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Review

Potentiality of Green Fluorescent Protein (GFP) from *Aequorea victoria* – A Mini Review

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Abstract Green fluorescent protein (GFP), a very important biological agent that involves shifting the color of bioluminescence from blue to green in luminous coelenterates and to increase the quantum yield of light emission. GFP discovered in medusa, *Aequorea victoria* is a key factor of various biotechnological and cell biological applications. Beside these applications, GFP of *A. victoria* is generally stable, which does not require co-factors for activity and can be functionally expressed in different bacterial species. This property of GFPs from *A. victoria* permits them to be a unique tool to monitor gene expression and protein localization in different organisms. The present review brings out the past milestones and future perspectives on GFPs, with an elaborative reviewing on its applications.

Key words : Green fluorescent protein; *Aequorea victoria*; bioluminescence

Introduction

Many of the marine invertebrates are possessing magical coloration mechanism adopted from nature called bioluminescence. This is the form of green light produced by the help of chemical reactions due to photon emission [1]. The jellyfish, *Aequorea victoria* is a bioluminescent coelenterate that produces a variety of coloration proteins e.g., aequorin [2], which is the active component of the bioluminescence. The light emission of purified aequorin peaked in the blue part of the visible spectrum, visualized as a bioluminescence in green color. In the quest to explain this situation, Shimomura and Johnson (1969, 1970) decided to isolate yet another protein found to be of blue colored luminescence, subsequently different from aequorin [3,4]. This luminescence matches with the long wavelength peak in the excitation spectrum of GFP, with an emission spectrum observed at 510 nm [5]. This data proves that the green light of *A. victoria* originates indirect, radiation-less transfer of the energy of the chemically excited electronic dipole in aequorin to excite the electronic dipole of GFP, which followed by green photon emission and the later returns to the ground state [6,7]. It clearly indicated that GFP is the acceptor and aequorin, which became the source for an energy transfer reaction in Forster Resonance Energy Transfer (FRET).

The strategic research of Shimomura (1979) focused on the fluorescent chromophore of GFP to clarify its chemical structure [8]. According to his postulations, the digested GFP with papain led to the disappearance of the fluorescence of the protein, but resulted in a peptide fragment with same absorption spectrum as that of the intact protein. The physico-chemical properties of this peptide were studied by comparing with those of a model compound and suggested that the chromophore is a p-hydroxybenzylideneimidazolinone moiety. With access to the primary structure of GFP [9], research continued on re-characterizing GFP chromophore by using Nuclear Magnetic Resonance (NMR) and it has been confirmed that the functional portion of the chromophore as p-hydroxybenzylideneimidazolinone. However, as suggested by Shimomura, the two re-identified amino acids, making up the imidazolone ring were Ser and Gly [10].

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Structure

The native GFP isolated from the *A. victoria* [2] was first named by Morin and Hastings in 1971 [6-7], which contains 238 amino acids [9]. Residues 65-67 (Ser-Tyr-Gly) in the GFP sequence spontaneously [11] form the fluorescent chromophore p-hydroxybenzylideneimidazolinone [10,12]. The excitation spectrum of GFP fluorescence has a dominant maximum at about 400 nm and a significantly smaller maximum at about 470 nm, while the emission spectrum has a sharp maximum at about 505 nm and a shoulder around 540 nm [13].

The crystal structure of GFP [14,15] is an eleven-stranded ß- barrel, threaded by an α-helix, running up along the axis of the cylinder. The chromophore is in the α-helix, very close to the center of the can-like cylinder. A very large part of the primary structure of the protein is used to construct the ß-barrel and the threading α-helix [11,16,17]. The N-terminal residue and the C-terminal residues 230-238, approximately corresponding to the maximal number of residues of GFP that can be removed from the N- (2 residues) and C-terminal (6 residues), respectively at retained fluorescence are disordered and therefore unresolved in this structural image [18].

The tripeptide motif Ser65-Tyr66-Gly67 in the primary structure of unfolded or denatured GFP does not display any striking feature [19]. However, as the GFP protein folds into its native conformation, these three amino acids are forced into a sharp turn, greatly favoring a nucleophilic attack of the amide of Gly67 on the carbonyl of Ser65, leading to imidazolinone formation by cyclization and dehydration [20,21]. At this point, GFP does not fluoresce [11] but, conditional on the presence of molecular oxygen, the a-ß bond of residue 66 is subsequently dehydrogenated into conjugation with the imidazolinone, which results in maturation of the GFP chromophore to its fluorescent form [11-16].

GFP is generally non-toxic and can be expressed to high levels in different organisms with minor effects on their physiology [22]. Furthermore, when the gene for GFP is fused to the gene of a protein to be studied in an organism of interest, the expressed respective protein retains its normal activity and, likewise, GFP retains its fluorescence, so that the location, movement and other activities of the studied protein can be followed by microscopic monitoring of the GFP fluorescence [23]. Considering both cases, the remarkable and unexpected properties of GFP from *Aequorea victoria* summarized in this section are essential for the usefulness of GFP for studies at the molecular level of dynamic processes in living cells.

Classification

Classification of spectral mutants of *Aequorea* GFP by chromophore type has provided a logical and useful guide to users of the GFPs since its introduction in a comprehensive form in 1998 [13]. GFP variants divided into seven classes based on the distinctive component of their chromophore.

Wild- type mixture of neutral phenol and anionic phenolate (wild type green fluorescent proteins-WGFP)

The wild-type *Aequorea* protein has the most complex spectra of all the GFPs. It has a major excitation peak at 395 nm that is about three times higher in amplitude than a minor peak at 475 nm. In normal solution, excitation at 395 nm gives emission peaking at 508 nm, whereas excitation at 475 nm gives a maximum at 503 nm [11].

Phenolate anion in chromophore (green fluorescent proteins-GFP)

GFPs with phenolate anions in the chromophore have become the most widely used class for routine cell biological use. The most commonly used mutation to cause ionization of the phenol of the chromophore is a replacement of Ser65 by Thr, or S65T [20], though several other aliphatic residues such as Gly, Ala, Cys and Leu have roughly similar effects [20,24,25]. In the wild-type 395-nm excitation peak due to the neutral phenol is suppressed and the 470- to 475-nm peak due to the anion is enhanced five- to sixfold in amplitude and shifted to 489-490 nm [20,24,26].

Neutral phenol in chromophore (sapphire fluorescent proteins-SFP)

Mutation of Thr203 to Ile in wild type, largely suppresses the 475-nm excitation peak, leaving only the shorter wavelength peak at 399 nm [11,27].

Phenolate anion with stacked π-electron system (yellow fluorescent proteins-YFP)

The longest wavelengths currently available by mutation result from stacking an aromatic ring next to the phenolate anion of the chromophore. So far the aromatic ring has always come from the side chain of residue 203 and residue 65 is Gly or Thr instead of Ser, to promote ionization of the chromophore. All four aromatic residues at that position 203 (His, Trp, Phe and Tyr) increase the excitation and emission wavelengths by up to 20 nm, with the shifts increasing in the stated order [28].

Indole in chromophore derived from Y66W (cyan fluorescent proteins-CFP)

Substitution of Trp for Tyr66 produces a new chromophore with an indole instead of a phenol or phenolate. Excitation and emission wavelengths are 436 and 476 nm, intermediate between neutral phenol and anionic phenolate chromophores [20,29].

Imidazole in chromophore derived from Y66H (blue fluorescent proteins-BFP)

Substitution of His for Tyr 66 puts an imidazole in the chromophore and shifts the wavelengths yet shorter than Trp66. The excitation and emission peaks are around 383 and 447 nm [20,30].

Phenyl in chromophore derived from Y66F

The very shortest wavelengths are obtained with Phe at 66. The excitation and emission peaks are around 360 and 442 nm. This mutant has been little studied because of no obvious practical use for proteins [16,30].

Applications

Biologically, GFP acts to shift the color of bioluminescence from blue to green in luminous coelenterates and to increase the quantum yield of light emission [31]. The stability of GFP from *A. victoria* renders no requirement of cofactors for its activity and can be functionally expressed in various bacterial species [22,32]. This properties permits green fluorescent protein to be a unique tool for cell biology applications to monitor gene expression and protein localization in different organisms [22,33-36]. For example in prokaryotes, GFP had a significant impact in the fields of bacterial development, pathogenesis [37-39] and ecology [40,41]. On the other hand, the development of spectral variants of GFP, including the CFP and YFP along with the discovery of a red fluorescent protein (RFP) from the coral *Discosoma*, have paved the way for *in vivo* co-localization and fluorescence resonance energy transfer (FRET) studies in yeast [13,42,43,].

Marker genes like β-glucuronidase (*gusA*) gene have proved as extremely useful in reporting gene expression in transformed plants [44]. Transformed cells or patterns of gene expression within plants can be identified histochemically, but this is generally a destructive test and is not suitable for assaying primary transformants, or for following the time course of gene expression in living plants, or as a means of rapidly screening segregating populations of seedlings [45]. The GFP from *A. victoria* is a void of all these problems, because its intrinsic fluorescence can be identified in living cells. In addition, GFP usage as a marker for transgenic plants has been of a great interest.

Unmodified GFP has been successfully expressed at high levels in tobacco plants using the cytoplasmic ribonucleic acid (RNA) viruses, potato virus X [46] and tobacco mosaic virus [47]. In these experiments, the gene was directly expressed as a viral mRNA in infected cells and significantly high levels of GFP fluorescence were exhibited during the expression.

The use of viral vector systems to express GFP has also been used to monitor the production and release of therapeutic molecules from mammalian cells and tissues. Bartlett et al. (1995) developed an adenovirus vector delivery system using GFP inserted downstream from the human muscle creatine kinase promoter and following the vector injection, efficient GFP expression was recorded in skeletal muscle [48]. Moreover, Moriyoshi et al. (1996) used an adenovirus vector to transfer GFP into post-mitotic neuronal cells *in vivo* to study cell migration and development of neuronal connections [49]. However viral vectors containing GFP have been used to monitor viral infection and pathogenesis with no need for processing of cells to detect infected cells. Using this approach, Dorsky et al. (1996) have identified human immunodeficiency virus (HIV)-1-infected cells in the tissues using GFP tagged HIV-1 [50]. In addition, Dhandayuthapani et al. (1995) used a mycobacterial shuttle-plasmid vector carrying

GFP cDNA to assess mycobacterial interactions with macrophages. Moreover, GFP has been used to comprehend the cytology of viral infection directly by visualizing cell uptake and viral factories *in vivo* [51-58].

The cytoskeleton can be "illuminated" by the use of GFP and not only the individual polymer subunits can be independently labeled, but associated proteins including motor proteins and binding proteins can be tagged along with GFP. Direct labeling of cytoskeletal subunits was initially used as a noninvasive label of the cytoskeleton with the aim of watching movements of vesicular intermediates or membranes [59-60] or of mitotic chromosomes [61] in living cells. However understanding of nuclear architecture and dynamics has been dramatically improved due to the use of GFP in the study of nuclear behavior. One of the most significant improvements in the field has been the realization that interphase nuclear organization, once thought to be comprised of stably associated components assembled into rigid arrays, is extremely dynamic and is capable of self-organization [59-61]. Using time-lapse imaging and photobleaching approaches to probe the behavior of different GFP-tagged nuclear components, researchers have found that many nuclear proteins are undergoing rapid movement within the nucleoplasm. The linker histone, H1, e.g., was found to undergo rapid association and dissociation with chromatin [59]. Likewise, only a transient association of the transcription factor, glucocorticoid receptor, with its promoter elements was observed [62]. Moreover, RNA polymerase I and II components, DNA topoisomerase II, as well as DNA repair complexes were found to be highly mobile in the nucleoplasm [63-68] and do not form the stable holo-complexes.

Because of the potential, fluorescent protein technology has become inevitable to drug discovery in recent years. Up to date, the greatest impact of such proteins has been within the compound screening phase of drug discovery for which fluorescent protein probes have been developed. In parallel, many instruments have become available which allow the detection of changes in the fluorescence characteristics of single or populated cells within microtiter plate formats [69]. It is now possible to determine the effect on the subcellular localization of a fluorescent protein fusion protein of 384 compounds within six minutes, a process which by confocal microscopy would have taken several days or weeks. In the coming years, with the development of more sensitive and faster detection apparatus and improved fluorescent protein probes especially with the help of improvements in GFP field, fluorescent protein screening assays will become integral to the high-throughput screening and pharmacology research in most pharmaceutical companies. These developments should permit drug screening to be performed on biologically relevant cell types, perhaps including primary human cells, at very high throughput. The application of GFP technology within the organized development of most drug companies is in its infancy. In the coming years, fluorescent protein technology will be applied to generate novel drugs and their application in various toxicology assays. The application of fluorescent protein assays for primary screening, development and toxicology should lead to the identification of molecules with improved efficacy, toxicity profiles and pharmacokinetics and thus should contribute to the reduction of both cycle time and attrition within the drug discovery process.

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References

1. Davenport, D. and Nicol, J. A. C. 1955. Luminescence in Hydromedusae. *Proc. R. Soc. London Ser. B* **144**, 399-411.
2. Shimomura, O., Johnson, F. H. and Saiga, Y. 1962. Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. *J. Cell. Comp. Physiol.* **59**, 223-239.
3. Shimomura, O. and Johnson, F. H. 1969. Properties of the bioluminescent protein aequorin. *Biochemistry* **8**, 3991-3997.
4. Shimomura, O. and Johnson, F. H. 1970. Calcium binding, quantum yield and emitting molecule in aequorin bioluminescence. *Nature* **227**, 1356-1357.
5. Cormier, M. J., Hori, K., Karkhanis, Y. D. anderson, J. M., Wampler, J. E., Morin, J. G. and Hastings, J. W. 1973. Evidence for similar biochemical requirements for bioluminescence among the coelenterates. *J. Cell. Physiol*. **81**, 291-298.
6. Morin, J. G. and Hastings, J. W. 1971. Biochemistry of the bioluminescence of colonial hydroids and other coelenterates. *J. Cell. Physiol*. **77**, 305-311.

7. Morin, J. G. and Hastings, J. W. 1971. Energy transfer in a bioluminescent system. *J. Cell. Physiol*. **77**, 313-318.
8. Shimomura, O. 1979. Structure of the chromophore of *Aequorea* green fluorescent protein. *FEBS Lett*. **104**, 220-222.
9. Prasher, D. C., Eckenrode, V. K., Ward, W. W., Prendergast, F. G. and Cormier, M. J. 1992. Primary structure of the *Aequorea victoria* green-fluorescent protein. *Gene* **111**, 229-233.
10. Cody, C. W., Prasher, D. C., Westler, W. M., Prendergast, F. G. and Ward, W. W. 1993. Chemical structure of the hexapeptide chromophore of the *Aequorea* green-fluorescent protein. *Biochemistry* **32**, 1212-1218.
11. Heim, R., Prasher, D. C. and Tsien, R. Y. 1994. Wavelength mutations and posttranslational autoxidation of green fluorescent protein. *Proc. Natl. Acad. Sci. USA* **9**, 12501-12504.
12. Shimomura, O. and Johnson, F. H. 1975. Regeneration of the photoprotein aequorin. *Nature (London)* **256**, 236-238.
13. Tsien, R. Y. 1998. The green fluorescent protein. *Annu. Rev. Biochem.* **67**, 509-544.
14. Ormö, M., Cubitt, A. B., Kallio, K., Gross, L. A., Tsien, R. Y. and Remington, S. J. 1996. Crystal structure of the *Aequorea victoria* green fluorescent protein. *Science* **273**, 1392-1395.
15. Yang, F., Moss, L. G. and Phillips Jr., G. N. 1996. The molecular structure of green fluorescent protein. *Nat. Biotechn*. **14**, 1246-1251.
16. Cubitt, A. B., Heim, R., Adams, S. R., Boyd, A. E., Gross, L. A. and Tsien, R. Y. 1995. Understanding, improving and using green fluorescent proteins. *TIBS* **20**, 448-455.
17. Ehrig, T., O'Kane, D. J. and Prendergast, F. G. 1995. Green-fluorescent protein mutants with altered fluorescence excitation spectra. *FEBS Lett.* **367**, 163-166.
18. Dopf, J. and Horiagan, TM. 1996. Deletion mapping of the *Aequorea victoria* green fluorescent protein. *Gene* **173**, 39-44.
19. Inouye, S. and Tsuji, F. I. 1994. Aequorea green fluorescent protein, expression of the gene and fluorescence characteristics of the recombinant protein. *FEBS Lett.* **341**, 277-280.
20. Heim, R., Cubitt, A. B. and Tsien, R. Y. 1995. Improved green fluorescence. *Nature. (London)* **373**, 663-664.
21. Niwa, H., Inouye, S., Hirano, T., Matsuno, T., Kojima, S., Kubota, M., Ohashi, M. and Tsuji, F. I. 1996. Chemical nature of the light emitter of the *Aequorea* green fluorescent protein. *Proc. Natl. Acad. Sci. USA* **93**, 13617-13622.
22. Chalfie, M., Tu, Y., Euskirchen, G., Ward, W. W. and Prasher, D. C. 1994. Green Fluorescent Protein as marker of gene expression. *Science* **263**, 802-805.
23. Wang, S. and Hazelrigg, T. 1994. Implications for *bcd* mRNA localization from spatial distribution of *exu* protein in *Drosophila* oogenesis. *Nature (London)* **369**, 400-403.
24. Delagrave, S., Hawtin, R. E., Silva, C. M., Yang, M. M. and Youvan, D. C. 1995. Red-shifted excitation mutants of the green fluorescent protein. *Bio/Technol.* **13**, 151-154.
25. Cormack, B., Valdivia, R. and Falkow, S. 1996. FACS-optimized mutants of the green fluorescent protein (GFP). *Gene* **173**, 33-38.
26. Cheng, L., Fu, J., Tsukamoto, A. and Hawley, R. G. 1996. Use of green fluorescent protein variants to monitor gene transfer and expression in mammalian cells. *Nat. Biotechnol.* **14**, 606-609.
27. González, D., Sawyer, A. and Ward, W. W. 1997. Spectral perturbations of mutants of recombinant *Aequorea victoria* green-fluorescent protein (GFP). *Photochem. Photobiol.* **65**, 21S.
28. Kolb, V. A., Makeyev, E. V., Ward, W. W. and Spirin, A. S. 1996. Synthesis and maturation of green fluorescent protein in a cell-free translation system. *Biotech. Lett.* **18**, 1447-1452.
29. Yang, T. T., Cheng, L. and Kain, S. R. 1996. Optimized codon usage and chromophore mutations provide enhanced sensitivity with the green fluorescent protein. *Nucleic Acids Res.* **24(22)**, 4592-4593.
30. Brejc, K., Sixma, T. K., Kitts, P. A., Kain, S. R., Tsien, R. Y., Ormo, M. and Remington, S. J. 1997. Structural basis for dual excitation and photoisomerization of the *Aequorea victoria* green fluorescent protein. *Proc. Natl. Acad. Sci. USA* **94**, 2306-2311.
31. Ward, W. W. and Cormier, M. J. 1979. An energy transfer protein in coelenterate bioluminescence, Characterization of the *Renilla* green-fluorescent protein (GFP). *J. Biol. Chem.* **254**, 781-788.
32. Inouye, S. and Shimomura, O. 1997. The use of *Renilla* luciferase, *Oplophorus* luciferase and apoaequorin as bioluminescent reporter protein in the presence of coelenterazine analogues as substrate. *Biochem. Biophys. Res. Commun*. **233**, 349-353.
33. Hassler, S. 1995. Green fluorescent protein, The next generation. *Bio/Technol.* **13**, 103.
34. Kain, S. R., Adams, M., Kondepudi, A., Yang, T.T. Ward, W. W. and Kitts, P. 1995. Green fluorescent protein as a reporter of gene expression and protein localization. *BioTechniques* **19**, 650-655.
35. Prasher, D. C. 1995. Using GFP to see the light. *Trends Genet.* **11**, 320-323.
36. Stearns, T. 1995. Green fluorescent protein. The green revolution. *Curr. Biol.* **5**, 262-264.
37. Dhandayuthapani, S., Via, L. E., Thomas, C. A., Horowitz, P. M., Deretic, D. and Deretic, V. 1995. Green fluorescent protein as a marker for gene expression and cell biology of mycobacterial interactions with macrophages. *Mol. Microbiol.* **17**, 901-912.
38. Kremer, L., Baulard, A., Estaquier, J., Poulain-Godefroy, O. and Locht, C. 1995. Green fluorescent protein as a

new expression marker in mycobacteria. *Mol. Microbiol.* **17**, 913-922.
39. Valdivia, R. H., Hromockyj, A. E., Monack, D., Ramakrishnan, L. and Falkow, S. 1996. Applications for the green fluorescent protein (GFP) in the study of host-pathogen interactions. *Gene* **173**, 47-52.
40. Christensen, B. B., Sternberg, C. and Molin, S. 1996. Bacterial plasmid conjugation on semisolid surfaces monitored with the green fluorescent protein (GFP) from *Aequorea victoria* as a marker. *Gene* **173**, 59-65.
41. Crameri, A., Whitehorn, E. A., Tate, E. and Stemmer, W. P. C. 1996. Improved green fluorescent protein by molecular evolution using DNA shuffling. *Nat. Biotechnol.* **14**, 315-319.
42. Campbell, R. E., Tour, O., Palmer, A. E., Steinbach, P. A., Baird, G. S., Zacharias, D. A. and Tsien, R. Y. 2002. A monomeric red fluorescent protein. *Proc. Natl. Acad. Sci. USA* **99**, 7877-7882.
43. Matz, M. V., Fradkov, A. F., Labas, Y. A., Savitsky, A. P., Zaraisky, A. G., Markelov, M. L. and Lukyanov, S. A. 1999. Fluorescent proteins from nonbioluminescent Anthozoa species. *Nat. Biotechnol.* **17**, 969-973.
44. Jefferson, R. A., Kavangh, T. A. and Bean, M. W. 1987. GUS fusions, b-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J.* **6**, 3901-3907.
45. Hu, W. and Cheng, C. L. 1995. Expression of *Aequorea green fluorescent protein in plant cells. FEBS Lett.* **369**, 331-334.
46. Baulcombe, D. C., Chapman, S. and Cruz, S. S. 1995. Jellyfish green fluorescent protein as a reporter for virus infections. *Plant J.* **7**, 1045-1053.
47. Heinlein, M., Epel, B. L., Padgett, H. S. and Beachy, R. N. 1995. Interaction of tobamovirus movement proteins with the plant cytoskeleton. *Science* **270**, 1983-1985.
48. Bartlett, R. J., Singer, J. T., Shean, M. K., Weindler, F. W. and Secore, S. L. 1995. Expression of a fluorescent reporter gene in skeletal muscle cells from a virus-based vector, Implications for diabetes treatment. *Transplant. Proc.* **27**, 3344.
49. Moriyoshi, K., Richards, L. J., Akazawa, C., O'Leary, D. D. and Nakanishi, S. 1996. Labeling neural cells using adenoviral gene transfer of membrane-targeted GFP. *Neuron* **16**, 255-260.
50. Dorsky, D. I., Wells, M. and Harrington, R. D. 1996. Detection of HIV-1 infection with a green fluorescent protein reporter system. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* **13**, 308-313.
51. Garcia del Portillo, F. and Finlay, B. B. 1995. The varied lifestyles of intracellular pathogens within eukaryotic vacuolar compartments. *Trends Microbiol.* **3**, 373-380.
52. Elliott, G. and O'Hare, P. 1999. Live-cell analysis of a green fluorescent protein-tagged herpes simplex virus infection. *J. Virol.* **73**, 4110-4119.
53. Chen, J. and Ahlquist, P. 2000. Brome mosaic virus polymerase-like protein 2a is directed to the endoplasmic reticulum by helicase-like viral protein 1a. *J. Virol*. **74**, 4310-4318.
54. Ward, B. M. and Moss, B. 2001. Vaccinia virus intracellular movement is associated with microtubules and independent of actin tails. *J. Virol.* **75**, 11651-11663.
55. Potel., C., Kaelin, K., Gautier, I., Lebon, P., Coppey, J. and Rozenberg, F. 2002. Incorporation of green fluorescent protein into the essential envelope glycoprotein B of herpes simplex virus type 1. *J. Virol. Methods* **105**, 13-23.
56. Taylor, T. J., McNamee, E. E., Day, C. and Knipe, D. M. 2003. Herpes simplex virus replication compartments can form by coalescence of smaller compartments. *Virol.* **309**, 232- 247.
57. La Boissière, S., Izeta, A., Malcomber, S. and O'Hare, P. 2004. Compartmentalization of VP16 in cells infected with recombinant herpes simplex virus expressing VP16-green fluorescent protein fusion proteins. *J. Virol.* **78**, 8002-8014.
58. Moradpour, D., Evans, M. J., Gosert, R., Yuan, Z., Blum, H. E., Goff, S. P., Lindenbach, B. D. and Rice, C. M. 2004. Insertion of green fluorescent protein into non-structural protein 5A allows direct visualization of functional hepatitis C virus replication complexes. *J. Virol.* **78**, 7400-7409.
59. Misteli, T., Gunjan, A., Hock, R., Bustin, M. and Brown, D. T. 2000. Dynamic binding of histone H1 to chromatin in living cells. *Nature* **408**, 877-881.
60. Janicki, S. M. and Spector, D. L. 2003. Nuclear choreography, Interpretations from living cells. *Curr. Opin. Cell Biol.* **15**, 149-157.
61. Bubulya, P. A. and Spector, D. L. 2004. "On the movements of nuclear components in living cells." *Exp. Cell Res.* **296**, 4-11.
62. McNally, J. G., Müller, W. G., Walker, D., Wolford, R. and Hager, G. L. 2000. The glucocorticoid receptor, Rapid exchange with regulatory sites in living cells. *Science* **287**, 1262-1265.
63. Houtsmuller, A. B., Rademakers, S., Nigg, A. L., Hoogstraten, D., Hoeijmakers, J. H. J. and Vermeulen, W. 1999. Action of DNA repair endonuclease ERCC1/XPF in living cells. *Science* **284**, 958-961.
64. Becker, M., Baumann, C., John, S., Walker, D. A., Vigneron, M., McNally, J. G. and Hager, G. L. 2002. Dynamic behavior of transcription factors on a natural promoter in living cells. *EMBO Rep.* **3**, 1188-1194.
65. Christensen, M. O., Larsen, M. K., Barthelmes, H. U., Hock, R. andersen, C. L., Kjeldsen, E., Knudsen, B. R., Westergaard, O., Boege, F. and Mielke, C. 2002. Dynamics of human DNA topoisomerases IIa and IIb in living cells. *J. Cell Biol.* **157**, 31-44.
66. Dundr, M., Hebert, M. D., Karpova, T. S., Stanek, D., Xu, H., Shpargel, K. B., Meier, U. T., Neugebauer, K. M., Matera, A. G. and Misteli, T. 2004. *In vivo* kinetics of Cajal body components. *J. Cell Biol.* **164**, 831-842.

67. Essers, J., Houtsmuller, A. B., van Veelen, L., Paulusma, C., Nigg, A. L., Pastink, A., Vermeulen, W., Hoeijmakers, J. H. J. and Kanaar, R. 2002. Nuclear dynamics of *RAD52* group homologous recombination proteins in response to DNA damage. *EMBO J.* **21**, 2030-2037.
68. Kimura, H. and Cook, P. R. 2001. Kinetics of core histones in living human cells, Little exchange of H3 and H4 and some rapid exchange of H2B. *J. Cell Biol.* **153**, 1341-1353.
69. Graham, D. L., Bevan, N., Lowe, P. N., Palmer, M. and Rees, S. 2001. Application of bgalactosidase enzyme complementation technology as a high throughput screening format for antagonists of the epidermal growth factor receptor. *J. Biomol. Scr*. **6**, 401-411.