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Introduction

Cells are the fundamental building blocks of all living organisms, including humans. Our bodies are made up of around 10^{14} cells, of which there are many different types that have particular characteristics that enable them to carry out a particular function. For example, red blood cells have the ability to transport oxygen molecules, whereas liver cells are capable of metabolising drugs and synthesising cholesterol. However, although these mature cell types display an enormous number of different features, they are all derived from a similar precursor cell, known as a stem cell, in a process called differentiation. More surprisingly, nearly all cells within a particular individual contain exactly the same ensemble of DNA sequences. Genes encoded within these DNA sequences are activated or deactivated during cellular differentiation in a controlled and regulated manner, and this results in cells receiving different subsets of genetic information that direct and enable them to perform specific functions.

Despite the many differences between fully differentiated cells in our bodies, there are also many similarities, in particular in the processes that determine the genetic information that is "read" within a particular cell type. This information is contained within DNA, which is located within a specific region of the cell known as the "nucleus". This region can be visualised under a microscope by using fluorescent stains that specifically interact with DNA (Figure 1). Other stains (antibody-based) can be used to reveal internal nuclear regions such as "nucleoli" and "Cajal bodies" that contain specific protein and DNA molecules, demonstrating that the nucleus is highly organised. There are also similarities in the processes that determine how cells respond to changes in their environment, such as stress, in order to either adapt and therefore survive the change, or die to minimise the level of unhealthy cells within an organism. Many of the cellular stress responses involve changes within the cell nucleus and ultimately result in changes to which genes are activated or not. These changes are mediated by protein molecules that have received signals to change either their structure, location within the cell or interactions with other molecules. Since these stress response pathways are fundamental processes that operate in a large variety of cell types, it is important to understand these at a highly detailed, molecular level.

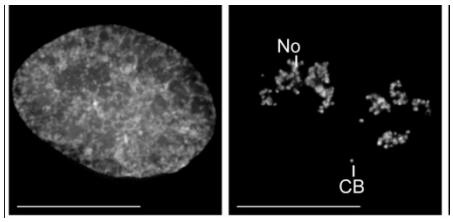

Figure 1: The cell nucleus can be visualised under the microscope using specific stains for DNA (left). The same nucleus can be co-stained to reveal subnuclear structures such as nucleoli (No) and Cajal bodies (CB). Scale Bar = 10microns.

Aim

The aim of this project was to understand changes that occur within the cell nucleus and subnuclear regions such as nucleoli in both normal growth conditions and in response to stress. One change that is known to mediate stress responses is the attachment (or

posttranslational modification) of particular protein molecules to another class of protein molecules collectively referred to as “SUMOs” (small ubiquitin-like modifiers). Therefore, we initially carried out a screen using the latest proteomic technology based on SILAC and mass spectrometry to identify proteins in nucleoli that can be modified by SUMO. The rest of the project aimed to confirm the results of this screen and to obtain molecular insight about the role of SUMOylation for the identified target proteins.

Results and conclusions

Our screen identified the proteins Nop58, dyskerin, Nhp2 and Nopp140 as major candidates for modification by SUMO in the nucleolus. These proteins are involved in one of the major functions of the nucleolus, namely to generate ribosomes. Ribosomes are machines that are able to use the genetic information contained within DNA-derived RNA molecules to synthesise new protein molecules. Although protein synthesis takes place outside the nucleus, ribosomes are largely put together in nucleoli, which likely prevents immature ribosomes from prematurely interacting with RNA or protein molecules.

We next confirmed that two of these proteins, namely Nop58 and Nhp2, are indeed modified by SUMO. This was demonstrated using a number of different experimental approaches, including both cell-free reactions and analyses within living cells. Nop58 and Nhp2 are made up of 529 and 153 amino acids, respectively, and we were next able to identify precisely which amino acids were able to be attached to SUMO, namely two (K467 and K497) in Nop58 and one (K5) in Nhp2. Interestingly, related proteins known as Nop56 and nhpx that are composed of very similar amino acid sequences to Nop58 and Nhp2, respectively, were shown not to be SUMOylated. This demonstrated that we had uncovered a very specific mechanism to regulate the function of proteins such as Nop58 and Nhp2. Finally, we discovered that although SUMO-Nop58 appeared to localise correctly to nucleoli and Cajal bodies, its interactions with one of its major binding partners (small nucleolar RNA) appeared to be stronger than that of Nop58 alone. This suggested that SUMOylation of proteins such as Nop58 could indirectly play a role in ribosome biogenesis, which provides a major new insight into this field. The successful conclusion of the project was underlined by these new results being accepted for publication in a leading international journal, “Molecular Cell”.

Impact, Target Groups and Socio-Economic Impact

We have discovered the existence of novel molecular pathways that link, proteins involved in stress, ribosome biogenesis, SUMOylation and the nucleus/subnuclear bodies. Since ribosome biogenesis occurs in nearly every human cell type, these findings advance our current understanding of fundamental cellular pathways. In the short term, this project will be of greatest interest for researchers interested in understanding basic molecular processes, such as ribosomal assembly and posttranslational modification of nuclear proteins. However, in the longer term, our findings could be useful also for pharmaceutical companies and/or academic researchers interested in developing therapeutic agents, especially if different levels of SUMOylated Nop58 are related to particular disease states, which is now an important topic for further research.