

for blast disease, encoding of a receptor-like protein kinase by disease resistance gene, molecular analysis of anthocyanin pigmentation pathway, manipulation of starch metabolism during seed development, characterization of mitochondrial genome, silencing of β -glucuronidase gene in transgenic rice.

It has been projected that by the year 2025, the global demand for rice would increase by 70%, requiring production of 810 million tons of unmilled rice compared to the present production of 480 million tons. This will have to be harvested from diminishing cultivated

area and availability of water due to competing demands on these resources. It was repeatedly emphasized that to meet this demand the rice yields will have to increase from the present mean of 4.5 tons ha^{-1} to 8.0 tons ha^{-1} under irrigation and from 1.9 tons ha^{-1} to 3.6 tons ha^{-1} in the rainfed areas. This will have to be achieved by increasing the yield ceiling, bridging the gap between the potential and the realized yield and sustaining the present levels. The major role of biotechnology towards this goal would be in the development of transgenic plants with resistance to biotic

and abiotic stresses. Therefore there is an urgent need to evolve procedures for field releases of transgenic plants and approval of grain harvested from such plants as safe food similar to those produced using conventional genetic tools. The marker-assisted selection and tissue culture methods would facilitate breeding programmes and reduce the breeding cycle time.

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RESEARCH NEWS

Designer molecules: A chromogenic sensor of creatinine

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Specific recognition of molecules by one another via shape complementarity and specific, weak non-covalent interaction like hydrogen-bonds, van der Waal's interaction, etc. is central to biology. Recognition of a chosen guest by specifically designed host molecules is currently a major area of chemical research.

Molecular and ionic sensors have always been in great demand for the easy detection/assay of a variety of molecules and ions¹. Such sensors find many applications in medical diagnostics and in detecting environmental pollutants. A variety of chemical sensors, including biosensors, can be designed². However, sensors based on synthetic molecular receptors are becoming more attractive since such molecules are usually much more stable compared to biomolecules. Of sensors, chromogenic sensors are the most valuable ones since the presence of the 'guest' is signalled by a change in colour. A number of chromogenic sensors have recently been designed and synthesized based on the principles of molecular recognition³.

In a recent communication to *Science* (1995, 269, 671), Bell and coworkers have designed a receptor which detects creatinine efficiently. Creatinine is one of the end products of nitrogen metabolism, and in healthy human beings it is

excreted in the urine. The transport of creatinine from the blood to urine is mediated by the kidneys, hence the blood level of creatinine is a key indicator of renal function. Present methods available for the assay of creatinine are tedious and expensive. Bell's work has produced a simple but sensitive assay of creatinine, in which the formation of a supramolecular complex is signalled by a colour change.

The $\text{H}_2\text{N}-\text{C}=\text{N}$ moiety of creatinine has a D-D-A array* for forming hydrogen bonds. The designed receptor however, in its neutral form, has a D-A-A surface for forming an H-bond. However, a zwitterionic structure is also possible for this receptor, in which the phenolic hydrogen is transferred to the nitrogen atom at the other end of the molecule, thereby converting the D-A-A surface to an A-A-D surface which is now complementary to the creatinine surface. In other words, of the two tautomeric forms only the zwitterionic form (the 'dye'-like form) should bind creatinine. In this example, that is what happens – the receptor exists predominantly in its neutral form, say in dichloromethane solution, which has

*D and A stand for donor and acceptor groups for forming a hydrogen bond, respectively.

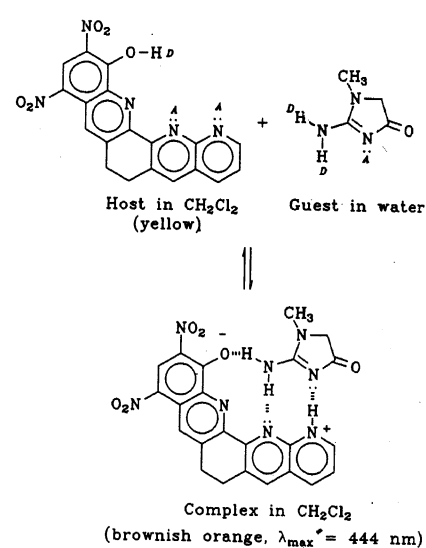


Figure 1

a yellow colour. However, when this solution is shaken with an aqueous solution of creatinine, the receptor extracts creatinine into the organic solution, forms the complex, and in that process undergoes a change from the neutral to the zwitterionic form. This causes a colour change from yellow to brownish orange (the complex shows an extra absorption band at $\lambda_{\text{max}} = 444 \text{ nm}$). This is schematically shown in

Figure 1. The authors have also carried out X-ray structural analysis of the receptor, creatinine, and the complex to demonstrate some of the finer details of the binding.

The authors have shown that this synthetic receptor is selective for creatinine, since no colour change was observed with creatine, urea, uric acid, proline or histidine. The estimated dissociation constant of the complex (0.5 μM) is also sufficiently low so that blood serum creatinine (typically 40–130 μM) can be sensed by it. Alkali metal ions were found to give some chromogenic response with the receptor,

but those were significantly weaker. The prospects for the use of synthetic receptors in medical diagnostics are bright.

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M., Pallavicini, P., Perotti, A. and Sacchi, D., *Angew. Chem. Int. Ed. Engl.*, 1994, 33, 1975; Vance, D. H. and Czarnik, A. W., *J. Am. Chem. Soc.*, 1994, 116, 9397; Chawla, H. M. and Srinivas, K., *Chem. Commun.*, 1994, 2593; Hamasaki, K., Ikeda, H., Nakamura, A., Ueno, A., Toda, F., Suzuki, I. and Osa, T., *J. Am. Chem. Soc.*, 1993, 115, 5035; Teixidor, F., Flores, M. A., Escriche, L., Vinas, C. and Casabo, J., *Chem. Commun.*, 1994, 963; Marsella, M. J. and Swager, T. M., *J. Am. Chem. Soc.*, 1993, 115, 12214.

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Pathogenesis: Generating new paths in understanding bacterial virulence

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The survival strategy of bacterial pathogens requires establishment in a suitable niche within another living organism, outwitting it by avoiding its formidable immune system, acquiring nutrients from it and multiplying, thus causing disease in the host. The host-pathogen interaction is a battlefield where the infecting organism encounters a range of adverse environmental conditions. These, along with other selective pressures, appear to have directed the evolution of specialized regulatory systems controlling the expression of virulence factors in the pathogen. To understand mechanisms by which the bacterial parasites circumvent or avoid the immune system of the host and cause disease, it is necessary to identify and characterize the bacterial genes and gene products responsible for the virulence at each stage of the development and progression of the disease. Traditionally, the strategies for identification of virulence factors included the three following approaches:

- (1) Brute force screens, that pinpoint the virulence genes on the basis of mutational loss of a virulence phenotype^{1,2}.
- (2) Cloning screens, that pick up novel virulence genes depending on their

ability to confer a virulence-associated phenotype in a heterologous bacterium³. (3) Regulatory screens, that identify potential virulence genes by their coordinate expression with other known virulence genes under defined laboratory conditions^{4,5}.

All these strategies, carried out *in vitro* were, however, very often limited by their inability to reproduce accurately the complex and changing environments encountered by the pathogens in their hosts. Moreover, there could also be certain factors which would be induced only under specific environments within a host and could never be expressed, or were expressed in extremely low and negligible amounts, *in vitro*. Therefore it remained quite possible for one to miss out these genes if only the traditional methods were followed.

To overcome these limitations, Mahan *et al.*⁶ developed, in 1993, a novel genetic system, which they termed 'in vivo expression technology' (IVET). What made this strategy unique was their use of simple laboratory techniques to randomly choose genes (or only their promoters) from virulent pathogens, induce their expression within the host (i.e. *in vivo*), and then compare this with their expression *in*

vitro. This then allowed them to select for those genes which were transcribed better or exclusively so only in the presence of the host environment. This, in fact, was the first attempt to study the induction of pathogenesis-related genes in the actual host environment conducive to disease development.

The first step in the technique consisted of the selection of a bacterial cell with a mutation in a biosynthetic pathway gene that greatly attenuated growth *in vivo*. For this purpose, Mahan *et al.* used a purine auxotroph (*purA*) of *Salmonella typhimurium*. Growth of such a strain *in vivo* was possible only if a wild-type *purA* gene could be introduced into it. A plasmid (pIVET1) was constructed by inserting a fusion of the wild-type *purA* gene with a marker gene (in this case, *lacZY*) into a broad host-range suicide vector (pGP704). The chromosomal DNA of *S. typhimurium* was fragmented by a specific restriction enzyme and cloned into the plasmid pIVET, 5' to the *purA-lacZY* operon, thus giving rise to a transcriptional fusion where a *S. typhimurium* promoter would drive the transcription of a wild-type *purA-lacZY* fusion. A pool of pIVET1 containing these transcriptional fusion constructs was introduced into purine-deficient *S. typhimurium* cells.