

Selected Reading

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Electrostatic Fasteners Hold the T Cell Receptor-CD3 Complex Together

Assembly of the T cell receptor includes the formation of trimers stabilized by electrostatic interactions inside the membrane (Call et al., 2003). Such interactions can strongly stabilize subunit associations while permitting conformation changes during signaling.

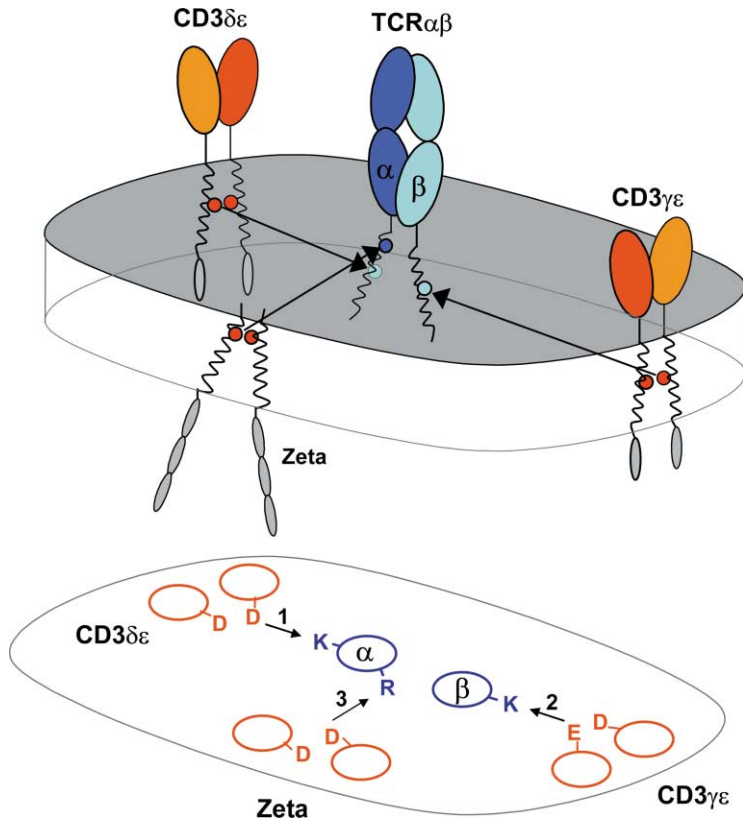
The simplest view of electrostatic interactions inside membrane lipid bilayers is that they have two important properties: strength and range. Compared with electrostatics in an aqueous environment, interactions are much stronger because of the lower dielectric environment, and, because the coulombic interaction potential falls off relatively slowly, the high-energy interactions in the low dielectric extend for considerable distances. Thus, the use of electrostatics in this environment gives a great opportunity for organizing strongly held structures but also poses a danger from nonspecific interactions that might occur. In their closely reasoned study of the T cell receptor complex, Call et al. (2003) have studied a case that is both exciting and provocative, creating new perspectives on the stabilization of membrane proteins and the biosynthetic pathways by which they must originate. Their basic finding is that the assembly of the receptor complex includes the formation of two trimeric complexes, each of which is stabilized through electrostatic interactions in their transmembrane regions. These interactions are asymmetric—two carboxyl groups on each of two subunits interact with the epsilon amino group of a lysine in the third, each of the amino acids being positioned deep in the membrane on separate transmembrane helices. The interactions are highly specific *in vivo* but much less specific *in vitro*, suggesting that the assembly process traps correct complexes in a process that does not result in thermodynamic equilibrium.

How strong can electrostatic interactions be? From a simple Coulomb's Law standpoint, opposite charges separated by 4 Å would have an interaction energy of about 1 kcal per mole in water and about 40 kcal per mole in a lipid bilayer interior. For perspective, the latter energy is comparable to the binding of biotin by streptavidin. If the charges were separated by 16 Å, they would still have an interaction of about 10 kcal per mole.

In a protein environment, other groups, including other side chains, water and the backbone, would modulate the interactions, and the energies could be smaller. But, these simplified views emphasize the potential strength and range of such interactions in a low dielectric environment. The finding that strongly polar groups, namely two carboxyl groups and one amino group on the side-chains of three helices, cause trimers to form during the assembly of the T cell receptor complex therefore exploits a strong interaction energy. Since the interactions are so strong, the idea that conformational changes consisting of altered tilts and relationships among the helices could be facilitated by such interactions is attractive. The regions where the strongly polar groups interact would form pivot points around which the helices could move without risking a disruption of their mutual interactions.

Because the interactions are so strong, there is an apparent risk that nonspecific interactions might occur during assembly, scrambling the subunits. Indeed, Call et al. (2003) observe that nonspecific complexes can form during assembly *in vitro*, whereas they are not observed *in vivo*. Thus, the assembly pathways that form trimers of helices based on two acidic and one basic amino acid engaging in electrostatic interactions requires an assembly step before the trimer reaches full immersion in a lipid bilayer. This is a fascinating observation and challenges the simple view that all subunits would simply emerge from the Sec61 complex directly into the bilayer where they would then assemble. Either there must be some kind of membrane chaperone that guides assembly, or assembly must take place, as the authors suggest, before the helices fully enter the bilayer environment. The conclusion from preassembly is that the three-helix complexes are not at thermodynamic equilibrium in the lipid bilayer; rather, they are kinetically trapped.

That there are two acidic and one basic amino acids interacting poses an interesting question concerning the nature of their interactions and which of the sidechains might or might not be charged. As Honig and Hubbell (1984) observed some time ago, the interactions can be strong whether charges are removed by protonation/deprotonation of the potentially charged groups or whether the charges are preserved and ion-pairs are present. That there would be a charge imbalance (from the two negatively charged carboxyl groups and one positively charged amino group) suggests that at least some reduction of formal charge may be present, although formal net charge can be tolerated if it is buried inside



Electrostatic interactions between side-chains of the transmembrane helices of the T cell receptor. The indicated sidechains have been shown to stabilize the associations of receptor subunits during the assembly of the receptor and are thought to be involved in electrostatic interactions. The figure is reproduced from the Call et al. (2003) article.

a helical complex, effectively creating a hydrophobic ion (essentially by giving the ion a large Born radius).

The Call et al. study is based on the idea that coimmunoprecipitation using digitonin can preserve native interactions present during assembly of the receptor complex in the endoplasmic reticulum. While the controls are impressive, and the reasoning elegant, a caveat is that other components, particularly the lipids, are likely to be part of the precipitated complexes. As lipids are often found to be structural elements of membrane protein complexes, and as they are often charged, the observed interactions could involve their participation (see Popot and Engelman, 2000).

The findings of Call et al. are important both from the perspective of elucidating the organization of the T cell receptor/CD3 complex and from the fascinating insight they give concerning organization using electrostatic interactions. The strength of the interactions would cer-

tainly be useful in maintaining the integrity of the receptor complex while allowing significant conformational changes during the transmembrane signaling process.

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The Forkhead Transcription Factor Foxo1: A Possible Link between Obesity and Insulin Resistance

The forkhead transcription factor Foxo1 has previously been shown to be a mediator of insulin action

in liver and pancreas. New data from Nakae et al. (2003) demonstrate that it also functions in adipose cells to couple insulin signaling to adipogenesis, which involves switching preadipocytes from proliferation to terminal differentiation.

The physiological role of insulin is to postprandially clear circulating glucose by suppressing hepatic glucose output and enhancing glucose uptake in skeletal muscle