all D-tri- and tetrapeptides. Peptide sequences that bound strongly to vancomycin could be recognized by the longer capillary electrophoresis retention times of these compounds, and on-line mass spectral analysis gave immediate identification of the compound sequences. This technique was studied for libraries of 500–1,000 components, but with more sensitive mass spectrometry detection larger libraries could be analysed directly.

Isotopic encoding of bead libraries

A novel approach to combinatorial library compound structure elucidation has been proposed by a group from Glaxo Wellcome [Geysen, M. *et al. Chemistry & Biology* (1996) 3, 679–688]. As mass spectrometry is a highly sensitive technique for the characterization and quantification of organic compounds, this approach has been used to encode for the individual compounds in a library. As organic compounds appear in the spectrum as a singly charged ion, any isotopic variation in the compound's composition will show up as a predictable shift in molecular weight. This principle has been used to generate several methods for the isotopic or mass encoding of bead libraries.

One approach uses a coding sequence of two glycine monomers each containing either zero, one or two ^{13}C-atoms. Using these monomers either alone or in equimolar quantities gives rise to 25 different highly predictable mass spectrum 'bar code' patterns generated over a very small region of the mass spectrum.

This and other isotope encoding strategies are most effective for 'one bead–one compound' libraries, and particular care needs to be taken in matching the encoding strategy with the bead size and the chemical loading being employed.

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Emerging molecular targets

Tissue-specific estrogen therapy

Estrogen replacement therapy prevents resorption of bone and provides a strong protective effect against cardiovascular disease in postmenopausal women. However, estrogen therapy is not without danger. Some cervical and breast tumor cells are stimulated to grow by estrogen, requiring women to make a difficult choice. If they take estrogen, they will benefit from its well-known protective effects while at the same time they increase their risks of developing cervical or breast cancer.

If they decide to forgo estrogen therapy, they will almost certainly have some bone loss, which puts them at risk of developing osteoporosis, and they will increase their risk of developing cardiovascular disease. Recent research on the molecular mechanism of action of estrogen suggests some new strategies for developing drugs that will provide only the beneficial actions associated with estrogen therapy.

Bone resorption occurs in postmenopausal women because the lack of estrogen permits the overgrowth of osteoclasts, large multinucleated cells that perforate the bone and resorb the minerals that make up the bone structure. Estrogen prevents the growth of osteoclasts in bone, but until the recent studies of David E. Hughes and coworkers at the University of Texas Health Science Center (San Antonio, TX, USA) and the University of Sheffield (Sheffield, UK) the mechanism of its action was unknown. They found that 17β-estradiol triggers apoptosis, or programmed cell death, of osteoclasts grown in cell culture, and *in vivo* studies in ovariectomized mice suggest that this is how estrogen controls osteoclast growth in bone [*Nat. Med.* (1996) 2, 1132–1136].

Apoptosis of osteoclasts is a newly discovered pathway for the action of estrogen, and most of the details remain to be established. The investigators believe that the induction of apoptosis by estrogen is mediated by transforming growth factor-β (TGF-β). They found that estrogen induces the synthesis of TGF-β and antibodies against TGF-β blocked the action of estrogen on the osteoclasts. The source of the TGF-β is unclear; the studies were conducted with cultured cells consisting of a mixture of osteoclasts and stromal cells.

Another recent discovery regarding the mechanism of action of an estrogen analogue, raloxifene, also suggests that tissue-specific estrogen analogs are possible. Raloxifene acts as an estrogen antagonist in the uterus and the breast, but it acts as an estrogen agonist in the preservation of bone and in reducing cholesterol levels. Na Yang and coworkers at Lilly Research Laboratories (Indianapolis, IN, USA) found that activation of the TGF-β3 gene by raloxifene in osteosarcoma cells requires the estrogen receptor, but the raloxifene-estrogen receptor complex does not act by binding to the estrogen response element of DNA as might be expected. Instead, the raloxifene–estrogen receptor complex utilizes a cellular adapter protein to bind to a newly discovered polypurine DNA sequence on the TGF-β3 gene [*Science* (1996) 273, 1222–1225]. This polypurine sequence has now been termed the raloxifene response element or RRE. The nature of the cellular adapter protein remains to be determined.

Both of these discoveries – that estrogen protects women from developing osteoporosis by limiting the lifespan of osteoclasts and the presence of multiple DNA response elements for the estrogen receptor – help explain the wide range of effects of estrogen on the female body. Most important, however, they provide a rational approach for the development of tissue-selective estrogen therapies that will allow postmenopausal women to enjoy the positive aspects of estrogen therapy without the danger of developing cervical or breast cancer.

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About Bob Wallace...

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