Phosphorylation of alcohol sidechains

Claire E. Grigglestone, Nina Hartrampf

Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland
claire.grigglestone@chem.uzh.ch

MYC is an intrinsically disordered protein (IDP) that acts as a regulator of gene transcription and is deregulated in over 50% of human cancers.[1–3] Its activity is largely modulated by phosphorylation and the protein-protein interactions (PPIs) they induce.[4] Changes in phosphorylation could be responsible for switching from a controlled (normal) state to a deregulated (cancer) state.[5] To date, mainly small fragments of MYC with minimal phosphorylations have been isolated and studied.[6,7]

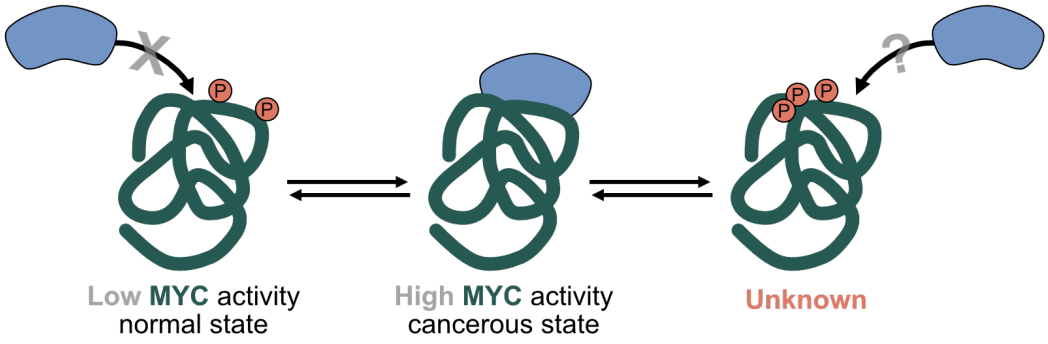

Figure 1. Phosphorylation dependence of MYC activity on cancer.

It is challenging to obtain peptides with multiple selective phosphorylations using the typical methods of a phosphorylated building block or P(III) global phosphorylation. The SPPS building block approach suffers from incomplete couplings that lead to decreased yield, limiting the number of phosphorylated residues that can be incorporated, and high costs. Meanwhile, P(III) phosphoamidite methods require a harsh oxidation step that can be incompatible with oxidation prone residues and destructive to the peptide.[8,9] To address this issue, a novel method for the on-resin (poly)phosphorylation of Ser, Thr & Tyr sidechains has been developed. This approach utilizes previously discovered, inexpensive and commercially available P(V) reagents, eliminating the need for oxidation and expensive SPPS building blocks.

[1] Meyer, N.; Penn, L. Z. Reflecting on 25 Years with MYC. *Nat. Rev. Cancer* **2008**, 8 (12), 976–990.
[2] Lorenzin, F.; Benary, U.; Baluapuri, A.; Walz, S.; Anna Jung, L.; von Eyss, B.; Kisker, C.; Wolf, J.; Eilers, M.; Wolf, E. Different Promoter Affinities Account for Specificity in MYC-Dependent Gene Regulation. *Elife* **2016**, 5 (e15161). https://doi.org/10.7554/eLife.15161.001.
[3] Dhanasekaran, R.; Deutzmann, A.; Mahauad-Fernandez, W. D.; Hansen, A. S.; Gouw, A. M.; Felsher, D. W. The MYC Oncogene — the Grand Orchestrator of Cancer Growth and Immune Evasion. *Nat. Rev. Clin. Oncol.* **2022**, 23–36. https://doi.org/10.1038/s41571-021-00549-2.
[4] Weber, L. I.; Hartl, M. Strategies to Target the Cancer Driver MYC in Tumor Cells. *Front. Oncol.* **2023.** https://doi.org/10.3389/fonc.2023.1142111.
[5] Kalkat, M.; De Melo, J.; Hickman, K. A.; Lourenco, C.; Redel, C.; Resetca, D.; Tamachi, A.; Tu, W. B.; Penn, L. Z. MYC Deregulation in Primary Human Cancers. *Genes.* **2017**, 2–30. https://doi.org/10.3390/genes8060151.
[6] Tu, W. B.; Helander, S.; Pilstål, R.; Hickman, K. A.; Lourenco, C.; Jurisica, I.; Raught, B.; Wallner, B.; Sunnerhagen, M.; Penn, L. Z. Myc and Its Interactors Take Shape. Biochim. Biophys. *Acta Gene Regul. Mech.* **2015**, 469–483. https://doi.org/10.1016/j.bbagrm.2014.06.002.
[7] Soucek, L.; Whitfield, J. The Myc Gene Methods and Protocols Second Edition Methods in Molecular Biology 2318, 2nd ed.; Soucek, L., Whitfield, J., Eds.; Springer Science+Business Media LLC: New York, 2021.
[8] Shapiro, G.; Btichler, D.; Dalvit, C.; Frey, P.; Del Carmen Fernfindez, M.; Gomez-Lor, B.; Pombo-Villar, E.; Stauss, U.; Swoboda, R.; Waridel, C. Combined Fmoc-Alloc Strategy for a General SPPS of Phosphoserine Peptides; Preparation of Phosphorylation-Dependent Tau Antisera. *Bioorg. Med. Chem.* **1997**, 5 (1), 147–156.
[9] Bilbrough, T.; Piemontese, E.; Seitz, O. Dissecting the Role of Protein Phosphorylation: A Chemical Biology Toolbox. *Chem. Soc. Rev.* **2022**, 5691–5730. https://doi.org/10.1039/d1cs00991e.