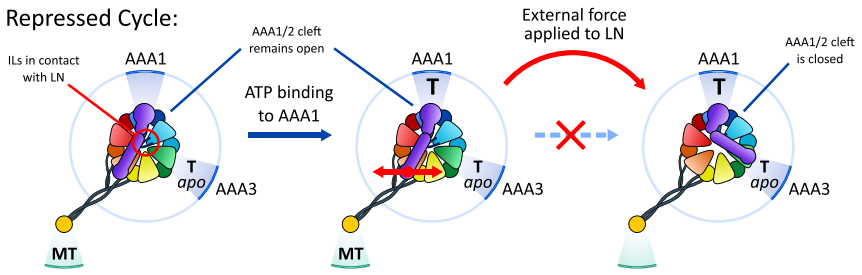

Figure 10. The Repressed Mechanochemical Reaction Cycle in Dynein

Dynein is in the MT-bound *apo* state. However, unlike in the normal cycle, the AAA3 site is either unoccupied or occupied by ATP. The ILs of AAA2 are in close contact with the LN domain (red circle in the bottom left figure). In this state the MD remains tightly bound to the MT, even after ATP binds to the AAA1 site. The AAA1/2 cleft remains partially open regardless of ATP binding. However, if an external force is applied at the N terminus of the LN domain, the MD domain can detach from the MT and complete the priming stroke.

post-power stroke state (see Figure S5). Our findings are supported by the observation that when a series of mutations is introduced to the ILs, the LN assumes an intermediate conformation between the pre- and post-power stroke states, just as we observe (Kon et al., 2012).

ILs in Dynein Motility

The importance of AAA2 ILs for dynein function is appreciated (Bhabha et al., 2014; Carter et al., 2011; Kon et al., 2012). The precise mechanisms, however, used by the ILs to regulate motor activity have not been fully resolved. Our simulations present a picture of the possible ways in which the AAA2 domain is involved in allosteric communication in the MD. We surmise that there are three important roles of the AAA2 IL: (1) stabilizing the LN-AAA5 interactions in the *apo* state; (2) preventing the closure of the AAA1/2 cleft in the repressed state; (3) serving as an external force sensor in the repressed state (see below).

Persistence of LN-AAA2 Interactions in the Repressed State

The LN-AAA2 interactions also play an important role in the repression of the allosteric pathway along the AAA1/2 and AAA5/6/S domains by the AAA3 unit. While it is clear that the conformational changes in the AAA3 domain are involved in the repression of dynein activity, the dynamical manner in which they do so is not clear because the changes are subtle (Bhabha et al., 2014). Our simulations reveal that AAA3 unit suppresses the detachment from MT by over-stabilizing the interactions between the LN and the ILs in AAA2 (see Figure S8). In the crystal structure of the repressed state, the AAA3 and AAA4 domains are slightly shifted relative to one another, compared with the other structures. This shift is sufficient to bring the AAA2 ILs closer to the LN. In addition to stabilizing the LN-AAA2 interactions, this shift may directly or indirectly destabilize the AAA1 and AAA2 interactions in the AAA1/2 cleft. As a result, the cleft is prevented from closing, even when ATP is bound at the AAA1/2-binding pocket. As shown in Figure 6, the LN and AAA2 ILs do come into contact regardless of the nucleotide state of the AAA3 domain. However, these interactions by themselves are not sufficient to prevent conformational changes in the AAA1/2 domains without contribution from the AAA3 domain. This can be seen from our simulations of the ATP-bound dynein in the non-repressed state (Figures 4A and 6B), in which the AAA1/2 cleft is able to close despite the LN interactions with the ILs.

Mechanical Forces Rescue the Motility of Dynein in the Repressed State

Studies show that even when ATP is bound to the AAA3, if a strong enough external mechanical force is applied at the N terminus of the LN, dynein resumes normal activity (Nicholas et al., 2015). The results of our simulations, which provide a molecular picture of communication between AAA1 and ATP-bound AAA3 needed for stepping, are consistent with experiments. We find that pulling on the LN does not immediately destabilize the interactions of the LN with the AAA2 domain (Figures 9A and 9B). Instead, breaking the contacts between the LN and the AAA5 domain is sufficient to reverse the effects of the AAA3 when dynein is in the repressed state (Figures 9C and 9D). This implies that the combination of LN interactions with both the AAA2 and AAA5 domains as well as the stabilization of these interactions by the ATP-bound AAA3 domain are required for repression. It is sufficient to disrupt interactions associated with one of these three elements to allow dynein to undergo the priming stroke and detach from MT. As we mentioned earlier, previous studies have examined mutations in AAA2 ILs (Bhabha et al., 2014; Kon et al., 2012). However, these studies focused on the effects of mutations on the ATPase activity and LN conformation but did not measure their effect on the affinity of dynein for MT. We propose that force-dependent unbinding experiments, such as those reported recently (Nicholas et al., 2015), be performed with mutations of the AAA2 IL to test our predictions regarding the role of the AAA2 IL in the repression mechanism.

Effect of Mechanical Force and Implications for Gating

In the context of gating, it is worth pointing out that, in our simulations, pulling in both the negative and positive directions along the MT axis had approximately the same effect. This implies that the AAA3 repression system does not work as a gating mechanism in the classical sense of creating an asymmetry between the leading and trailing MDs. Instead, it prevents dynein detachment from the MT in the absence of external strain, indicating that the repressed state, in addition to a potential regulation mechanism, may play an important in the context of processivity of dynein (Bhabha et al., 2014).

Since we know that dynein does respond asymmetrically to an external load, it might seem that our findings may not be correct (Cleary et al., 2014). This is, however, not necessarily the case as there are other possible reasons for the asymmetric response. One reason could be that, when dynein binds to MT, it does so at an angle (Redwine et al., 2012; Ueno et al., 2008). This, by itself, could be sufficient to elicit an asymmetric response to force,