Exploring the regulation of mitotic $PP2A^{Rts1}$ activity in *Saccharomyces cerevisiae*

Alain David, BSc

Thesis submitted to the University of Ottawa

in partial fulfillment of the requirements for the

Masters Degree in Biochemistry

Department of Biochemistry, Microbiology, and Immunology

Faculty of Medicine

University of Ottawa

ABSTRACT

Protein phosphorylation is an essential post-translational modification used in cells for regulating multiple biological processes in all organisms. Particularly, mitotic onset is regulated in all eukaryotes by an increase in cyclin-dependent kinase 1 (Cdk1) activity caused by the dephosphorylation of Cdk1 on a conserved tyrosine residue. $PP2A^{Rts1}$ is a phosphatase that participates in dephosphorylating the conserved tyrosine residue, tyrosine-19 (Y19). $PP2A^{Rts1}$ dephosphorylates phosphorylated serine and threonine residues. However, in vitro experiments suggest that in conjunction with the mammalian PP2A phosphatase activator (PTPA), PP2A gains phosphotyrosine specificity. My work indicates that Rrd1 and Rrd2 (the budding yeast homologs of PTPA) genetically interact with $PP2A^{Rts1}$ and the absence of these proteins cause a Swe1-dependent delay in mitosis. In parallel, utilizing a candidate approach to identify additional phosphatases specific to Cdk1-Y19, my work indicates that Ych1 and Arr2 act redundantly with Mih1 and Ptp1, and Ych1 may act downstream of $PP2A^{Rts1}$. In summation, my work provides the groundwork for how $PP2A^{Rts1}$ functions to dephosphorylate the conserved Y19 residue on Cdk1 and will lead to a better understanding of its role in regulating mitotic progression.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I would like to this as an opportunity to thank Adam Rudner for being the best and most enthusiastic mentor throughout my UROP, Honours, and MSc. I am forever grateful for you are the one who gave me a shot back in 2017, which propelled me into my journey in science.

My time in the Rudner Lab is filled with unforgettable and well-cherished memories as I have met many people throughout the years, some of whom I now have the pleasure of calling my friends. I am grateful for Sean Stephenson as he has been with me ever since my first day in the lab. Through the ups and downs, Sean and I have always had each other's backs. Being the same year as me, Ashley Janna is also someone I need to mention as she made me feel at ease whenever I felt unsure and uneasy of my progress in the lab. She made me realize that everyone is in the same boat and that we are all in this together.

My year of graduation is the unfortunate year in which COVID-19 took the world by storm. But it is because of this global pandemic do I have the greatest appreciation for my family and friends for helping me through the good and the bad. Without everyone's support, I would not have been able to do any of it.

LIST OF ABBREVIATIONS

ARR2	**Arsenicals resistance 2**
BUR1	**Suppressor of Gpa-Val50 mutation 1**
CDC25	**Cell division cycle 25**
CDC28	**Cell division cycle 28**
CDC55	**Cell division cycle 55**
CDK1	**Cyclin-dependent kinase**
CLB	**Cyclin B**
CTK1	**Carboxy-terminal domain kinase 1**
DNA	**Deoxyribonucleic acid**
ER	**Endoplasmic reticulum**
GSS	**Gamete-shedding substance**
KIN28	**Protein Kinase 28**
LH	**Luteinizing hormone**
MIH1	**Mitotic inducer homolog 1**
MIS	**Meiosis-inducing substance**
MPF	**Maturation Promoting Factor**
NPP	***para*-nitrophenyl phosphate**
OCA2	**Oxidant-induced cell cycle arrest 2**
ORF	**Open reading frame**
PHO85	**Phosphate metabolism 85**
PPase	**Protein phosphatase**

PP2A	**Protein phosphatase 2A**
PPH21	**Protein phosphatase 21**
PPH22	**Protein phosphatase 22**
PPH3	**Protein phosphatase 3**
PTM	**Post-translational modification**
PTP1	**Protein tyrosine phosphatase 1**
PTPA	**PP2A phosphatase activator**
PTPase	**Protein tyrosine phosphatase**
RRD1	**Resistant to rapamycin deletion 1**
RRD2	**Resistant to rapamycin deletion 2**
RTS1	**ROX3 suppressor**
PYP3	**Tyrosine-protein phosphatase 3**
SPD1	**S-phase delating protein 1**
SSN3	**Suppressor of Snf1**

LIST OF FIGURES

LIST OF TABLES

1.0 INTRODUCTION

1.1 Importance of the cell cycle

Every organism is made up of cells and new cells can only arise from pre-existing cells. With such principles discovered, this prompted the question of how cells divided. With the help of the pioneering work of Howard and Pelc (1953), they determined that for active eukaryotic cells to divide, they must undergo a series of events which are collectively known as the cell cycle[1]. This process allows the cell to delegate tasks into specific phases which are all crucial to maintain cellular size, organize their newly synthesized genome, and prepare for chromosome segregation[2,3]. The cell cycle can be separated into two stages: Interphase (G1, S, and G2) and Mitosis (M). More specifically, in eukaryotes, DNA replication is strictly confined in Synthesis (S), or S-phase, and chromosome segregation occurs in Mitosis, or M-phase. In the cell cycle, there also exists two Gap phases denoted as G1 and G2. They are not to be mistaken for periods of inactivity but rather points where cells gain sufficient mass, initiate growth signals, and organize their newly replicated genome in preparation for chromosome segregation[2]. The M-phase requires strict coordination of events as it is a process that determines cellular size which is important for proper cell function and survival[4,5]. Anaphase in M phase involves the irreversible event of sister chromatid separation. This stresses the imperativeness of the coordination of these events as mistakes can lead to aneuploidy and lethality[6].

1.1.1 Cell cycle checkpoints

Previous work has suggested that yeast cells use cellular checkpoints to coordinate processes relating to growth and division[7–11]. In several experiments, conducted in both fused

human cells and oocytes, crosstalk between S-phase and M-phase was observed[2,12–14]. That is, a cell undergoing S-phase could send signals to a nucleus in G2 to delay its entry into mitosis. Similarly, *Weinart and Hartwell* (1989) utilized DNA damage to arrest of the cell cycle and identified the first DNA damage checkpoints[15]. These checkpoints showed the dependency of mitosis on the completion of DNA replication and thus, in dividing cells, cell size checkpoints exist to delay cell cycle transitions to ensure proper growth has occurred.

1.2 Discovering and characterizing the master regulator of mitosis: Cdk1

1.2.1 Maturation promoting factor (*MPF*)

In the mid 1950s, it had been established that the luteinizing hormone (LH), a gonadotropin secreted by the pituitary gland, induces ovulation and oocyte maturation in vertebrates[13]. Later, in the 1960s, large strides into the reproductive endocrinology of starfish were made as they found that GSS, the first gonadotropin-like hormone demonstrated in invertebrates, not only prompted gamete shedding but also induced meiotic resumption in oocytes[16]. Shortly after this discovery, purification of the starfish MIS was achieved and identified as 1-methyladenine (1-MeAde). However, due to the finding that the microinjection of 1-MeAde into immature starfish oocytes failed to induce maturation, this gave rise to the idea that a third putative substance was responsible for this phenomenon.

Proceeding with such an idea, a cytoplasmic component, found in progesterone-treated frog oocytes, possessing mitotic promoting capabilities was found[17]. In this study, this factor was required to enable oocytes to mature into unfertilized eggs. In turn, this component was termed as maturation promoting factor (MPF). Shortly after, MPF was demonstrated to be detected periodically in cleaving blastomeres (highest at mitotic M-phase) of frogs and starfish[18–20],

mammalian cell cultures synchronized at M-phase[21], in mouse oocytes[22], and in extracts of budding yeast *cell division cycle* (*cdc*) mutants arrested at M-phase[19,21]. According to these studies, the concept emerged that MPF is the universal inducer of M-phase in all eukaryotic cells, whether it being mitosis or meiosis[13]. Hence, MPF was re-termed from 'maturation promoting factor' to 'M-phase promoting factor'[2].

1.2.2 Cyclins and cyclin-dependent kinase 1 (Cdk1)

In parallel to the numerous studies about MPF, a study involving sea urchins demonstrated that in addition to the rise in MPF levels during early embryonic cleavage (M-phase), a series of proteins also appeared and disappeared in synchrony as well[23]. Notably, upon maturation of sea urchin oocytes, Evans *et al*. observed that some proteins would increase and decrease in a periodical, or cyclic, manner – where maximal levels would be reached preceding the cleavage event[23]. At the time, these were the first proteins whose abundance varied during the cell cycle, and they were named as 'cyclins'.

The fluctuations of these cyclins serve as an important switching mechanism that triggers major cellular events in the cell division cycle[3]. These cyclins are necessary as they are activating partners of a highly conserved family of proline-directed kinases, cyclin-dependent kinases (CDKs)[24]. There are three main points that cyclin-Cdk complexes control; Start (G1/S), mitotic entry, and metaphase-anaphase transition[25]. The temporal degradation of cyclins and activation by phosphorylation are the two factors required for the full activation of CDKs. Generally, the concept of cyclins is conserved throughout the eukaryotes[3]. In budding yeast, there are a total of nine cyclins, three Cln cyclins (1-3) and six Clb (1-6) cyclins. More specifically, Cln1-3 are involved in Start events (e.g. G1 and G1/S transition), Clb5 /6 drive S-

phase, Clb3/4 promote early mitotic events, and Clb1/2 are responsible for the completion of mitosis[26,27]. Notably, in budding yeast, there are six conserved CDKs that exist: Cdk1 (originally named Cdc28), Pho85, Kin28, Ssn3, Ctk1, and Bur1[28–31]. However, only a single Cdk, Cdk1, is essential for cell cycle progression and interacts with all nine cyclins and mediates most transitions in the cell cycle[28]. Cdk1 is very well known as the master regulator of mitosis because even alone, it is both necessary and sufficient to drive cell cycle progression. In consequence, the gene encoding Cdk1 is essential[30]. In studies where *cdk1* mutants were generated, it was exhibited that cell cycle progression was arrested at Start when shifted restrictive temperatures[32,33]. Furthermore, defects in Cdk1 have been shown to have many downstream consequences such as defective post-mating nuclear fusion, mitotic chromosome stability, and meiosis[34–36].

1.2.3 Cdk1 as a kinase

Phosphorylation is a post-translation modification (PTM) that involves the process of adding a phosphoryl group onto a substrate whereas the inverse process, removing of a phosphoryl group, is called dephosphorylation. Reversible phosphorylation of proteins is an essential mechanism utilized by all organisms to regulate biological processes[1]. Moreover, approximately one third of the proteome in cellular organisms are reversibly phosphorylated, implicating the role of phosphorylation in many processes[37]. The phosphorylation status of substrates acts as an intricate form of regulation during mitotic events because it can affect their localization, interactions, and functions[38]. As previously mentioned, Cdk1 is a proline-directed kinase that preferentially phosphorylates the minimal consensus sequence S/T-P as well as the consensus sequence S/T-P-x-K/R (where x is any amino acid)[39]. Cdk1 interacts with all nine

cyclins as this interaction with the cyclins is important for its kinase activity and substrate specificity[39]. Additionally, some cyclins help determine the cellular localization of some cyclin-Cdk complexes, like Clb2-Cdk1[34]. Although there is significant overlap between the cyclins demonstrated by the rescue of *clb1,2,3,4,5,6*Δ via the over-expression of a single Clb (e.g. Clb1), it is worth noting that sequential synthesis and degradation of different cyclins are important for the ordering of cellular and phosphorylation events during the cell cycle[40]. Although the kinase activity of Cdk1 is influenced by multiple and complex controls, the protein abundance of Cdk1 remains unchanged throughout the cell cycle[41]. Moreover, given that over-expression of wild-type Cdk1 is well tolerated by the cell, transcriptional and translational regulation of Cdk1 has not been considered important[50]. Therefore, PTMs have been a large focus in determining the regulation and control of Cdk1. In yeast, inhibitory phosphorylation of Cdk1 has been previously shown to mediate the checkpoint that delays mitosis until sufficient cell growth has occurred in the events such as DNA damage and/or small cell size,[7,8,11,44].

1.2.4 Cell division cycle 2 (*cdc2*) and Cell division cycle 28 (*CDC28*)

Inspired from the initial demonstrations of MPF in several organisms, the question of the molecular identity was still unclear. After successfully purifying and isolating MPF biochemically from frog oocytes, it was discovered that MPF consisted of two major proteins that together demonstrated kinase activity phosphorylating a model substrate, histone H1[45]. These components were identified to be homologs of the fission yeast *cdc2* gene product and cyclin B. Hence, it was concluded that MPF is a histone H1 kinase consisting of the complex cyclin B-Cdc2. Both the fission yeast and budding yeast have been used extensively as model organisms for studies done on the eukaryotic cell cycle. Accordingly, such work in these

organisms have utilized temperature-sensitive *cdc* mutants, which arrest during the cell cycle at restrictive temperatures, to identify over 25 *cdc* genes in fission yeast and 40 *cdc* genes in budding yeast[46]. Among these, *cdc2* of fission yeast and *CDC28* of budding yeast were identified to be necessary for bud emergence and DNA replication[47,48]. Interestingly, across the two organisms, it has been found that isolated *cdc2*8 of budding yeast can complement mutations of *cdc2* in fission yeast and that human Cdc2 can complement fission yeast *cdc2*[47]. This suggested that these two proteins not only share similar properties, but also that cell cycle events in both organisms proceed under a similar molecular basis.

1.3 Regulation and control of Cdk1

1.3.1 Discovery of Wee1/Cdc25

In fission yeast, the Wee1 kinase is responsible for phosphorylating and inhibiting Cdk1 (Y15 in fission yeast) which delays mitosis[4,8,49,50]. Wee1 delays Cdk1 via inhibitory phosphorylation of a conserved tyrosine residue (Y15 in fission yeast ; Y19 in budding yeast) on the N-terminus[49–52]. Moreover, *Gould and Nurse* (1989) showed that activation of cyclin B-Cdc2 is preceded by the phosphorylation of the 15th residue (Y15) on the Cdc2 subunit[49]. By mutating this tyrosine residue to a non-phosphorylatable phenylalanine (*cdc2-F15*), mitosis was initiated earlier suggesting that this residue plays a key role in negatively regulating cyclin B-Cdc2 activation. Additionally, work by *Russell and Nurse* (1987) determined that the affect of Wee1 on cell size is dose-dependent, where decreased activity of Wee1 led to premature entry into mitosis at a reduced cell size, whereas increased activity of Wee1 led to delayed entry and increased cell size[8,53].

Conversely, the Cdc25 phosphatase acts as an inducer of mitosis by dephosphorylating and promoting Cdk1 activity[54–57]. Thus, both Wee1 and Cdc25 are targets for cell-cycle checkpoints because activating Wee1 or inhibiting Cdc25 will cause a delay in mitotic onset[58]. Recent work in budding yeast has shown that Swe1, the budding yeast homolog of Wee1, also delays mitotic entry and is required for cell-size control as *swe1*Δ cells enter mitosis prematurely and produce abnormally small cells[59,60]. Inversely, loss of Mih1, the budding yeast homolog of Cdc25, causes delayed entry into mitosis and produces abnormally large cells. Moreover, budding yeast Swe1 can rescue the temperature-sensitive phenotype of *wee1*Δ cells in fission yeast[61]. Altogether, these observations show that the basic functions of fission yeast Wee1 and Cdc25 are conserved in budding yeast[26].

1.3.2 Phosphorylation of a conserved tyrosine residue during DNA damage

In a similar way that checkpoints exist to monitor and ensure proper cell growth is proceeding, there are checkpoints resulting in the arrest of the cell cycle when the presence of DNA damage is detected. DNA damage could be the result of intrinsic factors such as telomeric failure, metabolic by-products, and DNA replication errors[2]. Alternatively, the cause of damage could be from extrinsic sources such as UV radiation, carcinogens, and DNA lesions. Whilst the DNA is under repair, a host of events must occur to prevent the activation of CDKs until the process is complete. Specifically, in fission yeast, key targets of the G2 DNA damage checkpoints are Cdc25 and Wee1[62].

1.4 Presence of redundant phosphatases with Mih1: Ptp1 and PP2A

1.4.1 Applications of tyrosine phosphorylation

Since tyrosine phosphorylation regulates a plethora of cellular events and is required for maintaining homeostasis, significant changes in tyrosine phosphorylation are often associated with diseases involving metabolic disorders, cancers, and cardiovascular diseases[63]. Moreover, phosphorylation of tyrosyl residues have been implicated in several cellular processes that include cell growth, differentiation, and oncogenic transformation The dynamics of tyrosine phosphorylation is tightly controlled by the opposing pair of protein-tyrosine kinases (PTKs) and protein-tyrosine phosphatases (PTPs)[64]. PTPs typically contain a 240-250 amino acid catalytic domain, which contains an essential cysteine residue, that specifically recognizes phosphotyrosine (pTyr) residues[65]. This high-level of specificity means that PTPs must be closely regulated and thus, are utilized by the cell to mark the initiation or termination of cell signals.

1.4.2 Ptp1B in humans

Protein-tyrosine phosphatase 1B (PTP1B) is an abundant and widely expressed non-receptor phosphatase that was first purified from human placenta by Charbonneau and colleagues (1989)[66]. Since PTP1B regulates several signalling pathways, it is under tight control to avoid random and aberrant signalling from occurring. Therefore, the PTMs and sub-cellular localization of PTP1B is important for modulating its function. PTP1B is localized to the cytoplasmic face of the endoplasmic reticulum (ER) because of its hydrophobic C-terminal[67]. This trait imposes a geometric constraint on PTP1B which restricts its access to substrates and, in effect, regulating its function via localization. On top of this regulation, numerous PTMs also

regulate PTP1B including phosphorylation, oxidation, nitrosylation, sulfyhydration, and sumoylation[63]. Importantly, when PTP1B is phosphorylated on tyrosine residues – PTP1B is phosphorylated in the presence of insulin on Y66, 152, and 153 – this results in increased phosphatase activity. Additionally, PTP1B can also be phosphorylated on serine residues which occurs during mitosis and conditions of stress[68].

1.4.3 Pyp3 in fission yeast

In 1992, Millar *et al*. observed that even in the absence of *cdc25* (in a temperature-sensitive *wee1* mutant), dephosphorylation of Y15 was still occurring[59]. Therefore, Millar *et al*. considered the plausible chance that a second *CDC25*-like gene existed in fission yeast. As a result, they found that a PTPase was encoded that showed a weak resemblance to Cdc25, equivalent to the level seen comparing Cdc25 and PTP1B. On such basis, this new PTPase gene was named *pyp3* as it was more reminiscent of the proteins Pyp1 and Pyp2. Interestingly, the Pyp3 protein was isolated and showed the ability to dephosphorylate and activate Cdc2 *in vitro*. Furthermore, Pyp3 over-expression showed identical results caused by the over-expression of Cdc25 where early initiation of mitosis was demonstrated. In summation of the data above, it suggested that Pyp3 was both structurally and functionally a member of the PTPase family.

1.4.4 Ptp1 in budding yeast

Concurrently, in 1991, Guan and colleagues were interested in studying the regulation of tyrosine phosphorylation in the budding yeast model organism[69]. By designing PCR primers that matched conserved amino acid sequences of mammalian PTPases, they were able to identify the yeast *PTP1* gene. The authors then purified Ptp1 from *Escherichia coli* through single affinity

chromatography and determined Ptp1 hydrolyzed artificial phosphotyrosine-containing substrates *in vitro* at roughly 1000 times faster than phosphoserine-containing substrates. Additionally, it was demonstrated that when increasing the expression of *PTP1* or any of the other homologs of *PTP1* in other organisms (i.e. *pyp3* in fission yeast), this suppressed the temperature-sensitivity observed in *cdc25-22* cells, whereas deleting these genes exacerbated the defects of *cdc25-22* cells, suggesting the conserved function of Ptp1 in dephosphorylating Cdk1[67,69,79]. In 2016, study of Ptp1 in budding yeast was continued by Kennedy and colleagues to further detail its role in dephosphorylating Cdk1. In this study, the authors were able to show that asynchronously growing *ptp1*Δ cells exhibited higher levels of Cdk1-Y19 phosphorylation proving a role *in vivo*. In addition, Kennedy *et al*. (2016), were able to show that Ptp1 was able to dephosphorylate Cdk1-Y19, through an *in vitro* phosphatase assay, confirming its specificity towards this residue.

1.4.5 Structure and Functions of PP2A

Protein phosphatase 2A (PP2A) is known as a major serine/threonine phosphatase that has been linked genetically and biochemically to a large set of biological functions including DNA replication, transcription, signal transduction, metabolism, cellular growth, and mitosis[71–74]. With such a myriad of functions, PP2A phosphatases can be found in all organisms whilst conserving their structure across all eukaryotes. This heterotrimeric holoenzyme is a combination of three different subunits: a structural scaffolding (A) subunit, a regulatory (B) subunit, and a catalytic (C) subunit. In mammalian systems, the B-type subunit's functionality can be carried out by one of three different types of proteins, B55 (Cdc55 in budding yeast), B56 (Rts1 in budding yeast), and B72 (B''). Studies have demonstrated that the substrate specificity and

subcellular localization of PP2A is heavily dictated by the B subunit. These two attributes of PP2A are what allows for the diverse regulation of activities that PP2A provides in the cell[71]. In budding yeast, the C subunits are encoded by three genes, *PPH21*, *PPH22*, and *PPH3*[75,76]. Despite individual knockouts of these genes having no adverse phenotypes, it has been shown that deletion of both *PPH21* and *PPH22* results in reduced growth rates and is synthetically lethal upon the deletion of the third gene, *PPH3*[76]. Thus, these results reinforce the importance and necessity of PP2A activity in cell viability. The A subunit in budding yeast is encoded by *TPD3* and is required for the association of the C subunit[73].

1.4.6 Regulators of PP2A: Cdc55 and PP2A

Given the multiple roles that PP2A undertakes in the cell, regulation of this holoenzyme must be closely monitored. One way that PP2A is regulated is through PTMs of its C subunits via reversible phosphorylation and methylation which mediates its ability to form heterotrimers[77]. Additionally, direct phosphorylation of the regulatory subunits of PP2A is another mechanism for regulating PP2A activity. Multiple pSer/Thr residues have been found on Cdc55 and Rts1 that can be phosphorylated, such as Thr242 by Cdk1, which inactivates PP2A activity. In terms of mitosis, both Cdc55 and Rts1 play a key role in regulating the G2/M transition. Knowing that phosphorylation of the conserved Y19 residue on Cdk1 triggers mitotic entry, recent studies have shown that inhibition of $PP2A^{Cdc55}$ is required for the activation of Cdk1[87]. This is because $PP2A^{Cdc55}$ regulates the phosphorylation status of Y19 indirectly by either dephosphorylating Mih1, which promotes Cdk1 activity, or dephosphorylating Swe1, which opposes Mih1 and inhibits Cdk1 activity[61]. Similarly, $PP2A^{Rts1}$ has also been shown to play a role in regulating the phosphorylation of Cdk1 as *rts1*Δ cells exhibit significant increases

in Y19 phosphorylation. Additionally, in combination with *mih1*Δ *ptp1*Δ, this triple delete mutant is inviable but is rescued upon the deletion of Swe1 arguing a role for $PP2A^{Rts1}$ in this regard.

1.5 Regulation of mitotic PP2A activity by PTPA

1.5.1 PP2A phosphatase activator (PTPA) in humans

In 1990, Cayla *et al*. were very aware of the active and important role that protein phosphatases have on the control of the cell cycle[78]. To find regulators of PTPases, Cayla *et al.* (1990) purified and identified a protein from *Xenopus laevis* ovaries that can change the residue specificity of PP2A and stimulate *in vitro* phosphotyrosine activity. Therefore, this new 40kDa protein was termed as a phosphotyrosyl phosphatase activator (PTPA). PTPA recently has also been demonstrated to not only play a critical role in determining residue specificity of PP2A but also in its assembly process[72]. This is because PTPA prompts the stabilization of the active site and helps facilitate the loading and chelating of catalytic metal ions. All of which are necessary for the acquisition of residue specificity of PP2A activity.

1.5.2 Rrd1 and Rrd2 in budding yeast

Although PP2A is mostly known for its functions as a pSer/Thr phosphatase, its metal-dependent form has been shown to have broadened substrate specificity as it enables the dephosphorylation of pTyr-containing substrates and para-nitrophenyl phosphate (NPP)[79–81]. Further supporting this function is the finding that PTPA can transiently activate pTyr phosphatase activity *in vitro*[78]. PTPA has been found to be a gene that is highly conserved throughout evolution, suggesting an important role for this protein[82,83]. In a functional analysis

conducted by Rempola *et al.* (2000), they found two novel yeast genes to two open reading frames (ORFs), *YIL153w* and *YPL152w*, that were 38% and 37% identical, respectively, to human PTPA[84]. Consequently, as deletion of these ORFs led to, among other phenotypes, rapamycin-resistance, these proteins were termed as *RRD1* and *RRD2* for rapamycin-resistance deletion[85]. This resistance often has been associated to potential roles involving the PP2A phosphatase. On the basis that the double deletion of both *RRD* genes is synthetically lethal, Rempola *et al*. (2000) concluded that one of the potential targets of Rrd2 is PP2A because over-expression of PP2Ac rescues this lethal phenotype. Shortly after this study, Fellner *et al.* (2003) conducted experiments involving the Rrd proteins to determine the elusive mechanism by which determines the specificity of PP2A *in vivo*[81]. In this study, the authors presented that both Rrd proteins are required for generating pSer/Thr-specific PP2A *in vivo*. In addition, on the basis that PTPA can convert the pSer/Thr PP2A into a pTyr phosphatase *in vitro* and that wild-type PP2A and PTPA interactions have been documented through a PTPA-affinity chromatography experiment, Fellner *et al.* (2003) wanted to further confirm this interaction[82]. Thus, through immunoprecipitation of MYC-tagged PTPA along with PP2Ac, they also observed the same interaction as seen by Cayla *et al.* (1994)[82]. To date, there has yet be any data supporting a role for these Rrd proteins in broadening the substrate specificity of PP2A *in vivo*.

Table 1: Human and budding yeast genes
A list of human and budding yeast genes discussed in this thesis.

Function	Human	Budding yeast
Wee1 tyrosine kinase	*WEE1*, *MYT1*	*SWE1*
Cdc25 protein phosphatase	*CDC25*	*MIH1*
B-subunit of PP2A	*B55* (4 isoforms)	*CDC55*
B-subunit of PP2A	*B56* (6 isoforms)	*RTS1*
Protein tyrosine phosphatase	*PTP1B*	*PTP1*
PP2A phosphatase activator	*PTPA*	*RRD1*, *RRD2*

2.0 Rationale, Hypothesis, and Objectives

2.1 Rationale

Findings from Kennedy et al., (2016) resulted in the identification of two new phosphatases participating in regulating the dephosphorylation of Cdk1-Y19, Ptp1 and PP2A[61]. Their work successfully determined that deletion of either of these phosphatases leads to increased levels of Cdk1-Y19 phosphorylation and that via an *in vitro* phosphatase assay have been shown to be specific against this tyrosine residue. Although $PP2A^{Rts1}$ plays a genetic role in dephosphorylating Cdk1-Y19, its modality in performing this function is still largely unknown. Previous *in vivo* studies have demonstrated that $PP2A^{Rts1}$ is specific against S/T-P residues which strongly suggests the possibility that $PP2A^{Rts1}$ is acting indirectly whereby it activates a downstream PTPase to dephosphorylate Cdk1-Y19[86,87]. Alternatively, based on *in vitro* experiments showing that PP2A gains phosphotyrosine-specificity when co-incubated with mammalian PTPA, it is possible that $PP2A^{Rts1}$ may acquire such activity against phosphorylated tyrosine residues by associating with PTPA[78,85] (a list of all the proteins discussed in this thesis can be found in **Table 1**). Thus, this thesis explores these two competing models for how $PP2A^{Rts1}$ regulates Cdk1-Y19 dephosphorylation.

2.2 Hypothesis

I hypothesize that both *RRD1* and *RRD2* genetically interact with Mih1, Ptp1, and Swe1 and are required to give $PP2A^{Rts1}$ phosphotyrosine specificity to induce mitotic onset. In parallel, I will also explore the possibility that $PP2A^{Rts1}$ indirectly dephosphorylates Cdk1-Y19 by activating downstream PTPases.

2.3 Objectives

i) Objective 1: Determining whether if the Rrd proteins genetically interact with the known phosphatases and kinase

ii) Objective 2: Identifying downstream PTPases of $PP2A^{Rts1}$ and if an interaction is observed with known phosphatases and kinase

3.0 MATERIALS AND METHODS

3.1 Strain and plasmid construction

All the relevant strains used, and their respective genotypes can be found in **Table 2**. All yeast strains are derivatives of W303-1a. All deletion or mutations were checked using immunoblotting, PCR, or phenotype assessment. The sequences of all the primers that were used can be given upon request. Strains were constructed by genetic cross (strains isolated through microscopic tetrad dissection) and transformation. For all PCR reactions, the reaction mixture consisted of 1X HF Buffer, 0.2mM DNTPs, 2% DMSO, 1mM of each oligonucleotide used, and Phusion polymerase (NEB). The templates for creating marker cassettes are detailed below and used at 0.1ng/µL. PCR checking of all incubation of yeast cells in 20mM NaOH at 100°C for 15 minutes, followed by a quick spin maximum speed in a microcentrifuge, 5 minutes of vortexing, and a second quick spin. 0.5µL of the supernatant was used as a DNA template for each 25µL reactions. PCR reaction conditions were as follows: 35 cycles / 20 seconds per kB of DNA, denaturation at 98°C, extension temperature at 72°C, and annealing temperature at 60°C. For plasmid amplifications, bacterial strains TG1 and DH5 α were used.

*mih1*Δ*::kanMX*, *rts1*Δ*::kanMX*, and *oca2*Δ*::kanMX* were constructed amplifying *kanMX* off pFA6a-kanMX6 and deleting MIH1, RTS1, and OCA2, respectively[88]. *rrd2-AID::kanMX* was constructed amplifying *kanMX* and *AID* off pAID1 and integrating the resulting fragment into *RRD2*[89]. *mih1*Δ*::LEU2* was constructed using pIP33 (P. Sorger, Harvard Medical School, Bostone, MA). *leu2::pGDP1-OsTIR1-LEU2* was constructed by digesting pTIR4 and pTIR6 with Stu1 and Nde1, respectively, to integrate at *LEU2* (plasmid was a gift from T. Eng and D. Koshland, UC Berkeley, CA)[89]. *mih1*Δ*::natMX* and *ptp1*Δ*::natMX* were constructed amplifying

natMX off pAG25 [90]. *arr2*Δ*::URA3* was constructed by amplifying *URA3* off PAR747[91]. The oligonucleotides used to make new and unpublished strains can be seen in **Table 3**.

3.2 Physiology

Unless stated otherwise, cells were grown in yeast extract peptone media with 2% dextrose (YPD) at 25°C. 100ng/mL α-factor (Biosynthesis) was used to arrest cells in G1. Following the arrest, cells were washed three times and suspended in YPD. Cells were re-arrested in 100ng/mL α-factor.

3.3 Serial dilution assays

When performing serial dilution assays, cells were grown to saturation in YPD at 25°C. 10-fold dilutions were then spotted using a multi-pronged serial dilution fork (DAN-KAR) onto specified plates and incubated at the indicated temperatures for one or two days. Afterwards, the plates were then imaged using BioRad imager system (BioRad). When indicated, strains were also plated onto YPD+Auxin (indole-3-acetic-acid ; Sigma-Aldrich) was used at 500μM.

3.4 Time-courses

Cells were grown at 25°C until an optical density (OD_{600}) between 0.6-0.8. Afterwards, the cells were arrested for three hours with 100ng/mL α-factor. After checking that the cells have been arrested under a microscope, the cells would be washed three times and resuspended in YPD. Cells would then be re-arrested with 100ng/mL α-factor at t = 60min or 1hr and samples would be taken at the indicated time-points. Addition of auxin in liquid media was done at 500μM.

3.5 Western blots

Cell extracts for western blots were prepped by bead beading frozen pellets at a cell density of 3.0 x 10^7 cells using the Mini-Beadbeater (BioSpec Products) into 1X SDS sample buffer (2% SDS, 80mM Tris-Cl pH 6.8, 10% glycerol, 10mM EDTA, 0.02% bromophenol blue, 1mM Na3VO4, 1mM PMSF and 5mM NaF) and an equal volume of acid washed glass beads (BioSpec Products) for 45-60 seconds. Samples were run on 12.5-15% protein gels and transferred to nitrocellulose membranes. These membranes were then stained with Ponceau S to assess protein loading, blocked for at least 30 minutes in tris-buffered saline (TBS) + 0.1% Tween-20 (TBS-T) and 4% fat-free milk powder (Carnation), or 2% BSA, at room temperature and incubated overnight at 4°C in a primary antibody solution. The use and construction of rabbit α-Swe1, α-Rts1, α-Mcd1, α-Pds1, α-Clb2, α-Clb5, and α-Cdk1 have been previously discussed in prior studies[61,92,93]. The mouse monoclonal α-Rrd2 primary antibody (a gift of Egon Ogris, Medical University of Vienna, AT) was used at a dilution factor of 1:2500 in 4% milk + TBS-T. Blots were then washed using TBS + 0.1% Tween-20 (TBS-T) for 25 minutes (5 x 5min) at room temperature and incubated in a secondary antibody solution (HRP-coupled α-mouse or α-rabbit ; BioRad) for at least 30 minutes at room temperature. Blots were washed with TBS-T for at least 30 minutes at room temperature and incubated in Clarity Western ECL (BioRad). Blots were then imaged using the ChemiDoc XRS+ System (BioRad).

4.0 RESULTS

4.1 Addition of auxin leads to rapid degradation of *rrd2-AID*

*mih1*Δ *ptp1*Δ is a double-mutant that has been shown to be viable and healthy[61,92]. Deletion of either or both these genes leads to an increase in Cdk1-Y19 phosphorylation. Additionally, the entry into mitosis is delayed and the duration of mitosis is also lengthened. Furthermore, when Kennedy *et al*. (2016) deleted the gene *RTS1* along with *mih1*Δ *ptp1*Δ, they discovered that this triple-mutant is inviable and synthetically lethal. Despite showing that $PP2A^{Rts1}$ genetically regulates Cdk1-Y19 phosphorylation and is a phosphatase, they were unable to show if this regulation was occurring in a direct or indirect manner.

Notably, $PP2A^{Rts1}$ substrates have been thought to be phosphorylated specifically on S/TP residues *in vivo*[71–74]. Interestingly, past work showing that PP2A can obtain phosphotyrosine-specificity *in vitro* in either specific reaction conditions or in the presence of PTPA, which is encoded by Rrd1 and Rrd2 in budding yeast[78,83,85]. Moreover, in support of these results, previous work in our lab has determined that deletion of either *RRD1* or *RRD2*, exhibit increased levels of Cdk1-Y19 phosphorylation (Kennedy *et al*., 2016; data not shown). Therefore, if the absence of both Rrd proteins would phenocopy the defects in growth and delays in mitosis displayed by *rts1*Δ cells, this would provide evidence that Rrd1 and/or Rrd2 change the substrate specificity of $PP2A^{Rts1}$ to target phosphotyrosine (**Figure 4-1A**). However, since the *rrd1*Δ *rrd2*Δ mutation is synthetically lethal, simply deleting both genes was not an option[81].

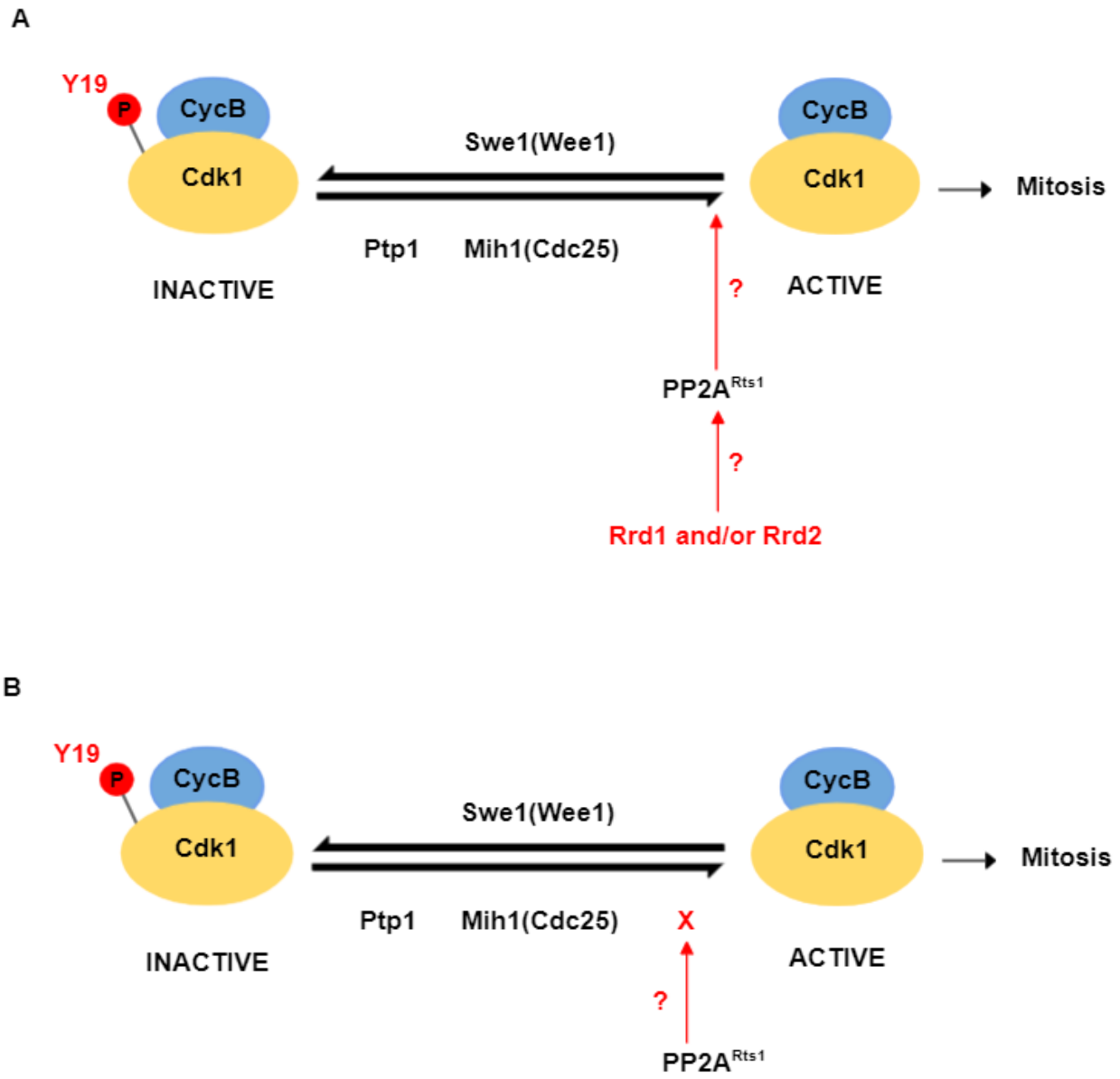
A
Y19
P
CycB
Cdk1
Swe1(Wee1)
Ptp1
Mih1(Cdc25)
INACTIVE
CycB
Cdk1
ACTIVE
Mitosis
?
PP2A^Rts1
?
Rrd1 and/or Rrd2
B
Y19
P
CycB
Cdk1
Swe1(Wee1)
Ptp1
Mih1(Cdc25)
X
INACTIVE
CycB
Cdk1
ACTIVE
Mitosis
?
PP2A^Rts1

Figure 4-1

Figure 4-1. **Potential regulation pathways of PP2A^{Rts1}: Direct vs Indirect.** (A) Rrd proteins are required for PP2A^{Rts1} to acquire phospho-tyrosine specificity against Cdk1-Y19. (B) PP2A^{Rts1} activating a downstream tyrosine phosphatase, denoted by X, which dephosphorylates Cdk1-Y19.

To avoid this lethality, conditional knockout of one of these genes must be achieved. To do so, I utilized an auxin-inducible degron (AID) system paired with an integrated *OsTIR1* which is a system known to directly and rapidly deplete targeted proteins (**Figure 4-2**)[89,94]. This system consists of three key components: 1) the plant hormone auxin (indole-3-acetic acid, IAA) which promotes the degradation of auxin transcriptional repressors through a ubiquitin proteasome pathway, 2) the auxin-receptor (Transport Inhibitor Response 1, TIR1) that is exogenously expressed via an integrating plasmid to form the ubiquitin ligase complex, and 3) *Arabidopsis thaliana* IAA17 protein which is used as an AID-tag. By incorporating these components into my strains, I created *rrd1*Δ *rrd2-AID OsTIR1*, so that I can rapidly degrade Rrd2-AID and assess the short-term consequences of the loss of Rrd1 and Rrd2.

By utilizing the AID tag system and tagging Rrd2 in an *rrd1*Δ strain, I am able to assess the immediate consequences of inactivating Rrd2 and thus, allowing the observation of cells that phenotypically behave like "*rrd1*Δ *rrd2*Δ" cells, which inviable[81,89,94]. Prior to performing a serial dilution assay to determine the growth defects in the absence of the Rrd proteins, I wanted to test the efficacy of the AID system. To determine the efficacy of the degradation of Rrd2-AID, I performed a time-course where I induced the indicated strains with auxin and collected samples at the specified time-points after which I then observed the rate of degradation of *rrd2-AID* in the samples through a western blot (**Figure 4-3**). In both time-courses, as quickly as after 5 minutes post-induction with auxin, Rrd2-AID levels are significantly lower. Notably, similar to results obtained by Lianga *et al*. (2018) for Pds1-AID and Leitao *et al*. (2019) for Rts1-AID, ~10-15% of the Rrd2-AID protein is produced and is significantly much lower compared to wild-type (**Figure 4-3B**) [93,95]. This is important to note because when compared to endogenous Rrd2 levels, Rrd2-AID levels are significantly lower suggesting a partial loss-of-function. Regardless, I

utilized this system for the rest of my experiments as it is the strain that most resembled a "*rrd1*Δ *rrd2*Δ" strain.

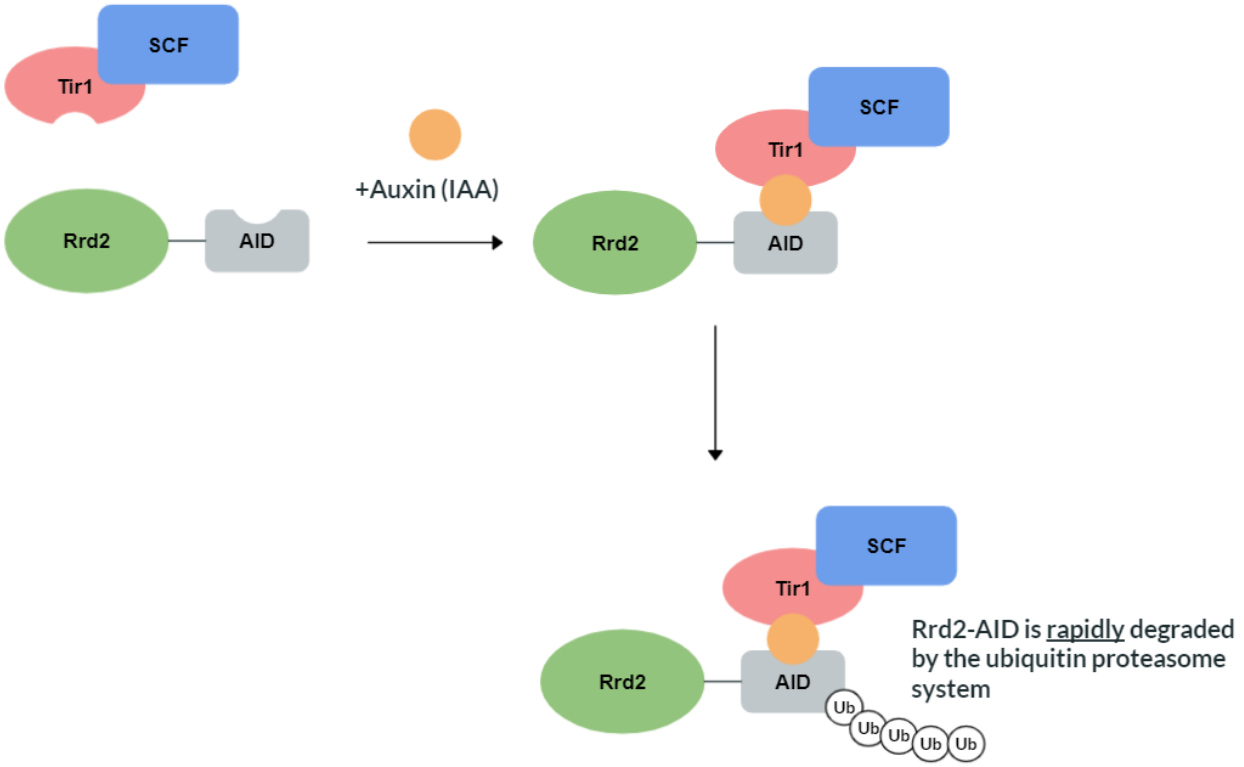
SCF
Tir1
Rrd2
AID
+Auxin (IAA)
SCF
Tir1
Rrd2
AID
SCF
Tir1
Rrd2
AID
Ub
Ub
Ub
Ub
Ub
Rrd2-AID is rapidly degraded by the ubiquitin proteasome system

Figure 4-2

Figure 4-2. **Degradation of Rrd2-AID using AID system.**
Stepwise diagram depicting the AID system: 1) Addition of auxin, 2) In the presence of auxin, Tir1 is recruited, and 3) AID-tagged protein (Rrd2-AID) is degraded by ubiquitin proteasome.

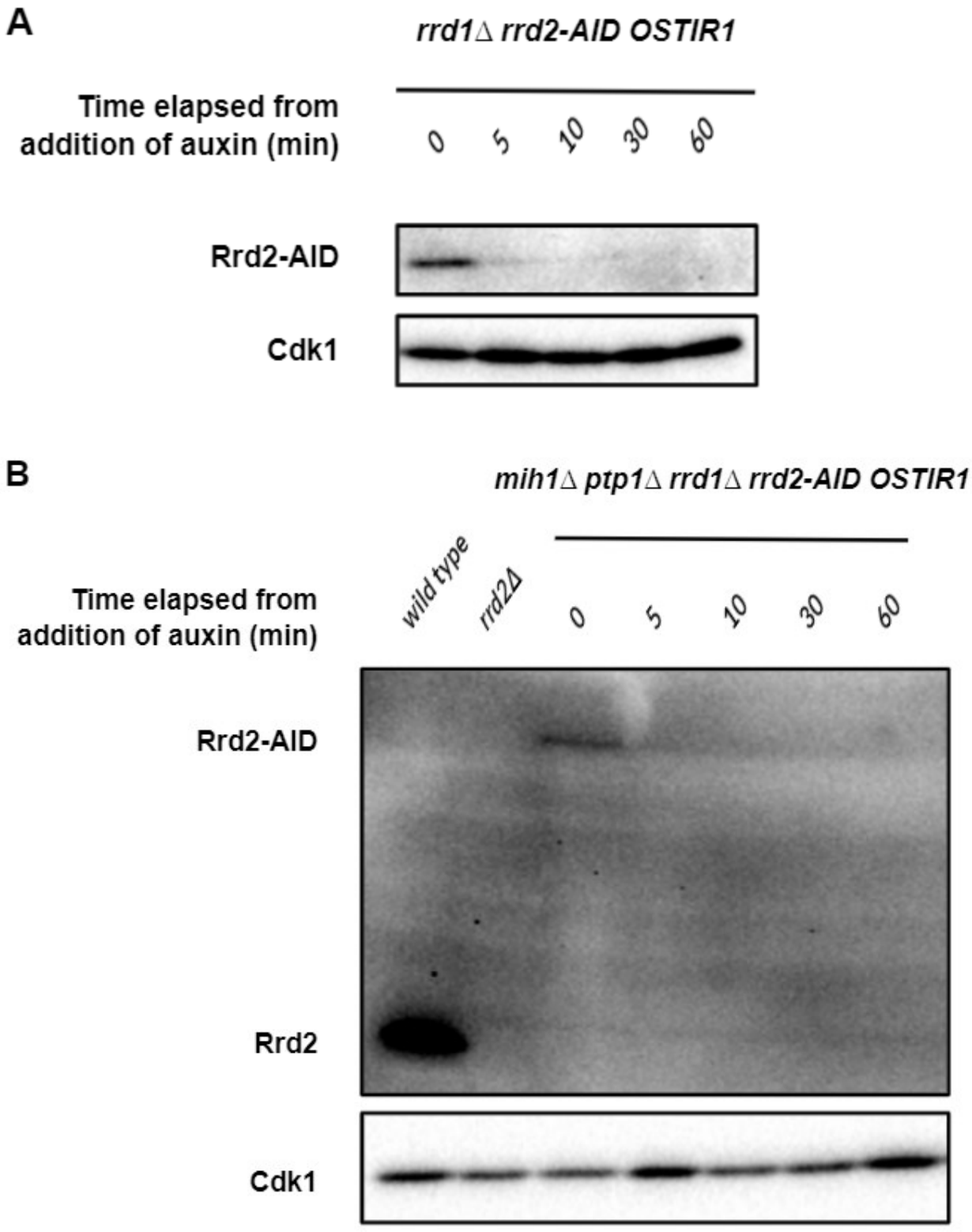
A
rrd1△ rrd2-AID OSTIR1
Time elapsed from addition of auxin (min)
0
5
10
30
60
Rrd2-AID
Cdk1
B
mih1△ ptp1△ rrd1△ rrd2-AID OSTIR1
wild type
rrd2Δ
Time elapsed from addition of auxin (min)
0
5
10
30
60
Rrd2-AID
Rrd2
Cdk1

Figure 4-3

Figure 4-3. **Addition of Auxin leads to rapid degradation of *rrd2-AID*.**
(A) *rrd1*Δ *rrd2-AID OsTIR1* (ADR11082) was grown to log phase and subjected to auxin (t = 0min). Samples were taken for immunoblotting at the time-points indicated. α-Rrd2 antibody used to blot against Rrd2-AID. (B) Wild type (ADR10331), *rrd2*Δ *swe1*Δ (ADR10971), and *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1* (ADR11072) were grown to log phase and subjected to auxin (t = 0min). Samples were taken for immunoblotting at the time-points indicated. α-Rrd2 antibody used to blot against Rrd2-AID.

4.2 *RRD1* and *RRD2* genetically interact with *MIH1*, *PTP1*, and *SWE1*

Because the deletion of these genes led to higher Cdk1-Y19 levels, I wanted to test if *RRD1* and/or *RRD2* genetically interacted with the phosphatases, *MIH1* and *PTP1*, and the kinase, *SWE1* which are known to participate in this pathway. To assess if there are interactions that exist between these genes, I performed a serial dilution assay and assessed any differences in viability and growth rate (**Figure 4-4**). In this assay, I spotted the indicated strains onto two conditions, YPD and YPD+Auxin, to determine the effects of the loss of both Rrd proteins. From this assay, I primarily observed that *rrd1*Δ *rrd2-AID OsTIR1* showed defects in both growth and viability in the presence of auxin especially when compared to the two negative controls, wild type and *mih1*Δ *ptp1*Δ, which exhibited no defects when plated onto both YPD and YPD+Auxin. Importantly, I observed that deletion of *mih1*Δ *ptp1*Δ in these Rrd mutants, the cells became more sick suggesting that *RRD1* and *RRD2* are genetically interacting with *MIH1* and *PTP1*. Moreover, it is important to take note of the partial rescue of viability that is observed upon the deletion of *SWE1* in both *rrd1*Δ *rrd2-AID OsTIR1* and *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1*.

*rts1*Δ cells are very sick when combined with *mih1*Δ but are rescued upon the deletion of *SWE1*[96,97]. Similarly, since the lethality of *rrd1*Δ *rrd2-AID OsTIR1* and *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1* are partially suppressed upon the deletion of *SWE1*, this is evidence that *RRD1* and *RRD2* are regulating the same pathway regulated by *SWE1* – the Cdk1-Y19 pathway. Lastly, as the third and last negative control, I wanted to examine if the AID tag alone, without expression of *TIR1* via the TIR1-containing plasmid, had any unexpected effects. Even without the expression of *TIR1*, *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID* seems to have a slight growth defect. This is unlikely due to *rrd1*Δ alone as no growth defects have been observed when *RRD1* is deleted

alone. This defect can likely be attributed to either the genetic interaction between *mih1*Δ *ptp1*Δ with *rrd1*Δ and/or the lower basal levels observed in Rrd2-AID strains (**Figure 4-3**).

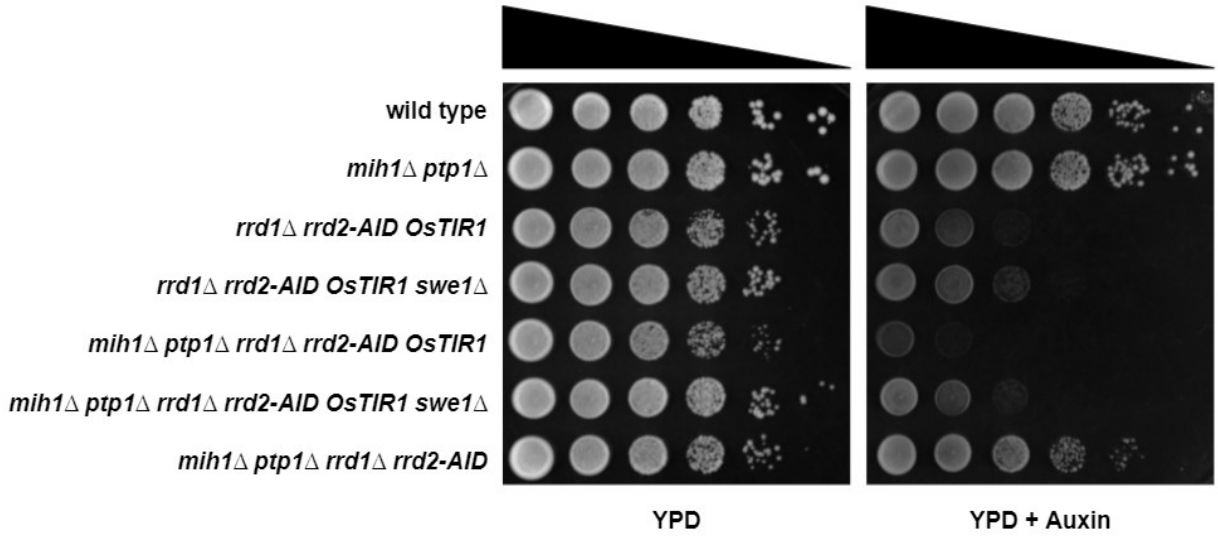
wild type
mih1Δ ptp1Δ
rrd1Δ rrd2-AID OsTIR1
rrd1Δ rrd2-AID OsTIR1 swe1Δ
mih1Δ ptp1Δ rrd1Δ rrd2-AID OsTIR1
mih1Δ ptp1Δ rrd1Δ rrd2-AID OsTIR1 swe1Δ
mih1Δ ptp1Δ rrd1Δ rrd2-AID
YPD
YPD + Auxin

Figure 4-4

Figure 4-4. **Rrd proteins genetically interact with Mih1, Ptp1, and Swe1.**
Ten-fold serial dilutions of wild type (ADR10331), *mih1*Δ *ptp1*Δ (ADR10852), *rrd1*Δ *rrd2-AID OsTIR1* (ADR11079), *rrd1*Δ *rrd2-AID OsTIR1 swe1*Δ (ADR11075), *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1* (ADR11073), *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1 swe1*Δ (ADR11072), and *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID* (ADR11084). Cells were spotted onto the indicated plates, pre-grown and grown at 25°C.

4.3 Absence of Rrd proteins causes mitotic delays

*mih1*Δ cells display higher levels of Cdk1-Y19 phosphorylation and also show delayed degradation of the mitotic cyclins Clb2 and Clb5, the anaphase inhibitor Pds1/Securin, and the cohesin subunit Mcd1/Scc1[69,104-108]. As aforementioned, by using the AID tag system I can examine the short-term effects on cells that lack Rrd1 and Rrd2. Given that $PP2A^{Rts1}$ plays a role in mitosis and that I hypothesize that Rrd1 and Rrd2 co-activate $PP2A^{Rts1}$, I wanted to investigate if the absence of Rrd1 and Rrd2 could influence the progression of mitosis or delay the degradation of mitotic substrates. To determine this, I compared cell cycle progression of *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1* (denoted as SWE1) and *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1 swe1*Δ (denoted as *swe1*Δ) cells after release from an arrest in the G1 phase of the cell cycle. Both strains enter mitosis with similar timing, but there are significant delays in mitotic progression in the *SWE1* strain. The degradation of the mitotic cyclins Clb2 and Clb5, and the anaphase inhibitor Pds1, are delayed by several hours (**Figure 4-5**). Looking at Mcd1, which is a protein which peaks at metaphase of M phase, it is arguable that there is a small delay in the *SWE1* strain compared to *swe1*Δ suggesting that mitotic exit is slightly delayed. However, when assessing the effects on the mitotic cyclins Clb2 and Clb5, there are significant delays in mitotic progression in the *SWE1* strain – particularly Clb2 displays a much more significant delay in degradation. These results suggest that the duration of mitosis is prolonged in the *SWE1* strain. Importantly, these delays are rescued by the deletion of *SWE1* which strongly suggests that Rrd1 and Rrd2 are playing an important role in regulating mitotic $PP2A^{Rts1}$ activity.

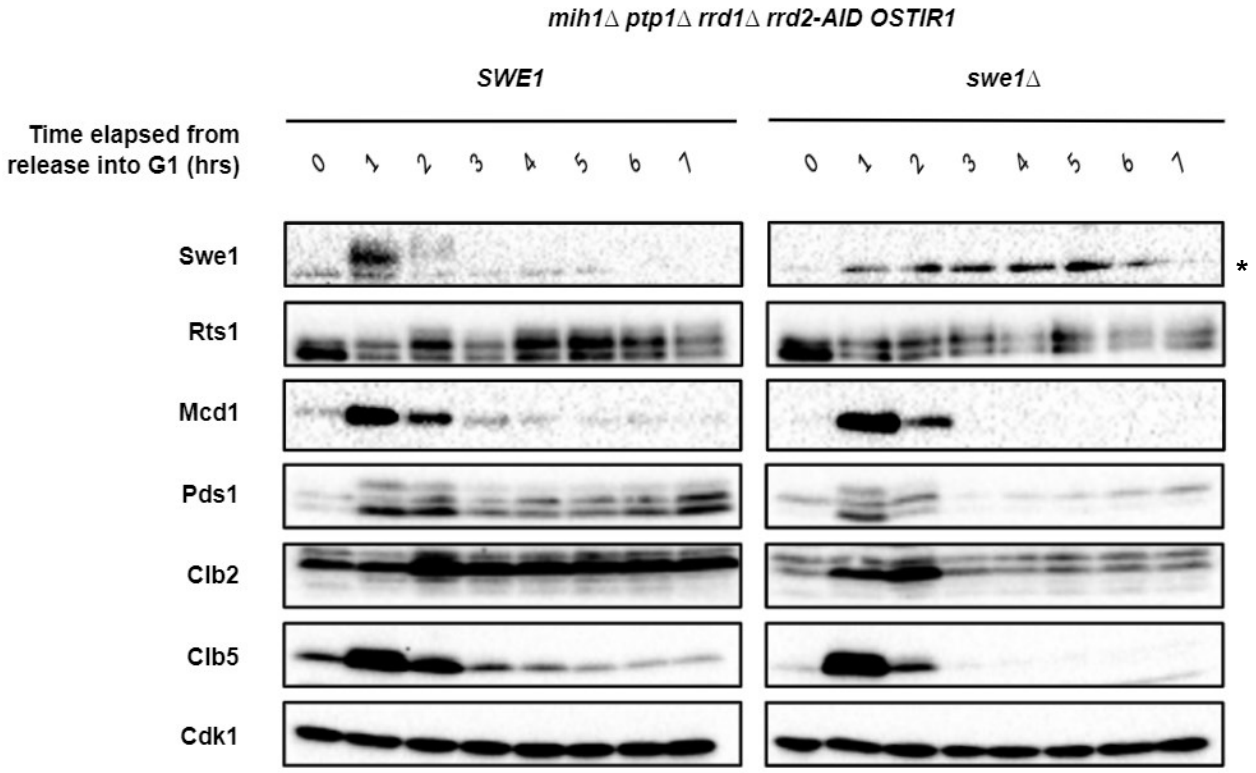
mih1Δ ptp1Δ rrd1Δ rrd2-AID OSTIR1
SWE1
swe1Δ
Time elapsed from release into G1 (hrs)
0 1 2 3 4 5 6 7
0 1 2 3 4 5 6 7
Swe1
Rts1
Mcd1
Pds1
Clb2
Clb5
Cdk1
*

Figure 4-5

Figure 4-5. **Absence of Rrd proteins causes mitotic delays.**
*mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1* (ADR11073 ; denoted as SWE1) and *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1 swe1*Δ (ADR11072 ; denoted as *swe1*Δ) were grown, arrested at G1 using α-factor and released into the cell cycle (t = 0hr). The cells are then re-arrested using α-factor at t = 1hr to re-arrest the cells in the following G1. Samples were taken for immunoblotting at the time-points indicated. Rts1 and Cdk1 are used as loading controls. Asterisk (*) denotes that the bands observed are background bands.

4.4 Ych1 and Arr2 act independently of Mih1 and Ptp1

Currently, all *in vivo* data suggests $PP2A^{Rts1}$ to be only phospho-specific against S/T-P residues which strongly suggests that $PP2A^{Rts1}$ regulates Cdk1-Y19 phosphorylation indirectly by activating a downstream protein (**Figure 4-1B**)[71–74]. My strategy to explore the indirect regulation is to mutate a candidate protein (or a combination of numerous proteins) with the aim to phenocopy *rts1*Δ: 1) synthetically lethal in combination with *mih1*Δ *ptp1*Δ, 2) exhibit suppression by *swe1*Δ and 3) be epistatic to *rts1*Δ. Compiling high-throughput analyses of proteins interacting with $PP2A^{Rts1}$ and comparisons of BLASTP sequences, I have created mutants knocking out the following candidates: *YCH1*, *ARR2*, *OCA2*, *SPD1*[103].

Out of the four candidate proteins, Ych1 and Arr2 were prioritized. Ych1 (yeast Cdc25 homolog 1) was prioritized as this phosphatase's protein sequence is very similar to Mih1, which is a member of the Cdc25p subfamily of PTPases and contains a rhodanese PTPase domain, HCX_5R, which is the active site of all Cdc25 PTPases (**Figure 4-6**)[104,105]. Similarly, although Arr2 is classified as an arsenate reductase, this protein also possesses the rhodanese catalytic motif seen in PTPase Cdc25[111,112]. From the BLASTP sequence alignment, it is evident that Ych1 and Arr2 share sequence similarities making them prime candidates to explore (**Figure 4-6**) – though Ych1 and Arr2 are significantly smaller proteins compared to Mih1 that only contains the PTPase domain[108].

To further explore these two candidates, I deleted *YCH1* and *ARR2*, and using genetic crosses, created strains to compare the growth rates of serial dilutions of single mutants and combinations of *mih1*Δ, *ptp1*Δ, *ych1*Δ, *arr2*Δ, and *swe1*Δ (**Figure 4-7**). This was done to determine if either *YCH1* and/or *ARR2* play a role in dephosphorylating Cdk1-Y19. Deletion of *YCH1* and/or *ARR2* (Lanes 1-3) cause no defects in growth of the cells. However, when these

mutants are combined with *mih1*Δ *ptp1*Δ and incubated at high temperatures, a loss in viability is observed (Lanes 5-7) demonstrating a synthetic genetic interaction with *MIH1* and *PTP1*. The reduction in viability is rescued upon the deletion of *SWE1*, suggesting that Arr2 and Ych1, like Mih1 and Ptp1, function to regulate Cdk1-Y19 phosphorylation. This data suggests that Ych1 and Arr2 act in parallel to Mih1 and Ptp1 but cannot distinguish if they work downstream of $PP2A^{Rts1}$ or in a pathway independent of PP2A. it suggests that these candidate PTPases are either being activated downstream or are acting in parallel of $PP2A^{Rts1}$.

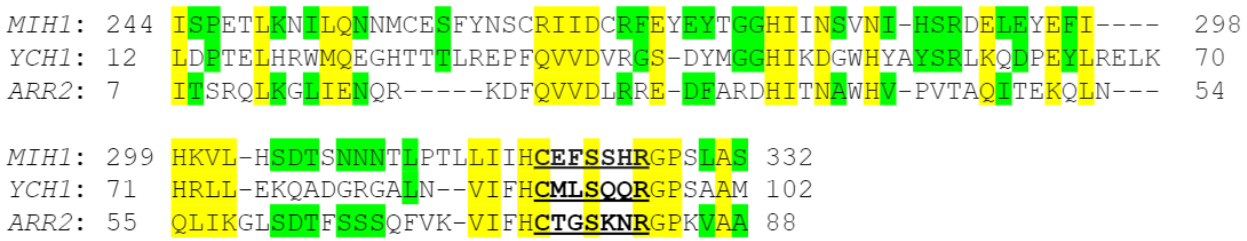
MIH1: 244 ISPETLKNILQNNMCESFYNSCRIIDCRFEYEYTGGHIINSVNI-HSRDELEYEFI---- 298
YCH1: 12 LDPTELHRWMQEGHTTTLREPFQVVDVRGS-DYMGGHIKDGWHYAYSRLKQDPEYLRELK 70
ARR2: 7 ITSRQLKGLIENQR-----KDFQVVDLRRE-DFARDHITNAWHV-PVTAQITEKQLN--- 54
MIH1: 299 HKVL-HSDTSNNNTLPTLLIIHCEFSSHRGPSLAS 332
YCH1: 71 HRLL-EKQADGRGALN--VIFHCMLSQQRGPSAAM 102
ARR2: 55 QLIKGLSDTFSSSQFVK-VIFHCTGSKNRGPKVAA 88

Figure 4-6

Figure 4-6. ***MIH1*, *YCH1*, and *ARR2* share structural motif.**
Sequences obtained from *Saccharomyces* Genome Database (SGD). BLASTP sequence alignment of *MIH1* (SGD:S000004639), *YCH1* (SGD:S000003435), and *ARR2* (SGD000006404) showing biochemical similarities between *MIH1* and two candidate proteins, *YCH1* and *ARR2*. Residues highlighted in yellow indicates high conservation among all three proteins and residues highlighted in green indicates partial conservation between *MIH1* and two candidate proteins. Bolded and underlined indicates the rhodanese catalytic site present in the PTPase Cdc25 (Mih1).

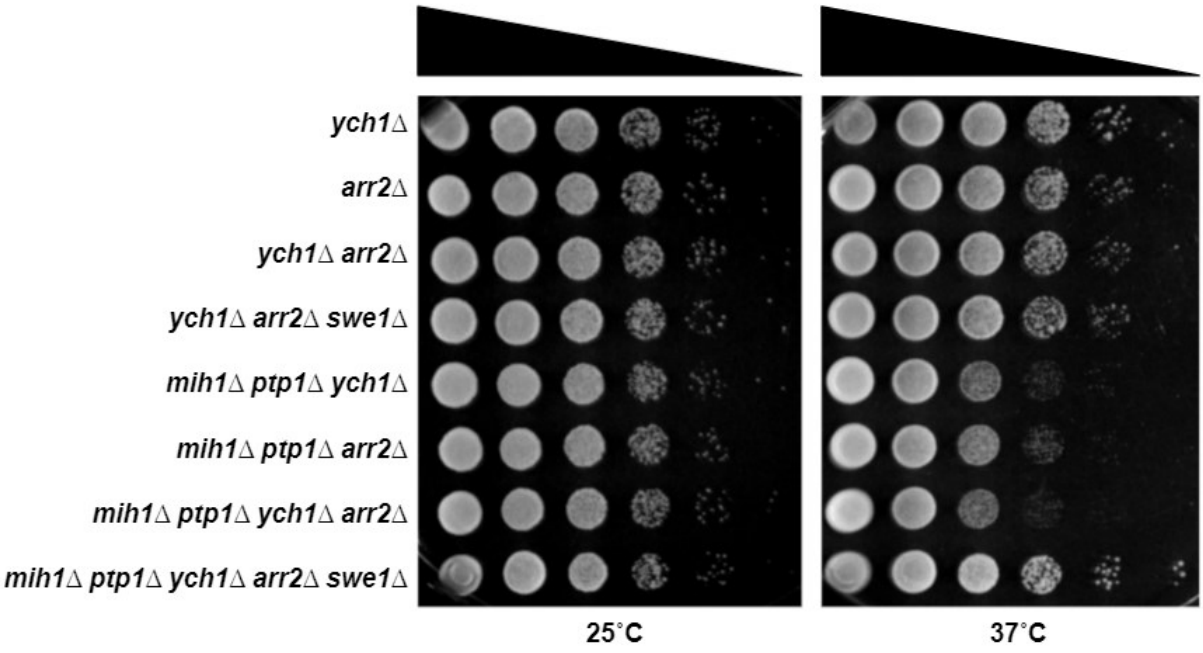
ych1Δ
arr2Δ
ych1Δ arr2Δ
ych1Δ arr2Δ swe1Δ
mih1Δ ptp1Δ ych1Δ
mih1Δ ptp1Δ arr2Δ
mih1Δ ptp1Δ ych1Δ arr2Δ
mih1Δ ptp1Δ ych1Δ arr2Δ swe1Δ
25˚C
37˚C

Figure 4-7

Figure 4-7. **Ych1 and Arr2 act independently of Mih1 and Ptp1.**
Ten-fold serial dilutions of *ych1*Δ (ADR11053), *arr2*Δ (ADR11054), *ych1*Δ *arr2*Δ (ADR11096), *ych1*Δ *arr2*Δ *swe1*Δ (ADR11098), *mih1*Δ *ptp1*Δ *ych1*Δ (ADR11092), *mih1*Δ *ptp1*Δ *ych1*Δ (ADR11094), *mih1*Δ *ptp1*Δ *arr2*Δ (ADR11101), *mih1*Δ *ptp1*Δ *ych1*Δ *arr2*Δ (ADR11102), *mih1*Δ *ptp1*Δ *ych1*Δ *arr2*Δ *swe1*Δ (ADR11103). Cells were spotted onto the indicated plates, pre-grown and grown at 25°C.

4.5 Deletion of *YCH1* has no effect on *rts1*Δ cells

Given the results in Figure 4-4 where *mih1*Δ *ptp1*Δ *ych1*Δ, *mih1*Δ *ptp1*Δ *arr2*Δ, and *mih1*Δ *ptp1*Δ *ych1*Δ *arr2*Δ all exhibit growth defects, this can be due to either Ych1 and Arr2 acting in parallel of $PP2A^{Rts1}$ or that these two proteins are acting downstream of $PP2A^{Rts1}$. To elucidate this question, I created strains in which I deleted *YCH1* and/or *ARR2* in combination with *rts1*Δ and compared the growth of serial dilutions of the indicated strains by performing a serial dilution assay (**Figure 4-8**). In this assay, I observed that when *ych1*Δ is combined with *rts1*Δ, the strain is unaffected when compared to *rts1*Δ alone suggesting that $PP2A^{Rts1}$ may be upstream to Ych1. Similar to combinations with *mih1*Δ *ptp1*Δ (**Figure 4-7**), when *SWE1* is deleted, *rts1*Δ and *rts1*Δ *ych1*Δ are partially rescued, and to an equal extent. Interestingly, *arr2*Δ combined with *rts1*Δ leads to an increase in viability when compared to *rts1*Δ alone. These growth defects I am observing with Arr2 may be due to a pathway independent of the Cdk1-Y19 pathway. But looking at how the phenotype of *rts1*Δ *ych1*Δ *arr2*Δ is like *rts1*Δ *ych1*Δ, there is the potential argument that Arr2 may be a negative regulator of Ych1 but no interaction between these two proteins has yet to be made.

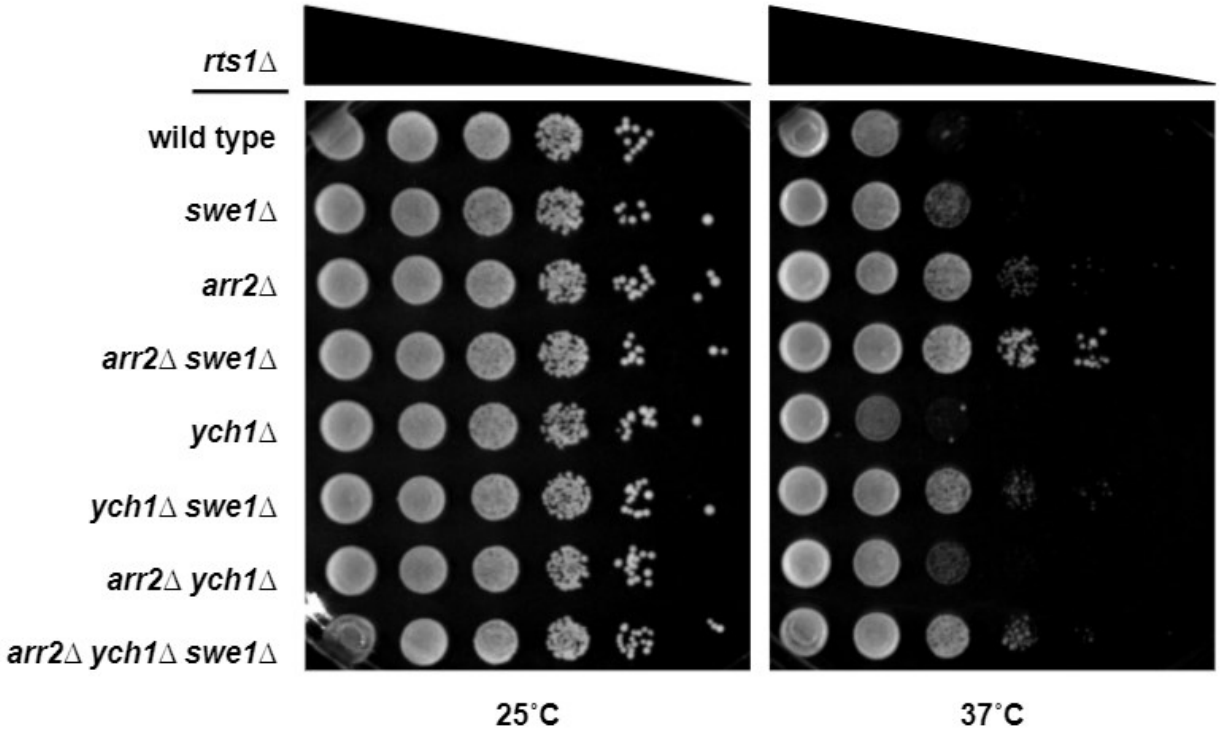
rts1Δ
wild type
swe1Δ
arr2Δ
arr2Δ swe1Δ
ych1Δ
ych1Δ swe1Δ
arr2Δ ych1Δ
arr2Δ ych1Δ swe1Δ
25˚C
37˚C

Figure 4-8

Figure 4-8. **Deletion of *YCH1* has no effect on *rts1*Δ cells.**
Ten-fold serial dilutions of *rts1*Δ (ADR9538), *rts1*Δ *swe1*Δ (ADR9534), *rts1*Δ *ych1*Δ (ADR11126), *rts1*Δ *ych1*Δ *swe1*Δ (ADR11127), *rts1*Δ *arr2*Δ (ADR11128), *rts1*Δ *arr2*Δ *swe1*Δ (ADR11129), *rts1*Δ *ych1*Δ *arr2*Δ (ADR11130), *rts1*Δ *ych1*Δ *arr2*Δ *swe1*Δ (ADR11131). Cells were spotted onto the indicated plates, pre-grown and grown at 25°C.

Figure 4-6. *SPD1* weakly interacts with *MIH1*, *PTP1*, and *SWE1* but *OCA1* does not

Like *YCH1*, *OCA2* and *SDP1* belong to a class of little studied tyrosine phosphatases. *OCA2* has been shown to encode a putative protein where upon its deletion, demonstrates caffeine sensitivity[109]. This phenotype is characteristic of mutants of the cell-integrity pathway[110]. Moreover, through a global analysis performed by Huh *et al*. (2003), they predicted Oca2 as being a PTPase as well[103]. As per *SDP1*, this gene encodes a PTPase that has been found to have dual-specificity for cysteine and tyrosine residues[111]. Therefore, I combined *oca2*Δ and *sdp1*Δ with *mih1*Δ *ptp1*Δ and compared the growth rates of serial diluted cultures (**Figure 4-9**). From this assay, *mih1*Δ *ptp1*Δ *spd1*Δ demonstrates a slight defect in growth as the colonies appear to be smaller when compared to *mih1*Δ *ptp1*Δ alone whereas *mih1*Δ *ptp1*Δ *oca2*Δ appears to show no phenotype.

I tested these proteins in parallel to Ych1 and Arr2 to determine if these enzymes also played a role in dephosphorylating Cdk1-Y19. To do so, I performed a serial dilution assay in which I observed no significant changes in growth or viability of the cells. Although the colonies at the lowest concentration are slightly sicker in *mih1*Δ *ptp1*Δ *spd1*Δ, if these Sdp1 was to be involved in dephosphorylating Cdk1-Y19, its role would be minimal. Thus, I refrained from continuing with these proteins any further.

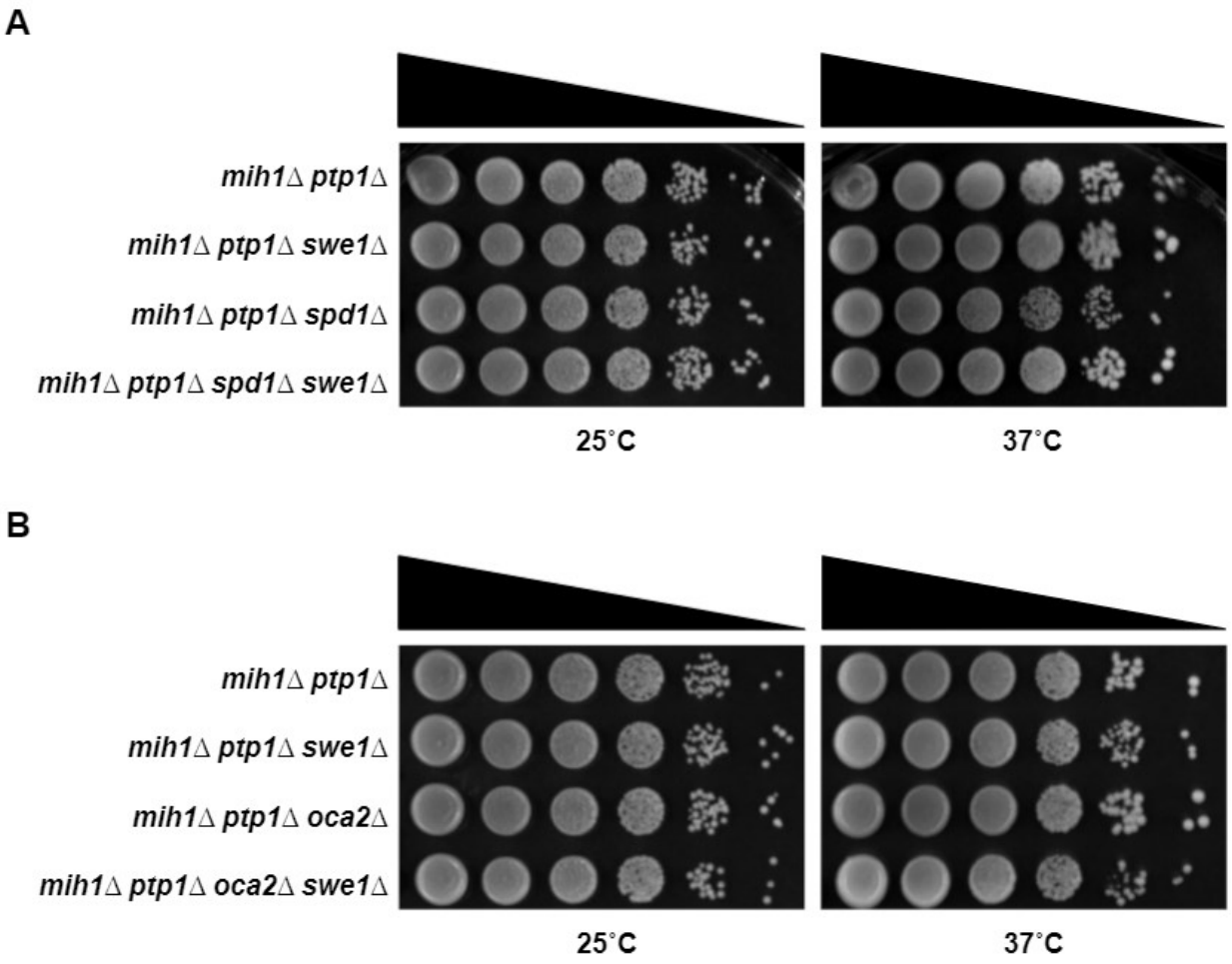
A
mih1Δ ptp1Δ
mih1Δ ptp1Δ swe1Δ
mih1Δ ptp1Δ spd1Δ
mih1Δ ptp1Δ spd1Δ swe1Δ
25˚C
37˚C
B
mih1Δ ptp1Δ
mih1Δ ptp1Δ swe1Δ
mih1Δ ptp1Δ oca2Δ
mih1Δ ptp1Δ oca2Δ swe1Δ
25˚C
37˚C

Figure 4-9

Figure 4-9. **SPD1 weakly interacts with *MIH1*, *PTP1*, AND *SWE1* but *OCA2* does not.** (A) Ten-fold serial dilutions of *mih1*Δ *ptp1*Δ (ADR10854), *mih1*Δ *ptp1*Δ *swe1*Δ (ADR10867), *mih1*Δ *ptp1*Δ *spd1*Δ (ADR10936), *mih1*Δ *ptp1*Δ *spd1*Δ *swe1*Δ (ADR10844) (B) Ten-fold serial dilutions of *mih1*Δ *ptp1*Δ (ADR10854), *mih1*Δ *ptp1*Δ *swe1*Δ (ADR10867), *mih1*Δ *ptp1*Δ *spd1*Δ *oca2*Δ (ADR10858), and *mih1*Δ *ptp1*Δ *spd1*Δ *oca2*Δ *swe1*Δ (ADR10806). Cells were spotted onto the indicated plates, pre-grown and grown at 25°C.

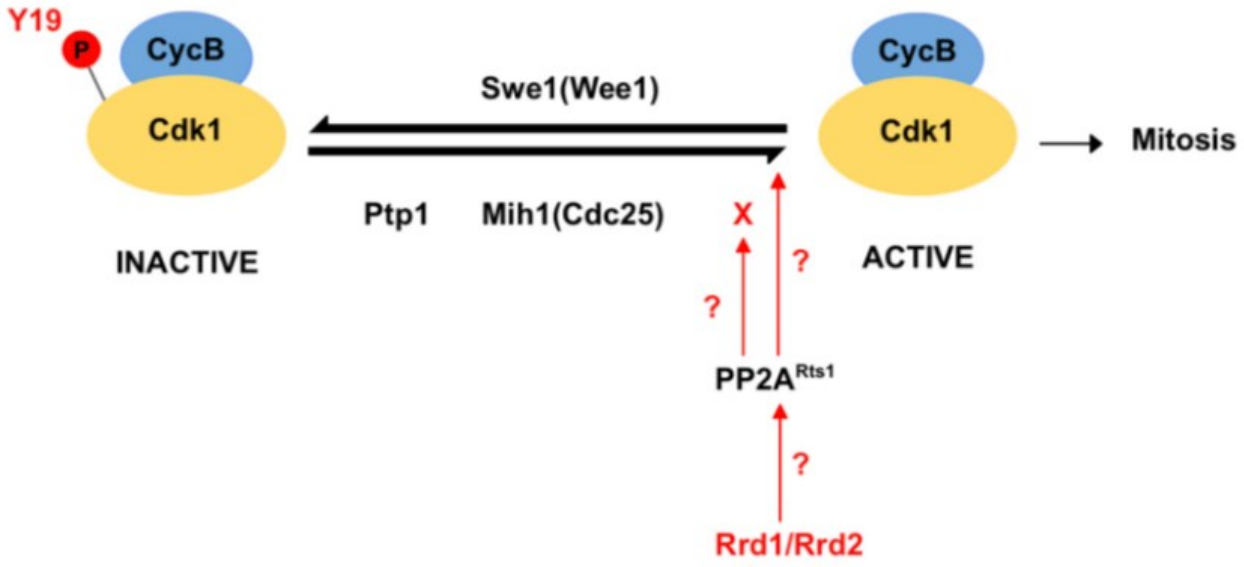
Y19
P
CycB
Cdk1
INACTIVE
Swe1(Wee1)
Ptp1
Mih1(Cdc25)
CycB
Cdk1
ACTIVE
Mitosis
X
?
?
PP2A^Rts1
?
Rrd1/Rrd2

Figure 4-10

Figure 4-10. **Potential regulation pathways of PP2A^{Rts1}: Direct and Indirect.** Combination of both direct and indirect Cdk1-Y19 dephosphorylation by PP2A^{Rts1}.

5.0 DISCUSSION

Notably, *Russell and Nurse* (1986) showed that the deletion of *CDC25* in fission yeast is lethal as it caused the cells to arrest in G2, indicating the necessity of Cdk1-Y15 dephosphorylation[64]. However, upon deletion of *MIH1*, even though budding yeast cells experience delayed mitotic onset and anaphase onset, these delays are only mild[92]. Such behaviour led Kennedy *et al*. (2016) to pursue the possibility that at least one additional phosphatase exists that is participating redundantly with Mih1 in this pathway[61]. This eventually led to the identification of two phosphatases, Ptp1 and $PP2A^{Rts1}$, which work redundantly with Mih1 to remove the inhibitory phosphorylation on Cdk1. Although Kennedy *et al*. (2016) found that $PP2A^{Rts1}$ plays a role in dephosphorylating Cdk1-Y19, its regulation and mode of function are still largely unknown. My objectives aimed to determine how mitotic $PP2A^{Rts1}$ activity regulates Cdk1-Y19 dephosphorylation and whether it functions via a direct and/or indirect pathway (**Figure 4-1**).

5.1 Rrd proteins play a role in dephosphorylating Cdk1-Y19

The growth assay presented in Figure 4-4 suggests that the Rrd proteins function with Mih1 and Ptp1 to dephosphorylate Cdk1-Y19. This is evident by the severe growth defect when *rrd1*Δ *rrd2-AID OsTIR1* is combined with *mih1*Δ *ptp1*Δ and grown in media containing auxin. The loss in viability is then partially rescued when *SWE1* is deleted arguing that the lethality is mediated through the Cdk1-Y19 pathway. It is important to note that PP2A has a plethora of functions outside of its regulation of the Cdk1-Y19 phosphorylation which could account for the partial rescue by Swe1 that is observed and the phenotype of *rrd1*Δ *rrd2-AID OsTIR1* and *rrd1*Δ

*mih1*Δ *ptp1 rrd2-AID OsTIR1* [80-83]. Notably, Rrd1 has other functions such as its involvement in Tap42-phosphatase complexes which are integral for activating Tor proteins[112]. And given that *rrd1*Δ *rrd2*Δ is synthetically lethal, this could explain why *rrd1*Δ *rrd2-AID OsTIR1* strains are sick in the absence of auxin. We also note that Rrd2-AID is expressed at lower levels compared to untagged Rrd2 prior to auxin addition observed in *rrd2-AID* mutants when testing the efficacy of the AID tag system (**Figure 4-3**), suggesting that the sick of *rrd1*Δ *rrd2-AID OsTIR1* may be due to partial loss-of-function of Rrd2. The premature degradation of the target protein even without the addition of auxin due to the high expression rate of the TIR1-containing plasmid is a limitation of the AID system[113–115]. Despite this limitation, the degradation of Rrd2 is significant when comparing conditions with auxin (**Figure 4-3**) and only has a subtle effect on viability when spotted on YPD (**Figure 4-4**).

5.2 Absence of Rrd proteins delays mitotic entry and anaphase onset/progression

In order to determine the effects of the *mih1*Δ *ptp1*Δ double-mutant, Kennedy and her colleagues performed a time-course where they observed a mild delay in mitotic entry, a prolonged mitosis, and an increase in Cdk1-Y19 phosphorylation which did not significantly vary from the deletion of *MIH1* alone[69,99]. Similarly, to evaluate the effects of the absence of Rrd proteins on mitotic entry and progression, I performed a time-course comparing *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1* and *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1 swe1*Δ. From this time-course, I observed that mitotic progression was significantly delayed in the *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1* strain and these delays were rescued upon the deletion of *SWE1*. I determined this by comparing the delays in degradation of several anaphase-promoting complex (APC)

substrates and mitotic cyclins between *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1* and *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1 swe1*Δ.

Given that a key characteristic of cyclins is that they are substrates which appear and disappear in a periodic manner, making them great substrates to track the cell cycle[99]. From Figure 4-4, I observed that *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1* is very sick in the presence of auxin. Therefore, I wanted to determine if this defect in growth would correlate with impediments in Clb2 and/or Clb5 degradation, suggesting delays in mitotic progression. Comparing the Clb2 and Clb5 levels between the two strains, it is evident that there are delays in both Clb2 and Clb5 degradation in *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1*, which is rescued upon the deletion of *SWE1*. This rescue by *swe1*Δ is very important as it strongly suggests that the Rrd proteins are meditating this process through Cdk1-Y19 dephosphorylation. Notably, these delays in mitotic cyclin degradation alone cannot give an approximation to where the delay in mitosis is occurring.

To further explore this delay, I also blotted against APC substrates, Mcd1 and Pds1, which are targeted for degradation by the APC upon anaphase onset[116]. Moreover, since cleavage of Mcd1 triggers sister chromatid separation and destruction of Pds1 (also known as Securin) inhibits anaphase by binding to Separin/Esp1, delay in degradation of either of these substrates would be indicative of a delay in anaphase onset/progression[102,108,123,124,125]. Looking at Figure 4-4, it is evident that there is a slight delay in the destruction of Mcd1, suggesting that there is a delay in anaphase onset. Furthermore, in addition to this delay, there is also a severe delay in Pds1 as well. Together, the results suggest that the sickness seen in *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1* is caused by a delay in the degradation of mitotic cyclins and APC substrates. And given that these defects are rescued upon the deletion of *SWE1*, this strongly suggests that the Rrd

proteins are regulating this process through Cdk1-Y19 dephosphorylation. Future directions will involve live microscopy to track spindle lengths to determine the stage of mitosis *mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1* cells are delayed at.

5.3 *YCH1* and *ARR2* genetically interact with *MIH1*, *PTP1*, and *SWE1*

As previously stated, $PP2A^{Rts1}$ has only ever been proven to be a phosphatase phospho-specific against S/T-P residues suggesting that $PP2A^{Rts1}$ is likely dephosphorylating Cdk1-Y19 through an indirect pathway[71–74](**Figure 4-1B**). Utilizing a candidate approach, I prioritized my attention on Ych1 and Arr2 because both of these proteins are biochemically similar to Mih1 (**Figure 4-6**)[104].

Although these proteins share structural motif with Mih1, firstly, I wanted to obtain preliminary evidence suggesting that these candidate phosphatases may be involved in dephosphorylating Cdk1-Y19. My approach was to look if either of these proteins showed genetic interactions with *MIH1* and *PTP1*. To determine this, I performed a serial dilution assay making different combinations as indicated in Figure 4-7. Based on the results, I observed that when either or both are deleted with *mih1*Δ *ptp1*Δ, growth defects and loss of viability is present which suggests that Ych1 and Arr2 are interacting with Mih1 and Ptp1. Notably, the sickness exhibited by *mih1*Δ *ptp1*Δ *ych1*Δ *arr2*Δ is fully rescued upon deletion of *SWE1*, suggesting that Ych1 and/or Arr2 are involved in mediating Cdk1-Y19 dephosphorylation.

5.4 $PP2A^{Rts1}$ is epistatic to Ych1

Since Figure 4-7 demonstrated that Ych1 and Arr2 are acting independent of Mih1 and Ptp1, I next wanted to determine if these proteins are acting independently of $PP2A^{Rts1}$ or in the

PP2A^{Rts1} pathway. As such, my approach was to create different mutant combinations of *ych1*Δ, *arr2*Δ, *swe1*Δ, and *rts1*Δ and to observe how these proteins interacted with one another. To test this, I performed another serial dilution assay (**Figure 4-8**) which I was interested in determining if deletion of *YCH1* and/or *ARR2* would modify the phenotype of *rts1*Δ. The rationale behind this experiment was if the deletion of *YCH1* and/or *ARR2* would cause *rts1*Δ cells to be sicker, it would argue that these proteins are working independently of PP2A^{Rts1}. However, if deleting these phosphatases would not affect the viability of *rts1*Δ cells, it would argue the opposite, in which these phosphatases function in the PP2A^{Rts1} pathway.

Based on the results I obtained in Figure 4-8, I observed that although *mih1*Δ *ptp1*Δ becomes sicker upon the deletion of *YCH1*, the same cannot be said with *rts1*Δ cells, suggesting that Ych1 requires PP2A^{Rts1} to perform its function. Based on these results, it argues that PP2A^{Rts1} influences the function of Ych1. Interestingly, *arr2*Δ *rts1*Δ combined with *swe1*Δ leads to an increase of suppression when compared to *rts1*Δ *swe1*Δ suggesting that the growth defects I am observing with Arr2 may be due to a pathway independent of the Cdk1-Y19 pathway.

5.5 Future Directions

Although the Rrd proteins (associated with PP2A^{Rts1}), Ych1, and Arr2 are arguably playing a role in dephosphorylating Cdk1-Y19, I have no evidence proving it has the specificity or activity against this phosphorylated residue. Therefore, to determine this, these proteins need to be purified and subjected to an *in vitro* phosphatase assay evaluating the ability of these proteins to dephosphorylate Cdk1. This will prove that these proteins are able to dephosphorylate Cdk1-Y19 strengthening the argument for their potential roles of the Rrd proteins as either necessary co-activators and/or Ych1 and Arr2 as downstream proteins activated by PP2A^{Rts1}.

6.0 CONCLUSION

The goal of my thesis was to explore the two competing models for how $PP2A^{Rts1}$ regulates Cdk1-Y19 dephosphorylation. From my results, I obtained evidence arguing for the direct pathway involving the Rrd proteins and the indirect pathway involving the activation of downstream PTPases specific to Cdk1-Y19. In this study, it is evident that the Rrd proteins play an important role in regulating Cdk1-Y19 dephosphorylation given the sickness observed in the serial dilutions (**Figure 4-3**) and the delay in degradation of mitotic cyclins and APC substrates (**Figure 4-4**). In an opposing manner, I also found upon deletion of *YCH1* in *mih1*Δ *ptp1*Δ, cells became sicker suggesting that Ych1 was working independently of Mih1 and Ptp1 (**Figure 4-7**). But given that *ych1*Δ *rts1*Δ cells were phenotypically indifferent from *rts1*Δ alone (**Figure 4-8**), this argues that Ych1 may be acting downstream of $PP2A^{Rts1}$. Although it is inconclusive of whether $PP2A^{Rts1}$ acts solely through a direct or indirect manner, my work has demonstrated the acknowledgement of the possibility that $PP2A^{Rts1}$ may be mediating the dephosphorylation of Cdk1-Y19 through a mix of both a direct and indirect manner (**Figure 4-10**). Given that the Wee1 inhibitor AZD1775 is currently in phase I and II clinical trials as a potential chemotherapeutic, my work will serve to better the understanding of how Wee1/Cdc25 is regulated and if other proteins, such as PP2A, also play a role in this regulation. Therefore, my work will be essential in determining new ways to design anti-mitotic treatments to enhance or repurpose current and experimental cancer therapeutics.

APPENDIX

Table 2: Yeast strains used in this thesis

All strains in this study are derivatives of W303 (*ura3-1 leu2-3,112 trp1-1 his3-11 ase2-1 can1-100)*

Strain	Mating type	Genotype
ADR21	MATa	
ADR22	MATα	
ADR4006	MATa	*bar1*Δ
ADR9534	MATa	*rts1*Δ *swe1*Δ
ADR9539	MATα	*rts1*Δ
ADR10331	MATα	*bar1*Δ
ADR10806	MATa	*mih1*Δ *ptp1*Δ *spd1*Δ *oca2*Δ *swe1*Δ
ADR10844	MATα	*mih1*Δ *ptp1*Δ *spd1*Δ *swe1*Δ
ADR10852	MATα	*mih1*Δ *ptp1*Δ
ADR10854	MATα	*mih1*Δ *ptp1*Δ
ADR10858	MATα	*mih1*Δ *ptp1*Δ *spd1*Δ *oca2*Δ
ADR10867	MATα	*mih1*Δ *ptp1*Δ *swe1*Δ
ADR10936	MATa	*mih1*Δ *ptp1*Δ *spd1*Δ
ADR10971	MATa	*rrd2*Δ *swe1*Δ
ADR11053	MATα	*ych1*Δ
ADR11054	MATa	*arr2*Δ
ADR11072	MATa	*mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1 swe1*Δ
ADR11073	MATα	*mih1*Δ *ptp1*Δ *rrd1*Δ *rrd2-AID OsTIR1*
ADR11075	MATα	*rrd1*Δ *rrd2-AID OsTIR1 swe1*Δ

ADR11079	MATa	*rrd1Δ rrd2-AID OsTIR1*
ADR11082	MATα	*mih1Δ ptp1Δ rrd1Δ rrd2-AID OsTIR1*
ADR11084	MATα	*mih1Δ ptp1Δ rrd1Δ rrd2-AID*
ADR11092	MATa	*mih1Δ ptp1Δ ych1Δ*
ADR11094	MATα	*mih1Δ ptp1Δ ych1Δ*
ADR11096	MATα	*ych1Δ arr2Δ*
ADR11098	MATα	*ych1Δ arr2Δ swe1Δ*
ADR11101	MATa	*mih1Δ ptp1Δ arr2Δ*
ADR11102	MATα	*mih1Δ ptp1Δ ych1Δ arr2Δ*
ADR11103	MATα	*mih1Δ ptp1Δ ych1Δ arr2Δ swe1Δ*
ADR11138	MATa	*rts1Δ ych1Δ*
ADR11139	MATα	*rts1Δ ych1Δ swe1Δ*
ADR11140	MATα	*rts1Δ arr2Δ*
ADR11141	MATa	*rts1Δ arr2Δ swe1Δ*
ADR11142	MATα	*rts1Δ ych1Δ arr2Δ*
ADR11143	MATa	*rts1Δ ych1Δ arr2Δ swe1Δ*

Table 3: Oligonucleotides used in this thesis
All oligonucleotides that created new and unpublished strains.

Oligo	Sequence
35 Pringle 5' check	GGACGAGGCAAGCTAAACAG
36 Pringle 3' check	TGGTCGCTATACTGCTGTCG
1504 RRD1-del UP	AAAGAACGCACATATGAACAAGCATTAAACGAGCAAAGAACAGCTGAAGCTTCGTACGC
1505 RRD1-del DS	TCATAATGCTTGTCATACACATTTATATGTTTAATTAATATACGACTCACTATAGGG
1506 RRD1 UP check	TGAGTCTCGTTCGCCTCTTT
1732 YCH1-del US	GCCAGATAGAAGCAAAAGAGAAGTCAATTGCAAAAAAAATAGCTGAAGCTTCGTACGC
1733 YCH1-del DS	TCATGGCAAATATATGATCACGTGCGATTGTGTAAACCTGTACGACTCACTATAGGG
1734 YCH1-del DS check	CTGAGTCTGGTCTGTTGCCT
2071 RRD2-AID US	TGAGATGAATAAAAAACATCATAAACCAATACCTTTTGAT
2072 RRD2-AID DS	GTGTATATACAATGAGATAGCATCTATAAATGGACTTTTC
2073 RRD2 DWN check	ATGGGGATTAGCAGAGGGGT
2169 ARR2-del US	AAACTAATATTGAGGAAAACTTGACAATCCAGCAAGCGTAAGCTGAAGCTTCGTACGC
2170 ARR2-del DS	GACTTTTACATTAATGCTGGAACAATAAAGCTTGAGGAGCTACGACTCACTATAGGG

2171 ARR2 check DWN	AAGTGTGACGGGAAGAAGGC
2172 ARR2 check UP	GGCCGCTCCTTAAAGACCAT
2173 OCA2-del US	TGAAGGCAAAATACCAATAGAGTGCACGTTTCTTGATGCTAGCTGAAGCTTCGTACGC
2174 OCA2-del DS	AATGACAGAAAATATTCTTTGTAACGGCTGGTTCTCATAATACGACTCACTATAGGG
2175 OCA2 check UP	GTGAGAAGGGAGGVTGTCAC
2176 OCA2 check DWN	GGTGTCGGTTCCTCTTTCGA
2177 SDP1-del US	AATAACACATACGGCTGCGCACTTGTAGCCATAATCTCGCAGCTGAAGCTTCGTACGC
2178 SDP1-del DS	AAAGCCTCATTGAATGCTATATCTTTTTTTTTTTTCTCTCTACGACTCACTATAGGG
2179 SDP1 check UP	AGGGCGCACACTATTGGAAT
2180 SDP1 check DWN	GACGAGTCACTTCCTGAGCC

REFERENCES

1. HOWARD A. Synthesis of deoxyribonucleic acid in normal and irradiated ceils and its relation to chromosome breakage. *Heredity Suppl*. 1953;6:261-273.

2. Barnum KJ, O'Connell MJ. Cell Cycle Regulation by Checkpoints. *Methods Mol Biol*. 2014;1170:29-40. doi:10.1007/978-1-4939-0888-2_2

3. Kõivomägi M, Valk E, Venta R, et al. Dynamics of Cdk1 Substrate Specificity during the Cell Cycle. *Mol Cell*. 2011;42(5-4):610-623. doi:10.1016/j.molcel.2011.05.016

4. Jorgensen P, Tyers M. How Cells Coordinate Growth and Division. *Current Biology*. 2004;14(23):R1014-R1027. doi:10.1016/j.cub.2004.11.027

5. Turner JJ, Ewald JC, Skotheim JM. Cell Size Control in Yeast. *Current Biology*. 2012;22(9):R350-R359. doi:10.1016/j.cub.2012.02.041

6. Griffiths AJ, Miller JH, Suzuki DT, Lewontin RC, Gelbart WM. Aneuploidy. *An Introduction to Genetic Analysis 7th edition*. Published online 2000. Accessed June 30, 2021. https://www.ncbi.nlm.nih.gov/books/NBK21870/

7. Nurse P. Genetic control of cell size at cell division in yeast. *Nature*. 1975;256(5518):547-551. doi:10.1038/256547a0

8. Fantes P, Nurse P. Control of cell size at division in fission yeast by a growth-modulated size control over nuclear division. *Experimental Cell Research*. 1977;107(2):377-386. doi:10.1016/0014-4827(77)90359-7

9. Hartwell LH, Unger MW. Unequal division in Saccharomyces cerevisiae and its implications for the control of cell division. *Journal of Cell Biology*. 1977;75(2):422-435. doi:10.1083/jcb.75.2.422

10. Johnston GC. Cell size and budding during starvation of the yeast Saccharomyces cerevisiae. *Journal of Bacteriology*. 1977;132(2):738-739.

11. Rupeš I. Checking cell size in yeast. *Trends in Genetics*. 2002;18(9):479-485. doi:10.1016/S0168-9525(02)02745-2

12. Rao PN, Johnson RT. Mammalian cell fusion. IV. Regulation of chromosome formation from interphase nuclei by various chemical compounds. *J Cell Physiol*. 1971;78(2):217-223. doi:10.1002/jcp.1040780208

13. Maller JL. MPF and cell cycle control. *Adv Second Messenger Phosphoprotein Res*. 1990;24:323-328.

14. Masui Y. From oocyte maturation to the in vitro cell cycle: the history of discoveries of Maturation-Promoting Factor (MPF) and Cytostatic Factor (CSF). *Differentiation*. 2001;69(1):1-17. doi:10.1046/j.1432-0436.2001.690101.x

15. Hartwell LH, Weinert TA. Checkpoints: controls that ensure the order of cell cycle events. *Science*. 1989;246(4930):629-634. doi:10.1126/science.2683079

16. Kanatani H. Spawning of Starfish: Action of Gamete-Shedding Substance Obtained from Radial Nerves. *Science*. 1964;146(3648):1177-1179. doi:10.1126/science.146.3648.1177

17. Masui Y, Markert CL. Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. *J Exp Zool*. 1971;177(2):129-145. doi:10.1002/jez.1401770202

18. Kishimoto T, Kuriyama R, Kondo H, Kanatani H. Generality of the action of various maturation-promoting factors. *Exp Cell Res*. 1982;137(1):121-126. doi:10.1016/0014-4827(82)90014-3

19. Wasserman WJ, Smith LD. The cyclic behavior of a cytoplasmic factor controlling nuclear membrane breakdown. *J Cell Biol*. 1978;78(1):R15-22. doi:10.1083/jcb.78.1.r15

20. Gerhart J, Wu M, Kirschner M. Cell cycle dynamics of an M-phase-specific cytoplasmic factor in Xenopus laevis oocytes and eggs. *J Cell Biol*. 1984;98(4):1247-1255. doi:10.1083/jcb.98.4.1247

21. Sunkara PS, Wright DA, Rao PN. Mitotic factors from mammalian cells induce germinal vesicle breakdown and chromosome condensation in amphibian oocytes. *Proc Natl Acad Sci U S A*. 1979;76(6):2799-2802.

22. Kishimoto T, Yamazaki K, Kato Y, Koide SS, Kanatani H. Induction of starfish oocyte maturation by maturation-promoting factor of mouse and surf clam oocytes. *Journal of Experimental Zoology*. 1984;231(2):293-295. doi:10.1002/jez.1402310215

23. Evans T, Rosenthal ET, Youngblom J, Distel D, Hunt T. Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell*. 1983;33(2):389-396. doi:10.1016/0092-8674(83)90420-8

24. Pines J. Protein kinases and cell cycle control. *Semin Cell Biol*. 1994;5(6):399-408. doi:10.1006/scel.1994.1047

25. Sullivan M, Morgan DO. Finishing mitosis, one step at a time. *Nat Rev Mol Cell Biol*. 2007;8(11):894-903. doi:10.1038/nrm2276

26. Cross FR. Starting the cell cycle: what's the point? *Curr Opin Cell Biol*. 1995;7(6):790-797. doi:10.1016/0955-0674(95)80062-x

27. Nasmyth K. At the heart of the budding yeast cell cycle. *Trends Genet*. 1996;12(10):405-412. doi:10.1016/0168-9525(96)10041-x

28. Enserink JM, Kolodner RD. An overview of Cdk1-controlled targets and processes. *Cell Division*. 2010;5(1):11. doi:10.1186/1747-1028-5-11

29. Liu J, Kipreos ET. Evolution of cyclin-dependent kinases (CDKs) and CDK-activating kinases (CAKs): differential conservation of CAKs in yeast and metazoa. *Mol Biol Evol*. 2000;17(7):1061-1074. doi:10.1093/oxfordjournals.molbev.a026387

30. Toh-e A, Tanaka K, Uesono Y, Wickner RB. PHO85, a negative regulator of the PHO system, is a homolog of the protein kinase gene, CDC28, of Saccharomyces cerevisiae. *Mol Gen Genet*. 1988;214(1):162-164. doi:10.1007/BF00340196

31. Lörincz AT, Reed SI. Primary structure homology between the product of yeast cell division control gene CDC28 and vertebrate oncogenes. *Nature*. 1984;307(5947):183-185. doi:10.1038/307183a0

32. Hartwell LH. Saccharomyces cerevisiae cell cycle. *Bacteriol Rev*. 1974;38(2):164-198.

33. Reed SI. The Selection of Amber Mutations in Genes Required for Completion of Start, the Controlling Event of the Cell Division Cycle of S. CEREVISIAE. *Genetics*. 1980;95(3):579-588.

34. Dutcher SK, Hartwell LH. The role of S. cerevisiae cell division cycle genes in nuclear fusion. *Genetics*. 1982;100(2):175-184.

35. Shuster EO, Byers B. Pachytene arrest and other meiotic effects of the start mutations in Saccharomyces cerevisiae. *Genetics*. 1989;123(1):29-43.

36. Lim HH, Goh PY, Surana U. Spindle pole body separation in Saccharomyces cerevisiae requires dephosphorylation of the tyrosine 19 residue of Cdc28. *Mol Cell Biol*. 1996;16(11):6385-6397.

37. Touati SA, Hofbauer L, Jones AW, Snijders AP, Kelly G, Uhlmann F. Cdc14 and PP2A Phosphatases Cooperate to Shape Phosphoproteome Dynamics during Mitotic Exit. *Cell Reports*. 2019;29(7):2105-2119.e4. doi:10.1016/j.celrep.2019.10.041

38. Hertz EPT, Kruse T, Davey NE, et al. A Conserved Motif Provides Binding Specificity to the PP2A-B56 Phosphatase. *Mol Cell*. 2016;63(4):686-695. doi:10.1016/j.molcel.2016.06.024

39. Nigg EA. Cellular substrates of p34(cdc2) and its companion cyclin-dependent kinases. *Trends Cell Biol*. 1993;3(9):296-301. doi:10.1016/0962-8924(93)90011-o

40. Haase SB, Reed SI. Evidence that a free-running oscillator drives G1 events in the budding yeast cell cycle. *Nature*. 1999;401(6751):394-397. doi:10.1038/43927

41. Mendenhall MD, Jones CA, Reed SI. Dual regulation of the yeast CDC28-p40 protein kinase complex: cell cycle, pheromone, and nutrient limitation effects. *Cell*. 1987;50(6):927-935. doi:10.1016/0092-8674(87)90519-8

42. Mendenhall MD, Hodge AE. Regulation of Cdc28 Cyclin-Dependent Protein Kinase Activity during the Cell Cycle of the Yeast Saccharomyces cerevisiae. *Microbiol Mol Biol Rev*. 1998;62(4):1191-1243.

43. Mendenhall MD, Richardson HE, Reed SI. Dominant negative protein kinase mutations that confer a G1 arrest phenotype. *Proc Natl Acad Sci U S A*. 1988;85(12):4426-4430.

44. Harvey SL, Charlet A, Haas W, Gygi SP, Kellogg DR. Cdk1-Dependent Regulation of the Mitotic Inhibitor Wee1. *Cell*. 2005;122(3):407-420. doi:10.1016/j.cell.2005.05.029

45. Lohka MJ, Hayes MK, Maller JL. Purification of maturation-promoting factor, an intracellular regulator of early mitotic events. *Proc Natl Acad Sci U S A*. 1988;85(9):3009-3013. doi:10.1073/pnas.85.9.3009

46. Beach D, Durkacz B, Nurse P. Functionally homologous cell cycle control genes in budding and fission yeast. *Nature*. 1982;300(5894):706-709. doi:10.1038/300706a0

47. Nurse P, Thuriaux P, Nasmyth K. Genetic control of the cell division cycle in the fission yeast Schizosaccharomyces pombe. *Mol Gen Genet*. 1976;146(2):167-178. doi:10.1007/BF00268085

48. Culotti J, Hartwell LH. Genetic control of the cell division cycle in yeast. 3. Seven genes controlling nuclear division. *Exp Cell Res*. 1971;67(2):389-401. doi:10.1016/0014-4827(71)90424-1

49. Gould KL, Nurse P. Tyrosine phosphorylation of the fission yeast cdc2 + protein kinase regulates entry into mitosis. *Nature*. 1989;342(6245):39-45. doi:10.1038/342039a0

50. Parker LL, Piwnica-Worms H. Inactivation of the p34cdc2-cyclin B complex by the human WEE1 tyrosine kinase. *Science*. 1992;257(5078):1955-1957. doi:10.1126/science.1384126

51. Featherstone C, Russell P. Fission yeast p 107 wee1 mitotic inhibitor is a tyrosine/serine kinase. *Nature*. 1991;349(6312):808-811. doi:10.1038/349808a0

52. Lundgren K, Walworth N, Booher R, Dembski M, Kirschner M, Beach D. mik1 and wee1 cooperate in the inhibitory tyrosine phosphorylation of cdc2. *Cell*. 1991;64(6):1111-1122. doi:10.1016/0092-8674(91)90266-2

53. Russell P, Nurse P. The mitotic inducer nim1+ functions in a regulatory network of protein kinase homologs controlling the initiation of mitosis. *Cell*. 1987;49(4):569-576. doi:10.1016/0092-8674(87)90459-4

54. Kumagai A, Dunphy WG. Regulation of the cdc25 protein during the cell cycle in Xenopus extracts. *Cell*. 1992;70(1):139-151. doi:10.1016/0092-8674(92)90540-S

55. Gautier J, Solomon MJ, Booher RN, Bazan JF, Kirschner MW. cdc25 is a specific tyrosine phosphatase that directly activates p34cdc2. *Cell*. 1991;67(1):197-211. doi:10.1016/0092-8674(91)90583-K

56. Russell P, Nurse P. cdc25+ functions as an inducer in the mitotic control of fission yeast. *Cell*. 1986;45(1):145-153. doi:10.1016/0092-8674(86)90546-5

57. Strausfeld U, Labbé JC, Fesquet D, et al. Dephosphorylation and activation of a p34 cdc2 /cyclin B complex in vitro by human CDC25 protein. *Nature*. 1991;351(6323):242-245. doi:10.1038/351242a0

58. Kellogg DR. Wee1-dependent mechanisms required for coordination of cell growth and cell division. *Journal of Cell Science*. 2003;116(24):4883-4890. doi:10.1242/jcs.00908

59. Jb M, G L, P R. Pyp3 PTPase acts as a mitotic inducer in fission yeast. *EMBO J*. 1992;11(13):4933-4941. doi:10.1002/j.1460-2075.1992.tb05600.x

60. Minshull J, Straight A, Rudner AD, Dernburg AF, Belmont A, Murray AW. Protein phosphatase 2A regulates MPF activity and sister chromatid cohesion in budding yeast. *Current Biology*. 1996;6(12):1609-1620. doi:10.1016/S0960-9822(02)70784-7

61. Kennedy EK, Dysart M, Lianga N, et al. Redundant Regulation of Cdk1 Tyrosine Dephosphorylation in Saccharomyces cerevisiae. *Genetics*. 2016;202(3):903-910. doi:10.1534/genetics.115.182469

62. Raleigh JM, O'Connell MJ. The G(2) DNA damage checkpoint targets both Wee1 and Cdc25. *J Cell Sci*. 2000;113 (Pt 10):1727-1736.

63. Bakke J, Haj FG. Protein-Tyrosine Phosphatase 1B Substrates and Metabolic Regulation. *Semin Cell Dev Biol*. 2015;0:58-65. doi:10.1016/j.semcdb.2014.09.020

64. Tonks NK. Protein tyrosine phosphatases: from genes, to function, to disease. *Nat Rev Mol Cell Biol*. 2006;7(11):833-846. doi:10.1038/nrm2039

65. Denu JM, Dixon JE. Protein tyrosine phosphatases: mechanisms of catalysis and regulation. *Curr Opin Chem Biol*. 1998;2(5):633-641. doi:10.1016/s1367-5931(98)80095-1

66. Charbonneau H, Tonks NK, Kumar S, et al. Human placenta protein-tyrosine-phosphatase: amino acid sequence and relationship to a family of receptor-like proteins. *Proc Natl Acad Sci U S A*. 1989;86(14):5252-5256. doi:10.1073/pnas.86.14.5252

67. Frangioni JV, Beahm PH, Shifrin V, Jost CA, Neel BG. The nontransmembrane tyrosine phosphatase PTP-1B localizes to the endoplasmic reticulum via its 35 amino acid C-terminal sequence. *Cell*. 1992;68(3):545-560. doi:10.1016/0092-8674(92)90190-n

68. Flint AJ, Gebbink MF, Franza BR, Hill DE, Tonks NK. Multi-site phosphorylation of the protein tyrosine phosphatase, PTP1B: identification of cell cycle regulated and phorbol ester stimulated sites of phosphorylation. *EMBO J*. 1993;12(5):1937-1946.

69. Guan KL, Deschenes RJ, Qiu H, Dixon JE. Cloning and expression of a yeast protein tyrosine phosphatase. *J Biol Chem*. 1991;266(20):12964-12970.

70. Hannig G, Ottilie S, Schievella AR, Erikson RL. Comparison of the biochemical and biological functions of tyrosine phosphatases from fission yeast, budding yeast and animal cells. *Yeast*. 1993;9(10):1039-1052. doi:10.1002/yea.320091002

71. Shu Y, Yang H, Hallberg E, Hallberg R. Molecular genetic analysis of Rts1p, a B' regulatory subunit of Saccharomyces cerevisiae protein phosphatase 2A. *Mol Cell Biol*. 1997;17(6):3242-3253.

72. Wlodarchak N, Xing Y. PP2A as a master regulator of the cell cycle. *Crit Rev Biochem Mol Biol*. 2016;51(3):162-184. doi:10.3109/10409238.2016.1143913

73. Ariño J, Velázquez D, Casamayor A. Ser/Thr protein phosphatases in fungi: structure, regulation and function. *Microb Cell*. 6(5):217-256. doi:10.15698/mic2019.05.677

74. Moyano-Rodriguez Y, Queralt E. PP2A Functions during Mitosis and Cytokinesis in Yeasts. *Int J Mol Sci*. 2019;21(1). doi:10.3390/ijms21010264

75. Sneddon AA, Cohen PT, Stark MJ. Saccharomyces cerevisiae protein phosphatase 2A performs an essential cellular function and is encoded by two genes. *EMBO J*. 1990;9(13):4339-4346.

76. Ronne H, Carlberg M, Hu GZ, Nehlin JO. Protein phosphatase 2A in Saccharomyces cerevisiae: effects on cell growth and bud morphogenesis. *Mol Cell Biol*. 1991;11(10):4876-4884.

77. Holt LJ, Tuch BB, Villén J, Johnson AD, Gygi SP, Morgan DO. Global analysis of Cdk1 substrate phosphorylation sites provides insights into evolution. *Science*. 2009;325(5948):1682-1686. doi:10.1126/science.1172867

78. Cayla X, Goris J, Hermann J, Hendrix P, Ozon R, Merlevede W. Isolation and characterization of a tyrosyl phosphatase activator from rabbit skeletal muscle and Xenopus laevis oocytes. *Biochemistry*. 1990;29(3):658-667. doi:10.1021/bi00455a010

79. Goris J, Pallen CJ, Parker PJ, Hermann J, Waterfield MD, Merlevede W. Conversion of a phosphoseryl/threonyl phosphatase into a phosphotyrosyl phosphatase. *Biochem J*. 1988;256(3):1029-1034. doi:10.1042/bj2561029

80. Alessi DR, Street AJ, Cohen P, Cohen PT. Inhibitor-2 functions like a chaperone to fold three expressed isoforms of mammalian protein phosphatase-1 into a conformation with the specificity and regulatory properties of the native enzyme. *Eur J Biochem*. 1993;213(3):1055-1066. doi:10.1111/j.1432-1033.1993.tb17853.x

81. Fellner T, Lackner DH, Hombauer H, et al. A novel and essential mechanism determining specificity and activity of protein phosphatase 2A (PP2A) in vivo. *Genes Dev*. 2003;17(17):2138-2150. doi:10.1101/gad.259903

82. Cayla X, Van Hoof C, Bosch M, et al. Molecular cloning, expression, and characterization of PTPA, a protein that activates the tyrosyl phosphatase activity of protein phosphatase 2A. *J Biol Chem*. 1994;269(22):15668-15675.

83. Van Hoof C, Janssens V, Dinishiotu A, Merlevede W, Goris J. Functional analysis of conserved domains in the phosphotyrosyl phosphatase activator. Molecular cloning of the homologues from Drosophila melanogaster and Saccharomyces cerevisiae. *Biochemistry*. 1998;37(37):12899-12908. doi:10.1021/bi980496l

84. Rempola B, Kaniak A, Migdalski A, Rytka J, Slonimski PP, di Rago JP. Functional analysis of RRD1 (YIL153w) and RRD2 (YPL152w), which encode two putative activators of the phosphotyrosyl phosphatase activity of PP2A in Saccharomyces cerevisiae. *Mol Gen Genet*. 2000;262(6):1081-1092. doi:10.1007/pl00008651

85. Van HOOF C, MARTENS E, LONGIN S, et al. Specific interactions of PP2A and PP2A-like phosphatases with the yeast PTPA homologues, Ypa1 and Ypa2. *Biochemical Journal*. 2005;386(1):93-102. doi:10.1042/BJ20040887

86. JANSSENS V, GORIS J. Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. *Biochemical Journal*. 2001;353(3):417-439. doi:10.1042/bj3530417

87. Shi Y. Serine/Threonine Phosphatases: Mechanism through Structure. *Cell*. 2009;139(3):468-484. doi:10.1016/j.cell.2009.10.006

88. Longtine MS, McKenzie A, Demarini DJ, et al. Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. *Yeast*. 1998;14(10):953-961. doi:10.1002/(SICI)1097-0061(199807)14:10<953::AID-YEA293>3.0.CO;2-U

89. Nishimura K, Fukagawa T, Takisawa H, Kakimoto T, Kanemaki M. An auxin-based degron system for the rapid depletion of proteins in nonplant cells. *Nat Methods*. 2009;6(12):917-922. doi:10.1038/nmeth.1401

90. Goldstein AL, McCusker JH. Three new dominant drug resistance cassettes for gene disruption in Saccharomyces cerevisiae. *Yeast*. 1999;15(14):1541-1553. doi:10.1002/(SICI)1097-0061(199910)15:14<1541::AID-YEA476>3.0.CO;2-K

91. Larin ML, Harding K, Williams EC, et al. Competition between Heterochromatic Loci Allows the Abundance of the Silencing Protein, Sir4, to Regulate de novo Assembly of Heterochromatin. *PLoS Genet*. 2015;11(11):e1005425. doi:10.1371/journal.pgen.1005425

92. Lianga N, Williams EC, Kennedy EK, et al. A Wee1 checkpoint inhibits anaphase onset. *Journal of Cell Biology*. 2013;201(6):843-862. doi:10.1083/jcb.201212038

93. Lianga N, Doré C, Kennedy EK, et al. Cdk1 phosphorylation of Esp1/Separase functions with PP2A and Slk19 to regulate pericentric Cohesin and anaphase onset. *PLoS Genet*. 2018;14(3):e1007029. doi:10.1371/journal.pgen.1007029

94. Nishimura K, Yamada R, Hagihara S, et al. A super-sensitive auxin-inducible degron system with an engineered auxin-TIR1 pair. *Nucleic Acids Res*. 2020;48(18):e108. doi:10.1093/nar/gkaa748

95. Leitao RM, Jasani A, Talavera RA, Pham A, Okobi QJ, Kellogg DR. A Conserved PP2A Regulatory Subunit Enforces Proportional Relationships Between Cell Size and Growth Rate. *Genetics*. 2019;213(2):517-528. doi:10.1534/genetics.119.301012

96. Pal G, Paraz MTZ, Kellogg DR. Regulation of Mih1/Cdc25 by protein phosphatase 2A and casein kinase 1. *Journal of Cell Biology*. 2008;180(5):931-945. doi:10.1083/jcb.200711014

97. Costanzo M, Baryshnikova A, Bellay J, et al. The genetic landscape of a cell. *Science*. 2010;327(5964):425-431. doi:10.1126/science.1180823

98. Guacci V, Koshland D, Strunnikov A. A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of MCD1 in S. cerevisiae. *Cell*. 1997;91(1):47-57. doi:10.1016/s0092-8674(01)80008-8

99. Surana U, Robitsch H, Price C, et al. The role of CDC28 and cyclins during mitosis in the budding yeast S. cerevisiae. *Cell*. 1991;65(1):145-161. doi:10.1016/0092-8674(91)90416-V

100. Schwob E, Nasmyth K. CLB5 and CLB6, a new pair of B cyclins involved in DNA replication in Saccharomyces cerevisiae. *Genes Dev*. 1993;7(7A):1160-1175. doi:10.1101/gad.7.7a.1160

101. Yamamoto A, Guacci V, Koshland D. Pds1p is required for faithful execution of anaphase in the yeast, Saccharomyces cerevisiae. *J Cell Biol*. 1996;133(1):85-97. doi:10.1083/jcb.133.1.85

102. Ciosk R, Zachariae W, Michaelis C, Shevchenko A, Mann M, Nasmyth K. An ESP1/PDS1 Complex Regulates Loss of Sister Chromatid Cohesion at the Metaphase to Anaphase Transition in Yeast. *Cell*. 1998;93(6):1067-1076. doi:10.1016/S0092-8674(00)81211-8

103. Huh W-K, Falvo JV, Gerke LC, et al. Global analysis of protein localization in budding yeast. *Nature*. 2003;425(6959):686-691. doi:10.1038/nature02026

104. Foster MW, Forrester MT, Stamler JS. A protein microarray-based analysis of S-nitrosylation. *Proc Natl Acad Sci U S A*. 2009;106(45):18948-18953. doi:10.1073/pnas.0900729106

105. Yeo HK, Lee JY. Crystal structure of Saccharomyces cerevisiae Ygr203w, a homolog of single-domain rhodanese and Cdc25 phosphatase catalytic domain. *Proteins*. 2009;76(2):520-524. doi:10.1002/prot.22420

106. Mukhopadhyay R, Shi J, Rosen BP. Purification and characterization of ACR2p, the Saccharomyces cerevisiae arsenate reductase. *J Biol Chem*. 2000;275(28):21149-21157. doi:10.1074/jbc.M910401199

107. Mukhopadhyay R, Rosen BP. Saccharomyces cerevisiae ACR2 gene encodes an arsenate reductase. *FEMS Microbiol Lett*. 1998;168(1):127-136. doi:10.1111/j.1574-6968.1998.tb13265.x

108. Mukhopadhyay R, Zhou Y, Rosen BP. Directed Evolution of a Yeast Arsenate Reductase into a Protein-tyrosine Phosphatase *. *Journal of Biological Chemistry*. 2003;278(27):24476-24480. doi:10.1074/jbc.M302610200

109. Alic N, Higgins VJ, Pichova A, Breitenbach M, Dawes IW. Lipid Hydroperoxides Activate the Mitogen-activated Protein Kinase Mpk1p in Saccharomyces cerevisiae *. *Journal of Biological Chemistry*. 2003;278(43):41849-41855. doi:10.1074/jbc.M307760200

110. Heinisch JJ, Lorberg A, Schmitz HP, Jacoby JJ. The protein kinase C-mediated MAP kinase pathway involved in the maintenance of cellular integrity in Saccharomyces cerevisiae. *Mol Microbiol*. 1999;32(4):671-680. doi:10.1046/j.1365-2958.1999.01375.x

111. Fox GC, Shafiq M, Briggs DC, et al. Redox-mediated substrate recognition by Sdp1 defines a new group of tyrosine phosphatases. *Nature*. 2007;447(7143):487-492. doi:10.1038/nature05804

112. Zheng Y, Jiang Y. The yeast phosphotyrosyl phosphatase activator is part of the Tap42-phosphatase complexes. *Mol Biol Cell*. 2005;16(4):2119-2127. doi:10.1091/mbc.e04-09-0797

113. Morawska M, Ulrich HD. An expanded tool kit for the auxin-inducible degron system in budding yeast. *Yeast*. 2013;30(9):341-351. doi:10.1002/yea.2967

114. Nishimura K, Fukagawa T. An efficient method to generate conditional knockout cell lines for essential genes by combination of auxin-inducible degron tag and CRISPR/Cas9. *Chromosome Res*. 2017;25(3):253-260. doi:10.1007/s10577-017-9559-7

115. Zasadzińska E, Huang J, Bailey AO, et al. Inheritance of CENP-A Nucleosomes during DNA Replication Requires HJURP. *Developmental Cell*. 2018;47(3):348-362.e7. doi:10.1016/j.devcel.2018.09.003

116. Rudner AD, Murray AW. Phosphorylation by Cdc28 Activates the Cdc20-Dependent Activity of the Anaphase-Promoting Complex. *J Cell Biol*. 2000;149(7):1377-1390.

117. Cohen-Fix O, Peters JM, Kirschner MW, Koshland D. Anaphase initiation in Saccharomyces cerevisiae is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. *Genes Dev*. 1996;10(24):3081-3093. doi:10.1101/gad.10.24.3081

118. Cohen-Fix O, Koshland D. The anaphase inhibitor of Saccharomyces cerevisiae Pds1p is a target of the DNA damage checkpoint pathway. *PNAS*. 1997;94(26):14361-14366. doi:10.1073/pnas.94.26.14361