colorectal cancer or adenomatous polyps in the general population. We also need information about the lowest effective dose, the optimal duration and drug, the cellular and enzymatic targets, the contraindications to NSAID use, and proof that at a certain dose the balance of benefits over risks is favorable in defined populations.

Summary

NSAIDs appear to offer much promise against colorectal cancer, yet this potential remains unproven. At present, there is not sufficient evidence to recommend aspirin for the prevention or treatment of cancer[8]. However, there is reason to believe that NSAIDs may have an important role in cancer prevention or treatment if we have the patience to define it.

Michael J. Thun

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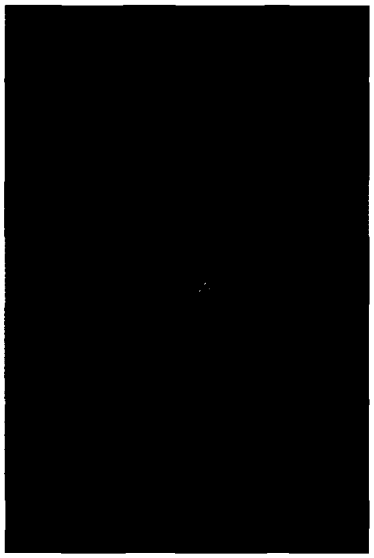
Green fluorescent protein – 'paint in a can'

A typical art store tempts us with row upon row of brillant paints, each with a distinct color that is ours to select. Just imagine the possibilities if a similar assortment of hues was available to illuminate the nooks, crannies and organelles of intracellular space or to serve as differently colored reporters to simultaneously monitor the output of a host of different genes.



A model of wild-type green fluorescent protein based on a structure solved by F. Yang, L. Moss and G. Phillips. Illustration produced by T.D. Romo.

Such choices may soon be ours, according to George Phillips, a biophysicist at Rice University (Houston, TX, USA) who leads one of the two research groups that recently determined the molecular structure of green fluorescent protein (GFP). He refers to GFP as 'paint in a can' and believes that mutagenesis of this most unusual protein paint will soon provide many different colors for labeling cells.

The novel GFP is folded into a cylinder composed of 11 strands of β-sheet with a central α-helix. This structure is novel, and Phillips has proposed that it should be called a β-can. The central helix is also unusual: three amino acids in the helix, serine-65, tyrosine-66 and glycine-67, undergo spontaneous cyclization and oxidation to form the fluorophore of the protein. Usually, protein fluorophores are prosthetic groups noncovalently bound to the protein. The formation of the GFP fluorophore from the amino acid backbone makes GFP an especially attractive candidate for mutagenesis. Slight alterations of the environment surrounding the fluorophore, or in the actual amino acids that make up the fluorophore, are expected to provide a family of GFPs that emit a rainbow of different colors.

Phillips and his student, Fan Yang, in collaboration with Dr Larry Moss at the New England Medical Center (Boston, MA, USA), determined the structure of the wild-type GFP [*Nature Biotechnology* (1996) 14, 1246–1251]. A collaboration between the research groups of Dr Roger Tsien (UCSD, La Jolla, CA, USA), Dr James Remington (University of Oregon, Eugene, OR, USA) and Dr Andrew Cubitt (Aurora Biosciences, La Jolla, CA, USA) led to the determination of the 3D structure of the mutant of GFP in which the normal serine in the fluorophore is replaced by a threonine residue. This mutant GFP has a brighter fluorescence than the wild-type protein [*Science* (1996) 273, 1392–1395]. The structures derived by the two groups are essentially identical.

The West Coast group also used site-directed mutagenesis to convert threonine-203, which is hydrogen-bonded to a phenolic group of the fluorophore, to a tyrosine residue. They reasoned that this alteration would perturb the fluorophore, resulting in a shift in its fluorescence properties. Indeed, they found that the mutated protein had a red-shift in both its excitation and emission spectrum, which they claim to be sufficient for differentiation from the original GFP in fluorescence microscopy.

The directed mutagenesis of GFP described above is not the first successful effort to produce useful GFP mutants. Previous efforts have used random mutation of GFP followed by the isolation of those mutants with desirable fluorescence properties. Aurora Biosciences, for example, already has mutants of GFP that emit ultrabright green, as well as yellow and two shades of blue fluorescence. They believe these mutants of GFP will be critical components for the development of ultra-high-throughput screening assays [*Drug Discovery Today* (1996) 1, 313–314]. The availability of the molecular structure of GFP will now provide a sound chemical understanding of the altered fluorescence properties of the mutant GFPs that have already been produced by random mutagenesis. Moreover, it will provide a rational approach to produce directed mutants that may never have been explored without the insight provided by the newly derived molecular structure.

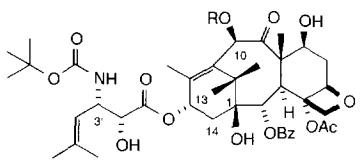
GFP is already proving to be a highly useful agent for exploring cell structure and for assay development. GFPs emitting different colors can be used as reporters to monitor the activation of multiple genes, and also as protein tags. The glowing protein can be attached to other proteins on either its amino or carboxyl terminus and used to monitor their transit or to follow biochemical reactions and interactions in living cells. GFP can be readily expressed in a variety of different cells including yeast, *Drosophila*, zebrafish, mammalian, plant, bacteria and slime mold cells. It can be directed to specific areas of the cell by fusion to targeting sequences, and it has utility as a tracer to determine the lineage of a particular cell. Once expressed, the protein is very stable and is resistant to degradation by proteases. In drug discovery it will undoubtedly be very useful in conjunction with imaging technologies for the development of ultra-high-throughput screening assays.

In nature, GFP dramatically illuminates marine coelenterates, such as the jellyfish *Aequorea victoria* found in the waters off the Pacific Northwest of the USA. It works in conjunction with aequorin, a Ca^{2+}-sensitive, luminescent protein that has also proved to be a highly useful tool for cell biologists in measuring the concentration of intracellular Ca^{2+}. In the jellyfish, GFP absorbs the blue chemiluminescence of aequorin and acts as a transducer by emitting it as green fluorescent light. Many of the marine invertebrates have evolved light-producing systems, but few of the molecular components of these systems have been isolated, cloned and made available for use in biotechnology. Perhaps the obvious utility of GFP will spur additional activity in this area.

Robert W. Wallace

Taxol moves on

Small and easy-to-carry-out changes in the basic structure of the drugs taxol and taxitere increase their potency against various types of cancer cells in the test tube, according to US researchers. The discovery could lead to a second generation of these powerful anticancer compounds that may also show less side effects than the present formulations.



One of the taxol derivatives with increased antitumour activity.

Dr Iwao Ojima (State University of New York, Stony Brook, NY, USA) described at a lecture at the University of Cambridge in October how he and his team have discovered that modifying the amino acid side chain at the C-13 position as well as the C-10 acyl group of the basic taxol structure (see Figure) boosts the drug's cytotoxic activity against ovarian, non-small-cell lung, colon and breast cancer cells.

He and his team set out to build a taxol derivative that would overcome taxol's side effects and multi-drug resistance, which has recently been reported in clinical trials. In early experiments they noticed that certain of their C-3′ substituted taxoids were more potent against a particular drug-resistant cancer cell line if the free hydroxyl (OH) group at the C-10 position was substituted with an acetyl group. They decided that this might provide a clue to building a second generation taxoid and began synthesizing various analogues with alkyl and alkenyl groups at the C-3′ position as well as different acyl groups at C-10.

According to Ojima, all but one of their new taxoid compounds were more effective than taxol (paclitaxel) or taxotere (docetaxel). He adds that changing the C-13 side chain as well as the C-10 acyl group quite significantly still leads to effectiveness against standard cancers.

Three of the taxoids were found to be more potent than either taxol or taxotere against drug-resistant breast and ovarian cancer cell types by two orders of magnitude. Ojima points out that the breast cancer type has 180-fold resistance to the drug doxirubicin and so alternatives are keenly sought.

The team has already found that one of their taxoids, dubbed SB-T-1213, shows strong antitumour activity in nude mice against melanoma, and the other compounds are currently being investigated in detail to uncover the relationship between structure and activity.

The team also described the synthesis and assaying of their taxoids in the *Journal of Medicinal Chemistry* (1996) 39, 3889.

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