**Post-synthetic processing**

mRNA often undergoes processing after synthesis

- during RNA splicing introns get excised out leaving exons behind
- same gene can be spliced in many different ways to produce different translated products

Proteins can also self-splice after synthesis

- intein gets spliced out, leaving exteins covalently linked together
- protein splicing can be used for protein purification
- chemical ligation can be used to synthesize protein in vitro bypassing the normal molecular biology route

Nobel prize in physiology, 1993
A) Protein Splicing:

DNA

N-extein    intein    C-extein

transcription

mRNA

N-extein    intein    C-extein

translation

protein precursor

N-extein    intein    C-extein

protein splicing

N-extein    C-extein

mature spliced protein

excised intein

B) RNA Splicing:

DNA

exon-1    intron    exon-2

transcription

RNA precursor

exon-1    intron    exon-2

RNA splicing

mRNA

exon-1    exon-2    intron

translation

exon-1    exon-2

mature protein

Discovery of protein splicing

TFP1 gene in S. cerevisiae encodes the 69 kDa catalytic subunit of the vacuolar proton-translocating ATPase and another 50 kDa protein.

Kane et al, Science 250, 651 (1990)
Protein splicing

Occurs in both prokaryotes, eukaryotes and archaea
All the information needed for splicing resides within the spacer (i.e. intein) region
  - exteins can be as short as 13 residues
  - splicing element can function when embedded in foreign proteins

Requires a combination of key residues
  - Cys/Ser/Thr at the extein boundaries
  - Asn is required at the C-terminal end of the intein

Splicing element from a hyperthermophilic organism inserted between foreign proteins yields a final product in a temperature dependent manner
  - Xu et al, Cell 75, 1371 (1993)

Cooper and Stevens, TIBS 20, 351 (1995)
Splicing mechanism

precursor

N-extein

intein

C-extein

Asn cyclization

MI> + P

linear ester intermediate

Step 1: N-X acyl shift

Step 2: transesterification

branched intermediate

H₂O

ester hydrolysis

M + IP

Step 3: Asn cyclization

Step 4: X-N acyl shift
Protein purification

- The intein domain is functionally independent and may be introduced in many different contexts
- Intein Mediated Purification with an Affinity Chitin-binding Tag (IMPACT)
ELP Intein Purification

- Elastin like peptide (VPGXG) undergoes a sharp reversible transition between soluble and insoluble phases
- ELP fusion constructs have been used to purify protein
  - Meyer and Chilkoti, Nat Biotech 17, 1112 (1999)
- ELP and intein can work together to rapidly purify recombinant protein
  - Banki and Wood, Nat Meth 2, 659 (2005)
Peptide ligation

- Solid phase synthesis vastly simplified the chemical synthesis of peptides
- Chemical synthesis works only up to a certain length due to coupling inefficiency
- Splicing two separately expressed protein fragments in vitro to achieve a longer peptide chain
- Peptide ligation and solid phase synthesis together make a novel engineering tool
- Non-natural amino acids can be easily incorporated in a protein
- Chemically similar to protein splicing

Chemistry Nobel 1984
intein mediated protein ligation

Dawson et al, Science 266, 776 (1994)
HIV d-protease

• How do enzymes made of D-amino acids differ from their natural counterparts?
• Enzymes operate exclusively on one enantiomer of a chiral substrate
• Chemical synthesis enables construction of the mirror image of HIV protease using D-amino acids