Modification and processing of eukaryotic pre-mRNAs

RNA Capping

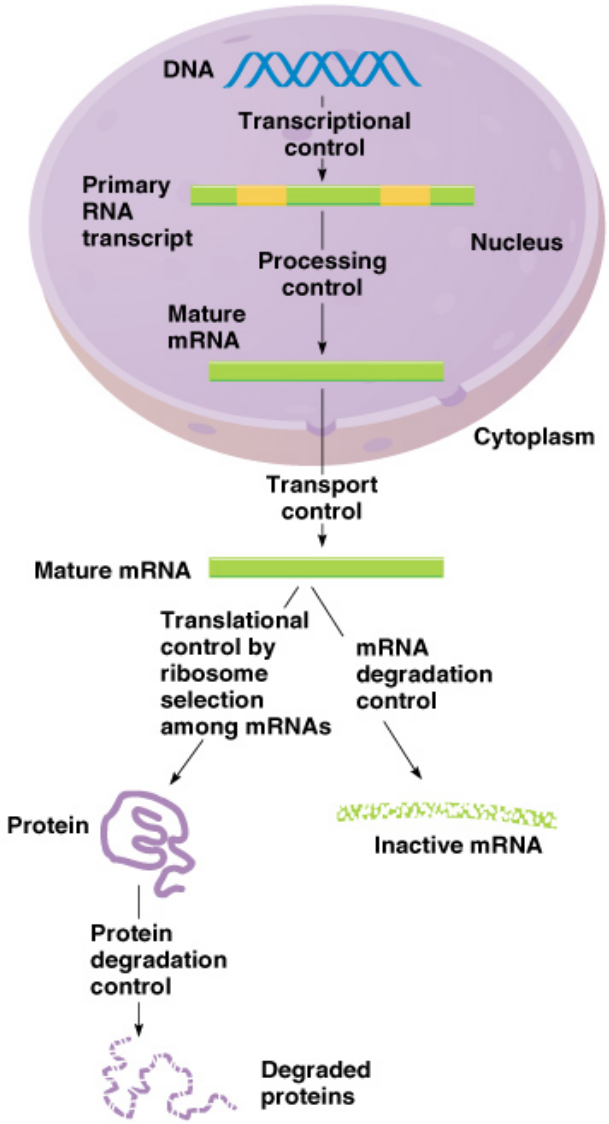
Gene Expression in General

Eukaryote gene expression is regulated at seven levels:

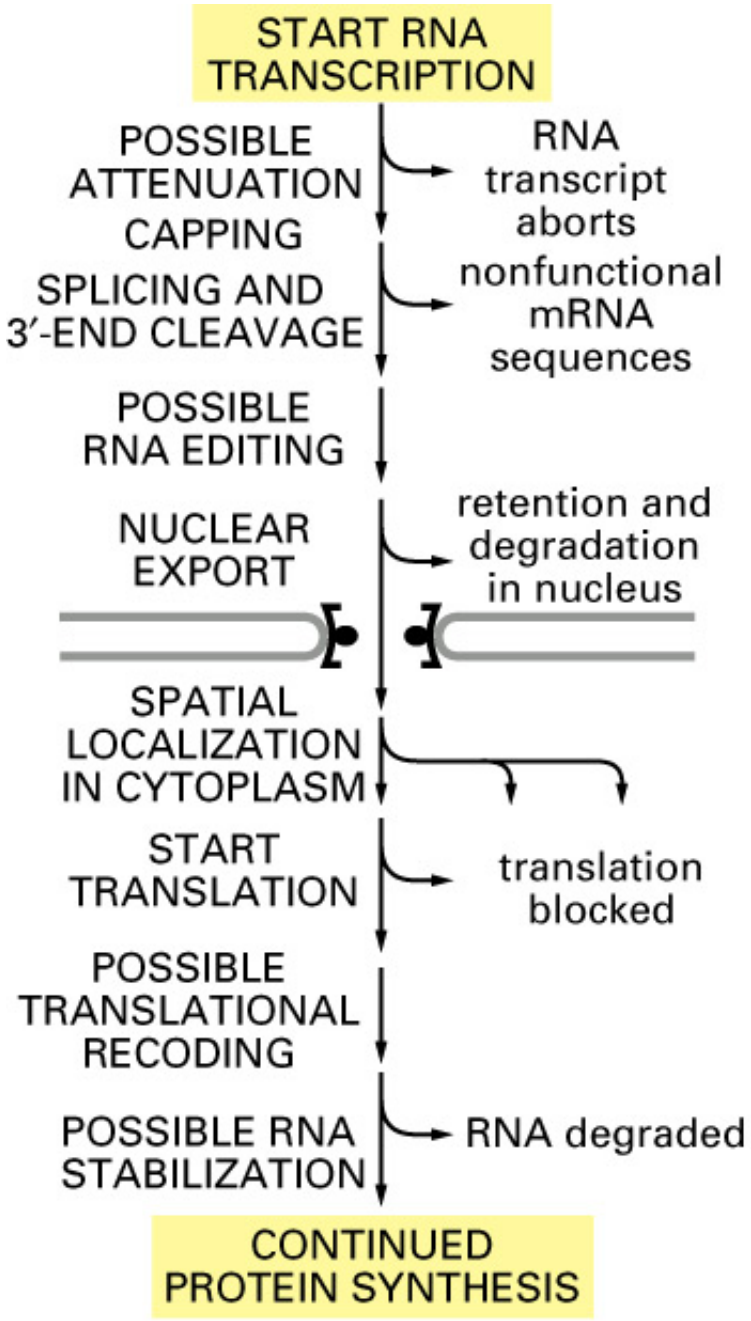
1. **Transcription**
2. **RNA processing**
3. **mRNA transport**
4. **mRNA translation**
5. **mRNA degradation**
6. **Protein targeting**
7. **Protein degradation**

Each of these levels contains multiple steps, which are also subject of control and regulation

Control vs. regulation



The Long and Winding Road…..



Processing of eukaryotic RNA polymerase II transcripts

Steps in pre-mRNA processing

- **i). Capping**
- **ii). Splicing**
- **iii). Cleavage and polyadenylation**

I. Capping

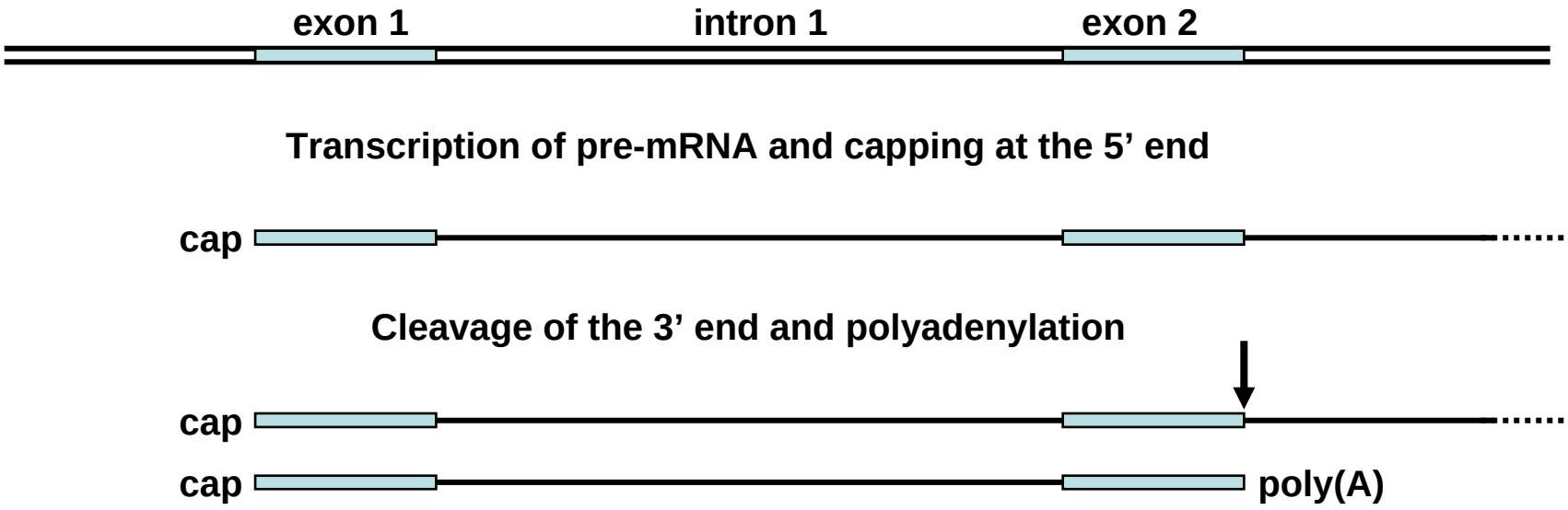
II. Splicing

- **a). Chemistry of mRNA splicing**
- **b). Donor and acceptor splice sites**
- **c). Spliceosome assembly and splice site recognition**
- **d). Small nuclear RNAs and RNPs**
- **e). Role of SR proteins in splicing**
- **f). Splicing regulation**
- **g). Alternative splicing**
- **h).Mutations that disrupt splicing**
- **i). AT-AC introns**
- **j). Trans splicing**

III. 3’ End Processing: Cleavage and Polyadenylation of Primary Transcripts

Steps in mRNA processing (hnRNA is the precursor of mRNA)

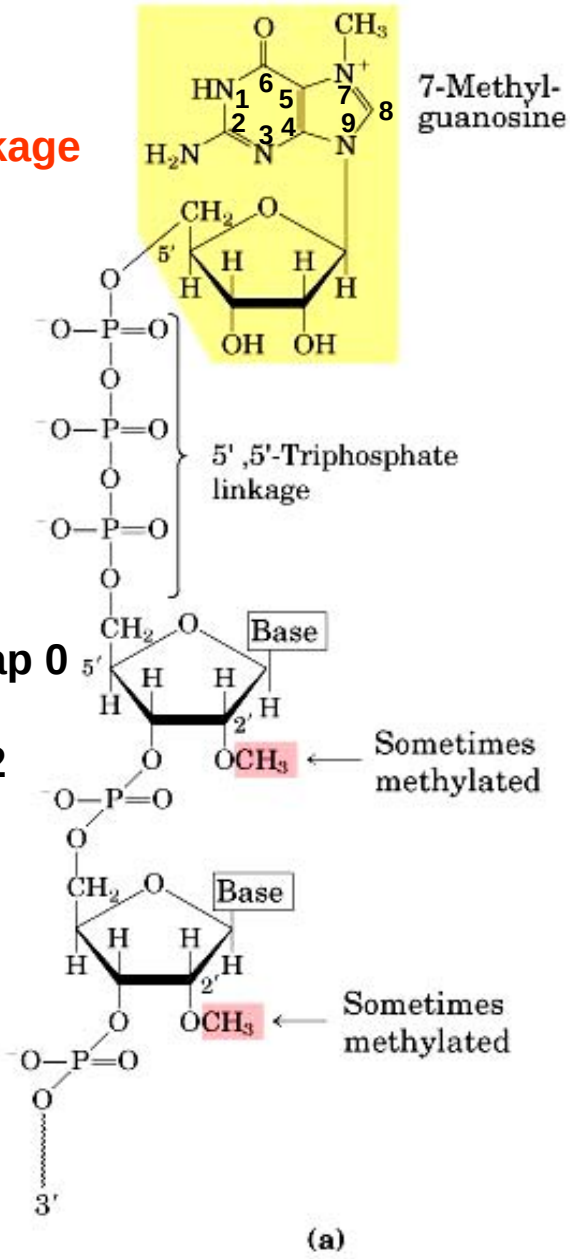
- capping (occurs co-transcriptionally)
- cleavage and polyadenylation (forms the 3’ end)
- splicing (occurs in the nucleus prior to transport)



RNA capping

5’ cap

Most eukaryotic mRNAs contain a 5' cap of 7-methylguanosine linked to the 5'-end of the mRNA in a novel 5', 5'-triphosphate linkage
This base is not encoded in the DNA template



<u>The cap may also have some additional modifications including:</u>
1.Methyl groups at 2'-O position of the 1st and 2nd template encoded nucleotides
No methyl at either 1st or 2nd template encoded nucleotides = Cap 0
Methyl at only 1st template encoded nucleotide = Cap 1
Methyl at both 1st and 2nd template encoded nucleotides = Cap 2

2.In some cases, if the 1st nucleotide is an adenine it may be methylated at N6

RNA capping

Addition of the cap involves several step:

1. pppN(pN)n —*Triphosphatase*→ **ppN(pN)n + P i**

2. ppN(pN)n + pppG —*Guanylyltransferase*→ **G(5')pppN(pN)n + pp i**

3. G(5')pppN(pN)n + AdoMet —*(guanine-7)methyltransferase*→ **m7G(5')pppN(pN)n + AdoHcy**

AdoMet = S-adenosylmethionine (common methyl donor) for number of methyl transfer reactions
AdoHcy = S-adenosylhomocysteine

Triphosphatase typically strongly purine specific without ribo- vs. deoxy-specificity

Activity on pyrimidine NTPs is 4-10% of that with GTP suggesting that first encoded nucleotide is likely (A or G)

Reaction involves a covalent intermediate of GMP-guanylyltransferase (phosphoamide bond to e-amino of lysine)

Additional modifications to the cap can occur at the first and second encoded nucleotides via addition of methyl groups through a 2'-O methyltransferase

RNA capping

Yeast

Bifunctional complex consisting of 2 polypeptides of 80 and 52 kDa

80 kDa subunit has RNA triphosphatase activity

52 kDa subunit has guanylytransferase activity

Methyltransferase activity is thought to be located on a separate polypeptide

Mutations in capping in yeast show pre-mRNA splicing defects suggesting a direct or indirect link between the cap and/or capping enzyme and splicing – this may be strongest evidence for relationship between cap and splicing

Higher eukaryotic cellular enzymes

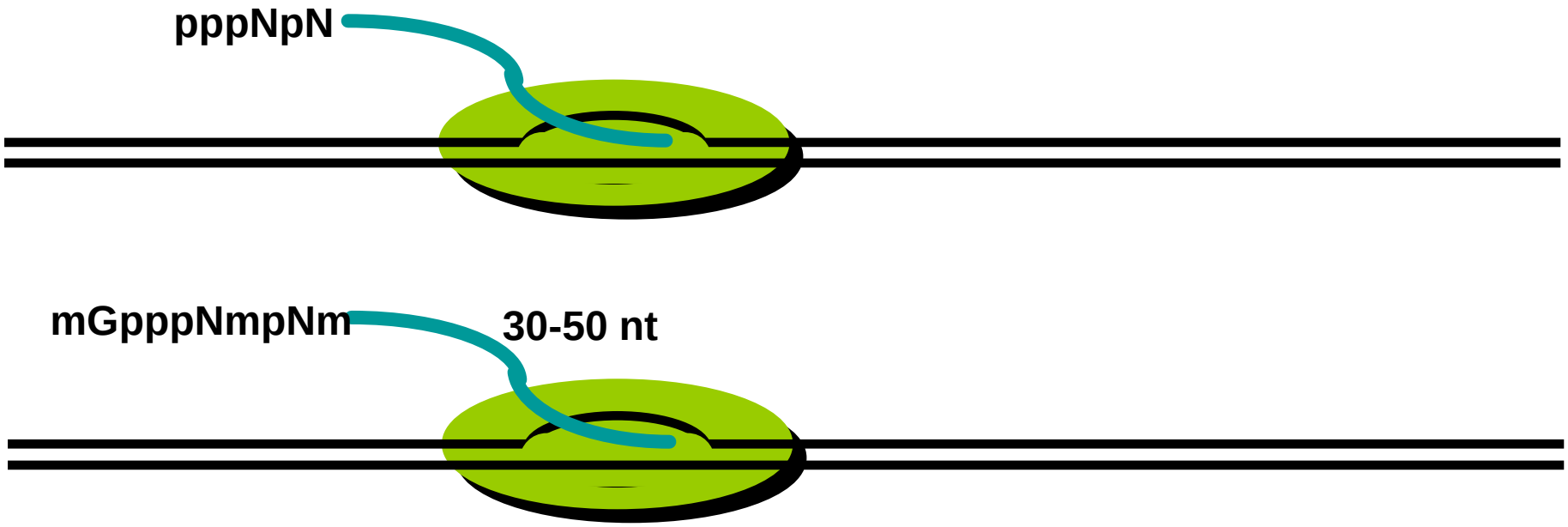
Do not show tight association between guanylytransferase and 7-methyltransferase nor have they been shown to be associated with transcription initiation/termination

Triphosphatase activity associated with guanylytransferase (except yeast) based on biochemical data in higher eukaryotes = variable size from 65-77 kDa

Recent identification of the cDNA for C. elegans guanylytransferase indicates that guanylytransferase/ triphosphatase are associated within same enzyme

Capping occurs co-transcriptionally shortly after initiation

- **guanylyltransferase (nuclear) transfers G residue to 5' end**
- **methyltransferases (nuclear and cytoplasmic) add methyl groups to 5' terminal G and at two 2' ribose positions on the next two nucleotide**



Capping in vivo is coordinated temporally and physically with transcription
Occurs between 20-74 nts based on analysis of prematurely terminated transcripts in vitro
20 nt nascent RNA uncapped
75 nt nascent RNA capped
Does not appear to be associated with transcription initiation

RNA capping

Capping occurs only on pol II transcripts including snRNAs (U1, U2, U4, U5)
But cap on U snRNAs is 2,2,7,methylguannosine

<u>What targets capping to only pol II transcripts?</u>
Interaction between capping enzyme and pol II or transcription complex?
Recent data suggest that an interaction between the CTD of RNA pol II and capping enzyme may target pol II transcripts for polyadenylation providing a mechanism for why only pol II transcripts are capped (no CTD on pol I and III)

Capping may also be important for mRNA identity to target transcript for mRNA processing and transport (see below)

RNA capping - functions

Processing (mRNA splicing and 3'-end formation)

5' cap also appears to target RNA for mRNA recognition and subsequent steps in processing including splicing and 3‘ end-formation

Cap functions in "exon definition" in splicing and 3'-end formation

(see splicing and polyadenylation lectures)

Cap may actually facilititate all of these processes via interaction with a second cap binding complex (CBC; 80 and 20 kDa proteins) that is located in the nucleus

Nuclear Transport/Export

5' cap required for mRNA recognition and is also important for nuclear transport and export (and possibly import U snRNAs);

A second cap binding nuclear complex (80 and 20 kd proteins) associated with transport/export

Translation

Cap methylation is critical for cap function in promoting translation -- bound by initiation factor (eIF4E) and associated with recruitment of mRNA to 40S ribosome during translation initiation

In Xenopus oocytes some cap modifications may have regulatory affects for translation

Stability

5' cap may stabilize mRNAs

Frog oocyte microinjection experiments with capped and uncapped in vitro transcripts

Although several indications that cap may play a role in processing, stability, and translation in vitro, only recently has there been direct or genetic evidence to support these suggestions in yeast- particularly for stability

What is potential role of other cap modifications? Cap 1 or Cap 2 or adenine methylation -Currently unclear