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The yeast kinetochore — structural insights from optical microscopy

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In eukaryotic organisms each chromosome is captured by spindle microtubules. This interaction is mediated by an evolutionarily conserved, multi-protein complex called kinetochore. The main function of the kinetochore is to maintain correct chromosome segregation during cell division by transporting each sister chromatid to the spindle pole bodies localized at the opposite sites of the yeast nucleus. The kinetochore of budding yeast (*Saccharomyces cerevisiae*) is often used as a model system due to its simple composition compared to higher eukaryotes. This review highlights results obtained using optical imaging that revealed relative positions and stoichiometry of the major components of the budding yeast kinetochore.

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Introduction

Cell division crucially depends on proper chromosome segregation [1]. During metaphase, chromosomes are lined up in the middle of the cell and subsequently, in anaphase, each sister chromatid is pulled apart toward opposite spindle poles (Figure 1a). Errors during segregation may lead to, for example, aneuploidy, where daughter cells receive an aberrant number of chromosomes [2]. In eukaryotic organisms each chromatid is captured by spindle microtubules and this interaction is mediated by the kinetochore.

This review focuses on recent advances in studying the structure of the budding yeast kinetochore by optical imaging techniques. After introducing the general architecture of the complex we will summarize current accomplishments in exploring relative localization and stoichiometry of kinetochore components.

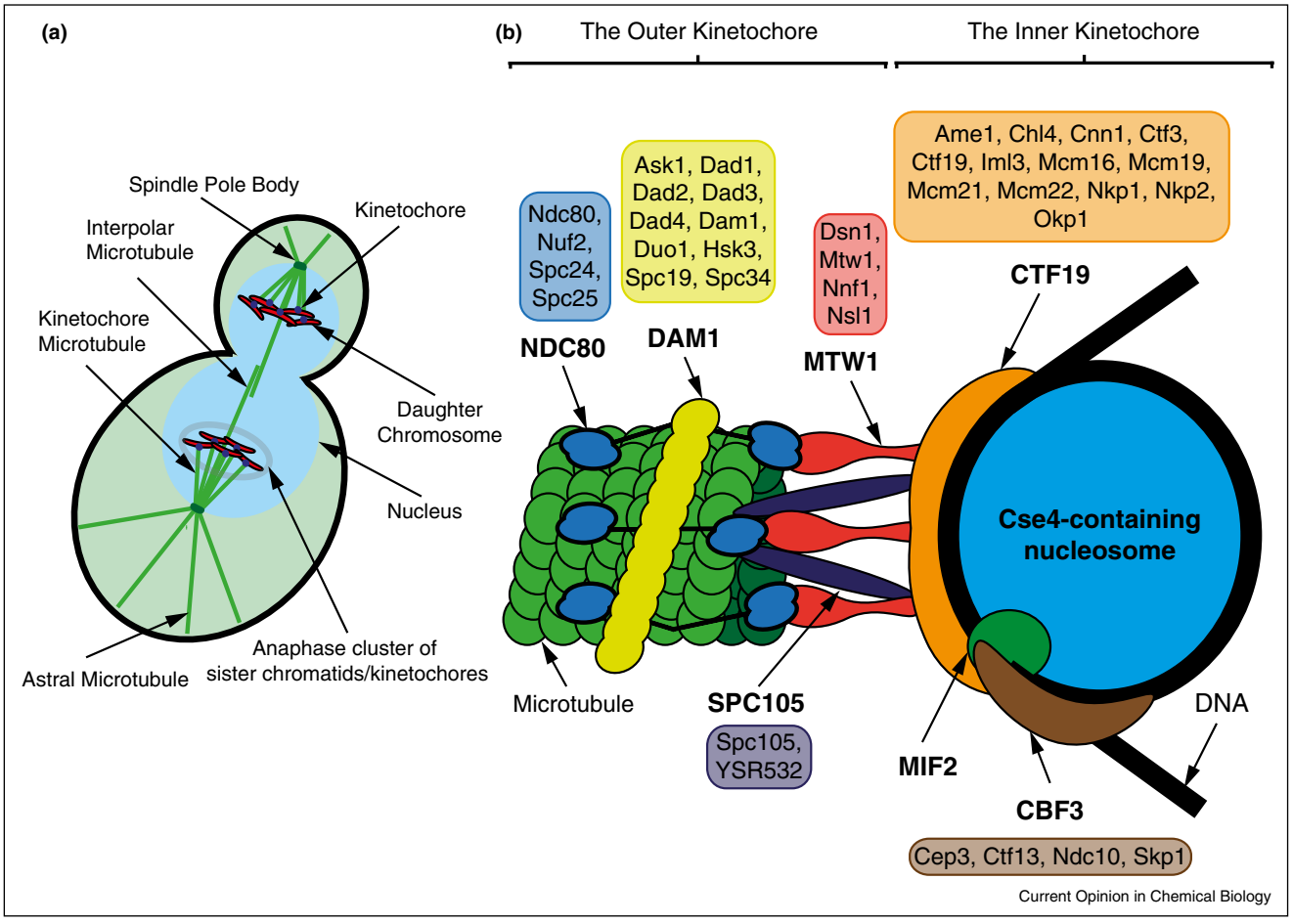
The kinetochore in budding yeast

The general architecture of the kinetochore is preserved in eukaryotes. A simple model to study its properties is *Saccharomyces cerevisiae*, where a single copy of the kinetochore is attached to one microtubule, whereas in higher organisms kinetochores have multiple attachment sites. The general concept assumes that single kinetochores in multicellular organisms and other fungi are composed of multiple copies of the budding yeast kinetochore [3].

The budding yeast kinetochore is a large protein complex with a size of roughly 125 nm [4]. It is composed of more than 50 proteins that may exist in many copies [5] (total molecular weight ~5 MDa [6]). Among them, there are many regulatory proteins including the major metaphase to anaphase progression gatekeeper Ipl1 kinase [7]. In general, the budding yeast kinetochore is bound to DNA via the centromeric nucleosome for most of the cell cycle but its composition changes over time [8]. In the very first step of cell division the kinetochore laterally and subsequently coaxially attaches to a microtubule. During metaphase, chromosomes establish bi-oriented interactions with microtubules from opposite poles and come under tension, which is one of the factors in decision making during cell division [9]. When chromosomes are attached to microtubules in an incorrect fashion, the lack of tension causes a distance decrease between Ipl1 and its substrates in the kinetochore complex [10]. Phosphorylation of kinetochore components reduces interactions between the kinetochore and microtubule, thus allowing the complex to establish proper attachment between the two cellular structures again [10]. In anaphase, sister chromatids are separated and subsequently transported toward the opposite spindle pole bodies (Figure 1a). The force is generated by the depolymerizing microtubule that is connected to the kinetochore complex, and has been described using two different models: 'biased diffusion' [11] and 'forced walk' [12]. The first model assumes that the kinetochore-microtubule binding results from multiple weak interactions and can move toward the microtubule's minus end due to its depolymerization. The second model describes the movement of the kinetochore that is tightly bound to microtubule. This motion results from sliding of the microtubule protofilaments that push the kinetochore [9].

The majority of its components serve as molecular scaffolds [13]. The function and architecture of the kinetochore are closely related. Therefore, the respective localization of the kinetochore components may provide a good understanding of the overall kinetochore role. It may also explain the contribution of each 'building block' to the kinetochore function in chromosome segregation [13].

Figure 1



(a) A schematic representation of chromosome segregation in budding yeast during anaphase. DNA and kinetochores of each cell are compacted together (anaphase chromosome/kinetochore cluster) and are seen as a single fluorescence spot in an optical microscope. **(b)** Simplified structure of a single budding yeast kinetochore bound to a microtubule. Subunit compositions are presented as color-coded frames. The stoichiometry and detailed architecture of each subunit are not shown.

Interestingly, some individual subunits or partially reconstituted kinetochores *in vitro* withstand substantially lower forces than complete kinetochores *in vivo*, possibly because of cooperative effects among kinetochore constituents [9]. Therefore, determining relative localizations of single components of the kinetochore and their stoichiometry *in vivo* will be key to understanding kinetochore function. This can be achieved with fluorescence imaging and electron microscopy along with indispensable techniques from fields such as structural biology and biochemistry. This review highlights progress in understanding the kinetochore structure with fluorescence microscopy.

The kinetochore structure can be divided into two regions, the inner kinetochore and the outer kinetochore, which will be discussed in this review. The inner kinetochore interacts with chromatin via Cse4-containing nucleosome and the CTF19, CBF3 and MIF2 complexes, which make up the CCAN (Centromere-associated Network of Proteins). The outer kinetochore that maintains interaction with microtubules consists of MTW1, SPC105, NDC80 and DAM1 complexes (Figure 1b).

The inner kinetochore

The yeast kinetochore is rather unique compared to that of other eukaryotes [14] because it is assembled on a 'point' centromere, which is a defined DNA sequence bound by the kinetochore-interacting centromeric nucleosome. This sequence was extensively studied by chromatin immunoprecipitation (ChIP). As a result, three regions were revealed: firstly, Centromere-Determining-Elements (CDE) I bound by Cbd1, secondly, CDE II occupied by the evolutionary conserved single histone H3 variant Cse4 [15], and finally, CDE III.