Assess the importance of regulation of translation in bacteria and eukaryotes. Are the mechanisms used common to both groups?

Both prokaryotes and eukaryotes translate mRNA transcripts to produce proteins for use in cellular processes. Translation, however, is not a simple mass production of protein from the entire transcriptome, but a process which is controlled in both bacteria and eukaryotes by a number of mechanisms.

The number of ribosomes available to perform translation sets an upper limit on the total amount of translation possible at any time, but is rarely the limiting factor in determining the rate of translation of a specific mRNA. It is however worth noting that the level of rRNA is regulated by the cell, so presumably may become limiting under certain circumstances.

The most obvious point at which translation can be regulated is the frequency of initiation. The first step in initiation involves the binding of a [met-tRNAimet] to the small ribosomal subunit. In bacteria this is formylated, but in both cases the tRNA is specific for a Start codon, AUG.

Bacterial initiation relies on a consensus sequence 5'-AGGAGGU-3', the Shine-Dalgarno sequence. A complementary region of the 16S rRNA pairs with this sequence, forming the initial association of ribosome with mRNA. IF-1 binds to the A site of the ribosome, blocking tRNA from associating, whilst and promoting binding of IF-2 associated fmet-$tRNA_i^{fmet}$. Finally, IF-3 is required to transiently mediate the association of the 30S subunit to the Shine-Dalgarno sequence. After IF1 and IF3 dissociate, the association of the 50S ribosomal subunit is followed by GTP hydrolysis and dissociation of IF2, leaving a completed ribosome, charged with fmet-$tRNA_i^{fmet}$ in the P site ready to begin protein synthesis.

Transcription occurs in a 5' to 3' direction, so translation can initiate as soon as the Shine-Dalgarno sequence is complete.

The trp attenuator is one very well-known example of a translation regulatory mechanism in bacteria, which regulates the translation of the trp operon. The trp operon contains a tryptophan-rich region, and three complementary base-pairing regions which for the attenuator. If the ribosome rapidly traverses the first domain when translating this leader section, indicating a plentiful supply of tryptophan, the complementary regions furthest downstream form a hairpin structure which results in termination of transcription, and prevention of further translation.

This mechanism is clearly impossible in eukaryotes, both because their genes are not composed into operons and because transcription and translation are seperated by the nuclear envelope. However, eukaryotes display other mechanisms of translational regulation, which will examine in more depth later.

In eukuaryotes the transcript is processed within the nucleus, splicing out introns and adding a methyl guanosine cap and polyadenylated tail before export to the cytoplasm. There eIF4E binds to the 5' cap, which lays within a hydrophobic slot on the protein's surface, and mediates association with eIF4G, which is responsible for recruitment of the 43S complex through eIF3.

The affinity of eIF4E binding to the mRNA cap has been suggested to be modulated by its phospohorylation state. In the proposed mechanism, Ser209 of eIF4E is reversibly phospohorylated by Mnk-1, forming a salt bridge with Lys159 and 'clamping' the hydrophobic grove onto its substrate. In favour of this mechanism, phosphorylation has been observed in vitro and in vivo, and Mnk-1 is regulated by the MAP kinase pathway, an integrator of growth signals.

Unfortunately, this clamping mechanism was proposed based on earlier structural data, which has since been thrown into question, placing the two residues too far apart for such an interation. More compellingly, when cap-binding activity was assayed in vitro, the phosphorylated form was shown to have a lesser affinity than the unphosphorylated form. This was suprising both because upregulation of translation would be the expected result of the terminal stage of growth signal pathway, and because of work done before isolation of Mnk kinase. Assays of each isoform as directly purified had suggested that the phosphorylated form bound with greater affinity, but with the availability of Mnk kinase, the degree of phosphorylation could be better experimentally controlled, and was demonstrated to have the opposite effect.

eIF4E is the least abundant translation factor, and has been shown to be rate-limiting for translation. Additionally, a family of proteins homologous to eIF4E compete for its binding site, and have also been shown to be regulated by phospohorylation. This points clearly to a central role for eIF4E in translational regulation.

If eIF4E is the limiting factor in ribosome assembly, eIF4G is the centrepiece. It acts as an adaptor between eIF4E on the mRNA cap, and the poly-A binding protein associated with the 3' tail. This interaction is required for recruitment of the 43S subunit, and has been suggested to act as a verification that the mRNA is complete before initiation of translation.

Once the 43S subunit, associated with eIFs 1, 2, 3 and 5 and met-tRNA$_i^{met}$ has assembled onto the 5' UTR it begins to scan along the mRNA, until a Start (AUG) codon is reached. The recognition of this codon, however, is not specific solely to the codon, but relies on a consensus sequence 5'-GCCRCCATGG-3' known as a Kozak sequence. These sequences have been observed to occur at various locations within the 5' region of an mRNA. This is not essential for initiation, but the more closely the area around a start codon conforms to this sequence, the greater its probability of being recognised for initiation. Obviously, if some start codons are passed over for initiation the resulting polypeptide will have a different N-terminus than one transcribed from an earlier AUG. This phenomenon is known as leaky scanning, and the variable N-terminus of proteins is commonly used as a tag, to direct proteins to a particular compartment of the cell.

Cap-independent translation initiation has also been observed in viral mRNA from eukaryotes, where a 5'-UTR forms a tertiary structure capable of binding eIF4G/4A in such a way that 43S subunits are recruited directly to the Start codon, followed by association of the 60S subunit, and continuation of translation. It may also be used by eukaryotes in situation of cellular stress, and during apopotosis.

Overall, it seems that there is very little overlap between the regulation of translation in prokaryotes and eukaryotes. The greater range of strategies used to regulate translation in eukaryotes suggest that it is a more important mechanism than in prokaryotes, in which the much simpler system of initiation leaves very few points of control.