**From CTG (Cal Tech Geneticist) – March 29, 2018**

Once again, the credibility of that mummy article among professional molecular biologists is very low, because: (1) it sat out in the desert for >100 years, and (2) it shows lots of “novel” mutations not found in medical databases, which are therefore probably artefactual. They may not think the mummy is “alien”, but they suspect that the paper is “wrong”.

Interestingly from a medical point of view, most of those critical mutations which Nolan cites seem to be heterozygous, or present on 1 chromosomal copy only (listed in his Supplemental Tables), which would be unusual for real mutations of developmental significance.

Finally here is a technical note which you might not wish to read in detail, quite long and boring, but which can refute anyone who says that Dr. Nolan used a computer program to “prove” there was no DNA damage?

Appendix 1. An indirect computer model of DNA damage was used by the Chilean mummy researchers: what is it really telling us?

The researchers who did full-genome sequencing on this small Chilean mummy used an indirect, statistical approach to estimate damage to the DNA bases in their 300 bp, partly-degraded sample: namely a computer program called “mapDamage2.0”. This program measures variations of base sequence, away from mean values, near the two ends of any 101 bp sequence-read. Such variations come about partly because damaged (or deaminated) cytosine C bases may become converted to thymine T by polymerase-reading errors. One or more C-to-T transitions near the end of a PCR product may reduce its efficiency of ligating to a synthetic linker used later in sequencing, if that double-helical end becomes too AT-rich.

We can see typically an excess of false C-to-T transitions near any 5’ end, and false G-to-A transitions near any 3’ end (see their Figure S1, lower part). The researchers of that small Chilean mummy did try to exclude some artefactual biases due to DNA damage from their final sequenced database, close to the two ends of any 101 bp sequence-read. Thus they “trimmed off” non-random sequence biases there of about 2% on each end. The program “mapDamage2.0” also suggested, for their final sequenced database, statistical estimates of C deamination as 2% near the ends of any 101 bp sequence-read, or 1% near their centres (meaning 1 altered base in every 50 or 100 bp respectively).

This program offers a fine way for researchers of ancient DNA to estimate approximately how “damaged” their DNA samples might be, by looking for artefactual biases of sequence away from random in large datasets. It also helps researchers to “trim away” some obvious sequencing artefacts near each end of any 101 bp product. Yet the slow, age-related deamination of C bases in ancient DNA may come about in two ways. Firstly because each end of a 300 bp mummy DNA fragment may have extended or frayed into a single-stranded form, which makes C bases there more exposed to damage. Secondly, because other internal parts of a 300 bp mummy fragment may have become chemically damaged in some way.

Even if we sequence some particular DNA strand 10 times over in excess, and trim away the two ends of wherever that 101 bp sequence might begin or end, still certain sequence errors will take place when deaminated C is copied to T in PCR, no matter where that might happen. The damaged base deaminated-C will be “read” by the polymerase as “U”. There is no way to avoid this, except by treating the original genomic sample with an enzyme such as UNG before PCR and sequencing. You cannot remove DNA base damage by “trimming”.

The very process of breaking down a long DNA double helix into small fragments of mean size 300 bp involves chemical damage to its A or G bases (called “depurination”). So we can be fairly sure that significant chemical damage to the DNA bases has taken place within this small Chilean mummy, which lay exposed in the desert for 100-500 years! Both to its A or G bases (“depurination”) and also to its C bases (“deamination”).

Why not measure such patterns of chemical damage directly to find out for sure? As opposed to all of these indirect measures, one could simply label the 5’ or 3’ ends of those 300 bp mummy DNA fragments with a fluorescent or radioactive marker, treat the labelled sample with an enzyme UNG, high heat, or other strand-breaking-at-damaged-base conditions, then look by gel or capillary electrophoresis to see whether the mean single-stranded length of that mummy sample was reduced? Such a quick and easy experiment would provide a direct measure of whether those 300 bp mummy DNA fragments were significantly damaged by C deamination. Yet no experiment of this kind was reported, despite many “novel” mutations being found in their final sequenced database, and being given great significance by bioinformatics strategies.

Jonsson et al., “mapDamage2.0: fast approximate Bayesian estimates of ancient DNA damage parameters”, Bioinformatics 29, 1682-1684, 2012)

Seguin-Orlando et al., “Ligation bias in Illumina Next-Generation Libraries: implications for sequencing ancient genomes”, PLoS ONE 8 (10), e78575, 2013.

THE END