actin structures in the nucleus and to discover how the architecture of chromatin and the nucleus affects the formation and remodelling of actin polymerization. Further studies are also required to unravel the mechanism by which polymerized actin facilitates chromatin mobility and DNA-end processing. Given that genomic instability is a major contributor to the development of cancer, working out how cells use actin to safeguard their genome will have implications for our understanding of the basic principles of cancer aetiology. ■

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STRUCTURAL BIOLOGY

Picturing a key brain protein

Proteins called $GABA_A$ receptors have a pivotal role in neuronal inhibition, and are targets of several drugs. Structures of the most abundant $GABA_A$ receptor in humans are now reported, and will aid future drug discovery. SEE ARTICLE P.67

ERWIN SIGEL

The daytime biological activity of humans is based on a delicate equilibrium between excitatory and inhibitory signalling in the brain. The neurotransmitter molecule that mediates the main inhibitory signal is γ-aminobutyric acid (GABA), which binds to and activates ion channels, including a family known as $GABA_A$ receptors[1] found in the cell membranes of neurons. The activity of these receptors is modulated by many drugs[2], including sedatives, anxiolytics and sleeping pills. $GABA_A$ receptors can be assembled from several types of subunit, and the most abundant $GABA_A$ receptors in the adult human brain consist of two α1 subunits, two β2 subunits and one γ2 subunit. On page 67, Zhu *et al.*[3] present two structures of this receptor isoform in complex with GABA and a drug molecule known as flumazenil, providing much-needed insight that will aid future drug-discovery efforts.

The $GABA_A$ receptors that mediate fast signalling are found in several locations, most importantly at the synaptic junctions between neurons. Once activated by GABA, the channels in the receptors open, and conduct chloride ions through their pores. Benzodiazepines are a widely used class of drug that acts at some subtypes of $GABA_A$ receptor by binding to a specific site[4] distinct from that at which GABA binds. These compounds do not induce receptor activity on their own, but increase activity triggered by GABA, a process known as positive allosteric modulation.

Zhu *et al.* prepared the receptors for their study by expressing them in human embryonic kidney (HEK) cells in the presence of GABA and flumazenil, which is an antagonist for the benzodiazepine binding site (that is, it blocks the site). The authors used cryo-electron microscopy to obtain two high-resolution structures of the synaptic α1β2γ2 $GABA_A$ receptor (see Fig. 1 of the paper[3]), which they propose are in non-conducting, desensitized states — closed states of the channel that occur when the receptors have had prolonged exposure to GABA. Crucially, the structure confirms that the number of each subunit type and the arrangement of the subunits in the assembled pentameric receptor correspond to what had previously been postulated[1].

The structures reveal differences between the GABA binding sites (which are found at the two β2–α1 subunit interfaces in the receptor), the benzodiazepine binding site (which is found at the α1–γ2 interface, at an equivalent position to the GABA binding site), and equivalent sites at the α1–β2 and γ2–β2 interfaces to which neither GABA nor benzodiazepines bind (Fig. 1). These differences help to explain why the different sites bind (or do not bind) GABA or benzodiazepines. Both the γ2–β2 and α1–β2 sites are potential targets for drug discovery. A compound that binds at the α1–β2 interface has already been reported[5], but no compounds have yet been found for γ2–β2. The new receptor structures will provide a

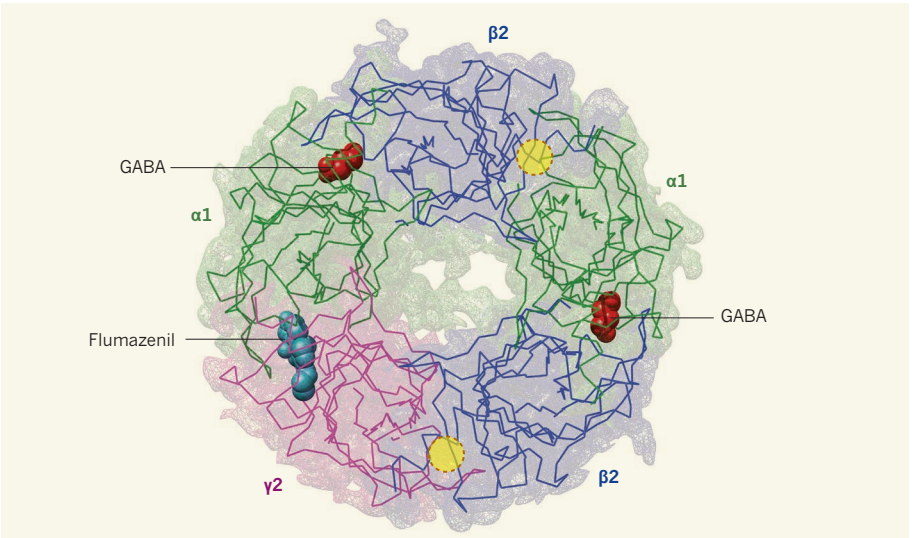

Figure 1 | Structure of the α1β2γ2 $GABA_A$ receptor viewed from the synapse. $GABA_A$ receptors are involved in neuronal inhibition and are targeted by several drugs. They form channels in the membranes of neurons, at different locations, most importantly at the synaptic junctions that connect pairs of neurons. Zhu *et al.*[3] report the structure of the most abundant $GABA_A$ receptor found in adult humans, which consists of two α1 subunits, two β2 subunits and one γ2 subunit. A molecule of γ-aminobutyric acid (GABA) — the naturally occurring ligand that activates the receptors — is bound at each of the GABA-binding sites, which are located at the two β2–α1 subunit interfaces. The drug flumazenil is bound at the benzodiazepine site at the α1–γ2 subunit interface (the site at which drug molecules from the benzodiazepine class bind). The γ2–β2 and α1–β2 interfaces contain pockets (yellow circles) that are at equivalent positions to the GABA and benzodiazepine sites, and which might be suitable targets for drug discovery. (Adapted from Fig. 2c of ref. 3.)

template for molecular-modelling studies aimed at discovering more compounds that bind at the different interfaces, or to any of the many other pockets present in the receptor.

The extracellular region of the receptor forms a wide chamber. Zhu *et al.* find that most of the space in this chamber is occupied by complex carbohydrate chains attached to some of the subunits, leaving relatively little space for ion permeation. If the same is true for receptors expressed in neurons, rather than in HEK cells, then this observation is mechanistically highly relevant. Ions might also enter the channel through openings found at the subunit interfaces. A structure of the α1β1γ2 $GABA_A$ receptor was recently published[6] on a preprint server, and the authors of that paper suggest that the attachment of carbohydrates to the α1 subunit has a key role in receptor assembly.

Understanding the variations between benzodiazepine binding sites found in different $GABA_A$-receptor isoforms might help researchers to find compounds that act selectively at just one isoform. This could allow researchers to realize the hope of developing drugs that produce desirable therapeutic effects (such as reducing anxiety) without the unwanted side effects (such as sedation) caused by existing drugs that target several isoforms of $GABA_A$ receptors. How diazepam — the archetypal benzodiazepine drug — binds to benzodiazepine sites is of particular interest. Computational studies[7] suggest that there are three possible binding modes (BM I–III) of benzodiazepines, of which BM I was proposed to actually occur. By contrast, an alternative approach has been used to identify the amino-acid residues in α1β2γ2 $GABA_A$ receptors that come into direct contact with diazepam[8], and this pointed instead to BM II. The information from that study was used to identify high-affinity ligands for the benzodiazepine site.

In Zhu and colleagues' structures, the position of flumazenil in the benzodiazepine site is similar to BM I. The authors suggest that docking of diazepam to the observed site would be possible through a binding mode similar to BM I, but that diazepam docking similar to BM II would lead to clashes between the molecule and the receptor. It should be noted, however, that the receptor conformations stabilized by flumazenil must be different from that stabilized by diazepam, so the idea that diazepam would dock into the reported structure in a similar way to flumazenil is questionable. A recent study[9] has shown that benzodiazepines whose structures are similar to that of flumazenil use BM I, whereas those similar to diazepam use BM II. Thus, the positioning of diazepam postulated by Zhu *et al.* will probably need to be revised.

In structural biology, there is always the possibility that the protein structure determined does not entirely correspond to a conformation adopted in nature. This is illustrated by one of Zhu and colleagues' structures (described as conformation A), in which the transmembrane part of the γ2 subunit is squeezed into the receptor's pore. This conformation is unlikely to occur in nature, and might have been caused by the type of detergent that Zhu *et al.* used to replace the natural membrane environment in their microscopy experiments. The water-soluble domain of the protein is not subject to this problem.

Proteins are dynamic structures that assume distinct conformational states. In each state, large parts of the proteins vibrate much like wobbly puddings. The binding of molecules to allosteric sites in proteins (such as the benzodiazepine site in $GABA_A$ receptors) can stabilize different conformational states. This explains how molecules can act as positive or negative allosteric modulators, or as antagonists. $GABA_A$ receptors exist in at least four conformational states: closed; ligand-bound but not open (known as the pre-activated state); open; and desensitized. Therefore, insight into allosteric modulation cannot be gained from structural studies alone, because protein structures are caught in static conformations. Structural-biology methods will need to be combined with other approaches to probe the dynamic properties of $GABA_A$ receptors.

Nevertheless, Zhu and colleagues' structures could well be the key to addressing some of the crucial issues in the study of $GABA_A$ receptors. For example, the work might help to unravel the numerous conformations of the α1β2γ2 $GABA_A$ receptor, and the differences in the benzodiazepine binding site between different isoforms of $GABA_A$ receptors, as well as providing information on all the possible modulatory sites. It is to be hoped that this will, in turn, lead to the development of improved drugs that target these receptors. ■ SEE EDITORIAL P.5

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NEUROSCIENCE

Social isolation's molecular signature

Extended social isolation causes debilitating effects in social mammals such as humans. A study in mice shows that the gene *Tac2* is upregulated throughout the brains of socially isolated animals, driving massive behavioural changes.

NOGA ZILKHA & TALI KIMCHI

Even the toughest prisoners fear solitary confinement. There is a growing awareness across the globe that we are facing an epidemic of loneliness. Prolonged social isolation and loneliness can lead to many profound physiological and neuropsychiatric conditions, including depression and heart disease, and to increased mortality rates[1]. In the United States, more than 50% of people over the age of 60 experience loneliness[2], and the United Kingdom has appointed a government minister to tackle the issue of loneliness. But the biological mechanisms underlying the effects of social isolation are poorly understood. Writing in *Cell*, Zelikowsky *et al.*[3] reveal a signalling mechanism that acts in several brain regions in mice to drive some of the harmful effects of the stress caused by chronic social isolation.

The authors examined the effects of two weeks of social isolation on the brains and behaviour of male mice (equivalent to more than a year in these conditions for humans[4]). First, the researchers used an array of behavioural tests to compare mice kept in isolation with control mice that had been housed in groups. These assays revealed widespread effects. Compared to control animals, isolated mice showed enhanced aggression and hypersensitivity to diverse stressful stimuli. For example, the socially isolated mice responded more aggressively to an unfamiliar mouse placed in their cage. In another assay, the researchers presented mice with a dark circle that loomed overhead, simulating an approaching predator. Control animals froze in response to the threat, but moved normally after the stressful stimulus was removed, whereas isolated mice remained frozen long after the apparent threat was removed.

Next, Zelikowsky *et al.* investigated the brain mechanisms underlying this behaviour. In a