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Macromolecular interactions: tracing the roots

In the 1990s there has been an explosion of articles in the biochemical literature focusing on various aspects of macromolecular interactions. Such heightened recognition of the significance of this subject has led some to hail the area as 'the new biochemistry'. In fact, this realm of biological research is quite 'old', with experimental and theoretical roots dating well over 50 years ago and with conceptual bases reaching back to the early 19th century. Revisionist history is a current (and controversial) literary development in the world of academia, which, when applied to biochemistry, seems to have resulted in the (re)interpretation of many of the discoveries in present-day biochemistry and cell biology. I believe that current researchers should be aware of the seminal ideas and findings of the various scientists of the past and so here, I present a brief history of work on macromolecular interactions.

Philosophical aspects

In philosophical terms, the significance of macromolecular interactions in cell biology is linked, ironically, to the notion of vitalism. Various debates concerning the role of a 'vital force' in characterizing the living state raged from the late 18th century well into the 20th century (see Ref. 1). The most extreme – and most (in)famous – vitalist position followed the late 18th-century 'design' argument that life is unique and cannot be explained as something acting physically *within* matter; life, in this view, must be accepted as a result of an imponderable 'force' operating *through* the system from *without*. This nonphysical conception led to the permanent (and negative) stigma commonly attached to the term 'vitalism' today. However, there was another belief that the expression 'vital force' simply connotes that ordinary physicochemical forces somehow act on the parts of the living being to achieve the organismal whole, following the Aristotelian concept that 'the whole is greater than the sum of its parts' (see Ref. 1). Today, 'complexity theory' has, indeed, solidified such a view of life (see Ref. 2). During the 19th century, though, this notion was advanced by the 'protoplasmic theory of life', which attempted to reduce the mechanistic definition of the living state to the cellular level and generated a variety of holistic physical models based on putative associations of the particulate matter within living cells (see Ref. 3).

In the late 19th century, the discovery of isolable enzymes, along with the finding by the Büchners that fermentation can occur in extracts of yeast cells, shifted attention away from the issue of the organization of cellular elements. There was, however, still discussion of organization versus nonorganization of enzyme action. For example, the eminent 19th-century physiologist Claude Bernard[4] differentiated '.... two kinds of fermentation, ... the one produced by .... an organized or *structured* ferment, the other produced by nonorganized ferments' [original italics].

The 'bag of enzymes' view of cells, nevertheless, held sway for the first half of the 20th century, as the reductionists pressed the philosophy that, if we know all the parts, we can conjure the whole – a conviction that is seriously flawed, if not completely wrong (see Ref. 5). Notably, many prominent biologists (Chambers, Cori, Krebs, Loeb, Peters, Wilson, to name a few) expressed the clear need to consider organizational ideas to explain differences between the observed behavior of cells and that of cell-free systems. For example, Krebs[6], in the 1930s, wrote that, 'Since all essential metabolic phenomena are bound to the cell structure, the tissue *Brei*, used so often in the past – in which the structure is destroyed – is unsuitable for metabolic investigations.' At about the same time, Rudolph Peters[7], arguing from theoretical grounds of physical chemistry, elaborated how, 'The view that is presented here differs from most others in the stress which is laid upon architecture. Its keynote is the complete (or nearly complete) structural organization of the cell. I believe this to be organized not only in respect of its grosser parts such as the nucleus, but also in regard to the actual chemical molecules of which it consists.'

Experimental beginnings

The demonstration of complete metabolic processes such as glycolysis, cholesterol synthesis and fatty acid synthesis in homogenized cell-free preparations was probably responsible for the belief that cellular integrity is essentially irrelevant to potential structural associations involved in metabolism. In the case of macromolecule synthesis, however, it was apparent early on that protein–protein, protein–nucleic acid and nucleic acid–nucleic acid interactions abound. The highly processive mechanisms observed in these pathways, catalysed by large proteinaceous complexes, implied the existence of almost perfect 'channeling' (i.e. molecular compartmentalization) of metabolic intermediates (see below).

David Green, in the late 1940s, reported the isolation of an aggregated system containing all of the enzymes of the Krebs tricarboxylic acid (TCA) cycle – which he dubbed the 'cyclophorase complex' (reviewed in Ref. 8). Green introduced the general term 'multienzyme complex' to designate 'an organized mosaic of enzymes in which each of the large number of component enzymes is uniquely located to permit efficient implementation of consecutive reaction sequences'. [More recently, I proposed the term 'metabolon' for such units of catalytic action (see Ref. 9).] It was

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found later[8] that what Green had isolated were, in fact, mitochondria and so, it was assumed that the enzymes within these organelles are unorganized. Confirmation of this view was thought to be at hand when disruption of the organelles *in vitro* yielded most of the Krebs TCA cycle enzymes free in solution. At about the same time, others, including Palade, Siekevitz, de Duve and Claude, established differential centrifugal methods for the isolation of cell organelles. Thus, cells were still viewed as 'bags of enzymes' – the essential modification of the prevailing view being the presence within cells of smaller bags of enzymes.

During the isolation and characterization of enzymes *in vitro*, several annoying experimental problems might or should have signaled that natural macromolecular interactions exist in cells, not only within organelles but also in the aqueous intracellular milieu surrounding them. These problems included findings of co-purification of proteins through several separation procedures and co-precipitation of proteins (thought to be contamination) when specific antibodies were used to isolate a specific protein. Nor was much attention paid to results that showed large differences between metabolic rates *in vitro* compared with those *in vivo*. Nevertheless, because the individual biochemical reactions that one assumes occur *in vivo* can be demonstrated *in vitro*, any idea of special properties arising from interacting systems was ignored. It is important to note, however, that *in vitro* studies of metabolic reactions often had little in common with their *in vivo* counterparts. Attempts were made to simulate bulk-phase cellular pH and ionic strength. But seldom did researchers use *in vitro* conditions that reflect cellular enzyme concentrations or total cellular macromolecule levels. Naturally, the absence of the latter conditions would militate against *in vitro* macromolecular interactions that occur *in vivo*. We now know, in fact, that TCA cycle complexes of the type envisioned by Green exist within mitochondria (Fig. 1).

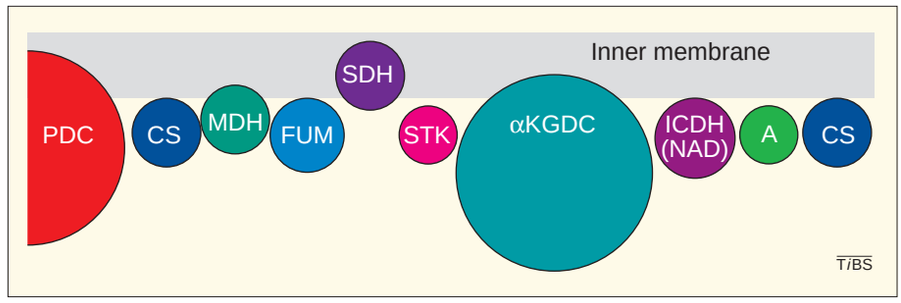

Figure 1

Schematic representation of the known binding of Krebs tricarboxylic acid (TCA) cycle enzymes to one another and to the inner mitochondrial membrane. The depth of protein 'burying' in the membrane is proportional to the presumed strength of binding. Membrane association might involve other proteins not depicted in this scheme. Abbreviations: A, aconitase; CS, citrate synthase; FUM, fumarase; ICDH (NAD), isocitrate dehydrogenase (NAD-dependent); αKGDC, α-ketoglutarate dehydrogenase complex; MDH, malate dehydrogenase; PDC, pyruvate dehydrogenase complex; SDH, succinate dehydrogenase; STK, succinate thiokinase. Figure modified, with permission, from Ref. 40.

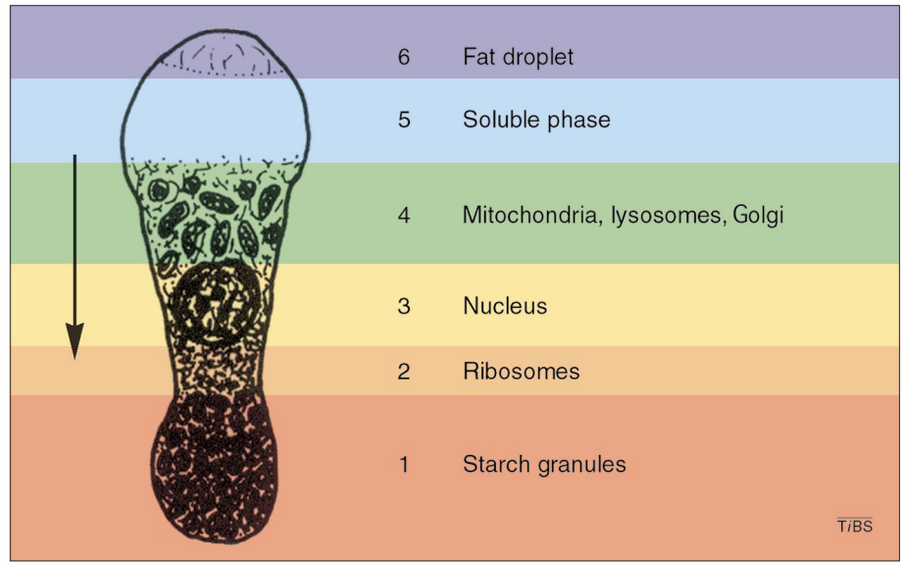

Figure 2

Euglena cell stratified by ultracentrifugation, from the work of Kempner and Miller. Figure modified, with permission, from Ref. 13.

Cell biology approaches

Early evidence indicating that the interiors of cells contain only small amounts of freely diffusing proteins came from a series of ingenious microinjection studies on marine invertebrate eggs by Chambers and by Kopac in the 1930s and 1940s (see Refs 10,11). Oil droplets introduced into healthy cells did not behave as though they were in contact with significant concentrations of diffusing globular proteins (absence of the 'Devaux effect'), but the latter could quickly be demonstrated in dead and dying cells. Further evidence was obtained in the 1960s from whole-cell centrifugation studies by Zalokar and by Kempner and Miller, who found that during centrifugation of intact *Neurospora*[12] and of intact *Euglena*[13] the cell contents become stratified (reversibly) into layers which roughly correspond to the separation of cell components by differential centrifugation of homogenates (i.e. nuclei, mitochondria, endoplasmic reticulum/microsomes and cytosol/aqueous cytoplasm) (see Fig. 2). One startling result of these experiments (wherein the cells remain viable) was that no enzymes of the central metabolic pathways, nor any proteins for that matter, were detected in the stratum identified as the cytosol. It was known, of course, that the standard *in vitro* fraction, called the 'cytosol', contains high concentrations of proteins that, according to prevailing thought, were assumed to exist freely in solution in the aqueous compartment of intact cells. The conclusion from these early studies was that most cellular proteins *in situ* are bound to (or reside within) structures that are very large, compared with individual macromolecules – a conclusion that has been ignored by many, even to the present time. Indeed, even the term 'cytosol' is ambiguous, having been coined originally to describe operationally the 100 000 × *g* supernatant from homogenates, but commonly employed since then to describe generally the aqueous phase(s) of intact cells (see Ref. 14).

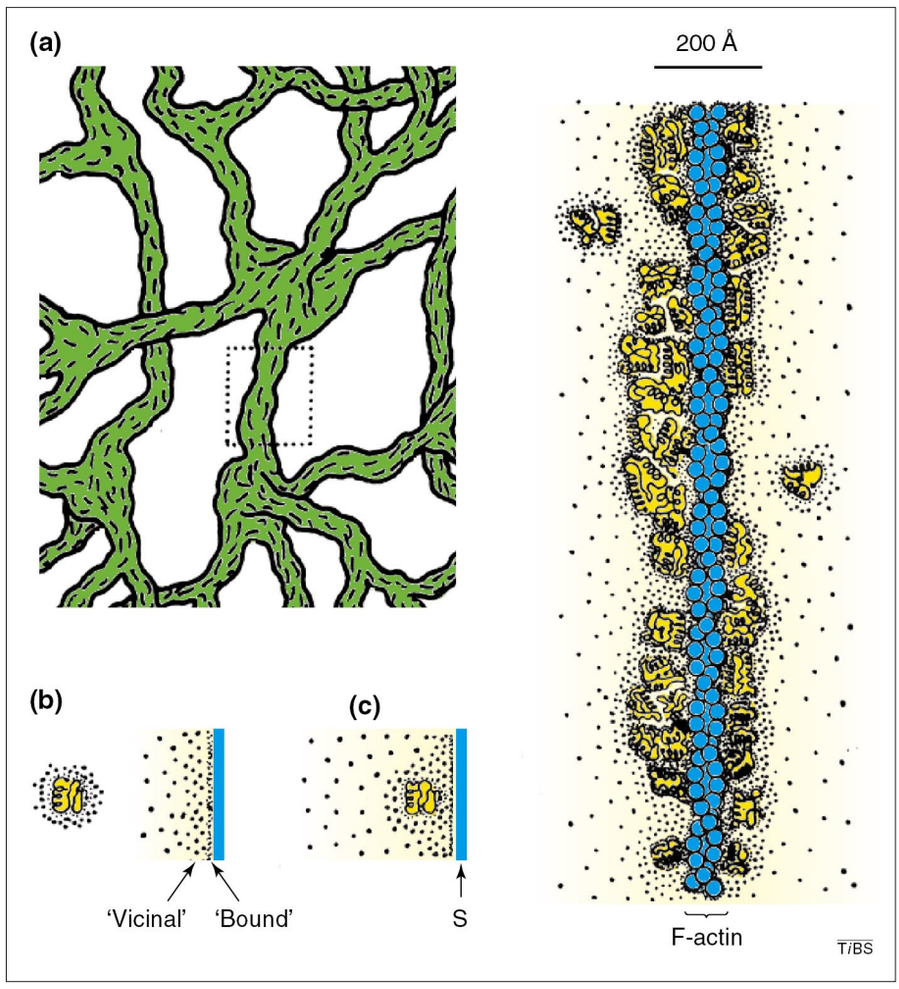

Figure 3

The microtrabecular lattice (MTL), shown in **(a)**, as suggested by electron micrographs. The inset at the right depicts a hypothetical structure of the lattice strands, in which 'soluble' globular proteins reversibly associate with a core of F-actin. In **(b)** and **(c)**, the reversible association of proteins with the lattice strand (S) is pictured under hydration conditions with 'vicinal' and 'bound' water molecules. Figure modified, with permission, from Ref. 41.

Almost 30 years ago, Keith Porter[15] and colleagues proposed, on the basis of their studies with the high-voltage electron microscope, that eukaryotic cells contain, in addition to the usual cytoskeletal elements, an intricate network of proteins, which he called the 'microtrabecular lattice' (MTL). This lattice is thought to permeate the cell with a dynamic scaffolding, which functions not only in such roles as organellar distribution but, importantly, in localizing multienzymic metabolic processes (Fig. 3). Although Porter's high-voltage electron microscope images are considered to be artifacts by some cell biologists, the presence in cells of something like the MTL is fully consistent with results obtained from a number of independent studies (see: http://gepasi.dbs.aber.ac.uk/dbk/canon.htm and Refs 16–20). For example, it has been shown that permeabilized cells do not release proteins anywhere near as rapidly as would be expected if the *in vivo* 'cytosol' were simply a concentrated protein 'solution' (see Ref. 21) and, furthermore, that these opened cells display metabolic processes thought to be conducted by 'soluble' enzymes (see Ref. 22).

In the 1940s, Straub and Szent-Györgyi showed that the viscous extracts of skeletal muscle are composed chiefly of two interacting proteins, actin and myosin (actomyosin). Today, the list of proteins known to be involved in the contractile apparatus has grown much larger. The early analyses indicated that the large actomyosin complex, when supplied with ATP, is responsible for the contraction of muscle. However, more recent studies (e.g. see Ref. 23) have shown that specific localization and binding of certain glycolytic enzymes to specific contractile proteins is a requirement for muscle function *in vivo*.

More recently, cytological studies involving the introduction of electron spin resonance (ESR) probes, as well as fluorescently labeled proteins [coupled with such techniques as fluorescence recovery after photobleaching (FRAP)], have shown in many cases that the diffusion coefficients of protein molecules are reduced substantially compared with their values in dilute aqueous solution, suggesting that they are bound in complexes, or otherwise associated with larger structures, rather than diffusing as free, individual molecules (see Refs 17,19).

Biochemical studies

From the 1970s, techniques such as counter-current distribution (using heterogeneous polymeric media like polyethylene glycol) have been used to demonstrate highly specific protein–protein interactions, involving metabolically sequential enzymes. Since then, many physical and chemical methods have been applied to the study of macromolecular interactions, including ultracentrifugation, affinity (and other forms of) chromatography, fluorescence polarization, electrophoresis, surface plasmon resonance and calorimetry. More recently, mass spectrometry and nuclear magnetic resonance have been used (for reviews, see http://gepasi.dbs.aber.ac.uk/dbk/canon.htm and Refs 24–26).

In the case of sequential enzyme reactions, protein–protein interactions have also been inferred from changes in kinetic behavior of the coupled enzyme system. Two phenomena that have been extensively studied are metabolite channeling (see:http://gepasi.dbs.aber.ac.uk/dbk/canon.htm) and the associated, experimentally measured 'transient time' parameter (see: http://gepasi.dbs.aber.ac.uk/dbk/canon.htm and Ref. 27). One of the first examples of the observed channeling phenomenon concerned the two-enzyme tryptophan synthase system. In 1958, Yanofsky[28] reported that the metabolic intermediate, indole, does not appear free in solution during the course of the overall reaction sequence. Subsequently, numerous studies, using a variety of biochemical and genetic techniques, have confirmed the channeling phenomenon in this system. Despite some 40 years of evidence on tryptophan synthase from several laboratories, many biochemists failed to accept channeling in this enzyme complex until just recently – when X-ray

crystallographic analysis[29] revealed an actual physical 'tunnel' between the two active sites that prevents indole from mixing with the bulk solution.

Similar analytical methods have been applied in a number of other systems, and considerable credence has now been given to the validity of kinetic experiments in revealing protein–protein interactions in various metabolic pathways (for reviews, see: http://gepasi.dbs.aber.ac.uk/dbk/canon.htm).

Recent genetic approaches

The supramolecular, interactive nature of enzyme action *in vivo* explains, in part, why enzymes seem much bigger than is necessary to carry out their enzymatic functions (see Ref. 30). Their surfaces must contain binding sites for localization on cytomatrix elements or for association with other enzymes, and a comparative study[31] suggests that such sites have been conserved during the course of evolution. Over 30 years ago, Munkres and Woodward[32] suggested the concept of 'genetics of locational specificity' to describe the multiplicity of genetic loci that influence enzyme superstructure *in vivo*, whereby there would be two classes of enzyme mutation: (1) 'structural', referring to amino acid residues affecting catalytic action *per se* and (2) 'integrational', denoting amino acid residues relating to the organizational state. They emphasized the importance of (re)assessing the (then) traditional kinds of complementation studies concerning the role of heterologous protein–protein interactions. Mutational pleiotropy (i.e. when a single mutation has multiple phenotypic effects) relating to enzyme organization was not readily revealed by the older analytical methodologies (e.g. nutritional auxotrophy). Stringent conditions are required if 'integrational' mutants are to be distinguished from the wild type in a competitive situation (see Refs 33,34).

Today, the rapidly emerging 'functional genomics' methodologies allow the simultaneous analysis of so many phenotypic variables at the level of the transcriptome[35], the proteome[36], and the metabolome[37]. Indeed, one might anticipate a much broader awareness of macromolecular interactions *in vivo* on the horizon than heretofore appreciated. Some ten years ago, Fields (see Ref. 38) introduced the 'two-hybrid method' for detecting protein–protein interactions *in situ*, and a number of more recent variations (e.g. see Ref. 39) have increased its usefulness. The *in vivo* aspect of this technique is unique and important. Coupled with the latest developments in 'functional genomics', such methods are likely to enrich our already-extensive understanding of macromolecular interactions *in vivo* immensely.

Conclusion

So, what is 'new' about 'the new biochemistry'? Certainly not macromolecular interactions. Rather, it is the realization that the role of macromolecular interactions is fundamental to all of the currently 'hot' research areas in biochemistry, including the cell cycle, receptor activation, signal transduction, apoptosis and molecular chaperone action. However, in the rush to be first, it is important to avoid the tendency to do so on the backs of our predecessors, rather than, as used to be the case, on their shoulders.

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* Deceased 11 July 1999 (Ref. 42). Paul Srere conceived the idea for this article from discussions at a Gordon Research Conference on 'Macromolecular Organization and Cell Function' held in 1998 in Oxford, UK. Not long before his death, he had circulated a draft to us for review. We submit it posthumously in his honor, with minor editorial changes for clarity and emphasis.

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