type of ATP-competitive inhibitor can achieve potent affinity levels for a target kinase, maintaining selectivity against the other protein kinase family members remains a major challenge (Grant, 2009). Fedorov et al. (2011) have shown that KH-CB19, an inhibitor with a noncanonical halogen interaction with the hinge backbone CO, can attain potent and selective affinity for members of the CLK family (Figure 1B). Contributing to the affinity is an inward binding of Phe172 in the P loop, which partly defines the pocket. Other pharmacophores with a halogen interaction to the kinase hinge region have been reported by other groups (De Moliner et al., 2003), including DRB, which binds to CDK9 through two chlorines. (Baumli et al., 2010).

It remains to be seen whether or not the replacement of the standard kinase inhibitor core with a novel halogenated ring system is a strategy that can be applied more broadly toward the elaboration of potent and selective kinase ligands. Yet, the results reported here by Fedorov et al. (2011) enhance the attractiveness of such an approach and highlight a potential emerging area of chemical space for the design of kinase inhibitors.

REFERENCES

Baumli, S., Endicott, J.A., and Johnson, L.N. (2010). Chem. Biol. *17*, 931–936.

De Moliner, E., Brown, N.R., and Johnson, L.N. (2003). Eur. J. Biochem. *270*, 1–8.

Fedorov, O., Huber, K., Eisenreich, A., Filippakopoulos, P., King, O., Bullock, A.N., Szklarczyk, D., Jensen, L.J., Fabbro, D., Trappe, J., Rauch, U., Bracher, F., and Knapp, S. (2011). Chem. Biol. *18*, this issue, 67–76.

Grant, S.K. (2009). Cell. Mol. Life Sci. *66*, 1163–1177.

Hagiwara, M. (2005). Biochim. Biophys. Acta *1754*, 324–331.

Morikawa, T., and Manabe, T. (2010). Neurochem. Int. *57*, 691–704.

Mott, B.T., Tanega, C., Shen, M., Maloney, D.J., Shinn, P., Leister, W., Marugan, J.J., Inglese, J., Austin, C.P., Misteli, T., Auld, D.S., and Thomas, C.J. (2009). Bioorg. Med. Chem. Lett. *19*, 6700–6705.

Muraki, M., Ohkawara, B., Hosoya, T., Onogi, H., Koizumi, J., Koizumi, T., Sumi, K., Yomoda, J., Murray, M.V., Kimura, H., Furuichi, K., Shibuya, H., Krainer, A.R., Suzuki, M., and Hagiwara, M. (2004). J. Biol. Chem. *279*, 24246–24254.

Nayler, O., Schnorrer, F., Stamm, S., and Ullrich, A. (1998). J. Biol. Chem. *273*, 34341–34348.

A Jump-Start for Planarian Head Regeneration

Amy K. Sater[1,*]
[1]Department of Biology and Biochemistry, University of Houston, Houston TX 77204-5001, USA
*Correspondence: asater@uh.edu
DOI 10.1016/j.chembiol.2011.01.002

Planaria are simple flatworms with an extraordinary ability to regenerate missing body parts. This makes them a unique model system for the study of regeneration. Extending an earlier chemical screen, Beane et al. (2011) now reveal a role for H^+/K^+ ATPase and membrane depolarization in anterior regeneration in planaria.

Biologists have known for decades that vital events in early embryonic development are accompanied by changes in ion flow, leading to biophysical signaling events such as membrane depolarization, spatial patterns of membrane current, or alterations in gap junctional communication. Tissues undergoing wound healing or regeneration show similar biophysical signals, which arise from changes in the activity of ion channels or transporters. Understanding how these biophysical signals promote the establishment of embryonic pattern, the specification of cell fate, or wound healing however, has been a major challenge. The chief difficulty has been in defining a functional link between altered ion channel or transporter activity and the changes in gene regulation that underlie developmental processes. Now Beane et al. (2011) have demonstrated that H^+/K^+ ATPase activity is essential for planarian head regeneration and identified a mechanism by which H^+/K^+ ATPase activity could activate expression of genes associated with head formation.

Planaria, free-living arrow-headed flatworms, are simple invertebrates with a surprisingly complex nervous system and an unparalleled capacity for regeneration. Amputation of either the head or the tail region leads to regrowth of the lost structures within several days. As with regeneration of the limbs or tail in lower vertebrates, regeneration proceeds by formation of an undifferentiated cell mass referred to as a blastema, which then undergoes coordinated differentiation to reconstitute the missing structures. While a vertebrate blastema forms via dedifferentiation and proliferation of the cells near the amputation site, the planarian blastema arises from the migration and proliferation of neoblasts, a highly pluripotent stem cell population that constitutes 30% of the cells of the adult flatworm. Blastema formation is followed by the establishment of regional identity along the anteroposterior axis. During anterior regeneration, the emergence of the brain rudiment, neuronal differentiation, and the establishment of neuronal connectivity rapidly follow, and specific behavioral responses are reestablished within five days after amputation. Planarian regeneration has emerged

as an outstanding model system for the investigation of stem cell function in vivo (see Pearson and Sánchez-Alvarado [2008] for review).

Regeneration in planaria is mediated by several highly conserved signaling pathways. One study indirectly implicates FGF signaling in promoting head regeneration, in that RNAi-mediated loss-of-function for *nou-darake*, which is thought to inhibit FGF signals, leads to ectopic head formation (Agata and Umesono, 2008). A role for the canonical wnt/β-catenin pathway in posterior regeneration has been well established (Petersen and Reddien, 2008; Adell et al., 2009; Gurley et al., 2010), and expression of wnt genes is under the control of the hedgehog (hh) pathway (Yazawa et al., 2009; Rink et al., 2009). Thus, the transcriptional programs underlying the establishment of anterior versus posterior identity during regeneration are governed in part by FGF and canonical wnt signals.

Further insight into the molecular requirements for regeneration arose from a chemical genetic (CG) screen that implicated H^+/K^+ ATPase as a regulator of anterior regeneration (Nogi et al., 2005). This CG screen was designed to uncover functions of ionic regulatory components, including ion channels and transporters such as the H^+/K^+ ATPase, in development and regeneration. Channels and transporters are generally composed of multiple subunits, and, in many cases, a subunit is encoded by more than one gene. As a result, there is considerable functional redundancy among genes encoding channel or transporter components, which can confound conventional loss-of-function strategies (e.g., RNAi). Thus, this CG screen provides an essential complement to genetic or reverse genetic approaches. Levin and colleagues have used variations on this screen to investigate the establishment of vertebrate left-right asymmetries (Fukumoto et al., 2005; Adams et al., 2006).

The H^+/K^+ ATPase is a two-subunit plasma membrane antiporter that maintains low gastric pH, among many other functions. Since the H^+/K^+ ATPase exchanges one cytoplasmic H^+ for a single extracellular K^+, it is electroneutral in isolation. Earlier studies, however, have demonstrated that H^+/K^+ ATPases can indirectly regulate membrane voltage when they act in combination with a class of K^+ channels (e.g., Lambrecht et al., 2005). The resulting increase in intracellular K^+ leads to the opening of specific K^+-efflux channels and the movement of K^+ out of the cell; this outwardly directed K^+ current is sufficient to initiate membrane depolarization.

To determine whether H^+/K^+ ATPase functions to regulate membrane voltage during anterior regeneration, Beane et al. (2011) used a high K^+ medium to depolarize the plasma membranes of regenerating fragments treated with the H^+/K^+ ATPase inhibitor SCH28080; this depolarization restored head regeneration in the absence of H^+/K^+ ATPase function. These results demonstrate that this membrane voltage function of the H^+/K^+ ATPase is essential for the establishment of anterior pattern during regeneration. Moreover, they also used voltage-sensitive dyes to visualize membrane polarization during regeneration, finding that fragments undergoing anterior regeneration have relatively depolarized plasma membranes while posterior regenerates have relatively hyperpolarized plasma membranes. These findings place the functions of membrane depolarization in the second phase of regeneration, in which anterior regional identity is established; they exclude the possibility that membrane depolarization is required for blastema formation. Finally, Beane et al. (2011) carried out a K^+-independent depolarization using ivermectin, which maintains the glutamate-gated chloride channel in an open state, permitting Cl^- ions to enter the cell. Remarkably, this pharmacologically-induced depolarization of doubly amputated fragments led to anterior regeneration at both ends, indicating that membrane depolarization is sufficient to initiate head regeneration.

These effects on membrane voltage offer a testable hypothesis regarding the mechanism by which H^+/K^+ ATPase activity might contribute to transcriptional regulation during anterior regeneration. Membrane depolarization can activate voltage-gated calcium channels, leading to an increase in intracellular free calcium (Ca^{2+}), which can in turn activate calcium-responsive transcription factors, such as cAMP/Ca^{2+} –responsive element binding protein (CREB) or nuclear factor of activated T cells (NFAT) (see Carrasco and Hidalgo [2006] for review). Beane et al. (2011) investigated the hypothesis that membrane depolarization leads to increased Ca^{2+}, showing that (1) levels of intracellular Ca^{2+} were elevated in anterior blastemas relative to posterior blastemas, and (2) treatment with Nicardipine, an inhibitor of voltage-gated calcium channels, led to defects in anterior regeneration. Pharmacological activation of voltage-gated calcium channels leads to the formation of ectopic heads during regeneration (Nogi et al., 2009). Taken together, these findings suggest the hypothesis that Ca^{2+}-dependent transcriptional regulatory events are required for the establishment of anterior identity during planarian regeneration.

Several questions emerge from this work; the most obvious will address the mechanisms of Ca^{2+}-dependent transcriptional control, the specific functions of Ca^{2+}-dependent transcriptional regulation in anterior regeneration, and the identification of transcriptional targets. Such future investigations would allow the integration of these results with the current gene regulatory framework for planarian regeneration; genes activated in response to elevated intracellular Ca^{2+} may synergize or act in parallel with genes activated by FGF-dependent transcription, while genes that are downregulated by Ca^{2+}-dependent transcriptional events might include components or targets of the canonical wnt/β-catenin pathway.

A larger set of questions concerns the role of biophysical signals in the regulation of stem cell activity, wound healing, and tissue repair. This study suggests that biophysical signals associated with these processes are worthy of renewed attention, in view of their capacity to direct patterning events. Given the involvement of conserved molecular regulatory mechanisms such as the wnt, FGF, and hh pathways in planarian regeneration, the central role of membrane depolarization may imply that similar biophysical signals may modulate conserved signaling pathways in mammalian tissues. The emergence of highly specific pharmacological probes such as those used in this screen may provide new avenues for the development of regenerative therapies via the integration of biophysical and molecular approaches.

REFERENCES

Adams, D.S., Robinson, K.R., Fukumoto, T., Yuan, S., Albertson, R.C., Yelick, P., Kuo, L.,