Making DNA from RNA – reverse transcriptase

(Extension material for Level 3 Biology Study Guide, ISBN 978-1-927194-58-4, page 368)

Making DNA from RNA – reverse transcriptase

A major problem that limits the incorporation of eukaryote DNA into prokaryote DNA is that of the length of the eukaryote DNA. A large proportion of eukaryotic DNA is **intron DNA**, while prokaryote DNA does not contain introns. This has two implications …

- Eukaryotic DNA can be too long to be incorporated into the prokaryote plasmid.

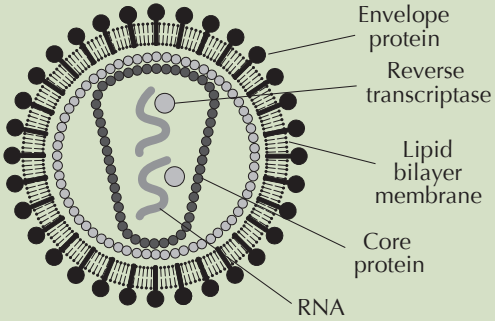
  ### Example

  The *Ti* plasmid in *Agrobacterium* is considered to be a relatively large plasmid at 200 kb. However, the length of the region of DNA coding for one of the human blood clotting factors, Factor VIII, is 190 kb, while the gene for Duchenne muscular dystrophy is over 1 000 kb.

- Eukaryote primary RNA transcript is modified before translation by having the non-coding introns removed so the mRNA consists only of coding regions. Prokaryotes lack the cell machinery necessary for this editing process, so the final protein made by the prokaryote will be non-functional (as it would contain stretches of 'junk' polypeptide corresponding to the introns). One solution is to use *reverse transcriptase* that occurs naturally in some viruses.

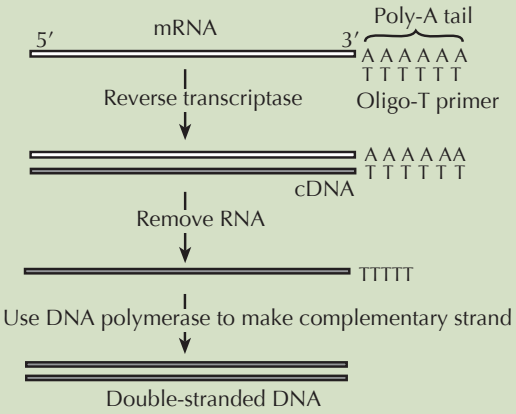
Reverse transcriptase is an enzyme produced by **retroviruses**, which include tumour viruses and HIV.

Their genetic material is RNA and this is replicated indirectly in the host cell via a DNA template – the reverse of what normally happens. The viral RNA is used as a template to make a complementary sequence of DNA (**cDNA**), which is then used to make more viral DNA. Genetic engineers use it to make DNA from messenger RNA *in vitro*.



HIV, a retrovirus

Reverse transcriptase needs a **primer**; the 3' end of all eukaryote mRNA has a poly-A tail, so an oligo-T sequence serves as primer. The mRNA is removed from the (single-stranded) cDNA and then used as a template to generate the other strand using DNA polymerase.



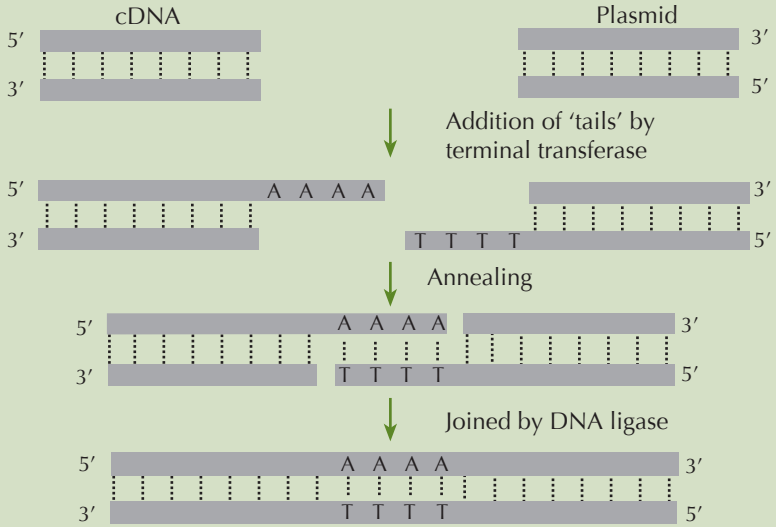
The production of the second strand of the cDNA is more complicated than shown.

Synthesis of DNA from mRNA using reverse transcriptase

The DNA produced in this way will have blunt ends – these must first be made sticky by addition of linkers that are complementary to the ends that will be created when the plasmid is cut. A common method is to add poly-A tails using the enzyme **terminal transferase**.

Example

cDNA is to be inserted into a plasmid. The bacterial plasmids are opened up using a restriction enzyme that generates blunt ends. These are also 'tailed' by addition of poly-T to make them complementary to the poly-A tails of the cDNA. After mixing the plasmids with the cDNA, they are covalently joined using DNA ligase.



Joining flush-ended DNA using terminal transferase to create 'linkers'