

# Molecular Imaging in the Age of Genomic Medicine

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## Abstract

The convergence of molecular and genetic disciplines with non-invasive imaging technologies has provided an opportunity for earlier detection of disease processes which begin with molecular and cellular abnormalities. This emerging field, known as molecular imaging, is a relatively new discipline that has been rapidly developed over the past decade. It endeavors to construct a visual representation, characterization, and quantification of biological processes at the molecular and cellular level within living organisms. One of the goals of molecular imaging is to translate our expanding knowledge of molecular biology and genomic sciences into good patient care. The practice of molecular imaging is still largely experimental, and only limited clinical success has been achieved. However, it is anticipated that molecular imaging will move increasingly out of the research laboratory and into the clinic over the next decade. Non-invasive *in vivo* molecular imaging makes use of nuclear, magnetic resonance, and *in vivo* optical imaging systems. Recently, an interest in Positron Emission Tomography (PET) has been revived, and along with optical imaging systems PET is assuming new, important roles in molecular genetic imaging studies. Current PET molecular imaging strategies mostly rely on the detection of probe accumulation directly related to the physiology or the level of reporter gene expression. PET imaging of both endogenous and exogenous gene expression can be achieved in animals using reporter constructs and radio-labeled probes. As increasing numbers of genetic markers become available for imaging targets, it is anticipated that a better understanding of genomics will contribute to the advancement of the molecular genetic imaging field. In this report, the principles of non-invasive molecular genetic imaging, its applications and future directions are discussed.

**Keywords:** molecular genetic imaging, reporter gene imaging, probe, PET, genomics

## Introduction

All human characteristics depend on a myriad of biochemical reactions that occur many millions of times per minute within the cells and tissues of the body. An unbalanced biochemical process can lead to diseases which exhibit structural abnormalities. Conventional imaging techniques have mostly relied on nonspecific macroscopic anatomical changes to detect pathological changes in tissues. In contrast, molecular imaging allows for identification of specific molecular events which can cause disease. This is a significant change in the imaging paradigm because earlier detection, characterization, and monitoring of treatment procedures can be achieved easily. The advent of molecular imaging is largely due to the recent convergence of molecular and genetic disciplines, and non-invasive imaging technologies that have established new standards for biomedical practice.

Molecular imaging offers distinct advantages when compared to *in vitro* tissue culture studies or *in vivo* animal experiments. It allows for the non-invasive, repetitive imaging of targeted macromolecules and biological processes in a quantitative manner in living organisms (Massoud *et al.*, 2007). This is an extremely powerful tool with numerous applications including evaluation of gene therapy, stem cell tracking studies, monitoring of endogenous gene expression, screening of transgenic animals, integrative biology, risk stratification, and drug discovery. One of the most exciting aspects of this emerging field is that real-time imaging of molecular-genetic processes can be made within the inherent spatial-resolution limits of existing imaging systems.

Advances in the design of imaging devices have also been crucial in the development of molecular imaging technologies. Over the past few decades, there have been huge advances in imaging devices such as nuclear (PET, Single Photon Emission Tomography [SPECT], gamma camera), magnetic resonance, and *in vivo* optical imaging systems. Coupled with these improvements, sophistication of non-invasive imaging strategies has enabled better spatial and real-time visualization of normal and abnormal cellular processes at the molecular and genetic level. Non-invasive imaging of molecular-genetic and cellular processes will undoubtedly complement established *ex vivo* molecular-biological assays that require tissue sampling. These will provide spatial as well as temporal elements to our understanding of various diseases

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(Blasberg *et al.*, 2002). This article will review the current status of molecular genetic imaging technologies, their applications and potential, with a focus on reporter gene imaging and the PET system.

## Definition of Molecular Imaging

Molecular imaging is a growing research area aimed at developing and testing novel tools, reagents, and methods to image specific molecular pathways *in vivo*, particularly those that are key targets in disease processes. In contrast to classical diagnostic imaging, molecular imaging probes the molecular abnormalities that are the basis of disease rather than imaging the result of these molecular alterations. Molecular imaging can be defined as the visualization and quantification of biological processes at the molecular and cellular levels within intact living organisms (Massoud *et al.*, 2003). Alternatively, it can be defined as the minimally invasive *in vivo* sensing, depiction, and characterization of spatially localized biologic processes at the cellular and molecular levels (Britz-Cunningham *et al.*, 2003). In a broader sense, molecular imaging encompasses non-invasive visualization of the molecular processes of life in organisms ranging in complexity from individual cells to human beings. Defined from a different perspective, molecular imaging is the combination of new molecular agents with traditional imaging tools to capture pictures of specific molecular pathways in the body, particularly those that are key targets in disease processes.

## Imaging Strategy

Recent progress in our understanding of the molecular and genetic mechanisms of disease has ushered in the age of molecular genomic medicine together with the application of diverse biologically-oriented therapeutics. New gene-based therapies are providing control over the level, timing, and duration of transgene expression, and non-invasive imaging modalities are proving to be useful tools in treating patients using such therapies. Indeed, non-invasive imaging of molecular genetic events will accelerate the development of molecular therapies that can lead to more effective treatment.

Currently, there are three different imaging strategies: direct, indirect and surrogate approaches (Blasberg *et al.*, 2003). Direct strategies are used by all three (nuclear, MR, and optical) imaging modalities. For example, monoclonal antibody (mAb)- or peptide-specific targeting of a particular cell membrane epitope can be imaged with a paramagnetic, fluorescent, or radionuclide-labeled probe. Imaging of cell surface-specific antigens or epitopes with radiolabeled

antibodies is an example of direct molecular imaging that has been developed over the past 30 years. Similarly, PET imaging of receptor density or occupancy using small radiolabeled molecular probes has been widely used in neuroscience research over the past two decades. These examples represent some of the first molecular imaging applications used in clinical nuclear medicine research. Other examples of direct-imaging paradigms that use radiolabeled analogues of naturally occurring compounds, such as 2-deoxy-2-[ $^{18}\text{F}$ ]fluoro-D-glucose (FDG) to image glucose utilization in the brain, have been used extensively in the past. FDG PET imaging of glucose utilization is based largely on the activity of the enzyme hexokinase and was described three decades ago (Sokoloff *et al.*, 1977; Revich *et al.*, 1977). Similarly, imaging of the activity of a particular transporter with a transport-specific probe has been described previously, and has been used to generate images of tumors (Haberkorn, 2001; Miyagawa, 1998).

A more recent direct-imaging strategy involves the use of antisense oligonucleotide and aptamer as *in vivo* diagnostic agents. Antisense oligonucleotides target gene expression at the RNA level, while aptamer oligonucleotides are designed to detect proteins of interest. Since these nucleotide probes can specifically hybridize to target mRNA or proteins *in vivo*, they enable molecular imaging at the level of gene expression. In principle, they could potentially target any specific mRNA or DNA sequence within the genome. In this context, imaging of specific mRNAs using radiolabeled antisense oligonucleotides or aptamers can produce direct images of specific molecular-genetic events. Some success for the imaging of endogenous gene expression using PET has also been reported (Tavitan *et al.*, 1998; Cong *et al.*, 2005).

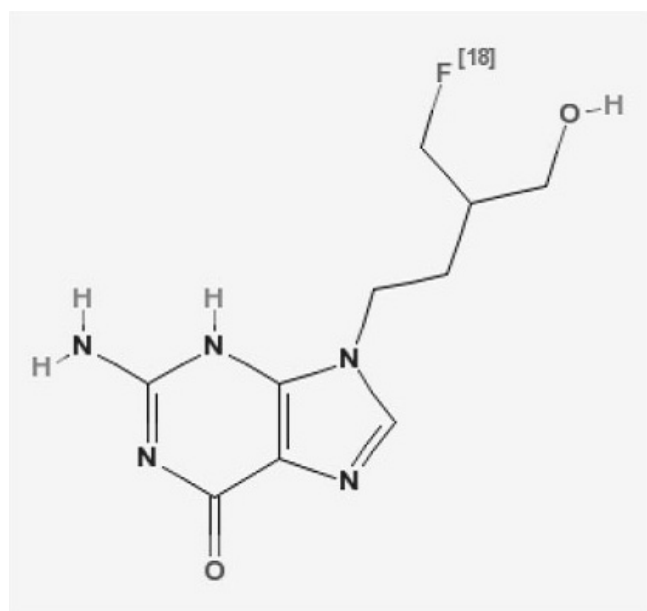
Despite the advantages of direct-imaging strategies, they are constrained by the necessity to develop specific probes for each molecular target, and then to validate both the sensitivity and specificity for the application of each newly developed probe-target imaging paradigm (Blasberg *et al.*, 2003). This can be very time-consuming and costly as has been demonstrated in the case of FDG PET imaging of glucose utilization in tumors which took over 20 years to develop, validate, and obtain regulatory approval. Nevertheless, this traditional approach will continue to be used to develop new probes for imaging specific molecular-genetic targets using radiolabeled, paramagnetic, or fluorescent small molecular probes. In addition to these direct imaging strategies, indirect and surrogate approaches are also being used. However, the most current *in vivo* molecular genetic imaging strategies are indirect and involve the coupling of a reporter gene with a complementary reporter probe.

## Reporter Gene Imaging

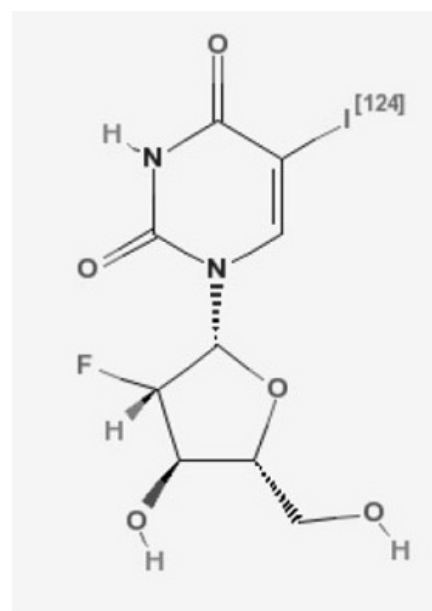
Indirect imaging strategies involve the coupling of a reporter gene with a complementary reporter probe such as FHBG ( $^{18}\text{F}$ -9-(4-fluoro-3-hydroxymethyl-butyl)guanine) and FIAU ( $^{124}\text{I}$ -2'-fluoro-2'-deoxy-1-beta-d-beta-arabinofuranosyl- 5-iodouracil) (Fig. 1). Imaging the level of probe accumulation provides indirect information related to the level of reporter gene expression. Reporter gene imaging can provide a molecular and biologic approach to assess genetic and cellular therapies. Reporter genes have been used to track the progress of patients with cancer (Penuelas *et al.*, 2005; Jacobs *et al.*, 2001) and to image viral gene transfer to the heart in rats and pigs (Bengel *et al.*, 2003). The technique of reporter gene imaging involves introduction of the reporter gene and its expression by a constitutively active promoter or tissue-specific promoter. The expressed reporter proteins then produce a quantifiable signal when exposed to a specific imaging probe.

Reporter gene technology has a number of advantages over direct labeling of cells with imaging agents (Chun *et al.*, 2007). Firstly, reporter gene imaging requires cellular viability. Direct labeling of cells does not discriminate cellular viability, as signals may still be present after cell death owing to local accumulation. Reporter gene expression can be constitutive leading to continuous transcription, can

be used to identify the site of transduction, and to monitor the level and duration of gene expression. Alternatively, reporter genes can be made inducible, enabling controlled gene expression. Gene expression can be made tissue or condition-specific (Lee *et al.*, 2006) and/or responsive to the level of endogenous promoters and transcription factors. Inducible promoters can also be used as sensors to regulate the magnitude of reporter gene expression, and can be used to provide information about endogenous cell processes. Reporter systems can also be constructed to monitor mRNA stabilization and specific protein-protein interactions. Secondly, reporter imaging can be performed repeatedly at later time points without being limited by the half-life of the labeling agents. In fact, the reporter probe can be injected on a daily or weekly basis to investigate the physiologic state of the cells expressing the reporter genes. Thirdly, if the reporter genes are integrated into the chromosomal structure and passed on to subsequent generations of cells, daughter cells can be tracked with equal efficiency as the parental cells. Fourthly, using recombinant DNA technologies, various promoters and reporter genes can be combined to create a construct that expresses multiple genes. Such a construct could be engineered not only to assess cellular viability, but also to determine cellular differentiation via the use of tissue-

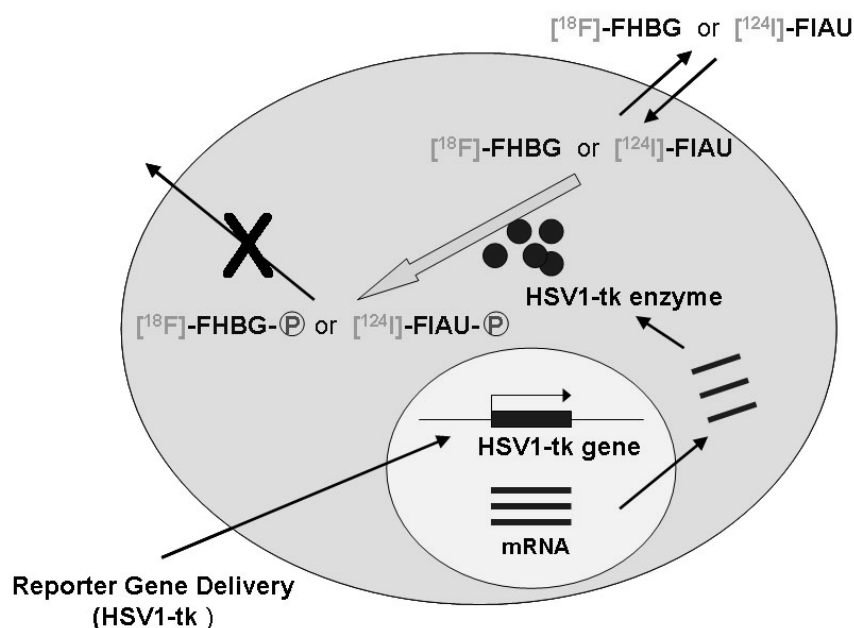


**[ $^{18}\text{F}$ ]-FHBG**



**[ $^{124}\text{I}$ ]-FIAU**

**Fig. 1.** Chemical Structures of FHBG and FIAU. These pictures were taken from a database of chemical molecules (PubChem, National Center for Biotechnology Information). FHBG: 2-amino-9-[3-(fluoromethyl)-4-hydroxy-butyl]-3H-purin-6-one, FIAU: 1-[(2R,3S,4R,5R)-3-fluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-iodo-pyrimidine-2,4-dione.



**Fig. 2.** Schematic for imaging herpes simplex virus type 1-thymidine kinase (*HSV1-tk*) reporter gene expression with reporter probes FHBG and FIAU. The *HSV1-tk* gene is transfected into target cells by a gene delivery vector. Inside the transfected cell, the *HSV1-tk* gene is transcribed to *HSV1-tk* mRNA and then translated to the HSV1-tk enzyme. After administration of a radiolabelled probe ( $[^{18}\text{F}]$ -FHBG or  $[^{124}\text{I}]$ -FIAU) and its transport into the cell, the probe is phosphorylated by HSV1-tk enzyme. The phosphorylated radiolabelled probe does not readily cross the cell membrane and remains “trapped” inside the cell, generating a signal in microPET. Thus, the magnitude of probe accumulation in the cell (level of radioactivity) reflects the level of HSV1-tk enzyme activity and the level of *HSV1-tk* gene expression.

specific promoters.

Two well-established examples of reporter gene assays are herpes simplex virus thymidine kinase (HSV-tk)-based PET imaging (Fig. 2) and firefly luciferase (Fluc)-based optical bioluminescence imaging. PET imaging using the HSV-tk reporter protein has been widely used with a number of radiolabeled thymidine analogs (Gambhir *et al.*, 2000; Tjuvajev *et al.*, 2002). Bioluminescence imaging using the Fluc reporter protein is based on the oxidation of the D-luciferin reporter probe to generate low-energy photons. The signal is emitted from the cell through the tissue and detected using ultra-sensitive charge-coupled device (CCD) cameras. More recently, a human-derived intrinsically non-immunogenic reporter gene, the human mitochondrial thymidine kinase type 2 (hTK2) reporter gene, was evaluated for PET imaging applications (Ponomarev *et al.*, 2007). The implication of human-derived reporter genes is that they may be non-immunogenic and therefore potentially allow long-term monitoring of different molecular- genetic processes by nuclear imaging techniques in humans. Using a well-characterized radiotracer such as  $[^{124}\text{I}]$ -FIAU, it should be possible to image human reporter gene expression with PET in preclinical and clinical studies.

Despite these advantages, reporter gene imaging still has several limitations. An ideal gene delivery system that enables target-specific stable expression must be developed. Moreover, the safety of vectors in relation to potential adverse effects and cytotoxicity must be guaranteed before such therapies can be used in clinical practice. Reporter gene imaging studies will be limited in human subjects, because each new vector requires extensive and time-consuming safety testing before regulatory approval for human administration. Similarly, radiolabeled probes for existing and new reporter systems, and the probes being developed for new direct imaging paradigms, will also require safety testing and regulatory approval. Therefore, the translation of molecular genetic imaging research from animal proof-of-principle experiments into patient studies and the subsequent clinical application of these imaging paradigms will need to be developed with careful monitoring. Nevertheless, the use of the reporter gene imaging strategy is supported by clinical studies using adenoviral-mediated gene transfer of HSV-tk into patients with glioblastoma and hepatocellular carcinoma for PET imaging (Penuelas *et al.*, 2005; Jacobs *et al.*, 2001).

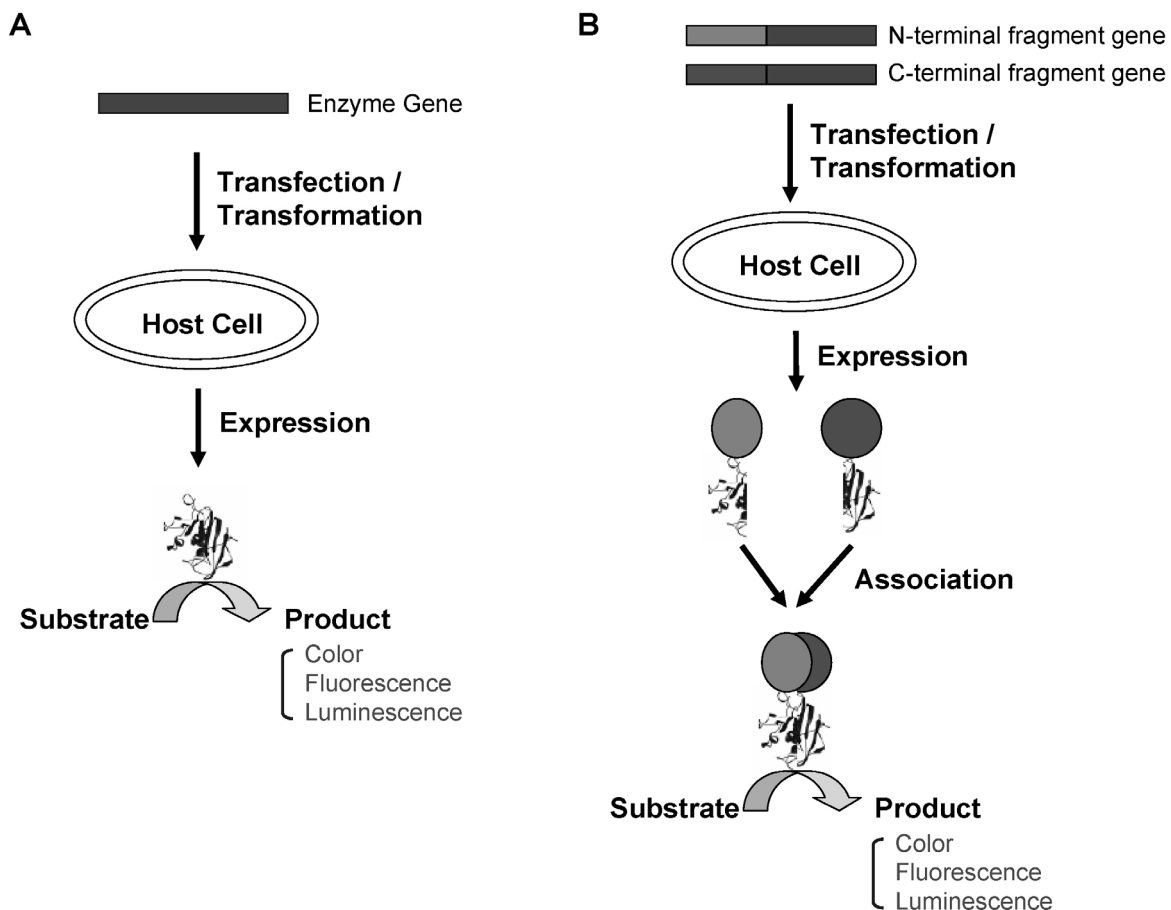
## Protein-Protein Interactions

Protein-protein interactions regulate a variety of cellular functions, including cell cycle progression, signal transduction, and metabolic pathways. On a whole organism scale, protein-protein interactions regulate signals that affect overall homeostasis, patterns of development, normal physiology, and disease in living animals. In addition, protein-protein interactions have considerable potential as therapeutic targets. However, compared with *in vitro* studies of protein interactions in cultured cells, *in vivo* investigation of protein-protein interactions in living organisms impose even greater constraints on reporter systems and detection mechanisms.

Most strategies for detecting protein-protein interactions are based on fusion of a pair of interacting molecules to the defined protein elements to reconstitute a biological or

biochemical function. The two-hybrid system is the most widely applied method used to identify and characterize protein interactions (Parrish *et al.*, 2006). Another method that is attractive for *in vivo* imaging of protein interactions is 'protein fragment complementation (PFC)' (Fig. 3). Variations of recruitment systems such as Ras recruitment (Staglar *et al.*, 1998) and interaction trap have also been developed (Eyckerman *et al.*, 2001). Recently, imaging using fluorescence resonance energy transfer (FRET) and bioluminescence resonance energy transfer (BRET) has been increasingly investigated.

Evidence has accumulated that the pathways of protein interactions in specific tissues produce regional effects that cannot be investigated fully with *in vitro* systems, and thus there is considerable interest in evaluating protein interactions in live animals. The *in vivo* protein-protein interaction was demonstrated by a reporter gene imaging



**Fig. 3.** General Features of Protein Fragment Complementation (PFC). **(A)** The gene encoding an enzyme can be transformed/transfected into a host cell and its activity detected by an *in vivo* assay that can detect color, luminescence and fluorescence. **(B)** Oligomerization domains are fused to N- and C-terminal fragments of the enzyme gene. Upon co-transformation/transfection of the fusion genes, spontaneous or drug-induced association of oligomerization domains results in reconstitution of enzyme activity. Reassembly of enzyme will not occur unless the oligomerization domains interact.

strategy that employed *id-gal4* and/or *myoD-vp16* fusion genes driven by the NF- $\kappa$ B promoter responsive to TNF- $\alpha$  or by PET and fluorescence imaging using a variant of the HSV1-tk/GFP dual-reporter fusion gene (Ray *et al.*, 2002; Luker *et al.*, 2002). These initial results are very encouraging, but they are also limited, since they demonstrate only constitutive interactions of the known pairs of interacting proteins. Further studies will be required to validate the approach with respect to the sensitivity and dynamic range of these reporter systems for monitoring the induction and inhibition of endogenous protein-protein interactions.

Recently, the potential of molecular imaging for monitoring conditional gene activation and gene deletion in the cardiovascular system was reported (Chang *et al.*, 2007). In their study, a silenced PET reporter gene (HSV1-tk) carried in a replication-deficient adenovirus was conditionally activated in cardiac tissue using the Cre-*loxP* system and cardiac-specific promoters in living animals. Since the ability to switch transgenes on and off would be of paramount importance not only when the therapy is no longer needed, but also in the case of the development of adverse side effects to the therapy, development of such technologies for monitoring and controlling gene expression *in vivo* could provide significant benefits.

## Two-Hybrid System

Two-hybrid systems exploit the modular nature of transcription factors, many of which can be separated into discrete DNA-binding and activation domains. Proteins of interest are expressed as fusion proteins with either a DNA-binding domain (BD) or activation domain (AD). If these hybrid proteins bind to each other as a result of interaction between the proteins of interest, the BD and AD domains are brought together within the cell nucleus and act as a transcription factor to drive expression of reporter gene. In the absence of specific interaction between the hybrids, the BD and AD do not associate and no transcription occurs (von Mering *et al.*, 2002).

This two-hybrid system can be used to image protein interactions in living mice with positron emission tomography (PET) or bioluminescence imaging. Because the production of signal in this system requires nuclear localization of the hybrid proteins, membrane proteins cannot be studied in their intact state. Also, indirect readout of the reporter may limit kinetic analysis due to the time delay associated with both transcriptional activation of the reporter gene and decay of the reporter mRNA and proteins.

In another approach termed the cytokine receptor-based interaction trap, a signaling-deficient receptor provides a scaffold for the recruitment of interacting fusion proteins that phosphorylates endogenous STAT3. Activated STAT

complexes then drive a nuclear reporter (Eyckerman *et al.*, 2001). This system permits detection of both modification-independent and phosphorylation-dependent interactions in intact mammalian cells, but the transcriptional readout can limit kinetic analysis.

## Protein Fragment Complementation

The protein fragment complementation (PFC) assay depends on the division of a monomeric reporter enzyme into two separate inactive components that can reconstitute function upon association (Fig. 3). To enable this, reporter fragments are first fused to interacting proteins, which, upon association, brings together the separated reporter fragments and restores the enzymatic activity. Enzymes which can be used for PFC strategies include beta-galactosidase, dihydrofolate reductase (DHFR), beta-lactamase, and luciferase (luciferase complementation imaging [LCI]) (Rossi *et al.*, 1999; Remy *et al.*, 1999). A fundamental advantage of PFC is that the hybrid proteins directly reconstitute enzymatic activity of the reporter. Therefore, in principle, protein interactions could be detected in any subcellular compartments. Also, monitoring in real-time of the assembly of protein complexes may be possible.

A disadvantage of the PFC approach is that reconstitution of enzyme activity may be susceptible to steric constraints imposed by the interacting proteins. Another potential limitation is that the amount of reconstituted enzyme may not be sufficient for non-invasive detection. However, this sensitivity problem may be overcome with technological developments in the detection system. Another aspect is that PFC systems can enhance the sensitivity of detection by potential amplification of the signals.

## PET: A Versatile Imaging Modality

PET-based molecular genetic imaging in living organisms has enjoyed exceptional growth over the past 7 years. PET is a versatile tool for molecular genetic imaging since it can detect the biochemical changes accompanying genetic and cellular processes. Although structural imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) offer excellent resolutions for visualization of anatomical changes, many diseases may already be in advanced stages by the time they are detected by MRI and CT. However, PET can detect the altered metabolism of disease and also provide highly accurate quantitative results as a functional molecular imaging technique.

The enormous potential of PET for the investigation of various biological processes opens up new possibilities for basic research and everyday clinical practice. With its unique ability to reveal biochemical and physiological processes, PET can provide crucial information about numerous diseases. PET imaging radiotracers are designed to provide insights about healthy, normal biology, the biological process of disease, and even the molecular errors that cause diseases. Radiotracers can even interact with biological processes such as bone mineral turnover (Frost *et al.*, 2007) and glucose metabolism in various organs. One of the primary applications of PET reporter gene imaging is likely to be the quantitative monitoring of transduction efficacy in gene therapy and animal research protocols by imaging of the location, extent, and duration of transgene expression.

Monitoring of cell trafficking, targeting, and replication in adoptive therapies, involving *ex vivo* transduction of harvested immune-competent cells and stem/progenitor cells will be another area for PET application. There are numerous examples of molecular imaging of the cells that are marked by the *ex vivo* gene transfer procedure and subsequently placed in living rodents. In one example, embryonic rat H9c2 cardiomyoblasts were transduced with an adenoviral vector containing the HSV1-sr39tk reporter gene, and injected into the myocardium (Inubushi *et al.*, 2003). Since current methods for the analysis of cell-based therapies are largely restricted to invasive measurements in animal models, there is a need for alternative methods to monitor the movement and location of specific cell populations *in vivo* through the development of innovative, high-resolution cellular tracking techniques. Particular emphasis needs to be placed on methods enabling the identification and characterization of cell mobilization, engraftment, and differentiation. Integration of emerging *in vivo* molecular genetic imaging capabilities with advances in stem cell biology will be required for effective development and utilization of cell-based therapies.

## PET Tracers

The future success of PET imaging will largely depend on the discovery of novel radiopharmaceuticals that seek specific molecular and genetic targets, the design of companion advanced scanners for creating meaningful images, and the new radiopharmaceutical treatments for intractable diseases including cancers. PET uses radioisotopes of naturally occurring elements, such as  $^{11}\text{C}$ ,  $^{13}\text{N}$ , and  $^{15}\text{O}$ , in order to perform *in vivo* imaging of biologically active molecules. Although there are no radioisotopes of hydrogen that can be used for PET, many

molecules can replace a hydrogen (or hydroxyl group) with Fluorine-18 ( $^{18}\text{F}$ ) without changing its biological properties. Fluorine-18 can also be used as a substitute in fluorine-containing compounds such as 5-fluorouracil (5-FU) (Mintun *et al.*, 1988). Most PET tracers utilize a radioisotope that has a short half-life and can be produced by a cyclotron. However, there are some radiotracers, such as copper-62, that can be manufactured in a nuclear generator. Radiopharmaceuticals are produced after the radioisotope has been generated and substituted into the compound of interest. Because of the short half-lives of most PET tracers, sequential scanning on the same day is not usually feasible.

Any biological function can be studied *in vivo* using appropriately labeled PET tracer molecules. The concentration of any PET tracer molecule should be significantly higher at the target sites than in the background. This allows biological function to be measured by determining tracer concentration over a specified target tissue or organ region. For example, physiological processes such as oxygen consumption, blood flow, and tissue metabolism can also be visualized *in vivo* using PET tracers. Receptors can be visualized and quantified by labeling receptor-binding ligands with PET tracers. New radiotracers based on messenger RNA for dynamic imaging of gene expression can also be developed for use in animals in real time (de Vries *et al.*, 2004).

At present, FDG is the most commonly used positron-emitting radiotracer for PET imaging. FDG is a radioactive analog of glucose, and is able to detect altered glucose metabolism in pathological processes. Like glucose, FDG is transported into cells by means of a glucose transporter protein and begins to follow the glycolytic pathway. FDG is subsequently phosphorylated by hexokinase to form FDG-6-phosphate, which then cannot continue through glycolysis because it is not a substrate for glucose-6-phosphate isomerase. FDG-6-phosphate is therefore trapped biochemically within the cell. This process of metabolic trapping constitutes the basis of PET imaging using FDG. Because there may be a substantial increase or decrease in glucose metabolism in diseased tissues, such differences can be visualized using FDG PET.

## Future Directions for PET Imaging

As more information about the relationship between genes and diseases is discovered, new molecular targets for imaging the biological activity of diseases will be elucidated. In the age of genomic medicine, drugs may be custom made for individual patients based on their genetic makeup (pharmacogenomics), and nuclear medicine will

play a crucial role in this pursuit. Since mice can be engineered biologically to carry genes that cause disease, novel molecular probes coupled with microPET may allow the imaging of disease initiation and progression in a living mouse. In concert with this trend, highly sophisticated drugs are likely to be designed to correct the molecular errors of disease. Combined with the explosive growth of knowledge from genome research, PET imaging could play a major role in the promising new era of molecular medicine. In the near future, PET may become the critical modality for diagnosing a variety of diseases and for selecting appropriate treatments when disease processes are still at the molecular level. With further development and validation, PET imaging for examining gene expression, signal transduction, and cellular metabolism may move into the clinical realm in the not-so-distant future.

## Multi-modality Imaging

The convergence of different imaging modalities is creating new opportunities for molecular genetic imaging. Multi-modality, non-invasive *in vivo* imaging is increasingly being used in molecular genetic studies, and will soon become the standard approach for reporter gene imaging studies in small animals. With this trend, multidisciplinary efforts from molecular and cellular biology, chemistry, bioinformatics, physics, engineering, and many clinical areas are becoming united. For example, the coupling of nuclear and optical reporter genes represents the beginning of a far wider application of multi-modality imaging in molecular genetic studies being performed in small animals. Optical reporter imaging systems have the advantage of cost effectiveness and time efficiency. Moreover, they require less resources and space than PET or MRI and are particularly well suited for small animal imaging and for *in vitro* assays to validate different reporter systems. However, optical imaging techniques do not currently provide optimal quantitative or tomographic information because they are limited by depth of light penetration and scatter. These issues are not limiting for PET- or MRI-based reporter systems, and PET- and MRI-based animal studies are more easily converted to human applications (Blasberg, 2003). Many of the shortcomings of each modality alone can be overcome by the use of dual- or triple-modality reporter constructs that incorporate the advantage of each imaging modality.

## Conclusions and Future Perspective

The emerging field of molecular genetic imaging is providing new opportunities for visualizing and quantifying

the biology of living organisms. To reach such goals all the major disciplines and imaging modalities must cooperate with this new field, each with its own mechanism for generating contrast, spatial resolution, and specificity. If molecular imaging can be broadly defined as the *in vivo* characterization and measurement of biologic processes at the molecular and cellular level, efforts such as the development of novel probes, signal amplification strategies, and imaging technologies clearly fit together with prior research efforts in the imaging field.

Future success of molecular genetic imaging will be facilitated by bringing the imaging disciplines closer together as can be seen in the complementary benefits of dual-modality reporters that incorporate both PET and optical imaging technology. Complementary instrumentation for imaging both animals and patients provides a unique opportunity for translational research. Animals bearing transduced xenografts that contain specific reporter constructs, and animals transduced with viral vectors that bear these reporter constructs, currently provide the main resource for further advances in non-invasive molecular genetic imaging research. Transgenic animals that express reporter constructs in a system-wide manner, or selectively in certain organs or cell types in the adult organism or during a particular phase of embryonic development, are the subject of active current research. Non-invasive molecular genetic imaging can assist in these studies by assessing the activation or suppression of specific endogenous genes, and the activity of specific signal transduction pathways and specific protein-protein interactions. This information could prove useful in defining and monitoring cell transformation to a more malignant state, or determining whether a specific drug effectively targets a specific signal transduction pathway.

For the time being, basic science investigations and preclinical research will be a mainstay of the molecular genetic imaging field. But an eye towards the design of clinical translational research will be necessary to ensure successful integration and practical use of molecular genetic imaging. As imaging schemes are growing more sophisticated, more precise genetic markers are needed to study more subtle forms of cancer progression and other diseases. Anatomic localization will become increasingly important as more biologically-targeted tracers are introduced. Thus, there will be an increasing need for hybrid or fusion imaging modalities such as PET-MRI. Consistent with these trends, the use of radio-, optical, and other forms of biological tracers or contrast agents to diagnose disease and assess efficacy of novel therapeutics is likely to grow in the next decade. More importantly, improvements in molecular target selection, probe developments, and imaging instrumentation will



remain crucial issues together with collaborative efforts between basic scientists, clinicians, and industry to optimize device design, and to identify new applications. In conclusion, in the age of genomic medicine and systems biology approaches to disease complexity, molecular genetic imaging promises to change the clinical landscape of future medicine by providing predictive, preventative, and personalized medicine.

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