S-palmitoylation of NOD2 controls its localization to the plasma membrane

Charneal L. Dixon and Gregory D. Fairn

1Keenan Research Centre for Biomedical Science, St. Michael’s Hospital, Unity Health Toronto, Toronto, Ontario, Canada; 2Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada; and 3Department of Pathology, Dalhousie University, Halifax, Ontario, Canada

Nucleotide-binding and oligomerization domain containing protein 2 (NOD2) is a cytosolic pattern-recognition receptor that detects intracellular peptidoglycan (muramyl dipeptide) from bacteria. Membrane association of NOD2 is essential for its ability to activate nuclear factor κB and mitogen-activated protein kinase signaling pathways via the kinase, receptor-interacting serine/threonine-protein kinase 2. The post-translational addition of palmitate to NOD2 results in an acylated protein with increased affinity for membrane bilayers (1). Palmitoylation of cysteine residues (shown in orange) at positions C395 and C1033 en face of the structural model (A) based on the crystal structure of rabbit NOD2 (PDB ID: 5IRL) (2) is mediated by the protein acyltransferase enzyme zDHHC5 (1). The model suggests that this face of the protein is juxtaposed to the plasmalemmal surface. Palmitoylation of the expressed GFP-NOD2WT and GFP-NOD2C395,1033S in HCT116 cells was further characterized using a biorthogonal chemical reporter assay (B) (3). Here, 15-hexadecynoic acid (15-HDYA), also referred to as alkynyl palmitic acid, was metabolically incorporated into cells. The 15-HDYA covalently attached to the immunocaptured GFP-NOD2 proteins was reacted with azide-PEG3-FLAG via a copper-catalyzed azide–alkyne cycloaddition reaction. The proteins were resolved by SDS-PAGE and subsequently detected by an anti-FLAG antibody. The metabolic label, which was incorporated into NOD2WT and was notably absent in the double cysteine mutant, was removed by treatment with 2.5% hydroxylamine that hydrolyzes the 15-DYA–protein thioester linkage. Finally, the palmitoylation-deficient GFP-NOD2C395,1033S does not localize to the plasma membrane in HCT116 cells (C). Mutations that disrupt S-palmitoylation are associated with severe immunologic and inflammatory diseases such as Crohn’s disease, whereas mutations associated with Blau syndrome demonstrate increased S-palmitoylation and membrane localization (1).

EQUIPMENT: The following equipment were used: A Zeiss Axiovert 200M microscope with a Hamamatsu ImageEM x2 camera, Yokogawa spinning disc, and a 63x1.4 NA oil objective.

*For correspondence: Gregory D. Fairn, gfairn@dal.ca.

Published, JLR Papers in Press, July 20, 2021
https://doi.org/10.1016/j.jlr.2021.100097

© 2021 THE AUTHORS. Published by Elsevier Inc on behalf of American Society for Biochemistry and Molecular Biology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
REAGENTS: Alkynyl-palmitate (Click Chemistry Tools), azide-PEG₃-FLAG (Jena Bioscience), hydroxylamine, and other copper-catalyzed azide–alkyne cycloaddition reagents (TCEP, CuSO₄, and TBTA) were obtained from MilliporeSigma.

SOFTWARE: The following software were used: MetaMorph, ImageJ, Python, PyMOL, and AutoDock (4).

Funding and additional information
This work is supported by the Canadian Institutes of Health Research Project Grants PJT166010 awarded to GDF.

Author ORCIDs
Charneal L. Dixon  https://orcid.org/0000-0003-4283-732X

REFERENCES