Reviews

PET Imaging of Transgene Expression

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A vital step in transgenic animal study and gene therapy is the ability to assay the extent of transgene expression. Unfortunately, classic methods of assaying transgene expression require biopsies or death of the subject. We are developing techniques to noninvasively and repetitively determine the location, duration, and magnitude of transgene expression in living animals. This will allow investigators and clinicians to assay the effectiveness of their particular experimental and therapeutic paradigms. Of radionuclide (single photon emission computed tomography, positron emission tomography [PET]), optical (green fluorescent protein, luciferase), and magnetic (magnetic resonance imaging) approaches, only the radionuclide approach has sufficient sensitivity and quantitation to measure the expression of genes in vivo. We describe the instrumentation involved in high resolution PET scanning. We also describe the principles of PET reporter gene/reporter probe in vivo imaging, the development of two in vivo reporter gene imaging systems, and the validation of our ability to noninvasively, quantitatively, and repetitively image gene expression in murine viral gene transfer and transgenic models. We compare the two reporter gene systems and discuss their utility for the study of transgenic animals and gene therapies. Finally, we mention alternative approaches to image gene expression by using radiolabeled antibody fragments to image specific proteins and radiolabeled oligonucleotides to image RNA messages directly. Biol Psychiatry 2000;48:337–348 © 2000 Society of Biological Psychiatry

Key Words: Transgene imaging, PET, gene expression, herpes simplex virus thymidine kinase, dopamine 2 receptor, reporter gene

Introduction

In this review we describe the technology involved in monitoring reporter gene expression in living animals, using positron emission tomography (PET). First we address the biological and clinical significance of studying gene expression in mouse model systems and how it applies to human diseases. Then we cover the technology involved in imaging gene expression using classic (e.g., β-galactosidase [βgal], alkaline phosphatase [AP], luciferase, fluorescent proteins) and radionuclide (e.g., PET) methods. We also describe and compare current PET reporter gene imaging methods presently in use to quantitatively assay gene expression in living animals. We then comment on potential new technologies to image gene expression and, finally, discuss the general state, utility, and future of using PET to image gene expression.

Human Diseases and Mouse Model Systems

Advances in molecular biology have allowed us to investigate the function of genes and the role of various regulatory/promoter regions in human diseases by studying their role in mouse model systems. For example, mutations in the p53 gene can lead to many cancers in both human and murine subjects (Culver and Blaese 1994; Dasika et al 1999; Lewin 1994). Studies of the promoter and regulatory regions of genes in mouse models have also led to an understanding of human diseases where the expression of the gene under consideration is either attenuated or accentuated. Following gene therapy in animal models of Parkinson’s and Alzheimer’s diseases could lead to treatments for human patients.

Reporter Genes

The role and function of the promoter and regulatory regions of genes are often measured by their regulated expression of a reporter gene (e.g., βgal and AP; Forss-Petter et al 1990; Lewin 1994; Naciff et al 1999). The reporter gene can either be fused to the gene of interest to make a chimeric protein of questionable function or be expressed as a separate protein. In the case where the reporter gene is expressed as a separate protein, its expression can be controlled by the same but separate promoter, or the reporter gene can be expressed as part of a bicistronic message through the use of an internal
ribosome entry site (IRES; Levenson et al 1998). Unfortunately, assaying these classic reporter gene methods requires biopsies or even death of the subject, and thus leaves out the possibility of true noninvasive longitudinal studies. Methods to image gene expression in animals that are small or largely transparent to visible light include the use of fluorescent proteins (e.g., green fluorescent protein; Misteli and Spector 1997) and luciferases (firefly and renilla luciferases; Nishiyama et al 1985). Technologies have also been developed to qualitatively image luciferase gene expression in small animals such as mice by using systemic delivery of an enzyme substrate (e.g., luciferin) and extremely sensitive cameras (Contag et al 1998; Sweeney et al 1999). A method applied more recently to noninvasively, repetitively, and quantitatively image gene expression uses PET reporter genes.
and radio-labeled PET reporter probes (for reviews, see Gambhir et al 1999a; Herschman et al 2000).

**PET Reporter Genes**

Positron emission tomography reporter genes encode receptors that bind positron-emitting ligand probes or enzymes that modify the positron-emitting substrate probes to produce sequestered positron-emitting products. Cells expressing the PET reporter gene will sequester the radiolabel of the PET reporter probe 1) as a ligand bound to the PET reporter receptor or 2) as a “trapped” product of the enzymatic reaction of the PET reporter enzyme (Figure 1; also reviewed in Gambhir et al 1999a; Herschman et al 2000). Ideally, those cells not expressing the PET reporter genes will not retain the PET reporter probe. Since positron-emitting radionuclides result in the creation of high-energy gamma rays (511 keV) by positron–electron annihilation, the animal is largely transparent to the wavelength of the radiation produced, and visualization of radiolabeled probe/ligand accumulation is readily obtained in even deep and visually opaque tissues. Emission computed tomography then allows quantitative imaging of the accumulation of the PET reporter probe and, in turn, the expression levels of the PET reporter gene. Since PET imaging does not require obtaining tissue samples from the subject, this system is noninvasive and can be used to repetitively measure reporter gene expression in vivo.

It is also important to note that radionuclide-based methods offer significant advantages over optical- (Contag et al 1998) and magnetic resonance imaging–based (Bogdanov and Weissleder 1998) approaches for imaging reporter gene expression. Radionuclide-based methods offer the highest level of sensitivity for imaging relatively low levels of reporter gene expression—as low as $10^{-12}$ mol/L of radiolabeled substrate (Phelps 1991; Phelps et al 1986). This high degree of sensitivity may allow the use of relatively weak promoters and the imaging of relatively low levels of gene expression. Furthermore, radionuclide-based methods are highly quantitative. Since PET allows for the quantitation of absolute levels of radionuclide probes, dynamic imaging and kinetic modeling allow us to obtain the rate constants of the underlying biochemical processes (Green et al 1998; Huang and Phelps 1986).

One of the most basic considerations is whether the reporter gene is endogenous or exogenous for the organism under scrutiny. Expression of endogenous genes has the advantage of not inducing an immune response and thus allowing for repeated studies. A possible complication in using an endogenous gene as a PET reporter transgene is a high background or erroneous signal due to its inherent expression. Exogenous genes, especially in gene therapy use, have the disadvantage of inciting an immune response that might limit their repeated application, unless, of course, one wishes to image the immune response itself. Exogenous PET reporter genes, with the appropriate probe, have the advantage of only producing signal in the tissues in which they are expressed. An ideal reporter gene for longitudinal studies should therefore produce no immune response and not be normally expressed in the organism—or at least in the organ(s) under consideration.

**Direct and Indirect Reporter Transgene Strategies**

Direct imaging strategies are based on imaging the transgene product directly by binding of a radiolabeled ligand or probe directly to the gene product (e.g., messenger RNA [mRNA], dopamine 2 receptor [D₂R]; MacLaren et
al 1999). Another “direct” approach involves an enzymatic strategy where a radiolabeled probe is modified and trapped in cells expressing the PET reporter gene (e.g., HSV1-tk; Gambhir et al 1998a, 1999d, 2000a; Tjuvajev et al 1995, 1998).

Most therapeutic transgenes do not lend themselves to direct imaging of the transgene product. This is because most therapeutic transgene products lack appropriate radiolabeled probes. In addition, it would be very time consuming and inefficient to develop and validate new probes for each therapeutic transgene. Alternatively, it is both feasible and reasonable to develop and validate “indirect” imaging strategies using a reporter gene in combination with a therapeutic gene. The advantage of this paradigm is that a given reporter gene can be coupled with any therapeutic gene. Two strategies have been discussed: one uses a fusion gene containing complementary DNA from both reporter and therapeutic genes, and the second strategy has a separate reporter gene and therapeutic gene on the same mRNA transcript (cis-linked). Both strategies are based on demonstrating a proportional and constant relationship in the coexpression of two transgenes over a wide range of expression levels.

Researchers have described and validated the proportional expression of two cis-linked genes, using an IRES element within a single bicistronic transcription unit (Tjuvajev et al 1999; Yu et al 2000). The IRES element enables translation initiation at a downstream start codon within the bicistronic mRNA, thus permitting gene coexpression by cap-dependent translation of the first cistron and cap-independent, IRES-mediated translation of the second cistron (Figure 2; Ghattas et al 1991; Jackson and Kaminski 1995; Pelletier 1988; Sachs et al 1997). These studies show that coexpression of the genes is proportional and quantitative and also demonstrate the potential for monitoring therapeutic gene transfer and expression by noninvasive imaging of cis-linked PET reporter genes (Tjuvajev et al 1999; Yu et al 2000).

**PET Instrumentation**

Because of their short breeding span, extensively characterized genetics, and readily manipulatable genome, mice have become the primary platform for most whole-animal research in gene expression, gene transfer, and models of human disease. The desire to image mouse models of human disease has led to rapidly increasing interest and efforts in developing imaging technologies that can measure the distribution of radiolabeled tracers in vivo in the mouse (Weber and Ivanovic 1999). Autoradiography is a well-established technique that requires killing the animal of interest (Lear 1986, 197–235) and placing tissue slices in direct contact with analog or digital film. Although autoradiography has and continues to play a key role, noninvasive approaches are highly desirable for applications in which the same animal needs to be repeatedly evaluated, or for applications in which it is too expensive to study large sets of animals at various time points. Furthermore, for human applications, although tissue biopsies can be performed, whole-body noninvasive imaging is much more compatible with the patient’s comfort and safety. In essence, the goal for radionuclide-based imaging instrumentation has been to develop a noninvasive in vivo analogue of autoradiography, with sufficient spatial resolution to resolve the structures of interest in a mouse and with sufficient sensitivity that high signal-to-noise images can be obtained.

Radiotracer imaging technologies that can measure the distribution of radiolabeled tracers in the human body are widely available and have a wide range of clinical and research applications. Two classes of clinical nuclear imaging systems exist—those designed to image single gamma-emitting radionuclides (e.g., technetium-99m, iodine-131) and those designed to image positron-emitting radionuclides (e.g., fluorine-18, carbon-11, nitrogen 13, oxygen-15, copper-64, iodine-124). The former is known as single photon imaging or, when performed tomographically, single photon emission computed tomography (SPECT). The latter is known as PET. In general, PET has greater spatial resolution and higher sensitivity and is easier to quantify than SPECT. For the sake of brevity, this review focuses on PET, though most of the discussion is also applicable to other radionuclide systems.

The realization of the potential power of using PET reporter genes in animal research, together with several technological innovations, has led to the development of dedicated animal PET scanners by a number of research centers in the past 5 years. The first system designed specifically for rodent imaging was the RAT-PET system developed at Hammersmith Hospital (Bloomfield et al 1995). Although this system was limited to a resolution in the 3–4-mm range, it established the principle of using a dedicated PET scanner for imaging small animals. RAT-PET, despite its relatively coarse spatial resolution, clearly demonstrated the ability of PET to obtain relevant biological information from the dopaminergic system of the rat using highly specific radiotracers (Fricker et al 1997; Hume et al 1996). These results encouraged a number of research groups worldwide to develop very high resolution PET systems (Cherry et al 1997), with a focus on the opportunities afforded by the sophistication of genetic manipulation techniques in the mouse (Fries et al 1997; Jeavons et al 1999; Marriott et al 1994; Weber et al 1997). The latest example of a dedicated small animal PET system is the microPET system (Cherry et al 1997).
microPET scanner has a reconstructed image resolution of 1.8 mm in all three axes and has been shown to be fully quantitative (Chatziioannou et al 1999). The volumetric resolution is more than an order of magnitude better than state-of-the-art clinical PET systems, as illustrated by the images in Figure 3.

**Gene Therapy and Reporter Genes**

Our understanding of molecular biology in the last decades allowed us to create transgenic animals as well as to introduce genes into living animals (i.e., gene therapy). A vital step in either of these processes is the ability to assay for the expression of the transgene. Thus, the use of PET reporter genes can play critical roles in developing gene therapies by allowing researchers to determine the location, duration, and expression level of the transferred DNA and, specifically, 1) develop vector modifications to improve delivery, 2) control expression levels, and 3) improve treatments to control duration of expression. Applications of somatic gene transfer technology to treat diseases are at the forefront of gene therapy applications; as a result, these issues are of great interest to life scientists and clinicians. The repeatability, quantifiability, and high sensitivity of PET reporter gene systems should lead to rapid advancements in science and medicine.

**Herpes Simplex Virus 1 Thymidine Kinase (HSV1-tk), an Enzymatic PET Reporter Gene**

Herpes simplex virus 1 thymidine kinase, like mammalian TKs, phosphorylates thymidine, but unlike mammalian TKs, HSV1-TK has relaxed substrate specificity and so phosphorylates thymidine analogues (e.g., 5-ido-2'-deoxy-1-β-D-arabinofuranosyluracil [FIAU]) as well as acycloguanosine analogues (e.g., acyclovir, ganciclovir [GCV], penciclovir [PCV]; Namavari et al 2000). Cellular enzymes then convert acycloguanosine monophosphates and the monophosphate of FIAU to di- and triphosphates, which have been shown to kill cells by incorporation as chain-terminating derivatives and/or by inhibition of DNA polymerase; however, at the concentrations of tracer used for imaging by PET, the derivatives have no discernable effect upon the cells or the health of patients (for review, see Gambhir et al 2000b). Herpes simplex virus 1 thymidine kinase has been extensively studied; it is nontoxic in humans and is currently being used as a “susceptibility” gene (in combination with GCV) in clinical gene therapy protocols. Herpes simplex virus 1 thymidine kinase can be used as a reporter gene as well as a therapeutic gene (Borrelli et al 1988; Culver et al 1992; Moolten 1997; Moolten and Wells 1990). In gene therapy protocols using HSV1-tk as a susceptibility gene, identifying the location and magnitude of HSV1-TK expression by noninvasive imaging would provide a highly desirable measure of expression (following successful gene transfection) and a basis from which the timing of GCV treatment can be optimized. This represents an ideal situation where the therapeutic and reporter genes are the same, and is an example of a direct imaging approach.

Two main categories of substrates have been investigated as reporter probes for imaging HSV1-tk reporter gene expression: derivatives of uracil nucleoside (e.g., FIAU radiolabeled with iodine; Morin et al 1997; Tjuvajev et al 1995) and derivatives of guanosine radiolabeled with fluorine-18 or carbon-11 (Alauddin et al 1996; Barrio et al 1996a, 1996b, 1996c, 1997; Monclus et al 1995). These two major classes of reporter probes share the ability to be phosphorylated by HSV1-TK, leading to their accumulation in cells by DNA polymerase.

**Thymidine Derivative Reporter Probes**

Using a cell culture model, researchers investigated three compounds (FIAU, iododeoxyuridine, and GCV) as potential HSV1-tk reporter probes and found FIAU to have the best imaging potential, based upon its in vitro characteristics for HSV1-TK and its ability to be labeled with several different nuclides (Tjuvajev et al 1995). Radiolabeled FIAU has been used in cell culture and in vivo as an agent for imaging gene expression by both SPECT and PET systems (Tachizawa et al 1981; Tjuvajev et al 1995, 1996). Imaging HSV1-TK expression in cancer patients undergoing combined HSV1-tk–GCV gene therapy with PET or SPECT has also been evaluated and shown to be feasible (Blasberg and Tjuvajev 1997).

**Guanosine Derivative Reporter Probes**

Another approach developed to image reporter gene expression also relies on the HSV1-tk reporter gene but utilizes acycloguanosine (e.g., radiolabeled GCV/PCV) derivatives as reporter probes (Gambhir et al 1998a, 1999d). The choice of acycloguanosines as potential probes was based on their ability to be radiolabeled with the short half-life (110 min) isotope fluorine-18, thus allowing rapid repeated PET imaging of HSV1-tk gene expression. Initial cell culture uptake experiments with 8-[18F]fluoracyclovir showed poor performance and were not pursued further (Srinivasan et al 1996). Subsequent investigations using 8-[14C]GCV and 8-[18F]Fluoroganciclovir (FGCV) as reporter probes showed better performance (Gambhir et al 1998a, 1999d). In further studies to test reporter probes in vivo for HSV1-tk, a replication-deficient adenovirus expressing the HSV1-tk PET reporter gene was injected into mice. Because adenovirus accumu-
lates on hepatocytes in large part due to the presence of coxsackie and adenoviral receptors (Haisma et al. 1999), the majority of the injected adenovirus (>95%) infects the liver. Administration of radiolabeled reporter probes showed accumulation of PET signal only in livers expressing HSV1-TK (Figure 4), thus validating the applicability of using radiolabeled acycloguanosine derivatives to image HSV1-tk as a PET reporter gene (Gambhir et al. 1998a, 1998b, 1999c, 1999e). Panels B and C of Figure 4 also show the linear relationship between the amount of FGCV signal to the HSV1-tk activity and mRNA levels.

Reporter probes using fluorine-18 in the side chain of GCV (FHPG; Alauddin et al. 1996; Bading et al. 1997; Monclus et al. 1997) and in the side chain of PCV (FHBG; Alauddin and Conti 1998) are also being studied and have shown that high specific activity (250–500 Ci/mmol)

Figure 5. Fluoropenciclovir (FPCV) and the side chain of PCV (FHBG) positron emission tomography (PET) images of a transgenic mouse expressing herpes simplex virus 1 thymidine kinase (HSV1-tk) in the liver. We studied a transgenic mouse in which the albumin promoter drives the HSV1-tk reporter gene. The mouse was imaged on day 0 with a microPET 1 hour after administration of FPCV and on day 1 with FHBG. Both images are displayed using the same common global maximum and illustrate the higher percent injected dose retained per gram of liver tissue (%ID/g) when utilizing FHBG. There is significantly greater hepatic accumulation when using FHBG (8–11% ID/g), as compared with FPCV (3–6% ID/g).

Figure 6. 3-(2'-[18F]Fluoroethyl)sipiperone (FESP) can be used as a reporter probe to image dopamine 2 receptor (D2R) positron emission tomography (PET) reporter gene expression in living mice. Nude mice were injected via the tail vein with 1 × 10^9 plaque-forming units of adenovirus expressing either β-galactosidase (Ad-βGal) (A) or the dopamine 2 receptor (Ad-D2R) (B). Two days later, both mice were injected with FESP and imaged using microPET. For each mouse, a whole-body coronal projection image of the [18F] activity distribution is displayed on the left. The liver outline, in white, was determined from both the FESP signal and cryostat slices. The second images from the left are coronal sections, approximately 2 mm thick, from the microPET. After their PET scans, the mice were killed, frozen, and sectioned. The next images are photographs of the tissue sections (0.2-mm thickness) corresponding to approximately the thickness of the microPET coronal section. The images on the right are autoradiographs of these tissue sections. The color scale represents the percent injected dose per gram of tissue (%ID/g). Images are displayed on the same quantitative color scale, to allow signal intensity comparisons among the panels.
[18F]fluorinated acycloguanosines can be synthesized in relatively high yields (5–15 mCi). The side chain of GCV and FHBG have been studied in cell culture models and in vivo, and are also well suited for imaging HSV1-tk gene expression (Gambhir et al 2000b).

**Comparison of HSV1-tk Reporter Probes**

Direct comparison of all the radiolabeled probes for in vivo imaging of HSV1-tk reporter gene expression is a necessary step for optimizing this PET reporter system. The UCLA group has compared uptake of FGCV, FPCV, FHBG, FHPG, and [14C]-FIAU in C6 cells expressing HSV1-tk. These preliminary data show that FIAU and FHBG are the better candidates for imaging HSV1-tk reporter gene expression because of their 1) long half-life in vivo, 2) low nonspecific retention, and 3) high specific retention. The true utility of alternative HSV-TK probes must ultimately be evaluated in whole animals, as issues such as stability, substrate competition, routes of clearance, and rates of cellular transport all come into play. A transgenic mouse model in which the albumin promoter drives the HSV1-tk reporter gene is being studied at UCLA (Gambhir et al 2000b; Herschman et al 2000). These transgenic mice have been imaged by PET and clearly demonstrate accumulation of the FPCV (3–6% injected dose retained per gram of tissue [ID/g]) and FHBG (8–11% ID/g) reporter probes in the mouse liver (Figure 5). Future studies that directly compare all prospective acycloguanosines and thymidine analogues in whole animals will help to better define the advantages and disadvantages for each probe.

**Mutant HSV1-tk Reporter Gene Approaches**

Researchers at UCLA are also investigating mutant HSV1-tk reporter genes to further enhance the sensitivity of the HSV1-tk reporter assays (Gambhir et al 2000a). We are using a mutated HSV1-TK enzyme (HSV1-sr39TK) that utilizes GCV and PCV substrates more effectively and thymidine less effectively than the wild-type HSV1-TK enzyme (Black et al 1996). Mouse models with an adenovirus expressing this mutant HSV1-TK enzyme demonstrated improved imaging sensitivity (equivalent to two- to threefold) with both FGCV and FPCV as PET reporter probes. Additional studies with FHBG show a further enhancement by a factor of ~2 as well. Taken together, the mutant HSV1-tk with FHBG should offer a greater than fourfold improvement in sensitivity and place it on par with the sensitivity of the D3R/FESP PET reporter system.

**D3R, a Receptor PET Reporter Gene**

Another PET reporter gene system investigated uses D3R and the radiolabeled ligand 3-(2′-[18F]fluoroethyl)spiperone (FESP), a positron-emitting analogue of the dopamine antagonist spiperone. The latter was originally developed as a probe to image, by PET, the D3Rs of the striatum (Barrio et al 1989), where concentrations of D3R as low as 2–20 nmol/L can be detected. This PET reporter gene system was also investigated because of the high binding affinities of D3R to FESP and, unlike substrates for HSV1-tk, the ability of FESP to diffuse into every tissue in the body (Barrio et al 1989).

As for the HSV1-tk system, we used adenovirus delivery of the D3R reporter gene to demonstrate the noninvasive, repetitive, and quantitative ability of the D3R/FESP PET reporter gene/PET reporter probe in vivo imaging system. To determine whether PET can quantitatively monitor hepatic D3R expression in adenovirus-infected mice, animals were injected with varying amounts of D3R-expressing or βgal-expressing (control) virus, then injected with FESP and imaged by PET (Figure 6; MacLaren et al 1999). After scanning, the mice were killed and liver samples were analyzed for 1) FESP retention by ether extraction and [fluorine-18] well counting, 2) functional D3R protein levels by [3H]spiperone binding using a conventional receptor binding assay, and 3) D3R mRNA levels by quantitative Northern blots. The fluorine-18 signal retained in liver, as determined by region of interest measurements of PET images of living mice, is proportional to both the amount of hepatic FESP present and functional D3R protein levels (Figure 7). In vivo PET analysis of hepatic D3R reporter gene expression accurately reflects in vitro determinations of D3R levels and validates the use of this PET reporter gene/probe system.

**Comparison of HSV1-tk and D3R Reporter Gene Systems**

The reporter gene assays developed to date fall into the two main categories enzyme based (e.g., HSV1-tk) and receptor based (e.g., D3R). Each of these assays has some distinct features that deserve special comment. An enzyme-based approach has the theoretical advantage of signal amplification, since one molecule of reporter enzyme is capable of acting on many molecules of reporter probes. Most receptor-based assays, such as the D3R system, are capable of only a one-to-one stoichiometric interaction of reporter ligand with receptor. Enzyme-based approaches will always require intracellular transport of the reporter substrate, and the rate of transport may change independent of levels of reporter.
gene expression, thereby making it more difficult to quantitate the signal observed. Receptor-based approaches in which the receptor is primarily limited to the cell surface have the unique advantage of not requiring reporter probe transport.

Our current mutant HSV1-tk/FHBG and D_{2R}/FESP PET reporter gene imaging systems now have essentially equivalent sensitivities, ~20% ID/g in the liver (unpublished data); however, each of these systems has distinct advantages and disadvantages. The HSV1-tk reporter gene does not meet all the requirements of an ideal system. Expression of HSV1-tk may lead to an immune response, which is not optimal from the perspective of gene therapy. An additional limitation of the HSV1-tk system is that reporter probes for this system do not significantly penetrate the intact blood–brain barrier, thus preventing the easy utilization of the HSV1-tk reporter gene imaging system for in vivo imaging of the brain. The probe used for imaging the D_{2R} gene, FESP, has no such barrier limitations and penetrates into all tissues. Because the D_{2R} gene is normally present in the mammalian genome there are no issues of an immune response, but it also does not meet all the requirements of an ideal system. Being an endogenous gene leads to a problem of background signal in the striatum where D_{2R} is normally expressed; however, this may be viewed as an advantage, as the signal in the striatum may serve as an internal control for measuring D_{2R} reporter gene expression in other tissues. Herpes simplex virus 1 thymidine kinase expression, in the absence of acycloguanosines, has little or no effect on cells and tissues. In contrast, occupancy by endogenous agonists of the ectopic wild-type D_{2R}, either in transgenic animals or in gene therapy delivery vehicles, might have physiologic consequences because ligand-activated D_{2R} regulates intracellular cyclic adenosine monophosphate levels. Fortunately, D_{2R} structure–function studies have identified amino acids that uncouple receptor occupancy from intracellular signaling (Cox et al 1992; Neve et al 1991; Woodward et al 1996), and adenovirus strains with these D_{2R} mutations are being developed to test the efficacy of these mutant receptors as PET reporter genes.

Alternative PET Gene Imaging Methods

There are other potential methods to image gene expression that warrant mention. One is by using radiolabeled modified oligonucleotides or small molecules to bind to the mRNA transcripts directly (Charlton et al 1997; Gambhir et al 1999b; Kobori et al 1999; Mannironi et al 1997; Pan et al 1998; Tavitian et al 1998). The mRNA transcripts themselves can also be engineered or selected so that sections of them bind to specific radiolabeled small molecules (e.g., RNA aptamers; Werstuck and Green 1998). A second method is to radiolabel antibodies or antibodylike constructs that target specific cell surface–expressed proteins (Govindan et al 1996; Hu et al 1996).

Discussion and Future Directions

There are a number of scenarios in which the use of two in vivo reporter gene imaging systems will be of consider-
able utility in research protocols. For example, the ability to image two distinct reporter genes in vivo will allow direct comparisons, in the same individual, of alternative gene delivery vehicles (e.g., viruses, liposomes) in somatic gene transfer protocols. Many combinatorial applications of these in vivo PET reporter gene imaging systems are likely to emerge as their availability and utility become apparent. The advantages of high sensitivity, quantitative capability, and direct ability to translate the developed assays from animal to human studies will keep radionuclide-based approaches at the forefront of imaging gene expression for transgenic and gene therapy studies.

Continued development of reporter genes and corresponding reporter probes will be needed to produce more optimal assays; however, for many applications the systems developed to date appear sufficiently robust. The major applications of PET reporter gene imaging in vivo are likely to be 1) repetitive, quantitative monitoring of the location, duration, and extent of gene expression from gene therapy vehicles, using bicistronic vectors that express therapeutic and reporter gene products from a common transcript, and 2) the evaluation of gene function and the role of promoter/regulatory elements by reporter gene expression in transgenic animals during longitudinal experiments.

There are also improvements in PET instrumentation—a “second-generation” microPET instrument, currently being developed, will provide a much higher resolution of 1 mm for each axis and be able to scan an entire mouse in 10 min. Positron emission tomography technology is still a long way from reaching the resolution and sensitivity limits imposed by geometric considerations and the physics of the positron annihilation process (Chatziioannou et al 1999; Cherry et al 1997). The performance of dedicated animal PET scanners is likely to improve substantially in the next few years and will be accompanied by a reduction in both their size and their cost.

The combination of improved PET reporter gene imaging systems and PET instrumentation will allow investigators to readily image and quantitatively evaluate gene expression in transgenic animals and gene therapy subjects. This will ultimately allow doctors to follow the effectiveness of gene therapy for many disorders, including neurodegenerative diseases such as Parkinson’s and Alzheimer’s.

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These studies were supported by Department of Energy contract DE-FC03-78ER60615, National Institutes of Health Grant No. ROI CA85472-01 (HRH), the University of California Biotechnology Program, the UCLA Gene Medicine Program, the Dana Foundation, and the UCLA–Jonsson Comprehensive Cancer Center.


