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Report

A Coordinated Global Control over Cellular Transcription

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Summary

Although much is known about the regulation of gene transcription in eukaryotes, it is not clear whether cells have global controls that determine overall rates of transcription. We have investigated the effects that the DNA-to-protein ratio has on both total transcription and the transcription of individual genes in the unicellular eukaryote fission yeast. Mutants altered in cell size and those blocked in cell-cycle progression were used to vary the DNA-to-protein ratio over a 5-fold range. We found that cells of sizes within 2-fold of the wild-type value regulated global transcription to maintain similar transcription rates per protein regardless of the cellular DNA content. These changes in total transcription correlated with coordinated changes in gene occupancy by RNA polymerase II. In cell-cycle-arrested mutants exceeding a certain size, total transcription rates plateaued as DNA became limiting for transcription at low DNA-to-protein ratios [1]. Unexpectedly, expression levels of individual genes remained tightly coordinated with each other over the entire range of cell sizes. We propose that there is a coordinated, global control that determines the rate of transcription of most genes and that this control plays a role in regulating growth rate of the cell.

Results and Discussion

To investigate more global cellular controls over transcription, we have determined the influence of changes in the DNA-to-protein ratio (DPR) or "gene concentration" [2, 3] on transcription in the fission yeast. Earlier studies have examined this problem during synchronous cultures when the DNA content doubles during S phase. Step-like changes in total and poly $(A)^+$ transcription rates were described to occur during the cell cycle [3–5], but different timings of the steps were reported depending on the procedure used to prepare the synchronous cultures [5–7]. To avoid potential artifacts produced by the use of synchronous cultures, we have reinvestigated the influence of DPR on transcription rate using unperturbed asynchronous cultures of wild-type fission yeast and two mutants dividing at a small size (*wee1-50*) [8] and at a large size (*cdc25-22*) [9] (Figure 1A).

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All three strains were grown in the same conditions at two temperatures and were found to have similar doubling times, with DPRs varying over a 2-fold range (Table 1). From the RNA content per cell and the doubling time, we calculated the net rate of RNA accumulation per genome for the three strains. This rate increased (Figure 1B) as cells increased in size. To determine whether net RNA accumulation rates reflected rates of transcription, we assayed rates of total RNA and mRNA transcription by [^{3}H]-adenine pulse labeling (Figures 1C and 1D) using labeling conditions that reflect mostly RNA synthesis rather than turnover [10]. We found that the rates of total RNA and poly$(A)^+$ RNA synthesis correlated with cell size and cellular protein content (Table 1; Figures 1C and 1D), resulting in the transcription rate per protein being similar between the three strains (Figure 1E; see also Figure S1A available online). The similarity in rates per protein between the strains and the fact that there is no evidence to suggest that the *wee1-50* and *cdc25-22* mutations have any direct effect on transcription lead us to conclude that it is the changes in cell size that are influencing total transcription rate. To test whether changes in RNA turnover might be having an effect, we examined the mRNA turnover rates of eight genes after inhibiting transcription and found that all but one (*SPBC1734.07c*) of the genes had very similar rates in the three strains (Figure S1B). We conclude that changes in RNA turnover are unlikely to have significant effects on mRNA accumulation. We also conclude that the fission yeast cell compensates for a 2-fold change in DPR largely by globally adjusting the transcript synthesis rate, assuming no changes in gene copy numbers between the strains, for example in the rDNA repeat number. Similar observations made with bacterial cells [2] and mammalian hepatocytes [11] indicate that this global control may be present in other organisms.

We next investigated how transcription of individual mRNA genes responded to changes in gene concentration. Using microarrays, we determined mRNA levels in the three strains for 4655 open reading frames (ORFs; see Experimental Procedures) [12]. In a plot comparing mRNA expression normalized to total mRNA in the two size mutants (Figure 2A), nearly all genes were found to be close to the diagonal, indicating that the contribution of mRNAs to total mRNA for the majority of genes is the same (Figures 1C and 1D; Figure S2A). Very few genes lie beyond the 1.5× change threshold shown by the gray lines in Figures 2A and 2B. This is supported by Figure S2B, which plots ratios of individual gene expression levels. Plotting the number of genes that apparently change above a particular threshold as a function of that threshold (Figure 2C) showed that the observed changes in relative expression between mutants and wild-type were similar to the noise in the self:self hybridization control (Figure S2C). To produce an upper estimate of the number of the genes changing expression, we chose a high p value of 0.1. Of the 4845 genes present in both mutant microarrays, only 101 (2.1%) changed their relative expression more than 1.5-fold with a $p < 0.1$. It should be noted that the expression of ribosomal protein genes was not changed. Thus, when total mRNA transcription adjusts to DPR changes, at least 97% of all genes remain closely coordinated in the rates of their expression.

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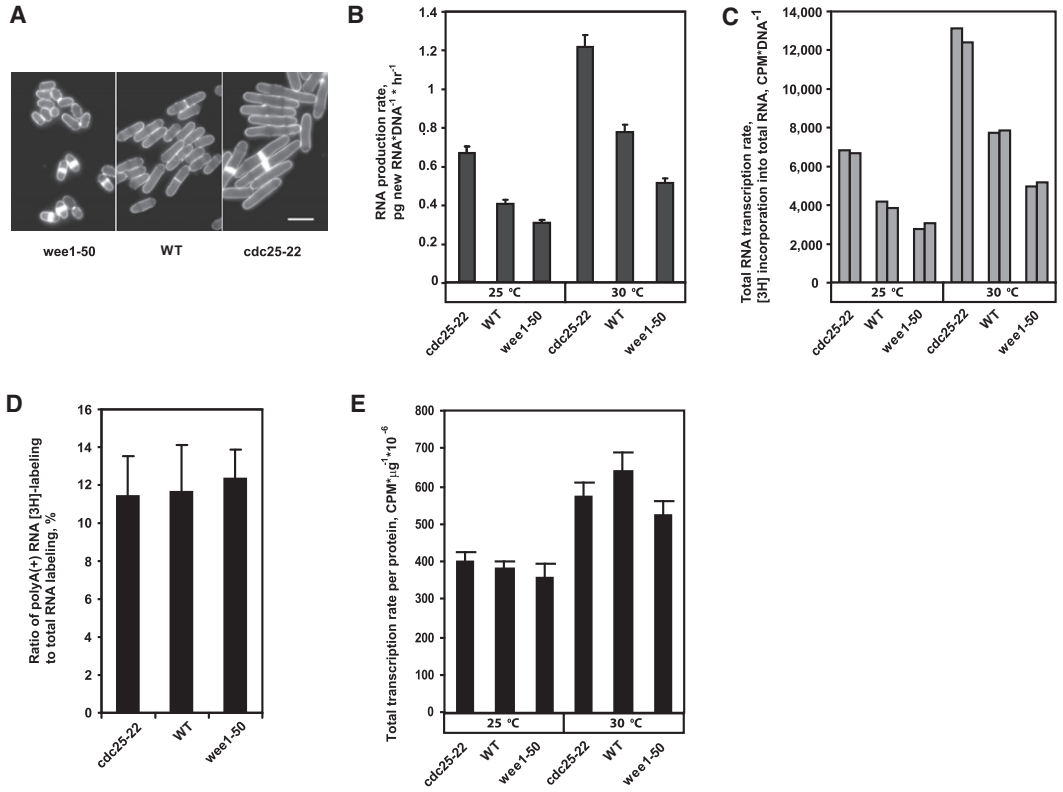

Figure 1. Changes in Cell Size Regulate Rates of Total and mRNA Transcription

(A) Size mutants *wee1-50*, *cdc25-22*, and wild-type (WT) grown at 30°C and stained with blancophor. Scale bar represents 10 μm.
(B) New total RNA synthesis per genome in *cdc25-22*, WT, and *wee1-50* strains; the mean (n = 3) and range are shown.
(C) [^{3}H]-adenine incorporation into total RNA. Two repeats are shown.
(D) [^{3}H]-adenine incorporation into poly(A)$^+$ RNA as percentage of incorporation into total RNA, mean (n = 2), and range.
(E) Rates of total transcription normalized to cellular protein content, counts per minute (CPM)/pg, mean (n = 2), and range. See also Figure S1.

Changes in global mRNA transcription rates are likely to be correlated with changes in RNA polymerase II (RNAPII) gene occupancy. To test this, we used chromatin immunoprecipitation with RNAPII antibodies followed by microarray (ChIP on chip; Figure 2D) or real-time polymerase chain reaction (Figure S2E) for 4638 genes in *cdc25-22* and *wee1-50* cells relative to wild-type cells. For nearly all genes, RNAPII occupancies increase in *cdc25-22* cells and decrease in *wee1-50* cells compared to wild-type cells (Figure 2D; Figures S2D and S2E). Only 1.8% (84 of the total 4638) of genes deviated with $p < 0.1$ more than 1.5-fold from the median change in RNAPII occupancy. Only 18 genes are common between the mRNA microarray and the ChIP experiments (Table S1), suggesting that most of 84 genes are false positives. We conclude that as gene concentration changes within this 2-fold range, there is a closely coordinated compensatory change in RNAPII occupancy of most genes, accounting for the similar changes in the transcription rates of individual genes and in total cellular transcription.

These data demonstrate that, over limited changes in DPR, transcription rates are mostly fully compensated. To investigate how a significant DPR drop affects transcription [1], we used the mutants *cdc10-M17* [9] and *cdc2-33* [9], which arrest mostly in G1 and G2, respectively, while RNA and protein synthesis continues [1, 9], generating DPRs around 5-fold lower than wild-type cells. We measured RNA and protein content, DPR, and the total transcription rate after shift from 25°C to the restrictive temperature of 36.5°C (Figures 3A–3D). Initially, total transcription rates per DNA in the mutant strains increased in proportion to the protein content of the

Table 1. Protein Contents, RNA Contents, and Doubling Times of *cdc25-22*, *wee1-50*, and Wild-Type

Strain	Temperature (°C)	DNA Per Cell (fg)	RNA Per Cell (pg)	Protein Per Cell (pg)	Doubling Time (hr)	DNA-to-Protein Ratio, 1 × 10^{-3}	RNA-to-Protein Ratio
cdc25-22	25	33.8	3.7 ± 0.27	17.02 ± 2.5	3.84 ± 0.02	1.89	0.22
WT	25	33.8	2.31 ± 0.23	10.5 ± 1.0	3.91 ± 0.03	2.94	0.22
wee1-50	25	33.8	1.86 ± 0.19	8.18 ± 0.4	4.22 ± 0.05	3.48	0.23
cdc25-22	30	33.8	5.04 ± 0.22	22.24 ± 1.3	2.87 ± 0.05	1.69	0.23
WT	30	33.8	2.97 ± 0.17	12.2 ± 0.6	2.63 ± 0.03	2.54	0.24
wee1-50	30	30.8	2.03 ± 0.18	9.74 ± 0.4	3.03 ± 0.06	3.24	0.21

For values shown in the table, at least two independent measurements have been performed. fg denotes femtogram; pg denotes picogram.

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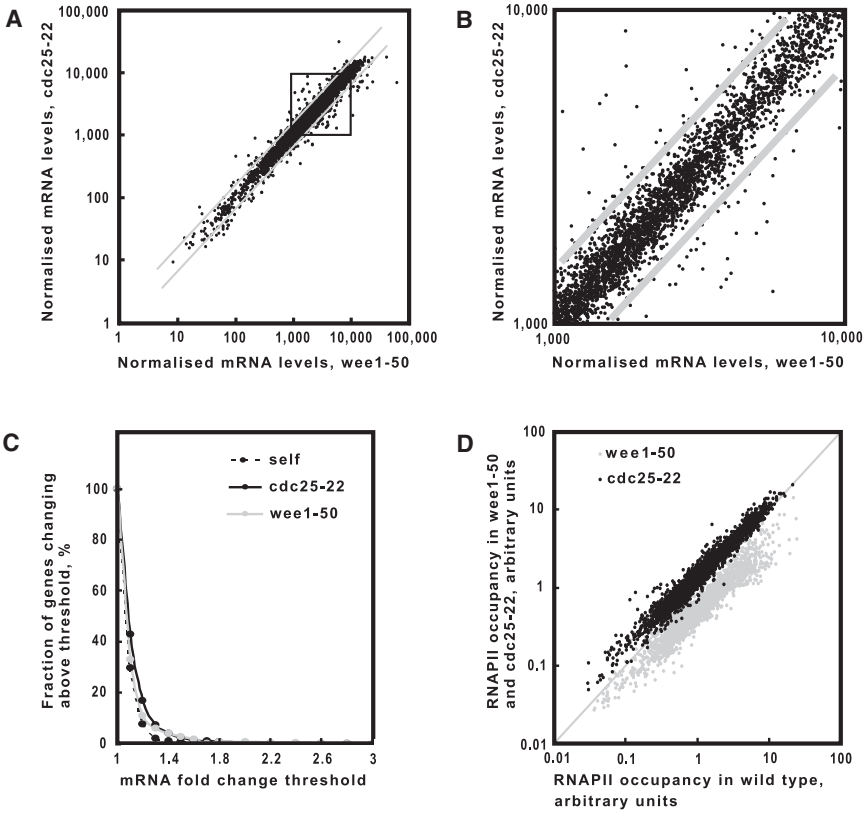

Figure 2. Transcriptional Response of Individual Genes to Cell Size Changes Is Globally Coordinated

(A) Similar expression of most of 4655 mRNA genes in *wee1-50* and *cdc25-22* cells grown at 30°C. Gray lines indicate 1.5× difference between the strains.

(B) Enlarged fragment of the graph in (A).

(C) The fraction of all genes that change between *cdc25-22* and wild-type, between *wee1-50* and wild-type, and within self:self hybridization experiment, as a function of a varying fold-change threshold.

(D) Global differences in RNA polymerase II (RNAPII) occupancy between *cdc25-22* and *wee1-50* cells as seen by ChIP on chip. See also Figure S2.

cell (Figure 3D). However, beyond 5 hr, transcription rates per DNA stopped increasing, generating a plateau between 5 and 7 hr (Figure 3D). During this 5–7 hr period, the two mutants had 2-fold higher net RNA accumulation rates per DNA compared with wild-type (Figure 3E; see Supplemental Experimental Procedures for calculation). At around 4–5 hr, the rate of increase in total cellular protein became slower than that of wild-type cells (Figure 3A), coinciding with the onset of the plateau in the rate of total transcription. These results suggest that large cells with a low DPR are unable to increase their total transcription to the levels required to support the continuing increases in protein synthesis.

To test whether the plateau in RNA transcription was due to the reduced DPR rather than the large cell size of the mutants, we measured transcription rate in a cytokinesis mutant *cdc11-119* [9], which, at 36.5°C, produces large multinucleate cells with a DPR similar to wild-type cells [9, 13]. After temperature shift, the average DNA content per cell gradually increased (Figure 3F), and the rate of transcription per cell reached much higher levels than in the arrested *cdc2-33* and *cdc10-M17* cells (Figure 3G). Therefore, enlarged cells that continue to increase their DNA content do not display a plateau in RNA synthesis. In a second experiment, we used a mutant *cdc10-V50* [14] that blocks in G1 at 36.5°C but that, after 5 hr at the restrictive temperature, resumes DNA synthesis (Figure 3H). Total transcription rate and DNA content were measured in *cdc10-V50* after the temperature shift to 36.5°C (Figures 3H and 3I). Early in the time course, *cdc10-V50* cells behave similarly to *cdc10-M17*, but when cells resumed DNA synthesis, transcription rate increased further in proportion to the increased amount of DNA (Figure 3I). We confirmed these results using another mutant allele *cdc10-129* (data not shown). From these results, we suggest that in cells twice the wild-type size or larger, the gene concentration drops to a level where genes become limiting for the rate of transcription.

To determine how this limit to total cellular transcription affects individual mRNA expression, we performed mRNA microarray analysis of the arrested *cdc2-33* and *cdc10-M17* mutants (Figure 4; Figure S4). Plots compared mRNA expression at 2 and 7 hr after the shift to correct for the temperature shift. Most genes were found to lie close to the diagonal (Figure 4), indicating that the majority of genes were coordinated in their expression despite the large decrease in DPR. Few points lie beyond the 1.5× change threshold marked by the gray lines (Figures 4A–4D). Only 7.2% (327 of 4555) of genes changed more than 1.5-fold in arrested *cdc2-33* cells, and 9.2% (451 of 4896) of genes changed more than 1.5-fold in arrested *cdc10-M17* cells (Tables S3–S5). Thus, at least 90% of genes remained coordinated in the cell-cycle-arrested mutants. This tight coordination is in contrast with the significant changes in relative gene expression described (1) in nutrient-limited cultures [15–20], (2) in response to histone deacetylation [21], and (3) in mutants with impaired TFIIH [22]. It appears that gene transcription responds very differently when external conditions are varied compared to DPR variations in constant external conditions. Finally, we investigated how increased gene dosage of a small number of genes influences their transcription by comparing gene expression in wild-type and cdc2-33 cells in the presence of an extra chromosome 3 fragment [23, 24]. We found that mRNA expression of genes on the fragment increased approximately 2-fold in the cells with the extra chromosome fragment (Figures S3E and S3F). We suggest that redistribution of a limiting factor among the increased number of genes may be responsible for this increase.

Our results indicate that there is a global cellular control that regulates the overall transcription in the cell and coordinates transcription rates of the majority of genes. We propose that this global control works through a factor (or factors) limiting gene transcription whose level is determined by the protein content of the cell. This control can compensate for moderate changes in gene concentration. However, it is unable to compensate for more extreme low gene concentrations

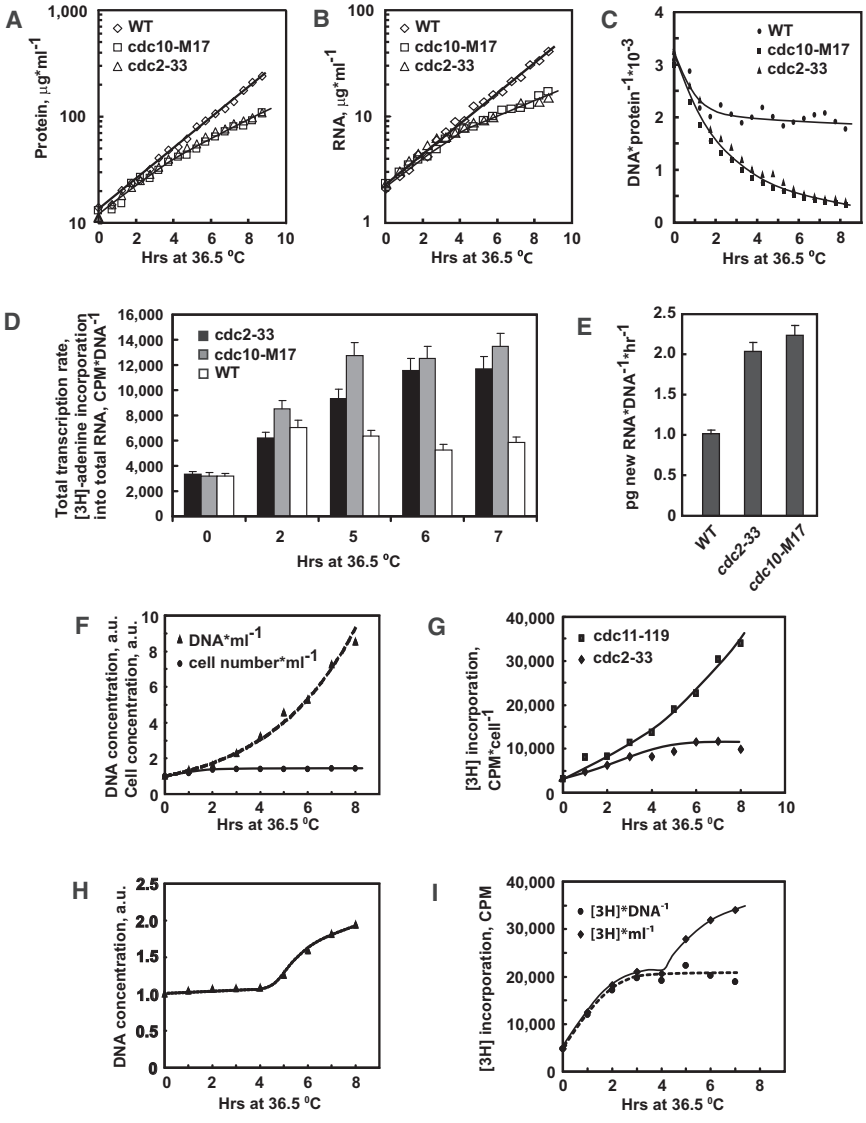

Figure 3. At Low DNA-to-Protein Ratio, DNA Becomes Limiting for Transcription

(A–C) *cdc2-33*, *cdc10-M17*, and wild-type (WT) growth after the shift to 36.5°C. Total protein (A), RNA (B), and DNA-to-protein ratio (DPR, C) are shown.

(D) [^{3}H]-adenine total RNA incorporation in *cdc2-33*, *cdc10-M17*, and wild-type normalized to cellular DNA after the shift to 36.5°C. Mean (n = 2) and range are shown.

(E) Rates of total RNA accumulation for wild-type, *cdc2-33*, and *cdc10-M17* between 5 and 7 hr after the shift to 36.5°C. Mean (n = 2) and range are shown.

(F) Normalized cell (●) and DNA (▲) concentration in *cdc11-119* cells after the shift to 36.5°C.

(G) [^{3}H]-adenine incorporation per cell into total RNA in *cdc11-119* (■) and *cdc2-33* (◆) cultures shifted to 36.5°C.

(H and I) *cdc10-V50* arrests at 36.5°C but resumes DNA synthesis after 5 hr. Normalized DNA concentration (H) and [^{3}H]-adenine total RNA incorporation per ml culture (◆) and per DNA (●) (I) are shown. a.u. denotes arbitrary units. See also Figure S3.

when DNA becomes limiting for transcription. There are many potential candidates for these factors, including transcription machinery components such as RNA polymerase [19, 25] and proteins determining chromatin accessibility [21, 26]. Competition for and subsequent partitioning of this common limiting factor between individual genes would coordinate gene expression in cell size mutants and cell-cycle-arrested cells with different levels of total cellular transcription. In addition to genes becoming limiting, it is possible that the transcription of a specific regulatory gene becomes limited, which restricts RNAPII transcription of most genes. We propose that this global cellular control determines the overall level of gene transcription in the cell, with individual gene circuits competing for one or more limiting factors that coordinate transcription of most genes, and that it is likely to be important for the overall growth of a cell.

Experimental Procedures

Standard *S. pombe* media and methods were used [27]. Strains used are listed in Table S2. For transcription rate analysis, 3.5 μCi of [^{3}H]adenine (21 μCi/nmol, Amersham) and 7.4 μM unlabeled adenine were added to 1 ml culture aliquots, and samples were incubated for 10 min at the respective temperatures noted in the text. The reaction was stopped by the addition of 10 ml ice-cold 10% trichloroacetic acid containing 0.74 mM adenine. Precipitated RNA was collected by filtering, and radioactivity was determined by scintillation counting. Poly(A)$^+$ RNA was isolated from acid phenol-extracted total RNA using PolyATtract magnetic beads (Promega). Fluorescence-activated cell sorting analysis of DNA content [28] was used to correct for differences in DNA per cell between strains. Cell numbers were determined using a Z-2 Coulter particle count and size analyzer (Beckman Coulter). Cellular protein contents were determined after overnight lysis in 1 M NaOH 1% Triton X-100 using BioRad Dc protein assay kit. Protein densities were found to be 0.13 ± 0.011 μg/femtoliter, 0.11± 0.009 μg/fl, and 0.12 ± 0.014 μg/fl for *wee1-50*, *cdc25-22*, and wild-type at 30°C. For mRNA expression analysis, ORF microarrays were used covering the coding regions, and signals were normalized to set the median expression ratios to 1 [10]. Analysis of the microarray results was performed using Genespring GX software (Agilent). To calculate individual mRNA levels, we multiplied mRNA levels in wild-type fission yeast [29] by the fold changes from the wild-type obtained from the microarray data. Chromatin immunoprecipitation was performed as described [30] using 4H8 antibody to RNA polymerase II.

Accession Numbers

The array data reported in this paper has been deposited in the ArrayExpress database with the accession number E–TABM-1075.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, four figures, and five tables and can be found with this article online at doi:10.1016/j.cub.2010.10.002.

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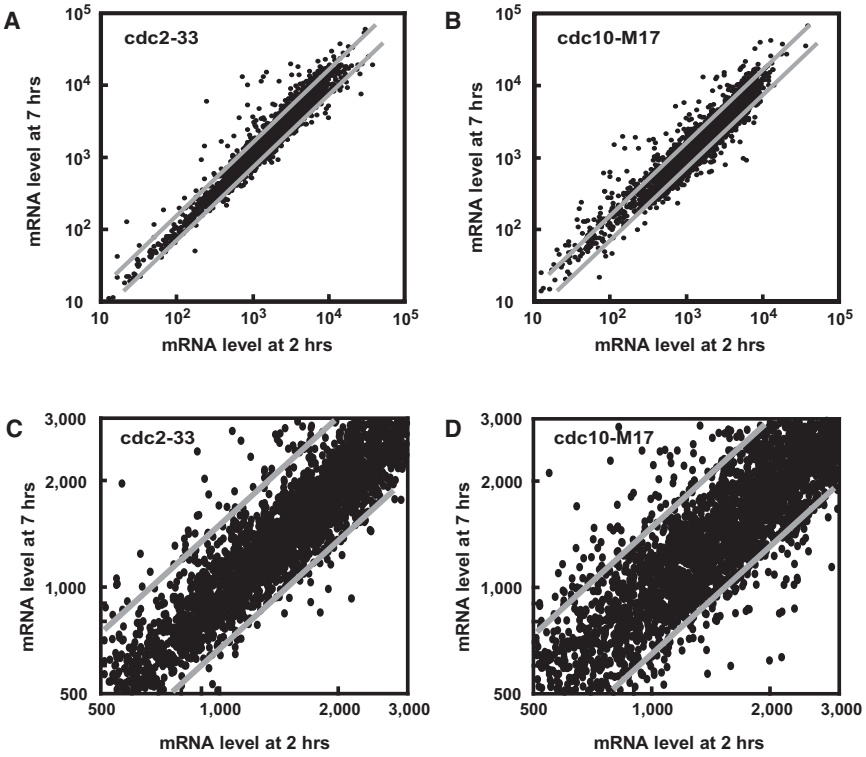

Figure 4. Tight Coordination of Gene Expression in Arrested *cdc2-33* and *cdc10-M17* Cells

(A and B) mRNA levels in *cdc2-33* (A) and *cdc10-M17* (B) cells at 7 hr after shift to 36.5°C were compared to cells at 2 hr after shift, before the plateau in transcription rates. Lines indicate 1.5× difference in mRNA expression.

(C and D) Enlarged fragments of the graphs shown in (A) and (B). See also Figure S4.

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