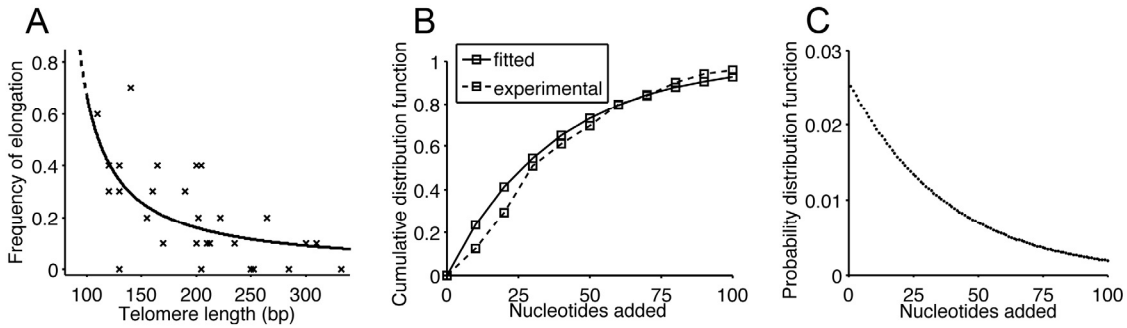

Figure S1 Numerical fit of the parameters using data from (Teixeira *et al.* 2004). *(A)* Numerical fit of the probability of elongation. The probability of elongation (continuous line) from equation 2 was plotted as a function of telomere length for parameters β = 0.048 and L_0 = 90 bp. This function was fitted to the data from (Teixeira *et al.* 2004) (dots). Goodness of fit was given by SSE = 0.6021 (summed square of residuals), R-square = 0.378, adjusted R-square = 0.3558 and RMSE = 0.1466 (root mean squared error). *(B)* Numerical fit of the law of the number of nucleotides b added per elongation. In continuous line, the cumulative distribution function of the geometrical law of parameter p was plotted and fitted to the empirical cumulative distribution extracted from experimental data from Teixeira et al., 2004 (dotted line). We found a value for p = 0.026. Goodness of fit was given by SSE = 0.03822, R-square = 0.9688, adjusted R-square = 0.9688 and RMSE = 0.06182. *(C)* Probability distribution function of b. The probability distribution function associated with the value p = 0.026 found in *(B)* was plotted.

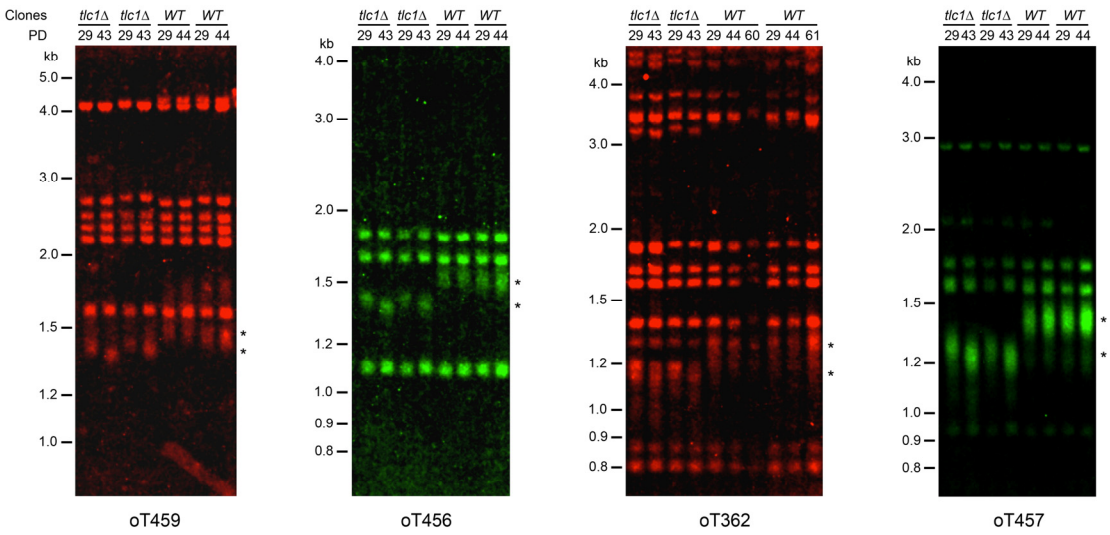

Figure S2 Other examples of single-telomere Southern blots using oT459, oT456, oT362 and oT457 fluorescent probes. Telomerase-positive (wild-type, WT) or negative (*tlc1Δ*) cells from the same tetrad were harvested at different population doublings (PD). Genomic DNAs were extracted, digested with the adequate enzyme (NdeI/BstEII or BstNI) and Southern blots were performed using the indicated probe. Different non specific bands could be observed for each probe. The positions of the telomere signals are marked with the asterisks.

File S1

Supporting Material and Methods

Numerical implementation and parameter estimation.

Numerical simulations and parameter estimation were performed using Matlab (R2012a version). To estimate the values of the parameters β and L_0 in equation 2, we fitted equation 2 to experimental data of (Teixeira *et al.* 2004) (Fig. S1*A*) using the fitting toolbox of Matlab. The parameter p for the elongation length was estimated from the empirical cumulative distribution of the elongation length (Teixeira *et al.* 2004), which we fitted with the cumulative distribution function of parameter p (Fig. S1*B*). The probability function of the increasing length b was plotted in figure S1*C*.

To approximate the probability $P(L)$ of elongation of a telomere of length L, we used the following function

$$P(L)=\frac{1}{1+\beta(L-L_0)}, \quad (*)$$

where β and L_0 are fitting constant parameters. This expression was inferred from the biochemical process in which telomerase and Rap1/Rif1/Rif2 compete for the same substrate, namely the telomere (Fig. 1*A*).

To obtain the equation (*), we shall now derive from the analysis of chemical reactions regulating telomere length expression. There are two opposite reactions:

First, when telomerase is recruited, the telomere elongates:

$$Telomere + Telomerase \xrightarrow{k_2} Elongated\ Telomere \qquad (3)$$

where k_2 is the rate constant of the reaction.

Conversely, a shortening event occurs if the amount of Rap1/Rif1/Rif2 in *cis* is enough to inhibit telomerase recruitment:

$$Telomere + Rap1/Rif1/Rif2 \xrightarrow{k_1(L_n-L_0)} Shortened\ Telomere \qquad (4)$$

where $k_1(L_n-L_0)$ is the rate constant of the reaction.

We consider that the number of telomere-bound Rap1/Rif1/Rif2 protein complexes is proportional to the telomere length (Marcand *et al.* 1997) and thus the rate constant in equation 4 is proportional to the telomere length L_n-L_0. Combining equations 3 and 4, we obtain the steady-state probability distribution $P(L_n)=\frac{1}{1+\beta(L_n-L_0)}$ used in equation 2, where $\beta=\frac{k_1}{k_2}$. We fitted this expression to experimental data, and we found $\beta=0.045$ and $L_0=90$ bp (Fig. S1*A* and Table 1).

In the present model, we consider that the correspondence between the amount of Rap1/Rif1/Rif2 protein complexes and the constant rate of equation 4 is linear over the entire range of telomere lengths used for our simulations. Indeed, this assumption is verified *a posteriori* since expression (*) was a good fit to the data on the entire length interval.

Statistical analysis.

All statistical analyses were performed using the statistical toolbox of Matlab. The means of senescence indices measured by the spot assay were tested for statistical difference by unpaired two-sample Student's *t*-test, with "the means of senescence indices are equal for the two considered sets" being the null hypothesis. The ratio of the number of tetrads with differential senescence between two *tlc1Δ* spores to the total number of tetrads was compared to theoretical ratios using Pearson's χ^2 goodness-of-fit test, which is commonly used to assess whether an observed distribution differs from a theoretical one, with "the observed ratio is equal to the theoretical one" being the null hypothesis. We also used Pearson's χ^2 goodness-of-fit test to compare simulated distributions to the experimental one (Fig. 1*F*), with "the frequency distribution of telomere lengths in the same in the simulation and the experiment" as the null hypothesis. For both statistical tests, we used the *p*-value to evaluate the probability of obtaining the test statistic assuming the null hypothesis. 0.05 was chosen as a threshold for the *p*-value, below which we rejected the null hypothesis.

Oligonucleotide probe design.

The probes used for single-telomere Southern blot were designed using the sequence of the S288C strain from Saccharomyces Genome Database (SGD) version R64-1-1 and information about W303 strain telomeres from Ed Louis's group's website (http://www.nottingham.ac.uk/biology/people/louis/telomere-data-sets.aspx). All analyses were performed using Geneious Pro 5.5.2. To design specific probes, we tested divergent regions among telomeres within 1.5 kb of the telomeric repeats. Each candidate was blasted against the whole genome using the blastn algorithm and selected for its specificity, although cross-reactivity with nonterminal restriction region was allowed. To confirm specificity, we used each probe as a primer to amplify the corresponding telomeric region by PCR, with another specifically designed primer located at the junction of the telomeric repeats and the specific subtelomeric region. We only used probes for which PCR amplified a unique band. The PCR product was then sequenced (Eurofins MWG Operon), and the sequence was compared to the S288C genome. With the exception of the XIV-R telomere (Table S1), which failed to be sequenced, all sequences either were completely identical to S288C sequences or displayed some point mutations (only for the XI-L telomere), which was expected since the yT337 background is different from S288C. As IX-L and X-L subtelomeres were identical, the oT457 probe was not specific to a unique telomere (Table S1).

Quantitative senescence assay.

The quantitative senescence assay used in this study was based on the semi-quantitative senescence assay described in (Abdallah *et al.* 2009). In most experiments, yT337 tetrads were dissected and spores were grown for 2 days at 30°C on a YPD plate. A third of each colony was resuspended in 5 µl water, boiled at 95°C for 5 min, and used as a substrate in PCR for genotyping *TLC1* locus. Colonies grown from single spores were resuspended in water in microtiter plates, and their concentrations were normalized at 800,000 cells per ml after OD (optical density) at 600 nm using an Epoch spectrophotometer (Biotek). For each cell suspension, eight replicates of 10-fold serial dilution spots were grown on a YPD plate, so as to assess the intraclonal variation that could arise and to statistically compare cell growth from different spores. The spots were grown for 2 days at 30°C. For each replicate, the most concentrated spot was then resuspended, normalized at 800,000 cells per ml, and re-spotted with serial dilutions. The same procedure was repeated for the next passage as well. Compared to the quantification procedure in (Abdallah *et al.* 2009), a new senescence index was used in this study. After 2 days of growth, spot plates were scanned at 1200 dpi using an Epson Perfection V750 Pro scanner. Images were processed with ImageJ. After background subtraction, all the spot intensities were measured and plotted against the logarithm of the 10-fold dilutions. The senescence index corresponded to the logarithm of the 10-fold dilution needed to grow the cells up to a set standard intensity value, which corresponded to the median intensity reached by wild-type telomerase-positive cells in 2 days of growth. For example, an index value of 1.3 for a given spore at passage 2 meant that, at passage 2, these cells would have needed to be diluted to $10^{-1.3}$ (starting with 800,000 cells per ml concentration) in order to reach, after 2 days, the set standard intensity value.

File S2

Supporting Text

Calculation of the ratio of tetrads displaying different senescence for their telomerase-deficient spores to the total number of tetrads.

We made the assumption that telomeres are independently regulated regarding their length, as suggested in figure 1*D* where two telomeres in different clones did not show correlated lengths and in (Shampay and Blackburn 1988). We considered a *TLC1/tlc1Δ* diploid cell with 64 independent telomeres. Let us order these 64 telomeres by their length: $L_1 \leq L_2 \leq \ldots \leq L_{64}$, corresponding to the telomeres T_1, T_2, …, T_{64}, respectively. At prophase I of meiosis, all 64 telomeres are replicated. For a given telomere T_n ($1 \leq n \leq 64$), we assumed that the length of the two replicated strands was the same: if we called the two telomeres T_n and T_n', we assumed that $L_n = L_n'$ ($1 \leq n \leq 64$). In theory, there should be a difference of about the overhang length (~10 nt) between L_n and L_n'. As shown in figure 5*A*, however, this difference has no phenotypic consequence in the senescence onset, as mother and daughter cells always displayed similar senescence, even though mitotic replication should generate the same difference in length for a given telomere. Therefore, this assumption seems valid considering the sensitivity of the spot assay.

Meiotic crossing-overs would exchange telomeres between homologous chromosomes with the highest ratio as telomeres are located at the ends of chromosomes. Thus, meiosis randomly divides out these 128 telomeres (T_1, T_1', T_2, T_2', …, T_{64}, T_{64}') between the four spores.

First hypothesis: the senescence signal is controlled by a dominant telomere, likely the shortest.

Let us analyze how telomeres T_1 and T_1' segregate between the four spores. There are 12 possibilities (see below for the complete list). In two cases, both T_1 and T_1' fall into the two *tlc1Δ* spores (either T_1 into spore 1 and T_1' into spore 2, or the reverse). Thus, there is a 1/6 probability that T_1 and T_1' fall into the two *tlc1Δ* spores. Similarly, there is also a 1/6 probability that T_1 and T_1' fall into the two *TLC1* spores. That leaves a 2/3 probability that T_1 falls into a *tlc1Δ* spore and T_1' falls into a *TLC1* spore, or the reverse[*].

Under the hypothesis that a dominant telomere, likely the shortest shortest controls the senescence signal, there should be, for the two *tlc1Δ* spores:

1- No difference in senescence if both T_1 and T_1' fall into the two *tlc1Δ* spores (P = 1/6)

[*] The same result could be obtained by classical tetrad analysis where 1/6 are ditype parental (for instance, T_1 and T_1' in the two *tlc1Δ*), 1/6 ditype non-parental (T_1 and T_1' in the two *TLC1*) and 2/3 are tetratypes.

2- Differential senescence if either T_1 or T_1' falls into a *tlc1Δ* spore but not the other (P = 2/3)

3- Unknown result if both T_1 and T_1' fall into the two *TLC1* spores (P = 1/6), because senescence onset should then be controlled by T_2 and T_2'. We can then apply the same reasoning to T_2 and T_2' and show that with a 1/6 probability, the two *tlc1Δ* spores should display the same senescence onset; with a 2/3 probability, different senescence onsets; and with a 1/6 probability, a result that would depend on T_3 and T_3'.

Therefore, we recursively show that the two *tlc1Δ* spores should have the same senescence onset with the following probability:

$$P = 1/6 + (1/6)^2 + \ldots + (1/6)^{32} \approx 1/5 = 20\%$$

And the two *tlc1Δ* spores should display differential senescence with the complementary probability:

$$1 - P \approx 80\%$$

Second hypothesis: the senescent cell is not able to detect the shortest telomere.

In this case, a minimal postulate would be that the senescent cell cannot distinguish between the shortest and the second shortest telomeres, which is equivalent, for calculation simplicity, to $L_1 = L_2$ while keeping control by the shortest telomere as an assumption. Other postulates would lead to an even lower probability of different senescence onsets for the two *tlc1Δ* spores. This case is best illustrated by a two-way table with all 12 possible segregations of T_1/T_1' into the four spores along one dimension and all 12 possible segregations of T_2/T_2' on the other. The output in each square will be "=", meaning "same senescence onset for the two *tlc1Δ* spores", if the two *tlc1Δ* spores have either one of the four T_1, T_1', T_2, or T_2'; "≠", meaning "different senescence onsets for the two *tlc1Δ* spores", if one has T_1, T_1', T_2, or T_2' but the other none of these telomeres; or "?" if these four telomeres fall into the two *TLC1* spores.

The 12 possibilities for segregation are listed below:

	*tlc1*Δ #1	*tlc1*Δ #2	*TLC1* #1	*TLC1* #2
1	T	T'		
2	T'	T		
3	T		T'	
4	T			T'
5		T	T'	
6		T		T'
7	T'		T	
8	T'			T
9		T'	T	
10		T'		T
11			T	T'
12			T'	T

The rules we stated above lead to the following two-way table:

$T_2 \backslash T_1$	1	2	3	4	5	6	7	8	9	10	11	12
1	=	=	=	=	=	=	=	=	=	=	=	=
2	=	=	=	=	=	=	=	=	=	=	=	=
3	=	=	≠	≠	=	=	≠	≠	=	=	≠	≠
4	=	=	≠	≠	=	=	≠	≠	=	=	≠	≠
5	=	=	=	=	≠	≠	=	=	≠	≠	≠	≠
6	=	=	=	=	≠	≠	=	=	≠	≠	≠	≠
7	=	=	≠	≠	=	=	≠	≠	=	=	≠	≠
8	=	=	≠	≠	=	=	≠	≠	=	=	≠	≠
9	=	=	=	=	≠	≠	=	=	≠	≠	≠	≠
10	=	=	=	=	≠	≠	=	=	≠	≠	≠	≠
11	=	=	≠	≠	≠	≠	≠	≠	≠	≠	?	?
12	=	=	≠	≠	≠	≠	≠	≠	≠	≠	?	?

In these 144 squares, if we neglect the four "?" squares, we obtain the following probability of getting two *tlc1Δ* spores with the same senescence onset:

$$P \approx 76/140 \approx 54\%$$

And the probability for the two *tlc1Δ* spores to display different senescence onset would be:

$$1 - P \approx 46\%$$

We can notice that this calculation is also valid if the phenotypic assay, namely the spot assay, is not sensitive enough to distinguish between senescence onsets induced by the difference in length between T_1 and T_2. This might explain why there was a slight difference between our experimental 71% ratio and the theoretical 80% ratio.

Lastly, we also considered the uninvestigated possibility that telomerase may act at prophase I of meiosis before division on T_1 but not T_1' (or the reverse). Given the range of length of T_1 and T_1' in our simulations (around 180–200 bp), the probability of extension by telomerase is around 15% (Fig. S1*A*). This would generate a difference in length between T_1 and T_1' if telomerase acts on one but not the other, which corresponds to a probability of 0.15 × (1-0.15) = 0.1275. This has to be applied to all cases where we are supposed to observe a similar senescence for the two telomerase-deficient spores because they received T_1 and T_1'. Such cases amount to 2 out of the 12 possibilities in the previous table. Thus, these 2/12 ≈ 17% have to be corrected down by the 0.1275 probability of extension, which leads to 0.1275 × 2/12 ≈ 0.02. Overall, if we consider that telomerase can differentially act at prophase I on two sister chromatid telomeres, this would theoretically change the ratio of tetrads with similar senescence for the two telomerase-deficient spores from 20% to ~18%, and the ratio of tetrads with different senescence onsets for the two telomerase-deficient spores from 80% to ~82%.

SI REFERENCE

Shampay, J., and E. H. Blackburn, 1988 Generation of telomere-length heterogeneity in Saccharomyces cerevisiae. Proc Natl Acad Sci U S A 85**:** 534-538.

Table S1 Oligonucleotides probes used in single-telomere Southern blot.

Probe name	Telomere	Subtelomere elements	Restriction enzyme	Probe sequence	Size of terminal restriction fragment in bp	Quality[1]
oT355	I-L	X	NdeI	GCTTGTGGTAGCAACACTATCATGGTATCAC	965	+++
oT356	II-R	X	BstEII	CTAACACAATCCTAACAGTACCCTATTCTAACCCTGATG	1308	++
oT357	III-L	X	BstEII	CTCAATTTATACACACTTATGTCAATATAACCACAAAATCAC	828	+
oT358	XI-L	X	BstEII	TTTTACCTGTCTCCAAACCTACCCTCACATTACCCTA	964	+
oT360	VI-R	X	NdeI	CATTCCGAACGCTATTCCAGAAAGTAGTCCAGCC	688	+++
oT361	VII-L	X	BstEII	CTATTTTCTTGAACGGATGACATTTCATGTTG	840	++
oT362	XIV-R	X	NdeI	TTCTACAACTCCAACCACCATCCATCTCTCTACTTACCACTA	988	+
oT455	X-R	X	NdeI	CATGCCATACTCACTTGCACTTGTATACTGATATGG	391	+
oT456	I-R	X	BstNI	CAAGATGGTAAAAGATTGAAGGCGTATCGTGGTATGG	1171	+++
oT457	IX-L/X-L	XY'/XY'	BstNI	GTTGTTGTGGAAGCGCTCGAGAAAGG	1165	+++
oT459	XV-L	X	BstNI	GTGGTATTGATACGTAATTGAGTG	1149	+++

[1]Refers to the sensitivity and the specificity of the oligonucleotide probe:

+++ sensitive and specific telomeric signal; ++ less sensitive but specific telomeric signal; + presence of non specific bands that may hinder analysis