Search for small RNAs in *Dictyostelium discoideum*; a bioinformatical approach.

Introduction

The discovery of small functional RNAs (~22 nt) is one of the major scientific breakthroughs the recent years and has revolutionized the way we look at gene regulation (1). These small RNAs, *i.e.* microRNAs (miRNAs) and small interfering RNAs (siRNAs) act post-transcriptionally by binding to target mRNAs and thereby inhibiting translation, either by mediating degradation of their target mRNA or by hybridizing to the 3'UTR and prevent translation by an hitherto unknown mechanism (2). They are also involved in transcriptional control by inducing methylation of DNA or histones and thereby inactivate gene expression. Furthermore, the absence of certain miRNAs has been suggested to be implicated in different diseases, *e.g.* leukemia (3). MicroRNAs are present in animals, *i.e.* metazoans as well as in plants but no miRNAs have been found in unicellular organisms. Small interfering RNAs are more widespread and are found in lower as well as higher eukaryotes where they mainly seem to function as cellular immune systems, protecting the cells from viruses and transposones.

My research group is interested in different aspects of RNA biology and has recently focused on the role of small RNAs in control of development (4). For our studies we use *Dictyostelium discoideum*, a soil-living amoeba that grows as single cells but upon starvation enters a well-defined multicellular developmental program. It carries genes and regulatory pathways of high similarity to metazoans and the annotated sequence of the *Dictyostelium* genome is available in a public database; http://www.dictybase.org (5). This organism is therefore used worldwide to study fundamental cellular processes, *e.g.* cytokinesis, motility, phagocytosis, chemotaxis, and signal transduction.

The project

The aim is to investigate if miRNAs and siRNAs are present in *Dictyostelium*. If we can identify miRNAs in *Dictyostelium*, it will be of great evolutionary interest since this class of RNAs has so far not been identified in single-cell organisms. Furthermore, evolutionary *Dictyostelium* branched out after plants but before animals, hence the presence of miRNAs is likely. We are also interested in the putative role of siRNA in *Dictyostelium*.

In collaboration with Prof. Victor Ambros, one of the "founders" of miRNAs, we have constructed a cDNA library from *Dictyostelium* representing ~22 - 26 nt long RNAs – the size of miRNAs and siRNAs.

The cDNA library consists of 9000 sequences which we now need to analyze. A likely flowchart for the project would look like this:

1) Remove duplicate sequences.

2) Genomic location – are the RNAs derived from intergenic regions or annotated genes (ORFs)? This will indicate their functions as miRNAs or siRNAs, respectively. Can we see clustering of small RNAs, *i.e.* are many generated from a certain gene or class of gene? The identified small RNAs derived from ORFs – are they sense or antisense to the mRNA?

3) It is known from other organisms that miRNAs are generated from longer precursor molecules that have a consensus stem-loop structure. The secondary structure of the putative pre-miRNAs derived from intergenic regions identified in *Dictyostelium* has to be analyzed. This can be done by available folding programs.

The expression and developmental regulation will later be analyzed by Northern blot experiments. Preliminary data where a handful of the RNAs were analyzed indicates that siRNAs are common in *Dictyostelium* and most likely developmentally regulated. The presence of miRNAs has still to be investigated and the finding of this class in *Dictyostelium* would be of major scientific interest.

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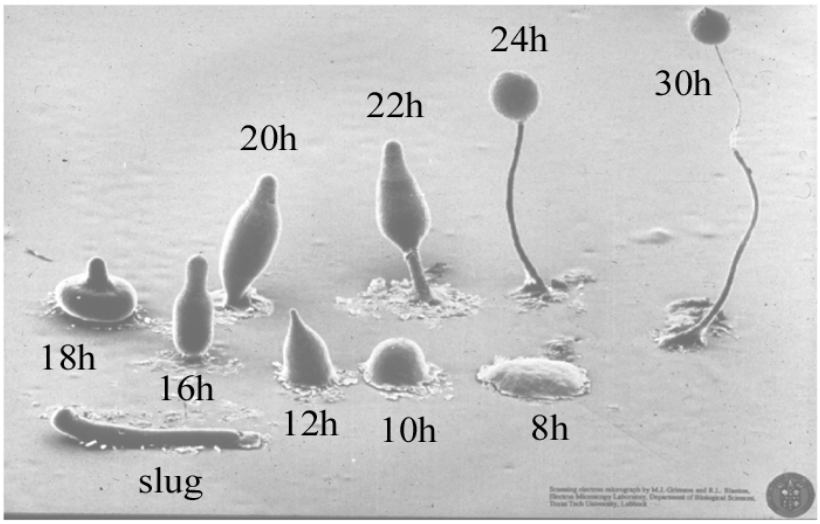

The different developmental stages of *Dictyostelium discoideum*.

References

1. Couzin, J. (2002) *Science*, **298,** 2296-2297.
2. Bartel, D.P. (2004) *Cell*, **116,** 281-297.

3. Calin, G.A., Dumitru, C.D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., Aldler, H., Rattan, S., Keating, M., Rai, K. *et al.* (2002) *Proc Natl Acad Sci U S A*, **99,** 15524-15529.
4. Aspegren, A., Hinas, A., Larsson, P., Larsson, A. and Soderbom, F. (2004) *Nucleic Acids Res*, **32,** 4646-4656.
5. Eichinger, L., Pachebat, J.A., Glockner, G., Rajandream, M.-A., Sucgang, R., Berriman, M., Song, J., Olsen, R., Szafranski, K., Xu, Q. *et al.* (2005), **435,** 43-57.