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# *Schistosoma mansoni*: Gene expression of the nucleotide excision repair factor 2 (NEF2) during the parasite life cycle, and in adult worms after exposure to different DNA-damaging agents

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**Abstract**

DNA is often damaged by many environmental agents, which lead to the up-regulation of several genes involved in different repair pathways. *Schistosoma mansoni* has a complex life cycle, being exposed to a subset of DNA-damaging agents, such as those present in the environment and host immune response. Recently, studies showed that nucleotide excision repair (NER) is an indispensable mechanism for removing a broad spectrum of different DNA lesions. In the present report, we showed the gene expression of nucleotide excision repair factor 2 (NEF2) SmRad23 and SmRad4, in different developmental stages of *S. mansoni*, as well as the differential expression of these genes in *S. mansoni* adult worms treated with DNA-damaging agents. Furthermore, it was revealed the correlation of these genes with their orthologues in other eukaryotes. Our reports suggest that NER is an important repair pathway during the complex life cycle of *S. mansoni*.


*Keywords:* *Schistosoma mansoni*; DNA repair; Nucleotide excision repair; Proteasome; Rad23; Rad4

## 1. Introduction

*Schistosoma mansoni* has a complex life cycle involving a mammalian definitive host, a molluscan intermediate host and a free aquatic environment. In mammals, adult male and female worms mate and deposit eggs, which are excreted through the feces to the environment and then hatch releasing the miracidium stage in water. Miracidia infect the snail and differentiate into sporocysts that multiplicate by asexual reproduction. Another free aquatic stage is cercaria, which infects

*Abbreviations:* NER, nucleotide excision repair; TCR, transcription-coupled repair; GGR, global genomic repair; NEF, nucleotide excision repair factor; UBL, ubiquitin-like; UBA, ubiquitin-associated; RB4, Rad4-binding; TMA, tetramethylammonium; MMS, methylmethanesulphonate; XP, xeroderma pigmentosum; Png1, peptide *N*-glycanase; ERAD, endoplasmic reticulum – associated protein degradation; CPDs, cyclobutane pyrimidine dimmers; DDB, damaged-DNA binding

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the mammalian host through active penetration of intact skin, losing its forked tail and becoming schistosomulum. The schistosomula migrate to their final location in the mesenteric veins of the mammalian host and then differentiate into adult worms (Wilson and Lawson, 1980; El-Ansary, 2003). In this process, *S. mansoni* undergoes several modifications in its life cycle, being exposed to a subset of DNA-damaging agents, such as those present in the environment and host immune response, and therefore, similar to other organisms, it is likely to be provided for efficient repair mechanisms.

In eukaryotes, nucleotide excision repair (NER) is the major cellular pathway responsible for the protection against mutagenesis, cytotoxicity and neoplasia, being conserved from yeast to humans (Hoeijmakers, 1995). The presence of NER genes was shown in the *S. mansoni* transcriptome project (Verjovski-Almeida et al., 2003). This repair system acts through removing a wide range of structurally unrelated DNA lesions such as those induced by UV light (cyclobutane pyrimidine dimmers (CPD) and 6-4 photoproducts), and bulky DNA adducts (*N*-acetyl-2-aminofluorene, cisplatin intra-strand crosslinks, polycyclic aromatic hydrocarbon adducts) (Huang et al., 1992; Wang et al., 1993).

At least five integrated steps can be discerned in NER: damage recognition, incision of the damaged strand on both sides of the lesion, excision of the lesion-containing oligonucleotide, synthesis of new DNA using the undamaged strand as a template, and DNA ligation (Huang et al., 1992; Hoeijmakers, 1995).

There are two NER subpathways: transcription-coupled repair (TCR) and global genomic repair (GGR). TCR is specific to the repair of transcribed strand of active genes, and GGR is responsible for the repair of the untranscribed regions within overall genomic DNA (Masutani et al., 1994; Sweder and Madura, 2002; Cleaver, 2005). NER is mediated by protein complexes called nucleotide excision repair factors (NEFs). NEF1 binds to damaged DNA and contains a damage recognition protein Rad14 and the 5′ endonuclease complex Rad1/Rad10. NEF2 (Rad4, Rad23) and NEF4 (Rad7, Rad16) also recognize the DNA lesion. NEF3 is composed for a 3′ endonuclease (Rad2) and the RNA polymerase II transcription factor complex, TFIIH (helicases Rad25/Rad3), responsible for DNA unwinding and opening around the lesion (Guzder et al., 1998; Araújo and Wood, 1999; Prakash and Prakash, 2000).

The NEF2 complex has been extensively studied in eukaryotes, and its role emphasized in NER (Huang et al., 1992; Sugasawa et al., 1998; Sweder and Madura, 2002). Several studies in yeast and human have been developed emphasizing NEF2 complex and its role connecting NER and proteasome (Schauber et al., 1998; Russell et al., 1999; Sweder and Madura, 2002; Ortolan et al., 2004). The human homologue of yeast Rad23/Rad4 complex is hHR23/XPC (Masutani et al., 1994). Rad23 protein has four domains, one N-terminal ubiquitin-like (UBL), two ubiquitin-associated (UBA), and one Rad4-binding (RB4, located between UBA1 and UBA 2) that permit its interaction with both NER and proteasome. It is known that UBL and UBA domains link Rad23 proteins to the proteasome and polyubiquitinated substrates, respectively, and RB4 domain promotes the linkage between Rad23 and Rad4 (Masutani et al., 1997; Schauber et al., 1998; Walters et al., 2004; Miller and Gordon, 2005).

The complex assembled by Rad23 and Rad4 proteins, NEF2, is indispensable to the optimal activity of NER, being responsible for the damage recognition and recruiting of other repair proteins to the sites of DNA lesions (Huang et al., 1992; Sugasawa et al., 1998; Sweder and Madura, 2002). However, reports have demonstrated that the main function of Rad23 in DNA repair is to stabilize Rad4, protecting it from the proteasome degradation, and therefore constitutes only an accessory protein in NER (Lommel et al., 2002; Ng et al., 2003; Ortolan et al., 2004; Xie et al., 2004). Additionally, the direct role by Rad23 in repair is supposed to be exerting a stimulatory effect on Rad4-mediated damage binding/recognition (Xie et al., 2004).

The precise function of Rad23 in NER remains unclear. According to Xie et al. (2004) Rad23 constitutes an accessory NER protein. In addition to its repair participation, this protein has a well-defined activity in the 26S proteasome, in which exhibits a cooperative role in the recognition and translocation of polyubiquitinated proteins to the proteolytic core – 20S proteasome (Chen and Madura, 2002; Saeki et al., 2002; Lambertson et al., 2003; Kim et al., 2004). Thus, Rad23 presents a dual role, establishing a link between NER and ubiquitin–proteasome pathway (Schauber et al., 1998; Russell et al., 1999).

According to Sugasawa et al. (1996), the fraction of hHR23 bound to XPC is small in comparison to the total amount of hHR23 in the cell, which suggests that the protein has some functions other than those related to NER. Furthermore, Kim et al. (2006) have proposed a novel function of the XPCB (RB4) domain outside of DNA repair, in which the interaction between Rad23 and Png1 (peptide *N*-glycanase) facilitates not only the recognition of Rad23 and/or Png1 substrate but also the transference direction of deglycosylated ERAD (endoplasmic reticulum – associated protein degrada-