

# Policing Cells under Stress: Noncoding RNAs Capture Proteins in Nucleolar Detention Centers

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In this issue of *Molecular Cell*, Audas et al. (2012) demonstrate that a class of stress-induced noncoding RNAs immobilizes proteins in the nucleolus in response to a specific stimulus.

The cell nucleus is organized into specific subnuclear domains that are generally characterized by the presence of a unique set of proteins and RNAs within them. These nuclear domains help to provide a high local concentration of proteins with related functions so as to facilitate intermolecular interactions within a restricted area (Dundr and Misteli, 2010). Such an organization also prevents unwanted interaction with other nuclear factors that could compete or inhibit the desired function. Unlike cytoplasmic organelles, subnuclear domains lack a membrane enclosure. It has been demonstrated that the factors present in these domains show continuous and rapid exchange with the surrounding nucleoplasm. This supports a model of stochastic self-organization, where high-affinity interactions among molecules within a domain help to establish a steady-state residency time within these domains (Dundr and Misteli, 2010).

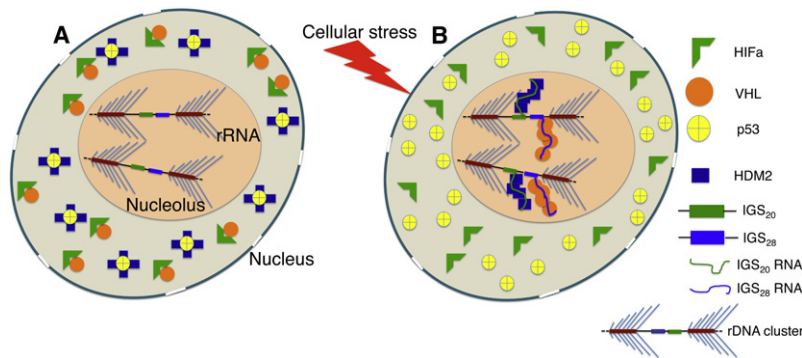
The nucleolus is one of the well-studied subnuclear domains and is involved primarily in ribosomal-subunit biogenesis (Boisvert et al., 2007). Recent large-scale proteomic analyses have demonstrated that the nucleolus contains ~4500 proteins, of which only ~30% have been linked directly to ribosome biogenesis (Boulon et al., 2010). It is now clear that other than its bona fide role in ribosome biogenesis, the nucleolus also coordinates several other vital cellular functions, including cell-cycle control, DNA replication and repair, and the stress response (Boulon et al., 2010). The nucleolus modulates several of these processes by sequestering or releasing proteins upon specific physiological signals. For example, it has been documented that upon cellular stress and oncogenic sig-

nals, P14<sup>ARF</sup> physically sequesters HDM2 into nucleoli, thereby relieving nucleoplasmic p53 from HDM2-mediated degradation (Boulon et al., 2010). Similarly, cellular changes in pH during hypoxia or acidosis result in the nucleolar sequestration of several proteins, including von Hippel-Lindau (VHL), a factor that, under normal oxygen tension, facilitates the degradation of hypoxia-inducible factor- $\alpha$  (HIF $\alpha$ ) (Figure 1). HIF $\alpha$  is a transcription factor that activates genes involved in hypoxic stress response (Mekhail et al., 2004). Earlier studies revealed that the stimulus-induced nucleolar-detained pool of VHL and HDM2 do not shuttle and are completely immobilized within the nucleolus (Mekhail et al., 2005). These results clearly demonstrated that the nucleolar detention of proteins is a key cellular mechanism that maintains cellular homeostasis during the stress response (Boulon et al., 2010). However, the mechanisms that control stress-mediated nucleolar immobilization of proteins remained to be elucidated. In this issue of *Molecular Cell*, Audas and coworkers demonstrate the role of long noncoding RNAs (lncRNAs) in the stimulus-induced detention of nucleolar proteins (Audas et al., 2012).

The mammalian genome encodes a large number of noncoding RNAs (ncRNAs) that do not code for proteins but function directly as RNA molecules (Zong et al., 2011). ncRNAs can be broadly classified into small, typically 20–200 nt in length (examples include microRNAs, siRNAs, and piRNAs), and long ncRNAs (lncRNAs) ranging in size from 200 nt to 100 kb. Although several functions have been attributed to small ncRNAs, the biological relevance of the

vast majority of the lncRNAs remains incompletely determined. The defined subcellular localization and spatial and temporal expression pattern of a large number of lncRNAs support the argument that they are a vital part of an extensive RNA control system that coexists along with proteins (Mercer et al., 2009). Some of the lncRNA-regulated functions include facilitating the recruitment of proteins to specific chromatin sites, acting as structural scaffolds of subnuclear domains, and controlling the activity and subcellular distribution of proteins (Zong et al., 2011).

To better understand the components that are responsible for the stimulus-specific nucleolar detention of proteins, Audas and coworkers conducted a chromatin immunoprecipitation (ChIP) analysis using probes scanning the entire rDNA cassette (Audas et al., 2012). In hypoxic cell extracts, both VHL and the catalytic subunit of DNA polymerase (POLD1) were found associated with intergenic spacer chromatin (IGS), 28 kb (IGS<sub>28</sub>) downstream of the rRNA transcriptional start site. Furthermore, the IGS<sub>28</sub> locus was found to transcribe an lncRNA only under hypoxic conditions; this lncRNA was further processed into an ~325 nt ncRNA (IGS<sub>28</sub> RNA). The non-polyadenylated IGS<sub>28</sub> RNA was transcribed by RNA polymerase I, localized to nucleoli, and associated with VHL and POLD1, and it was essential for the stimulus-induced nucleolar detention of VHL and POLD1. Interestingly, when the IGS<sub>28</sub> RNA was transiently expressed from a plasmid under neutral pH conditions, the ncRNA sequestered VHL in the cytoplasm as large aggregates, indicating that the IGS RNA-mediated detention of proteins does not necessarily require



**Figure 1. Proposed Model Depicting the Role of IGS RNAs in the Stress-Mediated Nucleolar Sequestration of Proteins**

(A) In unstressed cells, the nuclear and cytoplasmic pools of VHL and HDM2 interact with HIF $\alpha$  and p53 respectively, facilitating the degradation of HIF $\alpha$  and p53.

(B) Various cellular stress signals activate the transcription of noncoding IGS RNAs that immobilize NoDS-containing proteins in the nucleolar compartment. Low cellular pH activates IGS<sub>28</sub> RNA that immobilizes VHL, whereas transcriptional stress-induced IGS<sub>20</sub> RNA detains HDM2. Note that the figures are not drawn to scale.

nucleolar architecture. This could suggest that, under acidosis conditions, the endogenous IGS RNAs never leave the nucleoli and, as a result, detain proteins within the nucleolar compartment. Alternatively, the IGS RNA-mediated nucleolar immobilization of proteins might require other factors that are induced only upon a specific stimulus and are absent in cells under normal physiological conditions.

Besides acidosis, several other cellular stress signals also induce nucleolar detention of proteins (Emmott and Hiscox, 2009). Interestingly, Audas et al. (2012) demonstrated that various stress stimuli activated ncRNAs from different IGS regions, and each IGS RNA specifically interacted with a particular protein and controlled its nucleolar immobilization. For example, heat shock-induced IGS<sub>16</sub> and IGS<sub>22</sub> RNAs controlled the nucleolar association of HSP70, whereas several other IGS RNAs, including the one transcribed from IGS<sub>20</sub>, regulated the transcriptional stress-induced nucleolar immobilization of HDM2 (Figure 1). The transcriptional activation of individual IGS loci was tightly linked to a specific stimulus (e.g., IGS<sub>28</sub> RNA was activated only by acidosis and not by heat shock, whereas the heat shock-induced IGS<sub>16</sub> and IGS<sub>22</sub> RNAs did not respond to low pH conditions). Furthermore, it was revealed that different stress-stimuli could

activate the nucleolar immobilization of the same protein through its interaction with different IGS RNAs. This was observed in the case of HSP70, which was captured by IGS<sub>28</sub> during acidosis and by IGS<sub>16</sub> and IGS<sub>22</sub> RNAs upon heat shock.

The study by Audas et al. (2012) has raised a number of interesting questions. In general, the IGS regions of mouse and human show limited sequence conservation (~40%) and were previously thought to consist of nontranscribed sequence elements. It now remains to be determined how various stress signals activate transcription from a specific IGS region, as well as what specific factors and regulatory elements control stimulus-specific induction of IGS RNAs. It is also not clear whether various stress signals activate the transcription of different IGS RNAs from a single IGS or are induced from separate IGS regions. Alternatively, it is possible that different stress signals activate a single long primary transcript that is further processed into a specific IGS RNA, and the association with nucleolar detained protein determines the stability of IGS RNA.

Based on their results, Audas and coworkers propose that, upon various extracellular signals, ncRNAs are transcribed from the IGS region and then capture nucleolar detention sequence

(NoDS)-containing proteins to the rDNA cassettes in the nucleolus, even though the exogenously expressed IGS RNA could immobilize NoDS-containing proteins outside of the nucleolus. This indicates that the stimulus-induced IGS RNAs are somehow tethered to nucleoli by unknown factor(s) or sequence elements and that such an arrangement facilitates the nucleolar immobilization of proteins. It is possible that the newly synthesized IGS RNAs are tethered to IGS DNA elements at the sites of transcription by forming RNA-DNA hybrids. The ChIP data showing the interaction of VHL and POLD1 with DNA from the IGS regions further support this notion. Future studies using the recently described chromatin isolation by RNA purification (ChIRP) technique could test this model (Chu et al., 2011).

In conclusion, the study by Audas and colleagues elucidates another vital function of nuclear-retained 1ncRNAs and provides crucial evidence for the involvement of ncRNAs in regulating protein activity by controlling intranuclear protein distribution.

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