Viable RNase H1 knockout mice: The end of an enigma

Human cells have two RNase H enzymes, HI and H2. RNase H enzymes degrade RNA but only when an RNA strand is hybridized to a DNA strand forming an RNA-DNA heteroduplex. These enzymes are present in almost all animal and bacterial cells and are highly conserved, suggesting that they must play important roles in the cells. Although it has been known for some time that RNase H2 is important for de-novo DNA synthesis, i.e. replication of genes because synthesis of new genes employ old genes as a template and the process is primed by a small strand of RNA that must be removed, the roles of RNase H1 have been more difficult to unravel.

Interest in RNase H1 intensified and because of a more practical concern when efforts to create a new drug discovery technology (antisense technology) that targets RNA rather than protein (the targets of most of the types of drugs) showed that DNA-like antisense drugs when bound to their target RNAs caused degradation of the RNA by RNase H1 (and not RNase H2).

Cells and animals in which a gene is “knocked out”, i.e. disabled, so it can no longer make the protein it codes for are useful in determining what role the proteins (and therefore the genes) play in the cell of the animal. An earlier attempt to create RNase H1 knockout mice resulted in the death of the embryos. This demonstrated once again how important RNase H1 must be and showed that it is vital in the formation of the energy producing organelles in mammalian cells, mitochondria, but since all the fetuses died *in utero*, it provided no insights into the biological roles of RNase H1 in mature animals.

The strategy that we employed used a system that inactivated the RNase H1 gene near the birth of the knockout mice and to knock out the enzyme only in the liver rather than the whole animal. We hoped that such an approach would result in viable animals with RNase H1 knocked out in only one type of cell, liver cells, and this would afford us the opportunity to understand the enzyme’s role in mature cells and animals.

Fortunately the idea worked (it took almost 3 years of work before we knew that it worked) and we showed that the loss of RNase H1 in liver cells is lethal to those cells because their energy forming units, the mitochondria, stop functioning. We were able to map out the time course (14 week) of the liver failure, then induce new RNase H1 to be made and watch recovery. Using modern genomic methods we were also able to see how the liver cells first tried to compensate for the loss of RNase H1 and eventually died.

Thus, our study is the first to be able to address the various functions of RNase H1 in mature cells and provides a tool for many investigations. The primary defect in these mice is that they lose mitochondria because RNase H1is necessary not for DNA synthesis, but to facilitate transcription of the genetic information in to messenger RNA. Sometimes during the conversion of genetic information in genes to RNA (the process called transcription) the long nascent strand of RNA can loop back on itself forming almost a knot which is called an R Loop. If these mistakes are not

rigorously edited, transcription stops and, of course, that is fatal. RNase H1 is present in mitochondria, nuclei, and cytoplasm. It corrects R Loops in mitochondria and collaborates with Topoisomerase 1 in the nucleus to do the same job i.e. remove nuclear R Loops.

So now we know the primary functions of both RNase H1 and H2 and we know that when we administered DNA-like antisense, we take advantage of a natural cellular component, RNase H1, to produce therapeutic gain.

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