The effect of dynamic instability of microtubules on growth cone dynamics

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Microtubules (MTs) play an important role in growth cone dynamics and neurite outgrowth. To study the effect of the dynamic instability of MTs, we constructed a Monte Carlo model of MT dynamics in a closed compartment, where MT (de)polymerization changes the free tubulin concentration in the medium. We show that (i) the transition times between MT elongation and shrinkage are non-exponentially distributed, which has previously been attributed to MT "memory"; and (ii) MTs are more dynamic than in a medium with a constant tubulin concentration. These features are important for the explorative behavior of growth cones.

The microtubule cytoskeleton is an important structural component of neurites and plays a crucial role in neurite outgrowth. Elongation (retraction) of neurites is dependent on tubulin assembly (disassembly). Tubulin assembly and disassembly mainly take place in the growth cone, the most distal part of an outgrowing neurite. The effect of dynamic instability of microtubules (i.e., the irregular switching between microtubule polymerization and depolymerization) on growth cone dynamics, however, is unclear.

So far, microtubule dynamics has been modelled assuming a fixed free tubulin concentration, which corresponds to a situation in which the medium, or the concentration of free tubulin in it, is large. In a growth cone this may not be the case, since its volume is small, and the amount of tubulin available for microtubule polymerization is probably also small. Therefore, the fluctuations in free tubulin concentration could have a significant effect on the microtubule dynamics in the growth cone.

To investigate this, we created a Monte Carlo model of the dynamics of one microtubule (MT) in a closed compartment, so that assembly and disassembly of tubulin changes the free tubulin concentration in the medium. Every step of the simulation process consisted of making a list of possible events in the system, assigning the rates for these events, randomly picking one of them, and then executing it. The possible events were: (i) the association of a tubulin molecule to the MT and the consequent decrease in the free tubulin concentration in the compartment; (ii) the dissociation of a tubulin molecule from the MT and the consequent increase in the free tubulin concentration; and (iii) a catastrophe event (if the MT is in the elongation state) or a rescue event (if the MT is in the shrinking state). Every event was assigned a first order rate constant k_i (time^{-1}), using experimentally determined rates for in vitro MT growth, shrinking, catastrophe, rescue, and their dependencies upon free tubulin concentration. In accordance with the experimental data, we assumed that the association rate of a

tubulin molecule with the MT is dependent on the free tubulin concentration, whereas the dissociation rate is independent of the tubulin concentration. Kinetically, transitions of the MT from the elongation to the shrinking state and back are first-order events (the probability of transition is constant with constant free tubulin concentration). The transitions switch between two sets of association-dissociation constants so that the MT prefers tubulin association in the elongation state and dissociation in the shrinking state. We did not model the GDP-GTP tubulin conversion dynamics and assumed that this process happens very fast, so that it does not influence the MT dynamics. For each event, the random implementation time $t_i = -(1/k_i)logk_i$ was calculated, and the event with the smallest time t_i was selected (the event with a faster rate had a higher chance to be selected) and executed. Then the total simulation time was extended and the cycle repeated again. Thus, the total simulation consisted of alternating association, dissociation, catastrophe or rescue events.

We show that the transition times of MTs in a closed compartment are non-exponentially distributed. The experimental data for both in vitro and in vivo MTs have been shown to fit gamma distributions. Getting this distribution was attributed to several consecutive events for transition to happen or some other kind of structural MT "memory" that remembers the time that MTs have already spent in a growing or shrinking phase. However, our results show that MTs in a closed compartment can have non-exponential transition times without such requirements. In our model, the memory required for getting such a distribution is achieved by changes in free tubulin (or available free tubulin) concentration in the medium.

A gamma distribution means that very short and very long transition times are hardly possible for MTs in the growth cone and, consequently, for saltatory neurite outgrowth. This feature could be very important for the explorative behavior shown both by MTs inside the cell and by growth cones in neural tissue. We show that mean elongation time and mean shrinking time of MTs are smaller (that is, catastrophe and rescue rates are higher; in other words, the MTs are more dynamic) in a closed compartment than in a medium with a constant tubulin concentration (with the same concentration as the average tubulin concentration in the closed compartment). This shows the possibility of modulating the dynamics of MTs in closed compartments not only by MT associated proteins but also by decreasing or increasing the amount of available free tubulin. To better compare the dynamics of MTs with the dynamics of neurite outgrowth, we are now simulating a population of MTs in a closed compartment. In addition, we are investigating the effects of MT associated proteins and motor proteins on MT dynamics and neurite outgrowth.