Plasticity of the asialoglycoprotein receptor deciphered by ensemble FRET imaging and single-molecule counting PALM imaging

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AUTHOR SUMMARY

The plasma membrane contains numerous membrane receptors that assemble into complexes of differing complexity that influence their activity and ligand binding. Here, to better understand the relationships between receptor structure and function, we performed visualization studies on a prototypic two-subunit plasma membrane receptor, the asialoglycoprotein receptor, which binds and internalizes diverse classes of desialylated glycoproteins. The asialoglycoprotein receptor is crucial for removing thrombogenic material (i.e., blood clotting factors and platelets) in sepsis or after transfusion of long-term stored platelets. Using ensemble FRET imaging and single-molecule counting photoactivated localization microscopy (PALM), we tested whether cells display different forms of the asialoglycoprotein receptor, what the subunit stoichiometries of these forms are, and whether they have distinct ligand specificities. Our results reveal that plasma membrane receptors, such as the asialoglycoprotein receptor, may be far more plastic and modular systems than previously thought. This finding may help in better understanding other receptor systems and in the case of the asialoglycoprotein receptor, better clarify its role in the rapid uptake of thrombogenic material in disease.

Using fluorescence microscopy, we first showed that expression levels of the two subunits of the asialoglycoprotein receptor [rat hepatic lectin 1 (RHL1) and RHL2] modulate a cell’s ability to bind different desialylated ligands, including asialofetuin and lactoferrin. Although asialofetuin binding required both receptor subunits on the plasma membrane, lactoferrin binding occurred either in the presence of only RHL1 or when the levels of RHL1 were higher than levels of RHL2. To test whether this differential ligand binding was caused by receptor subunit assembly into different complexes consisting of, for example, RHL1→RHL1, RHL1→RHL2, or RHL2→RHL2, we used FRET, a radiationless transfer of energy from an excited dye, the donor, to another nearby chromophore, the acceptor, typically used to determine inter- or intramolecular distances from 1 to 10 nm (1). Our FRET approach permitted the quantitative analysis of receptor subunit assembly (Fig. P1A). We tested for the presence of homo-oligomerization and found that RHL1 self-associates extensively, whereas RHL2 does not and instead, distributes mainly as homo-dimers in the plasma membrane. This differential tendency to homo-associate was mediated by specific protein domains. Furthermore, the use of a photoactivatable fluorescent protein for FRET experiments suggested that the presence of RHL2 limits the extent of RHL1 homo-oligomerization, although the reverse is not true and RHL2 remained homo-dimeric. We then used FRET to examine RHL1 and RHL2 hetero-oligomerization. The FRET data fit an analytical model that suggests a receptor subunit composition of 2:1 (RHL1: RHL2), with a basic building block of 2× RHL1 dimers and 1× RHL2 dimer.

To directly visualize receptor composition and stoichiometry in situ, we used PALM for single-molecule counting. In this single molecule-based imaging technique, repeated activation and sampling of individual molecules permits densely expressed fluorescent proteins to be resolved in time (2). PALM is typically used to create superresolution images of cellular structures. Using a rigorous new protocol for PALM that included spectrally distinct fluorophore calibrators, we showed that this technique could also be used to reproducibly count single events corresponding to photoactivation of single fluorescent proteins (Fig. P1B). The counting calibrators, consisting of distinct ratios of green and red photoactivatable proteins, ensured that activation and detection efficiency were constant during single-molecule measurements. This con-

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sistency made our counting of molecules reproducible, which is essential for obtaining quantitative information about the assembly and stoichiometry of membrane proteins in homo- and hetero-oligomeric states in situ, even in areas of the membrane with a high density of receptors. Using this approach, we visualized different coexisting receptor subunit assemblies, higher-order RHL1 homo-association, and RHL1/RHL2 hetero-association into distinct clusters that preserved a 2:1 stoichiometry in the presence of free RHL2 subunits. Moreover, we showed that addition of exogenous ligands shifts coexisting receptor states at the plasma membrane of a cell, leading to the preferential internalization of receptor complexes.

In conclusion, we showed that the two subunits of the asialoglycoprotein receptor can assemble into homo- and hetero-oligomeric complexes with distinct ligand specificities that coexist on the plasma membrane and can be modulated by exogenous ligands (Fig. P1C). The coexistence of distinct oligomerization states of the asialoglycoprotein receptor may provide a versatile means to efficiently bind different glycoproteins as they suddenly arise, for instance, in septic conditions. The plastic modularity of the receptor system, in contrast, may allow rapid shifts in specificity by changing the relative abundance of its different oligomeric states and thereby, account for the rapid depletion of blood clotting factors or platelets in disease. In turn, the variety of potential ligands for asialoglycoprotein receptor and the propensity of its subunits to form distinct oligomers may explain previous inconsistent biochemical results regarding receptor stoichiometry, underscoring the importance of deciphering oligomerization in a single cell and at the single-molecule level.