Genome-wide transcription map of an archaeal cell cycle

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Relative RNA abundance was measured at different cell-cycle stages in synchronized cultures of the hyperthermophilic archaeon *Sulfolobus acidocaldarius*. Cyclic induction was observed for >160 genes, demonstrating central roles for transcriptional regulation and cell-cycle-specific gene expression in archaeal cell-cycle progression. Many replication genes were induced in a cell-cycle-specific manner, and novel replisome components are likely to be among the genes of unknown function with similar induction patterns. Candidate genes for the unknown genome segregation and cell division machineries were also identified, as well as seven transcription factors likely to be involved in cell-cycle control. Two serine-threonine protein kinases showed distinct cell-cycle-specific induction, suggesting regulation of the archaeal cell cycle also through protein modification. Two candidate recognition elements, CCR boxes, for transcription factors in control of cell-cycle regulations were identified among gene sets with similar induction kinetics. The results allow detailed characterization of the genome segregation, division, and replication processes and may, because of the extensive homologies between the archaeal and eukaryotic information machineries, also be applicable to core features of the eukaryotic cell cycle.

Archaea | chromosome replication | microarrays | mitosis | *Sulfolobus*

Coordination of chromosome replication, genome segregation, and cell division with cell growth is central to all organisms. Precise temporal and spatial control of the cell-cycle processes ensures that daughter cells receive a full complement of the genetic material in each generation and that production of nonviable offspring is minimized. Mapping of cell-cycle-regulatory mechanisms and identification of genes and proteins involved in cell-cycle processes is, consequently, crucial for understanding the basic organization of all living cells.

In organisms belonging to the Archaea domain of life, most proteins involved in information processing (replication, recombination, repair, transcription, and translation) are homologous to the corresponding eukaryotic gene products (1, 2). In terms of the main cell-cycle processes, many components of the archaeal chromosome replication machinery have been characterized and shown to be homologous to eukaryotic counterparts (3). In contrast, limited information is available about genes and proteins involved in archaeal cell division and genome segregation (4–6). The FtsZ division protein, originally identified in bacteria, has been found in organisms belonging to the Eur-yarchaeota phylum, and formation of protofilaments and division ring structures has been demonstrated, but in the other main archaeal phylum, Crenarchaeota, no division proteins are known. In both phyla, little information is available about chromosome segregation mechanisms. The few putative segregation proteins identified include distant members of the ParA family of bacterial partition proteins, and of the bacterial/eukaryotic Smc family, but the extent to which they participate in archaeal genome segregation, if at all, is not known.

Analysis of gene expression in populations that progress in synchrony through the cell cycle is a key strategy for identification of cell-cycle genes, and for mapping regulatory circuits. Microarray-based whole-genome analysis has greatly expanded the scope of such studies, and provided possibilities for global investigations. Several species have been analyzed in this respect, eukaryotic (ref. 7 and references therein; Arabidopsis thaliana, Homo sapiens, Saccharomyces cerevisiae, Schizosaccharomyces pombe) as well as bacterial (ref. 8; Caulobacter crescentus). In these studies, 400–600 genes were found to be expressed in a cyclic manner, underscoring transcriptional regulation as an important cell-cycle control mechanism in bacteria and eukaryotes. No equivalent study of cell-cycle-specific gene expression in archaea has been reported.

The cell cycle of organisms belonging to the archaeal genus *Sulfolobus* is characterized by a short G1 phase, an S phase of about a third of a cell generation, and an extensive G2 phase lasting more than half the generation time, followed by rapid genome segregation and cell division (9–11). The cell cycle displays several eukaryotic-like features, including synchronous replication initiation from multiple origins (12, 13), a ten-fold lower replication rate than in bacteria (12), and a distinct G2 phase between termination of chromosome replication and initiation of genome segregation (10, 11). It is, consequently, possible that the mitotic and division machineries also display similarities to eukaryotic counterparts (6).

We have developed several techniques for synchronization of *Sulfolobus acidocaldarius* (11, 14), and a method based on acetate treatment has proven convenient and efficient (12). Using synchronized cultures, we previously demonstrated cell-cycle-specific synthesis and degradation of Cdc6/Orc1 (denoted Cdc6 in the following) replication initiation proteins in *S. acidocaldarius*, whereas the level of Tbp (TATA-binding protein) remained constant (13). The Cdc6-1 and Cdc6-3 paralogues were shown to be present during S phase, whereas Cdc6-2 was detected only in nonreplicating cells, suggesting positive and negative functions in replication control, respectively.

We have constructed whole-genome DNA microarrays for two *Sulfolobus* species (15). Here, we report a global analysis of relative RNA abundance at different cell-cycle stages in *S. acidocaldarius*, using synchronized populations and microarray-based transcript profiling.

**Results**

**Synchronization and Sampling.** Exponentially growing *S. acidocaldarius* cultures were synchronized in G2, in triplicate biological replicates [supporting information (SI) Figs. 5 and 6]. After release, newly divided cells first appeared 80 min after resuspension, and >50% of the population consisted of newly divided cells after another 30 min. Initiation of genome segregation and cell division were estimated to 60–70 and 70–80 min after
resuspension, respectively, because each process occupies \(\leq 5\%\) of the cell cycle (10, 11). After division, the cells proceeded through a short G1 period followed by rapid entry into S phase (9). Samples for RNA extraction were removed at eight time points during a 170 min interval, initiated 30 min after resuspension to reduce possible initial effects related to the synchronization treatment.

**Data Sets and Expression Patterns.** After data filtering, expression profiles were obtained for GSTs (gene-specific tags) corresponding to 1,677 (73\%) of the 2,292 protein-coding genes in *S. acidocaldarius* (16). Individual expression profile plots for each gene are at www.egs.uu.se/molev/suppl.data/lundgren2007.pnas/. Principal component analysis (PCA; SI Fig. 7) showed that data set pairs from adjacent time points clustered together, and demonstrated a general cyclic trend in the entire data set.

A vast majority of the genes showed minor variation over the cell cycle, as expected e.g., for basal transcription and translation machineries, metabolic genes and other house-keeping functions. In contrast, the GSTs that showed the largest changes in RNA abundance displayed \(>50\) fold induction. Because the level of synchrony was \(\approx 50\%\) (above), the magnitudes of the changes were even higher in individual cells than indicated by the population data. The sharpness of the induction peaks are also underestimated, as the return to lower levels were obscured by cells that gradually resumed cell-cycle progression after the first synchronous wave.

The responses were classified into either gradual or cyclic changes. The gradual effects, usually minor, were assumed partly to reflect continuous adaptation to postsynchronization conditions and were not considered further, except where specifically indicated. Instead, we focused on the \(>160\) GSTs, or 10\% of the genes analyzed, that displayed clear cyclic transcript abundance patterns, suggesting involvement in chromosome replication, genome segregation or cell division. Details are limited to a selected subset of particular relevance; a comprehensive list of genes considered to display interesting differential expression is provided in **SI Table 1**.

**Cyclic Induction.** The distribution of cyclically induced genes around the chromosome is shown in **SI Fig. 8**.

Within the replication category, the *cdc6-1* (Saci0722) and *cdc6-3* (Saci0001) replication initiation genes displayed strong cell-cycle-specific induction around the early G1 stage (Fig. 1A). Because a corresponding protein induction at this stage previously was demonstrated for both gene products (13), the results serve as an internal control for the data set, in addition to showing that the increases at the protein level result from an increased transcriptional activity. The induction occurred slightly earlier for *cdc6-1*, and also levelled off earlier, whereas the induction magnitude was significantly higher for *cdc6-3*. In contrast, the *cdc6-2* transcript (Saci0903) displayed an increase in abundance as the synchronized cell population approached G2 (Fig. 1B). Two genes of unknown function, Saci0002 and Saci0721, situated immediately adjacent to *cdc6-3* and *cdc6-1*, respectively, displayed clear cyclic induction patterns with similar timing as their adjoining genes (Fig. 1A. B, E, and F) and are, thus, likely to be coexpressed with these genes.

Two of the three *S. solfataricus* chromosome replication origins identified by marker frequency analysis (12) were fine-mapped by 2-dimensional gel electrophoresis (13), whereas the location of the third origin was restricted to within a 40 kb chromosome region. Three repeat elements within this region were suggested as a possible origin (12), but a conserved ORF across the repeats conflicted with the suggestion. In light of this finding, it is striking that the conserved gene, Saci1405, displayed strong cyclic induction around the G1/S transition (Fig. 1A).

An additional set of replication-associated genes also displayed clear cell-cycle-specific cyclic induction around the G1/S transition (Fig. 1 C and D), including *mcn* (Saci0900), *gins23* (Saci0901; presumably cotranscribed with Saci0900), DNA primase small subunit (Saci1279), *pemA* (Saci1280; presumably cotranscribed with Saci1279), ribonucleotide reductase large subunit (Saci1353), DNA polymerase I (Saci1537), deoxyuridylate deaminase (Saci1672), and thymidylate synthase (Saci2347). In contrast, a few genes whose annotation indicate an involvement in DNA precursor synthesis displayed an unexpected decrease in S phase and were instead induced toward the S/G2 transition, including phosphoribosyl transferase and phosphoribosylaminomimidazole carboxylase ATPase (Saci0925 and Saci1191; see www.egs.uu.se/molev/suppl.data/lundgren2007.pnas/), as well as two genes encoding ribonucleotide reductase small subunits (Saci1112 and Saci2220; Fig. 3B). The DNA polymerase II (Saci2156) transcript also displayed cyclic induction peaking toward the S/G2 transition (Fig. 3A). Yet another group of replication genes encompassing Flap endonuclease (Saci0775), DNA ligase (Saci0788), Rfc small subunit (Saci0907), ribonuclease HII (Saci0958), single strand DNA-binding protein (Saci0975), and DNA primase large subunit (Saci1542), displayed no differential expression (SI Fig. 9A).

A set of genes encoding proteins involved in DNA supercoiling or chromatin structure showed cyclic induction patterns (Fig. 2A). A previously unannotated orf homologous to topR-2 was
In accordance with a function in genome segregation, the first significantly, the gene was among the very first to be induced, in interestingly, the gene showed strong cyclic induction (Fig. 2 process). A promoter and an involvement in repair- and replication-related identical to that of \( \text{Saci1366} \) transcript displayed an induction pattern virtually implicating these genes in replication-associated repair. The genome segregation/cell division proteins in \( \text{Saci1321} \) and \( \text{Saci1322} \), \( \text{Saci0129} \), \( \text{Saci0604} \), \( \text{radA} \) (Saci0715) and \( \text{ogg} \) (Saci1367), implicating these genes in replication-associated repair. The cDNA products from the \( \text{Saci0064} \) and \( \text{Saci0362} \) genes, for which no \( S. \ \text{acidocaldarius} \) GSTs were present, cross-hybridized to the highly similar \( Sulfobolus \ \text{solfa-} \) taricus \( \text{SSO9180} \) GST on the dual-genome arrays, and displayed strong cyclic induction around the \( S/G_2 \) transition, demonstrating that one, or both, of these \( S. \ \text{treplica} \) rial cell-cycle functions. This result also shows that the population synchrony was maintained enough to detect induction at all main cell-cycle transitions. The \( \text{Saci0046} \) gene, which shares similarity with chromosom segmentation and DNA repair ATPases, as well as with \( \text{SpH} \), a protein suggested to be involved in cell division in the \( euryarchaeon \ Halobacterium \ \text{salinarum} \) (18). Orthologs to the \( \text{Saci0053} \) gene product have been suggested as archaeal functional analogues to the bacterial \( \text{FtsK} \) family of genome-partitioning proteins (19). Both \( \text{Saci0053} \) and the adjacent \( \text{mreI} \) repair nuclease \( \text{Saci0052} \) displayed induction around the \( S/G_2 \) transition (see www.egs.uu.se/molev/suppl.data/lundgren2007pnas/). The genes for the \( \text{Saci0055} \) gene product, which have been suggested as archaeal functional analogues to the bacterial \( \text{FtsK} \) family of genome-partitioning proteins (19). Both \( \text{Saci0053} \) and the adjacent \( \text{mreI} \) repair nuclease \( \text{Saci0052} \) displayed induction around the \( S/G_2 \) transition (see www.egs.uu.se/molev/suppl.data/lundgren2007 pnas/). The \( \text{Saci0049} \) protein, and for the \( \text{Pelota} \) protein (Saci0072), also displayed \( S/G_2 \) induction. The \( \text{Saci2119} \) gene product, a coiled-coil protein (COILS 2.2 prediction; ref. 20) which displays similarity to chromosome segregation and cytoskeletal proteins from both pro- and eukaryotes, also showed clear cyclic induction around the \( S/G_2 \) transition, and thereby constitutes an

![Fig. 2. Cyclic induction of genes known, or inferred, to be involved in chromatin structure, DNA repair, genome segregation, and cell division, and of genes with partial annotation. (A) DNA supercoiling and chromatin structure genes. (B) DNA repair genes. (C) Genome segregation and cell division genes. (D) Early and strongly induced genes with partial annotation. (E) Genetic map of the katanin chromosome region, with tags illustrating expression profiles. The scale is in kilobases.](image-url)

![Fig. 3. Cyclic induction of genes at the \( S/G_2 \) transition, and of regulatory genes. (A and B) Genes with partial annotation induced at the \( S/G_2 \) transition. (C and D) Cyclic induction of protein serine-threonine kinases and transcription factors.](image-url)
additional GSTs displayed G2 induction, including Saci108 (acyl-Coa dehydrogenase), Saci116 (acetyl esterase) and DNA polymerase II (Saci2156), as well as the Saci1854, Saci1855, Saci1882, Saci1930 (AAA* ATPase) and Saci2219 genes without detailed annotation (SI Fig. 9E).

The genes that showed cyclic induction included several candidates for cell-cycle-regulatory functions (Fig. 3C and D), a striking example being the Saci1193 protein serine-threonine kinase which was among the first to be induced, in parallel with parA (above). Also the Saci1694 serine-threonine kinase showed distinct cyclic induction, slightly later than Saci1193 and at a significantly lower magnitude. One of the three transcription factor B paralogues (tfb-2; Saci3141) also displayed strong early cyclic induction and, thus, seems specifically devoted to cell-cycle regulation. At least six additional putative transcription regulators showed clear cyclic abundance patterns at different cell-cycle stages, including Stal (Saci102), Saci0800, belonging to the ArsR family, Saci0942, with similarity to CopG plasmid copy number regulators, Saci1012, which shares similarities with Mn-dependent transcription factors, Saci1107, a TetR family regulator, and Saci2136, an AsnC-family transcription factor.

Gradual Increase or Decrease. The GSTs that showed a gradual increase or decrease in transcript abundance (SI Fig. 9F–I), included several genes related to flagellar synthesis (Saci1174-Saci1178), the Saci1944 conserved membrane protein with a strong gradual increase throughout S phase, and the Saci2230 gene for a predicted nucleic-acid-binding protein, with strong gradual increase throughout the early S phase. A majority of the purine biosynthesis genes (Saci0124; Saci 0709; Saci1607-Saci1613) were also among those that showed coordinated gradual transcript increase in S phase, in accordance with an increased requirement for DNA building blocks. A gradual increase in abundance was observed for the strongly repressed Saci1090 ATP-dependent helicase, the Pat acetyltransferase (Saci2284), and the Saci1223 transcription regulator of the Lrs14 family.

Quantitative PCR. Quantitative PCR (qPCR) was performed on genes representing all main expression categories, to validate the microarray results (SI Table 2 and SI Fig. 10). The results were highly similar to the microarray data, with a Pearson correlation coefficient of 0.98. A set of genes considered to be cell cycle related, but for which the microarray data were poor or missing, were also analyzed by qPCR. The pcnaA (Saci 0826) and pcnaB (Saci0817) genes displayed induction in early S, although at a lower magnitude than pcnaC (above). The Saci1340 gene displayed strong cyclic induction at the G1/S transition, similar to the adjacent tfb-2 (Saci3141) gene (above), indicating cotranscription and a cell-cycle role. Expression of the DNA polymerase III gene (Saci0074) decreased across the time series, confirming microarray data from a single biological replicate, whereas the gene for DNA polymerase IV (Saci0554) displayed low-magnitude cyclic induction in late S.

CCR Elements. The upstream regions of cyclically induced genes were searched for common regulatory elements. The CCTCTC-CCTA consensus sequence, which we denote CCR-1 (Cell Cycle Regulation 1 box), was identified ∼50 nt upstream of the Saci0743, Saci0942, Saci1012, Saci1237, Saci1340-Saci1341, and Saci1372-Saci1374 genes. A second consensus element (CCR-2; TGTAT-TAT) was found ∼45 nt upstream of the first codon of another set of genes (Saci0203-Saci0204; Saci0969; Saci1193; Saci1340-Saci1341). The genes in each set showed near-identical timing, kinetics and magnitude of induction (Fig. 4). Genes with similar induction profiles that lacked both elements were, however, also present (SI Fig. 9F), indicating additional regulatory complexity.

Discussion

Cell-Cycle Regulation. The analysis demonstrates that transcriptional regulation is fundamental for archaeal cell-cycle progression, and several putative cell-cycle-regulatory factors were identified. Elucidation of phosphorylation targets for the two serine-threonine protein kinases will be highly interesting: could these targets, e.g., include Cdc6/Orc1, orthologs of which have been shown to autophosphorylate on serine residues in archaea (21)? In addition to regulation at the protein level, at least seven transcription factors displayed cyclic transcript abundance. These factors are likely to define regulons specific for archaeal cell-cycle progression, including identification of promoter binding sites and target genes will be essential for delineation of regulatory networks. Prominent among the regulators is the Tfb-2 basal promoter recognition protein, providing an explanation for the presence of multiple Tfb paralogues in Sulfolobus. Furthermore, eukaryotic CDKs (cyclin-dependent kinases) also belong to the serine-threonine family, and Tfb-2 proteins contain a cyclin-box fold (22). In combination with the distinct cyclic expression patterns of kinases, cdc6 genes and tfb-2, these observations may even hint at the existence in archaea of regulatory features reminiscent of those of eukaryotic cyclin/CDK complexes.

Successive activation of transcription factors provides temporal control of different gene sets through cascade-type regulation, and a tentative order of activation in S. acidocaldarius is apparent in Fig. 3C and D. The presence of the CCR-1 and CCR-2 elements in the promoter regions of gene sets with similar induction profiles provides further support for this type of mechanism. Identification of the transcription factor in control of the respective CCR regulon will be important for elucidation of regulatory features, and for correlating the gene sets to execution of specific cell-cycle processes.

One of the transcription factors, Stal, has also been recruited by the SIRV1 virus (23). The use of a cell-cycle-induced transcription factor could provide a means to couple viral multiplication with host cell entry into S phase, thus ensuring adequate supplies of host replication factories and DNA precursors for SIRV1 replication.

Chromosome Replication. Many replication genes displayed cyclic activation around the G1/S transition. Induction of cdc6-1 seemed to occur even earlier, raising interesting questions about coordination of division termination with initiation of chromosome replication. It is possible that successful cell division could provide a cue to initiate cdc6-1 transcription, thereby ensuring coordination of the two processes. However, a newborn Sulfolobus cell clearly goes through a G1 period before replication is
initiated (ref. 9; SI Fig. 6), indicating that additional requirements need to be fulfilled before a commitment to replication can be made.

The cdc6-3 gene showed a slightly later induction and plateau, in agreement with a secondary role relative to Cdc6-1. It is also possible that the two proteins represent different functions, e.g., origin recognition and helicase loading, analogous to models derived from recent structural data for the bacterial DnaA and DnaC proteins (24), but the previously demonstrated differential recognition of replication origins (13) is difficult to reconcile with such a model. The gradual induction of the cdc6-2 gene as the cultures approached G2 contrasts sharply with the transcript level. The transcript level did not increase until after 80 min and showed significant induction already at 60 min (Fig. 4), and those profiles for the other two paralogues, and agrees well both with protein abundance data and with a suggested negative regulatory role in chromosome replication (13).

The data set is the first to differentiate between the in vivo roles of the at least 4 different Sulfolobus DNA polymerases, and an important outcome is the strong implication of Pol I as the main replicative archaeal polymerase. The induction magnitude was moderate, either indicating low-level expression also at other cell-cycle stages, or that only a small number of gene products are required. In theory, 12 molecules would be enough to support leading and lagging strand DNA polymerization at the 6 replication forks (12) in actively replicating Sulfolobus cells, assuming that Pol I performs both functions. Other DNA polymerases either showed decreased expression in S phase (Pol III and Pol IV), or gradual induction culminating at the S/G2 transition (Pol II), indicating auxiliary, or subsequent, functions.

All three DNA polymerase sliding clamp (Pcna) subunits displayed increased expression in early S, in agreement with a heterotrimERIC sliding clamp complex (25), although the induction magnitude differed between pcaNA and pcaNB on the other. Gins23, together with the associated Gins15 and RecJdbh proteins, have been suggested to couple leading and lagging strand replication in archaea (26). The gins23 gene displayed one of the strongest cyclic induction patterns in the data set, whereas recJdbh (Saci0177) and gins15 (Saci1278) only showed minor changes (see www.egs.uu.se/molev/suppl.data/lundgren2007/pnas/), demonstrating that Gins23 is the differentially regulated member of the complex.

Several other genes central for replication and DNA precursor synthesis also displayed cyclic induction at the beginning of S phase, whereas a second set of replication-associated genes showed no detectable cell-cycle-specific expression. The archaenal replication machinery is, thus, divided into a constitutively expressed set, which includes most lagging-strand replication proteins, and a set specifically induced at the onset of chromosome replication, suggesting that lagging-strand functions may be required for repair-related DNA synthesis to a significantly larger extent than the leading strand machinery.

Several genes believed to be specifically associated with DNA repair showed cyclic induction in early to middle S, including exonuclease III, ogg, radA and xpf, associating these enzymes with DNA damage restoration during the main replication process, in addition to repair of lesions in nonreplicating DNA. The Xpf endonuclease has previously been shown to interact with Pena in S. solfataricus (27), in support of an active role at the replication fork.

The data set also implicates a set of uncharacterized genes in the replication process, based upon expression profiles and induction timing. Two genes immediately adjacent to cdc6-1 and cdc6-3, respectively, are prime candidates to encode previously unrecognized replication functions, as is the gene across the previously suggested third replication origin, whose induction profile is consistent with a replication initiation function. Importantly, a clear distinction was apparent between genes that showed significant induction already at 60 min (Fig. 4), and those in which the transcript level did not increase until after 80 min (Fig. 1A). The latter category included previously known replication genes such as cdc6-1 and cdc6-3, and the similar induction kinetics for the Saci0276-Saci0277, Saci0344, Saci0947, Saci0969-Saci0970 and Saci1130 genes therefore implicate these gene products as novel replication-associated functions.

**Genome Segregation, Cell Division, and Chromatin Organization.** A main aim of this study was identification of candidate genes for the unknown genome segregation and cell division machineries. The strong early cyclic induction of the parA gene served as a marker in the search, and provides support for involvement of ParA in archaeal mitosis. A set of other genes showed similar early induction and therefore emerge as putative novel segregation, mitotic spindle-like or division functions. This inference is further supported by the presence of the CCR-1 and CCR-2 boxes in the promoter regions of two gene subsets, implicating these gene sets as coordinately controlled mitosis/division regulators. The CCR-1 regulated genes include the Saci1372-Saci1374 operon, with Saci1372 encoding a homologue of eukaryotic p60 katanin protease, involved in disassembly of microtubules (28).

Together with the striking induction pattern, this similarity implicates this transcription unit as a prime candidate for an archaenal mitosis-associated operon. The Saci0203 gene also emerges as a putative novel genome segregation protein based on the similar expression timing as that of parA, and on the adjacent location. The two genes may thus constitute an operon, analogous to the soj (ParA family) and spo0F partition gene pair in Gram-positive bacteria (29). More than 10 additional novel candidate genome segregation and/or cell division genes were identified (see Results) based upon immediate cyclic induction, as compared with the 20 min delay observed for replication genes (above).

A number of genes were induced at the S/G2 transition, indicating cell-cycle-specific involvement in postreplicative events. SRSR clusters have been suggested to act as possible centromer-like elements during archaenal chromosome segregation (30, 31), and the induction of an SRSR-binding protein agrees with this suggestion, although an RNAi-like function for SRSR clusters recently was proposed (32). The Pelota protein (33) is implicated in spindle formation and nuclear envelope breakdown in Drosophila (34), and the late induction is in line with a cell-cycle-related function also in archaea, as is the induction of the Saci0053 gene, suggested to be involved in chromosome segregation (19), in addition to DNA repair. The postreplicative induction of the Saci0046 gene, and of the gene for the Saci2119 coiled-coil protein, may indicate cytoskeletal roles that could be related to cell-cycle-specific chromatin rearrangements after replication termination.

Several genes involved in chromatin organization (17) displayed no expression changes over the cell cycle (see www.egs.uu.se/molev/suppl.data/lundgren2007/pnas/ and SI Fig. 9A), including those encoding both Alba paralogues (Saci1321; Saci1322), and the Sir2 Alba deacetylase (Saci0381). In contrast, the Pat acetyltransferase, which also acts upon Alba (35), displayed strong down-regulation in early S, indicating that acetylation is suppressed during chromosome replication. Furthermore one, or both, of the two Sac7 DNA-binding proteins displayed strong cyclic induction around the S/G2 transition, indicating involvement in cell-cycle-specific chromatin rearrangement. The chromosome DNA in Sulfolobus cells seems to occupy a smaller relative intracellular space during genome segregation (10, 11). This finding indicates that the chromatin condensation state changes during the cell cycle, and it is possible that altered acetylation status, or differences in the abundance of DNA-binding proteins, could influence higher-order DNA structure.

Among genes that affect chromatin structure, the unannotated topR-2 gene displayed strong early cyclic induction,
whereas topR-1 showed constitutive expression, suggesting differential roles for the two reverse gyrase paralogues. The top64 (SacI314) and top6B (SacI315) genes for the two subunits of TopoVI topoisomerase showed little differential expression (see www.ebi.ac.uk/molevel/suppl.data/lundgren2007pnas/), unexpected if the enzyme mainly functions in postreplicative chromosome decatenation (36). In contrast, the gene for the XerD recombinase displayed strong cyclic expression with induction around the G2/S transition, suggesting a role in cell-cycle-specific resolution of chromosome dimers during replication. The data provide a basis for further investigation into the roles of different topoisomerases and recombinases during the cell cycle, a topic that clearly requires further study.

Concluding Remarks. We have generated a genome-wide transcription map of the cell cycle, providing a basis for detailed characterization of the chromosome replication, genome segregation and cell division processes in archaea. Comprehensive comparisons with other organisms will provide insights into whether the induction patterns may be coupled to a prokaryotic mode of cell and chromosome organization, or whether the similarities between archaea and eukaryotes in terms of the information processing are also reflected in principles for cell-cycle control. Because cell-cycle-specific gene expression patterns vary considerably even within the eukaryotic lineage (7), similarities to the evolutionarily distant archaea will provide useful indicators of features that are central for cell-cycle regulation. Systematic investigations into the extent with which the S. acidocaldarius cell-cycle-regulated genes are present not only in other archaea, but also in eukaryotes and bacteria, will provide further insights into the conservation of core cell-cycle characteristics over the immense evolutionary distances that separate the three domains of life.

Materials and Methods

Strains, Growth Conditions, and Synchronization. S. acidocaldarius DSM639 (Deutsche Sammlung von Mikroorganismen) 500-ml cultures were grown in shaking water baths in 2-liter flasks at 79°C, in modified Allen mineral base medium (37) supplemented with 0.2% tryptone. Growth was monitored by optical density measurements at 600 nm. Synchronized cultures were generated by acetate treatment, as described (12). Three independent biological replicates of the entire experiment were carried out.

Sampling for RNA Extraction and Flow Cytometry. Samples (30–60 ml) for RNA extraction were taken at 30, 60, 80, 100, 120, 140, 170, and 200 min after acetate-treated cultures had been centrifuged and resuspended in fresh, acetate-free medium. The samples were mixed with an equal volume of ice-cold medium, centrifuged at 2,700 × g for 15 min at 4°C, and snap-frozen in liquid N2. Samples for flow cytometry were removed every hour in acetate-treated cultures, every 2 h in control cultures, and every 10 min in synchronized cell populations. Sampling and flow cytometry were performed as described (9), except that an A40 Analyzer instrument (Apogee Flow Systems, Hemel Hempstead, U.K.) was used.

RNA Extraction. RNA was extracted as described (15) and stored in 30–60 μl of RNA storage solution (Ambion, Austin, TX). RNA concentration and quality were determined by using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and by agarose gel electrophoresis.

Microarray Hybridization, Scanning, and Data Processing. Microarray design and construction has been described (15). Labeled cDNA probes were hybridized to the successive sample in the time series, rather than including dye swaps for all samples compared. Each sample was hybridized to the successive sample in the time series, rather than a common reference. The microarrays were scanned by using a GenePix Personal 4100 scanner ( Molecular Devices, Sunnyvale, CA), and data were extracted by using GenePix Pro 5.1 software (Molecular Devices). Low-quality spots were excluded by using filtering parameters described (15), along with removal of saturated spots. All microarrays were visually inspected and the automated filtering procedure was complemented with manual spot removal, or inclusion, when necessary.

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Figure S1.
Figure S2.
Cyclic expression change:

High, Medium, Low

oriC2

oriC1

oriC3
Figure S6.