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MicroCommentary

Membrane RNAs in bacteria

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Ornate large extremophile (OLE) RNAs constitute a family of large, well-conserved non-coding transcripts of unknown function. In this issue of *Molecular Microbiology*, Kirsten Block, Ron Breaker and co-workers begin to decipher some of the functional mysteries surrounding OLE RNA. This class of RNA is approximately 610 nucleotides in length, with an average nucleotide identity of 65%. The OLE RNA family was discovered using comparative sequence analysis of bacterial genomes. It was found in *Bacillus halodurans* and in extremophiles of the *Clostridiae* order. So far, OLE RNA is confined to anaerobic bacteria from the environment and the human gut. The large number of well-conserved OLE RNA sequences has made it possible to use sequence covariation analysis to accurately predict OLE RNA secondary structures (Puerta-Fernandez *et al*., 2006; Weinberg *et al*., 2007; 2009; Tseng *et al*., 2009). OLE RNAs are among the most complex non-coding RNAs currently known to exist in bacteria (Weinberg *et al*., 2009).

Ornate large extremophile RNA is part of a large and complex transcriptional unit (Fig. 1A) that contains 10 other genes including OLE-associated protein (OAP). The OAP protein is encoded by a gene located just downstream of the OLE RNA gene. To begin to understand the biological role of OLE RNA, Block and co-workers (2010) analysed the interaction between the RNA and OAP of *B. halodurans in vitro*. Using gel shift assays, they found that OAP and OLE form a Ribo-Nucleo-protein particle. By systematically removing domains of the OLE RNA, they were able to map the binding site of OAP in the RNA. This analysis yielded a sequence motif that is highly conserved. The fact that OLE RNA and OAP are co-transcribed and form a Ribo-Nucleo-protein particle suggests that this interaction is functionally important.

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Ornate large extremophile-associated protein has several transmembrane domains, raising the possibility that OAP is a membrane protein that recruits OLE RNA to the cell membrane. To test this, the authors performed fluorescent *in situ* hybridization on OLE RNA to determine its sub-cellular localization. Indeed, they found that OLE RNA localized to the membrane in *Escherichia coli*. The localization of OLE to the membrane raises the possibility that it has a novel cellular function.

The remarkable 'ornate' nature of OLE RNA's secondary structure is so highly conserved that it could be deduced solely by covariation analysis. The conserved structure indicates that the RNA has functional significance. Further evidence for this conjecture comes from the analysis of global expression levels of *B. halodurans* RNAs. Breaker and co-workers measured the relative expression level of all *B. halodurans* transcripts using deep sequencing and found that OLE RNA is the 11th most abundant RNA in the cell. Its expression level is 10% of the ubiquitous signal recognition particle (SRP).

We plot in Fig. 1B data provided in the supplement of the manuscript, which reports on the relative expression of all transcripts in *B. halodurans*. The fact that OLE RNA is one of the most highly expressed RNAs provides further support for a central functional role. Bacteria expressing OLE must expend significant energy producing such large quantities of RNA, meaning that it must confer an evolutionary advantage. Furthermore, because OLE is found in extremophiles, it is possible that its function is related to the adaptation to extreme environments. Combined with its recruitment to the membrane by OAP, it is likely that OLE may have a novel function in the cell.

Bacillus halodurans has approximately 4200 genes and as is illustrated in Fig. 1B, 4000 of those genes are identified by deep sequencing RNA transcripts. The result reveals the abundance of RNAs that are continuously produced. The fact that some of the most abundant RNAs are non-coding (e.g. OLE, SRP, tmRNA and RNASE P) provides a clear illustration of the functional importance of non-coding RNA in the cell. This study also reveals how inexpensive next generation sequencing is revolutionizing the field of molecular microbiology. Indeed, OLE RNA was identified from an analysis of thousands of bacterial

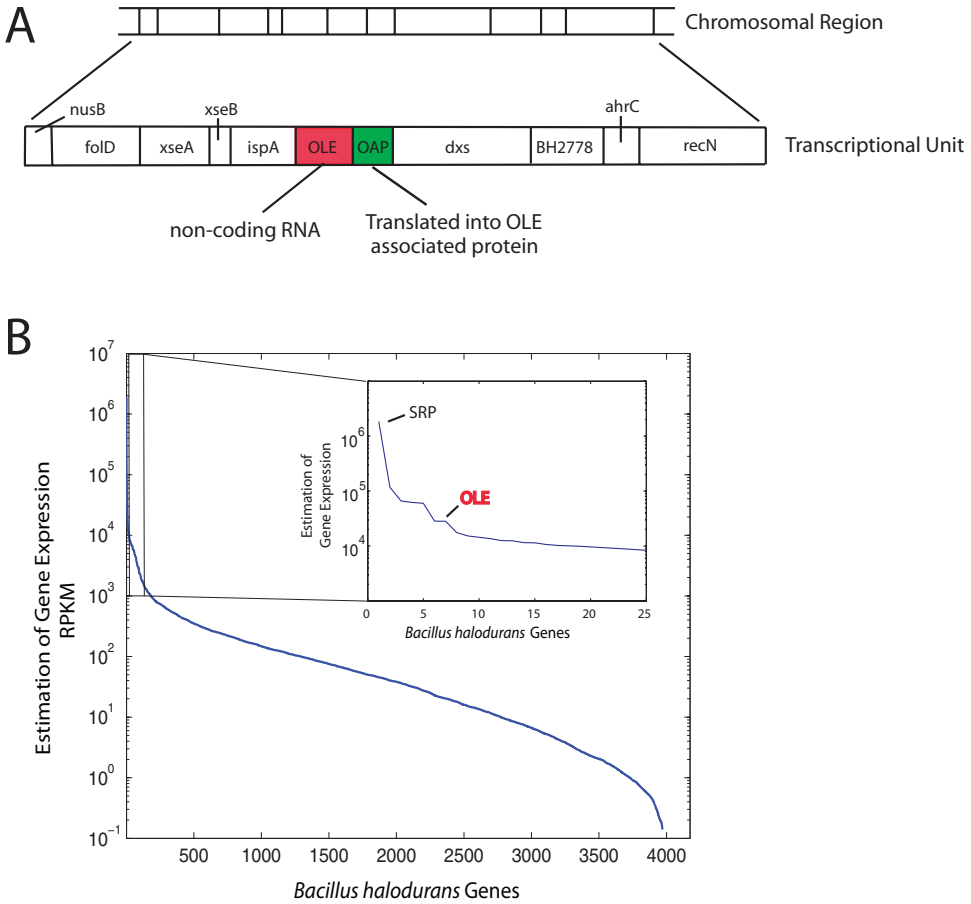

Fig. 1. A. Schematic representation of the transcript containing OLE RNA (red) as well as OAP (green) as transcribed from genomic DNA. OLE is co-transcribed with OAP.
B. Rank ordered plot of the RNA expression levels (as measured by Reads Per Kilobase per Million Reads) for all genes in *B. halodurans* (measured by deep sequencing, based on *Supporting information* data provided by Block *et al*., 2011). OLE RNA (indicated in the inset) is in the top 10 of the most highly expressed RNAs, and its expression level is ≈ 10% of that of the ubiquitous SRP.

genomes. Furthermore, it is now possible to characterize an entire bacterial transcriptome in a single experiment (Fig. 1B). We can therefore expect to discover many new non-coding and functionally important RNAs with these new techniques. The challenge will be to unravel their biological function.

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