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Live Imaging Mouse Embryonic Development: Seeing Is Believing and Revealing

Sonja Nowotschin and **Anna-Katerina Hadjantonakis**

Abstract

The use of genetically encoded fluorescent proteins has revolutionized the fields of cell and developmental biology and redefined our understanding of the dynamic morphogenetic processes that work to shape the embryo. Fluorescent proteins are routinely used as vital reporters to label tissues, cells, cellular organelles, or proteins of interest and in doing so provide contrasting agents enabling the acquisition of high-resolution quantitative image data. With the advent of more accessible and sophisticated imaging technologies and abundance of fluorescent proteins with different spectral characteristics, the dynamic processes taking place in situ in living embryos can now be probed. Here, we provide an overview of some recent advances in this rapidly evolving field.

Keywords

Mouse embryo; Transgenic; Gene targeted; Reporter; Genetically encoded fluorescent protein; Green fluorescent protein; GFP; Red fluorescent protein; RFP; Live imaging; 3D; Time-lapse

Introduction

A goal of developmental biology is to formulate an understanding of the carefully orchestrated stereotypical cell behaviors, as well as the molecular and physical mechanisms that underlie the complex morphogenetic processes that shape a multicellular organism. Even though genetic manipulations and the analysis of mutants have identified many genes important for regulating key developmental events in the mouse embryo, we are still a long way from understanding how many key morphogenetic events are regulated.

The details of critical events in the development of the mammalian embryo, such as, early lineage commitment and segregation at preimplantation, the establishment of the germ layers and elaboration of the axes at early postimplantation, and the subsequent morphogenesis of individual organ systems remain obscure. Intrinsic cell behavior is not only determined by gene expression but also by intercellular interactions and physical forces generated between cells, as well as between cells and their substrata. It is therefore critical to move analyses of sequentially staged dead embryos forward and place them into a dynamic context so as to reveal the cell behaviors underlying normal embryonic development. Only with such information at hand can we begin to appreciate how mutations can result in an aberrant course of events.

Microscopy is a central tool in developmental biology, both for determining the normal course of developmental events and for investigating the consequences of experimental perturbations. Traditionally (dead) embryos have been visualized at single time points either in whole mount, to reveal gross morphological features, or after sectioning and histological staining, to provide cellular resolution. Dynamics and the sequence of events are usually inferred by analyzing multiple sequentially staged embryos. The ability to observe a single specimen continuously and at cellular resolution holds great promise and represents the

evolution of classical histology into digitized multidimensional information documenting actual developmental progression. Recent advances in microscopy techniques for data acquisition as well as in computational methods for data processing in combination with the ever-increasing repertoire of reporter strains expressing spectrally distinct genetically encoded fluorescent proteins (FPs) provide powerful tools for the generation of 3D time-lapse (i.e., 4D) information. Moreover such data promises to provide insight not only to the field of developmental biology but also beyond, by furthering our understanding of homeostasis and disease progression.

2 Live Imaging Mouse Embryonic Development

A fertile period of methodological innovation has spearheaded the rapid evolution of the live imaging field. This rapid progress is a reflection of technical improvements and the increased affordability of digital microscopy techniques in parallel with the advent of genetically encoded fluorescent proteins with different spectral characteristics. By exploiting on-stage whole embryo or explant cultures and utilizing appropriate environmental climate control, short periods of normal development can be fostered ex utero [1–4]. While various imaging modalities can be used to study the tissue architecture and dynamic processes taking place in biological samples at high resolution, there is no single approach that is ideal for all applications, and a discussion of the various modalities is beyond the scope of this review. Certain applications, for example, determining gross tissue organization, are better served by imaging of large samples (e.g., whole embryos) at lower resolution, while others require investigations at the subcellular level. As a consequence while imaging modalities such as optical projection tomography (OPT), optical coherence tomography (OCT), and magnetic resonance imaging (MRI) provide insight into embryogenesis and the global organization of structures, they lack the cellular or subcellular resolution of laser-scanning microscopy (LSM) [5].

LSM, including confocal point-scanning, multi-point and slit scanning modalities in addition to multi-photon excitation, has found widespread use for many live high-resolution imaging applications. Data is usually acquired as *x*–*y* images in the *z* dimension (3D) and in 3D over time (3D time-lapse or 4D). Thus fluorescently tagged objects can be analyzed quantitatively in situ using 3D time-lapse imaging with a high spatial and temporal resolution. LSM can provide information at single-cell resolution by revealing information on cell position, morphology, division, and death in situ. The main limitations of LSM are limited imaging depth in the tissue being sampled, and cell death resulting from phototoxicity. New developments in light sheet microscopy have partially addressed some of these issues, but have yet to be applied in the mouse [6, 7].

Once image data is collected computational methods are used to quantify and segment data. This generates high-resolution information on, for example, cellular organelles, which can be used as descriptors of cell position (e.g., nuclei), and cell morphology (e.g., plasma membrane labels). A detailed discussion of the software available is beyond the scope of this introduction; however, a short list is provided for the interested reader to follow up on. Commercially available, general purpose image analysis software packages include Amira from Mercury Computer Systems (http://3dviz.mc.com/), Imaris from Bitplane (http://www.bit-plane.com), MetaMorph from Molecular devices (http://www.moleculardevices.com/pages/software/metamorph.htm), and Volocity from Perkin-Elmer (http://www.cellularimaging.com/products/volocity/). While open source academic packages include the general purpose ImageJ which is based on the NIH Image (http://rsb.info.nih.gov/ij/ rashband et al. 2006), as well as software for image segmentation methods used for the identification and tracking of individual cells which include 3D-DIAS [8], STARRYNIGHT [9, 10], and cell division analysis [11].

Irrespective of imaging modality used, observation of tissues or individual cells of interest and their functional properties is not often possible without molecular tagging. Typical requirements for tagging agents include developmental neutrality, fluorescence intensity and stability, and the possibility of multiplexing. Numerous agents for molecular labeling are available. They range from fluorescent organic dyes, to quantum dots and molecular beacons to genetically encoded reporters [12–14]. Genetically encoded reporters comprise two main categories: chromogenic enzyme-based reporters (e.g., beta-galactosidase and alkaline phosphatase) and vital autofluorescent proteins (e.g., the Green Fluorescent Protein, GFP). In this chapter we focus on the latter as it is currently the most prevalent reporter system used in the mouse.

Strains of mice expressing genetically encoded reporters are routinely generated by gene targeting or gene trapping in embryonic stem (ES) cells and germline transmission through chimeras or by transgenesis (in ES cells or by pronuclear injection of DNA into zygotes) through modification of large insert genomic DNA containing vectors (e.g., Bacterial Artificial Chromosomes—BACs) or by using defined *cis*-regulatory elements. A key advantage of using autofluorescent proteins (FPs) for cell labeling is the lack of exogenous compounds necessary for reporter visualization and the attractive possibility to image noninvasively. However, a disadvantage is that since there is no enzyme-based amplification, what you see is what you get, and therefore low levels of reporter expression may not be readily detectable, and cannot easily be improved upon.

FPs can either be used in their native form or they can be incorporated into protein fusions to follow protein dynamics or interactions or to label specific subcellular compartments or organelles, such as the nucleus, plasma membrane, cytoskeleton, or mitochondria. The increasing number of FPs provides an assortment of probes with increased fluorescence intensities to mix and match for multiplexing and simultaneous detection. As a consequence, such variety can also represent a confusing and sometimes overwhelming choice. Importantly, choice is often best dictated by application. Since no review can exhaustively cover the panoply of available genetically encoded fluorescent proteins, we discuss some of the more commonly used ones and present some of the rationale behind their use.

Given how quickly the field of FPs is evolving, the information presented here will likely be obsolete as soon as it sees the light of publication. With this in mind, the discussion provided is intended for use as a guide, rather than a definitive collection.

3 Reporters for Live Imaging: A Color-Palette of Genetically Encoded Fluorescent Proteins

3.1 Green Fluorescent Protein (GFP) and Its Variants

The cloning of the green fluorescent protein, wtGFP from *Aequorea victoria* [15] the first genetically encoded fluorescent protein, spearheaded a revolution in the use of fluorescent markers as autofluorescent protein tags to study cell function, morphology, and protein–protein interactions. GFP protein and its spectral variants are the favored choice in many applications and are used in many different organisms. GFP and its variants possess a unique structure, which consists of 11 beta sheets and an internal alpha helix. The fluorophore, which is located in the center of the barrel, is formed by three amino acid residues of the alpha helix which form a cyclic tripeptide, Ser65-Tyr66-Gly67, during a process of maturation [16, 17].

Its stability over a wide range of pH and temperatures, as well as the lack of a need for a cofactor to fluoresce, made wtGFP attractive for use in molecular and biochemical applications [18, 19]. Improved versions of GFP with increased levels of fluorescence and

photostability, and include enhanced GFP (EGFP) which contains a point mutation that leads to a S65T substitution in the cyclic tripeptide [20], Emerald GFP (EmGFP) [21], Superfolder GFP [22], TagGFP2 [23], and new green FPs such as mWasabi [24], Azami Green (AG) [25] have been discovered or developed through mutagenesis. EGFP was one of the first improved GFPs and has so far been the green FP of choice for most applications in mice. It remains to be seen if any of the newly identified green FPs will perform comparatively to EGFP in mice.

Spectral GFP variants include the blue fluorescent proteins (BFP), cyan fluorescent proteins (CFP), and yellow fluorescent proteins (YFP). These GFP-based variants exhibit different spectral characteristics and were obtain through site-directed mutagenesis of GFP [26]. To date improved versions of all color variants have become available exhibiting improved quantum yields, higher extinction coefficients, and brighter fluorescence than their predecessors. Of the YFPs, Venus [27], Citrine [28] and TagYFP (Evrogen) are less acid sensitive and brighter than YFP, and so are better suited for labeling proteins in secretory organelles. Cerulean [29], the successor of CFP, as well as TagCFP (Evrogen) are characterized through their increased brightness and faster maturation, and so are preferable for use in FRET experiments together with YFPs. The availability of spectrally distinct FPs allows simultaneous visualization of two or more FPs expressed in different tissues, or even in the same cell, and can be used for quantitative dual imaging applications such a fluorescence resonance energy transfer (FRET) and FRET-based sensors [27, 29–31].

The advent of spectrally distinct FPs has afforded multiplexing for simultaneous detection in different tissues while under the control of different *cis*-regulatory elements or when targeted to different locations within the same cell (e.g., labeling nucleus vs. plasma membrane) when placed under the control of the same *cis-regulatory* elements. However, it should be noted that, when using newly isolated fluorescent proteins, new variants of established fluorescent proteins or newly generated fluorescent protein fusions, their biological compatibility, particularly their developmental neutrality must first be carefully evaluated. This is usually achieved by generating strains of mice exhibiting widespread expression of an FP, or FP fusion, of interest. For example, even though DsRed and its early variants were extensively used in cells, only the monomer mRFP1 was proven to be developmentally neutral when widely expressed in mice [32]. Consideration of existing fluorescent reporter strains, which are mainly green, should also be taken into account when generating new strains that might be used in combination [33].

Of great promise are genetically encoded fluorescent protein reporters that can be used as sensors to monitor the activity of proteins or provide a readout and so act as sensors of a functional change in proteins acting within an intracellular network. These reporters have several advantages over widely used synthetic indicators. They are noninvasive and allow monitoring of a specific subset of cells and specific subcellular compartments, respectively. The probes are constantly produced hence permanently label the cell of interest, which makes them suitable for long-term imaging in vivo. Pioneering work has been achieved regarding using indicators for calcium (cameleons, camgaroos, pericams, and G-CaMP) in invertebrates [34–36] as well as in zebrafish [37, 38]. In zebrafish a G-CaMP transgene, expressed under a cardiac specific promoter, was used to live image (optical mapping) the development of the cardiac conduction system. The same transgenic line was also used to identify mutants of an ENU screen by optical methods [38]. Developing similar transgenes in mice would be desirable. Indeed, use of the improved genetically encoded calcium indicator, G-CaMP2, has been reported in mice. When expressed in pyramidal neurons action potentials lead to changes in fluorescent intensity facilitating the detection of bursts of high-frequency action potentials and synaptic currents in vivo [39].

3.2 Orange and Red Fluorescent Proteins

Bright orange and red fluorescent proteins are much sought after for live cell or whole animal imaging. These red-emitting FPs, due to their long wavelength emission, are less toxic for the cell and possess a greater depth penetration. Approaches to mutagenize GFP to obtain FPs with farther red-shifted spectra than YFP have been unsuccessful. To date only few FPs emitting in the orange and red wavelengths (560–650 nm) have been isolated or engineered, with most far-red FPs having been isolated from different sea anemone and coral species.

DsRed from the sea anemone *Discosoma sp*. was the first RFP that was discovered [40]. Since then many variants have been engineered to obtain brighter more photostable and monomeric forms. One of them, mRFP1, was the first true monomeric variant of DsRed [41]. mRFP1 has been successfully used in mice [32], as has as one of its brighter successors, mCherry [42]. Widespread expression of the native form of mRFP1 or mCherry does not affect cell morphology, developmental potential, or animal viability. A tandem dimer variant of mRFP, (td)RFP has been used to generate a Cre recombinase reporter strain of mice for lineage tracing studies [43]. It should be noted that attempts at widespread expression of several mRFP1 fusions in mice, including a myristoylated-mRFP1 and a human histone H2B-mRFP1 fusion that labels active chromatin, have not been successful, suggesting that some mRFP1 fusion reporters might not be neutral (our unpublished observations).

Additional orange and red FPs have been reported, but to date these not been used to generate mouse strains. Kusabira Orange, a tetrameric protein was isolated from the coral *Fungia concinna*. The monomeric versions, mKO [44] and its enhanced version mKO2 [45], have been generated through site-directed mutagenesis. Their brightness is similar to that of EGFP, and they exhibit high photostability making them particularly suitable for live imaging applications [44]. Variants of mKO are commercially available from MBL International (http://www.mblintl.com/). Another variant resulting from the site-directed mutagensis of DsRed was mOrange, its successor mOrange2, and the tandem dimer (td) tomato. Compared to mOrange, (td)tomato has greater photostability and brightness [46]. Other orange-red FPs that are commonly available include TagRFP [47], its enhanced version TagRFP-T [48] and variants including the dimer TurboRFP [47], as well as the monomeric form of TagRFP exhibits high brightness and photostability. In contrast to TurboRFP, the monomeric TagRFP is well tolerated in a variety of fusion proteins [47]. TagRFP and TurboRFP (as well their cyan, green, and yellow couterparts) are commercially available from Evrogen (http://www.evrogen.com/).

Such observations underscore the need for alternative red FPs or variants of existing ones. A series of spectral variants, named after fruits, have been engineered through a large-scale mutagenesis of mRFP1. These variants exhibit excitation/emission maxima between 537 and 610 nm and range from a green FP (mHoneydew) to several reds (mCherry, mStrawberry) through to a far-red (mPlum). For live imaging the tandem dimer tdTomato, and the monomers mCherry and mStrawberry are considered as the most applicable red FPs of this series [46]. mRuby, a recently engineered monomeric RFP, exhibits increased brightness as compared mStrawberry and mCherry, but it is not as photostable. However, its stability at extreme pH values makes it ideal for labeling subcellular organelles for live imaging [49]. Given its photostability, mCherry is currently one of the better choices for time-lapse imaging applications in the mouse. mCherry has also been shown to be incorporated in protein fusions that are correctly targeted, labeling the plasma membrane and the nucleus without affecting the developmental potential [50, 51]. But, as with mRFP1, constitutive widespread expression of an H2B-mCherry fusion is always not compatible with normal

embryonic development [52]. However this does not necessarily preclude the use of H2B-mCherry fusions from use a conditional or lineage-specific reporters.

3.3 Far-Red Fluorescent Proteins

Given their physical properties, very bright far-red FPs will be difficult to generate, however, FPs with bright near-infrared spectra are eagerly anticipated as they should provide greater tissue penetration, reduced autofluorescence and less phototoxicity. The fare-red FPs, mPLum and mRasberry, were the first genetically engineered monomeric proteins generated through iterative somatic hypermutation [53]. However, their brightness is only small fraction of that of GFP. Over the recent years additional far-red proteins have become available and include the dimeric Katushka (available from Evrogen as TurboFP635), its monomeric version mKate (available from Evrogen as TagFP635) [54], and its successor mKate2 [55], as well as the unrelated mNeptune [56]. The monomer mKate2 is currently considered to be a preferable far-red FPS for most applications due to its considerable brightness and high photostability making it ideal for cell labeling or protein tagging in the far-red spectrum [55].

A recent report has introduced two new near-infrared dimeric FPs, eqFP650, and eqFP670, where the former has been reported as the brightest far-red FP so far, and the latter is characterized as exhibiting the most red-shifted emission spectrum and high photostability, thereby making them attractive for whole body imaging experiments [57].

3.4 Photomodulatable Fluorescent Proteins

Photomodulatable proteins represent novel tools for cell lineage tracing and fate mapping cells in vivo. Up until now pulse-chase labeling or fate mapping cells are predominantly performed using invasive techniques like the injection of dyes, grafting tissues, or genetically using the binary Cre/*lox*P system. Photomodulatable FPs facilitate the labeling and tracking of single or cohorts of cells noninvasively. In addition, fused to a protein of interest they provide information on localization, turnover and tracking of a protein in specific cell types.

Photomodulatable FPs change their spectral characteristics when exposed to short wavelength illumination. This class of fluorescent proteins comprises two main groups: (1) photoactivatable proteins that change their state from nonfluorescent to a fluorescent upon irradiation with short wavelength light (referred to as photoactivation) and (2) photoconvertible proteins that can be converted from one fluorescent state (i.e., color) to another when irradiated with short wavelength light (referred to as photoconversion). With the growing numbers of proteins that belong to each particular group one has powerful tools at hand to noninvasively label cohorts of cells, single cells, subcellular organelles, or individual proteins in vivo and track them over time. The choice of most appropriate PMFP for the planned experiment depends on the specific characteristics of the PMFP, as well as on the imaging modalities available. The structure of the photomodulatable protein must also be considered for its application. Monomeric ones can be used in fusion proteins to label intracellular molecules. Whereas tetrameric photomodulatable proteins by their nature are not ideal for use in fusion proteins since they could potentially disrupt the function and localization of the protein [58]. Below we briefly review some of the most common PMFPs.

3.5 Photoactivatable Proteins

PA-GFP, a variant of GFP, is a photoactivatable protein that changes irreversibly from a weak green fluorescent state (excitation peak at 400 nm, emission peak at 515 nm) to a bright green fluorescent state (excitation peak at 504, emission peak at 517) with a 100-fold increase in green fluorescence upon irradiation with short wavelength light [59]. Red

variants, including PA-RFP, of irreversible photoactivable proteins that change from a nonfluorescent to a red fluorescent state were obtained from the red FP DsRed through mutagenesis; however first generation variants were dim [60]. Their successors PA-mCherry1, -2, and -3 are more promising for broader spectrum of applications including use in the mouse due to their enhanced brightness, photostability, and faster photoactivation [61].

3.6 Reversible Photoactivatable Fluorescent Proteins

This group encompasses the proteins KFP1 [62] and Dronpa [63, 64]. KFP1, kindling fluorescent protein, is derived from the chromoprotein asFP595 of the Anthozoa *Anemonia sulcata* [65]. It converts from a nonfluorescent to a green fluorescent state upon exposure to green light. Depending on the intensity and duration of the activating light this photoactivation can be reversible or irreversible [62, 66]. Dronpa from the coral *Pectiniidae sp*. can be activated from a green to a nonfluorescent state upon activation with blue light [63, 64]. Such reversible proteins have been used in a number of applications for protein tracking [67, 68] and superresolution imaging [69].

3.7 Photoconvertible Proteins

Another variant of GFP, PS-CFP, fluoresces cyan in its neutral state and changes, upon activation with short wavelength light, irreversibly to a green fluorescing state. The recently engineered PS-CFP2 succeeds PS-CFP due to its increase in brightness [70, 71] and has been proven useful for live cell tracking for several hours up to days in the chick due to its photostability [72].

A large group of photoconvertible proteins comprise FPs that change from a green to a red fluorescent state upon irradiation with short wave length light. Among them are Dendra and its successor Dendra2 [70], Kaede [73, 74], the tandem dimer EosFP and its monomeric variant mEos2 [75] as well as KikGR [76] and its monomeric variant mKikGR [77].

In the mouse, Dendra has been used in an actin fusion to investigate the role of actin in axonal transport in axonogenesis in hippocampal neurons [78]. Dendra2, along with mEos2, finds widespread use in super-resolution microscopy [79, 80], as well as in protein tracking studies [81].

Kaede and KikGR have been shown to be attractive photomodulatable markers for efficiently labeling and tracking single cells or subpopulations of cells in the chick embryo [72, 82], as well as in the mouse embryo [83–85]. Transgenic mouse strains expressing KikGR in a widespread fashion in combination with 3D time-lapse imaging have been shown to be useful tools for cell tracking in various morphogenetic processes [84]. Such strains have been used to demonstrate that the specification of the embryonic–abembryonic axis in the mouse blastocyst is independent of early cell lineage [85]. Since both, Kaede and KikGR, form tetramers, neutral protein fusions comprising these photoactivatable proteins have not been reported in the chick or in mice [58], with the exception of a recent study using electroporation of a Kaedecentrin1 fusion to elegantly visualize mother vs. daughter centrosomes in mammalian neocortical neural progenitors [86]. Even so, the recently developed monomer of KikGR, mKikGR [77], represents a promising new variant that should be tested in protein fusions, and if successful will likely find a frequent application.

A unique recently reported FP is IrisFP. IrisFP is the only FP that can undergo irreversible photoconversion from a green to a red fluorescent state, as well as a reversible photoswitching from a fluorescent to a nonfluorescent form in both the green and the red fluorescent states [87]. The monomeric variant, mIrisFP has already been successfully

applied in pulse-chase experiments in combination dual color photoactivation localization microscopy (PALM) [88].

4 Visualizing Mouse Embryonic Development

Live imaging cell behaviors together with mouse genetics represent a powerful approach to gain a deeper understanding of the mechanisms that play a role embryonic development and disease. Engineered mouse strains that express genetically encoded FPs are an essential step in towards fulfilling this goal.

Widespread expressed native fluorescent proteins often do not provide the desired cellular resolution in imaging. Therefore, cells are marked with a tag so one can track and identify individual cells in 3D space. The most prominent of such applications are the incorporation of FPs into protein fusions that serve as vital labels for subcellular compartments [89]. The most commonly used protein fusions are the histone fusions that mark the cell nucleus, signal sequence tagged fusions for the plasma membrane of the cell, cytoskeletal fusion, i.e., actin or tubulin FP fusion proteins and mitochondrial fusions. Besides its low autofluorescence the nucleus is an ideal organelle to be labeled with a fluorescent tag since it is optimal for tracking cells. Histone fusion proteins are bound to chromatin and stay bound during cell divisions. This makes them suitable to visualize dividing cells over time [90–92]. Cells in mitosis can be easily distinguished from cells in interphase. Individual cells can be tracked and identified in a subpopulation of cells. In addition, cells that undergo apoptosis can be visualized. To gain information on cell morphology, lipid modified fusions act as tags for the plasma membrane and secretory pathway. These tags can be fused to different spectrally distinct FPs used to gain information on both, cell morphology and cell position, simultaneously [90, 91, 93–95]. Moreover, a recently published global double Cre reporter expressing membrane tagged (td)Tomato prior to Cre-mediated excision and membrane-tagged GFP after excision is a useful reporter to trace cell lineages and gain information on cell morphology in the context of recombined and nonrecombined cells [95].

Increasingly, sophisticated approaches exploiting the copious FPs are being used to label and trace cells in mice. The combinatorial expression of FPs to label individual neurons and trace their origin in the mouse brain was demonstrated using a Cre/*lox*P strategy, called Brainbow. Brainbow results in the Cre-mediated stochastic expression multiple FPs from a single transgene and simultaneously labels neurons in as many as 90 different colors [96]. A different strategy employing CFP, GFP, YFP, and RFP was chosen to analyze the contributions of clonal progenitors to yolk sac blood islands, the initial sites of hematopoetic and endothelial cells, to reveal that these cell lineages do not arise from a single clonal precursor [97].

Such reporter fusions have already been successfully used in other diverse organisms including nematode worms, fruit flies, zebrafish, and chick embryos [94, 98–101]. Several transgenic and gene-targeted mouse strains expressing histone fusions and various membrane fusions in a variety of colors have been made and are used to perform high-resolution imaging of cell function and behavior. Besides widespread expression, these fusions are also used in a tissue-specific context when they are expressed under defined *cis*-regulatory elements to provide further understanding of specific gene activity or protein function, for example, the H2B-GFP knock-in into the *Pdgfr*α locus, which recapitulates PDGFRα expression and can be used as a marker for PDGFRα expression as well as the tissues in which is expressed *Pdgfr*α is in refs. [102, 103]. The visualization of live cell behaviors in these models will pave the way for an in-depth knowledge of the mechanisms that control embryonic development, homeostasis, and disease progression.

5 Imag(in)ing the Future

Imaging is unique in its ability to capture quantitative data at single cell resolution in living embryos. Improvements in both optical imaging modalities and the characteristics of fluorescent proteins are spearheading the increased popularity of live imaging approaches to investigate mouse embryonic development. Novel reporter strains are continually being developed for diverse live imaging applications. It is likely that the future holds more color variants, brighter, faster maturing, and more photostable proteins. Such FPs will be better suited, for longer time-lapse imaging of mouse embryos or explants in culture. Photomodulatable FPs represent a new class of genetically encoded reporter and further engineering will produce brighter and monomeric versions, more suitable for protein fusions and less toxic for cells. In addition, photoconversion at wavelengths that are less toxic and shifting emission wavelengths to far-red or near infrared regions are desirable and likely will be achieved. One can predict with certainty, is that whatever the future holds, live imaging using genetically encoded fluorescent reporters will be center stage.

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