

# PROTEIN TRANSDUCTION AS A TOOL FOR DELIVERING OF DRUGS INTO THE CELLS

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Delivery of macromolecular drugs (e.g. antisense oligonucleotides, polymer-drug conjugates, etc.) designed to work in specific sites inside cells is complicated, because macromolecules typically have access to fewer biological compartments.

Because of that, the potential for intracellular therapeutic use of proteins, peptides and oligonucleotides has been limited by the impermeable nature of the cell membrane to these compounds. Thus protein transduction has been overcome that obstacle, and it has been widely used to analyze biochemical processes in living cells.

TAT peptides derived from the HIV-1 TAT protein facilitate intracellular delivery of proteins and small colloidal particles. TAT fusion protein enters into the cells when added to the surrounding media, in concentration depended manner.

## Aim

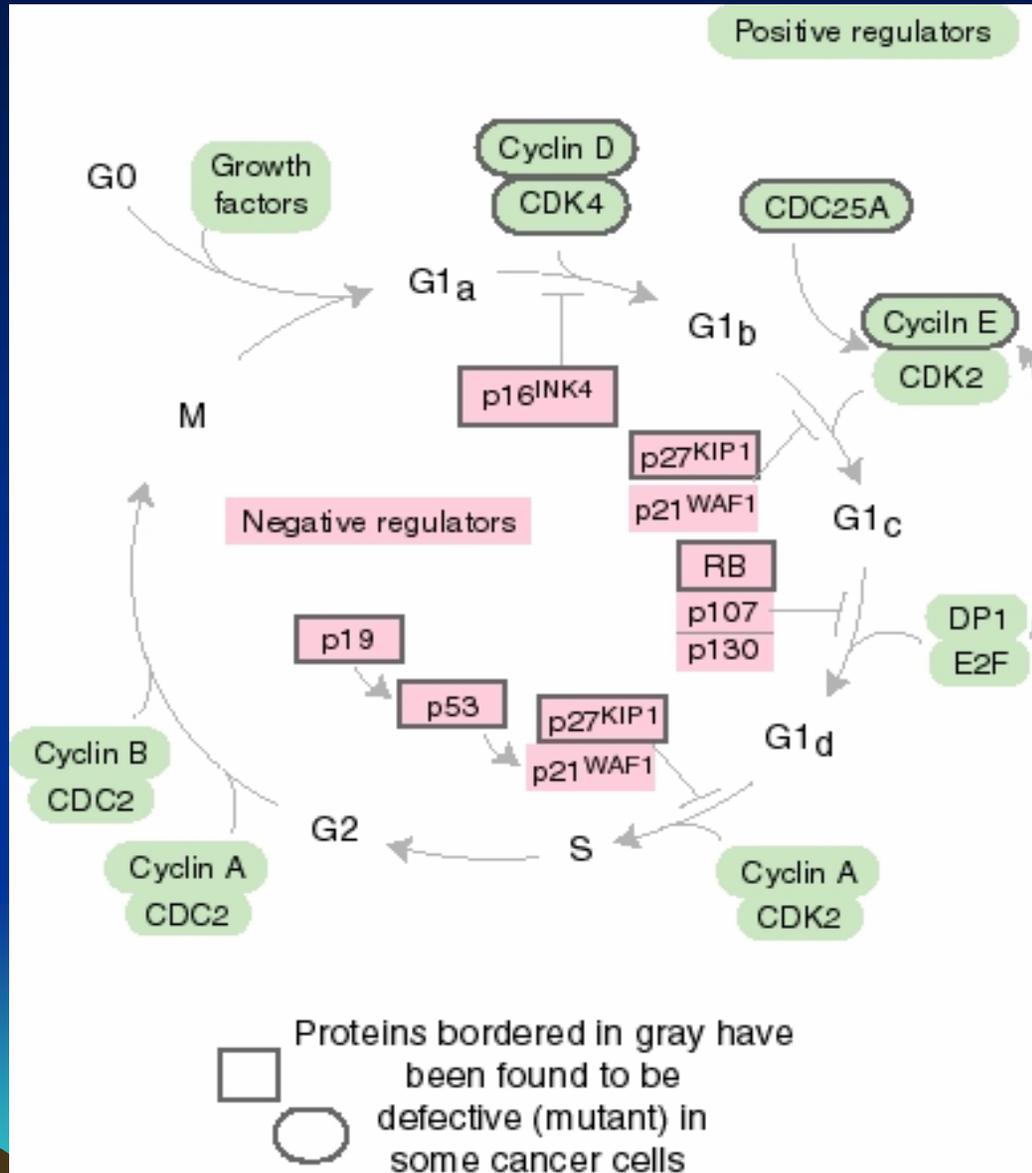
- how transduction of the proteins responsible for regulation of cell cycle, effects on its regulation

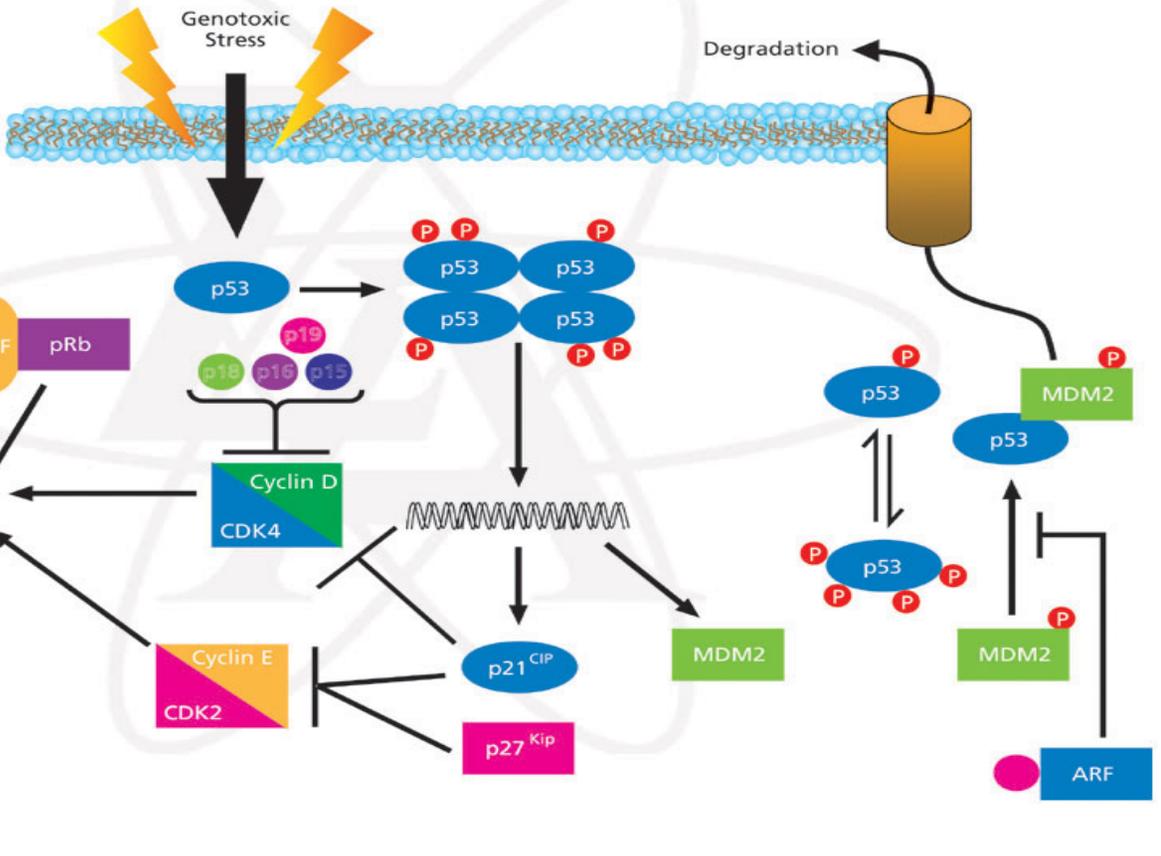
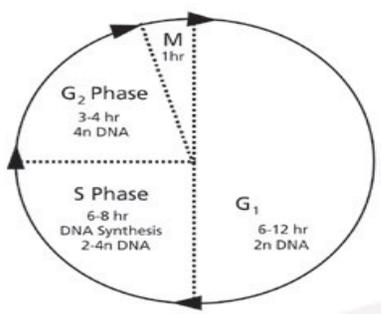
Cell cycle – cell proliferation

Cancer – increased proliferation - disorder in apoptosis

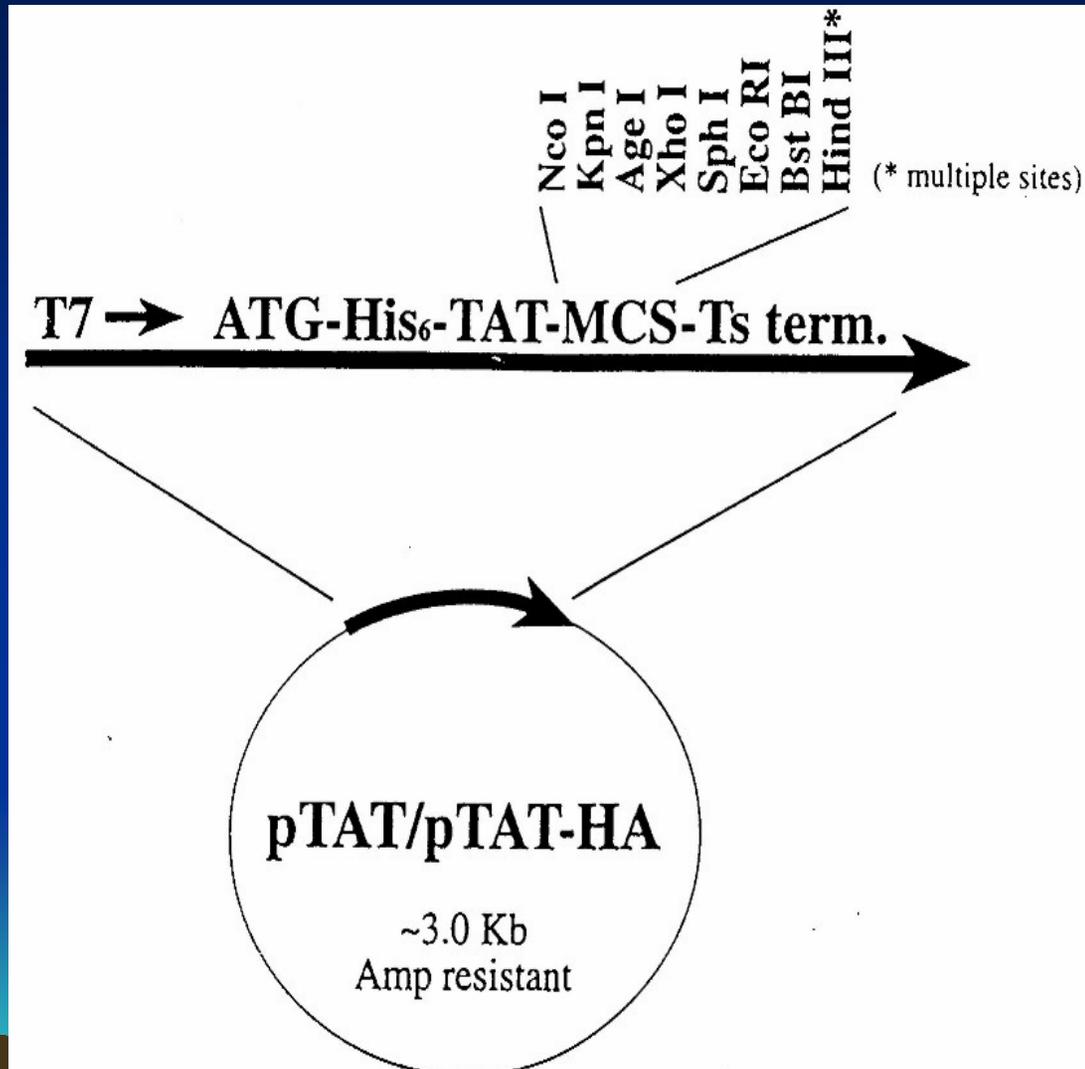
B-CLL – accumulation in late G1 phase – increased level of p27 prevent cells to go in apoptosis

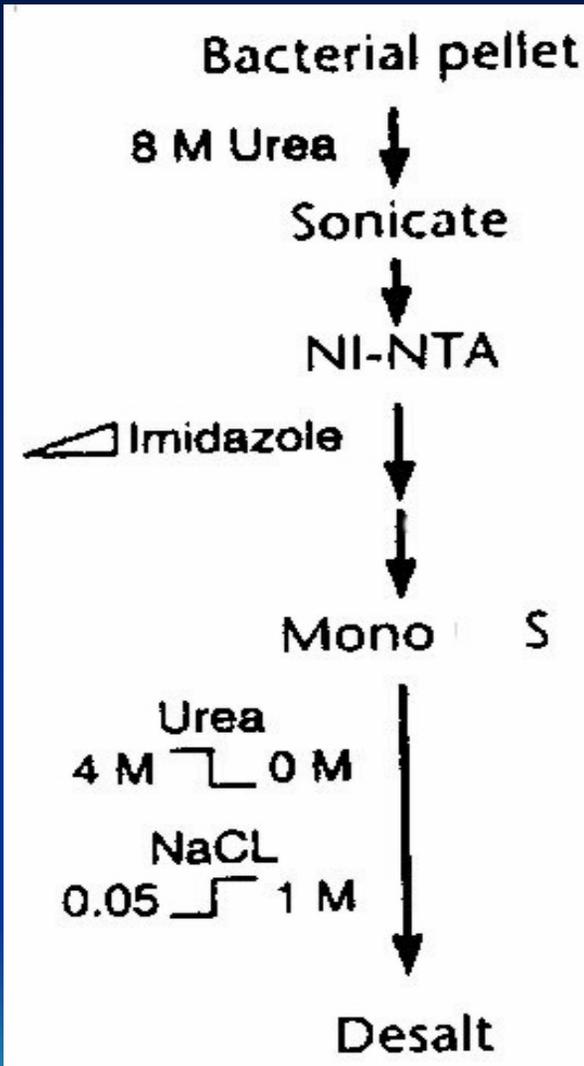
# Cell cycle regulators





# cDNA – TAT-protein

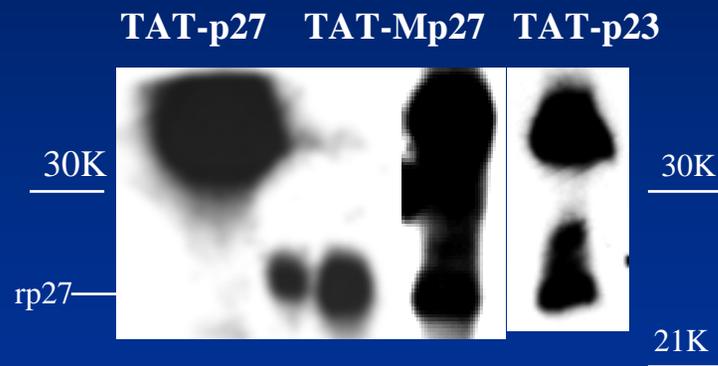




## Purification of TAT-fusion proteins

- Expression in bacteria
- Isolation from bacteria
  - Affinity chromatography
  - Ion exchange chromatography
  - Desalting (PD-10)

# Purified TAT-proteins



## Cell lines:

Raji – B lymphoma

SuDHL – myelogenous leukemia

NALM – B lymphoma

MOLT – T lymphoblastic leukemia

K562 – chronic myelogenous leukemia

## Transduction:

$2 \times 10^6$  cells/ml

100 nm TAT-protein

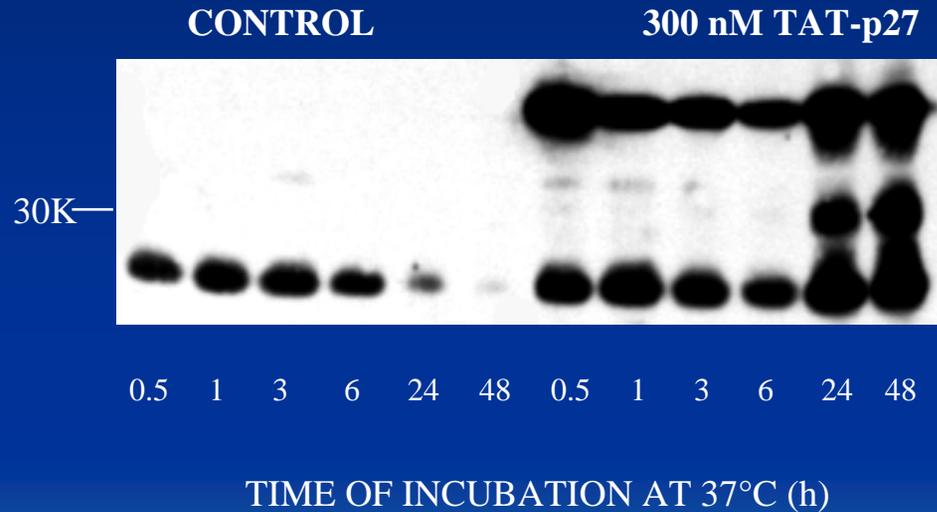
## Detection:

- Viability 72 hrs (MTT)

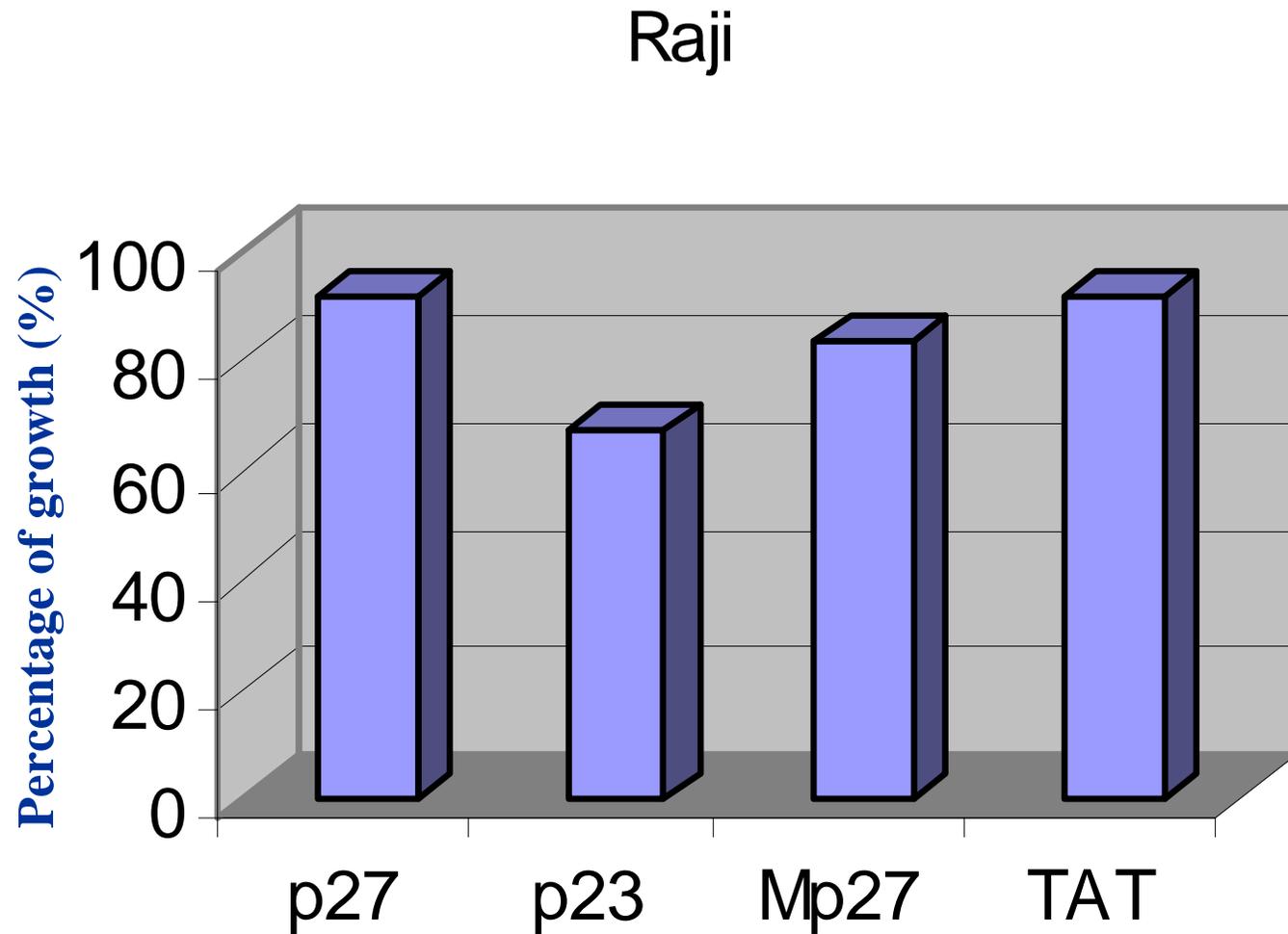
- PAGE, Western blot

# Detection of transduced TAT-p27 by PAGE and Western blot

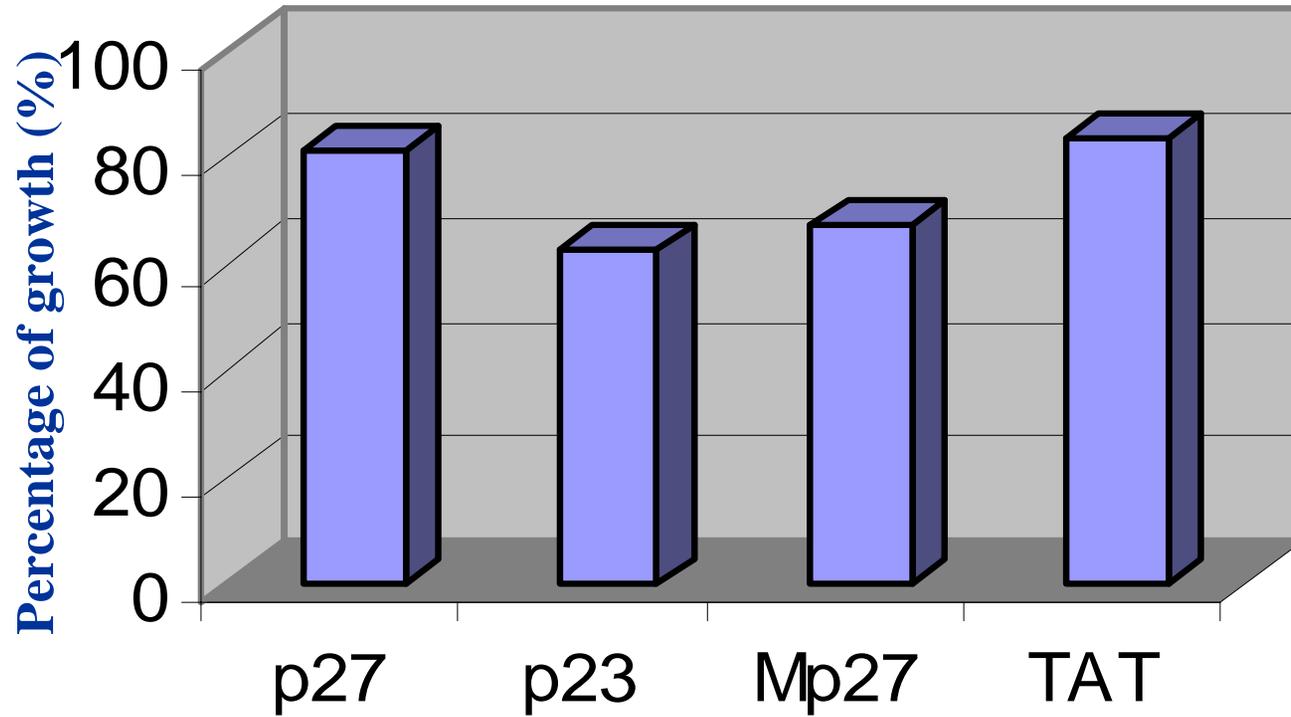
**Raji**  
(p27 Ab)



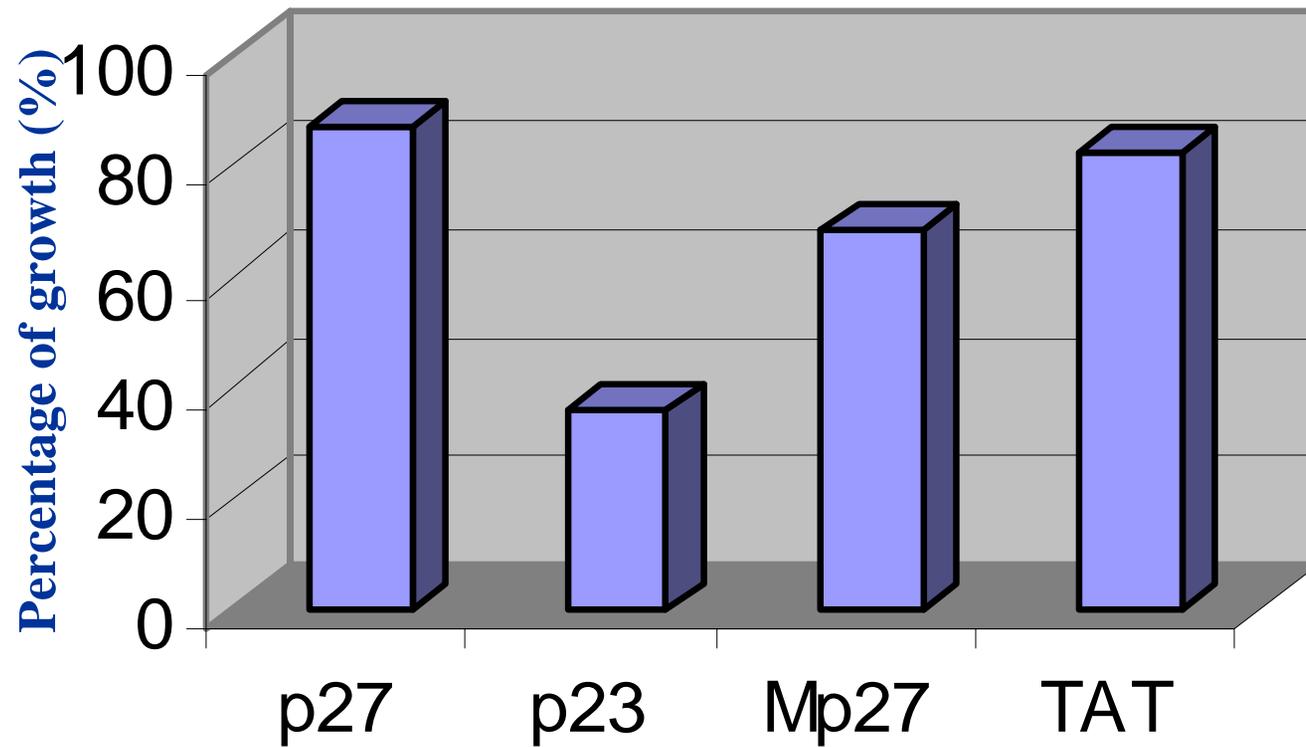
# Influence of transduced proteins on cell viability



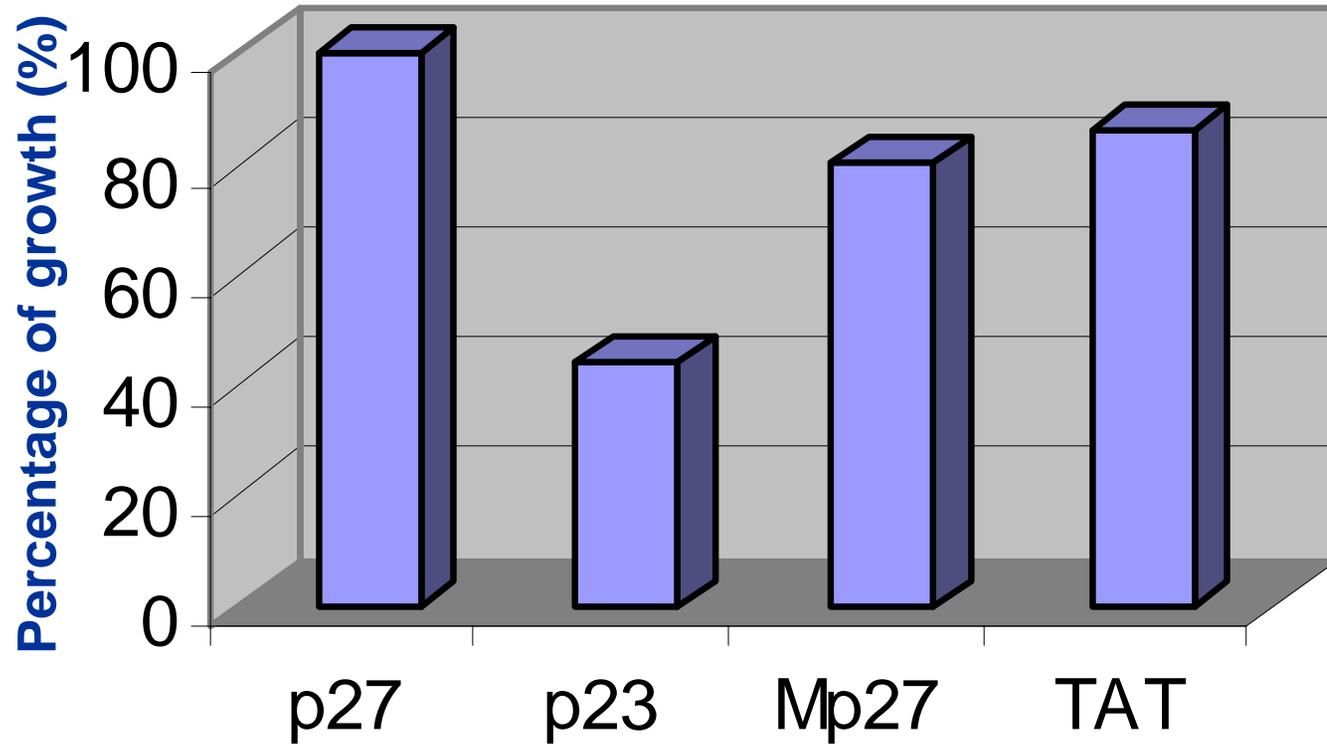
# SuDHL



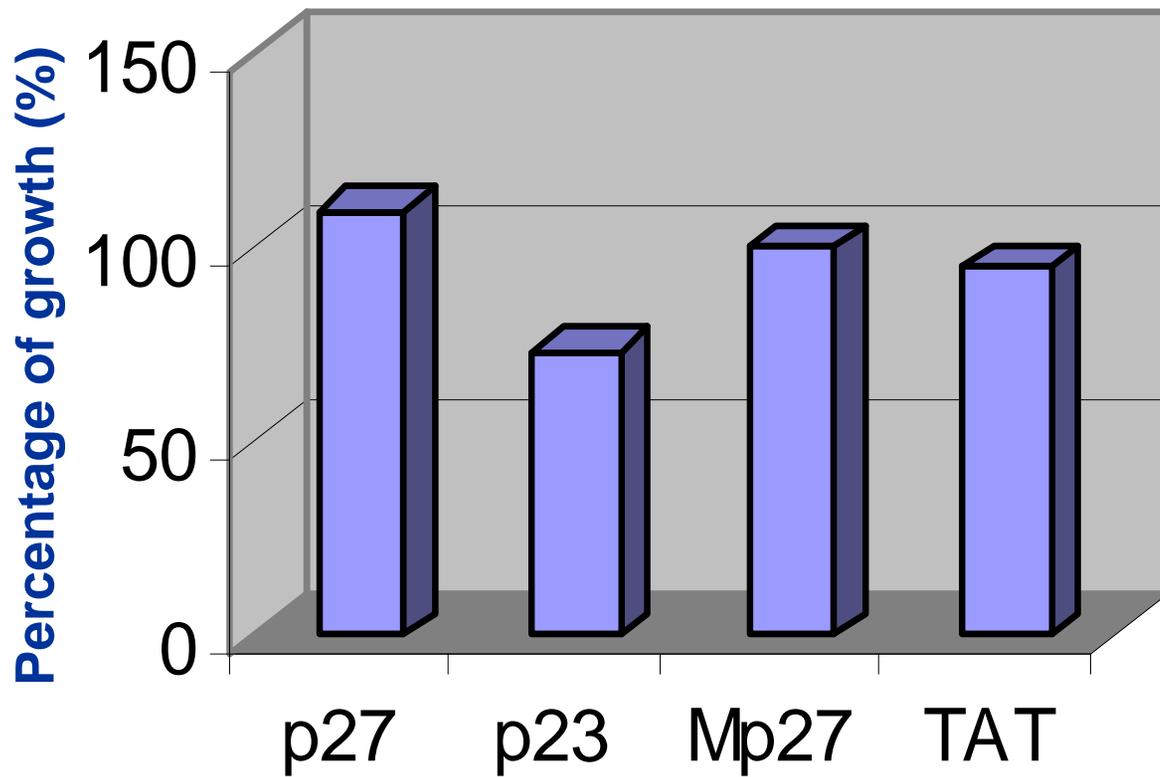
# NALM



# MOLT

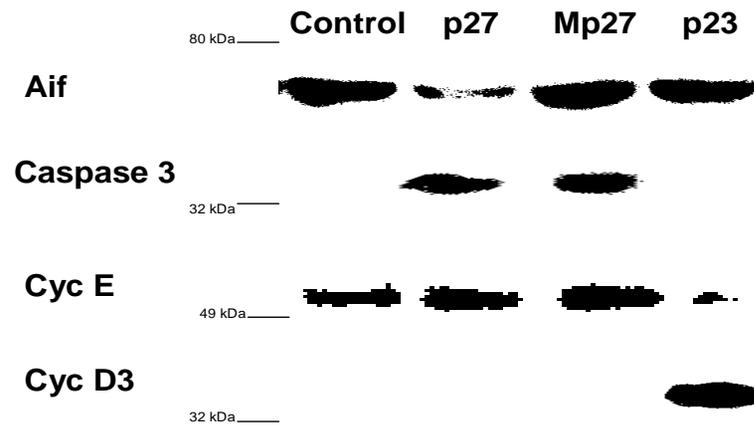


# K562

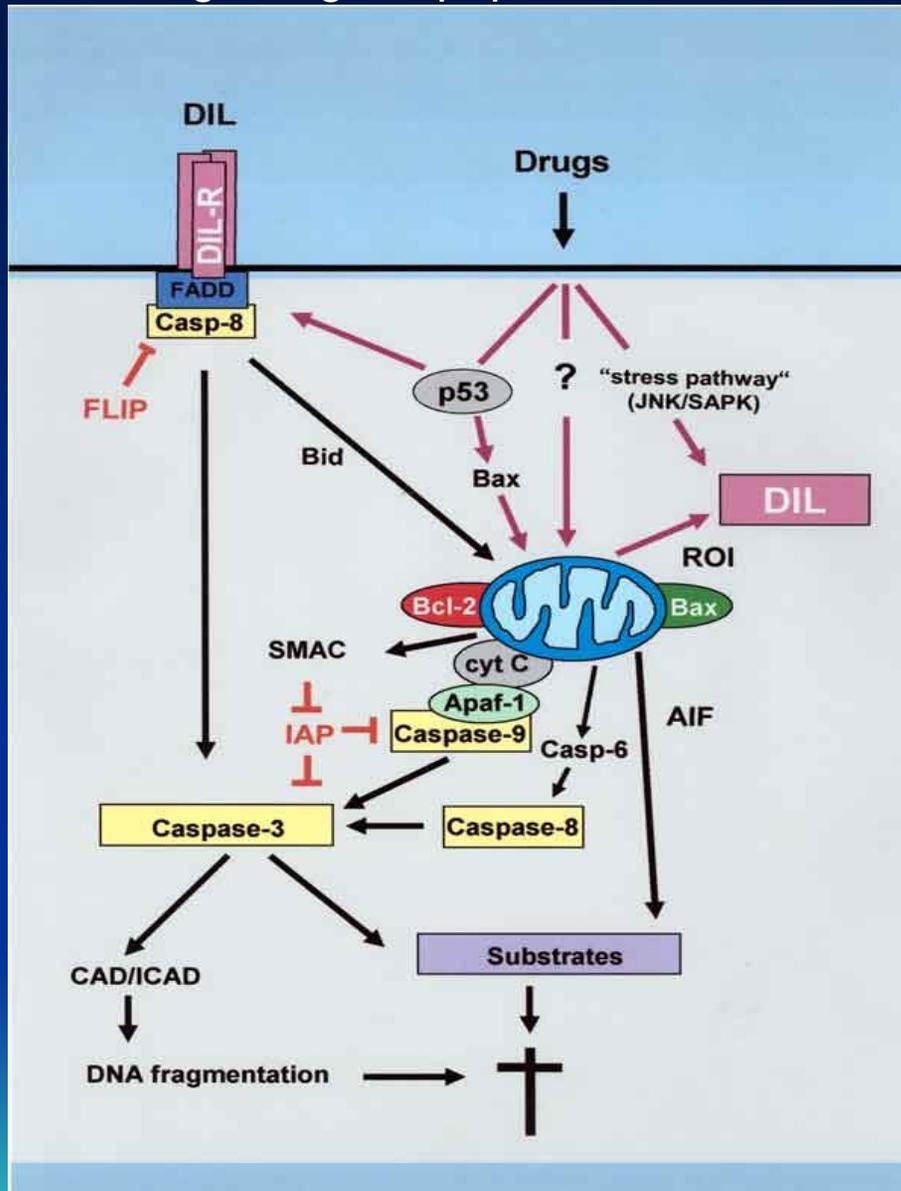


SuDHL cells transduced with 100 nM TAT-p27 or 100 nM TAT-Mp27

or 50 nM TAT-p23, for 21 hrs

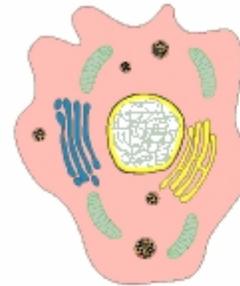


# Signaling to apoptosis



## Morphological Changes during Apoptosis

①



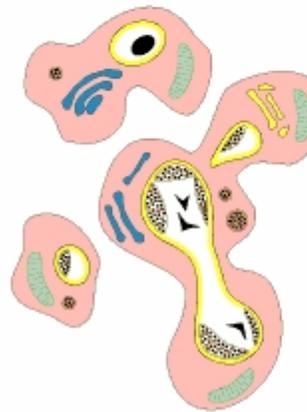
normal

②



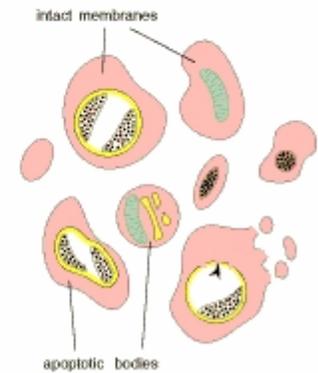
condensation (cell blebbing)

③



fragmentation

④



secondary necrosis

Transduction of proteins responsible for cell cycle regulation (p27, p23, Mp27) affected the proliferation of NALM, MOLT, Raji, SuDHL, and K562. A transduced p27 did not remarkable influence on proliferation of examined cell lines.

Mutated p27 inhibited the proliferation of examined cell lines up to 30 %. On the other hand, a transduction of p23 protein, truncated form of p27, inhibited the proliferation all of examined cell lines between 30-60 %.

Transduced proteins did not remarkable influence on the expression of cell cycle regulatory proteins. It seems that transduced p27 and Mp27 induced apoptosis, through the activation of caspases.

**This new method could give some promise for delivering of a drug into determined cell compartment and acting on specific target.**