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COMMENTARY

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Dynamic tandem proximity-based proteomics—Protein trafficking at the proteome-scale

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new methodology, proximity labeling, TransitID

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Over the last six decades, protein fate in cells and tissues has been mostly investigated using imaging and biochemical approaches. Even though these approaches have produced major advances in the understanding of protein trafficking within and between cells, they have often focused on a very limited number of proteins and small-scale experiments. Experiments investigating protein trafficking mostly relied on (i) pulse-chase metabolic labeling with radioactive amino acids (e.g., ^{35}S-methionine, ^{3}H-leucine) followed by either immunoprecipitation or electron microscopy and autoradiography,[1,2] (ii) protocols of transport synchronization based on temperature blocks or on temperature sensitive cargoes; (iii) live-imaging using single fluorescent proteins,[3] or (iv) reconstitution/complementation assays.[4]

More recently, several approaches have been developed to investigate protein trafficking on a larger scale. These include inference from high-throughput mass-spectrometry or antibody-based proteomics studies,[5–7] functional imaging screens using fluorescent proteins[8] or from high-throughput screens for exploring protein trafficking using engineered genetic tools (e.g., based on transcriptional readout reporters).[9]

Approaches that have provided a better understanding of global molecular mechanisms underlying protein trafficking in cells have been complemented by additional studies based on proximity labeling (PL) strategies. Indeed, recent developments in the use of PL approaches (with BirA*, miniTurbo, turboID, or APEX fusion proteins) have produced comprehensive PL-based mapping in mammalian cells[10] and even proximity-based labeling of protein trafficking in vivo in Drosophila.[11] Several other PL-based approaches combined with subcellular fractionation have also extended these approaches to in vivo studies in mice.[12,13]

In a very recent resource article in Cell, Qin, et al.[14] describe a very elegant and versatile methodology, named TransitID, which enables the monitoring of protein trafficking from a donor to an acceptor compartment or cell, on a proteome-wide scale. The approach relies on the use of proximity labeling mediated by two kinds of PL enzymes, namely TurboID and APEX2 (see Figure 1). The procedure involves the following steps:

First, the proteome in a donor compartment (localization #1, Figure 1) is biotin-labeled with a compartment-resident TurboID-tagged protein. Then, the fraction of the proteome that traffics to the acceptor compartment (localization #3) is labeled with alkyne phenol (AP) by an APEX2 protein resident in the acceptor compartment.

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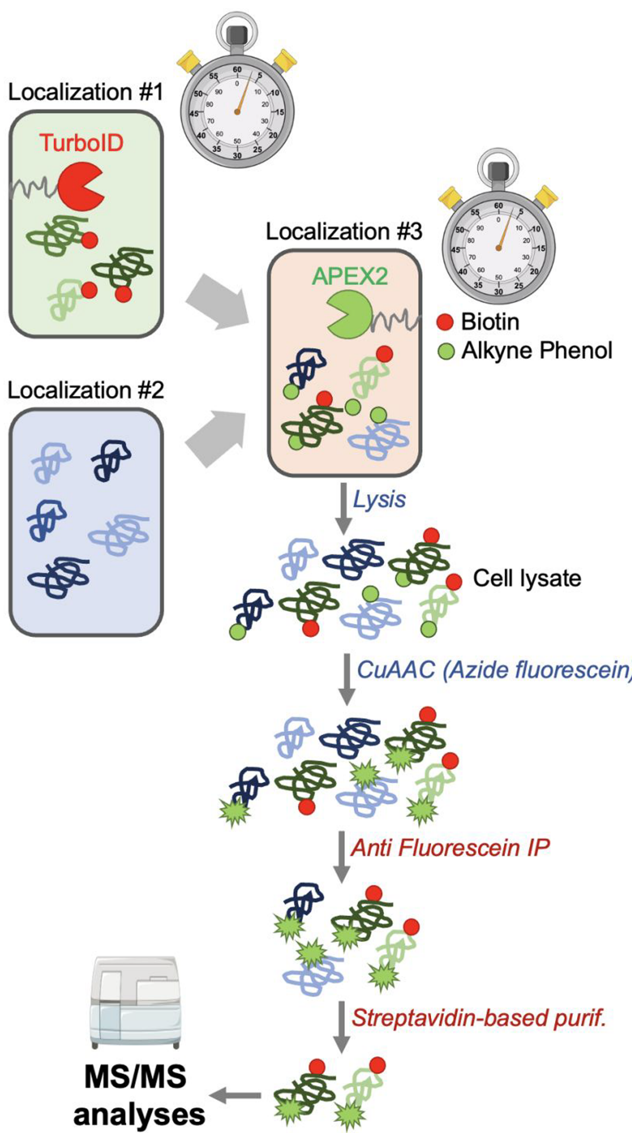

FIGURE 1 Schematic representation of TransitID. Proteins from two donor compartments (localizations #1 and #2) with only one bearing a TurboID (localization #1) traffic to an acceptor compartment (localization #3) containing APEX2 and are doubly labeled with biotin and alkyne phenol over a specific time frame (chronometers). Labeling with biotin should precede alkyne phenol addition (different pulse-kinetics with biotin and alkyne phenol). The presence of alkyne allows for click-based modification with an azide fluorescein tag in the cell lysate. Fluorescein-tagged proteins are first purified using anti-fluorescein-based immunoprecipitation of the lysate followed by streptavidin coated bead-based pull-down of the first eluate resulting in the enrichment of doubly-labeled proteins, corresponding to their presence in both donor and acceptor compartments. These purified proteins can then be analyzed using tandem mass spectrometry.

Proteins may also traffic from other donor compartments (e.g., localization #2) but are not biotin-labeled. Of note, the development of the double-labeling was facilitated by the use of AP as an APEX2 substrate rather than biotin phenol (BP). APEX2-mediated transfer of AP appears to be five times more efficient than that of BP.

Next, proteins with the alkyne tag (i.e., APEX2-labeled proteins) undergo click-based derivatization by azide-containing tags. The authors demonstrated that in this case the most efficient and selective tag was azide fluorescein. Subsequently, proteins labeled with both fluorescein and biotin are enriched using sequential immunoprecipitation and pulldown with anti-fluorescein antibodies and streptavidin-conjugated beads, respectively. The enriched proteins are then analyzed using tandem mass spectrometry (Figure 1).

Using this very elegant approach, Qin, et al. investigated protein trafficking between subcellular compartments such as the nucleus and mitochondria, the cytosol and nucleus (upon stress), or between stress granules and nucleoli (upon stress and recovery). Inter-cellular transfer was demonstrated between tumor cells and macrophages.

The authors critically analyzed TransitID, specifically by pointing out limitations related to the labeling times of TurboID and APEX2. This restricts the dynamic range of the time course that can be used, as well as the depth of labeling coverage when both enzymes are used in tandem. However, it is reasonable to expect that future improvements of proximity labeling will overcome these limitations. This might involve expanding labeling schemes, improving TransitID sensitivity, exploring new combinations of donor and acceptor compartments, testing other model systems (cellular, plant, animal) and finally expanding the repertoire of the trafficked material. In conclusion, Qin, et al.[14] have developed a very versatile methodology that will certainly be of great value to dissect trafficking events on a global scale.

AUTHOR CONTRIBUTIONS

Eric Chevet wrote a first draft of the manuscript. Hesso Farhan, Maria Antonietta De Matteis, Eeva-Liisa Eskelinen read and modified the text.

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CONFLICT OF INTEREST STATEMENT

Eric Chevet is a founding member of Thabor Therapeutics.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/tra.12914.

DATA AVAILABILITY STATEMENT

Data sharing not applicable - no new data generated.

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REFERENCES

1. Warshawsky H, Leblond CP, Droz B. Synthesis and migration of proteins in the cells of the exocrine pancreas as revealed by specific activity determination from radioautographs. *J Cell Biol*. 1963;16(1):1-24.
2. Berridge MV, Lane CD. Translation of Xenopus liver messenger RNA in Xenopus oocytes: vitellogenin synthesis and conversion to yolk platelet proteins. *Cell*. 1976;8(2):283-297.
3. Lippincott-Schwartz J, Cole N, Presley J. Unravelling Golgi membrane traffic with green fluorescent protein chimeras. *Trends Cell Biol*. 1998;8(1):16-20.
4. Calì T, Brini M. Quantification of organelle contact sites by split-GFP-based contact site sensors (SPLICS) in living cells. *Nat Protoc*. 2021;16(11):5287-5308.
5. Gilchrist A, Au CE, Hiding J, et al. Quantitative proteomics analysis of the secretory pathway. *Cell*. 2006;127(6):1265-1281.
6. Thul PJ, Åkesson L, Wiking M, et al. A subcellular map of the human proteome. *Science*. 2017;356(6340):eaal3321.
7. Shin JJH, Crook OM, Borgeaud AC, et al. Spatial proteomics defines the content of trafficking vesicles captured by golgin tethers. *Nat Commun*. 2020;11(1):598.
8. Simpson JC, Joggerst B, Laketa V, et al. Genome-wide RNAi screening identifies human proteins with a regulatory function in the early secretory pathway. *Nat Cell Biol*. 2012;14(7):764-774.
9. Coukos R, Yao D, Sanchez MI, et al. An engineered transcriptional reporter of protein localization identifies regulators of mitochondrial and ER membrane protein trafficking in high-throughput CRISPRi screens. *Elife*. 2021;10:e69142.
10. Go CD, Knight JDR, Rajasekharan A, et al. A proximity-dependent biotinylation map of a human cell. *Nature*. 2021;595(7865):120-124.
11. Droujinine IA, Meyer AS, Wang D, et al. Proteomics of protein trafficking by in vivo tissue-specific labeling. *Nat Commun*. 2021;12(1):2382.
12. Spence EF, Dube S, Uezu A, Locke M, Soderblom EJ, Soderling SH. In vivo proximity proteomics of nascent synapses reveals a novel regulator of cytoskeleton-mediated synaptic maturation. *Nat Commun*. 2019;10(1):386.
13. Uezu A, Kanak DJ, Bradshaw TW, et al. Identification of an elaborate complex mediating postsynaptic inhibition. *Science*. 2016;353(6304):1123-1129.
14. Qin W, Cheah JS, Xu C, et al. Dynamic mapping of proteome trafficking within and between living cells by TransitID. *Cell*. 2023;186(15):3307-3324.e30.

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