RNAi as tool to study molecular mechanisms of metabolic adverse reactions in *Caenorhabditis elegans*

Manuela Hörnle [1)2)], Isabelle Hellwig [1)], Wolfgang P. Kaschka [1)2)] & Steve Hodgkinson [2)]
[1)] Zfp Südwürttemberg, Ravensburg, Germany
[2)] Clinic for Psychiatry I, University Hospital Ulm, Ulm, Germany

Introduction:

The use of second generation antipsychotic (SGA) drugs for the treatment of bipolar disorders has been associated with metabolic adverse reactions. Many studies cite significant weight gain as a common side effect, which is often attributed to dysfunction of glucose homeostasis and the development of dyslipidaemia. This in turn may trigger the early pathogenesis observed for example in type-2-diabetes. Given that factors controlling energy metabolism are largely conserved between mammals and the nematode *Caenorhabditis elegans (C.elegans)*, C. elegans provides a powerful model system to delineate changes in signalling pathways that lead to metabolic disorders.

Methods:

We have established a tissue-specific molecular method (RNA Interference; RNAi) in our lab for controlled and celltype-specific silencing of protein synthesis in *C.elegans.* With this method it is possible to block protein synthesis in specific development stages of the worms. We used different *C.elegans* mutant strains which lack key regulator proteins in the Insulin- and TGF-β-signaling pathways to study the effects of their absence on tissue lipid accumulation and other metabolic markers. A very promising candidate for this metabolic effect is the Daf-3.

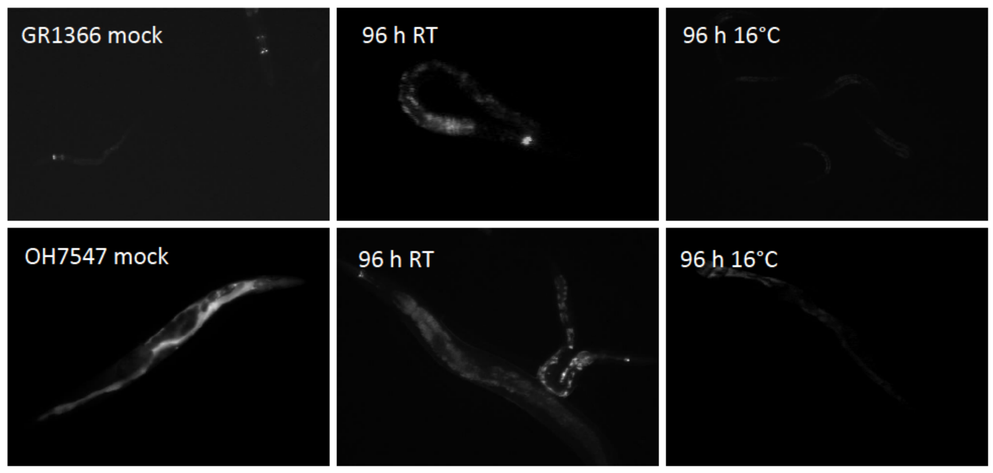

Fig 1: representative images of RNAi-method using an GFP-construct; *C.elegans* was fed with Bacteria carrying a dsRNA-containing vector targeting GFP-sequence. 96h later, in GFP-expressing mutants GFP-intensity was documented by fluorescence- microscopy.
GR1366: (tph::GFP) defective synthesis of serotonin; stains **ADF neurons**
OH7547 (cat::GFP) GFP within all **dopaminergic neurons**

Results:

Using GFP we show the suitability of the RNAi-method for studying the molecular mechanisms at a single-cell-level (figure 1). Further, studies in our lab support the conservation of metabolic signaling pathways between humans and Nematodes as treatment of *C.elegans* with a common antipsychotic drug (Olanzapine) resulted in massive weight gain just as in human patients (data not shown). In addition, visualisation of fat deposits with the lipophilic dye Sudan Black confirmed the role of Daf-3 in storage of fat as animals lacking this protein exhibit around about 20 % less staining compared to wildtype animals (figure 3).

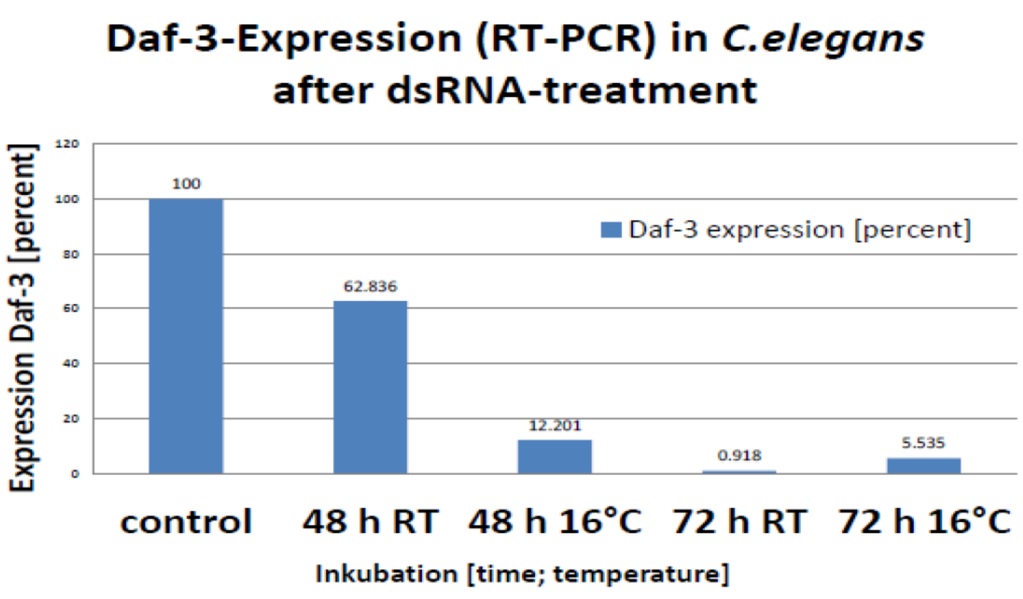

Fig 2: diagram of Daf-3 silencing after dsRNA-feeding specific for daf-3-sequence. *C.elegans* was fed with bacteria containing feeding vector specific for Daf-3-Sequence. 48 h respectively 72 h after incubation at room temperature or at 16°C, daf-3-expression was determined using specific transcription-primers in RT-PCR. Feeding *C.elegans* with the empty backbone vector served as control; Expression level of daf-3 in control was assumed as 100 % expression.

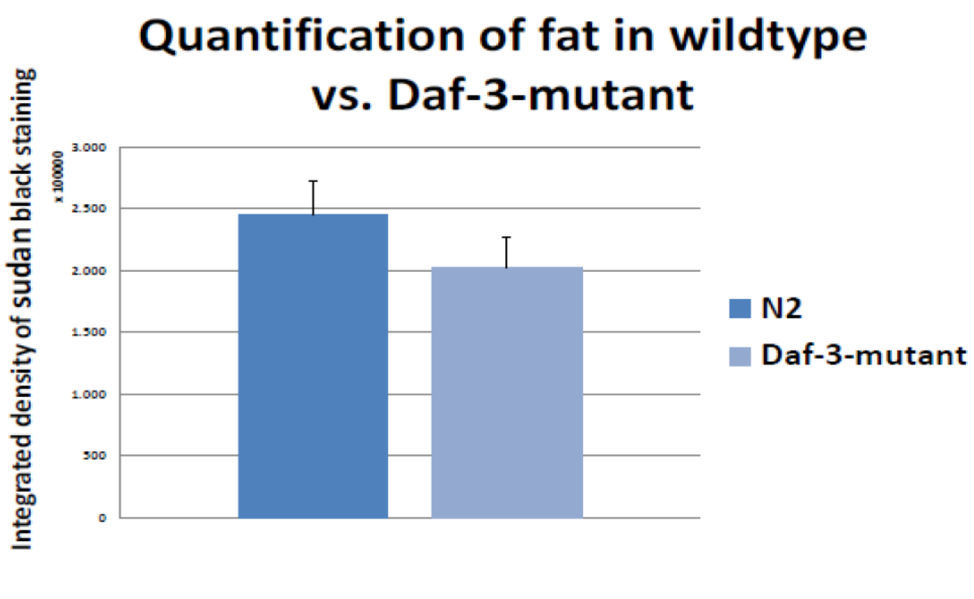

Fig 3: quantification of fat storage in wildtype *C.elegans* compared to a mutant strain lacking Daf-3-protein using Sudan Black-staining. Fat deposits of *C.elegans* wildtype animals (N2) and an Daf-3-lacking mutant strain were stained using the lipophilic dye Sudan Black. After documentation of animals via micoscropy, image analysis was undertaken using ImageJ.

Conclusion:

C.elegans represents a powerful model-system for medical research. Molecular mechanisms leading to excessive storage of fat as a side effect of antipsychotic medication are very complex. Daf-3 does act a part in regulating the storage of fat, but is not the only player. At least two or more proteins operate in concert to cause this metabolic adverse reaction.

ESPE 2015 845--P3
Fat 1
Manuela HORNLE
DOI: 10.3252/pso.eu.54espe.2015
Poster presented at: 54th Annual Meeting 1-3 October ESPE 2015 BARCELONA European Society for Paediatric Endocrinology
PosterSessionOnline.com