Analysis of the repair activity of enzymes involved in the Base Cleavage Repair pathway.

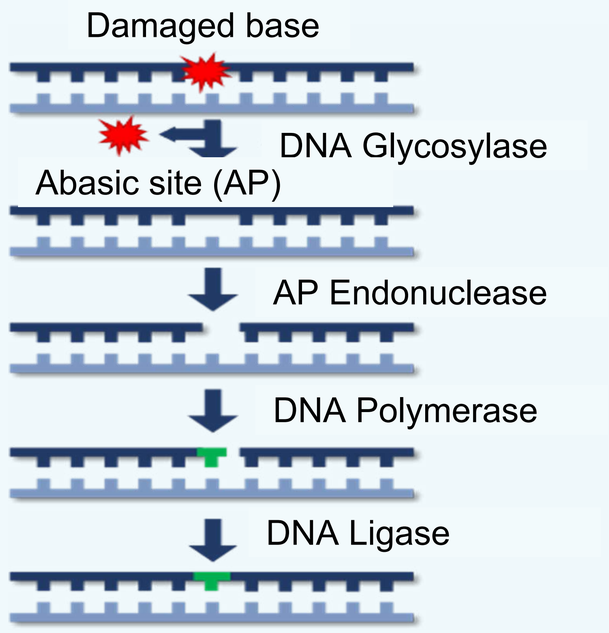
PUPILS: López- Rico,E.[1] Cabezas-Ordoñez,N.[1] Posadas- Zaragoza, E.[2] Rodríguez- Cruz,P.[2] 1ºBachillerato.
RESEARCHERS: León-Rodríguez,E.[1] Moreda- Moreno,M.[1] Jordano-Raya, M.[3]
CENTRES: 1 IES FIDIANA; 2 CES LOPE DE VEGA SCA; 3 DEPARTAMENTO DE GENÉTICA "GRUPO GC22 EPIGENÉTICA" IMIBIC.

1 INTRODUCTION

The Base Excision Repair pathway is a crucial mechanism for the maintenance of genome stability. The BER (Base Excision Repair) mechanism consists of several steps and is initiated by the action of a specific class of repair enzymes called DNA glycosylases, which catalyse the hydrolysis of the N-glycosyl bond linking the altered base to the sugar-phosphate backbone.

There are different types of DNA glycosylases, each of which recognises a specific type of lesion or range of chemically related lesions. After this initial step, which releases the damaged base as a free base, an abasic site (AP) is generated. This AP site is processed by an endonuclease that catalyses the hydrolysis of the phosphodiester bond to the 5' side of the base-free residue, generating a 3'-OH end that will be used at a later stage of repair. In addition, a 5' end is produced with a deoxyribose phosphate residue without base (dRP), which is removed by the action of a deoxyribose phosphate diesterase (dRPase).

Finally, continuity of the damaged strand is restored by the action of a DNA polymerase and a ligase.



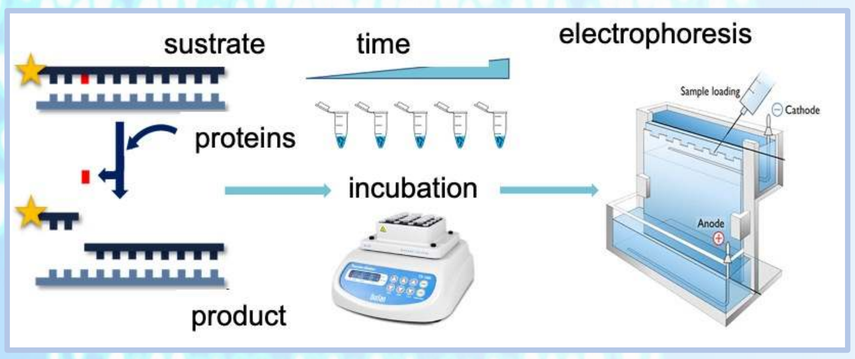
2 RESEARCH OBJECTIVES

The aims of this research are:

- To analyse the repair activity of two enzymes, Uracil DNA Glycosylase and Human AP endonuclease 1 at different times.
- To obtain two enzyme kinetics curves, one for each enzyme as a function of the times used.

3 MATERIALS AND METHODS

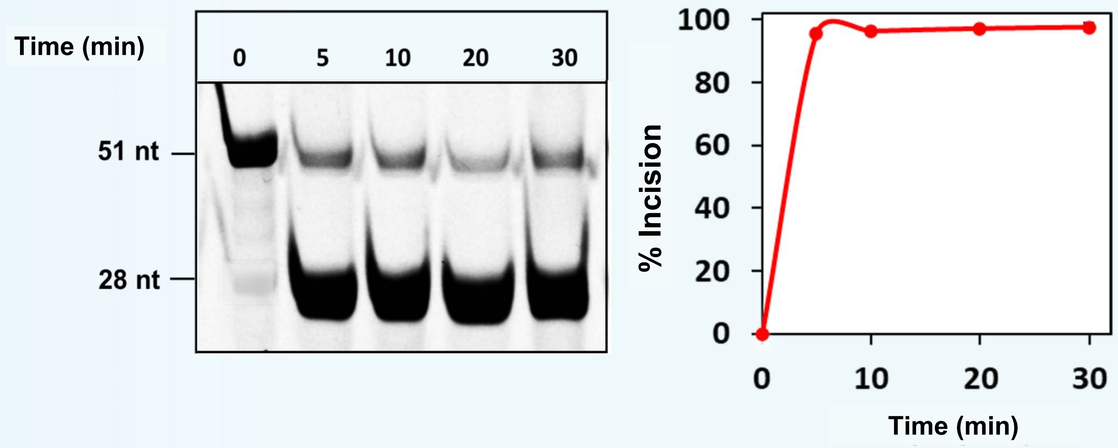
DNA substrates damaged by a uracil or a basic site are synthesised and fluorescently labelled. Subsequently, enzyme activity assays were performed with the enzyme AP Endonuclease and DNA Glycosylase, analysing the efficiency by the appearance of product at different times of the enzymatic reaction. After the reaction, DNA was purified and electrophoresis was performed on acrylamide gels.



After electrophoresis, the data were revealed using a fluorescence scanner and subsequently analysed and quantified using Multigauge software to obtain the enzyme kinetics. Finally, the unprocessed substrate of 51 nt and the accumulation of processed product of 28 nt with respect to time were observed.

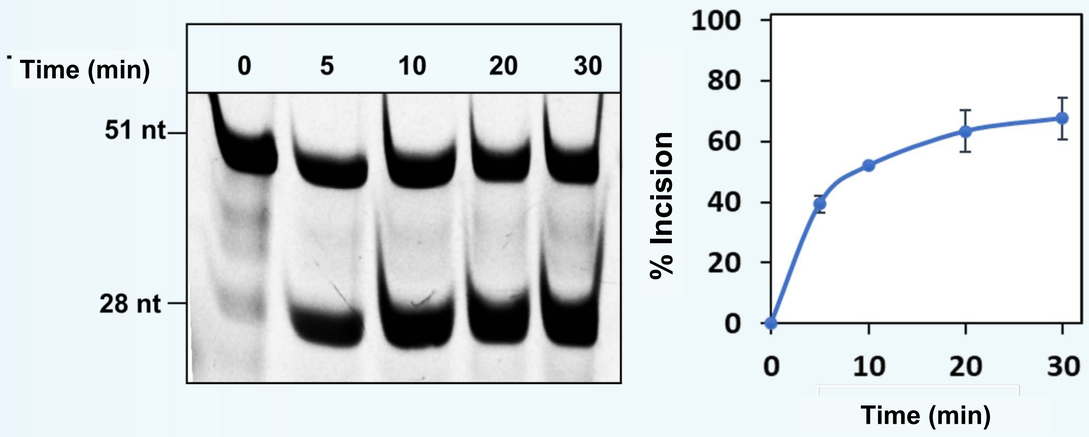
4 RESULTS

Initially, 20 nM of damaged DNA substrate was incubated with a uracil at position 29 in the presence of uracil dna glycosylase (UDG) 0.2 U at 5, 10, 20 and 30 minutes. After analysis of the data, the results indicated that this enzyme is highly efficient, reaching 100% of the substrate processed within 5 minutes of the start of the enzymatic reaction.



Graph 1: a) Electrophoresis. Variation of the amount of product over time with Uracil DNA glycosilase. b) Enzyme kinetics.

In addition, human AP endonuclease 1 (APE1), 0.2 U activity was analysed using substrates (20 nM) that were damaged by an abasic site at position 29. After processing, the data was analysed and it was observed that this enzyme presents a lower efficiency than UDG, since after 5 minutes we obtained an incision of 40%.



Graph 2: a) Electrophoresis. Variation of the amount of product over time with AP Human endonuclease 1. b) Enzyme kinetics.

5 CONCLUSIONS

- The amount of substrate and enzymes used were adequate for the study of the Base Excision Repair (BER) mechanism.
- The enzymes studied, Uracil DNA glycosylase and AP Human endonuclease 1, are able to recognise and process the substrates tested.
- Uracil DNA glycosylase is more efficient than AP human endonuclease 1.

6.ACKNOWLEDGEMENTS

- To the researcher Marina Jordano Raya
- To the tutors Elena León and Mª del Mar Moreda
- To IES Fidiana and CES Lope de Vega
- To the Educational Innovation Programme Fidiciencia 2.0
- To Erasmus+ Programme
- To the Department of Didactics of Experimental Sciences and the Department of Genetics of the University of Cordoba "Group GC22 EPIGENETICS" IMIBIC.