is being pried open under force. Vinculin in its open state then inhibits a key upstream regulator of CLIC/GEEC pathway,therebyregulating endocytosis in response to the membrane tension.

Thus, like a perfect air conditioner maintaining the room temperatureby sensing increase or decrease in temperature and responding to it, each cell senses the force and regulates the CLIC/GEEC pathway to maintain membrane homeostasis. If the force goes higher, the CLIC/GEEC pathway is shut down helping the membrane relax while if tension goes lower, endocytosis increases and extra membrane is taken in. Cell does this by employing vinculin that can open up and close in response to tension. Thus each and every moment the cell is constantly measuring and responding to tension through this pathway to maintain membrane homeostasis without which a cell, and thus life, won't exist.CLIC/GEEC endocytosis is also hijacked by viruses to enter cells. This pathway is also important for cell migration which is employed by cancer cells undergoing metastasis to spread to different organs. This study shows the importance of understanding forces in regulating these processes and thus would be important for fighting them effectively. For these cells, Do or Do Not, there is no try!