The GUS reporter gene system

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The GUS reporter gene system is already a powerful tool for the assessment of gene activity in transgenic plants. Further developments may lead to routine *in vivo* analysis and fusion genetics.

THE GUS (β-glucuronidase) gene fusion system is now being used extensively as a reporter gene in plant and agricultural molecular biology. Here, I review the properties of GUS that have led to its widespread use and discuss possible developments of the system.

The enzyme β-glucuronidase (GUS E.C.3.2.1.31) catalyses the hydrolysis of a wide variety of glucuronides. Its substrates consist of D-glucuronic acid conjugated through a β-*O*-glycosidic linkage to virtually any aglycone. Most naturally occurring substrates for GUS are generated by the main detoxification pathway of vertebrates; thousands of endogenous and xenobiotic compounds, including steroid hormones, bilirubin, plant secondary products and antibiotics, are conjugated to glucuronic acid in the liver and other organs of vertebrates to effect detoxification, and are then excreted in the urine and bile[1,2].

Escherichia coli, one of the most abundant aerobic constituents of the vertebrate large intestine, is unusual among bacteria in possessing a highly efficient mechanism for the assimilation and metabolism of the many β-glucuronides with which it is presented. The gene encoding β-glucuronidase from *E. coli* K12, *gusA* (formerly *uidA*) has been sequenced and the gene product shown to be a stable homoteramer of subunit relative molecular mass of 68,000[3].

Advantages of GUS over other reporter systems include the robustness of the enzyme, the simplicity of the assays and the variety of substrates available. These include sensitive histochemical substrates such as 5-bromo-4-chloro-3-indolyl β-D-glucuronide (X-Gluc; Fig. 1), and numerous other chromogenic and fluorogenic substrates for quantitative analysis. Expression of the gene is not deleterious to plant hosts[4]. In addition, virtually all aspects of the GUS gene fusion system can be reduced to very low technology as many GUS substrates can be prepared biosynthetically using the conjugation and excretion systems of vertebrates.

A key advantage of GUS is the absence of GUS activity in many organisms other than vertebrates and their attendant microflora. Lower and higher plants and most bacteria, fungi and many insects that exist in the phyllo- and rhizosphere are largely, if not completely lacking in GUS activity[4]. Minute quantities of GUS activity can therefore be accurately measured — even in single cells[5] — when *gusA* is used as a reporter gene in these systems.

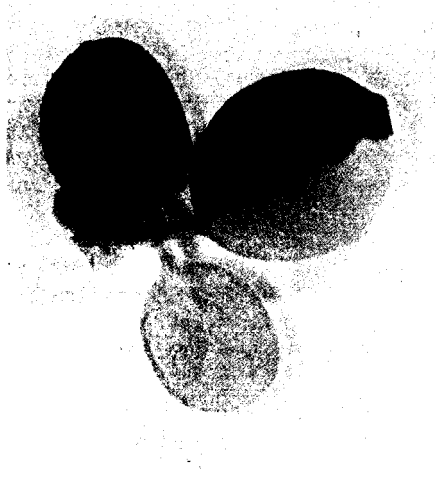

FIG. 1 GUS expression in leaves of a R$_0$ soybean plant transformed with a CaMV 35S promoter — GUS fusion. The leaf was incubated with the substrate X-Gluc, which is cleaved in GUS-expressing cells resulting in deposition of a blue indigo dye. The pattern of GUS expression indicates that this plant is a chimaera, and this has been confirmed by DNA hybridization. The lack of background activity in non-transformed cells is clear. (Courtesy of P. Christou and W. Swain Agracetus.)

Applications

Since the development of the GUS system, many thousands of transgenic plants have been generated expressing GUS. In such plants, the spatial distribution of gene activity can be visualized in the absence of any background signal. This has been instrumental in the development of new methods of plant transformation such as particle bombardment[6-8], and in the successful transformation of many crop plants including soybean and monocots such as rice[8-10]. The power and simplicity of GUS histochemical methods is also demonstrated in assays for transposon excision[11], lineage analysis[6,12] (Fig. 1), developmental analyses[13] and discrimination between gene family members[14].

GUS fusions are now widely used to study plant-pathogen and plant-symbiont interactions. They can be used both to study expression of particular genes and to mark and monitor populations of microorganisms in soil or in association with plants. GUS offers a promising route for transformation, monitoring and molecular genetic analysis of the numerous important epi- and endophytic organisms that are not readily cultured, such as oomycetes and mycorrhizal fungi. In addition GUS fusions can be used in many laboratory model systems lacking endogenous activity, including *Saccharomyces, Schizosaccharomyces, Dictyostelium*, some strains of *Caenorhabididtis elegans*[15] and *Drosophila*.

Unlike *lac Z*-encoded β-galactosidase, *gus A*-encoded GUS can readily traverse membranes when an appropriate transit or signal sequence is fused to its amino terminus. GUS fusions have been used to study targeting and transport of chimaeric proteins into chloroplasts[16], mitochondria[17] and the endoplasmic reticulum (ER)[18]. The N-terminal addition of an ER targeting sequence may lead to the secretion of GUS from cells: however, in the ER, GUS becomes glycosylated at two cryptic N-linked glycosylation sites and is thereby inactivated[18]. Work is in progress to eliminate these cryptic sites to allow fully active GUS to be secreted. Secreted GUS may then accumulate in the space between the plant cell membrane and cell wall, where it can act upon those soluble substrates that can cross the cell wall.

Glucuronide permease

E. coli has evolved not only the *gusA*-encoded hydrolase, but a general mechanism for transmembrane transport of β-glucuronides. The glucuronide permease was first described biochemically by Stoeber[19], and has recently been characterized genetically and molecularly (R.A.J. unpublished). It can accumulate a wide variety of β-glucuronide substrates ranging from simple aliphatic to large, complex heterocyclic conjugates. The 49 kDa permease is encoded by a single gene, *gusB*, which is cocistronic with *gusA* (R. A.J. and W. Liang, in preparation), and is an integral membrane protein that appears to be a symporter coupled to the H^+/Na^+ gradient.

It seems likely that glucuronide permease will also function in heterologous systems, including plants. This would permit the accumulation of GUS substrates in cells, and would enable quantitative, non-destructive, real-time imaging of gene activity within the organism. New fluorogenic substrates for GUS, such as trifluoromethylumbelliferyl glucuronide and resorufin glucuronide (Molecular Probes, Inc.) that are efficiently trans-

ported by the permease and fluoresce maximally at the pH of the cell, together with recent developments in epifluorescence microscopy and imaging, should be of value in this respect.

Fusion genetics

Application of genetic selection to obtain *cis*- and *trans*-acting mutations that affect gene fusion activity in genetically complex eukaryotes has been described by Bonner *et al.*[20] in *Drosophila*. The potential use of GUS for this approach hinges on the ability to prepare hundreds of different substrates that could release bioactive aglycones on hydrolysis by GUS, so giving the possibility of implementing almost any conceivable selection.

For example, plant growth regulator glucuronide conjugates have been synthesized and shown to provide hormonal effects only to those tissues expressing GUS (R. A. J., unpublished). Conversely, cytotoxic aglycones such as cycloheximide or a herbicide could be conjugated to give a non-toxic but toxigenic suicide substrate that kills GUS-expressing cells specifically. One can envisage targeting GUS activity to discrete cells, tissues, plants or developmental stages, thereby targeting the effects of a generally applied substrate. This would provide not only genetic selections but may allow new means of modifying the physiology and/or agronomic traits of a plant. □

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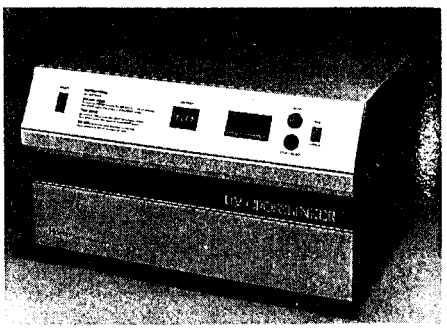
DNA technology transfer

This week's focus on recombinant DNA technology features a protocol for the rapid separation of plasmid DNA, a baculovirus expression system and customized RNA-oligos.

HydroLink is the new alternative to agarose and acrylamide gels, introduced by Biochem and developed specifically for the electrophoretic **separation of double-stranded DNA and DNA sequencing** (*Reader Service No. 101*). HL-dsDNA is a novel polymer supplied as a ready-to-pour stable liquid, which can be used to separate dsDNA from 100–7,000 bp, with nonlinear separation up to 23,000 bp. Biochem says that compared with polyacrylamide and agarose gels, HydroLink offers a ten-fold increase in loading capacity, higher resolution, picomole sensitivity, and increased gel strength. The HydroLink DNA Sequencing Gel Electrophoresis System (HL-SEQ) comes with high pH buffer for denaturing DNA, eliminating the need for urea. Due to the chemical and thermal stability of HL-SEQ, sequencing parameters can be extended with respect to solvent, ionic strength, pH denaturing additives and temperature.

Using the new **protocol for plasmid separation**, plasmid DNA can be separated in 1.5 hours, says Jouan (*Reader Service No. 102*). Jouan, distributors of Hitachi's CS120 ultracentrifuge, has developed a method using CsCl gradients that cuts down on processing time and allows several runs per day. High-density CsCl is loaded into a tube and low-density CsCl is loaded on top. The plasmid DNA mixture is added and the tubes are spun in the CS120 at 120,000 r.p.m., 600,000 *g* for 1.5 hours. The two CsCl solutions form density gradients, but the boundary between the two solutions presents a significant step in densities, which, Jouan says, ensures good separation between the sharply defined bands of linear DNA (upper band) and circular DNA (lower band), which would normally be difficult to separate.

Using the UVC 1000 UV Crosslinker, **DNA and RNA can be crosslinked to membranes** in seconds not hours, says Hoefer Scientific Instruments (*Reader Service No. 103*). The UVC 1000 can accommodate a variety of membrane sizes, including blots from DNA sequencing gels. It can be used to crosslink to nitrocellulose, nylon, or nylon-reinforced nitrocellulose membranes for multiple probing of Southern, Northern and dot/slot blots. The machine's monitoring device can be preset to measure either energy in microjoules or time in minutes, with values displayed on the LED countdown display monitor. If set to measure energy, the UV light is automatically deactivated when the energy level has been reached. The $1,295 (US) UVC 1000, measuring 37 × 15 × 49 cm, is fitted with a door that is safety interlocked to prevent accidental exposure to UV.



UVC 1000: crosslinks DNA and RNA to membranes in moments.

Recombinant reagents

For researchers studying the mechanisms controlling cellular proliferation, Immunodiagnostic Systems Ltd is selling **recombinant transforming growth factor** (TGF-Beta 1) (*Reader Service No. 104*). TGF-Beta 1 is prepared from CHO cells using recombinant DNA techniques. It is an acid-stable, disulphide-linked 25 kDa dimer that is made up of two identical 12.5 kDa subunits, each comprising 112 amino acids. IDS says the recombinant TGF-Beta 1 has a purity of greater than 95 per cent when assayed by an amino acid sequencer and SDS-PAGE, and is free of the biohazards associated with natural TGF-Betas. Recombinant TGF-Beta 1 will be available in 1- and 5-μg quantities in early 1990.

The Japanese company Nacalai Tesque, Inc. is offering **recombinant human prolactin** (hPRL) (*Reader Service No. 105*). This simple polypeptide of 200 amino acids and molecular weight of 23 kDa is produced in *Escherichia coli* cells. As the hPRL is produced by recombinant techniques, it has, says Nacalai, a purity of greater than 99 per cent on SDS-PAGE and is free of contaminating protein, such as growth hormone, which may contaminate hPRL derived from the anterior pituitary gland. The recombinant hPRL is supplied in 100-μg, 1-, 10- and 100-g quantities as a lyophilized reagent that is stable at −20 °C.

In kit form

Invitrogen has introduced the MaxBax **baculovirus expression system** for the pro-