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A new versatile system for rapid control of gene expression in the fission yeast *Schizosaccharomyces pombe*

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Abstract

The ability to regulate the expression of a gene greatly aids the process of uncovering its functions. The fission yeast *Schizosaccharomyces pombe* has so far lacked a system for rapidly controlling the expression of chromosomal genes, hindering its full potential as a model organism. Although the widely used *nmt1* promoter displays a wide dynamic range of activity, it takes >14–15 h to de-repress. The *urg1* promoter also shows a large dynamic range and can be induced quickly (<2 h), but its implementation requires laborious strain construction and it cannot be used to study meiosis. To overcome these limitations we constructed a tetracycline-regulated system for inducible expression of chromosomal genes in fission yeast, which is easily established and implemented. In this system the promoter of a gene is replaced by simple one-step substitution techniques with a tetracycline-regulated promoter cassette (*tet*O_7-$TATA_{CYC1}$) in cells where TetR/TetR′-based transcription activators/repressors are also produced. Using *top1* and *nse6* as reporter genes, we show that Top1 and Nse6 appear after just 30 min of activating *tet*O_7-$TATA_{CYC1}$ and plateau after ~4–6 h. The amount of synthesised protein is comparable to that produced from the attenuated *nmt1* promoter P_{nmt8}, which should be closer to wild type levels for most genes than those generated from excessively strong promoters and can be controlled by changing the concentration of the effector antibiotic. This system also works efficiently during meiosis, thus making it a useful addition to the toolkit of the fission yeast community.

Keywords

Schizosaccharomyces pombe; fission yeast; tetracycline; gene expression; inducible promoter

Introduction

Being able to control the expression of a gene is an extremely powerful approach to uncovering its functions. An ideal controllable gene expression system should allow the user to turn on, or off, the transcription of a gene in a rapid, tight and reversible manner. It should also be quick and easy to implement. The unicellular eukaryote *Schizosaccharomyces pombe*, also known as fission yeast, is a very valuable model organism because in some respects it shows a greater degree of conservation of key molecular pathways with higher eukaryotes than those found in the distantly related, but more widely employed, budding yeast *Saccharomyces cerevisiae* (Moreno, *et al.*, 1991; Nurse, 2000). Although a few systems for regulating gene expression have been developed for fission yeast they display several limitations. For instance, the commonly used *nmt1* promoter (P_{nmt1}) is induced ~70-fold upon removal of thiamine from the growth medium and it can be easily used to replace the endogenous promoter of a gene of interest by routine one-step PCR integration protocols (Bahler, *et al.*, 1998; Maundrell, 1993). Attenuated versions of this very strong promoter,

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called P_{nmt4} and P_{nmt8}, have also been created, which allow the user to control the levels of gene transcription (Basi, *et al.*, 1993). Yet, $P_{nmt1/4/8}$ take at least 14–15 h to induce (Kumar and Singh, 2006). The basal levels of gene expression under non-induced conditions are also relatively high, especially in the case of the full-strength P_{nmt1}, and to a lesser extent for P_{nmt4}. In addition, when cells are cultured in liquid medium, inducing $P_{nmt1/4/8}$ requires extensive washes to ensure that thiamine is completely removed from the medium, which is time-consuming and can induce cellular stress responses. Similarly, the recently developed *urg1* promoter system, P_{urg1}, allows quick (< 2 h) induction of gene expression, but its implementation involves relatively complicated strain construction: the gene whose expression has to be controlled must be integrated, by Cre-loxP site-specific recombination, at the *urg1* locus in place of $urg1^+$ itself (Watson, *et al.*, 2011). Although this system is certainly useful for controlling the expression of a xenogene, it poses complications when used to control the transcription of an endogenous fission yeast gene. For example, if the copy of the gene inserted at the *urg1* locus is to be the sole source of its expression, which is often the case, then the one found at the endogenous locus must be removed. Lastly, P_{urg1} cannot be used for studying meiosis because it is induced upon nitrogen starvation, which is a pre-requisite for triggering mating and meiosis in fission yeast.

A general drawback of using endogenous promoters to alter the transcription of a gene is that controlling them often requires changes in the growth medium, *e.g.* removal of thiamine and uracil for P_{nmt} and P_{urg1} respectively, which can interfere with cellular metabolism. A solution to this problem is to use heterologous promoters in combination with synthetic transcription factors, such as tetracycline-regulated promoters, where addition of the effector antibiotic tetracycline can either induce (Tet-On) or repress (Tet-Off) gene expression. This system relies on the ability of the *Escherichia coli* tetracycline repressor TetR, or its "cousin" TetR′, to bind to the *tet*O sequence only under specific conditions, *i.e.* either in the presence or absence of tetracycline, respectively (Gossen and Bujard, 2002). Sophisticated Tet-On and Tet-Off systems, which allow for tight and fast regulation of gene expression, have been developed by fusing TetR/TetR′ to certain transcription activation/repression domains and substituting the promoter of a desired gene with *tet*O arrays (Belli, *et al.*, 1998a; Belli, *et al.*, 1998b; Yen, *et al.*, 2003). Tetracycline-regulated expression systems have been developed for fission yeast but they show limitations. The approach developed by Faryar and Gatz (1992) can be used to control the expression of plasmid-borne genes only. The same applies to the system devised by Erler *et al.* (2006), with the difference that their plasmids can be genomically integrated and the selection marker they contain can be recycled by Cre-loxP site-specific recombination. It follows that the endogenous copy of the gene whose expression has to be controlled must be knocked out to ensure that the tetracycline-regulated one is the sole source of its transcription. This requires complicated and time-consuming strain manipulation that could be avoided. Thus, we set out to develop a tetracycline-regulated system to control the expression of chromosomal genes in fission yeast in a rapid, tight and reversible manner, which is both easy and quick to implement.

Materials and Methods

Strains and growth conditions

Standard fission yeast culture and handling were used as described by Moreno *et al.* (1991). All strains are *ura4-D18 leu1-32*. Table 1 contains the list of strains used to prepare this report.

PCR amplification of integrative cassettes, transformation and verification of integration

Integrative cassettes for promoter replacement were generated by PCR with Ex Taq DNA polymerase (Clontech, Mountain View, CA) following the manufacturer's instruction.

Fission yeast cells were transformed essentially as described by Suga and Hatakeyama (2001). Integration of gene targeting cassettes or linearised plasmids at a specific genomic locus was confirmed by PCR directly from isolated yeast colonies.

Spot assay

For spot assays, cells were propagated in YES medium at 30 °C to logarithmic phase, spotted on YES agar supplemented with the relevant drug in five-fold dilutions from a starting OD_{600} of 0.5, and then grown at 30 °C for 2–3 days. Irradiation with UV light at 365 nm was carried out in a Stratalinker-1800 crosslinker instrument (Stratagene, La Jolla, CA).

Preparation of whole-cell extracts and western blotting

For western blotting, ~1–3 10^8 cells were lysed by beating four times at 5.0 m s^{-1} for 20 s in a FastPrep-24 instrument (MP Biochemicals, Solon, OH) in 150 μL of 8 M urea, 50 mM Tris-HCl pH 6.8, 1× Complete Protease Inhibitor Cocktail, 2 mM PMSF supplied with 100 μL of silica-zirconia beads (BioSpec Product, Bartlesville, OK). The lysates were cleared by centrifugation at 16,000 g for 10 min at 4 °C. The protein concentration of the cleared lysates was measured using Bio-Rad's protein assay (Bio-Rad, Hercules, CA) and equalized to 3 μg μL^{-1}. These samples were supplemented with LDS Sample Buffer (Life Technologies, Carlsbad, CA) and DTT to a final concentration of 1× and 50 mM, respectively, and denatured at 70 °C for 15 min. 20 μg of protein was resolved on 4–20% Tris-Glycine gels (Expedeon, San Diego, CA or Life Technologies, Carlsbad, CA) and transferred to a nitrocellulose membrane using an iBlot Dry Blotting Transfer System (Life Technologies, Carlsbad, CA). The membrane was blocked in 5% w/v non-fat milk in Tris-buffered saline solution with 0.1% v/v Tween-20 before being blotted with antibodies against FLAG or tubulin (Sigma-Aldrich, Saint Louis, MO). After incubation with a horseradish peroxidase- or IRDye-conjugated secondary antibody (Pierce, Rockford, IL or Li-Cor, Lincoln, NE, respectively), the membrane was subjected to enhanced chemiluminescence (Thermo Scientific, Waltham, MA) or imaged on an ODYSSEY scanner (Li-Cor, Lincoln, NE), respectively.

Construction of plasmids

To create fission yeast-compatible pFA6a-*tet*O_7-$TATA_{CYC1}$ plasmids the *tet*O_7-$TATA_{CYC1}$ cassette was amplified by PCR from pCM325 (Yen, *et al.*, 2003) with oNZ3 and oNZ4 (Table 2) and used to replace $P_{nmt1/4/8}$ from existing pFA6a vectors (Bahler, *et al.*, 1998; Noguchi, *et al.*, 2008), using *Bgl*II and *Pac*I.

To construct the pDM291-(*tet*R/*tet*R′)-*tup11Δ70* plasmids, a DNA fragment containing the *tup11* open reading frame, missing the region encoding for its first 70 amino acids, was generated from wild type genomic DNA using oNZ5 and oNZ6, and inserted into pCM217 or pCM223 (Belli, *et al.*, 1998b) with *Kpn*I. Next, a DNA fragment containing the *adh1* terminator (T_{adh1}) was amplified from pFA6a-*hphMX6*-$P3_{nmt1}$-$FLAG_3$ with oNZ14 and oNZ15 and fused by PCR to a DNA molecule encompassing a region located 3′ of the *ura4* locus (oNZ16 and oNZ17). The resulting product was cloned into pCM217-*tup11Δ70* with *Kas*I and *Bst*XI. pCM217-*tet*R′-*tup11Δ70* was created by extracting a fragment comprising part of the CMV promoter and *tet*R′-*tup11Δ70* from pCM223-*tup11Δ70* with *Nco*I and *Bgl*II and cloning it into pCM217-*tup11Δ70*. The DNA molecule encompassing the (*tet*R/*tet*R′)-*tup11Δ70* cassette was generated from pCM217-*tup11Δ70* or pCM217-*tet*R′-*tup11Δ70* by PCR with oNZ33 and oNZ17, digested with *Sma*I and ligated into a pDM291 plasmid (Grimm, *et al.*, 1988) that had been cut with *Hpa*I and whose *Bam*HI site had been disrupted. In this construct the (*tet*R/*tet*R′)-*tup11Δ70* fusion is transcribed in the opposite direction from the *ura4*$^+$ marker.

The pUG6SP-(tTA/tTa′) vectors are derivatives of pUG6-tTA (Yen, *et al.*, 2003). pUG6-tTA′ was created by replacing *tet*R in pUG6-tTA with a DNA fragment containing *tet*R′ from pCM251 (Belli, *et al.*, 1998b), which had been digested with *Sph*I and *Bbs*I. The *nmt1* terminator was generated by PCR from pREP1 with oNZ27 and oNZ28 and fused to a ~600-bp region that is located between the *arg3* and *arg11* loci that was amplified from genomic DNA with oNZ29 and oNZ30. The resulting DNA fragment was inserted into pUG6-tTA/tTA′ with *Pvu*II.

Analysis of meiosis

For meiotic studies, ~1–2 10^7 cells of each relevant haploid strain were mixed in 10 μL of water and plated on sporulation SSA medium lacking or supplied with anhydrotetracycline. After 2 days at 25 °C, the cell mixture was observed on an Eclipse E800 microscope (Nikon Metrology, Brighton, MI) and images were acquired with a Quantix camera (Photometrics, Tucson, AZ). Staining with 4′,6-diamidino-2-phenylindole (DAPI) and the measurement of spore viability were performed as described in Wehrkamp-Richter *et al.* (2012).

Results

In order to engineer a tetracycline-inducible promoter for fission yeast we built upon the systems developed by Belli *et al.* (1998b) and Yen *et al.* (2003) for budding yeast, because of their versatility, ease of use and how quickly they can be activated. In such systems the endogenous promoter of a gene is replaced, by one-step PCR integration, with a cassette that contains an array of two or seven *tet*O sequences (*tet*O_2 or *tet*O_7) immediately upstream of the TATA and leader regions of the *CYC1* gene from *S. cerevisiae* (TATA$_{CYC1}$). Co-producing fusions of TetR and TetR′ to specific transcription activation (*e.g.* VP16) and repression (*e.g.* Tup1) modules in the same cell allows for low basal transcription under non-induced conditions and good expression upon induction. In order to adapt and improve this system for *S. pombe*, we engineered various pFA6a-family vectors that are commonly used to substitute the endogenous promoter of a gene with P$_{nmt}$ (Bahler, *et al.*, 1998; Noguchi, *et al.*, 2008) to contain the *tet*O_2/*tet*O_7-TATA$_{CYC1}$ cassette in place of P$_{nmt}$ itself (Figure 1A). We also constructed fission yeast-compatible vectors for the constitutive production of fusions of TetR/TerR′ to either the transcription activation domain of the herpes simplex virus protein VP16 or a truncation of the transcription co-repressor Tup11, Tup11Δ70. This construct lacks the first 70 amino acids found in the full-length Tup11, which is the region that in the budding yeast orthologue Tup1 mediates interactions with Ssn6 (Figures 2A and 2B). We used Tup11Δ70 because Tup11 does not need to interact with Ssn6 to repress gene expression. In fact in the Ssn6-Tup1 repressor complex, it is Tup1 that executes the silencing role while Ssn6 acts as a bridge for the promoter-specific DNA-binding subunit (Tzamarias and Struhl, 1994; Tzamarias and Struhl, 1995). The chimeras we generated include a λ cI linker between their constituent moieties to increase flexibility. Both sets of plasmids contain a recyclable selection marker, *his*G-*ura4*$^+$-*his*G or loxP-*KanMX6*-loxP, which can be removed by either plating cells on 5-fluoro-orotic acid-containing medium or by producing the Cre recombinase, respectively. The vectors we constructed also contain a region that is homologous to a specific locus in the fission yeast genome, which allows them to be chromosomally integrated following digestion with the relevant restriction enzyme. In all of the strains presented in this report, TetR/TetR′, or their derivatives, are produced from a chromosomally integrated form of the relevant expression vector. Having created fusions of Tup11Δ70 and VP16 to both TetR and TetR′ means that, depending on which set of chimeras are introduced in a cell, tetracycline can be used either to turn on gene expression or to turn it off.

Initially we explored whether *tet*O_7-TATA$_{CYC1}$ can be used to effectively control gene expression. We replaced the endogenous promoter of *top1*$^+$, which codes for topoisomerase

I, with the *tet*O$_7$-TATA$_{CYC1}$-FLAG$_3$ cassette. Western blotting analysis of extracts from *tet*O$_7$-TATA$_{CYC1}$-FLAG$_3$-*top1* cells showed the presence of an anti-FLAG antibody-reactive band that migrated as the FLAG$_3$-tagged Top1 produced from previously validated P$_{nmt4/8}$-FLAG$_3$-*top1* strains (Figure 3; Heideker, *et al.*, 2011). These data also showed that the amount of FLAG$_3$-Top1 generated from an uninhibited *tet*O$_7$-TATA$_{CYC1}$ was comparable to that produced from the induced P$_{nmt8}$. Such an extent of expression is likely to be closer to wild type levels for most genes than those driven by the overly powerful P$_{nmt4}$, P$_{nmt1}$ or P$_{urg1}$. Thus, *tet*O$_7$-TATA$_{CYC1}$ is an ideal promoter for all those experiments where "physiological" amounts of a protein need to be produced in a regulated manner. Unless the amount of required gene expression significantly exceeds the intrinsic activity of TATA$_{CYC1}$, it follows that producing the tetracycline-regulated activator TetR/TetR′-VP16 should not be necessary in most cases; for instance, see below. This is advantageous because it reduces the number of manipulations that are necessary to construct the relevant "tet" strains, *i.e.* integration pUG6-tTA/tTa′ and removal of its selection marker.

Constitutively producing tetR alone from an expression vector chromosomally integrated near the *ura4* locus, in the absence of an effector antibiotic, was sufficient to reduce the amount of FLAG$_3$-Top1 to very low, but not undetectable, levels in the *tet*O$_7$-TATA$_{CYC1}$-FLAG$_3$-*top1* cells (Figure 3). Conversely, TetR-Tup11Δ70 reduced the amount of FLAG$_3$-Top1 to imperceptible levels, which was reverted by addition to the growth medium of the tetracycline analogue anhydrotetracycline (ahTet). We used ahTet instead of doxycycline, which is more commonly employed to control Tet-On/Tet-Off systems, because we, as previously reported by Erler, *et al.* (2006), also found that the former antibiotic worked more efficiently than the latter in fission yeast (data not shown). Fusions of TetR to transcription repressors other than Tup11, such as fission yeast Ssn6 or Sir2, did not repress gene expression from *tet*O$_7$-TATA$_{CYC1}$ as well as TetR-Tup11Δ70 did (data not shown), and therefore they were not used further in this study. Poor repression of *top1* expression was also observed when *tet*O$_2$-TATA$_{CYC1}$ was used instead of *tet*O$_7$-TATA$_{CYC1}$ (data not shown). In conclusion, in the absence of ahTet TetR-Tup11Δ70 efficiently represses transcription from *tet*O$_7$-TATA$_{CYC1}$, while allowing it to become active following addition of the effector antibiotic. The *tet*O$_7$-TATA$_{CYC1}$-FLAG$_3$-*top1* + *tet*R-*tup11Δ70* strain will be hereafter referred to as *tet*O$_7$-*top1* for simplicity.

We also examined how quickly the expression of a gene could be turned on from *tet*O$_7$-TATA$_{CYC1}$. We found that FLAG$_3$-Top1 appeared after only 30 min of adding ahTet to the growth medium. The amount of this protein rose steadily thereafter and plateaued within 4–6 h (Figure 4A). This pattern of induction was not unique to Top1 but was also observed for FLAG$_3$-Nse6 produced from a *tet*O$_7$-*nse6* strain (Figure 4B). Nse6 is a component of the structural maintenance of chromosome complex Smc5-6 (Pebernard, *et al.*, 2006). Thus, *tet*O$_7$-TATA$_{CYC1}$ can be used to rapidly express various genes in fission yeast.

Next, we asked whether our tetracycline-regulated promoter could be used to investigate questions of biological relevance. To address this, we examined how the *tet*O$_7$-*top1* strain responded to the topoisomerase I inhibitor camptothecin (CPT) in the presence of different concentrations of ahTet *vs.* wild type and *top1Δ* cells (Figure 5A). We observed that, as expected, the wild type was unable to proliferate in the presence of 30 μM CPT. Instead, the *top1Δ* cells grew uninhibited. Uninhibited growth was also observed for the *tet*O$_7$-*top1* strain that was grown on medium lacking ahTet, that is, under conditions of gene repression, thus indicating that little or no Top1 was produced in these cells. In the presence of increasing amounts of ahTet, which leads to activation of transcription from *tet*O$_7$-TATA$_{CYC1}$ in the presence of TetR-Tup11Δ70, the *tet*O$_7$-*top1* strain became progressively more sensitive to CPT, which implies that the concentrations of effector antibiotic determined the amount of Top1 produced by these cells. As expected producing TetR′-

Tup11Δ70, instead of TetR-Tup11Δ70, in the *tet*O$_7$-TATA$_{CYC1}$-FLAG$_3$-*top1* cells yielded a reverse growth pattern on CPT in the presence of increasing concentrations of ahTet to the one described above. These results show that both the tet-off and tet-on systems work equally well. They also demonstrate that the amount of ahTet added to the growth medium can be adjusted to control the extent of expression from *tet*O$_7$-TATA$_{CYC1}$ to a desired level, which is a great advantage over the "all-or-none" promoters P$_{nmt}$ and P$_{urg1}$. Analogously, adding increasing amounts of ahTet to the growth medium of the *tet*O$_7$-*nse6* cells reverted their exquisite sensitivity towards the replication fork stalling agent hydroxyurea (HU) from an *nse6Δ*-like phenotype to a wild type-like one (Figure 5B).

For the *tet*O$_7$-*nse6* strain we observed that, in the absence of ahTet, its viability was greatly impaired following irradiation by UV light, too. However, this phenotype was not as penetrant as that observed for the *nse6Δ* mutant (Figure 5B, Pebernard, *et al.*, 2006), which suggests that some, minimal, transcription of *nse6* must occur from *tet*O$_7$-TATA$_{CYC1}$ even under repressed conditions. This leakiness was more obvious when the cells were challenged by UV light, instead of HU, probably because the minimal amount of Nse6 required to counteract UV light-induced DNA damage, being an acute form of genotoxic stress, is likely to be less than that required to counteract HU, which was used as a chronic source of insults to DNA in Figure 5B.

Having shown that, in the presence of TetR-Tup11Δ70, *tet*O$_7$-TATA$_{CYC1}$ can be used to induce expression of a desired fission yeast gene from its endogenous locus very quickly, we asked whether this system could be used to induce gene expression during meiosis. This is an important question because although P$_{urg1}$ can also be quickly activated it cannot be used to study meiosis: it is strongly activated by nitrogen starvation (Watt, *et al.*, 2008), which is required to induce mating and meiosis in fission yeast. Endogenous promoters whose activation state changes upon entry into meiosis do exist, however their activation/repression cannot be controlled at will and is restricted to a specific stage of the meiotic cycle, *e.g.* the *rec12* promoter is activated during pre-meiotic DNA synthesis (Farah, *et al.*, 2009; Lin and Smith, 1994; Mata, *et al.*, 2002). In order to address the above-mentioned question we used the *tet*O$_7$-*nse6* strain because *nse6* is critical to carry out meiosis (Wehrkamp-Richter, *et al.*, 2012). When *nse6Δ* cells are induced to undergo meiosis they produce morphologically aberrant asci and spores with very low viability (Figure 6, Wehrkamp-Richter, *et al.*, 2012). When *tet*O$_7$-*nse6* cells were stimulated to undergo mating and sporulation with an *nse6Δ* strain in the absence or presence of ahTet we observed that, under conditions of gene repression, they produced mostly aberrant asci with inviable spores to an extent that was similar to, but not as penetrant as, that observed for the *nse6Δ* mutant (Figure 6). Again, this phenotype was reverted to a wild type-like behaviour by the addition of ahTet. Such results indicate that *tet*O$_7$-TATA$_{CYC1}$ can be used to study biology associated with meiosis.

In conclusion, we report the construction and validation of a new system for rapidly controlling the expression of chromosomal genes in the fission yeast *S. pombe*, which relies on the use of a heterologous promoter, *tet*O$_7$-TATA$_{CYC1}$, in combination with tetR-based synthetic transcription factors. This system is easy and rapid to establish because it can be implemented using ordinary gene targeting techniques. It also allows marker recycling, so that the number of genetic modifications to a strain is unrelated to the number of available markers. We have generated all the reagents necessary to establish both Tet-On and Tet-Off systems, including several pFA6a-*tet*O$_7$-TATA$_{CYC1}$ plasmids that can be used to generate PCR fragments for promoter replacement and gene tagging.

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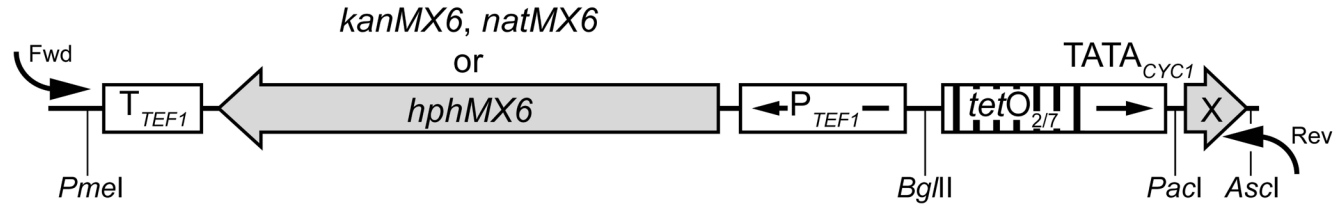
A



B

TAG (X)	Reverse primer	Vector name	PCR product size (kbp)
No tag	5'-(gene-specific)-CATGATTTAACAAAGCGACTATA-3'	pFA6a-*kanMX6*-*tet*O_7-$TATA_{CYC1}$	~2.2
$FLAG_3$	5'-(gene-specific)-TCCGCCTCCTTTATCATCATCGTCCTTATAG-3'	pFA6a-*kanMX6*-*tet*O_7-$TATA_{CYC1}$-$FLAG_3$	~2.3
		pFA6a-*hphMX6*-*tet*O_7-$TATA_{CYC1}$-$FLAG_3$	~2.4
		pFA6a-*natMX6*-*tet*O_7-$TATA_{CYC1}$-$FLAG_3$	~2.1
HA_3	5'-(gene-specific)-GCACTGAGCAGCGTAATCTG-3'	pFA6a-*kanMX6*-*tet*O_7-$TATA_{CYC1}$-HA_3	~2.3
GFP (S65T)	5'-(gene-specific)-TTTGTATAGTTCATCCATGC-3'	pFA6a-*kanMX6*-*tet*O_7-$TATA_{CYC1}$-GFP	~2.9
GST	5'-(gene-specific)-ACGCGGAACCAGATCCGATT-3'	pFA6a-*kanMX6*-*tet*O_7-$TATA_{CYC1}$-GST	~2.9

Figure 1.

The pFA6a-*tet*O_7-$TATA_{CYC1}$ module. (A) Schematic representation of the pFA6a-*tet*O_7-$TATA_{CYC1}$ module employed to generate gene-targeting cassettes for promoter replacement/N-terminal tagging. The position of the restriction sites used for cloning is shown. Arrows inside boxes show the direction of transcription. Arrows outside boxes indicate the position where the forward (Fwd) and reverse (Rev) PCR primers anneal. X = Tag. (B) Reverse primers for amplifying the promoter replacement/tagging cassettes from pFA6a-*tet*O_7-$TATA_{CYC1}$ vectors. The forward primer is common to all vectors and should conform to the following sequence 5′-(gene-specific sequence)-GAATTCGAGCTCGTTTAAAC-3′. The reverse primer is specific to each unique template. The gene-specific part of the primer typically corresponded to 80 bp of sequences upstream (Fwd) and downstream (Rev) of the start codon of the relevant gene. The expected sizes of the PCR products are shown.

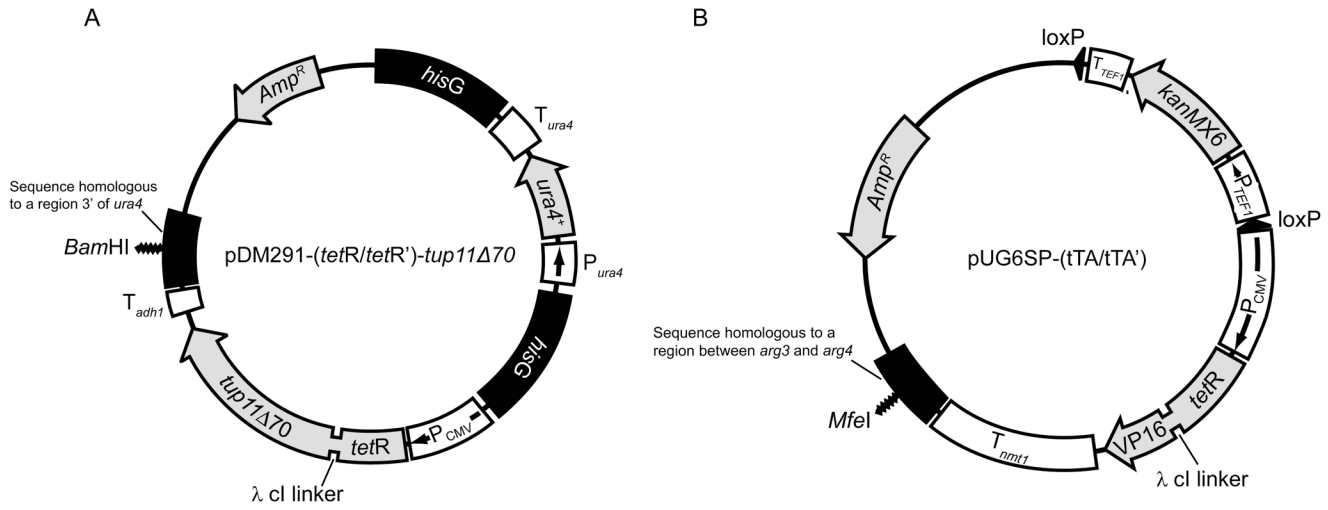


Figure 2.

The pDM291-(*tet*R/*tet*R′)-*tup11Δ70* and pUG6SP-(tTA/tTA′) vectors. Schematic representations of (A) pDM291-(*tet*R/*tet*R′)-*tup11Δ70* and (B) pUG6SP-(tTA/tTa′). The position of the restriction sites, (A) *Bam*HI and (B) *Mfe*I, which can be used to linearise the plasmids prior to transformation into yeast cells is shown. Arrows inside boxes show the direction of transcription. pDM291-*tet*R′-*tup11Δ70* and pUG6SP-tTA′ are identical to pDM291-*tet*R-*tup11Δ70* and pUG6SP-tTA except for the fact they contain *tet*R′ instead of *tet*R.

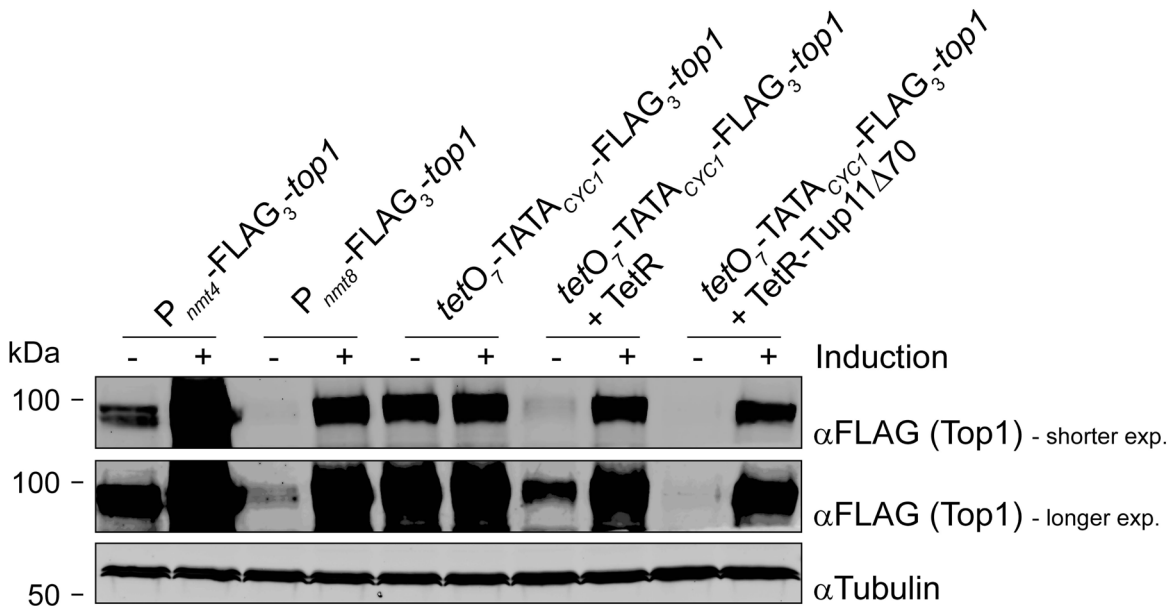


Figure 3.

On and off states of *tet*O_7-$TATA_{CYC1}$ *vs.* P_{nmt4} and P_{nmt8}. A comparison of *top1* expression driven from P_{nmt4}, P_{nmt8} and *tet*O_7-$TATA_{CYC1}$ under repressed and induced conditions, as determined by analyzing $FLAG_3$-Top1 protein levels by western blotting. $P_{nmt4/8}$ were induced by growing cells in medium lacking thiamine for at least 24 h. *tet*O_7-$TATA_{CYC1}$ was de-repressed by propagating cells in 2.5 μg mL^{-1} ahTet for at least 6 h.

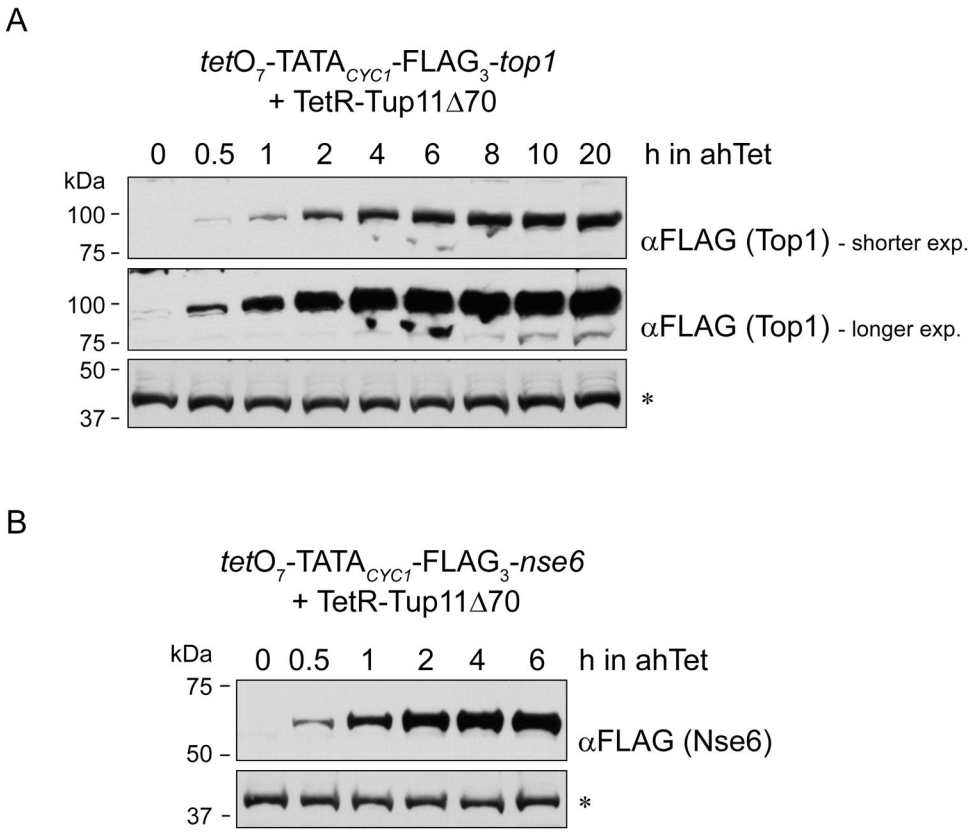


Figure 4.

Kinetics of protein accumulation after induction of *tet*O_7-$TATA_{CYC1}$. Time-course experiments showing the accumulation, by western blotting, of (A) $FLAG_3$-Top1 and (B) $FLAG_3$-Nse6 following induction of *tet*O_7-$TATA_{CYC1}$ by addition of 2.5 μg mL ^{1}ahTet to the growth medium. A yeast protein of unknown identity that cross-reacted with the anti-FLAG tag antibody (*) is used as a loading control.

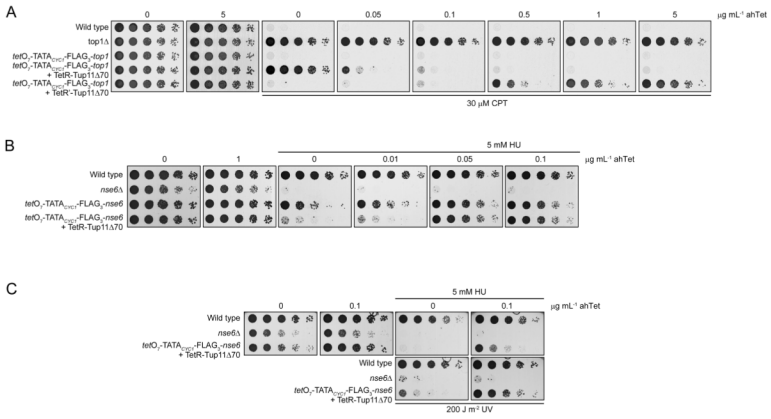


Figure 5.

The phenotypes of the *tetO*$_7$-*top1* and *tetO*$_7$-*nse6* strains. The relevant yeast strains were serially diluted onto rich medium in the presence of the indicate drugs and grown at 30 °C.

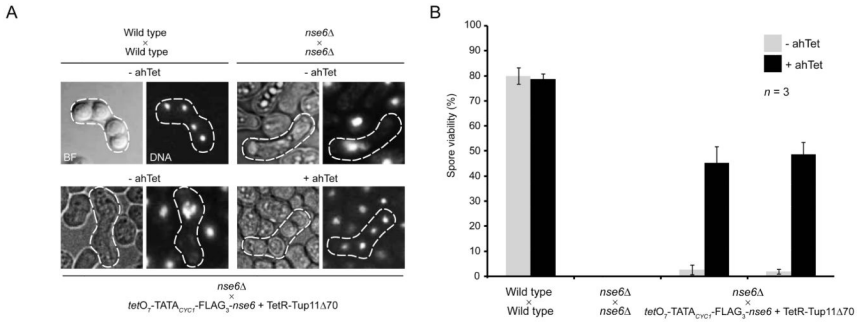


Figure 6.

*tet*O$_7$-TATA$_{CYC1}$ can be induced during meiosis. Wild type and *nse6Δ* cells or a cross between the *tet*O$_7$-*nse6* and *nse6Δ* strains were stimulated to undergo meiosis in the presence or absence of 2.5 μg mL^{-1} ahTet. The resulting cell-ascus mixtures were: (A) imaged by microscopy to determine ascus morphology and the DNA content of spores by DAPI staining, and (B) processed to isolate individual spores whose viability was measured by plating them on rich medium. Relevant asci are highlighted by dashed white lines. Error bars indicate the standard error of the mean from three independent crosses. Crosses between the *tet*O$_7$-*nse6* and *nse6Δ* cells were carried out using both h^+ (leftward set of bars in B) and h^- (rightward set of bars in B) *tet*O$_7$-*nse6* strains and the matching *nse6Δ* mutants. BF = bright field, DNA = DAPI.

Table 1

A list of the yeast strains used in this study. All strains are *ura4-D18 leu1-32*.

Strain #	Genotype	Source
NBY780	h^+	
NBY781	h^-	
NBY3770	*tet*O_7-$TATA_{CYC1}$-$FLAG_3$-*top1:hphMX6*, h^-	This study
NBY4461	*tet*O_7-$TATA_{CYC1}$-$FLAG_3$-*top1:hphMX6*, pDM291-*tet*R:*ura4*$^+$, h^-	This study
NBY3771	*tet*O_7-$TATA_{CYC1}$-$FLAG_3$-*top1:hphMX6*, pDM291-*tet*R-*tup11Δ70:ura4*$^+$, h^-	This study
NBY2553	P_{nmt4}-$FLAG_3$-*top1:hphMX6*, h^+	Heideker *et al.* (2011)
NBY2554	P_{nmt8}-$FLAG_3$-*top1:hphMX6*, h^+	Heideker *et al.* (2011)
NBY670	*top1::kanMX6*, h^+	Heideker *et al.* (2011)
NBY3992	*tet*O_7-$TATA_{CYC1}$-$FLAG_3$-*nse6*, h^+	This study
NBY3912	*tet*O_7-$TATA_{CYC1}$-$FLAG_3$-*nse6:hphMX6*, pDM291-*tet*R-*tup11Δ70:ura4*$^+$, h^-	This study
NBY871	*nse6::kanMX6*, h^-	Pebernard *et al.* (2006)
NBY835	*nse6::kanMX6*, h^+	Pebernard *et al.* (2006)
NBY4209	*tet*O_7-$TATA_{CYC1}$-$FLAG_3$-*nse6:hphMX6*, pDM291-*tet*R-*tup11Δ70:ura4*$^+$, h^+	This study
NBY4756	*tet*O_7-$TATA_{CYC1}$-$FLAG_3$-*top1:hphMX6*, pDM291-*tet*R′-*tup11Δ70:ura4*$^+$, h^-	This study

Table 2

A list of the oligonucleotides used in this study.

Primer #	Sequence
oNZ3	5′-CGCAGATCTGGGTAATATAGATCAATTCCTCGA-3′
oNZ4	5′-CGCATTTTAATTAACCTCCAGGATGATAAACGGATCCCCCG-3′
oNZ5	5′-GGAGGTACCTACGAAAAAGATATCAACAAGTTG-3′
oNZ6	5′-CGTGGTACCTCAAGGAGATGCAGGGTC-3′
oNZ14	5′-CCGGGCGCCCGCGCCACTTCTAAATAAGCG-3′
oNZ15	5′-CAATTTTAAAACCTATTTGCACCCCCTAGCGGATCTGCCGGTAG-3′
oNZ16	5′-GGTGCAAATAGGTTTTAAAATTG-3′
oNZ17	5′-ATGCATCCAACCCACCTAAATGGCCCGGGCATGGTACGATATATAATTAG-3′
oNZ33	5′-CCATACCCGGGGGTCGAGGAGCTTGGCCC-3′
oNZ27	5′-CCGGCCAGCTGGGGTAAAAGGAATGTCTCCC-3′
oNZ28	5′-GAATGCTTTCATGTATAAATCAAGGAGCTCGCATTACTAATAGAAAG-3′
oNZ29	5′-CTTGATTTATACATGAAAGCATTC-3′
oNZ30	5′-GGCCGCAGCTGCATGGTAATATTGATACAGAACG-3′