Evidence for a Role of MCM (Mini-chromosome Maintenance)5 in Transcriptional Repression of Sub-telomeric and Ty-proximal Genes in Saccharomyces cerevisiae*

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Renata Dziak‡§, David Leishman‡§, Maja Radovic‡, Bik K. Tye†, and Krassimir Yankulov‡

From the ‡Department of Molecular Biology and Genetics, University of Guelph, Guelph, Ontario N1G 2W1, Canada and the §Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York 14853

The MCM (mini-chromosome maintenance) genes have a well established role in the initiation of DNA replication and in the elongation of replication forks in Saccharomyces cerevisiae. In this study we demonstrate elevated expression of sub-telomeric and Ty retrotransposon-proximal genes in two mcm5 strains. This pattern of up-regulated genes resembles the genome-wide association of MCM proteins to chromatin that was reported earlier. We link the altered gene expression in mcm5 strains to a reversal of telomere position effect (TPE) and to remodeling of sub-telomeric and Ty chromatin. We also show a suppression of the Ty phenotype of a mcm5 strain by the high copy expression of the TRA1 component of the chromatin-remodeling SAGA/ADA (SPT-ADA-GCN5 acetylase ADAptor). We propose that MCM proteins mediate the establishment of silent chromatin domains around telomeres and Ty retrotransposons.

MCM1 (mini-chromosome maintenance) genes had been identified in screens for Saccharomyces cerevisiae mutants, which displayed high rates of minichromosome loss (1). MCM2-MCM7 homologues were subsequently found in all eukaryotes. It is believed that MCM proteins act as a component of the licensing machinery, which limits the occurrence of DNA replication to once per cell cycle (2, 3). MCM proteins form pre-replicative complexes at active as well as silent origins in S. cerevisiae or on un-replicated DNA in higher eukaryotes in a manner dependent on origin recognition complex (ORC) and Cdc6 (2, 3). Activation of these pre-replicative complexes by protein kinases is required for initiation of replication (2, 3). Removal of the MCM complex from origins at the time of initiation is believed to limit origin firing until the next cell cycle when the pre-replicative complex is re-established (2, 3). MCM proteins also seem to have a post-initiation role in DNA replication as indicated by their association with moving replication forks (4) and by the requirement for their uninterrupted function for fork progression in S. cerevisiae (5). In vitro assembled MCM complexes have also been shown to have DNA helicase activity consistent with a role in both melting of origin DNA and unwinding during fork elongation (3).

Some of the properties of MCM proteins suggest that they may have functions that extend beyond the regulation of DNA replication. They are 100- to 1000-fold more abundant than the estimated number of origins in S. cerevisiae (6, 7) and mammals (8, 9). Mammalian MCMs have been reported to associate with large complexes containing or contacting RNA polymerase II and general pol II transcription factors (10, 11), with the MAT1 component of CAK/TFIHII (12), with the transcriptional activator STAT1 (13, 14), and with the tumor suppressor p53 (15, 16) thus suggesting a function of MCMs in pol II transcription and gene expression. Other studies have linked MCM proteins to chromatin remodeling based on the interaction between the MCM proteins and histone H3/H4 dimers (17, 18) and between MCM2 and the histone acetyltransferase HBO1 (19).

 Genome-wide analyses of initiation of DNA replication in S. cerevisiae (20, 21) mapped 332 origins of replication. However, genome-wide mapping of the association of ORC and MCM proteins to chromatin (22) indicated at least 429 binding sites for these complexes. Only 79% of the ORC-MCM binding sites on chromosome X serve as origins. Another notable feature of ORC-MCM distribution is the clustering of ORC-MCM at telomeres, near Ty-transposable elements as well as solo Ty-LTR elements. Although telomeric X and Y elements are known to contain inactive or late replicating ARS elements where pre-replicative complexes could assemble (23), Ty and solo Ty-LTR elements are not known to associate with replication origins. It was suggested that this association of ORC-MCM with extensive repetitive elements could influence chromatin organization and genome stability (22).

Gene expression in the proximity of telomeres is reversibly repressed to produce either actively transcribed or completely silent chromatin, a phenomenon referred to as TPE (telomere position effect) (24). Mutations in the major regulators of TPE, the SIR genes and RAP1, also have an effect on the silent mating type loci (HM) and on rRNA gene repression (25, 26). On the other hand, HDP1 and HDP2 are required for silencing at telomeres, but not at the HM loci (27, 28). Thus, silencing of different regions appears to involve non-identical regulatory mechanisms that have some common features. It is notable that many of the genes that affect silencing at telomeres and the HM loci are also involved in some aspects of DNA replication (29–31). However, a number of studies suggest that DNA replication itself is not required for silencing (29, 30, 32). For example, analysis of the two components of ORC, ORC2 and
MCM5 Regulates Transcriptional Silencing

As in the mechanism, which requires expression of genome RNA (33, 35–37). Re-establishment of the silent state in all stress can be induced to produce as much as 10% of the total elements are dormant under normal conditions but under stress can be induced to produce as much as 10% of the total elements are expressed (33, 34).

Another transcriptional repression phenomenon was recently described for Ty1-transposable elements (33, 34). Ty elements are dormant under normal conditions but under stress can be induced to produce as much as 10% of the total elements are expressed (33, 34).

In this report we analyzed global gene expression in four mcm5 mutants. We found that a considerable number of genes in the vicinity of telomeres and Ty elements were up-regulated at the restrictive temperature in two of the mutants. This up-regulation correlated with chromatin remodeling of the affected genome regions and reversal of TPE.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Plasmids**—The strains used in this study are listed in Table I. Complemented merodiploid mcm5-1::MCM5, mcm5-2::MCM5, mcm5-3::MCM5, and mcm5-461::MCM5 were produced by transforming the corresponding mutant mcm5 strains with YIP122CDC46 (38) linearized by BspRI to produce tandem MCM5 duplications. Positive clones were selected on SC-Leu plates and then on YPD (1% yeast extract, 2% peptone, 2% glucose) plates at 37 °C. Telomere reporter strains, mcm5-461::Ura3-tel, mcm5-461::VR-UA3-tel, and mcm5-461::MCM5-VIIL-UA3-tel were produced by transforming mcm5-461::MCM5 and mcm5-461::YIpADH4UCAIV (39) linearized by Sall and EcoRI or by YIpUCAV (39) linearized by EcoRI, respectively, and selecting on SC-Ura. URA3 is inserted next to the partially truncated telomeric Tq1 repeats of the left arm of chromosome VII (VIIL-UA3-tel) and the right arm of chromosome V (VR-UA3-tel), respectively (39). Positive clones were streaked on SC-Ura, FOA, and SC-Leu plates at 30 °C and on YPD medium at 37 °C to select for strains that exhibit true TPE and display the expected phenotypes. The pAS-TRA1 plasmid (2 μm, TRP1, TRA1) was described in a previous study (40).

**Microarray Analysis of Gene Expression**—Mutant mcm5 and complemented mcm5::MCM5 strains were grown in YPD at 30 °C or 37 °C to A600 = 0.2–0.5. Probes were synthesized by PCR from S. cerevisiae genomic DNA with primer pairs, which amplified fragments as follows: a 500-bp fragment from HXK1, a 570-bp fragment from NCE103, a 430-bp fragment from ACT1, a 460-bp fragment from HXK1, a 430-bp fragment from ACT1, a 330-bp fragment from Ty1B protein. The HXK1, ACT1, DAN3, NCE103, and Ty1B

**Statistical Evaluation of the Data**—The evaluation was performed according to the textbook "Introductory Statistics" (41) and was based on the information provided in mips.gsf.de/proj/yeast and Refs. 22 and 42.

**Northern Blot Analysis**—Northern blots were performed with 10 μg of total RNA isolated from cells grown at 30 °C or 37 °C to A600 = 0.2–0.5. Probes were synthesized by PCR from S. cerevisiae genomic DNA with primer pairs, which amplified fragments as follows: a 500-bp fragment from COS1, a 400-bp fragment from YFL068, a 460-bp fragment from HXK1, a 430-bp fragment from ACT1, a 330-bp fragment from DAN3, a 570-bp fragment from NCE103, and a 400-bp fragment from the Ty1B protein. The HXK1, ACT1, DAN3, NCE103, and Ty1B

**FIG. 1.** Growth kinetics of mcm5 mutant and complemented strains at 37 °C. Cells were grown in YPD medium at 37 °C for 24 h. The A600 was measured at regular intervals and then plotted.
The normalized values from four microarray experiments (two mcm5–1 versus mcm5–1::MCM5 at 37 °C and two mcm5–461 versus mcm5–461::MCM5 at 37 °C) are shown. Systematic and common names of the ORFs are given in the left columns. ORFs that are up- or down-regulated at least 2-fold in at least three out of four experiments were obtained with GeneSpring version 5.0. The genome position of each ORF was obtained from mips.gsf.de/proj/yeast. Genes are listed according to their chromosome location. Genes that belong to a cluster (i.e. ORF is within 3 kb of a rRNA cluster) are indicated with a “R” in the column “ORF is within 3 kb of a rRNA cluster”. Genes that were obtained from Ref. 42. M/G, G1, G2/M, and S: genes with expression at peak at the corresponding stage of the cell cycle (42).

### Table II

| Systematic name | Common name | I. Genome position | II. Next to ARS | III. Cell cycle regulation | IV. Normalized values in individual experiments |
|-----------------|-------------|--------------------|-----------------|-----------------------------|-----------------------------------------------|
| YAR010C         | TY, CEN     | 4.2                | 25.0            | 2.6                        | 1.5                                           |
| YBL065W-A       | PDR3        | 4.8                | 20.0            | 2.9                        | 2.2                                           |
| YBL099W         | ATP1        | 3.1                | 2.0             | 1.4                        | 2.1                                           |
| YBL101C         | ECM21       | 2.7                | 2.2             | 2.1                        | 0.9                                           |
| YBL111C         | TEL         | 2.9                | 7.1             | 2.0                        | 1.7                                           |
| YBR012W-B       | TY          | 4.5                | 16.7            | 4.5                        | 2.7                                           |
| YBR068C         | BAP2        | 2.1                | 2.3             | 2.1                        | 2.2                                           |
| YBR072W         | HSP26       | 20.0               | 12.5            | 16.7                       | 3.1                                           |
| YBR080C         | SEC18       | 3.4                | 2.6             | 5.0                        | 2.0                                           |
| YBR218C         | FYC2        | 2.6                | 3.4             | 2.6                        |                                               |
| YBR236W         | SNF5        | 5.0                | 2.5             | 2.9                        | 1.7                                           |
| YBR301W         | DAN3        | 3.1                | 2.7             | 2.9                        |                                               |
| YCL020W         |              | 2.6                | 3.8             | 2.9                        | 1.7                                           |
| YCL040W         | GLK1        | 4.5                | 5.0             | 8.3                        | 0.9                                           |
| YDL124W         |              | 3.6                | 4.0             | 4.8                        | 1.1                                           |
| YDL248W         | COST7       | 2.4                | 4.0             | 2.5                        |                                               |
| YDR077W         | SED1        | 5.3                | 10.0            | 3.3                        | 3.4                                           |
| YDR171W         | HSP42       | 3.8                | 1.6             | 8.3                        | 0.8                                           |
| YDR298C         | ATP5        | 2.4                | 1.1             | 2.7                        | 1.5                                           |
| YDR342C         | HXT7        | 3.8                | 1.9             | 5.6                        | 2.6                                           |
| YDR368C         | TY          | 3.8                | 7.7             | 3.6                        | 2.4                                           |
| YEL039C         | CYC7        | 3.6                | 0.6             | 3.1                        | 1.2                                           |
| YEL060C         | PRB1        | 8.3                | 6.7             | 4.3                        | 1.1                                           |
| YEL077C         |              | 4.2                | 8.3             | 2.2                        | 1.9                                           |
| YER004W         | CEN         | 2.6                | 2.0             | 2.4                        | 1.5                                           |
| YFL014W         | HSP12       | 2.0                | 3.7             | 1.7                        | 2.4                                           |
| YFL062W         | COS4        | 2.3                | 2.4             | 2.3                        | 1.9                                           |
| YFL068W         | TEL         | 2.3                | 2.4             | 2.7                        | 1.2                                           |
| YFR053C         | HXK1        | 4.0                | 2.4             | 10.0                       | 1.1                                           |
| YGL058W         | OLE1        | 2.2                | 6.3             | 0.7                        | 2.3                                           |
| YGR298W         |              | 2.3                | 2.7             | 2.0                        | 1.2                                           |
| YGR161C         | TY          | 3.1                | 2.4             | 2.8                        | 1.2                                           |
| YGR180C         | RNR4        | 11.1               | 5.9             | 4.2                        | 1.7                                           |
| YGR296W         | TEL         | 0.6                | 2.1             | 2.6                        | 2.0                                           |
| YGR295C         | COS6        | 2.3                | 3.6             | 3.3                        | 2.4                                           |
| YGR296W         | YRF1-3      | 2.6                | 9.1             | 2.5                        | 1.8                                           |
| YHL021C         | tRNA, LTR,  | 3.3                | 3.2             | 4.5                        | 0.7                                           |
| YHL056C         |              | 2.9                | 3.0             | 1.3                        | 2.5                                           |
| YHR059C         | SLY         | 2.2                | 2.3             | 2.4                        | 2.0                                           |
| YHR057W         | TEL         | 6.7                | 2.2             | 9.1                        | 1.1                                           |
| YHR218W         | TEL         | 2.3                | 5.9             | 2.4                        | 1.5                                           |
| YIL107C         | PFK26       | 2.0                | 2.7             | 3.1                        | 1.2                                           |
| YJL028W         | RNR2        | 10.0               | 3.6             | 5.6                        | 1.8                                           |
| YJL158C         | CIS3        | 2.6                | 3.1             | 1.4                        | 2.5                                           |
| YJL159W         | HSP150      | 7.1                | 2.7             | 3.7                        | 1.9                                           |
| YJR026W         | TY          | 3.6                | 20.0            | 2.3                        | 1.5                                           |
| YJR039W         | TY          | 2.1                | 4.5             | 2.3                        | 2.6                                           |
| YJR045C         | SSC1        | 4.2                | 3.6             | 3.0                        | 0.8                                           |
| YKK067W         | YNK1        | 2.3                | 1.1             | 2.0                        | 2.0                                           |
| YKL109W         | HAP4        | 2.3                | 5.0             | 2.6                        | 2.3                                           |
| YKL151C         |              | 3.0                | 1.8             | 2.7                        | 0.8                                           |
| YKL163W         | PBR3        | 4.8                | 5.9             | 4.8                        | 2.3                                           |
| YLL026W         | HSP104      | 3.3                | 2.1             | 7.7                        | 0.8                                           |
| YLR121C         | YPS3        | 2.7                | 3.8             | 1.9                        | 1.6                                           |
| YLR142W         | PUT1        | 2.2                | 2.3             | 2.4                        | 1.1                                           |
| YLR155C         | ASP3–1      | 2.0                | 2.2             | 2.8                        | 1.3                                           |
| YLR194C         | TY          | 1.9                | 3.7             | 2.9                        | 1.9                                           |
| YLR216C         | CPR6        | 2.5                | 3.3             | 2.0                        | 0.7                                           |
| YLR327C         | tRNA        | 2.4                | 3.3             | 2.2                        | 1.4                                           |
| YLR334C         | tel, LTR    | 2.9                | 2.2             | 2.5                        | 2.5                                           |
| YLR391W         | SSM         | 2.8                | 3.3             | 3.6                        | 2.7                                           |
| YLR466W         | YRF1–4      | 2.9                | 6.3             | 2.0                        | 1.5                                           |
| YML100W         | TSL1        | 2.9                | 4.3             | 5.6                        | 1.1                                           |
| YML128C         | MSC1        | 2.2                | 2.0             | 4.3                        | 5.6                                           |
| YML132W         | COS3        | 1.6                | 3.3             | 2.8                        | 2.2                                           |
| YMR051C         | TY, tRNA    | 3.8                | 11.1            | 3.4                        | 1.9                                           |
**RESULTS**

**Analysis of Differential Gene Expression in mcm5 Strains**—An earlier analysis of gene expression of the mcm5-461 strain has indicated that several COS genes, which are positioned next to telomeres, were up-regulated at 37 °C compared with 30 °C (Ref. 38 and data not shown). It was unclear if the observed effect was determined by the position of the COS genes or it is a normal response to elevated temperature. To systematically correlate MCM5 function to gene expression we selected four previously characterized mcm5 strains (43, 44) and produced isogenic complemented strains (Table I) by inserting MCM5-Leu2 alleles in the corresponding mcm5 alleles. mcm5-2 and mcm5-461 displayed significant temperature sensitivity at 37 °C relative to their corresponding complemented strains (Fig. 1). mcm5-1 showed intermediate and mcm5-3 showed mild Ts phenotypes, respectively (Fig. 1). All complemented strains grew indistinguishably from unrelated wt strains at both 30 °C and 37 °C (data not shown).

We used these strains to conduct micro-array analysis of global gene expression. Two independent experiments were performed with each mutant versus its complemented strain at both 30 °C and 37 °C. We defined differentially expressed genes as 2-fold up- or down-regulated. Using this criterion we looked for common ORFs in different experiments by the clustering algorithm of the GeneSpring version 5.0 software. These analyses identified no consistent differences between complemented and mutant strains at 30 °C (analysis not included). At the same time, the same criteria pointed to considerable similarities in the expression patterns of mcm5-1 and mcm5-461 versus their isogenic complemented counterparts at 37 °C (Table II). In these strains we found 85 genes that were up-regulated and five genes that were down-regulated in three out of the four experiments. Comparison of all mcm5 mutants at 37 °C did not reveal other clusters of common genes (analysis not included). There were no significant similarities between mcm5-2 and mcm5-3 (analysis not included). In summary, we observed a common pattern of differentially expressed genes in the mcm5-1 and the mcm5-461 strains at the restrictive temperature. It is important to note that mcm5-1 and mcm5-461 were identified in different screens using different parent strains (43, 44).

**Characteristics of the Up-regulated Genes in the mcm5-1 and mcm5-461 Strains**—We defined several genome elements and areas of interest and examined the positions of the up-regulated genes in the mcm5-1 and mcm5-461 strains relative to these elements (Table II, column I). We also examined if the ORFs are immediately next to a pro-ARS (binds MCM proteins and has ARS activity (22)) or a false ARS (binds MCM-ORC proteins but has no ARS activity (22)) sites (Table II, column II). Because some mcm mutants arrest in early S or before M phase of the cell cycle, we examined if the listed ORFs were previously reported as cell cycle-regulated genes (42) (Table II, column III). We noticed a high representation of ORFs within 5 kb of Y' or X telomere elements, within 3 kb of Ty and within 2 kb of solo Ty-LTR elements or tRNA genes as well as genes whose expression is at its peak in M/G1 and G2 phases of

| Systematic name | Common Name | I. Genome position | II. Next to ARS | III. Cell cycle regulation | IV. Normalized values in individual experiments |
|-----------------|-------------|--------------------|-----------------|---------------------------|-----------------------------------------------|
| YMR105C        | PGM2        | tRNA               | False ARS       |                           |                                               |
| YMR186C        | ALD3        |                   |                 |                           |                                               |
| YMR186W        | HSC82       |                   |                 |                           |                                               |
| YMR273C        | ZDS1        | LTR                |                 |                           |                                               |
| YNL036W        | NCE103      | TY                 |                 |                           |                                               |
| YNL055C        | POR1        | TY                 |                 |                           |                                               |
| YNL180W        | YGP1        | M/G1               |                 |                           |                                               |
| YNL192W        | CHS1        | Pro-ARS            |                 |                           |                                               |
| YNL208W        |             |                   |                 |                           |                                               |
| YNL336W        | COS1        | TEL                |                 |                           |                                               |
| YOL016C        | CMR2        | LTR                |                 |                           |                                               |
| YOL053C-A      | DDB2        | LTR                |                 |                           |                                               |
| YOR374W        | ALD4        |                   |                 |                           |                                               |
| YOR385W        |             |                   |                 |                           |                                               |
| YPL058C        | PDR12       | TY                 |                 |                           |                                               |
| YPL154C        | PEP4        | False ARS          |                 |                           |                                               |
| YPL217W        | ATP15       | tel                |                 |                           |                                               |
| YPR160W        | GPH1        | TY                 |                 |                           |                                               |
| YPR204W        |             |                   |                 |                           |                                               |
| YVR016C        | TEF4        |                   |                 |                           |                                               |
| YOR333C        |             |                   |                 |                           |                                               |
| YDL051W        | LHP1        | tRNA               |                 |                           |                                               |
| YHR016C        | YSC84       | tRNA, LTR          |                 |                           |                                               |
| YCR304W        |             |                   |                 |                           |                                               |

At least 2x down-regulated ORFs in mcm5 versus complemented strains

| Table II—continued |
The estimated number of ORFs with the described characteristics in the entire genome are listed in column I. The numbers of genome elements and the size of the genome are derived from mips.gsf.de/proj/yeast. The number of cell cycle-regulated genes is from Ref. 42. Column II shows the numbers of ORFs with the described characteristics that are up-regulated genes in the mcm5 mutants (see Table II). The expected (random) frequency (E) is shown in column III. The observed frequency (O) in the cluster of up-regulated genes in the mcm5 mutants is shown in column IV. (O − E)^2/E values are shown in column V. A value close to 0 indicates random distribution.

| Characteristics of ORF | I. Estimated number of ORFs | II. Up-regulated ORFs in the mcm5 strains | III. E (%) expected frequency | IV. O (%) observed frequency | V. (O − E)^2/E |
|------------------------|-------------------------------|-------------------------------------------|-----------------------------|-----------------------------|----------------|
| 5 kb from a Y/X telomere element | 78 | 18 | 1.33 | 21.18 | 297 |
| 20 kb from a Y/X telomere element | 312 | 8 | 5.30 | 9.41 | 3 |
| 3 kb from a Ty element | 152 | 17 | 2.58 | 20.0 | 117 |
| 1.5 kb from a solo LTR element | 307 | 11 | 5.22 | 12.94 | 11 |
| 1.5 kb from a tRNA gene | 401 | 15 | 6.82 | 17.65 | 17 |
| 3 kb from a rRNA gene | 3 | 1 | 0.05 | 1.18 | 25 |
| 5 kb from a CEN element | 78 | 2 | 1.33 | 2.35 | 1 |
| Next to a pro-ARS element | 429 | 7 | 7.29 | 8.24 | 0 |
| Next to a false ARS element | 215 | 6 | 3.86 | 7.06 | 3 |
| M/G1 genes | 113 | 11 | 1.92 | 12.94 | 63 |
| S genes | 71 | 2 | 1.21 | 2.35 | 1 |
| S/G2 and G1/M genes | 316 | 4 | 5.37 | 4.71 | 0 |
| M/G1/TEL/TY | 3 | 3 | 3.53 | 5.88 | |
| G1/TEL/TY | 5 | | | | |

* M/G1/TEL/TY, ORFs close to a telomere or Ty element and at peak in the M/G1 phase of the cell cycle; G1/TEL/TY, ORFs close to a telomere or Ty element and at peak in the G1 phase of the cell cycle.

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* M/G1/TEL/TY, ORFs close to a telomere or Ty element and at peak in the M/G1 phase of the cell cycle; G1/TEL/TY, ORFs close to a telomere or Ty element and at peak in the G1 phase of the cell cycle.

Next we investigated the possible relationship between the members of the three groups of genes that showed the most significant increase in the observed relative to the expected frequency. The distribution of up-regulated ORFs and Ty elements along chromosomes (performed by the GeneSpring) indicated that they tend to co-localize in the central part of chromosomes and not around telomeres (analysis not shown). This observation argues against the idea that Ty de-repression is an indirect effect of de-repression of telomeres. Another possibility was that the up-regulation of genes in the mcm5 strains was a consequence of cell cycle deregulation. Indeed, we noticed a significantly higher than expected frequency of cell cycle genes with a peak in M/G1. We used a chi-square independence analysis to test if the appearance of TY and M/G1 genes or the appearance of TEL and M/G1 genes in the analyzed cluster are statistically related events (41). The calculations did not support the assumptions that the TY and M/G1 or TEL and M/G1 clusters are statistically dependent. In addition, only three ORFs were both close to telomeres or Ty elements and were up-regulated in M/G1 and only five ORFs were both close to telomeres or Ty elements and were up-regulated in the G1 phase. There is less than 20% overlap between the TEL/TY group of genes (Table II) and the M/G1, G1/TEL/TY, or G1/TEL/TY genes (42). These observations indicated that some of the differential gene expression in the mcm5-1 and mcm5-461 strains was linked to a cell cycle arrest (or delay) before or during mitosis. Such a conclusion would be in tune with the observed arrest (or delay) of different mcm mutants in late S or G2 phases of the cell cycle (45–47). However, it is more important that the cell cycle-regulated genes and the genes close to telomeres and Ty elements did not seem statistically dependent, and there was no significant overlap between these groups of genes.

In summary, the microarray analyses indicated that genes close to telomeres and to Ty elements are up-regulated at the restrictive temperature in two mcm5 strains. We concluded that the immediate proximity to a pro-ARS/false ARS elements (22) or cell cycle regulation of gene expression (42) cannot account for the number of up-regulated genes in these genome areas.

**Confirmation of the Microarray Results by Northern Blot**—We tested the validity of our microarray analysis by Northern blot with probes specific for genes that represent the TY and TEL groups of up-regulated ORFs in the mcm5 strains (Table III). The Ty probe is complementary to the Ty1B ORF sequence and will recognize all Ty RNAs. NCE108 is positioned next to a Ty2B element in the middle of chromosome XIV. These two probes represent the group of Ty-associated genes. HXX1 is 15 kb away from the VIR telomere. DAN3 is 1 kb away from the centromere.
The TPE analyses showed that the insertion of the Ty1B retrotransposon expression in the mcm5-1 and mcm5-461 strains at 37 °C. The strains indicated on the top of the figure were grown in YPD at 30 °C or 37 °C to \( A_{600} = 0.2–0.5 \). Total RNA (10 μg) was analyzed by Northern blot with the probes indicated on the left. Images of the ethidium bromide-stained gels prior to transfer to membranes are also shown (rRNA).

Expression of URA3 renders the cells sensitive to FOA thus selecting only cells in which URA3 is completely repressed. If TPE is reversed, the number of colonies on FOA plates will decrease. To minimize the effects on the reporter from specific individual telomeres, we inserted URA3 next to the left telomere of chromosome VII (VIIL-URA3-tel) and the right telomere of chromosome V (VRII-URA3-tel) of the mcm5-461 and mcm5-461::MCM5 strains (Fig. 3A). We used these strains to investigate the relationship between TPE and the elevated expression of telomere-proximal genes. TPE assays were performed with cells that were grown for about 12 generations at 30 °C or 37 °C. The numbers in column I represent average values of two independent experiments with three independent isolates with each of the strains.

The TPE analyses showed that the insertion of URA3 next to the VIIL telomere resulted in a moderate repression, which from the IIR telomere. These two probes are derived from unique genomic sequences as determined by BLAST. The COS probe is complementary to a region shared by the highly conserved COS1 through COS8 genes, which are positioned immediately next to telomeres. The YFL068 probe shares a significant homology with at least 10 other sub-telomeric genes. DAN3, COS1, and YFL068 represent 22% of all genes within 5 kb from telomeric repeats and 21% of the up-regulated genes in Table II. ACT1 has a unique sequence that is not close to a telomere or a Ty element.

Northern blot analyses with the Ty1B, NCE103, HXX1, DAN3, COS, and YFL068 probes detected very weak to moderate signals in samples from all cells grown at 30 °C (Fig. 2, lanes 1, 2, 5, and 6) and in the samples from the two complemented strains at 37 °C (Fig. 2, lanes 4 and 8). These data are consistent with the tight repression of most sub-telomeric genes under normal conditions. In all cases there was a significant increase in the signals in the two mutant strains at 37 °C only (Fig. 2, lanes 3 and 7). This increase is not a consequence of a general mRNA accumulation or overloading as indicated by the approximately equal signals detected by the ACT1 probe and the approximately equal amounts of rRNA loaded in all lanes (Fig. 3, lower part). We concluded that NCE103, HXX1, and DAN3 expression was elevated in the mcm5-1 and mcm5-461 strains at 37 °C. Similarly, the COS and YFL068 probes detected stronger signals in the same samples. Whereas the conserved repetitive nature of many sub-telomeric genes does not allow unambiguous conclusions on the expression of individual genes, it was clear that at least some COS and some YFL068-homologous genes were up-regulated in the mcm5-1 and mcm5-461 strains at 37 °C. These results are in agreement with the data in Table II.

The several bands that interacted with the Ty1B probe represent the expression of estimated 50 Ty elements in the genome. In the mcm5-1 and the mcm5-461 samples at 37 °C we noticed a significant decrease in the lower mobility bands plus the appearance of higher mobility species that were not detected at 30 °C. In addition, a slight smearing in these samples suggested a partial degradation of the Ty RNAs (Fig. 2, lanes 3 and 7). These new species of Ty RNAs were not a result of general degradation as indicated by the integrity of rRNA in these samples (Fig. 2, lanes 3 and 7). Currently we do not have a good explanation for the appearance of these RNAs. Nevertheless, the higher expression of Ty RNA as suggested by the microarray analyses was confirmed. Northern blot analysis of the mcm5-2 and mcm5-3 strains showed no increase of the analyzed RNAs at 37 °C (not shown).

In conclusion, the gene expression profiles of mcm5-1 and mcm5-461 in comparison to their complemented isogenic strains showed an up-regulation of telomere and Ty-proximal genes during growth at 37 °C. A similar gene expression pattern was not observed for the mcm5-2 and mcm5-3 strains. There was no obvious relationship between this expression pattern and the temperature sensitivity of the strains.

Reversal of TPE in mcm5-461—Genes close to telomeres are repressed by a mechanism referred to as TPE (telomere position effect) (24). The increased expression of sub-telomeric genes as determined by microarray and Northern blot analyses strongly suggested that a reversal of TPE takes place in the mcm5-1 and mcm5-461 strains at 37 °C. We tested this hypothesis by independent genetic TPE analyses. TPE confers a fully transcribed or completely repressed state of the genes thus leading to a mosaic of cells with different gene expression. A frequently used TPE assay exploits the expression of URA3 inserted next to a telomere (39). TPE is then assessed by growth of the recombinant cells on FOA-containing plates.
produced between 11 and 14% FOAR cells at 30 °C in the mcm5-461 and mcm5-461::MCM5 strains, respectively (Fig. 3B, column 1). When inserted next to the VR telomere, URA3 expression produced only about 0.1% FOAR cells in both the merodiploid and the mutant strains (Fig. 3B, column 1). Importantly, when the cells were grown at 37 °C, the proportion of FOAR cells in the mutant mcm5-461 strain significantly decreased (Fig. 3B, column 1). The ratio of FOAR cells in mcm5-461 relative to mcm5-461::MCM5 decreased from 0.82 at 30 °C to 0.12 at 37 °C in the case of VII-URA3-tel and from 1.21 to 0.02 in the case of VR-URA3-tel, respectively. These data showed that the elevated expression of telomere-proximal genes in mcm5-461 correlated to a reversal of TPE at two different telomeres.

**Modifications in Chromatin Structure Correlate with the Up-regulation of Sub-telomeric and Ty-proximal Genes—**
Repression by chromatin is a major regulatory mechanism of TPE (24). Similarly, Ty elements are also repressed by chromatin structures (34). It is possible that the transcriptional effects we observed at 37 °C are mediated by de-repression of chromatin. We tested this hypothesis by estimating the sensitivity of sub-telomeric chromatin (represented by the analysis of YFL068, COS, HXX1, and DAN3) and Ty chromatin to nuclease digestion. The mcm5-461 and mcm5-461::MCM5 strains were grown at 37 °C, and the cells were lysed and treated with micrococcal nuclease for 0, 2.5, 5, 10, and 20 min. Analyses of the resulting DNA are shown in Fig. 4. COS, DAN3, and HXX1 chromatin was significantly more susceptible to nuclease digestion in the mcm5-461 than in the mcm5-461::MCM5 strain (Fig. 4, compare lanes 1–5 to lanes 6–10). In these cases a substantial chromatin digestion was evident after 2.5 min of nuclease treatment in the mcm5-461 strain (Fig. 4, lane 2) but after 10–20 min in the wild type strain (Fig. 4, lanes 9 and 10). Similarly, the YFL068 and Ty1B probes detected degraded DNA after 2.5 min of nuclease treatment in the mcm5-461 strain (Fig. 4, lane 2), but 5 min in the mcm5-461::MCM5 strain (Fig. 4, lane 8). The control ACT1 probe did not detect major differences between the nuclease digestion pattern in the mcm5-461 and mcm5-461::MCM5 strains (Fig. 4, lower part). These observations lead to the conclusion that all tested sub-telomeric and Ty chromatin was more sensitive to nuclease treatment in the mcm5-461 as compared with the mcm5-461::MCM5 strain.

**Suppression of the Ts Phenotype of mcm5-461 by High Copy Number of TRA1**—The observed correlations between gene expression, chromatin structure, and mcm5 mutations suggested that MCM5 could functionally interact with chromatin remodeling factors. Experiments directed toward the identification of factors that suppress the Ts phenotype of mcm5 strains had identified such an interaction. In Fig. 5 we show that the expression of TRA1 from a high copy plasmid partially reversed the temperature sensitivity of mcm5-461. TRA1 is a component of several related histone-acetyl transferase complexes referred to as SAGA/ADA (40). It is important that mutations in TRA1 or other SAGA/ADA components have positive but also negative effects on the expression of different genes (48). Although the suppression of the Ts phenotype of mcm5-461 by TRA1 does not provide insights into the mechanism of suppression of sub-telomeric and Ty-proximal genes, it suggests that MCM proteins may interact with chromatin remodeling factors that could potentially influence gene expression.
DISCUSSION

In this report we show that mutations in \textit{MCM5} lead to de-repression of genes that are close to telomeres and \textit{Ty} transposable elements. We estimated that these effects are not secondary to cell cycle regulation of gene expression and are not a consequence of immediate positioning of the affected ORFs next to ARS elements (Table III). We also show that the gene expression pattern correlates with chromatin remodeling in the sub-telomeric and \textit{Ty}-occupied regions of the genome and with reversal of TPE. These findings are significant for two reasons.

First, the results suggest an unexpected role for the \textit{MCM} genes in transcriptional repression in addition to their well characterized function in initiation of DNA replication and in replication fork elongation (2, 3). Our findings are consistent with a recent study showing that Mcm7 may act as a repressor of several early cell cycle genes.\footnote{M. Fitch and B. K. Tye, personal communication.} Second, we demonstrate a similarity in the repression of \textit{Ty} retrotransposons and telomeres. We suggest that there may be a common mechanism in the variegated gene expression observed near telomeres and \textit{Ty} retrotransposons. Finally, we propose that \textit{MCM5} is yet another DNA replication factor that affects transcriptional silencing.

A key question in our study is whether the observed gene expression effects are secondary to deregulation of DNA replication or retardation of the cell cycle. In general, there are two views on the relationship between DNA replication and chromatin silencing. The first one postulates that chromatin silencing and especially TPE require DNA replication or a closely related event during S phase (24, 49, 50). Therefore, mutations that prolong S phase could have a secondary effect on TPE (24) and possibly other silenced domains. However, there is evidence at variance with this model. DNA replication itself is not required to establish silencing at the mating type loci (32) and telomeres can switch their transcriptional state during G1 arrest (51). There seems to be no parallel in the effect of mutations that affect DNA replication and their ability to suppress silencing defects at HMR (29). In the case of ORC2 and ORC5, their functions in silencing and DNA replication are completely separable (30, 31). There is also evidence suggesting both S- and M-phase requirements for the establishment of silencing (52). These and other studies propose the alternative view that replication proteins have a direct role in silencing perhaps by communicating with factors that establish silenced chromatin (22, 29, 32, 53). Consequently, some other S phase event independent of DNA replication mediates the establishment of silent chromatin. In agreement with the second model, we did not see any strong correlation between DNA replication deficiency and cell growth on one hand and gene de-repression on the other. For example, all \textit{mcm5} strains we analyzed have a strong mini-chromosome maintenance deficiency (43, 44, 54) that is indicative of poor DNA replication (1), but only \textit{mcm5-461} and \textit{mcm5-461::MCM5} showed up-regulation of telomere-proximal and \textit{Ty}-proximal genes (Table III). Similarly, there was no correlation between cell growth and presumably severity of the genome replication deficiency (Fig. 1) and gene de-repression (Table III).

We paid special attention to the possible relationship be-
between cell cycle regulation of gene expression and de-repression of sub-telomeric and Ty-proximal genes. In our experiments we observed higher than the expected number of ORFs with expression at peak in M/G1 (Table III and Ref. 42). It is possible that an arrest or a prolonged late S or G2 phase, which are characteristic for many mcm mutants (45–47), could contribute to this gene expression pattern in the mcm5-1 and mcm5-461 mutants (Table III). It is important that most of the M/G1 genes in Table II are not close to telomeres or Ty elements and that most of the Ty-proximal and sub-telomeric genes had not been characterized as cell cycle-regulated genes (Table II). In addition, none of the de-repressed genes corresponded to DNA damage of DNA replication checkpoint-regulated genes. It is therefore unlikely that a late S/G2 checkpoint jointly regulates the M/G1 genes and the Ty-proximal and sub-telomeric genes. To our knowledge there is no report linking Ty expression to cell cycle checkpoints that could support further speculation in this direction.

On the other hand, the distribution of up-regulated genes in the mcm5 mutants (Table III) is reminiscent of the genome binding of ORC-MCM complexes to chromatin (22). In this study a significantly higher than expected association of MCM-ORC complexes with sub-telomeric chromatin and chromatin around Ty and solo LTR elements was found (22). Only 77% of the genome-ORC/MCM binding sites serve as origins and a subset of them are inefficient (22). We found a higher than expected number of up-regulated genes in the mcm5 mutants in the same regions of the genome that show higher association of MCM proteins (Table III and (22)). Finer scrutiny indicated that only a small proportion of the up-regulated genes in the mcm5-1 and mcm5-461 strains (Table II) are positioned immediately next to a mapped MCM-ORC binding sites (22). Again, we could not explain our results with effects of active or dormant ARS sequences on neighboring genes. We therefore propose that at least some of the ORC/MCM binding sites in sub-telomeric chromatin and close to Ty retrotransposon elements serve in chromatin silencing. Our model suggests that MCM proteins could interact with chromatin remodeling factors that might influence chromatin structure of broader regions of the genome. As a result, de-repression and up-regulation of gene expression in the affected regions would take place. This effect is exerted on a larger scale along discrete genome regions rather than on individual genes. It is important that we observed up-regulation of ORFs next to 15 telomeres and 13 Ty elements. It seems that de-repression of sub-telomeric and Ty-proximal chromatin is a characteristic of many but probably not all of these genome areas. The actual mechanism of this proposed function of the MCM proteins is unclear. The observed genetic interaction of mcm5 with TRA1 points toward the involvement of SAGA/ADA. This complex is a major histone-acetyltransferase that is required for activation of many genes. However, a recent report showed the requirement of SAGA and its histone-acetyltransferase activity for gene repression as well (48). Involvement of other chromatin factors should also be considered.

We also found a higher than expected frequency of up-regulated genes within 1.5 kb from solo LTR elements and tRNA genes and 3 kb from rRNA genes (Table III). Although the difference in the observed versus the random frequency of these genes is obvious, the increase is smaller than in the case of sub-telomeric and Ty elements (Table III). It should be noted that chromatin at these genomic sites also tends to bind ORC-MCM complexes at higher incidence (22). At this point we do not exclude a connection between MCM binding and repression of transcription around LTR and tRNA elements, but the evidence is tenuous. An obvious entry missing in the list of up-regulated ORFs in Table II are the genes in the mating type loci. Suppression of these loci is regulated by ARS-containing silencers that are known to bind ORC (55) and probably MCMs (22). One possibility is that our microarray analysis had missed them. An equally good explanation is that suppression of these loci is indifferent to mutations in MCM5. The latter idea is in tune with an earlier study in which the mcm5-461 allele (44) failed to restore silencing at HCMR (29).

Previous studies have pointed out interactions of MCM proteins with a transcriptional activator (13, 14) or RNA pol II and general transcription factors (10, 11) in higher eukaryotes. A gene interaction between RNA pol II and MCM5 was also recently documented (38). We favor the idea that the effect of mutations in MCM5 on gene expression in the sub-telomere and Ty-proximal regions is mediated by alterations of chromatin structure. Nevertheless, in light of the published studies it is possible that the observed effects are partially mediated by the interactions of the MCM proteins with factors that are directly involved in pol II transcription. Further analyses should address the relationship between MCM proteins, chromatin factors, and gene expression.

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MCM5 Regulates Transcriptional Silencing

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Evidence for a Role of MCM (Mini-chromosome Maintenance) in Transcriptional Repression of Sub-telomeric and Ty-proximal Genes in *Saccharomyces cerevisiae*

Renata Dziak, David Leishman, Maja Radovic, Bik K. Tye and Krassimir Yankulov

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