From Department of Bioscience and Nutrition (BioNut)
Karolinska Institutet, Stockholm, Sweden

Genetic analysis of cell cycle and chromatin regulation in quiescent fission yeast cells

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Stockholm 2022

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2022.

ISBN 978-91-8016-641-6
Cover illustration by Yasaman Zahedi and Rajitha Indukuri, using Shutterstock sample

Genetic analysis of cell cycle and chromatin regulation in quiescent fission yeast cells

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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The thesis will be defended in public at Gene room on floor 5, NEO building, KI south campus, 141 57 Huddinge

Monday, June 13th, 2022 at 9:00 AM

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Who believes you, is one step ahead of someone who loves you

To my beloved parents and dear uncle Masoud, who is in heaven

ABSTRACT

During proliferation, cells produce their genetic materials to increase the number of cells, while in the absence of nutrients or by the induction of stimulus, the proliferative phase is stopped and entry into quiescence is triggered to increase their chance of survival. Quiescence is a reversible resting phase where cells enter, in case of nutrient deprivation or damage and induced by stimuli. In cancer development, the shift between proliferation and quiescence stage is critical since, for example, tumor cells in dormancy are more resistant to cancer treatments. In the resting phase, energy sources are saved by minimizing or stopping the metabolism and cell division in order to use energy for maintaining cell survival. In this case, cells adapt to the new conditions by gene expression reprogramming, which is mediated by chromatin remodeling mechanisms. Therefore, there is a need to investigate mechanisms to understand genes and pathways affecting quiescence entry and maintenance.

To investigate the role of genes in quiescence, using high-throughput flow cytometry analysis, we developed the projects to discover new genes and pathways involved in the vegetative or quiescence stages. To achieve this end, we utilized the fission yeast and *Schizosaccharomyces pombe* which is a convenient model to study both vegetative and quiescence stages. Then, we performed both DNA content and cell survival analysis on the haploid deletion mutant library. Through these original approaches, gene-deleted mutants were classified according to their phenotypes to disclose mechanisms involved in vegetative and quiescence stages.

In the present study, different remodeler complexes such as INO80 C, SWR1 C, and SAGA C were investigated and the effect of these complexes on quiescence entry or maintenance was observed. The results demonstrate the effect of remodeler complexes for reprogramming gene expression patterns, that lead cells to enter quiescence or viability of cells during quiescence. The most interesting complex mainly observed was Ino80.
Ino80 ATPase-dependent remodeling complex mediates chromatin remodeling by removing histone variant H2A.Z from chromatin. This remodeler complex is required for the regulation of quiescence-related genes. More remodeler complexes and effective genes, that are related to quiescence entry and maintenance, are explained more in details in the result and discussion section.

LIST OF SCIENTIFIC PAPERS

I. Abo1 is required for the H3K9me2 to H3K9me3 transition in heterochromatin

Wenbo Dong, Eriko Oya, Yasaman Zahedi, Punit Prasad, J. Peter Svensso, Andreas Lennartsson, Karl Ekwall & Mickaël Durand-Dubief. Scientific Reports, Article number: 6055 (2020), Published: 08 April 2020.

II. High-Throughput Flow Cytometry Combined with Genetic Analysis Brings New Insights into the Understanding of Chromatin Regulation of Cellular Quiescence

Yasaman Zahedi, Mickael Durand-Dubief and Karl Ekwall, International Journal of Molecular Sciences, 2020, 21, 9022; doi:10.3390/ijms21239022.

III. An essential role for the Ino80 chromatin remodeling complex in regulation of gene expression during cellular quiescence

Yasaman Zahedi and Karl Ekwall
Submitted to Chromosome Research (Springer Nature), *under revision*

LIST OF ABBREVIATIONS

Act	Actin
Arp	Actin related protein
ATP	Adenosine triphosphate
bp	Base pairs
CDK	Cyclin-dependent kinase
CenH3	Centromere H3 variant
CH3	Methyl group
CpG	5'-C Phosphate G-3'
CRC	Chromatin remodeler complex
DNA	Deoxyribonucleic Acid
DNA	Deoxyribonucleic acid
DNA-A	DNA-width
DNA-W	DNA-Area
DNAMTs	DNA methyltransferase enzymes
DSBs	Double strand break
DSR	Determinant of selective removal
EMM	Edinburgh Minimal media
FSC	Forward light scatter
G0	Gap phase 0
G1	Gap phase 1
G2	Gap phase 2
GE	Gene Expression
GO	Gene Ontology
H3ac	H3 hyperacetylation
H3K9me2	Histone H3 lysine 9 di-methylation
H3K9me3	Histone H3 lysine 9 tri-methylation
HAT	Histone acetyltransferase
HDAC	Histone deacetylases
HMT	Histone methyltransferases
HP1	Heterochromatin protein 1
Ino80 C	Ino80 complex
IP4	Inositol tetra-kisphosphate
IP5	Inositol penta-kisphosphate
IP6	Inositol hexa-kisphosphate
IP7	Inositol hepta-kisphosphate
IP8	Inositol octa-kisphosphate
Ipk	Inositol polyphosphate kinase
KAT	Lysine acetyl transferase
KDa	Kilodaltons
KDMs	Lysine demethylases
KMT	Lysine methyltransferase

M phase	Mitosis
MB	Megabases
MBF	MluI cell-cycle box binding factor
MBF C	MluI cell-cycle box binding factor complex
Me2	Di-methylation
Me3	Tri-methylation
MMS	Methyl methane sulfonate
Nhp	Non-histone protein
Paf1 C	Paf1 complex
PMG	Pombe Glutamate Medium
PPIP5K	Pentakisphosphate kinase
PTM	Post-translational modification
PTR	Post-transcriptional regulation
RISC	RNA-induced silencing complex
RITS	RNA-induced transcriptional gene silencing
RNA	Ribonucleic acid
RNA pol II	RNA polymerase II
RNAi	RNA interference
RNAi-CTG	RNAi co-transcriptional gene silencing
RNAi-TGS	RNAi transcriptional gene silencing
Rpd3 C	Rpd3 complex
RSC	Remodeling the Structure of Chromatin
SAGA C	SAGA complex
S phase	Synthesis
S. cerevisiae	Schizosaccharomyces cerevisiae
S. pombe	Schizosaccharomyces pombe
SGA	Synthetic genetic array
SiRNA	Small interfering RNA
SPA	Sporulation Agar
SSC	Side light scatter
SWR C	SWR complex
TF	Transcription factor
TSG	Tumour suppressor genes
V.5	Version 5
YES	Yeast Extract with Supplements
5mC	5-methylcytosine

CONTENT

1 INTRODUCTION

1.1 CHROMATIN STRUCTURE

1.1.1 Chromatin assembly and organization

Chromatin organization is the process of packing and condensing genomic DNA within the cell nucleus. In the eukaryotic cell, genomic DNA is combined with histone octamers (H2A, H2B dimer and H3, H4 tetramer), and forms the nucleosome units in order to pack and protect the genome, to form chromatin structure, and regulate gene expression (GE). In each nucleosome unit, 146 base pairs (bp) of double-strand DNA is wrapped around the histone core (histones octamer complex), which are small conserved proteins measuring 10-15 kilodaltons (KDa) (Clapier CR & Cairns BR. 2009). Each nucleosome becomes more stable when the linker histone (H1) is added to it in order to form chromatosome (Izzo A & Schneider R. 2016). This structure helps the progress of packing and increases chromatin stability. In order to compress genomic DNA within the nucleus, chromatin is packed into 30- 70 nm fibers and is then folded into chromosome formation (Ou HD et al. 2017) (Figure 1). Histone protein and DNA synthesis begin from in the S phase (Harris ME et al. 1991).

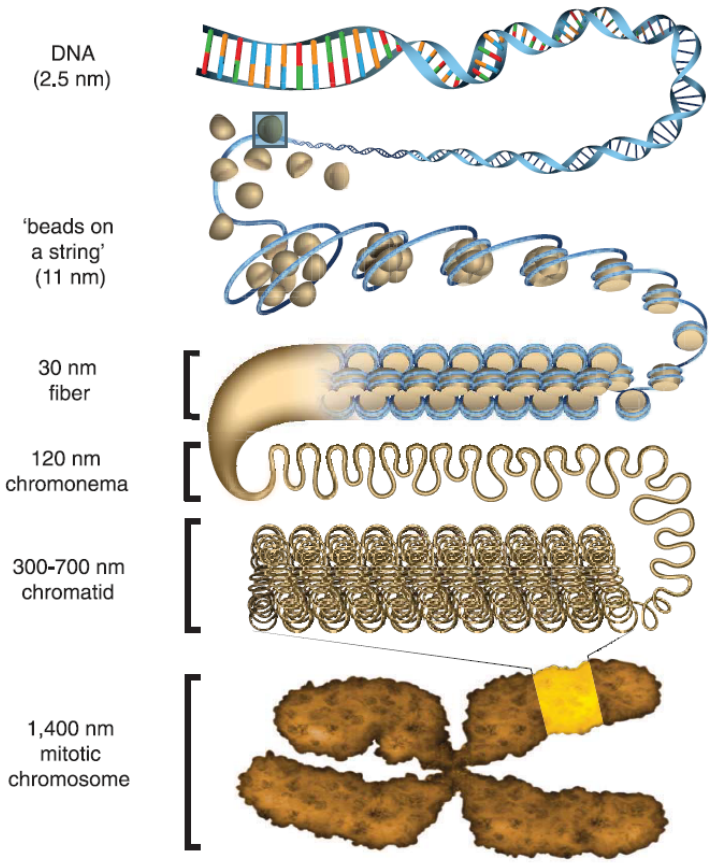


Figure 1. Eukaryotic chromatin organization. Nucleosome is formed by wrapping the double-helix DNA around the histone octamer, then further compacted to structure chromatin. In order to organize the whole genome in the nucleus, a series of compacting processes and folding of chromatin is performed. Subsequently, 30 nm fibers are compressed into the chromatin structure, and then the mitotic chromosome is formed (Ou HD et al. 2017).

1.1.2 Chromatin types and dynamics

According to the different levels of transcription and gene expression in various biological processes, two types of chromatin are formed based on the different density patterns (Hübner MR. 2010). Euchromatin and heterochromatin are functionally and structurally distinct. Modification of histones and DNA causes the change of chromatin density pattern and regulates formation of euchromatin or heterochromatin.
Euchromatin is more active transcriptionally and is less dense. In contrast, heterochromatin is more compact, therefore the possibility of DNA-transcription factor (TF) interaction is decreased and gene expression is deactivated (Penagos-Puig A & Furlan-Magaril M. 2020; Allshire RC and Madhani HD. 2017).

In euchromatin, the level of histone acetylation, H3K4 methylation, and H3K9 methylation increases (Penagos-Puig A & Furlan-Magaril M. 2020; Biterge B & Schneider R. 2014). Moreover, the distance between nucleosome units and the lack of

H1 cause a higher level of DNA accessibility to transcription factors, hence gene expression becomes active (Penagos-Puig A & Furlan-Magaril M, 2020). To allow chromosome segregation, chromatin condenses is exist during mitosis and it condensed in interphase for gene transcription (Babu A & Verma R. 1987).

The condensed chromatin regions throughout the cell cycle are defined as heterochromatin and do not activate transcriptionally (Elgin SC. 1996). Heterochromatin formation is involved in several biological processes like gene expression regulation, the DNA repair process, and chromosome segregation in mitosis (Allshire RC & Madhani HD. 2017). Moreover, the presence of heterochromatin regions increases genome stability and the integrity of chromosomes (Penagos-Puig A & Furlan-Magaril M. 2020). The heterochromatin pattern is epigenetically inherited. The constitutive and facultative heterochromatin regions are the two types of heterochromatin.

Constitutive heterochromatin is a region that is permanently silent and includes tandem repeats that are made satellite repeats (Saksouk N et al. 2015; Rego A et al. 2008). Constitutive heterochromatin is found in telomeres, centromeres, and pericentromeric chromatin regions (Saksouk N et al. 2015). Facultative heterochromatin includes the transcriptionally silent regions while they have the potential to be converted into euchromatin and become active. X chromosome activation and deactivation are the best examples of facultative heterochromatin mechanisms (Rego A et al. 2008). The determinant of selective removal (DSR) island is another example of a facultative heterochromatin region that includes mitotic genes, which activate only during mitosis. This region becomes transcriptionally deactivated via RNA degradation machinery (Zofall M et al. 2012; Harigaya Y et al. 2006).

Chromatin dynamics include mechanisms that mediate the mobility of heterochromatin and euchromatin such as histone modification, ATP-dependent chromatin remodelling, non-coding RNA etc.

1.1.3 Histone variants

As mentioned, four canonical histones (H2A, H2B, H3, and H4) are located in a histone core of nucleosome and are important for chromatin formation (Biterge B & Schneider R. 2014). However, these histones can be replaced with histone variants, hence affect the structure and function of chromatin (Henikoff S & Smith MM. 2015; Biterge B & Schneider R. 2014). Histone variants are expressed along the cell cycle, while canonical histones are expressed in the S phase (Maxouri S et al. 2018).
For instance, H3.3 in mammals involved in genome integrity during development (Jang CW et al. 2015). Mammalian CenH3 (centromere H3 variant, or CENP A in human), which is homologous with Cnp1 in *S. pombe* and Cse4 in *S. cerevisiae*, is crucial for

centromere localization during cell division (Choy JS et al. 2012; Lermontova I et al. 2006).

In fission yeast, there are three histone H3 genes (*hht1*$^+$, *hht2*$^+$, and *hht3*$^+$). The three genes encode the same H3 protein but they show different expression patterns during the cell cycle. The genes *hht1*$^+$ and *hht3*$^+$ are expressed in the S phase, while *hht2*$^+$ is constantly expressed throughout the cell cycle (Takayama Y & Takahashi K. 2007). Moreover, histone H2A has the largest number of variants for example H2A.Z, which is the most conserved member of this family and is encoded by the *pht1*$^+$ gene in *S. pombe* (Brewis HT et al. 2021; Kim HS et al. 2009). The H2A.Z variant is involved in several biological mechanisms such as regulation of gene expression and transcription, heterochromatin formation, chromatin stability, and DNA repair (Giaimo, B.D et al. 2019). A previous study demonstrated the effect of H2A.Z in the segregation process and the presence of this variant increases chromatin stability and mobility (Rudnizky, S et al. 2016). The strong correlation between the presence of H2A.Z and the level of transcription is reported in animals (Hardy S et al. 2009), while the H2A.Z presence in the promoter region of yeast is not connected with the level of transcription (Millar CB et al. 2006). In fact, a decrease in H2A.Z results in drops of growth rate, and it is not lethal for *S. cerevisiae* (Millar CB et al. 2006). Additionally, the absence of H2A.Z increases the damage effect of UV and MMS (Methyl methane sulfonate) on DNA in *S. cerevisiae* (Mizuguchi G et al. 2003). Interestingly, H2A.Z regulates transcription both positively and negatively (Marques M et al. 2010).

According to the effect of H2A.Z, the process of histone H2A variants exchanging has been an important and interesting subject to study. Different chromatin remodeler enzymes such as Ino80 and SWR1 complexes mediate the replacement of histone H2A variants, hence regulating gene expression and transcription.

1.2 PRINCIPLE OF GENE REGULATION

Gene regulation is a general term that defines how gene expression is controlled and programmed by different biological mechanisms. This regulation is performed during the whole transcription process (from transcription initiation until post-transcriptional protein modification and stability). Regulation of gene expression is a key strategy for differentiation, morphogenesis, development, and biological adaptation processes (Singh KP et al. 2018; López-Maury L et al. 2008). Different chemical and structural modification mechanisms are involved in regulation of gene expression that mediate post-translational modification and chromatin remodelling etc. (Singh KP et al. 2018).

1.2.1 Epigenetic changes and regulation of gene expression

Epigenetic modification defines the heritable changes that alter gene expression without affecting the sequences of genes. Two mechanisms, DNA methylation and histones modification, control gene expression level by silencing and activating the genes (Krishnakumar R & Blelloch RH. 2013). Different epigenetic mechanisms can activate or de-activate the expression of genes by post-transcriptional regulation (PTR) (O'Kane CJ & Hyland EM. 2019; Krishnakumar R & Blelloch RH. 2013; Torok MS & Grant PA. 2004). For example, methyl, phosphate or acetyl groups can be covalently added or removed by specific enzymes to histone proteins. Hence, the structure of chromatin is modified, and thereby the DNA accessibility for DNA binding proteins, such as transcription factors, is altered (Kagohara LT et al 2018; Krishnakumar R & Blelloch RH. 2013; Grønbaek et al. 2007) (Figure 2).

In general, gene expression is regulated by two main remodeling processes involving covalent histone modification and nucleosome remodeling via the ATP-dependent chromatin remodeler complexes (CRCs) (Tang L et al. 2010).

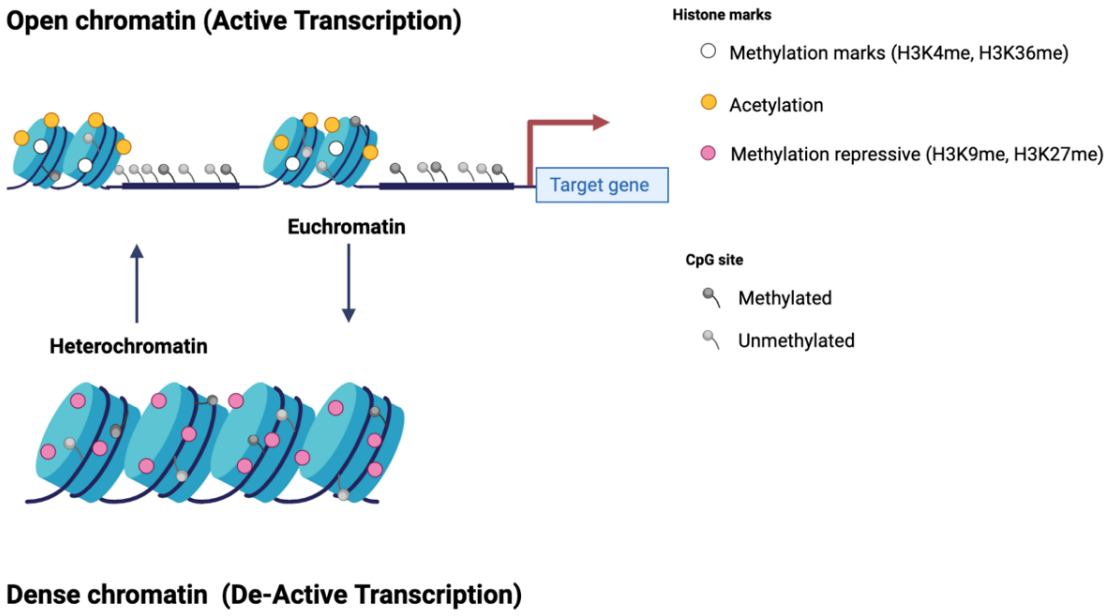


Figure 2. Regulation of gene expression by epigenetic modification. Methylation (H3K4me and H3K36me), acetylation of histones, and patterns of unmethylated CpG sites leave chromatin structures open and increase the accessibility of DNA for transcription factors. Hence, transcription of the gene is activated. On the other hand, H3K9me, H3K27me and methylation of CpG sites induce denser chromatin structure therefore deactivating gene transcription (Kagohara LT et al. 2018).

1.2.1.1 DNA methylation

DNA methylation is a common mechanism in eukaryotes that silences genes by the methylation of cytosine in the 5'-C Phosphate G-3' (CpG) region of the promoter using DNA methyltransferase enzymes (DNAMTs) (Li E & Zhang Y. 2014). Through this biological process, methyl group (CH3) is covalently added to the cytosine ring in DNA and forms 5-methylcytosine (5mC), which restricts the access of transcription factors to DNA and inhibits the transcription process (Moore, L et al. 2013).

1.2.1.2 Histone modification

Histone proteins are altered by post-translational modification (PTM) and this process is mediated by different biological mechanisms, such as acetylation, phosphorylation, ubiquitination, de-methylation, and methylation. The lysine methyltransferase (KMT), lysine demethylases (KDMs), lysine acetyl transferase (KAT) and histone deacetylases (HDACs) are enzymes involved in histone modification (Jambhekar A et al. 2020; Lee, K. & Workman, J. 2007; Wood A & Shilatifard A. 2004). The N-terminal tails of histone proteins are the target of post-translation modification mechanisms (Tolsma TO & Hansen JC. 2019).
Nucleosome alteration plays an important role in regulating the genes expression (O'Kane CJ & Hyland EM. 2019; Torok MS & Grant PA. 2004). In fact, the modification of nucleosomes leads to the electronegativity of histones, followed by the alteration of DNA-core histone complex interaction and nucleosome remodeling, therefore directly affecting expression patterns of genes.

The methylation of histones is interfered by an enzymes family called histone methyltransferases (HMTs), that add methyl group into arginine or lysine amino acids of histones (Bannister AJ & Kouzarides T. 2011; Kouzarides T. 2002). Usually, the modification of histone, with the absence of methylation on H3 on lysine 4, and the presence of methylation on lysine 9 (H3K9me), defines heterochromatin structure. (Bannister AJ & Kouzarides T. 2011; Kouzarides T. 2002). The heterochromatin protein 1 (HP1) family, as a conserved heterochromatin proteins group, bind to H3K9me, hence recognizes heterochromatin regions. Both forms of H3K9 me2 and me3 provide the binding of HP1 proteins in order to silence heterochromatin (Lachner M et al. 2001). Additionally, Clr4, which is H3K9 methyltransferase is involved in heterochromatin formation and gene silencing in *S. pombe* (Allshire RC & Ekwall K. 2015).

Histone acetylation (the addition of the acetyl group to the histone) and de-acetylation (the removal of the acetyl molecule) are two other epigenetic mechanisms involved in

control gene expression mediated by HATs and HDACs respectively (Torok MS & Grant PA. 2004). In general, histone acetylation induces the activation of the gene. The role of this epigenetic modification in DNA damage response and repair mechanisms in yeast is reported (Torok MS & Grant PA. 2004).

1.2.1.3 RNAi mediated epigenetic changes

In fission yeast, gene expression is also regulated by other post-transcriptional mechanisms, such as RNA interference (RNAi). This mechanism allows the degradation of small interfering RNA (siRNA) through the RNA-induced silencing complex (RISC) or RNA-induced transcriptional gene silencing (RITS) (Djupedal I & Ekwall K. 2009; Moazed D. 2009).
In fission yeast, most epigenetic mechanisms such as histone modifications and RNAi mediated mechanisms with the exception of CpG DNA methylation at the promoter are conserved to mammals (Allshire RC and Ekwall K. 2015; Moazed D. 2009; Djupedal I & Ekwall K. 2009).

1.3 CHROMATIN REMODELING AND GENETIC REPROGRAMMING

1.3.1 What the genetic reprogramming is?

Cell reprogramming generally refers to the remodeling patterns of gene expression by epigenetic changes. Cell reprogramming through epigenetic changes is essential for cellular processes such as development, differentiation, and aging (Krishnakumar R & Blelloch RH. 2013). In fact, the remodeling of chromatin, that leads to the reprogramming of gene expression patterns, plays a crucial role in the adaptation of cells to the new biological situation. For instance, downregulation of gene expression involved in metabolism pathways during the cellular resting phase serves to save the cell's energy in the absence of nutrition and also to maintain cell survival (Jishage M et al. 2020). The mechanisms involved in processes of exploiting the DNA accessibility can be various, such as the expression of specialized histone and transcriptional factors or nucleosome modifications (Kane AE & Sinclair D. 2019; Krishnakumar R & Blelloch RH. 2013).

1.3.2 ATP-dependent chromatin remodelers

Chromatin structure determines the gene expression due to the accessibility of DNA for transcription machinery proteins. Additionally, the transcription pattern is regulated and altered according to the biological conditions, or mechanisms that cells are following. Therefore, modification of chromatin structure is a smart regulation strategy of cells in

order to adapt to new conditions, for instance DNA replication or DNA damage, quiescence, proliferation, etc.

Chromatin remodeling complexes, such as the ATP-dependent chromatin remodelers, include essential factors that participate in the transition between chromatin states and modulate gene expression (Clapier, C et al. 2017; Gangaraju VK & Bartholomew B. 2007).
ATP-dependent chromatin remodeling complexes modify the structure of chromatin by hydrolysis of ATP as a source of energy (Gangaraju VK & Bartholomew B. 2007). In fact, ATP-dependent CRCs have a conserved ATPase domain (SNF2 subunit), that provides energy for the remodeling process of the chromatin (Ryan DP & Owen-Hughes T. 2011; Sen P et al. 2011).

In general, the SNF2 remodeler family consists of a different group of enzymes that mediate the distances between nucleosomes and alter chromatin density. This affects transcription regulation that ultimately causes activation or silencing of genes. Hence, ATPase remodellers play an important role in the development and differentiation process. Therefore, misfunction or absence of them can result in the development of diseases such as cancer (Längst G & Manelyte L. 2015; Narlikar GJ et al. 2013; de la Serna IL et al. 2006).

The remodeller complexes control different mechanisms that result in the re-structure of chromatin by two main strategies: 1, Movement of histones along the DNA strand through eviction, sliding, assembly, and unwrapping of the nucleosome, as well as nucleosome editing, and histone dimer ejection. 2, Replacement of histone variants that affect chromatin structure (Singh R et al. 2018; Henikoff S & Smith MM. 2015).

ATP-dependent chromatin remodelers are categorized into different families based on their activities, including SWI/SNF, ISWI, NURD-MI2, CHD and INO80 complex etc. Most of the studies have investigated the role of CRCs in proliferation but little is known about their roles during the quiescence resting phase. Interestingly, the most extensive studies have been carried out on yeast, as a proper model, to investigate the function and structure of remodeler complexes. Additionally, CRCs have been conserved from yeast to human (Eriksson, P.R. & Clark, D.J. 2021; Prajapati HK et al. 2020).

1.3.2.1 SWI/SNF chromatin remodeler family

SWI/SNF is an ATP-dependent chromatin remodelling complex that affects the structure of chromatin via the ejection and sliding of nucleosomes. In the assembly of this complex, 8 to 14 conserved and non-conserved protein components are involved. The SNF2 subunit is an ATPase domain in yeast SWI/SNF complex (Sen P et al. 2011; Laurent BC et al. 1993). RSC (Remodeling the Structure of Chromatin) is another

complex in fission yeast with a similar function to SWI/SNF (Cairns BR et al. 1996). SWI/SNF and RSC complex in fission yeast have 12 common subunits that are mentioned in Table 1 (Collected based on Gene Ontology from *S. pombe* gene source, https://www.pombase.org).

Systematic ID	Gene name	Product description
SPAC23D3.09	arp42	SWI/SNF and RSC complex subunit Arp42
SPAC1071.06	arp9	SWI/SNF and RSC complex subunit Arp9
SPAC17G6.10	ssr1	SWI/SNF and RSC complex subunit Ssr1
SPAC23H3.10	ssr2	SWI/SNF and RSC complex subunit Ssr2
SPAC23G3.10c	ssr3	SWI/SNF and RSC complex subunit Ssr3
SPBP23A10.05	ssr4	SWI/SNF and RSC complex subunit Ssr4
SPCC1620.14c	snf22	SWI/SNF ATP-dependent chromatin remodeler Snf22
SPAC23G3.07c	snf30	SWI/SNF complex subunit Snf30
SPAC2F7.08c	snf5	SWI/SNF complex subunit Snf5
SPBC26H8.09c	snf59	SWI/SNF complex subunit Snf59
SPBC30B4.04c	sol1	SWI/SNF complex subunit Sol1
SPAC22H12.02	tfg3	TFIID, TFIIF, Ino80, SWI/SNF, and NuA3 complex YEATS family subunit Tfg3

Table 1. Collection of common subunit of SWI/SNF and RSC complexes in fission yeast from Pombase (Fission yeast gene bank). Arp4, which is actin related protein, is a synonym of ARP42 which is an actin related subunit of SWI/SNF complex. *Tfg3* is a synonym of *taf14*. Both SWI/SNF and RSC complexes are involved in the chromatin remodelling process.

1.3.2.2 Ino80 chromatin remodeller complex

Ino80 is a member of the SNF2 family and is an ATP-dependent chromatin remodeler, which is involved in histone variant exchange, nucleosome eviction from DNA, spacing mechanisms, regulation of transcription (both activation and repression of genes) chromatin remodeling, and DNA repair (Zhou CY et al. 2018; Liu B et al. 2012). The subunits of this complex have been conserved between yeast to human (Bao Y & Shen X. 2007). In yeast, ATP complex includes core ATPase subunits that are encoded by *Rvb1* and *Rvb2* (homologous with *tip49a* and *tip49b* in mammals), Act1 subunit which is actin, Arp4 (homologous to mammalian Baf53a), Arp5 and Arp8 as the actin related proteins, non-histone protein Nhp10, TATA-binding protein-associated factor Taf14, and Ino80 subunits Ies1, Ies3, Ies4 and Ies5 (Jin J et al. 2005). Ino80 complex subunits of fission yeast are mentioned in Table 2. Comparing the Ino80 complex in fission yeast, budding yeast and human illustrates that subunits of this complex are highly conserved (Hogan CJ et al. 2010; Jin J et al. 2005; Kobor, M. S. 2004; Krogan, N. J et al. 2003; Shen, X et al. 2000**)** (Table 3).

Systematic ID	Gene name	Product description
SPBC32H8.12c	act1	actin Act1
SPBP23A10.08	alp5	actin-like protein Arp4
SPAPB8E5.09	rvb1	ASTRA/Swr1/Ino80 complex AAA family ATPase Rvb1
SPBC83.08	rvb2	ASTRA/Swr1/Ino80 complex AAA family ATPase Rvb2
SPBC11B10.10c	pht1	histone H2A variant H2A.Z Pht1
SPBC365.10	arp5	Ino80 complex actin-like protein Arp5
SPAC664.02c	arp8	Ino80 complex actin-like protein Arp8
SPAC10F6.08c	nht1	Ino80 complex HMG box subunit Nht1
SPAC144.02	iec1	Ino80 complex subunit Iec1
SPCC1259.04	iec3	Ino80 complex subunit Iec3
SPAPB1E7.14	iec5	Ino80 complex subunit Iec5
SPAC6B12.05c	ies2	Ino80 complex subunit Ies2
SPAC23G3.04	ies4	Ino80 complex subunit Ies4
SPAC222.04c	ies6	Ino80 complex subunit Ies6
SPCC16C4.20c	hap2	Ino80 complex, HMG box protein Hap2
SPAC29B12.01	ino80	SNF2 family ATP-dependent chromatin remodeller Ino80
SPAC23D3.09	arp42	SWI/SNF and RSC complex subunit Arp42
SPAC22H12.02	tfg3	TFIID, TFIIF, Ino80, SWI/SNF, and NuA3 complex YEATS family subunit Tfg3

Table 2. Summary of Ino80 submits in fission yeast which is collected from Pombase (Fission yeast gene bank).

S. pombe	*S. cerevisiae*[a]	Human[b]	Description
Ino80 (SPAC29B12.01)[c]	**INO80**	**hIno80**	**SNF-like helicase**
Arp8 (SPAC664.02)	ARP8	Arp8	Actin-related protein
Arp5 (SPBC365.10)	ARP5	Arp5	Actin-related protein
Alp5	ARP4	BAF53a/Arp4	Actin-related protein
Rvb1 (SPAPB8E5.09)	RVB1	TIP49a	AAA^+ ATPase
Rvb2 (SPBC83.08)	**RVB2**	**TIP49b**	**AAA^+ ATPase**
Ies6 (SPAC222.04)	IES6	C18orf37	Related to YL-1 family
Ies2 (SPAC6B12.05)	IES2	PAPA-1	PAPA_1 domain
Iec1		**YY1**	**Zinc finger transcription factor**
HMG-box like protein (*nht1*)	NHP10		HMG-type domain; binds DNA
Taf14/tfg3	TAF14		Transcription factor
Act1	**ACT1**		**Actin**
SPAC23G3.04 (*ies4*)[c]	IES4		Ino80 complex subunit
SPCC1259.04 (*iec3*)[c]			Sequence orphan
SPCC16C4.20[c,d]			Sequence orphan
	IES1		Ino80 complex subunit
	IES3		Ino80 complex subunit
	IES5		Ino80 complex subunit
		Amida/TCF3	b-ZIP-type protein; DNA binding
		NFRKB	Metazoan specific; nuclear factor
		MCRS1	Forkhead-associated domain protein
		FLJ90652	Conserved in metazoa
		FLJ20309	Conserved in metazoa
		UCH37	Ubiquitin C-terminal hydrolase

Table 3. Comparison of Ino80 complex in *S. pombe*, *S. cerevisiae*, and human (Jin J et al. 2005).

In response to DNA damage and during homologous recombination repair, the Ino80 complex is recruited. In yeast, the phosphorylated histone H2AX (γH2AX) is formed under double strand break (DSBs), then Ino80 C interacts with H2AX. This interaction is Nhp10 (HMG box subunit of Ino80 C and homologous of Nht1 in fission yeast) dependent and induces synthetic genetic interaction of Ino80 subunits with the Rad52 pathway. Rad52 is a main yeast DSB repair pathway (Morrison AJ et al. 2004). On the other hand, the presence of the Ino80 complex induces nucleosome eviction at a location of damage, and the assembly of factors involved in repair mechanisms at the damaged region (Morrison AJ et al. 2004). One of the crucial Ino80 roles is the exchange of histone variants that causes the restructuring of chromatin. This complex replaces H2A.Z with histone variant H2A and causes the increase of promoter

accessibility for TFs. It then induces the activation of gene expression (Papamichos-Chronakis M et al. 2011). SWR1 C (SWR complex) is another ATP-dependent protein complex involved in the remodelling of chromatin. SWR1 includes 14 subunits in yeast, and Swi2/Snf2 acts as a catalytic domain in this complex. SWR C cooperates with Ino80 to re-assemble and dis-assemble the chromatin structure. In fact, its function is opposite to the function of Ino80 and deposes H2A.Z in chromatin, while Ino80 removes H2A.Z from chromatin (Poyton MF et al. 2022; Brahma, S et al. 2017; Chen L et al. 2013; Yen K et al. 2013; Papamichos-Chronakis M et al. 2011). Different probable theories have been suggested and investigated regarding SWR1C and Ino80 C cooperation in this histone exchange mechanism. The SWR1 enzyme is usually located in nucleosome -1, or close to a promoter which is the site of RNA polymerase II (RNA pol II) localization (Yen K et al. 2013; Venters BJ & Pugh BF. 2009) (Figure 3). SWR1 deposits H2A.Z in a promoter location, therefore blocking physical interaction between transcription factors and the promoter region and hence restricting transcription (Papamichos-Chronakis M et al. 2011).

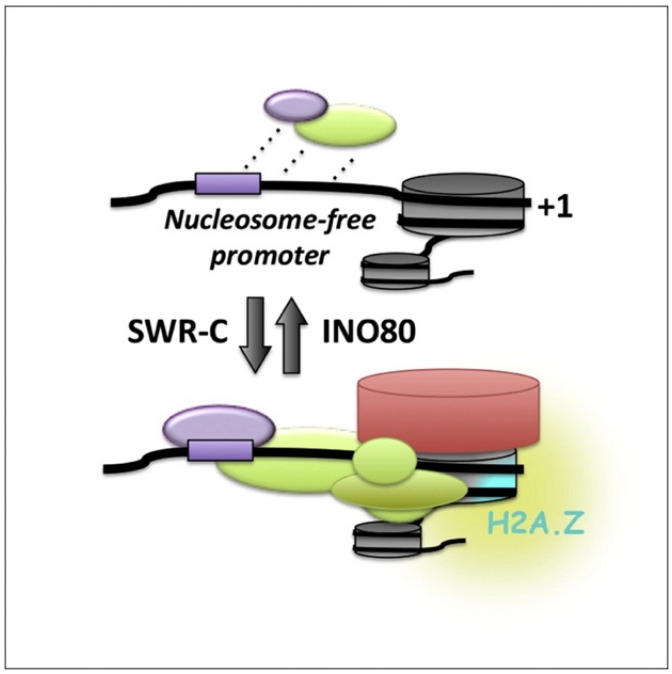


Figure 3. Cooperation of SWR C and Ino80 C regulate gene expression by histone A variant exchange. Cooperation of SWR C and Ino80 C regulate gene expression by histone A variant exchange. SERC deposes the histone H2A.Z variant in the promoter region and blocks the transcription process, while Ino80 C makes the promoter free by removing H2A.Z (Yen K et al. 2013).

On the other hand, the interaction of RNA pol II with transcription elongation complex can target Ino80 C in a coding region (Venters BJ & Pugh BF. 2009). Moreover, Ino80 C can mediate H2A.Z removal by stability of replication fork or elongation of replication fork related process (Yen K et al. 2013; Papamichos-Chronakis M et al. 2011; Shimada K et al. 2008).

As mentioned before, H2A.Z is replaced with H2A and rearranges the structure of chromatin (Poyton MF et al. 2022; Brahma, S et al. 2017; Chen L et al. 2013; Papamichos-Chronakis M et al. 2011).

1.3.2.3 How does ASP1 affect Ino80 complex?

ASP1 (diphosphoinositol pentakisphosphate kinase/InsP8 pyro phosphatase) is an inositol kinase and a member of the diphosphoinositol pentakisphosphate kinase (PPIP5K)/ Vip1 family. Asp1 (Vip1) mediates the conversion of Inositol polyphosphates, inositol hepta-kisphosphate (IP7) to inositol octa-kisphosphate (IP8) (Pascual-Ortiz et al. 2021) (Figure 4). The absence of *asp1* reduces the IP8 level and increases the level of IP6 and IP7 that are precursors to IP8 production (Pascual-Ortiz et al. 2021). Asp1 plays a crucial role in the adaptation process under the limitation of nutrients in fission yeast that allows cells to grow invasively in this condition (Pöhlmann J & Fleig U. 2010). Inositol polyphosphates affect the function of different chromatin remodeling complexes in vitro. Inositol hexa-kisphosphate (IP6) prevents the function of Ino80, NURF and ISW1 remodeling complexes in nucleosome mobility. Inositol tetra-kisphosphate (IP4) and inositol penta-kisphosphate (IP5) induce nucleosome mobility mediated by the SWI/SNF complex, which illustrates the role of inositol phosphate on the chromatin remodelling process (Shen X et al. 2003).

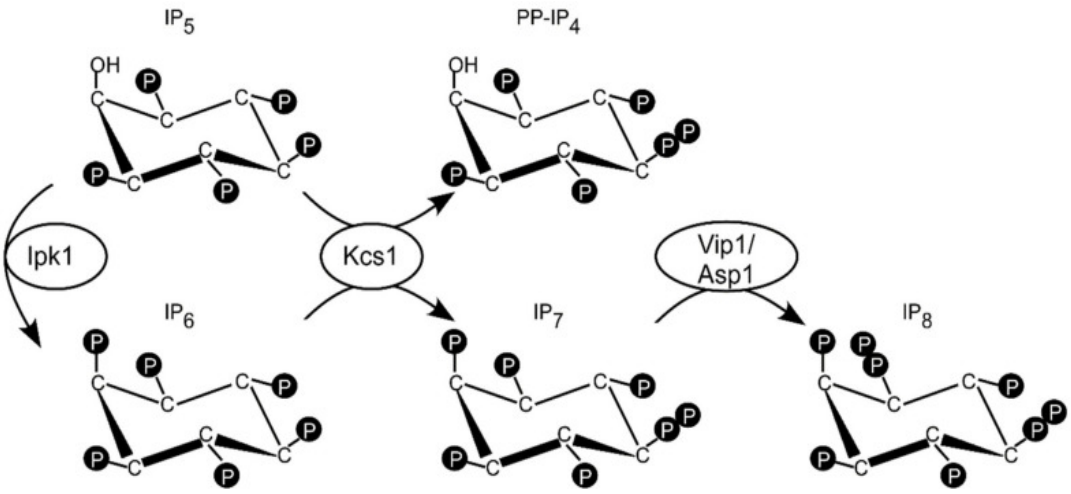


Figure 4. Inositol Pyrophosphate metabolic pathways in fission yeast and budding yeast. In both yeast families, the Inositol polyphosphate kinase 1 (Ipk1) enzyme converts IP5 to IP6 and the Kcs1 enzyme produces PP- IP4 from IP5 and IP7 from IP6. IP7 is a precursor of IP8, produced by VIP1 in budding yeast and Asp1 in fission yeast (Pascual-Ortiz et al. 2021).

Additionally, Ino80 C activity is modulated by Inositol polyphosphates in budding yeast (Shen X et al. 2003), and *asp1* is required for quiescence survival in fission yeast (Sajiki,K et al. 2018).

Asp1 and Ino80 C functional interaction is not well known yet and understanding the role of Inositol polyphosphates and histone exchange by Ino80 C was an interesting aspect to investigate during my last project.

1.4 CELL CYCLE AND QUIESCENCE

1.4.1 Proliferative cell cycle process and quiescence step in eukaryotes

In eukaryotic cells, the cell cycle is principally divided into four stages that include Gap1 (G1), synthesis (S), Gap2 (G2) in interphase, and mitosis (M). During interphase (G1, S, G2), chromosome replication and cell preparation for division are performed, and in the mitotic phase two identical daughter cells are produced (Nurse P. 2020). In interphase, cells become ready for division in G1, which is then followed by the synthesis of genetic material in the S phase. Before the M phase entry, organelles are produced and genetic material condensed to start cell division during mitosis (Cooper GM. 2000). In order to have an entire cell cycle, the mechanisms and progress of the cycle are monitored and investigated by checkpoints in each step of the proliferation cycle (Cooper GM. 2000). Cell proliferation and division occur when environmental conditions are favourable, in cases such as the presence of nutrients and growth factors, genome integrity, and cell cycle checkpoint approval (Forsburg SL et al. 1991). When cells are under stress, caused by factors, such as the lack of nutrition (glucose, phosphate, and nitrogen) or damage, cells exit the cycle through G1 and enter a resting non-vegetative phase called Gap 0 (G0) (Gómez EB & Forsburg SL. 2004). Cells in the G0 phase can be irreversible through senescence/differentiation or reversible through quiescence (Blagosklonny MV. 2011; Takeda K & Yanagida M. 2010).

The quiescence state is characterized by a low RNA expression, a decrease of DNA and RNA content, and the absence of proliferative markers (Gómez EB & Forsburg SL. 2004). The transcriptional profiles and cellular pathways change drastically to survive in these new conditions (Oya E et al. 2019; Sajiki K et al. 2018; Yanagida M. 2009). Under the absence of nutrients quiescence optimizes the odds of survival by maintaining mitotic competence and the ability to re-start the cell cycle upon nutrition compensation (Sajiki K et al. 2018; Sajiki K et al. 2013; Blagosklonny MV. 2011; Takeda K & Yanagida M. 2010). The proper regulation of this non-replicative stage is essential for cell adaptation and survival. For example, cancer cells enter into a resting phase, which is called ‘dormancy’ under stress in order to maintain cell survival. Therefore, they escape treatment and have a chance to re-enter the cell cycle and have the potential to develop malignant cancer types (Zhang J et al. 2019).

1.4.2 How chromatin regulation controls cell cycle progression in eukaryotes?

The expression of each gene can play a crucial role in controlling cell cycle progression and cell differentiation in order to avoid producing abnormal cells such as cancer cells. For example, at the end of the G1 phase, the cell cycle is controlled by the expression of tumour suppressor genes (TSGs) to inspect DNA damage and cell integrity. Cells can either be directed in the absence of damage to the next phase of the cell cycle or be temporarily stopped for DNA repair mechanisms. When necessary, cells are directed to senescence or apoptosis pathways to avoid abnormal cell proliferation (Terzi MY et al. 2016).

The regulation of gene expression involved in each phase of the cell cycle is an important aspect of cell cycle progression. For example, in *S. pombe*, the cyclin-dependent kinase (CDK) *Cdk2* is differently expressed during the cell cycle and increased in M and S phases (Stern B & Nurse P. 1996).

Chromatin regulation is essential to regulate both step-specific gene expression and global gene expression (for example RNA pol II) to continue the cell cycle progression (Zhurinsky J et al. 2010). SWI/SNF enzymes regulate the cell cycle during the muscle cell differentiation process (Sendinc E et al. 2015). As mentioned above the ATPase-dependent remodeler, RSC, restructures chromatin by altering the nucleosome structure in budding yeast. RSC is required for growth and mitotic cell cycle division (Muchardt C & Yaniv M. 2001). Progression of the cell cycle is controlled by the regulated expression of specific proteins during the different cell cycle phases (Moser BA & Russell P. 2000). In addition, growth factors, hormones, and checkpoint pathways also control cell cycle progression (Moser BA & Russell P. 2000; Stern B & Nurse P. 1996).

1.4.3 Investigation into the challenges of *S. pombe* cell cycle analysis in proliferation and quiescence

During rapid growth, *S. pombe* cells are mainly in the G2 phase. During proliferation of fission yeast, the septum is not formed until the G1/S phase and cytokinesis begins in the M phase and terminating at the end of the S phase (Balazs A et al. 2012). After nuclear division (mitosis, M), the cells go through G1 and enter the S phase before the cells complete cytokinesis (Balazs A et al. 2012) (Figure 5). Therefore, cells in the G1 phase contain two nuclei each, with a single completed genome amount (1C DNA content), hence these cells contain the same total amount of 2C DNA content. In the S phase, the genome is duplicated (4C DNA content). When cells enter the G2 phase, cells contain their DNA within a single nucleus (2C) (Balazs A et al. 2012). Due to singularities in fission yeast, distinguishing the cell population by measuring the cellular DNA content (histogram analysis) remains not straightforward.

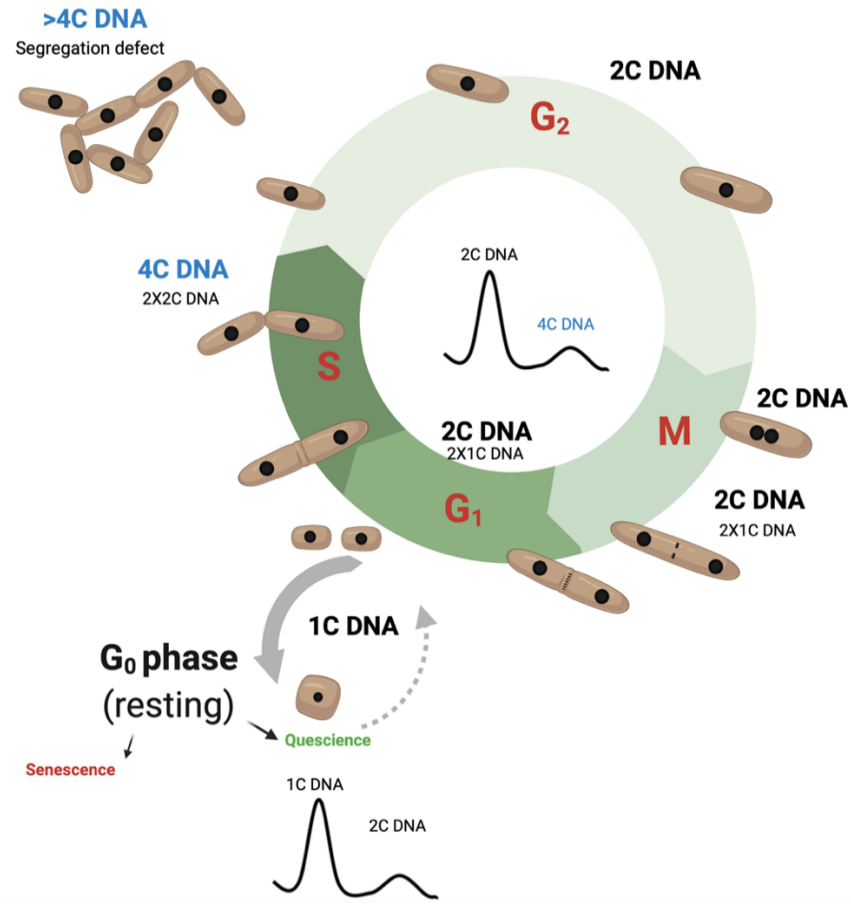


Figure 5. ***S. pombe*** **in proliferation and quiescence cell cycle process**. Overview of the fission yeast cell cycle in vegetative (green cycle) and quiescence phases explained via histograms. The incomplete cytokinesis caused the formation of binuclear 2C DNA content (2 x 1C DNA) in mitosis. Furthermore, DNA content is not decreased in G1. The 2C peak demonstrates the cells in G1, late M, and G2. DNA content in the S phase will be 4C after DNA synthesis and placed in a separated flow cytometry peak in the vegetative phase (created by BioRender software).

In *S. pombe*, the lack of nitrogen or glucose in the media can induce cell arrest in order to maintain cell survival within a non-proliferative and reversible G0 phase (Sajiki K et al. 2013; Yanagida M. 2009). For example, in the absence of glucose, cells stop their division and start to lose their viability within 32 hours (Sajiki K et al. 2018). Under nitrogen deprivation in the media and in the absence of mating partners, cells proliferate two rounds of the cell cycle and become G0 quiescence cells with 1C DNA content after 12-24 hours. Proliferative cells are rod shaped, while quiescence cells become small and round-shaped (Sajiki K et al. 2009) (Figure 6).

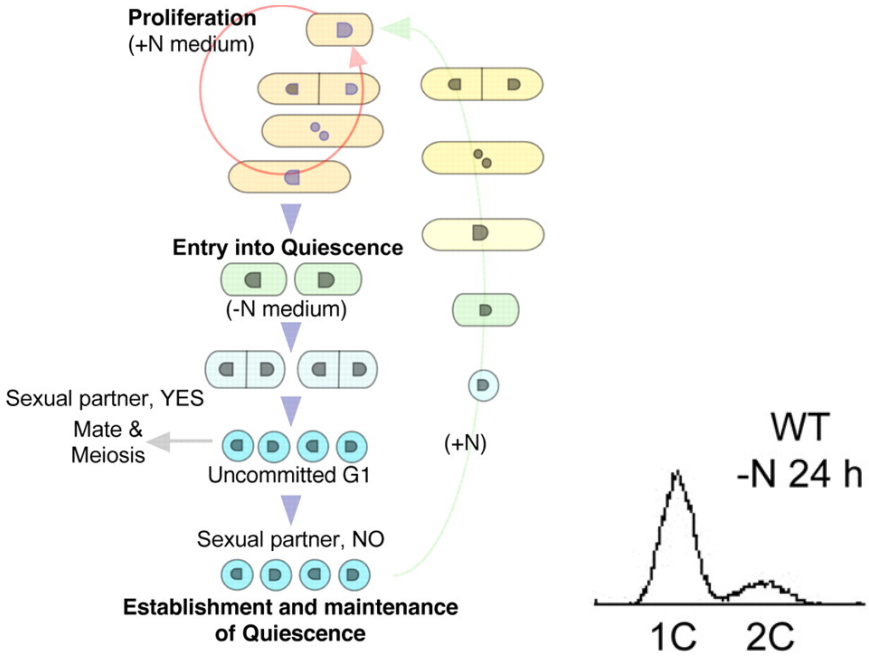


Figure 6. *S. pombe* in proliferation and quiescence stages. In the presence of nitrogen in the media, cells proliferate with a normal size and a rod shape. In the absence of nitrogen and mating partners, cells undergo two cellular divisions and enter into quiescence G0. Quiescence cells are small and round shaped. After 24 hours, the DNA content histogram of quiescence cells (WT) under nitrogen starvation detected 1C DNA content cells by flow cytometry analysis. Quiescence cells are revealed by the increased number of cells with a 1C DNA content peak (Sajiki K et al. 2009).

1.5 ADVANTAGE OF FLOW CYTOMETRY TO ANALYSE *S. POMBE* CELL CYCLE

Flow cytometry is an accurate and popular technique that can be used to investigate many aspects of cell behaviour such as cell size, DNA content, or metabolic activities using various detectors. Flow cytometry is used for various purposes, for example to investigate blood cell marks, cell viability, and cell cycle analysis. Detection of proliferative cell stages in eukaryotes, such as G1 (2C DNA content), S, or G2/M (4C DNA content) phases can be determined using a flow cytometry histogram in cell cycle studies. In *S. pombe* cells, quiescence (G0) can be detected via flow cytometry by the characteristic 1C DNA content. However, in vegetative fission yeast cells, the histogram-peak analysis cannot distinguish G1 (binuclear) from G2 (mononuclear) cells, since both cellular stages take place within the 2C peak due to incomplete cytokinesis at this stage (Knutsen JH et al. 2011).

To solve this issue, the cell population can be analysed in detail using a specific flow cytometry gating strategy instead of histogram analysis (Knutsen JH et al. 2011) (Figure 7). Additionally, in general, population analysis is more accurate than histogram analysis in flow cytometry-based assay. Recent technological advances allow for high-throughput analysis using 96- or 384-well plate formats. Additionally, in quiescence cells, three main populations of G0, G1 (two attached binuclear cells), G2 (single

mononuclear cell) and M can be identified. In this case, DNA-A determines the total area of DNA obtained as a signal when cells pass a laser, and DNA-W shows a total width of DNA signal, which is increased in binuclear cells in comparison with mononuclear cells (Knutsen JH et al. 2011). Therefore, flow cytometry is an appropriate technique to investigate cell cycle and vegetative cells in fission yeast.

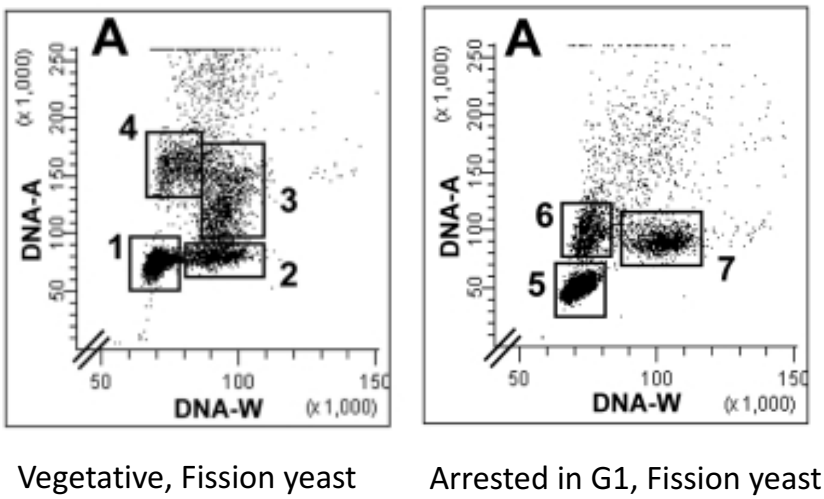


Figure 7. Specific population flow cytometry analysis to investigate *S. pombe* cell cycle and arrested cells. Population analysis of cells in vegetative stages G2 (DNA-A negative - DNA-W negative), are selected in population 1, G1, late M (DNA-A negative -DNA-W positive), are selected in population 2, S (DNA-A positive -DNA-W positive, population 3), and Population 4 demonstrates cells in duplets. Thr arrested cells (G0) are selected in population 5 (DNA-A negative -DNA-W negative). The cells in G1 and G2 are located in population 6, and cells that are still in S phase selected in population 7. DNA-W/DNA-A negative cells are mononuclear and DNA-W positive cells demonstrates cells in binuclear (two nuclei in one cytoplasm) (Knutsen JH et al. 2011).

1.6 THE FISSION YEAST SCHIZOSACCHAROMYCES POMBE

The *Schizosaccharomyces pombe* (fission yeast) is an excellent and popular model organism for chromatin studies due to the basic chromatin organization, which is very similar to human cells (Piel M & Tran PT. 2009; Wilhelm BT et al. 2008; Yanagida M. 2002). Additionally, it is a proper model to study the cell cycle due to its rapid division cycle, regular size, and the way it grows by elongation with the formation of a septum (Daga RR & Chang F. 2005; Wixon J. 2002).

This unicellular eukaryote is a non-pathogenic organism that grows quickly in both solid and liquid media, making it convenient for various studies. The genome of fission yeast has a size of 14 megabases (MB) with only three chromosomes containing approximately 5000 coding genes. About 70% of *S. pombe* protein-coding genes have human orthologue and have a high range of linkage with human diseases (Wood V et al. 2012).

2 RESEARCH AIMS

In this thesis, the effect of genes encoding proteins involved in the alteration of chromatin and mediating epigenetic reprograming, is explored in fission yeast. Moreover, our genetic analysis of the cell cycle aiming to discover the new genes involved in the proliferation of *S. pombe,* is presented.

Study I: Study the role of Abo1 in the forming of different chromatin structures, as well as, the mechanism of H3K9me2 to H3K9me3 transition in heterochromatin regions.

Study II: Investigation of the new genes and complexes that are involved in chromatin regulation, and required for cellular quiescence entry and maintenance, such as Ino80 C and SAGA C.

Study III: The exploration of Ino80 remodelling complex, which mediates chromatin remodelling and regulates gene expression, in cellular quiescence

Study IV: high-throughput Investigation of cell cycle analysis to discover the new genes involved in the cell cycle progress

3 MATERIALS AND METHODS

In this study, different methods were designed and used to investigate the cell cycle in the vegetative state and at different time points of quiescence. Additionally, the level of RNA production was measured in both vegetative state and quiescence to explore the effect of chromatin remodelling complexes on gene expression in the fission yeast model.

3.1 CELL CULTURE

In all projects, fission yeast (*Schizosacchromyces pombe*) was used as a great laboratory model in chromatin structure and cell cycle study.
For vegetative state investigation and normal culturing, yeast cells were cultured in full nutrition (Yeast Extract with Supplements) YES media (5g/l yeast extract, 20g/l glucose with 225g/l supplements: adenine, histidine, lysine hydrochloride, leucine, and uracil and 2% Bacto agar for solid culture).

To perform the quiescence experiment, first the fission yeast cells were cultured in Pombe Glutamate Medium (PMG), consisting of 3g/l potassium hydrogen phthalate, 2.2g/l Na2HPO4, 3.75g/l L-glutamic acid- monosodium salt (Sigma G-5889) as a nitrogen source, 20g/l glucose, 20ml/l salt, 1ml/l vitamin, and 0.1 ml/l mineral. To starve the cells in nitrogen-free media, PMG minus nitrogen source was used to arrest the cells.

In high throughput experiments, liquid culture in a high-volume 96-well plate was used to grow the cells. To avoid evaporation of liquid media, plates were covered by a permeable layer to allow cells to breathe and were incubated under a humidity chamber in shaker incubators.

3.2 LIBRARIES AND STRAINS

1- Single strains that were used in paper 1, were selected from Karl Ekwall group's strains bank.
2- The main source of the cells in the vegetative state was Bioneer library version five (Bioneer company) and this was used directly.
3- To design a small specific library that included a number of genes involved in chromatin remodelling and DNA repair, the main Bioneer library was used. The selection of strains of interest was done based on gene ontology. The small library

was crossed after selection to produce the main source of the cells for the quiescence projects.

3.3 PREPARATION OF SMALL LIBRARY

The vegetative stage in the whole Bioneer library was investigated in the sub-project, while for the quiescence study we narrowed this to a selection of around 750 mutants from the Bioneer library. The Bioneer library is auxotrophic which cannot survive under the absence of a nitrogen source, therefore we must produce a prototrophic library to investigate cells under these conditions.

To produce this library, we crossed the auxotrophic Bioneer V.5 library which has a *mat1-P* mating type linked to the *leu1-32* (leucine auxotrophic) marker and carries gene deletions marked by the resistant antibiotic cassette *KanMX4*, with a prototrophic *mat1-M smt-0* strain. Next, we selected prototrophic strains from this cross that carried the *KanMx4* gene deletion and the *mat1-M smt-0* mating type. The prototrophic strains are able to survive during nitrogen starvation (Sideri T et al. 2014).

Bioneer V.5 library was crossed with the *mat1-M smt-0* strain in the Sporulation Agar (SPA) plate using a ROTOR robot (used for high-throughput screening, Singer company), and incubated at 25°C for 3 days to produce spores. To eliminate the rest of the vegetative cells plates were incubated at 42°C for 3 days. Spores were transferred to YES plates (as a rich media) to regrow prototrophic deletion mutants. Finally, the selection of prototrophic mutants marked by the *kanMX4* cassette was performed by re-growing the mutants on Edinburgh Minimal Media (EMM) and YES + G418 respectively (Sideri T et al. 2014) (Figure 8).

During this procedure, we eliminated 1.7 % of the V.5 library that could not mate with Smt0. All mutants were placed again into a 96-well plate (YES liquid media + 20% glycerol) via ROTOR and stored at -80°C (large library for quiescence study).

After crossing and prototroph selection, mutants of interest were manually selected to produce a smaller library from the main library. During this selection, mutants involved in chromatin or transcription regulation processes were selected from the generated prototroph library using Gene Ontology (GO) terms such as “chromatin binding”, “DNA binding”,” chromosome binding”, “chromosome”, and “transcription”.

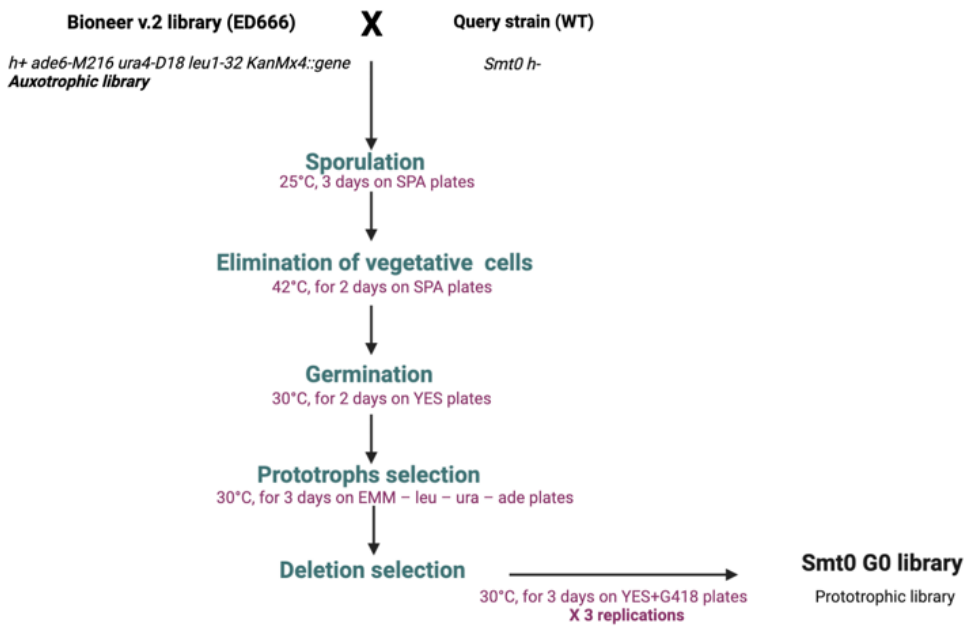


Figure 8. Overview of fission yeast mating strategy. The version 5 Bioneer library was mated with *smt-0* strain to produce a prototrophic library which is able to survive in the lack of nitrogen source (Bahler strategy, Sideri et al. 2014)

For each experiment, the specific rectangular cell plates (YES) were handled by ROTOR pads and 0.5µl of mutants from V.5 were transferred into each plate considering the control (empty well) and incubated at 30°C for 3 days. All mutants were then arranged in 8 plates (8 X 96-well plates). After 3 days, cells from agar plates were transferred into 96-well plates YES media with complements containing 20% glycerol using ROTOR, and stored at -80°C. This smaller library for quiescence study was named the''Small G0 Library''.

3.4 STARVATION OF THE CELLS AND CELL PREPARATION FOR FLOW CYTOMETRY ANALYSIS

Cells were transferred from the library directly into the solid SPA plates using the ROTOR robot and then incubated at 30°C for 3 days. Colonies were transferred from the solid plate to 96-well plates containing YES liquid media (via ROTOR) and incubated in a shaking incubator at 30°C, 200 RPM inside a humidity chamber for 12 hours. Serial dilutions were performed to reach $1x10^6$ cell/ml. Next, 3µl of cells from the YES culture were transferred into the PMG media containing nitrogen source and incubated to reach $1x10^7$ cells/ml (Yanagida M. 2009). In this step, 50µl of culture was harvested for the samples at T=0 (D0).

The rest of the culture was washed and then incubated in 1500µl with pre-warmed PMG minus nitrogen at 30°C, using a shaker (200 RPM), in the humidity chamber for 4 weeks. The starvation process was performed in high-volume 96-well plates with a 1500µl total volume of media (2 ml deep plate). Cells were collected at five points in time under starvation and prepared for viability and DNA content investigation using the plate mode of flow cytometry (Cytoflex) (second high-throughput step). For all time points (T=0, 24H, 7D, 14D, 21D, and 28D), cells were stained with a fixable viability kit (Live-or-Dye™ 640/662, APC emission filter/ VWR), then fixed with 70% ethanol (30 minutes incubation in ice) and stained (DNA) with Propidium Iodide (PI) (1% Sigma Aldrich) (30 minutes incubation) after sodium citrate-RNase A (Roche company) treatment (3 hours incubation at 37°C).
The preparation steps were performed for all mutants in separate wells. Samples were analysed and investigated by using the "slow" mode multiplex setting of the flow cytometry. Double staining and a specific gating strategy for flow cytometry allowed us to investigate both viability and DNA content at the same time and from the same sample (Figure 9).

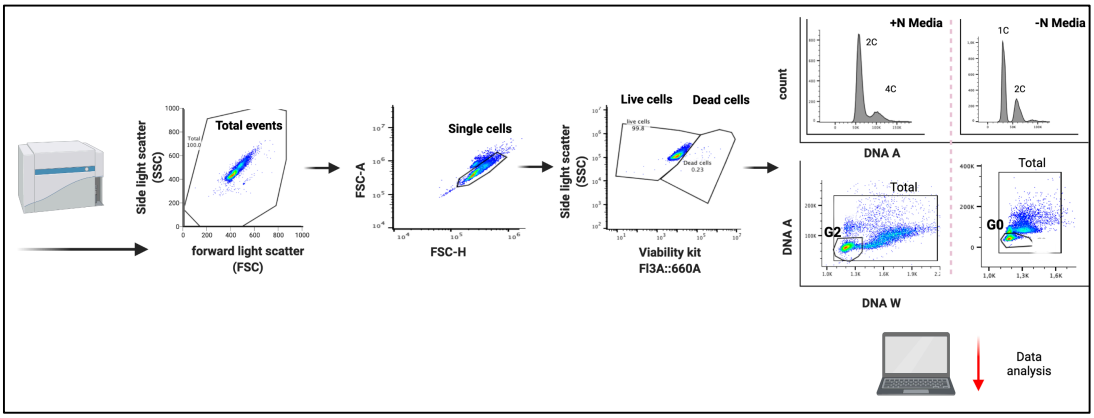


Figure 9. Gating strategy to investigate viability and DNA content. After excusing dead cells by Fl3A signal, DNA content (histogram analysis) and percentage of G2 cells in vegetative and G0 cells after arresting (-N media) were investigated from live cells.

3.5 FACS DATA ANALYSIS FOR VEGETATIVE AND QUIESCENCE CELL

Flowjo software (From Flowjo company, versions 9) was used to analyse the DNA content and viability rate. Due to incomplete cytokinesis, both population and histogram analyses were performed for all mutants in this project (Figure 9) (Knutsen JH et al. 2011). Generally, forward light scatter (FSC) vs side light scatter (SSC) were used to measure all cells and categories them in the different groups, based on their size and internal complexity of cells. Then these detection settings were used to select all flow events. FSC-A (total area of DNA signal) vs FSC-H (total height of DNA signal) is one of the popular gating strategy to discard doublets. Doublet cells, that

passed the cytometer, have the same high as single cells but the total area is two times larger. Alive and dead cells were collected from the single cell population by FSC-A vs FL3A (R660) which detected the labelled dead cells (labelled free intracellular amines of the dead cell). Therefore, live and dead cells could be distinguished. In each run, at least 20,000 events of living cells were recorded and dead cells discarded (positive cells stained fixable viability kit). DNA content analysis was performed from the live cell population for all time points. For DNA content analysis, the total Area of DNA signal (DNA-A) vs Width of DNA signal (DNA-W) displays the DNA content in both vegetative and arrested cells (Knutsen JH et al. 2011). Three populations for vegetative cells (in T=0) in G1- late M, G2 and S are collected by DNA-A vs DNA-W. Cells in the late M and G1 phases are binuclear and include two 1C DNA content particles, therefore, they have the same DNA content as G2 (2C DNA content). Cells with two nuclei show the higher DNA-W signal in comparison with mononuclear, additionally, cells with more DNA content have a higher total DNA signal (DNA-A) the S phase. After nitrogen starvation, DNA content decreased and G0 cells with lower DNA content were placed on the lower part of the DNA-A axis. On the other hand, two of the G1 cells that performed cytokinesis and are still attached to each other as well as G2 cells with 2C DNA content (higher DNA-A) placed above the G0 population. Cells that do not undergo cytokinesis (G1 or M) were located in the separated populations with higher DNA-W (Figure 9).

For all mutants, the analysis was performed in biological duplicate in paper 2 and triplicate in paper 3. The G2 percentage for each mutant illustrates the normal cell cycle progress in the proliferative phase and the G0 percentage shows the ability of each mutant to enter quiescence.

Each mutant of the small library was explored for one month in biological duplicate (paper 2). In paper 3, the same strategy was used to select mutants in biological triplicate format for two weeks.

3.6 STATISTICAL DATA ANALYSIS AND VISUALISATION PROGRAM

Raw data, which was collected by Flowjo software was analysed through statistical tools such as JMP (SAS) and Excel, then compiled and visualised using Tableau software and JMP. The next step in this study was an investigation of the expression levels of the selected genes. To this aim, we used the RNA-seq technique to investigate the level of transcription.

3.7 GENE EXPRESSION ANALYSIS VIA RNA ISOLATION

Wild type and mutant strains were grown in a liquid YES and then PMG+N medium using a shaking incubator (200 RPM at 30°C) to reach 10^6 cells/ml. Then, the culture was washed with pre-warmed PMG-N and incubated for 24 hours in 500 ml of pre-warmed PMG-N using a shaking incubator (200 RPM at 30°C).
For RNA extraction, cells were washed with ice-cold PBS and re-suspended in 500 µl of ice-cold RNA extraction buffer (10 mM Tris–HCl pH 8.0, 1 mM EDTA, 2% Triton X-100, 1% SDS, 100 mM NaCl). Then, 500 µl of Phenol (acidic phenol pH 4.5, Sigma) and 500 µl of glass beads (acid washed, Sigma) were added to each tube. Cells were vortexed and incubated at 65°C for 45-60 min and then incubated on ice for 5 min. Samples were centrifuged (1300 g, 5 min, 4°C). The upper aqueous part was collected and transferred to a tube with 500 µl of chloroform (Sigma Aldrich). It was then vortexed and centrifuged (1300 g, 5 min, 4°C). The upper phase was collected and subjected to RNA precipitation at -20°C overnight. The precipitated RNA was washed once with 70% ethanol then dissolved in 30 µl RNA-DNA free H2O and kept at -80°C for further study.

3.8 RNA-SEQ AND BIOINFORMATICS

All steps were performed in the BEA facility (Huddinge, Sweden). In the next step, 3 µg of excreted total RNA was treated with Ribominus Eukaryote System v.2 kit (Ambion, Thermo Fisher Scientific) To exclude rRNA from the purified RNA. For sequencing, library preparation was performed using the Illumina Stranded mRNA Prep Ligation kit (Illumina) and 100 ng rRNA-depleted stocks sample and Qubit (HS dsDNA) was used to quantify samples. Illumina Nextseq 2000 platform (P3 100 cycle kit, 58 + 58 cycles, paired-end sequencing) was used to sequence. Samples were normalized based on the number of cells before arresting by using ERCC RNA Spike-In Mix 1, dilution 1:100 (Invitrogen, Thermo Fisher Scientific). bcl2fastq v2.20.0.422 program was used to convert data from Nextseq 2000 (Bcl files). The Schizosaccharomyces_pombe reference genome (ASM294v2) and spike-in sequences were profiled and indexed by the STAR 2.7.9a program (Dobin A et al. 2013) to prepare fastq files. eatureCounts v1.5.1 (Liao Y et al. 2014) was used to count the exons. Gene expression analysis was performed by EdgeR package (Robinson MD et al. 2010) in a linear setting. TMM normalization was used to analyze data based on ERCC spike in the samples with genes higher than 1 per million in 3 or more samples.

4 RESULTS AND DISCUSSION

Study I: Abo1 is required for the H3K9me2 to H3K9me3 transition in heterochromatin

The presence of H3K9me2 and H3K9me3 play a crucial role in the stability and formation of heterochromatin, as well as the silencing mechanism. Two forms of facultative and constitutive heterochromatin have distinct heterochromatin patterns (Grewal SI & Jia S. 2007; Peters AH et al. 2002). In this study, we characterized the role of conserved bromodomain AAA/ATPase, Abo1 in heterochromatin formation and maintenance in *Schizosaccharomyces pombe* ''fission yeast'' (Gal C et al. 2016). We discovered the interaction of Abo1 with histone deacetylase Clr3, H3K9 methyltransferase Clr4, and HP1 homologous of Swi6 that are heterochromatin factors and are involved in silencing. The determinant of selective removal (DSR) as facultative heterochromatin, carries the genes involved in meiosis and becomes silent via Clr4 when cells are not in meiosis (Zofall M et al. 2012; Harigaya Y et al. 2006). Abo1 mediated the H3K9me2-me3 transition in different heterochromatin regions, such as the DSR island.

First of all, to investigate the genetic interaction of Abo1 with Clr3, Clr4 and Swi6, as the heterochromatin factors, the synthetic genetic array (SGA) was used to cross the Abo1 strain with a small single deletion library including 771 genes involved in chromatin regulation. Data demonstrated the strong negative genetic interaction of Clr3, Clr4 and Swi6 with Abo1, which reflected the effect of Abo1 in heterochromatin assembly. Clr4 alters the silencing pattern at the facultative heterochromatin island at a low temperature (18°C) (Gallagher, P.S et al. 2018). Furthermore, other bromodomain affect gene expression in heterochromatin under temperature stress (Col E et al. 2017). Hence, we investigated the behaviour of heterochromatin factors in *abo1*Δ under the heat shock. Viability analysis displayed a higher level of mortality in *abo1*Δ*clr3*Δ, *abo1*Δ*clr4*Δ, and *abo1*Δ*swi6*Δ in comparison with WT under temperature stress (25°C and 37°C and cells could not tolerate heat shock (Flow Cytometry analysis). Therefore, the negative genetic interaction of Abo1 and all heterochromatin factors demonstrated the effect of Abo1 in the formation of heterochromatin. Additionally, the Chip-Seq and RNA profiling data demonstrated a reduction of H3K9me2 and H3K9me3 in the subtelomeric region of chromosomes I and II, as well as an abundance of RNA expression in the same region in *abo1*Δ. However, in the right end of chromosome III, only H3K9me2 was increased slightly which did not affect the RNA expression level. Moreover, a reduction of silencing in the lack of *abo1* was observed with re growing *tel2L::ura4*$^{+}$ strains in minus and plus ura media. Therefore, Abo1 is involved in temperature-independent gene silencing regulation in the subtelomeric regions.

In fission yeast, an increase of H3K9me2 causes activation of transcription, while H3K9me3 induces transcription silencing (Ivanova AV et al. 1998). An increase of H3K9me2 and a decrease of H3K9me3 is reported in the pericentromeric region in *clr4*Δ (Jih, G et al. 2017). Additionally, in the pericentric region, *clr4*Δ blocks the transition to H3K9me3, which demonstrates the role of Clr4 on H3K9me3 transition.
To assess how Abo1-Clr4 interaction affects heterochromatin formation, the H3K9me2 level was investigated by Chip-qPCR in *abo1*Δ and WT. Then, data compared with available data for Clr4 mutant strains (*clr4*Δ, $clr4^{I418P}$, $clr4^{F449Y}$, and $clr4^{W31G}$) and other mutants involved in the heterochromatin association process (Jih, G et al. 2017; Zofall M et al. 2016; Zofall M et al. 2012). The result illustrated the strong decrease in the level of H3K9me2 in both *abo1*Δ and $clr4^{W31G}$ in subtelomeric regions. Therefore, *abo1*Δ may affect H3K9me2 to me3 transition, the same as $clr4^{W31G}$. Data was confirmed by Chip-qPCR and RT-PCR analysis that demonstrated a reduction of H3K9me2 and H3K9me3 in the absence of *Abo1,* in the genes that are located in the Tel1R and Tel1L subtelomeric region. However, gene expression analysis demonstrated an increase of H3K9me2 and a decrease of H3K9me3 in both *dhk* repeat (in centromeric region) and *dg-dh-like* repeat (in *tlh1*, telomeric region) in *abo1*Δ compared to WT. This data confirmed the role of Abo1 in silencing defect (decrease of H3K9me3 in the pericentric region).

The constitutive pericentromeric region becomes silent with a two step-process, firstly RNAi co-transcriptional gene silencing (RNAi-CTGS) and secondly, RNAi transcriptional gene silencing (RNAi-TGS). RITS complex is activated by siRNA (from RNAi-CTGS) and mediates H3K9me establishment via Clr4 methyltransferase recruitment. The dg-dh region can still be transcribed due to the presence of H3 hyperacetylation (H3ac) and H3K9me2. In the next step, swi6 interacts with H3K9me3 and induces silencing machinery (Jih, G et al. 2017; Zofall M et al. 2016). At the pericentric region of *S. pombe*, Clr4 SET domain I418P, F449Y, and W31G mutants block the transition of H3K9me2 to H3K9me3 with different mechanisms (Jih, G et al. 2017; Towbin BD et al. 2012; Bessler JB et al. 2010; Zhang, K et al. 2008). Furthermore, in the absence of *Clr4*, H3K9me2 increased and H3K9me3 decreased to activate dg-dh repeat in the pericentric region (Jih, G et al. 2017). This data strongly supported our hypothesis.

Combining what we know about the role of Clr4 in the organization of H3K9me2 and me3 regions with our data, we can draw a conclusion: in facultative heterochromatin region, Abo1 can mediate promotion of both H3K9me2/me3 by recruitment of Clr4 under the TGS mechanism.

Our data displayed a decrease of H3K9me2 and me3 in DSR islands in *abo1*Δ in comparison with WT. DSR includes meiotic genes and is silent when the cells are not in meiosis, and regulation of silencing is mediated by Clr4 and the RNA elimination process (Zofall M et al. 2016; Harigaya Y et al. 2006). To investigate the expression

of this island, RT-qPCR was performed for single and double deletion mutants *rrp6* (genes involved in RNA degradation) (*rrp6*Δ, *rrp6*Δ*abo1*Δ) and *abo1*Δ, and compared with WT. Gene expression decreased in *rrp6*Δ*abo1*Δ, while H3K9me2 and me3 were reduced. However, gene expression is high in *rrp6*Δ and is low in *abo1*Δ. Hence *abo1*Δ caused a gene expression reduction in *rrp6*Δ *abo1*Δ. This data is strong evidence to confirm the role of Abo1 in heterochromatin formation and transition from H3K9me2 to me3 in DSR islands. Moreover, Chip-qPCR analysis on subtelomeric regions demonstrated a decrease in Clr4 occupancy levels in Clr4 flag tagged-*abo1*Δ in comparison with Clr4 flag tagged, which was verified by decreasing the level of H3K9me3 in *abo1*Δ *in* the previous experiment. In the absence of Abo1, Clr4 occupancy decreased in telomeric repeats (*tlh1*), centromeric region (*dhk*) and in DSR island regions.

All data supports the role of Abo1 in the transition from H3K9me2 to H3K9me3 by recruitment of Clr4 as a methyltransferase in different types of heterochromatin regions, although several aspects of Abo1 in formation of heterochromatin in fission yeast is still unclear and further study is needed.

Study II: High-Throughput Flow Cytometry Combined with Genetic Analysis Brings New Insights into the Understanding of Chromatin Regulation of Cellular Quiescence

Cellular quiescence is a reversible resting phase that cells enter into due to cellular reprogramming in the biological adaptation process. In fact, when nutrition is limited, cells genetically reprogram to decrease metabolism and biological activity in order to save energy for survival (Valcourt JR et al. 2012). In this study, we discovered new genes and complexes involved in quiescence entry and survival in fission yeast. Data demonstrated the role of the Ino80 complex as an ATPase remodeler complex, in quiescence entry and maintenance. Additionally, the SAGA complex, which is involved in histone acetylation, as well as factors involved in DNA repair are required for survival in quiescence.

To start the investigation of genes involved in quiescence entry and survival, a small prepared auxotrophic library including genes involved in chromatin remodeling, DNA repair, and heterochromatin formation, was cultured in YES media to germinate. Then cells were re-grown in PMG+N and then starved in PMG-N media. After 24 hours, cells became quiescence G0 (Yanagida M. 2009), and the quiescence phenotype on all individual mutants was assessed. More information is mentioned in the material and method section. During the first part of the study, the percentage of DNA content and cells in G2 (before nitrogen starvation) and G0 (after nitrogen starvation), as well as the viability rate was analysed via flow cytometry specific gating strategy (Knutsen JH et al. 2011). All parameters were investigated at six time points (T-0 before starvation, 24 hours in starvation, 1 week, 2 weeks, 3 weeks and 4 weeks into starvation). In the proliferation step, the normal fission yeast is mainly in G2 (Peng X et al. 2005), which was monitored in our study as well. Therefore, the number of G2 cells before starvation represented how the cell cycle progresses and discovered cell cycle defects in the absence of each mutant in the whole library. After starvation, the G0 percentage and 1C DNA content represented the arrested cells, and cells in quiescence. In our study, the half-time (1/2) mortality rate represented the viability rate.

After analysis, all mutants were collected and classified based on 1/4 and 1/2 mortality rate, G2 percentage before starvation, and G0 percentage after nitrogen starvation in day 1 and day 7. Higher 1/2 mortality rate defines more viable cells. In clusters 1 and 2, a high half time mortality rate (higher viability) is correlated with high G0 entry which shows adaption in quiescence. In cluster 3, cells enter into G0 but cannot survive in quiescence (low half time mortality rate). Cells in cluster 4 demonstrate a high rate of mortality and low G0 percentage. Cluster 6 includes the mutants with a high mortality rate and low G0 percentage. In cluster 7 genes with different phenotypes are observed and the majority of them show a moderate rate of

mortality and G0 entry. The clusters with a low G0 entry rate and high mortality phenotype were selected for more analysis.

Clusters 4 and 5 demonstrated the phenotype in G0 entry. All 15 genes classified in cluster 4 showed strong G0 entry defects. The genes encoding subunits of the SAGA complex were highly enriched in this cluster (*ubp8, tra1,* and *sgf11*) and data suggested the clear effect of the SAGA complex in quiescence entry. However, in cluster 5, the mild G0 entry defect phenotype was observed. This cluster included genes encoding subunits of set1C/COMPASS (histone methyltransferase) complex. We can conclude that, the genes which are required for quiescence entry may need to be activated by SAGA, as a coactivator of transcription, and set1C/COMPASS, which is involved in H3K4 methylation (Cheon Y et al. 2020; Shilatifard A et al. 2012).
High enrichment of DASH complex genes and Fft3, which is an ATPase dependent nucleosome remodeling factor, was observed and indicated the role of DASH complex and Fft3 in quiescence entry in the absence of nitrogen. DASH complex is required for segregation and may be related to the effect of this complex in quiescence entry (Miranda JJ et al. 2005). Moreover, the clear quiescence phenotype in the absence of *fft3* can be connected to the role of this gene in the organization of telomeres and the effect of telomere organization on the maintenance of the nuclear periphery in quiescence (Maestroni L et al. 2020; Strålfors A et al. 2011).

In the next step, the genes involved in survival maintenance during quiescence were selected from clusters 2 and 3. Under the assessment of cluster 2, the six genes of Clr6 histone deacetylase complex with high enrichment were obtained. Additionally, in cluster 3, a high enrichment of genes, which encoded Ino80 C subunits remodeling complex, DASH C, and RITS complex was identified. Data showed a high mortality rate in quiescence in the absence of the subunits of all mentioned complexes.
RNA interference pathways play critical roles to maintain survival in fission yeast quiescence (Roche B et al. 2016), while heterochromatin formation, which is mediated by Clr4 methyltransferase activation, can be regulated by RNAi machinery mechanisms (Joh RI et al. 2016). Hence, the observation of the quiescence phenotype in the lack of RITS activity is explainable and logical.
Interestingly, the *hht2*Δ, encoding histone H3, was also in cluster 3, while no other histone's genes were found in any clusters belonging to the maintenance of quiescence. This data expressed the strong effect of *hht2* in the maintenance of quiescence, while the other histone mutants, *hht1* and *hht3*, showed a mild phenotype during starvation.

In this study, several complexes involving chromatin remodelling were investigated in quiescence. We assessed the behaviour of Paf1 C, Rpd3 C, SAGA C, and Ino80 C in quiescence. Data showed a high mortality percentage (low half time mortality rate) in MBF C and Paf1 C, while no G0 entry defect was observed in these two complexes

under the absence of nitrogen. Hence, these complexes are required for survival during quiescence, not for G0 entry. Furthermore, the lack of *alp13* and *cph2* genes (belonging to histone deacetylase Rpd3s complex) decreased G0 entry, while the rest of the genes belonging to Rpd3s and Rpd3L were only required for viability maintenance of quiescence cells in the absence of nitrogen. MBF (MluI cell-cycle box binding factor) is a transcription factor complex and Whi5, Yox1 and Nrm1 act as co-repressors of the MBF complex (Travesa A. 2013; Gómez-Escoda B et al. 2011; de Bruin RA et al. 2006). The transcription of genes mediated by the MBF complex is repressed by MBF's co-repressors. For instance, in fission yeast, Yox1 represses the gene transcription in the G1/S stage of the cell cycle (De Bruin RA et al. 2006). In this study, data demonstrated the significant mortality phenotype in quiescence in the absence of MBF complex co-repressors (Whi5, Yox1, and Nrm1), which is in line with the probable role of this complex to repress genes that are supposed to be silenced during quiescence stage.

Additionally, the lack of genes *spt20*, *ubp8*, *tra1*, *sgf11*, and *sgf73* in the SAGA complex led to the G0 entry defect. However, the rest of SAGA complex's subunits (*ada2*, *sgf29*, *gcn5*, and *ngg1*) were involved in survival maintenance of quiescence. Moreover, *ies6* and *tgf3* genes (of Ino80 complex) are involved in G0 entry. However, the rest of the components are involved in survival maintenance in quiescence. Therefore, both SAGA and Ino80 complexes are involved in G0 entry and maintenance of survival during quiescence.

Ino80 is the chromatin remodeler complex that mediates nucleosome eviction and H2A-H2A.Z histone exchange, and is implicated in some DNA damage repair processes in quiescence (Eustermann S et al. 2018; Brahma, S et al. 2017; Hogan CJ et al. 2010). Moreover, the effect of this complex on histone H3 turnover was discovered (Singh PP et al. 2020). As we mentioned and according to our data, *hht2* is required for quiescence maintenance, and considering the role of Ino80 on H3 turnover, we may conclude that H3 turnover which is mediated by Ino80 is required for survival in quiescence.

All genes encoded Ino80 C subunits illustrated the mortality phenotype in quiescence in our research. Therefore, we re-assessed the viability phenotype of all components and compared them with positive control *hht2*Δ and WT. Data validation approved the effect of *ies6* and *pht1* (encoding Histone variant H2A.Z) on mortality rate during quiescence. The highest mortality rate was observed in *ies6*Δ and *pht1*Δ in quiescence. In addition, histone variant H2A.Z is removed via the Ino80 C from chromatin and is deposed via the SWR1C (Brahma, S et al. 2017; Mizuguchi G et al. 2004). According to our data, the Ino80 C is required for G0 entry and maintenance of quiescence, and it displayed a stronger quiescence phenotype in comparison with SWR1. Hence, we can conclude that removing H2A.Z via Ino80 C is more crucial in comparison to deposing it via the SWR1C in quiescence maintenance.

In the last part of this study, we focused on the genes involved in the DNA repairing process and the quiescence phenotype was specified in several cases. A defect of G0

entry was reported in two following complexes. The *mms1*Δ and *rad51*Δ strains, that belong to the Homology-dependent repair (HR) complex, as well as in *mhf1*Δ and *mhf2*Δ (from complex synthesis-dependent strand annealing- SDSA), which is involved in the double-strand break repair process. Moreover, Rad3 (ATR-like checkpoint-kinase), Xrc4 (non-homologous end-joining factor and the member of NHEJ pathway), Rad50 and Mer1 (from MRN complex), and Nht1 from Ino80 are required for quiescence maintenance, and all of them are involved in DNA repair.

The presence of Rad3 as an ATR-like checkpoint-kinase, which is involved in the NHEJ repair pathway, is essential for the G0 process under UV irradiation (Mochida S and Yanagida M. 2006). Additionally, in DNA double-strand break, the MRN complex is involved in the activation of ATR kinase before the HR and NHEJ pathways (Lee JH et al. 2014). According to our data, all mentioned genes, which showed the quiescence phenotype, are involved in different DNA repair pathways.

Study III: An essential role for the Ino80 chromatin remodeling complex in regulation of gene expression during cellular quiescence

Cellular quiescence is a resting phase that stops cell division in order to proliferate, respond to stress, and repair DNA. Ino80 C (INOsitol requiring nucleosome remodelling factor) is an ATP-dependent remodelling complex, which is required for quiescence survival. This study investigates the role of Ino80 in regulation of gene expression during quiescence in fission yeast. The reduction of gene transcription levels was observed in Ino80 subunits *Arp42* and *Iec1*, inositol *Asp1*, as well as *Pht1* which encoded histone variant H2A.Z. Moreover, the lack of Ino80 subunits affects the activation of several genes that are localized in the subtelomeric region. In this study, we investigate the Ino80 C, which is regulated by Asp1 inositol kinase, and is required for H2A.Z exchange in the subtelomeric region. Our results suggest that removal of H2A.Z via Ino80, which is modulated by inositol kinase, causes a restructuring of chromatin and the activation of genes that are required for survival during quiescence.

The Ino80 C removes H2A.Z from the nucleosome and replaces it with H2A due to restructuring of chromatin and regulation of gene expression. Opposite, SWR1 C deposits H2A.Z (Papamichos-Chronakis M et al. 2011). Interestingly, the lack of SWR1 C subunits has less of an effect on quiescence survival when compared to the absence of Ino80 C subunits, which highlights the crucial role of Ino80 C in the maintenance of quiescence (Zahedi Y et al. 2020). Previous studies show the effect of Ino80 subunits (Iec1, Arp8, Iec3, Nht1, Ies4 and Ies2) in quiescence maintenance and chronological aging in fission yeast (Romila CA et al. 2021; Zahedi Y et al. 2020). Asp1, as an inositol kinas, modulates Ino80 C activity in *Saccharomyces cerevisiae* (Shen X et al. 2003). Additionally, it is important for quiescence survival in *S. pombe* (Sajiki K et al. 2018). In this study, we assessed the effect of Asp1 on Ino80 C and the function of Iec1, H2A.Z and histone H3 to regulate gene expression during quiescence.

In this study, the viability rate, DNA content and gene expression level in five null mutants were compared with WT at different time points of quiescence induced by nitrogen starvation. The included mutants in this study were Ino80 C subunits point mutants, *arp42Δ* and *iec1Δ,* followed by *asp1Δ* (deletion on inositol kinase gene), *pht1Δ* (gene carries H2A.Z) and *hht2Δ* (deletion on histone H3 encoding genes).

Data demonstrated the reduced viability in Ino80 C subunits *iec1Δ* and *arp42Δ* as well as *pht1Δ* after one week, and viability reduction of *asp1Δ* cells after one to two weeks, which was already discovered previously (Sajiki K et al. 2018). However, a milder phenotype was observed for *hht2Δ* cells after two weeks. According to data, *iec1Δ* demonstrated a strong quiescence entry phenotype in comparison with control, which was already reported previously (Zahedi Y et al. 2020). Moreover, RNA-seq

data illustrated similar levels of gene expression in *hht2Δ* in comparison with WT, while global repression was reported in *arp42Δ, iec1Δ, asp1Δ,* and *pht1Δ* in 24 hours. In quiescence, gene transcription is downregulated in order to minimize the level of metabolism (Marguerat S et al. 2012), which corresponds to our data.

In WT (*smt-0*), 149 up regulated genes and 1208 downregulated genes were found at 24 hours. Then, in the upregulated gene category for WT, 16 'core quiescence genes' were identified that were common upregulated genes in three quiescence time points, and 9 out of 16 core quiescence genes were located near *tel1R* and *tel2L* in the subtelomeric regions. After verification of our data, induction of gene transcription was confirmed of all core quiescence genes which matches a previous study (Marguerat S et al. 2012). Moreover, the investigation of downregulated genes in different time points illustrated more than 97 percent of downregulated transcriptomes after two weeks of quiescence.
In the next step, transcriptional changes in different time points were assessed in all mutants. *hht2Δ* with 1286 downregulated genes in 2 weeks of quiescence showed the weakest phenotype, which is correlated with high viability of *hht2Δ* in the first two weeks of quiescence. This gene is expressed constitutively in the cell cycle, in contrast with *hht1* and *hht3* that are expressed in the S phase (Takayama Y & Takahashi K. 2007), therefore the only histone gene which is expressed in quiescence is *hht2*.
On the other hand, *pht1Δ,* which encodes H2A.Z, demonstrates a high mortality rate during quiescence and a high number of downregulated genes in one week, which matches the role of H2A.Z for regulating genes and the effect of H2A.Z in quiescence survival. Moreover, Ino80 C subunit point mutants (*arp42Δ* and *iec1Δ*) illustrated a decrease in transcriptome in the first day of G0 and also a high mortality rate during quiescence. This data supported the role of Ino80 C as a remodeler to remove H2A.Z and is matched with published data that demonstrates the function of Ino80 C on quiescence survival (Zahedi Y et al. 2020).

Asp1, which is inositol kinase mediates the production of Inositol polyphosphate, IP8, and in the absence of *asp1*, the level of IP8 decreased, while IP6 and IP7 levels increased (Pascual-Ortiz et al. 2021). Additionally, IP6 inhibits Ino80 C activity in budding yeast. The quiescence phenotype of *asp1Δ* and mutants of Ino80 C subunits (*arp42Δ* and *iec1Δ*) are similar, and considering the previous data we can conclude *asp1Δ* induces the accumulation of IP6 and IP7 and these inositol polyphosphates may inhibit Ino80 C in *S. pombe*.

Any of the upregulated genes in WT (149 genes) were downregulated in *arp42*Δ*, iec1*Δ*,* and *asp1*Δ cells in the first day of quiescence, meaning that the upregulation of these genes are required for quiescence survival. In fission yeast, peripheral clusters are formed in the telomere location of chromosome I and II (Maestroni L et al. 2020).

Moreover, subtelomeric regions include genes that are activated during meiosis and sporulation (Mata J et al. 2002).

None of the 149 genes that are upregulated in WT are enriched in the absence of H2A.Z (*pht1Δ* cells). Additionally, the strong overlap genome-wild between *pht1Δ* and Ino80 C subunits *(arp42Δ* and *iec1Δ*) was observed.

When considering the role of Ino80 C in removing H2A.Z and what we observed in our data, we can propose that it is important to present H2A.Z on the promoter (particularly located in the subtelomeric region) of genes that are required for quiescence. Fft3 is important to maintain H2A.Z boundaries (Steglich B et al. 2015). Therefore, we can propose the idea that the lack of *Fft3* induces downregulation of subtelomeric boundary elements, hence increasing H2A.Z occupancy in the subtelomeric regions. This background may be related to the function of Ino80 C in activating gene transcription by modulation of Asp1, to remove H2A.Z. Hence, this reorganises chromatin for regulation of gene expression in order to respond to nitrogen starvation.

Study IV: Introducing the novel group of genes that regulate cell cycle progression

Each step of the cell cycle is controlled via checkpoints and any defect during this process leads to abnormal cell proliferation, lack of the normal cell cycle product, or non-controlled cell division. The investigation of the cell cycle steps may introduce the role of the specific genes to lead a cell proliferation. In this study, we tried to assess the behaviour of selected genes in the vegetative stage to investigate the new genes involved in the progress of cell cycle and cell survival in proliferation, considering their growing speed.

We used deleted coding-gene haploid strains from version 5 (Bioneer) fission yeast library includes around 3000 deletion mutants. The cell cycle steps were studied and viability rate and DNA content of cells in whole library were analysed by Flow Cytometry (cytoflex), as well as considering the size of the colonies. All genes were assessed individually and in three biological replications. The FACS gating strategy was the same with project II.

After the analysis of genes, 6 different clusters were obtained based on the DNA content and mortality rate. Since fission yeast is mainly in G2 during the normal cell cycle, it was expected to see high 2C DNA content cells (G2) followed by a short peak of 4C (cells in S phase). Also, we analysed cells in each step of the cell cycle by population analysis that displayed the exact percentage of cells in different cell cycle steps (G2, G1-M, and S) for each mutant by considering the survival rate in the vegetative stage and colony size. Based on G2 percentage and mortality rate, around 3050 mutants from library were classified in 6 groups (Figure 10A). Cells with higher than 4 C DNA content may involve in segregation (Cluster 5 and 6) and lower percentage of G2 demonstrates defect of cell cycle progress (Figure 10A-D).

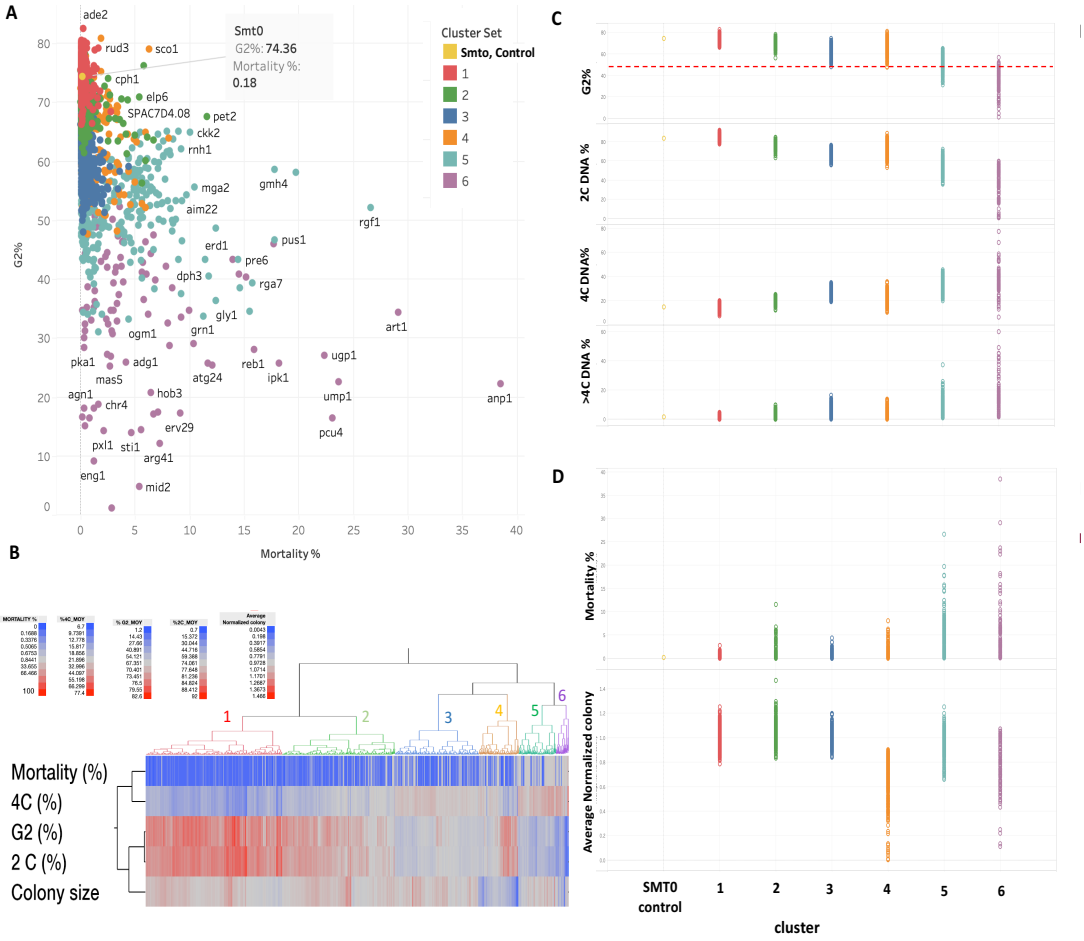


Figure 10. Hierarchical clustering and cluster description based on mutant phenotype patterns. **(A)** Representation of the cell cycle progression (percentage of cells in G2) and cell mortality for each mutant. Smt0 is used as a control **(B)** Heat map showing individual characterisation of mutants to select candidate involved in maintenance of viability and cell cycle process and growing intensity. **(C)** Intensities of the phenotypic data demonstrates high G2 and 2C DNA content %, lower number of cells in 4C DNA content in the first 2 clusters while cells with 2C DNA content and G2 decreasing and 4C and even cells with higher than 4C DNA rising step by step in other clusters. **(D)** Phenotypic data represents mortality rate and average colony size in each cluster comparing with control. Data for average colony size in control in not available but it shows less than 0.2 % of mortality. Cluster 4 identifying cells with smaller size and mutants with highest mortality rate classified in cluster 5 and 6.

Data shows the majority of the library demonstrated a normal G2 percentage and a high viability rate, and this data confirmed that, these genes are not essential for cell cycle progress and survival. Besides that, a lower percentage of cells in 2C or low viability rate demonstrates the critical roles of some genes in the progress of the cell cycle or survival of cells (all genes in cluster 5 and 6 and 2 genes from cluster 3 and 4) (Figure 11). During analysis of the whole library, 134 novel genes that are involved in cell cycle analysis were investigated. The deeper analysis of them identified the genes involved in the maintenance of viability during proliferation, the progress of the cell cycle, and segregation.

Characterization of the new genes involved in cell cycle progress

All investigated genes below 50% in G2 were selected from the whole library (n=207 were sorted based on cells below), and data on these was compared with 699 selected genes from the Pombase data set (abnormal cell cycle) (Figure 11A). In this selection, 134 new genes were found (Figure11A). All 134 genes were compared based on G2%, cells with 2C and 4C DNA content, cells with higher than 4C DNA content (>4C DNA), and mortality rate (Table 4). The average colony size data was not available for 9 out of 134 selected genes, therefore they were excluded for future analysis (Figure 11B) (Table 4). The other 125 (out of 134) genes were analysed based on G2 percentage, mortality rate and colony size (Figure 11C) (Table 5). The gene names with all information are mentioned in table 6.

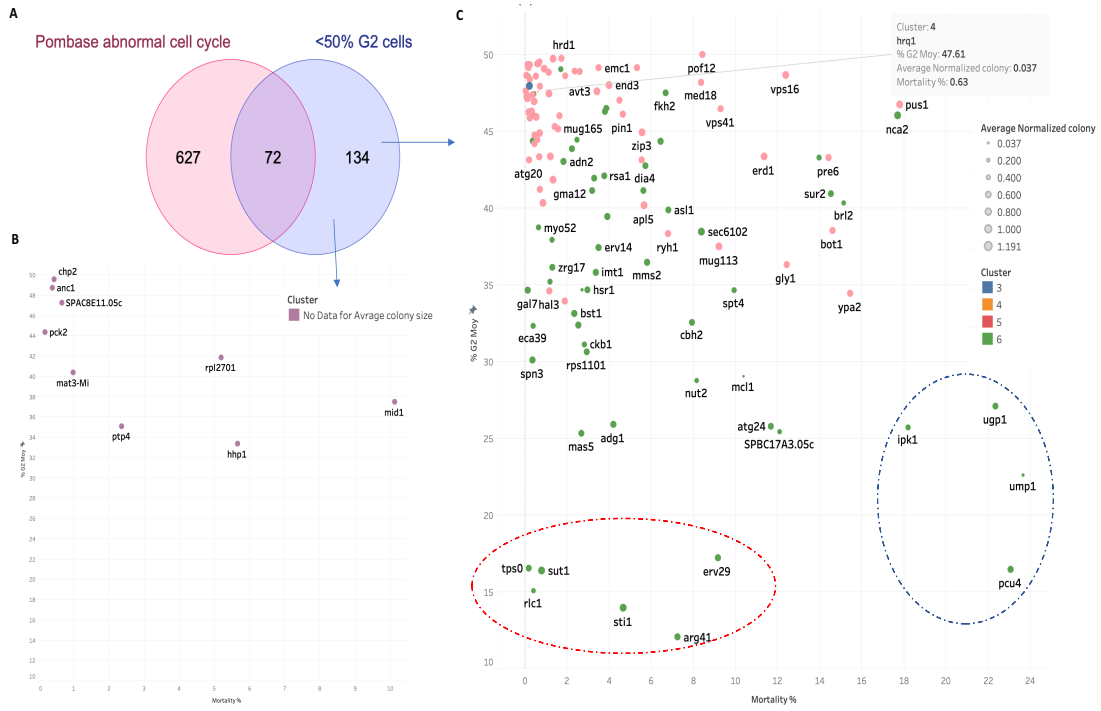


Figure 11. Identification of genes involved in cell cycle progress and survival. A. Comparing the genes involved in abnormal cell cycle (extracted form Pombase) with all genes with lower than 50 percent G2 in our analysis. 134 new genes with lower G2 % observed. B. excluded 9 genes from the selected 134 genes in order to the lack of data for average mortality rate. C selected genes that are involved in survival and cell cycle progress are located on the bottom of graph (red cycle), and mutants that lave less effect on survival and still high effect on cell cycle progress are collected in the blue cycle.

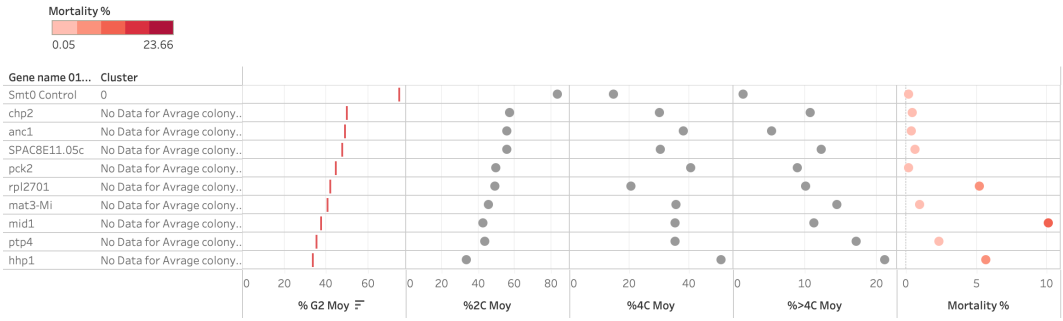
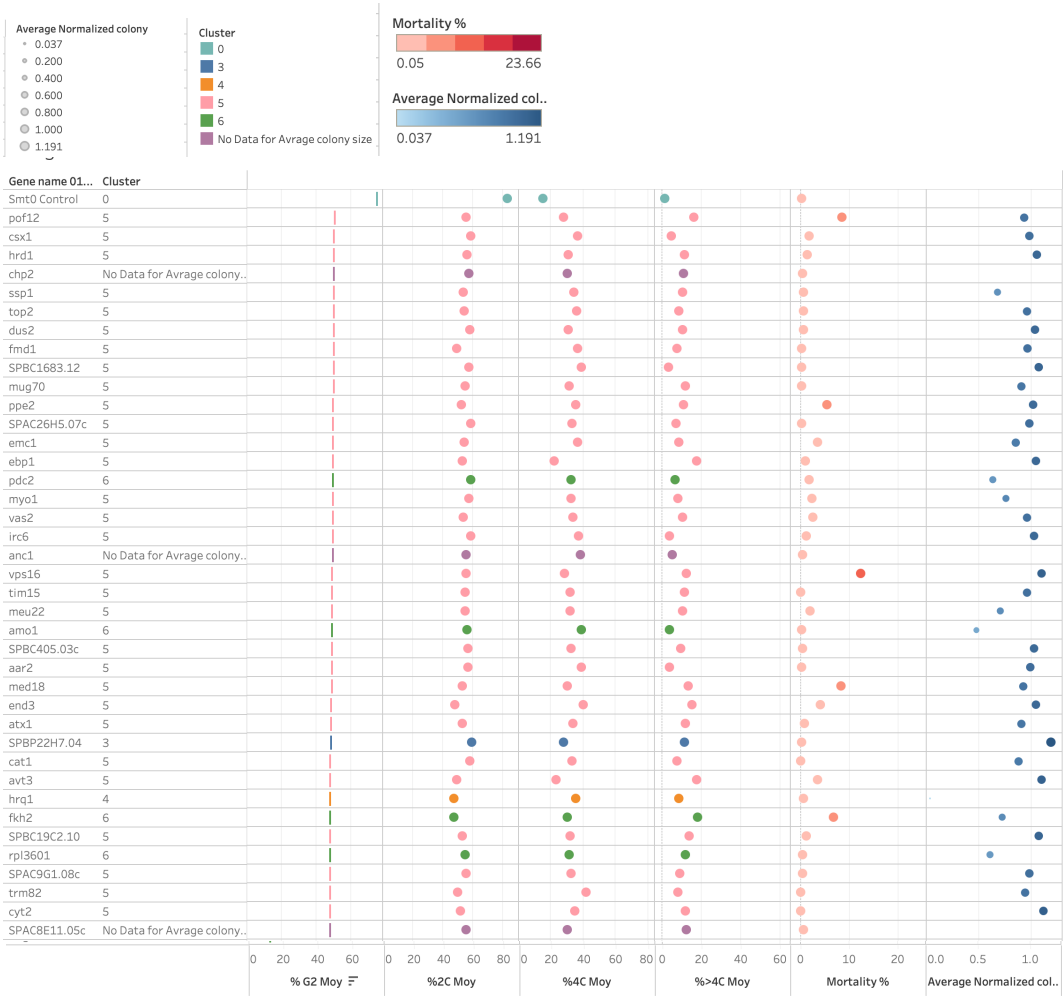


Table 4. Cell cycle analysis for 9 out of 134 the new genes with <50% G2 from whole library (Data for colony size is not available for these genes).



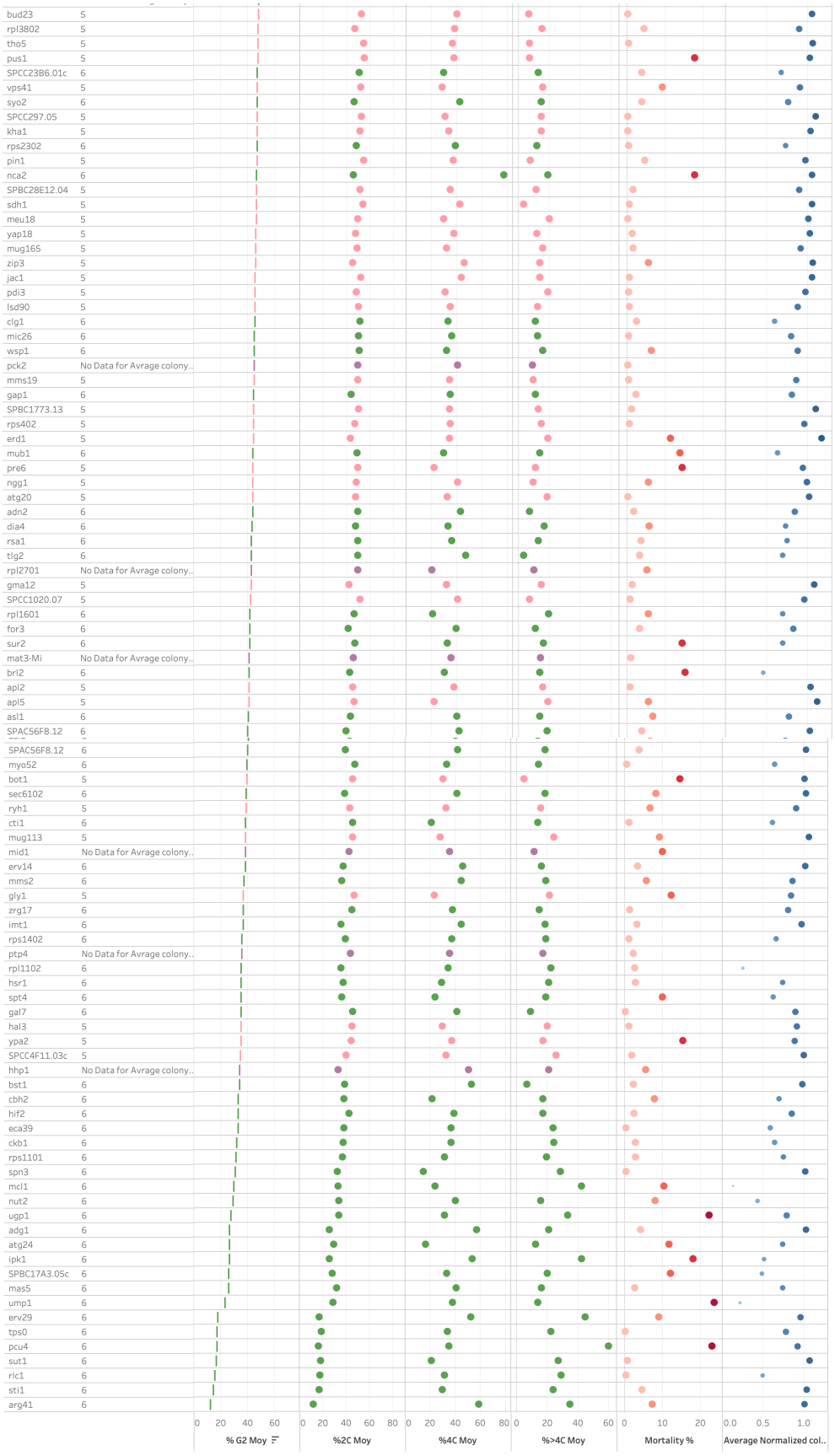


Table 5. Cell cycle analysis and average colony size rate for 125 out of 134 the new genes with <50% G2 from whole library.

Gene name	Information
aar2	SPAC3H5.04 chromosome_1 U5 snRNP-associated protein Aar2 Q6LA53 protein coding gene
adg1	SPAPJ760.03c chromosome_1 Schizosaccharomyces specific protein Adg1 Q9P7E7 protein coding gene
adn2	SPBC1289.10c chromosome_2 DNA-binding transcription factor Adn2 O94619 protein coding gene
amo1	SPBC15D4.10c chromosome_2 nuclear rim protein Amo1 O74315 protein coding gene
anc1	SPBC530.10c chromosome_2 mitochondrial carrier, ATP:ADP antiporter Anc1 Q09188 protein coding gene
apl2	SPBC947.02 chromosome_2 AP-1 adaptor complex beta subunit Apl2 O43079 protein coding gene
apl5	SPAC144.06 chromosome_1 AP-3 adaptor complex subunit Apl5 Q9UTL8 protein coding gene
arg41	SPBC1539.03c chromosome_2 argininosuccinate lyase P50514 protein coding gene
asl1	SPAC13G6.10c chromosome_1 cell wall protein Asl1, predicted O-glucosyl hydrolase Q09788 protein coding gene
atg20	SPCC16A11.08 chromosome_3 autophagy associated PX/BAR domain sorting nexin Atg20 Q9USM8 protein coding gene
atg24	SPAC6F6.12 chromosome_1 autophagy associated PX/BAR domain sorting nexin Atg24 O14243 protein coding gene snx4
atx1	SPBC1709.10c chromosome_2 copper chaperone Atx1 O74735 protein coding gene
avt3	SPAC3H1.09c chromosome_1 vacuolar amino acid transmembrane transporter Avt3 Q10074 protein coding gene
bot1	SPBC14C8.16c chromosome_2 mitochondrial ribosomal protein subunit S45 O60096 protein coding gene
brl2	SPCC970.10c chromosome_3 histone H2B-K119 ubiquitin ligase complex (HULC) subunit, ubiquitin-protein ligase E3 Brl2 O74563 protein coding gene rfp1
bst1	SPAC824.02 chromosome_1 GPI inositol deacylase Bst1 Q9UT41 protein coding gene
bud23	SPAC26A3.06 chromosome_1 rRNA (guanine-N7-)-methyltransferase Bud23 Q10162 protein coding gene
cat1	SPAC869.11 chromosome_1 plasma membrane arginine/lysine amino acid transmembrane transporter Cat1 Q9URZ4 protein coding gene SPAC922.08c
cbh2	SPBC14F5.12c chromosome_2 CENP-B homolog Cbh2 O60108 protein coding gene
chp2	SPBC16C6.10 chromosome_2 heterochromatin (HP1) family chromodomain protein Chp2 O42934 protein coding gene
ckb1	SPAC1851.03 chromosome_1 CK2 family regulatory subunit Ckb1 P40232 protein coding gene
clg1	SPBC1D7.03 chromosome_2 cyclin-like protein involved in autophagy Clg1 O14336 protein coding gene mug80,SPBC1D7.03c
csx1	SPAC17A2.09c chromosome_1 RNA-binding protein Csx1 O13759 protein coding gene
cti1	SPCC1739.07 chromosome_3 exosome C1D family subunit Cti1 O74469 protein coding gene lrp1,rrp47
cyt2	SPAC24C9.02c chromosome_1 cytochrome c1 heme lyase Cyt2 O13962 protein coding gene
dia4	SPAC25B8.06c chromosome_1 mitochondrial serine-tRNA ligase Q9UTB2 protein coding gene
dus2	SPBC1709.06 chromosome_2 tRNA/mRNA dihydrouridine synthase Dus2 O74731 protein coding gene
ebp1	SPAC6G9.15c chromosome_1 Ell binding protein Ebp1 Q92360 protein coding gene
eca39	SPBC428.02c chromosome_2 branched chain amino acid aminotransferase Eca39 O14370 protein coding gene SPBC582.12c
emc1	SPAC25H1.07 chromosome_1 ER membrane protein complex subunit Emc1 O13981 protein coding gene
end3	SPBC11G11.02c chromosome_2 actin cortical patch component End3 Q9USZ7 protein coding gene
erd1	SPAC227.01c chromosome_1 ER protein, Erd1 homolog, required for glycosylation and ER retention Erd1/Gmn2 Q9UTD8 protein coding gene SPAPB21F2.04c,gmn2,sgm2
erv14	SPAC30C2.05 chromosome_1 cornichon family protein Erv14 Q9P6K6 protein coding gene
erv29	SPCC970.06 chromosome_3 COPII adaptor Erv29 O74559 protein coding gene
fkh2	SPBC16G5.15c chromosome_2 DNA-binding forkhead transcription factor Fkh2 O60129 protein coding gene
fmd1	SPBC1539.07c chromosome_2 glutathione-dependent formaldehyde dehydrogenase P78870 protein coding gene
for3	SPCC895.05 chromosome_3 formin For3 O94532 protein coding gene
gal7	SPBPB2B2.10c chromosome_2 galactose-1-phosphate uridylyltransferase Gal7 Q9HDU5 protein coding gene
gap1	SPBC646.12c chromosome_2 Ras GAP Gap1 P33277 protein coding gene src1,sar1
gly1	SPAC23H3.09c chromosome_1 threonine aldolase Gly1 O13940 protein coding gene
gma12	SPCC736.04c chromosome_3 alpha-1,2-galactosyltransferase Gma12 Q09174 protein coding gene
gma13	SPAC15E1.04 chromosome_1 thymidylate synthase / phosphopantothenoylcysteine decarboxylase / protein phosphatase inhibitor moonlighting protein Hal3 Q9UTI7 protein coding gene
hhp1	SPBC3H7.15 chromosome_2 serine/threonine protein kinase (CK1 family) Hhp1 P40235 protein coding gene
hif2	SPCC1235.09 chromosome_3 Set3 complex subunit Hif2 O74845 protein coding gene
hrd1	SPBC17D11.02c chromosome_2 Hrd1 ubiquitin ligase complex E3 subunit Hrd1 O74757 protein coding gene
hrq1	SPAC23A1.19c chromosome_1 RecQ type DNA helicase Hrq1 O13983 protein coding gene SPAC26H5.01c
hsr1	SPAC3H1.11 chromosome_1 DNA-binding transcription factor Hsr1 Q10076 protein coding gene msn2
imt1	SPAC2F3.01 chromosome_1 mannosyltransferase Imt1 O14084 protein coding gene SPAC323.09
ipk1	SPCC4B3.10c chromosome_3 inositol 1,3,4,5,6-pentakisphosphate (IP5) kinase Q9USK0 protein coding gene
irc6	SPAC19A8.11c chromosome_1 clathrin coat adaptor Irc6 O13827 protein coding gene
jac1	SPAC144.08 chromosome_1 mitochondrial (2Fe-2S) cluster assembly co-chaperone Jac1 Q9UTL6 protein coding gene
kha1	SPAC105.01c chromosome_1 plasma membrane potassium ion/proton antiporter Kha1 Q9P7I1 protein coding gene
lsd90	SPBC16E9.16c chromosome_2 Lsd90 protein A9ZLL8 protein coding gene
mas5	SPBC1734.11 chromosome_2 Hsp40 family DNAJ domain protein Mas5 O74752 protein coding gene
mat3-Mi	SPBC1711.01c chromosome_2 mating type M-specific polypeptide Mi at silenced MAT3 locus P0CY14 protein coding gene matMi,mat3-Mm-silenced,mat3-Mm
mcl1	SPAPB1E7.02c chromosome_1 DNA polymerase alpha accessory factor Mcl1 Q9C107 protein coding gene slr3,cos1
med18	SPAC5D6.05 chromosome_1 mediator complex subunit Med18 O14198 protein coding gene pmc6,sep11
meu18	SPBC409.11 chromosome_2 Schizosaccharomyces specific protein Meu18 Q9UUB3 protein coding gene
meu22	SPBC19F8.06c chromosome_2 amino acid transmembrane transporter Meu22 O60170 protein coding gene
mic26	SPCC1442.05c chromosome_3 MICOS complex subunit Mic23/26/27 O94578 protein coding gene mic27,mic23
mid1	SPCC4B3.15 chromosome_3 anillin-related medial ring protein Mid1 P78953 protein coding gene dmf1
mms19	SPAC1071.02 chromosome_1 CIA machinery protein Mms19 Q9UTR1 protein coding gene
mms2	SPCC338.05c chromosome_3 ubiquitin conjugating enzyme E2 Mms2 O74983 protein coding gene spm2
mub1	SPBC31F10.10c chromosome_2 Armadillo-type fold protein, zf-MYND type zinc finger protein, Mub1-Rad6-Ubr2 ubiquitin ligase complex Mub1 P87311 protein coding gene
mug113	SPAC3F10.05c chromosome_1 GIY-YIGT nuclease superfamily protein Q10180 protein coding gene
mug165	SPAC5D6.02c chromosome_1 Clr6 histone deacetylase complex subunit Mug165 O14196 protein coding gene
mug70	SPAC24C9.05c chromosome_1 CBS and PB1 domain protein, conserved in fungi and plants, implicated in signalling Mug70 O13965 protein coding gene
myo1	SPBC146.13c chromosome_2 myosin type I Q9Y7Z8 protein coding gene

Gene name	Information
myo52	SPCC1919.10c chromosome_3 myosin type V O94477 protein coding gene myo4
nca2	SPBC4B4.02c chromosome_2 mitochondrial protein Nca2 O74963 protein coding gene
ngg1	SPBC28F2.10c chromosome_2 SAGA complex subunit Ngg1/Ada3 Q9USU8 protein coding gene kap1,ada3
nut2	SPBC31F10.09c chromosome_2 mediator complex subunit Med10 P87310 protein coding gene med10
pck2	SPBC12D12.04c chromosome_2 protein kinase C (PKC)-like Pck2 P36583 protein coding gene sts6,pkc1,mok3
pcu4	SPAC3A11.08 chromosome_1 cullin 4 O14122 protein coding gene pcu4,Cul-4
pdc2	SPBC19G7.10c chromosome_2 topoisomerase II-associated deadenylation-dependent mRNA-decapping factor Pdc2 O42958 protein coding gene ppo1
pdi3	SPBC3D6.13c chromosome_2 ER associated protein disulfide isomerase Pdi3 P87178 protein coding gene pdi2
pin1	SPCC16C4.03 chromosome_3 peptidyl-prolyl cis-trans isomerase Pin1 O74448 protein coding gene
pof12	SPBC56F2.01 chromosome_2 F-box protein Pof12 O60053 protein coding gene
ppe2	SPBC26H8.05c chromosome_2 serine/threonine protein phosphatase PP4 catalytic subunit Pph3 O74789 protein coding gene ppe2
pre6	SPBC106.16 chromosome_2 20S proteasome complex subunit alpha 4 Pre6 Q10329 protein coding gene mts7
ptp4	SPBC3D6.05 chromosome_2 phosphatidate cytidylyltransferase Ptp4/Dgk1 P87170 protein coding gene
pus1	SPAC637.10c chromosome_1 19S proteasome regulatory subunit Rpn10 O94444 protein coding gene pus1
rlc1	SPAC926.03 chromosome_1 myosin II regulatory light chain Rlc1 Q9UUG5 protein coding gene
rpl1102	SPBC17G9.10 chromosome_2 60S ribosomal protein L11 P0CT78 protein coding gene rpl11-2
rpl1601	SPBC839.13c chromosome_2 60S ribosomal protein L13/L16 O42991 protein coding gene
rpl2701	SPBC685.07c chromosome_2 60S ribosomal protein L27 O14388 protein coding gene rpl27-1
rpl3601	SPCC970.05 chromosome_3 60S ribosomal protein L36 Q92365 protein coding gene rpl36-1
rpl3802	SPAC30D11.12 Pchromosome_1 60S ribosomal protein L38 Q09900 protein coding gene rpl38-2,rps38
rps1101	SPAC31G5.03 chromosome_1 40S ribosomal protein S11 P0CT73 protein coding gene rps11-1
rps1402	SPBC18H10.13 chromosome_2 40S ribosomal protein S14 P0CT57 protein coding gene rps14-2
rps2302	SPBP4H10.13 chromosome_2 40S ribosomal protein S23 P0CT76 protein coding gene rps23-2
rps402	SPBC21B10.10 chromosome_2 40S ribosomal protein S4 Q9USW5 protein coding gene rps4-2
rsa1	SPBC16C6.03c chromosome_2 ribosome assembly protein Rsa1 O42927 protein coding gene
ryh1	SPAC4C5.02c chromosome_1 Rab family GTPase Ryh1 P17608 protein coding gene hos1,sat7
sdh1	SPAC1556.02c chromosome_1 succinate dehydrogenase Sdh1 Q9UTJ7 protein coding gene
sec6102	SPBC19G7.17 chromosome_2 translocon subunit Sec61 homolog O42965 protein coding gene SPBC36B7.01
SPAC26H5.07c	SPAC26H5.07c chromosome_1 seven transmembrane receptor protein O13989 protein coding gene
SPAC56F8.12	SPAC56F8.12 chromosome_1 DUF2434 family conserved fungal multispanning membrane protein Q10260 protein coding gene
SPAC8E11.05c	SPAC8E11.05c chromosome_1 DUF5102 family conserved fungal protein, associated with clathrin coated vesicles O42882 protein coding gene
SPAC9G1.08c	SPAC9G1.08c Pchromosome_1 palmitoyl-(protein) hydrolase O14304 protein coding gene
SPBC1683.12	SPBC1683.12 chromosome_2 carboxylic acid transmembrane transporter Q9P6J0 protein coding gene
SPBC1773.13	SPBC1773.13 chromosome_2 aromatic aminotransferase O94570 protein coding gene
SPBC17A3.05c	SPBC17A3.05c chromosome_2 DNAJ/DUF1977, human DNAJB12 homolog, Hsp70 co-chaperone O13633 protein coding gene
SPBC19C2.10	SPBC19C2.10 chromosome_2 BAR adaptor protein, human endophilin A1-like ortholog, implicated in endocytosis Q9UUD0 protein coding gene
SPBC28E12.04	SPBC28E12.04 chromosome_2 cytochrome b translation regulator Cbp7 O74361 protein coding gene
SPBC405.03c	SPBC405.03c chromosome_2 transmembrane transporter O94654 protein coding gene
SPBP22H7.04	SPBP22H7.04 chromosome_2 mitochondrial membrane protein, human TMEM186 ortholog, implicated in respiratory complex assembly Q9C0W3 protein coding gene
SPCC1020.07	SPCC1020.07 chromosome_3 pseudouridine-5'-phosphatase O59760 protein coding gene
SPCC23B6.01c	SPCC23B6.01c chromosome_3 sterol transfer protein Osh6 Q9UUA1 protein coding gene
SPCC297.05	SPCC297.05 chromosome_3 Rab GEF, implicated in endosomal transport Q9Y7Q7 protein coding gene
SPCC4F11.03c	SPCC4F11.03c chromosome_3 Schizosaccharomyces specific protein Q9UT68 protein coding gene
spn3	SPBC16A3.01 chromosome_2 mitotic septin Spn3 P48008 protein coding gene SPBC543.01c
spt4	SPBC21C3.16c Pchromosome_2 DSIF transcription elongation factor complex subunit Spt4 Q9P7K8 protein coding gene
ssp1	SPCC297.03 chromosome_3 calcium/calmodulin-dependent (CaMKK) protein kinase Ssp1 P50526 protein coding gene
sti1	SPCC645.14c Pchromosome_3 chaperone activator Sti1 Q9USI5 protein coding gene
sur2	SPBC887.15c chromosome_2 sphingosine hydroxylase/sphingolipid delta-4 desaturase activity Sur2 O94298 protein coding gene
sut1	SPAC2F3.08 chromosome_1 plasma membrane sucrose/maltose:proton symporter Sut1 O14091 protein coding gene
syo2	SPBC1703.03c chromosome_2 armadillo repeat protein, involved in nucleocytoplasmic transport Syo2 Q9P7W7 protein coding gene
tho5	SPBC577.04 chromosome_2 human THOC5 ortholog Tho5 Q9USR5 protein coding gene
tim15	SPAC24H6.02c chromosome_1 TIM23 translocase complex subunit Tim15 Q09759 protein coding gene
tlg2	SPAC823.05c chromosome_1 SNARE Tlg2 Q9P6P1 protein coding gene
top2	SPBC1A4.03c chromosome_2 DNA topoisomerase II P08096 protein coding gene ptr11
tps0	SPBC725.10 chromosome_2 mitochondrial outer membrane protein, TspO/MBR-related, implicated in lipid/sterol transport, tspO O94327 protein coding gene SpTSPO
trm82	SPCC18.13 chromosome_3 tRNA (guanine-N7-)-methyltransferase WD repeat subunit Trm82 O74863 protein coding gene
ugp1	SPCC794.10 chromosome_3 UTP-glucose-1-phosphate uridylyltransferase-like Ugp1 O59819 protein coding gene
ump1	SPCC14G10.03c chromosome_3 proteasome maturation factor Ump1 O74416 protein coding gene
vas2	SPAC4A8.08c chromosome_1 mitochondrial valine-tRNA ligase Vrs2/Vas2 O14160 protein coding gene vas2,ValRS2
vps16	SPAC824.05 chromosome_1 HOPS/CORVET complex subunit Vps16 Q9UT38 protein coding gene
vps41	SPAP27G11.05c chromosome_1 HOPS complex subunit Vps41 Q9P7N3 protein coding gene
wsp1	SPAC4F10.15c chromosome_1 WASp homolog O36027 protein coding gene
yap18	SPBC19F8.03c chromosome_2 ENTH/VHS domain protein O60167 protein coding gene
ypa2	SPAC1782.05 chromosome_1 serine/threonine protein phosphatase PP2A regulatory subunit, PTPA family Ypa2 Q9P7H4 protein coding gene pta2
zip3	SPAP8A3.03 chromosome_1 ER zinc exporter, ZIP family Zip3 Q9UT11 protein coding gene zrt2
zrg17	SPBC16E9.14c chromosome_2 Golgi zinc importer, CDF family, Zrg17 O14329 protein coding gene

Table 6. The list of gene names, systematic ID, and gene function for 134 the new genes (selected from Pombase)

According to the data, mutants *arg41* (argininosuccinate lyase), *sti1* (chaperone activator), *sut1* (plasma membrane sucrose), *tps0* (mitochondrial outer membrane protein, conserved unknown), *rlc1* (myosin II regulatory light chain) and *erv29* (COPII adaptor) from cluster 6, show the lowest G2 % in our screen. They illustrated below 8% of mortality and normal colony size, that demonstrates the role of these genes in the progress of cell cycle, while they are not severe effect on cell viability (Figure 11C). No data has been reported on the effective role of these genes in the progress of cell cycle, while our data demonstrate a significant drop of G2 %, as an evidence of cell cycle defect, in the absence of any of these genes (Figure 11C). On the other hand, *pcu4*Δ, *ipk1*Δ (IP5 kinase), *ugp1*Δ, and *ump1*Δ (proteasome maturation factor), from cluster 6, with low G2 % and high mortality affected both cell cycle progress and viability. Additionally, *ump1* is required for the normal speed

of growing (low average of mortality rate) (Figure 11C). *Pcu4* (cul4, cullin 4) and *ugp1* (UTP-glucose-1-phosphate uridylyltransferase) mutants can be considered as a control, because both of them affect viability (Hayles J et al. 2013; Kopanja D et al. 2009), and it is approved our data. In this screen *mcl1* (DNA polymerase alpha accessory factor), *ipk1*, and *ckb1*(CK2 family regulatory subunit) are required for segregation (Blyth J et al. 2018; Nie M et al. 2012; Tsutsui Y et al. 2005; Williams DR & McIntosh JR. 2002), and high percentage of cells with higher than 4C DNA content, in comparison with control (0 percent of >4C DNA content), in these genes demonstrates same behaviour. Segregation defect correlated with the highest mortality rate is observed for mutants *gly1* (threonine aldolase), *ugp1*, and *pcu4* (data not shown), which may suggest the role of these genes in cell cycle progress and the viability of cells, due to the segregation defect. Interestingly, *spn3* (mitotic septin Spn3), which codes mitotic septin and its important for septin ring assembly and heterochromatin septin complex that is involved in mitotic cell division (An H et al. 2004) and this mutant is also selected as a new gene involved in cell cycle progress.
Thus, in this project we have obtained huge amount of data and more time and deeper data analysis is needed to finalise this project. In this context, I have mentioned some mutants with stronger phenotype in proliferation and further studies will be needed to establish clear conclusions.

This paper is currently on the revision stage.

5 ACKNOWLEDGMENTS

Undoubtedly, it is the best opportunity for me to express my gratitude to all who have been with me during this journey. Without their support, it was not possible to achieve this goal.

Dear professor **Karl Ekwall**, the words cannot afford to explain your supports to all your students in the lab. I have been so lucky to have your professional supervision during my PhD studies. Being a student in your group was a great opportunity for me to learn a lot‹ as well as meeting intelligent scientists. Without your financial, educational, and mental support, I could not manage any challenges through my PhD. Thanks for all and I hope that I can have a chance to collaborate with you again in the future.

My co-supervisor **Michael Durand-Dubief** , I am so excited to work with you and have interesting scientific discussions. Thanks for your support in doing a huge data analysis during my PhD.
Thanks to all my colleagues in **KEK** group, **Lina**, I am so happy to have you as one of my close friends now. I will not forget all our nice memories and your support in the challenging moments during the last year. Thanks for all your kindness. **Vladimir**, I have learned a lot from exciting scientific discussion with you. I wish you all the best. **Eriko**, thanks a lot for sharing your knowledge as a talented postdoc researcher with me. Other group members, **Olga**, **Dorian**, and **Shengyuan** thanks for the nice memories.

Monica and **Eva**, having you as administration member, was the best of luck in my educational life during my PhD and especially in the last months of working at BioNut department. I saw the happiness in your eyes when everything was solved in my personal life, and I will not forget your effort to help me when I really needed it.
Dear **Susann** and **Tassos** and all **BEA** facility, thank you so much for all your effort in RNA-seq and data analysis on my project. It was a great collaboration with you. **Patrick**, you are such a nice person that we can enjoy talking about different topics together. I am so happy that I got a chance to see you sometimes in KI.
Thanks to **Andreas Lennartsson** and **Victoria Menendez Benito** group members. Thanks **Victoria** for nice scientific discussions that we had. **Wenbo**, we had a nice collaboration during our first project and learned a lot from each other, I hope to see you soon. **Sophia** and **Anna**, I am so happy to meet you here in Sweden. I will always remember our nice discussions together. **Alex**, thanks for lending me the ROTOR plates when I really needed them. Working with you in the same corridor was so interesting. I am so happy that you are almost finalizing your PhD. Good luck!

To all my Iranian friends at KI and the first to my dearest **Shabnam**, I am so happy to find you as one of my best friends in Sweden, and I am happier to work with you now every day at Next Cell Pharma Company. I have learned and still will learn a lot from you. I will never forget our nice memories at HERM. **Farzaneh**, my lovely FERI, many thanks for all the seconds that you were beside me when I was sick, for all birthdays that we celebrated at your apartment, for lovely and amazing memories that we created during the last 6 years, and for your number one Ranginak. I'll be your SEFR KILOOMETR forever. **Amineh**, you helped me during the most difficult moments of my life, your support melted my heart and I hope that I can do my best back to your kindness. **Parisa**, my lovely friend with the highest level of sympathy and support that I could not imagine. You proved that friendship can be saved even in distance. Good luck! **Arash**, thanks a lot for your nice support when I was so upset and for your scientific advice when I was applying for the job. **Laia** jan, I found you a bit late but I am so happy to have you as an amazing friend. **Yaser** and **Manizheh**, we are missing both of you here and I hope to see you soon. **Mohsen**, I have learned a lot from you as one of the best scientists that I have met in my life. It is a pity that I do not have you on my thesis nailing. I wish you the best in your life. **Soophie**, the nice girl with a lovely smile, good luck with your PhD. My dearest **Jelveh**, you are one of the best people that I have met in my life, with an amazing mind and pure heart. Thanks for all your kindness and understanding when I faced challenges in my personal life. **Mahin**, as one of my friends and the FACS facility supervisor, thanks for helping me to arrange my booking for a long time FACS analysis at the facility. Good luck with your new life in Sweden!. **Amir** thanks for nice discussion that we had and good luck with your future my friends.

Thanks to all my good friends at HERM department, Karolinska Institute. **Nadir** as my first supervisor at KI, I have learned everything about FACS from you, and this field changed my educational life. I was so lucky to meet you. **Denis**, you are my closest Swedish friend, and I am so happy and lucky to have a friend like you. I will not forget how you had empathy with me when I was upset as a foreign student. Our friendship is sweet like our homemade cakes, always fresh and tasty. **Rajitha**, we have been with each other as young researchers and good friends. Thanks for your helps to finalise my Kappa cover. I always enjoy talking to you and I wish you the best future.

To all my cousins who understood my situation as a PhD student during the last years when I was busy with my studies and could not visit them regularly. **Pouya**, I should have started this acknowledgment with your name since you were the person to change my life 10 years ago when I was so upset and hopeless. If I am here in Sweden and have had the chance to follow my dreams, is because of you. Thanks for your support during all these years. **Kiarokh**, you are one the most supporting and amazing guys I have ever seen in my life. Your empathy from the deepest of your heart was my mental support over the last few years. I am so happy to have you here.

Goli, **Keyrokh**, **Hanie**, **Arash**, and my lovely **Sophie** as the youngest member of our family here, I am so glad to have had all of you during the last 10 years of my life in Sweden. Thank you all for being a part of my life.
And my angel **SAYEH**, you make me happy when I am tired and upset. I am so lucky to be your aunt. Do not forget that " khale yasi hamisheye asheghete". I cannot wait to see you as a successful girl in future. I love you so much azizam.

To my Iranian friends in Sweden: **Ashkan**, I met you at the right time. With your help, I came to Sweden and this journey started then. I should thank you proudly and I will not forget your support never. I wish you all the best. **Marziyeh**, you were my first friend in Sweden, living with you was the most interesting period of my life in Sweden. I am so happy to have you, **Simon**, and **Vida** in my life. **Forough** and **Dariush**, with your kindness and support I felt you like my parents here. I will not forget your care and understanding when I really needed it. **Mehdi**, you are like an older brother to me, and I am so happy for the new chapter of your life which is recently opened. I wish you the best in following your dreams. **Mahyar**, your strong belief in me changed everything in my life. You motivated me to follow my dreams. Thank you for your critical and fundamental role in my life. Good luck my friend!

To My colleagues in **Next Cell Pharma**: **Matias** and **Chaz**, I should especially thank you for giving me a chance to be part of the lab. I hope I will be able to be a good member in your team. **Patrik**, I am so happy to start my days with the smell of your coffee and your positive energy. **Rebecka**, Thanks a lot for your amazing support during the pending time for my residency application. **Bahar,** working with you is one of the brilliant opportunities for me and I am so happy to meet you every day and have you as a friend with a big heart and real kind. To all my new friends in Next Cell Pharma, **Sofia**, **Maria**, **Sofie**, **Preeti**, **Klaudia**, **Giorgia**, **Sruthy**, and **Sakthi**, I am so glad to have all of you as my friendly colleagues. I enjoy learning from and collaborating with you.

To my close friends: **Negin**, you are the first and last one in the most painful and happiest moments of my life during the last 9 years. I was thinking for many days to write just theses lines for you, and it is still so hard for me to explain my feelings. I am glad for having you as a friend in my life. You have been beside me throughout all my sad and happy situations of my life; without any judgements. You are the right person that I can share all my happiness with, after all challenges. Thanks for all your supports my shining diamond. **Sophia**, I should thank Negin to introduce you to me. You are like a lovely older sister to me with bright emotions and a kind heart. Beside you and your lovely family, I always feel safe. **Farkhan**, you are like my older brother that I have always missed in my life. Thanks for being a great support all the time. You, Sophia and my sweet **Adreal** always make my heart warm and bring smile to my lips. My lovely **Marjan**, friendship with you is the best achievement of working at Bionut. Our PhD studies are going to be finished and it is so hard for me

to not to be able to meet you every day. I have learned a lot from you as a scientist and a person with a golden heart. Thanks for all your support, understanding, attention and love. You deserve the best and I wish you brilliant moments in your life. **Nadia**, 10 years are passed from the last time we hugged each other, and you are still my best friend. I really miss you and thanks for more than 20 years of being my trustworthy sister. I am glad to have you in my life to support and believe in me. **Hermineh**, I am always proud of you. You inspire me to be an independent and successful woman. Thanks for believing in me when I had no belief in me! **Nasrin**, I am just counting the second to have a free time after my defense to be more and more with you. You and **Mehdi** are the best gift for me from yaser. A couple with the golden hearts that have been beside me in a best and worst moments in Sweden. I do love both of you and have the best wishes for you from the bottom of my heart.

To my family: **Jan** and **Ingegerd**, you are my Swedish parents. Words do not afford to express my feelings about you. I remember you every day when I pass the house where we lived in. Living with you was an adorable chance for me to be a happy girl. Thanks for your support when I was sad, tired, and upset in my life. It is a pity that I cannot have you beside me on my nailing day and defense, but your love is always with me.

My cousins and my uncles have loved and always believed in me. Thank all of you for everything, all your support, and for spreading positive energy in my life from the UK, Canada, and IRAN. **Mani** and **Mazda**, my lovely twin uncles, it is so amazing that I have this love doubled. I am so happy to have both of you and the memories of **dayi Masoud** in celebrating this achievement. We will miss you dayi Mehdi and your family on my decertation party. My younger brothers, **Ardavan**, **Ahoura**, **Tiam, Arman**, **Arian** and **Aram**, I am the happiest sister in the world to have you as my brothers. **Tiam**, my special thanks to you for your effort and help during the last month. I hope you start your PhD soon, and then I can pay your kindness and support back to you.

I have grown up in the arms of my mom, dad, and my two brothers. Having a family like this is a dream for anybody. We have supported each other to achieve our goals and now we are the happiest family in the world. We have each other with our deepest sympathy, love, and support. My family, although I left you 10 years ago and started a new life far away from home, I have been living with you in my heart and mind every day. Without your support, none of my life challenges could be over. I am so thankful that I can share my success with you today.
My brothers **Siavash** and **Soroush**, I am so glad to be your sister. You are the best brothers and the most amazing supports that I have had in my life. You are always in my heart and Love You Both.

برای پدر ومادر فداکار و عزیزم

من از شما آموختم که روزهای سخت تمام میشوند ولی انسانهای سخت باقی میمانند.
شاید خیلی دشوار به آرزوهایم رسیدم اما همواره سخت جنگیدم چرا که هر دوی شما مرا برای روزهای پر چالش تربیت کردید. به داشتن شما افتخار میکنم و برای تلاشهای شبانه روزی و حمایت های شانه به شانه اتان از من و رویاهایم همواره سپاسگزارتان هستم. از اینکه حامی بلند پروازی های من بودید وهرگز بال های پروازم را نچیدید احساس خوشبختی میکنم و برای داشتن والدینی این چنین سخاوتمند و صبور خداوند را شاکرم. این کتاب نتیجه ۵ سال تلاش من برای یادگیری، و لبخند رضایت این روزهایم نتیجه ۳۵ سال عشق شما است برای پرورش فرزندی که همواره دغدغه اش ساختن وآموختن بوده است. امیدوارم انسان قابل ستایشی باشم برای جهان و بتوانم سهم کوچکی در رخدادهای مثبت دنیا داشته باشم تا غبار دوری ودلتنگی این سالها از روح مهربان شما زدوده شود.
با تمام عشق این کتاب ،که نتیجه ارزشمند ترین دوره زندگی ام است را به هر دوی شما تقدیم میکنم. دوستتان دارم و دستان پر مهر شما را میبوسم.

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