Transcription factor distribution in *Escherichia coli*: studies with FNR protein

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**ABSTRACT**

Using chromatin immunoprecipitation (ChIP) and high-density microarrays, we have measured the distribution of the global transcription regulator protein, FNR, across the entire *Escherichia coli* chromosome in exponentially growing cells. Sixty-three binding targets, each located at the 5' end of a gene, were identified. Some targets are adjacent to poorly transcribed genes where FNR has little impact on transcription. In stationary phase, the distribution of FNR was largely unchanged. Control experiments showed that, like FNR, the distribution of the nucleoid-associated protein, IHF, is little altered when cells enter stationary phase, whilst RNA polymerase undergoes a complete redistribution.

**INTRODUCTION**

An army of more than 250 transcription factors controls gene expression in *Escherichia coli*. Some of these factors are operon-specific while others, known as global regulators, coordinate the expression of scores of promoters in response to specific environmental cues [reviewed in (1–3)]. The advent of whole-genome DNA sequencing, and associated advances in DNA microarray technology, has enabled investigation of the battery of genes regulated by each of these global factors. The *E.coli* FNR protein (regulator of fumarate and nitrate reduction) is the global transcription factor that manages the distribution of RNA polymerase in response to oxygen starvation. FNR senses oxygen via an N-terminal iron–sulfur cluster. Hence, in anaerobic conditions, FNR is able to bind to specific DNA targets at promoters and modulate transcription. In aerobic conditions, FNR is converted to a form, unable to bind these targets [reviewed in (4,5)]. Bioinformatic analysis has been used to search the *E.coli* genome for DNA sequences that resemble known FNR binding sites (6,7) and DNA microarrays have been used to study differences in the transcriptome that arise when the fnr gene is deleted from the genome (8–10). These studies illustrate the complexity of the FNR regulon and predict that, while FNR directly regulates ~100 transcription units, it indirectly affects up to 1000 genes. In this study, we used chromatin immunoprecipitation (ChIP), in conjunction with high-density microarrays (ChIP-chip), to measure the binding of FNR across the *E.coli* chromosome directly, and in vivo, for the first time. This allowed us to identify 63 DNA targets for FNR, some of which are adjacent to poorly expressed genes where FNR has minor regulatory effects.

In the second part of the study, we studied the distribution of FNR as growing *E.coli* cells enter stationary phase, and found that it is largely unchanged. Recall that transcription patterns change dramatically when cells cease to grow (11,12) but little is known about the distribution and binding of transcription factors in stationary phase cells. In control experiments, we showed that the binding pattern of IHF, a nucleoid-associated protein, is also unchanged, whereas the distribution of RNA polymerase is radically altered.

**MATERIALS AND METHODS**

**Bacterial strains, plasmids and growth conditions**

Bacterial strains used in this work are described in Table 1, together with the oligos used to generate different promoter fragments. For ChIP-chip experiments with FNR, MG1655 and JCB1011 cells were grown anaerobically in Luria–Bertani (LB) medium supplemented with 0.4% glucose. Supplementary Figure 1A shows growth of MG1655 and JCB1011 under these conditions, and the time points at which cells were harvested for ChIP-chip experiments. For ChIP-chip experiments with IHF and RNA polymerase, MG1655 cells were grown aerobically in M9 minimal medium supplemented with 0.4% glucose. MG1655 and JCB1011 under these conditions, and the time points at which cells were harvested for ChIP-chip experiments. For ChIP-chip experiments with IHF and RNA polymerase, MG1655 cells were grown aerobically in M9 minimal medium supplemented with 0.4% glucose. MG1655 and JCB1011 under these conditions, and the time points at which cells were harvested for ChIP-chip experiments. For ChIP-chip experiments with IHF and RNA polymerase, MG1655 cells were grown aerobically in M9 minimal medium supplemented with 0.4% glucose. Supplementary Figure 1B. To compare the activity of different promoters: lacZ fusions in the presence and absence of FNR, we used *E.coli* JCB387 and the fnr derivative JRG1728.

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The table lists bacterial strains, plasmids and oligonucleotides used in this work. Segments of DNA amplified in PCR using the oligonucleotides listed in section C target was set, and all probes that had an intensity ratio greater than this value were selected as FNR targets. When several adjacent probes (i.e. probes forming one peak) passed the cut-off, the target position was defined as the centre of the probe with the highest Cy5/Cy3 ratio.

To identify overlapping peaks for ChIP-chip datasets obtained using nucleoprotein from cells growing in different conditions, we aligned the averaged Cy5/Cy3 signals obtained for each condition and applied an equivalent cut-off to both datasets. We then counted the number of probes that passed the cut-off for both datasets (a leeway of one probe in either direction was allowed). To generate the data in Figure 5C, we selected the top 50, 100, 200, 400, 800, 1600, 3200 and 6400 probes from each dataset and determined the distribution of these probes between coding and non-coding DNA.

Construction and assay of promoter::lacZ fusions

Intergenic regions containing putative FNR targets were amplified by PCR from MG1655 genomic DNA using primers listed in Table 1. Fragments were digested with EcoRI and HindIII and cloned into pRW50, a low-copy-number lac expression vector, to generate promoter::lacZ fusions. β-galactosidase levels in JCB387 and JRG1728 cells carrying these recombinants were measured by the Miller method (14). Activities shown are the average of three independent experiments, and error bars show one SD on either side of the mean. Cells were grown anaerobically in LB medium supplemented with 0.4% glucose. Assays were performed in triplicate.

Electrophoretic mobility shift assays (EMSA)

EMSA were carried out as detailed by Browning et al. (15). Purified promoter fragments were end-labelled with [γ-32P]ATP and ~0.5 ng of each fragment was incubated with varying amounts of purified FNR D154A, which allows binding in the presence of oxygen. The reaction buffer

### Table 1. Bacterial strains, plasmids and oligonucleotides

| Name | Description | Reference |
|------|-------------|-----------|
| (A) Strains | | |
| MG1655 | F- lambda- ibG- rph-50 rph-1 | (22) |
| JCB1011 | MG1655 encoding fur3aFLAG | (10) |
| JCB387 | Prototrophic F-, ΔnirB-cysG, lac, chl+ | (23) |
| JRG1728 | lacX74, galK, galU, rpsL, Δ(ara-leu), Δ(try for trg) | (24) |
| (B) Plasmids | | |
| pRW50 | Low copy lac expression vector | (25) |
| (C) Oligonucleotides | | |
| nohAI upstream | | |
| yccF upstream | | |
| helD downstream | | |
| dbpA downstream | | |
| mrr downstream | | |
| hsdR upstream | | |
| hsdR downstream | | |
| The table lists bacterial strains, plasmids and oligonucleotides used in this work. Segments of DNA amplified in PCR using the oligonucleotides listed in section C are illustrated in supplementary Figure 2.
Figure 1. Distribution of FNR binding across the E.coli chromosome. (A) The figure shows an overview of results from ChIP-chip experiments that measure the profile of FNR binding across the E.coli chromosome during exponential growth in anaerobic conditions. Binding signals (y-axis) are plotted against their location on the 4.64 Mb E.coli chromosome (x-axis). The locations of selected signals are labelled in plain typeface (newly identified FNR targets) or in bold face (known FNR targets). A complete list of FNR targets identified is presented in Table 2. (B) The figures show expansion of selected regulatory regions, quantifying FNR binding during growth in anaerobic conditions (black) of aerobic conditions (grey). (C) The figure shows a DNA sequence motif present at newly identified FNR targets. The DNA sequences from each of the 43 novel FNR targets (see Table 2) were combined and analysed using AlignACE (http://atlas.med.harvard.edu). The motifs identified were then aligned to create a sequence logo (http://weblogo.berkeley.edu). Individual motifs are shown in Table 2.
Table 2. FNR targets identified by ChIP-chip analysis

| Peak centre | Gene     | Identified by transcriptome analysis? | Sequence motif identified by AlignACE | Site centre | Distance from nearest transcription start site |
|-------------|----------|---------------------------------------|--------------------------------------|-------------|-----------------------------------------------|
| (A) Metabolism                                  |          |                                       |                                      |             |                                               |
| 30 774      | carB     | No                                    |                                      |             |                                               |
| 815 981     | ybbK/moaA| No                                    |                                      |             |                                               |
| 913 086     | hcp      | Yes                                   |                                      |             |                                               |
| 1 003 973   | pyrD     | No                                    |                                      |             |                                               |
| 1 297 552   | adhE     | Yes                                   |                                      |             |                                               |
| 1 545 174   | yddG/indG| Yes                                   |                                      |             |                                               |
| 1 934 484   | zwf/yebK | No                                    |                                      |             |                                               |
| 1 935 348   | pykA     | No                                    | 5'-ATGTTAGATGATTATAGCTAAATT-3'       | 1 935 377.5 | Unknown                                       |
| 2 411 269   | ybfV/acrA| Yes                                   | 5'-AAAAATTGGCAGTGTGGATGATTAA-3'      | 2 411 385.5 | Unknown                                       |
| 2 619 106   | upp/pmr  | No                                    | 5'-TTTCCCTGATATTAAAATAAGTA-3'       | 2 618 979.5 | -51.5/-192.5                                |
| 2 632 233   | guaB/aseA| No                                    | 5'-AGAATTGATGATCAGTCAGAT-3'         | 2 632 245.5 | -117.5/28.5                                 |
| 3 242 421   | uacCX cueT| Yes                                   | 5'-TTTTCAAGATTACACATACAAAC-3'       | 3 242 481.5 | Unknown                                       |
| 3 491 582   | nirB     | Yes                                   |                                      |             |                                               |
| 4 285 074   | asc/renA | Yes                                   |                                      |             |                                               |
| 4 365 790   | aspA/jxkA| Yes                                   |                                      |             |                                               |
| 4 380 005   | frdA/jyjA| Yes                                   |                                      |             |                                               |
| 4 460 414   | mrD      | Yes                                   |                                      |             |                                               |
| (B) Unknown function                            |          |                                       |                                      |             |                                               |
| 526 318     | yblZ     | No                                    |                                      |             |                                               |
| 541 128     | ybbZ     | No                                    |                                      |             |                                               |
| 579 197     | ybw      | Yes                                   | 5'-GTGTTAGATGATTATAGCTAAATT-3'       | 579 914.5   | Unknown                                       |
| 1 164 384   | ycfP     | No                                    |                                      |             |                                               |
| 1 311 745   | yclJ/lmpW| Yes                                   | 5'-AAAAATTGGCAGTGTGGATGATTAA-3'      | 1 311 887.5 | Unknown                                       |
| 1 396 615   | ydaA     | No                                    | 5'-TTTTCAAGATTACACATACAAAC-3'       | 1 396 756.5 | Unknown                                       |
| 1 457 773   | ydaN/ydaN| No                                    |                                      |             |                                               |
| 1 515 200   | ydeX     | No                                    | 5'-AAAAATTGGCAGTGTGGATGATTAA-3'      | 1 515 344.5 | Unknown                                       |
| 1 627 197   | ydfZ     | Yes                                   | 5'-TTTTCAAGATTACACATACAAAC-3'       | 1 627 144.5 | Unknown                                       |
| 1 665 352   | ydfK     | Yes                                   | 5'-AAAAATTGGCAGTGTGGATGATTAA-3'      | 1 665 337.5 | Unknown                                       |
| 1 717 809   | ydhH/ydxB| Yes                                   | 5'-AAAAATTGGCAGTGTGGATGATTAA-3'      | 1 718 014.5 | Unknown/215.5                                |
| 1 777 299   | ydhL/ydxQ| No                                    | 5'-TTTTCAAGATTACACATACAAAC-3'       | 1 777 183.5 | Unknown                                       |
| 2 066 717   | yeeH/yoeA| No                                    | 5'-TTTTCAAGATTACACATACAAAC-3'       | 2 066 535.5 | Unknown                                       |
| 2 415 046   | yfeC     | Yes                                   | 5'-TTTTCAAGATTACACATACAAAC-3'       | 2 415 030.5 | Unknown                                       |
| 2 558 454   | yffL     | Yes                                   | 5'-TTTTCAAGATTACACATACAAAC-3'       | 2 558 434.5 | Unknown                                       |
| 2 562 348   | yffS     | No                                    | 5'-TTTTCAAGATTACACATACAAAC-3'       | 2 562 425.5 | Unknown                                       |
| 2 714 514   | yfd/ung  | Yes                                   |                                      |             |                                               |
| 3 151 520   | ygbB     | No                                    | 5'-TTTCAAGATTACACATACAAAC-3'        | 3 151 585.5 | Unknown                                       |
| 3 265 311   | yhdB     | No                                    | 5'-TTTCAAGATTACACATACAAAC-3'        | 3 265 180.5 | Unknown                                       |
| 3 296 995   | yhBT/ybhU| Yes                                   | 5'-TTTCAAGATTACACATACAAAC-3'        | 3 299 038.5 | Unknown                                       |
| 3 351 934   | yhcCy/1B | Yes                                   | 5'-TTTCAAGATTACACATACAAAC-3'        | 3 351 753.5 | Unknown/395.5                                |
| 3 578 508   | yhxX/yhhB| No                                    |                                      |             |                                               |
| 3 635 245   | yhnIp/taA| Yes                                   | 5'-TTTCAAGATTACACATACAAAC-3'        | 3 635 162.5 | Unknown                                       |
| 4 248 287   | yhl      | No                                    | 5'-TTTCAAGATTACACATACAAAC-3'        | 4 248 420.5 | Unknown                                       |
| 4 368 072   | yjgH/groS| Yes                                   | 5'-TTTCAAGATTACACATACAAAC-3'        | 4 368 101.5 | Unknown/91.5                                 |
| (C) Transcription factors                       |          |                                       |                                      |             |                                               |
| 34 153      | yuaV/cyaF| Yes                                   |                                      |             |                                               |
| 70 210      | araB/araC| No                                    |                                      |             |                                               |
| 121 966     | aroP/aoD | Yes                                   |                                      |             |                                               |
| 1 719 041   | stn/ycltd| No                                    | 5'-ATTTAATTACCTAAAGGCGATACAT-3'     | 1 719 013.5 | Unknown/9.5                                   |
| (D) Membrane proteins                          |          |                                       |                                      |             |                                               |
| 747 050     | aboB     | No                                    |                                      |             |                                               |
| 770 354     | cydA     | Yes                                   |                                      |             |                                               |
| 940 149     | dmxA     | Yes                                   |                                      |             |                                               |
| 953 866     | focA     | Yes                                   |                                      |             |                                               |
| 1 165 181   | ndh      | Yes                                   |                                      |             |                                               |
| 1 277 131   | narX/mnkK| Yes                                   |                                      |             |                                               |
| 2 403 375   | nmoA     | No                                    |                                      |             |                                               |
| 2 583 617   | aecA/narQ| No                                    | 5'-ATTTAATTACCTAAAGGCGATACAT-3'     | 2 583 671.5 | Unknown                                       |
| 3 144 499   | hybO     | No                                    | 5'-ATTTAATTACCTAAAGGCGATACAT-3'     | 3 144 461.5 | -83.5                                        |
| 3 150 195   | exbB/mecC| No                                    |                                      |             |                                               |
| 3 928 854   | yihN/ykdD| No                                    | 5'-ATTTAATTACCTAAAGGCGATACAT-3'     | 3 928 956.5 | Unknown                                       |
| 4 346 720   | dcvB     | Yes                                   |                                      |             |                                               |
Table 2. Continued

| Peak centre | Gene | Identified by transcriptome analysis? | Sequence motif identified by AlignACE | Site centre | Distance from nearest transcription start site |
|-------------|------|--------------------------------------|--------------------------------------|------------|---------------------------------------------|
| (E) DNA/RNA manipulation | 579 727 | nohB | No | 5’-TTAAGTTGAAGATCAATTAAAT-3’ | 579 735.5 | Unknown |
| 1 023 647 | ycfF/heiD | No | 5’-AGTAATTAGAAGGAAACAAAGG-3’ | 1 023 811.5 | Unknown |
| 1 407 100 | dpnA | No | 5’-AAAGTTTGAAGGAATTAAATAC-3’ | 1 407 110.5 | Unknown |
| 1 634 803 | nohAidyD | No | 5’-TTAAGTTGCAACATCAATTAAAT-3’ | 1 634 713.5 | –85.5/unknown |
| 4 584 413 | hotR/mnr | No | 5’-CATCAGTTATTATCAACCCT-3’ | 4 584 337.5 | Unknown |

The table lists the locations of peaks for FNR binding identified using chip and high-density microarray analysis. All of the peaks identified fell in non-coding DNA or were adjacent to the 5’ end of a gene. Targets are grouped according to the function of the gene(s) adjacent to the FNR target and are listed in chromosomal order within these groups. Entries highlighted with a bold face are experimentally verified FNR targets present in the current version of the Ecocyc database. For newly identified FNR targets (plain typeface) the sequence of the FNR binding site identified by AlignACE is given along with its genomic coordinate and, if known, the distance from the nearest transcription start site. The transcriptome analysis of Constantinidou et al. (10) was used for comparison.

RESULTS

Isolation of DNA fragments associated with FNR in mid-log phase E.coli

Our aim was to use ChIP to measure the distribution of FNR across the chromosome of growing E.coli cells. To do this, we exploited strain JCB1011 whose fnr gene had been previously modified to encode FNR with a C-terminal 3× FLAG tag (10). Supplementary Figure 3A shows a western blot of total protein from strain JCB1011 and its parent, MG1655, probed with anti-FLAG or anti-FNR antibodies. The results show that intracellular levels of wild-type FNR and the FLAG-tagged FNR are indistinguishable from the background signal.

Identification and sequence analysis of FNR targets

To determine the location of peaks for FNR binding in an unbiased manner, a Cy5/Cy3 cut-off was applied to the ‘anaerobic’ dataset. A total of 204 probes passed this cutoff, corresponding to 63 separate peak locations, all of which were in non-coding DNA or close to the 5’ end of a gene (Table 2). Of the 63 peaks identified, 20 correspond to locations listed as FNR targets in the current version of the Ecocyc database (www.ecocyc.org, (16)) and 9 of the remaining 43 peaks locate to FNR targets predicted by Constantinidou et al. (10). To identify FNR binding sites at the 43 loci not currently listed by Ecocyc, we selected and then combined 500 bp DNA sequences corresponding to the centre of each peak. We then used AlignACE to search for sequence motifs present in these DNA sequences. A sequence logo representing the motif that we identified is shown in Figure 1C. The motif clearly matches the known FNR consensus binding motif of TTGAT(n)4ATCAA.

Association of FNR with some previously uncharacterized DNA targets

Five of the targets for FNR binding are adjacent to genes encoding proteins involved in the manipulation of RNA or DNA and, in each case, a likely FNR binding site was identified (see Table 2 section E; Figure 2A). DNA fragments covering each of these targets were amplified and end-labelled, or cloned into the lac expression vector pRW50 to create promoter::lacZ fusions. To detect the binding of FNR to the predicted target promoters in vitro, the radiolabelled DNA fragments were used in EMSA assays with purified FNR protein (Figure 2B). In all cases, addition of purified FNR retarded the migration of the purified DNA fragments. To investigate the effect of FNR on transcription from each target promoter, each of the pRW50 encoded...
promoter::lacZ fusions was transformed into E.coli strain JCB387 or the fnr derivative JRG1728. Expression of lacZ in each strain was measured. Our data (Figure 2C) show that deletion of fnr had marginal affects on transcription and that the activity of each cloned promoter was low in our conditions.

Distribution of FNR in stationary phase

The analysis of FNR binding was repeated using stationary phase cultures of JCB1011 and MG1655. The dataset for the FNR experiment is presented in Supplementary Table 1, alongside the data from growing cells, and an overview is shown in Figure 3A. The results show that the profile of
FNR binding in stationary phase is similar to the profile in growing cells (e.g. as shown in Figure 3B). As controls for this experiment, similar analyses were performed with the nucleoid-associated protein, IHF and with RNA polymerase, which is known to be redistributed in stationary phase E.coli. Note that previously we had used ChIP-chip to study IHF and RNA polymerase in exponentially growing cells (17). The datasets for IHF and RNA polymerase binding are shown in Supplementary Tables 2 and 3, respectively, alongside data generated using growing E.coli cells (17). The results show that the profile of IHF binding in stationary phase is similar to the profile in growing cells while the profile of RNA polymerase is radically altered.

Similarities between the datasets for FNR, IHF and RNA polymerase binding, in growing and stationary phase cells, were quantified by calculating correlation coefficients (Figure 4A) and by comparing the position of probes passing the cut-off for each dataset (Figure 4B). For FNR and IHF, the correlation between ChIP-chip datasets profiling binding in growing and stationary phase cells is high [Figure 4A (i) and (ii)]. Much less correlation is observed when ChIP-chip datasets for RNA polymerase binding in stationary phase and mid-log phase cells are compared [Figure 4A (iii)]. Consistent with this, for FNR and IHF, many of the same probes pass the cut-off for both datasets. In contrast, few probes pass the cut-off for both the mid-log and stationary phase RNA polymerase experiments (Figure 4B).

**DISCUSSION**

In this work, we have applied ChIP-chip technology to produce the first chromosome-wide direct analysis of DNA binding in vivo by the global E.coli transcription regulator, FNR. The advantage of this approach to studying the FNR
regulon is that it avoids complications due to genes that are indirectly controlled by FNR or genes that are regulated by multiple transcription factors. Moreover, FNR binding at sites adjacent to poorly transcribed genes, or genes where FNR has little impact on transcription, can be detected and the effects of environmental conditions can be studied. We identified 63 locations at which FNR binds to the E.coli chromosome, including a group of five targets adjacent to genes encoding proteins that manipulate DNA and RNA. None of these five targets were identified as FNR regulated by previous transcriptome analyses (8,9,10), none are listed as FNR targets by the Ecocyc database (16), and, to our knowledge, FNR-dependent regulation of such proteins has not been documented. At 10 of the 63 targets of FNR binding, we were unable to identify a match to the canonical FNR binding sequence. These may be locations at which FNR binds cooperatively with another factor. We note that the profile of FNR binding presented here consists of discrete peaks (Figure 1A). In contrast, the published binding profile for the related transcription activator, CRP, is far more complex, due to the existence of ~10,000 low affinity binding sites for CRP scattered throughout the genome (6,13). Consistent with

Figure 4. Comparison of chromosome-wide FNR, IHF and RNA polymerase distribution in growing and stationary phase E.coli. (A) Correlation between ChIP-chip datasets profiling the chromosome-wide distribution of FNR (i) IHF (ii) and RNA polymerase (iii) in growing and stationary phase cells. The log_{10} value of the mid-log phase (x-axis) binding signal measured at each probe by ChIP-chip analysis is plotted against the corresponding log_{10} ratio for stationary phase (y-axis). (B) Overlap of DNA targets occupied by FNR, IHF and RNA polymerase during rapid growth and stationary phase. The ChIP-chip datasets obtained for FNR, IHF and RNA polymerase binding during rapid growth and stationary phase were aligned and an equivalent cut-off was applied to each dataset. The number of probes passing the cut-off for both stationary phase and mid-log phase datasets was then determined (black bar). As a control, this analysis was repeated after the genomic position of probes in the stationary phase dataset had been randomized (grey bar).
this, Robison et al. (6) predicted only ~500 low affinity targets for FNR in the *E. coli* chromosome. Although our experiments identified 63 discrete targets for FNR binding, these targets include only 20 out of the 65 validated targets listed in the Ecocyc database (16). This highlights an important limitation of the ChIP-chip methodology. Its inability to detect all FNR-DNA interactions is likely to be due to inefficient crosslinking at some locations, or epitope masking (18).

Although major changes in gene expression and nucleoid structure occur when *E. coli* cells enter stationary phase

Figure 5. Increased association of RNA polymerase with non-coding DNA during stationary phase. (A) The figure shows an overview of results from ChIP-chip experiments that measure the profile of RNA polymerase binding across the *E. coli* chromosome during stationary phase. Binding signals (y-axis) are plotted against their location on the 4.64 Mb *E. coli* chromosome (x-axis). The locations of selected signals are labelled. (B) Skewed distribution of RNA polymerase across transcribed regions during stationary phase. The figure illustrates of selected regions highlighted in (A). Data for RNA polymerase binding during stationary phase are shown in black and RNA polymerase binding during mid-log phase is shown in grey. (C) Increased association of RNA polymerase with non-coding DNA during stationary phase. The ChIP-chip datasets for RNA polymerase binding during rapid growth (grey) and stationary phase (black) were aligned and a range of Cy5/Cy3 cut-offs were applied to select the upper 50, 100, 200, 400, 800, 1600, 3200 and 6400 probes for each dataset. We then determined the distribution of probes passing the cut-offs between coding and non-coding DNA.
(11,12,19), it is not known if changes in the distribution of global DNA binding proteins occur. Our observation that the DNA binding profile for FNR is largely unaltered in stationary phase cells shows that the compaction of the stationary phase chromosome does not occlude FNR binding sites. This surprising result prompted us to examine the nucleoid-associated protein IHF and we came to the same conclusion; the distribution of the protein is similar in growing and stationary phase cells. In sharp contrast, as expected, the binding profile of RNA polymerase was completely altered. In growing cells, most RNA polymerase is associated with ~90 transcription units encoding factors required for protein synthesis, motility and ATP production (13). In stationary phase, this RNA polymerase is liberated and is distributed more equitably between the different genes (Figure 5A). Interestingly, for most transcription units, this RNA polymerase is liberated and is distributed more equitably between the different genes.

In stationary phase, this RNA polymerase is liberated and is distributed more equitably between the different genes. This surprising result prompted us to examine the nucleoid-associated protein IHF and we came to the same conclusion; the distribution of the protein is similar in growing and stationary phase cells. In sharp contrast, as expected, the binding profile of RNA polymerase was completely altered. In growing cells, most RNA polymerase is associated with ~90 transcription units encoding factors required for protein synthesis, motility and ATP production (13). In stationary phase, this RNA polymerase is liberated and is distributed more equitably between the different genes.

**SUPPLEMENTARY DATA**

Supplementary Data are available at NAR online.

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**Conflict of interest statement.** None declared.

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