DESCRIPTION OF MODEL FOR NUP58 PROTEIN LOCALIZATION AND ITS FUNCTION DURING CYTOKINESIS

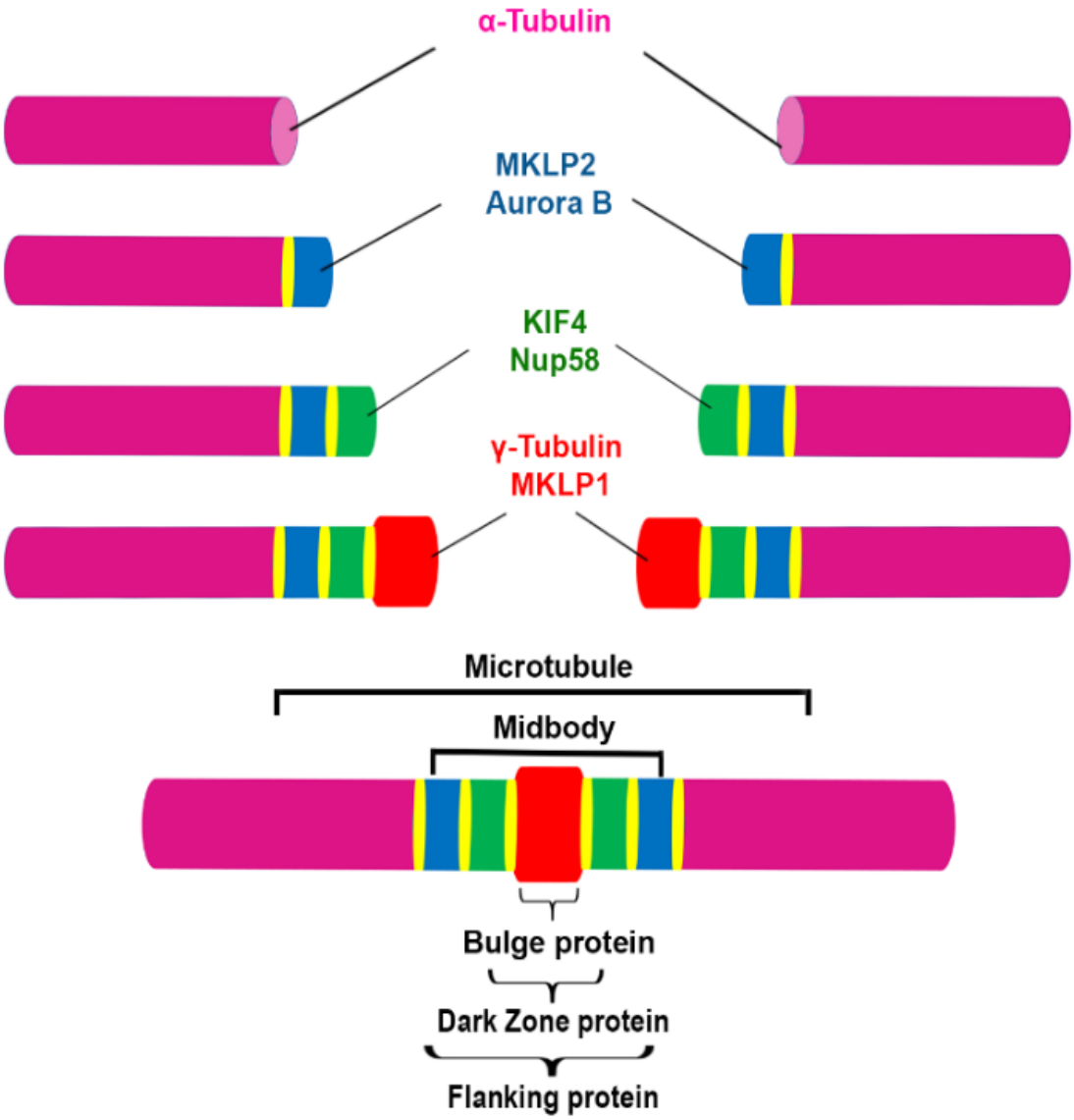

Figure 1. Speculative model for Nup58 protein localization in midbody structures based on deconvoluted confocal and STED nanoscopy images. Nup58 protein is localized at the dark zone of a midbody.

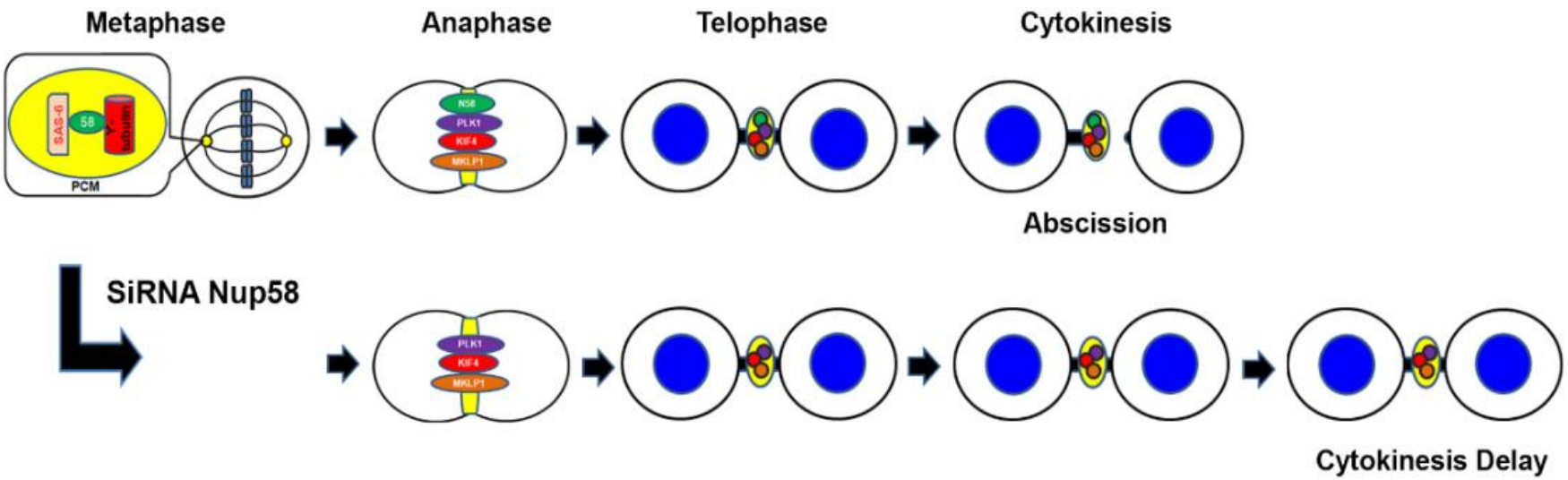

Figure 2. Speculative working model for Nup58 protein during mitotic progression and cytokinesis. Absence of Nup58 protein during cytokinesis caused delay from midbody maturation to final abscission.

Nucleoporin p58 (Nup58) is a protein that in humans is encoded by the *NUPL1* gene. This gene encodes a member of the nucleoporin family that shares 87% sequence identity with rat nucleoporin p58. The protein is localized to the nuclear rim and is a component of the nuclear pore complex (NPC). All molecules entering or leaving the nucleus either diffuse through or are actively transported by the NPC. Alternate transcriptional splice variants, encoding different isoforms, have been characterized (1).

In recent years, it has been revealed that nucleoporins play various alternative roles unrelated to nuclear transport (2, 3, 4), including mitotic roles (5-8). In the present study, we found evidence for an additional role of Nup58 protein, a nucleoporin member that form nucleoporin 62 complex, in nuclear pore biogenesis (9,10). It has unexpected roles during mitosis and cytokinesis; namely, temporal regulation of telophase, cytokinesis, and abscission. Using immunofluorescence assay, live cell imaging and STED nanoscopy, we discovered that Nup58 transiently localizes to the centrosomes and the midbody. In addition, it is particularly enriched near the midbody core and around dark zone/flanking regions, in patterns distinct from other nucleoporins (10). We propose a model of Nup58 protein localization in midbody among other protein markers (**Figure 1**).

During mitosis, we found that Nup58 protein gradually accumulated into spindle-like structures and colocalized with α-tubulin, a protein which plays critical roles during chromosome segregation. Nup58 protein was also detectable at mitotic spindle poles or centrosomal regions during prophase to anaphase and colocalized with some centrosome marker proteins such as γ-tubulin and SAS-6 (**Figure 1**).

Immunoblotting of anti-Nup58 protein immunoprecipitates of mitotic cells demonstrated coprecipitating γ-tubulin and SAS-6 but not the protein ninein. Figure 1 suggested that Nup58 protein physically interacts with γ-tubulin and SAS-6 at the spindle poles/centrosomes during mitosis. Consistently, our result showed that depletion of Nup58 gene also reduce the expression of SAS-6 (10).

Some studies previously reported that SAS-6 protein play very important roles during centriole duplication or biogenesis (11), therefore depletion of SAS-6 protein lead to block centriole duplication whereas overexpression causes centriole amplification. Based on our IP data and the fact that depletion of Nup58 lead to increase of centrosome abnormalities especially monopolar spindle, we hypothesize that in complex with SAS-6, Nup58 protein might have contributed to centrosome duplication or segregation (10).

The reduction of SAS-6 protein level after Nup58 depletion although the mRNA level of SAS-6 was not significantly reduced, lead us to assume that Nup58 does not affect SAS-6 gene expression but only is required to prevent SAS-6 from proteosomal degradation (10). Similarly, in the previous study Dewi *et al* (2018) found that the protein level of nucleoporin TPR was reduced after GSK3β inhibition although q-RT PCR analysis showed that mRNA level of TPR was not significantly changes following GSK3β inhibition (12). They proposed that GSK3β is required to prevent TPR from such proteosomal degradation.

Another attractive option is that Nup58 protein stabilizes microtubules in a specific arrangement to enable the formation of dark zone and/or flanking regions

with other midbody proteins (such as KIF4, PLK1, or ESCRT). In a previous study, we reported that Nup62 localized on the mitotic spindles and centrosomes during cell division (20). Consistent with this, it has been reported that the centrosome play roles in abscission and a number of centrosomal proteins localize to the midbody ring. Indeed, Nup62 protein also transiently localizes to midbody ring at the end of abscission, interacting with filamentous actin-capping protein CapG (23). This study is consistent with our result which has been shown in **Figure 1.**

In our study (**Figure 2**), we also revealed that Nup58 transiently localized to midbody during cytokinesis (10). The midbody, initially described by Walther Flemming in the 19th century, forms from the midzone—a bipolar microtubule array that assembles between separating sister chromatids during anaphase (13). Recently, it has been clear that the midbody helps as a polarity cue during spindle orientation, asymmetric cell division, and cell polarization by orchestrating vesicular transport, cytoskeletal organization, and localized cortical cues (13-16). Indeed, during cytokinesis, the cellular cortex changes into a cleavage furrow due to actomyosin ring contraction and then, the midbody which is a platform for the gathering of the abscission apparatus that regulates the final separation of daughter cells is constructed (17).

Although the overall program of cytokinesis is well-documented, many questions remain, particularly at the molecular level, in part because of the high spatiotemporal complexity of cytokinesis joined to the condition for significant force cohort during cleavage (17-19). We demonstrate here in **figure 2** that Nup58 depletion induced increased disorder in central spindle microtubules and changed

midbody widths. This may arise from the role of Nup58 protein in bundling and anchoring microtubules at the center of the midzone (19). Our finding that Nup58 depletion induces cells to be stuck in abscission for hours also raises the idea that Nup58 protein may contribute effectual abscission during cytokinesis (**Figure 2**) (10).

Previously, Hashizume *et al* reported that Nup62 protein knockdown induced significantly higher numbers of multipolar spindles compared with controls (20). In contrast, our present study revealed that Nup58 protein depletion primarily enhanced monopolar spindle formation. Our live-cell imaging also revealed that Nup58-depleted monopolar spindle cells induce mitotic catastrophe, aneuploidy, and eventually cell death; as these delayed processes (~300 min) happened before cytokinesis, midbody formations were often not found (Figure 2)(10). In mammals, aneuploidy has been linked to cancer progression, which, like cancer development, is a complex process involving functional and genetic abnormalities (21). Moreover, Nup58 protein aids metastasis and EMT in lung cancer (22).

Assuming that such a Nup58-Nup62-Nup54 protein subcomplex is required for correct progression of cytokinesis, then a missing partner might dissolve the complex and result in cytokinetic defects, as seen in the Nup58 depletion (**Figure 2**). In this perspective, we can also postulate that Nup54 protein may also localize at the centrosomes or the midbodies during mitosis. Several organelles, such as the mitotic spindle, the centrosome and the midbody, use microtubules as a structural constituent. Interactions between the microtubule-dependent and actin cytoskeleton organelles can also be categorized regulatory or structurally (23).

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