Where no RNA polymerase has gone before

Novel functional transcripts derived from the ribosomal intergenic spacer

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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. Although 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, which until recently had not been attributed any significant function. Early work postulated that this region either played a structural role within the nucleus 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 retroposons, 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 nuclear retention 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 (NolC), to the ribosomal cassettes. Recruitment of the NolC results in the transcriptionally-repressive histone modifications H3K9me1 and H4K20me3 and transcriptionally-repressive histone modifications H3K9me1 and H4K20me3 and in HP1 binding, ultimately inhibiting rRNA synthesis. Interestingly, the secondary structure of the pRNA is apparently more conserved than its sequence. Though mouse and human pRNA transcripts share less than 50% sequence identity, both possess a highly conserved stem-loop structure. In fact, expression of the human variant can rescue the phenotype of pRNA-depleted mouse cells, demonstrating the significance of the structure. The importance of secondary structure within the IGS is particularly striking considering the overall divergence in sequence similarity between mice and humans, as the IGS of these related species share only ~40% sequence similarity. Transcription of pRNA is driven by a spacer promoter that is 90% homologous.
to the rRNA promoter yet produces pRNA at a rate 1,000-fold lower. The resulting transcript is present at very low levels throughout most of the cell cycle, as it is rapidly degraded by the exosome. However, during mid-S phase pRNA levels transiently increase 2-fold, allowing it to repress rRNA synthesis in late replication. Whether pRNA originates from a region closely associated with rRNA, expression of these transcripts appears to be regulated independently, establishing pRNA as the first example of a functional ribosomal IGS-derived transcript (Fig. 2A).

**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, 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, the murine double minute 2 (MDM2)/promyelocytic (PML) protein complex in response to transcriptional stress as well as the heat shock protein 70 kDa (Hsp70) in response to heat shock. 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). 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). 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. IGS28RNA rapidly recruits and immobilizes NoDS-containing proteins at its site of transcription, thereby 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). This raises the possibility that novel editing pathways are involved and poses the pathways to collapse. Well-documented examples include the von-Hippel Lindau (VHL) tumor suppressor protein in response to anaerobic metabolism, the murine double minute 2 (MDM2)/promyelocytic (PML) protein complex in response to transcriptional stress as well as the heat shock protein 70 kDa (Hsp70) in response to heat shock. 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). 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). Bioinformatic studies suggest that up to 9% of all proteins encode an NoDS and consequently could be regulated by the nucleolus.

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question of the functional significance of post-transcriptional processing. The majority of IGS transcripts. In fact, transient overexpression of IGS RNAs has shown that the processed form of IGSrRNA is capable of inactivating NofRC-containing protein complexes, whereas its unprocessed counterpart failed to produce the same effect. This suggests that IGS RNA processing is an activatory step that regulates their function. Additional stresses like DNA damage have been shown to cause the nuclear sequestration of NoRS-containing protein complexes. 11,12 Given this diversity of stimuli, it is highly probable that the IGS contains additional sites of capture. Though they remain to be identified, novel IGS RNAs comparable to 16, 22 and 28 could be involved.

Concluding Remarks

The findings discussed here demonstrate that the IGS is not an inert region of the genome but in fact a complex and plurifunctional transcriptional unit organized into discrete loci. Some of these loci, located in close proximity to rDNA, serve to control rRNA transcription by recruiting chromatin remodeling complexes. Other loci, located deeper within the IGS, regulate molecular networks by capturing proteins in a stress-specific manner. The possibility that these two systems are related is not implausible. In fact, TIPs, the subunit of the NoRC, that interacts with pRNA, contains an NoDS. Likewise, sequestration of proteins at the IGS locus has been shown to trigger nuclear condensation.


and a decrease in precursor rRNA syn
thesis.9 The vast number of different pro
teins that appear to be regulated in trans by the IGS suggests that it is central to a variety of canonical cellular processes. In functional terms the bioinformatic data mentioned here, proteins empirically shown to be targets of the IGS include the catalytic subunit of DNA polymerase (POLD1), the DNA methyl transferase 1 (DNMT1), the E3 ubiquitin ligases VHL, MDM2 and RNF8, the anaphase promoting complex 2 (APC2), the 26S proteasome component Sagl and the chaperone Hsp70, among many others.13,14 Certainly, the IGS and its transcripts play an unforeseen yet central role in cell biology. Further characterization of the IGS may establish it as one of the cell’s primary regulatory systems.


42. Andressz K, Beninati WZ, Dong L, Myer DL, Li YQ, Tischfield JA. The novel mouse polo-like kinase 5 responds to DNA damage and localizes in the nucleolus. Nucleic Acids Res 2010; 38:2391-43; PMID:20103084; http://dx.doi.org/10.1093/nar/gkp151.