A Role for an Untranscribed Gene

Keeping the Distance

The transcription and expression of genes is regulated by transcription factors. The expression of some genes may cease in response to alterations in cellular or environmental conditions. Such untranscribed genes are usually lost by selection during evolution. Therefore, if an untranscribed gene is present, we speculate that it is not non-functional but is likely to have an as yet unidentified role in the cell. Here I will describe such roles that we recently identified for the ribosomal RNA gene cluster, rDNA [1].

Untranscribed Genes

Three unique regions stand out when one examines the eukaryotic genome. One is the telomere. Telomeres are located at the ends of chromosomes; they protect the ends from degradation by nucleases and prevent connections with other chromosomal ends. Another is the centromere; this region associates with microtubules and controls the segregation of the chromosome during cell division. In the cells of higher eukaryotes, the centromere has a very large repetitive structure. Finally, the largest unique region is the so-called ribosomal DNA (rDNA). rDNA consists of tandem repeats of the ribosomal RNA genes that encode the ribosomal RNAs that form the skeletal framework of the ribosome.

Human cells have five rDNA regions, located on chromosomes 13, 14, 15, 21 and 22. Each of these regions has approximately 70 copies of ribosomal RNA (rRNA) genes, giving a total of approximately 350 copies (~16 Mbp) per haploid genome. In budding yeast, the most well-studied eukaryotic cell, about 150 copies (1.4 Mbp) of the rRNA genes are located on chromosome XII. Plants generally have more copies. For example the pea has about 4000 copies (see Table). One reason for the presence of so many copies in the genome is to meet the huge demand for the product, rRNA. The ribosome, a complex of rRNAs and ribosomal proteins (rP), is a central player in gene expression, translating mRNA into protein. Ribosomes are present in very large numbers: rP accounts for approximately 50% of the total protein in a cell and rRNA represents approximately 60% of the total RNA [2]. Interestingly, however, about half of the rDNA copies are not transcribed in yeast.
and human cells [3].

In some plants, only a small percentage of the copies is transcribed. Therefore, it is a long-standing puzzle why there are so many untranscribed copies in the rDNA.

The repetitive nature of the rDNA region makes it highly recombinogenic and vulnerable to loss of copies after deleterious recombination events among the repeats. However, the copy number maintenance system ensures that the cell has a sufficient number of rDNA copies including many untranscribed copies [4]. In addition, each species has a characteristic "correct" copy number as shown in Table 1. This suggests that untranscribed copies are important and functional in the cell.

**rRNA Copy Number Maintenance**

Our earlier study of the mechanisms of rDNA copy number maintenance identified a unique recombination system for rDNA amplification. In this system, DNA replication is inhibited by a fork blocking protein, Fob1, generating double strand breaks that induce recombination [5].

During this recombination process, some rDNA copies are replicated twice and the copy number increases. Therefore, by inhibition of the expression of FOB1 during the amplification, we can fix the copy number at various levels. Using this approach, we succeeded in isolating yeast strains with 20, 40, 60, 80, or 110 copies of rDNA. Interestingly, the low copy number strains, those with 20 or 40 copies, grew normally and produced a similar level of rRNA as wild-type cells. In these low copy number strains, all of the rDNA repeats were strongly transcribed and there were no longer any untranscribed copies. We characterized the low copy number strains and found they had increased sensitivity to DNA damage by factors such as ultraviolet radiation and carcinogens. Moreover, there was a clear negative correlation between copy number and sensitivity to such DNA damaging factors, i.e. the lower the copy number the greater the sensitivity. Thus, the sensitivity of
the cells to damage was determined by the rDNA copy number, although the number did not affect growth rate.

Why does the cell show an increase in sensitivity to DNA damage when the rDNA copy number is reduced? To answer this question, we analyzed yeast strains with mutations affecting DNA damage repair and rDNA stability to identify the gene involved in copy number-dependent damage sensitivity. We found that rDNA transcription was necessary to make the cell more sensitive when the copy number was reduced. In addition, DNA double strand break repair genes were also required. These observations suggested that in the low copy number strains, all of the copies are actively transcribed and that transcription interferes with DNA repair. In other words, the untranscribed copies are working as a "footing space" where the DNA damage repair enzymes gather and do their jobs. We investigated the behavior of condensin, which is responsible for sister chromatid cohesion during DNA damage repair, and found that its association with rDNA was especially inhibited in strains with a low copy number (see fig. 1) [6].

**Untranscribed Genes and Damage Repair**

Our studies indicate that the untranscribed copies are necessary for damage repair. Therefore, in low copy number strains without untranscribed copies, the rDNA becomes increasingly unstable (as it is not repaired) and the cell cannot survive under DNA damaging conditions. More interestingly, this instability effect is not confined to the rDNA. We found that other chromosomal regions also showed instability in the low copy number strains. This may be a consequence of the activities of the repair enzymes being concentrated on the damaged rDNA to the detriment of other damaged regions for which efficient repair cannot be afforded. Damage to the non rDNA regions would also impair cell viability under stressful conditions.

Overall, as the rDNA is comprised of repeats of highly transcribed genes, it needs "space" for repair. Therefore, a higher rDNA copy number facilitates greater repair efficiency, resulting in better cell viability. This also implies that cells with higher number of rDNA copies would be positively selected during evolution, possibly explaining the development of the gene amplification system. It may also explain why plants have more rDNA copies - they are always exposed to ultraviolet radiation from the sun.

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References


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