

Protein Modeling Division C 2012 San Diego Science Olympiad

Mastering the Protein Modeling C Event

Jan 7, 2012

San Diego Supercomputer Center/UCSD

2:00 - 4:00 pm



Nic's Story

- Nicholas Volker has a mysterious bowel disease
- To find the cause of his disease researcher sequenced a portion (1.5%) of his genome
- This portion, called the exome, corresponds to genes that are expressed as proteins
- Nic's DNA sequences were compared with normal human reference sequences and 16,124 differences were found

Nic' Story continued

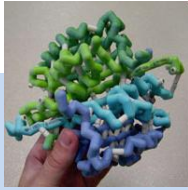
- One of the differences found is a G to A mutation at nucleotide 641 in Nic's XIAP gene
- This mutation results in the change of amino acid 203 in the XIAP protein from cysteine (CYS or C) to tyrosine (TYR or Y)
- It is known that XIAP is involved in the regulation of programmed cell death (apoptosis) and immune response

Nic's Story continued

- Nic's bowel disease may be related to a defect in regulating programmed cell death
- By replacing Nic's hematopoietic progenitor cells with those of a healthy donor, physicians hoped that the functional XIAP protein would
 - resolve his bowel disease
 - eliminate the risk of developing a potentially fatal blood cancer

Two Proteins Related to Apoptosis

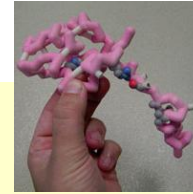
• Caspase-3



- A **cysteine protease** that uses a CYS residue in the active site to cleave target proteins
- It cuts proteins at a peptide bond following an **aspartic acid (ASP)**
- Called the “executioner”, it is activated during apoptosis and cleaves several target proteins including PARP

Pre-build model

• XIAP

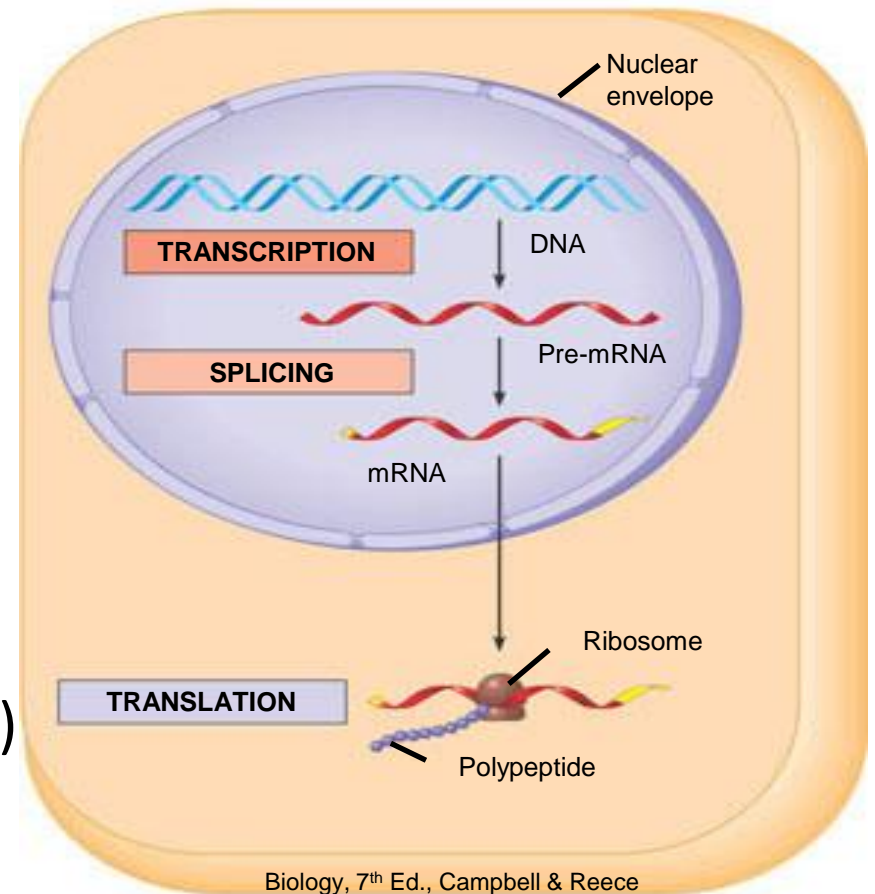


- In a healthy cells, XIAP binds to caspase-3 and keeps caspase-3 inactive
- The structure of XIAP bound to caspase-3 is in PDB entry 1i3o.pdb
- During apoptosis the protein SMAC/DIABLO binds to XIAP and sets free the activated caspase-3, which then cleaves proteins and leads to apoptosis

Onsite model

Flow of Genetic Information *

- Transcription
 - DNA → pre-mRNA
- Splicing
 - pre-mRNA → mRNA
- Translation
 - mRNA → polypeptide (protein)

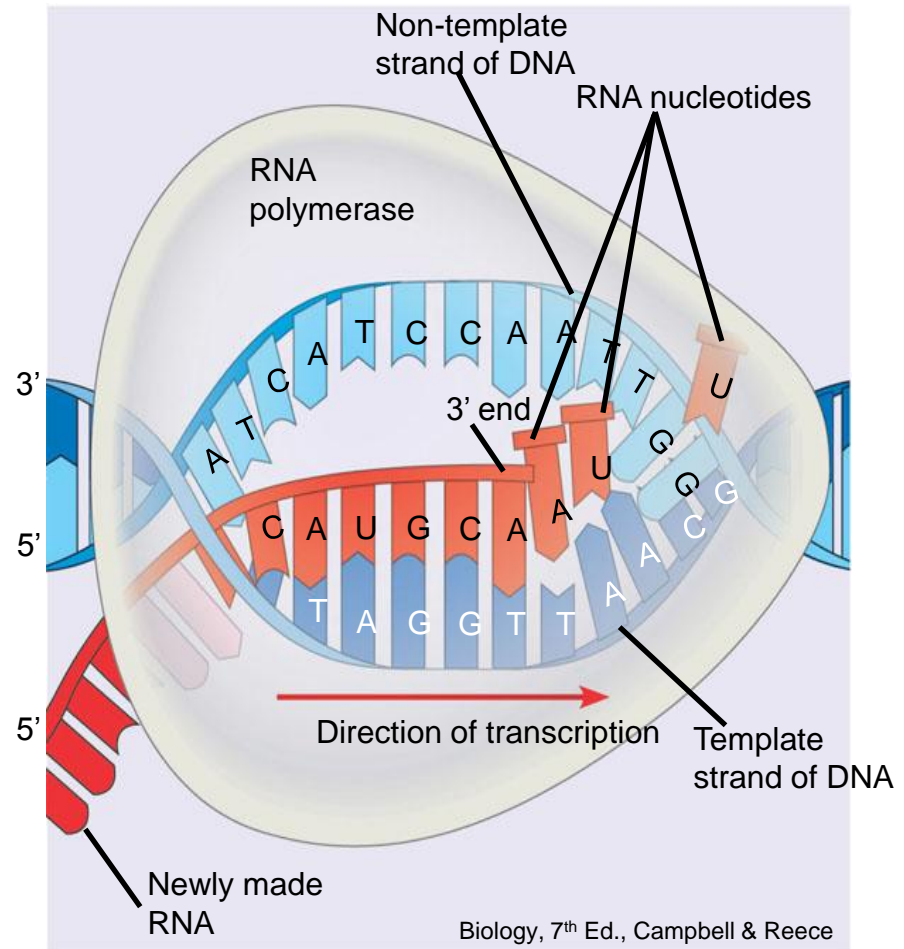


* In eukaryotes where genetic material is in the nucleus (for example humans, animals)

Transcription

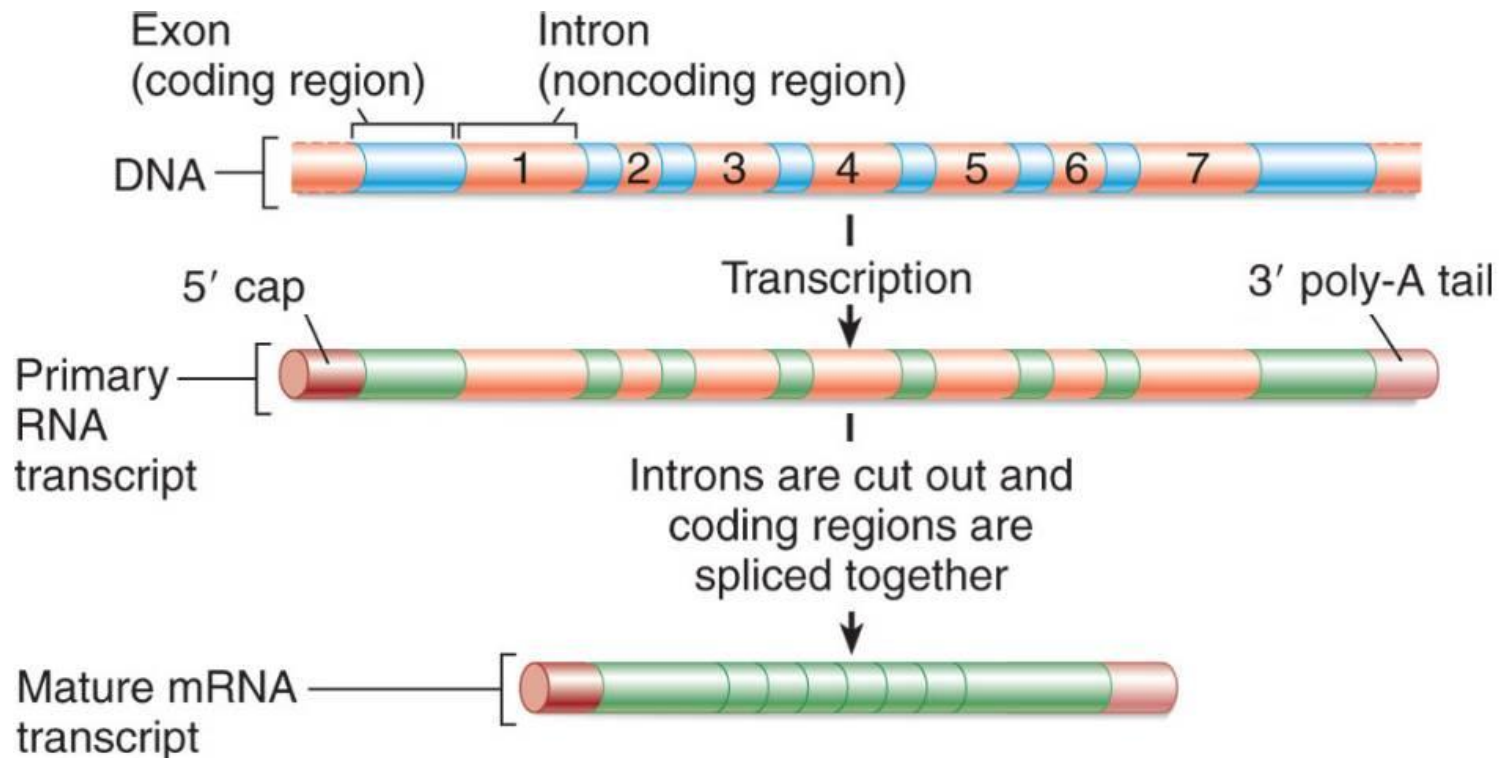
RNA Polymerase synthesizes a single strand of mRNA that is complementary to the template DNA strand

- DNA
 - A-T and C-G base pairs
 - double stranded
 - forms helix
- RNA
 - contains U (uracil) instead of T(thymine)
 - single stranded



Splicing

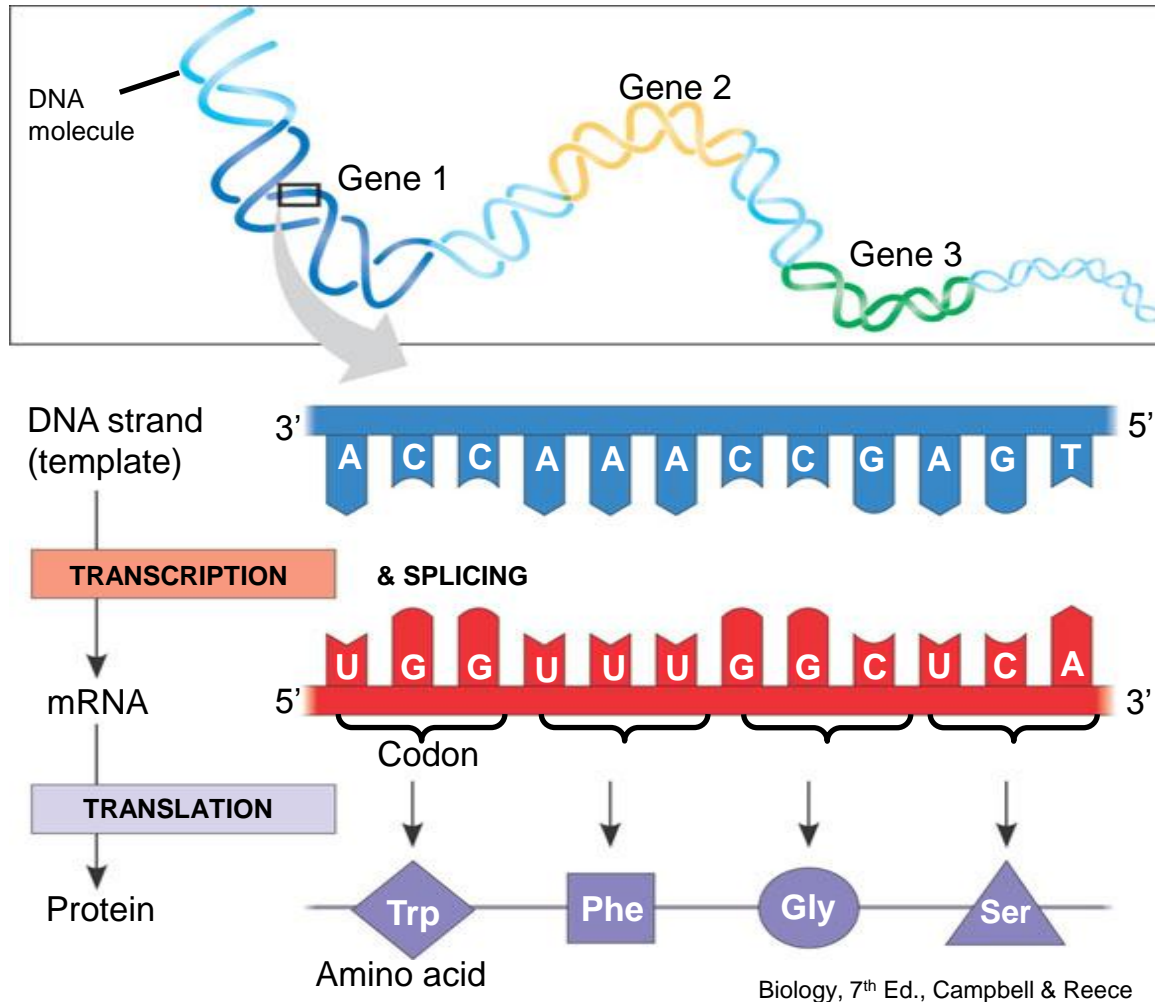
Spliceosomes recognize junctions between exons (coding regions) and introns (non-coding regions). They cut out introns and join the exons to form mature mRNA.



<http://bio1100.nicerweb.net/Locked/media/ch13/splice.html>

Translation

The ribosome synthesizes a polypeptide based on the mRNA sequence. Three nucleotides (codon) encode one amino acid.










Genetic Code

second mRNA base

		second mRNA base					
		U	C	A	G		
first mRNA base (5' end)	U	UUU → Phe F	UCU → Ser S	UAU → Tyr Y	UGU → Cys C	U	
		UUC → Phe F	UCC → Ser S	UAC → Tyr Y	UGC → Cys C	C	
		UUA → Leu L	UCA → Ser S	UAA → Stop	UGA → Stop	A	
		UUG → Leu L	UCG → Ser S	UAG → Stop	UGG → Trp W	G	
	C	CUU → Leu L	CCU → Pro P	CAU → His H	CGU → Arg R	U	
		CUC → Leu L	CCC → Pro P	CAC → His H	CGC → Arg R	C	
		CUA → Leu L	CCA → Pro P	CAA → Gln Q	CGA → Arg R	A	
		CUG → Leu L	CCG → Pro P	CAG → Gln Q	CGG → Arg R	G	
	A	AUU → Ile I	ACU → Thr T	AAU → Asn N	AGU → Ser S	U	
		AUC → Ile I	ACC → Thr T	AAC → Asn N	AGC → Ser S	C	
		AUA → Ile I	ACA → Thr T	AAA → Lys K	AGA → Arg R	A	
		AUG → Met M	ACG → Thr T	AAG → Lys K	AGG → Arg R	G	
	G	GUU → Val V	GCU → Ala A	GAU → Asp D	GGU → Gly G	U	
		GUC → Val V	GCC → Ala A	GAC → Asp D	GGC → Gly G	C	
		GUA → Val V	GCA → Ala A	GAA → Glu E	GGA → Gly G	A	
		GUG → Val V	GCG → Ala A	GAG → Glu E	GGG → Gly G	G	

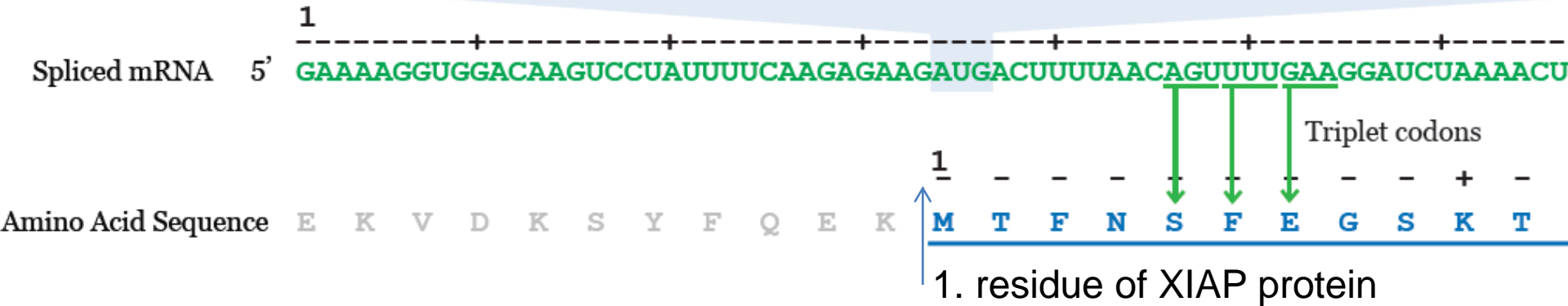
third mRNA base (3' end)

-  translation start codon
-  hydrophobic amino acids
-  negatively charged amino acids
-  cysteine
-  translation stop codon
-  hydrophilic non-charged amino acids
-  positively charged amino acids

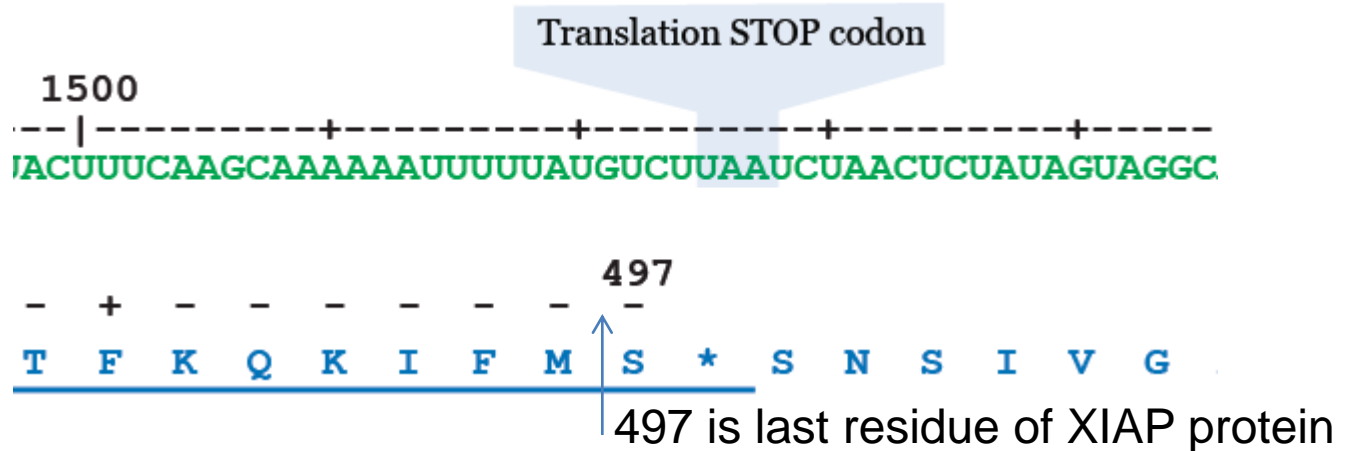
Translation of XIAP Protein

Ribosome begins translation at start codon: AUG

Note that the AUG translation initiation codon is not at the immediate 5' end of the mRNA



Translation ends at stop codon: UAA



Quiz

- Translate the following fragment of an mRNA sequence into the corresponding protein sequence

5' ... AAG AUG GAA ACG GAG CGU ...

Quiz

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5' ... AAG AUG GAA ACG GAG CGU ...

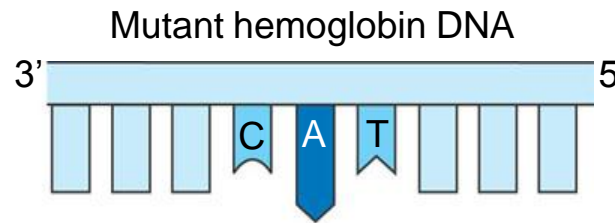
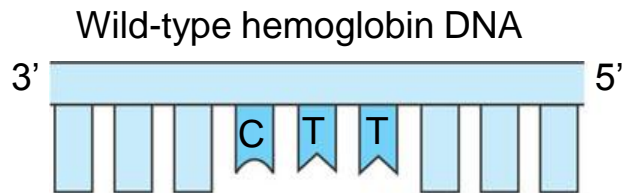
M E T E R ...

Start codon

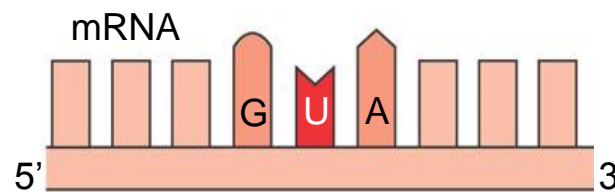
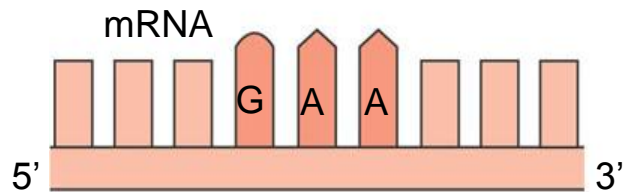
Mutation

Point mutation: a change of a single base pair in the genetic material

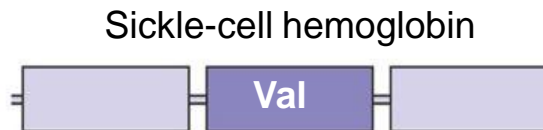
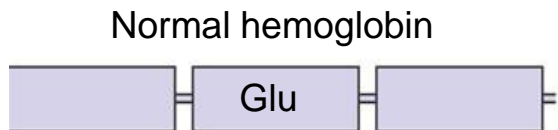
Can lead to the expression of a defective protein



In the DNA, the mutant template strand has an A where the wild-type template has a T.



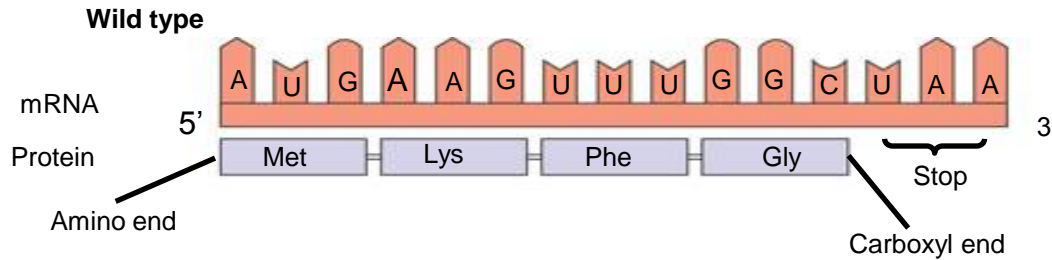
The mutant mRNA has a U instead of an A in one codon.



The mutant (sickle-cell) hemoglobin has a valine (Val) instead of a glutamic acid (Glu).

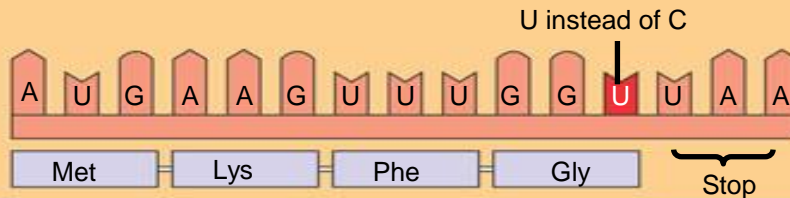
Biology, 7th Ed., Campbell & Reece

Effect of Mutation



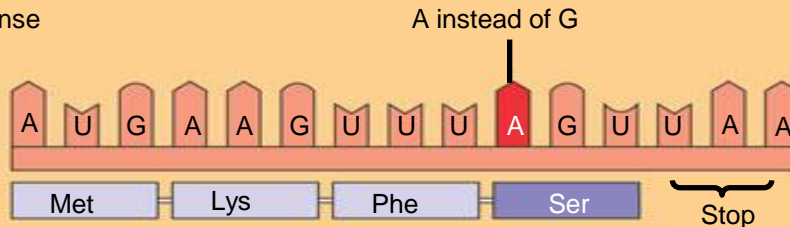
Base-pair substitution

Silent mutation



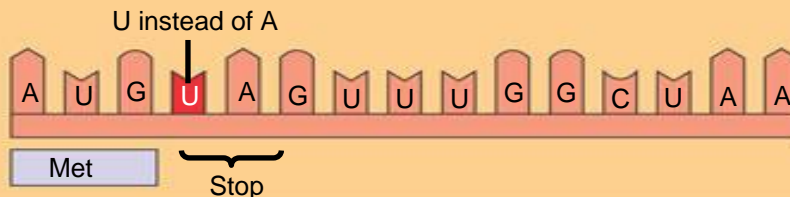
No effect on protein

Missense



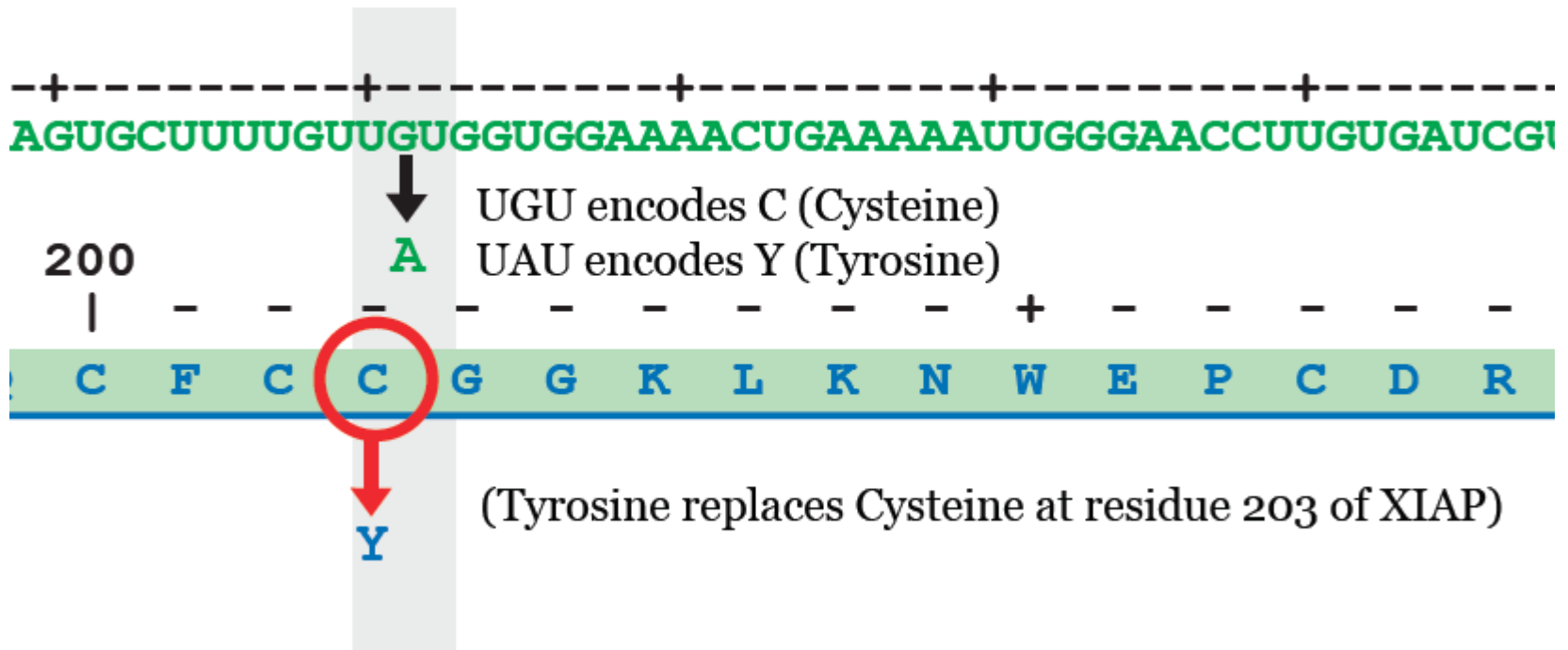
A modified protein is produced. This can lead to a disease if the change is in an important region of the protein.

Nonsense



A shortened protein is produced. This can lead to a disease if the missing region is important.

Mutation in Nic's XIAP Gene



Mutation: C203Y or CYS203TYR

Jmol Exercise

- We will use Jmol to figure out if CYS 203 is important for the function of XIAP
- Go to <http://cbm.msoe.edu/stupro/so/index.html>
- Click on 2012 Pre-build Competition Environment
 - This environment displays the structure of caspase-3 bound to the XIAP protein
 - Caspase-3: chains A and B (pre-build model)
 - XIAP: chain E (onsite model)

Setup

Display only chain E (XIAP protein)

```
restrict :E
```

Color by secondary structure

```
color structure
```

Change to a thicker backbone

```
backbone 200
```

What Interactions does CYS 203 make?

Display the side chain of CYS 203 in chain E

```
select 203:E and (sidechain or alpha)  
wireframe 75  
cpk 100  
color yellow
```

Center the display on residue 203:E

```
center 203:E
```

Display the zinc ion in the E chain as cpk

```
select Zn:E  
cpk 300
```

What other Interactions does the Zinc make?

Display the side chains of all CYS residues

```
select CYS:E and (sidechain or alpha)
wireframe 75
cpk 100
color cpk
```

Do the same for all HIS (histidine) residues

Conclusions

- 3 CYS and 1 HIS residue bind Zn in this Zinc finger motif (domain) of XIAP. These interactions stabilize the protein structure.
- Mutation of CYS 203 to TYR would disrupt the coordination to the Zinc and may change the structure (compare the size of CYS and TYR in the table of amino acids)
- Structural changes in the XIAP protein may lead to changes or disruption in its function (do you remember what these functions are?)

Amino Acid Sequence Alignment of a section of the XIAP protein

	195			200			205			210							
Nic's XIAP	G	D	Q	V	Q	C	F	C	Y	G	G	K	L	K	N	W	E
Human	G	D	Q	V	Q	C	F	C	C	G	G	K	L	K	N	W	E
Chimpanzee	G	D	Q	V	Q	C	F	C	C	G	G	K	L	K	N	W	E
Mouse	D	D	Q	V	Q	C	F	C	C	G	G	K	L	K	N	W	E
Dog	D	D	Q	V	Q	C	F	C	C	G	G	K	L	K	N	W	E
Cow	D	D	Q	V	Q	C	F	C	C	G	G	K	L	K	N	W	E
Shrew	G	D	Q	V	Q	C	F	C	C	G	G	K	L	K	N	W	E
Chicken	D	D	Q	V	Q	A	F	C	C	G	G	K	L	K	N	W	E
Zebra Fish	D	D	N	V	Q	C	F	C	C	G	G	G	L	S	G	W	E
Frog	R	D	H	V	K	C	F	H	C	D	G	G	L	R	N	W	E
House Fly	L	D	H	V	K	C	V	W	C	N	G	V	I	A	K	W	E

<http://cbm.msoc.edu/stupro/so/module2012/dnaSequencingVolker.html>

Cysteine 203 is highly conserved (no variation) across species. This may indicate an important role for this amino acid.

The On-Site Event



2011 Science Olympiad National Tournament

- Two parts
 - Build on-site model of a portion of XIAP (50% of score). The on-site environment will be provided on laptops running the Windows operating system.
 - Multiple choice test (50% of score) plus 1 written question. For example tests from the national tournaments check out: <http://cbm.msos.edu/stupro/so/SONTExamRubric.html>
- How much time do you have?
 - 50 minutes for model building and written test
- What can/should you bring to the event?
 - Pencils
 - Up to 5 double-sided 8.5" x 11" pages of notes (anything you think might help you during the event)
- What will the organizers provide?
 - Rulers
 - Sharpies
 - Mini-toobers, foam amino acid sidechains, cross-linkers, blue and red end caps

Parts List:



Written Test

- DNA & RNA
 - Transcription and splicing
 - Translation and genetic code
 - Disease mutations
- Proteins
 - Amino acids: chemical structure, chemical reactions, and properties
 - Primary, secondary, tertiary, and quaternary structure
 - Interactions that hold proteins together, protein folding
 - Protein conformation, geometric features of protein backbones
 - Protein structure determination: X-ray crystallography
 - Protein function, enzymes, enzyme inhibitors
 - Protein visualization in Jmol
- All about the 2012 Story
 - Genome sequencing
 - The cell and its components
 - Regulation of apoptosis (programmed cell death) and connection to genetic disease
 - Immune system
 - Proteins: Caspase-3, XIAP
 - Sequence, structure, function, relationship to disease