

CTLA-4 may be expressed at functionally significant levels even by unactivated T cells or be very quickly induced by antigen receptor engagement. Even at low levels of expression, CTLA-4, by virtue of its high affinity for B7, might be dominant over CD28 when limited amounts of B7 are available. This would raise the threshold for activation by self antigens. The absence of the damping effects of CTLA-4 in the knockout mice could account for the observed generalized proliferation. It seems likely that there is a dynamic interplay between antigen receptor signals and the modulating effects of CD28 and CTLA-4 throughout the early and late stages of T cell activation.

The existence of alternative pathways that regulate T cell activation offers exciting clinical prospects. Inhibition of CD28 co-

stimulation with a soluble form of CTLA-4 can suppress immune responses, prolong organ graft survival, and ameliorate experimental autoimmune disease (16). Tumor cells transfected with B7 are often rejected and can induce immunity to the parental tumor cells, demonstrating that provision of costimulation can enhance host immunity to tumors (17). Antibody to CTLA-4 greatly enhances the clonal expansion of antigen-specific T cells in vivo (13). Thus, blockade of the inhibitory effects of CTLA-4 might also prove useful in augmenting T cell responses in clinical situations.

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Trimeric G Proteins: Surprise Witness Tells a Tale

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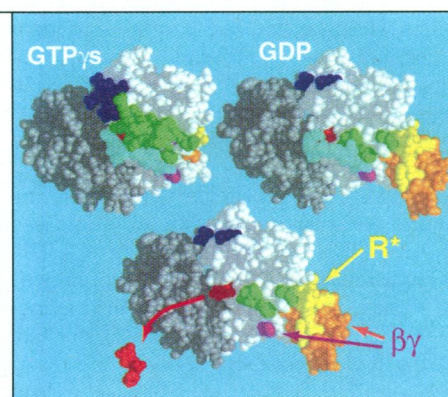
Like key witnesses in a detective story, protein crystal structures can surprise, intrigue, mislead, or instruct us—and often do all four at once. On page 954 of this issue, Mixon *et al.* (1) serve up an intriguing surprise: Crystals of the guanosine diphosphate (GDP) form of α_{i1} , a G protein α subunit that mediates hormonal inhibition of adenylyl cyclase, reveal G α monomers linked, head-to-tail, in endless polymers. I suspect that the inference that similar G α polymers exist and function in vivo is a red herring. Instead, at least to this armchair sleuth, this polymer and other clues hint at a solution to a fascinating molecular puzzle at the heart of G protein signaling: How do receptors, in combination with G $\beta\gamma$, trigger GDP release and G protein activation?

The 16 trimeric ($\alpha\beta\gamma$) G proteins of vertebrates relay messages from hormone and sensory receptors to effector targets that mediate or regulate heart rate, contraction of smooth muscle, synaptic transmission, endocrine secretion, olfaction, vision, and many other functions. In each case the receptor turns on the G protein by promoting exchange of guanosine 5'-triphosphate (GTP) for GDP bound to the G α subunit, followed by dissociation of α GTP and the

G $\beta\gamma$ dimer from the receptor and from each other. The appropriate effector protein is then regulated by α GTP or G $\beta\gamma$ (or both) until bound GTP is converted to GDP, allowing α GDP to reassociate with G $\beta\gamma$ and turn off the signal (2, 3).

Previous G α crystal structures provided three-dimensional (3D) views of GTP-induced conformational change (4, 5) and the guanosine triphosphatase (GTPase) catalytic mechanism (6, 7) but no evidence for any kind of polymer. The α_{i1} GDP polymer is made possible by a small folded domain, composed of sequence from both the amino (orange) and carboxyl (yellow) termini of each monomer (the N-C domain) (see figure). Previous G α structures showed only the Ras-like (white) and α -helical (gray) domains. In the α_{i1} GDP polymer, the new domain tucks neatly into a crevice in the α -helical domain of the next monomer. Neither the polymer nor the N-C domain was seen in α_{i1} bound to GTP γ S (see figure, upper left) (6), suggesting that occupation of the nucleotide binding site by the GTP analog somehow promotes disorder of the amino and carboxyl termini, whereas the polymer promotes stable folding of the same sequences in the GDP-bound form. No N-C domain was seen in the GDP- or GTP γ S-bound structures of α_t (4, 5), a retinal G α , presumably because the α_t amino termini were proteolytically removed before crystallization.

Is the head-to-tail polymer biologically relevant or an artefact of crystallization? I



Three faces of G α_{i1} . G α_{i1} bound to GTP γ S (upper left); G α_{i1} bound to GDP (as in the polymer); imaginary G α_{i1} structure from which GDP can exit quickly.

suspect the latter, although Mixon *et al.* (1) cite reports suggesting that G protein polymers can be detected biochemically under certain conditions in vitro. Indeed, polymeric G α might serve as a storage form for the protein or somehow facilitate activation of many G protein molecules by a single receptor. A reason for doubt, however, is that each monomer in the polymer is rotated 180° with respect to the one preceding (1), allowing only alternate monomers to attach to the plasma membrane.

More disturbing is the fact that the N-C domain seen in the α_{i1} GDP polymer, if it existed in vivo, would probably prevent crucial interactions of G α with pertussis toxin, which catalyzes adenosine 5'-diphosphate ribosylation at Cys³⁵¹ (2), receptors (8, 9), and G $\beta\gamma$, which binds to the G α amino terminus. Each of these would encounter steric obstacles in the polymer.

Even misleading testimony can furnish valuable clues. This is perhaps the case with the α_{i1} GDP polymer, which may not exist in cells but nonetheless reveals structural changes in the monomer (see figure, upper right) that suggest a way to resolve a para-

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dox in our understanding of how receptors activate G proteins: Experimentally identified contact points (2) of $G\alpha$ with receptor (yellow) and $G\beta\gamma$ (orange and magenta) are more than 30 Å from the interdomain pocket that cradles GDP (red), suggesting that the receptor and $\beta\gamma$ must "act at a distance" to catalyze GDP-GTP exchange. Definitive resolution of this paradox—a 3D structure of the receptor- $G\alpha\beta\gamma$ complex from which GDP is released—will take time. In the meantime, we need to identify a mechanism that can transmit conformational change from one end of the protein to the interdomain cleft in the middle.

Differences between the GTP γ S- and GDP-bound structures of α_{i1} (figure, compare upper left and upper right) indicate a route for transmitting just such a conformational change. The three $G\alpha$ "switch regions" (cyan, green, and purple), first identified as sites of GTP-induced conformational change in α_t (4), are well defined in α_{i1} GTP γ S (6). In contrast, two of these, switch 2 (green) and switch 3 (purple), are almost completely disordered in the GDP form of α_{i1} (1). In precise reciprocity, the amino and carboxyl termini are disordered in α_{i1} GTP γ S but well ordered in α_{i1} GDP, presumably by their interaction (not shown) with the α -helical domain of the next monomer. The interdomain cleft is also affected, owing to lost contacts between the disordered switch 3 and the α -helical domain (1). Mixon *et al.* infer that the widening in α_{i1} GDP results from weak or broken contacts between the disordered switch 3 and the α -helical domain (1). Thus, structural stability and disorder of different parts of the protein appear to be concerted, with stability of the amino and carboxyl termini and disorder of two switch regions in α_{i1} GDP and exactly the opposite in α_{i1} GTP γ S.

These apparently concerted, reciprocal transitions between order and disorder suggest a parallel scenario for action at a distance in G protein activation (see figure, lower panel): Like the next α_{i1} GDP monomer in the polymer, activated receptor and $G\beta\gamma$ bind to and stabilize the $G\alpha$ amino and carboxyl termini, albeit in conformations that must differ from those depicted in the figure, for reasons outlined above. Stability of the termini in turn destabilizes switch regions 2 and 3 and alters the GDP binding pocket in the cleft. Rapid release of GDP probably requires a wider exit route (red arrow), which can be opened by displacing or creating disorder in switch 1 (cyan; removed in the lower panel). Such a change can plausibly be effected by $G\beta\gamma$ because allele-specific complementation in *Saccharomyces cerevisiae* (10) indicates a direct interaction between $G\beta$ and a $G\alpha$ side chain corresponding to that of His¹⁸⁸ in α_{i1} (magenta), located just at the end of switch

1. After GDP escapes through the wider opening, GTP enters the empty site to complete the exchange reaction; its γ -phosphate reverses the conformational changes by reorganizing all three switch regions (4, 5), reciprocally destabilizing the amino and carboxyl termini to promote departure of α GTP from $G\beta\gamma$ and the receptor, and in addition converting $G\alpha$ into the right shape for stimulation of an effector.

This speculative scenario ties together several otherwise puzzling clues, although more detective work is clearly in order. The stakes are high because the molecular

mechanism in question accounts for much of the regulatory and sensory information received by every cell.

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Remembering X-rays

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On the evening of 8 November 1895, Wilhelm Conrad Röntgen immediately recognized a remarkable new phenomenon. Fluorescent material lying on a bench some distance from the cathode ray tube with which he was experimenting lit up in his darkened laboratory. Like many others around the world, he was studying the beam of electrons emanating from the cathode in a low-pressure gas discharge (1). The electron beam could emerge from the thin window of the tube, where its range was a few millimeters, but fluorescence at a substantial distance was most surprising.

In fact, the fluorescence was not caused by electrons but by an entirely new form of radiation. Röntgen, 50 years old, and professor of physics at the University of Würzburg, went to work on this phenomenon with great intensity. He told no one about it until 22 December, when he told his wife and made the famous x-ray photograph of her hand. During the 7 weeks that followed his initial discovery, he did many careful experiments and wrote the results in the paper "Eine Neue Art von Strahlen" (1), calling the new radiation X-rays. He submitted it to the *Sitzungsberichte der Physikalischen-medizinischen Gesellschaft zu Würzburg* on 28 December, and within 4 days received printed copies of the publication. Röntgen refused to patent x-rays, preferring to put his discovery into the public domain for all to benefit. And indeed, the imagination of the public was captured by the ability to see bones in a living person and its obvi-

ous potential applicability to medical diagnosis (2).

On 23 January, at his first and only public lecture on the discovery, Röntgen made an x-ray picture of the hand of Dr. Albert von Kölliker, who in turn suggested that the new phenomenon be called Röntgen rays, the name used to this day in much of the world. The first attempt to treat cancer with x-rays was reported to have been carried out by E. H. Grubbe in Chicago on 29 January. The idea of using the technique to search baggage was put forward in a cartoon by the French *Journal La Nature* in May. At the same time, the harmful effects of the radiation became manifest very rapidly, with numerous injuries resulting from an almost complete lack of concern.

In 1896, Nobel prizes did not yet exist. However, when the prizes were first awarded 5 years later, Röntgen was the recipient in physics. Even today, 100 years after the discovery, we see continuing developments in x-ray sources (third-generation synchrotrons, x-ray lasers, and so on), as well as optical elements and detectors. These devices are opening up new fields in areas from microscopy to astronomy, from micromachining to the study of the dynamics of biological macromolecules. And so, this week we celebrate the centenary of a truly singular event in the history of science.

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