Coin Tossing Explains Activity of Opposite Motors on Phagosomes

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We, The Mallik Lab, at Tata Institute of Fundamental Research are working towards understanding how tiny cellular material moves over large distances inside a living cell.

Cellular environment is extremely crowded and busy. Cells have several smaller compartments called organelles and various biomolecules, each of varying shapes and sizes. If we put a live cell under a microscope, we see that there is constant exchange and movement of material from one part of the cell to the other. This trafficking of biomolecules, or "cargoes", as we call them is required for various life processes, such as cell division, uptake of nutrients, and migration of cells to the site of wound healing. Defects in transport of key molecules can often result in death or it can manifest in a number of diseases such as Alzheimer's and Huntington's.

We study the transport process with respect to infection. In our day-to-day life, we encounter a variety of infectious agents. Our body has developed defense mechanisms to fight disease-causing agents without affecting our normal functions. When a foreign particle infects our body, our immune cells ingest these particles and trap them in a double-layered membrane structure, which is called a phagosome. This process is called phagocytosis. Phagosomes eventually move to the centre of the cell for degradation. Motion of a phagosome from the site of engulfment to its site of killing is extremely important for effective clearance of the pathogen.

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How does this motion of phagosome or other cargoes occur in the cell? This is brought about by the action of cellular "motors". Motor proteins walk along pre-existing roads to deliver cargoes at their required locations. For simplicity, let us imagine the cargo as a cart, which requires a motor- say a horse, to drive its motion in one direction. There are two main types of motors that carry out long distance transport – like the horse and the bullock, which are Kinesin and Dynein. Both Kinesin and Dynein motors walk on same kind of tracks, however, they are quite different in their size, structure, as well as the direction in which they walk. Kinesin motors move cargoes towards cell periphery (a horse-cart moving in one direction) while Dynein generally moves them towards the centre (a bullock-cart in the opposite direction).

To add to the cellular complexity, a large number of cargoes have both kinds of motors and actually move back and forth. So, we now imagine a cart with horses on the one end and bullocks on the other, both pulling back and forth to drive its motion in opposite directions. How these opposite motors work together to bring about molecules to the right place at the right time is hotly debated. We have recently addressed this question in our manuscript published in "Current Biology" in May 2018.

In our study, we examined the motion of phagosomes in their early stages of phagocytosis. These Early Phagosomes (EPs) display bidirectional back and forth motion due to presence of both Kinesin and Dynein motors. To study motor function on EPs, we extracted them from immune cells using well-established protocols and made them walk on artificially constructed tracks. We analyzed their motion outside the cell using a special kind of microscopy called optical trapping. This technique provides tremendous amount of information as to how far motors can walk; the forces exerted by each motor type, their speed. Such experiments allow us to decipher properties of motors at a single phagosome level, a resolution that cannot be achieved when looking at an entire cell.

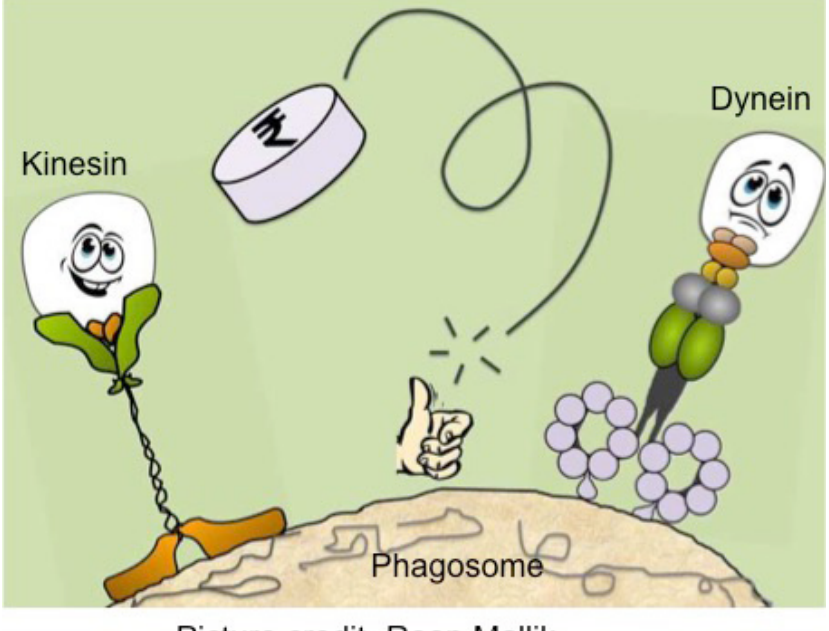
We investigated how could we explain back and forth motion of EPs? Do opposite motors depend on each other or do they pull against each other like in a tug of war? To make sense of how opposite motors behave on an EP, we specifically removed Dynein motors from the EP membrane. Surprisingly, upon Dynein removal, Kinesin neither performed better nor did it become worse. This suggested that both types of motors act independent of each other and do not require the opposite motor for their function.If motors function independently, what governs the choice of active motor – when do horses pull and when do bullocks pull the cart? Is there a pattern these motors follow to bring about motion?

To answer this, we analyzed a number of events where pulling force was generated by Kinesin (horses) and Dynein (bullocks) motors. We focused on even pairs, for instance, a KK pair where Kinesin was followed by another Kinesin event, or KD pair where a Kinesin was followed by a Dynein event, DK pair where Dynein was followed by Kinesin event and DD pair where Dynein was followed by another Dynein event. On performing statistical analysis of these pairs, we found that the number of each type of event pair is more or less similar. This suggests that all four types of event are equally likely to occur. This is similar to tossing of a coin where the probabilities of getting two heads (HH) or one head and then a tail (HT) or two tails (TT) or one tail and then a

head (TH) are more or less equal. Thus, once a pulling event occurs, the choice between Dynein and Kinesin for the next event is a random process. The system does not have any memory of the first event and thus, activation by either type of motor is equally likely.

We next asked whether this random choice of active motor explains bidirectional EP motion? If this is random, can we simulate this motion using modeling? Interestingly, we found that the back and forth EP motion is accurately explained by mathematical modeling when we take into account motor numbers on EP, their binding and unbinding rates and the geometry of phagosome where motors bind the track. These parameters somehow ensure that both Dynein and Kinesin events are equally possible resulting in back and forth EP motion. Such motion allows EPs to sample more intracellular space and interact with other organelles for exchange of components.

Thus, from our studies we have obtained basic parameters that make choice of motors a fair process. This may also be true for many other cellular cargoes. Further levels of regulation such as change in membrane composition; motor numbers or organization can bias this fair coin and accordingly change motion properties of phagosomes in the later stages. Our work, in general, addresses some fundamental questions by using a variety of approaches – we employ biological methods, biophysical force measurement techniques as well as mathematical modeling. Our work provides a holistic view in understanding of bidirectional cargo transport duringearly stages of phagosome motion as well as in situations when things go awry and result in infections.



Cartoon depicting a coin toss between Kinesin and Dynein motors on the phagosome