lacZ fusions report gene expression, don’t they?

Reporter genes – the basics

To study regulation of gene expression in bacteria, most, if not all, geneticists use constructs in which a gene of interest is fused to a reporter gene, for reasons of convenience, the Escherichia coli lacZ gene encoding β-galactosidase is most popular. Two types of lacZ reporter fusions are routinely constructed: transcriptional (sometimes called operon) fusions and translational (gene) fusions (10, 11). Transcriptional fusions join a transcriptional unit (a gene or operon) to a promoterless lacZ gene containing its own translational start codon and RBS. This creates a single transcriptional unit, but no hybrid proteins. In this case, β-galactosidase expression should be proportional to the rate of transcription of the gene or operon of interest cloned upstream of lacZ. In lacZ translational fusions, a gene of interest is joined, in-frame, to the eighth codon of lacZ lacking its transcriptional and translational start sites. Thus, the specific activity of the resulting β-galactosidase hybrid protein should reflect the rates of both transcription and translation of the gene studied, and such are our textbook rules (8).

The question is: can we be sure that transcriptional fusions report promoter strengths and that translational fusions monitor the combined transcriptional and translational expression levels of genes of interest? In principle, yes, but if all else fails, read the instructions (11). It is our impression that complications caused by mRNA secondary structures and processing are not always fully appreciated.

mRNA stabilizing or destabilizing sites that influence the expression of transcriptional reporters

Suppose that we are interested in a gene of great opportunity (gogO) and that we wish to study the regulation of its promoter (P) (Fig. 1a). Depending on where a transcriptional lacZ fusion is made, however, we may not measure promoter strength only.

We will illustrate this point by giving two examples. The first example deals with a case involving an orphan RBS upstream of the introduced reporter. The cryIIA toxin gene of Bacillus thuringiensis was fused to a transcriptional lacZ reporter. A deletion removing a region located between 148 and 121 nucleotides upstream of the lacZ fusion site reduces β-galactosidase activity about 12-fold, in comparison with the parental construct (1). In the absence of transcript analysis, one might have concluded that the deleted region is an important part of the cryIIA promoter. However, transcription is initiated further upstream and in reality the sensitive region carries an mRNA-stabilizing determinant called STAB-SD, i.e. a Shine–Dalgarno sequence which is capable of binding the 30S ribosomal subunit and thereby protects the mRNA from nucleolytic degradation, probably in the absence of translation from this Shine–Dalgarno sequence (1). In Fig. 1(b), this mRNA stabilizing site is designated X.

The second example demonstrates the importance of a site promoting mRNA destabilization by processing. The rnc (RNase III) operon of E. coli is transcribed from a single promoter. Transcriptional lacZ fusions were made in the rnc gene and in the downstream era gene. Point mutations lying in the rncO (‘operator’) sequence located some 40 nucleotides upstream of the rnc translation start site enhance β-galactosidase activities of both fusions two- to threefold (9). Again, without transcript analysis, one could assume that the ‘operator’ mutations enhance transcription. However, these mutations block nucleolytic processing by RNase III and thereby increase the stability of the 5’ untranslated leader of rnc (9). In Fig 1(b), this processing site is indicated by Y. A good method to avoid complications caused by regulated mRNA degradation is to join the transcriptional lacZ reporter to the gene of interest (gogO) precisely at the transcription start (+1) site (Fig. 1c).

Translational lacZ reporters that affect mRNA stability or initiation of translation

Interpretation of translational lacZ fusion data may also be less straightforward than is sometimes assumed. The site at which a fusion is made can be very important. For instance, in the arcDABC operon of Pseudomonas aeruginosa, which encodes the enzymes of the arginine deiminase pathway, segmental differences in mRNA stability contribute to differential gene expression (6). Shortly after transcription initiation, the arc mRNA is stabilized by processing. The first example is given by the authors to illustrate the potential of translational lacZ gene expression.
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Fig. 1. How the sites of transcriptional and translational lacZ fusions can influence the expression of the reporter. (a) The gene to be studied (gogO), its mRNA and encoded protein. (b) Transcriptional lacZ fusion made within gogO: β-galactosidase expression depends on mRNA stabilizing or destabilizing sites. (c) Transcriptional lacZ fusion made at the transcription start site measures promoter strength. (d) Translational lacZ fusions may be differentially expressed depending on the fusion site: removal of negative translational control or processing sites will result in increased β-galactosidase activity. gogO, gene of great opportunity; +1, transcription start site; P, promoter of gogO; RBS, ribosome-binding site; X, upstream mRNA stabilizing site (e.g. orphan RBS); Y₁, Y₂, mRNA processing sites (destabilizing); Z, translational control sequence (e.g. masking RBS by formation of double-stranded mRNA region); lacZ, β-galactosidase structural gene.

Transcriptional and translational regulation are not always easy to separate

Combined use of transcriptional and translational reporter gene fusions can be helpful to distinguish between transcriptional and translational regulation (8, 11). For example, in Pseudomonas spp., the conserved two-component regulatory system GacS/GacA controls the production of secondary metabolites and exoenzymes (7). The question as to whether the GacA system mediates the regulation of target genes such as those specifying cyanide synthesis (hcn) at the level of transcription or translation has been examined in Pseudomonas fluorescens. A translational hcnA-lacZ fusion was found to be strongly GacA-dependent (by a factor of 60), while a transcriptional hcnA-lacZ fusion was not; this transcriptional fusion was made precisely at the mapped transcription start site (+1). From this experiment it can be concluded that the major impact of the GacA system occurs at the post-transcriptional level (3). This interpretation is supported by the complete GacA-dependence of lacZ translational fusion processed in the distal part of arcD by an RNaseE-like enzyme. Although the ArcD integral membrane protein is synthesized in much smaller amounts than is the ArcA enzyme, correlating with differences in concentration and chemical half-life of arcD and arcA mRNAs, an arcD’-lacZ fusion is expressed at higher levels than is an arcA’-lacZ fusion (5). This paradoxical discrepancy is due to the fact that in the arcD’-lacZ construct the ‘lacZ fusion was made upstream of the major RNaseE cleavage site(s). As a consequence, the expression of arcD’-lacZ is considerably higher than that of native arcD. Thus, the arcD’-lacZ and arcA’-lacZ expression levels do not reflect efficiencies of transcriptional and translational initiation of arcD and arcA only; the lacZ reporter used masks the regulation by mRNA processing, whose site is indicated schematically by Y₂ in Fig. 1(d).

Depending on the site of insertion, the introduction of a reporter gene can disrupt an mRNA secondary structure which is important for translational regulation (4) and which is indicated by Z in Fig. 1(d). If Z normally restricts translation initiation and if the translational ‘lacZ fusion is made upstream of Z, β-galactosidase expression will be increased in comparison with the initial situation (Fig. 1(d)). Finally, intrinsically stable and, therefore, highly expressed mRNAs can be destabilized by a lacZ insertion, whose mRNA has a relatively short half-life, resulting in reduced expression (12). In conclusion, reporter gene fusions themselves can occasionally have profound effects on mRNA processing and secondary structure, with dramatic consequences for the expression of the reporter gene.
constructs in which the hcn promoter has been exchanged for the tac promoter precisely at the transcription initiation site (2, 3). However, a transcriptional fusion constructed within the coding region of hcnA, i.e. at the same site as the translational hcnA–lacZ fusion, exhibits some apparent GacA control (by a factor of 2–3). This might be caused by mRNA destabilization during translational repression of the target gene (our unpublished results). This is a caveat that has been pointed out before by Silhavy and Beckwith (11): translational control can favour mRNA degradation, leading to the false impression of transcriptional control.

**Reporters fusions: simple to measure, but not always simple to interpret**

The facts and artefacts recalled here are not novel. We are bringing them up mainly with the intention of providing some cautionary notes for which there is not enough space in textbooks. Our main conclusions are that the interpretation of reporter gene fusion data becomes more reliable when the precise transcription start site is known and that the introduced reporter gene can substantially interfere with certain post-transcriptional regulatory mechanisms.

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