The Intrinsic Fragility of DNA

Nobel Lecture, December 8, 2015

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All macromolecules are, to some extent, unstable. My own work has focussed on the inherent lability of DNA [1, 2].

In my early studies as a postdoc at Princeton University in the 1960s we investigated heat-induced shape changes and unfolding of the macromolecular structure of purified transfer RNA, the small RNA molecules that are key components in protein synthesis [3]. In these time-consuming experiments, I was surprised to observe that my purified tRNA not only unfolded at elevated temperatures, but also very slowly decomposed in an irreversible way [4]. I was advised by colleagues that human fingers often have substantial amounts of ribonuclease on their surface, that is, the enzyme that degrades RNA, and that the problem might disappear if I improved my laboratory technique. But that was not the problem; I observed that different preparations of tRNA obtained by different methods still retained their property of apparently unprovoked slow decomposition in the same way. I extended this work to show that the decomposition of tRNA involved destruction of individual base residues and also involved slow cleavage of the phosphodiester bonds that link the RNA nucleotide building blocks together. I even published a short report on the heat-induced decomposition of tRNA that nobody found particularly interesting [4]. So, I moved on to other experimental work on ligation and processing of strand-breaks in DNA by previously unknown mammalian enzymes such as DNA ligases and exonucleases [5, 6]. But I had not forgotten the puzzling spontaneous decomposition of tRNA. When I moved back to Sweden and obtained my own research laboratory in Stockholm...
a couple of years later, I wanted to investigate if DNA, like tRNA, was susceptible to slow decomposition.

This was a rather far-fetched idea, because DNA, as the carrier of genetic information in our cells, was believed to be very stable in the intracellular environment (Fig. 1). In order to support such non-conventional work, I did not apply for a research grant, which may well not have been funded, but used some Swedish funds I had already been awarded to study enzymatic processing of DNA strand breaks in mammalian cells. The initial strategy was to perform some
pilot experiments on DNA instability, and if the results did not seem promising, quietly bury the project. But it turned out that although DNA was considerably more stable than RNA, it still underwent very slow, but relevant decomposition in neutral aqueous buffers.

Together with my meticulous laboratory assistant, Barbro Nyberg, I then devised a series of time-consuming experiments to attempt to quantify and characterise the very slow degradation of DNA solutions under physiological conditions. This meant investigating the stability of DNA at different pH values not too dramatically removed from neutral pH, at various elevated temperatures, and at different ionic strengths and levels of charge neutralisation. In order to facilitate our analyses, most studies were performed with DNA radioactively labelled in individual base residues; such DNA can be prepared from various bacterial mutant strains with defects in synthesis of precursors of DNA, grown in the presence of commercially obtained radioactive base residues. DNA from either *B. subtilis* or *E. coli* was used to avoid possible complications due to the presence of the modified bases 6-methyladenine and 5-methylcytosine. Moreover, DNA labelled with $^{14}$C rather than $^{3}$H was employed to avoid any possible exchange of $^{3}$H with the aqueous solvent during the prolonged incubations.

Aliquots of such DNA solutions were incubated for several days, and then analysed by chromatography. The most conspicuous change was that small numbers of base residues were lost from the DNA, in particular the purine bases guanine and adenine [7].

Figure 2 shows a summary of the different changes that were detected: a section of one of the two strands of DNA is illustrated with arrows indicating the sites of change. Cleavage of a base-sugar bond results in the loss of genetic information and formation of an abasic site in DNA. The abasic sites resulting from the loss of the bases guanine or adenine are chemically identical and were introduced at similar rates, so to know the identity of a missing base one has to consult the information in the opposite strand of the DNA molecule.

There are also some changes to the remaining DNA bases, the most important of these is the deamination of cytosine residues to uracil. This changes the coding specificity of DNA, that is, a mutation has occurred [8].

When I quantified all these losses or changes of information in DNA, the numbers were surprisingly high (Ref. 1, Fig. 3). In a single mammalian cell, there are 10 to 20 thousand changes per day. This is for double-stranded DNA.

There is some protection of bases by the double helical structure of DNA. While double-stranded and single-stranded DNA are depurinated at similar rates with only a 3- to 4-fold difference, single-stranded DNA is 150 times more susceptible than double-stranded DNA to deamination of cytosine and
FIGURE 2. DNA lability.

FIGURE 3. Spontaneous DNA lesions in a mammalian cell.
5-methylcytosine, and also formation of 1-methyladenine and 3-methylcytosine residues. This means that in a transcriptionally active, replicating cell, there are about 300 potentially mutagenic cytosine and 5-methylcytosine deamination events per day. This decay of the cellular DNA would lead to an unacceptable deleterious loss and alteration of genetic information. The answer to this dilemma must be that there is a correction mechanism.

In a search for such mechanisms, we established that abasic sites can be removed and replaced by an excision mechanism [9]. The same general excision-and-repair strategy is used for other types of DNA lesions, such as DNA damage induced by ultraviolet light, described by Dr Sancar and others, or to correct replication errors in the DNA, as discovered by Dr. Modrich.

If the DNA contains an altered base, such as a uracil which may be a deaminated cytosine, a previously unknown class of repair enzymes is employed, DNA glycosylases, that cleave base-sugar bonds in DNA [10]. In contrast, nucleases cleave phosphodiester bonds. We reconstituted the base excision repair pathway with purified enzymes, first with bacterial enzymes [11] and then with human enzymes [12] (Fig. 4).

A stretch of synthetic double-stranded DNA is made that contains a uracil residue in the centre of one of the two DNA strands. It can then be visualised by gel electrophoresis under conditions where the DNA strands have been separated. If there is uracil in DNA, the DNA strand remains intact after removal of this base, but an abasic site has been generated which is susceptible to cleavage by the next enzyme in the pathway, the endonuclease for abasic sites. The sugar-phosphate residue at the site of damage is then removed, DNA polymerase fills in the small gap and finally the DNA is ligated. (Fig. 5) In mammalian cells, the gap-filling enzyme DNA polymerase β (beta) has a separate domain that promotes the release of the base-free sugar-phosphate.

Models for aspects of the pathway have been proposed by several groups, including us. It is not a simple task for the DNA glycosylase to find a single uracil base that has replaced a chemically similar cytosine (or thymine) residue in a large excess of DNA, so this enzyme scans the DNA and usually flips out the altered base, and then initiates the repair process [13].

So far, the repair enzymes that can restore damaged DNA have been discussed. But occasionally an organism can also use induced changes in the DNA structure to generate helpful genetic diversity. A striking case is the efficient diversion of antibodies.

In order to improve the repertoire of antibodies, an antibody-producing cell can have the ability to actively change the structure of genes encoding antibodies.
by targeted deamination of cytosine in DNA. This idea, and understanding the further processing, were due to the brilliant insight of the late Michael Neuberger of the MRC Laboratory of Molecular Biology in Cambridge, UK (Fig. 6). I had the pleasure to collaborate briefly with the Neuberger group [14]. One specific deaminase AID, discovered by T. Honjo, apparently causes targeted deamination of antibody genes, and the uracil-DNA glycosylase then processes this DNA and

FIGURE 4. Repair of Abasic Sites in DNA.
The Intrinsic Fragility of DNA

triggers local mutational changes, which are reflected in an expanded and more efficient antibody response.

So far, hydrolytic DNA damage has been discussed, but there are other types of DNA damage, some of which are caused by the oxygen we breathe and metabolise. One particularly sinister form of DNA damage caused by reactive oxygen species is the oxidation of guanine residues in DNA to 8-hydroxyguanine, which

![Figure 5](image1.png)

**FIGURE 5.** Reconstruction of base excision repair with purified human proteins.

![Figure 6](image2.png)

**FIGURE 6.** When DNA damage is a good thing: Generation of antibody diversity by somatic hypermutation.
FIGURE 7. DNA lability: Oxidative damage.

FIGURE 8. DNA lability: Alkylation.
The Intrinsic Fragility of DNA

is a miscoding base (Fig. 7). This lesion is excised by a specific DNA glycosylase distinct from the enzyme that removes uracil [15].

There are other endogenous agents in cells besides water and oxygen that can cause DNA damage. We showed that one important example is the reactive coenzyme S-adenosylmethionine, SAM, which is an alkylating agent that can cause methylation damage to DNA [16]. There are several susceptible sites in DNA, and they are different from the targets of water or oxygen (Fig. 8). Furthermore, there are intricate DNA repair mechanisms that deal with such damage employing different chemical mechanisms and strategies. There are three main different approaches (Fig. 9) to deal with methylation damage [15].

A base in DNA can be methylated in such a way that it blocks replication of the DNA, this could be a lethal change, but a special repair enzyme excises the methylated base to trigger a base excision-repair event (17,18). This is analogous to the removal of uracil from DNA. In another approach, the very mutagenic base O<sup>6</sup>-methylguanine is directly demethylated by a methyltransferase that removes the offending methyl group by transferring it to itself, to generate a methylated cysteine residue in the repair protein (19). The term suicide inactivation has been used for this event, because the whole repair protein is destroyed by the methylation. Methylcysteine is a chemically very stable entity that could

**FIGURE 9.** Three mechanisms for repair of methylated DNA bases.
not be easily cleaved to regenerate an unmethylated repair protein. So, this is an energetically costly but effective form of DNA repair.

More recently, we found another type of DNA repair enzyme that can remove methyl groups from the toxic residues 1-methyladenine and 3-methylcytosine in DNA [20, 21]. It took us many years to find this enzyme because it has very unusual cofactors, that is, iron and the small metabolite alpha-ketoglutarate (Fig. 9). It turns out that this unexpected demethylation reaction with DNA using these cofactors also is employed for demethylation of histones, which is important for regulation of cell growth [20].

In conclusion, there are several common molecules in cells that can damage DNA, and which are impossible to avoid (Fig. 10).

Water is a weak reagent, but it is present in cells at a very high concentration. Several other commonly occurring small molecules may also damage DNA. Probably not all of them have even been identified yet as DNA damaging agents, which suggests that there are more DNA repair enzymes waiting to be discovered. But the fact that water is a damaging agent for tissues has been known for over 400 years, because William Shakespeare points this out in the graveyard scene in Hamlet, (Fig. 11) This scene is immediately followed by the famous monologue on life and death. Hamlet shows himself to be an excellent scientist by asking a series of logical and penetrating questions. Note that Shakespeare pinpointed the deleterious effect of water on the soft components of the human body, including the DNA [22].
The Intrinsic Fragility of DNA

FIGURE 11. From Shakespeare’s Hamlet.

REFERENCES


