Virtosomes

Possible intracellular messenger

Mariapia Viola
Fondazione "Enrico Puccinelli"
ONLUS Research Center

Presence in various tissue

Virtosomes have been demonstrated in human and other mammalian, avian amphibian and tumour cells

They can be isolated from body fluids.
They may be involved in inter-cellular signalling

Circulating DNA in body fluids

First demonstration in blood in 1948

Many researchers have confirmed these results

They are being exploited for:

- Fetal disorder diagnosis
- Markers for different tumours
- Markers for pathological disorders
- Monitoring of treatment

Possible origin of circulating DNA

Nucleated erythrocytes
White blood cell breakdown
Breakdown of bacteria and viruses
Leucocytes surface DNA
Cell and tissue necrosis
Cell apoptosis
Cellular release of exosomes
Cellular release of transposons and retro-transposons
Parasite nucleic acids
Cellular release of **virtosomes**

Virtosome

The virtosomes were separated:

- from the cytoplasm using very mild homogenization;
- from the medium in which the cells are placed for at least 4 hrs.

The virtosomes were isolated from other components by ultra- centrifugation.

Virtosome structure

They are a cytosolic complex formed from newly synthesized:

- DNA
- RNA
- Proteins
- Phospolipids

Composition of virtosomes

	Cytosolic	Released
Protein:	41.01%	37.91%
DNA:	3.45%	4.65%
RNA:	35.09%	53.41%
PLs:	19.90%	3.94%

Cytosolic v Released Virtosomal Phospholipids

	Cytosolic	Released
	Mean values %	
PS	12.50	14.78
PI	19.23	16.25
SM	19.23	27.57
PC	22.58	20.69
PE	22.50	20.69

Sphingomyelin of cytosolic and released virtosomes

- P-Ls are associated with chromatin structure
- SM forms a large percentage
- SM's role in DNA transcription is documented
- SM is also linked to an RNase resistant RNA
- SM may aid the transfer of RNA to the cytoplasm

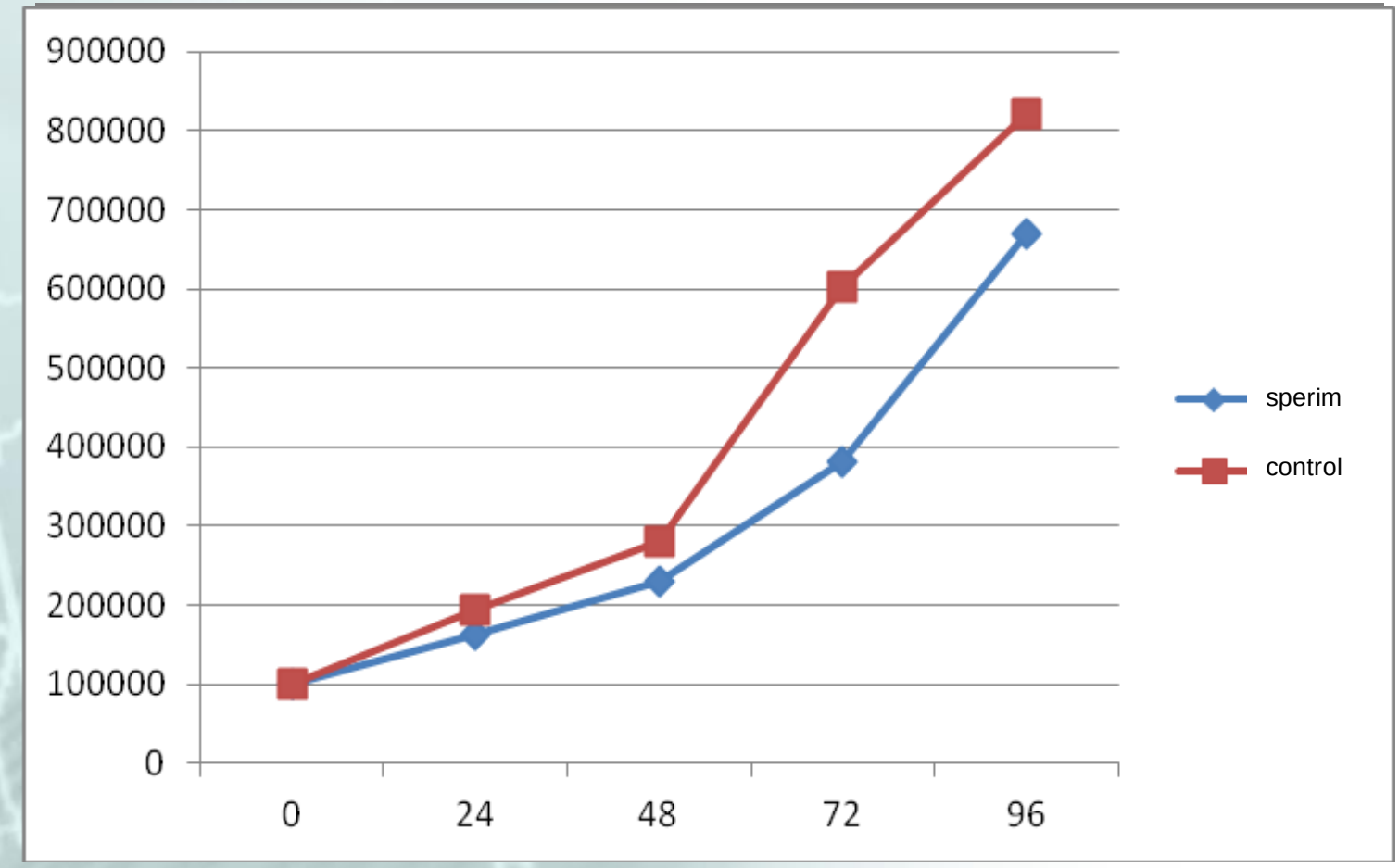
Virtosomal Function

Appears to act as intercellular messengers.

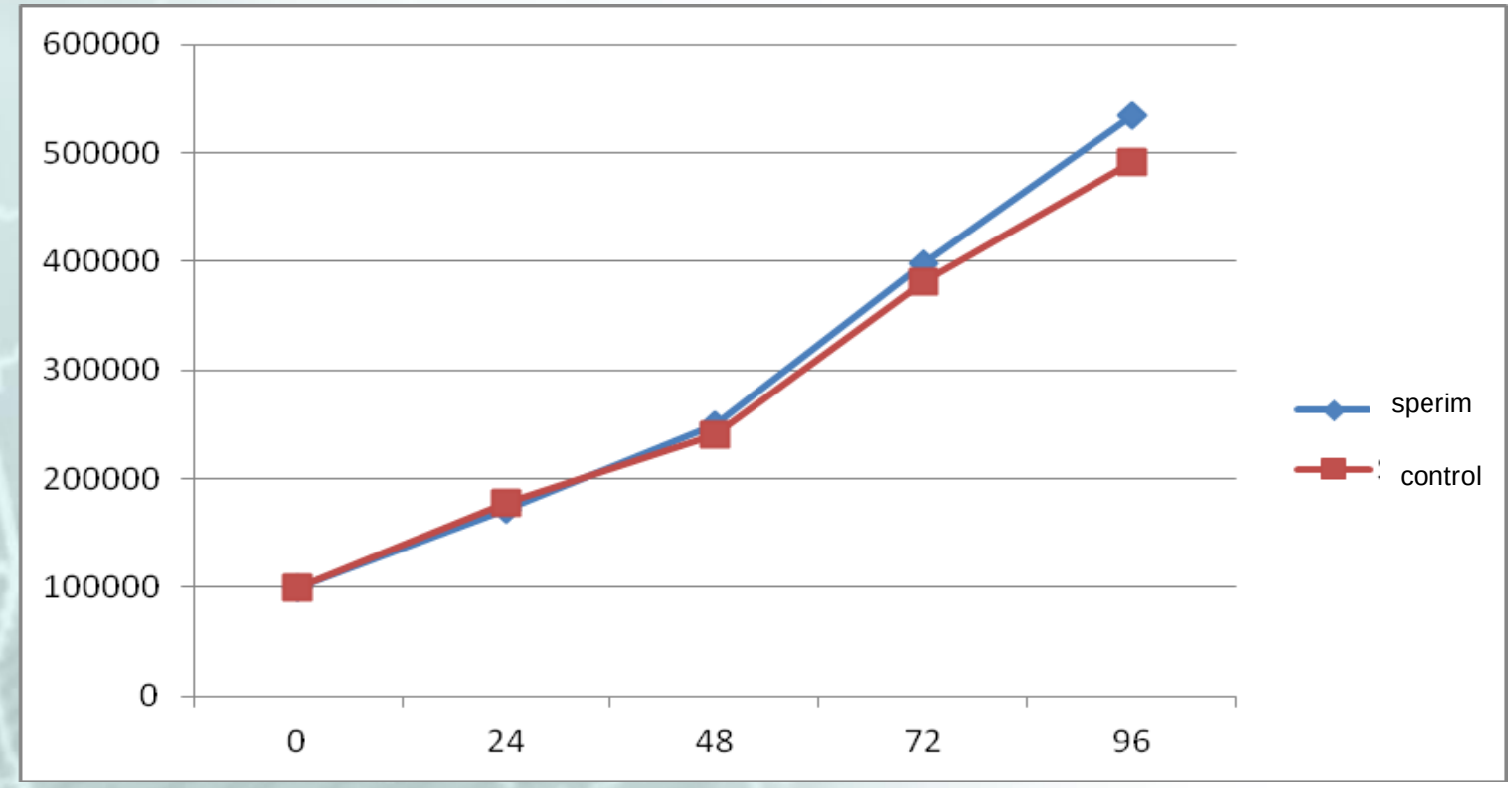
Virtosomes from non-dividing, non-stimulated lymphocytes block cell division in dividing stimulated lymphocytes, and vice versa.

We will use non-stimulated and stimulated lymphocytes in this study in order to compare the virtosomes from dividing and non-dividing cells as well as the differences induced on stimulation.

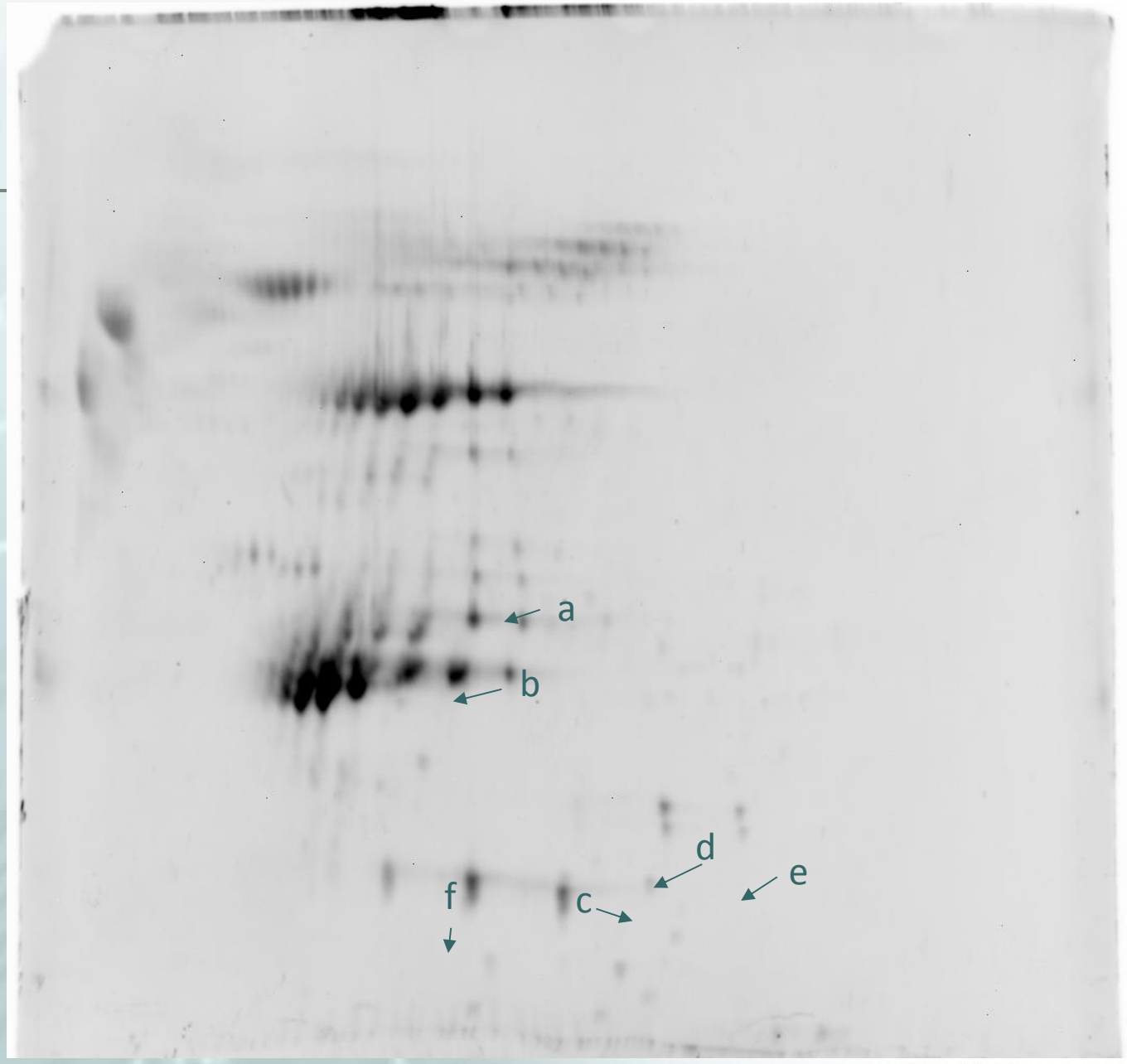
Effect of virtosomes derived from PHA stimulated cells



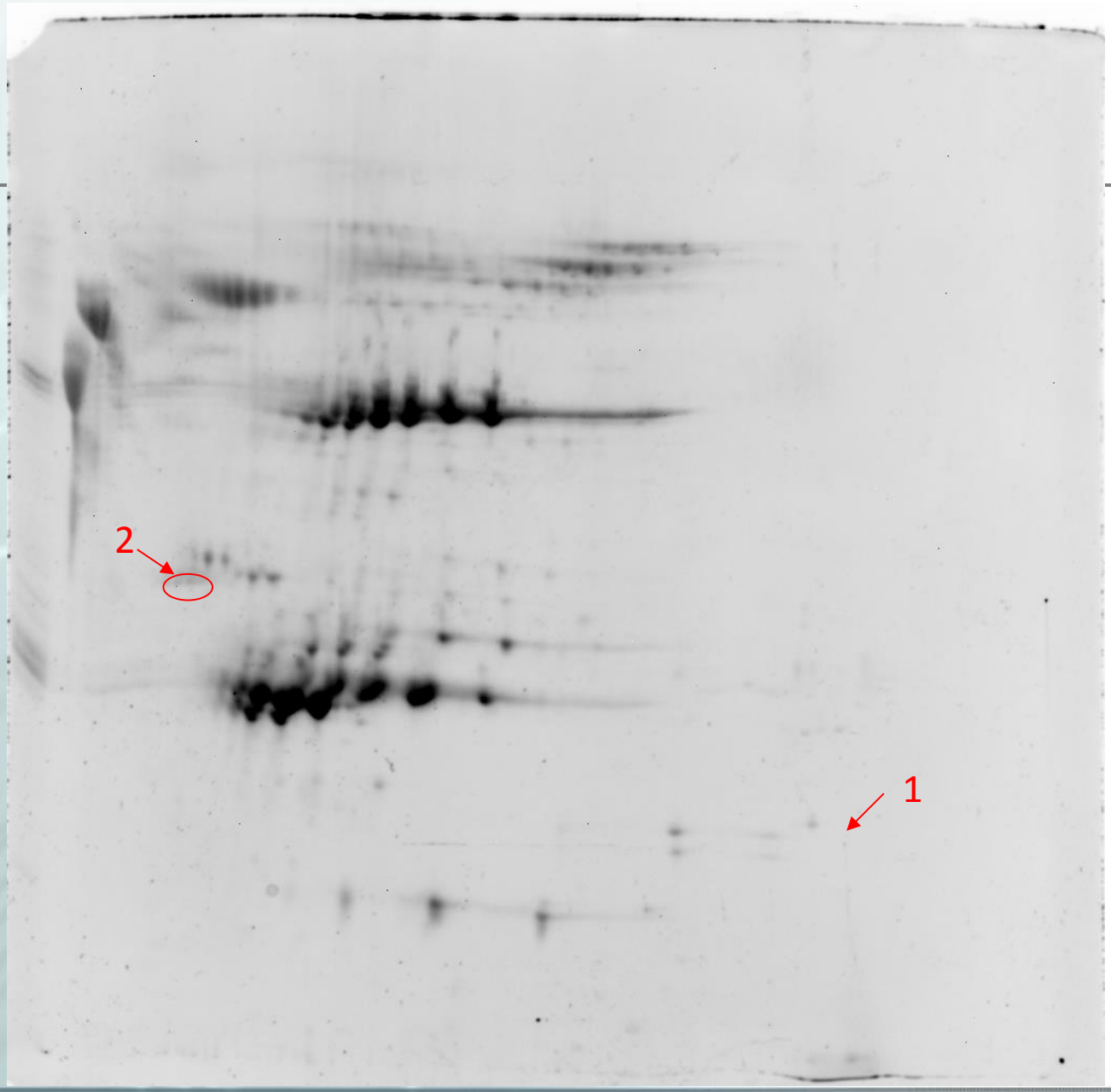
Effect of virtosomes from non stimulated cells on PHA Lymphocytes



Lymphocytes not Stimulated



Stimulated Lymphocytes



Two-dimensional gel electrophoresis (2D-electrophoresis) RESULTS

Stimulated lymphocytes, spot 1:

NFRKB_HUMAN Nuclear factor related to kappa-B-binding protein

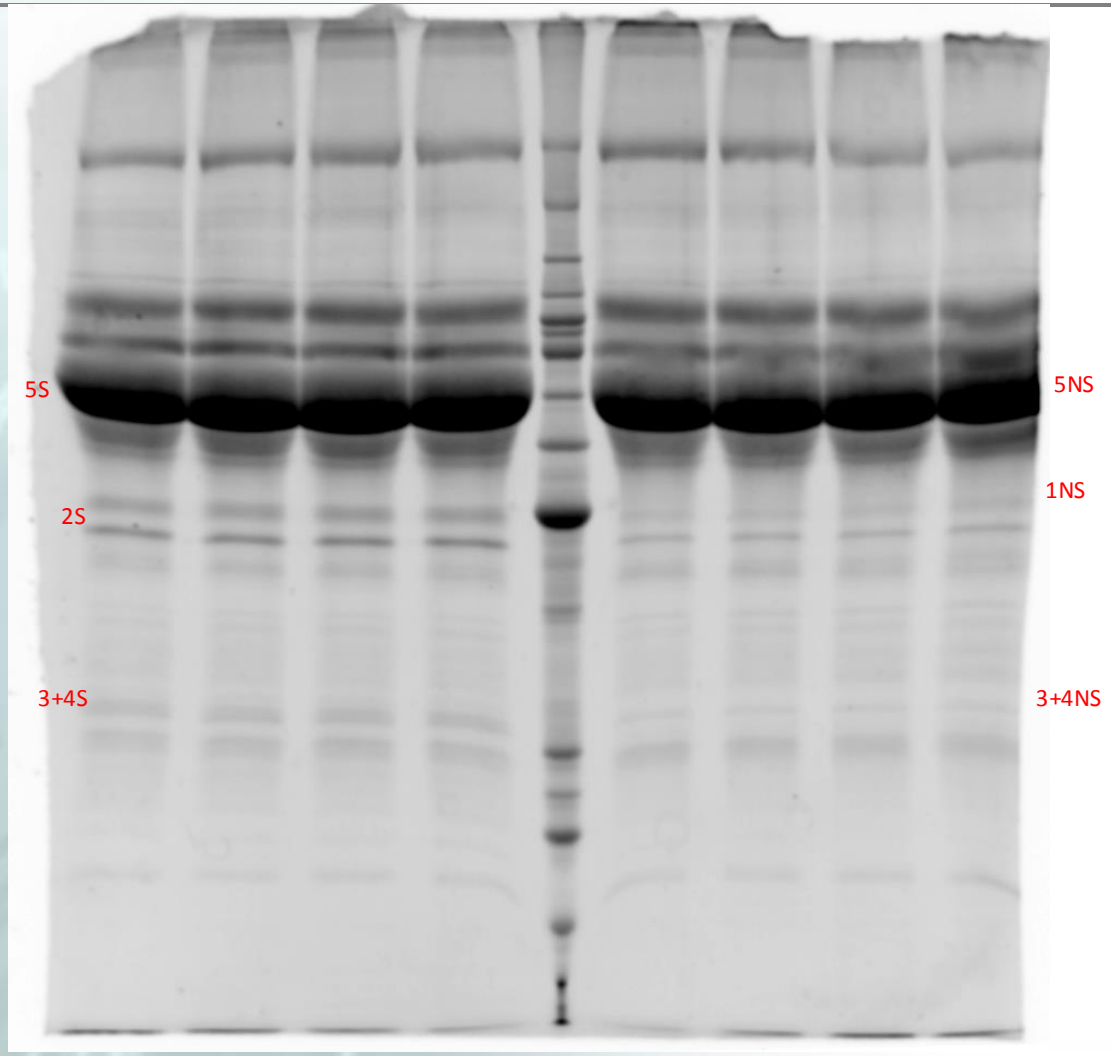
Non-stimulated lymphocytes, spot f:

RAI1_HUMAN Retinoic acid-induced protein

Gel Mono 50 ug /lane

Stimulated

NotStimulated



Specific protein in stimulated lymphocytes

The most interesting are:

- ZZNF 160 zub-nc finger protein 160 regulating transcription with affinity to the DNA and Zn present in another finger protein,
- PNPH purine nucleoside phosphorylase with a central role in purine metabolism

DNA duplication

This mobile DNA is most likely synthesized during G1/G0 phases.

It is synthesized in non dividing cells.

It may be related to differentiation.

It is synthesized possibly in relation to cell function

DNA changes during differentiation

Rat Hepatocytes :

amount of DNA/nucleus

- Foetal life 4.7^{x10-12}
- Neonatal period 7.7^{x10-12}

Non premitotic DNA synthesis

The synthesis of DNA happens during the first day of life and is accompanied by loss and new synthesis in the second day.

In this period the mitotic activity is very low (0.4%) in comparison to labelled cells (20%).

The synthesis of specific enzymes, like thyrosin transaminase, start from the second day of life

The virtosomes may explain the hepatocytes expression?

In the second day of rat life the hepatocytes are able to express new specific proteins.

In which way they comunicate without division?

The loss of DNA of the second day may be due to the release of virtosomes which favour the differentiation of the other cells

Contacts:

Fondazione ONLUS "Enrico Puccinelli"
Presidente/President
Prof. Mariapia Viola
Sede legale/Legal adress
Piazza Università, 1 Perugia 06123
ITALY
Sede operativa/ headquarters
Via Pietro Cestellini, 3
Ponte San Giovanni 06135 Perugia
ITALY
Tel. e Fax: 0039 075 572 21 94
Website: www.fondazionepuccinelli.org/
E-mail: presidente@fondazionepuccinelli.org
Skype: Mariapia Viola
For your 5 x 1000 CF: 94043090540