Translation is not the end of the story

Translation finishes with the release of the newly synthesized polypeptide chain from the ribosome. However, several more processes are needed to convert the nascent polypeptide into a fully functional protein.

- Folding the chain into the correct three-dimensional structure requires no additional information - the amino acid sequence potentially dictates the folding. However, until they are correctly folded, proteins are unstable and vulnerable. It has also recently become apparent that partially folded or incorrectly folded proteins can be toxic to the cell. A number of 'chaperone' molecules assist the folding process and protect the polypeptide during folding, while misfolded proteins are detected and degraded.

- Many proteins incorporate chemical modifications to the basic polypeptide. Often sugars are attached (glycosylation) or a variety of other small molecules. The chain may be cleaved; cysteines may be cross-linked to form S-S disulfide bridges that lock the structure in place; other amino acid residues may be chemically modified, for example, pralines may be hydroxylated. All polypeptides initally have an N-terminal methionine, incorporated in response to the AUG initiation codon, but very often this is cleaved off.

- Proteins must be transported to an appropriate location. The destination is often specified by a short N-terminal signal peptide that is removed during the process of protein sorting. In other cases the signal is a sequence of amino acids located somewhere within the chain, and is not removed. In the case of Joanne Brown patient, it will turn out that one of her two CFTR genes carries a mutation that prevents the protein being correctly located in the cell membrane (her other copy of the gene carries a different mutation - that is, she is a compound heterozygote).
- Structural proteins may be further modified in their final location.

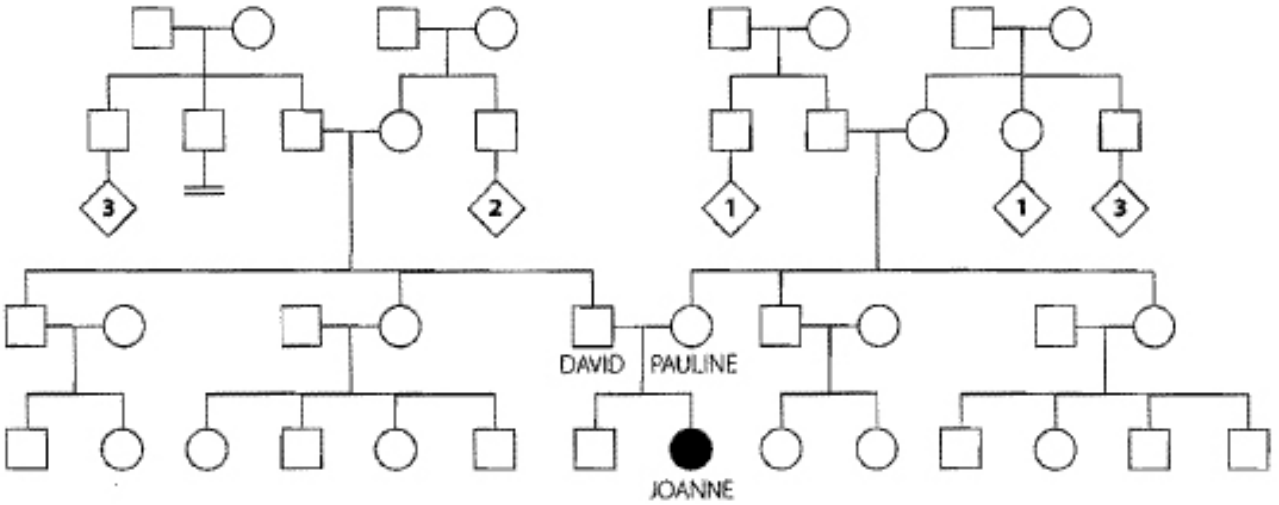

Figure 1.8 – Pedigree of Joanne Brown's family.
Note the complete absence of any family history of cystic fibrosis. Autosomal recessive conditions commonly present as a single isolated case.

The pedigree shows that there is no family history of cystic fibrosis or other evident genetic problems. "How can it be genetic, when nobody in either family has ever had it?" In fact this pedigree is typical of the way autosomal recessive diseases present in societies where consanguinity is not common. The affected child is usually the only affected case born to a non-consanguineous couple with no relevant family history. Thus the pedigree gives no clue that the condition is genetic. Identifying the cause usually starts with making a clinical diagnosis. Sometimes the condition is so unmistakable, and the genetics so unambiguous, that a clinical diagnosis provides an adequate degree of certainty. More often the clinical diagnosis is really a hypothesis, more or less plausible. Ideally it would be confirmed by a molecular test demonstrating a mutation.

In most cases of cystic fibrosis the clinical history and a positive sweat test (showing characteristically raised sodium) make the diagnosis fairly secure. Genetically, cystic fibrosis is always autosomal recessive, and always caused by mutations in the CFTR gene on chromosome 7. A molecular test to demonstrate the mutation is needed for advising relatives about their carrier status, for prenatal testing, and to confirm the diagnosis in atypical cases.

Mutations that cause amino acid substitutions

A single nucleotide change within a coding sequence will change on codon into another. Assuming the changed codon is not a stop codon, there are two possible results.

- *synonymous substitutions* are single-nucleotide changes that replace a codon with a different one that encodes the same amino acid - for example, T$\geq$G change in the DNA that converts a UUU codon in the mRNA to UUC. Both code for phenylalanine.
- *Mis-sense mutations* replace one amino acid with another by changing a codon. Use Table 6.1 to identify the effect of a codon change on the amino acid sequence.

Table 6.1 – The genetic code

1st base in codon	2nd base in codon				3rd base In codon
	U	**C**	**A**	**G**	
U	Phe	Ser	Tyr	Cys	**U**
	Phe	Ser	Tyr	Cys	**C**
	Leu	Ser	STOP	STOP	**A**
	Leu	Ser	STOP	Trp	**G**
C	Leu	Pro	His	Arg	**U**
	Leu	Pro	His	Arg	**C**
	Leu	Pro	Gln	Arg	**A**
	Leu	Pro	Gln	Arg	**G**
A	Ile	Thr	Asn	Ser	**U**
	Ile	Thr	Asn	Ser	**C**
	Ile	Thr	Lys	Arg	**A**
	Met	Thr	Lys	Arg	**G**
G	Val	Ala	Asp	Gly	**U**
	Val	Ala	Asp	Gly	**C**
	Val	Ala	Glu	Gly	**A**
	Val	Ala	Glu	Gly	**G**

Some amino acids such as serine and arginine have multiple codons, whereas tryptophan and methionine have only one eac. AUG doubles as the initiator codon and as the codon for internal methionines. Note the three stop codons.

It is important to remember, as mentioned above, that seemingly innocuous synonymous or mis-sense changes may have a major pathogenic effect by disrupting splicing.
The next Table gives a general guide to the likely effect of the various types of mutation.

Table 6.3 – Common types of mutation and their likely effects

Type of mutation	Likely effect on a gene
Large deletion or inversion	Most likely to completely abolish function
Duplication of whole gene	Will increase the amount of product by 50% (from 2 to 3 gene copies); this will generally have no phenotypic effect, unless the precise level of the gene product is critical (dosage-sensitive gene); see for example Aitman et al. (2006)
Change in promoter or regulatory sequence	May reduce or increase the level of transcription, or alter the response to control signals; any protein produced has the normal structure and function
Change in intron	Most likely to have no effect – but can sometimes affect splicing
Change in 5' or 3' untranslated region of mRNA	Most likely to have no effect – but can sometimes affect the stability or translation efficiency of the mRNA
Splicing mutation	Mutation of the canonical GT …AG splice sites is likely to abolish function of that allele. Other mutations may have more subtle effects, causing a proportion of transcripts to be incorrectly spliced or changing the pattern of alternative splicing. This can produce a partial loss of function. A change deep within an intron may activate a cryptic splice site.
Frameshift mutation	Likely to abolish function of that allele. The polypeptide downstream of the frameshift bears no resemblance to the correct sequence. Usually a stop codon will be encountered quite soon as the ribosomes read the frameshift codons. The effect will then be the same as a nonsense mutation.
Nonsense mutation	Likely to abolish function of that allele. Most mRNAs containing premature termination codons are not translated to produce a truncated protein. Instead, they are degraded and not used at all (nonsense-mediated mRNA decay)
Mis-sense mutation	Effect very variable, depending on the nature and function of the amino acids concerned. Could be loss or gain of function, or no effect. Replacing an amino acid by a chemically very similar one is likely to have less effect than a more radical change. Some amino acids in a protein are essential to its structure or function, others are not. Apparent mis-sense mutations may actually be pathogenic because of an effect on splicing
Synonymous substitution	Most likely to have no effect – but can sometimes affect splicing

Nonsense mutations. The three codons UAG, UAA and UGA in mRNA are stop codons (Table 6.1). A single nucleotide change that converts any other codon into a stop codon (TAG, TAA or TGA in the DNA) causes the ribosome to detach and protein synthesis to terminate at that point. Such mutations are called **nonsense mutations**. Contrary to common belief, mRNAs containing nonsense mutations do not normally produce truncated proteins. Cells have a very interesting mechanism (**nonsense-mediated decay**) for detecting and degrading mRNAs that contain premature termination codons (*Figure 6.3*). In some cases a certaina mount of a truncated protein may be produced, but the usual effect of a nonsense mutation is the same as a complete deletion of the gene.

Nonsense-mediated decay probably evolved to protect cells against possibly toxic dominant negative effects of truncated proteins (see below). It presumably explains why the last exon of many genes is very large - you can't split the 3' untranslated region between several exons. Some rather mystifying differences between the effects of nonsense mutations in different parts of the same gene are the result of nonsense-mediated decay operating process. i