Steady state growth of Hela cells propagated in sonoperfused fedbatch (cytostat) mode Influence of the cell concentration on proteome, the splicing-and translation activities.

Lilia Ayadi[2], Christine Branlant[2], Hans Voshol[3], David Sergeant[4], Michael Moser[1], Alain Miller[1]

[1]CILBIOTECH sa, Mons, Belgium, [2]Laboratoire de maturation des ARN et Enzymologie Moleculaire, Vandoeuvre-les-Nancy, France, [3]Novartis Institutes for Biomedical Research, Basel, Switzerland, [4]IPRATECH sa, Mons, Belgium

Using an automated perfusion rate control system (Sergeant, D., 2007) comprised of an on-line capacitance probe (Fogale Nanotech, France) and a model 50L acoustic cell filtering device (Applisens, the Netherlands), Hela cells have been cultivated for extended periods successively at 5.10⁶-, 10.10⁶- and 25.10⁶ cells/ml (low-, middle and high cell concentrations respectively) in sonoperfused fedbatch (cytostat) mode (Fig.1).

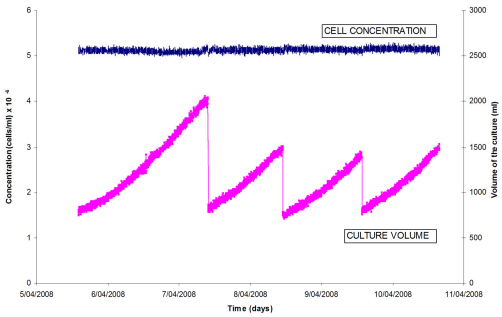

Fig.1: Hela cells continuously grown in sonoperfused cytostat mode at 5.10⁶ cells/ml. Because of the limited capacity of the bioreactor (3L), suspension must regularly be withdrawn.

The quality of cells grown under these steady state conditions is reproducible (Fig.2A).

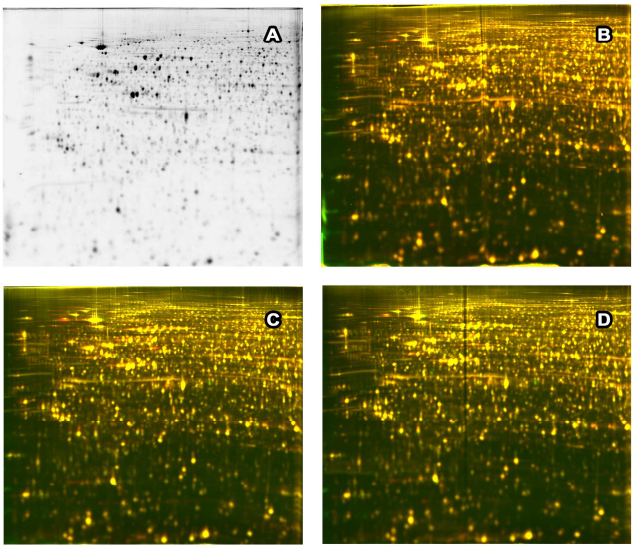

Fig.2: High resolution 2D PAGE proteome profile of whole Hela cell lysates:
A: representative proteome profile of a series of successive lysates from cells grown at 5.10⁶ cells/ml as described in Fig.1
B: 13.10⁶ cells/ml (green) and 27.10⁶ cells/ml (red)
C: 10.10⁶ cells/ml (green) and 13.10⁶ cells/ml (red)
D: 10.10⁶ cells/ml (green) and 27.10⁶ cells/ml (red)

For both the HIV-C2 (Ropers et al., 2004) and Adeno-Sp1 (Marchand et al., 2002) truncated transcripts, used in this work splicing activity as measured by the M/P (Messenger/premessenger) ratio is highest at 25.10⁶ cells/ml, Adeno-Sp1 being spliced more efficiently than HIV-C2. (Table I, II and Fig.3)

Table I: Influence of the cell concentration at which Hela Nuclear Extract (HNE) are prepared on the mean M/P ratio (HIV-C2 premessenger)

Table I: Influence of the cell concentration at which Hela Nuclear Extract (HNE) are prepared on the mean M/P ratio (HIV-C2 premessenger)

Cell concentration at sampling (cells/ml)		
Low: ≤ 5.10⁶ cells/ml	Medium: 10.10⁶ cells/ml	High: 25.10⁶ cells/ml
0.52 (n=14)	0.45 (n=3)	1.43 (n=3)

Table II: Influence of the type of premessenger on the splicing efficiency

Cell concentration (cells/ml)	HIV-C2	Adeno-Sp1
Low (≤ 5.10⁶ cells/ml)	0.28	3.14
Medium (10.10⁶ cells/ml)	ND	4.44
High (25.10⁶ cells/ml)	1.57	4.22
	1.38	4.06

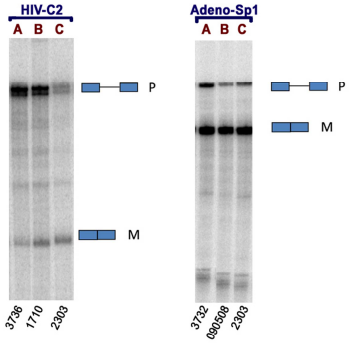

Fig.3: PAGE-based evaluation of the splicing activity of HNE prepared from cells grown in sonoperfused cytostat at low (A), middle (B), high (C) concentration.

For a given premessenger, the splicing activity very much depends upon the cell concentration at which the HNE is prepared. It increases some 5.6 fold and only 1.3-fold for HIV-C2 and Adeno-Sp1 respectively when the cells concentration shifts from low to high cell density.

Translation of capped tailed or untailed mRNA using the Hela cell-free system (Bergamini et al., 2000) shows the synergistic interplay between the 5'-m7GpppN-mRNA cap and its polyA tail (Fig.4). Maximum translation occurs at 10.10⁶ cells/ml.

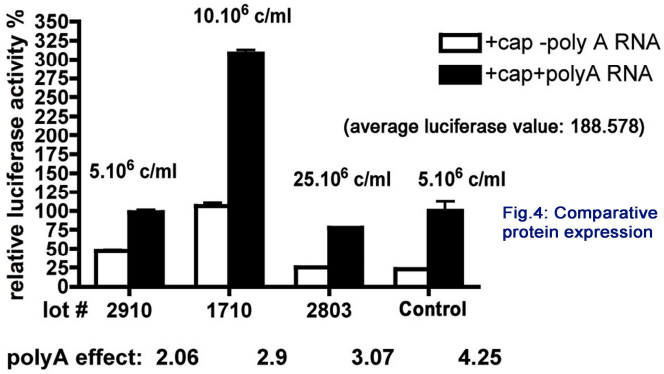

Fig.4: Comparative cell-free protein expression

Irrespective of the cell concentration at which the Hela cells are sampled, the cell-free translation activity is very labile. The reasons for this are sought.
High resolution co-electrophoresis of whole cell lysate stained proteins obtained from cells grown at 10.10⁶-, 13.10⁶- and 27.10⁶ cells/ml (Fig.2 B,C,D) shows the perfect match between the proteomes of 13.10⁶- and 27.10⁶ cells/ml samples (Fig.2B The overlapping red and green spots yield yellow spots only). Non-overlapping red and green spots in Figure 2C and 2D exemplify the difference between the proteome of 10.10⁶ Hela cells/ml with that of cells cultivated at 13.10⁶ (Fig.2C) and 27.10⁶ cells/ml (Fig.2D).

Conclusions:

Hela cells have been cultivated in sonoperfused fedbatch mode, maintaining for long periods the cell concentration constant at 5-,10- or 25 million cells/ml. Splicing- and cell-free translation maxima are observed with nuclear and cytoplasmic extracts prepared from cells grown at 25- and 10 million cells/ml respectively. There is a perfect coincidence between the high resolution 2D PAGE proteome profile of lysates from cells grown at 13.10⁶- and 25.10⁶ cells/ml which contrasts with the proteome of cells propagated at 10.10⁶ cells/ml. Cultivation of Hela cells at constant concentration under steady state conditions offers new means to study the molecular mechanisms underlying translation and splicing.

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