Name	FOLKERT VAN WERVEN
Position	Group Leader (2nd 6)
Year joined *(Crick or founder institute)*	2013

Career History

2004 – 2009 - Ph.D. biochemistry
University Medical Center Utrecht (UMCU)/University of Utrecht, Netherlands
2009 – 2013 - Postdoctoral Associate/Fellow
Massachusetts Institute of Technology, Cambridge, USA
David H. Koch Institute for Integrative Cancer Research and Howard Hughes Medical Institute
2013 – 2015 - Junior Group Leader
Cancer Research UK, London Research Institute, London, UK
2015 – 2020 - Junior Group Leader
The Francis Crick Institute, London, UK
2020 - Group Leader
The Francis Crick Institute, London, UK

Major Awards, Honours and Prizes

Membership of external committees, editorial boards, review panels, SABs etc

Lab Name	***Cell fate and gene regulation laboratory***

Research programme and achievements

Understanding how a multicellular organism arises from a single cell is a major challenge in biology. Each cell fate or cell differentiation programme is controlled by multiple regulatory signals that drive changes in gene expression. Dissecting the mechanisms of gene regulation is key to the understanding of development, but also to diseases such as cancer. As such, how gene expression is regulated during cell differentiation remains poorly understood. In my laboratory we use budding yeast and the yeast gametogenesis or sporulation programme as a basic model for deciphering principles of regulating gene expression in general and during cell fate changes. The research can be summarised by three key questions: How do signals integrate and control the promoter of a cell fate regulator? How do noncoding RNAs and alternative mRNA isoforms control gene expression? How does a highly conserved RNA modification, *N6*-methyladenosine (m6A), regulate gene expression? The main findings from this research over the past five years can be summarised as follows:

We have deciphered multiple regulatory mechanisms describing how the promoter of a master regulator controls the decision to enter meiosis. Specifically, we identified key regulatory functions for a cluster of transcription factors, a co-repressor complex and two noncoding RNAs. The regulatory principles identified in this work show features of how enhancers in mammalian cells are controlled. For example, many enhancers are primed for activation, integrate various signals, and multiple transcription factors associate with enhancers.

We found new gene regulatory functions for transcription of noncoding RNAs and mRNA isoforms, and a novel regulatory mechanism that ensures repression of aberrant transcription in intergenic regions. We have developed technology to quantify transcript heterogeneity by measuring transcript start and end site usage, and have demonstrated that changes in transcription start site usage form a major regulatory feature of cell fate transitions in yeast. Our results give new and important insights into how gene expression is controlled when cells transition from one state to another and may shed new light on how gene expression is mis-regulated in diseases such as cancer and neurodevelopment disorders, which display pervasive mis-regulation of alternative transcription start sites and alternative promoters.

We investigated the function of the N6-methyladenosine (m6A) modification in mRNAs. This modification is highly abundant, and present in eukaryotes from yeast to mammals, and it alters the fate of transcripts in various ways: it can stimulate turnover of transcripts, promote translation and RNA splicing, alter transcript localisation, and more. The m6A mark also has essential functions in development; for example, the m6A writer and reader machinery is critical for stem cell differentiation and oocyte development. We developed a research programme to dissect the functions and regulation of the m6A modification in mRNAs in yeast, and have developed various techniques to measure the abundance and regulation of the m6A mark. We have identified a conserved protein that associates with the m6A mark, which we are currently studying further. The work will be the starting point for several new investigations, and the outcome from the research will provide important new insights on the function of the m6A mark and the highly conserved m6A machinery.

My future research programme will centre on two questions:

1) How does transcript heterogeneity shape gene expression? We propose that a primary function for transcript heterogeneity is to regulate and tune the transcription of main protein-coding mRNA isoforms often through transcription coupled chromatin changes. A long term aim of my laboratory is to identify the regulatory principles required for regulation of gene

expression via these alternative transcription events. The results from our studies will reveal how transcript heterogeneity shapes gene expression and how this controls cell state changes in yeast and mammalian cells. Our work may provide important new insights into how mis-regulation of alternative transcription events drives the onset of diseases such as cancer or neural developmental disorders, which often display pervasive mis-regulation of alternative promoters

2) How does the m6A machinery regulate gene expression and cell fate? The long-term aim is to decipher the functions and mechanisms by which the m6A machinery controls gene expression. It is our hope that the work in yeast will provide insights onto the function and regulation of m6A in human cells and help to understand how mis-regulation of m6A contributes to the onset of diseases such as cancer.

Research outputs

Moretto F., N.E. Wood, M. Chia, C. Li, N.M. Luscombe, F.J. van Werven. (2021) *Transcription levels of a long noncoding RNA orchestrate opposing regulatory and cell fate outcomes in yeast*. Cell Reports. 34(3):108643. DOI: 10.1016/j.celrep.2020.108643
This work demonstrates how yeast cells have put in place a robust regulatory circuit to ensure that the cell fate decision leading to meiosis is tightly controlled, and that only diploid but not haploid cells enter meiosis. We identified a new mechanism by which transcription of noncoding RNAs can activate gene expression. Our findings have important implications for the gene regulatory potential of long non-coding RNAs (lncRNAs) in general. We propose that transcription level dependent chromatin changes may explain the opposing regulatory activities exerted by lncRNAs.

Chia M.*, C. Li*, S. Marques, V. Pelechano, N.M. Luscombe and F.J. van Werven (*equal contributors). (2021) *High-resolution analysis of cell-state transitions in yeast suggests widespread transcriptional tuning by alternative starts*. Genome Biology. 22, 46. DOI: 10.1186/s13059-021-02274-6.
The start and end sites of messenger RNAs (TSSs and TESs) are highly regulated, often in a cell-type specific manner. Yet the contribution of transcript diversity in regulating gene expression remains largely elusive. We performed an integrative analysis of multiple highly synchronized cell-fate transitions and quantitative genomic techniques in Saccharomyces cerevisiae to identify regulatory functions associated with transcribing alternative isoforms. Increased upstream alternative TSS usage is linked to various effects on canonical TSS levels, which range from co-activation to repression. We identified two key features linked to these outcomes: an interplay between alternative and canonical promoter strengths, and distance between alternative and canonical TSSs. These two regulatory properties give a plausible explanation of how locally transcribed alternative TSSs control gene transcription. Our integrative analysis of multiple cell fate transitions suggests the presence of a regulatory control system of alternative TSSs that is important for dynamic tuning of gene expression.

Tam J., and F.J. van Werven. (2020) *Regulated repression, and not activation, governs the cell fate promoter controlling yeast meiosis*. Nature Communications. 11, 2271. DOI: 10.1038/s41467-020-16107-w.
Our findings provide important new insights into how the decision to enter meiosis and produce gametes is regulated; environmental signals regulate the association and disassociation of transcription factors that recruit Tup1-Cyc8 to the *IME1* promoter. In

addition, the work on the *IME1* promoter gives novel insights into how Tup1-Cyc8 regulates gene transcription in general. Transcription factors designated to recruit Tup1-Cyc8 and transcriptional activators prime the *IME1* promoter, but these proteins are likely not the same proteins as have been proposed before. The regulatory principles identified in this work show features of how enhancers in mammalian cells are controlled.

Wu, A.C.K., H. Patel, M. Chia, F. Moretto, D. Frith, A.P. Snijders, F.J. van Werven. (2018) *Repression of Divergent Noncoding Transcription by a Sequence-Specific Transcription Factor*. Molecular Cell 72(6):942-954. DOI: 10.1016/j.molcel.2018.10.018.
Transcription factors typically activate transcription by recruiting cofactors, but our data in this paper illustrate a new function. We show that the sequence-specific transcription factor Rap1 prevents regulatory elements from initiating transcription in the divergent direction. We define a novel mechanism for providing directionality towards productive transcription, as Rap1 promotes directionality, at least in part, by directly interfering with transcription initiation in the divergent direction. Our study reveals a new important layer of regulation, describing how genomes restrict the accumulation of aberrant transcripts and ensure productive coding gene expression.

Chia M., A. Tresenrider, J. Chen, G. Spedale, V. Jorgensen, E. Ünal* and F.J. van Werven* (*co-corresponding authors). (2017) *Transcription of a 5' extended mRNA isoform directs dynamic chromatin changes and interference of a downstream promoter*. Elife 6:e27420. DOI: 10.7554/eLife.27420.
This work demonstrates that 5' extended mRNA isoforms can act as repressors of gene expression. During meiotic prophase in *S. cerevisiae,* the kinetochore complex subunit Ndc80 is downregulated by transcription from a distal *NDC80* promoter directing histone H3K4 and H3K36 methylation to establish a repressive chromatin state on the downstream canonical *NDC80* promoter. This mechanism may be conserved, suggesting that such simple switches may affect meiosis in higher eukaryotes. The findings have potentially wide implications for interpreting gene expression studies, which often report changes in expression levels without taking into account changes in isoform length; genes that appear up-regulated may actually be repressed.