Chapter-1
INTRODUCTION
1.1: Background

In mid of 19th century “The Cell Theory” was proposed; which becomes one of the foundations of biology (Karling 1939). According to Henri Dutrochet (1824) “The cell is the fundamental element of organization”. New cells are formed from pre-existing cells; this doctrine proposed by German pathologist Rudolf Virchow in 1858 carried with it a profound message for the continuity of life.

The cell cycle is an arrangement of macromolecular interactions of various proteins. The master molecules of the cell cycle are cyclin-dependent protein kinases (CDKs). As their name implies, CDKs require a cyclin partner to be active. When associated with appropriate cyclins, CDKs trigger major events of the cell cycle (DNA replication, nuclear envelope breakdown, chromosome condensation, spindle assembly) by phosphorylating certain target proteins on chromosomes and elsewhere (John et al 2002).

The regulation of cell cycle is critical for the normal development of multicellular organisms and loss of control ultimately leads to cancer and neurodegeneration. To permit the completion and correct progression of the cell cycle, a variety of mechanisms exist by which the cell actively halts cell-cycle progression until earlier processes have been completed. These mechanisms, known as “checkpoints,” monitor key processes and can delay cell-cycle progression in the event of a problem (Weinert and Hartwell 1988, May and Hardwick 2006, Callegari and Kelly 2007). The cell can not proceed to the next phase until checkpoint requirements have been met. Cell-cycle checkpoint pathways contribute to genomic stability, which is dependent on the DNA replication and chromosome segregation. During the course of evolution a variety of cell cycle checkpoints were evolved to prevent the genomic stability because genomic stability is under constant assault of many chemicals, physical factors like radiation and normal DNA metabolism.

Accumulation of mutations and chromosomal aberrations is one of the hallmarks of cancer cells. This enhanced genetic instability is fueled by defects in the genome maintenance mechanisms including DNA repair and cell cycle checkpoint pathways. If these cell cycle checkpoints are not in place then inappropriate proliferation can occur. It is a well known fact that probably all human tumors harbor genetic alterations in the genes that control cell cycle progression and checkpoint function. Therefore to understand the links between cell cycle checkpoints and cancer, we must
Introduction

first understand the roles of various genes, which have any connection with cell cycle progression.

A conditional synthetic lethal mutation in organisms or cells makes it possible to study genes whose total inactivation would be lethal. The usual mechanism of temperature sensitivity is that the mutated gene codes for a protein with a temperature dependent conformational instability, so that it possesses normal activity at the permissive temperature, but is inactive at restrictive temperature. Such temperature sensitive mutations can provide information about the effect of reversible switching by temperature changes in expression of the mutated gene. Temperature sensitive mutants provide powerful tools to study in vivo gene function. They often serve as start points to unravel gene networks controlling any biological process of interest. Such conditionally lethal mutants have been much used in the genetic analysis.

1.2: Rationale of present study

Cancer has emerged as the potential threat for mankind even in the developed countries. Perturbation in cell division cycle leads to uncontrolled cell proliferation which is the root cause of tumor either benign or malignant. Time to time various researchers contributed for understanding the cell cycle, but cancer is a multifactorial disease. Despite the tremendous amount of work on cancer, still there are roles of various genes and their interaction with other genes yet to be deciphered. Present study focused towards the role of such a gene wat1/pop3.

Fission yeast wat1/pop3 is a homologue of lst8 of budding yeast. The lst8 encodes an essential protein with WD-repeats and has a closely related human orthologue. The lst8 gene is essential in budding yeast and mammalian cells (Guertin et al 2006, Roberg et al 1997). Depletion of Lst8 in budding yeast cells results in a rapid arrest of cell growth (Loewith et al 2002, Roberg et al 1997). Lst8/Wat1/Pop3 is conserved among all eukaryotes, including D. melanogaster, S. pombe, S. cerevisiae, C. elegans, A. thaliana (Kim et al 2003, Ochotorena et al 2001, Roberg et al 1997). A number of lst8 mutants were studied in many organisms (Liu et al 2001, Díaz-Troya et al 2008), provide the data how much essential is this gene in those organisms but only two mutants were described in fission yeast (Kemp et al 1997, Ochotorena et al 2001). So this thesis is an effort to understand the role of temperature sensitive mutant of wat1/pop3 and its synergistic interaction with chk1 null mutant. This mutant was found during a temperature sensitive reverse genetic screen where chk1 null mutant
was used as query mutation, screening this against other genes for studying complex haplo-insufficient interactions and the role of those genes.

Chk1 is an evolutionarily conserved protein kinase from simple unicellular eukaryotes to metazoans, which regulates cell cycle progression in response to checkpoint activation. Chk1 was first identified in fission yeast as an essential component of the DNA damage checkpoint (al-Khodairy et al 1994, Walworth and Bernards 1996). Chk1 is a component of signaling pathways that respond to structures characteristic of DNA damage and/or incomplete DNA replication. In fission yeast, Chk1 responds to DNA damage induced either by IR or methyl methanesulfonate as well as UV (Walworth et al 1993, Walworth and Bernards 1996). The cell cycle delay induced by DNA damage is lost in fission yeast chk1 null cells treated with UV or MMS, causes the accumulation of single stranded DNA at telomere-proximal regions and thus generates a DNA damage signal (Garvik et al 1995, Sanchez et al 1999).

During this study we identify mutants defective in cell cycle control in a temperature sensitive mutant screen. As wat1 and chk1 are dispensable in fission yeast, our study shows the chk1 null mutant and wat1 mutant together poses a grave effect on survival of fission yeast cells, when exposed to restrictive temperature, UV and different drugs. We examine the localization of Wat1 protein in the fission yeast cell by immunofluorescence study. Furthermore, we have demonstrated that Wat1 protein physically interacted with Prp2 and the interaction was disrupted in Wat1 mutant protein. We further have shown that Wat1 protein is constitutively phosphorylated.