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Minireview

Free Radical Induced Oxidative damage to DNA: Relation to Brain Aging and Neurological Disorders

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Free radicals are produced in cells by cellular metabolism and by exogenous agents. These species react with biomolecules in cells and one of the important targets is DNA. This kind of damage, often referred to as oxidative DNA damage, has consequences in various organs and particularly in brain, in view of its high metabolic activity and oxygen consumption. The consequences include mutagenesis of various kinds ranging from simple oxidation of bases to large deletions through single and double strand breaks. In brain, because of its post-mitotic nature, oxidative damage to DNA is seen more often at the level of bases. A major route for repairing oxidative damage to bases is base excision repair (BER). It is increasingly becoming apparent that defects in repairing oxidative DNA damage can lead to a number of neurological disorders like Alzheimer and Parkinson. Our recent studies have clearly demonstrated that BER is highly compromised in brain cells with increasing age and this could well be one of the major causative factors for normal aging and the associated deteriorating mental conditions, including certain neurological abnormalities.

Keywords: Free radical, Oxidative damage, DNA, Brain aging, Neurological disorders, Base excision repair (BER), Nucleotide excision repair (NER)

Introduction

One of the most intriguing aspects of the origin of life and its evolution is the care taken by the living organisms, no matter primitive or highly evolved, to preserve the structural integrity of the cellular DNA, so that transfer of genetic information occurs within the reasonable limits of perturbation. Thus, with the advent of choosing DNA to be the repository of genetic information, evolution, as it were, had to develop tools to ensure the fidelity of information transfer. Since no foolproof technique could be evolved to ensure that the molecular structure of DNA is not altered, organisms have developed pathways that would immediately repair the damage that could possibly occur to DNA. Interestingly, there are multiple ways in which cellular DNA can be altered from its structural integrity. Every day a number of single-strand breaks (SSBs) and double-strand breaks (DSBs) arise in every cell as a consequence of DNA molecule being attacked by various reactive oxygen species (ROS) and other redox cycling molecules (RCM). A number of intrinsic and extrinsic mutagens cause structural changes in cellular DNA that could eventually lead to alteration in the coding properties of DNA and cellular toxicity. In aerobic organisms, the life sustaining reactions that occur naturally by themselves produce reactive species and these can damage macromolecules including DNA. Thus, reactions that are needed to sustain life may themselves cause damage to DNA.

Based on the existing knowledge, the many ways in which DNA can be structurally altered are depicted in Fig. 1. Generally, as many as 75,000 to 100,000 DNA damage events might occur in each cell per day (Table 1)[1-5]. However, organisms have also evolved many intricate mechanisms to detect and repair these damages in DNA. Just as the normal metabolic

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Abbreviations: APE1, apurinic/apyrimidinic endonuclease; BER, base excision repair; DSBs, double-strand breaks; FEN1, 'Flap' structure-specific endonuclease 1; NER, nucleotide excision repair; NHEJ, non-homologous end joining repair; dNTPs, deoxynucleoside triphosphates; PNKP, polynucleotide kinase 3'-phosphatase (also known as PNK, or DNA-kinase), pol λ, DNA-polymerase λ; RCM, redox cycling molecules; ROS, reactive oxygen species; SSBs, single-strand break.

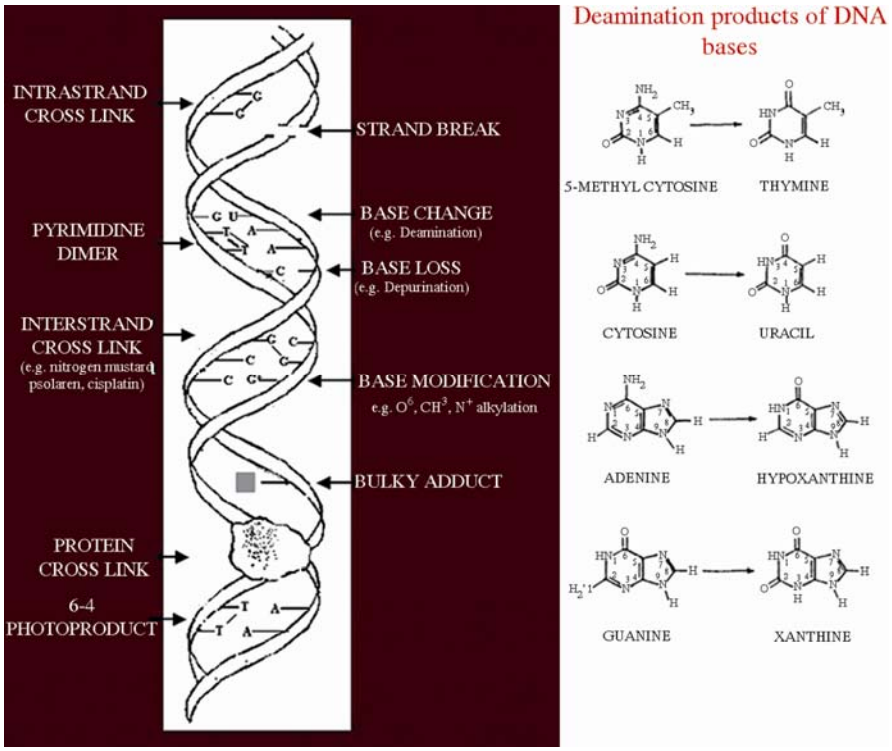

Fig. 1—Different forms of DNA damage due to various endogenous and exogenous sources [On the right side spontaneous deamination of the bases in DNA known to occur, is shown. The products of deamination, if not removed could result in mutations because of the changed pairing properties]

Table 1—Approximate rates of occurrence of endogenous DNA damages in mammalian cells

Damage	Events per cell per day	Ref.
Depurination	10,000	1
Depyrimidination	600	2
Deamination	100-300	3
Single-strand breaks (Including all types of base damage viz., oxidative damage, adduct formation with reducing sugars, methylation, cross-links, and so forth)	20,000-40,000	4
Double-strand breaks	9	5
Interstrand cross-links	8	5
DNA Protein cross-links	Unknown	5

reactions themselves can induce structural damage to macromolecules including DNA, many DNA repair pathways have been evolved to counteract the various types of damage. Essentially, in mammalian cells, there are four major DNA repair pathways: (i) a simple reversal of the damage, (ii) nucleotide excision repair (NER), (iii) base excision repair (BER), and (iv) recombination repair including the end joining (RR). Detailed discussion of these DNA repair pathways is beyond the scope of this article. However, many recent reviews on these pathways may be consulted for further reading[6-10]. In brief, reversal of an alteration in the structure of DNA, for example methylation of guanine, can take place by an enzyme like O^6-methyl guanine methyl transferase which removes the methyl group from O^6-methyl guanine, thereby avoiding the possibility of a mismatch formation, as O^6-methyl guanine can pair with both C or T. Similarly, in some organisms, monomerization of pyrimidine dimers is catalyzed by an enzyme photolyase, and this simple reversal repair is dependent upon light of wavelength above 300 nm.

Nucleotide excision repair (NER), which is rather a complex and a multi-step pathway to take care of the repair when DNA is damaged in such a manner, so as to create a major distortion in its structure. Formation of pyrimidine dimmers, following exposure to UV light is a classical example of causing such major distortion in DNA structure. Also, inter and intrastrand crosslinks and interstrand adduct formation with some drugs and antibiotics lead to major distortion in the structure of DNA, affecting its normal information transfer function (Fig. 1 and Table 1). Essentially, NER involves four major steps: (i) recognition of the damage, (ii) removal of the damaged portion of the

DNA strands containing the damage, (iii) filling up of the gap created by an appropriate DNA polymerase using the other strand as template, and finally (iv) ligation of the newly synthesized strand with down stream sequence. NER also includes mismatch repair, when a reasonably lengthy fragment of DNA containing the mismatch is removed and the fragment is resynthesized using the other strand as template. Also NER includes another type of repair sub pathway, the transcription coupled DNA repair (TCR). As the name indicates, those damages that are in that part of DNA which is actually being transcribed, are repaired preferentially over the structural alterations that are situated anywhere in DNA (global DNA repair, GR).

Recombination repair is a mode of DNA repair that is most prevalent in rapidly dividing tissues/cells, where the damaged or mutated portion of DNA is removed and replaced with a homologous portion from a sister DNA molecule. The precise mechanism of this type of DNA repair is still to be understood and it appears that this mode of repair is not greatly relevant to quiescent and non-replicating cell populations. However, there is a highly error-prone version of recombinant repair, referred to as non-homologous end joining repair (NHEJ) seen in many tissues, irrespective of their replicative capacity. This process simply involves rejoining of loose DNA ends quite often resulting in the loss of original sequence at the site.

Finally BER, closely concerned with repair of oxidative damage to DNA, is a simpler version of NER. This mode of repair would come into operation when the damage is a simple one and confined to a base. For example, as already outlined in Table 1, bases can be modified in a number of ways. Alkylation, spontaneous deamination and loss of bases can cause mutations. More importantly, oxidative damage of bases is a major event since free radicals generated internally due to metabolism itself or exposure to ionizing radiations from outside can cause extensive oxidative damage to macromolecules and in particular, the genetic material DNA[11]. For example, free radicals can damage the nucleobases or the sugar units. Hydroxyl radical, being the most reactive species interacts with C-8 of guanine (8-oxo-guanine), which is one of the most commonly found oxidized base in DNA[12]. Several oxidized products of bases and sugar moieties (5-OH cytidine, hypoxanthine, oxidized deoxy ribose, formamidopyrimidine, fragmented thymine and thymine glycol etc.) are formed and they are all capable of stalling the replicative and transcriptional processes[13]. Even bulky lesions are reported to be formed by the action of ROS on DNA which require to be removed before transcription process can proceed[14].

BER is the pathway that is equipped to handle such damage to DNA. Thus, this pathway has a close link to the free radical metabolism and has an impact on health and disease including aging and neurodegenerative diseases. Fig. 2 depicts some details about the BER pathway on the basis of the existing information. This pathway, like NER essentially consists of four steps and can be divided into two sub pathways: one concerned with “short patch” or single nucleotide replacing pathway and the other “long patch pathway”. In the long patch pathway, as many as up to 13 nucleotides may be inserted, whereas the short patch pathway involves mostly filling up of a single nucleotide gap. In the first step of short patch repair (left panel of Fig. 2), the altered base (A) is recognized, at times quite specifically, and cleaved from the sugar phosphate moiety by an appropriate DNA-glycosylase[15]. At the same time, AP endonuclease (APE1) attaches itself to the 5’ side of the base to break the chain. The main endonuclease that carries out this function in humans is APE1, generally referred to as AP endonuclease. In step 3, the pol β fills up the one nucleotide gap and also releases the 5’-deoxyribose phosphate (dRp). At the same time, DNA-ligase III - XRCC1 (X-ray repair cross complementing gene 1) complex arrives at the site. Step 4 consists of DNA-ligase III sealing the nick and pol β dissociating from the site. Subsequently, the XRCC1 and ligase III come off from the site leaving behind repaired DNA (Fig. 2, left panel, B).

The predominant route for BER is the ‘short patch or single nucleotide pathway’ discussed until now. In some cases, where the terminal sugar phosphate at step 2 develops a complex structure and cannot be removed. The repair synthesis would nevertheless continue, but in a strand displacement manner (Fig. 2, right side panel), this long patch synthesis is catalyzed either by pol β itself or a bigger polymerase like pol δ/ε with associated proof reading activity. Also, certain additional factors like PCNA, FEN1 are needed for this pathway. The involvement of other proteins in BER, such poly (ADP-ribose) polymerase-1 (PARP-1), polynucleotide kinase 3’-phosphatase (PNKP, also known as PNK, or DNA-kinase), DNA-

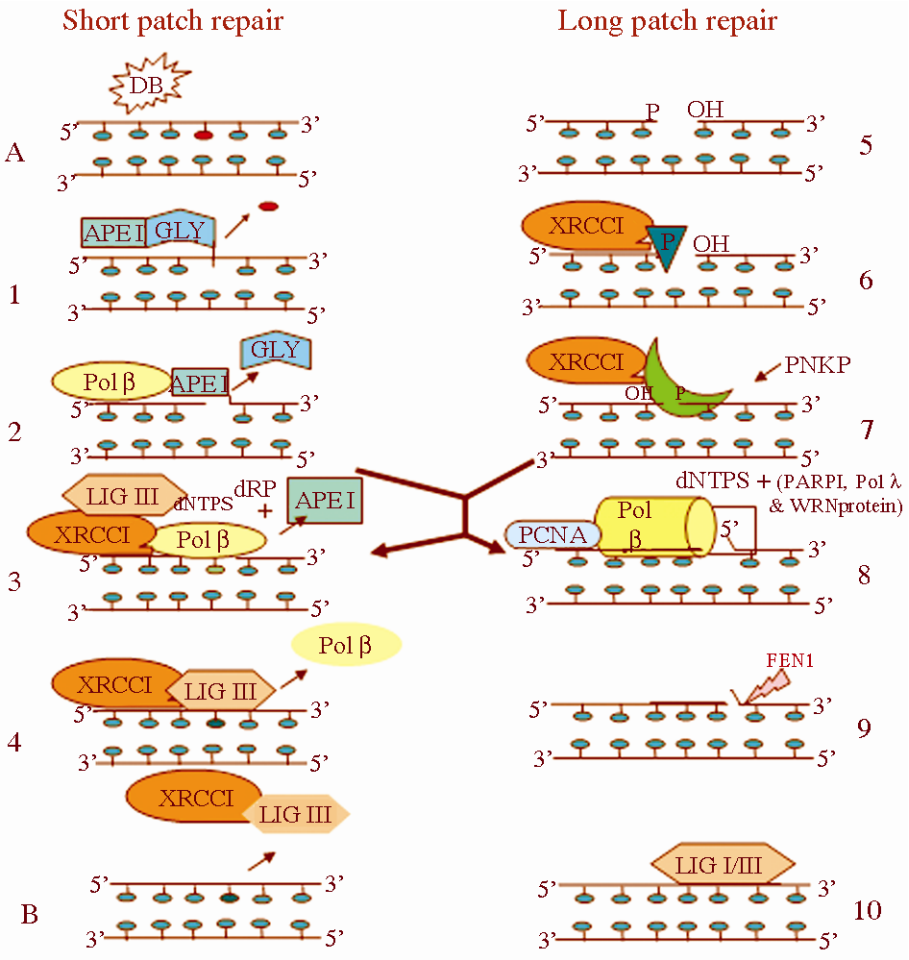

Fig. 2—Pathways of BER: On the left and right side are the 'Short patch' or single nucleotide pathway and the 'long patch' pathway respectively [Crossing-over of the pathways can occur at points 2 and 7. For detailed discussion of each step, please see text. *Abbreviations*: DB, damaged base; APE1, human apurinic/apyrimidinic endonuclease 1; GLY-DNA, glycosylase DNA; Pol β/δ/ε- DNA, polymerase β/δ/ε respectively; dRP, deoxyribose-5'-phosphate; XRCC1, X-ray repair cross-complementing gene 1; LIGI/III, DNA-ligase I/III; PARP1, poly (ADP-ribose) polymerase 1; PNKP, polynucleotide kinase 3-prime phosphatases; FEN1, 'Flap' structure-specific endonuclease 1; dNTPs, deoxynucleoside triphosphates]

polymerase λ (pol λ) and Werner syndrome protein is also reported. These enzymes are envisaged to help the long patch repair process, entry of damaged substrates into BER pathway, providing a substitute for pol β activity respectively and aiding the pol β activity by providing the proof reading. For a detailed discussion of these aspects, the reader is referred to a recent review[16].

Free radical damage, BER and aging

Free radicals are routinely referred to as ROS in biological sciences, since most of the biologically important free radicals are all oxygen-centered, although not all free radicals are ROS. Free radical-induced damage to biological systems has gained importance when Dr. Denham Harman first proposed the free radical theory of aging more than 50 years ago[17]. Noting that radiation causes mutation, cancer and aging, Harman argued that oxygen free radicals produced during normal respiration would cause cumulative damage which would eventually lead to loss of functionality, and ultimately to death. In later years, the free radical theory was expanded not only include aging per se, but also age-related diseases. More recently, the relationship between free radicals and aging and disease has brought in other phenomena like DNA damage and its repair . To day, one of the most attractive theories of aging is the DNA damage and DNA repair theory which exhorts that accumulation of DNA damage and decreae of DNA repair capacity to remove that damage would result in loss of cell's proper functionality, eventually leading to death. Naturally, free

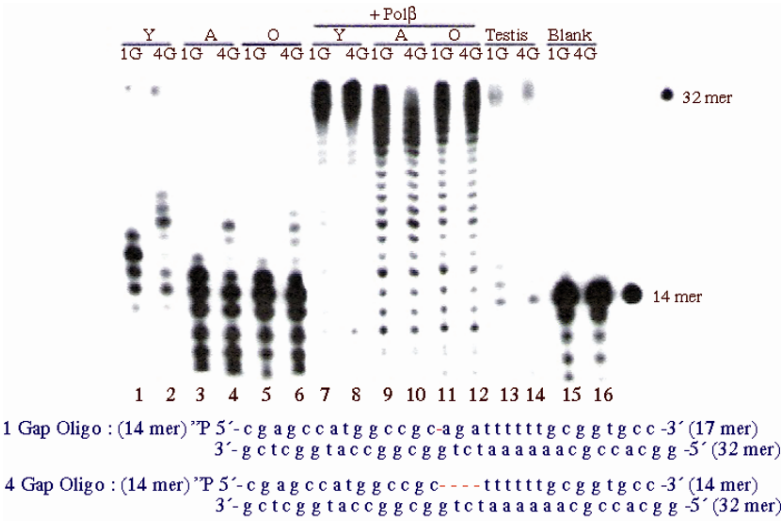

Fig. 3—Gap repair activity in 'Young', 'Adult' and 'Old' neuronal extracts supplemented with recombinant pure rat liver pol β or pol β alone [A typical autoradiogram from three different experiments is shown. Lanes 1-6 neuronal extracts from young brain (Y, 5 days postnatal), adult brain (A, 6 months) old brain (O, ≥ 2 years); lanes 7-12, neuronal extracts supplemented with 1 unit of polβ. Unit of pol β activity is defined as that which will affect incorporation of 1 nanomole of total nucleotides into acid insoluble fraction. Lanes 13 and 14, testis extracts alone as positive control; and lanes 15 and 16, without any neuronal extracts (enzyme blanks). The mobility of labeled standard 14-mer and 32-mer are also shown. Lanes 1,3,5,7,9,11,13,15 are with 1-gap substrate (1G), while lanes 2,4,6,8,10,12,14,16 are with 4-gap substrate (4G). The oligoduplexes with 1 and 4 nucleotide gap used as substrates in the study are also shown. As can be seen one of the strands has a gap of either I or 4 nucleotides. These strands are ^{32}P-labeled with 5'-kinase for the subsequent identification on the sequencing gel. Also, in either case the downstream primer after the gap is phosphorylated with non-radioactive phosphate by 5'-kinase before annealing as it is known that it would enhance the binding of pol β to gapped substrate and promotes the next step of ligation[20]. Fig. taken from reference[16]]

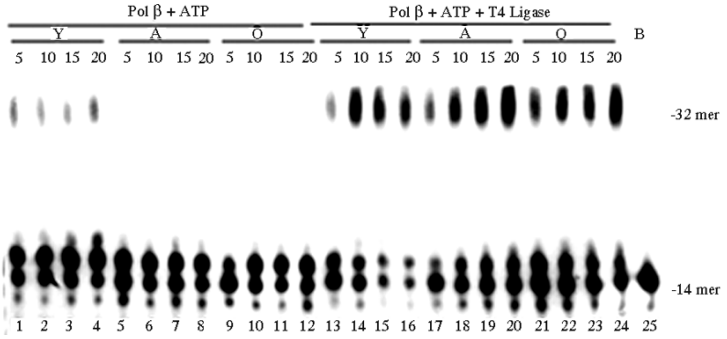

Fig. 4—Restoration of the gap repair activity in adult and old rat neuronal extracts when supplemented with limited amounts of pol β (0.2 units) and 20 units of T4 DNA ligase [The spots corresponding to 32-mer without any intermediary spots indicate that the gap repair has been performed efficiently. All the experimental details and notations are as in Fig. 3]

radical production and their targeted action on DNA to cause damage would have a tight link to aging process and age-associated disabilities.

Our laboratory has been interested in assessing the DNA repair capacity of brain with respect to the age of the animal. It is the hypothesis that DNA repair capacity in higher animals decreases with age, particularly from the point of attaining puberty, in a predetermined manner which would eventually lead to senescence and death. Our opinion further delineates this argument to the level of BER which is a DNA repair pathway that is conserved as well as evolved to take care of the minor and simple damages that can occur to DNA bases due to the very cellular metabolism itself. As already mentioned, oxidative damage to DNA is repaired by the BER pathway and thus may play a more emphatic role in the phenomenon of aging and age-associated diseases. For this reason, we have measured BER potential in isolated neuronal cells from brains of rats of different ages to examine the validity of our thinking. Synthetic deoxyoligo duplex substrates with one or four nucleotide gaps in one of the strands were used and the ability of neuronal extracts to fill the gap was examined. The first set of results are presented in Fig. 3.

Gap repair involves two steps: the filling of the gap by the addition of the required number of nucleotides followed by the ligation with the 5'-phosphorylated downstream primer. If the repair process is completed properly, a radioactive spot on the sequencing gel corresponding to the 32-mer should be seen since the inserted nucleotide is radio-labeled[16,18]. However, it is seen that only addition of nucleotide has occurred with adult and old neuronal extracts. In the young, addition of nucleotides was seen and ligation to downstream primer also occurred, although at a low level. On the other hand, when the extracts were supplemented with pol β, addition of nucleotides occurred all the way to extend the upstream primer to a 32-mer apparently in a distributive strand displacement manner. On the other hand, when low amounts of pol β were added, addition of just the required number of nucleotides occurred. Even then, ligation was achieved only in young extracts and no ligation could be visualized in adult and old. Finally, efficient gap filling followed by ligation, i.e. complete gap repair was achieved and for this to happen, conditions required are the presence of 5'-PO_4 on the downstream primer, and supplementation of aging neuronal extracts with both pol β and DNA ligase (Fig. 4). These studies, thus demonstrated that aging neurons are unable to affect BER, due to deficiency of pol β and DNA-ligase and fortifying the neuronal extracts from aged animals with these two factors can restore the lost BER activity.

Neurological diseases associated with aging

It is becoming increasingly clear that a number of neurodegenerative disorders/syndromes make their appearance more often in the later stages of life span (viz., Alzheimer, Parkinson and Huntington disease, amyotrophic lateral sclerosis etc.). It is also seen that the severity of these diseases is on the increase with advancing age. Further, there are some syndromes that are characterized by accelerated aging right from the early stages of life span and these subjects also display some attributes of mental insufficiency (viz., Down syndrome, Progeria, Werner's syndrome). In majority of these syndromes, it is found, among other things, DNA repair is compromised. More importantly, out of the 15 neurological disorders that are found to have an etiological link to DNA repair defect, 6 of them (spinocerebellar ataxia with axonal neuropathy-1, Huntington, Alzheimer and Parkinson disease, Down syndrome and amyotrophic lateral sclerosis) seem to result from increased oxidative stress and the inability of BER pathway to handle adequately the oxidative damage inflicted upon DNA[19]. It, therefore, appears that aging and the associated neurological disorders have a link to BER efficiency, thereby pointing out that repair of oxidative damage to DNA plays an important role in aging as well as age-associated neurodegenerative disorders.

Epilogue

DNA damage is an extremely common event in all living cells. The damage is caused by both intrinsic, as well as extrinsic factors. Free radical-induced damage to DNA, generally referred to as oxidative damage is a major form of damage that could be deleterious, if not repaired. As organisms age, their DNA repair capacity decreases and this coupled with accumulating damage to DNA may eventually lead to breakdown of the cellular machinery culminating in disease and finally death. Among the various pathways of DNA repair, BER appears to have a specialized role in that this mode of repair is more related to the maintenance of the cellular activities as opposed to the other repair pathways which are linked to major damage to DNA, transcription and replication. Our results show that BER is compromised in brain cells with age and the two limiting factors appear to be pol β and DNA ligase. What is a bit refreshing is the finding that the BER activity could be restored at least *in vitro* by supplementing the neuronal extracts with pol β and DNA ligase. This lends hope that attempts could be made to extend these observations to *in vivo* situation. The BER potential of a person, particularly during advancing age may well be a major determining factor for the health and the vulnerability to neurodegenerative diseases.

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