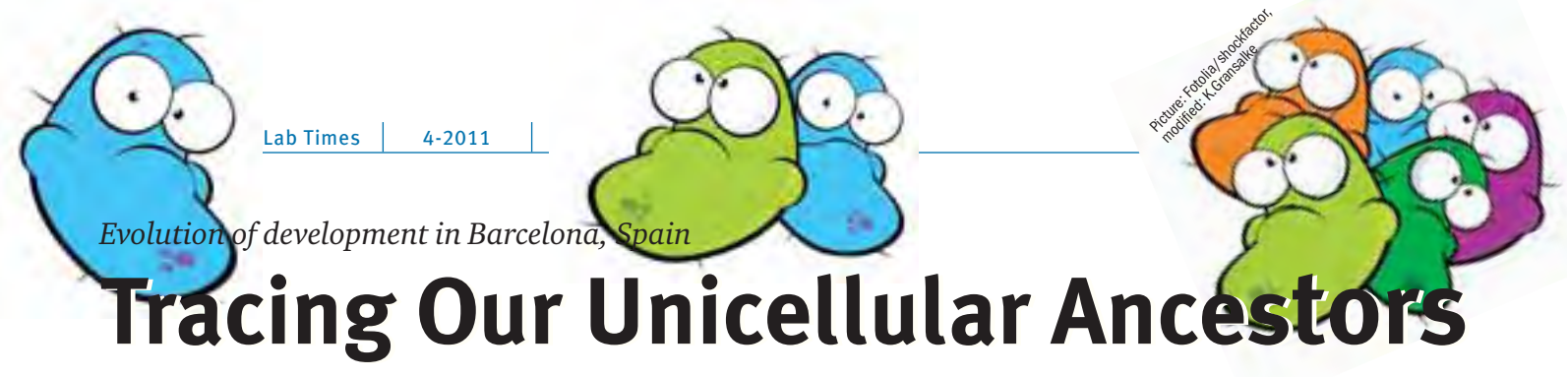


Evolution of development in Barcelona, Spain

Tracing Our Unicellular Ancestors

It was one of the 'major transitions' in evolution: the origin of multicellularity. Iñaki Ruiz-Trillo's lab, together with an international team, is starting to unravel how single-celled ancestors once might have evolved into multicellular organisms.

Which critical events of the ancient past determine our existence today? Of course, you might immediately think about your mother giving birth to you but if you go even further back in time you are sure to find some episodes in the history of life that define not only you but *all* the biodiversity there is today.

Iñaki Ruiz-Trillo, currently group leader in the Department of Genetics at the 'Universitat de Barcelona', has always been interested in these evolutionary transitions. After finishing his PhD, he particularly became more curious about the origins of multicellular organisms. So he moved to Halifax, Canada, to start a postdoc in the Andrew Roger laboratory, where his adventures deciphering the unicell-to-multicell transition began.

UNICORN research

While Ruiz-Trillo was still in Canada, various laboratories from all over the world, including the one in which he was working, came up with an interesting proposal. Scientists from Montreal, Halifax, Oxford (UK) and Berkeley, all concerned with the transition to multicellularity from unicellular ancestors, proposed a 'multi-taxon genome-sequencing initiative' named UNICORN (unicellular opisthokont research). The Opisthokonta forms a eukaryotic supergroup comprising animal (Metazoa), fungi and other unicellular organisms related to each and both of them. The purpose of the initiative was to focus on the unicellular opisthokonts and sequence their genomes, in order to compare the gene contents between unicellular and multicellular species. The results, so UNICORN's rationale, should yield further insights into how multicellularity evolved – once in Metazoa and possibly many times in Fungi.

Before this initiative started, the only unicellular organism closely related to animals and whose genome had been completely sequenced was the choanoflagellate *Monosiga brevicollis*, a free-living eukaryote that resembles the choanocytes of sponges. But UNICORN set forth the necessity to study other opisthokonts. Ruiz-Trillo explains that they chose the organisms from their list based on their phylogenetic relevance and cultivability, "There are very few unicellular organisms closely related to animals that can be grown in a lab and from which we are able to extract enough DNA to make a genome – so there was not much choice." In the end, the final set included unicellular organisms closely related to either animals or fungi, some fungal groups, and, in addition, one species belonging to the phylum Apusozoa, protozoic flagellates, which probably are the closest relatives to the opisthokonts.

The initiative was accepted for funding by the US National Human Genome Research Institute (NHGRI) and The Broad Institute, based in Harvard/USA, became the genome centre in charge of the sequencing. The analysis of every piece of data produced by the ambitious UNICORN project is currently being worked out by its many proponents – and Ruiz-Trillo is still part of it, now as a research group leader in Barcelona. More than that, the project has just started to shed some light on the transition to multicellularity.

Seven multicellular organisms, including Ruiz-Trillo, second from right.

Unicellular cousins

One of the unicellular species chosen for sequencing was *Capsaspora owczarzaki,* an amoeboid symbiont living inside a freshwater snail. Phylogenetic studies suggested that it is the closest relative to choanoflagellates and animals, so Ruiz-Trillo and colleagues looked into its genome to search for genes relevant to the metazoan lineage. Their first results have recently been published and, as the title of their paper "Unexpected Repertoire of Metazoan Transcription Factors in the Unicellular Holozoan *Capsaspora owczarzaki*" suggests, the outcome was certainly surprising (*Mol. Biol. Evol.* 28(3): 1241-54).

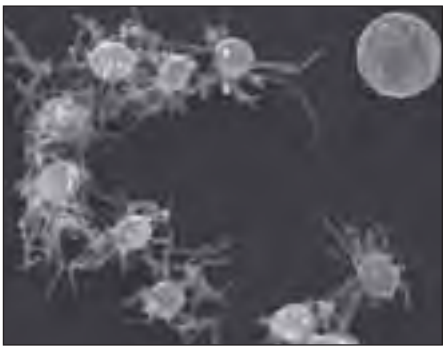
Most important times in one's life

Ruiz-Trillo and co. identified *C. owczarzaki* genes coding for homologues of three transcriptional factors (TFs), previously considered to be exclusively present in animals: Runx, T-box and NF-kappaB, all of them involved in metazoan development and various other associated mechanisms. The NF-kappaB family, for example, plays an additional role in processes of the animal immune system, while the Runx family has the task of activating or repressing the transcription of genes related to diverse developmental pathways.

Even more striking was the identification of a well-conserved T-box homologue to *Brachyury*. This TF is known to control gene expression during gastrulation, one of the most important events in early embryonic development of most animals. Lewis Wolpert, a renowned developmental biologist, once rather humorously declared that "*it's not birth, marriage, or death, but gastrulation which is truly the most important time in your life*". Indeed, during this phase the embryo undergoes dramatic changes in form and at the same time it is also the period where, in most animals, the gut is formed. But what is a *Brachyury* homologue doing in a unicellular protist? Or... what are *all* those TFs doing there?

Difficult question. Ruiz-Trillo confesses that they don't have any idea, yet but they are currently working to understand

the roles of those proteins in a unicellular context. Arnau Sebé-Pedrós, one of the first authors of the paper and a PhD student in Ruiz-Trillo's lab, explains that, before starting their work with *C. owczarzaki*, its life cycle was basically unknown. Now, one of the priorities clearly is to draw a better picture of this eukaryote's biology.



Study object *Capsaspora owczarzaki* with and without filopodia.

As developmental processes involve cell differentiation, one might think that these TFs could be playing some role in defining cell types in a temporal, instead of a spatial context. Sebé-Pedrós affirms that they indeed have observed some type of differentiation in *C. owczarzaki*, such as "the formation of resistant cystic cells, alternating with its common phase as an amoeboid adhered to substrate and with multiple filopodia". Alex de Mendoza, the other first author of the paper and also a PhD student with Ruiz-Trillo, comments that they are also performing comparative transcriptomics for the different phases of the *C. owczarzaki* life cycle. This might give them a clue, about which genes are expressed during different periods but then it still remains to be shown, whether these genes are actually regulated by the TFs found. There could also be a simpler explanation: TFs have the function of regulating expression, so the target genes could also be doing something else.

Compact complexity

Apart from looking into the presence or absence of genes, it will therefore also be important to understand how the regulatory networks are wired. Although there is nothing clear yet, de Mendoza believes that the diversity of the TFs present in *C. owczarzaki* genome points, indirectly, to a very complex regulation. But in spite of this apparent complexity, as Sebé-Pedrós affirms, the *C. owczarzaki* genome looks very compact, with only very small intergenic regions that are more similar to other unicellular eukaryotes than to animals. "This, in turn, suggests that the regulation might be not as complex as in animals. Although the quantity of TFs in *C. owczarzaki* is certainly high, we could also think of a simpler regulatory environment with many TFs having simpler downstream networks, which eventually could have gained more complexity later in animal evolution."

He adds that these genes found in *C. owczarzaki* might be telling us something about the origin of the basic elements of gene regulatory networks in Metazoa but probably without a high level of interaction. "We still have to experimentally confirm these assumptions, using ChIP-Seq and other techniques in order to describe the downstream networks of some of the genes observed."

A good environment for *evo-devo*

Hence, a clear hypothesis for explaining the presence of these TFs in this unicellular creature is still lacking. But as unexpected as these findings can be, they do say something about our unicellular ancestors and suggest that multicellularity didn't arise from scratch. Instead, the results, together with other discoveries, related to the study of transitions, have pointed out that, as in many other cases, parts of the genetic machinery that would define the 'new' trait were already present; however, those gene families were apparently less diverse and probably used for something different. Therefore, as Sebé-Pedrós suggests, "The crux of the matter now is to identify the functions of these genes in their unicellular context, i.e. their 'original' function, and then describe the history of their co-option for other functions in animals. Or, more shortly, how they evolved from one function to the other."

And apparently, Barcelona is a great place to do this. Ruiz-Trillo's lab is part of a group in the Department of Genetics at the 'Universitat de Barcelona' which is completely focussed on studying the evolution of development (evo-devo). Sebé-Pedrós believes that this university is indeed the only one in Spain with such a diversity of groups working on this kind of problems. He explains that, even though the research lines among all labs in this group might be different, they all do work and discuss together under the evo-devo framework.

Therefore, it wouldn't be a great surprise if we were soon to hear about more findings related to our very early ancestors, coming out of this vibrant city.

Alejandra Manjarrez