MILESTONE 5

Maximum control

These days, the lactose system of *Escherichia coli* is regarded as the classic example of an operon. It is used by every modern molecular biology or genetics textbook worth its salt to illustrate the basic principles of gene transcription. The regulation of the structural genes *lacZ*, *lacY* and *lacA* through binding of the *lacI*-encoded repressor to the operator site (*lacO*) was famously uncovered by Jacob and Monod at the beginning of the 1960s (see Milestone 2). However, it would take the rest of the decade, and some complex bacterial genetics, before their fellow scientists uncovered the key *cis*-regulatory element of transcription — the promoter.

Even when they are fully induced, different enzymes are present in bacterial cells in widely varying amounts. So what controls the maximum rate of enzyme production? Scaife and Beckwith subscribed to the idea that gene expression could be controlled by a specific chromosomal site that is able to limit the rate of initiation of either transcription or translation. They therefore tried to identify such a site by searching for mutations that would decrease or abolish the potential of the *lac* operon to be expressed. Several mutations that resulted in reduced levels of all three *lac* structural genes were picked up in a mutagenesis screen. The researchers showed that these mutations were *cis*-dominant by confirming that their effects were not relieved by the introduction of a second *lac* region into the cell. Furthermore, using gene-repression experiments and detailed genetic analysis, they showed that these mutations were separate from the repressor–operator system, and mapped outside *lacI* and probably also outside *lacO*.

By 1968, Ippen and colleagues had built on these initial results using a series of mutants in which increasing amounts of the *lac* operon and its upstream region had been deleted in a stepwise fashion. Recombination analysis showed that the mutations mapped to just before the beginning of the *lac* operon. The authors designated the site of the mutations as the ‘promoter’ (reintroducing a term that had been used previously, with a different meaning, by Jacob, Ullman and Monod). So did the promoter control transcription or translation?

Ippen and co-workers suggested that it is transcription that begins at the promoter.

The *lacI*-encoded repressor binds to the *lacO* operator sequence and prevents transcription of the *lac* structural genes (top). Binding of an inducer prevents the repressor from binding *lacO* and the structural genes are transcribed (bottom). Studies using the *lac* operon identified the promoter (*p*) as a *cis* controlling element for gene transcription in the 1960s.

Moreover, as there was no evidence that the *lacO* region is translated, they proposed that translation begins just downstream of *lacO*, in a region that Jacob, Ullman and Monod had previously suggested was important for gene expression.

The function of the promoter was elegantly confirmed by Eron and Block in 1971 using an *in vitro* transcription system. They showed that mutations in the *lac* promoter altered the levels of transcription initiation, and confirmed the functional significance of transcription initiation *in vitro* by showing that it required the auxiliary sigma factor (see Milestone 6), was negatively regulated by the *lac* repressor, and was positively regulated by cyclic AMP (cAMP) and cAMP-binding protein (see Milestone 4). Furthermore, confirming extensive genetic work from the Beckwith group, they showed that the *lac* promoter had two parts: one that mediated transcription initiation and one that mediated positive regulation. So, by the start of the 1970s, the concept of the promoter, and its potential for complexity of both regulation and structure, had emerged.

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MILESTONE 6

Sigma, from the start

It's easy to forget that somewhere between the now high-tech fields of genomics and transcriptomics once lay the fundamental problem of how DNA is transcribed. Perhaps most important was the question of how RNA polymerase is guided to the right promoters and prevented from binding unproductively to random sequences. This question was answered almost 40 years ago for bacteria with the finding that sigma factors lead RNA polymerase to its target sequences, thereby kick-starting a flurry of biochemical studies of transcription.

In 1969, knowledge about bacterial transcription was based largely on *in vitro* assays using purified RNA polymerase and a (typically bacteriophage) template DNA. Such experiments had shown that the polymerase was specific about the sequences it transcribed *in vivo*, but was less choosy *in vitro* — in fact, its specificity *in vitro* varied depending on the template used and how the polymerase was purified.

The solution to this confusing state of affairs depended on having a reliable means

MILESTONE 7

And then there were three

In bacteria, a single RNA polymerase core enzyme is known to be responsible for synthesizing all RNAs, including messenger RNAs (mRNAs), ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs). However, until the early 1970s, it was unclear whether the same was true for eukaryotic cells.

At the time, several lines of evidence implied that a distinct polymerase transcribed rRNAs. The (G+C)-rich base composition of rRNAs was distinct from other RNA classes, their genes were heavily redundant and localized in the nucleolus, and rRNA synthesis was regulated independently from the other RNA types.

In late 1969, Roeder and Rutter reported the discovery of three chromatographically separable forms of eukaryotic RNA polymerase from sea urchin embryos (I, II and III) and two species from rat liver (I and II). Further