Perspective

The Origins of Allostery: From Personal Memories to Material for the Future

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Abstract

This introductory text evokes personal memories about the origins and definition of the word allosteric that is meant to qualify an "indirect" interaction between topographically and stereospecifically distinct sites, mediated by a discrete and reversible conformational change of the protein. The allosteric transition paradigm adds a new dimension and creates new openings to understanding receptors, signal transduction and drug design.

Introduction

This introductory chapter does not aim at presenting an objective historical account on the origins of the concept of allosteric interactions and of the subsequent models. Such an attempt would require the complete elucidation of the rich network of interactions that existed at the time between the concerned scientists at the international scale. This document should better be viewed as an evocation of personal memories about my contributions to the work and the anticipated subjective bias. I was then a modest, though motivated, graduate student struggling to initiate a new scientific topic in an environment of scientific giants constantly communicating—far above my head—with multiple international authorities and personal friends. It may nevertheless bring the unconventional testimony of a freshman scientist that is not so often considered in the official history of scientific discoveries.

From Marine Biology to *Escherichia coli* (1958)

As far as I can remember, since my early age, I was fascinated by natural history. Adolescent, I owe to my Biology Professor at the Lycée Montaigne the initiative, in 1954 and 1955, to enroll in summer courses in marine biology at the laboratories of Arcachon and Banyuls-sur-Mer in the south of France. They gave me the opportunity to have my first "physical" contact with the electric discharge of the *Torpedo marmorata* fish and to learn about the work of David Nachmansohn on acetylcholine and neurotransmission. This initiation had critical importance in the later evolution of my work. Accepted as an independent worker in the laboratory of Banyuls-sur-Mer, my first publications were primarily about a new species and genius of parasitic copepod that I discovered in holothurian echinoderms.[1] I became an enthusiast of marine biology. However, thinking to find a laboratory for a PhD thesis, I decided to broaden my interests and visited Jean Brachet for a few weeks in Brussels in the fall of 1958 where I also attended Christian de Duve's lectures. Inspired by their philosophy that the solution of the great problems of biology had ultimately to be found in elementary biochemical mechanisms, my passion shifted to the chemistry of early embryonic development and, specifically, the activation of the oocyte by the fertilizing sperm cell. My naïve theory was that the enzyme activations that follow the entry of the spermatozoon in the egg were due to a burst of subcellular particles called lysosomes by Christian de Duve. Back in Paris, I tried to test this hypothesis.

Confronted by concrete difficulties in enzyme assays, I asked for help from Jacques Monod, whom I had briefly met by chance. After a seminar that I gave at the Pasteur Institute on my project, Jacques Monod suggested that I, in the future, enter his laboratory as long as I am ready to abandon my project and work with *E. coli*. After 3 months of reflection, I adopted this solution, keeping my interest in animal biology for the future!

My entry in Jacques Monod's laboratory, at the beginning of 1959, put me in a rather stressful position. To improve my experience in biochemistry, he suggested that I work first on the expression of *E. coli Lac* operon genes transferred by François Jacob into the *Salmonella typhimurium* genome. As expected, I did not find any difference between the two.[2] However, this short venture turned out to be an exceptional training on the basic methods of molecular biology under the direct supervision of Jacques Monod.

The Mechanism of Feedback Inhibition and the Origins of the Word "Allosteric" (1961)

The moment came, in the spring of 1959, to select a project for my PhD thesis. Jacques Monod and François Jacob suggested several research themes, most of them dealing with their ongoing work on the operon. They did not suit me. I wanted to have a more personal and independent project. A particularly original one, mentioned by François Jacob, held my attention. Edwin Umbarger had shown that, in certain bacterial biosynthetic pathways, the first enzyme is inhibited, in an "apparently competitive" manner, by the end product of the pathway.[3] The issue was to understand the molecular mechanism of this elementary regulatory operation. This topic fitted with the spirit of my first theoretical enthusiasm about enzyme activations following fertilization. I selected the subject for my thesis. The experimentation was difficult for a beginner and I felt rather isolated. François Jacob and Jacques Monod were fully occupied by the construction of the operon model,[4] mentioning *en passant* that the regulation of protein biosynthesis they were concerned with was of primary importance compared to the mechanism of activity control I was engaged in.

My first idea was that, if a special molecular device mediated the feedback inhibition by the enzyme, one should be able to find a way of identifying its molecular constituents, for instance, by dissociating *in vitro* the regulatory interaction from the catalytic activity. I started with the first enzyme of the valine pathway—acetolactate synthetase—that Umbarger and Brown[5] had shown to be feedback inhibited by valine and with the help of *E. coli* mutant strains identified by François Jacob that excreted valine and were interpreted as having an acetolactate synthase no longer feedback inhibited by valine. This was the first result of its type but was never published (1959). Acetolactate synthase was in fact a difficult enzyme to work with and I decided to switch to L-threonine deaminase with a closer supervision by Jacques Monod who made Edwin Umbarger aware of it (June 1959). First I confirmed Umbarger's *in vitro* experiments that L-threonine deaminase was apparently competitively inhibited by L-isoleucine and that it displayed "bimolecular" cooperative kinetics toward both the substrate and the feedback inhibitor.[3] However, soon I noticed (end of 1959, beginning of 1960) that the sensitivity of enzyme preparations to L-isoleucine changed with time and progressively decreased, specifically in the course of purification. Heating the enzyme up to 55 °C accelerated the process and resulted in a complete loss of sensitivity to L-isoleucine, without significant decline of enzymatic activity. The effect was blocked by the chelator magnesium titriplex suggesting the implication of a heavy metal in the process. Moreover, *p*-chloromercuribenzoate, a commonly used reagent of –SH groups, gave a similar effect in the absence of heat treatment. Interestingly, the loss of L-isoleucine feedback regulation was also accompanied by the abolition of the unusual "bimolecular" kinetics of the enzyme toward its substrate. The complex cooperative kinetics of the enzyme thus seemed to be an integral part of the regulatory properties of the enzyme.

In the spring of 1961, I was invited as a discussant to the 26th Cold Spring Harbor Symposium on Quantitative Biology entitled Cellular Regulatory Mechanisms (1961). Jacque Monod pressed me to write, by myself, the paper I would read in my oral communication.[6] I first wrote a presentation of the data and the kinetic paradox they raised. Then I briefly discussed the two plausible models that might account for the "apparent competitive" antagonism between the feedback inhibitor L-isoleucine and the substrate L-threonine (Fig. 1). According to the first model, the binding sites for the substrate and regulatory inhibitor are partially "overlapping" and the interaction is thus a classic competition by steric hindrance. In the second, "new", model referred to as "no overlapping", the two sites are separated from each other and the interaction between ligands takes place between topographically distinct sites. I favored the second model that the substrate and the regulatory effector were to bind topographically distinct sites particularly on the basis of the argument that loss of feedback inhibition was accompanied by a normalization of the kinetics.[6] I wrote in my communication "it seems inevitable to assume the existence of two distinct sites which we would respectively designate as activity site (A) and inhibition site (I) and to further assume that the

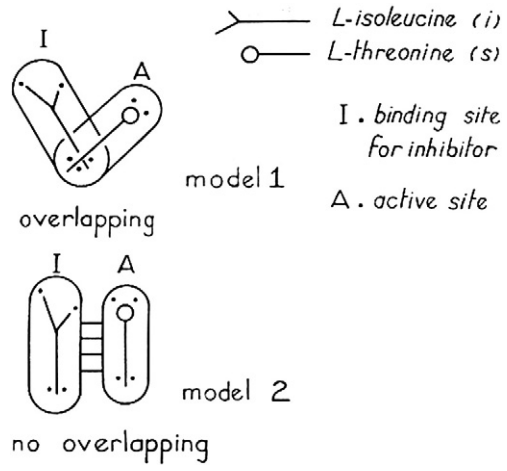

Fig. 1. The two plausible models proposed for the "apparent competitive" antagonism between the feedback inhibitor L-isoleucine and the substrate L-threonine on L-threonine deaminase (from Changeux).[6]

properties of the active site are influenced by the compound bound at the inhibition site".

I had the opportunity to show a first draft of the paper to Jacques Monod and to discuss with him the relationship between the non-hyperbolic shape of the substrate saturation curve and the inhibition by regulatory ligand. According to him, the partial structural analogy between threonine and isoleucine (both are amino acids) would allow the substrate to bind non-specifically to the site of the inhibitor and vice versa. In other words, a one substrate–one regulatory site monomer would suffice. I was not pleased with this scheme that, to some extent, attenuated the principal message of my findings. However, I did not feel confident enough to defend the alternative simple model I had in mind (two active sites and two regulatory sites) against the views of my respected and, to some extent, feared supervisor. In any case, this was, in my opinion, an important moment in the dialog with Jacques Monod whose views subsequently evolved possibly as a consequence of personal exchanges with me and also with Arthur Pardee who briefly visited the laboratory before the meeting.

At the end of the conference, Jacques Monod presented orally the General Conclusions of the Symposium. In the section dealing with the regulation of enzyme activity, he reported my results and mentioned that "closely similar observations have been made independently and simultaneously by Pardee (private communication) on aspartate carbamyl transferase" (see Ref. 7). He did not use orally the word allosteric but instead "Novick-Szilard-Umbarger" effect that emphasized the cybernetics of the feedback inhibition process. Yet, his presentation showed a full appreciation of the novelty and generality of the concept of an interaction between topographically distinct and stereospecific sites. These General Conclusions were an important moment of the meeting where Jacques Monod displayed his exceptional ability to brilliantly synthesize current ideas into organized concepts. Only in Monod and Jacob's subsequently written publication of the Conclusions does the word "allosteric" appears as composed of two Greek roots expressing the difference (αλλο–) in (στερεο–) specificity of the two binding sites to qualify and generalize the "no overlapping sites" mechanism and its mediation by a conformational change of the protein.[8] This was the birth date of the word allosteric. The appearance of the word also consolidates, in the written text, the shift of emphasis from the cybernetics of feedback inhibition to the more molecular and biochemical aspects of allosteric interactions. With the work on the operon being completed with this 26th Cold Spring Harbor meeting, the reflection on allosteric proteins became progressively a privileged topic in Jacob and Monod scientific interests.

The Concept of Allosteric Transition and Koshland Induced Fit (1963)

Both in the reference list of my written communication (p. 318) and in Monod and Jacob printed conclusions (p. 391), the name of Daniel Koshland appears and the "induced-fit" theory suggested to account for the indirect interaction between distinct sites. Yet, initially, Koshland's theory did not address the regulation of enzyme activity by a metabolic signal but the specificity of enzyme catalysis. His view was that the enzymatic reaction occurs "only after a change in shape of the enzyme molecule had been induced by the substrate".[9]

Our contribution was to extend and generalize Koshland idea on enzyme catalysis to the indirect allosteric interaction between active site and regulatory site that would then be mediated by a reversible conformational change of the enzyme "induced" by the ligand. In other words, the local catalytic process was extended to the global signal transduction mechanism mediated by the protein. This was the origin of the concepts of allosteric *interaction* and allosteric *transition* that have, since then, been broadly applied not only to regulatory proteins in general but also to the mode of action of pharmacological agents (see Refs. 10 and 11).

My research on the properties of L-threonine deaminase was developing in parallel with the work from different laboratories, for instance, on aspartate transcarbamylase (ATCase),[7] eukaryotic glutamic dehydrogenase,[12–16] acetyl-CoA carboxylase[17] and phosphorylase b,[18] among many others. The data collected substantiated the initial proposal of distinct sites for substrate and regulatory ligand. François

Jacob and Jacques Monod became more and more concerned by the reflections on allosteric sites and interactions, to the extent that they decided to write, together with me, a general review entitled "Allosteric proteins and cellular control systems".[19] Its aim was to further specify the definition of the word "allosteric" on the basis of the available data and to introduce, as we shall see, an important issue about the molecular regulation of the newborn operon. Concerning the specification of the concept of allostery, the evidence that the allosteric effectors do not directly participate in the reaction they control was emphasized. In addition, on the basis of observations such as the activation of phosphorylase b by 5′ AMP[18] and of glutamate dehydrogenase by various effectors (ADP, diethylstilbestrol),[20] a change in the state of aggregation of the protein was proposed, among others, as a plausible mechanism for the allosteric transition. Yet, the question was raised as to whether or not "this alteration is induced directly by the binding of the nucleotide, the dimerisation being then a result of this primary effect" (p. 319). Still, in the general discussion, the "induced-fit" theory of Koshland is suggested (p. 323) as contributing to the allosteric transition. Moreover, I was personally not pleased by the emphasis on a change of the state of aggregation associated with ligand binding, enthusiastically defended by Gordon Tomkins and others. During 1962 and in the spring of 1963, I tried to experimentally test this possibility with L-threonine deaminase. I found repeatedly that the sedimentation coefficient of the enzyme did not change in the presence of either the substrate or the allosteric effectors and remained close to 8.6 s.[21] This was confirmed in the laboratory by the ongoing work of Agnes Ullmann and Roy Vagelos on phosphorylase b.[22] For the time being, we had to think about a general mechanism of allosteric transition that would *not* involve a change of aggregation.[21,23]

At the end of my first public presentation at the 1961 Cold Spring Harbor meeting, Bernard Davis stood up and noted the analogy between the cooperative binding properties of L-threonine deaminase and oxygen binding to hemoglobin. This was a highly relevant comment and, for me, the beginning of an exciting story, even though generations of most distinguished scientists including Linus Pauling, Jeffries Wyman and others had being producing major contributions to it in the past 50 years. Max Perutz's structural work on hemoglobin had recently made major progress. In the 1963 review, it was mentioned that "the four haem groups are actually wide apart (40A) excluding any possibility of direct interaction"[24] and that "the recent crystallographic work of Muirhead and Perutz (personal communication) has indicated that the *distance* between certain –SH residues in the molecule may be shifted by about 19% upon oxygenation, providing direct though still tentative evidence of a conformational alteration" (p 320). At the end of 1962, I had the privilege to meet for the first time Max Perutz whom I invited for a lecture in Paris as secretary of the "Club de Biologie Moléculaire". I remember very well the dinner discussion with his wife at the restaurant "La pie qui chante", which followed his talk. He was interested by the idea of allosteric interaction and was aware of my recent data on threonine deaminase…In this context, he was amazed that at 5.5 Å resolution "only the quaternary structure of hemoglobin did change upon oxygenation". This was remarkable. I argued that these data were consistent with our views but that "the tertiary structure of the globin chain must also change upon oxygen binding…". The mention of these results in the 1963 review emphasized our early interest in the relationship between subunits' tertiary conformation and the quaternary structure of regulatory proteins. However, they were further specified only in the subsequent 1965 model.

The 1963 review ends with an attempt to extend the concept of allosteric proteins to both hormone action (thyroxine and estrogens) and gene repressors "possessing two sites, one of which binds the operator, the other the (positive or negative) effector" (p. 328). In 1961, Jacob and Monod had published that the repressor was a polyribonucleotide. However, as a result of the development of the work on regulatory enzymes in the laboratory, the analogies with the repressor binding properties, the failure to identify the repressor as a RNA and other observations, including the possible protein nature of lambda repressor,[25] the idea was abandoned. It was evident that the main reason to write the 1963 review was for François Jacob to abandon the RNA hypothesis and to propose that gene repressors were *bona fide* allosteric proteins.

Meanwhile, my thesis work was further progressing. Among the many observations, new ligands of L-threonine deaminase were discovered, which, for instance, bind to the regulatory site but stabilize the active conformation (valine, norleucine and the substrate analog L-allothreonine) instead of shutting it off (1962), and chemical conditions (some reversibly similar to pH) that uncouple regulatory and active site and abolish cooperative interactions were further specified (1963). Yet, at variance with Gerhart and Pardee,[7,26] the protein did not split into subunits as a consequence of uncoupling. Also, mutations that differently alter the response to regulatory ligands were uncovered (some had lost their sensitivity to isoleucine, others have increased it, some had degrees of cooperation between regulatory sites altered and their interaction with the active sites and so on).[21]

At the end of 1963, beginning 1964, I began to write down and organize all the data I had collected for my thesis. It took me several months of considerable efforts, fearing too many criticisms from my respected

and severe advisor. Last, following my "innate" tendency to build theories, I wanted to end my thesis by a mathematical model that would globally account for all the observations I had made. In particular, I wanted to account for the well-defined relationships between homotropic and heterotropic interactions also noted with several other systems, on the basis of simple structural principles. I discussed the topic with Adam Képès, an outstanding specialist in enzyme kinetics working in the laboratory. The result came soon yet in the format of huge equations that I found extremely difficult to use even if based upon simple Michaelis–Menten principles. Later (mid 1964), I handed Jacques Monod the first typed version of my thesis work[23] and mentioned to him the difficulties I had encountered. He was especially interested with my data and even more with the theoretical challenge I had raised.

The MWC Model (1965)

As a consequence of this encounter, daily lively debates with Jacques Monod followed. I specially appreciated these privileged moments. Facing the whiteboard, I reviewed for him, in a systematic manner, the available data. In particular, I came back to observations I had made about the effect of urea (1962), which, at an adequate concentration, inactivates reversibly threonine deaminase, an inactivation interpreted as a split of the enzyme into subunits (Fig. 2). Quite interestingly, in this system, the allosteric activators (such as L-norleucine, L-valine and L-allothreonine) facilitated inactivation and thus subunit dissociation while the feedback inhibitors (such as L-isoleucine) protected against inactivation and thus strengthened the assembly of the subunits in the protein. In the communication I presented to the 1963 Cold Spring Harbor meeting, I even "surmised that the allosteric transitions involve weakening or increasing interactions between subunits. Such a situation is actually known in the case of hemoglobins (p. 503)"[21] (see Ref. 27). Continuing on the discussions with Jacques Monod, to formally account for the observed effects, I mentioned the possibility of "three" possible conformational states, one for each experimental condition (presence of activator, presence of inhibitor and no effector). Then, Jacques Monod with his reductionist mind pressed me, as usual, to limit the number of hypotheses. Two states should suffice! I immediately agreed and further documented the virtues of the "two states" with a tight (to become T) or relaxed (to become R) mode of packing the subunits, having different intrinsic affinities for both substrate and allosteric effectors. This would altogether account for the signal transduction mechanism and the relationships between homotropic and heterotropic interactions. This is, to my opinion, the birth date of the "two-state mechanism" of pre-existing conformational states (R ↔ T) and, by consequence, of a "selective" rather than an "instructive" effect of the ligands. It allowed a remarkable economy of means and explained simply a large number of kinetic properties of the native enzyme that we progressively discovered during the following months. All this was far from any deep theoretical consideration…just for sake of simplicity! These views led, after many writings and re-writings,[28] to the 1965 MWC (*M*onod–*W*yman–*C*hangeux) model.[29] In parallel, I had the responsibility to compose (and often re-compose) nearly all of the figures. For me, this "quasi-artistic" activity was a most enjoyable responsibility that Jacques Monod himself quite surprisingly was reluctant to take. The final version was handed by Jacques Monod directly to Sydney Brenner and Francis Crick in Cambridge and readily accepted. MWC statements have been since then abundantly re-formulated and discussed in terms of "conformational shift" or "shape shifting" by various groups.[30–34]

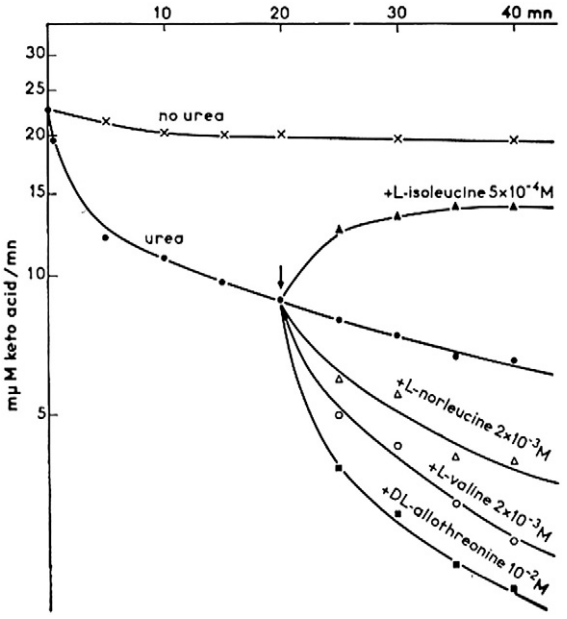

Fig. 2. Opposite effects of activators and inhibitors on the inactivation of L-threonine deaminase in the presence of urea (from Changeux).[21]

An important part of the final version of the MWC paper deals with the quaternary organization of proteins and specifically with their symmetry properties. It developed mainly not only from the personal reflections of Jacques Monod about the three-dimensional organization of proteins in relation with genetic complementation but also from abundant exchanges, with Francis Crick and Jeffries Wyman (among others), but that usually—and unfortunately—did not involve me as a participant. Yet, I fully appreciated the issue and became much concerned

by the oligomeric status of most well-identified regulatory proteins (even if a few exceptions do exist).[33,34] The final version of the manuscript included at the end a section about thermodynamic considerations on symmetry that I found far from my way of thinking. The versions of the model that were introduced in my PhD thesis or that I presented by myself at the 1964 Brookhaven symposium did not include an extensive discussion on the quaternary organization of proteins. However, both mentioned what I considered the fundamental mechanistic issue of the model: the cooperative symmetrical assembly of the subunits within an allosteric oligomer and the quaternary constraint established between subunits that affect their tertiary organization yielding an all-or-none molecular switch as the structural mechanism of the cooperative effect of ligands binding.

The model postulates the following:

(1) regulatory proteins are symmetrically organized from a small number of subunits associated into a cooperative structure forming closed "microcrystals", or oligomers, and the tertiary organization of the individual subunits is constrained within the oligomer by its quaternary organization;
(2) protein oligomers exist in a few discrete conformations (R, T, …) in thermal equilibrium in the absence of a regulatory signal;
(3) the regulatory ligands merely shift the equilibrium between the conformations, selectively stabilizing the one for which they display the highest affinity; and
(4) the conformational transitions take place in an all-or-none concerted fashion for all subunits and thus with conservation of symmetry.

Writing the basic expression of the model was started together with Jacques Monod. In the absence of ligand, the two states, R_0 and T_0, were assumed to spontaneously establish an equilibrium characterized by an intrinsic equilibrium constant, $L_0 = T_0/R_0$, called the allosteric constant. The ligands would differentially bind to each state with microscopic dissociation constants, K_R and K_T, that "are the same for all homologous sites in each of the two states" independent of their ligand occupancy and with $c = K_R/K_T$. The development of the formalism was continued by Jacques Monod himself. However, the final, correct, version of the equations was derived by Robert Baldwin who was present for a sabbatical at the Pasteur Institute.

The model fundamentally distinguishes a "function of state", "R", which describes the conformational equilibrium, and a "binding function", "Y", which distinctly evolves as a function of ligand concentration. The signal transduction mechanism then results exclusively from the displacement, or conformational shift, of the spontaneous equilibrium between the R and T states, including the observed cooperativity between sites.

A year following the MWC publication, Koshland, Nemethy and Filmer, reactualizing the Pauling scheme for O_2 cooperativity in hemoglobin,[35] proposed an alternative instructive [KNF (*K*oshland–*N*emethy–*F*ilmer)] model[36] that implies a graded "induced" change of biophysical parameters and the superimposition of the "state" and "binding" functions. The debate is still lively today.[33,34,37]

Testing the MWC Model

The first molecule with which the model could be tested was, of course, hemoglobin. During my military service, I had the opportunity to establish friendly relationships with another draftee François Bernède who was doing sophisticated computations on the military supercomputers. He tried to fit the O_2 binding curves of horse hemoglobin by R. Lyster with the MWC model. The fit was remarkable with plausible values of the MWC parameters ($L = 9054$ and $c = 0.014$) to the extent that the figure was integrated as Fig. 3 in the final version of the paper.

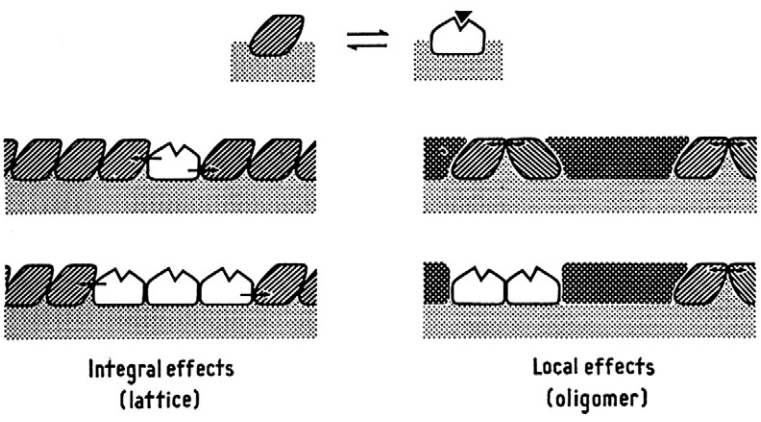

Fig. 3. Lattice and oligomeric models for cooperative interactions between receptor units in a biological membrane (from Changeux).[38]

These early hemoglobin data were followed by attempts by Edelstein,[39] Perutz *et al.*,[40] Hammes *et al.*,[32] Fischer *et al.*[41] and Viappiani *et al.*[42] to confront hemoglobin data with the respective predictions of the MWC *versus* KNF model.

In 1965, liberated from my military obligations, I decided to further challenge the MWC model by myself outside the Pasteur environment. I took a postdoctoral position with John Gerhart and Howard Schachman, in Berkeley's Molecular Biology Virus Laboratory, who had in hands large amounts of highly purified ATCase. I had in mind to test one of the crucial propositions of the MWC model that the conformational equilibrium of the protein becomes established independently of the binding of the ligand. In other words, the state function R should differ from the binding function Y. The binding experiments I did with ATCase together with the conformational data collected by Gerhart and Schachman demonstrated that this was the case.[43,44]

Yet, to fit the data with the original MWC equations, Merry Rubin, a student of Howard specially competent in computational sciences, helped me to extend the model to the general situation where regulatory ligands nonexclusively bind to both R and T states (the case of nonexclusive binding of the substrate was present in the original model) and the consequences on the cooperativity and shift of the R–T equilibrium. The paper was accepted by Sydney Brenner for the *Journal of Molecular Biology*,[45] and similar findings were also reported with phosphofructokinase.[46] The general formulation it proposes has two important consequences:

(a) the new equations adequately fit the ATCase data despite an uncertainty on the number of sites per ATCase molecule (six rather than four as initially thought), with the substrate analog succinate almost exclusively bound to the R state ($c < 0.001$), while the feedback inhibitor CTP is nonexclusively bound (1.7-fold stronger) to the T state, and with $L = T/R = 4$; the data unambiguously demonstrate the anticipated distinction between the state and binding function.[44,45]

(b) the generalization of nonexclusive binding offers a plausible (among others) mechanism for partial agonism, an important issue in pharmacology (see Refs. 10 and 47).

In the past 50 years, the MWC model has led to considerable structural and biophysical work on the conformational transitions of a large spectrum of regulatory proteins. It has been recently re-examined in the framework of molecular dynamics[48–51] and contrasted again with the sequential induced-fit scheme, in particular, for hemoglobin[41,52] (for review, see Ref. 34).

Extension to Membrane Assemblies: A Subunit-Based General Model for Cooperative Interactions

In the conclusion of my thesis (1964),[23] I considered explicitly the possibility of extending the MWC model to the "membrane phenomena involved in the recognition of communication signals and their transmission (synaptic transmission, for example)". Before leaving Pasteur, I decided to challenge the plausible extension of the model to brain proteins involved in synaptic transmission. I had been acquainted with acetylcholinesterase (AChE) and *Torpedo* electric organ during my early experience in Arcachon. In the absence of a well-identified neurotransmitter receptor, I decided to investigate the kinetic properties of AChE taking benefit of the many compounds left by Daniel Bovet when he was working at the Pasteur Institute (in particular, derivatives of flaxedil, the first synthetic curare he discovered). Interestingly, some of these compounds did not behave as steric competitors but as allosteric modulators of AChE activity suggesting a possible (though only superficial) analogy with the authentic receptor.[53]

In the spring of 1966 while in Berkeley working with Howard Schachman, I received a letter from Max Delbrück, who, aware of my thesis work, invited me to give a series of three lectures at Caltech on allostery, the last one mentioning cooperativity in membrane proteins. Inspired by 1963 Peter Weiss' play Marat/Sade, Max amazingly introduced my lectures as being "the persecution and assassination of 'Jean-Paul' Changeux as performed by the inmates…". The lectures and the questions went quite well. Commenting my last lecture, Max was struck by a slide I showed where nearest-neighbor cooperative interactions between receptor molecules were suggested to take place in a membrane. I said that I wanted to develop a mathematical model of such interaction, but he mentioned that he was personally interested by the project. He nevertheless suggested that I meet the solid-state physicist Charles Kittel when back in Berkeley. The relationship with Kittel was extremely positive, and together with one of his students and a friend Jean Thiéry, the MWC model was re-examined and reformulated[54] (Fig. 3). In addition to the classical oligomeric case of membrane receptors, the model was applied to larger—unlimited—cooperative assemblies of membranes proteins, in a two-dimensional lattice[54] (Fig. 3). At variance with MWC, the thermodynamic formulation was based on the conformational transition of single units (or protomers) between a minimum of two states modulated (or not) by the interaction with other protomers with the isomerization constant: $(s)/(r) = l'$. Several formulations were proposed.

According to a particularly simple one (referred to as the Weiss molecular field approximation), in a system of interacting protomers, for instance, within a membrane lattice, the free energy F of the transition ($s \leftrightarrow r$) is proposed to depend on the fraction of protomers that are already in the r state and expressed as $\Delta F = (\varepsilon - \eta(r))$.

The isomerization constant $l' = (s)/(r)$ is then simply $l' = \exp[\beta\Delta F] = l/\backslash^{(r)}$.

Depending on the value of the free energy of the interaction between protomers, the model predicts the existence of various classes of responses to specific regulatory signals exhibited by biological systems: from a graded response of a single-receptor protomer or oligomeric receptor (MWC model) to an all-or-none phase transition response in large and periodic protein assemblies.

In more general terms, the model by Changeux *et al.*[54] lays the groundwork for a general and simple thermodynamic mechanism of conformational selection (see Refs. 34, 37 and 42). Independently, a general formulation covering both MWC and KNF for oligomers was presented by Eigen.[55] As in Changeux *et al.*,[54] the induced-fit KNF model appears as a limit case in which the unbound active R_0 state of MWC is simply omitted.[30–32]

The lattice model was rediscovered and further developed—30 years later—by Dennis Bray and colleagues with the experimental system of bacterial chemoreceptors.[56,57] Yet, no such cooperative interaction has ever been identified in a native synaptic membrane…but other supramolecular assemblies might possibly be interpreted in those terms from the chaperonins to flagellar motion and even the gene transcription complex! (see Refs. 34 and 58).

Conclusion: The Nicotinic Acetylcholine Receptor, Allosteric Modulation and Drug Design

I deliberately close this chapter on the "Origins of allostery" with the Changeux *et al.* model.[54] It has been a concrete stimulus for decades of research I subsequently did on membrane receptors. The moment had come for me to experimentally test the suggestion from my PhD thesis (1964) that allosteric mechanisms might operate in the nervous system and specifically mediate synaptic transmission. The early work I did on acetylcholine esterase from *Torpedo* in 1965,[53] before I left to the US, convinced me that, to be efficient, the work had to be done on a real receptor. However, at that time, no authentic receptor for a neurotransmitter had ever been identified. I thus decided to leave Berkeley (February 1967) for a second postdoctoral study with David Nachmansohn at Columbia University, in New York, then world expert on the chemistry of cholinergic systems from electric fishes. I wanted to train myself in the dissection and recording of the electrophysiological response of the single electroplaque from *Electrophorus electricus* electric organ to nicotinic agents with the ultimate aim to establish a rigorous strategy linking the *in vivo* physiological and pharmacological response to the chemistry of the receptor molecule I was planning to identify from the electric organs, an extremely rich source of nicotinic synapses.

I stayed only a few but very productive months with David and returned to the Pasteur Institute at the end of 1967. There I established the electroplaque setup. A fundamental step in the strategy then became to reduce the electroplaque system to the subcellular and last molecular levels without losing its characteristic physiology and pharmacology. Having practiced with my hands the assay of bacterial permeases, I developed, together with Michiki Kasai, an *in vitro* system of excitable membranes or "microsacs" from the electric organ that responded to acetylcholine in a manner very similar to that recorded with the electroplaque.[59,60] This step was essential to me since it demonstrated unambiguously that the regulatory interaction mediating channel opening operates in the absence of energy source, in a chemically defined environment. It was a strong suggestion in favor of an allosteric transition of the receptor. It also paved the way to the chemical identification of the receptor protein from these membranes,[61] using nicotinic ligands in conjunction with a snake venom toxin α-bungarotoxin.[62] Bungarotoxin blocked, altogether, the physiological response to nicotinic agonist on the electroplaque *in vivo* and on the microsacs *in vitro* and also bound decamethonium to a protein isolated from these membranes.[61] All subsequent molecular biology and biophysical work has confirmed that this protein is *bona fide* the nicotinic acetylcholine receptor protein.[34,63]

The nicotinic receptor became a favorite model of the subsequently identified pentameric, tetrameric or trimeric, often hetero-oligomeric, ligand-gated ion channels, including the GABA receptor[63–65] and even G-protein-coupled receptors.[34,66] When the genomic analysis of Tasneem *et al.* that sequences homologous to the nicotinic receptor were present in bacteria appeared,[67] I heartily encouraged Pierre-Jean Corringer to functionally express as a ligand-gated ion channel and purify one of these molecules.[68] His success opened the field to the X-ray crystallography of pentameric receptors[69–71] and their conformational transitions.[69,72,73] After reaching the brain, I was back to prokaryotes!

The presently available data are consistent with the notion that the pentameric receptors, including the nicotinic receptor, behave as authentic allosteric proteins yet with features of their own. They demonstrate, in particular, the following[74,75]:

(1) an oligomeric pentameric organization with an axis of symmetry (or pseudosymmetry) perpendicular to the plane of the membrane;

(2) a topological distinction between the neurotransmitter (ACh) sites (distant by 40 Å between ACh sites) in the extracellular domain and the ion channel (distant by 60 Å from the ACh sites) in the transmembrane domain, demonstrating that the interaction between the neurotransmitter (ACh) and the channel is an indirect—allosteric—interaction;
(3) the occurrence of several discrete conformational changes (in general, more than two) mediating activation (channel opening) and desensitization (slow inactivation toward a high-affinity state); and
(4) the presence of multiple allosteric "modulatory sites" in the synaptic (e.g., Ca^{2+}) and transmembrane domain (e.g., ivermectin, general anesthetics, …).

Further, the allosteric transition paradigm adds a new dimension to receptor understanding, signal transduction and drug design, in particular, with the concept of "allosteric modulator",[11] which is abundantly used with ligand-gated ion channels,[76–79] G-protein-coupled receptors[66,80] or tyrosine kinase receptors,[81,82] among many regulatory proteins. In the classical pharmacology paradigm, both agonist and antagonist drugs were designed to fit a site present on a unique receptor conformation. By contrast, the allosteric scheme posits that agonists and antagonists, as well as positive and negative allosteric modulators, select and stabilize several structurally different conformations, each with its own pharmacological specificity. Many new openings for drug design are thus emerging from this new and simple approach fueled by the abundant structural and molecular dynamics studies that emerged from the broad diversity of recently discovered neurotransmitter receptors, following the lead of the nicotinic receptor.

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Abbreviations used:
ATCase, aspartate transcarbamylase;
AChE, acetylcholinesterase.

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