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REVIEW

Membrane organization and dynamics of the serotonin$_{1A}$ receptor in live cells

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Abstract

The G-protein coupled receptor (GPCR) superfamily is one of the largest classes of molecules involved in signal transduction across the plasma membrane. The serotonin$_{1A}$ receptor is a representative member of the GPCR superfamily and serves as an important target in the development of therapeutic agents for neuropsychiatric disorders such as anxiety and depression. In the context of the pharmacological relevance of the serotonin$_{1A}$ receptor, the membrane organization and dynamics of this receptor in the cellular environment assume relevance. We have highlighted results, obtained from fluorescence microscopy-based approaches, related to domain organization and dynamics of the serotonin$_{1A}$ receptor. A fraction of serotonin$_{1A}$ receptors displays detergent insolubility, monitored using green fluorescent protein, that increases upon depletion of membrane cholesterol. Fluorescence recovery after photobleaching measurements with varying bleach spot sizes show that lateral diffusion parameters of serotonin$_{1A}$ receptors in normal cells are consistent with models describing diffusion of molecules in a homogenous membrane. Interestingly, these characteristics are altered in cholesterol-depleted cells. Taken together, we conclude that the serotonin$_{1A}$ receptor exhibits dynamic confinement in the cellular plasma membranes. Progress in understanding GPCR organization and dynamics would result in better insight into our overall understanding of GPCR function in health and disease.

Keywords: cholesterol, detergent insolubility, dynamic confinement, FRAP, G-protein coupled receptor; serotonin$_{1A}$ receptors.

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The G-protein coupled receptor (GPCR) superfamily represents the largest class of molecules involved in signal transduction across the plasma membrane (Pierce *et al.* 2002; Rosenbaum *et al.* 2009). GPCRs are prototypical members of the family of seven transmembrane domain proteins and include > 800 members which together constitute ~ 5% of the human genome (Zhang *et al.* 2006). GPCRs dictate physiological responses to a diverse array of stimuli. As a consequence, these receptors mediate multiple physiological processes. GPCRs therefore have emerged as major targets for the development of novel drug candidates in all clinical areas (Heilker *et al.* 2009). It is estimated that up to 50% of clinically prescribed drugs act as either agonists or antagonists of GPCRs with several ligands of GPCRs among the top 100 globally selling drugs, which points out their immense therapeutic potential (Schlyer and Horuk 2006).

The major paradigm in GPCR signaling is that their stimulation leads to the recruitment and activation of heterotrimeric GTP-binding proteins (G-proteins). These initial events, which are fundamental to all types of GPCR signaling, occur at the plasma membrane *via* protein-protein interactions. An important consequence is that the organization of molecules such as receptors and G-proteins in the membrane represents an important determinant in GPCR signaling (Ostrom and Insel 2004; Pucadyil and Chattopadhyay 2006). In this regard, the observation that GPCRs are not uniformly present on the plasma membrane, but are concentrated in specific membrane domains that are enriched

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Abbreviations used: 5-HT$_{1A}$R-EYFP, 5-hydroxytryptamine-1A receptor tagged to EYFP; EYFP, enhanced yellow fluorescent protein; FRAP, fluorescence recovery after photobleaching; GFP, green fluorescent protein; GPCR, G-protein coupled receptor.

in cholesterol assumes significance (Ostrom and Insel 2004). The role of membrane domains in GPCR function therefore represents a challenging aspect of GPCR signaling. Receptor-G-protein interactions may be dependent on their organization in membranes and not solely on the binding sites present on the interacting proteins. The restricted mobility of receptor, G-protein, and effector on the cell surface in addition to the selectivity of receptor-G-protein interaction, is now believed to be an important determinant of spatiotemporal regulation of GPCR signaling (Hur and Kim 2002; Ostrom and Insel 2004; Ganguly *et al.* 2008). In this context, we have previously shown that dynamics of GPCRs could be modulated by G-protein activation (Pucadyil *et al.* 2004). The heterogenous distribution of GPCRs into membrane domains has given rise to new challenges and complexities in receptor signaling. Signaling in such a case has to be understood in the context of three dimensional organization of various signaling components which includes receptors and G-proteins.

The serotonin$_{1A}$ receptor is an important member of the GPCR superfamily and is the most extensively studied among serotonin receptors for a variety of reasons (Pucadyil *et al.* 2005). The serotonin$_{1A}$ receptor agonists and antagonists have been shown to possess potential therapeutic effects in anxiety or stress-related disorders. As a result, the serotonin$_{1A}$ receptor serves as an important target in the development of therapeutic agents for neuropsychiatric disorders such as anxiety and depression. Interestingly, mutant (knockout) mice lacking the serotonin$_{1A}$ receptor exhibit enhanced anxiety-related behavior, and represent an important animal model for genetic vulnerability to complex traits such as anxiety disorders and aggression in higher animals (Gardier 2009). Keeping in mind the pharmacological relevance of the serotonin$_{1A}$ receptor, the localization, organization and dynamics of this receptor in the cellular milieu assume significance. This is particularly true as the influence of membrane order and heterogeneity on cellular signaling represents one of the challenging problems in contemporary cell biology (Groves and Kuriyan 2010). While considerable information is available regarding the pharmacology and neurobiology of the serotonin$_{1A}$ receptor, molecular details regarding its organization and dynamics in the membrane remain largely unexplored. In this review, we have highlighted results obtained from confocal fluorescence microscopy based imaging and dynamic approaches such as fluorescence recovery after photobleaching (FRAP) in order to explore membrane organization and dynamics of the serotonin$_{1A}$ receptor. For this, we visualized serotonin$_{1A}$ receptors stably expressed in mammalian cells by its fusion to enhanced yellow fluorescent protein (EYFP). A schematic diagram indicating the location of the EYFP tag on the serotonin$_{1A}$ receptor (5-HT$_{1A}$R-EYFP) is shown in Fig. 1(e) (inset). This fusion protein was found to be essentially similar to the native receptor in pharmacological assays and therefore can be used to reliably explore aspects of receptor biology such as cellular distribution and dynamics (Pucadyil *et al.* 2004).

Domain localization of the serotonin$_{1A}$ receptor: monitoring detergent insolubility using green fluorescent protein

Contemporary understanding of the organization of biological membranes involves the concept of lateral heterogeneities in the membrane, collectively termed as membrane domains. Many of these domains (sometimes termed as 'lipid rafts') are believed to be important for the maintenance of membrane structure and function, although characterizing the spatiotemporal resolution of these domains has proven to be challenging (Edidin 2001; Munro 2003; Mukherjee and Maxfield 2004; Jacobson *et al.* 2007). These specialized regions are believed to be enriched in specific lipids and proteins, and facilitate processes such as trafficking, sorting, signal transduction and pathogen entry (van der Goot and Harder 2001; Mukherjee and Maxfield 2004; Jacobson *et al.* 2007; Pucadyil and Chattopadhyay 2007a). It is therefore important to understand the dynamic organization of membrane-bound molecules in order to arrive at a comprehensive view of cellular signaling (Simons and Toomre 2000; Jacobson *et al.* 2007).

Insolubility of membrane components in non-ionic detergents such as Triton X-100 at low temperature represents an extensively used biochemical criterion to identify, isolate and characterize certain types of membrane domains (Brown and Rose 1992; Hooper 1999; Chamberlain 2004). Evidence from model membrane studies shows that enrichment with lipids such as sphingolipids (with high melting temperature) and cholesterol serves as an important determinant for the phenomenon of detergent resistance (Schroeder *et al.* 1998). The tight acyl chain packing in cholesterol-sphingolipid rich membrane regions is thought to confer detergent resistance to membrane regions enriched in these lipids and to the proteins which reside in them. Several glycosylphosphatidylinositol-anchored proteins, few transmembrane proteins and certain G-proteins have been found to reside in detergent resistant membrane domains, popularly referred as DRMs (Brown and Rose 1992; Chamberlain 2004). In spite of concerns on the possibility of membrane perturbation because of the use of detergents (Edidin 2001; Heerklotz 2002), resistance to detergent extraction continues to be a principal tool to study membrane domains as the need for relatively simple and straightforward biochemical methods for detecting membrane domains persists. Information obtained from this extensively used biochemical approach can often form the basis for a more detailed analysis of membrane domains utilizing other specialized techniques.

Detection of proteins in detergent resistant membranes is usually performed either by immunoblotting or ligand

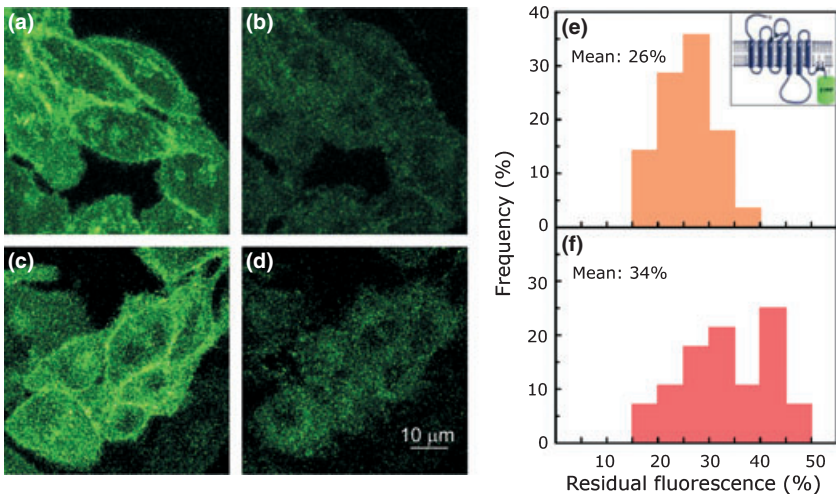

Fig. 1 Effect of cholesterol depletion on detergent insolubility of 5-HT_{1A}R-EYFP. Experiments were carried out on CHO-5-HT_{1A}R-EYFP cells stably expressing the $serotonin_{1A}$ receptor tagged to EYFP at the C-terminal (see inset in panel e). CHO-5-HT_{1A}R-EYFP cells under normal (a and b) and cholesterol-depleted (c and d) conditions are shown before (a and c) and after (b and d) treatment with cold Triton X-100. Cholesterol depletion was achieved by treatment of cells with methyl-β-cyclodextrin. Images represent projected mid-plane confocal sections of the same group of cells before and after detergent extraction. The scale bar represents 10 μm. Frequency distribution profiles of detergent insolubility of 5-HT_{1A}R-EYFP determined under normal (e) and cholesterol-depleted (f) conditions are shown. Fluorescence intensity of the same group of cells, before and after detergent treatment, was quantitated using the Meridian DASY Master program. Detergent insolubility of 5-HT_{1A}R-EYFP was estimated by measuring the residual fluorescence following detergent extraction. The histograms shown represent 28 different data points of residual fluorescence measurements each for the normal and cholesterol-depleted conditions. The frequency of occurrence of various values has been normalized to the total number of measurements. Adapted and modified from Kalipatnapu and Chattopadhyay (2005).

binding. However, these approaches are not suitable in cases where ligand binding of the protein is compromised in presence of the detergent (Banerjee *et al.* 1995) and/or is limited by availability of antibodies with high specificity (Zhou *et al.* 1999). Membrane proteins tagged with green fluorescent protein (GFP) provide an alternative that can overcome these limitations. We have previously designed a GFP-fluorescence based approach to directly determine detergent insolubility of the $serotonin_{1A}$ receptor (Kalipatnapu and Chattopadhyay 2004). This method is based on quantitating fluorescence of the membrane protein in cells before and after detergent treatment (see Fig. 1a and b). Interestingly, this method of analysis of detergent insolubility could be potentially useful in exploring membrane localization of other GPCRs. This approach has the potential to be used in large scale screenings of detergent insolubility of membrane proteins by making fluorescent fusion proteins and testing for insolubility by an automated fluorescence imaging system capable of handling multiple samples.

Utilizing this GFP-based approach, ~ 26% of fluorescence of 5-HT_{1A}R-EYFP was shown to be retained upon extraction with Triton X-100 (Kalipatnapu and Chattopadhyay 2004; Fig. 1e). This represents the fraction of the $serotonin_{1A}$ receptor that is resistant to detergent treatment. These results imply that the domain organization of $serotonin_{1A}$ receptors is heterogenous and only a small (~ 26%) population of receptors resides in detergent resistant domains (Kalipatnapu and Chattopadhyay 2004). These results have been supported by later experiments utilizing western blot and co-patching (Renner *et al.* 2007), and a detergent-free approach (Kalipatnapu and Chattopadhyay 2007).

Cholesterol is often found enriched in detergent-insoluble fractions isolated from many natural sources (Brown and Rose 1992). It is considered an essential constituent of such fractions and studies carried out in model membrane systems have addressed specific molecular requirements for sterols in detergent resistance (Schroeder *et al.* 1998; Xu and London 2000). As mentioned above, the tight packing of membrane regions enriched in lipids such as cholesterol and sphingolipids (with high melting temperature) is thought to confer detergent resistance of these regions and to proteins that reside in them (Schroeder *et al.* 1998; Xu and London 2000; Mukherjee and Maxfield 2004). According to this model, depletion of cholesterol is generally believed to cause disruption of such domains resulting in an increased extraction of proteins residing in the domain (Edidin 2001). Several examples are known in the literature where reduced detergent insolubility of membrane proteins has been observed upon depletion of membrane cholesterol (Harder *et al.* 1998). In contrast, the detergent insolubility of the

serotonin$_{1A}$ receptor exhibits an increase (from ~ 26% to ~ 34%) upon cellular cholesterol depletion (Kalipatnapu and Chattopadhyay 2005; see Fig. 1c, d and f).

It has been previously reported that cholesterol depletion from lipid vesicles, originally present in a uniform liquid phase, leads to separation of phases as monitored by the distribution of fluorescent lipid probes (Veatch and Keller 2003). Similar observations were reported in mammalian cells (Hao *et al.* 2001). These authors showed that while the cell membrane was uniformly labeled under normal conditions by lipid probes with preferential phase partitioning properties, reduction in membrane cholesterol content induced formation of visible micrometer-scale domains on the cell membrane (Hao *et al.* 2001). These results, in combination with evidence from model membrane studies (Veatch and Keller 2003), have given rise to the proposal that cholesterol, while maintaining domain organization in membranes, could also be involved in reducing immiscibility of domains. Reduction in cholesterol levels therefore could induce domain segregation (Mukherjee and Maxfield 2004).

The increase in detergent insolubility of the serotonin$_{1A}$ receptor could be interpreted on the basis of the above model of formation of large sized ordered domains upon cholesterol depletion. In such a scenario, cholesterol depletion could lead to segregation of ordered domains on the cell membrane, into which a slightly greater fraction of the serotonin$_{1A}$ receptor may be included (a proposal supported by independent measurements utilizing bleach area-dependent FRAP, see later), resulting in an increase in the relative detergent insoluble fraction. Interestingly, the requirement of cholesterol for detergent insolubility of membrane components has earlier been critically assessed (Pucadyil and Chattopadhyay 2004). This study suggests that the presence of long chain saturated lipids (lipids that pack into ordered domains), and not necessarily cholesterol, contributes to detergent insolubility. In addition, there have been reports indicating that detergent insolubility is unaltered upon cholesterol depletion (Rivas and Gennaro 2003; Romanenko *et al.* 2004). Thus, it is possible that cholesterol depletion leads to differential effects on detergent insolubility of membrane components (Hao *et al.* 2001).

Membrane dynamics of the serotonin$_{1A}$ receptor utilizing bleach area-dependent FRAP: dynamic confinement of the receptor upon cholesterol depletion

An interesting source of heterogeneity (domain) in cell membranes is the confinement of diffusion of membrane components. Cellular signaling mediated by proteins could be viewed as a consequence of differential mobility of the various interacting partners (Peters 1988). Lateral diffusion of membrane-bound molecules represents a powerful approach to understand their membrane organization. FRAP is a widely used approach to quantitatively estimate diffusion properties of molecules in cells. This approach provides information on the diffusion behavior of an ensemble of molecules, as the area monitored is large (in the order of micrometers) (Lagerholm *et al.* 2005). FRAP involves generating a concentration gradient of fluorescent molecules by irreversibly photobleaching a fraction of fluorophores in the sample region. The dissipation of this gradient with time owing to diffusion of fluorophores into the bleached region from unbleached regions in the membrane is an indicator of mobility of fluorophores in the membrane. As fluorescence recovery kinetics in FRAP measurements contains information on the area being monitored, a comprehensive understanding of the spatial organization of molecules in the plasma membrane can be obtained by systematically varying the area monitored in FRAP experiments (Edidin 1992). Deviations in diffusion characteristics of molecules obtained from FRAP experiments performed with bleach spots of different sizes can be correlated to the presence of domains on the cell membrane with dimensions that fall in the same range as the area monitored in these measurements (Yechiel and Edidin 1987; Edidin and Stroynowski 1991; Salome *et al.* 1998; Cézanne *et al.* 2004; Baker *et al.* 2007; Saulière-Nzeh *et al.* 2010). This interpretation is based on the model described below, and has been previously validated by Monte Carlo simulations and FRAP experiments performed on physically domainized model membrane systems (Salome *et al.* 1998).

The recovery of fluorescence into the bleached spot in FRAP experiments is described by two parameters, an apparent diffusion coefficient and mobile fraction. The rate of fluorescence recovery provides an estimate of the apparent diffusion coefficient of molecules, while the extent to which fluorescence recovers provides an estimate of mobile fraction of molecules. In reality, the mobile fraction is an estimate of the fraction of molecules mobile at the time scale of the FRAP experiment. In general, for molecules diffusing laterally in a homogenous membrane, the diffusion coefficient is independent of the dimensions of the bleach spot in FRAP measurements. A small bleach spot (Fig. 2a) results in faster recovery of fluorescence while a large bleach spot (Fig. 2b) results in slower fluorescence recovery. Nonetheless, the rate of fluorescence recovery is same in both cases, irrespective of bleach spot size. This essentially means that the diffusion coefficient remains same in both cases. Moreover, if the bleached area is significantly smaller than the total area of the membrane (i.e., condition of an infinite reservoir), the extent of fluorescence recovery is the same in both cases resulting in a constant mobile fraction (Fig. 2e).

In contrast, if diffusion was confined to closed domains of dimensions of the same scale as that of the bleach spot, and static in the time scale of FRAP experiments, diffusion coefficient would no longer be a constant. A small bleach spot (Fig. 2c) would tend to monitor diffusion properties of

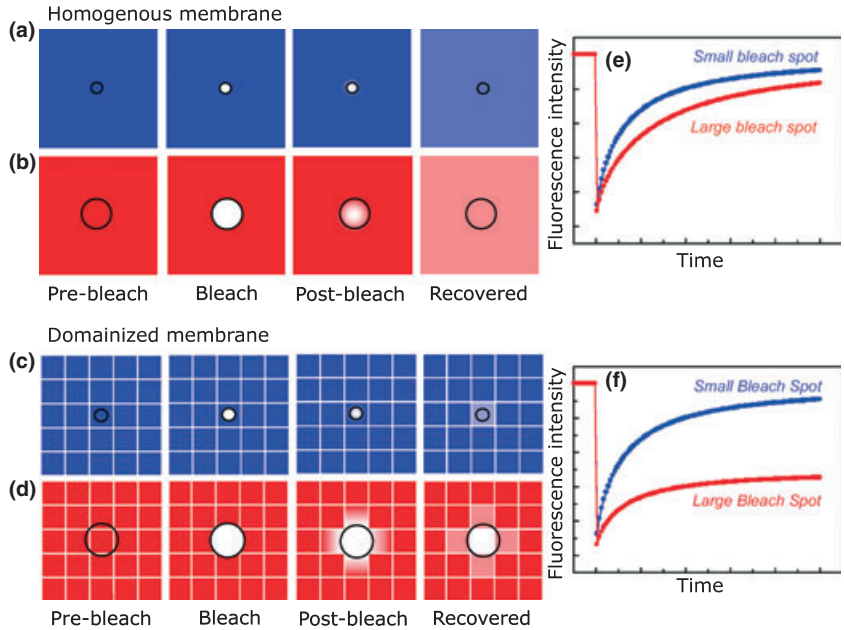

Fig. 2 Possible fluorescence recovery plots of FRAP measurements with a small or large bleach spot performed on homogenous or domainized membranes. The region of interest for FRAP is represented by a circle. The homogenous membrane is characterized by free molecular diffusion throughout the total area of the membrane in the experimental time scale. In contrast, molecular diffusion on the domainized membrane is confined to closed areas (of comparable dimension as that of the bleached area) termed as 'domains' present on the membrane. The diffusion coefficient and mobile fraction in homogeneous membranes (panels a and b) would be independent of the size of the bleach spot (see panel e). On the other hand, these parameters would depend on the bleach spot size in case of a domainized membrane (panel c and d). FRAP measurements on such a domainized membrane therefore show an increase in diffusion coefficient and decrease in mobile fraction with increasing bleach spot size (panel f). It is important to mention here that these domains are assumed to be static in the time scale of FRAP measurements. See text for other details. Adapted and modified from Pucadyil and Chattopadhyay (2007b).

molecules within domains. Kinetics of fluorescence recovery with a small bleach spot on a domainized membrane therefore would be similar to that observed in a homogenous membrane. On the other hand, a large bleach spot (overlapping different domains to varying extents, as shown in Fig. 2d) would result in non-uniform bleaching of domains as the bleached area would be partial for a few and complete for others. As a result of this, fluorescence recovery kinetics in the entire region of observation would not be proportional to the actual size of the bleach spot. While kinetics of fluorescence recovery within domains would be proportional to the area bleached in these domains, the apparent diffusion coefficient would show an increase (as diffusion coefficient is calculated taking into account the actual bleach spot size). Importantly, a large bleach spot would reduce mobile fraction as it could bleach an entire domain resulting in total loss of fluorescence in such a domain (Fig. 2d and f).

Analysis of fluorescence recovery kinetics of 5-HT_{1A}R-EYFP in normal cells with bleach spots of different sizes showed a relatively constant diffusion coefficient and mobile fraction (Pucadyil and Chattopadhyay 2007b; see Fig. 3). The relatively constant values of diffusion coefficient and mobile fraction over a range of bleach spot size in normal cells suggest that serotonin$_{1A}$ receptors experience a membrane environment that appears homogenous, at least in the spatiotemporal resolution of FRAP measurements. Interestingly, FRAP experiments performed on cholesterol-depleted cells with an identical range of bleach spot size showed a marked dependence of diffusion coefficient and mobile fraction of serotonin$_{1A}$ receptors on the size of the bleach spot (see Fig. 3). This type of dependence of diffusion coefficient and mobile fraction in cholesterol-depleted membranes is consistent with a model describing confined diffusion in a domainized membrane (see Fig. 2c and d) (Yechiel and Edidin 1987; Edidin and Stroynowski 1991; Salomé *et al.* 1998; Cézanne *et al.* 2004; Baker *et al.* 2007; Saulière-Nzeh *et al.* 2010). The dependence of the lateral diffusion parameters of the serotonin$_{1A}$ receptor on the bleach spot size in cholesterol-depleted cells indicates that cholesterol depletion induces dynamic confinement of the serotonin$_{1A}$ receptor resulting in confined diffusion of the receptor into domains (see model in Fig. 4). Importantly, this model is further supported by our previous results based on non-ionic detergent insolubility of serotonin$_{1A}$ receptors that suggested that cholesterol depletion led to a possible reorganization of the receptor into domains that could

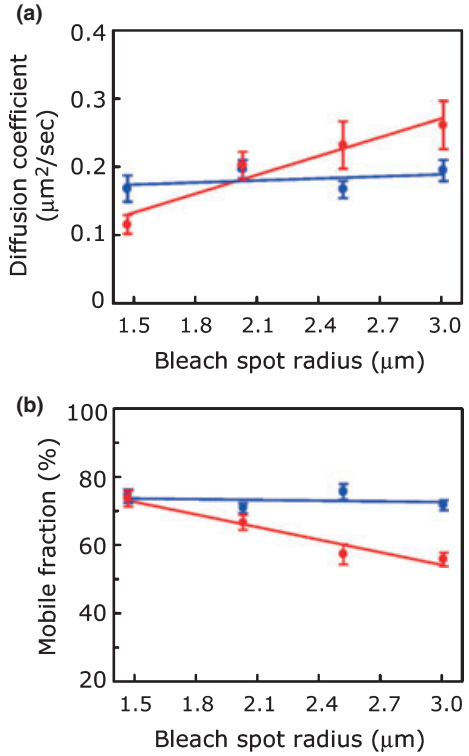

Fig. 3 Diffusion parameters from FRAP measurements with bleach spots of different sizes for 5-HT$_{1A}$R-EYFP. The fluorescence recovery plots were analyzed based on a two-dimension diffusion model. The diffusion coefficient (a) and mobile fraction (b) of 5-HT$_{1A}$R-EYFP obtained are shown for normal (blue line) and cholesterol-depleted (red line) cells. Adapted and modified from Pucadyil and Chattopadhyay (2007b).

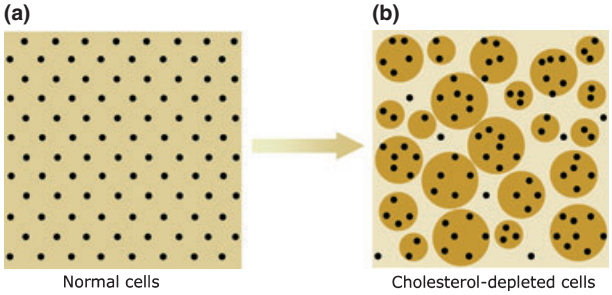

Fig. 4 A model depicting the possible organization of the serotonin$_{1A}$ receptor in normal and cholesterol-depleted plasma membranes. The distribution of serotonin$_{1A}$ receptors (black dots) in the plasma membrane of normal cells (a) was found to be homogenous at the spatiotemporal resolution of FRAP measurements, based on the relatively constant diffusion coefficient and mobile fraction of receptors with increasing bleach spot size (see Fig. 3). Cholesterol depletion of cells (b) results in dynamic confinement of receptors into membrane domains (brown circles) on the plasma membrane. These domains restrict receptors within their boundaries, resulting in dependence of diffusion coefficient and mobile fraction of the receptor on the bleach spot size (Fig. 3). The distribution of receptors has been depicted to be similar in normal and cholesterol-depleted cells because of the absence of any obvious spatial reorganization of the receptor on the cell membrane in response to cholesterol depletion. FRAP experiments performed on a domainized membrane with a small bleach spot provide information on the diffusion properties of molecules on a relatively local scale (within domains). The lower value of diffusion coefficient of serotonin$_{1A}$ receptors in the plasma membrane of cholesterol-depleted cells obtained for a small bleach spot indicates that the receptors experience a relatively ordered environment in domains (brown circles) that confine the receptor. Receptors and domains are not drawn to scale. Adapted and modified from Pucadyil and Chattopadhyay (2007b).

represent an ordered membrane phase (see above; Kalipatnapu and Chattopadhyay 2005). This proposal was based on the observation that serotonin$_{1A}$ receptors exhibit a higher degree of detergent insolubility in cholesterol-depleted cells (Kalipatnapu and Chattopadhyay 2005).

Conclusion and future perspectives

We discuss here the change in the membrane organization of the serotonin$_{1A}$ receptor upon cholesterol depletion. The organization of the serotonin$_{1A}$ receptor under low cholesterol condition is relevant as reduced membrane cholesterol results in manifestation of several physiological effects. For example, it has been previously shown that cholesterol depletion affects sorting (Hansen *et al.* 2000), distribution (Pike and Casey 2002), endocytosis (Subtil *et al.* 1999) and trafficking (Pediconi *et al.* 2004) of membrane proteins. Importantly, we recently reported that chronic cholesterol depletion impairs the function of the serotonin$_{1A}$ receptor, which could have important implications in mood disorders (Shrivastava *et al.* 2010).

As mentioned above, the GPCR superfamily is the largest and most diverse protein family in mammals, involved in signal transduction across membranes. Because of their role in mediating multiple physiological processes, GPCRs have emerged as major targets for the development of novel drug candidates (Heilker *et al.* 2009). Interestingly, although GPCRs represent ~ 50% of current drug targets, only a small fraction of all GPCRs are presently targeted by drugs (Lin and Civelli 2004). This points out the exciting possibility that the receptors which are not recognized yet could be potential drug targets for diseases that are difficult to treat by currently available drugs. A major challenge in designing future drugs that would act on GPCR targets is the lack of understanding of structure, lipid specificity and membrane organization of GPCRs.

Interestingly, membrane cholesterol has been reported to have a modulatory role in the function of a number of GPCRs (reviewed in Paila *et al.* 2009; Paila and Chattopadhyay 2010). Recently reported crystal structures of GPCRs have shown structural evidence of cholesterol binding sites

(Cherezov *et al.* 2007). Against this backdrop, we proposed a novel mechanism by which membrane cholesterol could affect structure and function of GPCRs. According to our hypothesis, cholesterol binding sites in GPCRs could represent 'non-annular' binding sites. Previous work from our laboratory has demonstrated that membrane cholesterol is required for the function of the serotonin$_{1A}$ receptor, which could be because of specific interaction of the receptor with cholesterol. Based on these results, we envisage that there could be specific/non-annular cholesterol binding site(s) in the serotonin$_{1A}$ receptor (Paila *et al.* 2009). We have further analyzed putative cholesterol binding sites from protein databases in the serotonin$_{1A}$ receptor. Our analysis shows that cholesterol binding sites are inherent characteristic features of serotonin$_{1A}$ receptors and are conserved over natural evolution (Paila and Chattopadhyay 2010). With progress in deciphering details of the membrane localization and organization of GPCRs, our overall understanding of GPCR function in health and disease would improve significantly, thereby enhancing our ability to design better therapeutic strategies to combat diseases related to malfunctioning of these receptors.

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