Gene expression homeostasis and chromosome architecture

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In rapidly growing populations of bacterial cells, including those of the model organism *Escherichia coli*, genes essential for growth - such as those involved in protein synthesis - are expressed at high levels; this is in contrast to many horizontally-acquired genes, which are maintained at low transcriptional levels.¹ This balance in gene expression states between 2 distinct classes of genes is established by a galaxy of transcriptional regulators, including the so-called nucleoid associated proteins (NAP) that contribute to shaping the chromosome.² Besides these active players in gene regulation, it is not too far-fetched to anticipate that genome organization in terms of how genes are arranged on the chromosome,³ which is the result of long-drawn transactions among genome rearrangement processes and selection, and the manner in which it is structured inside the cell, plays a role in establishing this balance. A recent study from our group has contributed to the literature investigating the interplay between global transcriptional regulators and genome organization in establishing gene expression homeostasis.⁴ In particular, we address a triangle of functional interactions among genome organization, gene expression homeostasis and horizontal gene transfer.

Xenogene Silencing and the Molecular Players Involved

Horizontal gene transfer is a major mode of evolution in bacteria.⁵ The term *xenogene* refers to foreign, horizontally-acquired genes. They are key drivers of phenotypic innovation and represent a significant portion of the total gene content of many bacteria including *E. coli*:

estimates of the number of horizontally-acquired genes in any genome are generally not precise because of difficulties in identifying such genes, but hover around 15% for laboratory *E. coli*.⁶ As an example, the genome of laboratory *E. coli* K12 typically harbours 9 cryptic prophages, which together account for ~5% of the organism's ORF content. Genetic screening has indicated that many of these genes may have little role to play under standard growth conditions, but offer selective advantages under alternative situations including certain stresses.⁷ Given their dispensability during rapid growth, it is not surprising that they are expressed at low levels, or kept in a transcriptionally silent state under such conditions.¹

The central protein player in xenogene silencing in *E. coli* and other enterobacteria is the nucleoid-associated global transcriptional repressor H-NS.^{8–11} H-NS is an ~140 amino acid protein, containing a DNA-binding domain and an oligomerisation domain separated by a linker region.¹² A+T-richness of a DNA sequence,¹³ which correlates with DNA curvature,¹⁴ is a major predictor of H-NS binding affinities, and it is not entirely clear whether it is A+T-richness or DNA curvature that determines H-NS binding. H-NS also binds to non-canonical DNA structural motifs including Holliday junctions.¹⁵ A consequence of the affinity of H-NS to A+T-rich sequences is that it binds to loci that are predicted to be horizontally-acquired. A curious facet of many enterobacterial genomes is that horizontally-acquired genes, which are predicted based on their atypical oligonucleotide usage, tend to be abnormally high A+T- rather than high G+C-rich.¹¹ Why this should be so is not clear, and the subject of this commentary is not appropriate for a

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discussion of applicable hypotheses. Gene silencing by H-NS is part of an intricate network of interactions¹⁶ involving H-NS homologs such as StpA,¹⁷ and antagonists including LeuO,¹⁸ as well as the transcriptional terminator Rho,¹⁹⁻²² which is part of the core transcriptional machinery. This has been reviewed and researched elsewhere, and the rest of this commentary will deal with the core silencing set-up represented by H-NS and its homolog StpA.

Molecular Back-Ups for Xenogene Silencing

ChIP-chip and ChIP-seq studies of H-NS have shown that this protein binds in the form of long tracts to A+T-rich DNA.^{11,23} Supporting evidence from transcriptomics¹¹ and RNA polymerase ChIP^{8,10,11} favors the exclusion of RNA polymerase from H-NS bound loci, as well as their low levels of transcription in wildtype *E. coli* (and *Salmonella*). In Δhns , these genes are differentially up-regulated, indicating a direct functional consequence of H-NS-DNA interactions. A ChIP-chip study of StpA in laboratory *E. coli* showed that it binds to the same loci as H-NS.²⁴ Whereas $\Delta stpA$ leaves H-NS binding largely unaffected *in vivo*, loss of H-NS abrogates the binding of StpA to up to 2-thirds of its binding sites. A more recent transcriptome analysis from our laboratory supported these findings.²⁵ Whereas Δhns results in a significant alteration of *E. coli*'s transcriptional state, $\Delta stpA$ does not; however, deletion of *stpA* in a Δhns background (i.e. $\Delta stpA-hns$), results in a unique transcriptome that is distinct from that of Δhns alone. These differences in the transcriptome between Δhns and $\Delta hns-stpA$ are consistent with the findings of the afore-mentioned ChIP-chip study of the 2 proteins. Thus, the back-up for H-NS function by StpA in *E. coli* is partial. Further computational analysis showed that the retention of StpA binding to a subset of its sites in Δhns occurs at loci with potentially high affinity for H-NS. It was also shown that the partial backup of H-NS function by StpA is concentrated at the most dispensable loci. Further, and potentially most

importantly, it was shown that H-NS-silenced genes, especially those where its function is backed-up by StpA, are highly transcribable in the absence of the gene silencing system. In other words, genes which are suppressed by H-NS (and StpA) are expressed at low levels in the wildtype, but transit to higher-than-average expression levels in $\Delta stpA-hns$. More recent analysis of individual loci regulated by H-NS in *Salmonella* supported their high transcribability.²⁶ Detailed studies from David Grainger's lab showed that gene expression from these loci may not necessarily produce full-length transcripts; instead pervasive transcription occurs from myriad promoter-like elements commonly found in these A+T-rich sequences.²⁷ Antibiotic-mediated inhibition of Rho, which results in a transcriptional state similar to that of Δhns , results in the production of antisense transcripts from many otherwise-silent loci.²⁸ Taken together, H-NS function might indicate selection for silencing dispensable but highly transcribable genes, wherein high transcribability is a function of pervasive transcription.

Whereas the above deals with the effect of $\Delta stpA-hns$ on genes directly bound by these proteins, the extreme increase in the expression levels of these genes results in severe collateral damage wherein many other genes – not known to be bound by H-NS – are differentially down-regulated.⁴ That many of these downregulated genes have high expression levels in wildtype *E. coli* indicates that en-masse desilencing of horizontally-acquired genes results in a global imbalance in gene expression states, extending to core genes. It might not be surprising that *E. coli* $\Delta stpA-hns$ shows a strong growth defect.²⁹

Chromosome Organization and Gene Expression States

The central theme of this commentary is the gene expression imbalance caused by the disruption of the xenogene silencing system and its interplay with chromosome organization. A fundamental feature of circular bacterial chromosomes is the presence of a single origin of replication (Ori).³ Replication initiates at this site and

proceeds bidirectionally until it terminates at the approximately diametrically-opposite terminus (Ter). Over the duration of the S-phase (replication period), there is a 2-fold difference in copy number between Ori-proximal and Ter-proximal loci. In fast-growing *E. coli* where the population doubling time is less than the replication period, the Ori fires multiple times per cell cycle, resulting in larger gradients in gene dosage between the Ori and the Ter. As a result, many highly-expressed genes, including rRNA genes, are encoded closer to the Ori than to the Ter; to some extent high gene expression correlates with gene essentiality. It has been shown in contrast that horizontally-acquired and stress-responsive genes tend to be encoded around the Ter,^{3,30} at least in *E. coli*. In summary, there exists a polarity in gene organization on circular bacterial chromosomes, whereby essential and highly-expressed genes are encoded around the Ori, whereas stress-responsive and horizontally-acquired genes are located closer to the Ter.

Xenogene Silencing and Chromosome Organization

H-NS is a nucleoid-associated protein (NAP) that plays a role in shaping the chromosome. Super-resolution microscopy has shown that the many binding sites of H-NS condense into a few foci in *E. coli* cells.³¹ The *E. coli* nucleoid becomes highly compacted when H-NS is over-produced.³² To our knowledge, high resolution, genome-wide chromosome conformation capture maps have not been generated for mutants in *E. coli* NAPs, and are indeed a need of the hour. At a simpler level, statistical preferences for NAPs – including H-NS – to bind to specific segments of the *E. coli* chromosome have been analyzed. In line with the evidence that many horizontally-acquired genes are encoded close to the Ter, H-NS binding sites are significantly enriched in the Ter-half of the chromosome.³³ Our analysis showed -in contrast to xenogenes directly silenced by H-NS – that genes, which were down-regulated indirectly in $\Delta stpA-hns$ were more likely to be found in the Ori half of the chromosome.⁴

Toward an exploration of links between chromosome architecture and xenogene silencing, we resorted to laboratory evolution – a form of suppressor genetics experiment – in which the *E. coli* Δ *stpA-hns* strain was repeatedly passaged through exponential growth to select for and identify mutants that confer a growth advantage.⁴ Earlier screens for suppressors of the growth defect of Δ *stpA-hns* had identified additional global regulatory networks around the protein CRP and the alarmone guanosine tetraphosphate.²⁹ It had also been shown that growth defects resulting from perturbations of H-NS can be suppressed by inactivation of RpoS, the sigma factor responsible for the general stress response.³⁴ This emerges from the overlap in the regulatory targets of RpoS and H-NS, wherein many genes that are repressed by H-NS can be activated by RpoS. Our laboratory evolution experiment recapitulated this effect. A similar laboratory evolution experiment of Δ *hns* in *Salmonella* showed that suppressors included excisions of various horizontally-acquired pathogenicity islands – consistent with previous directed experiments – indicating the role of xenogene silencing in potentiating virulence.³⁵

We found that the duplication of ~40% of the *E. coli* chromosome, centered around the Ori, could partly suppress the growth defect and the transcriptional imbalance of Δ *stpA-hns*.⁴ The duplication was mediated by repeated transposable elements, located symmetrically on either side of the Ori. That the 2 transposable elements were equidistant from the Ori is important as replication forks, which are hotspots for recombination, would be concurrent at these elements. Further, on the basis of genetic studies interrogating the topology of the *E. coli* chromosome, the 2 repeats are located in what are called non-structured segments of the chromosome, which are capable of recombination with distal loci.³⁶ Using publicly-available microarray data for ~300 conditions for *E. coli*, we also showed the presence of 2 large gene expression domains in the *E. coli* genome – one corresponding to the Ori half and other to the Ter half. Genes within the Ter half of the chromosome are more

likely to be co-expressed among each other, and anti-correlated in expression to those in the Ori half of the chromosome. Most remarkably, the boundaries marking these 2 expression domains were proximal to the transposable elements marking the aforementioned duplication.

Chromosome Architecture, its Evolvability and Gene Expression States

What establishes the 2 domains of gene expression? We do not know the answer; data of the transcriptional regulatory network of *E. coli*, as documented in the RegulonDB database does not explain this pattern, at least on first glance. A trivial explanation could be based on the nature of genes encoded on the Ori-half versus those on the Ter-half of the chromosome: it is possible that conditions favoring the expression of growth-associated genes, typically encoded proximal to the Ori, are mutually exclusive of those under which stress-responsive Ter-proximal genes are expressed; this would potentially be mediated by a whole constellation of regulatory molecules. A second, but not necessarily exclusive explanation could be the topology of the chromosome: loci that are spatially proximal could be co-expressed. RNA polymerase molecules located within the confines of a chromosomal domain could be locked within this space, and prevented from accessing the rest of the chromosome. This would suggest the presence of 2 large topologically isolated domains in the *E. coli* chromosome, one encompassing the Ori-centered half and the other including the Ter-centered segment. Theoretical studies have shown that a combination of macromolecular crowding and DNA bridging elements could condense the chromosome into multiple-lobed structures.³⁷ One might start exploring this with recently-generated chromosome conformation capture maps for *E. coli*.³⁸⁻⁴⁰

That the 2 gene expression domains coincide with the boundaries of the segmental duplication, which suppresses the gene expression imbalance caused by

the disruption of xenogene silencing, suggests selection for the positioning of repetitive elements in favor of *evolvability*. There could be several situations that are not best handled by the regulatory architecture of *E. coli*. For example, the classical switch between the housekeeping sigma factor RpoD and the stress-responsive RpoS is best suited for feast-famine switches and not ideal for environments supporting prolonged slow growth.⁴¹ These situations select for the mutational inactivation of RpoS, enabling decreased expression of its targets, many of which are Ter-proximal.⁴³ Large segmental duplications were discovered in *Salmonella* several decades ago during growth in low nutrient environments,⁴² and these could be similar in principle and structure to that defined at a higher resolution in our study. Our study has shown convergence in the transcriptional outputs of RpoS inactivation and the large duplication in the Δ *stpA-hns* background⁴; this suggests that alteration in chromosome organization can at least in part recapitulate gene expression changes effected by perturbations of classical transcriptional networks. Therefore, precise positioning of elements favoring certain large structural variations, which result in dramatic alterations of gene expression states, is a possible example of a genomic feature that enables evolvability.

Conclusion

Together, gene expression homeostasis and chromosome architecture are intertwined. Mechanisms that ensure evolvability by which gene organization can be transiently altered to maintain gene expression states might be hardwired into the genome. Finally, silencing of horizontally-acquired and stress-responsive genes when they are not required is essential for the maintenance of gene expression homeostasis on a genome-wide scale.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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