

# Integrating nuclear receptor mobility in models of gene regulation

Laurent Gelman, Jerome N. Feige, Cicerone Tudor, Yves Engelborghs, Walter Wahli and Beatrice Desvergne 

 Corresponding Author: Beatrice.Desvergne@unil.ch

Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland [LG, JNF, WW, BD] and Laboratory of Biomolecular Dynamics, Katholieke Universiteit Leuven, Leuven, Belgium [CT, YE]

**The mode of action of nuclear receptors in living cells is an actively investigated field but much remains hypothetical due to the lack, until recently, of methods allowing the assessment of molecular mechanisms *in vivo*. However, these last years, the development of fluorescence microscopy methods has allowed initiating the dissection of the molecular mechanisms underlying gene regulation by nuclear receptors directly in living cells or organisms. Following our analyses on peroxisome proliferator activated receptors (PPARs) in living cells, we discuss here the different models arising from the use of these tools, that attempt to link mobility, DNA binding or chromatin interaction, and transcriptional activity.**

Received October 21st, 2005; Accepted March 1st, 2006; Published April 28th, 2006 | **Abbreviations:** **CHIP:** chromatin immunoprecipitation; **DBD:** DNA binding domain; **ER:** estrogen receptor; **FCS:** fluorescence correlation spectroscopy; **FRAP:** fluorescence recovery after photobleaching; **FRET:** fluorescence resonance energy transfer; **GR:** glucocorticoid receptor; **MMTV:** mouse mammary tumor virus; **NR:** nuclear receptor; **PPAR:** peroxisome proliferator-activated receptor; **PPRE:** peroxisome proliferator response element; **RAR:** retinoic acid receptor; **RE:** response element; **RXR:** retinoid X receptor; **TR:** thyroid hormone receptor | Copyright © 2006, Gelman et al. This is an open-access article distributed under the terms of the Creative Commons Non-Commercial Attribution License, which permits unrestricted non-commercial use distribution and reproduction in any medium, provided the original work is properly cited.

**Cite this article:** Nuclear Receptor Signaling (2006) 4, e010

## Introduction

Recent developments in live cell imaging have greatly challenged our view of nuclear receptor (NR) action *in vivo*. In a global approach combining fluorescence recovery after photobleaching (FRAP), fluorescence correlation spectroscopy (FCS) and fluorescence resonance energy transfer (FRET), we recently started to characterize the behavior in living cells of the peroxisome proliferator-activated receptors (PPARs) [Feige et al., 2005a] (Figure 1). We first demonstrated that PPARs do not form speckles in living cells, in opposition to the estrogen receptor, and that the subnuclear structures that may be observed under some experimental conditions result from overexpression of the protein. Moreover, our immunolabeling experiments suggest that these structures are subjected to degradation by the proteasome. One of our most unexpected results was that PPAR diffusion coefficients in the nucleus are not compatible with a model where PPAR/RXR heterodimers freely diffuse in the nucleus, even in the absence of ligand. We choose here to further discuss this issue and to point out the questions that arose from our and other recently published works on the link between NR mobility and transcriptional activation.

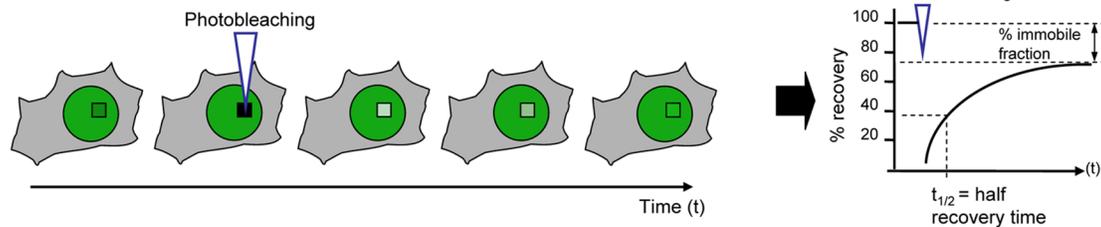
## FRAP and FCS are complementary techniques with different time resolutions

FRAP facilitates the study of the mobility of a fluorescent component in a living cell. A region of interest is first bleached with a high intensity laser beam, and fluorescence recovery is then monitored and modeled to derive the fraction of immobilized molecules and the diffusion parameters of the fraction of mobile molecules (Figure 1). When monitored by FRAP, PPARs, as well

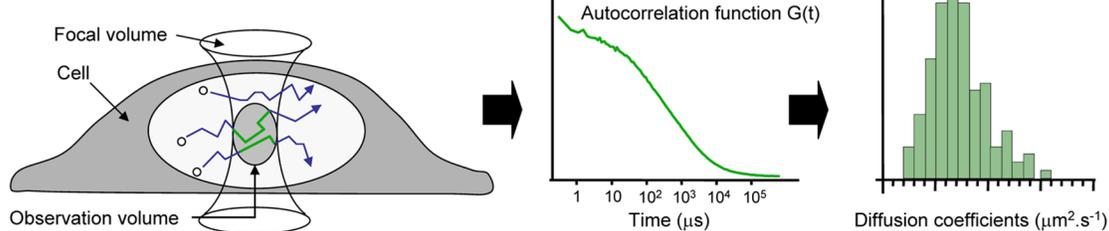
as the retinoic acid receptor (RAR) and the thyroid hormone receptor (TR), appear as very mobile nuclear receptors, which are neither immobilized or slowed down upon ligand binding [Feige et al., 2005a; Martone et al., 2003; Maruvada et al., 2003]. In contrast, the estrogen receptor (ER) and the glucocorticoid receptor (GR) behave differently as, upon ligand binding, a subpopulation of ER and GR is immobilized, and the remaining diffusing receptors display significantly reduced recovery times [Maruvada et al., 2003; Reid et al., 2003; Schaaf and Cidlowski, 2003; Stenoien et al., 2001b]. Although it is tempting to speculate that these differences reflect the dimerization properties of these receptors (PPAR, RAR and TR form heterodimers with RXR and ER and GR form homodimers), we showed that these results are biased by the difficulty in quantifying half-recovery times in the order of hundreds of milliseconds with FRAP.

FCS overcomes this limitation, as it provides quantitative information on the diffusion of fluorescent molecules in the microsecond time range (Figure 1). FCS revealed that PPAR mobility is actually significantly impaired by ligand binding, and that both liganded and unliganded PPARs have diffusion coefficients which are much smaller than those expected if the receptors were freely diffusing in the nucleus as monomers or heterodimers [Feige et al., 2005a]. A straightforward explanation would be that, in the absence of ligand, PPARs associate with corepressors [Dowell et al., 1999; Guan et al., 2005; Shi et al., 2002], and that ligand binding induces the recruitment of larger coactivator complexes [Robyr et al., 2000]. However, the sizes of the complexes deduced from the diffusion coefficients are huge and suggest that, beside cofactor docking, PPARs may also transiently interact with much less mobile components, such as chromatin (Figure 2).

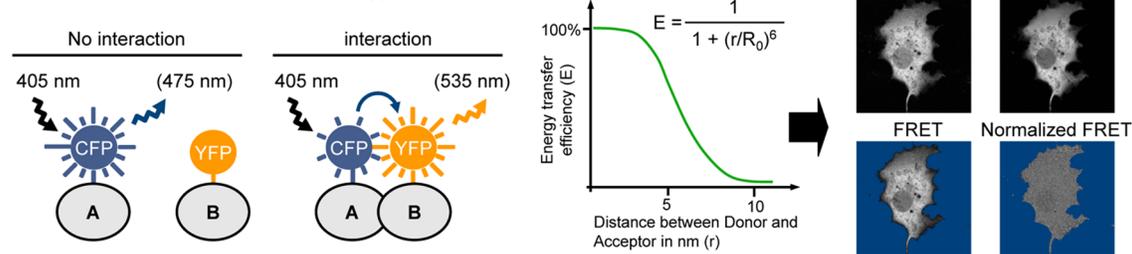
### Fluorescence Recovery After Photobleaching



### Fluorescence Correlation Spectroscopy



### Fluorescence Resonance Energy Transfer



**Figure 1. Imaging methods to study protein behavior in living cells.** Fluorescence recovery after photobleaching (FRAP). A small region of interest is photobleached by application of a high power laser beam. Fluorescence recovery is then calculated based upon quantification of fluorescence intensity in the region of interest (ROI) as a function of time, normalized to the global fluorescence in the cell to take into account photobleaching outside the ROI and during imaging. If a fraction of the molecules are not mobile, recovery does not reach 100%. The half-recovery time (i.e. the time required to get half of the maximal recovery) is often used to describe the mobility of the fluorescent molecule. Fluorescence correlation spectroscopy (FCS). FCS is a method based on the analysis of fluctuations of fluorescence intensity due to the diffusion of a labeled protein through a very small volume (in the range of  $1 \mu\text{m}^3$ ) [Brock et al., 1998; Dittrich et al., 2001]. It allows study of the diffusion of mobile molecules at a higher spatial and temporal (microsecond range) resolution than FRAP. A mathematical transformation of the signal allows one to draw an autocorrelation curve  $G(t)$ , from which the concentration of fluorescent particles and their average diffusion coefficient can be derived. Fluorescence Resonance Energy Transfer (FRET). FRET is the non-radiative transfer of energy between two fluorophores. The energy transfer efficiency decreases proportionally to the sixth power of the distance between the two fluorophores, and hence occurs only when the molecules harboring the fluorophores are in contact or very close proximity. Different methods and programs, such as PixFRET, can be used to calculate and map FRET in a cell or in a cell population [Berney and Danuser, 2003; Feige et al., 2005b]. PixFRET is available for download at <http://www.unil.ch/cig/page16989.html>.

## The "free diffusion" and the "three-dimensional genome scanning" models

Transient interactions with chromatin affect the diffusion of numerous transcription factors [Phair et al., 2004]. A model was proposed where these interactions reflect a dynamic three-dimensional (3D) scanning of the genome until transcription factors reach a genuine enhancer site, where they may reside for longer periods and promote pre-initiation complex assembly. It is tempting to extrapolate such a model to PPARs, but the number of PPAR target genes in a cell is probably low compared to the number of receptors. Hence, few EYFP-PPARs interact with bona fide PPREs, while most of them transiently contact non-specific sites resembling PPREs, but not located within active promoters. "Non-specific" interactions at numerous sites along a chromosome, which are not genuine response elements, has been previously uncovered for NF- $\kappa$ B by chromatin immunoprecipitation (ChIP) [Martone et al., 2003].

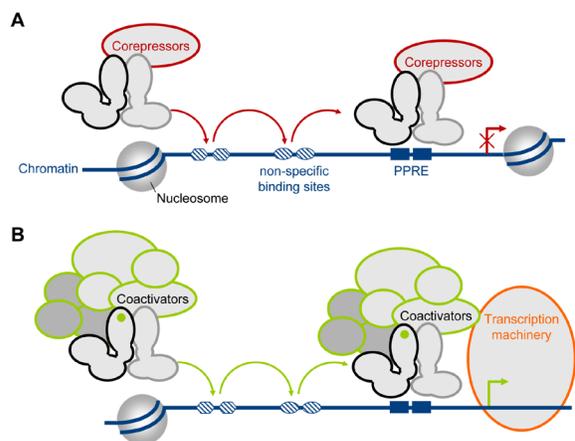
The 3D scanning model actually predicts that the mobility of the transcription factors in the nucleus is correlated with chromatin density. Recently, the interaction of GR with DNA was visualized in situ by FLIM-FRET [Cremazy et al., 2005] and was shown to occur all over the nucleus. However, the authors did not precisely map FRET intensity as a function of DNA concentration. Moreover, a drawback of this approach is that cells need to be fixed, accumulating interaction events over the fixation time and overestimating the proportion of bound receptors. In this regard, FCS is an interesting alternative approach to investigate the relationship between PPAR diffusion and chromatin concentration.

Mutation of the DNA binding domains (DBD) of several transcription factors results in dramatically faster recovery times after photobleaching [Parada and Misteli, 2002]. We have undertaken a systematic analysis of the mobility of different PPAR mutants to understand the contribution of chromatin and cofactor binding to that process. Preliminary experiments indicate that transient DNA

binding in the nucleus impairs receptor mobility, but that the major determinant is the association with other nuclear factors, both in the absence and in the presence of ligand (our unpublished results). However, it is not possible so far with FCS to determine the exact proportion of receptors which are present as DNA-bound heterodimers and those which diffuse in association with huge cofactor complexes. The development of cross-correlation microscopy will allow the monitoring of both receptors and cofactors labeled with distinct fluorophores at the same time, and will be of great help in this regard.

## The "hit and run" and the "cycling on promoter" models

Beside the transient and "non-specific" or "non-productive" interactions of transcription factors with chromatin, another very exciting question is how stable is the association between activated receptors and genuine response elements. At present, NRs are often represented as being stably associated with DNA and interacting with corepressors, until ligand binding induces the release of the corepressors and the successive recruitment of a series of cofactors by the very same receptor. However, this model was challenged by FRAP experiments showing that the glucocorticoid receptor rapidly exchanges with regulatory sites in living cells, the recovery time of fluorescence after photobleaching on an array of response elements being 10 seconds approximately [McNally et al., 2000]. Moreover, the measure of the residence time of the receptor on the promoter in this "hit-and-run" model is probably biased by the high concentration of binding sites in the array of response elements. The residence time of a receptor on a single and isolated response element might thus be even smaller. A rapid exchange of cofactors on nuclear receptors bound to DNA has also been shown by FRAP [Becker et al., 2002; Stenoien et al., 2001a], further supporting the model of a highly dynamic turnover of transcription complexes at promoters. This rapid turnover measured by FRAP is often opposed to the results obtained by ChIP, which revealed a cyclical association to promoters with periods of 20 to 90 minutes [Burakov et al., 2002; Metivier et al., 2003; Reid et al., 2003; Shang et al., 2000]. Actually, the ChIP experiment measures a phenomenon at the scale of the cell population and indicates the probability that a given complex interacts with the promoter at a given time, rather than the time spent by each individual complex on the promoter. The probability of a given factor binding to the promoter may increase simply because of the ATP-dependent remodeling of nucleosomes, their covalent modification, the binding of another transcription factor in the neighborhood or covalent modifications of the receptor itself, all of which potentially translate into a much higher affinity of the NR for its response element. Hence, a 20-minute cycle of ER does not mean that the same ER molecule binds for 20 minutes to the promoter, but that during this period, the configuration of the promoter greatly favors the association of ER. Other factors may also bind during this period in a stochastic manner, but these associations are not detected by ChIP.



**Figure 2. A new hypothetical model for PPAR action in living cells.**

This model is based on the results reported in the present article and in [Feige et al., 2005a; McNally et al., 2000; Phair et al., 2004]. (A) in the absence of ligand, PPAR and RXR are heterodimerized, recruit corepressors, and roam the nucleus where they interact transiently with chromatin, both on genuine PPRES and unspecific binding sites. (B) Upon ligand binding, PPAR mobility is reduced due to its AF-2-dependent binding to cofactors. PPAR/cofactor complexes may transiently bind to "non-specific" sites on chromatin, performing a three dimensional-scanning of the genome, until they encounter a genuine response element in a promoter, at which chromatin remodeling and transcription are initiated.

The exchange rate of receptors at promoters is not solely governed by the equilibrium between the bound and unbound fractions of the receptor and the affinity for its binding site. It also involves numerous other factors and is an energy-driven process. Indeed, the cycling of GR and PR on the MMTV promoter is governed by the action of at least three different complexes requiring ATP for their function: the chromatin remodeling complex SWI/SNF, the hsp90, hsp70 and p23 chaperone complex, and the proteasome [Nagaich et al., 2004; Rayasam et al., 2005; Stavreva et al., 2004]. The binding of the receptor to DNA may also be stabilized by cooperation with another chromatin binding factor, as recently demonstrated by FRAP for GR and HMGB1 [Agresti et al., 2005]. All these factors, and probably others so far unidentified, determine the residence time of the receptor on the promoter, a parameter which seems to be correlated with transcriptional output [Stavreva et al., 2004].

## Conclusion

Altogether, our view of NR action on gene activation has tremendously evolved over the past 5 years, in combination with the development of live cell microscopy techniques. The "hit-an-run", the "three-dimensional scanning of chromatin" and the "cycling on promoter" models for transcription factors represent the most interesting breakthroughs in the field. However, we have only started to describe the behavior of NRs in the nucleus of living cells, and understanding the molecular mechanisms underlying NR mobility remains a great challenge and will necessitate the combination of microscopy techniques such as FRET, FRAP and FCS, with ChIP experiments.

## Acknowledgements

The work performed in the authors' laboratory was supported by grants from the Swiss National Research Project 50, the Swiss National Science Foundation and the Etat de Vaud (L.G., J.F., W.W. and B.D.), and by the Research Council (C.T.) and the grant GOA 2001/2 (Y.E.) of the Katholieke Universiteit Leuven.

## References

- Agresti, A., Scaffidi, P., Riva, A., Caiolfa, V. R. and Bianchi, M. E. (2005) GR and HMGB1 interact only within chromatin and influence each other's residence time *Mol Cell* **18**, 109-21.
- 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**, 1188-94.
- Berney, C. and Danuser, G. (2003) FRET or no FRET: a quantitative comparison *Biophys J* **84**, 3992-4010.
- Brock, R., Hink, M. A. and Jovin, T. M. (1998) Fluorescence correlation microscopy of cells in the presence of autofluorescence *Biophys J* **75**, 2547-57.
- Burakov, D., Crofts, L. A., Chang, C. P. and Freedman, L. P. (2002) Reciprocal recruitment of DRIP/mediator and p160 coactivator complexes in vivo by estrogen receptor *J Biol Chem* **277**, 14359-62.
- Cremazy, F. G., Manders, E. M., Bastiaens, P. I., Kramer, G., Hager, G. L., van Munster, E. B., Verschure, P. J., Gadella, T. J., Jr. and van Driel, R. (2005) Imaging in situ protein-DNA interactions in the cell nucleus using FRET-FLIM *Exp Cell Res* **309**, 390-6.
- Dittrich, P., Malvezzi-Campeggi, F., Jahnz, M. and Schwille, P. (2001) Accessing molecular dynamics in cells by fluorescence correlation spectroscopy *Biol Chem* **382**, 491-4.
- Dowell, P., Ishmael, J. E., Avram, D., Peterson, V. J., Nevriy, D. J. and Leid, M. (1999) Identification of nuclear receptor corepressor as a peroxisome proliferator-activated receptor  $\alpha$  interacting protein *J Biol Chem* **274**, 15901-7.
- Feige, J.N., Gelman, L., Tudor, C., Engelborghs, Y., Wahli, W. and Desvergne, B. (2005a) Fluorescence imaging reveals the nuclear behavior of PPAR/RXR heterodimers in the absence and presence of ligand *J Biol Chem* **280**, 17880-90.
- Feige, J. N., Sage, D., Wahli, W., Desvergne, B. and Gelman, L. (2005b) PixFRET, an ImageJ plug-in for FRET calculation that can accommodate variations in spectral bleed-throughs *Microsc Res Tech* **68**, 51-58.
- Guan, H. P., Ishizuka, T., Chui, P. C., Lehrke, M. and Lazar, M. A. (2005) Corepressors selectively control the transcriptional activity of PPAR $\gamma$  in adipocytes *Genes Dev* **19**, 453-61.
- Martone, R., Euskirchen, G., Bertone, P., Hartman, S., Royce, T. E., Luscombe, N. M., Rinn, J. L., Nelson, F. K., Miller, P., Gerstein, M., Weissman, S. and Snyder, M. (2003) Distribution of NF-kappaB-binding sites across human chromosome 22 *Proc Natl Acad Sci U S A* **100**, 12247-52.
- Maruvada, P., Baumann, C. T., Hager, G. L. and Yen, P. M. (2003) Dynamic shuttling and intranuclear mobility of nuclear hormone receptors *J Biol Chem* **278**, 12425-32.
- McNally, J. G., Muller, W. G., Walker, D., Wolford, R. and Hager, G. L. (2000) The glucocorticoid receptor: rapid exchange with regulatory sites in living cells *Science* **287**, 1262-5.
- , Penot, G., Hubner, M. R., Reid, G., Brand, H., Kos, M. and Gannon, F. (2003) *Cell* **115**, 751-763.
- Nagaich, A. K., Walker, D. A., Wolford, R. and Hager, G. L. (2004) Rapid periodic binding and displacement of the glucocorticoid receptor during chromatin remodeling *Mol Cell* **14**, 163-74.
- Parada, L. and Misteli, T. (2002) Chromosome positioning in the interphase nucleus *Trends Cell Biol* **12**, 425-32.
- Phair, R. D., Scaffidi, P., Elbi, C., Vecerova, J., Dey, A., Ozato, K., Brown, D. T., Hager, G., Bustin, M. and Misteli, T. (2004) Global nature of dynamic protein-chromatin interactions in vivo: three-dimensional genome scanning and dynamic interaction networks of chromatin proteins *Mol Cell Biol* **24**, 6393-402.
- Rayasam, G. V., Elbi, C., Walker, D. A., Wolford, R., Fletcher, T. M., Edwards, D. P. and Hager, G. L. (2005) Ligand-specific dynamics of the progesterone receptor in living cells and during chromatin remodeling in vitro *Mol Cell Biol* **25**, 2406-18.
- Reid, G., Hubner, M. R., Metivier, R., Brand, H., Denger, S., Manu, D., Beaudouin, J., Ellenberg, J. and Gannon, F. (2003) Cyclic, proteasome-mediated turnover of unliganded and liganded ER $\alpha$  on responsive promoters is an integral feature of estrogen signaling *Mol Cell* **11**, 695-707.
- Robyr, D., Wolffe, A. P. and Wahli, W. (2000) Nuclear hormone receptor coregulators in action: diversity for shared tasks *Mol Endocrinol* **14**, 329-47.
- Schaaf, M. J. and Cidlowski, J. A. (2003) Molecular determinants of glucocorticoid receptor mobility in living cells: the importance of ligand affinity *Mol Cell Biol* **23**, 1922-34.
- Shang, Y., Hu, X., DiRenzo, J., Lazar, M. A. and Brown, M. (2000) Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription *Cell* **103**, 843-52.
- Shi, Y., Hon, M. and Evans, R. M. (2002) The peroxisome proliferator-activated receptor  $\delta$ , an integrator of transcriptional repression and nuclear receptor signaling *Proc Natl Acad Sci U S A* **99**, 2613-8.
- Stavreva, D. A., Muller, W. G., Hager, G. L., Smith, C. L. and McNally, J. G. (2004) Rapid Glucocorticoid Receptor Exchange at a Promoter Is Coupled to Transcription and Regulated by Chaperones and Proteasomes *Mol Cell Biol* **24**, 2682-2697.
- Stenoien, D. L., Patel, K., Mancini, M. G., Dutertre, M., Smith, C. L., O'Malley, B. W. and Mancini, M. A. (2001a) FRAP reveals that mobility of oestrogen receptor- $\alpha$  is ligand- and proteasome-dependent *Nat Cell Biol* **3**, 15-23.
- Stenoien, D. L., Nye, A. C., Mancini, M. G., Patel, K., Dutertre, M., O'Malley, B. W., Smith, C. L., Belmont, A. S. and Mancini, M. A. (2001b) Ligand-mediated assembly and real-time cellular dynamics of estrogen receptor  $\alpha$ -coactivator complexes in living cells *Mol Cell Biol* **21**, 4404-12.