Small RNA dynamics in cholinergic systems

Dissertation
zur Erlangung des Doktorgrades
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in Frankfurt am Main

von
Sebastian Lobentanzer
aus Schlüchtern

Frankfurt 2019

Small RNA dynamics in cholinergic systems

Abstract

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Contents

This is the dedication.

»Ever tried. Ever failed. No matter.
Try again. Fail again.
Fail better.«

Simon Beckett

Acknowledgments

Thanks are due, for every scientist is not only standing on the shoulders of giants, but also on those of very real persons, without whom this dissertation would not have been possible. consectetuer adipiscing elit. Morbi commodo, ipsum sed pharetra gravida, orci magna rhoncus neque, id pulvinar odio lorem non turpis. Nullam sit amet enim. Suspendisse id velit vitae ligula volutpat condimentum. Aliquam erat volutpat. Sed quis velit. Nulla facilisi. Nulla libero. Vivamus pharetra posuere sapien. Nam consectetuer. Sed aliquam, nunc eget euismod ullamcorper, lectus nunc ullamcorper orci, fermentum bibendum enim nibh eget ipsum. Donec porttitor ligula eu dolor. Maecenas vitae nulla consequat libero cursus venenatis. Nam magna enim, accumsan eu, blandit sed, blandit a, eros.

0 Introduction

0.1 Cholinergic Systems

Nary a process in the mammalian body can commence without participation of cholinergic systems. Acetytcholine was chemically described by Henry Dale more than 100 years ago (Dale, H.H. (1914) J. Pharmacol., 6 147). A short time later, Otto Loewi published the first proof of signal transmission by small molecules: he transferred physiological solutions from electrically stimulated frog hearts to naive hearts and observed their reactions; the solution that provoked a parasympathetic response he proposed to contain a "parasympathetic substance" (Loewi, O (1921) Pflügers Arch. Ges. Physiol., 189 239). Finally, in 1929, Henry Dale completed the picture by isolating acetylcholine from mammalian tissue and identifying it as the molecule responsible for the parasympathetic response (Dale H.H. and Dudley, H.W. (1929) J. Physiol., 68 97). Their joint effort in "Discoveries in Chemical Transmission of Nerve Impulses" was rewarded with the Nobel Prize in Physiology or Medicine in 1936.

Although we have learned much about cholinergic systems in these past 100 years, our understanding of the mammalian nervous system still is fairly limited. Even when disregarding peripheral nervous systems, the complexity of cholinergic transmission is immense, and a myriad of functions have been attributed to cholinergic circuits in the central nervous system. Central nervous projections of cholinergic fibres were extensively mapped by M. Marsel Mesulam and others in the 1990s, with a majority of long projection neurons originating in one of the eight cholinergic nuclei, Ch1-Ch8. While many of these anatomical structures have been filled with meaning by associations with both rudimentary as well as higher brain functions, there are still as many cholinergic pathways whose function is entirely unclear (Figure 1, from XXX).

This holds particularly true for the only recently discovered cortical cholinergic interneurons, which, in comparison to their projecting counterparts, are very small and numerically vastly inferior to other

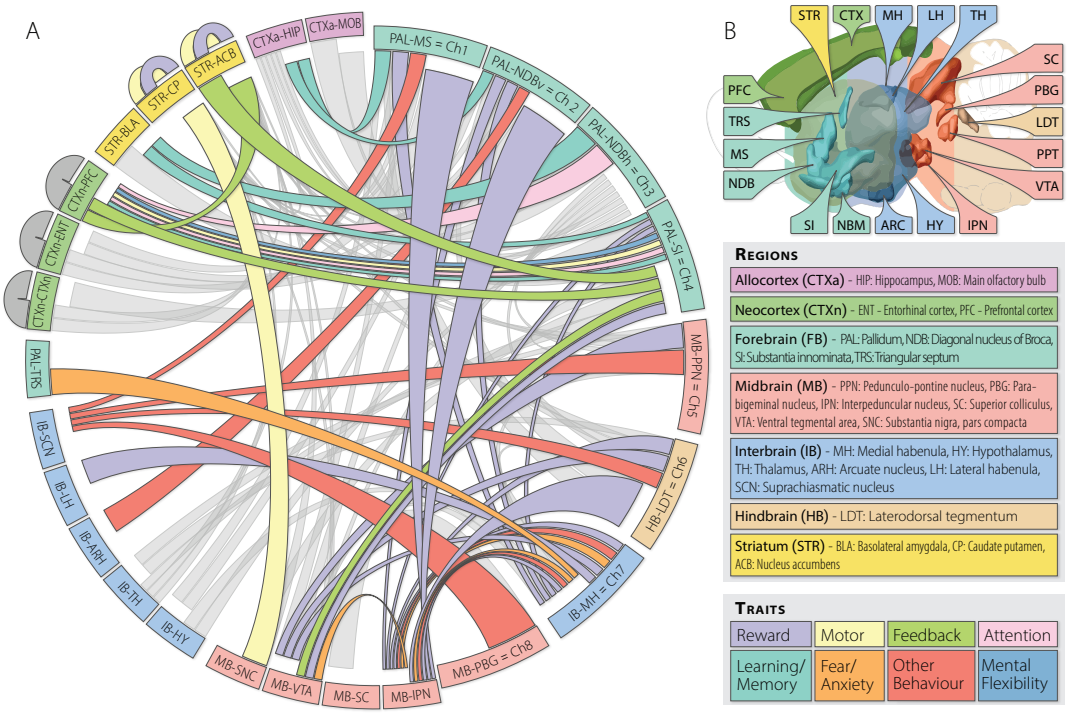

Figure 1: This is a figure that floats inline and here is its caption.

neuron types in the cortex. Thus, their detection and analysis with current methods is challenging.

0.2 Transcriptional Connectomics

No matter their location, cholinergic neurons are defined by their ability to synthesize ACh and release it to neighboring cells to a certain effect. To fulfil this capacity, two particular proteins are essential: the choline acetyltransferase (ChAT) to synthesize ACh from choline and acetyl-CoA, and the vesicular acetylcholine transporter (vAChT, also known as SLC18A3), which concentrates ACh in vesicles for later release. A notable genetic feature connects these two proteins beyond their functional association: the small *SLC18A3* gene (size XXX) sits inside the first intron of the *CHAT* gene and thus is already included in its primary transcript, and is subject to the *CHAT* promoter. However, oftentimes the (mature) transcript levels of *CHAT* and *SLC18A3* mRNA seem to be independently regulated; from the perspective of the organism, the possibility of differential regulation between these two genes makes sense. Since *SLC18A3* does not possess its own promoter, this differential regulation has to be conveyed epigenetically. This dissertation deals in large parts with approaches aiming to decipher these interactions; and while its primary topic revolves around cholinergic systems, the methods described in the following are designed to be applicable to the entirety of the genome/epigenome. Four particular types of cellular actors are subject of these methods and therefore will be briefly introduced: genes in the classical sense as the conveyors of cellular function by encoding for proteins; transcription factors, a special kind of protein coding gene that is able to regulate the expression of other genes; microRNAs (miRs), a class

of small non-coding RNA that has been known for approximately two decades and is reasonably well described in terms of function and mechanisms; and transfer RNA fragments (tRFs), a second class of small non-coding RNA that has only recently been rediscovered and is significantly less well described regarding its functionality.

0.2.1 Nested Multimodal Transcriptional Interactions - The Need for Connectomics

Complex tissues - complex function

0.2.2 Transcription Factors

history, Marbach

0.2.3 microRNA

biological background

0.2.4 Transfer RNA Fragments

novelty

0.3 Neurokines

McManaman, CDF Neuronal activity is profoundly modified by cytokines Li Gan - NFkB activation by tau - Ikkbeta knockout corrects STAT1 DE Oleg Butovsky - TGFbeta as master glia regulator

Four particular types of cellular actors are subject of these methods and therefore will be briefly introduced: genes in the classical sense as the conveyors of cellular function by encoding for proteins; transcription factors, a special kind of protein coding gene that is able to regulate the expression of other genes; microRNAs (miRs), a class of small non-coding RNA that has been known for approximately two decades and is reasonably well described in terms of function and mechanisms; and transfer RNA fragments (tRFs), a second class of small non-coding RNA that has only recently been rediscovered and is significantly less well described regarding its functionality.

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Creation of a Comprehensive Connectomics Database

(Move since it fits implementation better) The need for bioinformatical support in connectomics is immediately obvious from the sheer multitude of possible interactions between the participating factors. For any biological question to be asked, the effectiveness of the bioinformatical query determines the practicality of the approach. Since computational resources are limited, the database that is queried should be organised in a way that facilitates retrieval of the desired information without excess processing of useless information. In the simple case of only miRNAs interacting with genes in one direction (miRNA to gene), this means retrieval of only those interactions relevant for the queried genes or miRNAs. Traditional table-based approaches such as SQL ("Structured Query Language") cannot provide such an implementation, since individual entries for genes and miRs (rows or columns) have to be accessed in their entirety, whether there is a connection between gene and miRNA (1) or not (0). This results in a computation time of XXX, example?

1.1 Materials

1.1.1 microRNA Interactions

miRWalk2, Diana miRTarBase Prediction aggregation

1.1.2 Gene Annotation

ENSEMBL, NCBI

1.1.3 Transcription Factor Targeting

Marbach

1.1.4 De-novo Prediction of tRF Interaction

Targetscan

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1.2 Implementation

1.2.1 Infrastructure

Neo4j

1.2.2 High-throughput Database Generation

Java

1.2.3 Maintenance and Quality Control

Java/RNeo4j

1.3 Usage

1.4 Statistical Approach to Connectomics

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microRNA Dynamics in Cholinergic Differentiation of Human Neuronal Cells

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2.1 Background? - Intro?

2.2 The Cellular Model

2.3 Small RNA Sequencing and Differential Expression Analysis

2.3.1 microRNA Family Enrichment

2.4 Network Generation

2.5 The Cholinergic/Neurokine Interface

2.6 Application to Schizophrenia and Bipolar Disorder

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Dynamics Between Small and Large RNA in the Blood of Stroke Victims

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Pain?!

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4.1 Background

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5 Discussion

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5.1 Methods

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Conclusion

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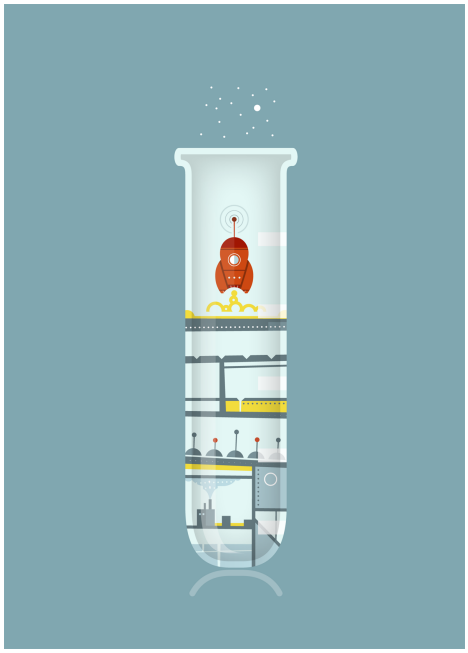

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