Gene function is regulated on several levels. Among others, it depends on the spatial order of chromatin in the cell nucleus. Chromosome occupy specific territories in the nucleus and can be further divided into compartments A (euchromatic; transcriptionally active) and B (heterochromatic, inactive). On a lower level, topologically associated domains and gene loops can be determined. The correct structure of those domains is essential for the optimal gene expression determining the proper function of cells and organisms. Knowledge on the 3D chromatin structure was gained mostly in humans and animals. It is known that proteins belonging to the CTCF family are crucial for the maintenance of the domain structure. However, those proteins are absent from plants and no functionally similar proteins have been identified in plants, while plant chromatin is also spatially organized. Knowledge on such organization is currently limited to model plant species, thus it has been postulated to broaden the research and include non-model agriculturally important species.

Mobile genetics elements, commonly referred to as “jumping genes”, are defined as DNA segments capable of changing their chromosomal localization in a process named ‘transposition’. They can be mobilized in certain conditions, giving rise to novel genetic variability. Every such change (mutation) can significantly affect functioning of the host genome. It is assumed that most transposition events are neutral or deleterious, however there is a number of reports on advantageous transposition events, e.g. leading to better adaptation to stress conditions or emergence of traits enabling crop domestication or increasing the agronomic value of crops. As mobile elements form families comprising many similar copies scattered across the host genome, they are also described as dispersed repetitive DNA. Thus, if certain families can modify physical conformation of chromatin, the effect would be genome-wide. There are reports on the enrichment of particular groups of mobile elements in some spatial structures. In plants, it was shown that miniature inverted-repeat transposable elements (MITEs) are enriched near domain boundaries, however the extent to which they are engaged in the formation of such domains is not recognized. Also, it is not known if they interact with any proteins involved in the process of domain formation.

We are going to investigate the 3D organization of the carrot genome. Carrot is a vegetable crop of high agricultural significance. 3D organization of its genome will be characterized by means of Hi-C technology based on high-throughput DNA sequencing of physically interacting regions staying is spatial proximity. The Hi-C map allows identification of topologically associated domains and their boundaries, followed by the identification of MITE copies residing close to those boundaries. As Hi-C maps for few carrot genomes differing in terms of the distribution of MITE copies will be developed, it will be possible to verify the hypothesis on the global significance of those elements to the 3D structure of the nuclear chromatin. In parallel, we will search for proteins binding to DNA motifs attributed to MITE families enriched around the domain boundaries. We will use a yeast system in which a gene governing antibiotic resistance (allowing yeast to survive on the medium supplemented with the antibiotic) will be put under control of the MITE element. In that way, only those yeast cells in which a protein binding to a DNA motif in the MITE would become antibiotic-resistant, which in turn allows identification of the carrot gene encoding that protein. As MITEs are usually rich in A/T nucleotides, we are particularly interested in proteins binding to AT-rich motifs.

Novel knowledge on the 3D structure of the carrot genome, identification of proteins binding to MITEs and the evaluation of the global effect of the localization of MITE copies in the genome on the spatial conformation of chromatin will be the final results of the project.