Annu. Rev. Genet. 1998. 32:307–37
Copyright © 1998 by Annual Reviews. All rights reserved

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

KINETOCHORES AND THE CHECKPOINT MECHANISM THAT MONITORS FOR DEFECTS IN THE CHROMOSOME SEGREGATION MACHINERY

Robert V. Skibbens[1] *and Philip Hieter*[2]
[1]Carnegie Institute of Washington, Department of Embryology, Baltimore, Maryland 21210; [2]The Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, Canada V5Z 4H4; e-mail: skibbs@welchlink.welch.jhu.edu

KEY WORDS: checkpoint, kinetochore, mitosis, APC, chromosome segregation, anaphase onset

ABSTRACT

Whether we consider the division of the simplest unicellular organisms into two daughter cells or the generation of haploid gametes by the most complex eukaryotes, no two processes secure the continuance of life more than the proper replication and segregation of the genetic material. The cell cycle, marked in part by the periodic rise and fall of cyclin-dependent kinase (CDK) activities, is the means by which these two processes are separated. DNA damage and mistakes in chromosome segregation are costly, so nature has further devised elaborate checkpoint mechanisms that halt cell cycle progression, allowing time for repairs or corrections. In this article, we review the mitotic checkpoint mechanism that responds to defects in the chromosome segregation machinery and arrests cells in mitosis prior to anaphase onset. At opposite ends of this pathway are the kinetochore, where many checkpoint proteins reside, and the anaphase-promoting complex (APC), the metaphase-to-interphase transition regulator. Throughout this review we focus on budding yeast but reference parallel processes found in other organisms.

0066-4197/98/1215-0307$08.00

CONTENTS

INTRODUCTION 308
Mitosis: Chromosome Segregation and the Cell Cycle Machinery 308
Checkpoint Pathways: Chromosome Segregation Insurance Policies 310
CHECKPOINT PROTEINS 311
Genetic Assays Used to Identify Checkpoint Proteins 311
Localization of Checkpoint Proteins 313
Centromere and Kinetochore Structure 314
CHECKPOINT MECHANISMS 316
Organization of the Checkpoint Pathway 317
Effectors of Cell Cycle Arrest 318
Sensors of the Checkpoint Pathway: Looking for Lesions 322
Putting It Together: Assembly of Kinetochore and Checkpoint Complexes 325
Compromising the Checkpoint Response: Adaptation and Cancer 328

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

INTRODUCTION

Mitosis: Chromosome Segregation and the Cell Cycle Machinery

Proper chromosome segregation during mitosis is a complicated process that requires the faithful execution of several events (125, 133). For example, chromosome segregation requires that centrosomes (spindle pole bodies in budding yeast) duplicate and separate. Each centrosome nucleates a polarized array of dynamic microtubules, with microtubule minus-ends situated near the centrosome. Microtubule plus-ends constantly probe the nucleoplasm, growing and shrinking by tubulin addition and loss. Microtubule plus-ends are captured and stabilized by kinetochores, the proteinaceous complex assembled onto the centromere DNA of chromosomes. Kinetochore capture of a microtubule from one pole mono-orients that chromosome. Kinetochores attached to microtubules are typically quite mobile, oscillating toward and away from the centrosome. The majority of this motion is tightly coupled to microtubule plus-end assembly reactions at the kinetochore attachment site (23, 62, 125). Replicated chromosomes are composed of two chromatids, each with its own kinetochore. Sister kinetochore capture of a microtubule emanating from the opposite pole bi-orients that chromosome. In a process that is not well understood, sister kinetochore movements become coordinated and align bi-oriented chromosomes near the spindle midzone (75, 135, 141, 147, 149, 171, 180). Bi-oriented chromosomes are continuously subjected to kinetochore/spindle forces that act to separate sister chromatids (141, 147, 171). To prevent precocious sister chromatid separation, protein-protein interactions help keep sister chromatids paired until all of the chromosomes have correctly bi-oriented. In budding yeast, sister chromatid cohesion is regulated by Pds1p and requires other cohension/condensation factors including Mcd1p/Scc1p, Scc2p, Smc1p, and Smc3p (45, 102). Some period after all the chromosomes have bi-oriented,

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

sister chromatids disjoin (anaphase onset), allowing for movement of each chromatid to its respective pole (anaphase A). Spindle elongation (anaphase B) separates the centrosomes, further segregating sister chromatids to mother and daughter cells.

The rise and fall of cyclin-dependent kinase (CDK) activities separate DNA replication from chromosome segregation and help regulate cell cycle progression (24, 77, 106, 109, 116). Minimally, CDKs are composed of a regulatory cyclin subunit and a catalytic kinase subunit. In a simple system such as budding yeast, there are roughly three classes of cyclins, each specific for a portion of the cell cycle (G1, S-phase, and G2/mitosis), but a single catalytic kinase subunit, Cdc28p (3a). Activation of mitotic CDKs or MPF (M-phase/maturation promoting factor) requires that mitotic cyclins Clb1p-Clb4p associate with Cdc28p. Activated MPF drives cells through G2 and into mitosis, during which time chromosomes condense, the mitotic spindle assembles, and the chromosome segregation machinery becomes active. MPF inactivation (exit from mitosis) facilitates chromosome decondensation, spindle disassembly, inactivation of the chromosome segregation machinery, and cytokinesis, thus compartmentalizing the segregated chromosome masses into two distinct cells.

Anaphase onset (sister chromatid separation) and exit from mitosis (MPF inactivation) are two key regulatory events that comprise the metaphase-to-interphase transition and require the degradation of two protein classes: Pds1p and the mitotic cyclins. Both harbor a 9-amino acid destruction box that targets them for proteolysis. In budding yeast, Pds1p (and the functional equivalent Cut2p in fission yeast) is required for sister chromatid cohesion and degraded at the metaphase-to-anaphase transition (18, 35, 182). The importance of Pds1p and Cut2p proteolysis for cell cycle progression was revealed by the inability of cells containing mutations in the Pds1p (or Cut2p) destruction box to initiate anaphase onset (18, 35). In fact, cells containing nondegradable Pds1p remained in mitosis with relatively high MPF activity, indicating that Pds1p proteolysis is required for both sister chromatid separation and MPF inactivation. Consistent with this model, loss-of-function mutations in *PDS1* allow for both anaphase onset and exit from mitosis in the presence of either DNA or spindle damage, conditions that normally arrest cells in mitosis prior to anaphase onset (18, 181, 182). As stated earlier, mitotic cyclins (Clb1p-Clb4p) associate with and activate the catalytic kinase subunit encoded by Cdc28p (3a). Cyclin degradation results in MPF inactivation. Deletions that remove the cyclin destruction box cause cells to arrest in mitosis (39) but allow for sister chromatid separation (54, 127, 143, 161), indicating that cyclin proteolysis normally occurs after Pds1p proteolysis.

Pds1p and cyclins are targeted for degradation by a ubiquitin-conjugating system (24, 77, 106, 110). In a bucket brigade, ubiquitin-activating enzymes

(E1s) transfer ubiquitin to ubiquitin-conjugating enzymes (UBCs or E2s) that, in cooperation with a ubiquitin ligase (E3), covalently attach ubiquitin to Pds1p and cyclin. In mitosis, the E3 complex is known as the anaphase promoting complex (APC) or cyclosome. The APC is composed of 8–12 subunits, many of which are conserved across kingdoms and five of which (encoded by *CDC16*, *CDC23*, *CDC26*, *CDC27*, and *DOC1*) participate in the formation of a 20S complex in budding yeast. Strains harboring APC mutations fail to target cell cycle transition inhibitors for degradation and so arrest in mitosis prior to anaphase onset (57, 63, 78, 82, 160, 166, 185–187), indicating that the APC is critical for this cell cycle transition. Strains harboring mutations in *PDS1* bypass certain aspects of the cell cycle arrest normally imposed by mutations in APC component genes *CDC16* or *CDC23* (18, 181, 182), placing Pds1p and cyclin downstream of APC function. Multi-ubiquitinated Pds1p and cyclins are in turn shuttled to the 26S proteosome for degradation (77), thereby facilitating entry into interphase.

APC substrate specificity for Pds1p and cyclin is facilitated by APC cofactors Cdc20p and Hct1p/Chd1p, a Cdc20p homolog: Both of these are members of a growing family of WD-40 proteins implicated in cell cycle progression (p55CDC, fizzy, fizzy-related, and β transducin) (21, 22, 57, 73, 76, 91, 118, 136, 139, 140, 143, 144, 167, 173). Budding yeast cells that harbor mutated Cdc20p accumulate in mitosis with aberrant spindle morphologies (118, 140). Visintin and coworkers (167) took advantage of the observation that cells arrested in G1 contain active APC capable of mediating the degradation of cyclin and Pds1p (both were expressed after the metaphase-to-interphase transition using inducible promotors) (3). In G1-arrested *cdc20* mutant strains, Pds1p levels were stable relative to the levels of mitotic cyclins. Furthermore, overexpression of Cdc20p selectively targeted Pds1p, but not mitotic cyclins, for degradation. Hct1p/Chd1p was independently identified as an APC cofactor by two groups (139, 167). In G1-arrested *hct1*/*chd1* strains, mitotic cyclins, but not Pds1p, were stabilized. Finally, overexpression of Hct1p/Chd1p resulted in proteolysis of mitotic, but not G1, cyclins (139, 167). Although this division of labor in target specificity is less clear in other systems (21, 22, 143, 144), the above observations in budding yeast show that Pds1p and mitotic cyclins are targeted for APC-dependent proteolysis by Cdc20p and Hct1p/Chd1p, respectively.

Checkpoint Pathways: Chromosome Segregation Insurance Policies

Defects happen. Spindle pole bodies may fail to duplicate or separate or nucleate microtubules. Defective kinetochores might be incapable of either capturing microtubules, maintaining that association, or moving poleward during anaphase A. MPF activity may fall prematurely, adversely affecting spindle

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

structure or cytokinesis. Cell cycle progression (anaphase onset or MPF inactivation) that occurs in the presence of such defects typically results in the unequal partitioning of chromosomes between daughter cells: aneuploidy. Fortunately, cells have evolved mechanisms that respond to such damage by stopping the cell cycle engine, allowing time for repair or correction. These surveillance mechanisms are now commonly referred to as checkpoint pathways (51). In recent years, dramatic advances in our knowledge have been made concerning the surveillance mechanism that monitors for defects in the chromosome segregation machinery.

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

CHECKPOINT PROTEINS

Genetic Assays Used to Identify Checkpoint Proteins

In budding yeast, eight proteins that monitor for defects in the chromosome segregation machinery have been identified. Six of these were identified in two similar but subtly different screens (108, 130, 176). Cells respond to the loss of spindle microtubules by arresting in mitosis prior to anaphase onset. The arrest is reversible: When spindles are allowed to assemble and chromosomes become bi-oriented, cells properly segregate their chromosomes and proceed through the cell cycle. In this way, chromosome loss is averted and cells retain high viability. Hoyt and coworkers (56) used substantive amounts of benomyl (70 μg/ml is about three times the amount of this microtubule depolymerizing drug required to inhibit growth) to disrupt spindles within cells. One class of mutant strains that died in response to transient microtubule depolymerization were found not to have arrested but progressed through mitosis. Li & Murray (86) used low levels of benomyl (15 μg/ml) that diminished, but did not abolish, spindle assembly. Wild-type cells delayed cell cycle progression in response to slow spindle assembly, whereas some mutant strains exited mitosis before assembling a functional spindle apparatus and perished. Mutant strains from both screens, *b*udding *u*ninhibited in *b*enzimidazole (*bub1–bub3*) (56) and *m*itotic *a*rrest *d*eficient (*mad1–mad3*) (86), appeared to have normal microtubule function and spindle formation, which suggests that benomyl sensitivity was not due to structural microtubule defects but rather to defects in a surveillance mechanism. Both groups showed that cells exited mitosis as assayed by decreased MPF activity, DNA re-replication, and bud re-emergence and that cell cycle progression was required for cell death.

All six checkpoint genes are nonessential, but mutations in them cause elevated chromosome loss or slow growth phenotypes. In particular, *bub1* strains are severely compromised for growth such that second site mutations or reversions are presumed to occur in these strains to facilitate cell growth (128). These findings reveal that even under optimum laboratory growth conditions, Bub and

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

Mad proteins function in some capacity every cell division. Checkpoint proteins may be essential in larger eukaryotic cells. In vertebrate cells or cell extracts, introduction of either antibodies directed against Bub1p or Mad2 proteins or peptides that affect checkpoint function resulted in precocious exit from mitosis, dramatic chromosome missegregation, or both (16, 43, 89, 163, 170). The importance of a cell cycle arrest in response to spindle perturbation was further revealed in the following manner. Mitosis in *Ptk1* cells lasts, on average, 50 min but can extend well over an hour (126). This period typically increases to over 6 h in taxol-treated cells (taxol stabilizes microtubule plus-ends). *Ptk1* cells arrested in mitosis with taxol were injected with antibodies directed against Mad2p. Almost immediately (12 min), those cells disjoined their sister chromatids (170). Surprisingly, 12 min is approximately half the time (23 min) required for cells to separate sisters after the final kinetochore associates with microtubules in normal mitoses (126). These findings show that the processes of chromosome bi-orientation and checkpoint inactivation that lead to anaphase onset individually require more time than is required for sister chromatids to separate in the absence of checkpoint function. Thus, checkpoint function is most likely essential in larger eukaryotes each and every cell division.

Mps1p (*m*ono*p*olar *s*pindle) was the seventh protein identified as a checkpoint component and is essential for spindle pole body duplication during G1. *mps1-1* was isolated from a temperature-sensitive collection to identify additional factors required for spindle pole body duplication (179) and, when held at the restrictive temperature, form an enlarged half-bridge but not a sister spindle pole body. Unlike other monopolar mutants that arrest in the first cell cycle in G2/M (*cdc31* and *mps2-1*), *mps1* strains failed to arrest cell cycle progression, even in the absence of spindle microtubules (174, 178). Both defects, monopolar spindles and loss of checkpoint function, appear tightly linked: Five of five independent *mps1* mutants tested both formed monopolar spindles and failed to arrest in the presence of nocodazole. Similarly, overexpression of Mps1p affects both spindle pole body integrity and checkpoint activity (50). These observations suggest that Mps1p functions in both spindle pole duplication and the checkpoint mechanism that monitors the integrity of the chromosome segregation machinery (174).

Modulating MPF activity can also alter the timing of exit from mitosis. Cdc55p is the protein most recently identified as a putative checkpoint component. Cdc55p is a regulatory subunit of *p*rotein *p*hosphatase 2A (PP2A) and has been implicated in a variety of cell processes including, but not limited to, regulating exit from mitosis (41, 53). In terms of checkpoint function, *CDC55* was identified in two ways: *cdc55* mutant strains fail to arrest cell cycle progression in response to spindle perturbations (104) and *cdc55* genetically interacts with a kinetochore mutation (169). However, cell cycle progression in *cdc55* strains

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

is different from that which occurs in *bub*, *mad*, or *mps1* strains. Minshull and coworkers (104) demonstrated that MPF inactivation occurred without cyclin degradation: *cdc55* mutant cells released into nocodazole contained little MPF activity but accumulated near wild-type levels of mitotic cyclins (Clb2p and Clb3p). Instead, MPF inactivation was shown to occur by inhibitory phosphorylation of Cdc28p: *cdc55* strains contained substantially phosphotyrosinated Cdc28p, whereas *cdc55* strains harboring *cdc28VF* (Cdc28p mutated at sites where inhibitory phosphorylation normally occurs) retained high MPF activity in the presence of nocodazole. A similar mechanism for exiting mitosis without cyclin degradation has been suggested for Sic1p, a CDK inhibitor (139, 167); this indicates that there are multiple pathways for regulating MPF activity to facilitate cell cycle progression.

Localization of Checkpoint Proteins

Numerous checkpoint proteins have recently been localized to kinetochores and other structures in mitotic vertebrate cells. In vertebrate cells, kinetochores are easily visualized such that even kinetochore substructures are resolvable (92). Work from several labs reveals that *MAD1*, *MAD2*, and *BUB1* encode proteins that have homologs conserved across kingdoms, both in terms of amino acid sequence and checkpoint function. In vertebrate cells, Mad2p localizes to kinetochores in a cell cycle–dependent manner; kinetochores free of or recently attached to microtubules are decorated with Mad2p, whereas kinetochores associated with a full complement of microtubules fail to exhibit Mad2p association (16, 89, 170). Mad2p also localizes at centrosomes, along spindle microtubules, and diffusely in the cytoplasm (43, 170). Data from several cell systems indicate that Mad2p physically interacts with Mad1p and Mad3p (57, 69, 130). A human homolog of the *Saccharomyces cerevisiae* (Sc)Mad1p, known as TXBP181 or *Homo sapiens* (Hs)Mad1p, has been identified and also localizes to kinetochores in a cell cycle–dependent manner. HsMad1p resides at kinetochores in interphase but then moves to centrosomes during mitosis (69). Thus, there is currently no evidence that Mad1p associates with Mad2p except at centrosomes, but these interactions need to be more clearly defined. Both ScMad1p and HsMad1p are hyperphosphorylated during mitosis. ScMad1p is known to be phosphorylated by Mps1p kinase in vitro (50) and thereby provides circumstantial evidence for the association of another checkpoint protein to this kinetochore complex. Mouse Bub1p and at least two human Bub1p isoforms localize to kinetochores during mitosis (13, 163; G Chan, B Schaar & T Yen, personal communication). Interestingly, one of the human isoforms (referred to as either huBUB1A or HsBUB1R)(13; G Chan, B Schaar & T Yen, personal communication) localizes to kinetochores early in prophase prior to nuclear envelope breakdown and remains at the kinetochore throughout

mitosis. This isoform was identified by virtue of its binding CENP E (G Chan, B Schaar & T Yen, personal communication), the kinesin-like microtubule motor that resides at the kinetochore, suggesting a link between kinetochore motility, checkpoint function, and assembly reactions (see below). Studies in budding yeast show that Bub1p kinase both binds and phosphorylates Bub3p (128), suggesting that Bub3p may also reside at the kinetochore. Bub1p residues 141–609 are required to bind Bub3p. The amino-terminal region of all three (human, mouse, and yeast) Bub1 proteins overlap this Bub3p binding domain and exhibit significant (25–44%) identity to residues 76–202 of Mad3p (128, 163; G Chan, B Schaar & T Yen, personal communication), providing circumstantial evidence that Mad3p interacts with Bub3p. These results suggest that at least six checkpoint proteins (Mad1p–Mad3p, Bub1p, Bub3p, and Mps1p) reside at kinetochores.

Although the vast majority of data argue that the checkpoint machinery assembles on kinetochores, it is worth noting evidence to the contrary. First, Mad1p and Mad2p interact in a complex but as yet they are not known to temporally coexist at the kinetochore. Second, at least one component (Mad1p) is known to exist in both a soluble and insoluble complex. In vertebrate cells where localization has been possible, most of these proteins appear in vast excess of kinetochore binding sites and localize to other regions of the cell: Mad1p, Mad2p, and Bub1p exhibit diffuse nuclear staining or colocalize to centrosomes in addition to kinetochores (69, 163, 170). Often, localization is cell-cycle specific such that these proteins migrate from one organelle to another over time. Thus, which set of complexes or protein populations are checkpoint competent has yet to be rigorously determined.

Currently, there is no strong evidence that checkpoint proteins reside at budding yeast kinetochores. However, localization of checkpoint proteins to vertebrate cell kinetochores portends that such proteins will be identified as we continue to build out from the budding yeast kinetochore. Indeed, most data from both budding yeast and larger eukaryotes indicate that the checkpoint mechanism is activated by defects in kinetochore structure.

Centromere and Kinetochore Structure

Kinetochores are nucleated on a specialized region of DNA termed the centromere (27, 34, 55, 61, 137). The centromere of the yeast *Saccharomyces cerevisiae* is composed of three centromere DNA elements (CDE I, II, and III) and requires only 125 bp to function in vivo. The CDE I 8-bp sequence is bound by CBF1/CP1/CBF1/CEP1, a protein that contains a helix-loop-helix DNA-binding domain and is involved in both transcription regulation and chromosome segregation. Neither CDE I nor *CBF1* is essential for cell viability. The 78–86-bp region of CDE II is composed of AT-rich DNA and appears to act as

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

a spacer between the conserved CDE I and CDE III DNA elements. Altering either CDE II's base composition or sequence length reduces the fidelity of plasmid segregation. CDE III is highly conserved among budding yeast centromeres and is comprised of a 25-bp sequence that contains regions of inverted dyad symmetry. Although not sufficient for kinetochore function, CDE III is absolutely essential. Mitotic stability, centromere function, and protection from nuclease degradation (an assay for kinetochore assembly) are abolished if the central C/G or portions of the inverted palindromic sequences of CDE III are mutated. Together, these three *cis*-acting elements nucleate the formation of the kinetochore, the organelle responsible for tethering chromosomes to spindle microtubules.

The microtubule attachment site for yeast chromosomes appears relatively simple. CBF3 is a multiprotein complex that binds CDE III in a DNA sequence–specific manner. The CBF3 complex is composed of at least four proteins: Ctf14p/Ndc10p/CBF2 (25, 40, 66, 101), Ctf13p (25), Cep3p/CBF3d (83, 159), and Skp1p/CBF3d (4, 19, 157). Together, these proteins form a 240-kDa complex that is essential for centromere function in vivo. Cbf5p, Mck1p, Kar3p, and Spt4p have also been implicated in kinetochore function. Cbf5p is essential, binds microtubules in vitro, and at elevated levels suppresses *ndc10* mutant strain temperature sensitivity (68), although recent information suggests Cbf5p may function in nucleolar processes (12, 99). Mck1p is a serine/threonine protein kinase that interacts with CBF3 proteins in a two-hybrid assay. At high levels, Mck1p rescues strains harboring mutations in *NDC10*, *CBF5*, and centromere DNA (67, 142). The kinesin-like minus-end-directed Kar3p microtubule motor copurifies with the CBF3 complex using CDE III DNA as an affinity matrix (60, 103), although it is presently unclear that Kar3p is integral to the kinetochore complex or responsible for chromosome motion in vivo. *spt4* mutations cause elevated rates of chromosome loss and genetically interact with mutations in either kinetochore proteins or centromere elements (6). Many of these proteins contain motifs or regions of identity conserved across species and have been the topic of several reviews (61, 122, 137).

Skp1p, as opposed to other CBF3 subunits, plays multiple roles in the cell (24). In terms of kinetochore function, Skp1p is a CBF3 component required for CDE III binding and is a high-copy suppressor of the kinetochore mutation *ctf13-30* (19, 157). In addition, Skp1p appears to play a critical role in regulating Ctf13p levels during kinetochore assembly (74). Surprisingly, Skp1p is also involved in the ubiquitin-mediated destruction of the G1 CDK inhibitor Sic1p (33, 150). Both Connelly & Hieter (19) and Bai et al (4) found that the terminal cell cycle arrest phenotypes in *skp1* mutant strains are allele specific (i.e. *skp1-3* strains arrest in G1 while *skp1-4* strains arrest in mitosis prior to anaphase onset), consistent with a model whereby Skp1p plays distinct (but perhaps

functionally related) roles during the cell cycle. Skp1p is highly conserved in both sequence and function; human *SKP1* complements the temperature sensitivity of *skp1-12* in yeast cells (4). In vertebrate cells, Skp1p associates with the cyclin A-CDK complex required for S-phase entry (189) and binds a new family of proteins, including cyclin F, Cdc4p, Skp2p, and others, based on an F-box binding motif (4).

Recently, three other proteins have been identified as residing at kinetochores: Mif2p, Cse4p (122), and Ctf19p (K Hyland, P Meluh, D Koshland, P Hieter, personal communication). *MIF2* was identified over a decade ago in an assay for proteins that, when expressed at high dosage, resulted in chromosome instability (97). Subsequent studies revealed that this 65-kDa protein is essential for proper spindle morphology (11). In addition, *mif2* mutations genetically interact with CDE I and kinetochore mutations (100). Mif2p contains an A-T hook motif shared by other chromatin proteins that associate with A/T-rich DNA and exhibits conserved regions of identity to CENP-C, a vertebrate cell kinetochore protein (132). Most recently, Meluh & Koshland (101) used a chromatin immunoprecipitation method to show that Mif2p binds CDE III in vivo, possibly through cooperatively binding CDE III and other kinetochore proteins such as Ndc10p. A chromatin cross-linking strategy was also used to show that Cse4p associates with CEN DNA in vivo (P Meluh, M Smith, P Yang, L Glowczekski, D Koshland, personal communication). *CSE4* encodes an essential histone H3 variant similar to the vertebrate centromere protein CENP-A (119a) and was identified both in screens assaying for increased rates of chromosome nondisjunction and suppression of a temperature-sensitive histone H4 mutant (153, 158). Although Cse4p is at the centromere, it is unclear whether Cse4p is an integral component of the yeast kinetochore or facilitates kinetochore nucleation on specialized centromere nucleosome substructures.

Chromatin immunoprecipitation methods also revealed that epitope-tagged Ctf19p specifically binds to higher-order centromere complexes in vivo (K Hyland, P Meluh, D Koshland, P Hieter, personal communication). *ctf19* mutant strains are inviable in the presence of high levels of Ctf13p (81, 155) and *ctf19* genetically interacts with mutations for both kinetochore and some, but not all, checkpoint proteins. An intriguing observation is that extracts made from *ctf19* mutant strains exhibit greatly reduced levels of minichromosome binding to microtubules in vitro (K Hyland, J Kingsbury, D Koshland, P Hieter, personal communication). Thus, ctf19p may represent an exciting step forward in coupling the centromere binding CBF3 complex to microtubule binding activity.

CHECKPOINT MECHANISMS

Surveillance or checkpoint mechanisms are modeled as having roughly three components: sensors, signal transducers, and effectors of cell cycle arrest.

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

Below we discuss the observations that led to models regarding how checkpoint proteins interact to form a coherent pathway and follow with how that pathway halts cell cycle progression.

Organization of the Checkpoint Pathway

Biochemical and genetic interactions of checkpoint proteins in budding yeast cells have led several investigators to propose linear models for this feedback pathway (28, 49, 176). The more cogent findings that have led to those models are presented here. *BUB1* and *BUB3* exhibit several genetic interactions with each other and Bub1p/Bub3p physically interact (56, 128). Overexpression of a dominant allele of *BUB1* (*BUB1-5*) arrests cells in mitosis. This arrest is absent in *mad1–mad3*, *mps1*, and *bub3* mutants (the arrest is also absent in *bub2* strains, although a significant delay occurs). Because this *BUB1-5* arrest depends on the other six checkpoint proteins, Bub1p (and by inference Bub3p) must act very early in the checkpoint pathway (32). Mad1p levels do not fluctuate within the cell cycle but Mad1p modifications are cell cycle dependent. Mad1p is constitutively phosphorylated throughout the cell cycle and hyperphosphorylated in mitotic cells or in cells containing spindles disrupted using either microtubule depolymerizing drugs or mutations in tubulin genes. Hyperphosphorylation is greatly reduced or absent in *bub1*, *bub3*, and *mad2* mutant strains, suggesting that *MAD1* functions downstream of *BUB1/3* and *MAD2* (49). *mps1* mutant strains treated with nocodazole fail either to hyperphosphorylate Mad1p or arrest (50, 174). Based on this observation, Hardwick and coworkers (50) showed that Mps1p exhibits kinase activity and that Mad1p is an Mps1p substrate in vitro. Overexpression of Mps1p results in Mad1p hyperphosphorylation even in *bub1* strains, arguing that Mad1p is not a Bub1p substrate (50). These and other findings have been used to formulate a linear checkpoint pathway: Bub1p/3p → Mad2p → Mps1p/Mad1p → Bub2p/Mad3p.

More recent evidence suggests that Bub, Mad, and Mps1 proteins may not function in a linear pathway. As described above, Mad1p, Mad2p, and Bub1p all reside at (but are not limited to) kinetochores in vertebrate cells. In addition, Mad1p, Mad2p, and Mad3p physically interact, although whether they interact in single or multiple complexes is less well established (57, 69, 130). Similarly, Bub1p binds Bub3p, and the N-terminal portion of mammalian and yeast Bub1p that is sufficient to direct binding to Bub3p is 35% identical to the amino-terminal of Mad3p (128; G Chan, B Schaar & T Yen, personal communication). These physical interactions suggest that these proteins are not separated along a linear pathway, as previously proposed. Genetic interactions similarly confuse the issue: High levels of Mps1p, Mad2p or a dominant form of Bub1p (Bub1-5p) arrest cells in mitosis (32, 50, 52, 76). However, Mad1p is hyperphosphorylated in combination with elevated Mps1p but not in combination with Bub1-5p. Not only is Bub1p supposed to act upstream of Mad1p (and therefore predicts that

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

Mad1p should be hyperphosphorylated), these results also show that cells can both arrest in mitosis without Mad1p hyperphosphorylation and exit mitosis in the presence of hyperphosphorylated Mad1p (32, 50), suggesting Mad1p modification may be irrelevant to checkpoint function. These results attest to the complicated network of interactions that comprise the checkpoint pathway.

One response to the above observations is to tie the linear pathway into a knot and place it on the kinetochore as a single multi-interacting checkpoint complex (32, 130). In this model, the kinetochore checkpoint complex (*A*) could interact with its downstream effector (*B*) in one of three ways: *A* goes to *B*, *B* comes to *A*, or *A* talks to *B* through *C*. In the first case, checkpoint protein or protein subsets could leave the kinetochore to transduce the checkpoint signal to effector molecules that halt the cell cycle. An excess soluble pool of proteins would quickly fill in this vacancy, keeping the checkpoint active. In the second case, the checkpoint complex remains at the kinetochore and recruits/activates effector molecules that directly halt the cell cycle. In the third case, a kinetochore checkpoint complex could modify soluble components that in turn carry the signal to effector molecules. One possible transduction pathway involves the ERK2/mitogen-activated protein (MAP) kinase cascade. Studies performed using *Xenopus* extracts revealed that MAP kinase is active in arrested *Xenopus* extracts but that a MAP kinase-specific inhibitor allowed for abrogation of the arrest (105). A second response to the genetic and physical interactions described in the preceding paragraph could be that there are parallel and often overlapping pathways that may reside at various sites within the cell.

Effectors of Cell Cycle Arrest

A major advance in our understanding of cell cycle arrest is that checkpoint proteins interact with components of the APC to inhibit Pds1p and cyclin degradation. Work from fission yeast revealed that high levels of Mad2p arrest cells in mitosis with unseparated condensed chromosomes (52). Mad2p-arrested cells decondensed their chromosomes after MPF inactivation (these cells harbored a temperature-sensitive *cdc2* mutation that encodes the catalytic subunit of MPF) in the absence of nuclear division (i.e. sister separation). Motivated by observations that strains harboring mutations in APC subunits (*cut4*, *cut9*, and *nuc2*) exhibit similar phenotypes (decondensed chromosomes, absence of nuclear division), He and coworkers (52) determined that APC mutant strains were exquisitely sensitive to elevated Mad2p levels: Cells progressed through the cell cycle and died. These results demonstrated a genetic interaction between a checkpoint protein and APC subunits, suggesting that checkpoint-mediated arrests occur via APC inhibition.

Li et al (90) observed that Mad2p and APC components exist in a complex in HeLa cells. Mad2p was found to coimmunoprecipitate with human homologs

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

of APC components Cdc16p and Cdc27p. This association was checkpoint dependent: Extracts made from mitotic, but not G1 or S-phase, cells resulted in both Mad2p and Cdc16p association with Cdc27p. Of interest is that Mad2p preferentially associated with slower migrating forms of APC components. A functional link to APC activity was also demonstrated: *Xenopus* extracts arrested in mitosis using nondegradable cyclin were unable to degrade endogenous cyclins when recombinant Mad2p was present (90). But where does this interaction occur? When they asked, "Your place or mine?", Jorgensen et al (72) discovered that Tsg24, the mammalian homolog of fission yeast *Cut4p*, localized to centromeres in both CHO and 3T3 mouse fibroblast cells. Similarly, p55CDC, a candidate Cdc20p mammalian homolog, also localizes to kinetochores (and other structures) throughout mitosis (73). Although these findings lend credence to a model whereby effector molecules are recruited to the kinetochore checkpoint complex (see above), this model is tempered by observations that other APC components have not been localized to centromeres but instead reside on chromosomes, centrosomes, or along spindle microtubules (72, 73, 82, 166).

Genetic evidence suggests that checkpoint and APC machineries are coupled by Cdc20p. In budding yeast, Cdc20p promotes APC-dependent proteolysis of Pds1p (167). Recently, *CDC20-50* cells were reported to *p*erish in the *a*bsence of *CIN8* (PAC screen), a nonessential kinesin-like microtubule motor protein implicated in anaphase B spindle elongation. This finding suggested that cells containing this dominant allele of *CDC20* failed to regulate cell cycle progression in response to spindle assembly defects. The link to checkpoint function was confirmed by the observation that *CDC20-50* strains exhibit dramatic rebudding and DNA re-replication in the presence of microtubule depolymerizing drugs (37, 136). Dominant mutations that can rescue loss-of-function mutations often are constitutively active. Consistent with this model, cells overexpressing wild-type Cdc20p failed to arrest in the presence of spindle defects, APC mutations (*cdc16-1* and *cdc23-1*), or high levels of either Mps1p or Pds1p (136). All of these findings make for a rich array of genetic interactions between Cdc20p, the APC, and checkpoint functions.

Working in parallel systems, researchers in several labs found that Cdc20p homologs physically interact with checkpoint proteins. Slp1p and Cdc20p are fission and budding yeast homologs, respectively. Kim and coworkers (76) showed that Slp1p binds Mad2p and identified a 66–amino acid polypeptide sufficient for Slp1p-Mad2p association. Interestingly, this 66–amino acid region, and a 29–amino acid domain required for Slp1p-Mad2p interactions, both reside outside of the 7 WD-40 repeats in Slp1p (76). This result reveals that Slp1p-Mad2p association can occur independently of this protein binding motif. Hwang et al (57) showed that Cdc20p physically associates with Mad1,

Mad2, and Mad3 checkpoint proteins. Although Mad3p produced the strongest binding reaction by two-hybrid analysis, Mad2p-Cdc20p association required Mad1p but not Mad3p. Given that Mad1p and Mad2p interact with each other (57, 69, 130), the combination of these results suggests that a Mad1p-Mad2p complex associates with nascent Cdc20p and further recruits Mad3p. Mad2p and Mad3p bound Cdc20p in cells arrested using either nocodazole or an APC mutant, indicating that these interactions are not necessarily mediated by checkpoint activation (57). Interestingly, Cdc20p levels are dramatically increased in *mad3* strains, which may provide a molecular explanation for Mad3p-dependent arrest defects.

To explicitly link checkpoint and Cdc20p protein functions, both groups went on to identify dominant *SLP1*(*S.p.*)/CDC20(S.c.) mutations (57, 76) that abrogated the mitotic arrest normally induced by either nocodazole or high levels of Mad2p or Mps1p (50, 52). In each case, dominant-mutant Slp1/Cdc20 proteins no longer associated with Mad2p (and Mad3p in budding yeast), indicating that uncoupling communication between Slp1p/Cdc20p and Mad2p or Mad3p results in loss of checkpoint function. Inclusion of mutations in APC proteins conferred cell cycle arrest to cells containing either the dominant-mutant Slp1p or high Cdc20p levels, revealing that APC function occurs after that of Slp1p/Cdc20p. This communication forum extends to vertebrate cell systems. p55CDC, a candidate mammalian homolog of Cdc20p, coimmunoprecipitates with both Mad2p and Cdc27p in extracts made from mitotic HeLa cells. A link between p55CDC and APC function was proposed based on the observation that microinjection of antibodies directed against p55CDC generally resulted in a cell cycle arrest (73). In summary, these studies provide compelling evidence using different cell systems that Cdc20p (and homologs) is the effector and direct target/substrate for the kinetochore/spindle assembly checkpoint signal.

Cells containing high levels of Cdc20p fail to arrest in response to either nocodazole or the replication mutant *cdc13* (which results in incomplete telomere replication) (36), suggesting that Cdc20p is a target for both spindle and DNA damage checkpoint pathways. However, different *CDC20* dominant alleles abrogate the spindle checkpoint pathway but not, or only in a limited fashion, the DNA damage checkpoint pathway (57, 136). Thus, it is likely that the DNA damage pathway does not directly target Cdc20p. Pds1p, however, is a target for the DNA damage checkpoint pathway and is an APC substrate. But if Pds1p is downstream of APC function, how does Pds1p stop APC-mediated degradation of cyclin in DNA damaged cells? Unpublished findings reveal that the nondegradable form of Pds1p blocks cyclin degradation but not the degradation of endogenous Pds1p (O Cohen-Fix & D Koshland, personal communication). Note that nondegradable cyclin does not block degradation of endogenous cyclin, indicating that cyclin stabilization by nondegradable Pds1p

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

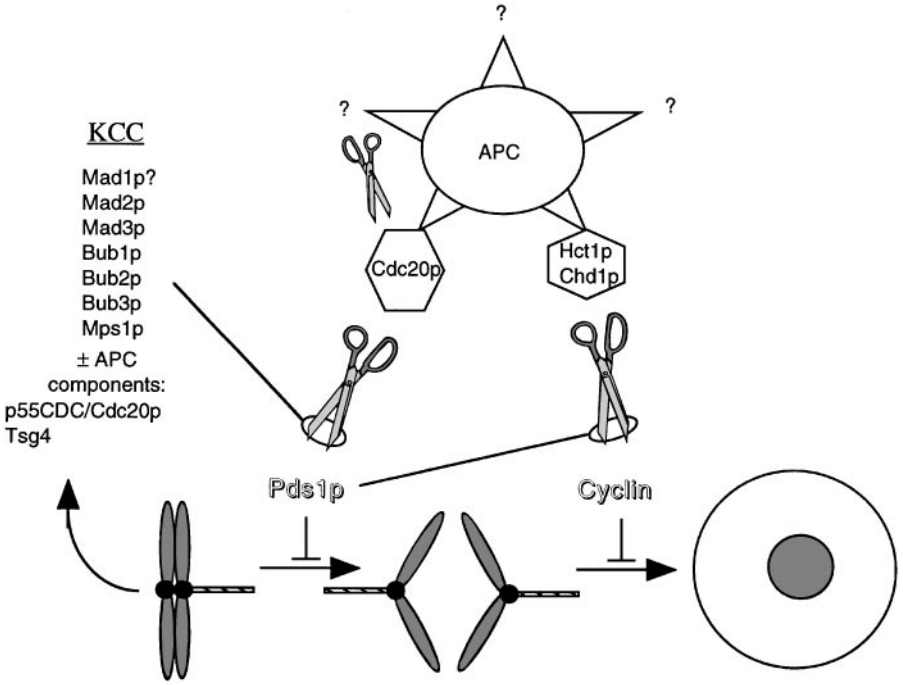

Figure 1 An unattached kinetochore blocks cell cycle progression. One model is that a kinetochore checkpoint complex (KCC) at the kinetochore directly associates with and inhibits (*lasso*) Cdc20p activation of APC-mediated Pds1p degradation (*scissors*). Pds1p in turn inhibits Hct1p/Chd1p-dependent cyclin degradation. See text for details.

is not simply due to substrate competition. These findings are consistent with a model whereby Pds1p functions in two ways: It inhibits both sister chromatid separation (anaphase onset) and Hct1p function, the APC cofactor that mediates cyclin degradation and MPF inactivation (exit from mitosis). This model predicts a sequential order to the mitotic-to-G1 transition (Figure 1): In the absence of spindle or DNA damage, activated Cdc20p facilitates Pds1p degradation and allows for sister chromatid separation. Pds1p degradation allows for activation of Hct1p, which can now promote MPF inactivation and exit from mitosis. Such a pathway provides a kinetic explanation for an old observation by cell biologists. Separation of different sister chromosomes at anaphase onset is precise, coordinated, and very rapid. If Pds1p is both an inhibitor of anaphase A and of APC function, any reduction in Pds1p may increase the rate of both cyclin and Pds1p degradation, continuing in a positive feedback loop that results in logarithmic Pds1p degradation, cyclin degradation, and sister separation (17, 29, 57).

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

Sensors of the Checkpoint Pathway: Looking for Lesions

The checkpoint pathway that responds to defects in the chromosome segregation machinery is well documented, but it is not clear exactly what defect is being monitored. Proposed models include kinetochore/microtubule attachment, tension at kinetochores or along centromeres, chromosome alignment to the metaphase plate, centrosome/spindle pole body disposition, the ratio of tubulin:microtubule polymer, length of preanaphase spindles, and others (42, 48, 114, 176). Given this array of possible modes of activation, it is not surprising how many names have been given to this checkpoint: the spindle assembly checkpoint (107, 152), metaphase checkpoint (42), mitotic checkpoint (89, 163), and kinetochore attachment checkpoint (152). Part of this confused morass of terminology may occur because evolutionarily diverse cell systems may monitor for predominantly different defects. On the other hand, a single cell type may be capable of responding to a variety of unrelated defects.

A wealth of information obtained from large eukaryotic cells suggests that the checkpoint system monitors the status of the kinetochore (107, 114, 130, 176). Kinetochores not properly associated with microtubules emit a "WAIT" signal (70, 71, 126, 177). That the kinetochore is responsible for this signal has been elegantly demonstrated. Mono-oriented autosomal chromosomes elicit a cell cycle arrest in invertebrate meiotic cells. If all the chromosomes are removed from the cell by micromanipulation, that cell exits mitosis with apparently normal kinetics (188). In vertebrate cell systems, laser ablating the last remaining centromere of a mono-oriented chromosome relieves the delay normally associated with unattached kinetochores and again facilitates sister chromatid separation and exit from mitosis (123). The above results are consistent with a model whereby improperly or unattached kinetochores are sufficient to halt cell cycle progression. Dispersement of this kinetochore WAIT signal is less clear. Hela cells that contained two spindles, one spindle in which all chromosomes had bi-oriented while the second contained mono-oriented chromosomes, proceeded to separate all of the sister chromosomes (124). Two of many explanations for this precocious exit from mitosis is that the inhibitory WAIT signal is not diffusible (i.e. maybe it is propagated along and therefore limited to spindle microtubules) or processes set into motion after inactivation of the WAIT signal in one spindle are dominant once set into action.

In *Saccharomyces cerevisiae*, most evidence also identifies the kinetochore as a key target for the checkpoint surveillance system. Checkpoint proteins exhibit genetic interactions with a variety of proteins (microtubule motors, tubulin, spindle pole body determinants, etc) whose ultimate defect could impair or retard kinetochore attachment to microtubules (37, 46, 86, 129, 174, 175). In addition, cells arrest in mitosis in response to both centromere- and kinetochore-specific defects. Cells similarly arrest in the presence of short linear

chromosomes, although the defect that causes this arrest is not clear. Regardless, all of these arrests are abolished in strains harboring mutations in any of the checkpoint proteins tested (56, 86, 120, 156, 168, 175). Interestingly, cells containing dicentric chromosomes exhibit a mid-anaphase delay that is partially abrogated in combination with mutations in either DNA or spindle damage checkpoint proteins, suggesting that dicentric chromosomes generate two forms of damage (10, 111).

Given that unattached kinetochores activate the checkpoint pathway, two opposing models have gained favor. McIntosh (94) first proposed that the checkpoint machinery is sensitive to tension at centromeres generated by opposing mitotic kinetochore/spindle forces. This model has gained strong support. Micromanipulation studies in meiotic cell systems have shown that exogenous tension exerted along chromosome arms both decreases kinetochore phosphorylation and facilitates precocious anaphase onset (87, 88). Kinetochore phosphorylation was monitored using the antibody 3F3 (20, 44). Kinetochore phosphorylation was correlated to checkpoint function by two observations. First, 3F3 decorates unattached kinetochores but not kinetochores saturated with microtubules or associated with bi-oriented chromosomes aligned at the metaphase plate (44). Second, microinjected 3F3 antibody delayed anaphase onset in cells in which all of the chromosomes were bi-oriented and aligned on the metaphase plates. Cells progressed into anaphase only after the antibody was cleared from the kinetochores (14).

On the other hand, there is a growing body of evidence that the checkpoint pathway monitors the availability of kinetochore microtubule binding sites. First, kinetochore-microtubule interactions are stabilized by tension (113, 148), blurring the distinction between the effects of tension per se and the role of tension in facilitating the cooperative binding of additional kinetochore microtubules. Second, the functional correlation between 3F3 antibody binding and anaphase onset observed is not universal. In newt lung cells, 3F3 antibodies stain with equal intensity both free and attached kinetochores in prometaphase and kinetochores of cells in mitosis and anaphase (171). One interpretion of this result is that, at least in these cells, 3F3 decorates kinetochores regardless of changes in tension or microtubule attachment. On the other hand, the 3F3 antibody is poorly defined (20) such that 3F3 staining cannot be attributed to recognition of any one single protein or protein function. Third, the staining intensity of Mad2p at kinetochores is correlated with the number of kinetochore microtubules: On congressing chromosomes, Mad2p was present on the sister kinetochore associated with fewer microtubules (16, 43, 170). More important, a loss in tension did not equate to re-association of normal Mad2p levels to kinetochores in cells treated with taxol. At these taxol levels, cells typically retain their kinetochore microtubules but tension across the centromere (as measured

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

by the distance between sister kinetochores) is dramatically reduced (93, 170), suggesting that the rare Mad2p re-association could be due to the equally rare microtubule turnover and not loss of tension. Given that checkpoint complexes and microtubules may compete for similar kinetochore binding sites, one pleasing aspect of the above model is that it correlates chromosome bi-orientation with checkpoint inactivation. As a free kinetochore captures microtubules, fewer sites are available for kinetochore checkpoint complexes. When all of the microtubule sites are occupied with microtubules, the checkpoint complex is pushed off and any soluble components are blocked from re-associating with the kinetochore, thus inactivating the checkpoint mechanism and allowing for anaphase onset.

Surveillance systems that ensure the accurate completion of chromosome segregation are probably not universal in mechanistic detail (107, 114, 130, 176). For instance, unpaired sex chromosomes delay the cell cycle in mantid but not grasshopper spermatocytes, whereas free kinetochores on autosomal chromosomes will arrest cells in both. Sea urchin zygotes and mouse oocytes (but not spermatocytes) appear unconcerned about unattached kinetochores, whereas the checkpoint sensory mechanism in *Drosophila* appears quite different from any of those described above (85, 87, 95, 115, 151). In addition, checkpoint proteins may be involved in processes unrelated to cell cycle transition. For instance, HsMad2p interacts with the receptor for insulin using both two-hybrid and coimmunoprecipitation methods (117). This interaction was inversely proportional to insulin levels, consistent with the finding that receptor activation resulted in decreased Mad2p binding. Parenthetically, *HsMAD2* maps to a region that is associated with insulin resistance, a predecessor to noninsulin-dependent diabetes mellitus (80). Bub2p (and the fission yeast homolog Cdc16p) (31) contains regions of significant homology to a large family of GTP-activating proteins implicated in regulating vesicle trafficking and cell morphogenesis (112), although the implications of this homology are not clear. Sorting out the specifics of different surveillance mechanisms and checkpoint protein functions may only be resolved step-by-step on a species-by-species basis.

There is evidence from budding yeast that a surveillance mechanism exists that monitors for defects in addition to kinetochore abnormalities. As previously described, overexpression of Mps1p arrests cells in mitosis with morphologically wild-type looking spindles (50). In combination with any of the *bub* or *mad* mutations, cells expressing high levels of Mps1p fail to arrest. However, in combination with *mad1* or *mad2* (but not *mad3*, *bub1*, *bub2*, or *bub3*), cell cycle progression is fatal. When Mps1p is overexpressed after spindle pole body duplication, these double mutants again fail to arrest but now retain high viability (50). These observations are intriguing: (*a*) High levels of Mps1p cause an

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

arrest that depends on each checkpoint protein in cells with morphologically wild-type looking spindles (and by inference, normal kinetochore-microtubule interactions) and (*b*) *MAD1* and *MAD2* rescue overexpressed *MPS1*-dependent inviability whereas *MAD3* and *BUB* gene products do not. A likely model that explains these observations is that Mad1p and Mad2p monitor for both spindle pole body and kinetochore defects. Alternatively, Mad1p and Mad2p may act parallel to or downstream of Mps1p checkpoint function. Similarly, high levels of the dominant allele *BUB1-5* arrest cells in mitosis with wild-type-looking spindles (32). Cells no longer arrested when high levels of *BUB1-5* were combined with mutations in *mad1-mad3*, *bub3*, or *mps1* (*bub2* gave an intermediate result). Interestingly, cell cycle progression in these double mutants yielded very different cell viabilities: Cells with high levels of BUB1-5 combined with *mad1*, *mad2*, or *mps1* exhibited about a twofold decrease in cell viability, whereas cells with high levels of *BUB1-5* combined with *bub1* or *bub3* exhibited roughly a twofold increase in cell viability compared with wild-type cells. Although there is no evidence that Bub1p is involved in spindle pole body function, *BUB1-5* clearly generates lesions visible to some but not all components of the checkpoint pathway. It is hoped that further investigation will provide more compelling data regarding alternative sensory checkpoint mechanisms.

Putting It Together: Assembly of Kinetochore and Checkpoint Complexes

All of the information obtained to date reveals that, in every aspect, kinetochore and checkpoint functions are intricately linked. Thus it is not surprising that mutations that affect kinetochore function can also have profound effects on checkpoint mechanisms. Often, kinetochore defects are incurred early in the cell cycle during S-phase. Studies from vertebrate cell systems indicate a clear temporal order of protein recruitment/assembly on the kinetochore that includes chromatin scaffold, microtubule motors, and checkpoint proteins. Defects incurred during kinetochore assembly result in cell cycle arrest (7, 8, 14, 90a, 135, 145, 164, 180, 183; G Chan, B Schaar & T Yen, personal communication). For instance, injection of antibodies directed against a subset of kinetochore proteins altered kinetochore structure, inhibited chromosome movement, and ultimately arrested cells prior to anaphase. In most cases, the timing of injection was critical: Cells arrested in mitosis only if they were injected early in the cell cycle at or before the S/G2 border. The fact that cells injected during prophase or prometaphase failed to arrest argued that the action of the antibody was to inhibit assembly of a complex important for cell cycle transitions (26).

In yeast, kinetochore assembly is also staged such that kinetochore protein subcomplexes exist at discrete steps (30, 101). Both Mif2p and Ndc10p interact

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

with solitary CDE III. CDE III–bound Mif2p is decreased in *ndc10* mutant strains, which suggests that Mif2p association with CDE III occurs in cooperation with Ndc10p (101). Analysis of UV cross-linked CDE III DNA-protein complexes further revealed that three of four CBF3 components (Ctf13p, Ncd10p, and Cep3p) bind CDE III directly and that binding requires the activity of all four proteins, including Skp1p (30). Interestingly, even a minimal CBF3-CDE III complex appears functional in that inclusion of CDE III on a 2-μm plasmid reduced the plasmid copy number, whereas cell growth on selection media that increased CDEIII plasmid copy number incurred increased chromosome instability in vivo (138). Cbf1p, which binds CDE I in vitro (5), requires CDE I–III for association in vivo (101), which suggests that Ndc10p, Cep3p, Mif2p, and Ctf13p recruitment to CDE III facilitates further protein association, possibly by changes in chromatin conformation (101, 154). As found for vertebrate cell kinetochores, perturbations of early assembly events in yeast cells have drastic effects on kinetochore function: Higher-order structures associated with centromeres are abolished or chromosome stability decreased by mutations in *cis* (34), limited levels or mutations in histone-related proteins (98, 134, 153, 158), or factors implicated in chromatin remodeling (6). In fission yeast, mutations that perturb heterochromatin formation or derepress silencing also impair kinetochore function (1). These findings suggest that processes that occur early during DNA replication or chromatin remodeling are critical for proper kinetochore assembly.

Interestingly, ubiquitin-dependent processes regulate facets of kinetochore assembly reactions for Ndc10, Ctf13, Cep3, and Mif2 kinetochore proteins. Ndc10p not associated with CEN DNA is ubiquitinated while *ndc10* strains are rescued by overexpression of ubiquitin-conjugating enzymes Ubc6p and Cdc34p or second site mutations in *UBC6* or *UBC7* (79, 184). Because Ndc10p is phosphorylated (67) and CBF3 phosphorylation is correlated with CEN binding activity (84, 184), phosphorylation and ubiquitination may form opposing pathways to kinetochore assembly reactions: Phosphorylated Ndc10p is activated to bind CEN DNA while unbound Ndc10p is ubiquitinated and, presumably, targeted for degradation (74, 79, 184). Just such a mechanism has been proposed for Ctf13p: Phosphorylated Ctf13p is activated to bind CEN DNA while Ctf13p ($+/-$ phosphorylation) unbound to CEN DNA is ubiquitinated in a Cdc34p-dependent manner (74). The kinase that phosphorylates Ctf13p is unidentified, but kinases already implicated in centromere function are likely candidates (65, 67, 142). In addition to Ndc10p and Ctf13p, there is evidence that Cep3p is also regulated by ubiquitination: Cep3p physically interacts with Ubc9p, another ubiquitin-conjugating enzyme (65). Finally, Meluh & Koshland (100) identified a suppressor of *mif2* (*SMT3*) that was independently cloned and found to encode the yeast homolog of a small ubiquitin-like modifier

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

(SUMO). SUMO-modification has been observed for a variety of proteins and may represent a new class of posttranslation modifications required for protein activation (131). As yet, a role for ubiquitin or ubiquitin-like proteins in regulating checkpoint assembly has not been defined.

There is evidence from budding yeast that assemblies of chromosome segregation and checkpoint complexes are linked. As opposed to other kinetochore mutations (*ctf13*, *cep3*, *skp1*), *ndc10* strains fail to arrest in mitosis but allow for spindle elongation, cytokinesis, and DNA re-replication in the absence of nuclear division. The point of execution for the *ndc10* lesion occurs early in the cell cycle during DNA replication. One interpretation of these findings is that mutant Ndc10p abolishes both kinetochore and checkpoint assembly reactions during S-phase and allows cells to precociously exit mitosis (40, 79). Alternatively, faulty Ndc10p may significantly weaken the kinetochore-microtubule interaction and allow for mitotic forces to rip the kinetochore away from the bulk of the chromosomes. Just such a phenomenon has been reported for vertebrate cells that enter mitosis in the absence of DNA replication (9).

Further evidence suggests that kinetochore and checkpoint assembly pathways are coordinated early in the cell cycle. First, Mad1p can become hyperphosphorylated prior to a rise in Clb2p levels, suggesting that this pathway can be activated early in the cell cycle. Mad1p appears refractory to hyperphosphorylation in G1, giving a temporal specificity to this reaction and suggesting that the Mad1p-kinase is either inactive or not properly targeted in G1. In addition, checkpoint proteins genetically interact with two gene products implicated in early cell cycle events: Chl1p/Ctf1p and Ctf7p. For instance, *mad2-1* severely compromises viability in combination with *chl1*/*ctf1*. Chl1p/Ctf1p (38, 47) shares short regions of homology to the ATPase and helicase domains of Rad3p, a nucleotide excision repair protein. Putative human homologs of yeast Chl1p/Ctf1p (hCHLR1 and hCHLR2) bind DNA and localize predominantly in nucleoli (2, 38). Weiler and coworkers (172) observed that *chl1*/*ctf1* mutant strains switch mating types by random usage of *HML* and *HMR* loci, and both Weiler et al (172) and Amann et al (2) have proposed that Chl1p/Ctf1p functions in assembling higher-order chromatin structures. *CTF7* encodes a nuclear protein and is essential for proper chromosome segregation, and the mitotic delay observed for *ctf7* strains depends on Mad2 and Bub3 checkpoint proteins. As for *ndc10* alleles, the point of execution in *ctf7* strains occurs during S-phase. Interestingly, high levels of Pol30p (the yeast DNA replication processivity factor PCNNA) suppress *ctf7* phenotypes and *ctf7* mutations genetically interact with alleles of both *POL30* and *CHL1*/*CTF1* (R Skibbens, L Corson, D Koshland & P Hieter, personal communication). One interpretation of these genetic interactions is that kinetochore and checkpoint assemblies are coordinated with PCNA-dependent DNA replication.

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

As revealed by mutations in several DNA replication proteins (Cdc6p, Dbf4p, Cdc7p, etc), replication appears to be essential for establishing the checkpoint mechanism that monitors for defects in chromosome segregation (121, 162, 165). For instance, Dbf4p and Cdc7p form a complex and are essential for initiation of DNA replication (64). *dbf4* mutant strains fail to replicate their DNA but undergo a reductional anaphase such that spindles elongate and MPF is inactivated. However, *dbf4* cells first arrested in S-phase with hydroxyurea and then released at the restrictive temperature arrested in mitosis, suggesting that processes occurring coordinately with replication were required for the establishment of a functional checkpoint (165). Based on these observations, Tavormina and coworkers (162) looked at strains harboring *dbf4* in combination with a mutation either in the kinetochore protein Ctf13p or in the presence of nocodazole to ask whether cells that failed to initiate replication could arrest in response to spindle/kinetochore damage. Strains harboring both *dbf4* and *ctf13-30* failed to arrest while *dbf4* strains treated with nocodazole arrested in mitosis. Understanding the mechanism that separates these effects is key: Unreplicated but wild-type kinetochores retain the capability of assembling a checkpoint complex; unreplicated but defective kinetochores fail to assemble a checkpoint complex and thus fail to elicit arrest in mitosis (162). In combination with the studies presented above, these findings suggest that chromosome segregation and checkpoint functions during mitosis are licensed in S-phase, analogous to the formation of prereplication complexes, by coupling replication to kinetochore and checkpoint assembly reactions.

Compromising the Checkpoint Response: Adaptation and Cancer

In response to defects in the chromosome segregation machinery, multicellular organisms face a complicated decision. To remain arrested is certain death; to divide and generate aneuploid daughter cells could have grave consequences for the organism—not an enviable choice for a cell. Taylor & McKeon (163) presented evidence that organisms outrank cells: Prolonged nocodazole treatment resulted in exit from mitosis but also in the subsequent activation of an apoptotic pathway, killing the cells. A key finding was that cells that expressed a dominant negative allele of *BUB1* exited mitosis without activating the apoptotic pathway, leading the authors to propose that cells exiting mitosis with activated checkpoints respond by committing suicide and thus saving the organism. Is there any evidence that defects in the checkpoint pathway could result in cancer? The correlative evidence is loud and clear. Colon cancers are thought to arise by two mechanisms: (*a*) Defects in mismatch repair lead to increased mutation rates and microsatellite instability (MIN), and (*b*) improper sister chromosome segregation (or some related mechanism) leads to aneuploidy or chromosomal instability (CIN) (119). Most colorectal cancers incur

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

chromosome instability. Based on findings that Bub1p was diminished in cancer cells and that dominant *BUB1* alleles allowed for cell cycle progression without subsequent activation of apoptosis (163), Cahill et al (13) sequenced the *BUB1* coding regions from tissues obtained from two colorectal cancer patients and found that both contained mutations in the checkpoint open reading frame. Functional analysis showed that these *BUB1* mutations exhibited dominant negative effects: CIN cells that normally retain functional checkpoint controls were severely compromised for checkpoint function when expressing the dominant negative allele. Although the causal mechanism of cancer in CIN cells remains to be clearly established, evidence is growing that checkpoint gene products are cancer-related targets.

Recently, the gene for the human homolog of Sc Mad1p, TXBP181 or Hs*MAD1*, was cloned and found to be conserved in both sequence and function (69). HsMad1p interacts with itself and HsMad2p. In hopes that perturbing these protein-protein interactions would abrogate checkpoint function, a 174-polypeptide fragment of HsMad1p was identified that associated with endogenous HsMad1p but not HsMad2p. Expression of this fragment resulted in the accumulation of multinucleated cells, the frequency of which dramatically increased when cells were treated with nocodazole. These results suggest that this fragment acts as a dominant negative mutation similar to that employed by Taylor & McKeon (163) for Bub1p and ascribes checkpoint function to human Mad1p. More important, however, is that HsMad1p checkpoint function appears to be another cancer-related target. HsMad1p also interacts with Tax, a phospho-oncoprotein encoded by human T cell leukemia virus type 1 (HTLV-1). The region that directs HsMad1p dimerization is required for HsMad1p-Tax heterodimer formation, suggesting that these are competitive interactions. Expression of either Tax or the 174-residue fragment of HsMad1p increased the frequency of multinucleated cells. However, coexpression of HsMad1p reduced the number of multinucleated cells in Tax-expressing cells. Based in part on this evidence, Jin and coworkers (69) proposed that the HTLV-1 protein Tax compromises checkpoint function by inhibiting HsMad1p homodimer formation. Indeed, Tax-expresssing cells had decreased levels of HsMad1p. Similarly, Taylor & McKeon (163) found that the level of Bub1p was diminished in samples harvested from cancer patients. The culmination of findings reported by Cahill et al (13) and Taylor & McKeon (163) for Bub1p and Jin et al (69) for Mad1p suggest that checkpoint proteins will be a recurring theme as oncogenic and cancer-inducing targets. Researchers in the checkpoint field have an open door and a vast body of knowledge to carry with them.

ACKNOWLEDGMENTS

Writing a current review requires the cooperation of the research community as a whole. We are deeply indebted to the many collegues who made accessible

data prior to publication: JC Waters, R-H Chen, AW Murray, ED Salmon, K Farr, A Hoyt, G Chan, B Schaar, T Yen, M Kallio, J Daum, J Weinstein, D Burke, G Gorbsky, L Corson, D Koshland, O Cohen-Fix, K Hyland, P Meluh, M Smith, P Yang, L Glowczekski, J Kingsbury, F Spencer, R Krishnan, B Goodman, D-Y Jin, K-T Jeang, C Collins, and G Stetten, to all we say thanks. Few tasks are as thankless as reviewing manuscript drafts. No greater appreciation can we acknowledge to our readers—Drs. Forrest Spencer, Cindy Dougherty, and Orna Cohen-Fix—other than to state that you are responsible for any information that is correct or presented clearly. We take the blame for the rest. RVS extends special thanks to D Koshland for his patience during the writing of this review and to M Kenna, JW Skibbens, and family for their love and support.

Visit the *Annual Reviews home page* at http://www.AnnualReviews.org

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org by Lehigh University on 07/27/05. For personal use only.

Literature Cited

1. Allshire RC. 1997. Centromeres, checkpoints and chromatid cohesion. *Curr. Opin. Gen. Dev.* 7:264–73
2. Amann J, Kidd VJ, Lahti JM. 1997. Characterization of putative human homologues of the yeast chromosome transmission fidelity gene, CHL1. *J. Biol. Chem.* 272:3823–32
3. Amon A, Irniger S, Nasmyth K. 1994. Closing the cell cycle circle in yeast: G2 cyclin proteolysis initiated at mitosis persists until the activation of G1 cyclins in the next cycle. *Cell* 77:1037–50

3a. Andrews B, Measday V. 1998. The cyclin family of budding yeast: abundant use of a good idea. *Trends Genet.* 14:66–72

4. Bai C, Sen P, Hofmann K, Ma L, Goebl M, et al. 1996. SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. *Cell* 86:263–74
5. Baker RE, Fitzgerald-Hayes M, O'Brien TC. 1989. Purification of the yeast centromere binding protein CP1 and a mutational analysis of its binding site. *J. Biol. Chem.* 264:10843–50
6. Basrai MA, Kingsbury J, Koshland D, Spencer F, Hieter P. 1996. Faithful chromosome transmission requires Spt4p, a putative regulator of chromatin structure in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 16:2838–47
7. Bernat RL, Borisy GG, Rothfield NF, Earnshaw WC. 1990. Injection of anticentromere antibodies in interphase disrupts events required for chromosome movement at mitosis. *J. Cell Biol.* 111:1519–33
8. Bernat RL, Delannoy MR, Rothfield NF, Earnshaw WC. 1991. Disruption of centromere assembly during interphase inhibits kinetochore morphogenesis and function in mitosis. *Cell* 66:1229–38
9. Brinkley BR, Zinkowski RP, Mollon WL, Davis FM, Pisegna MA, et al. 1988. Movement and segregation of kinetochores experimentally detached from mammalian chromosomes. *Nature* 336: 251–54
10. Brock JA, Bloom K. 1994. A chromosome breakage assay to monitor mitotic forces in budding yeast. *J. Cell Sci.* 107:891–902
11. Brown MT, Goetsch L, Hartwell LH. 1993. MIF2 is required for mitotic spindle integrity during anaphase spindle elongation in *Saccharomyces cerevisiae*. *J. Cell Biol.* 123:387–403
12. Cadwell C, Yoon HJ, Zebarjadian Y, Carbon J. 1997. The yeast nucleolar protein Cbf5p is involved in rRNA biosynthesis and interacts genetically with the RNA polymerase I transcription factor RRN3. *Mol. Cell Biol.* 17:6175–83
13. Cahill DP, Lengauer C, Yu J, Riggins GJ, Willson JKV, et al. 1998. Mutations of

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

mitotic checkpoint genes in human cancers. *Nature* 392:300–3
14. Campbell MS, Gorbsky GJ. 1995. Microinjection of mitotic cells with the 3F3/2 anti-phosphoepitope antibody delays the onset of anaphase. *J. Cell Biol.* 129:1195–204
15. Deleted in proof
16. Chen RH, Waters JC, Salmon ED, Murray AW. 1996. Association of spindle assembly checkpoint component XMAD2 with unattached kinetochores. *Science* 274:242–46
17. Cohen-Fix O, Koshland D. 1997. The metaphase-to-anaphase transition: avoiding a mid-life crisis. *Curr. Opin. Cell Biol.* 9:800–6
18. Cohen-Fix O, Peters JM, Kirschner MW, Koshland D. 1996. Anaphase initiation in *Saccharomyces cerevisiae* is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. *Genes Dev.* 10:3081–93
19. Connelly C, Hieter P. 1996. Budding yeast SKP1 encodes an evolutionarily conserved kinetochore protein required for cell cycle progression. *Cell* 86:275–85
20. Cyert MS, Scherson T, Kirschner MW. 1988. Monoclonal antibodies specific for thiophosphorylated proteins recognize *Xenopus* MPF. *Dev. Biol.* 129:209–16
21. Dawson IA, Roth S, Akam M, Artavanis-Tsakonas S. 1993. Mutations of the *fizzy* locus cause metaphase arrest in *Drosophila melanogaster* embryos. *Development* 117:359–76
22. Dawson IA, Roth S, Artavanis-Tsakonas S. 1995. The Drosophila cell cycle gene *fizzy* is required for normal degradation of cyclins A and B during mitosis and has homology to the CDC20 gene of *Saccharomyces cerevisiae*. *J. Cell Biol.* 129:725–37
23. Desai A, Mitchison TJ. 1995. A new role for motor proteins as couplers to depolymerizing microtubules. *J. Cell Biol.* 128:1–4
24. Deshaies RJ. 1997. Phosphorylation and proteolysis: partners in the regulation of cell division in budding yeast. *Cur. Opin. Gen. Dev.* 7:7–16
25. Doheny KF, Sorger PK, Hyman AA, Tugendreich S, Spencer F, Hieter P. 1993. Identification of essential components of the *S. cerevisiae* kinetochore. *Cell* 73: 761–74
26. Earnshaw WC, Bernat RL, Cooke CA, Rothfield NF. 1991. Role of the centromere/kinetochore in cell cycle control. *Cold Spring Harbor Symp. Quant. Biol.* 56:675–85
27. Earnshaw WC, Pluta AF. 1994. Mitosis. *BioEssays* 16:639–43
28. Elledge SJ. 1996. Cell cycle checkpoints: preventing an identity crisis. *Science* 274:1664–72
29. Elledge SJ. 1998. Mitotic arrest: Mad2 prevents *sleepy* from waking up the APC. *Science* 279:999–1000
30. Espelin CW, Kaplan KB, Sorger PK. 1998. Probing the architecture of a simple kinetochore using DNA-protein crosslinking. *J. Cell Biol.* 139:1383–96
31. Fankhauser C, Marks J, Reymond A, Simanis V. 1993. The *S. pombe* cdc16 gene is required both for maintenance of p34cdc2 kinase activity and regulation of septum formation: a link between mitosis and cytokinesis. *EMBO J.* 12:2697–704
32. Farr KA, Hoyt MA. 1998. Bub1p kinase activates the *Saccharomyces cerevisiae* spindle assembly checkpoint. *Mol. Cell. Biol.* 18:2738–47
33. Feldman RM, Correll CC, Kaplan KB, Deshaies RJ. 1997. A complex of Cdc4p, Skp1p, and Cdc53p/cullin catalyzes ubiquitination of the phosphorylated CDK inhibitor Sic1p. *Cell* 91:221–30
34. Fitzgerald-Hayes M. 1987. Yeast centromeres. *Yeast* 3:187–200
35. Funabiki H, Yamano H, Kumada K, Nagao K, Hunt T, Yanagida M. 1996. Cut2 proteolysis required for sister-chromatid seperation in fission yeast. *Nature* 381:438–41
36. Garvik B, Carson M, Hartwell L. 1995. Single-stranded DNA arising at telomeres in cdc13 mutants may constitute a specific signal for the RAD9 checkpoint. *Mol. Cell. Biol.* 15:6128–38
37. Geiser JR, Schott EJ, Kingsbury TJ, Cole NB, Totis LJ, et al. 1997. *Saccharomyces cerevisiae* genes required in the absence of the CIN8-encoded spindle motor act in functionally diverse mitotic pathways. *Mol. Biol. Cell* 8:1035–50
38. Gerring SL, Spencer F, Hieter P. 1990. The CHL1 (CTF1) gene product of *Saccharomyces cerevisiae* is important for chromosome transmission and normal cell cycle progression in G2/M. *EMBO J.* 9:4347–58
39. Glotzer M, Murray AW, Kirschner MW. 1991. Cyclin is degraded by the ubiquitin pathway. *Nature* 349:132–38
40. Goh PY, Kilmartin JV. 1993. NDC10: a gene involved in chromosome segrega-

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

tion in *Saccharomyces cerevisiae*. *J. Cell Biol.* 121:503–12
41. Gomes R, Karess RE, Ohkura H, Glover DM, Sunkel CE. 1993. Abnormal anaphase resolution (aar): a locus required for progression through mitosis in Drosophila. *J. Cell Sci.* 104:583–93
42. Gorbsky GJ. 1997. Cell cycle checkpoints: arresting progress in mitosis. *BioEssays* 19:193–97
43. Gorbsky GJ, Chen R-H, Murray AW. 1998. Microinjection of antibody to Mad2 protein into mammalian cells in mitosis induces premature anaphase. *J. Cell Biol.* 141:1193–205
44. Gorbsky GJ, Ricketts WA. 1993. Differential expression of a phosphoepitope at the kinetochores of moving chromosomes. *J. Cell Biol.* 122:1311–21
45. Guacci V, Koshland D, Strunnik A. 1997. A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of MCD1 in *S. cerevisiae*. *Cell* 91:47–57
46. Guenette S, Magendantz M, Solomon F. 1995. Suppression of a conditional mutation in alpha-tubulin by overexpression of two checkpoint genes. *J. Cell Sci.* 108: 1195–204
47. Haber JE. 1974. Bisexual mating behavior in a diploid of *Saccharomyces cerevisiae*: vidence for genetically controlled nonrandom chromosome loss during vegetative growth. *Genetics* 78: 843–58
48. Hardwick KG. 1998. The spindle checkpoint. *Trends Genet.* 14:1–4
49. Hardwick KG, Murray AW. 1995. Mad1p, a phosphoprotein component of the spindle assembly checkpoint in budding yeast. *J. Cell Biol.* 131:709–20
50. Hardwick KG, Weiss E, Luca FC, Winey M, Murray AW. 1996. Activation of the budding yeast spindle assembly checkpoint without mitotic spindle disruption. *Science* 273:953–56
51. Hartwell LH, Weinert TA. 1989. Checkpoints: controls that ensure the order of cell cycle events. *Science* 246:629–34
52. He X, Patterson TE, Sazer S. 1997. The *Schizosaccharomyces pombe* spindle checkpoint protein mad2p blocks anaphase and genetically interacts with the anaphase-promoting complex. *Proc. Natl. Acad. Sci. USA* 94:7965–70
53. Healy AM, Zolnierowicz S, Stapleton AE, Goebl M, DePaoli-Roach AA, Pringle JR. 1991. CDC55, a *Saccharomyces cerevisiae* gene involved in cellular morphogenesis: identification, characterization, and homology to the B subunit of mammalian type 2A protein phosphatase. *Mol. Cell Biol.* 11:5767–80
54. Holloway SL, Glotzer M, King RW, Murray AW. 1993. Anaphase is initiated by proteolysis rather than by the inactivation of maturation-promoting factor. *Cell* 73:1393–402
55. Hoyt MA, Geiser JR. 1996. Genetic analysis of the mitotic spindle. *Annu. Rev. Genet.* 30:7–33
56. Hoyt MA, Totis L, Roberts BT. 1991. *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. *Cell* 66:507–17
57. Hwang LH, Lucius LF, Smith DL, Mistrot CA, Hardwick KG, et al. 1998. Budding yeast Cdc20: A target of the spindle checkpoint. *Science* 279:1041–44
58. Deleted in proof
59. Deleted in proof
60. Hyman AA, Middleton K, Centola M, Mitchison TJ, Carbon J. 1992. Microtubule-motor activity of a yeast centromere-binding protein complex. *Nature* 359:533–36
61. Hyman AA, Sorger PK. 1995. Structure and function of kinetochores in budding yeast. *Annu. Rev. Cell Dev. Biol.* 11:471–95
62. Inoue S, Salmon ED. 1995. Force generation by microtubule assembly/ disassembly in mitosis and related movements. *Mol. Biol. Cell* 6:1619–40
63. Irniger S, Piatti S, Michaelis C, Nasmyth K. 1995. Genes involved in sister chromatid separation are needed for B-type cyclin proteolysis in budding yeast. *Cell* 81:269–78
64. Jackson AL, Pahl PM, Harrison K, Rosamond J, Sclafani RA. 1993. Cell cycle regulation of the yeast Cdc7 protein kinase by association with the Dbf4 protein. *Mol. Cell Biol.* 13:2899–908
65. Jiang W, Koltin Y. 1996. Two-hybrid interaction of a human UBC9 homog with centromere proteins of *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* 251:153–60
66. Jiang W, Lechner J, Carbon J. 1993. Isolation and characterization of a gene (CBF2) specifying a protein component of the budding yeast kinetochore. *J. Cell Biol.* 121:513–19
67. Jiang W, Lim MY, Yoon HJ, Thorner J, Martin GS, Carbon J. 1995. Overexpression of the yeast MCK1 protein kinase suppresses conditional mutations in centromere-binding protein genes CBF2 and CBF5. *Mol. Gen. Genet.* 246:360–66

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

68. Jiang W, Middleton K, Yoon HJ, Fouquet C, Carbon J. 1993. An essential yeast protein, CBF5p, binds in vitro to centromeres and microtubules. *Mol. Cell Biol.* 13:4884–93
69. Jin D-Y, Spencer F, Jeang K-T. 1998. Human T cell Leukemia Virus Type 1 oncoprotein Tax targets the human mitotic checkpoint protein Mad1. *Cell* 93:81–91
70. Jordan MA, Thrower D, Wilson L. 1992. Effects of vinblastine, podophyllotoxin and nocodazole on mitotic spindles. Implications for the role of microtubule dynamics in mitosis. *J. Cell Sci.* 102:401–16
71. Jordan MA, Toso RJ, Thrower D, Wilson L. 1993. Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations. *Proc. Natl. Acad. Sci. USA* 90:9552–56
72. Jorgensen P-M, Brundell E, Starborg M, Hoog C. 1998. A subunit of the anaphase-promoting complex is a centromere-associated protein in mammalian cells. *Mol. Cell Biol.* 18:468–76
73. Kallio M, Daum J, Weinstein JR, Burke DJ, Gorbsky G. 1998. Mammalian p55CDC mediates association of the spindle checkpoint protein Mad2 with the cyclosome/anaphase-promoting complex and is involved in regulating anaphase onset and late mitotic events. *J. Cell Biol.* 141:1393–406
74. Kaplan KB, Hyman AA, Sorger PK. 1997. Regulating the yeast kinetochore by ubiquitin-dependent degradation and Skp1p-mediated phosphorylation. *Cell* 91:491–500
75. Khodjakov A, Rieder CL. 1996. Kinetochores moving away from their associated pole do not exert a significant pushing force on the chromosome. *J. Cell Biol.* 135:315–27
76. Kim SH, Lin DP, Matsumoto S, Kitazono A, Matsumoto T. 1998. Fission yeast Slp1: an effector of the Mad2–dependent spindle checkpoint. *Science* 279:1045–47
77. King RW, Deshaies RJ, Peters JM, Kirschner MW. 1996. How proteolysis drives the cell cycle. *Science* 274:1652–59
78. King RW, Peters JM, Tugendreich S, Rolfe M, Hieter P, Kirschner MW. 1995. A 20S complex containing CDC27 and CDC16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B. *Cell* 81:279–88
79. Kopski KM, Huffaker TC. 1997. Suppressors of the ndc10–2 mutation: a role for the ubiquitin system in *Saccharomyces cerevisiae* kinetochore function. *Genetics* 147:409–20
80. Krishnan R, Goodman B, Jin D-Y, Jeang K-T, Collins C, et al. 1998. Map location and gene structure of the Homo sapiens mitotic arrest deficient 2 (MAD2L1) gene at 4q27. *Genomics* 49:475–78
81. Kroll ES, Hyland KM, Hieter P, Li JJ. 1996. Establishing genetic interactions by a synthetic dosage lethality phenotype. *Genetics* 143:95–102
82. Lamb JR, Michaud WA, Sikorski RS, Hieter PA. 1994. Cdc16p, Cdc23p and Cdc27p form a complex for mitosis. *EMBO J.* 13:4321–28
83. Lechner J. 1994. A zinc finger protein, essential for chromosome segregation, constitutes a putative DNA binding subunit of the *Saccharomyces cerevisiae* kinetochore complex, Cbf3. *EMBO J.* 13:5203–11
84. Lechner J, Carbon J. 1991. A 240 kd multisubunit protein complex, CBF3, is a major component of the budding yeast centromere. *Cell* 64:717–25
85. LeMaire-Adkins R, Radke K, Hunt PA. 1997. Lack of checkpoint control at the metaphase/anaphase transition: a mechanism of meiotic nondisjunction in mammalian females. *J. Cell Biol.* 139:1611–19
86. Li R, Murray AW. 1991. Feedback control of mitosis in budding yeast. *Cell* 66:519–31
87. Li X, Nicklas RB. 1995. Mitotic forces control a cell-cycle checkpoint. *Nature* 373:630–32
88. Li X, Nicklas RB. 1997. Tension-sensitive kinetochore phosphorylation and the chromosome distribution checkpoint in praying mantid spermatocytes. *J. Cell Sci.* 110:537–45
89. Li Y, Benezra R. 1996. Identification of a human mitotic checkpoint gene: hsMAD2. *Science* 274:246–48
90. Li Y, Gorbea C, Mahaffey D, Rechsteiner M, Benezra R. 1997. MAD2 associates with the cyclosome/anaphase-promoting complex and inhibits its activity. *Proc. Natl. Acad. Sci. USA* 94:12431–36
90a. Liao H, Winkfein RJ, Mack G, Rattner JB, Yen TJ. 1995. CENP-F is a proteini od the nuclear matrix that assembles onto kinetochores at late G2 and is rapidly degraded after mitosis. *J. Cell Biol.* 130:507–18
91. Matsumoto T. 1997. A fission yeast homolog of CDC20/p55CDC/Fizzy is required for recovery from DNA damage

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

and genetically interacts with p34cdc2. *Mol. Cell Biol.* 17:742–50
92. McEwen BF, Arena JT, Frank J, Rieder CL. 1993. Structure of the colcemid-treated PtK1 kinetochore outer plate as determined by high voltage electron microscopic tomography. *J. Cell Biol.* 120: 301–12
93. McEwen BF, Heagle AB, Cassels GO, Buttle KF, Rieder CL. 1997. Kinetochore fiber maturation in PtK1 cells and its implications for the mechanisms of chromosome congression and anaphase onset. *J. Cell Biol.* 137:1567–80
94. McIntosh JR. 1991. Structural and mechanical control of mitotic progression. *Cold Spring Harbor Symp. Quant. Biol.* 56:613–19
95. McKim KS, Jang JK, Theurkauf WE, Hawley RS. 1993. Mechanical basis of meiotic metaphase arrest. *Nature* 362: 364–66
96. Deleted in proof
97. Meeks-Wagner D, Wood JS, Garvik B, Hartwell LH. 1986. Isolation of two genes that affect mitotic chromosome transmission in *S. cerevisiae*. *Cell* 44:53–63
98. Megee PC, Morgan BA, Smith MM. 1995. Histone H4 and the maintenance of genome integrity. *Genes Dev.* 9:1716–27
99. Meier UT, Blobel G. 1994. NAP57, a mammalian nucleolar protein with a putative homolog in yeast and bacteria. *J. Cell Biol.* 127:1505–14
100. Meluh PB, Koshland D. 1995. Evidence that the MIF2 gene of *Saccharomyces cerevisiae* encodes a centromere protein with homology to the mammalian centromere protein CENP-C. *Mol. Biol. Cell* 6:793–807
101. Meluh PB, Koshland D. 1997. Budding yeast centromere composition and assembly as revealed by in vivo cross-linking. *Genes Dev.* 11:3401–12
102. Michaelis C, Ciosk R, Nasmyth K. 1997. Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. *Cell* 91:35–45
103. Middleton K, Carbon J. 1994. KAR3–encoded kinesin is a minus-end-directed motor that functions with centromere binding proteins (CBF3) on an in vitro yeast kinetochore. *Proc. Natl. Acad. Sci. USA* 91:7212–16
104. Minshull J, Straight A, Rudner AD, Dernburg AF, Belmont A, Murray AW. 1996. Protein phosphatase 2A regulates MPF activity and sister chromatid cohesion in budding yeast. *Curr. Biol.* 6:1609–20
105. Minshull J, Sun H, Tonks NK, Murray AW. 1994. A MAP kinase-dependent spindle assembly checkpoint in Xenopus egg extracts. *Cell* 79:475–86
106. Murray A. 1995. Cyclin ubiquitination: the destructive end of mitosis. *Cell* 81: 149–52
107. Murray AW. 1994. Cell cycle checkpoints. *Curr. Opin. Cell Biol.* 6:872–76
108. Murray AW. 1995. The genetics of cell cycle checkpoints. *Curr. Opin. Gen. Dev.* 5:5–11
109. Nasmyth K. 1993. Control of the yeast cell cycle by the Cdc28 protein kinase. *Curr. Opin. Cell Biol.* 5:166–79
110. Nasmyth K. 1996. Viewpoint: putting the cell cycle in order. *Science* 274: 1643–45
111. Neff MW, Burke DJ. 1992. A delay in the *Saccharomyces cerevisiae* cell cycle that is induced by a dicentric chromosome and dependent upon mitotic checkpoints. *Mol. Cell Biol.* 12:3857–64
112. Neuwald AF. 1997. A shared domain between a spindle assembly checkpoint protein and Ypt/Rab-specific GTPase-activators. *Trends Biochem. Sci.* 22:243–44
113. Nicklas RB. 1971. Mitosis. *Adv. Cell Biol.* 2:225–97
114. Nicklas RB. 1997. How cells get the right chromosomes. *Science* 275:632–37
115. Nicklas RB, Ward SC, Gorbsky GJ. 1995. Kinetochore chemistry is sensitive to tension and may link mitotic forces to a cell cycle checkpoint. *J. Cell Biol.* 130:929–39
116. Nurse P. 1994. Ordering S phase and M phase in the cell. *Cell* 79:547–50
117. O'Neill TJ, Zhu Y, Gustafson TA. 1997. Interaction of Mad2 with the carboxyl terminus of the insulin receptor but not with IGFIR. Evidence for release from the insulin receptor after activation. *J. Biol. Chem.* 272:10035–40
118. O'Toole ET, Mastronarde DN, Giddings TH Jr, Winey M, Burke DJ, McIntosh JR. 1997. Three-dimensional analysis and ultrastructural design of mitotic spindles from the cdc20 mutant of *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 8:1–11
119. Orr-Weaver TL, Weinberg RA. 1998. A checkpoint on the road to cancer. *Nature* 392:223–24
119a. Palmer DK, O'Day K, Tron HL, Charbonneau H, Margolis RL. 1991. Purification of the centromere-specific protein

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

CENP-A and demonstration that it is a distinctive histone. *Proc. Natl. Acad. Sci. USA* 88:3734–38

120. Pangilinan F, Spencer F. 1996. Abnormal kinetochore structure activates the spindle assembly checkpoint in budding yeast. *Mol. Biol. Cell* 7:1195–208
121. Piatti S, Lengauer C, Nasmyth K. 1995. Cdc6 is an unstable protein whose de novo synthesis in G1 is important for the onset of S phase and for preventing a 'reductional' anaphase in the budding yeast *Saccharomyces cerevisiae*. *EMBO J.* 14:3788–99
122. Pluta AF, Mackay AM, Ainsztein AM, Goldberg IG, Earnshaw WC. 1995. The centromere: hub of chromosomal activities. *Science* 270:1591–94
123. Rieder CL, Cole RW, Khodjakov A, Sluder G. 1995. The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitory signal produced by unattached kinetochores. *J. Cell Biol.* 130:941–48
124. Rieder CL, Khodjakov A, Paliulis LV, Fortier TM, Cole RW, Sluder G. 1997. Mitosis in vertebrate somatic cells with two spindles: implications for the metaphase/anaphase transition checkpoint and cleavage. *Proc. Natl. Acad. Sci. USA* 94:5107–12
125. Rieder CL, Salmon ED. 1994. Motile kinetochores and polar ejection forces dictate chromosome position on the vertebrate mitotic spindle. *J. Cell Biol.* 124: 223–33
126. Rieder CL, Schultz A, Cole R, Sluder G. 1994. Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. *J. Cell Biol.* 127:1301–10
127. Rimmington G, Dalby B, Glover DM. 1994. Expression of N-terminally truncated cyclin B in the Drosophila larval brain leads to mitotic delay at late anaphase. *J. Cell Sci.* 107:2729–38
128. Roberts BT, Farr KA, Hoyt MA. 1994. The *Saccharomyces cerevisiae* checkpoint gene BUB1 encodes a novel protein kinase. *Mol. Cell Biol.* 14:8282–91
129. Roof DM, Meluh PB, Rose MD. 1991. Multiple kinesin-related proteins in yeast mitosis. *Cold Spring Harbor Symp. Quant. Biol.* 56:693–703
130. Rudner AD, Murray AW. 1996. The spindle assembly checkpoint. *Curr. Opin. Cell Biol.* 8:773–80
131. Saitoh H, Pu RT, Dasso M. 1997. SUMO-1: wrestling with a new ubiquitin-related modifier. *Trends Biochem. Sci.* 22:374–76
132. Saitoh H, Tomkiel J, Cooke CA, Ratrie Hd, Maurer M, et al. 1992. CENP-C, an autoantigen in scleroderma, is a component of the human inner kinetochore plate. *Cell* 70:115–25
133. Salmon ED. 1989. Microtubule dynamics and chromosome movement. In *Mitosis Molecules and Mechanisms*, ed. JS Hyams, BR Brinkely, pp. 119–82. London: Academic
134. Saunders MJ, Yeh E, Grunstein M, Bloom K. 1990. Nucleosome depletion alters the chromatin structure of *Saccharomyces cerevisiae* centromeres. *Mol. Cell. Biol.* 10:5721–27
135. Schaar BT, Chan GK, Maddox P, Salmon ED, Yen TJ. 1997. CENP-E function at kinetochores is essential for chromosome alignment. *J. Cell Biol.* 139:1373–82
136. Schott EJ, Hoyt MA. 1998. Dominant alleles of *Saccharomyces cerevisiae* CDC20 reveal its role in promoting anaphase. *Genetics* 148:599–610
137. Schulman I, Bloom KS. 1991. Centromeres: an integrated protein/DNA complex required for chromosome movement. *Annu. Rev. Cell Biol.* 7:311–36
138. Schulman IG, Bloom K. 1993. Genetic dissection of centromere function. *Mol. Cell. Biol.* 13:3156–66
139. Schwab M, Lutum AS, Seufert W. 1997. Yeast Hct1 is a regulator of Clb2 cyclin proteolysis. *Cell* 90:683–93
140. Sethi N, Monteagudo MC, Koshland D, Hogan E, Burke DJ. 1991. The CDC20 gene product of *Saccharomyces cerevisiae*, a beta-transducin homolog, is required for a subset of microtubule-dependent cellular processes. *Mol. Cell Biol.* 11:5592–602
141. Shelby RD, Hahn KM, Sullivan KF. 1996. Dynamic elastic behavior of alpha-satellite DNA domains visualized in situ in living human cells. *J. Cell Biol.* 135: 545–57
142. Shero JH, Hieter P. 1991. A suppressor of a centromere DNA mutation encodes a putative protein kinase (MCK1). *Genes Dev.* 5:549–60
143. Sigrist S, Jacobs H, Stratmann R, Lehner CF. 1995. Exit from mitosis is regulated by Drosophila *fizzy* and the sequential destruction of cyclins A, B and B3. *EMBO J.* 14:4827–38
144. Sigrist SJ, Lehner CF. 1997. Drosophila *fizzy*-related down-regulates mitotic cy-

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

clins and is required for cell proliferation arrest and entry into endocycles. *Cell* 90:671–81
145. Simerly C, Balczon R, Brinkley BR, Schatten G. 1990. Microinjected centromere [corrected] kinetochore antibodies interfere with chromosome movement in meiotic and mitotic mouse oocytes. *J. Cell Biol.* 111:1491–504
146. Deleted in proof
147. Skibbens RV, Rieder CL, Salmon ED. 1995. Kinetochore motility after severing between sister centromeres using laser microsurgery: evidence that kinetochore directional instability and position is regulated by tension. *J. Cell Sci.* 108:2537–48
148. Skibbens RV, Salmon ED. 1997. Micromanipulation of chromosomes in mitotic vertebrate tissue cells: tension controls the state of kinetochore movement. *Exp. Cell Res.* 235:314–24
149. Skibbens RV, Skeen VP, Salmon ED. 1993. Directional instability of kinetochore motility during chromosome congression and segregation in mitotic newt lung cells: a push-pull mechanism. *J. Cell Biol.* 122:859–75
150. Skowyra D, Craig KL, Tyers M, Elledge SJ, Harper JW. 1997. F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. *Cell* 91:209–19
151. Sluder G, Miller FJ, Thompson EA, Wolf DE. 1994. Feedback control of the metaphase-anaphase transition in sea urchin zygotes: role of maloriented chromosomes. *J. Cell Biol.* 126:189–98
152. Sluder G, Thompson EA, Miller FJ, Hayes J, Rieder CL. 1997. The checkpoint control for anaphase onset does not monitor excess numbers of spindle poles or bipolar spindle symmetry. *J. Cell Sci.* 110:421–29
153. Smith MM, Yang P, Santisteban MS, Boone PW, Goldstein AT, Megee PC. 1996. A novel histone H4 mutant defective in nuclear division and mitotic chromosome transmission. *Mol. Cell Biol.* 16:1017–26
154. Sorger PK, Severin FF, Hyman AA. 1994. Factors required for the binding of reassembled yeast kinetochores to microtubules in vitro. *J. Cell Biol.* 127:995–1008
155. Spencer F, Gerring SL, Connelly C, Hieter P. 1990. Mitotic chromosome transmission fidelity mutants in *Saccharomyces cerevisiae*. *Genetics* 124:237–49
156. Spencer F, Hieter P. 1992. Centromere DNA mutations induce a mitotic delay in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 89:8908–12
157. Stemmann O, Lechner J. 1996. The *Saccharomyces cerevisiae* kinetochore contains a cyclin-CDK complexing homologue, as identified by in vitro reconstitution. *EMBO J.* 15:3611–20
158. Stoler S, Keith KC, Curnick KE, Fitzgerald-Hayes M. 1995. A mutation in CSE4, an essential gene encoding a novel chromatin-associated protein in yeast, causes chromosome nondisjunction and cell cycle arrest at mitosis. *Genes Dev.* 9:573–86
159. Strunnikov AV, Kingsbury J, Koshland D. 1995. CEP3 encodes a centromere protein of *Saccharomyces cerevisiae*. *J. Cell Biol.* 128:749–60
160. Sudakin V, Ganoth D, Dahan A, Heller H, Hershko J, et al. 1995. The cyclosome, a large complex containing cyclin-selective ubiquitin ligase activity, targets cyclins for destruction at the end of mitosis. *Mol. Biol. Cell* 6:185–97
161. Surana U, Amon A, Dowzer C, McGrew J, Byers B, Nasmyth K. 1993. Destruction of the CDC28/CLB mitotic kinase is not required for the metaphase to anaphase transition in budding yeast. *EMBO J.* 12:1969–78
162. Tavormina PA, Wang Y, Burke DJ. 1997. Differential requirements for DNA replication in the activation of mitotic checkpoints in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 17:3315–22
163. Taylor SS, McKeon F. 1997. Kinetochore localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage. *Cell* 89:727–35
164. Tomkiel J, Cooke CA, Saitoh H, Bernat RL, Earnshaw WC. 1994. CENP-C is required for maintaining proper kinetochore size and for a timely transition to anaphase. *J. Cell Biol.* 125:531–45
165. Toyn JH, Johnson AL, Johnston LH. 1995. Segregation of unreplicated chromosomes in *Saccharomyces cerevisiae* reveals a novel G1/M-phase checkpoint. *Mol. Cell Biol.* 15:5312–21
166. Tugendreich S, Tomkiel J, Earnshaw W, Hieter P. 1995. CDC27Hs colocalizes with CDC16Hs to the centrosome and mitotic spindle and is essential for the metaphase to anaphase transition. *Cell* 81:261–68
167. Visintin R, Prinz S, Amon A. 1997. CDC20 and CDH1: a family of substrate-specific activators of APC-dependent proteolysis. *Science* 278:460–63

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

168. Wang Y, Burke DJ. 1995. Checkpoint genes required to delay cell division in response to nocodazole respond to impaired kinetochore function in the yeast *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 15:6838–44
169. Wang Y, Burke DJ. 1997. Cdc55p, the B-type regulatory subunit of protein phosphatase 2A, has multiple functions in mitosis and is required for the kinetochore/spindle checkpoint in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 17:620–26
170. Waters JC, Chen R-H, Murray AW, Salmon ED. 1998. Localization of Mad2 to kinetochores depends on microtubule attachment, not tension. *J. Cell Biol.* 141:1181–91
171. Waters JC, Skibbens RV, Salmon ED. 1996. Oscillating mitotic newt lung cell kinetochores are, on average, under tension and rarely push. *J. Cell Sci.* 109:2823–31
172. Weiler KS, Szeto L, Broach JR. 1995. Mutations affecting donor preference during mating type interconversion in *Saccharomyces cerevisiae*. *Genetics* 139:1495–510
173. Weinstein J, Jacobsen FW, Hsu-Chen J, Wu T, Baum LB. 1994. A novel mammalian protein, p55CDC, present in dividing cells is associated with protein kinase activity and has homology to the *Saccharomyces cerevisiae* cell division cycle proteins Cdc20 and Cdc4. *Mol. Cell Biol.* 14:3350–63
174. Weiss E, Winey M. 1996. The *Saccharomyces cerevisiae* spindle pole body duplication gene MPS1 is part of a mitotic checkpoint. *J. Cell Biol.* 132:111–23
175. Wells WA, Murray AW. 1996. Aberrantly segregating centromeres activate the spindle assembly checkpoint in budding yeast. *J. Cell Biol.* 133:75–84
176. Wells WAE. 1996. The spindle-assembly checkpoint: aiming for a perfect mitosis, ever time. *Trends Cell Biol.* 6:228–34
177. Wendell KL, Wilson L, Jordan MA. 1993. Mitotic block in HeLa cells by vinblastine: ultrastructural changes in kinetochore-microtubule attachment and in centrosomes. *J. Cell Sci.* 104:261–74
178. Winey M, Baum P, Goetsch L, Byers B. 1991. Genetic determinants of spindle pole body duplication in budding yeast. *Cold Spring Harbor Symp. Quant. Biol.* 56:705–8
179. Winey M, Goetsch L, Baum P, Byers B. 1991. MPS1 and MPS2: novel yeast genes defining distinct steps of spindle pole body duplication. *J. Cell Biol.* 114: 745–54
180. Wood KW, Sakowicz R, Goldstein LS, Cleveland DW. 1997. CENP-E is a plus end-directed kinetochore motor required for metaphase chromosome alignment. *Cell* 91:357–66
181. Yamamoto A, Guacci V, Koshland D. 1996. Pds1p is required for faithful execution of anaphase in the yeast, *Saccharomyces cerevisiae*. *J. Cell Biol.* 133:85–97
182. Yamamoto A, Guacci V, Koshland D. 1996. Pds1p, an inhibitor of anaphase in budding yeast, plays a critical role in the APC and checkpoint pathway(s). *J. Cell Biol.* 133:99–110
183. Yen TJ, Compton DA, Wise D, Zinkowski RP, Brinkley BR, et al. 1991. CENP-E, a novel human centromere-associated protein required for progression from metaphase to anaphase. *EMBO J.* 10: 1245–54
184. Yoon HJ, Carbon J. 1995. Genetic and biochemical interactions between an essential kinetochore protein, Cbf2p/Ndc10p, and the CDC34 ubiquitin- conjugating enzyme. *Mol. Cell Biol.* 15: 4835–42
185. Yu H, Peters JH, King RW, Page AM, Hieter P, Kirschner MW. 1998. Identification of a cullin homology region in a subunit of the anaphase-promoting complex. *Science* 279:1219–22
186. Zachariae W, Shevchenko A, Andrews PD, Ciosk R, Galova M, et al. 1998. Mass spectrometric analysis of the anaphase-promoting complex from yeast: identification of a subunit related to cullins. *Science* 279:1216–19
187. Zachariae W, Shin TH, Galova M, Obermaier B, Nasmyth K. 1996. Identification of subunits of the anaphase-promoting complex of *Saccharomyces cerevisiae*. *Science* 274:1201–4
188. Zhang D, Nicklas RB. 1996. 'Anaphase' and cytokinesis in the absence of chromosomes. *Nature* 382:466–68
189. Zhang H, Kobayashi R, Galaktionov K, Beach D. 1995. p19Skp1 and p45Skp2 are essential elements of the cyclin A-CDK2 S phase kinase. *Cell* 82:915–25

Annual Review of Genetics
Volume 32, 1998

CONTENTS

Alfred D. Hershey, *Allan Campbell, Franklin W. Stahl* 1

The Role of the *FHIT/FRA3B* Locus in Cancer , *Kay Huebner, Preston N. Garrison, Larry D. Barnes, Carlo M. Croce* 7

Regulation of Symbiotic Root Nodule Development, *M. Schultze, A. Kondorosi* 33

Targeting and Assembly of Periplasmic and Outer-Membrane Proteins in *Escherichia coli* , *Paul N. Danese, Thomas J. Silhavy* 59

The Genetics of Breast Cancer Susceptibility, *Nazneen Rahman, Michael R. Stratton* 95

Nonsegmented Negative Strand RNA Viruses: Genetics and Manipulation of Viral Genomes, *Karl-Klaus Conzelmann* 123

The Genetics of Disulfide Bond Metabolism, *Arne Rietsch, Jonathan Beckwith* 163

Comparative DNA Analysis Across Diverse Genomes, *Samuel Karlin, Allan M. Campbell, Jan Mrázek* 185

The Ethylene Gas Signal Transduction Pathway: A Molecular Perspective, *Phoebe R. Johnson, Joseph R. Ecker* 227

Molecular Mechanisms of Bacteriocin Evolution, *Margaret A. Riley* 255

Alternative Splicing of Pre-mRNA: Developmental Consequences and Mechanisms of Regulation, *A. Javier Lopez* 279

Kinetochores and the Checkpoint Mechanism that Monitors for Defects in the Chromosome Segregation Machinery, *Robert V. Skibbens, Philip Hieter* 307

The Diverse and Dynamic Structure of Bacterial Genomes, *Sherwood Casjens* 339

Recombination and Recombination-Dependent DNA Replication in Bacteriophage T4, *Gisela Mosig* 379

Natural Selection at Major Histocompatibility Complex Loci of Vertebrates, *Austin L. Hughes, Meredith Yeager* 415

Evolution and Mechanism of Translation in Chloroplasts, *Masahiro Sugiura, Tetsuro Hirose, Mamoru Sugita* 437

Genetics of Alzheimer's Disease, *Donald L. Price, Rudolph E. Tanzi, David R. Borchelt, Sangram S. Sisodia* 461

THE CRITICAL ROLE OF CHROMOSOME TRANSLOCATIONS IN HUMAN LEUKEMIAS, *Janet D. Rowley* 495

Early Patterning of the *C. elegans* Embryo, *Lesilee S. Rose, Kenneth J. Kemphues* 521

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.

Genetic Counseling: Clinical and Ethical Challenges, *M. B. Mahowald, M. S. Verp, R. R. Anderson* 547

Mating-Type Gene Switchingin *Saccharomyces cerevisiae* , *James E. Haber* 561

Epitope Tagging, *Jonathan W. Jarvik, Cheryl A. Telmer* 601

The Leptotene-Zygotene Transition of Meiosis, *D. Zickler, N. Kleckner* 619

Annu. Rev. Genet. 1998.32:307-337. Downloaded from arjournals.annualreviews.org
by Lehigh University on 07/27/05. For personal use only.