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ORGANIZATION AND REGULATION OF THE METABOLIC FACTORY

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Anyone who visits a large factory is likely to be astonished by the astonishing chaos of machines, products, components and workers, not to mention computers, bar codes, light signals, warehouses and recycling sites. Even with knowledge of what final products are being manufactured, and even with the help of a good guide, the apparent chaos can be disconcerting, specially if one is interested in analysing how this “chaos” can be controlled. If it were possible to enter a eukaryotic cell in the same manner, the large amount of detailed information available about cell metabolism would likewise not prevent us from feeling a similar sensation of apparent chaos. We should observe a large number of different enzymes working and generating continuous fluxes of final products and intermediate metabolites, a high degree of compartmentalization generated by the different organelles and internal membranes, a high degree of exchange through them, transport channels, metabolite storage areas, sophisticated material supports to store instructions for controlling fluxes, etc. Both visits could be just as overwhelming as they would interesting; as we shall see in this chapter, we can profit from what we have seen in the industrial visit to understand better what we have seen in the cell.

It is a constant feature in the history of science that anything that has not been sufficiently analysed tends to appear very complicated, and the study of metabolism is an excellent example of this. One hundred years after the experiments of Buchner (1897), we have an accurate knowledge of the functioning of enzymes — the genuine cellular machines: we know many of their individual mechanisms very well; yet we still have a very incomplete understanding of how the thousands of different metabolic reactions combine into a whole and how fluxes are regulated. We still not know the *fundamental rules* that govern metabolism.

In the manufacturing world, everybody realizes that the mere fact of expertise in the functioning of a single machine does not imply any knowledge or understanding of how to organize a factory with hundreds of machines so as to optimize the production. For the factory manager, it is evident that the person who organizes and controls productivity, and has the best understanding of the working of the factory as a whole, does not have to be expert in the working of each machine. In the same manner, 100 years after Buchner it is clear that the detailed characterization of the mechanisms of many individual enzymes has not allowed us to understand how metabolism is organized or how production of metabolites is optimized. Trying to achieve a deep understanding of metabolism from the study of its components now seems a labyrinth or a search without end, and perhaps it is time to try new approaches. The complexity of metabolism is so overwhelming that we should not neglect the help that could come from other areas of knowledge, bringing clues about the correct approach for understanding its organization, control and regulation. Moreover, we think that to go deeply into the no man's land between the different scientific disciplines is often fruitful for scientific progress. In this chapter we shall present what has been a surprising discovery for us, that studying metabolism in the light of current knowledge of industrial control can lead to new and interesting concepts not previously taken into account; the approach can be highly fruitful for the scientific community devoted to the study of supramolecular organization, control and regulation of metabolism.

Parallels between systemic variables in metabolism and manufacturing. Living organisms resemble industrial factories in being open systems with significant inputs, throughputs and outputs of various sorts of matter, energy and information (Miller, 1978). In both cases energy is continuously consumed to generate highly ordered and complex structures from simple components, to store information for making choices between different options, and to transmit adequate instructions to the correct places. In short, both types of organization involve the creation and maintenance of information systems.

Let us make a visit to the interior of one of these systems. We choose a factory because this is a macroscopic system that we can enter in reality, because we know its functioning very well and we can modify it, and finally because we hope that it will permit us to enlarge our knowledge of "metabolic factories". We shall start the visit at one of the more exciting places in a factory, the warehouse, often a large polyhedral building near the factory, at an airport or along a main roads close to the industrial zone. Inside the impressive windowless structure is a beehive full of shelves crowded with products that brought in or out automatically.

We may be astonished at the perfect order inside the enormous building but, if we ask the head of the company if he is proud of his wonderfully organized warehouse, he is likely to answer that he wished he did not need a warehouse at all. This is because the warehouse collects together the consequences of the limitations and deficiencies of the manufacturing organization. Hidden within it are the inefficient management, the poor forecasting, problems of quality and bad design, errors in the information system, etc., in short the incompetence and incapacity of the factory to produce exactly what is needed in the moment when it is needed. The ideal would be to have no inventory anywhere that is not actively in process, whereas the material in the warehouse is not in process. Inventory not only hides factory limitations; it also ties up capital, uses storage space, requires handling; it deteriorates, sometimes becomes obsolete, requires insurance, it incurs taxes, as well as risks of theft, accidental damage, or loss (Hall, 1983). A good analysis of the inventory of a factory will give us an accurate diagnostic about the deficiencies of its organization. Without a measure of inventory, therefore, investment management is operating in the dark. However, to start the inventory analysis by looking at each of the thousands of different components is as difficult as trying to understand the whole metabolism by analysing each enzyme in isolation. It is necessary to define a variable to evaluate inventory correctly, which should react with minimum lag to changed conditions and which can be measured and monitored.

The absolute number of items in stock is not by itself informative about the system fitness; it is better to relate inventory to sales. The appropriate variable, called the *inventory turnover,* is obtained dividing sales (throughput) by inventory (stocks) over a given period of time; it provides the number of times that the inventory has turned over or been replaced during that period:

sales/inventory = inventory turnover

The inverse of the inventory turnover give us the average time that an item remains in store. This time must be minimized if we are to have the maximum production flux with the minimum accumulation of intermediate products inside the system. There are many industrial examples to illustrate this point but we prefer to use an example from the domestic life. Let us imagine a family that consumes an average of 10 pots of yoghurt per day. If they go to the supermarket every two days, the stock of yoghurt in the refrigerator will be around 15 pots. However if they do the shopping only once per month, they will need to buy another refrigerator to store all the pots of yoghurt; this will consume a lot of energy; they will need more time to arrange them, and they will have a large amount of money tied up in the form of yoghurt that they cannot use for anything else; probably some of the yoghurt will become outdated and will finish in the rubbish. Even though the consumption has been the same and everybody has had his yoghurt, the efficiency in the second case is lower and the resources are used less efficiently, and consequently turnover is much lower.

Some metabolic pathways can be viewed as "manufacturing chains" inside a "cell factory", allowing the production of fundamental components (ATP, NADH, etc.) with the greatest yield and efficiency possible. In recent decades there have been several attempts to develop a theoretical framework to understand the design of metabolism and quantify its control [see, for example, Savageau (1976), Voit (1991), Cornish-Bowden and Cárdenas (1990) and Fell (1997)]. Among the various proposals, *metabolic control analysis* (see Fell, 1996, for a review) and *biochemical systems theory* (see Voit, 1991, for a review) have been the most widely used. Both are based on sensitivity analysis and despite their different original formulations it has been proved that they give equivalent results (Cascante *et al.,* 1989). Both metabolic control analysis (Kacser and Burns, 1973; Heinrich and Rapoport, 1974) and biochemical systems theory (Savageau, 1969) have been fully extended to allow analysis of any steady-state characteristics of complex metabolic networks. These theories have focussed mainly on the need to quantify the distribution of control of flux and metabolic concentration among the different steps of the pathway by means of *control coefficients* (metabolic control analysis) or *logarithmic gains* (biochemical systems theory); either can be defined as the fractional change in a systemic variable y that results from an infinitesimal fractional change in the amount e_i of enzyme:

$$C^{y}_{e_i} = \frac{\partial \ln y}{\partial \ln e_i}$$

Metabolic flux (J) and the metabolite concentrations (each individual concentration S_j, or their sum $\sigma = \sum S_j$) have been the system variables traditionally considered as being of interest in metabolic pathways.

If we consider a metabolic system as a black box for which σ is the steady-state mass inside it and J the net flux through it, we can define the average time that a molecule remain in stock (mean passage time) simply, as follows:

$$\tau = \sigma / J$$

Moreover, even though τ defined in this manner is a steady-state property of the metabolic pathway, it can be informative about some aspects of its temporal behaviour (Easterby, 1981; Cascante *et al.,* 1996):

1. It is related to the *lag time* of the metabolic pathway, the time necessary to fill the system from a state in which there are no intermediate metabolites to the steady state.

2. It is related to the *transition time,* the time necessary to move from one steady state to another.

So even though τ so defined is not exactly the same as the transient time between the two steady states, in general it turns out that the transient time necessary to reach a new steady state is lower for a system in an steady state with a low value of τ than for a system in an steady state with a high value of τ.

Another important aspect of τ is that it can be viewed as a relative measure of intermediate levels with regard to the flux that is more informative than σ itself, because the metabolite intermediate levels are difficult to compare directly for a given metabolic pathway in different steady states or in different tissues. This difficulty arises from the fact that flux through the pathway can be very different depending on the physiological condition in which it is operating and consequently the minimum intermediate metabolite pools necessary to maintain the system operative can also be very different. In this perspective τ can also be viewed as a performance index of the metabolic pathway, and we can conclude that it is better to express intermediate metabolite pools normalized with regard to the flux ($\tau = S_j / J$ or $\tau = \sigma / J$) in order to better describe, evaluate or compare the performance of the system.

We recently showed that a possible parallel exists between cell metabolism and factories (Cascante *et al.,* 1996). We realized, not without surprise, that despite the great complexity of both systems, with a large number of enzymes/machines, products and control systems, the number of variables of interest for describing the productivity of the system is very small, and in fact there exists a very nice equivalence between these variables in the two systems. In terms of this parallel it is easy to see that throughput is equivalent to metabolic flux through the pathway (J) and that τ defined as $\tau = \sigma/J$ (the total of metabolic intermediates inside the system divided by the flux through it), is equivalent to the inverse of the inventory turnover. So we suggest that even though the primary characteristics of a metabolic pathway can be described in terms of its steady-state flux J and intermediate metabolite concentrations σ or S_j, the system is better described in terms of J and τ (or τ_j). In fact, we can also define for a metabolic pathway a quantity as J/σ or $1/\tau$ equivalent to the *metabolic stock turnover.*

Even though in classical enzymology the turnover of an enzyme has often been used to characterize its activity, we are not aware of a previous definition or use of this concept of the turnover of a metabolic pathway. However, this quantity could be very interesting: some intermediate metabolites may be quite labile and can be degraded into unwanted products, but a high turnover can prevent chemical degradation of intermediates. It is important to note that applying the concept of turnover to an isolated machine inside the factory is not a useful way of evaluating the efficiency of the factory, because if there are numerous fast and efficient machines that are inappropriately located the result will be that the whole set of machines is badly organized; much of what is produced will need to be stored in the warehouse and the factory inventory turnover (the economically important magnitude) will be very low. It is likewise not useful to apply the concept of turnover to an isolated enzyme as a means of evaluating the efficiency of the whole metabolic system. The concept of turnover becomes very useful only when we refer to metabolic pathways, the cell as a whole, an organ, or even an entire organism.

Which metabolites should be stored in the cellular warehouse? We have seen in the above section how two fields as apparently distant as inventory control and metabolic control use the same concepts. We now take this further to examine in detail the concept of inventory turnover. What we call inventory in a factory is in fact the *cost* of the inventory, the money that is invested in the form of components, products, etc., and what we call sales is in fact the value of all final products that have been sold. When we evaluate the inventory of a factory, we are interested not only in the number of items in stock but also in the cost of each. The inventory cost is therefore calculated by considering that each different item has a given cost. Maintaining an inventory of 1000 units of an expensive item will give us a worse inventory turnover than having an inventory of 1000 units of an inexpensive article. Thus although achieving high efficiency of "industrial metabolism" requires us to maintain all stocks at low level, this is especially true for expensive items.

If we compare the inventory turnover of a factory with the metabolite turnover, defined as $1/\tau = J/\sigma$, we see that equating the total metabolic stocks with σ, i.e. expressing it as the sum of the concentrations of the different metabolites ("items"), implies that all metabolites are considered equally important in evaluating the turnover of stocks. If we compare this with how inventory turnover in a factory is evaluated, taking account of cost, it becomes evident that the concept of σ defined as the sum of metabolite concentrations is perhaps too simple: it is easy to see that storing ATP is not equally expensive as storing lactate, for example. We can conclude therefore that it will also be interesting to define the "fabrication cost" of each metabolite appropriately, and to take this into account in the study of the metabolism. In this light the concept of metabolite turnover (or its inverse, τ), could be improved by treating σ as a weighted sum of metabolite concentrations with weights based on "fabrication costs". How to define "fabrication cost" adequately is one of the current objects of our research.

The optimization of cellular productivity: analogy with industrial factories. Inspired by this parallel with a real factory, we can hypothesize that in the cell the performance functions that we can expect have been optimized during evolution must be J, τ or both simultaneously, for any pathway whose main purpose is to produce metabolites with maximum efficiency. In principle, one might expect that minimization of τ (or maximization of metabolite turnover) would be sufficient to optimize the performance of the system, because minimum τ can be achieved by minimizing σ and maximizing J. However, in a previous study (Cascante *et al.,* 1996) we demonstrated, using a linear sequence of two enzyme-catalysed reactions as an example, that the converse is not true: minimization of τ does not guarantee the maximization of flux, because minimizing τ produces a state in which metabolite concentrations are extremely low but the flux is also very low. In this metabolic state the "stock" of metabolites is below the minimum necessary for maintaining the system fully operative. As enzymes need a "minimum stock" of their substrates to operate, it is not a good idea just to minimize τ, as this allows τ to approach zero. In a metabolic pathway the stocks can never be zero unless intermediates are transferred directly from one enzyme to the next enzyme in the pathway. We also demonstrated that maximization of flux results in accumulation of metabolic stocks within the system (high τ).

To compromise between maximizing flux and minimizing τ we defined a new function, the *productivity performance index,* as $\psi = J/\tau$. We demonstrated that this function has a maximum, which means that there exists an optimal set of kinetic constants for the enzymes of the system that simultaneously results in optimal values of J and τ in the metabolic pathway. We concluded that a high value of ψ implies flexibility, quality and responsiveness in a metabolic system whose main purpose is productivity. Consequently, ψ permits a straightforward comparison between the productivity of the same pathway with the same purpose in different living organisms. Moreover, we can hypothesize that metabolic pathways whose main purpose is the production of a fundamental metabolite have evolved towards attaining an optimal value of ψ.

New insights on the supramolecular organization of metabolism by analogy with industrial factories. If after visiting a factory warehouse we enter a production plant, our attention will immediately be drawn by the machines devoted to the incessant creation of objects from materials that are often very different from the final result: thermoplastic injection machines that can produce a bumper from minute grains of material, enormous metal hammer machines that transform an aluminium band into an elegant lipstick container, robots that assemble the complicated tools used as modern toys.

Nonetheless, if we ask the director of the factory whether the most important characteristic of an efficient factory is to have the fastest machines possible, his answer will be emphatically negative. Of course, it is very important that the machines are fast, but it is at least as important that their arrangement inside the factory is correct. Taking an example from daily life, if all the cars in a traffic jam were to start together to run at the same velocity the car queue would disappear, but as in practice they are not well organized it continues endlessly. In a factory we have to avoid the creation of queues, so that all the machines work at the optimal velocity for which they have been designed without generating queues of material. This is very difficult, often more difficult than increasing the working rate of the individual machines; it is only possible by organizing the factory correctly, putting the machines in appropriate positions, making the internal transport simple and efficient, and setting up rapid and reliable systems of information.

At the present time the importance of “lay-out” of enzymes, and in general of molecules inside the cell, has not yet been taken very much into account. However, it is highly probable that the spatial arrangement of enzymes is at least as important as the individual characteristics of each one of these molecules. For this reason it is interesting to get a glimpse of how "industrial metabolism" is organized, to get clues of how cellular metabolism might be organized. Before designing a factory a factor that must be take into account, as it determines the main features of its organization, is the nature of the product (or products) that will be manufactured there. There are many characteristics on which attention could be focussed, but there are two principal ones, the quantity and the variety of the product (Hayes and Wheelwright, 1984). We shall therefore examine how factories are organized in relation to these two factors.

We start with the extreme case of a factory that produces a single type of product in a very high quantity, such as a refinery that refines crude oil into petroleum; other examples of continuous flow manufacturing processes are pipelines of water or natural gas. Because only a single product is produced, at a rate that varies little, such a facility can be very specialized and can operate at very low cost. However, a permanent maintenance service is essential, to prevent any breakdown in the line that could halt it. So the lay-out is a production line made with highly specialized technology but acting as a single machine. In such a case the production planning, the control system, is very simple and is driven by the rate of flow. If it is necessary to stop the line this must be for a long period because the set up is long and costly.

What happens when the variety increases? Let us consider a bottling plant that handles lager, light and alcohol-free beer. The quantity of each product does not justify a dedicated plant for each kind of beer, and anyway the market may change unpredictably, converting one line into a pile of obsolete machines. In this case it is better to have a line less specialized and optimized than in the former example, but much more versatile and it can handle three kinds of beer.

The main difference between the two lines is that managing the beer line requires decisions about which beer to produce and in what quantities. This is a very critical decision that greatly increases the complexity of the control. Even if we optimize the sequence of kinds of beer to minimize the number of changeovers without ever running out of stock, it will be inevitable to generate stocks because there will be simultaneous demand for the three kinds even though only one is being produced at any moment. This type of process is called *batch flow,* and implies a high degree of control, and above all a system for calculating the quantity per batch and the sequence of batches. If we increase the variety and we expect to fill 20 kinds of different liquids in the same filling plant we will find that the line is not sufficiently versatile for so many changeovers at low cost. Continuing to work with batch flow with 20 products will result in such a huge stock level that the survival of the company will be jeopardized.

Consider now an example familiar from any economic discussion, the car industry. The market, i.e. the ordinary customers who buy cars, demand many options from manufacturers in the form of different colours, upholstery, engines, accessories, etc. A manufacturer who adopts the batch flow system described earlier will find it nearly impossible to predict the quantity needed of every option, yet the goal is to produce just what the customer wants. Among the important factors needed to be taken into account for achieving this the equipment must be general purpose, and parts should not be forwarded to the next operation until they are requested. A consequence of this is that production lines can no longer be linear, but are organized in a U-shape to facilitate communication and permit workers to circulate inside the U, transferring components one at a time from machine to machine. This allows different combinations of options for a single product to emerge from the same production line in U-form according to the combination of processes used and the starting materials chosen. These production units will be composed of machines of different technologies, and the better communication achieved with this type of organization allows one to avoid forwarding parts to the next operation until they are requested.

With this system customers order what they require from the factory; we can say that they will “pull” cars from the factory instead of the factory “pushing” cars to the warehouse. The control systems will be based on signals that go from the customer to the production lines and from the lines to the suppliers of materials, with the aim that only the production flux needs to be taken into account, as the variety will not be an obstacle as in the case of the bottling line.

In many cases, systems with simple labels are used that are transferred from production cell to production cell. One of the critical points of this system is that the material suppliers must be very reliable as a break in the line could be very damaging. A very important way of maintaining this organization successfully is to try to manufacture the maximum variety of products using the minimum number of common components. But when the variety of products is very large (hundreds or thousands), we cannot have a production line for each article or model.

Let us imagine a toy factory. The variety of products is huge but the relative quantity of each model is quite small and the technologies used are also few: thermoplastic injection, assembly machines, hot stamping, offset, etc., but probably no more than 10 different technologies to manufacture all the variety of final products. In this cases, the lay-out needs to support a manufacturing environment in which there can be a great diversity of flow among products (Fogarty *et al.,* 1991). It is called the *job shop* process, and is characterized by the organization of similar equipment by function (milling, drilling, assembly, etc.). As jobs flow from work centre to work centre, a different type of operation is performed. Orders may follow similar or different paths through the plant, suggesting one or several dominant flows. Thus very different products are produced simultaneously. The equipment is multipurpose: a very clear case is thermoplastic injection, where the same machine can manufacture a shoe sole or a car door according to the mould that is installed. The control is very complex because the loads of the work centres differ greatly and the critical capacity of each work centre tends to generate queues that have to be minimized. This complexity has the result that the amount of work-in-process material tends to be high relative to that in a flow process, due to the queues and the long in-process time (Fogarty *et al.,* 1991).

Finally, consider the other extreme case, a shipyard. As it is then not possible to move the “in-process product” from one work centre to another, the tools and workers must be displaced wherever they are needed, and these tools are very simple compared with the final product that will be manufactured: this is exactly the opposite of the petroleum refinery case we examined earlier. Now it is not necessary to have production plans or scheduling of work centres, but it is necessary to have a very well defined “project” of the final product before starting, which must be perfectly detailed about what will be done, how, when and where. Once the manufacturing work starts, what we need to control is that the work plan thus defined in the project is followed without any deviation. The characteristic of this type of factory is that it requires very specialized workers and that the price of the final product is very high. (This again contrasts with the refinery case, in which the cost of the product to be manufactured is relatively low).

It is obvious that in general we will not find factories that are pure examples of each case; probably all will have some characteristics of one or another of the different cases described. It is even highly probable that inside the same factory two or more of the cases described may coexist independently.

We now examine whether this very simplified overview of the organization of factories can help us in any way to elucidate the laws that govern the supramolecular organization of metabolism. Supramolecular organization of metabolism can involve interactions of the enzymes (machines) with organelles, membranes, the cytoskeletal matrix, nucleic acids, or other enzymes. In the last decades, strong experimental evidence of a high degree of organization of metabolism has been reported (Ovádi, 1995), but the theoretical framework to permit us to fully understand its logic and its role in the regulation of metabolism does not yet exist. If we take account of what we have summarized in the first section of this chapter about the importance of maximizing inventory turnover and also what we have just seen on the importance of the quantity and variety of the final products, we can guess that the laws that govern the optimal supramolecular organization of metabolism must consider at least these three factors as crucial.

In this light, considering the cell as a "metabolic factory", we can discuss what one should expect to be the supramolecular organization of metabolic pathways by analogy with the rules for organizing factories. Considering the different organs and tissues as big metabolic factories, we can also hypothesize that the supramolecular organization will depend, among other factors, on the quantity and variety of products that need to be produced in each type of cell or tissue. Even for a single metabolic pathway, the degree of organization will depend on the physiological role of the pathway in each tissue. Let we start for two "metabolic factories" that we know very well and which differ in the quantity and variety of final products produced: the liver and skeletal muscle.

In liver there are more than 500 different metabolic functions, including various biosynthetic processes that need some common intermediates; that is to say it is an organ in which a large variety of different and very important final products are manufactured in relatively small quantities, and in which intermediate metabolites are used for a variety of purposes. Thus, by analogy with the organization factories as a function of quantity and variety of final products we should expect to find in liver some of the characteristics typical of a job-shop organization of metabolism.

In contrast, skeletal muscle is a much more specialized tissue with only a few different products to be manufactured. What is not predetermined, however, is how much of the final product will be needed, and at what rhythm; for this reason muscle must be prepared to work at different rates, and it needs to have reliable "suppliers". We can predict that muscle will work with very low stocks and that its production is regulated by the needs of its “customers”, i.e. it will work “just in time”; like a car factory, muscle will tend to manufacture just what is ordered by its “customers”. In contrast, for liver to work with the type of organization found in a car factory would be much more difficult. As we have suggested, we predict that it must approach more to an organization closer to the job-shop process in a factory, with a consequent generation of more stocks than in muscle. Liver has a “high metabolic responsibility”, and is usually regarded as the “big factory” of the body. It does not have to respond as quickly as muscle, but it must never be out of stock if it is to ensure production at all times of all the important final products that it needs to supply to the rest of the body. Forecasting the demand of the final products is quite uncertain, as also is the supply of materials. As a result, the liver needs a much more complex regulation system to optimize what must be manufactured, and in which quantity, to avoid “metabolite queues”. As liver works with uncertainty about the availability of suppliers, and it needs always to be ready to manufacture a high variety of final products, it needs a “safety stock” and cannot work at “zero stock”. Thus it is necessary not only to have a warehouse but also to ensure that what is stored in it has the lowest possible cost; therefore evolution has probably tended to optimize the inventory turnover of these stocks, resulting in the storage of low-cost components and construction of different final products from common components as much as possible.

The prediction from the parallel with real factories that liver and skeletal muscle will have different types of organization leads to a further prediction that the organization of the same pathway may also be different in the two tissues. Let us compare glycolysis in liver and muscle, for example. Even though the pathway is the same in both tissues, the “environment” in which it operates is not the same. In muscle (pull), the absence of a requirement to manufacture so many final products as liver means that the intermediate metabolites are not simultaneously necessary for other purposes, so it will be not so necessary to maintain stocks of them when they are not actively in process. In liver (push), on the other hand, the several different final destinations of the metabolites, in very variable quantities makes it much more efficient to have them always available even when they are not actively in process, despite the fact that that this will generate stocks.

An other important aspect is that metabolic control in job-shop systems will be much more complex that in just-in-time systems, as the loads of the work centres, i.e. metabolic pathways, vary greatly and the critical capacity of each pathway tends to generate queues of metabolites or, at the other extreme, to generate stockout. The existence of a great number of metabolic machines that are needed to manufacture different final products simultaneously using some common metabolites implies that is not possible to organize the "liver factory" with an independent controlled manufacturing line to make each final product; instead it is necessary to interrelate all the manufacturing lines. The control system needs not only to regulate the rate flow at which a single final product is produced, but also to regulate the quantity and rate flow of all the different products that must be produced. In muscle we can expect a much less complex system, structured more as a "dedicated line".

It is interesting to point out that in muscle glycolysis the existence of dynamic enzyme–enzyme interactions (corresponding to juxtaposition of machines) has been described that may allow *channelling* of a metabolite in the reaction sequence, i.e. the transfer of the reaction product of one enzyme to the next without equilibrating with the bulk solution. Channelling is quite prevalent in metabolism, to an extent that varies with the physiological state [see Ovádi (1995), and Agius and Sherratt (1997) for reviews]. However, despite the accumulating evidence about its existence, its significance and relevance to metabolic regulation remains controversial. As enzyme–enzyme interactions are weak in many cases, the resulting complexes are "dynamic", i.e. short-lived, and characterizing them is complicated as they may be lost when the cell is disrupted. Srere and Ovádi (1990) have pointed out that organized systems are easier to demonstrate (i.e., there exist stronger interactions between enzymes) in highly processive pathways. These include pathways such as macromolecular biosynthesis, fatty acid synthesis, and oxidation and nucleotide metabolism.

On the other hand, weakly interacting systems occur in amphibolic pathways such as glycolysis that have several flow bifurcations along them, the intermediates being used for other pathways inside the cell. It has been in these pathways traditionally regarded as cytosolic, i.e. constituted of enzymes in free solution, that investigators have encountered most difficulties for demonstrating metabolite channelling unequivocally, and they have stimulated most of the criticisms and discussions of the physiological relevance of organizational aspects of metabolism (Srere and Ovádi, 1990; Ovádi, 1995).

In the light of the parallels with manufacturing factories, we can also hypothesize that the degree of channelling will depend on the quantity and variety of products that need to be produced in each type of cell or tissue. Moreover, even for a single metabolic pathway, the degree of channelling will depend on the physiological role of the pathway on each tissue. Thus, we can expect that a higher degree of channelling will exist in just-in-time or pull pathways, i.e. in muscle glycolysis, than in job-shop or mainly push pathways, i.e. in liver glycolysis. Thus in general we can predict that the existence of some degree of channelling in a pathway need not be an intrinsic characteristic of the pathway but that it will depend on the characteristics (including quantity and variety of final products) of the metabolic factory in which it is operating. However, much work remains to be done to fully explore the parallels between organization of factories and cells in order to try to achieve a better understanding of the supramolecular organization of metabolism from this new prespective.

In this chapter we have presented only some ideas emerging from the current knowledge of a discipline apparently as different as industrial manufacturing: the importance of the turnover of stocks in metabolic pathways, the need for an adequate unit for quantifying the cost of stocks, the need to maximize flux and minimize stocks simultaneously, and the possible influence of the quantitity and variety of the final product on the supramolecular organization of metabolism. We now need to explore these ideas more deeply and to confirm them experimentally. We are confident that this interdisciplinary field can provide new insights for a better comprehension of cell metabolism.

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