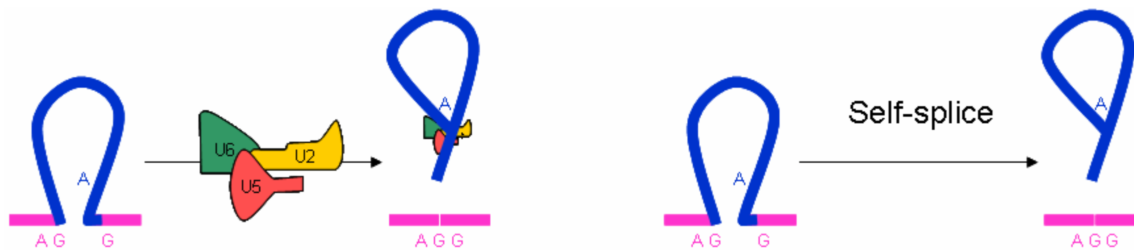
Post-synthetic processing

mRNA often undergoes processing after synthesis

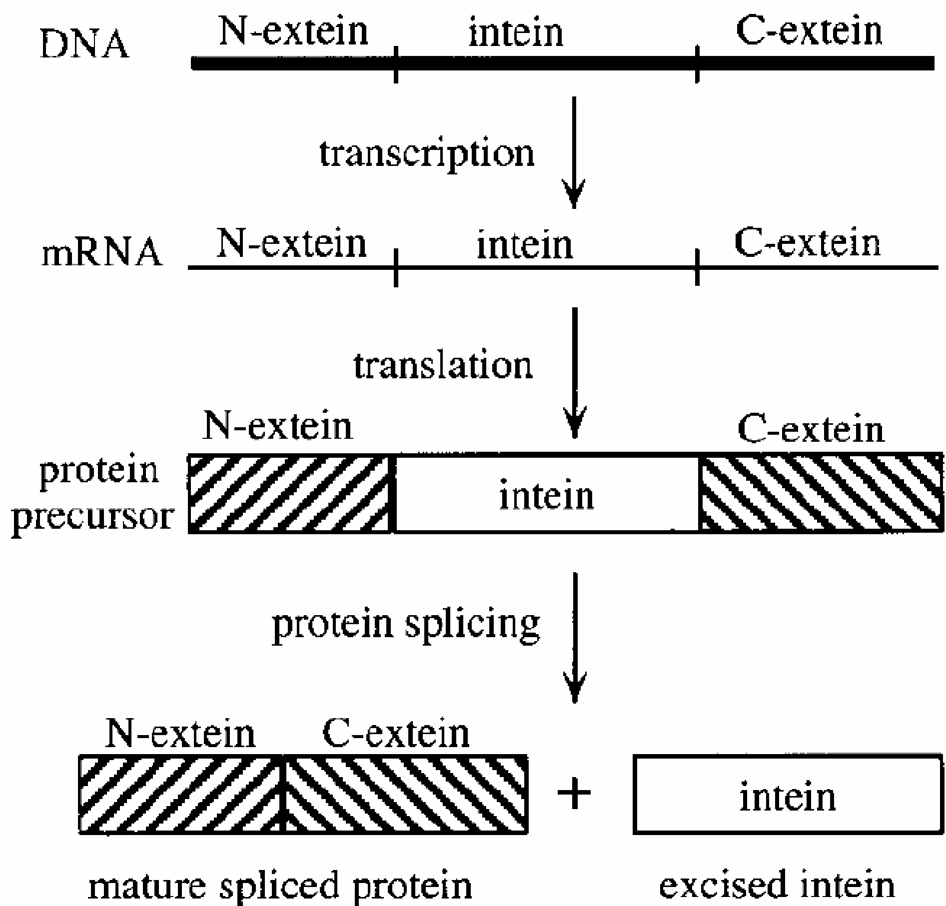
- during RNA splicing introns get excised out leaving exons behind
- same gene can be spliced in many different ways to produce different translated products

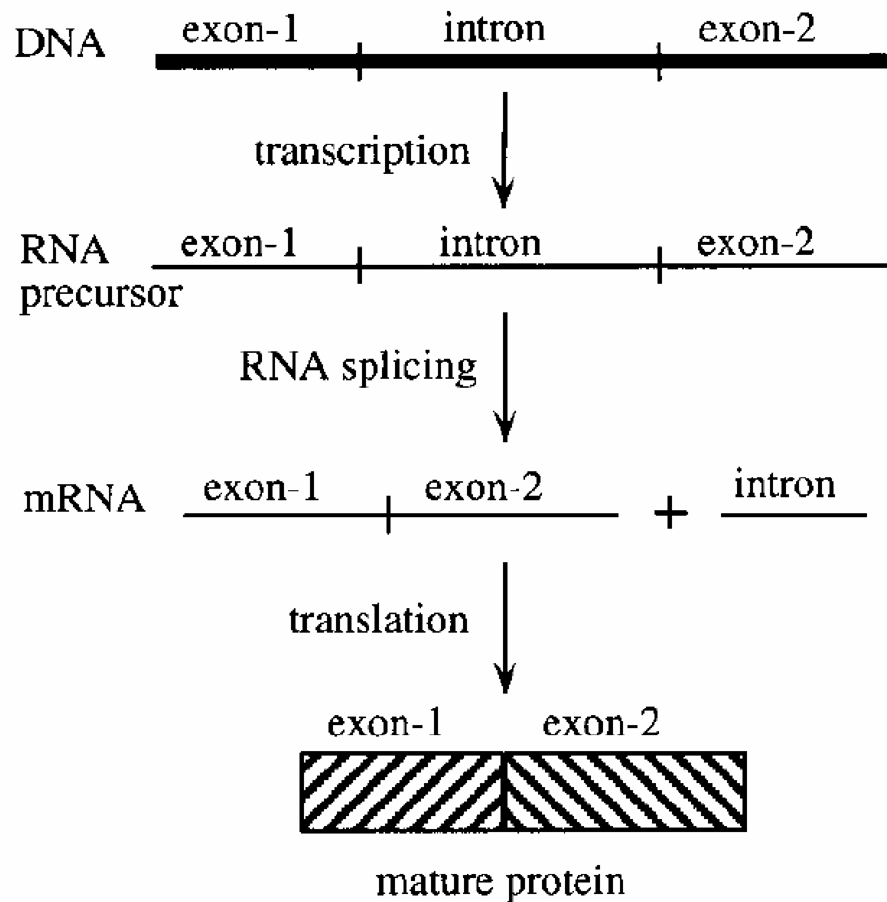


Nobel prize in physiology, 1993

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

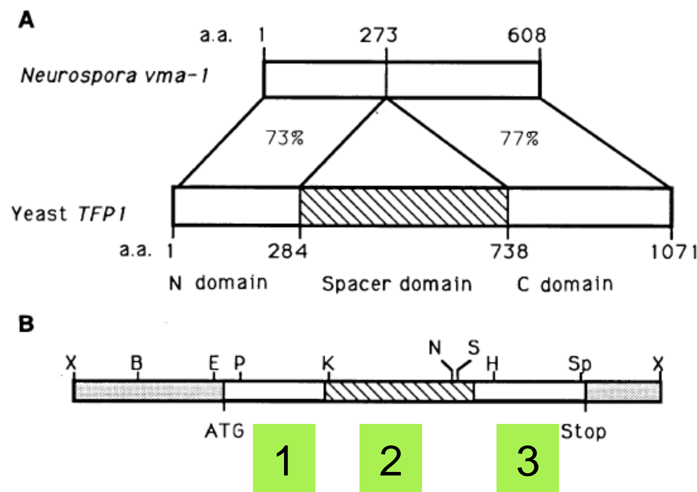


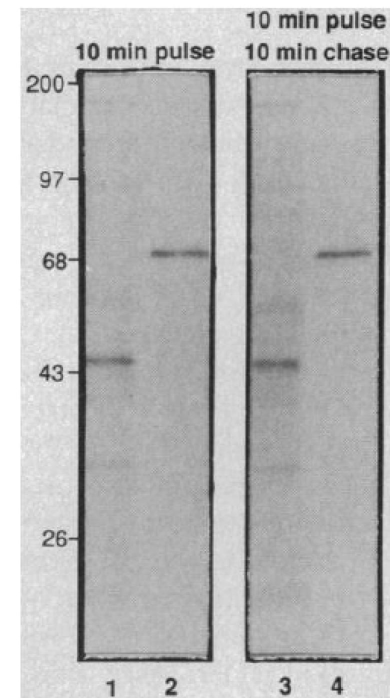


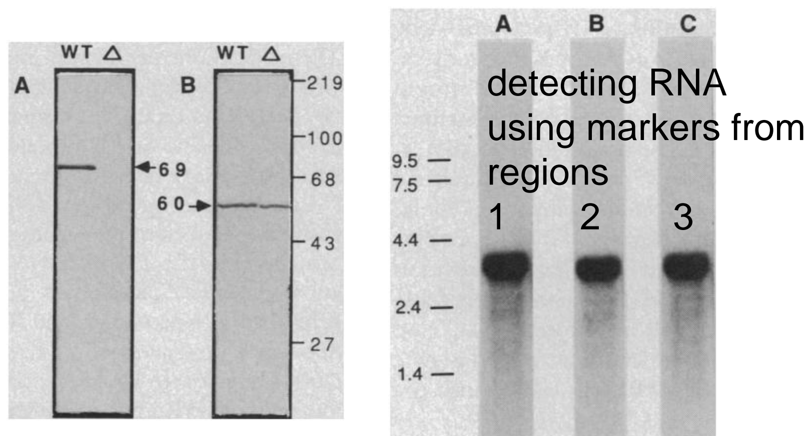
Perler et al, Angew Chem 39, 450 (2000)

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







69 kDa ans 50 kDa fragments are produced at the same rate

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

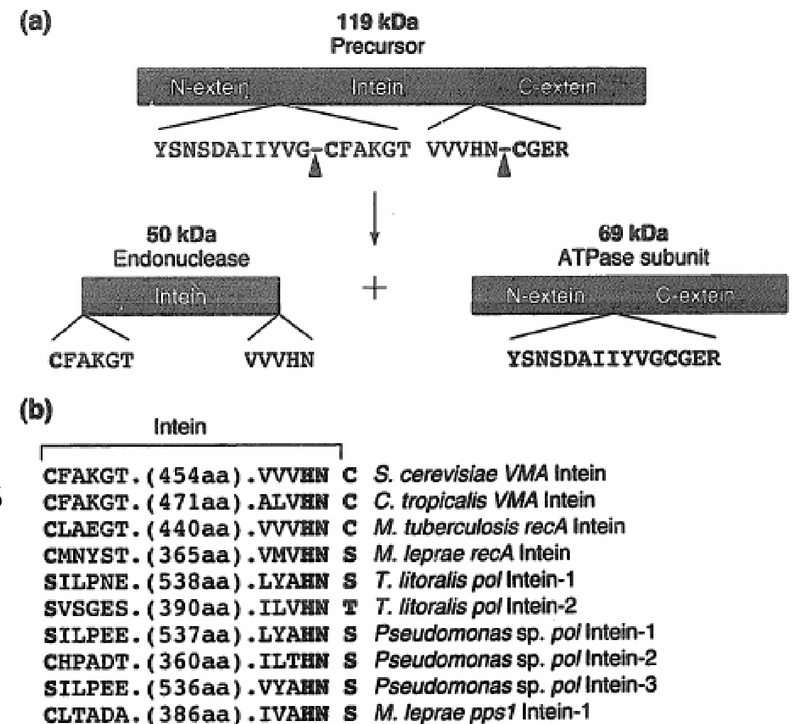
- exteins can be as short as 13 residues
- splicing element can function when embedded in foreign proteins

Requires a combination of key residues

- Cys/Ser/Thr at the extein boundaries
- Asn is required at the C-terminal end of the intein

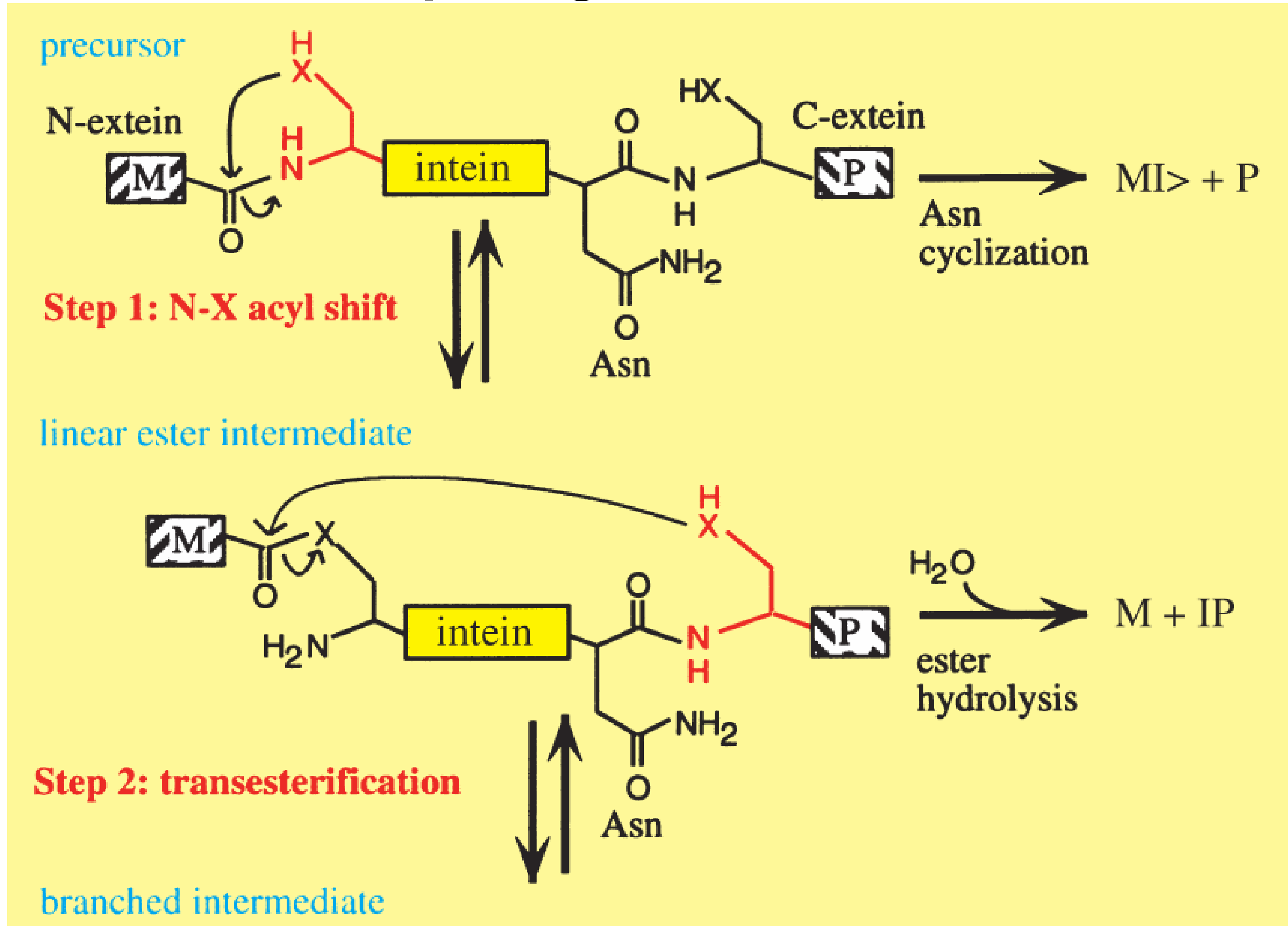
Splicing element from a hyperthermophilic organism inserted between foreign proteins yields a final product in a temperature dependent manner

- Xu et al, Cell 75, 1371 (1993)

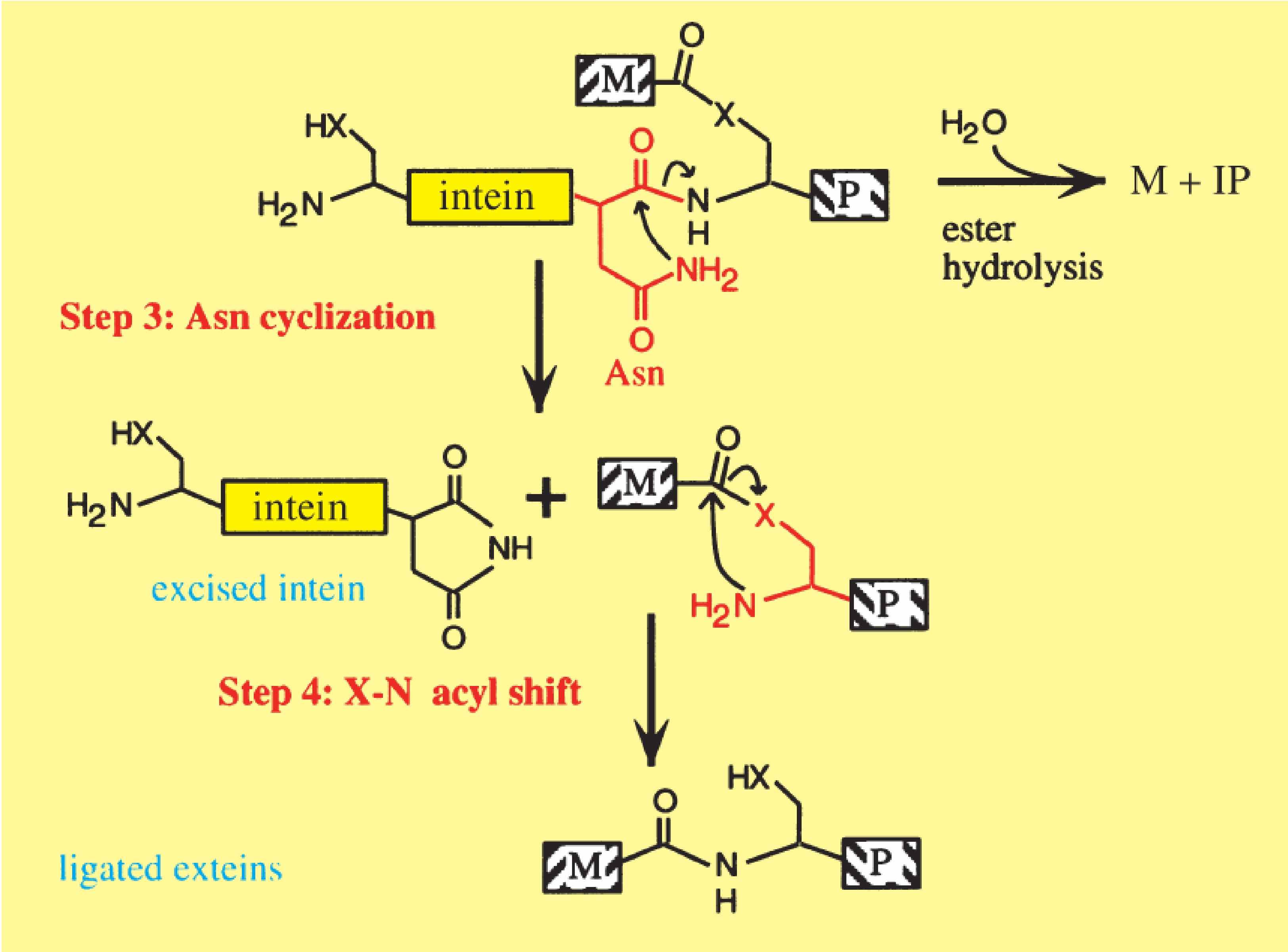


Cooper and Stevens, TIBS 20, 351 (1995)

Splicing mechanism

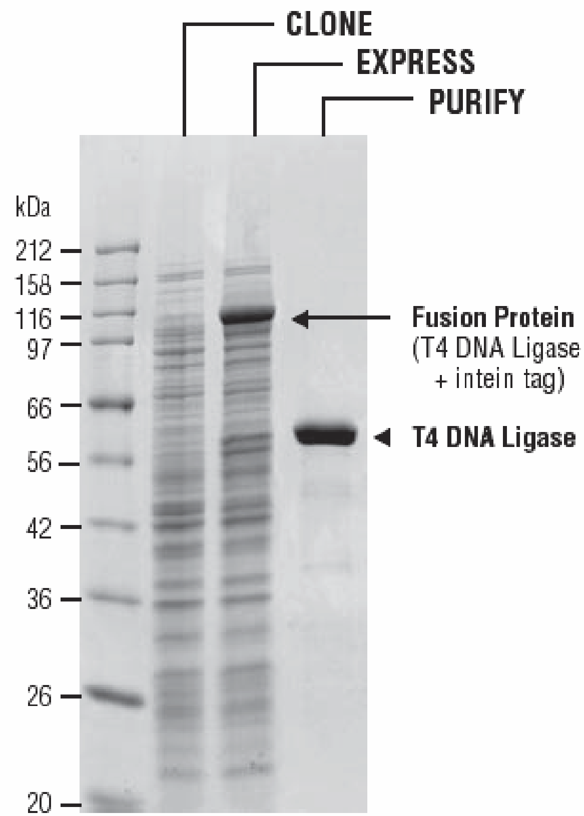


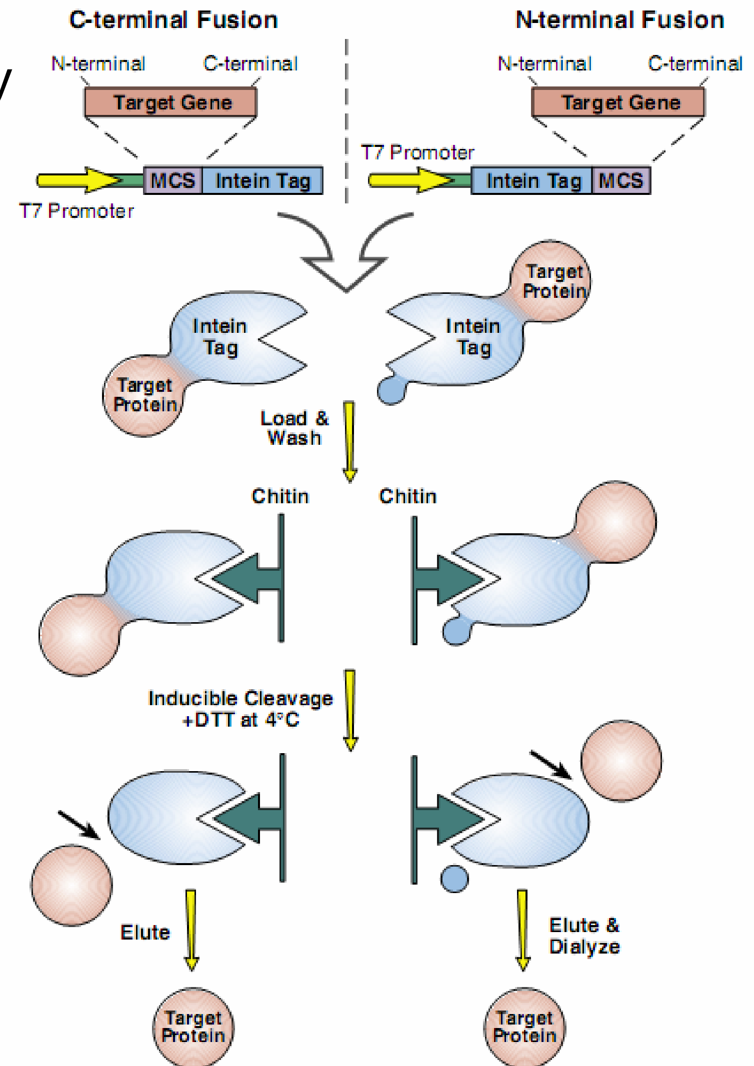
Perler et al, Angew Chem 39, 450 (2000)


M
O
X
HX
O
H2O
H2N
intein
N
H
P
M + IP
NH2
ester
hydrolysis
Step 3: Asn cyclization
O
Asn
HX
O
O
H2N
intein
NH
+
M
X
excised intein
H2N
P
O
Step 4: X-N acyl shift
HX
O
M
N
H
P
ligated exteins

Protein purification

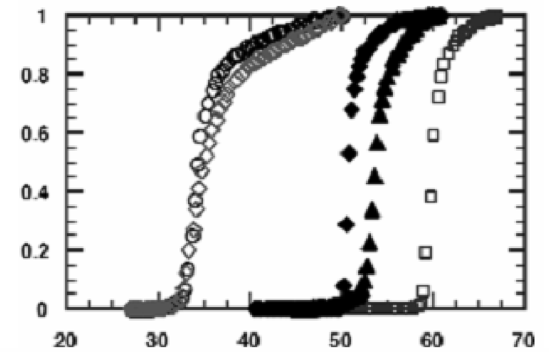
- The intein domain is functionally independent and may be introduced in many different contexts
- Intein Mediated Purification with an Affinity Chitin-binding Tag (IMPACT)



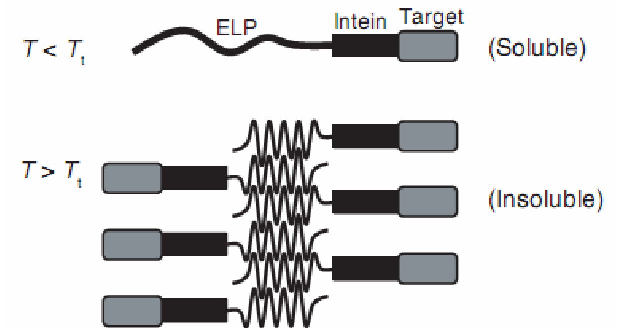


New England Biolabs

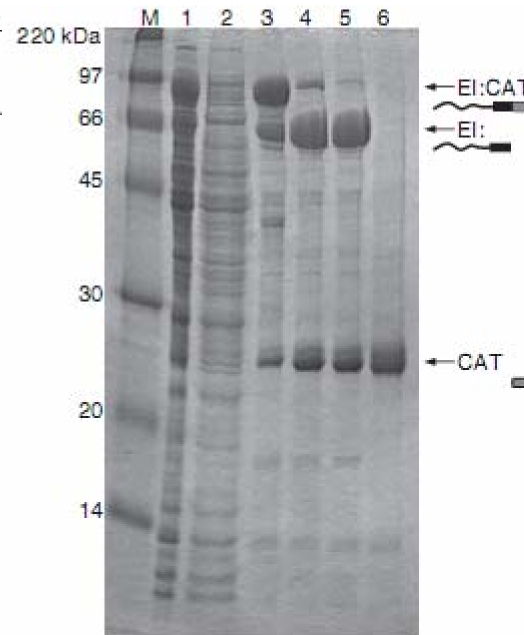
ELP Intein Purification

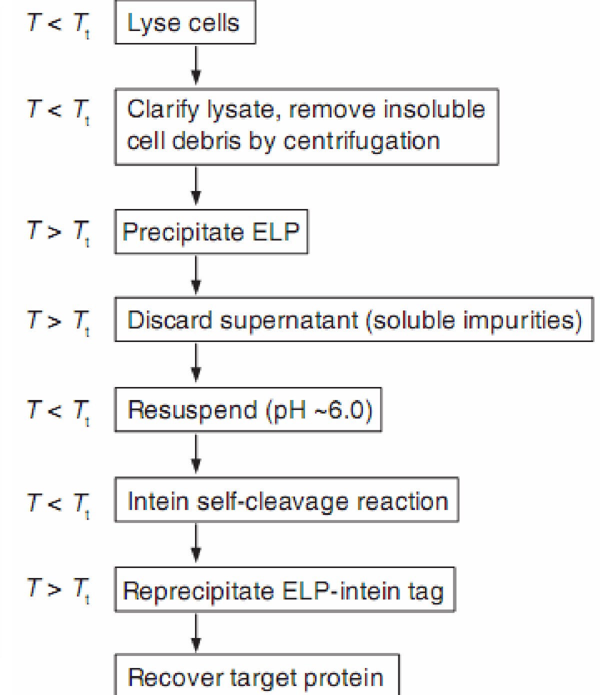


- Elastin like peptide (VPGXG) undergoes a sharp reversible transition between soluble and insoluble phases
- ELP fusion constructs have been used to purify protein
  - Meyer and Chilkoti, Nat Biotech 17, 1112 (1999)
- ELP and intein can work together to rapidly purify recombinant protein



Product protein (molecular weight)	Quantity of purified protein[a] (µg/ml)
α-hemoglobin stabilizing protein (AHSP) (12 kDa)	104.1 ± 9.1
β-lactamase (29 kDa)	70.3 ± 5.1
β-galactosidase (β-gal) (116 kDa)	122.3 ± 10.9
Catalase (80 kDa)	79.8 ± 7.8
Glutathione S-transferase (GST) (26 kDa)	118.0 ± 17.8
Green fluorescent protein (GFP) (27 kDa)	110.2 ± 6.1

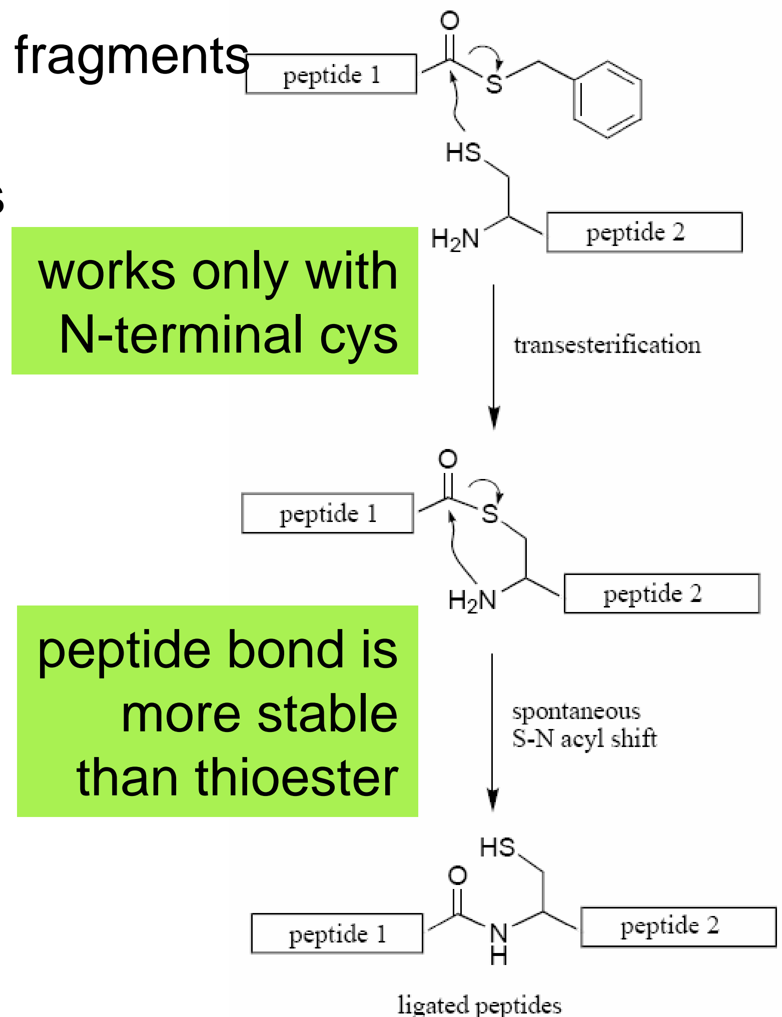




Banki and Wood, Nat Meth 2, 659 (2005)

Peptide ligation

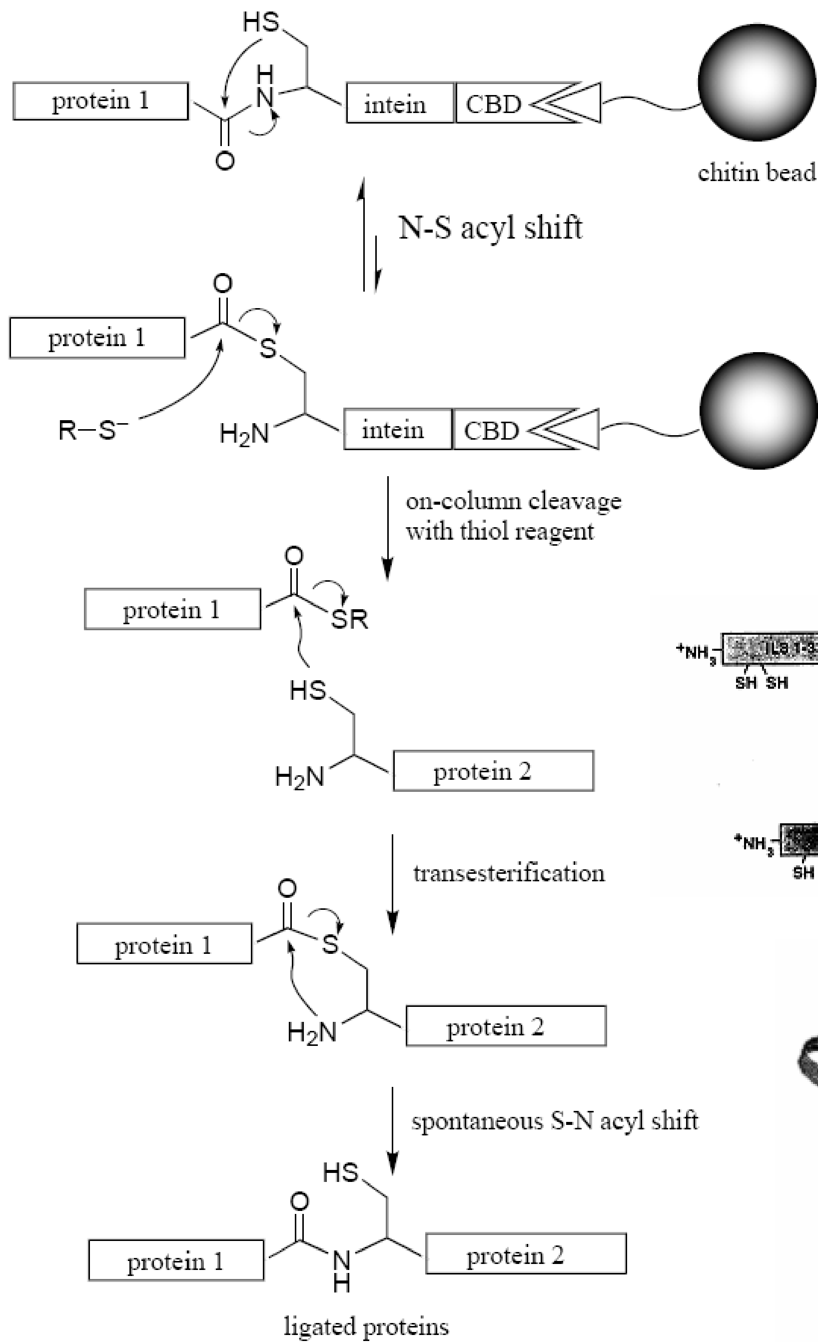
- Solid phase synthesis vastly simplified the chemical synthesis of peptides
- Chemical synthesis works only up to a certain length due to coupling inefficiency
- Splicing two separately expressed protein fragments in vitro to achieve a longer peptide chain
- Peptide ligation and solid phase synthesis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

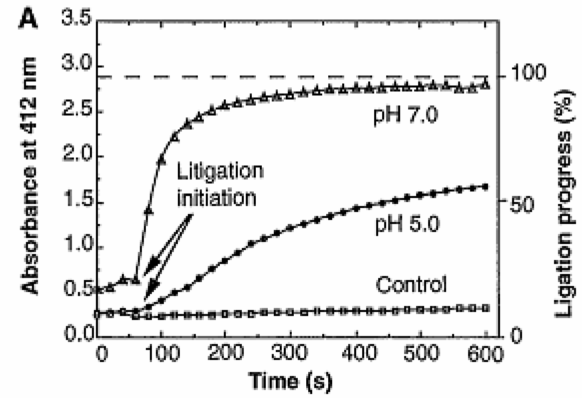


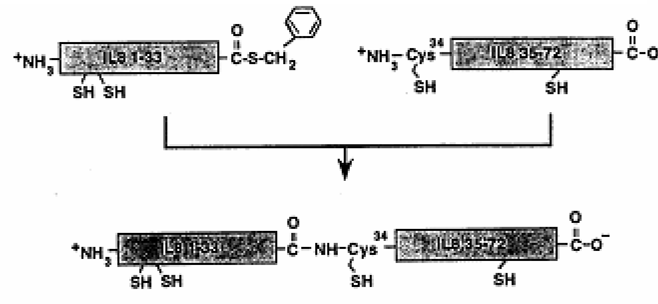
works only with N-terminal cys

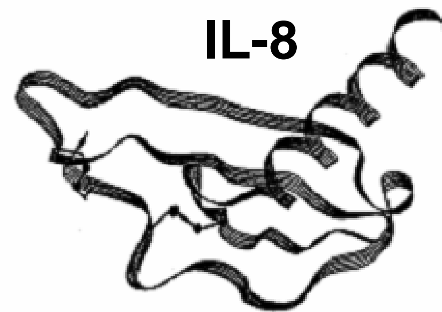
peptide bond is more stable than thioester

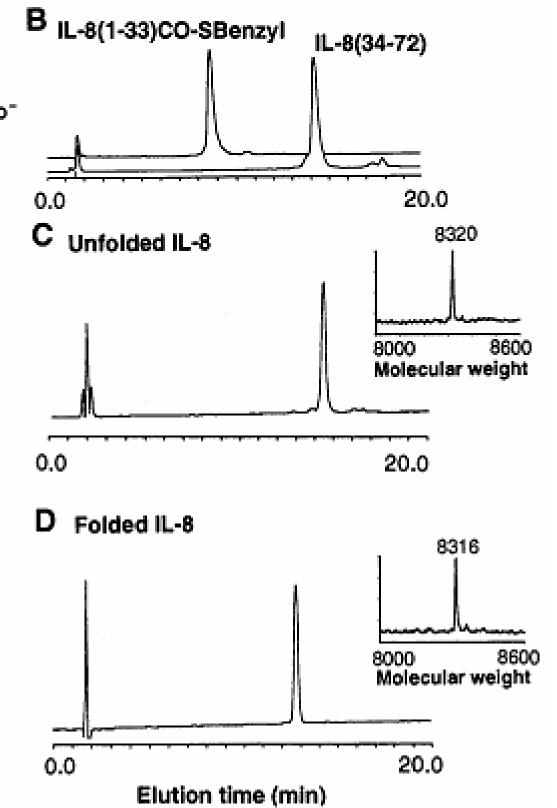
Chemistry Nobel 1984









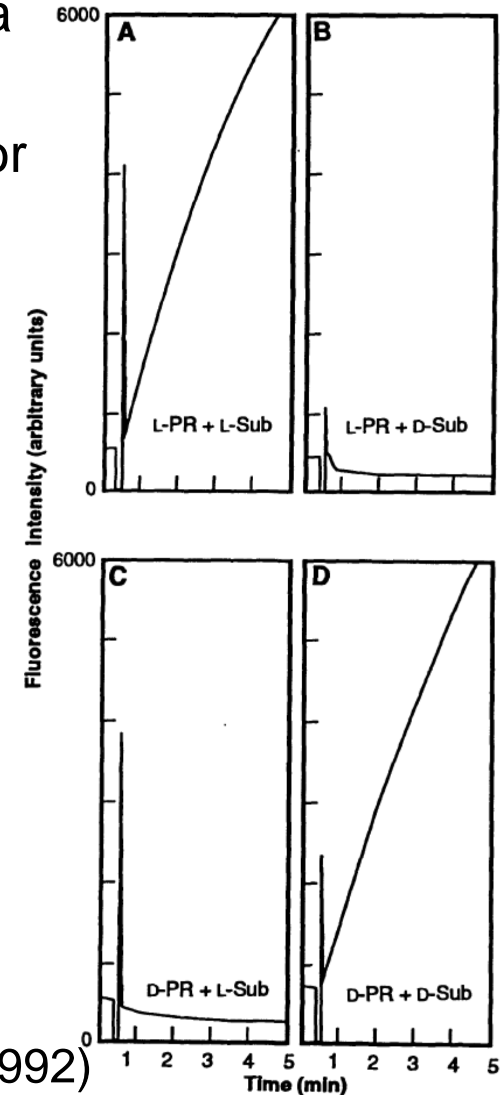


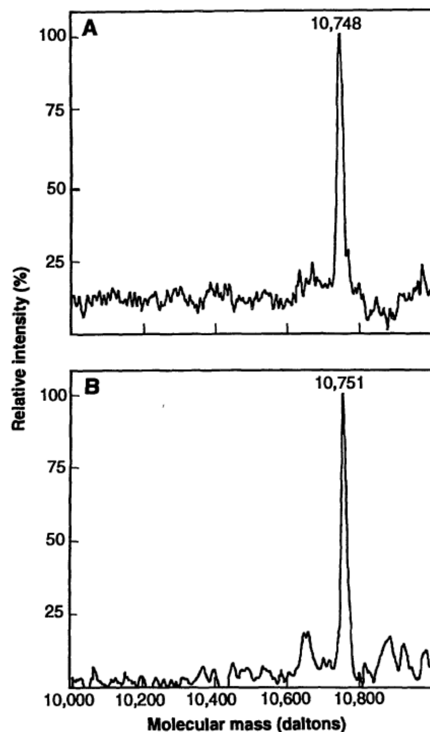
intein mediated protein ligation

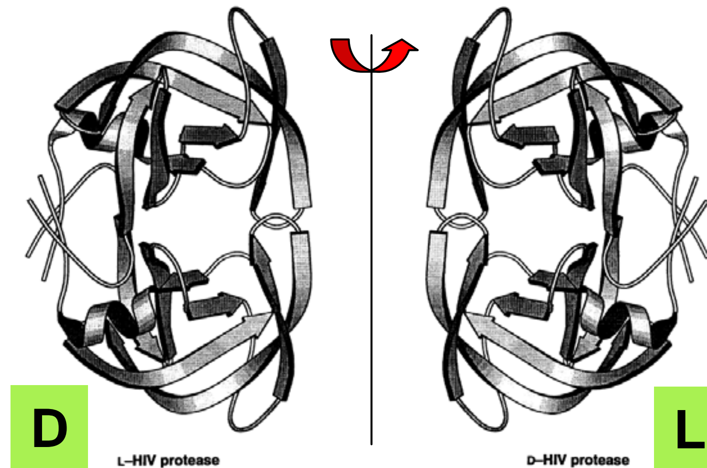
Dawson et al, Science 266, 776 (1994)

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







Enzyme	L-MVT101	D-MVT101
L-HIV PR	+	–
D-HIV PR	–	+

deL Milton et al, Science 256, 1445 (1992)