SCREENING METHOD FOR CELL AGEING

Field of Invention

The present invention relates to methods of screening to identify compounds which have an effect on ageing of a cell, more particularly chronological ageing of a cell, methods of diagnosing disorders related to a change in the chronological life span of a cell.

Background

The target of rapamycin complex, TORC1, is conserved from yeast to man and has critical roles in sensing and signalling the nutrient and stress status of the cell, thus controlling the balance between cell growth[1-5] and cell survival[6-11]. In budding yeast TORC1 promotes fermentative growth on glucose and down regulates respiration[12,13]. TORC1 contains a phosphatidylinositol kinase (PI3-K)-related kinase, either Tor1 or Tor2. The macrolide rapamycin[14], in a complex with Fpr1 (Fk506-sensitive Proline Rotamase), binds to Tor1/2 causing cells to enter a state that resembles nutrient limitation[15] probably due to a change in the substrate specificity of the Tor kinase[16]. This new state of the cell is associated with changes in patterns of gene expression, particularly genes required for respiration and stress resistance[6,10,17,18]. The expression of many TORC1 genes is dependent on the SAGA family of transcriptional co-activator complexes including SAGA (Spt-Ada-Gcn5-Acetyltransferase)[19,20], SLIK (SAGA-like)[21] and SALSA (SAGA altered, Spt8 absent)[22-24]. SAGA, SLIK and SALSA contain the lysine acetyltransferase (KAT) Gcn5[21-23], with lysine 14 on histone H3 (H3K14ac) as a substrate, but differ in their abundance, the genes they regulate and subunit composition[19,24]

The inventors have discovered that H3K18 acetylation, is central to a mechanism that controls the balance between cell growth and longevity. They have also identified a number of genes involved in the SAGA SLIK and SALSA complexes whose disruption results in an increase in chronological lifespan.

Summary

In a first aspect, there is provided a method for increasing the chronological lifespan of a cell comprising disrupting the function of the SAGA complex in said cell, wherein the complex is disrupted by disrupting the function of the Spt7 gene or a homologue thereof.

According to the present disclosure, there is provided a method for increasing the chronological lifespan of a cell comprising disrupting the function of at least one of the SAGA, SLIK and/or SALSA complexes in said cell.

According to the present disclosure, there is also provided a method for identifying a potential modulator of the chronological life span (CLS) of a cell, comprising the steps of

i) contacting a cell having a known Histone 3 Lysine 18 (H3K18) acetylation status with a test compound; and

ii) determining if said compound has an effect on the acetylation status of H3K18 in said cell;

wherein, a change in the acetylation status of H3K18 in the cell indicates that the compound modulates CLS.

According to the present disclosure, there is also provided a modulator of the CLS of a cell identified by the method defined above.

According to the present disclosure, there is also provided a method for identifying the replication status of a cell comprising identifying the acetylation state of H3K18 , wherein the presence of an acetyl modification of H3K18 indicates that the cell is an actively replicating cell and the absence of an acetyl modification of H3K18 indicates a cell which is no longer replicating.

According to the present disclosure, there is also provided a method of identifying a change in the CLS of a cell comprising identifying the acetylation state of H3K18 in the cell and comparing this to the acetylation state of a control cell, wherein loss of H3K18Ac when compared to the control cell indicates an increased CLS and acquisition of H3K18Ac when compared to the control cell indicates a reduced CLS.

According to the present disclosure, there is also provided a method of diagnosing a disorder associated with a change in the CLS of a cell, said method comprising identifying the acetylation status of H3K18 in a cell previously isolated from a subject and comparing said acetylation status to the acetylation status of a control cell.

Detailed description of the invention

It will be understood that any preferred embodiments described herein in relation to one aspect of the present invention can, where appropriate, be equally applicable to any other aspect of the invention.

According to a first aspect there is provided a method for increasing the chronological lifespan of a cell comprising disrupting the function of at least one of the SAGA, SLIK and/or SALSA complexes in said cell.

As used herein the term chronological life span refers to the time cells in a stationary phase culture remain viable.

It will be understood that the function of the at least one of the SAGA, SLIK and/or SALSA complexes may be disrupted directly or indirectly. These complexes play a crucial role in controlling of the acetylation state and CLS of a cell, but differ in their levels depending upon the status of the cell and its environment.

As used herein the terms directly and indirectly in relation to interaction with the recited complexes refer to an interaction with either the complex itself, or with a gene product from a gene encoding a peptide which forms part of the complex, or with the gene product from a gene which allows the complex to form.

Preferably, disruption is effected through disruption of at least one gene or a product of at least one gene selected from the group consisting of Spt3, Rtg2, Gcn5, Ubp8, Spt7, Spt8 and/or Snf1 or their homologues.

The term homologue as used herein refers to an analogous gene from a different organism which performs the same function and in general shows some degree of sequence homology. The skilled person will understand that the above genes from *S. cerevisiae* have homologues in other organisms including mammels. For example, Spt3 shows homology to human SUPT3H-203; Gcn5 shows homology to human KAT2B-001 and KAT2A-001; Spt7 shows homology to human SUPT7H and SNF1 shows homology to PRKAA1 and PRKAA2.

It will be understood that these genes encode products which form part of the SAGA, SLIK and/or SALSA complexes, or interact with said complexes in manner so as to affect acetylation of histones in a cell.

Preferably, the disruption is effected through disruption of SPT7 (SEQ ID NO:11) or SPT7-217 (SEQ ID NO:19).

As used herein the term "disrupting the function", "disruption of the function" or "disrupts the function" when used in relation to a gene or gene product refers to disrupting the expression of the gene or disrupting the activity of the encoded polypeptide. It will be further understood that any stage of gene expression between initiation of transcription and production of a mature protein can be disrupted. The skilled person will understand that this will include epigenetic means of controlling gene expression through controlling chromatin structure as well as transcriptional, translational and post translation means of controlling gene expression.

It will be understood that by disrupting expression of a gene as used herein is meant preventing or inhibiting production of a functional polypeptide by any means known in the art and that disrupting the activity of the encoded polypeptide refers to disrupting interaction of the functional polypeptide with one or more of it's binding partners such that the polypeptide does not perform it's function. The production or function may be fully or partially prevented. In one embodiment, preferably the production or function of the gene product is fully prevented, i.e. there is no active gene product. In some instances the production or function of the gene product may be disrupted such that there is only about 5%, about 10% about 20%, about 30%, about 50%, about 60%, about 70%, about 80%, about 90% or about 95% of the wild type level of expression remaining.

As used herein by inhibiting production of a functional polypeptide it is meant that the production of the gene product may be prevented or inhibited by (a) knocking out said gene; (b) post-transcriptionally silencing said gene through for example the use of iRNA or antisense RNA (gene silencing); (c) transcriptionally silencing said gene by, for example, epigenetic techniques; (d) preventing or altering the function of the gene product by the introduction of at least one point mutation; (e) post translationally inactivating the gene product.

In one preferred embodiment, expression of the gene or homologue is disrupted by iRNA.

Preferably, the cell is transformed with a plasmid/vector encoding an iRNA under control of a promoter. It will be apparent that this promoter may be a constitutive promoter and/or a tissue specific promoter.

As used herein the term iRNA refers to RNA interference (RNAi). This is a method of post-transcriptional gene silencing (PTGS) in eukaryotes induced by the direct introduction of dsRNA (Fire A, *et al.*, (1998)).

In a further preferred embodiment expression of the gene is disrupted at the transcriptional / DNA level. Preferably, said disruption is effected by insertion of at least one nucleotide into the gene or deletion of at least one nucleotide from the gene.

In a further embodiment, the disruption of the gene is effected by introduction of at least one point mutation.

It will be understood that in the case of disruption of the interaction of the polypeptide with one or more of it's binding partners. this disruption can be by any suitable means, for example, competitive inhibition, non-competitive inhibition, mixed inhibition or uncompetitive inhibition.

The present invention encompasses the use of sequences having a degree of sequence identity or sequence homology with amino acid sequences of the polypeptides defined herein or of any nucleotide sequence encoding such a polypeptide (hereinafter referred to as a "homologous sequence(s)"). Here, the term "homologous" means an entity having a certain homology with the subject amino acid sequences and the subject nucleotide sequences. Here, the term "homology" can be equated with "identity".

The homologous amino acid sequence and/or nucleotide sequence should provide and/or encode a polypeptide which retains the functional activity and/or enhances the activity of the enzyme.

In the present context, a homologous sequence is taken to include an amino acid sequence which may be at least 50, 60, 70, 75, 80, 85 or 90% identical, preferably at least 95%, 97%, 98% or 99% identical to the subject sequence. Typically, the homologues will comprise the same active sites etc. as the subject amino acid sequence. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions).

In the present context, a homologous sequence is taken to include a nucleotide sequence which may be at least 50, 60, 70, 75, 80, 85 or 90% identical, preferably at least 95%, 97%, 98% or 99% identical to a nucleotide sequence encoding a polypeptide of the present invention (the subject sequence). Typically, the homologues will comprise the same sequences that code for the active sites etc. as the subject sequence. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions).

Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate % homology between two or more sequences.

% homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause the following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed.

Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the the Vector NTI (Invitrogen Corp.). Examples of software that can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel et al 1999 Short Protocols in Molecular Biology, 4th Ed - Chapter 18), BLAST 2 (see FEMS Microbiol Lett 1999 174(2): 247-

50; FEMS Microbiol Lett 1999 177(1): 187-8 and tatiana@ncbi.nlm.nih.gov), FASTA (Altschul et al 1990 J. Mol. Biol. 403-410) and AlignX for example. At least BLAST, BLAST 2 and FASTA are available for offline and online searching (see Ausubel et al 1999, pages 7-58 to 7-60).

Suitably, the degree of identity with regard to a nucleotide sequence is determined over at least 20 contiguous nucleotides, preferably over at least 30 contiguous nucleotides, preferably over at least 40 contiguous nucleotides, preferably over at least 50 contiguous nucleotides, preferably over at least 60 contiguous nucleotides, preferably over at least 100 contiguous nucleotides.

Suitably, the degree of identity with regard to a nucleotide sequence may be determined over the whole sequence.

As used herein, the term fragment refers to a fragment of the sequence which provides and/or encodes a polypeptide which retains the functional activity and/or enhances the activity of the enzyme.

When referring to a polypeptide fragment, preferably, the fragment is at least 50 amino acids in length. More preferably, the fragment comprises at least 100, 200, 300, 400 or 500 600, 700, 800, 900 or 1000 continuous amino acids from the subject sequence, for example SEQ ID NO:19, up to and including a polypeptide comprising one amino acid less than the full length protein.

When referring to a polynucleotide fragment, preferably the fragment comprises at least 100 nucleotides, more preferably, at least 200, 500, 800, 1000, 1500 or more nucleotides, up to and including a polynucleotide comprising one nucleotide less than the full length polynucleotide.

It will be understood by the skilled person that polynucleotides encoding a particular polypeptide can differ from each other due to the degeneracy of the genetic code. Included herein are the use of such polynucleotides encoding the polypeptide of the present invention.

It will be further apparent to the skilled person that the term homologous sequence in relation to a polynucleotide sequence can refer to a sequence which binds under stringent conditions to the polynucleotide sequence.

Hybridisation conditions are based on the melting temperature (Tm) of the nucleotide binding complex, as taught in Berger and Kimmel (1987, Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol. 152, Academic Press, San Diego CA), and confer a defined "stringency" as explained below.

Maximum stringency typically occurs at about Tm-5°C (5°C below the Tm of the probe); high stringency at about 5°C to 10°C below Tm; intermediate stringency at about 10°C to 20°C below Tm; and low stringency at about 20°C to 25°C below Tm. As will be understood by those of skill in the art, a maximum stringency hybridisation can be used to identify or detect identical nucleotide sequences while an intermediate (or low) stringency hybridisation can be used to identify or detect similar or related polynucleotide sequences.

In a preferred aspect, the present invention covers nucleotide sequences that can hybridise to the nucleotide sequence of the present invention under stringent conditions (e.g. 65°C and 0.1xSSC {1xSSC = 0.15 M NaCl, 0.015 M Na_3 Citrate pH 7.0). Where the nucleotide sequence of the invention is double-stranded, both strands of the duplex, either individually or in combination, are encompassed by the present invention. Where the nucleotide sequence is single-stranded, it is to be understood that the complementary sequence of that nucleotide sequence is also included within the scope of the present invention.

Nucleotide sequences which are not 100% homologous to the sequences of the present invention but fall within the scope of the invention can be obtained in a number of ways. Other variants of the sequences described herein may be obtained for example by probing DNA libraries made from a range of sources. In addition, other viral/bacterial, or cellular homologues particularly cellular homologues found in mammalian cells (e.g. rat, mouse, bovine and primate cells), may be obtained and such homologues and fragments thereof in general will be capable of selectively hybridising to the sequences shown in the sequence listing herein. Such sequences may be obtained by probing cDNA libraries made from or genomic DNA libraries from other animal species, and probing such libraries with probes comprising all or part of the nucleotide sequence set out in herein under conditions of medium to high stringency. Similar considerations apply to obtaining species homologues and allelic variants of the amino acid and/or nucleotide sequences of the present invention.

In another aspect of the present invention there is provided a method for identifying a potential modulator of the chronological life span (CLS) of a cell, comprising the steps of

i) contacting a cell having a known Histone 3 Lysine 18 (H3K18) acetylation status with a test compound; and

ii) determining if said compound has an effect the acetylation status of H3K18 in said cell;

wherein, a change in the acetylation status of H3K18 in the cell indicates that the compound modulates CLS.

It is known that modification of the histone components of chromatin often reflect whether genes are active or repressed and these changes are globally regulated by enzymes that deposit or remove specific modifications. On active genes, the chromatin is often modified by lysine (K) acetylation (ac) or methylation (me), particularly of histone H3. The inventors have identified a new lysine in histone H3 whose modification status appears to play a critical role in determining the lifespan of a cell.

As used herein, the term modulator of the chronological life span refers to a compound which has an effect on the CLS of the cell. This effect may be to increase the CLS of the cell or to decrease the CLS of the cell. It will be understood that, dependent upon the purpose to which the compound is to be put, either effect may be desirable.

It will be understood that the compound referred to herein may be any suitable compound and may be, for example, a small molecule compound or equally a biological molecule such as a peptide or nucleic acid.

Preferably, the compound interacts with at least one gene or a product of at least one gene selected from the group consisting of Spt3 (SEQ ID NO: 22), Rtg2 (SEQ ID NO: 4), Gcn5 (SEQ ID NO: 6), Ubp8 (SEQ ID NO: 10), Spt7 (SEQ ID NO: 12), Spt8 (SEQ ID NO: 14) and/or Snf1 (SEQ ID NO: 16) or their homologues.

It will be apparent to the skilled person that the gene with which the compound interacts can be identified through the use of various knock out mutant strains.

Methods of producing such strains are well known to the skilled person and include for example, insertion of one or more nucleotides into the coding region of the gene. It will be understood that, as used herein, the term product of at least one gene refers to either a nucleic acid, e.g. mRNA, or peptide product.

In a further preferred embodiment, the compound interacts with the gene designated Acs1 (SEQ ID No: 18) or a product of the gene designated Acs1.

It will be further apparent to the skilled person that the acetylation status of H3K18 can be identified by any suitable means known in the art.

In one embodiment, the acetylation status is determined by measurement of mitochondrial respiration.

It will be understood by the skilled person that any suitable method for measuring mitochondrial respiration can be used. For example, mitochondrial respiration can be measured by incubating the cells in the presence DIOC6 and visualising the cells.

In an alternative embodiment, the acetylation status is determined by indirect immunofluorescence with monoclonal antibodies against H3K18ac on live or fixed cells.

The present invention also provides methods for identifying the replication status of a cell or identifying a change in the CLS of a cell.

As used herein, the term identifying the replication status refers to identifying whether a particular cell or population of cells is actively dividing, or capable of actively dividing or whether the cell or population of cells are no longer able divide.

As used herein, the term identifying a change in the CLS of a cell refers to identifying a step change in a cell or population of cells from a state in which it/they is/are capable of actively dividing to a state in which it/they can no longer divide or *vice versa*.

It will be understood that this change can be deliberately induced or can occur naturally or through exposure to environmental factors.

Preferably, the cell is a mammalian cell. More preferably, the cell is a human cell.

In one preferred embodiment, the cell is an induced pluripotent stem cell.

The skilled person will understand that an induced pluripotent stem cell is typically a somatic cell which has been caused to regress to a pluripotent state either by exposure to certain chemicals or through transfection with, for example, various viruses.

In a further preferred embodiment the cell is a cell suspected of being neoplastic and/or cancerous. Preferably, the cell is a cell from a sample which has previously been isolated from a patient suspected of having or at risk of developing cancer.

In a further aspect, their is provided a method of diagnosing a disorder associated with a change in the CLS of a cell, said method comprising identifying the acetylation status of H3K18 of a cell previously isolated from a subject and comparing said acetylation status to the acetylation status of a control cell.

As used herein, the term control cell refers to a cell of the same tissue type as that isolated from the subject, the control cell being isolated from healthy tissue and having a known acetylation status.

Preferably, said disorder is selected from the group comprising an age related disorder, cancer, a blood disorder, Parkinson's disease or Alzheimer's disease.

The invention will be further described with reference to the figures. References to strains in the figures refer to the strains disclosed in Table 1. In the figures:-

Figure 1 H3K14ac by SAGA reflects growth. Figure 1 shows Western blots showing levels of various post- translational modifications to histone H3, in various backgrounds including HA-Spt7 and Gcn5 in total cell extract prepared from cells mock-treated or treated with 10μM rapamycin for up to 180 minutes in the BY4741 background (**a**, **b**, **c**, **d**), FY168 and FY571 (**e**, **h**), FY2 and FY2030 (**f**) and JR-52A (**g**). In panel **e** The version of Spt7 expressed from the *spt7-217* allele in FY571 is truncated at amino acid 1119 and has lost 213 C-terminal residues.

Figure 2 SAGA and K14ac influence ageing. *Western blots showing levels of* K14ac (**a**) and HA-Spt7 (**b**) in total protein prepared from 1 x 10^8 cells of the FY2030 background (**a**, **b**) or FY168 and FY571 (**c**) , subject to biotinylation, growth for 10 or 20 generations in exponential culture (YPD) and isolation using magnetic streptavidin beads. Young cells (majority less than 5 generations old) were prepared from the remaining non-biotinylated cells. * indicates a processed version of histone H3.

Figure 3 Control of SAGA, SLIK and K14ac. Western blots showing levels of H3K14ac Gcn5 or HA-Spt7 in total cell extracts prepared from the strains indicated (genotype shown in Table 1) after growth in the presence of 10μM rapamycin (+) or mock-treated (-) for 3 hours. WT strains are BY4741.

Figure 4 Rtg2 and SLIK determine chronological lifespan. a FY168 (WT), FY571 (Spt7-217) and *rtg2Δ* derivatives in exponential phase stained with DiOC6 to assess mitochondrial membrane potential (ψ). Scale bar is 10μm. **b** Serial ten-fold dilutions of cells from strains indicated grown with aeration to stationary phase in CSM containing 3% glucose and plated onto YDP on the day shown to assess viability. The average lifespan (time in days to 50% drop in viability) was calculated from colony counts. **c** Fluorographs of total protein extracts in exponential phase treated without or with cyclohexamide to inhibit cytoplasmic translation and pulse labelled for 15 minutes with 35S methionine.

Figure 5 shows Western blots showing levels of various post-translational modifications to histone H3 in total cell extract prepared from BY4741 in exponential or early stationary phase.

Figure 6 a shows the effect of expressing a C-terminally truncated version of Spt7 (Spt7-217) in strain FY571 and derivatives on K14ac and gene expression. Figures 12 b-i show the effect of growth phase and the presence of RTG2 on the induction of various genes.

Figure 7 shows HA-Spt7 undergoes C-terminal processing in cells entering stationary phase.

Figure 8 shows K14ac is reduced as cells age.

Figure 9 shows the effect of Rapamycin on K14ac at *CIT2* (SLIK induced) or *HMS2* (not induced) by ChIP normalised to histone H3. ChIP monitored by real time PCR[53], expressed as a percentage of input and normalised to levels of histone H3 in three preparations of chromatin, at the 5' region of the genes shown.

Figure 10 shows that Snf1 is required for the rapamycin dependent reduction in K14ac on rapamycin treatment. **a-d** Western blots showing levels of modifications at H3 on total cell extracts prepared from the strains indicated in the LPY8056 background (**d**), BY4741 (**a-b, f-g**) or FY3 (**c**). n=3 for all experiments shown. **e** Indirect immunofluorescence with FITC tagged anti-HA antibody (right panel) or DAPI (DNA) (left panel) of Gal83-HA. Cells were treated +/- 10μM rapamycin for up to 3 hours.

Figure 11 shows Rtg2 is required for optimal levels of K14ac but K14ac is rapamycin sensitive in a rtg2Δ strain . Western blots showing levels of modifications at H3 (**a**) and Gcn5 (**b**) in total cell extracts prepared from the strains indicated in the BY4741 background. Cells were treated +/- 10μM rapamycin for up to 3 hours. Rtg2 is negatively regulated by the Lst8 component of TORC1[66] and this repression is relieved by loss of TORC1 signalling or rapamycin treatment. Rtg2 is a component of SLIK[11] required for the induction of the retrograde responsive genes in quiescent cells.

Figure 12 shows the effect of loss of Sch9 on the inducibility of *CIT2*, *ATG1* and *ACS1* in stationary phase. This figure shows reverse transcription real time PCR quantitation of RNA for the genes shown. The results suggest that Sch9 is required to maintain the integrity of SALSA and SLIK in stationary phase cultures. Consistent with this we show that the induction of *ACS1* is independent of Sch9 (data in figure 9 suggests that this gene is dependent on Rtg2 dependent nuclear uptake of Rtg1/3 but not on SLIK). By contrast, *CIT2* (SLIK/Rtg2 –dependent) and *ATG1* (SALSA but not SLIK dependent) require Sch9 for their expression.

Figure 13 is a western blot showing that disruption of the SAGA complex results in an increase H3K18 acetylation.

Figure 14 is a graph showing that disruption of SAGA extends the chronological lifespan of yeast cells.

Figure 15 is a graph showing that disruption of H3K18 acetylation results in a significant reduction in chronological lifespan of yeast cells.

Materials and Methods

Details of strains are provided in the Table 1. Yeast were grown at 30°C in rich medium (YPD), 1% bactpopeptone, 1% Difco yeast extract (BD and Co.), 2% glucose to a density of 0.4 x 10^6 cells/ml and treated with 10μM rapamycin in 90% ethanol/10% Tween20 or mock treated for up to three hours. Details for preparation of whole cell extracts, western blotting and antibodies used, preparation of RNA and RNA quantitation, chromatin immunoprecipitation (ChIP), protocols for ageing, assessment of ERCs and chronological ageing assays are set out below.

Table 1

Strain	Parent	Genotype	Origin
RMY200 WT		*MAT***a**; *ade2-10;1 his3Δ200; lys2-801; trp1Δ901; ura3-52; hht1,hhf1::LEU2; hht2,hhf2::HIS3* plus pRM200 *(CEN TRP1 HHF2 HHT2)*	Michael Grunstein
H3 K14R	RMY200	Plus pRM200 (*hht2 K14R*)	Michael Grunstein
H3 K18R	RMY200	plus pRM200 (*hht2 K18R*)	Michael Grunstein
YSL151 WT		*ura3-5; his3Δ20; leu2Δ; trp1Δ63 lys2-128Δ(hht1-hhf1)::LEU2; (hht2-hhf2)::HIS3; pTRP1-HHT2-HHF2*	Shelley Berger
H3 K4A	YSL151	Plus pTRP1 (*hht2 K4A*)	Shelley Berger
YZS276		*MAT***a**; *hta1-htb1Δ::LEU2 hta2-htb2Δ leu2-3,-112 his3-11,-15 trp1-1 ura3-1 ade2-1 can1-100* (pZS145 *HTA1-Flag-HTB1 CEN HIS3*)	David Allis
H2B K123R	YZS276	Plus pZS14 (*htb1 K123R*)	David Allis
LPY8056		*MAT***a**; *his3Δ200; leu2Δ1; ura3-52; trp1Δ63; lys2-128δ; (hht1-hhf1)Δ::LEU2* plus pRS314B (*HHF2 HHT2*)	Shelley Berger
H3 S10A	LPY8056	Plus PRS314B (*hhf2 S10A*)	Shelley Berger

H3 K14A	LPY8056	Plus PRS314B (*hhf2 K14A*)	Shelley Berger
H3 S10A K14A	LPY8056	Plus PRS314B (*hhf2 S10A K14A*)	Shelley Berger
BY4741		*MATa; his3Δ; leu2Δ; met15Δ; ura3*	Euroscarf
gcn5Δ	BY4741	*gcn5*::KanMX	Euroscarf
spp1Δ	BY4741	*spp1*::KanMX	Euroscarf
S288c		*MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	Open Biosystems
fpr1Δ	BY4741	*fpr1*::KanMX	Euroscarf
fpr2Δ	BY4741	*fpr2*::KanMX	Euroscarf
fpr3Δ	BY4741	*fpr3*::KanMX	Euroscarf
fpr4Δ	BY4741	*fpr4*::KanMX	Euroscarf
rim15Δ	BY4741	*rim15*::KanMX	Euroscarf
rtg2Δ	BY4741	*rtg2*::KanMX	Euroscarf
msn2Δ	BY4741	*msn2*::KanMX	Euroscarf
msn4Δ	BY4741	*msn4*::KanMX	Euroscarf
snf1Δ	BY4741	*snf1*::KanMX	Euroscarf
sch9Δ	BY4741	*sch9*::KanMX	Euroscarf
dot1Δ	By4741	*dot1*::KanMX	Euroscarf
L5487		*MATa, ura3-52, leu2::hisG*	Aaron Mitchel
sch9Δ	L5487	*sch9::URA3*	Paul Nutton
Gal83-HA	BY4741	*GAL83-HA*-His3MX6	This study
Gcn5-HA	JR-52A	Plus pRS314 (*GCN5 3HA::his5+*)	Shelley Berger
spt20Δ	BY4741	*spt20*::KanMX	Euroscarf
spt8Δ	BY4741	*spt8*::KanMX	Euroscarf
FY3		*MATa ;ura3Δ0*	Fred Winston
FY2030 HA-Spt7	FY3	*MATa; ura3Δ0 ; leu2Δ1 ; trp1Δ63 ; his4-917 δ ; lys2-173R2 HA-SPT7-URA3*	Fred Winston
HA-Spt7 *snf1Δ*	FY2030	*snf1*::KanMX	This study
HA-Spt7 *sch9Δ*	FY2030	*sch9*::KanMX	This study

FY168		*MATa ; leu2Δ1 ; his4-917 δ ; lys2-173R2*	Fred Winston
FY168 *rtg2Δ*		*rtg2*::KanMX	This study
FY571 *spt7-217*		*MATa; ura3Δ0 ; leu2Δ1 ; trp1Δ63 ; his4-917 δ ; lys2-173R2 spt7-217*	Fred Winston
FY571 *rtg2Δ*	*spt7-217*	*rtg2*::KanMX	This study

Preparation of yeast whole cell extracts.

25 ml of cells were grown in YPD to an OD of ~ 0.4 A_{600} and harvested by centrifugation. For rapamycin treated cells, cells were grown to mid-log followed by the addition of 10 μM rapamycin (Sigma R0395-1MG) for up to 3 hours and harvested by centrifugation. Cell pellets were resuspended in 300 μl 8 M urea and broken by vortexing for 5 mins following the addition of 200 μl acid-washed glass beads (Sigma). Lysates were boiled for 5 mins in standard laemmli loading buffer.

Western blotting.

Protein extracts were subject to electrophoresis on polyacrylamide gels using standard Tris-glycine running buffer (40% (w/v) glycine, 0.25 M Tris-base, 10% (w/v) SDS) following heating at 90°C for 3 min. Proteins were transferred onto a nitrocellulose membrane using semi-dry transfer (Bio-Rad). Successful transfer of protein was verified by Ponceu S staining (0.1 % Ponceu S, 5 % acetic acid). Membranes were then blocked in PBS containing 5 % dry milk or BSA for 1 hour, followed by incubation with primary antibody: 1:3000 anti-H3 (Abcam ab1791), 1:5000 anti-H3 K9ac (Upstate 07-352), 1:3000 anti-H3 K14ac (Upstate 07-353), 1:5000 anti-H3 K18ac (Upstate 07-354), 1:10,000 anti-H3 K23ac (Upstate 07-355), 1:3000 anti-H3 K27ac (Upstate 07-360), 1:5000 anti-H3 K4me1 (Upstate 07-436), 1:2000 anti-H3 K4me2 (Upstate 07-030), 1:5000 anti-H3 K4me3 (Upstate 07-473), 1:500 anti-Gcn5 (Santa Cruz sc-9078), 1:5000 anti-Tubulin (Abcam ab6160), 1:1000 Anti-HA (Roche clone 3F10 11867423001) in 5 % dry milk/PBS/0.5% Tween 20. Membranes were then washed for 6 x 5 min in PBS and incubated for 1 hour with horseradish peroxidase conjugated secondary antibody in 5 % dry milk/PBS/0.5% Tween 20, and washed for 6 x 5 min in PBS/0.5% Tween 20. Bound antibody was visualised using a Pico West chemiluminescence kit (Pierce Biotechnology Ltd) according to manufacturer's instructions. Multiple exposures of each film were made

to ensure signals detected were not saturated. Each experiment was repeated at least 3 times.

RNA extraction and Northern blotting.

Extraction of RNA was performed using hot phenol extraction. 15µg of total RNA was separated on 1.1% formaldehyde gels and transferred to Magna nylon membranes and baked at 80°C for 2 hours. The membranes were blocked by incubation for 2 hours at 65°C with PerfectHyb Plus (Sigma). Membranes were typically exposed for 24 hours unless otherwise stated. Levels of total RNA loaded was monitored by the rRNA species, which are equal across samples unless indicated.

Isolation of yeast at 10 or 20 generations of growth.

1×10^8 cells from a culture at OD_{600} of 0.2 were washed in PBS, biotinylated with 3mg of sulfo-NSH-LC-biotin at room temperature for 15 minutes, washed 6 times with PBS and added to 1 litre of pre-warmed YPD containing 2.5% glucose and incubated for 10 generations. Harvested cells were washed in PBS. 400ul of strepavidin beads were added and incubated with the cells on ice for 2 hours in PBS. A magnetic sorter was used to select beads with biotinylated cells attached for 20 minutes on ice with occasional mixing. The mixture was washed and reselected five times using PBS. The sorted cells were added to a second litre of prewarmed YPD and grown for an additional 10 generations, sorted and washed exactly as before. Protein or DNA was isolated from the yeast using urea and glass beads (see above) for analysis by Western blotting or by preparing sphaeroplasts and extracting total DNA by phenol chloroform extraction exactly as described [51]. The total DNA extract was separated on a 0.8% agarose gel. DNA was visualized by hybridization to radiolabelled probes.

Labelling yeast with 35S methionine.

Exponential cultures in synthetic complete medium with glucose were treated with or without cycloheximide (250 µg/ml in 10 ml of culture), and the incubation was *continued for 5 min prior to the 15-min incubation with 100 µCi of [^{35}S]methionine* (PerkinElmer Life Sciences). Total protein was separated on a 10 or 15% SDS-PAGE *gel. The gel was then treated with Enlightening* (PerkinElmer Life Sciences), *dried*, and exposed to x-ray film for 40-72 h.

Microscopy.

Cells in exponential growth or after 2.5 days in culture (stationary phase) were incubated with the membrane-potential-sensitive dye 3,3'-dihexyloxacarbocyanine iodide (DiOC6) obtained from Molecular Probes at a concentration of 20 ng/ml for 30 minutes, washed in PBS and visualised using exposure of 1000ms (exponential cells) or 250ms (stationary phase cells) the FITC channel on an Olympus IX-81 fluorescence microscope with a 150 W xenon-mercury lamp and an Olympus 150X Plan NeoFluor oil-immersion objective. Brightfield images (DIC) were captures for each field.

Optimizing conditions for treating cells with rapamycin

Cells in exponential phase of growth were treated with 10μM rapamycin in 90%ethanol/10%Tween 20 for up to 180 minutes and levels of H3K14ac and histone H3 examined. Alternatively, cells were treated with up to 20μM rapamycin for 30 minutes. A standard set of conditions were determined and for all work in this paper involved treatment of exponentially growing cells (0.4 x 10^7 cell/ml) for 2 to 3 hours with 10μM rapamycin.

Assay showing the dependency of post-translational modifications to histone H3 on the integrity of factors known to influence modifications on histone H3.

Total cell extracts were prepared from LPY8056 cells expressing histone H3 with alanine (A) substitutions at S10 or K14 or both residues, BY4741 carrying deletions of *SPP1*, encoding a factor required specifically for H3K4me3[52] or *DOT1*, the methyltransferase for H3K79 [53,54], or YZS276 carrying a substitution at H2BK123 [55], required for H3K4me2 and H3K4me3. The modifications of lysines on histone H3 were monitored by Western blotting of total cell protein extracts using antibodies specific for each modification.

HA-Spt7 undergoes C-terminal processing in cells entering stationary phase or treated with rapamycin.

Strain FY2030, expressing an N-terminally tagged version of Spt7 from the *SPT7* locus and FY3, an untagged control were used for these experiments (n=9 for **a**). **a** Cells were grown in YDP to mid-log phase, post-diauxic phase or early stationary phase and total protein extracts prepared, subject to western blot using the 3F10 monoclonal antibody to reveal the HA epitope. Positions of the molecular weight markers are shown and a blot developed to reveal histone H3 levels to act as a loading control. Three high molecular weight form of HA-Spt are present, consistent

with full length Spt7 in SAGA, a C-terminally truncated form missing approx 200aa found in SLIK and form 3 who function is not known[27,25]. In addition a form that migrates at 50kDa is also evident in these and other preparations when levels of full length Spt7 drop. b A repeat of the experiment shown above showing more extensive C-terminally truncated version of Spt7 in all three growth conditions. About three of nine experiments show a profile such as this while six show more discrete bands as in a.

Indirect Immunofluoresence

The acetylation/methylation staus of a cell was assessed using indirect immunoflurescence according to the following protocol. 10-50 ml of a fresh mid-log culture of cells per sample was used. Make fresh 30% formaldehyde (3g p-formaldehyde in 5ml PEM, add 4M NaOH until dissolved and make up to 10 ml with PEM) and add 1/10th volume of 30% formaldehyde to the culture with agitation (in conical flask). 30s later add gluteraldehyde solution to a final concentration of 0.2% (w/v). Shake at incubation temp for 90 min. Spin cells 2K 5min then wash 3X in PBS or PEM (100mM Pipes pH 6.9; 1mM EGTA, 1mM Mg2SO4). Resuspend cells in 10ml of PEMS (PEM in 1M Sorbitol) and add 500µl of ICN Yeast Lytic Enzyme (10µg/ml). Incubate at 37°C until ~80% of cells are digested (about 15 min). Wash 3X in 10ml of PEMS. Resuspend in 10ml of 1% Triton X100 in PEM for 30s. Wash 3X in 10ml PEM. Roughly assess the volume of the final pellet. Resuspend in 2ml of PEMBAL (PEM, 0.1M L-lysine, 1% BSA (globulin free), 0.1% Na Azide) and transfer a volume which will give a 20-30µl pellet upon a subsequent spin to each of 2 Eppendorf tubes. Put on a rotating wheel for 30 min at room temp. Spin for 10 sec. Resuspend in 50µl of primary antibody in PEMBAL (test suitable dilution) and incubate for 16 hours on rotating wheel. Wash 3X in 1ml PEMBAL. Resuspend in 1ml of PEMBAL and rotate on a wheel for 30 min. Resuspend in 50µl of Goat anti-mouse Texas Red at 20mg/ml in PEMBAL. Incubate 16 hours on rotating wheel. Wash 3X in PEMBAL. Resuspend pellet in 100µl PEMBAL and mount on poly L- Lysine coated coverslips. Dry with hairdryer and invert on 1µg/ml DAPI in 100% glycerol if required. Alternatively use a FITC secondary antibody at 1/200 and incubate for 1 hour at room temperature on a wheel. N.B. cover tubes with foil during incubations with secondary antibody. The cells were then visualised

Examples

H3K14ac by SAGA reflects growth

The type of post-translational modification on the histone components of chromatin often reflects whether genes are active or repressed and these changes are globally regulated by enzymes that deposit or remove specific modifications. On active genes, the chromatin is often modified by lysine (K) acetylation (ac) or methylation (me), particularly on histone H3[27]. In order to identify post-translational modifications on histone H3 that reflect cell growth, we prepared total protein extracts from yeast in exponential or early stationary phase. Large and reproducible differences in the signals on Western blots allow us to correlate changes in acetylation and methylation with cell physiology. Cells in stationary phase show reductions in K14ac, K18ac and trimethylation (me3) of K4 that are not a consequence of cell-cycle arrest (Fig. 5). These changes are similar in exponentially growing cells treated with the macrolide rapamycin, which blocks growth and proliferation[14] (Fig. 1a and 10-11), suggesting that the presence of these modifications reflects the proliferative capacity of the cells.

Gcn5 is the major acetyltransferase for K14 and K18 (Fig. 1b). Furthermore, the rapamycin-sensitive K14ac detectable by Western blotting is mediated by Gcn5 in SAGA (Fig 1c). Strains lacking Spt8, specific to SAGA[22] or Spt20, required for the integrity of SAGA and SLIK/SALSA[23,31,32], have low levels of K14ac that do not detectably change when treated with rapamycin. In contrast, K14ac is resistant to rapamycin in a strain lacking Ubp8, a component of SAGA with ubiquitin protease activity required for processing the C-terminal region of Spt7[23,25] (Fig. 1b). Western blots showing levels of modifications at H3 on total cell extracts prepared from the strains indicated all in the BY4741 background are shown in figures 1c- e. Cells were treated +/- 10μM rapamycin for up to 3 hours. A strain expressing Spt7 lacking the C-terminal 213aa, known as Spt7-217[33] also shows rapamycin resistant K14ac, suggesting that SLIK and SALSA are resistant to rapamycin (Fig. 1e). It is important to note that in this strain, the truncated Spt7 is expressed at levels similar to full length Spt7[33], hence the high levels of K14ac. Levels of truncated Spt7 in SLIK and SALSA *are normally much lower than full length Spt7 and make a minimal* contribution to global levels of K14ac[22,23,25]. Moreover, this resistance to rapamycin is consistent with roles in the activated, but not basal, expression of TATA box genes[34] that function to promote growth when glucose is depleted[18,21] (Fig. 6). Furthermore, this implicates the C-terminal 213aa of Spt7 in the rapamycin sensitivity of K14ac by SAGA as Spt8, a SAGA specific subunit, is recruited through this region[22,23]. Thus

rapamycin may have differential effects on SAGA and SLIK/SALSA. SAGA is active in glucose grown cells while SLIK and SALSA are active in nutrient limited cells when TORC1 signalling is reduced (Fig. 6).

The FY168 WT strain has been engineered to express only Spt7 containing a C-terminal truncation (FY571 Spt7-217) similar to that found naturally in the SLIK/SALSA complex. The Spt7 protein is expressed at similar level to full length Spt7[10]. we investigated levels of K14ac in this strain and the influence of Rtg2, the retrograde regulator and component of SLIK on the activity of this strain. Figure 6a shows that the K14ac is not significantly reduced in this strain when a deletion of *RTG2* is introduced. Rtg2 is required for the H3 directed HAT activity of the SLIK complex[11]. This may reflect the naturally low levels of SLIK in cells compared to this complex. In addition we tested transcriptional responses (using reverse transcription coupled to real time PCR using primers to the loci indicated; No indicates reaction with no RTase added to reaction to control for DNA contamination) in this strain in exponential growth (log) and in early stationary phase (Stat or SP). Levels of transcript were normalised to U4snRNA. Levels of this transcript drop by half in stationary phase cells (data not shown). b Levels of RDNA transcription, monitored using a primer set to the intergenic region between the 25S and 18S regions, are reduced over 7 fold in stationary phase. c The retrograde responsive gene *CIT2*[12] is induced in the stationary phase cells and is dependent on Rtg2 in the WT Spt7(FY168) and Spt7-217 (FY571)backgrounds, as expected. d Induction of *ACS1*, encoding mitochondrial acetyl CoA synthase is induced in stationary phase and is Rtg2-dependent. In cells containing high levels of the SLIK/SALSA complex the gene is not induced. SLIK/SALSA may repress *ACS1* expression or alternatively, the high levels of SLIK/SALSA may sequester Rtg2 creating an *RTG2* null. e The induction of *ATG1*, a regulator of the autophagy [13], another starvation induced response, shows no dependence on Rtg2. Instead, the strains expressing Spt7-217 show a more than two fold increase in *ATG1* mRNA levels under starvation conditions suggesting a role for the SALSA complex. The patterns of expression of these three genes may define how the SLIK/SALSA complexes contribute to gene regulation. We propose that *ATG1* is dependent on SALSA and independent of SLIK and Rtg2. By contrast, *CIT2* requires the SLIK complex for its activation while *ACS1* is dependent on Rtg2 but not SLIK (The Rtg2 function to regulate nuclear uptake of Rtg1/3 as activators). Expression at a number of other loci is also monitored (f-i) in log phase. Modifications of lysines on histone H3 are monitored by Western blotting of total protein extracts using antibodies specific to the modification or protein indicated. n=2

for each experiment. Total protein and RNA were prepared from the same cultures for the experiment shown.

We used an N-terminally HA tagged version of Spt7 to examine its levels and integrity in rapamycin treated (Fig. 1f) or stationary phase cells (Fig. 7). Reduced levels and C-terminal truncation of Spt7 occurs in both conditions and, by compromising the integrity of SAGA[23], explains the reduced K14ac. Thus the integrity of SAGA is controlled by C-terminal truncation of Spt7 that occurs when cells enter stationary phase or on rapamycin treatment. Levels of Gcn5, but not its RNA[18], also drop significantly in rapamycin treated WT cells (Fig. 1g). In contrast, Gcn5 levels are higher in rapamycin-treated cells expressing Spt7-217 (Fig. 1h). Thus the reduction in Gcn5 is likely to be a consequence of the C-terminal truncation and reduction in levels of Spt7.

SAGA decreases with age in growing cells

The data suggests that SAGA activity is a marker of growth and proliferation. As cells age both proliferative capacity and mitochondrial function are reduced. Experiments were undertaken to assess if SAGA changes during ageing by assessing levels in young cells (generally <5 generations old) compared to cells after 10 or 20 generations of growth. As cells age, levels of K14ac drop (Fig. 2a) and this is associated with an overall decrease in HA-Spt7 levels, in particular a drop in full length HA-Spt7 and increased truncated forms of HA-Spt7 supporting loss of SAGA function during ageing (Fig. 2b). By contrast, in the strain expressing only C-terminally truncated Spt7 (Spt7-217) K14ac does not drop in old cells (Fig. 2c). Total protein preparations were made from young cells or biotinylated cells after 20 generations of growth in exponential phase in rich medium, isolated using streptavidin magnetic beads. Western blot of levels of K14ac in total protein extracts prepared from FY168 (WT) and FY571 expressing Spt7-217. Levels of histone H3 were assessed to control for loading. It can be seen from Fig. 8 that there are differences in the amount of protein isolated. Levels of K14ac drop in the old WT strain but not in the strain expressing Spt7-217 suggesting that the C-terminal region of Spt7 is required for the reduction in K14ac and that SAGA is the target of this regulation. Note in this preparation there is less histone "clipping" evident than in other experiments (See Figure 2a). These cells also contain increased levels of a smaller form of histone H3, possibly clipped[36]. This suggests that the mechanism by which SAGA and K14ac are reduced as cells age is similar to that occurring in rapamycin treated cells and involves processing of the C-terminal region of Spt7.

TORC1 maintains K14ac in growing cells

We sought to define how rapamycin influences acetylation by SAGA. There are four targets of rapamycin in yeast, Fpr1-4[37]. In the presence of rapamycin, Fpr1 inhibits functions associated with the PI3-related kinases Tor1 or Tor2 within the TORC1 complex[38]. This supports TORC1-dependent signalling controlling the global levels of K14ac, K18ac and K4me3 by maintaining SAGA function in proliferating cells. Inhibition of TORC1 by rapamycin during the early stages of growth results in upregulation of SLIK/SALSA regulated genes that promote efficient respiration of glucose and stress resistance (Fig. 9)[20,39]. As can be seen in Fig 9b, levels of *CIT2* expression, regulated by the TORC-1 complex are increased upon addition of rapamycin.

AMPK is generally considered to negatively regulate mammalian mTOR, resulting in down regulation of TORC1 signalling when glucose becomes scarce and intracellular levels of AMP increase[63]. The yeast AMPK Snf1 as can be seen from figure 10 may function in a similar way as it is required for the rapamycin-dependent reduction in K14ac (**a**). Levels of K14ac in a *snf1Δ* strain are reduced to about 50% of those in a WT strain, due to Snf1 directed phosphorylation of serine 10 on histone H3 that promotes K14 acetylation by Gcn5[64]. Importantly, K14ac (**a**) and Gcn5 (**b**) and some of the HA-Spt7 in the cell (**c**) are resistant to rapamycin in the *snf1Δ* strain. Note that track five* is under loaded in **c**. Note that the integrity of S10 on histone H3, phosphorylated by Snf1, does not influence the rapamycin sensitivity of K14ac although as with the *snf1Δ*, level of K14ac is reduced in this background (**d**). We asked is Snf1 is functioning in the nucleus or cytoplasm. Gal83 is required for the nuclear uptake of Snf1[65] and HA tagged-Gal83 moves from the cytoplasm to the nucleus in rapamycin treated cells as demonstrated by indirect immunofluorescence in fixed cells (**e**). However, the relationship between Gal83 and Snf1 is not straightforward as *gal83Δ* strains show WT levels of K14ac in untreated cells and some resistance to rapamycin (**f**). Similar results are observed for Gcn5 protein in the *gal83Δ* strain (**g**). Thus it appears that Gal83 is required for the rapamycin dependent reduction in K14ac and Gcn5 suggesting that this is a nuclear function for Snf1.

SLIK controls CLS through Rtg2

We examined mitochondrial membrane potential (ψ) and CLS in the strain expressing only truncated Spt7 (Spt7-217), and thus expressing high levels of SLIK/SALSA complexes during exponential growth. Both ψ (Fig. 4a) and average

CLS (Fig. 4b) are increased compared to WT but the strain then appears to undergo a rapid and complete loss of viability around day 12 in culture that may reflect imbalances in patterns of gene expression. Rtg2 is repressed by the TORC1 complex[26] and has at least two distinct functions, one as a regulator of retrograde response, and a second as a component of SLIK[13]. The high levels of truncated Spt7 might result in sequestration of Rtg2 into a SLIK complex, resulting in an *rtg2* null for other functions. In support of this, an *rtg2* strain shows increase ψ in exponential phase (Fig. 4a), increased mitochondrial protein synthesis (Fig. 4c) and enhanced CLS (Fig. 4b). This suggests that Rtg2 functions to repress mitochondrial function when TORC1 is active and that the formation of SLIK is linked to reduced TORC1 signalling, leading to truncation of Spt7 and relief of Rtg2-dependent repression of respiration. This provides an additional way to extend CLS. Interestingly, both Spt7 and Rtg2 are reported to be mitochondrially associated proteins[13,47]. Finally, we show that the most marked increase in CLS is observed when *RTG2* is knocked out of FY571(Spt7-217) (Fig. 4b), perhaps reflecting strong induction of genes for autophagy, known to prolong lifespan, by SALSA, as this is Rtg2 independent. It should be noted that this *rtgΔ* phenotype can also be produced by the addition of inhibitors of mitochondrial respiration.

In summary, we show that the SAGA family of transcriptional regulators control the balance between growth and chronological lifespan. Metabolic changes resulting in up- or down- regulation of respiration are differentially controlled by TORC1 and Sch9 signalling to these complexes. TORC1 coordinates mitochondrial function with gene expression through the activities of Spt7 and Rtg2 and the chromatin modification at K14 on histone H3, providing a TORC1 signalling to SAGA and SLIK highly efficient mechanism by which cells switch fate in order to control the balance between growth and longevity.

Disruption of SAGA results in increased H3K18 acetylation and an extension in chronological lifespan.

Figure 13 is a western blot showing the increase in H3K18 acetylation in strains in which the SAGA complex has been disrupted. As can be seen in the top rows of both panels, the amount of H3K18ac present in whole cell yeast extracts in stains in which the SAGA complex has been disrupted are increased compared to wildtype. The strains used in these experiments were either ΔSPT8 or Spt7 truncated.

Figure 14 shows that *S. cerevisiae* strains having a disrupted SAGA complex have an increased chronological lifespan. As show in the figure strains FY631 and FY2030 are wild type, strain FY571 expresses a truncated Spt7 protein which lacks the SAGA specific Spt7 region, strain FY2037 is ΔSPT8 (Wu, P.Y. and Winston, F., [2002] Mol Cell Biol., 22(15), p5367-5379). Lifespan was determined as described in Murakami, C. and Kaeberlein, M., (2009) J. Vis. Exp., 27. Briefly, chronological lifespan of yeast refers to the profile of viability of an ageing yeast culture over time. A yeast culture is grown in liquid media until the glucose carbon source is exhausted and the cells stop dividing. At this point the proportion of cells which are alive and able to divide is measured by observing the outgrowth characteristics of a fresh inoculate of the aging culture using a Bioscreen C machine. Viabilities at various time points are compared to determine the chronological lifespan of the culture.

Figure 15 shows that in a H3K18Q mutant in which acetylation at this position is disrupted chronological lifespan, as measured using the method above, is reduced compared to wild type. In the H3K18Q yeast strain, both endogenous copies of the *H3 gene have been deleted and replaced by a single copy of the H3 gene containing* a substitution of lysine 18 with glutamine. In the wild type strain shown in the figure, the deleted H3 genes have been replaced with a single wild type copy of the gene.

All publications mentioned in the above specification are herein incorporated by reference in their entirety. Various modifications and variations of the described methods and system of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the present invention. Although the present invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in biochemistry and biotechnology or related fields are intended to be with in the scope of the following claims.

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Sequence Listing

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601 LIFEMCCGWS PFFAENNQKM YQKIAFGKVK FPRDVLSQEG RSFVKGLLNR NPKHRLGAID
661 DGRELRAHPF FADIDWEALK QKKIPPPFKP HLVSETDTSN FDPEFTTAST SYMNKHQPMM
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SEQ ID No:9
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61 LQCGFCGCWN HSHFLSHSKQ IGHIFGINSN NGLLFCFKCE DYIGNIDLIN DAILAKYWDD
121 VCTKTMVPSM ERRDGLSGLI NMGSTCFMSS ILQCLIHNPY FIRHSMSQIH SNNCKVRSPD
181 KCFSCALDKI VHELYGALNT KQASSSSTST NRQTGFIYLL TCAWKINQNL AGYSQQDAHE
241 FWQFIINQIH QSYVLDLPNA KEVSRANNKQ CECIVHTVFE GSLESSIVCP GCQNNSKTTI
301 DPFLDLSLDI KDKKKLYECL DSFHKKEQLK DFNYHCGECN STQDAIKQLG IHKLPSVLVL
361 QLKRFEHLLN GSNRKLDDFI EFPTYLNMKN YCSTKEKDKH SENGKVPDII YELIGIVSHK
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SEQ ID No:10
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SEQ ID No:11
Spt7

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121 VENEIEDKGD NAIANEDNFV NNDESDNVEE DLFKLDLEDL KQQISGTRFI GNLSLKIRYV
181 LWQCAIDYIY CDRNEFGDEN DTEYTLLDVE EKEEEEIGKN EKPQNKEGIS KFAEDEDYDD
241 EDENYDEDST DVKNVDDPPK NLDSISSSNI EIDDERRLVL NISISKETLS KLKTNNVEEI
301 MGNWNKIYHS FEYDKETMIK RLKLEESDKM IEKGKKKRSR SDLEAATDEQ DRENTNDEPD

361 TNQKLPTPEG STFSDTGNKR PKQSNLDLTV NLGIENLSLK HLLSSIQQKK SQLGISDYEL
421 KHLIMDVRKN RSKWTSDERI GQEELYEACE KVVLELRNYT EHSTPFLNKV SKREAPNYHQ
481 IIKKSMDLNT VLKKLKSFQY DSKQEFVDDI MLIWKNCLTY NSDPSHFLRG HAIAMQKKSL
541 QLIRMIPNIT IRNRADLEKE IEDMEKDKDY ELDEEEEVAG SGRKGLNMGA HMLAKENGKV
601 SEKDSSKTVK DEAPTNDDKL TSVIPEGEKE KDKTASSTVT VHENVNKNEI KENGKNEEQD
661 MVEESSKTED SSKDADAAKK DTEDGLQDKT AENKEAGENN EEEEDDDDED EDEDMVDSQS
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781 LFLEYKEQKA LESYRQKIEQ NSIMKNGFGT VLKQEDDDQL QFHNDHSLNG NEAFEKQPND
841 IELDDTRFLQ EYDISNAIPD IVYEGVNTKT LDKMEDASVD RMLQNGINKQ SRFLANKDLG
901 LTPKMNQNIT LIQQIRHICH KISLIRMLQS PLSAQNSRSN PNAFLNNHIY NYTIIDDSLD
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1081 LRALLRPTLQ ELSERNFEDE SQSFFTGDFA SELTGEDFFG FRELGLEKEF GVLSSSVPLQ
1141 LLTTQFQTVD GETKVQAKKI QPEESDSIVY KKITKGMLDA GSFWNTLLPL LQKDYERSKA
1201 YIAKQSKSSA NDKTSMTSTE DNSFALLEED QFVSKKTATK ARLPPTGKIS TTYKKKPIAS
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1321 SSFSLSLPRL NQ

SEQ ID No:12
Spt7

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SEQ ID No:13
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121 KLSDVDGGVG SREASSSTHE ASANGEVYEY YKHMLNAAQI ADSYNIYPTA AIPIQTHVNA
181 LAVSRGLKYL FLGGSDGYIR KYDLLNTLEG KLSLTILQKH SLAESIQNAG ILQSYWENEI
241 PQKKSEMKLS ANKTDYEPKV SPVHSLEVQS ECLFILSGLQ NGGITMQGVR YMEGSIAHYF
301 KGRNGHTQIV NILRLNGQED RFLSGSWDKR LLEWDLQTGD IVNEFKKSRS ELSSLEMRPL
361 YSSVDVSGNV NSGKENENAD DDMDSLFGDE DEDEKQDAGN EPVETGDGSN GEENKEQISE
421 ESLNIVYDES VFMTSGLNGS VHIWDRRMTQ SPALSLERGA GVPPWCLSAC WGVDGDHVYA
481 GRRNACVEQF DLKMPSKPIH NLKLPSISGP VSCVKAMPNN KHLLCASRDN IRLYNVEIAV
541 DASNSTTKSS KVPFLIVPGH HGGIISNLYL DPTSRFIIST SGNRGWQGNS TDTTLIYDID
601 LE

SEQ ID No:14
Spt8

>YLR055C Chr 12
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SEQ ID No:15
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1 MSSNNNTNTA PANANSSHHH HHHHHHHHHH GHGGSNSTLN NPKSSLADGA HIGNYQIVKT
61 LGEGSFGKVK LAYHTTTGQK VALKIINKKV LAKSDMQGRI EREISYLRLL RHPHIIKLYD
121 VIKSKDEIIM VIEYAGNELF DYIVQRDKMS EQEARRFFQQ IISAVEYCHR HKIVHRDLKP
181 ENLLLDEHLN VKIADFGLSN IMTDGNFLKT SCGSPNYAAP EVISGKLYAG PEVDVWSCGV
241 ILYVMLCRRL PFDDESIPVL FKNISNGVYT LPKFLSPGAA GLIKRMLIVN PLNRISIHEI
301 MQDDWFKVDL PEYLLPPDLK PHPEEENENN DSKKDGSSPD NDEIDDNLVN ILSSTMGYEK
361 DEIYESLESS EDTPAFNEIR DAYMLIKENK SLIKDMKANK SVSDELDTFL SQSPPTFQQQ
421 SKSHQKSQVD HETAKQHARR MASAITQQRT YHQSPFMDQY KEEDSTVSIL PTSLPQIHRA
481 NMLAQGSPAA SKISPLVTKK SKTRWHFGIR SRSYPLDVMG EIYIALKNLG AEWAKPSEED
541 LWTIKLRWKY DIGNKTNTNE KIPDLMKMVI QLFQIETNNY LVDFKFDGWE SSYGDDTTVS
601 NISEDEMSTF SAYPFLHLTT KLIMELAVNS QSN

SEQ ID No:16
Snf1

YDR477W Chr 4

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SEQ ID No:17
ACS1

1 MSPSAVQSSK LEEQSSEIDK LKAKMSQSAA TAQRKKEHEY EHLTSVKIVP QRPISDRLQP
61 AIATHYSPHL DGLQDYQRLH KESIEDPAKF FGSKATQFLN WSKPFDKVFI PDPKTGRPSF
121 QNNAWFLNGQ LNACYNCVDR HALKTPNKKA IIFEGDEPGQ GYSITYKELL EEVCQVAQVL
181 TYSMGVRKGD TVAVYMPMVP EAIITLLAIS RIGAIHSVVF AGFSSNSLRD RINDGDSKVV
241 ITTDESNRGG KVIETKRIVD DALRETPGVR HVLVYRKTNN PSVAFHAPRD LDWATEKKKY
301 KTYYPCTPVD SEDPLFLLYT SGSTGAPKGV QHSTAGYLLG ALLTMRYTFD THQEDVFFTA
361 GDIGWITGHT YVVYGPLLYG CATLVFEGTP AYPNYSRYWD IIDEHKVTQF YVAPTALRLL
421 KRAGDSYIEN HSLKSLRCLG SVGEPIAAEV WEWYSEKIGK NEIPIVDTYW QTESGSHLVT
481 PLAGGVTPMK PGSASFPFFG IDAVVLDPNT GEELNTSHAE GVLAVKAAWP SFARTIWKNH
541 DRYLDTYLNP YPGYYFTGDG AAKDKDGYIW ILGRVDDVVN VSGHRLSTAE IEAAIIEDPI
601 VAECAVVGFN DDLTGQAVAA FVVLKNKSSW STATDDELQD IKKHLVFTVR KDIGPFAAPK
661 LIILVDDLPK TRSGKIMRRI LRKILAGESD QLGDVSTLSN PGIVRHLIDS VKL

SEQ ID No:18
Acs1

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SEQ ID No:19
Spt7-217

1 MTERIPIKNY QRTNAKALLK LTEKLFNKNF FDLYLTSQQL VVLEYLLSIS SEEDKLKAWD
61 YFLKGNIALN VEKSFPLTQE EEHHGAVSPA VDTRSDDVSS QTIKDNNNTN TNTSISNENH
121 VENEIEDKGD NAIANEDNFV NNDESDNVEE DLFKLDLEDL KQQISGTRFI GNLSLKIRYV
181 LWQCAIDYIY CDRNEFGDEN DTEYTLLDVE EKEEEEIGKN EKPQNKEGIS KFAEDEDYDD
241 EDENYDEDST DVKNVDDPPK NLDSISSSNI EIDDERRLVL NISISKETLS KLKTNNVEEI
301 MGNWNKIYHS FEYDKETMIK RLKLEESDKM IEKGKKKRSR SDLEAATDEQ DRENTNDEPD
361 TNQKLPTPEG STFSDTGNKR PKQSNLDLTV NLGIENLSLK HLLSSIQQKK SQLGISDYEL
421 KHLIMDVRKN RSKWTSDERI GQEELYEACE KVVLELRNYT EHSTPFLNKV SKREAPNYHQ
481 IIKKSMDLNT VLKKLKSFQY DSKQEFVDDI MLIWKNCLTY NSDPSHFLRG HAIAMQKKSL
541 QLIRMIPNIT IRNRADLEKE IEDMEKDKDY ELDEEEEVAG SGRKGLNMGA HMLAKENGKV
601 *SEKDSSKTVK DEAPTNDDKL TSVIPEGEKE KDKTASSTVT VHENVNKNEI KENGKNEEQD*
661 MVEESSKTED SSKDADAAKK DTEDGLQDKT AENKEAGENN EEEEDDDDED EDEDMVDSQS
721 YLLEKDDDRD DLEISVWKTV TAKVRAEICL KRTEYFKNGK LNSDSEAFLK NPQRMKRFDQ
781 LFLEYKEQKA LESYRQKIEQ NSIMKNGFGT VLKQEDDDQL QFHNDHSLNG NEAFEKQPND
841 IELDDTRFLQ EYDISNAIPD IVYEGVNTKT LDKMEDASVD RMLQNGINKQ SRFLANKDLG
901 LTPKMNQNIT LIQQIRHICH KISLIRMLQS PLSAQNSRSN PNAFLNNHIY NYTIIDDSLD
961 IDPVSQLPTH DYKNNRELIW KFMHKNISKV AMANGFETAH PSAINMLTEI AGDYLSNLIK
1021 *TLKLHHETNS LNRGTNVEML QTTLLENGIN RPDDLFSYVE SEFGKKTKKL QDIKQKLESF*
1081 LRALLRPTLQ ELSERNFEDE SQSFFTGDFA SELTGEDFF

SEQ ID No:20
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SEQ ID No:21
Spt3

1 MMDKHKYRVE IQQMMFVSGE INDPPVETTS LIEDIVRGQV IEILLQSNKT AHLRGSRSIL
61 PEDVIFLIRH DKAKVNRLRT YLSWKDLRKN AKDQDASAGV ASGTGNPGAG GEDDLKKAGG
121 GEKDEKDGGN MMKVKKSQIK LPWELQFMFN EHPLENNDDN DDMDEDEREA NIVTLKRLKM
181 ADDRTRNMTK EEYVHWSDCR QASFTFRKNK RFKDWSGISQ LTEGKPHDDV IDILGFLTFE
241 IVCSLTETAL KIKQREQVLQ TQKDKSQQSS QDNTNFEFAS STLHRKKRLF DGPENVINPL
301 *KPRHIEEAWR VLQTIDMRHR ALTNFKGGRL SSKPIIM*

SEQ ID No:22
Spt3

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CLAIMS

1. A method for increasing the chronological lifespan of a cell comprising disrupting the function of the SAGA complex in said cell, wherein the complex is disrupted by disrupting the function of the Spt7 gene or a homologue thereof.

2. The method according to claim 1 wherein the SAGA complex is directly or indirectly disrupted.

3. The method according to any preceding claim, wherein the function of a gene is disrupted by iRNA.

4. The method according to any one of claims 1 to 3, wherein the function of a gene is disrupted at the transcriptional / DNA level.