Chapter 6

Study of Cellular Processes in Higher Eukaryotes Using the Yeast *Schizosaccharomyces pombe* as a Model

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Abstract

Schizosaccharomyces pombe (Sz. pombe), or fission yeast, is an ascomycete unicellular fungus that has been used as a model system for studying diverse biological processes of higher eukaryotic cells, such as the cell cycle and the maintenance of cell shape, apoptosis, and ageing. *Sz. pombe* is a rod-shaped cell that grows by apical extension; it divides along the long axis by medial fission and septation. The fission yeast has a doubling time of 2–4 hours, it is easy and inexpensive to grow in simple culture conditions, and can be maintained in the haploid or the diploid state. *Sz. pombe* can be genetically manipulated using methods such as mutagenesis or gene disruption by homologous recombination. Fission yeast was defined as a micro-mammal because it shares many molecular, genetic, and biochemical features with cells of higher eukaryotes in mRNA splicing, post-translational modifications as N-glycosylation protein, cell-cycle regulation, nutrient-sensing pathways as the target of rapamycin (TOR) network, cAMP-PKA pathway, and autophagy. This chapter uses *Sz. pombe* as a useful model for studying important cellular processes that support life such as autophagy, apoptosis, and the ageing process. Therefore, the molecular analysis of these processes in fission yeast has the potential to generate new knowledge that could be applied to higher eukaryotes.

Keywords: Yeast, *Schizosaccharomyces pombe*, cellular model, autophagy, apoptosis, ageing, chronological lifespan

1. Introduction

1.1. General features of *Schizosaccharomyces pombe*

Schizosaccharomyces pombe (*Sz. pombe*) is a yeast found in sugar-containing fermentations of alcohol involved in the production of beer in subtropical regions; the yeast was first described in 1893 by P. Lindner who isolated yeast from millet beer of East Africa and gave it the name "pombe" from the Swahili word for beer. *Sz. pombe* is a free-living fungus, classified with ascomyctes such as *S. cerevisiae* (Table 1). However, fission yeast is taxonomically and as evolutionarily distinct from budding yeasts as it is from humans. *Sz. pombe* reproduces by fission, a process similar to that used by higher eukaryotic cells. Despite the variation in the predicted time of divergence, phylogenetic analysis, and experimental evidence, it can be shown that the gene sequences of *Sz. pombe* are often more similar to its counterpart in mammals than the equivalent of genes of *S. cerevisiae* [1].

Sz. pombe is described as a fission yeast because it grows by apical extension and it divides along the long axis by medial fission and septation. The fission yeast has a doubling time of 2–4 hours, and can be maintained in the haploid or the diploid state. The yeast has a cylindrical rod-shape 3–4 μm in diameter and 7–14 μm in length [2]. The cell wall of *Sz. pombe* provides mechanical strength and protection from environmental stresses. The cell wall determines cellular morphology during the different stages of the life cycle, and is continuously remodelled during the cell cycle to allow cellular growth. The cell wall of *Sz. pombe* is composed of 74–82% glucan and 9–14% galactomannan, and has a chemical composition of β-1, 3-glucan, β-1, 6-glucan, α-1, 3-glucan, and α-galactomannan. Viewed by electron microscopy, the cell wall is organized in a three-layered structure: the outer layer is electron-dense, the adjacent layer is less dense, and the third layer bordering the cell membrane is dense [4, 5].

1.2. Genome organization of *Sz. pombe*

The whole genome of *Sz. pombe*, distributed over three chromosomes, is estimated at 13.8 Mb [6]. The genome of *Sz. pombe* was fully sequenced using 452 cosmids, 22 plasmids, 15 BAC clones, and 13 PCR products, in a project under the control of Schizosaccharomyces f European Sequencing Consortium (EUPOM), the Wellcome Trust Sanger Institute (United Kingdom), and Cold Spring Harbor Laboratory (USA). The collaborating bodies were Funding Agency Cancer Research UK (Cambridge Institute United Kingdom) and the European Union (EU) [7]. The genome is organized in the chromosomes I (5.7 Mb), II (4.6 Mb), and III (3.5 Mb), and a 20-kb mitochondrial genome. The three centromeres are 35, 65, and 110 kb long for chromosomes I, II, and III, respectively. On the centromeres are 174 tRNAs genes, 45 of which have introns. The genome of the yeast has at least 4, 940 protein-coding genes (including 11 mitochondrial genes) and 33 pseudogenes. *Sz. pombe* introns average only 81 nucleotides in length. The gene density for the complete genome is one gene every 2, 528 bp, which is similar in chromosomes II and I (one gene every 2, 483 and 2, 457 bp, respectively) and is less dense for chromosome III (one gene every 2, 790 bp). The spliceosomal RNAs (U1±U6) are located with 16 small nuclear RNA genes (snRNAs) and 33 small nucleolar RNAs (sno-RNAs). The 5.8S, 18S, and 26S ribosomal RNA genes are grouped as 100±120 tandem repeats in two arrays

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on chromosome III, with the 5S ribosomal RNA genes being distributed throughout the genome [7]. In silico analysis of the *Sz. pombe* genome has made it known that there are genes highly conserved between the vegetable and animal kingdoms, some of which have been implicated in cancers in humans [8, 9].

2. Mating-type locus, heterothallic, and homothallic phenotype

Sz. pombe has been studied extensively since the 1950s. Urs Leupold isolated the *Sz. pombe* strains widely used in research from a culture of *Sz. pombe* Lindner str. *liquefaciens* obtained by Osterwalder [10] from grape juice in France, in 1921. In its normal life cycle, *Sz. pombe* cells are haploids, although they can grow in a diploid state. Leupold described the haploid character and the complexity of the mating-type genetics of *Sz. pombe*, by culturing the haploid and diploid yeast [11, 12, 13], which were both homothallic and heterothallic (h) strains. The mating of *Sz. pombe* works by controlling *mat* genes located on the mating-type locus in the chromosome II [14], which is organized by one expression locus (*mat1*) and two donor loci *mat2* and *mat3* that encode the *mat P* and *mat M* genes, respectively. There are three alleles *mat* for the mating-type: h^+ (strain 975) or *P* (Plus, +), h^- (strain 972) or *M* (Minus, -), and h^{90} (strain 968). The mating-type is determined by the *mat1* locus, cells that present the segment called *mat1-P* determine the sexual type *P* or h^+ via the two genes *mat-Pi* and *mat-Pc* [15]. Cells with the segment *mat1-M* define the sexual type *M* or h^- by two genes *mat-Mi* and *mat-Mc*. The *P* and *M* alleles determine the mating reactions of the heterothallic strain. The homothallic strains with cells of both the *P* and *M* mating type can mate and form asci with four ascospores.

The heterothallic strain with only one mating type, are able to mate with heterothallic *Sz. pombe* strains of the opposite mating type. The allele h^{90} determine the homothallism [11], derived from *P* and *M* by recombination and mutation by the crossing of heterothallic strains. Homologous sequences flank the mating-type locus support site-specific recombination between the expression locus and the donor loci (*mat1* and *mat2*, or *mat1* and *mat3*) [16]. Heterothallic strains in *Sz. pombe* originated from strains having spontaneously rearranged their mating type *loci*. For homothallic segregants with the markers h^+ parent and recombination for two marker genes (*his7* and *his2*) flanking the locus, two mating-type genes are generated and named initially as *h1* and *h2* and later renamed *mat1* and *mat2*, respectively [17].

GENERAL FEATURES	
Doubling time of 2–4 hours	
Cylindrical rod-shaped	
3–4 μm in diameter and 7–14 μm in length	
Ascospores arranged linearly	
Easy genetic manipulation and mutant generation	
ORGANISM TAXONOMY	
NCBI Taxonomy ID: 284812	
Kingdom:	*Eukaryota*

Phylum:	*Ascomycota*
Class:	*Schizosaccharomycetes*
Order:	*Schizosaccharomycetales*
Family:	*Schizosaccharomycetaceae*
Genus:	*Schizosaccharomyces*
Species:	*Schizosaccharomyces pombe*

CELL CYCLE	
Phase	Percentage in the full cell cycle
G1	10%
S (*Cytokinesis*)	
G2	70%
M	

MATING TYPES	
Fission yeast can exist stably in either haploid or diploid states.	
Phenotype	Symbol
Homothallic	h^{90}
Heterothallic P	h^{+}
Heterothallic M	h^{-}

GENOME				
	Haploid Diploid	Gene density	Coding (%)	
	Length (Mb)			
Whole genome	13.8			
Chromosome	5.7	2483	58.6	Centromere I 35 kb
Chromosome	4.6	2457	57.5	Centromere II 65 kb
Chromosome	3.5	2790	54.5	Centromere III 110 kb
Mitochondrial genome	20 kb			

PEPTIDASES MEROPS
Count of known and putative peptidases121
non-peptidase homologues 39

PomBase (http://www.pombase.org) is the model organism database for the fission yeast *Schizosaccharomyces pombe*.

Table 1. General features of *Schizosaccharomyces pombe*.

3. Cell cycle of *Sz. pombe*

Sz. pombe coordinate their growth with the cell cycle. Exponentially growing cells are of a similar cell size and double in mass before entering the next round of cell division. Cell length is a measure of the stage of the cell cycle that the cell has reached [18]. The cells are commonly cylindrical with rounded ends, but under conditions of starvation cells become shorter. *Sz.*

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pombe is a single-celled, haploid organism with a cell cycle typical of a eukaryote, organized in the same stages as those of higher eukaryotes: interphase (G1, S, and G2 phases) and mitosis by mechanisms that are similar to those in multicellular animals. *Sz. pombe* usually proliferates in a haploid state. In a medium rich in nutrients, wild-type cells undergo a mitotic division approximately every two hours, with a short G1 phase taking less than 10% of the cell cycle. Next, the cells enters into the S phase following cytokinesis; the longer stage of the cell cycle is the G2 phase (70% of the cell cycle) [19]. Cells sense their size and use this information to coordinate cell division with cell growth to maintain a constant cell size by Pom1 protein and Nif1, localized to the cell ends of DNA, and inhibits mitotic activators in the middle of the cell acting as sensors of cell size controlling the onset of mitosis [20]. When starved of nitrogen, cells stop the division and arrest the cycle in G1, which promotes the sexual phase of the life cycle through conjugation of pairs of P and M cells to form diploid zygotes in a process known as karyogamy. In nitrogen-starved conditions, *Sz. pombe* haploid cells expressing the *mat P* and *mat M* genes enters into meiotic division after forming zygotes [21, 22]. After mating and nuclear fusion, premeiotic replication occurs, DNA content is duplicated from 2N to 4N, there is pairing and recombination of homologous chromosomes, and ocurrs the reductional meiosis I and the meiosis II division for the production of four haploid nucleic (1N), which are separated into four spores in an ascus, where the ascospores of *Sz. pombe* are arranged linearly [13]. *Sz. pombe* is normally haploid, but it is possible to select diploid strains, which can then divide by vegetative mitotic growth until starved of nitrogen, when they undergo meiosis and form azygotic asci. The provision of a rich nutrient source induces new vegetative growth and mitotic cell division. In 1957, Mitchison measured changes in the volume of cells along the yeast cell cycle. He found that volume increased slightly in the interphase, then changed about an hour before the cell split and increased just after cleavage. *Sz. pombe* extended at one end only, usually the older one [23, 24]. The cell cycle of *Sz. pombe* is described in Figure 1.

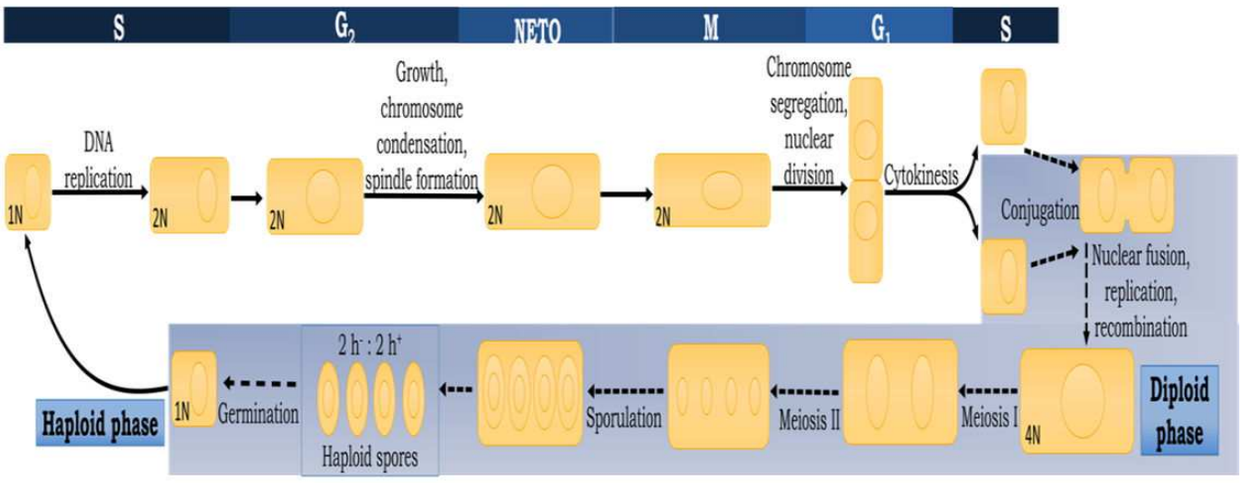

Figure 1. ***Sz. pombe cell cycle***. DNA replication starts in S-phase then continues in G2-phase. *Sz. pombe* growth is accompanied by chromosome condensation and spindle formation. During G2, *Sz. pombe* starts bipolar growth known as New End Take Off (NETO), in M-phase chromosome segregation and nuclear division occurs, and in G1 cytokinesis is accomplished so two daughter cells are produced and it can undergo S-phase. When cells are starved, they undergo nuclear fusion, replication, and recombination, entering a diploid-phase which ends in the formation of four haploid spores that, under normal conditions, enter into S-phase.

4. Cellular process studied using *Sz. pombe*

Sz. pombe is an excellent model for the study of common eukaryotic cellular processes. The yeast shares important biological aspects with metazoans, such as chromosomal structure and metabolism, relatively large chromosomes and centromeres, low-complexity replication origins, epigenetic mechanisms for regulation of genetic expression, G2/M cell cycle control, cytokinesis, mitosis and meiosis, DNA repair and recombination, the mitochondrial translation code, and spliceosome components, among others [2, 25].

Fission yeast presents a short cell cycle and is easy to manipulate by genetic classical and molecular analysis. *Sz. pombe* is a particularly useful model for the study of the function and regulation of genes from more complex species [26]. A model organism is any species used for scientific research in order to answer a specific question that cannot be studied in any other way. A model must be amenable to experimental manipulation in the laboratory, require low costs of culture and maintenance, have a short generation time, be amenable to genetic manipulation to produce mutants and allow the study of their effects, and show high conservation of mechanisms or specific cellular process. A system model extensively used in microbiology, biochemistry, biotechnology, and molecular biology is *Escherichia coli* (*E. coli*). Although this bacterium can be grown and be cultured easily and inexpensively in a laboratory setting, *E. coli* lacks the mechanisms for post-transcriptional on mRNA or post-translational modifications on proteins of eukaryotic origin. *Saccharomyces cerevisiae* (*S. cerevisiae*) is other model used due to its quick, inexpensive, and easy growth in control conditions of the laboratory. However, like *E. coli*, *S. cerevisiae* has a limited capability for performing post-transcriptional or post-translational modifications such as N-glycosylations. *Drosophila melanogaster* is other model system in the cellular biology field widely used for classical genetics analysis. *Caenorhabditis elegans* is the model of choice for understanding the genetic control of development, physiology, and the study of cellular process such as apoptosis and ageing. *Mus musculus* and *Rattus norvegicus* are two mammalian models used for the study of several diseases that affect humans; produced by highly conserved genes, its main disadvantage is slower reproduction than other models allow. In contrast, fission yeast is an ideal system for the analysis of cellular processes common in eukaryotic cells; since it shares many molecular, genetic, and biochemical characteristics with multicellular organisms — such as the splicing of mRNA, post-translational modifications, cell division, and cell cycle control [13, 27, 28] — it has a high rate of reproduction in a short period.

4.1. Model for studying the eukaryotic cell cycle

Sz. pombe, the fission yeast, has been used as a model for the study of the eukaryote's cell cycle. Fission yeast is a powerful tool for the study of the cell cycle, since it has a typical cycle cell eucarionte G1, S, G2, and M. G2 occupies approximately 70% and the remaining stages on 30% of the total duration of the cell cycle [29, 30]. Since it has a typical cell shape, it is a useful system for identifying genes involved in the cell cycle because mutants are easily identifiable when compared with the wild type [31]. In the 1970s, using *Sz. pombe* P. Nurse demonstrated the cyclin/cyclin-dependent kinase complex as the key regulator of cellular cycle. *Sz. pombe* has a

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single mitotic cyclin-dependent kinase (CDK), highly conserved, named cdc2p, homologous to CDC28 from *S. cerevisiae* and CDK1 from human cells [32]. *Sz. pombe* has been used for the molecular sexual differentiation analysis. In this system, it was discovered that a cluster of genes associated with the main biological events of the differentiation process are up-regulate: genes induced in response to environmental changes (starvation and pheromone-induced genes), early genes (pre-meiotic S phase and recombination), middle genes (meiotic divisions and early steps of spore formation), and late genes (spore maturation) [33]. In addition, the use of the fission yeast has contributed to the knowledge of other areas such as cytokinesis, the formation of microtubules, differentiation, cell morphogenesis and polarity, mechanisms of response to stress, and response to damage of the DNA [34, 35].

4.2. Cellular ageing

The identification of evolutionarily conserved mechanisms that determine long lifespans has been of great interest, making the fission yeast *Sz. pombe* a novel model for the study of ageing. Ageing is a genetically programmed event [36, 37] and can be defined as a decline in biological functions due to the accumulation of molecular and cellular damage imposed by intrinsic and extrinsic factors on living organisms, having mortality as a consequence. Lifespans of humans are measured chronologically from birth to death. Ageing can considered from the perspectives of two types of cellular lifespans: the replicative lifespans (RLS) of continuously dividing cells and the chronological lifespans (CLS) of post-mitotic cells, from terminally differentiated cells. RLS is the number of mitotic divisions a cell can undergo before senescence, and CLS is the length of time a non-dividing cell can remain viable. CLS measures how long cells can remain viable in a non-dividing state, allowing a continuous decline in viability without detectable re-growth until all cells in the culture are dead. The complexity of human bodies and the length of the human lifespan make research on the biology of human ageing difficult. Model organisms such as yeast, worms, and flies have been extensively utilized because of their short lifespans and their accessibility to classical genetics techniques, as well as the high degree of conservation of genes, processes, and signalling pathways that they share with humans and other mammals [38]. Some aspects of ageing studied in fission yeast have been mainly focused on the effect of calorie restriction on ageing and on the detection of nutrient signalling pathways [39, 40]. The protein kinase target of rapamycin (TOR) and insulin/insulin-like growth factor (IGF)-1 pathway are the well-studied nutrient sensing pathways that determine the length of lifespan [41]. TOR exerts its function through two distinct complexes: TOR complex 1 (TORC1) and TOR complex 2 (TORC2), which regulate translation, cell growth, and the organization of actin cytoskeleton [42, 43]. Inactivation of TORC1 increases the lifespan in model organisms such as budding yeast, worms, flies, and mice [44, 45]. The insulin/IGF-1 signalling pathway, and their components such as PI3 kinase and AKT kinase, affect the longevity of flies, worms, and mice [39]; interestingly, this pathway has also been found in yeasts with the AKT kinase. In *S. cerevisiae*, nutrient signalling via the kinases Sch9/Tor/PKA pathway has a strong pro-ageing effect. Down-regulation of this pathway is partially responsible for CLS increase. In fission yeast, there are two homologues of these kinases with a similar pro-ageing activity in *Sz. pombe*: pka1 y sck2 [46]. In this model, deletion of *pka1* or *sck2* extended CLS by diminishing the accumulation of ROS and increasing oxidative stress resistance [47,

48]. AKT kinase orthologues of *Sz. pombe* regulate CLS: loss of *sck1* extended the lifespan in the condition with the excess of nutrients and loss of both genes showed that *sck1* and *sck2* control different longevity pathways. In addition, there are four genes identified as regulators of ageing in *Sz. pombe*: 1. *psp1+*/*sds23+*/*moc1+*, a gene required for long-term survival in the stationary phase. Psp1 is phosphorylated in the stationary phase by the cyclin-dependent kinase complex Cdc2/Cdc13. 2. Casein kinase *cka1+*, an orthologue of the α-subunit of casein kinase II in mammals, whose function is essential but the details of the effect of its deletion are not known. 3. *adh1+*, encoding the alcohol dehydrogenase that converts acetaldehyde to ethanol. 4. *rpb10+*, a protein subunit shared by RNA polymerases I, II, and III. It is noteworthy that lifespan extension from TOR-inhibition, Sch9/Akt, or caloric restriction is an effect highly conserved between yeast, worms, and flies [39]. Research of ageing on fission yeast has greatly benefited the knowledge of molecular process since the data obtained can be extrapolated to mammalian cells.

4.3. Autophagy

Autophagy is a catabolic mechanism that regulates the intracellular turnover of unfolded/misfolded, long-lived, or damaged proteins, lipids, and organelles (such as mitochondria and the endoplasmic reticulum (ER), through its sequestration within a double-membrane and delivery to lysosomes for degradation and recycling of biocomponents. Under basal conditions, autophagy is a housekeeping programme, but it can also be activated by nutrient starvation, low cellular energy levels, amino acid deprivation, growth factor withdrawal, ER stress, hypoxia, oxidative stress, and infection [50–54]. Autophagy deregulation in higher eukaryotes leads to muscle atrophy, myopathy, and cardiac and immune disease [52–54], and plays a dual role in the pathogenesis of cancer [50, 52, 55, 56].

Duve [57] introduced the term autophagy from the Greek *auto* (itself) and *phagy* (eat) which means that cells degrade their cytoplasmic components through lysosomal degradation. Clark described the autophagy process in 1957 through electron microscopy of the kidneys of newborn mice [58]; he discovered that inside lysosomes there were mitochondria. Unfortunately, it was very difficult to study autophagy via electron microscopy and biochemical analysis; thanks to the introduction of molecular techniques, it was later discovered that the yeast *S. cerevisiae* was a tractable model of eukaryotes. Thirty Atg proteins (autophagy-related proteins) were also discovered later, and it was found that they possess mammalian orthologues [50–52, 54, 59–61]. Four types of autophagy have been described in mammals (Figure 2): macroautophagy (hereafter referred to as autophagy), microautophagy, chaperone-mediated autophagy (CMA), and the more recently described RNautophagy [61–64]. In autophagy, the cytoplasmic components (also known as cargo) are first encapsulated in a double-membrane structure synthesized *de novo*, which at the beginning is the phagophore and when it is completely formed is called autophagosome (AP), after which it is trafficked and fused to the lysosome. In microautophagy the cargo is selected through an invagination in the lysosome where it is degraded. In the CMA, specific cytosolic proteins containing the motif KFERQ are recognized by the heat shock cognate protein 70 (HSC70), and are then delivered to the lysosomal associated membrane protein (LAMP2) of type A receptor that is

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located in the lysosome membrane, where the proteins are translocated inside and degraded [62–64]. In 2013, Fijiwara [65] described RNautophagy, where several RNAs were recognized by the LAMP2C receptor and degraded in the lysosome. However, autophagy is a very complex process in metazoans, making it important to have simplest biomodels that share with mammals the core of the autophagic machinery, such as the budding yeast *S. cerevisiae* and the fission yeast. In the budding yeast autophagy, microautophagy and cytoplasm to vacuole targeting (CVT) have been outlined; likewise in the fission yeast, autophagy shares almost the same conserved core of autophagic machinery (Figure 2). However, microautophagy, CVT, and RNautophagy have not been described and further investigation is needed in order to unravel the conservation of these mechanisms.

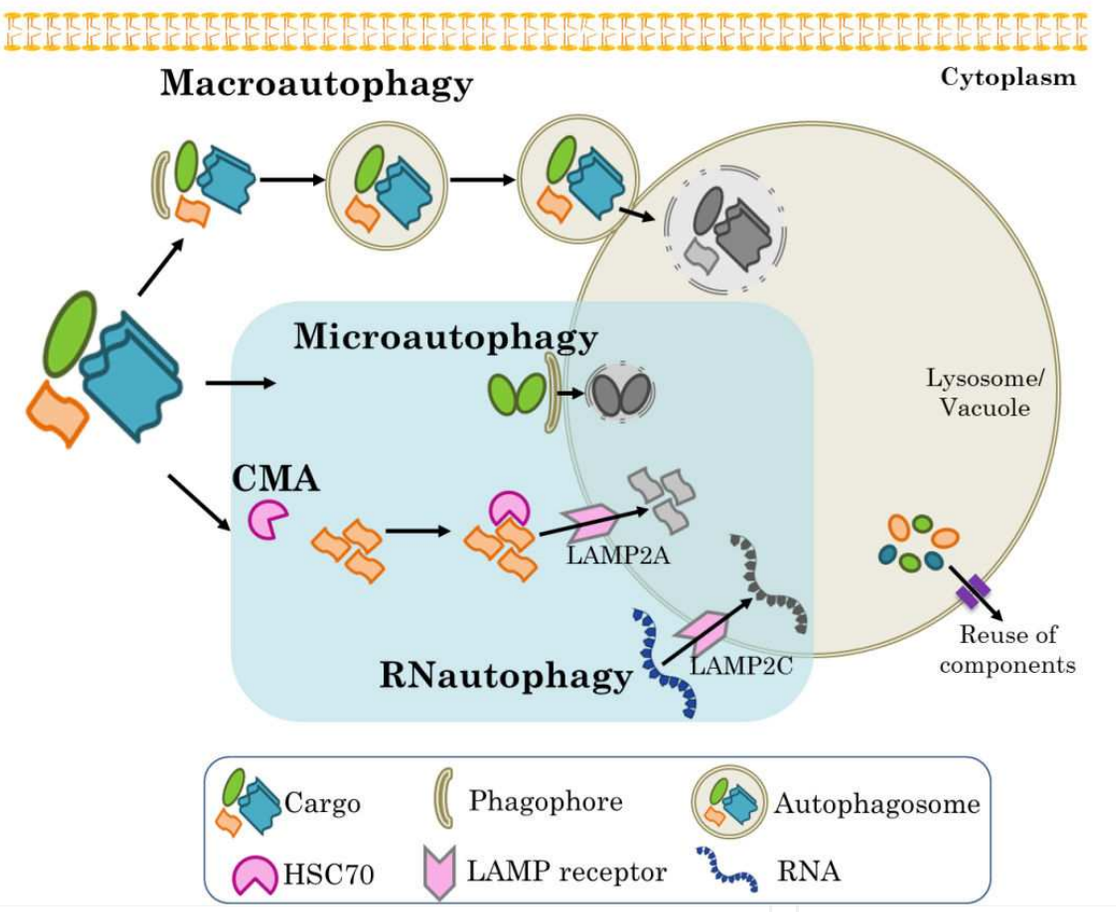

Figure 2. Types of autophagy. In mammals (*H. sapiens*, *M. musculus*, *and R. norvergicus*), macroautophagy, microautophagy, cytoplasm to vacuole targeting (CVT), and RNautophagy have been described. In macroautophagy, cargo is sequestered by an invagination of a lipid bilayer which at the beginning is called phagophore, before it elongates and madurates, forming the autophagosome, then it fuses to the lysosome. In microautophagy, cargo is selected by an invagination of the lysosome. In CVT, proteins with the motif KFERQ are recognized by the HSC70, delivered, and translocated inside the lysosome. In RNautophagy (in *Sz. pombe*), macroautophagy is highly conserved; however, microautophagy, CVT, and RNautophagy (in the blue square) have not yet been elucidated.

The Atg machinery was classified into five groups according to function in each step of the autophagic process (Table 2 and Figure 3) [1–6, 11, 12, 50–55, 60, 63, 64]: ULK1 kinase and its

regulators that signal the autophagosome biogenesis, the phosphatidylinositol (PtdIns) 3-kinase complex controls the nucleation step that recruitsother Atg proteins hierarchically, the elongation of the phagophore mediated by the Atg12 and LC3 conjugation systems, and a subgroup of proteins with unknown functions. In mammals under nutrient rich conditions, insulin or growth factors activate the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway. When PI3K is activated, it converts phosphatidylinositol (3, 4)-bis phosphate (PIP2) to phosphatidylinositol-3 phosphate (PIP3), then Akt is activated and inactivated by phosphorylation to TSC which leads to mTOR complex 1 (mTORC1) activation. mTORC1 negatively regulates ULK1 and Atg13, so when mTORC1 is inactivated by nutrient depletion, low concentration of insulin, deprivation of amino acids, or the addition of its antagonist rapamycin, the ULK1 kinase and Atg13 are then activated, forming the ULK1 kinase complex (Table 2), which signals the induction of the AP formation. There is another inductor of autophagy, the AMP activated protein kinase (AMPK), that senses low intracellular energy status and directly activates by phosphorylation to ULK1, and by inhibiting mTORC1 via phosphorylation of Raptor (a subunit of mTORC1) [66–69]. ULK1 phosphorylates Beclin1 at serine 14, which is a component of the PtdIns 3- kinase complex (Table 2) and enhances Vps34 activity — this step is crucial in the AP formation because Vps34 inhibition by 3-methyladenine (3-MA) or wortmannin disrupts the biogenesis. Vps34 synthesizes PIP3 at the sites where AP are assembled; it has been suggested that PIP3 recruits additional factors for AP formation, but its role remains unclear. Beclin1 binding proteins that activate or inhibit AP biogenesis have been identified: the UV radiation resistance-associated gene protein (UVRAG) and Bcl-2 or Bcl-X_L, respectively. Beclin-UVRAG interacts with the class C Vps complex proteins, which are part of the endosomal fusion machinery, so this interaction induces AP fusion with lysosomes [50, 53, 70–71].

The next step in AP building is the elongation of the phagophore, which requires membrane input from organelles (such as ER, mitochondria, cytoplasmic membrane, or possibly from *de novo* synthesis); however, the exact membrane source is unknown. In mammals, Atg9 is a transmembrane protein that probably carries membrane expansion of the phagophore but its downstream effectors have not yet been elucidated. However, there are two ubiquitin-like complexes that are required for elongation; the first one is the Atg12 conjugation system (Table 2), in which the ubiquitin-like protein Atg12 is covalently joined to Atg5. This must first be activated by Atg7 (E1 ubiquitin activating enzyme-like or E1). Atg7 uncovers Atg12 glycine carboxyl-terminus, then Atg12 is transferred to Atg10 (E2 ubiquitin conjugation enzyme-like or E2), after which it is covalently attached by its terminal glycine to lysine 130 (Lys 130) of Atg5.

Finally, Atg12-Atg5 conjugates with Atg16L1 forming a tetramer; this tetramer is essential for the elongation but when AP has been completed it dissociates [76–78]. The second ubiquitin-like complex is the microtubule-associated protein 1 light chain 3 (LC3) conjugation system (Table 2). LC3 is a precursor of LC3-I, it is obtained by the cleavage of the protease Atg4B to its carboxyl terminus. LC3-I is attached to phosphatidylethanolamine (PE) by the enzymes E1 and E2- ubiquitin-like Atg7 and Atg3, respectively, obtaining the LC3-II product which forms part of the AP. Notice that the LC3 complex lacks an E3 ubiquitin ligase-like enzyme that could

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facilitate LC3I-PE conjugation; however, a crosstalk between these two ubiquitin-like systems has been demonstrated by the tetramer Atg12-Atg5-Atg16L1 acting as E3-like enzyme. When AP is formed, LC3 can be recycled from the form LC3II; this is achieved through Atg4 which breaks apart LC3I from PE, and LC3-I and LC3-II assays are widely used for monitoring autophagy [60, 77]. Finally AP moves bidirectionally along microtubules towards the microtubule organizing centre (MTOC) where lysosomes are enriched, then AP fuses with lysosomes forming autolysosomes where the content is degraded and the components are recycled [50]. Atg2 is required for the AP location and can possess a different role when binding to Atg18 in order to target the complex to autophagic membranes [79]. In *Sz. pombe*, depletion of nitrogen from the culture medium triggers autophagy. Unlike mammals, the addition of AMPK does not induce autophagy, nor can rapamycin induce it. It has been reported that rapamycin has a partially inhibitory effect on TOR activity, moreover, the addition of rapamycin plus caffeine prompts a starvation-specific gene expression pattern and autophagy [80–81]. Atg 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 18a, 18b, 18c, Vps34 homologues have been found (Table 2) [73–75], and their deletion inhibits AP formation.

	Components		**Features**
	Mammals **% identity**	***Sz. pombe*** **(% identity)**	
ULK1 kinase complex	**ULK1** *M. musculus*: 89% *R. norvergicus*: 89%	**Atg1** **38%**	Kinase ser/thr induces autophagy and PA assembly [72, 73–75]
	Atg13 *M. musculus*: 94% *R. norvergicus*: 89%	**Atg13** **23%**	Regulator and binding protein of ULK1 complex [72, 73–75]
	FIP200 *M. musculus*: 89% R. norvergicus: 89%	**Atg17** **47%**	Binding protein and regulator of ULK1, induces PA biogenesis [72, 73–75]
	Atg101 *M. musculus*: 99% *R. norvergicus*: 98%	**Atg101** **25%**	Binds to Atg13 in the ULK-Atg13-FIP200 complex, which is important for the stability and basal phosphorylation of Atg13 and ULK1 [72, 73–75]
PtdIns 3- kinase complex	**Vps15** *M. musculus*: 96% *R. norvergicus*: 96%	**Vps15** **44%**	Regulatory kinase subunit of the PtdIns 3- kinase complex [72, 73-75]
	Atg14 *M. musculus*: 93% *R. norvergicus*: 98%	**Atg14** **32%**	Necessary for the function of the PtdIn2-kinase complex [72, 73–75]
	Vps34 *M. musculus*: 97% *R. norvergicus*: 98%	**Vps34** **52%**	PtdIns 3- kinase produces PI3P and allows the recruitment of PI3P binding proteins like WIPI1/2 and the two conjugation systems [72, 73–75]
	Beclin1 *M. musculus*: 98%	**Atg6** **29%**	Modulates AP biogenesis by binding to Vps34 [72, 73–75]

	Components		Features
	R. norvergicus: 97%		
	UVRAG *M. musculus*: 88% *R. norvergicus*: 94%	**Vps38** **25%**	Binds Beclin1 enhancing AP biogenesis [72, 73–75]
Atg12 conjugation system	**Atg12** *M. musculus*: 76% *R. norvergicus*: 71%	**Atg12**	Ubiquitin-like protein covalently joined to Atg5 [72, 73–75]
	Atg5 *M. musculus*: 97% *R. norvergicus*: 95%	**Atg5** **28%**	Ubiquitin-like protein covalently attached to Atg12 and interacts with Atg16 [72, 73–75]
	Atg7 *M. musculus*: 89% *R. norvergicus*: 89%	**Atg7** **38%**	E1-like enzyme for Atg12-Atg5 and LC3-PE formation [72, 73–75]
	Atg10 *M. musculus*: 72% *R. norvergicus*: 79%	**SpAtg10** **21%**	E2-like enzyme required for Atg12-Atg5 complex In *Sz. pombe*, SpAtg10is also required for normal cell cycle progression and for responses to several stress agents but has no role in autophagy [72, 73–76]
	Atg16 *M. musculus*: 82% *R. norvergicus*: 82%	**Atg16** **0%**	Necessary for the assembly of the tetramer Atg12-Atg5-Atg16 [72, 73–75]
LC3 conjugation system	**LC3** *M. musculus*: 83% *R. norvergicus*: 83%	**Atg8** **37%**	Conjugated with PE forms part of the AP membrane [72, 73–75] [22, 23–25]
	Atg3 *M. musculus*: 91% *R. norvergicus*: 93%	**Atg3** **36%**	E2-like enzyme [72, 73–75] [22, 23–25]
	Atg4 *M. musculus*: 92% *R. norvergicus*: 91%	**Atg4** **28%**	Induces LC3-II then helps to recycle LC3 [72, 73–75]
	Atg7 *M. musculus*: 89% *R. norvergicus*: 98%	**Atg7** **38%**	E1- like enzyme [72, 73–75]
Atg2-Atg18 complex and Atg9	**Atg2** *M. musculus*: 85% *R. norvergicus*: 84%	**Atg2A** **27%**	Required for AP localization [72, 73–75]
		Atg2B	Required for AP localization [72, 73–75]
	WIPI *M. musculus*: 94% *R. norvergicus*: 94%	**SpAtg18a** **34%**	In *Sz. pombe* is a protein required for the targeting of Atg12-Atg5-Atg16 complex [72, 73–75]
		SpAtg18b	

	Components		Features
		29%	
		SpAtg18c	
		25%	
	Atg9	**Atg9**	Transmembrane protein that might carry membrane
	M. musculus: 98%	**29%**	expansion of phagophore [72, 73–75]
	R. norvergicus: 98%		

*% of identity respect to Atg *Homo sapiens* proteins, obtained using Blast http://blast.ncbi.nlm.nih.gov/Blast.cgi

Table 2. Autophagic machinery in mammals and *Sz. pombe.*

Finally, Atg12-Atg5 conjugates with Atg16L1 forming a tetramer; this tetramer is essential for the elongation but when AP has been completed it dissociates [76–78]. The second ubiquitin-like complex is the microtubule-associated protein 1 light chain 3 (LC3) conjugation system (Table 2). LC3 is a precursor of LC3-I, it is obtained by the cleavage of the protease Atg4B to its carboxyl terminus. LC3-I is attached to phosphatidylethanolamine (PE) by the enzymes E1 and E2- ubiquitin-like Atg7 and Atg3, respectively, obtaining the LC3-II product which forms part of the AP. Notice that the LC3 complex lacks an E3 ubiquitin ligase-like enzyme that could facilitate LC3I-PE conjugation; however, a crosstalk between these two ubiquitin-like systems has been demonstrated by the tetramer Atg12-Atg5-Atg16L1 acting as E3-like enzyme. When AP is formed, LC3 can be recycled from the form LC3II; this is achieved through Atg4 which breaks apart LC3I from PE, and LC3-I and LC3-II assays are widely used for monitoring autophagy [60, 77].

AP moves bidirectionally along microtubules towards the microtubule organizing centre (MTOC) where lysosomes are enriched, then AP fuses with lysosomes forming autolysosomes where the content is degraded and the components are recycled [50]. Atg2 is required for the AP location and can possess a different role when binding to Atg18 in order to target the complex to autophagic membranes [79]. In *Sz. pombe*, depletion of nitrogen from the culture medium triggers autophagy. Unlike mammals, the addition of AMPK does not induce autophagy, nor can rapamycin induce it. It has been reported that rapamycin has a partially inhibitory effect on TOR activity, moreover, the addition of rapamycin plus caffeine prompts a starvation-specific gene expression pattern and autophagy [80–81]. Atg 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 18a, 18b, 18c, Vps34 homologues have been found (Table 2) [73–75], and their deletion inhibits AP formation.

4.4. Apoptosis

The maintenance of homeostasis in pluricellular and unicellular organisms is achieved through lots of mechanisms, one of the most important of which is related to the death of the cell itself. In this way, cell death regulates the number of cells in a tissue or a colony, and the removal of damaged cells. In 1963, Lockshin introduced the term Programmed Cell Death (PCD) in order

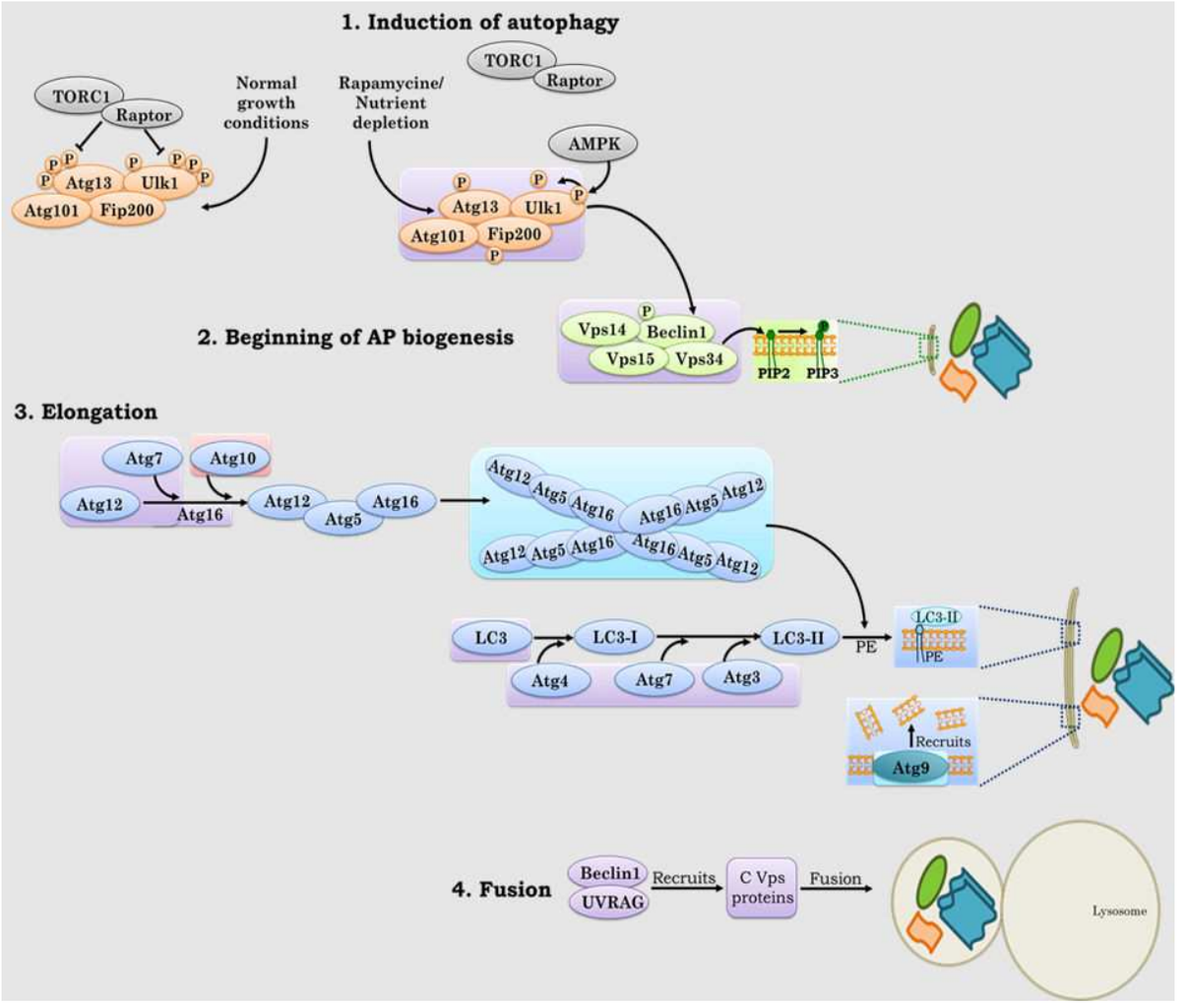

Figure 3. Mechanism of the core Atg proteins in autophagy. Atg proteins with a purple rectangular background have been identified in *Sz. pombe*, the ones in blue rectangles have not yet been described, and the ones in a red rectangle have been identified but have no role in autophagy.

to define the programmed and controlled self-destruction process in a local and temporal way [83–84]. There are many ways for a cell to die; one of them is via apoptosis. The term apoptosis was introduced by Kerr and colleagues in 1972, which is defined as a highly coordinated cellular suicide programme controlled principally by zymogens (i.e., caspases and metacaspases), proteins of the Bcl-2 family, and mitochondria [85]. The Nomenclature Committee on Cell Death established the morphological and biochemical changes in the apoptotic cell, such as membrane blebbing, cell shrinkage (pyknosis), chromatin condensation plus fragmentation (karyorrhexis), formation of membrane bound cell fragments (apoptotic bodies), decrease in mitochondrial inner transmembrane potential, selective cleavage of various cellular proteins, and the translocation of phosphatidylserine from the inner to the outer leaflet of the plasma membrane [86].

The importance of Bcl-2 proteins relies on Cyt C, which in basal conditions works at the respiratory chain but plays a different role in the cytoplasmic space: Cyt C oligomerizes with

http://dx.doi.org/10.5772/60720

the adaptor protein Apoptosis protease activating factor-1 (Apaf-1), forming the complex known as apoptosome [98, 99]. The apoptosome activates by proteolytic cleavage to caspase-9 which is one of the cascade cysteine-aspartate proteases (caspases); in other words, caspases are liberated from their inhibitory prodomain [98, 99]. Once caspase-9 is activated, the downstream zymogens procaspase 3/7 are activated as caspase 3/7, then they attach to specific substrates in the cell leading to cell dismantling but they must first be released from their endogenous inhibitor X-linked inhibitor of apoptosis (XIAP). XIAP activity is inhibited by SMAC/ DIABLO and HtrA2/Omi, which should be remembered as mitochondrial proteins that were released along Cyt C [94, 95, 100]. In *C. elegans* the mitochondrial permeabilization and the release of Cyt C have no role in CED-3 activation, unlike its mammalian counterparts. Another difference between the nematode and mammals is that CED-4 lacks a Cyt C interaction motif, this means that some of the apoptotic machinery are conserved among them. However, some protein-protein interactions differ from each other.

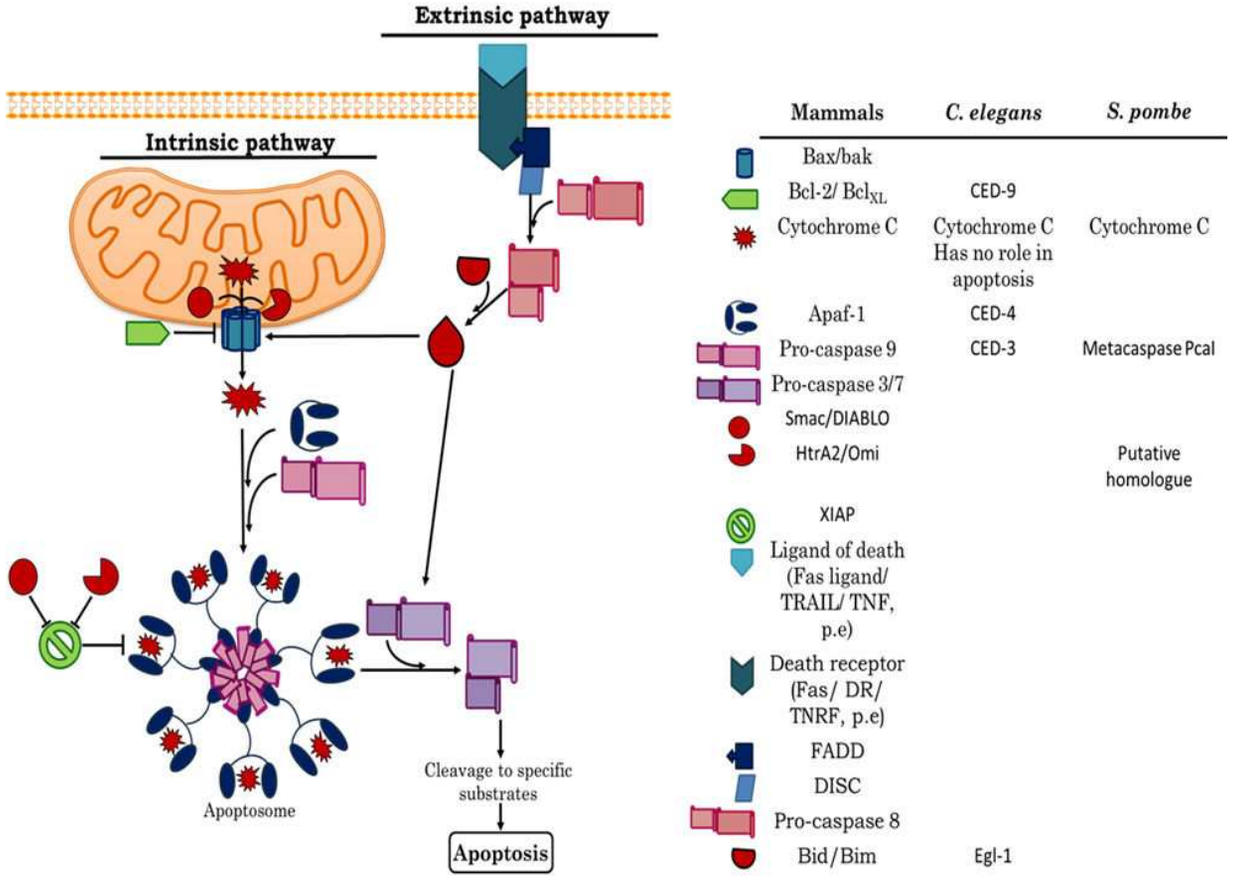

Figure 4. Apoptosis in eukaryotic cells. The factors involved in the basic molecular machinery executing cell death are conserved in yeast, including the yeast caspase YCA1. Apoptosis in yeast is composed of molecules shared with other model systems. This figure shows the intrinsic and extrinsic pathways as well as the components from the *C. elegans*, mammalians, and *Sz. pombe*.

On the other hand, the extrinsic pathway start when death ligands such as tumour necrosis factor (TNF), Fas ligand (FasL), TNF-related apoptosis-inducing ligand (TRAIL), among

others, join and activate their respective transmembrane death receptor, such as Fas. The interaction of death ligands with death receptors result in the recruitment of adaptor proteins like the Fas-associated death domain protein (FADD), which recruits, aggregates, and promotes procaspase-8 activation. Caspase-8 switch on procaspase 3/7 and the Bid protein through is proteolysis. At this point, a crosstalk between the extrinsic and intrinsic pathways are mediated by Bid activation since truncated Bid (tBid) promotes Cyt-C by interacting with Bax, leading to Bax insertion in the mitochondrial membrane as an oligomer pore [93–95].

Study of apoptosis is difficult in higher eukaryotes due to the complexity of the phenomena itself, which is why it was thought until recently that unicellular organisms could not have PCD machinery because it would mean that the organism could orchestrate its suicide, so yeast were employed as purely naïve backgrounds for studying proteins' interaction. In 1997, B. Ink and JM. Jurgensmeier independently performed a yeast two-hybrid system in *Sz. pombe* to study the proteins of the apoptotic cascade. It was found that the expression of the proteins Bax and Bak lead to the induction of chromatin condensation and DNA cleavage [101–102]. Moreover, the lethal phenotype was obliterated by the co-expression of the antiapoptic Bcl-$_{XL}$ and Bcl-2, therefore, yeasts undergo mammalian-like apoptosis. Likewise, C. James proved that CED-4 expression in the fission yeast induced chromatin condensation and it was abolished by CED-9 interaction [103]. Induction of a yeast apoptotic programme can be triggered by exogenous and intrinsic stresses, such as low doses of H_2O_2, acetic acid, valproic acid, HCl, and NaOH in the media, cell ageing, and temperature stress [104–107]; these stimuli induce ROS that also enable an apoptosis programme. Additionally, DNA damage-responsive proteins are highly conserved among fission yeast and metazoans; hence, defects in DNA replication promote high levels of ROS, promoting apoptosis. Interestingly, SpRad9 — the orthologue of the human Rad9 — controls cell cycle checkpoints, and also possess a BH3 domain which enables binding with Bcl-2 and Bcl-XL in mammal cells, prompting cell death. This proves that the Bcl-2 family of proteins may be evolutionarily conserved from fission yeast to mammals [108–112].

Caspases are the main components of the apoptotic pathway, and are not found in *Sz. Pombe*; however, it possess an orthologue in the metacaspase PcaI (Figure 4). Unlike mammalian caspase activation, PcaI undergoes auto proteolytic cleavage activation just as in metazoan caspases; however, until now there has been no evidence of any substrate of PcaI [104, 105, 113]. Notwithstanding, overexpressing PcaI in *Sz. pombe* does not induce an apparent cell death phenotype, moreover it appears to stimulate growth. Also, PcaI expression was up-regulated by oxidative and heavy metal toxicity, heat, hyperosmotic stress and DNA damage conditions, H_2O_2, sodium nitroprusside, and CdCl, giving cytoprotection; this evidence suggests that the metacaspase could be involved in growth and stress adaptation of *Sz. pombe*. It has also been demonstrated that the level of oxidized proteins is proportional to the presence of the metacaspase, possibly due to a cleaning function within the population. Apparently, PcaI has pro-survival and pro-apoptotic roles and further investigation is needed to understand its function [105, 113, 114]. The Htra2/Omi-like protein Nma11p (Nuclear mediator of apoptosis) is found in *S. cerevisiae*, which is a serine protease, having a pro-apoptotic function, and which aggregates in the nucleus after cellular stress conditions; in *Sz. Pombe*, its putative homologue has

been found [115]. In mammals, an apoptotic marker is the release of CytC; as in yeasts, its presence in the cytoplasm is found after the induction of the apoptotic programme. Just as in mammals, the release of CytC decreases mitochondrial inner transmembrane potential, but unlike metazoans the upstream and downstream effectors of PcaI have not been elucidated. The release of CytC has been correlated with the induction of cell death in *S. cerevisiae* [116], which correlates highly with the mammalian intrinsic pathway; nevertheless, CytC release has yet to be investigated in *Sz. pombe*.

5. *Sz. pombe*: Heterologous expression systems of proteins

Fission yeast is a useful system for studying the function and regulation of genes, and an excellent host for heterologous expression of molecules derived particularly from higher eukaryotes because it produces high cell densities, short fermentation times, and the use of chemically defined media [117]. Difficulties in the production and purification of heterologous proteins are related mainly to proteolytic degradation of gene products by specific proteases of the host [118]. This makes it highly important to obtain strains that have specific mutations eliminating the activity of proteases. Success of this expression is based on the use of genome-reduced strains with the deletion of unnecessary genes. Gene-deletion technology used for this purpose is the LATOUR (latency to universal rescue) method, which eliminates a DNA segment of more than 100 kb [119, 120]. This strategy of gene deletion is useful for identifying genes that are essential to the yeast [121]. The *Sz. pombe* proteolytic system has been seldom studied. Currently, two proteolytic systems have been identified with different subcellular localizations: a vacuolar one (where can be found the Proteinase yspA, Proteinase yspB, Carboxipeptidasa Y1p, Aminopeptidase yspI, Dipeptidyl aminopeptidase DPK, and DPC) and another Cytosolic level (Leucin Aminopeptidase yspII, peptidase multicorn, and proteasome 26S) [122, 123]. From full-genome sequence data which are deposited in the MEROPS database, an output of 121 peptidases and probable peptidases, being non-peptidase homologues 39 proteases, has been identified [124].

6. Conclusions

This chapter discussed some topics related to the employment of *Sz. pombe* as a model system for the study of the cellular processes of eukaryotes. Despite the complexity of mammalian cells with respect to single-celled yeasts, many intracellular processes are highly conserved, such as cell cycle, autophagy, and ageing; there are even similarities at the level of the mechanisms of the processes carried out in the cell. In some cases, there is only the sharing of some genes, as in the apoptosis process. However, their genetic regulation could be similar in triggering the specific cellular responses. The use of yeast *Sz. pombe* as a model of study allows the generation of new knowledge with direct application to mammalian cells such as human ones.

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