Size of animals dating back 100-350 million years ago inferred from resurrected proteins

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David De Sancho, Raul Pérez-Jimenez and Aitor Manteca, three of the researchers who participated in the research. Credit: CIC nanoGUNE

Titin is one of the proteins that make up the muscles of all vertebrates; it is an elastic protein that acts as a spring by refolding and returning to its original state. "Protein evolution has been studied from many points of view: its thermal stability, function and structure, but no one had ever studied the evolution of the mechanical properties of a protein. For titin this is a particularly appropriate approach given its function," said Pérez-Jiménez.

For this research, they selected over thirty animals from different taxonomic groups and of different sizes. "The complete genome of many animals was already available, so the first thing we did was to build a phylogenetic tree with the titin sequences of around thirty tetrapods. This tree enabled us to calculate the most probable sequences of the protein titin of four common ancestors of the taxonomic groups to which these animals belong: placental mammals, dating back about 100 million years; all mammals, dating back 170-180 million years; the common ancestor of the sauropsids, including all birds, reptiles and also dinosaurs, and which lived about 280 million years ago; and the common ancestor of all the animals we have studied, which would be the common ancestor of the tetrapods, dating back about 350 million years," said Pérez-Jiménez.

Once they had the sequences, they synthesised the most elastic fragment of the proteins in the laboratory, and using an atomic force microscope available at nanoGUNE, they were able to measure the mechanical resistance of each of the proteins. "This instrument allows us, literally, to take a protein and stretch it, unfold it mechanically using force, which is something similar to what happens to titin in the muscle," said the researcher. They were able to compare the resistance or stability of all the titins being studied. "In this study, we realised that the mechanochemical stability of the proteins depended on the number of disulphide bridges displayed by the titin, which are sulphur-sulphur bonds between two cysteine residues."

They were able to see that the ancestral proteins were more resistant than those of today's animals, and they had more disulfide bridges than the modern ones. "Yet this difference was not so big compared with a small animal such as a finch." This fact led them to think that there could be a link between the mechanochemical properties of titin and the size of the animals. "We saw a pretty good correlation: The larger animals had less stable proteins and the smaller ones more stable proteins. And that enabled us to predict the size of the ancestral animals."

Once they had inferred the size of the common ancestors, the group compared them with fossil records and the scientific literature available in this respect. "We were able to see that there was substantial agreement; the ancestors of mammals, birds and tetrapods in general were really small, weighing less than 100 g; although we do, of course, have a margin of error inherent in the techniques themselves. This may not be surprising, because one could consider that it is information that was already known, but what is new here is that we did not use a fossil, but started from a reconstructed protein, a purely molecular piece of information," said the researcher.

"The interesting thing is that we have how the titin gradually changed throughout evolution, and we have been able to reconstruct it. We would like to see, for example, whether this correlation with size is truly global, whether it exists in all animal groups," he said.

More information: Aitor Manteca et al. Mechanochemical evolution of the giant muscle protein titin as inferred from resurrected proteins, *Nature Structural & Molecular Biology* (2017). DOI: 10.1038/nsmb.3426

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