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Genome-wide Nuclear Morphology Screen Identifies Novel Genes Involved in Nuclear Architecture and Gene-silencing in *Saccharomyces cerevisiae*

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Organisation of the cell nucleus is crucial for the regulation of gene expression but little is known about how nuclei are structured. To address this issue, we designed a genomic screen to identify factors involved in nuclear architecture in *Saccharomyces cerevisiae*. This screen is based on microscopic monitoring of nuclear pore complexes and nucleolar proteins fused with the green fluorescent protein in a collection of approximately 400 individual deletion mutants. Among the 12 genes identified by this screen, most affect both the nuclear envelope and the nucleolar morphology. Corresponding gene products are localised preferentially to the nucleus or close to the nuclear periphery. Interestingly, these nuclear morphology alterations were associated with chromatin-silencing defects. These genes provide a molecular context to explore the functional link between nuclear architecture and gene silencing.

Keywords: nuclear architecture; gene-silencing; nuclear envelope; nucleolus; yeast

Introduction

During interphase, many functions of the eukaryotic cell nucleus are organised into sub-compartments. Particular sub-regions in the nucleus are associated with molecular complexes dedicated to replication, transcription, RNA splicing and transport.[1,2] The emerging view is that chromosomes are packed into discrete territories in the interphase nuclei of mammalian cells, and this facilitates gene regulation and coordinate patterns of gene expression.[3,4] Microscopy studies suggest that these sub-nuclear compartments are highly dynamic, with constant exchanges of proteins between sub-compartments and the nucleoplasm.[5,6] In yeast, localisation of the various nuclear machineries is relatively unexplored. It is likely that nuclear territories are not defined as precisely as in mammalian nuclei, although some nuclear sub-compartments, such as the nucleolus and silent chromatin, are well conserved between yeast and mammals.[7,8]

In the yeast *Saccharomyces cerevisiae*, telomeres are clustered at the nuclear periphery.[9] These telomeric domains behave like silent heterochromatin-like DNA regions, similar to the rDNA repeats and mating-type switch loci *HMR* and *HML*. Silencing is achieved by the recruitment of Sir proteins through the combined action of *cis*-acting silencer elements and DNA-binding factors.[10] Mutations disrupting gene silencing also affect the localisation of telomeric proteins and can alter spatial distribution of telomeres.[11–13] Although not sufficient in itself, peripheral positioning of these loci might facilitate the establishment of silencing,[14,15] suggesting that certain regions of the nucleus concentrate *trans*-acting silencing factors, necessary for efficient formation of heterochromatin.

The structural basis for the functional organisation of yeast and mammalian interphasic nuclei is not well understood. Macromolecular self-assembly and the existence of a putative nuclear matrix or skeleton have been proposed to organise nuclear functions.[6,16] Self-assembly was first suggested for organisation of the nucleolus.[17] This compartment serves mainly as the site of rRNA transcription, initiated from the tandem arrays of rRNA genes sequestered in the region and for pre-ribosome formation.[18] Microscopically, the

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Abbreviations used: GFP, green fluorescent protein; NPC, nuclear pore complex; ORF, open reading frame; Pol II, RNA polymerase II.

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yeast nucleolus appears as a single crescent-shaped structure juxtaposed directly to the nuclear envelope.[8] Although yeast strains deleted for chromosomal rDNA repeats can be complemented by a single rRNA gene driven by an RNA polymerase II (Pol II) promoter carried on a multicopy plasmid, the nucleolus is disassembled in these conditions.[19] The nucleolar compartment might therefore result from the coalescence of transcription units, with the subsequent recruitment of processing factors by nascent RNA.[18] This mechanism is proposed to be a paradigm for the formation of other nuclear structures.

Nevertheless, self-assembly itself is not sufficient to explain all observations made in *S. cerevisiae*. An alternative hypothesis has been proposed, in which nuclear matrix or nucleoskeleton organises the nuclear space; similar to the cytoskeleton.[16] Nuclear pore complexes (NPCs) are large proteinaceous structures that mediate regulated transport of macromolecules between the nucleus and the cytoplasm.[20] They are embedded in the nuclear envelope and might provide anchor sites for the nucleoskeleton and/or chromatin domains at the nuclear envelope.[21] The yeast nucleus remains intact during mitosis and structural orthologues of lamina coding genes are not found in the *S. cerevisiae* genome. However, two NPC-associated myosin-like proteins (Mlp1p and Mlp2p) have been proposed to form a structural link between telomeres and the nuclear envelope.[11] Actin and actin-related proteins are found within the yeast nucleus in association with chromatin-remodelling complexes.[22,23] Whether nuclear actin exists as a polymer remains unclear, but short nuclear filaments may provide a scaffold helping chromatin positioning. Notably, a mutant allele of nuclear actin-related protein Act2p affects NPC morphology[17] and Act3p interacts with core histones.[24]

Using *S. cerevisiae* as an experimental model, we designed a screen for novel genes involved in nuclear architecture. Previous studies of nucleolar, as well as nuclear envelope mutants have demonstrated that nuclear morphology defects can be observed without growth phenotypes.[12,17,25,26] However, a systematic genetic approach for defective nuclear morphology has never been reported. Our screen was based on microscopic monitoring of two green fluorescent protein (GFP) fusion proteins; Nop1p a nucleolar protein involved in rRNA processing and maturation[27] and Nup49p an NPC component.[28] This approach led to the identification of 12 novel proteins that provide a molecular link between nuclear organization and chromatin silencing.

Results

A screen for mutations affecting nuclear architecture

We screened a library of yeast strains with deletions in non-essential genes (see Materials and Methods) for nuclear morphology phenotypes by monitoring the nuclear markers Nop1p and Nup49p fused to GFP. These fusion proteins were functional as determined by correct nuclear localisation (see Figure 1) and complementation of corresponding Δ*nup49* and Δ*nop1* mutant strains (data not shown).[28] We used 417 haploid deletion strains (*MAT*a and their *MAT*α counterparts) for transformation with Nop1p-GFP and Nup49p-GFP expressing plasmids. As a control, a wild-type strain and a mutant Δ*nup145C* strain, affecting nuclear envelope and nucleolar morphologies, were included in the test.[29] Nuclear architecture defects were scored by monitoring GFP fluorescence in the transformants. Strains were scored as positive when more than approximately 12% of the cells showed either a GFP-Nup49p localisation different from the ring-like shape typically observed in wild-type cells, or a GFP-Nop1p fluorescence distinct from the typical crescent-like signal observed for wild-type nucleoli (see Figure 1, *WT*). Out of the 417 mutant strains tested, three presented a defect in GFP-Nup49p distribution, 16 in GFP-Nop1p and ten were defective in the localisation of both markers (Table 1). Phenotypes associated with GFP-Nup49p corresponded either to clustering or irregular distribution (Figure 1(a) and (c)). We did not observe mutants in which the GFP signal could not be detected or was delocalised from the envelope. Defects observed in the GFP-Nop1p signal corresponded to a difference in signal intensity (stronger), or shape (diffused or fragmented; Figure 1(b)–(d)).

In a second step, we verified that these phenotypes were reproducible in a *MAT*a or *MAT*α background. Phenotypic discordances between strains carrying the same deletion, but with opposite mating-types have been observed.[30] These differences might be explained by secondary mutations occurring during the transformation process. Consequently, a total of 29 *MAT*a strains selected in the first step, and their *MAT*α counterparts carrying the same deletions, were transformed *de novo* with plasmids encoding GFP-Nop1p or GFP-Nup49p. Mutants in which phenotypes could not be reproduced or were reproduced, but not in both mating types, were discarded (Table 1). Thirteen pairs of deletion strains showed similar phenotypes and are illustrated in Figure 1.

To verify that the observed phenotypes were linked to the gene deletions, we transformed the mutants with plasmids encoding their corresponding wild-type genes obtained from EUROSCARF (see Materials and Methods). As a control, pRS316 (vector alone) was transformed. Direct observation of GFP-Nup49p and GFP-Nop1p in this set of strains produced the quantifications reported in Table 2. Most mutant strains reproduced the phenotype(s) for which they were scored. The single exception was Δ*yll040c*, which was therefore discarded from the following studies (Table 2D). We confirmed that phenotypes were restored to wild-type levels by complementation (Table

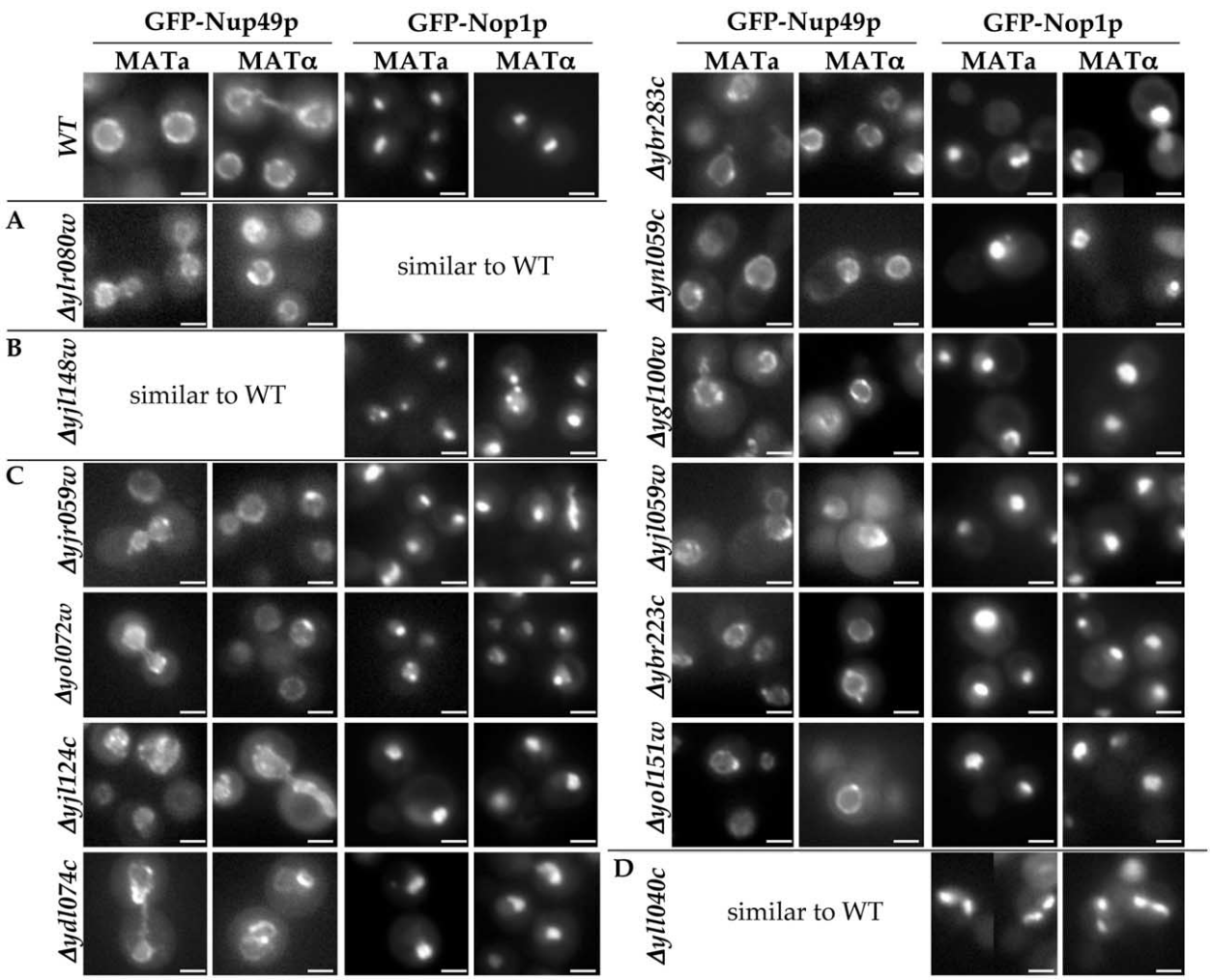

Figure 1. Direct fluorescence microscopy analysis of GFP-Nup49p and GFP-Nop1p localisation in different mutant strains in *MAT*a and *MAT*α backgrounds. Cells were grown overnight in selective medium, diluted and transferred for three hours at 37 °C. Each image was taken at the same exposure time (two seconds for GFP-Nup49p signal, and one second for GFP-Nop1p signal), except for GFP-Nop1p in Δ*ybr283c*, where the exposure time was reduced twofold. Mutants are classified as in Table 2. The scale bar represents 1 μm.

2A–C). For the Δ*ygl100w* mutant strain, complementation with the corresponding open reading frame (ORF) did not rescue the mutated phenotype (Table 2C, Δ*ygl100w*). One possible interpretation is that the wild-type gene cloned into the rescue plasmid received a mutation during acquisition of the gene by the gap repair process. Since the phenotype was reproduced in two different genetic backgrounds, this mutant was retained for further studies.

Together, these results show that deletion of each of the 12 selected genes leads to reproducible defects in GFP-Nup49p and GFP-Nop1p localisation, indicating that these 12 genes might be involved, directly or indirectly, in nuclear architecture.

Gene product localisation

In order to determine the localisation of the proteins encoded by the genes selected in the screen, all ORFs were C-terminally tagged with *GFP*. Tagged genes were placed under the control of the Pol II-transcribed *CYC1* promoter regulated by the tetOn operator (see Materials and Methods).[31] Original deletion strains were transformed with their corresponding tagged genes. *YOL151w* was C-terminally tagged at its chromosomal locus (see Materials and Methods). GFP fusions were not toxic when expressed and produced full-length GFP fusion proteins as verified by Western blot analyses (data not shown). After four hours of induction, GFP was localised in living cells (Figure 2). Most of the GFP fusions were found in the nucleus (Ydl074p, Ynl059p, Ybr223p), the nucleolus (Yjl148p) and the nuclear envelope (Ygl100p, Yol072p, Ybr283p, see also Table 3). The latter distribution resembles that of the ER markers Sec63p-GFP and GFP-HDEL in living yeast cells.[32] In three cases (Yjr059p, Yj124p, and Yol151p) localisation was not restricted to the nucleus but the GFP fusions were found in the

Table 1. List of EUROSCARF strains used in this screen

ORF	Strain
YBL001c	10367A
YBL006c	10369A
YBL009w	10370A
YBL010c	10371A
YBL019w	10113A
YBL024w	10114A
YBL025w	10190A
YBL028c	10189A
YBL029w	10188A
YBL031w	10187A
YBL032w	10186B
YBL036c	10185A[b]
YBL042c	10115A
YBL043w	10116A
YBL046w	10117A
YBL047c	10055A
YBL048w	10372A
YBL051c	10130A
YBL052c	10131A
YBL054w	10132A
YBL055c	10133A
YBL056w	10134A[b]
YBL057c	10361A
YBL059w	10362A
YBL064c	10135A
YBL066c	10136A
YBL067c	10363A
YBL071c	10364A
YBR008c	10008A
YBR014c	10057B
YBR016w	10058A
YBR028c	10149A[b]
YBR041w	10009A
YBR042c	10010A
YBR043c	10011A
YBR071w	10392A
YBR073w	10393A
YBR074w	10394A
YBR075w	10395A
YBR076w	10396B
YBR078w	10349A
YBR128c	10412A
YBR129c	10350A
YBR130c	10351A
YBR131w	10413A
YBR133c	10414A
YBR137w	10415A
YBR138c	10416A
YBR161w	10059A
YBR162c	10060A
YBR175w	10061A
YBR180w	10012A
YBR182c	10062A
YBR194w	10461B
YBR197c	10462B
YBR201w	10464B
YBR203w	10465A
YBR204c	1036B
YBR207w	10466B
YBR209w	10467B
YBR210w	10468B
YBR214w	10341A
YBR216c	10342A
YBR217w	10470B
YBR220c	10343A
YBR222c	10344A[b]
YBR223c	10345A[a]
YOR154w	10329B
YOR155c	10330A
YOR161c	10377A

ORF	Strain
YBR225w	10346A
YBR228w	10471B
YBR229c	10347A[b]
ORF	Strain
YBR255w	10254A
YBR258c	10256A
YBR259w	10257A[a]
YBR260c	10019A
YBR264c	10020A
YBR266c	10258A
YBR281c	10379A
YBR283c	10380A[a]
YBR284w	10381A
YBR285w	10382A
YBR287w	10383A
YBR288c	10384A
YDL005c	10171A
YDL010w	10169A
YDL012c	10168A
YDL018c	10446A
YDL019c	10445A
YDL021w	10444A
YDL024c	10352A
YDL025c	10106A
YDL048c	10489A
YDL057w	10167A
YDL074c	10053A[a]
YDL082w	10196A
YDL085w	10194A
YDL086w	10193B
YDL089w	10212A
YDL091c	10211A
YDL100c	10355A
YDL109c	10443B
YDL117w	10051A
YDL131w	10488A
YDL136w	10486A
YDL144c	10411A
YDL146w	10478A
YDL158c	10475A
YDL161w	10474A
YDL171c	10472B
YDL171c	10476A
YDL175c	10483A
YDL176w	10482A
YDL177c	10481A
YDL179w	10480A
YDL180w	10479A
YDL183c	10283A
YDL189w	10434B
YDL199c	10432A
YDL201w	10431A
YDL202w	10430A
YDL213c	10218A
YDL214c	10217B
YDL216c	10216A
YDL218w	10215A
YDL219w	10214A
YDL222c	10213A
YDL223c	10052A
YDL224c	10410A
YDL233w	10409B
YDL234c	10408A
YDL237w	10093A
YDL238c	10092A
YDL239c	10091A
YDL243c	10386A
YDR014w	10174A
YOR162c	10331A
YOR164c	10332A
YOR165w	10378A

ORF	Strain
YDR015c	10175A
YDR018c	10176A
YDR020c	10177A
YDR022c	10448A
YDR027c	10449A
YDR030c	10450A
ORF	Strain
YDR032c	10451B
YDR033w	10452B
YDR036c	10453A
YDR063w	10419A
YDR066c	10420A
YDR067c	10421B
YDR071c	10422A[b]
YDR072c	10423A
YGL078c	10326B[b]
YGL094c	10252A
YGL096w	10249A
YGL099w	10248A
YGL100w	10250A[a]
YGL101w	10251A
YGL121c	10239A
YGL121c	10240A
YGL124c	10490A
YGL128c	10238A
YGL129c	10237A
YGL131c	10236A
YGL133w	10303A
YGL134w	10302B
YGL138c	10296A
YGL139w	10286A
YGL140c	10285A
YGL141w	10284A
YGL144c	10295A
YGL179c	10324A
YGL180w	10323A
YGL183c	10387A[b]
YGL184c	10388A
YGL185c	10389A
YGL186c	10095A
YGL194c	10160A[b]
YGL196w	10159A
YGL197w	10390A
YGL231c	10366A
YGR106c	10310A
YGR136w	10056A
YGR187c	10241A
YGR189c	10242A
YGR194c	10243A
YGR196c	10245A
YGR205w	10491A
YGR210c	10492A
YGR221c	10045B
YGR223c	10046B
YGR224w	10007A
YGR225w	10047A
YGR226c	10048B
YGR231c	10049B
YGR232w	10050A
YGR241c	10494A
YGR284c	10288A
YJL027c	10402A
YJL029c	10403A
YJL037w	10406A
YJL038c	10079A
YJL045w	10077A
YJL046w	10076A
YJL047c	10068A
YOR166c	10333A
YOR172w	10334A
YOR267c	10158A

ORF	Strain
YJL048c	10067A
YJL049w	10066A
YJL051w	10064A
YJL055w	10073A
YJL056c	10072A
YJL057c	10071A
YJL058c	10070A
YJL059w	10069A[a]
YJL062w	10075A
ORF	Strain
YJL065c	10096A
YJL066c	10101A
YJL068c	10102A
YJL070c	10104A
YJL071w	10105A
YJL094c	10204A
YJL105w	10203A
YJL107c	10202A
YJL112w	10224A
YJL118w	10437A
YJL122w	10438A
YJL123c	10439A
YJL124c	10440B[c]
YJL126w	10225A
YJL131c	10226B
YJL132w	10227B
YJL134w	10424A
YJL135w	10228A[c]
YJL137c	10229A
YJL145w	10425A
YJL146w	10426A
YJL147c	10427A
YJL148w	10428A[a]
YJL149w	10429A
YJL169w	10054A
YJL198w	10201A
YJL199c	10032A
YJL201w	10031A
YJL204c	10030A
YJL206c	10029A
YJL207c	10028A
YJL213w	10027A
YJR001w	10206A
YJR015w	10107A
YJR036c	10150A
YJR044c	10151A[b]
YJR054w	10152A
YJR056c	10153A[b]
YJR059w	10154A[c]
YJR155w	10327A
YLL038c	10337A
YLL040c	10338A[b,d,e]
YLL042c	10339A
YLL044w	10340A
YLR020c	10328B
YLR036c	10080A
YLR040c	10407A
YLR042c	10074A
YLR049c	10353A
YLR070c	10137A
YLR072w	10138A
YLR073c	10139A
YLR074c	10140B
YLR077w	10141A
YLR080w	10142A[a]
YLR102c	10317A
YLR104w	10319A
YOR301w	10234A
YOR306c	10235A
YOR311c	10003A

ORF	Strain
YLR108c	10321A
YLR114c	10322A
YNL008c	10108A
YNL010w	10109A
YNL011c	10110A
YNL050c	10314A
YNL051w	10313A
YNL054w	10018A
YNL056w	10017A
YNL058c	10016A
YNL059c	10373A[a]
YNL063w	10015A
YNL065w	10014A
ORF	Strain
YNL066w	10013A
YNL083w	10356B
YNL086w	10357A
YNL087w	10358A
YNL091w	10210A
YNL092w	10209A
YNL094w	10208A
YNL095c	10207A
YNL097c	10359A[b]
YNL099c	10459B
YNL100w	10458B
YNL101w	10457A[b]
YNL106c	10456A
YNL107w	10312A
YNL148c	10374A
YNL155w	10118A
YNL159c	10120A
YNL164c	10121A
YNL166c	10122B
YNL168c	10123B
YNL196c	10039A
YNL200c	10040A
YNL206c	10041B
YNL208w	10026A
YNL211c	10043A
YNL212w	10025A
YNL213c	10024A
YNL214w	10023A
YNL215w	10022A
YNL217w	10044A
YNL218w	10021A
YNL227c	10289A
YNL230c	10290A
YNL242w	10455B
YNL270c	10354A
YNL273w	10263A
YNL274c	10262A
YNL275w	10261A
YNL278w	10260A
YNL279w	10259A
YNL281w	10086A
YNL283c	10084A
YNL284c	10124A
YNL285w	10083A
YNL286w	10082B
YNL288w	10081A
YNL292w	10125A
YNL293w	10126A
YNL294c	10127A
YNL295w	10128A
YNL297c	10129A
YNL299w	10184A
YNL300w	10183A
YNL303w	10182B
YOR315w	10268A
YOR320c	10269A
YOR322c	10004A

ORF	Strain
YNL304w	10181A
YNL305c	10180A
YNL306w	10292A
YNL310c	10179A
YNL311c	10293A
YNL325c	10294A
YNR004w	10278A
YNR007c	10279A
YNR008w	10280A
YNR009w	10281A
YNR013c	10282A
YNR018w	10143A
YNR020c	10144A
YNR021w	10145A
YNR028w	10146A
YNR030w	10147A
YNR031c	10148A
ORF	Strain
YNR039c	10111A
YNR047w	10315A
YNR049c	10316A
YNR051c	10305A
YNR068c	10306A
YNR069c	10307B
YNR070w	10308B
YNR071c	10309A
YNR074c	10264A
YOL018c	10033A
YOL022c	10223A
YOL025w	10222A
YOL029c	10221A
YOL032w	10220A
YOL036w	10219A
YOL072w	10166A[a]
YOL083w	10267A
YOL087c	10002A
YOL088c	10001A
YOL091w	10005A
YOL093w	10266A
YOL095c	10265A
YOL098c	10034B
YOL100w	10156A
YOL101c	10035B
YOL104c	10037B
YOL105c	10038A
YOL107w	10157A
YOL111c	10165A
YOL112w	10164B
YOL113w	10155A
YOL114c	10163A
YOL115w	10162A
YOL117w	10161A
YOL118c	10233A
YOL119c	10232A
YOL124c	10496A[b]
YOL125w	10089A
YOL132w	10090B
YOL137w	10275A
YOL138c	10274A
YOL141w	10273A
YOL151w	10495A[a]
YOL152w	10087A
YOL154w	10231A
YOL155c	10230B
YOR007c	10436A
YOR086c	10360A
YOR109w	10454A
YOR152c	10376A
YOR324c	10270A

Strains showing a phenotype in all steps of the screen are dark shaded. Strains showing a phenotype only in the first step of the screen are light shaded.

[a] Strains affected for GFP-Nup49p localisation.

[b] Strains affected for GFP-Nop1p localisation.

[c] Strains affected for both.

[d] Cells exhibiting morphological defects.

[e] Cell division defects could be observed.

Table 2. Quantification of affected cells for GFP-Nup49p or GFP-Nop1p localisation in the presence or in the absence of complementing plasmid

Strains	% Affected cells for GFP-Nup49p localisation		% Affected cells for GFP-Nop1p localisation	
	pRS416	Complementing plasmid	pRS416	Complementing plasmid
WT	12.6 ± 2.3	12.6 ± 2.3	12.4 ± 2.7	12.4 ± 2.7
A. *Strains affected only for GFP-Nup49p localisation*				
Δ*ylr080w*	32.0 ± 5.7	16.5 ± 0.7	6.8 ± nd	7.7 ± nd
B. *Strains affected only for GFP-Nop1p localisation*				
Δ*yjl148w*	13.4 ± 3.7	18.7 ± 4.7	26.4 ± 2.0	15.6 ± 1.9
C. *Strains affected for both localisations*[a]				
Δ*yjr059w*	42.3 ± 5.2	8.1 ± nd	40.0 ± 11.1	8.3 ± nd
Δ*yol072w*	39.8 ± 5.9	16.5 ± 10.6	35.7 ± 0.04	14.3 ± 2.9
Δ*yjl124c*	37.7 ± 4.6	11.9 ± 0.8	37.2 ± 21.3	19.1 ± 4.3
Δ*ydl074c*	35.0 ± 12.7	13.3 ± 0.6	26.4 ± 10.1	13.1 ± 2.7
Δ*ybr283c*	32.6 ± 10.1	12.8 ± 5.1	43.7 ± 26.8	22.2 ± 8.2
Δ*ynl059c*	29.4 ± 1.8	6.9 ± nd	45.5 ± 3.8	13.9 ± 1.5
Δ*ygl100w*	24.0 ± 2.8	23.1 ± 11.0	16.0 ± 13.8	21.1 ± 14.7
Δ*yjl059w*	23.9 ± 5.5	7.5 ± nd	24.0 ± 3.4	7.3 ± nd
Δ*ybr223c*	20.8 ± 5.4	10.7 ± 1.9	32.0 ± 14.4	18.8 ± 2.8
Δ*yol151w*	16.7 ± 4.7	11.3 ± 7.5	38.9 ± 7.5	13.0 ± 1.9
D. Strains not affected (see the text)				
Δ*yll040c*	11.5 ± 2.1	nd	9.7 ± 1.0	nd

Strains were grown in selective media overnight at 30 °C, diluted and transferred to 37 °C for three hours. Quantification of affected cells was determined visually under the epifluorescence microscope. The average values ± one standard error for 150 cells inspected in two independent experiments are presented. nd, not determined.

[a] Strains are classified according to increasing values for GFP-Nup49p defects.

cytoplasm. In two cases, the gene product was vacuolar (Yjl059p) or associated with a structure resembling the Golgi apparatus (Ylr080p) (Figure 2 and Table 3).

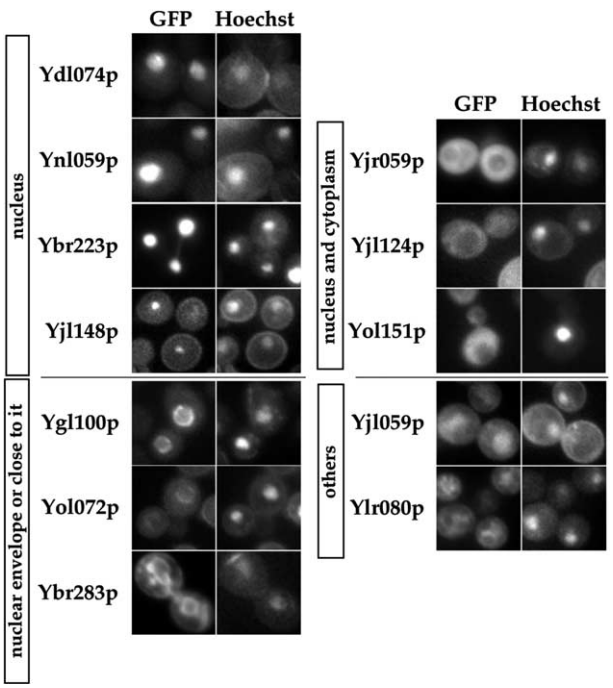

Figure 2. Localisation of GFP-tagged ORF products. After four hours of induction, GFP fusion proteins were localised in living cells. Nuclear staining with Hoechst 33342 is shown. In some cases, DNA was difficult to stain. Mutants are classified according to the localisation of the GFP-tagged protein (see the text).

These results indicate that, in most cases, nuclear architecture defects could be linked to the lack of a nuclear, nucleolar or nuclear envelope protein.

The 12 selected mutants are defective in gene-silencing

If nuclear morphology is related to the organisation of nuclear functions, one can predict that silent chromatin might be disturbed in our mutants. To test for this, we used a strain in which three silent loci contain relevant markers under the control of Pol II-driven promoters (rDNA :: *MET15*, telomere VIIL :: *URA3*, HMR :: *TRP1*).[33] We individually deleted the 12 genes selected in our screen in this new background (see Materials and Methods) and tested for gene-silencing defects at the three loci (Figure 3). As controls, two dominant negative mutants of *SIR2* (*sir2-143* and *sir2-88*) were used. Sir2p is an NAD-dependent histone deacetylase involved in chromatin silencing.[34] The class I mutant, *sir2-143*, is defective for silencing at the left telomere of chromosome VII and at *HMR*, but displays normal rDNA-silencing. The class II mutant, *sir2-88*, is defective in silencing only at rDNA repeats.[33] We found that all gene deletion mutants selected for their defects in Nup49p and/or Nop1p localisation were defective in silencing of at least one of the three loci (Figure 3). A defect in GFP-Nup49p localisation correlated nine times out of 11 with a telomeric or mating-type silencing defect (Figure 3(a) and Table 3). The two exceptions were Δ*yol072w* and Δ*yjl059w*. Although a clustering of Nup49p signal was observed in these mutants, no correlation

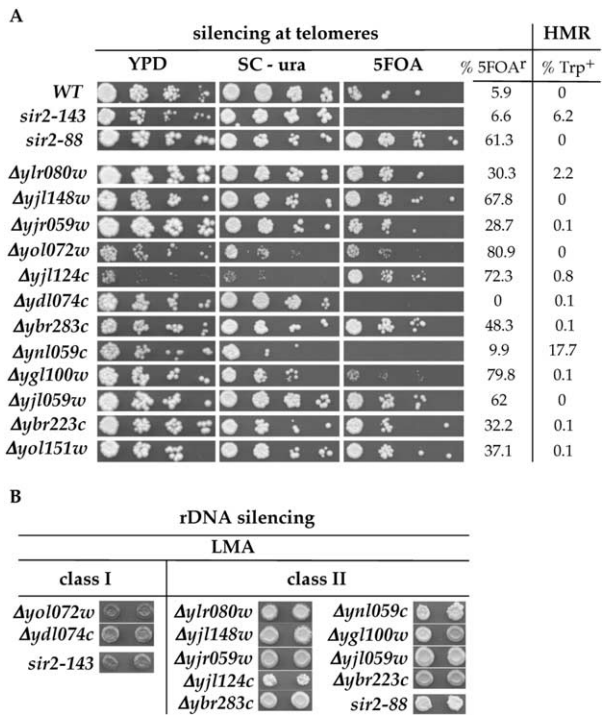

Figure 3. Silencing at the left telomere VII, HMR and rDNA. (a) After growth of deletants on YPD, tenfold dilutions of cells were spotted on YPD, SC-Ura, 5FOA (to test silencing at telomeres). In parallel, ca 300 cells of two independent mutants were plated on YPD, 5FOA and SC-trp (to test silencing at HMR locus). The percentage of colony-forming units (CFU) ((mean number of 5FOA-resistant or Trp^+ colonies/mean number of colonies on YPD) × 100) of two independent colonies are indicated. (b) After growth of deletants on YPD, non-diluted cells were spotted onto lead nitrate-containing medium (see Materials and Methods) to test rDNA-silencing. Dominant negative mutants of *SIR2* are shown as controls. See the text for details.

could be made with telomeric or *HMR* silencing. This indicates either that in these mutants Nup49p is affected specifically, or that nuclear envelope organisation and silencing at telomeres and *HMR* are not always linked. The most severely affected mutants for silencing at telomeres were Δ*ydl074c* and Δ*ynl059c* (Figure 3(a), 5FOA), the latter showing a derepression also at the mating-type locus (Figure 3(a), Trp^+). Contrary to the *sir2-143* class I mutant, linkage between both types of silencing was not always observed.[33]

Most mutants defective for Nop1p localisation were deficient in rDNA-silencing, similar to observations with the *sir2-88* class II mutant (Figure 3(b) and Table 3). There were however, three exceptions: the Δ*ydl074c* strain, in which the lack of rDNA-silencing is comparable with the class I *sir2-143*; the strain Δ*ylr080w*, in which rDNA silencing defects were observed in the absence of Nop1p delocalisation; and the Δ*yol072w* strain, in which rDNA-silencing seems to be stronger than the *sir2-88* mutant. In this latter case, we excluded the possibility that this could be due to the loss of the reporter gene, as cells were capable of growth on minimal medium lacking methionine (data not shown). In the case of Δ*yol151w*, rDNA-silencing could not be tested, because the single mutant obtained was auxotrophic for methionine (data not shown).

In conclusion, these results indicate that defects in Nup49p and Nop1p localisation are linked to chromatin-silencing defects. Defects in nuclear envelope morphology tend to correlate with derepression of telomeric and/or mating-type loci chromatin, whereas disassembly of nucleolus is related preferentially to rDNA derepression.

Discussion

Our genome-wide screen took advantage of the complete genome sequence and the availability of a non-random collection of mutant strains deleted for ORFs of unknown function, to investigate the functional organisation of the *S. cerevisiae* nucleus in a non-biased manner. About 5600 ORFs are predicted in the *S. cerevisiae* genome,[35] but most (82%) of these genes are not required for survival under standard laboratory conditions.[36] This has provided a challenge to assign function to each gene. Our efforts were focused on these viable mutants, with the assumption that genes involved in nuclear architecture are not necessarily essential for viability under normal growth conditions. Previous examples exist in which nucleolar or nuclear envelope-altered structures do not affect cell growth.[12,25,26] Our results demonstrate that a significant fraction (3%) of the non-essential genes tested here have nuclear morphology defects that are, in all cases, linked to specific defects in chromatin-silencing. In addition, the majority (10/12) of the genes encode proteins that localise to the nucleus, or to structures associated with it, pointing to their putative role in nuclear functional architecture.

During the course of this work, some of the genes identified in this screen were characterised by independent studies and were shown to be involved in different pathways (see Table 3). However, none of these genes was previously associated with defects in nuclear morphology and/or silencing.

Although we cannot exclude the possibility that some of these genes affect nuclear morphology and chromatin-silencing indirectly, it is remarkable that four genes connected directly to chromosome metabolism were selected (Table 3). This result suggests the existence of a tight link between chromatin structure, compaction or dynamics and global nuclear morphology. It is established that both telomeric and HM-silencing requires DNA-binding proteins that in turn recruit the Sir complex.[37] Histone modifications participate in the formation of repressed chromatin, as well as chromatin assembly factors.[38] In this respect, our observation that *YNL059c* and *YBR223c* deletions

Table 3. Classification of genes found in this work

Yeast gene[a]	NEV[b]	No[c]	TEL[d]	HMR[e]	rDNA[f]	Loc[g]	Fam[h]
Chromosome metabolism							
YNL059c/ARP5	+++	+++	+++	+++	+++	N[1]	8
YBR223c/TDP1	++	+++	++	++	+++	N	1
YDL074c/BRE1	+++	+++	+++	=	=	N	9
YJL148w/RPA34/CST2	+	+++	=	=	+++	No	1
mRNA metabolism							
YJL124c/LSM1/SPB8	+++	+++	=	++	+++	N, C[1]	7
YOL072w/THP1	+++	+++	=	=	++++	NEV[2]	1
YGL100w/SEH1	++	++	++	++	+++	NEV[1]	2
Secretory protein pathway							
YBR283c/SSH1	+++	+++	++	=	+++	NEV/ER[1]	2
YLR080w	+++	+	++	++	+++	G[3]	2
Small molecule transport							
YJR059w/PTK2/STK2	+++	+++	++	++	+++	N, C[1]	1
YJL059w/YHC3/BTN1	++	++	=	=	+++	V[1]	1
Others							
YOL151w/GRE2	++	+++	++	++	nd	N, C	4

[a] Gene functions in these groups refer to literature and experiments summarised in the Yeast Protein Database (see www.proteome.com for references) and are based on (i) structural similarity with genes of known function (*YNL059c/ARP5*; *YJL124c/LSM1*; *YDL074c/BRE1*; *YJL059w/BTN1*; *YBR283c/SSH1*), (ii) transcriptome analysis (*YOL151w/GRE2*), (iii) protein complex purification (*YNL059c/ARP5*; *YJL124c/LSM1*; *YJL148w/RPA34*) and (iv) specific functional screens (*YJR059w/PTK2*; *YOL072w/THP1*; *YBR223c/TDP1*). *YLR080w* was classified according to its homology with Emp47p (data not shown).

[b] Nuclear envelope defects as detected by GFP-Nup49p localisation. +++, more than 25% of affected cells; ++, between 15% and 25% of affected cells; +, between 10% and 15% of affected cells.

[c] Nucleolar defects as detected by GFP-Nop1p localisation. +++, more than 25% of affected cells; ++, between 15% and 25% of affected cells; +, between 10% and 15% of affected cells.

[d] Telomeric silencing defects as detected by growth on 5FOA. +++, like *sir2-143* (class I) mutant; ++, between *sir2-143* (class I) and *sir2-88* (class II) mutant; =, like *sir2-88* (class II) mutant.

[e] Mating-type silencing defects as detected by growth on SC-trp. +++, like *sir2-143* (class I) mutant; ++, between *sir2-143* (class I) and *sir2-88* (class II) mutant; =, like *sir2-88* (class II) mutant.

[f] rDNA silencing defects as detected by colour on lead nitrate-containing medium. ++++, stronger than *sir2-88* (class II) mutant; +++, like *sir2-88* (class II) mutant; = like *sir2-143* (class I) mutant; nd, not determined.

[g] Localisation of GFP-tagged ORF products. N, nuclear; NEV, nuclear envelope or ER; No, nucleolus; C, cytoplasm; G, Golgi-like structures; V, vacuolar. [1]These localisations are in agreement with published data (see YPD for references). [2]N-terminally tagged protein, although poorly fluorescent, was found in the nucleus.[56] [3]Ylr080p shows similarity with Emp47p, a Golgi protein.

[h] Gene families, according to Blandin *et al*.[35] The number of members found in each family is indicated.

affect silencing at all tested loci is of particular interest. On one hand, Ynlo59p is an actin-related protein that has been shown to be part of INO80.-com, a chromatin-remodelling complex,[23] indicating that silencing is particularly sensitive to chromatin compaction and dynamics. On the other hand, Ybr223p is a Tyr-DNA phosphodiesterase that repairs topoisomerase I complexes,[39] suggesting that DNA topological changes and repression of chromatin are interconnected. In addition, it is noteworthy that a gene required for spermidin transport was selected (*YJR059w*). This would be in accordance with the proposal that polyamines, in concert with histones, modulate chromatin structure.[40] The case of Δ*ydl074c* is particularly intriguing, since this protein shows a preferential defect in telomeric silencing and thus might have a specific role in the establishment and/or maintenance of silencing at the telomeres.

The links between nuclear envelope integrity and establishment of silenced chromatin are not well understood. Yet, rare mutations were previously identified that affected both the spatial distribution of telomeres and telomeric silencing (i.e. the yeast ku heterodimer[12]). Nuclear envelope integrity is required for local concentration of Sir proteins at telomeric chromatin. In the present screen, we found the nucleoporin encoded by *YGL100w*. This nucleoporin belongs to a complex of NPC proteins [29,41] involved in the tethering of telomeres at the nuclear periphery.[11] Although it is not known whether Sir proteins are mislocalised in this mutant, it provides an additional link between nuclear envelope integrity and establishment of repressive chromatin.

We have found that nucleolus morphology was often altered and correlated with a derepression of the Pol II-driven *MET15* marker integrated in the rDNA locus. This is the case when *YJL148w* is deleted. Yjl148p is likely needed for Pol I to overcome topological constraints imposed on ribosomal DNA during transcription.[42] One interpretation is that structural alterations in rDNA chromatin structures may have direct consequences on nucleolar integrity. It is possible, however, that reduced rRNA affects the nucleolar localisation of rRNA-processing factors such as

Nop1p. Interestingly, a genetic screen for rDNA-silencing defects recently identified genes associated with DNA replication and chromatin-modulating factors.[43] Although nucleolar morphology was not analysed in this study, it implies that rDNA-silencing mechanisms share common features with other forms of silencing in *S. cerevisiae*.

It is notable that all these genes are not required for cell viability under laboratory conditions. According to the functional categories defined by MIPS,[44] 761 genes (13.5%) are involved in nuclear organisation. Among these, and after crossing data from YPD,[45] MIPS[44] and the Eurofan project (A. Thierry & B.D., unpublished results), approximately 55% are not essential for cell growth. Although the number of genes belonging to this functional category is meant to evolve, the failure of a number of mutants to reveal a phenotype points to the importance of assessing their role quantitatively rather than qualitatively. About half of the non-essential MIPS genes involved in nuclear organisation are found to belong to gene families (data not shown). Two-thirds of the genes selected in our screen also belong to gene families (Table 3). Genetic redundancy can partially account for the lack of phenotypes for cell growth in these deleted strains, since other members of the family can contribute to cell viability.

Phenotyping yeast gene knockout collections remains a challenging task. Several genome-wide approaches have been developed, such as comparison of cell fitness before and after gene deletion[46] or analysis of changes in expression patterns, subsequent to gene inactivation.[47] Although extremely powerful, they do not always solve the question of protein function. Furthermore, functional screens on a genomic scale remain rare.[48] The functional screen established here relies on microscopic observation of individual mutants, without any pressure of selection. Thus, even mildly affected mutants, or phenotypes with low penetrance, could be scored. Because of this, it was important to determine that the phenotypes observed were reproducible, significantly different from the wild-type counterpart and linked directly to gene deletions (Table 2). Although significant and reproducible, phenotypes are seen in only approximately 15–40% of cells derived from a single colony. It is possible that the remaining cells are affected but too weakly to be detected. Alternatively, as is observed during gene variegation, cell-cycle or aging, some variations could occur in one cell, offering a selective growth advantage within a colony.

In conclusion, our screen reveals a striking correlation between nuclear structural defects and deregulation of chromatin-silencing. Since silencing occurs preferentially at the nuclear periphery, one hypothesis is that the perinuclear space is disrupted and, as a consequence, structural proteins of repressed chromatin are displaced. Some of the nuclear proteins found here are thus good candidates for being components of the perinuclear space. In this respect, spatial distribution of telomeres should be analysed, as well as functional relationships with proteins involved in silencing maintenance. Another possible, non-exclusive, mechanism is revealed by the selection of a number of genes involved in chromatin dynamics. It is indeed possible that chromatin organisation itself is involved in the organisation of the nuclear space. Subsequent analysis of protein functions of each of the genes selected in this screen should give more insight into this issue.

Materials and Methods

Yeast strains

The first set of 417 viable haploid yeast deletion strains issued from the EUROFAN initiative was used in this study (Table 1).[49] This collection includes individual complete deletions of ORFs that were of unknown function at the time of the program (1996). All deletions were performed with a *KANMX4* marker in *MAT*a and *MAT*α strains, isogenic to the strain used for the sequencing project (FY1679, a S288c derivative). FYBL1-3D, FYBL1-17D[50] and GCY273[33] are described elsewhere.

DNA constructs

pRS316-*GFP-NUP49* (centromeric plasmid containing *URA3*[51] was constructed by digesting pUN100-*GFP-NUP49*[28] by *Sac* I-*Bam* HI and ligation of the fragment into pRS316 digested by the same enzymes. The gene fusion is under the control of the *NUP49* promoter, and N-terminally located *GFP* consists of the S65T/V163A variant of wild-type *GFP*. Similarly, pRS316-*GFP-NOP1* was constructed by digesting pUN100-*GFP-NOP1* (kindly provided by V. Doye) by *Sac* I-*Hin*dIII. In this case, the *GFP-NOP1* is under the control of the *NOP1* promoter. The fusion genes were functional, since they could complement the deletions of *NOP1* and *NUP49*, which are essential[28] (and not shown).

Rescue plasmids, issued from the EUROFAN project, were obtained from EUROSCARF. Each contains a wild-type ORF, cloned by gap-repair, under the control of its own promoter and terminator on a centromeric *URA3*-carrying plasmid.[51]

Expression plasmid pE207 was derived from pCM189[31] by inserting a PCR fragment encoding *GFP* between *Bam* HI and *Pst* I restriction sites. The upstream oligonucleotide contained in addition a *Not* I restriction site. Selected ORFs were amplified by PCR using Pfu DNA polymerase (Stratagene) and inserted between *Bam* HI and *Not* I sites. In the case of *YJL059w*, which contained an internal *Bam* HI site, a *Bgl* II-*Not* I PCR fragment was generated.

Large scale yeast transformation

The one-step yeast transformation protocol[52] was modified as follows. Haploid yeast strains stored in micro-titer plates at −80 °C, were thawed, diluted ten-fold in micro-titer plates in 100 μl of YPGlu and grown overnight at 30 °C. After low-speed centrifugation, the supernatant was discarded by quickly flipping the plate and cell pellets were resuspended in the remnant culture medium and 70 μl of 40% (w/v) polyethylene glycol

4000, 0.2 M lithium acetate, 0.1 M DTT, containing 0.5 μg/μl of sonicated salmon sperm DNA and 10 ng/μl of transforming DNA was added. After incubation of the micro-titer plate for 30 minutes at 45 °C, 10 μl of the individual suspensions were spotted onto solid synthetic complete medium (SC; 0.17% (w/v) Yeast Nitrogen Base (Difco) without amino acids, 0.015% (w/v) all amino acids, 2% (w/v) glucose, 2% (w/v) agar) lacking uracil. Transformant colonies were visible after two days of incubation at 30 °C. One to ten transformants per transformation were usually obtained. *MAT*a transformants were first studied and, when not available, a corresponding *MAT*α transformant was picked. In the few cases in which no transformants were obtained in both mating-types derivatives, transformation was carried out as described.[52]

Fluorescence observation

All strains transformed with *GFP-NUP49* or *GFP-NOP1* encoding plasmids were used for observation of GFP fluorescence directly on living cells. After overnight growth in micro-titer plates in 100 μl of selective medium (same as above but lacking agar) at 30 °C, cells were diluted in fresh medium and grown for three hours at 37 °C. Then 3 μl of the suspension was observed directly with a Leitz DMRB microscope. Strains transformed with expression plasmids were grown under repressive conditions to exponential phase, washed in water, diluted in fresh inducible medium (SC-Ura without doxycycline) and grown for four hours at 30 °C. For DNA staining, cells were incubated for 15 minutes with 1 μg/ml of Hoechst 33342 (Molecular Probes) before observation. Images were captured with a cooled CCD C4880 camera (Hamamatsu). The CCD camera was controlled by HiPic32 (Hamamatsu) software and images were processed using Adobe Photoshop software.

Construction of deletion strains

Deletions of selected ORFs in FYBL1-3D (*MAT*a, *ura3-Δ851*, *trp1Δ63*, *leu2Δ1*, *his3Δ200*) and in FYBL1-17D (*MAT*α, *ura3-Δ851*, *leu2Δ1*, *his3Δ200*, *lys2Δ202*)[50] were performed because original mutant strains were not necessarily auxotrophic for the two markers present on the rescue plasmids (*URA3*) and on the GFP-expressing plasmid (in this case associated with *LEU2*). Reproducibility of the phenotypes in this novel context is a further validation of the screen. ORFs were deleted in FYBL1-3D, FYBL1-17D from the ATG to the STOP codon using an *HIS3* marker *via* PCR-mediated homologous recombination.[53] Primer sequences are available on request. His^+ transformants were selected and proper integration of the *HIS3* marker was checked by Southern blot. Deletions in GCY273 were generated similarly. However, complete deletion of the *his3-11* locus was achieved with *KANMX4* in some cases, in order to reduce recombination at the *his3-11* locus. Strains are available upon request.

Construction of the tagged strain

Tagging of *YOL151w* was made using a C-terminal GFP cassette,[54] using a PCR product generated from plasmid pFA6a GFP(S65)-TRP1 that encoded the 3′ end of *YOL151w* in-frame with GFP followed by *TRP1* gene.

Silencing assays

HMR, telomeric and rDNA-silencing assays were performed as described.[33] When rDNA-silencing was tested, strains were first checked for their prototrophy towards methionine, because *MET15* is unstable.[43] This must be performed on a minimal medium supplemented with required amino acids (A. Thierry, personal communication). In a second step, Met^+ cells were grown in YPGlu (1% (w/v) yeast extract (Difco), 1% (w/v) proteose peptone (Difco), 2% (w/v) glucose, 2% (w/v) agar) and spotted onto plates containing lead nitrate (0.1% (w/v) $Pb(NO_3)_2$, 0.3% (w/v) peptone, 0.5% (w/v) yeast extract, 4% (w/v) glucose, 0.02% (w/v) ammonium acetate, 2% (w/v) agar). Met^- colonies are dark in the presence of Pb^{2+}, while Met^+ colonies are white on this medium.[55]

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