

One protein—Many functions

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The concept of one enzyme-one activity had influenced biochemistry for over half a century. Over 1000 enzymes are now described. Many of them are highly 'specific'. Some of them are crystallized and their three-dimensional structures determined. They range from 12 to 1000 kDa in molecular weight and possess 124 to several hundreds of amino acids. They occur as single polypeptides or multiple-subunit proteins. The active sites are assembled on these by appropriate tertiary folding of the polypeptide chain, or by interaction of the constituent subunits. The substrate is held by the side-chains of a few amino acids at the active site on the surface, occupying a tiny fraction of the total area. What is the bulk of the protein behind the active site doing? Do all proteins have only one function each? Why not a protein have more than one active site on its large surface? Will we discover more than one activity for some proteins? These newer possibilities are emerging and are finding experimental support. Some proteins purified to homogeneity using assay methods for different activities are now recognized to have the same molecular weight and a high degree of homology of amino acid sequence. Obviously they are identical. They represent the phenomenon of one protein—many functions.

A review on proteins with more than one activity, called multifunctional, was available in 1976 (ref. 1). Some examples are described for a single protein with multiple catalytic sites in distinct domains. In other words, a protein can be visualized as a collection of domains on the surface fused together! A protein may be associated with other proteins(s) in hetero-oligomeric structure, or may be embedded in a membrane and exhibit another activity. The number of such proteins is increasing and some of these are described here (Table 1).

Protein disulphide isomerase (PDI)

This enzyme, discovered by Anfinsen and coworkers² in liver microsomes, catalyses the rearrangement of disulphide bonds during protein folding. Given the name protein disulphide isomerase, it has 'unimpressive' *k*_{cat} value (0.4/min) but occurs ubiquitously in mammalian tissues at high concentration. It is a homodimer of 58-kDa protein located on the lumen side of endoplasmic reticulum³. Each monomer has two identical, independently active sites with a sequence—Try.Cys.Gly.His.Cys.Lys (WCGHCK), first Cys being essential. This sequence is similar to that of thioredoxin and β -subunit

of follicle stimulating and lutenizing hormones. PDI has thioredoxin-like activity and it can act as a substrate for thioredoxin reductase⁴.

PDI has the ability to bind to peptides of various lengths and sequences with little specificity. These peptides act as competitive inhibitors of its isomerase activity⁵. The protein-folding activity was shown to be inhibited by Cd⁺⁺ and arsenite, both specific dithiol-complexing compounds⁶, possibly by blocking the two cysteines in the thioredoxin-like active sites. The protein-binding activity in the cadmium-treated protein was found to remain unaffected⁷. The two actions are therefore carried out by two distinct domains of the protein. In an yeast mutant, a truncated PDI protein, without a third of its C-terminal portion, possessed the isomerase activity but the peptide-binding activity and the viability of the cells decreased⁸. It appears that the peptide-binding activity of PDI is more important in the cell. Does this protein have other more relevant functions?

Although PDI is ubiquitous, it occurs with high specific activities (concentrations?) in collagen-synthesizing cells, and interestingly correlates with prolyl-4-hydroxylase (P4H). The β -subunit of P4H has 94% homology with PDI⁹. Could this then be the primary function of this protein wrongly identified as PDI? P4H is a 240 kDa-protein with two 64 kDa α -subunits and two β -subunits of PDI. In the complex the PDI expresses only half its isomerase activity.

A thyroxine-binding protein (*K*_D for T₃ 57 nM)^{10,11} and another protein with thyroxine-5'-deiodinase activity¹² in liver microsomes were found to be 58 kDa-proteins. In both cases the amino acid sequences of the respective purified proteins were independently determined and were found to be identical with PDI.

The binding-protein of the glycosylation site, Asn.aa.Ser/Thr, a component of oligosaccharyl transferase was found to be same as PDI¹³. But it was not involved in the N-glycosylation process. However, its apparent molecular weight increased to 60 kDa by glycosylation¹⁴.

Being a component of microsomal triglyceride-transfer protein (MTP), PDI plays a role in the exchange of triglyceride between membranes, and possibly plasma lipoproteins¹⁵. This protein, a heterodimer of a 58 kDa-protein (PDI) and a 88 kDa-protein, was isolated from lumen of microsomes of liver and intestines where such exchanges occur, but was not detected in brain, heart and kidney. Association of the two proteins drastically decreased the isomerase activity of PDI and increased the lipid-transfer activity. The 88 kDa-protein was not

Table 1. One protein—Many functions

Primary identity	Other activities
Protein disulfide isomerase 58 kDa	Thioredoxin-like activity Peptide-binding (sequence non-specific) Prolyl-4-hydroxylase- subunit Thyroxine-binding Oligosaccharyl transferase component Microsomal triglyceride transfer protein-subunit
58 kDa-cytosolic proteins (a) Pyruvate kinase (b) Non-specific lipid transfer protein (c) Fermodulin	T ₃ -binding protein Sterol carrier protein 2 Fe-binding protein
Peptidyl-prolyl- <i>cis-trans</i> isomerase 17 kDa	Cyclophilin Cyclosporin A-binding
Glyceraldehyde-3-phosphate dehydrogenase 37 kDa	Acyl phosphatase Esterase ADP-ribosylation Microtubule bundling Protein-kinase Uracil-DNA glycosylase (nuclear) tRNA-binding (sequence specific) Amyloid protein (Amy C)-binding Membrane-binding
Lens crystallins: delta epsilon eta lambda mu rho tau sigma	Arginine-succinate lyase Lactate dehydrogenase-B Aldehyde dehydrogenase Hydroxylacyl CoA dehydrogenase Ornithine cyclodeaminase NADPH-quinone reductase Enolase GSH-S-transferase
Aconitase (cytosolic with Fe-S cluster)	Iron-response element binding protein (Fe-free)
(a) Isocitrate dehydrogenase (NAD ⁺ specific) (b) Isocitrate dehydrogenase (NADP ⁺ specific) 40 kDa	Mitochondrial mRNA-binding Vanadate-dependent NADH oxidation
Lactoferrin (Fe-binding) 80 kDa	Ribonuclease
Heat shock proteins (hsp)	Peroxidase (Mn) Alpha-subunit of F ₁ -ATPase
(a) Oxygenase from <i>P. syringe</i> (b) Mitochondrial protease (c) Amylase/Trypsin inhibitor	Mono-oxygenase/dioxygenase reactions Non-overlapping specificities of peptidase action Independent inhibition of two activities
Leukocyte antigen CD 38 46 kDa	NAD glycohydrolase ADP-ribose cyclase Cyclic ADP-ribose hydrolase

detected in abetalipoproteinemia¹⁶.

PDI was found to be the major auto-immune antigenic protein in LEC strain of rats prone to spontaneous, hereditary hepatitis¹⁷.

The various functions of this 58 kDa-protein are schematically shown in Figure 1.

Pyruvate kinase

An extranuclear tri-iodothyronine (T₃)-binding protein was identified as a 58 kDa-protein in the cytosol possessing an amino acid sequence entirely different

from PDI¹⁸. This sequence was found to be homologous to the subunit of pyruvate kinase subtype M₂, found in many tissues, with its amounts increasing in proliferating liver tissue. The purified monomeric 58 kDa-protein showed only about 5% of pyruvate kinase activity. It is converted to the tetramer with full activity in the presence of fructose-1, 6-bisphosphate. Binding of thyroid hormones to the tetrameric protein dissociated it to the monomeric form with loss of the kinase activity. T₃ was most efficient with a K_i = 30 nM. Both forms of the protein may thus exist simultaneously depending on the concentrations of the two ligands.

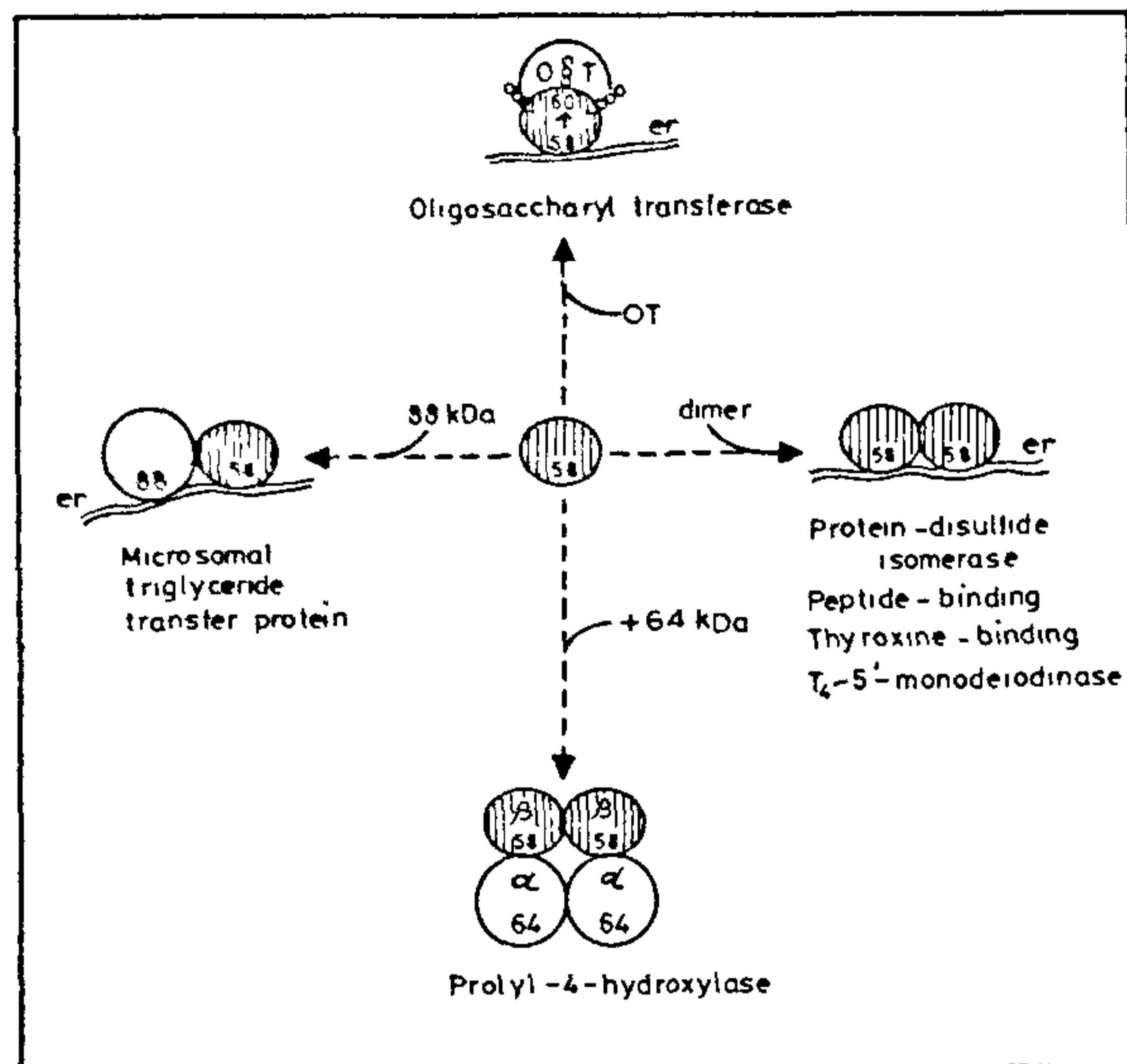


Figure 1. Multiple ways of utilizing a 58-kDa protein

Two other ubiquitous cytosolic 58 kDa-proteins were found¹⁹. One is identified as non-specific lipid-transfer protein (sterol-carrier protein 2) whose amino acid sequence is different from both PDI and pyruvate kinase. The other 58 kDa-protein named fermodulin, is a weak iron-binding protein. It inhibited in the presence of ferrous ions the activity of 3-hydroxy-3-methylglutaryl-CoA reductase, the regulatory enzyme in cholesterol biosynthesis²⁰. It bears some relationship to pyruvate kinase but no clues are available on its identity with the other 58 kDa-proteins.

Peptidyl-prolyl cis-trans isomerase (PPI)

A 17 kDa-protein which can catalyse the isomerization of cis and trans forms of peptidyl-prolines is widespread in mammalian tissues, insects and micro-organisms. It is also able to activate protein folding. The action of PDI, being part of P4H, was initially thought to be through proline isomerization but the two actions were later found to be independent.

Proline isomerase was found to be identical with cyclophilin. Cyclophilin is a cytosolic protein that specifically binds the undecapeptide of fungal origin, cyclosporin A, an immuno-suppressive drug that prevents rejection of organ transplants. Cyclophilin and PPI have 96% homology of amino acid sequence. Thiol-dependent binding of cyclosporin A inhibits the isomerase activity of PPI^{21, 22}.

Glyceraldehyde-3-phosphate dehydrogenase (GAPD)

GAPD is a key enzyme in glycolysis and is responsible for the conservation of energy on oxidation by NAD^+ of an aldehyde group to acetylphosphate. It occurs widely and is abundant (10% in muscle and 20% in yeast cytosolic proteins). It occurs as a homotetramer with subunits of 37 kDa-protein (about 330 amino acids). Each subunit has distinct domains for NAD^+ -binding and for catalytic site with a highly reactive cys-149 (see ref. 23 and references therein). This protein is found to have versatile functions given below:

- (a) GAPD oxidizes glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate and exhibits half-of-the sites activity²³.
- (b) GAPD shows a low activity of hydrolysis of acetylphosphate when the active -SH group is blocked²³.
- (c) When bound NAD^+ is removed, GAPD shows iodoacetate-sensitive hydrolysis of aryl esters²³.
- (d) On treatment with NO-yielding nitroprusside, a tiny fraction of GAPD becomes competent for auto-ADP-ribosylation. This effect was obtained with the proteins from muscle²⁴ and brain²⁵, but not yeast²⁵ or the dimeric form in erythrocytes²⁶.
- (e) A 37 kDa-protein that regulates microtubule bundling was found to be identical to GAPD²⁷.
- (f) GAPD can act as a phosphokinase and phosphorylate proteins in skeletal muscle microsomes²⁸.
- (g) A monomeric 37 kDa-protein was obtained from human erythrocyte GAPD by electro-elution after separation on 10% SDS-PAGE followed by dialysis. This preparation of monomer was inactive as the dehydrogenase but showed another activity, a nuclear uracil-DNA glycosylase (UDG) by which uracil residues adventitiously occurring in DNA, as a result of deamination of cytosine or incorporation of dUTP, are excised²⁹.
- (h) GAPD has the ability for sequence-specific binding to t-RNA and its translocation³⁰.
- (i) GAPD in rat brain interacts with C-terminus of Alzheimer's amyloid protein, a constituent of abundant senile plaque and aggregate of beta-A4 peptide derived from amyloid precursor protein (APP)³¹. This appears to be part of a general phenomenon of its binding to proteins such as lens fibre, cardiac muscle, skeletal muscle microsomes, synaptosomes and nerve endings.

Lens-crystallins

Crystallins are a set of proteins used in the lens. They are not lens-specific or special structural proteins. The crystallins from different sources are identified by Greek letters, and are related or identical to the proteins with a variety of activities (see ref. 32 and references therein).

Some examples are given below: delta in reptiles—arginosuccinate lyase; epsilon in birds—lactate dehydrogenase-B; zeta in camel—NADPH-quinone oxidoreductase; eta in elephants—aldehyde dehydrogenase; lambda in rabbit—hydroxyacyl CoA dehydrogenase; mu in kangaroo—ornithine cyclodeaminase; rho in frogs—NADPH-acceptor reductase; tau in turtle—enolase; sigma in cephalopods—GSH-S-transferase. These proteins are recruited from the available enzyme-proteins in the cell and then structured into the transparent lens. It is amazing how such bulk protein is accumulated at the site and folded differently from its native enzyme structure apparently by some guided process.

Aconitase sans Fe-S cluster

Aconitase catalyses the interconversion of citrate and isocitrate, involving a dehydration-hydration reaction. This non-oxidative enzyme has an Fe-S cluster with labile sulphide and is also identified as the high potential iron-sulphur protein (HiPIP). Full activity of this enzyme needed addition of iron in the assay, known to convert [3Fe-4S] to-[4Fe-4S] cubane cluster.

The most obvious function of aconitase is in citric cycle and therefore it is in mitochondria. But a cytosolic form of the enzyme is known for a long time which is distinct and coded by a gene on a different chromosome. The importance of this protein in the cytosol became apparent when it was found to have high degree of homology with the binding protein of iron-responsive elements (IRE). These are present in the messenger RNAs of transferrin receptor and of ferritin in the form of RNA stem-loop structures located in the untranslated regions. The iron-sensing is carried out by the protein IRE-BP which on binding to IRE with high affinity 'exerts an iron-dependent, dual, and reciprocal control of transferrin receptors and ferritin concentrations'. The cytosolic aconitase without the Fe-S clusters is identical with IRE-BP, and the two forms of the protein coexist³³.

Isocitrate dehydrogenase

The NAD⁺-dependent isocitrate dehydrogenase in yeast mitochondria and a 40 kDa-protein that binds to the 5'-untranslated leaders of mitochondrial mRNA are the same³⁴.

The NADP⁺-specific isocitrate dehydrogenase protein was able to oxidize NADH, but not NADPH, in presence of polyvanadate³⁵.

Human lactoferrin

Human milk was found to have potent ribonuclease activity which turned out to be 80 kDa-Fe-binding glyco-

protein, an isoform of lactoferrin sharing physical, chemical and antigenic properties but differing from it in the lack of significant iron-binding capacity³⁶.

ATPase and stress-induced peroxidase

F₁-ATPase in mammalian mitochondria has multiple subunits of which the α -subunit is considered regulatory and the β -subunit binds to nucleotides. The α -subunit, a 57 kDa-protein, was also found to occur in peroxisomes, and to respond to heat shock. It belongs to the stress proteins of hsp-60 family³⁷.

A manganese-dependent peroxidase induced in *Neurospora crassa* by H₂O₂, ethanol, arsenite or CdCl₂ was identified as a heat shock protein³⁸.

Single enzyme catalysing two independent reactions

It is possible that two genes are 'fused' and give a single mRNA translated into the product as a fusion protein having two independent activities. Two subunits having different activities may thus form a single protein. Some examples are available in this category.

Two reactions were found to be catalysed by a single enzyme isolated from *Pseudomonas syringe*. Acting on 2-oxoglutarate in the presence of arginine, the products produced by mono-oxygenase reaction were succinate, hydroxy arginine and CO₂, and that by dioxygenase were ethylene and 3CO₂ (ref. 39).

Maturation of imported proteins into mitochondria is carried out by signal peptidases. A mitochondrial protease with 'two catalytic subunits of non-overlapping specificities' was recently described⁴⁰.

On the same basis as activities, multifunctional or multiheaded inhibitors representing a sum of individual capabilities are also known to act on more than one enzyme with separate non-overlapping binding site for each⁴¹⁻⁴³. A ragi amylase/trypsin inhibitor (RATI), stable to exposure to 8M urea or heating at 90°, inhibiting amylase and trypsin activities⁴³ is an example.

Human leukocyte antigen CD 38

The 46 kDa-protein on the outer surface of human erythrocytes identified immunologically as the antigen CD 38 was found to have three related NAD⁺-metabolizing enzymes—NAD⁺ glycohydrolase, ADP-ribose cyclase and cyclic ADP-ribose hydrolase^{44,45}—involved in the formation and degradation of cyclic ADP-ribose, emerging as an important calcium-mobilizing molecule. This glycoprotein, bound to the membrane by a transmembrane domain and a short cytoplasmic tail, acts as a surface

antigen and is involved in activation and proliferation of lymphocytes. A vignette of a general phenomenon of two related activities resident in a protein is obvious.

Perspective remarks

The number of proteins present in any cell is limited whereas the functions performed by them are many. About fifty bands are visualized on SDS-PAGE analysis of proteins of tissues. About 20 of these are major and some may occur in too small concentrations to be detected. At the best the total number of individual proteins may not far exceed 100. With some of these, the cell has obviously devised ways of structuring them to multiple active forms.

The proteins are large and their surfaces can provide multiple active sites of different catalytic potential. The examples available already indicate specific, individually active domains fused together into a protein showing multifaceted activities. With minor chemical modifications of the amino acids some proteins gain or lose activities that can be used also as part of metabolic regulation. Binding of effector molecules alter the subunit structures and show totally different activities. By cooperative hetero-oligomer formation between two or more proteins, an entirely new activity can emerge. Embedding in the membranes gives the proteins additional potential for altered forms and activities. Indeed the process of guided folding, now an active field of research, can provide multiple stable structures that can coexist with multiplicity of activities. This is exemplified by the phenomenon of one mutation and multiple syndromes⁴⁶.

Three distinct ways of using a protein become apparent from the examples available: multiple catalytic sites, binding properties and structural roles. Thus, a protein active as an enzyme can be 'recruited' to do other functions. It can bind to its own substrate or other metabolites (e.g. tRNA) and transport them. Complexing with distinct structures in mRNA or DNA, it can play a regulatory role. The regulatory elements and transcription factors may turn out to be known enzyme proteins. It can become a structural element like in lens crystallins or in the subunit or membrane structures. Indeed, the possibilities are legion.

One other perspective is emerging. How valid is the enzyme nomenclature in the light of these developments? In the foregoing examples, it can be seen that a 58 kDa-protein is identified with at least four distinct catalytic activities, and there are at least two distinctive 58 kDa-proteins with other activities. It is probably time to consider developing a 'protein nomenclature' based on domain-level amino acid sequence, structural motifs and their arrangements in polypeptides. More and more

proteins will now be identified with multiple functions and the author will be grateful if the readers draw the attention to other examples.

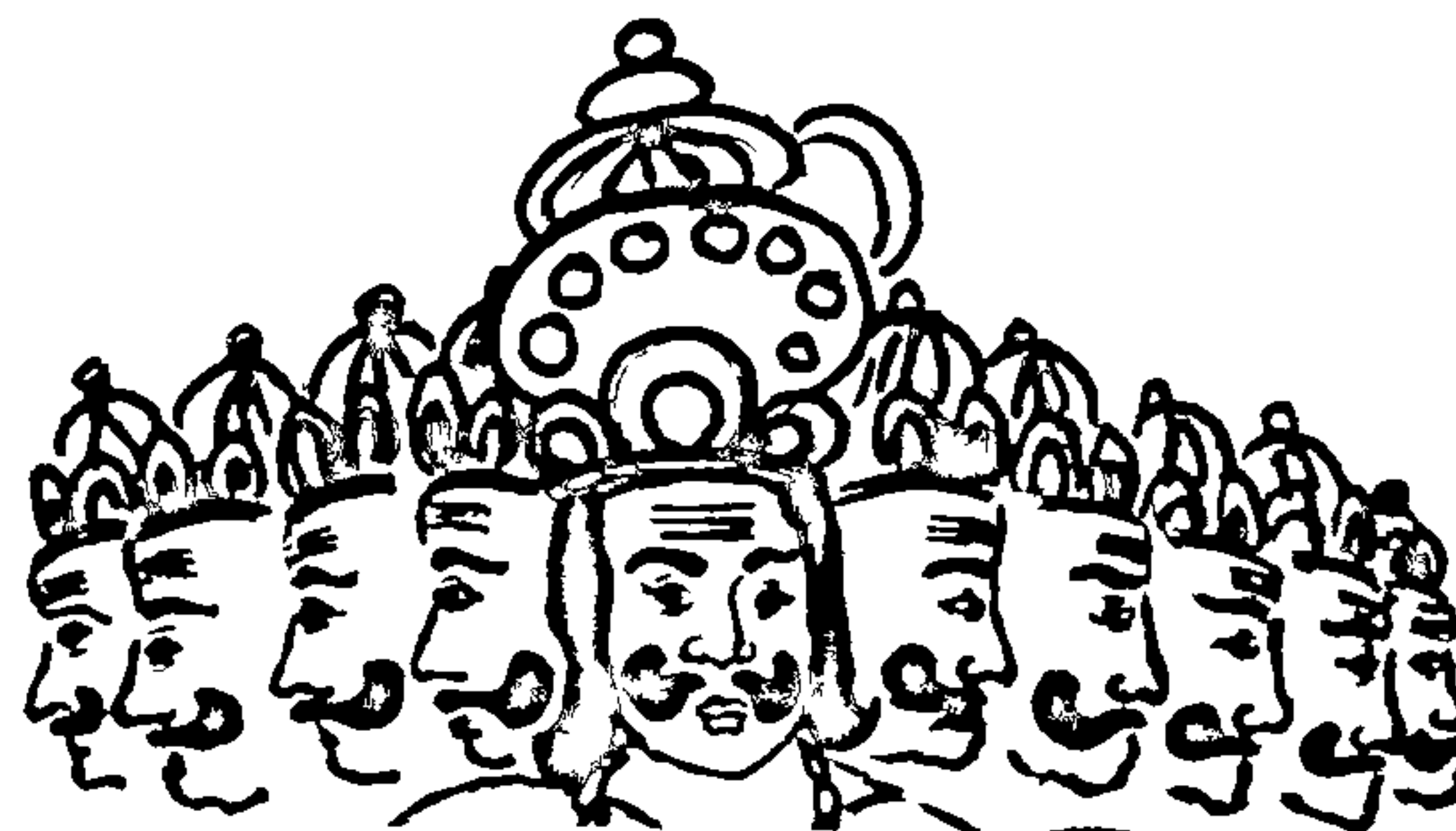
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In the epic *Ramayana*, the king of Lanka, Ravana, depicted to have ten faces, is a great warrior, a scholar of scriptures and an accomplished veena player—a multifaceted personality! (idea conceived by V. V. Vaidyanathan and drawn by M. Anil Kumar).

Leaf gas exchange in lightflecks of plants of different successional range in the understorey of a Central European beech forest

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Young seedlings of two vines, in particular *Clematis vitalba* L. and *Rubus fruticosus* agg, and of seven other plants all co-occurring in a European beech forest, were subjected to lightflecks. We study their induction dynamics in particular, since induction state controls light use efficiency, light quantum yield and carbon gain. Distinct differences have been observed in the velocity of photosynthetic induction increase and loss. Pioneer species take a longer time to reach half induction in continuous saturating light as compared to late successional representatives. Despite the identical growth conditions, photosynthesis is induced faster in the pioneer species and the induction is lost slower. This is important for seedling establishment in the understorey.

THE light environment in forest understoreys typically consists of long periods of low PFD¹ separated by periods of short but frequent sunflecks lasting from less than a second up to a few seconds, and rarely sunflecks lasting up to several minutes^{1–4}. In a variety of forests it has been shown that sunflecks contribute between 35% and 75% to total daily photon flux^{1,3,5}. Current evidence suggests that a large portion of carbon gain is attributable to these higher light phases in the understorey.

For the first lightflecks after darkness or a prolonged dim light phase, photosynthetic carbon gain by a leaf is likely to be limited by low photosynthetic induction. This limitation, however, is gradually removed during subsequent lightflecks^{6,7}. Understorey plant species accomplish a considerable portion of their daily carbon