C. R. Biologies 338 (2015) 372–379

Contents lists available at ScienceDirect

Comptes Rendus Biologies

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Jacques Monod – A theorist in the era of molecular biology / Un théoricien à l'ère de la biologie moléculaire

A faith in the coherence of the living world

La foi dans la cohérence du monde vivant

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ARTICLE INFO

Article history:
Available online 11 April 2015

Keywords:
Transcriptional control
Allosteric control
Jacques Monod

ABSTRACT

In this review, I compare the development of Monod's intellectual leadership in two fields, the regulation of enzyme biosynthesis and the control of enzymatic activity. I characterize the comings and goings between his scrupulous analysis of a given model system, his ability to compare the outcome with very distant experimental results, his audacity in formulating, then a physical interpretation of this convergence through a unifying mechanism. Finally, I briefly discuss how his attitude has durably impacted the whole field of molecular biology.

Mots clés :
Contrôle transcriptionnel
Contrôle allostérique
Jacques Monod

RÉSUMÉ

Dans ce texte, je compare comment s'est établie la prééminence intellectuelle de Jacques Monod dans deux champs de recherches, la régulation de la biosynthèse des protéines et le contrôle de l'activité enzymatique. Je caractérise les allers et retours qu'il a pratiqués entre une analyse scrupuleuse de quelques systèmes particulièrement bien choisis, sa capacité à les mettre en relation avec des résultats expérimentaux de natures tout à fait différentes, son audace pour formuler alors une interprétation physique des convergences observées, à travers des mécanismes unificateurs. In fine, je discute comment cette attitude a durablement affecté le champ entier de la biologie moléculaire.

1. Introduction

In one of the most quoted passages of *The statue within*, François Jacob explained that he learned from Francis Crick "not to quail about the boldness of a hypothesis; the process of experimental science does not consist in explaining the unknown by the known, as in certain mathematical proofs. It aims on the contrary to give an account of what is observed by the properties of what is imagined. To explain the visible by the invisible". Few pages below, he characterizes Monod's style as "a mixture of logic and passion, of tenacity along a single track, and probing thrusts in every direction. [Monod was] haunted by the need to look for the truth of nature and to make it known... More than confidence, he had faith in this nature, in its coherence, its unity" [1].

The purpose of this essay is to characterize the boldness with which Jacques Monod proceeded in two of the most significant discoveries he made with his close collaborators, the regulation of enzyme biosynthesis, the indirect control of enzymatic activity by effectors. These episodes

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http://dx.doi.org/10.1016/j.crvi.2015.03.007
1631-0691/

are well known and have been extensively commented (see, for example, [2,3]). I am revisiting here not only his famous final papers, but also the documents we have kept on their elaboration inquiring how tightly are linked in his case the audacity of his style of investigation and his faith in the coherence of the living processes.

2. From enzyme induction to the repressor hypothesis

Microorganisms adapt their metabolism according to the carbon source to which they are exposed. The phenomenon of enzyme adaptation – how the enzymatic activity enabling nutrient assimilation is established – was studied by Monod on the specific case of lactose utilization. True induction operated immediately, triggered by components bearing some chemical similarity with the substrate to be degraded. In the so-called constitutive strains, a specific mutation could restore the ability of the bacteria to produce the enzymatic system, even in the absence of an added inducer. For Monod, the challenge at stake was to understand "the respective roles of the inducing substrate and of the specific gene (or genes) in the formation and the structure of the enzyme" [4].

Monod devoted all his energy to this simple but impressive challenge. He assembled around him a team of competent people, bringing to the Institut Pasteur all the skills they could provide in a generous and friendly atmosphere. Though the experimental work of the group was not exclusively devoted to the adaptation of lactose utilization, one can follow his methodology by focusing on this sole example. On the biochemical side, he showed that expression of β-galactosidase enzymatic activity – the main protein responsible for lactose utilization – reflected the completion of its synthesis from its amino acid components. Chemistry was then solicited: synthesis of dozens of analogs of the enzymatic substrate was performed, and each of them was assayed as a potential substrate, inhibitor or inducer, leading to the first great surprise: there was no correlation whatsoever between the catalytic efficiency displayed by a given compound and its regulatory power, as if the two functions were under the command of two different templates in the cell. These two experiments were inconsistent with a common view prevailing at the time, where the role of the inducer acting as a substrate analog was supposed to convert an inactive precursor of β-galactosidase into an active enzyme.

On the genetic side, the fabulous expertise developed in Lwoff's unit was systematically adapted to the study of the lactose system. The lambda bacteriophage had already been used as a gene carrier to generate transient diploids for mapping purposes. Transposition of this methodology to the *lac* system by Jacob and Monod's group showed that the *z* and *y* genes responsible for lactose utilization were distinct from *i*, the one conferring sensitivity to the inducer. The synergy between genetic and biochemical studies culminated in the Pajamo experiment: bacteriophages carrying the various combinations of *i* and *z* genes were injected into recipient bacteria possessing the complementary set of alleles. The analysis of the ensuing expression of β-galactosidase activity showed that it was the i^+ allele that was functional. It maintained the system in the "off state", exerting a dominant effect on its impaired i^- allele. Furthermore, in this experiment, switching off the system could be either immediate or could take time, depending on the precise disposition of the two sets of alleles, present on the chromosome of the recipient cell and on the injected phage. When the phage injected the i^- and z^- alleles into a recipient i^+z^+ bacterium, no β-galactosidase activity was observed, the recipient i^+ gene exerting permanently its negative effect on enzymatic expression. When the combination of donor and recipient genes was inverted in the assay, the injected phage triggered β-galactosidase enzymatic activity at a maximal rate in a first phase. The negative regulation exerted this time by the injected i^+ gene took place along a second, slow phase. This slow process was completely abolished if an inducer had been previously added to the culture medium. The only difference between the two assays was the previous history of the recipient bacteria. Their cytoplasm had to contain an active principle in the first case, while the synthesis of this agent took time in the second experiment. In summary, the i^+ gene had to exert a repressive action, arising from a cytoplasmic product. This action was relieved when an inducer molecule was present in the culture medium.

This dry account misses an important point; these experiments did not develop in isolation. From the very beginning, a parallel was systematically drawn by Monod between the control of the induction process in the *lac* system and that exerted on amino acid biosyntheses. Experiments and comparisons were simultaneously performed on these different systems. The final explanation reached on the *lac* system through the Pajamo experiment applied as well to these other cases if the control gene was still supposed to encode a repressor, but if the role of the regulating metabolite was inverted, it had no longer to act as an anti-repressor, but as a co-repressor, required for turning off the biosynthesis of the specific enzymes involved. Indeed, recalling his attitude during this exploratory phase, Monod claimed: "faith (was) established a long time before I would be able to achieve certainty" [4]. He progressively established a common experimental strategy to reach this goal in all these different cases. As pointed out by Jon Beckwith, "the approaches (followed in the *lac* case) presented a model not only for a mechanism for gene regulation but also for *how* to study gene regulation" ([5], see also [6]).

Furthermore, it also appeared in the same period that the repressor model not only accounted for the regulation of protein syntheses in repressible and in inducible systems, but also for the biosynthesis of specific phage proteins early expressed after the induction of lysogenic bacteria. As it became clear to Jacob a few months afterwards, the Pajamo experiment paralleled an earlier observation made by Wollman and himself in 1956 as they were performing reciprocal crosses between non-lysogenic bacteria and bacteria carrying a prophage. The outcome strictly depended on whether the prophage was carried by the donor or by the recipient bacteria, as in the initial phase of the Pajamo experiment. The prophage was at once induced if and only if it was the male cell that carried the prophage. Why not then to assume, that again the presence

of several specific types of repressor molecules were required for the maintenance of the lysogenic state in the recipient cells? Their rate of synthesis was again probably too slow to prevent lysis when their structural genes were injected during mating. When Jacob came to this supposition, the initial resistances that Monod opposed to this hypothesis are marvelously reported in *The statue within* [1]. But, once "Jacques" had accepted Jacob's point of view, he was the one who pushed it to its extreme consequences: does the correlation extend down to the molecular level? If so, Monod reasoned that the control exerted by the postulated repressor gene(s) would involve in both cases not only structural genes coding for their syntheses, but also operator sequences able to bind the signalling molecules and to turn off the structural genes abutted to them. Fortunately, Jacob and Adelberg had improved the mating technologies so that the relevant tests of dominance could be performed in the *lac* case, using mutant candidates, O^c, that had been previously isolated in the laboratory. The mapping of this specific operator sequence could be performed. And indeed the prediction made on its mode of action – a local receptor of the signal brought by the repressor – was found to hold!

This brief account focuses on the initial stage of the discovery process. As reminded by Peter Medawar, in real life, discovery and justification are almost always different processes [7]. The famous paper, "Genetic regulatory mechanisms in the synthesis of proteins", is the first overall account of the whole enterprise as well as its initial justification [8]. It was published two years after the completion of the Pajamo experiment. There, *all* the experimental facts arising from the systems under consideration appear required to justify the introduction of the new concepts. They definitively invalidate the previous conception of a bacterial genome as a monotonous string of structural genes. The ensuing proposed mechanism necessarily involves the intervention of a cytoplasmic product in gene regulation, while the kinetics of expression of the structural genes implies the existence of an unstable intermediate in protein synthesis, the messenger RNA. The final section of this paper clearly distinguishes experimentally established conclusions from speculations. However, the justification given in the final paper hardens the reasoning followed during the investigation process on a crucial point: by mere chance, all the analyzed cases of regulation could be accounted for by an inhibition of the transcription process, a coincidence that was unhappily accepted as a rule for too long [5].

We admire the economy displayed in the design of a chain of experiments that led to inescapable conclusions. We seldom notice that the underlying strategy did not rest on pure logical considerations, but also on an intense search for coherence, the hope that a simple output will ultimately prevail (this point is incisively developed in [9]). During this exploration, Monod's audacity consisted in expecting that the similarity will extend down to the mechanistic level and in searching for it. As in the quest for Cipango, the lost continent, by the Spanish adventurers in the sixteenth century, efficient investigation does not simply require an impeccable logic, but human virtues that Monod did not lack.

3. Allostery, from the concept to the model

The comings and goings between the analysis of specific systems and a search for generality can be better appreciated in this case by considering three major papers, the initial report given at the Cold Spring Harbor symposium (CSHS) of 1961, the review written in cooperation with François Jacob and Jean-Pierre Changeux, published in 1963, and the model proposed for allosteric transitions or, rather, the succession of manuscripts written by Monod in the 1963–1964 academic year during its elaboration [10–12].[1]

Bernard D. Davis opened the CSHS symposium on cellular regulatory mechanisms by a penetrating review on the regulations controlling cell physiology. Monod and Jacob's concluding remarks took another viewpoint, an analysis of the underlying mechanisms, including obviously their recent findings on the control of gene expression. In particular, the analysis of the process of feedback inhibition led to the proposal of a unifying mechanism, the control of enzymatic activity at a distance *via* an interaction between stereo specific sites.

This theme was introduced in 1959 in Monod's laboratory, when Jean-Pierre Changeux chose to work on the regulation of *E. coli* biosynthetic threonine deaminase. At a diverging branch of a metabolic pathway, the first enzyme that initiates the new path appeared to be specifically inhibited by the end-product of the reaction chain, though its stereochemical structure was strikingly different from that of the entering substrate. Concurrent, but scattered experimental arguments indicated that the underlying mechanism could not be a competition between the substrate(s) and the effector at the catalytic site involved. For this reason, this type of inhibition was called allosteric. Monod insisted that the allosteric concept, a principle of indirect action, would have a broader impact in biology than the mere control of metabolic pathways. His reasoning went like this: in these specific cases, evolution had favored the emergence on a common template of two mutually interacting stereo specific sites. According to that same principle, completely different sites could be connected on the same module, allowing the mutual control of their specific functions. Any signal, transduced *via* a stereo specific molecule, could now affect the performances of any unrelated machinery. In the last section of their conclusions, Monod and Jacob presented five molecular "circuits" where negative controlling elements operated in cooperation at the two general levels of enzyme biosynthesis and of feedback inhibition. Their proper functioning was "able to account in principle for any type of differentiation". Conversely, their dysfunction would be a major cause of malignancy. Thus, a major source of diversity in the living world could rest on the same principle, an assembly process, occurring randomly, selected according to its performances and used over and over again during evolution [10].

From the very beginning, and in contrast with what happened in the field of the control of protein

[1] Monod's characteristic handwriting is shown in Fig. 1.

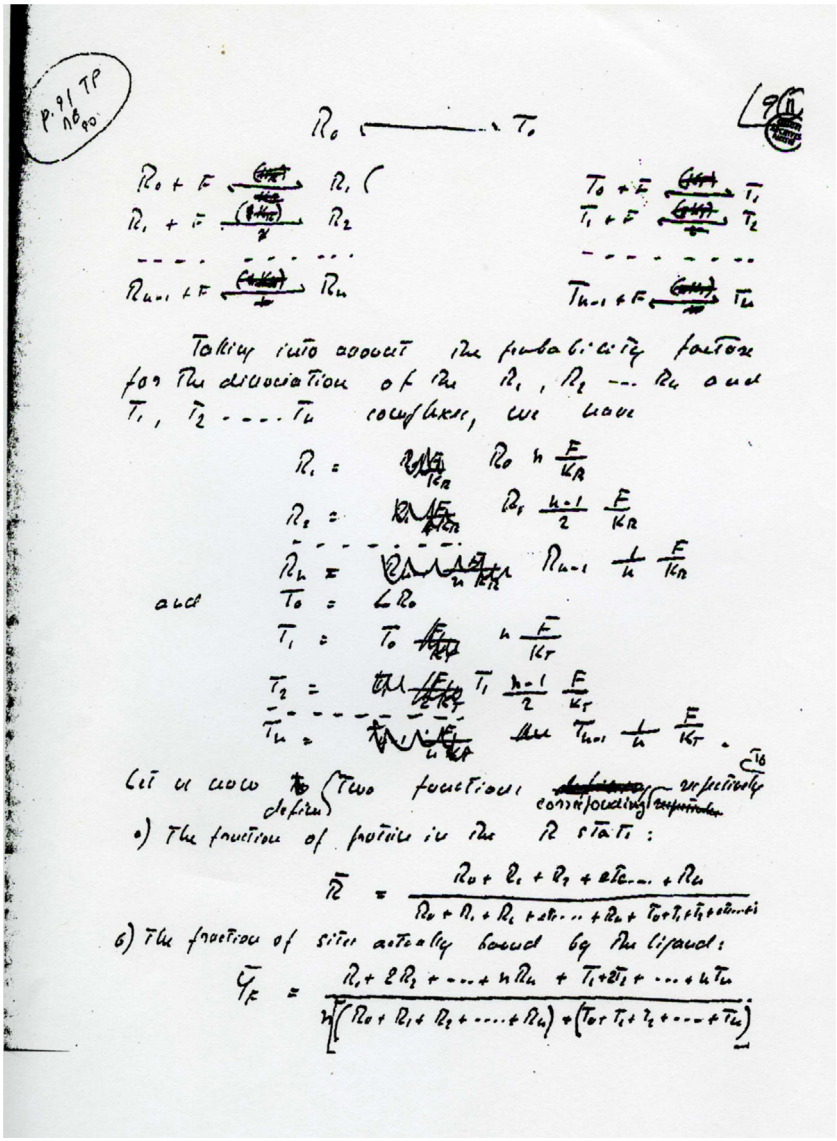

Fig. 1. The establishment of the MWC model. A fragment of a manuscript referred to as 6B, written by Jacques Monod. Fonds Jacques Monod, Archives de l'Institut Pasteur.

biosynthesis, allostery was viewed in a very global prospect, a product of evolution acting in all the kingdoms of life. Yet, the progression and the critical appraisal of the experimental work developed in a manner quite similar in the two cases. A restricted number of bacterial systems emerged from the work of the small community of the molecular biologists interested; genetics and enzymology were generally used in concert; two systems played again a dominant role, as it had been the case for lambda and *lac*. A similar set of experiments were performed almost simultaneously on the regulation of threonine deaminase by Jean-Pierre Changeux at the Institut Pasteur, and on aspartyl transcarbamylase by John Gerhart in Arthur Pardee's laboratory [13–15]. In both cases, mild treatments of the regulated enzyme led to the loss of its sensitivity to the inhibitor (desensitization). Simultaneously, it was losing a cooperative kinetic response to increasing concentrations of its substrate(s) (a phenomenon called normalization of kinetics by Jean-Pierre Changeux). In his communication at the CSHS of 1961, Changeux advanced a model where the binding sites for the substrate and for the inhibitor were distinct, each one exerting a negative effect on the binding of the other in the native enzyme [16]. In both systems again, specific activators were discovered after the meeting; they behaved phenotypically as mild desensitizers. The convergence of the methods led to similar inferences: a constellation of stereo specific sites was thought to interact *via* a mechanism similar to the one invoked by Koshland in his induced-fit theory to explain the

interactions of two substrates at their catalytic site. The flexible protein structure responded to the binding of the various effector molecules by increasing or decreasing the fit of the substrate within its active site.

In September 1962, when the French group decided to write a review on the possible generality of the concept of allosteric effects, it faced a difficult challenge. The original concept had emerged from the experimental evidence collected on five bacterial enzymes subjected to feedback inhibition. It assumed a conformational change, an allosteric transition for which there was still no experimental evidence. On the other hand, for mammalian enzymes, conformational changes associated with the presence or absence of physiological effectors had been reported, but the precise physiological role of these interactions was far from being clear. Despite this extreme diversity, was it possible to formulate any kind of generalization? At this time, it was solely in the comparison of chemical processes occurring at the catalytic sites of homologous enzymes that unifying principles were emerging. To draw a parallel between bacterial and mammalian proteins subject to grossly similar regulations, but devoid of any catalytic similarity, was a total novelty.

The challenge was not really fulfilled. The arguments based on the comparison of the five bacterial enzymes were mere extensions of previous reports. The kinetic analysis of the various types of inhibition profiles observed experimentally was rather crude; it was good enough, however, to conclude that "distal interactions between stereo specific sites, and certainly not a direct interference between them, account for the reported effects" [11]. By contrast, the section on eukaryotic regulatory proteins was constantly revised, a point that is particularly clear if one compares the two versions of the paper kept in the archives with previous papers written by Jacob and Monod on the subject [17]. Many of the selected enzymes turned out to be subject to covalent modifications, a factor that was not properly controlled in these early times. It was not clear whether this modulation of enzyme activity in response to a physiological signal reflected an alteration of an association–dissociation process or an isomerization of the protein assembly without a change in its molecular mass. Looking in retrospect, two proteins only, among the dozen that were examined, provided precise insights, rabbit muscle glycogen phosphorylase, and hemoglobin.

The impact of the mechanistic studies on hemoglobin on the evolution of this field of research cannot be underestimated. The protein had been intensively and quantitatively studied by physicochemists since the beginning of the century. Mechanistic conclusions relied on equilibrium data, not on kinetic profiles established at the steady state, the latter being more difficult to interpret. The equilibrium association curves for oxygen molecules displayed cooperativity, though their receptors, the hemes, are wide apart. These binding isotherms were markedly affected by other ligands, notably protons. During the fall of 1962, Max Perutz interacted with Changeux and Monod and communicated to Monod the first evidence of a true conformational change of the protein upon oxygen binding: based upon crystallographic evidence, the distance between two labeled cysteinyl residues located at equivalent positions in the β chains of the same tetramer decreased by 20% upon oxygenation ([18], see also [19]). For the first time, a regulatory effect, the cooperative binding of oxygen at four distant sites of a protein, was very likely linked to an internal change in the assembly of the corresponding subunits. From then on, it was accepted that "the hemoglobin system provided the most valuable model from which to start in the further analysis and interpretation of allosteric effects in general" [11].

In a separate section of the review, inserted very late after the general conclusions, it was suggested that the repressor was a protein. It operated as a switch commuting between two mutually exclusive modes, the recognition of the inducer in solution or the binding of the free protein on a specific DNA sequence, the operator locus, where it prevented transcription. Eighteen months after the Cold Spring Harbor Symposium, allosteric proteins were still primarily considered as "potential molecular receivers and transducers of chemical signals... allowing selection to interconnect the immensely complex circuitry of living organisms irrespective of their catalytic function" [11]. Attention had however shifted to the determination of the detailed mechanisms by which these performances could be accomplished. As the discussion on the nature of the repressor shows, it was inconceivable for the authors to imagine that entities other than proteins could assume such a function.

The publication of this text and its reception illustrate the cleaving effects generated by any revolutionary proposal. In this case, the scientific community interested was much larger than the initial circle of molecular biologists, familiar with their specific modes of reasoning, notably deductions based upon the agreement between biochemical and genetic experiments, and accustomed to the elaboration of global concepts, the messenger, the repressor, or allosteric effects. The most reluctant intellectual community was the one constituted by experimental biophysicists[2]. A whole school of physical biochemists, led by John Edsall, had adapted the experimental tools used in the theory of solutions to biochemical objects. In the biophysical tradition of the 1950s, experimental results were expressed through rigorous thermodynamic formulations [21]. In particular, for proteins subjected to physiological controls, conformational changes were frequently analyzed (an extensive review, written on the subject in 1963, reports more than forty examples, to be compared with the very few retained in the paper by Monod, Changeux and Jacob [22]). But from the angle adopted by this community at the time, diversity appeared to be the rule. A clarifying overview

[2] Ironically, when physicochemistry emerged as a scientific discipline, decades earlier, a similar burst of *ad hoc* rational entities took place. François Gros reminded me that the quotation mentioned in the introduction, justifying the need to "account for what is observed by the properties of what is imagined". "To explain the visible by the invisible" was borrowed, word for word, by François Jacob from Jean Perrin, the founder of the atomistic theory [1,20]. It applies as well to the birth of molecular biology as to the creation of the atomistic theory.

had probably to wait until the obtainment of more precise structural data [3]. Thus, in many circles, the reality appeared much more complex, the formulation of the allosteric concept was too vague, the synthesis too hasty [23].

Meanwhile, in the camp of the molecular biologists, work progressed at an accelerating pace. Within one year, glycogen phosphorylase b, threonine deaminase, aspartyl transcarbamylase were shown to respond to their physiological effectors by an isomerization of their oligomeric structure. And some major biophysicists endorsed the new prospect. As early as January 1963, in a letter to Monod [24], Jeffries Wyman explained how the thermodynamic formalism he had developed for hemoglobin would be useful to account for the reciprocal effects observed in the case of threonine deaminase. In a late but moving testimony, Perutz mentioned how the French publication enlarged his personal prospect [25]. Clearly, the dilemmas posed by distal regulations in enzymatic systems and in hemoglobin were sharing some interesting similarities.

At the Institut Pasteur, one was now looking for a model, a unifying principle that might overcome the critics of the biophysicists [4]. Indeed, for Monod, "a model is not a symbolic representation of experimental results, but an attempt to physically interpret the properties of a given system" [24]. What type of model was looked for? A mechanism that generated marked positive cooperativity in response to the binding of a given ligand (homotropic effects) and where strong modulating effects were exerted on this phenomenon upon addition of allosteric effectors (heterotropic effects). In the spring of 1963, Monod and Changeux had long discussions on this topic on the blackboard, particularly before Changeux exposed his findings on threonine deaminase in the incoming CSHS [27].

It was initially envisaged that each one of these effectors was generating a new conformation of the regulatory protein. But, after inspection of the new data on threonine deaminase, it appeared sufficient to take two conformations (or "states") into consideration, one binding preferentially the inhibitor, the other binding more tightly both the substrate and the activator [13,28]. This simplification had a deep conceptual impact: instead of conceiving that any ligand was *informing* the protein structure – as in the induced-fit concept – the various effectors were simply viewed as *selecting* conformations pre-existing to any binding process. The group at Pasteur was shifting to another intellectual tradition represented by Wyman: how the various equilibria occurring between a polymer and various ligands were linked together through the laws of mass action.

Changeux started to write his thesis, a work that involved a considerable reformulation of his results and interpretations. As for Monod, he entered an intense period of reflections on a single subject, the diverse ways protein subunits of identical structure could be assembled into a polymer having a finite number of components, an "oligomer" [29]. For this purpose, he played with pieces of cardboards, ping-pong balls and dices and arrived at a simple solution to create the same interface between all subunits. They were all related to each other by rotations around axes of symmetry. In fact, he just rediscovered some known principles of crystallography, the various classes of groups of symmetry that allow the formation of closed assemblies of identical asymmetrical objects (Fig. 2). He then envisaged how the binding of a stereo specific effector on such a tight assembly could markedly affect its structure, this structural change provoking in turn the appearance of a strong cooperativity in the corresponding binding curve. This would have occurred if the effector had caused a total dissociation of the protein upon its binding. The experimental evidence suggested an isomerization from one state to another one, rather than a full dissociation; the following scenario was then imagined: upon binding, a restricted set of the bonds gluing the monomers together in the original state were simultaneously and symmetrically broken. The original symmetry of the oligomer was then preserved. Under these assumptions, as computations showed, the concerted change in the quaternary structure of the protein upon ligand binding manifested itself by a strong cooperativity in the corresponding binding curve. These major points were already developed in the first manuscript at our

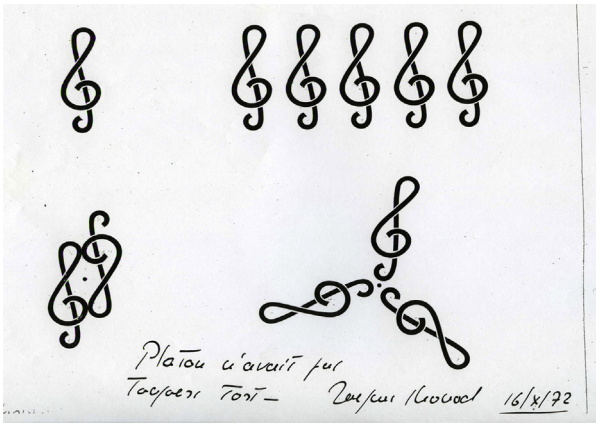

Fig. 2. Different ways of assembling asymmetrical objects figuring protein subunits. Monod realized that closed assemblies containing a finite number of components are easily constituted if, in the final structure, each element is related to the others by well-defined symmetry operations. J. Monod discussed this problem in several manuscripts. Later on, he related it to the creation of the famous Platonic solids. Fonds Jacques Monod, Archives de l'Institut Pasteur.

[3] An innovative study program in biophysical science had been elaborated in Boulder in 1958. It presented biological problems "as viewed through physical spectacles". The thermodynamic approaches developed by Wyman had already established the quantitative relations existing between the affinities of ligands for distinct sites present on a polymer undergoing a conformational change [21]. These advances did not however uncover a common cause for the appearance of cooperative responses at equilibrium and under steady state conditions. It does not seem then fair to claim that the principles of allosteric regulation were already established at this point [21,23].

[4] The elaboration of this model took place between the summer of 1963 and the end of 1965. It can be traced back through the numerous recollections of the actors [26] and through the manuscripts and letters kept in the archives of our institute, the six manuscripts or "typos" written by Jacques Monod over the period, and the thesis that Jean-Pierre Changeux defended in June 1964.

disposal, dated September or October 1963. It took time however to translate these proposals into a correct quantitative model (and here the help of R. Baldwin was considerable) and to confront the available experimental data to the equations derived from this model. This was achieved in the spring of 1964.

But, at this point, Monod's quest for coherence was not satisfied. Conservation of symmetry appeared necessary for the model to hold. Perhaps this hypothesis, in turn, rested on a powerful and yet hidden physical law? He wrote to Wyman: "I have thought a lot about the possibility of a general demonstration of the proposition according to which, if symmetrical and dissymmetrical structures were accessible to an oligomer, the first ones would, in general, be more stable" [24]. Despite Wyman's reluctance, Monod maintained his point of view on this topic. He reconsidered, however, the question from a more fruitful point of view, the evolutionary one.

Two months before the submission of the manuscript, he started to write the last section of the text, his personal summary of the essence of the model and of the way evolution has worked to attain these goals. He envisaged the case where the *N* subunits of a given assembly were coded by the same gene: the effect of any mutation will then be operating *N* times by reason of symmetry, Therefore, "symmetrical oligomers should constitute particular sensitive targets for molecular evolution, allowing much stronger selective pressures to operate in the random pursuit of functionally adequate structures". The general conclusion of the final text, the now famous MWC model, is in fact an answer to the challenge raised at the CSHS, three and a half years earlier: "A general and initially simple relationship between symmetry and function may explain the emergence, evolution and properties of oligomeric proteins as molecular amplifiers both of random structural accidents and of highly specific, organized, metabolic interactions" [12].

The formulation of a precise model helped to dissipate the impression that the concept of allosteric transitions was too vague and could "explain away" almost everything [9]. However, here too, diversity had to be taken into account, ruling out the emergence of a unique solution to respond to a functional challenge. Covalent modifications soon appeared as another efficient way to modulate the catalytic competence of a given protein, notably in the eukaryotic kingdom. Counterproposals, backed by new experimental findings, emerged from diverse scientific fields. Specialists of enzyme kinetics argued that the MWC model worked at equilibrium, though enzymes operate far from it. They specify the conditions where this restriction generates new sources of positive cooperativity. Symmetry had seemed crucial to generate the system of equations defining the model; it turned out not to be strictly necessary [29]. There was no justification whatsoever to assume that symmetrical states are intrinsically more stable than dissymmetrical ones. Evolution can optimize the stability of very dissymmetrical assemblies – transient conformations encountered during the translocation of specific molecular motors – as well as fulfil the opposite challenge, the elimination of very dissymmetrical intermediates during the concerted transitions undergone by regulatory proteins. Through an intense controversy implying Wyman, Crick and Monod, the "essence of the model" was also more precisely reformulated. More precise tests were elaborated to evaluate up to what point the behavior of real systems approached the limit case formulated in the MWC model (discussed in [30,31]).

Yet, the MWC article of 1965 is still fascinating to read. Its more tangible message is very easy to grasp. From simple postulates, that were counterintuitive at the time, an elegant model was derived, predicting the most concerted transition that could possibly arise at equilibrium in regulatory enzymes and, consequently, the most drastic effects that stereospecific ligands could possibly trigger. But part of the seduction of the paper rests also on its speculative character. Proteins are considered there not only as transducers working close to their theoretical limit of efficiency, but also as historical objects that evolution has optimized. In its two facets, radiant and convincing, or speculative and questionable, the article bears Monod's label, an amateur in physicochemistry, a visionary so far as molecular Darwinism is concerned [31].

4. General discussion

Over time, Monod markedly evolved in his quest for coherence within the living world. At the very start, it was essentially a very successful comparative approach, which met a rapid acceptance from the small community involved. As Jon Beckwith wrote about the scheme that emerged from the Pajamo experiment, "the beauty of the original model and its apparently powerful explanatory qualities not only generated this field, but also constrained thinking about alternative models. This may be inevitable in any case of powerful new concepts. Part of their strength ironically lies in their ability to channel research in a way that restricts speculation" [32]. The only solution proposed in early times for the control of protein biosynthesis – negative regulation affecting the initiation of transcription – was progressively enlarged, despite some harsh resistances from Monod [5]. As we have discussed, the quest for coherence appeared decisive to delineate efficient strategies of research in a world where clear experimental evidence was scarce.

On the other hand, allostery was formulated at the CSHS symposium as a very general proposal. It was immediately realized that the concept provided an efficient way for coupling different biochemical pathways. In the following years, research in Monod's laboratory focused on the conformational changes that could be involved. This led to the formulation of a very elegant but restricted mechanism on which the efficient role of mutational events could be easily perceived. The allosteric model did not exert a very strong "channelling" effect on the field concerned. Rather, it was considered as a limit scheme, built under restrictive hypotheses, a useful reference rather easy to challenge. Its discussion put in close communication two communities who were previously rather ignorant of their respective way of performing research. From now on, the field evolved quickly. Data were accumulating at an accelerated pace, brought in particular by a new type of scientists, the crystallographers. From their work, it was easier to

visualize how the spatial relationship existing between structural domains in a given protein could play a major role in explaining the interaction patterns occurring between the sites they carry.

Despite their different impacts on their respective fields, the negative control of transcription and the allosteric concept have kept an exceptional status in modern biology. In *Chance and necessity*, it is essentially through these two notions that Monod provided a clear overview of the way molecular biology relates structures and functions. This quasi-iconic status could however hide to the students the challenges we are presently facing in research, in particular a comprehensive account of biological diversity. This does not mean to abandon Monod's aspiration for coherence, but to put it in a prospect compatible with the current analysis of an ever-increasing amount of published data, displaying an incredible diversity in their mechanisms and in their interaction patterns. Refined phylogenetic analyses tell us in more precise terms how they did historically appear. Do new regularities emerge as this field of research is undergoing such drastic changes? For example, is it true that all the significant improvements in regulatory performances arose by the "tinkering" of pre-existing regulatory complexes or from the regulatory sequences implicated? Is it true, as argued in [33], that these regulatory processes generally operate *via* conformational transitions requiring only weak energetic changes? These modern types of molecular analyses open new questions about the way evolution unfolded, an historical domain that Monod thought to be definitively inaccessible to scientific inquiry, a challenge for scientists inspired by his style of research.

A reflection on Monod's major achievements might close by meditating the comment Peter Medawar wrote on intuition and induction in scientific thought: "Like other exploratory processes, (scientific method) can be resolved into a dialogue between fact and fancy, the actual and the possible; between what could be true and what is in fact the case. The purpose of scientific enquiry is not to compile an inventory of factual information, nor to build up a totalitarian world picture of Natural Laws in which every event that is not compulsory is forbidden. We should think of it rather as a logically articulated structure of justifiable beliefs about nature. It begins as a story about a Possible World – a story which we invent and criticize and modify as we go along, so that it ends by being, as nearly as we can make it, a story about real life" [7].

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