Evidence that post-translational modifications control mammalian cell differentiation

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We know that the Central Dogma, "DNA makes RNA makes Protein," is the basis for all life on earth [1], and that DNA mutations are the root cause of cancer [2]. However, as noted by Roehrl et al., "Proteins are the true machines of life. Protein enzymes carry out virtually all complex chemical transformations in living organisms such as nucleic acid synthesis and replication, post-translational modifications (PTM), carbohydrate and lipid metabolism, hormone biosynthesis proteolysis, and many more" [3]. PTMs such as tyrosine phosphorylation (pTyr) are responsible in turn for animal multicellularity [4].

Considerable evidence has accrued showing expressed protein levels are mostly unrelated to mRNA levels. Cao et al. reported that for a proteogenomic characterization of 140 pancreatic cancer samples, the median correlation (Pearson's correlation coefficient) between mRNA and protein levels was 0.35 [5]. Stetson et al. reported that the average mRNA/protein agreement for 27 snap-frozen glioblastoma tumors was 0.22 [6]. Even for normal tissues the agreement between mRNA and protein expression is poor. Wang et al. reported that for a proteome/transcriptome analysis of 13,413 protein coding genes in 29 healthy human tissues, the median correlation was 0.35. Some proteins could not be detected for highly expressed mRNAs, and some mRNAs could not be detected for highly expressed proteins [7].

The discrepancy between mRNA and protein levels is likely the consequence of metazoan evolution over two billion years [8]. The four human tissue types: epithelial, muscle, nervous, and connective, are made up of about 200 distinct cell types [9], all with the same nuclear genome [10] but different sets of expressed proteins. Thus, "When and where a protein is synthesized in an organism is as important as the protein's function" [11, 12].

Genome analysis has revealed the existence of multiple gene families whose expressed proteins have similar functions as shown in Table 1. The family members, scattered throughout the genome, are likely expressed at different times and in different tissues during embryonic development. About 5% of human genes (588 proteases and 377 E3 ubiquitin ligases) code for proteins that break down other proteins. This speaks to the importance of protein turnover in mammalian tissues.

Post-translational modifications (PTMs) are key to protein turnover timing. Protein phosphorylation is the most common PTM [13]. Interestingly, while there are many more serine/threonine kinases (428) than phosphatases (30),the reverse is true for tyrosine: there are more tyrosine *phosphatases* (107) than kinases (90). Since trans-phosphorylation of key tyrosine residues on RTKs creates binding sites that control the location/actions of many cytoplasmic signaling proteins [2], dephosphorylation of pTyr-RTKs is an important regulatory event. Malfunctions of tyrosine phosphatases are likely oncogenic, as has been reviewed by Zhao et al. [14].

The fact that the serine-threonine kinase/phosphatase ratio (428/30) is so high suggests that this post-translational modification (PTM) is common and mostly irreversible. This hypothesis is supported by the fact that serine/threonine phosphorylation is the most abundant PTM of the human

proteome. About 13,000 (68%) of the ~19,000 proteins in the human genome are phosphorylated on either serine or threonine residues [15].

Human protein families that perform key primary functions (RBP, TF, GPCR and IF), or activate/inactivate other proteins via post-translational modifications (PTMs, shaded)	Number of family members
RNA-binding proteins (RBP) [16]	2,961
Transcription factors (TF) [17]	1,639
G protein-coupled receptors (GPCR) [18]	826
Intermediate filaments [19] (IF, cell shape)	70
Proteases [20]	588
Serine/threonine kinases [21]	**428**
Serine/threonine phosphatases [22]	**30**
E3 ubiquitin ligases [23]	377
Glycosyltransferases [24]	244
Tyrosine phosphatases [25]	**107**
Tyrosine kinases (58 RTK & 32 non-R-TK [26]	**90**
Histone acetylation modulator proteins [27]	73
2-OG-dependent dioxygenases [28]	70
Histone lysine methyltransferases [29]	50
DHHC3 Palmitoyltransferases [30]	23
Glutathione S-transferases [31]	16
Sulfotransferases [32]	13
NSD protein methyltransferases [33]	3
EGLN prolyl hydroxylase [34]	3
Total	7611 (40%)
Total shaded	2115 (11%)
Total protein-coding genes in human genome [35]	19,202

Table 1. Gene families whose expressed proteins regulate cell differentiation by controlling activity, expression, and/or PTMs (shaded rows) of other proteins. TFs control which genes are transcribed into mRNA. RBPs regulate the translation of mRNA transcripts into proteins. GPCRs transduce extracellular signals into physiological effects via G proteins. IFs control cell shape and migration. The next fifteen rows (shaded) show protein families with 2115 members that post-translationally modify other proteins. This table is incomplete, showing only 40% of protein-coding genes.

In the textbook "Molecular Biology of the Cell" (7th edition, p 401), Alberts et al. discuss seven control points for pathways that bring about cellular differentiation as shown in Table 2 [36]. The fact that the PTM protein families in Table 1 match well to the seven control points described by Alberts et al. (Table 2) suggests that PTM timing is key to cell differentiation.

Control Point	Cell Process from Molecular Bio textbook [36]	Controlling Protein Family	# of Family Members	Ref
1	DNA transcription	Transcription Factors	1,639	[17]
2	RNA-processing including alternative splicing	RNA Binding Proteins	2,961	[16]
3	mRNA transport/localization, from nucleus to cytosol			
4	mRNA translation into protein			
5	mRNA degradation			
6	protein degradation	Proteases	588	[20]
		E3 ubiquitin ligases	377	[23]
7	protein activity control	G protein-coupled receptors	826	[18]
		serine/threonine kinases	428	[21]
		serine/threonine phosphatases	30	[22]
		tyrosine kinases	**90**	[26]
		tyrosine phosphatases	**107**	[25]
		glycosyl transferases	244	[24]
		Histone acetylation	73	[27]
		2-OG-dependent dioxygenases	70	[28]
		Histone lysine methyltransferases	50	[29]
		Glutathione S-transferases	16	[31]
		Sulfotransferases	13	[32]
		NSD methyltransferases	3	[33]
		EGLN prolyl hydroxylase	3	[34]
	Cell shape & migration	Intermediate filaments	70	[19]
		Total	**7,611 (40%)**	
		Total protein-coding genes in the human genome	19,202	[35]

Table S2. Correlation of cell processes described in the textbook by Alberts et. al. [36] by protein PTM families comprising 40% of the human genome. code for proteins that regulate one or more of the seven control points to achieve differentiation of 200 cell types. A huge family of 1639 transcription factors bind DNA to stimulate or inhibit gene transcription (control point 1). An even larger family of 2961 RNA binding proteins regulates mRNA alternative splicing and subsequent translation into proteins (control points 2-5). The protease gene family (588 members), along with ubiquitin ligases (377 members) regulate protein degradation (control point 6). Finally, various protein families including some not shown, regulate protein activity especially serine/threonine phosphorylation by 428 serine/threonine kinases along with tyrosine phosphorylation regulated by 90 tyrosine kinases (control point 7).

While the root cause of cancer is DNA alterations that lead to uncontrollable growth [37], linking specific DNA “driver” mutations to drug targets is often difficult because, as noted, mRNA

transcript abundances correlate poorly with protein abundances for both healthy [7] and cancerous [6, 38] human tissues.

At last count, 40 cancer drugs are available that target receptor tyrosine kinase proteins [39]. Regardless of NGS mutation status and drug availability, however, RTKs must be expressed, dimerized by the presence of the corresponding growth factor (e.g., epidermal growth factor) and specific tyrosines trans-phosphorylated to allow binding of signaling proteins containing Src Homology 2 (SH2) and phosphotyrosine-binding (PTB) domains. Mutated RTK proteins lacking tyrosine phosphorylation will be inactive, and the matching cancer drug will fail. Thus, correlation of **pTyr-RTK proteoforms** in cancer biopsies with NGS mutation patterns might assist in finding new genome biomarkers that predict drug response.

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