

Cell growth arrest by nucleotides, nucleosides and bases

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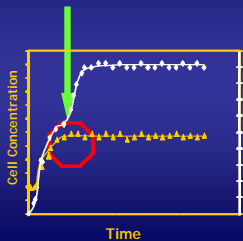


GOAL

A tool for improved production of recombinant proteins

HOW

Cell growth arrest ON

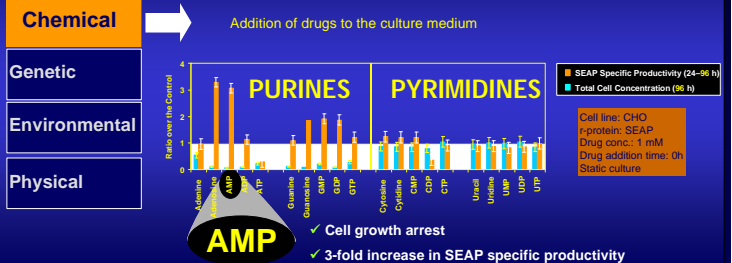


Advantages:

- Decrease of cell division
- More available cell energy for product synthesis
- Decrease the chance for genetic drift

BACKGROUND

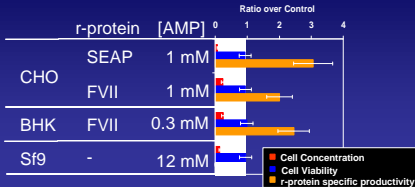
Strategies to arrest cell growth 1,2,3,4,5,6



MAIN QUESTIONS

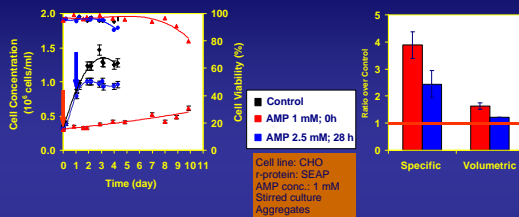
1 Can cell growth arrest by AMP be a valid strategy for r-protein production improvement?

Influence of rec-protein synthesis, r-protein type and cell line



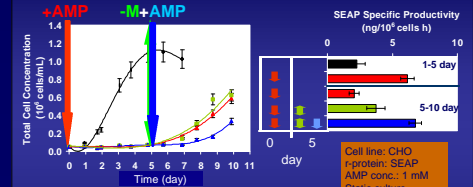
- AMP effect on r-protein specific productivity did not depend upon:
 - r-protein (SEAP and Factor VII- CHO cells)
 - cell line (FVII- CHO and BHK cells)
- AMP effect on cell concentration did not depend upon:
 - r-protein (SEAP and Factor VII; CHO cells)
 - cell line (CHO, BHK and Sf9)
- AMP optimum concentration depended upon:
 - Cell line (CHO, BHK and Sf9)

High cell density



- AMP addition in stirred cultures:
 - sharply decreased cell growth
 - viability of cells in AMP cultures were kept high for long periods (216 hours)
 - led to a 2.5-fold increase in SEAP specific productivity
- Optimum AMP concentration 3 μmol / 10⁶ cells

Control for long periods

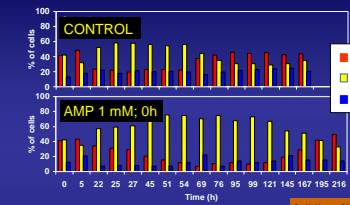


- Multiple AMP addition maintains cell growth arrest and high r-protein productivity

- YES, since AMP:**
- is a valid cell growth arrest strategy
 - improves r-protein productivity
 - is highly promising for larger scale application

2 Is it advantageous to consider a relationship between cell cycle and r-protein production?

AMP effect upon cell cycle



- S-phase accumulation
- SEAP specific productivity increase

Different cell growth arrest strategies- Same cell line

Strategy	Chemical	Environmental	
Cell concentration (fold lower)	2	2	
Cell cycle phase accumulation	S extension	S extension	
Increase of SEAP specific productivity (fold higher)	1.0	0.5	
Adenosine	9	S	3.3
AMP	14	S	3.1
ADP	12	S	1.1
ATP	5	S	0.3
Guanine	9	S	1.1
Guanosine	11	S	1.8
GMP	5	S	1.9
GDP	11	S	1.9
GTP	4	S	1.2
Aphidicolin	10	S	1.2
Doxorubicin	4	G2	2.2
Na Butyrate	5	negligible	2.5
Lactate	2	negligible	0.5
Ammonia	2	S extension	0.5
Glc	3	none	0.5
Gln	4	none	0.7
Glc and Gln	6	none	0.7
Serum	13	G1	0.2

- S-phase accumulation → purines → SEAP specific productivity increase
- Cell growth arrest *per se* was not necessarily associated with an increase in SEAP specific productivity
- Accumulation of cells in the S-phase was not always associated with an increase in SEAP specific productivity
- Nevertheless, in the case of cell growth arrest by purines, whenever SEAP specific productivity increased an accumulation of cells in the S-phase was observed

- YES, but for a particular cell growth arrest strategy/producer cell line system**

EXPERIMENTAL

Cells: The CHO cells producing SEAP were kindly supplied by Prof. James Bailey from ETH, Zurich, Switzerland. SEAP expression is under control of the tetracycline-responsive promoter. The CHO and BHK clones expressing factor VII were kindly supplied by Dr. Karen Hansen from Novo Nordisk, Denmark. Sf9 cells (ECACC no. 8907101) adapted to serum free medium.
Culture medium: CHO/SEAP: FMX-8 medium (Dr. Messi Cell Culture Systems, Switzerland) supplemented with 10% FBS, 4.5 g/l of glucose, 4 mM glutamine, 5 mg/l of puromycin and 400 mg/l of G418. CHO expressing FVII: ExCell 325 (JHR, USA) supplemented. BHK expressing FVII: DMEM/FHAM's F12 (1/1) (Gibco, UK) with 10% FBS, 4.5 g/l of glucose, 4 mM glutamine. Sf9 cells: Sf900I medium (Gibco).
Culture Systems: Static culture systems of 25, 75 and 175 cm² (Starsted, Germany) operated as batch cultures. Stirred cultures in spinners tanks of 125, 250 ml and 500ml (Wheaton and Corning, USA), agitation rate of 80 rpm.

Flow cytometric analysis: Cells stained in Vindelov's solution (1 g/l trisodic citrate (pH 7.6); 50 mg/l propidium iodide; 0.1% NP 40; 700 U/l RNase; 0.01 M NaCl). Flow cytometer: FACScalibur (Becton Dickinson). The laser was tuned to 488 nm and bandpass of 585/42 filter (FL2) was used for red fluorescence detection. Cell cycle was collected at FL2-H and the double discriminating module (DDM) was used. Cell cycle analysis was performed using FlowJo software (Tree Star Inc, USA); i) in order to discriminate debris and doublets the data was gated at FCS/SSC and FL3-A/FL3-W; ii) fitting with Watson Pragmatic algorithm; iii) CV all lower than 8%.



References

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