2. Turnover of proteins

The word “turnover” is used widely in many fields including biology and economics. For proteins, it is used as a concept of replacement of ‘old’ proteins by degradation with ‘new’ ones by synthesis. Here, we discuss protein turnover without detail mechanisms of degradation and synthesis.

In early works of turnover, radio-labeled amino acids were used (Borsook, H., Physiol.Rev., 1950; Lajtha, A., et al., J.Neurochem., 1957), and half-lives (τ_h) of histone were reported to be 54 and 104 days in brain, and 18, 56, and 93 days in liver (Piha, R.S., et al., J.Biol.Chem., 1966). The development of technologies enabled analyses of larger population of proteins showing log-normal distribution of τ_h with median of 43 min for proteins of 3,751 budding (Belle, A., et al., PNAS, 2006). They also reported that proteins with shorter and longer τ_h was serin- and valine-rich, respectively, and length of amino acids and τ_h were negatively correlated. τ_h of 43 min is short, which may be correlated with a short cell cycle of budding east (~1.5 hrs).

Further technological advancement enabled analyzing 8,000 protein species in a single cell resolution of HEK-293, and showed bimodal distribution of τ_h with 0.5 and 2 hrs (Yen, H.-C. S., et al., Science, 2008). They saw strong positive correlation between τ_h and length of amino acids. Shorter and longer τ_h proteins were rich in amino acids W, C, L, T, F, W, Y, V and E, D, K, N, R, Q, respectively. Their result of correlation between amino acid length and τ_h is contradictory to a report by Belle, 2006, and it seems that the mechanism and physiological significance for this correlation are not clear.

Relation of τ_h and mouse organs and protein functions were reported using a method called SILAC (Price, J.C., et al., PNAS, 2010). They reported τ_h of 9.0, 3.0, and 3.5 days for brain, hepatis, and blood, respectively. Cambridge, et al. reported that there was a high correlation in τ_h between orthologs of human and mice by measuring 4,106 and 3,528 protein species in HeLa and C2C12 cells (Cambridge, S.B., et al., J.Proteome Res., 2011).

In addition to a report by Price, et al. above, additional review on a relation between τ_h and protein function appeared, showing that 1) τ_h of 0.5-35 hrs and 43 hrs for mammalian dividing and non-dividing cells, respectively, 2) average τ_h of 3-9 days in brain, liver, and blood of mice (life time ~1.5 yeas), 3) whereas the peripheral Nups of the NPC (nuclear pore complex) are continuously turned over, the scaffold of the NPC built during embryogenesis lasts the lifetime of the worm (Toyama, B.H., et al., Nat.Mol.Cell Biol., 2013). Mathieson, et al. reported wide range of τ_h from 10-1,000hrs in mice, showing 1) τ_h for B cells, NK cells and monocytes were similar, 2) τ_h for hepatocytes and embryonic nerve cells were in large difference, 3) τ_h for histone complex was longer with some exceptions, 4) τ_h for proteasome and NPC were dependent on their components (Mathieson, T, et al., Nat.Comm., 2018). Choi, et al, reported τ_h of 0-144 hrs for 3T3-L1 adipocytes with longer or shorter τ_h for proteins of PPARc pathway, energy metabolism, ECM, ubiquitin-dependent degradation, splicing, Golgi

complex, and lysosome-related proteins (Choi, S., et al., J. Proteome Res., 2023).

Resolution of analyses advanced, and cell type-dependent, subcellar compartment-dependent, and protein function-dependent τ_h were analyzed showing that 1) τ_h for membrane protein was short compared to that for synaptic proteins, 2) τ_h for mitochondrial proteins was longer, 3) τ_h for glia was shorter than neuron, and 4) glial cells had some effect on the τ_h of neurons (Dörrbaum, A.R., et al., eLife, 2018). Recently, τ_h of more than 3,000 protein species in liver, skeletal muscle, cartilage, mucosa, and blood in young adult rats were analyzed using isotope-labeling and mass spectrometry combined with original analyzing program (Rolfs, Z., et al., Nat.Commn., 2021). They found that 1) τ_h distributed within a range from <1 - several hundred days, 2) shortest in liver with single peak (median 3.6 days), 3) longest in skeletal muscle with dual peak (median 10 days). Importantly, they found significant alteration in τ_h by post-transcriptional modification of acetylation, methylation, phosphorylation, hydroxylation, and carboxylation.

Regulation of τ_h by environmental signals and/or specific stimulus is important. On the contrary, a protein turnover could alter signal transduction in cells (Yen, H.-C, S., et al., Science, 2008). Relation between τ_h and diseases is also reported, in which τ_h was altered by cancer, neural degeneration, cell senescence, and aging (Basisty, N., et al., 2018; Savas, J.N., et al., Methods Mol.Biol., 2016; Toyama, B.H., et al., Nat.Mol.Cell Biol., 2013; Rolfs, Z., et al., Nat.Commn., 2021).

From these reports, we find advances in the research on protein turnover from limited to comprehensive view, that is, organs/animals to single cells/cell compartments, bulk protein species to single protein species/post-transcriptionally modified proteins, phenomenal to disease/aging/drug-discovery specific. Synthesis and degradation of proteins are ultimate methods to regulate their activities. It is interesting to find usage discrimination of this type of regulation from post transcriptional regulation.

Concept of protein turn over: Protein turnover is related to a concept of "Dynamic Equilibrium", in which an internal state is kept constant if temporal integration of inward and outward flows is equal, irrespectively to their speed. Thus, this concept shed little light on the speed of replacement. Since I didn't realize the limitation of this concept clearly, I was skeptical about a hypothesis by E.R.Kandel (Nobel Prize in Physiology or Medicine, 2000), who proposed that protein synthesis was involved in synaptic plasticity triggered by stimulus in a time scale from sub-second to minute*. In late 1980s, J.C.Eccles (Nobel Prize in Physiology or Medicine, 1963) visited Japan, and I had a chance to talk to him asking his view on this hypothesis. If I remember, he had also some skepticism on the hypothesis. However, Park et al. reported the protein increase 5 min after stimulation which induced synaptic plasticity (Ashall, L., et al., Science, 2009). Our understanding on cells is advancing.

* If I remember, this hypothesis was proposed for the maintenance phase of synaptic plasticity. Thus, gene expression and proteins were assumed to be stable for some period of time.