Luminescence 2002;17:43–74
DOI: 10.1002/bio.676

REVIEW

Imaging of light emission from the expression of luciferases in living cells and organisms: a review

Lee F. Greer III and Aladar A. Szalay*

Department of Biochemistry, School of Medicine and Department of Natural Sciences—Biology Section; Loma Linda University, Loma Linda, CA, USA

Received 13 August 2001; accepted 16 October 2001

ABSTRACT: Luciferases are enzymes that emit light in the presence of oxygen and a substrate (luciferin) and which have been used for real-time, low-light imaging of gene expression in cell cultures, individual cells, whole organisms, and transgenic organisms. Such luciferin–luciferase systems include, among others, the bacterial *lux* genes of terrestrial *Photorhabdus luminescens* and marine *Vibrio harveyi* bacteria, as well as eukaryotic luciferase *luc* and *ruc* genes from firefly species (*Photinus*) and the sea panzy (*Renilla reniformis*), respectively. In various vectors and in fusion constructs with other gene products such as green fluorescence protein (GFP; from the jellyfish *Aequorea*), luciferases have served as reporters in a number of promoter search and targeted gene expression experiments over the last two decades. Luciferase imaging has also been used to trace bacterial and viral infection *in vivo* and to visualize the proliferation of tumour cells in animal models. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: luciferases; gene expression; low-light imaging; luciferase expression constructs

INTRODUCTION

From time immemorial, seamen and fishermen have observed 'lights' on the water. In the nineteenth century it was realized that the most frequent cause of such luminous oceanic phenomena are minute marine organisms emitting light—bioluminescence. About 35 years ago, various luciferases began to be characterized (1, 2) which, in their many forms, in the presence of a substrate, a luciferin, emit light in the visible range under physiological conditions. Some eukaryotic organisms, such as the firefly (*Photinus*), have their own luciferin–luciferase light-emitting systems. Many marine organisms, however, such as mid-depth fishes and invertebrates such as molluscs, emit light because of symbioses with luciferase-producing bacteria occurring in highly specialized light organs. These luminescent bacteria include taxa such as *Photobacterium phosphoreum, P. leiognathi, Vibrio logei, V. harveyi* and *V. fischeri*.

It is to be expected that a costly characteristic like biological production of light would be retained only if luminescent visualizing were advantageous. Bioluminescence is used as a disguise for fleeing prey, for ventral light emission to efface an organism's shadow and render it invisible from below (3, 4), for luring prey (ceratioid fish), for signalling for courtship and mating, and in stress-induced light emission (bioluminescent plankton). One could argue that ever since such metazoan bioluminescent bacteria symbioses and other bioluminescent organisms appeared in the oceans with their unique light emission systems, there has been *in vivo* luminescent 'imaging' or visualization.

*Correspondence to: A. A. Szalay, Department of Biochemistry, School of Medicine and Department of Natural Sciences—Biology Section; Loma Linda University, Loma Linda, CA 92354, USA.
Email: aszalay@som.llu.edu

Contract/grant sponsor: Dept. Natural Sciences, Loma Linda University, CA, USA.
Contract/grant sponsor: Basic Science Research Grant from Loma Linda University, USA.

NATURAL LUMINESCENT 'VISUALIZATION'

Marine bioluminescence may be considered one of the most widespread forms of communication on the planet. Organisms emit light that other organisms detect or 'visualize' and to which they give some behavioral response (5). Behavior based on natural bioluminescence imaging may be classified under three general headings (5): offence (luring, baiting); defence (startle, camouflage); and communication (courtship and mating). Some striking uses of natural bioluminescent 'visualization' include the following: some squids with bacterial symbionts use shadow-effacing, or modulation of their ventrally-emitted light, to match ambient sunlight or moonlight; crustaceans, similar to fireflies, may use a repetitive mating 'Morse code' of blinks; some jellyfishes deposit an adhesive glow upon contact with predators, leaving the predator visible and vulnerable; some squids flee, leaving a luminescent cloud of 'ink' in a predator's face; some dragonfishes (Malacosteidae) emit blue-green light, but also emit a 'night-vision' long-wavelength red light by which they can

detect prey (using reverse fluorescence energy transfer) without their prey seeing them (6–12).

The purpose here is to review the representative scientific imaging applications to which these naturally occurring visible light bioluminescent systems, the genes encoding the proteins and their modifications have been put. However, we first present an overview of the luciferin–luciferase light emission systems.

PHYLOGENY AND EVOLUTION

Luciferase is a generic name because none of the major luciferases share sequence homology with each other (5). Luciferases occur in bacteria, fungi, dinoflagellates, radiolarians and about 17 metazoan phyla and 700 genera, mostly marine (5, 12, 13). These include Annelida (segmented worms), Chordata (some elasmobranchiomorphs or sharks, many teleosts or bony fishes), Cnidaria (jellyfishes, anthozoans such as the sea pansy, *Renilla*), Chaetognaths (one species of arrow-worm), Crustacea (many, including ostracods and euphausiid shrimps or krill), Ctenophora (comb jellies), Echinodermata (sea stars, brittle stars), hemichordate worms, Insecta (fireflies, click beetles), Mollusca (squids, octopods, nudibranchs), Nemertean worms (one species), Pycnogonids (sea spiders), Urochordata (larvaceans, pyrosomes, and one tunicate), millipedes and centipedes (12). Phylogenetic analyses suggest that luciferin–luciferase systems have had more than 30 independent origins (5, 14–16).

LUCIFERIN–LUCIFERASE–PROTEIN LIGHT-EMITTING SYSTEMS

Bioluminescence is a chemiluminescent reaction between at least two molecules produced under physiological conditions within or in association with an organism. The substrate molecule reacted upon, which emits light in such a reaction, is called a luciferin. Luciferases are a wide range of enzymes that catalyse the oxidation of substrate luciferins to yield non-reactive oxyluciferins and the release of photons of light (17–21). As luciferin substrates are used, they must be replenished, which usually occurs through diet. Some luciferins require the presence of a co-factor to undergo oxidation, such as $FMNH_2^+$, Ca^{2+} or ATP (22). Complexes that contain a luciferase, a luciferin, and generally requiring O_2 are also called photoproteins (12).

Although luciferin–luciferase bioluminescence is found in hundreds of taxa across many phyla, there are five basic luciferin–luciferase system (12):

- Bacterial luciferin is a reduced riboflavin phosphate ($FMNH_2$) that is oxidized by a luciferase in association with a long-chain aldehyde and an oxygen molecule. It is found in luminescent bacteria, certain fish, pyrosomes, and in some squids (e.g. *Euprymna*).
- Dinoflagellate luciferin resembles, and may be derived from, the porphyrin of chlorophyll. In the dinoflagellate *Gonycaulax*, this luciferin is conformationally shielded from luciferase at the basic pH of 8 but becomes free and accessible to oxidation near the more acidic pH of 6. A modification of this luciferin occurs in a herbivorous euphausiid shrimp, where it is apparently acquired by ingestion.
- Another luciferin, from the marine ostracod *Vargula*, is called vargulin. It also seems to be acquired by ingestion. It is also found in some fish species.
- Coelenterazine is the most widely known luciferin. It occurs in cnidarians, copepods, chaetognaths, ctenophores, decapod shrimps, mysid shrimps, radiolarians, and some fish taxa. Coelenterate luciferase activity is controlled by the concentration of Ca^{2+} and shares homology with the calcium-binding protein calmodulin (5).
- Firefly luciferin (a benzothiazole) is found exclusively in fireflies (*Photinus* or *Luciola*). It has the unique property of requiring ATP as a co-factor to convert it to an active luciferin (5). It was realized early that firefly luciferin–luciferase could be used to determine the presence of ATP (23). This has become a standard ATP assay. For one example, since nickel alloys have been shown to have an adverse effect on respiratory metabolism in eukaryotic cell lines, the firefly luciferin–luciferase system has been used to document depressed levels of ATP in cells exposed to the alloys (24).

The mechanisms of bioluminescence utilized by amphipods, bivalves, earthworms, fresh-water limpets, fungus gnats, larvaceans, nemertean worms, polychaete worms and tunicates are currently unknown. Luciferin–luciferase bioluminescence systems are multiform phenomena and polyphyletic in origin.

GENES AND cDNAs ENCODING DIFFERENT LUCIFERASES

Science has entered into the field of bioluminescent visualization in far more recent times. In the last few decades, many luciferase genes have been isolated, sequenced at least in part, and used to build DNA vectors. In Table 1 we summarize the DNA fragments and cDNAs that encode the different luciferases significant in scientific imaging.

The luciferases most commonly used in experimental bioluminescent imaging applications include the bacterial

Table 1. A summary of known luciferase genes, cDNAs, and proteins. Among these are the prokaryotic luciferases (Lux), eukaryotic luciferases (Luc, Ruc and their regulatory proteins) both of which are commonly used in imaging of luciferase expression in living cells, tissues, and organisms

Taxa	Gene – cDNA (size in bp)	Protein product (size in number of amino acids)	Gen Bank accession no. (DNA and amino acid)	Reference
Vibrio harveyi	*luxA*, 1067 bp	α subunit, 355 aa	M10961 AAA88685	32
Vibrio harveyi	*luxB*, 947 bp	β subunit, 324 aa	M10961.1 AAA88686	223
Vibrio harveyi	*luxE*, 1136 bp	acyl-protein synthetase, 378 aa	M28815.1 AAA27531	223
Vibrio fischeri	*luxA*, 1064 bp	alkanal mono-oxygenase α-chain, 354 aa	X06758 CAA29931	224
Vibrio fischeri	*luxB*, 980 bp	alkanal mono-oxygenase β-chain, 326 aa	X06797 CAA29932	224
Vibrio fischeri	*LuxRICDABEG* operon		AF170104	Knight T, Papadakis N. *Vibrio fischeri lux* operon *Sal I* digest (unpublished—direct submission to GenBank, 1999)
	• *luxR*, 752 bp	• regulatory protein LuxR, 250 aa	• AAD48473	
	• *luxI*, 581 bp	• autoinducer synthesis protein LuxI, 193 aa	• AAD48474	
	• *luxC*, 1439 bp	• acyl-CoA reductase LuxC, 479 aa	• AF170104	
	• *luxD*, 923 bp	• acyl transferase LuxD, 307 aa	• AAD48476	
	• *luxA*, 1077 bp	• alkanal mono-oxygenase α-chain LuxA, 354 aa	• AAD48477	
	• *luxB*, 993 bp	• alkanal mono-oxygenase β-chain LuxB, 326 aa	• AAD48478	
	• *luxE*, 1136 bp	• long chain fatty acid luciferin component ligase LuxE, 378 aa	• AAD48479	
	• *luxG*, 722 bp	• probable flavin reductase LuxG, 236 aa	• AAD48480	
Photorhabdus luminescens = *Xenorhabdus*; since 1999 reduced to synonymy (225)	*LuxCDABE* operon		M62917	34
	• *luxC*, 1442 bp	• fatty acid reductase LuxC, 480 aa	• AAA63563	
	• *luxD*, 923 bp	• acyl transferase LuxD, 307 aa	• AAA63564	
	• *luxA*, 1088 bp	• alkanal mono-oxygenase α-chain LuxA, 362 aa	• AAA63565	
	• *luxB*, 974 bp	• alkanal mono-oxygenase β-chain LuxB, 324 aa	• AAA63566	
	• *luxE*, 347 bp	• acyl-protein synthetase LuxE, 116 aa	• AAA63567	

Table 1. Continued

Taxa	Gene – cDNA (size in bp)	Protein product (size in number of amino acids)	Gen Bank accession no. (DNA and amino acid)	Reference
Photinus pyralis	*luc*, 2387 bp	Luciferase, 550 aa	M15077 AAA29795	226
Luciola cruciata	*luc*, 1985 bp	Luciferase, 548 aa	M26194 AAA29135	227
Vargula hilgendorfii (sea firefly)	• *vuc*, 1834 bp • *vuc* mRNA, 1818 bp	Vargulin, 611 aa Vargulin, 555 aa	E02749 M25666 AAA30332	228 39
Aequorea victoria	*aeq1*, 672 (590) bp *aeqprec*, 568 bp *aeq2*, 531 bp *aeq3*, 531 bp *aq440*, 925 bp *aqua*, 587 bp	Aequorin 1; calcium-binding protein, 196 aa Aequorin precursor, ~189 aa Aequorin 2, 177 aa Aequorin 3, 177 aa Aequorin, calcium binding - 196 aa Luminescent protein Aqualine, ~196	M16103 AAA27719 M11394 AAA27716 M16104 AAA27717 M16105 AAA27718 L29571 AAA27720 E02319	 229 230 231
Oplophorus gracilorostris	*luc*, 590 bp *luc*, 1079 bp	Oplophorin, oxygenase, imidazopyrazinone luciferase, 196 aa Oplophorin, oxygenase, imidazopyrazinone luciferase ~359 aa	AB030246 BAB13776 AB030245 BAB13775	52
Renilla muelleri	*ruc*, 1208 bp	*Ruc*, 311 aa	AY015988 AAG54094	Szent-Gyorgyi CS, Bryan BJ. cDNA encoding *Renilla muelleri* luciferase (unpublished manuscript).
Renilla reniformis	*ruc*, 1196 bp	*Ruc*, oxygenase, 311 aa	M63501 AAA29804	59

luciferases (*lux*) from the marine genera *Photobacterium* and *Vibrio*, firefly luciferase (*Photinus*), aequorin (luciferase from the jellyfish *Aequorea*), vargulin (luciferase from the marine ostracod *Vargula*), oplophoran luciferase (deep-sea shrimp *Oplophorus*) and *Renilla* luciferase (anthozoan sea pansy, *Renilla reniformis*).

- *Bacterial luciferase*. Bacterial luciferase proteins were purified and isolated from the light organs of mid-depth fishes in the ocean (25, 26). It was known early that the catalytic site was on the α subunit (27). Belas *et al.* (1982) isolated and expressed luciferase genes from *Vibrio harveyi* in *E. coli* (28). Olsson *et al.* (1988) characterized the activity of the LuxA subunit of *Vibrio harveyi* luciferase by visualizing various *luxA* and *luxB* truncations, as well as a *luxAB* fusion expressed in *E. coli* (29). Olsson *et al.* (1989) furthermore made monomeric luxAB fusions and expressed them also in *E. coli* (30). The *Vibrio harveyi luxA* and *luxB* cDNAs were cloned and sequenced in the mid-1980s (31–33). The *luxCDABE* operon from the terrestrial bacterium *Photorhabdus luminescens* was cloned and sequenced and its product, luciferase, was characterized and published in 1991 (34).
- *Firefly luciferase*. The active sites and properties of firefly luciferase (*Photinus*) began to be characterized about 35 years ago (35–37). Firefly luciferase was purified and characterized in 1978 (19). The cDNA encoding the luciferase (*Luc*) from the firefly *Photinus pyralis* was cloned and expressed in *E. coli* by De Wet *et al.* (1985) (38).
- *Vargulin*. A cDNA for the luciferase gene from the marine ostracod *Vargula hilgendorfii* was cloned, sequenced and expressed in mammalian cells by Thompson *et al.* (1989) (39). They also discovered that *Vargula* luciferase expression requires only its substrate and molecular oxygen (but no co-factors), thus making it potentially more useful for mammalian expression systems (40). The activity of *Vargula* luciferase is not dependent on a pyrazine structure, as has been demonstrated by cross-reaction experiments with the *Oplophorus* luciferin (41).
- *Aequorin*. The aequorin protein was first extracted from the hydromedusa *Aequorea*, purified and characterized in part by Shimomura *et al.* (1962) (42). In 1975, Shimomura and Johnson described what was known about the mechanisms of various coelenterate luciferins, including aequorin (22). Ward and Cormier (1975) reported the isolation of various *Renilla*-type luciferins, including aequorin (43). A few years later, it was discovered that *Renilla* luciferin analogues were catalysed by luciferase to excited energy states to transfer energy to a green fluorescence protein or GFP (44). Ward and Cormier (1979) characterized the *Renilla* green fluorescence protein (RGFP) and showed that a natural energy transfer was occurring from the isolated *Renilla* luciferase (Ruc) bioluminescence to RGFP (45). In 1985, the cDNA for aequorin was cloned, sequenced and expressed in heterologous systems (46, 47). The aequorin gene from the jellyfish *Aequorea victoria* was cloned in 1990 (48). It is now known that many cnidarians have GFPs that serve as energy-transfer acceptors fluorescing in response to excited oxyluciferin–luciferase complexes or to a Ca^{2+}-activated phosphoprotein. The cDNA encoding the GFP of *Aequorea victoria* has also been cloned and sequenced (49).
- *Oplophorus luciferase*. The general reaction mechanisms and properties of the luciferin–luciferase system of the deep-sea shrimp *Oplophorus gracilorostris* were reported by Shimomura *et al.* (1978) (50). An empirical formula and structure has been suggested for *Oplophorus* luciferin using spectroscopy and cross-reaction with the luciferase of the ostracod *Vargula hilgendorfii* (40). By 1997, *Oplophorus* luciferase was known to have a more intense light emission than either *Renilla* luciferase or the recombinant aequorin. However the *Oplophorus* luciferase cDNA, not yet cloned, could not be used as a reporter gene (51). Recently, Inouye *et al.* (2000) succeeded in cloning the *Oplophorus* luciferase cDNA (52).
- *Renilla luciferase*. In 1966, Hori and Cormier described some of the properties and a hypothetical partial structure for the *Renilla reniformis* luciferase protein (Ruc) (53). Kreis and Cormier (1967) showed that light could inhibit the activity of Ruc (54). The isolation of Ruc was first done and further properties elucidated by Karkhanis and Cormier (1971) (55). DeLuca *et al.* (1971) demonstrated that the *Renilla* bioluminescent system involves the oxidative production of CO_2 (56). It was further shown that Ca^{2+} triggered a luciferin binding protein, thus inducing the Ruc system (57). Ruc was first purified and characterized by Matthews *et al.* (1977) (58). The cDNA of *ruc* was isolated and later expressed in *E. coli* by Lorenz *et al.* (1991) (59). The *ruc* cDNA was also expressed in a number of transgenic plant tissues (60). In 1996, Lorenz *et al.* expressed Ruc in simian COS-7 cells and in murine C5 cells (61).

In retrospect, it might be noted that since their discovery, Luc (*Photinus*), aequorin (*Aequorea*) and GFP have been used in a multitude of successful experiments. In combination the three have even been useful in assaying or imaging the spatial–temporal concentrations of Ca^{2+} (62). Combinations of multiple

Table 2. A summary of selected luciferase constructs and vectors useful for imaging. (Construct/vector nomenclature not standardized in the literature).

Construct/vector	Luciferase genes or cDNAs	Promoters/enhancers	Organism/cells	Substrate requirement	Imaging application and reference
pB101; pB102; pB105; pB110; pB123; pB128; pGMC12	*luxA*, *luxB* (*Vibrio harveyi*)	Phage λ promoters P_L and P_R	*Escherichia coli*	Decanal	Expression of *luxA*, *luxB* in *E. coli* (28)—first transgenic expression of *lux*
Transposon mini-*Mu*lux	*luxE*, *B*, *A*, *D*, *C* (*Vibrio parahaemolyticus*)	*lac* promoter	*E. coli*	None	Visualization of *Vibrio lux* genes in *E. coli* (26)
pFIT001; pPALE001	*luxA*, *luxB* (*V. harveyi*)	Anti-Tet (P1), *nifD*, *nifH* promoters	*E. coli; Bradyrhizobium japonicum in Glycine max*	Decanal	Visualization of N-fixation in soybean nodules via *Bradyrhizobium japonicum* (210)
Agrobacterium binary vector	*luxA* & *B* (*V. harveyi*)	T_L promoter	*Daucus carota; Nicotiana tabacum*	Decanal	Visualization of tissue-specific chimaeric *lux* expression (169)
pDO432; pDO435; pDO446; pDO445	*luc* (*Photinus*)	*CaMV 35S* RNA promoter	*Nicotiana tabacum*	*Photinus* luciferin and ATP in solution (topically delivered)	Visualization of *luc* expression in tobacco plants (168)
pPCV701luxA & B	*luxA*; *luxB* (*V. harveyi*)	T_R-DNA P^1 and T_R-DNA P^2 *mas* promoters	*Nicotiana tabacum; Daucus carota via Agrobacterium tumefaciens*-mediated gene delivery	Decanal (injected) Decanal + $FMNH_2$	Assembly and expression of functional luxA and B genes in plants (170)
pRSVL	*luc* but imaged by immunoflurescence	Promoter in Rous Sarcoma Virus long terminal repeat (RSV LTR)	Monkey kidney cells (CV-1)	NA	Luciferase peroxisomal localization visualized by immunofluorescence (109)
pMRP1; pMRD2	*luxAB* fusion	*P1* promoter	Soybean (*Glycine max*)	Decanal	Successful imaging of LuxAB fusion expression in soybean root nodules using photographic film and low-light intensified video microscopy (211)
pSCLUC →, pSCLUC ⟶, rVV-luc	*luc*	7.5 kDa viral promoter, Vaccinia *tk* gene fragments	BSC-40 cells (African green monkey kidney cells)	Firefly luciferin	Film imaging of recombinant vaccinia infection of monkey cells (212)
pLX vector series	*luxA*; *luxB*; *luxAB* (*luxF*)	*T7* promoter	BE21(DE3) cells	Decanal	Imaging of various *lux* truncations and LuxF fusion in cells (29)
pRS1105	*luxA*, *luxB* (*V. harveyi*)	EndoH, *bldA* (leu tRNA), *WhiG* (uncharacterized), *SapA* (all *Streptomyces* promoters)	*Streptomyces coelicolor*	Decanal	Visualizing *luxA* & *B* expression in *Streptomyces* (91)
pLX, pICLX, pCV702 and p35Slux vector series	*luxAB*; *luxBA* fusions	*T7* gene 10 and *CaMV 35S* promoter	*E. coli*; tobacco calli	Decanal	Showed that a LuxAB fusion is much more active than LuxBA (30)
pLX vector series	*luxAB*	*T7* gene 10 promoter	*E. coli*	Decanal	Bacterial luciferase $\alpha\beta$ fusion functional as a monomer (65)
pMW41	*luc*	*CMV* intermediate–early enhancer/promoter; trans-activated by *HIV-1 Tat* protein	COS-7 cells	Luciferin (firefly) and derivatives: ethoxyvinyl ester, 2-hydroxyethyl ester, 3-hydroxy-*n*-propyl ester, ethyl ester	Visualization of Luc expression in individual mammalian cells (112)
pSV2-vl	*luc* (*Vargula* luciferase)	SV40 promoter	CHO cells	Luciferin	Visualization of luc secretion in CHO cells real time using an image-intensifying technique (114)
pPCVGLuxA and B	*luxA* and *B*	Promoterless *luxA* pCaMV 35S RNA (*luxB*), i.e. promoter search vector assay	Tobacco	Decanal	Imaging of constitutive and organ-specific Lux expression in transgenic tobacco (172)
pAM1224; psbAI::luxAB construct	*luxAB* (*V. harveyi*)	*luxAB* promoterless, (to be inserted downstream of cyanobacterial promoter); *psbAI*, *psbAIII* and *purF* promoters	*Synechococcus* sp., vectored by conjugation with *E. coli*	Decanal	Cooled-CCD detection and documentation of circadian rhythms in cyanobacteria (98–100)
pPCVG luxA and B	*luxA* and *B* (*V. harveyi*)	T-DNA promoter search vector; *luxA* promoterless, but enhanced by CaMV 35S promoter in front of *luxB*	*Agrobacterium tumefaciens* T-DNA-mediated transfer of lux into transgenic *Nicotiana tabacum cv. SR1* (especially root stem, leaf and flower tissues)	Decanal	Imaging used to locate developmentally regulated promoters in tobacco (96)
pWH1520–xylA–luxF (*Bacillus* Gram positive) expression vector	*luxAB* (*luxF*; *V. harveyi*)	Xylose-inducible promoter–*luxF* fusion	*XylA–luxF*-transformed *B. thuringiensis* injected into *Manduca sexta* arvae (6th instar of the tobacco hornworm)	Decanal	Use of photon-counting to visualize (13): • Inducible *luxF* expression from *B. thuringiensis* and *B. megaterium* infection in lepidopteran insect larvae and environment (and of *luxF* in *E. coli*)

NA, not applicable.

Table 2. Continued

Construct/vector	Luciferase genes or cDNAs	Promoters/enhancers	Organism/cells	Substrate requirement	Imaging application and reference
Recombinant AcNPV–luc (*Autographica californica* nuclear polyhedrosis virus with Pluc)	*luxF* (*V. harveyi*); Pluc	*Arabidopsis* phenylalanine ammonia lyase (*PAL*) promoter–*luxF* gene fusion; AcNPV polyhedron late promoter (pNI101)–*luc*	Recombinant AcNPV–*luc*-infected *Trichoplusia ni* (386) cells	Decanal; luciferin (firefly)	Use of photon-counting imaging to visualize (93): • AcNPV–*luc* expressing plaques in *T. ni* cells
pPCVGluxA and B	*luxA* and *luxB* (*V. harveyi*)	*luxA* promoterless, but enhanced by CaMV 35S promoter in front of *luxB* (i.e. promoter search vector)	*Agrobacterium tumefaciens* T-DNA-mediated transfer of *lux* into transgenic *Nicotiana tabacum* cv. *SR1* for expression in response to vir and avir infection by *Pseudomonas syringae*	Decanal	Use of photon-counting imaging to visualize (93): • Promoter search expression of LuxA and LuxB in *N. tabacum*
pPCVGluxA and B	*luxA* and *luxB* (*V. harveyi*)	*luxA* promoterless, but enhanced by *CaMV 35S* promoter in front of *luxB* (i.e. promoter search vector)	*Agrobacterium tumefaciens* T-DNA-mediated transfer of *lux* into transgenic *Arabidopsis thaliana* (RLD) for expression in response to *vir* and *avir* infection by *Pseudomonas syringae*	Decanal	Use of photon-counting imaging to visualize (93): • Promoter search expression of LuxA and B in *N. tabacum*
pLTu–luxF	*luxF* (*V. harveyi*)	*Craterostigma plantagineum* drought and ABA-regulated promoter–*luxF*	*Agrobacterium tumefaciens* T-DNA-vectored *lux* in transgenic *Nicotiana tabacum* cv. *SR1*	Decanal	Use of photon-counting imaging to visualize (93): • Stress and ABA-induced LuxF expression in *N. tabacum*
pPCV701–luxAB	*luxAB* (*V. harveyi*)	*Agrobacterium tumefaciens* auxin-regulated mannopine synthase bi-directional promoters (mas P_1 and P_2)	Transgenic *Nicotiana tabacum* cv. SR1	Decanal	Photon-counting visualization of auxin-induced activation of *mas* promoters of *luxAB* in transgenic tobacco (94)
pWH1520–xylA–luxF, called pWH1520SF; pLX703-fab9	*luxAB* (*V. harveyi*)	*B. megaterium* xylose isomerase gene (*xylA*) promoter	*lux*-transformed *Bacillus thuringiensis* and *B. megaterium*	Decanal	Measured xylose induction of *xylA–luxAB* in transformed *Bacillus thuringiensis* and *B. megaterium* (95)
Bacterial prokaryotic promoter search vector: 35 bp luxF–Ori_T–Ori_V–NPT2–transposase from TN5 with kan^r—35 bp	*luxF* (*V. harveyi*)	Random prokaryotic promoter regions	*E. coli*	Decanal	Image-intensified low-light single-photon video imaging (and X-ray autoradiography) of transgenic organisms using bacterial luciferases (97): *E. coli*
Vector containing Gal4 promoter–luxF	*luxF* (*V. harveyi*)	*Gal4* promoter	*Saccharomyces cerevisiae*	Decanal	Image-intensified low-light single-photon video imaging (and X-ray autoradiography) of transgenic organisms using bacterial luciferases (97): yeast
Transgenic rhizobia (bacteriods) containing rhizobium nitrogenase P1 promoter–luxF linkage	*luxF* (*V. harveyi*)	Constitutive *P1* promoter	*Glycine max* (soybean plant) nodules infected with transgenic *Bradyrhizobium*	Decanal	Image-intensified low-light single-photon video imaging (and X-ray autoradiography) of transgenic organisms using bacterial luciferases (97): transgenic rhizobium in soybean nodules
Plant expression vector: L_B–p–NPT II (neomycin phosphotransferase II)–pA–p–luxF–pA–R_B pWH1520SF–xylR–luxAB	*luxF* (*V. harveyi*)	Auxin-activated mannopine bidirectional *1′*, *2′* (*mas*) promoters; nopaline synthase promoter	*Agrobacterium*-delivered expression vector into explants of *Nicotiana tabacum*	Decanal	Image-intensified low-light single-photon video imaging (and X-ray autoradiography) of transgenic organisms using bacterial luciferases (97): transgenic tobacco
pCV701–luxF	*luxF* (*V. harveyi*)	Auxin-activated *mas* promoter	*Agrobacterium*-delivered expression vector into *Lycopersicon esculentum* (transgenic tomato tissues)	Decanal	Image-intensified low-light single-photon video imaging (and X-ray autoradiography) of transgenic organisms using bacterial luciferases (97): transgenic tomatoes
Plant promoter search vector B: *Agrobacterium* L_B–luxA–NOS_{pA}–a $P_{CaMV\ 35S}$–luxB–NOS_{pA}–P_{NOS}–HPT–$g7_{pA}$–Ap^r–R_B	*luxAB* (*V. harveyi*)	Auxin-activated *mas 1′*, *2′* promoters	*Agrobacterium*-delivered expression vector into *Solanum tuberosum* (potato)	Decanal	Image-intensified low-light single-photon video imaging (and X-ray autoradiography) of transgenic organisms using bacterial luciferases (97): transgenic tomatoes

NA, not applicable.

Table 2. Continued

Construct/vector	Luciferase genes or cDNAs	Promoters/enhancers	Organism/cells	Substrate requirement	Imaging application and reference
Plant promoter search vector A: *Agrobacterium* T–DNA left border, L_B–luxF–OS_{pA}–P_{NOS}–HPT–NOS_{pA}–Ap^r–R_B	*luxF* (*V. harveyi*)	Auxin-activated *mas 1′*, *2′* promoters	*Agrobacterium*-delivered expression vector into *Solanum tuberosum* (potato)	Decanal	Image-intensified low-light single-photon video imaging (and X-ray autoradiography) of transgenic organisms using bacterial luciferases (97): transgenic tomatoes
Plant promoter search vector B: *Agrobacterium* L_B–luxA–NOS_{pA}–a $P_{CaMV\ 35S}$–luxB–NOS_{pA}–P_{NOS}–HPT–$g7_{pA}$–Ap^r–R_B	Mas promoter–*luxAB* (*V. harveyi*) fusion	Auxin-activated *mas 1′*, *2′* promoter; *CaMV 35S* promoter	*Agrobacterium*–delivered expression vector into *Lycopersicon esculentum* (transgenic tomato fruit)	Decanal	Image-intensified low–light single-photon video imaging (and X-ray autoradiography) of transgenic organisms using bacterial luciferases (97): transgenic tomato fruit
Plant promoter search vector A: *Agrobacterium* T–DNA left border, L_B–luxF–OS_{pA}–P_{NOS}–HPT–NOS_{pA}–Ap^r–R_B	Mas promoter–*luxF* (*V. harveyi*) fusion	Auxin-activated *mas 1′*, *2′* promoter	*Agrobacterium*-delivered expression vector into *Nicotiana tabacum*	Decanal	Image-intensified low-light single-photon video imaging (and X-ray autoradiography) of transgenic organisms using bacterial luciferases (97): transgenic tobacco stems
Plant promoter search vector A: *Agrobacterium* T–DNA left border, L_B–luxF–OS_{pA}–P_{NOS}–HPT–NOS_{pA}–Ap^r–R_B	Mas promoter–*luxF* (*V. harveyi*) fusion	Auxin-activated *mas 1′*, *2′* promoter	*Agrobacterium*-delivered expression vector into *Datura stramonium*	Decanal	Image-intensified low-light single-photon video imaging (and X-ray autoradiography) of transgenic organisms using bacterial luciferases (97): transgenic *Datura*
Bacterial prokaryotic promoter search vector: 35 bp luxF–Ori_T–Ori_V–NPT2–transposase from Tn5 with kan^r—35 bp	*luxF* (*V. harveyi*)	Random prokaryotic promoter regions	*Pseudomonas solanacaerum* via conjugation with an *E. coli* bearing Tn5	Decanal	Image-intensified low-light single-photon video imaging (and X-ray autoradiography) of transgenic organisms using bacterial luciferases (97): transgenic *Pseudomonas*—visualizing
Plant promoter search vector B: *Agrobacterium* L_B–luxA–NOS_{pA}–a $P_{CaMV\ 35S}$–luxB–NOS_{pA}–P_{NOS}–HPT–$g7_{pA}$–Ap^r–R_B	Heterodimeric *luxAB* (*V. harveyi*)	Auxin-activated *mas 1′*, *2′* promoter or phenylalanine ammonia lyase (PAL) promoter	Transgenic *Solanum tuberosum* (potato) infected with *Erwinia herbicola* (avirulent), *E. carotovora* (virulent), *Pseudomonas syringae* strain tomato (avirulent)	Decanal	Image-intensified low-light single-photon video imaging (and X-ray autoradiography) of transgenic organisms using bacterial luciferases (97): transgenic *Pseudomonas*—monitoring the virulence of pathogen strains
pPCV701–luxAB	*luxAB* (*V. harveyi*)	Auxin-activated *mas 1′*, *2′* promoters	Transgenic *Solanum tuberosum* (potato) infected by *Pseudomonas syringae, Erwinia carotovora carotovora, Erwinia carotovora atroseptica, Erwinia herbicola*	Decanal	Image-intensified low-light single-photon video imaging (and X-ray autoradiography) of transgenic organisms using bacterial luciferases (97): transgenic potato
pWH1520SF–xylR–xylA–luxAB	*luxAB* (*V. harveyi*)–*xylA* promoter fusion	*xylA* promoter	*Bacillus thuringiensis* and *Bacillus megaterium*	Decanal	Image-intensified low-light single-photon video imaging (and X-ray autoradiography) of transgenic organisms using bacterial luciferases (97): *Bacillus*
AcNPV–luc	*luc*	AcNPV polyhedrin late promoter	*Autographa californica* nuclear polyhedrosis virus-vectored Luc in *T. ni* 368 cells and *Trichoplusia ni* 3rd instar larvae	Firefly luciferin	Image-intensified low-light single-photon video imaging (and X-ray autoradiography) of transgenic organisms using bacterial luciferases and firefly luciferase (97): *T. ni* 368 cells and *T. ni* 3rd instar larvae
	luxA and *B* (*V. harveyi*), *Fab9*, Fab_cb_c9	–	*E. coli*	Decanal	Imaging of overexpression of GroEL and GroES-mediated folding of Fab9–bacterial luciferase fusions at different temperatures (97): *E. coli*
pCEP4–luc	*luc*	*CMV* promoter	*Brachydanio rerio* (zebrafish)	Luciferin (firefly; 0.1 mmol/L)	Transgenic zebrafish with Luc visualized by low-light video-image analysis (195)
pPVC701–ruc	*ruc* (*Renilla reniformis* luciferase)	*pCaMV 35S* RNA	Alfalfa (*Medicago sativa*), tomato, potato, tobacco	Coelenterazine	Imaging of Ruc in tissues of transgenic plants (60)
pMW54; pMV53; pMV16; pOGS213	*luc*	*HIV-1*, *HIV-1 LTR* enhancer–promoter elements and *CMV* promoter	HeLa cells	Luciferin (firefly)	Visualization of HIV- and hCMV-promoted expression of Luc in single mammalian (HeLa) cells (139)
pCol.luc; pCMV.luc; pCMV.Aqm; pcDNAAIneo.CL100	*luc*; aequorin	Collagenase and *CMV* promoters	CHO.T cells	Coelenterazine and beetle luciferin (for Aeq and Pluc respectively)	High-intensity real-time photon-counting imaging of insulin-induced MAP kinase signaling in single cells (137)

NA, not applicable.

Table 2. Continued

Construct/vector	Luciferase genes or cDNAs	Promoters/enhancers	Organism/cells	Substrate requirement	Imaging application and reference
pGL101	*luxF* (luxAB fusion)	*Arabidopsis PAL1* (phenylalanine ammonia-lyase) promoter	*Arabidopsis thaliana*	Decanal	Successful photon-counting imaging of localized activation of PAL1 (187)
pCEP4	*ruc–gfp* fusion, and individually	*CMV* and human *β*-actin promoters	LM-TK$^-$ cells; murine embryonic stem (ES) cells growth-supported by STO feeder cells	Coelenterazine	Visualization of Ruc–GFP expression in ES cells and embryos (69)
pCEP4–Ruc; pCEP4–Ruc/GFP; pCEP4–GFP/Ruc; pGEM–5z(+)–Ruc/GFP; pGEM-5z(+)–GFP/Ruc	*ruc*; 'humanized' *gfp* (*Aequorea*)	*CMV*, *β*-actin, (for pCEP4 vector), and *T7* (pGEM-5zf$^+$ vector) promoters	LM-TK$^-$ (murine fibroblast cell line with thymidine kinase missing because of mutation)	Coelenterazine	Imaging Ruc–modified GFP fusion in murine cells (70)
Mitochondrially targeted aequorin vectors	*aeq* (*Aequorea*)	–	CHO.T cells	Coelenterazine	Imaged intramitochondrial Ca^{2+} in cells using recombinant aequorin with a CCD camera (138)
Recombinant baculovirus constructs BmNPVluc (*Bombyx mori* nuclear polyhedrosis virus) and AcNPVluc [2]	*luc*	[2] In reverse orientation to the *Autographa californica* nuclear polyhedrosis virus (AcNPV) polyhedrin viral promoter, possibly under ORF629 promoter	*Bombyx mori* N-4 and Sf 9 cells, *Trichoplusia ni* 368 cells	Luciferin (firefly)	Imaging of baculovirus-vectored Luc in insect cells (218)
pRLuc6 and pRLuc6.1; pMCT–Ruc	*ruc*	*AdV* major late promoter; *hCMV* intermediate–early enhancer/promoter	COS-7 cells; C5 cells	Coelenterazine	Imaging of Ruc expression in simian and murine cells (61)
Recombinant herpes/ pseudorabies virus PrV A916	*luc*	Glycoprotein *gG* early promoter	African green monkey kidney (VERO) cells	Luciferin	Visualizing of PrV infection in culture via Pluc using a photon- counting camera (217)
CHS::luc construct	*luc*	Fragment of chalcone synthase promoter; Ω translational enhancer of the Tobacco Mosaic Virus (TMV)	*A thaliana* seedlings (Columbia g/1) and in an *A. thaliana* C24 cell line	Luciferin	Comparisons between air-cooled CCD and intensified CCD cameras (178)
LTR–luc in pGL3	*luc*	*SV40* promoter and enhancer in LTRs	Neonatal rats (*Rattus*); mice (*Mus*); human T cells	Luciferin (firefly)	Induction of SV40 promoter–luciferase expression in neonatal rats, in mice/and in human T-cells (157)
pLPKLuc$_{FF}$, truncated pΔL4–LPKLuc$_{FF}$	*ruc*	L-pyruvate kinase normalized by constitutive *CMV* promoter, requiring upstream stimulatory factor2 (USF2)	Human islet *β*-cells, derived INS-1 cells	Beetle luciferin, eoelenterazine	Single-cell CCD imaging of Ruc-marked necessary upstream stimulatory factor activity (136)
pCK218	*luxAB* (*Vibrio fischeri*)	Promoterless in *V. fischeri*; unidentified strong constitutive promoter in *Pseudomonas putida*	*Vibrio fischeri* MJ-1; *lux*-marked *Pseudomonas putida*	Decanal	Comparison of single- bacterium low-light imaging using a cryogenically-cooled CCD camera and a photon-counting camera (141)
pTKEluc; pTK6WEluc; pSVEluc; pMiwluc; pMiwEluc	*luc*	*6W* enhancer, *TK*; *SV40*; RSV (Rous Sarcoma Virus) LTR, *β*-actin promoters	Bovine embryos	Luciferin (firefly)	Detection of light emission from Luc in transgenic bovine embryos (200)
–	*ruc* (*Renilla reniformis*)	Promoterless Ruc expression–promoter search vector	Transgenic *Arabidopsis, Tabacum* or other plant calli, roots, leaf, stem, flower tissue; potato tubers; sedimented transformed plant protoplasts (*lux*, *ruc*); transgenic	Decanal; luciferin 2-benzyl coelenterazine	Imaging of plant promoter expression using Lux and Ruc reporters in plant cells (182)
pSP–Luc ± phPRL	*luc*	*hPRL* (human prolactin) promoter	Rat pituitary tumour GH3 cells	Luciferin (firefly)	CCD photon-counting imaging of PRL promoter activation of Luc in individual cells (121)
RD29A–Luc construct	*luc*	*RD29A*; *COR47*; *COR15A*; *KIN1*; *ADH*; *RAB18*; *RD22*; *RD29B*; *LUC*; actin—all cold sensitive gene promoters	*Arabidopsis thaliana*	Luciferin (firefly)	Visualized mutant seedlings with mutant cold-response gene *HOS-1* (181)
Synthetic RBCSB gene	*luc* (promoterless in construct)	*RBCSB* promoter juxtaposed upstream of *luc* by cross-over	*Arabidopsis thaliana*	Luciferin (firefly)	Visualized cross-over seedlings (184)
p260Ins.Luc$_{FF}$; pF711fo.Luc$_{FF}$; pCMV–Ren	*luc*; *ruc*	*SRE* and *CRE* of the human insulin promoter, *Herpes simplex* minimal *TK*, *c-fos* and *CMV* promoters	MIN6 *β*-cells; CHO cells; anterior pi tuitary-derived AtT20	Luciferin (firefly); coelenterazine	Real-time intensified CCD camera imaging of constitutive glucose enhancement of insulin promoter-activated luciferase activity (123)
pcLuc (non-targeted, cytosolic luciferase); pmLuc (plasma membrane targeted luciferase)	*luc*	*CMV* and *SV40* intermediate–early promoters	Primary rat islet *β*- cells; derived MIN6 cells	Luciferin (firefly)	Luciferase photon-counting imaging of subcellular compartmentalization of ATP (124)

NA, not applicable.

Table 2. Continued

Construct/vector	Luciferase genes or cDNAs	Promoters/enhancers	Organism/cells	Substrate requirement	Imaging application and reference
RD29A–Luc	*luc*	*RD29A* promoter	Transgenic *Arabidopsis* (ecotype C24) transformed by *Agrobacterium tumefaciens*	Luciferin (firefly)	Real-time thermoelectrically cooled CCD camera visualization of Luc-marked stress response in *Arabidopsis* (185, 186)
pGL3	Modified *luc*	*SV40* promoter	HeLa-luc cells in young CB17 SCID mice	Luciferin (firefly)	Visualization of HeLa–Luc tumours resulting from intraperitoneal injection (160)
pGL3—source of Luc	*luc*	Murine heme oxygenase 1 (*HO-1*) promoter	Transgenic mice with (HO–luc; murine heme oxygenase 1–Pluc)	Luciferine (firefly)	Intensified CCD camera monitoring of Pluc expression assayed levels of tissue oxygenation in transgenic mice (201)
pMK4 luxABCDE	*luxABCDE* (interspersed with Gram-positive ribosome binding sites)	Randomly integrated into *S. aureus* genome—differing expression levels—promoters	*Staphylococcus aureus* in mice	None necessary—full lux operon (screening done with *n*-decyl aldehyde)	Successful imaging of Gram-positive bacterial Lux in mice (202)
pHAL119; mini-Tn10luxABcam/ Ptac–ATS	*luxAB* (*V. harveyi*)	Promoterless in plasmid, but transduced downstream of *E. coli* promoters	*Escherichia coli* O157:H7	Decanal	Visualizing *E. coli* colonies transduced by Tn and Lux expression AB by image quantifier (101)
pLPK–Luc_{FF}; pΔL4–LPK–Luc_{FF}; pINS–luc_{FF}; pGL3 basic; pRL-CMV	*luc*; humanized *luc*; *ruc*	Rat *L–PK* (liver-type pyruvate kinase); human insulin; *Herpes simplex* minimal *TK* and *CMV* promoters	Pancreatic (MIN6) β-islet cells	Beetle luciferin, coelenterazine	Imaging of AMP-activated PK in single cells using luciferase expression (126)
pLPK-Luc_{FF}; p(−150) LPK-Luc_{FF}; pINS- Luc_{FF}; pRL-CMV; pβGK4-Luc; AdCMVcLuc; pPPI- Luc_{FF}	*luc*; humanized *luc*; *ruc*	Rat *L-PK*, human insulin, *Herpes simplex* minimal *TK*, *CMV* immediate-early, rat β-cell glucokinase, and human *PPI* promoters	Pancreatic (MIN6) β-islet cells	Beetle luciferin, coelenterazine	Single-cell imaging of glucose-activated insulin secretion and activation of phosphatidylinositol 3-kinase using luciferase expression (127)
pND2-Ruc/GFP; pND2-Sruc/GFP	*ruc/gfp*; secreted *ruc/gfp* (Sruc/*gfp*)	*CMV* promoter	COS-7; CHO cells	Coelenterazine	Imaging and quantifying of Ruc–GFP fusion protein secretion (73)
pGL3	*luc*	–	$9L_{Luc}$ rat gliosarcoma cells in vivo in Fischer 344 rats	Luciferin (firefly)	Assessment of chemotherapeutic progress in treating Luc-bearing tumor cells through imaging in rats (208)
Tn4001 luxABCDE Km^r; pAUL-A Tn4001 luxABCDE Km^r (Gram+)	*luxABCDE* Km^r operon (*Photorhabdus luminescens*; Gram+)	Promoterless - a promoter search vector: Random transposon insertion into the *S. pneumoniae* genome behind stronger or weaker promoters	Lux-transformed Gram + *Streptococcus pneumoniae* infection in BALB/c mice	NA—*lux* operon produces its own substrate	Transformed luminescent, kanamycin resistant bacteria were non-invasively visualized in vivo in mice; CCD imaging of longer-term pneumococcal lung infections in mice using bacteria transformed with the Gram-positive *lux* transposon (214)
AdCMVLuc; Ad5LucRGD	*luc*	*CMV* promoter	A549 cells	Cell membrane-permeable acetoxy methyl ester derivative of D-luciferin	Imaging of Pluc expression to assay differences in signal transduction efficiencies of two Ad vectors with different cell binding affinities (219)
AAV–EF1α–luciferase (rAAV); pSSV9–E1α–luciferase; pXX2; pXX6	*luc*	*EF1α* promoter	Mice (CD-1)	Luciferin (firefly)	Imaging of long-term intraperitoneal Luc expression vectored by rAAV in mice (165)
pET–G2R; pET–RG2; pC–IGF–II–GFP; pC–IGFBP6–Ruc; pC–IGFBP6–Ruc; pC–INS–GFP	*ruc–gfp* (*Aequorea*) fusion	*T7* and *CMV* promoters	Simian COS-7 cells	Coelenterazine	Intensified CCD camera maging of luminescence resonance energy transfer (LRET) from Ruc to AeqGFP (72)
AdCMVcLuc; pcLuc; AdPPIcLuc; pmtAEQ; AdCMVmLuc; pAdTrackCMV; pADCMVmAq	Aequorin (*aeq*); *luc* (humanized)	*CMV* and human preproinsulin (*PPI*) promoters	MIN6 β-islet cells	Luciferin (firefly)	Imaging of changes in Ca^{2+} and ATP^c in individual cells via luciferase and aequorin AdCMVcLuc (130)
rVV–RG [rVV–PE/L–ruc–gfp]	*Renilla* luciferase (*ruc*)	Vaccinia strong synthetic early–late (*PE/L*) promoter	CV-1 African green monkey kidney cells; athymic *nu/nu* mice	Coelenterazine	Low-light imaging of recombinant Vaccinia viral infection in cell culture and immune-compromised mice (222)
pAM401ASGX pSB2035 [–xylA–gfp–luxABCDE]	*luxCDABE* (*Photorhabdus luminescens*) fused to *gfp* (*Aequorea*) *luxCDABE* (*Photorhabdus luminescens*); *Aequorea gfp*	*SOD* (superoxide dismutase) promoter *xylA* promoter	*E. coli* and other Gram-negative bacteria; visualized in *nu/nu* mice Bovine mammary epithelial MAC-T cells	None None	Imaging of bacterial infection in mice and rats (221) Visualizing the expression and temporal induction of the quorum-sensing accessory gene regulator (Agr) in *S. aureus* in MAC-T cells (133)
Ad–CMV–Luc	*luc*	*CMV* promoter	Muscles of immunocompetent Swiss Webster mice		Visualizing the location, magnitude and persistence of Luc expression in mice by CCCD imaging (203)
AdSV40/Luc; AdHIV/Luc; rLNC/Luc; pLNC/Luc	*luc*	*CMV*, *C/EBPβ* (P_{LAP}), *SV40*, *CMV*, *BGLAP* (osteocalcin), *HIV*–LTR, *SV40* and *CMV*, *SV40* and *H19* promoters	Transfected cell lines: HepG2/Luc (human hepatocellular carcinoma); PC3.38/Luc (clone of human human prostate adenocarcinoma); T50/Luc	Beetle luciferin	ICCD and CCCD visualization of Luc expression in living animals under various conditions and parameters to optimize luciferase *in vivo* imaging (209)

NA, not applicable.

photoproteins as simultaneous reporters will probably become more common in the future.

Fusions

The papers cited above show that luciferases have been very useful as reporter genes in living cells and in bioluminescent immunoassays (63). In fact there were about 30 photoprotein fusions and conjugates reported between 1988 and 2000 (63–65). The first such fusion was that of the *Vibrio harveyi luxA* and *luxB* into the luxAB (luxF) fusion, which was expressed as a monomer in *E. coli, Bacillus*, yeast and plant systems (30, 66). A *luxAB* fusion has also been made from the luciferase of *Photorhabdus luminescens* (67). A *gfp–luxAB* fusion construct expressed in *E. coli* DH5α and *Pseudomonas fluorescens* SBW25 was used to assay bacterial numbers and nutrient-based bacterial metabolic activity in soil samples (68). Assaying the luxAB expression required luminometry (For more information, see section on Bacterial luciferase imaging in environmental health assays, below).

An *ruc–gfp* fusion construct was first engineered and expressed in murine LM–TK$^-$ fibroblast cells, in embryonic stem (ES) cells and in early stage embryos by Wang *et al.* (1996) (69). A *ruc*-modified *gfp* fusion was found to be functionally expressed in murine LM–TK$^-$ cells, whereas a reverse modified *gfp–ruc* fusion showed no GFP expression, probably because of misfolding (70). In 1999, Wang *et al.* suggested that chemiluminescent energy transfer Ruc to GFP could be used to image protein–protein interactions (71).

Using an *ruc–gfp* fusion construct, Wang *et al.* (2001) imaged the luminescence resonance energy transfer (LRET) phenomenon from *Renilla* luciferase (Ruc) emission to a humanized *Aequorea* GFP to document protein–protein interaction in eukaryotic cells (72). cDNAs of *ruc* and insulin-like growth factor binding protein 6 (Ruc–IGFBP-6) were expressed, along with fused cDNAs for *gfp* and insulin-like growth factor II (*gfp–igf-II*). The interaction of the recombinant IGF-II and IGFBP-6 resulted in LRET (luminescence resonance energy transfer) from Ruc to GFP. In 2000, Liu *et al.* visualized and quantified protein secretion using an Ruc–GFP fusion in the COS-7 and Chinese hamster ovary (CHO) cell lines (73). In simian COS-7 cells, intensified CCD camera imaging has been used to detect LRET from Ruc to aequorin GFP in an Ruc–GFP fusion protein (72).

An important recent development is the use of *P. luminescens luxCDABE* gene fusions in a collection of 8066 individual *E. coli* transformants to assay genome-wide expression profiles in response to environmental stress (74).

LOW-LIGHT IMAGING TECHNOLOGY

In 1989, Wick (75) reviewed the growing usage of single photon-counting visualization being used to assay luminescence in microtitre plates, to image metabolite distribution in tumour tissues, to visualize single cell gene expression, and even to visualize the faint chemiluminescence resulting from oxidative metabolism in phagocytes.

In a series of papers from 1992 to 2000, Stanley reviewed the commercially available luminometers, radiometers, low-light imaging CCD cameras, immunoassays, ATP rapid microbiology, hygiene monitoring, molecular probes, labels, nucleic acid hybridizations and reporter genes available for bio-imaging applications, as well as units and standards of bioluminescence (76–86).

Hill and Stewart (1994) reviewed the applied developments of bacterial luciferases as real-time, non-invasive reporters using low-light and photon-counting video cameras (87). They noted their sensitivity and real-time, non-invasive nature and their amenability to imaging by photon-counting and low-light video cameras. Contag *et al.* published two reviews on the various molecular imaging technologies for the detection and tracking of molecules and cells *in vivo* (88, 89). They also described briefly the work done in imaging tumours using luciferase expression *in vivo*.

It is to the imaging experiments that we now turn (see Table 2).

APPLICATIONS OF LUCIFERASE IMAGING

We attempt to summarize the imaging of luciferase expression in individual cells and cell cultures, in individual bacteria, yeasts, algae, insect cells, plant cells, or mammalian cells. We also focus on some of the applications and types of questions that can be answered by such imaging. These imaging experiments include the imaging of luciferases in transformed cells and cell systems in real time.

Next, we consider imaging of luciferase expression in multicellular organisms *in vivo*. This includes the expression and visualization of luciferase in permanently transformed or transgenic organisms.

Finally, we present representative studies using luciferase expression imaging to investigate host–pathogen interactions in whole plant and animal models.

IMAGING OF LUCIFERASE EXPRESSION IN TRANSFORMED SINGLE CELLS AND CELL CULTURES

Imaging of prokaryotic (bacterial) luciferases in cells and cell cultures

Imaging of bacterial luciferase in bacteria and cell culture has proved to be a fruitful venture. Some representative

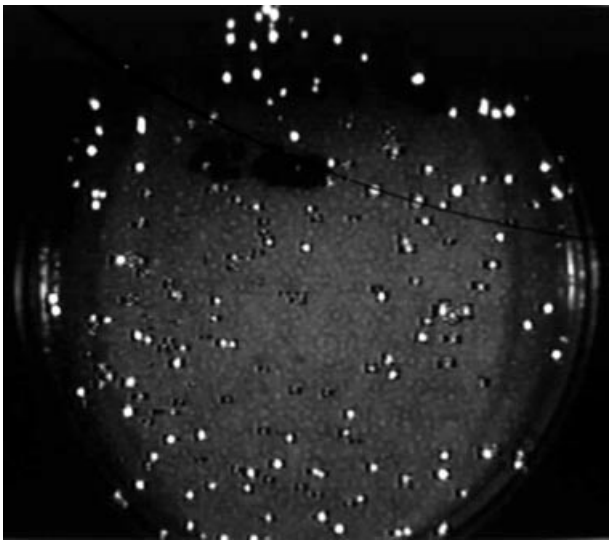

Figure 1. Visualization of individual *Escherichia coli* colonies transformed by *Vibrio harveyi lux* genes (cf. 97).

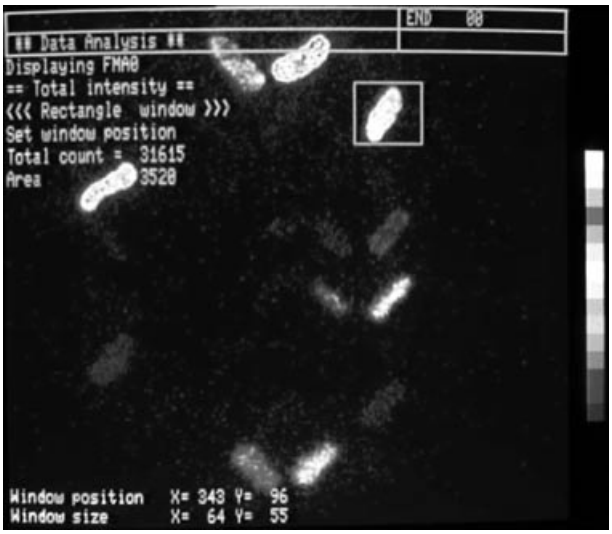

Figure 2. Visualization of *lux*-transformed *Pseudomonas* patches grown on agar medium using the Argus-100 low-light imaging system. (cf. 97).

papers are overviewed below. The monitoring of bioluminescent bacterial pathogens and symbionts in hosts is discussed in the section on symbiosis and host–pathogen interactions.

In a landmark paper, Engebrecht and his co-workers showed, in 1985, that the bacterial luciferase operon *luxCDABE* (i.e. *luxICDABE* under the control of *luxR* and *luxI*) from *Vibrio fischeri* could be vectored by a transposon and expressed in *E. coli* without the need to add the lux substrate, decanal, because of the presence of the full operon (26). Ulitzur and Khun (1987) also discussed the use of introduced luciferase genes in bacteria as an assay for the presence of particular bacteria and their susceptibility to a given antibiotic (90). In 1988, Schauer *et al.* visualized *Vibrio harveyi luxA* and *luxB* expression in *Streptomyces coelicolor* (91). Olsson *et al.* (1988) have shown that fusion gene products can be added to the *luxA* of *Vibrio harveyi* as long as the N-terminal hydrophobic sequences of the α-subunit are preserved intact, in order to retain enzymatic activity (29).

Olsson *et al.* (1989) constructed *luxAB* and *luxBA* fusions of the *V. harveyi* luciferase genes and expressed them in *E. coli* (Fig. 1) and in calli of *Nicotiana tabacum*, indicating their possible application as reporter genes in eukaryotic cells (30). The authors showed that *luxAB* has higher expression levels than *luxBA*. This was an important first. Escher *et al.* (1989) described a fusion of the *luxAB* genes of *V. harveyi* and showed it to be capable of functioning as a monomer in *E. coli* (66). Using video-imaging and spectroscopy, they found that *luxAB* has an emission spectrum comparable to the wild-type *luxA* and *luxB*, but is more sensitive to elevated temperature. In 1991, Langridge *et al.* provided an overview of the bacterial luciferase gene expression system and its applications, using low-light imaging in other bacteria and in eukaryotic cells, namely plant cells (92).

In a series of studies (93–97) it was shown that *Vibrio harveyi luxA*, *luxB* and a *luxAB* fusion could be successfully expressed under various bacterial, mammalian and viral promoters and visualized by photon-counting imaging in Gram-positive *Bacillus thuringiensis* and *B. megaterium* (cf. Fig. 2), *Arabidopsis thaliana, Nicotiana tabacum, Trichoplusia ni* (cabbage looper) (386) cells and *Manduca sextans* (tobacco hornworm; Fig. 3a, b; see the section a Imaging of host–pathogen interactions, below) (95). These *lux* constructs were vectored into transgenic plants by *Agrobacterium tumefaciens* T-DNA. Through T-DNA integration, promoterless constructs bearing *luxA* and *luxB* were randomly inserted into transgenic *Nicotiana tabacum*. Lux activity was visualized at different developmental stages in different organs (Fig. 4) (96). Bacterial luciferase expression was also successfully imaged in tomato leaves and fruit (Fig. 5a, b) (97). Firefly luciferase (Pluc) was also expressed in some of these experiments.

When promoterless *V. harveyi* luciferase (*luxAB*) was introduced downstream of the promoter for the cyanobacterium *Synechococcus psbAI* gene (a photosystem II protein), its varying expression under constant bioluminescent imaging revealed that prokaryotes also have circadian rhythms (98). Furthermore, when *luxAB* was inserted randomly by conjugation and subsequent hom-ologous recombination into the *Synechococcus* genome and transformed clones were monitored by the then newly developed cooled-CCD camera system, it was found that luciferase expression in these cyanobacteria exhibited not only circadian rhythmicity but a

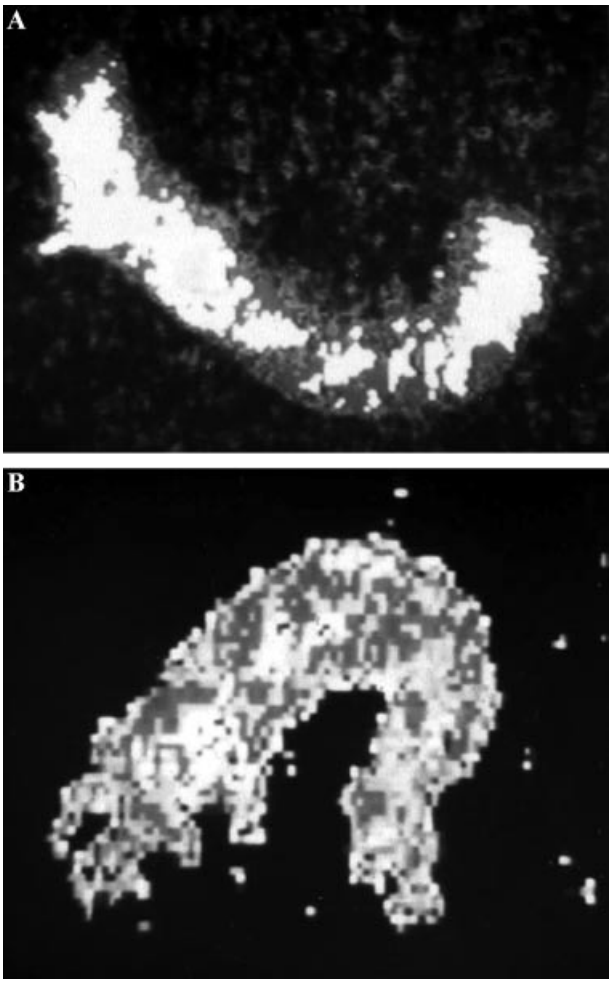

Figure 3. Visualization of the progression of an infection of *Bacillus thuringiensis*, labelled with the *xylA* promoter–*luxAB* fusion gene construct, in tobacco hornworm (*Manduca sextans*) larvae (A) through feeding or (B) after injection into the haemolymph (95).

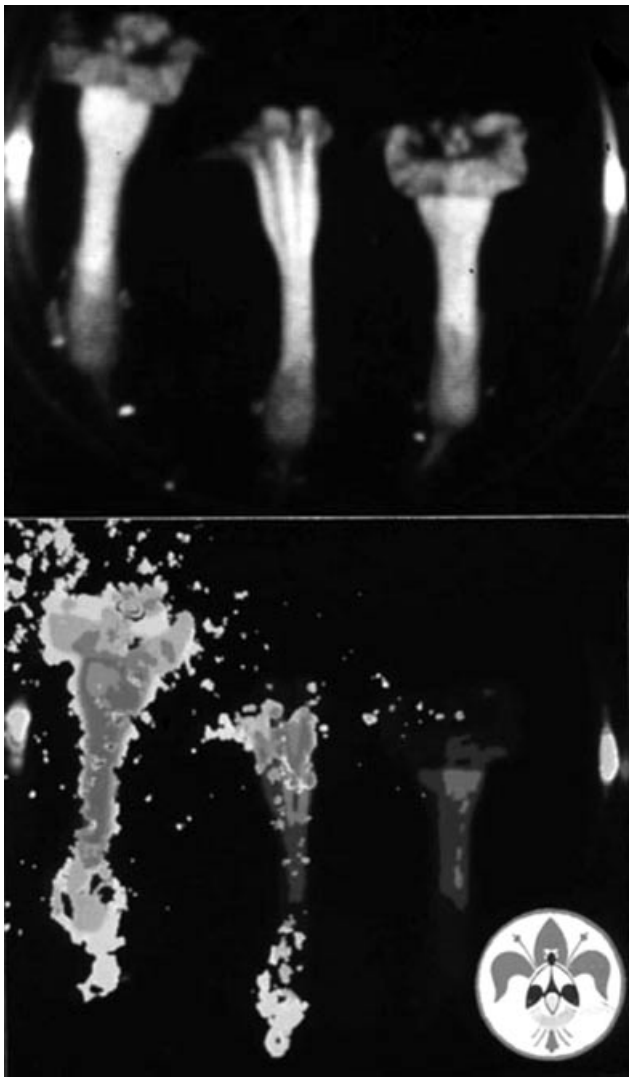

Figure 4. Visualization of randomly inserted luxA and luxB containing construct in transgenic *N. tabacum* corollas, indicating *N. tabacum* promoter-driven expression at different developmental stages (96).

wide variety of amplitude and waveform cyclicity (99). Liu *et al.* also discovered that random insertion of promoterless luciferases by homologous recombination is an extremely sensitive assay for differential gene expression levels. Thus, bacterial luciferase imaging was useful in documenting a complex gene expression phenomenon such as cyanobacterial circadian rhythms (100).

In 2000, Kunert *et al.* transfected *Synechocystis* with *gfp* and *luxAB* (64). Under different media conditions, luminescence imaging revealed that GFP expression initiated and dissipated at a slower rate, while LuxAB expression had a much more rapid response reaction time. The advantage of GFP in *Synechocystis* is that it has no substrate requirement. The advantage of LuxAB is its rapid expression response time. So, in combination with another reporter gene, such as GFP, LuxAB can be used as a sensitive measure of gene expression in bacteria.

Waddell and Poppe (2000) developed a mini-transposon bearing *Vibrio harveyi* luciferase (*luxAB*) in order to produce a luciferase-transfecting bacteriophage to detect *E. coli* strain 0157:H7 (101). The *E. coli* colonies of interest could be visualized by image quantifier about 1 h post-transfection. The ability to use a vectored *luxAB* to detect the presence of bacteria has led to important environmental and food safety applications, of which we review a few.

Bacterial luciferase imaging in environmental health assays

One of the earliest uses of luciferase imaging to detect microbial contamination involved eukaryotic firefly (*Photinus*) luciferase (Luc), not bacterial luciferase

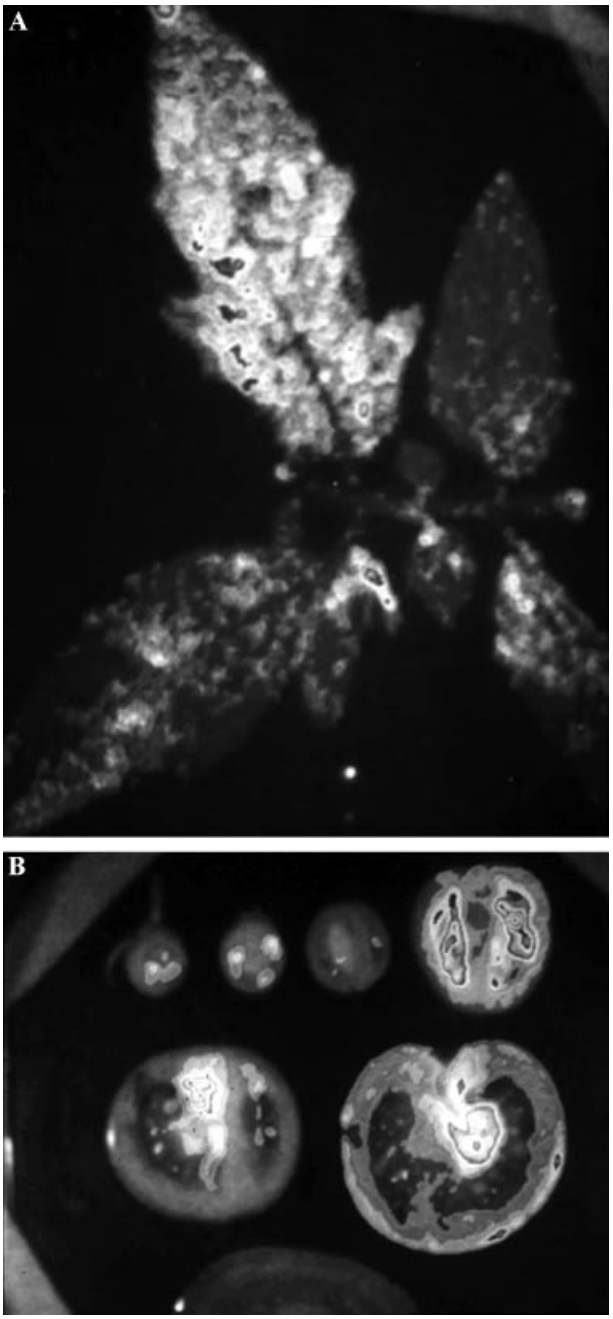

Figure 5. Visualization of bacterial luciferase expression in the leaves (A) and fruit (B) sections of transgenic tomato *Lycopersicon esculentum*) plants (97, 172).

(102). Since that time, both *luc* and bacterial *luxAB* assays have been used in environmental sampling.

Prosser *et al.* (1996) reviewed how luminometry and CCD image-enhanced microscopy may be useful in detecting naturally luminescent bacteria in environmental samples, to monitor their growth and metabolism on soil particles, microbial survival and recovery, microbial predation, plant pathogenicity, rhizosphere colonization and the reporting of gene expression in environmental samples (103). They suggested that this technology may also be used to distinguish genetically modified bacteria from indigenous bacteria in environmental samples.

Using a mycobacteriophage T5–*luc* construct, Sarkis *et al.* (1995) infected *Mycobacterium smegmatis* to produce luciferase expression both in the bacteria and in the post-infection lysogen (104). Such luciferase reporter phages may be used to test for the presence of drug-resistant or drug-sensitive *M. smegmatis* strains, as well as for the rapid identification of other effective antimycobacterial agents.

Loessner *et al.* showed (1996, 1997) that the *Listeria monocytogenes*-specific bacteriophage A511, when transformed with the *Vibrio harveyi luxAB* gene, can be a sensitive detector of viable *Listeria* cells in environmental and food samples within 24 h, rather than the usual 3 days required for traditional culturing (105, 106). Also, competitive PCR and imaging analysis can be used to quantify the number of luciferase gene copies in sediment samples to which *Synechocystis* 6803-luc cells were added (107). The authors suggest that this technique should have applications for quantifying genetically modified cyanobacteria in nature.

Although not directly imaging luciferase expression, Unge *et al.* used a mini-transposon construct bearing a *gfp–luxAB* fusion to simultaneously assay bacterial cell numbers and populational metabolic activities in specific populations of *E. coli* DH5α and *Pseudomonas fluorescens* SBW25 in soil samples (68). Bacterial numbers were determined by flow cytometric monitoring of GFP-expressing cells. LuxAB expression, as determined by luminometry, was shown to be dependent on nutrient levels and hence metabolic activity.

Bacterial luciferase imaging in food contamination assays

A bioluminescent reporter strain of *Escherichia coli* (O157:H7) containing the full bacterial luciferase (*luxABCDE*) operon was inoculated in buffer and in fecal slurry, which were both placed on surfaces of beef carcases to determine the interaction between potential bacterial pathogens and human food animal tissues (108). A sensitive photon-counting camera was used to visualize the presence of bacteria in real time. The full *lux* operon in O157:H7 renders substrate addition unnecessary.

Imaging eukaryotic luciferase expression in cells and cell cultures

Imaging of luciferase expression in plant and animal cell lines has found more diverse applications than has imaging of luciferase in bacteria. These applications include the imaging of protein site-specific secretion, protein trafficking and protein targeting to the imaging of

transgenic promoter expression, real-time gene activation, cell injury-induced expression and regulation, the determination of ATP and free Ca^{2+} concentrations, and the visualization of immune response.

Keller *et al.* showed in 1987 that a firefly luciferin–luciferase cDNA construct could be expressed in monkey kidney cells and that the gene product was targeted to the peroxisomes via a putative peroxisomal targeting protein translocation sequence (109). Using cDNAs of *luc* with the peroxisomal targeting sequences, Gould *et al.* helped to demonstrate that such sequences for protein transport are conserved from yeasts to plants, insects and mammals (110, 111). The expression of firefly luciferase (Luc) has been imaged in single COS-7 cells by White *et al.* (1990) (112).

A method for photographic film detection of firefly luciferase expression regulated by the simian virus 40 promoter in mammalian cells was developed for use with a polyester mesh replica plating technique to determine luciferase expression in mammalian cells (113).

Site-specific protein secretion from transformed Chinese hamster ovary (CHO) cells, containing luciferase from the marine crustacean ostracod *Vargula hilgendorfii* (Luc), was visualized in real time using an image-intensifying system (114).

Transient expression of luciferase fusion proteins has been an important tool in cell imaging. Luciferase cDNA fused to the 5′-flanking region of the rabbit collagenase gene containing a wild-type promoter showed an increased expression after mechanical injury to the smooth muscle Rb-1 cell line (115). In hormone studies, a modified luciferase expression system was used for real-time measuring of gene expression in endocrine cells (116). Jausons-Loffreda *et al.* (1994) used single photon-counting technology to document steroid hormone activity in transformed cell lines expressing firefly luciferase, activated by chimeric constructs of the binding domain of the Gal4 yeast protein fused to the hormone-binding domains of various steroid receptors (117). Using promoter–luciferase reporter constructs, dynamic gene expression was visualized in real time and quantified in nursing rat lactotrope cells by Castano *et al.* (118), who proposed that similar constructs could be used to visualize gene expression in any normal cell type. The *Renilla reniformis* luciferase cDNA was expressed transiently in simian COS-7 cells and stably in murine C5 fibroblasts and in extracts (61). A firefly luciferase–aequorin fusion protein was used in HeLa cells to detect rapid changes in ATP and free Ca^{2+} levels, based on light emission in response to C9 complement attack (119). Quantification of ATP concentrations has also been carried out in rat cardiac myocytes by Dorr *et al.* (1989) (120).

Rat pituitary tumour cells (GH3) were transformed with a construct containing the firefly luciferase gene and 5000 bp of the 5′ flanking region of the human PRL (prolactin) gene, subjected to luciferin, and then imaged by CCD photon-counting for time periods up to 72 h (121). Basal PRL promoter–luciferase activity was compared to stimulated activity after the addition of such stimuli of the PRL promoter as thyrotropin-releasing hormone (TRH), forskolin, calcium channel agonist Bay K8644, and basic fibroblast growth factor (bFGF). Individual cells could be imaged.

Visualization of longer-term transient luciferase expression in mammalian cells has also been possible. Various modified polylysine constructs with coupling reagent sulpho-LC SPDP were transfected with luciferase into HuH7 human hepatoma cells and imaged 2–16 days after transfection (122).

Real time-intensified CCD camera imaging of firefly luciferase (Luc) expression has been utilized to confirm that glucose may induce insulin gene transcription through increases in intracellular Ca^{2+} concentration in MIN6 β-cells when glucose, insulin or the Ca^{2+} channel inhibitor verapamil were added (Kennedy *et al.* 1999a) (123). Furthermore, Kennedy *et al.* (1999b) used recombinant firefly luciferases and photon-counting imaging to visualize concentration changes in free ATP in subdomains of single living MIN6 and primary β-cells (124). When control was made for pH, free ATP levels could be visualized in real time in the cytosol, at the plasma membrane and in the mitochondrial matrix by using luciferases specifically targeted to these three subdomains (cLuc, pmLuc, and mLuc), respectively. This was an excellent example of subcellular imaging of luciferase expression.

Chinese hamster ovary (CHO) cells with stable expression of both a CRE–luciferase reporter construct and the human pituitary adenylyl cyclase-activating peptide (PACAP) receptor were exposed to receptor agonists for each. Visualization of luciferase light emission, as well as fluorescence, was used to visualize Ca^{2+} mobilization and the induction of adenylyl cyclase activity (125).

Pancreatic β-cell activation of phosphatidylinositol 3′-kinase has been visualized by photon-counting imaging with an intensified CCD camera, using intranuclear injection of recombinant promoter fused to firefly and *Renilla* luciferase cDNAs (126). Elevated glucose levels induced pre-proinsulin (PPI) and liver-type pyruvate kinase (L-PK) promoters fused to firefly and *Renilla* luciferase cDNAs, thus producing light emission which was used to visualize single cells (127).

By constructing a less stable (and fainter) luciferase with a shorter functional half-life and implanting it into human breast cancer T-47D cells under the control of oestrogen response elements, Leclerc *et al.* (2000) were able to observe real-time gene expression in single living human breast cancer T-47D cells (128).

Increases in Ca^{2+} concentrations in HeLa cells and skeletal myotube cells caused by agonist addition have been shown to raise levels of ATP production (129). Ainscow and Rutter (2001) have shown the significance

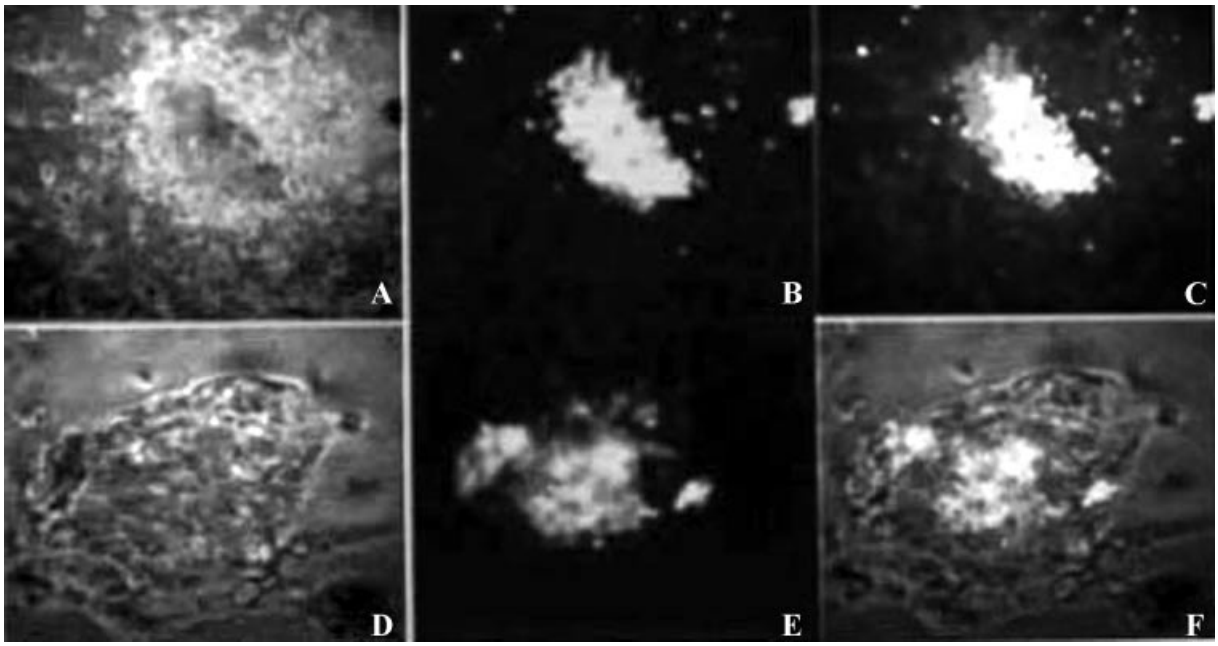

Figure 6. Visualization of mouse embryonic stem (ES) cells transformed with a cDNA construct expressing the Ruc–GFP fusion protein (69). A and D are the light images; B and E are the images of green fluorescence under UV light. C and F are overlays of A and B, D and E, respectively.

of this by quantifying (via continuous photomultiplier monitoring and intensified CCD imaging of aequorin and firefly luciferase) the expression introduced by adenoviral vectors in rat pancreatic islet (MIN6) cells and stimulated by the addition of glucose (130). This further indicated that the expression of aequorin and Pluc is dependent on Ca^{2+} and ATP concentrations.

Synthetic esters of luciferin, a number of which were substrates for purified esterases, apparently hydrolysed into luciferin in intact mammalian cells, where they caused peaks in expression at levels six-fold higher than wild-type luciferin (131). This suggests that such esters may be used to assay for luciferase activity in mammalian cells where the concentration of luciferin would be the rate-limiting factor.

Although imaging luciferase in individual plant cell lines and cultures is more difficult, and therefore has not been done as often as in animal cell lines, several reports are available. Polyadenylated luciferase mRNA electroporated into tobacco protoplasts has been imaged by video at a wide range of levels of expression (132).

Another advance was the transformation of mouse embryonic stem (ES) cells with a cDNA construct expressing the Ruc–GFP fusion protein, and its visualization (Fig. 6) (69).

Recently a *gfp–luxABCDE* reporter construct, under the control of the XylA promoter, was used to monitor the expression and temporal induction of the quorum-sensing accessory gene regulator (*agr*) in *S. aureus* infecting bovine mammary epithelial MAC-T cells (133). The reporter gene expression was occasioned by the virulence factor-mediated escape of *S. aureus* from the host endosome and its ensuing intracytoplasmic growth.

A further note on low-light imaging of luciferase expression in single cells

A most exciting feature of luciferase expression imaging is the ability to visualize gene expression in individual cells in real time. Wood and DeLuca (1987) showed that vectors can be checked for functional coding sequences by including a firefly luciferase gene probe, expressing it in *E. coli*, and detecting it by photographic film after the appropriate luciferin is added (134). Back in 1990, Hooper *et al.* reviewed low-light imaging with particular emphasis on charge couple device (CCD) imaging of single cells expressing luciferase (135). Recombinant *Vaccinia* virus bearing firefly luciferase were added to cell culture. Virally transduced cells could be detected by imaging of Luc expression at a level of one infected cell per million. Hooper *et al.* further suggested that imaging of luciferase-expressing viruses could be used to detect virus deletion mutants. Photon-counting CCD imaging of firefly luciferase activity was used by Kennedy *et al.* to visualize glucose L-pyruvate kinase (L-PK) promoter activity in single living pancreatic islet β-cells at different glucose levels (136). Detection of the L-PK promoter-driven firefly luciferase activity was standardized using CMV promoter-controlled *Renilla reniformis* luciferase activity. High intensity real-time photon-counting imaging was able to

detect firefly luciferase and aequorin reporter genes activated by insulin-induced MAP kinase signalling in single CHO.T cells, even when under the control of weak promoters, according to the findings of Rutter *et al.* (1995) (137). Rutter *et al.* (1996) also used CCD camera imaging to visualize intramitochondrial Ca^{2+} concentrations in CHO.T cells, using recombinant, Ca^{2+}-sensitive aequorin (138). Regulation of human cytomegalovirus (hCMV) and human immunodeficiency virus (hHIV) gene expression in individual, intact HeLa cells has been imaged using constructs with a firefly luciferase reporter gene downstream of viral promoters (139).

Single bacterial cell microscopic imaging also is an emerging field. Hill *et al.* (1994) were the first to report the imaging of bioluminescence in individual bacterial cells (140). Using photon-counting, they were not only able to visualize individual bacteria (such as *Pseudomonas fluorescens*) that had been transformed with *luxAB*-bearing plasmids and transposons, but were also able to image naturally luminescent *Photobacterium phosphoreum*. Furthermore, they were able to show that expression varied over time, due to cell cycle-related changes in metabolic activity.

Two low-light imaging systems were compared to assess their efficiency in visualization of single cells of *Vibrio fischeri* (MJ-1) and of a strain of *Pseudomonas putida* both of which were expressing *V. harveyi luxAB* (141). The authors found that a slow scan liquid N_2 cooled CCD (C-CCD) camera was preferable for higher resolution of single cell signal at longer exposure times, but that a photon-counting CCD (PC-CCD) camera was to be preferred for living cells at shorter exposure times, even though the resolution was somewhat lower. Phiefer and colleagues (1999) quantified relative photon flux from individual cells of *Vibrio fischeri* and *V. harveyi* using photon-counting microscopy (142). *Vibrio fischeri* luciferase was found to be more stable in expression, while *V. harveyi* luciferase was found to be much more variable in its light emission.

Even the rapid flashing of individual bioluminescent organelles (scintillons), within individual dinoflagellates of the species *Pyrocystis noctiluca*, has now been successfully imaged using video image intensifier light microscopy (143). The flashing, which occurs with fraction of a second rapidity, was induced by concentrated citric acid stimulation.

In another review (1994), Hooper *et al.* summarized in general the new improvements in low-light imaging technology. They discussed the hardware and software available, noted the rapid non-invasive advantages of low-light imaging in reporter (*luc*, *lux*) gene expression, in intracellular expression and in analysis of tissue sections, as well as immunoassays, gels and blots (144). Also in 1994, Nicolas reviewed the varied applications in the biological sciences of low-light photon-counting imaging, from the large-scale (immunoassays, DNA probes and *in vivo* imaging of expression and promoter activity) to the small scale (*in situ* hybridization and cellular luciferase expression) (145).

IMAGING OF LUCIFERASE EXPRESSION IN TISSUES AND ORGANISMS

In some ways, the goal of luciferase imaging has always been to monitor processes in living, multicellular organisms non-invasively.

Imaging luciferase expression in animals

As in cell and tissue cultures, a very wide variety of applications have been made of luciferase expression imaging in multicellular animals—both living and post-mortem.

In 1989, Hohn-Berlage *et al.* showed that ATP in intact brain cryosections can be imaged using firefly luciferin–luciferase, and that lactate concentrations can be likewise imaged using *V. fischeri* luciferase and lactate dehydrogenase (146) (see also Paschen, 1985) (147). Luciferin–luciferase assays can also be used to image distributions and abundances of respiratory metabolites such as glucose, ATP, and lactate in tissue sections (148).

Following liposome-mediated transformation of luciferse expression vectors, luciferase expression has been monitored in normal and atherosclerotic external iliac rabbit arteries (149). Replication-deficient adenovirus-bearing firefly luciferase and β-galactosidase genes were successfully used to test the comparative efficiencies of transgenic gene delivery to cultured Sprague–Dawley rat thoracic artery and aortic artery smooth muscle tissue cultures (150). Luminometer readings confirmed the luciferase assay differential.

In 1993, Mueller-Klieser and Walenta showed the spatial distribution and concentration of respiratory metabolites (ATP, glucose and lactate) in rapidly frozen tissue in absolute unit concentrations and at single-cell level resolution, using photon-counting visualization of luciferase expression coupled with any particular enzyme of interest (151). Single-photon count imaging of luciferase assays was used to spatially quantify the distribution of respiratory metabolites (ATP, glucose, and lactate) in cryosections of tumours and normal tissue (152). Luciferase light emission was proportional to metabolite concentration. These results were confirmed using other metabolite quantification methods.

Rembold *et al.* (1997) used a replication-deficient adenoviral vector carrying an apo-aequorin cDNA with a sarcoplasmic reticulum (SR) targeting sequence to infect intact rat tail arteries (153). In this way the authors were able to measure fluctuation in the presence of free Ca^{2+} in the SR in the presence of coelenterazine, the apo-aequorin luciferin substrate.

Using a luciferase reporter, Thierry *et al.* (1997) showed that when a lipopolyamine, a neutral lipid and a plasmid DNA are associated in the formation of lamellar vesicles, they can be used to compare *in vitro* and *in vivo* transfection efficiencies in mouse tissues (154). These lamellar vesicle–DNA complexes have a higher *in vitro* transfection efficiency than that of previously reported liposome transgenic delivery systems.

Making use of the fact that muscles, injected with a promoter–firefly luciferase cDNA fusion construct, retain Luc activity for up to 60 days, Davis *et al.* (1997) showed that, when a luciferase construct was co-injected with a hepatitis B virus surface antigen (HBsAg)-expressing DNA, the luciferase expression time is shortened to about 5 days of strong expression and a cut-off after 20 days (155). These findings suggest that luciferase can be used to image indirectly the progress of immune-mediated destruction of muscle myofibre tissue.

The subcutaneous injection of a lipid gadolinium contrast complex containing Luc was imaged by Wisner *et al.* (1997) using MRI (156). The Luc was also imaged. The successful insertion of Luc with the complex into the cell provided a possible method for the visualizing of a transfection event. MRI imaging provided an independent check on the luciferase imaging.

In 1997, Contag *et al.* successfully monitored *in vivo* transient expression of SV40 promoter/enhancer–Luc fusion constructs, using intensified CCD imaging on the lungs of neonatal rats (157). Cationic liposome delivery of the vectors was used. The expression vector was also induced in mice and in human T-cells.

Endogenous ATP was measured simultaneously with the release of acetylcholine from the isolated superior cervical ganglion of a rat using firefly luciferin–luciferase (158). Using imaging of luciferase light emission, March *et al.* (1999) showed the feasibility of catheter-based pericardial local delivery of adenoviral vectors for gene therapy in dogs (159). One of the recombinant adenoviral expression vectors encoded luciferase in the cytoplasm. This was visualized post mortem in pericardial tissues in sacrificed animals.

Similar imaging precision has been possible in living animals. In a landmark study by Edinger *et al.* (1999), HeLa cells stably expressing firefly luciferase were introduced via subcutaneous, intraperitoneal and intravenous injection into SCID mice (160). Tumour cell kinetics and growth were monitored by whole-body photon-counting visualization. Immediately postinjection, the following tumour cell numbers could be observed by low-light imaging: 1×10^3 cells in the peritoneal cavity; 1×10^4 cells at subcutaneous sites; and 1×10^6 circulating cells.

Using a bispecific antibody, Reynolds *et al.* (2000) targeted an adenoviral vector encoding firefly luciferase to the pulmonary endothelium of rats (161). Targeting led to a 20-fold increase in target area luciferase expression and a reduction in expression in non-targeted organs. Orson *et al.* (2000) imaged luciferase expression localized in mice lungs by using an intravenously injected, artificial, lung-targeting macroaggregated polyethyleneimine–albumin protein conjugates binding a firefly luciferase cDNA (162).

Sugihara *et al.* (2000) transfected the chloramphenicol acetyltransferase (CAT) gene and firefly luciferase (*luc*) expression cassettes by electroporation into the testicles of living chickens (163). The authors were able to image luciferase expression in and around the injection site. A self-replicating sequence of the Epstein–Barr virus was added to stabilize *luc* expression *in vivo*.

Using a tetracycline-inducible promoter construct, Hasan *et al.* (2001) have shown that transgenic firefly luciferase is suitable as a visualizing marker to monitor induction of the expression of a second transgene in living mice, in this case, Cre recombinase (164).

Recently, Lipshutz *et al.* (2001) tested prenatal, *in utero* delivery of recombinant adeno-associated virus (rAAV) vectors carrying firefly luciferase via intraperitoneal injection in mice (165). Luciferase expression was visualized by whole-body imaging in all injected animals. At birth, the highest Luc expression was in the peritoneum and liver, with lower expression levels in the heart, brain and lung. Expression persisted for as long as 18 months in the peritoneum. No antibodies against Luc or rAAV were detected and no liver cell damage was reported. These data suggest that *in utero* DNA delivery is a safe and effective method of prenatal gene therapy in animal models.

Muramatsu *et al.* (2001) have demonstrated nutritionally-regulated transgene expression in mouse liver using a liver-type phosphoenolpyruvate carboxykinase (PEPCK) gene promoter driving firefly luciferase expression (166). Fasting induced a PEPCK-driven 13-fold increase of luciferase expression in the liver, but no similar induction was found in muscle tissue for either the PEPCK promoter or a control SV40 promoter.

Yu *et al.* used the 'Gene Switch' progesterone antagonist (RU486)-inducible system co-transformed with a *Renilla* luciferase–gfp fusion construct (CMV–*ruc–gfp*) to visualize inducible gene expression in COS cell culture, and also intramuscularly in male *nu/nu* mice (167). RU486-induced Ruc–GFP expression was visualized using low-light imaging.

In short, numerous studies have shown the efficacy of luciferase imaging in modelling and observing complex gene activation events taking place *in vivo* in live animals and in tissue sections.

IMAGING OF LUCIFERASE EXPRESSION IN TRANSGENIC ORGANISMS

In many ways, the ultimate goal of luciferase imaging is

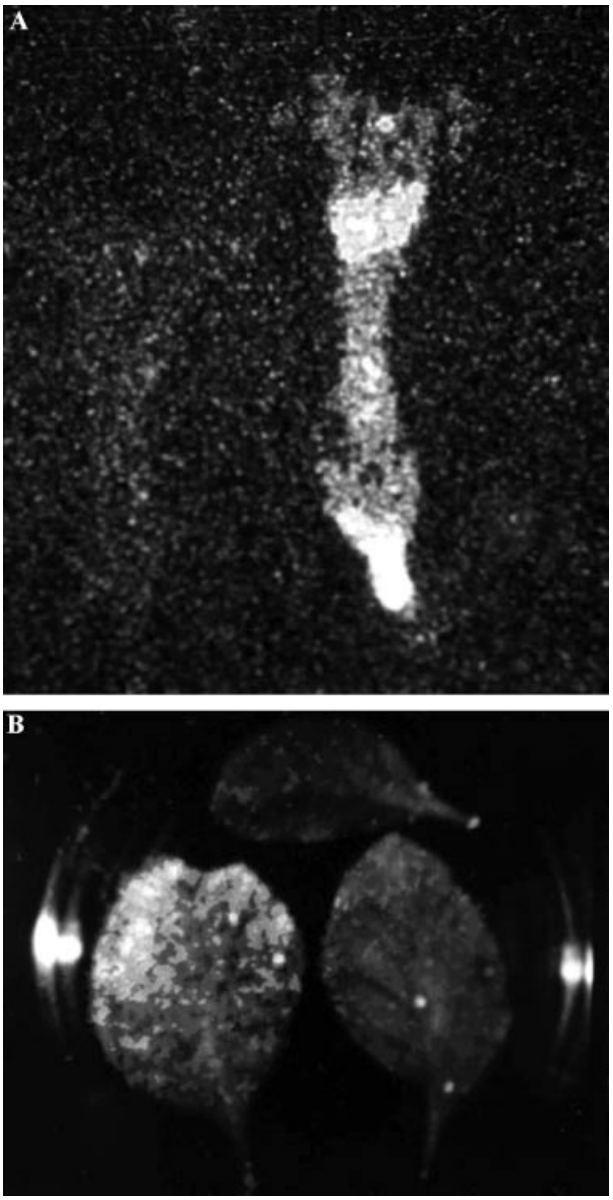

Figure 7. Visualization of the expression levels of a bidirectional *mas* P_1,P_2 promoter–*luxA* and *luxB* gene construct within specific tissues of transformed *N. tabacum*, such as (A) in flowers (non-transformed flower image on the left) and (B) in leaves. Placement of transgene accomplished via *Agrobacterium*-mediated T-DNA or direct DNA transformation (172).

not only to monitor gene expression non-invasively and in real time in living, multicellular organisms, but also to reveal and document spatial, tissue and cell type-specific expression in genetically altered (transgenic) organisms. Such applications of luciferase imaging have advanced rapidly especially in plants. By 1994, Langridge *et al.* had addressed the usefulness and convenience of using the bacterial luciferase (*luxAB*) system in a variety of eukaryotic transgenic organisms and reporter gene applications (97).

Imaging luciferase in transgenic plants

Global imaging of luciferase-expressing transgenic plants has served further research in many gene expression studies, from simple expression of luciferases in transgenic plants, to regulation of developmental genes to the gene expression of wound response, and to the detection of expression in response to thermo-osmotic stress. Photographic film was used by Ow *et al.* (1986) to visualize transient and stable firefly luciferase (Pluc) expression in *Nicotiana tabacum* (168). In 1986, Koncz and Schell imaged tissue-specific chimaeric LuxA and LuxB expression in *Daucus carota* and *Nicotiana tabacum* (169). By 1987, Koncz *et al.* (170) had demonstrated the successful assembly and expression of LuxA and LuxB from *Vibrio harveyi* in transgenic carrot (*Daucus carota*) and tobacco (*Nicotiana tabacum*) plants via *Agrobacterium*-mediated T-DNA or direct DNA transformation. Furthermore, Koncz *et al.* (1990) discussed the advantages and disadvantages of expressing bacterial (Lux) and firefly (Luc) luciferases as reporter genes in transgenic plants (171).

A promoterless *luxA* gene was fused to the 5′ end of a T-DNA, adjacent to a cauliflower mosaic virus 35S promoter-driven selectable marker, and inserted into tobacco leaf explants to generate transgenic tobacco plants. Some of these transformed plants were found, by low-light imaging, to express luciferase in only one organ, e.g. a floral corolla. This system thus served as a promoter search assay to find organ-specific promoters (172, cf. 96) (Fig. 7a, b). Sequencing of linked genomic DNA from these plants allowed for the isolation of developmental genes and their regulatory elements.

By 1994, Mayerhofer *et al.* had visualized stable *Renilla* luciferase expression in transgenic tobacco leaves, tomato fruit, and potato tubers (173). In a follow-up study, Mayerhofer *et al.* (1995) used *Agrobacterium*-mediated transfection to create transgenic alfalfa (*Medicago sativa*), tomato (*Lycopersicon esculentum*), tobacco (*Nicotiana tabacum*) and potato (*Solanum tuberosum*) plants (Fig. 8) with high levels of *Renilla* luciferase (Ruc) (60). The authors found that Ruc expression levels are substantially higher than that of either firefly (*luc*) or bacterial (Lux) luciferase.

Firefly luciferase (*luc*) was fused with an *Arabidopsis* circadian regulator, *cab2* (chlorophyll binding protein 2) promoter, and the activity of Cab2 was inferred by visualizing *luc* expression both spatially and temporally in seedlings by low-light video-imaging (1992) (174). A *luc* fusion construct carried by transgenic *Arabidopsis* plants was later (1995) used to identify plants with mutant long- and short-period circadian cycle genotypes, namely plants with mutations in *toc1*, a gene involved in the timing of Cab regulation (175). Another group (1998) used luciferase Cab promoter–luciferase fusion vectors and video-imaging to visualize transgenic expression in

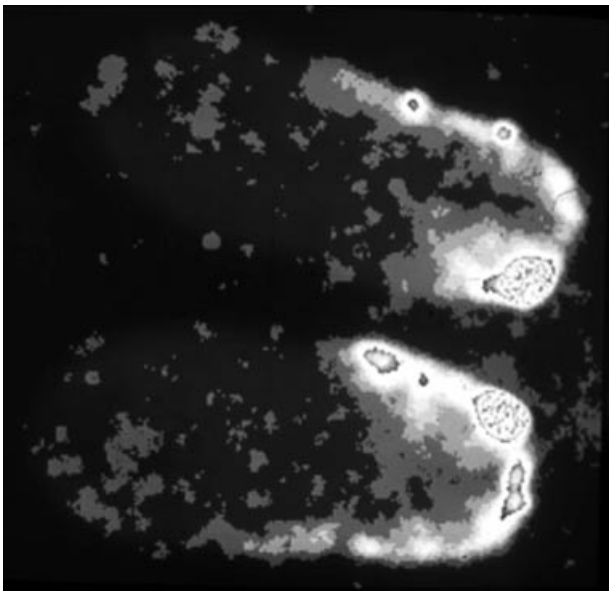

Figure 8. Visualization of *Renilla* luciferase gene expression in transformed potato tubers (*Solanum tuberosum*) (cf. 60, 173).

tobacco seedlings, to determine how the photoreceptor phytochrome circadian oscillator regulates expression of *cab* genes, which drive early seedling development (176). Recently, Schutz and Furuya (2001) monitored Cab signalling in the cotyledons of *Nicotiana tabacum* using *cab*-luciferase reporter genes (177).

Luciferase expression in transgenic plants has been used to evaluate imaging equipment and technology. Mutants of transgenic seedlings of *Arabidopsis thaliana*, containing native promoter-inserted luciferase constructs, were monitored by low-light imaging (178). When compared, they found that a cooled CCD camera was more efficient than an intensified CCD camera in detecting mutants in the screen.

Firefly luciferase (*luc*) under the control of the stress-responsive RD29A promoter was introduced into *Arabidopsis* plants by Ishitani *et al.* (179, 180). Induction of the endogenous RD29A gene was visualized using high throughput *in vivo* luminescence imaging. Under different conditions, expression of the various stress/osmotic response pathways was monitored. These pathways included those that are both phytohormone abscisic acid (ABA)-dependent and ABA-independent. Further work by Ishitani *et al.* (1998) established that inducible luciferase expression in *Arabidopsis* could be used to find a temperature cold-response gene (HOS-1) mutant (181).

The general utility of imaging luciferase reporters in transgenic plants has been amply demonstrated, not only for eukaryotic Luc but also for bacterial Lux. Langridge and Szalay (1998) used low-light intensified photon-counting imaging to visualize bacterial luciferase (*Vibrio harveyi*) and eukaryotic luciferase (*Renilla reniformis*) light emissions as markers for transformation and reporters of gene expression in transgenic *Arabidopsis thaliana* and *Nicotiana tabacum*. Both *lux* and *ruc* served as promoter search reporter genes (182). Langridge *et al.* have also imaged bacterial luciferase expression in the tomato, *Lycopersicon esculentum* (see Fig. 5a, b), and in the potato, *Solanum tuberosum* (97).

The success of luciferase expression in plants has been extended to unicellular algae. Anthozoan *Renilla reniformis* luciferase (Ruc) has been successfully expressed in the chloroplast of the alga *Chlamydomonas reinhardtii* (183). A cryogenic CCD camera was used to image the light-emitting transgenic algal colonies.

Firefly luciferase (Luc) imaging has been used in evolutionary studies in *Arabidopsis*. Jelesko *et al.* (1999) documented unequal meiotic crossing over in *Arabidopsis thaliana* plants with a synthetic *rbcsb* gene cluster composed of Δ*rbcs1b::luc–rbcs2b–rbcs3b*, instead of the wild-type *rbcs1b–rbcs2b–rbcs3b* (184). Over 1 million F_2 generation seedlings screened by low-light photon-counting imaging yielded three light-emitting seedlings with a chimeric Δ*rbcs1b::luc–rbcs3b* gene cluster, expressing luciferase and a predicted *rbcs26* duplication. These results were confirmed by molecular methods. Luciferase imaging was thus used to assay directly the frequency of evolutionary gene conversion in *A. thaliana* ($\approx 3 \times 10^{-6}$).

Xiong *et al.* (1999a, b) utilized an efficient method of high throughput genetic screening of hormone and environmental stress signal transduction mutants of *Arabidopsis thaliana*, using the firefly luciferase (*luc*) gene regulated by a cold, osmotic stress and ABA-responsive promoter (185, 186). A thermoelectrically-cooled CCD camera was used to image the plants under addition of ABA and change of temperature conditions. The system allowed screening and recognition of high- and low-expression mutants.

Luciferase imaging, in addition to being used to visualize plant promoter–gene response to thermo-osmotic pressures, has also been used to visualize plant gene response to infection. In 1996, Giacomin and Szalay utilized *Pseudomonas* infection of *Arabidopsis thaliana* to induce expression of the phenylalanine ammonia-lyase (*PAL1*) promoter fused to the *luxF* gene (187). In another study, transgenic *Arabidopsis* carrying a *gst1::luc* transgene were used to image the spatial and temporal concentrations of reactive oxygen intermediates (ROIs) in response to an assault by infectious *Pseudomonas syringae* pv. *tomato* (188) (for more details, see the section on Imaging of host–pathogen interactions, below).

Transgenic seedlings were engineered by Urwin *et al.* (2000) to carry a bicistronic gene with both GFP and firefly luciferase ORFs linked by the encephalomyocarditis (ECM) IRES element and regulated by the CaMV 35S promoter (189). Both GFP and Luc were expressed

and detected by *in vivo* imaging, indicating that the ECM IRES element facilitates the translation of the second ORF *in vivo*. Northern blot analysis also confirmed the presence of both GFP and luciferase products.

Transgenic tobacco plants (*Nicotiana tobacum* L.) expressing firefly luciferase (*luc*) driven by the *Arabidopsis* phenylalanine ammonia lyase 1 (*PAL1*) promoter have been imaged *in vivo* for up to 24 h after the addition of exogenous luciferin (190). Wounding enhanced the *luc* expression in these plants, suggesting that there are barriers in plants to ordinary luciferin uptake in transgenic luciferase assays.

Using a fusion of the *GA5* promoter (growth phytohormone gibberellin) and firefly luciferase (*ga5–luc*) cDNA, Meier *et al.* (2001) used imaging to show that *GA5* promoter regulation of GA occurs at the level of transcription (191). Imaging allowed the investigators to identify recessive mutants with high Pluc expression.

Recently, van Leeuwen and colleagues (2001), using the *CaMV 35S*, modified *CaMV 35S* and the *Arabidopsis thaliana* lipid transfer protein gene promoters in conjunction with the firefly luciferase (*luc*) gene, were able to show that variant levels of transgene promoter activation result not only from the integration site (position effect) but also from spatial and temporal promoter regulation (192). These patterns are inherited by the next generation. Expression levels were monitored in leaves of individual transgenic plants during a 50 day period, both by imaging and by assaying local mRNA levels. Further work has shown that matrix-associated regions (MAR) elements in proximity to the transgene cause a varying effect on the temporal regulation of the transgene expression between individual plant transformants (193).

In summary, luciferase imaging of transgenic plants has thus been used in the study of whole plant gene expression and regulation, as well as in the screening of mutants.

Imaging luciferase in transgenic animals

Whole-body imaging of luciferase expression in transgenic animals has not proceeded as rapidly as in plants, largely because of the greater difficulty of generating transgenic animals. However, during the past decade, significant work has been carried out, particularly in imaging of gene expression during development in transgenic embryos.

In 1990, Tamiya *et al.* first imaged the distribution of firefly luciferase expression in transgenic zebrafish (*Brachydanio rerio*) (194). Zebrafish have been a favourite transgenic model because zebrafish eggs are easily accessible to DNA injection. Mayerhofer *et al.* visualized the spatial distribution of firefly luciferase expression in transgenic zebrafish using low-light video-image analysis (195).

Using photon-counting imaging, Matsumoto *et al.* (1994) found that mouse embryos from transgenic parents carrying the paternally inherited chicken β-actin promoter–*luc* construct emitted detectable light at the two-cell stage (196). Luciferase mRNA was found even at the one-cell stage. Thus, luciferase imaging helped to demonstrate the very early onset of embryonic gene expression.

Expression of secreted *Vargula* luciferase (Vuc) was imaged in live pre-implantation mouse embryos from homozygous transgenic mice containing *luc* and *vuc* cDNAs, using image intensifiers connected to a CCD camera (197). In this way, developmental gene expression modulation could be observed and assessed in individual embryos by two different luciferases.

Transgenic mice (adults and neonates) with HIV LTR-*luc* (firefly luciferase) constructs were found to express *luc* luminescence after intraperitoneal, topical absorption, or topical electroporative delivery of D-luciferin substrate in DMSO (198). Both near the surface and deeper, visualization of *luc* expression was possible using intensified CCD (ICCD) and cooled CCD (cCCD) imaging.

Transgene integration efficiency was determined by bioluminescent visualization screening in microinjected bovine embryos (199). A murine HSP70.1 promoter was linked to a firefly luciferase (*luc*) cDNA and microinjected into zygote pronuclei produced *in vitro*. Pluc expression was then visualized in the resulting embryos.

Various promoter/enhancer–Pluc constructs were microinjected into pre-implantation bovine embryos and their expression assayed by luminometer and photon-imaging at 2 and 6 days postinjection (200). These experiments tended to document the persistence of early somatic cell promoter activation during embryonic development.

Utilizing transgenic mice that carry an integrated murine heme-oxygenase 1 (*HO-1*)-*luc* cistron, Zhang *et al.* (1999) were able to visualize levels of tissue oxygenation in real time using intensified CCD camera imaging (201). The changing O_2 concentrations were triggered by intraperitoneal injections of $CdCl_2$. Both hypoxic and hyperoxic tissue conditions altered HO-1 promoted luciferase expression.

Taking advantage of the recently discovered fact that the Cytomegalovirus immediate–early gene 1 (*CMV IE-1*) enhancer–promoter is selectively expressed in only certain brain cells, Sigworth *et al.* imaged Pluc expression in brain sections from two lines of transgenic C3H/B6 mice, using a liquid nitrogen-cooled CCD camera (202). One line of mice contained the human *CMV::luc* firefly luciferase construct and the other contained the human *c-fos::luc* firefly luciferase construct. The *CMV::luc* mice brain sections maintained at 30C or 36C showed discrete expression patterns, especially in the dorsal suprachiasmatic nucleus circadian pacemaker of the hypothalmus.

Making use of an E1-deletion adenovirus expressing Pluc under the control of a cytomegalovirus promoter, Wu *et al.* (2001), utilizing cooled CCD imaging, were able to visualize the location, magnitude and persistence of Pluc expression in Swiss Webster mice (203).

IMAGING OF TUMORS IN VIVO VIA LUCIFERASE EXPRESSION

Using photoproteins such as luciferase and GFP to image the progression of tumour growth *in vivo* is an important and rapidly advancing field (204–206). We summarize selected papers published recently. Contag *et al.* (2000) have discussed the use of GFP and firefly luciferase as reporter in real time, *in vivo* imaging of tumours. They also discussed the use of these reporter genes in studying cellular and molecular aspects of neoplastic disease, growth and regression under therapy (207). The authors show that photon imaging is sensitive enough now to detect 1000 luciferase-labelled tumour cells spread throughout a mouse peritoneal cavity. The authors further note that, while GFP is adequate for high-resolution analyses after tumour localization *in vivo*, luciferase-labelling is superior in tracing the progress of neoplastic growth from a few cells to extensive metastases. In future, luciferase reporter monitoring of cancer gene therapy is a suggested application.

In a recent tumour imaging study, Rehemtulla *et al.* (2000) used 9L rat gliosarcoma cells stably transfected with firefly luciferase ($9L_{Luc}$) to produce orthotopic brain tumors (208). Other luciferase-transformed tumour lines have been used in intraperitoneal, subcutaneous and intravascular models to visualize the kinetics of tumour growth and response to therapy. Cooled CCD camera and magnetic resonance imaging (MRI) showed an excellent 0.91 correspondence between imaged photon emission and MRI-measured tumour volume. In evaluating chemotherapeutic treatment modalities, CCD and MRI confirmed each other to a $p = 0.951$ confidence level. It is significant and promising that luciferase *in vivo* imaging compared so favourably with MRI as a tool for assessing the spatial extent of *in vivo* tumours.

In a recent important luciferase imaging study, Honigman *et al.* tested a number of parameters of *in vivo* imaging in mice and rats (209). After injecting various plasmid vectors, recombinant viruses and transfected tumour cell-lines (see Table 2), the authors imaged bladder, bone, dermis, liver, muscle, peritoneum, prostate, salivary glands, teeth and testis in mice and rats. They used visualization of Luc expression to check organ specificity, efficiency of substrate delivery, long-term monitoring of tumour growth, promoter specificity, and efficiency of injection methods using image-intensified (ICCD) and cooled (CCCD) charge-couple device cameras. Location, magnitude and duration of Luc expression were simply and reproducibly determined by CCCD photon-counting methods. Luminometry served to monitor Luc activity within organ and cell extracts.

In spite of the remarkable progress made, much more remains to be done with luciferase visualization of tumours *in vivo*. Tumour imaging in general is an important interface between basic research and the clinical applications. Luciferase imaging promises a significant role in this burgeoning field, perhaps eventually in the visualization of tumours in humans.

IMAGING OF HOST–PATHOGEN INTERACTIONS IN HOST ORGANISMS USING LUCIFERASE EXPRESSION

Many of the marine bioluminescent organisms are found to exist in symbiotic metazoan–bacterial interactions and so are involved in natural bioluminescent *in vivo* 'visualization' (3). However, most of the applied scientific uses of luciferase imaging directed at such interactions have tended to study host–pathogen relationships.

One of the earliest and most exciting areas of luciferase *in vivo* imaging has been the real-time visualization of bacterial infection in living organisms, both plants and animals. Back in 1986, Legocki and co-workers used transformed *Bradyrhizobium japonicum* carrying *luxAB* (*V. harveyi*) under the control of the *B. japonicum* nitrogenase *nifD* promoter to monitor the presence of N-fixing bacteria in soybean root nodules, and so indirectly to visualize N-fixation (*Glycine max* var. Wilkin) (210). Cell extracts of root nodules grown on plants without N_2 were assayed for LuxAB activity by luminometer, indicating *nif*-driven LuxAB synthesis. *LuxAB*-transformed *E. coli* bacteria were used as a negative control. LuxAB fusion expression in transformed *B. japonicum*-infected soybean root nodules was successfully visualized using photographic film (Fig. 9a, b) and low-light intensified video microscopy (211). Using *N. tabacum* transformed with an auxin-stimulated bidirectional *mas* promoter–*luxAB* gene fusion construct, it was possible to visualize the spread of *Pseudomonas syringiae* infection in *N. tabacum* leaves (Fig. 10) and of *Agrobacterium*-induced crown gall in *N. tabacum* stem sections (Fig. 11) (97).

In 1993 Wang *et al.* imaged the expression of *luxAB*-bearing *Bacillus thuringiensis* within sixth instar larvae of *Manduca sextans* (tobacco hornworm) 10 min post-injection and also after larval ingestion of the bacteria (Figure 3a, b) (95). The *B. thuringiensis* bacteria were transformed with a plasmid containing *luxAB* under the control of the *XylA* promoter (*B. megaterium*). A recombinant baculovirus (polyhedrin promoter–*luc*) was developed for use as a rapid luminescent plaque assay to optimize concentrations of recombinant baculoviral infection in insect cell cultures and larvae (93).

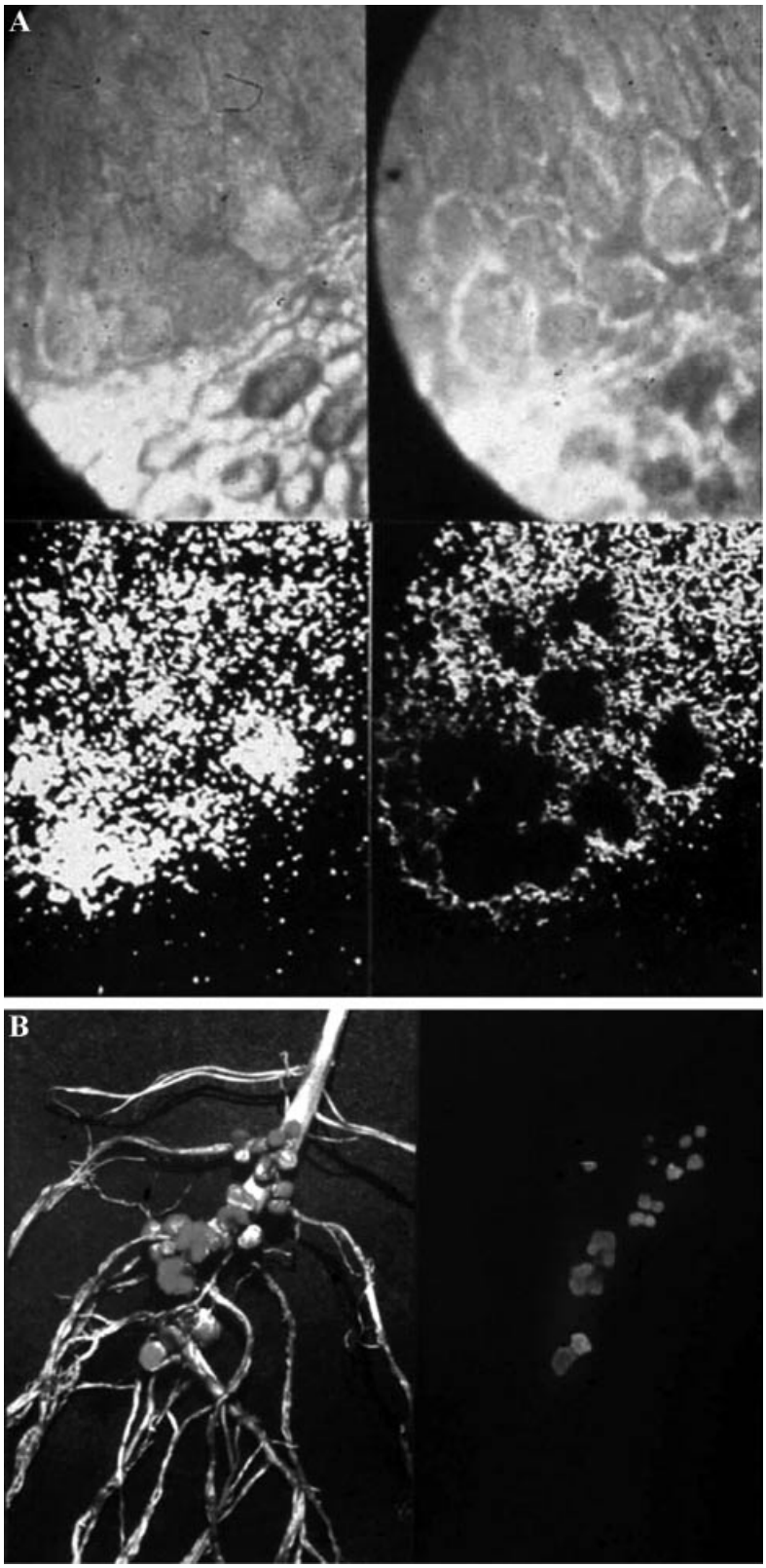

Figure 9. (A) Visualization of transformed *Bradyrhizobium*-infected soybean plant cells (*Glycine max*) in cross-sections of root nodules through LuxAB light emission (211). (B) Visualization of light emission in root nodules of soybean plants *Glycine max* grown in the absence of N_2 in the gross medium. The bacterial inoculant was *Bradyrhizobium japonicum*, stably transformed with the *nifD* promoter–*luxAB* fusion gene construct (211, cf. 210).

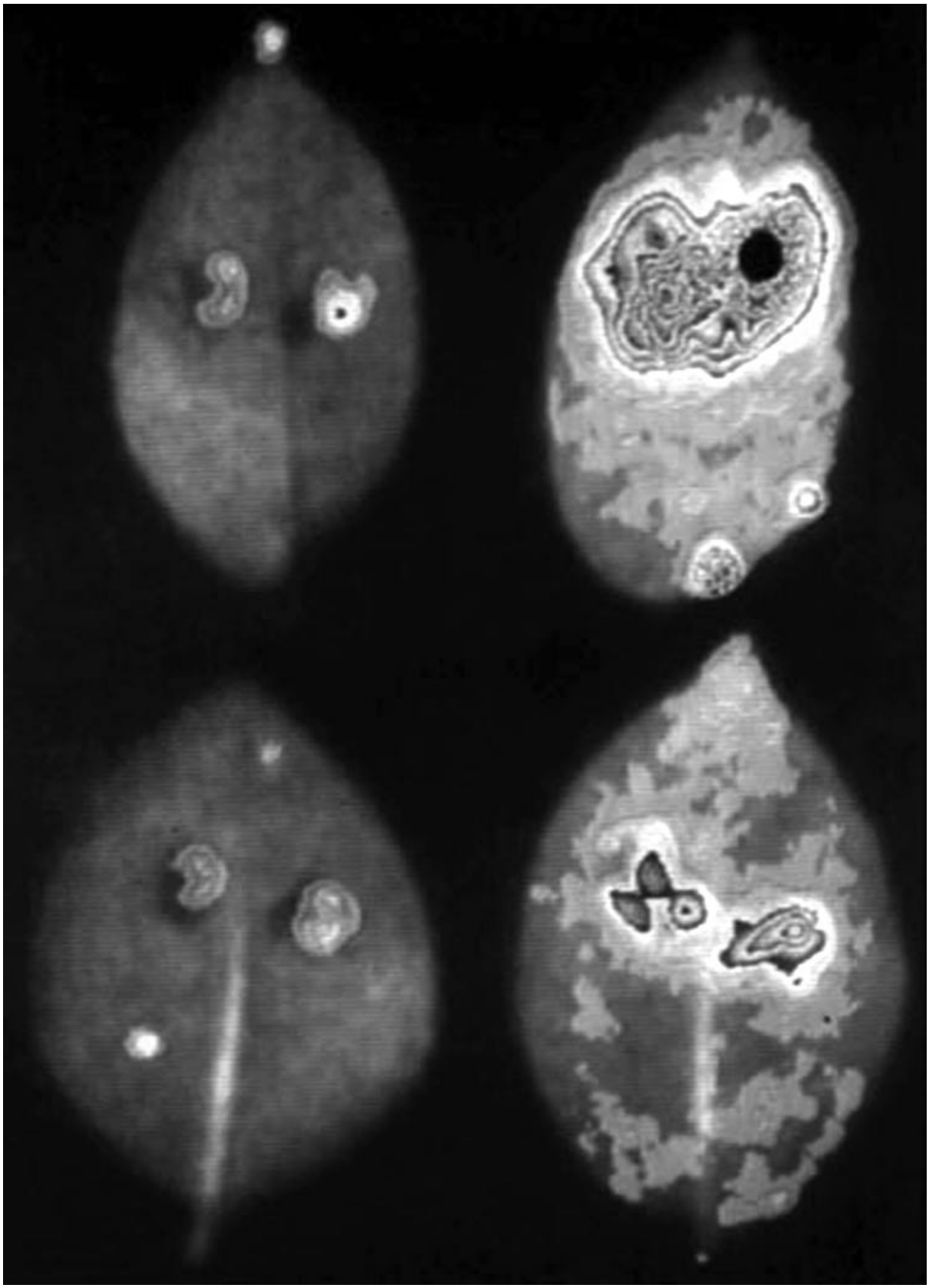

Figure 10. Visualization of the spread of pathogens, *Pseudomonas syringiae*, in *N. tabacum* carrying the bidirectional *mas* promoter-*luxAB* gene fusion construct (97, 170).

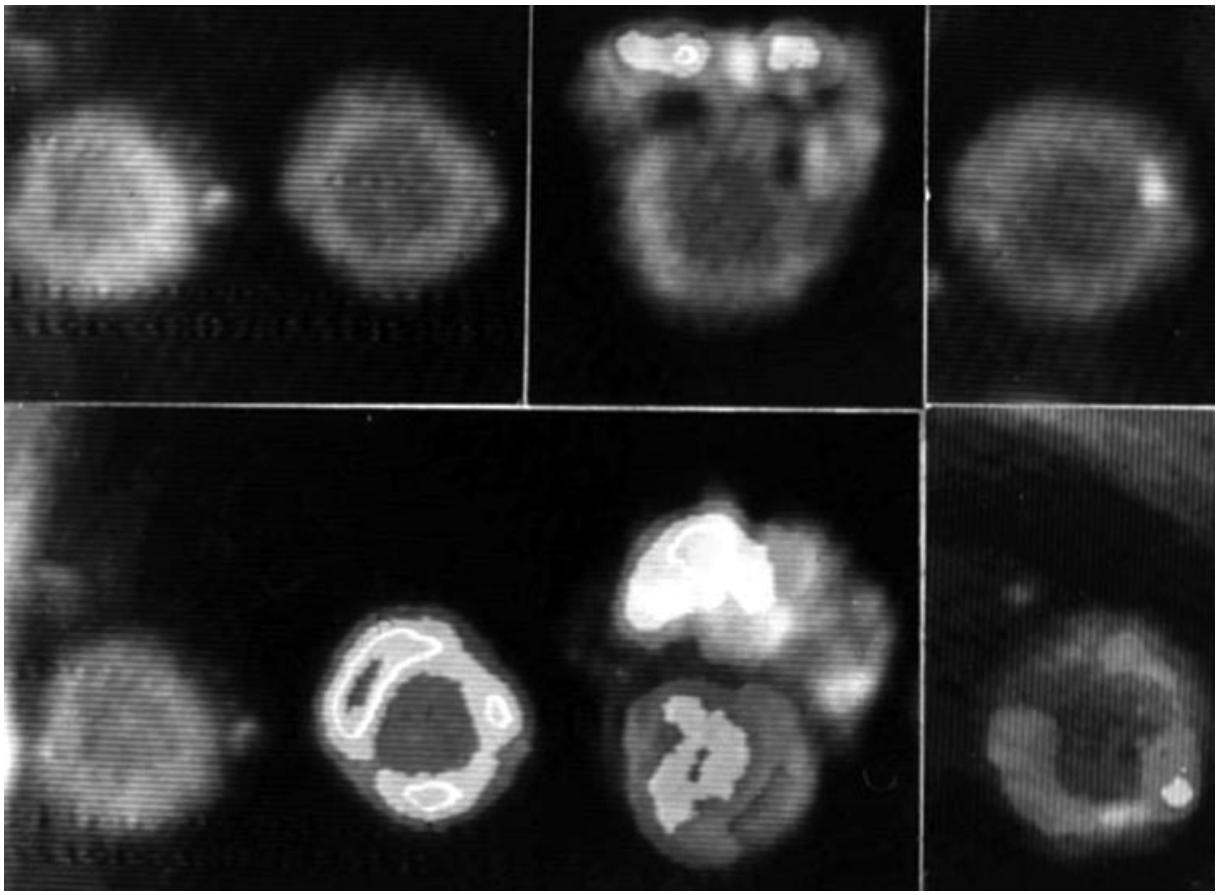

Figure 11. Visualization of the auxin-activation of the bidirectional *mas* promoter-*luxAB* fusion construct in the stem sections of transgenic *N. tabacum*, which carry a crown gall tumour caused by the wild type *Agrobacterium tumefaciens*, strain C58 (93).

Expression of Pluc within invading recombinant vaccinia virus has been imaged in African green monkey BCS-40 kidney cells (212). Time exposure with sensitive film was used to capture the images of the light-emitting viral plaques in cell culture. Although not imaged, the recombinant *luc*-bearing vaccinia virus could be assayed in target organs in BALB/c mice.

Luciferase expression in transgenic *Arabidopsis thaliana* transformed with a phenylalanine ammonia-lyase promoter (PAL1)–*luxF* fusion was used to image localized activation of PAL1 by infection of *Pseudomonas syringae* pathovar *tomato*, using photon counting (187). The *PAL* gene encodes phenylalanine ammonia lyase, which catalyses the first step in a plant development pathway responding to environmental stresses, such as infectious invasion.

In 1995, Contag *et al.* showed that bacterial infections could be traced non-invasively *in vivo* in mice using *Staphylococcus typhimurium* transformed with a vector carrying constitutively expressed bacterial luciferase (lux) (213). Recently, Francis *et al.* (2000) showed that *Staphylococcus aureus* transformed with the rearranged *Photorhabdus luminescens lux* operon (*luxABCDE*) can be used to visualize the presence of infection and the effectiveness of antibiotic treatment by direct whole-body imaging of mice after intramuscular injection of the recombinant bacteria (214). A similar method for *Streptococcus pneumoniae* has since been developed by Francis *et al.* (2001), using a Gram-negative transposon bearing the *luxABCDE* and the kanamycin resistance gene (kan^r) in one promoterless operon allowing transformed, luminescent, kanamycin-resistant bacteria to be non-invasively visualized *in vivo* in murine models (215). Rocchetta *et al.* (2000) have used clinical *E. coli* EC14 transformed with the *P. luminescens lux* operon to visualize bacterial infection in rat thigh muscles, using an intensified CCD camera system (ICCD) (216). The imaging system was sensitive enough to achieve good statistical correlation between luminescence and viable bacterial cell numbers, both with and without the presence of antimicrobial agents. Rocchetta *et al.* propose the use of this technology for *in vivo*, therapeutic testing of antimicrobial agents.

Viral infection has also been visualized by luciferase imaging. By using recombinant herpes/pseudorabies virus bearing *luc*, it is possible with ultra-high-sensitivity

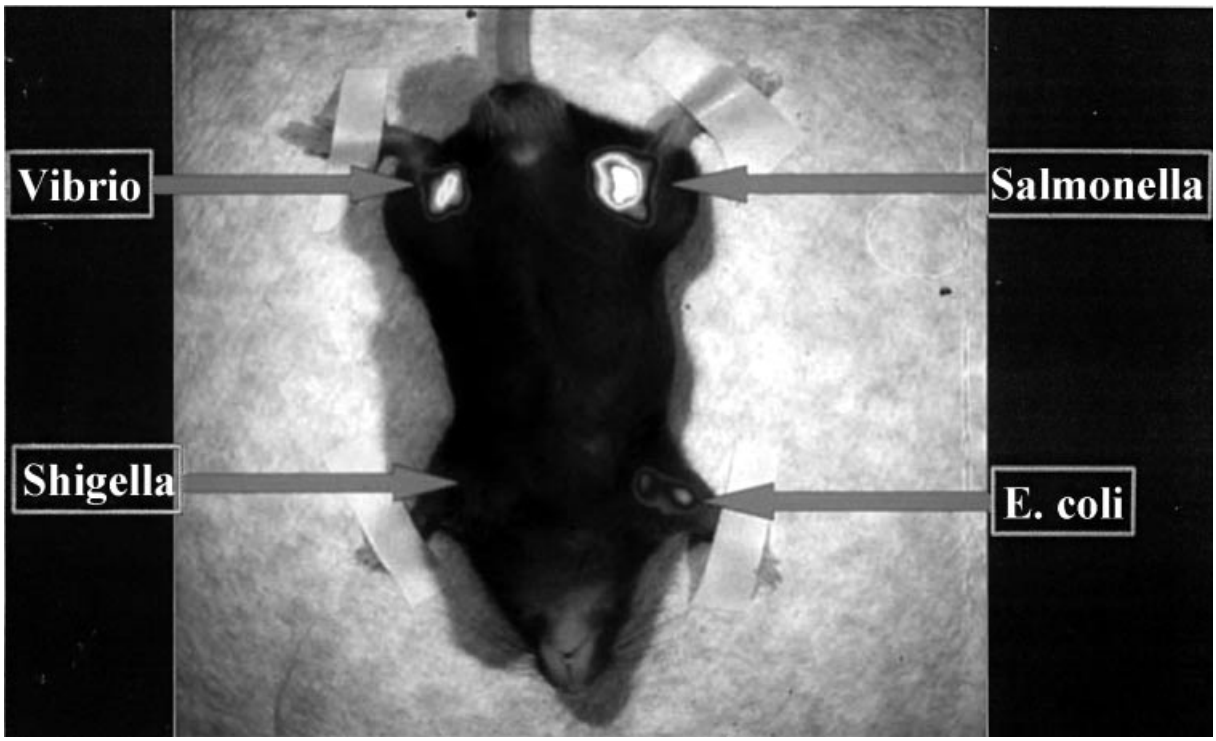

Figure 12. Visualization of intramuscularly-injected bacteria carrying the complete *luxCDABE* operon from *P. luminescens* in a C57 mouse (221).

photon-counting enhanced video imaging to visualize in real-time the progress of viral infection and destruction of mammalian cells in cell culture, even down to single cell resolution, according to Mettenleiter and Gräwe (1996) (217) who used recombinant herpes/pseudorabies virus in African green monkey kidney (VERO) cells. They suggest that *in vivo* monitoring of the spread of viral infection in a living animal is now feasible. This has in fact been done in insects. Langridge *et al.* (1996) successfully imaged not only recombinant baculovirus expression of firefly luciferase in insect cell culture (*Trichoplusia ni*) 386 cells and *Bombyx mori* N-4 and SF 9 cells, but also tracked *in vivo* the progress of a Pluc-transformed baculovirus infection in cabbage looper larvae (*Trichoplusia ni*), using low light photon-counting video imaging (218).

Digital imaging microscopy has also been used (in 2001) to determine the difference in transduction efficiencies on human A549 cells between two recombinant adenoviral vectors, AdCMVLuc and Ad5LucRGD (219).

Another recent development has been the direct *in vivo* imaging of bioluminescent $CD4^+$ T cells in a murine model (220). In a murine analogue of multiple sclerosis, experimentally-induced autoimmune encephalomyelitis, T lymphocytes transduced to express luciferase by pGC retroviral vectors were visually tracked by low-light imaging cameras into the central nervous system. Long-term transgene expression in the central nervous system was confirmed by histology.

Recent work has shown that injected bacterial infections of *Vibrio*, *Salmonella* and *E. coli* transformed with an expression construct containing the *P. luminescens luxCDABE* operon can be visualized over many days in C57 mice (Fig. 12) and Sprague–Dawley rats (221). Low-light imaging was used to visualize bacterial infections in muscles, in specific organs such as the kidney and liver *in vivo*, and in excised hearts. Luminescent bacteria could also be observed through the mouse skull *in vivo* and through excised rat tibia.

Recombinant vaccinia virus (rVV-RG, LIVP strain), bearing a *Renilla* luciferase–*gfp* fusion gene under the control of the vaccinia strong synthetic early–late promoter (rVV–*PE/L–ruc–gfp*), have been used to image rVV infection in CV-1 African green monkey kidney cells and in athymic *nu/nu* mice (222).

SUMMARY AND CONCLUSIONS

Natural *in vivo* luciferase 'visualization' has taken place in marine organisms since time immemorial in the oceans of the world. Scientific instrumental imaging of luciferase expression in living cells, tissues and organisms has made significant advances over the last few decades. This progress was only possible because of fundamental

research. The mechanisms of the luciferin–luciferase bioluminescence systems are being elucidated and their phylogenetic relationships are being worked out. Various luciferase genes and cDNAs have been isolated and cloned. Advancement in visualization of luciferase expression in individual cells, in somatic plant and animal tissues and in transgenic plants and animals has been made possible because of: (a) recombinant DNA construction of various promoter–luciferase gene constructs and fusion gene products; (b) more efficient and precise delivery of transgenic DNA and exogenous substrate; and (c) development of highly sensitive and versatile imaging technologies. Luciferase has been a useful reporter gene for imaging singly and in concert with other photoproteins, notably green fluorescence protein (GFP). Luciferase imaging has also begun to play a crucial role in imaging of tumours and metastases. Other possible applications include the imaging of LRET between luciferases and GFPs in documentation of intracellular protein–protein interactions. It seems likely that the most significant step forward, although still in its infancy, is the ability of luciferase imaging technology to resolve real-time gene expression in individual cells. Perhaps the imaging of real-time gene expression *within* individual cells *within* living organisms may be a wave of the future.

A perspective

One chimaeric dream of modern science has been to observe the world without disturbing it. In a small way, *in vivo*, non-invasive, real-time imaging of luciferase expression and light emission in living cells and organisms is at least in the spirit of that dream.

Acknowledgements

We would like to thank former and present members of the Szalay laboratory, some of whose unpublished imaging data we have included here; Dr Phil Hill for providing us with a pre-publication manuscript on the work in his laboratory with intracellular infections of *Staphylococcus aureus*; and various members of the Szalay laboratory for their help during the preparation of the manuscript, as well as Dr Bruce Wilcox, Dr William Langridge, Dr William Hayes and others who made helpful suggestions. LG was the recipient of a graduate research assistantship from the Department of Natural Sciences, Loma Linda University. The imaging research in A. A. Szalay's laboratory was supported by different grants cited in earlier publications. The present research and unpublished images were supported with the help of funding by BSRG research grants provided by Loma Linda University to A. Szalay. We apologize to the authors whose papers we were not able to include due to our own limitations.

REFERENCES

R = review

1. McElroy WD, Seliger HH, White EH. Mechanism of bioluminescence, chemiluminescence and enzyme function in the oxidation of firefly luciferin. *Photochem. Photobiol.* 1969; **10**(3): 153–170.
2. Johnson FH, Shimomura O. Enzymatic and non-enzymatic bioluminescence. Review. *Photophysiology* 1972; **7**: 275–334.
3. Ruby EG, Lee KH. MiniReview. The *Vibrio fischeri–Euprymna scolopes* light organ association: current ecological paradigms. *Appl. Environm. Microbiol.* 1998; **64**: 805–812.
4. Visick KL, McFall-Ngai MJ. Review. An exclusive contract: specificity in the *Vibrio fischeri–Euprymna scolopes* partnership. *J. Bacteriol.* 2000; **182**(7): 1779–1787.
5. Hastings JW. Bioluminescence. In *Cell Physiology*, 2nd edn, Sperelakis N (ed.). Academic Press: New York, 1998; 984–1000.
6. Widder EA, Latz MF, Herring PJ, Case JF. Far-red bioluminescence from two deep-sea fishes. *Science* 1984; **225**: 512–514.
7. Partridge JC, Douglas RH. Far-red sensitivity of dragon fish. *Nature* 1995; **375**: 21–22.
8. O'Day WT, Fernandez HR. *Aristostomias scintillans* (Malacosteidae): a deep-sea fish with visual pigments apparently adapted to its own bioluminescence. *Vision Res.* 1974; **14**: 545–550.
9. Douglas RH, Partridge JC, Marshall NJ. The eyes of deep-sea fish. I: lens pigmentation, tapeta and visual pigments. *Prog. Ret. Eye Res.* 1998; **17**: 597–636.
10. Douglas RH, Partridge JC, Dulai K, Hunt D *et al.* Dragon fish see using chlorophyll. *Nature* 1998; **393**: 423–424.
11. Douglas RH, Partridge JC, Dulai KS, Hunt DM *et al.* Enhanced retinal longwave sensitivity using a chlorophyll-derived photosensitiser in *Malacosteus niger*, a deep-sea dragon fish with far red bioluminescence. *Vision Res.* 1999; **39**: 2817–2832.
12. Haddock SHD, McDougall CM, Case JF. The Bioluminescence Web Page. http://lifesci.ucsb.edu/∼biolum/2000.
13. Thomson CM, Herring PJ, Campbell AK. The widespread occurrence and tissue distribution of the imidazolopyrazine luciferins. *J. Biolumin. Chemilumin.* 1997; **12**(2): 87–91.
14. Hastings JW. Biological diversity, chemical mechanisms, and the evolutionary origins of bioluminescent systems. *J. Mol. Evol.* 1983; **19**: 309–321.
15. Wilson T, Hastings JW. Bioluminescence. *Ann. Rev. Cell Dev. Biol.* 1998; **14**: 197–230.
16. Herring PJ. Systematic distribution of bioluminescence in living organisms. *J. Biolumin. Chemilumin.* 1987; **1**: 147–163.
17. Bowie LJ, Irwin R, Loken M, De Luca M, Brand L. Excited-state proton transfer and the mechanism of action of firefly luciferase. *Biochemistry* 1973; **12**(10): 1852–1857.
18. DeLuca M, McElroy WD. Purification and properties of firefly luciferase. In *Methods in Enzymology*, vol LVII, DeLuca M (ed.). New York: Academic Press, 1978; 3–15.
19. Gates BJ, DeLuca M. The production of oxyluciferin during the firefly luciferase light reaction. *Arch. Biochem. Biophys.* 1975; **169**(2): 616–621.
20. Henry JP, Monny C, Michelson AM. Characterization and properties of *Pholas* luciferase as a metalloglycoprotein. *Biochemistry* 1975; **14**(15): 3458–3466.
21. Deluca M. Firefly luciferase. Review. *Adv. Enzymol. Relat. Areas. Mol. Biol.* 1976; **44**: 37–68.
22. Shimomura O, Johnson FH. Chemical nature of bioluminescence systems in coelenterates. *Proc. Natl Acad. Sci. USA* 1975; **72**(4): 1546–1549.
23. Neufeld HA, Towner RD *et al.* A rapid method for determining ATP by the firefly luciferin–luciferase system. *Experientia* 1975; **31**(3): 391–392.
24. Baumgardner JD, Doeller J *et al.* Effect of nickel-based dental casting alloys on fibroblast metabolism and ultrastructural organization. *J. Biomed. Mater. Res.* 1995; **29**(5): 611–617.
25. Hastings JW, Nealson KH. Bacterial bioluminescence. *Ann. Rev. Microbiol.* 1977; **31**: 549–595.
26. Engebrecht J, Simon M, Silverman M. Measuring gene expression with light. *Science* 1985; **227**: 1345–1347.
27. Cline TW, Hastings JW. Mutationally altered bacterial luciferase.

Implications for subunit functions. *Biochemistry* 1972; **11**: 3359–3370.
28. Belas R, Mileham A, Cohn D, Hilmen M, Simon M, Silverman M. Isolation and expression of the luciferase genes from *Vibrio harveyi*. *Science* 1982; **218**: 791–793.
29. Olsson O, Koncz C, Szalay AA. The use of the *luxA* gene of the bacterial luciferase operon as a reporter gene. *Mol. Gen. Genet.* 1988; **215**: 1–9.
30. Olsson O, Escher A, Sandberg G, Schell J, Koncz C, Szalay AA. Engineering of monomeric bacterial luciferases by fusion of *luxA* and *luxB* genes in *Vibrio harveyi*. *Gene* 1989; **81**: 335–347.
31. Baldwin TO, Berends T, Bunch TA *et al.* Cloning of luciferase structural genes from *Vibrio harveyi* and expression of bioluminescence in *Escherichia coli*. *Biochemistry* 1984; **23**: 3663–3667.
32. Cohn DH, Mileham AJ *et al.* Nucleotide sequence of the luxA gene of *Vibrio harveyi* and the complete amino acid sequence of the a subunit of bacterial luciferase. *J. Biol. Chem.* 1985; **260**: 6139–6146.
33. Johnston TC, Thompson RB *et al.* Nucleotide sequence of the luxB gene of *Vibrio harveyi* and the complete amino acid sequence of the B subunit of bacterial luciferase. *J. Biol. Chem.* 1986; **261**: 4805–4811.
34. Xi L, Cho KW, Tu SC. Cloning and nucleotide sequences of lux genes and characterization of luciferase of *Xenorhabdus luminescens* from a human wound. *J. Bacteriol.* 1991; **173**(4): 1399–1405.
35. Travis J, McElroy WD. Isolation and sequence of an essential sulfhydryl peptide at the active site of firefly luciferase. *Biochemistry* 1966; **5**(7): 2170–2176.
36. Lee R, McElroy WD. Role and reactivity of sulfhydryl groups in firefly luciferase. *Biochemistry* 1969; **8**(1): 130–136.
37. DeLuca M. Hydrophobic nature of the active site of firefly luciferase. *Biochemistry* 1969; **8**(1): 160–166.
38. De Wet JR, Wood KV, Helinski DR, DeLuca M. Cloning of firefly luciferase cDNA and the expression of active luciferase in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* 1985; **82**(23): 7870–7873.
39. Thompson EM, Nagata S *et al.* Cloning and expression of cDNA for the luciferase from the marine ostracod *Vargula hilgendorfii*. *Proc. Natl Acad. Sci. USA* 1989; **86**(17): 6567–6571.
40. Thompson EM, Nagata S *et al. Vargula hilgendorfii* luciferase: a secreted reporter enzyme for monitoring gene expression in mammalian cells. *Gene* 1990; **96**(2): 257–262.
41. Yamaguchi I. *Oplophorus* oxyluciferin and a model luciferin compound biologically active with *Oplophorus* luciferase. *Biochem. J.* 1975; **151**(1): 9–15.
42. Shimomura O, Johnson FH, Saiga Y. Extraction, purification, and properties of aequorin, a bioluminescent protein from the luminous hydromedusan *Aequorea*. *J. Cell Comp. Physiol.* 1962; **59**: 223–229.
43. Ward WW, Cormier MJ. Extraction of *Renilla*-type luciferin from the calcium-activated photoproteins aequorin, mnemiopsin, and berovin. *Proc. Natl Acad. Sci. USA* 1975; **72**(7): 2530–2534.
44. Hart RC, Matthews JC *et al. Renilla reniformis* bioluminescence: luciferase-catalyzed production of non-radiating excited states from luciferin analogues and elucidation of the excited state species involved in energy transfer to *Renilla* green fluorescent protein. *Biochemistry* 1979; **18**(11): 2204–2210.
45. Ward WW, Cormier MJ. An energy transfer protein in coelenterate bioluminescence: characterization of the *Renilla* green fluorescence protein. *J. Biol. Chem.* 1979; **254**: 781–788.
46. Inouye S, Noguchi M *et al.* Cloning and sequence analysis for the luminescent protein aequorin. *Proc. Natl Acad. Sci. USA* 1985; **89**: 3901–3907.
47. Prasher D, McCann R, Cormier MJ. Cloning and expression of the cDNA coding for aequorin, a bioluminescent calcium-binding protein. *Biochem. Biophys. Res. Commun.* 1985; **126**: 1259–1268.
48. Campbell AK, Sala-Newby G *et al.* From Luc and Phot genes to the hospital bed. *J. Biolumin. Chemilumin.* 1990; **5**(2): 131–139.
49. Prasher DC, Eckenrode VK *et al.* Primary structure of the *Aequorea* victoria green fluorescent protein. *Gene* 1992; **111**(2): 229–233.
50. Shimomura O, Masugi T *et al.* Properties and reaction mechanism of the bioluminescence system of the deep-sea shrimp *Oplophorus gracilorostris*. *Biochemistry* 1978; **17**(6): 994–998.
51. Inouye S, Shimomura O. The use of *Renilla* luciferase, *Oplophorus* luciferase, and apoaequorin as bioluminescent reporter protein in the presence of coelenterazine analogues as substrate. *Biochem. Biophys. Res. Commun.* 1997; **233**(2): 349–353.
52. Inouye S, Watanabe K *et al.* Secretional luciferase of the luminous shrimp *Oplophorus gracilirostris*: cDNA cloning of a novel imidazopyrazinone luciferase(1). *FEBS Lett* 2000; **481**(1): 19–25.
53. Hori K, Cormier MJ. Studies on the bioluminescence of *Renilla reniformis*. VI. Some chemical properties and the tentative partial structure of luciferin. *Biochim. Biophys. Acta* 1966; **130**(2): 420–425.
54. Kreis P, Cormier MJ. Inhibition of *Renilla reniformis* bioluminescence by light: effects on luciferase and its substrates. *Biochim. Biophys. Acta* 1967; **141**(1): 181–183.
55. Karkhanis YD, Cormier MJ. Isolation and properties of *Renilla reniformis* luciferase, a low molecular weight energy conversion enzyme. *Biochemistry* 1971; **10**(2): 317–326.
56. DeLuca M, Dempsey ME *et al.* Mechanism of oxidative carbon dioxide production during *Renilla reniformis* bioluminescence. *Proc. Natl Acad. Sci. USA* 1971; **68**(7): 1658–1660.
57. Anderson JM, Charbonneau H *et al.* Mechanism of calcium induction of *Renilla* bioluminescence. Involvement of a calcium-triggered luciferin binding protein. *Biochemistry* 1974; **13**(6): 1195–1200.
58. Matthews JC, Hori K, Cormier MJ. Purification and properties of *Renilla reniformis* luciferase. *Biochemistry* 1977; **16**: 85–95.
59. Lorenz WW, McCann RO, Longiaru M, Cormier MJ. Isolation and expression of a cDNA encoding *Renilla* luciferase. *Proc. Natl Acad. Sci. USA* 1991; **88**: 4438–4442.
60. Mayerhofer R, Langridge WHR, Cormier MJ, Szalay AA. Expression of recombinant *Renilla* luciferase in transgenic plants results in high levels of light emission. *Plant J* 1995; **7**(6): 1031–1038.
61. Lorenz WW, Cormier MJ *et al.* Expression of the *reniformis* luciferase gene in mammalian cells. *J. Biolumin. Chemilumin.* 1996; **11**: 31–37.
62. Magalhaes PJ, Rizzuto R. Mitochondria and calcium homeostasis: a tale of three luminescent proteins. *Luminescence* 2001; **16**(2): 67–71.
63. Kricka LJ, Stroebel J, Stanley PE. Bioluminescent fusion conjugates and bioluminescent immunoassays: 1988–1998. *Luminescence* 1999; **14**(1): 39–46 (R).
64. Kunert A, Hagemann M, Erdmann N. Construction of promoter probe vectors for *Synechocystis* sp. PCC 6803 using the light-emitting reporter systems Gfp and LuxAB. *J. Microbiol. Methods* 2000; **41**(3): 185–194.
65. Wang D, Yang W *et al.* MGSA/GRO-mediated melanocyte transformation involves induction of Ras expression. *Oncogene* 2000; **19**(40): 4647–4659.
66. Escher A, O'Kane DJ, Lee J, Szalay AA. Bacterial luciferase $\alpha\beta$ fusion protein is fully active as a monomer and highly sensitive *in vivo* to elevated temperature. *Proc. Natl Acad. Sci. USA* 1989; **86**: 6528–6532.
67. Hill PJ, Throup JP, Steward GSAB. Novel bacterial luciferases. In *Bioluminescence and Chemiluminescence: Status Report. Proceedings of the VIIth International Symposium on Bioluminescence and Chemiluminescence 1993*, Szalay AA, Kricka LJ, Stanley P (eds). Wiley: Chichester, 1994; 122–126.
68. Unge A, Tombolini R, Molbak L, Jansson JK. Simultaneous monitoring of cell number and metabolic activity of specific bacterial populations with a dual *gfp-luxAB* marker system. *Appl. Environ. Microbiol.* 1999; **65**(2): 813–821.
69. Wang G, Czeh S, Wang Y, Szalay AA. Imaging of luciferase fusion gene expression in transformed cells and embryos. In *Bioluminescence and Chemiluminescence: Molecular reporting with photons. Proceedings of the 9th International Symposium 1996*, Hastings JW, Kricka LJ, Stanley PE (eds). Chichester: Wiley, 1997; 415–418.
70. Wang Y, Wang G, O'Kane DJ, Szalay AA. The *Renilla* luciferase-modified GFP fusion protein is functional in trans-

formed cells. In *Bioluminescence and Chemiluminescence: Molecular Reporting with Photons. Proceedings of the 9th International Symposium 1996*, Hastings JW, Kricka LJ, Stanley PE (eds). Chichester: Wiley, 1997; 419–422.
71. Wang Y, Wang G, O'Kane DJ, Szalay AA. The study of protein–protein interactions using chemiluminescence energy transfer. In *Bioluminescence and Chemiluminescence: Perspectives for the 21st Century 1999*. Roda APM, Kricka LJ, Staley PE (eds). Chichester: Wiley, 2000; 475–478.
72. Wang Y, Wang G *et al.* A study of protein–protein interactions in living cells using luminescence resonance energy transfer (LRET) from *Renilla* luciferase to *Aequorea* GFP. *Mol. Gen. Genet.* 2001; **264**(5): 578–587.
73. Liu J, Wang Y, Szalay AA, Escher A. Visualizing and quantifying protein secretion using a *Renilla* luciferase–GFP fusion protein. *Luminescence* 2000; **15**(1): 45–49.
74. Van Dyk T, Gonye G, Reeve M *et al.* Genome-wide expression profiling with *luxCDABE* gene fusions. In *Proceedings of the 11th International Symposium on Bioluminescence and Chemiluminescence 2000*, Case JF, Herring PJ, Robison BH, Haddock SHD, Kricka LJ, Stanley PE (eds). Chichester: Wiley, 2001; 461–464.
75. Wick RA. Photon counting imaging: applications in biomedical research. *Biotechniques* 1989; **7**(3): 262–269.
76. Stanley PE. A survey of more than 90 commercially available luminometers and imaging devices for low-light measurements of chemiluminescence and bioluminescence, including instruments for manual, automatic and specialized operation, for HPLC, LC, GLC and microtitre plates. Part 1: Descriptions. *J. Biolumin. Chemilumin.* 1992; **7**(2): 77–108 (R).
77. Stanley PE. A survey of more than 90 commercially available luminometers and imaging devices for low-light measurements of chemiluminescence and bioluminescence, including instruments for manual, automatic and specialized operation, for HPLC, LC, GLC and microtitre plates. Part 2: Photographs. *J. Biolumin. Chemilumin.* 1992; **7**(3): 157–169 (R).
78. Stanley PE. A survey of some commercially available kits and reagents which include bioluminescence or chemiluminescence for their operation: including immunoassays, hybridization, labels, probes, blots and ATP-based rapid microbiology. Products from more than forty companies. *J. Biolumin. Chemilumin.* 1993; **8**(2): 51–63 (R).
79. Stanley PE. Commercially available luminometers and imaging devices for low-light measurements and kits and reagents utilizing bioluminescence or chemiluminescence: survey update I. *J. Biolumin. Chemilumin.* 1993; **8**(5): 237–240 (R).
80. Stanley PE. Commercially available luminometers and imaging devices for low-light level measurements and kits and reagents utilizing bioluminescence or chemiluminescence: survey update 2. *J. Biolumin. Chemilumin.* 1994; **9**(2): 51–53 (R).
81. Stanley PE. Commercially available luminometers and imaging devices for low-light level measurements and kits and reagents utilizing bioluminescence or chemiluminescence: survey update 3. *J. Biolumin. Chemilumin.* 1994; **9**(3): 123–125 (R).
82. Stanley PE. Commercially available luminometers and imaging devices for low-light level measurements and kits and reagents utilizing bioluminescence or chemiluminescence: survey update 4. *J. Biolumin. Chemilumin.* 1996; **11**(4–5): 175–191 (R).
83. Stanley PE. Commercially available luminometers and imaging devices for low-light level measurements and kits and reagents utilizing bioluminescence or chemiluminescence: survey update 5. *J. Biolumin. Chemilumin.* 1997; **12**(2): 61–78 (R).
84. Stanley PE. Commercially available fluorometers, luminometers and imaging devices for low-light level measurements and allied kits and reagents: survey update 6. *Luminescence* 1999; **14**(4): 201–213 (R).
85. Stanley PE. Some brief notes on nomenclature and units and standards used in bioluminescence and chemiluminescence. *Methods Enzymol.* 2000; **305**: 47–50 (R).
86. Stanley PE. Commercially available luminometers and low-level light imaging devices. *Methods Enzymol.* 2000; **305**: 96–103 (R).
87. Hill PJ, Stewart GS. Use of *lux* genes in applied biochemistry. *J. Biolumin. Chemilumin.* 1994; **9**(3): 211–215 (R).
88. Contag CH, Fraser S, Weissleder R. Strategies in *in vivo* molecular imaging. *NeoReviews* 2000; **1**: 225–232 (R).
89. Contag CH, Weissleder R, Bachmann MH, Fraser SE. Applications of *in vivo* molecular imaging in biology and medicine, *NeoReviews* 2000; **1**: 233–240 (R).
90. Ulitzur S, Khun J. Introduction of *lux* genes into bacteria, a new approach for specific detection of bacteria and their antibiotic susceptibility. In *Bioluminescence and Chemiluminescence: New Perspectives* Chichester: Wiley 1987; 463–472.
91. Schauer A, Ranes M, Santamaria R *et al.* Visualizing gene expression in time and space in the filamentous bacteria *Streptomyces coelicolor*. *Science* 1988; **240**: 768–772.
92. Langridge WH, Escher A, Koncz C, Schell J, Szalay AA. Bacterial luciferase genes: a light emitting reporter system for *in vivo* measurement of gene expression. *Technique* 1991; **3**(3): 91–97.
93. Langridge W, Jiang C, Wang G *et al.* Use of a luciferase marker gene system to monitor gene expression in bacteria, plant and virus infected animal cells. In *Bioluminescence and Chemiluminescence: Status Report. Proceedings of the VIIth International Symposium on Bioluminescence and Chemiluminescence 1993*, Szalay AA, Kricka LJ, Stanley P (eds). Chichester: Wiley, 1994; 222–226.
94. Langridge WHR, Baga M, Ayer W, Koorneef M, Szalay AA. Use of an auxin-stimulated promoter–luciferase gene fusion to monitor hormone stimulated gene expression in transgenic plants. In *Bioluminescence and Chemiluminescence: Status Report. Proceedings of the VIIth International Symposium on Bioluminescence and Chemiluminescence 1993*, Szalay AA, Kricka LJ, Stanley P (eds). Chichester: Wiley, 1994; 227–231.
95. Wang G, Mayerhofer R, Langridge WHR, Szalay AA. Expression of a bacterial luciferase marker gene in *Bacillus* species. In *Bioluminescence and Chemiluminescence: Status Report. Proceedings of the VIIth International Symposium on Bioluminescence and Chemiluminescence 1993*, Szalay AA, Kricka LJ, Stanley P (eds). Chichester: Wiley, 1994; 232–236.
96. Jiang C, Langridge WHR, Szalay AA. Isolation of developmentally regulated promoter elements from tobacco by T-DNA insertional activation with promoterless luciferase marker gene. In *Bioluminescence and Chemiluminescence: Status Report. Proceedings of the VIIth International Symposium on Bioluminescence and Chemiluminescence 1993*, Szalay AA, Kricka LJ, Stanley P (eds). Chichester: Wiley, 1994; 217–221.
97. Langridge W, Escher A *et al.* Low-light image analysis of transgenic organisms using bacterial luciferase as a marker. *J. Biolumin. Chemilumin.* 1994; **9**(3): 185–200.
98. Kondo T, Strayer CA, Kulkarni RD *et al.* Circadian rhythms in prokaryotes: luciferase as a reporter of circadian gene expression in cyanobacteria. *Proc. Natl Acad. Sci. USA* 1993; **90**: 5672–5676.
99. Liu Y, Golden SS, Kondo T, Ishiura M, Johnson CH. Bacterial luciferase as a reporter of circadian gene expression in cyanobacteria. *J. Bacteriol.* 1995; **177**(8): 2080–2086.
100. Liu Y, Tsinoremas NF, Johnson CH *et al.* Circadian orchestration of gene expression in cyanobacteria. *Genes Dev.* 1995; **9**(12): 1469–1478.
101. Waddell TE, Poppe C. Construction of mini-*Tn101uxABcam/Ptac-ATS* and its use for developing a bacteriophage that transduces bioluminescence to *Escherichia coli* O157:H7. *FEMS Microbiol. Lett* 2000; **182**(2): 285–289.
102. Levin GV, Chen CS *et al.* Development of the firefly bioluminescent assay for the rapid, quantitative detection of microbial contamination of water. *Amrl. Tr.* 1967; 1–73.
103. Prosser JI, Killham K *et al.* Luminescence-based systems for detection of bacteria in the environment. *Crit. Rev. Biotechnol.* 1996; **16**(2): 157–183.
104. Sarkis GJ, Jacobs Jr. WR, Hatfull GF. L5 luciferase reporter mycobacteriophages: a sensitive tool for the detection and assay of live mycobacteria. *Mol. Microbiol.* 1995; **15**: 1055–1067.
105. Loessner MJ, Rees CE, Stewart GS, Scherer S. Construction of luciferase reporter bacteriophage *A511::luxAB* for rapid and sensitive detection of viable *Listeria* cells. *Appl. Environ. Microbiol.* 1996; **62**: 1133–1140.
106. Loessner MJ, Rudolf M, Scherer S. Evaluation of luciferase

reporter bacteriophage *A511::luxAB* for detection of *Listeria monocytogenes* in contaminated foods. *Appl. Environ. Microbiol.* 1997; **63**: 2961–2965.
107. Moller A, Jansson JK. Quantification of genetically tagged cyanobacteria in Baltic Sea sediment by competitive PCR. *Biotechniques* 1997; **22**(3): 512–518.
108. Siragusa GR, Nawotka K, Spilman SD, Contag PR, Contag CH. Real-time monitoring of *Escherichia coli* O157:H7 adherence to beef carcass surface tissues with a bioluminescent reporter. *Appl. Environ. Microbiol.* 1999; **65**: 1738–1745.
109. Keller GA, Gould S *et al.* Firefly luciferase is targeted to peroxisomes in mammalian cells. *Proc. Natl Acad. Sci. USA* 1987; **84**(10): 3264–3268.
110. Gould SJ, Krisans S *et al.* Antibodies directed against the peroxisomal targeting signal of firefly luciferase recognize multiple mammalian peroxisomal proteins. *J. Cell Biol.* 1990a; **110**(1): 27–34.
111. Gould SJ, Keller GA *et al.* Peroxisomal protein import is conserved between yeast, plants, insects and mammals. *EMBO J* 1990b; **9**(1): 85–90.
112. White MRH, Craig FF, Watmore D, McCapra F, Simmonds AC. Applications of the direct imaging of firefly luciferase expression in single intact mammalian cells using charge-coupled device cameras. In *Bioluminescence and Chemiluminescence: Current Status. Proceedings of the VIth International Symposium on Bioluminescence and Chemiluminescence 1990*. Chichester: Wiley, 1990; 357–360.
113. Hornsby PJ, Yang L *et al.* A modified procedure for replica plating of mammalian cells allowing selection of clones based on gene expression. *Biotechniques* 1990; **12**(2): 244–251.
114. Inouye S. Ohmiya Y *et al.* Imaging of luciferase secretion from transformed Chinese hamster ovary cells. *Proc. Natl Acad. Sci. USA* 1992; **89**(20): 9584–9587.
115. James TW, Wagner R *et al.* Induction of collagenase and stromelysin gene expression by mechanical injury in a vascular smooth muscle-derived cell line. *J. Cell Physiol.* 1993; **157**(2): 426–437.
116. Frawley LS, Faught WJ *et al.* Real-time measurement of gene expression in living endocrine cells. *Endocrinology* 1994; **135**(1): 468–471.
117. Jausons-Loffreda N, Balaguer P *et al.* Chimeric receptors as a tool for luminescent measurement of biological activities of steroid hormones. *J. Biolumin. Chemilumin.* 1994; **9**(3): 217–221.
118. Castano JP, Kineman RD *et al.* Dynamic monitoring and quantification of gene expression in single, living cells: a molecular basis for secretory cell heterogeneity. *Mol. Endocrinol.* 1996; **10**(5): 599–605.
119. Sala-Newby GB, Taylor KM *et al.* Imaging bioluminescent indicators shows Ca^{2+} and ATP permeability thresholds in live cells attacked by complement. *Immunology* 1998; **93**(4): 601–609.
120. Dorr RT, Bozak KA *et al. In vitro* rat myocyte cardiotoxicity model for antitumor antibiotics using adenosine triphosphate/protein ratios. *Cancer Res.* 1988; **48**(18): 5222–5227.
121. Takasuka N, White MR *et al.* Dynamic changes in prolactin promoter activation in individual living lactotrophic cells. *Endocrinology* 1998; **139**(3): 1361–1368.
122. Ziady AG, Ferkol T *et al.* Ligand substitution of receptor targeted DNA complexes affects gene transfer into hepatoma cells. *Gene Ther.* 1998; **5**(12): 1685–1697.
123. Kennedy HJ, Rafiq I *et al.* Glucose enhances insulin promoter activity in MIN6 β-cells independently of changes in intracellular Ca^{2+} concentration and insulin secretion. *Biochem J.* 1999a; **342**(Pt 2): 275–280.
124. Kennedy HJ, Pouli AE *et al.* Glucose generates sub-plasma membrane ATP microdomains in single islet β-cells. Potential role for strategically located mitochondria. *J. Biol. Chem.* 1999b; **274**(19): 13281–13291.
125. Goetz AS, Liacos L *et al.* A combination assay for simultaneous assessment of multiple signaling pathways. *J. Pharmacol. Toxicol. Methods* 1999; **42**(4): 225–235.
126. da Silva Xavier G, Varadi A *et al.* Regulation of gene expression by glucose in pancreatic beta-cells (MIN6) via insulin secretion and activation of phosphatidylinositol 3′-kinase. *J. Biol. Chem.* 2000; **275**(46): 36269–36277.
127. da Silva Xavier G, Leclerc I *et al.* Role of AMP-activated protein kinase in the regulation by glucose of islet beta cell gene expression. *Proc. Natl Acad. Sci. USA* 2000; **97**(8): 4023–4028.
128. Leclerc GM, Boockfor FR *et al.* Development of a destabilized firefly luciferase enzyme for measurement of gene expression. *Biotechniques* 2000; **29**(3): 590–591, 594–596, 598 passim.
129. Jouaville LS, Pinton P, Bastianutto C, Rutter GA, Rizzuto R. Regulation of mitochondrial ATP synthesis by calcium: evidence for a long-term metabolic priming. *Proc. Natl Acad. Sci. USA* 1999; **96**(24): 13807–13812.
130. Ainscow EK, Rutter GA. Mitochondrial priming modifies Ca^{2+} oscillations and insulin secretion in pancreatic islets. *Biochem. J.* 2001; **353**(pt 2): 175–180.
131. Craig FF, Simmonds AC, Watmore D, McCapra F, White MRH. Membrane-permeable luciferin esters for assay of firefly luciferase in live intact cells. *Biochem. J.* 1991; **276**: 637–641.
132. Gallie DR, Lucas WJ *et al.* Visualizing mRNA expression in plant protoplasts: factors influencing efficient mRNA uptake and translation. *Plant Cell* 1989; **1**(3): 301–311.
133. Qazi SN, Counil E, Morrissey J *et al. agr* expression precedes escape of internalized *Staphylococcus aureus* from the host endosome. *Infect. Immun.* 2001; **69**(11): 7074–7082.
134. Wood KV, DeLuca M. Photographic detection of luminescence in *Escherichia coli* containing the gene for firefly luciferase. *Anal. Biochem.* 1987; **161**(2): 501–507.
135. Hooper CE, Ansorge RE *et al.* CCD imaging of luciferase gene expression in single mammalian cells. *J. Biolumin. Chemilumin.* 1990; **5**(2): 123–130.
136. Kennedy HJ, Viollet B *et al.* Upstream stimulatory factor-2 (USF2) activity is required for glucose stimulation of L-pyruvate kinase promoter activity in single living islet β-cells. *J. Biol. Chem.* 1997; **272**(33): 20636–20640.
137. Rutter GA, White MR *et al.* Involvement of MAP kinase in insulin signalling revealed by non-invasive imaging of luciferase gene expression in single living cells. *Curr. Biol.* 1995; **5**(8): 890–899.
138. Rutter GA, Burnett P, Rizzuto R *et al.* Subcellular imaging of intramitochondrial Ca^{2+} with recominbinant targed aequorin: significance for the regulation of pyruvate dehydrogenase activity. *Proc. Natl Acad. Sci. USA* 1996; **93**: 5489–5494.
139. White MR, Masuko M *et al.* Real-time analysis of the transcriptional regulation of *HIV* and *hCMV* promoters in single mammalian cells. *J. Cell Sci.* 1995; **108**(pt 2): 441–455.
140. Hill PJ, Eberl L, Molin S, Stewart GSAB. Imaging bioluminescence in single bacteria. In *Bioluminescence and Chemiluminescence: Fundamentals and Applied Aspects. Proceedings of the 8th International Symposium on Bioluminescence and Chemiluminescence 1994*, Campbell AK, Kricka LJ, Stanley PE (eds). Chichester: Wiley, 1994; 629–632.
141. Sternberg C, Eberl L, Poulsen LK, Molin S. Detection of bioluminescence from individual bacterial cells: a comparison of two different low-light imaging systems. *J. Biolumin. Chemilumin.* 1997; **12**: 7–13.
142. Phiefer CB, Palmer RJ, White DC. Comparison of relative photon flux from single cells of the bioluminescent marine bacteria *Vibrio fischeri* and *Vibrio harveyi* using photon-counting microscopy. *Luminescence* 1999; **14**: 147–151.
143. Behrmann G, Hardeland R. Intracellular imaging of bioluminescence in *Pyrocystis noctiluca*. In *Proceedings of the 11th International Symposium on Bioluminescence and Chemiluminescence 2000*, Case JF, Herring PJ, Robison BH, Haddock SHD, Kricka LJ, Stanley PE (eds). Chichester: Wiley, 2001; 19–22.
144. Hooper CE, Ansorge RE *et al.* Low-light imaging technology in the life sciences. *J. Biolumin. Chemilumin.* 1994; **9**(3): 113–122.
145. Nicolas JC. Applications of low-light imaging to life sciences. *J. Biolumin. Chemilumin.* 1994; **9**(3): 139–144.
146. Hohn-Berlage M, Okada Y *et al.* Imaging of brain tissue pH and metabolites. A new approach for the validation of volume-selective NMR spectroscopy. *NMR Biomed* 1989; **2**(5–6): 240–245.
147. Paschen J. Regional quantitative determination of lactate in brain sections. A bioluminescent approach. *Cereb. Blood Flow Metab.* 1985; **5**(4): 609–612.

148. Paschen W. Imaging of energy metabolites (ATP, glucose and lactate) in tissue sections: a bioluminescent technique. *Prog. Histochem. Cytochem.* 1990; **20**(4): 1–122.
149. Leclerc G, Gal D *et al.* Percutaneous arterial gene transfer in a rabbit model. Efficiency in normal and balloon-dilated atherosclerotic arteries. *J. Clin. Invest.* 1992; **90**(3): 936–944.
150. Yao A, Wang DH. Heterogeneity of adenovirus-mediated gene transfer in cultured thoracic aorta and renal artery of rats. *Hypertension* 1995; **26**(6, pt 2): 1046–1050.
151. Mueller-Klieser W, Walenta S. Geographical mapping of metabolites in biological tissue with quantitative bioluminescence and single photon imaging. *Histochem. J.* 1993; **25**(6): 407–420.
152. Tamulevicius P, Streffer C. Metabolic imaging in tumours by means of bioluminescence. *Br. J. Cancer* 1995; **72**(5): 1102–1112.
153. Rembold CM, Kendall JM *et al.* Measurement of changes in sarcoplasmic reticulum [Ca^{2+}] in rat tail artery with targeted apoaequorin delivered by an adenoviral vector. *Cell Calcium* 1997; **21**(1): 69–79.
154. Thierry AR, Rabinovich P *et al.* Characterization of liposome-mediated gene delivery: expression, stability and pharmacokinetics of plasmid DNA. *Gene Ther.* 1997; **4**(3): 226–237.
155. Davis HL, Millan CL *et al.* Immune-mediated destruction of transfected muscle fibers after direct gene transfer with antigen-expressing plasmid DNA. *Gene Ther.* 1997; **4**(3): 181–188.
156. Wisner ER, Aho-Sharon KL *et al.* A modular lymphographic magnetic resonance imaging contrast agent: contrast enhancement with DNA transfection potential. *J. Med. Chem.* 1997; **40**(25): 3992–3996.
157. Contag CH, Spilman SD, Contag PR *et al.* Visualizing gene expression in living mammals using a bioluminescent reporter. *Photochem. Photobiol.* 1997; **66**(4): 523–531.
158. Vizi ES, Liang SD *et al.* Studies on the release and extracellular metabolism of endogenous ATP in rat superior cervical ganglion: support for neurotransmitter role of ATP. *Neuroscience* 1997; **79**(3): 893–903.
159. March KL, Woody M *et al.* Efficient *in vivo* catheter-based pericardial gene transfer mediated by adenoviral vectors. *Clin. Cardiol.* 1999; **22**(1, suppl 1): 123–129.
160. Edinger M, Sweeney TJ *et al.* Noninvasive assessment of tumor cell proliferation in animal models. *Neoplasia* 1999; **1**(4): 303–310.
161. Reynolds PN, Zinn KR *et al.* A targetable, injectable adenoviral vector for selective gene delivery to pulmonary endothelium *in vivo*. *Mol. Ther.* 2000; **2**(6): 562–578.
162. Orson FM, Kinsey BM *et al.* Genetic immunization with lung-targeting macroaggregated polyethyleneimine–albumin conjugates elicits combined systemic and mucosal immune responses. *J. Immunol.* 2000; **164**(12): 6313–6321.
163. Sugihara K, Park HM *et al. In vivo* gene electroporation confers strong transient expression of foreign genes in the chicken testis. *Poult. Sci.* 2000; **79**(8): 1116–1119.
164. Hasan MT, Schonig K *et al.* Long-term, non-invasive imaging of regulated gene expression in living mice. *Genesis* 2001; **29**(3): 116–122.
165. Lipshutz GS, Gruber CA *et al. In utero* delivery of adeno-associated viral vectors: intraperitoneal gene transfer produces long-term expression. *Mol. Ther.* 2001; **3**(3): 284–292.
166. Muramatsu T, Ito N *et al. In vivo* gene electroporation confers nutritionally-regulated foreign gene expression in the liver. *Int. J. Mol. Med.* 2001; **7**(1): 61–66.
167. Yu Y, Caltharp S, Szalay AA. Inducible gene expression *in vivo* using a *Renilla* luciferase–GFP fusion construct. In *Proceedings of the 11th International Symposium on Bioluminescence and Chemiluminescence 2000*, Case JF, Herring PJ, Robison BH, Haddock SHD, Kricka LJ, Stanley PE (eds). Chichester: Wiley, 2001; 465–468.
168. Ow DW, Wood KV, Deluca M, De Wet JR, Helinski DR, Howell SH. Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. *Science* 1986; **234**: 856–859.
169. Koncz C, Schell J. The promoter of TL-DNA gene 5 controls the tissue-specific expression of chimaeric genes carried by a novel type of *Agrobacterium* binary vector. *Mol. Gen. Genet.* 1986; **206**: 383–396.
170. Koncz C, Olsson O, Langridge WHR, Schell J, Szalay AA. Expression and assembly of functional bacterial luciferases in plants. *Plant J.* 1987; **7**(6): 1031–1038.
171. Koncz C, Langridge WHR, Olsson O, Schell J, Szalay AA. Bacterial and firefly luciferase genes in transgenic plants: advantages and disadvantages of a reporter gene. *Dev. Genet.* 1990; **11**: 224–232.
172. Jiang C, Langridge WHR, Szalay AA. Protocol: identification of plant genes *in vivo* by tagging with T-DNA border-linked luciferase genes followed by inverse polymerase chain reaction amplification. *Plant Mol. Biol. Reporter*, 1992; **10**(4): 345–361.
173. Mayerhofer R, Wang G, Hua D *et al.* 1994. Visualization of light emission from different luciferases in transgenic organisms. In *Bioluminescence and Chemiluminescence: Fundamentals and Applied Aspects. Proceedings of the 8th International Symposium on Bioluminescence and Chemiluminescence 1994*, Campbell A, Kricka L, Stanley P (eds). Chichester: Wiley, 1995; 607–612.
174. Millar AJ, Short SR *et al.* A novel circadian phenotype based on firefly luciferase expression in transgenic plants. *Plant Cell* 1992; **4**(9): 1075–1087.
175. Millar AJ, Carre IA *et al.* Circadian clock mutants in *Arabidopsis* identified by luciferase imaging [see comments]. *Science* 1995; **267**(5201): 1161–1163.
176. Kolar C, Fejes E *et al.* Transcription of *Arabidopsis* and wheat Cab genes in single tobacco transgenic seedlings exhibits independent rhythms in a developmentally regulated fashion. *Plant J.* 1998; **13**(4): 563–569.
177. Schutz I, Furuya M. Evidence for type II phytochrome-induced rapid signalling leading to *cab*::luciferase gene expression in tobacco cotyledons. *Planta* 2001; **212**(5–6): 759–764.
178. Michelet B, Chua NH. Improvement of *Arabidopsis* mutant screens based on luciferase imaging in planta. *Plant Mol. Biol. Reporter* 1996; **14**(4): 320–329.
179. Ishitani M, Xiong L *et al.* Genetic analysis of osmotic and cold stress signal transduction in *Arabidopsis*: interactions and convergence of abscisic acid-dependent and abscisic acid-independent pathways. *Plant Cell* 1997a; **9**(11): 1935–1949.
180. Ishitani M, Xiong LM, Stevenson B *et al.* Isolation of stress signal transduction mutants by luciferase imaging in *Arabidopsis thaliana*. *Plant Physiol.* 1997b; **114**(3): 1378–1378.
181. Ishitani M, Xiong L *et al. HOS1*, a genetic locus involved in cold-responsive gene expression in *Arabidopsis*. *Plant Cell* 1998; **10**(7): 1151–1161.
182. Langridge WH, Szalay AA. Bacterial and coelenterate luciferases as reporter genes in plant cells. *Methods Mol. Biol.* 1998; **82**: 385–396.
183. Minko I, Holloway SP *et al. Renilla* luciferase as a vital reporter for chloroplast gene expression in *Chlamydomonas*. *Mol. Gen. Genet.* 1999; **262**(3): 421–425.
184. Jelesko JG, Harper R *et al.* Rare germinal unequal crossing-over leading to recombinant gene formation and gene duplication in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA* 1999; **96**(18): 10302–10307.
185. Xiong LM, David L, Stevenson B *et al.* High throughput screening of signal transduction mutants with luciferase imaging. *Plant Mol. Biol. Reporter* 1999; **17**(2): 159–170.
186. Xiong L, Ishitani M, Zhu JK. Interaction of osmotic stress, temperature, and abscisic acid in the regulation of gene expression in *Arabidopsis*. *Plant Physiol.* 1999; **119**: 205–211.
187. Giacomin LT, Szalay AA. Expression of a PAL1 promoter luciferase gene fusion in *Arabidopsis thaliana* in response to infection by phytopathogenic bacteria. *Plant Sci.* 1996; **116**: 59–72.
188. Grant JJ, Yun BW *et al.* Oxidative burst and cognate redox signalling reported by luciferase imaging: identification of a signal network that functions independently of ethylene, SA and Me-JA but is dependent on MAPKK activity. *Plant J.* 2000; **24**(5): 569–582.
189. Urwin P, Yi L *et al.* Functional characterization of the EMCV IRES in plants. *Plant J.* **24**(5): 583–589.
190. Nass N, Scheel D. Enhanced luciferin entry causes rapid wound-induced light emission in plants expressing high levels of luciferase. *Planta* 2001; **212**(2): 149–154.
191. Meier C, Bouquin T *et al.* Gibberellin response mutants identified by luciferase imaging. *Plant J.* 2001; **25**(5): 509–519.

192. van Leeuwen W, Ruttink T, Borst-Vrenssen AW, van Der Plas LH, van Der Krol AR. Characterization of position-induced spatial and temporal regulation of transgene promoter activity in plants. *J. Exp. Bot.* 2001; **52**(358): 949–959.
193. van Leeuwen W, Mlynarova L, Nap JP, van Der Plas LH, van Der Krol AR. The effect of MAR elements on variation in spatial and temporal regulation of transgene expression. *Plant Mol. Biol.* 2001; **47**(4): 543–554.
194. Tamiya E, Sugiyama T *et al.* Spatial imaging of luciferase gene expression in transgenic fish. *Nucleic Acids Res.* 1990; **18**(4): 1072.
195. Mayerhofer R, Araki K *et al.* Monitoring of spatial expression of firefly luciferase in transformed zebrafish. *J. Biolumin. Chemilumin.* 1995; **10**(5): 271–275.
196. Matsumoto K, Anzai M *et al.* Onset of paternal gene activation in early mouse embryos fertilized with transgenic mouse sperm. *Mol. Reprod. Dev.* 1994; **39**(2): 136–140.
197. Thompson EM, Adenot P *et al.* Real-time imaging of transcriptional activity in live mouse preimplantation embryos using a secreted luciferase. *Proc. Natl Acad. Sci. USA* 1995; **92**(5): 1317–1321.
198. Contag CH, Spilman SD, Contag PR *et al.* Visualizing gene expression in living mammals using a bioluminescent reporter. *Photochem. Photobiol.* 1997; **66**(4): 523–531.
199. Menck M, Mercier Y *et al.* Prediction of transgene integration by noninvasive bioluminescent screening of microinjected bovine embryos. *Transgen. Res.* 1998; **7**(5): 331–341.
200. Nakamura A, Okumura J *et al.* Quantitative analysis of luciferase activity of viral and hybrid promoters in bovine preimplantation embryos. *Mol. Reprod. Dev.* 1998; **49**(4): 368–373.
201. Zhang W, Contag PR, Madan A, Stevenson DK, Contag CH. Bioluminescence for biological sensing in living mammals. *Adv. Exp. Med. Biol.* 1999; **471**: 775–784.
202. Sigworth L, Chandler T, Liao L, Geusz M. Luciferase imaging reveals distinct patterns of gene regulation in live brain slices. In *Proceedings of the 11th International Symposium on Bioluminescence and Chemiluminescence 2000*, Case JF, Herring PJ, Robison BH, Haddock SHD, Kricka LJ, Stanley PE (eds). Chichester: Wiley, 2001; 185–188.
203. Wu JC, Sundaresan G, Iyer M, Gambhir SS. Noninvasive optical imaging of firefly luciferase reporter gene expression in skeletal muscles of living mice. *Mol. Ther.* 2001; **4**(4): 297–306.
204. Chishima T, Miyagi Y *et al.* Cancer invasion and micrometastasis visualized in live tissue by green fluorescent protein expression. *Cancer Res.* 1997a; **57**(10): 2042–2047.
205. Chishima T, Miyagi Y *et al.* Visualization of the metastatic process by green fluorescent protein expression. *Anticancer Res.* 1997b; **17**(4A): 2377–2384.
206. Chishima T, Miyagi Y *et al.* Metastatic patterns of lung cancer visualized live and in process by green fluorescence protein expression. *Clin. Exp. Metastasis* 1997c; **15**(5): 547–552.
207. Contag CH, Jenkins D *et al.* Use of reporter genes for optical measurements of neoplastic disease *in vivo*. *Neoplasia* 2000; **2**(1–2): 41–52 (R).
208. Rehemtulla A, Stegman LD *et al.* Rapid and quantitative assessment of cancer treatment response using *in vivo* bioluminescence imaging. *Neoplasia* 2000; **2**(6): 491–495.
209. Honigman A, Zeira E *et al.* Imaging transgene expression in live animals. *Mol. Ther.* 2001; **4**(3): 239–249.
210. Legocki RP, Legocki M, Baldwin TO, Szalay AA. Bioluminescence in soybean root nodules: demonstration of a general approach to assay gene expression *in vivo* by using bacterial luciferase. *Proc. Natl Acad. Sci. USA* 1986; **83**: 9080–9084.
211. O'Kane DJ, Lingle WL, Wampler JE, Legocki M, Legocki RP, Szalay AA. Visualization of bioluminescence as a marker of gene expression in *Rhizobium*-infected soybean root nodules. *Plant Mol. Biol.* 1988; **10**: 387–399.
212. Rodriguez JF, Rodriguez D, Rodriguez JR, McGowan EB, Esteban M. Expression of the firefly luciferase gene in vaccinia virus: a highly sensitive gene marker to follow virus dissemination in tissues of infected animals. *Proc. Natl Acad. Sci USA* 1988; **85**(5): 1667–1671.
213. Contag CH, Contag PR *et al.* Photonic detection of bacterial pathogens in living hosts. *Mol. Microbiol.* 1995; **18**(4): 593–603.
214. Francis KP, Joh D, Bellinger-Kawahara C, Hawkinson MJ, Purchio TF, Contag PR. Monitoring bioluminescent *Staphylococcus aureus* infections in living mice using a novel *luxABCDE* construct. *Infect. Immun.* 2000; **68**(6): 3594–3600.
215. Francis KP, Yu J, Bellinger-Kawahara C *et al.* Visualizing pneumococcal infections in the lungs of live mice using bioluminescent *Streptococcus pneumoniae* transformed with a novel gram-positive *lux* transposon. *Infect. Immun.* 2001; **69**(5): 3350–3358.
216. Rocchetta HL, Boylan CJ, Foley JW *et al.* Validation of a noninvasive, real-time imaging technology using bioluminescent *Escherichia coli* in the neutropenic mouse thigh model of infection. *Antimicrob. Agents Chemother.* 2001; **45**: 129–137.
217. Mettenleiter TC, Gräwe W. Video imaging of firefly luciferase activity to identify and monitor herpesvirus infection in cell culture. *J. Virol. Methods* 1996; **59**(1–2): 155–160.
218. Langridge WH, Krausova VI *et al.* Detection of baculovirus gene expression in insect cells and larvae by low light video image analysis. *J. Virol. Methods* 1996; **61**(1–2): 151–156.
219. Kratzer S, Mundigl O *et al.* Digital imaging microscopy of firefly luciferase activity to directly monitor differences in cell transduction efficiencies between AdCMVLuc and Ad5LucRGD vectors having different cell binding properties. *J. Virol. Methods* 2001; **93**(1–2): 175–179.
220. Costa GL, Sandora MR, Nakajima A *et al.* Adoptive immunotherapy of experimental autoimmune encephalomyelitis via T Cell delivery of the IL-12 p40 subunit. *J. Immunol.* 2001; **167**(4): 2379–2387.
221. Shabahang S, Szalay AA. Visualization of bacteria in live animals using luciferase labeling. In *Proceedings of the 11th International Symposium on Bioluminescence and Chemiluminescence 2000*, Case JF, Herring PJ, Robison BH, Haddock SHD, Kricka LJ, Stanley PE (eds). Chichester: Wiley, 2001; 449–452.
222. Timiryasova T, Yu Y, Shabahang S, Fodor I, Szalay AA. Visualization of vaccinia virus infection using the *Renilla* luciferase–GFP fusion protein. In *Proceedings of the 11th International Symposium on Bioluminescence and Chemiluminescence 2000*, Case JF, Herring PJ, Robison BH, Haddock SHD, Kricka LJ, Stanley PE (eds). Chichester: Wiley, 2001; 457–460.
223. Johnston TC, Hruska KS, Adams LF. The nucleotide sequence of the luxE gene of *Vibrio harveyi* and a comparison of the amino acid sequences of the acyl-protein synthetases from *V. harveyi* and *V. fischeri*. *Biochem. Biophys. Res. Commun.* 1989; **163**: 93–101.
224. Foran DR, Brown WM. Nucleotide sequence of the luxA and luxB genes of the bioluminescent marine bacterium *Vibrio fischeri*. *Nucleic Acids Res.* 1988; **16**(2): 777.
225. Fischer-Le Saux M, Viallard V, Brunel B *et al.* Polyphasic classification of the genus *Photorhabdus* and proposal of new taxa: *P. luminescens* subsp. *luminescens* subsp. nov., *P. luminescens* subsp. *akhurstii* subsp. nov., *P. luminescens* subsp. *laumondii* subsp. nov., *P. temperata* sp. nov., *P. temperata* subsp. *temperata* subsp. nov. and *P. asymbiotica* sp. nov. *Int. J. Syst. Bacteriol.* 1999; **49**: 1645–1656.
226. de Wet JR, Wood KV, DeLuca M *et al.* Firefly luciferase gene: structure and expression in mammalian cells. *Mol. Cell. Biol.* 1987; **2987**(7): 725–737.
227. Masuda T, Tatsumi H, Nakano E. Cloning and sequence analysis of cDNA for luciferase of a Japanese firefly, *Luciola cruciata. Gene* 1989; **77**: 265–270.
228. Furederitsuku IT, Osada J, Eritsuku MT. DNA compound coding luciferase and manifestation vector containing the same compound. Patent: JP 1991030678-A 1.08 Feb 1991.
229. Prasher DC, McCann RO, Longiaru M, Cormier MJ. Sequence comparisons of complementary DNAs encoding aequorin isotypes. *Biochemistry* 1987; **26**: 1326–1332.
230. Inouye S, Noguchi M, Sakaki Y, *et al.* Cloning and sequence analysis of cDNA for the luminescent protein aequorin. *Proc. Natl. Acad. Sci. USA* 1985; **82**: 3154–3158.
231. Takagi Y, Sakaki Y, Inoue S, *et al.* Biosynthesis of photoprotein aequorin. Patent: JP 1990096597-A 1.09 Apr-1990; CHISSO CORP.

