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The Nucleolus and Viral Infection*

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Abstract: The nucleolus is a subnuclear structure of eukaryocytes. It was thought that nucleolus only participates in the biogenesis and processing of rRNA. However, more and more evidence shows that it has many other functions, such as tRNA precursor processing, stress sensing and it is also involved in gene silencing, senescence and cell cycle regulation. Here, we summarize the recent understandings about the nucleolar functions, the regulation of nucleolar localization of proteins and the role that the nucleolus plays in virus infection, in which some related studies of Herpes simplex virus type 1 (HSV-1) US11, UL24 and bovine herpesvirus-1 infected cell protein 27 (BICP27) carried out in our lab will also be included.

Key words: Nucleolus; Stress; Cell cycle regulation; Virus infection.

Eukaryotes have a specialized non-membranous subnuclear cell structure, the nucleolus, which is relatively large, dynamic, highly organized and is visible under the light microscope. It can be divided into three separate parts that reflect its role in the ribosome subunit synthesis. These regions are structures formed by the functional association of macromolecules and comprise fibrillar centers (FC), dense fibrillar components (DFC) and a granular component (GC). The FC is organized around multiple rRNA genes in tandem arrays which can be found at several chromosomal loci. The DFC is a compartment usually associated with FC, and contains newly synthesized pre-rRNAs and some proteins; and the GC is a region of the nucleolus that contains nearly complete preribosomal particles which will be transported to the cytoplasm later. The number of FCs, GCs and DFCs located in the nucleolus depends on the cellular metabolic status [9].

NUCLEOLAR PROTEOME DYNAMICS

Researchers have used three different metabolic inhibitors which can affect nucleolar morphology to treat cells and characterized the flux of 489 endo genous nucleolar proteins. Their data shows that when cells grow in varied conditions the proteins in nucleoli

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change significantly. Although many proteins stay in the nucleolus only in the presence of rRNA substrates, the fact that in response to transcription inhibitors, many other proteins either remain at nearly the same levels, or even increase within nucleoli, indicates that not all resident nucleolar proteins require rRNA substrate synthesis [1].

The nucleolus is usually thought of as the site for rRNA synthesis, processing and maturation. Recently, it has become more clear that the nucleolus also has a role in regulating the cell cycle, tumor suppression, oncogenic activities, assembly of signal recognition particle (SRP), aging control and modulation of telomerase functions [18~20] and sensing cellular stress. Some of these functions are mediated through sequestration of transcription factors that control the cell cycle.

Ribosome biogenesis is regulated by gene silencing and by modulating the transcriptional apparatus [16]. In many eukaryotic cells, significant parts of the tandemly repeated rRNA genes are silenced. Although studies of the nucleolus have focused on precursor of rRNA, many other RNA species have been found in the nucleolus including several precursor tRNA species and the signal recognition particle (SRP). It is interesting that some Arabidopsis nucleolar proteins could also be found in the exon junction complex, which play a role in mRNA processing, export and other downstream events such as nonsense mediated decay [21] [a]and there are aberrantly spliced mRNA transcripts in the nucleoli.

There is increasing evidence that the nucleolus can sense stress and control the function of p53 [22]. It has been proposed that p53 is stable in a cell unless the nucleolus promotes its degradation, and the nucleolus seems to have close relationships with the degradation of p53 via the ubiquitin-proteasome pathway. Thus, there seems to be mutual interaction between p53 and the nucleolus: p53 can determine the function of the nucleolus under normal growth conditions, however, the nucleolus influences p53 function in response to stress.

It is striking that glyceraldehyde phosphate dehydrogenase (GAPDH) could also be detected in nucleoli of both the Arabidopsis and human. Recent evidence has shown that in addition to its glycolytic activity, GAPDH is a protein having multifunction both in cytoplasm and nucleus, one of which is the regulation of mammalian histone H2B expression, and GAPDH functions as an essential component of a transcriptional activator complex [17,28].

THE MULTIFUNCTIONAL NUCLEOLUS

Post translational modifications may be the most dynamic changes that take place throughout the cell cycle and these modifications can regulate many activities of proteins. Some of these protein modifications, sumoylation and phosphorylation for example, are regulated by the nucleolus. Connection with small protein chains, such as the small ubiquitin like modifier (SUMO) protein[26], is a process which is both dynamic and reversible, and several SUMO-specific proteases that remove SUMO chains from protein substrates have been described[13]. A new SUMO-specific protease, SENP5, was recently identified and found to be predominantly localized in the nucleolus [8]. Knock down of SENP5 by RNA interference results in defects in cell division and aberrant nuclear morphology. This suggests a role for the nucleolus in regulating sumoylation of proteins that affect progression of cell division.

The sequestration of specific proteins into the nucleolus during the cell cycle is another mechanism used by this region to regulate the functions of these proteins. An example is telomerase reverse transcriptase, a RNP enzyme which maintains telomere by addition of the nucleotide repeats to the ends of chromosomes, that is sequestered in nucleoli until the late stages of S phase when the telomeres are replicated. The nucleolar sequestration of telomerase is reportedly mediated through its binding to the nucleolin which is a nucleolar protein. Therefore, the regulated release from the nucleolar region might ensure the appropriate function of telomerase during DNA replication. Interestingly, the cell cycle dependent sequestration of telomerase into nucleolous could not be detected in either cells that have experienced DNA damage or in cancer cells, which indicates that the loss of telomerase localization into the nucleolus might be an important feature of abnormal cells. Proteomic studies have shown that many proteins of the helicase family have nucleololar localization, some of which accumulate at certain cell cycle stages or as a result of a specific stimulus. It is also interesting that many helicases which are found in the nucleolus can causes human genetic disorders when mutated. Although it's possible that other non-nucleolar functions are likely to be affected, the nucleolar localization of these helicases implies that these specific functions in the processing of DNA or RNA that occur in nucleoli are very important for normal cell growth.

CONTROL OF NUCLEOLAR LOCALIZATION

Identifying the molecular pathways for targeting proteins to the nucleolus is an important part of understanding nucleolar function and its regulation. Researchers have discovered the first detailed molecular mechanism --GTP-driven cycle-- for targeting a protein to the nucleolus [25]. Nucleostemin accumulates in the nucleolus. Its N-terminal basic domain is essential and sufficient for this localization. In addition, the protein harbours two GTP-binding domains, which prompted researchers to test whether these domains determine protein localization. Sure enough, mutations in the GTP-binding sites resulted in decreased binding of GTP to nucleostemin and dispersed the protein from the nucleolus leading to its diffuse nuclear distribution. This research directly shows that GTP binding is critical for nucleolar accumulation of nucleostemin. But how general is regulation of nucleolar targeting by GTP binding? A search of the Lamond database shows that about 3% of the over 700 known nucleolar proteins habour a GTP-binding or closely related domain. This indicates that direct regulation by GTP binding is a relevant but not a major mechanism for nucleolar targeting. On the other hand,Tsai and Mckay have found that GTP binding-controlled localization of some nucleolar proteins may indirectly affect the localization of other proteins [25]. For example the B23 protein does not have any obvious GTP binding motifs, but its dynamic distribution can be affected by changes of GTP levels in cells, indicating that nucleolar proteins can mutually affect their localization. Also nucleolar localization can also be regulated by phosphorylation of the proteins, such as LIM Kinase 2 and Hand1.

Unlike the nucleus, there is no evidence for the existence of a barrier separating the nucleolus from the surrounding nucleoplasm. As a result, any soluble molecule could principally spread in and out of the nucleolar region. Sequence comparison of nucleolar

proteins could not identify a general NoLS (Nucleolar localization/retention signal). Localization of the proteins to the nucleolus might be achieved by one of three different types of interactions; (a) with nucleolar or nucleolar-associated proteins and (b) with rDNA and (c) nucleolar RNA consisting mainly of rRNA. Arginine/lysine-rich RNA binding domains could be found in many viral proteins that relate with the nucleolus.

Nucleolar localization/retention signals (NoLSs) and pathways have not been well characterized, and the NoLSs could vary, but are usually rich in lysine and arginine, although there is no obvious consensus motif, such as the MQRKPTIRRKNLRLRRK sequence found in survivin-deltaEx3 protein, or the RSRKYTSWYVALKR residues of the fibroblast growth factor-2. Our lab has identified an arginine rich domain RPRRPRRRPRRR as a functional NoLS in bovine herpesvirus-1 infected cell protein 27 (BICP27), which localizes predominantly in the nucleolus [12]. Compared with NESs in which the leucine rich export motif (for CRM1 type nuclear export receptors) is available for interaction with the carrier proteins, the structural setting of a NoRS has not been well understood. In many cases, proteins localizing to both the cytoplasm/nucleus/nucleolus contain multiple signals to determine their subcellular localization. This increases the difficulty in identifying NoRSs, in that, many proteins which localize to the nucleolus also localize to the nucleus and contain both classical nuclear and nucleolar signals which can also overlap.

VIRAL PROTEINS LOCALIZE TO THE NUCLEOLOUS

The interaction between viruses and the nucleolus is a common phenomenon for many viruses, which is exhibited by DNA viruses, retroviruses and RNA viruses. They interact with the nucleolus to take over host cell functions and recruit nucleolar proteins to help with virus replication.

Adenovirus infection changes nucleolar structure and function. B23 is a nucleolar protein present in two isoforms (B23.1 and B23.2) and both isoforms have been identified as stimulatory factors that can facilitate adenovirus DNA replication. It has been shown that in adenovirus infected cells B23.1 and B23.2 interact and co-localize with different viral proteins pTP and DBP that participate in viral DNA replication. Thus, the mechanism by which the two proteins facilitate the replication of viral DNA is likely to differ [10].

Soon after infection of herpes simplex virus type 1 (HSV-1), nucleoli increase in size, move close to the nuclear membrane and then become fragmented into small pieces. In addition, several herpesviral proteins, including ICP0, ICP4, ICP27, US11 and gamma 34.5, localize at least transiently to nucleoli during infection. It's reported that the 88-125 peptide of US11 could have a role in nucleolar retention [7], moreover, researchers in our lab have found another motif, the 126-152 peptide of US11 also has a role in nucleolar targeting (Xing, *et al*., unpublished data). In addition, we also found HSV-1 UL24 targeting the nucleolus in transfection Cos-7 cells (Li, *et al*., unpublished data).

Using siRNA technology, researchers have shown that replication of HSV-1 requires high level of nucleolin expression, demonstrating for the first time a direct role of a nucleolar protein in HSV-1 biology.

They also show that the three abundant nucleolar proteins: nucleolin, B23 and fibrillarin are dispersed out of the nucleoli as a result of HSV-1 infection and a nucleolar protein, such as nucleolin, co-localizes with ICP8 in the viral replication regions, at the time when viral replication is effective, indicating an participation of nucleolin in the HSV-1 DNA replication process [4]. Furthermore, UL12, a viral alkaline nuclease, which is involved in viral DNA maturation and nuclear egress of nucleocapsids, can localize with nucleolin during HSV-1 infection and knock down nucleolin in the infected cells which could result in decreased amounts of viral capsid in the cytoplasm [23].

In addition, nucleolin is also involved in the viral DNA synthesis in HCMV infected cells. During infection, the viral DNA polymerase processivity factor UL44 could co-localize with nucleolin. Treating HCMV infected cells with siRNA targeting nucleolin mRNA could impair viral DNA synthesis, and there is a correlation between the efficacy of knock-down and effect on virus replication [3].

The bovine herpesvirus-1 infected cell protein 27 (BICP27) is targeted predominantly in the nucleolus and contains a NoLS. Our data has added BICP27 to the growing list of transactivators which localize to the nucleolus [12].

The coronavirus infectious bronchitis virus (IBV) nucleoprotein (N) which is involved in many aspects of virus replication, localizes to the nucleolus and might repress host cell translation through disrupting the biogenesis of new ribosomes [11].

It is reported that the PRRSV N protein co-localizes with nucleolar protein fibrillarin and specifically interacts with it. Yoo *et al* also show that N protein could bind RNA and suggest a possible role of N as a potential competitor for fibrillarin [27].

The viral component that has attracted the most attention is the HIV-1 Rev protein, which is predominantly localized to the nucleolus [14] and interacts with the nucleolar protein B23 [6]. Rev regulates the splicing pattern of the HIV-1 mRNA by promoting unspliced or partially spliced mRNA export to the cytoplasm. Studies suggest that a complex comprising Rev, two nucleoporin proteins, and the nuclear export factor CRM1 (Chromosome region maintenance protein 1) assemble within the nucleolus, which could facilitate movement of this complex to the nuclear pore. The multimerization pattern of Rev in the nucleolus also indicates that it plays a role in nucleocytoplasmic transport [5]. It has been demonstrated that the HIV-1 mRNA actually passes part of its life cycle in the nucleolus. Finally, studies by Michenzi *et al*. suggest that the HIV-1 Tat protein also localizes to the nucleolus and that nucleolar trafficking by Tat is essential for viral replication. Thus, there are strong hints that the nucleolus participates in HIV infection in a specific way, possibly as one station in the HIV mRNA transport process. However, the exact role is still poorly understood.

FUTURE RESEARCH DIRECTIONS

An online database containing data about 700 proteins detected in purified human nucleoli is now available (www.lamondlab.com/Nopdb)[2]. A proteome database of the *Arabidopsis thaliana* nucleolus has also been made accessible recently (http://bioinf.scri.sari.ac.uk/cgibin/atnopdb/home [15]. One striking discovery from these proteomic studies is that about 30% of the

nucleolar proteins are encoded by previously uncharacterized genes. This suggests that in spite of the previous extensive research on the nucleolus over almost two centuries, there is still much to be studied about its structure and function. Moreover, with the recent availability of databases of human and plant nucleolar proteins, bioinformatic techniques could be applied to study new functions of nucleolar proteins[24] and the evolution of this subnuclear region.

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