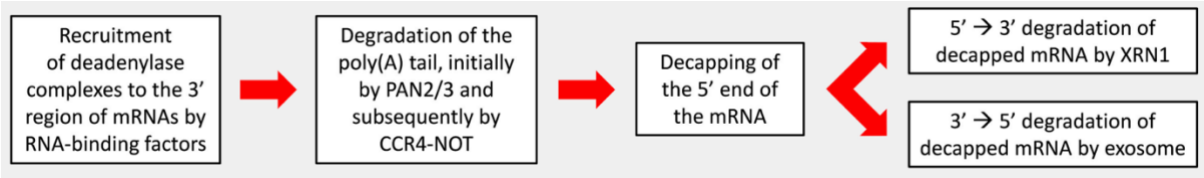
mRNA Degradation

- mRNA is degraded if it is damaged (nonsense mediated decay -NMD), if there is too much present in the cell (form of regulating gene expression by degrading before translation) or to defend against viruses
- mRNA is protected from degradation by the 5' cap and poly A tail at 3' end
- poly A binding protein (PABP) helps stabilise and protect the mRNA at 3' end
- Cells repress gene expression via mRNA decay pathways (degradation of existing mRNA transcripts so they are not converted to protein)

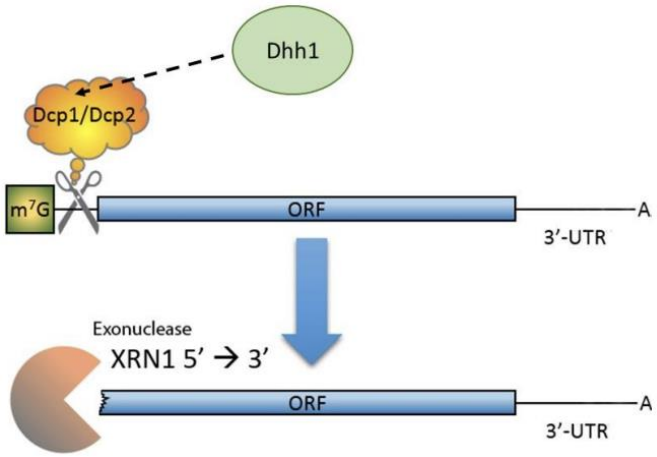
Decapping Mediated mRNA Decay Pathway

Steps

- mRNA decay is initiated by **deadenylation**: shortening/degradation of the 3' poly(A) tail
  - CCR4/POP2/NOT is inhibited by the PABP so it cannot degrade the poly A tail
  - PABP stimulates PAN2 and PAN3 which destabilises PABP
  - Once destabilised, CCR4/POP2/NOT can then degrade the poly A tail
- Deadenylation is followed by mRNA **decapping**: removal of the protective 5' cap: a guanosine methylated at position 7
  - The 5' cap is also termed the 7-methylguanylate cap, or m7G
  - Decapping yields a 7m-GDP molecule and an mRNA fragment phosphorylated at the 5' end
- This phosphorylated mRNA can undergo **exonuclease digestion** by Xrn1p from the 5' to 3'
  - 3' to 5' exonuclease digestion can occur by the exosome (ATP dependent)
  - RNA is fed through the ring channel to Rrp44 for degradation



- Cleavage of the 5' cap is catalysed by a holoenzyme consisting of two proteins, DCP1 and DCP2
- **DCP1** (mRNA DeCaPping protein 1) enhances the activity of catalytic subunit DCP2
- **DCP2** (mRNA DeCaPping protein 2/m7GpppN-mRNA hydrolase) is a metalloenzyme which catalyses the cleavage of the 7-methyl guanylate cap
- **DHH1** is an ATP-dependent RNA helicase that acts by activating the DCP1/DCP2 complex



Nonsense Mediated Decay Pathway (NMD)

- Ensures mRNA encodes the correct protein
- Degrades mRNA that encodes for a nonsense stop codon (point mutation) which results in pre-mature termination

- Presence of cap binding complex (CBC) and exon junction complex (EJC) during translation allows for quality control check to determine where stop codon should be
- Stop codon outside 50-55nt from the last exon-exon boundary can be recognised by nonsense mediated decay machinery
- If the stop codon is inside the 50-55nt, it will not be recognised and will escape nonsense mediated decay
- If a pre-mature stop codon is found, the SURF complex is recruited and phosphorylates UPF1
- UPF1 can then recruit SMG6, an exonuclease that can cleave the mRNA in half → making it vulnerable to degradation at the cleavage site
- UPF1 can also recruit SMG5 and SMG7 which recruit CCR4/POP2/NOT, DCP1 and DCP2 → deadenylation, decapping, exonuclease digestion

RNAi (RNA interference)

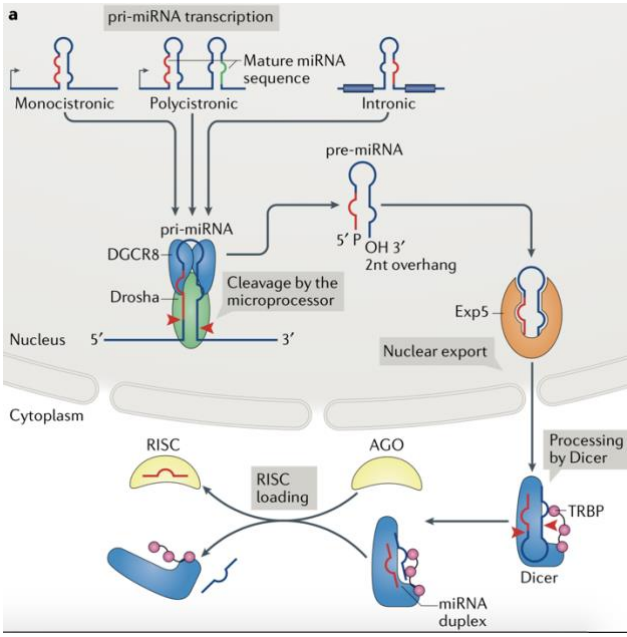
- RNA interference (RNAi) silences genes by mRNA degradation by cleaving the mRNA or preventing binding of ribosomal subunit
- Silencing of mRNA → less protein is translated → detect amount of protein
- siRNA and microRNA negatively regulate gene expression -transcriptionally

siRNA

- siRNA can be naturally derived from long double stranded RNA or can be delivered into cells experimentally
- Double stranded precursor of siRNA binds to dicer (an endonuclease) in the cytosol and cleaves RNA into short segments known as siRNA
- The anti-sense strand of siRNA then binds to an argonaut protein and is unwound and the anti-sense strand remains whilst the other strand is degraded
- The anti-sense strand and argonaut protein form the RNA induced silencing complex (risc)
- The anti-sense strand of siRNA directs. Argonaut in risc to the target mRNA
- The gene can then be silenced/knocked down by the cleavage of mRNA catalysed by the argonaut protein
- The mRNA is then degraded by nonsense/decapping mediated pathway

miRNA

- miRNA genes found on all chromosomes except Y
- Transcribed by RNA polymerase II and have a poly A tail and 5' cap
- mRNA genes can be **monocistronic** or **polycistronic** (several miRNA genes in the one mRNA transcript)
- miRNA can also be **intronic** (found in introns)
- pri-miRNAs are processed by the RNase III enzyme Drosha and DGCR8 (dsRNA binding protein) to produce pre-miRNAs
- DGCR8 measures 11nt from the base of the hairpin which determines the Drosha cleavage site



- Drosha and DGCR8 are part of the Drosha Microprocessor Complex
- pre-miRNAs are exported to the cytoplasm (by exportin 5) through the NPC for further processing to become miRNA
- **Mirtrons** are pri-miRNAs encoded by introns that do not contain them 11nt segment at the base for Drosha cleavage
- Mirtrons are processed by spliceosomes instead of Drosha and are released as lariat structures
- Lariat structures are first linearized by a debranching enzyme (DBR1 hydrolyzes the 2–5 phosphodiester linkage); then they fold into hairpins (pre-miRNA)
- They are then exported by exportin 5 through the NPC for further processing to become miRNA
  When exportin 5 is bound by Ran GTP, it can export the pre-miRNA out of the nucleus
- GTPase converts GTP to GDP, which releases exportin 5 and the pre-miRNA
- Exportin and Ran can then be recycled back into the nucleus for export again

miRNA Knockdown

- RNase III enzyme Dicer and TRBP (dsRNA binding protein) cleave the pre-miRNA into shorter segments to produce mature miRNA (approximately 21bp long)
- miRNA is then unwound at the end with the lowest thermodynamic stability by a helicase argonaut protein (AGO)
- The strand that has its 5' terminus at this end is the future miRNA; the other strand is degraded
- The selected (guide) strand is bound to an RNA-induced silencing complex (RISC)
- miRNA sequence (seed sequence nt 2-8 at 5') acts as a guide to direct the Argonaute protein in the RISC to the target mRNA
- miRNA sequence is not completely complimentary (except for seed region) to the target mRNA sequence → a single miRNA can target multiple mRNAs because their 3'-UTRs contain the same seed sequence
- siRNA is more specific
- miRNA can knock down genes in two ways;
  - Argonaut in risc catalyses cleavage of mRNA which is then degraded (like siRNA) by nonsense/decapping mediated pathway
  - Directly inhibiting translation of the mRNA by preventing the ribosomal subunit from binding due to incomplete base pairing (reversible)
    - Blocking translation can lead to aggregation of mRNA in P bodies

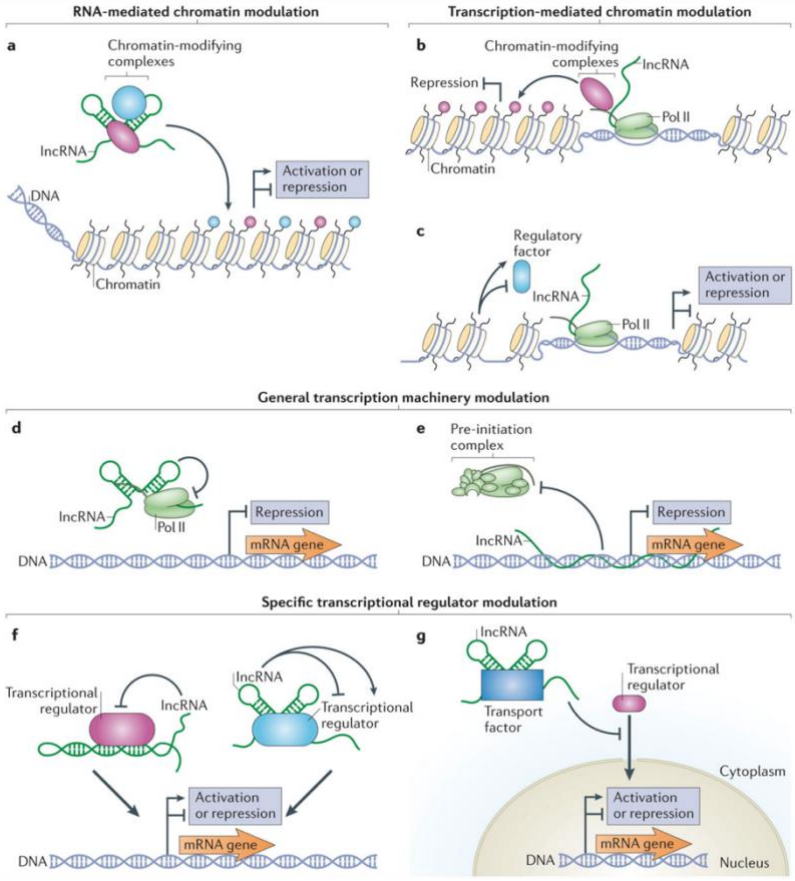
lncRNAs (long noncoding RNAs)

- >200 nt long
- Similar histone marks as protein-coding genes: H3K4me3 (promoters) & H3K36me3 (gene body)
- Mostly transcribed by RNA polymerase II
- May undergo splicing or have a single exon (small open reading/coding frame)
- Some/most are processed like mRNAs (cap and polyA tail)
- Limited protein coding potential

Function

- Transcriptional → recruit epigenetic modifiers to alter chromatin state for transcription

- Post-transcriptional → capping, splicing (exon skipping), editing, translation, transport, degradation
- Post-translational → protein ubiquitination, acetylation, phosphorylation



HOTAIR

- HOTAIR lncRNA is part of the HOXC cluster
- It can bind PRC2 and LSD1 and regulate other chromosomes (it's a trans-acting modulator)
- PRC2 recruits H3K27me3 which promotes gene silencing
- LSD1 demethylates H3K4me2 to H3K4me1

XIST

- 17 kb lncRNA
- Master regulator of X-inactivation
- Cis-acting
- X-inactivation center (Xic) encodes Xist RNA which is transcribed/expressed on the inactive X chromosome
- XIST RNA binds PRC2 via conserved repeat motif (Repeat A, RepA) and spreads along the entire chromosome (methylates the chromosome) → repression of chromosome
- Tsix is antisense to Xist & blocks recruitment & binding of PRC2 to XIST → prevents inactivation of X chromosome