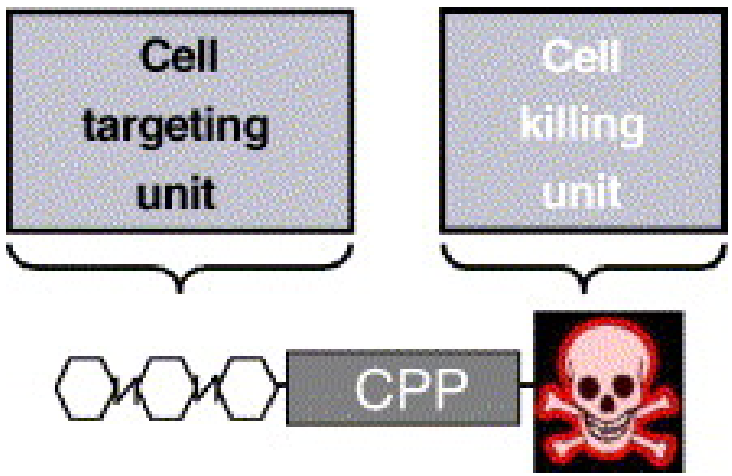
Trends of protein therapy technology



Byeong Hwa Jeon
Chungnam National University

“Protein Transduction”

- During the last decade, several proteins and peptides have been found to traverse through the cellular membranes in a process called “Protein Transduction”, delivering their cargo molecules into the cytoplasm and/or nucleus.

- Trojan horse approach

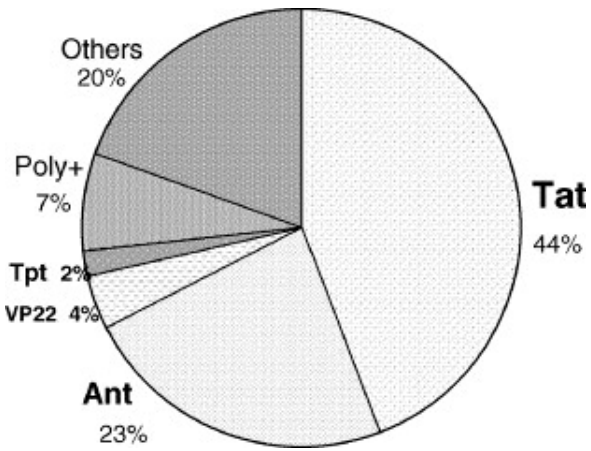
- More precisely, their ability to translocate across the plasma membranes is confined to short sequences of less than 20 amino acids, which are highly rich in basic residues.

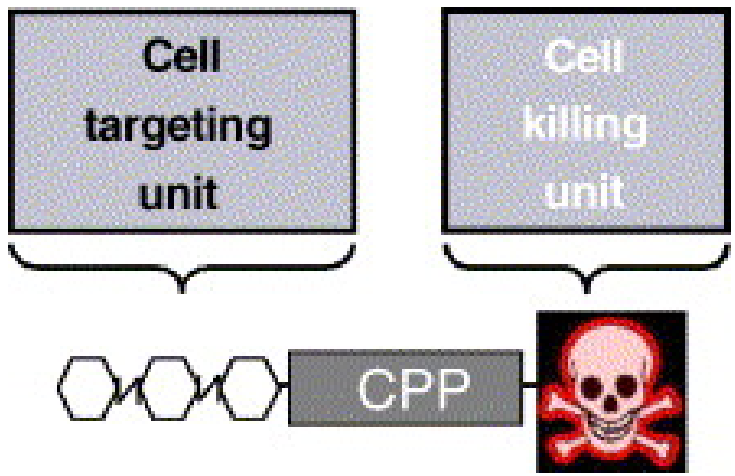
- Such sequences are called
  - “Protein Transduction Domains (PTDs)”
  - or “Cell-Penetrating Peptides (CPPs)”.

Cell-penetrating peptides (CPPs)

- Trans-activating transcriptional activator (TAT)
- Homeodomain of Antennapedia (Antp)
- Herpes simplex virus type 1 (HSV-1) protein VP22
- Transportan
- Model amphipathic peptide (MAP)
- Signal sequence-based peptides
- Other transducing peptides

Protein	PTD amino acid sequence[b]
Cationic	
HIV-1 TAT (47–57)	YGRKKRRQRRR
Drosophila Antennapedia (43–58)	RQIKIWFQNRRMKWKK
Poly-arginine (R7) (synthetic)	RRRRRRR
PTD-5 (synthetic)	RRQRRTSKLMKR
Amphipathic	
Transportan (chimeric, galanin fragment plus mastoparan)	GWTLNSAGYLLGKINLKALAALAKKIL
KALA (synthetic)	WEAKLAKALAKALAKHLAKALAKALKACEA





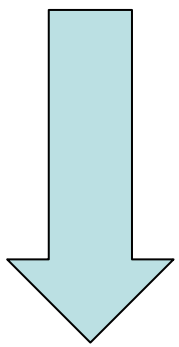
Trans-activating transcriptional activator (TAT)

- TAT is a transcription activating factor with 86 amino acids
  - a cluster of basic amino acids 49–57, i.e. minimal protein transduction domain (PTD),
  - the protein transduction sequence for TAT includes residues 49–57 (9-mer; Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg).

Mechanisms of translocation

- The internalization of TAT
  - non-saturable, dose-dependent kinetics.
  - the uptake of these peptides was believed to occur efficiently both at 37 °C and at 4 °C, excluding the possibility of endocytosis.

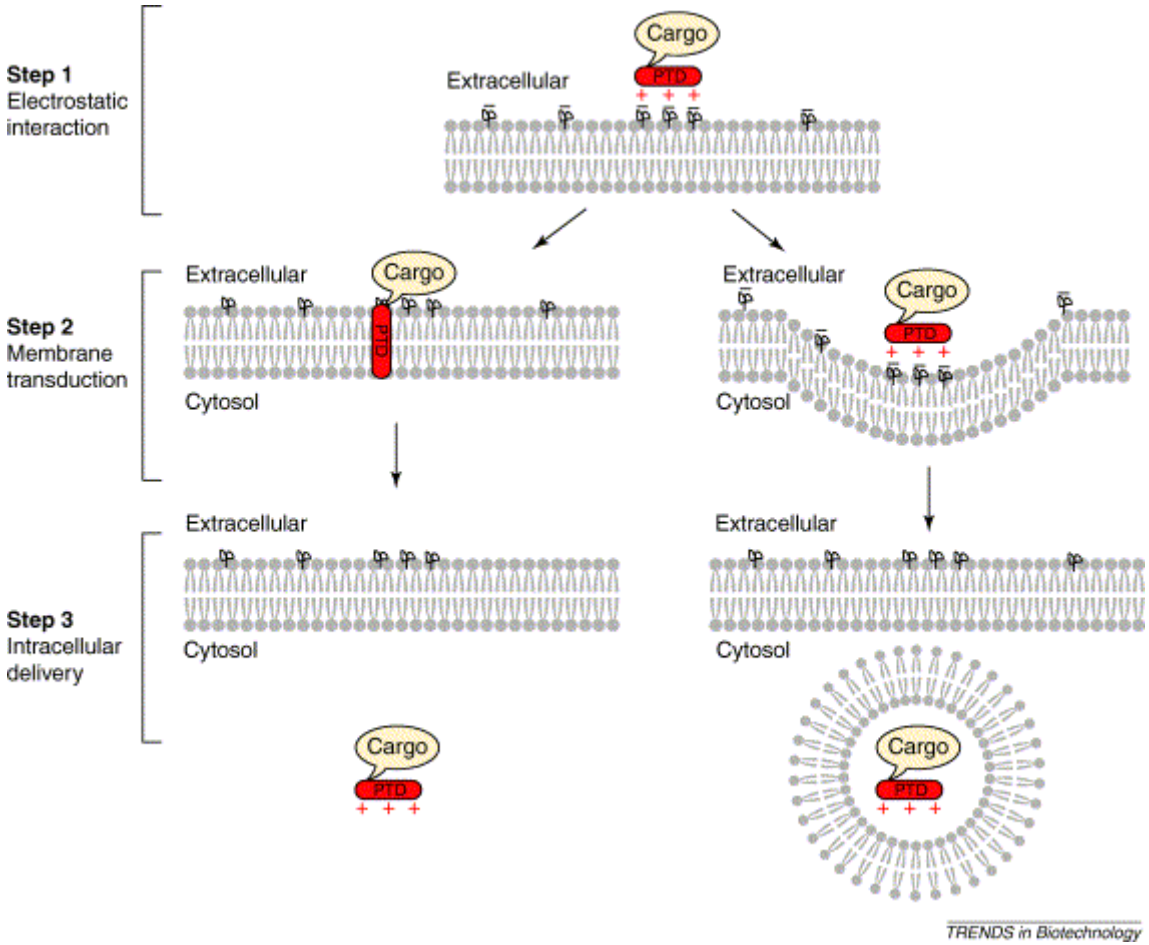
- While studying the intracellular trafficking of TAT-fusion proteins, it was demonstrated that TAT-fusion protein entered the cell via endocytic pathway, circumvented lysosomal degradation, and was then sequestered in the periphery of the nucleus (*Biochem. Biophys. Res. Commun.* **319** (2004), pp. 12–20)



- Collectively, the recent data assume more than one mechanism for CPP-mediated intracellular delivery of various molecules and particles

  – TAT-mediated intracellular delivery of large molecules and nanoparticles proceed via the energy-dependent macropinocytosis with subsequent enhanced escape from endosome into the cell cytoplasm

  – While individual CPPs or CPP-conjugated small molecules penetrate cells via electrostatic interactions and hydrogen bonding and do not seem to depend on energy.


Step 1
Electrostatic interaction
Step 2
Membrane transduction
Step 3
Intracellular delivery
Extracellular
Cargo
PTD
Cytosol
Extracellular
Cargo
PTD
Cytosol
Extracellular
Cargo
PTD
Cytosol
Extracellular
Cytosol
Cargo
PTD
Cargo
PTD
TRENDS in Biotechnology

Delivery of proteins

- TAT-β-galactosidase chimeras when tested in vivo resulted in delivery to several tissues, with high levels in heart, liver, and spleen, low-to-moderate levels in lung and skeletal muscle, and little or no activity in kidney and brain.
- Full-length TAT fusion proteins ranging in size from 15 to 115 kDa demonstrated transduction in a variety of cells
- Protein transduction occurred in a concentration-dependent manner, achieving maximum intracellular concentrations in less than 10 min.
- Also delivered the protein across the blood–brain barrier.

Application of TAT-PTD

- TAT PTD fusion protein displayed potential to treat disorders pertaining to oxidative stress.
  - TAT–SOD fusion protein
  - TAT-CAT
  - TAT-HSP70
- TAT-mediated protein delivery showed potential as a therapeutic and prophylactic vaccine. (such as TAT-ovalbumin conjugate)
- TAT PTD was also applied to eradicate tumor by utilizing dendritic cell (DC)-based immunotherapy
  - TAT PTD-TRP2
- TAT transduction domain also showed a potential for application in inflammatory conditions
- TAT PTD was also used to transduce a biologically active neuroprotectant Bcl-xL in cerebral ischemia
  - PTD-HA-Bcl-xL
- Delivery of antibodies