Zusammenfassung

Der Ursprung der Entwicklung der Mehrzelligkeit ist immer noch weitgehend unbekannt. Allgemein wird angenommen, dass sich aus einem Choanoflagellaten- ähnlichen Organismus innerhalb der Opisthokonta das mehrzellige Leben entwickelt hat. Bereits vor 150 Jahren entdeckten Forscher die Ähnlichkeit zwischen Choanozyten (Schwämme) und Choanoflagellaten. Die Verbreitung von einzelligen Organismen könnte zur Artbildung geführt haben und ist daher ein wichtiges Untersuchungsgebiet. Deshalb wurde in dieser Arbeit im ersten Kapitel die Populationsgenetik von Schwämmen im Süßwasser (Sieg) untersucht, um Rückschlüsse auf die Verbreitungsmechanismen zu erhalten. Die Analyse von 11 Mikrosatellien- Sequenzen von *Ephydatia fluviatilis* ergab, dass es sich um eine passive Verbreitung flussabwärts handelt. Dabei wurde ein signifikanter Mikrosatelliten- Polymorphismus zwischen den verschiedenen Populationen im Flusslauf gefunden und das sogenannte "isolation-by-distance"- Muster nachgewiesen. Schwämme wurden hier als Modellorganismen für die Verbreitung primitiver Opisthokonta im Freiland herangezogen, da sie morphologisch einfach zu erkennen sind und das Sammeln im Flusslauf möglich ist.

Für die Laborversuche wurden Choanoflagellaten herangezogen, die eine kurze Reproduktionsdauer haben und damit sehr gute Modellorganismen für die weiteren Versuche darstellen.

Im zweiten Kapitel wurde eine neue Methode zur Transfektion von Choanoflagellaten mittels "Cell-penetrating peptides" entwickelt, da bis heute keine Möglichkeit bestand, die Funktion von Genen mittels Deaktivierung (genesilencing) durch siRNA zu überprüfen. Die neuentwickelte Methode wurde an einem Gen getestet, das an der Ausbildung der Lorica, einem Gehäuse aus feinen Silikatstäben, wesentlich beteiligt ist. Die Unterdrückung dieses Gens bewirkt, dass die nächste Generation keine Hülle besitzt, der Nachweis erfolgte mittels qPCR und Mikroskopie. Es handelt sich hierbei um eine hocheffiziente Methode mit geringer Toxizität, die es in Zukunft ermöglicht, andere Kandidatengene, die für die Entwicklung von Mehrzelligkeit verantwortlich sind, zu überprüfen.

Die Vorraussetzung für die Geninaktivierung mittels siRNA ist das Vorhandensein des

miRNA- und RNAi- Signalwegs. In den Genomdaten von zwei Choanoflagellaten, *Salpingoeca rosetta* und *Monosiga brevicollis*, wurden bisher keine Komponenten nachgewiesen. Im dritten Kapitel wurden in Transkriptomdaten von zwei Choanoflagellaten und einer *Ministera*-Art eine Dicer- ähnliche Helikase und Exportin-5 nachgewiesen, beides wichtige Bestandteile des untersuchten Signalwegs. Damit konnte gezeigt werden, dass die Geninaktivierung mittels siRNA auch bei Choanoflagellaten eine praktisch anwendbare Methode ist und in Zukunft die Evolution der Mehrzelligkeit an Choanoflagellaten als Modellorganismen untersucht werden kann.

Abstract

The origin of multicellularity is still under discussion. Current opinion is that within the Opisthokonta, metazoans evolved from a choanoflagellate-like ancestor. The striking similarity between choanocytes of sponges and choanoflagellates and the ability of choanoflagellates to form colonies lead already almost 150 years ago to the conclusion that there is a close relationship. Up to now, little is known about the reasons for the step from unicellular organism to metazoans. Another blank space in our knowledge is concerning the distribution and as a consequence the speciation of unicellular organisms like choanoflagellates. This mechanism might have played an essential role in the evolution of metazoans.

Considering the arguments given above and the fact that sponges and choanoflagellates both play a crucial role in aquatic ecosystem, the thesis focused first on the population genetics of freshwater sponges (**Chapter 1**) to elucidate the distribution and dispersal of primitive metazoans, closely related to choanoflagellates. Sponges were selected as, compared to choanoflagellates, different populations can easily be collected in the field. In addition, marker genes for population genetic studies are available, in contrast to choanoflagellates. The hypothesis for this part of the thesis was that, there is an inactive dispersal upstream of this non-motile organism. The result allows to gain insight into possible distribution mechanisms and to study if the anthropogenic influence would cause a uniform distribution of population structures. We assessed data on distribution patterns of freshwater sponges to study the connectivity of genotypes of *Ephydatia fluviatilis* in a river system. The genetic structure of *Ephydatia fluviatilis* populations was analyzed with a set of eleven microsatellite loci from seven locations in River-Sieg system in Germany. Besides of *Ephydatia fluviatilis,* we also found three other sponge species (*Ephydatia mülleri, Spongilla lacustris, Eunapius fragilis*). In contrast to our hypothesis, we observed an overall correlation between genetic and geographic distances among populations of this sessile species, which follows a clear isolation-by-distance pattern. A significant microsatellite polymorphism and high levels of genetic divergence between populations (F_{ST}) in upstream reaches were present. These results confirm the hypothesis of an inactive dispersal of non-motile

opisthokont organisms, like sponges but also choanoflagellates. The colonization of a new habitat and the different environmental parameters might have triggered the formation of a colony and hence have given this species a certain advantage to survive and maybe even conquer this habitat. Based on these findings, choanoflagellates as a model for unicellular opisthokonts were studied in the lab to establish a tool in order to verify the function of key genes responsible for the evolution of multicellularity. In the second part of the thesis the focus was on the transfection of choanoflagellates to allow gene silencing of candidate genes. Choanoflagellates are the closest sister group to metazoan and essential for understanding the evolution of multicellularity. Genes regarding of cell adhesion, cell-cell communication and cell signaling are encoded in choanoflagellates and shared with Metazoa. However, up to now the research on the origin of multicellularity is mainly hindered by the lack of a reliable transfection method (**Chapter 2**). The aim of this part was to establish a transfection method based on cell penetrating peptides (CPP) and to silence a gene responsible for a clear morphological characteristic, to prove the method. In a series of experiments with the choanoflagellate *Diaphanoeca grandis* we proof for the first time that silencing target genes in choanoflagellates with siRNA mediated by CPP is a reliable and highly efficient method. We were able to silence the silicon transporter gene (SIT), which is responsible for constructing the lorica (characteristic siliceous basket). Hence the lorica formation of *Diaphanoeca grandis* in the second generation was suppressed efficiently. Cells without lorica (naked) were observed and high gene suppressed efficiency was determined and measured by light microscopy and RT-qPCR. In addition, only low cytotoxic effects of the CPP (NE5-hCT(18-32)-k7) were detected and the internalization of the CPP in other choanoflagellates, for instance, in *Salpingoeca euryoecia* and *Salpingoeca rosetta*, was successful with low cytotoxicity. Our new technique allows scientists in future to verify the function of genes in choanoflagellates, which are involved in cell adhesion or cell signaling by silencing them based on RNAi machinery. This is a step stone for the research on the evolution of multicellularity.

Finally, in the third part of the thesis the RNAi machinery of choanoflagellates, a prerequisite for the use of siRNA to silence target genes, was studied in **chapter 3**. The presence/absence of core-components of the miRNA and RNAi pathway was analyzed using transcriptome data of one ministeriid species (*Ministeria vibrans*) and two

additional choanoflagellates (*Salpingoeca euryoecia* and *Salpingoeca sp.* HFCC 164), together with the published data (genome and transcriptome) of *Salpingoeca rosetta* and *Monosiga brevicollis*. For the first time, we reported Dicer-like helicase, Class I RNase III and Exportin-5 in choanoflagellates and ministeriids, which were previously believed to have been lost. These findings open up the field of silencing genes by siRNA via RNAi machinery with the technique reported in **chapter 2** and enrich the possibility to study the functions of target genes in other choanoflagellates. Together, these results will be usable for studying the evolution of multicellularity via the model organisms, sponges and choanoflagellates.