

**Uncovering the secret life of the protein gnomes in our cells**

*As a child, have you ever been told stories of little gnomes, trolls or fairies that live in the hidden? Maybe sometimes things randomly appeared or disappeared and you thought it was by the action of one of these omnipresent but invisible little creatures? Once we grow older, we often stop believing in the world of gnomes. However, several scientists around the world revisited these thoughts and are now setting out to discover the secret world of gnomes, more specifically of protein gnomes. They identified a new type of proteins, which are really small and contribute to the functioning (and thus likely also disease) of the cells in our body. While researchers have hints that thousands of these small protein gnomes, called microproteins, might exist, only few are known at the moment. The rest are hidden to the eye and are waiting to be discovered— in a hunt for the hidden microproteins in our cells!*

Our body is made up of trillions of cells, the proper functioning of which is vital for us to stay healthy. One of the key players in the cell are proteins. Proteins are long chains of amino acids, encoded by the genetic material, the DNA. If we imagine the cell as a city, proteins are the workers that ensure the city is functioning— builders, teachers, policemen, doctors. The DNA is the blueprint for making the workers. We know many of these protein workers and how exactly they function. This means that if one of them is unwell and makes the cell sick, we might be able to help by giving the right medicine— if the baker cannot bake bread because his oven is broken, we fix it so the city does not starve.

However, next to the known protein workers, there also seem to be many unknown workers— the protein gnomes, so-called microproteins! They have largely been missed until now because they are really small (maximally a hundred amino acids) compared to ‘standard’ proteins (which often contain several hundreds of amino acids). Thus, they frequently escaped detection by the usual protein discovery methods and only very few microprotein examples are known. Some of these however, were shown to be important for example in processes like heart development or fertilization. In recent years and through refined technologies, researchers finally started to notice many more protein gnome footprints in the city, and found many hidden blueprints in the DNA, suggesting that there are potentially thousands of tiny so-far invisible gnomes helping, along with the regular workers, to keep the city running! Thus, scientists now want to uncover the secret life of these gnomes and make them visible: firstly, to understand cellular function but in the future, potentially also for new angles to therapy— since of course, first you need to know the baker exists, before you can fix his oven!

So how does one expose the functions of thousands of potentially existing protein gnomes? Scientists are sending the gnome candidates on vacation, one by one, and then assess any malfunction in the city. For example, maybe suddenly trash accumulates everywhere, then the gnome likely was a bin collector. For this, the researchers make use of a technology called CRISPR/Cas9. This method allows for creating small, targeted cuts in the DNA (the city blueprint), which disturb production of a single small protein within a cell (gnome goes on vacation). The researchers then test for defects in cells that are now missing the protein gnome (trash everywhere). The amazing thing about the CRISPR/Cas9 method is that one cannot just disturb one, but thousands of proteins simultaneously- one in each cell- to separate out their function. This means that one can screen thousands of gnomes in various cities at the same time, one gnome per city, in order to check if they are involved in a certain task. While this has previously been done to describe in more detail the functions of known, larger proteins, only a few microprotein screens have been carried out before. A research team at SciLifeLab is performing one of them and has set out to discover microproteins that are important for cell survival— uncovering gnomes who are so vital for city life that if you send them on vacation, the city will eventually die out.

Indeed, after a few months of screening, the researchers found various microproteins which seem to be essential for the cell to live. Now the scientists are wanting to validate these findings to ensure that they have not accidentally sent a regular worker rather than a protein gnome on vacation. Subsequently, the researchers want to test which cellular pathway the microprotein functions in. This can be done, for example, by recording which known workers the protein gnome meets on a regular basis— if you find a gnome interacting with millers, milk men, bakers and fika lovers Monday to Friday, it is likely that your protein gnome is in the baking business!

While the SciLifeLab team so far was looking for gnomes ensuring city survival, one can take the same approach to identify microproteins with different roles— the baker, builder and police gnomes of the city. Thus, scientists hope that this is just a starting point for identifying many more important microproteins and characterizing their function. This will help us in better understanding how cells work, but might potentially also aid in developing new therapies in the more distant future. Because of course, once you identify a gnome, you also can help him get better once he runs into trouble.