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Laying Tracks for Poison Delivery to "Kiss of Death": Search for Immune Synapse by Microtubules

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"Search-and-capture" (SAC) is a common phenomenon: how does a lion search for a prey and capture it? In prehistoric times, hunter-gatherer humans also searched for food and shelter. Many activities of humans, even in the current information age, rely on a successful search for target information using search engines like Google. In the most general abstract formulation of a SAC problem, a searcher uses a search strategy to hit a specific target located in the search space as efficiently and as reliably as possible.

SAC phenomena occur at multiple levels of biological organization. Organisms search and acquire not only food and mates but also other necessary resources that are crucial for their own survival and reproduction. Bacteria search for nutrients, whereas sperm cells search for egg cells (oocytes). The axon of a neuron targets the dendrites of another in a way that is similar to the way a pollen tube targets a pistil. In this issue of the *Biophysical Journal*, Sarkar et al. (1) develop a theoretical model for an SAC phenomenon that occurs at the subcellular level and analyze it quantitatively by a combination of analytical and numerical techniques.

There are two extreme limiting modes of search. In the "ambush" mode, the searcher may simply "sit and wait" for the target to come to it while wandering in the search space. Search for cognate aminoacyl-transfer RNA by a ribosome during translation falls in this category. In the other extreme is the wandering searcher that scans the search space looking for the static target. Most real SAC fall somewhere in between these two extremes. Moreover, based on the rules used by the wandering searcher for scanning, search strategies can be classified into two broad categories, namely deterministic (systematic) or stochastic (random). In (1), the authors have modeled a stochastic search by a searcher wandering in the three-dimensional search space.

The core of the SAC mechanism used in (1) is based on the phenomenon of dynamic instability of microtubule (MT) filaments. A polymerizing MT keeps growing in length till it suffers a "catastrophe" that triggers its rapid depolymerization. If its depolymerization is stopped by a "rescue" event before its complete disappearance, it resumes polymerization till it suffers the next catastrophe. Thus, the dynamics of an MT alternates between phases of growth and shrinkage.

Discoverers of dynamic instability made the bold proposition that an MT, which grows from a centrosome, can search for a target at a distance, exploiting its dynamic instability. If a growing MT successfully hits its target, which is capable of capturing it and suppressing its dynamics after capture, then a stable connection (or, a transient connection with sufficiently long lifetime) can be formed between the centrosome and the target. In the context of the assembly of the mitotic spindle, in which this mechanism of SAC was first proposed, the targets were a proteinous complex called kinetochore (2). Even if a search in any specific direction by an MT ends unsuccessfully by its eventual complete disappearance, a search in other directions can be carried out by other MTs.

Almost all the earlier theoretical works on SAC in molecular cell biology (see (3–5) for reviews) focused on the formation of MT-kinetochore attachment during mitotic spindle assembly. In (1), the authors have studied a different system involved in an immune response. A T cell binds specifically with an antigen-presenting cell (APC) forming an adhesive junction. This junction is referred to as an immunological synapse (IS) (6), in analogy with a neural synapse in the central nervous system. After establishment of this junction, dynamic MTs growing from the centrosome of the T cell search for the IS and eventually get captured by the IS. The force generated by the interaction of the MTs with the IS pulls the centrosome close to the IS. Finally, MTs also serve as motorized transport of lytic materials that kill the APC adhered to the T cell at IC in a "kiss of death."

In (1), the authors have developed a minimal theoretical model quantifying

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the concept of "MT-end-on-capture" by the IS. This was an alternative to another mechanism of pulling the centrosome with force generated by cortical sliding of MTs by dynein motors (7). The model developed in (1) focuses almost exclusively on the search of the IS by dynamic MTs and their eventual capture by the IS.

In all the quantitative calculations on SAC reported so far, it has been assumed that the attachment is formed just when the searcher successfully hits the target for the first time. In the theory of stochastic processes, there is a huge amount of published literature on analytical methods of calculating such first passage times (8). In (1), the mean first-passage time (MFPT) to the IS has been identified as the average search time taken by an MT, nucleated from the centrosome. In reality, the first encounter of the searcher and target may not lead to their immediate binding, just as a predator may not be able to catch a prey in the very first encounter; the actual binding may require multiple hit and trials. Therefore, the MFPT might underestimate the average time actually needed to connect the centrosome with the IS.

Obviously, the MFPT depends not only the kinetic properties of an individual MT (rates of growth, shrinkage, catastrophe, and rescue) but also on the number of MTs simultaneously engaged in the search. This time depends crucially also on the size of individual targets as well as the space and time dependence of the distribution of the targets in the search space.

One complexity in the search by MT for a kinetochore is that none of the kinetochores remains static during the search process; thus, the problem is that of hitting a tiny mobile target that changes position stochastically. An additional complexity is that the search space is populated by more than one kinetochore, and therefore, there is a possibility of erroneous attachment with a nontarget kinetochore, thereby leading to a potential mitotic error. Most of these complexities do not arise in the context of MT-IS attachment because, barring a few exceptional situations (9), the T cell forms a single unique and practically immobile IS with the APC. Therefore, compared to the models of MT-kinetochore attachment formation, a simpler model of MT-IS can provide a reliable estimate of the mean time for the formation of the attachment.

For simplicity, the authors of (1) model the T cell and the nucleus as two concentric rigid spheres. The centrosome is located at a fixed position in the annular space between the two spheres on the upper hemisphere along the axis passing through the two poles. The target is represented by the center of a circular ring, which represents the IS, on the cell surface, and its position is specified by the corresponding polar and azimuthal angles. Over a range of the polar angles, the target IS is screened by the nucleus; the larger the size of the nucleus, the stronger is the screening. An IS that is screened by the nucleus remains accessible only by the MTs that glide along the cortex, bypassing the nucleus.

To speed up the search process, in some systems, specific cues guide the search; these cues could be chemical or physical in nature (2). However, because, at present, no experimental evidence in favor of any cue has been reported in the context of a search for IS by MT, only shooting MTs from the centrosome randomly in all possible directions and the (de-)polymerization kinetics of individual MTs constitute the search strategy in this model. The authors report the dependence of the MFPT on 1) the angular position of the IS on the cell surface, 2) the size of the IS, 3) the difference in the sizes of the cell and the nucleus, 4) the mean length of a MT (that, in turn, depends on the four rates of the polymerization kinetics of an MT), and 5) the number of MTs.

The most notable result of this article is that when the target is located at the south pole, the corresponding mean search time is shorter compared to that when the target is located near the equator. This may appear counterintuitive at first sight because it implies that shorter time is needed to hit a target located farther from the centrosome. However, this is a consequence of the fact that all MTs gliding along the cell cortex reach the south pole, irrespective of the initial direction, if growth persists for a sufficiently long time. In contrast, for all other locations of the IS, the probability of reaching the target even ballistically depends on the ratio of the radius of the IS and that of the T cell.

SAC mechanism is not restricted only to the formation of cytoskeletal structures in which searching MTs are captured by a kinetochore or the cell cortex. A similar SAC mechanism is involved also in the search for specific binding sites on the nucleic acid strands by proteins in key genomic processes like transcription and translation (10). One common feature of the attempts of a search exploring different regions or different directions in the search space is that the searcher "resets" its state and resumes the search. If an MT is the searcher, its complete disappearance by depolymerization resets the search by the fresh nucleation of an MT that grows, and explores, a different direction for the target. In the context of a search on a nucleic acid, a complete detachment of a searching protein from the nucleic acid strand, followed by diffusion in the surrounding medium and the subsequent reattachment with the strand, resets the search process.

It will be interesting to extend the model of (1) in the future by including 1) the effects of static and dynamic protein crowding (11,12) as well as a cross talk between the MT and actin mesh and 2) force generation by the MTs, leading to the repositioning of the centrosome closer to the IS (7).

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