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Unveiling the non-repair face of the Base Excision Repair pathway in RNA processing: A missing link between DNA repair and gene expression?

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ABSTRACT

The Base Excision Repair (BER) pathway, initially studied as a mere DNA repair pathway, has been later found to be implicated in the expression of cancer related genes in human. For several years, this intricate involvement in apparently different processes represented a mystery, which we now are starting to unveil.

The BER handles simple alkylation and oxidative lesions arising from both endogenous and exogenous sources, including cancer therapy agents. Surprisingly, BER pathway involvement in transcriptional regulation, immunoglobulin variability and switch recombination, RNA metabolism and nucleolar function is astonishingly consolidating. An emerging evidence in tumor biology is that RNA processing pathways participate in DNA Damage Response (DDR) and that defects in these regulatory connections are associated with genomic instability of cancers. In fact, many BER proteins are associated with those involved in RNA metabolism, ncRNA processing and transcriptional regulation, including within the nucleolus, proving a substantial role of the interactome network in determining their non-canonical functions in tumor cells. Maybe these new insights of BER enzymes, along with their emerging function in RNA-decay, may explain BER essential role in tumor development and chemoresistance and may explain the long-time mystery. Here, we would like to summarize different roles of BER pathway in human cells. First, we will give a short description of the classical BER pathway, which has been covered in detail in recent reviews. We will then outline potential new roles of BER in gene expression and RNA metabolism. Although recent works have provided tremendous amount of data in this field, there are still lot of open questions.

1. Relevance of the canonical BER pathway and open questions

The BER pathway (Fig. 1) is an essential DNA repair system in higher eukaryotes and gene deletions of the core BER factors (apurinic/apyrimidinic endonuclease 1 – APE1, DNA polymerase β − Polβ, X-ray repair cross-complementing 1 – XRCC1, DNA ligase I − LigI and DNA ligase III − LigIII) results in embryonic or early post-natal lethality [1]. The pathway is comprised of five major steps, in which enzymatic and non-enzymatic components cooperate to carry out a highly integrated set of reactions: i) recognition and excision of the damaged base; ii) incision of the resulting AP site to generate a nick on the DNA backbone; iii) processing of the nick ends; iv) filling of the nucleotide gap; and v) sealing of the nick (Fig. 2).

Different specific DNA glycosylases scan the DNA substrate, recognize, through a flipping out mechanism, and excise the damaged base in a lesion-specific manner. Two kinds of DNA glycosylases are known: mono- or bi-functional, depending on their mechanism of action. While monofunctional DNA glycosylases (e.g. the uracil-DNA glycosylase − UNG) simply cleave the C1′-N-glycosidic bond, generating an AP-site, bifunctional enzymes also possess an associated β-lyase activity (e.g. the 8-oxoguanine DNA glycosylase − OGG1) deputed to cleave the DNA backbone leaving a 3′-α,β-unsaturated aldehyde blocking group. An additional family of DNA glycosylases, represented by the human NEIL1 and NEIL2 enzymes, is also able to operate a β,δ-elimination reaction, leaving a 3′-phosphate nick [2]. Higher eukaryotes are provided with a vast array of DNA glycosylases with a significant redundancy in their damage selectivity for this reason, single knockout of several DNA glycosylases is not lethal *per se*, although an accumulation of unrepaired DNA lesions occurs [3].

The glycosylase-catalyzed reaction generally produces an AP-site, which is immediately processed by APE1 in metazoans. APE1 cleaves at the 5′ termini of the abasic site, generating a nick on the DNA backbone

Abbreviations: AP-site, apurinic/apyrimidinic site; APE1, apurinic/apyrimidinic endonuclease 1; BER, Base Excision Repair; DDR, DNA damage response; miRNA, microRNA; ncRNA, non coding RNA; NIR, Nucleotide Incision Repair; PARP, poly(ADP-ribose) polymerase; RBP, RNA binding protein; rNMPs, ribonucleotides; ROS, reactive oxygen species; SSBR, Single Strand Break Repair

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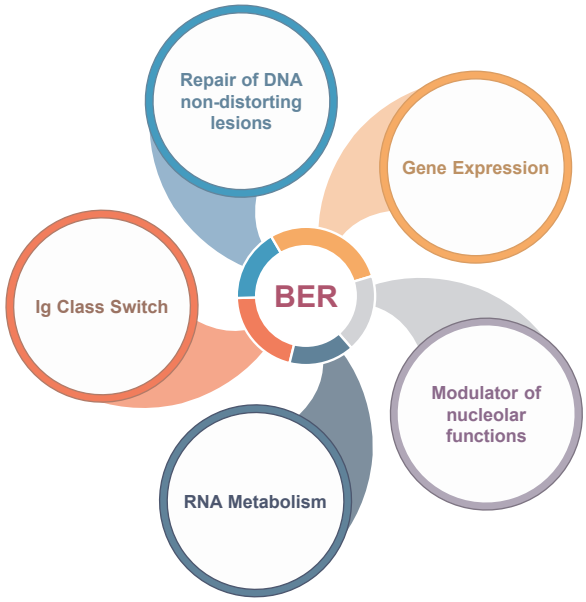

Fig. 1. BER exerts different functions in human cells.
Schematic representation of different biological functions of Base Excision Repair (BER) pathway in human cells including DNA repair player of alkylated and oxidative DNA lesions; regulator of expression of genes involved in response to genotoxicants; regulator in RNA metabolism, control of nucleolar function and regulation of immunoglobulin Class Switching together with AID.

and producing a 3′-OH and a 5′-dRP (deoxyribonucleotide-phosphate) termini. Usually, APE1-incision activity is sufficient to generate the DNA ends required for the completion of the DNA repair process. Once further oxidation of the DNA termini or base-excision operated by bifunctional glycosylases occurs, other end-processing enzymes may be involved, such as tyrosyl-DNA phosphodiesterase 1 (TDP1), aprataxin (APTX) or polynucleotide kinase 3′-phosphatase (PNKP). The dRP-lyase activity of Polβ, along with APE1 3′-phosphodiesterase activity, contributes to the "end-cleaning" process, ultimately generating a single-nucleotide gap that can be efficiently filled in and re-ligated [4].

BER is then completed via a "short-patch" (SP) or a "long-patch" (LP) pathways, depending on the 5′-moiety generated. In the SP-BER, Polβ is engaged to replace the missing nucleotide, and then is followed by the XRCC1-LigIII complex, which is responsible for the ligation of the nick [5]. In the presence of a 5′-moiety refractory to the Polβ lyase activity, low ligation efficiency, or during the S-phase of the cell cycle (i.e. when replication-associated proteins are more abundant), BER can be completed through the LP-BER which involves a strand displacement-dependent gap filling process [1]. Replicating polymerases, such as DNA polymerase δ and ε, act in concert with the sliding clamp PCNA (proliferating cell nuclear antigen) in the LP-BER, generating a stretch of 2–12 nucleotides, which is removed by the flap endonuclease 1 (FEN1). Finally, intervention of the PCNA-associated DNA Ligase I seals the nick [6].

Notably, BER protein components are involved in at least two subpathways, namely Single Strand Break Repair (SSBR) and Nucleotide Incision Repair (NIR) [7]. SSBs are generated by different sources including reactive oxygen species (ROS), radiomimetic drugs, ionizing radiation, topoisomerase-mediated DNA cleavage or they are unavoidable intermediates generated during BER processing. The SSBR pathway initiates through recruitment of the poly(ADP-ribose) polymerase PARP1, which recognizes exposed SSBs and modulates the repair process through enzymatic ADP-ribosylation of protein substrates. Tight connection between BER and SSBR has been highlighted by the observation that many BER proteins (e.g. XRCC1, Polβ) interact with PARP1 [6] and by the fact that PARP1 has been shown to orchestrate the BER processing of uracil and AP-sites [8]. Interestingly, it has been recently demonstrated that APE1 has glycosylase-independent NIR activity on particular modified bases, see below. Although very intriguing, the physiological impact of the NIR pathway is still under investigation, as within the intracellular milieu the presence of specific DNA glycosylases would likely dampen the efficiency of the NIR process on DNA.

Overall, BER is currently regarded as a dynamic intertwining of different enzymes and auxiliary proteins that operate in a highly coordinated manner to allow temporal and spatial modulation. The relevance of this coordination is remarked by several observations, which still deserve further studies:

- imbalanced expression of BER components has been linked to genomic instability. In particular, overexpression of core elements of the pathway is a hallmark of cancer progression and resistance to therapy. Increased expression of a single BER factor may result in competition or in excessive enzymatic activity, which is not compensated by equimolar amounts of other BER proteins. This has been formally demonstrated, in the case of APE1 [9–11] and Polβ [12].
- abortive intermediates of the pathway are intrinsically cytotoxic since unprotected intermediates (e.g. SSBs) are much more toxic than the initial damaged base [13]. Therefore, the fine-tuning and coordination of the pathway is possibly the result of an evolutionary tradeoff between the rapid repair of mutagenic lesions and the potentially hazardous intermediates that such repair may generate.

In order to explain mechanisms evolved to optimize the repair efficiency of the BER pathway, several models have been proposed including those of the 'passing the baton' and the 'BERosome' [7,8,14]. Despite the apparent divergence amongst models that have been put forward to explain the complexity of BER, each of them probably describes different aspects of a unique and highly dynamic integrated process. However, it is clear how it is modulated through a complex network of more or less stable DNA-mediated or protein-protein interactions, among BER enzymes and non-enzymatic scaffold proteins (e.g. XRCC1, PCNA) and PTMs (Post-translational modifications). Phosphorylation, acetylation, methylation, SUMOylation, as well as ubiquitination of almost every BER component have been suggested to play a role in the modulation of the pathway [7].

An emerging concept in this field is the role of some non-canonical regulatory proteins as BER modulators. Several proteins, apparently unrelated to the pathway, have recently been discovered as novel unexpected coordinators of BER [15]. p53, for instance, has been implicated in the modulation of both APE1 and Polβ [6], whereas our laboratory discovered nucleophosmin (NPM1) as a modulator of the APE1 enzymatic activity [16]. Additional regulation of the BER pathway is also achieved through evolutionarily acquired disordered extensions of some BER components [17,18]. These accessory proteins were proposed to be important for stabilizing large complexes, "repairsome", by providing extended interaction surface area [19].

An interesting link between DNA damage sensing and modulation of BER protein amount has been recently demonstrated. Indeed, BER proteins amount, which is generally abundant with a relatively long half-life, is constantly oscillating in response to the DNA damage load at the steady-state level. This equilibrium is strictly controlled by the ubiquitin-proteasome system [4]. It is possible that the high level of them (such as APE1 and Polβ) observed in several tumors is the result of perturbations of this equilibrium. Understanding these aspects will shed light on the role of BER proteins in cancer development.

2. NIR activity of APE1 on non-canonical substrates

In the last decade, Nucleotide Incision Repair (NIR) pathway has been described as a new function of APE1 which works as back up of BER pathway ensuring a correct removal of damaged bases as a result of oxidative stress [20–25]. NIR activity by APE1 consists of an incision at