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Review

Non-coding transcription by RNA polymerase II in yeast: *Hasard* or *nécessité*?

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ABSTRACT

Recent developments of microarrays and deep sequencing techniques have unveiled an unexpected complexity of the eukaryotic transcriptome, demonstrating that virtualy the entire genome is transcribed by RNA polymerase II (RNAPII). Transcription occurring outside of annotated regions is generally referred to as pervasive transcription and leads to the production of several classes of non-coding RNAs (ncRNAs). In this review we will discuss the metabolism and functional significance of these ncRNAs in the yeast *Saccharomyces cerevisiae*. We will discuss the mechanisms that the cell has adopted to prevent potentially disruptive interference between pervasive transcription and the expression of canonical genes. We will explore the possible reasons that justify the evolutionary conserved maintenance of extensive genomic transcription.

1. Introduction

Correct and efficient synthesis of functional RNAs is one of the main pillars of cellular metabolism. Messenger RNAs (mRNAs), that are essential for protein synthesis, are the first and best studied products of RNA polymerase II (RNAPII). RNAPII, however, also produces various non-coding RNAs (ncRNAs) such as small nuclear/small nucleolar (sn-/snoRNAs), small interfering (siRNAs), microRNAs (miRNAs) and long intergenic non-coding RNAs (lincRNAs), which greatly contribute to correct gene expression by regulating synthesis, processing or translation of various transcripts. Efficient and error-free synthesis of these ncRNA is thus vital for the cell and often tightly supervised. Thanks to novel high throughput techniques we have recently learned that virtually the whole genome is transcribed. Many newly identified ncRNAs originate from coding regions in a sense and antisense orientation as well as from intergenic regions. These RNAs seldomly have assigned functions, although the act of their transcription can influence gene expression. Their widespread production is conserved in *Eukaryotes*, leading to the notion of 'pervasive transcription'. Such ubiquitous initiation of ncRNA synthesis, sometimes useful for regulatory purposes, is potentially dangerous for the cell because it could interfere with « canonical » transcription events. Also, the ncRNA produced could sequester factors involved in processing or translation, or bind to complementary transcripts, thus affecting their function. Controlling pervasive transcription and the levels of ncRNAs produced is therefore vital for the cell.

Transcription termination and 3′-processing of the RNA are important tools used by the cell to control pervasive transcription and the production of ncRNA. These steps not only spatially limit the extent of transcription itself, but also have a major impact on the sub-cellular localization of the RNAs produced. Importantly, in some instances they ensure the handover of the nascent transcript to RNA quality control systems, which enhances the detection and degradation of pervasive transcripts. Degradation takes place both in the nucleus and in the cytoplasm, where dedicated systems exist to exonucleolytically destroy unwanted molecules.

In this review we will present different classes of ncRNAs produced by RNAPII in *Saccharomyces cerevisiae*, focusing on pervasive transcripts. We will discuss the mechanisms by which their expression is controlled by quality control systems that implicate transcription termination and RNA degradation pathways.

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2. RNA polymerase II transcribes a variety of non-coding RNAs

In budding yeast, non coding RNAs produced by RNAPII can be divided into two main classes: ncRNAs that are engaged in RNA modification or processing, such as small nuclear and small nucleolar RNAs (sn-/snoRNAs), and products of pervasive transcription.

Members of the first class influence a number of biological processes. SnRNAs participate in nuclear mRNA splicing. SnoRNAs confer specificity to proteins that introduce modifications in rRNAs, mainly pseudouridylation and methylation. In yeast cells, sn- and snoRNAs are generally transcribed as mono- or polycistronic transcripts that are processed by nuclear enzymes (including the nuclear exosome, see below) to their mature length and associate with specific sets of factors to form small nuclear or nucleolar ribonucleoprotein particles (snRNPs and snoRNPs).

Members of the second class, referred here as pervasive transcripts, profoundly differ from sn- and snoRNAs in that they are generally non functional. Their discovery was only made in recent years, when the transcriptome was analyzed by high throughput techniques, both for the distribution of steady state RNAs (e.g. by tiling arrays and, more recently, RNAseq) and transcribing polymerases [1–6]. Use of these techniques showed that transcription is not limited to canonical genes and that virtually all of the genome is transcribed. These novel ncRNAs are often synthesized as independent transcription units possessing their own promoter and termination signals; however, many originate from promoters of protein coding genes and are transcribed in the opposite direction [4,5].

These RNAs have been historically classified based on their stability and their sensitivity to different degradation pathways. CUTs (Cryptic Unstable Transcripts) were the first characterized class of non-coding RNA and were identified in a strain defective for nuclear degradation [1]. They are on average short (200-800 nt) and do not possess defined 3′ ends. SUTs (Stable Unannotated Transcripts), on the contrary, can be detected in wild type strains [2]. They are similar to mRNAs in size and have defined 3'ends. The distinction between CUTs and SUTs is sometimes blurry, as many SUTs are stabilized in nuclear RNA degradation mutants [7,8]. XUTs (Xrn1-dependent Unstable Transcripts) are long non coding transcripts similar to SUTs and are degraded in the cytoplasm by the 5′-3′ exonuclease Xrn1 [9]. NUTs (Nrd1 Unterminated Transcripts) are defined based on their sensitivity to the NNS termination pathway [see below, [10]] and largely overlap CUTs. Lastly, MUTs (Meiotic Unstable Transcripts) are only detected during meiosis [11].

The susceptibility to degradation of a given ncRNA depends on its sequence and on the pathway of transcription termination and 3'-end processing that are employed for its production. These factors will be detailed in the following sections.

3. RNA degradation limits the steady state levels of pervasive transcripts

Yeast pervasive transcripts are very heterogeneous in terms of stability and are extensively degraded both in the nucleus and the cytoplasm (Fig. 1). The exosome is implicated in RNA degradation in both of these compartments [for a recent review see Ref. [12]]. This conserved complex is composed of six core structural subunits forming a ring associated with three cap subunits responsible for RNA binding. The catalytic activity of the exosome relies on the Dis3 subunit, which associates with the core ring on the opposite side of the cap, and possesses two distinct nucleolytic activities, 3′ to 5′ exonuclease and endonuclease. In addition, in the nucleus, the exosome associates with a second conserved 3′ to 5′ exonuclease, Rrp6, which is a distributive enzyme as opposed to Dis3 that is processive. The activity of the exosome is stimulated by many co-factors among which the TRAMP4/5 complexes in the nucleus [1,13,14]. These complexes contain an RNA-binding protein (either Air1 or Air2), a polyA polymerase (Trf4 or Trf5, respectively in TRAMP4 and TRAMP5), and a helicase (Mtr4). The exact mechanism by which the TRAMP stimulates exosome activity is not completely elucidated. The addition of a poly(A) tail by Trf4 is thought to provide an unstructured RNA extension that favors threading of

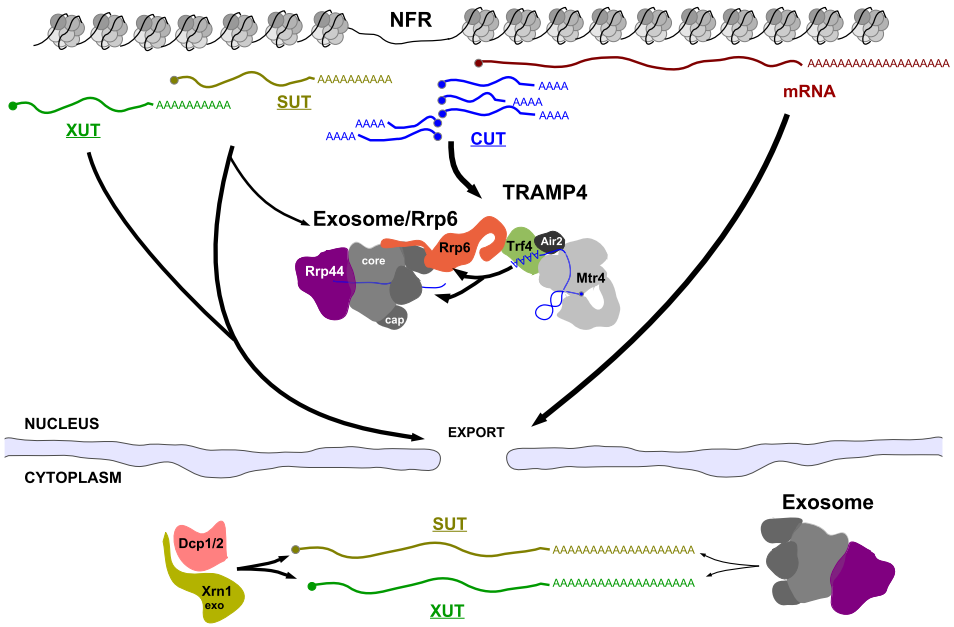

Fig. 1. Schematic representation of nuclear and cytoplasmic RNA degradation pathways. Several classes of coding and non-coding RNAs are transcribed from different regions of the genome. While mRNAs are directly exported and translated, different classes of non-coding transcripts are subject to nuclear and/or cytoplasmic degradation. CUTs are almost exclusively degraded by the nuclear exosome. SUTs and XUTs are preferentially exported to the cytoplasm, but, in the case of SUTs, a small portion is degraded in the nucleus. After export, SUTs and XUTs can either be subject to decapping by Dcp1/2 and degradation by Xrn1, or, less prominently, be directly degraded by the cytoplasmic exosome.

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