

Organizational properties of T cell surface molecules studied using BRET

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An important step in determining how lymphocytes respond to environmental cues is to understand how their cell surfaces are organized. The stoichiometric arrangements of T cell surface molecules were analysed by Bioluminescence Resonance Energy Transfer (BRET), a powerful new technology that allows lateral protein-protein interactions to be monitored at the living cell surface. The transfer of energy between a luminescent donor (*Renilla* luciferase - Rluc) and a fluorescent acceptor (GFP) protein is assessed, and if GFP is in close proximity (i.e. 10 to 100 Å) allowing energy transfer to occur between Rluc and GFP, a signal is emitted by the latter that can be detected. The BRET ratio is a measure of the efficiency of energy transfer and reflects the proximity of Rluc to GFP. Some of the most important T-cell surface receptors, all of which contribute to signal transduction or are involved in T cell recognition, were selected for analysis and genetically fused to either Rluc or GFP and co-expressed in a suitable cell line. This allowed us to determine whether dimerization is a relatively rare property that confers a specific functional role to a protein, or rather that many proteins can participate in homotypic interactions that have yet to be linked to their functions. The study included the adhesion molecule CD2, the co-receptor CD4, the accessory molecule CD5, the highly O-glycosylated surface protein CD43, the ligand of CD6, CD166 and two ligands of the B7 family, PDL-1 and PDI-2. The co-receptor CD4 and the accessory molecule CD5, somewhat unexpectedly, exhibit a tendency to dimerize, with the former being the stronger interaction. CD4 dimerization in 293T cells appears to be dependent on the presence of its cytoplasmic domain and the protein assumes a monomeric configuration when it is complexed with Lck. The remaining molecules tend to be monomers but nevertheless give a significant BRET signal, presumably due to diffusion-based non-specific interactions within the membrane. This substantial baseline signal suggests that BRET could be useful for studying the segregation of molecules during leukocyte activation.

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