THE Renilla LUCIFERASE-MODIFIED GFP FUSION PROTEIN IS FUNCTIONAL IN TRANSFORMED CELLS

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1. SUMMARY

The cDNA of Renilla reniformis luciferase (ruc) has been cloned and used successfully as a marker gene in a variety of transgenic species. Similarly, the transfer and expression of green fluorescent protein (GFP) cDNA (gfp) and its mutants from Aequorea victoria resulted in high levels of GFP in transformed cells, allowing convenient visualization of gene expression under the microscope.

Here we present the construction of four fusion genes from the cDNAs of Renilla luciferase and Aequorea GFP mutants (gfp2 and gfph, which have been engineered specifically for expression in prokaryotic organisms and mammalian cells, respectively). The fusion gene I (rg2) contains the Renilla luciferase cDNA linked through a 15-nucleotide (5 amino acid) spacer added to its 3' end to the 5' end of the intact gfp2. When the gfph fragment replaces gfp2, fusion gene II (rg) was formed. In fusion gene III (g2r), the positions of the ruc and gfp2 are reversed with a linker composed of seven amino acids in length. In fusion gene IV (gr), gfph replaced gfp2 with a linker of nine amino acids. The fusion gene cassettes I and III were placed into pBluescript KS II (+) and the fusion gene II and IV into mammalian expression vector pCEP4. The above plasmids were transformed into E.
coli, different mammalian cell lines, and mouse embryos (microinjection). Fusion proteins with an apparent molecular weight around 65 kDa were detected by Western blotting using either anti-Renilla luciferase antibody or anti-Aequorea GFP antibody. Proteins RG2 and G2R extracted from the transformed E. coli have both green fluorescence activity and luciferase activity when expressed in E. coli. RG and GR are active in mammalian cells, ES cells, and mouse embryos. Fluorescence resonance energy transfer (FRET) between Renilla luciferase (emission at 478 nm) and Aequorea GFP (emission 510 nm) was detected by spectrofluorimetry, only if the two proteins were linked.

The Renilla luciferase-GFP fusion proteins offer a novel marker system for photosynthetic microorganisms and plants. This fusion protein helps to overcome the problems in quantifying GFP fluorescence. The determination of fusion protein in cells can be quantified based on luciferase activity. Furthermore, the system may be useful in the study of protein-protein interactions in vivo.

2. INTRODUCTION

The cDNA from Renilla luciferase (ruc) has been isolated and sequenced (Lorenz et al., 1991). By providing appropriate promoters, the cDNA gene cassettes were expressed in bacteria, transformed plant cells, and mammalian cells (Mayerhofer et al., 1995; Lorenz et al., 1995). The documented high efficiency of the Renilla luciferase is a useful and novel trait for a marker enzyme for gene expression studies. The usefulness of the green fluorescent protein from the jellyfish Aequorea victoria was documented as a reporter in prokaryotes and animal cell systems (Chalfie et al., 1994). The UV light stimulated GFP fluorescence does not require cofactors and the gene product alone is sufficient to allow detection of living cells under the light microscope. Bioluminescence in Renilla reniformis is produced by the reaction between coelenterazine (luciferin) and oxygen, a reaction that is catalyzed by Renilla luciferase. The reaction yields blue light with an emission wavelength maximum of 478 nm using the purified enzyme. In Renilla reniformis cells, this reaction is shifted toward the green with a maximum of 510 nm. This wavelength transition is due to an energy transfer to a green fluorescent protein. In this paper, we describe the engineering of a novel protein with dual functions combining characteristics for Renilla luciferase and GFP molecules. This task is accomplished by construction of a fusion gene between the cDNA of Renilla and the cDNA of the modified Aequorea GFP molecules (Zolotukhin et al., 1996; Cormack et al., 1996).

3. EXPERIMENTAL

3.1. Vectors and Cells

The vectors used for cloning and expression of the gene constructs in E. coli and mammalian systems were pBluescriptKS II (Strategen) and pCEP4, respectively. The cDNA of Renilla luciferase and gfp2 were in plasmids designed pCEP4-RUC provided by Dr. Cormier and pBcGFP2 by Dr. Cormack, and GFPβ was in pTR-βcatin-GFP. E. coli strains used included DLT101 and DH5α. Mammalian cell line LM-TK− was used as a recipient for gene expression.

3.2. Primers

The following six primers were designed for cloning of RG and GR gene constructs.