When a gene is worth 2

March 22 2013

The notion that each gene can only codify for a single protein has been challenged for some years. Yet, the functional outcomes that may result from genes encoding more than one protein are still largely unknown.

Now, in a study published in the latest issue of the *Plant Cell* journal, a group of scientists led by Paula Duque at the Instituto Gulbenkian de Ciência (IGC, Portugal) discovered a gene – ZIFL1 – that has the particularity of producing two different proteins with completely distinct locations and functions in the plant. The researchers observed that in the root ZIFL1 codifies a protein that is important for the transport of auxin, a hormone essential for the correct growth and development of the plant. However, in the leaves the same gene originates a protein that promotes tolerance to drought. The gene presented in this study is one of the few identified to produce two proteins with such different biological roles.

ZIFL1 belongs to a family of transporter genes known to be present in all classes of organisms, but the functional role of most of its members remains unknown. What is known is that these transporter genes encode proteins that are integrated into cell membranes and act by allowing the passage of small molecules across them. By undergoing genetic and cell biology studies in the plant model *Arabidopsis thaliana*, Paula Duque's team was able to study the role of the ZIFL1 gene.

What surprised the scientists was that mutant plants unable to produce the ZIFL1 transporter presented specific defects in different organs and functions. On one hand, their roots exhibited problems of growth, ramification and orientation when compared to normal plants. These observations suggested that the ZIFL1 gene was involved in the transport of the auxin hormone, which plays an important role in the development of the root. But the researchers also found out that the mutant plants had problems in tolerating drought. They realized that the leaf pores that regulate transpiration – the stomata – were more open in the mutants than in normal plants, resulting in the loss of higher quantities of water. This suggested a role for ZIFL1 in the closure of stomata and in the control of water loss by the plant, which can be critical under drought conditions.

Intrigued by these observations, the researchers investigated whether the ZIFL1 gene could be originating two proteins that would act differently in distinct tissues. Alternative splicing is a key mechanism allowing the same gene to produce multiple proteins. When genes are activated to give rise to proteins, they first originate an intermediate molecule of RNA that can be processed differently, with some parts being removed. This cut and paste process may originate different RNA molecules that can then be converted into different proteins. Estelle Remy, investigator at Duque's laboratory and first author of this work, observed that in the case of the ZIFL1 gene, alternative splicing originates two RNA molecules that differ in just two chemical residues. However, this small difference has a huge impact on the proteins that are generated, with one of them being shortened by 67 amino acids. In collaboration with Isabel Sá-Correia's group at Instituto Superior Técnico, the researchers then tested the activity of the two proteins in yeast cells and found that both transport potassium ions.

Having different size but similar transport activity, Estelle looked for the reason why these two proteins had such distinct biological functions. Surprisingly, she observed that root tissues only present the longer form of the protein, whereas the shorter protein can only be found in the leaves. Furthermore, the location of these two proteins also differs inside the cells of the root and leaves, being integrated into different cell membranes. According to Estelle, "the fact that we cannot find both proteins being expressed either in roots or leaves suggests that these tissues may have specific factors that somehow influence the splicing of the ZIFL1 RNA into the form that confers the biological role necessary for that tissue."

Says Paula Duque, "To our knowledge, there are not many known cases of proteins with such different biological functions being codified by the same gene. What is most fascinating is how the inclusion or removal of just two chemical residues in the RNA molecule results in the production of two proteins that play essential roles either in hormone transport or in tolerance to drought."

Alternative splicing is a crucial mechanism to generate protein diversity. In humans, about 20,000 to 25,000 genes codify proteins. However, recent studies indicate that over 90% of these genes undergo alternative splicing, with scientists estimating that there may be up to 500,000 or more different proteins in the human body.

More information: *Plant Cell* doi: 10.1105/tpc.113.110353.

Provided by Instituto Gulbenkian de Ciencia