Abstract

Transcriptional regulation of gene expression in eukaryotes has evolved over millions of years. The regulatory pathways of nuclear receptors represent an evolutionarily ancient, but conserved mechanism with associated accessory proteins, many of them forming a functional nexus known as the Mediator complex involved in transcription. Despite the versatility of the pathway, e.g. through the adoption of new regulatory functions in phylogenetically more recent Metazoa, we hypothesise that the intrinsic potential of the NR-Mediator axis to directly translate a stimulus to a biological response is conserved across species, and additional regulation could also be achieved through secondary functions of its essential members.

To support the hypothesis, we assessed the ligand-binding capability of retinoic X receptor in *Trichoplax adhaerens* and provided evidence to support the concept that this capability was already present at the base of metazoan evolution.

With regards to the potential secondary functions, we took inspiration from previous research and identified the Mediator subunit 28 (MED28) as the only known member having documented nuclear and cytoplasmic dual roles, and thus possessing the potential to transmit signals from the cellular structural states to the nucleus. Due to the lack of significant sequence conservation and a robust experimental toolset, we chose to characterise the presumed MED28 orthologue W01A8.1 in *Caenorhabditis elegans*. Our results suggest that W01A8.1 is in fact, with a high degree of certainty, a member of the Perilipin family thus, unveiling the previously unknown Perilipin-dependent regulation of lipid metabolism in Nematoda. This effort led to the reannotation as PLIN-1 (PeriLIpiN).

Keeping in line with the hypothesis, we subsequently identified F28F8.5 as the most probable orthologue of MED28 in *C. elegans*, which also consequently led to its reannotation as MDT-28 (MeDiaTor).

The work showed that the Mediator subunit 28 is a conserved member of the Mediator complex, which has a potential to connect regulation of transcription with cytoplasmic events. Together with the conserved NR signalling, it supports the hypothesis that the general architecture of the NR-Mediator signalling axis has been conserved throughout evolution of Metazoa. Additionally, I argue and make a case for Perilipin having a potential indirect and direct role in the regulation of gene expression. This incentivises further research on 'Proteome' signalling as a general principle.