DNA repair and mutations during quiescence in yeast

Serge Gangloff, Benoît Arcangioli

DNA REPAIR AND MUTATIONS DURING QUIESCENCE IN YEAST

Serge Gangloff * and Benoit Arcangioli

Genome Dynamics Unit - Department of Genomes & Genetics - Institut Pasteur

UMR 3525 CNRS – Institut Pasteur, Paris, France

serge.gangloff@pasteur.fr

One sentence summary: Quiescence unveils a novel mutational force

*: correspondence: serge.gangloff@pasteur.fr

ABSTRACT

Life is maintained through alternating phases of cell division and quiescence. The causes and consequences of spontaneous mutations have been extensively explored in proliferating cells, and the major sources include errors of DNA replication and DNA repair. The foremost consequences are genetic variations within a cell population that can lead to heritable diseases and drive evolution. While most of our knowledge on DNA damage response and repair has been gained through cells actively dividing, it remains essential to also understand how DNA damage is metabolized in cells which are not dividing. In this review, we summarize the current knowledge concerning the type of lesions that arise in non-dividing budding and fission yeast cells, as well as the pathways used to repair them. We discuss the contribution of these models to our current understanding of age-related pathologies.

INTRODUCTION

Age-related diseases including cancer and neurodegeneration are the major threats to human health in developed countries and represent a challenge for individual patient care and public health systems. Somatic mutations cause not only cancer but also abnormalities in the brain and recent findings indicate that a range of age-related diseases is linked to defective DNA repair pathways (Garinis *et al.* 2008; Burtner and Kennedy 2010). Genetic instability has been implicated in several neurodegenerative disorders, including amyotrophic lateral sclerosis (Julien 2001) and ataxia (Lim *et al.* 2006), and it has been proposed that the highly metabolically active neurons are particularly sensitive to DNA damage and that their post-mitotic lifespan relies on specific DNA surveillance and repair systems. Therefore, the stability and homeostasis of the genetic material in post-mitotic tissues may play a greater role than anticipated, and raises the question of how DNA damage is repaired in non-dividing cells.

All organisms exist in two cell states: proliferation, whereby the cell number increases by division, and quiescence, which sustains life during the non-dividing phase. The majority of the cells in human body tissues and organs are non-dividing**,** so quiescence is a common life form for the cell. However, this latter state contains two separate conditions depending upon their capacity to re-enter the cell cycle. For example, the yeast model systems and stem cells sustain a reversible quiescent state and can resume proliferation, whereas post-mitotic cells are metabolically active, but irreversibly arrested.

In addition to being the most common cell state on earth (Lewis and Gattie 1991), quiescence (or G0) plays a critical role in normal metazoan development and disease (Kim *et al.* 2005; Suda, Arai and Hirao 2005; Abbott 2006). In complex eukaryotes, quiescence is essential for stem-cell maintenance (Suda, Arai and Hirao 2005) as well as for activating cells for wound healing (Chang *et al.* 2002) and sexual reproduction, and this is particularly important for female gametes. Both wound healing and sexual reproduction requires regulated exit from G0 (Gouge *et al.* 1998). Quiescent cells are directly involved in the establishment and persistence of microbial infectious diseases such as tuberculosis (Parrish, Dick and Bishai 1998), cryptosporosis (Alexander and Perfect 1997) and anthracis (Murray 1999; Gray *et al.* 2004). It is also important in the environment, since the vast majority of all microbes exist in an unculturable, quiescent state (Kaeberlein, Lewis and Epstein 2002). Fortunately, several recent developments, including the ability to use genome-scale tools to understand this state, have facilitated studies into the quiescent state, especially in yeast (Allen *et al.* 2006; Yang, Ren and Zhang 2006).

Nonetheless, genetic studies of post-mitotic cells face several complications: with the exception of germ and stem cells, post-mitotic cells do not generate descendants and thus do not transmit their genetic traits. Despite the ongoing progress of DNA sequencing technologies (Kalisky, Blainey and Quake 2011), mutations in individual post-mitotic cells remain difficult to identify. Indeed, in response to excessive DNA damage or mutations, post-mitotic cells activate a cell death program that reduces the window for molecular genetic studies (Behrens *et al.* 2014). Control of cell proliferation and quiescence remains a central problem in biology. The switches from proliferation to quiescence and vice versa have a broad range of implications for development, differentiation, cancer and longevity. To understand these important biological functions better, the mechanisms of quiescence and particularly those pertaining to the repair capabilities of the quiescent cells must be studied. Indeed, cells must have evolved strategies to protect and neutralize physical and chemical

attacks as well as DNA repair mechanisms to prevent the accumulation of DNA damage responsible for mutations and cell death, allowing them to survive through division or arrest.

In this review, we will examine our current knowledge concerning the repair mechanisms at work in non-dividing yeast cells as well as the consequence of a natural time-related mutagenesis. We will focus on budding and fission yeast chronological lifespan (CLS) that measures the reproductive capacity of the cells as a function of time (Fabrizio and Longo 2003; Kaeberlein 2010) and highlight the approaches taken in each of these model systems.

QUIESCENCE INDUCTION IN YEAST MODELS

Budding and fission yeasts have been excellent model organisms for studying cell proliferation. The pioneering studies of the genetic control of the cell division cycle in *Saccharomyces cerevisiae* by Hartwell (Hartwell *et al.* 1973) and in *Schizosaccharomyces pombe* by Nurse (Nurse 1975) testify to it. These two yeasts are evolutionarily distant and the features found in both species are often also found in human cells. A similar assumption can be made for quiescence as well, for which a striking conservation of the regulatory strategies used to exit the cell cycle and enter a reversible quiescent state has been observed (Miles and Breeden 2016). Although the cause for quiescence might greatly vary between yeast and human, the underlying mechanisms for quiescence require the same set of genes and similar mechanisms. Quiescence in multicellular organisms is difficult to study because of the multiple environmental signals that control it. By contrast, in budding and fission yeast, quiescence entry and exit are solely conditioned by nutrient availability (Figure 1). The quiescence entry program relies greatly on the information transmitted by two nutrient signaling pathways. These are the Target Of Rapamycin Complex 1 (TORC1) pathway, regulated by the available carbon and/or nitrogen source, and the glucose-responsive protein kinase A (PKA) pathway (Gray *et al.* 2004; De Virgilio 2012; Bontron *et al.* 2013). The target of rapamycin complex (TORC) is important for the transition between proliferation and quiescence from yeast to humans. Genes controlling quiescence are conserved from yeast to humans, and support the use of yeast model systems to study the mechanisms of diseases affecting human dormant cells.

Severe reduction of the nutritional conditions in the environment trigger microbial quiescence to adapt the cells for survival during stress. During this stage, cells stop growing and acquire a distinct array of physiological, biochemical, and morphological traits that confer on cells both the ability to survive extended periods of starvation and to transit back to the proliferating state upon feeding (Lillie and Pringle 1980).

Most *S. cerevisiae* stationary phase cells are produced in limited glucose conditions. Cell growth is transiently arrested and the cells switch to a respiratory mode of energy production at the 'diauxic shift' (Herman 2002). The cellular responses initiated at the diauxic transition include the transcriptional induction of genes whose products are involved in respiration, fatty acid metabolism, and glyoxylate cycle reactions, and, likely as a consequence of turning on respiratory activity, of genes encoding antioxidant defenses that allow scavenging and/or the destruction of reactive oxygen species (ROS) (Jamieson 1998; Costa and Moradas-Ferreira 2001). In the post-diauxic growth period, only non-fermentable carbon sources are available to support growth and cells slowly grow and utilize the ethanol produced during an earlier stage of culture fermentation. Finally, when this ethanol is exhausted, the cells enter the stationary phase where cell proliferation stops (Werner-Washburne *et al.* 1993; Gray *et al.* 2004). However, the majority of early studies have assumed that cultures in stationary phase are homogeneously composed of a single cell type, and it was shown that a significant fraction of cells begin to differentiate into quiescent and non-quiescent cell fractions that can be separated by centrifugation on Percoll gradients (Allen *et al.* 2006; Werner-Washburne, Roy and Davidson 2012). Quiescent cells contain sequestered mRNAs that are released into the cytoplasm in a stress-specific manner, survive for up to three weeks, and retain a high level of reproductive competence, genome stability, and low reactive oxygen species (ROS). Additionally, the vast majority of these cells are daughter cells that have never budded (Aragon *et al.* 2006; 2008). Thus, the structure and heterogeneity of the stationary phase cells have important implications on a variety of levels for aging studies, especially studies of CLS.

In *S. pombe*, the diauxic shift does not occur because *S. pombe* lacks the glyoxylate cycle enzymes required for the utilization of ethanol for growth after glucose depletion (Heslot, Goffeau and Louis 1970; de Jong-Gubbels, van Dijken and Pronk 1996; Flores *et al.* 2000). *S. pombe* cells enter the stationary phase with a 2C DNA content and lose viability rapidly (Costello, Rodgers and Beach 1986). In this regard, one must be careful when comparing stationary phase cell results from both yeasts before extending the comparisons to higher eukaryotes.

In the wild, nitrogen removal triggers meiosis in yeast. However, if the nitrogen source is removed from the medium of heterothallic cells, budding (Figure 1A) and fission yeast cells (Figure 1B) require autophagy to enter quiescence (Takeda and Yanagida 2010; An *et al.* 2014). *S. pombe* divides rapidly twice with no cellular elongation, becomes short and spherical to enter in G1 with a 1C DNA content before reaching the quiescent state (Baker *et*

al. 1976; Nurse and Bissett 1981; Egel 1989) (Figure 1B). Cells, upon nitrogen removal, immediately repress the growth-related mRNAs while transiently inducing stress-related mRNAs, which help to adjust the proteome for extended quiescence (Marguerat *et al.* 2012). After several days of nitrogen starvation, cells acquire heat resistance similar to that observed in spores, but differ from spores in their requirement of glucose for viability (Egel 1989). In this mode of quiescence entry, the cells are viable for months provided the medium is exchanged for fresh medium every other week, metabolically active, exhibit stress-responsive signaling and are highly efficient in DNA damage repair (Mochida and Yanagida 2006). Also, they contain transcripts that are distinct from proliferating cells and the cells greatly alter their constituents upon the replenishment of the nitrogen source to restore proliferation (Shimanuki *et al.* 2007). In these quiescent cells, protein translation is diminished but sugar catabolism, protein and nucleic acid recycling mechanisms, trafficking, and vesicle fusion is highly active (Yanagida 2009). TORC1 promotes protein translation and ribosome biogenesis and is required for the exit from quiescence, suggesting that diminished protein biosynthesis is important for maintaining the G0 phase (Shimanuki *et al.* 2007).

The major advantage of inducing quiescence of prototrophic cells by removing nitrogen is that it is a synchronized process working on low density cultures that survive for a few months in a controlled medium. Indeed, prototrophy has surfaced as an important factor when comparing transcriptome analyses since it has recently been shown that deletions of the same metabolic gene in a different background, even when supplemented, could provoke profoundly altered transcriptomic, proteomic and metabolic profiles (Jacquier 2016; Alam *et al.* 2016). Thus, quiescence of *S. pombe* is precisely defined under a simple nutritional change so that results can be interpreted and scrutinized rigorously.

MAJOR SOURCES OF DNA DAMAGE

DNA damage can originate in extrinsic and intrinsic sources (Gensler and Bernstein 1981; Lombard *et al.* 2005; Hoeijmakers 2009). Extrinsic sources include chemicals, radiation, and viruses. Intrinsic sources include spontaneous chemical reactions and reactive oxygen species (ROS). Endogenous causes of DNA damage, “the enemy from the inside” are the major threats reducing CLS (Gensler and Bernstein 1981) (Figure 2).

Reactive Oxygen Species

A common cause of damage to DNA and other macromolecules in non-dividing cells is exposure to ROS, which include superoxide, hydrogen peroxide, hydroxyl radicals and singlet

oxygen. In DNA, oxidized bases, abasic sites and single- and double-strand breaks result from exposure to ROS (Jackson and Loeb 2001; De Bont and van Larebeke 2004). Organisms cope with ROS through antioxidant enzymes that eliminate ROS or convert them to less harmful molecules. However, the production of ROS can overpower those defense mechanisms, resulting in oxidative stress (reviewed in Friedberg *et al.* 2005). ROS can produce many different kinds of damage and mutations in DNA. For instance, the cytosine base alone can undergo oxidative damage producing at least 40 different modified species (Jackson and Loeb 2001). Some oxidized bases are mispaired and lead to base substitutions when DNA replicates during the exit of quiescence. Several studies have indicated that damage arising from mitochondrial production of reactive oxygen species contributes to the chronological ageing process (Burhans and Weinberger 2009). Consistent with this view, oxidative damage to proteins and mitochondria accumulates with chronological age (Fabrizio and Longo 2007; Rockenfeller and Madeo 2008).

Cyclobutane Pyrimidine dimers (CPDs)

Among the causes of extrinsic damage to quiescent yeast cells, UV light triggers the formation of CPDs that is characterized by covalent linkages between adjacent pyrimidines in the same DNA strand. It is the most frequent type of photoproduct produced when DNA is exposed to UV irradiation. The type of CPD most frequently found in DNA consists of a thymine dimer, which is known to be mutagenic (Friedberg *et al.* 2005).

DNA strand breaks

DNA strand breaks can be caused by intrinsic oxidative damage to DNA or by extrinsic ionizing radiation (Friedberg *et al.* 2005). Strand breaks can occur during metabolic processes like transcription relaxation by topoisomerase I or endonucleolytic cleavage. Interestingly, the disruption of pathways involved in single-strand break repair often results in neurodegeneration rather than carcinogenesis or progeria. Because ROS are one of the major causes of single-strand breaks, it is possible that the oxygen consumption in the nervous system makes it more susceptible to defects in single-strand break repair, thus contributing to neurological decline. In support of this view, the number of single- and double- strand breaks in the neurons of rat cerebral cortex is considerably increased with age (Rao 2007; Wei *et al.* 2016).

Abasic (AP) sites, depurination and depyrimidination

An abasic site (AP) site is formed when a base is lost from the DNA by cleavage of an N-

glycosyl bond, leaving the sugar-phosphate chain intact (Friedberg *et al.* 2005). At normal physiological conditions, it has been estimated that 50,000–200,000 AP site lesions persist at a steady-state level in mammalian cells (Nakamura and Swenberg 1999). Abasic sites are potentially mutagenic and can be produced by a spontaneous loss of bases from DNA. Abasic sites can also be produced by ROS (Nakamura and Swenberg 1999), as well as being produced in intermediate steps of the base excision repair pathway. Inefficient or incomplete base excision repair might leave abasic sites in DNA.

Deamination

Deamination involves the loss of amino groups from DNA bases. Almost all DNA bases undergo deamination in spontaneous reactions, with the exception of thymine – which does not have an amino group. Most types of deamination produce a base that does not naturally occur in DNA (the only exception is the deamination of 5-methylcytosine), and this facilitates the identification and excision of the deaminated base by a DNA glycosylase.

The most common type of deamination event in cells is deamination of cytosine into uracil, which leads to the transformation of G:C to G:U that is potentially mutagenic. This event occurs at a rate of about 100–500 bases per cell per day in mammalian cells, in spontaneous deamination reactions (Friedberg *et al.* 2005). Interestingly, one possible reason why the genetic code, which is thought to have been initially carried in RNA bases (A, C, G, U), was replaced by the current code carried in DNA bases is so a deaminated C converted to a U can be easily recognized as damage (Alberts *et al.* 2007).

DNA can also contain 5-methylcytosine, which base pairs with guanine and is involved in silencing gene expression at CpG sequences. The deamination of 5-methylcytosine into thymine leads to the transformation of a G:C to G:T base pair, which is also potentially mutagenic. Interestingly, although only about 3% of the C bases in human DNA are methylated, G:C to A:T transitions at the sites of those methylated cytosines account for about one-third of the single-base mutations in inherited human diseases (Cooper and Youssoufian 1988; Alberts *et al.* 2007). Note that cytosine or methyl-cytosine deamination leads to C:G to T:A mutations as well.

As quiescent cells are metabolically and transcriptionally active, transcription is most likely an important contributor to the accumulation of DNA damage in the absence of replication (Kim and Jinks-Robertson 2012). During transcription, the non-templated strand is exposed, and cytosines in the context of ssDNA have >200x higher kinetic of deamination (Lindahl

1993).

IMPORTANCE OF DNA REPAIR PATHWAYS IN QUIESCENCE

Overview

When the cells arrest cell division and become quiescent, repair of the damaged components becomes crucial to maintain viability. This is particularly true for post-mitotic neurons that are metabolically and transcriptionally active, consuming a large proportion of oxygen and glucose, thus generating high levels of reactive oxygen species (ROS). In 1956 Harman proposed that oxygen species with one unpaired electron (free radicals) may cause aging (Harman 1956). The free radical theory of aging became one of the most widely accepted theories after the overexpression of antioxidant enzymes was shown to extend longevity and after most long-lived model organisms were shown to be resistant to oxidative stress (Longo 1999; Finkel and Holbrook 2000).

In complex organisms, the activity of some DNA repair pathways was found to decline with age (Freitas and de Magalhães 2011), but it has not be formally demonstrated that enhanced DNA repair extends lifespan although the evidence from many lines of investigation suggests that DNA damage is a significant causal factor in normal aging (Burhans and Weinberger 2012). In non-dividing cells, DNA repair machinery is continuously required to maintain the genetic message intact and functional, and accumulating evidence supports the idea that DNA damage, pathological DNA or protein-RNA/DNA structures or defects in DNA damage response or repair can lead to recurrent genomic disorders (Garinis *et al.* 2008). Accumulation of proteins damaged by oxidation with chronological age has also been observed in both fission and budding yeasts.

This situation is best illustrated in recessive ataxia caused by mutations in genes involved in DNA repair, such as *mre11* (**A**taxia **T**elangiectasia-**L**ike **D**isorder - ATLD), *nbs1* (**N**ijmegen **B**reakage **S**yndrome - NBS1), *tdp1* (**S**pino**C**erebellar **A**taxia with **N**europathy **1** - SCAN1), *aptx* (**A**taxia with **O**culomotor **A**praxia **1** - AOA1), *senx* (**A**myotrophic **L**ateral **S**clerosis **4** – ALS4, **A**taxia with **O**culomotor **A**praxia **2** – AOA2) and *ighmbp2* (**S**pinal **M**uscular **A**trophy with Respiratory **D**istress **1** – SMARD1) (overviewed in Jayadev and Bird 2013). Mre11 and Nbs1 are members of the MRN complex participating to the DNA damage repair/response and telomere maintenance (Williams, Hetrick and Foster 2010). Human tyrosyl DNA phosphodiesterase (TDP1) repairs Topoisomerase1-DNA covalent complexes that can be induced by replication- and transcription-mediated DNA damage (Caldecott 2008). Aptx

(aprataxin) removes AMP from 5'-termini and is implicated in short-patch repair of oxidative single-strand breaks (Ahel *et al.* 2006). Senx (Senataxin) is a RNA/DNA helicase that has been implicated in transcription termination of Pol II transcription at snoRNAs and other noncoding RNAs in yeast (Kim *et al.* 2006; Arigo *et al.* 2006). Recently, budding yeast Sen1 has been shown to restrict the occurrence of RNA/DNA hybrids that may naturally form during transcription, when nascent RNA hybridizes to DNA prior to its packaging into RNA protein complexes (Mischo *et al.* 2011). These hybrids displace the non-transcribed strand and create R—loop structures whose accumulation can be prevented by Sen1. Finally, the Ighmbp2 helicase shares significant similarity with Senataxin, which is mutated in human SMARD1 and in mouse neuromuscular degeneration (Cox, Mahaffey and Frankel 1998). The activities of these genes support the idea that defects in transcription-related DNA lesions and single-strand breaks may play a role in the generation of ataxia and motor neuron diseases (El-Khamisy and Caldecott 2006). This hypothesis is consistent with the fact that post-mitotic neurons are facing high levels of oxidative stress and transcriptional activity (Caldecott 2008). Taken together, these data suggest that DNA repair pathways are essential for maintenance of the neurons by protecting these metabolically and transcriptionally highly active non-proliferating cells against free radical-induced DNA damage and genomic instability (Figure 3).

ROS detoxification and Base Excision Repair (BER)

Endogenous ROS are metabolic byproducts disseminated from many subcellular compartments, in particular during respiration and incorporation of uracil (Arcangioli and Ben Hassine 2009). The ROS produced in the mitochondria represent the predominant group of chemicals attacking DNA. Endogenous and exogenous DNA alterations may cause simple base changes or more complex and clustered lesions (Sage and Harrison 2011). The complex DNA lesions are more difficult to repair and can spontaneously or enzymatically lead to single or double strand breaks that can generate point mutations, deletions, duplications, translocations, aneuploidy or cellular death.

Studies have suggested that DNA damage or genomic instability may directly cause neuronal degeneration. Mutations in superoxide dismutase 1 (SOD1), a major cytoplasmic antioxidant enzyme that metabolizes superoxide radicals to molecular oxygen and hydrogen peroxide, account for 10 to 20% of all familial forms of ALS (Cleveland and Rothstein 2001). Expansion of CAG triplet repeats in the androgen receptor gene is responsible for **S**pinal and **B**ulbar **M**uscular **A**trophy (SBMA) (La Spada *et al.* 1991) and expansion of hexanucleotide

GGGGCC repeats in the C9ORF72 gene for some forms of ALS and **F**ronto-**T**emporal **D**ementia (FTD) (Renton *et al.* 2011; DeJesus-Hernandez *et al.* 2011). Finally, expansion of unstable triplet repeats (Friedreich ataxia (GAA), X fragile (CGG) and Huntington (CAG)) and deficits in DNA repair can cause related neurodegenerative disorders (Rass, Ahel and West 2007; Orr and Zoghbi 2007). The instability of the repeats reflects their unfaithful repair, generating a persistent signal to the DNA damage checkpoint. Unstable repeats in quiescence are to some extent similar to the lesions persisting in the absence of the dedicated DNA repair machinery, a field that is still not yet actively studied.

Like mammalian cells, *S. cerevisiae* expresses a cytosolic (Sod1) and a mitochondrial (Sod2) superoxide dismutase that are required for long-term survival (Longo, Gralla and Valentine 1996). The expression of human SOD1 in yeast *sod1* null mutants completely reverses the survival defects, suggesting that the function of this enzyme is conserved from yeast to mammals, although Sod1 is only required under a high concentration of oxygen or superoxide (Fabrizio and Longo 2003). Superoxide toxicity thus contributes to aging and death in model organisms.

BER is the major pathway for the repair of damaged bases (Wallace 1998). As a first step, oxidized bases are removed by specialized glycosylases, leaving behind abasic sites (AP apurinic/apyrimidinic). However, AP sites can occur spontaneously after hydrolytic attack of the N-glycosidic bond between base and sugar (Çağlayan and Wilson 2015). AP sites are a major threat to genomes because of their abundance and capacity to block DNA replication and transcription and to cause mutations (Boiteux and Guillet 2004). The subsequent step is the cleaving of the DNA backbone by an AP-endonuclease (or alternatively by an AP lyase), resulting in the formation of a single strand break (SSB). BER proceeds by a removal of the baseless DNA backbone residue and a gap-filling DNA synthesis of one or a few nucleotides. After 8 days of lysine-depletion induced quiescence in budding yeast, the amount of spontaneous AP sites in the cells was found increased about tenfold compared to the exponential cells (Steinboeck *et al.* 2010). Similarly, AP endonuclease function was involved in the optimal survival of a mixed population of stationary cells. However, overexpression of AP endonucleases does not extend this survival (Maclean *et al.* 2003). Increased concentrations of ROS cause oxidative damage to DNA (reviewed in Cooke *et al.* 2003). Depending on the dose, cycling yeast cells display a wide range of responses to ROS (Temple, Perrone and Dawes 2005), from adaptation to low doses (Jamieson 1992; Collinson and Dawes 1992) up to apoptotic death (Laun *et al.* 2001; Fabrizio *et al.* 2004). For the case

of lysine starvation-induced cell-cycle arrest, only the accumulation of hydrogen peroxide has been reported so far (Eisler, Fröhlich and Heidenreich 2004). Such cytotoxic DNA lesions may stimulate local DNA processing or turnover in non-replicating cells, thereby providing the chance for potentially error-prone repair or translesion synthesis. Some evidence for the latter processes has been found (Heidenreich *et al.* 2003; Heidenreich and Eisler 2004; Heidenreich, Holzmann and Eisler 2004; Heidenreich, Eisler and Steinboeck 2006; Halas *et al.* 2009; Heidenreich *et al.* 2010).

Single-strand break repair and checkpoint response

In fission yeast, Topoisomerase I (Top1) introduces transient SSBs to relax the DNA during DNA replication and transcription. If a DNA polymerase or RNA polymerase encounters the DNA–Top I intermediate complex before DNA ligation, then Top1 remains covalently trapped to the 3' ends of the DNA breaks (Pommier *et al.* 2003). Tyrosyl-DNA phosphodiesterase 1 (TDP1) is a well-conserved SSB repair enzyme (Pouliot *et al.* 1999) hydrolyzing a variety of 3' lesions including the phosphodiester bond linking Top1 to the 3'-end of DNA ends (Yang *et al.* 1996; Interthal, Pouliot and Champoux 2001) leaving ends which are further processed (Vance and Wilson 2001). As a consequence, eukaryotic cells mutated in *tdp1* are sensitive to the anticancer drug camptothecin (CPT), which specifically traps Top1 on DNA (Pouliot *et al.* 1999; El-Khamisy *et al.* 2005; Miao *et al.* 2006). Current models propose that physiological Top1 or ROS- induced SSB is responsible for neuronal dysfunction in the absence of the DNA end processing enzyme, Tdp1, but there is as yet no conclusive evidence for this hypothesis (Rass, Ahel and West 2007; El-Khamisy, Hartsuiker and Caldecott 2007).

The contribution of Tdp1 to genome stability in non-dividing has been investigated in *S. pombe* cells where it was shown that *tdp1* mutant cells progressively accumulate unrepaired oxidative DNA damage, which ultimately induces an ATM/Tel1-dependent apoptotic-like program in G0 (Ben Hassine and Arcangioli 2009). Indeed, quiescent fission yeast compromised for DNA repair exhibits a DNA-damage checkpoint response (Tel1 and Rad3) leading to nuclear DNA degradation and cell death (Arcangioli and Ben Hassine 2009). Among the cells that do not form colonies, about one-half were enlarged, indicating an attempt to re-enter into the vegetative cycle, whereas the other half did not change their appearance, suggesting that they died during the G0 period. DAPI staining of the nuclei revealed that DAPI-negative cells accumulate in the *tdp1* population and FACS analysis

revealed a new population of propidium iodide-negative *tdp1* cells that reaches 21% after 8 days in G0. This loss in viability does not depend on the function of Top 1. Analysis of the re-entry into the vegetative cycle through the microscopic observation of Rad52-YFP foci (Lisby, Rothstein and Mortensen 2001) as a function of time indicated that *tdp1* mutants exhibit a progressive and dramatic increase in the proportion of nuclei containing Rad22–YFP foci during the first DNA replication that fades during the second DNA replication. This indicates that the mutant cells accumulate DNA damage in G0, that is massively repaired during the first round of replication. The involvement of the HR process in DNA repair in the *tdp1* mutant was confirmed by the rapid and extensive death of the *tdp1 rhp51* double mutant strain. However, the lack of cancer predisposition in SCAN1 (Takashima *et al.* 2002) suggests that most of the lesions are repaired without gross genetic alterations. In addition, it was shown that the production of endogenous ROS by-products by mitochondria is a major cause for the accumulation of DNA lesions in the absence of Tdp1 (Ben Hassine and Arcangioli 2009).

Although DSBs and blocked replication forks are known to alert the DNA damage checkpoints (Bartek, Lukas and Bartkova 2007; Lavin, Gueven and Grattan-Smith 2008), little is known about whether and how simple lesions, such as SSBs, alert the DNA damage checkpoints, but it was reported in human cells that XRCC1 is phosphorylated in an ATM-dependent manner, suggesting that SSBs can also trigger checkpoint responses in G1 (Chou *et al.* 2008).

Double-strand breaks

When DSBs in cell cycle-arrested budding yeast cells were investigated by pulsed field gel electrophoresis no significant number of broken chromosomes resulting from spontaneously occurring DSBs was observed (Heidenreich, unpublished results). However, with a specific H2A antibody, a progressive accumulation of DSBs during lysine starvation-induced cell-cycle arrest was observed, a part of which is NAC (N-acetyl cysteine – a radical scavenger) sensitive suggesting that it originates from ROS-induced DNA damage. A similar increase in spontaneous DSB formation in carbon source-starved yeast cells has also been documented by Pawar et al. (Pawar *et al.* 2009). It should nonetheless be kept in mind that, although sensitive, γH2A phosphorylation can also be triggered by lesions that are not DSBs (Marti *et al.* 2006).

Repair of DSBs triggered by γ-ray irradiation was monitored by pulsed field gel

electrophoresis (PFGE) on *S. pombe* chromosomes. Restoration of chromosomes in G0 cells is 2 to 3-times slower than in growing cells. Accordingly, the γ-ray sensitivity of G0 cells was considerably more perceptive, suggesting a slower rejoining of DSBs in G0 than in vegetative cells (Mochida and Yanagida 2006).

The involvement of NHEJ was examined in cells lacking Ku80 after γ-ray irradiation. Mutant cell viability was more sharply decreased in G0 than in cycling cells. In G0 cells after γ-ray irradiation, the repair of broken DNA took 24 h. In the Δ*pku80* mutants, the bulk of broken chromosome DNA still remained unrepaired after 24 h. In fission yeast, when the UVDE endonuclease is present, UV irradiation can also cause DNA strand breaks whose repair is slower in G0 cells. These results suggest that the repair of broken DNA formed by UV or γ-ray in G0 cells depends on NHEJ. The repair of breaks in G0 and cycling cells requires the Rad3/ATR checkpoint. Interestingly, while the breaks were mostly repaired in quiescent cells they are not at all in cycling cells deleted for Crb2 or Chk1 (Mochida and Yanagida 2006).

Nucleotide excision repair (NER)

The involvement of NER was determined in budding yeast by using antibodies against thymine dimer (McCready and Cox 1993). Subsequently, this method was used in the first experiment showing that fission yeast cells do actually repair lesions in quiescence and not only during the first round of replication when they exit from G0 (Mochida and Yanagida 2006). Although slower than in cycling cells, the level of thymine dimer rapidly decreases as a function of time in non-dividing cells. Accordingly, G0 cells are much more sensitive to UV irradiation, suggesting that a damage repair process other than the elimination of thymine dimer is very inefficient in G0. Addition of nitrogen to the medium triggers re-entry into the cell cycle, and cells become resistant to UV irradiation after DNA replication (Mochida and Yanagida 2006). *S. pombe* Crb2 is required for the activation of a checkpoint kinase Chk1 and the repair of damaged DNA (Du *et al.* 2003; Nakamura *et al.* 2004; Sanders *et al.* 2004). In G0 cells, the signal for Chk1 hyper-phosphorylated by Cdc2 and Rad3 (Saka *et al.* 1997; Esashi and Yanagida 1999) after irradiation was greatly diminished, and absent in *chk1* mutants (Mochida and Yanagida 2006). Additionally, G0 cells deleted for Rad3 and Crb2 are hypersensitive to UV, and the rate of thymine dimer repair was only slightly slower in *rad3* cells, suggesting an additional role for Rad3 in G0.

RNA interference

In recent years, RNA interference has been shown to be an important contributor in DNA

repair, closely associated with HR proteins, in many organisms including humans (d'Adda di Fagagna 2014; Francia *et al.* 2016). RNA interference was shown to play an important role in long term quiescence in *S. pombe*. RNAi-guided heterochromatic silencing is a major epigenetic pathway in *S. pombe*, which has one copy of each of the key enzymes involved: Dicer (Dcr1), Argonaute (Ago1) and RNA-dependent RNA polymerase (Rdp1) (Roche, Arcangioli and Martienssen 2016). RNAi mutants are viable but lose silencing of the pericentromeric repeats (Volpe *et al.* 2002) resulting in chromosome segregation defects (Volpe *et al.* 2003). RNAi both promotes heterochromatin formation at centromeres allowing proper chromosome segregation during G0-entry, and prevents heterochromatin formation at the rDNA locus during quiescence maintenance.

In RNAi mutants, loss of viability is linked to an over-accumulation of H3K9me2 at rDNA as a consequence of a stalled RNA pol I. The rDNA locus plays a central role in aging (Ganley and Kobayashi 2014) and H3K9me is a hallmark of rDNA silencing in plants (Pontvianne *et al.* 2012) as well as in mammalian cells (Murayama *et al.* 2008). The role of RNAi at rDNA may thus be evolutionarily conserved, as it was shown that Dicer physically associates with rDNA in mammalian cells (Sinkkonen *et al.* 2010). Interestingly, there is evidence that during evolution, RNAi and heterochromatin proteins (H3K9me, HP1) have been lost together, a loss which has happened independently in distinct fungal lineages such as budding yeasts (Drinnenberg *et al.* 2009). In budding yeasts, the loss of RNAi is correlated with the ability to acquire Killer RNA viruses (Drinnenberg, Fink and Bartel 2011).

In *Cænorhabditis elegans* (Ketting *et al.* 2001) and *Drosophila melanogaster* (Jin and Xie 2007), Dicer mutants affect germ cells, which also spend long periods in quiescence. Given the importance of quiescence in the life cycle of unicellular as well as multicellular organisms, it is likely that epigenetic pathways will be found to be essential in neurons, germ cells and cancer stem cells, which can spend many years in a quiescent state.

MUTATIONS IN G0

Overview

Despite occasional reports on stationary-phase mutations in bacteria (Ryan 1955; Grigg and Stuckey 1966), it was long believed that spontaneous mutations generally arise in the course of genome duplication, either by DNA polymerase errors or by replication of damaged DNA. Intermittent times of environmentally enforced quiescence was believed to be merely resting stages, also regarding spontaneous mutagenesis. In the last few decades, however, it has

become increasingly evident that mutations occur readily during cell cycle arrest or slow proliferation. Since the majority of cells in nature spend large parts of their lifetimes under proliferation-limiting conditions, spontaneous mutations arising in cells in this state might even outnumber the incidence of proliferation-dependent mutations.

In the absence of DNA replication, the introduction of mutations into a genome must result from some form of DNA processing or turnover, including transcription. Undeniably, the accuracy of DNA repair enzymes is not absolute and errors occur during the necessary repair of spontaneous DNA damage as a consequence of a decay of the DNA or the attack by reactive endogenous molecules. Replication-independent mutations reflect the mechanistic difference with proliferation-dependent mutagenesis. This term is, however, infrequently used, and many researchers prefer “adaptive mutation” (Steele and Jinks-Robertson 1992; Baranowska, Policińska and Jachymczyk 1995; Heidenreich and Wintersberger 1997). Adaptive or selection-induced mutations are defined as mutations that occur in non-dividing cells as a response to prolonged non-lethal selective pressure such as starvation for an essential amino acid. Adaptive mutation in the broad sense also designates the analogous phenomenon pioneered in *Escherichia coli* (early work reviewed by Foster 1993; Rosenberg 1997; Hall 1998).

The importance of adaptive mutations comes from the assumption that it may trigger an accelerated evolution of microorganisms and in multicellular organisms contribute to a breakout of somatic cells from negative growth regulation to carcinogenesis.

During cell division, the spontaneous mutation rate is expressed as the probability of mutations *per* generation and is measured by fluctuation assay (Luria and Delbrück 1943; Lea and Coulson 1949). More direct measurements using next generation sequencing, including mutation accumulation (MA) (Halligan and Keightley 2009) and *de novo* mutations (Conrad *et al.* 2011) improved the mutation rate estimations and led to the definition of their spectrum in budding and fission yeast (Zhu *et al.* 2014; Behringer and Hall 2015; Farlow *et al.* 2015). Conversely, during quiescence it is expressed *per* unit of time. The major sources include errors of DNA replication and DNA repair and the foremost consequences are genetic variations within a cell population that can lead to heritable diseases and drive evolution. Knowledge of the rate and spectrum of spontaneous mutations is of fundamental importance to understand their origin.

The efficiency and accuracy of the repair of DNA lesions in quiescence remain unknown, and lesions are converted into mutations either during quiescence or when cells re-enter the vegetative cycle.

Budding yeast

Adaptive mutation experiments using *S. cerevisiae* rely on the starvation for an essential amino acid or nucleobase. After depletion of internal reserves, the cell cycle arrests and only cells that have already acquired prototrophy manage to continue to grow. In this system, it is presently unclear whether the cells actually enter a true G0 phase or whether the mutations are truly produced in quiescence and not during the last round of DNA replication. This inherent problem of adaptive mutation assays is that the experiment starts by a culture during which proliferation-dependent revertants arise that are carried over to the starvation plates (Heidenreich *et al.* 2003). Because pre-existing revertants proliferate faster, it is possible to somehow discriminate between proliferation-dependent revertants and adaptive revertants by their time of colony appearance. Crossfeeding, the ability to restart proliferation feeding on nutrients excreted by older revertant colonies, can also bias the experimental interpretations and was reported to be troublesome when using tryptophan auxotrophy alleles (Storchová *et al.* 1997; Storchová and Vondrejs 1999; Rojas Gil and Vondrejs 1999) or the *his1–7* allele (Lax, Fogel and Cramer 1979; Borstel *et al.* 1998; Marini, Matmati and Morpurgo 1999). In other systems, crossfeeding only infrequently occurs after 2 weeks of starvation (Steele and Jinks-Robertson 1992).

Using this approach, the mutation frequencies were determined in strains affected in the major DNA repair pathways. Mutations in many pathways increase the incidence of adaptive mutation, suggesting that they contribute directly or indirectly to the suppression of adaptive mutation.

Mutations in the Double Strand Break Repair pathway (DSBR) (Haber 2000; Symington 2002) have shown that the genes of the Rad52 epistasis group required for Homologous Recombination (HR) have no effect on the production of adaptive mutations in a frameshift detection assay, either in haploid or diploid cells. However, the deletion of the genes coding for the obligatory constituents of the Non-Homologous End Joining (NHEJ) pathway exhibit a 50% reduction of the replication-independent mutation frequency in haploid cells. Sequencing the mutations showed that the events are small deletions within mononucleotide repeats (Heidenreich *et al.* 2003).

The analysis of the single Translesion Lesion Synthesis (TLS) polymerases Rev1p, Rev3p and polymerase η has indicated that they do not significantly change the incidence of adaptive frameshift mutations. The knockout of all three TLS enzymes did not have an effect either. A deficiency in Nucleotide Excision Repair (NER) elevates adaptive mutations in non-irradiated cells and depends on a functional Rev3 polymerase. Rev3 is also required for the occurrence of increased frequencies of adaptive mutants in the NER-proficient cells following UV irradiation. In the absence of UVs, some activity of NER is required in non-dividing cells to repair poorly understood types of spontaneously occurring lesions (Heidenreich, Holzmann and Eisler 2004).

The role of replicative Pol δ and Pol ε in adaptive mutagenesis was studied using temperature-sensitive or proofreading-deficient alleles of the essential genes coding for the catalytic subunits of these polymerases. Temperature-sensitive alleles in DNA polymerase δ revertant colonies accumulated in a time-dependent manner in the absence of any detectable increase in cell number. Therefore, when the proofreading activity of DNA polymerase δ is impaired under restrictive conditions, the frequency of adaptive mutations is markedly enhanced (Baranowska, Policińska and Jachymczyk 1995). Similar results, but to a lesser degree, were obtained with the proofreading exonuclease mutant of Pol ε (Babudri *et al.* 2001), suggesting that the proofreading activities play a role in the avoidance of mutation formation not only during replication, but also during cell-cycle arrest.

The major pathway for correction of errors that escaped proofreading is the Mismatch Repair (MMR) pathway that recognizes the mismatch and corrects it (Jiricny 2006). The crucial factor for this process is the ability to discriminate between the original template strand and the new altered strand. In eukaryotes, the necessary information is most likely provided by direct interaction with the DNA replication machinery and/or the presence of DNA termini in the newly synthesized strand (Pavlov, Mian and Kunkel 2003). If this information is not available, as supposed in arrested cells, MMR proteins might potentially just as well lead to a fixation of a mutation instead of a correction to the original sequence. In addition, a mismatch can also occur by the spontaneous deamination of 5-methylcytosine to thymine or of a cytosine to uracil, and thus may cause base substitutions but no frameshifts.

A transient deficiency in the MMR capacity during prolonged starvation was associated with adaptive or stress-induced mutagenesis in *E. coli* (Longerich *et al.* 1995; Harris *et al.* 1997; Bjedov *et al.* 2003) and *Bacillus subtilis* (Pedraza-Reyes and Yasbin 2004) and prompted its investigation in *S. cerevisiae*. As anticipated, the frequency of *LYS2* revertants was found to

be extremely elevated in MMR mutants (The influence of the mismatch-repair system on stationary-phase mutagenesis in the yeast Saccharomyces cerevisiae. 2002).

Finally, the hypothesis of the existence of a hypermutable subpopulation was proposed (Hall 1990) in adaptive mutation studies in *E. coli*. A testable prediction of this hypothesis is that the frequency of nonselected additional mutations should be higher in adaptive revertant clones than in clones derived from nonreverted cells. This idea was substantiated by several studies in *E. coli* (reviewed by Hall 1998; Foster 1999; Galhardo, Hastings and Rosenberg 2007). In budding yeast, Steele and Jinks-Robertson (Steele and Jinks-Robertson 1992) reported that they failed to detect additional auxotrophies in over 1500 adaptive revertants. However, since it is uncertain whether an effect could have been expected with this number of probes, it is hard to estimate the significance of this result and it is too early to reject the possibility of a transiently hypermutable subpopulation in starving yeast populations.

Fission yeast

In fission yeast, we have developed many approaches to determine survival and mutagenesis (Figure 4). Mutation frequencies of quiescent cultures have been resolved by plating samples from prototrophic strains taken at various time points onto medium containing 5-fluoroorotic acid (5-FOA) that selects for *ura4*$^-$ and *ura5*$^-$ loss-of-function mutants (Grimm *et al.* 1988). The *ura*$^-$ mutants remain fully viable for two weeks of quiescence, which indicates that colonies resistant to 5-FOA (FOA^R) arising early are not counter-selected and that the phenotypic accumulation assay is unbiased during the course of the experiment. It is thus possible to extract their DNA and identify the causal mutations and define their spectrum by Sanger sequencing.

After one day in quiescence, the mutation frequency and the spectrum is similar to previously published results in cycling cells for *URA3* in budding yeast (Lang and Murray 2008) and for *ura4*$^+$ and *ura5*$^+$ in fission yeast (Fraser, Neill and Davey 2003). Interestingly, the number of mutations resulting in FOA^R colonies increases linearly as a function of time, and several quantitative and qualitative mutational differences were found in the mutational landscape of dividing and quiescent cells. First, elevated levels of indels are detected (>50% indels after two weeks as compared to 15 to 20% in cycling cells), almost exclusively small deletions and duplications (micro-indels). Second, two recent MA studies (Behringer and Hall 2015; Farlow *et al.* 2015) with fission yeast have shown that in dividing cells insertions outnumber deletions, whereas the reverse is observed in quiescence (manuscript submitted).

This suggests that, in addition to the previously hypothesized gene conversion, nucleotide modifications or transposition, the equilibrium of size and composition of the *S. pombe* genome depends as well on the relative strength of the opposing forces applied during growth and quiescence, and that growth and quiescence explore different landscapes of protein variation and genome organization.

Using the capacity of fission yeast cells to survive in quiescence for several months, a phenotypic survey in conditions that affect a broad range of cellular functions was conducted. Although no phenotypes were found upon examination of 384 colonies after 1 day or 1 month of quiescence, increasing numbers of colonies displaying phenotypes were observed at 2 months (1.1%) and 3 months of quiescence (1.8%). Genetic crosses confirm that the phenotype observed in all the detected colonies derives from a single mutated locus (manuscript submitted).

CONCLUSIONS AND PERSPECTIVES

Here we reviewed the current knowledge on the genetics of quiescence compared to growth and focused on two single cell model organisms. During growth, a preference for insertions at the expense of deletions has been reported in numerous studies along with a universal substitution bias toward AT (*E. coli*, *S. cerevisiae*, *S. pombe*, *C. elegans* and humans). The fact that the replication-driven mutational bias has not yet reached an equilibrium strongly suggests the existence of forces capable of counterbalancing it. DNA replication-dependent mutations, DNA repair errors, nucleotide modifications, recombination or transposition have been hypothesized to impact on genome size and composition.

During its life cycle, *S. pombe* sequentially experiences two modes of mutational processes that alternatively work on genes required for proliferation and/or quiescence. Since the mutations in gametes are central for evolution, it is tempting to propose that in higher eukaryotes the germ cells are sequentially selecting for high genetics qualities of quiescence in females and for high genetics qualities of proliferation in males. These qualities will eventually merge in the diploid zygote prior to the initiation of a new life cycle, suggesting that the fundamental difference in the lifestyle of the two gametes contributes to the genetic performance of the dividing and quiescent somatic cells and the homeostasis of adult tissues with aging. Conversely, it may explain the size dimorphism of the two complementary gametes found in many multicellular organisms.

The genetics of quiescence underscores the importance of a replication-independent but time-dependent process (Kumar and Subramanian 2002; Ségurel, Wyman and Przeworski 2014; Goldmann *et al.* 2016; Hazen *et al.* 2016) to the overall mutation spectrum. This time-dependent process should help to fine-tune the accuracy of the "molecular clock" that scales absolute time of divergence to evolutionary distance of two related species (Reis, Donoghue and Yang 2016). Such hypotheses are accessible to experimental and modeling approaches and are of great interest for evolutionary, developmental and human-health perspectives.

ACKNOWLEDGMENTS

We thank Stefania Francesconi for her critical reading of the manuscript. Work is supported by the FRM, ANR-Blanc SVSE8, CNRS and Institut Pasteur.

REFERENCES

Abbott A. Cancer: the root of the problem. *Nature*. August 17, 2006:742–3.

Ahel I, Rass U, El-Khamisy SF *et al.* The neurodegenerative disease protein aprataxin resolves abortive DNA ligation intermediates. *Nature* 2006;**443**:713–6.

Alam MT, Zelezniak A, Mülleder M *et al.* The metabolic background is a global player in *Saccharomyces gene* expression epistasis. *Nat Microbiol* 2016;**1**:15030.

Alberts B, Johnson AD, Lewis J *et al.* ***Molecular Biology of the Cell, 5th Edition***. New York: Garland Science, 2007.

Alexander BD, Perfect JR. Antifungal resistance trends towards the year 2000. Implications for therapy and new approaches. *Drugs* 1997;**54**:657–78.

Allen C, Buttner S, Aragon AD *et al.* Isolation of quiescent and nonquiescent cells from yeast stationary-phase cultures. *J Cell Biol* 2006;**174**:89–100.

An Z, Tassa A, Thomas C *et al.* Autophagy is required for G_1/G_0 quiescence in response to nitrogen starvation in *Saccharomyces cerevisiae*. *Autophagy* 2014;**10**:1702–11.

Aragon AD, Quiñones GA, Thomas EV *et al.* Release of extraction-resistant mRNA in stationary phase *Saccharomyces cerevisiae* produces a massive increase in transcript abundance in response to stress. *Genome Biol* 2006;**7**:R9.

Aragon AD, Rodriguez AL, Meirelles O *et al.* Characterization of differentiated quiescent and nonquiescent cells in yeast stationary-phase cultures. *Mol Biol Cell* 2008;**19**:1271–80.

Arcangioli B, Ben Hassine S. Unrepaired oxidative DNA damage induces an ATR/ATM apoptotic-like response in quiescent fission yeast. *Cell Cycle* 2009;**8**:2326–31.

Arigo JT, Carroll KL, Ames JM *et al.* Regulation of yeast NRD1 expression by premature transcription termination. *Mol Cell* 2006;**21**:641–51.

Babudri N, Pavlov YI, Matmati N *et al.* Stationary-phase mutations in proofreading exonuclease-deficient strains of the yeast *Saccharomyces cerevisiae*. *Mol Genet Genomics* 2001;**265**:362–6.

Baker BS, Carpenter AT, Esposito MS *et al.* The genetic control of meiosis. *Annu Rev Genet* 1976;**10**:53–134.

Baranowska H, Policińska Z, Jachymczyk WJ. Effects of the CDC2 gene on adaptive mutation in the yeast *Saccharomyces cerevisiae*. *Current Genetics* 1995;**28**:521–5.

Bartek J, Lukas J, Bartkova J. DNA damage response as an anti-cancer barrier: damage threshold and the concept of 'conditional haploinsufficiency'. *Cell Cycle* 2007;**6**:2344–7.

Behrens A, van Deursen JM, Rudolph KL *et al.* Impact of genomic damage and ageing on stem cell function. *Nat Cell Biol* 2014;**16**:201–7.

Behringer MG, Hall DW. Genome wide estimates of mutation rates and spectrum in *Schizosaccharomyces pombe* indicate CpG sites are highly mutagenic despite the absence of DNA methylation. *bioRxiv* 2015:025601.

Ben Hassine S, Arcangioli B. Tdp1 protects against oxidative DNA damage in non-dividing fission yeast. *EMBO J* 2009;**28**:632–40.

Bjedov I, Tenaillon O, Gérard B *et al.* Stress-induced mutagenesis in bacteria. *Science* 2003;**300**:1404–9.

Boiteux S, Guillet M. Abasic sites in DNA: repair and biological consequences in *Saccharomyces cerevisiae*. *DNA Repair (Amst)* 2004;**3**:1–12.

Bontron S, Jaquenoud M, Vaga S *et al.* Yeast endosulfines control entry into quiescence and chronological life span by inhibiting protein phosphatase 2A. *Cell Rep* 2013;**3**:16–22.

Borstel von RC, Savage EA, Wang Q *et al.* Topical reversion at the *HIS1* locus of *Saccharomyces cerevisiae*. A tale of three mutants. *Genetics* 1998;**148**:1647–54.

Burhans WC, Weinberger M. Acetic acid effects on aging in budding yeast: are they relevant to aging in higher eukaryotes? *Cell Cycle* 2009;**8**:2300–2.

Burhans WC, Weinberger M. DNA damage and DNA replication stress in yeast models of aging. *Subcell Biochem* 2012;**57**:187–206.

Burtner CR, Kennedy BK. Progeria syndromes and ageing: what is the connection? *Nat Rev Mol Cell Biol* 2010;**11**:567–78.

Caldecott KW. Single-strand break repair and genetic disease. *Nat Rev Genet* 2008;**9**:619–31.

Chang E, Yang J, Nagavarapu U *et al.* Aging and survival of cutaneous microvasculature. *J Invest Dermatol* 2002;**118**:752–8.

Chou W-C, Wang H-C, Wong F-H *et al.* Chk2-dependent phosphorylation of XRCC1 in the DNA damage response promotes base excision repair. *EMBO J* 2008;**27**:3140–50.

Cleveland DW, Rothstein JD. From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. *Nat Rev Neurosci* 2001;**2**:806–19.

Collinson LP, Dawes IW. Inducibility of the response of yeast cells to peroxide stress. *J Gen Microbiol* 1992;**138**:329–35.

Conrad DF, Keebler JEM, DePristo MA *et al.* Variation in genome-wide mutation rates within and between human families. *Nat Genet* 2011;**43**:712–4.

Cooke MS, Evans MD, Dizdaroglu M *et al.* Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J* 2003;**17**:1195–214.

Cooper DN, Youssoufian H. The CpG dinucleotide and human genetic disease. *Hum Genet* 1988;**78**:151–5.

Costa V, Moradas-Ferreira P. Oxidative stress and signal transduction in *Saccharomyces cerevisiae*: insights into ageing, apoptosis and diseases. *Mol Aspects Med* 2001;**22**:217–46.

Costello G, Rodgers L, Beach D. Fission yeast enters the stationary phase G0 state from either mitotic G1 or G2. *Current Genetics* 1986;**11**:119–25.

Cox GA, Mahaffey CL, Frankel WN. Identification of the mouse neuromuscular degeneration gene and mapping of a second site suppressor allele. *Neuron* 1998;**21**:1327–37.

Çağlayan M, Wilson SH. Reprint of "Oxidant and environmental toxicant-induced effects compromise DNA ligation during base excision DNA repair". *DNA Repair (Amst)* 2015;**36**:86–90.

d'Adda di Fagagna F. A direct role for small non-coding RNAs in DNA damage response. *Trends Cell Biol* 2014;**24**:171–8.

De Bont R, van Larebeke N. Endogenous DNA damage in humans: a review of quantitative data. *Mutagenesis* 2004;**19**:169–85.

de Jong-Gubbels P, van Dijken JP, Pronk JT. Metabolic fluxes in chemostat cultures of *Schizosaccharomyces pombe* grown on mixtures of glucose and ethanol. *Microbiology (Reading, Engl)* 1996;**142 (Pt 6)**:1399–407.

De Virgilio C. The essence of yeast quiescence. *FEMS Microbiol Rev* 2012;**36**:306–39.

DeJesus-Hernandez M, Mackenzie IR, Boeve BF *et al.* Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 2011;**72**:245–56.

Drinnenberg IA, Fink GR, Bartel DP. Compatibility with killer explains the rise of RNAi-deficient fungi. *Science* 2011;**333**:1592–2.

Drinnenberg IA, Weinberg DE, Xie KT *et al.* RNAi in budding yeast. *Science* 2009;**326**:544–50.

Du L-L, Nakamura TM, Moser BA *et al.* Retention but Not Recruitment of Crb2 at Double-

Strand Breaks Requires Rad1 and Rad3 Complexes. *Mol Cell Biol* 2003;**23**:6150–8.

Egel R. *2 Mating-Type Genes, Meiosis, and Sporulation*. Elsevier, 1989:31–73.

Eisler H, Fröhlich K-U, Heidenreich E. Starvation for an essential amino acid induces apoptosis and oxidative stress in yeast. *Exp Cell Res* 2004;**300**:345–53.

El-Khamisy SF, Caldecott KW. TDP1-dependent DNA single-strand break repair and neurodegeneration. *Mutagenesis* 2006;**21**:219–24.

El-Khamisy SF, Hartsuiker E, Caldecott KW. TDP1 facilitates repair of ionizing radiation-induced DNA single-strand breaks. *DNA Repair (Amst)* 2007;**6**:1485–95.

El-Khamisy SF, Saifi GM, Weinfeld M *et al.* Defective DNA single-strand break repair in spinocerebellar ataxia with axonal neuropathy-1. *Nature* 2005;**434**:108–13.

Esashi F, Yanagida M. Cdc2 phosphorylation of Crb2 is required for reestablishing cell cycle progression after the damage checkpoint. *Mol Cell* 1999;**4**:167–74.

Fabrizio P, Battistella L, Vardavas R *et al.* Superoxide is a mediator of an altruistic aging program in *Saccharomyces cerevisiae*. *J Cell Biol* 2004;**166**:1055–67.

Fabrizio P, Longo VD. The chronological life span of Saccharomyces cerevisiae. *Aging Cell* 2003;**2**:73–81.

Fabrizio P, Longo VD. The chronological life span of *Saccharomyces cerevisiae*. *Methods Mol Biol* 2007;**371**:89–95.

Farlow A, Long H, Arnoux S *et al.* The Spontaneous Mutation Rate in the Fission Yeast *Schizosaccharomyces pombe*. *Genetics* 2015;**201**:737–44.

Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature* 2000;**408**:239–47.

Flores CL, Rodríguez C, Petit T *et al.* Carbohydrate and energy-yielding metabolism in non-conventional yeasts. *FEMS Microbiol Rev* 2000;**24**:507–29.

Foster PL. Adaptive mutation: the uses of adversity. *Annu Rev Microbiol* 1993;**47**:467–504.

Foster PL. Mechanisms of stationary phase mutation: a decade of adaptive mutation. *Annu Rev Genet* 1999;**33**:57–88.

Francia S, Cabrini M, Matti V *et al.* DICER, DROSHA and DNA damage response RNAs are necessary for the secondary recruitment of DNA damage response factors. *J Cell Sci* 2016;**129**:1468–76.

Fraser JLA, Neill E, Davey S. Fission yeast Uve1 and Apn2 function in distinct oxidative damage repair pathways in vivo. *DNA Repair (Amst)* 2003;**2**:1253–67.

Freitas AA, de Magalhães JP. A review and appraisal of the DNA damage theory of ageing. *Mutat Res* 2011;**728**:12–22.

Friedberg EC, Walker GC, Siede W *et al. DNA Repair and Mutagenesis*. 2005.

Galhardo RS, Hastings PJ, Rosenberg SM. Mutation as a stress response and the regulation of evolvability. *Crit Rev Biochem Mol Biol* 2007;**42**:399–435.

Ganley ARD, Kobayashi T. Ribosomal DNA and cellular senescence: new evidence supporting the connection between rDNA and aging. *FEMS Yeast Research* 2014;**14**:49–59.

Garinis GA, van der Horst GTJ, Vijg J *et al.* DNA damage and ageing: new-age ideas for an age-old problem. *Nat Cell Biol* 2008;**10**:1241–7.

Gensler HL, Bernstein H. DNA damage as the primary cause of aging. *Q Rev Biol* 1981;**56**:279–303.

Goldmann JM, Wong WSW, Pinelli M *et al.* Parent-of-origin-specific signatures of de novo mutations. *Nat Genet* 2016;**48**:935–9.

Gouge RC, Marshburn P, Gordon BE *et al.* Nitric oxide as a regulator of embryonic development. *Biol Reprod* 1998;**58**:875–9.

Gray JV, Petsko GA, Johnston GC *et al.* "Sleeping beauty": quiescence in *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev* 2004;**68**:187–206.

Grigg GW, Stuckey J. The reversible suppression of stationary phase mutation in *Escherichia coli* by caffeine. *Genetics* 1966;**53**:823–34.

Grimm C, Kohli J, Murray J *et al.* Genetic engineering of *Schizosaccharomyces pombe*: a system for gene disruption and replacement using the ura4 gene as a selectable marker. *Mol Gen Genet* 1988;**215**:81–6.

Haber JE. Partners and pathways: repairing a double-strand break. *Trends in Genetics* 2000;**16**:259–64.

Halas A, Baranowska H, Podlaska A *et al.* Evaluation of the roles of Pol zeta and NHEJ in starvation-associated spontaneous mutagenesis in the yeast *Saccharomyces cerevisiae*. *Current Genetics* 2009;**55**:245–51.

Hall BG. Spontaneous point mutations that occur more often when advantageous than when neutral. *Genetics* 1990;**126**:5–16.

Hall BG. Activation of the bgl operon by adaptive mutation. *Mol Biol Evol* 1998;**15**:1–5.

Halligan DL, Keightley PD. Spontaneous mutation accumulation studies in evolutionary genetics. *Annual Review of Ecology* 2009;**40**:151–72.

Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 1956;**11**:298–300.

Harris RS, Feng G, Ross KJ *et al.* Mismatch repair protein MutL becomes limiting during stationary-phase mutation. *Genes Dev* 1997;**11**:2426–37.

Hartwell LH, Mortimer RK, Culotti J *et al.* Genetic Control of the Cell Division Cycle in Yeast: V. Genetic Analysis of cdc Mutants. *Genetics* 1973;**74**:267–86.

Hazen JL, Faust GG, Rodriguez AR *et al.* The Complete Genome Sequences, Unique Mutational Spectra, and Developmental Potency of Adult Neurons Revealed by Cloning. *Neuron* 2016;**89**:1223–36.

Heidenreich E, Eisler H, Lengheimer T *et al.* A mutation-promotive role of nucleotide excision repair in cell cycle-arrested cell populations following UV irradiation. *DNA Repair (Amst)* 2010;**9**:96–100.

Heidenreich E, Eisler H, Steinboeck F. Epistatic participation of *REV1* and *REV3* in the formation of UV-induced frameshift mutations in cell cycle-arrested yeast cells. *Mutat Res* 2006;**593**:187–95.

Heidenreich E, Eisler H. Non-homologous end joining dependency of gamma-irradiation-induced adaptive frameshift mutation formation in cell cycle-arrested yeast cells. *Mutat Res* 2004;**556**:201–8.

Heidenreich E, Holzmann V, Eisler H. Polymerase zeta dependency of increased adaptive mutation frequencies in nucleotide excision repair-deficient yeast strains. *DNA Repair (Amst)* 2004;**3**:395–402.

Heidenreich E, Novotny R, Kneidinger B *et al.* Non-homologous end joining as an important mutagenic process in cell cycle-arrested cells. *EMBO J* 2003;**22**:2274–83.

Heidenreich E, Wintersberger U. Starvation for a specific amino acid induces high frequencies of rho- mutants in *Saccharomyces cerevisiae*. *Current Genetics* 1997;**31**:408–13.

Herman PK. Stationary phase in yeast. *Current opinion in microbiology* 2002;**5**:602–7.

Heslot H, Goffeau A, Louis C. Respiratory metabolism of a “petite negative”yeast *Schizosaccharomyces pombe* 972h-. *Journal of Bacteriology* 1970;**104**:473–81.

Hoeijmakers JHJ. DNA damage, aging, and cancer. *N Engl J Med* 2009;**361**:1475–85.

Interthal H, Pouliot JJ, Champoux JJ. The tyrosyl-DNA phosphodiesterase Tdp1 is a member of the phospholipase D superfamily. *Proc Natl Acad Sci U S A* 2001;**98**:12009–14.

Jackson AL, Loeb LA. The contribution of endogenous sources of DNA damage to the multiple mutations in cancer. *Mutat Res* 2001;**477**:7–21.

Jacquier A. Systems biology: Supplementation is not sufficient. *Nat Microbiol* 2016;**1**:16016.

Jamieson DJ. *Saccharomyces cerevisiae* has distinct adaptive responses to both hydrogen peroxide and menadione. *Journal of Bacteriology* 1992;**174**:6678–81.

Jamieson DJ. Oxidative stress responses of the yeast *Saccharomyces cerevisiae*. *Yeast* 1998;**14**:1511–27.

Jayadev S, Bird TD. Hereditary ataxias: overview. *Genet Med* 2013;**15**:673–83.

Jin Z, Xie T. Dcr-1 maintains Drosophila ovarian stem cells. *Curr Biol* 2007;**17**:539–44.

Jiricny J. The multifaceted mismatch-repair system. *Nat Rev Mol Cell Biol* 2006;**7**:335–46.

Julien J-P. Amyotrophic Lateral Sclerosis. *Cell* 2001;**104**:581–91.

Kaeberlein M. Lessons on longevity from budding yeast. *Nature* 2010;**464**:513–9.

Kaeberlein T, Lewis K, Epstein SS. Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. *Science* 2002;**296**:1127–9.

Kalisky T, Blainey P, Quake SR. Genomic analysis at the single-cell level. *Annu Rev Genet* 2011;**45**:431–45.

Ketting RF, Fischer SE, Bernstein E *et al.* Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in *C. elegans*. *Genes Dev* 2001;**15**:2654–9.

Kim CFB, Jackson EL, Woolfenden AE *et al.* Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* 2005;**121**:823–35.

Kim M, Vasiljeva L, Rando OJ *et al.* Distinct pathways for snoRNA and mRNA termination. *Mol Cell* 2006;**24**:723–34.

Kim N, Jinks-Robertson S. Transcription as a source of genome instability. *Nat Rev Genet* 2012;**13**:204–14.

Kumar S, Subramanian S. Mutation rates in mammalian genomes. *Proc Natl Acad Sci U S A* 2002;**99**:803–8.

La Spada AR, Wilson EM, Lubahn DB *et al.* Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* 1991;**352**:77–9.

Lang GI, Murray AW. Estimating the per-base-pair mutation rate in the yeast *Saccharomyces cerevisiae*. *Genetics* 2008;**178**:67–82.

Laun P, Pichova A, Madeo F *et al.* Aged mother cells of *Saccharomyces cerevisiae* show markers of oxidative stress and apoptosis. *Mol Microbiol* 2001;**39**:1166–73.

Lavin MF, Gueven N, Grattan-Smith P. Defective responses to DNA single- and double-strand breaks in spinocerebellar ataxia. *DNA Repair (Amst)* 2008;**7**:1061–76.

Lax C, Fogel S, Cramer C. Regulatory mutants at the his1 locus of yeast. *Genetics* 1979;**92**:363–82.

Lea DE, Coulson CA. The distribution of the numbers of mutants in bacterial populations. *J Genet* 1949;**49**:264–85.

Lewis DL, Gattie DK. *The Ecology of Quiescent Microbes.* ASM American Society for Microbiology News, 1991.

Lillie SH, Pringle JR. Reserve carbohydrate metabolism in *Saccharomyces cerevisiae*: responses to nutrient limitation. *Journal of Bacteriology* 1980;**143**:1384–94.

Lim J, Hao T, Shaw C *et al.* A protein-protein interaction network for human inherited ataxias

and disorders of Purkinje cell degeneration. *Cell* 2006;**125**:801–14.

Lindahl T. Instability and decay of the primary structure of DNA. *Nature* 1993;**362**:709–15.

Lisby M, Rothstein R, Mortensen UH. Rad52 forms DNA repair and recombination centers during S phase. *Proc Natl Acad Sci U S A* 2001;**98**:8276–82.

Lombard DB, Chua KF, Mostoslavsky R *et al.* DNA repair, genome stability, and aging. *Cell* 2005;**120**:497–512.

Longerich S, Galloway AM, Harris RS *et al.* Adaptive mutation sequences reproduced by mismatch repair deficiency. *Proc Natl Acad Sci U S A* 1995;**92**:12017–20.

Longo VD, Gralla EB, Valentine JS. Superoxide dismutase activity is essential for stationary phase survival in Saccharomyces cerevisiae. Mitochondrial production of toxic oxygen species in vivo. *J Biol Chem* 1996;**271**:12275–80.

Longo VD. Mutations in signal transduction proteins increase stress resistance and longevity in yeast, nematodes, fruit flies, and mammalian neuronal cells. *Neurobiol Aging* 1999;**20**:479–86.

Luria SE, Delbrück M. Mutations of Bacteria from Virus Sensitivity to Virus Resistance. *Genetics* 1943;**28**:491–511.

Maclean MJ, Aamodt R, Harris N *et al.* Base excision repair activities required for yeast to attain a full chronological life span. *Aging Cell* 2003;**2**:93–104.

Marguerat S, Schmidt A, Codlin S *et al.* Quantitative analysis of fission yeast transcriptomes and proteomes in proliferating and quiescent cells. *Cell* 2012;**151**:671–83.

Marini A, Matmati N, Morpurgo G. Starvation in yeast increases non-adaptive mutation. *Current Genetics* 1999;**35**:77–81.

Marti TM, Hefner E, Feeney L *et al.* H2AX phosphorylation within the G1 phase after UV irradiation depends on nucleotide excision repair and not DNA double-strand breaks. *Proc Natl Acad Sci U S A* 2006;**103**:9891–6.

McCready S, Cox B. Repair of 6-4 photoproducts in *Saccharomyces cerevisiae*. *Mutat Res* 1993;**293**:233–40.

Miao Z-H, Agama K, Sordet O *et al.* Hereditary ataxia SCAN1 cells are defective for the repair of transcription-dependent topoisomerase I cleavage complexes. *DNA Repair (Amst)* 2006;**5**:1489–94.

Miles S, Breeden L. A common strategy for initiating the transition from proliferation to quiescence. *Current Genetics* 2016:1–8.

Mischo HE, Gómez-González B, Grzechnik P *et al.* Yeast Sen1 helicase protects the genome from transcription-associated instability. *Mol Cell* 2011;**41**:21–32.

Mochida S, Yanagida M. Distinct modes of DNA damage response in *S. pombe* G0 and vegetative cells. *Genes Cells* 2006;**11**:13–27.

Murayama A, Ohmori K, Fujimura A *et al.* Epigenetic control of rDNA loci in response to intracellular energy status. *Cell* 2008;**133**:627–39.

Murray PJ. Defining the requirements for immunological control of mycobacterial infections. *Trends Microbiol* 1999;**7**:366–72.

Nakamura J, Swenberg JA. Endogenous apurinic/apyrimidinic sites in genomic DNA of mammalian tissues. *Cancer Res* 1999;**59**:2522–6.

Nakamura TM, Du L-L, Redon C *et al.* Histone H2A phosphorylation controls Crb2 recruitment at DNA breaks, maintains checkpoint arrest, and influences DNA repair in fission yeast. *Mol Cell Biol* 2004;**24**:6215–30.

Nurse P, Bissett Y. Gene required in G1 for commitment to cell cycle and in G2 for control of mitosis in fission yeast. *Nature* 1981;**292**:558–60.

Nurse P. Genetic control of cell size at cell division in yeast. *Nature* 1975;**256**:547–51.

Orr HT, Zoghbi HY. Trinucleotide repeat disorders. *Annu Rev Neurosci* 2007;**30**:575–621.

Parrish NM, Dick JD, Bishai WR. Mechanisms of latency in *Mycobacterium tuberculosis*. *Trends Microbiol* 1998;**6**:107–12.

Pavlov YI, Mian IM, Kunkel TA. Evidence for preferential mismatch repair of lagging strand DNA replication errors in yeast. *Curr Biol* 2003;**13**:744–8.

Pawar V, Jingjing L, Patel N *et al.* Checkpoint kinase phosphorylation in response to endogenous oxidative DNA damage in repair-deficient stationary-phase *Saccharomyces cerevisiae*. *Mech Ageing Dev* 2009;**130**:501–8.

Pedraza-Reyes M, Yasbin RE. Contribution of the mismatch DNA repair system to the generation of stationary-phase-induced mutants of *Bacillus subtilis*. *Journal of Bacteriology* 2004;**186**:6485–91.

Pommier Y, Redon C, Rao VA *et al.* Repair of and checkpoint response to topoisomerase I-mediated DNA damage. *Mutat Res* 2003;**532**:173–203.

Pontvianne F, Blevins T, Chandrasekhara C *et al.* Histone methyltransferases regulating rRNA gene dose and dosage control in *Arabidopsis*. *Genes Dev* 2012;**26**:945–57.

Pouliot JJ, Yao KC, Robertson CA *et al.* Yeast gene for a Tyr-DNA phosphodiesterase that repairs topoisomerase I complexes. *Science* 1999;**286**:552–5.

Rao KS. DNA repair in aging rat neurons. *Neuroscience* 2007;**145**:1330–40.

Rass U, Ahel I, West SC. Defective DNA repair and neurodegenerative disease. *Cell* 2007;**130**:991–1004.

Reis dos M, Donoghue PCJ, Yang Z. Bayesian molecular clock dating of species divergences in the genomics era. *Nat Rev Genet* 2016;**17**:71–80.

Renton AE, Majounie E, Waite A *et al.* A hexanucleotide repeat expansion in C9ORF72 is

the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 2011;**72**:257–68.

Roche B, Arcangioli B, Martienssen RA. RNA interference is essential for cellular quiescence. *Science* 2016;**354**:aah5651.

Rockenfeller P, Madeo F. Apoptotic death of ageing yeast. *Exp Gerontol* 2008;**43**:876–81.

Rojas Gil AP, Vondrejs V. Development of papillae on colonies of two isopolyauxotrophic strains of *Saccharomyces cerevisiae* allelic in *RAD6* during adenine starvation. *Folia Microbiol (Praha)* 1999;**44**:299–305.

Rosenberg SM. Mutation for survival. *Current Opinion in Genetics & Development* 1997;**7**:829–34.

Ryan FJ. Spontaneous Mutation in Non-Dividing Bacteria. *Genetics* 1955;**40**:726–38.

Sage E, Harrison L. Clustered DNA lesion repair in eukaryotes: relevance to mutagenesis and cell survival. *Mutat Res* 2011;**711**:123–33.

Saka Y, Esashi F, Matsusaka T *et al.* Damage and replication checkpoint control in fission yeast is ensured by interactions of Crb2, a protein with BRCT motif, with Cut5 and Chk1. *Genes Dev* 1997;**11**:3387–400.

Sanders SL, Portoso M, Mata J *et al.* Methylation of histone H4 lysine 20 controls recruitment of Crb2 to sites of DNA damage. *Cell* 2004;**119**:603–14.

Ségurel L, Wyman MJ, Przeworski M. Determinants of mutation rate variation in the human germline. *Annu Rev Genomics Hum Genet* 2014;**15**:47–70.

Shimanuki M, Chung S-Y, Chikashige Y *et al.* Two-step, extensive alterations in the transcriptome from G0 arrest to cell division in *Schizosaccharomyces pombe*. *Genes Cells* 2007;**12**:677–92.

Sinkkonen L, Hugenschmidt T, Filipowicz W *et al.* Dicer is associated with ribosomal DNA chromatin in mammalian cells. Aramayo R (ed.). *PLoS ONE* 2010;**5**:e12175.

Steele DF, Jinks-Robertson S. An examination of adaptive reversion in *Saccharomyces cerevisiae*. *Genetics* 1992;**132**:9–21.

Steinboeck F, Hubmann M, Bogusch A *et al.* The relevance of oxidative stress and cytotoxic DNA lesions for spontaneous mutagenesis in non-replicating yeast cells. *Mutat Res* 2010;**688**:47–52.

Storchová Z, Gil AP, Janderová B *et al.* Accumulation of Ade+ reversions in isoauxotrophic stains of *Saccharomyces cerevisiae* allelic in *RAD6* during adenine starvation. *Folia Microbiol (Praha)* 1997;**42**:47–51.

Storchová Z, Vondrejs V. Starvation-associated mutagenesis in yeast *Saccharomyces cerevisiae* is affected by Ras2/cAMP signaling pathway. *Mutat Res* 1999;**431**:59–67.

Suda T, Arai F, Hirao A. Hematopoietic stem cells and their niche. *Trends Immunol* 2005;**26**:426–33.

Symington LS. Role of *RAD52* Epistasis Group Genes in Homologous Recombination and Double-Strand Break Repair. *Microbiol Mol Biol Rev* 2002;**66**:630–70.

Takashima H, Boerkoel CF, John J *et al.* Mutation of TDP1, encoding a topoisomerase I-dependent DNA damage repair enzyme, in spinocerebellar ataxia with axonal neuropathy. *Nat Genet* 2002;**32**:267–72.

Takeda K, Yanagida M. In quiescence of fission yeast, autophagy and the proteasome collaborate for mitochondrial maintenance and longevity. *Autophagy* 2010;**6**:564–5.

Temple MD, Perrone GG, Dawes IW. Complex cellular responses to reactive oxygen species. *Trends Cell Biol* 2005;**15**:319–26.

Vance JR, Wilson TE. Uncoupling of 3'-phosphatase and 5‘-kinase functions in budding yeast. Characterization of *Saccharomyces cerevisiae* DNA 3’-phosphatase (TPP1). *J Biol Chem* 2001;**276**:15073–81.

Volpe T, Schramke V, Hamilton GL *et al.* RNA interference is required for normal centromere function in fission yeast. *Chromosome Res* 2003;**11**:137–46.

Volpe TA, Kidner C, Hall IM *et al.* Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. *Science* 2002;**297**:1833–7.

Wallace SS. Enzymatic processing of radiation-induced free radical damage in DNA. *Radiat Res* 1998;**150**:S60–79.

Wei P-C, Chang AN, Kao J *et al.* Long Neural Genes Harbor Recurrent DNA Break Clusters in Neural Stem/Progenitor Cells. *Cell* 2016;**164**:644–55.

Werner-Washburne M, Braun E, Johnston GC *et al.* Stationary phase in the yeast *Saccharomyces cerevisiae*. *Microbiol Rev* 1993;**57**:383–401.

Werner-Washburne M, Roy S, Davidson GS. Aging and the survival of quiescent and non-quiescent cells in yeast stationary-phase cultures. *Subcell Biochem* 2012;**57**:123–43.

Williams AB, Hetrick KM, Foster PL. Interplay of DNA repair, homologous recombination, and DNA polymerases in resistance to the DNA damaging agent 4-nitroquinoline-1-oxide in *Escherichia coli*. *DNA Repair (Amst)* 2010;**9**:1090–7.

Yanagida M. Cellular quiescence: are controlling genes conserved? *Trends Cell Biol* 2009;**19**:705–15.

Yang H, Ren Q, Zhang Z. Chromosome or chromatin condensation leads to meiosis or apoptosis in stationary yeast (*Saccharomyces cerevisiae*) cells. *FEMS Yeast Research* 2006;**6**:1254–63.

Yang SW, Burgin AB, Huizenga BN *et al.* A eukaryotic enzyme that can disjoin dead-end covalent complexes between DNA and type I topoisomerases. *Proc Natl Acad Sci U S A* 1996;**93**:11534–9.

Zhu YO, Siegal ML, Hall DW *et al.* Precise estimates of mutation rate and spectrum in yeast. *Proc Natl Acad Sci U S A* 2014;**111**:E2310–8.

The influence of the mismatch-repair system on stationary-phase mutagenesis in the yeast *Saccharomyces cerevisiae*. 2002;**42**:140–6.

Figure 1

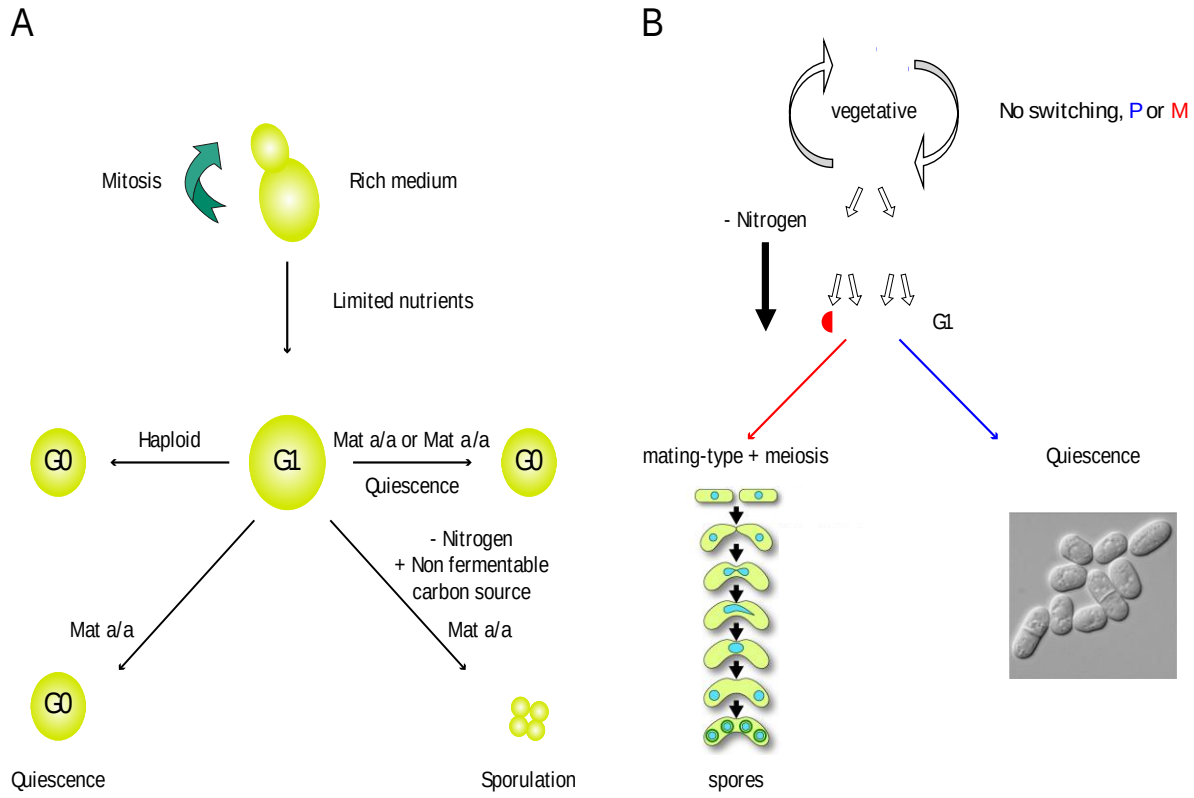
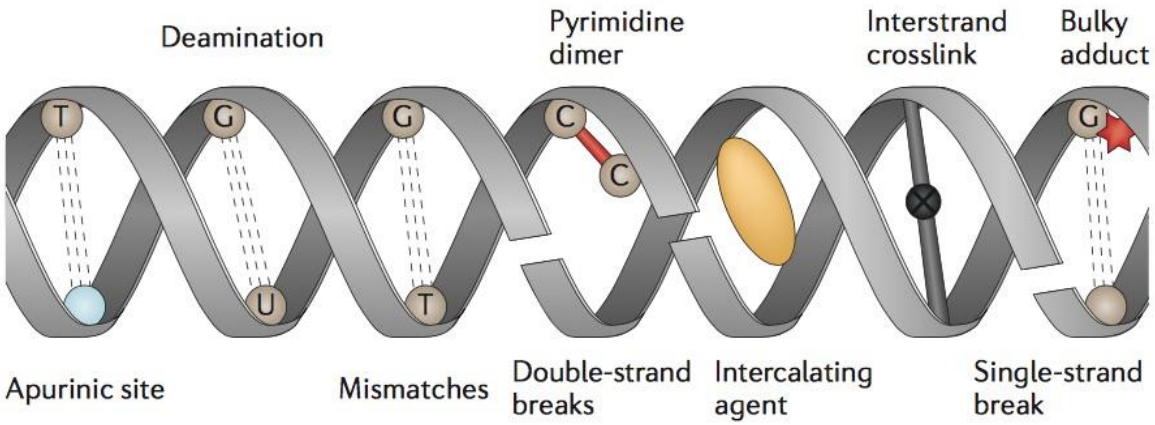


Figure 1: Entry into quiescence in budding and fission yeast.

A. Under the shortage of essential nutrients, cells by default arrest in G1 and proceed to G0. Meiotic entry requires the expression of an inducer gene (*IME1*) whose expression is controlled by mating type heterozygosity, nitrogen starvation and a non-fermentable carbon source. **B.** Upon nitrogen starvation, cells mate and sporulate. If no mating partner is available (heterothallic situation), cells enter quiescence.

Figure 2



Helleday T, Eshtad S, Nik-Zainal S. Nat Rev Genet 2014;15:585–98.

Figure 2: An overview of types of DNA damage and causal agents (Helleday, Eshtad and Nik-Zainal 2014)**.**

Sources of DNA damage include endogenous factors such as spontaneous or enzymatic conversions. The *N*-glycosidic bond that links a nucleobase and a pentose sugar to form a nucleoside is labile. This fact underlies the common occurrence of spontaneous base loss in DNA (~10000 bases per cell per day), which results in the formation of apurinic or apyrimidinic sites. Other types of endogenous DNA damage include deamination, polymerase errors and free radical species which can induce the formation of double-strand breaks. By contrast, ultraviolet radiation is responsible for the formation of pyrimidine dimers, which can be mutagenic when left unrepaired. Other external agents that are known to cause DNA damage include chemical compounds which can cause bulky adducts or interstrand and intrastrand crosslinks, intercalating agents, DNA alkylating agents and psoralens.

Figure 3

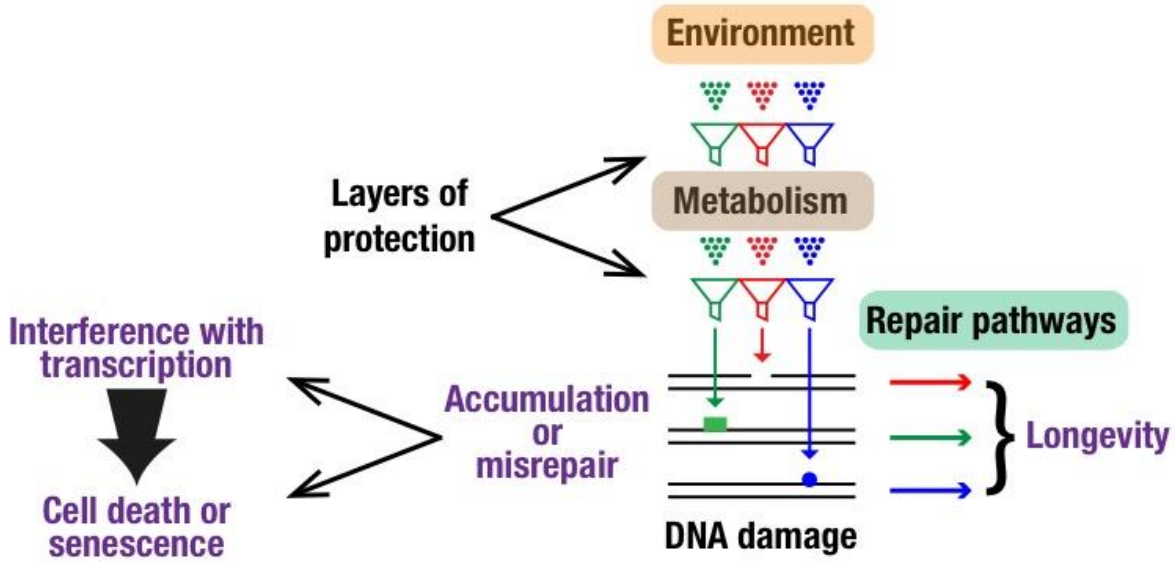


Figure 3: Preservation of the genetic message during quiescence

Cells suffer insults from the environment but also from their own metabolism. Several layers of protection exist to limit their damaging effects. Because no biological system is foolproof, longevity relies on performant repair systems. When a DNA repair pathway makes an error, or is absent, lesions accumulate and affect transcription, which in turn trigger cell death or senescence.

Figure 4

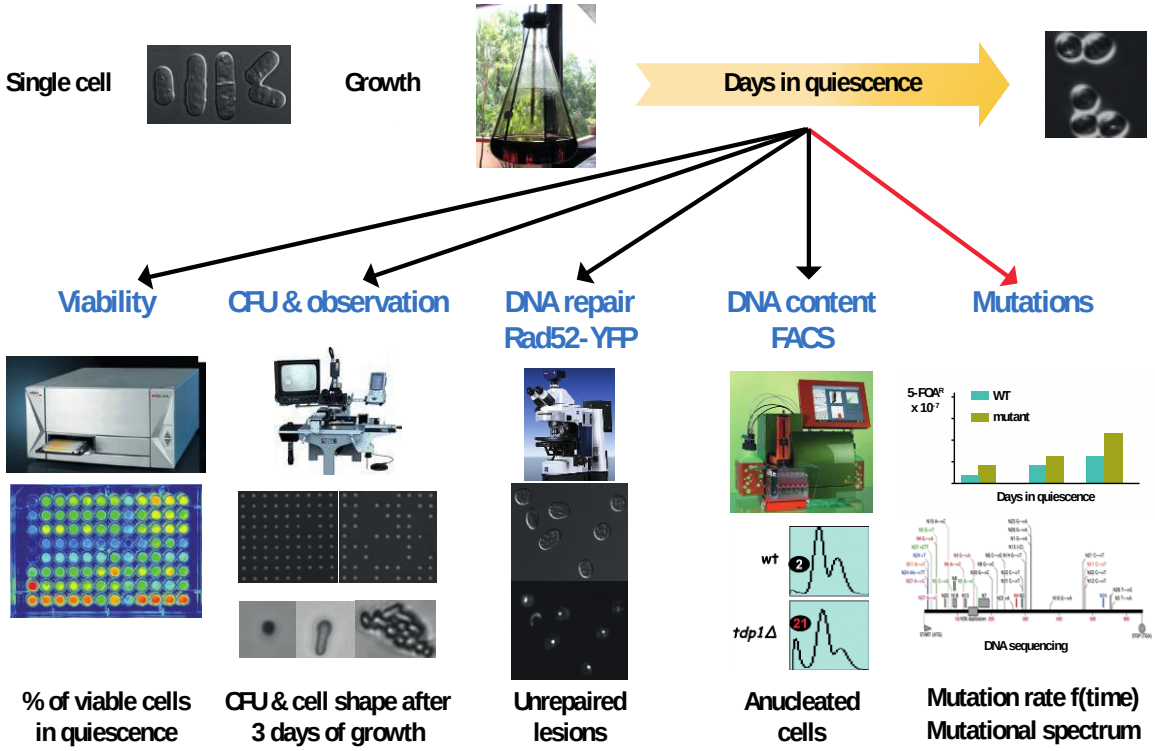


Figure 4: Approaches used to study viability and mutations during quiescence in *S. pombe*.

Quiescent cells in quiescence can be monitored by multiple methods. Viability of a population can be assessed by measuring free ATP. Individual cells can be reseeded on solid medium to determine their viability. This technique allows to determine how the cells that do not form colonies have died. GFP-fusion proteins can be used to observe foci under normal and stress-induced conditions, either during quiescence or immediately following the exit from quiescence. FACS analysis allows to detect the fraction of anucleated cells in a quiescent population (apoptosis). DNA sequencing of a reporter gene or entire genomes of cells that have survived prolonged periods of quiescence can be used to determine the frequency and the spectrum of mutations in quiescent populations.