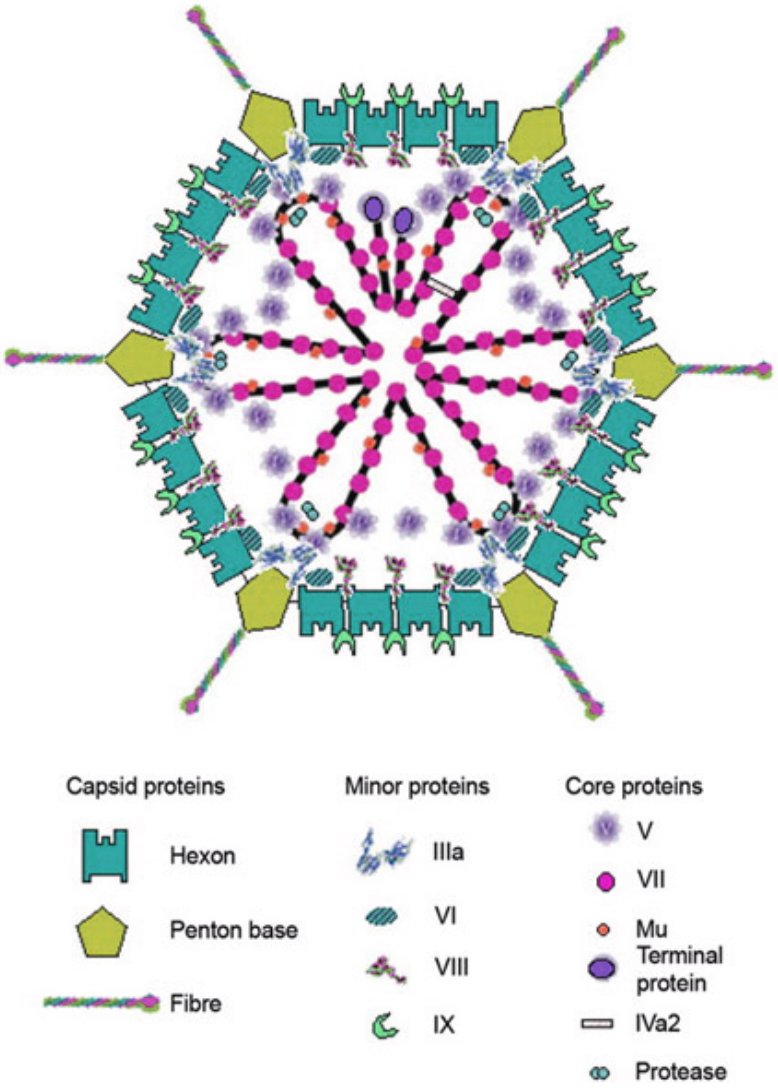
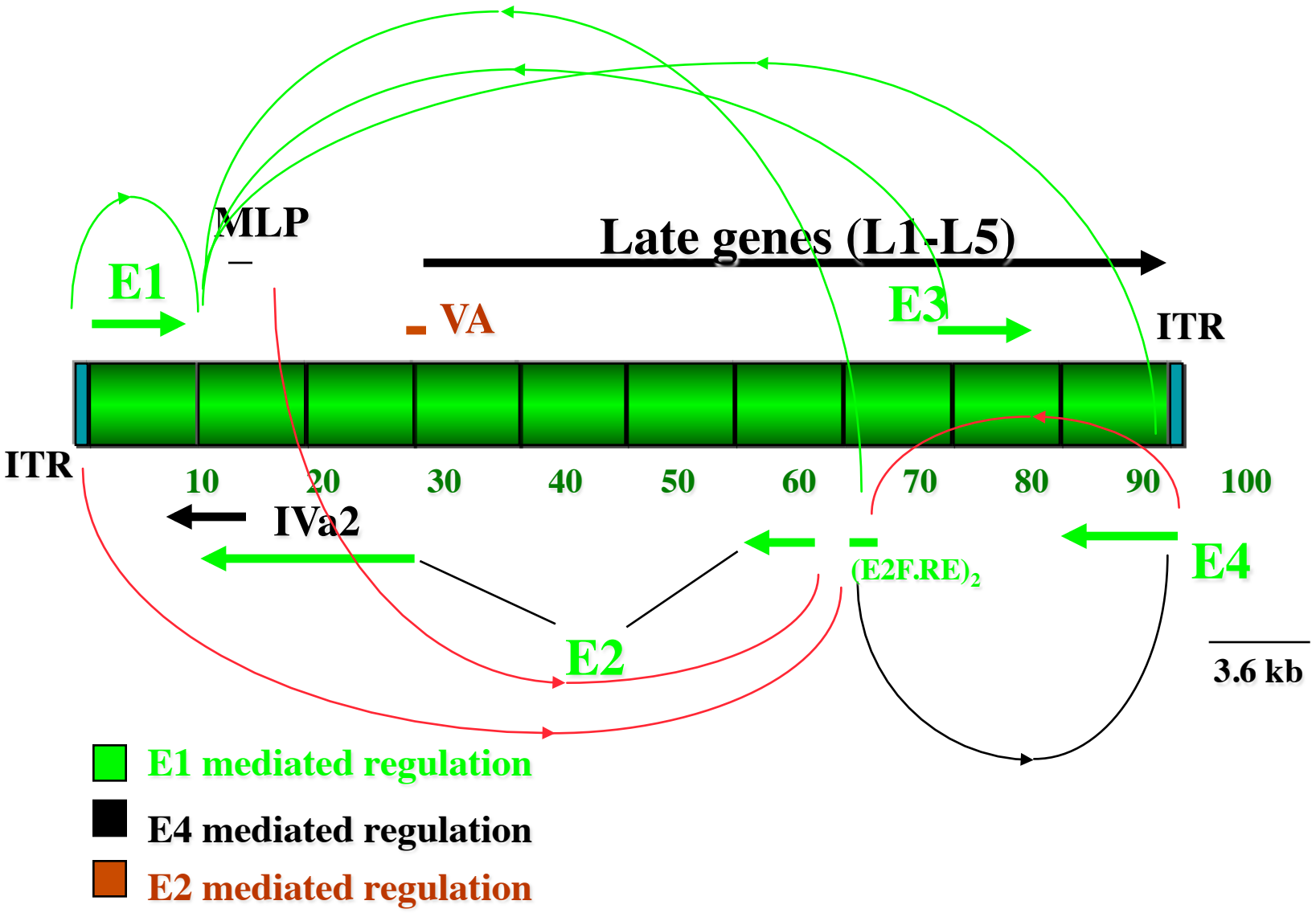
Cherish your doubts, for doubt
is the attendant of the truth

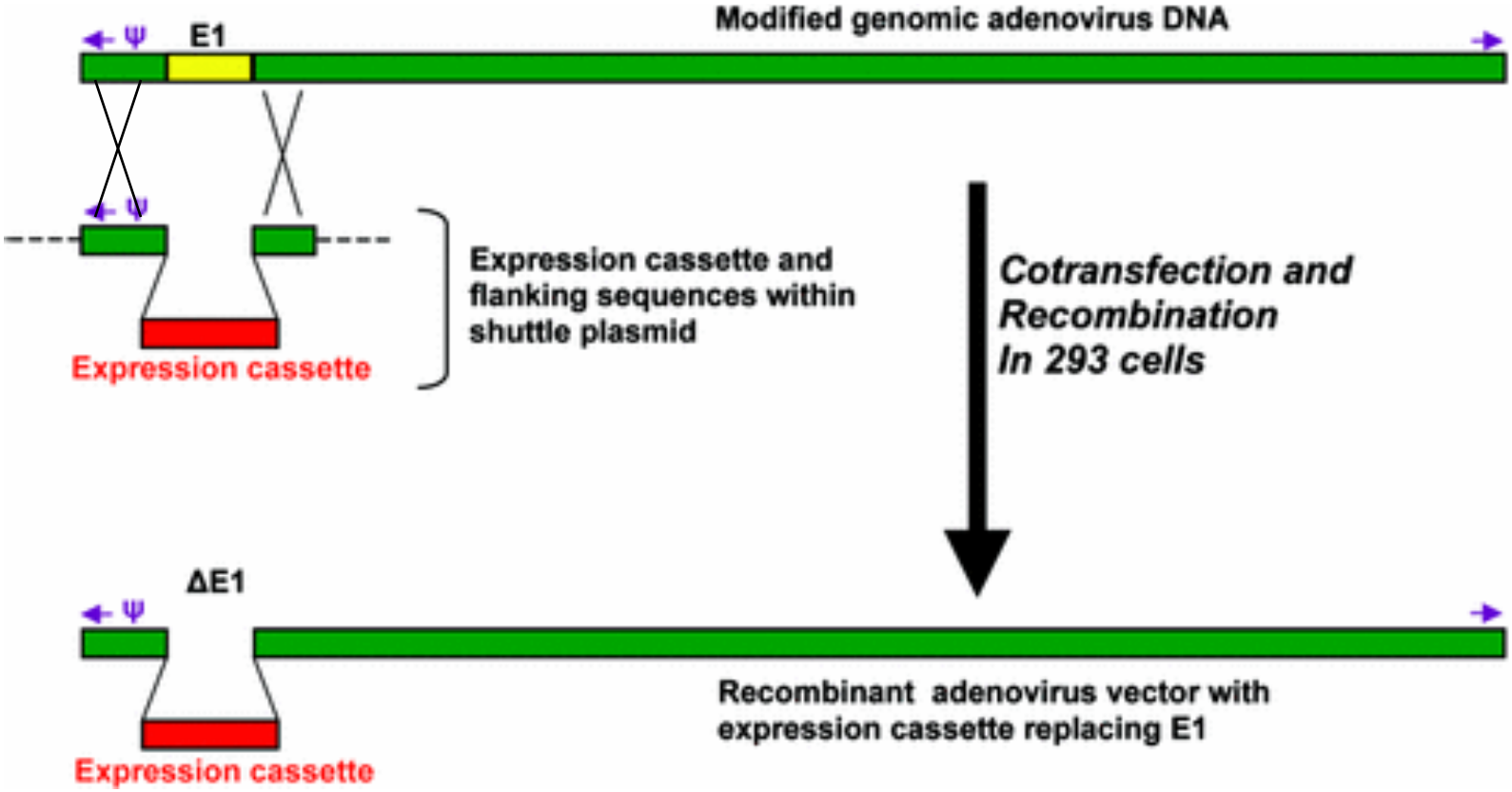
Adenovirus


Capsid proteins
Hexon
Penton base
Fibre
Minor proteins
IIIa
VI
VIII
IX
Core proteins
V
VII
Mu
Terminal protein
IVa2
Protease

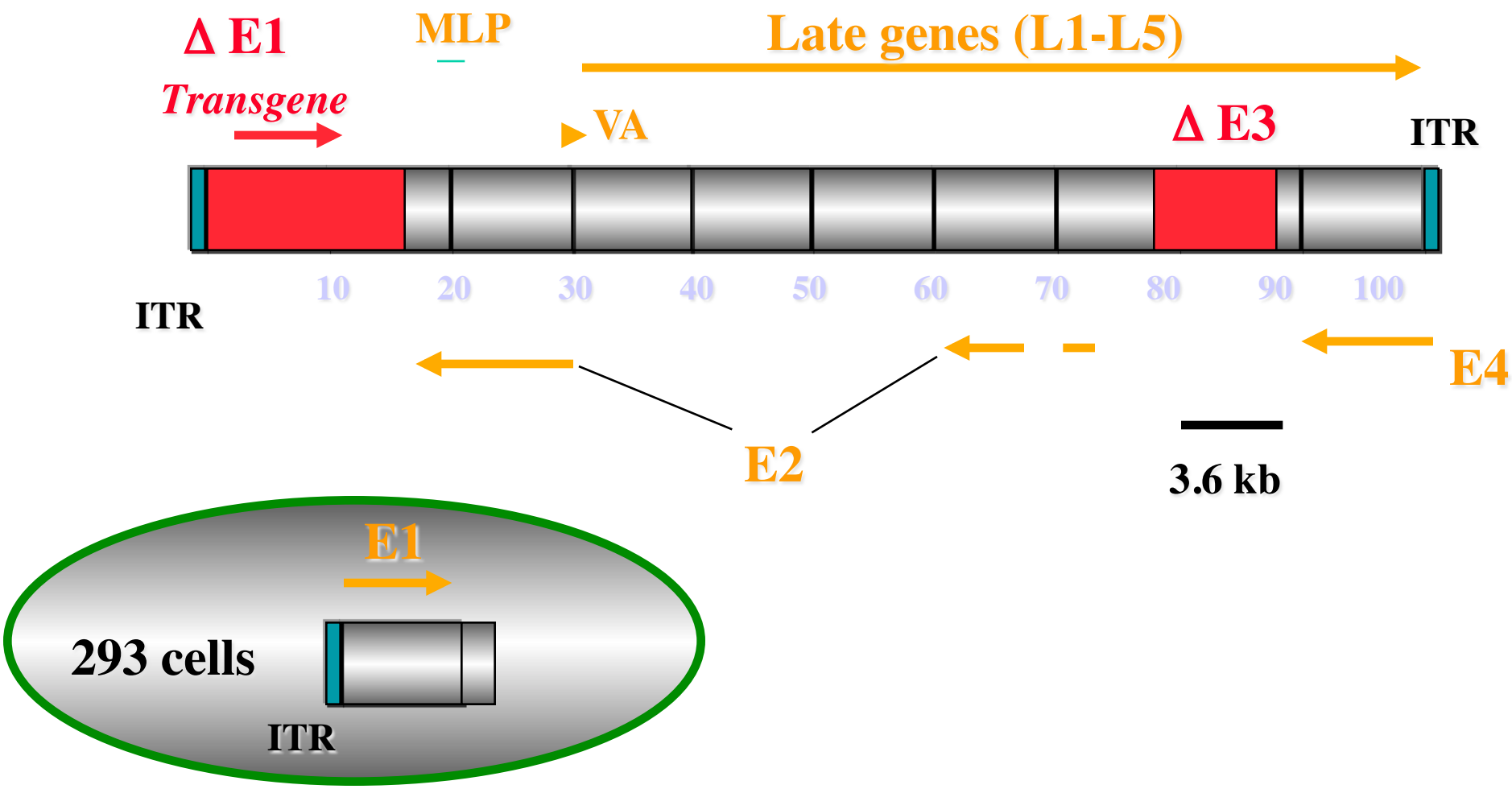
Adenovirus genome



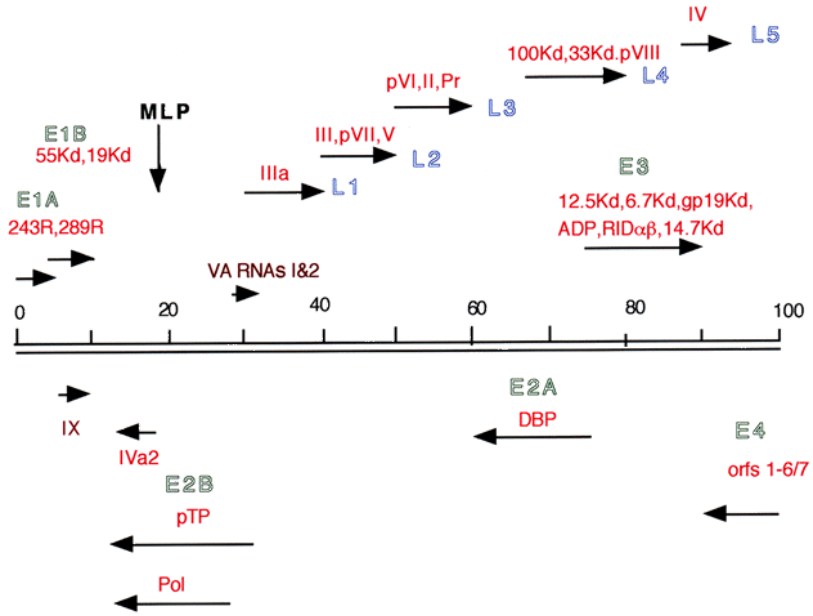
1st generation adenoviral vectors

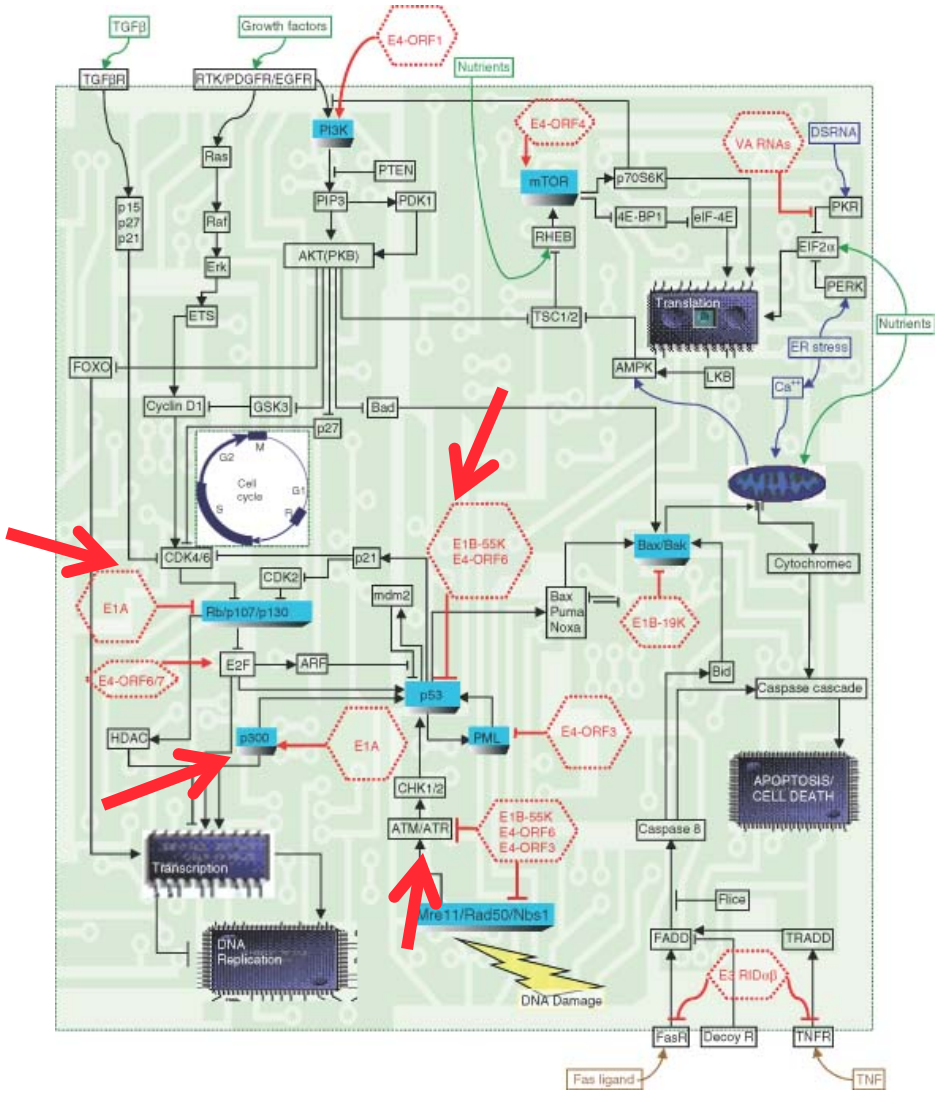


1st generation adenoviral vectors



Ad modulates cell functions

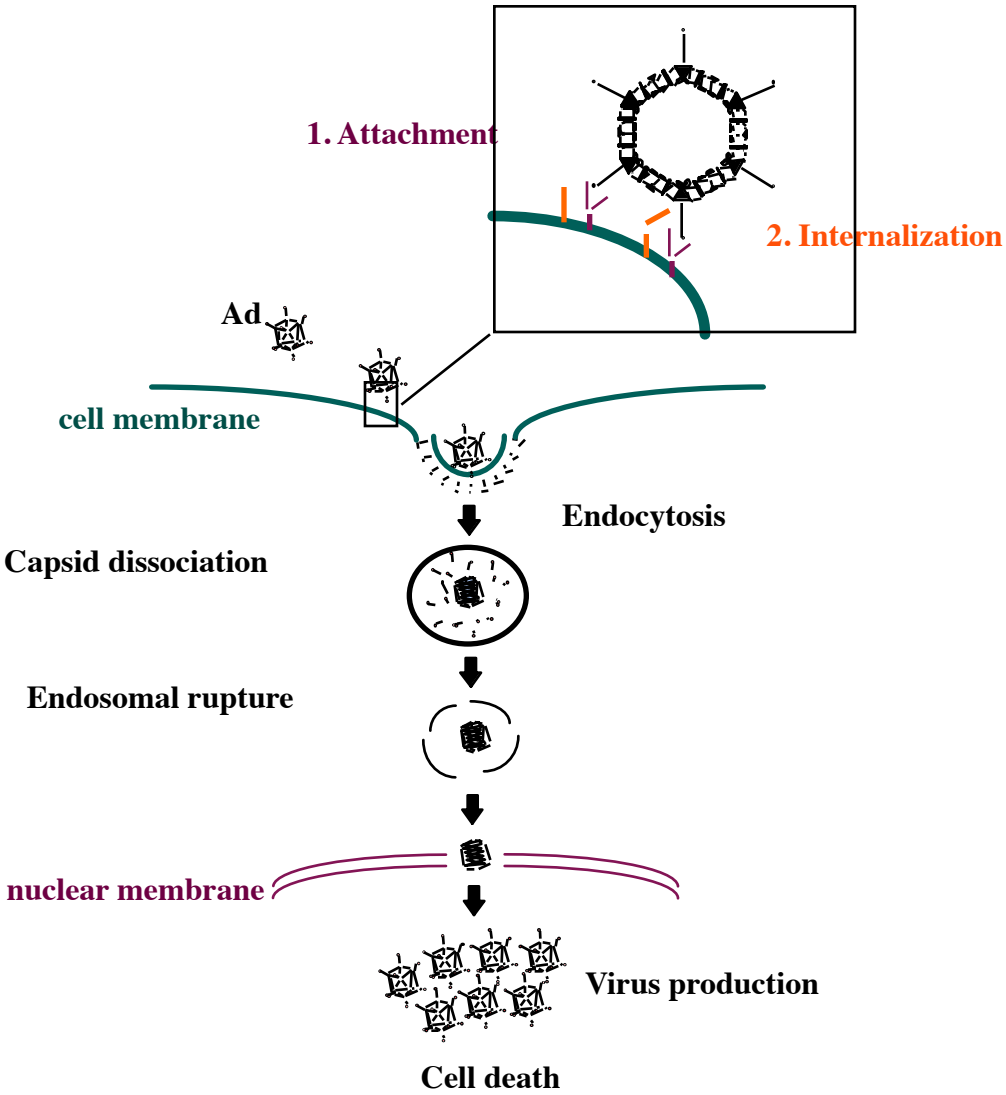




Problems and ameliorations of Ad vectors

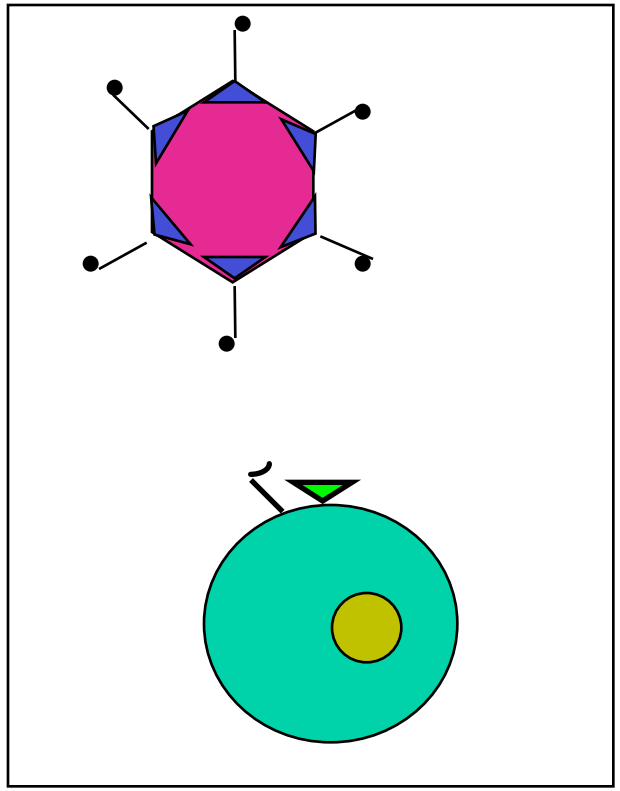
- no integration => chimaeres AAV/ Retro
- seropositivity to Ad => change of serotype, higher doses, immunosuppression
- large tropism => **targeted transduction**, targeted expression
- immunogenicity => **immuno-suppression**, **new vectors**
- size of the insert => new vectors
- short term expr. => chimaeres AAV/Retro, immuno-suppression, new generation vectors
- RCA => new lines, new vectors
- transcomplementation => new vectors

Ad entry into cells

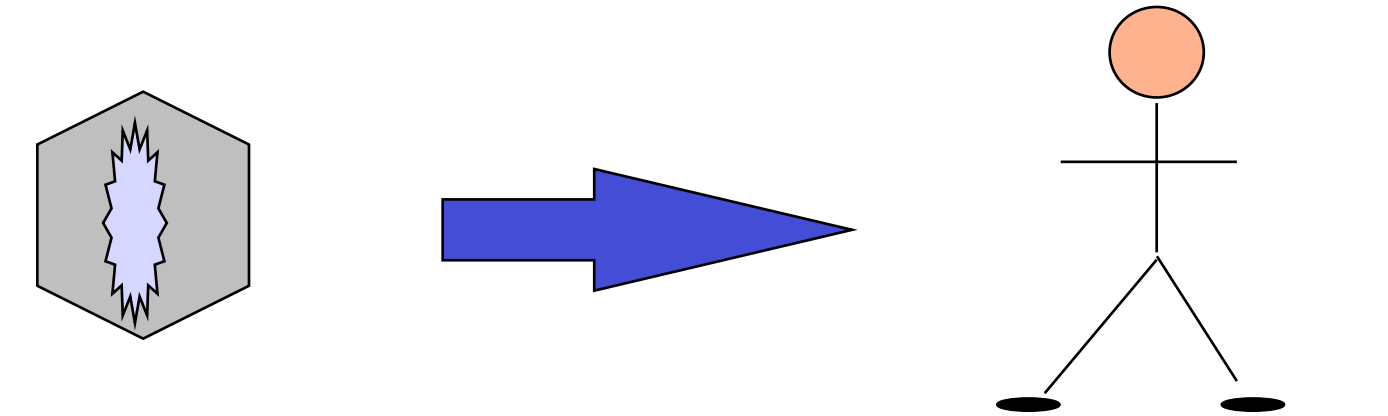


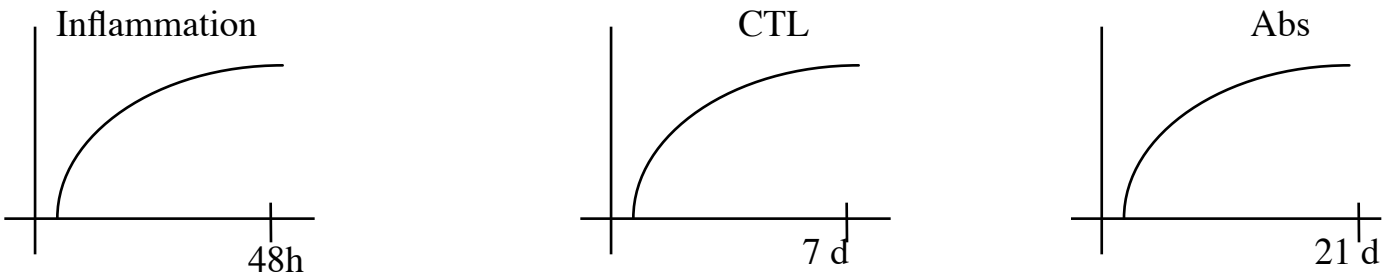
Ad modifications for targeting

- bispecific ABs antifiber/ antireceptor (nabs)
- bispecific abs anti fiber insert/ antireceptor (antiflag/ antireceptor)
- fiber inserts (RGD)
- hexon inserts
- penton base inserts



Immune response to adenoviral vectors



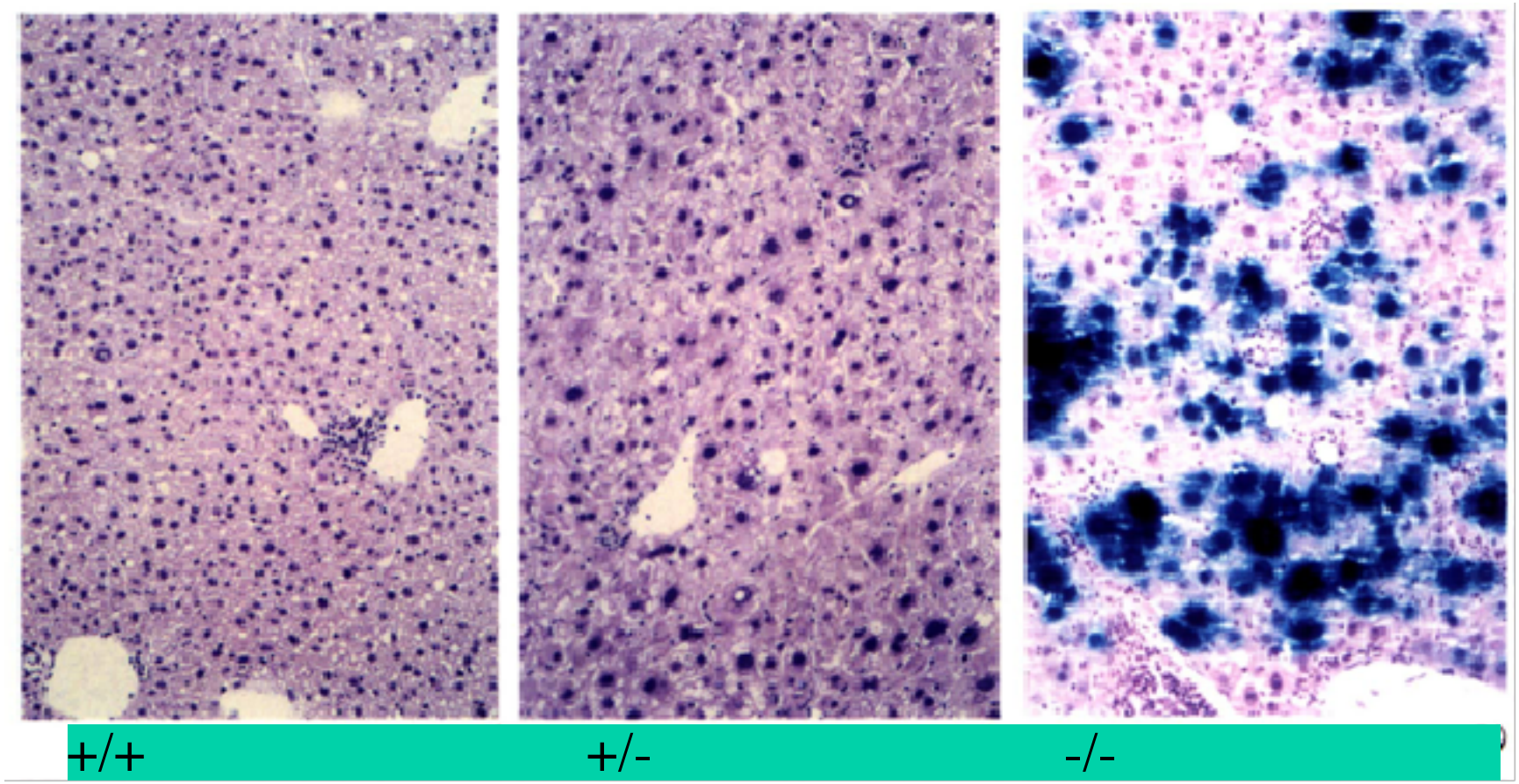


IL-6 and TNF-α in the immune response to Ad

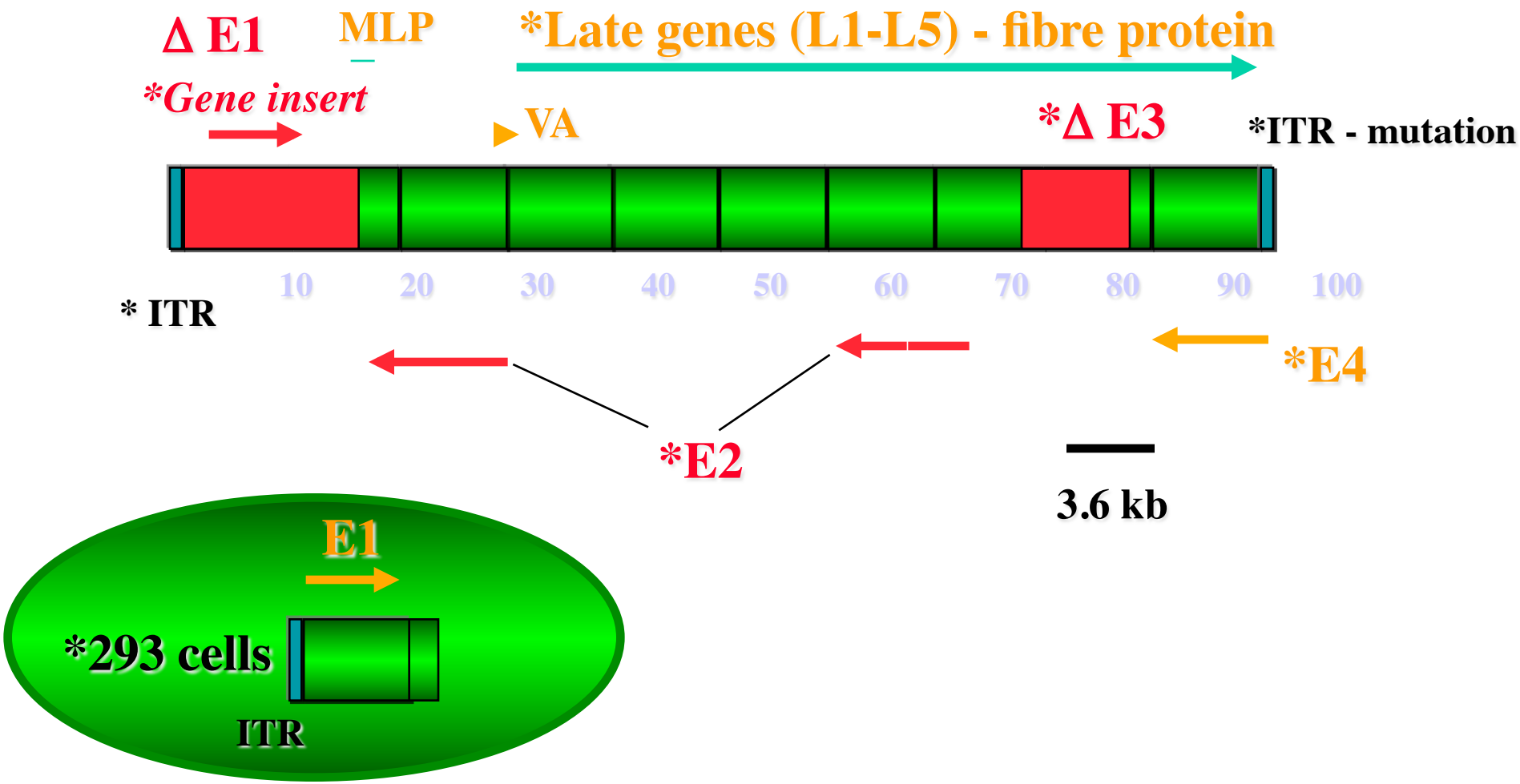
- IL-6 inflammatory cytokine
- IL-6 in rabbit model of Ad induced pneumonia
- IL-6 in Ad injected patients

- TNF-α inflammatory cytokine
- anti-TNF genes in Ad

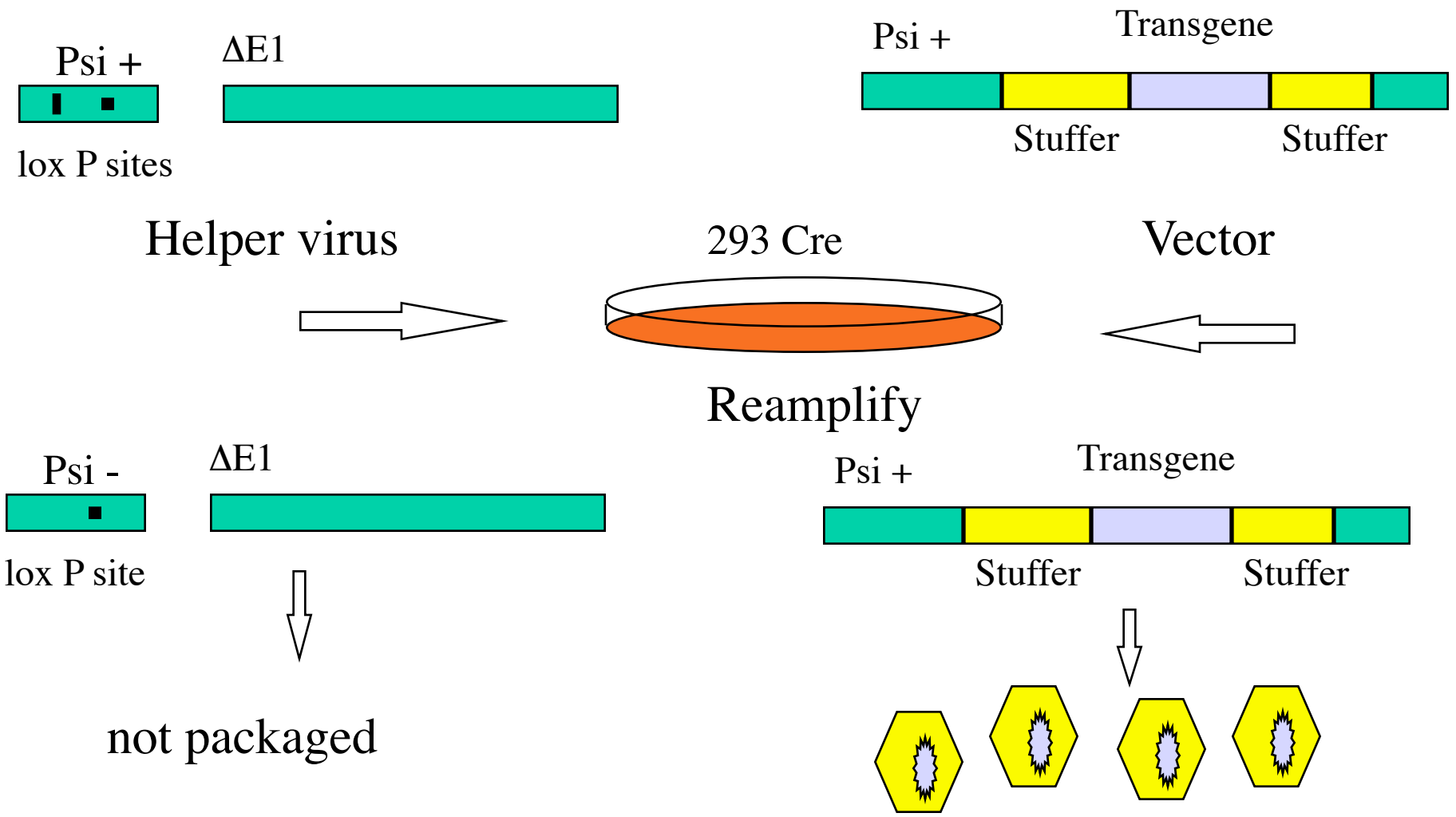
Ad reinjection in TNF KO



2nd generation Adenoviral vectors



3rd generation Adenoviral vectors



3rd generation Ad vectors: advantages

- size of the insert (36kb)
- low immunogenicity (no viral sequences)
- long term expression

3rd generation ad vectors: disadvantages

- titers
- instability
- helper contaminations
- stuffer?

Adenovirus mediated gene therapy: history

- *Welsh Cell 1993* Adenovirus mediated gene transfer transiently corrects the chloride transport defect in nasal epithelia of patients with cystic fibrosis
- *Wilson Nature Genetics1993* Gene therapy in a xenograft model of cystic fibrosis lung corrects chloride transport more effectively than the sodium defect
- *Peschanski Nature Genetics 1993* Transfer of a foreign gene into the brain using adenovirus vectors
- *Wilson 1993* Direct gene transfer of human CFTR into human bronchial epithelia of xenografts with E1-deleted adenoviruses

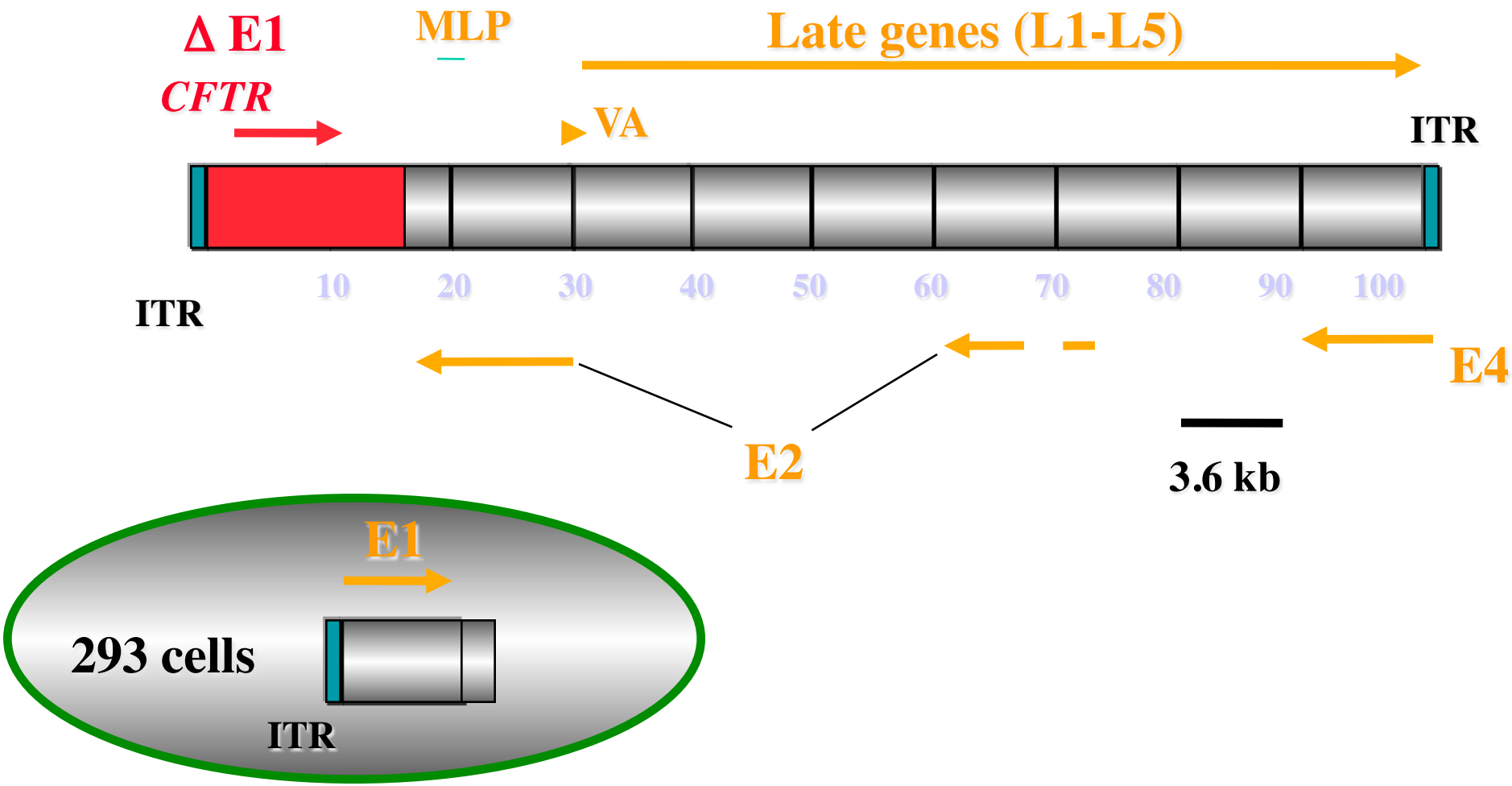
Ad-mediated gene therapy: history (follows)

- *Crystal Nature Genetics 1994* Administration of an adenovirus containing the human CFTR cDNA to the respiratory tract of individuals with cystic fibrosis
- *Wilson Nature Genetics 1996* Effective treatment of familial hypercolesterolaemia in the mouse model using adenovirus-mediated transfer of the VLDL receptor gene
- *McCormick Science 1996* An adenovirus mutant that replicates selectively in p53 deficient human tumor cells

Cystic fibrosis gene therapy

- Autosomal recessive disease caused by mutations in the transmembrane conductance regulator (CFTR)
- The Cl- channel is deregulated => defective Cl- transport => lung disease

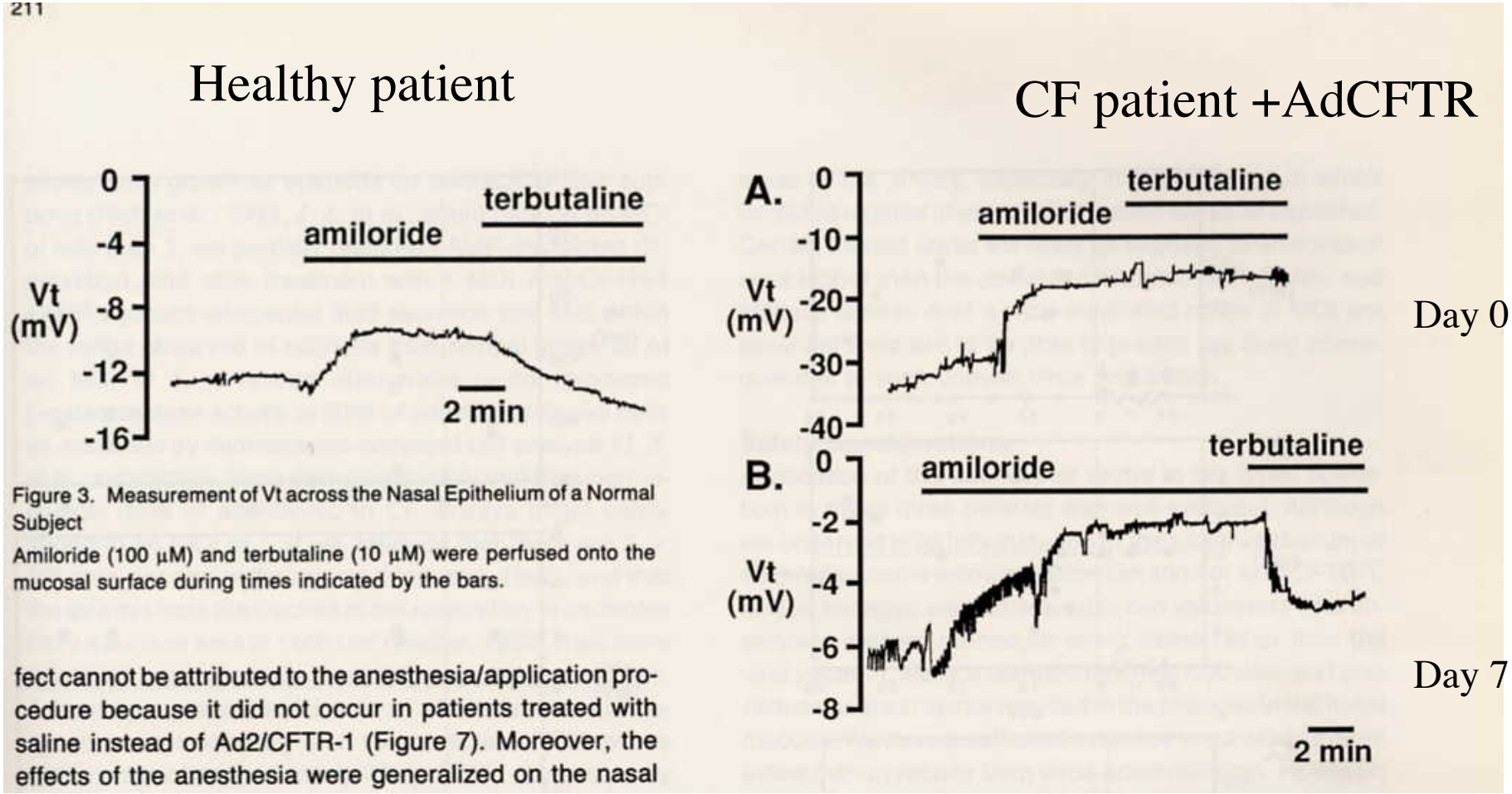
1st generation Ad-CFTR



Cell 93: AdCFTR in human patients

Check of ion transport:

amiloride creates a gradient and if the channel works, terbutaline makes Cl^- going out



Ad-CFTR

Nature Genetics 93/94

"S*emi-in vivo*"
AdCFTR in human
bronchial xenografts

Bronchus ->

Xenograft ->

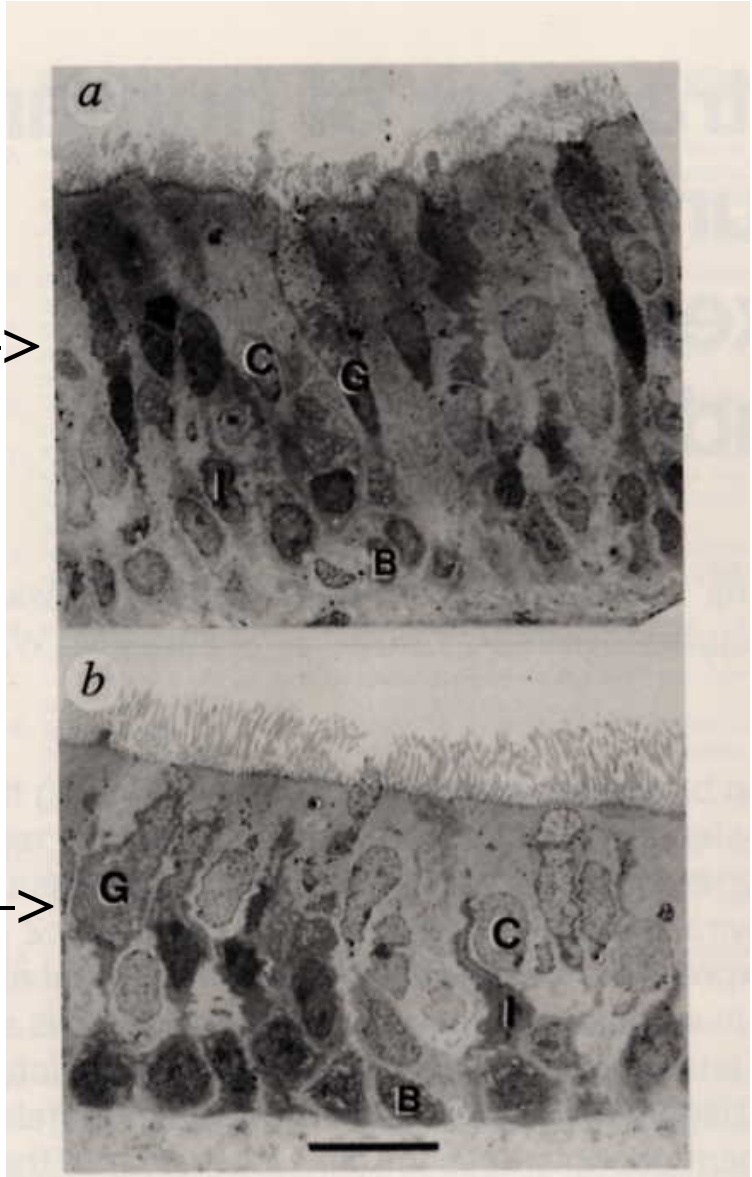
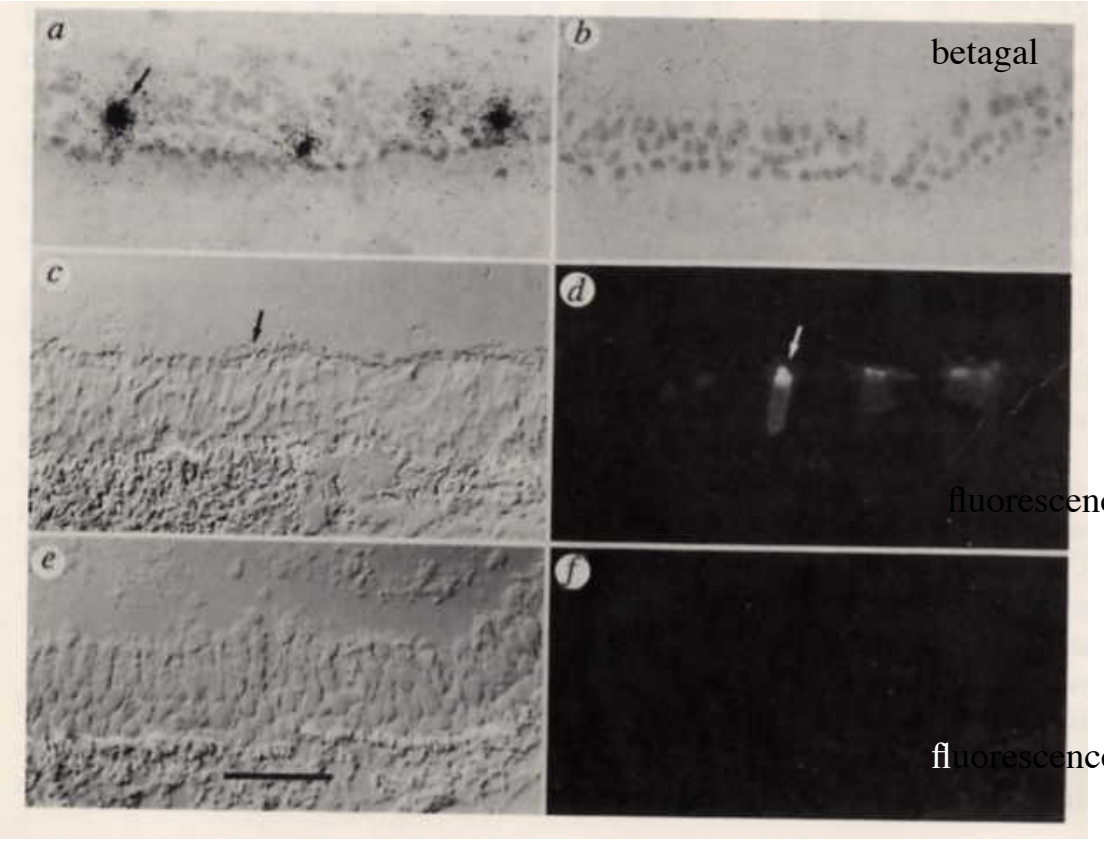


Fig. 1 Electron micrographs of bronchial epithelia from human bronchus and a xenograft. Micrograph of human bronchial epithelium *a*, and epithelium from a xenograft seeded with human bronchial epithelial cells and harvested at 42 days *b*. C ciliated cell; G, goblet cell; B, basal cell and I, intermediate cell

AdCFTR in human bronchial epithelial xenografts; 1 week after infection



Adbgal

AdCFTR

Adbgal

Nat Gen 93 Wilson/Perricaudet

Ion function in AdCFTR infected human bronchial xenografts

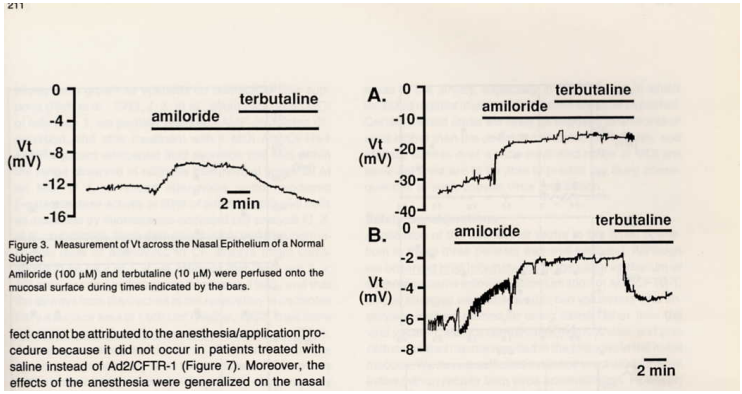


Figure 3. Measurement of Vt across the Nasal Epithelium of a Normal Subject

Amiloride (100 μM) and terbutaline (10 μM) were perfused onto the mucosal surface during times indicated by the bars.

fect cannot be attributed to the anesthesia/application procedure because it did not occur in patients treated with saline instead of Ad2/CFTR-1 (Figure 7). Moreover, the effects of the anesthesia were generalized on the nasal

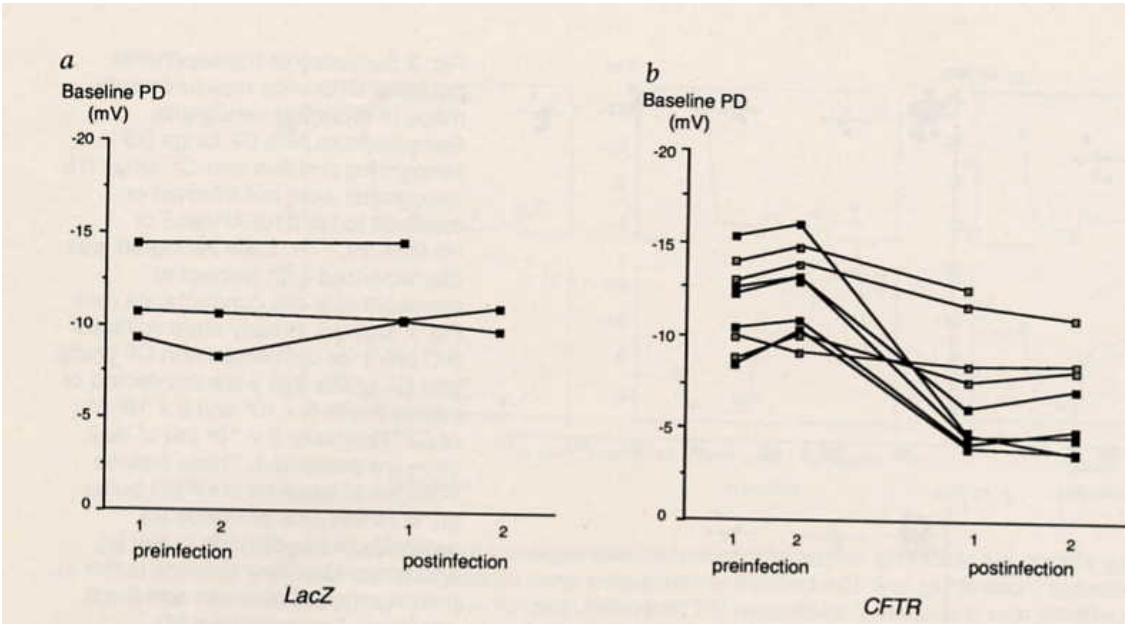


Fig. 5 Baseline PD (mV) in xenografts infected with *lacZ* and CFTR virus. CF xenografts infected with 5×10^9 total pfu of H5.010CMV*lacZ* (*a*) and H5.020CB*CFTR* (*b*). In *b*, closed squares (n=5) represent responders and open squares (n=4) nonresponders. Baseline PD in mV was measured twice over seven day intervals before and after gene transfer.

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Ad-residual activity in bronchial xenografts

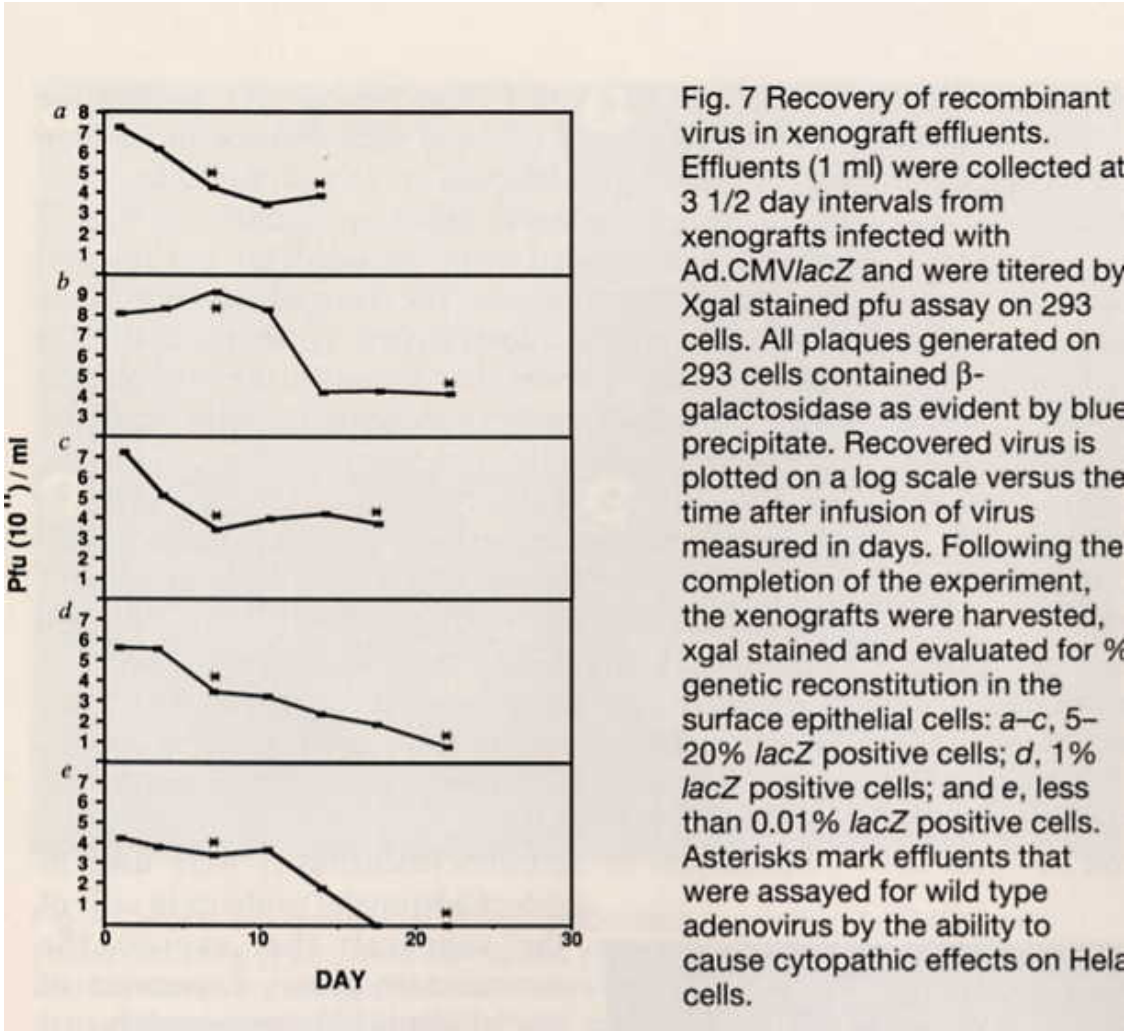

Fig. 7 Recovery of recombinant virus in xenograft effluents. Effluents (1 ml) were collected at 3 1/2 day intervals from xenografts infected with Ad.CMV*lacZ* and were titered by Xgal stained pfu assay on 293 cells. All plaques generated on 293 cells contained β-galactosidase as evident by blue precipitate. Recovered virus is plotted on a log scale versus the time after infusion of virus measured in days. Following the completion of the experiment, the xenografts were harvested, xgal stained and evaluated for % genetic reconstitution in the surface epithelial cells: *a–c*, 5–20% *lacZ* positive cells; *d*, 1% *lacZ* positive cells; and *e*, less than 0.01% *lacZ* positive cells. Asterisks mark effluents that were assayed for wild type adenovirus by the ability to cause cytopathic effects on Hela cells.

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AdCFTR in humans: immunocytochemical detection of CFTR in human bronchial cells

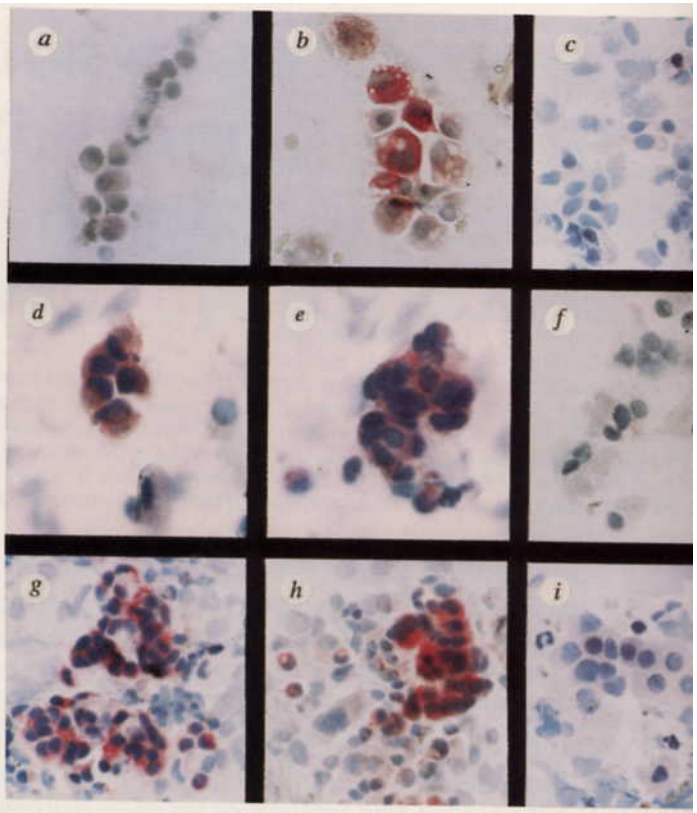

Fig. 3 Immunocytochemical detection of human CFTR in bronchial epithelial cells before and after *in vivo* administration of Ad*CFTR* to the bronchial epithelium. Low level endogenous expression of *CFTR* in the bronchial epithelium pre-therapy (*a–e*) is not detected; for *f–i*, the cells were exposed to the substrate for a longer period, and minimal pink staining is observed in the control (*f*). *a*, Bronchial epithelial cells from individual 3A recovered pre-therapy and maintained in culture under conditions identical to *b*. *b*, as (*a*), but infected with Ad*CFTR in vitro* as a positive control. *c*, Fresh (not cultured) bronchial epithelial cells of the same individual obtained pre-therapy immediately prior to intrabronchial administration of Ad*CFTR*. *d* and *e*, Left bronchial epithelium 4 d after intrabronchial administration of Ad*CFTR* (2×10^6 pfu) to the airway epithelium of the left lower lobe. *f* and *g*, same individual as in (*c–e*), but with the cells exposed to the colorimetric substrate for a longer time to increase the sensitivity of the assay. *f*, Bronchial epithelial cells obtained prior to intrabronchial administration of Ad*CFTR*. *g* and *h*, Evaluation similar to (*f*) of the left bronchial epithelium 4 d after intrabronchial administration of Ad*CFTR*. In (*h*), the morphology of one "positive" cell (at 9 o'clock, far left) is indeterminate. *i*, as (*g*) and (*h*), but with an irrelevant isotype control antibody. The positive epithelial cells following therapy include ciliated, non-ciliated columnar or basal cells. For *a–e*, the time of exposure to the alkaline phosphatase substrate was 9 min; for *f–i*, the time was 17 min. For the short exposure to the substrate, quantification of the "% positive cells" demonstrated in the pre-therapy period 0% epithelial cells were positive (n=800 cells), and 0% of inflammatory cells were positive (n=200), while in the post-therapy period 5.0% of epithelial cells were positive (n=500) and 0% inflammatory cells were positive (n=500). For the longer exposure, in the pre-therapy period 0% epithelial cells were positive (n=500), and 0% inflammatory cells were positive (n=200), while in the post-therapy period 14.0% epithelial were positive (n=500), and 0.4% inflammatory cells were positive (n=500). In all panels, the samples were counterstained with hematoxy panels 630× except *d* and *e*, 1,000×.

A: pretherapy
B: prether inf in vitro
C: pretherapy
D: 4 d after AdCFTR
E: 4 d after AdCFTR
F: pretherapy
G: 4d after AdCFTR
H: 4d after AdCFTR
I : control ab

Crystal Nat Gen 94