Introduction
Cell division or mitosis is the process by which a cell divides to give rise to two identical daughter cells. The life of any cell between two successive divisions is known as the cell cycle. The eukaryotic cell cycle is a co-ordinate sequence of events where chromosome duplication is followed by chromosomal separation, which can be broadly categorized into four phases — G1, S, G2 and M. In a mitotic cell cycle DNA replication occurs in the S phase, and the equal segregation of genetic material into the nuclei of two progeny cells occur in the M phase of cell cycle. These two events are separated by two ‘gap phases’ — G1 and G2. In these two gap phases, a cell prepares itself for DNA replication and cell division. The G1 and G2 phases are marked by an increase in cellular dimensions also. Throughout the four phases, the genetic material is under strict surveillance. This helps it to maintain genomic stability and fidelity of the cell division process. Damage to the genetic material often halts the cell cycle arrest, and the cell attempts to repair the damage. Appropriate regulation and co-ordination of all these events — DNA replication, chromosome segregation, DNA repair, cell growth and cell cycle arrest is essential for proper cell division. Aberrations in any of the processes will lead to formation of non-identical progeny cells violating the basic goal of cell division. Needless to say, such aberrations are frequently associated with survival defects and have clinical manifestations. Often the loss of cell division control leads to cancer. Thus regulation of mitotic cell division in eukaryotes constitutes an important area of research.
Studies in model organisms like yeasts (budding and fission), Xenopus, sea urchins and mammals have contributed immensely to the understanding of cell cycle regulation. The initiation of DNA replication at specific origins was first identified in budding yeast (1). The cyclins were first identified in sea urchins (2). While the Mitosis Promoting Factor (MPF) required by the cells to enter into M phase was identified in *Xenopus laevis* (3).

The fission yeast, *Schizosaccharomyces pombe*, has served as an excellent model in the field of cell cycle research. The basic cell cycle machinery is a conserved mechanism present in all the eukaryotes from yeasts to humans. Thus, understanding cell cycle regulation in a simpler organism helps a lot in discovering similar processes existing in higher eukaryotes.

As discussed later in Section 1.5 periodic transcription is one of the important determinants of cell cycle progression. However, a lot is still not known about the co-ordination between transcriptional regulation and cell cycle phase transitions. Many transcription factors have been shown to be associated with cell division defects. A prominent name is the list of such transcription factors is Activating Transcription Factor 2 (ATF2). ATF2 has emerged as a target for cancer management therapy. Thus a better understanding of ATF2’s role in the cell cycle is very important.

ATF2 has a well-characterized homolog in *Schizosaccharomyces pombe*, namely Atf1. As the transcription factors are the ultimate regulators of gene expression. This thesis reports the results of the study on the transcriptional regulation of cell
cycle in *S. pombe* by Atf1. Our study mainly involves the role of a bZIP transcription factor, Atf1 in the cell cycle phase transition of fission yeast.

### 1.1. Eukaryotic cell cycle

The cell cycle events are more or less conserved in all eukaryotes from yeasts to mammals. The mitotic phase of cell cycle gives rise to two identical daughter cells from a single parent cell.

The cell cycle of eukaryotes consists of two major events – the replication of the chromosome and the separation of them into two daughter cells. For mammalian cells, the two phases are known as the interphase and the mitotic (M) phase respectively. The M phase is again subdivided into – prophase, metaphase, anaphase and telophase. And the interphase too is divided into – G1, S and G2 phases (4). During the interphase, the cell duplicates its genetic material and cellular components and prepares the cell for mitosis. The DNA replication occurs mainly in the S phase, at G1 the cell prepares itself for replication whereas at G2 gap phase the cell prepares itself for mitosis. It is in G1 that the cell takes the decision to commit to the division.

G1 cells hence need to cross the Restriction point (mammalian cells) or START (yeasts) in order to enter into cell division. The cell makes its decision to cross the restriction point depending on a number of factors like mitogens, the growth rate of the cells, internal stimulation, cell size, and availability of nutrients (5). The cells which do not commit to division stay back in G1 and ultimately enter the G0 or
quiescent state. For dividing cells, entry into the S phase is marked by the onset of DNA replication. Replication begins at multiple origins. After completion of one round of DNA replication, further replication is prevented until the cell reaches at G1 once again. At the end of replication, cells enter the G2 phase and continue to increase in volume. Attainment of a particular threshold size triggers entry into mitosis or M phase (Fig 1.1) (6).

The M phase is divided into following phases – prophase, prometaphase, metaphase, anaphase, and telophase (7). At prophase, the chromosome condensation begins. The nuclear envelope disappears at the prometaphase (8). A complex protein structure assembles at the centromere of the chromosomes. The chromatids get attached to the microtubules emanating from the each pole with the help of the kinetochores. At metaphase, the chromosome completely condenses. They then become ready to endure the tension caused by the separation of the sister...
chromatids to the opposite poles of the cell. This tension acts opposite to the cohesive force between the two sister chromatids. The chromosomes are then aligned at the central region of the spindle along the metaphase plate. At anaphase, the sister chromatids separate and spindle elongation takes place. Finally, at telophase, the newly separated chromosomes decondense, the interphase cytoskeleton is reformed and the mitotic spindles dissolve. Gradually the nuclear envelope is formed. This is followed by cytokinesis, where the cytoplasm is divided to physically give rise to two daughter cells (9). The mitotic phases have been depicted in Fig 1.2.

The eukaryotic cell cycle can go through two different pathways – mitotic or meiotic cycle. In mitotic pathway, two daughter cells are produced which are identical to the parent cell. In meiosis, four daughter cells are produced which are dissimilar from the parent cell. The mitotic cycle has four different cell cycle phases as discussed earlier. One of the most crucial necessities of the cell cycle in any organism is its regulation. There are multiple events which need to be regulated in a cell cycle like – maintenance of genetic material, replication of the DNA and cell division. Unless these events are regulated the survival of the cell becomes difficult. In the case of multicellular organisms, perturbations in any of these events lead to mutations which at times end up with cancer. The cell cycle is a major event occurring in a cell as it controls the survival probability of the same. The cell has got stringent checkpoints which govern the maintenance and proper control over cell division.
Fig1.2. **Stages of mitosis in cell cycle**: During mitosis a single parent cell divides into two daughter cells. At prophase, the condensation of the chromosome begins. In prometaphase the nuclear envelope breaks down and the spindle microtubules starts originating from the opposite poles of the cell. At metaphase the condensed chromosomes align at the equatorial plane. In the following anaphase the cohesins holding the two sister chromatids together are cleaved and then each of them move to the opposite poles of the cell. At telophase the chromosome decondenses again, the nuclear envelope is reformed and the mitotic spindles disappear. This phase is followed by the cytokinesis, where the two daughter cells are finally separated (10).
The cyclin-dependent kinase (CDK), a Serine/Threonine (2) class of proteins has been found to be the key regulators of the cell cycle. They function in phosphorylating and activating proteins in various phases of the cell cycle in a cell cycle specific manner. These proteins include the ones in DNA replication, prevention of re-replication of DNA, chromosome condensation, and reorganization of the microtubules. The CDKs are inhibited by CDK inhibitors which are also known as CKI (11-13). Though the levels of the CDK remain constant throughout the cell cycle, but it is the formation of an active cyclin-CDK complex which governs the phase transition. Formation of this cyclin-CDK complex provides substrate specificity to the CDK proteins. CDK are inactive as monomers but their formation of a heterodimeric cyclin-CDK complex makes them active. There are majorly four classes of cyclin-CDK complexes operating throughout the cell cycle (14). The level of the cyclin–CDK complexes oscillate. The phase-specific expression of the four cyclin-CDK complexes has been depicted in Fig 1.3.

**Fig 1.3. Oscillation of the expression of cyclin–CDK complexes in cell cycle:** The expression and degradation of the cyclin CDK complexes are phase specific. The increase in the expression of the complex initiates entry on the cell into that phase, whereas degradation marks the exit. Delay in both expression or degradation of these complexes will cause cycle delay (14).
In mammals, there are four cyclins – Cyclin A, B, D and E, and there are three kinds of CDK- Cdk2, 4 and 6. The Cyclin A and Cyclin B function in the S phase, G2 phase, and early mitosis. Cyclin D and cyclin E are the G1 cyclins. Thus, the mammalian cyclins fall into two major groups the G1 cyclins and the B-type cyclins.

All the B-type cyclins contain a conserved sequence which is required for its recognition by the Anaphase Promoting Complex (APC) for destruction. Such conserved sequence is absent in G1 cyclins. The former contains a consensus sequence of RXXLXX (I/L) (S/T) N, termed as Destruction box or D-box. This sequence is responsible for the interaction between the cyclins and the co-activator of the APC/C, which ultimately leads to the ubiquitin-mediated proteasomal degradation of the later, by 26S proteasome (Fig 1.4) (15).

In *S. pombe* the CDK is known as Cdc2 and *S. cerevisiae* it is Cdc28. The fission yeast has got four types of cyclins- Cig1, Puc1, Cig2, and Cdc13. A comparison between the formation of the major cyclin –CDK complexes have been shown in Table1.1. The activation of the CDK is dependent on its phosphorylation status at specific amino acid residues. For fission yeasts, phosphorylation at Tyr15/ Thr 14
inhibits the activation of the CDK, whereas phosphorylation at Thr161/Thr167 activates it (11).

### Table 1.1 – Comparison of the cyclin–CDK complexes in mammals and *S. pombe*

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<tr>
<th>Cyclin-CDK complex</th>
<th>Mammals</th>
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<td></td>
<td>Cyclin</td>
<td>CDK</td>
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<td>G1-Cdk</td>
<td>Cyclin D</td>
<td>Cdk4, Cdk6</td>
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<td>G1-S Cdk</td>
<td>Cyclin E</td>
<td>Cdk2</td>
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<td>S- Cdk</td>
<td>Cyclin A, Cyclin E</td>
<td>Cdk1, Cdk2</td>
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<td>M</td>
<td>Cyclin B</td>
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<th><em>S. pombe</em></th>
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<td></td>
<td>Cyclin</td>
<td>CDK</td>
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<td>Puc1</td>
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<td>Puc1, Cig1</td>
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<td>Cdc13</td>
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Many molecules control the cell cycle phase transition process. Among them, the Cyclin-dependent kinases, CDKs, can be the most significant one to play a pivotal role. Cell cycle progression is delayed with the help of the CDKs, whenever the cell senses any kind of damage to its DNA. They help the cell to preserve its genome integrity. These halting mechanisms are called checkpoints. As mentioned in the next section checkpoints do not merely sense DNA damage, but also any irregularity that might interfere with perfect DNA replication, segregation and faithful completion of cell division.
1.2. Cell cycle checkpoints

The transition from one cell cycle phase to the other requires the activity of the cyclin-CDK complexes. The cells have their own machinery to delay a phase if the events in one phase are incomplete or if there is any DNA damage, replication defect, or missegregation of chromosomes. The delay in the cell cycle occurs due to the activation of a particular checkpoint. Checkpoints function by modulating the activity of important cell cycle regulatory molecule, like the CDKs. Mostly, by delaying the cell cycle it provides time to the recovery machinery of the cell to repair the damage. Other than the CDKs, the checkpoints may also target the proteins in DNA replication and the transcriptional program. The final property of the checkpoints is to ensure the re-entry of the cell into the cell cycle after the defect has been repaired. In certain case, when the cell is unable to repair the defect then the checkpoints direct the cells to apoptosis. Generally, all checkpoint responses are initiated by the recognition of the stress by sensor proteins, followed by apical kinases. This is followed by the activation of relay kinases. Then finally the effector proteins are activated which finally shows a response to that specific stimulation, as shown in Fig 1.5.

The cells do have checkpoints at numerous phases of cell cycle. The cause for checkpoint activation is hence discussed. Cells failing to replicate their chromosome do not enter into mitosis. This leads to the activation of the DNA replication checkpoint. Generally, the cell gets arrested in the intra-S phase.
The cell recognizes the presence of unreplicated DNA and stalled replication fork which prevents the activation of the Mitosis-promoting Factor (MPF). This shows that the mitosis of the cell is dependent on DNA replication, which should be properly completed before the initiation of the next phase of cell cycle. The cell can also sense DNA damage caused due to chemical agents, irradiation, UV, and gamma rays, with the help of the DNA damage checkpoint proteins. Cell cycle arrest in the G1 and S phase helps the cell to repair any mutation in the genome. Replication of mutated base can lead to the onset of cancer. During double strand breaks in DNA, the checkpoints arrest the cell at G2 phase of cell cycle. Entry of cells into mitosis with this defect will lead to improper segregation of chromosome. The cell size checkpoint prevents entry of the cell into mitosis until the cell reaches a proper volume before separation. As cells dividing with improper volume will lead to defects in the progeny cells which will hamper their viability.
During M phase, the spindle assembly checkpoint prevents the cell from entering into anaphase unless all the kinetochores of the chromatin are associated with the spindle microtubules. Cells entering into anaphase with unattached kinetochores lead to missegregation of the chromosomes into the daughter cells, which finally die.

<table>
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<tr>
<th>Checkpoint</th>
<th>Purpose</th>
<th>Effect</th>
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<tr>
<td>DNA replication</td>
<td>Ensuring all DNA replication is complete before entering M Phase</td>
<td>Inactivation of cyclin-CDKs and blocking early M phase events</td>
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<tr>
<td>DNA damage</td>
<td>Detects DNA damage throughout cell cycle</td>
<td>Inactivates all cyclin-CDKs to cause cell cycle arrest</td>
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<tr>
<td>Spindle assembly</td>
<td>Ensuring attachment of all chromosome kinetochores to the spindle microtubules before anaphase</td>
<td>Prevents onset of anaphase</td>
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<tr>
<td>Spindle orientation</td>
<td>Ensuring all chromosomes are properly aggregated to daughter cells before telophase and cytokinesis</td>
<td>Prevention of degradation of mitotic cyclins and blocking late mitotic events</td>
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<tr>
<td>Cell size</td>
<td>Detects defect in cell volume and mass</td>
<td>Prevents the onset of G1 and M phase</td>
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The initiation of the telophase of cell cycle occurs after the segregation of the chromosomes is complete. The exit from mitosis and entry into cytokinesis takes place at telophase. This requires the degradation of the MPF. The inactivation of the MPF depends on the APC/C (Anaphase-promoting complex/ Cyclosome). The activation of the APC/C occurs at late anaphase. The checkpoint which plays a
pivotal role here is the Spindle orientation checkpoint. This monitors the location of the segregating daughter chromosome and the activation of the APC/C to promote mitotic exit. Table 1.2 shows the various checkpoints in the cell cycle, its purpose for activation, and its effect.

The basic cell cycle and the role of checkpoints in the cell cycle are more or less conserved in all eukaryotes. To understand the regulation of cell cycle we used the fission yeast, *Schizosaccharomyces pombe*, as a model organism for our study. But before discussing its cell cycle regulation, it is very necessary to understand the cell cycle of fission yeast, which has been discussed in the next section.

**1.3. Cell cycle of fission yeast**

*Schizosaccharomyces pombe* is a unicellular microorganism. It is also known as fission yeast due to its mode of cell division. *S. pombe* was first isolated by Paul Lindner in 1893, from East African millet beer. The name ‘pombe’ is derived from the Swahili word for beer. The brightfield and DAPI stained images of *S. pombe* cells are shown in Fig 1.6.

The life cycle of *S. pombe* is mainly haploid having two kinds of mating types P (*h+*) and M (*h-*). Diploid cells or zygotes are formed during conjugation and nuclear fusion of two haploid cells of opposite mating type. The zygotes enter into meiosis and they sporulate to form a four-spored ascospore. The ascospores are surrounded by ascus wall.
During favorable growth conditions, the ascus wall ruptures to generate four individual haploid cells. This condition permits the cell to go through the vegetative life cycle. The cells then grow at their tips and divide by medial fission. In poor growth conditions, the presence of only one mating type drives the cells towards stationary phase. But when cells of both mating types are present then they generally undergo zygote formation followed by meiosis and sporulation (5). This helps in the survival of the organism in challenged environment by being in a dormant state. But the diploid cells can have mitotic life cycle when they are transferred to a rich media. Thus the diploid state of the cells is very transient. The shape of the ascus formed from a haploid or diploid cell can be differentiated morphologically. The azygotic asci from the haploid cells are rod-shaped whereas
the other zygotic asci are banana shaped (18-19). Both the meiotic and mitotic life cycle of *S. pombe* are inter-connected with each other.

In the mitotic phase of the life cycle, cell growth is fundamental to carry out cell cycle progression. Cell growth occurs in all the phases except in the M phase. Cell cycle checkpoints are present to check the size of the cells before they enter the M phase.

**Fig1.7. The life cycle of *Schizosaccharomyces pombe*:** Fission yeasts survive as haploids and divide by mitotically. During nutrition limitation they exit from the mitotic life cycle and enter a stationary phase. In this condition if opposite mating types are present then they conjugate to form a diploid and they undergo meiosis to form a zygote containing four haploid ascospores. In certain conditions the diploid zygote grows vegetatively to yield an azygotic ascus. During the presence of nutrients the haploid spores enter the mitotic cell cycle again. (18)
In fission yeasts, it has been seen that by blocking the cell cycle progression and, measuring the growth rate of the cells, the big cells grow faster compared to the small cells. They always maintain a constant cell-size distribution throughout the cell cycle. Thus it is evident that fission yeasts do have cell size checkpoint operating with the cell cycle progression. Hence, the cells that are born larger than the normal size have a faster cell cycle progression rate than the ones which are born smaller. The cell-size checkpoints thus ensure the return of such larger or smaller cells to the mean size before they go to cell division.

The growth of the fission yeast occurs by the polarized extension at its tips. The tip extension occurs from a length of 7 um to 14um. Cells then divide by assembling an actomyosin contractile ring at the geometrical center of the cell. Then two daughter cells are formed with each having a length of about 7um. Each daughter cell is initially grown from its old tip from which the separation had taken place.
until the cell reaches S phase. Thus growth initially is monopolar. After this, the growth takes place at two ends of the cell, and is termed as NETO, ‘new end take off’. NETO is generally patches of F-actin. At mitotic entry, these patches translocate to the middle of the cell forming cytokinetic actomyosin ring (CAR) (20). Constriction of the CAR at the end of anaphase is the driving force for cell division (21).

**Fig1.9. Structural distribution of actin microtubules during cell cycle of fission yeast:** After cytokinesis at the S phase the F-actin patches localize at one end of the cell, the place from where it has divided from the mother cell. The ‘new end take off’ (NETO) takes place at the G2 phase. At G2 the F-actin patches localize at both ends of the cell causing bipolar growth. At M phase, the F-actin patches form a cytokinetic actomyosin ring (CAR) near the centre of the cell. This promotes cell division (24).
In spite of the fact that the cell cycle of *S. pombe* is very much similar to that in higher eukaryotes, there are noticeable differences in it. The generation time of mammalian cells is 24 hours, whereas that in fission yeast is around 2.5 hours in rich media. The time spent in the G1 phase is the maximum in mammalian cells. It is here that the cell prepares its complete machinery for mitotic commitment before it decides to enter G0 or the S phase. In fission yeast, the G1 phase is the shortest. After completion of the M phase, the cells rapidly enters into G1 followed by the S phase. Thus unlike mammalian cells, cytokinesis of *S. pombe* occurs in the S phase. Fission yeast does lack certain homologous proteins present in higher eukaryotes. Like, it does not possess any p53 homolog of a mammalian cell. Thus p53 mediated cell cycle progression is absent in the former. It also lacks in having a homolog of RB proteins which act as cell cycle repressors in mammals.

It was long back in the 20th century that the cell cycle regulation of fission yeast was elucidated. The genes that were important for cell division were termed as cell division cycle (cdc) genes. In *S. pombe*, cdc2 is the only cell cycle regulator, CDK, and is the key regulator of the cell cycle. Cdc2 is the mammalian homolog of the mammalian CDK1 and *S. cerevisiae* Cdc28. The primary regulation of the cell cycle in fission yeast is executed by the phase-specific oscillation of the four cyclins - Cig1, Cig2, Puc1, and Cdc13. Cig2 is the major cyclin of G1-S phase, and associated with Cdc2 in the late G1 phase. The active Cdc2 – Cig2 complex helps the cell to pass the ‘START’ and enter into S phase. At S phase Cig2 gets degraded, thus preventing re-entry into the S phase. The level of the mitotic cyclin remains low in the G1 phase,
then starts rising in the S phase, after which it reaches its peak in the G2 phase. This level is then maintained throughout the M phase. The Cdc2- Cdc13 activity is kept low in the S and G2 phase by the inhibitory phosphorylation of Cdc2 at its Tyr15 residue by the two inhibitory kinases Mik1 and Wee1. The removal of this phosphorylation at the G2-M transition by the phosphatase Cdc25 activates the complex.

At the end of mitosis, the degradation of Cdc13 begins. The low activity of Cdc2 in G1 is due to the presence of a CDK inhibitor, Rum1. It prevents the activity of Cdc2-Cdc13 complex at G1 and at the same time promotes the degradation of Cdc13 at G1. This helps the cell to prevent premature entry into mitosis at G1 phase. The cyclin – CDK complexes operating throughout the cell cycle is shown in the Fig 1.10. The details of the biochemical events associated with each phase are as follows-

**Fig1.10. Formation of cyclin – CDK complex in different phases of cell cycle of S. pombe:** The Cdc2- Cdc13 complex is activated in the M phase entry, Cig1-Cdc2 and the Puc1-Cdc2 complexes are activated in the G1 phase, whereas, the Cig2- Cdc2 complex is activated in the S phase of cell cycle.
1.3.1. G1 phase

In fission yeast, at the end of mitosis, the unseparated cell enters into the G1 phase. Here the phase marks the upregulation of the transcription factors of the MBF/DSC1 complex and at the same time abolishes the activity of Cdc2. The expression of the CKI, Rum1 begins at the anaphase, reaches a peak at the G1 phase and is again degraded at S phase. Rum1 associates with the cyclin, Cdc13, and targets it for degradation. The association of Rum1 with Cdc13 inhibits the association of Cdc2 with the cyclin.

The transition of G1-S phase requires the transcription of the genes of the MBF/DSC1 complex. The expression of the genes like the S phase cyclin Cig2, the larger subunit of ribonucleotide reductase Cdc22, the proteins required for DNA replication Cdc18 and Cdt1 are required for the cells to onset S phase. The MBF/DSC1 complex is made up of – Cdc10, Res1, Res2, and Rep2. The transcription factor binds to the MCB (Mlu1 cell cycle box) region at the promoter. The consensus sequence of this region is ATGCGT (21, 22).

The loss of the mitotic activity of Cdc2 occurs by the degradation of the mitotic cyclin; Cdc13. Cdc13 is degraded by a 26S proteasome by ubiquitination. The 26S proteasome complex is composed of E1, E2, and E3, where E1 is the ubiquitin-activating enzyme, E2 is the ubiquitin-conjugating enzyme and E3 is the ubiquitin ligase. Ubiquitination of specific substrates targets it for degradation (23). In fission yeast, the Anaphase-promoting complex (APC/C) is the E3 ligase. The
phase-specific activation of APC/C thus controls the degradation of the B-type cyclins allowing the cell cycle to proceed (23). There are several proteins of the WD40 repeat family whose association with the APC/C leads to the activation of the complex. Other than these, there are two proteins Slp1 and Ste9 which are involved in the activation of the APC/C. The levels of Slp1 and Ste9 are also maintained throughout the cell cycle. At S and G2 phase these proteins are phosphorylated by Cdc2 and remain inactivated. The activation of Slp1 occurs at the late M-phase (24). The activation of APC/C by Slp1 leads to the degradation of securin (Cut2). The proteolysis of Cut2 helps in the separation of the two sister chromatids (25). The other protein Ste9 is activated in the G1 phase. The Ste9 mediated activation of APC/C helps in the degradation of the cyclins (23). The G1 phase assures the complete degradation of the M-phase cyclins. At the same time, it prepares the cell to enter into S phase for DNA replication. In the absence of favorable conditions, the S phase entry is prevented and the cells enter the G0 state. At G0 the cell stops growing and might enter into a meiotic phase of life cycle. The transition from G1- S phase occurs in a very short period of time. Hence, cytokinesis of S. pombe cells mainly occurs at the S phase.

At S phase, the DNA replicates to form an exact copy of the one present in the parent cell. Replication begins at multiple origins in the genome, but the cell has an intrinsic mechanism to control the replication in such a way that only one copy of the DNA is replicated in one cell cycle.
1.3.2. S phase

Cig2 is the S phase cyclin of fission yeast. The Cdc2- Cig2 complex formation occurs at the G1 phase itself, but it remains inactive due to the inhibition by Rum1. As the cell progresses through G1 phase, the expression of Cig1 and Puc1 increases. The formation and activation of Cdc2- Cig1 and Cdc2- Puc1 complex is independent of the Rum1 inhibition. This complex then phosphorylates and degrades Rum1. Removal of Rum1 initiates the activation of Cdc2- Cig2, which marks the onset of S phase (26).
The beginning of replication at the replication origin is termed as ‘firing’ or ‘licensing’ the DNA. This process is highly conserved in all the eukaryotes and is initiated by the formation of an active pre-replicative complex (pre-RC). The activation of the pre-RC requires the association of a complex known as the Origin of Replication Complex (ORC) to the replication origins. In *S. pombe* the replication origins are large about 500-1500bp in size (27). They are rich in AT regions and are very close to the promotor region of the genes. Along with this they also possess short stretches of AT regions asymmetric in nature (28, 29).

The ORC which associates with the replication origin are six in number and are Orc1-Orc6 (30). Orc4 mainly associates with the replication origin. The C-terminal
of Orc4 has the ATP binding site and the N-terminal binds to the AT region of the DNA. The Orc4 remains bound to the replication origins throughout the cell cycle, but the activation of the pre-RC is dependent on several other factors. The ORC helps in the recruitment of another heterohexameric complex to the DNA, and they are known as the minichromosome maintenance complex (MCM). The MCM is composed of the proteins Mcm2-Mcm7 (31). The MCM proteins have a DNA binding domain and have got replicative helicase activity (32). Thus, MCM binds to the DNA and unwinds it for replication initially and then continues along with the replication fork. The loading of the MCM to the replication origin requires two replication initiation factors, Cdc18 and Cdt1 (33). Cdc18 associates with the ORC directly, whereas Cdt1 interacts with Cdc18 for its association with the pre-RC assembly. The expression of both Cdc18 and Cdt1 are under tight cell cycle regulation to mark the activation of a single replication origin during each phase of the cell cycle (8). The expression of Cdc18 and Cdt1 are dependent on the transcription via the MBF/DSC1 complex. Their expression begins at the M phase, peaks at G1 and then they are degraded at the S phase. Thus the increase in the level of Cdc18 and Cdt1 is also a marker of the G1- S phase transition (23). Cdc18 also possesses ATPase activity like the other members of the ORC family. The phase-specific degradation of the Cdc18 and Cdt1 prevents any further activation of a pre-RC in one cycle. Cdc18 is phosphorylated by the Cdc2-cyclin complex which leads to its degradation by the SCF. At the same time, Cdc2-cyclin complex phosphorylates the Orc2 at the rest of the phases of the cell cycle, in order to
inactivate the ORC. Cdt1 is degraded in the S phase by E3 ubiquitin ligase. This ligase is composed of Cul4 and Ddb1 complex. Cdt2, a member of the WD40 repeat family helps in the recognition of the Cdt1 as a target.

The initiation of replication begins at the S phase which requires the transformation of the pre-RC to the pre-IC (pre-initiation complex). For this, the cell activates two complexes at the S phase: one is the Cdc2- Cig2 complex and the other is the Hsk1- Dfp1 complex. Hsk1 belongs to the DDK (Dbf1 dependent kinase) family of proteins. These two complexes help in the loading of Sna41 to the pre-RC. This interaction leads to the recruitment of Pol α to the DNA, hence leading to the formation of the pre-IC. The other proteins which get associated with the replication origin are – Drc1, Cdc23, Sld3, and Cut5. Among them, the Sld3 and Cdc23 are required for the binding of Sna41 with the origin, and Cut5 and Drc1 associate with each other with the help of Cdc2 mediated phosphorylation. The activation association of Sna 41 helps in the formation of pre-IC and facilitates unwinding of DNA. Then the replication protein A (RPA) associates with the single stranded DNA and recruits Polα , Polε, and primase to initiate DNA synthesis.

The primase and Polα majorly lead to the formation of small RNA- DNA primers at the replication fork. This is followed by the DNA elongation phase which requires the recruitment of Polδ and Polε, proliferating cell nuclear antigen (PCNA) and replication factor C (RFC) (35). PCNA acts as sliding clamp and prevents the falling of pol δ pol ε from the DNA.
RFC acts as clamp loader binding the PCNA to the DNA. After the completion of cytokinesis at S phase, the cell starts replicating its DNA for the next round of cell cycle. At the end of DNA replication, the cell enters the G2 phase, by preventing further initiation of DNA replication. The S-G2 transition is prevented during activation of DNA damage or replication checkpoints.
1.3.3. G2 phase

As DNA replication is complete, the cell then spends a considerable time at G2 and prepares itself for the M phase. The absolute necessary complex for this transition is an active Cdc2-Cdc13 complex (36). The levels of Cdc2 remain almost equal throughout the cell cycle, but it is the level of Cdc13 which oscillates in a cell cycle. The expression of Cdc13 begins in the middle of G2, and peaks at the mitotic entry point, after which is gradually degraded in the G1 phase (37). The formation of an inactive Cdc2-Cdc13 complex occurs at the S phase. The inactive state of the complex is maintained by the phosphorylation of the Cdc2 in its Tyr15 residue by Wee1 and Mik1 kinases. In mammals, the phosphorylation of Thr14 is also necessary to inhibit the CDK, Cdk1 along with Tyr15 residue (34). Both of this phosphorylation is done by inhibitory kinases. Wee1 kinase needs to be inactivated before the cell enters into mitosis. The phosphorylation of Wee1 leads to its inactivation. Nim1/Cdr1 and Cdr2 are responsible for the phosphorylation of Wee1. These two are Ser/Thr kinases which act as mitotic inducers. On the other hand, the expression of Mik1 peaks at the S phase under the regulation of the MBF/DSC1 transcription factors. The increase in the level of the inhibitor Mik1 ensures the complete inhibition of the Cdc2/Cdc13 complex during the replication of the DNA in the S phase. It also reported that during DNA damage, the checkpoint proteins Chk1 and Cds1 are involved in increasing the Mik1 levels in the cell in order to prevent mitotic entry until the damage is recovered. As the cell progresses through the S phase, the levels of Mik1 drop and the inhibitory role is then played by Wee1.
Other than the inhibitory phosphorylations, Cdc2 requires an activating phosphorylation at its Thr167 residue to be active. This phosphorylation provides substrate specificity to the Cdc2-Cdc13 complex (38). The inhibitory phosphorylation on Cdc2 needs to be removed in order to allow mitotic entry. The Cdc25 phosphatase dephosphorylates Cdc2 at Tyr15 at the onset of mitosis, leading to the formation of an active Cdc2-Cdc13 complex. During DNA damage, Chk1 directly phosphorylates Cdc25 (39). This phosphorylation leads to the interaction of Cdc25 with 14-3-3, Rad24 proteins. This association translocates Cdc25 from nucleus to cytosol, thus preventing mitotic entry. The activation of Cdc25 is again dependent on a positive feedback loop. An activated Cdc2 leads to the basal level phosphorylation and activation of Cdc25. Once activated, Cdc25 leads to further activation of the Cdc2-Cdc13 complex. The activation of the CDK is also dependent on CDK-activating kinase (CAK). In *S. pombe* there are two CAKs, the Cdk, Mcs6.

**Fig1.14. Activation of the MPF at G2 phase:** The MPF is maintained in an inactive state by inhibitory phosphorylation by Wee1 kinase at the Tyr15 residue. Removal of this phosphorylation by Cdc25, and by the activating phosphorylation by CAK (CDK activating kinase) at Thr161 (T161), activates the MPF at the G2-M transition of cell cycle.
and the cyclin, Mcs2. The other kind is Csk1. The phosphorylation activation of Cdc2 by Csk1 and Mcs2- Mcs6 complex is not so important; hence the latter is not under the regulations of cell cycle control.

1.3.4. M phase

As the cell reaches an appropriate mass and size, activation of the M-phase cyclin- CDK complex drives the cell to the M phase. Cdc2-Cdc13 has got several mitotic substrates to promote mitosis. It phosphorylates Dis1 to change its localization from microtubules to SPB to promote sister chromatid separation. It is also involved in the phosphorylation of Cut3, a subunit of the condensin complex to help in the condensation of the chromosome. Cut3 phosphorylation changes its localization to the nucleus. The phosphorylation of Dis2, a homolog of the mammalian PP1 phosphatase promotes the cells towards the mitotic exit and enters G1 (40). The Dis2 phosphorylation is an inhibitory one. The Plo1, Ark1 and Fin1 kinases belonging to the Polo, Aurora and NIMA family also play important role in the mitotic phase of cell cycle.

Plo1 plays an important role in the formation of mitotic spindles and in cytokinesis. Plo1 is also responsible for the expression of the genes in M-G1 phase of cell cycle. Plo1 is essential for spindle formation, actomyosin ring formation, and septum formation. Plo1 can be found to be localized to the SPB from the beginning of M phase up to the anaphase. It has also been found to be present in the medial ring and mitotic spindles (41). Thus Plo1 is activated during both the events of
medial ring formation and septation. (42). Plo1 mediates the positioning of the actomyosin ring by interacting with Mid1/Dmf1. Mid1/Dmf1 which otherwise resides in the nucleus localizes into the cell cortex to initiate the formation of the actomyosin ring. (43). Plo1 facilitates this change in localization of Mid1/Dmf1 by phosphorylating it. Plo1 regulates the PBF (PCB Binding Factor) which is made up of Fkh2, Sep1, and Mbx1. Fkh2 and Sep1 belong to the Forkhead family of transcription factors, whereas Mbx1 belongs to the MADS-box family of a transcription factor. The PBF binds to the PCB (Pombe Cell cycle Box) motif in the promoter region of the genes that are specifically transcribed in the M-G1 phase. The genes that are expressed are – $plo1^+$, $spo12^+$, $ppb1^+$, $slp1^+$, $fin1^+$, $ace2^+$, $sid2^+$, $mid1^+$, and $dmf1^+$ (43).

Fin1 belongs to the family of NIMA proteins plays an important role in mitosis. The expression of Fin1 peaks at the mitotic phase and its function include – chromosome condensation, spindle organization and cytokinesis. Fin1 helps in the recruitment of Plo1 to the SPB. At the same time, it plays a role in spindle formation and spindles pole maturation. Fin1 plays an active role in septation initiation network, hence causing cytokinesis. (44).

Ark1 in fission yeast belong to the family of Aurora kinases. Ark1 phosphorylates Ser10 of histone H3 to facilitate chromosome condensation. The expression of Ark1 remains constant throughout the cell cycle, but its localization within the cell differs in cell cycle specific manner. It gets localized in the centromeres in the central region of the spindles during mitosis (45).
The M phase is followed by cytokinesis. The M-G1 phase transition up-regulates the genes required for cytokinesis. As the G1 phase is very short in fission yeasts, thus the cytokinesis majorly occurs at the S phase of cell cycle.
1.3.5. Cytokinesis

Late mitosis is followed by the cytokinesis which should be a coordinate event, otherwise, it may lead to aneuploidy and polyploidy. Mitotic exit occurs with inactivation of the CDK activity. After chromosome segregation, the Cdc13 is degraded by the APC/C with the help of Slp1 (46). At G1, the cells maintain inactivated Cdc2 with the help of another APC/C activator Ste9. The Clp1/Flp1 phosphatase inactivates Cdc25 to inactivate the complex again (47, 48). Exit from the mitotic phase requires a cascade of proteins known as the Septation Initiation Network (SIN). This network is similar to the MEN (mitotic exit network) in budding yeast (49). The microtubules at this stage are involved in the separation of the two copies of the chromosome and the actomyosin is involved in the cell wall cleavage. The actomyosin ring is composed of F-actin, myosin II, which accumulates in the medial plane to separate two cells. The localization of F-actin keeps on varying with the stage of cell cycle. At interphase, F-actin is mainly found in the one pole of the cell. This position is the one from where the new cell has been separated from the parent cell. Thus the growth of the cell before it enters the mitosis is predominantly monopolar. As the cell enters G2, the F-actin patches are found in both the poles of the cell thus causing the bipolar growth of the cell. At G2, the F-actin accumulates in the medial region of the cell to form a ring-like structure which is also known as Cytokinetic Actomyosin Ring (CAR) (50). At the end of telophase, the CAR constricts to separate the two daughter cells. The constriction of the CAR signals the beginning of cytokinesis. New cell wall materials are deposited
on the inside of the cell cortex and behind the CAR which leads to the formation of the primary division of the septum. The primary septum is made up of sugar polymers, and secondary septum forms at the sides of it. Degradation of the septum is the final step of cytokinesis. This leads to the formation of two daughter cells (51).

Fig1.16. **Cytokinesis**- During cytokinesis the activation of the APC/C leads to the degradation of the M phase Cyclin, Cdc13. D Dephosphorylation of Cdc25, further inactivates the Cyclin-CDK complex. Activated Clp1/Flp1 dephosphorylates Cdc25, this leads to inactivation of Cdc25. The onset of the Septation Initiation Network (SIN), leads to division of the cell into two daughter cells.

The cell cycle of *S. pombe* is at times perturbed due to activation of checkpoint proteins. The mechanism of checkpoint control in the cell cycle of *S. pombe* has been discussed in the next section.
1.4. Cell cycle checkpoints in *S. pombe*

Fission yeast has got several checkpoints activated at different cell cycle phases. The presence of double-stranded breaks in DNA or unreplicated DNA prolongs the G2 phase and initiates the DNA repair by activating the DNA damage checkpoints. Incomplete replication of the DNA at S phase also arrests mitotic entry of the cells by activating the DNA replication checkpoint.

Fig 1.17. *Arrangement of checkpoint at different phases of cell cycle in S. pombe*: DNA damage at G1 or G2 phase delays cell cycle at both the phases by inhibiting the activity of MBF and MPF respectively. DNA replication checkpoint is activated during DNA replication defects at S phase. The mitotic checkpoint is activated to prevent premature entry into M phase. The SAC/SOC ensures the proper arrangement of the chromosomes before cytokinesis.
DNA damage of any kind can also activate the checkpoint at intra-S – checkpoint hence inhibiting the progression through S phase. Before entering into mitosis if the cells do not reach the appropriate length then the mitotic entry of the cells is delayed by the mitotic checkpoint proteins. During mitosis, the attachment of the sister chromatids to the spindles is monitored by the spindle assembly checkpoint (SAC) and spindle orientation checkpoint (SOC). Among these checkpoints, those are termed as the ‘classic checkpoints’ which have sensors, transducers, and effectors in their signaling pathway. Other than SAC most of the checkpoints in fission yeast fall into the category of classic checkpoints. Most of the genes in the classic checkpoint machinery were identified in radiation sensitive screenings and hence they are named as ‘rad’ genes. The six rad genes in the *S. pombe* are – rad1+, rad3+, rad9+, rad17+, rad26+, and hus1+. Rad1, a protein of 194 amino acids is homologous to RAD17 in *S. cerevisiae*. Rad1 has got structural similarity with the PCNA. Hus1 is a protein of 304 amino acids and also has got PCNA related motifs. In response to DNA damage, Hus1 is phosphorylated by Rad3 in the presence of Rad1 and Rad9. Rad9 is a protein of 427 amino acids, and also has got PCNA like motif. The structural similarity of Rad1, Hus1, and Rad9 with PCNA indicate that this heterotrimeric complex might help in forming a sliding clamp-like structure to attach to the DNA. Rad17 is homologous to RAD24 of *S. cerevisiae* and is required for S phase arrest of the cells in response to DNA damage. Rad3 is a protein of 2396 amino acids and is similar in structure with the phosphatidylinositol kinase family of proteins.
Rad3 has got its kinase domain at the C-terminal end and is required for the checkpoint response to DNA damage and both S and intra-S phase arrest. Rad3 plays a role in the checkpoint activation signal and is required for the phosphorylation of Hus1, Chk1, Rad26 and Cds1 during DNA damage stress. Rad3 also has a role in DNA replication checkpoint response. Rad26 is a protein having a
putative coiled-coil motif and of 615 amino acids. Rad26 plays an active role in preventing M phase entry of the cells in response to DNA damage or S phase arrest. It is phosphorylated and activated by Rad3 independently of the presence of other checkpoint proteins (52).

DNA damaging agents like methyl methane sulfonate (MMS), UV-C, Hydrogen peroxide lead to the activation of the DNA damage checkpoint in the G1 phase. In mammalian cells, DNA damage in G1 phase stabilizes the tumor suppressor p53 protein. This p53 helps in the up-regulation of the CDK inhibitor p21. But this p53 mediated cell cycle arrest takes place in late G1. As soon as the DNA gets damaged by any genotoxic stress a p53 independent checkpoint arrest gets initiated. The ATM/ATR sensor kinases activate the transducers Chk1/Chk2. The activated Chk1/Chk2 targets Cdc25A and phosphorylates it. This signal Cdc25A for ubiquitination-mediated degradation. This prevents the activation of CDK2 thus halting S phase entry of the cells. The inactivation of CDK2 prevents the association and activation of Cdc45 to the DNA, hence preventing the formation of pre-RC.

Activation of any classic checkpoint requires the activation of the sensor kinases. In mammals, the ATM/ATR kinases are activated depending upon the kind of damage in the cell. Double-stranded break (DSB) in DNA induces an intra-S phase checkpoint. Though this is similar to the signaling of the G1 arrest where it begins with ATM and ends in Cdc45. Other than this cascade, ATM also activates BRCA1 (isolated in breast cancer) and Nbs1. The ATM/ATR bind with the BRCA-1
associated genome surveillance complex (BASC) which is composed of the proteins Mre11- Nbs1- Rad50 (52). This complex is recruited in the damaged DNA, where they repair the same by non-homologous end joining. DNA damage also induces transcriptional repression of genes by Rb proteins. On a separate study, it has been reported that p53 induces the upregulation of apoptotic genes leading to G2 arrest in the cells, which has been elaborately discussed later in the transcriptional control of cell cycle regulation part of this thesis.

In fission yeast, a single sensor kinase is present. It is the Rad3, an ATR homolog. Rad3 is associated with Rad26, an ATRIP homolog in mammalian cells, and Rad9- Rad1- Hus1 (9-1-1) complex. Upon DNA damage, the two complexes are
recruited independently to the damage site. The checkpoint activation occurs after the recruitment of both the complexes. Rad17 loads the 9-1-1 complex to the damaged DNA site. The 9-1-1 complex is loaded at the 5’ end of the DNA by a process which is dependent on the presence of ssDNA and RPA. A similar scenario is present in the mammals where the RPA loads the ATR-ATRIP complex to the ssDNA. The checkpoints lead to the association of Cut5 to the 9-1-1 complex to mediate the activation of Rad3. Activated Rad3 phosphorylates and activates the checkpoint transducers Cds1 and Chk1 (53). This activation requires the adapter proteins Crb2 and Mrc1.

The association with the adaptor protein is dependent on which phase the cell is present and the kind of damaged signal being sensed. Mrc1 is expressed only in the S phase of cell cycle. Thus the Mrc1 mediated signaling phosphorylates and activates Cds1, the S phase kinase. Crb2, though expressed throughout the cell cycle but carries out the checkpoint-mediated signaling in the late S and G2 phase. Crb2 leads to the activation of Chk1 in these phases. The major downstream targets of Chk1 and Cds1 are Cdc25, Mik1, and Wee1. Cdc25 is primarily targeted by Chk1, whereas Cds1 targets Mik1 and Wee1 to a certain extent. This leads to the transcription of the G1-S phase genes which will help in repairing the DNA damage.

The Chk1 dependent phosphorylation of Cdc25 associates with Rad24, a 14-3-3 protein. Rad24 carries nuclear export signal. It hence localizes Cdc25 in the cytosol
preventing mitotic entry of the cells. This mechanism is similar to the one present in mammalian cells to arrest cells in G1 and S phase during genotoxic stress.

**Fig1.20. DNA damage leading to cell cycle arrest at G2-M phase:**
The DNA damage leads to the recruitment of the 9-1-1 complex (Rad9-Rad1- Hus1) to the ssDNA along with Rad17 and rad26. This leads to the association of activated Rad3 with the complex which leads to the downstream activation of Crb2 and Chk1. Chk1 phosphorylates Cdc25, and facilitates its export to the cytosol upon association with 14-3-3 (Rad24). Activation of Cds1 by Rad3 leads to the activation of Wee1 and Mik1, the MPF inhibitory kinases.

Activation of DNA replication checkpoint occurs at the intra-S phase of cell cycle.

The presence of unreplicated DNA or stalled replication forks leads to the activation
of this checkpoint. In mammals, ATR associates with both unreplicated DNA and stalled replication fork. This then phosphorylates and activates the Chk1 kinase. The activation of Chk1 causes phosphorylation-dependent inactivation of Cdc25c. Inactivation of Cdc25c will lead to the inhibition of the activation of the CDK, hence preventing the mitotic entry of the cells. This ultimately results in the increase in the inhibitory phosphorylation of Cdc2 at Tyr15 (54).

The absence of an active Cdc2- cyclin complex prevents the phase transition in the cell cycle. Cds1 also inhibits the activity of Cdc25, and at the same time maintains replication fork stability by phosphorylating the proteins like Mus81-Eme1, Rqh1, and Rad60 of the stalled replication fork. At the end of S phase, the MBF dependent transcription of genes is repressed by the recruitment of the inhibitor Yox1 by Nrm1 in the MBF promoter sites. During the presence of stalled replication forks, Cds1 phosphorylates Yox1 at Ser114 and Thr115, inhibiting the interaction between Yox1 and MBF (55).

**Fig1.21.DNA replication checkpoint.** Presence of unreplicated DNA or stalled replication fork is first detected by Rad3. It phosphorylates and activates Cds1. The activated Cds1 phosphorylates Yox1, and prevents it from associating with MBF. The continuation of transcription by MBF delays the cell cycle at S phase.
In *S. pombe* cell size is coordinate with cell cycle progression. The cell size checkpoint exists at two phases of cell cycle, one at G1 and the other at G2-M transition. The mitotic regulatory proteins, Cdc25 and Wee1 control the mitotic entry of the cells. Wee1 and Cdc25 are inhibitory kinases and activating phosphatase respectively which maintains the activity state Cdc2 by controlling its phosphorylation at Tyr15 residue. Other than maintaining genome stability, Wee1 maintains the cell size checkpoint at G2-M boundary. Fission yeasts divide by medial fission. At the mid zone of the cell, a protein called Pom1 accumulates. Before M phase, as the cell grows from the tip ends, the concentration of the Pom1 at the midzone region starts decreasing. Pom1 regulates a Wee1 inhibitory kinase Nrm1/Cdr1. The decrease in the Pom1 concentration makes Nrm1 actively phosphorylate Wee1. Phosphorylation of Wee1 leads to its degradation hence proceeding cells to mitosis. Another protein PP2A also plays a role in the mitotic entry of the cells by dephosphorylating Cdc25 (56).

At the end of every mitotic cycle, the cell proceeds to segregate its replicated chromosomes into two daughter cells. The sister chromatids attach themselves to the bipolar mitotic spindles in the prometaphase, they are aligned in the metaphase and then in the anaphase they are separated to the opposite poles of the cell. Perturbations in the attachment step of the chromatids to the spindles delay the onset of anaphase and activation of a checkpoint known as spindle assembly checkpoint.
During mitosis, the spindle microtubules bind to protein structures called kinetochores. The chromosomes are attached to the spindles through these kinetochores. The Mad and Bub proteins are attached to the outer kinetochore early in the mitotic phase. After attachment is over these proteins are localized in the unattached kinetochores, which activates the checkpoint. When chromosomes are attached to the microtubules, tension is generated from the opposite poles to pull them to the two ends. Kinetochores lacking this tension when the sister chromatids are attached to the microtubule from the same pole (syntelic attachment), also generate checkpoint activation even though the kinetochores microtubule attachment are present. The lack of tension is sufficient for checkpoint activation. Mad1 and Mad2 are the two proteins that are attached to the unattached kinetochores, and Bub1, BubR1/Mad3 are attached to kinetochores lacking tension or microtubule attachment (57, 58). The protein Aurora B is known to promote bipolar attachment by destabilizing the kinetochore-microtubule interactions that are not under tension. By doing this Aurora kinase B signals the Mad/Bub proteins for checkpoint activation. The downstream target of the spindle checkpoint is the anaphase-promoting complex / cyclosome (APC/C), an E3 ubiquitin ligase. Securin is the regulator of the anaphase onset. APC/C degrades Securin, due to which Separase gets released. Separase degrades the cohesion holding the two sister chromatids together and hence promotes their separation to the opposite poles. Cdc20 and Cdh1 help APC/C to recognize its substrates. The APC/C subunits are
thought to localize to the kinetochores in a checkpoint-dependent manner and also to centrosomes and mitotic spindles.

In the beginning, Mad1 and Bub1 are attached to the unattached kinetochores which allow the attachment of Mad2 and BubR1/Mad3. Mad2 bind to Cdc20, which then inhibits the APC/C. BubR1/Mad3 and Bub3 form complex with Mad2-Cdc20. This complex is known as mitotic checkpoint complex (MCC). MCC is a potent inhibitor of APC/C. the BubR1/Mad3 and Mad 2 act synergistically, showing that both of them are required for inhibition. The levels of MCC increase upon checkpoint activation and that both APC/C and MCC are phosphorylated in the mitotic phase up on checkpoint activation. Bub1 can phosphorylate Cdc20 to inhibit it. Mps1 is the upstream regulator of the checkpoint cascade and its kinase activity peaks in the metaphase causing checkpoint activation (59, 60).

**Fig 1.22. Spindle assembly checkpoint:** Presence of unattached kinetochores lead to the activation of the spindle assembly checkpoint (SAC). The activation of the APC/C is inhibited which blocks the cell cycle at metaphase to anaphase transition.
Once all the kinetochores are attached to the bipolar spindles the checkpoint proteins needs to be removed. Mad1, Mad2, and BubR1 are removed from the kinetochore by dynein. Association of microtubules to CENP-E downregulate the kinase activity of BubR1. Again phosphorylation of Mad2 disrupts further MCC formation. The checkpoint inhibitor Cmt2 binds to Mad2 inhibiting the formation of new MCC, and at the same time disrupts the older ones by forming a complex with Mad2–Cdc20 (57-59).

Fig 1.23. Spindle orientation checkpoint (SOC). Absence of spindle orientation occurs due to the perturbations in the interaction between the astral microtubule and actin cytoskeleton. Bub1, Bub3, Mad3 and Mps1 are activated in the SOC pathway, which leads to the inactivation of APC/C, causing cytokinetic delay in the cells.
In fission yeast, as the cells divide by medial fission, the orientation of the spindles is dependent on the interaction between the astral microtubules with the cortical actin cytoskeleton. Any kind of perturbations in the actin cytoskeleton gives rise to the spindle orientation checkpoint (SOC). Bub1 is found to be attached to the kinetochores during SOC activation. Bub1 is responsible for the metaphase delay in the cells. It has also been reported that Atf1 plays an important role in cell cycle delay in response to SOC activation. Cells lacking Atf1 lost their viability when treated with actin depolymerizing agent due to its inability to inhibit nuclear division.

Another report states the role of MAPK cascade in maintaining the cell polarity along with the Tea4-Tea1 complex. Even the recruitment of the polo-like kinase to the spindle pole body is dependent on the MAPK proteins. In response to SAC, both Mad2 and BubR1 inhibit APC/C in response to unattached kinetochores or lack of spindle tension (59). But they do so independently of each other. It has been seen that Mad 3 along with Bub1 is responsible for preventing monopolar attachment independently of Mad2. Bub1 also associates with the kinetochores during the perturbation of the astral microtubule contact with medial cell cortex. The checkpoints monitor the timing of the onset of the anaphase depending on the positioning of the spindle and the tension at kinetochores (59).

Activation of checkpoints in response to stimulations needs a proper regulation. As uncontrolled activation of checkpoint proteins often leads to unwanted changes to the cellular material. The regulation of the mitotic cell cycle is necessary to
prevent the activation of checkpoint pathways. A proper cell cycle engine thus depends on phase-specific regulation of the cell cycle proteins in a time-dependent manner.

1.5. Transcriptional regulation of cell cycle

With the advancement of molecular biology, transcription of the cell cycle genes has been studied extensively. The pattern of cellular expression of the genes has been found to be phase specific. The oscillatory pattern of gene expression reflects the existence of a transcriptional control in the cell cycle. The regulation of few particular transcription factors dictates the continuous transcriptional wave throughout the cell cycle.

1.5.1. Transcriptional regulation of cell cycle in mammals

In mammalian cells, the transcription of multiple genes occurs at the onset of each phase. These genes fall into two categories, one that are transcribed early (early response genes) and one that are transcribed late (delayed – response genes). At G0 phase, growth factors induce the expression of the early response genes at a very short time. The early transcription factors cause the induces the transcription of the transcription factors c- Fos and c-Jun which helps in the transcription of the delayed genes. Delayed response genes encode the transcription of transcription factors like E2F. The E2F class of transcription factors along with their dimerizing partners (E2F-DP) and the pocket proteins bind to the promoter sequence of the genes which are expressed in cell cycle phase-specific manner. During early G1, the
E2F proteins are inhibited by forming complex with RB proteins, and E2F4 and E2F5 are associated with p130 and p107 (61). This complex formation occurs at the promoter region of the genes, hence repressing their transcription. The association of E2F4 and E2F5 with the pocket proteins localizes them in the nucleus. The phosphorylation of the pocket proteins by the G1-S cyclin –CDK complexes abolishes the association hence localizing them at the cytoplasm. The promoters of the genes are then occupied by the activating transcription factors E2F1, E2F2, and E2F3A. It is from this point that transcription of G1-S related genes take place. As the cell proceeds into the S phase, the transcription of the E2F dependent genes needs to be turned off. Cyclin A- Cdk2 phosphorylates E2F1, which prevents the latter from binding to DNA.

As the transcription of Cyclin A is itself dependent E2F, hence a negative feedback loop exists. p27, a CDK inhibitor, inhibits cyclin A- Cdk2 complex. The cyclin E- Cdk2 and Cyclin A- Cdk2, on the other hand is responsible for the degradation of p27. The ubiquitin ligase SCF, S phase kinase-associated protein (SKP2) – cullin (1-F-box protein) regulates the stability of E2F1 in the G2 and M phase, the expression of which is again dependent on E2F proteins. The transcriptional repression is also dependent on E2F6, E2F7, and E2F8. The expression of these repressors is dependent on the E2F proteins itself. These repressors do not need pocket proteins for their activity, and hence they function in the S, G2 and M phases when the pocket proteins are absent. Like E2F6 is involved in the transcriptional inactivation in the late S phase.
A cell in which E2F6 is absent, the similar role is compensated by E2F4 (62-64). Thus in whole, the transcriptional program in the cell suggests the existence of a very stringent feedback loop. The series in which the transcription will occur is also specific. The product of the genes related to the positive feedback loop is transcribed first, and then the ones related to the negative feedback loop.

As the cells enter the S phase replication of DNA begins. At this stage to ensure proper replication of DNA two checkpoints are associated with it – the DNA damage...
checkpoint and the DNA replication checkpoint. These two checkpoints when activated a separate transcriptional program is stimulated to repair the damage and to delay the cell cycle in order to repair it. During replication stress, E2F6 is phosphorylated and inactivated by the checkpoint kinase 1 (CHK1). As a result, it is removed from the promoters of the genes, which are otherwise repressed in the S phase. The E2F proteins are not inhibited and the transcription of the G1-S phase genes continues causing a delay in the S phase entry of the cells. The targets of the E2F proteins help to prevent replication fork collapse. During DNA damage, a different checkpoint, CHK2 gets activated, which stalls the cell cycle at any point in the interphase (65-67). Activation of the DNA damage checkpoint generally leads the cell to programmed cell death or apoptosis, when the damage becomes irreversible. One of the major genes which are upregulated during DNA damage is p53. The activation of p53 leads to the transcription of the various genes which are involved in apoptosis like – BAX, NOXA, PIG3, PUMA and p21/waf1, GADD45 and 14-3-3 which are responsible for growth arrest (68). P21/waf1 is an inhibitor of Cdk2. Cdk4 and Cdk6. This prevents the G1- S phase transition of the cells. P21/waf1 can cause G2 arrest by inhibiting the activating phosphorylation at Thr161 of CDC2. GADD45 induces cell cycle arrest at G2 by preventing the CDC2-Cyclin B complex formation by binding with CDC2 (69). On the other hand 14-3-3σ associated with the CDC2-Cyclin B complex and anchors it to the cytoplasm where it is unable to promote mitosis. In response to DNA damage, the genes which are
down---regulated in a p107 and p130 dependent manner are –Cdc2, CyclinB1, Cdc25c, Plk1 (70).

Other than these the genes which are involved in the chromosome arrangement in the metaphase plate, mitotic spindle formation, and cytokinesis are also downregulated in the same fashion. There are multiple mechanisms which ensure the cell cycle transition from G2 to M phase. The regulation mainly deals with the activity of CDC2, which is largely dependent on the transcriptional regulation of the cell. Stable G2 arrest helps to protect the genome against damages and suppresses tumorigenesis. Compared to rest of the transition phases, the transcriptional regulation of M- G1 phase is much less revealed.

1.5.2. Transcriptional regulation of cell cycle in *S. pombe*

In fission yeast, the cell cycle is regulated by a specific transcriptional wave. Both mitosis and meiosis are regulated by the transcription factors and each phase transition is governed by a single wave. The first wave begins at the G1-S phase, the second at S phase, the third at G2 phase and the end of M-G1 phase. They are described in the following section. The genes that are involved in G1-S phase transition are – *cdc22*, *cdc18*, *cdt1*, *cdt2*, *cig2*, *rad11*, *rad21*, *ste9*, *mik1*, *eng1* and *mid2*. Among which *cdc22*, *cdc18* and *cdt1* are involved in DNA replication, *cig2* is the G1 phase cyclin.

All of these genes have got a conserved DNA motif in the promoter region, the sequence of which is ACGCGT. This motif is named as the MCB (Mlu1 cell cycle
The binding of the transcription factor complex DSC1 (DNA synthesis control 1) also known as MBF (Mlu1 binding factor) controls the expression of these genes. The MBF is composed of Cdc10, Res1, Res2, and Rep2. Cig2 phosphorylates Rep2, which inhibits MBF dependent transcription (73-75). The combination of the transcription factors binding to the common promoter sequence determines the induction or inhibition of the transcription.

The S phase wave has the core histone genes – $H2A^+$, $H2B^+$, $H3^+$, and $H4^+$. The promoter sequence of all of the genes has a sequence – AACCCT. This sequence gets repeated for a stretch of 17bp. No transcription factor as such has been identified for the transcription of these genes (76).

The genes expressed in G2-M transition phase are – $spd1^+$, $psu1^+$, and $rds1^+$. The promoter sequence of the motifs are – ACCNCGCT, known as Novel 3, but no transcription factors as such have been identified yet (76).

The product of the genes at M-G1 transition phase is required for spindle formation, septation and cytokinesis (76, 77, and 78). The genes are – $cdc15^+$, $plo1^+$, $srd2^+$, $fin1^+$, $slp1^+$, $fkh2^+$, $ppb1^+$, $dmf1^+$, $cdc19^+$ and $ace2^+$, $cdc15^+$ and $dmf1^+$ control cytokinetic ring formation, $plo1^+$ and $fin1^+$ control spindle formation, chromosome separation and septation. The DNA sequence at the promoter is called the Pombe Cell cycle Box, PCB, controls the expression of these genes (79).

The transcription factor which binds to this sequence is known as forkhead transcription factors – Fkh2, Sep1, and Mbx1 (80). Here Fkh2 is a transcriptional
repressor and Sep1 is a transcriptional activator. Plo1 also regulates the transcription of the genes at M-G1 phase. The *ace2* gene encodes for the protein Ace2, which is a transcription factor.

**Fig1.25. Transcriptional regulation of cell cycle in S. pombe:** Cell cycle phase specific expression of the genes is dependent on the binding of the transcription factors to specific sequence of the DNA. The regulation of the cell cycle thus lies primarily in the transcriptional level. The known transcription factors are PBF, Ace2 and MBF acting in the M-G1, G1 and G1-S phase respectively (76).

This Ace2 helps in the transcription of the genes whose expression occurs at the same time of the G1-S wave (81). The overlapping the two waves suggests that all the phases of the cell cycle are connected. The transcription of the genes requires
transcription factors. So on narrowing down the search for the principle regulators of the eukaryotic cell cycle, the transcription factors appear to take the center stage. The role of transcription factors elucidates a lot about the progression of cell cycle phases.

1.5.3. Cell cycle regulation by the bZIP transcription factors

One of the classes of the transcription factor is the bZIP transcription factors whose DBD remains folded by hydrophobic interactions. The DNA binding of the bZIP proteins helps in their dimerization too. Otherwise, neither the basic region nor the leucine zipper region is stabilized. The bZIP DBD generally has 55-65 residues. The basic region of almost 30 bZIP transcription factors have been identified and among all the residues Asn243 and Arg245 have been found to be very conserved among most of the eukaryotes. Ala-238 and Ala-239 are found in most of the bZIP regions. The Ser242 is known to form Van der Waals interaction with the methyl group of Thy3. The reported binding site of the bZIP proteins are mostly AP-1 or ATF/CREB binding sites.

The bZIP transcription factors associated with cell cycle regulation in mammalian cells include the following:

**CHOP/GADD153**- These classes of transcription factors are activated by genotoxic stresses. The bZIP, CCAAT enhancer binding protein (c-EBP) family of transcription factors, also known as c-EBP homologous proteins (CHOP) and growth arrested DNA damage- 155 (GADD- 153) are activated by phosphorylations at
Ser78 and Ser81 (82). They as activators for stress-induced genes, at the same time they act as repressors for many cAMP related genes. The p38α are directly involved in the phosphorylation activation of these transcription factors. They are responsible for the cell cycle arrest of the cells at G1/ S phase in response to DNA damage. The advantage of this arrest helps the cell to repair the DNA damage before entering into the S phase (83).

**NFAT** - The nuclear factor of activated T – cells (NFAT) bears similarities with the Rel/NFkB family of proteins. They are localized in the cytosol due to inhibitory phosphorylation by Casein Kinase Iα and glycogen synthase kinase (GSK3). Extracellular stimulations increase the levels of Calcium ions in the cells. The Calcium-dependent activation of Calcineurin dephosphorylates NFAT, which hence helps in its localization into the nucleus. The DNA binding regions of NFAT are near to the AP-1 sites. This helps in the trans-activation of various genes (84, 85).

**AP-1** - The JNKs and p38 are mainly responsible for the recruitment of the heterodimeric activator protein, AP-1 transcription factors. The AP1 dimers are mainly of the bZIP class of transcription factors of the Jun, Fos, and the activating transcription factor ATF2. They are involved in various cellular processes like proliferation, differentiation, apoptosis and oncogenesis. The dimerization is of the following types- Jun- Jun, Jun- Fos (86, 87).

The Fos and Jun class of proteins bind to the AP-1 regions in the promoters and enhancers of the mammalian gene. The Jun family of proteins forms both homo and
heterodimers, whereas the Fos can form heterodimers only for its role as a transcription factor. The DNA binding and the dimerization domain of these proteins are well conserved. The Fos –Jun dimers generally bind to a consensus sequence in DNA TGA(C/G) TCA, which is the AP1 site. It might weakly bind to CRE regions TGACGTCA of the DNA too (88). At the same time, the weak binding affinity of Fos-Jun at some regions of the DNA suggests that the involvement of some other transcription factors in the upregulation of that gene. The affinity and selection of DNA binding by Fos-Jun are dependent on the cell type and stimulation.

Fos and Jun belong to the large group of bZIP transcription factors, they possess a highly conserved basic region required for DNA binding and a leucine zipper region for its dimerization. This dimerization helps in the binding of the dimer to the DNA where each monomer binds to the half site of the major groove of DNA. Both Fos and Jun adopt alpha-helical conformation. The N-terminal region, which is the basic region of each protein penetrate into the major groove of the DNA by a hydrogen bond and Van der Waals interactions. These interactions provide sequence specificity, and the positively charged residues are responsible for its interaction with the phosphate backbone of the DNA. The dimerization between the proteins favors electrostatic interactions (90).

Regulation of the AP-1 transcription factors occurs at various levels, including phosphorylation – dephosphorylation, activation and its expression depending on the transcription factors. Phosphorylation of c –Jun immediately above the DBD at
Thr231, Thr239, Ser 243, Ser249, inhibits DNA binding. The c-Jun – ATF2 complex leads to transactivation due to the presence of transactivation domains at its –NH2 terminal. It is Ser63 and Ser73 for c-Jun and Thr69 and Thr71 for ATF2. According to our study in this thesis, a detailed discussion about ATF2 has been given further.

**Activating transcription Factor2 (ATF2)**

ATF2 also known as activating transcription factor 2 is a member of the mammalian ATF/CREB family of transcription factors. It is also known as cAMP-Responsive Binding Protein 2 (CREB 2) or CRE – Binding Protein – 1 (CREBP -1). The ATF/CREB family of proteins share the ability to recognize and bind to the ATF/CRE consensus site 5’- TG/TACGTCAn- 3’ and contains a bZIP domain which is required for its DNA binding and dimerization (91). Most of the ATF/CREB family proteins are involved in the stress response like ATF2- activation and ATF3 are involved in stress response, whereas ATF4 and ATF6 are involved in the protein unfolding response generated by the endoplasmic reticulum. But the CREB family proteins are also involved in the cAMP response.

ATF2 is located in the chromosome 2q32 of the genome. The length of the protein is 505 amino acids. It has a transactivation domain (TAD) in its N-terminal and a basic leucine (bZIP) domain in its C-terminal. The two terminals are connected by a long stretch of Proline that is followed by a histone acetyltransferase (HAT). ATF2 has 2 nuclear localization signal and one nuclear export signal. The
modifications in ATF2 occur by site-specific phosphorylation, ubiquitination, and glycosylation (92).

Fig 1.26. Schematic diagram of ATF2: Mammalian ATF2 is a 505 amino acids containing protein. It has got a trans-activating domain (TAD), Proline enriched stretch, histone acetyl transferase (HAT) and a bZIP domain (92).

Structural analysis shows that the TAD domain stretching from 1-105 can be subdivided into two subdomains- (1) Met19- Gly 56 contains a Zn- finger motif required for binding to specific DNA sequences. The residues which are responsible for the DNA binding are Cys27, Cys32, His45 and His 49.

Fig 1.27 General structure of a dimer of bZIP transcription factor (95).
This binding is almost similar to the rest of the transcription factors (2) the second subdomain Pro57 – Lys 105 is somewhat flexible and phosphorylation at specific sites are responsible for bringing conformational changes in the protein. Especially, phosphorylation at Thr69 and Thr71 are responsible activation of the protein by bringing conformational change in it (121-124).

ATF2 can acetylate histones H2B and H4. The HAT domain of ATF2 is located between 289 – 311 amino acids (95). The bZIP domain of ATF2 is located between Pro351 and Asp417, has got the basic region at Pro351- Lys 381 and the zipper region at Lys382 – Asp417. This region has also got two nuclear localization signals and one nuclear export localization signal. This region is involved in DNA binding and dimerization (Fig 1.27). ATF2 can form both homodimers and heterodimers. It can form heterodimers with the proteins belonging to the ATF/CREB and Fos/Jun families. Like ATF3, c-Jun and Jun D. Distinct alternative splicing and alternative promoter usage produces various isoforms of ATF2, that differ mostly in the TAD regions, the Zn finger and the Proline stretch linking the TAD and the HAT regions.

ATF2 can have post-translational modifications like phosphorylation, ubiquitination, and glycosylation. The activity of ATF2 mainly depends on the phosphorylation at the Thr69 and Thr71 residues (95). This phosphorylation cause an enhancement in the transcriptional activity of ATF2 (96). Though there are other phosphorylation sites in ATF2 like Ser490 and Ser498 by ATM, but the actual activity of these phosphorylations are not yet known. The activation of ATF2
involves the transactivation, which is again dependent on the phosphorylation of the Thr69 and Thr71 residues. The MAPK members and the c-Jun N terminal kinase (JNK) phosphorylate ATF2 at both these sites. The phosphorylation of ATF2 in response to growth factors occurs by two pathways. The Ras-dependent ERK1/2 pathway causes phosphorylation at Thr69, and the phosphorylation at Thr71 occurs by p38 or JNK. In normal condition, the ATF2 remains inactive by the binding between the C-terminal DNA binding domain and the N-terminal activation domain (92).

ATF2 has oncogenic activity in melanoma and tumor suppressor role in non-malignant skin and breast tumor. It has been reported that ATF2 plays two different roles depending on its subcellular localization and dimerizing partner. When it is localized in the nucleus then it helps in the transcription of the genes which repair DNA damage, and in cell proliferation and cell death. But upon cytosolic localization it disrupts the membrane potential of mitochondria which leads to mitochondrial based cell death. Not only that, the role of ATF2 as a transcriptional inducer or repressor depends on its dimerizing partner, cell type and the stimulation (96). Thus ATF2 is often referred to have a role in ‘oncogene addiction’ in the cell. This variation in the role of ATF2 in cell cycle and cell death program has been depicted in the following table.
Table 1.3: Transcriptional regulation of ATF2 in cell cycle and cell death

<table>
<thead>
<tr>
<th>ATF2 binding partner</th>
<th>Cell type</th>
<th>Stimulus</th>
<th>Transcriptional regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATF2, JunD</td>
<td>Rat chondrosarcoma cells, MEFs</td>
<td>Serum</td>
<td>TI*</td>
</tr>
<tr>
<td>BRCA1, Oct-1, Neurofibromin-1</td>
<td>MEFs</td>
<td>Anisomycin, hypoxia</td>
<td>TI</td>
</tr>
<tr>
<td>BRCA1, Oct-1, Neurofibromin-1</td>
<td>MEFs</td>
<td>Anisomycin, hypoxia</td>
<td>TI</td>
</tr>
<tr>
<td>CREB1</td>
<td>Murine chondrocytes</td>
<td>TGFβ, PTHrP</td>
<td>T1</td>
</tr>
<tr>
<td>JunD</td>
<td>Intestinal epithelial cells</td>
<td>Polyamines</td>
<td>TR**</td>
</tr>
<tr>
<td>Jun</td>
<td>Transformed human embryonic kidney (293T)</td>
<td>Oxidative stress (H2O2)</td>
<td>TI</td>
</tr>
<tr>
<td>Jun</td>
<td>Endothelial (HUVEC)</td>
<td>Growth factors (VEGF, EGF)</td>
<td>TI</td>
</tr>
<tr>
<td>Jun</td>
<td>Rat cerebellar granule neurons (CGN), murine immortalized gonadotrope cell lines (αT3-1)</td>
<td>K+ withdrawal</td>
<td>TI</td>
</tr>
<tr>
<td>JDP2</td>
<td>HeLa, HEPG2, MEFs</td>
<td>Amino acid deprivation</td>
<td>TR</td>
</tr>
</tbody>
</table>

*TI: Transcriptional Induction; **TR: Transcriptional Repression (96)

bZIP transcription factors in *S. pombe*

**Atf1** - Atf1 is the homolog of the mammalian ATF2. Atf1 is phosphorylated and activated by Sty1 at Ser/Thr residues in response to environmental stresses. Atf1 is activated by the MAPK, Spc1 and is degraded by Fbh1. The role of Atf1 has been discussed in detail in Chapter 6.1 of this thesis.
**Pcr1** - Pcr1 is homologous to the mammalian CREB proteins. It belongs to the family of bZIP transcription factors. Pcr1 specifically binds to the CRE- ATF sites of the DNA. The role of the transcription factor Pcr1 has been discussed in Chapter 6.3 of this thesis.

**Pap1** - This transcription factor is a redox sensor and has been found to be activated in low levels of H2O2 (around 0.05 mM) stress in the cells. The activation of Pap1 is independent of Sty1 (97).

**Zip1** - the Zip1 transcription factor is involved in the stress response of the cells during Cadmium and Arsenite stress. Zip1 is highly unstable protein. It is degraded by an F- box protein Pof1. Activated Zip1 upregulates glutathione genes to combat cadmium stress. Zip1 has the ability to induce cell growth arrest. The two possibilities for this growth arrest are – one Zip1 might induce the transcription of growth inhibitory genes or the intracellular Sulfur deficiency in cells due to Cadmium stress (98).

**Atf21** - Atf21 was first isolated as a multicopy suppressor of Δsty1 cells in response to osmotic stress. The atf21 gene is upregulated in sorbitol treatment and helps the cells to survive in Ca stress in datf1 cells. Although, Atf21 deleted cells can grow in high osmolarity conditions, and they have been reported to play a significant role in spore formation. Atf21 deleted cells can’t lead to mature spore formation. They have been identified as transcriptional activators. The level of Atf21 remains high in the cells even after 5 hours of nitrogen starvation. Meiotic genes like Mei4 are
responsible for the transcriptional activation of Atf21 by binding to its FLEX site in the promoter region (99).

**Atf31** - Atf31 is also related to meiosis in the cells. Atf31 binds to the CRE sequence of DNA. It is not essential for the viability of the cells (99). It is also involved in the expression of the late genes.

Thus we find that the bZIP transcription factors play a varied role in the cell. ATF2, one of the most studied bZIP transcription factor. Characterization of the role of ATF2 in cell cycle will thus open new insights to cancer research because it has already been reported to play a major role in ‘oncogene addiction’ in the cell. The inter-relation between numerous mechanistic pathways in the mammalian system often hides the proper understanding of the actual role of ATF2. As there is a lot of homology between the fission yeast bZIP transcription factor Atf1 and ATF2, thus deciphering the role of Atf1 in cell cycle might help to understand the underlying complexity in the mammalian system.

This thesis aims to study the regulation of cell cycle in fission yeast by the transcription factor, Atf1. Our investigation aims to decipher the role of Atf1 in the mitotic entry of cell cycle. Earlier studies report that Atf1 promotes mitotic exit by degrading Cdc13 by the APC/C. Thus it is essential to characterize the functional switch of Atf1 in the cell cycle. The regulation of Atf1 is dependent on its dimerizing partner Pcr1. This study also elucidates how Pcr1 regulates the level of Atf1 throughout the cell cycle. Unambiguously the immense utility of ATF2 in cancer
research, it is important to decipher the actual targets of ATF2. Investigation on its homolog Atf1 might open some new mechanistic approaches to cancer therapy.