New and Notable

Motor Mutants bring Wild-Type Motors to a Halt Stochastically

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Characterizing the collective functions of cytoskeletal motors has become increasingly important to elucidating mechanisms governing intracellular transport processes in eukaryotic cells (1,2). A variety of organelles, vesicles, and signaling complexes are known to be transported by collections of interacting actin and microtubule-dependent motors. Cooperation among similar types of motors may be important for transporting cargos past specific physical barriers in the cytoplasm requiring the production of large forces. Numerous intracellular transport processes and regulatory mechanisms also depend fundamentally on how collections of dissimilar motors either cooperate or compete antagonistically (1). For example, cargos are often outfitted with multiple copies of oppositely directed kinesin and dynein motors and exhibit saltatory or bidirectional motions. Such behavior appears to play important roles in processes that control the spatial distribution of motor cargos in the cytoplasm. Similar competitions between kinesin, dynein, and myosin motors can influence the trafficking responses at actin and microtubule filament junctions (3), and, hence, the partitioning of cargos at different locations within cells, especially neurons (4).

Evaluations of collective motor behaviors are also important to characterizing various disease pathologies stemming from motor and other transport-related protein mutations. The impact of these mutations on cargo transport will ultimately depend on the extent to which they perturb the composite dynamics of motor systems that also contain groups of wild-type motors. Such behavior is explored in an exciting new study by Scharrel et al. (5) in this issue of *Biophysical Journal* that examines how distributions of microtubule filament gliding velocities are influenced by the ratio of fully functional, wild-type, and dysfunctional mutant kinesins immobilized on a stiff cover slip substrate. The inactive mutants contained a point mutation within kinesin's catalytic domain that is known to interfere with ATP binding. This mutation also renders the motors inactive and causes them to associate with microtubules in a strongly bound rigor-like state. Microtubules naturally glide at fast velocities when the ratio of active to inactive motors was high, and gliding motions were largely arrested when the inactive mutants were the dominant species on the support surface. Yet, they also observed novel salutatory motions at an intermediate wild-type/mutant ratio regime where the filaments appeared to switch stochastically between fast and slow gliding modes. Moreover, velocity distributions were bimodal in this regime and exhibited distinct coexistent peaks at high and low velocities. Thus, the transport behavior was somewhat of an all-or-nothing response, where collections of either the full active or inactive motors dominated filament motion.

The coexistence of fast and slow velocities is also reproduced in Monte Carlo simulations and by a mean-field theoretical model. These analyses predict that the motor system will only have two stable steady state configurations: a stalled and a fast state, but only at specific ratios of inactive and active motors. Spontaneous fluctuations in motor stepping, binding, and detachment cause the system to switch between these two states (Fig. 1). States where the system moves with intermediate velocities are unstable and short-lived and, hence, do not influence gliding velocity distributions.

Analogous behavior has been predicted by Müller et al. for two teams of opposing motors (6). In this case, cargos can switch their transport direction spontaneously via a mechanism involving motor unbinding reaction cascades, a process where most or all of the motors of a single motor type detach in rapid succession, leaving their opposing motor team largely unobstructed. Such behavior is fostered by the strong nonlinear force dependence of filament detachment rates and the fact that the force (or strain) experienced by motors on the detaching team will continually increase as their partners release from the filament. Again, spontaneous fluctuations in the system can shift force distributions within the system in either direction, resulting in stochastic switching between different cargo transport directions.

Recognition of such behaviors is important to interpretations of various collective motor behaviors. For example, prior to the predictions of Müller et al., physical mechanical competitions between motors were expected to produce "tug-of-war" like behaviors where cargos move with a range of velocities in either direction, stalled at times, and switched their transport directions slowly. Rapid switching of transport directions and velocities were presumed to indicate that the activities of the motors are somehow coordinated, potentially by a biochemical factor. Yet, these predictions show that unbinding reaction cascades can be sufficient to explain fast transitions between plus- and minus-end directed motion and pausing.

It is important to note that the coexistence of high and low velocity modes was not observed in previous experiments that examine how motors with
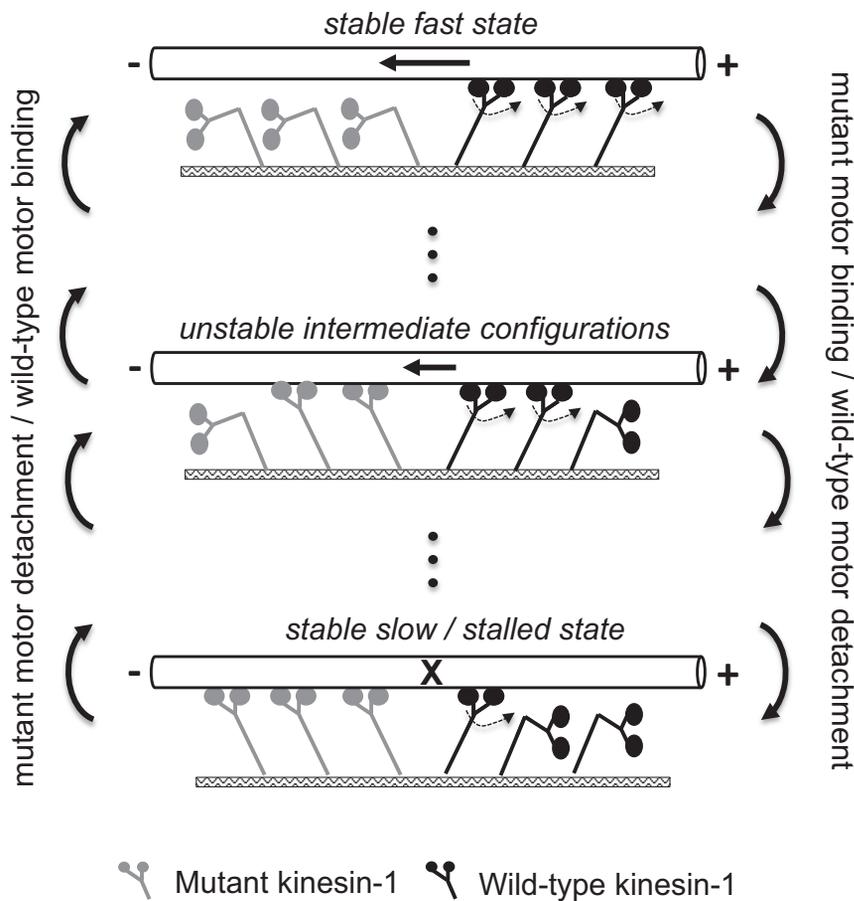
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FIGURE 1 The emergence of distinct stable states/configurations when dissimilar cytoskeletal motors function collectively. While the binding of inactive mutant motors can slow the gliding motions of filaments driven by wild-type motors, many motor bound configurations of the system will be kinetically unstable in such circumstances. Consequently, processes such as motor unbinding reaction cascades can cause the entire system to transition between two different steady states. For the case of rigid wild-type and inactive mutant kinesins, microtubules are found to transition between a largely unobstructed or fast motile state and a stalled state.

different levels activities functioned collectively. A similar study by Larson et al. found that filament gliding velocities of wild-type kinesin dropped rapidly as the fraction of slow, but not inactive, mutant kinesins increased (7). Coexistence of velocity states was not observed in this case. Pan et al. examined interactions between kinesin-2 and OSM-3, a slow nonprocessive kinesin with low ATPase activity (8). Consistent with a more standard model of mechanical competition, OSM-3 was found to attenuate the velocity of the kinesin-2 motors, and these motor systems were able to move with intermediate velocities. Similarly diverse behaviors have been reported for collections of oppositely-directed motors. There are cases where the bidirectional transport behaviors of cargos appear to be consistent with the stochastic tug-of-war mechanism (9), whereas others are reflective of more traditional notions of tugs-of-war competitions, as is the case when the filament affinity of one type of motor in a system far outstrips that of their opposing motors (10). Specific coordination among dissimilar motors has also been reported (11). Collective motor mechanics can therefore be very system dependent, and all options should generally be considered when evaluating collective behaviors.

Scharrel and coworkers also emphasize that their ability to model the coexistence of distinct velocity states requires assumptions of force balance and an equal sharing of loads among the motors responsible for driving filament motion. This treatment is likely appropriate for their motor systems, because both its inactive and active motors were very short and rigid (1 pN/ nm) truncated versions of kinesin-1. These properties could, for example, restrict the number of bound configurations the motor system is capable of adopting, and, therefore, the probability that its motors will associate in configurations where forces are distributed unequally. Inter-motor tension will also change rapidly as the bound configuration of the system changes due to motor stepping, binding, and detachment, which could help to support processes like unbinding reaction cascades by making certain states less stable. Full-length kinesins are much longer, and their linkages to cargos are generally expected to be much more compliant, at least at low to moderate extension distances. Optical trapping analyses of collective motor force production have shown that compliant motor systems spend significant portions of time in states where loads are distributed unevenly (12,13). The time required for motor systems to relax to steady states can also be quite large in these circumstances (14). It is therefore possible that the coexistence of slow and fast velocities due to the predominance of select states would be washed out by such effects.

Despite these potential caveats and the need for additional studies, the new observation that mixtures of impaired and normal motors will move via distinct stable states as opposed to a spectrum of states is an important addition to the growing list of emergent collective motor behaviors that already includes that stochastic switching of cargo transport directions (6,9) and the generation of spontaneous mechanical oscillations (15). This work also further highlights the importance of merging theory with experiment to dissect the highly rich and complex behaviors collections of

interacting motors are capable of producing.

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