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Decoding neuroproteomics: integrating the proteome with the transcriptome and genome

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but almost all have reported values between 50-70%;

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aim to more tightly integrate analyses of the proteome with the transcriptome. Experimental methodologies such as ribosome profiling, single molecule RNA-sequencing, and top-down MS/MS profiling of intact proteins enable greater selectivity and sensitivity to molecules that are actively involved in the process of protein production and greater specificity to the exact structure of these transcripts and isoforms. Similarly,

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Utilising ribosome footprinting to identify coding sequences and translation initiation sites has produced moderate increases in the yield of peptide spectra in the same samples[58].

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information obtained from various

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dynamics cellular processes through

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Alternative RNA-splicing (AS) is well known to be a highly tissue-specific process[9] that greatly increases the complexity of the potential set of RNA molecules produced from multi-exon genes. Splicing in the brain has been extensively profiled using transcriptomics and specific instances of alternative transcript usage have been implicated in neuropsychiatric disorders[78]. During CNS cell development, for example,

alternative splicing (AS) in neuronal progenitor stem cells has revealed different isoform usage at different stages of maturation to the final neuronal state[79]. The differential expression of DISC1 isoforms that are associated with schizophrenia[80], not to mention the translocation itself[81]. Validation of splicing events by MS/MS is quite common, either in terms of determining the contribution of known isoforms in a given experiment [ref], or verifying the existence of novel exon junctions identified by RNA-sequencing[82].[1]

A recent result that potentially has a significant impact on both the future analyses of the CNS and interpretation of proteomics data is the finding that most genes, in a given cell-type or tissue, tend to express only a single dominant transcript/isoform[83]. The myelin basic protein (MBP), for example, expresses a completely different isoform in the brain compared to all other healthy human tissues[83]. This has a direct impact on the so-called interactome, in which differential isoform usage between CNS cell types and during the progression of neurological disorders affects protein-protein interactions, as exemplified in the Autism Spliceform Interaction Network[84]. Currently full-length transcript[85,86] and isoform[87] profiling technologies are immature[88], however an early example of full-length transcript profiling in the brain directly observed the various isoforms of neurexin, showing their production mediates distinct protein interactions across the synapse[89]. Such investigations will only become more common, and have great potential to significantly simplify the process of integrating and interpreting genome-wide measurements of RNA and protein in the near future.

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There have been many proteomic studies of the CNS by MS/MS, and these have been enumerated in previous reviews[88-90]. More recent applications of MS/MS to the CNS include the profiling of neural tissue in model organisms such as the fruit fly[91] and neurodegeneration in zebrafish[92], as well as profiling cultures of specific CNS cell-types in primary culture such as neurons[93] and oligodendroglial cells[94]. Fluorescence activated cell sorting (FACS) of microglia has provided more than 100 genes that are enriched compared to neurons and other oligodendrocytes[95] and enabled MS/MS assessment of synaptic proteins[96]. A novel method, fluorescence activated nuclei sorting (FANS), in combination with an antibody against the neuronal-specific splicing protein, NeuN, has been successfully applied to purify neuronal nuclei from primary tissue in order to provide a quantitative comparison of the abundance of nuclear proteins with astrocytes and oligodendrocytes[97].

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Monitoring rates of protein production and degradation can be achieved fairly straightforwardly in cultured cells by pulse-labelling followed by SILAC proteomics[98]. Similarly, nascent peptide chains can be captured as they are synthesised by the ribosome using a biotin-puromycin labelling approach[99]. However these approaches do not lend themselves to assessment of rates of protein turnover in mammalian and human tissues. Integration with transcriptome profiling, specifically ribosome profiling, is an increasingly attractive proxy to direct measurement of protein synthesis. Similarly, non-invasive imaging methods are potentially valuable methods by which differential rates of protein synthesis can be repeatedly observed in the mammalian CNS[100], both over long and relative short periods of time.

The major obstacle to any genome/transcriptome/proteome wide study of neuronal cells derives from their immense heterogeneity.

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There are methods to monitor protein dynamics, but these are not particularly applicable to the mammalian CNS.

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