

dimmed alarmingly with the indefinite extension of the NPT in 1995. So this year, coinciding with the Golden Jubilee celebrations of our Independence, we were compelled to re-define the parameters of our security requirements. As a developing country, India hopes that the developing world notices that the countries which have chosen to vehemently criticize the recent tests are either the established Nuclear Weapon States, who like to preserve their exclusive position, or are those who have already addressed their nuclear-related national security concerns of the kind India has. This is not surprising because they are either not placed in a hostile neighbourhood or they enjoy the security of a nuclear umbrella of a Nuclear Weapon State. The political or geographical proximity of some of the latter to a friendly supportive Nuclear Weapon State is so easily recognizable that they can perhaps be looked upon as surrogate Nuclear Weapon States. Interestingly, none of them is a developing country and worryingly the attitude of many of them smacks of a new kind of colonialism through technology control.

'We find it strange and contradictory that we, who have never violated any treaty obligation, are being accused by some countries of violating the norms of CTBT, a Treaty which is yet to enter into force, and to which we are not a party. It is worthwhile recalling the Partial Test Ban Treaty (PTBT), a Treaty which prohibited atmospheric tests and which entered into force in 1963. Having been among the first to

sign this treaty, we carried out our first test underground in 1974. Two states did not sign the PTBT and continued to carry out atmospheric test long after the entry into force of the Treaty. One State, China, carried out its first test in the atmosphere one year after the entry into force of PTBT. In comparison, it needs to be noted that CTBT is yet to enter into force....

'From the point of view of developing countries, the focus of this Conference should be on the statutory technical issues like nuclear power and not on extraneous political issues related to nuclear weapons, a subject dealt with by the Conference on Disarmament in Geneva.

'The IAEA GC 41 Resolution of Strengthening of Technical Cooperation has made special mention of improving technical capabilities of developing countries in nuclear power. With this in view, IAEA has decided to hold an International Seminar on "Nuclear Power in Developing Countries: Its Potential Role and Strategies for its Development" in India in October 1998.

'The Agency needs to find methodologies so that scientific cooperation in this field is not inhibited by the commercial interests of the vendors. The Agency must also be a prime mover in ensuring that safety-related equipment and the information on Research and Development in safety-related issues are readily disseminated without being hindered by arbitrary and politically motivated export control regimes. Safeguards, while necessary, must

obviously be confined to the respective State's obligations. The hesitation in developing countries to initiate a nuclear power programme often is due to unfamiliarity with steps needed for it. A situation must not be created where the leadership and the public in developing countries, planning to introduce nuclear power for the first time, feel frightened by Safety and feel threatened by Safeguards. The Agency must play a key role in removing such inhibitions while, of course, ensuring safety of nuclear power and implementing effectively and economically its safeguards responsibilities.'

Chidambaram concluded with a call to, 'restoring the original scientific-technical character of the IAEA. The IAEA, a specialized UN agency, used to be such an organization and we must not allow it to degenerate into a shadow political forum repeating the debate in the United Nations General Assembly. It is time that the IAEA should aim at an 'International nuclear heritage'. Unbiased dissemination and deployment of the vast scientific and technical knowledge that has been accumulated through thousands of meetings and conferences should be used to meet the objectives laid out in the Statute, namely, to accelerate and enlarge the contribution of atomic energy to peace, health, and prosperity throughout the world. There is much that the IAEA can do to drive out pessimism, to encourage a culture of nuclear safety and to ensure that safeguards implementation does not inhibit technology development in the area of nuclear power'.

## RESEARCH NEWS

### p53 degradation by Mdm2 – A novel mechanism for regulation of p53 stability

*/ K. Nampoothiri*

Tight regulation of p53 function is critical for normal cell growth and development. The p53 tumour-suppressor protein exerts antiproliferative effects, including growth arrest and apoptosis, in response to various types of stress. The activity of p53

is abrogated by mutations that occur frequently in tumours, as well as by several viral and cellular proteins. The Mdm2 oncoprotein is a potent inhibitor of p53. It is a large protein (491 amino acids for the human protein) of which only the

N-terminal 100 amino acids are involved in the interaction with p53. The results of the present experiments show that binding of Mdm2 to p53 is not sufficient for degradation and suggest a role for other domains of Mdm2 protein. It is well

established that the Mdm2 regulates p53 stability by binding at the transcriptional activation domain of p53 and blocks its ability to regulate target genes and to exert antiproliferative effects. On the other hand, p53 activates the expression of the *mdm2* gene in an autoregulatory feedback loop. The interval between p53 activation and consequent Mdm2 accumulation defines a time window during which p53 brings about its effect. But now it has been reported that Mdm2 also promotes the rapid degradation of p53 under conditions in which p53 is otherwise stabilized. This effect of Mdm2 requires binding of p53, moreover a small domain of p53, encompassing the Mdm2-binding site, confers Mdm2-dependent destabilization upon heterologous proteins. Raised amounts of Mdm2 strongly repress mutant p53 accumulation in tumour-derived cells. During recovery from DNA damage, maximal Mdm2 induction coincides with rapid p53 loss. Hence it is proposed that the Mdm2-promoted degradation of p53 provides a new mechanism to ensure effective termination of the p53 signal<sup>1</sup>.

The p53 tumour suppressor gene is a key target for inactivation in human cancer. Loss of p53 function contributes to tumour development<sup>2</sup>. The product of p53 gene is a 53 kD cell cycle regulator protein. It is a 393 amino acid phosphoprotein. One of the main biological functions of p53 protein is the positive regulation of apoptosis in response to signals such as genomic damage and the aberrant activation of certain oncogenes. Sequence specific transcriptional activation (SST) function of p53 is essential for apoptosis in certain cell types, but not in others. Typically both mechanisms (SST dependent and SST independent) may be triggered simultaneously and their co-operation may be required for maximal apoptotic effects.

Identification of p21 as a target was pivotal for unravelling the mechanism by which p53 can induce a G1 growth arrest. p21 is an universal inhibitor of cyclin dependent kinases. Inactivation of the cyclin D1-Cdk4 and cyclin D1-Cdk6 complexes by p21 prevents the phosphorylation of their major target, the retinoblastoma protein (pRb). Hence, pRb remains in its hypophosphorylated active form, tightly bound to the E2F transcription factor. When bound to pRb, E2F cannot activate genes required for the transition of cells from G1 into and

through the S phase. Proteins encoded by the adenoviral E1A and E7 genes bind to and inactivate pRb and p53 respectively. Activation of *bax* can induce apoptosis by antagonising *bcl-2*. However, the induction of apoptosis by p53 does not always correlate with the activation of *bax* expression. Transcription activation of *Fas/Apo-1* by p53 has also been shown in different cell types<sup>3</sup>. p53-mediated apoptosis could be inhibited by the protein encoded by the *mdm2* oncogene. This inhibition required the formation of complexes between the Mdm2 protein and p53 and appeared only in SST dependent apoptosis but not SST-independent apoptosis. p53 induces apoptosis through the activation of multiple biochemical pathways and the efficiency of the process is dictated by the cellular context. Modulation of p53 activity by the Mdm2 oncoprotein contributes to the oncogenic effects of Mdm2 in certain cell types. Mdm2 can inhibit the transcriptional activity of p53 and was shown to abrogate the ability of p53 to induce growth arrest and to suppress cell transformation. In normal tissues, wild type (wt p53) protein is present at low levels due to its short half-life<sup>4</sup>. But numerous stimuli, including reactive oxygen intermediates and nitric oxide, give rise to the accumulation of high nuclear levels of wt p53 due to protein stabilization<sup>5</sup>. The p53 gene product accumulates in response to DNA damage causing a G1 block of the cell cycle<sup>6</sup>.

An important regulator of p53 activities is the Mdm2 protein. wt p53 induces both a G1 and G2 cell cycle block and activates the transcription of several genes including the murine double minute oncogene (*mdm2*)<sup>6</sup>. Amplification of the *mdm2* gene has first been identified in a mouse tumour cell line<sup>7</sup>. Mdm2, the product of *mdm2* gene is a nuclear protein which forms a complex with tumour suppressor protein p53 and inhibits p53 mediated gene expression by concealing its transactivating domain<sup>8</sup>. Thus the *mdm2* and p53 genes are involved in a feed-back regulatory loop. The *mdm2* gene has been shown to enhance the tumorigenic potential of cells and abrogates the wt p53-induced cessation of cell proliferation<sup>9</sup>. Tissue culture experiments have demonstrated that wt p53 positively regulates the expression of the *mdm2* gene. The functional role for the *mdm2* gene product is to inactivate wt

p53 activity by forming p53/Mdm2 complex<sup>10</sup>. Therefore, the direct interaction of the Mdm2 protein with wt p53 represents a potential negative feed-back control of p53 function. Infact it has been shown that after irradiation of mammalian cells with UV light and after serum stimulation of resting cells, Mdm2 may reverse the possible negative regulatory effects of p53 in the G1 phase of the cell cycle, thus promoting the entry of cells into S phase<sup>11</sup>.

Mice deficient in Mdm2 die early in embryonic development, whereas mice deficient in both Mdm2 and p53 develop normally and are viable indicating the pivotal role of Mdm2 to interfere with p53 activity<sup>12</sup>. Hence it was concluded that embryonic lethality was due to an inability to down regulate p53 by Mdm2 and that deregulated p53 may lead to cell death by apoptosis. The precise role of *mdm2* expression remains to be determined and the fate of the cells may depend not only on the presence of Mdm2, but also on the relative ratios of Mdm2 and p53 proteins in individual cells.

The modulation of p53 mediated apoptosis by Mdm2 depends upon the mechanisms by which p53 induces apoptosis, which in turn are dictated by cellular context. This modulation is most effective when SST is obligatory for p53-mediated apoptosis. Cells which respond efficiently to the SST-independent apoptotic signals are likely to be more resistant to the inhibitory effects of Mdm2. The recent observation is that the interaction of p53 with Mdm2 can also result in large reduction of p53 protein levels through enhanced proteasome-dependent degradation. Endogenous levels of Mdm2 are sufficient to regulate p53 stability, and overexpression of Mdm2 can reduce the amount of endogenous p53. Because Mdm2 is transcriptionally activated by p53, this degradative pathway may contribute to the maintenance of low p53 concentrations in normal cells. Furthermore, mechanisms regulating the Mdm2-induced degradation of p53 may play a role in controlling the extent and duration of the p53 response. The mechanism by which proteolytic degradation of p53 takes place, is identified by examining the effect of a specific inhibitor of proteasome, lactacystin, on degradation of p53 by Mdm2. Inhibition of proteasome function in *mdm2*-expressing mouse embryoblasts resulted in a clear defect in the ability

to degrade exogenous p53, although this effect was not seen in cells lacking Mdm2. Treatment of human cells expressing endogenous wild type p53 and Mdm2 with this proteasome inhibitor resulted in enhanced stability of the endogenous p53. An inhibition of Mdm2-targeted p53 degradation could also be seen following cotransfection of these cells with *mdm2* and p53. These results strongly indicate that Mdm2 targets p53 for degradation through the proteasome, and suggests that Mdm2 is involved in the normal regulation of p53 stability.

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## OPINION

### Why does object technology hasten slowly?\*

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The entire evolution in programming languages and programming strategies seems to point to one major effort—how to cordon the scope of data and functions so that they can be accessed, changed and used without causing unintentional side-effects. In short, software engineering is progressively trying to enforce, among software developers, the discipline that mathematicians bring naturally to their tasks while solving problems. Object technology (OT) is the currently fashionable effort in this direction. At its heart is the requirement that the software developer be able to see (and invent) concepts and patterns and translate them into user-defined data types, better known in the literature as abstract data types (ADTs), in terms of which one can effectively describe a problem and its solution.

All programming languages form a branch of mathematics. All requirements analysis is essentially an exercise in mathematical modelling. All software design is a further detailed elaboration of that analysis with a view to developing an implementation strategy; and coding is the step which spells out exactly how the design will be executed. Intellectual control at every step is the key to software development. Analysis, design and coding, although occurring at different levels of abstraction, must ideally be equivalent in a mathematical sense.

Mathematics has developed over many centuries, has a rich tradition, and at any given time it is served by a good number of extraordinarily talented people devoted to furthering the subject. Mathematicians work without text-editors, debuggers, case-tools and so on. They do not generally commit errors of syntax, type mismatch or make a religion out of reusability even though a very high percentage of their work is in reusable form (theorems, lemmas, methods, data types, etc.). They conceptualize, create abstract data types, encapsulate, inherit and reuse. They go about their job quietly, methodically, carefully, rigorously, painstakingly, and above all, knowledgeably. After completing a piece of work, they take the time to sit back, reflect and make it elegant. They provide a polished document at the end of their endeavours stating what they have done so that others may review, criticize and finally use. Mathematicians generally avoid self-inflicted complexity in their work by being very systematic. They usually focus on resolving domain knowledge complexity. Indeed they already practise what practitioners of OT hope to achieve in the future. *So why are software developers so different from mathematicians?*

#### The reasons

There are perhaps several reasons. First is the multidisciplinary nature of the field—hardware, software and applica-

tions. They involve practitioners from very different academic fields with different traditions, training, prejudices and goals. Indeed, many managers in the software industry have none or very little formal training in software engineering. Consequently, there are problems of depth, focus, traditions, customs, role definitions, etc., all of which remain rather hazy today. What is really needed is an interdisciplinary approach. That is, software practitioners must acquire a broad range of skills that at least span the core areas of hardware, software and applications so as to develop a wider perspective. In particular, professional grade software developers must be trained in operating system design, compiler design, programming language design, functional analysis, logic and theoretical physics. Such a training will provide them with the competence to understand and deal with both domain knowledge as well as software development complexities at appropriate levels of abstraction.

Second is the pretence that software engineering can largely manage without a great deal of training in mathematics. It simply cannot in the future.

Third is the poor repertoire of symbols in software engineering. Much of human civilization's efforts in building knowledge has gone into devising expressive symbols to convey concepts and relations among them so that knowledge generation and its transmission can be handled with

\*The views expressed are those of the author.