from 22 computational models of the Earth system, ultimately finding that it is a good predictor of the inherent ECS of each of the models.

Cox *et al.* then used the relationship between the metric and the ECS found in the models as a constraint on ECS in the real world. Their analysis revealed that only climate models that produce relatively small values of ECS match the variability seen in the historical temperature record. It turns out that, in general, climate models have considerable memory in their climate systems, so if one year is abnormally hot, for example, then the next year is likely also to be hot. The historical temperature record, however, does not seem to have as much system memory as most models. This means that some models have both autocorrelations and ECS values that are too high.

These new findings must be interpreted carefully. ECS is arguably the main factor that governs uncertainty in projected temperatures, but is not the only factor. For example, Earth-system feedbacks such as the effects of permafrost melting are expected to increase warming. Climate models often exclude these feedbacks, reducing the projected warming. In models that have an ECS that is too high, such exclusions could potentially compensate for the effects of the inflated ECS value.

It is also crucial to examine other lines of evidence when assessing ECS. The best estimates of ECS that have been made by analysing Earth's energy budget (the balance of the energy received by Earth from the Sun and the energy radiated back to space) are relatively low, at around 2 °C (ref. 7). But recent work[8] is helping us to understand that ECS values inferred from energy-budget changes over the past century are probably low, and shows that a higher value is more applicable when projecting future change. Applying such a correction to the original estimates[9] brings their values very much in line with Cox and co-workers' estimate (Fig. 1).

By contrast, analyses[3] of present climate conditions (particularly cloud properties) produced by models show that the models that best represent today's climate have ECS values greater than 3 °C. Indeed, one of the most recent of these analyses[4] showed that models with an ECS of around 4 °C best captured today's climate across nine emergent constraints (Fig. 1). In my view, Cox and colleagues' estimate and the estimates produced by analysing the historical energy budget carry the most weight, because they are based on simpler physical theories of climate forcing and response, and do not directly require the use of a climate model that correctly represents cloud. To resolve which estimates are most accurate, more research is needed to compare the different lines of evidence and to improve the representation of clouds in models.

"The idea underpinning this work is so enviably simple that it will make climate scientists ask, "Why didn't I think of that?"

I hope that a much more refined estimate of ECS can be made from the different lines of evidence by the time the next IPCC assessment is published in 2021. If the upper limit of ECS can truly be constrained to a lower value than is currently expected, then the risk of very high surface-temperature changes occurring in the future will decrease. This, in turn, would improve the chances of keeping the temperature increase well below 2 °C above pre-industrial levels, the target of the Paris Agreement under the United Nations Framework Convention on Climate Change. So, rather than be jealous, I should thank Cox and colleagues for helping me to sleep a little easier in my bed at night. ■

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MOLECULAR BIOLOGY

Limitless translation limits translation

Evidence has now been found that ribosomes — the cell's translational apparatus — can pass beyond the main protein-coding region of messenger RNAs to form 'traffic jams' that inhibit protein expression. SEE LETTER P.356

PETRA VAN DAMME

During the process of translation, molecular machines in the cell called ribosomes use sequences encoded by messenger RNAs as templates for protein synthesis. On page 356, Yordanova *et al.*[1] propose an intriguing mechanism that might limit the number of protein molecules that can be synthesized from a single mRNA. It involves the formation of a queue of ribosomes on the mRNA, downstream of the main protein-coding region.

The conventional view of translation in eukaryotes — organisms such as fungi, plants and animals — is that each mRNA consists of a stretch of nucleotides that contains an open reading frame (ORF), which encodes a single protein containing more than 100 amino-acid residues. But over the past decade, the advent of technologies such as ribosome profiling[2] has revealed that a more-diverse range of ORF sequences can, in fact, be translated. For example, numerous small upstream ORFs (uORFs) have been identified whose translation might regulate expression of the main ORF.

Ribosome profiling has also revealed a wealth of events in which translation is initiated at alternative start codons[3] (triplets of nucleotides other than the triplets at which translation is normally assumed to initiate), and read-through events[4] in which translation continues beyond the stop codon (the nucleotide triplet at the end of the ORF). Not only do these two types of event increase the overall diversity of proteoforms (molecular forms of proteins produced from genes)[5], but they have also emerged as regulatory mechanisms for hundreds of genes in eukaryotic genomes. Other regulatory mechanisms for translation are also known, including ribosome stalling, in which obstacles impede ribosome movement along mRNAs.

Yordanova *et al.* now propose another evolutionarily conserved mechanism for translational control. They suggest that sporadic stop-codon read-throughs can lead to the formation of ribosome queues at downstream stalling sites, such that the queue length is proportional to the number of protein molecules that have been synthesized. The authors define the region between the end of the main ORF and the next in-frame stop codon (that is, the next nucleotide triplet that would be recognized as a stop codon by a

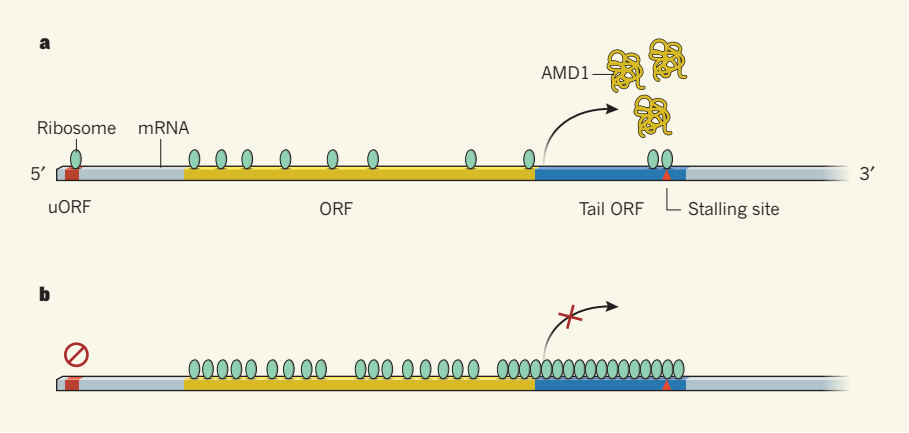

Figure 1 | A proposed regulatory mechanism for translation of the *AMD1* gene. **a**, The messenger RNA for the AMD1 protein contains an open reading frame (ORF) that encodes the protein's amino-acid sequence, and also an upstream open reading frame (uORF), the translation of which regulates translation of the ORF (ref. 6). Grey regions of mRNA are not translated. Yordanova *et al.*[1] propose that ribosomes (the cellular machinery responsible for translation) can sporadically enter and translate a region called the tail ORF, rather than stopping at the end of the main ORF. The ribosomes eventually halt at a stalling site (a nucleotide sequence that halts translation) in the tail ORF. **b**, When uORF-mediated regulation is blocked, translation initiation at the main ORF increases, so that ribosomes accumulate more quickly in the tail ORF, forming a queue. When the queue extends beyond the tail ORF, translation of the main ORF is impaired, limiting the number of protein molecules that can be synthesized from a single *AMD1* mRNA template.

ribosome translating beyond the main ORF's stop codon) as the tail ORF. They suggest that translation is halted when queuing ribosomes in the tail ORF extend into the main ORF (Fig. 1).

The authors were inspired to propose this mechanism after inspecting publicly available ribosome-translation profiles for a protein called adenosylmethionine decarboxylase 1 (encoded by the *AMD1* gene), the translation of which is tightly controlled. The profiles revealed translation of a uORF in the *AMD1* mRNA, as previously reported[6], but also a high density of ribosomes in a region known as the 3′ trailer of the mRNA, downstream of the *AMD1* stop codon. This suggested that a stop-codon read-through had occurred, allowing ribosomes to accumulate in the tail ORF of *AMD1*.

Yordanova and colleagues performed experiments showing that stable peptidyl–transfer RNA complexes (which form between tRNA and the nascent protein chain during translation) are generated when tail-ORF sequences are translated, and that complex formation occurs before translation reaches the stop codon at the end of the tail ORF. This confirmed that the proposed read-through could occur, and that translation could stall in the tail ORF. The authors also constructed a mutant mRNA in which the *AMD1* stop codon was replaced by a sense codon (a nucleotide triplet that encodes an amino acid), in the expectation that translation would occur uninterrupted through the mutated sequence. However, almost no *AMD1* translation occurred with this mutant — the expected extended proteoform was produced in nearly undetectable levels.

To explore the mechanisms that affect the levels of expressed protein, Yordanova *et al.* used a strategy[7] known as StopGo, which allows the cleavage and release of nascent protein chains at chosen positions during translation, but then allows ribosomes to resume translation of the downstream sequence. The authors used StopGo to cleave nascent proteins before translation of the *AMD1* stop codon in the wild-type mRNA, and before translation of the sense codon in the mutant mRNA. They observed that the amount of AMD1 protein subsequently produced from the mutant mRNA was lower than the amount produced from the wild-type mRNA — even though the amino-acid sequences of the proteins were identical.

This result suggests that the tail ORF must lower protein expression by influencing translation, rather than by reducing the stability of the produced protein. The finding is at odds with previous work[8] showing that proteins are generally destabilized when their sequences are extended by a stop-codon read-through. Yordanova and colleagues' experimental data collectively show that the effects of translation of the *AMD1* tail ORF are independent of the main coding sequence, mRNA stability, common protein-degradation and cleavage pathways, or whether the expressed protein is secreted by cells.

The findings are therefore consistent with the idea that translation is halted when a ribosome queue in the tail ORF extends into the main *AMD1* coding region. Note that such regulation could work only if *AMD1* translation is dysregulated and high, and would not apply under standard conditions. The authors also observed that translational output was more strongly reduced in experiments that increased the efficiency of read-throughs, thereby accelerating queue formation — in agreement with the model.

A note of caution is warranted, because Yordanova and co-workers have not directly observed long ribosome queues. The proposed ribosome stalling might also occur only transiently, thereby increasing the time required to attain full ribosome coverage of the tail ORF and so decreasing the overall impact of ribosome-queue formation on translation. Furthermore, besides the experimentally validated traffic-jam model[9] (in which ribosomes collide and form queues, blocking translation initiation), other models for how ribosome stalling interferes with translation have been proposed. For example, it has been suggested that stalled ribosomes fall off mRNA following collision with a trailing ribosome; this model conflicts with the idea that long ribosome queues could form[10]. Peculiarities of the expression systems used by Yordanova *et al.* might also underlie some of the authors' observations. Finally, their data do not rule out the possible involvement of other factors that could cause the downregulation of protein expression, such as protein-degradation pathways that occur at the same time as translation.

Nevertheless, the authors' findings will surely inspire future endeavours to obtain concrete proof of the proposed mechanism, and to assess how widely it is used to limit protein synthesis. Single-molecule imaging of translation on individual mRNA molecules, in real time and in live cells, might eventually allow simultaneous observation of mRNAs and their protein products[11]. ■

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