

Tyrosines in the Kinesin-5 head domain are necessary for phosphorylation by Wee1 and for mitotic spindle integrity

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Mitotic spindle assembly and maintenance relies on Kinesin-5 motors that act as bipolar homotetramers to cross-link microtubules [1-5]. Kinesin-5 motors have been subject to extensive structure-function analysis [5], but the regulation of their activity in the context of mitotic progression remains less well understood [2]. We report that *Drosophila* Kinesin-5 (KLP61F) is regulated by *Drosophila* Wee1 (dWee1). Wee1 tyrosine kinases are known to regulate mitotic entry via inhibitory phosphorylation on Cdk1 [6-10]. Recently, we showed that dWee1 also plays a role in mitotic spindle positioning through γ -tubulin and spindle fidelity through an unknown mechanism [11]. Here we investigated whether a KLP61F-dWee1 interaction could explain the latter role for dWee1. We found that dWee1 phosphorylates KLP61F *in vitro* on three tyrosines within the head domain, the catalytic region that mediates movement along microtubules. *In vivo*, KLP61F with tyrosine-to-phenylalanine mutations fails to complement a *klp61f* mutant and dominantly induces spindle defects similar to ones seen in *dwee1* mutants. We propose that phosphorylation of the KLP61F catalytic domain by dWee1 is important for the motor's function. This study identifies a second substrate for a Wee1 kinase and provides evidence for phospho-regulation of a kinesin in the head domain.