17. Loubat-Hugel, C., Tritsch, D., and Biellmann, J.F. (1994). C. R. Acad. Sci. III *317*, 299–303.

18. Gale, E.F. (1984). Macrolide Antibiotics, S. Omura, ed. (Orlando, FL: Academic Press), pp. 425–455.

19. Anton, N., Mendes, M.V., Martin, J.F., and Aparicio, J.F. (2004). J. Bacteriol. *186*, 2567–2575.

20. Omura, S., Ikeda, H., Ishikawa, J., Hanamoto, A., Takahashi, C., Shinose, M., Takahashi, Y., Horikawa, H., Nakazawa, H., Osonoe, T., et al. (2001). Proc. Natl. Acad. Sci. USA *98*, 12215–12220.

21. Seco, E.M., Perez-Zuniga, F.J., Rolon, M.S., and Malpartida, F. (2004). Chem. Biol. *11*, 357–366.

When an Inhibitor Promotes Activity

Michel Bouvier[1,*]
[1] Université de Montréal, Institute for Research in Immunology and Cancer, Marcelle-Coutu Pavillion, C.P. 6128 Succursale Centre-Ville, Montreal, Quebec H3C 3J7, Canada
*Correspondence: michel.bouvier@umontreal.ca
DOI 10.1016/j.chembiol.2007.03.004

In a recent issue of *Chemistry & Biology*, Tropak et al. [1] reported a high-throughput screening assay to identify β-hexosaminidase inhibitors that act as pharmacological chaperones and are potential drug candidates for the treatment of Tay-Sachs disease.

Genetic conformational diseases are often caused by modest mutations in proteins that lead to unnecessary recognition of the mutant protein by the endoplasmic reticulum (ER) quality control system as misfolded. These mutations classically occur outside of the predicted functional domain of the affected proteins such that interventions to allow their release from the quality control system and their targeting to their proper site of action could restore function [2]. Classic examples of such diseases include cystic fibrosis, nephrogenic diabetes insipidus, hypogonadotropic hypogonadism, and various lysosomal storage disorders such as GM2 gangliosidosis, Fabry, Gaucher, and Morquio B diseases.

Pharmacological chaperones are small molecules that can bind and stabilize the folding intermediates of the mutant proteins thus favoring their proper folding, ER export, and subsequent trafficking to their normal subcellular location [3]. In most instances, pharmacological chaperones that can rescue folding, trafficking, and function of mutant proteins have been found among ligands that were already known to bind to the affected proteins thus conferring a measure of selectivity. For example, specific receptor antagonists in the case of diseases resulting from mutations in G protein-coupled receptors (like V2-vasopressin and gonadotropin-releasing hormone receptors for nephrogenic diabetes insipidus and hypogonadotropic hypogonadism, respectively [4,5]) and competitive enzyme inhibitors for lysosomal storage disorders [6] were found to increase the levels of active receptors and enzymes in cellular systems, indicating that such compounds could have therapeutic value. Consistent with this notion, a small scale clinical trial carried out in nephrogenic diabetes insipidus patients confirmed the therapeutic activity of a pharmacological chaperone, as administration of a vasopressin antagonist significantly improved kidney functions in five patients harboring three distinct mutations [7].

The use of an antagonist or inhibitor to treat a disease resulting from a loss of function mutation may appear contradictory, but is based on the idea that the high affinity binding of these compounds to the mutant protein provides sufficient interaction time to promote proper folding and escape from the ER quality control system. Obviously, the inhibitors then need to dissociate from the rescued enzyme/receptor to permit their activity. It follows that detailed pharmacokinetic studies are needed to find a drug administration regimen that would result in the optimum therapeutic activity of individual pharmacological chaperones.

Based on the idea that inhibitors can act as pharmacological chaperones and rescue the activity of protein function, Tropak et al. [1] developed a real time, high-throughput assay to identify new inhibitors of human β-hexosaminidase that could have pharmacological activity in the treatment of two forms of GM2 gangliosidosis, infantile Tay-Sachs disease (ISD) and adult Tay-Sachs disease (ATSD). These diseases result from mutations in the gene encoding β-hexosaminidase that render the enzyme unstable most likely due to misfolding, thus making them potential targets for the therapeutic action of pharmacological chaperones. The fluorescence-based in vitro assay developed by the authors uses purified enzyme and identified 24 confirmed inhibitors of the enzymatic activity out of a commercially available 50,000 compound library. Three of these compounds, belonging to distinct classes of chemical structures, were then selected to study their potential action as pharmacological chaperones in treatment of Tay-Sachs disease.

In a cell culture system, the three compounds were found to increase the amount and activity of β-hexosaminidase in fibroblasts obtained from patients with ISD and ATSD. Analysis of the mode of action of the inhibitors revealed that they most likely function by stabilizing the enzyme, and thus qualify to be called pharmacological chaperones. Interestingly, the observed increase in activity (3-fold in the best case) would be predicted to confer sufficient function to have beneficial effects in patients, making them good candidates for the development of clinically useful drugs. The three novel pharmacological chaperones displayed better selectivity profiles than the known β-hexosaminidase inhibitor that was previously shown to have pharmacological chaperone activity [8], leading to the hope that they could have fewer undesirable off-target effects. Also of significant interest, some of the identified compounds share chemical scaffolds with drugs that have already been approved by the FDA, increasing the likelihood that they could meet the criteria for good drug candidates.

In addition to identifying novel pharmacological chaperones with therapeutic potential for the treatment of Tay-Sach diseases, the present study represents a proof of principle that high-throughput assays can be used to identify new chemical entities with pharmacological chaperone activity—a path that will undoubtedly be followed by investigators in search of novel therapeutic avenues for treating conformational diseases. Although the present screening campaign was searching for inhibitors, there are no a priori theoretical reasons why other types of ligands (agonists, allosteric regulators, etc.) that bind and stabilize misfolded proteins could not also act as pharmacological chaperones. Thus, high-throughput screens based on the ability of compounds to restore normal subcellular targeting, independent of their intrinsic signaling activities, are also likely to be carried out in the near future.

Upcoming and ongoing clinical trials for Tay-Sachs disease and other lysosomal storage disorders such as Fabry disease will soon tell us if the initial clinical results for pharmacological chaperones [7] can be generalized. It will be interesting to see if screening for novel pharmacological chaperones will become a common approach in the search for conformational disease treatments.

REFERENCES

1. Tropak, M.B., Blanchard, J., Withers, S.G., Brown, E., and Mahuran, D. (2007). Chem. Biol. *14*, 153–164.

2. Arakawa, T., Ejima, D., Kita, Y., and Tsumoto, K. (2006). Biochim. Biophys. Acta. *1764*, 1677–1687.

3. Bernier, V., Lagace, M., Bichet, D.G., and Bouvier, M. (2004). Trends Endocrinol. Metab. *15*, 222–228.

4. Morello, J.P., Salahpour, A., Laperrière, A., Bernier, V., Arthus, M.F., Lonergan, M., Angers, S., Petaja-Repo, U., Morin, D., Bichet, D.G., et al. (2000). J. Clin. Invest. *105*, 887–895.

5. Janovick, J.A., Goulet, M., Bush, E., Greer, J., Wettlaufer, D.G., and Conn, P.M. (2003). J. Pharmacol. Exp. Ther. *305*, 608–614.

6. Fan, J.Q. (2003). Trends Pharmacol. Sci. *24*, 355–360.

7. Bernier, V., Morello, J.P., Zarruk, A., Debrand, N., Salahpour, A., Lonergan, M., Arthus, M.F., Laperriere, A., Brouard, R., Bouvier, M., et al. (2006). J. Am. Soc. Nephrol. *17*, 232–243.

8. Tropak, M.B., Reid, S.P., Guiral, M., Withers, S.G., and Mahuran, D. (2004). J. Biol. Chem. *279*, 13478–13487.

Reprogramming the Histone Code

Salam A. Shaaban[1,*] and Mark T. Bedford[2,*]
[1] OSI Pharmaceuticals Inc., 1 Bioscience Park Drive, Farmingdale, NY 11735, USA
[2] The University of Texas M.D. Anderson Cancer Center, Science Park-Research Division, Park Road 1C, Smithville, TX 78957, USA
*Correspondence: sshaaban@osip.com (S.A.S.), mtbedford@mdanderson.org (M.T.B.)
DOI 10.1016/j.chembiol.2007.03.001

Histone lysine methyltransferases, like G9a, play central roles in the regulation of gene expression. A current study by Kubicek et al. [1] reports the identification of a G9a small-molecule inhibitor, thereby opening the way to new epigenetic cancer therapies.

An important part of the pathogenesis of cancer lies in the inactivation of tumor suppressor genes, which can be achieved either genetically or epigenetically. Epigenetic alterations refer to changes in gene expression that do not result from alterations in the DNA sequence. Epigenetic mechanisms include DNA methylation, and histone protein acetylation and methylation. Both DNA methylation and histone acetylation have been the target of small-molecule therapies [2, 3], while the development of compounds that target lysine and arginine methyltransferases has lagged behind. In a recent issue of *Molecular Cell*, Kubicek et al. [1] described screening for and identification of a highly specific, small molecular weight histone lysine methyltransferase (HKMT) inhibitor that