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Review

Mapping the cleavage sites on mammalian pre-rRNAs: Where do we stand?

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ABSTRACT

Ribosomal RNAs are produced as lengthy polycistronic precursors containing coding and non-coding sequences, implying that extensive pre-rRNA processing is necessary for the removal of non-coding spacers. Remarkably, this feature is conserved in all three kingdoms of life and pre-rRNA processing has even become more complex during the course of evolution. While the need for such extensive processing remains unclear, it likely offers increased opportunities to finely regulate ribosome synthesis and to temporally and spatially integrate the various components of ribosome synthesis. In this review we discuss our current understanding of pre-rRNA processing pathways in mammals (human and mouse), with a particular focus on the known and putative cleavage sites, and we compare it to budding yeast, the best eukaryotic model, thus far, regarding ribosome synthesis. Based on the emerging research, we suggest that there are likely more pre-rRNA processing sites and alternative processing pathways still to be identified in humans and that a certain level of functional redundancy can be found in the *trans*-acting factors involved. These features might have been selected because they increase the robustness of pre-rRNA processing by acting as "back-up" mechanisms to ensure the proper maturation of rRNA.

1. Introduction

The eukaryotic ribosome is a large, evolutionarily conserved ribonucleoprotein complex composed of four rRNA molecules (18S rRNA in the small subunit and the 25/28S, 5.8S, and 5S rRNAs in the large subunit) and approximately 80 ribosomal proteins. Three of the four rRNAs are transcribed by RNA polymerase (Pol) I in the nucleolus as a single polycistronic transcript comprising the 5′-external transcribed spacer (ETS)–18S rRNA–internal transcribed spacer (ITS) 1–5.8S rRNA–ITS2–25/28S rRNA–3′-ETS (Fig. 1). The 5S rRNA gene is transcribed separately by RNA Pol III [1,2]. Maturation of the pre-rRNA transcripts includes cleavage (processing) steps, chemical modification (pseudouridylation and methylation) of specific residues, assembly (folding of the RNA and association with proteins into pre-ribosomes), and transport (within the nucleus and through the nuclear pore complexes). All of these processes involve scores of protein *trans*-acting factors and small nucleolar (sno) RNAs [1,3] (see Box 1).

For two decades, research in the field of ribogenesis has been dominated by work on budding yeast. Thirty years of genetics, followed by the recent purification of pre-ribosomes, have led to the identification and characterization of about 200 ribosome synthesis factors; much fewer human ribosome synthesis factors have been characterized to date [4–6]. There are several reasons why ribogenesis is bound to be more complex in humans than in budding yeast. Firstly, human ribosomes are larger and contain additional ribosomal proteins and non-conserved rRNA extensions [7]. The mature human rRNAs are comparable in size to yeast, except for human 28S rRNA which has increased in size by ~1.5 fold. A greater difference in size is seen in the non-coding spacers, which underwent remarkable expansion, being extended 5-fold or more (see Fig. 1). The transcribed spacers contain a plethora of mono- and di-nucleotide repeats that act as hotspots for expansions potentially caused by replication slippage [8]. Secondly, human nucleoli, the sites of rRNA transcription and early pre-rRNA processing, have three, rather than two, subcompartments [9,10]. Thirdly, the human nucleolar proteome comprises at least ten times more proteins than its yeast counterpart (~200–300 estimated nucleolar proteins in budding yeast and up to ~4500 in human) [11,12]. Despite these differences, it was generally assumed, until recently, that extrapolation of the work carried out in yeast could be sufficient to understand how ribosome are synthesized and assembled in human cells, but this prediction is probably an

Abbreviations: Pol, polymerase; ETS, external transcribed spacer; ITS, internal transcribed spacer; RACE, random amplification of cDNA ends; RPS, proteins of the small subunit; PCR, polymerase chain reaction; siRNA, small interfering RNA.

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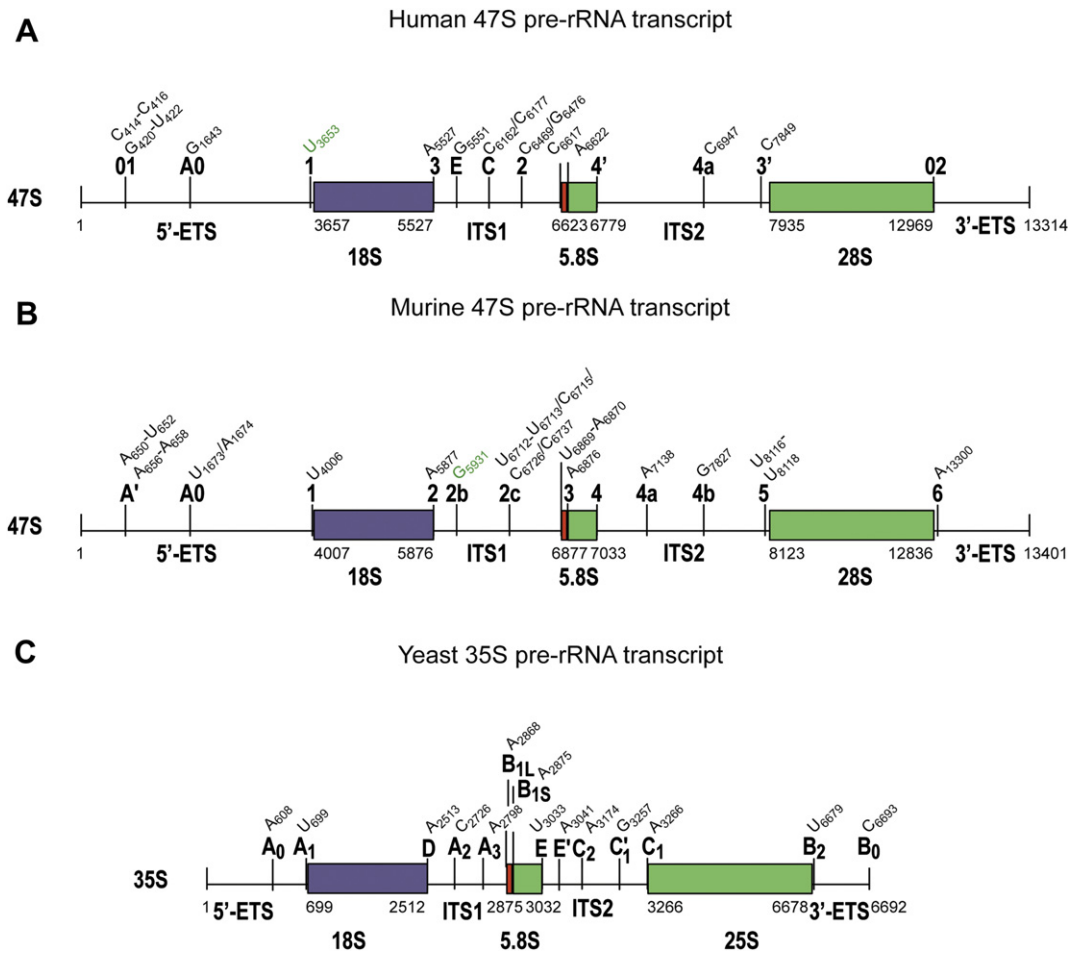

Fig. 1. Schematic of the human, mouse and yeast RNA polymerase I transcript. A, Human 47S pre-RNA; B, Mouse 47S pre-rRNA; C, Yeast 35S pre-rRNA. Three out of the four ribosomal RNAs, the 18S, 5.8S, and 25S (in yeast)/28S (in human and mouse), are encoded in a single long RNA Pol I transcript (47S in human and mouse; 35S in yeast). The fourth rRNA (5S) is synthesized independently by RNA Pol III (not represented). The coding sequences for the mature rRNAs are embedded in non-coding spacers, namely the 5′- and 3′-external transcribed spacers (5′- and 3′-ETS) and the internal transcribed spacers 1 and 2 (ITS1 and ITS2). Relative positions of known and predicted processing sites are indicated. The size of the mature rRNAs is largely conserved, except for the 28S which is about ~1.5 fold longer than the 25S. The size of the non-coding spacers has witnessed a dramatic expansion during evolution being up to ~5 fold larger in human than yeast. All processing sites were determined experimentally *in vivo*, except those color-coded in green, which were established in *in vitro* reconstituted systems.

oversimplification because of the frequent functional redundancy and increased complexity in higher eukaryotes.

Even though much is still unknown regarding the protein factors involved in pre-rRNA processing in humans, a number of the cleavage sites have been mapped over the last thirty years and a consensus processing pathway is available (Fig. 2A [13,14], reviewed in [4,15]). Using a combination of biochemical approaches, including Northern blotting, primer extension, and 3′ random amplification of cDNA ends (RACE) in combination with DNA sequencing techniques, processing sites have been mapped in the 5′-ETS, ITS1, ITS2, and 3′-ETS regions, yet recent analyses indicate that additional cleavage sites likely exist (e.g. [16–19]). In this review we discuss the known cleavage sites in mammalian (human and murine) rRNA transcripts, compare these sites to those known in budding yeast, and highlight the sites that require further study to resolve the location at the nucleotide level.

2. General overview of pre-rRNA processing in human cells

Pre-rRNA processing begins on the 47S primary transcript by snipping both ends of the molecule at sites 01 (sometimes called A′) and 02 in the 5′- and 3′-ETS segments, respectively, generating the 45S pre-rRNA (Fig. 2A). Previous studies indicated that the 45S precursor is matured following two major alternative pre-rRNA processing pathways [13,14]. In pathway 1, the initial cleavage occurs in the 5′-ETS at site A0 and is soon followed by cleavage at site 1. In pathway 2, the first cleavage event takes place at site 2 within ITS1. In cultured HeLa cells, the major contributor to ribosome synthesis is pathway 2. Each pathway offers an additional, optional "loop" that results from uncoupling at sites A0 and 1 (Fig. 2A). These "loops" generate the 43S and 26S pre-rRNAs in pathways 1 and 2, respectively. As far as we know, the two major pathways appear to differ not in the nature of the actual processing sites used but rather in the kinetics and order of cleavage. Similarly, two major pathways have been described in mouse (Fig. 2B). In addition to this post-transcriptional processing, it is quite possible that a fraction of the pre-rRNA molecules are cleaved co-transcriptionally, akin to the situation recently described in budding yeast. In fast-growing yeast cells up to 70% of pre-rRNA molecules are cleaved at site A_2, midway through ITS1 (see Fig. 3), while RNA Pol I is still actively transcribing the rDNA and approaching the 5′-end of the 25S gene [20–22]. Yeast site A_2 separates the precursors destined to be incorporated into the small and large subunit and is equivalent to site 2 in human and site 2c in mouse (Figs. 2 and 3). While co-transcriptional cleavage remains to be documented in mammals, we suggest that it might increase the overall efficiency of ribosome synthesis and, as such, be