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Original article

# Cell-cycle involvement in autophagy and apoptosis in yeast

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ABSTRACT

Regulation of the cell cycle and apoptosis are two eukaryotic processes required to ensure maintenance of genomic integrity, especially in response to DNA damage. The ease with which yeast, amongst other eukaryotes, can switch from cellular proliferation to cell death may be the result of a common set of biochemical factors which play dual roles depending on the cell's physiological state. A wide variety of homologues are shared between different yeasts and metazoans and this conservation confirms their importance. This review gives an overview of key molecular players involved in yeast cell-cycle regulation, and those involved in mechanisms which are induced by cell-cycle dysregulation. One such mechanism is autophagy which, depending on the severity and type of DNA damage, may either contribute to the cell's survival or death. Cell-cycle dysregulation due to checkpoint deficiency leads to mitotic catastrophe which in turn leads to programmed cell death. Molecular players implicated in the yeast apoptotic pathway were shown to play important roles in the cell cycle. These include the metacaspase Yca1p, the caspase-like protein Esp1p, the cohesin subunit Mcd1p, as well as the inhibitor of apoptosis protein Bir1p. The roles of these molecular players are discussed.



**Contents**



*Abbreviations:* AIF, apoptosis-inducing factor; APC, anaphase promoting complex; ATG, autophagy-related; BH, Bcl-2 homology domain; BIR, baculovirus IAP repeat; CAK, CDK-activating kinase; CKI, CDK inhibitor; CPC, chromosomal passenger complex; CSC, checkpoint slide clamp; CVT, cytoplasm-to-vacuole; DDR, DNA damage response; IAP, inhibitor of apoptosis protein; ORC, origin recognition complex; PCD, programmed cell death; RNR, ribonucleotide reductase; ROS, reactive oxygen species; SCF, skp cullin f-box containing complex; rad, radiation-sensitive; TOR, target of rapamycin.

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## 1. Introduction

The eukaryotic cell cycle encompasses an evolutionarily conserved sequence of events that occur in a growing cell to duplicate all of its components. These include not only the nuclear genome but all intracellular organelles, which are divided between the two daughter cells so that they are suitably equipped to repeat the replication process (Imoto et al., 2011; Nurse, 2000; Tyson and Novak, 2008).

### 1.1. Cell-cycle regulation and apoptosis

The cell cycle of eukaryotic cells ensures maintenance of ploidy level and cell size (Imoto et al., 2011; Nasmyth, 1996). Its regulation is essential for the orderly and timely progression of the cell-cycle phases (Hartwell and Weinert, 1989). This is mainly dependent on a highly varied molecular set containing important regulatory motifs which are tightly regulated by complex transcriptional controls mainly at the G1-S and G2-M stages of the cell cycle (Glotzer et al., 1991; Kaizu et al., 2010; Mendenhall and Hodge, 1998; Nasmyth, 1996; Pines, 1999).

Cyclin-dependent kinases (CDKs) are a group of serine-threonine protein kinases essential in controlling the alternation between DNA synthesis and DNA segregation during the cell cycle (Kaizu et al., 2010). The highly conserved *Saccharomyces cerevisiae CDC28* is implicated in both G1-S and G2-M transitions (Harashima et al., 2013; Mendenhall and Hodge, 1998) similar to the CDK Cdc2p in *Schizosaccharomyces pombe* (Harashima et al., 2013; Mendenhall and Hodge, 1998).

CDK activity is highly dependent on the periodic succession of different combinations of CDK-cyclin complexes during the various phases of the cell cycle (Kõivomagi et al., 2011). When complexed to CDKs, cyclins impart substrate specificity and enable the complex's correct sub-cellular localisation (Gazitt and Erdos, 1994 Kõivomagi et al., 2011). Orderly cyclin expression and degradation are necessary for CDKs to tightly control cell-cycle progression. Furthermore, the unidirectionality of the cell cycle ensures fidelity between successive generations by checking that a particular phase of the cell cycle is complete prior to initiation of a new subsequent phase, thereby preventing the formation of genetically defective cells (King and Cidlowski, 1998). This is brought about by the correct transcriptional control that regulates cyclin synthesis, as well as the timely ubiquitin-mediated degradation of protein substrates, mainly cyclins or CDK inhibitors, *via* the anaphase promoting complex (APC) or the Skp, Cullin, F-box containing complex (SCF) (as reviewed by Vodermaier, 2004).

In addition, activity of CDKs may also be affected by their phosphorylation at specific amino acid residues (Espinoza et al., 1998). CDK-activating kinases (CAKs) facilitate substrate binding by opening up the protein substrate binding region and hence increasing the number of contacts between the CDK and the cyclin (Russo et al., 1996). The budding yeast CAK, Cak1p, is dissimilar to the relatively similar and homologous mammalian and fission yeast CAKs (Damagnez et al., 1995; Espinoza et al., 1996). Contrary to the function of cyclins and CAKs, cell-cycle regulation can also be brought about by inhibition of CDK activity through cyclin-dependent kinase inhibitors (CKIs) or other inhibitory protein kinases. The most studied CKI in budding yeast is Sic1p (Mendenhall and Hodge, 1998), whereas the only CKI in fission yeast is Rum1p (Moreno and Nurse, 1994). Both Sic1p and Rum1p show weak similarities in their inhibitory domains (Sánchez-Diaz et al., 1998). The inhibitory protein kinases Swe1p and Wee1p in budding and fission yeast respectively (Asano et al., 2005; Perry and Kornbluth, 2007; Stark and Taylor, 2004) phosphorylate CDKs in response to incomplete DNA synthesis, unrepaired DNA damage, bud formation failure or other unfavourable intrinsic conditions, causing the G2-M checkpoint to be activated by arresting the cell cycle prior to mitosis (Liu and Kipreos, 2000; O'Connell et al., 2000).

Any failure associated with cell-cycle regulation may result in the uncontrolled proliferation of cells (Evan and Vousden, 2001). To mitigate for such eventualities, organisms have evolved programmed cell death (PCD), which is another highly conserved mechanism whereby they eliminate defective or excess cells in an orderly manner without eliciting an immune response (Madeo et al., 2002a; Pucci et al., 2000; White and McCubrey, 2001).

Programmed cell death, such as apoptosis, is an effective way for cells to commit suicide in response to key apoptotic signals (King and Cidlowski 1998; White and McCubrey, 2001). In unicellular organisms, the scope of apoptosis in offsetting any imbalance in cell-cycle progression may be less obvious than in multicellular organisms. Regardless of being unicellular, it is known that yeast cells naturally co-exist in colonies and have the ability to form biofilms when nutrients are depleted (Váchová and Palková, 2005; Zara et al., 2002). Therefore, apoptosis may be a good mechanism to clear old, infertile or otherwise damaged yeast cells. In addition, it redistributes nutrients from chronologically aged or stressed cells to young, healthier cells, thereby increasing their odds of survival (Büttner et al., 2006; Herker et al., 2004; Knorre et al., 2005). Apoptotic triggers in yeast may be both exogenous (including reactive oxygen species (ROS), pheromones, heavy metals and drugs) as well as endogenous (including ageing and mutations which cause mitotic catastrophe and replication failure) (Carmona-Gutierrez et al., 2010; Madeo et al., 2002a). Concurrent incompatible signals for proliferation and cell-cycle arrest may also induce apoptosis (Yonish-Rouach et al., 1993).

#### 1.1.1. Checkpoints biochemically link the cell cycle to apoptosis

Many authors have suggested the use or control of a shared set of biochemical factors which interconnect cell-cycle regulation to apoptosis at cell-cycle checkpoints (Alenzi, 2004; King and Cidlowski, 1995; Madeo et al., 2002a; Pearce and Humphrey, 2001; Weinberger et al., 2003). Even before such common factors were identified, cytologists had already observed common morphological features in apoptotic and mitotic cells (Alenzi, 2004; King and Cidlowski, 1995; Pucci et al., 2000). During both cellular events, cells lose substrate attachment, become more rounded and decrease their cell volume. In addition, in both cases, cells display membrane blebbing and show chromatin condensation (Alenzi, 2004; Pucci et al., 2000), though the latter may be easily distinguished microscopically between apoptotic and mitotic events by