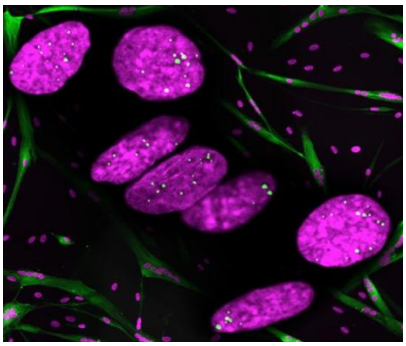
Elucidating the composition of toxic RNA-protein complexes in myotonic dystrophy

- Student Internship Project -

Background - Myotonic dystrophy type 1 (*dystrophia myotonica*; DM1) is an autosomal dominant, multisystemic disorder, which can affect all age groups and involves many different organs, including skeletal muscle, heart and brain. At the basis of the disease is a (CTG)n trinucleotide repeat expansion mutation located in the 3' UTR of the *DMPK* gene and the promoter area of the *SIX5* gene. Above a threshold of ~37 triplets the CTG repeat becomes unstable between generations and during ageing in somatic cells. Hence, length of the (CTG)n repeat is highly variable.

How does a non-coding (CTG)n repeat cause the highly variable symptoms of DM1? Most of the current experimental evidence supports a toxic RNA gain-of-function model for *DMPK* RNA carrying a long expanded (CUG)n repeat. The (CUG)n segment aberrantly binds RNA-binding proteins like MBNL and CUGBP1, which in turn leads to dysregulation of splicing, polyadenylation and transcription in cells where the *DMPK* gene is expressed. (CUG)n transcripts with aggregated proteins can be visualized as so-called foci in the cell nucleus (see Fig.).



Van Agtmaal et al. Molecular Therapy Jan. 2017 (Cover image)

Techniques:
- Cell culture
- Fluorescence *in situ* hybridisation
- Immunofluorescence staining
- Widefield microscopy
- Confocal microscopy
- Image analysis in FIJI
- RT-qPCR and western blotting

Goal - Our aim is to image the ribonuclear complexes (foci) in DM1 with super resolution microscopy (STORM) to identify their form, structure, and molecular and spatial composition. It is highly challenging to prepare cells for STORM and keep both the RNA and the proteins preserved and detectable.

Your project - In this project, you will apply a variety of microscopy techniques combined with image analysis using FIJI software. You will explore different cell fixation methods and antibody and oligonucleotide-based fluorescent probes. Immunofluorescence assays are combined with fluorescence *in situ* hybridization (FISH) to identify the protein as well as the RNA composition of DM1 foci. You will directly apply these techniques to patient muscle cells. You will first use widefield and confocal microscopy to evaluate the sample preparation. When this is successful you will be involved in experiments using super resolution microscopy. These findings will provide more inside in the complex composition of the RNA foci in DM1 patient cells and concurrently pave the way for the introduction of super resolution microscopy in the DM1 field.

Contact - Are you interested to investigate the composition of these fascinating foci in myotonic dystrophy? Contact Remco van Cruchten (PhD student; remco.vancruchten@radboudumc.nl), Marieke Willemse (Technician; marieke.willemse@radboudumc.nl) or Rick Wansink (PI, rick.wansink@radboudumc.nl), Department of Cell Biology (283), RIMLS, Radboudumc.