

## Transcriptional reprogramming in cellular quiescence

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### ABSTRACT

Most cells in nature are not actively dividing, yet are able to return to the cell cycle given the appropriate environmental signals. There is now ample evidence that quiescent G0 cells are not shut-down but still metabolically and transcriptionally active. Quiescent cells must maintain a basal transcriptional capacity to maintain transcripts and proteins necessary for survival. This implies a tight control over RNA polymerases: RNA pol II for mRNA transcription during G0, but especially RNA pol I and RNA pol III to maintain an appropriate level of structural RNAs, raising the possibility that specific transcriptional control mechanisms evolved in quiescent cells. In accordance with this, we recently discovered that RNA interference is necessary to control RNA polymerase I transcription during G0. While this mini-review focuses on yeast model organisms (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*), parallels are drawn to other eukaryotes and mammalian systems, in particular stem cells.

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### Introduction

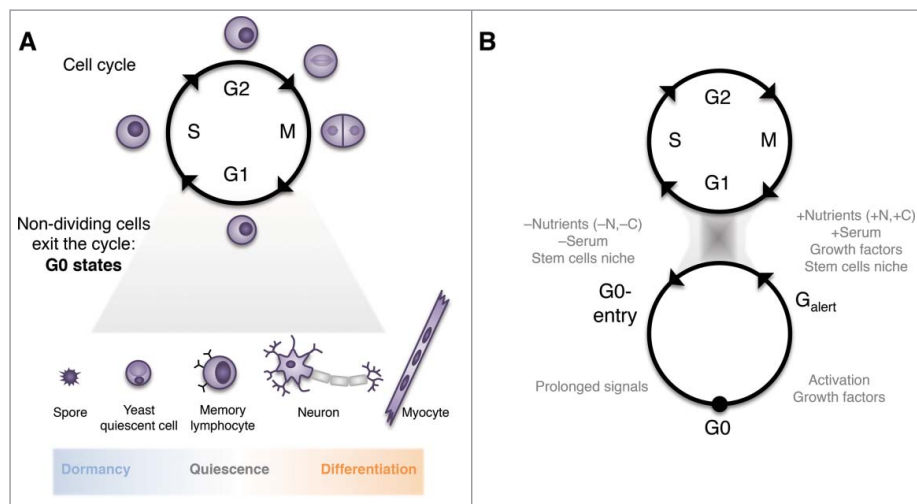
Cells only divide under appropriate conditions. In unicellular organisms such as microbes, this is usually linked to the availability of appropriate nutrients in the environment. Outside of the laboratory, these are often limiting. As a result, most microorganisms are present in a non-dividing (hereafter referred to as “G0”) state in nature.<sup>1–3</sup> Typical examples of non-dividing microbial cells include “viable but non-culturable” (VBNC) cells<sup>4–6</sup> and endospores in bacteria, as well as asexual and sexual spores in fungi. Multi-cellular microbes can also induce large resting structures such as sclerotia and cysts. In multi-cellular organisms possessing different cell types and functions, coordination between cells is important to ensure proper development and requires a tight control on cell proliferation. In the human body, most cells are not actively dividing but either terminally differentiated, such as neurons, or quiescent, such as most stem cells and memory lymphocytes.<sup>7–12</sup> Reactivation of quiescent stem cells is essential for tissue regeneration, for example during wound healing.<sup>13–14</sup>

It is important to note that several types of non-dividing states can exist (Fig 1A). Typically, ‘quiescence’ is defined by metabolic activity and full ability to return to the cell cycle. On the other hand, the absence of metabolic activity is a hallmark of ‘dormancy’, for example in spores. The inability to revert to the cell cycle characterizes post-mitotic cells, and when this inability is irreversibly acquired over-time constitutes a key feature of cellular senescence. For example, progressive loss of this reversibility hinders the self-renewal capability of a stem cell niche, resulting in its depletion.<sup>15–20</sup> A consequence of this phenotypic definition is

that distinct types—and depths—of quiescence can exist. The existence of different depths of cellular quiescence is illustrated for example in muscular stem cells for which cells can enter a ‘poised’ state in response to certain environmental signals, in which they are more responsive to the signal triggering quiescence-exit. A proposed name for this intermediary state has been ‘G-alert’ in stem cells<sup>21</sup> and ‘G<sub>0(A)</sub>’ in T lymphocytes.<sup>22</sup> This has led to the proposal of a ‘quiescence cycle’ alongside the cell cycle (Fig 1B).<sup>21,23</sup> Other examples include the morphological and phenotypic differences in fission yeast between early and late G0 cells,<sup>24,25</sup> and the transcriptome differences in human fibroblasts when G0 is induced by different signals.<sup>26</sup>

### Controversies

Many efforts in the field have sought a molecular basis underlying quiescence—similarly to the molecular characterization of stem cells and of what constitutes ‘stemness’ (the concomitant presence of ‘self-renewal’ and ‘differentiation potential’).<sup>27,28</sup> In part because of the different types of quiescent cells, there are still important controversies surrounding the definition of cellular quiescence. Some are semantic, such as cancer dormancy—“dormancy” qualifies the cancer and not necessarily the cancer cells underlying the “dormant” phenotype, which are thought to be either quiescent cells, and/or an immune balance between growth of a micro-tumor and the immune system.<sup>29</sup> Second, the term “G0” has been used as a synonym of “non-dividing” cells in general, as a synonym for “quiescence,” as well as the notion that cells enter quiescence uniquely from G1.



**Figure 1.** The many facets of cellular quiescence and non-dividing cells. (A) Non-dividing cells exit the cell cycle in response to specific environmental cues to enter different types of G0 states. At one end of the spectrum, microbial spores are metabolically shut-down and dormant; at the other end, cells terminally differentiate in specialized post-mitotic states (such as neurons and myocytes). There is a continuum in terms of reversibility to the cell cycle, metabolic activity and differentiation/specialization. (B) The “quiescence cycle” model proposes a progressive entry into G0, and conversely that in response to environmental signals (such as stem cell activation) G0 cells can enter a poised G<sub>0</sub> alert state facilitating G0-exit.<sup>21–23</sup>

However, in certain conditions cells can enter quiescence from distinct points of the cell cycle<sup>30–33</sup> and doing so preferentially in G1 may be a consequence of metabolic slow-down rather than necessity.<sup>32</sup> This has led to a controversy over the term “G0” when understood as indicating an exit only from G1.<sup>32,34</sup> Third, the choice of the model system and of the signal to induce quiescence has to be considered, as different organisms show different responses to starvation of different nutrients, such as nitrogen-starvation in budding yeast haploids vs. diploids,<sup>35</sup> and in fission yeast.<sup>24,36</sup> In particular, the use of stationary-phase cultures does not necessarily accurately reflect quiescent cells, as these cultures are heterogeneous and most cells not long-lived.<sup>37</sup> Last but not least, it has proven difficult to obtain biomarkers specific for G0 cells.

### Markers of cellular quiescence

The first proposed quiescence-specific marker was ‘statin’ in quiescent fibroblasts (not to be confused with the statin class of drugs: statin was later revealed to be lamin A),<sup>38–39</sup> but was subsequently found to not be completely specific.<sup>40</sup> Nowadays, the most commonly used marker in mammalian cells is pKi-67, which can distinguish between quiescent and proliferating cells; however, pKi-67 is still expressed at low levels in quiescence as it acts in rRNA synthesis.<sup>41,42</sup> Other proposed markers in mammalian cells include CDK inhibitors such as p27<sup>CDKN1B/Kip1</sup>,<sup>43–46</sup> which led to a Venus-p27K construct discriminating G0 and G1 cells,<sup>47</sup> p21<sup>Cip1/Waf1/Sdi1</sup>,<sup>48</sup> as well as the retinoblastoma (RB) protein (p105, p107, p130) family of cell cycle inhibitors, which regulate the E2F transcription factors,<sup>49–52</sup> and the RB yeast analog Whi5.<sup>53,54</sup> Quiescent cells have often been defined negatively i.e. what the cell does not do, such as absence of PCNA (i.e., absence of S-phase), and Hoechst 33342+Pyronin Y staining (DNA+RNA content) based on low RNA expression in G0.<sup>55</sup> Transcriptomic studies in human fibroblasts,<sup>26</sup> and in fission yeast,<sup>56,57</sup> have identified potential core ‘quiescent

program’ genes. This will help identifying better markers for cellular quiescence.

### Quiescence: Conserved or specialized?

Despite the importance of quiescence in all organisms, the molecular mechanisms underlying transition between growth and quiescence are not very well understood, nor are the molecular mechanisms of quiescence maintenance over long periods of time. An important question is whether molecular mechanisms underlying cellular quiescence are conserved across evolution. Because states of quiescence can be seen in prokaryotes, unicellular eukaryotes and multicellular organisms, it is likely that some aspects of quiescence are conserved.<sup>36</sup> However, it has been proposed that because cycling cells, in any organism, use their energy predominantly to grow and divide, the most likely conserved mechanisms in G0 would be mechanisms of shutting-down growth and division, and that apart from this common aspect, quiescent cells are driven to specialization.<sup>58</sup> Further study of quiescent cells in different organisms will help us determine to what extent this may be the case; furthermore, this dual aspect of conservation/specialization highlights the importance of in-depth study of several model organisms.

### Quiescence in pathological contexts

Quiescence is also important in pathological contexts, such as cancer, degenerative diseases, and microbial infection. Two hallmarks of cancer are the inability to sustain quiescence, resulting in aberrant proliferation, and the inability to trigger apoptosis to stop these cells. It is therefore especially important to study this question not only from the viewpoint of uncontrolled growth and proliferation, but in the context of unwarranted quiescence-exit.<sup>36,58,59</sup> Most cancer-related deaths are caused by secondary tumors after treatment of the primary cancer; one factor in cancer persistence and resurgence is thought

to be the presence of quiescent cancer cells, which are less sensitive to antitumor drugs.<sup>29,60</sup> An improved understanding of cellular quiescence would therefore permit better targeting of G0 cancerous cells, and develop more specific drugs.<sup>61-64</sup>

In the context of human pathogens, the formation of quiescent cells contributes to persistence of infection within the body, such as for the bacterial pathogen *Mycobacterium tuberculosis*<sup>3</sup> and for fungal pathogens such as *Cryptococcus* spp., which can survive in a latent state inside host macrophages,<sup>65,66</sup> and *Candida albicans*.<sup>67</sup> Furthermore, the persistence of quiescent cells in the environment is a matter of human health, illustrated by the fact that VBNC cells of pathogens such as *Vibrio vulnificus* maintain their infectious capability.<sup>3,68-70</sup>

### **Fission yeast is an ideal model for cellular quiescence**

Several model organisms are commonly used for the study of cellular quiescence; the most notable are the budding yeast *Saccharomyces cerevisiae*,<sup>71,72</sup> the fission yeast *Schizosaccharomyces pombe*,<sup>36,73</sup> *in vitro* mammalian cell culture,<sup>46,74</sup> the wing cells of *Drosophila melanogaster*,<sup>75,76</sup> and stem cells.<sup>9,77</sup> In fission yeast, quiescence is induced by nitrogen deprivation of a prototrophic heterothallic strain. This results in 2 divisions without growth, resulting in haploid cells with 1c DNA.<sup>24</sup> An advantage in using this particular model and signal is that G0-induction is synchronous,<sup>78</sup> all cells retain full viability for extended periods of time,<sup>24</sup> and G0-exit is synchronous,<sup>56</sup> more so than when quiescence is brought about by glucose- or phosphate-starvation.<sup>36</sup> This improved homogeneity of G0 cells is a big advantage over stationary-phase cultures or *in vitro* cell cultures, which are a heterogeneous population consisting mostly of cells that are not long-lived.<sup>23,37</sup> Furthermore, this model is distinct to that of dormant spores, as nitrogen-deprived fission yeast quiescent cells still require a carbon source for viability.<sup>24</sup> Genetic requirements common to several model systems are more likely to be conserved in higher eukaryotes.<sup>36</sup>

### **Cellular quiescence is transcriptionally and metabolically active**

While the distinction between cellular quiescence and dormancy is not always trivial to assay, these 2 states are conceptually different: the maintenance of metabolic and transcriptional activity in quiescent cells implies the maintenance of an operational transcriptional machinery. In fission yeast, quiescent cells have distinctive transcriptomic signatures.<sup>56,57</sup> mRNA and rRNA levels are greatly reduced (to ~30% and ~20% respectively) yet display a high diversity of transcripts and of proteins.<sup>57</sup> Regulation of RNA pol I and RNA pol III is tightly correlated with growth in all organisms<sup>79</sup> and accordingly, we have found that the binding of RNA polymerase I to rDNA is decreased over time in G0, reaching 10% that of G0-entering cells after 8 d (= 1% of cycling cells in rich medium).<sup>80</sup> Conversely, the proportion of H3K9-methylated silent rDNA repeats is increased.<sup>80</sup>

Quiescent fission yeast cells display an extensive metabolomic change,<sup>81,82</sup> reflecting their shift in metabolism and specialization in recycling of nutrients. In this model, the maintenance of quiescence requires glucose<sup>24</sup> and accordingly, several of the

most abundant transcripts in G0 code for proteins involved in glycolysis,<sup>56</sup> reflecting increased oxidative metabolism.<sup>57</sup> One of the immediate changes caused by nitrogen-starvation is a reduction in the free amino acid pool.<sup>82</sup> Autophagy is required for quiescence maintenance for recycling of amino acids<sup>83-87</sup> and nucleotides via degradation of RNAs.<sup>88,89</sup> Increased catabolism is also reflected by the vast size increase of the vacuole in quiescent cells,<sup>24</sup> and many vacuolar genes are essential for quiescence establishment.<sup>73</sup> Furthermore, new organelles are formed in quiescent fission yeast and budding yeast cells, such as actin bodies,<sup>90</sup> proteasome storage granules<sup>91</sup> rapidly re-imported into the nucleus upon quiescence-exit,<sup>92</sup> and a quiescent microtubule bundle.<sup>93,94</sup>

Another indication of the active state of quiescent *S. pombe* cells is that they are still able to repair DNA damage.<sup>95,96</sup> Importantly, transcription itself is a source of damage.<sup>97</sup> Transcription of genes exposes single-stranded DNA, which is more prone to several types of damage including cytidine deamination.<sup>98</sup> Quiescent cells also retain the ability to repair double-strand DNA breaks and do so preferentially by non-homologous end-joining.<sup>95</sup> However, certain DNA lesions may persist in G0 as an increase of DNA repair is seen in several types of G0-exiting cells, such as haematopoietic stem cells<sup>99</sup> and fission yeast cells (where repair is detected as Rad52 foci).<sup>80,100</sup> In wild type, the proportion of Rad52 foci forming during the first S-phase when cells are exiting quiescence and during the following S-phases are similar, indicating that the level of unrepaired lesions after 48 hours of quiescence and between 2 replications are similar. The number of Rad52 foci dramatically increases in several DNA repair mutants, indicating that spontaneous DNA lesions are efficiently repaired during quiescence.<sup>96</sup>

Several large-scale screens have been conducted in fission yeast to identify genes important for maintaining viability during cellular quiescence.<sup>73,86</sup> Essentiality in dividing cells does not correlate with essentiality in quiescence, and it has been estimated that ~25% of essential genes are necessary for both ('super-housekeeping' genes).<sup>73</sup> An especially interesting set of genes identified is involved in transcription, such as specific alleles of the RNA pol II C-terminal domain phosphatase Fcp1 and of the common RNA pol I/III subunit AC40,<sup>73</sup> and the Krüppel-like family transcription factor Klfl.<sup>25</sup>

As the characterization of the transcriptome in early G0 cells has allowed identification of several genes essential for quiescence,<sup>56</sup> an important future work would be to characterize the transcriptome and proteome after longer times spent in quiescence, as these might help identify factors involved for long-term maintenance rather than early adaptations to the metabolic stress caused by quiescence induction.

### **Epigenetic mechanisms of quiescence maintenance?**

We propose that epigenetic mechanisms are key to maintaining cellular quiescence. Cells in the cell cycle or in quiescence possess the same genotype, yet exhibit distinct phenotypes. It is therefore possible to envision cellular quiescence as a kind of cellular differentiation.<sup>58</sup> Accordingly, early cloning experiments were greatly enhanced by the induction of fibroblasts into quiescence in advance of nuclear transplantation, leading to the realization of its role as a state of major epigenetic

reprogramming.<sup>101-103</sup> Accordingly, evidence for essential epigenetic processes in quiescent cells is increasing.

### RNA interference controls RNA polymerases

In *S. pombe*, the key proteins Dicer, Argonaute and RNA-dependent RNA polymerase (respectively Dcr1, Ago1 and Rdp1) are involved in co-transcriptional silencing of pericentromeric repeat transcripts, processing these transcripts into small interfering RNAs (siRNAs) and recruiting the silencing CLRC/Rik1 complex, harboring the H3K9 methyltransferase Clr4<sup>SUV39H1</sup>. This results in the formation of H3K9me heterochromatin.<sup>104-105</sup> Pericentromeric heterochromatin allows proper chromosome segregation in mitosis,<sup>104,106</sup> in meiosis<sup>107</sup> and in G0-entry mitoses.<sup>80</sup> More recently, RNAi has been proposed to also act in post-transcriptional silencing (PTGS) pathways in fission yeast,<sup>108</sup> and in control of stress-related genes.<sup>109</sup>

Transcriptional silencing is a result of the inhibition of RNA polymerase II. In fission yeast heterochromatin, H3K9 is methylated, and binds HP1-like proteins (Swi6 and Chp2). In turn, Swi6<sup>HP1</sup> recruits the SHREC complex, containing histone deacetylases,<sup>110,111</sup> resulting in hypoacetylation of H3 and H4 tails, limiting RNA pol II recruitment. In S-phase, Swi6<sup>HP1</sup> is displaced by H3S10 phosphorylation by Aurora kinase,<sup>112</sup> alleviating this silencing; this results in transient transcription of peri-centromeric repeats that will trigger RNAi and heterochromatin formation on both daughter strands. The activity of DNA polymerases and RNA polymerase II on the same template during S-phase requires both processes to be tightly co-regulated. Therefore, we hypothesized that RNAi may act more directly on polymerases, and discovered that RNAi releases RNA pol II from pericentromeric heterochromatin,<sup>113</sup> as well as at several highly-transcribed euchromatic loci, tDNAs, and rDNA.<sup>114</sup> This sheds new light on why certain specific RNA pol II mutants lose silencing in *S. pombe*, such as *rpb2-m203* (N44Y)<sup>115</sup> and *rpb7-G150D*.<sup>116</sup>

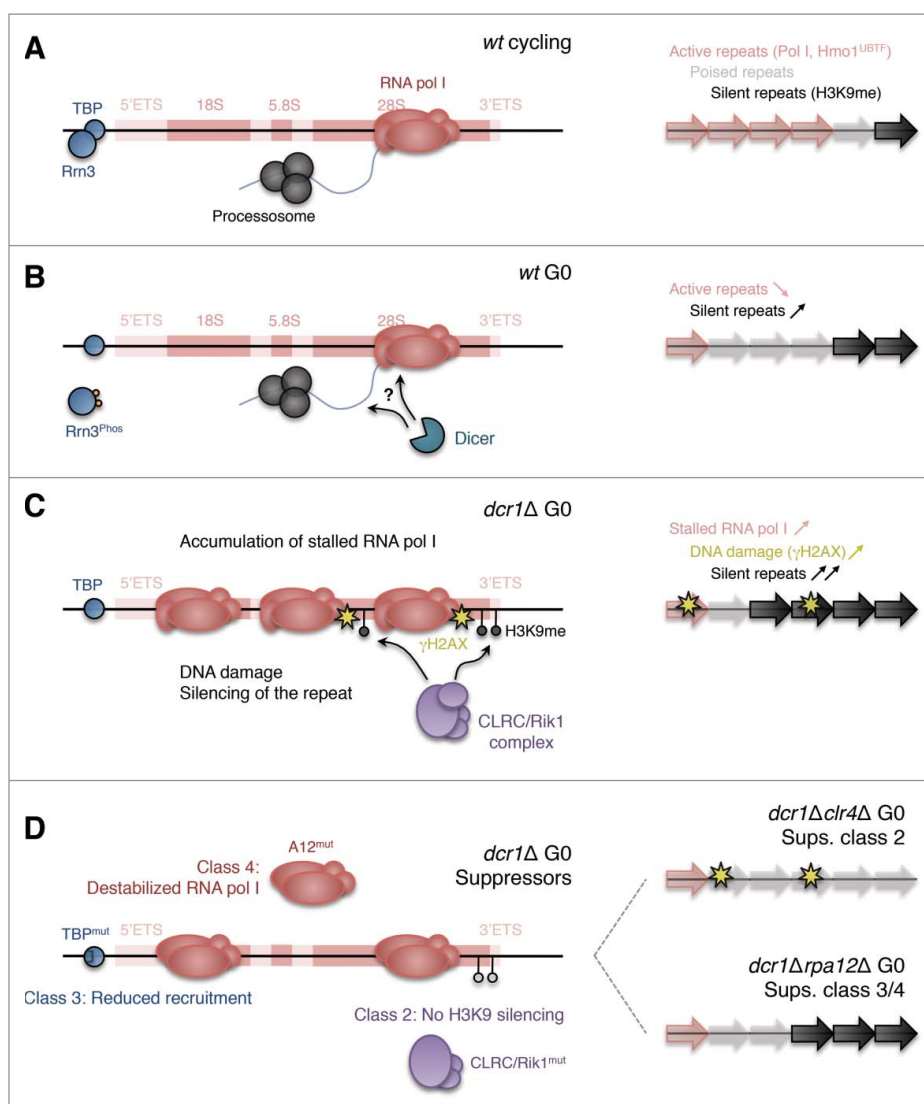
Finding an important role for RNAi at tDNAs and rDNA loci was one of the reasons that prompted us to investigate the response of RNAi mutants in quiescence. Indeed, we found that RNAi mutants have G0-entry defects and lose viability during quiescence maintenance. We found that RNAi releases RNA pol I from rDNA specifically in G0 (Fig 2ABC), and that the G0 defects of RNAi deletion mutants are suppressed by reducing RNA pol I binding (such as in a specific TBP mutant, *tbp1-D156Y*) or by destabilizing RNA pol I by deleting its non-essential subunit A12<sup>80</sup> (Fig 2D). Interestingly, mutants in equivalent subunits in RNA pol I (A12) and in RNA pol II (Rpb9 + TFIIS) specifically suppress, respectively, RNAi quiescence defects and RNAi silencing defects.<sup>80,117</sup> This strong parallel buttresses the proposition that RNAi proteins are indeed closely associated to RNA polymerase holocomplexes. Furthermore, the parallel role of A12 and TFIIS in polymerase backtracking<sup>118</sup> may indicate that in the absence of Dicer, this activity becomes detrimental. Pausing of RNA pol II during transcription termination results in polymerase backtracking, and the 3' end of the RNA is targeted by the RNA exosome.<sup>119</sup> Absence of TFIIS reduces the level of read-through, suggesting that TFIIS and the RNA exosome compete for the RNA 3' end<sup>119</sup> and that in the absence of Dicer, the activity of the RNA

exosome becomes essential. The fact that, in the case of RNA pol I, polymerase accumulation in Dicer mutants is not only seen at the 3'ETS region, but over the whole locus (rDNA promoter, 5'ETS and multiple sites in 18S, 5.8S and 28S),<sup>80</sup> suggests that Dicer may play a role during elongation in G0 rather than only during termination.

One difference is that Dicer's requirement in RNA pol II release from rDNA is independent of its catalytic activity and of Argonaute,<sup>114</sup> while all RNAi proteins are required for RNA pol I release in G0, including Dicer's catalytic activity.<sup>80</sup> Whether this reflects a difference in different RNA polymerase compositions or cell cycle stage would be an interesting aspect to investigate. Presumably, Dicer acts on a RNA template; and the genetic requirement for catalytic activity and RNAi proteins in this novel mechanism also points to specific small RNAs (sRNAs) being involved. However, we have not found Dicer-dependent novel sRNAs in G0.<sup>80</sup> What is the molecular target of Dicer in G0 cells? One possibility is that the rRNA itself is targeted by Dicer, potentially in a torpedo-like mechanism as used by the RNase III Rnt1.<sup>120</sup> In *Candida albicans*, the Dicer ortholog (although closer to the RNT1 family<sup>121</sup>) cleaves the 28S rRNA at the 3'ETS and the 3' tail of U4 snRNA,<sup>122</sup> but we did not detect signatures of this activity in our *S. pombe* sRNA-seq<sup>80</sup> (and unpublished observations). Another possibility is that Dicer may act indirectly on the rRNA, potentially through single-cleavage of a specific non-coding RNA (ncRNA) in G0, in a manner reminiscent of pRNA-mediated<sup>123</sup> and long ncRNA-mediated<sup>124,125</sup> rDNA silencing in mammals. Interestingly, the few genomic loci that are upregulated in Dicer mutants in G0 include several long ncRNAs of unknown function (unpublished observations); testing whether any of these ncRNAs plays any function in rDNA silencing is an important next step. Finally, similarly to its function controlling RNA pol I and RNA pol II, we hypothesize that RNAi may release RNA pol III through its C11 subunit at specific genomic loci.

### Histone marks in cellular quiescence

Dicer mutants cause over-activation of the CLRC/Rik1 silencing complex at the rDNA resulting in unchecked accumulation of H3K9 methylation (Fig 2C).<sup>80</sup> H3K9me causes cell death, as H3K9me mutants rescue RNAi mutants specifically in G0. In fact, our suppressor screen of G0 viability loss of *dcr1Δ* uncovered a large number of mutants involved in heterochromatin formation, and may potentially be used to uncover novel silencing factors. In addition to CLRC/Rik1 complex mutants (*rik1-V449G*, *rik1-K812\**, *rik1-A875P*, *rik1-T942K*, *clr4-R126\**, *clr4-C317F*, *clr4-Y451\**, *raf2-G37V*), we obtained Swi6<sup>HP1</sup> mutants (*swi6-T278K*, *swi6-W293\**).<sup>80</sup> In *S. pombe*, the CLRC/Rik1 complex associates with DNA polymerase  $\epsilon$  (Cdc20) through its replication targeting subunit Raf2, and several *cdc20* mutants lose silencing<sup>126,127</sup>; further, we uncovered a novel *cdc20-1* mutant required for Dicer mutant cells to enter G0 (unpublished observation). Proper regulation of H3K9me in G0 is important: wild-type cells show a physiologic increase of H3K9me at the rDNA to adjust the active/silent rDNA repeat ratio. Complete loss of H3K9me results in a mild defect in quiescence maintenance (although much milder than defects resulting from H3K9me over-accumulation).<sup>80</sup>



**Figure 2.** Model for the novel essential role for Dicer in RNA polymerase I release in G0. (A) In wild-type cycling cells, RNA pol I transcribes the rDNA repeats. (B) Wild-type G0 cells lower the recruitment of RNA pol I to rDNA, in part via phosphorylation of the Rrn3 initiation factor, shifting the ratio of active vs. silent rDNA repeats. Dicer contributes to RNA pol I release, although it is still unknown whether this occurs directly at the level of rRNA or via RNA pol I itself. (C) Dicer mutants in G0 are defective in RNA pol I release, resulting in accumulation of stalled RNA pol I, DNA damage, and the recruitment of the silencing CLRC/Rik1 complex at the repeat, causing a hyper-silencing of rDNA repeats via H3K9 methylation. (D) The Dicer defect is suppressed by mutants in the H3K9 methylation pathway (class 2: such as *dcr1Δclr4Δ*), by reducing RNA pol I transcription initiation (class 3: such as *dcr1Δtbp1-D156Y*), or by destabilizing RNA pol I itself (class 4: such as *dcr1Δrpa12Δ*).<sup>80</sup> (Note: class 1 suppressors are not represented, and concern chromosomal segregation during G0-entry).

Several other histone marks are thought to play an important role in quiescence maintenance, belonging to several classes: (i) marks to silence proliferation-genes, such as lack of histone H3 and H4 acetylation; (ii) bivalent marks at 'poised' genes, presumably for efficient G0-exit given the appropriate signal. Silencing marks cover a large part of the genome, and are thought to be an important factor in chromatin compaction, a hallmark of G0 cells in most organisms.<sup>24,128,129</sup> In budding yeast, condensin,<sup>129</sup> the H1-like Hho1 protein and H4K16 deacetylation<sup>130-133</sup> are thought to participate in this process; several quiescence-specific transcription factors (Xbp1 and Stb3) have been shown to recruit the class I histone deacetylase (HDAC) Rpd3 to half of gene promoters,<sup>134,135</sup> and to rDNA.<sup>136</sup> However, *Schizosaccharomyces* spp. do not have Rpd3 orthologs but only the Clr6 and Hos2 lineages of class I HDACs,<sup>137</sup> and their genomes do not encode H1 orthologs.

The contribution of condensin to G0 chromatin compaction has not yet been investigated in *S. pombe*. Quiescent naive T cells also display chromatin compaction by condensin.<sup>138</sup> Another mark of interest is H4K20 methylation, which is found at facultative heterochromatin in quiescent muscle stem cells,<sup>139</sup> in primary fibroblasts,<sup>140</sup> in serum-deprived NIH 3T3 cells,<sup>125</sup> and accumulates in quiescent brine shrimp encysted embryos.<sup>141</sup> H4K20 methylation is present in *S. pombe*, associates with histone turn-over rate at certain genes<sup>142</sup> and DNA repair via Crb2<sup>53BP1</sup> recruitment,<sup>143,144</sup> but has not yet been investigated in cellular quiescence. In *S. pombe*, it has been proposed that H3K9 methylation contributes to general silencing in G0.<sup>145</sup> The second class of marks believed to be pivotal for cellular quiescence is thought to signal a 'bivalent' state. Indeed quiescent ES cells display bivalent marks (H3K4 methylation and H3K27 methylation) at the promoters of many genes

important for lineage differentiation.<sup>146,147</sup> *S. cerevisiae* quiescent cells retain high levels of active marks such as H3K4me3, H3K36me3 and H3K79me3,<sup>148</sup> and H3K36 methylation by ASH1/Trithorax is important for quiescence and self-renewal of haematopoietic stem cells.<sup>18</sup> The contribution of these histone marks to quiescence maintenance has not yet been investigated in fission yeast.

An important question is whether specific novel histone marks are present during cellular quiescence. To our knowledge, this has not yet been investigated; while mass-spectrometry of histone post-translational modifications have allowed the identification of many novel marks of unknown function,<sup>149–153</sup> including in primarily non-dividing tissues such as the mouse brain,<sup>154</sup> such studies are usually conducted, in yeast, on growing cells.<sup>150,155,156</sup> An alternative, complementary approach would be to generate a library of histone mutants<sup>157,158</sup> to assay the effect of each amino acid on viability specifically during cellular quiescence. Toward this goal, we have designed a system for mutating H3 in *S. pombe*,<sup>80</sup> more physiological than previous systems<sup>159</sup> and therefore amenable to G0 study as well as making partial loss-of-function mutants. This approach has the potential to uncover novel histone marks of biological interest.

## Conclusion

The importance of quiescence makes it likely not only that many molecular mechanisms will be discovered for its establishment and regulation, but also that many of these basic mechanisms may be evolutionarily conserved.<sup>36</sup> Importantly, the essentiality of genes is usually determined using laboratory growth conditions, yet in nature, the prevalence of non-dividing states stresses the importance to identify genes essential specifically in quiescence (and genes essential for both growth and quiescence, often termed “super-housekeeping” genes<sup>73</sup>). Because of the reversible nature of quiescence and because it can be considered a kind of cellular differentiation, we think that epigenetic mechanisms may be key factors controlling maintenance of quiescence and rewiring its transcriptional program. Preliminary data suggests that indeed, other key chromatin genes are specifically essential in G0 in addition to key RNAi proteins<sup>80</sup> (and unpublished observations). In fact, it is important to test familiar mechanisms in quiescence as it appears—as is the case with RNAi—that quiescence can uncover novel mechanisms and functions that are important or essential. This also applies for numerous genes that are apparently devoid of function. Indeed mutants affected in growth are not necessarily affected in quiescence and vice versa.<sup>73,86</sup> Because of its advantages in studying cellular quiescence as well as in studying epigenetic pathways, we think that the fission yeast *S. pombe* is poised to continue to be a model system of choice. Accordingly, a lot of resources for fission yeast G0 have been developed recently, such as the G0 transcriptome<sup>56,57</sup> proteome,<sup>57</sup> metabolome.<sup>81</sup> Gene replacement in yeast also allows the use of molecular tools such as G0 over-expressing promoters,<sup>80,160</sup> and G0-shutoff systems.

Advances in fundamental research in cellular quiescence will have consequences in several fields relevant to human health, such as stem cell biology and cancer biology. For example, a strategy that has been successful in developing novel anti-

cancer drugs is to identify negative epistasis (synthetic lethality) networks.<sup>161–163</sup> Large-scale negative epistasis screens can be conducted on yeast before being tested on human cells, allowing more interactions to be tested<sup>164</sup>; importantly, there is good conservation of negative epistatic interactions. Using this type of approach to screen for synthetic lethality specific to quiescence would therefore be a promising approach to target quiescent cancer cells, an essential step toward effective treatment and avoiding relapse. We conclude that the investigation of cellular quiescence therefore opens not only a deeper understanding of fundamental biology, but also new avenues in medicine.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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