

Meeting report

Nuclear processes controlled by molecular machines

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A report on the 'Nuclear Structure and Function' symposium at the joint spring meeting of the British Society for Cell Biology, British Society for Developmental Biology and Genetics Society, York, UK, 20-23 March 2002.

The dynamic nucleus

Recent years have seen a dramatic increase in our understanding of the way the nucleus is organized; for example, we now know that many nuclear proteins, although restricted to particular compartments, are also very dynamic and can move rapidly between separate sites. Many of the new advances in the study of the nucleus stem from the use of proteins tagged with green fluorescent protein (GFP) and imaging of live cells. Several speakers used these techniques to ask new questions about nuclear organization and the control of gene expression.

Roel Van Driel (University of Amsterdam, Netherlands) has examined how gene expression is organized in the context of nuclear chromatin, using a stable cell line expressing GFP fused to histone 2B (GFP-H2B) to compare chromatin localization with transcription sites and transcription-factor localization. It is known that genes can be silenced epigenetically either by being in close proximity to centromeric heterochromatin (which is permanently condensed) or by the binding of arrays of Polycomb-group chromatin proteins. Using immunogold labeling and electron microscopy, Van Driel and colleagues have shown that Polycomb proteins are not localized to the condensed chromatin but rather are found in the inter-chromatin spaces, and to an even greater extent in the peri-chromatin spaces at the surface of the chromosome territories, where active transcription occurs. Initially this colocalization of Polycomb-group proteins and actively transcribed genes seems a surprising result, as it is generally thought that silenced genes are located in a different

part of the nucleus from active genes. Van Driel hypothesized that the cell uses the Polycomb-group proteins for silencing when it cannot use proximity to heterochromatin as a silencing method, that is, Polycomb-group proteins are used to silence genes that are interspersed with transcriptionally active genes.

Van Driel also used time-lapse imaging of cells stably expressing GFP-H2B as they completed mitosis to analyze chromatin decondensation (loosening of tightly packed mitotic chromatin). He reported an approximately five-fold increase in nuclear volume and a concomitant approximately five-fold decondensation of the chromatin at the end of mitosis, indicating that chromatin actually shows a surprisingly limited degree of overall decondensation. To study local chromatin decondensation, Van Driel used *Arabidopsis* as a model system. Subsets of specific chromosome regions were simultaneously labeled using different-colored probes, which showed that euchromatic (more active) regions were more extensively decondensed than heterochromatin. This indicates that specific regions of chromatin can unfold locally much more than is indicated by a global analysis of total decondensing chromatin.

Wendy Bickmore (MRC Human Genetics Unit, Edinburgh, UK) has also used tagged proteins, in this case by employing a gene-trap approach in which genes are fused with *lacZ* at random so as to identify the cellular localization pattern of the endogenous protein. This approach resulted in the identification of numerous new nuclear proteins that localize to different sub-nuclear compartments in human cells (see the Nuclear Protein Database [<http://npd.hgu.mrc.ac.uk/>]). As well as discussing these and other recently published results, Bickmore also presented an in-depth analysis of the three-dimensional locations of interphase chromosomes and gene loci in mammalian cells. Chromosomes are known to occupy distinct 'territories' within the nucleus. Following on from the observation that gene-rich chromosomes are preferentially located in the center of the nucleus and

gene-poor chromosomes at the periphery, Bickmore and colleagues have examined the organization of loci within a chromosome territory to see whether they correlate with gene expression patterns. Whole-chromosome painting using fluorescently labeled probes to each chromosome was used to define a chromosome territory, and defined sub-regions of a chromosome were independently labeled to observe their relative positions. Interestingly, although some sub-regions contained a mixture of gene-dense and gene-poor loci, their locations within the chromosome territory were consistent between experiments. For example, 11p15 was observed consistently at the edge of the chromosome 11 territory and 11p13 at the center. Moreover, this arrangement within the territory is conserved between mouse and humans, suggesting that it is significant. Questions from the audience focused on how knowledge of the location of genes and chromosomes can help to solve the question of how gene location relates to gene expression. The work of the Bickmore group on chromosome territories suggests that gene-rich areas are not found on the edge of the territory, contrary to the common view that transcription takes place at the edge or boundary of the territory. But given that these data correlate location with gene density, rather than with active versus inactive genes, the findings may not be generally applicable.

Nuclear transport

The focus of the session shifted briefly from nuclear organization to nuclear transport with a short talk by Richard Grant (Murray Stewart's group, Cambridge University, UK) on a structural analysis of Tap and its interaction with partner proteins. Tap is a shuttling transport receptor protein involved in exporting mRNA to the cytoplasm, carrying its cargo through the nuclear pore by interacting with different nucleoporins (the components of the nuclear pores). Grant described structures from nuclear magnetic resonance (NMR) and X-ray crystallography studies of the nucleoporin-binding domains of Tap in complex with different peptides that mimic the nucleoporin substrate.

Nuclear bodies

Many nuclear proteins and/or RNA molecules are organized within the interchromatin spaces of the nucleus in a number of discrete bodies, including nucleoli, Cajal bodies, gems, pro-myelocytic leukemia (PML) bodies and splicing speckles. These non-membrane-bound bodies are highly conserved, and many observations indicate that their maintenance is important for cellular function. In another application of GFP technology, Judith Sleeman (our group at Dundee University, UK) described the characterization of HeLa cell lines stably expressing survival of motor neurons (SMN) protein tagged with various fluorescent proteins. The gene encoding SMN is generally disrupted in patients suffering from spinal muscular atrophy (SMA), a common inherited human disorder. SMN is known to play a role in the biogenesis

of small nucleolar ribonucleoprotein (snRNP) particles needed for pre-mRNA splicing, although it remains unclear how this is connected to the SMA phenotype. By making stable cell lines, Sleeman was able to overcome problems caused by high expression levels of transiently expressed SMN; she found that tagged SMN localized to both the cytoplasm and in the Cajal bodies (which coincide with the gems in this cell type) in an identical pattern to endogenous SMN (Figure 1). Time-lapse analyses of mitotic FP-SMN cells showed that it is transiently found in a punctate pattern in the cytoplasm. Sleeman also described fluorescence recovery after photobleaching (FRAP) experiments showing that the SMN signal in Cajal bodies/gems recovered from bleaching more slowly than another Cajal-body protein, p80 coilin, whose role is unknown. These data suggest separate roles for SMN and p80 coilin within the nuclear bodies.

Greg Matera (Case Western Reserve University, Cleveland, USA) continued with the theme of nuclear bodies by describing an analysis of the direct molecular interaction between the SMN and p80 coilin proteins. Matera reviewed genetic and biochemical evidence indicating that p80 coilin can recruit SMN and snRNPs to the Cajal body. Analysis of the protein domains responsible for the SMN-p80 coilin interaction have led Matera to investigate the possibility that a type of methylation, symmetric dimethyl arginine (sDMA)

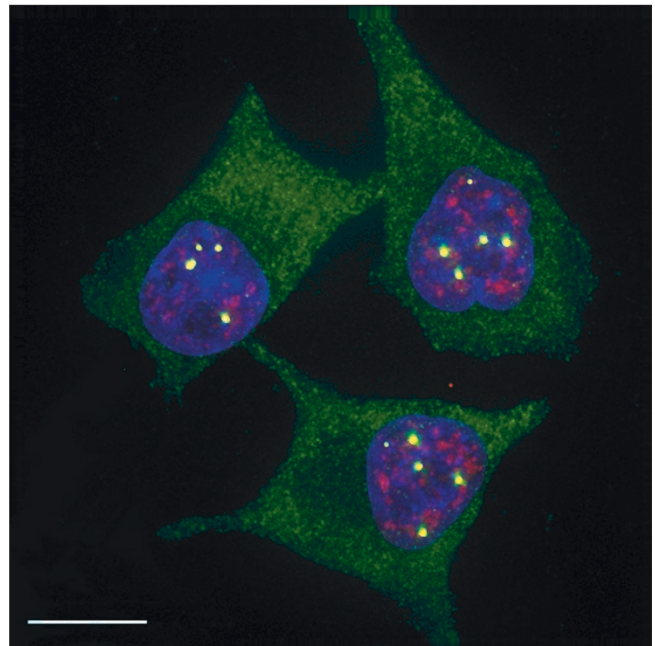


Figure 1
HeLa cells stably expressing GFP-SMN showing localization of GFP-SMN (green) to the cytoplasm and to Cajal bodies in the nucleus. Cells were fixed with paraformaldehyde and stained with the monoclonal antibody Y12, which recognizes Sm proteins found in splicing speckles and Cajal bodies (red) and with DAPI, which recognizes bulk chromatin (blue). The scale bar represents 10 μ m.

modification, is the key to this interaction. This modification can be detected using the well-known monoclonal antibody Y12, which was previously thought to recognize the core snRNP (Sm) proteins directly, but actually recognizes the sDMAs that are present in the Sm proteins. Y12 recognizes immunoprecipitated GFP-coilin from HeLa cells, but it does not recognize it when an inhibitor of the sDMA modification is used. SMN is found in distinct nuclear structures called gems in fetal tissues and in some cell lines, whereas in most adult tissues and most cell lines SMN localizes to Cajal bodies. Matera presented data that indicate that this difference may be due to the sDMA modification state of the p80 coilin, which affects its ability to recruit SMN to Cajal bodies.

The synthesis of ribosomes is one of the major functions of a cell, and this takes place in the nucleolus. David Tollervey (Edinburgh University, UK) reviewed how proteomic analyses of ribosome-particle assembly in yeast have led to a dramatic increase in our understanding of ribosome biogenesis. In a synthesis of data from several groups, the assembly of the 60S ribosomal particle is now thought to take place in several steps, each involving a different set of proteins. Assembly of the 40S particle is under scrutiny and, surprisingly, it appears that it has little in common with the 60S assembly pathway because the proteins involved do not overlap. Tollervey predicts that the next few years will see a greater understanding of where these assembly steps take place within the known structure of the nucleolus, providing a major new insight into structure-function relationships within the nucleus.