

to an assay system that has been pretreated with functional antagonists will produce less blue light emission as a result of decreased IP_3 activation caused by the presence of test compounds that are functional antagonists.⁶⁴

Since the generation of an assay signal does not depend upon irradiation with an outside light source, background fluorescence is generally not an issue. There are, however, other important limitations of aequorin assays. First and foremost, it is critical that the production of aequorin not interfere with cellular function. As with any assay system that depends on the addition of foreign genes, if insertion of the signaling genes significantly alters cellular function, then correlation to normal conditions (native cells) becomes problematic. In addition, compounds that interfere with the conversion of coelenterazine into coelenteramide will provide erroneous data, as this will block light production. Despite these limitations, aequorin-based assay systems are routinely employed in modern drug discovery research programs. Assay for a variety of GPCRs, ion channels, and transporters have been reported.⁶⁴

REPORTER GENE ASSAYS

Differential gene expression plays a key role in cellular functions such as communication between cells, cellular development, growth regulation, and proliferation. The process is tightly regulated and dysregulation can lead to a number of pathological conditions. Monitoring changes in gene expression has become an important aspect of drug discovery, and reporter gene assays have become an important tool for this purpose. Essentially, the expression of a native gene (one normally present in a cell) is coupled to the expression of a non-native gene, a reporter gene, whose protein product can be used to monitor changes in expression of the native gene. Every time the native gene is expressed, the non-native gene is also expressed (Figure 4.28).

FIGURE 4.28 In a reporter gene assay system, expression of target gene is tied to expression of a non-native gene that will produce a measurable signal upon expression.

