Where no RNA polymerase has gone before
Novel functional transcripts derived from the ribosomal intergenic spacer

Mathieu D. Jacob, Timothy E. Audas, Sahra-Taylor Mullineux and Stephen Lee*
Department of Cellular and Molecular Medicine; Faculty of Medicine; University of Ottawa; Ottawa, ON Canada

The nucleolus is organized around a scaffolding of rDNA tandem repeats. These repeats, known as ribosomal cassettes, are each composed of ribosomal RNA (rRNA) genes preceding a long intergenic spacer (IGS) that has been classically perceived to be transcriptionally silent. Recent study of the IGS has contradicted the dogma that these spacers are merely inert regions of the genome, instead suggesting they are biologically significant, complex and plurifunctional transcriptional units that appear central to proper cellular functioning. Through the timely induction of various ribosomal IGS noncoding RNA (IGS RNA) transcripts, the cell is capable of both regulating rRNA synthesis and sequestering large numbers of proteins, thereby modulating essential molecular networks. Here we discuss our current understanding of the organization and function of the IGS.

The human genome contains approximately 400 copies of ribosomal DNA (rDNA) found in tandem on the short arms of all five acrocentric chromosomes (13, 14, 15, 21 and 22). Actively transcribed rRNA cassettes are consolidated within the nucleolus, creating “ribosome factories” that account for the production of the majority of total cellular RNA. In mammals, nucleoli are highly dynamic structures that assemble and disassemble during each mitotic cycle. Reformation occurs during interphase, where all molecules necessary for its basic structure and functional organization are recruited.1-4

Under proliferative conditions the most commonly ascribed function of the nucleolus is the transcription, processing and modification of the rRNA as they are assembled into ribosomal subunits. rDNA is transcribed by RNA polymerase I (PolI) as a single 13.3 kb polycistrionic transcript comprising sequentially the 5'-external transcribed spacer (ETS), 18S rRNA, internal transcribed spacer (ITS) 1, 5.8S rRNA, ITS2, 28S rRNA and 3'-ETS (Fig. 1). The resulting pre-rRNA, known as 47S, is matured via a series of processing and chemical modification steps that include cleavage, pseudouridylation and methylation at selected residues.5 Ultimately, three distinct rRNA species (18S, 5.8S and 28S) are produced and assembled with proteins into pre-ribosomal subunits that take part in protein synthesis in the cytoplasm. As is the case with most metabolically important genes, transcription of rDNA can be modulated in response to a variety of cellular and pathological stimuli including amino acid starvation, aging, viral infection or toxic lesions.6,7 This modulation is accomplished by a complex series of enhancers, repressors and transcription factors that interact with PolI or its DNA regulatory elements. Though it can be initiated and interrupted, PolI-dependent transcription is usually considered a constitutive process in metabolically active cells, in contrast to many RNA Polymerase II-regulated transcripts that are rapidly induced as needed.

The plurifunctional nature of the nucleolus is evident in its response to physiological stimuli and stress conditions, such as anaerobic metabolism, heat shock or transcriptional stress. These conditions trigger significant structural
and functional changes that are important for cellular adaptation and survival. Hallmarks of nucleolar remodeling include a decrease or interruption in rRNA synthesis as well as the capture of numerous seemingly unrelated factors involved in a wide array of cellular functions. Thence, these phenomena have been observed for many years, they have only recently been mechanistically linked to the expression of novel species of non-coding RNA (ncRNA) originating from the ribosomal intergenic spacer (IGS). In this Commentary, recent and unexpected advances in our understanding of the transcriptional activity and biological role of the IGS are discussed.

The Ribosomal Intergenic Spacer

Human rRNA coding sequences are separated by a ~30 kb region formerly referred to as the non-transcribed spacer (NTS), which until recently had not been attributed any significant function. Early work postulated that this region either played a structural role within the nucleolus allowing for the formation of tightly packed nuclear-organizing regions or provided some form of genomic stabilization through the attachment of specific sequences to the nuclear matrix. Evolutionarily, the IGS appears to have increased in size over time, measuring only 2.5 kb in yeasts, 5.1 kb in Drosophila melanogaster, 5.7 kb in Xenopus laevis, while the chickens, mouse and primate sequences are all approximately 30 kb in length. At the sequence level the IGS differs considerably from the rRNA coding sequences, notably through its high level of variability both in length and nucleotide composition. Disparities in length have been attributed to unequal homologous exchanges, while sequence divergences are caused by the incorporation of a large number of retrotransposons, or alu elements and microsatellite variations thought to be caused by slipped-strand mispairing during DNA replication.

Despite the seemingly disorganized composition and a historical lack of evidence suggesting transcriptional activity, the ribosomal IGS appears to be more functionally relevant than previously believed. Promoter mapping studies of the Xenopus laevis IGS identified spacer promoters that share 90% homology with those of rRNA genes. These promoters were capable of activating PolI-mediated transcription, though the resulting nascent transcripts were rapidly terminated well upstream of the rRNA sequences by T3 termination sites. While similar promoters were also found in mice, no known function was conclusively attributed to these transcripts, though a potential role in rRNA transcription activation was speculated. Recently, several groups have confirmed the existence of a number of ncRNA transcripts originating from these regions of the genome. Functional studies of these molecules have revealed new mechanisms for regulating rRNA expression, as well as a novel post-translational regulatory pathway termed the nucleolar detention pathway.

IGS Transcript-Mediated Regulation of rRNA Synthesis

Analysis of the IGS region 2 kb upstream of the rRNA start site identified a 150–250 nucleotide PolI-mediated transcript, known as the promoter-associated RNA (pRNA). This molecule was shown to be involved in targeting TIP5, the large subunit of the nucleolar remodeling complex (NoRC), to the ribosomal cassettes. Recruitment of the NoRC results in the repression of rRNA transcription.

Figure 1. Synthesis of rRNA from the ribosomal cassette. The human ribosomal cassette is composed of a ~13 kb transcribed region followed by a ~30 kb intergenic spacer. RNA polymerase I is recruited to a promoter region, upstream of the ribosomal gene and transcribes a single polycistronic transcript that is then processed by either of two pathways into the 18S, 5.8S and 28S rRNAs.
leads to the accumulation of a ~400 bp transcript 28 kb downstream of the start of the rRNA genes (IGS28RNA) (Fig. 2A and B). Strikingly, this region has recently also been shown to possess prominent epigenetic markers typically linked to transcriptional activation.41 IGS 28RNA rapidly recruits and immobilizes NoDS-containing proteins at its site of transcription, thus inactivating them. Silencing of IGS28RNA allows proteins to evade this capture and to retain their dynamic profile under acidotic conditions. The IGS is therefore at the center of a systemic post-translational regulatory pathway that mediates cellular adaption to hypoxic stress.

In what appears to be a homologous pathway, heat shock induces the accumulation of two independent transcripts located at 16 kb and 22 kb, and referred to as IGS16RNA and IGS22RNA respectively. These RNAs appear to undergo post-transcriptional processing events, notably involving the removal of flanking external and internal spacers and the ligation of two RNA fragments (Fig. 2A and B).

IGS Transcript-Mediated Protein Immobilization

In addition to its function as a site of ribosomal biogenesis, the nucleolus is well known for the fundamental role that it plays in regulating molecular networks. In response to a variety of physiological and stress conditions, large numbers of proteins are captured and immobilized by the nucleolar architecture,12,13,16,63,64 effectively sequestering them away from their binding partners and causing downstream pathways to collapse. Well-documented examples include the von-Hippel Lindau (VHL) tumor suppressor protein in response to anaerobic metabolism,13,36-38 the murine double minute 2 (MDM2)/promyelocytic (PML) protein complex in response to transcriptional stress13,37,39 as well as the heat shock protein 70 kDa (Hsp70) in response to heat shock.13,40 These proteins share a consensus amino acid sequence that targets them to the IGS for static detention in the nucleolus, the nucleolar detention signal (NoDS).12,13 This discrete code is composed of an arginine motif (R-R-I/L-X3-r) along with several hydrophobic repeats (L-Φ/N-L/V; where Φ represents any hydrophobic residue).12 Bioinformatic studies suggest that up to 9% of all proteins encode an NoDS and consequently could be regulated by the nucleolus.

The mechanism by which NoDS-containing proteins are captured and immobilized by the nucleolus has recently been elucidated with the identification of novel species of stress-induced ribosomal IGS transcripts. In response to anaerobic metabolism, physiological acidification of the extracellular milieu to cell-specific pH leads to the accumulation of a ~400 bp transcript 28 kb downstream of the start of the rRNA genes (IGS28RNA) (Fig. 2A and B). Strikingly, this region has recently also been shown to possess prominent epigenetic markers typically linked to transcriptional activation.41 IGS28RNA rapidly recruits and immobilizes NoDS-containing proteins at its site of transcription, thus inactivating them. Silencing of IGS28RNA allows proteins to evade this capture and to retain their dynamic profile under acidotic conditions. The IGS is therefore at the center of a systemic post-translational regulatory pathway that mediates cellular adaption to hypoxic stress.

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This raises the possibility that novel editing pathways are involved and poses the

Figure 2. Novel transcripts of the IGS. (A) The ribosomal intergenic spacer encodes multiple stimulus-specific transcripts. These RNAs are transcribed as precursor molecules that are then processed into a functional transcript capable of interacting with proteins and other cellular elements. (B) IGS28RNA is induced in response to heat shock while IGS28RNA is specific to acidosis.
question of the functional significance of post-transcriptional modifications of rRNA transcripts. In fact, transient overexpression of IGS28 RNAs comparable to 16, 22 and 28 could promote association of RNP complexes.
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