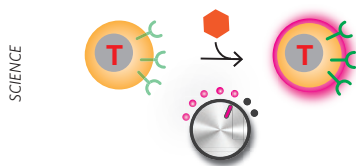


TUMOR IMMUNOLOGY

Assembling the T cell

Science 350, aab4077 (2015)



A patient's T cells can be modified to produce chimeric antigen receptors (CARs) that specifically recognize and target tumors that express a particular antigen. However, this approach is less successful in modulating immune activity in the temporal and spatial manner needed to prevent targeting of non-tumor cells and minimize the release of excess cytokines. To address these limitations, Wu *et al.* have developed a new system called "ON-switch" CARs that activates T cells only in the presence of both the antigen and a small-molecule dimerizer. They attached the FK506 binding protein (FKBP) domain to the extracellular antigen-recognition domain and fused a mutant version of the FKBP-rapamycin binding domain (FRB)

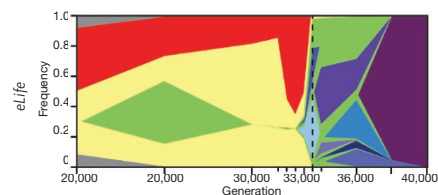
to the intracellular immunoreceptor tyrosine-based activation motifs (ITAMs). The introduction of the rapamycin analog AP21967 promoted the heterodimerization of the antigen-recognition and ITAM domains, which triggered antigen-mediated T cell activation. Although the authors detected colocalization of both components in T cells, high-resolution single-molecule imaging revealed that a physical interaction between components was only observable with AP21967 treatment. When these components were expressed in CD4⁺ helper T cells, the authors detected increased cytokine secretion and T cell proliferation only in the presence of both antigen-expressing target cells and AP21967. These cellular effects could be modulated by adjusting the concentration of the dimerizer. The use of the ON-switch CAR to modify CD8⁺ cells in culture and in a mouse xenograft model to target CD19⁺ cells resulted in decreased numbers of CD19⁺ cells in the presence of AP21967, while non-CD19⁺ cells were spared. Finally, the ability of the ON-switch CAR to utilize alternative modules, such as the gibberellin-induced dimerization system, provides increased flexibility for modulating T cell activity in a refined manner.

GM

METABOLISM

Some assembly required

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In 1988, Lenski and colleagues began a long-term experimental evolution project in which *E. coli* were continuously grown and samples collected every ~500 generations as a platform to explore evolutionary principles. After 31,500 generations of growth on glucose in the presence of citrate, which *E. coli* cannot typically metabolize under aerobic conditions, a citrate-utilizing population emerged; this population, called Cit⁺, was defined by duplication of the *citT* citrate:succinate antiporter gene and was followed by a stronger phenotype, Cit⁺⁺, based in part on activated expression of the *dctA* symporter gene. However, it was unclear what background changes were needed to facilitate the emergence of the Cit⁺ and Cit⁺⁺ populations. Quandt *et al.* now explore the role of the citrate synthase gene, *gltA*, in this process. In particular, the authors identified a *gltA1* mutation affecting citrate synthase prior to the evolution of Cit⁺; in Cit⁺⁺ clones, additional *gltA2* mutations were observed. Genetic reconstruction confirmed that *gltA2* mutations were needed to support the Cit⁺⁺ phenotype. In contrast, other strain combinations showed that *gltA2* mutations when citrate cannot be used as a carbon source, the *gltA1* mutation in the absence of a compensating *gltA2* mutation in a Cit⁺⁺ background, and the duplication of *citT* in the absence of the *gltA1* mutation were each deleterious for growth. Metabolic modeling suggested that growth on glucose would be constrained by low citrate synthase flux and that high levels of citrate synthase would improve growth on acetate (a byproduct of glucose metabolism), whereas growth on citrate would be optimal with no or low flux. Accordingly, the *gltA1* mutation likely provided a competitive advantage to the pre-Cit⁺ cells, while the *gltA2* mutations reversed the effect of the *gltA1* mutations by decreasing the amount of mRNA, reintroducing lost inhibitory mechanisms or directly reducing enzyme function. These 'metabolic epochs' provide a unique glimpse into the development of new functions.

CG

INTRINSICALLY DISORDERED PROTEINS

I like to move it, move it

Cell 163, 734–745 (2015)

The nuclear pore complex (NPC) is an enormous macromolecular structure that mediates the passage of proteins from the nucleus to the cytoplasm and vice versa. Phenylalanine-glycine-rich nucleoporins (FG-Nups) are intrinsically disordered proteins that line the pore of the NPC, preventing larger proteins (>40 kDa) that are not bound to nuclear transport receptors (NTRs) from moving through the nuclear pore. Milles *et al.* used NMR spectroscopy to show that the ProXxxPheGly-rich domain of Nup153 (Nup153FG^{PxFG}) is highly disordered in solution, with very little secondary structure. Only minor changes in the NMR spectrum were observed when importin β was present, indicating that the binding of this NTR to Nup153FG^{PxFG} does not markedly alter the disordered structure of the nucleoporin. Single-molecule Förster resonance energy transfer experiments on Nup153FG^{PxFG} and full-length Nup153FG confirmed that the overall structure of this nucleoporin does not change substantially when it is bound to importin β . Unbiased molecular dynamics simulations of different conformations of Nup153FG^{PxFG} and the N-terminal portion of importin β suggested that the presence of many solvent-exposed phenylalanines in the FG repeats means that importin β can easily bind to them without either protein undergoing substantial conformational changes. The authors then used fluorescence stopped-flow experiments and Brownian dynamics simulations to show that the binding of full-length Nup153FG to importin β is extremely rapid, even when high-ionic-strength buffers are used to diminish the effects of any potential long-range electrostatic interactions between the two proteins. These experiments indicate a transient and highly dynamic binding mode that allows importin β to quickly 'creep' through the FG motifs that line the nuclear pore until it reaches the nucleus. The authors found similar results for several other Nups and NTRs, suggesting that this may be a general mechanism by which this family of intrinsically disordered proteins is able to be selective for so many structurally diverse nuclear transport receptors on such a fast timescale.

JMF