Noncontiguous operon is a genetic organization for coordinating bacterial gene expression

S. Sáenz-Lahoya, N. Bitarte, B. García, S. Burgui, M. Vergara-Irigaray, J. Valle, C. Solano, A. Toledo-Arana, and I. Lasa

*Laboratory of Microbial Pathogenesis, Navarrebiomed, Complejo Hospitalario de Navarra-Universidad Pública de Navarra (UPNA), Instituto de Investigación Sanitaria de Navarra (IDISNA), 31008 Pamplona, Spain; and †Instituto de Agrobiotecnología (IDAB), Consejo Superior de Investigaciones Científicas (CSIC)-UPNA-Gobierno de Navarra, 31192 Mutriku, Navarra, Spain

Edited by Richard P. Novick, New York University School of Medicine, New York, NY, and approved December 11, 2018 (received for review July 26, 2018)

Bacterial genes are typically grouped into operons defined as clusters of adjacent genes encoding for proteins that fill related roles and are transcribed into a single polycistronic mRNA molecule. This simple organization provides an efficient mechanism to coordinate the expression of neighboring genes and is at the basis of gene regulation in bacteria. Here, we report the existence of a higher level of organization in operon structure that we named noncontiguous operon and consists in an operon containing a gene(s) that is transcribed in the opposite direction to the rest of the operon. This transcriptional architecture is exemplified by the genes menE-menC-MW1733-ytkD-MW1731 involved in menaquinone synthesis in the major human pathogen *Listeria monocytogenes*.

In a previous work, and through a genome-wide transcriptome profiling of the pathogen *Staphylococcus aureus* (21), we identified several examples of groups of genes that were apparently transcribed together despite that they were separated by gene(s) transcribed in the opposite direction. This transcriptional organization is an extreme example of an exclusion, since the mRNA encoded on the opposite strand of DNA to the operon would serve as a canonical mRNA that encodes for a protein while acting as an antisense RNA, base-pairing all along its length with an internal untranslated region of the polycistronic mRNA. Here, we report the existence of this transcriptional organization in an operon involved in the synthesis of menaquinone in *S. aureus*. Our results demonstrate that the expression of both overlapping transcripts is mutually regulated by transcriptional interference and endoribonuclease-mediated digestion. The existence of this genetic arrangement,

| Author contributions: A.T.-A. and I.L. conceived the idea; I.L. designed research; S.S.-L., N.B., B.G., S.B., and M.V.-I. performed research; J.V., C.S., A.T.-A., and I.L. analyzed data; and I.L. wrote the paper. The authors declare no conflict of interest. This article is a PNAS Direct Submission. Published under the PNAS license. 1To whom correspondence should be addressed. Email: ilasa@unav.es. This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1812746116/-/DCSupplemental. |  

---

**Significance**

In bacteria, functionally related genes are often cotranscribed in a single mRNA molecule under the same upstream promoter, forming a polycistronic operon unit. With this strategy, bacteria guarantee that production of all proteins related to a specific cellular process is simultaneously switched on or off. Here, we report the identification of a transcriptional organization consisting in operons that contain a gene(s) that is transcribed in the opposite direction to the rest of the genes of the operon. As a consequence, the resulting mRNA is fully complementary to the operon transcript. This genetic arrangement leads to mutual regulation of the overlapping transcripts expression and, thus, provides an additional strategy for coordinating the expression of functionally related genes within an operon.
which we named noncontiguous operon, confirms overlapping transcription as a specific mechanism for regulating gene expression within an operon. In addition, it underlines the relevance of reviewing operon structures in bacterial genomes to identify all protein partners whose expression is coordinated in a particular cellular process.

Results

Identification of "Noncontiguous Operons" in the S. aureus Genome.

We screened genome-wide the transcriptome data obtained from the clinical isolates S. aureus 15981 (21) and S. aureus MW2 to identify genes cotranscribed together despite being separated by a gene transcribed in the opposite direction. We found six examples that fit the predicted model (Fig. 1 and SI Appendix, Figs. S1 and S2). RNA sequencing data of published results from different laboratories (rnamaps.unavarra.es) confirmed the existence of identical transcriptional organizations in five other genetically unrelated S. aureus strains (Fig. 1 and SI Appendix, Figs. S1 and S2). The function of most of the proteins encoded by such operons is unknown. CoaD, which is part of the CoA biosynthesis pathway, MenADB and MenEC, required for menaquinone synthesis, and MoaABCED, required for molybdate transport, are among the proteins with known functions.

To explore the significance of these transcriptional organization, we chose the region comprising menE, menC, MW1733, ytkD, and MW1731 genes based on the size of the transcripts and the relevance of menaquinone synthesis during S. aureus infections (22) (Fig. 1). menE-menC and ytkD-MW1731 are listed as two independent operons in the prokaryotic operons database (csbl.bmb.uga.edu/DOOR/) (23). However, transcriptome data indicated that both operons are transcribed as a single transcriptional unit (Fig. 1). These results agreed with published results obtained by mapping of transcriptional start sites (TSS) by differential RNA-seq that revealed a unique TSS upstream the menE gene (24) (Fig. 1). To experimentally confirm the transcriptome results and because the environmental conditions controlling the expression of menE remain unknown, we first generated two derivatives of the wild-type strain in which the promoter region upstream the menE gene was deleted (∆men strain) or replaced by the constitutive blaZ promoter (PblaZ-men strain) (SI Appendix, Fig. S3). For each of these strains, we generated derivatives in which the chromosomal copy of either menC or MW1731 genes was tagged with the 3xFLAG sequence (SI Appendix, Fig. S3) and then examined MenC and MW1731 protein levels by Western blotting. Consistent with the transcriptome results, the ∆men mutation correlated with inhibition of both MenC and MW1731 proteins expression. Note that the ∆men deletion did not completely abolish protein production. On the contrary, the strains containing the constitutive blaZ promoter produced considerably higher levels of MenC and MW1731 compared with the wild-type strain (Fig. 2A). Thus, these findings suggested that expression of ytkD-MW1731 depends on the promoter region upstream the menE gene.

To further validate the cotranscription of menE-menC-ytkD-MW1731 genes, we performed Northern blot analysis using strand-specific riboprobes corresponding to menE-menC (probe A) and ytkD-MW1731 (probe B) coding regions with total RNA from exponentially growing cells of the wild-type strain, and its two isogenic derivatives, ∆men and PblaZ-men. Results showed that the mRNA expression levels are very low in the wild-type strain, because neither probe was able to detect the mRNA (Fig. 2B). In contrast, both probe A and B hybridizations with PblaZ-men RNA revealed an increased accumulation of a fuzzy band of ~4 kb that was compatible with cotranscription of menE-menC with the downstream genes ytkD-MW1731 (Fig. 2B). Note that probe B also clearly detects an additional processing band (∼1.2 kb). Together, these results strongly suggest that menE-menC and ytkD-MW1731 expression depends on the promoter located upstream menE, which is consistent with transcriptome data indicating that menE-menC-ytkD-MW1731 genes comprise a single transcriptional unit.

Transcriptome data also indicated that the MW1733 gene (258 bp long) is transcribed at high levels with a short 5′ UTR of 26 nucleotides and a 3′ UTR of 60 nucleotides that overlaps the 3′ end of the menC coding sequence. To confirm transcriptome data, we generated two additional strains in which 27 nucleotides of the promoter region upstream the MW1733 gene were deleted (∆PMW1733 strain) or replaced by the constitutive blaZ promoter (PblaZ-MW1733 strain) (SI Appendix, Fig. S3). For each of these strains, we generated a derivative in which the chromosomal copy of the MW1733 gene was tagged with the 3xFLAG sequence.

Fig. 1. Analysis of the noncontiguous operon architecture. JBrowser software images showing RNA-seq or TSS-seq mapped reads distribution in the region comprising menE-menC-MW1733-ytkD-MW1731 genes of seven unrelated S. aureus strains. The scale (log, or ×10^6) indicates the number of mapped reads per nucleotide position. A schematic representation of the structure under study is shown in the middle of the scheme. ORFs are represented as orange arrows for the genes that constitute the menE-menCytkD-MW1731 operon and as a blue arrow for the MW1733 gene. Promoters are shown as green triangles and transcriptional terminators as red rectangles. The transcript generated from the menE-menCytkD-MW1731 operon is represented as a dashed orange arrow, while the transcript generated from MW1733 is presented as a dashed blue arrow. The top line denotes the position in base pairs of the S. aureus MW2 genome. All genetic information about the start and the end of transcription was obtained from a previous study (21). RNA-seq data were obtained from 15981 (21), MW2 (this study), UAMS-1 (41), HG001 (42), WCH-SK2 (43), Homeland (24), and USA300-P23 (44).

References

S. aureus strains

15981 | VCH-SK2 | HG001 | Homeland
MW2 | UAMS-1 | USA300-P23

Fig. 2. Expression of MenC and MW1731 proteins in the wild-type strain and strains in which the promoter region upstream the menE gene was deleted (∆men strain) or replaced by the constitutive blaZ promoter (PblaZ-men strain). (A) Western blot analysis showing MenC and MW1731 protein levels. (B) Northern blot analysis showing the RNA expression levels.

Sáenz-Lahoya et al.
containing the chromosomal copy of the MW1733 gene tagged with a 3xFLAG epitope at the C terminus (SI Appendix, Fig. S4). Consistent with Northern blot results, MW1733 protein levels significantly decreased in PblaZ-men compared with those in the wild-type strain (Fig. 3A).

Next, we investigated the possibility of a reciprocal effect of MW1733 mRNA levels on the expression of the tetracistronic operon. To do so, we first analyzed by Northern blot, and with the use of probe A, menE-menc-ytkD-MW1731 mRNA levels in the wild-type, ∆PMW1733, and PblaZ-MW1733 strains. In agreement with the low level of expression of the tetracistronic mRNA in the wild-type strain (Fig. 2B), we could not find a significant difference in menE-menc-ytkD-MW1731 mRNA levels between strains when probe A was used (Fig. 3B). Thus, we repeated the Northern blot assay with the use of probe B, specific for ytkD-MW1731. Again, the ytkD-MW1731 transcript was undetectable in the wild-type and ∆PMW1733 strains. However, when MW1733 was overexpressed, a specific processing transcript was detected. The size of the discrete band (∼1.5 kb) is consistent with a transcript including ytkD-MW1731 that might be obtained upon processing of the menE-menc-ytkD-MW1731 mRNA (Fig. 3C). Next, we constructed derivatives of ∆PMW1733 and PblaZ-MW1733 harboring a chromosomal copy of either menc or MW1731 tagged with the 3xFLAG epitope in the carboxyl-terminal domain (SI Appendix, Fig. S4). Notably, constitutive expression of MW1733 caused a clear reduction in the levels of the Menc protein (Fig. 3B) and a significant accumulation of MW1733 protein levels in PblaZ-MW1733 compared with the wild-type strain (Fig. 3C).

Collectively, these results support the notion that in the noncontiguous operon, transcriptional units generated from opposite

Looking at the image, it seems to be a continuation of the previous discussion, mentioning MW1733 and its effects on transcriptional levels, with references to figures and specific probe analyses. The text continues to discuss experimental evidence showing that the region comprising menE-menc-MW1733-Δmen is under the control of the strains described in the strain, which is under the control of the transcriptional units generated from opposite.

The Expression of the menE-menc-CytkD-MW1731 Operon and the MW1733 Gene Is Reciprocally Regulated. To determine whether transcriptional levels of menE-menc-CytkD-MW1731 have an effect on the amount of MW1733 mRNA, we compared Northern blot the transcript levels of MW1733 in the wild-type, ∆men and PblaZ-men strains using probe C to detect MW1733 mRNA. Results showed that MW1733 transcript levels slightly increased when transcription of the operon was inhibited and, on the other hand, markedly decreased in PblaZ-men strain, which is under the control of the overlapping tetracistronic transcript (Fig. 3A). To confirm the regulation of MW1733 expression at a protein level, we constructed derivatives of ∆men and PblaZ-men.
strands regulate each other's expression. Thus, in the noncontiguous operon under study, an increase in tetracistronic operon transcription negatively regulates the expression of the interspersed MW1733 gene. Reciprocally, an increase in MW1733 mRNA discords expression within the overlapped operon, by strongly elevating ytkD-MW1731 mRNA levels while reducing menE-menC expression.

Analysis of the Mechanisms Underlying the Regulation of Noncontiguous Operons Expression. RNase III endoribonuclease is responsible for processing overlapping sense/antisense transcripts genomewide in bacteria (21, 25, 26). Thus, we examined the importance of RNase III activity in the reduction of MW1733 transcript levels when an excess of menE-menC-ytkD-MW1731 is transcribed. Deletion of RNase III both in the wild-type strain and in the strain overproducing the tetracistronic operon (PblaZ-men) (SI Appendix, Fig. S5) caused a slight increase in the amount of MW1733 mRNA (Fig. 4A). Consequently, MW1733 protein levels only moderately increased in mrc mutants compared with those in the respective RNase III producing strains (Fig. 4A). However, we studied the involvement of RNase III in menE-menC-ytkD-MW1731 mRNA processing when MW1733 is overexpressed. A Northern blot, using probe A, with RNA from cells of the wild type, PblaZ-MW1733, and their corresponding mrc mutants showed no significant differences between strains, given the low detectability of menE-menC-ytkD-MW1731 mRNA (Fig. 4B). Second, we carried out a similar Northern blot, but with the use of probe B, specific to detect ytkD-MW1731 mRNA. Results revealed that processing of the tetracistronic mRNA when an excess of MW1733 is transcribed still occurred in the absence of RNase III. However, in this case, the processing pattern of the operon changed, leading to a significant decrease in the amount of the discrete 1.5-kb transcript and to the appearance of two additional larger mRNA fragments (Fig. 4C).

Accordingly, MW1731 protein levels decreased in mrc mutants of the wild-type and PblaZ-MW1731 strains compared with those in

Fig. 4. RNase III processing at the overlapping region and transcriptional interference are involved in reciprocal regulation of the overlapping transcripts generated from the noncontiguous operon. (A, Upper) Northern blot showing MW1733 mRNA levels in the WT, ΔmenE-MenC, PblaZ-men, and PblaZ-men ΔmenC. A specific riboprobe (probe C) for MW1733 was used; Western blot showing MW1733 protein levels in the same strains producing a 3xFLAG-tagged MW1733 protein (A, Lower). (B, Upper) Northern blot showing menE-menC mRNA levels in the WT, ΔmenE-MenC, PblaZ-MW1733, and PblaZ-MW1733 ΔmenC. A specific riboprobe (probe A) for menE-menC was used; Western blot showing MenC protein levels in the same strains producing a 3xFLAG-tagged MenC protein (B, Lower). (C, Upper) Northern blot showing ytkD-MW1731 mRNA levels in the strains described in B. A specific riboprobe (probe B) for ytkD-MW1731 was used; (C, Lower) Western blot showing MW1731 protein levels in the same strains producing a 3xFLAG-tagged MW1731 protein. (D, Upper) Northern blot showing MW1733 mRNA levels in the WT, PblaZ-men, ΔPMW1733, ΔPMW1733 MW1733 trans and ΔPMW1733 PblaZ-men MW1733 trans. A specific riboprobe (probe C) for MW1733 was used; Western blot showing MW1731 protein levels in the same strains producing a 3xFLAG-tagged MW1731 protein (D, Lower). (E) Western blots showing MenC (Upper) and MW1733 (Lower) protein levels in the WT, WT pCN40, PblaZ-MW1733, and WT pCN40::MW1733-3xFLAG. Strains contained a chromosomal copy of either menC or MW1731 tagged with the 3xFLAG epitope. The positions of RNA standards are indicated, and 18S and 23S ribosome bands stained with ethidium bromide are shown as loading controls. The 3xFLAG-tagged proteins were detected with commercial anti-3xFLAG antibodies. Coomassie stained or stain-free gel portions are shown as a loading control. The strains used in this figure are depicted in SI Appendix, Fig. S5 for A–C and in SI Appendix, Fig. S6 for D and E.

their respective RNase III producing strains (Fig. 4C and SI Appendix, Fig. S5). Overall, these results indicated that RNase III explains, only to a certain extent, the MW1733-mediated cleavage of menE-menC-ytkD-MW1731 mRNA, suggesting that additional ribonuclease(s) might also be responsible for this processing.

Besides processing by RNase III, another possible explanation for the reciprocal regulation of overlapping transcripts described above might be transcriptional interference (27), defined as the suppressive influence that convergent RNA synthesis machinery from one DNA strand causes in cis on the transcription of the neighboring gene. Thus, we next sought to determine whether the observed antisense regulation of menE-menC-ytkD-MW1731 over MW1733 occurred when the MW1733 gene was expressed in another location of the chromosome. To do so, we inserted a 3xFLAG-tagged MW1733 gene under its own promoter next to the innocuous attB site of the lipase gene in both ΔPMW1733 and ΔPMW1733 PblaZ-men genetic backgrounds (SI Appendix, Fig. S6). Importantly, and contrary to what happens when MW1733 is located in its natural location, Northern blot analysis of MW1733 transcript levels showed that these were only slightly reduced in the presence of an excess of menE-menC-ytkD-MW1731 mRNA when the MW1733 gene was placed and expressed in trans (Fig. 4D). Note that there is a marked difference in the size and abundance of MW1733 mRNA when it is ectopically expressed from the attB chromosomal location. Consistent with Northern blot results, Western blot analysis showed that MW1733 protein levels were unaffected in the ΔPMW1733 PblaZ-men MW1733 trans strain compared with those in the wild-type strain (Fig. 4D). These results indicated that menE-menC-ytkD-MW1731–mediated suppressive influence on MW1733 expression requires cis localization of both transcripts. Lastly, to reinforce these results, we overexpressed a 3xFLAG-tagged MW1733 gene ectopically from a plasmid in the wild-type strain harboring a chromosomal copy of either menC or MW1731 tagged with the 3xFLAG epitope (SI Appendix, Fig. S6) and analyzed MenC and MW1731 levels by Western blot.
Overexpression of *MW1733* in trans did not have any impact on MenC or *MW1731* production, showing that *MW1733* effect in differocordinating menE-menC-YtkD-**MW1733** operon expression also requires cis localization of both transcripts (Fig. 4E). Overall, the above results indicate the existence of a transcriptional interference mechanism of gene regulation between the machinery that synthesizes the noncontiguous operon mRNA and the one synthesizing the mRNA of the interspersed gene.

**High Transcriptional Levels of the MW1733 Gene Can Lead to the Appearance of Small Colony Variants.** The experiments shown above demonstrated that overexpression of *MW1733* mRNA leads to reduced MenC protein levels (Fig. 3B). In *S. aureus*, the inhibition of the synthesis of menaquinone has been associated with a slowed growth phenotype, known as small colony variants (SCVs) (28). SCVs are frequently isolated from clinical samples obtained from patients experiencing chronic infections by *S. aureus*. We observed that the Δ*men* strain constructed in this work, which still shows some residual production of the MenC protein (Fig. 2A), produces colonies whose size are smaller than the ones corresponding to the wild-type strain although they are not as small as the SCVs generated by deletion of *menE-menC* genes (Δ*menEC*) (SI Appendix, Fig. S7A). Therefore, we wondered whether constitutive expression of *MW1733* might be followed by the appearance of SCVs phenotypic hallmarks. To test this hypothesis, the promoter of *MW1733* was replaced by the constitutive *blaZ* promoter in Δ*men* strain (SI Appendix, Fig. S8). The resulting strain produced colonies significantly smaller than the Δ*men* strain and exhibited several characteristics associated to *S. aureus* SCVs such as decreased pigmentation and increased resistance to aminoglycosides (tobramycin, streptomycin, gentamycin, and amikacin) than the wild-type strain (29) (SI Appendix, Fig. S7 B and C). These results suggest that overexpression of *MW1733* suppresses the expression of its convergent *menE-menC* genes, which, in turn, leads to suppressed menaquinone synthesis and the appearance of a SCV phenotype.

To confirm that appearance of SCVs by *MW1733* overexpression in Δ*men* strain exclusively happened when *MW1733* and *menE-menC-YtkD-MW1731* mRNAs were expressed in cis, we overexpressed the *MW1733* gene ectopically from a plasmid in Δ*men* strain and analyzed colony size on TSA plates. The resulting strain, Δ*men* pC40::*MW1733* (SI Appendix, Figs. S7A and S8), showed the same phenotype as the Δ*men* strain. Thus, we conclude that this noncontiguous operon transcriptional organization constitutes an effective mechanism for regulating gene expression and ultimately for controlling cell growth.

**Discussion**

The novelty introduced by the noncontiguous operon concept is that genes within an operon can be interspersed with genes divergently transcribed and that, consequently, they do not necessarily need to be contiguous in the genome. This transcriptional arrangement does not fit within the classical operon paradigm, explaining why it has passed previously unnoticed. It is important to note that in all of the examples of noncontiguous operons in the *S. aureus* genome, coding sequences of the operon never overlap the coding region of the interspersed gene. Thus, it appears that the noncontiguous operon transcriptional architecture may be a result of evolutionary pressure to minimize genome size and provide an additional strategy for coupling the expression of functionally related polypeptides. Our results provide evidence of two mechanisms by which the noncontiguous operon arrangement can coordinate gene expression. The first mechanism is related with the generation of double-stranded templates between complementary overlapping RNAs that can modify mRNA stability or translation (30, 31). We showed that RNase III digestion of the mRNA duplexes is partially responsible for both the repression of *MW1733* expression and also for the cleavage of the tetracistronic mRNA into two independent transcripts. The resulting two halves might be translated into proteins at a similar or different rate than before the cleavage. Our results indicate that transcriptional induction of the *MW1733* gene leads on one hand to a reduction in MenE protein levels, and on the other, to the stabilization of the ytkD-MW1733 half and, thus, to the accumulation of higher levels of *MW1731* protein compared with the wild-type strain. Specific RNase III cleavage at intercistronic regions with alternative outcomes for the resulting mRNAs has been previously reported in *Escherichia coli* (32). O’Daly et al. showed that binding of the cis noncoding RNA gadY to the intercistronic region of gadXW mRNA resulted in RNase III cleavage and monocistronic transcripts accumulation, probably due to increased stability of single transcripts. Similarly, binding of a cis-encoded noncoding RNA to the cII-O mRNA of λ phage has been shown to be responsible for an RNase III processing event that is followed by degradation of the upstream cII fragment while the downstream O mRNA remains stable. Because the sRNA partially overlaps the cII coding sequence at its 3’ end, it was concluded that degradation of the cII transcript is due to RNase III processing occurring at that region (33). Regarding the mechanisms underlying the stabilization process, it is possible that cleavage might alter the secondary structure of the transcripts so that they are less susceptible to degradation. RNase III is not the only endonuclease involved in *MW1733*-dependent processing of the *menE-menC-YtkD-MW1731* operon because discrete RNA fragments from the tetracistronic operon are still detected in the absence of RNase III when *MW1733* is overexpressed. An important direction for future studies should be to identify such additional endoribonuclease(s).

The second mechanism that contributes to coordinating mRNA expression within the noncontiguous operon is transcriptional interference. Because the distance between promoters of the tetracistronic operon and the *MW1733* gene is longer than 200 nucleotides, the most obvious explanation for transcriptional interference is the collision between the RNA synthesis machinery from one DNA strand with the transcription machinery from the other strand (34, 35). A major finding consistent with the existence of transcriptional interference is that tetracistronic operon overexpression did not cause any effect on *MW1733* mRNA levels when this was expressed in trans from a separate genomic location. Similarly, the expression of *menC* and *MW1731* was unaffected when *MW1733* was overexpressed in trans, which suggests that pairing between complementary transcripts can occur regardless of whether they are expressed in cis or trans, and therefore, digestion of overlapping transcripts by RNase III and additional endoribonucleases should take place when *MW1733* is produced in trans. Thus, we currently do not understand why *MW1733* overexpression in trans does not affect *menC* and *MW1731* expression. One possibility is that pairing and processing of the overlapping transcripts is less efficient when both complementary transcripts are produced from separate genomic locations.

What are the benefits of the noncontiguous operon organization compared with regular operons? The exact functions of overlapping transcription are still a matter of debate, and several authors defend that overlapping transcription are mainly the product of transcriptional noise, arising at spurious promoters throughout the genome (36). The existence and maintenance of noncontiguous operon transcriptional architecture is strong evidence that overlapping transcription represents a specific strategy for gene regulation. We can imagine a number of ways the noncontiguous operon may create higher-level organizational features that are adaptive compared with a regular operon. First, it enables a disordered expression within the genes of the operon upstream and downstream the overlapping gene, diminishing gene expression noise and ensuring a more precise stoichiometry. Second, it allows endoribonuclease-dependent removal of transcripts that escape the regular transcription repression process. Third, it allows down-regulation (exclusion) of the overlapping gene.
1. Jacob F, Monod J (1961) Genetic regulatory mechanisms in the synthesis of proteins. J Mol Biol 3:318–356.
2. Matteakis LC, Nomura M (1988) Feedback regulation of the sac operon in Escherichia coli: Translational coupling and mRNA processing. J Bacteriol 170:4484–4492.
3. Lim HN, Lee Y, Hussein R (2011) Fundamental relationship between operon organization and gene expression. Proc Natl Acad Sci USA 108:1026–1031.
4. Conway T, et al. (2014) Unprecedented high-resolution view of bacterial operon architecture revealed by RNA sequencing. MBio 5:e1042-e14.
5. Okuda S, et al. (2007) Characterization of relationships between transcriptional units and promoter structures in Bacillus subtilis and Escherichia coli. BMC Genomics 8:48.
6. Rocha EP (2008) The organization of the bacterial genome. Annu Rev Genet 42: 211–233.
7. Zheng Y, Szatzykowski JD, Fortnow L, Roberts RJ, Kasif S (2002) Computational identification of operons in microbial genomes. Genome Res 12:1221–1230.
8. Mao X, et al. (2014) DOOR 2.0: Presenting operons and their functions through dynamic and integrated views. Nucleic Acids Res 42:D654-D659.
9. Pertea M, Ayanbule K, Smedinghoff M, Salzberg SL (2009) OperonDB: A comprehensive database of predicted operons in microbial genomes. Nucleic Acids Res 37:D453–D454.
10. Toledo-Arana A, et al. (2009) The Listeria transcriptional landscape from saprophytism to virulence. Nature 459:950–956.
11. Cho BR, et al. (2009) The transcription unit architecture of the Escherichia coli genome. Nucleic Acids Res 37:1043–1049.
12. Guehl M, et al. (2009) Transcriptome complexity in a genome-reduced bacterium. Science 326:1268–1271.
13. Wurzel O, et al. (2010) A single-base resolution map of an archaeological transcriptome. Genome Res 20:133–141.
14. Beaufre M, et al. (2010) Cartography of methicillin-resistant S. aureus transcripts: Detection, orientation and temporal expression during growth phase and stress conditions. PLoS One 5:e10725.
15. Georgi L, et al. (2009) Evidence for a major role of antisense RNAs in cyanobacterial gene regulation. Mol Syst Biol 5:305.
16. Dornenburg JE, DeVita AM, Palumbo MJ, Wade JT (2010) Widespread antisense transcription in Bacillus subtilis. MBio 1:e00024-10.
17. Ruiz de los Mozos I, et al. (2013) Base pairing interaction between 5′- and 3′-UTRs controls icar mRNA translation in Staphylococcus aureus. PLoS Genet 9:e1004001.
18. Sesto N, Wurzel O, Archambaud C, Sorek R, Cossart P (2013) The exclusion: A new concept in bacterial antisense RNA-mediated gene regulation. Nat Rev Microbiol 11:75–82.
19. Thomason MK, Storz G (2010) Bacterial antisense RNAs: How many are there, and what are they doing? Annu Rev Genet 44:167–188.
20. Georgi J, Hess WR (2011) cis-antisense RNA, another level of gene regulation in bacteria. Microbiol Mol Biol Rev 75:286–300.
21. Sesto N, et al. (2011) A widespread antisense transcription drives mRNA processing in bacteria. Proc Natl Acad Sci USA 108:20172–20177.
22. Dean MA, Olsen RJ, Long SW, Rosato AE, Musser JM (2014) Identification of point mutations in clinical Staphylococcus aureus strains that produce small-colony variants autostable for methicillin. Infect Immun 82:1660–1665.
23. Mao F, Dam P, Chou J, Olman V, Xu Y (2009) DOOR: A database for prokaryotic operons. Nucleic Acids Res 37:D459-D463.

19. Thomason MK, Storz G (2010) Bacterial antisense RNAs: How many are there, and what are they doing? Annu Rev Genet 44:167–188.

20. Georgi J, Hess WR (2011) cis-antisense RNA, another level of gene regulation in bacteria. Microbiol Mol Biol Rev 75:286–300.

21. Sesto N, et al. (2011) A widespread antisense transcription drives mRNA processing in bacteria. Proc Natl Acad Sci USA 108:20172-20177.

22. Dean MA, Olsen RJ, Long SW, Rosato AE, Musser JM (2014) Identification of point mutations in clinical Staphylococcus aureus strains that produce small-colony variants autostable for methicillin. Infect Immun 82:1660-1665.

23. Mao F, Dam P, Chou J, Olman V, Xu Y (2009) DOOR: A database for prokaryotic operons. Nucleic Acids Res 37:D459-D463.

24. Koch G, et al. (2016) Evolution of resistance to a last-resort antibiotic in Staphylococcus aureus via bacterial competition. Cell 158:1060–1071.

25. Liolios E, et al. (2012) Global regulatory functions of the Staphylococcus aureus enod ribonucleocapsule III in gene expression. PLoS Genet 8:e1002782.

26. Lybecker M, Zimmermann B, Bilici C, Tukhtabauva N, Schroeder R (2014) The double-stranded transcriptome of Escherichia coli. Proc Natl Acad Sci USA 111:3314-3319.

27. Shearin KW, Callen BP, Egan JB (2005) Transcriptional interference–A crash course. Trends Genet 21:339–345.

28. Proctor RA, et al. (2006) Small colony variants: A pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol 4:295–305.

29. Sendi P, Proctor RA (2009) Staphylococcus aureus as an intracellular pathogen: The role of small colony variants. Trends Microbiol 17:54–58.

30. Brantl S (2007) Regulatory mechanisms employed by cis-encoded antisense RNAs. Curr Opin Microbiol 10:102–109.

31. Lasa I, Toledo-Arana A, Gingeras TR (2012) Am an effort to make sense of antisense transcription in bacteria. RNA 9:1039–1044.

32. Opdyke JA, Fozo EM, Hemm MR, Storz G (2011) RNase III participates in GadY-dependent cleavage of the gadY-gadW mRNA. J Mol Biol 406:29–43.

33. Krinke L, WuHL DL (1990) RNase III-dependent hydrolysis of lambda cII-O gene mRNA mediated by lambda OOP antisense RNA. Genes Dev 4:2223–2233.

34. Prescott EM, Proudfoot NI (2002) Transcriptional collision between convergent genes in budding yeast. Proc Natl Acad Sci USA 99:8796–8801.

35. Crampston N, Bonass WA, Kirkham J, Rivetti C, Thomson NH (2006) Collision events between RNA polymers in convergent transcription studied by atomic force microscopy. Nucleic Acids Res 34:5146–5152.

36. Llorens-Rico V, et al. (2016) Bacterial antisense RNAs are mainly the product of transcriptional noise. Sci Adv 2:e1501363.

37. Proctor RA, et al. (2014) Staphylococcus aureus small colony variants (SCVs): A road map for the metabolic pathways involved in persistent infections. Front Cell Infect Microbiol 4:99.

38. Johns BE, Purdy KJ, Tucker NP, Maddocks SE (2015) Phenotypic and genotypic characteristics of small colony variants and their role in chronic infection. Microbiol Insights 8:15–23.

39. Krinke L, Chastanet A, Débarbouillé M (2004) New vector for efficient allelic replacement in naturally nontransformable, low-GC-content, gram-positive bacteria. Appl Environ Microbiol 70:6887–6891.

40. Valle J, et al. (2003) SarA and not sigmaB is essential for biofilm development by Staphylococcus aureus. Genes Dev 17:1021–1031.

41. Fossum E, et al. (2008) Staphylococcus aureus small colony variants (SCVs): A road map for the metabolic pathways involved in persistent infections. Front Cell Infect Microbiol 4:99.

42. Franci C, Sorek R, Cossart P (2013) The exclusion: A new concept in bacterial antisense RNA-mediated gene regulation. Nat Rev Microbiol 11:75–82.

43. King AN, et al. (2018) Guanine limitation results in GadY-dependent and -independent alteration of Staphylococcus aureus physiology and gene expression. J Bacteriol, 10.1128/JB.00136-18.

44. Poupl E, Poupl C, Jagia B, Mudak T, Dubrac S (2018) SpdC, a novel virulence factor, controls histidine kinase activity in Staphylococcus aureus. PLoS Pathog 14:e1006917-e1006932.

45. Bui LMG, Hoffmann P, Turnidge JD, Zilm PS, Kidd SP (2015) Prolonged growth of a clinical Staphylococcus aureus strain selects for a stable small-colony-variant cell type. Infect Immun 83:470–482.

46. Yeo W-S, et al. (2018) The FDA-approved anti-cancer drugs, streptozotocin and fluorouracil, reduce the virulence of Staphylococcus aureus. Sci Rep 8:2521.