Microtubules and actin filaments: dynamic targets for cancer chemotherapy

Mary Ann Jordan* and Leslie Wilson†

Microtubules and actin filaments play important roles in mitosis, cell signaling, and motility. Thus these cytoskeletal filaments are the targets of a growing number of anti-cancer drugs. In this review we summarize the current understanding of the mechanisms of these drugs in relation to microtubule and actin filament polymerization and dynamics. In addition, we outline how, by targeting microtubules, drugs inhibit cell proliferation by blocking mitosis at the mitotic checkpoint and inducing apoptosis. The β-tubulin isotype specificities of new anticancer drugs and the antitumor potential of agents that act on the actin cytoskeleton are also discussed.

Addresses
Department of Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, CA 93106-9610, USA
*e-mail: jordan@lifesci.lscf.ucsb.edu
†e-mail: wilson@lifesci.lscf.ucsb.edu

Current Opinion in Cell Biology 1998, **10**:123–130

http://biomednet.com/elecref/0955067401000123

Abbreviations
MAD2 mitotic-arrest-deficient protein 2
OP18 oncoprotein 18

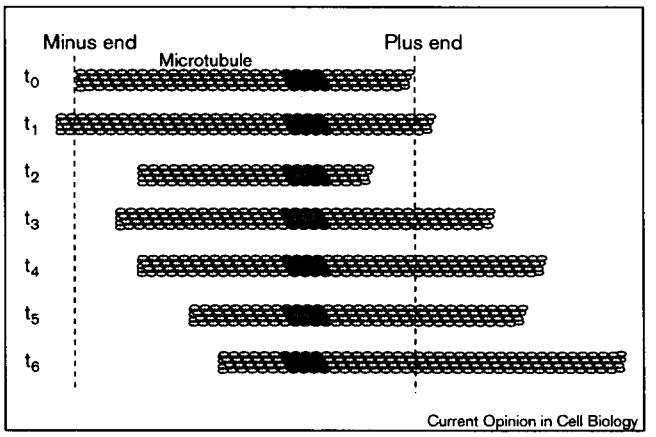
Introduction

Microtubules and actin filaments are cytoskeletal protein polymers critical for cell growth and division, motility, signaling, and the development and maintenance of cell shape (reviewed in [1]). Polymerization of actin monomers into actin filaments and of αβ-tubulin dimers into microtubules occur by similar nucleation-elongation pathways, in which formation of a short polymer 'nucleus' is followed by elongation of the polymer at each end by the reversible, noncovalent addition of subunits. Neither microtubules nor actin filaments are simple equilibrium polymers; instead both exhibit complex polymerization dynamics that use energy provided by the hydrolysis of nucleotide triphosphates. The non-equilibrium dynamics of these reactions are crucial to the cellular functions of the two cytoskeletal proteins.

Microtubule and actin filament polymerization dynamics

The polar nature of the microtubule polymer and the hydrolysis of GTP that occurs during microtubule polymerisation creates two unusual forms of dynamic behavior in cells and *in vitro*. One such form is *dynamic instability* [2], in which microtubule ends stochastically switch between episodes of prolonged growing and shortening. One microtubule end, the plus end, shows more dynamic instability behavior than the opposite or minus end. The other form of dynamic behavior, *treadmilling*, which is due to differences in the critical subunit concentrations at opposite microtubule ends [3,4], consists of net growing at microtubule plus ends and net shortening at minus ends. Treadmilling was shown, many years ago, to occur *in vitro* with microtubule populations rich in microtubule-associated proteins, but the rate of treadmilling was thought by some investigators to be intrinsically too slow to be of use in microtubule-mediated cell processes. However, rapid treadmilling of microtubules has recently been demonstrated in living cells [5•] and the notion that treadmilling is an important form of dynamic behavior in cells has been rekindled [6•].

Figure 1



Simultaneous dynamic instability and treadmilling in a single microtubule. The diagram shows consecutive 'snapshots' of a microtubule exhibiting episodes of growing and shortening at both plus and minus ends, with net growth at the plus end and net shortening at the minus end. The plus end shows more dynamic instability behavior than the minus end. The shaded subunits represent a marked segment of tubulin subunits, which is treadmilling or flowing from the plus to the minus end (t_0 through t_6 are arbitrary time points).

Microtubule dynamics are important for many microtubule-dependent processes in cells, the most dramatic of which is mitosis. When cells enter mitosis, the cytoskeletal microtubule network is dismantled and a bipolar, spindle-shaped array of microtubules is built that attaches to chromosomes and moves them to the two spindle poles. Microtubule dynamics are relatively slow in interphase cells, but increase 20- to 100-fold at mitosis. Both extensive dynamic instability and treadmilling (or flux) occur in mitotic spindles and the rapid dynamics of spindle

microtubules play critical roles in the intricate movements of the chromosomes.

The hydrolysis of ATP during polymerization of actin filaments also creates non-equilibrium dynamics. Actin filament treadmilling occurs both *in vitro* and in cells, with actin addition occurring at the 'barbed' ends of the filaments and actin loss occurring at the 'pointed' ends [1,7,8]. In principle, actin filaments could undergo dynamic instability; however, such behavior has not been observed.

While microtubules have long been valuable targets for cancer chemotherapy, recent evidence (discussed below) indicates that the dynamics of microtubules, not just their presence, are critical for cell proliferation [9–11]. The same may be true for actin filaments [12••].

Kinetic suppression (stabilization) of microtubule dynamics is a common and powerful mechanism of antimitotic agents

A large number of chemically diverse compounds, many of which are derived from natural products, bind to tubulin or microtubules and inhibit cell proliferation at the metaphase/anaphase transition of mitosis (see below) by acting on the mitotic spindle. Most such agents, including the vinca alkaloids and colchicine, inhibit microtubule polymerization at relatively high concentrations *in vitro* and in cells and thus have been thought to act solely by destroying the mitotic spindle (reviewed in [11]). More recently, a new class of anti-tumor drug, which includes taxol, taxotere, discodermolide and the epothilones, was also found to inhibit cell replication by acting on microtubules [13,14,15•–17•]. The mechanism of action of this class of compounds was initially considered to be fundamentally different from that of previous antimitotic compounds, in that the new drugs increased polymer mass and stabilized the microtubules. Recent video microscopic studies of the dynamics of individual microtubules *in vitro* and in cells, however, have revealed that, like taxol [18], antimitotic compounds that at high concentrations depolymerize microtubules, powerfully stabilize microtubule dynamics at low concentrations, with little or no accompanying microtubule depolymerization. For example, one or two molecules of vinblastine transiently bound at microtubule plus ends powerfully reduces both treadmilling and dynamic instability behaviors in the absence of significant changes in polymer mass (see [19,20]). Thus, the stabilizing effects of the vinca alkaloids [19,20], colchicine [21], cryptophycins [22], nocodazole [23•], and estramustine [24•]. on microtubule dynamics are much more similar to the stabilizing effects of taxol [25] than was previously recognized.

At their lowest effective concentrations, most of these compounds have been shown to inhibit the cell cycle at mitosis despite having little effect on microtubule polymer mass [18,19,26]. Inhibition of the cell cycle at mitosis occurs with only subtle alterations of the microtubule and chromosomal organization of the spindle. These observations, along with evidence that the drugs powerfully suppress microtubule dynamics (see above) led us to propose that the important common mechanism responsible for the potent ability of antimitotic compounds like taxol and vinblastine to inhibit cell proliferation and kill tumor cells is likely to be kinetic stabilization of spindle microtubule dynamics rather than depolymerization or excessive polymerization of the microtubules.

It is clear that all of the microtubule-targeted agents thus far examined powerfully stabilize microtubule plus ends *in vitro*, and that kinetic stabilization of spindle microtubules at plus ends by these agents is likely to play a major role in their antimitotic actions at the kinetochores. One poorly understood aspect of the action of microtubule-targeted compounds during mitosis is their effects at the spindle poles or centrosomes. These contain centriolar microtubules, tether the minus ends of spindle microtubules and are essential in most cells for spindle assembly [27]. Antimitotic drugs may have a number of effects on centrosomes: inducing abnormal centriole structure [28]; causing centrosomes to fragment [29]; and/or triggering inappropriate centrosome duplication in p53-lacking cells [30•]. In addition, recent work indicates that the effects of taxol [31•,32] and vinblastine [20] on microtubule dynamics differ at the plus and minus ends of microtubules. Since the consequences, for cell proliferation, of drug action on dynamics at spindle poles are unknown, exploring such consequences may prove valuable in the development of these drugs.

The antitumor mechanism of drugs acting on microtubules: inhibition of mitosis at the metaphase/anaphase transition induces apoptosis

Rapid microtubule dynamics during mitosis play an especially important role at metaphase. Disruption of spindle function with agents that suppress microtubule dynamics and, at high concentrations, either increase or destroy microtubules, blocks cell cycle progression in many cells at the transition from prometaphase/metaphase to anaphase — the mitotic checkpoint. Cell cycle arrest at the mitotic checkpoint is activated by a number of defects in microtubule-kinetochore interactions including microtubule depolymerization, stabilization of microtubule dynamics [18,19], absence of tension on kinetochore microtubules [33,34], and the presence of kinetochores that are not attached to microtubules [35]. Arrest for a prolonged period of time, caused by microtubule inhibitors, prevents the disappearance of staining for the checkpoint protein MAD2 (mitotic-arrest-deficient protein 2) from kinetochores [36••,37••], the destruction of mitotic cyclins, the inactivation of M-phase promoting factor (MPF) [38•], sister chromatid separation [39•], and cytokinesis (reviewed in [40]).

Laser ablation of an unattached kinetochore reverses arrest at the mitotic checkpoint suggesting that the mitotic block

is dependent upon a signal from unattached kinetochores [35]; however, examinations of the possibility that the number of attached microtubules (or empty attachment sites) per kinetochores on congressed chromosomes might be affected by antimitotic agents and contribute to mitotic block have yielded different results, depending upon the compound and incubation conditions used. Following brief exposure to micromolar concentrations of taxol, for example, the number of attached microtubules per kinetochore was unchanged [41••]; whereas, following prolonged incubation with nanomolar concentrations of vinblastine, the number was reduced [28].

So, what happens downstream from drug-induced mitotic block? Mitotic block persists for varying lengths of time, depending upon the cell type, and most cells ultimately exit mitosis and undergo apoptosis. Apoptosis may result from aberrant cell cycle control could and could be a consequence of conflicting growth-regulatory signals leading to an unsuccessful attempt to traverse the cell cycle [42]. Mitotic block by drugs, at concentrations that suppress microtubule dynamics [43•] or alter microtubule mass, induces apoptosis [44–48]. The biochemical events leading to apoptosis are complex, little understood and vary among cell types. Interesting new evidence indicates that apoptosis induced by microtubule-targeted compounds may involve phosphorylation of the protein kinase Raf-1 and the apoptotic regulator bcl2 [49•–51•].

On a related note, recent work indicates that, compared to normal cells, transformed and mutated cells respond differently to drug-induced mitotic block [52•,53•]. In addition, taxol appears to induce a temporary G1 block in nontransformed cells; however, transformed cells are not blocked, and undergo apoptotis [52•].

Novel antimitotic antiproliferative compounds

Several promising new compounds that interact with microtubules or tubulin, inhibit cell proliferation, and block mitosis have recently been characterized [54–58]. The search for taxol-like compounds has produced two interesting drug candidates: the natural products discodermolide and epothilone. Like taxol, these compounds block mitosis, stabilize microtubules, and induce formation of microtubule bundles in cells [15•–17•].

The cryptophycins are another recently-discovered class of remarkably potent natural compounds that are active against human solid tumors in murine xenographs. Cryptophycins bind tightly to tubulin and inhibit cell proliferation. At picomolar concentrations they slow or block mitosis and induce apoptosis, and at high concentrations they inhibit tubulin polymerization [59,60]. Cryptophycin 1 appears to bind at or near to the vinca site on tubulin [59–61], suppressing microtubule dynamics more potently than vinblastine or taxol without inducing net microtubule depolymerization [22].

Synergistic effects of combinations of antimitotic compounds

In addition to the development of novel compounds, a recent advance with potential clinical importance has been the observation that combinations of antimitotic drugs that stabilize microtubule dynamics, by binding to different sites on tubulin or microtubules, often have additive or synergistic anti-proliferative effects [24•,62•,63,64].

Tubulin isotypes, mutations, and drug efficacy

Important unsolved questions concerning the antitumor activity of microtubule-targeted drugs are, firstly, the basis of their tissue and tumor specificity, and secondly, the mechanisms involved in the development of drug-resistance. Recent evidence indicates that tubulin isotypes are an important determinant of drug efficacy. There are six α-tubulin isotypes and six β-tubulin isotypes in human cells. The β-tubulin isotypes, in particular, show marked differences in microtubule dynamics [65•] and drug binding [66•], and exhibit tissue-, cell- and tumor-specific patterns of expression. Taxol suppresses the dynamics of microtubules composed of purified αβIII- or αβIV-tubulin less strongly than the dynamics of control microtubules, suggesting that overexpression of βIII and βIV may lead to taxol resistance [65•]. In agreement with these findings, βIII and/or βIV isotypes are overexpressed in taxol-resistant ovarian tumor tissue [67••] and in human leukemia, prostate, and lung tumor cells in culture [67••,68,69•], in the absence of altered drug uptake. By contrast, taxol-resistant sarcoma cells derived by single-step exposure to taxol underexpressed the βIII and βIV isotypes [70•]. Another important mechanism of resistance, that is often ignored in studies of isotype-induced resistance, involves mutations in tubulin [71••,72]. Overall the present results suggest that alteration of tubulin isotype expression may be an important mechanism of tumor resistance to anti-mitotic drugs; if so, the development of drugs that target specific isotypes in tumors may be an attractive approach to achieving greater tumor specificity.

Cancer and oncoprotein 18, an endogenous destabilizer of microtubule dynamics

As described above, all antimitotic antitumor drugs examined to date suppress microtubule dynamics. A recently described endogenous phosphoprotein, oncoprotein 18 (Op18, stathmin, metablastin, p19) is overexpressed in a number of proliferating cell types and malignancies (discussed in [73•]). Op18 appears to modulate the mass of and increase the dynamics of microtubules in a cell-cycle-dependent manner [74•]. Alterations of Op18 levels or the phosphorylation state of its 4 serine residues confirm its potent effects on cellular microtubules, although many aspects of its mechanism of action remain to be elucidated [75•,76•].

Actin filaments in tumorigenesis: the actin cytoskeleton as a target for anticancer drugs

It has been known for 30 years that the actin cytoskeleton is substantially modified in transformed cells, and this occurs in concert with changes in a host of actin filament-associated regulatory proteins. These changes are thought be integrally involved in the abnormal growth properties of tumor cells, their ability to adhere to tissue, and their increased ability to metastasize. In the light of recent explosive work in this area (for reviews see [77–80]) the possibility that actin filaments and their many regulatory proteins could be selectively targeted in cancer chemotherapy is very attractive. For example, a recent study has, for the first time, quantitated the reduction in the ratio of polymerized actin to soluble actin that is apparent in transformed cells compared with non-transformed cells [81••]. The study also found that malignant cells were more sensitive to cytochalasin B than normal cells were [82••]. Since increased levels of soluble actin and decreased levels of polymerized actin appear to be relatively early events in tumorigenesis, assessment of the relative levels of these two forms of actin in cells might prove to be valuable in identifying individuals at risk from certain cancers [82••]. Interestingly, gelsolin, an actin capping/severing protein is commonly downregulated in many tumor cells and evidence is increasing that suggests gelsolin functions as a tumor suppressor [83••].

Compounds with antitumor potential that act on actin filaments or the actin cytoskeleton

The cytochalasins, the best studied and most widely used agents that act on actin, are mold metabolites that exert powerful effects on actin filament organization and function in cells [84]. Studies have indicated that a single molecule of cytochalasin, bound to the barbed end of an actin filament, caps the end so that the rates both of actin addition and loss are reduced. The cytochalasins do not affect dynamics at the pointed end. Cytochalasins both reduce actin filament mass and kinetically stabilize the barbed end dynamics of the filaments (reviewed in [85]). In important respects, this kinetic stabilization of the barbed end parallels the stabilizing actions of vinblastine and colchicine at microtubule plus ends.

It is curious that until recently, the cytochalasins were the only compounds known to affect actin filament polymerization and dynamics. During the past few years, however, a substantial number of powerful and chemically diverse new compounds, all derived from natural products, have been discovered that modulate actin polymerization and dynamics, often by unique mechanisms. They are likely to become valuable tools for the analysis of actin dynamics and functions in cells. Because they inhibit cell proliferation, they or their derivatives have potential as chemotherapeutic agents in the treatment of cancer.

Probably the best studied of the new actin-targeted compounds are the lantrunculins, natural marine products that bind to actin and disrupt actin organization in a wide variety of cells. They powerfully inhibit proliferation in a number of tumor cell lines, and appear to depolymerize actin filaments by sequestering actin subunits [86]. In addition to their anticancer potential, the lantrunculins are valuable probes of actin filament function in cells [87••].

Another new marine product with anticancer potential that acts on actin filaments is jasplakinolide, a cyclodepsipeptide. Jasplakinolide appears to act differently from the lantrunculins, binding to actin filaments, stabilizing them, and promoting their polymerization [88,89]. Swinholide A, a potent marine cytotoxic dilactone macrolide, destroys the actin cytoskeleton by severing actin filaments and sequestering actin dimers with a stoichiometry of one molecule of swinholide A per actin monomer. Its severing action appears cooperative and in some ways it resembles the severing actions of actophorin and gelsolin [90].

Misakinolide A is another novel compound; a dimeric lactone macrolide marine product that is similar in structure to swinholide A, it inhibits actin filament elongation at the barbed ends of actin filaments but, in contrast to swinholide A, it does not sever or stabilize the filaments [91]. Other compounds that appear to act by disrupting the actin cytoskeleton by a selective action on the contractile ring, which inhibits cytokinesis, are stypoldione [92] and pseudopterolide [93]. It is important to recognize that the mechanisms and the specificity for actin filaments of many of the aforementioned agents are still not well understood, and more efforts are required to elucidate them.

Finally, it should be emphasized that microtubules and actin filaments work in concert with a great many accessory proteins that regulate their organization and function. These accessory proteins also offer important targets for cancer chemotherapy. Agents, for example, designed to inhibit or mimic the actions of gelsolin might prove highly valuable in the treatment of cancer. One such class of agents presently generating considerable interest are inhibitors of farnesyltransferase, compounds that appear to selectively inhibit proliferation of Ras-transformed cells, without antiproliferative effects on normal cells, through a mechanism that may involve the actin cytoskeleton [94•,95–97].

Conclusions

Microtubule dynamics play an important role in cell proliferation, and inhibition of microtubule dynamics now appears to be the mechanistic basis underlying the antitumor effects of most antimitotic compounds. This advance in our understanding has led to renewed interest in the therapeutic potential of compounds that stabilize microtubule dynamics. Our developing knowledge of the importance of actin and actin-associated proteins in cancer could well lead to a similar expansion of interest in the therapeutic potential of compounds that target the actin cytoskeleton.

Acknowledgements

We sincerely apologize for any omissions; the task of summarizing the many exciting recent developments in this field was enormous. We gratefully acknowledge the support of the National Insitutes of Health (NIH grants CA57291 and NS13560).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Lodish H, Baltimore D, Berk A, Zipursky SL, Matsudaira P, Darnell J: *Mol Cell Biol*, edn 3. New York: Scientific American Books; 1995.

2. Mitchison TJ, Kirschner M: **Dynamic instability of microtubule growth.** *Nature* 1984, **312**:237-242.

3. Margolis RL, Wilson L: **Opposite end assembly and disassembly of microtubules at steady state *in vitro*.** *Cell* 1978, **13**:1-8.

4. Margolis RL, Wilson L: **Microtubule treadmills-possible molecular machinery.** *Nature* 1981, **293**:705-711.

5. • Rodionov VI, Borisy GG: **Microtubule treadmilling *in vivo*.** *Science* 1997, **275**:215-218.
This paper contains an elegant demonstration of rapid microtubule treadmilling in melanophore cell cytoplast preparations in the absence of detectable dynamic instability behavior.

6. • Waterman-Storer CM, Salmon ED: **Microtubule dynamics: treadmilling comes around again.** *Curr Biol* 1997, **7**:369-372.
This paper summarizes recent data on microtubule treadmilling in living cells.

7. Wang Y-L: **Exchange of actin subunits in the leading edge of living fibroblasts: possible role of treadmilling.** *J Cell Biol* 1985, **101**:597-602.

8. Theriot JA, Mitchison TJ: **Actin microfilament dynamics in locomoting cells.** *Nature* 1991, **352**:126-131.

9. Hayden JJ, Bowser SS, Rieder C: **Kinetochores capture astral microtubules during chromosome attachment to the mitotic spindle: direct visualization in live newt cells.** *J Cell Biol* 1990, **111**:1039-1045.

10. Rieder CL, Alexander SP, Rupp G: **Kinetochores are transported poleward along a single astral microtubule during chromosome attachment to the spindle.** *J Cell Biol* 1990, **110**:81-95.

11. Wilson L, Jordan MA: **Microtubule dynamics: taking aim at a moving target.** *Chem Biol* 1995, **2**:569-573.

12. •• Theriot JA: **Accelerating on a treadmill: ADF/cofilin promotes rapid actin filament turnover in the dynamic cytoskeleton.** *J Cell Biol* 1997, **136**:1165-1168.
This paper describes, in summary form, the results of recent papers showing that the dynamics of actin filaments are much more rapid in cells than *in vitro*, and that the rapid dynamics are promoted by a group of small actin binding proteins including cofilin, destrin, depactin, actophorin, and actin depolymerizing factor (ADF).

13. Schiff PB, Horwitz SB: **Taxol stabilizes microtubules in mouse fibroblast cells.** *Proc Natl Acad Sci USA* 1980, **77**:1561-1565.

14. Bissery M-C, Guenard D, Gueritte-Voegelein F, Lavelle F: **Experimental antitumor activity of taxotere (RP 56976, NSC 628503), a taxol analogue.** *Cancer Res* 1991, **51**:4845-4852.

15. • Hung DT, Chen J, Schreiber SL: **(+)-Discodermolide binds to microtubules in stoichiometric ratio to tubulin dimers, blocks taxol binding and results in mitotic arrest.** *Chem Biol* 1996, **3**:287-293.
Here, the authors report that discodermolide competes with taxol for binding to microtubules and binds with higher affinity than taxol. It also induces M phase arrest and bundles microtubules in cells.

16. • Ter Haar E, Kowalski R, Hamel E, Lin C, Longley R, Gunasekera S, Rosenkranz H, Day BW: **Discodermolide, a cytotoxic marine agent that stabilizes microtubules more potently than taxol.** *Biochemistry* 1996, **35**:243-250.
In the paper it is shown that discodermolide is a cytotoxic marine agent that induces mitotic arrest in Burkitt lymphoma cells, bundles microtubules in cells, and is more potent than taxol in inducing tubulin to form microtubules at low temperature, in the absence of GTP and/or microtubule binding proteins.

17. • Kowalski R, Giannakakou P, Hamel E: **Activities of the microtubule-stabilizing agents epothilones A and B with purified tubulin and in cells resistant to paclitaxel (Taxol(R)).** *J Biol Chem* 1997, **272**:2534-2541.
This paper shows that epothilones A and B block mitosis, induce tubulin to form microtubules at low temperature in the absence of GTP and/or microtubule binding proteins, and competitively inhibit the binding of taxol to tubulin polymers. Epothilone B is shown to be more potent in cells than epothilone A or taxol. Drug-resistant colon and ovarian cell lines retain sensitivity to the epothilones.

18. Jordan MA, Toso RJ, Thrower D, Wilson L: **Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations.** *Proc Natl Acad Sci USA* 1993, **90**:9552-9556.

19. Dhamodharan RI, Jordan MA, Thrower D, Wilson L, Wadsworth P: **Vinblastine suppresses dynamics of individual microtubules in living cells.** *Mol Biol Cell* 1995, **6**:1215-1229.

20. Panda D, Jordan MA, Chin K, Wilson L: **Differential effects of vinblastine on polymerization and dynamics at opposite microtubule ends.** *J Biol Chem* 1996, **271**:29807-29812.

21. Panda D, Daijo JE, Jordan MA, Wilson L: **Kinetic stabilization of microtubule dynamics at steady state *in vitro* by substoichiometric concentrations of tubulin-colchicine complex.** *Biochemistry* 1995, **34**:9921-9929.

22. Panda D, Himes RH, Moore RE, Wilson L, Jordan MA: **Unusually potent suppression of microtubule dynamics by cryptophycin 1: a mechanism for its antiproliferative action.** *Biochemistry* 1997, **36**:12948-12953.

23. • Vasquez RJ, Howell B, Yvon A-MC, Wadsworth P, Cassimeris L: **Nanomolar concentrations of nocodazole alter microtubule dynamic instability *in vivo* and *in vitro*.** *Mol Biol Cell* 1997, **8**:973-985.
Here the authors show that he compound nocodazole, a benzimidazole derivative that arrests mitosis, suppresses microtubule dynamics in living cells in interphase without depolymerizing microtubules.

24. • Panda D, Miller H, Islam K, Wilson L: **Stabilization of microtubule dynamics by estramustine by binding to a novel site in tubulin: A possible mechanistic basis for its antitumor action.** *Proc Natl Acad Sci USA* 1997, **94**:10560-10564.
Shows additive stabilization of microtubule dynamics by low concentrations of vinblastine and estramustine.

25. Derry WB, Wilson L, Jordan MA: **Substoichiometric binding of taxol suppresses microtubule dynamics.** *Biochemistry* 1995, **34**:2203-2211.

26. Jordan MA, Thrower D, Wilson L: **Mechanism of inhibition of cell proliferation by Vinca alkaloids.** *Cancer Res* 1991, **51**:2212-2222.

27. Zhang D, Nicklas R: **Chromosomes initiate spindle assembly upon experimental dissolution of the nuclear envelope in grasshopper spermatocytes.** *J Cell Biol* 1995, **131**:1125-1131.

28. Wendell KL, Wilson L, Jordan MA: **Mitotic block in HeLa cells by vinblastine: ultrastructural changes in kinetochore-microtubule attachment and in centrosomes.** *J Cell Sci* 1993, **104**:261-274.

29. Jordan MA, Thrower D, Wilson L: **Effects of vinblastine, podophyllotoxin and nocodazole on mitotic spindles. Implications for the role of microtubule dynamics in mitosis.** *J Cell Sci* 1992, **102**:401-416.

30. • Fukasawa K, Choi T, Kuriyama R, Rulong S, Vande Woude GF: **Abnormal centrosome amplification in the absence of p53.** *Science* 1996, **271**:1744-1747.
In this paper it is observed that in mouse embryonic fibroblasts lacking the p53 tumor suppressor protein, multiple copies of functionally competent centrosomes were generated during a single cell cycle, implicating p53 in the regulation of centrosome duplication and suggesting a mechanism by which loss of p53 may cause genetic instability.

31. • Waters JC, Mitchison TJ, Rieder CL, Salmon ED: **The kinetochore microtubule minus-end disassembly associated with poleward flux produces a force that can do work.** *Mol Biol Cell* 1996, **7**:1547-1558.
Here the authors show that taxol inhibits kinetochore-microtubule assembly/disassembly at kinetochores; however, minus-end microtubule disassembly continued.

32. Derry WB, Wilson L, Jordan MA: **The low potency of taxol at microtubule minus ends: implications for its therapeutic mechanism.** *Cancer Res* 1998, in press.

33. Li X, Nicklas RB: **Mitotic forces control a cell-cycle checkpoint.** *Nature* 1995, **373**:630-632.

34. Nicklas RB, Ward SC, Gorbsky GJ: **Kinetochore chemistry is sensitive to tension and may link mitotic forces to a cell cycle checkpoint.** *J Cell Biol* 1995, **130**:929-939.

35. Rieder CL, Cole RW, Khodjakov A, Sluder G: **The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitory signal produced by unattached kinetchores.** *J Cell Biol* 1995, **130**:941-948.

36. •• Chen R, Waters J, Salmon E, Murray A: **Association of spindle assembly checkpoint component XMAD2 with unattached kinetochores.** *Science* 1996, **274**:242-246.
This paper reports the isolation and expression of a cDNA encoding the Xenopus homolog of MAD2 protein (mitotic-arrest deficient 2 protein). Affinity-purified antibodies to XMAD2 recognized a 25-kD protein localized to unattached kinetochores at prometaphase in nocodazole-treated frog or human cells. The protein disappeared from kinetochores at metaphase in untreated cells, suggesting that XMAD2 plays a role in activation of the checkpoint by unattached kinetochores.

37. •• Li Y, Benezra R: **Identification of a human mitotic checkpoint gene: hsMAD2.** *Science* 1996, **274**:249-249.
Here, the human homolog of MAD2 (hsMAD2) was isolated and shown to be a necessary component of nocodazole-induced mitotic arrest in Hela cells, by electroporation of anti-hsMAD2 antibodies. As in [36••], hsMAD2 was localized at the kinetochore in prometaphase in nocodazole-treated cells, but disappeared from kinetochores at metaphase in untreated cells. Human breast tumor cells exhibited reduced hsMAD2 expression and failed to arrest in mitosis after nocodazole treatment, suggesting that defects in MAD2 expression may alter the responsiveness of tumors to antimitotic drugs.

38. • Minshull J, Straight A, Rudner AD, Dernburg AF, Belmont A, Murray AW: **Protein phosphatase 2A regulates MPF activity and sister chromatid cohesion in budding yeast.** *Curr Biol* 1996, **6**:1609-1620.
In this paper it is shown that in budding yeast the *cdc55* gene, which encodes a protein phosphatase 2A regulatory subunit, shows a checkpoint defect that allows sister chromatids to separate and cells to exit mitosis without inducing cyclin B destruction. This suggests that after drug-blockage at the mitotic checkpoint, cells may bypass the cyclin B proteolytic mechanism and inactivate MPF by phosphorylation of Cdc28.

39. • Straight A, Belmont A, Robinett CC, Murray AW: **GFP tagging of budding yeast chromosomes reveals that protein–protein interactions can mediate sister chromatid cohesion.** *Curr Biol* 1996, **6**:1599-1608.
In mitotic checkpoint-deficient yeast mutants *mad* and *bub*, this study shows sister chromatid separation can occur in the absence of microtubules (after microtubule depolymerization by high concentrations of the mitotic inhibitor nocodazole), indicating that sister chromatic separation is under the control of the spindle-assembly checkpoint and can occur in the absence of microtubule-dependent forces.

40. Rudner AD, Murray AW: **The spindle assembly checkpoint.** *Curr Biol* 1996, **8**:773-780.

41. •• McEwen B, Heagle A, Cassels G, Buttle K, Rieder C: **Kinetochore fiber maturation in PtK1 cells and its implications for the mechanisms of chromosome congression and anaphase onset.** *J Cell Biol* 1997, **137**:1567-1580.
By same-cell video light microscopy/serial section electron microscopy of PtK1 cells the authors of this report found that treatment of late metaphase cells with 1 μM taxol inhibits anaphase onset, but does not alter the number of kinetochore microtubules, suggesting that inhibition of anaphase onset by taxol involves suppression of microtubule dynamics rather than inhibition of microtubule attachment to kinetochores.

42. Colombel M, Olsson CA, Ng PY, Buttyan R: **Hormone-regulated apoptosis results from reentry of differentiated prostate cells onto a defective cell cycle.** *Cancer Res* 1992, **52**:4313-4319.

43. • Jordan MA, Wendell KL, Gardiner S, Derry WB, Copp H, Wilson L: **Mitotic block induced in HeLa cells by low concentrations of paclitaxel (Taxol) results in abnormal mitotic exit and apoptotic cell death.** *Cancer Res* 1996, **56**:816-825.
In this paper the authors triggered apoptosis in HeLa cells, following mitotic block, by low nanomolar concentrations of taxol that appear to suppress microtubule dynamics but do not bundle microtubules. Following removal of taxol, cells entered an interphase-like state without progressing through anaphase or cytokinesis and underwent apoptosis.

44. Bhalla K, Ilerado AM, Tourkina E, Tang C, Mahoney ME, Huang Y: **Taxol induces internucleosomal DNA fragmentation associated with programmed cell death in human myeloid leukemia cells.** *Leukemia* 1993, **7**:563-568.

45. Donaldson KL, Goolsby G, Kiener PA, Wahl AF: **Activation of p34cdc2 coincident with taxol-induced apoptosis.** *Cell Growth Differ* 1994, **5**:1041-1050.

46. Danesi R, Figg WD, Reed E, Myers CE: **Paclitaxel (taxol) inhibits protein isoprenylation and induces apoptosis in PC-3 human prostate cancer cells.** *Mol Pharmacol* 1995, **47**:1106-1111.

47. Martin SJ, Cotter TG: **Disruption of microtubules induces an endogenous suicide pathway in human leukaemia HL-60 cells.** *Cell Tissue Kinet* 1990, **23**:545-559.

48. Woods CM, Zhu J, McQueney PA, Bollag D, Lazarides E: **Taxol-induced mitotic block triggers rapid onset of a p53-independent apoptotic pathway.** *Mol Med* 1995, **1**:1076-1551.

49. • Blagosklonny MV, Schulte T, Nguyen P, Trepel J, Neckers L: **Taxol-induced apoptosis and phosphorylation of bcl-2 protein involves c-Raf-1 and represents a novel c-Raf-1 signal transduction pathway.** *Cancer Res* 1996, **56**:1851-1854.
In this paper the authors demonstrate taxol-induced phosphorylation and activation of the Raf-1 mitogen-stimulated protein kinase, coincident with phosphorylation of bcl-2. Depletion of Raf-1 protein by benzoquinone ansamycin GA prevented taxol-induced apoptosis and bcl-2 phosphorylation.

50. • Blagosklonny MV, Giannakakou P, El-Deiry WS, Kingston DGI, Higgs PI, Neckers L, Fojo T: **Raf-1/bcl2 phosphorylation: a step from microtubule damage to cell death.** *Cancer Res* 1997, **57**:130-135.
Here, it is reported that to taxol, vinca alkaloids, colchicine, epothilones and other microtubule active agents induce Raf-1/bcl-2 phosphorylation.

51. • Haldar S, Basu A, Croce CM: **Bcl2 is the guardian of microtubule integrity.** *Cancer Res* 1997, **57**:229-233.
The authors of this report observed that drugs that affect microtubules, including taxotere (1-100 nM), vinblastine, and vincristine (100 nM), induce phosphorylation of bcl2 in G2/M and induce apoptosis in human leukemia, lymphoma, breast and prostate cancer cells.

52. • Trielli M, Andreasson PR, Lacroix FB, Margolis RL: **Differential taxol-dependent arrest of transformed and nontransformed cells in the G1 phase of the cell cycle, and specific-related mortality of transformed cells.** *J Cell Biol* 1996, **135**:689-700.
In this paper evidence is presented showing that taxol, but not nocodazole, at micromolar concentrations, may induce a G1 block from which nontransformed rat and human fibroblasts recover and proliferate after release from taxol; however, transformed cells of the same lineage slip through the checkpoint and undergo apoptotis. The results suggest the existence of a microtubule-dependent checkpoint in G1 which is abrogated in transformed cells.

53. • Di Leonardo A, Khan S, Linke S, Greco V, Seidita G, Wahl G: **DNA rereplication in the presence of mitotic spindle inhibitors in human and mouse fibroblasts lacking either p53 or pRb function.** *Cancer Res* 1997, **57**:1013-1019.
This group found that normal mouse and human fibroblasts arrested with a 4N DNA content when treated with nocodazole and Colcemid, whereas isogeneic p53-deficient or pRb-deficient derivatives became polyploid indicating different susceptibilities of normal and mutated cells to anti-mitotic drugs.

54. Ter Haar E, Hamel E, Balachandran R, Day B: **Cellular targets of the anti-breast cancer agent Z-1,1-dichloro-2,3-diphenylcyclopropane: type II estrogen binding sites and tubulin.** *Anticancer Res* 1997, **17**:1861-1869.

55. Zhang X, Smith CD: **Microtubule effects of welwistatin, a cyanobacterial indolinone that circumvents multiple drug resistance.** *Mol Pharmacol* 1996, **49**:288-294.

56. Panda D, Singh JP, Wilson L: **Suppression of microtubule dynamics by LY290181.** *J Biol Chem* 1997, **272**:7681-7687.

57. Wood DL, Panda D, Wiernicki TR, Wilson L, Jordan MA, Singh JP: **Inhibition of mitosis and microtubule function through direct tubulin binding by a novel antiproliferative naphthopyran LY290181.** *Mol Pharmacol* 1997, **52**:437-444.

58. Kruczynski A, Barret J-M, Etievant C, Colpaert F, Fahy J, Hill BT: **Antimitotic and tubulin interacting properties of a novel fluorinated vinca alkaloid, vinflunine.** *Biochem Pharmacol* 1997, in press.

59. Bai R, Schwartz RE, Kepler JA, Pettit GR, Hamel E: **Characterization of the interaction of cryptophycin 1 with tubulin: binding in the Vinca domain, competitive inhibition fo dolastatin 10 binding, and an unusual aggregation reaction.** *Cancer Res* 1996, **56**:4398-4406.

60. Smith CD, Zhang X: **Mechanism of action of cryptophycin. Interaction with the vinca alkaloid domain of tubulin.** *J Biol Chem* 1996, **271**:6192-6196.

61. Kerksiek K, Mejillano MR, Schwartz RE, Georg GI, Himes RH: **Interaction of cryptophycin 1 with tubulin and microtubules.** *FEBS Letts* 1995, **377**:59-61.

62. Photiou A, Shah P, Leong L, Moss J, Retsas S: ***In vitro* synergy of**
• **paclitaxel (Taxol) and vinorelbine (navelbine) against human melanoma cell lines.** *Eur J Cancer* 1997, **33**:463-470.
Here the authors report that adding taxol and vinorelbine, a synthetic vinca alkaloid, at low nanomolar concentrations concurrently to cultures of human melanoma cell lines, resulted in supra-additive or synergistic antiproliferative effects.

63. Garcia P, Braguer D, Carles G, Briand C: **Simultaneous combination of microtubule depolymerizing and stabilizing agents acts at low doses.** *Anti-Cancer Drug* 1995, **6**:533-544.

64. Speicher LA, Barone L, Tew KD: **Combined antimicrotubule activity of estramustine and taxol in human prostatic carcinoma cell lines.** *Cancer Res* 1992, **52**:4433-4440.

65. Derry WB, Wilson L, Khan IA, Luduena RF, Jordan MA: **Taxol**
• **differentially modulates the dynamics of microtubules assembled from unfractionated and purified beta-tubulin isotypes.** *Biochemistry* 1997, **36**:3554-3562.
In this report the authors report that microtubules composed of purified αβ III or αβIV bovine brain tubulin were sevenfold less sensitive to the suppressive effects of bound taxol on the rate of shortening during excursions of dynamic instability, suggesting that cellular expression of increased levels of βIII or βIV tubulin isotypes may be an important mechanism of tumor cell resistance to taxol.

66. Laing N, Dahllof B, Hartley-Asp B, Ranganathan S, Tew KD:
• **Interaction of estramustine with tubulin isotypes.** *Biochemistry* 1997, **36**:871-878.
This paper documents the finding that binding of estramustine to the βIII isotype of tubulin occurs with reduced efficacy as compared with other β-tubulin isotypes, thus suggesting a mechanism for the resistance to estramustine of cancer cells overexpressing the βIII isotype of tubulin (see reference [69•]).

67. Kavalleris M, Kuo DY-S, Burkhardt CA, Regl DL, Norris MD, Haber
•• M, Horwitz SB: **Taxol-resistant epithelial ovarian tumors are associated with altered expression of specific beta-tubulin isotypes.** *J Clin Invest* 1997, **100**:1-12.
In this report the authors observed that taxol-resistant human ovarian tumors displayed significant increases in expression of class I, III and IVa β-tubulins, when compared to untreated primary ovarian tumors. Taxol-resistant cultured human lung cancer cells also showed increases in the levels of classes I,II,III, and IVa β tubulins, although MDR expression, total tubulin levels, and drug uptake were unchanged.

68. Jaffrezou J-P, Dumontet C, Derry WB, Duran G, Tsuchiya E, Wilson L, Jordan MA, Sikic B: **Novel mechanism of resistance of paclitaxel in human K562 leukemia cells by combined selection with PSC833.** *Oncology Res* 1995, **7**:517-527.

69. Ranganathan S, Dexter DW, Benetatos CA, Chapman AE, Tew
• K, Hudes GR: **Increase of βIII- and βIVa-tubulin isotypes in human prostate carcinoma cells as a result of estramustine resistance.** *Cancer Res* 1996, **56**:2584-2589.
The authors of this paper report that human prostate carcinoma cells (DU145), selected for resistance to estramustine, were moderately cross-resistant to paclitaxel, and overexpressed two β-tubulin isotypes three- to fourfold (classes IVa and III), suggesting that altered expression of tubulin isotypes may play a role in cellular resistance to antimicrotubule drugs.

70. Dumontet C, Duran G, Steger K, Beketic-Oreskovic L, Sikic B:
• **Resistance mechanisms in human sarcoma mutants derived by single-step exposure to paclitaxel (Taxol).** *Cancer Res* 1996, **56**:1091-1097.
In this report single-step exposure of MES-SA sarcoma cells to paclitaxel induced formation of 5 Mdr1-negative clones that were resistant to taxol, cross-resistant to vinca alkaloids, and underexpressed 2 β-tubulin isotypes, β5 (class IVa) and β4 (class III), as determined by RT-PCR, which suggests that altered microtubule function is responsible for resistance to taxol in these cells.

71. Giannakakou P, Sackett D, Kang Y, Zhan Z, Buters J, Fojo T,
•• Poruchynsky M: **Paclitaxel-resistant human ovarian cancer cells have mutant beta-tubulins that exhibit impaired paclitaxel-driven polymerization.** *J Biol Chem* 1997, **272**:17118-17125.
Here, the authors report that purified tubulin from two taxol-resistant sublines of human ovarian carcinoma cells exhibited resistance to taxol-induced polymerization which was attributed to acquired point mutations in the M40 isotype. The cell lines were sensitive to vinblastine, accumulated paclitaxel equally with the parental cells, expressed no detectable MDR-1 RNA, and expressed β-tubulin isotypes similarly, with a 1·5-fold increase in expression of M40 (betal) RNA.

72. Cabral F, Barlow SB: **Mechanisms by which mammalian cells acquire resistance to drugs that affect microtubule assembly.** *FASEB J* 1989, **3**:1593-1599.

73. Horwitz S, Shen H, He L, Dittmar P, Neef R, Chen J, Schubart U:
• **The microtubule-destabilizing activity of metablastin (p19) is controlled by phosphorylation.** *J Biol Chem* 1997, **272**:8129-8132.
In this experiment, microinjection of recombinant Op18 induced disassembly of microtubules in cells and *in vitro*. Aspartate substitution of a single serine residue (Ser63) abolished the effect on cellular microtubules.

74. Belmont LD, Mitchison TJ: **Identification of a protein that**
• **interacts with tubulin dimers and increases the catastrophe rate of microtubules.** *Cell* 1996, **84**:623-631.
This report documents the purification of Op18 from calf thymus extracts and the finding that it is a 17 kDa protein that it appears to bind tubulin dimers; destabilize microtubules, increase their catastrophe rates and decrease their growth rates. Op18 inhibited the taxol (300 μM)-induced polymerization of microtubules. Op18 is therefore a plausible regulator of microtubule dynamics during the cell cycle.

75. Marklund U, Larsson N, Graden H, Brattsand G, Gullberg M:
• **Oncoprotein 18 is a phosphorylation-responsive regulator of microtubule dynamics.** *EMBO J* 1996, **15**:5290-5298.
In this paper it is reported that increased expression of Op 18 resulted in microtubule depolymerization in interphase cells, but mitotic spindles were normal. Expression of Op18 mutated at serines 25 and 38 resulted in an absence of mitotic microtubules.

76. Melander Gradin H, Marklund U, Larsson N, Chatila T, Gullberg
• M: **Regulation of microtubule dynamics by Ca2+/calmodulin-dependent kinase IV/Gr-dependent phosphorylation of oncoprotein 18.** *Mol Cell Biol* 1997, **17**:3459-3467.
In this paper it is shown that Ca^{2+}/calmodulin-dependent kinase IV/Gr can suppress the microtubule regulating activity of Op18. Increased phosphorylation of Op 18 on serine residue 16 is associated with partial degradation of Op18 and an increase in cellular microtubule mass.

77. Janmey PA, Chaponnier C: **Medical aspects of the actin cytoskeleton.** *Curr Opin Cell Biol* 1995: **7**:111-117.

78. Sigmond SH: **Signal transduction and actin filament organization.** *Curr Opin Cell Biol* 1996, **8**:66-73.

79. Tapon N, Hall A: **Rho, Rac, and Cdc42 GTPases regulate the organization of the actin cytoskeleton.** *Curr Opin Cell Biol* 1997, **9**:86-92.

80. Assoian RK, Zhu X: **Cell anchorage and the cytoskeleton as partners in growth factor dependent cell cycle progression.** *Curr Opin Cell Biol* 1997, **9**:93-98.

81. Stournaras C, Stiakaki E, Koukouritaki SB, Theodoropoulos PA,
•• Kalmanti M, Fostinis, Y, Gravanis A: **Altered actin polymerization dynamics in various malignant cell types: evidence for different sensitivity to cytochalasin B.** *Biochem Pharmacol* 1996, **52**:1339-1346.
In this report the soluble : total actin ratio of malignant cells was found to be 1.37 to 1.81-fold higher than that of normal cells. The viability of malignant cells was also shown to be more strongly affected by cytochalasin B.

82. Hemstreet GP III, Rao JY, Hurst RE, Bonner RB, Waliszewski
•• P, Grossman HB, Liebert M, Bane B: **G-actin as a risk factor and modulatable endpoint for cancer prevention trials.** *J Cell Biochem* 1996, **25S**:197-204.
This review article describes evidence indicating that increased levels of unpolymerized actin and decreased levels of polymerized actin accompany development of cancer and that the soluble and polymeric actin levels in cells may be an important biomarker for early detection of cancer.

83. Asch HL, Head K, Dong Y, Natoli F, Winston JS, Connolly JL, Asch
•• BB: **Widespread loss of gelsolin in breast cancers of humans, mice, and rats.** *Cancer Res* 1996, **56**:4841-4845.
This paper expands on previous work showing decreased levels of gelsolin in tumor cells. Gelsolin was shown to be absent or greatly reduced in 70% of human breast cancers and in 100% of rat and mouse primary mammary tumors examined. The authors draw the potentially important conclusion that – across three species – partial or complete loss of gelsolin expression is one of the most frequently occurring defects in breast cancer, regardless of etiology. Insufficient gelsolin may impair regulation of the actin filament cytoskeleton and impair various signal transduction pathways involving the actin cytoskeleton, thereby leading to growth and behavioral defects in breast cancer cells by changing their response to external signals.

84. Tanenbaum SW (Ed): *Cytochalasins, Biochemical and Cell Biological Aspects.* Amsterdam: North Holland Publishing Company; 1978.

85. Cooper JA: **Effects of cytochalasin and phalloidin on actin.** *J Cell Biol* 1987, **105**:1473-1477.

86. Spector I, Shochet NR, Blasberger D, Kashman Y:
Lantrunculins – novel marine macrolides that disrupt microfilament organization and accct cell growth: 1. Comparison with cytochalasin D. *Cell Motil Cytoskeleton* 1989, **13**:127-144.

87. •• Ayscough KR, Stryker J, Pokala N, Sanders M, Crews P, Drubin DG: **High rates of actin filament turnover in budding yeast and roles for actin in establishment and maintenance of cell polarity revealed using the actin inhibitor lantrunculin-A.** *J Cell Biol* 1997, **137**:399-416.

This paper reports that the actin assembly-inhibitor lantrunculin A caused complete disruption of actin cytoskeleton in budding yeast within 2–5 min of addition, indicating that the actin cytoskeleton in this non-motile organism is highly dynamic. The study revealed the importance of actin dynamics in a number of yeast cell processes, including localization of secretory vesicles during localized exocytosis.

88. Bubb MR, Senderowicz AMJ, Duncan KIK, Korn ED: **Jasplakinolide, a cytotoxic natural product induces actin polymerization and competitively inhibits the binding of phalloidin to F-actin.** *J Biol Chem* 1994, **269**:14869-14871.

89. Senderowicz AM, Kaur G, Sainz E, Laing C, Inman WD, Rodriguez J, Crews P, Malspeis L, Grever MR, Sausville EA *et al.*: **Jasplakinolide's inhibition of the growth of prostate carcinoma cells *in vitro* with disruption of the actin cytoskeleton.** *J Natl Cancer Inst* 1995, **87**:46-51.

90. Bubb MR, Spector I, Bershadsky AD, Korn ED: **Swinholide A is a microfilament disrupting marine toxin that stabilizes actin dimers and severs actin filaments.** *J Biol Chem* 1995, **270**:3463-3466.

91. Terry DR, Spector I, Higa T, Bubb MR: **Misakinolide A is a marine macrolide that caps but does not sever filamentous actin.** *J Biol Chem* 1997, **272**:7841-7845.

92. O'Brien ET, Asai DJ, Jacobs RS, Wilson L: **Selective inhibition of cytokinesis in sea urchin embryos by low concentrations of stypoldione, a marine natural product that reacts with sulfhydryl groups.** *Ann Biochem* 1989, **181**:51-58.

93. Grace KJS, Medina M, Jacobs RS, Wilson L: **Selective inhibition of cytokinesis in sea urchin embryos by the marine natural product pseudopterolide.** *Mol Pharmacol* 1992, **4**:631-638.

94. • Lebowitz PF, Sakamuro D, Prendergast GC: **Farnesyl transferase inhibitors induce apoptosis of ras-transformed cells denied substratum attachment.** *Cancer Res* 1997, **57**:708-713.

These authors report that the farnesyl transferase inhibitor L739,749 potently activates apoptosis in Ras-transformed cells grown under conditions in which they cannot attach to the substrate, thus suggesting an explanation for tumour regression induced by farnesyl transferase inhibitors. Apoptosis is blocked by BCL-X_L, is independent of p53 status, and is inhibited by a myristylated form of RhoB.

95. Prendergast GC, Davide JP, deSolms SJ, Giuliani E, Graham S, Gibbs J, Oliff A, Kohl NE: **Farnesyltransferase inhibition causes morphological reversion of ras-transformed cells by a complex mechanism that involves regulation of the actin cytoskeleton.** *Mol Cell Biol* 1994, **14**:4193-4202.

96. Prendergast GC, Davide JP, Lebowitz PF, Wechsler-Reya R, Kohl NE: **Resistance of a variant ras-transformed cell line to phenotypic reversion by farnesyl transferase inhibitors.** *Cancer Res* 1996, **56**:2626-2632.

97. Gibbs J, Oliff A: **The potential of farnesyltransferase inhibitors as cancer chemotherapeutics.** *Annu Rev Pharmacol Toxicol* 1997, **37**:143-166.