Bacterial transcriptomics:

what is beyond the RNA horiz-ome?

成员分工：
胡汇丰 ：翻译文献，主讲PPT
骆 磊 ：翻译文献，制作PPT
史博文 ： 翻译文献

Prologue

Over the past 3 years, bacterial transcriptomics has undergone a massive revolution

Novel tools have made it possible to explore the bacterial transcriptome to an unprecedented depth

Revealed that the transcriptome is more complex and dynamic than expected

Prologue-----
how is the transcription start

Bacterial transcription is carried out by a single RNA polymerase (RNAP)

RNAP is consists of a core enzymatic machine and a σ-factor

Additional transcription factors--- Example:

Escherichia coli (314) 43% are repressors
35% are activators
22% are dual regulators

Prologue-----

oddities

RNAP-associated proteins affect the processivity of RNAP

sRNAs

riboswitches

leaderless mRNAs

Prologue

What is now on the horizon?
How can we integrate the newly acquired knowledge?
In this Review, we summarize the quantum leap that bacterial transcriptomics has taken over the past several years and suggest areas where the new technologies could have a major impact

Prologue

A technical revolution

Redefined Operon

sRNA and peptides

RNA pressing

Leaderless mRNAs and regulation by UTRs

3D DNA structure

Epigenetics

A technical revolution

From DNA microarray
to the RNA—seq
What is metatranscriptomes

Redefined operon

Example:

In the *E. coli* galactose operon, two promoters are separated by 5 bp. Transcription driven from the first promoter is terminated earlier than that from the second, suggesting that transcription termination could depend on transcription initiation

Redefined operon

sRNAs + **riboswitches** = **Operon polarity**

Chip + **ultra**sequencing = Chip---seq

Redefined operon

Example:

In *Caulobacter crescentus,* 6% of the genes are transcribed from multiple TSS
indicating that various activators and/or repressors can regulate transcription. Thus, an operon could respond to two or more different inputs

Redefined operon

To achieve a global view of such complex regulatory control in bacteria, all transcription factor binding sites will have to be mapped and all TSSs precisely determined

A recent study on *E. coli* that examined 600 combinations of promoter and coding regions from various genes found evidence that the regulatory information stored in the coding regions of genes strongly affects gene expression levels, indicating that this could be a more general phenomenon

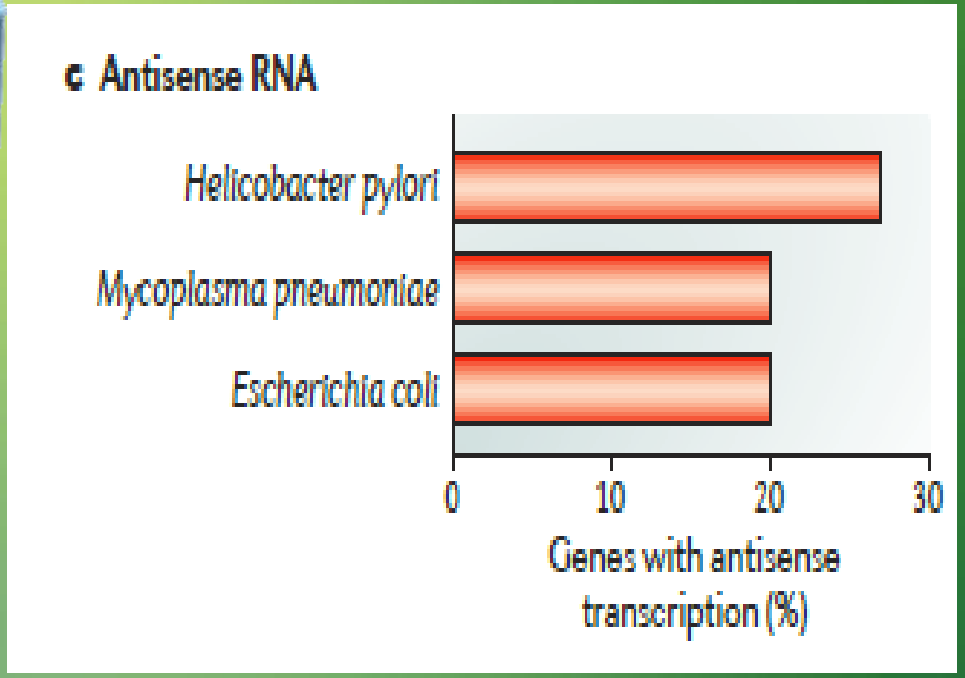
sRNAs

Transcriptional Interference

transcriptional activation

translational control

regulation of mRNA half-life



sRNAs

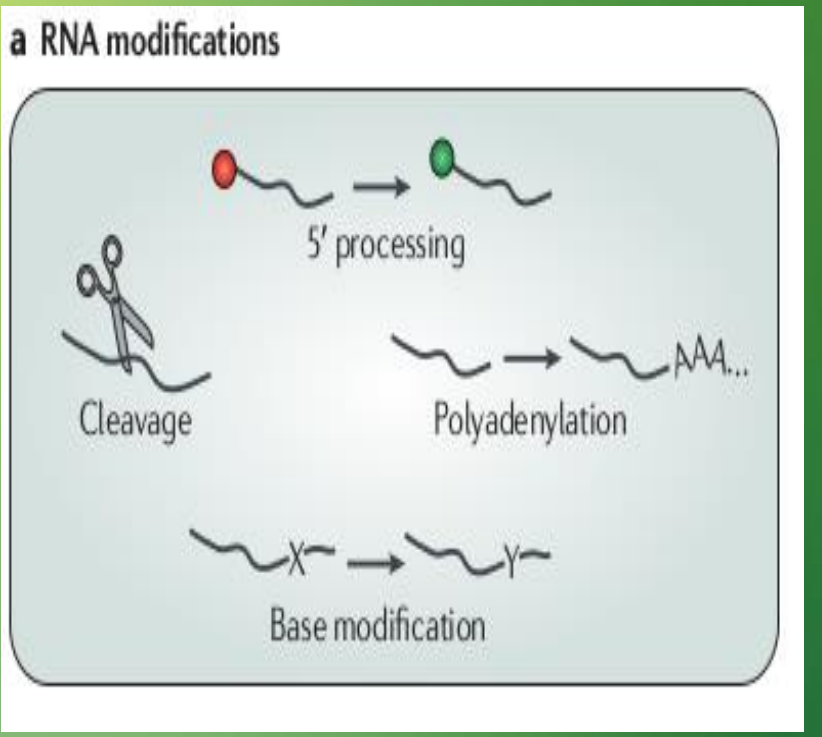
For example, in *M. pneumoniae*, genes with overlapping antisense transcripts have lower expression levels. *Trans*-encoded sRNAs typically interact with multiple mRNAs, as these sRNAs contact their target mRNAs in discontinuous patches. Thus, a single RNA can globally modulate particular physiological responses and networks in a manner similar to a transcription factor But at the post-transcriptional level and with varying degrees of stringency and outcomes

sRNAs

Metatranscriptomic studies of complex samples containing thousands of bacterial sequences have also corroborated the abundance of sRNAs within bacterial communities

RNA processing

在细菌中，也存在mRNA的修饰，5'端修饰，3'端加尾，剪切修饰等等，而且这些修饰是RNA成熟非常重要的环节。例如 3 '端的聚腺苷酸化，这种修饰与翻译的终止有关。另外，RNA拼接并不是真核生物所特有的，在某些共生体和噬菌体中也存在RNA拼接。



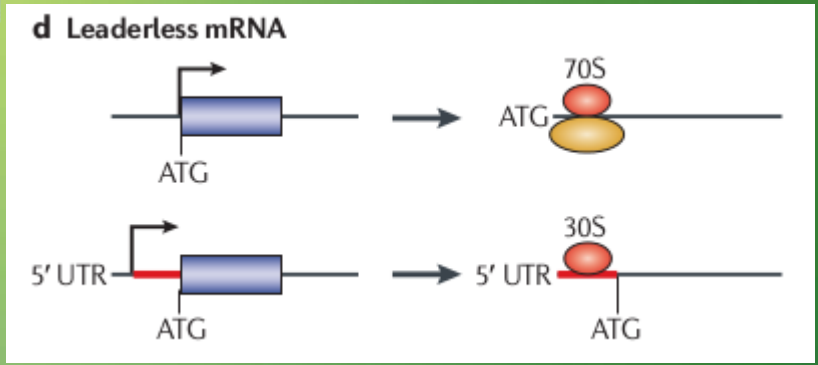
RNA processing

特征	真核中出现？	细菌中出现？
小片段RNA	是	是
复杂的启动子	是，真核有很多转录控制因素	是，但比真核简单
RNA加工	是	是，但是主要与降解的调控有关，而且缺乏具体的实例
RNA拼接	是	是，但受限于tRNA和rRNA
多聚腺苷酸	是，起稳定RNA的作用	是，使得RNA不稳定
定位翻译	是，RNA翻译时移动到特定的位点	是，mRNA在特定的拟核位点翻译
表观遗传修饰	是	是，但是可用的信息很少

Leaderless mRNA and UTRs

leaderless mRNA:
转录起始于起始密码子AUG的mRNA。
UTRs调控：非编码区的调控

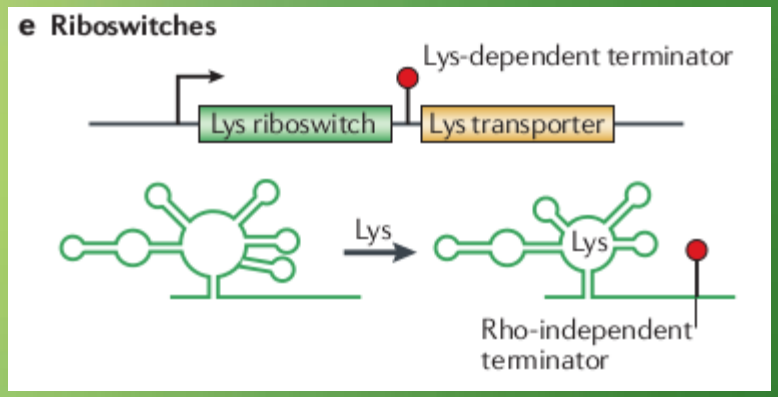
SD核糖识别序列长期以来被认为是mRNA上必不可少的与核糖体结合的非编码序列。最新的研究结果表明，一些mRNA是没有 5 '端非编码区的。在大肠杆菌的研究中发现，AUG密码子可以成为核糖体的结合序列.当大批mRNA不能翻译的时候，核糖体就会与起始密码子结合，开启翻译过程



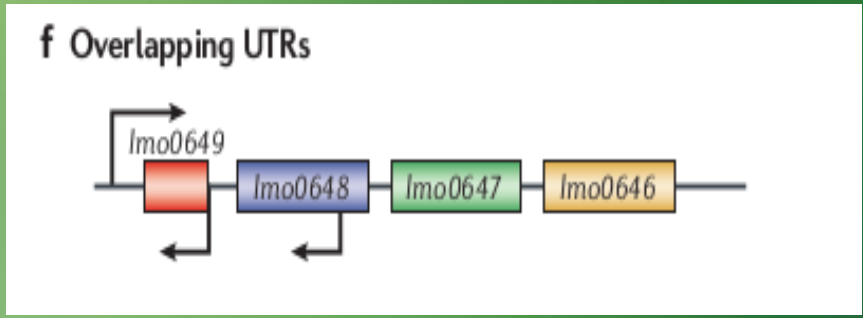
图为无5'UTR和有5'UTR启动翻译的过程

Leaderless mRNA and UTRs

转录组的研究显示，许多mRNA的5'端非编码区至少含有几百个bp，这些区域可以通过二级结构调节翻译过程。一个典型的例子是大肠杆菌色氨酸操纵子前导肽，它能根据色氨酸的效应控制转录终止位点。也可以控制核糖开关，影响mRNA的稳定性和翻译效率。



核糖开关的调控



重叠UTRs的调控

3D structure

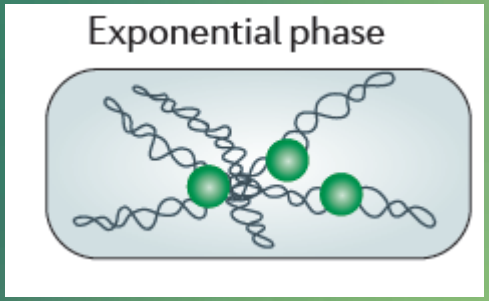
Like eukaryotes, bacteria contain nucleoid proteins that bind DNA in a sequence-specific or non-sequence-specific manner

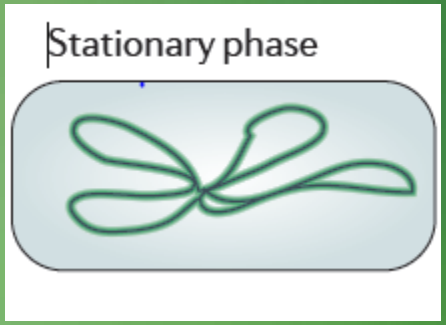
E. coli contains around 450 nucleoid structure domains, which have an average length of around 10 kbp, with variable boundaries and a random distribution along the chromosome

3D structure

High-throughput techniques have been applied to the determination of long-range DNA interactions and 3D DNA structure *in vivo* in eukaryotes: namely 3C(4C hi-C) They have been applied to regions of the human genome and in a genome-wide study in yeast

Similar studies in bacteria would help to unveil the structure and dynamics of the bacterial chromosome, as well as its role in transcription regulation



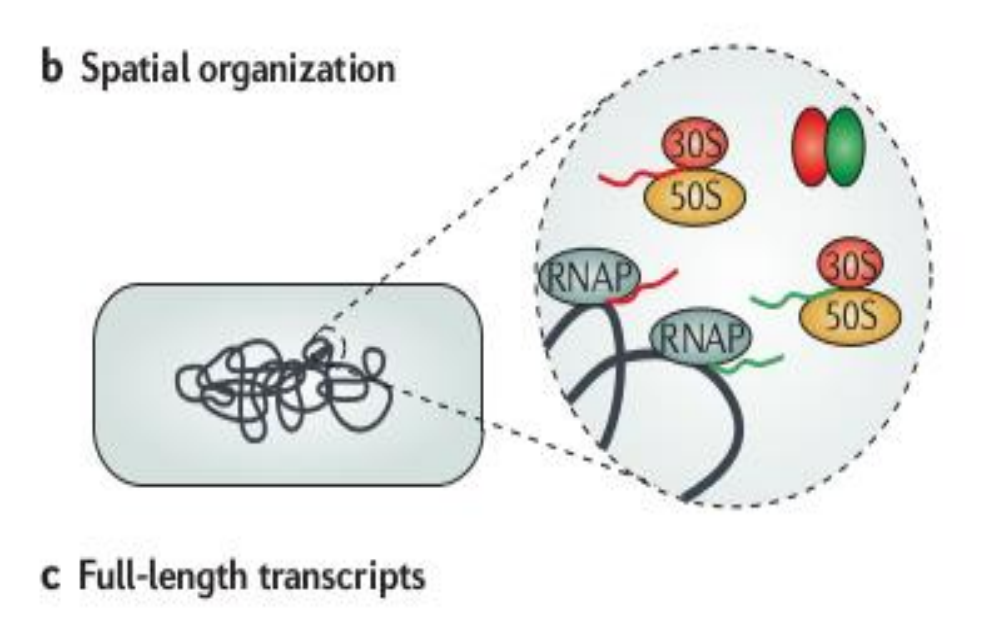


Epigenetics

细菌的DNA甲基化在之前一直被认为主要是用来区分外源DNA的。**事实上DNA甲基化在DNA复制起始、错配修复、细菌中寄主控制的修饰与限制以及转座子的失活等过程中对维持遗传信息的稳定性发挥着重要的作用。**例如，细菌细胞内有限制性内切酶，但是细菌的基因组上也有限制性内切酶的切割位点，为了不让基因组被限制性内切酶降解，细胞便将自己的这些序列甲基化，即在某些碱基上甲基化，这样限制性内切酶就不能识别了。这种系统在细菌中起到了免疫的作用，即外来入侵的DNA在限制性内切酶的作用下被水解，而细菌自身的DNA在甲基化酶的作用下被保护起来。

细菌mRNA的定位

在大肠杆菌内部某些信使RNA可以迁移到它们编码蛋白质的目的地,该过程由RNA跨膜序列所控制，在真核生物中也有这种过程。如果这种转运过程被应用与细菌内部所有RNA，　应用RNA分子可视化技术可以阐明信使RNA定位是否是一个普遍现象。可能可以通过特定序列来引导RNA定位，从而开启一个新的研究领域。



Perspecfices

data should be complemented with other levels of regulation
allowing full-length polycistronic transcripts to be sequenced and providing direct association of promoters and terminators
Genome-wide mappings with long reads will not only identify the different 5′ UTRs and 3′ UTRs but will also deconvolve the exact molecular nature of the operon by sequencing full-length transcripts

Perspecfices

The number of known sRNAs is increasing every day, but the functions of many of these remain unclear

New high-throughput methods for detecting RNA–protein interactions are expected to reveal important biological insights into RNA function and the regulation of protein activity by RNAs

THE END!

Thank you!

Any questions?