FEN-1 Nuclease in Genome Stability

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Doug Brutlag's Introsem

Introduction to FEN-1

- FEN-1, or Flap EndoNuclease 1, is a structure-specific 5' nuclease that plays a vital role in DNA repair, replication and recombination. It is found in all domains of life. Specifically, in DNA repair, FEN-1 has been implicated in the base excision repair and mismatch repair pathways (slide 6) for the removal of DNA lesions. During replication of DNA, FEN-1 cleaves the flap that results from the displacement of the RNA primer (slide 4). Deletions of FEN-1 in yeast cause a number of replication and repair defects including conditional lethality, increased sensitivity to UV light and chemical mutagens, genomic instability, increased levels of tri-nucleotide repeat expansion and destabilization of telomeric repeats. In humans, tri-nucleotide repeat expansion is implicated in recessive retinitis pigmentosa, lethal junctional epidermolysis bullosa, and familial hypertropic cardiomyopathy. In addition, FEN-1 homozygous knockouts are lethal in mice, and heterozygous knockout mutants display accelerated tumor growth.

Biochemical Properties of FEN-1

5′*GATGTCAAGCAGTCCTAAGTTTGAGGCAGAGTCC
3′GTGCAACTGATGGCAGAACTCCGTCTCAGG
5′CACGTTGACTACCGTC

Flap endonuclease substrate (flap sub)

34nt

FEN-1

21nt

19nt

5′*TTGAGGCAGAGTCC
3′GTGCAACTGATGGCAGAACTCCGTCTCAGG
5′CACGTTGACTACCGTC

Exonuclease substrate (exo sub)

16nt

3nt

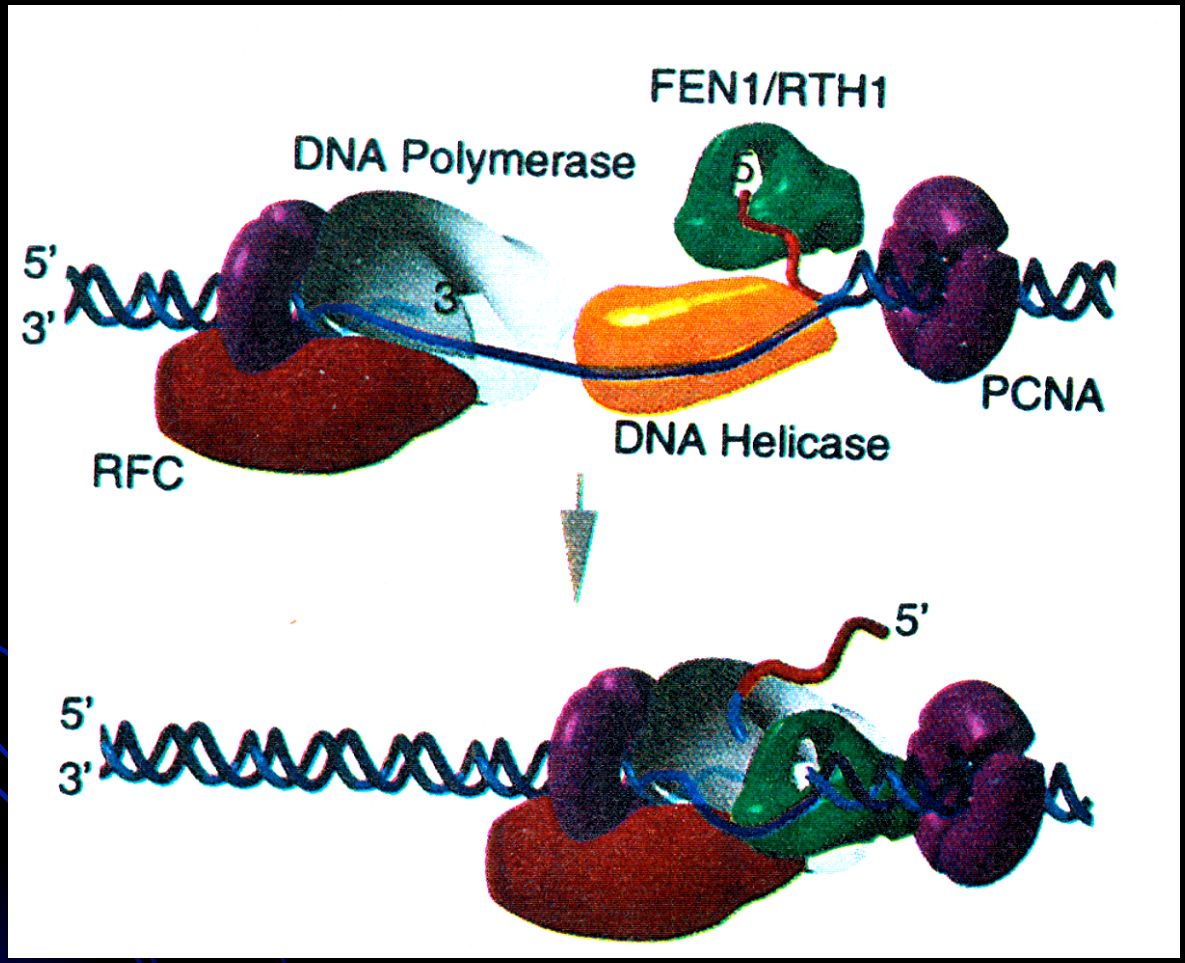
2nt

1nt

Biochemical Properties of FEN-1

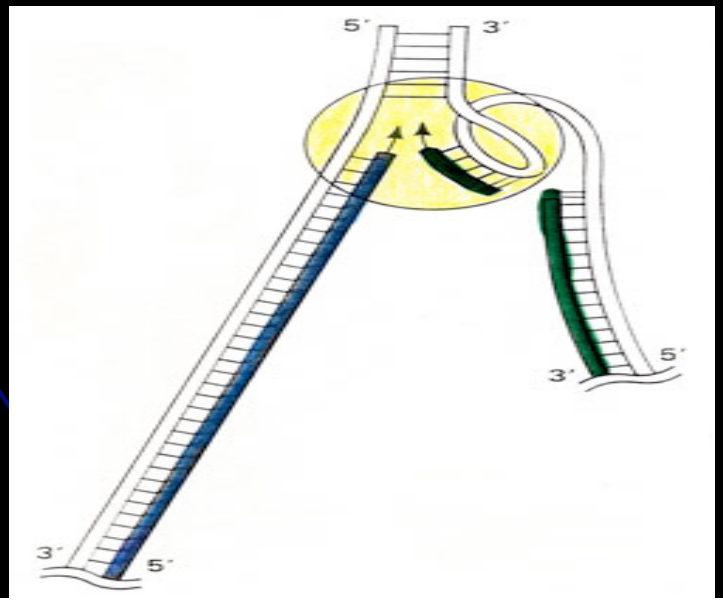
- FEN-1 is a 5' endonuclease as well as an exonuclease, meaning that it cleaves both endonuclease substrates (which possess a 5' flap) as well as exonuclease substrates. Cleavage of these substrates is integral to genomic integrity. The flap structure is simply a result of various biological mechanisms, such as Okazaki fragment processing. This flap structure needs to be cleaved, and FEN-1 comes to the rescue. The diagram above shows that FEN-1 cleaves the FEN-1 structure at 19 nucleotides and 21 nucleotides from the start of the 5' flap. As an exonuclease, FEN-1 recognizes and cleaves mismatched bases or extra bases. Although there are no mismatched bases in the exonuclease substrate, we are just using to diagram to show its properties. In the diagram, we intended for FEN-1 to cleave nucleotides 1, 2, and 3 nucleotides away from the 5' starred end.

FEN-1 in processing of Okazaki fragments

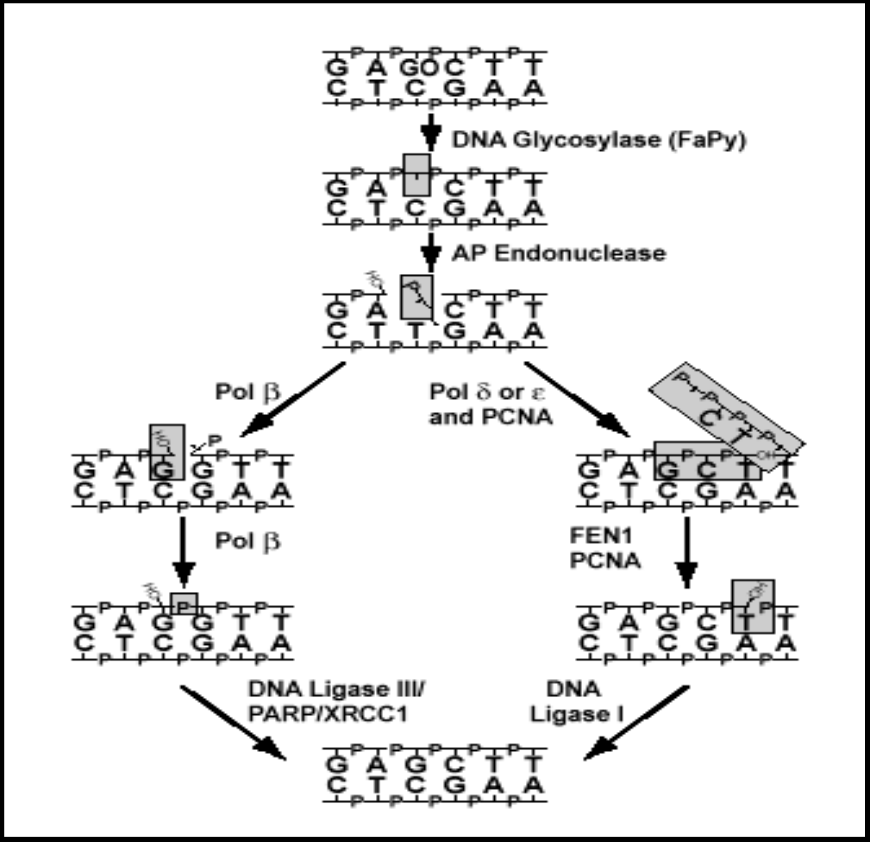


Okazaki fragment processing

- Since DNA can only be synthesized in the 5' to 3' direction, one of the strands, the 3' to 5' strand) can be copied continuously and is the leading strand, while the other, the lagging strand, is synthesized in fragments.
- FEN-1 cleaves the RNA primers and fills the gaps between Okazaki fragments with DNA



FEN-1 in base excision repair



In coordination with AP Endonuclease, Pol B, DNA Ligase, and other nucleases, FEN-1 helps to excise extra bases that are not complementarily paired.

The mutation of FEN-1 causes diseases

- http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=11260214&dopt=Abstract
- The link of mismatch repair defects with hereditary nonpolyposis colon cancer (HNPCC)
- Cockayne's syndrome-due to UV sensitivity

Evidence Suggesting that FEN-1 Helps Prevent Cancer

- First, Rad 27, which is a yeast analogue of FEN-1, is an important contributor to DNA metabolism, and the loss of its function causes severe biological defects such as temperature sensitivity, hypersensitivity to DNA-alkylating agents, strong mutator phenotype, cell cycle arrest, and genomic instability. Hypersensitivity to alkylating agents, which are anticancer drugs that interferes with the cell's DNA and inhibits cancer cell growth, means that the body reacts strongly against these alkylating agents. This likely hinders the effect of these drugs and thus makes the yeast more vulnerable to cancer. Strong mutator phenotype means that the yeast has a frequency of mutation 50 to 100 times that of a normal, healthy yeast, and increased mutations increase the possibility for disease and cancer. In addition, cell cycle arrest interferes with the normal growth of yeast and also makes diseases and cancers more likely. These potential defects in yeast arise from the failure of RNA primer removal during lagging strand DNA synthesis, or failure of FEN-1 during the mismatch repair and base excision repair pathways.

(Cont.-Trinucleotide expansion)

- One type of genomic instability results from the expansion of variable nucleotide repeat sequences, an excess of which cause of genetic diseases like Huntington's disease, myotonic dystrophy, and fragile X syndrome. The expansion of trinucleotide repeats in certain regions of the human genome are more likely to cause genetic disease than in other regions. Although the process by which trinucleotide expansion occurs is not clear so far, some researchers believe that realignment or slippage during Okazaki fragment processing is one cause for trinucleotide repeats. During lagging strand synthesis of DNA, there is a greater probability to form single-stranded regions as compared to leading strand synthesis. It has been suggested that the unusual structure of these single-stranded DNA, which tend to form hairpin loops, is more likely to result in replication errors at the spot of a trinucleotide repeat.

Trinucleotide Expansion (Cont.)

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FEN-1 as tumor suppressing gene

- There is also evidence supporting the role of FEN-1 as a tumor suppressor gene. In mice, homozygous knockout of FEN-1 is embryonically lethal, as blastocysts lacking the function of FEN-1 are arrested in the S-phase. Here, knockout refers to "knocking out" the function of FEN-1. Although heterozygous knockout mice are viable and seem to be healthy, heterozygous knockout mice that are also heterozygous for the adenomatous polyposis coli (*APC)* gene develop adenocarcinomas. This gene, found on chromosome number 5 in humans, causes the genetically inherited disorder known as Familial polyposis coli, which is characterized by polyps that can potentially turn into cancer later in life. The polyps develop mainly in the colon, but can affect all parts of the body. Even though the possibility of each individual polyp causing cancer is low, someone with this disease develops so many polyps that colon cancer is nearly inevitable by the age of 50. The fact that mice with fully functional FEN-1 suppress the heterozygous APC gene suggests that FEN-1 is a tumor suppressor gene as well.

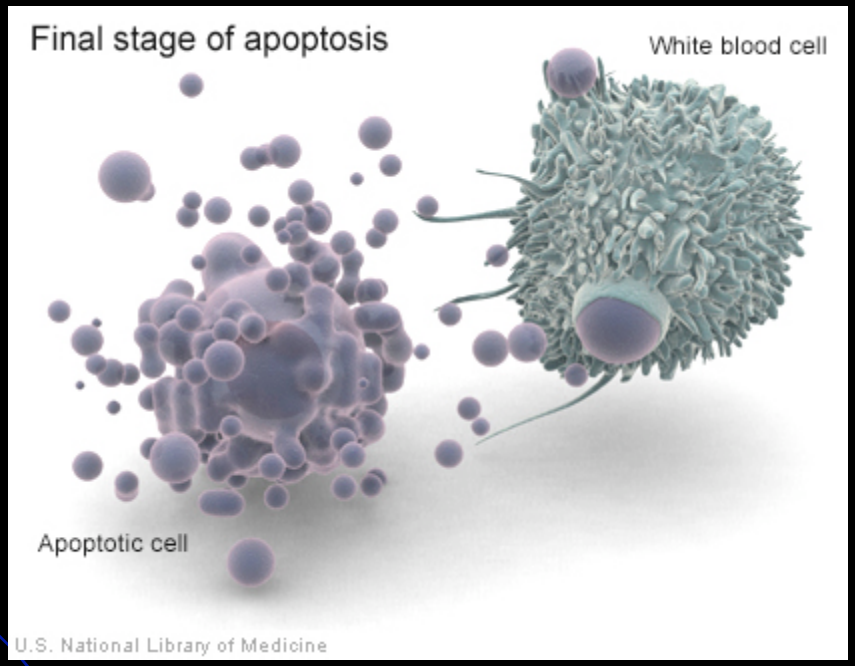
FEN-1 in apoptosis

- FEN-1 also plays critical roles in apoptosis. In one experiment, the reduction of Crn-1 (C. elegans FEN-1 homologue) activity, which was caused by RNA interference, resulted in resistance to DNA apoptosis in cells. Apoptosis, or programmed cell death, must occur in order to eradicate nonfunctional cells or cells infected by cancer or disease. If apoptosis did not occur, then the body would continue to produce more and more cells as a result of mitosis, without destroying any cells. In addition, the suppression of apoptosis can easily lead to the development of cancers. If an infected cell is allowed to stay in the body, the cancer can spread throughout the body much more easily. Thus, apoptosis is crucial in preventing the onset the cancer.

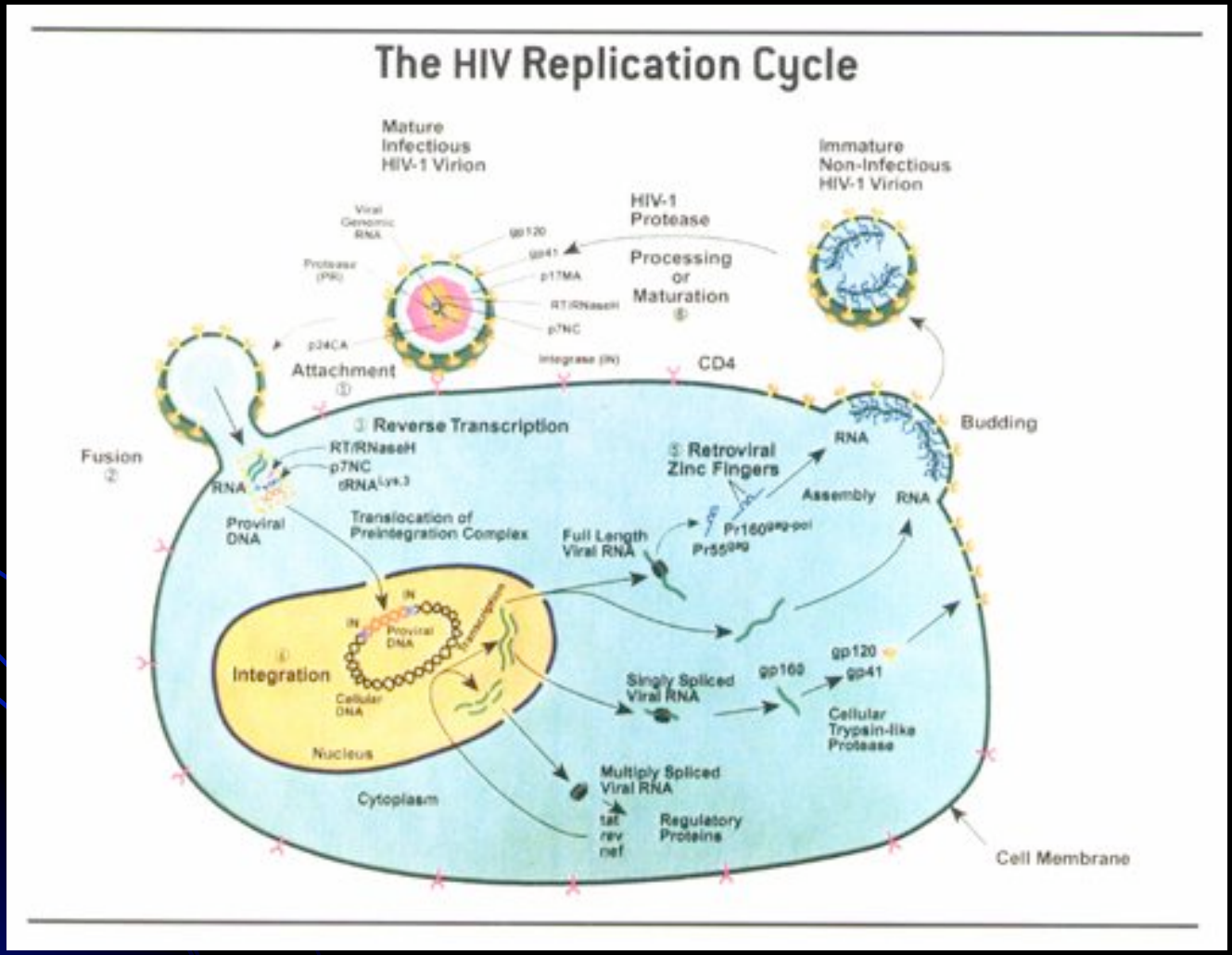
FEN-1: Potentially Harmful Effects

- However, recent evidence from the City of Hope lab I worked in last summer suggests that FEN-1 is a double-edged sword. The human immunodeficiency virus type 1 (HIV-1) may employ the endonuclease activity of FEN-1 to process its DNA during the replication cycle. The HIV-1 central DNA flap, known as the CDF, is formed during reverse transcription and is ninety-nine nucleotides long. No matter the secondary structure in the CDF, the CDF is a substrate for human FEN-1. Although results are not conclusive, HIV-1 integrase has been documented in interacting with human FEN-1. In addition, both HIV-1 integrase and human FEN-1 seem to work in a "symbiotic relationship," as each of the two stimulates the activities of the other.

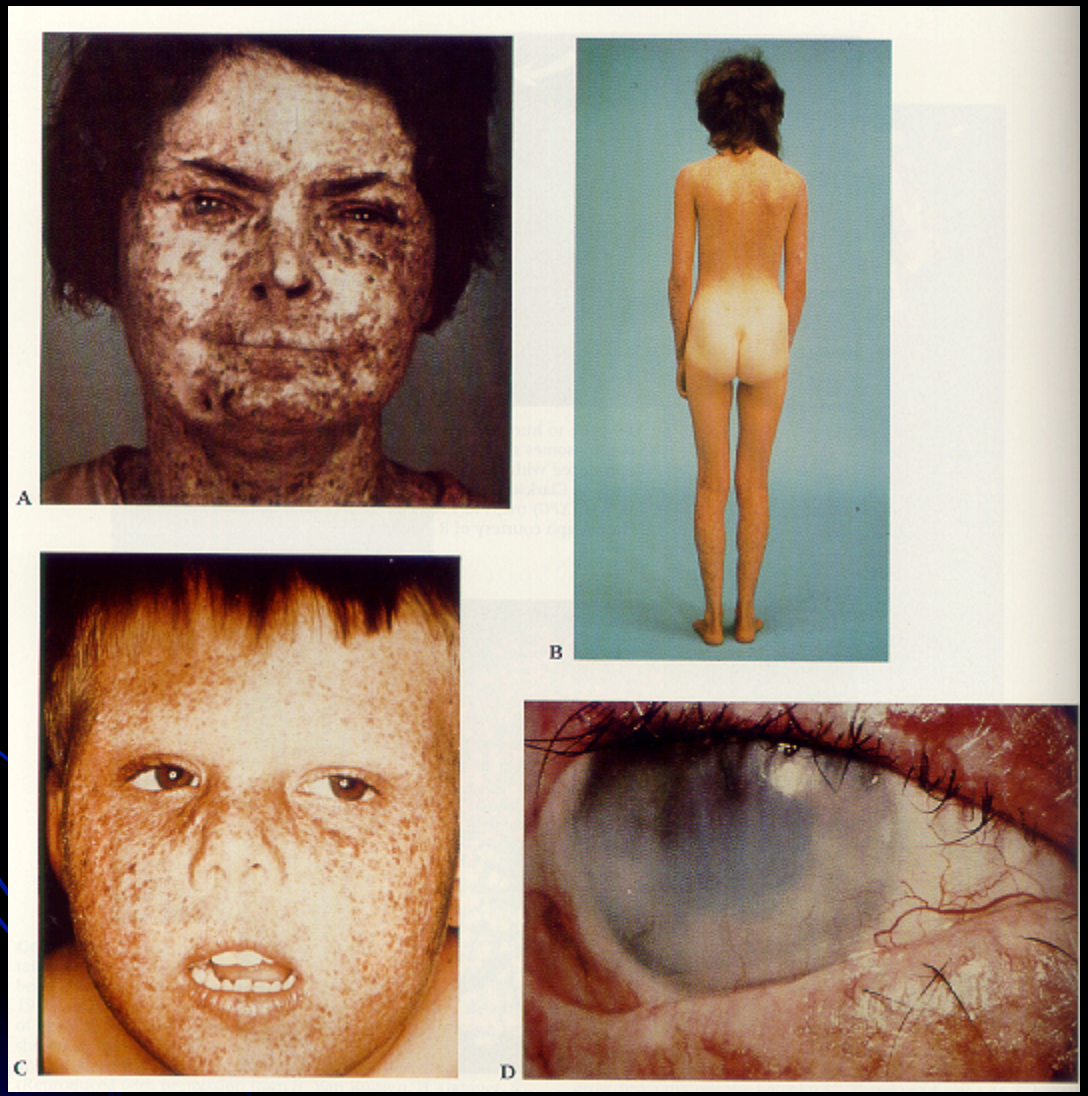
Apoptosis


Final stage of apoptosis
White blood cell
Apoptotic cell
U.S. National Library of Medicine

HIV Replication



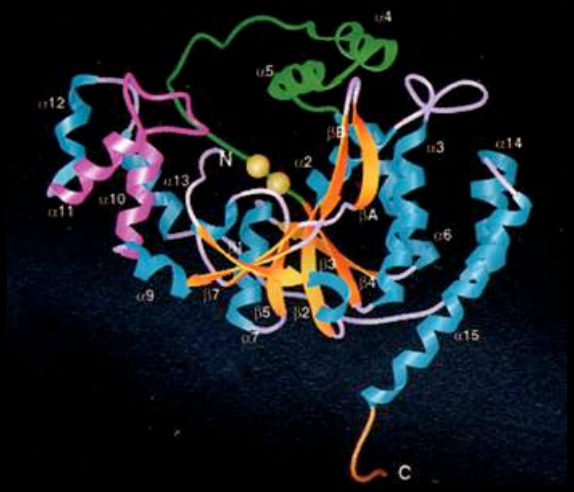
Xeroderma Pigmentosum

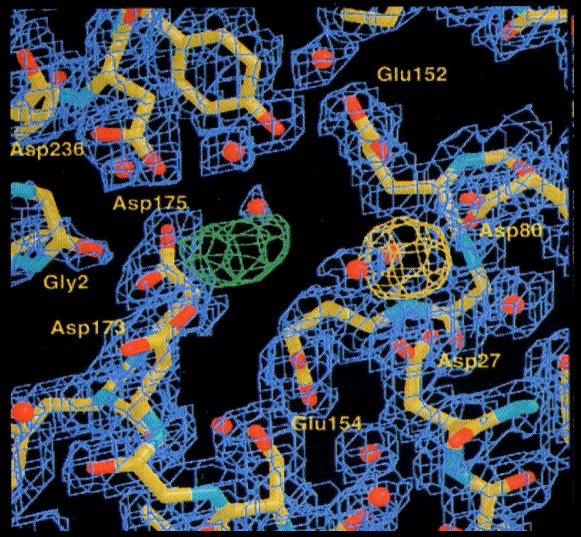


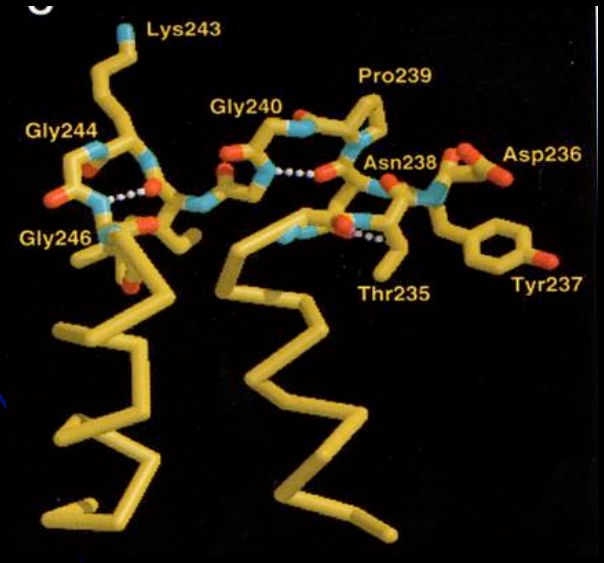
Xeroderma Pigmentosum

- The genetic disease xeroderma pigmentosum is caused by gene defectiveness in nucleotide excision repair. The disease has been classified into seven groups from A to G.
- Picture A. pigmentary disturbance; B. changes in parts of skin color; C. started at young age; D. clouding eyes. Causes mental defectiveness, is neurodegenerative, and leads to a short life span.
- The disorder is caused by photosensitivity, pigmentary changes, premature skin aging, malignant tumors, defective nucleotide excision repair, NER, defective DNA repair

Crystal Structure of P.furiosis FEN-1

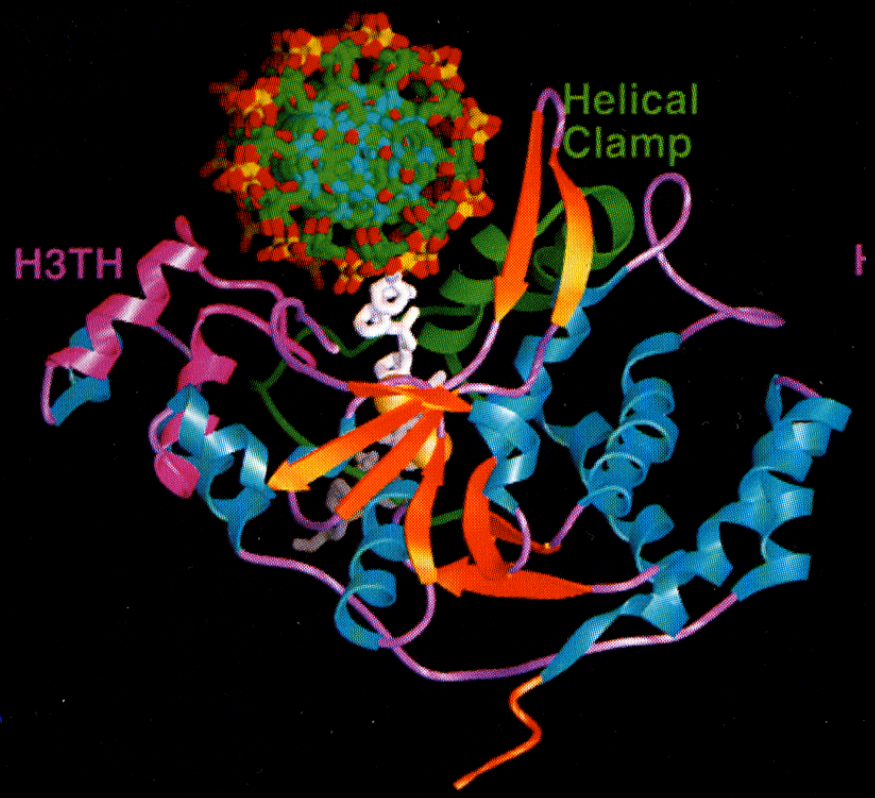




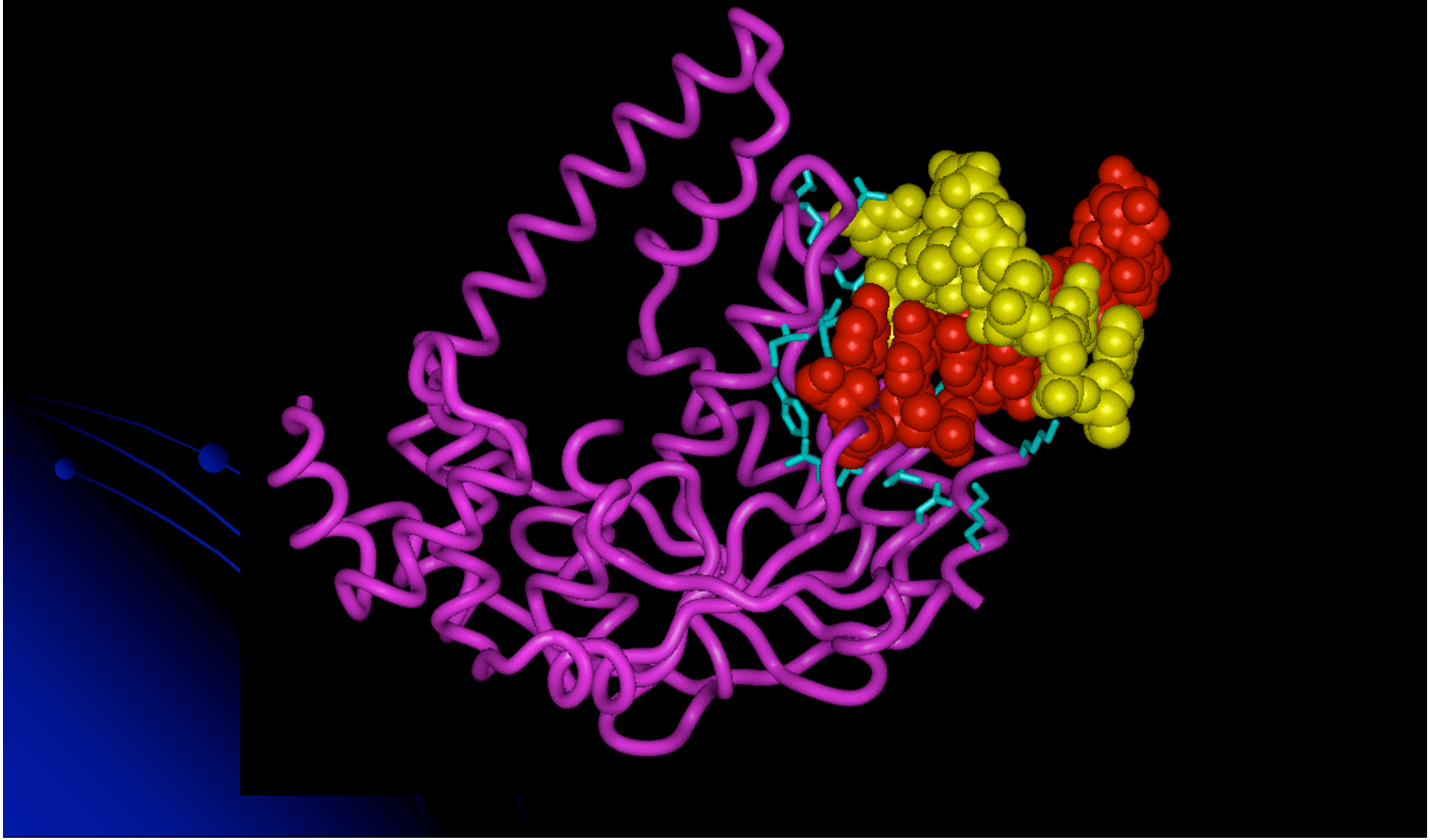


H3TH (helix 3 turn helix region)

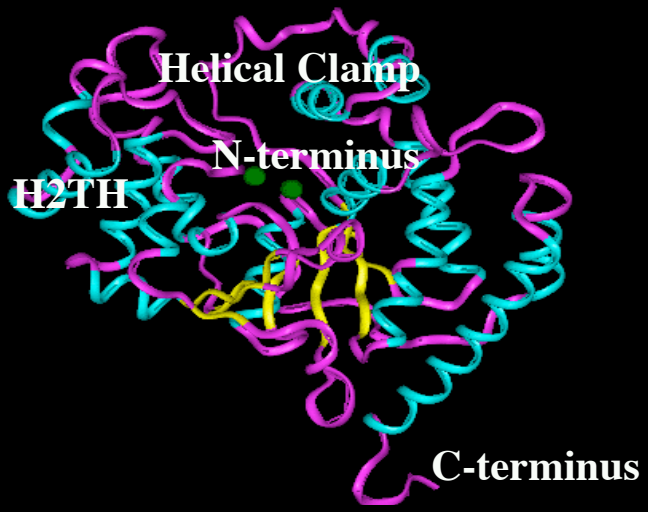
Model of FEN-1 Interaction



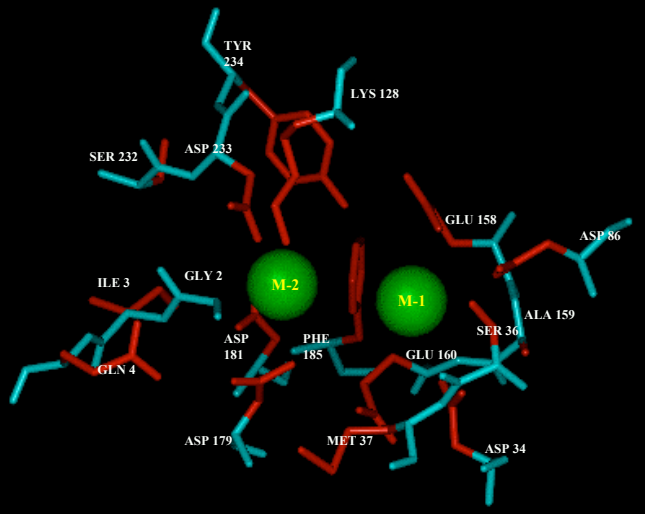
FEN1-DNA Crystal Structure



Human FEN-1



InsightII-generated model of human FEN-1 with Alpha Helices (aqua blue) and Beta Strands (gold)



Active center of Human FEN-1
Active site residues within 6.0 Å radius of metals

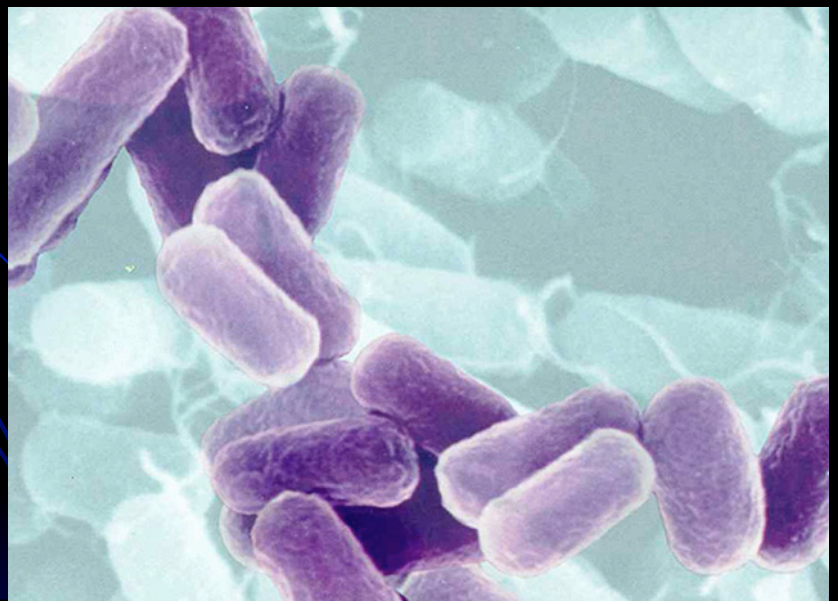
Human FEN-1

Why resolve crystal structures?

- By finding out the crystal structure of human FEN-1, we can do experiments to determine which amino acid residues of FEN-1 are critical to its function, and help protect against diseases.

Determining which Residues are Crucial in FEN-1's Functions

- Conduct mutagenesis (inducing the desired mutations in the amino acids
- Culture cells, overexpress the protein of interest using IPTG
- Purify the proteins using columns with nickel beads that attach to the histidine-tagged proteins
- After purifying the proteins, perform assays to determine the function of the proteins.



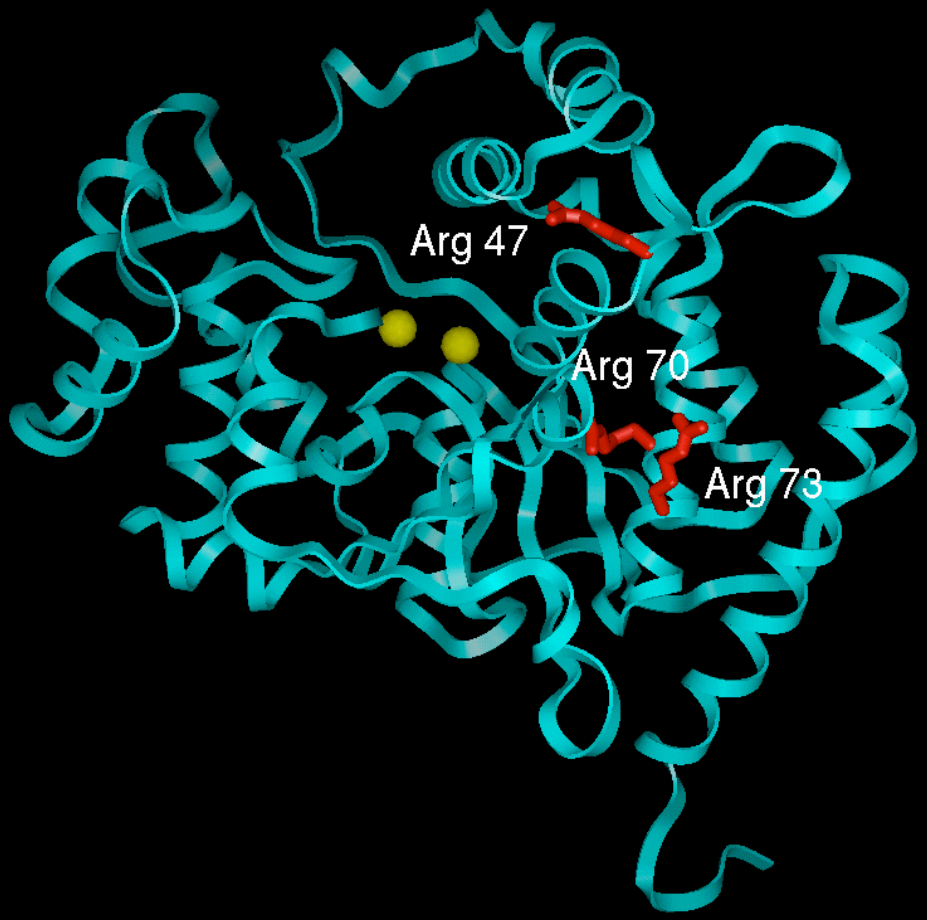
How to Purify Proteins

- To purify proteins, the method I used is called IMAC (immobilized metal affinity chromatography). This method is based on the principle that nickel beads in the Ni-NTA(nickel-nitrotriacetic acid) complex can bind effectively to the histidine tags located on the surface of a protein, which also immobilize the protein. We specifically constructed these six consecutive histidine tags on the carboxyl terminus of our protein of interest in order to facilitate the protein purification process. After allowing the desired his-tagged proteins to bind to the nickel beads, I washed away the unwanted proteins. The elution buffers, which I used to actually obtain the proteins, contain varying amounts of imidazol, which bind to the nickel beads and hence displace the proteins. The underlying reason is that the imidazol ring is part of the histidine structure, and imidazole has a greater affinity in binding to nickel than histidine. For each mutant, the desired protein can be eluted only above a certain point of imidazol concentration. Similarly, each protein can be eluted above a certain point of imidazol concentration and a certain imidazol concentration elutes all remaining proteins at or below that point.

Word of Caution

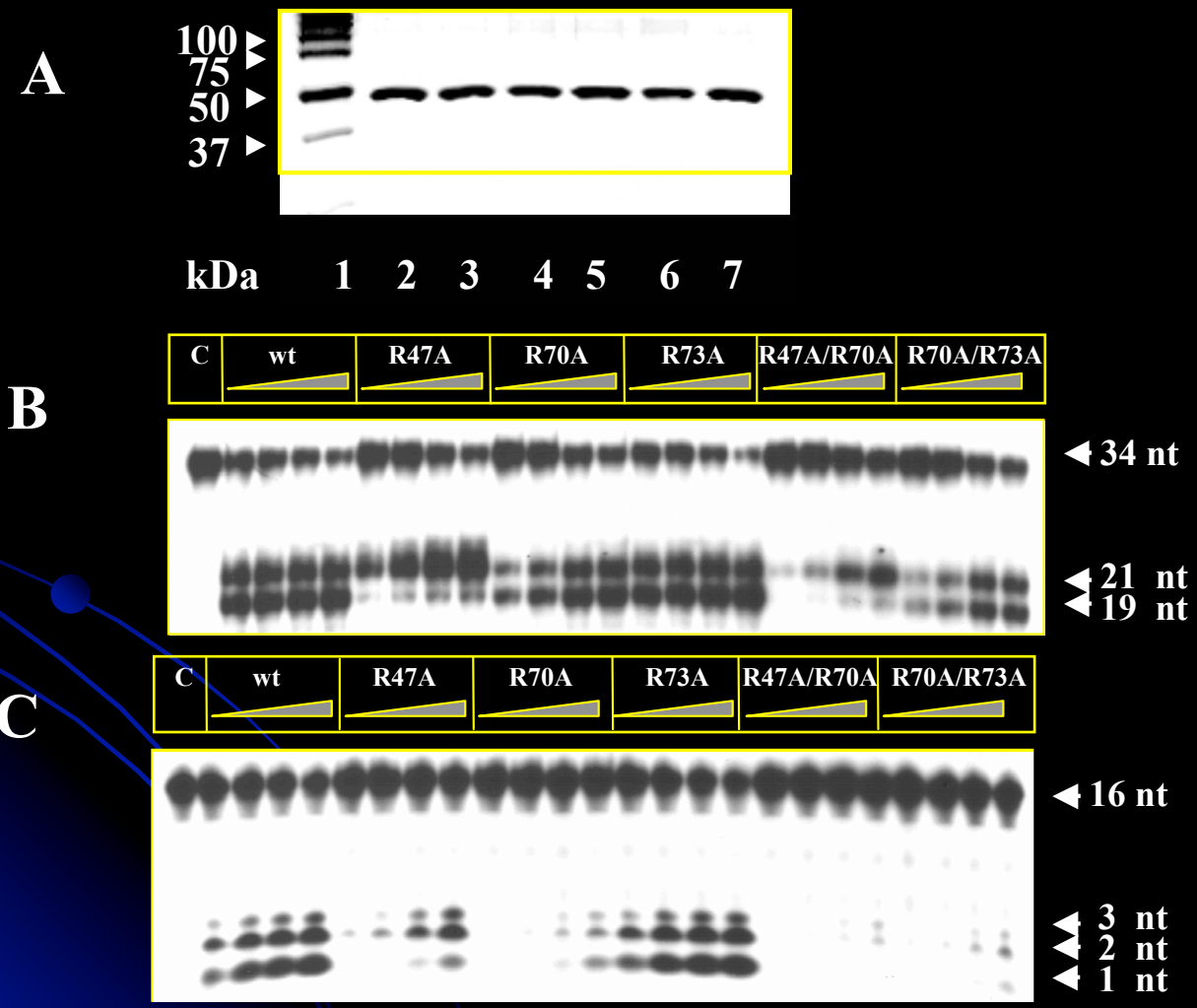
- But before I purify the protein, I must check to make sure the protein soluble and expressed in the cells. A soluble protein is a protein not located in an inclusion body and an expressed protein is a protein located in the cell. First, I ran a miniprep to check whether the protein of interest was overexpressed. I compared the bands on the protein gel of the control (without adding IPTG, which overexpresses the protein) with the bands of the actual sample to see whether the latter showed more of the protein of interest. If the latter were darker, that means that the protein is soluble and expressed in the cells.

R47, R70, R73

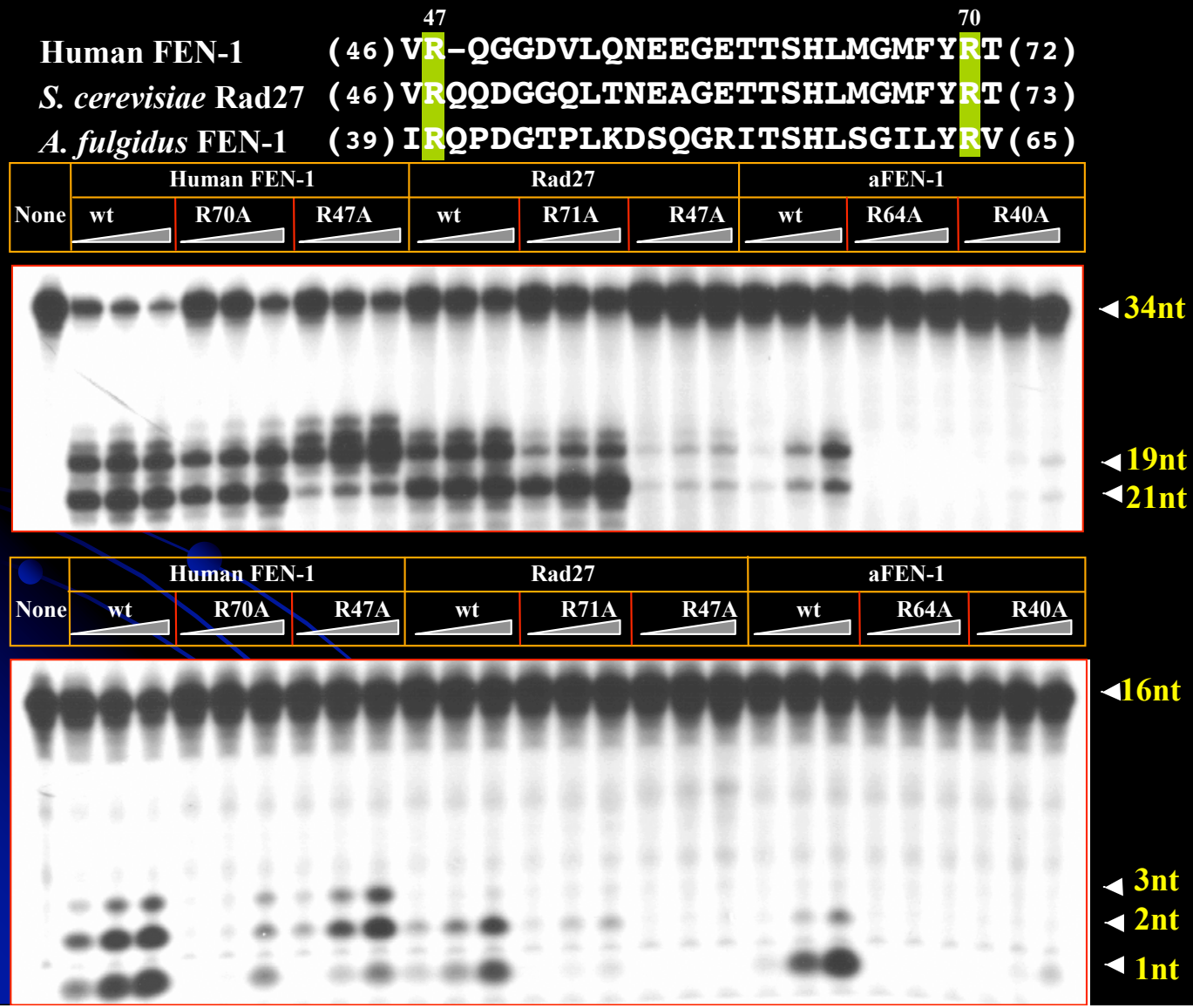


R47 and R70 appear to comprise a region for FEN-1 to interact with the down-stream double strand part of FEN-1 substrate

Mutation of R47 and R70 affect the enzyme activities of FEN-1



Function of R47A and R70A was conserved across 3 domains



R47A and R70A

- The finding that both R47A and R70A are conserved suggests that the two amino acid residues (R47A means the arginine at position 47 is mutated to alanine, wt, or wild type, means that the arginine is not mutant) are important in the function of FEN-1. Indeed, since both mutants R47A and R70A show a decrease in cleavage, as represented by the darkness in the assay shown, then R47 and R70 play crucial roles in the cleavage of DNA. Thus, R47 and R70 are two amino acid residues that are important in preventing diseases caused by deficiency of FEN-1.

Additional Readings

- http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=180932
- http://www.e-emm.org/article/article_files/emm34-4-9.pdf
- Chapados, Brian R., Structural basis for FEN-1 substrate specificity and PCNA-mediated activation in DNA replication and repair. (Accepted by Cell Magazine)
- Qiu, J. Bimston, D. Partikian, A., Shen, B., etc. Arginine Residues 47 and 70 of Human Flap Endonuclease-1 Are Involved in DNA Substrate Interactions and Cleavage Site Determination. The Journal of Biological Chemistry, 277, 24659-24666, 2002.
- Negritto MC, Qiu J, Ratay DO, Shen B, Bailis AM. 2001. Novel function of Rad27 (FEN-1) in restricting short-sequence recombination. Mol Cell Biol 21:2349-2358.
- Ruggiero BL, Topal MD. 2004. Triplet repeat expansion generated by DNA slippage is suppressed by human flap endonuclease 1. J Biol Chem 279:23088-23097.

More Readings

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