other emotional behaviours, too.

To investigate the neurobiology that underlies this tachycardia-induced anxiety, Hsueh *et al.* performed a screen of brain activity after 15 minutes of optically induced tachycardia. Whole-brain mapping of neurons revealed changes in gene expression in response to tachycardia. The authors found that neurons in two regions — the posterior insular cortex (posterior insula) and the brainstem — were strongly activated. Electrophysiological recordings in live mice also showed an increase in the firing rate of neurons in the posterior insula during optically induced tachycardia.

The insular cortex is involved in both interoceptive processing and anxiety-related behaviours[8–10]. By this stage, the authors had discovered a correlative increase in the activity of the posterior insula after an increase in heart rate. But any involvement of this region in the tachycardia-induced anxiety remained to be determined. To investigate this, Hsueh *et al.* optogenetically inhibited posterior insula neurons using a different opsin — the blue-light-sensitive protein iC++.

In so doing, they made a second discovery: inhibition of the posterior insula during optical pacing reduced the anxiety behaviours induced by tachycardia. This indicates that the posterior insula relays information about heart rate to affect anxiety. The attenuation was specific to the posterior insula, and was not observed with optogenetic inhibition of a different region, the medial prefrontal cortex.

Overall, then, Hsueh *et al.* have found that increases in heart rate promote anxiety-related behaviours in mice, and that this is mediated through the activation of specific brain structures that include the posterior insula. The authors' comprehensive study raises new questions, and opens up areas for research. For example, the neural circuits and mechanisms that allow the posterior insula to be activated by tachycardia — as well as the circuits that induce anxiety behaviours — have yet to be identified.

Another unexplored aspect is the long-term effect of days (or weeks) of optically induced tachycardia — a question with substantial clinical implications. This study lays the foundation for testing whether chronic increases in heart rate induce long-term changes in the brain, which could underlie harmful levels of anxiety. Testing this hypothesis would raise technical challenges, because the micro-LED vest used here is not suitable for such long periods of stimulation.

Finally, from a translational and therapeutic perspective, it might be possible to design experiments to slightly decrease heart rate. Would this change reduce anxiety-related behaviours? Hsueh and colleagues' work has provided the means to investigate this prospect.

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Structural biology

MicroRNA uses a gym to get fit for Dicer enzyme

Gunter Meister

The enzyme Dicer cleaves a type of RNA called a pre-microRNA to make the mature functional RNA. Structural evidence now sheds light on the catalytic mechanism involved and the role of a newly found RNA sequence termed GYM. **See p.323 & p.331**

Gene expression can be silenced through targeted pathways that depend on small RNAs. On pages 323 and 331, Lee *et al.*[1,2] provide insights into a key step in the maturation of such RNAs that is mediated by the enzyme Dicer.

RNA-dependent gene-silencing pathways are found in almost all eukaryotes (organisms whose cells contain a nucleus). Many of these systems are fuelled by immature versions of RNA that are often in the form of double-stranded RNA (dsRNA). Such dsRNA serves as the origin of small regulatory RNAs that include microRNAs (miRNAs) and short-interfering RNAs (siRNAs). In both cases, the dsRNA precursors are cleaved by a particular class of enzyme that is characterized by having what is termed an RNase III domain[3].

The miRNAs are processed from stem-loop-structured precursors, also described as a hairpin, and they require the consecutive action of two RNase III enzymes. In animals, the enzyme Drosha conducts the first cut and Dicer the second. Both enzymes define the ends of a short double-stranded miRNA intermediate from which one miRNA strand is selected and incorporated into the protein complex RISC, in which the RNA directly binds to a member of the Argonaute protein family.

The siRNAs are typically processed from the ends of long dsRNAs by only one enzyme, Dicer[4]. This scenario, however, requires that Dicer moves along the dsRNA. Therefore, Dicer enzymes can generally be divided into what are called non-processive and processive enzymes, depending on whether they generate more than one small RNA from a dsRNA

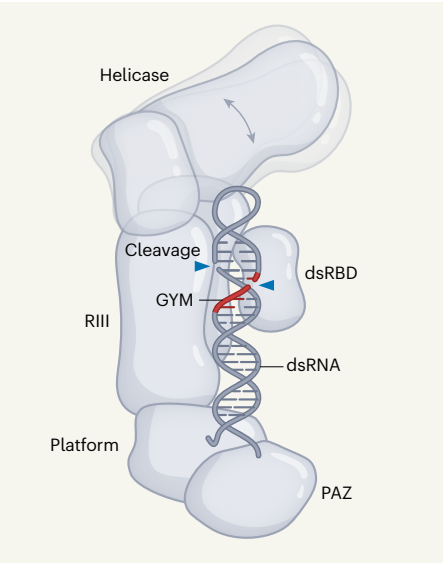

Figure 1 | Structural clues to how human Dicer enzyme functions. Some small RNAs, such as microRNAs, that function in gene silencing undergo a maturation step in which a double-stranded RNA (dsRNA) is cleaved by Dicer. Lee *et al.*[1] reveal that an RNA sequence that the authors term GYM has a role in facilitating this process. The authors' second paper[2] presents structural data obtained using cryo-electron microscopy, which captured the enzyme at the stage associated with RNA cleavage. This structure reveals the orientation of the dsRNA with respect to various domains of Dicer, a subset of which are shown here: helicase domain, RNase III (RIII) domain, dsRNA-binding domain (dsRBD), platform domain and PAZ domain. The helicase domain was not clearly visible in the structure, which suggests that it is in a flexible conformation at this step. (Adapted from Fig. 5 of ref. 2.)

molecule. Human Dicer (hDicer) is specialized for pre-miRNAs and is thus non-processive. Although structures of Dicer enzymes from various organisms are available, the new studies of hDicer clarify the catalytic cleavage step (also known as 'dicing'), as well as revealing evolutionarily conserved pre-miRNA features that are important for efficient RNA processing.

The multidomain protein hDicer (Fig. 1) includes two RNase III domains, a helicase domain, a dsRNA-binding domain (dsRBD) and a PAZ domain, which, together with the platform domain, accommodates the two ends of the pre-miRNA hairpin[4]. Dicer not only cleaves the pre-miRNA but also functions as a 'molecular ruler' by essentially measuring the distance between the end of the RNA stem and the catalytic centres of the enzyme to produce a dsRNA that is 20–23 nucleotides in length. The sequences of pre-miRNAs are highly diverse, but besides the common RNA features of the hairpin structure, a two-nucleotide 3′ overhang on one side of the RNA (its 3′ end) and a phosphate group on the other side of the RNA (its 5′ end), no specific sequences or extra local structural motifs that might guide cleavage had been found previously.

To find such features, the first study by Lee *et al.*[1] used a pre-miRNA-processing approach in which RNAs were engineered to randomize the sequence of the upper stem of the pre-miRNA. Then the authors tested RNA-processing efficiency by sequencing the small RNA products produced after cleavage by Dicer. They thereby found an evolutionarily conserved sequence of nucleotides that they term GYM. This nucleotide motif, located around the cleavage site, consists of a paired guanine (G, one of the four main bases found in RNA), a paired pyrimidine (Y, which can be either one of the bases cytosine and uracil) and a mismatched (M) nucleotide pair. When this motif is engineered onto a random short hairpin RNA, Dicer processing of the RNA is markedly increased compared with the case for RNAs lacking the motif.

In their second study, Lee *et al.*[2] used hDicer and a pre-miRNA with a GYM motif as the material for generating structural data by means of cryo-electron microscopy. Strikingly, the authors found complexes with hDicer in the dicing state, which had not been seen so far.

Compared with structures in the pre-dicing state, in which the helicase domain binds to the pre-miRNA loop and keeps the pre-miRNA away from the catalytic centre, the helicase domain is not visible in the dicing-state structure. This observation indicates that the helicase domain becomes very flexible in the dicing state (Fig. 1). It might no longer bind to the pre-miRNA, enabling the pre-miRNA to move into a cleavage-competent position. Furthermore, in this position, the dsRBD of Dicer recognizes the GYM motif and stabilizes the interaction between the RNA and Dicer for a more efficient cleavage reaction. This molecular interaction explains the evolutionary conservation of the GYM motif.

Although several Dicer structures are available from different organisms and in different states[5–9], there are still many unknowns about this fascinating molecular machine. First, and most intriguingly, how is the cleavage product released in a post-dicing state? A RISC-loading complex has been postulated [10], consisting of Dicer, its partner protein TRBP and an Argonaute protein that takes over handling the miRNA from Dicer after cleavage. Major structural rearrangements and long-distance movements of the cleavage product are obviously required for this step. The helicase domain might become more static in such a complex, and TRBP might also contribute to structural rearrangements. Another question is how a correctly loaded RISC might be released from this complex.

Furthermore, we have not even begun to understand the role of alterations (termed post-translational modifications) of Dicer, such as the addition of phosphate groups (phosphorylation), in this process. Many such modifications have been reported, but if and how they contribute to the dicing cycle, and particularly to the structural flexibility of Dicer, is unknown.

Notably, both studies highlight cancer-associated mutations that correspond to prominent structural positions in Dicer. For example, the sequence encoding a pocket in the platform domain that accommodates the 5′ end of the pre-miRNA is often mutated in cancer. In addition, cancer-associated mutations in the dsRBD lead to reduced binding of Dicer to the GYM motif.

It is tempting to speculate that many more such mutations exist, which might not only affect cleavage by Dicer but potentially also later, post-dicing steps. What are the consequences of such mutations for cell growth and cancer development? Is there solely a reduction of global miRNA levels owing to impaired processing, or could there even be pre-miRNA-specific processing effects? Future mechanistic and structural work will provide further functional insights into the fundamental cellular process of miRNA formation and its link to disease.

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Atmospheric chemistry

How wildfires deplete ozone in the stratosphere

V. Faye McNeill & Joel A. Thornton

Unexpected smoke-particle chemistry is shown to be the link between intense wildfires and stratospheric ozone loss. As the climate changes, more-frequent and more-intense fires might delay the recovery of the stratospheric ozone layer. **See p.259**

The devastating Australian bushfires of 2019–20 sent massive plumes of smoke high into the atmosphere, where it was transported around the world, affecting air quality as far away as South America[1]. Satellite data showed that this smoke also caused changes in the composition of the upper atmosphere, including a decline in stratospheric levels of ozone[2,3] — a gas that forms a protective layer around Earth, shielding terrestrial life from damaging short-wave ultraviolet radiation. But the mechanism by which wildfire smoke might enhance ozone depletion has remained uncertain. On page 259, Solomon *et al.*[4] make the case that particulate matter from wildfire smoke contributes to the destruction of stratospheric ozone, and suggest how it does this.

In 1974, modelling first pointed to increasing levels of chlorofluorocarbons (CFCs, which were often used in aerosol spray cans, foams and cooling devices) as a source of chlorine radicals in the stratosphere that could