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Minireview

Green fluorescent protein: applications in cell biology

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Abstract The green fluorescent protein (GFP) of *Aequorea victoria* is a unique in vivo reporter for monitoring dynamic processes in cells or organisms. As a fusion tag GFP can be used to localize proteins, to follow their movement or to study the dynamics of the subcellular compartments to which these proteins are targeted. Recent studies where GFP technology has revealed new insights regarding physiological activities of living cells are discussed.

Key words: Green fluorescent protein (GFP)

1. Introduction

Amongst the numerous bioluminescent organisms the jellyfish *Aequorea victoria* has been the most recent to come into the limelight. This Northern Atlantic organism can emit green fluorescent light from photocytes at the margin of its umbrella [1]. The fluorescence is generated by sequential activation of two photoproteins, aequorin and green fluorescent protein (GFP) [1]. Upon calcium binding, aequorin emits blue light which in turn excites GFP to fluoresce green [1]. This GFP has a unique property in that it forms a chromophore of three amino acids within its primary structure and, in contrast to other bioluminescent molecules, operates independently of cofactors [1]. Although the properties of the protein have been known for many years [2], it was not until the cloning of the cDNA of GFP in 1992 [3] and its subsequent heterologous expression in *E. coli* and *C. elegans* [4] that researchers from many fields became aware of the potential of this molecule. Up to this point the work of many laboratories had shown that GFP can produce green fluorescence in a variety of diverse organisms such as bacteria, slime molds, plants and mammals [2]. The numerous applications include: using GFP as a reporter for gene expression [4], as a marker to study cell lineage during development [5] and as a tag to localize proteins in living cells [6]. Here we focus on the use of GFP as a protein tag and upon those applications of this new tool in which GFP promises to be truely superior to conventional methods.

2. Properties of wildtype and mutant GFPs

GFP, as deduced from its cDNA, is a 238 amino acid protein [3] with an apparent molecular weight of about 27–30 kDa on SDS-PAGE [7]. Its chromophore is formed by cyclisation and oxidation of the three amino acids Ser65, Tyr66 and Gly67 [3,8]. This posttranslational modification (maturation) occurs within 2 to 4 h after synthesis and is probably autocatalytic [8]. The wildtype GFP (subsequently referred to as GFP) has two absorption maxima, a major peak at 395 nm and a minor one at 475 nm [2]. Excitation at either of the two wavelengths results in emission of green light at 508 nm (for photophysical and photochemical details see review [2]). These fluorescence properties have been changed by genetic engineering leading to several mutants [8–12]. Two of these mutants are of special interest for cell biologists. The Ser65 = > Thr (S65T) mutant of Heim et al. [9] has three advantages relative to GFP. First, a single excitation peak at 490 nm adapts this mutant to common fluorescein isothiocyanate (FITC) filter sets, second, excitation yields a six-fold stronger fluoresence which is more resistant to photobleaching [9] and third, this mutant matures fourfold faster than GFP [9], thus shortening the lag time between synthesis and photoactivity. The double mutant Y66H/Y145F [12], an improved version of a previously described mutant [8], emits blue light at 445 nm when excitated at 381 nm. Not only is this the first engineered GFP which emits light of a different colour, therefore called blue fluorescent protein (BFP) [12], but the blue light of BFP can also excite GFP thus allowing fluorescence resonance energy transfer (FRET) as has been elegantly demonstrated in vitro with the two mutants as donor and acceptor [12].

3. GFP-tagged proteins and their subcellular localisation

Expression of GFP on its own results in a diffuse distribution throughout the cytoplasm, in many cases including the nucleus [13–15]. Pioneering work by Wang and Hazelrigg [6] has demonstrated that GFP can be used as a fluorescent tag for the N- or C-termini of proteins. Although the chromophore is formed by just three amino acids, truncation of only a very few amino acids at either end of the full length GFP is tolerated if the protein is to maintain its fluorescence [2]. Despite the large size of such a GFP tag (238 amino acids versus 10 and 9 amino acids of the frequently used immunocytochemical tags myc and HA, respectively), it has been shown in numerous cases that the tagged proteins were functional and were targeted properly [2].

In this section we give an update of a recent review on GFP [2] in which successfully used GFP fusion proteins were comprehensively listed. We group the many examples of GFP-tagged proteins with respect to the cellular compartments and accentuate the use of GFP in the secretory pathway, the field of our own research. The discussed examples are listed in Table 1.

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Abbreviations: GFP, green fluorescent protein; BFP, blue fluorescent protein; TGN, trans-Golgi network; ER, endoplasmic reticulum; PM, plasma membrane

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3.1. Cytoskeleton

The cytoskeleton is a highly dynamic structure which is essential for intracellular transport processes as well as for cell growth, division, migration and differentiation. So far fluorescent-labeled tubulin or phalloidin have been injected into living cells to study the dynamics of the actin and microtubule based networks. Now with GFP, it is also possible to tag actin and microtubule binding proteins, or to label organelles interacting with the cytoskeleton. Moreover, a combination of both techniques is conceivable, e.g. one could visualize GFP-labeled organelles moving along rhodamine-labeled microtubules.

So far three interesting studies have dealt with GFP-tagged actin binding proteins. Gerisch and colleagues [16] elegantly showed that coronin, an actin-associated protein in the slime mold *Dictyostelium*, is involved in migration and phagocytosis. In cAMP-stimulated migrating cells coronin-GFP was seen at the leading edge [16] whilst in phagocytosing cells coronin-GFP was also concentrated at the rim of phagocytic cups [17]. Real-time recordings showed a competition between leading edge formation and phagocytic cup formation, a finding which would have been difficult to obtain with conventional methods. In a second study the actin-dependent motor protein myosin was tagged with GFP and analysed in *Dictyostelium* cells by videomicroscopy [18]. Unexpectedly, GFP-myosin was found to be transiently concentrated in the tips of retracting pseudopods. Third, the movement of cortical actin patches in living yeast cells was addressed. These cortical patches specify the location of polarized cell growth. One component of them, the capping protein (Cap2p), was fused to GFP and used as a reporter to monitor patch movement in living cells. Videomicroscopy revealed that changes in patch locations are due to movement of the patches and not to their disassembly and reassembly elsewhere [19].

The first microtubule binding protein to have been tagged with GFP was the exu protein of *Drosophila* [6]. This protein is involved in mRNA-localisation during oogenesis. Subsequently tubulin [20], Map4 [15], and tau [21] were tagged successfully. In a recent study a recombinant tobacco mosaic virus, expressing the viral movement protein (MP) fused to GFP, was used to infect plant leaves [22]. The MP-GFP was shown to interact with microtubules and promoted the cell-to-cell spreading of infection via plasmodesmata. This example demonstrates nicely how the use of a GFP-tagged molecule can give new insights into viral–host interactions.

Proteins indirectly linked to microtubules via the spindle

Table 1
GFP fusion proteins discussed in this review

Subcellular compartment	Chimera	Fusion partner	GFP version	[Ref.]
Cytoskeleton	coronin–GFP	coronin, *Dictyostelium*	wt	[16,17]
	GFP–myosin	myosin heavy chain, *Dictyostelium*	wt	[18]
	GFP–Cap2p	β-subunit of capping protein (Cap2p), *S. cerevisae*	S65T	[19]
	GFP–Exu	exuperantia (exu), *Drosophila*	wt	[6]
	GFP–tubulin	α-tubulin, *S. cerevisae*	wt	[20]
	GFP–MAP4	MAP4, *M. musculus*	wt	[15]
	Tau–GFP	tau, *Drosophila*	wt	[21]
	MP–GFP	movement protein (MP), tobamovirus	wt	[22]
	Nuf2–GFP	spindle pole body associated protein, *S. cerevisae*	wt	[23]
	GFP–mei-S332	centromere associated protein, *Drosophila*	wt	[24]
Secretory pathway	hCgB–GFP	human chromogranin B	S65T	[26]
	GFP–KDELr	KDEL-receptor	wt	[28]
	HmgRp–GFP	hydroxy-methylglutaryl-CoA reductase (Hmg1p, Hmg2p), *S. cerevisae*	wt	[29]
	GFP–z–α1Pi	α-1 proteinase inhibitor	wt	[30]
	TGN38–GFP, GFP–TGN38	rat TGN38	S65T	(a)
	Glut4–GFP, GFP–Glut4	insulin regulatable glucose transporter	wt	(b)
Plasma membrane	hGH–GFP–DAF	human growth hormone (GH), GPI-anchor of decay acceleration factor (DAF)	S65T	[31]
	GAP–GFP	membrane anchor signal of GAP-43	S65T	[32]
	GFP–Ras	membrane anchor signal of Ras	S65A	[32]
Nucleus	GFP–hGR	human glucocorticoid receptor (hGR)	wt	[13]
	nuGFP	rat glucocorticoid receptor	wt	[37]
	nuGFP(S65T)	rat glucocorticoid receptor	S65T	[37]
	H2B1–GFP	histone H2B1, *S. cerevisae*	wt	[34]
	Np13p–GFP	nuclear protein Np13p, *S. cerevisae*	wt	[35]
	GFP–nucleoplasmin	nucleoplasmin, *S. cerevisae*	wt	[27]
	Rev–GFP	Rev protein, HIV	wt	[36]
Mitochondrion	mtGFP	mitochondrial import signal of subunit VIII of cytochrome c oxidase	wt	[37]
	mtGFP(Y66H/Y145F)	same as mtGFP	Y66H/Y145F	[33]
Peroxisome	GFP–SKL	tripeptide Ser-Lys-Leu, peroxisomal import signal	S65T	(c)

The name of the chimera refers to the position of GFP in the fusion construct (GFP–X, N-terminal GFP; X–GFP, C-terminal GFP). The fusion partner is the protein or polypeptide tagged with GFP. GFP versions: wt: wildtype GFP; S65T, S65A, Y66H/Y145F, mutants of GFP, see Section 2. Ref., numbers refer to references; (a), G. Banting, unpublished; (b), J.M. Tavaré, unpublished; (c) W. Just, unpublished.

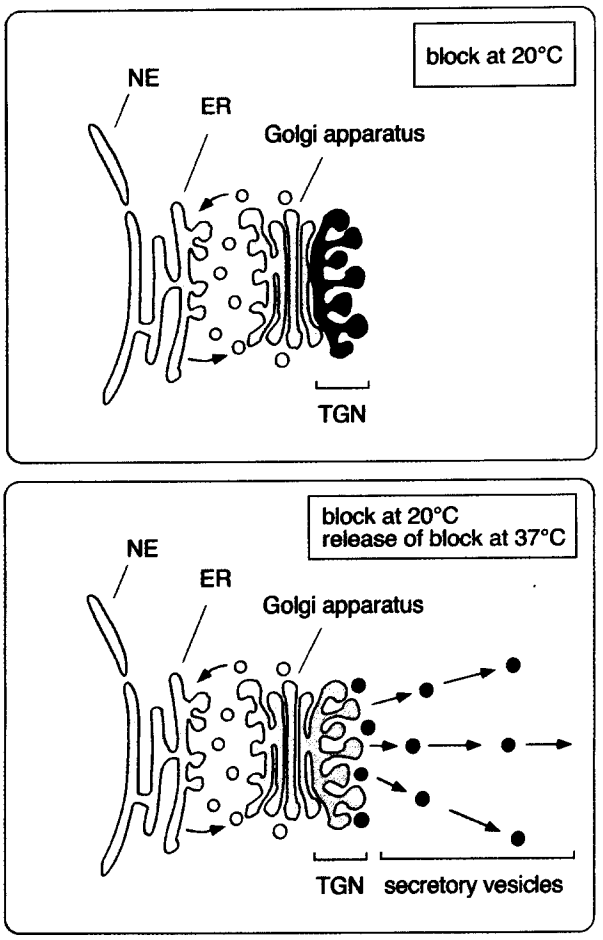

Fig. 1. Visualization of vesicular transport in living cells. GFP (S65T) was fused to the C-terminus of a secretory protein, chromogranin B, and expressed in mammalian cells [26]. Arrest of biosynthetic transport at the TGN by a 20°C temperature block led to green fluorescence in this compartment (top). Release of the temperature block by incubation at 37°C resulted in formation of GFP-containing transport vesicles (bottom). These vesicles can be followed in living cells with fluorescence videomicroscopy. NE, nuclear envelope; ER, endoplasmic reticulum; TGN, trans-Golgi network.

pole body [23] or centromere [24] have been tagged with GFP to study cell division. In the first case budding yeast cells were recorded in vivo by tagging the spindle pole body associated protein, Nuf2p. This led to the identification of previously unknown mitotic stages; in particular it was shown that the mitotic spindle has four sequential activities during anaphase [23].

3.2. Secretory pathway

The secretory pathway, composed of the endoplasmic reticulum (ER), the Golgi apparatus and the endosomal/lysosomal system, is the major intracellular membrane system. The highly dynamic process of shuttling proteins between these compartments in transport vesicles implies a complex and specific machinery [25]. Clearly here, as in the studies on the cytoskeleton, the application of GFP has the potential to extend the knowledge based on previous studies where 'snapshots' of fixed cells had to be interpreted. In the first study illuminating the secretory pathway with GFP, a tagged secretory protein was used to visualize vesicular transport steps en route to secretion [26]. In this case, no fluorescence was observed under normal cell culture conditions at 37°C. Green fluorescence could be obtained at the TGN after secretion was arrested by a 20°C temperature block (Fig. 1). In our opinion there are three reasons for this effect. First, a block prolongs the residence time of the GFP-tagged secretory protein in the cell, thus allowing acquisition of fluorescence before its secretion. Second, the lower temperature used for the block itself promotes proper maturation yielding a stronger fluorescence [13,14,27], and third, secretion arrest at defined stages in the pathway leads to higher local concentrations of fluorescent protein. The unique property of this system is that a defined population of GFP-labeled transport vesicles can be followed in a pulse/chase-like manner after the block is released (Fig. 1). Currently we are characterizing the movement of TGN-derived transport vesicles by videomicroscopy in collaboration with I. Wacker and W. Almers (Heidelberg).

Highly dynamic ER–Golgi interactions were studied by tagging the KDEL receptor, a membrane protein recycling between these two compartments [28]. Degradation of proteins in the ER was analysed both by tagging an ER resident membrane protein [29] and a mutated secretory protein [30]. The trans-Golgi network (TGN), a major sorting station of the secretory pathway, was not only visualized by following a tagged secretory protein [26] but also by fusing GFP to the TGN resident marker protein TGN38. Stably expressed N- or C-terminally tagged TGN38 were appropriately localized and recycled via the endosomal compartment (G. Banting, unpublished).

3.3. Plasma membrane

At the plasma membrane (PM), a continous flow of endocytic and exocytic vesicles occurs. In addition, intracellular pools of proteins like hormones, zymogens or transporters can be recruited by regulated vesicular transport. GLUT4, the insulin regulatable glucose transporter, is an example of a transporter which is temporarily exposed at the PM. The insulin-stimulated exposure to the surface and subsequent retrieval could be analysed in single living cells using GLUT4-GFP chimeras (J.M. Tavaré, unpublished). In other studies the PM was labeled with a GPI-anchored GFP [31], or with membrane-associated GFP, using the anchoring signal sequences of either GAP-43 or Ras [32].

3.4. Nucleus

The nuclear pore regulates bidirectional transport of proteins and RNAs across the nuclear envelope. One such class of transported proteins is the glucocorticoid receptors which are translocated into the nucleus upon steroid hormone binding. This translocation was monitored in living cells with GFP-tagged human or rat glucocorticoid receptors after dexamethasone induction [13,33]. Factors like small GTPases, which regulate translocation at the pore, were studied by following the import [27,34,35] or export [36] of GFP reporter proteins. In one study [27], the temperature-sensitivity of GFP was exploited in a similar pulse/chase-like manner as described for the secretory pathway (see Section 3.2).

3.5. Mitochondria and peroxisomes

Most peroxisomal and mitochondrial proteins are synthesized in the cytoplasm and imported posttranslationally via

well defined targeting signals. Pozzan and colleagues showed that GFP with a mitochondrial import signal intensively labeled mitochondria [37] and more recently his group demonstrated that the simultaneous expression of appropriately tagged GFP and BFP fusion proteins in HeLa cells labeled the nucleus and the mitochondria in green and blue, respectively [33]. Such a double-labeling could be useful to study organelle–organelle interactions or sorting of differentially tagged proteins.

In a similar approach, it has been possible to study microtubule dependent movement of peroxisomes, by labeling them with a GFP which includes the tripeptide peroxisome import signal (Ser-Lys-Leu) fused to its C-terminus (W. Just, unpublished).

4. Conclusions and perspectives

The many examples of GFP being used for the visualization of a variety of cellular compartments would suggest that there seems to be no principal obstacle preventing fluorescence in the majority of compartments. However, our GFP-tagged regulated secretory protein [26] exhibited fluorescence exclusively when expressed in cells which have only a constitutive pathway of protein secretion, but not in cells with an additional regulated pathway (C. Kaether and H.-H. Gerdes, unpublished).

Because GFP is such a powerful tool for uncovering dynamic cellular events it has become useful to display the data by video, giving rise to exciting and entertaining symposia such as those at the annual meeting of the ASCB in Washington 1995 and the annual meeting of the German Society for Cell Biology in Hamburg 1996. The traditional way of publishing unfortunately restricts these data to static pictures. However, with more and more journals going 'on line' this problem could well be circumvented in the near future. Two examples in which a 'green' movie can be accessed in a public domain folder are [http://www.comet.chr.va.us/quill/] (ref. [17]) and [http://www.cooperlab.wustl.edu/] (ref. [19]).

New applications of GFP can be expected from two fields of research. First, further genetic engineering might yield the long-sought-after red-emitting mutant (RFP). Second, demonstration of FRET by using a pair of GFP-mutants in vivo would open a new way for studying protein–protein interactions in living cells. At the moment it seems there will be few areas of biology onto which GFP could shed no light. Whether all the inital expectations are fulfilled remains to be seen, but the future promises to be illuminating.

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