# Metabolism of cell surface heparan sulfate proteoglycans

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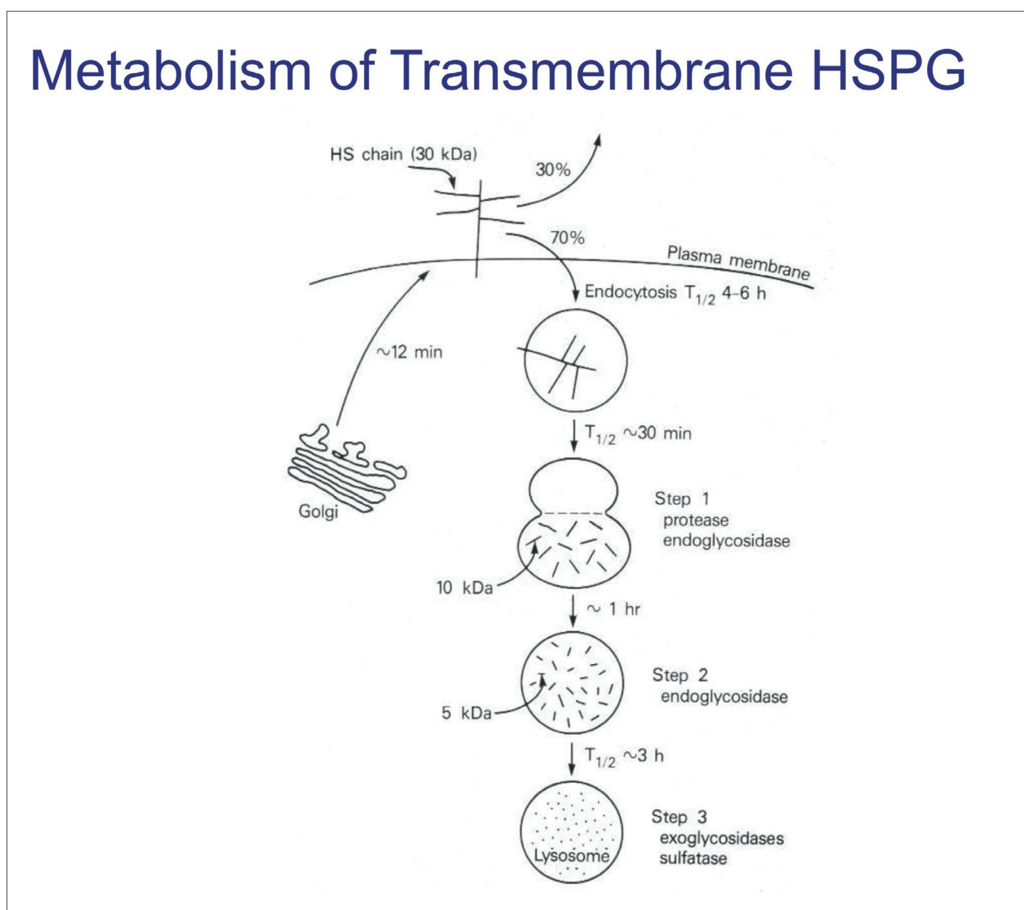
We have extensively studied anabolic and catabolic turnover of cell surface heparan sulfate proteoglycans using metabolic radiolabeling experiments in various cell culture systems. Following are some of the experimental, mostly technical, details which have not been discussed in publications but critically endowed significance to many of my works.

The use of radioisotopic labeling with [$^{35}$S]sulfate provided superb experimental tools in maneuverability, which was fundamentally based on small intracellular PAPS pools with a very rapid turnover. In addition, miniscule detection limits and vast dynamic ranges in radioactive detection allowed accurate monitoring of isotopes throughout the experiments. Furthermore, prominent molecular characteristics of proteoglycans, namely, high negative charges in glycosaminoglycans (plus additional luck in the differences in the extent of sulfation between heparan sulfate and chondroitin sulfate) and their large hydrodynamic sizes assisted easy chromatographic isolation of target molecules. For experimentalists, these molecular properties are




all fortunate yet natural attributes of the proteoglycans. Critically, however, without the help of a group of reagents, i. e., detergent, most of our proteoglycan metabolic studies were not practically meaningful, if not impossible. The important contribution of the detergents was their ability to improve recoveries of proteoglycans in chromatographic procedures even to quantitative levels. It consequently demanded the radioactive counting as a serious business; and the fallout was that we started to see what's really going on in the cell culture dishes and in the test tubes. Initially, the use of detergents in chaotropic solvents (many chromatographic analyses of proteoglycans were done in chaotropic solvents, e. g., 4 M guanidine HCl, to prevent their strong ionic interactions) was discouraged by convention. Fortunately, I was sufficiently unconventional to try experiments with unpromising prospects. Voila! Greatly improved chromatographic recoveries of proteoglycans were the results. Additional advantage of using detergents with chaotropic solvents included solubilization of cell surface, i.e., hydrophobic, proteoglycans in monomer forms. Thus, the use of detergents laid down important fundamentals required for the quantitative analyses of cell surface proteoglycans.

Another advantage of the experimental system I used worth mentioning was, I believe, the use of primary cell cultures; the (rat ovarian) cells were prepared every time for the experiment. I guessed that cells fresh from their physiological states are more stable in functions and could tell more about something related to physiology.