Current Biology, Vol. 13, R482–R484, June 17, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S0960-9822(03)00408-1

RNA Repair: Damage Control

Dispatch

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RNA in a cell is subject to many of the same insults as DNA. RNA damage can induce apoptosis and may be exploited for anti-cancer chemotherapy. It is a surprise, however, to learn that cells may repair RNA damage, suggesting a far greater significance of RNA in genotoxic stress.

Damage to DNA is a significant issue for all cells, particularly in cancer where DNA repair commonly fails. It is not widely appreciated that many agents that cause damage to DNA, such as radiation and certain cancer chemotherapy drugs, also damage RNA. Given that there is at least as much RNA in a cell as DNA, wherever DNA is damaged by such agents, RNA is surely damaged as well. When the damage to RNA is substantial, apoptosis is induced, which is the desired affect of anti-cancer chemotherapy [1]. Nevertheless, RNA has not been the major focus in investigating how cells cope with such insults (until very recently, 'RNA damage' and 'RNA repair' did not crop up in the PubMed index). There have been scattered insights over the years into the significance of RNA to genotoxic stress, but a recent report by Aas and colleagues [2] suggests that the cell has at least one specific mechanism to repair RNA damage, indicating a greater investment in the protection of RNA than previously suspected.

AlkB Reverses Alkylation Damage

Alkylating agents are endogenous and environmental compounds that cause mutations, tumors and neurotoxicity. Chemically, they add alkyl groups, like methyl or ethyl groups, to organic macromolecules, in particular to the ring nitrogens and oxygens of bases of nucleic acids. Cells have developed a host of repair systems to deal with alkylation damage of DNA. These include the removal of the damaged residues by DNA glycosylases, followed by replacement of the nucleotide by DNA polymerases using the opposite strand as template. Another mechanism is the direct reversal of the methylation damage, which does not require a template to make the repair. For example, the Ada and Ogt enzymes of *Escherichia coli* restore the normal base of an alkylated DNA by directly removing the offending chemical group in a suicidal reaction. The *E. coli* enzyme AlkB has long been known to act in alkylation damage repair, but until recently its mechanism of action was not clear, as biochemical assays failed to detect in the AlkB protein any of the enzymatic activities known to occur in DNA repair enzymes.

In a remarkable display of the power of bioinformatics applied to genome sequences, Aravind and Koonin [3] predicted, based on its relationship to another family of enzymes, that AlkB would cause hydroxylation of the methyl group on damaged DNA bases, and thus directly reverse alkylation damage. The Seeberg [4] and Sedgwick [5] labs independently confirmed this prediction and showed that, indeed, AlkB enzymatically demethylates the DNA bases adenine and cytosine, unlike the suicidal Ada and Ogt proteins, by oxidative demethylation: the methyl group is converted to an hydroxymethyl group which then leaves the base as formaldehyde.

Now there has been another twist to the plot. From the large number of AlkB homologues in plants and their RNA viruses, Aravind and Koonin [3] predicted that some members of this family might act on RNA bases modified by alkylating agents. Indeed, in their recent article, Krokan and colleagues [2] describe two human AlkB homologues, hABH2 and hABH3, and show that AlkB and hABH3 have RNA repair activity. Recombinant AlkB and hABH3 proteins demethylate methylated bases in RNA *in vitro*. Furthermore, expression of AlkB and hABH3 in an *alkB* mutant strain of *E. coli* reactivated a methylated RNA virus, providing functional proof of the relevance of RNA repair activity. While AlkB and hABH3 prefer single-strand DNA, hABH2 prefers double-strand DNA substrates and lacks significant RNA repair activity. It remains to be seen, however, whether hABH2 has activity on double-strand RNA.

Functional specialization is also reflected in the different subcellular localization of the two human enzymes [2,6]. hABH2 localizes to the nucleoplasm with some accumulation in nucleoli, indicating a possible involvement in the repair of ribosomal RNA, but in S phase it colocalizes with replication foci. hABH3 has a predominantly nucleoplasmic localization, with nucleolar exclusion and some cytoplasmic staining. This differential compartmentalization raises the intriguing question of what are the physiological targets of hABH2 and hABH3 in eukaryotic cells. This may be important, as some of the modifications induced by alkylating agents are within the spectrum of normal physiological RNA modifications [2].

Coping with RNA Damage

Cells may have mechanisms of dealing with RNA damage other than direct repair (Figure 1). RNA chaperones, for example, may help cope with broken or crosslinked RNA molecules so that they can be sequestered and degraded. For example, the protein YB-1, which binds nucleic acids, may be just such a chaperone. Several studies have connected YB-1 expression with chemotherapy resistance and disease prognosis in cancer [7]. Elevated expression of YB-1 can confer on cancer cells resistance to nucleic acid-damaging agents, such as cisplatin [8]. How YB-1 does this may not be entirely clear, because the initial suggestions that it does so by regulating other drug resistance genes have been challenged [9]. *In vitro*, this protein acts as an RNA chaperone by aiding in the unwinding and winding of

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RNA duplexes [10]. Perhaps YB-1 is part of a general RNA handling machinery that helps move the molecules from one complex to another.

Another gene with a potential role in RNA damage control is *LSM1* of budding yeast. Deletion of *LSM1* causes resistance to ultraviolet radiation [11]. *LSM1* encodes a protein involved in mRNA decapping and protection against trimming of 3′ untranslated regions [12], suggesting that regulation of mRNA stability may affect the response to exogenous RNA and DNA damage. Interestingly, the human *LSM1* orthologue is associated with the invasive potential of prostate cancer and perhaps other neoplasms [13].

Additional isolated suggestions that other general RNA binding proteins contribute to a cell's ability to deal with nucleic acid damaging agents have appeared [14]. There are many RNA binding proteins encoded in the genome with no known function. Perhaps one question to be asked of these is whether they play some caretaking role for the cell's RNA.

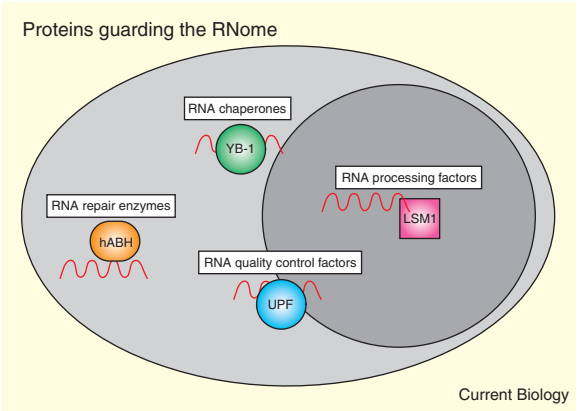

Figure 1. Proteins at all stages of an RNA's life may passively or actively protect it from damage.

Damaged RNA may simply interfere with a cell's normal activities, and/or it may induce checkpoints leading to apoptosis, as DNA damage does.

Anti-Cancer Chemotherapy and RNA Damage

It is well established that RNA damage caused by anticancer chemotherapy agents is an important component of their action. For example, the incorporation of 5-fluorouracil (5-FU) into newly synthesized RNA appears to be the primary determinant of its cytotoxicity [15]. Cisplatin, which forms adducts on both DNA and RNA, inhibits translation in cancer cells by crosslinking of mRNA to ribosomal RNA or ribosomal RNA to itself [16]. Adriamycin (doxorubicin), an anthracycline drug which intercalates between base pairs of double-helical nucleic acids, has been shown to bind RNA helices and inhibit RNA helicase, an activity essential for RNA synthesis, processing, transport and turnover [17].

Considering that there is generally more RNA in a cell than DNA, most of it in the form of ribosomes that turn over at relatively low rate, it is likely that there will be significant damage to cellular RNA when cells are treated with these drugs. Furthermore, the 'information content' of cellular RNA is greater than that of the chromosomal DNA: whereas only 3% of the human DNA has coding potential, almost all RNA sequences in the cytoplasm have functional significance, whether coding for proteins (mRNA) or performing translation (ribosomal RNA and tRNA). Therefore an agent that can affect RNA or DNA equally well from a chemical perspective is more likely to cause significant RNA damage in a living cell.

Importantly, a new class of anticancer agents that specifically damage RNA has been shown to be effective and is currently undergoing clinical trials. This cytotoxic ribonuclease — called Onconase — cleaves tRNA, and in so doing, induces apoptosis via a p53-independent mechanism [1]. Drugs that interfere with the synthesis of RNA and DNA precursors — for example hydroxyurea and methotrexate — or that block RNA polymerase — for example actinomycin D and α-amanitin — are also known to induce p53 and cause cell cycle arrest [18]. These examples show that RNA damage can lead to cell-cycle arrest and cell death, much as DNA damage does. The precise mechanisms by which apoptosis is induced by RNA damage deserves further study.

RNome Stability: A Link to Cancer?

Alterations of genome stability have an important role in tumorigenesis, particularly in the inherited predisposition to cancer. Typically, DNA repair genes mutated in sporadic and hereditary tumors have a double function: they effect DNA repair and also regulate cell cycle and apoptosis checkpoints. Inactivation of these genes increases the mutation rate and at the same time prevents engagement of checkpoints, resulting in a growth advantage. Is it possible that an ability of RNA damage to induce similar checkpoints means that defects in RNome stability might also play a role in tumorigenesis? One suggestion that this is so comes from the study of inherited predisposition to cancer. For example, the hereditary prostate cancer gene *HPC2/ELAC2* encodes a protein that shows homology to the mRNA cleavage and polyadenylation specificity factor CPSF73 [19], indicating a potential link between mRNA biogenesis and cancer predisposition.

A cell has a great investment in its RNAs — they are working copies of genomic information. The study of mRNA biogenesis in the last few years has revealed an elaborate surveillance mechanism involving factors such as the UPF proteins that culls aberrantly spliced mRNAs and mRNAs with premature termination codons. There might be a hint that such RNA quality control mechanisms go awry in cancers, just as DNA quality control mechanisms do, where aberrantly spliced transcripts accumulate in a tumor [20]. Now that the gates are open, we may have a flood of studies on RNome stability and cancer.

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