

Roles of phosphatases in eukaryotic DNA replication initiation control

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In eukaryotes, DNA replication is initiated at many sites (origins) across the genome. This process is strictly regulated to ensure that there is never more than a single initiation event at each origin per cell cycle. In addition, the number and location of origins that fire in S-phase are also regulated to ensure that the entire genome is replicated in a timely manner. Over- or under-replication causes genome instability [1], which appears to be an early event in cancer development [2–4]. The contributions of kinases to replication initiation control, both during the cell cycle and during DNA damage, are well established [5,6]. Here, we discuss how protein phosphatases can also contribute to DNA replication control: ensuring initiation only once per cell cycle and dictating the rate and location of origin firing. As many kinases involved in replication control are targets for new chemotherapies [7–9], understanding how these pathways are modulated by phosphatases may be critical to improve the clinical effectiveness of these drugs and may also provide insight into how tumours develop resistance to kinase inhibitors.

1. Protein dephosphorylation can modulate a kinase response

Protein phosphorylation is a key mechanism to control the events of the cell cycle, and large-scale protein dephosphorylation is required to reset the cell cycle during mitotic exit [10]. However, in *S. pombe* the majority of CDK substrates participate in rapid cycles of ATP turnover from S phase to mitosis, with a median phosphorylation half-life of just 2.2 minutes [11]. It is therefore likely that phosphatases are required to modulate the kinase response in other functionally important ways in addition to their role at mitotic exit [12]:

Creating a thresholded switch

The degree of coordination between kinase and phosphatase activities determines the amount of phosphorylated substrate (output) produced for a given change in enzymatic activity (input). Independent enzymatic regulation generates a hyperbolic response (Figure 1A, left), but reciprocal enzymatic regulation e.g. where the kinase inactivates the phosphatase or vice versa, generates an ultrasensitive, stepwise transition between a minimally and maximally phosphorylated substrate population, after a set activity threshold is exceeded (Figure 1A, middle). However, this regulatory configuration means that at input values close to the threshold, the transition may be inadvertently triggered by input 'noise'. A bistable switch averts this as the output is dependent upon the proportion of phosphorylated substrate already present (hysteresis, Figure 1A, right). The substrate/product feedback loops on enzymatic activity that underpin such bistable regulation are exemplified by key regulators of the G2-M and G1-S-phase transitions (Figure 1B) [13]. The robustness of the transition between stable states can be enhanced by multi-site phosphorylation of the substrate (Figure 2A, top), as in the case for Sic1 in budding yeast [14]. The speed of the transition between bistable states can be enhanced by phospho-regulated positive feedback on substrate-kinase or phosphatase binding-sites (Figure 2B, top), for example, sequential kinase priming regulates phospho-degrons in mitosis [15,16] and phosphorylation can inhibit PP1 phosphatase binding [17].

Ordering protein phosphorylation

For cell cycle and in particular DNA replication control, the order in which proteins are phosphorylated is critical (see section 2 below). Multi-site phosphorylation of a substrate can not only determine the threshold for a switch but can also act to ensure the protein is activated/inactivated with a delay, as is the case for Sld2 (Figure 2A, top) [18]. Several studies also demonstrate that competition between substrates for kinases and phosphatases can lead to the ordering of substrate phosphorylation/dephosphorylation [11,19,20]. In *S. pombe*, the earliest S phase substrates are preferentially phosphorylated due to their abundance and cyclin-binding motifs, while increasing CDK activity in interphase orders subsequent substrate phosphorylation [11]. In budding yeast, both PP2A^{B55} phosphatase's affinity for phospho-threonines in interphase [20], and the substrate's susceptibility to Cdc14 phosphatase in mitosis [19], are determinants of quantitative ordering. Therefore, differential substrate affinities for both kinases and phosphatases can play a crucial role in determining dephosphorylation and phosphorylation timings (Figure 2C).

Protein phosphorylation is not only ordered in time, but also in space. Spatial variations in phosphatase and kinase activity can produce a specifically localised signal for example the phosphorylation of KNL1 at the kinetochore [21] or create a phosphorylation gradient, for example the localisation of the PP1 phosphatase generates a Pom1 kinase gradient in *S. pombe* length-sensing [22], or even create a phosphorylation wave, for example the propagation of CDK1 activity by Wee1/Cdc25 in *Xenopus* eggs [23] and *Drosophila* embryos [24], as reviewed in [12].

Ensuring a transient response

While phosphorylation is often considered as switch, turning a protein on or off, dynamic phosphorylation turnover can also be inherently functionally important. For example, the dynamic Aurora B phosphorylation and PP2A^{B56} dephosphorylation of the kinetochore component NDC80 in mitosis promotes the association and dissociation of microtubule attachments, allowing for the selection of attachments that generate tension and the release

of those which do not [25–27]. A substrate can be dynamically phosphorylated by a delayed negative feedback mechanism, such as kinase-dependent phosphatase recruitment (Figure 2B, bottom), as has been shown for the PP2A^{B56} phosphatase [28].

In this review, we will investigate how DNA replication initiation is controlled and identify examples where phosphatases may be important to ensure the fidelity and regulation of this process.

2. **Once per cell cycle replication control and phosphatases**

Four sequential regulatory steps are required to achieve once-per-cell cycle DNA replication initiation [29]:

- (i) **Replication origin licensing:** a temporal period (late M/G1 phase) when the replicative helicase Mcm2-7 is loaded at origins in an inactive state
- (ii) **Licensing inactivation:** Further Mcm2-7 loading must be stopped before origin activation is permitted.
- (iii) **Replication origin activation:** Previously inactive ‘firing factors’ are activated in S-phase to trigger replication initiation at origins
- (iv) **Initiation inhibition:** Origin firing causes the loss of licensing and re-firing potential.

Protein kinases are critical to regulate these events, but how these processes are ordered and separated requires temporal control of substrate phosphorylation and dephosphorylation.

Kinase-regulation of origin licensing and firing

The licensing reaction requires the origin recognition complex (ORC), together with Cdc6 and Cdt1 to load Mcm2-7 complexes as head-to-head double hexamers around double-stranded (ds) DNA at origins [30,31]. The licensing reaction is restricted to late mitosis/G1 phase by inhibitory CDK phosphorylation. In yeast for example, S-phase CDK

phosphorylates Mcm3, resulting in Mcm2-7 nuclear export [32] , and this kinase also phosphorylates and inhibits ORC [33]. As early as late G1 phase and throughout S/G2 phase, Cdc6 is targeted for degradation by CDK-phosphorylation of a phospho-degron in Cdc6 [34–36]. This rapid and early targeting of multiple factors involved in licensing is critical to ensure licensing is switched off before initiation can begin.

To initiate DNA unwinding and DNA synthesis at a replication origin, the Mcm2-7 double hexamer must be converted into active CMG (Cdc45/Mcm2-7/GINS) helicases and additional factors must be recruited, such as DNA polymerases [5] (see Movie 1). This step is triggered in S-phase by cell cycle kinases: S-CDK and DDK whose activity is higher in S than G1 phase [37]. The essential role of DDK in yeast is the direct phosphorylation of loaded Mcm4 and Mcm6 [38,39] which allows the recruitment of the firing factors Sld3/Sld7 (Treslin/MTBP in humans) and Cdc45 to origins [40]. This DDK phosphorylation has recently been found to be functionally redundant with CDK1-dependent Mcm2-7 phosphorylation in mammals [41]. In yeast, CDK critically phosphorylates both Sld3 and another factor Sld2 (RecQL4 in humans), which allows the binding of the tandem BRCT repeat protein Dpb11/TopBP1 [42]. Distinct BRCT-repeats of Dpb11 bind CDK-phosphorylated Sld2 and Sld3, forming a transient intermediate called the pre-initiation complex (pre-IC) that recruits Pol ϵ (the leading strand polymerase) and the CMG component GINS to origins [43]. The CDK phosphorylation of Sld3/Treslin has been shown to be conserved and important for origin firing in several metazoa [44–46] and CDK phosphorylation of Sld2/RecQL4 has also been shown to be critical in *C. elegans* [47].

After pre-IC formation, and Mcm10 recruitment [48,49], several initiation factors including Sld3/Sld7, Dpb11 and Sld2 are released from origins and initiation occurs [50]. It is not clear how important this release step is for origin firing to occur, although reducing the affinity of Sld3 for Cdc45 can promote replication [51]. It is possible that phosphatase-assisted dissociation of the pre-IC may enable replisome formation, and dissolve unproductive pre-IC-like complexes such as those that form on Mcm2-7 tails [52].

Phosphatase-dependent mechanisms to prevent re-replication

Bistable cell cycle regulation by kinase and phosphatase activities is crucial to create distinct temporal windows for origin licensing and firing (Figure 3). It is also clear that to transition between periods of origin licensing and origin firing, the order of phosphorylation of key replication factors is critical. Licensing factors must be phosphorylated and inhibited before initiation factors are activated in S phase, and conversely, during mitotic exit, initiation factors must be deactivated and dephosphorylated before licensing factors. Any overlap between licensing and initiation could potentially lead to lethal re-replication.

Resetting the replication reaction during mitosis

To inactivate the kinases responsible for replication initiation from mitotic exit until G1/S phase, the cyclin subunits of CDK and the Dbf4 subunit of DDK are targeted for degradation by the APC/C [53–55]. Despite this, removal of the kinases to create a period of low DDK/CDK activity would not prevent replication initiation if their substrates remained phosphorylated during this period. Thus, phosphatases work together with ubiquitin-mediated degradation to ensure the resetting of CDK/DDK substrates during mitosis.

Several phosphatases directly oppose CDK phosphorylation during mitotic exit: PP2A^{B55}, Cdc14 (in budding yeast) and PP1 (in metazoa) [10,56,57]. Whereas Cdc14 is a single-subunit phosphatase with dual-specificity for Tyr and Ser/Thr phosphorylation [58], PP2A and PP1 are Ser/Thr phosphatases each with a diversity of regulatory subunits that greatly determine phosphatase specificity [59,60]. There are 15 PP2A regulatory subunit genes in human cells, while in dividing budding yeast there is only one B55 and one B56-type regulatory subunit expressed, Cdc55 and Rts1 respectively [59]. In yeast, both Cdc14 and PP2A^{B55} are tightly regulated in mitosis. Cdc14 is strictly sequestered and inhibited within the nucleolus prior to anaphase [10]. Cdc14 is released from inhibition in the nucleolus by the 'Cdc14 early anaphase release' (FEAR) network, and its activity maintained by the Mitotic Exit Network (MEN) until G1 phase [10]. Cdc14 appears to be selective for phospho-serine

CDK sites [61,62] in contrast to the PP2A^{B55} selectivity for phospho-threonines [20,63].

PP2A^{B55} is active in interphase but then largely inhibited in mitosis via the activity of Greatwall kinase [64,65] (Figure 1B and 3). PP2A^{B55} activity returns upon mitotic exit as CDK activity dissipates and PP1 counteracts Greatwall activity [66]. Other phosphatases, such as PP1 and PP2A^{B56}, appear to be active during mitosis [12] but their direct contributions to CDK/DDK site dephosphorylation is unclear.

Dephosphorylation during mitotic exit occurs in an ordered manner and substrate affinity for Cdc14 is a key determinant of this ordering in yeast [19]. As expected, firing factors are found to be inactivated prior to licensing factors [16]. For example, Sld2 in yeast is targeted by the sequential action of four kinases early in mitosis leading Sld2 to be targeted for degradation by Dma1/2 [16]. Conversely, phosphorylation that results in the degradation of the licensing factor Cdc6 by SCF^{CDC4} [34–36] is reversed by the sequential action of PP2A^{B55} and Cdc14 phosphatases in yeast [67,68]. Orc6 dephosphorylation is also a result of both Cdc14 and PP2A^{B55} action in yeast [69] and occurs strikingly late in mitotic exit [19], consistent with delayed re-activation of licensing factors. Finally, in humans, PP1 dephosphorylates Orc2 on its CDK sites at mitotic exit to allow ORC binding to chromatin in G1 phase [70–72]. Therefore, the binding and affinity of phosphatases towards licensing and firing factors is important for ordering dephosphorylation.

Whether the dephosphorylation of DDK-modified Mcm2-7 complexes occurs prior to the next cell cycle, is unclear. CMG complexes that have completed replication are ubiquitinated on the Mcm7 subunit by SCF^{Dia2} in yeast and CUL-2^{Lrr-1} and TRAP1/UBXN-3 ligases in metazoa [73]. Although this Mcm7 ubiquitylation does not appear to result in degradation, it does result in Cdc48 segregase dependent removal of the CMG from chromatin and CMG dissolution. In budding yeast, newly synthesised Mcm2-7 complexes are preferentially imported to the nucleus for licensing in G1 phase [74]. In human cells, although licensing of both new and parental Mcm2-7 occurs, parental Mcm2-7 form CMGs with higher efficiency [75] potentially indicating the presence of a replication-promoting modification from the

previous cell cycle. This may suggest that it is CDK substrate regulation that plays a predominant role in re-replication control.

Ordering replication protein phosphorylation in the G1/S phase transition

Cyclin specificity contributes to the ordering of licensing factor inactivation prior to firing factor activation at the onset of S phase. In budding yeast, licensing factors are targeted and inactivated by G1-CDK [36,76,77], while firing factors are only targeted and activated by S-CDK [16,78–80]. Furthermore, both Sld2 and Sld3 are predicted to have 10 CDK sites in addition to those directly essential for replication [18,78]. In the case of Sld2, it appears that phosphorylation of at least some of the other 10 CDK sites is required for T84 phosphorylation [18], perhaps safeguarding against premature activation [81]. The contribution of phosphatases to the ordering of replication factor phosphorylation in this transition is largely unexplored. However, in human cells, Rif1-PP1 suppresses Orc1 phosphorylation in G1 phase, which prevents Orc1 degradation and the inhibition of licensing [82].

3. The role of protein phosphatases in modulating replication in time and space

In most eukaryotic cells, the multitude of origins do not all fire at the beginning of S-phase. Although many origins are licensed in G1 phase, only a fraction of these (10% in mammalian cells) will be activated during a normal S-phase [83], with the rest remaining dormant, serving as back-up origins if local forks collapse due to DNA damage [84]. Origin activation (for those that fire) occurs throughout S-phase, resulting in differences in the temporal order of genome duplication, known as the replication timing (RT) programme. Importantly this RT programme is evolutionarily conserved between related species [85], is consistent within particular cell types [86] and changes dramatically during development [87] and differentiation [88]. This RT program also has a spatial component: origins of similar firing times seem to cluster into replication foci within the nucleus during S phase [89–92].

From yeast to metazoa, the signal for early or late replication initiation of an origin is established in G1 phase during licensing [93,94]. Several studies suggest that the establishment of RT is dependent on the regulation of the chromatin context of origins [95–98] and early origins differentially bind to ORC [99,100] and load more Mcm2-7 [101]. A critical determinant of origin timing is the ability of these licensed origins to compete for a limiting pool of initiation factors [102,103]. The activatory subunit of DDK, Dbf4, and the pre-IC initiation factors (Sld2, Sld3 and Dpb11) are stoichiometrically limiting for initiation in budding yeast and over expression of these factors, together with the Sld3 partner proteins, Sld7 and Cdc45, is sufficient to allow the early firing of late and dormant origins in yeast [102]. Importantly the vertebrate orthologues of these factors are also rate-limiting for replication initiation during early embryonic divisions [104].

An interesting feature of these limiting initiation factors is that apart from Cdc45, they are not incorporated into replisomes and are released from origins during the initiation reaction (see section 2). The local recycling of these limiting factors during initiation may therefore influence which origins fire next. Indeed, there is some evidence that origins fire sequentially in what's called the 'domino model' depending on local 3D chromatin structure [105], although the evidence for this model differs between studies [106–108].

Given the limiting nature of DDK (Dbf4) and CDK substrates (Sld2 and Sld3), the regulation of DDK and CDK activity or localisation can play an important role in the replication timing programme. Direct recruitment of Dbf4 to kinetochores and by the forkhead box transcription factors Fkh1/Fkh2 in yeast can drive the early firing of subsets of origins [109,110] while increased activation or inhibition of CDK alters replication initiation efficiency and RT progression [111,112].

Phosphatases are also important regulators of DDK and CDK activity and therefore can play a role in determining the RT programme. Clinically important Wee1 inhibitors, which promote Cdc25-dependent dephosphorylation and activation of CDK (Figure 1B), speed up S-phase

by increasing origin firing [113]. The chromatin binding protein Rif1 was also shown to regulate replication timing from yeast to mice/human cells [114–116]. A conserved feature of Rif1 is that it binds to the PP1 phosphatase [117] and initially in studies in yeast [118–120] and *Xenopus* extracts [121] it was shown that PP1 directly counteracts the DDK-dependent phosphorylation of Mcm2-7 at licensed origins. This Rif1-dependent recruitment of PP1 to chromatin delays the firing of subsets of origins [118,119]. DDK activity, which increases during S-phase, can disrupt the Rif1-PP1 interaction [118,119] and causes a feedback loop which allows for further origin firing as S-phase progresses (Figure 4). Rif1 also has roles in chromatin and nuclear organisation, which likely have additional effects on RT, independently of the regulation of licensed origins [117]. In yeast, Rif1 mutants also exhibit hyper-DDK phosphorylation of Sld3, although the physiological role of this regulation is not known [120]. In *Drosophila*, Rif1 is required to extend S-phase during the early embryonic divisions and the chromatin association of Rif1 is inhibited by Cdk1 [122]. In human cells the Rif1-PP1 interaction is counteracted by CDK [123], which together with the *Drosophila* studies provide direct linkage between the control of replication timing by CDK and DDK (through Rif1) (Figure 4).

Given the striking influence of PP1 for determining the order of origin firing by locally opposing DDK phosphorylation, could there be a function for a phosphatase that opposes the S-CDK phosphorylation during interphase? Knock-down of the PP2A inhibitor, ENSA, in human cell lines drives the degradation of unphosphorylated Treslin, slowing S phase [124]. Conversely, in *X. laevis* egg extracts PP2A has been implicated as a positive regulator of DNA replication initiation [125], acting after origin licensing to aid Cdc45 recruitment and promote origin firing [126]. The substrate for PP2A in replication initiation has not been identified, but it is possible that PP2A counteracts Chk1, a checkpoint effector kinase that can phosphorylate Treslin in an unperturbed cell cycle [127] (see section 4). Furthermore, DUE-B, a metazoan-specific Cdc45-recruitment factor [128] also requires PP2A activity to bind to Mcm2-7 [129]. Therefore, while Rif1-PP1 delays replication initiation at licensed

origins, PP2A may have positive and negative roles as a regulator of replication initiation through as yet unidentified targets. The activity of PP2A is influenced by nitrogen, carbon [130,131], methionine [132] and sphingolipid signalling [133,134], and responds to membrane growth and cell size in yeast [135,136]. Roles for PP2A in replication control might provide new links between metabolic pathways and genome duplication.

4. Checkpoint inhibition of origin firing

In the presence of DNA damage or fork stalling agents, checkpoint activation leads to inhibition of replication initiation [137]. In budding yeast, the effector checkpoint kinase Rad53 (analogous to Chk1 in humans) inhibits origin firing by targeting both kinase pathways (CDK and DDK) that activate origin firing [138,139]. Rad53 seems to inhibit DDK function by sterically blocking Dbf4 binding to Mcm2-7 in a phosphorylation-independent manner [140]. However, phosphorylation of the key CDK substrate, Sld3, by Rad53 prevents its interactions with other replication factors including Mcm2-7, Cdc45 and Dpb11, to prevent origin firing [40,138,139]. There are significant similarities in the mechanism of checkpoint inhibition of origin firing control in metazoa, including the checkpoint inhibition of the Sld3 orthologue Treslin [127,141] and inhibition of DDK [142,143]. By inhibiting origin firing in S-phase the checkpoint delays S-phase progression, preventing the accumulation of topological stress [144]. Interestingly, Dbf4/Sld3 are also inhibited by Rad53 outside of S-phase and this checkpoint control also prevents re-replication in G2 and precocious replication in G1 phase [145].

ATR-Chk1 and phosphatase-mediated inhibition of origin firing

Phosphatases play a role in determining the outcome of the DNA damage response towards replication control. The human checkpoint kinases ATR-Chk1 not only directly target replication factors, such as Treslin, but also regulate CDK activity through the activation of Wee1 and inactivation of the phosphatase Cdc25 [146]. As described above, changes in CDK activity in S-phase affect origin firing rates. This pathway explains how inhibitors of the

checkpoint kinases ATR-Chk1 and Wee1 stimulate origin firing, not only through increasing CDK activation, which regulates initiation directly, but also through CDK-dependent disruption of the Rif1-PP1 interaction [123] (Figure 4).

An additional link between ATR-Chk1 and phosphatase regulation of CDK activity was recently described in human cells [147]. Phosphorylation of Wee1, by CDK and Plk1, leads to Wee1 degradation, increasing CDK activity [148]. This Wee1 phosphorylation is counteracted by PP2A-B55 α [147]. In a CRISPR screen for genes that modulate the lethality of Chk1 inhibitors, loss of function of the PP2A-B55 α inhibitor FAM122A suppressed the effect of Chk1 inhibitors and FAM122A was itself inactivated by Chk1. As a result, Chk1 activation increases PP2A-B55 α activity (via inhibition of FAM122A), which in turn decreases CDK activity (in part by stabilising Wee1) [147] (Figure 4). Therefore, Chk1 inhibits CDK activity and regulates origin firing by multiple mechanisms including via the Cdc25 and PP2A phosphatases.

Phosphatases permit replication resumption after checkpoint activation

The checkpoint kinases inhibit replication and cell cycle progression to allow time to repair damage and remove DNA topological linkages [137,144]. The resumption of the cell cycle and DNA replication after repair has occurred must involve the inactivation of the checkpoint signalling cascade. In yeast, deactivation of the checkpoint-effector kinase Rad53 after DNA damage in S-phase requires the PP4 phosphatase Pph3 and the PP2C family phosphatases Ptc2/Ptc3 [149–151]. Failure to dephosphorylate and inactivate Rad53 prevents the restart of replication fork progression after DNA damage, inhibiting the completion of genome duplication [149]. The mechanism for this regulation of replisome progression and its reversal by phosphatases is not understood in mechanistic detail. PP2A phosphatases have also been implicated in the deactivation of Rad53 and the DNA damage response (DDR), which links DDR to metabolic pathways that regulate PP2A activity [152], but whether this regulation of the checkpoint kinases by PP2A is direct is not clear.

How ATR-Chk1 are deactivated after replication stress in human cells and how this deactivation facilitates the completion of genome duplication is poorly understood. The PP2C phosphatase PPM1D (also known as Wip1 - wild-type p53-induced phosphatase 1) has been shown to dephosphorylate Chk1 and p53 after DNA damage [153]. Inhibition of PPM1D reduces DNA synthesis but whether this is due to a defect in S-phase entry or a direct effect of hyperactive Chk1 on DNA replication control is not known [153]. As PPM1D/Wip1 not only negatively regulates the DNA damage response but is also involved in the dephosphorylation of several tumour suppressors including p53, this phosphatase acts as an oncogene and is over-expressed in several tumour types [154].

Conclusion.

Phosphatases ensure that kinase responses are robust, dynamic, appropriately timed and localised, which are critical features of DNA replication control. Although not fully understood, phosphatases have been shown to regulate multiple aspects of replication initiation. Many kinases involved in DNA replication control, such as ATR-Chk1 are important targets for the generation of new chemotherapies [155]. Phosphatases that counteract these kinase responses are emerging as important resistance genes and synthetic lethality targets, modulating the effectiveness of ATR/Chk1 kinase inhibitors in cancers [156,157]. Understanding how phosphatases regulate DNA replication will facilitate the discovery of new ways to exploit kinase inhibitors as a therapeutic strategy.

Conflict of interest statement.

The authors declare there are no conflicts of interest.

Acknowledgements.

We thank members of the Zegerman lab for critical reading of the manuscript. Work in the PZ lab was supported by AICR 10-0908, Wellcome Trust 107056/Z/15/Z and Cancer Research UK C15873/A12700. FJ was funded by AstraZeneca studentship CR-000608.

Figure legends

Figure 1 – The regulatory relationships between kinases, phosphatases and their substrates modulate phospho-signalling

A) The nature of the crosstalk between enzymes and substrates (top panel) generates different output responses to a range of input values (lower panel). In this instance, 'input' corresponds to an increase in kinase activity (or reduction in phosphatase activity) stimulated by factors outside the depicted network, and 'output' is the resulting proportion of phosphorylated substrate.

B) Examples of substrate feedback loops within the G2-M (upper panel) and G1-S (lower panel) transitions, which create bistable responses to CDK phosphorylation.

Figure 2 – Properties of the substrate can modulate phosphorylation dynamics.

A) Multisite phosphorylation can transform graded phosphorylation into a switch-like response (green arrow) or quantitatively modulate functionality as a rheostat (red arrow)

B) Reciprocal regulation of kinase/phosphatase binding sites on a substrate can promote bistability (green arrow) or disrupt it to promote rapid modification turnover (red arrow).

C) Binding site affinity for kinases and phosphatases contributes to (de)phosphorylation ordering in competition with other substrates.

Figure 3 – Regulation of DNA replication initiation in the cell cycle.

Dynamic changes in CDK, DDK and opposing phosphatase activities during the cell cycle in budding yeast create windows permissive to origin firing (black) or licensing (white).

Phosphorylation ordering of licensing and firing factors prevents these events from

occurring simultaneously at transitions (pink regions). For example at the G1-S transition G1-CDK inhibits licensing before S-CDK activates origin firing.

Figure 4 – Direct and indirect mechanisms of replication initiation control by kinases and phosphatases. See text for details.

Movie legend

Movie 1 - Replication initiation in *S. cerevisiae*

Replication origins, recognised by ORC, are licensed in G1 phase, by the Cdt1/Cdc6-facilitated loading of a double hexamer of Mcm2-7 proteins. Towards the end of G1, rising DDK can phosphorylate the double hexamer allowing the recruitment of Sld3 and Cdc45 at early firing origins. At some late firing origins, localised Rif1-PP1 phosphatase opposes initial DDK phosphorylation on MCMs which can only be overcome when DDK levels rise in late S phase. Upon S phase onset, S-CDK activity both inactivates licensing, and triggers firing by phosphorylating Sld3 and Sld2. Phosphorylated Sld2/Sld3 bind Dpb11 and recruit GINS and Polε to origins to form the transient Pre-Initiation Complex (Pre-IC). With the additional recruitment of Mcm10, the CMG helicase becomes activated and origin firing occurs. Sld2, Sld3 and Dpb11 are released from origins upon initiation and since they are limiting, they must be recycled to fire late origins: a step which remains CDK-dependent.

Bibliography

- [1] S.A. Hills, J.F.X. Diffley, DNA replication and oncogene-induced replicative stress, *Current Biology*. 24 (2014). <https://doi.org/10.1016/j.cub.2014.04.012>.
- [2] J. Bartkova, Z. Hořejší, K. Koed, A. Krämer, F. Tort, K. Zieger, M. Sehested, J.M. Nesland, C. Lukas, T. Ørntoft, J. Lukas, J. Bartek, DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis, 2005. www.nature.com/nature.

- [3] V.G. Gorgoulis, L.V.F. Vassiliou, P. Karakaidos, P. Zacharatos, A. Kotsinas, T. Liloglou, M. Venere, R.A. DiTullio, N.G. Kastrinakis, B. Levy, D. Kletsas, A. Yoneta, M. Herlyn, C. Kittas, T.D. Halazonetis, Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions, *Nature*. 434 (2005) 907–913. <https://doi.org/10.1038/NATURE03485>.
- [4] P. Kotsantis, E. Petermann, S.J. Boulton, Mechanisms of Oncogene-Induced Replication Stress: Jigsaw Falling into Place, *Cancer Discov.* 8 (2018) 537–555. <https://doi.org/10.1158/2159-8290.CD-17-1461>.
- [5] K. Siddiqui, K.F. On, J.F.X. Diffley, Regulating DNA replication in Eukarya, *Cold Spring Harbor Perspectives in Biology*. 5 (2013). <https://doi.org/10.1101/cshperspect.a012930>.
- [6] J. Bartek, C. Lukas, J. Lukas, Checking on DNA damage in S phase, *Nature Reviews Molecular Cell Biology*. 5 (2004) 792–804. <https://doi.org/10.1038/nrm1493>.
- [7] M.J. O'Connor, Targeting the DNA Damage Response in Cancer, *Molecular Cell*. 60 (2015) 547–560. <https://doi.org/10.1016/j.molcel.2015.10.040>.
- [8] R. Swords, D. Mahalingam, M. O'Dwyer, C. Santocanale, K. Kelly, J. Carew, F. Giles, Cdc7 kinase - A new target for drug development, *European Journal of Cancer*. 46 (2010) 33–40. <https://doi.org/10.1016/j.ejca.2009.09.020>.
- [9] U. Asghar, A.K. Witkiewicz, N.C. Turner, E.S. Knudsen, The history and future of targeting cyclin-dependent kinases in cancer therapy, *Nature Reviews Drug Discovery*. 14 (2015) 130–146. <https://doi.org/10.1038/nrd4504>.
- [10] C. Wurzenberger, D.W. Gerlich, Phosphatases: providing safe passage through mitotic exit, *Nature Reviews Molecular Cell Biology* 2011 12:8. 12 (2011) 469–482. <https://doi.org/10.1038/nrm3149>.
- [11] M.P. Swaffer, A.W. Jones, H.R. Flynn, A.P. Snijders, P. Nurse, CDK Substrate Phosphorylation and Ordering the Cell Cycle, *Cell*. 167 (2016) 1750–1761.e16. <https://doi.org/10.1016/j.cell.2016.11.034>.
- [12] L. Gelens, J. Qian, M. Bollen, A.T. Saurin, The Importance of Kinase–Phosphatase Integration: Lessons from Mitosis, *Trends in Cell Biology*. 28 (2018) 6–21. <https://doi.org/10.1016/j.tcb.2017.09.005>.
- [13] N. García-Blanco, A. Vázquez-Bolado, S. Moreno, Greatwall-endosulfine: A molecular switch that regulates PP2A/B55 protein phosphatase activity in dividing and quiescent cells, *International Journal of Molecular Sciences*. 20 (2019). <https://doi.org/10.3390/ijms20246228>.
- [14] M. Köivomägi, E. Valk, R. Venta, A. Iofik, M. Lepiku, E.R.M. Balog, S.M. Rubin, D.O. Morgan, M. Loog, Cascades of multisite phosphorylation control Sic1 destruction at the onset of S phase, *Nature*. 480 (2011) 128–131. <https://doi.org/10.1038/nature10560>.
- [15] N.A. Lyons, B.R. Fonslow, J.K. Diedrich, J.R. Yates, D.O. Morgan, Sequential primed kinases create a damage-responsive phosphodegron on Eco1, *Nature Structural and Molecular Biology*. 20 (2013) 194–201. <https://doi.org/10.1038/nsmb.2478>.
- [16] K.U. Reuswig, F. Zimmermann, L. Galanti, B. Pfander, Robust Replication Control Is Generated by Temporal Gaps between Licensing and Firing Phases and Depends on Degradation of Firing Factor Sld2, *Cell Reports*. 17 (2016) 556–569. <https://doi.org/10.1016/j.celrep.2016.09.013>.
- [17] M. Bollen, Combinatorial control of protein phosphatase-1, *Trends Biochem Sci*. 26 (2001) 426–431. [https://doi.org/10.1016/S0968-0004\(01\)01836-9](https://doi.org/10.1016/S0968-0004(01)01836-9).

- [18] Y.S. Tak, Y. Tanaka, S. Endo, Y. Kamimura, H. Araki, A CDK-catalysed regulatory phosphorylation for formation of the DNA replication complex Sld2-Dpb11, *EMBO Journal*. 25 (2006) 1987–1996. <https://doi.org/10.1038/sj.emboj.7601075>.
- [19] C. Bouchoux, F. Uhlmann, A quantitative model for ordered Cdk substrate dephosphorylation during mitotic exit, *Cell*. 147 (2011) 803–814. <https://doi.org/10.1016/j.cell.2011.09.047>.
- [20] M. Godfrey, S.A. Touati, M. Kataria, A. Jones, A.P. Snijders, F. Uhlmann, PP2ACdc55 Phosphatase Imposes Ordered Cell-Cycle Phosphorylation by Opposing Threonine Phosphorylation, *Molecular Cell*. 65 (2017) 393–402.e3. <https://doi.org/10.1016/j.molcel.2016.12.018>.
- [21] P. Ghongane, M. Kapanidou, A. Asghar, S. Elowe, V.M. Bolanos-Garcia, The dynamic protein Knl1 - a kinetochore rendezvous, *Journal of Cell Science*. 127 (2014) 3415–3423. <https://doi.org/10.1242/jcs.149922>.
- [22] O. Hachet, M. Berthelot-Grosjean, K. Kokkoris, V. Vincenzetti, J. Moosbrugger, S.G. Martin, A phosphorylation cycle shapes gradients of the DYRK family kinase pom1 at the plasma membrane, *Cell*. 145 (2011) 1116–1128. <https://doi.org/10.1016/j.cell.2011.05.014>.
- [23] J.B. Chang, J.E. Ferrell, Mitotic trigger waves and the spatial coordination of the *Xenopus* cell cycle, *Nature*. 500 (2013) 603–607. <https://doi.org/10.1038/nature12321>.
- [24] V.E. Deneke, A. Melbinger, M. Vergassola, S. di Talia, Waves of Cdk1 Activity in S Phase Synchronize the Cell Cycle in *Drosophila* Embryos, *Developmental Cell*. 38 (2016) 399–412. <https://doi.org/10.1016/j.devcel.2016.07.023>.
- [25] A. v. Zaytsev, J.E. Mick, E. Maslennikov, B. Nikashin, J.G. de Luca, E.L. Grishchuk, Multisite phosphorylation of the NDC80 complex gradually tunes its microtubule-binding affinity, *Molecular Biology of the Cell*. 26 (2015) 1829–1844. <https://doi.org/10.1091/mbc.E14-11-1539>.
- [26] S.J.E. Suijkerbuijk, M. Vleugel, A. Teixeira, G.J.P.L. Kops, Integration of Kinase and Phosphatase Activities by BUBR1 Ensures Formation of Stable Kinetochore-Microtubule Attachments, *Developmental Cell*. 23 (2012) 745–755. <https://doi.org/10.1016/j.devcel.2012.09.005>.
- [27] J.G. DeLuca, A. Musacchio, Structural organization of the kinetochore-microtubule interface, *Current Opinion in Cell Biology*. 24 (2012) 48–56. <https://doi.org/10.1016/j.ceb.2011.11.003>.
- [28] E.P.T. Hertz, T. Kruse, N.E. Davey, B. López-Méndez, J.O. Sigurðsson, G. Montoya, J. v. Olsen, J. Nilsson, A Conserved Motif Provides Binding Specificity to the PP2A-B56 Phosphatase, *Molecular Cell*. 63 (2016) 686–695. <https://doi.org/10.1016/j.molcel.2016.06.024>.
- [29] J.J. Blow, R.A. Laskey, A role for the nuclear envelope in controlling DNA replication within the cell cycle, *Nature* 332 (1988) 546–548. <https://doi.org/10.1038/332546a0>.
- [30] C. Evrin, P. Clarke, J. Zech, R. Lurz, J. Sun, S. Uhle, H. Li, B. Stillman, C. Speck, A double-hexameric MCM2-7 complex is loaded onto origin DNA during licensing of eukaryotic DNA replication, *Proc Natl Acad Sci U S A*. 106 (2009) 20240–20245. https://doi.org/10.1073/PNAS.0911500106/SUPPL_FILE/0911500106SI.PDF.

- [31] D. Remus, F. Beuron, G. Tolun, J.D. Griffith, E.P. Morris, J.F.X. Diffley, Concerted loading of Mcm2-7 double hexamers around DNA during DNA replication origin licensing, *Cell*. 139 (2009) 719–730. <https://doi.org/10.1016/J.CELL.2009.10.015>.
- [32] V.Q. Nguyen, C. Co, K. Irie, J.J. Li, Clb/Cdc28 kinases promote nuclear export of the replication initiator proteins Mcm2-7, *Current Biology*. 10 (2000) 195–205. [https://doi.org/10.1016/S0960-9822\(00\)00337-7](https://doi.org/10.1016/S0960-9822(00)00337-7).
- [33] S. Chen, S.P. Bell, CDK prevents Mcm2–7 helicase loading by inhibiting Cdt1 interaction with Orc6, *Genes & Development*. 25 (2011) 363–372. <https://doi.org/10.1101/GAD.2011511>.
- [34] G. Perkins, L.S. Drury, J.F.X. Diffley, Separate SCFCDC4 recognition elements target Cdc6 for proteolysis in S phase and mitosis, *EMBO Journal*. 20 (2001) 4836–4845. <https://doi.org/10.1093/emboj/20.17.4836>.
- [35] L.S. Drury, G. Perkins, J.F.X. Diffley, The Cdc4/34/53 pathway targets Cdc6p for proteolysis in budding yeast, *EMBO Journal*. 16 (1997) 5966–5976. <https://doi.org/10.1093/emboj/16.19.5966>.
- [36] L.S. Drury, G. Perkins, J.F.X. Diffley, The cyclin-dependent kinase Cdc28p regulates distinct modes of Cdc6p proteolysis during the budding yeast cell cycle, *Current Biology*. 10 (2000) 231–240. [https://doi.org/10.1016/S0960-9822\(00\)00355-9](https://doi.org/10.1016/S0960-9822(00)00355-9).
- [37] K. Labib, How do Cdc7 and cyclin-dependent kinases trigger the initiation of chromosome replication in eukaryotic cells?, *Genes Dev*. 24 (2010) 1208–1219. <https://doi.org/10.1101/GAD.1933010>.
- [38] L.I. Francis, J.C.W. Randell, T.J. Takara, L. Uchima, S.P. Bell, Incorporation into the prereplicative complex activates the Mcm2-7 helicase for Cdc7-Dbf4 phosphorylation, *Genes Dev*. 23 (2009) 643–654. <https://doi.org/10.1101/GAD.1759609>.
- [39] Y.J. Sheu, B. Stillman, The Dbf4-Cdc7 kinase promotes S phase by alleviating an inhibitory activity in Mcm4, *Nature*. 463 (2010) 113. <https://doi.org/10.1038/NATURE08647>.
- [40] T.D. Deegan, J.T. Yeeles, J.F. Diffley, Phosphopeptide binding by Sld3 links Dbf4-dependent kinase to MCM replicative helicase activation , *The EMBO Journal*. 35 (2016) 961–973. <https://doi.org/10.15252/embj.201593552>.
- [41] J.M. Suski, N. Ratnayeke, M. Braun, T. Zhang, V. Strmiska, W. Michowski, G. Can, A. Simoneau, K. Snioch, M. Cup, C.M. Sullivan, X. Wu, J. Nowacka, T.B. Branigan, L.R. Pack, J.A. DeCaprio, Y. Geng, L. Zou, S.P. Gygi, J.C. Walter, T. Meyer, P. Sicinski, CDC7-independent G1/S transition revealed by targeted protein degradation, *Nature*. (2022). <https://doi.org/10.1038/s41586-022-04698-x>.
- [42] P. Zegerman, Evolutionary conservation of the CDK targets in eukaryotic DNA replication initiation, *Chromosoma*. 124 (2015) 309–321. <https://doi.org/10.1007/s00412-014-0500-y>.
- [43] S. Muramatsu, K. Hirai, Y.S. Tak, Y. Kamimura, H. Araki, CDK-dependent complex formation between replication proteins Dpb11, Sld2, Pol ε, and GINS in budding yeast, *Genes and Development*. 24 (2010) 602–612. <https://doi.org/10.1101/gad.1883410>.
- [44] A. Kumagai, A. Shevchenko, A. Shevchenko, W.G. Dunphy, Direct regulation of Treslin by cyclin-dependent kinase is essential for the onset of DNA replication, *Journal of Cell Biology*. 193 (2011) 995–1007. <https://doi.org/10.1083/jcb.201102003>.
- [45] D. Boos, L. Sanchez-Pulido, M. Rappas, L.H. Pearl, A.W. Oliver, C.P. Ponting, J.F.X. Diffley, Regulation of DNA replication through Sld3-Dpb11 interaction is conserved

- from yeast to humans, *Current Biology*. 21 (2011) 1152–1157.
<https://doi.org/10.1016/j.cub.2011.05.057>.
- [46] V. Gaggioli, M.R. Kieninger, A. Klucnika, R. Butler, P. Zegerman, Identification of the critical replication targets of CDK reveals direct regulation of replication initiation factors by the embryo polarity machinery in *C. elegans*, *PLoS Genetics*. 16 (2020) e1008948. <https://doi.org/10.1371/JOURNAL.PGEN.1008948>.
 - [47] V. Gaggioli, E. Zeiser, D. Rivers, C.R. Bradshaw, J. Ahringer, P. Zegerman, CDK phosphorylation of SLD-2 is required for replication initiation and germline development in *C. elegans*, *Journal of Cell Biology*. 204 (2014) 507–522.
<https://doi.org/10.1083/jcb.201310083>.
 - [48] G. Watase, H. Takisawa, M.T. Kanemaki, Mcm10 plays a role in functioning of the eukaryotic replicative DNA helicase, Cdc45-Mcm-GINS, *Curr Biol*. 22 (2012) 343–349.
<https://doi.org/10.1016/J.CUB.2012.01.023>.
 - [49] F. van Deursen, S. Sengupta, G. de Piccoli, A. Sanchez-Diaz, K. Labib, Mcm10 associates with the loaded DNA helicase at replication origins and defines a novel step in its activation, *EMBO J*. 31 (2012) 2195–2206.
<https://doi.org/10.1038/EMBOJ.2012.69>.
 - [50] M. Miyazawa-Onami, H. Araki, S. Tanaka, Pre-initiation complex assembly functions as a molecular switch that splits the Mcm2-7 double hexamer, *EMBO Rep*. 18 (2017) 1752–1761. <https://doi.org/10.15252/embr.201744206>.
 - [51] T. Tanaka, T. Umemori, S. Endo, S. Muramatsu, M. Kanemaki, Y. Kamimura, C. Obuse, H. Araki, Sld7, an Sld3-associated protein required for efficient chromosomal DNA replication in budding yeast, *EMBO J*. 30 (2011) 2019–2030.
<https://doi.org/10.1038/EMBOJ.2011.115>.
 - [52] L. de Jesús-Kim, L.J. Friedman, M. Lööke, C.K. Ramsoomair, J. Gelles, S.P. Bell, Ddk regulates replication initiation by controlling the multiplicity of cdc45-gins binding to mcm2-7, *Elife*. 10 (2021) 1–83. <https://doi.org/10.7554/eLife.65471>.
 - [53] C.S. Sørensen, C. Lukas, E.R. Kramer, J.-M. Peters, J. Bartek, J. Lukas, A Conserved Cyclin-Binding Domain Determines Functional Interplay between Anaphase-Promoting Complex–Cdh1 and Cyclin A-Cdk2 during Cell Cycle Progression, *Molecular and Cellular Biology*. 21 (2001) 3692–3703. <https://doi.org/10.1128/mcb.21.11.3692-3703.2001>.
 - [54] P. Clute, J. Pines, Temporal and spatial control of cyclin B1 destruction in metaphase, *Nature Cell Biology*. 1 (1999) 82–87. <https://doi.org/10.1038/10049>.
 - [55] M. Godinho Ferreira, C. Santocanale, L.S. Drury, J.F.X. Diffley, Dbf4p, an Essential S Phase-Promoting Factor, Is Targeted for Degradation by the Anaphase-Promoting Complex, *Molecular and Cellular Biology*. 20 (2000) 242–248.
<https://doi.org/10.1128/mcb.20.1.242-248.2000>.
 - [56] S.A. Touati, L. Hofbauer, A.W. Jones, A.P. Snijders, G. Kelly, F. Uhlmann, Cdc14 and PP2A Phosphatases Cooperate to Shape Phosphoproteome Dynamics during Mitotic Exit, *Cell Reports*. 29 (2019) 2105–2119.e4.
<https://doi.org/10.1016/j.celrep.2019.10.041>.
 - [57] B.L. Powers, M.C. Hall, Re-examining the role of Cdc14 phosphatase in reversal of Cdk phosphorylation during mitotic exit, *Journal of Cell Science*. 130 (2017) 2673–2681.
<https://doi.org/10.1242/jcs.201012>.

- [58] A. Mocciaro, E. Schiebel, Cdc14: A highly conserved family of phosphatases with non-conserved functions?, *Journal of Cell Science*. 123 (2010) 2867–2876.
<https://doi.org/10.1242/jcs.074815>.
- [59] D.M. Virshup, S. Shenolikar, From Promiscuity to Precision: Protein Phosphatases Get a Makeover, *Molecular Cell*. 33 (2009) 537–545.
<https://doi.org/10.1016/j.molcel.2009.02.015>.
- [60] A. Bertolotti, The split protein phosphatase system, *Biochemical Journal*. 475 (2018) 3707–3723. <https://doi.org/10.1042/BCJ20170726>.
- [61] C.H. Gray, V.M. Good, N.K. Tonks, D. Barford, The structure of the cell cycle protein Cdc14 reveals a proline-directed protein phosphatase, *EMBO Journal*. 22 (2003) 3524–3535. <https://doi.org/10.1093/emboj/cdg348>.
- [62] B.K. Kaiser, Z.A. Zimmerman, H. Charbonneau, P.K. Jackson, Disruption of centrosome structure, chromosome segregation, and cytokinesis by misexpression of human Cdc14A phosphatase, *Molecular Biology of the Cell*. 13 (2002) 2289–2300.
<https://doi.org/10.1091/mbc.01-11-0535>.
- [63] M.J. Cundell, L.H. Hutter, R.N. Bastos, E. Poser, J. Holder, S. Mohammed, B. Novak, F.A. Barr, A PP2A-B55 recognition signal controls substrate dephosphorylation kinetics during mitotic exit, *Journal of Cell Biology*. 214 (2016) 539–554.
<https://doi.org/10.1083/jcb.201606033>.
- [64] S. Mochida, S.L. Maslen, M. Skehel, T. Hunt, Greatwall phosphorylates an inhibitor of protein phosphatase 2A that is essential for mitosis, *Science* (1979). 330 (2010) 1670–1673. <https://doi.org/10.1126/science.1195689>.
- [65] A. Gharbi-Ayachi, J.C. Labbé, A. Burgess, S. Vigneron, J.M. Strub, E. Brioudes, A. Van-Dorsselaer, A. Castro, T. Lorca, The substrate of Greatwall kinase, Arpp19, controls mitosis by inhibiting protein phosphatase 2A, *Science* (1979). 330 (2010) 1673–1677.
<https://doi.org/10.1126/science.1197048>.
- [66] A. Heim, A. Konietzny, T.U. Mayer, Protein phosphatase 1 is essential for Greatwall inactivation at mitotic exit, *EMBO Rep*. 16 (2015) 1501–1510.
<https://doi.org/10.15252/embr.201540876>.
- [67] Y. Zhai, P.Y.K. Yung, L. Huo, C. Liang, Cdc14p resets the competency of replication licensing by dephosphorylating multiple initiation proteins during mitotic exit in budding yeast, *Journal of Cell Science*. 123 (2010) 3933–3943.
<https://doi.org/10.1242/jcs.075366>.
- [68] J. Philip, M. Örd, A. Silva, S. Singh, J.F. Diffley, D. Remus, M. Loog, A.E. Ikui, Cdc6 is sequentially regulated by PP2A-Cdc55, Cdc14, and Sic1 for origin licensing in *S. cerevisiae*, *Elife*. 11 (2022). <https://doi.org/10.7554/ELIFE.74437>.
- [69] S.A. Touati, M. Kataria, A.W. Jones, A.P. Snijders, F. Uhlmann, Phosphoproteome dynamics during mitotic exit in budding yeast, *The EMBO Journal*. 37 (2018).
<https://doi.org/10.15252/emboj.201798745>.
- [70] K.Y. Lee, J.S. Bae, G.S. Kim, D.S. Hwang, Protein phosphatase 1 dephosphorylates Orc2, *Biochemical and Biophysical Research Communications*. 447 (2014) 437–440.
<https://doi.org/10.1016/j.bbrc.2014.04.029>.
- [71] K.Y. Lee, J.S. Bae, S. Yoon, D.S. Hwang, Dephosphorylation of Orc2 by protein phosphatase 1 promotes the binding of the origin recognition complex to chromatin, *Biochemical and Biophysical Research Communications*. 448 (2014) 385–389.
<https://doi.org/10.1016/j.bbrc.2014.04.109>.

- [72] K.Y. Lee, S.W. Bang, S.W. Yoon, S.H. Lee, J.B. Yoon, D.S. Hwang, Phosphorylation of ORC2 protein dissociates origin recognition complex from chromatin and replication origins, *Journal of Biological Chemistry*. 287 (2012) 11891–11898. <https://doi.org/10.1074/jbc.M111.338467>.
- [73] Y. Xia, The Fate of Two Unstoppable Trains After Arriving Destination: Replisome Disassembly During DNA Replication Termination, *Frontiers in Cell and Developmental Biology*. 9 (2021) 1772. <https://doi.org/10.3389/fcell.2021.658003>.
- [74] K.A. Braun, L.L. Breeden, Nascent transcription of MCM2-7 is important for nuclear localization of the minichromosome maintenance complex in G1, *Molecular Biology of the Cell*. 18 (2007) 1447–1456. <https://doi.org/10.1091/mbc.E06-09-0792>.
- [75] H. Sedlackova, M.B. Rask, R. Gupta, C. Choudhary, K. Somyajit, J. Lukas, Equilibrium between nascent and parental MCM proteins protects replicating genomes, *Nature*. 587 (2020) 297–302. <https://doi.org/10.1038/s41586-020-2842-3>.
- [76] S. Elsasser, Y. Chi, P. Yang, J.L. Campbell, Phosphorylation controls timing of Cdc6p destruction: A biochemical analysis, *Molecular Biology of the Cell*. 10 (1999) 3263–3277. <https://doi.org/10.1091/mbc.10.10.3263>.
- [77] K. Labib, J.F.X. Diffley, S.E. Kearsey, G1-phase and B-type cyclins exclude the DNA-replication factor Mcm4 from the nucleus, *Nature Cell Biology*. 1 (1999) 415–422. <https://doi.org/10.1038/15649>.
- [78] P. Zegerman, J.F.X. Diffley, Phosphorylation of Sld2 and Sld3 by cyclin-dependent kinases promotes DNA replication in budding yeast, *Nature*. 445 (2007) 281–285. <https://doi.org/10.1038/nature05432>.
- [79] S. Tanaka, T. Umemori, K. Hirai, S. Muramatsu, Y. Kamimura, H. Araki, CDK-dependent phosphorylation of Sld2 and Sld3 initiates DNA replication in budding yeast, *Nature*. 445 (2007) 328–332. <https://doi.org/10.1038/nature05465>.
- [80] H. Masumoto, S. Muramatsu, Y. Kamimura, H. Araki, S-Cdk-dependent phosphorylation of Sld2 essential for chromosomal DNA replication in budding yeast Cyclin-dependent protein kinases (Cdks) in eukaryotic cells work as a key enzyme at various points in the cell cycle 1,2. At the onset of S phase, active S-phase Cdks (S-Cdks) are essential for chromosomal DNA replication, 2002. www.nature.com.
- [81] A. Brümmer, C. Salazar, V. Zinzalla, L. Alberghina, T. Höfer, Mathematical modelling of DNA replication reveals a trade-off between coherence of origin activation and robustness against rereplication, *PLoS Computational Biology*. 6 (2010) 1–13. <https://doi.org/10.1371/journal.pcbi.1000783>.
- [82] S. Hiraga, T. Ly, J. Garzón, Z. Hořejší, Y. Ohkubo, A. Endo, C. Obuse, S.J. Boulton, A.I. Lamond, A.D. Donaldson, Human <sc>RIF</sc> 1 and protein phosphatase 1 stimulate <sc>DNA</sc> replication origin licensing but suppress origin activation, *EMBO Rep*. 18 (2017) 403–419. <https://doi.org/10.15252/embr.201641983>.
- [83] J.C. Rivera-Mulia, D.M. Gilbert, Replicating Large Genomes: Divide and Conquer, *Molecular Cell*. 62 (2016) 756–765. <https://doi.org/10.1016/j.molcel.2016.05.007>.
- [84] R.C. Alver, G.S. Chadha, J.J. Blow, The contribution of dormant origins to genome stability: from cell biology to human genetics, *DNA Repair (Amst)*. 19 (2014) 182–189. <https://doi.org/10.1016/j.dnarep.2014.03.012>.
- [85] C.A. Müller, C.A. Nieduszynski, Conservation of replication timing reveals global and local regulation of replication origin activity, *Genome Research*. 22 (2012) 1953–1962. <https://doi.org/10.1101/gr.139477.112>.

- [86] T. Ryba, I. Hiratani, J. Lu, M. Itoh, M. Kulik, J. Zhang, T.C. Schulz, A.J. Robins, S. Dalton, D.M. Gilbert, Evolutionarily conserved replication timing profiles predict long-range chromatin interactions and distinguish closely related cell types, *Genome Research*. 20 (2010) 761–770. <https://doi.org/10.1101/gr.099655.109>.
- [87] C. Kermi, E. Io Furno, D. Maiorano, Regulation of DNA Replication in Early Embryonic Cleavages, *Genes (Basel)*. 8 (2017) 42. <https://doi.org/10.3390/genes8010042>.
- [88] J.C. Rivera-Mulia, Q. Buckley, T. Sasaki, J. Zimmerman, R.A. Didier, K. Nazor, J.F. Loring, Z. Lian, S. Weissman, A.J. Robins, T.C. Schulz, L. Menendez, M.J. Kulik, S. Dalton, H. Gabr, T. Kahveci, D.M. Gilbert, Dynamic changes in replication timing and gene expression during lineage specification of human pluripotent stem cells, *Genome Research*. 25 (2015) 1091–1103. <https://doi.org/10.1101/gr.187989.114>.
- [89] D.A. Jackson, A. Pombo, Replicon clusters are stable units of chromosome structure: Evidence that nuclear organization contributes to the efficient activation and propagation of S phase in human cells, *Journal of Cell Biology*. 140 (1998) 1285–1295. <https://doi.org/10.1083/jcb.140.6.1285>.
- [90] R. Berezney, D.D. Dubey, J.A. Huberman, Heterogeneity of eukaryotic replicons, replicon clusters, and replication foci, *Chromosoma*. 108 (2000) 471–484. <https://doi.org/10.1007/s004120050399>.
- [91] I. Frouin, A. Montecucco, S. Spadari, G. Maga, DNA replication: A complex matter, *EMBO Reports*. 4 (2003) 666–670. <https://doi.org/10.1038/sj.embor.embor886>.
- [92] E. Kitamura, J.J. Blow, T.U. Tanaka, Live-Cell Imaging Reveals Replication of Individual Replicons in Eukaryotic Replication Factories, *Cell*. 125 (2006) 1297–1308. <https://doi.org/10.1016/j.cell.2006.04.041>.
- [93] M.K. Raghuraman, B.J. Brewer, W.L. Fangman, Cell cycle-dependent establishment of a late replication program, *Science* (1979). 276 (1997) 806–809. <https://doi.org/10.1126/science.276.5313.806>.
- [94] D.S. Dimitrova, D.M. Gilbert, The spatial position and replication timing of chromosomal domains are both established in early G1 phase, *Molecular Cell*. 4 (1999) 983–993. [https://doi.org/10.1016/S1097-2765\(00\)80227-0](https://doi.org/10.1016/S1097-2765(00)80227-0).
- [95] B.M. Ferguson, W.L. Fangman, A position effect on the time of replication origin activation in yeast, *Cell*. 68 (1992) 333–339. [https://doi.org/10.1016/0092-8674\(92\)90474-Q](https://doi.org/10.1016/0092-8674(92)90474-Q).
- [96] M. Vogelauer, L. Rubbi, I. Lucas, B.J. Brewer, M. Grunstein, Histone acetylation regulates the time of replication origin firing, *Molecular Cell*. 10 (2002) 1223–1233. [https://doi.org/10.1016/S1097-2765\(02\)00702-5](https://doi.org/10.1016/S1097-2765(02)00702-5).
- [97] N. Rhind, D.M. Gilbert, DNA replication timing, *Cold Spring Harbor Perspectives in Biology*. 5 (2013). <https://doi.org/10.1101/cshperspect.a010132>.
- [98] K. Yoshida, J. Bacal, D. Desmarais, I. Padioleau, O. Tsaponina, A. Chabes, V. Pantesco, E. Dubois, H. Parrinello, M. Skrzypczak, K. Ginalski, A. Lengronne, P. Pasero, The Histone Deacetylases Sir2 and Rpd3 Act on Ribosomal DNA to Control the Replication Program in Budding Yeast, *Molecular Cell*. 54 (2014) 691–697. <https://doi.org/10.1016/j.molcel.2014.04.032>.
- [99] T. Hoggard, E. Shor, C.A. Müller, C.A. Nieduszynski, C.A. Fox, A Link between ORC-Origin Binding Mechanisms and Origin Activation Time Revealed in Budding Yeast, *PLoS Genetics*. 9 (2013) e1003798. <https://doi.org/10.1371/journal.pgen.1003798>.
- [100] J.A. Belsky, H.K. Macalpine, Y. Lubelsky, A.J. Hartemink, D.M. Macalpine, Genome-wide chromatin footprinting reveals changes in replication origin architecture induced

- by pre-RC assembly, *Genes and Development*. 29 (2015) 212–224. <https://doi.org/10.1101/gad.247924.114>.
- [101] L. Dukaj, N. Rhind, The capacity of origins to load mcm establishes replication timing patterns, *PLoS Genetics*. 17 (2021). <https://doi.org/10.1371/journal.pgen.1009467>.
 - [102] D. Mantiero, A. MacKenzie, A. Donaldson, P. Zegerman, Limiting replication initiation factors execute the temporal programme of origin firing in budding yeast, *EMBO Journal*. 30 (2011) 4805–4814. <https://doi.org/10.1038/emboj.2011.404>.
 - [103] S. Tanaka, R. Nakato, Y. Katou, K. Shirahige, H. Araki, Origin association of Sld3, Sld7, and Cdc45 proteins is a key step for determination of origin-firing timing, *Current Biology*. 21 (2011) 2055–2063. <https://doi.org/10.1016/j.cub.2011.11.038>.
 - [104] C. Collart, G.E. Allen, C.R. Bradshaw, J.C. Smith, P. Zegerman, Titration of Four Replication Factors Is Essential for the *Xenopus laevis* Midblastula Transition, n.d. www.sciencemag.org.
 - [105] D. Lob, N. Lengert, V.O. Chagin, M. Reinhart, C.S. Casas-Delucchi, M.C. Cardoso, B. Drossel, 3D replicon distributions arise from stochastic initiation and domino-like DNA replication progression, *Nat Commun*. 7 (2016) 11207. <https://doi.org/10.1038/ncomms11207>.
 - [106] W. Wang, K.N. Klein, K. Proesmans, H. Yang, C. Marchal, X. Zhu, T. Borrmann, A. Hastie, Z. Weng, J. Bechhoefer, C.L. Chen, D.M. Gilbert, N. Rhind, Genome-wide mapping of human DNA replication by optical replication mapping supports a stochastic model of eukaryotic replication, *Mol Cell*. 81 (2021) 2975–2988 e6. <https://doi.org/10.1016/j.molcel.2021.05.024>.
 - [107] P.A. Zhao, T. Sasaki, D.M. Gilbert, High-resolution Repli-Seq defines the temporal choreography of initiation, elongation and termination of replication in mammalian cells, *Genome Biol*. 21 (2020) 76. <https://doi.org/10.1186/s13059-020-01983-8>.
 - [108] G. Guilbaud, A. Rappailles, A. Baker, C.L. Chen, A. Arneodo, A. Goldar, Y. d'Aubenton-Carafa, C. Thermes, B. Audit, O. Hyrien, Evidence for sequential and increasing activation of replication origins along replication timing gradients in the human genome, *PLoS Comput Biol*. 7 (2011) e1002322. <https://doi.org/10.1371/journal.pcbi.1002322>.
 - [109] T. Natsume, C.A. Müller, Y. Katou, R. Retkute, M. Gierliński, H. Araki, J.J. Blow, K. Shirahige, C.A. Nieduszynski, T.U. Tanaka, Kinetochore coordinate pericentromeric cohesion and early DNA replication by Cdc7-Dbf4 kinase recruitment, *Molecular Cell*. 50 (2013) 661–674. <https://doi.org/10.1016/j.molcel.2013.05.011>.
 - [110] D. Fang, A. Lengronne, D. Shi, R. Forey, M. Skrzypczak, K. Ginalska, C. Yan, X. Wang, Q. Cao, P. Pasero, H. Lou, Dbf4 recruitment by forkhead transcription factors defines an upstream rate-limiting step in determining origin firing timing, *Genes and Development*. 31 (2017) 2405–2415. <https://doi.org/10.1101/gad.306571.117>.
 - [111] A.M. Thomson, P.J. Gillespie, J.J. Blow, Replication factory activation can be decoupled from the replication timing program by modulating Cdk levels, *J Cell Biol*. 188 (2010) 209–221. <https://doi.org/10.1083/jcb.200911037>.
 - [112] L. Krasinska, E. Besnard, E. Cot, C. Dohet, M. Mechali, J.M. Lemaitre, D. Fisher, Cdk1 and Cdk2 activity levels determine the efficiency of replication origin firing in *Xenopus*, *EMBO J*. 27 (2008) 758–769. <https://doi.org/10.1038/emboj.2008.16>.
 - [113] H. Beck, V. Nahse-Kumpf, M.S. Larsen, K.A. O'Hanlon, S. Patzke, C. Holmberg, J. Mejlvang, A. Groth, O. Nielsen, R.G. Syljuasen, C.S. Sorensen, Cyclin-dependent kinase suppression by WEE1 kinase protects the genome through control of replication

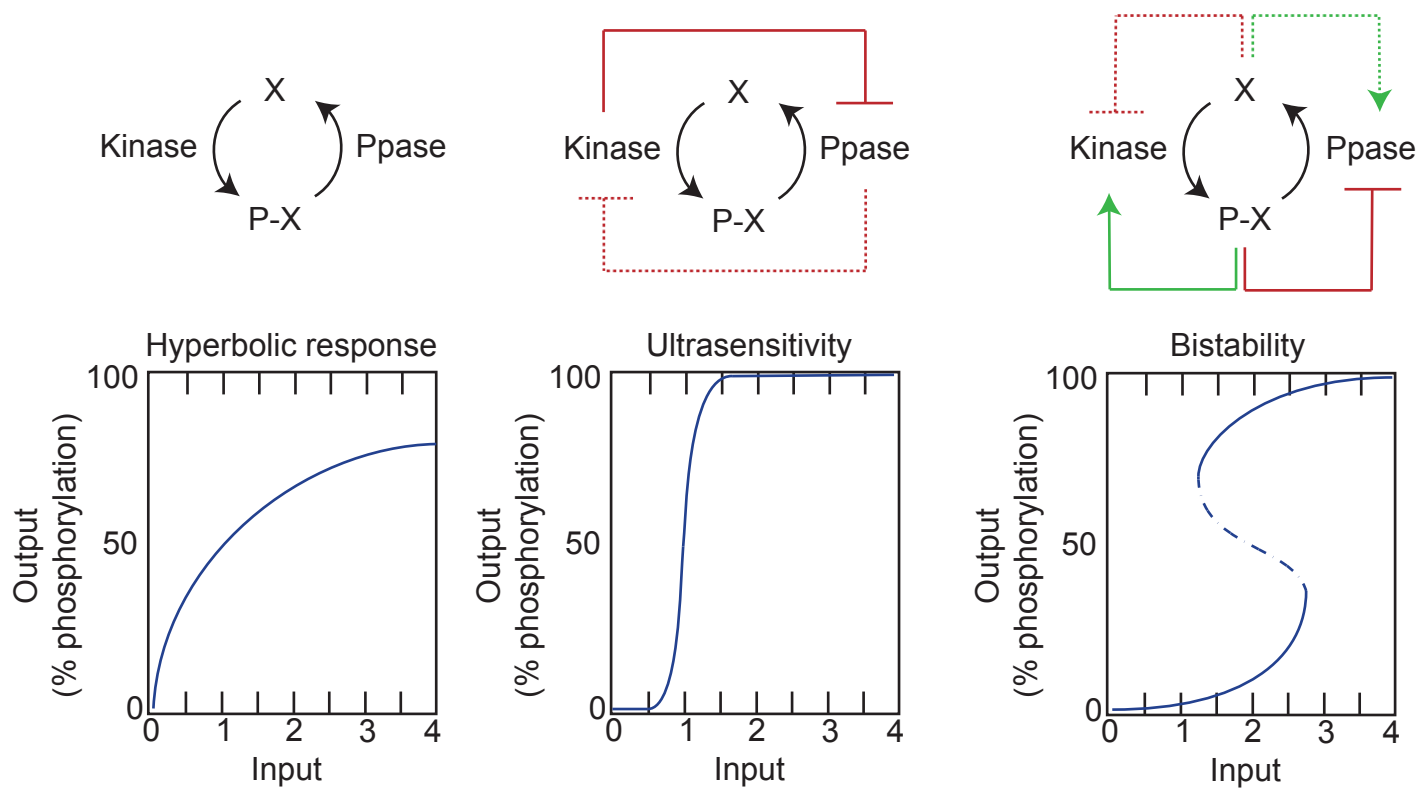
- initiation and nucleotide consumption, *Mol Cell Biol.* 32 (2012) 4226–4236.
<https://doi.org/10.1128/MCB.00412-12>.
- [114] D. Cornacchia, V. Dileep, J.P. Quivy, R. Foti, F. Tili, R. Santarella-Mellwig, C. Antony, G. Almouzni, D.M. Gilbert, S.B. Buonomo, Mouse Rif1 is a key regulator of the replication-timing programme in mammalian cells, *EMBO J.* 31 (2012) 3678–3690.
<https://doi.org/10.1038/emboj.2012.214>.
 - [115] M. Hayano, Y. Kanoh, S. Matsumoto, C. Renard-Guillet, K. Shirahige, H. Masai, Rif1 is a global regulator of timing of replication origin firing in fission yeast, *Genes Dev.* 26 (2012) 137–150. <https://doi.org/10.1101/gad.178491.111>.
 - [116] S. Yamazaki, A. Ishii, Y. Kanoh, M. Oda, Y. Nishito, H. Masai, Rif1 regulates the replication timing domains on the human genome, *EMBO J.* 31 (2012) 3667–3677.
<https://doi.org/10.1038/emboj.2012.180>.
 - [117] L. Richards, S. Das, J.T. Nordman, Rif1-Dependent Control of Replication Timing, *Genes (Basel)*. 13 (2022). <https://doi.org/10.3390/genes13030550>.
 - [118] A. Davé, C. Cooley, M. Garg, A. Bianchi, Protein Phosphatase 1 Recruitment by Rif1 Regulates DNA Replication Origin Firing by Counteracting DDK Activity, *Cell Reports*. 7 (2014) 53–61. <https://doi.org/10.1016/j.celrep.2014.02.019>.
 - [119] S.I. Hiraga, G.M. Alvino, F.J. Chang, H.Y. Lian, A. Sridhar, T. Kubota, B.J. Brewer, M. Weinreich, M.K. Raghuraman, A.D. Donaldson, Rif1 controls DNA replication by directing Protein Phosphatase 1 to reverse Cdc7- mediated phosphorylation of the MCM complex, *Genes and Development*. 28 (2014) 372–383.
<https://doi.org/10.1101/gad.231258.113>.
 - [120] S. Mattarocci, M. Shyian, L. Lemmens, P. Damay, D.M. Altintas, T. Shi, C.R. Bartholomew, N.H. Thomä, C.F.J. Hardy, D. Shore, Rif1 Controls DNA replication timing in yeast through the PP1 Phosphatase Glc7, *Cell Reports*. 7 (2014) 62–69.
<https://doi.org/10.1016/j.celrep.2014.03.010>.
 - [121] W.T. Poh, G.S. Chadha, P.J. Gillespie, P. Kaldis, J.J. Blow, *Xenopus* Cdc7 executes its essential function early in S phase and is counteracted by checkpoint-regulated protein phosphatase 1, *Open Biol.* 4 (2014) 130138.
<https://doi.org/10.1098/rsob.130138>.
 - [122] C.A. Seller, P.H. O'Farrell, Rif1 prolongs the embryonic S phase at the *Drosophila* mid-blastula transition, *PLoS Biol.* 16 (2018) e2005687.
<https://doi.org/10.1371/journal.pbio.2005687>.
 - [123] T.N. Moiseeva, Y. Yin, M.J. Calderon, C. Qian, S. Schamus-Haynes, N. Sugitani, H.U. Osmanbeyoglu, E. Rothenberg, S.C. Watkins, C.J. Bakkenist, An ATR and CHK1 kinase signaling mechanism that limits origin firing during unperturbed DNA replication, *Proc Natl Acad Sci U S A.* 116 (2019) 13374–13383.
<https://doi.org/10.1073/pnas.1903418116>.
 - [124] S. Charrasse, A. Gharbi-Ayachi, A. Burgess, J. Vera, K. Hached, P. Raynaud, E. Schwob, T. Lorca, A. Castro, Ensa controls S-phase length by modulating Treslin levels, *Nature Communications*. 8 (2017). <https://doi.org/10.1038/s41467-017-00339-4>.
 - [125] X.-H. Lin, J. Walter, K. Scheidtmann, K. Ohst, J. Newport, G. Walter, Protein phosphatase 2A is required for the initiation of chromosomal DNA replication, 1998. www.pnas.org.
 - [126] D.M. Chou, P. Petersen, J.C. Walter, G. Walter, Protein phosphatase 2A regulates binding of Cdc45 to the prereplication complex, *Journal of Biological Chemistry*. 277 (2002) 40520–40527. <https://doi.org/10.1074/jbc.M207226200>.

- [127] C. Guo, A. Kumagai, K. Schlacher, A. Shevchenko, A. Shevchenko, W.G. Dunphy, Interaction of Chk1 with treslin negatively regulates the initiation of chromosomal DNA replication, *Molecular Cell*. 57 (2015) 492–505.
<https://doi.org/10.1016/j.molcel.2014.12.003>.
- [128] A. Chowdhury, G. Liu, M. Kemp, X. Chen, N. Katrangi, S. Myers, M. Ghosh, J. Yao, Y. Gao, P. Bubulya, M. Leffak, The DNA Unwinding Element Binding Protein DUE-B Interacts with Cdc45 in Preinitiation Complex Formation, *Molecular and Cellular Biology*. 30 (2010) 1495–1507. <https://doi.org/10.1128/mcb.00710-09>.
- [129] Y. Gao, J. Yao, S. Poudel, E. Romer, L. Abu-Niaaj, M. Leffak, Protein phosphatase 2A and Cdc7 kinase regulate the DNA unwinding element-binding protein in replication initiation, *Journal of Biological Chemistry*. 289 (2014) 35987–36000.
<https://doi.org/10.1074/jbc.M114.589119>.
- [130] J.E. Hughes Hallett, X. Luo, A.P. Capaldi, State transitions in the TORC1 signaling pathway and information processing in *Saccharomyces cerevisiae*, *Genetics*. 198 (2014) 773–786. <https://doi.org/10.1534/genetics.114.168369>.
- [131] V. Ramachandran, P.K. Herman, Antagonistic Interactions Between the cAMP-Dependent Protein Kinase and Tor Signaling Pathways Modulate Cell Growth in *Saccharomyces cerevisiae*, *Genetics*. 187 (2011) 441–454.
<https://doi.org/10.1534/genetics.110.123372>.
- [132] B.M. Sutter, X. Wu, S. Laxman, B.P. Tu, XMethionine inhibits autophagy and promotes growth by inducing the SAM-responsive methylation of PP2A, *Cell*. 154 (2013) 403.
<https://doi.org/10.1016/j.cell.2013.06.041>.
- [133] J.T. Nickels, J.R. Broach, A ceramide-activated protein phosphatase mediates ceramide-induced G1 arrest of *Saccharomyces cerevisiae*, *Genes and Development*. 10 (1996) 382–394. <https://doi.org/10.1101/gad.10.4.382>.
- [134] V. Janssens, J. Goris, Protein phosphatase 2A: A highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling, *Biochemical Journal*. 353 (2001) 417–439. <https://doi.org/10.1042/0264-6021:3530417>.
- [135] V. Thai, N. Dephoure, A. Weiss, J. Ferguson, R. Leitao, S.P. Gygi, D.R. Kellogg, Protein kinase C controls binding of Igo/ENSA proteins to protein phosphatase 2A in budding yeast, *Journal of Biological Chemistry*. 292 (2017) 4925–4941.
<https://doi.org/10.1074/jbc.M116.753004>.
- [136] K. Artilles, S. Anastasia, D. McCusker, D.R. Kellogg, The Rts1 regulatory subunit of protein phosphatase 2A is required for control of G1 cyclin transcription and nutrient modulation of cell size, *PLoS Genetics*. 5 (2009).
<https://doi.org/10.1371/journal.pgen.1000727>.
- [137] P. Zegerman, J.F. Diffley, DNA replication as a target of the DNA damage checkpoint, *DNA Repair (Amst)*. 8 (2009) 1077–1088.
<https://doi.org/10.1016/j.dnarep.2009.04.023>.
- [138] P. Zegerman, J.F.X. Diffley, Checkpoint-dependent inhibition of DNA replication initiation by Sld3 and Dbf4 phosphorylation, *Nature*. 467 (2010) 474–478.
<https://doi.org/10.1038/nature09373>.
- [139] J. Lopez-Mosqueda, N.L. Maas, Z.O. Jonsson, L.G. Defazio-Eli, J. Wohlschlegel, D.P. Toczycki, Damage-induced phosphorylation of Sld3 is important to block late origin firing, *Nature*. 467 (2010) 479–483. <https://doi.org/10.1038/nature09377>.
- [140] S.A. Wahab, D. Remus, Antagonistic control of ddk binding to licensed replication origins by mcm2 and rad53, *Elife*. 9 (2020) 1–28. <https://doi.org/10.7554/eLife.58571>.

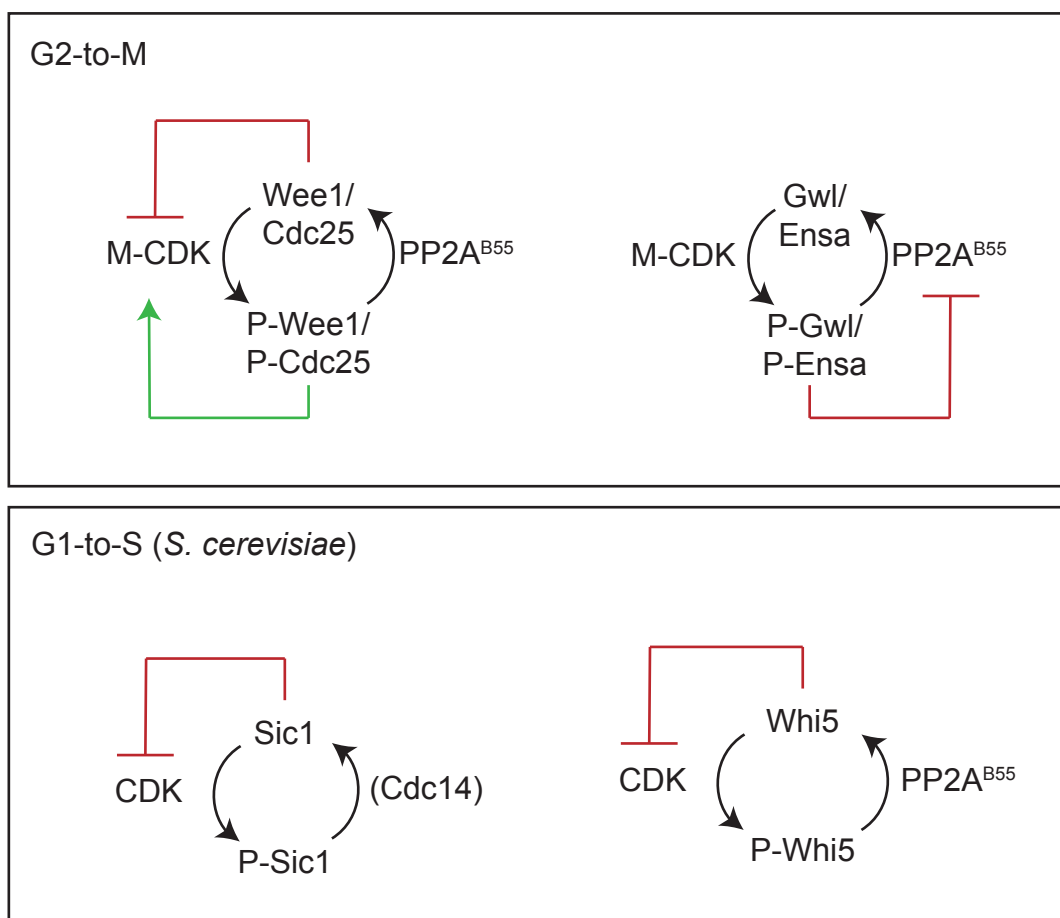
- [141] D. Boos, L. Sanchez-Pulido, M. Rappas, L.H. Pearl, A.W. Oliver, C.P. Ponting, J.F.X. Diffley, Regulation of DNA replication through Sld3-Dpb11 interaction is conserved from yeast to humans, *Current Biology*. 21 (2011) 1152–1157.
<https://doi.org/10.1016/j.cub.2011.05.057>.
- [142] V. Costanzo, D. Shechter, P.J. Lupardus, K.A. Cimprich, M. Gottesman, J. Gautier, An ATR- and Cdc7-dependent DNA damage checkpoint that inhibits initiation of DNA replication, *Molecular Cell*. 11 (2003) 203–213. [https://doi.org/10.1016/S1097-2765\(02\)00799-2](https://doi.org/10.1016/S1097-2765(02)00799-2).
- [143] A.Y.L. Lee, T. Chiba, L.N. Truong, A.N. Cheng, J. Do, M.J. Cho, L. Chen, X. Wu, Dbf4 is direct downstream target of ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) protein to regulate intra-S-phase checkpoint, *Journal of Biological Chemistry*. 287 (2012) 2531–2543.
<https://doi.org/10.1074/jbc.M111.291104>.
- [144] E.C. Morafraille, C. Hanni, G. Allen, T. Zeisner, C. Clarke, M.C. Johnson, M.M. Santos, L. Carroll, N.E. Minchell, J. Baxter, P. Banks, D. Lydall, P. Zegerman, Checkpoint inhibition of origin firing prevents DNA topological stress, *Genes Dev*. 33 (2019) 1539–1554. <https://doi.org/10.1101/gad.328682.119>.
- [145] M.C. Johnson, G. Can, M.M. Santos, D. Alexander, P. Zegerman, Checkpoint inhibition of origin firing prevents inappropriate replication outside of s-phase, *Elife*. 10 (2021) 1–16. <https://doi.org/10.7554/ELIFE.63589>.
- [146] C.J. Matheson, D.S. Backos, P. Reigan, Targeting WEE1 Kinase in Cancer, *Trends in Pharmacological Sciences*. 37 (2016) 872–881.
<https://doi.org/10.1016/j.tips.2016.06.006>.
- [147] F. Li, D. Kozono, P. Deraska, T. Branigan, C. Dunn, X.F. Zheng, K. Parmar, H. Nguyen, J. DeCaprio, G.I. Shapiro, D. Chowdhury, A.D. D’Andrea, CHK1 Inhibitor Blocks Phosphorylation of FAM122A and Promotes Replication Stress, *Mol Cell*. 80 (2020) 410–422 e6. <https://doi.org/10.1016/j.molcel.2020.10.008>.
- [148] N. Watanabe, H. Arai, J. Iwasaki, M. Shiina, K. Ogata, T. Hunter, H. Osada, Cyclin-dependent kinase (CDK) phosphorylation destabilizes somatic Wee1 via multiple pathways, *Proc Natl Acad Sci U S A*. 102 (2005) 11663–11668.
<https://doi.org/10.1073/pnas.0500410102>.
- [149] S.J. Szyjka, J.G. Aparicio, C.J. Viggiani, S. Knott, W. Xu, S. Tavare, O.M. Aparicio, Rad53 regulates replication fork restart after DNA damage in *Saccharomyces cerevisiae*, *Genes Dev*. 22 (2008) 1906–1920. <https://doi.org/10.1101/gad.1660408>.
- [150] A. Travesa, A. Duch, D.G. Quintana, Distinct phosphatases mediate the deactivation of the DNA damage checkpoint kinase Rad53, *J Biol Chem*. 283 (2008) 17123–17130.
<https://doi.org/10.1074/jbc.M801402200>.
- [151] B.M. O’Neill, S.J. Szyjka, E.T. Lis, A.O. Bailey, J.R. Yates 3rd, O.M. Aparicio, F.E. Romesberg, Pph3-Psy2 is a phosphatase complex required for Rad53 dephosphorylation and replication fork restart during recovery from DNA damage, *Proc Natl Acad Sci U S A*. 104 (2007) 9290–9295.
<https://doi.org/10.1073/pnas.0703252104>.
- [152] E. Ferrari, C. Bruhn, M. Peretti, C. Cassani, W. v Carotenuto, M. Elgendy, G. Shubassi, C. Lucca, R. Bermejo, M. Varasi, S. Minucci, M.P. Longhese, M. Foiani, PP2A Controls Genome Integrity by Integrating Nutrient-Sensing and Metabolic Pathways with the DNA Damage Response, *Mol Cell*. 67 (2017) 266–281 e4.
<https://doi.org/10.1016/j.molcel.2017.05.027>.

- [153] X. Lu, B. Nannenga, L.A. Donehower, PPM1D dephosphorylates Chk1 and p53 and abrogates cell cycle checkpoints, *Genes Dev.* 19 (2005) 1162–1174. <https://doi.org/10.1101/gad.1291305>.
- [154] Z.P. Wang, Y. Tian, J. Lin, Role of wild-type p53-induced phosphatase 1 in cancer, *Oncol Lett.* 14 (2017) 3893–3898. <https://doi.org/10.3892/ol.2017.6685>.
- [155] F.M. Barnieh, P.M. Loadman, R.A. Falconer, Progress towards a clinically-successful ATR inhibitor for cancer therapy, *Current Research in Pharmacology and Drug Discovery.* 2 (2021). <https://doi.org/10.1016/j.crphar.2021.100017>.
- [156] Z. Qiu, P. Fa, T. Liu, C.B. Prasad, S. Ma, Z. Hong, E.R. Chan, H. Wang, Z. Li, K. He, Q.E. Wang, T.M. Williams, C. Yan, S.T. Sizemore, G. Narla, J. Zhang, A genome-wide pooled shRNA screen identifies PPP2R2A as a predictive biomarker for the response to ATR and CHK1 inhibitors, *Cancer Research.* 80 (2020) 3305–3318. <https://doi.org/10.1158/0008-5472.CAN-20-0057>.
- [157] A. Khanna, O. Kauko, C. Böckelman, A. Laine, I. Schreck, J.I. Partanen, A. Szwajda, S. Bormann, T. Bilgen, M. Helenius, Y.R. Pokharel, J. Pimanda, M.R. Russel, C. Haglund, K.A. Cole, J. Klefström, T. Aittokallio, C. Weiss, A. Ristimäki, T. Visakorpi, J. Westermarck, Chk1 targeting reactivates PP2A tumor suppressor activity in cancer cells, *Cancer Research.* 73 (2013) 6757–6769. <https://doi.org/10.1158/0008-5472.CAN-13-1002>.

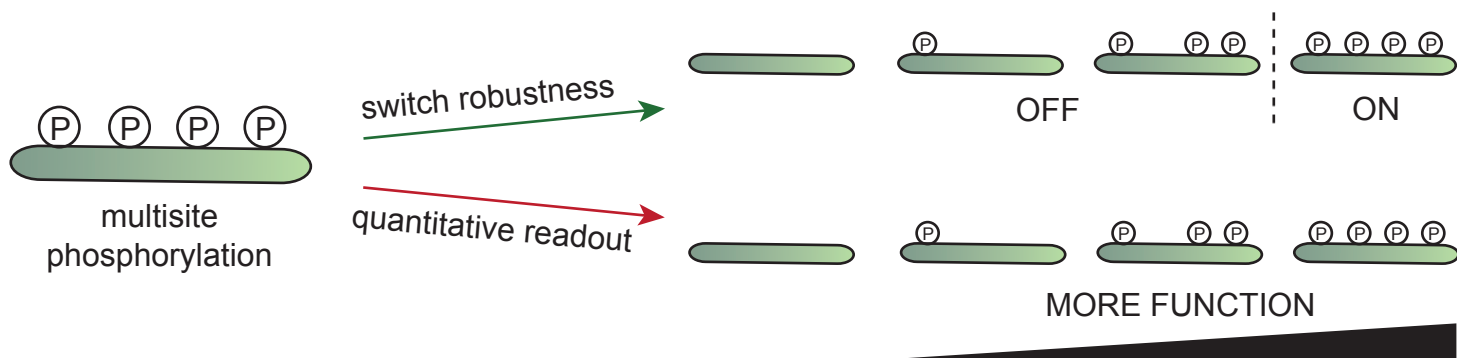
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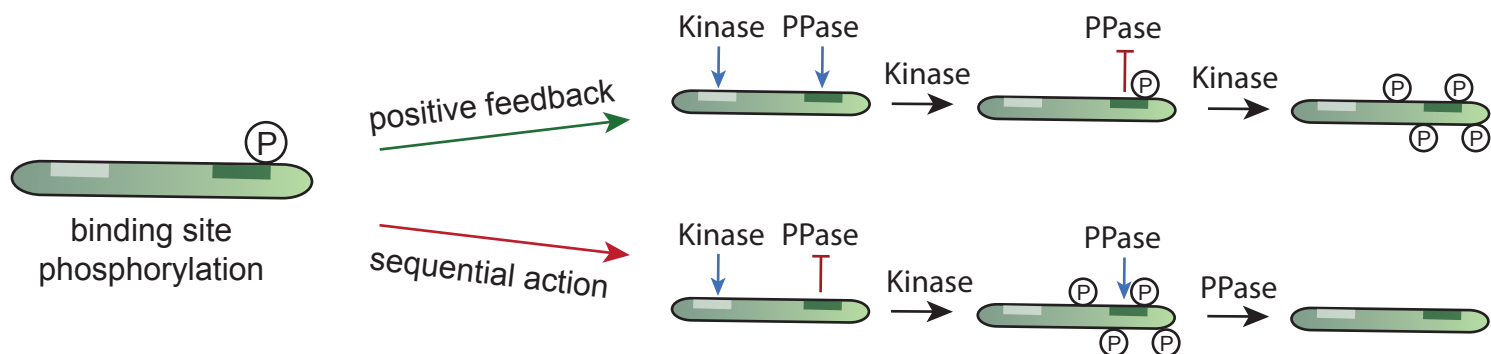
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B



C

