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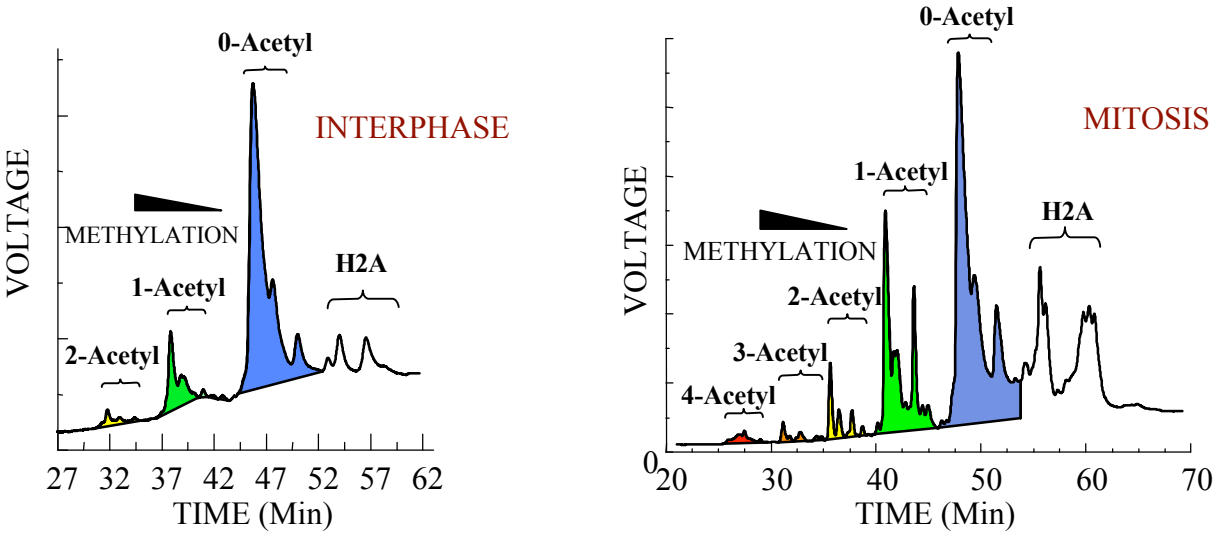
Bulletin for 2006

SEPARATE INTACT PROTEINS FOR PROTEOMICS

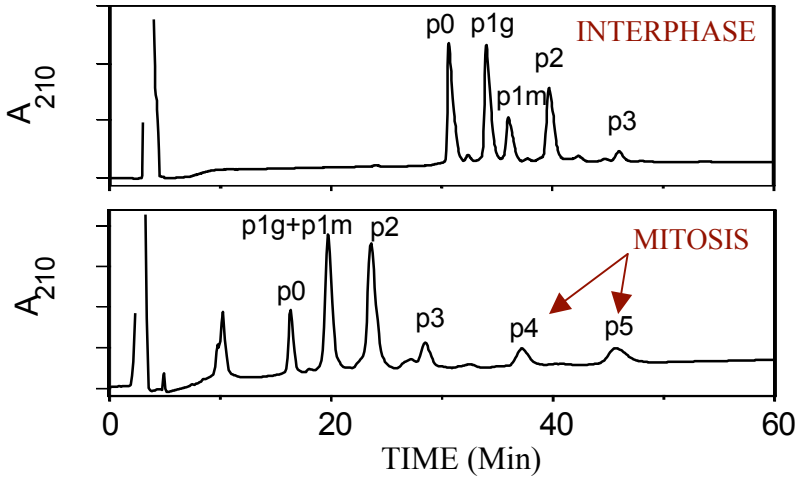
1) Histone H4 Acetylation & Methylation Variants



The most highly acetylated forms are present in the smallest amounts and for the shortest time, yet they are the most important in the cell cycle. It is only possible to detect and measure them by separating all forms by HPLC prior to digestion and MS analysis. COLUMN: PolyCAT A in CEX-HILIC mode.

- courtesy of James Pesavento and Craig Mizzen (Univ. of Illinois) -

2) Histone H1.5 Phosphorylation Variants



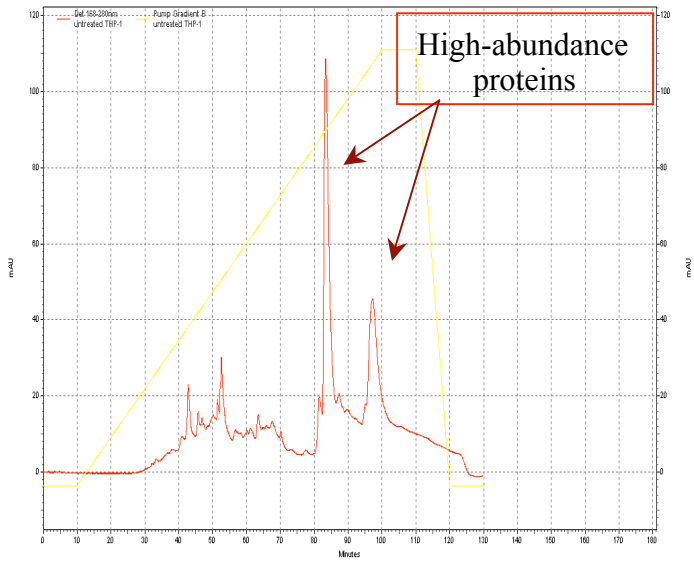
Again, the more highly phosphorylated forms, critical to the cell cycle, can only be detected and quantitated after separation. Phosphorylation proved to proceed in an obligate sequence of residues. COLUMN: PolyCAT A in CEX-HILIC mode.

- courtesy of Herbert Lindner (Univ. of Innsbruck) -

Why fractionate proteins prior to digestion?

1) Many extracts contain a few proteins of much higher abundance than the rest (as in the first example below). Fractionation before digestion permits you to collect those proteins in their own fractions. Their peptides will then not mask low-abundance peptides from the other fractions.

2) Suppose you distribute the proteins uniformly into ten fractions, then digest. The peptides from any protein within its fraction will represent 10x more of the total peptides than would have been the case with digestion of the unfractionated mixture. That greatly increases the chances of identifying more than one peptide from proteins of low abundance. Result: More rugged identifications.



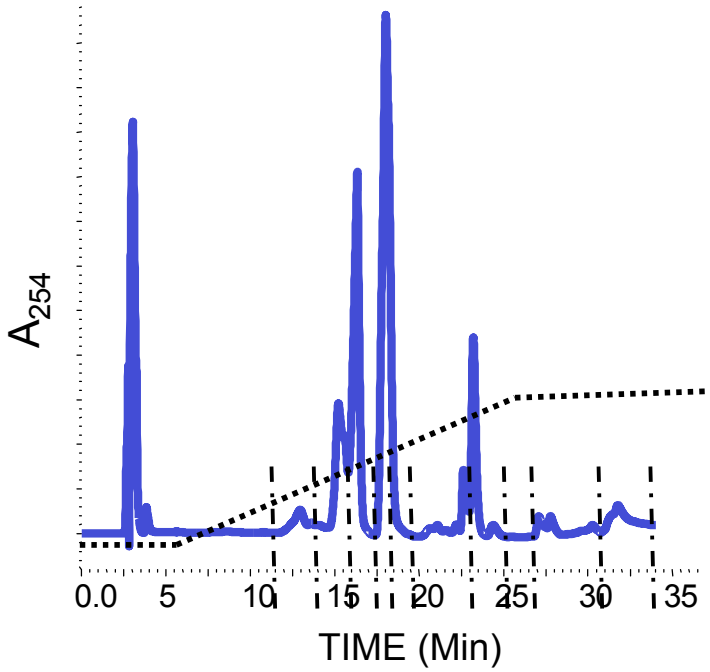
Water-soluble cell lysate.

Mixed-bed IEC of intact proteins from THP-1 Monocytes (~ $6x10^6$ cells)

Columns: PolyCAT A/ PolyWAX LP
(5 µm; 1000 Å)

Gradient: $NaClO_4$ A_{280}

- courtesy of Leticia Cano, City of Hope -



Water-insoluble membrane proteins

Pellet from mouse brain homogenate, solubilized with HFIP

Column: PolyHYDROXYETHYL A
(204HY0503; HILIC mode)

Gradient: 70-40% ACN in Tris.HCl,
pH 6.5, with 40 mM HFIP

PROTEINS IDENTIFIED (partial list):
Troponin I
Na/K ATPase α-1 chain
ADP/ATP carrier protein (isoforms T1 & T2)
Calpexin (pp90)
Neurofilament Triplet L

- courtesy Spiros Garbis, Academy of Athens -