Actin-Binding Toxin "Tail" Wags the Dog

Junichi Tanaka,[1] J. Craig Blain,[2] and John S. Allingham[2,*]
[1]Department of Chemistry, Biology, and Marine Science, University of the Ryukyus, Okinawa 903-0213, Japan
[2]Department of Biochemistry, Queen's University, Kingston, Ontario K7L 3N6, Canada
*Correspondence: allinghj@queensu.ca
DOI 10.1016/j.chembiol.2008.02.012

Actin-binding marine macrolides exhibit significant structural and functional diversity. In this issue, Perrins et al. demonstrate that a long stereochemically conserved aliphatic side chain, known as the "tail", found in many of these compounds is the functional determinant of cytotoxicity (Perrins et al., 2008).

The sources of cytotoxic marine natural products are both phylogenetically and geographically diverse, and their molecular structures are often exquisitely adapted to specific biological functions through eons of evolutionary refinement of their chemical space. Through this process, nature has provided "prevalidated" chemical scaffolds with vast pharmacological potential.

Actin is the target of a conspicuously large number of natural products that bind actin filaments and stop or accelerate their growth, chop them up, or inhibit their formation altogether (Figure 1). The structural features of these compounds can vary significantly and, until a short time ago, the details of their molecular interactions with actin, as well as their mode of action, were enigmatic. However, recent studies are beginning to shed light on unifying elements in certain classes of these compounds that determine their function (Allingham et al., 2006). These studies are also providing insight into how analogs of these compounds can be designed to retain the potency of the natural form, yet be tailored for specific uses in clinical or laboratory settings. Perrins et al. now provide significant momentum to rational actin-targeted drug design, reporting that only a portion of a natural product is required for activity when this component is elaborated with a simple aromatic group in place of the missing structural features of the natural form.

Actin is a globular protein that can polymerize into helical filaments (Figure 2), which form a three-dimensional network inside eukaryotic cells. Dynamic remodeling of actin filaments in the cytosol drives shape changes, cell locomotion, and chemotactic migration that are vital to tissue development, wound healing, neuron migration, immune responses, and maintenance of homeostasis. However, this activity of actin also facilitates division and migration of tumor cells, microbial infection, and other disease states when not properly regulated. Therefore, actin is recognized as a strategic target for the development of new anticancer drugs and other therapeutic agents.

There are at least six different natural product binding sites on actin, however, the majority of compounds appear to bind the barbed end of actin. This could indicate that this is the best place to target actin-binding drugs to produce an effect and that natural products that target this site may provide an excellent source of inspiration for drug design. Recently, a number of cytotoxic macrolides, such as reidispongiolides, sphinxolides, aplyronines, and ulapualides that bind to the

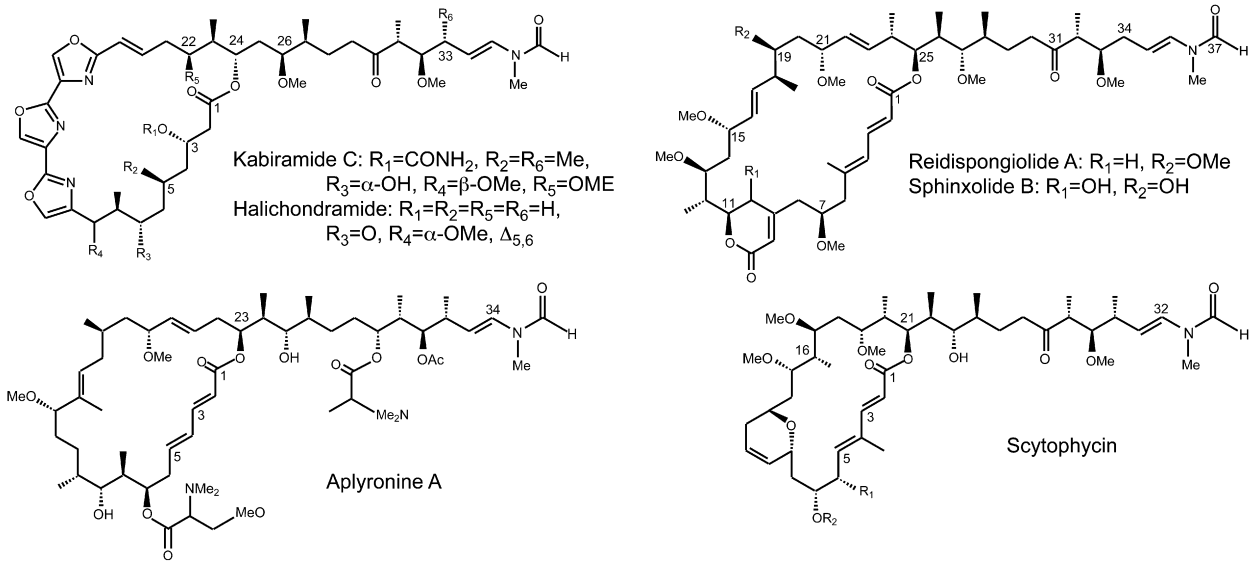

Figure 1. Barbed End-Targeting Macrolides
The chemical structures of representative members of known or inferred actin barbed end-targeting natural products are shown.

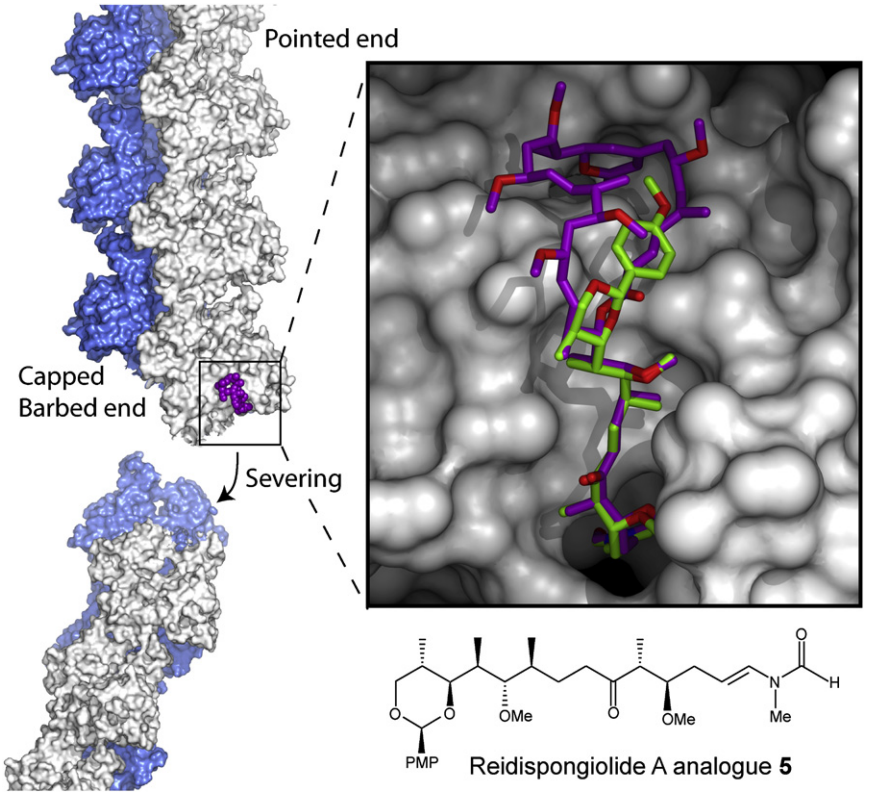

Figure 2. Model for Actin Binding, and Actin Filament Severing and Capping by Reidispongiolide A and a Synthetic Analog

The structure of reidispongiolide A (purple spheres) bound to actin (PDB code: 2ASM) is shown on the left superimposed onto a modified F-actin model (PDB code: 1O1E) depicting the hypothetical mechanism of filament severing and capping (left panel). Reidispongiolide A (purple ball-and-sticks) and manually docked reidispongiolide analog **5** (green ball-and-sticks) from Perrins et al. (2008) are shown on the top right superimposed onto a surface representation of actin (PDB code: 2ASM). The chemical structure of reidispongiolide analog **5** is shown below.

barbed end of actin with nanomolar affinity and disrupt actin filament dynamics have attracted significant attention as novel anticancer drug leads. These compounds are lethal to tumor cells, including those possessing multiple drug resistance, and several of them have been included in the National Cancer Institute's Molecular Targets Development Program (Braet et al., 2008; Fenteany and Zhu, 2003; Spector et al., 1999). So far, however, no actin-binding drugs have broken beyond the preclinical testing stage due to their extreme cytotoxicity and impartiality to normal and diseased cells. One route that holds promise in overcoming this obstacle is to design synthetic compounds that integrate the key elements producing the actin-targeting functions of compounds tailored by nature with cell-specific targeting elements. Fortunately, the complex structural features of the above macrolides attract synthesis chemists like Ian Paterson, who accomplished the total syntheses of several actin-binding macrolides (Paterson et al., 2007). Their achievements provide excellent opportunities to dissect the structural requirements for bioactivity of natural products by utilizing synthetic intermediates.

In order to define the key actin-targeting elements, a number of groups also have begun correlating the effects of different macrolide congeners, artificial analogs, and toxin fragments on purified actin filament dynamics and cell-based cytotoxicity assays (Allingham et al., 2005; Perrins et al., 2008; Suenaga et al., 1997; Vincent et al., 2007). X-ray crystal structures of several of these compounds bound to actin have aided enormously to this endeavor by revealing common actin-toxin binding interfaces and providing a molecular explanation for their mode of filament-destabilization (Allingham et al., 2005; Hirata et al., 2006; Klenchin et al., 2003). Collectively, these studies have uncovered unifying structure-cytotoxicity relationships for these macrolides.

Barbed end-binding macrolides typically possess a structurally variable hydrophobic macrocycle (the "ring") and a long and flexible aliphatic side chain (the "tail") with conserved stereochemistry that terminates with a highly conserved N-methyl-vinylformamide (MVF) moiety (Figure 1). The tail section intercalates into the narrow hydrophobic cleft splitting subdomains 1 and 3 at the barbed end of actin and forms critical stabilizing interactions via the MVF moiety (Allingham et al., 2005; Klenchin et al., 2003; Figure 2). Also, several conserved side groups along the length of the tail complement the irregular surface of the actin cleft to help impose the tail's extended conformation. Altogether, a tight interaction is created with a surface on actin that is involved in important actin-actin contacts formed during actin polymerization (Klenchin et al., 2003; Tanaka et al., 2003; Figure 2). While the ring of barbed end-binding macrolides also makes important interactions with actin at a separate surface that is exposed in both the polymerized and nonpolymerized state, there is significant variability in how the ring of different toxins engage this surface that appears to correlate with the structural variability of this component. These observations provided the first indication that the affinity of these compounds for actin and their cytotoxicity are determined by a subset of key structural features, and that most of their functionality resided in the tail region.

Perrins et al. (2008) now provide further clarification of the importance of the tail region by characterizing the structure-activity relationships of synthetic analogs of the tail portion of reidispongiolide. Their observation that analogs consisting primarily of the tail section alone retained actin-binding, polymerization inhibition, and filament severing activities demonstrates that the tail is the key determinant of actin filament dynamics inhibition, and thus confirms the importance of maintaining the tail in future actin-targeting drug design efforts. It also provides an optimistic outlook for the development of simplified natural product mimics whose molecular masses fulfill a parameter of Lipinski's Rule of Five (Lipinski et al., 2001).

Given that the tail comprises only ~40% of the total surface area of the toxins and the fact this feature is highly conserved across different cytotoxin families, the work by Perrins et al. raises important questions about why marine organisms go to the trouble to produce complex macrocycles. Their observation that the functionality of the tail segment was significantly enhanced with the addition of an aromatic group (a *para*-methoxyphenyl acetal protecting group), which is in actuality an artifact of the chemical synthesis, indicates that the macrocycle is not entirely superfluous, but can be modified or substituted with alternative hydrophobic moieties to help stabilize the actin-drug complex. Cursory docking of the most bioactive analog onto the structural coordinates of actin in its reidispongiolide A-bound conformation lends support to this hypothesis (Figure 2). The aromatic group could, in part, reconstitute important hydrophobic interactions with the shallow hydrophobic patch on actin that is contacted by analogous regions of many diverse barbed end-binding macrolides. Notably, this portion of the ring was previously been described as a likely component of the pharmacophore of these compounds (Allingham et al., 2006; Melville et al., 2007). Elaboration of the tail segment with other hydrophobic moieties in place of this portion of the ring could serve a dual role of stabilizing the interaction of the tail with actin, likely by reducing the entropy of binding, and providing a scaffold for appending cell-specific targeting ligands or optical probes.

In addition to the direct therapeutic applications, the work of investigators like Perrins et al. could also stimulate novel areas of interest in the development of actin-binding ligands as diagnostic or prognostic tools based on alterations of the cytoskeletal properties of tumor cells. On a more general note, such work may have important implications on the design of drugs that are intended to disrupt protein-protein interactions based on the filament-disrupting mechanism of these compounds.

REFERENCES

Allingham, J.S., Zampella, A., D'Auria, M.V., and Rayment, I. (2005). Proc. Natl. Acad. Sci. USA *102*, 14527–14532.

Allingham, J.S., Klenchin, V.A., and Rayment, I. (2006). Cell. Mol. Life Sci. *63*, 2119–2134.

Braet, H., Soon, L., Vekemans, K., Thordarson, P., and Spector, I. (2008). Actin-Binding Proteins and Disease (New York: Springer).

Fenteany, G., and Zhu, S. (2003). Curr. Top. Med. Chem. *3*, 593–616.

Hirata, K., Muraoka, S., Suenaga, K., Kuroda, T., Kato, K., Tanaka, H., Yamamoto, M., Takata, M., Yamada, K., and Kigoshi, H. (2006). J. Mol. Biol. *356*, 945–954.

Klenchin, V.A., Allingham, J.S., King, R., Tanaka, J., Marriott, G., and Rayment, I. (2003). Nat. Struct. Biol. *10*, 1058–1063.

Lipinski, C.A., Lombardo, F., Dominy, B.W., and Feeney, P.J. (2001). Adv. Drug Deliv. Rev. *46*, 3–26.

Melville, J.L., Moal, I.H., Baker-Glenn, C., Shaw, P.E., Pattenden, G., and Hirst, J.D. (2007). Biophys. J. *92*, 3862–3867.

Paterson, I., Ashton, K., Britton, R., Cecere, G., Chouraqui, G., Florence, G.J., and Stafford, J. (2007). Angew. Chem. Int. Ed. *46*, 6167–6171.

Perrins, R.D., Cecere, G., Paterson, I., and Marriott, G. (2008). Chem. Biol. *15*, this issue, 287–294.

Spector, I., Braet, F., Shochet, N.R., and Bubb, M.R. (1999). Microsc. Res. Tech. *47*, 18–37.

Suenaga, K., Kamei, N., Okugawa, Y., Takagi, M., Akao, A., and Kigoshi, H. (1997). Bioorg. Med. Chem. Lett. *7*, 269–274.

Tanaka, J., Yan, Y., Choi, J., Bai, J., Klenchin, V.A., Rayment, I., and Marriott, G. (2003). Proc. Natl. Acad. Sci. USA *100*, 13851–13856.

Vincent, E., Saxton, J., Baker-Glenn, C., Moal, I., Hirst, J.D., Pattenden, G., and Shaw, P.E. (2007). Cell. Mol. Life Sci. *64*, 487–497.

How to Identify a Pharmacophore

Heino Prinz[1,*]
[1]Max-Planck-Institut für Molekulare Physiologie, Otto-Hahn-Str. 11, D44227 Dortmund, Germany
*Correspondence: heino.prinz@mpi-dortmund.mpg.de
DOI 10.1016/j.chembiol.2008.02.013

The inhibition of chitinases by argifin and progressively dissected analogs had been studied by a combination of kinetic and crystallographic methods (Andersen et al., 2008). This work also leads to a general understanding of structure-activity relationships for inhibitors with one distinct pharmacophor.

In this issue of *Chemistry & Biology*, van Aalten and colleagues describe the identification of dimethylguanylurea as a pharmacophor for family 18 chitinases (Andersen et al., 2008). Dimethylguanylurea forms the terminus of the major side chain of argifin, a modified cyclopentapeptide which is a potent chitinase inhibitor. In hindsight, that finding may have been expected, since the molecule dimethylguanylurea is to the molecule argifin what the symbol + is to the symbol ♀. The beauty of this work lies in the complete set of methods applied. Five successively shortened linear fragments of argifin were synthesized. Their activity was tested against family 18 chitinases from three different species, fungus, human, and mouse. Crystals of one of these (chitinase B1 from the fungus *Aspergillus fumigatus*) were soaked with those fragments. The X-ray structures of the complexes were solved and compared to the known complex with argifin (Houston et al., 2002). All