

Protein AMPylation by an Evolutionarily Conserved Pseudokinase

Diana R. Tomchick^a, Anju Sreelatha^b, Samantha S. Yee^b, Victor A. Lopez^b, Brenden C. Park^b, Lisa N. Kinch^c, Sylwia Pilch^d, Kelly A. Servage^b, Junmei Zhang^b, Jenny Jiou^a, Monika Karasiewicz-Urbańska^e, Małgorzata Łobocka^{d,e}, Nick V. Grishin^{a,d}, Kim Orth^{b,c}, Roza Kucharczyk^d, Krzysztof Pawłowski^e, and Vincent S. Tagliabracci^b

^aDepartment of Biophysics, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA; Diana.Tomchick@UTSouthwestern.edu, Jenny.Jiou@UTSouthwestern.edu, Nick.Grishin@UTSouthwestern.edu

^bDepartment of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA; Anju.Sreelatha@UTSouthwestern.edu, Samantha.Yee@UTSouthwestern.edu, Victor.Lopez2@UTSouthwestern.edu, Brenden.Park@UTSouthwestern.edu, Kelly.Servage@UTSouthwestern.edu, Junmei.Zhang@UTSouthwestern.edu, Kim.Orth@UTSouthwestern.edu, Vincent.Tagliabracci@UTSouthwestern.edu

^cHoward Hughes Medical Institute, Dallas, TX 75390, USA; lkinch@chop.swmed.edu

^dInstitute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw 02-106, Poland; sylwia.pilch91@gmail.com, karasiewiczmonika@gmail.com, m.lobocka@upcpoczta.pl, rozakucharczyk@gmail.com

^eFaculty of Agriculture and Biology, Warsaw University of Life Sciences, Warsaw 02-776, Poland; krzysztof_pawlowski@sggw.pl

Approximately 10% of human protein kinases are believed to be inactive and named pseudokinases because they lack residues required for catalysis. Here, we show that the highly conserved pseudokinase selenoprotein-O (SelO) transfers AMP from ATP to Ser, Thr, and Tyr residues on protein substrates (AMPylation), uncovering a previously unrecognized activity for a member of the protein kinase superfamily. The crystal structure of a SelO homolog reveals a protein kinase-like fold with ATP flipped in the active site, thus providing a structural basis for catalysis. SelO pseudokinases localize to the mitochondria and AMPylate proteins involved in redox homeostasis. Consequently, SelO activity is necessary for the proper cellular response to oxidative stress. Our results suggest that AMPylation may be a more widespread post-translational modification than previously appreciated and that pseudokinases should be analyzed for alternative transferase activities.

