**Proteomics in Cell Division**

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**Abstract**

Cell division requires a coordinated action of the cell cycle machinery, cytoskeletal elements, chromosomes and membranes. Cell division studies have greatly benefitted from the mass spectrometry (MS)-based proteomic approaches for probing the biochemistry of highly dynamic complexes and their coordination with each other as a cell progresses into division. In this review, we first summarize a wide-range of proteomic studies that focus on the identification of sub-cellular components/protein complexes of the cell division machinery including kinetochores, mitotic spindle, midzone and centrosomes. We also highlight MS-based large-scale analyses of the cellular components that are largely understudied during cell division such as cell surface and lipids. Then, we focus on posttranslational modification analyses, especially phosphorylation and the resulting crosstalk with other modifications as a cell undergoes cell division. Combining proteomic approaches that probe the biochemistry of cell division components with functional genomic assays, will lead to breakthroughs toward a systems level understanding of cell division.

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**Proteomics in Cell Division**

Cell division is central to life, driving many vital cellular events such as proliferation, propagation, development and regeneration. The main components of cell division are ancient and highly conserved across eukaryotic cells. The evolution of the cell division machinery has led to multiple redundant pathways and checkpoints to monitor the coordination of numerous complex events for a faithful replication and cell division [1]. The recent development of genome-wide functional assays provided a list of genes that are required for proper cell division in various eukaryotic cells [2-5]. A high-throughput RNAi screen in mammalian cells targeted around 21,000 protein-coding genes; their phenotype profiling linked 1,249 genes in the human genome with cell division [6]. Of these candidate genes, 573 were further confirmed through a second validation screen and only less than half were previously implicated in mitosis [6, 7]. RNAi and genetic screens identified almost the complete list of main players of cell division. Recently developed CRISPR/Cas9 based knockout system [8] has been shown to be a powerful approach to study cell cycle. Inducible cell lines of gene knockouts generated by using CRISPR/Cas9 based genome engineering technology [9] are likely to expand this list further.

The MS-based proteomic field has made tremendous steps in the last decade, which allow quantitative analysis of biochemical changes during cell division [10]. Here, we review MS-based proteomic studies that probed the biochemistry of highly dynamic complexes and/or cellular processes during cell division. The near future should see the breakthroughs by shedding light on the unknown molecular mechanisms of cell division by combining functional genomic analyses with various proteomic approaches.

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## 1. Organelle Proteomics of Cell Division Machinery

During cell division, subcellular organelles dramatically reorganize to drive the faithful division of genetic material and other components of the cell interior. Combination of advanced cell biology and mass spectrometry techniques has led to the efficient isolation of organelles and detection of their global protein composition. Thus, optimal approaches allowed identifying and delineating even slight changes in the biochemical dynamics of organelles during cell division. In this section, we summarize the main organelle proteomics studies for cell division (**Figure 1**).

### 1.1 Centrosome

Centrosomes are the microtubule organization centers of cells and are composed of a pair of centrioles and pericentriolar material (PCM). Centrosomes carry out multiple important functions including microtubule nucleation, anchoring, formation of primary cilium in specific cell types, and positioning of the mitotic spindle that is crucial for proper cell division (reviewed in [11]). Defining the protein composition of centrosomes has great importance in elucidating the molecular mechanisms governed by centrosomes; yet, this has been challenging due to the difficulties in isolating the organelle. In the earlier days, classical genetic- and antibody-based approaches led to the identification of a significant number of core centrosomal proteins (reviewed in [12]). In recent years, multiple studies have combined sophisticated mass spectrometry techniques with traditional cell biology and microscopy methods.

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In a pioneering study, Andersen et al. [13] combined MS-based proteomic analysis with protein correlation profiling (PCP) to identify novel centrosomal proteins in the human lymphoblastic cell line KE-37. True centrosomal proteins were discriminated from the background of non-specific contaminants by correlating the protein abundance with known centrosomal proteins and by imaging GFP tagged novel proteins. Overall, 23 novel centrosomal components and 41 likely candidates were identified, representing a greater number of proteins when compared to previous studies [13]. In a combinatory research, Müller et al. [14] identified the protein composition of the embryonic *Drosophila* centrosome using immunoisolation followed by MS analysis. Identified components were functionally characterized by RNAi, where 13 of the novel centrosomal proteins were shown to be conserved in humans [14]. In a recent study, Jakobsen et al. [15] combined a novel quantitative proteomics approach, called PCP–SILAC (protein correlation profiling-stable isotope labeling by amino acids in cell culture), with BAC transgenomics and antibody-based screening to identify the composition of human centrosomes. In PCP–SILAC, a pool of centrosomal proteins was generated and labeled using SILAC method. This pool was used as a spike-in standard in centrosomal fractions to identify contaminant-free centrosomal proteins. In addition, pulsed-SILAC labeling determined the turnover of centrosomal proteins during cell cycle. In total, 126 known and 40 candidate-centrosomal proteins were identified, 22 of which were confirmed using microscopy. In parallel, they conducted an antibody-based screen covering 4,000 proteins and identified 113 new candidates for centrosomal localization [15]. These studies did not only extend the list of identified centrosomal proteins, but also provided an insight into the state of centrosomal proteins and their dynamics throughout the cell cycle.

Proteomics analysis of the specific subcomponents of centrosomes such as centrioles and PCM is crucial to dissect the properties and functions of different centrosomal layers. Size and protein composition of PCM is much bigger than the centrioles. Thus, it is likely that centriolar proteins

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might be masked by PCM when analyzed as a whole. In order to overcome this problem, Keller et al. [16] used basal bodies to study centriolar proteins. Basal bodies are closely related structures to centrioles that function in the formation of cilia and flagella during interphase [11]. Since they lack the surrounding PCM, basal bodies are good candidates to study centriolar proteins. Keller et al. [16] took the advantage of isolated basal bodies of the green alga *Chlamydomonas* to present the first proteomic analysis of the centriole by using MS-based multidimensional protein identification technology (MudPIT). Bioinformatical analysis characterized 61 of the identified proteins as either known or potentially centriole-associated proteins. Combining proteomic data with gene expression analysis and comparative genomics, they cross-validated 45 centriolar proteins. Human orthologs of three candidate centriole proteins were implicated in ciliary dysfunction and diseases [16].

The development of new technologies such as BioID (proximity-dependent biotin identification) advanced the capture of protein-protein interactions at specific cellular sites [17]. In the BioID method, the protein of interest is fused to a promiscuous *E. coli* biotin ligase (BirA*) that biotinylates the proximal proteins *in vivo*. Biotinylated proteins are then purified using streptavidin-based affinity chromatography and identified by LC-MS/MS [17]. Karalar et al. [18] used the BioID method [17] to identify the proximity interactors of centrosomal proteins. Two new regulators of centriole duplication were characterized in this study as components of centriolar satellites [18]. In a recent study, Gupta et al. [19] further extended this approach and created a centrosome-cilium interaction map by using the BioID method with 58 bait proteins. This interaction analysis generated a protein topology network consisting of more than 7,000 interactions among 1,405 unique proteins. They subsequently used a subset of their data in co-immunoprecipitation (IP) and FLAG-IP/MS analysis for validation and conducted functional screens to reveal their functions in centrosome and cilia biology. This broad analysis of the centrosomal and cilia interactome provided a powerful resource to advance our understanding of the centrosome biology [19].

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Development of combinatory approaches using mass spectrometry and other complementary techniques enabled the identification of hundreds of centrosomal components including scaffold proteins such as CPAP and CEP, kinases such as PLK1 and AurA [20], and the recognition of multiple interactions between these components. Further elucidation of functional relationships between identified complexes is critical for a deeper understanding of centrosome assembly.

### 1.2 Chromosome/Kinetochore

The centromeric region of chromosomal DNA directs the assembly of kinetochore, a proteinaceous structure which mediates the interaction between chromosomes and spindle microtubules [21]. Apart from being physical sites for the chromosome attachment, kinetochores also play important roles in "sensing" the unattached chromosome and triggering the spindle-assembly checkpoints to stop cell division in order to prevent the loss of genomic material [21]. Thus, analyzing the protein composition of kinetochores, and many other chromosomal protein complexes, has great importance for understanding the mechanism of chromosomal segregation during cell division. Earlier studies used antibody-based approaches and fluorescence microscopy to identify the core centromere proteins (reviewed in [21]). However, greater progress in understanding the protein composition of kinetochores and centromeres was only possible in the 1990s, when the applications of mass spectrometry in biological research began [22, 23].

Tandem affinity purification and immunoprecipitation techniques followed by MS analysis identified core kinetochore proteins in various model organisms such as budding yeast [24], fruit fly

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[25], worm [26] and in human [27-34] as well as other proteins that are in close proximity to the centromere such as the constitutive centromere associated network (CCAN) proteins [35, 36]. Development of alternative techniques to isolate chromosomal proteins substantially extended the list of identified novel components of mitotic chromosomal scaffold [37] that are important for the chromosomal function and structure [38]. A comprehensive study deciphered the chromosomal protein composition by taking a novel approach termed multiclassifier combinational proteomics (MCCP) [39]. By combining quantitative proteomics with bioinformatic analysis, this approach identified about 4,000 proteins in DT-40 cells, including all previously described chromosomal proteins and 560 uncharacterized proteins. Microscopy analysis revealed the chromosomal localization for most of the predicted proteins. As a result of this analysis, almost hundred novel putative centromeric proteins were discovered. This study represents a superior example for the use of mass spectrometry to identify the components of a complex structure and predict the functional relationship between them.

Another elegant study generated centromeric mini-chromosomes from budding yeast to enrich centrosome-bound kinetochores [40]. Mutant chromosomes without the ability to assemble kinetochores were used as negative controls. Among hundreds of identified chromosomal proteins, they characterized Fin1 as a kinetochore protein that mediates PP1 phosphatase activity for proper checkpoint activity at the kinetochores during chromosome segregation [40]. These results represent a prominent example on how a global proteomic analysis can reveal the specific players that govern the cell cycle progression. Nonetheless, MS-based approaches can also be used in a more targeted manner to identify the substrates of the master enzymes that govern the cell cycle progression.

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One of the master enzymes that regulates the kinetochore function through phosphorylation is AurB kinase. AurB is a component of the chromosomal passenger complex and plays a critical role in the correct attachment of kinetochores to microtubules [41]. Welburn et al. [42] purified and reconstituted outer kinetochore complexes and analyzed their phosphorylation dynamics by AurB *in vitro*, followed by mass spectrometry analysis with and without phosphopeptide enrichment. The authors were able to show a highly ordered spatial phosphorylation at the outer face of the kinetochore that is composed of the KNL1/Mis12 complex/Ndc80 complex (KMN) network. The study suggests a mechanism which spatially controls AurB activity to fine-tune kinetochore microtubule interaction [42].

Use of chemical cross-linking in combination with mass spectrometry is an emerging method for structural analysis of multiprotein complexes. This method was used to provide structural insight into human protein complexes with coiled-coil segments. Maiolica et al. [43] combined cross-linking mass spectrometry with bioinformatics and represented a new algorithm that finds and validates the cross-linked peptides using the fragmentation spectra. Their approach unraveled the structural organization of the NDC80 kinetochore complex and revealed the organization of a tetramerization region within this complex [43].

Considering the relatively low abundance of kinetochore and other centromere-associated proteins, identification and functional assessment of these key players of cell division have been challenging. Use of MS-based approaches has enabled the identification of a good number of kinetochore-associated proteins assembled into complex multiprotein structures at the inner and outer regions of kinetochores. Combining the proteomic approaches with *in vitro* reconstitution assays [44]

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will take us one step further for understanding the mechanistic of kinetochore assembly and its functional interaction with the master regulators of the cell cycle machinery.

### 1.3 Mitotic Spindle

The mitotic spindle, which is a self-assembled machinery consisting of microtubules that radiate from centrosomes, interacts with kinetochores on chromosomes [45]. The spindle mediates the physical separation of sister chromatids between daughter cells, determines the localization and orientation of cleavage furrow and finally, monitors the progression of cell division [46]. During late mitosis, the spindle re-organizes into the so-called central spindle, which bundles into a dense microtubule package, the midbody [46]. Different types of proteins are involved in the formation and proper functioning of the mitotic spindle, e.g. stabilizing proteins, motor proteins or regulatory proteins such as kinases and phosphatases [46].

Several MS-based studies have greatly contributed to our understanding of the spindle apparatus over the past years. Sauer et al. [45] identified a total of 795 proteins using gel electrophoresis followed by nanoHPLC and Q-TOF analysis. About 151 of these were already known to be associated with the spindle, centrosomes and/or kinetochores and 6 out of 17 previously uncharacterized proteins were identified as spindle-associated components. In contrast, Bonner et al. [46] used MudPIT to avoid the immense loss of proteins by gel-based separation methods. This allowed Bonner et al. a better recovery of a total of 1,155 potential spindle proteins including microtubule-, actin- and membrane-associated proteins, which are thought to be critical during mitosis and cytokinesis.

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The assembly of the mitotic spindle is a complex process continuously regulated by several phosphatases and kinases such as CDK1, PLK1 or AurA and AurB [47]. The phosphoproteome of the spindle was characterized by different groups over the past years as described in the part ‘phosphorylation’ (see [47-49]). Another relevant study is that of Nousiainen et al. [50] that reports the identification of a total of 736 phosphorylation sites in 260 proteins, revealing 279 novel sites of known spindle proteins. During the isolation process, mitotic spindles were stabilized by Taxol [51], the isolated proteins were in-gel digested and phosphoproteins were partly enriched by IMAC before analysis by ESI-MS/MS. This procedure allowed the identification of several known spindle proteins such as the kinesins KIF23, Kif20A, KIF11, KIF22 and KIF4A [52]. Furthermore, the structurally relevant proteins NuMA and Tpx2 as well as the protein complexes AurB, Survivin and Borealin were found [53].

As with all multiprotein systems, characterizing the components of the mitotic spindle apparatus such as kinetochores, centrosomes or microtubules is a relevant starting point for functional analysis, but it requires a global protein profile to understand the interplay of the involved proteins during spindle formation and development. Although the described studies led to the identification of a large number of spindle-associated proteins by MS, further attempts are required in order to comprehend the fundamental molecular mechanisms behind the cell division machinery. Future studies should therefore put emphasis on the development of *in vitro* reconstitution assays in order to properly simulate the complex spindle processes.

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**1.4 Midbody**

The midbody is a dense bundle of anti-parallel interdigitating microtubules first described by Flemming in 1891 [54]. It derives from the bipolar midzone structure in the anaphase of mitosis and forms a bridge between two dividing cells in cytokinesis [55]. The main function of the midbody is the localization of the abscission site where the plasma membranes of two daughter cells separate from each other leading to the accomplishment of cell division [55]. The midbody is surrounded by a complex tightly-packed matrix of mostly unknown components that stains darkly by electron microscopy. Only a small subset of proteins is known to participate in the assembly of the midbody such as the kinesins centralspindlin and KIF4, which recruit the microtubule cross-linker PRC1 to the midzone. This process is regulated by the kinases AurB and PLK1 [55].

Skop et al. [56] are the only researchers so far to report MS-based protein analysis of the midbody. Their isolation protocol is based on the stabilization of microtubules and actin filaments by Taxol and phalloidin and achieves a higher purity compared to the method described by Mullins and McIntosh in 1982 [57]. However, a drawback of this procedure is the poor recovery of several known midbody proteins such as PRC1, INCENP, syntaxin and RhoA, which are removed during the treatment. Nevertheless, Skop et al. identified a total of 160 potential midbody related proteins by MudPIT-based analysis in CHO cells. The protein panel constituted of secretory or membrane-trafficking proteins (33%), actin-associated proteins (29%), microtubule-associated proteins (11%) and protein kinases (11%). The rest were termed as ‘other’ proteins (16%) difficult to categorize by function. In general, 57 of these 160 proteins were previously known in different organisms as cytokinesis-related proteins. Among the remaining 103 proteins, which have not been associated with

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cell division so far, were 10 proteins that showed localization at the midbody. To functionally characterize the proteins, RNAi evaluations were performed using *C.elegans* homologs. Most of the identified proteins (88%) seem to be essential in germline cytokinesis and cleavage furrow initiation and termination, respectively. Moreover, Skop et al. assign lipid rafts and vesicle trafficking proteins a more central role in the process of abscission at midbodies, underlying molecular mechanisms remain to be further investigated.

### 1.5 Cell Surface

The reorganization of the cells' interior components during cell division is heavily studied. However, very little is known about the cell surface changes during cell division. Adherent cells undergo dramatic cell reshaping at the onset of mitosis. As cells enter mitosis, they transiently lose their adherence and round up (reviewed in [58]). At cytokinesis, the daughter cells spread back to regain their interphase morphology. Not only cultured cells, but also dividing cells in tissues round up and de-adhere during mitosis *in vivo* [59, 60]. Mitotic rounding is thought to be driven partly by actin-myosin cytoskeleton [61, 62] and its regulators including RhoA signaling [63, 64] and partly by downregulation of adhesive systems [65, 66] and upregulation of intracellular hydrostatic pressure [67]. It is not well understood how these different cellular systems act together to trigger cell rounding at the onset of mitosis. Another cellular system that internally changes and probably causes cell surface alterations is membrane trafficking. The endomembrane system is extensively remodeled during mitosis. At the onset of mitosis, Golgi is extensively fragmented and dispersed into the cytoplasm or fused into the ER (Endoplasmic Reticulum) (reviewed in [68]). Plasma membrane-endosome communication may also be regulated in a cell cycle dependent manner [69]. In adherent cancer cells, the balance between exo- and endocytosis is reported to be altered during mitosis:

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exocytosis was shown to decrease, while clathrin-mediated endocytosis remained constant as cells enter mitosis [70].

To investigate how the cell surface changes as a cell enters mitosis, we isolated cell surface exposed proteins by labeling the surface with cell impermeable biotin followed by affinity purification of biotinylated proteins. The surface proteins were quantitatively compared between the mitosis and interphase cell cycle stages using the SILAC method and analyzed by LC-MS/MS. Our analysis identified around 600 cell surface proteins, approximately 10% (>60 proteins) of these exhibited differential expressions at the cell surface as a cell progresses into mitosis [71]. The mitotic enriched or de-enriched proteins were mainly related to cell adhesion, receptor and endosome/lysosome biology. Interestingly, several interphase-enriched proteins were involved in clathrin-mediated endocytosis, but not all. Perhaps certain proteins are depleted on the cell surface through endocytosis to trigger cell surface remodeling for cell division. The cell cycle dependent surface changes are not limited to the proteome level. Eggert laboratory recently showed that the cellular lipidome changes during division relative to interphase (see "Lipidomics Analysis during Cell Division"). Further work is required to explore the precise mechanisms of cell surface changes during cell division. To this end, there is a critical need to develop diverse approaches to overcome challenges of efficient isolation and identification of plasma membrane components using mass spectrometry (reviewed in [72]) to further analyze dynamics of cell surface changes at different stages of cell division more extensively. Investigation of the underlying mechanism of biochemical behaviors observed in the global cell surface proteomic analyses would uncover possible communication between the extracellular environment and internal proteins that regulate the complex cell division process.

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## 2. Lipidomics Analysis during Cell Division

Serving as the building block of the plasma membrane and organelle membranes, lipids are of high importance for cellular stability and for the maintenance of biological processes. Cells produce tens of thousands of different lipid types displaying a wide range of physicochemical properties [73]. The rapid development of mass spectrometry made this instrument also accessible for lipidome analyses. The usage of soft ionization methods such as electrospray ionization (ESI) enabled gentle, efficient and highly selective procedures with minimal in-source fragmentation [74, 75]. To date, more than 500 lipids from different categories including sphingolipids, glycerolipids, glycerolphospholipids, sterols, fatty acids and prenols have been identified by MS-based approaches [74].

Despite the numerous studies on lipidomics, little is known about changes of the lipidome during cell division [74]. Lipids involved in cytokinesis are thought to occupy multiple functions and to act as structural, regulating and/or anchoring components [76]. Only a few lipids are known to be directly involved in cytokinesis, among them are phosphatidylinositol 4,5-bisphosphate (PIP2), phosphatidylinositol 3,4,5-triphosphate (PIP3), cholesterol and GM1 ganglioside [74, 76].

Atilla-Gökcümen et al. [76] were the first to study the cell division-related lipidome of an organism with the amenities of mass spectrometry. In their 2011 study, the enzyme glycosyl ceramide synthase (GCS) was inhibited by the small molecule inhibitor PPMP to perturb the synthesis of sphingolipids. The analysis was performed with a Q-TOF LC-MS system with ESI source. Their

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findings reveal that the treatment with PPMP causes an 8- to 10-fold accumulation of the sphingolipids C16- and C22-ceramide. RNAi and live imaging outcomes suggest that the affected lipids are essential for cytokinesis since the treated cells became binucleated and failed to divide properly although a cleavage furrow was formed.

A few years later, a more comprehensive approach allowed Atilla-Gökcümen et al. [73] to identify 11 lipid species which accumulate more than four times in dividing HeLa cells (**Figure 2**). Among these lipids, eight were sphingolipid subtypes with long fatty acid side chains (ceramides) and three belonged to other lipid species such as phosphaditolinositols, sterol derivatives and phospaditic acid ester/ether subgroups. Interestingly, some of the identified dihydroceramides were by then associated with nondividing cells. The findings of Atilla-Gökcümen et al. suggest a previously unknown role of these lipid species in the framework of mitosis and cytokinesis, which sheds new lights on the cell division field.

Furthermore, RNAi experiments targeting 23 lipid-synthesizing enzymes caused a failure in cytokinesis. These results confirmed their MS-data since 11 of the 23 enzymes are known to be involved in sphingolipid synthesis, enhancing the role of sphingolipids in cell division. Another interesting finding is that the lipid composition of the midbody alternates as well. LC-MS data revealed that four different lipid species accumulated in midbody-isolated extracts compared to purified cytokinesis lysates (**Figure 2**). On the contrary, five ceramide types accumulated at the midbody as well as during cytokinesis.

In contrast to proteomics, MS-based lipidomics remains a new field of research. Compared to proteins, standard methods for sample preparation and identification are not advanced [73]. Therefore,

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a comprehensive analysis of the cellular lipidome has not yet been achieved [74]. One of the biggest challenges is the immense diversity of lipid structures including subtypes ranging from low-molecular weight fatty acids to complex sphingolipids [74]. This challenge comes with diverse physicochemical properties making the extraction and comprehensive identification of lipid species more difficult. Advanced protocols would also allow a simultaneous proteomics and lipidomics analysis, which has been done in other fields [77, 78]. Considering the crosstalk of lipids and proteins during mitosis and cytokinesis, a combinatorial approach would enlighten the fundamental signaling mechanisms of the midzone with the cell cortex and the furrow ingression process. In the future, overcoming the technical challenges in the analysis of the whole lipidome will open new avenues for understanding cell division, in particular of the molecular mechanism of massive membrane rearrangements and lipid functions during cell division and cytokinesis.

## 3. Identification of Protein Complexes for Cell Division

The number of protein factors participating in a physiological process is important, but it is also the interactive networks of these factors that add a new layer of complexity to the system. An earlier estimation of the human interactome size puts it as 650,000 [79]. Cell division and mitosis are no exception to these protein-protein interaction networks. One of the contributing factors that would make the mapping of mitotic protein networks a challenge, is the fact that the networks are active for a relatively short period compared to the whole cell cycle duration. Many of these interactions are also made possible through transient post-translational modifications and/or controlled expression of individual components. Therefore, many experiments require a synchronization of the cell population and arrest in mitosis to enrich the signal in proteomics detection. Nevertheless, proteomics studies have been undertaken to identify and characterize protein complexes associated with cell division.

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A wide-range of affinity purification methods combined with downstream MS analysis have been facilitated extensive identification and analysis of interacting partners in multiprotein complexes (reviewed in [80]). Pagliuca and Collins et al. [81] employed affinity purification and performed a time resolved proteomics study to identify interaction partners of Cyclin-Cdk complexes that coordinate progress through cell cycle. YFP and FLAG-tagged cyclins A2, E1 and B1 were used for localization studies and affinity purification, respectively. Metabolic labeling with light, intermediate and heavy isotope of arginine and lysine, followed by cell cycle stage specific synchronization and immunoprecipitation for cyclins were performed. This study revealed that the cyclins bind to subsets of interacting proteins as cells transit through different stages, providing evidence that cyclins in the cyclin-cdk complexes contribute to the interactome specificity. The specificity of shared subset of cell cycle proteins in cyclin interactions is controlled both biochemically and temporally. For example, the majority of interacting partners found common between cyclin A and cyclin B exhibited interactions with cyclin A in interphase, and later as the cell enters into mitosis with cyclin B, revealing a coordination among cyclin-cdk modules during cell stage transition [81].

"BAC transgenomics" is an approach in which tagged-genes from a transspecies are transferred under their endogenous cis control elements to cells so as to promote their near physiological expression [82]. Such an approach makes large-scale screening of interactomes possible. Near physiological expression levels preclude the spurious presence of non-specific proteins that often co-precipitate as a result of overexpression of a protein of interest. An advantage of BAC transgenomics for functional characterization is the ease with which transgene expression can be maintained while endogenous expression is knocked down using RNAi (**Figure 3**).

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When combined with a quantitative proteomics approach (Quantitative BAC GFP interactomics - QUBIC), affinity purification can be performed maintaining the native interactions of the proteins [83]. This approach also provides a convenient method for imaging GFP-tagged version of the proteins. The MitoCheck consortium undertook a phenomenal effort to characterize over 100 mitotic protein complexes employed BAC transgenomics [84]. In this study, 504 unique proteins were tagged for both localization and affinity purification (LAP). LAP-tagged mouse transgenes were stably expressed in HeLa cells, and a subset showing potential association with mitotic structures were processed for localization trajectories during mitotic stages using time lapse imaging. Tagged proteins were processed for tandem affinity purification and LC-MS/MS analysis to identify interaction partners. New subunits of γ-TuRC and APC/C complexes were identified as part of this study [84].

Protein cross-linking using chemical agents coupled to MS is an increasingly popular method to study protein-protein interactions. Deciphering protein interactions at peptide level complements high-resolution structural biology techniques to reveal interacting interfaces between two proteins and within protein networks [85-89]. Owing to the transient nature of mitotic complexes and associated interactions, cross-linking coupled proteomics holds great potential to identify such interactions. A recent study characterized the molecular architecture of the Dam1 complex that interacts with microtubules at kinetochores, utilizing cross-linking mass spectrometry and previously published biochemical and electron microscopy data [90]. This study elucidated that it is exclusively the C-terminal regions of Duo1p and Dam1p components of the complex interacting with microtubules and altering structural arrangements upon microtubule interaction. The identification of only two proteins out of ten that constitute the Dam1 complex as microtubule binding components highlights the specificity of using cross-linking approach [90].

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For interactome targeting proteomic approaches, the limiting factor is to establish functional relationships among the many hits provided by large-scale analysis. A novel multiclassifier approach based on random forest analysis (RFA) in a recent study found chromosomal associated proteins [39]. The study has yielded an improved ability of finding real associates as well as establishing the relationships among the detected proteins at the chromosomes. This approach has the potential to eliminate random non-specific "hitchhikers" from a different pool, in this case the cytosolic pool [39]. Using this approach, previously known functionally related proteins exhibited a tendency to cluster. Assigning a kinetochore role to C13orf3, also known as Ska3/Rama1, this study revealed that Ska complex is required for chromosomal association of APC/C and RanBP2/RanGAP1 complexes [39]. Further, in a later work, a nano Random approach was employed to identify functional relationships among proteins involved in mitotic chromosomal assembly [91]. It revealed that only a small subset among identified proteins, 113 of 5,058 proteins, associated with mitotic chromosomes has functional relationships with chromosomal structure and segregation.

Despite of significant advances, better and more efficient methods targeting spatiotemporal dynamics of cell division need to be developed. Emerging techniques like APEX reporter [92] to target a cellular sub-compartment and isolate interactors within a controlled temporal span holds a good promise. The computational additives as described above predict a future of mitotic protein complex identifications with combinatorial approaches to rigorously minimize possibilities of false hits.

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## 4. Posttranslational Modification Analysis of Cell Division

Posttranslational modifications (PTMs) enable the space and time dependent control of proteins. During division, a cell undergoes dramatic reorganizations in a short time window. The highly dynamic nature of the cell division machinery makes PTM dependent control mechanisms common. MS-based global analysis of PTMs has made tremendous progress in the last decade and has greatly contributed to our understanding of the role of PTMs during cell division. In this section, we mainly focus on the studies about phosphorylation dynamics and its crosstalk with other modifications during cell division. The leading studies in the field are demonstrated in **Figure 4** as a timeline, and discussed in detail below.

### 4.1 Phosphorylation

In mitosis, CDK1, PLK1 and AurB are the major mitotic kinases responsible for orchestrating multiple aspects of cell division, and as the cell goes through cytokinesis, CDC25 and PP1 are the main phosphatases that interact with the mitotic kinases [93, 94].

Phosphorylation events have been intensely studied for more than 30 years, being part of the main switch mechanisms in cell division regulation [95]. Mass spectrometric strategies combined with phosphopeptide enrichment techniques (see [96] for details) enabled researchers to identify protein compositions and the underlying phosphorylation dynamics in an efficient way. Dephoure et al.'s study [97] was a breakthrough unraveling the global phosphoproteome of mitotic cells. Using SILAC and phosphopeptide enrichment methods, more than 14,000 different phosphorylation events were

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identified in the interphase to mitosis transition. In mitosis, more than 1,000 proteins became phosphorylated. Strikingly, many of these were multiply phosphorylated. Mapping the human protein-interaction networks with the high scored mitotic phosphorylations revealed the interaction of many mitotically phosphorylated proteins with the major mitotic kinases such as CDC2, PLK1, AurA and AurB [97]. Around the same time, by combining SILAC with selective kinase enrichment, Daub et al. [98] developed a chemical phosphoproteomics approach to investigate cell division dependent protein kinase phosphorylation events. This approach allowed the researchers to quantify 219 protein kinases and more than 1,000 phosphorylation sites on protein kinases. More than 50% of the phosphopeptides deriving from protein kinases were mitosis specific [98].

With the development of high-resolution, highly sensitive mass spectrometry and more selective phosphopeptide enrichment methods, the size of phophoproteomic data has increased enormously. Recently, Olsen et al. [99] performed the most comprehensive study on the human cell cycle proteome and phosphoproteome in which the authors developed a method to calculate the absolute phosphorylation site stoichiometry using SILAC. They quantified 6,027 proteins and 20,443 unique phosphorylation sites [99]; 60% of these were also identified in Dephoure et al.'s study [97]. This study predicted the proportion of the phosphoproteins in the proteome as 70%, demonstrating the power of more selective techniques to isolate phosphopeptides of low abundance proteins.

Subcellular structures during cell division have been also effectively analyzed using phosphopeptide enrichment empowered MS techniques. For instance, Malik et al. [47] investigated the spindle phophoproteomes of different mitotic stages. This study exhibited the first large-scale analysis of spindle phosphoproteome and different dephosphorylation dynamics of phospho-residues during mitotic progression. A total of 1,940 unique phosphorylation sites were identified; 47% of

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these sites were downregulated by the mitotic exit. CDK1 consensus phosphorylation sites demonstrated strong downregulation in late mitosis while those of AurA and PLK1 demonstrated modest changes [47]. Another study focused on the microtubule cytoskeleton during cytokinesis. Here, Ozlu et al. [100] examined the microtubule cytoskeleton changes in mitosis and cytokinesis using large-scale SILAC-based MS analysis. Twenty-five hits were identified as cytokinesis specific microtubule binding proteins including NUSAP, NPM1, CLIP1, CLIP2 and CLASP1. The phosphoproteome analysis in mitosis to cytokinesis transition revealed that AurB is one of the master kinases during cytokinesis and its activity regulates the interaction between microtubule-associated proteins and the cytoskeleton. The Aurora kinase inhibitor abolished the microtubule binding activity of these proteins in cytokinesis [100].

In addition to global phosphoproteomics, MS also served for dissecting the phosphorylation dynamics of specific proteins in a cell cycle dependent manner. A remarkable example is the analysis of histone phosphorylation inheritance during cell division conducted by Lin et al. [101]. FLEXIQuant (full-length expressed stable isotope-labeled proteins for quantification), as an alternative approach, can be used to investigate posttranslational modifications of a single protein [102]. For instance, Singh et al. [103] identified KIFC1 as the novel target of anaphase-promoting complex/cyclosome (APC/C) and using FLEXIQuant strategy (see [104] for details), they analyzed the phosphorylation dynamics of KIFC1 in mitosis. Overall, combining whole cell, subcellular or single protein phosphoproteome with quantitative methods provide a framework to globally characterize the phosphorylation-based biochemical regulations in cell division.

Combining chemical genetic analyses with mass spectrometry provided a convenient way to assign the huge number of phosphorylation sites identified by MS to specific kinases and thereby to

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map potential substrate-kinase interactions. Correspondingly, the major cell division kinases, AurA, AurB, CDK1 and PLK1 were the best-studied targets. Kettenbach et al. [48] used small molecule kinase inhibitors of AurA, AurB and PLK1 to assign target phosphorylation sites of these kinases by quantitative phosphoproteomic approaches using mitotic cells. Of 562 identified proteins, 64 mitotic spindle associated proteins were assigned as candidate substrates of AurA, AurB and PLK1 [48]. Our group focused on the potential targets of AurB kinase, specifically during cytokinesis by combining a monopolar cytokinesis approach with SILAC and small molecule inhibitors of Aurora kinases, VX680 and AZD1152 [105]. More than 20,000 phosphopeptides were identified upon Aurora inhibition; 246 of them showing significant downregulation; intermediate filaments, microtubules and the actin cytoskeleton-related proteins were among the potential Aurora kinase substrates during cytokinesis [105]. In another study, Santamaria et al. [49] performed a comparative analysis of the mitotic spindle phosphoproteome using both a small molecule inhibitor of PLK1 and inducible shRNA targeting PLK1. They identified 358 downregulated phosphorylation sites after PLK1 inhibition or depletion, verifying 102 of them by an *in vitro* kinase assay [49]. As an alternative approach to identify direct substrates of mitotic kinases, a kinase assay linked to phosphoproteomics (KALIP) has been deployed [106], which is a combination of *in vitro* kinase reaction and *in vivo* phosphoproteome analysis. KALIP provides an alternative approach for kinase analysis to identify their substrates using phosphoproteomics, especially for those that do not have specific small molecule inhibitors. In general, these kinds of studies reveal a more precise definition of consensus motifs of key kinases and provide a valuable resource to understand the complex signaling network of cell division.

Phosphoproteomics not only serves as an approach for the identification of phosphorylation events and the responsible kinases but also for the de-phosphorylation events and responsible phosphatases during mitotic exit. Cyclin dependent kinase (CDK) is the master mitotic kinase that

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activates hundreds to thousands mitosis players at the onset of mitosis. These players are gradually inactivated during mitotic exit through dephosphorylation by phosphatases and ubiquitin mediated degradation by APC/C [107]. To identify CDK substrates, Kuilman et al. [108] analyzed the phosphoproteome of budding yeast in mitotic exit and cytokinesis. They arrested cells in mitosis, overexpressed Cdc14 (phosphatase) and collected cells at eight time-points following Cdc14 induction. Overall, 1,808 proteins were assigned as stable during the mitotic exit process. The phosphopeptide changes of CDK substrates were mostly due to dephosphorylation events [108]. Another group, McCloy et al. [109], analyzed the phosphorylation changes of early mitotic exit. They arrested HeLa cells at the mitotic exit by using both protease and CDK1 inhibitors. Their results revealed that during early mitotic exit only 10% of the phosphosites were dephosphorylated, suggesting gradual dephosphorylation of mitotic substrates throughout the mitotic exit. Mitotic spindle, kinetochore and nuclear envelope associated proteins were among the firstly dephosphorylated proteins during early mitotic exit [109].

As a rapidly evolving field, proteomics has also started to integrate with structural biology studies. In order to investigate how protein structures affect the phosphorylation dynamics throughout cell division, Tyanova et al. [110] re-examined several thousand phosphorylation sites identified in the global phosphoproteomics analysis of Olsen et al. [99]. These phosphorylation sites were quantified for six time-points of cell division. The phosphorylation sites were categorized into two main groups, namely phosphosites within regular secondary structures (α-helix and β-sheet) and phosphosites within disordered regions. Their analysis revealed that the disordered regions displayed a significant enrichment of multiple phosphorylation sites and frequent dynamic phosphorylation , whereas the regular secondary structures exhibited more stable phosphorylation sites during cell cycle [110]. In addition, the phosphorylation sites within the regular structures were identified as more evolutionary conserved than in the disordered regions. Overall, the structural organization of the

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region, where the phosphorylation sites reside, exerts additional regulation on the phosphorylation dynamics of the cell cycle [110].

In conclusion, the MS-based phosphoproteome studies provide a valuable catalogue of protein phosphorylation events as a cell progresses into cell division. The next challenge is to integrate this large amount of information for a comprehensive understanding of complex signaling networks that coordinate the actions between the cell cycle machinery, cytoskeleton, chromosomes and membranes to drive cell division. Crosstalk between phosphorylation and other posttranslational modifications is part of this complex regulatory mechanism as discussed in the following sections.

**4.2 Glycosylation**

The addition of sugar moieties to specific amino acids of proteins as a PTM is called glycosylation. In comparison to the phosphorylation, glycosylation events during the cell cycle have been vastly understudied. However, a limited number of studies have provided evidence for the role of glycosylation in cell division. Various types of glycosylation occur in cells, namely N-, O- and C-linked glycosylation, glypiation and phosphoglycosylation. O-linked beta-N-acetylglucosaminylation (O-GlcNAcylation) has particularly attracted researchers' attention since it shares the same modification sites with phosphorylation; the sugar moiety O-GlcNAc is covalently attached to the serine and threonine residues from the hydroxyl group as in phosphorylation. This reversible modification is mediated by two enzymes: O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). Slawson et al. [111] revealed that in multiple cell lines, the increase of O-GlcNAc levels causes delayed $G_2$/M progression and mitotic phosphorylation changes, whereas the removal of O-GlcNAc enhances the mitotic exit phenotype. O-GlcNAcylation functions to regulate the cell cycle; however,

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the mechanism behind this regulation remains mostly unknown. In a recent study, Wang et al. [112] analyzed both glycoproteome and phosphoproteome of cytokinesis cells to illustrate the crosstalk between O-GlcNAcylation and phosphorylation on spindle and midbody proteins. They further showed that during mitosis OGT localizes to spindles and midbody, and its overexpression in HeLa cells causes cytokinesis errors and increases polyploidy [112]. To understand the mechanism behind this defect, they compared the modification site alterations in mitotic cells before and after OGT overexpression by combining O-GlcNAc and phosphopeptide enrichment methods with SILAC-based quantitative proteomics. Strikingly, OGT overexpression triggered inhibitory phosphorylation sites of CDK1 and decreased the phosphorylation of CDK1 substrates. In addition, OGT overexpression caused reduced mRNA translation and protein abundance of PLK1 [112].

In brief, the crosstalk of O-GlcNAcylation and phosphorylation is part of the regulatory circuitry behind the complex signaling network controlling proper cell division. The underlying mechanisms yet remain to be investigated.

**4.3 Ubiquitination**

A known function of ubiquitination is to target proteins for proteolytic degradation. Mitotic exit requires rapid ubiquitin mediated degradation of mitosis specific proteins such as cyclins and securins by ubiquitin ligase, APC/C, activity [113]. The advances in the enrichment techniques of ubiquitination enable researchers to compare ubiquitinated proteins of different cell cycle stages using mass spectrometry. Recently, three strategies have been developed for ubiquitinated-peptide enrichment: the use of tagged tandem ubiquitin binding domains, anti-gly-gly-lys antibodies, and chemically modified ubiquitin (see [114] for detailed information). For instance, Min et al. [115]

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deployed a chemically modified ubiquitin strategy to investigate the ubiquitin-mediated processes of mitotic exit on a large scale. Min and colleagues performed *in vivo* biotinylation of ubiquitin to isolate mono-/polyubiquitinated proteins from mitosis cells. They identified 470 ubiquitinated proteins of which 170 were enriched during mitotic exit. KIFC1 and RacGAP1 were two of the top-ranked proteins identified as APC/C substrates. Moreover, 17 of the mitotic exit specific proteins, such as anillin, sororin and TOME-1, were identified for the first time as ubiquitinated proteins [115].

In contrast to the well-established methods available to study phosphorylation at a proteome level, studying ubiquitin-like modifications remains challenging. Development of robust methods for identification of site-specific protein ubiquitylation would shed new light on biochemical mechanisms for the coordination between protein (de)-phosphorylation and ubiquitination during mitotic exit.

### 4.4 Acetylation

Acetylation on lysine residues of histones has been well studied for its regulatory role on gene expression. However, our knowledge about global acetylation events and their function in cell division is highly limited. Choudhary et al. [116] investigated the lysine-acetylome of different cell lines and the global acetylation changes of cells upon lysine deacetylase (KDAC) inhibition. Acetylated peptides were enriched using an anti-acetyl-lysine antibody and analyzed by mass spectrometry. For the quantitative analysis of acetylation changes, they combined SILAC labeling with inhibitor treatment. A total of 3,600 lysine acetylation sites on 1,750 proteins were identified. Many of the cell cycle proteins including the cell cycle kinases were subjected to acetylation. Acetylation of kinases suggests that in addition to the regulation of gene expression, lysine acetylations may also regulate the activity of kinases [116]. Since the transcription is silent during

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mitosis, acetylation of mitotic proteins suggests other functions than gene expression regulation. Chuang et al. [117] analyzed lysine acetylations particularly in mitosis. Cells were synchronized in mitosis using nocodazole and lysine acetylated proteins were immunoprecipitated using an anti-acetyl-lysine antibody. Besides histones, LC-ESI-MS/MS analysis identified 51 nonhistone proteins. Among those, proteins responsible for cell cycle regulation such as anillin, APC1 and NudC were subjected to acetylation during mitosis and cytokinesis. Since acetylation neutralizes the charge of lysine residues, it may cause conformational change and modulate protein-protein interactions. Thus, acetylation on cell cycle proteins may provide another layer to the regulation of cell division [117].

**4.5 Sumoylation**

A small ubiquitin-like modifier protein, SUMO conjugation to proteins (sumoylation) is another posttranslational modification found mainly on nuclear proteins. Similar to ubiquitination or acetylation, sumoylation targets lysine residues of proteins; thereby, it may result in competition with different PTMs. SUMO-interaction motif (SIM) bearing proteins interact with SUMO conjugated proteins, and sometimes the presence of bulky SUMO modification prevents the protein-protein interactions. Sumoylation is implicated in the regulation of many cellular events in mitosis similar to phosphorylation and ubiquitination [118].

Schimmel et al. [119] deployed a quantitative proteomics approach to investigate cell cycle dependent sumoylation alterations. They stably expressed Flag-SUMO2 containing Q87R mutation in cells. This mutation enabled the shortening of tryptic digest remnants and their detection in MS2 spectra. Cells were labeled with three distinct sets of SILAC isotopes and synchronized to different cell cycle stages. Of proteins with 203 SUMO2 acceptor lysines, 249 demonstrated significant

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alterations in SUMO2 conjugation throughout the cell cycle. Gene ontology (GO) enrichment analysis revealed that nuclear proteins were mostly enriched in SUMO2 substrates. For instance, sumoylation of FoxM1 reached the highest level in mitosis. Moreover, shRNA mediated depletion of FoxM1 resulted in the formation of polyploid cells. Their further investigation of the role of FoxM1 sumoylation revealed that FoxM1 transcription was increased by sumoylation [119]. Another group, Cubenas-Potts et al. [120] demonstrated that SUMO2/3 localizes to the centromeres of sister chromatids. Sumoylated proteins were isolated using SUMO2/3 specific antibody from mitotic chromosomes and analyzed by nLC-ESI-MS/MS. As a result, 149 proteins were identified as potential sumoylation substrates in mitotic chromosomes. For instance, TRIM28, SMC3, SAFB1, and KIF4A were among the validated SUMO substrates [120]. Moreover, Schou et al. [121] investigated global SUMO2/3 modifications in mitosis and upon mitotic exit. Mitosis and mitotic exit targets of SUMO2/3 modification were quantified by SILAC using stably Flag-His tagged SUMO2 expressing cells. Their analysis revealed that the levels of SUMO2/3 modified proteins such as KLN2 and Repo-Man increase upon mitotic exit. On the other hand, only a small number of proteins were SUMO2/3 modified in mitosis such as RhoGDIα [121].

Sumoylation and phosphorylation crosstalk may be one of the mechanisms regulating the cell cycle progression. Yao et al. [122] analyzed the effect of sumoylation changes on the global phosphoproteome. SILAC labeled cells were treated with ginkgolic acid to decrease the global level of sumoylation. Sumoylation inhibition led to the accumulation of cells in the $G_0/G_1$ phase and decreased the number of cells in the $G_2/M$ phase. A sumoylation dependent regulation of phosphorylation events was observed [122].

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In conclusion, the regulation of cell division depends on the combinatory complex activities and cross-talks between phosphorylation and other post-translational modifications. The absence of efficient enrichment methods for a vast number of PTMs, and the requirement of large amount of initial material have made PTM analysis challenging. Moreover, PTMs are rather dynamic and their cross-talks may occur in a very short time window that may not be traceable in a mix cell population, necessitating method development for single cell analysis [123] and robust synchronization methods at different cell cycle stages. Despite limitations, advancements in sample preparation techniques and mass spectrometry instruments provide necessary means for collecting a huge amount of PTM data at a proteome level. This makes numerous repositories and online databases with the appropriate bioinformatic support inevitable. **Table 1** summarizes the most significant posttranslational modification databases [124-128]. The integration of all PTM data in these databases and identification of putative underlying molecular pathways would lead to unprecedented biochemical mechanisms that govern essential cellular processes such as cell division. The analysis of dysregulation of PTM dynamics in cancer-like diseases having defects in cell division will help to unveil molecular mechanisms of diseases and to develop novel therapeutic strategies.

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**Conflict of Interests**

The authors have declared no conflict of interest.

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**References**

[1] Mitchison, T. J., Salmon, E. D., Mitosis: a history of division. *Nat Cell Biol* 2001, *3*, E17-21.

[2] Storchova, Z., Breneman, A., Cande, J., Dunn, J., *et al.*, Genome-wide genetic analysis of polyploidy in yeast. *Nature* 2006, *443*, 541-547.

[3] Kittler, R., Buchholz, F., Functional genomic analysis of cell division by endoribonuclease-prepared siRNAs. *Cell Cycle* 2005, *4*, 564-567.

[4] Gonczy, P., Echeverri, C., Oegema, K., Coulson, A., *et al.*, Functional genomic analysis of cell division in C. elegans using RNAi of genes on chromosome III. *Nature* 2000, *408*, 331-336.

[5] Sonnichsen, B., Koski, L. B., Walsh, A., Marschall, P., *et al.*, Full-genome RNAi profiling of early embryogenesis in Caenorhabditis elegans. *Nature* 2005, *434*, 462-469.

[6] Neumann, B., Walter, T., Heriche, J. K., Bulkescher, J., *et al.*, Phenotypic profiling of the human genome by time-lapse microscopy reveals cell division genes. *Nature* 2010, *464*, 721-727.

[7] David, R., Cell cycle: dissecting mitosis. *Nat Rev Mol Cell Biol* 2010, *11*, 310.

[8] Doudna, J. A., Charpentier, E., Genome editing. The new frontier of genome engineering with CRISPR-Cas9. *Science (New York, N.Y.)* 2014, *346*, 1258096.

[9] McKinley, K. L., Cheeseman, I. M., Large-Scale Analysis of CRISPR/Cas9 Cell-Cycle Knockouts Reveals the Diversity of p53-Dependent Responses to Cell-Cycle Defects. *Developmental cell* 2017, *40*, 405-420.e402.

[10] Walther, T. C., Mann, M., Mass spectrometry-based proteomics in cell biology. *J Cell Biol* 2010, *190*, 491-500.

[11] Doxsey, S., Re-evaluating centrosome function. *Nat Rev Mol Cell Biol* 2001, *2*, 688-698.

This article is protected by copyright. All rights reserved.

Accepted Article

[12] Kalt, A., Schliwa, M., Molecular components of the centrosome. *Trends Cell Biol* 1993, *3*, 118-128.

[13] Andersen, J. S., Wilkinson, C. J., Mayor, T., Mortensen, P.*, et al.*, Proteomic characterization of the human centrosome by protein correlation profiling. *Nature* 2003, *426*, 570-574.

[14] Muller, H., Schmidt, D., Steinbrink, S., Mirgorodskaya, E.*, et al.*, Proteomic and functional analysis of the mitotic Drosophila centrosome. *Embo j* 2010, *29*, 3344-3357.

[15] Jakobsen, L., Vanselow, K., Skogs, M., Toyoda, Y.*, et al.*, Novel asymmetrically localizing components of human centrosomes identified by complementary proteomics methods. *Embo j* 2011, *30*, 1520-1535.

[16] Keller, L. C., Romijn, E. P., Zamora, I., Yates, J. R., 3rd, Marshall, W. F., Proteomic analysis of isolated chlamydomonas centrioles reveals orthologs of ciliary-disease genes. *Curr Biol* 2005, *15*, 1090-1098.

[17] Roux, K. J., Kim, D. I., Raida, M., Burke, B., A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. *J Cell Biol* 2012, *196*, 801-810.

[18] Firat-Karalar, E. N., Rauniyar, N., Yates, J. R., 3rd, Stearns, T., Proximity interactions among centrosome components identify regulators of centriole duplication. *Curr Biol* 2014, *24*, 664-670.

[19] Gupta, G. D., Coyaud, E., Goncalves, J., Mojarad, B. A.*, et al.*, A Dynamic Protein Interaction Landscape of the Human Centrosome-Cilium Interface. *Cell* 2015, *163*, 1484-1499.

[20] Woodruff, J. B., Wueseke, O., Hyman, A. A., *Philos Trans R Soc Lond B Biol Sci* 2014.

[21] Cheeseman, I. M., Desai, A., Molecular architecture of the kinetochore-microtubule interface. *Nat Rev Mol Cell Biol* 2008, *9*, 33-46.

This article is protected by copyright. All rights reserved.

Accepted Article

[22] Chen, R. H., Shevchenko, A., Mann, M., Murray, A. W., Spindle checkpoint protein Xmad1 recruits Xmad2 to unattached kinetochores. *The Journal of cell biology* 1998, *143*, 283-295.

[23] Cheeseman, I. M., Brew, C., Wolyniak, M., Desai, A.*, et al.*, Implication of a novel multiprotein Dam1p complex in outer kinetochore function. *The Journal of cell biology* 2001, *155*, 1137-1145.

[24] Westermann, S., Cheeseman, I. M., Anderson, S., Yates, J. R., 3rd*, et al.*, Architecture of the budding yeast kinetochore reveals a conserved molecular core. *J Cell Biol* 2003, *163*, 215-222.

[25] Przewloka, M. R., Zhang, W., Costa, P., Archambault, V.*, et al.*, Molecular analysis of core kinetochore composition and assembly in Drosophila melanogaster. *PLoS One* 2007, *2*, e478.

[26] Desai, A., Rybina, S., Muller-Reichert, T., Shevchenko, A.*, et al.*, KNL-1 directs assembly of the microtubule-binding interface of the kinetochore in C. elegans. *Genes Dev* 2003, *17*, 2421-2435.

[27] Kline, S. L., Cheeseman, I. M., Hori, T., Fukagawa, T., Desai, A., The human Mis12 complex is required for kinetochore assembly and proper chromosome segregation. *J Cell Biol* 2006, *173*, 9-17.

[28] Ciferri, C., De Luca, J., Monzani, S., Ferrari, K. J.*, et al.*, Architecture of the human ndc80-hec1 complex, a critical constituent of the outer kinetochore. *J Biol Chem* 2005, *280*, 29088-29095.

[29] Cheeseman, I. M., Niessen, S., Anderson, S., Hyndman, F.*, et al.*, A conserved protein network controls assembly of the outer kinetochore and its ability to sustain tension. *Genes Dev* 2004, *18*, 2255-2268.

[30] Okada, M., Cheeseman, I. M., Hori, T., Okawa, K.*, et al.*, The CENP-H-I complex is required for the efficient incorporation of newly synthesized CENP-A into centromeres. *Nat Cell Biol* 2006, *8*, 446-457.

[31] Hayashi, T., Fujita, Y., Iwasaki, O., Adachi, Y.*, et al.*, Mis16 and Mis18 are required for CENP-A loading and histone deacetylation at centromeres. *Cell* 2004, *118*, 715-729.

This article is protected by copyright. All rights reserved.

Accepted Article

[32] Fujita, Y., Hayashi, T., Kiyomitsu, T., Toyoda, Y., *et al.*, Priming of centromere for CENP-A recruitment by human hMis18alpha, hMis18beta, and M18BP1. *Developmental cell* 2007, *12*, 17-30.

[33] Maddox, P. S., Hyndman, F., Monen, J., Oegema, K., Desai, A., Functional genomics identifies a Myb domain-containing protein family required for assembly of CENP-A chromatin. *J Cell Biol* 2007, *176*, 757-763.

[34] Van Hooser, A. A., Ouspenski, II, Gregson, H. C., Starr, D. A., *et al.*, Specification of kinetochore-forming chromatin by the histone H3 variant CENP-A. *J Cell Sci* 2001, *114*, 3529-3542.

[35] Hori, T., Amano, M., Suzuki, A., Backer, C. B., *et al.*, CCAN makes multiple contacts with centromeric DNA to provide distinct pathways to the outer kinetochore. *Cell* 2008, *135*, 1039-1052.

[36] Amano, M., Suzuki, A., Hori, T., Backer, C., *et al.*, The CENP-S complex is essential for the stable assembly of outer kinetochore structure. *J Cell Biol* 2009, *186*, 173-182.

[37] Gassmann, R., Henzing, A. J., Earnshaw, W. C., Novel components of human mitotic chromosomes identified by proteomic analysis of the chromosome scaffold fraction. *Chromosoma* 2005, *113*, 385-397.

[38] Uchiyama, S., Kobayashi, S., Takata, H., Ishihara, T., *et al.*, Proteome analysis of human metaphase chromosomes. *J Biol Chem* 2005, *280*, 16994-17004.

[39] Ohta, S., Bukowski-Wills, J. C., Sanchez-Pulido, L., Alves Fde, L., *et al.*, The protein composition of mitotic chromosomes determined using multiclassifier combinatorial proteomics. *Cell* 2010, *142*, 810-821.

[40] Akiyoshi, B., Nelson, C. R., Ranish, J. A., Biggins, S., Quantitative proteomic analysis of purified yeast kinetochores identifies a PP1 regulatory subunit. *Genes Dev* 2009, *23*, 2887-2899.

This article is protected by copyright. All rights reserved.

Accepted Article

[41] Ruchaud, S., Carmena, M., Earnshaw, W. C., Chromosomal passengers: conducting cell division. *Nat Rev Mol Cell Biol* 2007, *8*, 798-812.

[42] Welburn, J. P., Vleugel, M., Liu, D., Yates, J. R., 3rd*, et al.*, Aurora B phosphorylates spatially distinct targets to differentially regulate the kinetochore-microtubule interface. *Mol Cell* 2010, *38*, 383-392.

[43] Maiolica, A., Cittaro, D., Borsotti, D., Sennels, L.*, et al.*, Structural analysis of multiprotein complexes by cross-linking, mass spectrometry, and database searching. *Molecular & cellular proteomics : MCP* 2007, *6*, 2200-2211.

[44] Weir, J. R., Faesen, A. C., Klare, K., Petrovic, A.*, et al.*, Insights from biochemical reconstitution into the architecture of human kinetochores. *Nature* 2016, *537*, 249-253.

[45] Sauer, G., Korner, R., Hanisch, A., Ries, A.*, et al.*, Proteome analysis of the human mitotic spindle. *Molecular & cellular proteomics : MCP* 2005, *4*, 35-43.

[46] Bonner, M. K., Poole, D. S., Xu, T., Sarkeshik, A.*, et al.*, Mitotic spindle proteomics in Chinese hamster ovary cells. *PLoS One* 2011, *6*, e20489.

[47] Malik, R., Lenobel, R., Santamaria, A., Ries, A.*, et al.*, Quantitative analysis of the human spindle phosphoproteome at distinct mitotic stages. *Journal of proteome research* 2009, *8*, 4553-4563.

[48] Kettenbach, A. N., Schweppe, D. K., Faherty, B. K., Pechenick, D.*, et al.*, Quantitative phosphoproteomics identifies substrates and functional modules of Aurora and Polo-like kinase activities in mitotic cells. *Sci Signal* 2011, *4*, rs5.

[49] Santamaria, A., Wang, B., Elowe, S., Malik, R.*, et al.*, The Plk1-dependent phosphoproteome of the early mitotic spindle. *Molecular & cellular proteomics : MCP* 2011, *10*, M110 004457.

This article is protected by copyright. All rights reserved.

Accepted Article

[50] Nousiainen, M., Sillje, H. H., Sauer, G., Nigg, E. A., Korner, R., Phosphoproteome analysis of the human mitotic spindle. *Proc Natl Acad Sci U S A* 2006, *103*, 5391-5396.

[51] Zhou, X., Zhu, H., Liu, L., Lin, J., Tang, K., A review: recent advances and future prospects of taxol-producing endophytic fungi. *Appl Microbiol Biotechnol* 2010, *86*, 1707-1717.

[52] Cross, R. A., McAinsh, A., Prime movers: the mechanochemistry of mitotic kinesins. *Nat Rev Mol Cell Biol* 2014, *15*, 257-271.

[53] Carmena, M., Wheelock, M., Funabiki, H., Earnshaw, W. C., The Chromosomal Passenger Complex (CPC): From Easy Rider to the Godfather of Mitosis. *Nat Rev Mol Cell Biol* 2012, *13*, 789-803.

[54] Flemming, W., Neue Beiträge zur Kenntnis der Zelle. *Archiv für Mikroskopische Anatomie* 1891, *37*, 685-751.

[55] Hu, C. K., Coughlin, M., Mitchison, T. J., Midbody assembly and its regulation during cytokinesis. *Mol Biol Cell* 2012, *23*, 1024-1034.

[56] Skop, A. R., Liu, H., Yates, J., 3rd, Meyer, B. J., Heald, R., Dissection of the mammalian midbody proteome reveals conserved cytokinesis mechanisms. *Science (New York, N.Y.)* 2004, *305*, 61-66.

[57] Mullins, J. M., McIntosh, J. R., Isolation and initial characterization of the mammalian midbody. *J Cell Biol* 1982, *94*, 654-661.

[58] Champion, L., Linder, M. I., Kutay, U., Cellular Reorganization during Mitotic Entry. *Trends Cell Biol* 2016.

This article is protected by copyright. All rights reserved.

Accepted Article

[59] Jinguji, Y., Ishikawa, H., Electron microscopic observations on the maintenance of the tight junction during cell division in the epithelium of the mouse small intestine. *Cell Struct Funct* 1992, *17*, 27-37.

[60] Meyer, E. J., Ikmi, A., Gibson, M. C., Interkinetic nuclear migration is a broadly conserved feature of cell division in pseudostratified epithelia. *Curr Biol* 2011, *21*, 485-491.

[61] Cramer, L. P., Mitchison, T. J., Investigation of the mechanism of retraction of the cell margin and rearward flow of nodules during mitotic cell rounding. *Mol Biol Cell* 1997, *8*, 109-119.

[62] Ramanathan, S. P., Helenius, J., Stewart, M. P., Cattin, C. J.*, et al.*, Cdk1-dependent mitotic enrichment of cortical myosin II promotes cell rounding against confinement. *Nat Cell Biol* 2015, *17*, 148-159.

[63] Maddox, A. S., Burridge, K., RhoA is required for cortical retraction and rigidity during mitotic cell rounding. *J Cell Biol* 2003, *160*, 255-265.

[64] Matthews, H. K., Delabre, U., Rohn, J. L., Guck, J.*, et al.*, Changes in Ect2 localization couple actomyosin-dependent cell shape changes to mitotic progression. *Developmental cell* 2012, *23*, 371-383.

[65] Dao, V. T., Dupuy, A. G., Gavet, O., Caron, E., de Gunzburg, J., Dynamic changes in Rap1 activity are required for cell retraction and spreading during mitosis. *J Cell Sci* 2009, *122*, 2996-3004.

[66] Marchesi, S., Montani, F., Deflorian, G., D'Antuono, R.*, et al.*, DEPDC1B coordinates de-adhesion events and cell-cycle progression at mitosis. *Developmental cell* 2014, *31*, 420-433.

[67] Stewart, M. P., Helenius, J., Toyoda, Y., Ramanathan, S. P.*, et al.*, Hydrostatic pressure and the actomyosin cortex drive mitotic cell rounding. *Nature* 2011, *469*, 226-230.

This article is protected by copyright. All rights reserved.

Accepted Article

[68] Colanzi, A., Corda, D., Mitosis controls the Golgi and the Golgi controls mitosis. *Curr Opin Cell Biol* 2007, *19*, 386-393.

[69] Yeong, F. M., Multi-step down-regulation of the secretory pathway in mitosis: a fresh perspective on protein trafficking. *Bioessays* 2013, *35*, 462-471.

[70] Boucrot, E., Kirchhausen, T., Endosomal recycling controls plasma membrane area during mitosis. *Proc Natl Acad Sci U S A* 2007, *104*, 7939-7944.

[71] Ozlu, N., Qureshi, M. H., Toyoda, Y., Renard, B. Y.*, et al.*, Quantitative comparison of a human cancer cell surface proteome between interphase and mitosis. *Embo j* 2015, *34*, 251-265.

[72] Josic, D., Clifton, J. G., Mammalian plasma membrane proteomics. *Proteomics* 2007, *7*, 3010-3029.

[73] Atilla-Gokcumen, G. E., Muro, E., Relat-Goberna, J., Sasse, S.*, et al.*, Dividing cells regulate their lipid composition and localization. *Cell* 2014, *156*, 428-439.

[74] Brugger, B., Lipidomics: analysis of the lipid composition of cells and subcellular organelles by electrospray ionization mass spectrometry. *Annu Rev Biochem* 2014, *83*, 79-98.

[75] Han, X., Gross, R. W., Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics. *J Lipid Res* 2003, *44*, 1071-1079.

[76] Atilla-Gokcumen, G. E., Bedigian, A. V., Sasse, S., Eggert, U. S., Inhibition of glycosphingolipid biosynthesis induces cytokinesis failure. *J Am Chem Soc* 2011, *133*, 10010-10013.

[77] Didangelos, A., Stegemann, C., Mayr, M., The -omics era: proteomics and lipidomics in vascular research. *Atherosclerosis* 2012, *221*, 12-17.

This article is protected by copyright. All rights reserved.

Accepted Article

[78] Kreimer, S., Belov, A. M., Ghiran, I., Murthy, S. K., *et al.*, Mass-spectrometry-based molecular characterization of extracellular vesicles: lipidomics and proteomics. *Journal of proteome research* 2015, *14*, 2367-2384.

[79] Stumpf, M. P., Thorne, T., de Silva, E., Stewart, R., *et al.*, Estimating the size of the human interactome. *Proc Natl Acad Sci U S A* 2008, *105*, 6959-6964.

[80] Collins, M. O., Choudhary, J. S., Mapping multiprotein complexes by affinity purification and mass spectrometry. *Curr Opin Biotechnol* 2008, *19*, 324-330.

[81] Pagliuca, F. W., Collins, M. O., Lichawska, A., Zegerman, P., *et al.*, Quantitative proteomics reveals the basis for the biochemical specificity of the cell-cycle machinery. *Mol Cell* 2011, *43*, 406-417.

[82] Poser, I., Sarov, M., Hutchins, J. R., Heriche, J. K., *et al.*, BAC TransgeneOmics: a high-throughput method for exploration of protein function in mammals. *Nat Methods* 2008, *5*, 409-415.

[83] Hubner, N. C., Bird, A. W., Cox, J., Splettstoesser, B., *et al.*, Quantitative proteomics combined with BAC TransgeneOmics reveals in vivo protein interactions. *J Cell Biol* 2010, *189*, 739-754.

[84] Hutchins, J. R., Toyoda, Y., Hegemann, B., Poser, I., *et al.*, Systematic analysis of human protein complexes identifies chromosome segregation proteins. *Science* 2010, *328*, 593-599.

[85] Liu, F., Heck, A. J., Interrogating the architecture of protein assemblies and protein interaction networks by cross-linking mass spectrometry. *Curr Opin Struct Biol* 2015, *35*, 100-108.

[86] Herzog, F., Kahraman, A., Boehringer, D., Mak, R., *et al.*, Structural probing of a protein phosphatase 2A network by chemical cross-linking and mass spectrometry. *Science* 2012, *337*, 1348-1352.

This article is protected by copyright. All rights reserved.

Accepted Article

[87] Abad, M. A., Medina, B., Santamaria, A., Zou, J., *et al.*, Structural basis for microtubule recognition by the human kinetochore Ska complex. *Nat Commun* 2014, *5*, 2964.

[88] Zelter, A., Hoopmann, M. R., Vernon, R., Baker, D., *et al.*, Isotope signatures allow identification of chemically cross-linked peptides by mass spectrometry: a novel method to determine interresidue distances in protein structures through cross-linking. *J Proteome Res* 2010, *9*, 3583-3589.

[89] Leitner, A., Faini, M., Stengel, F., Aebersold, R., Crosslinking and Mass Spectrometry: An Integrated Technology to Understand the Structure and Function of Molecular Machines. *Trends Biochem Sci* 2016, *41*, 20-32.

[90] Zelter, A., Bonomi, M., Kim, J., Umbreit, N. T., *et al.*, The molecular architecture of the Dam1 kinetochore complex is defined by cross-linking based structural modelling. *Nat Commun* 2015, *6*, 8673.

[91] Ohta, S., Montano-Gutierrez, L. F., Alves, F. L., Ogawa, H., *et al.*, Proteomics analysis with a nano Random Forest approach reveals novel functional interactions regulated by SMC complexes on mitotic chromosomes. *Mol Cell Proteomics* 2016.

[92] Rhee, H. W., Zou, P., Udeshi, N. D., Martell, J. D., *et al.*, Proteomic mapping of mitochondria in living cells via spatially restricted enzymatic tagging. *Science (New York, N.Y.)* 2013, *339*, 1328-1331.

[93] Fededa, J. P., Gerlich, D. W., Molecular control of animal cell cytokinesis. *Nat Cell Biol* 2012, *14*, 440-447.

[94] Barr, F. A., Elliott, P. R., Gruneberg, U., Protein phosphatases and the regulation of mitosis. *Journal of Cell Science* 2011, *124*, 2323-2334.

[95] Suryadinata, R., Sadowski, M., Sarcevic, B., Control of cell cycle progression by phosphorylation of cyclin-dependent kinase (CDK) substrates. *Bioscience Reports* 2010, *30*, 243-255.

This article is protected by copyright. All rights reserved.

Accepted Article

[96] Ozlu, N., Akten, B., Timm, W., Haseley, N.*, et al.*, Phosphoproteomics. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine* 2010, *2*, 255-276.

[97] Dephoure, N., Zhou, C., Villen, J., Beausoleil, S. A.*, et al.*, A quantitative atlas of mitotic phosphorylation. *Proc Natl Acad Sci U S A* 2008, *105*, 10762-10767.

[98] Daub, H., Olsen, J. V., Bairlein, M., Gnad, F.*, et al.*, Kinase-selective enrichment enables quantitative phosphoproteomics of the kinome across the cell cycle. *Mol Cell* 2008, *31*, 438-448.

[99] Olsen, J. V., Vermeulen, M., Santamaria, A., Kumar, C.*, et al.*, Quantitative phosphoproteomics reveals widespread full phosphorylation site occupancy during mitosis. *Sci Signal* 2010, *3*, ra3.

[100] Ozlu, N., Monigatti, F., Renard, B. Y., Field, C. M.*, et al.*, Binding partner switching on microtubules and aurora-B in the mitosis to cytokinesis transition. *Molecular & cellular proteomics : MCP* 2010, *9*, 336-350.

[101] Lin, S., Yuan, Z. F., Han, Y., Marchione, D. M., Garcia, B. A., Preferential Phosphorylation on Old Histones during Early Mitosis in Human Cells. *J Biol Chem* 2016, *291*, 15342-15357.

[102] Singh, S., Springer, M., Steen, J., Kirschner, M. W., Steen, H., FLEXIQuant: a novel tool for the absolute quantification of proteins, and the simultaneous identification and quantification of potentially modified peptides. *Journal of proteome research* 2009, *8*, 2201-2210.

[103] Singh, S. A., Winter, D., Kirchner, M., Chauhan, R.*, et al.*, Co-regulation proteomics reveals substrates and mechanisms of APC/C-dependent degradation. *Embo j* 2014, *33*, 385-399.

[104] Singh, S., Kirchner, M., Steen, J. A., Steen, H., A practical guide to the FLEXIQuant method. *Methods Mol Biol* 2012, *893*, 295-319.

This article is protected by copyright. All rights reserved.

Accepted Article

[105] Polat, A. N., Karayel, O., Giese, S. H., Harmanda, B., *et al.*, Phosphoproteomic Analysis of Aurora Kinase Inhibition in Monopolar Cytokinesis. *Journal of proteome research* 2015, *14*, 4087-4098.

[106] Xue, L., Wang, W.-H., Iliuk, A., Hu, L., *et al.*, Sensitive kinase assay linked with phosphoproteomics for identifying direct kinase substrates. *Proceedings of the National Academy of Sciences* 2012, *109*, 5615-5620.

[107] Sanchez-Diaz, A., Nkosi, P. J., Murray, S., Labib, K., The Mitotic Exit Network and Cdc14 phosphatase initiate cytokinesis by counteracting CDK phosphorylations and blocking polarised growth. *Embo j* 2012, *31*, 3620-3634.

[108] Kuilman, T., Maiolica, A., Godfrey, M., Scheidel, N., *et al.*, Identification of Cdk targets that control cytokinesis. *Embo j* 2015, *34*, 81-96.

[109] McCloy, R. A., Parker, B. L., Rogers, S., Chaudhuri, R., *et al.*, Global Phosphoproteomic Mapping of Early Mitotic Exit in Human Cells Identifies Novel Substrate Dephosphorylation Motifs. *Molecular & cellular proteomics : MCP* 2015, *14*, 2194-2212.

[110] Tyanova, S., Cox, J., Olsen, J., Mann, M., Frishman, D., Phosphorylation Variation during the Cell Cycle Scales with Structural Propensities of Proteins. *PLoS Comput Biol* 2013, *9*, e1002842.

[111] Slawson, C., Zachara, N. E., Vosseller, K., Cheung, W. D., *et al.*, Perturbations in O-linked beta-N-acetylglucosamine protein modification cause severe defects in mitotic progression and cytokinesis. *J Biol Chem* 2005, *280*, 32944-32956.

[112] Wang, Z., Udeshi, N. D., Slawson, C., Compton, P. D., *et al.*, Extensive crosstalk between O-GlcNAcylation and phosphorylation regulates cytokinesis. *Sci Signal* 2010, *3*, ra2.

[113] Sivakumar, S., Gorbsky, G. J., Spatiotemporal regulation of the anaphase-promoting complex in mitosis. *Nat Rev Mol Cell Biol* 2015, *16*, 82-94.

This article is protected by copyright. All rights reserved.

Accepted Article

[114] Vertegaal, A. C., Uncovering ubiquitin and ubiquitin-like signaling networks. *Chem Rev* 2011, *111*, 7923-7940.

[115] Min, M., Mayor, U., Dittmar, G., Lindon, C., Using in vivo biotinylated ubiquitin to describe a mitotic exit ubiquitome from human cells. *Molecular & cellular proteomics : MCP* 2014, *13*, 2411-2425.

[116] Choudhary, C., Kumar, C., Gnad, F., Nielsen, M. L.*, et al.*, Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science (New York, N.Y.)* 2009, *325*, 834-840.

[117] Chuang, C., Lin, S.-H., Huang, F., Pan, J.*, et al.*, Acetylation of RNA Processing Proteins and Cell Cycle Proteins in Mitosis. *Journal of proteome research* 2010, *9*, 4554-4564.

[118] Jentsch, S., Psakhye, I., Control of nuclear activities by substrate-selective and protein-group SUMOylation. *Annu Rev Genet* 2013, *47*, 167-186.

[119] Schimmel, J., Eifler, K., Sigurðsson, Jón O., Cuijpers, Sabine A. G.*, et al.*, Uncovering SUMOylation Dynamics during Cell-Cycle Progression Reveals FoxM1 as a Key Mitotic SUMO Target Protein. *Molecular Cell*, *53*, 1053-1066.

[120] Cubenas-Potts, C., Srikumar, T., Lee, C., Osula, O.*, et al.*, Identification of SUMO-2/3-modified proteins associated with mitotic chromosomes. *Proteomics* 2015, *15*, 763-772.

[121] Schou, J., Kelstrup, C. D., Hayward, D. G., Olsen, J. V., Nilsson, J., Comprehensive Identification of SUMO2/3 Targets and Their Dynamics during Mitosis. *PLoS ONE* 2014, *9*, e100692.

[122] Yao, Q., Li, H., Liu, B. Q., Huang, X. Y., Guo, L., SUMOylation-regulated protein phosphorylation, evidence from quantitative phosphoproteomics analyses. *J Biol Chem* 2011, *286*, 27342-27349.

This article is protected by copyright. All rights reserved.

Accepted Article

[123] Polat, A. N., Ozlu, N., Towards single-cell LC-MS phosphoproteomics. *The Analyst* 2014, *139*, 4733-4749.

[124] Chen, T., Zhou, T., He, B., Yu, H., *et al.*, mUbiSiDa: a comprehensive database for protein ubiquitination sites in mammals. *PLoS One* 2014, *9*, e85744.

[125] Dinkel, H., Chica, C., Via, A., Gould, C. M., *et al.*, Phospho.ELM: a database of phosphorylation sites—update 2011. *Nucleic Acids Research* 2011, *39*, D261-D267.

[126] Gnad, F., Gunawardena, J., Mann, M., PHOSIDA 2011: the posttranslational modification database. *Nucleic Acids Res* 2011, *39*, D253-260.

[127] Hornbeck, P. V., Zhang, B., Murray, B., Kornhauser, J. M., *et al.*, PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. *Nucleic Acids Res* 2015, *43*, D512-520.

[128] Stark, C., Breitkreutz, B. J., Reguly, T., Boucher, L., *et al.*, BioGRID: a general repository for interaction datasets. *Nucleic Acids Res* 2006, *34*, D535-539.

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**Figure Legends**

**Figure 1.** Organelle proteomics studies for cell division.

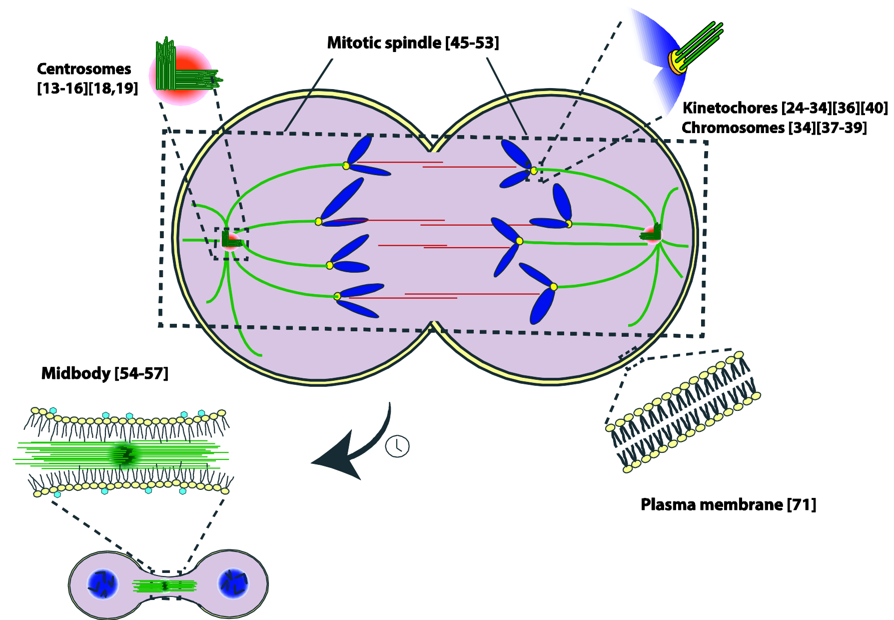


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**Figure 2.** Accumulating lipids during cytokinesis and at the midbody.

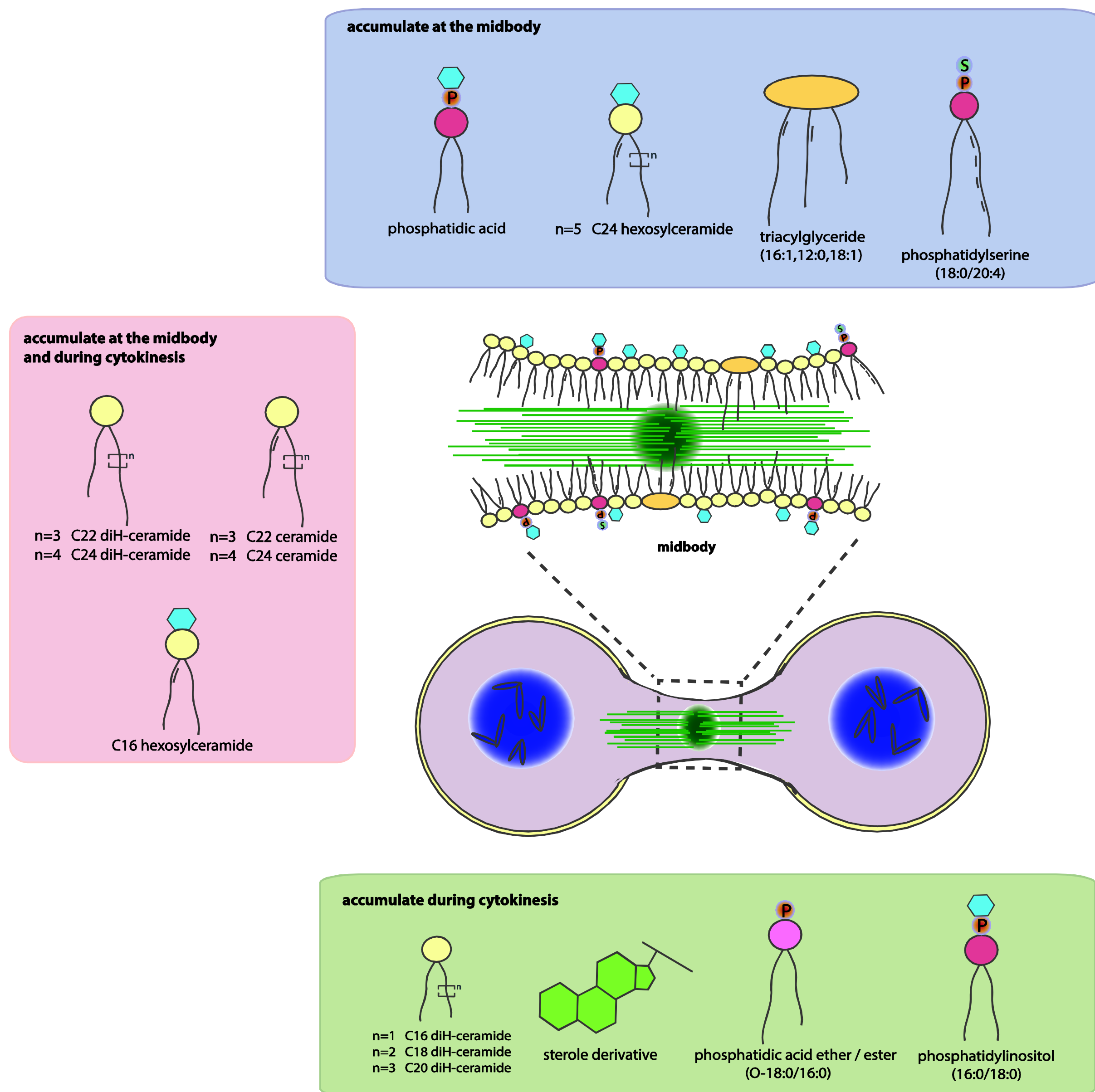


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**Figure 3.** Cartoon depicting BAC-transgenomics, in which GFP-tagged trans-species counterpart genes are expressed. Downregulation of endogenous genes is achieved through RNA interference targeting endogenous RNA. GFP pull down of trans-species gene is performed to identify interacting partners using mass spectrometry.

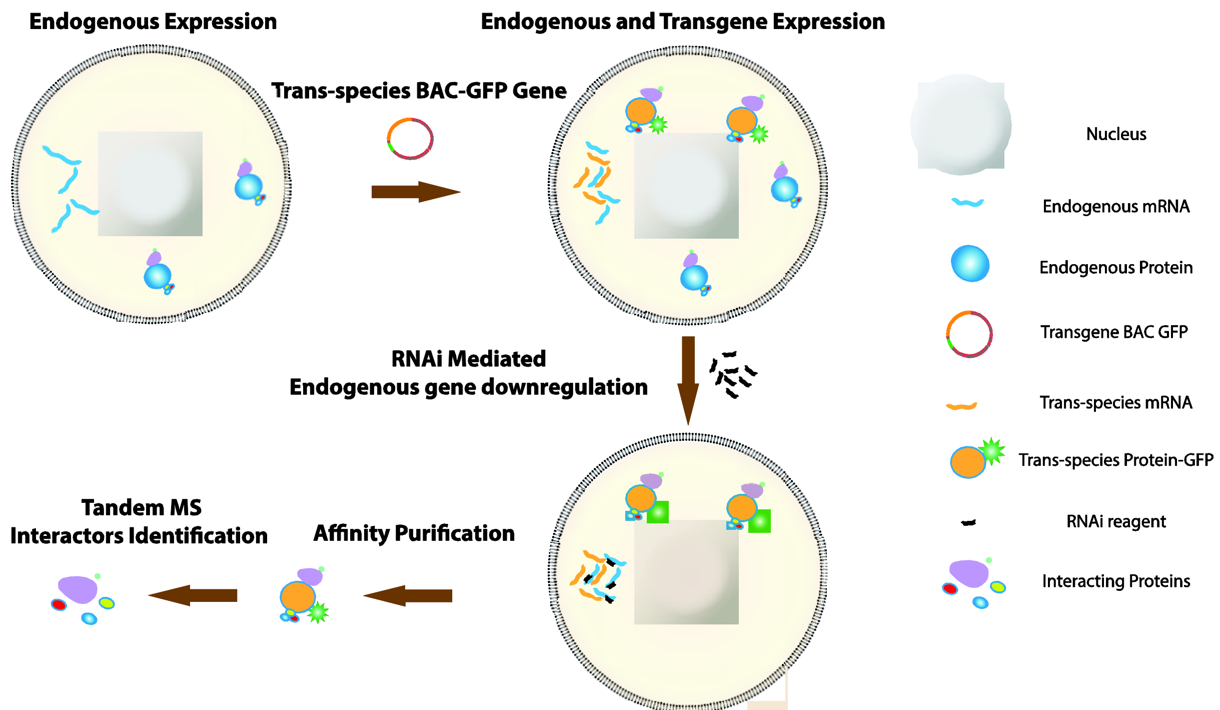


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**Figure 4.** Timeline of posttranslational modification analysis in cell division by mass spectrometry. For details see the text.

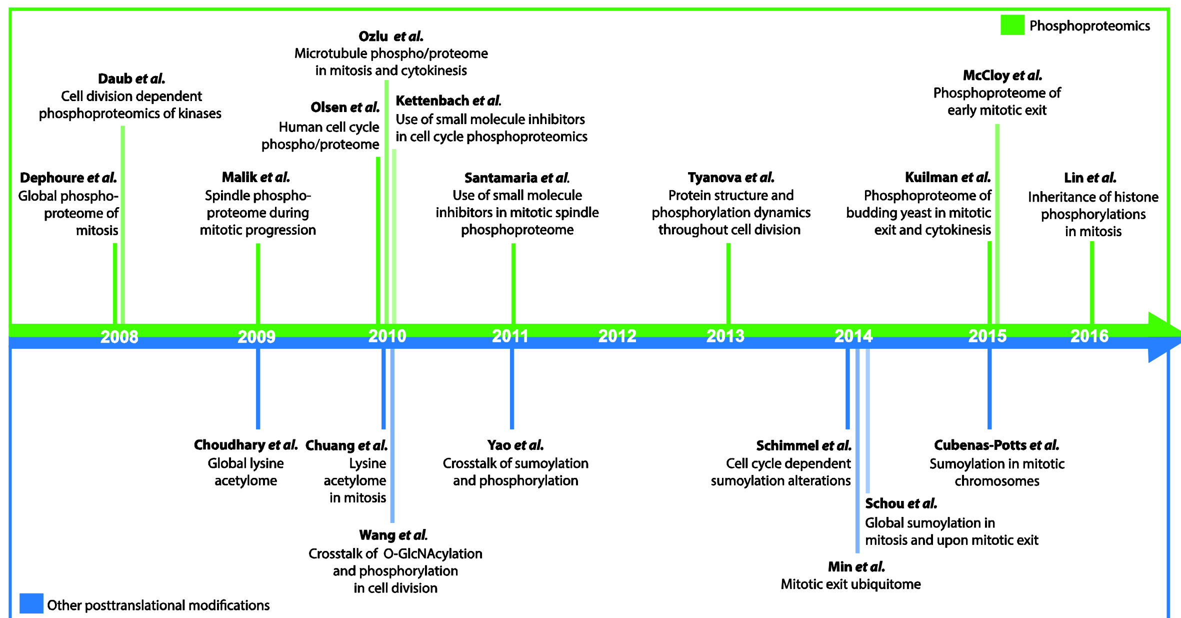


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**Table 1.** Common databases for posttranslational modifications.

**Table 1.** Common databases for posttranslational modifications.

| Database | Organism | Size | Type of PTM |
|---|---|---|---|
| PHOSIDA [126] (http://www.phosida.com/) | 9 different species including human | > 80,000 sites | phosphorylation, N-glycosylation, acetylation |
| PhosphoSitePlus (PSP) [127] (http://www.phosphosite.org/) | Human, mouse and closely related species | > 330,000 sites | phosphorylation, acetylation, methylation, ubiquitination, O-glycosylation |
| Phospho.ELM [125] (http://phospho.elm.eu.org) | Eukaryotic species | 42,574 sites | phosphorylation |
| BioGRID [128] (http://thebiogrid.org/) | Various prokaryotic and eukaryotic species including human | 38,559 sites | sumoylation, ubiquitination, neddylation |
| mubiSiDa [124] (http://reprod.njmu.edu.cn/mUbiSiDa) | 5 different species including human | 110,976 sites | ubiquitination |

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