imported scholars of high-motivation, dedication, commitment, track-record, etc. The New Yorkers also went to California because of attractive salaries and congenial 'academic' atmosphere. Therefore, motivation, commitment and such should also be obligatory on the part of the institutions and their management to create an atmosphere for creative endeavours.

Things have gone wrong in our academic institutions not due to any inherent fault in our concepts, and not due to lack of scientific culture and lack of understanding the spirit behind it, but due to our approach in their implementation. For example, generally academic institutions in Europe, with their reverence to traditions, might appear overbearing with Herr professors lording over their academic folks. In contrast, American universities might project nonchalance and irreverence to authority and protocol. Whatever may be the appearances, attitudes and the organizational set-up, they have never lost the sight of the primary objective, namely promotion of academic atmosphere. The loss of academic atmosphere is one of the major casualties in our approach to realize our objectives. Our academic institutions are fast becoming temples *sans* idols.

Probably, the approach to academic endeavours might be as business, as fun and as one's bounden duty in a Teutonic, American and Indian Institution, respectively. What could be the reason for such an attitude? All our institutions are fashioned and administered according to the blue-print of civil service rules and regulations. Our academic institutions in essence are extensions of bureaucracy-oriented governmental networks. In a 'command-control' system individual or collective endeavours must conform to the rigid bureaucratic constraints and protocols. Such an atmosphere would knock out any fun or curiosity and concepts such as dedication and commitment sound hollow. No wonder our country has excelled in producing 'managerial' and 'clerical' academicians. We may criticize the bureaucracy but we are all practising partners – using it for one's advantage when it suits and deriding it for public consumption.

It is argued that substantial funding is needed to carry out quality research. It is true for experimental sciences, but lack of funding does not explain the reasons for the dismal atmosphere prevailing in other areas of academics. It is also argued that we should invest sufficiently to build-up the infrastructures to cull our bright young students and train them towards achieving excellence. This, of course, we should and must continue doing. But, as the things stand today, can we say with confidence that *in a* decade or two the academics in Indian soil would be able to project Nobel-level scholars in science, medicine and economics? This loss of self-confidence, that our predecessors had, half a centuary ago, seems to be the crux of our problems. So, in order to reap the fruits, lest they might also turn sour, the existing ethos of our academic culture must be changed.

Introspection without a practical way out to the problems would make life more miserable and guilt-ridden. There is 'something basically wrong in the state of Denmark' and assertions that 'we are second to none' would not alter that situation. The voices from the Deep, such as 'thought of science and technology are receding from the ordinary folks', which should not come as a surprise to any of us, are the indicators of what is really happening at the grass-roots level in all walks of Indian academics.

In summary, we went wrong at the operational level of implementation of our goals, by imposing 'command-control' mode of approach to academics also and then adhering to that approach dogmatically all these decades. To mitigate our institutional problems we could/should evaluate the performance of institutions in other countries and find out why they are doing better than us. Our educational system is based on the British model; but the British are doing alright in science, technology and in other fields and they are receptive to the changing roles and functions of academic institutions to the changing times. It would be certainly worthwhile to look into the aspects that made our pre-Independence era so vibrant academically, socially and culturally and gave scope to the expression of native endeavours. It would also be pertinent to address, by way of introspection, whether we have been toppling the foundations in the process of building castles.

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COMMENTARY

Allosteric regulation in search of a role

J. Manjrekar

A near-casualty of the recent riots in Bombay was an international scientific meeting held at the Tata Institute of Fundamental Research (TIFR). Many participants braved the riots to gather to felicitate two stalwarts of Indian biology. The meeting, titled 'International Symposium on Contemporary Genetics', was organized in honour of Obaid Siddiqui and Pabitra Kumar Maitra of the TIFR Molecular Biology Unit, both of whom are nearing retirement.

Talks were by and large related to the areas in which Siddiqui and Maitra have been working, namely neurogenetics of *Drosophila* and biochemical genetics of yeast. While most of the presentations emphasized the invaluable contributions of genetic approaches to the study of a variety of problems in

Drosophila and yeast, one speaker chose to highlight an area in which, he argued, the potential of genetics to contribute to a better understanding has not been fully exploited. Describing himself as 'the skunk at the garden party', Dan Fraenkel of the Harvard Medical School (Boston, USA) claimed that the analysis of intermediary metabolism had not sufficiently benefited from the application of genetic techniques, with fundamental understanding of metabolic control not having advanced very significantly over the last two decades or so.

Fraenkel proceeded to discuss the properties of certain mutants obtained in microorganisms, in which the catalytic properties of enzymes held to be important in the regulation of glycolysis are altered. Such alterations include several-fold reductions in catalytic rates, and loss of sensitivity to allosteric effectors believed to be important *in vivo*. Mutants of this sort have been obtained, for example, in the yeast *Saccharomyces cerevisiae* (in, among others, Maitra's laboratory at TIFR) and the enteric bacterium *Escherichia coli*[1-5]. Some of these mutants show an astonishing phenotype, namely the absence of any apparent mutant phenotype: they are indistinguishable from the wild type with respect to growth and metabolite flux. This has been observed with mutants obtained by random mutagenesis, as well as by site-directed mutagenesis targeted at catalytic or regulatory regions of the concerned enzymes.

These results militate against some of our basic ideas about how cells manage their affairs. Most of us who have grown up on a biochemical diet liberally garnished with spices like 'rate-limiting step', 'control point', 'fine tuning', 'exquisite regulation' and the like, will find it difficult to digest the idea that cells don't seem to care particularly whether the enzymes supposedly entrusted with regulating the rate at which they use up or synthesize important metabolites, run that much faster or slower, with little regard for 'regulatory' inputs.

What, then, are we to make of these mutants? Clearly, they cannot be dismissed as oddities or aberrations. The enzymes affected include textbook examples of allosteric regulation controlling critical steps of glycolysis, such as phosphofructokinase (PFK) and pyruvate kinase (PYK). Their unexpectedly normal phenotypes demand explanations which must be incorporated into our picture of metabolic regulation. A number of possibilities which suggest themselves are explored here. Needless to say, these are not mutually exclusive.

Biochemical redundancy

There may be redundancy in the enzymatic regulation of biochemical pathways. Apart from the enzymes presently believed to constitute the control points of a pathway, additional enzymes of the same pathway, which have received less attention, could also be subject to regulation by physiological effectors. Redundancy of this sort could provide cells with reserve buffering capacity, and would be of even greater value to cells if alternative pathways exist through which intermediary metabolites can be fed into a pathway, analogous to the well-known anaplerotic channeling of α-ketoglutarate into the tricarboxylic acid cycle. It will be interesting to analyse the phenotypes of multiple mutants deficient in allosteric regulation of more than one enzyme of a pathway, where each mutant alone shows the wild type phenotype. If the multiple mutants have phenotypes different from those of the constituent single mutants, this would suggest redundancy in control by the known regulatory enzymes: as long as one, or some, enzymes function normally, adequate control is achieved, but if additional regulatory enzymes are affected, pathway control is perturbed to an extent that affects cell functioning. On the other hand, if multiple mutants do not differ phenotypically from single mutants, then allosteric regulation must be either altogether dispensable, or redundancy in regulation must extend beyond the known regulatory enzymes, involving additional enzymes of the pathway.

Another approach to detecting novel control points in a pathway might be to look for suppressor mutations for mutants in one of the known regulatory enzymes. Extragenic suppressor mutations might reveal new regulatory elements, such as other enzymes of the pathway with altered regulatory properties (ignoring mutations which simply result in permanently elevated or reduced enzyme activities). Apart from potential regulatory pathway enzymes, suppressor mutations could also show up other factors related to pathway control, such as enzymes involved in the synthesis of allosteric regulators, or in covalent modifications of proteins related to the functioning of the pathway.

In addition to the possibility of biochemical pathways being amenable to regulation at novel points, there may also be redundancy in the regulation of individual catalytic steps, through the availability of multiple allosteric effectors acting at distinct sites on an enzyme, and by virtue of the existence of isozymes. In the case of yeast PFK, around 25 allosteric ligands[6] and two isozymes[7] have been described. While the roles of effectors such as ATP, fructose-2, 6-bisphosphate (F2, 6bP) and fructose-1, 6-bisphosphate (FDP) have been fairly thoroughly investigated, the physiological significance of a number of other ligands is far from clear. It is conceivable that in mutants insensitive to 'major' effectors like ATP or F2,6bP, reasonably effective regulation is achieved by some of the numerous other substances known to affect PFK activity *in vitro*. There might also be entirely novel allosteric regulators; it is worth remembering that F2,6bP, which is credited with a central regulatory role in glycolysis and gluconeogenesis, was only discovered a little over a decade ago, despite the many years of attention PFK had enjoyed. What is true of PFK could well be true of some other metabolic enzymes. Among the glycolytic intermediates in particular, several are points of intersection with other metabolic pathways, serving for instance as precursors for the biosynthesis of a variety of substances[8]. This increases the likelihood that enzymes catalysing the synthesis of glycolytic intermediates are subject to regulation by substances associated with connected pathways, which are dependent on the activities of glycolytic enzymes. In rapidly growing and dividing cells, the demand for the synthesis of essential cellular constituents could drive the glycolytic pathway, even in the absence of positive regulation by effectors related to glycolysis and energy metabolism. Pyruvate, with some 16 different metabolic fates in

the cell[8], illustrates the point well. Intermediary metabolites of any of the different pathways into which pyruvate gets channelled could act as allosteric regulators of glycolytic enzymes; furthermore, a high flux of pyruvate into these pathways could help to maintain a high glycolytic flux by rapidly removing reaction products, thereby ensuring that the reactions of glycolysis–most of which are reversible–run predominantly in the forward direction. Conversely, under conditions of slow growth and cell division, pathways leading off glycolysis could exert negative effects on glycolytic enzymes.

A further level of redundancy in the control of individual catalytic steps may be the presence in cells of more than one enzyme able to catalyse the reaction. Isozymes have been reported for a number of glycolytic enzymes. Indeed, they seem to be more the rule than the exception. In *S. cerevisiae*, pyruvate decarboxylase and alcohol dehydrogenase have as many as three isozymes each. However, the metabolic roles of the isozymes are ill-defined. For instance, *E. coli* has two PFKs–a major and a minor form. If the major form is knocked out and the minor PFK overexpressed, cells grow normally. In yeast, there is a major soluble and a minor particulate form, which appear to share a common subunit. Disruption of either gene encoding the subunits of soluble PFK has little effect on cell growth, whereas double disruptants fail to grow on glucose.

What is the significance of two or more isozymes? Do they serve distinct, specialized functions? Do the minor forms complement the activity of major forms under 'normal' conditions, or do they enter the picture only in particular situations? These and other questions remain largely unanswered. The role of isozymes must be taken into consideration in genetic and biochemical analyses of the regulation of individual steps of metabolic pathways.

In general, redundancy in biological systems could be fairly common[9]. Mutational analysis of Drosophila has revealed a number of 'non-essential' loci encoding enzymes, which can be mutated without obtaining a (readily apparent) phenotype. There is also evidence for redundancy in the genetic control of early development in Drosophila and vertebrates. As more examples of redundancy in biological systems become known, it may turn out to be more widespread than is generally appreciated at present. Indeed, redundancy appears to be a property that can provide greater depth to the remarkable homeostatic capacities of biological systems.

Genetic regulation

Another level of redundancy in metabolic control could be the capacity for genetic regulation of metabolic enzymes. The expression of genes coding for these enzymes can be modulated at any of several levels—transcription, mRNA processing and stability, translational efficiency, post-translational modifications due to the products of other genes (themselves subject to various kinds of regulation), and protein stability. While regulatory mechanisms involving the control of gene expression are invariably slower than the very rapid responses which can be achieved through regulation of enzymatic activities, there seems to be no compelling reason why cells should respond on a millisecond to second time scale, rather than over seconds to minutes, to changes in physiological states. A number of constitutively expressed genes are not transcribed at uniformly high levels, but are capable of many-fold transcriptional activation. Further, the products even of uniformly transcribed genes can be regulated in numerous ways besides allosteric regulation. For instance, the *E. coli* hexose monophosphate shunt enzyme 6-phosphogluconate dehydrogenase is expressed in a growth-dependent manner. Different cloned alleles of the *gnd* gene expressed in a uniform genetic background were found to give different levels of enzyme activity which did not reflect corresponding differences in transcription rates. Sequence comparisons showed allelic differences predicted to affect mRNA secondary structure[10], so that differences in enzyme levels are due to different translational efficiencies for the allelic variants. Translational regulation has also been reported for pyruvate kinase and the PFK2-encoded subunit of PFK in *S. cerevisiae*[11]. A further level of regulation has been observed for gluconeogenic enzymes, which undergo inactivation by proteolytic degradation in the presence of glucose[12].

The role of transcriptional regulation in metabolic control can be studied by genetic manipulations resulting in altered rates of transcription. Experiments in this direction have been performed by overexpressing a number of glycolytic genes[13]. Overexpressing strains show no abnormal phenotypes, arguing against an important role for transcriptional regulation. The situation for decreased rates of transcription is not as clear. Although two-fold decreases in transcription are without effect[14], it must be remembered that basal levels of transcription are rather high[15], and probably not sensitive to smaller perturbations. Taken together, currently available information does not lend much support to the notion that transcriptional regulation of glycolytic enzymes plays a very major role. However, acting in combination with other mechanisms of regulation of gene expression (see above), transcriptional regulation could contribute to the regulatory landscape of metabolism. More data are required for a clearer picture to emerge.

Covalent modifications

Since they occupy a central position in cellular functioning, especially of eukaryotes, covalent modifications of proteins are considered separately from other post-transcriptional regulatory mechanisms. The importance of reversible protein phosphorylation in the regulation of every conceivable cellular activity is too well-known to require elaboration. Other modifications like methylation, glycosylation, acetylation, acylation, etc., though not as well understood, have also been found in some instances to profoundly affect the activities of proteins. Relatively little is known about the role of covalent modifications in the modulation of activities of metabolic enzymes, though some cases have been described[16–19]. Apart from the enzymes catalysing steps of a pathway, other proteins affecting its functioning could also be regulated by covalent modifications, as is the case with fructose 6-phosphate 2-kinase, which catalyses the addition of a phosphate group to fructose 6-phosphate, or its removal from F2,6bP. Remarkably, phosphorylation of mammalian liver[18] and heart[19] isozymes has opposite effects on their activities, illustrating the

varied and flexible ways in which covalent modifications of enzymes could contribute to metabolic regulation.

The role of covalent modifications could be genetically investigated, apart from the obvious biochemical studies. Mutations affecting individual steps of a pathway, but not the enzymes catalysing these steps, could provide useful leads. Generating suppressor mutations for mutant pathway enzymes could also be useful in identifying modifying systems, as discussed earlier.

Higher-level regulation

A more radical–and rather more exciting–possibility is that regulation of metabolic pathways at the level of individual enzymes, whether of their activities or gene expression, is not, in fact, of such critical importance. Cells might have homeostatic capacities broader and more extensive than generally imagined, with considerable flexibility to compensate for fluctuations in individual pathways. Indeed, perhaps 'individual pathways' are not nearly as individualistic as we conceive them to be, being more extensively tied into the cellular metabolic network than even those maddeningly intricate metabolic charts suggest. If this should turn out to be the case, then we might expect to find a considerable amount of genetic variability affecting the functional characteristics of enzymes. While high levels of protein and DNA sequence polymorphism have been demonstrated in a variety of microbial species and at a variety of loci[20–22], this variability has been visualized at the protein level primarily by electrophoretic and serotype analysis. It will be highly interesting to know whether comparable levels of functional polymorphisms exist in natural populations. The existence of such polymorphisms with respect to kinetic and regulatory properties of enzymes would constitute powerful support for the idea that these properties are of only limited importance in metabolic control, as suggested by the wild type phenotype of glycolytic mutants with altered catalytic properties. Given the amount of work that has been done on protein polymorphisms, it is somewhat puzzling that there is so little information on functional polymorphism at the population level. Even electrophoretic variation is often assumed to be limited to substitutions that leave protein function unaffected, confer heterozygous advantage in diploid organisms, or are maintained in a population by balancing selection. The wild type phenotypes of the enzyme mutants discussed here should convince us of the shakiness of such assumptions. What we find in natural populations regarding the variability of enzyme catalytic properties should tell us something about metabolic control.

What if natural populations fail to show significant functional variation? A lack of such polymorphisms would imply the operation of selection acting to conserve highly adaptive enzyme properties within a narrow range of values. This would suggest that the regulatory properties beloved of textbooks are about as important as they were thought and taught to be, though probably in more limited physiological contexts. This, of course, would not explain how the enzyme mutants do so well in laboratory culture. One kind of explanation could be along the lines of the arguments about redundancy and multiple levels of regulation: additional back-up mechanisms are available to cells if individual catalytic properties of enzymes are selectively altered. Judging by the number of 'non-essential' loci coding for enzymes[23], biochemical redundancy could be a phenomenon deserving serious attention. A different approach might be to assume that under standard culture conditions, the altered properties are not very important, and to ask under what conditions they do become crucial for cells. Laboratory culture conditions do not subject microorganisms to the range of stresses and fluctuations encountered in natural environments. While allosteric 'fine tuning' might not be critical in a culture medium, under certain conditions such as severe nutrient limitation, and a variety of other stresses, it might contribute significantly to the fitness of an individual. Furthermore, complex developmental changes involving major shifts in patterns of gene expression would occur in natural environments, but may be bypassed in culture. Before concluding that mutant enzymes have no effect on the fitness of their carriers, it would be prudent to analyse their phenotypes under a variety of conditions, and to carry out chemostat competition experiments under favourable as well as unfavourable conditions.

Some basic questions about metabolic control are raised by genetic analyses of glycolysis suggesting that microorganisms are not very sensitive to alterations in the kinetic and regulatory properties of important glycolytic enzymes.

A variety of factors could be responsible for the phenotypes of the glycolytic mutants, and much more detailed investigation is required. An immediate question to which an answer should be of considerable interest, is whether natural populations show large variations in kinetic and regulatory properties comparable to those obtained in mutants. An answer to this question may indicate what kinds of approaches can be adopted further on.

While I have frequently referred to 'metabolic pathways' in a general way, it must be emphasized that the mutants discussed here all affect fermentative energy metabolism. It will be interesting to see whether mutants with similar properties will eventually be found affecting enzymes of other metabolic pathways as well.

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Erratum

T. S. Suryanarayanan (PG Department of Botany, Ramakrishna Mission Vivekananda College, Madras 600 004) regarding G. Baskaran's article 'An encounter with genes' (*Curr. Sci.*, 1993, **64**, 216–218) writes: 'In the second paragraph, what should have been nucleic acid bases are termed nucleic acids; in the third paragraph, adenine is described as an amino acid. A more careful redaction could have averted such mistakes.'

G. Baskaran replies

'The encounter with genes left me spellbound. In that blissful state one mixes up names. Surely, this is no excuse for my writing "nucleic acids" instead of "nucleic acid bases" (2nd paragraph, 2nd line) or for that matter writing "amino acid adenine" instead of "nucleotide adenine" (3rd paragraph, 19th line) in my commentary. It is time I learn more about proof reading and error correction from genes (there seem to be fascinating phenomena in genes—error correction, proof reading, gene repair, error-prone gene repair, etc.!)'.

The editors themselves could have detected these errors. We thank T. S. Suryanarayanan for bringing these errors to our notice.

—*Ed.*

Correction

Phenology of seasonally dry tropical forest

J. S. Singh and V. K. Singh

(*Curr. Sci.*, 1992, **63**, 684-689)

In Table 1, few mistakes in the names of the families have occurred. The correct names of the families with species are given below.

Species	Family
Anogeissus latifolia	Combretaceae
Bauhinia racemosa	Caesalpiniacea
Boswellia serrata	Burseraceae
Briedelia retusa	Euphorbiaceae
Buchanania lanzan	Anacardiaceae