Bioinformatics and expression analysis of the Xeroderma Pigmentosum complementation group C (XPC) of Trypanosoma evansi in Trypanosoma cruzi cells

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Abstract

Nucleotide excision repair (NER) acts repairing damages in DNA, such as lesions caused by cisplatin. Xeroderma Pigmentosum complementation group C (XPC) protein is involved in recognition of global genome DNA damages during NER (GG-NER) and it has been studied in different organisms due to its importance in other cellular processes. In this work, we studied NER proteins in Trypanosoma cruzi and Trypanosoma evansi, parasites of humans and animals respectively. We performed three-dimensional models of XPC proteins from T. cruzi and T. evansi and observed few structural differences between these proteins. In our tests, insertion of XPC gene from T. evansi (TevXPC) in T. cruzi resulted in slower cell growth under normal conditions. After cisplatin treatment, T. cruzi overexpressing its own XPC gene (TcXPC) was able to recover cell division rates faster than T. cruzi expressing TevXPC gene. Based on these tests, it is suggested that TevXPC (being an exogenous protein in T. cruzi) interferes negatively in cellular processes where TcXPC (the endogenous protein) is involved. This probably occurred due interaction of TevXPC with some endogenous molecules or proteins from T.cruzi but incapacity of interaction with others. This reinforces the importance of correctly XPC functioning within the cell.

Keywords: Nucleotide excision repair (NER), Xeroderma Pigmentosum complementation group C (XPC), T. evansi, T. cruzi.

Resumo

O reparo por excisão de nucleotídeos (NER) atua reparando danos no DNA, como lesões causadas por cisplatina. A proteína Xeroderma Pigmentosum complementation group C (XPC) está envolvida no reconhecimento de danos pela via de reparação global do genoma pelo NER (GG-NER) e tem sido estudada em diferentes organismos devido à sua importância em outros processos celulares. Neste trabalho, estudamos proteínas do NER de Trypanosoma cruzi e Trypanosoma evansi, parasitos de humanos e animais respectivamente. Modelos tridimensionais das proteínas XPC de T. cruzi e T. evansi foram feitos e observou-se poucas diferenças estruturais entre estas proteínas. Durante testes, a inserção do gene XPC de T. evansi (TevXPC) em T. cruzi resultou em crescimento celular mais lento em condições normais. Após o tratamento com cisplatina, T. cruzi superexpressando seu próprio gene XPC (TcXPC) foi capaz de recuperar as taxas de divisão celular mais rapidamente do que T. cruzi expressando o gene TevXPC. Com base nesses testes, sugere-se que TevXPC (sendo uma proteína exógena em T. cruzi) interfere negativamente nos processos celulares em que TcXPC (a proteína endógena) está envolvida. Isso provavelmente ocorreu pois TevXPC é capaz de interagir com algumas moléculas ou proteínas endógenas de T.cruzi, mas é incapaz de interagir com outras. Isso reforça a importância do correto funcionamento de XPC dentro da célula.

Palavras-chave: Reparo por excisão de nucleotídeos (NER), Xeroderma Pigmentosum complementation group C (XPC), T. evansi, T. cruzi.
1. Introduction

Trypanosoma cruzi is the causative agent of Chagas disease or American trypanosomiasis, which infects about six to seven million people worldwide, mostly in Latin America (WHO, 2020; Bianchi et al., 2021), considered a typical disease of low-income population developing countries (Dutra et al., 2021). In fact, vectorborne transmission is limited to areas of South, Central and North Americas (Bern, 2015). Its transmission occurs mainly by blood-sucking reduviid insects through metacyclic trypomastigotes releaseasement in the feces during the insect blood meal and the entry of these parasites in mammalian host through skin wounds or mucosal membranes (Moretti et al., 2020).

Trypanosoma evansi is the causative agent of “Surra” disease. Despite this disease was endemic in the 1990s in China, affecting animals like buffaloes, cattle, camels and dogs, there are few epidemiological studies conducted on this subject in the past decade (Zheng et al., 2019). “Surra” presents non-specific clinical signs such as weight loss, anorexia, anemia, drop in milk production and reproductive disorders, impacting on economic business (Setiawan et al., 2021). Although this parasite can affect most of the mammals, human infection is rare (Desquesnes et al., 2013; Wabale et al., 2015). The mechanical transmission by biting insects represents the most important way of T. evansi transmission (Desquesnes et al., 2013). In Brazil, some studies emphasize that T. evansi is spreading, with new cases arising in regions previously free of infection, sustaining the need for precise measures of active surveillance for “Surra” (Reck et al., 2020).

Expanding knowledge about trypanosomatids biology is a way to better elaborate strategies for diseases control. An important study is the investigation of how these parasites maintain the integrity of their DNA. According to Sancar et al., (2004), DNA lesions in organisms can result in mutation, cancer, cell death or even organism failure. Nucleotide excision repair (NER) eliminates DNA lesions capable of causing double helix distortion, base pairing interference as well as blocking of transcription and replication (Costa et al., 2003). An example of an agent that causes DNA damage repaired by NER is cisplatin (Kang et al., 2010). Since cisplatin is a DNA-damaging agent and unrepaired DNA would lead to cell death, the ability of a cell to survive when exposed to this drug could depend on the efficiency of its DNA-repair mechanisms (Masters and Köberle, 2003).

NER system has two sub-pathways: (1) Global genome nucleotide excision repair (GG-NER) which recognizes damage from both expressed or silent parts of the genome and (2) Transcription-coupled NER (TC-NER), which recognizes damages that interfere in the elongation of RNA polymerase (Hanawalt and Spivak, 2008). Xeroderma Pigmentosum complementation group C (XPC) protein acts on the recognition process of ultraviolet (UV) DNA lesions and other bulky DNA adducts on the GG-NER, dependent of XPA, RPA and Transcription Factor II-H (TFIIH) proteins at the damage site, where the latter unwinds the double helix of DNA around the lesions (Kemp and Hu, 2017). Subsequently, XPG and XPF/ERCC1 are recruited to remove the damaged fragment (Staresinic et al., 2009). A new DNA strand is synthesized at the site of the incision and ligated to the DNA duplex (Kemp and Hu, 2017). In TC-NER, the initial lesion recognition is made through the stalled RNA Pol at lesions during transcription, and this increases the polymerase interaction with Cockayne syndrome type B (CSB) protein (Vessoni et al., 2020). Thus, RNA Pol translocation along the DNA strand is stimulated and the TFIIH complex accesses the lesion site (Hanawalt and Spivak, 2008). The next steps are similar for TC-NER and GG-NER (Schärer, 2013).

Regarding NER in trypanosomatids, the genomes of T. cruzi, Trypanosoma brucei and Leishmania major contain the majority of the NER components, but the biochemical mechanisms of this pathway may present some minor differences as compared to more complex eukaryotes (Passos-Silva et al., 2010). In T. brucei it has been shown that XPC gene silencing by RNA interference (RNAi) resulted in cell death, suggesting a vital role of this gene in T. brucei (Machado et al., 2014). Studies about NER genes and proteins from parasites contribute to the development of disease control strategies by understanding how these microorganisms deal with adversities that damage their DNA. In view of this, the goals of this work were to perform bioinformatic analysis in NER proteins from some trypanosomatids and evaluate the expression effects of XPC from T. evansi (TevXPC) in T. cruzi.

2. Material and Methods

2.1. Bioinformatics analysis

Gene ID’s of trypanosomatids NER proteins studied in this work are presented in Table 1 which was adapted from Genois et al. (2014). Sequences of NER proteins were selected from TriTrypDB (Aslett et al., 2010) and NCBI (NCBI Resource Coordinators, 2018) databases. Proteins from T. evansi were aligned with their orthologues in T. brucei, T. cruzi, Leishmania major, Saccharomyces cerevisiae, and humans through Protein Blast from NCBI. Alignment between TCXPC and TevXPC was also performed using a tool that can align virtually protein sequences called Clustal Omega (Sievers et al., 2011). Protein domains were located using InterPro (Blum et al., 2021), which contains integrated databases as Pfam (Mistry et al., 2021), PROSITE (Hulo et al., 2006) and SMART (Letunic et al., 2021). Alignments of NER proteins domains in T. cruzi and T. evansi were performed through NCBI Protein Blast. Motifs in proteins were investigated through ScanProsite, a tool for detecting PROSITE signature matches in protein sequences (de Castro et al., 2006). Three-dimensional models of proteins were performed by Phyre2, a bioinformatic tool for prediction and analysis of protein structure, function, and mutations (Kelley et al., 2015). The amino acid sequences found in TriTrypDB were placed into Phyre2 and the “normal modelling mode” was chosen for generation of three-dimensional models. This tool predicted TcXPC, TevXPC, and TbXPC protein’s structure based on a crystal structure of Rad4-Rad23 bound to a mismatched DNA performed by Min and Pavletich (2007) and available in RCSB Protein
The XPC gene of T. evansi and T. cruzi can be accessed through the PDB ID: 2QSH. The TcXPB, TevXPB, TcXPB-R and TevXPB-R proteins structures were performed based on a cryogenic electron microscopy structure of the human TFIIH core complex performed by Greber et al. (2019). This structure can be accessed through the PDB ID: 6NMI. All three-dimensional models generated were visualized and color edited through the PyMOL (www.pymol.org). The size space between parts of the protein models were measured through Jmol bioinformatic tool (jmol.sourceforge.net).

### 2.2. In vivo culture of T. evansi and DNA extraction

This work was approved by the Comitê de Ética em Experimentação Animal (Committee of Ethics in Animal Experimentation; CETEA) of the Santa Catarina State University (UDESC) under protocol number 2014 1.28.11. For parasite multiplication, one rat (Rattus norvegicus) was intraperitoneally infected with T. evansi cells (donated by Dr. Silvia Gonzalez Monteiro, UFSM, Rio Grande do Sul). Parasitemia was estimated by microscopic examination of tail blood smears stained with Panótico Rápido® (Laborclin, Solabia Group, Brazil). When 40 parasites/field in optical

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**Table 1.** Trypanosomatids genes involved in nucleotide excision repair (NER). The ellipsis indicates that there are not homologs found (...). Adapted from Genois et al. (2014).

| Name (other name) | T. evansi | T. brucei | T. cruzi | L. major | S. cerevisiae | Human |
|-------------------|-----------|-----------|----------|----------|---------------|-------|
| XPA               | ...       | ...       | ...      | ...      | ...           | YMR201C NM_000380 |
| XPC               | TevSTIB805.9.8560 | Tb297.9.11930 | TcCLB.507011.140 | LmjF.35.3450 | YER162C NM_004628 |
| RAD23             | TevSTIB805.6.4810 | Tb297.6.4650 | TcCLB.511731.10 | LmjF.30.3300 | YEL037C NM_005053 |
| TFIIH-XPB         | TevSTIB805.3.5380 | Tb297.3.5100 | TcCLB.510149.50 | LmjF.29.0590 | YIL143C NM_000122 |
| XPB-R             | TevSTIB805.11.01.16840 | Tb297.11.16270 | TcCLB.511527.20 | LmjF.32.3920 | ... ... |
| TFIIH-XPD         | TevSTIB805.8.6240 | Tb297.8.5980 | TcCLB.511075.30 | LmjF.24.2280 | YER171W NM_000400 |
| TFIIH-Tb1 (p62)   | TevSTIB805.11.01.9710 | Tb297.11.9430 | TcCLB.511589.29 | LmjF.36.3110 | YDR311W NM_005316 |
| TFIIH-Tb2 (p52)   | TevSTIB805.10.5510 | Tb297.10.5210 | TcCLB.510297.80 | LmjF.36.0800 | YPL122C NM_001517 |
| TFIIH-Ss1 (p44)   | TevSTIB805.8.6840 | Tb297.8.6540 | TcCLB.511907.30 | LmjF.24.1680 | YLR005W NM_001515 |
| TFIIH-TSP1        | TevSTIB805.11.01.1030 | Tb297.11.1080 | TcCLB.511423.40 | LmjF.20.0400 | ... ... |
| TFIIH-TSP2        | TevSTIB805.11.01.14590 | Tb297.11.14110 | TcCLB.511727.150 | LmjF.32.0860 | ... ... |
| TFIIH-Tb4 (p34)   | TevSTIB805.11.01.16620 | Tb297.11.16070 | TcCLB.509073.60 | LmjF.32.2885 | YPR056W NM_001516 |
| TFIIH-Tb5         | TevSTIB805.10.14950 | Tb297.10.14210 | TcCLB.511283.50 | LmjF.16.1145 | ... ... |
| XPG               | TevSTIB805.9.8390 | Tb297.9.11760 | TcCLB.507009.120 | LmjF.35.3590 | YGR258C NM_000123 |
| ERCC1             | TevSTIB805.7.2130 | Tb297.7.2060 | TcCLB.510165.20 | LmjF.22.0070 | YML095C NM_001983 |
| XPF               | TevSTIB805.5.4190 | Tb297.5.3670 | TcCLB.509779.10 | LmjF.08.0140 | YPL022W NM_005236 |
| RPA1              | TevSTIB805.11.01.9370 | Tb297.11.9130 | TcCLB.510901.60 | LmjF.28.1820 | YAR007C NM_002945 |
| RPA2              | TevSTIB805.5.1910 | Tb297.5.1700 | TcCLB.510821.50 | LmjF.15.0270 | YNL312W NM_002946 |
| RPA3              | ... ... ... ... | ... ... ... ... | ... ... ... ... | YJL173C NM_002947 |
| Pol ε             | TevSTIB805.9.7440 | Tb297.9.10440 | TcCLB.506147.180 | LmjF.35.4360 | YNL262W NM_006231 |
| Pol δ             | TevSTIB805.2.970 | Tb297.2.1800 | TcCLB.510259.6 | LmjF.33.1690 | YDL102W NM_002691 |
| DNA ligase I      | TevSTIB805.6.4950 | Tb297.6.4780 | TcCLB.509645.80 | LmjF.30.3440 | YDL164C NM_000234 |
| DDB1              | TevSTIB805.6.5280 | Tb297.6.5110 | TcCLB.509165.49 | LmjF.30.3710 | ... ... |
| DDB2              | ... ... ... ... | ... ... ... ... | ... ... ... ... | YDL156W NM_000107 |
| CSA               | ... ... ... ... | ... ... ... ... | ... ... ... ... | YDR030C NM_000082 |
| CSB               | TevSTIB805.7.4360 | Tb297.7.4080 | TcCLB.508675.20 | LmjF.14.0840 | YJR035W NM_000124 |
| Def1              | ... ... ... ... | ... ... ... ... | ... ... ... ... | YKL054C NM_004084 |
| HuF2              | TevSTIB805.11.01.8320 | Tb297.11.8010 | TcCLB.504041.29 | LmjF.28.0760 | YBR114W NM_003594 |

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Data Bank (Berman et al., 2000). This crystal structure can be accessed through the PDB ID: 2QSH. The TcXPB, TevXPB, TcXPB-R and TevXPB-R proteins structures were performed based on a cryogenic electron microscopy structure of the human TFIIH core complex performed by Greber et al. (2019). This structure can be accessed through the PDB ID: 6NMI. All three-dimensional models generated were visualized and color edited through the PyMOL (www.pymol.org). The size space between parts of the protein models were measured through Jmol bioinformatic tool (jmol.sourceforge.net).
100x was reached, the rat was anesthetized with xylazine (13 mg/kg) and ketamine (90 mg/kg). The blood containing the parasites was collected by cardiac puncture and first it was separated by Percoll® (GE Healthcare, Illinois, USA) gradient (17,500xg, 25 min, 4 °C), (Grab and Bwayo, 1982). The supernatant obtained was washed twice with PBS with 2% glucose (PBS-G) (6,000xg, 10 min, 4 °C). After this, DEAE-celullose (Sigma-Aldrich, Missouri, USA) chromatography was performed in a 10 mL syringe to the isolation of the parasites from blood cells (Lanham and Godfrey, 1970). Genomic DNA was extracted using the “Quick-DNA Universal Kit” (Zymo Research, California, USA) following the manufacturer’s instructions.

2.3. TevXPC coding region amplification

The coding region of the TevXPC gene was amplified by Polymerase Chain Reaction (PCR) (Mullis et al., 1986). For this, the TevXPC gene sequence was found in TriTrypDB and we designed the following primers: TevXPC Fwd HindIII (5’- CCAAGCTTATGGGGCAGCAGAAAAAA -3’) and TevXPC Rev Xhol (5’- CCGCTGCTAGTCAGTGAGAGGAAAGATG -3’). To amplify a 2307-bp fragment of TevXPC, the PCR was performed in accordance with the instructions from Q5® High-Fidelity DNA-Polymerase manufacturer (New England Biolabs® Inc., Massachusetts, USA). The reaction mixture contained Q5 Reaction Buffer and Q5 High GC Enhancer at 1X final concentration, 1U Q5® High-Fidelity DNA Polimerase, 0.5 μM of each primer, 200 μM deoxynucleotide triphosphates (dNTPs), 111 ng of T. evansi DNA and nuclease-free water to make up the final volume of 50 μL. The temperature conditions included initial denaturation at 98°C for 30 seconds, 32 cycles of 98°C for 10 seconds, 64°C for 30 seconds, and 72°C for 90 seconds, followed by 2 minutes at 72°C. Amplicons were stained with GelRed® Nucleic Acid Gel Stain (Biotium, Inc., California, USA), analyzed through 1.5% gel agarose electrophoresis, and visualized under UV light in a gel documentation system (MiniBiS Pro (DRN Bio Imaging System, Neve Yamin, Israel)). To determine molecular weight, was used 1 Kb DNA Ladder (Invitrogen™, Massachusetts, USA).

2.4. Gene cloning into pGEM®-T Easy vector

Amplicons were purified with PureLink Quick Gel Extraction Kit (Invitrogen™, Massachusetts, USA) and were A-tailed. The A-tailing reaction was conducted in a final volume of 10 μL containing reaction buffer at 1X final concentration, 2.5 mM magnesium chloride, 0.2 mM deoxyadenosine triphosphate (dATP), 5U Hot Start Platinum™ Taq DNA Polymerase (Invitrogen™, Massachusetts, USA) and 6.5 μL of purified PCR fragment. The reaction mixture was incubated at 94°C for 2 minutes and 30 seconds, 60°C for 30 seconds and 72°C for 30 minutes. Amplicon A-tailed was inserted into the pGEM®-T Easy vector (Promega™, Madison, USA) following the manufacturer’s instructions. The ligation reaction was incubated at 4°C overnight and 5 μL of this mixture was inserted into Escherichia coli DH10B electrocompetent cells that were transferred on LB agar plates containing 100 μg/ml sodium ampicillin, 0.25 mM IPTG (Isopropyl-β-d-1-thiogalactopyranoside), and 30 μg/ml X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). Plates were incubated at 37°C overnight and positive clones (selected by PCR as described before) were grown in liquid LB containing 100 μg/ml sodium ampicillin at 37°C with shaking overnight. DNA plasmidial was extracted from bacteria using PureLink™ Quick Plasmid Miniprep Kit (Invitrogen™, Massachusetts, USA). The construct was confirmed by Sanger sequencing (Ludwig Biotecnologia Ltda, Brazil) using BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Massachusetts, USA). For this, recombinant plasmid was sequenced using two pGEM®-T Easy primers (T7 promoter and plUC/M13 Reverse primers), and three TevXPC specific primers, TevXPC Fwd HindIII, TevXPC Rev Xhol, and TevXPC Int (5’- CCGCTTGGTGGTGGTTTTG -3’). Basic Local Alignment Search Tool (BLAST) of TriTrypDB was used to corroborate that recombinant plasmid contains a sequence corresponding to TevXPC gene.

2.5. Gene cloning into pROCK expression vector

Recombinant plGEM®-T Easy::TevXPC and pROCK expression vector (DaRocha et al., 2004) were digested with restriction enzymes HindIII (New England Biolabs® Inc., Massachusetts, USA) and Xhol (Promega™, Madison, USA). Samples were analyzed by electrophoresis with 1% low melting gel agarose and fragments of interest were cut and separately purified by the PureLink Quick Gel Gel Extraction Kit (Invitrogen™, Massachusetts, USA). Ligation was performed with a reaction buffer at 1X final concentration, 40 ng of pROCK expression vector, 42 ng of TevXPC gene and 2.5 U T4 DNA Ligase (Ludwig Biotecnologia Ltda, Brazil). The ligation product was inserted in Escherichia coli DH10B electrocompetent cells and transferred on LB agar plate containing sodium ampicillin (100 μg/mL). One clone was grown in liquid LB also containing sodium ampicillin at 37°C shaking overnight, followed by plasmidial DNA extraction using PureYield™ Plasmid Maxiprep System (Promega™, Madison, USA) following the manufacturer’s instructions. Recombinant plasmid pROCK::TevXPC was sequenced by Sanger method using TevXPC Fwd HindIII, TevXPC Rev Xhol and TevXPC Int. primers. Confirmation that the sequences corresponded to the TevXPC gene was performed using BLAST TriTrypDB.

2.6. Parasite transfection

Epimastigotes forms of T. cruzi strain CL Brener were grown at 28°C in liver infusion tryptose (LIT) medium (pH 7.4) supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100U/ml), and streptomycin (100 μg/ml). A total of 4 x 10^7 of these parasites were centrifuged (3,000 rpm, 10 min.) and the pellet was resuspended in electroporation buffer. For transfection, 20 μg of pROCK::TevXPC recombinant plasmid was linearized by digestion with restriction enzyme NotI (New England Biolabs® Inc., Massachusetts, USA). DNA was precipitated with isopropanol, solubilized in 20 μL of 1X electroporation buffer and added to T. cruzi cells for electroporation. One control sample with parasites, but without DNA was also prepared, and parasites were grown as described above at 28°C for 24 hours. After this, neomycin was added to a final concentration of 200 μg/ml for T. cruzi selection during four weeks.
2.7. Confirmation of TevXPC mRNA transcription in T. cruzi cells

RT-PCR was performed to confirm mRNA transcription of TevXPC in transfected cells of T. cruzi (here named Tc-TevXPC cells). We tested Tc-TevXPC in comparison with WT (wild type) and Tc-TcXPC cells (T. cruzi transfected with pROCK::TcXPC plasmid which were kindly donated by Dr. Isabela Cecília Mendes, UFMG, Minas Gerais, Brazil). For this, total cellular RNA from Tc-TevXPC, WT, and Tc-TcXPC cells was treated with DNase and tested for non-DNA contamination by PCR. The mixture reaction contained 1.5 U GoTaq® DNA Polymerase (Promega™, Madison, USA), Colorless GoTaq® Reaction Buffer at 1X final concentration, 1.5mM MgCl2, 200 μM dNTPs, 0.5 μM of each primer specific for TcXPC gene (also donated by Dr. Isabela Cecília Mendes), 150 ng of RNA and nuclease-free water to make up the final volume of 20 μL. Positive and negative controls contained the same components except that RNA was replaced with T. cruzi DNA and nuclease-free water, respectively. The temperature conditions included initial denaturation at 94°C for 2 minutes, 30 cycles of 94°C for 30 seconds, 64°C for 1 minute, and 72°C for 2 minutes and 40 seconds, followed by 10 minutes at 72°C. Amplicons were analyzed through electrophoresis with 1% gel agarose. SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen™, Massachusetts, USA) was used for cDNA synthesis following manufacturer’s instructions with oligo(dT) primer. PCR amplification was performed using cDNA from WT, Tc-TcXPC and Tc-TevXPC with the same protocol for non-DNA contamination test in RNA samples. However, this protocol was performed with the following exceptions: positive control contained T. evansi DNA and the specific primers TevXPC Fwd HindIII and TevXPC Rev Xhol were used on reactions.

2.8. Growth curve under normal conditions and after cisplatin treatment

In this work, we evaluate the growth of Tc-TevXPC cells in comparison with the growth of WT and Tc-TcXPC cells under normal conditions. Cultures with the same initial parasite concentration were incubated at 28°C in liver infusion tryptose (LIT) medium (pH 7.4) supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100U/ml), and streptomycin (100 μg/ml) for approximately seven days. Cells were daily counted in a Neubauer chamber using erythrosine vital stain. Experiments were performed in triplicate.

The growth after cisplatin treatment was evaluated in Tc-TevXPC and compared with the growth of WT and Tc-TcXPC cells also treated with this drug. For this, cultures with the same initial parasite concentration were treated with 300 μM of cisplatin and incubated at 28°C for 1 hour. After this period, the cells were removed from the LIT medium containing cisplatin and incubated for six days in a new LIT medium without the drug. The cell number was measured by counting as described above. The results were compared to untreated cultures. Experiments were performed in triplicate.

2.9. Statistical analysis

Growth and treatment curves graphs were generated in the GraphPad Prism 7.00 program (GraphPad Software Inc., CA, USA). The statistical analyzes were performed using the unpaired t test and p-value < 0.05 was considered as significant.

3. Results

3.1. Percentage of identity between NER proteins from T. evansi and other organisms

Percentage of identity between NER proteins from T. evansi and its orthologues from T. brucei, T. cruzi, L. major, S. cerevisiae, and Homo sapiens is shown in Table 2. Among trypanosomatids, T. evansi NER proteins show a higher percentage of identity with their orthologues from T. brucei than T. cruzi and L. major. The highest percentage of identity between NER proteins from T. evansi and T. brucei was shown by Pol Delta, RPA1, and TFIIH-Tfb5 (three proteins presented 100% of identity with E-values 0.0, 0.0 and 8e-119, respectively). The lowest identity percentage was shown by TFIIH-TSP1 protein (98.14%, E-value: 0.0). In T. evansi and T. cruzi comparison, the highest percentage of identity between NER proteins was showed by TFIIH-XPD (81.09%, E-value: 0.0), while the lowest was shown by RAD23 (40.92%, E-value: 3e-78). The highest percentage of identity between NER proteins from T. evansi and L. major was shown by Pol Delta (74.05%, E-value: 0.0), and the lowest percentage was presented by p34 (28.37%, E-value: 2e-28).

3.2. Comparative analysis of TcXPC, TevXPC, and TbXPC proteins

Sequences of TevXPC and TbXPC proteins were aligned and displayed 99.61% identity (E-value: 0.0). Sequences of TcXPC and TevXPC proteins showed 52.94% identity (E-value: 0.0) between them. The TcXPC model presented 58% of coverage, 21% of identity and 100% of confidence. For TevXPC, the values were 58% of coverage, 20% of identity and 100% of confidence while TbXPC presented 61% of coverage, 18% of identity and 100% of confidence. In TcXPC, TevXPC, and TbXPC model comparisons was observed that the distance between the “arms” involved in DNA lesion recognition is approximately 16.59 Å in TcXPC (see Figure 1a), 17.36 Å in TevXPC (see Figure 1b) and 23.93 Å for TbXPC (see Figure 1c). We also performed the scheme of TevXPC protein bound to a mismatch DNA (see Figure 1d) and TcXPC, TevXPC and TbXPC models overlay (see Figure 1e). The crystal structure of Rad4-Rad23 bound to a mismatched DNA performed with Min and Pavletich (2007) that was used as template for generation of three-dimensional models is shown in Figure 1f. In domain analysis of TcXPC and TevXPC, three RAD4 beta–hairpin domains were found: BHD1 (predicted by InterPro, Pfam and SMART), BHD2 (predicted by InterPro and SMART) and BHD3 (predicted by InterPro, Pfam and SMART). Furthermore, was found one RAD4/PNGase transglutaminase-like fold (TGD) predicted by InterPro and Pfam. Domain TGDs from TcXPC and TevXPC...
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3.3. Comparative analysis of TcRAD23 and TevRAD23 proteins

Full-length alignment between TcRAD23 (RAD23 from *T. cruzi*) and TevRAD23 (RAD23 from *T. evansi*) revealed 40.92% identity (E-value: 3e-78). Domain analysis showed that TcRAD23 (361 amino acids length) contains one ubiquitin-like domain (UBL) located on the amino acid 1 to 74 (predicted by InterPro and PROSITE), one XPC-binding domain (R4BD) from the amino acid 236 to 289 (predicted by InterPro and Pfam), and ubiquitin-associated domains (UBA). About the last, InterPro predicted that TcRAD23 contains only one UBA (amino acid 149 to 358) while two UBA domains were predicted by SMART (amino acid 151 to 189 and 317 to 357), Pfam (amino acid 151 to 187 and 320 to 352) and PROSITE (amino acid 149 to 190 and 314 to 358). TevRAD23 (356 amino acids length) also presents one domain UBL predicted by InterPro and PROSITE (amino acid 1 to 74), SMART (amino acid 1 to 70) and Pfam (amino acid 5 to 71). One domain UBA was predicted by InterPro and PROSITE (amino acid 149 to 190), SMART (amino acid 151 to 187) and Pfam (amino acid 143 to 179). One R4BD domain was predicted by InterPro and Pfam (amino acid 151 to 189 and 317 to 357). The R4BDs from TcRAD23 and TevRAD23 presented 41.18% identity (E-value: 4e-12). The three-dimensional models for TcRAD23 and TevRAD23 were performed, but more than 50% of the sequences were predicted disorderly and these regions cannot be meaningfully predicted.

3.4. Comparative analysis of Tcp62 and Tevp62 proteins

Tcp62 (p62 from *T. cruzi*) and Tevp62 (p62 from *T. evansi*) alignment revealed 51.09% identity (E-value: 4e-112) between them. In analysis using ScanProsite, was not found in TevXPC and TcXPC proteins sequences studied in this work, a sequence motif involved in binding with p62 as Okuda et al. (2015) describe for Human XPC and...
yeast RAD4: D/E-F/W-E-D/E-V. However, we noticed in TevXPC (see Figure 2b), one sequence (E-W-E-E-V) with high similarity with the sequence previously described. About TcXPC, the candidate sequence (E-W-D-E-V) contains one amino acid change in the third position that differs from the motif already described by Okuda et al. (2015).

3.5. Comparative analysis of TcXPB, TevXPB, TcXPB-R, and TevXPB-R proteins

Alignment of proteins TcXPB (XPB from *T. cruzi*) and TevXPB (XPB from *T. evansi*) showed a 68.06% identity (E-value: 0.0) between them. The three-dimensional models of TcXPB (see Figure 3a) and TevXPB (see Figure 3b).
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**Figure 2.** (a) Alignment between TcXPC and TevXPC proteins (mismatches highlighted) and its domains. Green: RAD4/PNGase transglutaminase-like fold. Blue: RAD4 beta-hairpin domain 1. Red: RAD4 beta-hairpin domain 2. Yellow: RAD4 beta-hairpin domain 3. (b) Candidate sequence motif involved in p62 interaction (highlighted by brown rectangle) found in TcXPC and TevXPC. This sequence is suggested based on the sequence motif described for Human XPC and yeast RAD4: D/E-F/W-E-D/E-V.

**Figure 3.** (a) TcXPB protein model. (b) TevXPB protein model. (c) TcXPB (green) and TevXPB (red) models overlay.
proteins were performed. The TcXPB model presented 62% of coverage, 36% of identity and 100% of confidence while the TevXPB model presented 61% of coverage, 38% of identity and 100% of confidence. In model overlays of TcXPB and TevXPB (as shown in Figure 3c), the proteins showed high similarity.

Alignment of proteins TcXPB-R (XPB-R from *T. cruzi*) and TevXPB-R (XPB-R from *T. evansi*) showed 65.61% identity (E-value: 0.0) between them. Three-dimensional models of TcXPB-R (Figure 4a) and TevXPB-R (Figure 4b) were performed. The three-dimensional model of TcXPB-R presented 77% of coverage, 38% of identity and 100% of confidence while the TevXPB-R model presented 73% of coverage, 36% of identity and 100% of confidence. In overlapping models of TcXPB-R and TevXPB-R (Figure 4c), the proteins showed high similarity.

3.6. *Tc-TevXPC* cells present growth difficulties

*TevXPC* gene was amplified by PCR and cloned into pGEM®-T Easy vector (Promega™, Madison, USA) followed by Sanger sequencing using two primers designed to be annealed to pGEM®-T Easy and three gene specific primers. The Figure S1a shows the result obtained using one vector specific primer (T7 promoter) as an example. The cloned *TevXPC* gene is 100% identical to the database *TevXPC* sequence (Supplementary Material Figure S1b). Thereafter, *TevXPC* was subcloned in pROCK vector (DaRocha et al., 2004) and recombinant plasmid was sequenced by Sanger method using three gene specific primers. Figure S2a shows the result obtained using the *TevXPC* Int. primer as an example. The sequencing result confirmed the insertion of *TevXPC* in pROCK vector (Figure S2b). Recombinant plasmid pROCK::*TevXPC* was introduced in *T. cruzi* cells and the resultant recombinant strain presented growth difficulties after transfection. Analysis by RT-PCR (Figure 5) showed *TevXPC* amplification from cDNA of the Tc-TevXPC cells. There was no amplification from cDNA of WT and Tc-TcXP cells as expected.

For testing the growth curve, parasite cultures of Tc-TcXP and Tc-TevXPC cells were analyzed in comparison to WT growth (Figure 6). The Tc-TcXP cell grew more slowly than WT in a period of the curve: in three points of the curve, Tc-TcXP grew significantly less than WT (p < 0.05), but there was no significant difference in growth between them on others four points (p > 0.05). Tc-TevXPC cells showed a remarkable growth rate slower than WT: only at day 1 of the curve there was no significant difference in growth between Tc-TevXPC and WT (p > 0.05), but all other days Tc-TevXPC grew significantly less than WT (p < 0.05). After cisplatin treatment (Figure 7), WT and Tc-TevXPC treated cells presented significantly lower growth in comparison with the same cells untreated: only at day 1 of the curve there was no significant difference (p > 0.05) between growth of WT treated and WT untreated cells or Tc-TevXPC treated and Tc-TevXPC untreated, but in all other days, cells treated with cisplatin grew significantly less than its respective untreated cells (p < 0.05). Tc-TcXP cells treated with cisplatin also grew less than Tc-TcXP untreated cells. However, the growth curve difference between these two cultures was not significant in all points of the curve (p > 0.05).

Figure 4. (a) TcXPB-R protein model. (b) TevXPB-R protein model. (c) TcXPB-R (blue) and TevXPB-R (orange) models overlay.
4. Discussion

Trypanosomatids present several highly diverged biological processes and T. cruzi, T. brucei, and Leishmania sp are among the most studied species of this family (Machado et al., 2014). T. cruzi, T. brucei and L. major show some remarkable NER differences from the higher eukaryotes. For instance, they do not present a recognizable ligase III, DDB2, XPA, CSA, and RPA3 genes (Passos-Silva et al., 2010). In humans’ cells, severe consequences are observed if there is misfunction in ligase III (Kohutova et al., 2019), DDB2 (Yoon et al., 2005), XPA (Messaoud et al., 2012; Manandhar et al., 2017), CSA (D’errico et al., 2007) or RPA3 (Dai et al., 2017) protein. Further, T. cruzi, T. brucei and L. major have duplication of XPB gene (Passos-Silva et al., 2010). On the TriTrypDB database we verified that in T. evansi two XPB-like proteins were also present while DDB2, XPA, CSA, RPA3 and ligase III were not found. Furthermore, T. cruzi, T. brucei and L. major contain two trypanosomatid-specific subunits of TFIH: TSP1 and TSP2 (Passos-Silva et al., 2010) that are also present in T. evansi.

We verified the percentage of identity between NER proteins from T. evansi, T. brucei, T. cruzi, L. major, S. cerevisiae, and human (as shown in Table 2) and the majority of trypanosomatids NER proteins studied in this work have orthologues in S. cerevisiae and humans. Among trypanosomatids, NER proteins from T. evansi show a higher percentage of identity with their orthologues in T. brucei than T. cruzi and L. major. It is important to emphasize that in genus Trypanosoma, the T. evansi and T. brucei (that presented the highest identity between their NER proteins) are classified into the subgenus Trypanozoon, while T. cruzi is classified in subgenus Schizotrypanum (Baral, 2010). The L. major, whose NER proteins presented the lowest identity with the orthologs in T. evansi, is classified in the same family as T. evansi, T. cruzi and T. brucei, but not in the same genus (Hamilton et al., 2004).

Figure 5. TevXPC amplification by RT-PCR with the cDNA from cell cultures. Lanes: (1) 1Kb DNA Ladder; (2) WT; (3) Tc-TcXPC; (4 and 5) Tc-TevXPC; (6) positive control (DNA from T. evansi); (7) negative control.

Figure 6. Growth assessment of T. cruzi: wild type (WT), TcXPC superexpressor (Tc-TcXPC) and TevXPC expressor (Tc-TevXPC). Statistical student’s t test: (*) On that point, only Tc-TevXPC presented a statistically significant lower growth in relation to WT (p < 0.05); (**) On that point, both Tc-TcXPC and Tc-TevXPC presented a significant lower growth in relation to WT (p < 0.05). All parasites were at same initial concentration, grown on LIT medium and were counted daily. Representative results of three independent experiments.

Figure 7. T. cruzi growth assessment after cisplatin treatment (300 μM). (a) Wild type (WT). (b) TcXPC superexpressor (Tc-TcXPC). (c) TevXPC expressor (Tc-TevXPC). The solid lines represent the untreated cells, while the dotted lines represent the cells treated with cisplatin. Statistical student’s t test: (*) On that point, cells treated with cisplatin presented a statistically significant lower growth in relation to untreated cells (p < 0.05). Representative results of three independent experiments.
Another focus of this work was the investigation about XPC protein in T. evansi and T. cruzi. This protein acts on the recognition of UV DNA lesions and other bulky DNA adducts on the GG-NER (Kemp and Hu, 2017). It is known that human XPC plays a role in many pathways within cells, such as proteolysis and post-translational modifications, transcription regulation, signal transduction, metabolism, DNA repair and replication (Lubin et al., 2013). Further, studies have found that XPC is also involved in base excision repair, chromatin remodeling, cell signaling, proteolytic degradation, and cellular viability (Nemzow et al., 2015).

In TcXPC, TevXPC and TbXPC model comparisons it was observed that the distance between the “arms” involved in DNA lesion recognition is approximately 16.59 Å for TcXPC (see Figure 1a), 17.36 Å in TevXPC (see Figure 1b) and 23.93 Å for TbXPC (see Figure 1c). The fact that these distances are more similar between TevXPC and TcXPC is surprising since TevXPC and TbXPC amino acid sequences showed 99.61% identity (E-value: 0.0), while TevXPC and TcXPC showed 52.94% identity (E-value: 0.0). It is important to highlight that the few differences between amino acid sequences from TevXPC and TbXPC are not located in the domains of these proteins. However, it seems that these changes were enough to present differences between the structures of the lesion recognition site in models performed for these two proteins. We performed a scheme of TevXPC protein bound to a mismatched DNA (see Figure 1d) and an overlapping model of TcXPC, TevXPC and TbXPC (see Figure 1e). Overlapping showed that the three models present a highly similar structure between them with few differences. Furthermore, these three models also presented similarity with Crystal structure of yeast Rad4-Rad23 bound to a mismatched DNA (see Figure 1f) performed by Min and Pavleitch (2007). Rad4-Rad23 is the yeast ortholog of human XPC-RAD23B complex (Krasikova et al., 2013). Likewise, Machado et al. (2014) also performed a TbXPC model and observed a distance of 27 Å between “arms” on the lesion recognition site. The author suggested that because the DNA double helix is approximately 20 Å, it is plausible that TbXPC may bind to two DNA strands while RAD4 that presents 13 Å can only recognize one DNA strand. Considering this, we suggest that is possible that the DNA binding mechanism has differences between TcXPC, TevXPC and TbXPC, because it is probable that TcXPC and TevXPC can recognize only one DNA strand while TbXPC can recognize both DNA strands.

Evaluation of TevXPC expression effects on T. cruzi cells was performed through TevXPC gene insertion in T. cruzi. The growth of modified cells was analyzed in comparison to WT cell growth. The tests were performed under normal conditions of culture as well as after cisplatin treatment. Under normal conditions, Tc-TevXPC cells presented remarkable growth difficulty in relation to WT. This suggests that TevXPC gene insertion in T. cruzi cells may have altered one or more cellular processes of this parasite. Although TcXPC and TevXPC are orthologous, it is believed that exogenous TevXPC protein interacts in one or more T. cruzi cellular processes wherein endogenous TcXPC protein plays a role. However, TevXPC is not able to work efficiently in T. cruzi cells. Therefore, it must have occurred some disorder in the cellular processes of T. cruzi wherein XPC protein is involved, resulting in altered cell growth.

In addition to remarkable difficulty growth of Tc-TevXPC under normal conditions, a slight lower cell growth was also observed in Tc-TcXPC cells when compared with WT. It is possible that excessive XPC protein in Tc-TevXPC and Tc-TcXPC cells results in the greater interaction of this protein with DNA, which can delay normal cell growth.

In the growth curve after cisplatin treatment, Tc-TevXPC cells presented more difficulty in growth when compared with other cells. While treated WT and Tc-TcXPC cells showed an increase in the cellular population during the days of observation, the treated Tc-TevXPC cells did not show an increase in the number of cells within 124 hours. This suggests that exogenous TevXPC can recognize DNA lesions in Tc-TevXPC cells, the possible lack of interaction between exogenous TevXPC and endogenous NER proteins in Tc-TevXPC cells results in no repair of DNA damage. Besides not working efficiently in DNA repair, exogenous TevXPC when binds to damaged DNA, takes the place of endogenous TcXPC on the site of lesion and does not allow that endogenous protein works in DNA repair.

We analyzed the amino acids sequences of TcXPC and TevXPC as well as the amino acids sequences from NER proteins that interact with them. Domain analysis showed three RAD4 beta-hairpin domains (BHD1, BHD2, and BHD3) and one RAD4/PNGase transglutaminase-like fold (TGD) in TevXPC and TcXPC proteins (see Figure 2a). It is predicted that RAD4/XPC proteins do not have transglutaminase enzymatic activity due to the disruption of the active site triad (Anantharaman et al., 2001), but TGD acts in DNA repair (Min and Pavleitch, 2007). In RAD4 protein, the domains TGD and BHD1 bind to DNA undamaged segments, while BHD2 and BHD3 bind to DNA segments that contain cyclobutane pyrimidine dimer lesion. In addition, TGD from RAD4 also interacts with UBA2 and R4BD of RAD23 protein (Min and Pavleitch, 2007). During GG-NER, mammalian RAD23 protein enhances the binding of XPC to DNA damage besides stabilizing XPC (Bergink et al., 2012). Domain TGDs from TcXPC and TevXPC presented 67.53% identity (E-value: 2e-42) while R4BDs from TcRAD23 and TevRAD23 presented 41.18% identity (E-value: 4e-12). In domain analysis, InterPro predicted a single UBA domain in TcRAD23, but Pfam, PROSITE and SMART predicted two UBA domains. It is important to note that the single UBA predicted by InterPro covers the regions of two UBA domains predicted by other databases. Regarding TevRAD23, only one UBA was predicted by InterPro, Pfam, PROSITE and SMART in this protein. Considering the differences between these domains of XPC and RAD23 proteins from T. cruzi and T. evansi, it is possible that in Tc-TevXPC cells, exogenous TevXPC protein could not interact with endogenous TcRAD23.

After DNA lesion recognition, XPC prepares the damaged site for TFIIH recruitment and excision of damaged DNA (Compe and Egly, 2012). TFIIH is recruited by XPC through interactions involving the p62 and XPB subunits of TFIIH (Yokoi et al., 2000). According to Badjatia et al. (2013), kinetoplastid organisms contain two divergent XBP paralogues (XBP and XBP-R). In other hand, in T. brucei only XBP was identified as a TFIIH subunit and is also suspected that in trypanosomes the NER machinery is
separate from the TFIIH complex while XPB-R does not function in transcription but acts in NER (Badjatia et al., 2013). Three-dimensional models of TcXPB (see Figure 3a) and TevXPB (see Figure 3b) were performed and in overlapping these two models it was observed that TcXPB and TevXPB structures are highly similar between them (as shown in Figure 3c). In our alignment analysis, proteins TcXPB and TevXPB showed 68.06% identity (E-value:0.0). Three-dimensional models of TcXPB-R (see Figure 4a) and TevXPB-R (see Figure 4b) also present high similar structure between them (as shown in Figure 4c). Alignment of proteins TcXPB-R and TevXPB-R showed 65.61% identity (E-value:0.0) between these two proteins. It is possible that although XPB proteins form T. cruzi and T. evansi are similar, TFIIH was not recruited due to impairment in TevXPB interaction with TcXPB or TcXPB-R proteins.

Regarding XPC interaction with p62, human XPC and yeast RAD4 contain a common motif involved in binding with p62 (Okuda et al., 2015). We found a candidate sequence motif for interaction with p62 in TevXPB. However, in TcXPB the corresponding sequence contains one amino acid change in the third position that differs from the motif already described (as shown in Figure 2b). Proteins Tcp62 and Tevp62 display 51.09% identity (E-value: 4e–112). It is possible that if exogenous TevXPB could recognize a DNA lesion in T. cruzi cells, it was not able to interact with endogenous Tcp62 due to some differences in relation to Tevp62. This lack of interaction between TevXPB and Tcp62 also may have occurred because motifs of interaction with p62 and surrounding regions in TcXPB and TevXPB have some differences. Thus, if TevXPB did not interact with Tcp62, probably TFIIH was not recruited for damage excision. Therefore, DNA damage recognition by TcXPB in T. cruzi cells is effective in TFIIH recruitment and DNA repair, but if TevXPB (being an exogenous XPC protein) binding to a DNA distortion, it is probably that TFIIH is not recruited due impaired interaction of exogenous XPC with the T. cruzi NER proteins and DNA repair does not occur.

5. Conclusion

In conclusion, although the three-dimensional models performed for TcXPB and TevXPB showed a highly similar structure between them, it is suggested that the identity between these proteins was not sufficient for establishing TevXPB function in T. cruzi. The TevXPB gene insertion in T. cruzi cells decreased their growth. This suggests that TevXPB protein acted as a dominant negative element within T. cruzi cells due decreased their growth. This suggests that TevXPB protein acted as a dominant negative element within T. cruzi cells due taking the place of endogenous TcXPB protein in one or more cellular processes, but is not able to continue the process (or processes). Probably because TevXPB is able to interact with some molecules, but is unable to interact with other factors due to some three-dimensional and/or chemical incompatibility. Another fact observed in our study was that Tc-TevXPB cells did not respond to cisplatin treatment similarly to Tc-TcXPB cells, possibly suggesting that TevXPB recognizes lesions in DNA, but is not able to direct de damage for repair. Therefore, these tests show the importance of correctly XPC functioning within the cell.

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Supplementary Material

Supplementary material accompanies this paper.

**Figure S1** (a) Sanger sequencing electropherogram of recombinant plasmid pGEM-T-Easy::TevXPC. For this result, one vector specific primer (T7 promoter) was used. (b) BLAST result showing that the TevXPC gene inserted in pGEM-T-Easy vector is 100% identical to the database TevXPC sequence. In this illustration, only a portion of the alignment is shown. This result was obtained by joining the sequencings performed with two primers that annealing to pGEM®-T Easy and three gene specific primers.

**Figure S2** (a) Sanger sequencing electropherogram of recombinant plasmid pROCK::TevXPC. For this result, the TevXPC Int. primer was used. (b) BLAST result of the sequencing obtained with plasmid pROCK::TevXPC and TevXPC Int primer.

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