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Proteins in the kinetochore generate forces to drive chromosomes movement

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Abstract

To accomplish the precise segregation of chromosomes, the kinetochore must be form incentive interaction with motor proteins. Any error happens in chromosome segregation led to aneuploidy that consider as main factor to cause the cancers and many birth defects. Kinetochore is a proteinous complex structure consist of a hundred of proteins. It's performed many functions such as it connects movement of chromosomes to microtubule, it acts as observer for chromosomes biorentation, beside the main role in chromosomes segregation. In this review we described the proteins content of kinetochore and the important role of these proteins in generating forces to drive the movement of chromosomes, also we clarified some experiments that used to identify these proteins and support their functions.

Keywords: Kinetochore; Chromosomes; Aneuploidy; KMN; FRAP

1. Introduction

Kinetochore is multi protein's structure which forms several connections between the centromere and kinetochore microtubules. The inner kinetochore contains an essential protein that are existed in the centromere called constitutive centromere-associated network (CCAN) which present through cell cycle, while the outer kinetochore proteins assemble at beginning of prophase and leave at the end of mitosis [1]. The outer kinetochore comprises from three complexes (Knl1, Misl2 and Ndc80), which all these named KMN network as indicated in Figure 1. These proteins create a stable attachment site with microtubule, and they have a controlling role in the polymerization state of kinetochore microtubule (KMT) as described here in Figure 2 [2].

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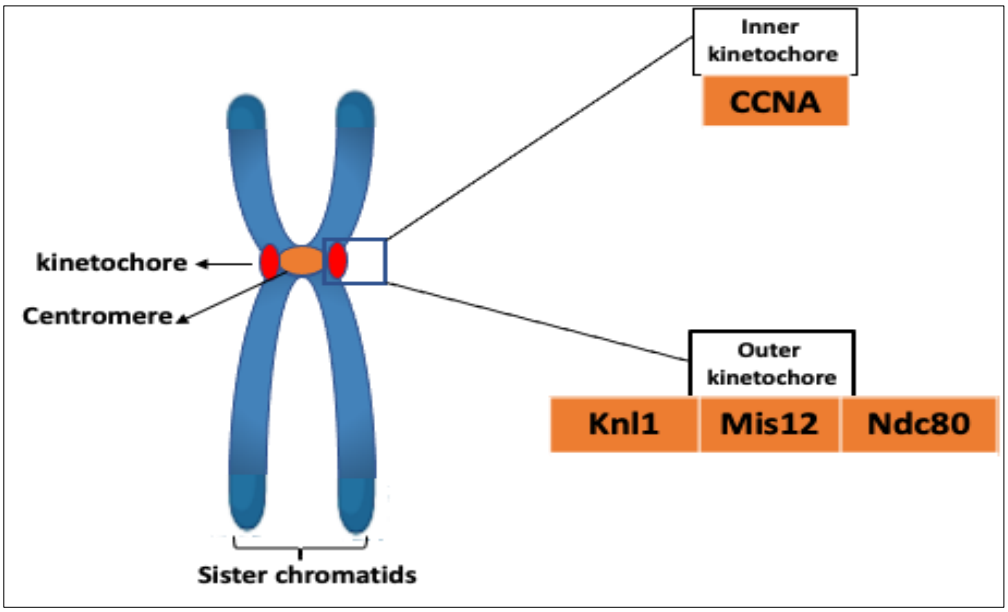

Figure 1 The structure of the kinetochore proteins. The schematic diagram illustrates the structure of the chromosome and the proteins structure of kinetochore

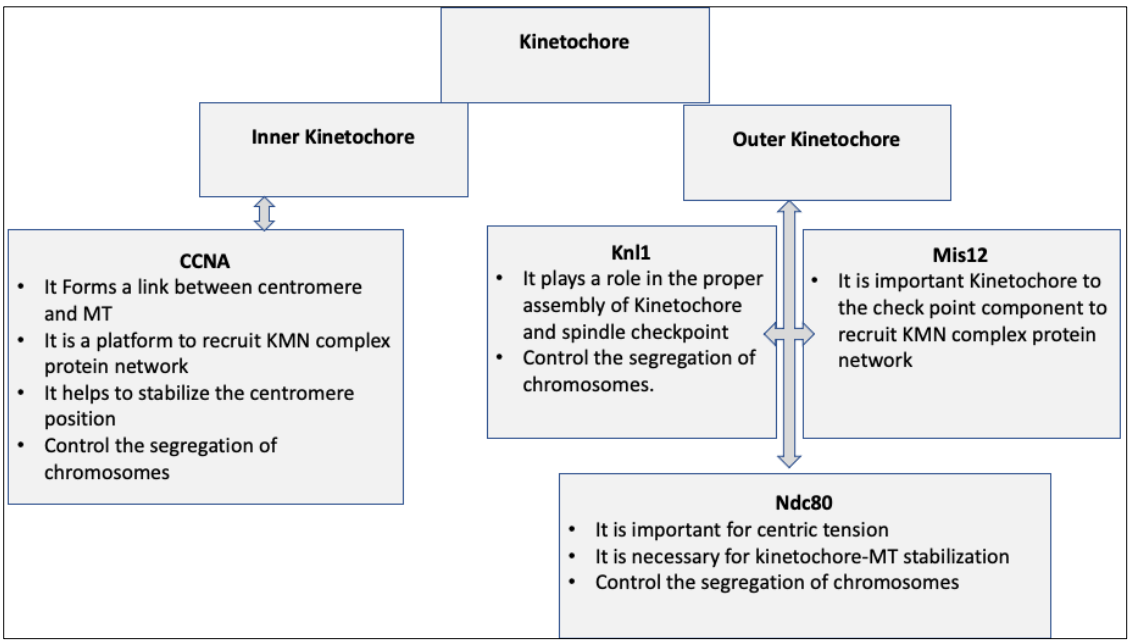

Figure 2 Functions of kinetochore proteins. The above graphic figure shows the functions of each protein in the kinetochore protein complex

2. Forces to drive the chromosome movement

Three main forces are believed to be an important to drive the chromosome movement. The first forces come from kinetochore proteins that consider the major forces. It pulls KT and associated chromosome to the spindle pole; KT moves poleward when the depolarization happen at the plus end of MT. The second forces generate by MT flux and its present in some types of cells. The polar injection is the third forces, and the chromosome movement occurs when interpolar MT interacts with Kinesin family that are present in the chromosome arm [3].

Kinetochore generates the forces by three ways, either by coupling of KT to depolarized MT, or from the tugging action of minus end motor protein (dynein) and, from the motor enzymes have an important role in tethering KT to MT [4]. Additionally, studies in *Drosophila* proposed the reduction of KT dynein this will lead to reduce chromosomal movement rate at anaphase. The reduction process in the *Drosophila* was happened by two ways, the first way by making mutation in Drosophila ZW10 and rod to disrupt RZZ components and the second way by inhibition cytoplasmic dynein via using p50 or by anti DHC antibody, also the confocal microscopy was showed the KTs alignment were not fully completed and unattached and set outside the mitotic spindle in the absences of the dynein [5-7].

3. Approaches to identify the protein contents of kinetochore

Three major kinetochore proteins were identified by different approaches as summarized in the Table 1, using human antibodies, the combination of genetic, RNA interference (RNAi) and biochemical methods were used to identify others kinetochore proteins that have an important role in chromosome segregation [8-13]. In last decade a large of kinetochore proteins were identified by using mass spectrometry in model organisms [14]. Other researchers used the Delta Assay to investigate the subunits that formed the Ndc80 complex, which is consider the main MT binding components of outer kinetochore. The results depended on the average of separation between two kinetochores inner and outer [15&16].

Additionally, a new technique named Elegant Fluorescence Microscopy used for showing the whole composition of kinetochore and it concluded the kinetochore is a tri-complex assembly and outer kinetochore has an attachment site with microtubule [17]. In addition, some researchers were used the mitotic arrest cells for identification some particles in kinetochore that have ability to bind with MT via multiple binding site [18].

There are many an important protein that play an impressive role in cell cycle events including EBI, CLIP170, and MAP215. These are plus end direct proteins and have a controlling role in the polymerization state of KT-MT attachment site and their functions are accomplished in budding yeast by Dam1 complex [19]. EBI has a binding and stabilizing role in MT seam [20]. CLIP170 helps in creating the primary connection between MT and kinetochore [21]. MAP250 acts to enhance the MT polymerization that is necessary for chromosome segregation [22&23].

Recently, GFP-tagged fusion model used to identify a new protein in human kinetochore named Ska1. Ska1 plays a several roles in coupling of chromosome movement, depolarization of MT and chromosome segregation [24&25]. Other researchers were used high pressure freezing method to study kinetochore proteins depend on preparing the samples for electron microscopy and it was better than chemical fixation because it has ability to preserve ultrastructure of the samples. This study proved the poleward chromosome movement result due to forces that arise in kinetochore [26&27].

Fluorescent Recovery after Photo bleaching (FRAP) technique was applied to investigate the dynamic property of MT by observing a bleached spot made on the lattice of fluorescent MT of Integral spindle. The searchers showed in the epithelial cells of kidney the photo bleached section stayed fixed on fluorescent MT of KT while the chromosome continued and crossed the bleached region, and these events proposed the MT depolarization happens at the KT [28]. Another study was revealed the KT contains a small members of motor proteins such as dynein, ZW10 and CENP-E and these motor proteins have an important role in chromosome movement to poleward [29 & 30].

Cells free approach were used to detect the role of the Ndc80 and dynein/dyactin, the inhibition of Ndc80 complex lead to delay dynein/dyactin rapid expression through prometaphase that depend on the KT poleward movement. Thus, dynein/dyactin movement continue without completing chromosome alignment and segregation at anaphase because the Ndc80 complex was absent [31]. A recent study showed the Ndc80 complex in the KT may act as a forcer transducer and it's stretched along the KT axis [32]. Similarly, the researchers described the Dam1 complex in budding yeast and their results clarified Dam1 complex role in the enhance the Ndc80 ability to mediate KT-MT attachment [33].

Conversely, in *Drosophila* the researchers were used Fluorescent Speckles Microscopy (FSM) technique to clarify the role of microtubule influx in producing the forces that drive chromosome movement to poleward. FSM is a useful method to following MT influx movement in the polymer, and their result showed the MT flux is the main forces for chromosome movement and this conclusion came by monitoring the speckles movement of injected labeled tubulin along MT [34]. Similarly, in the spermatocyte of insect, the results revealed the MT flux of kinetochore is the main forces to initiate the movement of chromosome [35]. Also, in fission yeast *Schizosaccharomyces pombe* the studies found it contains a member of motors proteins and none of these are necessary or involved for viability, so the chromosomes movement accomplish without motor proteins [36].

Table 1 Approaches used to identify the protein contents of kinetochore. Table shows the different methods can be used to identify the proteins structure the kinetochore, and the principle of each method summarizes here:

Name of Assay	**Principle**
Delta Assay	Measure the distance of florescence spot between proteins
Human Antibody	Specific Antibody to recognize the target protein
Genetic, biochemical and RNAi	Depend on the inhibition or activation specific protein
Mass Spectrometry	Measure the ionized content of the protein
Fluorescence Microscopy	Detect the fluorescence illuminated by protein
GFP-Tagged fusion	Mark a protein with specific tag
Fluorescent Recovery After Photobleaching (FRAP)	Measure the motility of fluorescence protein
Electron Microscopy (High Pressure Freezing)	Detect the electron contents of a protein
Cells Free	Depend on Protein-Protein interaction
Fluorescent Speckles Microscopy (FSM)	Measure the movement and the dynamic of a protein

4. Conclusion

From all above we concluded there were many models or mechanisms, which have ability to control the movement of chromosome during cell cycle. Chromosomes movement may be happened by forces generated from the depolarization of the MT minus end at the spindle poles and the source of energy emanate from hydrolysis of GTP through MT assembly, or from the KT motor proteins and these used the ATP as source of energy to support the mechanical forces to complete the movement of chromosomes, or by supporting roles of spindle matrix in the interaction between the motor protein through the generation of forces.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

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