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Minireview

The *Saccharomyces cerevisiae* kinetochore

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Abstract Accurate chromosome segregation is dependent on a specialized chromosomal structure, the kinetochore/centromere. The only essential constituent of the *S. cerevisiae* kinetochore established today is CBF3, a multisubunit complex that binds to *S. cerevisiae* centromere DNA. Therefore CBF3 and its four components, Cbf3a, Cbf3b, Cbf3c and Cbf3d, will form the centerpiece of this review. In addition, we will describe proteins that are putatively involved in kinetochore function specifically in the context with CBF3 interaction. Furthermore, we discuss the role of the *S. cerevisiae* kinetochores in a putative cell cycle checkpoint control and in microtubule attachment.

Key words: Centromere; Kinetochore; CBF3; *Saccharomyces cerevisiae*

1. Introduction

Chromosome segregation in mitosis and meiosis depends on specialized chromosomal structures, the centromere and kinetochore. Whereas the kinetochore of animal cells can be visualized as a sub-structure of the centromere, this is not possible in *S. cerevisiae*. Therefore both terms will be used synonymously through out the review.

An extraordinarily short and conserved centromere DNA sequence, in an organism that is amenable for biochemical and genetic analysis, makes the kinetochore of the budding yeast particularly suitable for molecular dissection and reconstitution. The centromere DNA (CEN DNA) consists of 125 bp in *S. cerevisiae* [1] and 250 bp in *Kluyveromyces lactis* [2]. In contrast, the centromere DNA of mammalian chromosomes is estimated to contain at least 1 Mb and functional centromere DNA isolated from *Drosophila melanogaster* [3] or *Schizosaccharomyces pombe* [4] consists of at least 220 kb or 40 kb respectively.

The functional centromere DNA of *S. cerevisiae* is organized into three domains: CDE I, CDE II, and CDE III (Fig. 1). CDE I represents an 8 bp consensus sequence, CDE III a 26 bp consensus sequence and CDE II consists of 78–86 bp A/T rich (90%) DNA. Mutational analysis of the CEN DNA is reviewed in [1]. In summary, this work revealed that CDE III and at least part of CDE II are essential for centromere function. In particular, certain point mutations in the central CCG triplet of CDE III inactivate the *S. cerevisiae* centromere completely. In contrast, a deletion of CDE I results in only diminished centromere function (increase in chromosome missegregation frequency by a factor of 10).

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Recent reviews that describe the *S. cerevisiae* kinetochore are available [1,5,6]. This review focuses on the protein components of the *S. cerevisiae* kinetochore. First we describe proteins that physically interact with the CEN DNA and we emphasize the analysis of CBF3 (centromere DNA binding factor), a key component of the *S. cerevisiae* kinetochore. Second we describe proteins that are putatively involved in kinetochore function (as structural components or regulators) as deduced from genetic interactions with CEN DNA or CEN DNA binding proteins. Finally we discuss the role of the *S. cerevisiae* kinetochore as a putative cell cycle checkpoint and in microtubule interaction.

2. CEN DNA binding proteins

The small size and the conserved sequence elements of the *S. cerevisiae* centromere DNA made it feasible to look for proteins that could interact with the *S. cerevisiae* CEN DNA in vitro. This has led to the identification and purification of proteins that bind to the CDE I or CDE III element of the CEN DNA.

The first CEN DNA binding protein identified, Cbf1 (Cpf1, Cp1), forms a homodimer that interacts with the CDE I element through helix-loop-helix DNA binding domains (for review see [1]). Cbf1 is not essential for cell viability. However, disruption of *CBF1* (similar to the deletion of CDE I, see above) results in a 10-fold increase in chromosome missegregation. In addition to chromosome segregation, Cbf1 is involved in transcriptional regulation of methionine biosynthetic genes [7].

A multisubunit protein complex, CBF3, binds to CDE III, the CEN DNA element that is absolutely essential for centromere function in vivo (see above). However, CBF3 does not bind to CDE III if this element contains a point mutation that interferes with chromosome segregation in vivo [8,9]. Analysis of affinity purified CBF3 revealed that CBF3 consisted of the subunits Cbf3a (110 kDa), Cbf3b (64 kDa) and Cbf3c (58 kDa) [9]. In addition, a fourth CBF3 component, Cbf3d (29 kDa), was recently identified by co-purification with Cbf3c [10]. The genes of Cbf3a (*CBF2*) [11], Cbf3b (*CBF3B*) [12] and Cbf3d (*CBF3D*) have been isolated from partial amino acid sequence information and characterized. The Cbf3a gene (*NDC10*) was also isolated from the non-disjunction (ndc) mutant collection [13] in a visual screen for abnormal spindle morphology. Furthermore, the genes of Cbf3a (CTF14) and Cbf3c (CTF13) were isolated from the chromosome transmission fidelity (ctf) mutant collection in secondary screens [14]. These screens exploited the observation that a partially defective kinetochore stabilizes a dicentric chromosome (dicentric stabilization) or allows transcription through a CEN DNA sequence (transcriptional readthrough). The

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Cbf3b gene (*CEP3* for centromere protein) was also identified in a screen for mutants that interfere with the centromere imposed copy number control on 2μ plasmids [15]. Finally, the Cbf3d gene (*SKP1* for suppressor of kinetochore protein) was also identified as a high copy number suppressor of a temperature sensitive Cbf3c mutation (ctf13-30) (Connelly and Hieter, personal communication).

All four CBF3 components are essential for cell growth. Moreover, mutations in either of the CBF3 components result in cells that suffer from defects in chromosome segregation as has been shown by chromosome fragment loss assays or flow cytometry analysis ([11–15], Connelly and Hieter, personal communication). Thus, the convergent results obtained by the biochemical and genetic approaches not only produced a heterogeneous terminology (see Table 1), but also convincingly established CBF3 as a component of the *S. cerevisiae* kinetochore that is essential for chromosome segregation. The significance of CBF3 has further been strengthened by the finding that CBF3 is necessary for CEN DNA/microtubule interaction in vitro and that CBF3 mutations can result in a cell cycle arrest at G2/M (see below).

3. CBF3-CEN DNA complex structure

Cbf3b is the only one of the CBF3 components that contains a known DNA binding domain, a Zn_2Cys_6 type zinc finger domain that is essential for chromosome segregation and CBF3-CEN DNA complex formation [12]. However, Cbf3b does not bind to CEN DNA by itself [10]; neither do isolated Cbf3a, -c and -d and neither does any combination of two or three CBF3 components. The cooperation of all four CBF3 components is absolutely required to constitute an activity that specifically interacts with CEN DNA [10]. *S. cerevisiae* therefore relies on an unusually complex protein-DNA recognition to form the inner core of its kinetochore. This may reflect the fact that it is absolutely essential to avoid the formation of a dicentric chromosome with its fatal consequences.

It is not clear yet which CBF3 components interact directly with the CEN DNA and how the CBF3 components interact to form the CBF3-CEN DNA complex. However, from the data available a working model can be deduced (Fig. 1). The zinc finger protein Cbf3b is most likely involved in the specific interaction of CBF3 with CDE III. Since zinc finger domains are known to recognize base triplets [16], it can be speculated that Cbf3b directly interacts with the essential CCG triplet of CDE III described above. CBF3 complex formation may allosterically activate Cbf3b for interaction with CDE III. In addition cooperative DNA binding of other CBF3 components may be required. Some of these interactions should be unspecific as was concluded from the finding that CBF3-CEN DNA complexes include 30 bp of DNA to the right of CDE III that exhibit no sequence similarities among different *S. cerevisiae* CEN DNAs [9]. Cbf3a is the only CBF3 component that shows unspecific DNA binding in vitro (Stemmann and Lechner, unpublished) and therefore may be responsible for the unspecific protein-DNA interaction within the CBF3-CEN DNA complex.

At high salt conditions two CBF3 sub-complexes can be fractionated by gel filtration chromatography [10]. One appears as a Cbf3a oligomer (apparent molecular weight of about 550 kDa). The second is a complex of Cbf3b, -c and

Table 1
Kinetochore proteins

Name (alternative name)	Homologues/motifs	Genetic interaction	Function	References
CEN binding				
Cbf1 (Cp1; Cpf1, Cep1p)	Helix-loop-helix	*MIF2*[b,c]	Not essential. CDE I binding. Activator of transcription.	[1,7]
CBF3			CDE III binding. Required for *CEN*-microtubule interaction.	[9]
Cbf3a (Cbf2p, Ndc10p, Ctf14p)	Nucleotide binding	*MCK1*[a], *MIF2*[c], *CDC34*[a]	Essential.	[11,13]
Cbf3b (Cep3p)	Zn_2Cys_6 zinc finger		Essential. CCG interaction?	[12,15]
Cbf3c (Ctf13p)		*CBF3D*[a]	Essential.	[14]
Cbf3d (Skp1p)	$p19^{SKP1}$ (CDK2/cyclinA binding)	*CBF3C* (*CTF13*)[b]	Essential. Anchor for cell cycle regulated factors?	[10]
Putative structural				
Cbf5p	KKE/D repeats (microtubule binding)	*MCK1*[a]	Essential. Microtubule binding?	[18,19]
Cse4p	Histone H3, CENP-A (mammalian kinetochore)	CDE II[c]	Essential. Centromere-specific nucleosome?	[21]
Mif2p	CENP-C (mammalian kinetochore)	CDE I[c], *CBF1*[a,c], *CBF3A* (*NDC10*)[c]	Essential. Formation of higher order CEN chromatin? CDE II interaction?	[24]
Putative regulatory				
Mck1p	Protein kinase domain	CDE III[b], *MDS1*[a] (kinase)	Not essential. Cbf3a phosphorylation?	[20,30,31]
Cdc34p		*CBF3A* (*NDC10*)[b]	Essential. Cbf3a ubiquitination?	[32]
Motor				
Kar3p	Kinesin type motor		Not essential. Microtubule based, minus end-directed chromosome movement?	[40]

[a]The gene denoted is a dosage suppressor of a mutant allele of the protein described.
[b]The protein described is a dosage suppressor of a mutant allele of the gene or DNA sequence denoted.
[c]A double mutant with a defect in the allele denoted and the protein described exhibits a synthetic chromosome segregation defect or synthetic lethality.

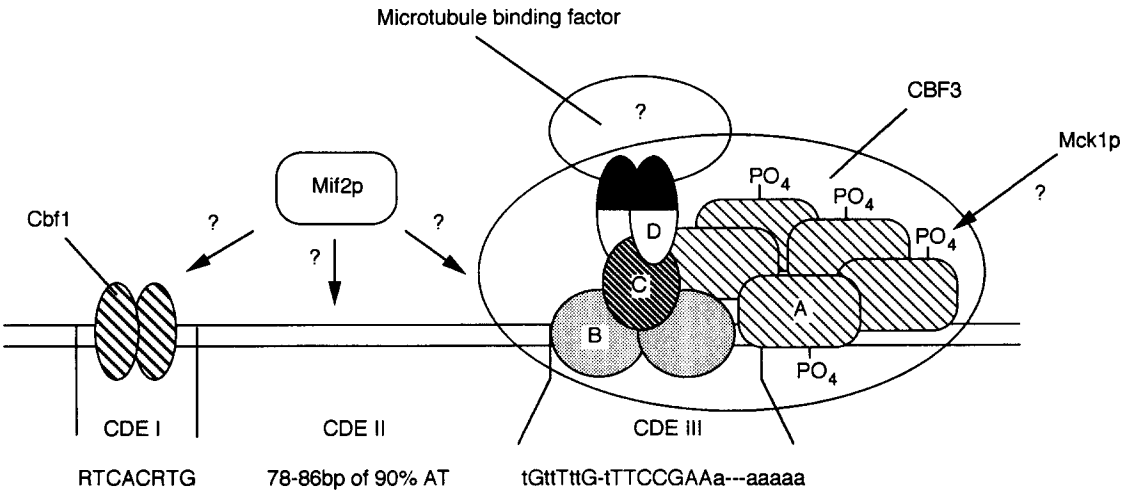

Fig. 1. CBF3-CEN DNA complex: working model and interaction with other putative kinetochore proteins. The organization of the *S. cerevisiae* CEN DNA into the three elements, CDE I, II and III and consensus sequences of CDE I and III are according to [1]. Capital or small letters indicate base pairs that occur in at least 14 or 9 CEN DNAs respectively. Dashes indicate non-conserved positions. The protein complex, CBF3, consists of four different components, Cbf3a, -b, -c and -d, that cooperate to form an activity that selectively binds to the CDE III element of the *S. cerevisiae* CEN DNA. Specific CDE III recognition may be achieved by Cbf3b which contains a zinc finger domain. Cbf3a may be involved in unspecific interaction to the right of CDE III as predicted by DNase I footprint analysis of CBF3-CEN DNA complexes. The stoichiometry of the CBF3 components shown is speculative (see text for further explanation). The protein kinase, Mck1p, may be involved in Cbf3a phosphorylation. Cbf3d, the only CBF3 component with a strong homology with a known protein of higher eukaryotes, may serve as a recognition site for cell cycle regulated factors. These factors may confer microtubule binding and/or regulation of other kinetochore activities. Mif2p may be involved in the formation of a higher order chromatin structure by interacting with CBF3, CDE II and Cbf1, the kinetochore protein that binds to CDE I.

-d with an apparent molecular weight of 250 kDa. This exceeds the size expected for a complex composed of monomers. In addition, Cbf3b and Cbf3d each form oligomers in vivo (Ortiz and Lechner, unpublished). Therefore the Cbf3b/c/d complex may contain one molecule of Cbf3c and two molecules of Cbf3b and Cbf3d each. In any case the size of the complete CBF3 complex seems to be considerably larger (around 800 kDa) than originally assumed [9]. Moreover, differently sized Cbf3a oligomers may result in a range of CBF3 sizes in vitro [17]. However, the significance of this observation in vivo is unclear.

4. Other putative structural kinetochore proteins

Cbf5p had been isolated by affinity chromatography on CEN DNA similar to the CBF3 components [18], however, the stringency of the method was significantly reduced. Unspecific DNA binding of the positively charged Cbf5p was not excluded and Cbf5p has never been shown to be part of protein-CEN DNA complexes detected by EMSA. Therefore to describe Cbf5p as 'centromere binding' seems inappropriate although circumstantial evidence exists that Cbf5p is involved in kinetochore function. This stems from the fact that Mck1p, a protein kinase that is involved in the suppression of certain centromere defects (see below), also is a dosage suppressor of a *CBF5* ts allele [19]. There is some evidence that Cbf5p is an essential microtubule-associated protein. Cbf5p contains 10 KKE/D in tandem similar to the microtubule-binding proteins MAP1A and MAP1B and Cbf5p has been shown to bind to microtubule in vitro [18].

Three putative kinetochore proteins, Cse1p (chromosome segregation), Cse2p [20], and Cse4p [21], have been identified in a genetic screen that assayed synthetic chromosome segregation defects as a result of CDE II mutations in conjunction with a second mutation in trans. Of these, Cse4p is the most reasonable candidate for a kinetochore component. The C-terminal half of Cse4p is 64% identical to the 'histone fold' of histone H3 and CENP-A, a histone-like protein that localizes in the centromere region of animal chromosomes. The N-terminal half of Cse4p is unique. Therefore Cse4p could be part of a specialized nucleosome that participates in the formation of the kinetochore [21,22]. However, it has yet to be addressed whether Cse4p physically interacts with the CEN DNA or with the CEN DNA binding proteins and whether the centromere chromatin is altered in *cse4* mutants. Therefore the kinetochore localization of Cse4p has yet to be established. Alternatively Cse4p may be involved in chromosome segregation by other means, for example as a specialized nucleosome necessary for chromosome condensation.

Mif2p, originally assigned to have a role in spindle integrity during anaphase [23], may also be a component of the *S. cerevisiae* kinetochore [24]. This is supported by two lines of evidence. First, *MIF2* shows genetic interaction with other kinetochore components. *MIF2* mutations result in a synthetic chromosome segregation defect in combination with CDE I mutations and are synthetically lethal with a *CBF1* deletion or a Cbf3a mutation (*ndc10-42*). Second, Mif2p exhibits limited homologies with CENP-C, a structural component of the mammalian kinetochore [25], and contains proline-rich sequences similar to proteins that bind to A/T-rich sequences. Therefore one possible model is that Mif2p assembles CDE I and CDE III bound components to a higher order chromatin complex. Clearly, biochemical verification of this hypothesis is necessary.

5. Regulation of kinetochore activity

In higher eukaryotes kinetochore activity is cell cycle-regu-

lated [5] and in *S. cerevisiae* the kinetochore-microtubule interaction appears cell cycle-dependent (see below) [28,39]. In addition, the *S. cerevisiae* kinetochore should contain bipartite factors that link *S. cerevisiae* specific CEN DNA-binding proteins with (cell cycle-regulated) proteins that interact with microtubules and therefore should have counterparts in the kinetochore of other eukaryotes. The CBF3 component, Cbf3d, may serve this purpose. Cbf3d exhibits a strong homology (54% identity) with $p19^{SKP1}$ [10], a human protein that is part of active cyclin A-CDK2 complexes predominantly found in transformed human fibroblasts [26]. A non-conserved region in the N-terminal half of Cbf3d could be essential for CBF3 formation. In contrast, the C-termini of Cbf3d and $p19^{SKP1}$ are particularly similar (68% identity over 76 amino acids). Therefore the Cbf3d C-terminus could provide an anchor that retrieves cell cycle-regulated factors to the kinetochore. Cyclin A-CDK2 has been connected to the regulation of DNA replication [27] whereas the kinetochore should be regulated by mitosis-specific cyclins. However, $p19^{SKP1}$ interacts with cyclin A/CDK2 only in the presence of a partner, $p45^{SKP2}$ [26]. Therefore, Cbf3d in combination with different partners may specifically recognize mitosis-specific cyclin/CDK complexes.

Dephosphorylation of CBF3 inactivates its CEN DNA binding activity [9]. This indicated that formation of the *S. cerevisiae* kinetochore could be regulated by phosphorylation. However, G1- and G2/M-arrested cells appear to contain similar amounts of active CBF3 [28]. This leaves two cell cycle windows where kinetochore disassembly (induced by CBF3 dephosphorylation) could be essential. In S-phase this may allow CEN DNA replication and at the end of anaphase this may facilitate spindle detachment. Alternatively CBF3 phosphate residues may be involved in signaling kinetochores, not attached to microtubules, to checkpoint control systems, as has been proposed for higher eukaryotes [29]. Why is the formation of the CBF3-CEN DNA complex dependent on CBF3 phosphorylation in this case? This could be a way to prevent unphosphorylated CBF3 that has become part of the kinetochore from incorrectly signaling a microtubule-attached kinetochore. The protein serine/threonine kinase, Mck1p, may be involved in CBF3 phosphorylation. Mck1p is a dosage suppressor of weak CDE III mutations [30] and Cbf3a mutations [20]. In addition, Mck1p can phosphorylate Cbf3a in vitro [20]. However, the phosphorylation observed in vitro is unlikely to reflect CBF3 activation because already active CBF3 was used as a source for Cbf3a in this experiment. In any case, if Mck1p is involved in CBF3 activation its role should be redundant because Mck1p is not essential for viability. This is supported by the observation that *MDS1*, a second putative protein kinase, is a dosage suppressor of ts phenotypes associated with the disruption of *MCK1* [31].

Despite the possibility that CBF3 activity is not cell cycle-dependent (see above) there is evidence that the level of uncomplexed Cbf3a could be regulated by poly-ubiquitination and putative subsequent proteolysis. This stems from the observation that *CDC34*, a ubiquitin conjugating enzyme (E2), is a dosage suppressor of the Cbf3a ts allele *ndc10-1* [32]. Furthermore there is evidence that Cbf3a is mono-ubiquitinated by Cdc34p in vitro and that *CDC34*-dependent poly-ubiquitination of uncomplexed Cbf3a occurs in vivo (when E3-type ubiquitin ligases are present) [32]. However, since Cbf3a which is part of the CBF3-CEN DNA complex is not ubiquitinated [32], it is unclear how restoring or increasing poly-ubiquitination (and degradation) of uncomplexed Cbf3a can compensate for the absence of CBF3-CEN DNA complex formation [33] observed for the *ndc10-1* mutant at the restrictive temperature.

6. The kinetochore checkpoint

Checkpoint control systems have been described [34] that halt the progression of the *S. cerevisiae* cell cycle before the onset of anaphase A as a result of damaged or incompletely replicated DNA or the failure to assemble an intact spindle. The finding that animal kinetochores that lack spindle attachment result in a signal to halt mitosis at metaphase [35] implies that checkpoint control systems also supervise the successful kinetochore-spindle interaction. The existence of a possible kinetochore checkpoint in *S. cerevisiae* has been proposed because mutant alleles of Cbf3b [12,15], Cbf3c [14] and Cbf3d (Connelly and Hieter, personal communication) and certain CEN DNA mutations [36] result in a G2/M delay of the cell cycle. In contrast, the Cbf3a mutants described do not delay at G2/M but proceed through mitosis with fatal consequences [13]. This may indicate that the putative control system recognizes kinetochore defects selectively. Alternatively, the Cbf3a defect may result in the destruction of the kinetochore feature that signals a free or defective kinetochore. In any case, the observation that certain kinetochore defects result in a G2/M delay, whereas others do not, diminishes the possibility that the observed G2/M arrest is just the consequence of crippled mitotic mechanics. Finally, the most important evidence for a kinetochore checkpoint stems from the finding that double mutants containing the *ctf13-30* (Cbf3c) allele and *mad1*, *mad2*, *bub1* or *bub3* mutation fail to show the G2/M delay at the restrictive temperature normally seen for *ctf13-30* mutants [37]. This strongly indicates that Mad1p, Mad2p, Bub1p and Bub3p (but not Bub2p), proteins that originally were associated with checkpoint controls supervising the correct spindle assembly [34], are also (or only) involved in transmitting the cell cycle arrest signals installed by a free or defective kinetochore.

7. Kinetochore-microtubule interaction

Video microscopy with taxol-stabilized microtubules and *S. cerevisiae* kinetochores that were partially reconstituted on CEN DNA-coated latex beads revealed that CEN DNA-affinity purified CBF3 preparations support weak CEN DNA binding to microtubules and minus end-directed movement of CEN DNA along microtubules [38]. CBF3 is necessary but not sufficient (even in combination with Cbf5p) to mediate this CEN DNA-microtubule interaction. Further factors detectable in crude yeast extracts (that probably contaminated the CBF3 preparations) confer a cell cycle-regulated microtubule binding activity to the kinetochore [28] as had been suggested before because of the co-precipitation of CEN DNA chromatin with microtubules [39]. However, these factors do not support kinetochore movement [28]. Instead, the microtubule-dependent kinetochore movement observed with the CBF3 preparations is due to the kinesin-related molecular motor, Kar3p, which exhibits unspecific DNA binding and thus can contaminate CBF3 preparation [40]. Kar3p, which has been associated with karyogamy and mitosis [41], is a

minus end-directed motor and could account for the chromosome movement at anaphase A in redundancy with other motor proteins (since Kar3p is not essential for viability). However, the co-purification of Kar3p and CBF3 appears accidental and therefore the in vivo role of Kar3p as a kinetochore-localized motor has yet to be established. Furthermore, an ATP-driven motor activity may not be imperatively required for chromosome movement. As has been shown for animal chromosomes, a kinetochore containing multiple binding sites for a microtubule may be moved solely by microtubule dynamics [42]. Therefore, it will be of great interest to examine the interaction of partially reconstituted *S. cerevisiae* kinetochores and dynamic (not stabilized) microtubules.

The experiments described above clearly demonstrated that CBF3-CEN DNA complex formation is necessary for kinetochore-microtubule interaction. Consequently the CDE III element is essential. In addition, the presence of a CDE II element to the left (actual site) of CDE III increases the stability of the kinetochore-microtubule interaction [28]. At present it is unclear whether the CDE II effect is due to direct or indirect microtubule-CDE II interaction or whether CDE II helps to present the CBF3-CEN DNA complex for optimal interaction. Also it has not been absolutely ruled out that CDE II just alters the CBF3-CEN DNA complex stability.

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