Review

Actin-targeting natural products: structures, properties and mechanisms of action

J. S. Allingham, V. A. Klenchin and I. Rayment*

Department of Biochemistry, University of Wisconsin, 433 Babcock Drive, Madison, Wisconsin 53706 (USA),
Fax: +1 608 262 1319, e-mail: Ivan_Rayment@biochem.wisc.edu

Abstract. Natural small-molecule inhibitors of actin cytoskeleton dynamics have long been recognized as valuable molecular probes for dissecting complex mechanisms of cellular function. More recently, their potential use as chemotherapeutic drugs has become a focus of scientific investigation. The primary focus of this review is the molecular mechanism by which different actin-targeting natural products function, with an emphasis on structural considerations of toxins for which high-resolution structural information of their interaction with actin is available. By comparing the molecular interactions made by different toxin families with actin, the structural themes of those that alter filament dynamics in similar ways can be understood. This provides a framework for novel synthetic-compound designs with tailored functional properties that could be applied in both research and clinical settings.

Introduction

Actin is the most abundant protein in eukaryotic cells and its ability to reversibly assemble into long and flexible polymers, or filaments, provides the foundation upon which many essential cellular functions rely [1]. For example, the interaction between actin filaments and the molecular motor myosin drives dynamic functions such as locomotion, division and growth, while their interaction with membrane-associated proteins gives eukaryotic cells their shape and mechanical strength, and facilitates attachments to other cells or substrates [2, 3]. Performing these functions demands strict regulation of the spatial and temporal organization of the actin cytoskeleton. This is facilitated on many levels by a myriad of accessory proteins [4]. These proteins influence every aspect of actin filament dynamics: the

rate of actin polymerization and depolymerization, the balance between the concentration of monomeric and filamentous actin forms within the cell, interfilament interactions and filament branching [5–7]. Certain proteins bind monomeric actin and physically inhibit its addition to growing filaments, and are therefore known as 'sequestering' proteins (e.g. thymosin *β*4, profilin, vitamin D binding protein). There are those that bind to filament ends and inhibit both monomer addition and dissociation, called 'capping' proteins (e.g. CapZ), as well as those that stimulate polymerization from pools of actin monomers in a process known as 'nucleation' (e.g. the Arp2/3 complex and formins). Other actin-binding proteins actively break or 'sever' actin filaments [e.g. actin-depolymerizing factor (ADF) and cofilin]. Finally, many actin-binding proteins exhibit multiple actin-modulating functions, where for example gelsolin is able to both sever actin filaments and cap the newly severed ends.

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* Corresponding author.

Besides endogenous actin filament-regulating proteins, numerous natural product small molecules also display potent abilities to affect the polymeric state of actin filaments [8–10]. This property has earned these compounds significant recognition as valuable molecular probes for dissecting complex cellular pathways that are dependent upon the actin cytoskeleton. It has also drawn attention to the possibility of their use as promising leads for anti-cancer drug development [8, 9, 11]. Indeed, extensive evidence has shown that the actin cytoskeleton plays a fundamental role in the cellular properties of oncogenic transformed cells. Here, the molecular composition, organization and polymeric state of the actin cytoskeleton is altered as a result of changes in the abundance or activity of some of the actin filament-regulating proteins described above [12–15]. This creates the characteristic cellular morphologies as well as the aggressive phenotypes of cancer cells, including anchorage-independent cell growth, cell migration and invasion [11, 16–18]. Such characteristics are facilitators of metastasis and angiogenesis, two hallmarks of malignant cell progression and the primary contributors to most cancer-related deaths [10, 18–21]. Thus the ability of certain actin-targeting natural products to manipulate actin filament structure may provide a valuable strategy to compensate for, or partially reverse, some of the changes to the cytoskeleton in malignant cells that are created by abnormal actin regulatory protein levels or activities, thereby controlling cancer proliferation.

An undeniable limitation to the use of actin-targeting natural products as therapeutic drugs is their inability to distinguish between the actin of normal and transformed cells, making them too toxic for direct clinical use due to the ubiquity of actin and its importance in non-neoplastic cells. However, this caveat can also be applied to microtubule-targeting drugs, such as Taxol, which is a successful and widely used anti-cancer drug [22, 23]. Furthermore, this does not rule out the possibility that the natural forms of actin-targeting small molecules can provide valuable functional and chemical information to inspire the design of more efficacious chemotherapeutics for cancer treatment. In the past few years, our knowledge of the complete chemical structures and mode of actin binding, and our understanding of the mechanisms of action of actin-targeting natural products have advanced tremendously. The purpose of this review is to outline these recent advances and to apply information about their molecular interactions with actin to cytotoxins with similar chemical structures and functional properties. By considering the connection between conserved structural features of particular toxin families, their manner of actin interaction, and the potency with which they affect the polymeric state of actin filaments, a framework is established for the design of specifically tailored functional analogues by chemical synthesis. When combined with an ability

to exploit the differences in the molecular composition and organization of actin filaments in cancer cells, this information may allow actin-targeting drugs to have discriminating effects on normal and transformed cells [12, 24]. This would increase the technological potential of these compounds for both biological and pharmaceutical applications.

Monomeric and filamentous actin structures

The primary structure of actin is highly conserved among eukaryotic organisms. Its monomeric form, known as globular or G-actin, is 43-kDa in size with four subdomains (Fig. 1a). ATP, along with Mg^{2+}, binds within a deep cleft between subdomains 2 and 4, and can be hydrolyzed to ADP + Pi. In physiological conditions, G-actin can self-assemble into a long double-stranded helical polymer, known as filamentous or F-actin. Within the filament, the position of each monomer is related to the preceding one by a translation of ~27.5 Å and a rotation of ~ −166°, giving a two-stranded filament with a right-handed helical twist [4].

Actin polymerization occurs in an organized manner, involving head-to-tail interactions, producing filament polarity (Fig. 1b). The end that is comprised of subdomains 2 and 4 is called the pointed or minus end. The opposite end is known as the barbed or plus end. The assembly of three actin monomers, known as 'nucleation', occurs spontaneously at physiological ionic strength and initiates filament growth. Filament elongation can then continue from both ends; however, the rate of monomer addition at each end is asymmetric, such that the barbed end elongates roughly five- to tenfold faster than the pointed end. The likely basis of this asymmetry is that after incorporation into the filament, actin monomers hydrolyze ATP, resulting in a conformational change in subdomain 2 [25]. This change probably weakens the intermolecular interactions made at the pointed end of actin monomers, leading to increased dissociation from the pointed end where ADP actin accumulates, while new ATP-bound actins are predominantly added to the barbed end. This process is referred to as 'treadmilling', and is a fundamental component of actin filament dynamics, as well as the functional target of actin-binding natural products [4].

Classification of actin-targeting natural products

A diverse group of natural products found to specifically target and demonstrate cytotoxic activity against the actin cytoskeleton have been isolated from a variety of sources: terrestrial plants, sponges, marine nudibranchs, algae, fungi and bacteria. Excellent reviews depicting the detailed structures of actin-targeting compounds and
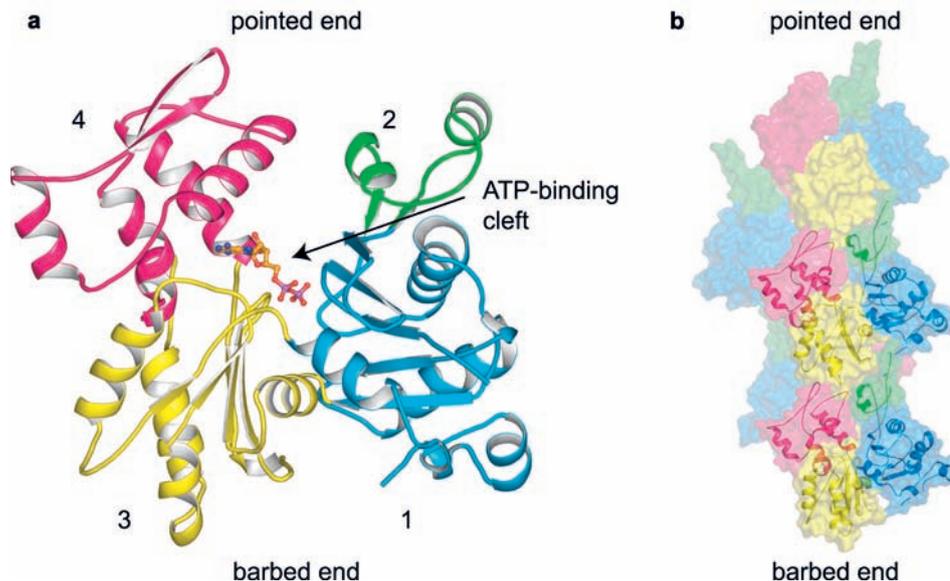
Figure 1. Structure of monomeric and filamentous actin. (*a*) Monomeric actin is shown as a ribbon representation and the bound nucleotide is shown as a stick representation. Actin subdomains 1–4 are labeled and colored individually (PDB accession 1J6Z [25]). (*b*) A model of the actin filament is shown as a surface representation of five actin monomers. The subdomains for each actin monomer are colored as in (*a*). The structure is based upon the filament model by Tirion et al. [59].

their effects on specific cell lines are available [8, 10, 26]. Their common structural features include a primarily hydrophobic component and stereochemically complex side groups. In general, these compounds can be divided into two groups: (1) those that inhibit filament assembly or destabilize filaments, and (2) those that stabilize actin filaments.

Filament-blocking/destabilizing compounds

Toxins that block or destabilize actin filaments have been shown to do so by binding two distinct regions of the actin monomer: (i) the ATP-binding cleft and (ii) the barbed end (Fig. 1a). Due to the dynamic nature of actin filaments, filament-destabilizing compounds can be further subdivided into those which merely sequester actin monomers when they passively dissociate from filaments, and those that also actively promote filament severing by binding directly to the filament and disrupting interactions between adjacent actin monomers. Since both mechanisms can lead to the apparent disassembly of actin filaments, the inherent mechanism of disassembly used by each toxin is outlined below.

Actin-binding mode of ATP-binding cleft-targeting compounds

Latrunculin cytotoxins are potent inhibitors of actin filament formation isolated from the Red Sea sponge *Negombata magnifica* (Fig. 2a) [27, 28]. The crystal struc-

ture of latrunculin A in complex with actin shows that this compound binds within the interface between actin subdomains 2 and 4, above the ATP-binding site (Fig. 2b) [27–30]. Here, the 2-thiazolidinone group is inserted into a pocket lined by residues Tyr69, Asp157, Arg183, Thr186, Arg206 and Arg210, where specific contacts involving the NH, OH, CO, and O-ester are formed.

Altogether, eight forms of latrunculin have been isolated to date, all of which contain the biologically rare thiazolidinone ring (Fig. 2a) [27, 31]. Comparisons of the structure-activity relationships for each latrunculin isoform have demonstrated the importance of the NH group on the thiazolidinone ring, as well as the OH side group on the pyrone ring for potent microfilament inhibition [31, 32]. Based on the orientation of latrunculin A in complex with actin, insertion of the 2-thiazolinone group is facilitated, and stabilized, by the rigid macrocyclic ring, which makes primarily hydrophobic contacts with actin (Fig. 2b). Interestingly, latrunculin B, which is also isolated from *N. magnifica* and differs from latrunculin A by the absence of two carbons in the macrocyclic ring, displays slightly lower cytotoxicity toward yeast and hamster fibroblast cell growth than latrunculin A [31, 33, 34]. This reduced activity likely results from the loss of hydrophobic interactions normally made by these two carbons and residue Gln59 in the latrunculin A-actin complex. This illustrates that the shape and size of the ring are also important determinants in the overall binding affinity of these cytotoxins for actin. An additional latrunculin-like compound, named latrunculeic acid, which lacks the macrocyclic and thiazolidinone rings, has recently been isolated. Not surprisingly, this compound is inactive toward actin [31].

Mechanism of filament destabilization of ATP-binding cleft-targeting compounds

The effect of latrunculins on actin is strictly limited to monomer sequestration [28, 33]. Comparison of actin filament models derived from fiber diffraction analysis and the binding mode of latrunculin A with actin in the crystal structure suggest that this compound inhibits assembly of actin monomers into filaments allosterically [29]. That is, latrunculin binding may act as a wedge between subdomains 2 and 4 (Figs. 1a, 2b), restricting conformational changes that normally allow the formation of stable interactions between actin monomer interfaces, thereby sequestering actin monomers from polymerization. What is not clear at this point is the exact nature of the conformational changes in actin that normally facilitate filament formation and whether the effects of latrunculin binding

a

Latrunculin A   Latrunculin B   Latrunculin C

b

Figure 2. Latrunculin chemical structures and actin-binding site. (*a*) The chemical structures of latrunculins A, B, and C are shown. (*b*) Latrunculin A is shown as green ball and sticks bound between subdomains 2 and 4 of actin. Amino acid residues in contact with latrunculin A are shown as purple sticks. ATP is shown as yellow sticks (pdb accession 1ESV [29]).

have a greater influence over interactions between axial or lateral actin monomer contacts (Fig. 1b).

Barbed end-targeting compounds

Unlike nucleotide-binding cleft-targeting toxins, those that bind to the barbed end of actin are numerous and their chemical structures are highly variable. There is also more structural information available for these compounds. In general, this class of toxins tends to have a larger and more diverse ring structure than latrunculins, as well as a long, stereochemically complex side chain. At least seven different classes of natural products target the barbed end (Fig. 3). This conclusion is based upon direct observations of toxin-actin interactions through X-ray cocrystallization data of five of these classes and the close similarities of their chemical structures to the remaining classes. (i) The trisoxazole toxins are isolated from Pacific sponges and nudibranch egg masses and contain many structural analogues. Over 30 forms of these compounds have been identified so far including mycalolides, ulapualides, kabiramides and halichondramides [8, 35–37]. (ii) Reidispongiolides and sphinxolides are isolated from the New Caledonian marine sponges *Reidispongia coerulea* and *Neosiphonia superstes*, respectively, and consist of numerous congeners [38–40]. (iii) Aplyronines are isolated from the Pacific sea hare *Aplysia kurodai* and exhibit structural differences from reidispongiolide/sphinxolide and trisoxazole macrolides primarily in the main chain of their ring structures [41]. Other structural differences characteristic of this group include the addition of the amino acid-like moieties, trimethylserine and dimethylalanine, on the ring and tail regions, respectively. (iv) Swinholide and misakinolide (bistheonellide) are dimeric macrolides isolated from the marine sponge *Theonella swinhoei*. Swinholide consists of a 44-membered dimeric cyclic lactone [42], while misakinolide has a 40-membered dimeric cyclic lactone that differs from swinholide by the absence of two double-bonded carbons from each repeated lactone moiety [43, 44]. (v) Scytophycins, such as tolytoxin, which are isolated from the terrestrial cyanobacterium *Scytonema pseudohofmanni*, resemble monomeric versions of swinholide, with tail sections similar to those of trisoxazole and reidispongiolide/sphinxolide toxins [45]. (vi) Lobophorolide is a recently identified 22-membered cyclic lactone isolated from the brown alga *Lobophora variegata*. Its ring and tail moieties together are essentially the structural equivalent for half of the dimeric cyclic lactone of swinholide A [46]. (vii) Finally, bistramides are a unique family of dilactam polyethers isolated from the marine ascidian *Lissoclinum bistratum* that have recently been shown to target actin with high affinity and inhibit filament formation and promote filament disruption [47–49]. Interest-

Figure 3. Barbed end-targeting macrolides. The chemical structures of representative members of known or inferred barbed end-targeting natural products are shown.

ingly, there is virtually no structural similarity between bistramides and other known G-actin-binding toxins.

Actin binding mode of barbed end targeting compounds

Recent crystallographic studies have determined the structures of representative members of the trisoxazole, reidispongiolide/sphinxolide, aplyronine, swinholide and bistramide classes of cytotoxins in their respective complexes with G-actin (Fig. 4) [50–55]. These studies have provided important details of the molecular interactions that confer their high affinity for actin. For each of the trisoxazole and reidispongiolide/sphinxolide compounds, this has also allowed assignment of the absolute stereochemical configuration for all stereocenters. In the structures of kabiramide C, jaspisamide A and ulapualide, their large hydrophobic ring lies against a large hydrophobic patch on the surface of actin that leads into the narrow cleft separating subdomains 1 and 3 at the barbed end (Fig. 5a). The ring portion of reidispongiolide A and C, and aplyronine A contacts this hydrophobic patch as well. However, the conformation of their ring appears to be flipped to the opposite face and interacts primarily with subdomain 3, while the trisoxazoles lie against subdomain 1 (Fig. 5b). This can be attributed to the opposite stereochemistries of the carbon at the ring-tail junction for these toxin families [54].

Sphinxolide B displays the same side of its ring to the solvent as reidispongiolide A and C and aplyronine A (Fig. 5c). However, here the replacement of a hydrogen atom at C10 (R_2) with a hydroxyl group and the absence of a methyl group at O19 (R_3) relative to reidispongiolide A and C produce limitations in the conformational freedom of the ring compared with the other toxins (Fig. 3). This results in a rotation of the plane of the ring away from the surface of actin in a manner that displaces over half of the ring-actin contact sites [53]. Loss of these contacts would substantially weaken the interaction between the toxin and actin. A reduction in the filament severing and capping activity of sphinxolide B relative to other toxins observed in *in vitro* studies using purified actin supports this conclusion [53].

Several aspects of the binding mode of swinholide A on actin are qualitatively similar to the other macrolides [52]. Here, the macrolide ring adopts a figure eight-like conformation that allows each half of the large ring to interact with the same hydrophobic patch on each actin monomer as the other toxins (Fig. 4e). Interestingly, the
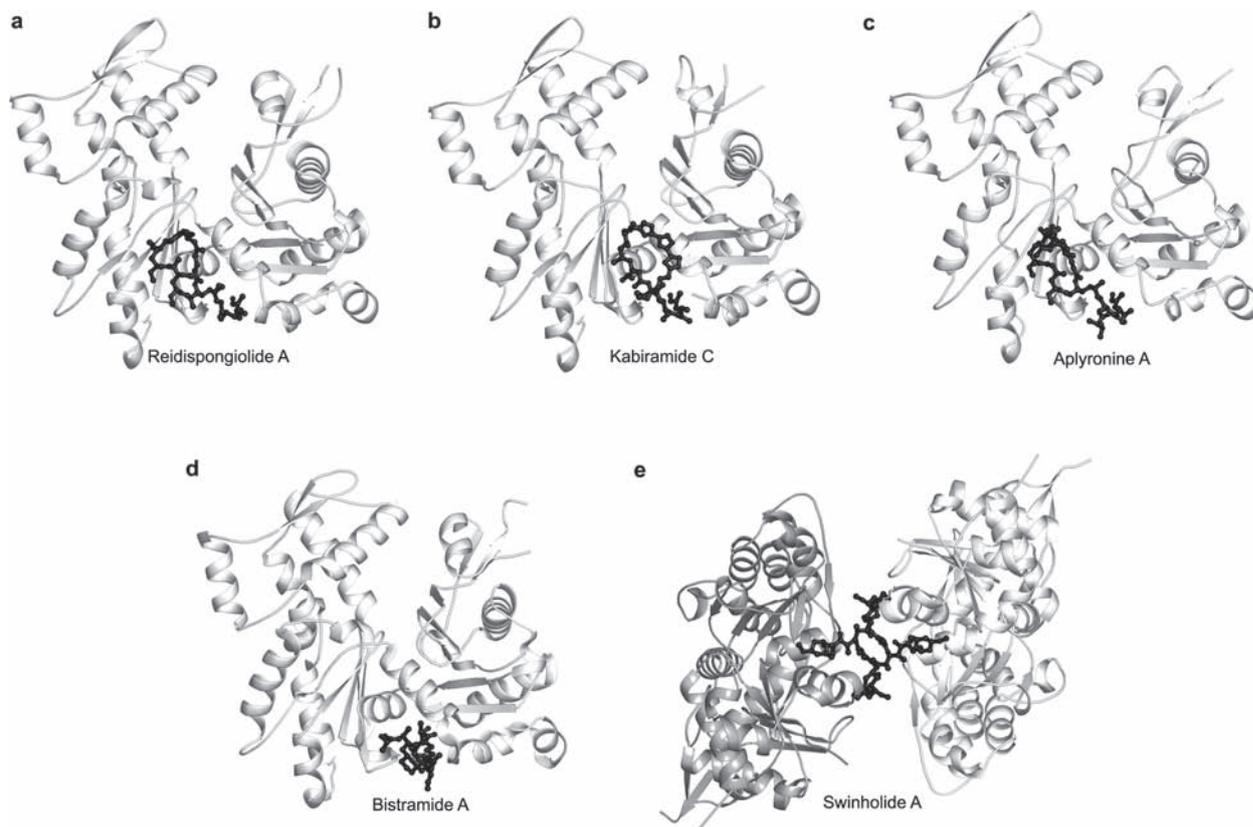

Figure 4. Structures of representative members of the trisoxazole, reidispongiolide/sphinxolide, aplyronine, bistramide and swinholide classes of cytotoxins in their respective complexes with actin. Actin is shown as a ribbon representation. Toxins are shown as ball and sticks. (*a*) Reidispongiolide A (PDB accession 2ASM [53]). (*b*) Kabiramide C (PDB accession 1QZ5 [50]). (*c*) Aplyronine A (PDB accession 1WUA [54]. (*d*) Bistramide A (PDB accession 2FXU [55]). (*e*) Swinholide A is shown bound to two actin monomers (PDB accession 1YXQ [52]).
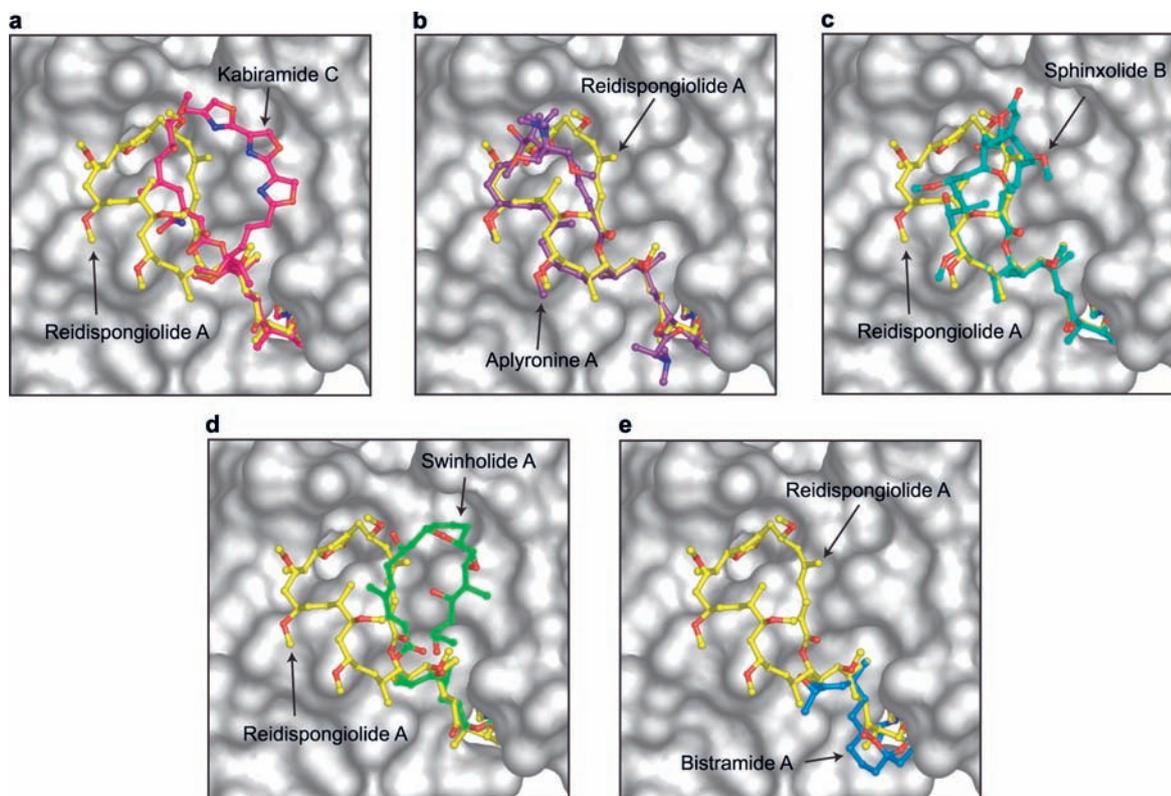
Figure 5. Binding modes of toxin ring moieties. (*a*) The actin-binding conformation of reidispongiolide A (yellow) and kabiramide C (magenta) are shown as an overlay using the actin coordinates from each structure as a reference. Actin is shown as a surface representation in white. (*b*) Reidispongiolide A (yellow) and aplyronine A (purple). (*c*) Reidispongiolide A (yellow) and sphinxolide B (teal). (*d*) Reidispongiolide A (yellow) and half of the dimeric lactone of swinholide A (green). (*e*) Reidispongiolide A (yellow) and bistramide A (blue).

conformation of each half of the ring resembles the mirror image of the conformation of the reidispongiolide A and C ring, and lies against subdomain 1 (Fig. 5d). The interaction between actin and misakinolide A will likely be quite similar, although the smaller ring size may limit the conformational freedom, such that acquisition of the twisted configuration observed in swinholide A is more difficult [52].

The strikingly different chemical structure of bistramide A creates a unique actin-binding mode relative to the other barbed end-targeting toxins [55]. Like the other toxins, bistramide A is situated within the cleft between subdomains 1 and 3. However, it does not contact the large hydrophobic patch on the surface of actin that leads into this cleft (Fig. 5e). Instead its interaction with actin spans the entire length of the cleft, beyond the termination point of the other toxins' tails. Its interactions with actin also involve considerably more polar contacts than the other toxins.

Based on this collective structural and functional information, a pharmacophore model of barbed end-targeting toxins can begin to be developed. In toxins containing the large hydrophobic ring, this component serves to maximize contacts with the comparatively large hydrophobic patch on the surface of actin (Fig. 5). It also provides con-

formational constraints that ensure appropriate presentation of key atoms to a discrete patch of residues on this surface. Within this binding surface, amino acid contacts common to each of these classes of toxin include Tyr143, Ala144, Gly146, Ile341 and Ile345. These contacts are made by a stretch of atoms that branch from analogous sides of the intersection of the ring and tail of all toxins and exist in an extended conformation (Fig. 6). This region could represent the pharmacophore component of the ring. Also, the conservation of this binding position among the majority of the toxin structures determined thus far indicates that this region on actin is a critical binding determinant for the toxins. It also implies that it is a critical site for axial actin monomer interactions within the filament. The region surrounding this surface appears to be large enough and amenable to a variety of chemical configurations and is thus less likely to be a critical contact surface for filament assembly.

Establishment of the observed interactions between the toxin ring and actin also aligns the tail section of the toxins for intercalation into the hydrophobic cleft separating domains 1 and 3. In nearly all of the structures of barbed end-targeting macrolides available to date, the conformation and actin interactions of the tail are virtually identical (Figs. 6, 7). Common amino acid
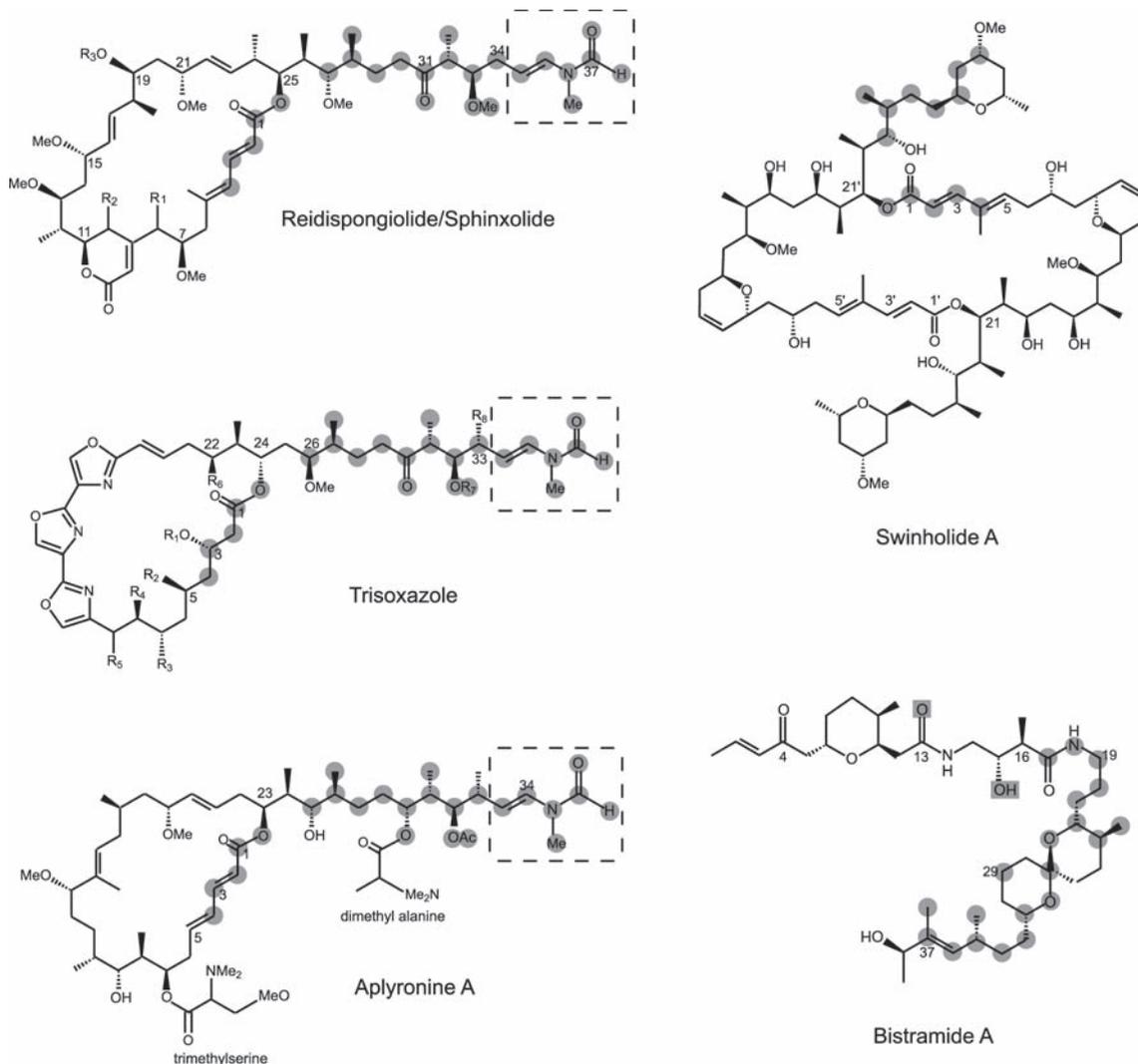
Figure 6. Pharmacophore model of barbed end-targeting toxins. The chemical structures of reidispongiolide/sphinxolide, trisoxazole, aplyronine A, swinholide A and bistramide A toxins are shown. Atoms covered by dots interact with the same residues on actin or overlap within van der Waals radii for all toxin families. Atoms covered by boxes on bistramide A spatially overlap with the water molecules coordinated to the N-methyl-vinylformamide (MVF) moieties of the reidispongiolide/sphinxolide, trisoxazole, aplyronine A toxins (dashed boxes).

residue interactions between the tails of the trisoxazole, reidispongiolide/sphinxolide and aplyronine toxins include Tyr133, Val139, Tyr143, Gly146, Arg147, Thr148, Gly168, Tyr169, Thr351 and Met355. For most family members of these toxins, there are also hydrogen-bonding interactions that occur between the highly conserved *N*-methyl-vinylformamide (MVF) moiety located at the end of the tail and two water molecules that are co-ordinated by the backbone amide hydrogens of Ile136 and Ala170 (Fig. 7a). Importantly, crystal structures of toxins lacking the MVF component, such as reidispongiolide C, or toxins whose tail contains side groups that interfere with this interaction, such as ulapualide, have a highly disordered tail terminus [51, 53]. They also display dramatically reduced filament severing and capping potency [53]. Although analyses of the cytotoxicity and *in vitro* actin-depolymerizing effects of synthetic analogues of

aplyronine A lacking the MVF showed partial recovery of activity when it was replaced by a different hydrophilic group, the level of activity was nevertheless significantly low relative to the natural form [56, 57]. Taken together, this would suggest that the MVF moiety makes a significant contribution toward stabilizing the intercalation of the tail deep within the barbed end cleft. Thus, along with the backbone of the rest of the tail, this moiety makes up the remainder of the pharmacophore of these toxins (Fig. 6).

All known forms of swinholide, misakinolide, lobophorolide and bistramide lack the MVF group. However, numerous atoms within the tail segments of swinholide A, and a large portion of the atoms in bistramide A, occupy the same space and interact with a number of the same residues within the cleft of actin as the tail of the other toxins [55]. In the case of swinholide A, the terminal py-
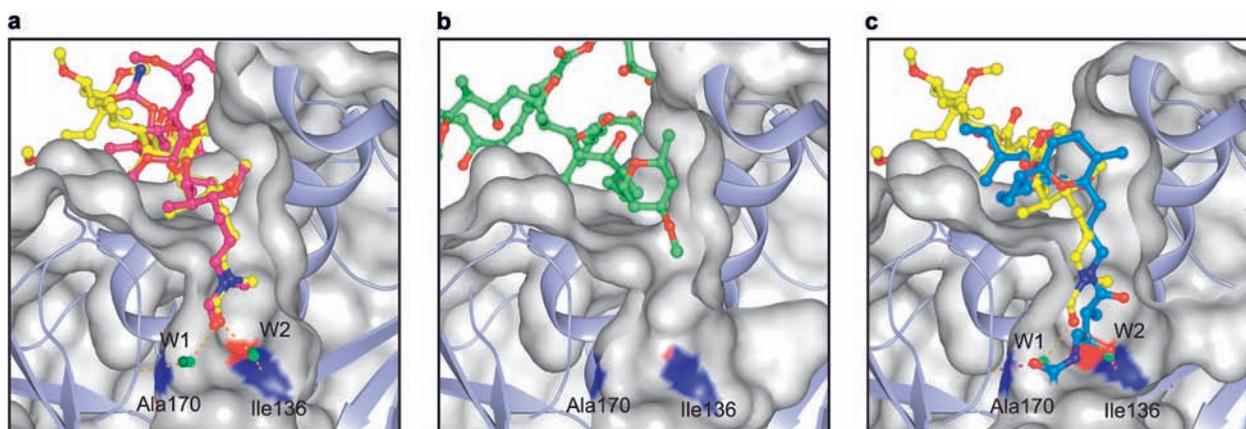
Figure 7. Binding modes of toxin tail moieties. (*a*) The tail conformations of reidispongiolide A (yellow) and kabiramide C (magenta) are shown within the cleft separating domains 1 and 3 of actin. Hydrogen-bonding interactions between the terminal MVF moiety and waters bridging actin residues Ile136 and Ala170 are shown. (*b*) The tail conformation of swinholide (green) is shown. (*c*) The tail conformation of reidispongiolide A (yellow) and C13 to C39 of bistramide A. Hydrogen-bonding interactions between the terminal MVF of reidispongiolide A and waters bridging actin residues Ile136 and Ala170 are shown as are the hydrogen-bonding interactions of the C13 carbonyl oxygen and C15 hydroxyl of bistramide A.

rone ring of their side chains may provide compensatory hydrophobic binding strength to stabilize their interaction with actin (Fig. 7b) [52]. Verification of this hypothesis will require direct comparisons of the capping and severing activities of these toxins to synthetic analogues lacking the pyrone ring, and to monomeric toxins containing the MVF group. For bistramide A, superposition of its structural coordinates onto those of reidispongiolide A reveal that the central hydroxyl and carbonyl groups occupy the same spatial position as the bridging water molecules found to interact with the MVF group of trisoxazoles, reidispongiolides, sphinxolides and aplyronines (Fig. 6). Furthermore, like their water counterparts, these groups also make direct hydrogen-bonding interactions with Ile136 and Ala170 of actin (Fig. 7c). This re-emphasizes the functional importance of formation of these specific polar interactions for toxin binding.

Considerations of the structure-activity relationship of toxin side groups other than the tail itself have not been characterized in detail either structurally or functionally for any of the barbed end-targeting toxins, with the exception of aplyronine. Comparisons of the cytotoxicity and actin depolymerization activity of natural and synthetic aplyronine analogues have produced a number of interesting observations about the various chemical modifications of these compounds [56, 57]. In particular, loss of the conjugated diene and trimethylserine components of the macrolide ring (Fig. 6), and the dimethylalanine on the tail, correlated with a dramatic reduction in cytotoxicity against HeLa S$_3$ cells. Conversely, the absence of the amino acid esters had little effect, and loss of the conjugated diene only had a moderate effect on actin depolymerization measurements by flow birefringence, relative to their cytotoxicity. A possible explanation for this discrepancy is that within a cellular environment these components facilitate uptake and retention within

the cell. Alternatively, they may prevent compartmentalization and/or metabolism to less active forms. Another possibility is that the amino acid additions, in particular, may disrupt the function of proteins other than actin, since they do not contribute significantly to the primary actin-binding surface of the toxin, as evidenced in the crystal structure. The fact that there was a moderate reduction in actin depolymerization of analogues lacking the conjugated diene (44% of aplyronine A) is undoubtedly related to the functional importance of this part of the ring, as previously described. This chemical form not only exists in aplyronines, but is also found in reidispongiolides, sphinxolides, swinholides, scytophycins and lobophorolide (Fig. 3). In each of these compounds, its purpose may be to reduce the conformational freedom of the ring and to facilitate the appropriate interaction with the hydrophobic surface on actin. Its absence in trisoxazole macrolides can be accounted for by the presence of the trisoxazole component on the opposite side of the ring, which provides compensatory ring rigidity.

Mechanism of filament-destabilization of barbed end-targeting compounds

While latrunculins bind within the ATP-binding pocket, barbed end toxins mainly interact at the surface of actin and are solvent exposed (Figs. 2b, 4, 5). Therefore, although it is possible that the effect of both classes of toxins on actin is allosteric, it seems more likely that the mechanism of action of the latter group involves the creation of steric clashes between axial actin monomers in the filament (Fig. 1b) [50, 53, 58]. Although the atomic details of actin-actin interactions within the filament are not entirely established, an examination of the Tirion et al. [59] model of F-actin derived from X-ray fiber diffraction

studies indicates that the position of bound toxin creates a steric clash between a stretch of hydrophobic residues found in the DNase I-binding loop of the 'lower' actin monomer and the hydrophobic cleft separating domains 1 and 3 of the axial 'upper' monomer (Fig. 8) [50, 59, 60]. If this model is correct, a conformational change involving a loop-to-helix transition in the DNase I-binding loop observed upon conversion from the ATP- to ADP-bound state actin would provide an elegant explanation for the higher critical concentration for polymerization of MgADP-actin [25, 61]. Furthermore, the toxin-binding site is also targeted by a number of actin-binding proteins, such as gelsolin, CapG and profilin, which sequester and sever actin, respectively, demonstrating the importance of accessibility of this site for intermonomer contacts [58, 62–64].

All of the barbed end-targeting toxins described above possess actin monomer-sequestering abilities. Not all of them, however, possess the ability to sever actin filaments [53, 65, 66]. The primary reason for this discrepancy is probably related to differences in the binding affinity of the particular toxin for actin due to its chemical structure. As outlined above, the capacity to sever is likely related
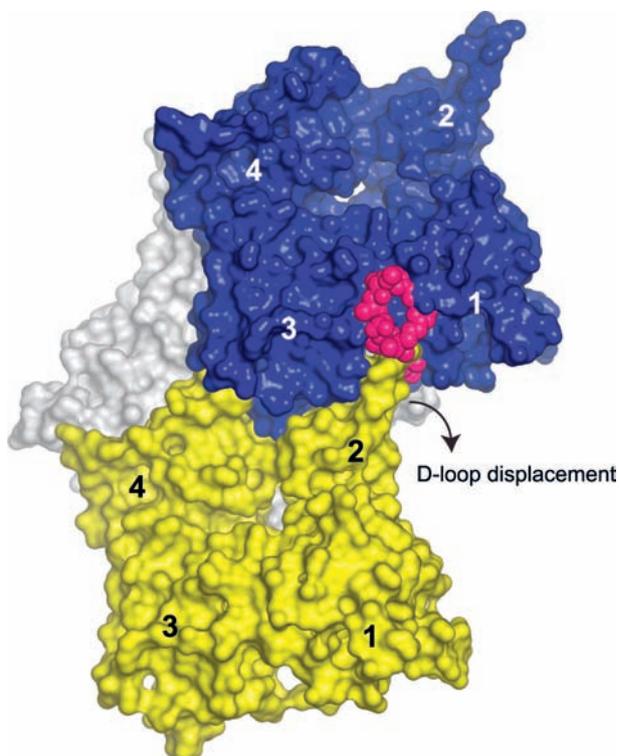

Figure 8. Proposed mechanism of filament-destabilization by barbed end-targeting macrolides. A surface representation of the structure of three monomers within the structural model for F-actin is shown with kabiramide C superimposed onto the barbed end of one actin monomer. The model shows the steric clash created between the DNase I-binding loop (D-loop) of the lower actin monomer and the bound toxin on the upper monomer, leading to displacement of the DNase I-binding loop from the cleft separating subdomains 1 and 3 of the upper monomer.

to the ability of the toxin to compete with the DNase I-binding loop once the intermonomer interaction has been formed. To do this, it has been proposed that, in ring-containing toxins, the ring makes an initial interaction with the hydrophobic patch on the side of actin, which is predicted to be exposed in the filament [50, 58]. This in turn orients and directs the long flexible tail into the cleft between subdomains 1 and 3 where hydrophobic contacts and, in the case of MVF-containing toxins, hydrogen bonds are formed, simultaneously displacing the DNase I-binding loop from the upper monomer (Fig. 8). Disruption of actin monomer contacts by what is, comparatively speaking, such a small molecule indicates that the interaction between axial monomers in the filament is relatively weak. Because filament integrity is largely dependent upon co-operativity, involving actin monomer interactions within and between filament strands, disruption of axial contacts in one filament strand would be sufficient to cause complete filament severing. Once this occurs, the toxin would prevent both monomer addition and dissociation by remaining attached to its actin-binding site, thereby capping the newly formed barbed end [53, 58].

With the markedly different overall chemical structure of swinholide A and misakinolide B, one might presuppose an alternate mechanism of filament disruption relative to monomeric barbed end-targeting macrolides (Fig. 3) [52, 65, 66]. However, because their binding mode on actin bears such remarkable similarity to monomeric macrolides (Figs. 5d, 7b), their mode of filament disruption is undoubtedly the same. What remains to be determined is how bistramide A, which lacks the discrete ring and tail components common to the other toxins, promotes F-actin depolymerization.

Structurally uncharacterized filament-destabilizing compounds

The current actin toxin structures may provide a model for the interactions of other toxins, like scytophycins and lobophorolide, with actin as well as their mechanism of filament-destabilization (Fig. 3) [45, 46, 67]. Although attempts to map the binding site of tolytoxin, a member of the scytophycin family, indicated that a region near the nucleotide-binding cleft on the back face of subdomains 2 and 4 was a likely site of contact, its ring resembles part of the monomeric unit of swinholide A [68]. Furthermore, its tail is remarkably similar to the trisoxazole, reidispongiolide/sphinxolide and aplyronine macrolides, terminating with an MVF moiety. Therefore, the binding mode and mechanism of filament-destabilization of this class of compound is undoubtedly the same.

Pectenotoxins are given separate mention from the other filament-destabilizing cytotoxins due to the unusual nature of their chemical structures (Fig. 9). Pectenotoxins
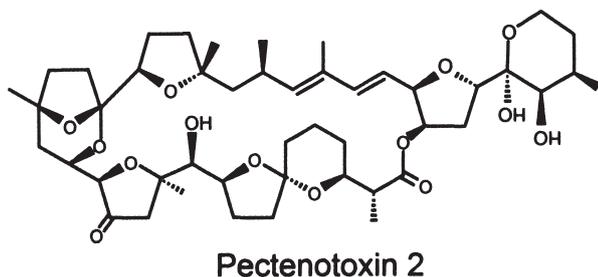
Pectenotoxin 2

Figure 9. Chemical structure of pectenotoxin-2.

represent a family of cyclic polyether macrolides produced by dinoflagellates such as *Dinophysis fortii* [69, 70]. They have also been found in the digestive glands of the toxic shellfish *Patinopecten yessoensis*, *Perna canalicus*, and *D. acuta*, where they are converted to other pectenotoxin chemical forms [71–73]. It has been reported that pectenotoxin-2 does not exhibit actin filament severing or capping activity, but only sequesters monomeric actin [8, 74]. Such activity is also reported for pectenotoxin-6, a derivative of pectenotoxin-2 [70]. Although pectenotoxins are structurally quite distinct from the other compounds that contain both ring and tail moieties, they contain a number of chemical features similar to those of bistramides. They are therefore likely to bind within the cleft separating actin subdomains 1 and 3 as well. What will be interesting is to see how their interaction with actin compares with the other actin-destabilizing compounds.

Filament-forming/stabilizing compounds

Natural compounds that stabilize actin filaments represent a separate class of cytotoxins that have been extremely useful as tools to understand actin filament assembly and organization, as well as actin-mediated cellular functions. As with the filament-destabilizing cytotoxins, elucidation of the molecular interactions made between these compounds and actin is important for understanding their mechanism of action as well as the structural properties of actin filaments. However, actin polymerization in the presence of these compounds makes crystallization impossible and thus the primary sources of information about the interaction of these toxins with actin have been derived from X-ray fiber diffraction data, electron microscopy and biochemical analyses [75–78].

The most common chemical feature of these cytotoxins is the cyclic depsipeptide, which is a polymeric compound containing both amino acids and hydroxy acids joined by peptide and ester bonds, respectively. Representative members of six different families of these compounds are shown in Figure 10. The best-known actin filament stabilizing compound is phalloidin, a phallotoxin synthesized by the poisonous 'Death Cap' mushroom *Amanita*

phalloides [79, 80]. Its chemical structure consists of a bicyclic heptapeptide that contains several uncommon amino acids [9]. The ring containing Cys3-Pro(OH)4-Ala5-Trp6 is essential for the cytotoxicity of the compound [76, 81].

Jasplakinolide is another actin filament stabilizer, as well as a potent inducer of actin polymerization, that is synthesized by the marine sponge *Jaspis johnstoni* [82]. Functionally, this cyclic depsipeptide behaves like phalloidin and even competes with its binding site. However, its structure is significantly different and it is membrane permeable, unlike phalloidin [82, 83]. Jasplakinolide contains a single 19-membered macrocyclic ring with branching aromatic side chains in the form of uncommon amino acids N-methyl-2-(bromo)-L-tryptophan and β-tyrosine, as well as L-alanine [8, 84, 85]. The L-tryptophan derivative is particularly important for actin binding in jasplakinolide, as is its analogue in phalloidin [82]. Jasplakinolide is closely related to the geodiamolide and neosiphoniamolide families of cytotoxins [86, 87].

Chrondramides are 18-membered cyclic depsipeptides from the myxobacterium *Chondromyces crocatus* and are remarkably similar in structure to jasplakinolide [88, 89]. These compounds also compete with phalloidin for actin binding [90, 91].

The dolastatin family of cytotoxins, isolated from the Indian Ocean sea hare *Dolabella auricularia*, are gaining recognition as useful F-actin stabilizers and potential anti-cancer agents [92]. Congeners of these compounds include both linear and cyclic forms. Studies of dolastatin-11 in particular, a 30-membered cyclic depsipeptide, indicate that its *in vitro* and cellular effects are similar but more potent than those of jasplakinolide and phalloidin, although it does not compete with phalloidin for actin-binding [93].

Doliculide is a 16-membered cyclic depsipeptide with a D-tyrosine derivative that is also isolated from *D. auricularia*, but in animals found off the coast of Japan [94, 95]. Biochemical studies on the (–)-doliculide isoform indicate its activity is identical to that of jasplakinolide and that it competitively inhibits phalloidin binding [96].

Finally, amphidinolides represent a unique group of cytotoxic compounds in that their interaction with actin, at least for amphidinolide H, involves formation of a residue-specific covalent bond [78]. Isolated from *Amphidinium* sp. dinoflagellates that live off the coasts of Japan and the U.S. Virgin Islands, these potent 26-membered macrolides display unique structural features including an allyl epoxide [97]. Studies by Kobayashi and colleagues have determined that the allyl epoxide, *S*-cis-diene moiety, and the ketone at C20 are important for the cytotoxicity of this compound [98].

One of the few examples of a non-depsipeptide that stabilizes actin filaments is the hectochlorin family of cytotoxins (Fig. 10). Compounds in this family come in the form
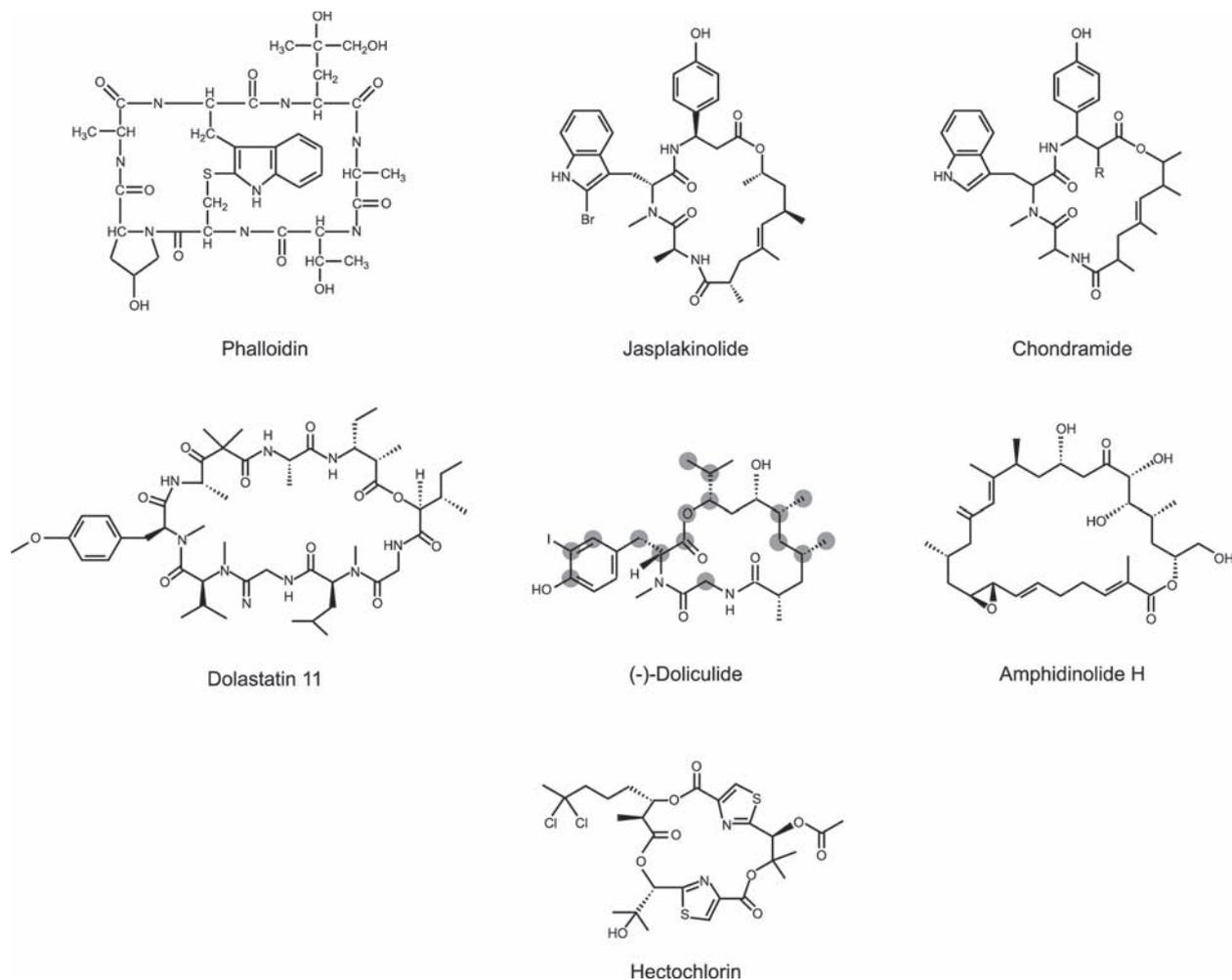
Figure 10. Filament-stabilizing macrolides. The chemical structures of representative members of known or inferred filament-stabilizing natural products are shown. Dots show the predicted pharmacophore of actin-stabilizing toxins based on shape matched alignment studies of phalloidin, jasplakinolide, dolastatin and (–)-doliculide [96].

of cyclic lipopeptides isolated from the cyanobacterium, *Lyngbya majuscula*, and have been demonstrated to be quantitatively identical to jasplakinolide in their ability to promote actin polymerization [99]. However, like dolastatin, hectochlorins do not compete with phalloidin for F-actin binding. Toxins bearing structural resemblance to hectochlorins include the lyngbyabellin class of natural products [100, 101].

Actin-binding mode

The phalloidin-binding site on actin has been characterized by microscopic and X-ray fiber diffraction methods and appears to interact with three actin monomers simultaneously via three different binding sites (Fig. 11) [75–77]. In particular, contacts between the weakly interacting two long-pitch strands of the filament appear to be stabilized by phalloidin binding between the loop containing residues 198–201 of the lower actin subunit, and the loop

of residues 73–75 and residue 179 of the diagonal subunit of actin [76]. Similarly, the binding site of dolastatin 11 has also been derived from X-ray fiber diffraction of actin filaments and, like phalloidin, appears to enhance filament stability by bridging the two long-pitch strands between subdomain 4 of one actin subunit and subdomain 1 of the diagonal subunit (Fig. 11) [102]. However, these studies demonstrated that within the filament, dolastatin 11 occupies a position that is clearly different from that of phalloidin. This is in agreement with the observation that this cytotoxin is incapable of displacing the phalloidin fluorescent derivative from actin polymers [93].

Identification of the binding site of amphidinolide H within the filament has been made possible by determining the site of the covalent interaction formed between this compound and actin [78]. Formation of this interaction seems to occur between the epoxide and Tyr198 (Tyr200 in mammalian actin) on subdomain 4 of actin (Fig. 11), such that only one toxin molecule is bound per actin monomer [78]. Because the site of covalent attach-
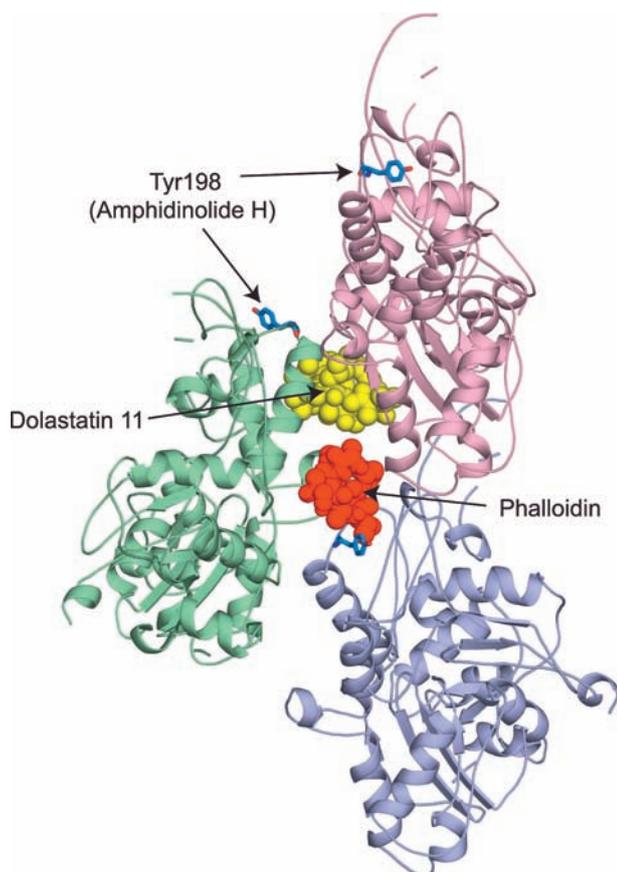
Figure 11. Binding locations of actin filament-stabilizing macrolides. Three monomers of G-actin are shown associating as in their F-actin form. The phalloidin- (red spheres) binding site within the filament shows it to interact with three actin monomers simultaneously [76]. Dolastatin 11 (yellow spheres) binds between subdomain 4 of one actin subunit and subdomain 1 of the diagonal subunit [102]. Amphidinolide H is not shown, although its interaction with actin is known to involve formation of a covalent bond with Tyr198 (blue sticks) [78].

ment of amphidinolide H is near the actin-actin contact between subdomain 4 of one subunit and subdomain 1 of the diagonally located subunit in F-actin, this suggests that this compound acts in a manner similar to phalloidin, jasplakinolide and dolastatin.

Based on the similar effects of phalloidin, jasplakinolide, dolastatin and doliculide on actin filament stabilization [82, 90, 93, 96, 103, 104], as well as their apparent interactions with a similar region of the actin filament, shape-matched alignment studies have recently been employed to identify a common pharmacophore within these diverse compounds [96]. This work concluded that a segment of the macrolide ring spanning from the benzyl group to the isopropyl side chain of (−)-doliculide overlapped significantly with analogous sections of the other filament-stabilizing macrolides. This segment was therefore considered the core pharmacophore component of these compounds (Fig. 10).

Mechanism of filament stabilization

A vast amount of structural, biochemical and theoretical analysis has been devoted to understanding the nature of monomer-monomer interactions during nucleation, filament elongation, and propagation of conformational changes through F-actin [105–108]. A key finding of these studies is that, as with many polymeric macromolecules, actin filaments rely on structural co-operativity for their assembly and stability. Based on their binding site and biochemical effects on polymeric actin, filament-stabilizing cytotoxins appear to take advantage of this co-operativity by binding to several actin monomers simultaneously (Fig. 11). Furthermore, under substoichiometric concentrations, their effects of stabilizing discrete locations of the filament are propagated through large distances via co-operativity within the filament [108–110]. Like the filament-destabilizing toxins, the affinity of these toxins for actin is determined by a subset of key structural features. All actin-stabilizing toxins consist of a ring component that limits the conformational flexibility of the compound. They also contain specific side groups whose configurations are undoubtedly important for molecular interactions with discrete actin residues. The necessity of these interactions re-emphasizes the indispensability of the ring, as its defined shape ensures appropriate presentation of these functional groups to the toxin-binding surface on actin. Part of what remains to be learned about this class of complex and fascinating molecules are the details of their molecular interactions with actin, as well as their allosteric effects on filament structure. Deciphering these key elements will require higher-resolution structural studies than those obtained by fiber diffraction.

Conclusions

The technological potential of natural product pharmacology is vast. Realization of this potential is considerably facilitated by understanding how these chemically diverse natural products interact with their biological targets and the specific molecular surfaces involved. X-ray crystallography, in combination with biochemical studies, has provided a powerful means of revealing this information. By characterizing the functional importance of both conserved and variable regions within these cytotoxic compounds, the structural themes that determine their bioactivity begin to emerge. This provides a useful framework from which synthetic chemistry can be used to create analogues with tailored chemical features to improve specificity and targeting toward malignant cells. This also reduces the necessity for chemical prospecting in delicate terrestrial and marine ecosystems that produce these rare compounds, providing much needed protection of biodiversity.

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