MOLECULAR BIOLOGY

Entry signals control development

Certain structural elements allow messenger RNAs not usually processed by the protein-synthesis apparatus to be translated. It now seems that they also control the expression of genes involved in embryonic development. SEE ARTICLE P.33

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The discrepancy between organismal complexity and genome size, known as the C-value paradox[1], is a classic problem in molecular biology. The solution to achieving complexity from a few genes is to control their expression at multiple levels. The genes responsible for driving complex pattern formation in highly developed organisms, called homeobox (*Hox*) genes, are particularly subject to layers of regulation[2]. In this issue, Xue *et al.*[3] (page 33) reveal a surprising mechanism by which *Hox* expression is controlled during embryonic development to ensure proper formation of the body plans of multicellular animals.

There is a common misconception that gene expression begins and ends with the synthesis of messenger RNA, perhaps because of the preponderance of studies based on yeast and tumour-derived cells grown in the presence of unlimited nutrients. This is akin to drawing conclusions about driving from observing cars on the Autobahn. Real life, however, is more like cross-town traffic: although mRNA transcription is the first and essential step in gene expression, the journey to protein synthesis can be complex and elaborate. This is particularly evident in developmental programs and responses to environmental cues or stresses, and in cases where proteins must be localized to specific regions of a cell. mRNA stability is also an important factor in governing protein synthesis. The regulation of gene expression at levels beyond mRNA synthesis is generally referred to as post-transcriptional control[4].

Like a good story, protein synthesis has a beginning (initiation), a middle (protein elongation) and an end (termination). Because it lies at the beginning, initiation represents a crucial control nexus. Initiation of protein synthesis from most mRNAs in eukaryotes (organisms that include plants, animals and fungi) requires covalent modifications called 5′ caps and poly(A) tails to be added to the beginnings and ends of mRNAs, respectively[5]. These are recognized by a preinitiation complex (PIC), which contains the small subunit of the protein-synthesizing ribosome apparatus, together with an initiator transfer RNA and additional accessory factors. The PIC scans along the mRNA in the 5′-to-3′ direction until it encounters the nucleotide sequence (AUG) that specifies where translation should begin. Such 'cap-dependent' translation confers several opportunities for control: it enables cells to distinguish between self and non-self mRNAs to subvert viral RNA infection; it can be used to modify mRNA stability; and structural barriers between the cap and AUG can be used to control initiation.

Not having caps and tails can also be advantageous. For example, many RNA viruses, especially positive-sense RNA viruses, whose genomes resemble mRNAs, directly deposit their genomes into a host cell's cytoplasm to be used as mRNAs. Because these genomes do not have access to the host cell's nucleus (which harbours the machinery that attaches 5′ caps and poly(A) tails to the host's mRNAs), many viruses have evolved alternative ways of recruiting ribosomes that use highly structured RNA elements called internal ribosome entry signals (IRESs). In the presence of viral enzymes that inactivate cap-dependent translation, cellular ribosomes are driven to the viral IRES-containing mRNAs. But IRESs are not just used by viruses: IRES-containing cellular mRNAs are also preferentially translated under conditions in which cells inactivate cap-dependent translation. This happens in response to a variety of stresses and during cell division. The list of IRES-containing cellular mRNAs continues to grow[6].

Xue and colleagues now show that IRESs are used to control gene expression during mammalian development. Vertebrate genomes harbour four clusters of *Hox* genes, *HoxA*–*HoxD*. The chromosomal region corresponding to the *HoxA* cluster contains 11 genes encoding transcription factors, each of which is involved in the development of different parts of the vertebrate body, from

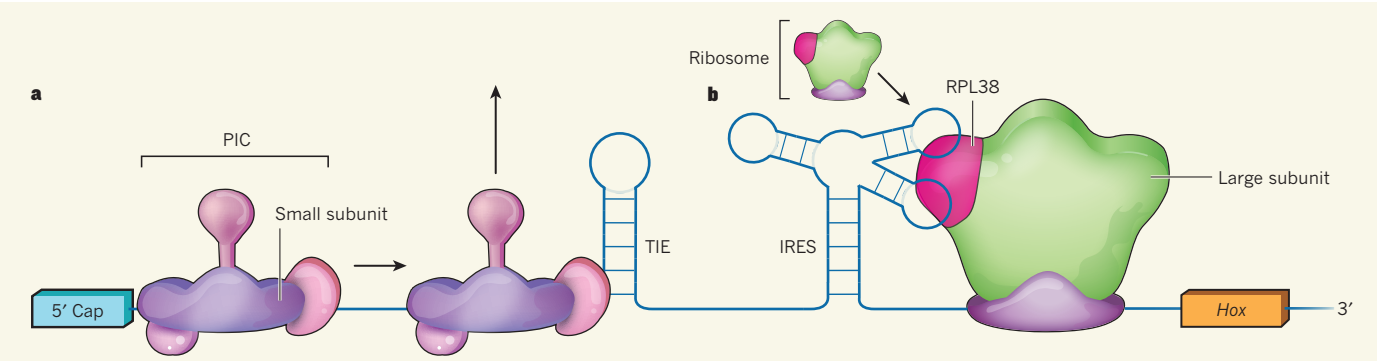

Figure 1 | Regulating the translation of certain *Hox* genes. **a**, A preliminary step in the translation of many messenger RNAs is the loading of a preinitiation complex (PIC, which includes the small subunit of the ribosomal protein-synthesis apparatus) onto cap structures at the 5′ end of the mRNAs. The PIC then scans along the mRNA in the 5′-to-3′ direction (horizontal arrow) searching for a nucleotide sequence that specifies where translation should begin. Xue *et al.*[3] report that translation inhibitory elements (TIEs) located in the 5′ untranslated regions of selected *Hox* mRNAs block the PIC's progress, and may cause it to detach from the mRNA (vertical arrow). **b**, They also find that RNA elements called internal ribosome entry signals (IRESs), located downstream of TIEs, directly recruit ribosomes to initiate translation of these *Hox* genes, perhaps with assistance from ribosomal protein RPL38 on the ribosome's large subunit.

the hindbrain to the sacrum (the bone at the base of the spine)[7]. Although the mRNAs that encode these *HoxA* genes are equipped with 5′ caps and poly(A) tails, they are poor substrates for translation. Previous work[8] demonstrated that ribosomal protein RPL38 facilitates translation of a subset of *Hox* mRNAs, suggesting that translation of some of the *HoxA* mRNAs may require direct interaction with the ribosome.

In their study, Xue and co-workers used a series of ingenious experiments not only to characterize IRES activity in 5 of the 11 *HoxA* mRNAs, but also to identify mRNA motifs that they call translation inhibitory elements (TIEs), located between the 5′ caps and IRESs. These motifs seem to impede scanning by the PIC (Fig. 1). The combination of TIEs and IRESs enables a previously unknown mode of translational regulation in these key developmental regulatory genes.

The authors used state-of-the-art methods to structurally characterize the IRESs and to demonstrate that, at least in cell lysates, ribosomes can be directly recruited to the mRNAs. Most intriguingly, they found that complete loss of the IRES in the *Hoxa9* gene, which has a crucial role in effecting the transition between the thoracic and lumbar regions of the spine, results in loss of the terminal rib anchored to the thirteenth thoracic vertebra (T13) but does not affect the distribution of the *Hoxa9* mRNA in the developing mouse fetus. Indeed, Xue *et al.* observed that T13 is partly transformed into L1 (the first lumbar vertebra) in mice carrying only one IRES-containing *Hoxa9* gene rather than the normal two. This finding emphasizes the part played by the newly discovered regulatory mechanism in the development of the mammalian body plan.

Xue and colleagues' groundbreaking study raises many questions. Does ribosome recruitment involve the whole ribosome or only the large subunit? If the latter is true, then how is the small subunit engaged for initiation of translation? Structurally, RPL38 lies along the solvent-accessible 'back side' of the large ribosomal subunit[9]; are other ribosomal proteins or soluble factors that interact with the protein-synthesis apparatus involved in the translation of IRES-containing *Hox* mRNAs, and what might they be?

RPL38 is not essential in yeast[10], so another question is whether RPL38-containing ribosomes in multicellular organisms are specialized to decode IRES-containing mRNAs. And are such *Hox* mRNAs ever translated by cap recognition and scanning? If so, how are the TIEs inactivated? If not, are these mRNAs translated only at specific stages in development when cap-dependent translation is suppressed? These questions will drive a diverse set of research efforts in the future. ■

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1. Mirsky, A. E. & Ris, H. *J. Gen. Physiol.* **34,** 451–462 (1951).
2. Mallo, M. & Alonso, C. R. *Development* **140,** 3951–3963 (2013).
3. Xue, S. *et al. Nature* **517,** 33–38 (2015).
4. Hershey, J. W. B., Sonenberg, N. & Mathews, M. B. *Cold Spring Harb. Perspect. Biol.* **4,** a011528 (2012).
5. Sonenberg, N. & Hinnebusch, A. G. *Cell* **136,** 731–745 (2009).
6. Mokrejš, M. *et al. Nucleic Acids Res.* **38** (Suppl. 1), D131–D136 (2010).
7. Alexander, T., Nolte, C. & Krumlauf, R. *Annu. Rev. Cell Dev. Biol.* **25,** 431–456 (2009).
8. Kondrashov, N. *et al. Cell* **145,** 383–397 (2011).
9. Jenner, L. *et al. Curr. Opin. Struct. Biol.* **22,** 759–767 (2012).
10. Giaever, G. *et al. Nature* **418,** 387–391 (2002).

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MATERIALS SCIENCE

Like cartilage, but simpler

The properties of articular cartilage, which lines bones in joints, depend partly on repulsion between components of the material. A new synthetic gel that mimics this feature has rare, direction-dependent properties. SEE LETTER P.68

ANNE LADEGAARD SKOV

The work of materials scientists usually focuses on attractive forces, which underpin the reinforcement of polymers by strong fibres or particles[1], and the self-repairing abilities of rubbery materials through hydrogen bonding[2]. But on page 68 of this issue, Liu *et al.*[3] report their use of repulsive forces in the design of a hydrogel — a water-swollen polymer network — that exhibits fascinating direction-dependent behaviour. The material might be useful in applications that require a reduction of vibrations.

The new hydrogel contains nanometre-scale sheets of a titanium oxide known as titanate(IV) nanosheets (TiNS), arranged cofacially (in planes with their faces aligned towards each other). TiNS consist of only surface atoms, and adopt an ultrathin (7.5-ångström)

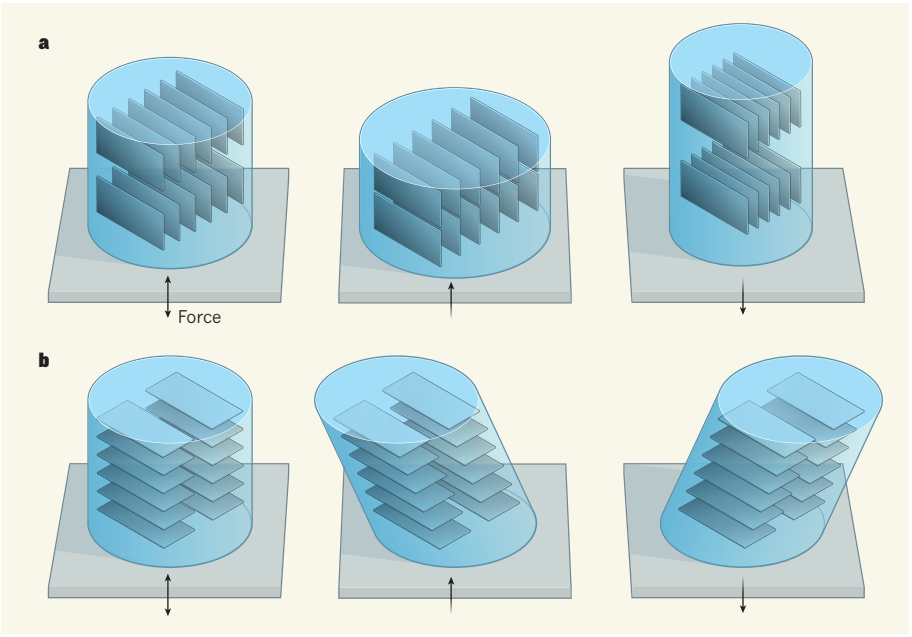

Figure 1 | Direction-dependent behaviour of a hydrogel. Liu *et al.*[3] have prepared a hydrogel — a water-swollen polymer network — that contains nanoscale sheets of a titanium oxide, aligned in planes with their faces parallel to each other. **a**, When an oscillatory force is applied up and down to cylindrical samples of the material parallel to the nanosheet planes, the hydrogel undergoes cycles of compression and expansion. Single-headed arrows indicate movement of the platform on which the sample rests. **b**, But when the same force is applied orthogonal to the nanosheet planes, the material undergoes almost completely horizontal deformation. Nanosheets are not shown to scale.