understand the details of the complex process of star formation[8]. Current numerical and theoretical models of star formation can reproduce the form of the IMF observed locally. One way to distinguish between this myriad of models is to see whether any can reproduce the types of variation reported by Cappellari and colleagues.

There is a long tradition of studies claiming that the IMF varies as a function of environment, especially in distant galaxies where individual stars cannot be identified directly and secondary tracers must be used. Most of these claims have fallen by the wayside when newer and better data became available, or as our understanding of the intricacies of the tracers has improved. However, the observations and analysis of Cappellari and colleagues[2] present a promising avenue that may lead to fundamental changes in the way that we understand how stars form and galaxies evolve. ■

Nate Bastian *is at the Excellence Cluster 'Universe', Technische Universität München, Garching 85748, Germany.*
e-mail: bastian@usm.lmu.de

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PROTEIN ENGINEERING

Tighter ties that bind

A stepwise process of mutation and structural analysis has modulated a flexible binding interface of an immune-cell signalling protein, interleukin-2, and generated mutant proteins with enhanced anticancer activity. **SEE ARTICLE P.529**

ERIC T. BODER

Interleukin-2 is a cytokine protein that signals to cells of the immune system, such as T cells and natural killer cells, to regulate their growth, activation and differentiation. This multifaceted role means that interleukin-2 (IL-2) is the subject of intense investigation as a potential therapeutic agent to boost immune responses in the treatment of diseases such as cancer. On page 529 of this issue, Levin *et al.*[1] describe the engineering of enhanced versions of IL-2 that more potently stimulate T-cell proliferation and antitumour immune responses. The authors also provide a compelling structural explanation for the mutated proteins' superior activity.

The cell-surface receptor for IL-2 comprises three protein subunits: the IL-2 receptor α-chain (IL-2Rα, usually called CD25), the IL-2 receptor β-chain, and the common γ-chain (γ_c), which is shared by numerous cytokine receptors. IL-2 binding bridges the β-chain with the γ-chain; this is sufficient to form an active signalling complex and thus yield a cellular response[2]. Complicating this picture is the CD25 chain, which is variably expressed on IL-2 target cells. CD25 binds at a site on the IL-2 molecule distinct from that bound by the β-chain and γ_c-chain[3], generating a tripartite receptor (CD25–IL-2Rβ–γ_c) that binds more tightly to IL-2 than does the IL-2Rβ–γ_c complex alone (Fig. 1a).

The story, however, does not end there. In addition to helping to capture IL-2 on the cell surface, IL-2 binding by CD25 increases the strength of binding between IL-2 and the β-chain[4]. Structural comparison of IL-2 and the IL-2–receptor complexes[3] using X-ray crystallography provides an explanation for this behaviour: the β-chain binding site on IL-2 is structurally flexible, and CD25 binding causes this site to become less mobile and to adopt a conformation more favourable to β-chain binding. Thus, although CD25 has no direct role in signalling, it greatly enhances the effect of IL-2 signalling on those cells that express high levels of CD25; these include T cells in the early stages of an immune response against, for example, a tumour.

However, a class of cell called regulatory T cells, which suppress T-cell-mediated immune responses[2], routinely express high levels of CD25, whereas the precursors to one of the cell types that is largely responsible for antitumour immunity — cytotoxic T cells — normally express little or no CD25 prior to stimulation. Although IL-2 is already approved for the treatment of melanoma and renal cancer, a version of the cytokine that can bind tightly to the β-chain, without requiring CD25 enhancement, could be a highly potent and useful therapeutic immune stimulator.

Levin and colleagues[1] applied a two-step strategy to generate such a protein. The researchers used yeast cells to express a library of randomly mutated IL-2 proteins, from which they identified one variant protein that showed enhanced binding to the isolated β–γ_c receptor. A single amino-acid change in this variant, at a

50 Years Ago

We have obtained what we believe to be a unique photograph of a pinched lightning discharge … Over the years we have taken hundreds of photographs of lightning discharges, and this is our first photographic evidence of a pinched lightning. We estimate the distance of the lightning to the camera at

between 200 to 1,000 metres, and thus, the transverse dimension of the lightning between 1 and 5 m. Somewhat puzzling is the apparent much larger intensity of the integrated luminosity of the pinched lightning when compared with the luminosity of the aforementioned standard lightning stroke.

From *Nature* 28 April 1962

100 Years Ago

Prof. Milne … has now further increased the debt of seismologists to him by compiling, at the cost of several years' labour, a "Catalogue of Destructive Earthquakes from A.D. 7 to A.D. 1899," … Though containing only half as many entries as the earlier version, its value, it may be anticipated, will be even greater. Being confined to shocks of an intensity sufficient to damage buildings, it deals with those movements which are of chief consequence in the moulding of the earth's crust. An analysis of the catalogue for different epochs should reveal to us some of the laws which govern the distribution of seismic energy within extensive regions, such, for instance, as the Pacific coast of the America continent.

From *Nature* 25 April 1912

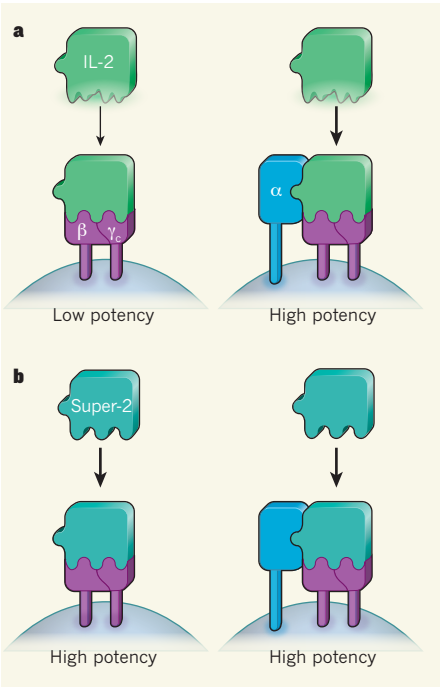

Figure 1 | Signalling superpowers. **a**, The cell-signalling protein interleukin-2 (IL- 2) binds to two versions of its receptor (IL-2R), both of which include the β- and γ_c-chains, and one that also includes the α-chain (also called CD25). These receptors are variably expressed on the surface of target cells, including T cells of the immune system. The IL-2 surface that makes contact with IL-2Rβ undergoes structural changes upon the binding of IL-2 to CD25, which leads to tighter IL-2Rβ–γ_c binding. Thus, IL-2 stimulates cells that have high levels of CD25 more potently than cells with low CD25 levels. **b**, Levin *et al.*[1] engineered IL-2 proteins, mutated to create a β-receptor-binding interface that allows tight binding to β–γ_c in the absence of CD25. These "super-2" proteins stimulate enhanced antitumour responses owing to their increased potency towards cells lacking CD25.

location in the protein remote from the β-chain binding site and within the protein's hydrophobic core, pointed to the importance of this core region in modulating β-chain recognition by IL-2. The investigators therefore designed and screened a second, smaller library that was biased towards mutations in the hydrophobic core. In this manner they identified several IL-2 variants, called "super-2s", that demonstrate superior potency in stimulating cellular activation in the absence of CD25.

Several of these super-2 variants bind tightly to the β-chain. Using a combination of X-ray crystallography and computer simulation of protein structure, the researchers verified that the mutations in one representative super-2 altered the structure and dynamics of key elements of the IL-2 surface that contacts the receptor β-chain. In effect, these mutations cause 'pre-organization' of the normally flexible binding site, in a manner that mimics the effect of CD25 binding. The authors show that, in contrast to normal IL-2, the super-2s activate T cells and natural killer cells with or without CD25 with almost equal potency (Fig. 1b). This potency is roughly equivalent to that of normal IL-2 on cells with CD25. The approach used to identify the super-2s thus represents a powerful demonstration of the use of 'directed evolution' to elucidate structure–function relationships to such an extent as to allow highly effective, rationally guided molecular engineering — an approach that is particularly challenging in a system with such complex structural regulation as that of IL-2 receptor binding.

Levin *et al.*[1] then evaluated their super-2s' ability to inhibit tumour growth, and found the mutant proteins to be superior agents in treating mice bearing any of three different types of human tumour. In comparison with normal IL-2, the super-2s also promoted greater proliferation of cytotoxic T-cell precursors in normal mice, but equivalent expansion of regulatory T cells, which suggests that an increase in cytotoxic T-cell number may be the mechanism for the improved antitumour responses. Another advantage of Levin and colleagues' super-2s is that they cause, somewhat paradoxically, significantly less fluid accumulation (oedema) than normal IL-2 — pulmonary oedema related to activation of CD25-lacking natural killer cells arises in patients treated with IL-2 and limits the dose that can be used[5]. Further investigation of this result might help us to better understand the biological basis of this side effect.

In addition to regulating the activity of T cells and natural killer cells, IL-2 contributes to the development of regulatory T cells in the thymus[2]; the multiple roles of this cytokine mean that further studies are required to fully understand the basis of super-2 activity *in vivo*.

Promising behaviour of anticancer agents in mouse experiments is not always predictive of success in human patients. Nonetheless, these IL-2 variants have exciting potential as investigational therapeutic agents for cancer and other diseases, such as HIV. Furthermore, the protein-engineering approach taken by Levin and colleagues, which iteratively combined structural analysis with directed evolution, might point the way to tailored versions of other proteins that have their activity regulated by the structural effects of complex binding interactions. Structural knowledge of the super-2 molecules might even aid the design of small-molecule drugs that modulate IL-2 behaviour in a similar manner. ■

Eric T. Boder *is in the Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville, Tennessee 37996-2200, USA.*
e-mail: boder@utk.edu

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