To What Extent is Junk DNA "Junk", and Why?

"[Junk DNA] had little specificity and conveys little or no selective advantage to the organism", stated Francis Crick and Leslie Orgel in an article for "Nature" in 1980[1]. The pufferfish, *Fugu rubripes*, is seemingly living proof of the veracity of this statement. Although the pufferfish genome contains roughly the same number of genes as the human one, it is approximately 9 times smaller with a genome of only 340 megabases, compared to the human 3.1 gigabases[2]. For this to occur, the majority of the pufferfish genome must be genes, and a very small proportion is therefore non-coding or "junk" DNA. The pufferfish may have very little junk DNA compared to other vertebrates, but it does not seem to be at any obvious disadvantage: although it may be slow moving, it can deter predators by ingesting large quantities of water to make it seem larger and more threatening, and can even produce a toxin (called tetrodoxin) which is 1,200 times more poisonous than cyanide[3]. However, if junk DNA is worthless and really has "no selective advantage", why has evolution not removed it to prevent precious energy and resources from being wasted on its transcription? What is the point of such a large DNA helix if only 2%[4] of which are protein coding genes and the rest is "junk"? Evolution is ruthless, and 3.5 billion years[5] of it would not have let junk DNA to continue existing if it was not an asset to the organism.

Chromosomal Junk: Telomeres and Centromeres

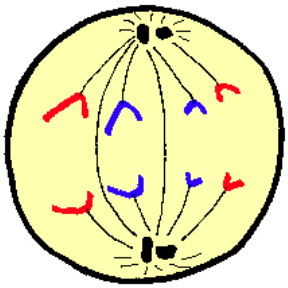

Fig. 1 Spindles fibres pull sister chromatids to opposite poles by the centromeres

One of the most important processes for an organism to complete is cell division. Mitosis is integral for growth, and to repair dead or damaged cells, and meiosis allows for sexual reproduction as it produces cells, gametes, with a haploid number of chromosomes so that the diploid number can be regenerated at fertilisation. This means that genes can be maintained and passed on to future generations, but to do this a piece of junk DNA is vital. Centromeres are an internal piece of junk DNA within a chromosome and are the site at which CENP-A (Centromeric Protein-A) binds[6]. Spindle fibres, "aggregates of microtubules that move chromosomes during cell division"[7], then recognise and adhere to these proteins in anaphase, before contracting and pulling the sister chromatids to opposite poles in the nucleus (Fig. 1). Once this has occurred, telophase and cytokinesis can complete the cell cycle.

Furthermore, with each cell division, the chromosomes begin to shorten by roughly 30-200 base pairs[8] as the very ends of it are left single stranded and unreplicated for enzymes to remove. However, another type of junk DNA, in the form of telomeres (Fig. 2), which are constructed of repeating TTAGGG units (AATCCC on the complementary DNA strand)[9], prevents actual genes from being lost with repeated cell division. These telomeres are placed as buffers on the ends of the chromosomes, and are removed in cell division in the place of actual genes. When all the telomeres have been used up, although an enzyme called telomerase does rebuild them to some extent, the cell becomes either senescent or undergoes apoptosis so that incomplete genes cannot be passed on to subsequent generations.

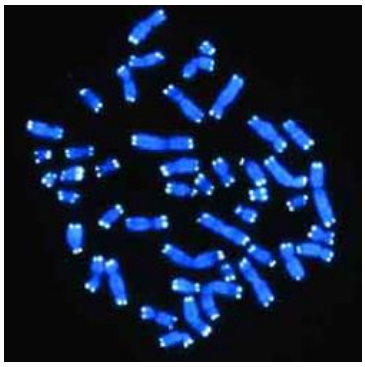

Fig. 2 The highlighted loci indicate telomeres

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From this it can perhaps be inferred that the longer the telomeres in an individual, the more times cell division can occur, and thus the longer they would be expected to live. A study completed at the University of Utah, USA, compared ""Healthy" centenarians (n=19) with physical function in the independent range[10] and the absence of hypertension, congestive heart failure, myocardial infarction, peripheral vascular disease, dementia, cancer, stroke, chronic obstructive pulmonary disease, and diabetes….to centenarians with physical function limitations and ≥2 of the above conditions (n=19)."[11] The mean telomere length of individuals with healthy characteristics was 5770.8 ± 3132.3 base pairs, and that of those who were "unhealthy" was 3657.2 ± 2019.2 base pairs. Although there is overlap in the error margins of the data, it can still be concluded that "Healthy centenarians had significantly longer telomeres than… unhealthy centenarians". As good health increase the potential for longevity, it may also be concluded that a long life is, at least, partially reliant on telomeres, and therefore junk DNA.

In addition, telomeres also act as buffers between two chromosomes, allowing for repair to be correctly carried out if there is more than one break in a chromosome (or chromosomes) in the DNA sequence. Usually, if there is only one break, a repair mechanism will identify it and rejoin the sections of DNA as quickly as possible. However, the repair mechanism is unable to tell the difference between the ends of the chromosomes and the breakages, meaning that there is a high possibility that strands of DNA will be incorrectly rejoined, causing mutations and possibly even death of the cell. On the other hand, with telomeres at the ends of the chromosomes, the repair mechanism is then able to differentiate between these, and the strands can be correctly joined again.

X-chromosome Inactivation and Transcription Management

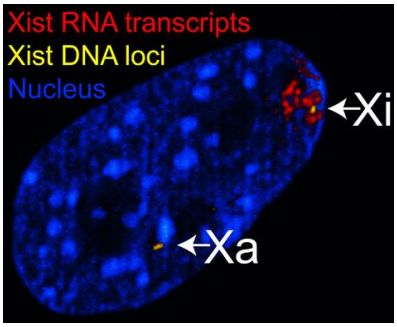


Fig. 3 Xi = the inactivated X-chromosome at the periphery of the nucleus. Xa = the active chromosome

In almost every female mammal, there are two X-chromosomes, which means that there is the potential of double the amount of X-chromosome linked genes being expressed. Therefore, one X-chromosome must be inactivated and excluded to the periphery of the nuclear envelope[12] to maintain a genetic balance within the cell (Fig. 3), and to do this, our body needs another piece of junk DNA or, more precisely, long non-coding RNA (lncRNA). XIST (X-inactive specific transcript) is used to initiate this X-chromosome inactivation process, targeting and spreading over the future inactive chromosome in the "painting" process[13], before silencing complexes are then attracted to the site to aid it and to ensure the job is completed irreversibly. Before X-chromosome inactivation, TSIX, the complementary RNA strand to XIST, is expressed at both chromosomes; however, after the process starts, TSIX is only expressed at the active X-chromosome. As the relationship between the two lncRNAs is inversely proportional[14], when the expression of TSIX is increased at a chromosome, the expression of XIST decreases, meaning that only one X-chromosome is shut down and the other remains active[15].

Moreover, silencing elements are not used exclusively for this one isolated incident: they are part of the transcription elements employed to regulate gene expression. Genes are either kept reversibly "off" and not transcribed as chromatin, which produces a shape that transcription factors (proteins) cannot bind to, or they are left untouched and "on"[16]. Junk DNA, in the form of transcription elements can manage gene expression on a much more sensitive level, allowing for transcription to be varied to many more degrees than being just "off" and "on". Enhancers are short, cis-acting pieces of DNA, only 50-100 base pairs in length[17] that can

both improve the speed and the frequency of transcription. They do this by binding to a transcription factor, forming an enhanceosome, which attracts chromatin-remodelling proteins and RNA polymerase, allowing for transcription to be "turned up"[18]. Silencers, on the other hand, cause the opposite effect. These can slow or stop transcription by working with repressor proteins to ensure the DNA is tightly packed and supercoiled around histone proteins. Finally, insulators assure genes are preserved from the effects of both of the other two elements.

An example of the importance of insulators is illustrated by the protection of pseudoautosomal regions in X-chromosome inactivation, mentioned above. It is important to note that Y-chromosomes also contain pseudoautosomal regions, so males as well as females feel the same consequences of their loss; however, focus has been given here to X-chromosomes because of the link they share with the effect of insulators. During X-chromosome inactivation, XIST spreads down the chromosome, causing most of it to be silenced, but there are some areas that are preserved by the presence of insulator regions – regions of junk DNA. These are the pseudoautosomal regions (PAR1 and PAR2 in X- and Y-chromosomes specifically[19]), which are important for a number of reasons. The X- and Y-chromosomes recognise these areas in meiosis, allowing them to pair up correctly: without PAR1 infertility can result. Mutation or loss of the SHOX (short-stature homeobox-containing) gene, located on PAR1 is also primarily linked to Léri-Weill dyschondrosteosis, Langer mesomelic dysplasia, Turner syndrome which is only prevalent in females, and idiopathic short syndrome, which are all connected to bone growth disorders and abnormal height. Langer mesomelic dysplasia, which is closely linked to Léri-Weill dyschondrosteosis[20], is a bone growth disorder affecting the radius (in the forearm) and tibia (lower leg) often making them shorter, thicker and more curved than in a normal human. Similarly, both the ulna (forearm) and fibula (lower leg) may be underdeveloped or not even present at all, leading to shorter legs and arms and a short stature[21].

DNA Methylation

Insulation and repression of specific genes can also be seen in irreversible DNA methylation, as junk DNA and epigenetics, the study of the changes caused by external factors on gene expression, are combined. Methylation in eukaryotes can occur every time there is a cytosine nucleotide base next to a guanine in the DNA sequence. This is also called a CpG motif, while a large collection of unmethylated units are referred to as CpG islands. DNA methyltransferase (DNMT) enzymes either bond the methyl group to the cytosine, converting it to 5-methylcytosine (Fig. 4), or maintain a previous methylation in DNA replication[22]. To "switch off" the genes, the methylated CpG units bind to MeCP2 (Methyl CpG binding protein 2)[23], which in turn attracts the transcription silencing, histone deacetylase repressive machinery[24]. In essence, this creates a positive charge on the protein, giving it a greater affinity for DNA, which is negatively charged, and histone proteins, resulting in more highly compacted chromatin. This then blocks transcription factors and can prevent RNA polymerase from binding, and thus transcription is limited[25].

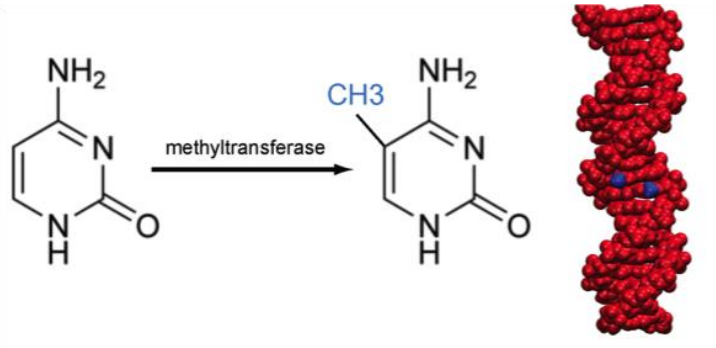


Fig. 4 DNA methylation of cytosine

It is universally believed that to make a zygote, a female nucleus and a male nucleus need to fuse in fertilisation, but perhaps two female or two male nuclei could fuse to produce the same result as the zygote would contain the same genetic information. To test this, researchers at the "Department of Molecular Embryology", Cambridge, used mouse eggs that they had extracted the nuclei from and then replaced them with either nuclei from eggs or sperm[26]. These modified eggs were planted back into the uterus of a mouse and left to develop for the normal gestation period. For the embryos with exclusive maternal genetics, normal gestation and development was apparent for eight days, but "the number of embryos surviving thereafter declined rapidly, and only a few of them reached Day 10 midgestation stage", and those that did had "very sparse extraembryonic tissues"[27]. Similarly, paternal nuclei produced roughly the same results, although some tissues in these embryos were better developed than in their female counterpart. This effect was later concluded to be as a result of genetic imprinting: the gametes contain epigenetic modifications on some regions of DNA, which produces a "parent-of-origin" effect. There are not that many other types of protein-coded genes which are imprinted, at least 80 in humans[28], but most of these contain an integral piece of junk DNA, called ICE, the imprinting control element, and are dependent on the lncRNA it controls the expression of. Methylation, mentioned above, is the key to the imprinting process. If the ICE is methylated, no lncRNA is produced, meaning that the protein coding genes in the same cluster are activated, and vice versa[29]. Therefore, if the parental gene is imprinted, only the maternal allele is expressed, and if the maternal gene is imprinted, it is silenced, and the paternal one is expressed.

Individuality: Transposable elements, Splicing and VNTRs

Interestingly, roughly 99.999 % of the DNA in any two *homo sapiens* is identical. This is simply because it contains the same genes, codes for all the same amino acids, with very similar proportions of raw elements, and creates the same basic "human" structure, but every individual within the species is unique. Although chromosomal crossing over in prophase I during meiosis does to some extent provide genetic diversity, this is not the only way the triplet code can be varied. In the 1940s, Geneticist Barbara McClintock discovered the presence of transposable elements, or "jumping genes" as they can sometimes be known colloquially. Although she did win a Noble prize for her research in 1983[30], transposable elements were dismissed as "useless" junk DNA; however, it has now become apparent that these make up almost 50 % of the human DNA[31] and have the unique ability to insert themselves into various positions in the genome and also to make multiple copies of themselves. There are two main strains of these transposable elements: retrotransposons and DNA transposons (Fig. 5).

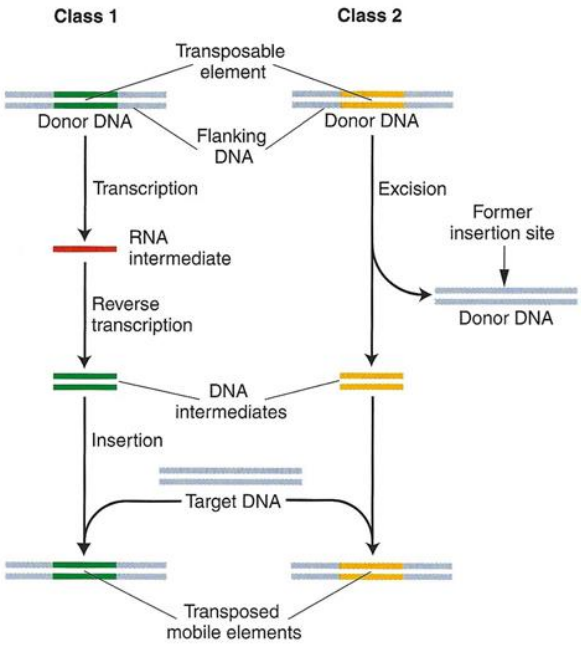
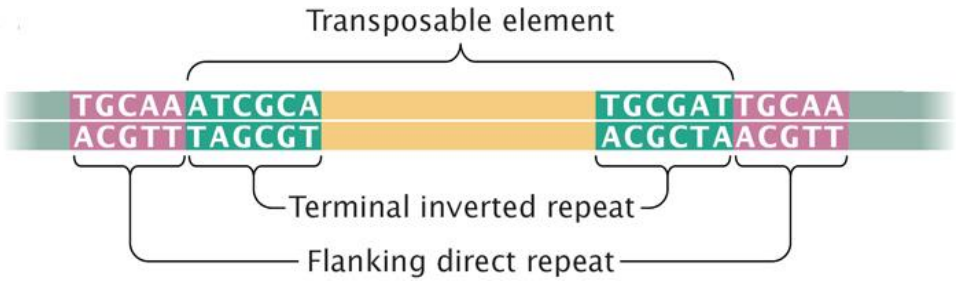


Fig. 5 The two classes of transposable elements shown in a scheme to highlight their similarities and differences.

Roughly 90% of transposable elements in the human genome are retrotransposons[32], with the rest being DNA transposons, while the proportions of these vary greatly in other species. Retrotransposons are classified as Class I and, as the name suggests, use reverse transcription to insert copies of themselves into loci in the genome. Transcription turns the original DNA into RNA, before reverse transcription, with the aid of the enzyme reverse transcriptase, produces a copy of the original DNA that can then be inserted into another section of the genome. Within Class II, the DNA transposons, instead of having multiple steps to the method, use a simple "cut and paste" mechanism. At both ends of each transposon, there are 9-40 base pair long terminal inverted repeats[33], which are recognised by the transposase enzyme encoded for in the transposon itself. This enzyme cuts out the transposon, leaving it with single stranded sticky ends. For reinsertion, both DNA ligase and DNA polymerase are used to secure the transposon in its new site. On top of terminal inverted repeats, there are also flanking direct repeats positioned on the outside at the 3' (downstream) and 5' (upstream) ends, which are not removed with the transposon, but are instead left behind as "footprints" (Fig. 6).

Fig. 6



Splicing is another way in which the genetic code can be edited and rearranged, and occurs straight after transcription, as the mRNA is unable to leave the nuclear envelope before it has happened. Pre-RNA (mRNA which is yet to be spliced) transcribed from the original DNA still contains introns, the "intervening" sequences of junk non-coding DNA, as well as exons, which are expressed as proteins. The beginning of the intron sequence at the 5' end is marked with the presence of the bases guanine and uracil, while there is an adenine at the A-branch point, and the bases adenine and guanine to cap the sequence[34] (Fig. 7). To initiate the basic splicing process, the spliceosome, a combination of small nuclear ribonucleoproteins (snRNPs) and five RNAs[35], binds to the beginning GU sequence, cutting the upstream exon away from the 5' end of the intron. A loop called a lariat is created as a transesterification reaction occurs between the guanine and adenine at the A-branch point – the lone pair of electrons on the hydroxyl group of the adenine ribose attacks the phosphorous in the phosphate group on the guanine, forming a new P-O bond, and cleaving the bond which attaches the exon to the phosphorous. In a second step, the 3' end hydroxyl on the upstream exon attacks the phosphorous, resulting in a covalent bond between the two exons[36]. The intron is cut out and released. See Fig. 8 on the next page for a clearer graphical representation of the mechanism.

Fig. 7 hnRNA stands for heavy molecular weight nuclear RNA and is equivalent to pre-RNA.

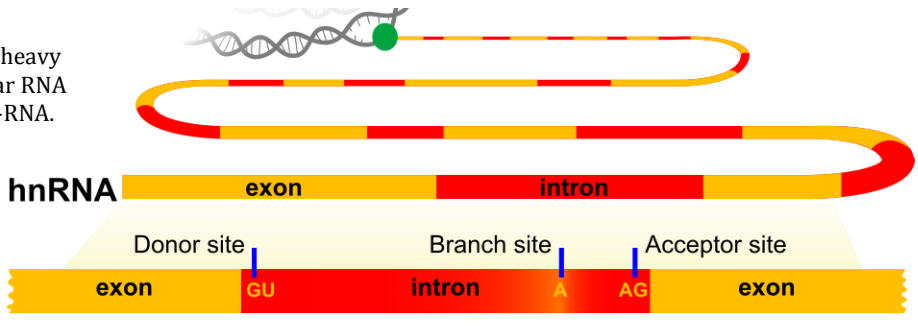
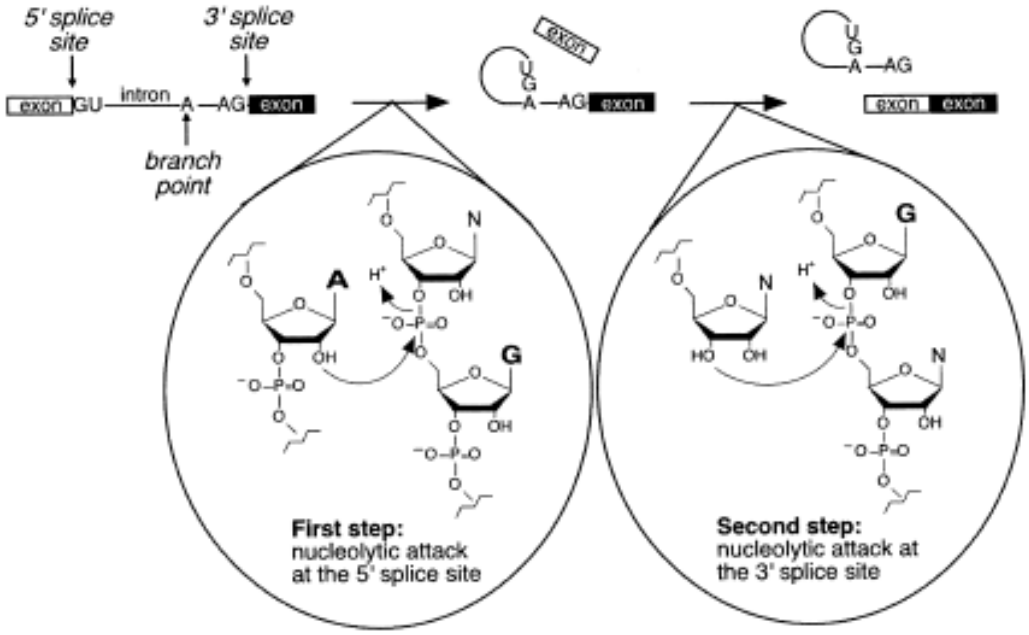
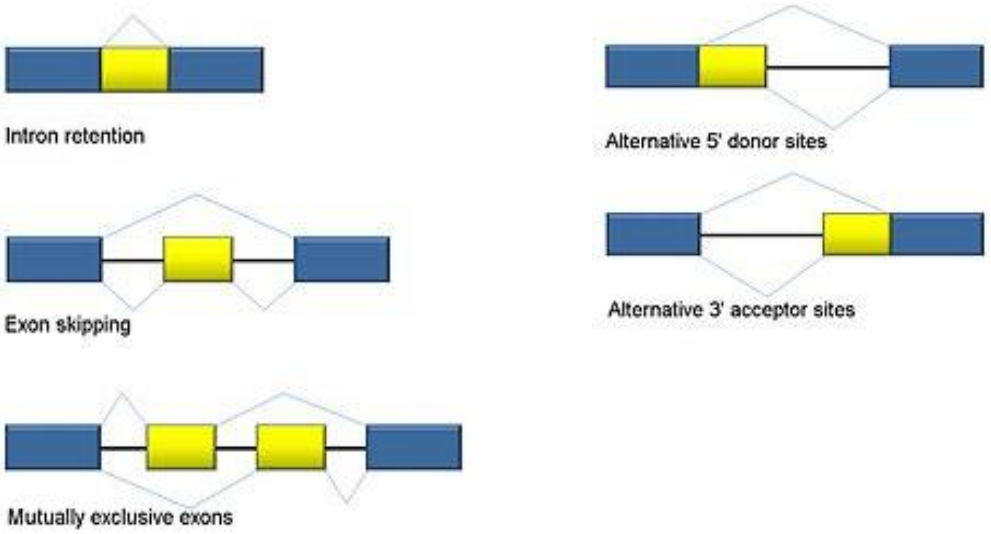


Fig. 8



In addition to this, alternative splicing may also occur, although this is mainly found in eukaryotes. There are five generally excepted methods included in alternative splicing[37] (Fig. 9), the simplest of which are exon skipping and intron retention. These are fairly self-explanatory: in exon skipping, an exon is spliced out of the sequence rather than being retained, while intron retention is the process used to preserve introns. Exons may also be mutually exclusive, meaning that any combination of introns and exons can be produced. There are several theories as to why this happens, among them the possibility that the spliceosome is not compatible with all the different mutually exclusive exons, so they cannot all be spliced at the same time. Similarly, there is evidence to suggest that with introns of less than 60 base pairs, there is steric hindrance, resulting in an exon being skipped out in order to create a longer intron that can then be spliced[38]. Finally, either there can be a change in the 5' prime splice site, causing a difference in the length of the 3' boundary of the upstream exon, or a similar thing can be produced with a revision of the 3' splice site. These alternatives may also explain why it is possible for only 22,000 human genes to produce 90,000 different proteins[39], and this is all available due to junk DNA.

Fig. 9



The last group of junk DNA that gives rise to individuality are variable number tandem repeats (VNTRs), which are short sequences of repeated bases that can occur from 70 to 450 times in a single genome[40]. For example, a normal stretch of junk DNA could be coded "TGCTCATTCACGGTCA", whereas a variable number tandem repeat would have a code much similar to "ACTACTACTACTACT", although an actual VNTR would be roughly a 10-100 base pair sequence long[41]. These repeats are inherited by offspring, so the length and frequency of them can be very similar to parents, which is why genetic fingerprinting is used to resolve paternity cases, as well as in forensic investigations. Having said this, there is, even between very close relatives, some variability. During normal DNA replication, errors or mutations occur once every 10^9 nucleotides[42], but when there are repeated "junk" sequences, the DNA polymerase sometimes becomes confused and adds or subtracts nucleotides. This means that all eukaryotic organisms, except those that are identical twins, have different genetic sequences and DNA fingerprints (Fig. 10).

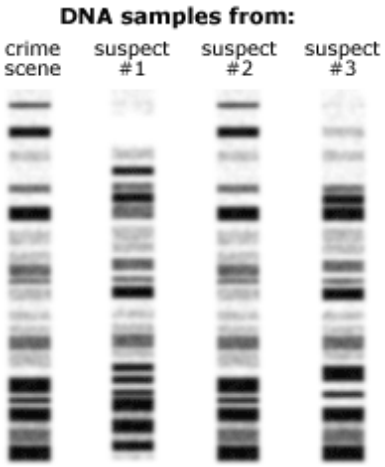


Fig. 10 VNTRs are used in DNA fingerprinting.

Junk DNA *is* "Junk"

However, even though there is evidence that junk DNA is not "junk" and is functional, there is still opposition, particularly against ENCODE (the encyclopaedia of DNA elements), which was a project launched in 2012 and funded by the National Human Genome Research Institute to classify junk DNA and to explore its functional possibilities. ENCODE concluded that 80% of all the junk DNA had at least one biochemical activity connected to it[43], but issues have been raised with this: biochemical activity does not prove functionality, nor is it conclusive evidence that it has an exclusive purpose within the genome as "the same phenotypic functions are often determined in a more diffuse fashion in larger-genome organisms"[44]. To illustrate this point, consider the example of a heart. The heart does lots of things, including pumping oxygenated and deoxygenated blood around the body, producing sounds and a pulse, and increasing in capacity with exercise and use, but the function of the heart is simply to pump blood around the body. The other things are "causal role" functions, which are functions that are "ahistorical and nonevolutionary"[45]. In the same way, junk DNA may just be "background noise" that appears to be functional, but has not evolved for a specific purpose.

Similarly, another aspect of the theory of junk DNA is brought to light by "the onion test" and its connection to the c-value paradox. In a similar way that the pufferfish genome was highlighted in the introduction, "the onion test" compares the genome of a common onion, *allium cepa*, with any other organism. When compared to the genome of a human, it is 5 times larger[46] and has much more junk DNA, but if junk DNA has functionality, logic would suggest that the onion would have the greater complexity. This is obviously not the case as humans have conscious thought, communication abilities and a larger spectrum of movement than an onion, so junk DNA cannot be what determines complexity and it therefore cannot play any significant role in the genome.

ENCODE also seems to have published optimistic and potentially scientifically inaccurate data, as illustrated by the example of transposable elements. The majority of transposons are

actually inactive, but a small, specific fraction of them do have a biochemical function. The rest have been labelled as parasitic or "selfish" as it could be that they exploit the genome for their own replication and proliferation. Nevertheless, the idea of function should not be extrapolated and applied to the whole group of transposable elements as this gives the impression that none of them are "junk", when there is no scientific evidence to support this. Moreover, the 80% estimate of functionality is arguable exaggerated and potentially impossible for an organism to reach. Mutations are always occurring, but these are usually either harmless or kept under control by natural selection. However, if the rate of deleterious mutations in each generation is greater than the speed at which natural selection works in order to remove them, then "the collective genomes of the organisms in the species will suffer a meltdown as the total number of deleterious alleles increases with each generation."[47] This means that there has to be an upper limit to the amount of functional DNA present in the genome. If this was at the 80% value that has been advertised by ENCODE, the probability of harmful mutations occurring in, or affecting, an integral proportion of the genome would be much higher, leading to disastrous results on the survival of the species. As it is, the human population has shown none of the effects that would be expected if such a high percentage of functionality was present.

The word "junk" is colloquially used to describe something useless or worthless, but in Biochemistry it is more often associated with biochemical function, so in determining the extent to which junk DNA is actually "junk", terminology and definitions must be more clearly determined. Not all the non-coding sequences uncovered by ENCODE may be classically defined as functional, but perhaps that is, rather paradoxically, the point and the reason for its existence: it prevents other important coding sequences from being lost, as demonstrated by telomeres, or from suffering potentially harmful mutations. With all the sensational headlines in the news like "Breakthrough study overturns theory of "junk DNA"[48] and "DNA junkyard yielding gold"[49], it is very easy to get overwhelmed and persuaded by all the media promotion and over-representation of the abilities and uses of junk DNA. Despite this, I still believe that it is important and that organisms do need it, particularly as the sequences that have already been studied, even if they are in the minority, have been proven to be active and to have set roles within the genome. Even though there might not be as much functional junk DNA as originally expected, I think the search should continue based on the evidence we already have. Winston Churchill is famously quoted saying "the optimist sees opportunity in every danger; the pessimist sees danger in every opportunity". Optimist or not, this is an opportunity that should be taken.

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