Molecular Cell *24*, 747–757, December 8, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.molcel.2006.10.030

The Transition between Transcriptional Initiation and Elongation in *E. coli* Is Highly Variable and Often Rate Limiting

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Summary

We perform a genome-wide analysis of the transition between transcriptional initiation and elongation in *Escherichia coli* by determining the association of core RNA polymerase (RNAP) and the promoter-recognition factor σ^{70} with respect to RNA transcripts. We identify 1286 σ^{70}-associated promoters, including many internal to known operons, and demonstrate that σ^{70} is usually released very rapidly from elongating RNAP complexes. On average, RNAP density is higher at the promoter than in the coding sequence, although the ratio is highly variable among different transcribed regions. Strikingly, a significant fraction of RNAP-bound promoters is not associated with transcriptional activity, perhaps due to an intrinsic energetic barrier to promoter escape. Thus, the transition from transcriptional initiation to elongation is highly variable, often rate limiting, and in some cases is essentially blocked such that RNAP is effectively "poised" to transcribe only under the appropriate environmental conditions. The genomic pattern of RNAP density in *E. coli* differs from that in yeast and mammalian cells.

Introduction

Transcription in *Escherichia coli* is performed by a single RNAP. Five subunits (α_2, β, β', and ω) form the catalytic core of RNAP that is sufficient for transcription in vitro. The core RNAP enzyme (E) must associate with a σ subunit to form a holoenzyme (Eσ) capable of binding promoter DNA and initiating transcription from a discrete site (Gross et al., 1998). The predominant σ factor during normal growth is σ^{70}, and RNAP:σ^{70} holoenzyme (Eσ^{70}) initiates transcription from the majority of promoters.

Extensive biochemical analysis has subdivided the process of transcriptional initiation into three steps: (1) Eσ^{70} recruitment, (2) isomerization from a closed to an open complex, and (3) promoter escape (McClure, 1985). The rate of each step is dependent on promoter sequence and is subject to regulation by transcriptional activators. However, it is unclear what determines the rates of isomerization and promoter escape. Transcription of a full-length RNA in vitro is usually preceded by multiple rounds of abortive transcription of 2–15 nt RNAs. Abortive initiation is influenced by the promoter sequence (Hsu et al., 2003; Vo et al., 2003b) and contributes to the rate of promoter escape. RNAP can also form unproductive complexes at promoters that can be converted to productive complexes, but the rate of conversion is promoter dependent (Susa et al., 2002; Vo et al., 2003a). It is unclear whether these in vitro events occur in living cells and which steps of transcription initiation are rate limiting in vivo.

σ^{70} is not required for transcriptional elongation or termination in vitro. Structural studies suggest that elongation of nascent RNA to >6 nt creates a steric clash with σ^{70} (Murakami et al., 2002), and various biochemical studies indicate that σ^{70} is released very rapidly from elongating RNAP (Mooney et al., 2005). However, σ^{70} release has also been proposed to occur in two steps: rapid triggering and a slow stochastic dissociation with a rate constant of 0.2 s^{-1} (Shimamoto et al., 1986). When free σ^{70} is absent in vitro, RNAP can recognize a σ^{70}-dependent pause site located 18, but not 37, bp downstream of the transcription start site (Mooney and Landick, 2003; Brodolin et al., 2004; Nickels et al., 2004), suggesting that σ^{70} may associate with RNAP after at least 18 nt of transcription. At high concentrations, σ^{70} is able to rebind to elongating RNAP in vitro (Mooney and Landick, 2003; Brodolin et al., 2004). More recent biochemical studies have shown that σ^{70} can associate with elongating RNAP, although the fraction and stability of σ^{70} association with elongating RNAP vary considerably among different studies (Bar-Nahum and Nudler, 2001; Mukhopadhyay et al., 2001; Nickels et al., 2004; Kapanidis et al., 2005).

Very little is known about the transition from transcriptional initiation to elongation in vivo. In principle, this transition can be addressed by using chromatin immunoprecipitation (ChIP) to determine RNAP density at promoters and their corresponding transcribed regions. If the transition is fast, RNAP levels should be constant across the transcribed regions; this situation typically occurs in the yeast *Saccharomyces cerevisiae* (Liu et al., 2005; Steinmetz et al., 2006 [this issue of *Molecular Cell*]). In contrast, if the transition to elongation is slow, one predicts higher levels of RNAP at the promoter than in coding regions, and there are several such examples in *Drosophila* and mammalian genes (Rougvie and Lis, 1988; Krumm et al., 1995; Cheng and Sharp, 2003; ENCODE Project Consortium, 2004; Carroll et al., 2006). In an initial small-scale study, RNAP association in *E. coli* was found to be higher at the promoter than in the corresponding coding sequence for some, but not all, transcription units (Wade and Struhl, 2004). Genome-wide analysis of RNAP association in *E. coli* cells treated with rifampicin, an inhibitor of transcriptional elongation, identified numerous promoters but did not provide information on elongating RNAP (Herring et al., 2005). A more recent study of RNAP association in growing cells used low-density microarrays that did not permit analysis of this issue (Grainger et al., 2005).

The fate of σ^{70} during the transition from initiation to elongation in vivo has been addressed with ChIP. We showed that σ^{70} rapidly dissociates from elongating

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RNAP, and in two cases examined in detail, we were unable to detect σ^{70} at position +50 relative to the transcription start point (Wade and Struhl, 2004). Interestingly, a fraction of σ^{70} can associate with elongating RNAP throughout the *melAB* operon, but only during stationary phase (Wade and Struhl, 2004). A more recent study on the ribosomal (rDNA) locus concluded that σ^{70} is stochastically released from elongating RNAP with a half-life of 7 s (Raffaelle et al., 2005).

To investigate the transition from initiation to elongation on a genome-wide scale and at high resolution, we combined ChIP with high-density, fully tiled microarrays (ChIP-chip) to determine the genomic binding profiles of β and σ^{70}. In addition, we generated an unbiased transcript map from the same samples by using the same microarray design. Finally, we determined the precise association of β and σ^{70} with three specific transcribed regions using ChIP and quantitative PCR. Our results indicate that the majority of σ^{70} is released very rapidly from RNAP after the transition to elongation at all transcribed regions, although a small fraction of σ^{70} can associate with elongating RNAP throughout the transcription cycle in a gene-dependent, condition-dependent manner. Furthermore, we demonstrate that the distribution of RNAP across transcribed regions is gene dependent, that postrecruitment steps of transcription initiation are often rate limiting, and that "poised" RNAP is observed at a substantial number of *E. coli* promoters with no detectable transcriptional activity in vivo. The pattern of RNAP in *E. coli* differs from that in *Saccharomyces cerevisiae* (Liu et al., 2005; Steinmetz et al., 2006) and human (ENCODE Project Consortium, 2004; Carroll et al., 2005), suggesting that these organisms differ significantly in the manner in which the transition between transcriptional initiation and elongation is controlled.

Results

Genome-Wide Transcriptional Activity and Identification of Novel Transcripts

Due to bias in microarray design, previous whole-genome analyses have been limited to the discovery of novel transcripts in intergenic regions (Tjaden et al., 2002). Using microarrays with 382,177 50-mers tiled every ~12 bp on alternate strands, we determined the *E. coli* transcript map. We identified 1815 transcription fragments, or "transfrags" (Kampa et al., 2004), that correspond to RNAs with a median length of 950 nt and a 140-fold linear dynamic range of expression (Table S1 in the Supplemental Data available with this article online). We identified 58 previously unannotated transcripts (Table S2), including some that are intragenic (Figure 1A), antisense (Figures 1B and 1C), and intergenic (Figure 1D; Supplemental Results).

Genome-Wide Binding Profile of σ^{70}

Using the same microarray format as above, we determined, for the same duplicate samples used for RNA analysis, the genome-wide binding profile of RNAP β subunit and σ^{70} (Figure S1 and Tables S3–S6). We identified 1286 reproducible peaks of σ^{70} association, which should represent Eσ^{70} promoters. Wavelet analysis of the σ^{70} ChIP peaks shows a statistically significant periodicity of ~700 kb^{-1} (Figure S2) similar to that observed previously (Herring et al., 2005). Of the 570 σ^{70} sites that could be associated with a unique transcript start, 165 σ^{70} are upstream of genes not known or predicted to be transcribed by Eσ^{70}, with 51 of these lying within experimentally known Eσ^{70} operons, indicating that many genes are transcribed both from a dedicated promoter immediately upstream and as a downstream gene in an operon. We also identified 161 σ^{70} promoters within the coding sequences of genes or between convergently transcribed genes (Figure 2A; Supplemental Results).

Core RNAP Is Often Poised at Promoters

Analysis of the RNAP profile across transcribed regions reveals a few cases in which β association is constant across a promoter and transcribed region (Figures 2B and 2C), but RNAP association is typically higher at the promoter than within the coding sequence (Figures 2D and 2E). Unexpectedly, both β and σ^{70} are clearly bound to promoters that are not associated with detectable transcriptional activity (Figures 2F and 2G). Quantitative PCR analysis confirms robust association of β with the *hepA*, *deoB*, and *yjiT* promoters but substantially reduced or nondetectable association with the corresponding coding sequences (Figure S3), indicating that ChIP-chip data are reliable.

Strikingly, 300 σ^{70} peaks (23% of all peaks) are not associated with transcribed regions (Table S5), indicating that a significant proportion of promoters bound by Eσ^{70} lack detectable transcriptional activity. RNAP is bound in an effectively poised fashion at these sites, although the term poised is not intended to imply a specific molecular mechanism or precise location at or near the promoter. Importantly, genes that bind poised RNAP do not have lower than average transcript half-lives (Figure 3 and Supplemental Results; Selinger et al., 2003). On average, peaks not associated with transcribed regions are of lower σ^{70} ChIP signal, but there is an extensive region of overlap between the peak-height distributions of the two classes (Figure 3), demonstrating that transcriptional activity is not completely dependent on the level of promoter-bound RNAP.

Relationship between Poised RNAP and Promoter T_m

Promoters in *E. coli* and other prokaryotes are enriched in relatively low melting temperature (T_m) regions that we refer to as T_m troughs (Kanhere and Bansal, 2005). We hypothesized that an important difference between σ^{70} peaks that are or are not associated with transcribed regions is that the DNA sequence around and including the promoters of the latter type might be more difficult for RNAP to melt than the former. Accordingly, we selected σ^{70} peaks that also had a β peak and could be unambiguously associated with the 5′ end of an annotated gene. We then compared those that were associated with transcribed regions to those that were not. Specifically, we determined whether a T_m trough, a local minimum in the smoothed genomic T_m profile often proximal to promoters, lay within ±200 bp of a given β/σ^{70} copeak (see Supplemental Experimental Procedures). We found that the median T_m trough depth of nontranscript-associated peaks is 4.86°C and that of transcript-associated peaks is 3.41°C (and that of all peak data is 3.78°C).

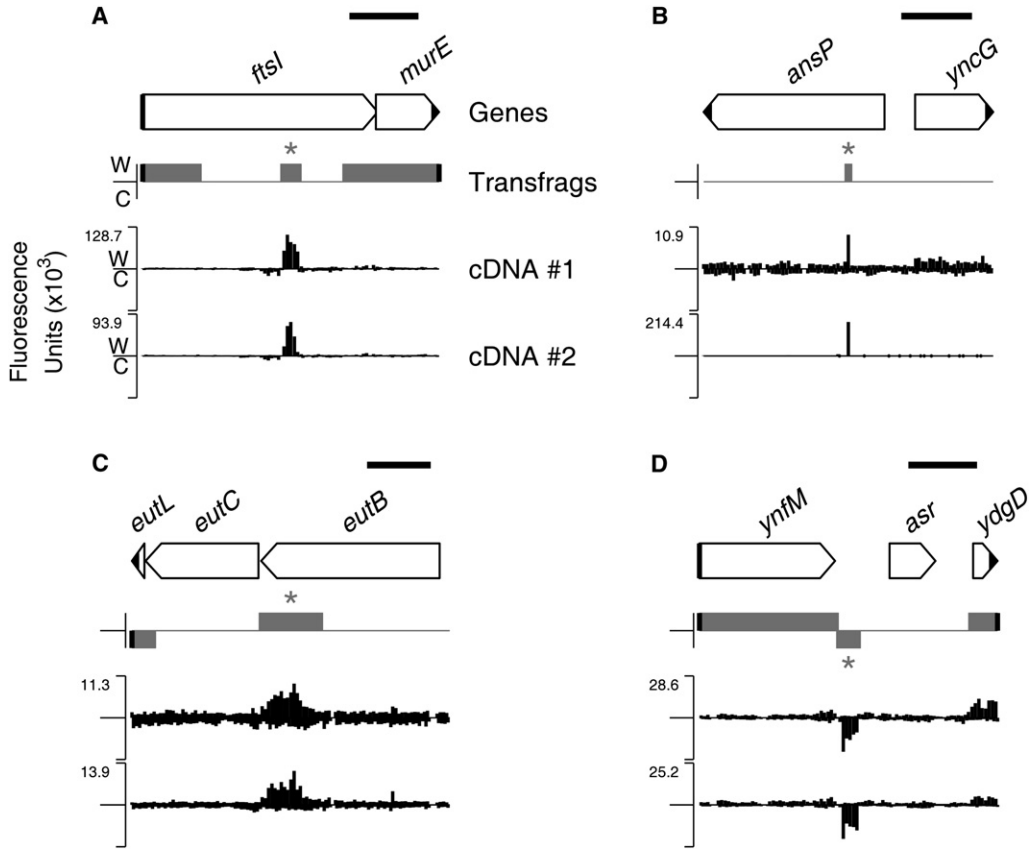

Figure 1. Novel Transcripts in *E. coli*

(A) Novel sense transcript found within *ftsI*.

(B) Novel antisense transcript found within *ansP*.

(C) Novel antisense transcript found within *eutB*.

(D) Novel transcript found in *ynfM*/*asr* intergenic region.

In (A)–(D), genes are indicated as arrowed boxes; transfrags are indicated as gray boxes on either the Watson (W) or Crick (C) strand. Raw data (fluorescence units × 10^3) from duplicate microarrays are plotted; the horizontal black bar represents 500 bp. Novel transcripts are indicated by an asterisk.

The difference between T_m trough depths of nontranscript-associated peaks and the T_m trough depths of transcript-associated peaks is statistically significant (Mann-Whitney p = 1.7e − 3). For 100 gene-map-rotated controls, we never observed a corresponding p- value below 0.1 (Supplemental Experimental Procedures). Thus, nontranscript-associated peaks are significantly enriched in being proximal to larger T_m troughs relative to transcript-associated ones.

Postrecruitment Steps of Transcriptional Initiation Are Highly Variable and Often Rate Limiting

If the transition between initiation and elongation is rate limiting, RNAP association should be higher at the promoter, whereas RNAP density should be constant across the transcription unit if the transition is relatively rapid. To address this issue, we analyzed the profile of β association across a "high-quality" set of 59 transfrags (see Supplemental Experimental Procedures and Table S3). We calculated a traveling ratio (TR) as the level of β or σ^{70} association 800 bp downstream of the peak (downstream signal) relative to the level at the peak (promoter signal; Figure 4A). A histogram of TR values is shown in Figure 4B.

The TR distribution for β shows a broad spread of values from 0 to 1, with a median value of 0.43. There is a significant correlation between transfrag expression level and β TR (Spearman r = 0.29 and p = 0.035). Thus, for the majority of actively transcribed Eσ^{70} transcription units, RNAP association is significantly higher at the promoter than within the coding sequence, with a TR of 1 being only rarely observed. We conclude postrecruitment steps of transcription initiation are often rate limiting in *E. coli*.

σ^{70} Rapidly Dissociates from RNAP after the Transition to Elongation

To investigate the timing of σ^{70} release from RNAP, we determined the level of σ^{70} retention at positions immediately downstream of the same promoters used for the TR analysis. We reasoned that immediate or very rapid release of σ^{70} from RNAP would result in symmetric peaks of σ^{70} association, whereas slower release over >100 bp would result in peaks of σ^{70} association skewed in the direction of transcription. For each transcribed region, we determined the ratio of the σ^{70} ChIP-chip signal 50 bp downstream of the σ^{70} peak relative to the direction of transcription, to that 50 bp upstream (the

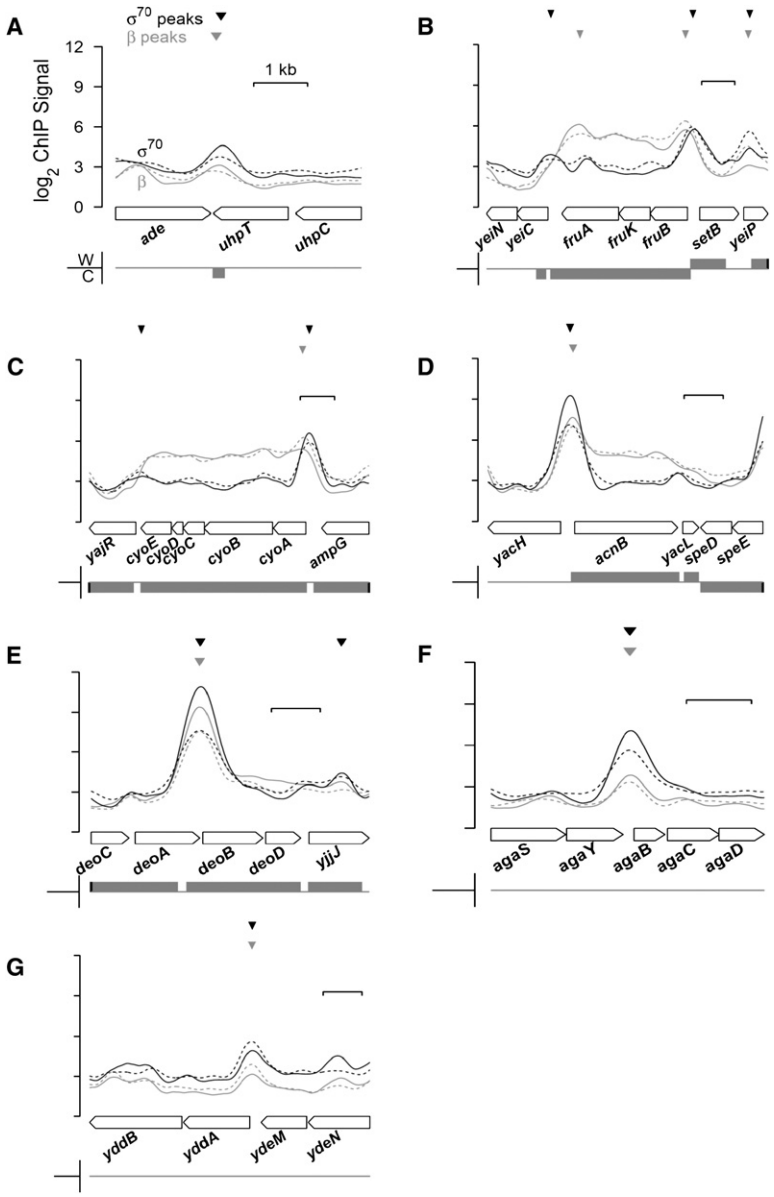

Figure 2. Representative Smoothed ChIP-Chip Profiles of β and σ^{70}

In (A)–(G), genes are indicated as arrowed boxes, transfrags are indicated as gray boxes on either the Watson (W) or Crick (C) strand, the location of ChIP-chip peaks are indicated with downward-facing arrowheads (black for σ^{70} and gray for β) at the top of each plot, the solid and dashed black lines represent the replicate σ^{70} ChIP-chip profiles, and the solid and dashed gray lines the replicate β ChIP-chip profiles. ChIP-chip profiles of interest are shown in the following genomic locations: between *ade* and *uhpT* genes (A), *fruBKA* operon (B), *cyoABCDE* operon (C), *acnB* gene (D), *deoBD* operon (E), *agaB* promoter (F), and *yddA* promoter (G).

"retention ratio"; see Supplemental Experimental Procedures and Figure 5A). We repeated this at 50 bp intervals from the σ^{70} peak. A retention ratio >0 indicates peak skew in the direction of transcription and, hence, association of σ^{70} with elongating RNAP. As a control, we calculated the level of β retention in the same way, as well as the predicted level of σ^{70} retention, assuming a stochastic release model with a half-life of 7 s and a transcription elongation rate of 30 nt/s (Raffaelle et al., 2005). Our data (Figure 5B) show that σ^{70} peaks are highly symmetric, with retention values not significantly different from 0 (Supplemental Experimental Procedures), thereby demonstrating that the majority σ^{70} is released very rapidly from RNAP—within 100 nt—after the transition to elongation at virtually all transcribed regions.

To provide independent evidence for this conclusion, we examined the association of β and σ^{70} at three well-transcribed regions (the *oppABCDF* operon, *adhE* gene, and rDNA locus) before and after rifampicin addition in either minimal or rich media during exponential growth (Figures 6A–6F and Figure S3). This approach has been used previously to assess σ^{70} association with elongating RNAP at the rDNA locus in vivo (Raffaelle et al., 2005), and it is based on the ability of rifampicin to inhibit early transcriptional elongation by blocking the path of the extending RNA (Campbell et al., 2001). If σ^{70} is retained by elongating RNAP at a given location, the level of σ^{70} association should be reduced at that position after rifampicin treatment. However, rifampicin does not significantly affect the level of σ^{70} association at several positions in the initial transcribed regions relative to that at the promoter. Furthermore, we detect significantly less σ^{70} association in the initial transcribed regions than predicted by stochastic dissociation with a half-life of 7 s (Raffaelle et al., 2005). These observations are consistent with our high-resolution mapping of σ^{70} association at the *melAB*

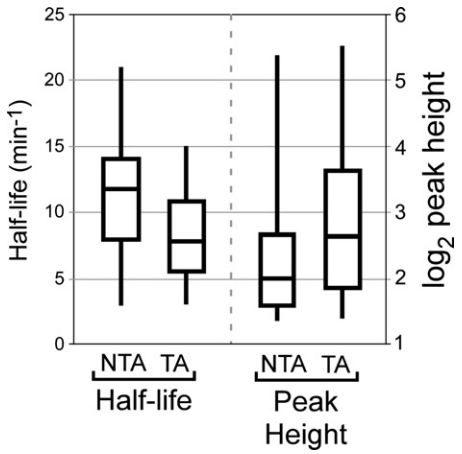

Figure 3. mRNA Half-Life and σ^{70} Peak Height Distributions of Non-transcript-Associated and Transcript-Associated σ^{70} Peaks Overlap Significantly with One Another

Box plot of mRNA half-life (min^{-1}) and peak-height distributions for nontranscript-associated (NTA) and transcript-associated (TA) σ^{70} peaks. Boxes indicate 25th–75th percentile, vertical lines indicate 5th–95th percentile, and horizontal lines indicate the median value. Half-lives are from Selinger et al. (2003).

and *lacZYA* operons during exponential growth (Wade and Struhl, 2004) and confirm that σ^{70} is in general released very rapidly from RNAP after the transition to elongation.

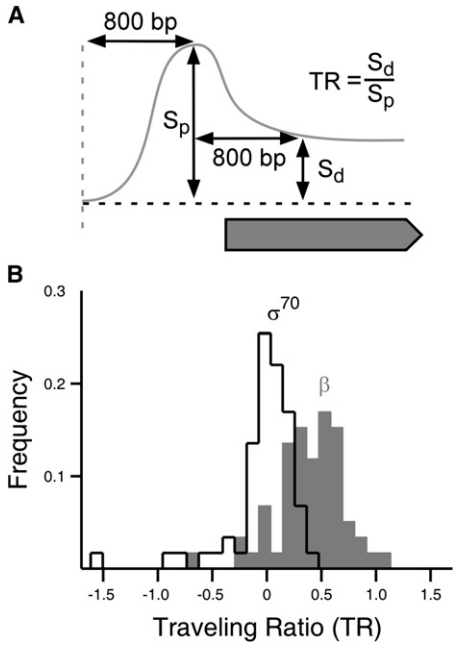

Figure 4. Distribution of Traveling Ratios for β and σ^{70}

(A) Schematic showing the method for calculating the traveling ratio (TR) for each of the high-quality transfrags selected for this analysis (see text). TR is the ratio S_d:S_p where S_p and S_d are the ChIP signals at the peak and +800 bp relative to the peak, respectively, using the ChIP signal at position −800 bp relative to the peak as background. TR > 0 indicates preferential binding of a protein downstream of the peak.

(B) Frequency distribution of TRs for β (gray area) and σ^{70} (black line) for 59 high-quality transfrags.

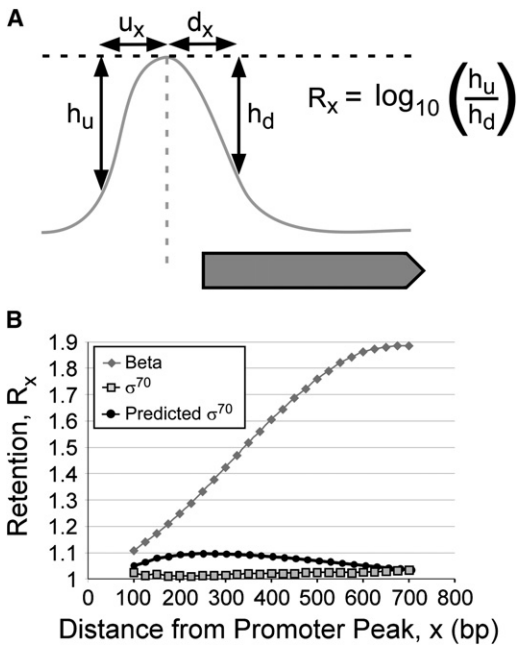

Figure 5. σ^{70} Is Rapidly Released from RNAP after the Transition to Elongation

(A) Schematic showing the method for calculating "retention" at the same individual loci as in Figure 4B. Retention is the $\log_{10}$ of the ratio h_u:h_d where h_d and h_u are the differences in ChIP signal at distance x upstream and downstream of the peak, respectively, to the ChIP signal at the peak. A retention ratio >0 indicates peak skew and thus binding of a protein downstream of the peak.

(B) Average retention values for a selection of high-quality transfrags. Retention is shown for β (gray diamonds), σ^{70} (gray squares), and the predicted values for σ^{70} assuming a stochastic release rate of 7 s (black circles). The σ^{70} profile is statistically indistinguishable from a random model (see Supplemental Experimental Procedures).

A Low-Level σ^{70} Can Associate with Elongating RNAP throughout Some Coding Regions

It is possible that a small fraction of σ^{70} could remain associated with RNAP throughout the transcription cycle that could not be detected by ChIP-chip due to the noise of the experiment. Indeed, we have shown previously that a small fraction of σ^{70} is retained by elongating RNAP throughout the transcribed region of the *melAB* operon at the onset of stationary phase (Wade and Struhl, 2004). Analysis of the same set of high-quality transfrags used for the β TR calculations reveals that the median TR value for σ^{70} is 0.03 (Figure 4) and is statistically indistinguishable from 0 (see Supplemental Experimental Procedures), suggesting that σ^{70} can associate with position +800 at very few transcribed regions.

To independently assess the association of σ^{70} with elongating RNAP, we analyzed the effect of rifampicin on σ^{70} association at a position in the distal transcribed regions (>1 kb downstream of the transcription start site) of the *oppABCDF* operon, the *adhE* gene, and the rDNA locus. We detected a small but significant fraction of σ^{70} in the distal transcribed regions of *adhE*, but not *oppABCDF*, when cells were grown in rich medium, but not minimal medium (Figures 6A–6F and Figure S4). The level of σ^{70} association in the distal *adhE* coding sequence is ~10% of that at the promoter, and it is not observed after rifampicin treatment, demonstrating

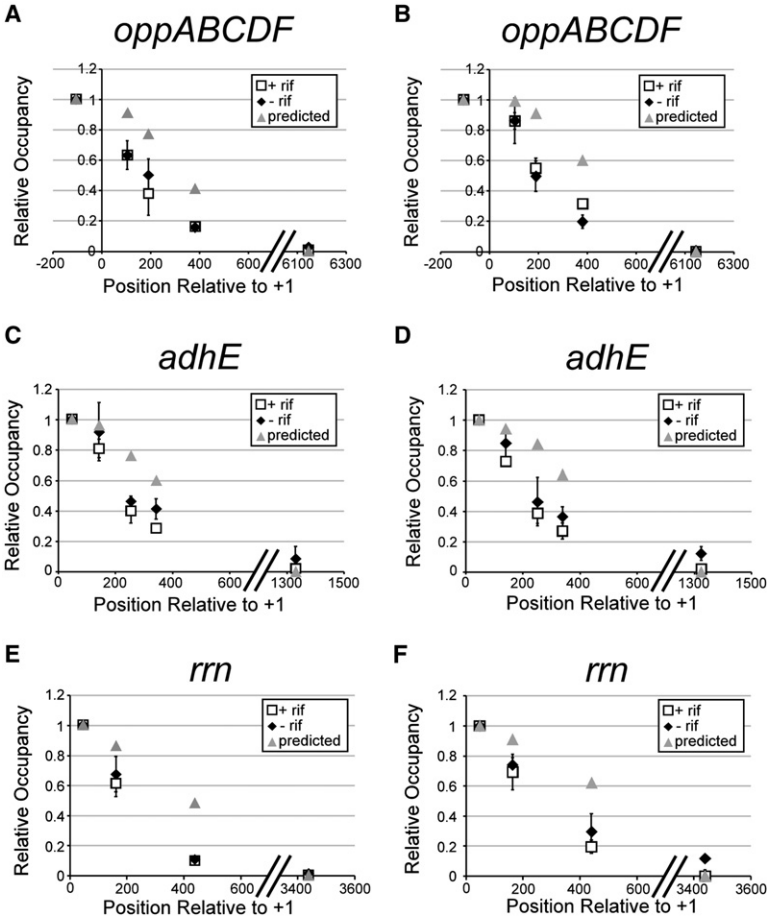

Figure 6. High-Resolution Mapping of σ^{70} at Three Transcribed Regions

(A and B) Relative occupancy values for σ^{70} at indicated positions throughout the *oppABCDF* operon before (black diamonds) and after (white squares) rifampicin treatment. Predicted values for σ^{70}, assuming a stochastic release rate of 7 s, are also shown (gray triangles). Positions are indicated relative to the transcription start point. Values are normalized to *bglB* coding sequence and plotted relative to the value at the most upstream position.

(C and D) Relative occupancy values for σ^{70} at indicated positions throughout the *adhE* gene before (black diamonds) and after (white squares) rifampicin treatment. Predicted values for σ^{70}, assuming a stochastic release rate of 7 s, are also shown (gray triangles). Positions are indicated relative to the transcription start point. Values are normalized to *bglB* coding sequence and plotted relative to the value at the most upstream position.

(E and F) Relative occupancy values for σ^{70} at indicated positions throughout the rDNA locus before (black diamonds) and after (white squares) rifampicin treatment. Predicted values for σ^{70}, assuming a stochastic release rate of 7 s, are also shown (gray triangles). Positions are indicated relative to the *P1* transcription start point. Values are normalized to *bglB* coding sequence and plotted relative to the value at the most-upstream position (*P1* promoter). Regions tested: promoter, +163, +440, and +3440. Note that the two most-upstream PCR products correspond to only four out of the seven rDNA loci, whereas the downstream two PCR products correspond to all seven. Note also that the rate of transcription elongation at the rDNA locus is ~90 nt/sec.

(A), (C), and (E) show data collected from cells grown in M9 minimal media at 30°C. (B), (D), and (F) show data collected from cells grown in LB media at 37°C.

Error bars represent one standard deviation from the mean.

that σ^{70} associated with elongating RNAP at these regions (note that values in distal coding regions are inherently more reproducible, as the ChIP signal here can be due only to elongating, and not initiating, RNAP). We also detected a small but significant fraction of σ^{70} in the distal transcribed region of the rDNA locus in rich medium in the absence, but not in the presence, of rifampicin. This gene-specific, condition-specific, low-level association of σ^{70} with elongating RNAP is consistent with our previous study (Wade and Struhl, 2004). Based on our genome-wide analysis, it is possible that a low level of σ^{70} associates with the coding sequences of a small fraction of transcribed regions. However, on average, this association is not detectable, or σ^{70} is unable to associate with elongating RNAP in minimal media.

Discussion

Tiled Arrays Identify Novel Transcripts and Novel Promoters in *E. coli*

Previous methods to identify *E. coli* transcripts have used microarrays that do not cover all genomic regions equally (Selinger et al., 2000; Tjaden et al., 2002). Here, using fully tiled, high-density microarrays, we identify 58 RNAs that, to the best of our knowledge, are novel, including many antisense transcripts that could not have been identified by previous experimental analyses. Our ability to identify so many novel transcripts highlights the remarkably compact nature of the *E. coli* genome.

Using ChIP-chip, we have also identified many σ^{70} promoters that, to the best of our knowledge, are novel. These include 165 promoters that are located upstream of genes not previously shown or predicted to be transcribed by Eσ^{70} (Bockhorst et al., 2003; Keseler et al., 2005). There are 161 σ^{70} promoters not associated with the 5′ ends of annotated genes (Tables S5 and S6), although we have shown that these are highly likely to be enriched in genuine promoters (Supplemental Results). This suggests the existence of many novel σ^{70}-dependent transcripts. Indeed, some of the novel transfrags we identify are associated with a novel σ^{70} ChIP peak. Interestingly, in a separate study, we have shown that ~25% of σ^{32}-dependent promoters are not associated with the 5′ ends of annotated genes (Wade et al., 2006).

Transition between Initiation and Elongation Is Rate Limiting at Many Promoters In Vivo

Recruitment of RNAP to promoters is the rate-limiting step at many *E. coli* promoters in vitro (Ptashne and

Gann, 1997), and artificial recruitment of RNAP to a promoter is sufficient to direct transcription in vivo (Dove et al., 1997). However, postrecruitment steps of transcription initiation can be rate limiting in vitro, and the rates of isomerization and promoter escape vary considerably between promoters (Hsu, 2002). Very little is known about the rate-limiting steps of transcriptional initiation in vivo and whether rate-limiting steps observed in vitro are physiologically relevant.

Our analysis of 59 high-quality transfrags indicates that, rather than being evenly distributed across the transcribed region, RNAP association is significantly higher at the promoter in almost all cases (Figures 2 and 4). The most-extreme example of this phenomenon is the 23% of $E\sigma^{70}$ promoters that is not associated with a transfrag. In addition, the level of RNAP association within the coding sequence of transcribed regions varies from 0% to 100% of that associated with the promoter. The ratio of RNAP association at the promoter and coding sequence is correlated significantly with the RNA level. Thus, postrecruitment steps of transcription initiation are often rate limiting in vivo, and the extent to which they are rate limiting varies dramatically, depending on the promoter.

Our conclusions are based on the assumption that RNAP crosslinking efficiency is similar at all stages of the transcription cycle. This is highly likely to be the case because RNAP is in close proximity to large regions of DNA throughout the transcription cycle. Importantly, we show that the coding sequence:promoter ratio of RNAP ChIP signal varies from 0 to 1, strongly suggesting that ChIP signal is reflective of real RNAP association and is unaffected by changes in RNAP conformation. Based on our results, we can estimate the amount of time that RNAP spends in the vicinity of the promoter in vivo. There are approximately ten different base pair positions within a promoter region with which RNAP could associate prior to escaping into the elongation phase, because ~10 nt is the size of abortive transcripts. However, due to the nature of ChIP, there are several hundred positions where elongating RNAP can contribute to a ChIP signal for a given genomic region being analyzed. Thus, a 2-fold-higher ChIP signal at the promoter requires that RNAP spends 40- to 80-fold more time in the vicinity of the promoter than at a given position within the coding region. As RNAP spends 0.03–0.04 s per nucleotide position during elongation, this means that RNAP spends ~1–3 s at the typical promoter during the transition between initiation and elongation. We note that this value not only represents an average promoter but also an average time at a given promoter, i.e., our results do not address the properties of individual RNAP molecules at a given promoter. Our experiments cannot address the possibility that subsets of RNAP molecules behave differently at a given promoter.

RNAP Is Poised at Many Transcriptionally Inactive Promoters

Twenty-three percent of $E\sigma^{70}$ promoters is not associated with detectable RNA transcripts, and we refer to RNAP at these promoters as poised. Poised RNAP likely accounts for the observation that some RNAP binding sites in cells treated with the elongation inhibitor rifampicin are not associated with RNAs (Herring et al., 2005). Promoters with poised RNAP have significantly larger T_m troughs between the promoter and corresponding coding sequence than promoters as a whole. This observation suggests that an energetic barrier blocking promoter escape is involved in the formation of poised RNAP. However, the size distributions of T_m troughs for promoters bound by poised and nonpoised RNAP overlap, suggesting that other factors are important.

One possibility is that RNAP bound at transcriptionally inactive promoters is due in part to "nonfunctional" RNAP binding sites. Computational analysis of the *E. coli* genome has revealed multiple potential binding sites for RNAP in individual promoter regions, but in most cases, the initiation site does not correspond to the region with the highest predicted affinity (Huerta and Collado-Vides, 2003). Alternatively, unproductive RNAP complexes (Susa et al., 2002; Vo et al., 2003a) and abortive RNA synthesis (Hsu et al., 2003; Vo et al., 2003b), both of which vary depending on the promoter, might contribute to increased association of RNAP with promoter regions in vivo. However, it seems unlikely that either phenomenon would result in completely poised RNAP, as this is not the case in vitro.

Lastly, poised RNAP might be generated by transcriptional repressors. Although most repressors are believed to occlude RNAP promoter elements and hence block RNAP association, some block postrecruitment steps of transcription initiation. For example, GalR appears to repress the *gal*P1 promoter by trapping RNAP in an intermediate state between the closed and open complex (Choy et al., 1997), phage *psi29* protein p4 blocks promoter escape via a very tight interaction with *Bacillus subtilis* RNAP (Monsalve et al., 1997), and H-NS represses the *rrnB*P1 promoter by trapping RNAP in a promoter loop (Dame et al., 2002). H-NS is extremely abundant in exponentially growing cells and binds to many promoters. Hence, it could be responsible for creating poised RNAP at many promoters.

Poised RNAP and Transcriptional Regulation

We propose that poised RNAP, transcriptionally inactive under the condition tested, is able to transcribe under the appropriate environmental condition. Stimulation of poised RNAP in vivo might involve contacts with transcriptional activator proteins, as some activators can increase the rates of isomerization and promoter escape in vitro (Eichenberger et al., 1997; Dove et al., 2000; Liu et al., 2003; Roy et al., 2004). This is the mechanism by which activators stimulate transcription by the σ^{54} holoenzyme (Buck et al., 2000), although σ^{54} shares no homology with σ^{70}, and the mechanisms of transcription initiation by σ^{54} and σ^{70} family holoenzymes are believed to be fundamentally different.

There is one described example of poised RNAP in *E. coli* involving a σ^{70} family holoenzyme. σ^{38} holoenzyme binds to the *osmY* promoter but does not transcribe under conditions of low salt. It has been proposed that high concentrations of potassium glutamate unwrap the promoter DNA, thereby altering the conformation of RNAP such that it escapes the promoter and transcribes the *osmY* gene (Lee and Gralla, 2004). Thus, in addition to activator-mediated mechanisms, it is possible that changes in the cellular chemical environment might

directly regulate the transcriptional activity of poised RNAP. Whatever the precise mechanisms involved, our results suggest that regulation of poised RNAP is a relatively common phenomenon.

Unlike the situations involving poised RNAP, many transcriptionally inactive genes are not detectably bound by σ^{70} in vivo. In some cases, σ^{70} association is likely to be stimulated by an activator that regulates transcription at the level of RNAP recruitment. In other cases, transcription might occur at only a low level, thereby precluding detection of σ^{70} at the promoter. However, as transcriptional repressors often occlude RNAP binding, it is likely that many inactive promoters not bound by poised RNAP are bound instead by repressors.

The Transition between Initiation and Elongation Differs among Species

In *E. coli*, the transition between initiation and elongation is often rate limiting, and a significant number of promoters bind poised RNAP. In striking contrast, our analysis of a ChIP-chip study of RNA polymerase II (Pol II) in the yeast *S. cerevisiae* (Liu et al., 2005) reveals a median TR of 0.9 for nine genes analyzed. This observation indicates that the transition between initiation and elongation is rapid, and it is consistent with the conclusion that preinitiation complex formation is the major rate-limiting step for transcription from the vast majority of promoters in vivo (Kuras and Struhl, 1999; Li et al., 1999). Interestingly, during stationary phase, Pol II appears to be poised at a number of promoters in *S. cerevisiae* (Radonjic et al., 2005), suggesting that posttranscriptional steps of transcription initiation may be an important regulatory target under different conditions. In fly and mammalian cells, there are well-characterized examples of poised or paused RNAP complexes (Rougvie and Lis, 1988; Krumm et al., 1995; Cheng and Sharp, 2003), and ChIP-chip analysis reveals promoter-localized Pol II at numerous locations (ENCODE Project Consortium, 2004; Carroll et al., 2006). These observations indicate that there are significant species-specific differences in rate-limiting steps during the transition from initiation to elongation.

σ^{70} Is Rapidly Released from RNAP during the Transition from Initiation to Elongation In Vivo

Our results demonstrate that, at essentially all transcribed regions in vivo, the majority of σ^{70} is rapidly released from elongating RNAP complexes after the transition to transcription elongation. Specifically, the profile of σ^{70} association upstream and downstream of the promoter is virtually symmetric (Figure 5), indicating that the vast majority of σ^{70} release occurs within the first 100 nucleotides of transcription. These genome-wide results are consistent with our previous analysis of the *melAB* and *lacZYA* operons, in which we could not detect σ^{70} at +50 (Wade and Struhl, 2004). Given an elongation rate of 30 nt/s, our results indicate that the half-life of σ^{70} release from elongating RNAP is <1 s.

Our conclusions differ from those of a recent study of the rDNA locus suggesting that the half-life of σ^{70} release from elongating RNAP is 7 s (Raffaelle et al., 2005). We do not know why Raffaelle et al. (2005) detected significant levels of σ^{70} in the initial transcribed region of the rDNA locus, whereas we did not (Figures 6E and 6F), but it is unlikely to be related to the antibody used for ChIP, because we obtained similar results at the rDNA locus by using a strain containing an epitope-tagged derivative of σ^{70} (Figure S5). Consistent with our previous study (Wade and Struhl, 2004), we also detect low-level association of σ^{70} with some downstream transcribed regions under certain conditions.

The conformation of regions 3 and 4 of σ^{70} must change with respect to core RNAP as the RNA leaves the exit channel (Murakami et al., 2002; Nickels et al., 2005). Although it is possible that this conformational change in RNAP holoenzyme alters the crosslinking efficiency of σ^{70}, a significant effect on crosslinking efficiency is unlikely. First, σ^{70} that is associated with elongating RNAP must remain in close proximity to the DNA because RNAP is able to recognize σ^{70}-dependent pause sites in vitro at positions as far downstream as +440, under conditions where the σ^{70} concentration is high or where σ^{70} has been fused to β (Mooney and Landick, 2003). Second, direct biophysical experiments suggest that changes in the relative position of σ^{70} within RNAP holoenzyme between transcription initiation and elongation of an 11 nt RNA are subtle (Mukhopadhyay et al., 2001). Third, proteins that associate with RNAP but do not associate directly with DNA, e.g., NusA, the ω subunit of RNAP, crosslink to DNA with high efficiency (Wade and Struhl, 2004; data not shown).

Crucially, we are able to detect σ^{70} that is associated with elongating RNAP. This association can be at least as high as 25% of that seen at the promoter, as is the case at the *melAB* operon (Wade and Struhl, 2004). The association of σ^{70} with the *melAB* coding sequence was only detected in the presence of melibiose, the inducer of *melAB* transcription, and therefore can only be due to association of σ^{70} with elongating RNAP. Therefore, if σ^{70} crosslinking efficiency is reduced in coding regions, it can be by no more than 4-fold, and this also makes the unlikely assumption that 100% σ^{70} is retained by elongating RNAP. Aside from being hypothetical, such a quantitatively modest effect on crosslinking efficiency would not affect our conclusions regarding σ^{70} release from elongating RNAP.

We note that σ^{70} association with elongating RNAP could be due to rebinding of σ^{70} (Mooney and Landick, 2003; Brodolin et al., 2004), although such rebinding would have to depend on the operon and the environmental conditions. We also note that our data are based exclusively on highly transcribed regions, because these are the only regions that permit detection of sufficient levels of σ^{70} by ChIP. Hence, it is possible that there are differences in σ^{70} retention that depend on the level of transcription that we are unable to investigate.

Functional Implications of σ^{70} Association with Elongating RNAP

Association of σ^{70} with elongating RNAP could have significant effects on transcription elongation, termination, and reinitiation (Bar-Nahum and Nudler, 2001; Mooney et al., 2005). For example, if σ^{70} release occurred stochastically with a 7 s half-life (Raffaelle et al., 2005), at least some σ^{70} would associate with RNAP

throughout the transcription cycle at many transcribed regions. This might allow for rapid rebinding of RNAP holoenzyme to promoters and for σ^{70}-dependent transcriptional pausing during transcription elongation. However, our results presented here and elsewhere are indicative of much more rapid release of σ^{70} at the majority of transcribed regions (half-life <1 s), arguing that such recycling of RNAP holoenyzme can only occur to a limited extent. Nevertheless, there is a general agreement that σ^{70} has the potential to associate with elongating RNAP. We therefore suggest that the rapid dissociation of σ^{70} from elongating RNAP in vivo is an active process, perhaps facilitated by other cellular factors.

Experimental Procedures

Strains and Media

E. coli strain MG1655 was used for all experiments, with the exception of the experiment described in Figure S5. In this case, the strain used was a derivative of DY330 that contained a C-terminally TAP-tagged copy of the *rpoD* (σ^{70}) gene (Butland et al., 2005). Cells were grown to exponential phase (OD_{600} = 0.2–0.4) at 30°C in M9 minimal media supplemented with fructose and casamino acids or at 37°C in LB. Fifty micrograms/milliliters rifampicin was added to selected cultures for 15 min prior to harvesting. Both transcriptional and ChIP microarray experiments were performed with the same cell culture.

mRNA Analysis

To purify mRNA, cells were harvested by centrifugation and resuspended in 400 μl RNA lysis buffer (20 mM Tris [pH 7.5], 150 mM NaCl, 4 mg/ml lysozyme, and 0.1% SDS) and incubated at 65°C for 1 min. Sodium acetate (pH 5.2) was added to a final concentration of 100 mM. Samples were extracted with acid phenol (pH 4.3) at 65°C for 10 min and precipitated with ethanol. Total RNA was purified by using Qiagen RNeasy columns with DNase I treatment. Ribosomal RNA was then removed by using the MicroExpress kit (Ambion). mRNA purification was performed in duplicate. Ten micrograms of purified RNA was reverse transcribed with SuperScript II reverse transcriptase (Invitrogen), 2 μg random hexamer, and dNTP mix containing aminoallyl-dUTP, according to the manufacturer's instructions.

ChIP

ChIP was performed as previously described (Wade and Struhl, 2004). For the experiment described in Figure S4, ChIP was performed with the TAP tag as previously described (Wade et al., 2004). ChIP experiments were performed at least twice. Note that the antibody against σ^{70} crossreacts in a western blot with the flagellar σ factor FliA (Breyer et al., 1997). Hence, it is possible that a small fraction of the targets identified for $E\sigma^{70}$ is in fact due to holoenzyme containing FliA. However, this is likely to be a very small fraction, as there are believed to be very few promoters bound by FliA (currently only 15 annotated promoters [Keseler et al., 2005]). Quantitative PCR was performed as previously described (Wade and Struhl, 2004) by using the coding sequence of *bglB* as a background control. Occupancy units represent a background-subtracted value for the association of a particular protein with a target region (Aparicio et al., 2004). Primer sequences are available on request.

Microarray Analysis

We used a custom-tiled NimbleGen microarray format for both mRNA and ChIP analysis. Probes consisted of all the *E. coli* MG1655 (U00096.2) 50-mer sequences spaced on average 12 bp apart, with consecutive probes being on opposite strands; three regions (*sdhCDAB*, *lexA-dinF*, and *osmE*) were tiled in this way at a resolution of 1 bp. The resulting 382,177 50-mers were positioned randomly over the microarray. To analyze RNA transcripts, aminoallyl-dUTP-containing cDNA was labeled with Cy3 or Cy5 (Amersham). As a control, genomic DNA was purified with a 20/G genomic-tip kit (Qiagen) and was sonicated to an average size of ~500 bp. Four micrograms of sheared genomic DNA was then incubated with Klenow DNA polymerase, 10 μg random hexamer, and dNTP mix containing aminoallyl-dUTP for 4 hr at 37°C. Amplified genomic DNA was then purified with a PCR purification kit (Qiagen) and labeled with Cy3 or Cy5 (Amersham). ChIP samples were amplified and labeled with Cy3 and Cy5 (Amersham) as described previously (Wade et al., 2004).

Supplemental Data

Supplemental Data include Supplemental Results, Supplemental Experimental Procedures, Supplemental References, five figures, and six tables and can be found with this article online at http://www.molecule.org/cgi/content/full/24/5/747/DC1/.

Acknowledgments

We thank David Grainger, Zarmik Moqtaderi, Joseph Geisberg, and Marc Schwabish for helpful discussions and David Shore for primer design. We acknowledge Peter Kharchenko for suggesting the T_m analysis. J.T.W. was supported by a Charles A. King Trust Postdoctoral Fellowship, Bank of America, Co-Trustee (Boston, MA). This work was supported by National Institutes of Health grant GM30186 (K.S.).

Received: June 14, 2006
Revised: October 10, 2006
Accepted: October 24, 2006
Published: December 7, 2006

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Accession Numbers

Raw microarray data are deposited in GEO under accession numbers GSE6060, GSE6061, and GSE6069.