Control of gene expression

1. transcription
2. **mRNA processing**
3. **mRNA stability**
4. **translation**
5. post-translational events (protein stability, modification, sequestration etc.)

Literature:
Lodish, Chapter11

Alternative Splicing:
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NMD:
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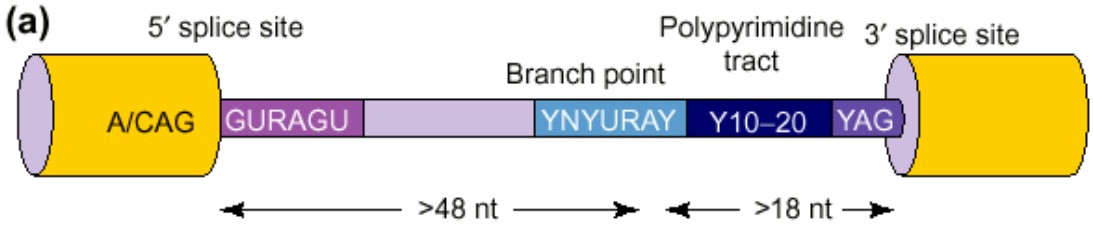
1. Pre-mRNA processing

The transcripts produced by RNA polymerase II undergo several modifications before being exported to the cytoplasm. Even before the transcript is completed, the 5' end is modified by covalent joining of a 7-methyl-guanylate residue known as the 5' cap. The 3' end of mRNA is produced by an endonucleolytic cleavage followed by the addition of about 250 adenylate residues. The cap and poly(A) tail are important determinants of the efficiency of mRNA export from the nucleus and, in the cytoplasm, they both play roles in translational initiation and in mRNA degradation. Finally, introns are removed and exons are stitched together.

1.1. **Regulation of splicing** (alternative splicing)

Before considering the **regulation of splicing**, it is helpful to recapitulate the splicing reaction. The splice sites are located at the two exon-intron boundaries. The location of splice sites is determined by comparing the genomic and cDNA sequences of a given gene. Analysis of a large number of pre-mRNAs reveals short, somewhat conserved sequence elements. Just 3' of the 5' splice junction, a **GU dinucleotide is 100% conserved**, with some base preference following into the intron; just 5' of the 3' splice junction an **AG dinucleotide is 100% conserved.** In higher organisms, a pyrimidine-rich region just upstream of the 3' splice site is common. A specific adenine residue is used as **a branch point** in the splicing reaction.



Since every exon-intron-exon unit has the same sequences that specify splice junctions, and the sequences themselves are rather simple, how does splicing proceed in the proper order; what ensures that the correct pairs of sites are spliced, especially given that pairs of sites may be separated by introns as large as 10 kb?

All 5' splice sites and all 3' splice sites are functionally equivalent, so splicing has to follow some "rule" to ensure a 5' site is always connected to the next 3' site. In 95% of the cases, splicing occurs only between the 5' and 3' sites of the same intron. How?

Splicing occurs in two steps: In the first stage of the process, a cleavage occurs at the 5' splice site. The right intron-exon molecule then forms a lariat (branched structure) in which the 5' terminus generated at the end of the intron becomes linked in a 5'-2' bond to a specific residue within the intron, the branch point. In the second stage, cleavage at the 3' splice site releases the free intron as a lariat while the right exon is joined to the left exon. The cleavage and joining reactions actually occur in a coordinated manner.

The fact that the excised intron is a branched structure rather than a linear molecule led to the realization that splicing occurs by two trans-esterification reactions rather than conventional cleavage and religation. In each reaction, one phosphoester bond is exchanged for another.

Molecular machinery:

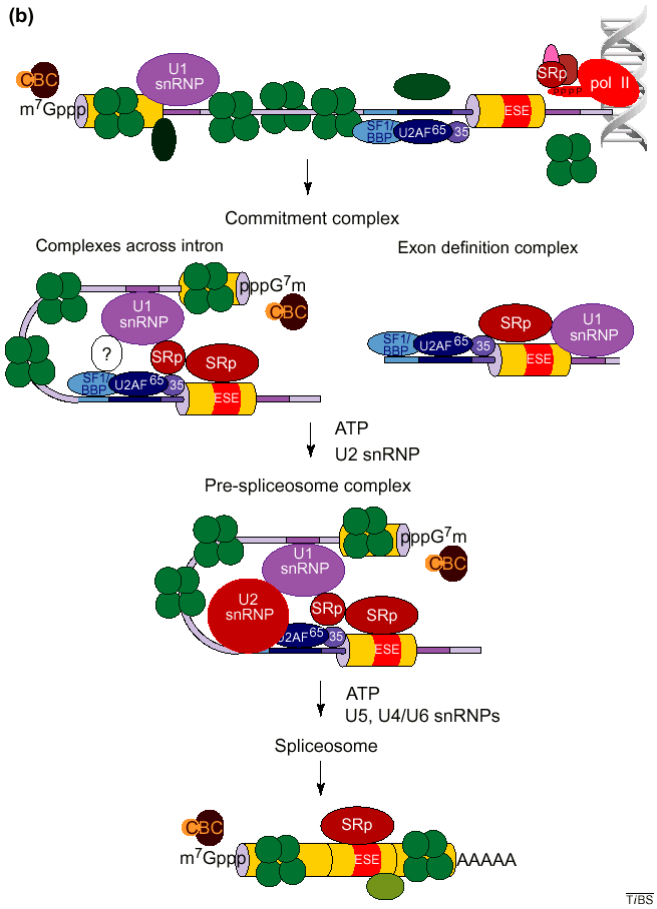
HnRNP-proteins (heterogenous nuclear ribonucleoprotein- proteins)

SnRNPs (snurps): Composed of snRNAs (small nuclear RNAs and proteins).

SR proteins (have Arg/Ser dipeptide repeats*)* as auxillary factors

The complete set of snRNAs involved are U1, U2, U4, U5, and U6. Along with a number of other splicing protein factors the snRNPs assemble on the pre-mRNA into the very large complex called the **spliceosome.**

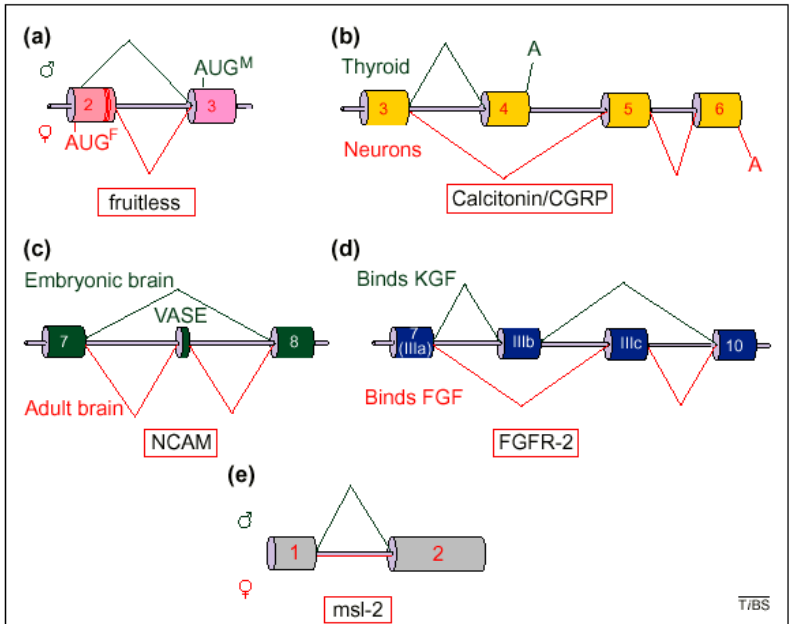
First, the U1 and U2 snRNAs, as part of their corresponding RNPs, base pair with the 5' splice site and the branch point, respectively. In yeast, where the branch point sequence is completely conserved, U2 forms 6 base pairs; in higher eukaryotes where the branch sequence is less conserved, the association of U2 with pre-mRNA is facilitated by a protein called U2AF, which binds the pyrimidine-rich region near the 3' splice site. Binding of U2 snRNP also requires ATP hydrolysis. Following the association of U1 and U2 snRNP, a complex consisting of U4/U6/U5 binds and completes the spliceosome.



Alternative splicing as a means of regulating gene expression

As mentioned at the outset, all 5' splice junctions are functionally equivalent as are all 3' junctions. In principle cells could vary the use of splice junctions so that a single gene could give rise to multiple mRNAs and, indeed, alternative splicing is a mechanism used to produce different polypeptides from a single gene. Some alternative splicing events appear to be constitutive whereas others are regulated in response to developmental and physiological cues (tightly regulated in a cell-type or developmental stage-specific manner; e.g. nervous system differentiation or apoptotic cell death).

Exons tend to encode distinct functional domains of proteins and alternative splicing can be used as a means to include or exclude one or more functions of a protein.Alternative splicing can lead to:
a. usage of alternative initiation codons
b. usage of alternative polyadenylation sites¨
c. exon skipping/ inclusion
d. use of alternative exons
e. retention of intron and inhibition of translation



Examples to a – e:

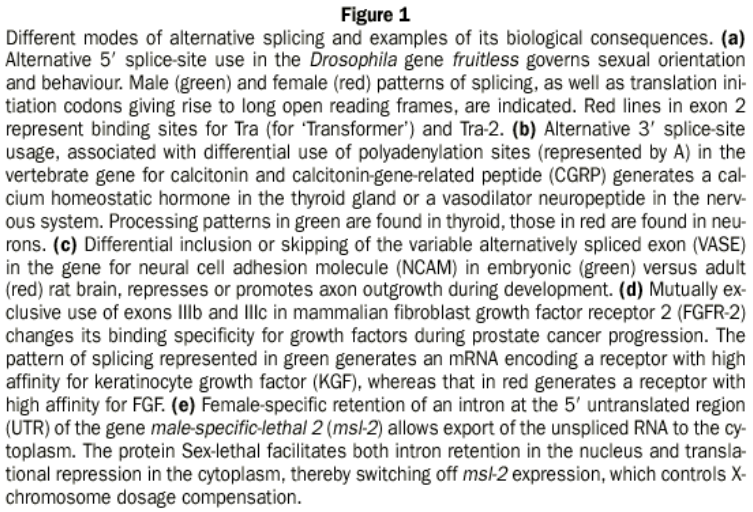


Figure 1

Different modes of alternative splicing and examples of its biological consequences. **(a)** Alternative 5′ splice-site use in the *Drosophila* gene *fruitless* governs sexual orientation and behaviour. Male (green) and female (red) patterns of splicing, as well as translation initiation codons giving rise to long open reading frames, are indicated. Red lines in exon 2 represent binding sites for Tra (for 'Transformer') and Tra-2. **(b)** Alternative 3′ splice-site usage, associated with differential use of polyadenylation sites (represented by A) in the vertebrate gene for calcitonin and calcitonin-gene-related peptide (CGRP) generates a calcium homeostatic hormone in the thyroid gland or a vasodilator neuropeptide in the nervous system. Processing patterns in green are found in thyroid, those in red are found in neurons. **(c)** Differential inclusion or skipping of the variable alternatively spliced exon (VASE) in the gene for neural cell adhesion molecule (NCAM) in embryonic (green) versus adult (red) rat brain, represses or promotes axon outgrowth during development. **(d)** Mutually exclusive use of exons IIIb and IIIc in mammalian fibroblast growth factor receptor 2 (FGFR-2) changes its binding specificity for growth factors during prostate cancer progression. The pattern of splicing represented in green generates an mRNA encoding a receptor with high affinity for keratinocyte growth factor (KGF), whereas that in red generates a receptor with high affinity for FGF. **(e)** Female-specific retention of an intron at the 5′ untranslated region (UTR) of the gene *male-specific-lethal 2* (*msl-2*) allows export of the unspliced RNA to the cytoplasm. The protein Sex-lethal facilitates both intron retention in the nucleus and translational repression in the cytoplasm, thereby switching off *msl-2* expression, which controls X-chromosome dosage compensation.

Mechanism of alternative splicing

Alternative splicing patterns result from the use of alternative 5' splice and 3' splice sites. Most alternative splicing decisions involve competition among potential splice sites; thus splicing patterns can be controlled by any mechanism that alters the relative rates of splice site recognition. Recognition of a particular splice site can be ensured by a strong match the consensus sequence or by the assistance of ***cis*-acting elements** and ***trans*-acting factors**. Frequently, regulated sites exhibit poor matches to the consensus and are inherently weak or their recognition is hampered by secondary structure. These features can contribute to positive regulation by rendering splice sites dependent on enhancement by *trans*-acting factors whose amounts or activities vary during development.

POSITIVE REGULATION:

***cis*-acting enhancer elements**:

a.) purine-rich splicing enhancers

often in exons, activate splicing of the upstream intron by promoting use of a weak 3' splice site (e.g. repeats of GARGAR (R is a purine), function as binding sites ***trans*-acting factors** such as SR-proteins)

b.) pyrimidine-rich enhancers

often in introns close to the 5' splice site, help to recruit U1 snRNP to the 5' splice site

NEGATIVE REGULATION:

Negative regulation could be achieved by simply providing a binding site for a factor that blocks access to a 5' or 3' splice site, but most cases seem more complicated. Many examples of inhibitory *cis*-acting elements have been described showing that splice sites can be blocked by

- secondary structure
- specific or general factors binding to regulatory elements within introns or exons

Example: **sexual differentiation in *Drosophila***:

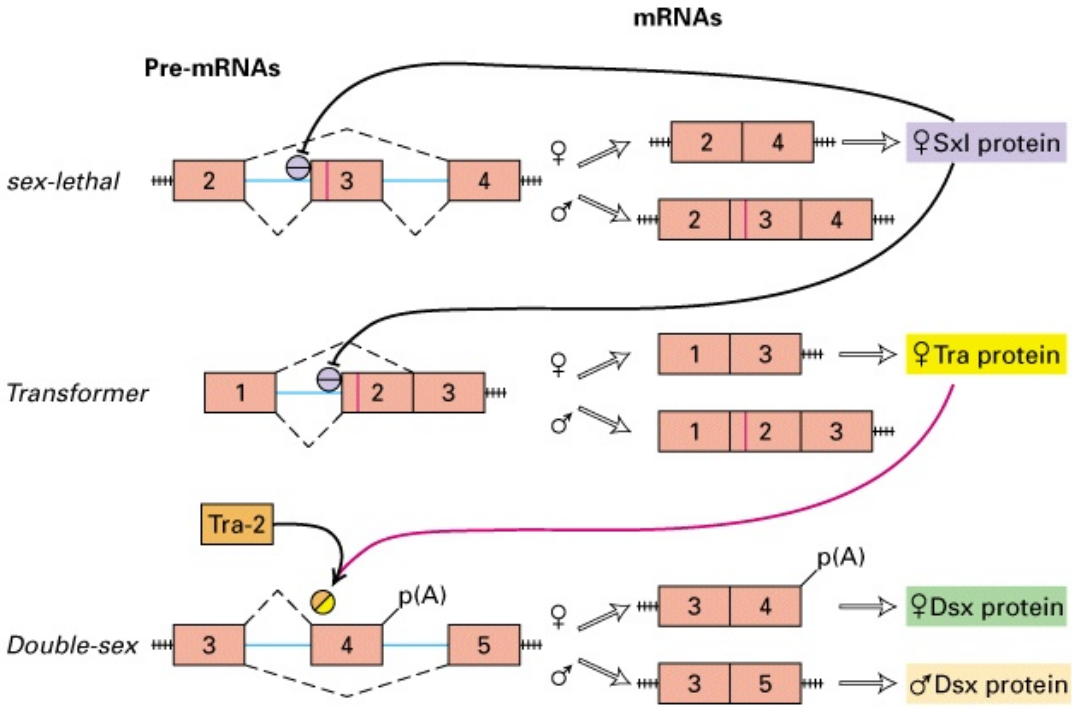
The primary target of the sex-determination pathway is the female-specific switch gene ***Sex-lethal (Sxl)*** which coordinately controls sex determination and dosage compensation. Sex is determined by the ratio of X chromosomes to autosomes: 2X female, 1X male. Gene dosage compensation is accomplished by enhancement of transcription of genes on the one X-chromosome in males.

The presence of two X chromosomes in female flies triggers expression of SXL early in development.
SXL is an RNA-binding protein (splicing and translational regulator exclusively produced in female flies) that **negatively** regulates three known alternative splicing events:

1. alternative splicing of its own mRNA
   SXL represses inclusion of an exon, which would hinder its translation (positive feed-back loop).
2. alternative splicing of *male-specific-lethal 2* mRNA *(msl2)*
   MSL proteins are required for dosage compensation (lead to hyperactivation of X-linked transcription) and are expressed only in males. In females, SXL blocks splicing of an intron, which interferes with translation of MSL2 protein.
3. alternative splicing of *transformer (tra)* mRNA
   SXL binds to the pyrimidine rich region of *tra* blocking the binding of U2AF and U2 snRNP and preventing splicing of exon 1 to exon 2, which contains a stop codon. Splicing therefore occurs to exon 3, producing a functional **TRA** protein only in female flies.

TRA is not an RNA-binding protein but it forms a complex with another protein, *tra2*. This complex functions as splicing activator in 2cases: mRNA, ***double sex*** **(*dsx*)**.

1. alternative splicing of mRNA ***double sex*** **(*dsx*)**
   In this case, the bound complex **activates** an upstream 3' splice site that is otherwise not used because of a poor sequence context. Since males produce no functional *tra* protein, this site cannot be used in male embryos. The alternative dsx mRNAs encode structurally and functionally distinct transcription factors (DSX^F and DSX^M). These transcription factors establish appropriate gene expression patterns for most somatic characters in females and males, respectively.
   TRA contains an RS domain and TRA2 contains an RNA binding domain and two RS domains. This complex recruits other SR proteins to the splicing enhancer such as RBP1.
2. alternative splicing of the *fruitless (fru)* mRNA
   TRA/TRA2 also affect splicing of the *fruitless (fru)* mRNA by activating the female-specific 5' splice site. The FRU protein is only produced in males. There it is responsible for male sexual orientation and court-ship behaviour.



DOSE-DEPENDENT REGULATION BY GENERAL FACTORS

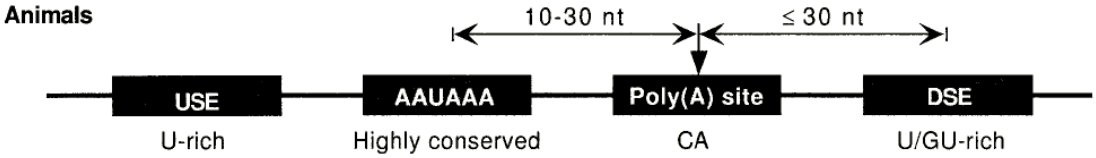
Highly specific alternative splicing factors have not yet been identified in vertebrate cells, and few have been identified in invertebrates. Numerous observations point at the involvement of general factors in regulation of splicing.
Specificity of alternative splicing is believed to arise from variations in the relative concentrations or activity of different competing or cooperating factors as well as from the strength and arrangement of binding sites for regulators and constitutive splicing factors.

Further, recent developments propose that the underlying chromatin status and transcription influence splice site selection, both by kinetic and splice factor recruiting mechanisms.

1.2. Regulation of pre mRNA 3' end processing

Transcription termination by eukaryotic RNA polymerase II is tightly linked to the 3' end processing and polyadenylation of pre-mRNAs. In eukaryotes, the efficiency of transcription termination generally correlates with the strength of the polyadenylation signal and the presence of a termination site or region located downstream of the poly (A) signal.

The mechanism of cleavage/polyadenylation has been determined largely by *in vitro* assays. Most Pol II transcripts contain the sequence **AAUAAA** 10-30 nucleotides upstream from the poly(A) tail and it is now known that this sequence directs cleavage and polyadenylation. A second signal downstream from the cleavage site is also required; this signal is not a specific sequence but is either a GU or U-rich region about 50 nucleotides 3' of the cleavage site.



Other sequences elements can modulate the efficiency of 3' end processing in a positive or negative fashion (e.g **USE: upstream element**).

Processing begins with the association of a large protein complex, the cleavage and polyadenylation specificity factor (CPSF), which binds the AAUAAA. Then a number of other proteins including cleavage factor (CF) and cleavage stimulatory factor (CstF) bind to the CPSF-RNA complex; one of these additional factors interacts with a G-U rich sequence downstream of the cleavage site resulting in stabilization of the entire complex. Next, a poly(A) polymerase (PAP) binds to the complex, before cleavage can occur, thereby linking cleavage to polyadenylation. Following cleavage, polyadenylation proceeds in two steps. Addition of about the first 12 residues occurs slowly and is followed by rapid addition of another 200-250 residues. This rapid addition requires a poly(A)-binding protein called PABII. PABII binds to the short poly(A) tail added initially by PAP, stimulating polymerization of the additional residues. PABII causes the polymerase to cease after about 250 residues, but the mechanism by which actual length is measured is not known.

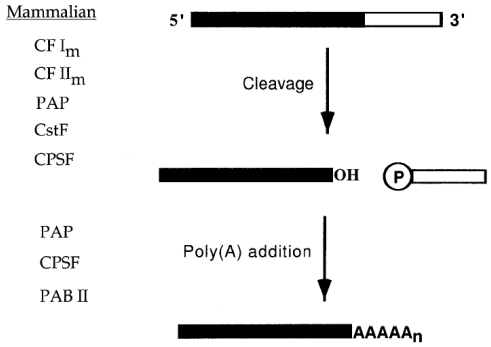


FIG. 2. mRNA precursors are processed at their 3′ ends in a two-step reaction. A primary transcript is cleaved endonucleolytically at the poly(A) site, and this is followed by the addition of adenylate residues to the 3′ end of the upstream fragment to form a poly(A) tail. The factors responsible for each step of the reaction in mammals and in the yeast *S. cerevisiae* are indicated on each side.

At the level of 3' end formation, two types of decisions can be made:

1. whether to process the transcript or not
2. where to process and to place the 3' end
   a) tandem arrays of poly(A) sites within a single 3' UTR
   b) composite 3' exons whose 3' end is formed by either a 5' splice site or a poly(A) site
   c) alternative 3' exons

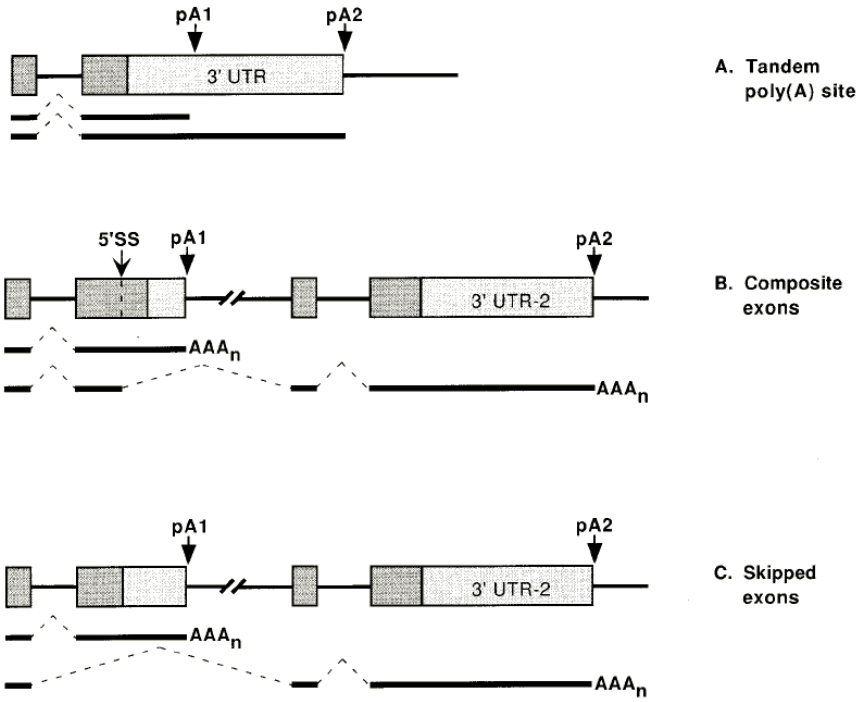


FIG. 9. Types of alternative polyadenylation choices include multiple poly(A) sites in the 3′ untranslated region (3′UTR) (A), a choice in defining the end of an exon by a 5′ splice site (5′SS) or a poly(A) site (B), and a choice of two different 3′-terminal exons (C). Adapted from reference 139.

Two mechanisms are commonly used to execute these decisions:

1. regulation of the efficiency with which the processing complex assembles on a poly(A) site: this depends on the stability with which the 3' end processing complex forms
   - many regulated poly(A) site are weak (poor match to the consensus or unfavorable sequence context)
   - efficiency at which the processing complex forms will be the combined effect of the concentration of polyadenylation factors and the additional stabilizing influence of other factors recruited onto polyadenylation enhancers

2. activity of processing enzymes which can be regulated by post-translational modifications or by interacting repressor proteins

Note, that tissue specific or transcript specific factors have not yet been discovered.

In vivo, the reactions of pre-mRNA transcription, capping, splicing and polyadenylation are tightly coupled. This is reflected by the fact, that interaction between the spliceosome and the polyadenylation machinery is required to define the 3' terminal exon.

Examples:

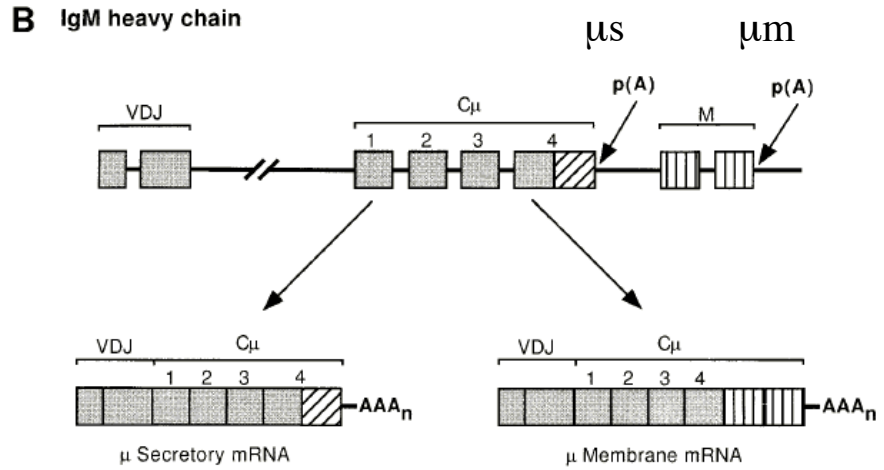


FIG. 10. (A) Organization of exons and introns in calcitonin/CGRP pre-mRNA and the structure of the primary mRNA products produced in thyroid cells or neurons. (B) Alternative processing choices in the immunoglobulin M (IgM) heavy-chain (μ) precursor. (C) Structure of the integrated proviral DNA of the HIV-1 retrovirus and the primary transcript. The transcription initiation site is indicated by the arrow. USE, upstream polyadenylation enhancer; LTR, long-terminal repeat sequence; TAR, TAT-binding site; MSD, major splice donor used by all spliced transcripts. The promoter proximal and promoter-distal poly(A) sites and alternative splice donor (◇) and acceptor (△) are also indicated. For ease of representation, the elements are not in correct size scale with respect to each other.

The regulation of the IgM heavy chain synthesis during B cell differentiation represents the best characterized example of how changes in the concentration of a general polyadenylation factor can modulate 3' end formation. This leads to the production of either membrane bound IgM (in pre-B cells, right) or secreted IgM (in plasma cells, left). Determination of the choice of poly(A) sites is regulated by the concentration of CstF-64 subunit. CstF-64 has a higher affinity for the mm poly(A) site. In preB cells, CstF-64 is the limiting factor for 3' end formation and it binds preferentially to the μm poly(A) site. In plasma cells, the CstF-64 concentration is higher and the use of the weak affinity μs poly(A) site is favored.

2. Regulation of mRNA stability

The steady state concentration of an mRNA is determined by the rates of synthesis and decay. The half-life of individual mRNAs within a given eukaryotic cell may vary by several orders of magnitude, from a few minutes to many hours and, in some cases, to days.
mRNAs are protected from degradation by the 5' cap and the 3' poly(A) tail. A major mRNA decay pathway is initiated by shortening of the poly(A) tail, decapping and subsequent 5' to 3' exonucleolytic degradation of the mRNA (3' to 5' exonucleolytic decay may also occur) .

NMD – Nonsense mediated decay

The nonsense-mediated mRNA decay (NMD) pathway is a post-transcriptional process that rapidly degrades mRNAs with premature translation termination codons (PTCs). This limits the expression of truncated polypeptides. The NMD pathway is initiated during translation when the responsible proteins discriminate premature from normal translation termination and mark the transcript for rapid decay.

There are several models to explain how cells recognize mRNAs harboring PTCs, including the 'Downstream marker model' and the 'Faux 3' UTR model'.

In spliced mRNAs in mammals, a PTC is recognized by its position relative to the last exon-exon junction (Downstream marker model). As a general rule, mammalian transcripts that contain a non-sense codon more than 50 nucleotides upstream of the last exon-exon junction will be subjected to NMD. In human NMD, human Upf (hUpf) proteins cooperate with an exon-junction complex (EJC) to identify mRNAs with premature stop codons. The EJC consists of multiple proteins that are deposited 20–24 nucleotides upstream of exon–exon junctions after splicing. Components of the EJC have been proposed to recruit hUpf proteins to trigger NMD.

It is estimated that 30% of inherited genetic disorders in humans result from premature termination codon (PTC) mutations. NMD is thought to underlie the recessive nature of many of these diseases. For example, recessive forms of ß-thalassemia result from PTC mutations occurring in the first or second exon of the ß-globin gene. The NMD pathway rapidly destroys the resultant mRNAs, such that heterozygous individuals are healthy. However, when a PTC is present in the third and final exon, it escapes detection by the decay machinery, leading to translation of a truncated protein that heterodimerizes with α-globin to produce a dominant-negative protein. Individuals with this type of mutation suffer from a form of anemia characterized by the presence of insoluble inclusion bodies in erythroid cells.

The mechanisms by which PTC-containing mRNAs are recognized in *S. cerevisiae* and mammals differ as most yeast mRNAs lack introns. In *S. cerevisiae*, a *cis*-acting element, the downstream sequence element (DSE) appears to be required for recognition of a stop codon as premature. The yeast protein Hrp1p has been shown to bind to a DSE as well as Upf proteins. This mechanism also follows the 'Downstream marker model'.

Further, both in yeast and mammals the distance between the poly(A) tail and a termination codon might be measured by a mechanism positing a positive influence of the poly(A) binding protein Pab1 (PABC in mammals). An unusually long distance between the poly(A) tail and the 'PTC' might define a termination codon as premature.

Regulated mRNA turnover

Specific internal sequence elements can influence mRNA stability. These destabilisation elements can be anywhere in the transcript, but mostly reside in the 3' untranslated region. The very short half-lifes observed for transiently expressed genes, including early response genes such as lymphokines, cytokines and transcription factors, correlate well with the presence of adenylate, uridylate-rich **(AU-rich) instability elements (AREs)** in their 3' UTRs. AREs enhance both deadenylation rates and subsequent mRNA degradation. A number of ARE-binding proteins have been identified, the best characterised of which are the human AUF1/ hnRNP D and HuR/HuA proteins. HuR and hnRNP D may have antagonistic effects on the stability of ARE containing mRNAs. While depletion of hnRNP D leads to a strong stabilisation of diverse ARE-containing mRNAs, overexpression of HuR stabilizes these RNAs.

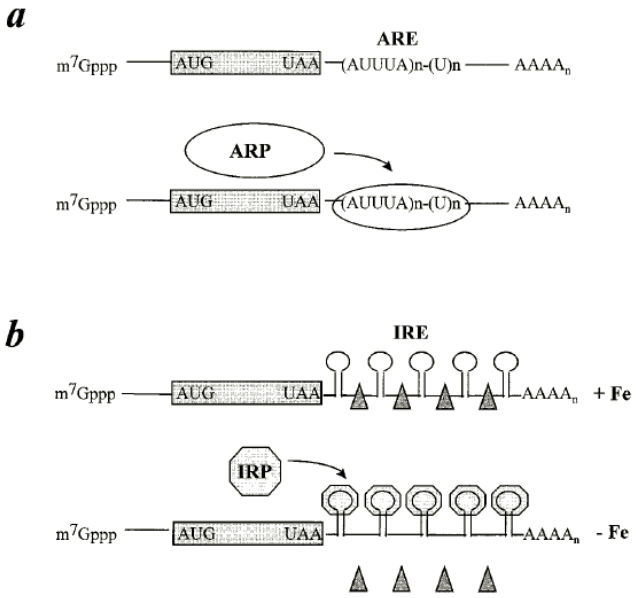


Figure 2 RNA-binding proteins that regulate mRNA stability. (*a*) A large number of unstable mammalian mRNAs contain AREs in their 3′ untranslated regions (3′ UTR), and may be bound by regulatory proteins (ARE-binding proteins, ARP), which promote poly(A) tail shortening. (*b*) The transferrin receptor (*TfR*) mRNA contains five IREs and a functional cleavage site within its 3′ UTR (▲). In response to low intracellular concentrations of iron, the IRP has a high affinity for the IREs and prevents access of the nuclease to the cleavage sites. Thus binding by the IRP stabilises the transcript by preventing endonucleolytic cleavage.

3. Regulation of translational initiation

Translational initiation is an important step in both global and mRNA-specific gene regulation. Global regulation of protein synthesis is generally achieved by the modification of eukaryotic initiation factors (eIFs), several of which are phosphoproteins. Translational control of individual mRNAs often depends upon the structural features of the transcript itself, and may include structures in the 5' UTR that inhibit translation directly, by acting as receptors for a regulatory RNA-binding protein.

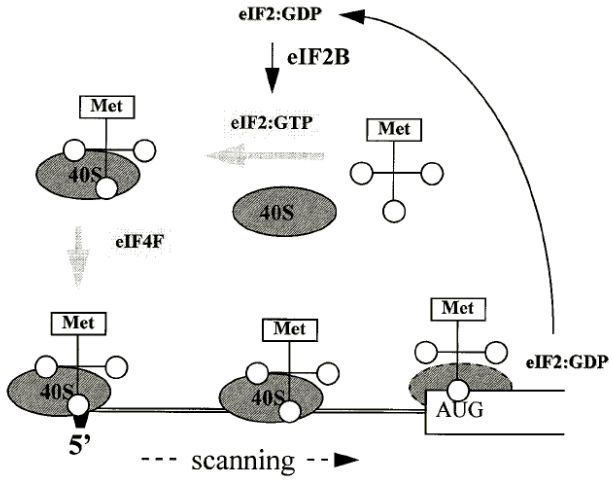


Figure 3 The initiation of translation on eukaryotic mRNAs, identifying the steps at which either global or mRNA-specific regulation is exerted. See text for a full explanation.

In eukaryotic cells, eIF-2 forms a complex with GTP and the initiator Met-tRNA$_i$ (ternary complex). The Met-tRNA$_i$/eIF-2/GTP complex then binds to a 40S-eIF3 complex to form a 43S complex. The 5' cap of the mRNA attracts the eIF4F complex, which is composed of the cap-binding protein eIF4E, the RNA-dependent ATPase eIF4a and the modular factor eIF4G. Then, the 43S complex binds to physical 5' end of the mRNA and the pre-initiation complex is formed. Under consumption of ATP the small ribosomal subsequently migrates in a 3' direction until it encounters the first AUG codon.

5' and 3' end interactions

The poly(A) tail is important for initiation of protein synthesis. This fact was known for a long time. Test mRNAs that are capped but which lack poly(A) are translated in vitro, but initiation occurs with poor efficiency. Similarly, test mRNAs with poly(A) but no cap are initiated poorly (test mRNAs with neither modification are essentially dead). One might understand why a 5' element, near the initiation codon might be important, but how can an element at the end of the mRNA be important for events taking place at the beginning?
The link was eventually revealed by studies of a second poly(A) binding protein, PABI. PABI binds the poly(A) in the cytoplasm and at the same time it binds to a subunit of eIF4 called eIF4G. eIF4G in turn is bound to eIF4E. eIF4E directly binds the 7mG cap. So the net effect is that the mRNA is circularized during the process of translation. Indeed, test mRNAs having both a cap and poly(A) tail are initiated with efficiencies greater than the simple sum of efficiencies produced by each modification individually.

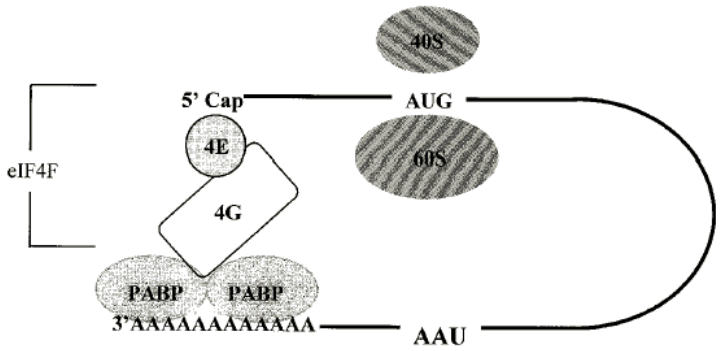


Figure 6 A model for the proposed interaction of the 5′ and 3′ ends of an mRNA by binding of PABP with eIF4G. The initiation factors, eIF4E and eIF4G (denoted 4E and 4G respectively), may form a bridge between the 5′ cap structure and one or more PABP molecules bound at the 3′ end of the mRNA.

This also supports mRNA stability.

Regulation of eIF2 activity

eIF2 is a multi-subunit complex. In mammals, phosphorylation of the alpha-subunit (eIF2α) on Ser51 is induced in response to a number of different stress conditions:

- iron (häm) deprivation
- heat-shock
- viral infection
- interferon

Four different kinases have been identified :

- haem-controlled repressor (**HCR**)
- **PKR** (protein kinase activated by ds RNA)
- **GCN2**
- **PERK**

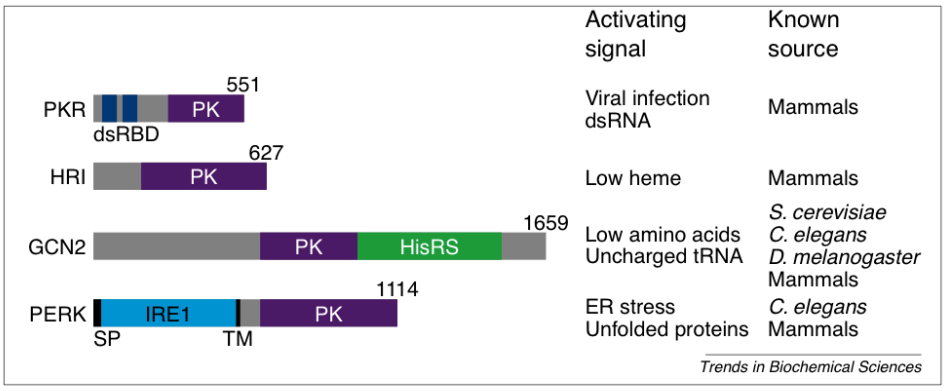


Figure 4

Structure of the four identified stress-responsive eIF2α kinases. The numbers refer to the size (in amino acids) of the primary translation products for each protein. The conserved eIF2α kinase domain (PK) is shown in purple. Also shown are the regulatory domains in the kinases: two double-stranded RNA binding domains (dsRBD) are present in the N-terminus of PKR, a histidyl-tRNA synthetase domain (HisRS) is present immediately C-terminal to the kinase domain in GCN2, and the N-terminus of PERK has a domain that shows similarity to the regulatory domain of IRE1 another kinase involved in the unfolded protein response. PERK also contains a signal peptide (SP) and transmembrane (TM) domain.

Phosphorylation of eIF2α inhibits guanine nucleotide exchange by eIF2B, which is the responsible GEF. The result is a general down-regulation of protein synthesis under various stresses.

Regulation of eIF4E activity

eIF4E is the cap-binding initiation factor that facilitates 43S complex binding to the mRNA.

3 ways of regulation:

1. limiting concentrations of eIF4E
2. phosphorylated eIF4E has a threefold greater affinity for both the cap structure and eIF4G; phosphorylation in response to hormones and growth factors, dephosphorylation in response to heat-shock and viral infection
   (exception: HSP mRNAs are efficiently translated after heat-shock as initiation of their synthesis is not strictly dependent on eIF4E)
3. two translational repressors (4E-BP1 and 2) regulate eIF4E function; their binding to eIF4E is regulated by phosphorylation: e.g. 4E-BP1 becomes phosphorylated in cells treated with hormones or growth factors and subsequently dissociates from eIF4E:

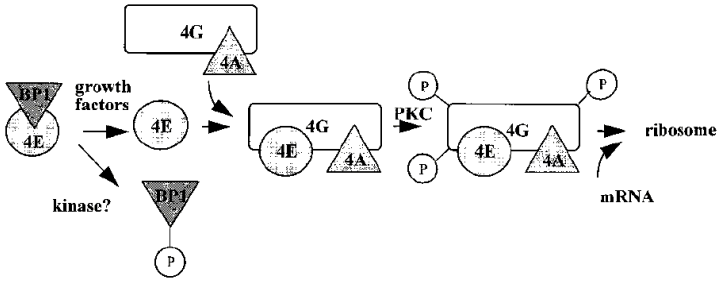


Figure 4 Regulation of eIF4E function in mammalian cells. 4E-BP1 becomes hyperphosphorylated by hormones or growth factors and subsequently dissociates from eIF4E. This, in turn, enables eIF4E to bind to eIF4G and to become phosphorylated by PKC. The phosphorylated eIF4E-eIF4G complex is now able to bind to the 5′ cap structure (Whalen *et al.* 1996).

TOR signaling: mTOR (target of rapamycin) in response to amino acids and growth factors controls the mammalian translation machinery by activation of p70S6kinase ($p70^{s6k}$) and via inhibition of 4E-BP1. mTOR also phosphorylates 4E-BP1 directly. Thus, mTOR also controls eIF4E function.

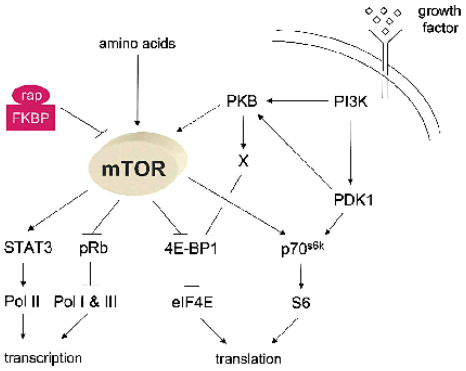


Figure 3. Model of mTOR Effectors and Signaling Pathways in Mammalian Cells

Arrows indicate activation; bars indicate repression. The dashed arrow between PKB and mTOR reflects the uncertainty as to whether PKB controls mTOR (see text). rap is rapamycin; X refers to an unidentified kinase.

Regulation by structure of the mRNA

5' UTR elements

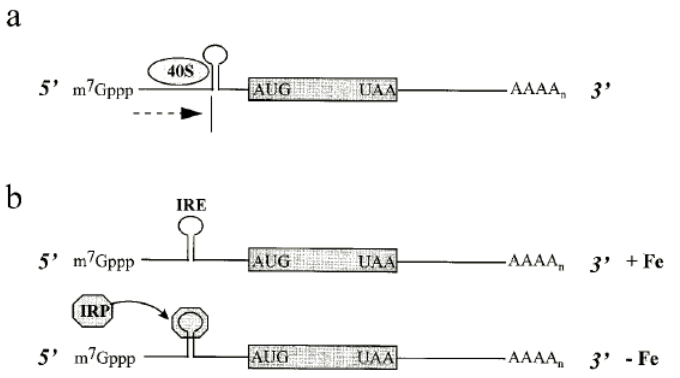


Figure 5 Regulation of translation by RNA–RNA and RNA–protein interactions. (a) Secondary structures within the 5′ UTR can act as a barrier to the scanning 43S ribosomal complex. (b) Ferritin mRNA contains an IRE in its 5′ UTR to which the IRE binding protein (IRP) binds when intracellular concentrations of iron are low, and inhibits its translation. The reduced ferritin concentration leads to a decrease in iron storage. When iron concentrations are high, translation of ferritin is enhanced, resulting in enhanced iron storage. (c) The presence of the selenocysteine insertion sequence, SECIS, within the 3′

3' UTR elements

example:
- 15-lipoxygenase (LOX) expression is translationally silenced in early erythroid precursor cells by a specific mRNA–protein complex formed between the differentiation control element in the 39 untranslated region (UTR) and hnRNPs K and E1. The UTR regulatory complex prevents the joining of the 60S ribosomal subunit at the AUG codon to form a translation competent 80S ribosome

- Translational regulation plays a prominent role in Drosophila body patterning. Normal progression through oogenesis and embryogenesis in Drosophila requires coupling between translational control and mRNA localisation to achieve proper temporal and spatial protein expression. For instance, translation of maternal *oskar* mRNA (Oskar (osk) protein directs the deployment of nanos (nos), the posterior

body-patterning morphogen in Drosophila. Oskar mRNA is silenced during transport to the posterior pole of the oocyte and until Oskar protein is required. The *Bruno* protein recognises repeated conserved 3' UTR sequences of oskar mRNA, the Bruno reponse elements (BRE), and thereby prevents premature translation.