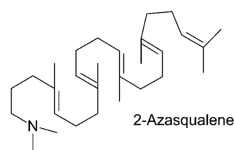

Figure 3. The Structure of 2-Azasqualene

nal design of sterol cyclase inhibitors as hypercholesteremic drugs.

Energetic and kinetic aspects of the SHC reaction are profoundly discussed in a very recent paper by Rajamani and Gao [17]. Reinert et al. [13] and Rajamani and Gao should be read together, because they nicely complement each other. The first article gives us a more static and less dynamic view of the reaction as compared to the second paper, which attempts to simulate the enzyme dynamics and energetics. Both papers agree that the 6,6,5-cyclic carbocation is not an intermediate during cyclization, but they disagree on the role played by the 6,6,6,5-cyclic carbocation. For the Schulz group, it represents an intermediate side product, and for Rajamani and Gao, a minor dead-end side product.

Two questions remain to be solved for oxidosqualene cyclases producing sterols. (1) How do sterol cyclases induce the boat conformation in ring B? (2) How do the sterol cyclases manage to orient the substrate oxidosqualene when it enters the catalytic cavity? Substrate orientation should not be problematic for the SHC, because the substrate is symmetrical and therefore it does not matter which end reaches the protonation site first. But in contrast, if one offers SHC oxidosqualene as an alternative substrate, cylization always begins from the oxido end [18]. Surprisingly, the enzyme retains a mechanism to select for the "correct" site.

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New Probes for Microtubule Dynamics

A phenotype-based screen identifies a purine analog, named diminutol, that perturbs the microtubule cytoskeleton in cells. An affinity-based approach identifies a protein target of this small molecule and leads to the characterization of a new pathway that may regulate cytoskeleton dynamics.

The treatment of cells with the small molecule colchicine, a natural product, results in dramatic phenotypes in dividing cells. In early studies these phenotypes were described as "explosions" of the mitotic apparatus (reviewed in [1]). Ed Taylor and coworkers used an affinity-based approach to identify the protein target of colchicine, and their research led to the discovery of tubulin [2, 3]. This landmark work, carried out in the 1960s, involved the use of a small molecule to unravel a key biological mechanism. In appreciation of the similarity between such a strategy and conventional genetics, in which one modulates protein function by introducing mutations in genes rather than by using cell-permeable small molecules, the term "chemical genetics" has been coined [4, 5]. Recently, several examples of the successful application of chemical genetics in the examination of a range of biological processes have been reported (for example, see [6]). Using phenotype-based screens and a battery of powerful in vitro and cell-based assays,

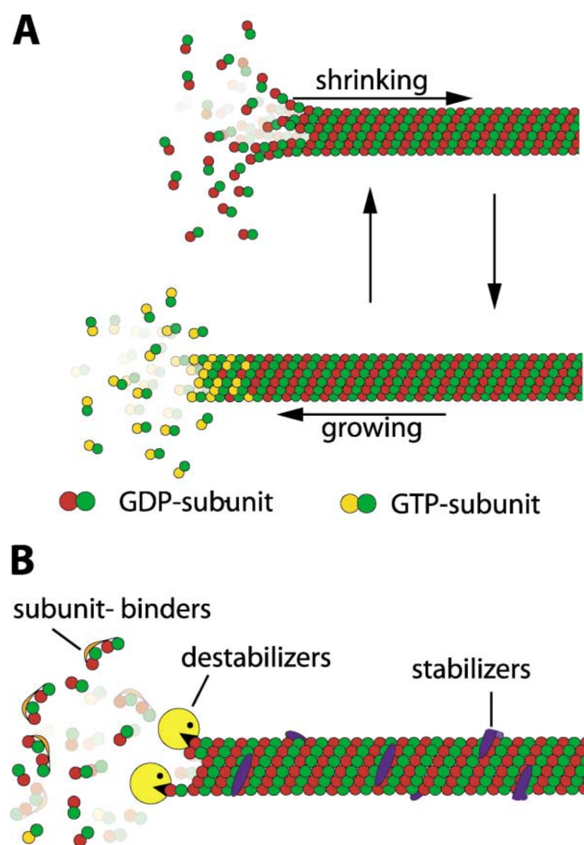

Figure 1. Regulation of Tubulin Polymerization

Polymerization depends on mechanisms intrinsic to tubulin (A) and on other cellular proteins that can bind tubulin subunits or microtubules (B). The schematic shows a microtubule consisting of protofilaments (typically 13). $\alpha\beta$-tubulin dimers are the building blocks that are added to or removed from the ends to grow or shrink the filament. GTP hydrolysis plays a critical role in regulating polymerization. There are proteins known to stabilize the polymer lattice, catalyze its depolymerization, or sequester subunits to influence polymer dynamics.

combined with systematic chemical synthesis and state-of-the-art mass spectroscopy, Wignall et al. now report the discovery of a new pathway that may regulate tubulin polymerization in cells [7].

Several small molecules that can destabilize or stabilize polymers of tubulin by directly binding to it are now known (reviewed in [8]). Not surprisingly, these small-molecule modulators have played a central role in defining the functions of tubulin and the importance of regulating its polymerization dynamics in cellular contexts. For example, modest perturbations of tubulin polymerization by small molecules during cell division activate a "checkpoint" mechanism, a signaling pathway that arrests a cell in mitosis and prevents the cell from improperly segregating its DNA into daughter cells (reviewed in [9]). This sensitivity of cell cycle progression to tubulin polymer dynamics is the likely basis for the efficacy of tubulin-targeting chemotherapeutic agents used in treating diseases such as cancer. Screening for genetic mutations that override the cell cycle arrest induced by small-molecule inhibitors of tubulin polymerization has allowed identification of the molecular components of the "checkpoint" pathway. The analysis of these signaling proteins is providing insight into mechanisms underlying human disorders, ranging from Down syndrome to cancer (reviewed in [10]).

Since the discovery of tubulin, the molecular basis of its complex polymerization dynamics has been examined in vitro and in vivo (reviewed in [11]). The $\alpha\beta$-tubulin subunit addition or loss from the ends of a microtubule results in its growth or shrinkage. Studies reconstituting polymerization with pure tubulin have led to detailed analyses of the kinetic parameters and nucleotide dependence of polymerization (Figure 1A). Electron microscopy has revealed structural differences between polymers that are growing and those that are shrinking [12]. Higher-resolution structural studies have provided atomic details of tubulin organization in polymeric forms and, when combined with computational approaches, have yielded insight into how tubulin interacts with small molecules such as taxol [13]. In the last decade or so, several proteins that can bind tubulin and influence its dynamics inside cells in at least three different ways have been identified (reviewed in [14]) (Figure 1B). Like the small molecules taxol and colcemid, some proteins are known to stabilize and destabilize microtubules. There are also proteins known to influence polymerization equilibrium by sequestering tubulin subunits in conformations that cannot assemble into a polymer. As an indication of our understanding of tubulin polymerization dynamics, key physiological features have been reconstituted in vitro with three pure components: tubulin, a microtubule-stabilizing protein, and a microtubule-destabilizing protein [15]. However, such experiments represent only the first steps toward the recapitulation of complex microtubule-dependent processes, such as movements of chromosomes during cell division. Additionally, the list of proteins and the different mechanisms by which tubulin polymerization and organization can be regulated in cells remains far from complete.

Wignall et al. screened a library of purine derivatives by using a cell-free system to select small molecules that modulate tubulin dynamics without binding tubulin directly. This cell-free system employs cytoplasm derived from unfertilized frog eggs, was first described by Masui and coworkers [16], and has since been used to identify several proteins and characterize mechanisms underlying a range of cellular processes, including the regulation of tubulin dynamics. The use of this system, as described in this study, represents a promising solution to a key challenge faced in phenotype-based chemical genetic screens. Recent advances in combinatorial or parallel synthesis allow for the generation of large collections of small molecules that can be efficiently screened via automation in hardware and analysis to select compounds that yield a desired biological phenotype. However, identification of protein targets of these small molecules can often be difficult. An important aspect of the frog egg extract system is that it can be prepared in quantities sufficient for biochemistry experiments and directly used in phenotypic assays as well as affinity-based target-identification experiments.

Guided by systematic SAR (structure-activity relationship) analysis, Wignall et al. identified active and inactive

derivatives of the lead compound they named diminutol, a purine analog that destabilized tubulin in the cell-free extracts and in cells. Active and inactive analogs were covalently attached to agarose beads for affinity-based target identification experiments. Although affinity-matrix based approaches have been used to identify protein targets of potent natural products (e.g., FK506 and myriocin, with activities on cells at nanomolar or lower concentrations [17, 18]) and also bioactive purine derivatives (e.g., purvalanol, cell growth inhibition, and GI_{50} 2.5 μM [19]), a noteworthy aspect of the report by Wignall et al. is the use of this strategy to identify the target of a less-potent compound. In cellular contexts, diminutol is active at 50 μM but is not very active at 10 μM. Using state-of-the-art mass spectrosocopy, the authors were able to identify the target protein from rather complex mixtures of proteins that selectively bind the affinity matrices. These data bode well for other phenotype-based screens that identify small molecules with interesting biological activities, often with activities in the micromolar range, but whose molecular targets are not readily apparent through phenotypic analysis or functional assays.

A surprising result of this study is that one target of diminuntol is NQO1, an NADP-dependent oxidoreducase that can catalyze the two-electron reduction of quinone compounds and is known to be overexpressed in certain tumors (reviewed in [20]). NQO1 has not previously been linked to regulation of microtubule dynamics, in dividing or nondividing cells. This finding presents an exciting opportunity for further research to examine how this enzymatic pathway regulates microtubule function in different cellular contexts. Dimuntol will no doubt be a valuable tool for these studies.

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Targeting FOXO Kills Two Birds with One Stone

PTEN deficiency activates Akt signaling and results in a variety of human malignancies. Encouragingly, recent studies demonstrate that small molecules can regulate FOXO1a, an Akt target, to suppress tumor growth, and FOXO1a is therefore a promising anticancer drug target.

Molecular targeting of tumor-specific signal transduction pathways is a promising strategy for discovering and developing novel potent anticancer drugs. Small-molecule libraries have been intensively screened for compounds that block ligand-receptor interaction [1], those that block signaling transduction pathways [2], and those that block master regulators of cell cycle arrest or apoptosis [3]. Among many possible targets, there is little doubt that phospho-Akt has a critical role in many human cancers, often as a consequence of PTEN inactivation [4]. PTEN, both a lipid phosphatase and a protein phosphatase, is frequently mutated in a wide range of human malignancies, including glioma, prostate and breast tumors, melanoma, squamous cell carcinoma, and thyroid tumors [5, 6]. Animal models have shown that PTEN knockout is embryonic lethal and that heterozygous PTEN-depleted mice are prone to the development of various cancers [7, 8]. Restoring PTEN function in mutant PTEN-containing tumor cells by expressing exogenous PTEN can largely reverse the malignant phenotype. The main mechanism of cancer development due to PTEN inactivation is constitutive activation of Akt function. Akt is a kinase that has numerous targets, many of which are important for regulating the balance between cell survival and apoptosis [9, 10]; such targets include MDM2, Bad, Bcl-2, CDK inhibitors, caspase 9, and forkhead transcription factors such as