MicroCommentary

Regulation of transcription by DNA supercoiling in *Mycoplasma genitalium*: global control in the smallest known self-replicating genome

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Summary

The mollicute *Mycoplasma genitalium* causes sexually transmitted disease in humans. It has recently come to widespread public attention through its involvement in pioneering synthetic biology experiments. The 580-kilo-base-pair genome of *M. genitalium* contains just 470 genes, few of which seem to encode conventional transcription regulators. This raises the important question of how does this simple organism control its gene expression? The finding that the transcription of an osmotically inducible gene encoding a lipoprotein is sensitive to antibiotics that inhibit the activity of DNA gyrase, the enzyme that introduces negative supercoiling into DNA, raises the possibility that changes in DNA supercoiling provide a regulatory mechanism for controlling transcription in *M. genitalium*.

Understanding how gene regulation operates in the smallest self-replicating genome is highly desirable both from the perspective of synthetic biology and from the point of view of understanding the evolution of natural gene regulatory circuits. Classically, transcriptional control has been considered chiefly in terms of the activities of *trans*-acting DNA-binding proteins that activate or inhibit gene promoters. More recently, the potential for small regulatory RNA (sRNA) molecules to act *in trans* to modulate transcript stability has been shown to add an additional level of control (Gripenland *et al.*, 2010). The relatively tiny *Mycoplasma genitalium* genome contains no genes capable of encoding obvious candidates for conventional transcriptional proteins and the situation regarding sRNA is unclear

(Fraser *et al.*, 1995). There is just one sigma factor, so the option of reprogramming RNA polymerase using alternative sigma factors seems not to be available. In this issue of *Molecular Microbiology*, Zhang and Baseman (2011a) provide evidence that changes to the structure of the genetic material itself are exploited by *M. genitalium* to control transcription.

The potential for transcription to be influenced by the supercoiling of the DNA has been appreciated for decades (Dorman, 1991). Supercoiling arises when the DNA duplex is underwound; the molecule adopts a minimal energy configuration to adjust to the torsional stress due to underwinding. This configuration can be made manifest in terms of writhing at the level of the DNA duplex axis, something that approximates to one's intuitive sense of 'supercoiling'. It can also be made manifest in terms of a change to the twist of the DNA, with one possible outcome being a loss of local base pairing (Fig. 1). In this case, the consequences of underwinding the DNA duplex for key events in transcription initiation such as open complex formation are obvious.

Most bacteria routinely maintain their DNA in an underwound state. While this situation can clearly assist transcription initiation, how can it be invoked as part of a regulatory process that sets and resets the level of transcription to different values? This is possible because the degree to which the DNA is underwound is not only **variable**, but is also **responsive** to changes in the external environment of the bacterium. Changes in aeration, osmotic pressure, temperature, pH, carbon source and growth phase have all been shown to result in adjustments to the superhelical density of bacterial DNA (Cameron *et al.*, 2011).

Hitherto, variations in the degree to which DNA is supercoiled in response to changes in specific environmental parameters have been studied in model bacteria with very sophisticated gene regulatory networks. In organisms such as *Escherichia coli* or *Salmonella enterica*, variable and environmentally responsive DNA supercoiling influences transcription and other DNA-based transactions in

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Fig. 1. Environmentally wrought changes in DNA supercoiling influence transcription. Physical and/or chemical changes in the environment (1) alter the metabolic flux within the bacterial cell resulting in a rebalancing of the ratio of ATP to ADP (2). This modulates the activity of the ATP-dependent type II topoisomerase DNA gyrase ('Gyr', 3) causing a change to the global level of DNA supercoiling in the genome that has the potential, *inter alia*, to assist promoter open complex formation through a loss of base pairing (4). The resulting adjustment to its transcriptional profile helps the cell to adapt to the altered environment (5).

the background while the regulatory foreground is dominated by transcription factors, nucleoid-associated proteins and alternative sigma factors. In contrast, *M. genitalium* possesses a simplified regulatory landscape from which DNA-binding proteins of many kinds are largely absent. However, it does possess DNA gyrase and DNA topoisomerase I, providing it with the potential to supercoil negatively and to relax its DNA (Zhang and Baseman, 2011a). Mycoplasmas are also known to vary their transcriptional profile in response to environmental stimuli (Madsen *et al.*, 2008; Zhang and Baseman, 2011b).

Zhang and Baseman (2011a) have examined the response of the MG_149 lipoprotein gene to changes in growth medium osmolarity and shown that its transcription is strongly enhanced by hyperosmotic stress and that this upregulation can be reversed by treating the bacterium with the DNA gyrase inhibitor novobiocin. Sensitivity to this treatment regime resides in the -10 region of the MG_149 promoter, an observation that is consistent with an influence of DNA topology on transcription initiation.

The finding that variable DNA supercoiling forms part of the gene regulatory repertoire of such a small genome is consistent with a role for environmentally responsive DNA topology acting as a primitive regulatory switch. A geneti-

cally simple organism without recourse to the regulatory refinements provided by large numbers of DNA-binding proteins can achieve a degree of control over its gene expression through changes in the local structure of the DNA. The sensitivity of DNA gyrase to the ratio of intracellular ATP to ADP provides a link between DNA supercoiling and metabolic flux (Hsieh *et al.*, 1991; Sonnenschein *et al.*, 2011). Changes in growth phase, nutrition, other chemical or physical stimuli originating internally or externally to the cell all have the potential to modulate gyrase activity and hence the degree to which the DNA in the genome is underwound. There is extensive evidence that bacteria with larger genomes, including pathogens, exploit this effect as part of their transcriptional regulatory machinery (Dorman, 1991; Cheung *et al.*, 2003; Cameron *et al.*, 2011). DNA supercoiling contributes to the compaction of the genetic material, allowing it to be accommodated within the bacterial cell. It forms a link between nucleoid structure and the modulation of DNA-based transactions such as transcription. It is tempting to speculate that, following the emergence of DNA as the repository of genetic information in cellular organisms, changes to the free energy of DNA supercoiling formed the basis of an early regulatory switch, providing a mecha-

nism by which gene expression can be influenced on both a global and a local level. In modern mollicutes such as *M. genitalium*, one may be seeing a continuing role for variable DNA supercoiling as a primary mechanism for the control of the transcriptome. Given the power of modern synthetic biology (Gibson *et al.*, 2008), this is now a testable hypothesis. With their tiny genomes, *M. genitalium* and its relatives are obvious candidates for use as experimental models in which to investigate the evolution of gene regulatory systems.

Acknowledgements

I thank Niamh Ní Bhriain for valuable comments on the manuscript. Research in the author's laboratory is supported by Science Foundation Ireland.

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