

Ancient tweaking

Twenty years ago, scientists knew nothing of the scraps of RNA that are now known to influence just about every process in our bodies. Back then, the textbooks were simpler: genes code for proteins via the intermediate of RNA, and proteins called transcription factors regulate other proteins. This recipe was so entrenched in the basic orthodoxy of molecular biology that it was even given the name ‘the central dogma’ by the co-discoverer of DNA, Francis Crick.

Scientists now know, however, that this classic view of protein regulation is far too blunderingly inefficient for evolution to settle for. At some point hundreds of millions of years ago, the generation of a small stretch of RNA that could tweak this process gave an individual the edge over everyone else. And so regulatory RNA was born. These scraps of RNA – on average only 22 nucleotides long and now dubbed microRNAs, or miRNAs for short – bind to some messenger RNAs and label them for inactivation or destruction.

So far thousands of miRNAs have been identified in animals. These superintendents of protein regulation are involved in the earliest stages of an animal’s development, determining which cell types grow where and when, and how these cells differentiate into the different body parts. However, since the discovery of miRNAs, many scientists have wondered whether the same miRNAs govern specific tissues in different animals. Knowing this would not only give clues to the age of these different miRNAs, but also to the age of the cells in which they are found.

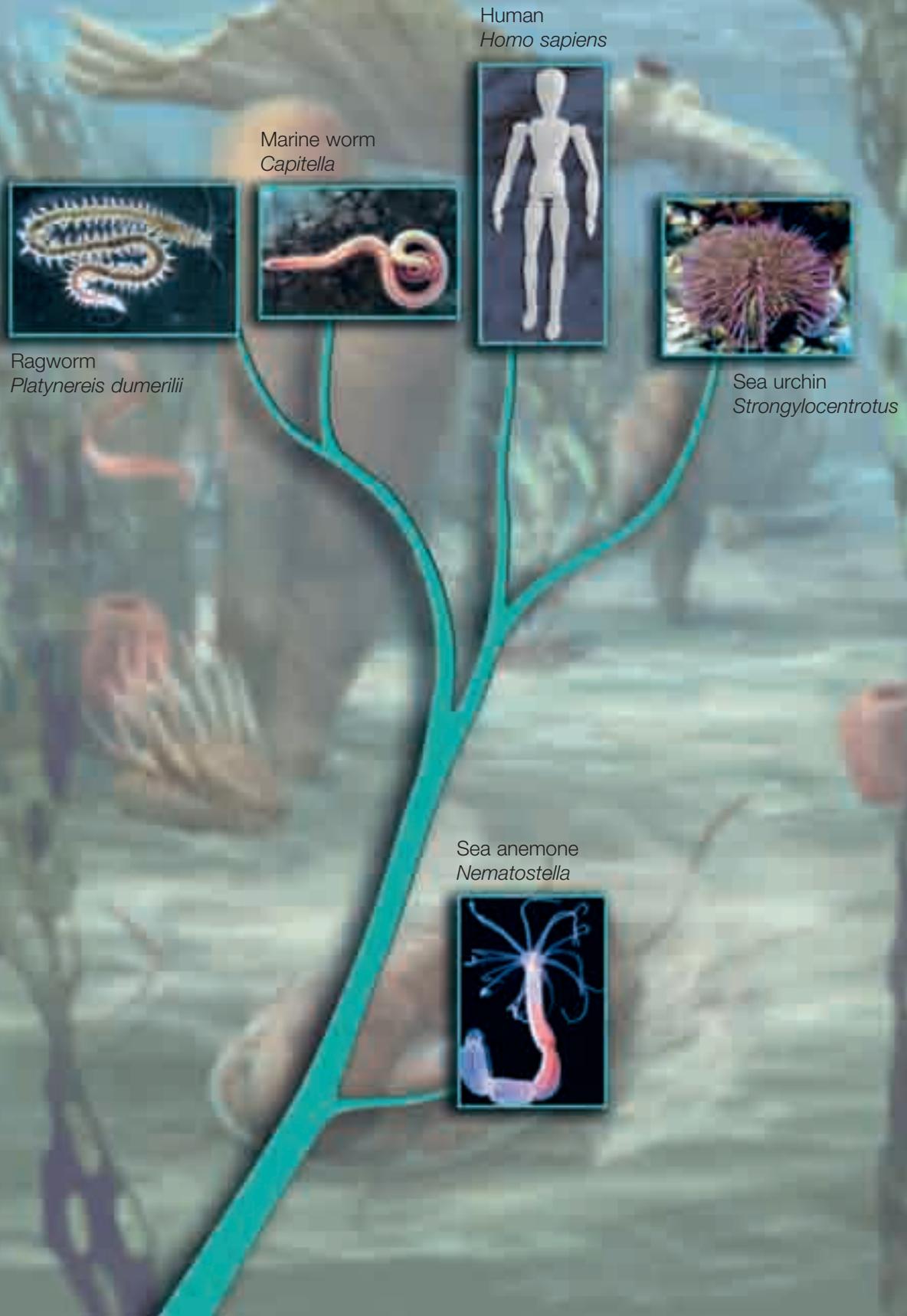
Developmental biologist Detlev Arendt from EMBL Heidelberg, whose work has recently traced the evolutionary origins of the brain and other organs, wondered whether he could study the oldest known animal miRNAs in a group of animals that at least look old themselves. In doing so, he hoped to investigate whether miRNA expression is conserved across the animal kingdom.

Studying ancient-looking animals is crucial for this process because “all animals evolve, but the speed with which they change differs,” explains Detlev. “Animals living near the coastline today exist in a similar environment to that which their ancestors thrived in, and so some of these animals haven’t been forced to change their body plans, or the genes that control these plans, because they are already well-adapted to this ancient environment.” By studying these animals, biologists can glean clues to ancient morphologies.

Detlev and Fay Christodoulou, who was a PhD student in Detlev’s group, used one such ‘living fossil’, the marine ragworm *Platynereis dumerilii*, which is thought to have changed little over the past 600 million years. In collaboration with an American team from Cold Spring Harbor Laboratory who sequenced the ragworm’s miRNAs, and Peer Bork’s group at EMBL who contributed to the bioinformatic analysis, they probed for 34 ancient miRNAs in the bodies of young worms.

By tracing a blue dye that becomes trapped when specific probes bind to the miRNA, Fay was able to see where the miRNAs were expressed in the worm embryos. “Initially the high temperatures required for the probes to bind

MicroRNAs present in the ragworm, sea urchin and worm probably existed already in their last common ancestor, which separated from the sea anemone's branch of the evolutionary tree around 600 million years ago.



destroyed the embryo's intricate body structures, but then I realised that I could reduce the temperature and still see binding," notes Fay.

After four months of developing this approach, and over a year of analysing the tissue samples, Fay showed that many miRNAs are highly specific for certain tissues and cell types in the worms. She then looked at the expression of these same miRNAs in three other marine species – a sea anemone, a worm and a sea urchin. Fay explains that sea anemones are radially symmetrical, whereas worms, like humans, are bilaterally symmetrical because they have a back and a belly, and their right side is the mirror image of their left. Sea urchins are more complex but they are still thought to stem from animals with bilateral symmetry. By comparing miRNA expression in these animals with that in the ragworm, the researchers hoped to resolve in which tissue particular miRNAs were first active when they evolved more than half a billion years ago.

For many of the miRNAs included in the study, Fay saw similar patterns of miRNA tissue specificity in the other marine species as she had seen in *Platynereis*. This suggests that these regulatory RNAs fulfill a similar role today as they did hundreds of millions of years ago in the common ancestor of these animals. "It seems that the evolution of miRNA and tissue identities are closely coupled," remarks Detlev.

Overall, miRNA expression patterns in the musculature, gut and nervous system are the most conserved, the researchers discovered. "In all bilaterians we found the same set of three miRNAs in cells that form the hair-like cilia that propel the animals forward," says Fay. "And we found the oldest known family of animal miRNAs – miR-100, miR-125 and let-7 – encircling the gut of each of these animals, where they are thought to have a role in developmental timing," she notes.

Detlev adds that "let-7 expression is perfectly timed with the transition of the worm from its immature free-swimming larval stage into its sedentary adult stage." Let-7 could therefore be important for regulating the timing of transitions towards the later stages of development, a suggestion that is supported by other studies of let-7's role in the development of molluscs, flies and zebrafish. "This is an important find as it demonstrates how miRNAs, which are responsible for regulating the developmental timing of one tissue, can keep their ancient roles even when body plans get more complex," remarks Detlev.

This gives clues to how tissues specialise and build complex structures within animals' bodies that help them to adapt to new environments, especially if they differ considerably from those of their ancestors. "What is interesting is that animals on different branches of the evolutionary tree adapt in very different ways, but keep an ancient set of tissues and cell types

that they re-shape according to the needs of their new environment. In some animals like insects these old components are sometimes modified beyond recognition, while in others, like the ancient-looking marine species, they are quite easy to distinguish," explains Detlev.

In addition, the pattern of miRNAs in the brain allowed the researchers to deduce that worms and humans share some miRNAs that are specific to the ancient parts of the central nervous system that secrete hormones into the blood. This offers a solution to the ongoing dispute over whether the last common ancestor of all bilaterians had a brain at all.

Detlev explains that the position of the central nervous system differs between invertebrates and vertebrates. "The brain and nerve cord are on the underside of invertebrates, while in vertebrates they are found towards the back," he explains. In the past, these observations convinced many scientists that the common ancestor of vertebrates and invertebrates was brainless, and that the nervous systems had evolved independently in both lineages. "But if it had, you would not expect the same miRNA in identical regions within the brains and nerve cords of descendants from different branches," explains Detlev. And this is exactly what the EMBL researchers found.

This example demonstrates how these findings are helping to establish miRNAs as an important new tool for reconstructing ancient animal body plans at important evolutionary junctures.

In future work Detlev's group would like to investigate the regulatory role of each of the conserved miRNAs by interfering with their expression. He explains that when you consider that within the human genome there are almost 700 miRNAs, which affect the expression of around 30% of the human genome, it is clear that we need to know what they are doing. If scientists can learn to block these tiny RNAs, or mimic their effects, they could use them to develop new treatments for cancer, help repair damaged organs and slow the process of ageing. But for now, the contribution of a handful of marine-dwelling creatures to scientists' understanding of how miRNA regulate gene expression should at the very least instil intrigue into the already colourful world of rock pools.

Christodoulou F, Raible F, Tomer R, Simakov, O, Trachana K, Klaus S, Snyman H, Hannon GJ, Bork P, Arendt D (2010) Ancient animal microRNAs and the evolution of tissue identity. *Nature* **463**: 1084-1088