Critical Review

Phylogenetic Challenges to the Evolutionary Origin of the Eukaryotic Cell Cycle

Jonathan McLatchie*[1]

[1]Discovery Institute, Seattle, WA

Abstract

The origin of the eukaryotic cell represents one of the most significant innovations in the history of life. Prokaryotic and eukaryotic cells have distinct modes of cell division both in the mechanisms of cell bifurcation and DNA segregation and in the underlying protein components that drive those processes. Recent phylogenomic analysis shows that most mitotic components were already present in the last eukaryotic common ancestor (LECA), Given the presence of modern-like cell cycle complexity in LECA, it is expected that homologues exist among prokaryotes for at least some of the components involved. This study used the Basic Local Alignment Search Tool (BLAST) to survey the proteomes of prokaryotes (in particular, members of the Asgard superphylum) for potential homologues of components of the anaphase promoting complex / cyclosome (APC/C) and its substrates and the mitotic checkpoint complex (MCC) and kinetochore network. Although the data gathered have failed to definitively show the presence of full homologues for most of the proteins investigated, this analysis reveals that the mitotic machinery is closely associated with eukaryogenesis. While the time window available for the origin of these mechanisms is unclear, even a large window (e.g., 2–3 billion years of time) would be insufficient for blind evolutionary mechanisms to produce such complex systems. This analysis raises significant questions about the feasibility of evolutionary processes to account for such an abrupt transition.

Cite as: McLatchie J (2024) Phylogenetic Challenges to the Evolutionary Origin of the Eukaryotic Cell Cycle. BIO-Complexity 2024 (4):1–19 **doi:**10.5048/BIO-C.2024.4.

Editor: Douglas D. Axe

Received: January 24, 2024; **Accepted:** July 26, 2024; **Published:** November 7, 2024

Copyright: © 2024 McLatchie. This open-access article is published under the terms of the Creative Commons Attribution License, which permits free distribution and reuse in derivative works provided the original author(s) and source are credited.

Notes: A Critique of this paper, when available, will be assigned **doi:**10.5048/BIO-C.2024.4.c.

*jmclatchie@discovery.org

INTRODUCTION

An important aspect of Darwin's treatment of evolution by natural selection in *The Origin* is "*natura non facit saltus*", a Latin expression meaning "nature does not make jumps" [1]. Yet the history of life is characterized by discontinuous 'jumps'. This is seen clearly in the fossil record, perhaps most notably the Cambrian explosion (approximately 530 million years ago), in which the vast majority of the animal body plans (phyla) appear to have emerged abruptly and explosively onto the world scene with no obvious predecessors among the Precambrian animals [2,3]. It is also seen in molecular biology, including the origins of life via inorganic chemistry, the transition from prokaryotic cells to eukaryotic ones, and the origins of multicellularity [4]. The discovery of taxonomically restricted genes and proteins, which have no annotated precursors from which they could have evolved, also illustrates a pattern of 'jumps' in the history of life [5,6,7]. The number of these apparently taxonomically restricted genes has not diminished with more genome sampling, but rather has tended to increase [8,9]. In this paper, we will limit our focus to an important innovation associated with the prokaryote-eukaryote transition—the evolution of the eukaryotic mode of cell division.

The mechanisms underlying mitotic cell division in eukaryotes are highly complex and tightly orchestrated. Though eukaryotes are thought to have evolved from prokaryotes, the mode of cell division employed by prokaryotic cells (bacteria and archaea) is highly distinct from that of eukaryotes, both in terms of the mechanism and of the protein components involved. This raises the question of the origins of the machinery that governs the progression of the eukaryotic cell through division. It has been said that the origins of the eukaryotic cell represents "the most intense phase of gene invention since the origin of life" [10]. The purpose of this paper is to compare the

similarities and differences between the eukaryotic and prokaryotic division machineries, ascertain how abruptly the transition occurred and determine whether evolutionary mechanisms or design can reasonably be inferred to be the best causal explanation for the origins of the eukaryotic cell division apparatus.

Overview of the Eukaryotic Cell Cycle

The purpose of the eukaryotic cell cycle is to ensure the faithful duplication of the genome and the segregation of the replicated DNA into two daughter cells. Failure to properly regulate the segregation of chromosomes often leads to aneuploidy, i.e., deviations from the typical karyotype. The result can be diseases such as cancer or genetic abnormalities such as Down syndrome [11,12].

A typical mammalian cell possesses a cell cycle comprising four distinct phases: growth phase 1 (G_1), synthesis phase (S), growth phase 2 (G_2)—these three phases are collectively known as interphase—and mitosis (M phase). The cell size increases at G_1 and G_2. S phase is the stage at which DNA replication occurs. The climax and culmination of the cell cycle is mitosis, or M phase, at which time the genetic material is segregated, and the cell differentiates into two identical daughter cells.

Cell cycle progression is driven by cyclin-dependent kinases (Cdks), which are activated by cyclin molecules [13] and subsequently phosphorylate their target substrates [14]. Cyclin molecules are synthesized in response to chemical signals only at specific cell cycle stages and are degraded by ubiquitin-mediated proteolysis. Cdks, on the other hand, are constitutively expressed [15].

The activity of Cdk1 is regulated by cyclin B1. The cyclin B1-Cdk1 complex is known as the maturation or mitosis promoting factor (MPF).It is responsible for driving the cell's entry into mitosis [16] as well as promoting early mitotic events such as nuclear disintegration and chromosome condensation [17]. The cell's levels of MPF increase during the cell cycle until prometaphase, at which point the cyclin B1 is degraded and its levels sharply decline [16,18,19]. Ubiquitin-mediated degradation of cyclin B1 is essential for the onset of anaphase: the transition from metaphase to anaphase can be blocked by the introduction of even low levels of a non-degradable form of cyclin B1 [19].

Mitotic Cell Division

Mitosis is the phase of the cell cycle at which the segregation of sister chromatids and cytokinesis takes place. Mitosis can be broken down into five stages. During prophase, the replicated chromosomes begin to condense. The mitotic spindle assembles between the two duplicated centrosomes, which have migrated towards the poles of the cell. The centrosomes are the microtubule organizing centers (MTOCs) and are made up of two orthogonally arranged centrioles surrounded by a mass of protein called the pericentriolar material from which the microtubules radiate. The chromosomes also form two kinetochores at the centromere, one bound at each sister chromatid.

Prometaphase is characterized by nuclear envelope breakdown. The kinetochore microtubules begin "searching" for kinetochores to which they can attach. This "search-and-capture" mechanism is facilitated by microtubule dynamic instability [20,21,22,23]. When a microtubule encounters a kinetochore, the kinetochore is "captured" via side-on attachment.

The sister chromatids are positioned at one of the cell poles, where they become associated with more microtubules. When the kinetochore has attached to the microtubule from the other pole, the chromosomes migrate towards the equator. Kinetochores are composed of at least 80 different proteins, which assemble at the centromere of each chromatid [24].

The nucleosomes of centromeric chromatin contain histone H3 variant CENP-A [25], which recruits a large protein complex called the constitutive centromere-associated network (CCAN) [26]. The CCAN, in turn, assembles the conserved KNL1/Mis12 complex/Ndc80 complex (KMN) network, which forms the outer kinetochore and is necessary for microtubule-kinetochore interactions [27,28]. Another complex, called the RZZ complex, regulates microtubule attachment and is essential for recruitment of checkpoint components Mad1 and Mad2 [29].

In metaphase, the condensed chromosomes are arranged at the equatorial/metaphase planeof the cell, located midway between the spindle poles. Microtubules that radiate from the centrosome bind to the kinetochores and thereby attach to the sister chromatids. The opposing pulling forces generated by the kinetochore microtubules result in the even alignment of chromosomes along the metaphase plate. A multimeric protein ring structure, cohesin, encircles and binds together the sister chromatids [30,31,32]. Cohesin is formed of four protein subunits—SMC3, SMC1, SCC1 and SCC3. SMC (structural maintenance of chromosomes) proteins are also found in bacteria, where they play a critical role in organizing and compacting the chromosome [33].

At anaphase, the sister chromatids are synchronously separated and pulled towards opposite spindle poles. The final phase is telophase, which is characterized in mammals by the formation of a new nuclear envelope around each chromosome cluster, which return to their more extended form.

The transition from metaphase to anaphase is achieved by the ubiquitin-mediated proteolysis of cyclin B1 the factor which is responsible for driving entry into mitosis by binding to Cdk1. Another protein, securin, which is responsible for inhibiting a protease called separase, also must be degraded [34]. Upon degradation of securin, separase cleaves the cohesin subunit Scc1, resulting in chromatid separation and each chromatid's migration towards opposite poles [35]. In both cases, the degradation is targeted to the 26S proteasome by the covalent attachment of polyubiquitin chains to lysine residues of the substrate, a process that is mediated by an E3 ubiquitin ligase, the anaphase promoting complex / cyclosome (APC/C). To prevent aneuploidy, the activity of the APC/C is inhibited by the spindle assembly checkpoint (SAC) which is active between prophase and metaphase until the kinetochores have all properly attached to the microtubules. Upon satisfaction of the SAC, the APC/C polyubiquitylates its substrates to mediate progression into anaphase.

Ubiquitin-Mediated Proteolysis

Ubiquitin-mediated destruction of cell cycle proteins plays a crucial role in the coordination of cell cycle progression. As previously mentioned, it is necessary for cyclin B1 and securin to be degraded in order to facilitate entry from metaphase to anaphase. The Skp, Cullin, F-box containing (SCF) complex and APC/C are two important E3 ubiquitin ligase enzymes that target different substrates for destruction in the cell cycle [36].

Ubiquitin peptides are transferred to the ubiquitin-activating enzyme (E1) and subsequently to the ubiquitin-conjugating (E2) enzyme [37]. An E3 ligase catalyzes the binding of ubiquitin to a lysine on the target protein. Monoubiquitylation influences protein-protein interactions and protein localization [38]. The binding of further ubiquitin peptides results in the assembly of polyubiquitin chains, which can be linked through different lysine residues to give different structures which serve different functions. Polyubiquitin chains linked to residues K11 and K48 of the substrate protein result in its proteasomal degradation [39.40].

The SCF complex is a multi-protein E3 ubiquitin ligase that is responsible for marking proteins for destruction [41]. It is responsible for ubiquitylation of cyclin-dependent kinase inhibitors (CKIs) (thereby marking them for destruction), including Wee1 (which acts to inhibit Cdk1, thereby preventing entry into mitosis and thus regulating cell size) [42] p27 (which inhibits the activation of cyclin E-Cdk2 or cyclin D-Cdk4 complexes, thereby regulating the progression of the cell cycle at G1) [43] and p21 (which regulates the activity of cyclin-Cdk1, -Cdk1, and -CDK4/6 complexes) [44,45], as well as the G1/S-specific cyclin E to regulate the transition from G1 to S and G2 to M.

The APC/C is a 1.5 MDa E3 RING-cullin protein complex responsible for the progression from metaphase to anaphase during mitosis [46,47]. The APC/C was first identified in 1995 as a mitosis-specific E3 ubiquitin ligase in budding yeast [48], clam [49], and *Xenopus laevis* [50]. Two substrate-specific co-activators, Cdc20 and Cdh1, are responsible for activating the APC/C. The APC/C^{Cdc20} complex targets a 9-residue motif (RxALGxlxN), the destruction box (D box), present on cyclin B1 [51]. The APC/C^{Cdh1} complex targets both the D-box and the KEN box motif (which possesses the consensus sequence KENxxxN) [52]. In human cells, the APC/C switches from using Cdc20 as its co-activator to using Cdh1 upon exiting mitosis [53]. This switch is dependent on degradation of Cdc20 and dephosphorylation of Cdh1 and the APC/C. Recent work has also demonstrated the involvement in human cells of the APC/C inhibitor Mad2L2 in this process, which inhibits free Cdh1 from interacting with the APC/C during prometaphase [54]. Upon progression to anaphase, Mad2L2 is degraded by the APC/C^{Cdc20}, thereby releasing Cdh1 to activate the APC/C.

The vertebrate APC/C is comprised of 14 different subunits and is organized into three structural domains: the platform, the catalytic core and the tetratricopeptide repeat (TPR) lobe. The platform subcomplex forms a base that connects the other APC/C components. The APC/C subunits in the TPR lobe are stabilized by three TPR accessory factors [55].

The Spindle Assembly Checkpoint (SAC)

Maintenance of genomic integrity is partly contributed by delaying degradation of cohesin and cyclin B, and thereby the segregation of sister chromatids, until all chromosomes are properly attached to the microtubule spindle apparatus. The unattached kinetochores are thought to send out a diffusible "wait anaphase" inhibitory signal that activates the SAC, a quality control mechanism which inhibits cell cycle progression until the kinetochores become stably bound to the microtubules, leading to the extinguishing of the checkpoint signal [56]. Just a single unattached kinetochore is thought to generate a sufficiently large signal to delay the progression from metaphase to anaphase [57,58]. The essential SAC component proteins include Mps1, BubR1, Bub1, Bub3, Mad1, Mad2 and Aurora B kinase. The protein complex responsible for generating the SAC signal is known as the mitotic checkpoint complex (MCC) [56,60]. These proteins include BubR1, Bub3, Mad2 and Cdc20. BubR1, Bub3 and Mad2 are inhibitors of the APC/C, and Cdc20 is a co-activator of the APC/C. Thus, the principal target of the SAC is Cdc20 [61].

It is generally thought that the MCC signal is generated by binding the Mad and Bub families at unattached kinetochores where they are catalyzed to form the Mad2-Cdc20 and the Mad3/BubR1Bub3 subcomplexes [62,63,64,65,66,67]. (Mad3 is the yeast homologue of the mammalian BubR1). The MCC forms from these subcomplexes as Cdc20 has specific and distinct binding sites for Mad2 and Mad3, although the exact mechanism of MCC assembly is not well understood. One possible scenario is that Mad2-Cdc20 and BubR1-Bub3-Cdc20 independently form a complex which come together to assemble the MCC. It is likely that the Mad2-Cdc20 complex initiates MCC formation, since binding of Mad2 to Cdc20 is a necessary prerequisite for BubR1 binding to Cdc20 [65,68]. BubR1 is essential for checkpoint activation, although reduced levels of BubR1 result in only a small decrease in Mad2-Cdc20 levels.

It was once thought that the SAC operates in a manner akin to an 'on/off switch' [57]. However, more recent studies using drugs to modulate spindle-microtubule dynamics have shown that the SAC operates more like a 'rheostat', that is, on a continuum, with the SAC response correlating with the number of unattached kinetochores [69]. Moreover, a long-standing controversy was recently resolved by experiments that revealed that the SAC is satisfied by kinetochore-microtubule attachments, facilitating the initiation of anaphase (rather than the SAC sensing forces caused by sister kinetochore biorientation) [70,71].

The Rod-Zwilch-Zw10 (RZZ) complex also plays an important role in the metazoan cell cycle. Through a poorly understood mechanism, the RZZ complex functions to recruit Mad1-Mad2 and the dynein-dynactin complex [12, 72]. The function of the RZZ complex in SAC signaling finishes with corona shedding [29], which silences the SAC by depleting Mad1 and Mad2 from the kinetochores. An additional component, Spindly, which also is dependent on RZZ for localization

to the kinetochore, is required for the recruitment of dynein-dynactin to the kinetochore.

It has been shown that the spindle assembly checkpoint is inactivated by p31^{comet}, which binds to Mad1 or Cdc20-bound Mad2 so as to prevent activation of Mad2 and promote the dissociation of the Mad2-Cdc20 complex [73,74]. p31^{comet}-mediated disassembly of the MCC is ATP-dependent and thus involves an AAA^{+}-ATPase protein called Trip13, which interacts and works in conjunction with p31^{comet} [75,76,77]. *In vitro* and structural studies have recently shown that p31^{comet} functions as an activator and adaptor for Trip13 for promoting conversion from C-Mad2 to O-Mad2 [78].

Given the crucial role played by the APC/C and MCC across all known branches of the eukaryotic tree, it might be predicted on evolutionary theory that homologues of these proteins would be found among prokaryotes, particularly archaea (that are believed to be more closely related to eukaryotes than are bacteria).

The Chromosome Passenger Complex

The chromosomal passenger complex (CPC) is a complex that functions to ensure the accurate segregation of chromosomes and proper progression through the various stages of mitosis. It is comprised of four subunits: a kinase component, Aurora B, and the regulatory components INCENP, Survivin and Borealin. Aurora B belongs to a family of three serine-threonine kinases first identified in *Drosophila melanogaster* [79], including Aurora A, which functions at the spindle poles; Aurora B, which functions at the anaphase spindle, cell cortex and centromere; and Aurora C, which functions during early development to regulate mitosis and meiosis [80].

The platform on which the CPC assembles is called the inner centromere protein (INCENP) [81]. Its N-terminus is necessary for the localization of the CPC to centromeres. INCENP residues 1–58 create a triple-helix bundle with Survivin and Borealin that is necessary for their localization to the centromere, as well as the anaphase spindle midzone and telophase midbody [82,83,84,85].

Assembly and Structure of the Kinetochore

The kinetochore is a multi-subunit complex, which assembles on the centromere and functions to attach sister chromatids to the spindle microtubules, thereby facilitating their partition to the daughter cells.

A centromere-specific histone H3 variant, CenpA, is believed to serve a crucial function in directing the assembly of the kinetochore [86,87]. It functions as an epigenetic marker, as well as the foundation for kinetochore assembly.

CenpA-containing nucleosomes direct the recruitment of a 16-subunit complex, the constitutive centromere-associated network (CCAN), which is divided into four subcomplexes: CenpHIKM, CenpLN, CenpOPQRU, and CenpTWSX. CenpLN and CenpHIKM are organized via CenpC, which functions as a scaffolding protein [24,88,89]. Together, they bind to and stabilize CenpA [90]. The CenpTWSX complex subunits have been proposed to create a unique centromeric chromatid structure [91]. Recruitment of the CenpOPQRU complex is dependent on CenpCHIKMLN [92]. CenpQ has been demonstrated to play a role in chromosome congression in association with the motor protein CenpE [93]. CenpU has also been shown to be involved in Plk1 recruitment [94]. Strikingly, evolutionary studies have found that, with the exception of CenpC, the repertoire of kinetochore proteins has undergone recurrent evolutionary turnover with losses in some organisms of kinetochore proteins that are essential in other lineages and the emergence of new kinetochore proteins in other organisms [95].

The principal microtubule-binding module of the kinetochore is the Knl1-C/Mis12-C/Ndc80-C (KMN) network, comprising ten subunits [96]. The four-subunit Mis12 complex comprises a Y-shaped structure composed of two heads that are formed by the heterodimers Dsn:Nsl1 and Mis12:Nnf1 and a stalk created from the long C-terminal coiled coil segments of all four subunits [97]. This stalk points away from the centromere, and phosphor-motifs in the C-termini serve as a landing platform for the RWD domains in the KMN subunits Spc24 and Spc25 (subunits of the Ndc80 complex) and Knl1 [98,99]. The Ndc80 complex (containing four subunits Ndc80, Nuf2, Spc25 and Spc24) has structural roles in kinetochore assembly, and also functions in spindle checkpoint signaling [100].

The Role of Motor Proteins

Another feature of mitotic cell division is the role of motor proteins in spindle assembly and function [101]. These motor proteins may be divided into four main classes: kinesin-5, kinesin-14, kinesins-4 and 10, and dynein. Kinesin-5 is plus-end directed and slides apart antiparallel microtubules (i.e., microtubules that have their polarities oriented in opposite directions). By moving these microtubules apart, kinesin-5 helps to push the spindle poles apart, contributing to spindle elongation and bipolarity [102].

Kinesin-14 proteins, by contrast, are minus-end–directed motor proteins that possess a single motor domain as well as other domains that bind to a different microtubule [103]. They tend to pull the poles together as they cross-link antiparallel interpolar microtubules at the spindle midzone. Kinesin-4 and kinesin-10, known as chromokinesins, play a crucial role in chromosome positioning and segregation during mitosis [104]. They assist in the movement of chromosomes to the correct positions within the cell, ensuring that each daughter cell receives the appropriate number of chromosomes during cell division.

Finally, dyneins are minus-end–directed motor proteins. They have their cargo-binding domain bound to proteins embedded in the cell cortex, while their motor domains bind to the minus ends of the microtubules, emanating from the centrosomes. These dyneins utilize ATP hydrolysis to 'walk' along the microtubules towards the minus ends, which are anchored at the centrosomes. Dynein's movement along the microtubules exerts a pulling force on the minus ends, effectively pulling the centrosomes (spindle poles) towards the cell cortex [105]. This movement ensures that the chromosomes are segregating

properly such that each daughter cell receives an accurate and complete set of chromosomes.

Overview of Prokaryotic Systems of Cell Cycle Control

Having briefly surveyed the major features of eukaryotic cell division, let us now turn our focus to the mode of cell division among prokaryotes (bacteria and archaea). While this section will lay out the basic mechanisms of cell division among prokaryotes, the purpose is to foster a comparison of eukaryotic and prokaryotic cell division and an assessment of the evolvability of eukaryotic systems from those in prokaryotes.

Comparing Bacterial and Archaeal Modes of Cell Division

Bacterial cell division normally depends on monomers of FtsZ, which polymerize around the division septum to form a structure known as the Z ring. First, the Z ring contracts, driven by GTP hydrolysis [106]. The invagination of the bacterial cell inner membrane is mediated by FtsZ and other components that form a protein complex: the divisome [107]. The FtsZ system of cell division is found in most bacteria and archaea. However, members of the archaeal phylum Crenarchaeota do not possess FtsZ and instead possess an actin-based ESCRT-III cell division system [108]. FtsZ is also absent in some other organisms, such as in members of the Plantomycetes-Verrucomicrobia-Chlamydia (PVC) bacterial superphylum [109]. Archaea also often encode more than one division system, which suggests a complex scenario with respect to cell division origins in prokaryotes [110].

The best understood archaeal cell cycle is that of *Sulfolobus*, a genus of the crenarchaeotal phylum [111]. A *Sulfolobus* spp. cell undergoing exponential growth goes through a brief G1 phase prior to entering S phase. This is followed by a prolonged G2 phase, which occupies more than half of the cell cycle. Following this, the genome segregation and cell division phases (M and D phases respectively) occur [112,113,114]. Previous work has also reported checkpoint-like inhibition of cell division and genome segregation as a result of blockage of upstream processes [115]. The mechanisms of cell division in *Sulfolobus* spp. are thought to be common among members of the crenarchaeal phylum, including *Acidianus hospitalis*, *Aeropyrum pernix*, *Pyrobaculum aerophilum* and *Pyrobaculum calidfontis* [116].

Tethering the Bacterial FtsZ Ring

The first step in the formation of the divisome is the polymerization of thousands of FtsZ molecules to form the FtsZ ring [117,118,119]. Two additional proteins that are recruited independently of each other, ZipA and FtsA, function to tether the FtsZ ring to the inner membrane [120,121]. Both proteins also play a role in the recruitment of other division proteins [120]. The FtsZ ring assembly occurs provided that at least one of those two proteins is present [122]. ZipA homologues are not found outside of the gamma-proteobacterial family, but a deficiency in ZipA can be sufficiently compensated for by a modification in a conserved residue of FtsA [123]. There is also evidence to suggest that elevated concentrations of FtsA (which is far more widely distributed) can compensate for a lack of ZipA. For instance, FtsA is present in significantly higher concentrations in the Firmicute bacterium *Bacillus subtilis* than in *Escherichia coli* [124].

The FtsZ ring assembles following replication of the bacterial DNA (the nucleoids block FtsZ ring assembly prior to segregation). Location of the cell's center occurs by an elegant mechanism: The proteins MinC and MinD oscillate from pole to pole, inhibiting assembly of the FtsZ ring [125,126,127]. Since the oscillation cycle of MinC and MinD entails that they spend more time at the poles of the cell than the middle, the cell's center has, on average, a lower concentration of these proteins than elsewhere. Midcell suppression of MinC and MinD is facilitated by an additional protein, MinE [128,129]. Consequently, the cell's mid-point is most conducive to FtsZ ring assembly.

Prokaryotic DNA Segregation Machinery

Bacterial DNA segregation is mediated by a DNA translocase, FtsK, a motor protein that converts chemical energy of binding and hydrolyzing ATP into translocation of DNA [130,131]. Its N-terminus localizes the protein to the division septum, whereas the C-terminus forms the translocation motor needed for DNA segregation. A homologue of FtsK (HerA) has been identified in archaea [132,133]. In bacteria, two proteins are important in DNA segregation: ParA and ParB. ParB is a DNA-binding protein, whereas ParA is an ATPase. ParB binds to a centromere-like DNA-binding site called *parS*, and ParA binds to ParB before assembling into dynamic filaments [134]. The most well-characterized system in bacteria is that of *Caulobacter crescentus*, wherein depolymerization of the ParA filaments creates a pulling mechanism that facilitates the translocation of the *parS*-containing region of one chromosome copy [135].

The segregation machinery in archaea is not well understood, although one study reported on an initial characterization of the genome segregation machinery in *Sulfolobus solfataricus* [136]. As in bacteria, two genes are responsible for genome segregation: *segA* and *segB*. SegB was reported to have no sequence similarity to bacterial ParB proteins, whereas SegA has approximately 30% sequence identity to ParA family proteins [136]. SegA, like ParA, polymerizes in an ATP-dependent manner. SegB appears to act as a functional analogue of ParB. Exactly how the SegAB system works, however, has not yet been fully elucidated.

Cell Wall Growth During Division

Bacterial cells prepare for cell division by elongating, a process that is critically dependent upon severing and resynthesis of the peptidoglycan cell wall. In rod-shaped cells (such as *Escherichia coli*), this occurs at multiple locations along the cell, whereas in coccus-shaped cells (such as *Staphylococcus aureus*) the cell wall grows outward in opposite directions from the FtsZ ring [137]. Crucial for the growth of the cell wall is the controlled cleavage of the existing peptidoglycan layer.

Enzymes called autolysins hydrolyze the β-1,4 glycosidic bonds that link N-acetylglucosamine and N-acetylmuramic acid. Since these can result in programmed cell death, they

have to be very carefully regulated [138,139]. The autolysins are targeted to the site of cell division by specific control mechanisms, where they catalyze the cleavage of the peptide cross-links [140,141,142]. This peptidoglycan at the division site is thereby weakened, facilitating controlled breakage of the cell wall.

The initial stage of cell wall biosynthesis is the synthesis of N-acetylglucosamine from glucose through a series of enzymatic steps and of N-acetylmuramic acid by the addition of a phosphoenolpyruvate-derived, lactic acid substituent. A pentapeptide is added to N-acetylmuramic acid, and a peptidoglycan precursor is created by the attachment of N-acetylmuramic acid to its end [143,144].

A lipid molecule, called bactoprenol, plays a crucial role in cell wall synthesis, serving as a carrier molecule for the precursor building blocks of peptidoglycan (i.e., N-acetylglucosamine and N-acetylmuramic acid, which are linked together to form glycan chains) across the inner cytoplasmic membrane [145,146]. The hydrophilic precursors cannot readily traverse the hydrophobic membrane by themselves, so bactoprenol possesses a hydrophobic tail, which allows it to move through the hydrophobic interior of the phospholipid bilayer that comprises the cell membrane. Once in the periplasmic space (gram-negative bacteria) or the exterior of the cell (gram-positive bacteria), the peptidoglycan precursors are transferred to the site of peptidoglycan assembly.

The peptidoglycan precursors are polymerized into glycan chains by glucosyltransferase enzymes and cross-linked by penicillin-binding proteins (also called transpeptidases), which form peptide bonds between adjacent amino acid chains in the peptidoglycan layer, thereby conferring upon the cell wall its structural integrity [147,148]. In *E. coli*, the transpeptidase FtsI is the key contributor to the cross-linking at the division septum. Crucial to FtsI localization is an intact N-terminal membrane anchor, as well as the division proteins FtsZ, FtsA, FtsQ, FtsW and FtsL [150,151].

Methods Used to Search for Homologues

Basic Local Alignment Search Tool (BLAST) was used to perform a protein-protein search (BlastP) for sequence homology among archaea and bacteria for each of the APC/C, MCC, and kinetochore network components (using the NCBI NR protein database). These mitotic components were selected for analysis based on prior phylostratigraphic analysis (to be discussed later) which inferred the presence of these proteins in LECA [152,153,154]. For each respective component, BLAST searches were performed using as query the annotated homologous APC/C component from *Homo sapiens* (opisthokonta), *Dictyostelium discoideum* (amoebozoa), *Naegleria gruberi* (excavate), *Phytophthora infestans* (heterokonta) and *Arabidopsis thaliana*—each selected to represent the breadth of eukaryotic diversity and for their conservation of the proteins of interest [155].

Alignment of sequences is effective in distinguishing homologous proteins from nonhomologous ones when the pairwise identity between the alignments is high (>40% for long alignments). The signal, however, becomes more ambiguous in the so-called twilight zone of 20–35% sequence identity. Rost (1999) analyzed more than a million sequence alignments between protein pairs of known structure and inferred a line for effective discrimination between true and false positives for homology detection, referred to as the Homology-derived Secondary Structure of Proteins (HSSP) curve, shown in Figure 1 [156].

Figure 1 plots the percentage sequence identity (X-axis) against the number of aligned residues (Y-axis). The standard HSSP curve (shown by the grey line in Figure 1) gives the threshold for accepting a hit from a BLAST search as a true positive. The higher above the standard HSSP curve a hit is, the more confidence one can have that it indeed represents a true positive rather than a false one. The HSSP distance is the distance in percentage points from the standard HSSP curve.

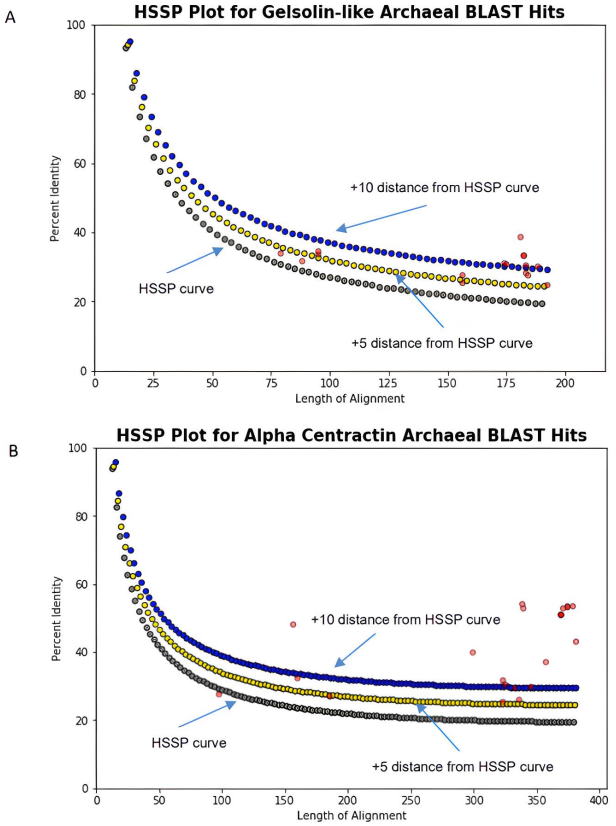

Figure 1. HSSP plots for two eukaryotic signature proteins. Scatter plots representing (A) Gelsolin-like (*Stegastes partitus*, accession XP_008276815.1, length 720 amino acids) and (B) Alpha-centractin (*Dictyostelium fasciculatum*, accession EGG13303, 385 amino acids) archaeal BLAST hits, plotting length of alignment (subtracting gaps) against percentage identity. The purpose of the HSSP curve is to determine if the degree of similarity is greater than what would be expected from randomness, thereby indicating true homology. Data points from BLAST output are plotted in red. Darker shades of red indicate multiple dots stacked on top of one another. Hits are only considered "homologous" if they are above the grey HSSP curve. The grey curve represents the HSSP curve; the yellow curve represents a distance from the HSSP curve of +5; the blue curve represents a distance from the HSSP curve of +10. The graph in both cases shows that the vast majority of hits are above the threshold of +5 above the HSSP curve (represented by the yellow line). **doi:**10.5048/BIO-C.2024.4.f1

Python scripts were used to compute HSSP scores for protein pairs and to plot the HSSP curve and the relative position on the graph of each of the protein hits listed by the BLAST output files.

Multiple assessments of this script were performed to determine its suitability for performing an analysis on BLAST output data. For this purpose, two examples of eukaryotic signature proteins (ESPs)—proteins that are limited to the eukaryotic domain—were used that have been previously identified to possess remote homologues in Asgard archaea [157]. A BLAST search against the entire Archaea domain for gelsolin-like protein (*Stegastes partitus*, accession XP_008276815.1), a protein of 720 amino acids, yielded a total of 16 hits all from the Asgard superphylum. Running the algorithm for preserving entries with a distance from the HSSP curve of +5 or greater preserved 10 out of 16 hits. A graph was generated to show the positioning of the BLAST hits relative to the HSSP curve. The position of the entries relative to the HSSP curve is given in Figure 1. A similar analysis was performed for Alpha-centractin (*Dictyostelium fasciculatum*, accession EGG13303), a 385-amino acid protein. The BLAST search against the entire Archaea domain again yielded only hits from the Asgard superphylum. There were 22 hits, 20 of which passed the criteria set by the HSSP script. The position of the entries relative to the HSSP curve is given in Figure 1.

RESULTS

Data are summarized graphically in Figure 2 below. Figures 2A, B and C show three phylogenies representing the commonly accepted evolutionary relationship of the domains of life including archaeal groups Asgard, TACK (Thaumarchaeota, Aigarchaeota, Crenarchaeota and Korarchaeota) and DPANN (Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota and Nanohaloarchaea). Next to each phylogeny is shown an absence/presence heatmap for each of the proteins involved, produced using an online tool, "interactive tree of life", or itol (https://itol.embl.de/).

DISCUSSION

Comparison of Eukaryotic and Prokaryotic Cell Cycle Control Systems and Evaluation of Evolvability

As summarized in Table 1, the analysis failed to identify homologues for the large majority of the eukaryotic cell division cycle proteins among prokaryotes, including among the Asgard archaea, believed to be the closest living relatives of eukaryotes. The results are therefore consistent with the mitotic components being eukaryotic innovations that arose after the split between archaeal and eukaryotic lineages. With the sole exceptions of the cohesin proteins SMC1 and SMC3, the AAA$^+$-type

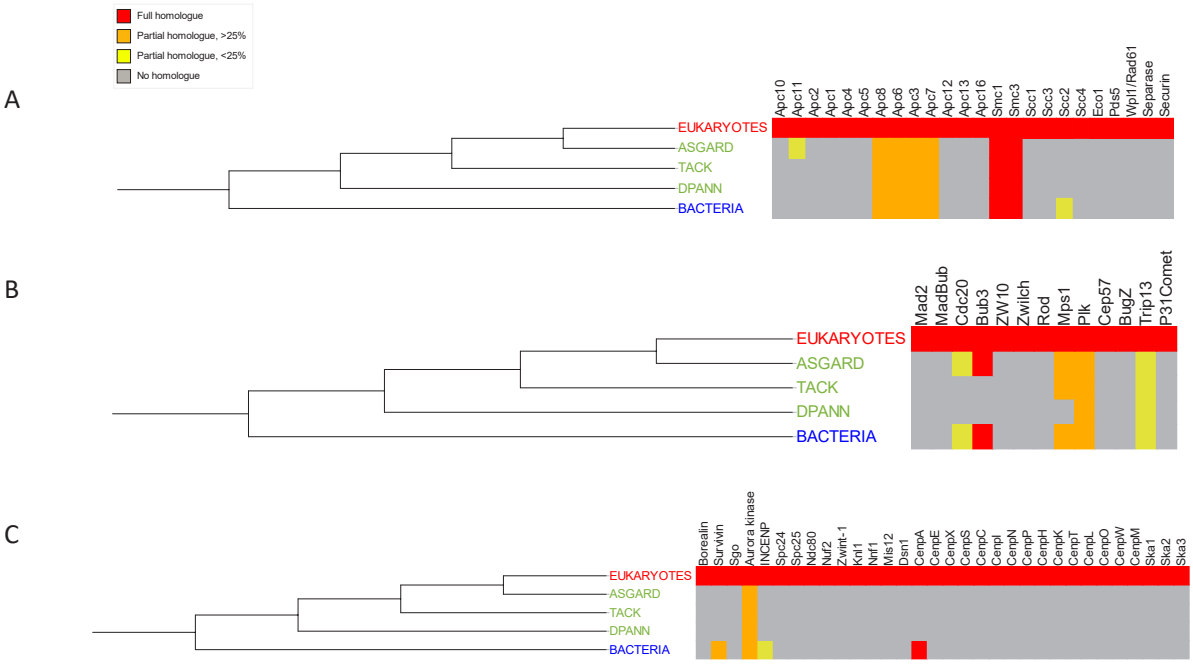

Figure 2. Homologue presence-absence data for mitotic proteins across phylogeny of domains of life. Phylogeny of domains of life includes archaeal groups Asgard, TACK (Thaumarchaeota, Aigarchaeota, Crenarchaeota, and Korarchaeota), and DPANN (Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota, Nanohaloarchaea).Each part shows an absence/presence map for each of the proteins involved: (A) in the APC/C and its substrates, (B) in the SAC, and (C) in the kinetochore network.Most of the components are taxonomically restricted to the eukaryotic domain and full remote homologues cannot be detected in bacteria or archaea, although partial homologues can be found in several cases. Key: red represents the presence of a component; grey represents the absence of a component; yellow represents the presence of a remote homologue. Eukaryotes are labelled in red font, archaeal groups in green font, and bacteria in blue font. Direct homologues of SMC1 and SMC3 can be identified among bacteria, as well as among the Asgard, TACK and DPANN archaeal groups.
doi:10.5048/BIO-C.2024.4.f2

ATPase Trip13, the centromere protein CenpA and checkpoint protein Bub3, full homologues of the mitotic proteins cannot be identified among archaea or bacteria, including the Asgard archaeal superphylum, the closest living archaeal relatives yet identified of the eukaryotic domain.

However, for a number of the proteins investigated that contain TPRs, WD40-repeats or kinase domains, a portion of the protein is often found to exhibit homology to bacterial or archaeal proteins. This is hardly surprising, since TPRs, WD40s and kinase domains are found throughout the tree of life. Indeed, kinases are distributed across all three domains of life and are thought to share a common evolutionary origin [158]. TPRs typically consist of 34 residues and of tandem arrays of typically 3–16 repeats. The TPR is composed of two α-helices which form an α-helix-turn-α-helix motif [159]. TPRs are involved in mediating protein-protein interactions and thus serve as modules for the assembly of multi-protein complexes [160]. TPR-containing proteins have been identified in organisms across the diversity of the tree of life [161]. For example, in bacterial pathogens, TPR-containing proteins have been shown to be involved in virulence-associated functions [162]. The TPR profile is well defined (Pfam clan CL0020). Thus, it is possible to predict the repertoire of TPR-containing proteins in organisms whose genomic sequences are annotated.

The WD40 repeat is a short structural motif of approximately 40 residues, often ending in a tryptophan-aspartic acid dipeptide [163]. They consist of a variable region of approximately 20 amino acids followed by repeated residues. Proteins containing a WD40 domain possess 4 to 16 repeating units which form a beta-propeller structure [164,165]. WD40 repeats function as a scaffold for protein-protein interactions and complex assembling [166]. Although WD40 proteins have been most frequently been reported in eukaryotes, a published analysis investigated prokaryotic WD40 proteins and performed a detailed comparison with eukaryotic WD40 proteins [167]. This work identified 4,187 bacterial and 63 archaeal WD40 proteins, which accounts for 6.5% of all WD40 proteins. It is widely proposed that WD40 propeller domains formed by duplications of ancestral repeat-encoding peptide genes [168].

From the comparison given above, it can be seen that the cell division machinery employed by prokaryotes (both bacteria and archaea) and eukaryotes are almost entirely distinct. Almost all of the mitotic components appear to be taxonomically restricted to the eukaryotic domain. A notable exception is that FtsZ (a bacterial cell division protein) is thought to be a homologue of tubulin, which plays a crucial role in eukaryotic chromosome segregation during division [169]. However, this identification of homology is based largely on structural similarity, since the sequence identity is relatively weak (less than 20% overall). The sequence identity is strongest over the N-terminal GTP-binding domains but is almost completely absent over the C-terminal domains. Moreover, the function of FtsZ and tubulin is significantly different. The former assembles to form the contractile ring that facilitates bifurcation of the parent cell, while the latter is involved in chromosome segregation during mitosis.

Furthermore, the proteins that perform the analogous task to FtsZ in eukaryotes are quite different. The invagination of the bacterial cell inner membrane is mediated by FtsZ and the other proteins that together comprise the divisome. In eukaryotic cells, by contrast, a contractile ring forms from actin filaments and myosin motor proteins, which pinches the cell's membrane to form two daughter cells [170]. The mechanisms of segregating DNA in prokaryotes is also significantly different from the manner of segregating genetic material in eukaryotes. During eukaryotic mitosis (described in more detail previously), the cell's replicated DNA condenses into distinct chromosomes. These chromosomes are then equally divided and segregated into two daughter cells through a process guided by the spindle apparatus, ensuring each cell receives a complete and identical set of genetic information. The underlying apparatus of these processes, therefore, are quite distinct between prokaryotes and eukaryotes. Table 1 highlights key differences in mode of cell division between prokaryotes and eukaryotes.

Several features of the eukaryotic cell division cycle exhibit irreducible complexity and thus challenge evolutionary accounts of their origin. For example, the absence of the condensins would result in chromosomal disorganization and difficulty in proper segregation during cell division. The absence of the kinetochores would result in improper attachment of the chromosomes to the spindle apparatus, leading to unequal distribution of genetic material. (Indeed, the kinetochore network is found in all known eukaryotic organisms and is essential for the proper segregation of chromosomes during cell division). Without the separase, the sister chromatids would fail to separate, resulting in a failure of chromosome segregation during anaphase. Experiments knocking out separase result in embryonic lethality [171,172]. In the absence of the APC/C, cell cycle progression would be halted, preventing the transition from metaphase to anaphase. Experiments knocking out APC2 (a core subunit of APC/C) in mice, for instance, resulted in a lethal bone marrow failure within seven days following the procedure [173].

Likewise, loss of the Aurora kinase activity would disrupt the proper spindle formation and chromosome segregation during mitosis. Indeed, "Aurora A null mice die early during embryonic development before the 16-cell stage. These Aurora A null embryos have defects in mitosis, particularly in spindle assembly, supporting critical functions of Aurora A during mitotic transitions," [174]. Without the microtubules, the formation of the mitotic spindle would be impaired, which would in turn prevent the proper alignment and segregation of chromosomes. Experiments with mice embryos that are deficient in γ-tubulin show that a characteristic mitotic arrest halts development at the morula/blastocyst stages [175]. The contractile ring, which is composed of actin and myosin, is also crucial for cytokinesis. Its absence would lead to the failure of cell division, resulting in binucleated cells and other abnormalities. The absence of the dynein and kinesin motor proteins (which are particular to eukaryotes and lack homologues in prokaryotic cell division) would also compromise the transport and positioning of chromosomes, leading to misalignment of chromosomes

Table 1. Overview of the differences in cell division mechanism in prokaryotes and eukaryotes.

Processes	Prokaryotes	Eukaryotes	Comments and Comparisons
Cell Division	• Binary fission	• Mitosis / meiosis	• Completely different mechanisms of cell replication are used in prokaryotes vs. eukaryotes.
Chromosome Segregation	• Bidirectional segregation of circular chromosomes. ParA and ParB are involved in the partitioning of the bacterial chromosome. ParB binds to specific DNA sequences (parS) on the chromosome, organizing the DNA into a structure that facilitates segregation. ParA forms filaments and helps to move the ParB-bound DNA to opposite ends of the cell. • MukB, MukE and MukF are part of the MukBEF complex, which aids in organizing and segregating the bacterial chromosome.	• Bipolar attachment of linear chromosomes. • Condensins play a crucial role in condensing the chromatin into visible chromosomes at the onset of mitosis. They help in the proper compaction and organization of chromosomes. • Kinetochores are protein structures on the centromeres of each chromosome. Proteins like CENP-A, CENP-B and CENP-C are involved in its formation and help in the attachment of microtubules from the spindle apparatus during mitosis. • Separase is an enzyme that cleaves the protein cohesion, allowing sister chromatids to separate during anaphase of mitosis. • Anaphase-Promoting Complex (APC / C) is a large multiprotein E3 ubiquitin ligase complex that helps regulate the progression of the cell cycle, including the transition from metaphase to anaphase. It targets specific proteins for degradation, including securin (inhibitor of separase), allowing chromatids to separate. • Aurora Kinases: These kinases play a role in various stages of mitosis, including regulating chromosome segregation, centrosome maturation, and cytokinesis.	• Completely different mechanisms of chromosome segregation are used in prokaryotes vs. eukaryotes.
Membrane Invagination	• FtsZ forms a contractile ring-like structure at the site of division, guiding the assembly of other proteins necessary for septum formation. Other proteins, such as FtsA, ZipA and FtsK, also play critical roles in orchestrating the formation and constriction of the septum.	• During cleavage, a contractile ring composed mainly of actin and myosin filaments forms just beneath the plasma membrane at the equatorial region of the cell. Actin-binding proteins like myosin-II and other regulatory proteins aid in the formation and contraction of the ring, pinching the cell into two daughter cells.	• FtsZ, used in bacterial cell division, is a homologue of tubulin, which plays a critical role in organizing the eukaryotic mitotic spindle and in sister chromatid separation. However, the functions of FtsZ and tubulin are distinct, meaning this is a case of homologous parts performing different functions. FtsA is homologous to actin, which is part of the cytoskeleton in eukaryotes. Although actin is involved in membrane invagination in eukaryotes, its function is distinct from that of FtsA (which tethers the FtsZ ring to the inner membrane). • Apart from these examples, proteins involved in bacterial membrane invagination have no known homologues among eukaryotic proteins. The mechanisms of membrane invagination in eukaryotes and prokaryotes are highly distinct.

Table 1. Overview of the differences in cell division mechanism in prokaryotes and eukaryotes.

Processes	Prokaryotes	Eukaryotes	Comments and Comparisons
Checkpoints	Initiation and Control of DNA Replication • The initiation of DNA replication in bacteria is tightly regulated to prevent over-replication. Key regulatory proteins (such as DnaA in Escherichia coli) control the initiation of DNA replication by binding to specific sites on the chromosome. This can be considered analogous to the G1 checkpoint in eukaryotic cells, which ensures the cell is ready to enter the DNA synthesis (S) phase. *DNA Damage* • Bacteria possess mechanisms to detect and repair DNA damage. When DNA damage is detected, cell cycle progression can be halted or slowed down to allow for repair. This is akin to the DNA-damage checkpoints (G2 checkpoint) in eukaryotic cells. *Cell Division* • Bacteria have mechanisms to ensure that the cell is ready for division. Proteins such as FtsZ and Min system play crucial roles in determining the site of cell division, ensuring that the genetic material is properly segregated and that the division occurs accurately. This can be likened to the mitotic checkpoint in eukaryotic cells, which ensures proper chromosome alignment and segregation during cell division.	*G1 Checkpoint (Restriction Point)* • Purpose: Ensures the cell is ready to commit to the cell cycle. • Key Proteins: Cyclin-dependent kinases (CDKs) and cyclins. *S Checkpoint* • Purpose: Verifies DNA replication is complete and accurate. • Key Proteins: DNA replication checkpoint proteins (e.g., ATM, ATR, CHK1, CHK2). *G2 Checkpoint* • Purpose: Ensures DNA replication and repair are successful before mitosis. • Key Proteins: CDKs, cyclins, and proteins involved in DNA repair. *Mitotic Checkpoint (Spindle Checkpoint)* • Purpose: Confirms proper spindle fiber attachment to chromatids. • Key Proteins: Mitotic checkpoint proteins (e.g., BUB1, BUBR1, MAD1, MAD2, CDC20).	• Prokaryotes have mechanisms to monitor and regulate their cell cycle, but they are simpler and less structured compared to the well-defined checkpoints found in eukaryotic cell cycles, and the involved proteins and mechanisms are quite different. There are no known homologues between bacterial proteins that serve as checkpoints vs eukaryotic proteins that perform similar functions.
Ubiquitylation	• No analogue in prokaryotes	• SCF is involved in targeted protein degradation during the cell cycle. • APC/C targets specific proteins for degradation during cell cycle progression. • MCC inhibits APC/C activity until all chromosomes are properly attached.	• Prokaryotes have no equivalent, and there are no homologues for eukaryotic proteins involved in ubiquitylation among bacterial proteins. Asgard archaea does employ ubiquitylation, but there are no known homologues of the SCF or APC/C among the Asgard.
Driving Cell Cycle Progression	• In *Caulobacter crescentus*, CtrA controls the transition from the swarmer to the stalked cell. CtrA acts as both a transcription factor and a phosphoregulatory protein, influencing the expression of genes involved in cell cycle progression. • CtrA activity is regulated by DivK and DivL. DivK is a response regulator that can be phosphorylated, and DivL is a histidine kinase that phosphorylates DivK.	• Cyclins and CDKs regulate the cell cycle progression by phosphorylating target proteins.	• Prokaryotes have no functional equivalent to cyclins and CDKs, and there are no homologues to cyclins and CDKs found in prokaryotes. The closest analogue is perhaps the CtrA system in *Caulobacter crescentus*, but this is very different to the control of cell cycle progression in eukaryotic cells. CtrA has no known homologue among eukaryotic proteins.

during metaphase and difficulty in establishing a proper mitotic spindle and errors in chromosome segregation during anaphase.

The cyclin and Cdk molecules are not necessarily all individually essential due to redundancy. Mouse knockouts of Cdk 2, 3, 4 or 6 are still viable [176,177,178,179,180,181,182]. Moreover, yeast cells only have a single Cdk, namely Cdk1 [183]. Curiously, double knockouts involving Cdk2 and 4, or Cdk4 and 6, result in embryonic lethality, though a double knockout of Cdk2 and 6 does not [182, 184]. This indicates that the pair Cdk2 and 4 and the pair Cdk4 and 6 can substitute for one another [185]. However, the absence of Cdk1 has been shown to result in arrested development at the blastocyst stage and, thus, appears to be essential [186].

The timely progression from one phase of the cell cycle to the next also depends crucially on the activation of these Cdks by cyclin molecules. In their absence, the Cdks would be inactive, leading to a halt in the cell cycle. Though it is not necessarily the case that all of the cyclin molecules are essential for successful replication due to redundancy, the absence of cyclin B (which activates Cdk1 to driveentry into mitosis), would result in an impairment of the G2 to M phase transition. The cell would fail to enter mitosis. This is supported by experimental knockout of cyclin B in mouse embryos, which arrested the cell cycle in G2 after only two divisions [187,188].

The various regulatory checkpoints are also crucial for successful cell division. For example, the loss of the mitotic checkpoint complex would abolish the cell's ability to monitor spindle assembly, significantly increasing the likelihood of cells proceeding through division with defective spindles and resulting in chromosome missegregation and aneuploidy. The loss of the G1 checkpoint would allow damaged DNA to enter S phase, potentially leading to the propagation of mutations and genomic instability. Absence of the G2 checkpoint would permit cells with DNA damage to progress into mitosis, resulting in the division of cells with unrepaired genetic material and an increased risk of chromosome aberrations. Absence of the DNA damage checkpoint in S phase would allow the replication of damaged DNA, leading to the propagation of mutations and an elevated risk of genetic abnormalities in the daughter cells.

Given the irreducibly complex nature of the eukaryotic cell division cycle, which requires multiple different proteins and complexes to work together in unison to bring about the higher-level objective of cell division, it is difficult to envision it being assembled by blind evolutionary processes without conscious knowledge of where the target is. This problem is accentuated by the wide gulf that exists between eukaryotic and prokaryotic cell division machinery.

In the next section, we shall seek to determine the time frame in which the eukaryotic cell division machinery must have arisen.

Estimating an Upper Limit for the Time Available for the Transition in Cell Division Machinery

The difference in eukaryotic and prokaryotic modes of cell division is striking, and it raises the question of whether the time available for the transition between the two sets of machineries was sufficient to attribute it to the standard processes of evolution— random variation acted on by natural selection and other undirected processes. To determine this, we examine how long ago the eukaryotic division machinery can be inferred to have been present (from phylostratigraphic evidence) and the latest point in evolution that we can confidently say that the machinery was *not* present. This analysis assumes that the paradigm of common ancestry is true, and (given that assumption) the critique is limited to the efficacy of the neo-Darwinian mechanism to accomplish the transition between the two modes of division within the maximum time inferred to be available.

As should be apparent from the foregoing review, the prokaryotic system of cell division and chromosome segregation is vastly different from that found in eukaryotic organisms. There is essentially nothing in common, either in terms of the molecular mechanisms or the underlying protein components.

Phylogenomic reconstructions of the Last Eukaryotic Common Ancestor (LECA) indicate that LECA was highly sophisticated, possessing a complex genome [189], complex coat/tether systems that organize vesicle trafficking between membrane-bound organelles [190,191], and cellular systems such as cilia and flagella [192]. Such studies have revealed that the prokaryote-eukaryote transition was accompanied by a significant level of *de novo* innovation. However, there is still a lack of understanding of the tempo and mode of eukaryogenesis.

With respect to the mitotic apparatus, recent phylogenomic analysis has revealed that at least 24 out of 37 known subunits, co-activators and targets of the APC/C were present in LECA [152]. The researchers surveyed complete or near-complete genome sequences in public databases available at the time to retrieve homologues of the various components. Phylogenetic methods were used to infer the origin and evolution of each respective component. Their conclusions revealed the highly complex and controlled character of the cell cycle possessed by LECA.

Similar phylogenomic analysis has been conducted by Vleugel et al. on the constituents of the mitotic checkpoint and their respective functional domains and motifs, leading them to likewise conclude that "most checkpoint components are ancient and were likely present in the last eukaryotic common ancestor," [153]. A yet further analysis also provided support for the notion that the dynactin complex, the activator of cytoplasmic dynein (important for mitosis) is also a very ancient complex and that most likely all of its subunits were present in LECA [193].

Another study, analyzing 90 different eukaryotic lineages, inferred the evolutionary histories of the proteins involved in the kinetochore network by applying Dollo parsimony (allowing only a single invention and inferring subsequent losses based on maximum parsimony) [154]. The study inferred that as many as 49 out of the 70 proteins examined (70%) were present in LECA.

From the data surveyed above, it appears that the vast majority of the mitotic components were present in LECA. Unfortunately, inferring anything about eukaryotic organisms

living between the first and last eukaryotic ancestor is highly murky and speculative territory. Therefore, to gain a handle on the upper limit for the time available to accomplish such a radical innovation, we need to determine the most closely-related living relative of eukaryotes among prokaryotes and to establish to what extent (if any) we can detect remote homologues of the components involved in mitosis.

A recently discovered archaeal superphylum called Asgard is thought to represent the closest known living relatives of eukaryotes among prokaryotes [157,194]. Its members include Lokiarchaeota, Thorarchaeota, Odinarchaeota and Heimdallarchaeota. Strikingly, the Asgard archaeal organisms were found to possess a number of proteins which were hitherto believed to be eukaryotic signature proteins (ESPs).

Since Asgard archaea are thought to be the most closely related prokaryotes to the eukaryotic cell and they possess various otherwise-believed-to-be ESPs, Asgard archaea are thus the most promising candidates for detection of remote homologues of the components involved in mitosis.

Given that almost all of the mitotic components lack homologues among the Asgard archaea (or other prokaryotic groups), this suggests a scenario in which the proteins arose *de novo* after the separation of the archaeal and eukaryotic lineages. This means that the mitotic protein components represent true ESPs based on current taxonomic sampling.

These results support the hypothesis that the components involved in eukaryotic cell division are substantially *de novo* eukaryotic innovations that arose sometime after the split with the archaeal lineages. There seems to be no *prima facie* evidence that the highly distinct cell replication machinery of these two systems are related through descent with modification. The fact that the majority of the components have also been inferred from phylostratigraphic analysis to have been present in LECA (estimated to have lived 1.1 to 2.3 billion years ago [195]) suggests that all eukaryotic proteins associated with cell division came to exist sometime after the eukaryotic split with the archaea but before LECA. While the timing of these events is unclear [196], it has been proposed that eukaryotes may have diverged very early in the history of life [197].

How much time would be needed for such a transition, and does the answer to this question even matter? Using very rough but liberal estimates, if life arose around 4 billion years ago and if LECA lived 1.1 to 2.3 billion years ago, this suggests that a window of somewhere between 1.7 to 2.9 billion years would have been available for this transition. While 1.7 to 2.9 billion years is by any estimate a very long period of time, population genetics studies have shown that even within bacteria (which can evolve rapidly compared to eukaryotes) systems which require just 7 (or more) mutations before providing an advantage would be highly unlikely to evolve during the entire history of life on earth [198]. In the window of time available for the origin of eukaryotic cell division control, multiple proteins not only need to evolve into their specifically crafted structures for the purpose of mediating the cell cycle, but they would need to replace the bacterial cell division proteins as well as be assembled into a highly coordinated system—all while maintaining the integrity of the cell division and DNA segregation process.

A standard waiting times analysis begins with some starting system (A) and then asks how long it would take to evolve some target system (A'), which would require making improvements x, y, and z to A. In this case, however, our target system (eukaryotic cell division) contains many components not even present in the starting system A (prokaryotic cell division). In that regard, the waiting times scenario we are presented with here is not simply a matter of improving system A until it becomes A', but rather evolving an entirely new system with completely new components—something so different from A that it does not warrant being called A', but rather should be called B. Thus, our waiting times analysis presents us with the challenge of determining how long it would take to produce from scratch quite a few enzymes and molecular machines necessary for eukaryotic cell division which are not present in the machinery of prokaryotic cell division. Given the hundreds if not thousands of coordinated mutations that would be necessary to evolve such functional systems, it seems likely that the amount of time required for such complexity to arise by standard evolutionary mechanisms would exceed the age of life on Earth, if not the entire universe [198,199].

It may of course be argued in response that the mitotic protein components did in fact arise from prokaryotic protein precursors, but that the sequences have subsequently diverged to such a degree that they are no longer detectable by a BLAST search. For instance, HSP70 and actin are known to be structurally similar even though their sequences are so widely divergent that they do not return hits for one another in a BLAST search [200,201]. Indeed, even among eukaryotes some of the mitotic components examined in this study do not share substantial sequence identity among different taxa. It must be admitted that this scenario cannot be easily ruled out by empirical or bioinformatic techniques. However, there is no positive empirical evidence to date that this is the case, and the burden of proof must surely be borne by those who maintain that such molecular predecessors did in fact exist. A model which provides no evidence for these relationships is not scientifically tenable.

Furthermore, many of the protein components involved in facilitating eukaryotic cell division are functional only in the context of cell division and are not known to have uses in other biological contexts. Additionally, there is no evidence that these proteins are being used in prokaryotic cell division. Given how starkly different the molecular mechanisms and protein components are between prokaryotic and eukaryotic cell division, it is evident that hypothetical homologues of the eukaryotic proteins, if they existed, must have participated in functions other than the mediation of cell division. Even given that such components existed, how they came to be fitted and assembled to form the elegant and tightly regulated process we know as eukaryotic cell division must still be explained.

Given the lack in any extant organisms of any transitional intermediates between the modes of prokaryotic and eukaryotic

cell division, it is also striking that, despite the wide gulf that exists between prokaryotic and eukaryotic modes of cell division (and hence the immense feat of rewiring that is required to facilitate such an evolutionary transition), no trace has been left of the intermediate steps between prokaryotic and eukaryotic cell division.

Implications for Intelligent Design

If blind evolutionary mechanisms are not causally adequate to bridge the gap between the world of the prokaryotic and eukaryotic cell, is there any other cause that is? The transition from prokaryotes to eukaryotes is inextricably associated with the creation of new information in the form of genes necessary to code for the expression of the numerous associated proteins (most of which are absent in prokaryotes). Furthermore, the functions of those proteins must be tightly regulated and controlled through various checkpoint mechanisms. To make matters worse for the standard processes of evolutionary biology, the transition must occur through many small and steady incremental steps, each yielding some functional advantage while also retaining the integrity of the cell division apparatus. Yet, as we have seen, many of the necessary processes are irreducibly complex, meaning that many mutually co-dependent changes are needed before a fitness advantage could be realized.

We know from experience that intelligent agents are capable of rapidly introducing new information into a system in order to radically change its fundamental components into a new set of integrated parts that perform some function. Thus, in every other realm of experience, we would routinely attribute such engineered systems to intelligent agency—a cause that possesses foresight and which can plan for the future, visualize complex endpoints and consciously bring together everything needed to actualize a complex endpoint. The famed 18th and 19th century philosopher William Paley is well known especially for his book *Natural Theology* and the watchmaker analogy that he develops therein. Paley wrote [202]:

> In crossing a heath, suppose I pitched my foot against a stone, and were asked how the stone came to be there; I might possibly answer, that, for anything I knew to the contrary, it had lain there forever: nor would it perhaps be very easy to show the absurdity of this answer. But suppose I had found a watch upon the ground, and it should be inquired how the watch happened to be in that place; I should hardly think of the answer I had before given, that for anything I knew, the watch might have always been there. ... There must have existed, at some time, and at some place or other, an artificer or artificers, who formed [the watch] for the purpose which we find it actually to answer; who comprehended its construction, and designed its use. ... Every indication of contrivance, every manifestation of design, which existed in the watch, exists in the works of nature; with the difference, on the side of nature, of being greater or more, and that in a degree which exceeds all computation.

There is an unexpected similarity between Paley's watch and the biological mechanisms that underlie cell division: both employ machinery and apparatuses that use carefully orchestrated mechanisms in order to control the timing of key events. How vindicated Paley would have felt had he had a chance to peer into the molecular wonder that is cell division, whether in prokaryotes or in eukaryotes. He would have observed intricate nanomachinery against which a human-made watch pales in comparison, providing compelling evidence for intelligent design.

The burden of proof should rest with the one who would claim that such systems exhibit in fact only the deceptive illusion of design and came to be by the mindless forces of nature—chance and necessity. To the contrary, the more one investigates the scientific evidence, the more apparent the limitation of neo-Darwinian mechanisms become, and the more it becomes apparent that intelligent design as a theory of biological origins succeeds at precisely the point at which neo-Darwinian processes break down, that is, when it comes to explaining the origins of meaningful information. The engineering prowess of living systems is well-attested by the apparatus of eukaryotic cell division.

The overall lack of prokaryotic homologues for proteins involved in controlling the eukaryotic cell cycle challenges their blind evolutionary origin, given that the cell division apparatus are so different that it seems highly unlikely that the transition from prokaryotic binary fission to eukaryotic mitosis could have taken place via unguided evolutionary mechanisms. This finding could also have implications not only for the unguided evolution of these systems but also for the standard view of common ancestry between eukaryotes and prokaryotes because only guided descent could overcome the problem of maladaptive intermediate stages. If eukaryotes and prokaryotes do not share a common ancestor, then the many points of similarity that exist between the domains of life (such as commonality of the genetic code, as well as metabolic pathways including glycolysis and citric acid cycle) require an alternative explanation. Besides common ancestry, the only known alternative phenomenon that can result in deep similarity is common intelligent design.

1. Darwin C (1859) *The Origins of Species by Means of Natural Selection, Or, the Preservation of Favoured Races in the Struggle for Life.* John Murray (London).
2. Erwin D, Valentine J (2013) *The Cambrian Explosion: The Construction of Animal Biodiversity.* Roberts and Company (Greenwood Village, CO).
3. Meyer SC (2013) *Darwin's Doubt: The Explosive Origin Of Animal Life And the Case For Intelligent Design.* HarperOne (New York).
4. Koonin EV (2007) The Biological Big Bang model for the major transitions in evolution. *Biol Direct* 2:21. **doi:**10.1186/1745-6150-2-21
5. Khalturin K, Hemmrich G, Fraune S, Augustin R, Bosch TC (2009) More than just orphans: are taxonomically-restricted genes important in evolution? *Trends Genet* 25(9): 404-13. **doi:**10.1016/j.tig.2009.07.006
6. Tautz D, Domazet-Lošo T (2011) The evolutionary origin of orphan genes. *Nat Rev Genet* 12(10): 692–702. **doi:**10.1038/nrg3053
7. Beiko RG (2011) Telling the whole story in a 10,000-genome world. *Biol Direct* 6:34. **doi:**10.1186/1745-6150-6-34
8. Zielezinski A, Dobrychlop W, Karlowski WM (2023) TRGdb: a universal resource for the exploration of taxonomically restricted genes in bacteria. *Database (Oxford)* 2023:baad058. **doi:**10.1093/database/baad058
9. Koonin EV, Krupovic M, Dolja VV (2023) The global virome: How much diversity and how many independent origins? *Environ Microbiol* 25(1): 40–44. **doi:**10.1111/1462-2920.16207
10. Lane N, Martin W (2010) The energetics of genome complexity. *Nature* 467: 929–934. **doi:**10.1038/nature09486
11. Bharadwaj R, Yu H (2004) The Spindle Checkpoint, Aneuploidy, and Cancer. *Oncogene* 23(11): 2016–2027. **doi:**10.1038/sj.onc.1207374
12. Kops GJ, Weaver BA, Cleveland DW (2005) On the Road to Cancer: Aneuploidy and the mitotic checkpoint. *Nature Reviews Cancer* 5(10): 773–785. **doi:**10.1038/nrc1714
13. Malumbres M, Barbacid M (2009) Cell cycle, CDKs and cancer: a changing paradigm. *Nature Reviews* 9(3): 153–166. **doi:**10.1038/nrc2602
14. Suryadinata R, Sadowski M, Sarcevic B (2010) Control of cell cycle progression by phosphorylation of cyclin-dependent kinase (CDK) substrates. *Bioscience Reports* 30(4): 243–255. **doi:** 10.1042/BSR20090171
15. Glotzer M, Murray AW, Kirschner MW (1991) Cyclin is degraded by the ubiquitin pathway. *Nature* 349 (6305): 132–8. **doi:**10.1038/349132a0
16. Porter LA, Donoghue DJ (2003) Cyclin B1 and CDK1: nuclear localization and upstream regulators. *Progress in Cell Cycle Research* 5: 335–347.
17. Heald R, McKeon F (1990) Mutations of phosphorylation sites in lamin A that prevent nuclear lamina disassembly in mitosis. *Cell* 61(4): 579–589. **doi:**10.1016/0092-8674(90)90470-y
18. Hershko A (1999) Mechanisms and regulation of the degradation of cyclin B. *Philosophical Transactions of the Royal Society B* 354(1389): 1571–1576. **doi:**10.1098/rstb.1999.0500
19. Chang DC, Xu N, Luo K (2003) Degradation of Cyclin B Is Required for the Onset of Anaphase in Mammalian Cells. *The Journal of Biological Chemistry* 278: 37865–37873. **doi:**10.1074/jbc.M306376200
20. Kirschner M, Mitchison T (1986) Beyond self-assembly: From microtubules to morphogenesis. *Cell* 3(9): 329–342. **doi:**10.1016/0092-8674(86)90318-1
21. Biggins S, Walczak CE (2003) Captivating Capture: how microtubules attach to kinetochores. *Current Biology* 13: 449–460. **doi:**10.1016/s0960-9822(03)00369-5
22. Mimori-Kiyosue Y, Tsukita S (2003) "Search-and-Capture" of Microtubules through Plus-End-Binding Proteins (+TIPs). *The Journal of Biochemistry* 134(3): 321–326. **doi:**10.1093/jb/mvg148.
23. Hauf S, Watanabe Y (2004) Kinetochore orientation in mitosis and meiosis. *Cell* 119: 317–327. **doi:**10.1016/j.cell.2006.12.040
24. Cheeseman IM, Desai A (2008) Molecular architecture of the kinetochore-microtubule interface. *Nature Reviews Molecular Cell Biology* 9: 33–46. **doi:**10.1038/nrm2310
25. Bailey A, Panchenko T, Sathyan KM, Petkowski JJ, Pai PJ et al. (2013) Posttranslational modification of CENP-A influences the conformation of centromeric chromatin. *Proceedings of the National Academy of Sciences USA* 110(29): 11827–32. **doi:**10.1073/pnas.1300325110
26. Hori T, Shang WH, Takeuchi K, Fukagawa T (2012) The CCAN recruits CENP-A to the centromere and forms the structural core for kinetochore assembly. *Journal of Cell Biology* 200(1): 45. **doi:**10.1083/jcb.201210106
27. Gonen S, Akiyoshi B, Iadanza MG, Shi D, Duggan N et al. (2012) The structure of purified kinetochores reveals multiple microtubule-attachment sites. *Nature Structural and Molecular Biology* 19: 925–929. **doi:**10.1038/nsmb.2358
28. Varma D, Wan X, Cheerambathur D, Gassmann R, Suzuki A, Lawrimore J, Desai A, Salmon ED (2013) Spindle assembly checkpoint proteins are positioned close to core microtubule attachment sites at kinetochores. *Journal of Cell Biology* 202(5): 735–746. **doi:**10.1083/jcb.201304197
29. Karess R (2005) Rod–Zw10–Zwilch: a key player in the spindle checkpoint. *Trends in Cell Biology* 15(7): 386–392. **doi:**10.1016/j.tcb.2005.05.003
30. Peters JM, Tedeschi A, Schmitz J (2008) The cohesin complex and its roles in chromosome biology. *Genes and Development* 22: 3089–3114. **doi:**10.1101/gad.1724308
31. Nasmyth K, Haering CH (2009) Cohesin: Its roles and mechanisms. *Annual Review of Genetics* 43: 525–558. **doi:**10.1146/annurev-genet-102108-134233
32. Mehta GD, Rizvi SMA, Ghosh SK (2012) Cohesin: A guardian of genome integrity. –*BBA Molecular Cell Research* 1823(8): 1324–1342. **doi:**10.1016/j.bbamcr.2012.05.027
33. Graumann PL (2001). SMC proteins in bacteria: condensation motors for chromosome segregation? Biochimie. 83(1): 53–59. **doi:**10.1016/s0300-9084(00)01218-9
34. Zur A, Brandeis M (2001) Securin degradation is mediated by fzy and fzr, and is required for complete chromatid separation but not for cytokinesis. *The EMBO Journal* 20(4): 792–801. **doi:**10.1093/emboj/20.4.792
35. Uhlmann F, Lottspeich F, Nasmyth K (1999) Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. *Nature* 400(6739): 37–42. **doi:**10.1038/21831
36. Cardozo T, Pagano M (2004) The SCF ubiquitin ligase: Insights into a molecular machine. *Nature Reviews: Molecular Cell Biology* 5: 739-751. **doi:**10.1038/nrm1471
37. Nakayama KI, Nakayama K (2006) Ubiquitin ligases: cell-cycle control and cancer. *Nature Reviews Cancer* 6: 369-381. **doi:**10.1038/nrc1881
38. Hicke L (2001) Protein regulation by monoubiquitin. *Nature Reviews Molecular Cell Biology* 2:195201. **doi:**10.1038/35056583
39. Grice GL, Nathan JA (2016) The recognition of ubiquitinated proteins by the proteasome. *Cellular and Molecular Life Sciences* 73: 3497–3506. **doi:**10.1007/s00018-016-2255-5
40. Yau RG Doerner K, Castellanos ER, Haakonsen DL, Werner A et al. (2017) Assembly and function of heterotypic ubiquitin chains in cell-cycle and protein quality control. *Cell* 171(4): 918–933. **doi:**10.1016/j.cell.2017.09.040
41. Zheng N, Schulman BA, Song L, Miller JJ, Jeffrey PD et al. (2002) Structure of the Cul1 – Rbx1 – Skp1 – F box Skp2 SCF ubiquitin ligase complex. *Nature* 416: 703–709. **doi:**10.1038/416703a
42. Kellogg DR (2003) Wee1-dependent mechanisms required for coordination of cell growth and cell division. *Journal of Cell Science* 116(24): 4883–90. **doi:**10.1242/jcs.00908

43. Sgambato A, Cittadini A, Faraglia B, Weinstein IB (2000) Multiple functions of p27(Kip1) and its alterations in tumor cells: a review. *Journal of Cell Physiology* 183(1): 18–27. **doi:**10.1002/(SICI)1097-4652(200004)183:1<18::AID-JCP3>3.0.CO;2-S
44. Deng C, Zhang P, Harper JW, Elledge SJ, Leder P (1995) Mice Lacking p21CIP1/WAF1 undergo normal development, but are defective in G1 checkpoint control. *Cell* 82(4): 675–684. **doi:**10.1016/0092-8674(95)90039-x
45. Gartel AL, Radhakrishnan SK (2005) Lost in transcription: p21 repression, mechanisms, and consequences. *Cancer Research* 65(10): 3980–3985. **doi:**10.1158/0008-5472.CAN-04-3995
46. Peters JM (2006) The anaphase promoting complex/cyclosome: A machine designed to destroy. *Nature Reviews Molecular Cell Biology* 7(9): 644–656. **doi:**10.1038/nrm1988
47. Barford D (2011) Structural insights into anaphase-promoting complex function and mechanism. *Philosophical Transactions of the Royal Society B*. 366: 3605–3624. **doi:**10.1098/rstb.2011.0069
48. 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: 269–278. **doi:**10.1016/0092-8674(95)90337-2
49. 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. *Molecular Biology of the Cell* 6: 185–197. **doi:**10.1091/mbc.6.2.185
50. King RW, Peters JM, Tugendreich S, Rolfe M, Hieter P et al. (1995) A 20S complex containing Cdc27 and Cdc16 catalyses the mitosis-specific conjugation of ubiquitin to cyclin B. *Cell* 81: 279–288. **doi:** 10.1016/0092-8674(95)90338-0
51. Yamano H, Gannon J, Mahbubani H, Hunt T (2004) Cell cycle-regulated recognition of the destruction box of cyclin B by the APC/C in Xenopus egg extracts. *Molecular Cell* 13(1): 137–147. **doi:**10.1016/s1097-2765(03)00480-5
52. Pfleger CM, Kirschner MW (2000) The KEN box: an APC recognition signal distinct from the D box targeted by Cdh1. *Genes and Development* 14: 655–665.
53. Izawa D, Pines J (2011) How APC/C–Cdc20 changes its substrate specificity in mitosis. *Nature Cell Biology* 13: 223–233. **doi:**10.1038/ncb2165
54. Listovsky T, Sale JE (2013) Sequestration of CDH1 by MAD2L2 prevents premature APC/C activation prior to anaphase onset. *Journal of Cell Biology* 203(1): 87–100. **doi:**10.1083/jcb.201302060
55. Chang L, Zhang Z, Yang J, McLaughlin SH, Barford D (2014) Molecular architecture and mechanism of the anaphase promoting complex. *Nature* 513: 388–395. **doi:**10.1038/nature13543
56. Lara-Gonzalez P, Westhorpe FG, Taylor SS (2012) The spindle assembly checkpoint. *Current Biology* 22: 966–980. **doi:**10.1016/j.cub.2012.10.006
57. 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. *Journal of Cell Biology* 130: 941–948. **doi:**10.1083/jcb.130.4.941
58. Li X Nicklas R B (1995) Mitotic forces control a cell-cycle checkpoint. *Nature* 373: 630–632. **doi:**10.1038/373630a0
59. Musacchio A, Salmon E D (2007) The spindle-assembly checkpoint in space and time. *Nature Reviews Molecular Cell Biology* 8: 379–393. **doi:**10.1038/nrm2163
60. Pines J (2011) Cubism and the cell cycle: the many faces of the APC/C. Nature reviews. *Molecular cell biology* 12: 427–438. **doi:**10.1038/nrm3132
61. Yu H (2002) Regulation of APC-Cdc20 by the spindle checkpoint. *Current Opinion in Cell Biology* 14: 706–714. **doi:**10.1016/s0955-0674(02)00382-4
62. Tang Z, Bharadwaj R, Li B Yu H (2001) Mad2-Independent inhibition of APCCdc20 by the mitotic checkpoint protein BubR1. *Developmental Cell* 1: 227–237. **doi:**10.1016/s1534-5807(01)00019-3
63. Hardwick KG, Johnston RC, Smith DL, Murray AW (2000) MAD3 encodes a novel component of the spindle checkpoint which interacts with Bub3p, Cdc20p, and Mad2p. *Journal of Cell Biology* 148(5): 871–882. **doi:**10.1083/jcb.148.5.871
64. Fraschini R, Beretta A, Sironi L, Musacchio A, Lucchini G, Piatti S (2001) Bub3 interaction with Mad2, Mad3 and Cdc20 is mediated by WD40 repeats and does not require intact kinetochores. *EMBO Journal* 20: 6648–6659. **doi:**10.1093/emboj/20.23.6648
65. Fang G (2002) Checkpoint protein BubR1 acts synergistically with Mad2 to inhibit anaphase promoting complex. *Molecular Biology of the Cell* 13: 755–766. **doi:**10.1091/mbc.01-09-0437
66. Poddar A, Stukenberg P T Burke DJ (2005) Two complexes of spindle checkpoint proteins containing Cdc20 and Mad2 assemble during mitosis independently of the kinetochore in Saccharomyces cerevisiae. *Eukaryotic Cell* 4: 867–878. **doi:**10.1128/EC.4.5.867-878.2005
67. Nilsson J, Yekezare M, Minshull J, Pines J (2008) The APC/C maintains the spindle assembly checkpoint by targeting Cdc20 for destruction. *Nature Cell Biology* 10(12):1411–1420. **doi:**10.1038/ncb1799
68. Kulukian A, Han JS, Cleveland DW (2009) Unattached kinetochores catalyze production of an anaphase inhibitor that requires a Mad2 template to prime Cdc20 for BubR1 binding. *Developmental Cell* 16: 105–117. **doi:**10.1016/j.devcel.2008.11.005
69. Collin P, Nashchekina O, Walker R, Pines J (2013) The spindle assembly checkpoint works like a rheostat rather than a toggle switch. *Nature Cell Biology* 15: 1378–85. **doi:**10.1038/ncb2855
70. Etemad B, Kuijt TE, Kops GJ (2015) Kinetochore–microtubule attachment is sufficient to satisfy the human spindle assembly checkpoint. *Nature Communications* 6(8987). **doi:**10.1038/ncomms9987
71. Tauchman EC, Boehm FJ, DeLuca JG (2015) Stable kinetochore–microtubule attachment is sufficient to silence the spindle assembly checkpoint in human cells. *Nature Communications* 6(10036). **doi:**10.1038/ncomms10036
72. Buffin E, Lefebvre C, Huang J, Gagou ME, Karess RE (2005) Recruitment of Mad2 to the kinetochore requires the Rod/Zw10 complex. *Current Biology* 15:856–861. **doi:**10.1016/j.cub.2005.03.052
73. Yang M, Li B, Tomchick DR, Machius M, Rizo Jet al. (2007) p31comet blocks Mad2 activation through structural mimicry. *Cell* 131: 744-755. **doi:**10.1016/j.cell.2007.08.048
74. Westhorpe FG, Tighe A, Lara-Gonzalez P Taylor SS (2011) $p31^{comet}$-mediated extraction of Mad2 from the MCC promotes efficient mitotic exit. *Journal of Cell Science* 124: 3905–3916. **doi:**10.1242/jcs.093286
75. Eytan E, Wang K, Miniowitz-Shemtov S, Sitry-Shevah D, Kaisari S et al. (2014) Hershko Disassembly of mitotic checkpoint complexes by the joint action of the AAA-ATPase TRIP13 and p31(comet) *Proceedings of the National Academy of Sciences USA* 111: 12019-12024. **doi:**10.1073/pnas.1412901111
76. Wang K, Sturt-Gillespie B, Hittle JC, Macdonald D, Chan GK et al. (2014) Thyroid hormone receptor interacting protein 13 (TRIP13) AAA-ATPase is a novel mitotic checkpoint-silencing protein. *Journal of Biological Chemistry* 289: 23928-23937. **doi:**10.1074/jbc.M114.585315
77. Ma HT, Poon RYC (2016) TRIP13 regulates both the activation and inactivation of the spindle-assembly checkpoint. *Cell Reports* 14(5): 1086–1099. **doi:**10.1016/j.celrep.2016.01.001
78. Ye et al., (2015) TRIP13 is a protein-remodeling AAA+ ATPase that catalyzes MAD2 conformation switching. *eLife* 4. **doi:**10.7554/eLife.07367

79. Glover DM, Leibowitz, MH, McLean, DA, Parry, H (1995) Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles. *Cell* 81: 95–105. **doi:**10.1016/0092-8674(95)90374-7
80. Carmena M, Ruchaud S, Earnshaw WC (2009) Making the Auroras glow: regulation of Aurora A and B kinase function by interacting proteins. *Current Opinion in Cell Biology* 21: 796–805. **doi:**10.1016/j.ceb.2009.09.008
81. Cooke CA, Heck, MM, Earnshaw, WC (1987) The inner centromere protein (INCENP) antigens: movement from inner centromere to midbody during mitosis. *Journal of Cell Biology* 105: 2053–67. **doi:**10.1083/jcb.105.5.2053
82. Ainsztein AM, Kandels-Lewis SE, Mackay AM, Earnshaw WC (1998) INCENP centromere and spindle targeting: Identification of essential conserved motifs and involvement of heterochromatin protein HP1. *Journal of Cell Biology* 143: 1763–1774. **doi:**10.1083/jcb.143.7.1763
83. Vader G, Kauw JJ, Medema RH, Lens SM (2006) Survivin mediates targeting of the chromosomal passenger complex to the centromere and midbody. *EMBO Reports* 7: 85–92. **doi:**10.1038/sj.embor.7400562
84. Klein UR, Nigg EA, Gruneberg U (2006) Centromere targeting of the chromosomal passenger complex requires a ternary subcomplex of Borealin, Survivin, and the N-terminal domain of INCENP. *Molecular Biology of the Cell* 17: 2547–2558. **doi:**10.1091/mbc.e05-12-1133
85. Jeyaprakash AA *et al.* (2007) Structure of a Survivin-Borealin-INCENP core complex reveals how chromosomal passengers travel together. *Cell*. 131: 271–285. **doi:**10.1016/j.cell.2007.07.045
86. McKinley KL, Cheeseman IM (2016) The molecular basis for centromere identity and function. Nature Reviews Molecular and Cell Biology 17: 16–29. **doi:**10.1038/nrm.2015.5
87. Musacchio A, Desai A (2017) A Molecular View of Kinetochore Assembly and Function. *Biology* 6(5). **doi:**10.3390/biology6010005
88. Gascoigne KE, Takeuchi K, Suzuki A, Hori T, Fukagawa T, Cheeseman IM (2011) Induced ectopic kinetochore assembly bypasses the requirement for CENP-A nucleosomes. *Cell* 145:410–422. **doi:**10.1016/j.cell.2011.03.031
89. Gascoigne KE, Cheeseman IM (2013) CDK-dependent phosphorylation and nuclear exclusion coordinately control kinetochore assembly state. *Journal of Cell Biology* 201: 23–32. **doi:**10.1083/jcb.201301006
90. Weir JR *et al.* (2016) Insights from biochemical reconstitution into the architecture of human kinetochores. *Nature* 537:249–253. **doi:**10.1038/nature19333
91. Nishino T, Takeuchi K, Gascoigne KE, Suzuki A, Hori T, Oyama T, Morikawa K, Cheeseman IM, Fukagawa T (2012) CENP-T-W-S-X forms a unique centromeric chromatin structure with a histone-like fold. *Cell* 148: 487–501. **doi:**10.1016/j.cell.2011.11.061
92. McKinley KL, Sekulic N, Guo LY, Tsinman T, Black BE, Cheeseman IM (2015) The CENP-L-N Complex Forms a Critical Node in an Integrated Meshwork of Interactions at the CentromereKinetochore Interface. *Molecular Cell* 60: 886–898. **doi:**10.1016/j.molcel.2015.10.027
93. Bancroft J, Auckland P, Samora CP, McAinsh AD (2015) Chromosome congression is promoted by CENP-Q- and CENP-E-dependent pathways. *Journal of Cell Science* 128: 171–184. **doi:**10.1242/jcs.163659
94. Kang YH., Park CH, Kim TS, Soung NK, Bang JK, Kim BY, Park JE, Lee KS. (2011) Mammalian polo-like kinase 1-dependent regulation of the PBIP1-CENP-Q complex at kinetochores. *Journal of Biological Chemistry* 286: 19744–19757. **doi:**10.1074/jbc.M111.224105
95. Drinnenberg IA, Henikoff S, Malik HS (2016) Evolutionary Turnover of Kinetochore Proteins: A Ship of Theseus? *Trends in Cell Biology* 26: 498–510. **doi:**10.1016/j.tcb.2016.01.005
96. Varma D, Salmon ED (2012) The KMN protein network -- chief conductors of the kinetochore orchestra. *Journal of Cell Science* 125(24): 5927-5936. **doi:**10.1242/jcs.093724
97. Petrovic A et al. (2016) Structure of the MIS12 Complex and Molecular Basis of Its Interaction with CENP-C at Human Kinetochores. *Cell* 167: 1028–1040. **doi:**10.1016/j.cell.2016.10.005
98. Malvezzi F, Litos G, Schleiffer A, Heuck A, Mechtler K, Clausen T, Westermann S (2013) A structural basis for kinetochore recruitment of the Ndc80 complex via two distinct centromere receptors. *EMBO Journal* 32: 409–423. **doi:**10.1038/emboj.2012.356
99. Petrovic A et al. (2014) Modular Assembly of RWD Domains on the Mis12 Complex Underlies Outer Kinetochore Organization. *Molecular Cell* 53: 591–605. **doi:**10.1016/j.molcel.2014.01.019
100. Ciferria C, Musacchioa A, Petrovica A (2007) The Ndc80 complex: Hub of kinetochore activity. *FEBS Letters* 581: 2862-2869. **doi:**10.1016/j.febslet.2007.05.012
101. Goshima G, Vale RD (2003) The roles of microtubule-based motor proteins in mitosis: comprehensive RNAi analysis in the Drosophila S2 cell line. *J Cell Biol.* 162(6): 1003-16. **doi:**10.1083/jcb.200303022
102. Ferenz NP, Gable A (2010) Wadsworth P. Mitotic functions of kinesin-5. *Semin Cell Dev Biol.* 21(3): 255-9. **doi:**10.1016/j.semcdb.2010.01.019
103. Mitra A, Meißner L, Gandhimathi R, Renger R, Ruhnow F, Diez S (2020) Kinesin-14 motors drive a right-handed helical motion of antiparallel microtubules around each other. *Nat Commun.* 11(1): 2565. **doi:**10.1038/s41467-020-16328-z
104. Mazumdar M, Misteli T (2005) Chromokinesins: multitalented players in mitosis. *Trends Cell Biol.*: 349-55. **doi:**10.1016/j.tcb.2005.05.006
105. Raaijmakers JA, Medema RH (2014). Function and regulation of dynein in mitotic chromosome segregation. *Chromosoma*. 123(5): 407-22. **doi:**10.1007/s00412-014-0468-7
106. Lutkenhaus J, Pichoff S, Du S (2012) Bacterial cytokinesis: from Z ring to divisome. *Cytoskeleton* 69: 778–790. **doi:**10.1002/cm.21054
107. Söderström B, Daley DO (2017) The bacterial divisome: more than a ring? *Current Genetics* 63(2): 161-164. **doi:**10.1007/s00294-016-0630-2
108. Hartel T, Schwille P (2014) ESCRT-III mediated cell division in Sulfolobus acidocaldarius -- a reconstitution perspective. *Frontiers in Microbiology* 5(257). **doi:**10.3389/fmicb.2014.00257
109. Bernander R, Ettema TJ (2010) FtsZ-less cell division in archaea and bacteria. *Current Opinion in Microbiology* 13: 747-752. **doi:**10.1016/j.mib.2010.10.005
110. Makarova KS, Yutin N, Bell SD, Koonin EV (2010) Evolution of diverse cell division and vesicle formation systems in Archaea. *Nature Reviews Microbiology* 8(10):731-741. **doi:**10.1038/nrmicro2406
111. Bernander R (2007) The cell cycle of Sulfolobus. *Molecular Microbiology* 66, 557–562. **doi:**10.1111/j.1365-2958.2007.05917.x
112. Bernander R, Poplawski A (1997) Cell cycle characteristics of thermophilic archaea. *Journal of Bacteriology* 179: 4963–4969. **doi:**10.1128/jb.179.16.4963-4969.1997
113. Poplawski A, Bernander R (1997) Nucleoid structure and distribution in thermophilic Archaea. *Journal of Bacteriology* 179: 7625–7630. **doi:**10.1128/jb.179.24.7625-7630.1997
114. Hjort K, Bernander R (1999) Changes in cell size and DNA content in Sulfolobus cultures during dilution and temperature shift experiments. *Journal of Bacteriology* 181: 5669–5675. **doi:**10.1128/JB.181.18.5669-5675.1999
115. Bernander R (2000) Chromosome replication, nucleoid segregation and cell division in archaea. *Trends in Microbiology* 8: 278–283. **doi:**10.1016/s0966-842x(00)01760-1

116. Lundgren M, Malandrin L, Eriksson S, Huber H, Bernander R (2008) Cell cycle characteristics of Crenarchaeota: unity among diversity. *Journal of Bacteriology* 190: 5362–5367. **doi:**10.1128/JB.00330-08
117. Stricker J, Maddox P, Salmon ED, and Erickson HP (2002) Rapid assembly dynamics of the Escherichia coli FtsZ-ring demonstrated by fluorescence recovery after photobleaching. *Proceedings of the National Academy of Sciences USA* 99(5): 3171-3175. **doi:**10.1073/pnas.052595099
118. Adams DW, Errington J (2009) Bacterial cell division: assembly, maintenance and disassembly of the Z ring. *Nature Reviews Microbiology* 7: 642-653. **doi:**10.1038/nrmicro2198
119. Fu G et al., (2010) In Vivo Structure of the E. coli FtsZ-ring Revealed by Photoactivated Localization Microscopy (PALM). *PloS ONE* 5(9): e12680. **doi:**10.1371/journal.pone.0012680
120. Pichoff S, Lutkenhaus J (2002) Unique and overlapping roles for ZipA and FtsA in septal ring assembly in Escherichia coli. *The EMBO Journal* 21(4): 865-693. **doi:**10.1093/emboj/21.4.685
121. Huang KH, Durand-Heredia J, Janakiraman A (2013) FtsZ Ring Stability: of Bundles, Tubules, Crosslinks, and Curves. *Journal of Bacteriology* 195(9): 1859-1868. **doi:**10.1128/JB.02157-12
122. Pichoff S, Lutkenhaus J (2005) Tethering the Z ring to the membrane through a conserved membrane targeting sequence in FtsA. *Molecular Microbiology* 55(6): 1722-1734. **doi:**10.1111/j.1365-2958.2005.04522.x
123. Geissler B, Elraheb D, and Margolin W (2003) A gain-of-function mutation in ftsA bypasses the requirement for the essential cell division gene zipA in Escherichia coli. *Proceedings of the National Academy of Sciences USA* 100(7): 4197-4202. **doi:**10.1073/pnas.0635003100
124. Feucht A, Lucet I, Yudkin MD, and Errington J (2001) Cytological and biochemical characterization of the FtsA cell division protein of Bacillus subtilis. *Molecular Microbiology* 40(1): 115-125. **doi:**10.1046/j.1365-2958.2001.02356.x
125. Johnson JE, Lackner LL, de Boer PA (2002) Targeting of (D) MinC/MinD and (D)MinC/DicB complexes to septal rings in Escherichia coli suggests a multistep mechanism for MinC-mediated destruction of nascent FtsZ rings. *Journal of Bacteriology* 184(11): 2951-2962. **doi:**10.1128/JB.184.11.2951-2962.2002
126. Shih YL, Le T and Rothfield L (2003) Division site selection in Escherichia coli involves dynamic redistribution of Min proteins within coiled structures that extend between the two poles. *Proceedings of the National Academy of Sciences USA* 100(13): 7865-7870. **doi:**10.1073/pnas.1232225100
127. Dajkovic *et al.* (2008) MinC Spatially Controls Bacterial Cytokinesis by Antagonizing the Scaffolding Function of FtsZ. *Current Biology* 18: 235-244. **doi:**10.1016/j.cub.2008.01.042
128. Raskin DM, de Boer PAJ (1997) The MinE Ring: An FtsZ-Independent Cell Structure Required for Selection of the Correct Division Site in E. coli. *Cell* 91(5): 685-694. **doi:**10.1016/s0092-8674(00)80455-9
129. Shen B, Lutkenhaus J (2011) Differences in MinC/MinD Sensitivity between Polar and Internal Z Rings in Escherichia coli. *Journal of Bacteriology* 193(2): 367-376. **doi:**10.1128/JB.01095-10
130. Bigot S, Sivanathan V, Possoz C, Barre FX, Cornet F (2007) FtsK, a literate chromosome segregation machine. *Molecular Biology* 64(6): 1434-1441. **doi:**10.1111/j.1365-2958.2007.05755.x
131. Crozat E, Grainge I (2010) FtsK DNA translocase: the fast motor that knows where it's going. *Chembiochem* 11(16): 2232-2243. **doi:**10.1002/cbic.201000347
132. Iyer LM, Makarova KS, Koonin EV, Aravind L (2004) Comparative genomics of the FtsKHerA superfamily of pumping ATPases: implications for the origins of chromosome segregation, cell division and viral capsid packaging. *Nucleic Acids Research* 32: 5260–5279. **doi:**10.1093/nar/gkh828
133. Hanson PI, Whiteheart SW (2005) AAA+ proteins: have engine, will work. *Nature Reviews Molecular Cell Biology* 6: 519–529. **doi:**10.1038/nrm1684
134. Gerdes K, Howard M, Szardenings F (2010) Pushing and pulling in prokaryotic DNA segregation. *Cell* 141: 927–942. **doi:**10.1016/j.cell.2010.05.033
135. Ptacin JL et al. (2010) A spindle-like apparatus guides bacterial chromosome segregation. *Nature Cell Biology* 12: 791–798. **doi:**10.1038/ncb2083
136. Kalliomaa-Sanford AK et al. (2012) Chromosome segregation in Archaea mediated by a hybrid DNA partition machine. *Proceedings of the National Academy of Sciences USA* 109: 3754–3759. **doi:**10.1073/pnas.1113384109
137. Margolin W (2009) Sculpting the bacterial cell. *Curr Biol.* 19(17): R812-22. **doi:**10.1016/j.cub.2009.06.033
138. Zoll S, Pätzold B, Schlag M, Götz F, Kalbacher H, Stehle T (2010) Structural basis of cell wall cleavage by a staphylococcal autolysin. *PLoS Pathog.* 6(3): e1000807. **doi:**10.1371/journal.ppat.1000807
139. Mitchell SJ, Verma D, Griswold KE, Bailey-Kellogg C (2021) Building blocks and blueprints for bacterial autolysins. *PLoS Comput Biol.* 17(4): e1008889. **doi:**10.1371/journal.pcbi.1008889
140. Zielińska A, Billini M, Möll A, Kremer K, Briegel A, Izquierdo Martinez A, Jensen GJ, Thanbichler M. (2017) LytM factors affect the recruitment of autolysins to the cell division site in Caulobacter crescentus. *Mol Microbiol.* 106(3): 419-438. **doi:**10.1111/mmi.13775
141. Mueller EA, Iken AG, Ali Öztürk M, Winkle M, Schmitz M, Vollmer W, Di Ventura B, Levin PA (2021) The active repertoire of Escherichia coli peptidoglycan amidases varies with physiochemical environment. *Mol Microbiol.* 116(1): 311-328. **doi:**10.1111/mmi.14711
142. Schlag M, Biswas R, Krismer B, Kohler T, Zoll S, Yu W, Schwarz H, Peschel A, Götz F (2010) Role of staphylococcal wall teichoic acid in targeting the major autolysin Atl. *Mol Microbiol.* 75(4): 864-73. **doi:**10.1111/j.1365-2958.2009.07007.x
143. Garde S, Chodisetti PK, Reddy M (2021). Peptidoglycan: Structure, Synthesis, and Regulation. *EcoSal Plus.* 9(2). **doi:**10.1128/ecosalplus.ESP-0010-2020
144. Schleifer KH, Kandler O (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev.* 36(4): 407-77. **doi:**10.1128/br.36.4.407-477.1972
145. Thorne KJ, Kodicek E (1966) The structure of bactoprenol, a lipid formed by lactobacilli from mevalonic acid. *Biochem J.* 99(1): 123-7. **doi:**10.1042/bj0990123
146. Barker DC, Thorne KJ (1970) Spheroplasts of Lactobacillus casei and the cellular distribution of bactoprenol. *J Cell Sci.* 7(3): 755-85. **doi:**10.1242/jcs.7.3.755
147. Sauvage E, Kerff F, Terrak M, Ayala JA, Charlier P (2008) The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. *FEMS Microbiol Rev.* 32(2):234-58. Erratum in: *FEMS Microbiol Rev.* 2008 May;32(3): 556. **doi:**10.1111/j.1574-6976.2008.00105.x
148. Sauvage E, Terrak M (2016) Glycosyltransferases and Transpeptidases/Penicillin-Binding Proteins: Valuable Targets for New Antibacterials. *Antibiotics (Basel).* 5(1): 12. **doi:**10.3390/antibiotics5010012
149. Wissel MC, Weiss DS (2004) Genetic analysis of the cell division protein FtsI (PBP3): amino acid substitutions that impair septal localization of FtsI and recruitment of FtsN. *J Bacteriol.* 186(2): 490-502. **doi:**10.1128/JB.186.2.490-502.2004
150. Mercer KL, Weiss DS (2002). The Escherichia coli cell division protein FtsW is required to recruit its cognate transpeptidase, FtsI (PBP3), to the division site. *J Bacteriol.* 184(4): 904-12. **doi:**10.1128/jb.184.4.904-912.2002
151. Weiss DS, Chen JC, Ghigo JM, Boyd D, Beckwith J (1999) Localization of FtsI (PBP3) to the septal ring requires its membrane anchor, the Z ring, FtsA, FtsQ, and FtsL. *J Bacteriol.* 181(2): 508-20. **doi:**10.1128/JB.181.2.508-520.1999

152. Eme L., Trilles A., Moreira D. and Brochier-Armanet C. (2011). The phylogenomic analysis of the anaphase promoting complex and its targets points to complex and modern-like control of the cell cycle in the last common ancestor of eukaryotes. *BMC Evolutionary Biology* 11: 265. **doi:**10.1186/1471-2148-11-265
153. Vleugel M, Hoogendoom E, Snel B, Kops GJPL (2012). Evolution and Function of the Mitotic Checkpoint. *Developmental Cell* 23: 239-250. **doi:**10.1016/j.devcel.2012.06.013
154. van Hoof JJE, Tromer E, van Wijk LM, Snel B, Kops GJPL (2017) Evolutionary dynamics of the kinetochore network in eukaryotes as revealed by comparative genomics. *EMBO Reports* 18(8): 1265-1472. **doi:**10.15252/embr.201744102
155. Adl SM et al (2012) The Revised Classification of Eukaryotes. *The Journal of Eukaryotic Microbiology* 59(5): 429-493. **doi:**10.1111/j.1550-7408.2012.00644.x
156. Rost B (1999) Twilight zone of protein sequence alignments. *Protein Engineering* 12(2): 85-94. **doi:**10.1093/protein/12.2.85
157. Zaremba-Niedzwiedzka K, Caceres EF, Saw JH, Bäckström D, Juzokaite L., et. al(2017) Asgard archaea illuminate the origin of eukaryotic cellular complexity. *Nature* 541(7637): 353-358. **doi:**10.1038/nature21031
158. Stancik IA, Šestak MS, Ji B, Axelson-Fisk M, Franjevic D, Jers C, Domazet-Lošo T, Mijakovic I (2018) Serine/Threonine Protein Kinases from Bacteria, Archaea and Eukarya Share a Common Evolutionary Origin Deeply Rooted in the Tree of Life. *J Mol Biol.* 430(1): 27-32. **doi:**10.1016/j.jmb.2017.11.004
159. Das AK, Cohen PW, Barford D (1998) The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein-protein interactions. *EMBO J.* 2;17(5): 1192-9. **doi:**10.1093/emboj/17.5.1192
160. Blatch GL, Lässle M (1999) The tetratricopeptide repeat: a structural motif mediating protein-protein interactions. *Bioessays*. 21(11): 932-9. **doi:**10.1002/(SICI)1521-1878(199911)21:11%3C932::AID-BIES5%3E3.0.CO;2-N
161. Jernigan KK, Bordenstein SR (2015) Tandem-repeat protein domains across the tree of life. *PeerJ.* 3: e732. **doi:**10.7717/peerj.732
162. Cerveny L, Straskova A, Dankova V, Hartlova A, Ceckova M, Staud F, Stulik J (2013) Tetratricopeptide repeat motifs in the world of bacterial pathogens: role in virulence mechanisms. *Infect Immun.* 81(3): 629-35. **doi:**10.1128/IAI.01035-12
163. Neer EJ, Schmidt CJ, Nambudripad R, Smith TF (1994) The ancient regulatory-protein family of WD-repeat proteins. *Nature*. 371(6495):297-300. Erratum in: *Nature* 1994 Oct 27;371(6500): 812. **doi:**10.1038/371297a0
164. Smith TF, Gaitatzes C, Saxena K, Neer EJ (1999) The WD repeat: a common architecture for diverse functions. *Trends Biochem Sci.* 24(5): 181-5. **doi:**10.1016/s0968-0004(99)01384-5
165. Li D, Roberts R (2001) WD-repeat proteins: structure characteristics, biological function, and their involvement in human diseases. *Cell Mol Life Sci.* 58(14): 2085-97. **doi:**10.1007/pl00000838
166. Xu C, Min J (2011) Structure and function of WD40 domain proteins. *Protein Cell* 2(3): 202-14. **doi:**10.1007/s13238-011-1018-1
167. Hu XJ, Li T, Wang Y, Xiong Y, Wu XH, Zhang DL, Ye ZQ, Wu YD (2017) Prokaryotic and Highly-Repetitive WD40 Proteins: A Systematic Study. *Sci Rep.* 7(1): 10585. **doi:**10.1038/s41598-017-11115-1
168. Chaudhuri I, Söding J, Lupas AN (2008) Evolution of the beta-propeller fold. *Proteins*. 71(2): 795-803. **doi:**10.1002/prot.21764
169. Margolin W (2005) FtsZ and the division of prokaryotic cells and organelles. *Nature Reviews Molecular Cell Biology* 6: 862–871. **doi:**10.1038/nrm1745
170. O'Shaughnessy B, Thiyagarajan S (2018) Mechanisms of contractile ring tension production and constriction. *Biophys Rev.* 10(6): 1667-1681. **doi:**10.1007/s12551-018-0476-6
171. Kumada K, Yao R, Kawaguchi T, Karasawa M, Hoshikawa Y, et. ak(2006) The selective continued linkage of centromeres from mitosis to interphase in the absence of mammalian separase. *J Cell Biol*. 172(6): 835-46. **doi:**10.1083/jcb.200511126
172. Wirth KG, Wutz G, Kudo NR, Desdouets C, Zetterberg A, et. al(2006) Separase: a universal trigger for sister chromatid disjunction but not chromosome cycle progression. *J Cell Biol.* 172(6): 847-60. **doi:**10.1083/jcb.200506119
173. Wang J, Yin MZ, Zhao KW, Ke F, Jin WJ, et. al(2017) APC/C is essential for hematopoiesis and impaired in aplastic anemia. *Oncotarget*. 8(38): 63360-63369. **doi:**10.18632/oncotarget.18808
174. Lu LY, Wood JL, Ye L, Minter-Dykhouse K, Saunders TL, Yu X, Chen J (2008) Aurora A is essential for early embryonic development and tumor suppression. *J Biol Chem.* 283(46): 31785-90. **doi:**10.1074/jbc.M805880200
175. Yuba-Kubo A, Kubo A, Hata M, Tsukita S (2005) Gene knockout analysis of two gamma-tubulin isoforms in mice. *Dev Biol.* 282(2): 361-73. **doi:**10.1016/j.ydbio.2005.03.031
176. Berthet C, Aleem E, Coppola V, Tessarollo L, Kaldis P (2003) Cdk2 knockout mice are viable. *Curr Biol*. 13: 1775–1785. **doi:**10.1016/j.cub.2003.09.024
177. Ortega S, et al. (2003) Cyclin-dependent kinase 2 is essential for meiosis but not for mitotic cell division in mice. *Nat Genet.* 35: 25–31. **doi:**10.1038/ng1232
178. Ye X, Zhu C, Harper JW (2001) A premature-termination mutation in the Mus musculus cyclin-dependent kinase 3 gene. *Proc Natl Acad Sci USA.* 98: 1682–1686. **doi:**10.1073/pnas.98.4.1682
179. Rane SG, et al. (1999) Loss of Cdk4 expression causes insulin-deficient diabetes and Cdk4 activation results in β-islet cell hyperplasia. *Nat Genet.* 22: 44–52. **doi:**10.1038/8751
180. Tsutsui T, et al. (1999) Targeted disruption of CDK4 delays cell cycle entry with enhanced p27Kip1 activity. *Mol Cell Biol.* 19: 7011–7019. **doi:**10.1128/MCB.19.10.7011
181. Hu MG, et al. (2009) A requirement for cyclin-dependent kinase 6 in thymocyte development and tumorigenesis. *Cancer Res.* 69: 810–818. **doi:**10.1158/0008-5472.CAN-08-2473
182. Malumbres M, et al. (2004) Mammalian cells cycle without the D-type cyclin-dependent kinases Cdk4 and Cdk6. *Cell.* 118: 493–504. **doi:**10.1016/j.cell.2004.08.002
183. Enserink JM, Kolodner RD (2010) An overview of Cdk1-controlled targets and processes. *Cell Div.* 5: 11. **doi:**10.1186/1747-1028-5-11
184. Berthet C, Kaldis P (2007) Cell-specific responses to loss of cyclin-dependent kinases. *Oncogene* 26: 4469–4477. **doi:**10.1038/sj.onc.1210243
185. Satyanarayana A, Kaldis P (2009) Mammalian cell-cycle regulation: Several Cdks, numerous cyclins and diverse compensatory mechanisms. *Oncogene*. 28:2925–2939. **doi:**doi.org/10.1038/onc.2009.170
186. Diril MK, Ratnacaram CK, Padmakumar VC, Du T, Wasser M, Coppola V, Tessarollo L, Kaldis P (2012) Cyclin-dependent kinase 1 (Cdk1) is essential for cell division and suppression of DNA re-replication but not for liver regeneration. *Proc Natl Acad Sci U S A.* 109(10): 3826-31. **doi:**10.1073/pnas.1115201109
187. Berthet C, *et al.* (2006) Combined loss of Cdk2 and Cdk4 results in embryonic lethality and Rb hypophosphorylation. *Dev Cell*. 10: 563–573. **doi:**10.1016/j.devcel.2006.03.004
188. Strauss B, Harrison A, Coelho PA, Yata K, Zernicka-Goetz M, Pines J (2018) Cyclin B1 is essential for mitosis in mouse embryos, and its nuclear export sets the time for mitosis. *J Cell Biol.* 217(1): 179-193. **doi:**10.1083/jcb.201612147
189. Koonin EV (2010) The Incredible Expanding Ancestor of Eukaryotes. *Cell* 140: 606-608. **doi:**10.1016/j.cell.2010.02.022
190. Dacks JB, Field MC, Buick R, Eme L, Gribaldo S, Roger AJ, Brochier-Armanet C, Devos DP (2016) The changing view of eukaryogenesis – fossils, cells, lineages, and how they all came together. *Journal of Cell Science* 129(20): 3695-3703. **doi:**10.1242/jcs.178566

191. Rout MP, Field MC (2017) The Evolution of Organellar Coat Complexes and Organization of the Eukaryotic Cell. *Annual Review of Biochemistry* 86: 637-657. **doi:**10.1146/annurev-biochem-061516-044643
192. Carvalho-Santos Z, Aximzadeh J, Pereira-Leal JB, Bettencourt-Dias, M. (2011) Evolution: Tracing the origins of centrioles, cilia, and flagella. *Journal of Cell Biology* 194: 165-175. **doi:**10.1083/jcb.201011152
193. Hammesfahr B, Kollmar M (2012). Evolution of the eukaryotic dynactin complex, the activator of cytoplasmic dynein. *BMC Evolutionary Biology* 12: 95. **doi:**10.1186/1471-2148-12-95
194. Spang A, Saw JH, Jørgensen SL, Zaremba-Niedzwiedzka K, Martijn J, et. al(2015). Complex archaea that bridge the gap between prokaryotes and eukaryotes. *Nature* 521(7551): 173-179. **doi:**10.1038/nature14447
195. Chernikova D, Motamedi S, Csürös M, Koonin EV, Rogozin IB (2011) A late origin of the extant eukaryotic diversity: divergence time estimates using rare genomic changes. *Biol Direct*. 6: 26. **doi:**10.1186/1745-6150-6-26
196. Gribaldo S, Poole AM, Daubin V, Forterre P, Brochier-Armanet C (2010) The origin of eukaryotes and their relationship with the Archaea: are we at a phylogenomic impasse? *Nature Reviews Microbiology*. 8: 743-752. **doi:**10.1038/nrmicro2426
197. Hedges SB, Chen H, Kumar S, Wang DYC, Thompson AS, Watanabe H (2001) A genomic timescale for the origin of eukaryotes. *BMC Ecology and Evolution* 1:4. **doi:**10.1186/1471-2148-1-4
198. Axe DD (2010) The Limits of Complex Adaptation: An Analysis Based on a Simple Model of Structured Bacterial Populations. *Bio-Complexity*. 2010. **doi:**10.5048/BIO-C.2010.4
199. Reeves MA, Gauger AK, Axe DD (2014) Enzyme Families – Shared Evolutionary History or Shared Design? A Study of the GABA-Aminotransferase Family. *Bio-Complexity.* 2014. **doi:**10.5048/BIO-C.2014.4
200. Flaherty KM, McKay DB, Kabsch W. and Holmes KC (1991) Similarity of the three-dimensional structures of actin and the ATPase fragment of a 70-kDa heat shock cognate protein. *Proceedings of the National Academy of Sciences USA* 88(11): 5041-5045. **doi:**10.1073/pnas.88.11.5041
201. Hurley (1996) The Sugar Kinase/Heat Shock Protein 70/Actin Superfamily: Implications of Conserved Structure for Mechanism. *Annual Reviews* 25: 137-162. **doi:**10.1146/annurev.bb.25.060196.001033
202. Paley W (1802) *Natural Theology: or, Evidences of the Existence and Attributes of the Deity* (1st ed.), London: J. Faulder