venues. They brought out a conference daily called *Scan '98*, largely produced by students of the Osmania University Department of Journalism.

Some critical observations

In the past few years, the character of the annual meeting of the Science Congress Association has changed. The leadership has passed on from academics – university professors – to bureaucrats. Look at the general presidents of ISCA. Last year it was S. K. Joshi, formerly Director General of CSIR and Secretary, DSIR. This time around it was P. Rama Rao, former Secretary, DST, and currently Chairman, Atomic Energy Regulatory Board. Next year it will be Manju Sharma, Secretary, DBT. The year after it will be R. A. Mashelkar, Secretary, DSIR. Is it all for the good, asked a delegate from Delhi. Our universities are downgraded not only from outside but also from within the scientific and scholarly community, he opined. Could it be because bureaucrats are in a position to fund such associations and meetings and laboratory scientists, however competent they may be in their work, have to depend on the bureaucrats for their survival? Also look at the awardees in this year's Science Congress. There were thirteen of them including those who received the five newly instituted awards. Asked A. Mahadevan, who spoke in the Botany Section on the last day, why was it that we kept on speaking of encouraging young scientists if the major awards of the country had to go to the same set of people. This question was also raised by reporters covering the Congress. Aren't we giving the impression to the large number of young research scholars attending the conference that one has to be a science administrator to win an award or be elected general president of ISCA? This year's award winners included – you wouldn't get a prize for guessing it right – M. G. K. Menon and Yash Pal, both of whom delivered lectures without a prepared text. Neither the organizers nor the media centre could provide a summary of their talks let alone the full text versions. If scientists want their views – especially about dwindling support – to be heard and acted upon, they should take such meetings seriously. Perhaps, the ISCA general president should insist on the submission of the text well in advance.

Several speakers touched upon the important issue of the exodus of the youth away from science not only to careers in engineering and medicine but also to management and marketing. It is clear that science education is being neglected and that science is no longer an attractive career option. The exodus is not restricted to the entry level. A substantial number of scientists, it was pointed out, were leaving the Departments of Space, Atomic Energy, Defence and CSIR for lucrative jobs in the globalized private sector. P. N. Tandon, the eminent neurosurgeon and former president of INSA, asked scientists to stop deserting the ship (of science in India) and sailing abroad. He urged young scientists to stay back, work on problems relevant to India and pursue excellence. But the Government is seriously considering to treat higher education as a non-merit good!

There were many disruptions caused by power failure. The first technical session on the second day of the conference, when the presidents of different sections were to deliver their presidential addresses, was delayed by nearly an hour. When Roald Hoffmann, the star attraction at the meeting, was delivering his special lecture on molecular beauty there were many disruptions to the great discomfiture of the organizers. The Andhra Pradesh Government had given Rs 10 million to the Osmania University but the entire sum seems to have been spent on giving a facelift to the campus. A part of it could have been used to improve the infrastructure.

These shortcomings notwithstanding, one got the feeling that efforts were afoot to change the perception that the annual meeting was a mela. Will the Madras session be better than the one at Hyderabad?

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RESEARCH NEWS

Green fluorescent protein: A novel reporter gene

Sonia, P. K. Jaiwal, A. Ahad and Lingaraj Sahoo

Plant transformation systems involve the stable insertion of the functional DNA directly into the genome of plant cells and their regeneration into transgenic plants. During the last decade, a wide range of methods and different approaches have been used to produce transgenic plants with many desirable traits in many important crop plants and some of them are moving to market for the end-users[1]. However, the main bottleneck in transgenic research is the low efficiency of stable gene transfer. The success of any transformation experiment depends upon the selection of the transformed cells, which are limited in number, from the whole plant tissue. To increase the frequency of transformed cells, it is essential to standardize all the factors (depending on the gene transfer technique used) using transient expression of reporter genes whose expression can be measured visually or biochemically and then to carry out transformation experiments using these optimized parameters. A large number of reporter genes are presently available. Among them, the most widely used is the *gus* reporter gene system and others are *luc*, *cat* and anthocyanins. These reporter genes suffer from various

limitations like low enzyme stability, low sensitivity, high cost, substrate requirement or lethal assay technique (see Table 1).

In the nineties, a novel reporter gene, green fluorescent protein (GFP) isolated from the Pacific jellyfish, *Aequorea aequorea*[2], has been cloned[3] which codes for a protein that fluoresces spontaneously in heterologous cells. *In vivo* light is produced by the bioluminescent jellyfish *Aequorea victoria*, when calcium binds to photoprotein aequorin which oxidizes bound coelentrazine. Second protein GFP derives its excitation energy from this oxidation and transduces it to produce green light[4] (Figure 1).

GFP is composed of 238 amino acids and is characterized by the presence of highly fluorescent chromophore. At positions 65–67, Ser–Tyr–Gly tripeptide is cyclized, which oxidizes to form *p*-hydroxybenzylidene-imidazolidinone chromophore[3]. Recent analysis of molecular structure shows GFP to consist of eleven strands of β-sheet forming the shape of a cylinder capped with α-helices on the top and bottom. These α-helices also form a scaffold for the chromophore, which is located near the geometric centre of the protein. This new protein structure has been named β-can[5,6]. The amino acid folding and formation of chromophore is oxygen dependent and requires almost 4 h.

The GFPs are unusually stable proteins and remain fluorescent up to 65°C, pH 11, 1% sodium dodecyl sulphate (SDS) or 6 M guanidinium chloride and resist most proteases for several hours[7]. Purified GFP absorbs blue light (maximally at 395 nm with a minor peak at 470 nm) and emits green light (peak emission at 509 nm with a shoulder at 540 nm)[8].

The jellyfish *Aequorea victoria* GFP possesses a number of desirable traits and has great potential to be used as reporter system especially in living tissues (Table 2). First, the expression of GFP is cell autonomous and independent of cell type and location. The mechanism of fluorescence is self-contained in the protein and does not require any cofactors to fluoresce. Secondly, the detection of gene expression is very simple and employs irradiation using standard long wave UV light sources. Introduction of any exogenous substrate is not required unlike other commonly used reporter genes (*gus*, *luc*, *cat*). The gene product or the assay technique are not lethal to the tissue. Thirdly, its relatively small molecular size (26,900 kDa) makes protein fusions manageable and also does not affect native protein structure, compartmentation as well as function. Fourth, amino acid replacements in the polypeptide yield light of different colours[9].

GFP can be used as long-term lineage marker provided it can be expressed from a strong, cell type specific promoter. The use of different cloned GFPs to track multiple cell types enables differences in cell movement or migration to be visualized in real time without the need to add additional agents or fix or kill the cells[10]. The ideal fusion of GFP with a host protein preserves both the fluorescence of GFP and all the targetting and physiological functions of the host protein[10]. Both carboxy and amino termini of GFP have been fused to a variety of proteins preserving both the fluorescence of GFP and physiological functions of proteins. Therefore, GFP can be used as a protein tag[10]. Different coloured GFP mutants with overlapping absorption spectra may be employed to study protein–protein interactions using fluorescence-resonance-energy-transfer (FRET)[10].

However, wild type GFP has several properties that need optimization like low brightness, a significant delay between protein synthesis and fluorophore development and complex photoisomerization. Improvement in GFP fluorescence signals has been achieved either by synthesizing GFP gene with optimized codon usage[11] or oligonucleotide-mediated site-directed mutagenesis[12] or DNA shuffling[9]. Random and directed point mutations in which single amino acid substitution of the chromophore site and other regions of protein have produced improved GFP isoforms. One form emits blue instead of green light (by substituting histidine or tryptophan for tyrosine at position 66 in the chromophore). A red-shifted variant is obtained by replacing serine at 65 threonine, cysteine, leucine, valine or alanine and another isoform significantly brighter than the wild-type by exchanging amino acid serine 65 to cysteine and threonine and phenylalanine 64 to leucine. DNA shuffling, a PCR-based *in vitro* recombination technique that combines useful mutations from individual genes, has been used widely to improve GFP in terms of brightness, codon usage, folding, proteolytic stability and location. Improved versions of GFP show 42-fold increase in brightness and fluorescence, single excitation and emission peaks[9,13]. Intensity-based on-line sensors have also been developed to quantify gene expression *in vivo*[14].

As far as we are aware, GFP has been successfully expressed at least once in every species that it has been tried in. A cDNA for *Aequorea victoria* green fluorescent protein produced fluorescent product when expressed in prokaryote *Escherichia coli* as well as a eukaryote *Caenorhabditis elegans*, was first demonstrated by Chalfie *et al.*[15]. In *E. coli*, the cDNA expressed under the control of T7 promoter and green fluorescence was observed on illumination with long

Table 1. Various reporter genes commonly used for transformation studies

Reporter gene	Substrate	Mode of assay	Limitations
β-glucuronidase (*gus*)	X-gluc	Detection of fluorescent blue product, histochemical assay	Lethal to tissues, high cost of substrate
Luciferase (*luc*)	Luciferrin	Detection of bioluminescence	High cost of substrate and equipment, very low sensitivity
Anthocyanins	—	Visible reporter (pigmentation)	Low sensitivity, poor quantitation, adverse effect on plant metabolism
Chloroamphenicol transferase (*cat*)	Chloroamphenicol acetyl-CoA	Quantified by acetylation of chloroamphenicol by densitometry/scintillation counting	Cumbersome assay technique, background problems in plant tissues

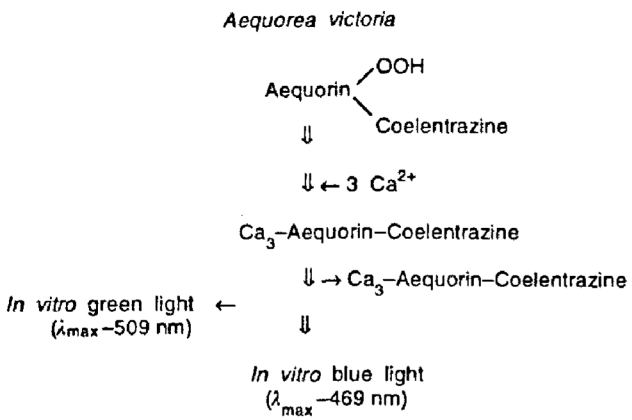

Figure 1. Bioluminescent pathway in *Aequorea*. The photoprotein responds directly to Ca^{2+} by oxidizing the bound coelentrazine. The energy thus released stimulates green fluorescent protein to emit green light *in vivo*.

wave UV source. The cells grew well in the presence of inducer IPTG and GFP did not appear to have a toxic effect on cells. Transformation of the nematode *Caenorhabditis elegans* also resulted in the production of fluorescent GFP. GFP was expressed in a small number of neurons under the control of a promoter for the *mec-7*-gene.

There are two major uses of GFP in plants: monitoring gene expression and protein localization, and providing an easily scored genetic marker in living plants[16]. To employ GFP successfully in plants, three major steps need to be taken: (a) GFP apoprotein must be produced in suitable amounts in plant cells, (b) the apoprotein must undergo efficient post-translational cyclization and oxidation to produce the mature GFP, (c) the fluorescent protein may need to be suitably targeted within the cell, to allow efficient post-translational processing, safe accumulation to high levels or to facilitate detection of expressing cells[16].

GFP permits direct selection of live transformed cells and allows them to be regenerated into transgenic plants. It has been used as a marker in a variety of plant cells and protoplasts derived from *Arabidopsis*[17], *Citrus sinensis*[18], *Nicotiana tabacum*[19], *Zea mays*[19] and onion epidermal cells[11]. This new reporter gene can be used to monitor *in vivo* gene expression, signal transduction, co-transfection, protein–protein interactions, cell separation, purification and cell lineage in higher plants; something now impossible with current reporter genes that require membrane impermeable substrates or cumbersome equipment.

GFP is suitable as a reporter gene for studying inducible gene expression in living cells. Various inducible promoters like heat-shock[17], light-inducible[17], ABA-regulated[17] and drought-inducible[11] promoters have been fused to GFP. The modified GFP will also be an invaluable reporter for monitoring plant responses to other environmental stimuli such as pathogens, wounding, touch, stresses as well as internal physiological metabolic and developmental activities in living cells and plants.

Ultrastructure and cytoplasm dynamics can be studied in whole mounts of plant cells illluminated by GFP using laser scanning microscope[16]. GFP has also been used to study various stages of pathogenesis during viral infection. For example, the movement of potato virus X (PVX) was monitored by replacing the coat protein genes by GFP[19]. Fusion of GFP to viral movement proteins determines the spread of virus[20,21]. GFP offers an excellent tracking system to monitor the spread of fitness-enhancing genes from transgenic crop plants to their wild relatives in the environment[22,23]. In this way, it will be of great help in protecting our natural ecosystems and sustainability of agriculture.

In higher animals also, GFP offers a wide range of applications. GFP has been successfully transfected into mouse embryos to generate transgenic green mice, without any toxic side effects[24,25]. Microinjection of GFP mRNA into blastocysts of mice enables to track the fate of cell lineage in the developing mouse embryo[26]. Injection of GFP-labelled tumour-forming cells into nude mice allowed the real time visualization of metastasis of tumours[27]. GFP under control of HIV-1 long terminal repeat when activated for transcription HIV-GFP reporter allows monitoring of viral titres and provides a system to screen anti-retroviral drug susceptibility[28,29]. A further application of GFP is in the identification of compounds that affect a particular enzyme activity of interest, for example GFP-based enzyme activity based assay for calcium-binding to calmodulin[30].

In conclusion, GFP is a sensitive and versatile tool of immense value in all areas of biological research (Table 3). This novel gene can be used for elucidating the mechanisms of gene regulation, signal transduction, cell biology, development and pathology in both plants and animals.

Table 2. Advantages of GFP

- Gene expression independent of cell type and location
- Detection simple and easy
- No exogenous substrate or sophisticated equipment required
- Small molecular size
- No adverse effect on cell metabolism
- Colour of light may be changed

Table 3. Applications of GFP cDNA

- Identification of transformed cells
- Measure gene expression *in vivo*
- Label and locate fusion proteins
- Study intracellular traffic
- Label unicellular organisms
- Label specific cell in multicellular organisms
- Elucidating ultra structure of cells
- Unravelling different events during developmental stages and fate of cell lineages
- Tracking the spread of transgenes in the environment
- Study stages of pathogenesis

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SCIENTIFIC CORRESPONDENCE

A tektite fragment discovered in the Central Indian Basin

On the basis of various lines of evidence[1,2], tektites are now considered as glassy objects generated by meteorite impacts, distributed in four known strewn fields of which, the Australasian tektite strewn field is the youngest (0.77 Ma) and the largest encompassing 1/10 of the Earth's surface[3]. Tektites of this strewn field exhibit morphological differences in different geographic domains, such as the blocky, layered Muong Nongtype tektites in the Indo-China region in the north, splash form types in the centre and the aerodynamically ablated forms in Australia in the south[4]. Although microtektites, which are microscopic counterparts of tektites (< 1 mm) have been found in over 40 sites in the oceans[5], its occurrences are known only on land but for rare exceptions[6,7]. We report here the finding of a tektite fragment from the surface sediments of the Central Indian Basin (CIB).

After the discovery of the australite button in the ocean floor of the CIB[7], we embarked upon a massive search of the samples collected for the Indian Polymetallic Nodule Programme during the last decade. We searched samples from 9507 operations of free fall grabs, located between 1° and 21°S and 70° to 88°E in the CIB. This tektite was discovered in a grab sample from station 74 A of the second cruise of *MV Skandi Surveyor* (23 Sept.–6 Nov. 1982) at the location 17°00′S; 78°001′E (depth 4990 m) (Figure 1). Along with the tektite, ~ 250 g of small (< 2 cm average size) manganese nodules have been recovered, and since the grab sampler (sampler penetration 15 cm) has a net of 6 mm mesh, the associated sediment has been filtered away during the ascent of the grab to the sea surface, effectively no sediment from this location was recovered.

A ~ 4 mm piece of the tektite was broken off, mounted in epoxy, polished and electron microprobe analysis was carried out with a Cameca CAMEBAX model 571 electron probe microanalyser for seven spots (Table 1) using silicate and oxide standards. ZAF corrections were made with an online computer using the method of Bence and Albee[8]. A synthetic tektite standard (USNM 2213) made by Corning Glass Company and analysed by the USGS which was kindly provided by the Smithsonian Institution (Washington), was also analysed to check the accuracy of analysis. Scanning electron microscopy was done with a JEOL JSM-T330A electron microscope.

This tektite fragment measures 14.5 × 7.5 mm, and weighs 0.44 g. It appears to be a part of a large tektite which fragmented while it was hot, possibly upon contact with the cool sea surface. It has a 'ledge' around it having sharp edges, outlining the border from which it was fragmented from the original tektite (Figure 2 *a*). The smooth exterior has a convex shape and represents the original tektite surface (Figure 2 *b*). The interior of the tekitite is uneven, has conchoidal fracturing and the overhanging ledge shows flow lines at the equatorial region which could be schlieren exposed