Adopting the Orphan: Determining the Role of the Motor Protein KIF9 During the Cell Cycle

By

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This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Nicholas J. Quintyne, and has been approved by the members of his supervisory committee. It was submitted to the faculty of the Honors College and was accepted in partial fulfillment of the requirements for the degree of Bachelor of Arts in Liberal Arts and Sciences.

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Abstract

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Institution: Wilkes Honors College of Florida Atlantic University

Thesis Advisor: Dr. Nicholas J. Quintyne

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The kinesin superfamily of microtubule motor proteins is subdivided into families based upon structure and function. KIF9 is the founding member of the Kinesin-9 family, which is a largely uncharacterized group of kinesins. It was originally identified by sequence homology to other kinesins. Subsequent studies have shown that KIF9 interacts with proteins involved in cell shape remodeling, cell migration and proper centrosomal positioning. We have examined KIF9 function in mammalian cells using shRNA-mediated knockdown and GFP-plasmid overexpression. By knocking down KIF9 expression in these cells, we have seen several effects on normal cell cycle progression. Using various cell cycle markers, we have observed an increase in the number of cells in late S phase. Conversely, by overexpressing KIF9 we notice a decrease in the number of cells in late S phase. In addition, there is a marked increase in the number of cells in early mitosis in unexpected time intervals. We propose that KIF9 is required for proper cell cycle progression, via a potentially novel checkpoint mechanism.

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Introduction

The cytoskeleton is an integral component of the cell. A number of significant functions of the cell rely on a functional cytoskeleton in order to perform numerous functions, such as maintenance of cellular morphology and polarity, cell motility, organelle transport and mitosis (reviewed in de Souza, 2012). The complexity and diversity of these functions require that the cytoskeleton remain a dynamic component of the cell, responding to various physiological and environmental changes in its environment (reviewed in Löwe and Amos, 2008). The cytoskeleton consists of three discrete fibrous structures: actin microfilaments, microtubules and intermediate filaments.

Intermediate filaments (IFs) were named because of their intermediate size (approximately 10 nm) as compared to the two other cytoskeletal structures. IFs consist of a large protein family that includes approximately 70 unique gene products and are divided into six types (types I-VI) based on similarities in amino acid sequence and protein structure. IFs can be found in monomeric forms, but are more frequently found in dimeric and tetrameric forms, where they are generally found in characteristic combinations of the different types of IFs. Historically, IFs have been fully documented in their roles in providing structural and mechanical support to the cell, but contemporary research has implicated IFs in multiple cellular functions, including signaling, apoptosis and migration (Eriksson et al., 2009; Herrmann et al., 2009; Liem and Messing, 2009; Omary , 2009; Hertel, 2011).

Actin microfilaments (F-actin) are comprised of globular actin subunits (G-actin), as well as several associated proteins. Depending on the form in which it is found, G-actin or F-actin, the

actin cytoskeleton is involved in a number of diverse cellular functions ranging from maintenance and reorganization of cellular morphology, chemotaxis, cell motility, cell polarity and signal transduction. Of particular biophysical importance, F-actin is the scaffold on which myosin proteins generate force to support muscle contraction (Valderrama et al., 2001; Xiao and Yang; 2007; Van den Broeke and Favoreel, 2011).

Microtubules (MTs) are long, hollow cylinders composed of polymerized tubulin heterodimers. The heterodimers, which are themselves composed of noncovalently bound α-tubulin and β-tubulin subunits, polymerize end to end to form protofilaments. Thirteen protofilaments then associate laterally to form a single MT. This deceptively rigid structure can easily be elongated (a process known as rescue) or shortened (catastrophe) by the respective accumulation or elimination of additional protofilaments. These processes are facilitated by the particular nucleoside expression found at the dynamic ends of the MTs, with GDP β-tubulin prone to catastrophe (or depolymerization) and GTP β-tubulin associated with rescue (polymerization). This overall process is known as dynamic instability and allows for the MT cytoskeleton to reorganize and to relocate quickly to where it is needed throughout the cell. In general, MTs have an essential role in mitosis where they form the mitotic spindles involved in the search and capture process of metaphase. Most relevant to my research involves their role in intercellular transport. Because of the unique α-β tubulin composition of the MTs, it is possible for us to determine distinct areas of the MTs. Within interphase cells, the minus end is defined as the location in which the MTs nucleate and is associated with the centrosome (also referred to as the microtubule organizing center or MTOC), while the plus end extends outward from the MTOC and refers to the cell periphery. This polarization allows for the retrograde (toward the minus end) and anterograde (toward the plus end) transport of associated motor proteins along

the lengths of the microtubules (reviewed in Mandelkow and Mandelkow, 1994; reviewed in Winsor and Schiebel, 1997; reviewed in Wade, 2009).

There are two main classes of MT associated motor proteins: dyneins and kinesins (Figure 1). Both of these convert the chemical energy contained within ATP to mechanical movement (Berg et al., 2002). The traditionally minus end directed dynein has been implicated in a number of intracellular processes, such as mitotic chromosome movements, nuclear positioning, retrograde vesicular transport and perinuclear localization of the Golgi complex (Paschal and Vallee, 1987; Hyman and Mitchison, 1991; Corthésy-Theulaz et al., 1992; McGrail et al., 1995). Dynein associates with the multi-subunit protein, dynactin, which aids in its vesicular transport efficiency (Schroer and Sheetz, 1991).

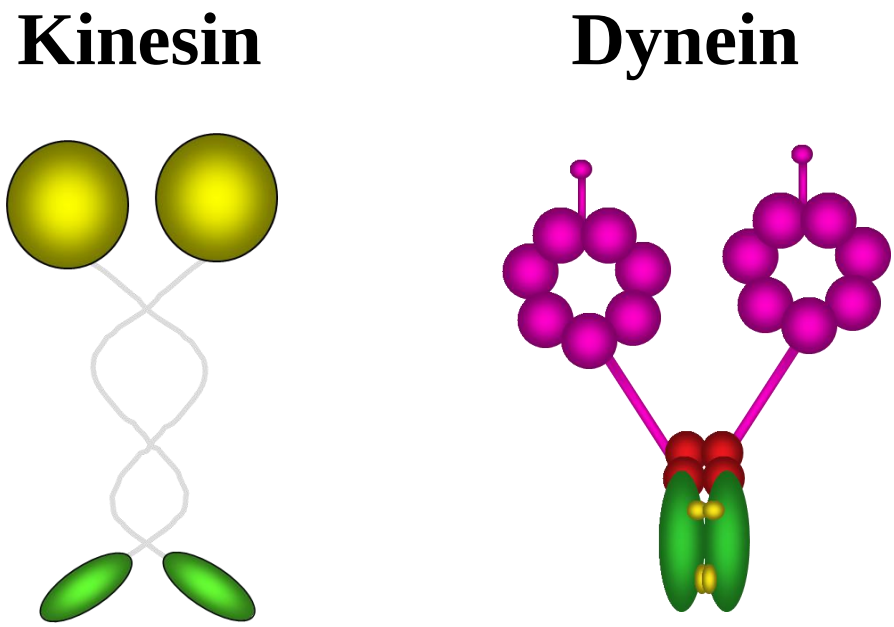

Figure 1: Stylized schematics of kinesin and dynein motor proteins (Courtesy of N. Quintyne)

The kinesin superfamily of motor proteins is divided into approximately 14 families (depending on the system of nomenclatures being utilized). The 45 distinct kinesin-like family proteins (KIFs), which constitute the kinesin superfamily, are united by a highly conserved ATPase motor domain, referred to as the head (Miki et al., 2001; Demonchy et al., 2009). Unlike

dynein, which is traditionally minus end directed, kinesin constitutes a bidirectional superfamily of proteins. Historically, kinesin was classified as the characteristic plus end directed motor protein, but the discovery of the minus end directed kinesin family (with nonclaret disjunctional, *ncd*, as the founding member), quickly challenged the traditionally accepted function of kinesin's head as more complex (reviewed in Woehlke and Schliwa, 2000). The motor domain, or head, consists of two heavy chains, which utilize the intrinsic polarization of the MT to associate consecutively as highlighted in Figure 2. Initially one of the heavy chains binds to the MT and then the next one binds, resulting in a highly processive motor protein, as there is always at least one chain bound. The motion of the motor protein is a direct result of a conformational change in the protein that occurs upon hydrolysis of the associated ATP molecule to ADP. Figure 2 shows that this results in an overall hand-over-hand movement across the MT (reviewed in Woehlke and Schliwa, 2000; Berg et al., 2002).

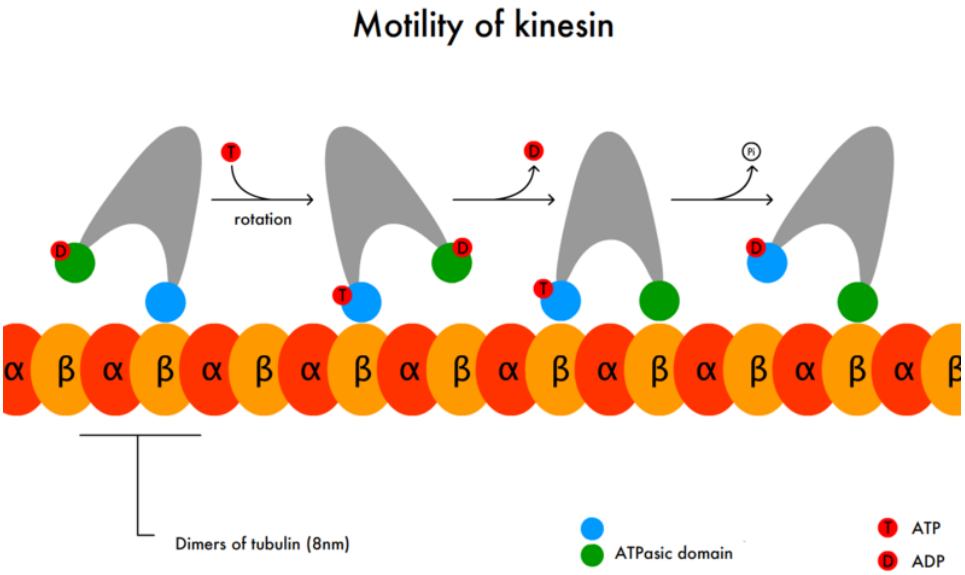

Figure 2: Mechanism of kinesin motion (Brian Jordan, San Diego Biotechnology Connection)

A typical kinesin consists of the head, a central stalk and a tail responsible for binding to different cargo (refer to Figure 1). The position of the motor protein varies, however, and can be found on either the NH_2 terminus (N-kinesins), the COOH terminus (C-kinesins), or somewhere in the middle (M-kinesins). The position of the motor domain is correlated with the directionality of the protein, with minus end directed kinesins being predominantly C-kinesins (Woehlke and Schliwa, 2000). It is the variable cargo binding domain that confers the specific identities to the individual kinesins. They have been implicated in a number of different cellular processes, including multidirectional transport of cargo proteins, ciliar and flagellar motility and chromosomal and spindle movements in mitosis and meiosis (Reviewed in Miki et al., 2001).

Based on these differences in morphology and function a number of standardized nomenclatures have been developed (Hirokawa, 1998; Miki et al., 2001; Lawrence et al., 2004; Wickstead and Gull, 2006). Based on the system developed by Miki et al. (2001) the kinesin superfamily is divided into 14 families. Figure 3 reveals that C-kinesins are classified into two classes (C-1 and C-2) and M-kinesins into a third (M), while the other 11 classes are associated with the more abundant N-kinesins (N-1 to N-11).

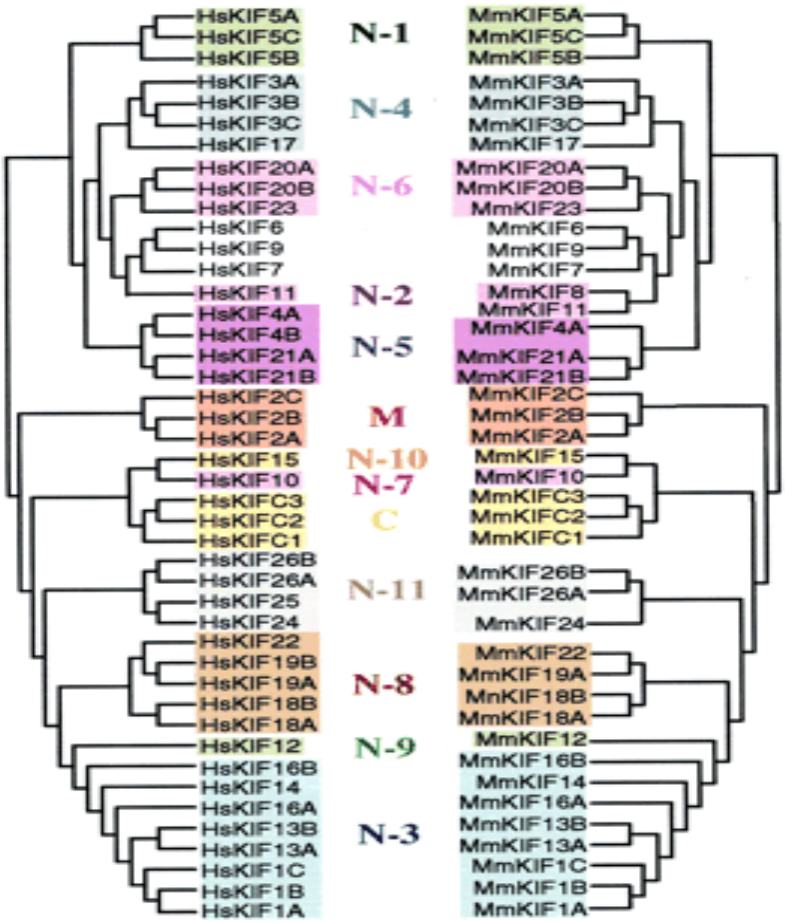

Figure 3: System of kinesin nomenclature (Miki et al., 2001)

Members of the N-2 kinesins are involved in bipolar spindle formation, members of the N-7 kinesins are involved in chromosome segregation and members of the M-kinesins are involved in MT destabilization. In Figure 3, three kinesins (KIF6, KIF7 and KIF9) constitute the orphan class of kinesins, which contain no counterparts in *Drosophila melanogaster* or *Caenorhabditis elegans*. According to the more recent systems of nomenclature (Miki et al., 2005; Wickstead and Gull, 2006), these kinesins are redistributed among sixteen different kinesin families. Specifically, KIF9 has been classified as the founding member of the Kinesin-9 family, which also includes Klp1 and KIF6 (Wickstead and Gull, 2006). The overall function of the members of the Kinesin-9 family are equivocal, as many of its member have yet to be fully defined. The association of Klp1 with the axonemal central pair (an integral component of the

flagella) of *Chlamydomonas* (green algae) suggests a role in ciliary and flagellar movement (Bernstein et al., 1994; Wickstead and Gull, 2006), while a Trp719Arg polymorphism in the KIF6 protein has been implicated as a predictor for risk of coronary heart disease (Li et al., 2010). In addition, the absence of the Kinesin-9 family member in higher plants strongly supports a function in cilia or flagella (Miki et al., 2005). Of the various Kinesin-9 family members, KIF9 in particular is poorly characterized and understood. So far, current research into Klp1 and KIF6 suggest only possible roles for KIF9 based on their morphological similarity, therefore it is important to perform further research on the functions of KIF9 in order to determine its uses within various organisms as well as the various roles of the Kinesin-9 family.

Demonchy and coworkers (2009) analyzed the functions of two Kinesin-9 subfamilies, KIF9A (which consists of Klp1 and KIF9) and KIF9B (which consists of KIF6) in *Trypanosoma brucei* (a protist with a single flagellum). Separate functions for KIF9A and KIF9B in the flagellum were identified via RNAi, suggesting roles in motility and paraflagellar rod formation, a component of the flagellum's axoneme (Demonchy et al., 2009). Piddini et al. (2001) first observed mammalian KIF9 while investigating Gem, a GTPase, implicating a function in MT and F-actin interaction, which is necessary for normal mitotic progression. Recent research by Cornfine et al. (2011) suggests a role in the regulation of podosomes, which are actin-based matrix contacts characterized by their ability to lyse extracellular matrix material. In addition, Kinesin-9 family members have been implicated in the proper positioning of the MTOC and timely mitotic entry in *Dictyostelium discoideum* (Tikhonenko et al., 2009).

Previous Honors College research has further investigated the role of mammalian KIF9. Initial research by Alsina and Billow suggests that KIF9 is involved in healthy mitotic progression, as they observed a noticeable delay in mitotic entry upon knockdown of KIF9 via

siRNA transfection. Alsina also implicated a possible role in mitotic spindle formation (2010). More importantly for my research, Alsina's work reported noticeable cell cycle irregularities in the cells treated with siRNA. Billow, who continued the research proposed by Alsina, reported defects in treated interphase cells, however, she went a step further and suggested that KIF9 is involved in the timely initiation and formation of the contractile ring, which is required for cytokinesis (Alsina, 2010; Billow, 2011).

My thesis project examines the roles of KIF9 with regards to the cell cycle as a whole. I manipulated KIF9 expression levels via knockdown and overexpression, in order to find its function. I utilized specific cell cycle markers, PCNA (which is involved in DNA replication and peaks in earl S phase) and Nek2 (which is involved in centrosome duplication and peaks in late S phase) to examine the effects of KIF9 knockdown and overexpression throughout the various stages of the cell cycle. I calculated the percentage of cells that were PCNA-nuclear positive and that expressed Nek2, where appropriate, in unsynchronized and synchronized cell populations, which effectively indicated in what stage of S phase the cells were. Cells were synchronized via a double thymidine block, which halts the cells at the G1/S boundary. We found that the percentage of cells that are PCNA-nuclear positive remains relatively constant when KIF9 is both knocked down and overexpressed. The percentage of cells that expressed Nek2 increased when KIF9 was knocked down, but decreased when KIF9 was overexpressed. This is highly suggestive of a role in a potentially novel cell cycle checkpoint mechanism that prevents the cell from proceeding into mitosis when activated.

Methods

Cell Culture: COS-7 cells (African Green monkey kidney fibroblasts, ATCC, Manassas, VA) grown in Dulbecco's Modified Eagle's Medium (Sigma Chemical Company, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS, Sigma) and 1% penicillin/streptomycin (MP Biomedicals, LLC, Solon, OH) were used in these experiments. The cells were kept at 37˚C in 5% CO_2. The cells were passaged whenever the individual plates approximated 80% confluency. Prior to passaging, the cells were washed with 1x phosphate buffered saline (PBS), treated with 2 mL of 0.05% trypsin-ethylenediaminetetraacetic acid (Trypsin-EDTA, Sigma) and subsequently incubated at 37˚C for 5 minutes. Finally, the cells were resuspended in 8 mL of media (to a total volume of 10 mL) and plated at a ratio of 1:4.

Antibodies: The following primary antibodies were used: mouse anti-PCNA (1:100 dilution, BD Transduction Laboratories, Franklin Lakes, NJ,), mouse anti-Nek2 (1:100 dilution, BD Transduction Laboratories), mouse anti-α-tubulin (1:200 dilution, Sigma), rabbit anti-γ-tubulin (1:500 dilution, Sigma). The following secondary antibodies were used: Texas-Red-X anti-mouse or anti-rabbit (1:250 dilution, Invitrogen, Carlsbad, CA) and Alexa488 conjugated goat anti-mouse or anti-rabbit (1:250 dilution, Invitrogen).

Lipid Transfections: 3 μL of FuGene 6 (Roche Diagnostics, Indianapolis, IN) was added to 100 μL of Opti-MEM (Sigma). The mixture was then incubated for 5 minutes at room temperature. 3 μL of each shRNA-plasmid was added to the transfection reagent mixture. Combinations of shRNA plasmids were used to knock down KIF9 expression from the set NM_02234 (Sigma). The anti-sense KIF9 shRNA sequences are:

5’-

CCGGCGCCAGTACCTTAAAGGACAACTCGAGTTGTCCTTTAAGGTACTGGCGTTTTT

G-3’

and

5’-

CCGGCCCAGTTAGAAGAAACGCTATCTCGAGATAGCGTTTCTTCTAACTGGGTTTTT

G-3’.

The plasmid vector pLKO.1-puro confers a resistance to puromycin, allowing for the stable selection for the presence of the shRNA plasmid after transfection. The resultant mixture was incubated for 15 minutes and 100 μL was subsequently added dropwise per coverslip. Finally, the cells were incubated for 72 hours.

Indirect Immunofluorescence: Fixed cells were stained with antibodies as described in Quintyne et al. (1999). Cells were grown on 22 mm^2 coverslips in six-well cell culture plates. The media within the wells was aspirated and then washed with PBS. After the PBS was aspirated, methanol was added and the coverslips were placed in the -20 °C freezer for approximately 5 minutes. Then, the methanol was aspirated and the cells are blocked with approximately 2 mL of a PBST/BSA (phosphate buffered saline using 1.5% Tween-20 and bovine serum albumin) mixture for 15 minutes at room temperature. From this point forward, the coverslips were kept in a damp chamber and were incubated at room temperature. The PBST/BSA was aspirated and 150 μL of the appropriate primary antibody, which was diluted in PBST/BSA, was added to each well (refer to the antibodies section) for 30 minutes. Afterwards, the primary antibodies were aspirated and the cells were washed three times with 1xPBS for 5 minutes each, with the PBS

being aspirated after each wash. Next, 150 μL of the secondary antibodies, which were diluted in PBST/BSA, was added to the coverslips. After 15 minutes the secondary antibodies were aspirated and the cells were once again washed three times with 1xPBS for 5 minutes each, with the PBS being aspirated after each wash. Afterwards, 100 μL of 4',6-diamidino-2-phenylindole (DAPI, Sigma) was added to the coverslips, which stains for chromatin. After 30 seconds the DAPI was aspirated. The cells were washed with water three times, with the water being aspirated the first two times only. Each coverslip was then mounted onto a slide along with mounting medium of p-phenylenediamine (Ultra, North Kingston, RI) in gylcerol. The coverslips were sealed to the slides with nail polish and stored at -20 °C. The immunofluorescent cells were then analyzed with an Olympus IX-81 Inverted Fluorescence Microscope with a 100X, 1.65 N.A. objective (Olympus America, Center Valley, PA) running Slidebook 5.0 software (Intelligent Imagine Innovations, Denver, CO). Images were imported into Adobe Photoshop CS2 (San Jose, CA) for contrast enhancement and figure assembly. Approximately 300 cells were analyzed per slide and the experiments were duplicated for accuracy.

Cell Synchronization: In order to synchronize cells at the G1/S boundary, a double thymidine block was performed according to the protocol described in Quintyne and Schroer (2002). Three 6-well plates were used per experiment, with 15 wells of the total 18 receiving coverslips. Trypsinized cells were resuspended with regular media, to a total volume of 10 mL, and 500 μL of a 95% confluent population was added to 1 mL of media in each well. 24 hours later, 2 mL of media supplemented with 2 mM thymidine (Acros Organics, Morris Plains, NJ) was added to each well and the plates were incubated for 12 hours. Then, the thymidine media was replaced with 2 mL of regular media for an additional 12 hours. After this time, the second thymidine

block was performed and the regular media was replaced with 2 mL of thymidine for 12 more hours. Finally, the cells were once again washed with regular media. This constituted the zero-hour time point. From this point on, coverslips were removed from the media and fixed in 2 mL of methanol for 5 minutes at -20 °C every hour for 10 hours (starting at 0 hours) and then every half hour for two additional hours, which results in 15 time points. Afterwards, immunofluorescence was performed on each coverslip as described in the Indirect Immunofluorescence section.

Primary Antibody Percentage: The primary antibodies utilized were Nek2 and PCNA (see the Antibodies section for more details). To calculate the expression level within a particular cell line of the respective primary antibodies, the number of cells that expressed Nek2 or that were PCNA-nuclear positive were counted in synchronized and unsynchronized populations. This value was divided by the total number of cells counted (approximately 300) and multiplied by 100 to obtain a percentage.

$$\frac{PCNA - Nuclear\ Positive\ or\ Nek2\ Expression}{Total\ Cells} \times 100\%$$

$$= Primary\ Antibody\ Percentage$$

Results

KIF9 is a largely uncharacterized member of the Kinesin-9 family of motor proteins (Wickstead and Gull, 2006). Other members of the Kinesin-9 family have reported roles in ciliar and flagellar movement and formation (Bernstein et al., 1994; Wickstead and Gull, 2006; Miki et al., 2005) as well as are implicated as predictors of coronary heart disease (Li et al., 2010). Although knowing the roles and functions of the other Kinesin-9 family members does shed some light on KIF9's potential functions due to morphological similarities, it is necessary to further characterize KIF9 itself in order to determine its roles within the cell, which will later lead to its potential roles in various disease pathways. In order to find the role of KIF9 during the cell cycle, we initially analyzed the effect of shRNA-mediated knockdown and GFP-plasmid overexpression of KIF9 in unsynchronized populations of COS7 cells. I utilized two cell cycle markers to identify specific stages of the cell cycle: PCNA, which corresponds to early S phase, and Nek2, which corresponds to late S phase. PCNA or proliferating cell nuclear antigen is involved in DNA replication, therefore its abundance in early S phase is a logical extension of its function. Figure 4A and B both show cells that are PCNA-nuclear positive versus PCNA-nuclear negative. Normally, approximately 60% of cells in a given population are PCNA-nuclear positive. When we knocked down KIF9, there was a significant increase in the percentage of cells that were PCNA-nuclear positive, with a p value < 0.02 (Figure 4C).

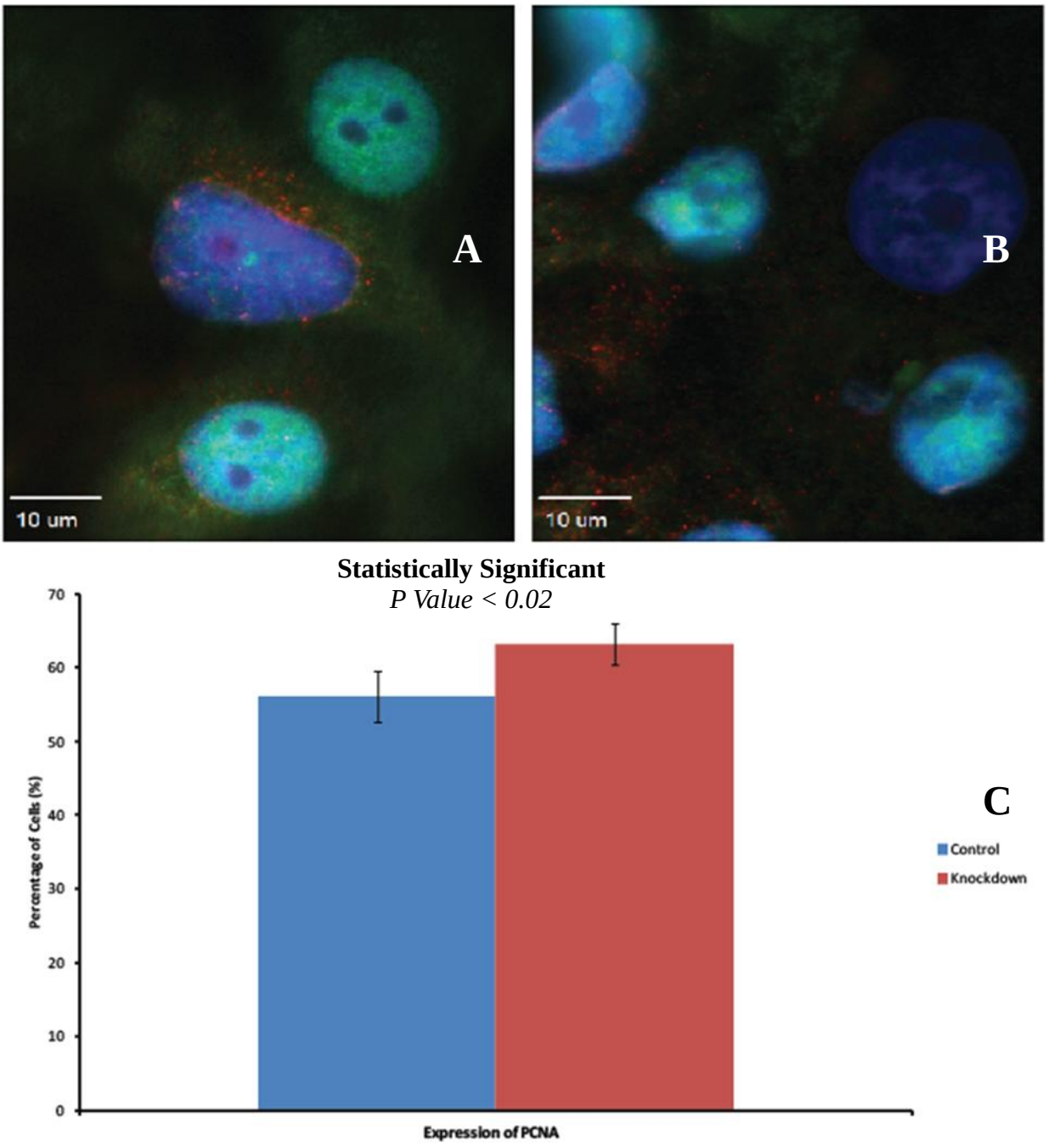

Figure 4: Analysis of PCNA-nuclear positive cells after KIF9 knockdown in unsynchronized cell populations. (A) and (B) Examples of PCNA-nuclear positive cells versus PCNA-nuclear negative cells. γ-tubulin: red, chromatin: blue, PCNA: green. Bar = 10 µm. (C) Percentage of PCNA-nuclear positive cells were quantified for each condition.

Upon the observation that knockdown of KIF9 leads to an increase in cells that are PCNA-nuclear positive in unsynchronized cell populations and therefore an increase in cells in early S phase, we examined the effect of KIF9 knockdown on synchronized cell populations. Figure 5 tracks the percentage of cells that are PCNA-nuclear positive over a 12 hour time interval, with t=0 indicating the initial release of the cells from the double thymidine block. Therefore, Figure 5 follows the cells as they progress through the cell cycle, from the beginning of S phase onwards. We noticed no significant trends between the transfected and untransfected cell populations.

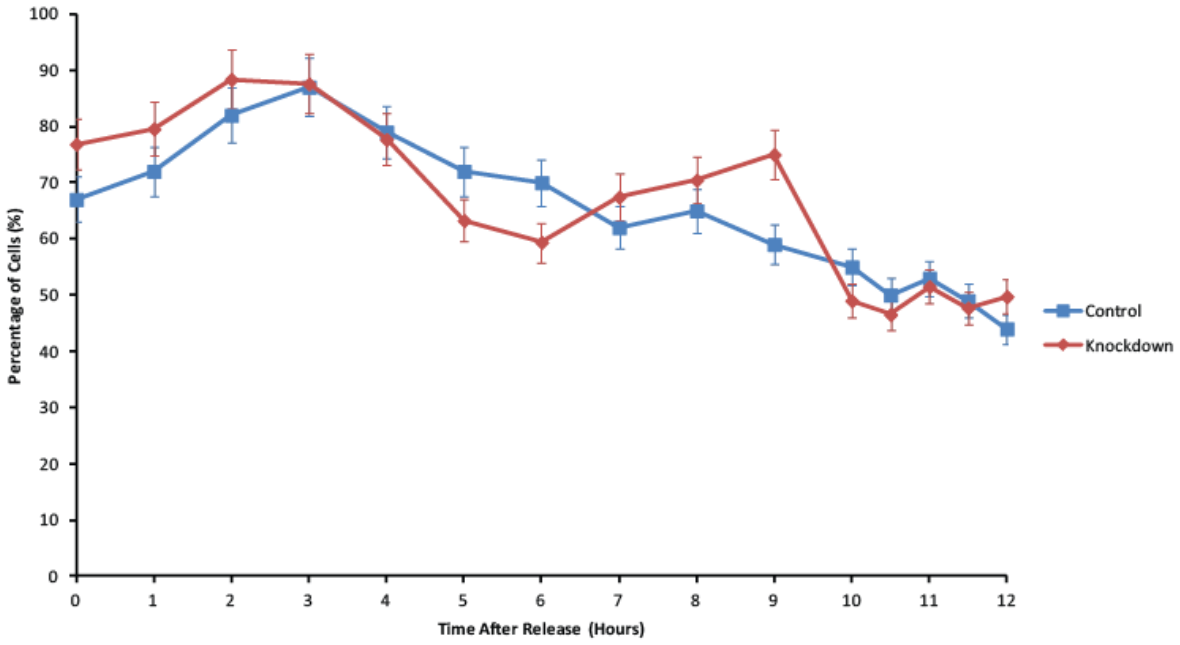

Figure 5: Analysis of PCNA-nuclear positive cells after KIF9 knockdown in synchronized cell populations over a 12 hour time interval. Time t=0 equates to the beginning of S phase, with the cells continuing through the cell cycle regularly afterwards.

Nek2 or never in mitosis gene A (NIMA)-related kinase 2 is involved in centrosome duplication. Figure 6A shows that Nek2 expression is characterized by the absence or presence of foci. Figure 6B and C show more clearly that Nek2 colocalizes with the centrosome. Normally, approximately 50% of cells in a given population express Nek2. When we knocked down KIF9, there was a highly significant increase in the percentage of cells that expressed Nek2, with a p value < 0.0001 (Figure 6D).

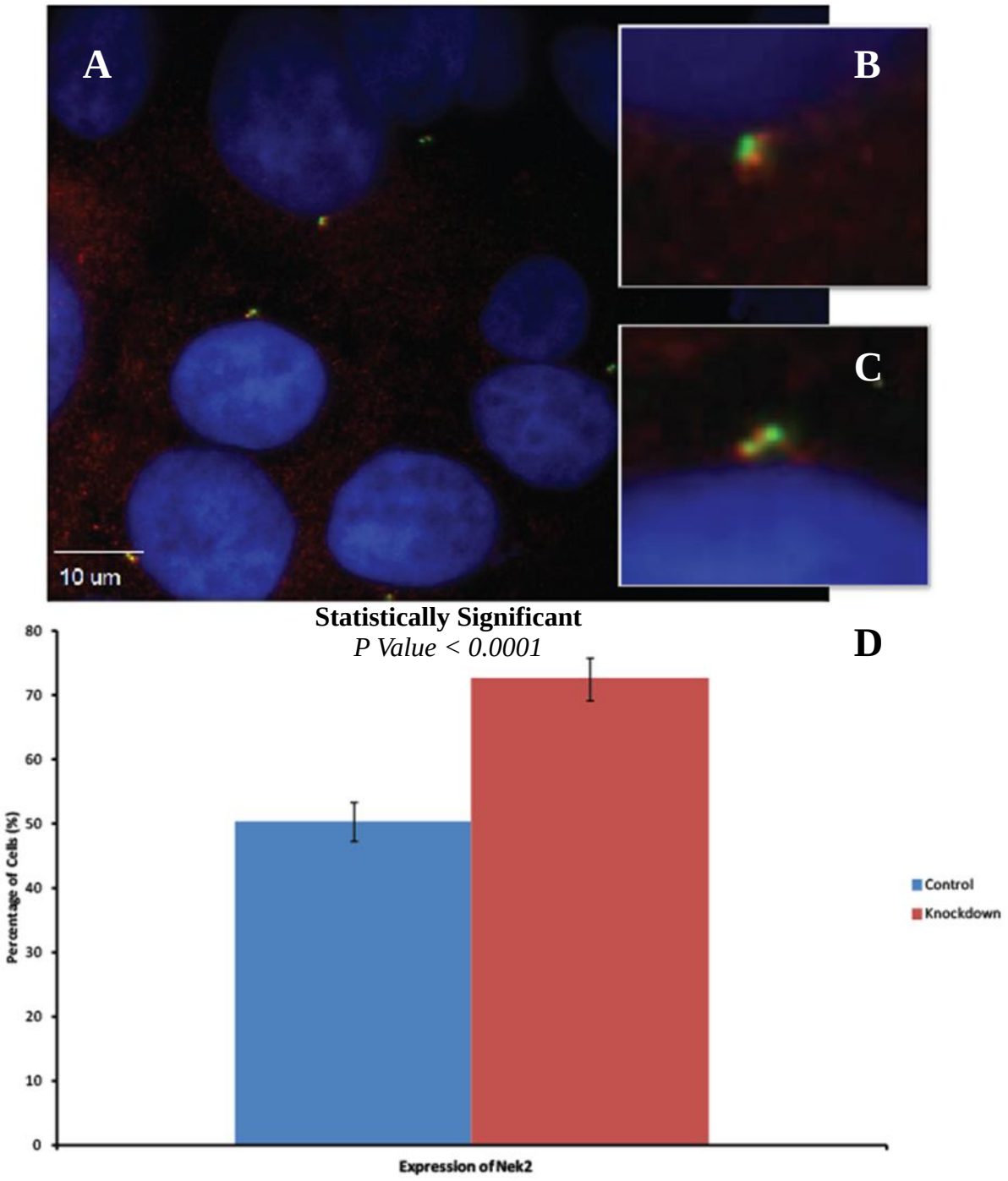

Figure 6: Analysis of cells that express Nek2 after KIF9 knockdown in unsynchronized cell populations. (A) Examples of Nek2 expression versus no Nek2 expression. (B) and (C) 3X zoom of selected cells. γ-tubulin: red, chromatin: blue, Nek2: green. Bar = 10 µm. (D) Percentage of cells that express Nek2 were quantified for each condition.

Upon the observation that knockdown of KIF9 leads to an increase in cells that express Nek2 in unsynchronized cell populations and therefore an increase in cells in late S phase, we examined the effect of KIF9 knockdown on synchronized cell populations. Figure 7 tracks the percentage of cells that express Nek2 over a 12 hour time interval. When compared to the untransfected populations, we noticed that there was an increased percentage of cells that express Nek2 earlier than expected. In other words, between t=0 and t=5 a larger number of cells than the untransfected populations were in late S phase, when they were expected to still be progressing through early S phase. In addition, the percentages of cells that express Nek2 remained relatively constant from t=6 onwards, when compared to the untransfected populations.

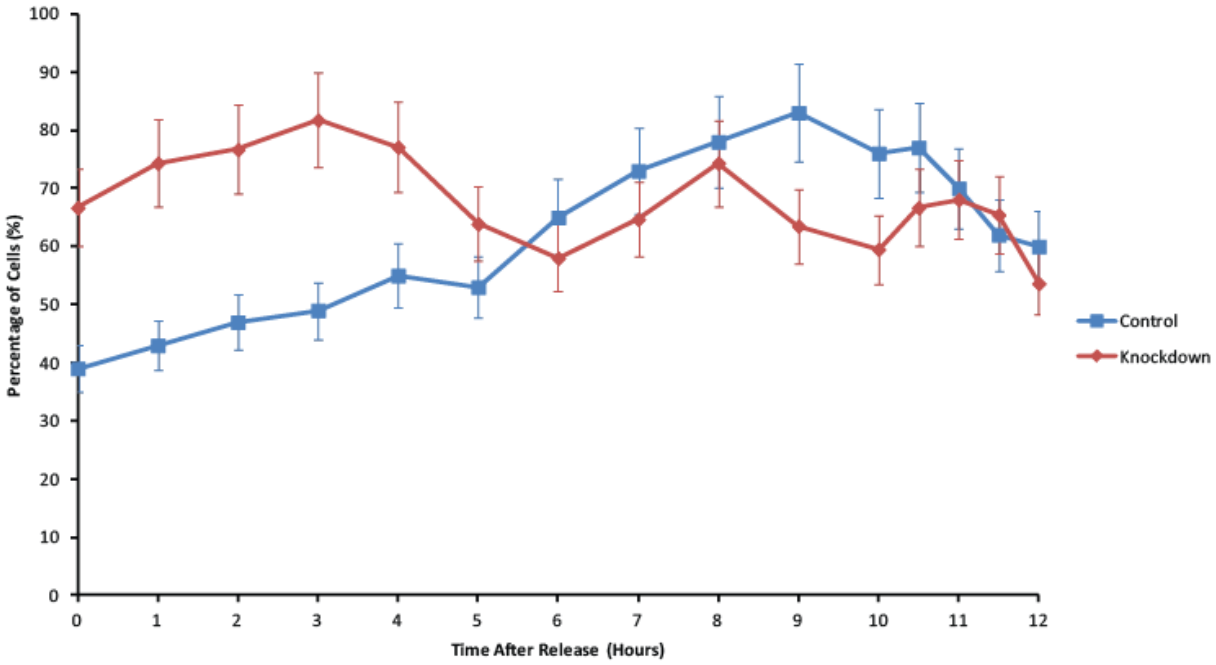

Figure 7: Analysis of cells that express Nek2 after KIF9 knockdown in synchronized cell populations over a 12 hour time interval. Time is measured after the double thymidine block is released, so that cells at time t=0 are at the beginning of S phase.

Although there was inconsistency in the data obtained from the cell populations in which KIF9 was overexpressed a number of noticeable trends were observed. First, there was an increase in the number of cells that were undergoing the initial stages of mitosis (namely prophase and prometaphase) in the earlier time points of the synchronized cell populations. Because cell synchronizations rely on the random incorporation of thymidine into the replicating DNA strands, there will always be a small percentage that remains unsynchronized, therefore any number of cells within a synchronized population may be undergoing mitosis. Figure 8 highlights the unusual increase in mitotics in the synchronized cell populations that overexpress KIF9. Second, there was a noticeable decrease in the number of cells that expressed Nek2 in both synchronized and unsynchronized cell populations.

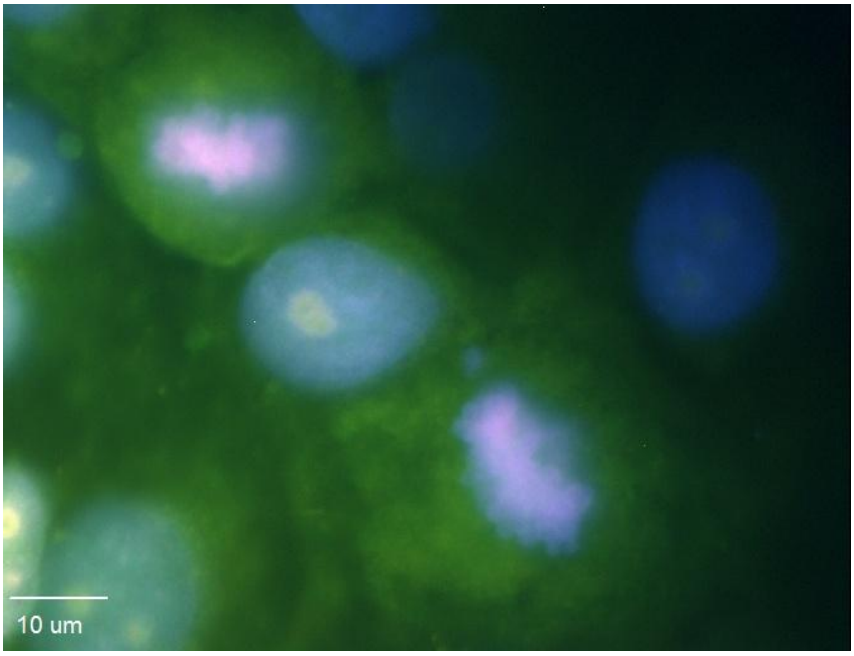

Figure 8: Examples of increased amount of cells in early mitosis, namely prophase, when KIF9 is overexpressed. Nek2: red, chromatin: blue, KIF9: green. Bar = 10 µm.

Discussion

Previous Honors College theses (Monroy, 2008; Alsina 2010; Billow, 2011) have identified mitotic defects associated with KIF9 knockdown. In addition to these mitotic defects, however, specific cell cycle irregularities were identified by Alsina (2010). The aim of this research was to further investigate the roles and functions of KIF9 during the cell cycle. Although there is still much to be done to further characterize KIF9's role during specific phases of the cell cycle, our data suggest that KIF9 is involved in a potentially novel checkpoint mechanism that prevents that cell from progressing into mitosis when activated.

We have seen that KIF9 knockdown and overexpression lead to altered S phase progression. More specifically, KIF9 knockdown leads to an increase in the number of cells in late S phase, while KIF9 overexpression results in the decrease of cells in late S phase (Figures 6-8). This suggests that KIF9 is integral to progression past a checkpoint, which triggers when KIF9 is absent. Cell cycle checkpoints are control mechanisms that ensure the fidelity of chromosomal segregation and cell division. For example, DNA double strand breaks are major factors that induce the various checkpoint mechanisms, such as the G1/S and G2/M checkpoints (Kempler, 2007; Deckbar, 2010). In addition, while some cell cycle checkpoints are thought to be complete blocks to cell cycle progression to provide time for the correction of any identified defects, the intra-S phase checkpoint is thought to function through the downregulation of inactivated origins of replication (Cann and Hicks, 2006). Based on this knowledge of cell cycle checkpoints, we propose that KIF9's absence induces a checkpoint mechanism, which does not necessarily result in a complete block of cell cycle progression, indicating that the intra-S phase checkpoint is an attractive possibility. If the checkpoint that KIF9 is involved in were to result in the complete block of cell cycle progression, we would expect to see a decrease of cells that

express Nek2 rather than the observed increase when KIF9 is knocked down. Similarly, KIF9 overexpression would result in the opposite effect than is experimentally observed. To better understand the proposed mechanism, recall that the identifying features of S phase or DNA replication and centrosome duplication. The cell cycle markers utilized for these experiments were chosen because of their involvements with DNA replication (PCNA) and centrosome duplication (Nek2). We propose that the absence of KIF9 inhibits DNA replication, while allowing for the cell to proceed directly with centrosome duplication, which reflects the increase in observed Nek2 expression.

Because PCNA is intimately related to DNA replication we would expect to see a trend in the number of cells that are PCNA-nuclear positive that corroborates this data. Before we can assess the validity of the experimental results presented in Figure 5, we must first examine PCNA's role in DNA replication. Proliferating cell nuclear antigen or PCNA acts as a processivity factor for DNA polymerase δ. It is an example of a DNA clamp that encircles the DNA in order to create a link between the DNA and the replication machinery. Our current model for how KIF9 acts during the cell cycle is that it is involved in a checkpoint mechanism that halts or slows down DNA replication. It is important to note that KIF9 downregulation does not necessarily block cell cycle progression entirely, nor is KIF9 likely directly interacting with the DNA replication machinery. This accounts for the observed increase in Nek2 expression, as the cells will forgo DNA replication in favor of centrosome duplication while the cell attempts to correct the defects. Taking into account the known functions of PCNA, the fact that the percentage of cells that are PCNA-nuclear positive remains relatively unchanged between transfected and untransfected populations is not damaging to our proposed model, as the

association of PCNA may not necessarily be affected by the halt or slowing down of DNA replication.

Further research is required to investigate some of the irregularities associated with KIF9 knockdown and overexpression and the presence of PCNA-nuclear positive cells (Figure 4 and 5). Although there appeared to be no noticeable trends, more precise examinations of the cell cycle may elucidate some novel functions of KIF9. Initial KI9 overexpression experiments shed some light on overall effects of KIF9 overexpression on cell cycle progression, such as an increase in cells in early mitosis, which supports the work done by Alsina (2011) and the decrease in percentage of cells that express Nek2. However, KIF9 overexpression research must be performed again to observe the true trends associated with overexpression, as when the aforesaid experiments were undertaken for this project a number of inconsistencies prevented us from obtaining accurate data. In addition, as this project mainly focused on irregularities in S phase progression through KIF9 knockdown and overexpression, similar research should be done in regards to the other phases of the cell cycle, namely G1 and G2. Finally, the construction of a temperature sensitive KIF9 mutant could be useful in future studies as this could isolate the knockdown or overexpression of KIF9 to even more specific points along the cell cycle.

Works Cited

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