Review

Emerging roles of the neuronal nucleolus

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Although, the nucleolus has been observed for almost 200 years in neurons, studies that directly address the neuronal roles of this subnuclear structure have appeared only recently. The aim of this review is to discuss recent progress and identify some critical questions that remain to be answered. As expected for the cellular center of ribosome biogenesis, the nucleolus is essential for the growth of developing neurons, including neurite morphogenesis and long-term maintenance of mature neurons. In addition, the nucleolus contributes to neuronal stress responses, including the regulation of apoptosis. Hence, disrupted neurodevelopment or neurodegeneration are among the likely consequences of nucleolar dysfunction. Conversely, the presence of active nucleoli may determine the potential for neurorepair.

Introduction

Almost two centuries ago, microscope observations of neurons resulted in the identification of a subnuclear structure that in 1839 was named the nucleolus by Gabriel Valentin [1]. Subsequent work done mostly in transformed cells revealed that the nucleolus is an initiation site for ribosomal biogenesis and as such determines cellular translation capacity and regulates cell growth [2–5]. Additional nucleolar functions have also been identified, including various stress responses [6,7]. Although neurons are post-mitotic cells, which on reaching maturity have limited growth potential, they often display prominent nucleoli. However, until recently the neuronal functions of nucleoli have not been addressed by direct experimentation. The aim of this review is to discuss the results of several studies from the past few years that have directly addressed the significance of neuronal nucleoli, and to identify some important questions that remain to be addressed.

Biology of the nucleolus

The nucleolus is assembled around several hundred copies of the repeated 45S rRNA gene (ribosomal DNA, rDNA) that are located in several clusters at various chromosomal locations throughout mammalian genomes [2,3]) (Figure 1a). rDNA is transcribed by a specialized nucleolar RNA polymerase, RNA polymerase-1 (Pol1) [3]. Transcription of rDNA initiates ribosomal biogenesis (Figure 1a). Additionally, Pol1 activity promotes the formation and maintenance of the nucleolus, including that of post-mitotic neurons [8,9] (Figure 1b). Hence, it is unsurprising that the size and structural organization of the nucleolus is directly related to the rate of ribosomal biogenesis [2].

Numerous additional steps in the maturation of precursors of the small and large ribosomal subunits (SSU and LSU, respectively) are also nucleolar [2,4,5]). The primary Pol1 transcript, 45S pre-rRNA, undergoes extensive cotranscriptional modification including ribose methylation and uridine isomerization to pseudouridine that may be required for the proper structural organization and activity of mature rRNA [4,5]. Ribosomal proteins begin to assemble on the nascent transcript, which, together with a vast array of accessory factors, stimulate subsequent steps of ribosomal subunit maturation including pre-rRNA processing and nuclear export. The pre-LSU assembly in the nucleolus also involves incorporation of 5S rRNA that is transcribed from non-nucleolar genes by RNA polymerase-3 [10]. Eukaryotic ribosomal biogenesis is a highly complex process that involves 80 ribosomal proteins, more than 200 non-ribosomal proteins and 75 small nucleolar RNAs (snoRNAs) [4,5]. In a rapidly dividing cell, several thousand ribosomes are generated every minute [4]. rRNA transcription accounts for at least one-half of the total transcriptional output of a cell during active proliferative growth [3,4]. Hence, ribosomal biogenesis represents a major item in the energy budget of proliferating cells [11]. High rates of ribosomal biogenesis are also expected in cells that do not divide but, instead, rapidly increase their volume, such as maturing oocytes, neurite-extending neurons and myelinating oligodendrocytes.

Multiple mechanisms are employed to adjust the rate of ribosomal production to cellular demand [3]. The primary target for such regulation is rDNA transcription. Several studies have shown that stimulation of rDNA transcription is sufficient to increase the entire process of ribosomal biogenesis, accelerating proliferation [12,13]. Transcription of rDNA is regulated at the level of Pol1 activity and also by epigenetic mechanisms that affect the number of transcriptionally active rDNA genes [3,14]. Thus, growth factors, nutrients and stressors increase or decrease Pol1 activity, respectively. Many of their effects on Pol1 are mediated by signaling kinases, including positive regulation by the mammalian target of rapamycin (mTor) and extracellular signal related kinases-1/2 (ERK1/2), and negative regulation by the c-Jun N-terminal

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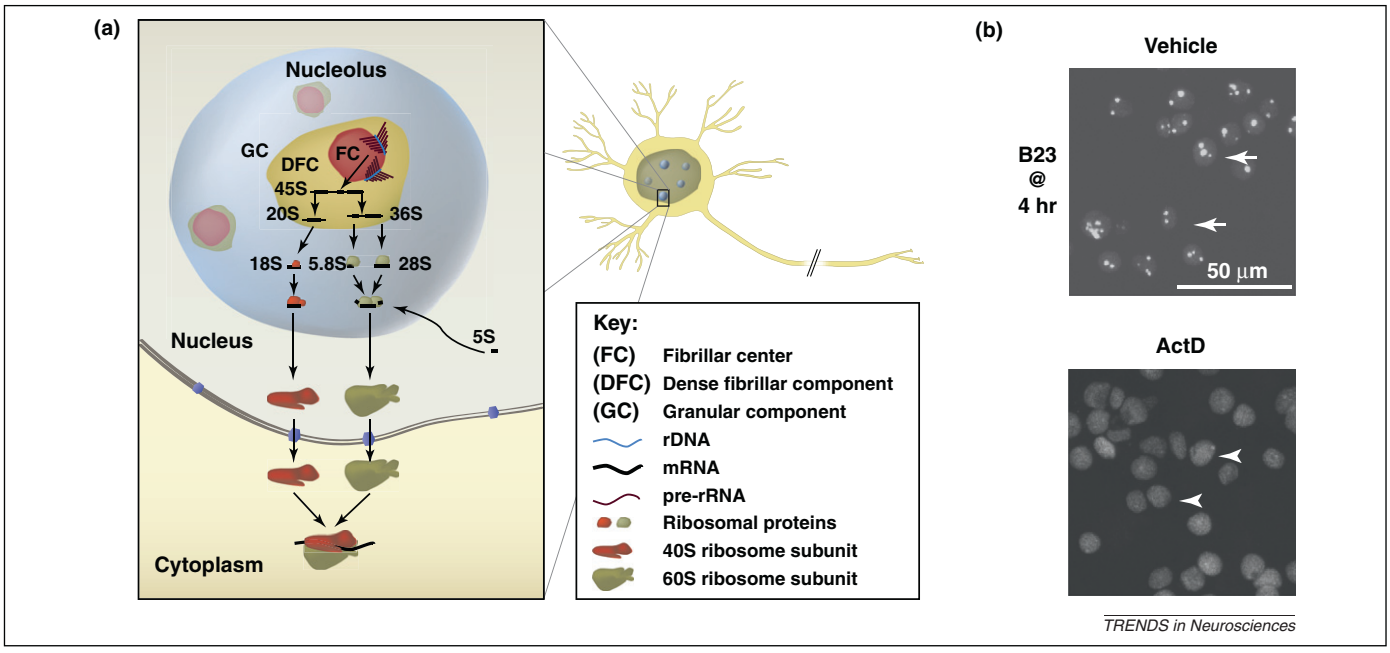

Figure 1. The nucleolus is a subnuclear structure that performs ribosomal biogenesis and is generated by Pol1-dependent transcription of the nucleolar rRNA genes (rDNA). **(a)** Ultrastructurally, the nucleolus has a tripartite organization that reflects the spatial separation of various steps of ribosomal biogenesis [2]. That process is initiated in the fibrillar centers (FC), where the 45S rRNA (pre-rRNA) is transcribed by Pol1 from rDNA. Note the 'Christmas tree' structure of the transcribed rDNA loci/elongating pre-rRNA molecules at the edges of the FC. Further steps of ribosomal biogenesis including pre-rRNA modifications and processing to mature rRNAs (18S, 5.8S and 28S), as well as rRNA assembly with ribosomal proteins and the 5S rRNA, occur in the dense fibrillar component (DFC) and granular component (GC) of the nucleolus. The final products of this process, the 40S and 60S ribosome subunits, are exported out of the nucleus, where, following final maturation, they may form ribosomes. **(b)** The nucleolus is a transcription-dependent structure. On 1 h of treatment with actinomycin D to block Pol1 (0.05 μg/ml), the nucleoli of cultured rat cortical neurons disintegrate, as demonstrated by nucleoplasmic translocation of the immunofluorescent nucleolar chaperone B23 [9]. Arrows (top panel) indicate neurons with intact nucleoli, as suggested by the predominantly nucleolar presence of B23; arrowheads (bottom panel) indicate neurons with nucleolar disruption, as indicated by diffusion of B23 throughout the nucleoplasm. Reproduced with permission from [9].

kinase (JNK) and AMP-activated protein kinase (AMPK) [12,15–20]. In addition, acetylation of regulators of rDNA transcription provides another positive input to Pol1 activity [21–23]. Finally, epigenetic regulation is believed to play a role in the reduction of ribosomal biogenesis when differentiation follows rapid growth such as that during embryonic development [14] or when cells are depleted of energy [11]. Epigenetic silencing of the nucleolus includes CpG methylation of rDNA promoters [14].

In addition to ribosomal biogenesis, maturation of tRNAs and small nuclear RNAs (snRNAs) that participate in mRNA splicing has been shown to occur in nucleoli [24,25]. Furthermore, nucleolar disruption is a rapid consequence of Pol1 inhibition following DNA damage or oxidative injury that has allowed the nucleolus to evolve as a sensor of cellular stress [19,26,27]. Thus, the disintegrating nucleolus releases several proteins that activate the stress response transcription factor p53, resulting in apoptosis [6,7]). Other proteins with dedicated functions in the stress response, such as several DNA repair enzymes, are localized to the nucleolus in the absence of genotoxic stress and released on DNA damage to restore the integrity of the genome [28,29]. Nucleolar sequestration is also a regulatory mechanism for several proteins that serve as molecular switches during cell cycle progression or differentiation [30]. In addition, the nucleolus is involved in nuclear export of ubiquitinated nuclear proteins such as p53, enabling their degradation by the proteasome system in the cytoplasm [31,32]. Finally, at least in yeast, the inherent instability of rDNA repeats determines the life span by linking the aging-associated loss of rDNA to the activation of DNA damage response and cell senescence [33]. Taken together, the highly dynamic nucleolus is the key controller of cell growth and an important sensor of cellular stress.

Role of the nucleolus in neuronal growth

Despite being non-dividing cells, neurons are able to grow. During development, neurotrophic factors stimulate neuronal growth including increases in the size of the perikarion as well as the length, caliber and complexity of neurites [34–36]. The large increase in cell volume that is associated with such growth suggests that its dependence on ribosome production is similar to that observed in the growth of dividing cells. This view is supported by observations from various regions of the nervous system indicating that the neuronal nucleolar morphology is increasingly active during neuronal development until the cells reach their final dimensions [8,37,38]. Some growth abilities are also retained in the mature nervous system, such as the growth of axons that occurs during peripheral nerve regeneration after injury [36]. There is a good correlation between regenerative growth of injured neurons and increased nucleolar activity in these cells [39,40]. Taken together, morphological observations suggest a link between neuronal growth and dynamic changes in the nucleolus indicative of increased ribosomal biogenesis. Recent work suggests that such a relationship is likely to be due to a requirement of ribosomal production for neuronal growth [41] (Figure 2).