INSTITUT DE LA VIE

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"DE LA PHYSIQUE THEORIQUE A LA BIOLOGIE"

VERSAILLES : 4-8 JUILLET 1988

RESUMES DES RAPPORTS

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HIGH TEMPERATURE SUPERCONDUCTIVITY: CURRENT THEORETICAL SITUATION

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Abstract

The discovery of superconductivity in ceramic oxides has led to significantly higher superconducting transition temperatures. Dozens of theoretical proposals have been made, but at present, there is no consensus on the underlying mechanism causing the high transition temperatures. After a brief review of the properties of superconductors and the microcopic BCS theory of superconductivity, the present status of the theory of high temperature superconductivity will be discussed.

Introduction

The rapid recognition and large response to the discovery [1] of superconductivity in ceramic oxides in 1986 are exemplified by the award of the 1987 Nobel Prize in Physics to Bednorz and Mueller and the large number of new research papers written in this area. The original observation [1] of superconductivity in La-Ba-Cu-O around 35K was followed by a breaking of the "nitrogen barrier" with the discovery [2] of superconductivity in Y-Ba-Cu-O above the boiling point of nitrogen in the 95K range. Now the superconducting transition temperature, T_c, has been raised [3] above 120K. A number of higher temperature observations of superconductivity have been reported, but at present, these are not reproducible.

On the experimental side, the properties of both the normal and superconducting phases of the oxides have been studied extensively [4,5]. Theoretical explanations for the higher T_c's have been suggested [4], but at this point, there is no general agreement on a theoretical explanation of the phenomena. This is contrasted with the situation before 1986 when it was felt that the BCS theory of superconductivity [6] explained almost all the observations in this field, and it became possible recently to predict the existence of new superconductors [7].

There is considerable motivation in this area to achieve room temperature superconductivity in the future and to develop applications now with liquid nitrogen replacing liquid helium as a coolant. These technological considerations have added to the perceived importance of this field. From a more basic scientific point of view, it is difficult to assess the impact of this discovery. The BCS theory [6] and other concepts related to the microscopic understanding of superconductivity gave great insight into the properties of matter ranging from elementary particles to nuclei to atoms, molecules, and solids. If the new superconductors are shown to be similar to their predecessors, except for some simple scale changes, the discovery

would still be important but not scientifically "earth shaking." Investigations at this point suggest that there are significant differences between the new and the "old" superconductors. Scaling takes us to the very limits of the domains of the BCS theory, and there is belief by some that radical changes and new ideas are needed. The possible influence of the correct theory explaining the new phenomena depends on the form and content of the theory, but even if it is unexpectedly dull, the search for it so far has raised so many interesting and challenging questions that at the least we are achieving greater understanding of interactions in solids and a deeper knowledge about materials.

Some Properties of Superconductors

A superconductor has zero resistance. This is a necessary but not sufficient condition to define superconductivity. For a hypothetically perfect conductor, according to Maxwell's equations, magnetic fields present in the conductor when it loses its resistance will be trapped inside the material. This is contrasted with the expulsion of internal magnetic fields for superconductors discovered first by Meissner. The Meissner effect and zero resistivity are the two phenomena most often used to establish the existence of superconductivity.

Other important macroscopic properties of superconductors include the destruction of superconductivity by magnetic fields (critical fields), persistent currents in superconducting loops, and the trapping of magnetic flux in holes within a superconductor. The observation of trapped flux bears on the microscopic picture of superconductivity because the trapped flux is quantized in units of hc/2e where h, c, and e are Planck's constant, the velocity of light, and the charge on the electron. The factor 2e illustrates the importance of electron pairs. The binding of electron pairs is also responsible for the infrared and thermal properties of superconductors. For example, a superconducting gap Δ can be determined from optical studies for frequencies $\omega \sim 2\Delta/\hbar$. For most superconductors $2\Delta \sim 3.5\ K_B T_c$, where k_B is Boltzmann's constant and the low temperature heat capacity depends exponentially on Δ.

An experiment which was important to uncovering the microscopic mechanism of superconductivity is the isotope effect. It was found that by varying the isotopic mass M, T_c varied as $M^{-\alpha}$ with $\alpha \sim 0.5$ for most superconductors. Since lattice vibrations vary in this same way, the "isotope effect" showed that superconductivity depended on electron-lattice interactions. Experiments on the tunneling of electrons between superconductors and normal metals or other superconductors verified this and the details of the theory of the interactions.

BCS Theory

Bardeen, Cooper, and Schrieffer (BCS) proposed their theory [6] in 1957, and applications of the theory were successful in almost all areas where it was tested. The theory was refined and extended using field-theoretic approaches, but the basic ideas remained the same. In particular, the theory proposes that in a superconductor electrons form pairs through a mutual attraction caused by the lattice. The mates in a pair are far apart in space compared to the separation of the pairs. For example, in superconducting Al it is estimated that between the mates in an average pair, there

a million electrons participating in other pairs.

The attractive pairing interaction is characterized by a dimensionless coupling constant λ caused by the electron-lattice interaction. A measure of the Coulomb repulsion between electrons is given by μ. In the original BCS formulation, if $\lambda > \mu$, then

$$T_c = 1.13\ T_D\ e^{-\frac{1}{\lambda-\mu}} \qquad (1)$$

where T_D is the Debye temperature characterizing the lattice vibrations. Since $T_D \sim M^{-0.5}$, this explains the isotope effect.

Equation (1) is approximate. Since T_c depends very sensitively on λ and μ, these couplings must be determined very accurately, and the equation for T_c must be capable of evaluating the effects of the coupling with great precision. This has been achieved only in the last few years, and some successful predictions have been made.

High T_c

There were many proposals for increasing T_c before 1986 and theories related to the maximum possible T_c resulting from electron-lattice interactions. These theories were revived after the recent discoveries, and new ones have been added. Some experimental properties of the oxides indicated that new theories were necessary [4]. Among these, the observations [4] that the isotope parameter $\alpha \sim 0.02$ for Y-Ba-Cu-O and $\alpha \sim 0.15$ for La-Sr-Cu-O encouraged the view that electron-lattice interactions are not alone in pairing the electrons. In addition, estimates [8] of the large λ's needed for these systems were discouraging for phonon mechanisms.

Since all the important interactions in solids arise from the Coulomb interactions among the electrons and ions, the mechanisms investigated focused on electron pairs resulting from excitations of: plasma-like oscillations of the electron sea; excitations involving electron-hole pairs (excitions); acoustic-like waves in a system having electrons of different angular momentum or band character (demons); magnetic excitations including spin fluctuations and antiferromagnetic correlations in a spin liquid (resonating valence bonds); and others. Many of these proposals [4] have been examined and refined in the current literature, but as yet the experimental data obtained does not rule out these mechanisms. In addition, there are suggestions that the unusual behavior results from the particular nature of the crystal structure and vibrational structure of these oxide systems. Hence, anharmonic lattice vibrations; defect enhancements of coupling; the two-dimensional character of the Cu-O planes; and one-dimensional Cu-O chains have been suggested. Again, experiment has not distinguished among these, but at present, chain theories are thought to be inadequate.

Conclusions

In the end, decisions in science are made by experiment. It is, therefore, essential that the theoretical proposals be evaluated and their predictions clarified. There are possible measurements to narrow the field, and these may help filter the theories or parts of theories which are applicable.

It is hoped that close experimental-theoretical collaborations will yield answers in the near future.

Acknowledgements

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References

1. J. G. Bednorz and K. A. Mueller, Z. Phys. B 64, 189 (1986).
2. W. K. Wu, J. R. Ashburn, C. J. Torng, P. H. Hor, and C. W. Chu, Phys. Rev. Lett. 58,908 (1987).
3. C. C. Torardi, M. A. Subramanian, J. C. Calabrese, J. Gopalakrishnan, K. J. Morrissey, T. R. Askew, R. B. Flippen, U. Chowdhry, and A. W. Sleight, Science 240, 631 (1988).
4. Proceedings of the International Workshop on Novel Mechanisms of Superconductivity, eds. V. Kresin and S. Wolf, Plenum, New York (1987).
5. High Temperature Superconductors, eds. D. U. Gubser and M. Schltuer, MRS Society, Pittsburgh (1987).
6. J. Bardeen, L. M. Cooper, and J. R. Schrieffer, Phys. Rev. 1-6, 162 (1957); 108, 1175 (1957).
7. M. L. Cohen, Science 234, 549 (1986).
8. W. Weber, Phys. Rev. Lett. 58, 1371 (1987).

LUNDI 4 JUILLET 1988

14 h 30 - 18 h

DEVELOPPEMENTS NOUVEAUX EN PHYSIQUE NON LINEAIRE

NEW DEVELOPMENT IN NON LINEAR PHYSICS

STABILISATION OF NON-LINEAR EXCITATION IN BIOLOGICAL SYSTEMS

by

H. Fröhlich

In the first meeting of the Institut de la Vie, about twenty years ago it has been shown that owing to the extraordinary dielectric properties of biological materials, the supply of random energy above a certain rate $S > S_o$ may lead to the coherent excitation of an electric vibration in the region of 10^{11}Hz. taking the form of a phase transition analogous to an Einstein-Bose condensation. Essentially this requires existence of certain non-linear interactions. This paper has recently been listed as a "most frequently cited work".

At the same meeting, Prigogine without specifying a particular type of interaction introduced his systems of "Dissipative Structures" showing also that when certain conditions are satisfied, non linear interactions may yield coherent excitations.

Far reaching developments have taken place since then, and a large book entitled Biological Coherence and Response to External Stimuli, ed. H. Fröhlich, Springer Verlag is at present in print.

From the point of view of physics, biological systems have three general characteristics

1. They are rather stable but far from equilibrium.

2. They exhibit a non-trival order.

3. They have extraordinary dielectric properties.

Three kinds of coherent excitation can then arise:

A. Excitation of a single mode of vibration.

B. Excitation of a metastable highly polar ferroelectric state.

C. Excitation of limit cycles or Lotka-Voltera oscillations in complex systems.

Fairly recently the existence of "Deterministic Chaos" in conjunction with bifurcations has also arisen, and experimental confirmation is now available.

To show reproducibility then requires a considerable number of experiments, and quite likely a relevant general concept has not been found yet.

Experimental evidence for the three types of excitation has been available for some time as discussed e.g. in the above mentioned book. It will be noted that solitons belong to B and a minimum energy is required for this type of excitation. Excitation of type A, however, requires an energy flux S to exceed a minimum S_o, $S > S_o$. It has been shown recently that this is available when temperature differences exist, and examples have been given (Phys. Lett. 110A, 480, 1985) where a small temperature difference yields effects where an overall change in temperature does not.

Most remarkable is a Russian finding according to which very sharp frequency dependent stimulations exist in certain humans at positions that are also active to acupuncture. From personal communications I understand that these effects have been confirmed in thousands of cases and should be interpreted in terms of the theory of coherent excitation.

A further aspect of the importance of coherent excitations arises in the theoretical discussion of cancer as first mentioned in the meeting on Biophysical Aspects of Cancer Prague 1987, Charles University. Assume that each cell of an organ, or tissue, can be excited coherently at the same frequency characteristic for the particular organ as first suggest by F. Fröhlich (Cooperative Phenomena, Ed. H. Haken & M. Wagner, Springer Verlag, 1973). When so excited, through long range coherent interaction, a collective normal mode is established; each cell contributes to it and reversly it keeps each cell excited at the appropriate phase and strength. Now preceding cell division, certain changes must take place in that particular cell which thus may lose its resonance with the normal mode. This requires the supply of energy which may not be available. The coherently excited mode thus controls cell division.

The interaction of the cell vibrating with the correct frequency thus has two consequences: It contributes to the magnitude of the amplitude of the excited mode, and, by it, is kept in the correct phase and strength. If now by external circumstances its frequency is changed, then the total amplitude is reduced, but forces on the particular cell try to bring it back to the correct state. If a great number of cells loose their correct frequency, however, then the excited normal mode will collapse. Control of cell division will no longer exist and cancer may arise.

The situation thus resembles an order-disorder transition. The order parameter is the amplitude of the coherently excited mode; cancer is the disordered state.

Recently it has been reported (P. Newmark, Nature 14 May 1987, 327, 101) that normal cells can exert control over those of their number that have been transformed into cancer cells, a characteristic to be expected in an order-disorder transition, as suggested above. It remains to be verified that the relevant interaction arises from long range coherent excitation.

Biophysics, Cell Physiology, Biometeorology, Engineering, Environmental Physiology

Of interest to biophysicists, cell physiologists, biometeorologists, engineers, environmental physiologists.– Level: Research Monograph

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H.F. FRÖHLICH, Liverpool, UK (Ed.)

Biological Coherence and Response to External Stimuli

1988. 97 figs. Approx. 340 pp. Hard cover DM 228,–.
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Contents: Theoretical Physics and Biology.– Theory of Non-Linear Excitations.– Structures, Correlations and Electromagnetic Interactions in Living Matter; Theory and Applications.– Resonant Cellular Effects of Low Intensity Microwaves.– Biological Effects of Low Intensity Microwaves.– Metastable States of Biopolymers.– Photosynthesis.– Emission of Radiation by Active Cells.– Physiological Signalling Across Cell Membranes and Cooperative Influences of Extremely Low Frequency Electromagnetic Fields.– The Interaction of Living Blood Cells.– The Genetic Code as Language.– Electromagnetic Effects in Humans.– Coherent Properties of Energy – Coupling Membrane Systems.– Coherent Excitations in Microtubula; Implications for Biological Information Processing.

This book presents an extensive treatment of the introduction of modern physical concepts into biology. In particular, the concept of coherence finds wide applications and yields novel results in context with multiple problems as they arise in biology: these include long range resonant cellular effects and resonant interactions of biological tissues with low intensity electro-magnetic radiation. Extensive experimental support of the theoretical concept is presented.

Dieses Buch befaßt sich mit der Anwendung moderner physikalischer Konzepte auf die Biologie. Schwerpunkt sind dabei durch schwache elektromagnetische Felder induzierte Resonanz-Effekte in den Zellen von Lebewesen.

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NON LINEAR I.R. EFFECTS IN ACETANILIDE, A MODEL SYSTEM FOR PROTEINS

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Abstract

Measurements of infrared absorption and Raman scattering on crystalline acetanilide, a model system for proteins, show a new band close to the conventional amide I band. Equilibrium properties and spectroscopy data rule out explanation based on conventional assignments. A detailed analysis show that a soliton model, similar to that proposed by Davydov for the α helix in protein, is in satisfactory agreement with the experimental data. Spectroscopy results supporting such assignment are presented and some possible biological implications are briefly mentioned.

An infrared active soliton in a model crystal

Hydrogen bonding is widespread in biomacromolecules, presenting physical features that vary greatly from one case to another. In weak bonds this interaction can be treated as a problem in electrostatics involving a set of fixed and localized interacting charges. On the other hand, in the case of very strong hydrogen bonds, one is faced with a delocalized charge distribution to be treated according to valence theory. Betweeen these two extremes, there are cases where the bonds are of intermediate strength. In these cases, one can model the complex state of affairs by assuming that the local charges depend upon their mutual distances and that these distances in turn depend upon the local charges. Thus in these intermediate cases one can visualize the microscopic source of the anharmonic coupling which gives rise to the non-linear terms in the equations describing the dynamics of the system. This is the case of the N-H ... O=C bond in proteins, for R(NO) distances close to 2.80 A°.

Acetanilide ($CH_3CONHC_6H_5$), or ACN, is an interesting solid because two close chains (spines) of nearly planar hydrogen-bonded amide groups run through the crystal, providing an interesting model for an array of hydrogen-bonded amides in one direction. Moreover, the bond distances in ACN are very close to those found in alpha-helices, where three similar spines are coiled along the helix axis. Since the physical properties of hydrogen-bonded systems are very sensitive to bond distances, we thought ACN would be a useful model system to be used in

searching for new physical features of extended polypeptide chains and perhaps also proteins. We found that a new amide-I band appears at low temperature in crystalline ACN, red-shifted by 15 cm^{-1} from the primary amide-I band at 1665 cm^{-1}, and a large number of other experiments by Raman, X-rays, specific heat and isotropic substitution pointed out that the new band at 1650 cm^{-1} is characteristic of the amide group of ACN in crystal form. A detailed analysis by the usual exiton model cannot account for this new band.

Having excluded conventional explanations we (1), (2), (3), (4) consider the possibility of assigning it to a collective excitation similar to the soliton proposed by Davydov for alpha-helix in proteins (5), (6). Davidov's soliton arises from a cooperative interaction between localized amide-I bond energy and lattice distortion. The bond energy acts through non-linear coupling, as a source of lattice distortion. This lattice distortion reacts, again through non-linear coupling, as a potential well to trap the bond energy and prevent its dispersion via dipole-dipole coupling effects. We followed the same theory with one important difference: for lattice distortion we substituted displacement of the hydrogen-bonded proton. The distinction is vital, because Davydov has shown that photon absorption by his intermolecular vibrational soliton is ruled out by the Frank-Condon principle. Here I shall limit myself to outlining the major points and presenting some conclusions. The main idea was that the effect of introducing localized amide-I energy could displace the ground state of the adjacent hydrogen-bonded proton. This displacement of the proton acts to trap the

amide-I band energy and prevent its dispersion via dipole-dipole interaction effects. The combined excitation was proved to be a soliton, and we assigned the binding energy of this soliton to the experimentally observed red shift of 15 cm^{-1} from the conventional amide-I band to the unconventional amide-I band. A more recent theoretical work (7) has identified this unconventional band as a vibronic analog of a small Holstein polaron.

Biological implications

The work of ACN reported above has been motivated by a few relevant biological implications that we believe it would be appropriate to mention. Biochemical events involving the reactions or changes of state of a macromolecule may be supposed to occur one step at a time (8). Thus they may often be represented in terms of a network of closed pathways or loops. At equilibrium there will be no net circulation around the network, the conditions of detailed balance being fulfilled. A living system requires process, as in the form of a one-way circulation around the network. In the fully-developed organism this state of affairs, which provides for the transduction of free energy from one chemical reaction to another, is made possible by the presence of highly developed polyfunctional enzymes. In an effort to provide a mechanism for this one-way circulation, we have recently introduced the concept of a trapped soliton as an energy packet created at one point of the network and liquidated at another (9). This mechanism can play a role in many biochemical

cycles and perhaps also in prebiotic recations (10). We postulated the formation of a soliton trapped as a ligand on the protein matrix, for which the surrounding heat bath serves as a sink. This soliton is assumed to display physical features quite similar to those observed experimentally in ACN. All this follows from the intrinsic structure of the hydrogen-bonded polypeptide chain itself, because of its capability to let the vibrational soliton be stable or decay in different backbone conformations.

References

1 - Careri G., Buontempo U., Carta F., Gratton E. and Scott A.C. 1983 - Phys. Rev. Lett. 51, 304.

2 - Careri G., Buontempo U. Galluzzi F., Scott A.C., Gratton E. and Shymsunder E., 1984. Phys. Rev. B, 30, 4689.

3 - Scott A.C., Gratton E., Shymsunder E. and Careri G. 1985. Phys. Rev. B32.

4 - Careri G., Gratton E. and Shyamsunder E. 1988. Phys. Rev. A (in press).

5 - Davydov A.S. and Kishlukha N.I. 1973. Phys. Status Solidi B 59, 465.

6 - Davydov A.S. 1973. J. Theor. Biol. 38, 559.

7 - Alexander D.M. and Krumhausl J.A. 1986. Phys. Rev. A (in press)

8 - Wyman J. 1975. Proc. Natl. Acad. Sci. U.S.A. 72, 3983-3987.

9 - Careri G. and Wyman J. 1984. Proc. Natl. Acad. Sci. U.S.A. 81, 4386-4388.

10 - Careri G. and Wyman J. 1985. Proc. Natl. Acad. Sci. U.S.A. 82, 4115.

Energy Transfer in Nonlinear Vibration Systems

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The nonlinear energy channeling among the normal modes of a vibration system is analysed on the basis of the Manley - Rowe energy relations.

Introduction

Frohlich derived a kinetic equation (FKE) describing the basic principles of the excitation of the coherent states and the energy transfer among the normal vibration modes [1 - 5]. The fundamental terms in the FKE contain one- and two-quantum processes representing the energy suply, the energy losses to the heat bath, and the energy channeling between the normal modes with the help of the heat bath. Livshitz and Yushina pointed out the significance of some additional terms in the FKE [6 - 8]. Coherent vibration systems are very likely located in cellular membranes. Due to the membrane potential the vibration systems have very likely highly nonlinear properties. The multiple quantum processes of the energy transfer, therefore, may be significant as is described in [9 - 10].

Manley - Rowe Energy Equations

Manley and Rowe [11] derived the energy relations (MRR) for a nonlinear elecric oscillator with a wide spectrum of the mode frequencies. They assumed the total energy of the oscillator to be constant. The MRR are valid for any nonlinear oscillator [9 - 10]. For a classical nonlinear oscillator the MRR may be easily derived using the Fourier expansion. We get

$$(1)\qquad \sum_{n=-\infty}^{+\infty}\sum_{m=0}^{+\infty}\frac{mP_{m,n}}{m\omega_1 + n\omega_2} = 0\,, \qquad \sum_{m=-\infty}^{+\infty}\sum_{n=0}^{+\infty}\frac{nP_{m,n}}{m\omega_1 + n\omega_2} = 0\,,$$

where $P_{m,n}$ is the derivative of the energy with respect to the time in the mode with the circular frequency $m\omega_1 + n\omega_2$ (m, n are integers).

The quantum anology of Eqs. (1) is derived in [9]. The Hamiltonian of the vibration system may be given as

$$(2)\qquad H = H_0 + H_1 + H_2\,,$$

where H_0 and H_1 are given by [12 - 13]

$$(3) \qquad H_0 = \sum_i \hbar\omega_i a_i^\dagger a_i + \sum_k \hbar\Omega_k c_k^\dagger c_k + \sum_g \hbar\Omega_g P_g^\dagger P_g \ ,$$

$$(4) \qquad H_1 = \sum_{i,k} (\varphi a_i c_k^\dagger + \varphi^* a_i^\dagger c_k) + \sum_{g,i} (\xi P_g a_i^\dagger + \xi^* P_g^\dagger a_i) +$$

$$+ \sum_{i,j,k} (\chi a_i^\dagger a_j c_k^\dagger + \chi^* a_i a_j^\dagger c_k) \ ,$$

and the multiple quantum term H_2 is given by [9 - 10]

$$(5) \qquad H_2 = \sum_{i,j,\ell} \sum_{m,n} [\varkappa a_\ell^\dagger (a_i)^m (a_j^\dagger)^n + \varkappa^* a_\ell (a_i^\dagger)^m (a_j)^n +$$

$$+ \sigma a_\ell^\dagger (a_i^\dagger)^m (a_j)^n + \sigma^* a_\ell (a_i)^m (a_j^\dagger)^n +$$

$$+ \mu a_\ell^\dagger (a_i^\dagger)^m (a_j)^n + \mu^* a_\ell (a_i^\dagger)^m (a_j)^n] \ .$$

Here ω_i, Ω_k, Ω_g are the circular frequencies, $a_i^\dagger$, $c_k^\dagger$, $P_g^\dagger$ are the creation operators, and a_i, c_k, P_g are the anihilation operators of the vibration system, of the heat bath, and of the energy source, respectively; φ, ξ, χ, $\varkappa$, σ, μ are the coupling coefficients. Using the time dependent perturbation theory the MRR may be derived in the form

$$(6) \qquad \sum_{n=-\infty}^{+\infty} \sum_{m=0}^{+\infty} \frac{m\langle \dot{N}_q \rangle \omega_q}{m\omega_i + n\omega_j} = 0 \ , \qquad \sum_{m=-\infty}^{+\infty} \sum_{n=0}^{+\infty} \frac{n\langle \dot{N}_q \rangle \omega_q}{m\omega_i + n\omega_j} = 0 \ ,$$

where $\langle \dot{N}_q \rangle$ is the expectation value of the first derivative (with respect to the time) of the number of the energy quanta in the qth mode. Eqs. (6) represent the interactions between the modes without the help of the heat bath and express the principle of the conservation of the energy.

Nonlinear Energy Channeling

The expectation values $\langle \dot{N}_q \rangle$, $\langle \dot{N}_i \rangle$, $\langle \dot{N}_j \rangle$ are given by

$$(7) \qquad \langle \dot{N}_q \rangle = -B \ , \quad \langle \dot{N}_i \rangle = \sum_{m=0}^{+\infty} \sum_{n=0}^{+\infty} mB \ , \quad \langle \dot{N}_j \rangle = \sum_{m=0}^{+\infty} \sum_{n=0}^{+\infty} nB \ ,$$

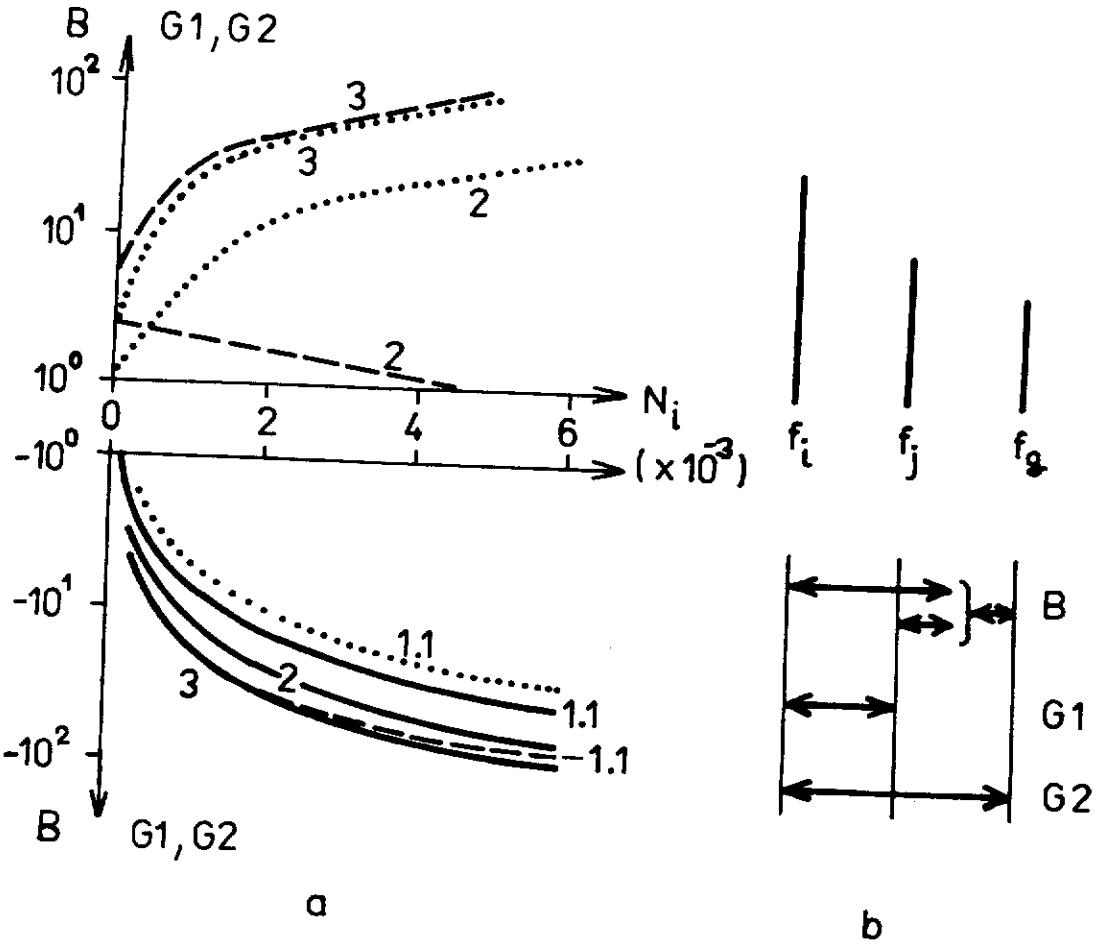

Fig. 1: a) B, G_1 (the dashed curves), and G_2 (the dotted curves) as a function of N_i (the ith mode has the lowest frequency).
b) A schematic picture of the energy channeling among the modes with the frequencies f_i, f_j, and f_q.

where

$$(8) \qquad B = -(2\pi/\hbar)|x|^2[(\langle N_q\rangle + 1)\alpha_i(m)\beta_j(n) - \langle N_q\rangle\beta_j(m)\alpha_j(n)] ,$$

$$\alpha_i(m) = \langle N_i\rangle(\langle N_i\rangle - 1)\ldots(\langle N_i\rangle - m + 1) ,$$

$$\beta_j(n) = (\langle N_j\rangle + n)(\langle N_j\rangle + n - 1)\ldots(\langle N_j\rangle + 1) .$$

Eqs. (7 - 8) are valid for $\omega_q = m\omega_i + n\omega_j$ and represent the x term in the H_2.

Similar equations may be derived for the σ and the μ terms in the H_2. (The σ and the μ terms represent the processes with the frequency relations $\omega_q = -\omega_i + \omega_j$, and $\omega_q = \omega_i - \omega_j$.) These relations describe the energy channeling among the vibration modes the frequencies of which are in convenient relationships.

The direction of the energy channeling depends on the energy excitations of the modes in question. All the equations represent only an approximate solution as the multiple quantum terms with the help of the heat bath were neglected.

We evaluated the B coefficients for a simple model of three interacting modes with the frequencies f_i = .1 THz, f_j = .2 THz, and f_q = .3 THz. The energy levels of the qth mode, and of the jth mode are 1.1, 2, and 3 times greater then the levels in the thermal equilibrium. The nonlinear process in question channels the energy from the ith mode and the jth mode to the qth mode (i.e. upwards) as follows from Fig. 1a. The χ process of the FKE represented by the G_1 and G_2 coefficients (Fig. 1b) channels the energy downwards if the energy levels of the jth mode and of the qth mode are sufficiently high. B, G_1, and G_2 are given in relative values.

Conclusions

1) The nonlinear energy transfer among the normal modes of a vibration system includes the multiple quantum processes with the help of the heat bath and those without the help of the heat bath. We analysed the processes without the help of the heat bath.

2) The direction of the energy flow may be downwards as well as upwards. The direction of the energy flow depends on the energy levels and on the frequencies of the vibration modes.

3) The electromagnetic field generated by the coherent vibrations in living cells has very likely a fundamental biological function. The changes of the field may be connected with the nonlinear interactions.

References

[1] Frohlich H.: Phys. Letters 26A(1968)402.
[2] Frohlich H.: Int. J. Quantum Chem. 2(1968)641.
[3] Frohlich H.: Riv. Nuovo Cimento 7(1977)399.
[4] Frohlich H.: IEEE Trans. MTT-26(1978)613.
[5] Frohlich H.: Adv. Electron. Electron Phys. 53(1980)85.
[6] Livshitz M. A.: Biofizika 17(1972)694.
[7] Livshitz M. A.: Biofizika 22(1977)744.
[8] Yushina M. Yu.: Phys. Letters 91A(1982)372.
[9] Pokorny J., Vacek K., Fiala J.: Phys. Letters 114A(1986)339.
[10] Pokorny J., Vacek K., Fiala J.: Czech. J. Phys., B36(1986)1443.
[11] ManleyJ. M., Rowe H. E.: Proc. IRE 44(1956)904.
[12] Wu T. M., Austin S. J.: Phys Letters 64A(1977)151.
[13] Wu T. M., Austin S. J.: J. theor. Biol. 71(1978)209.

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RESUME DE LA COMMUNICATION "L'ORDRE CHAOTIQUE"

par P. Bergé

Le but de la communication est de montrer que le comportement très compliqué d'un système n'est pas nécessairement à rattacher à une complexité inhérente à ce dernier.

Il est naturel d'associer une évolution erratique, imprévisible, à la conjonction d'une multiplicité d'évènements indépendants. C'est ainsi que les numéros que l'on tire au loto sortent de manière parfaitement imprévisible -erratique donc- du fait du très grand nombre de chocs que subissent les boules avant d'être extraites. Il en est ainsi de beaucoup de phénomènes dont la nature aléatoire est à relier à une loi des grands nombres : en effet, une quantité dont la grandeur résulte de l'effet d'une multitude de variables indépendantes aura nécessairement une variation irrégulière.

A l'opposé, les mathématiques nous donnent des exemples d'équations très simples dont la solution est, néanmoins, parfaitement irrégulière. C'est ainsi qu'on peut tirer des nombres au hasard, aussi efficacement qu'à la loterie, par une transformation simple que l'on recommence répétitivement. Par exemple, on prend un nombre X_0 entre 0 et 1 (pas trop simple !) ; on calcule l'image X_1 de ce nombre par la relation $X_1 = 4\ X_0\ (1 - X_0)$ puis on calcule l'image X_2 de X_1 par la même relation et ainsi de suite.

On obtient ainsi une série de nombres X_0, X_1, X_2 qui se succèdent au hasard, c'est-à-dire sans que l'on puisse déterminer un ordre dans leur apparition (on dit que la série est chaotique). Et pourtant, quoi de plus déterministe que la relation qui les engendre ? C'est ce paradoxe (apparent) qui traduit un nouveau concept : celui de chaos déterministe. Quittant les mathématiques et abordant les systèmes "réels", nous pouvons dire que point n'est besoin d'une complexité inhérente au système pour que son évolution soit erratique. C'est ainsi que les systèmes les plus simples peuvent avoir des comportements chaotiques, ce qui repose le problème plus philosophique de la vraie nature du hasard.

.../....

Dans ce cas, le comportement chaotique, c'est-à-dire l'impossibilité de faire des prévisions, provient d'une amplification incessante des plus infimes incertitudes dans les conditions initiales (S.C.I.). Entendons par là que si nous considérons deux conditions initiales extrêmement voisines, l'écart minime qui les sépare initialement va augmenter sans cesse (exponentiellement) quand le temps passe. C'est ainsi que les deux évolutions correspondantes, un moment semblables, vont devenir rapidement très différentes. C'est ce qui interdit, en pratique, toute prévision dans un système doté de SCI. Cette SCI peut intervenir dès que des non-linéarités sont présentes et que le nombre de variables du système (appelées degrés de liberté) est au moins égal à 3. C'est ainsi qu'un pendule sollicité périodiquement peut présenter des mouvements chaotiques.

On comprend toute l'importance de ce nouveau concept qui peut remettre en question bien des interprétations et des idées reçues. Citons, par exemple, l'évolution fort désordonnée de la concentration dans certaines réactions chimiques particulières. On aurait pu les attribuer à une mauvaise stabilisation de l'un des paramètres entraînant la présence de fluctuations. En fait, il n'en est rien et on a pu montrer que ce "chaos chimique" résultait de l'évolution déterministe d'une simple oscillation de la réaction. Beaucoup de phénomènes, incompréhensibles de par leur comportement erratique, ont peut-être une origine fort simple et déterministe...

Références

P. Bergé, Y. Pomeau, C. Vidal
"L'ordre dans le chaos", Hermann 1984.
Traduction anglaise "Order within chaos", Hermann-Wiley 1986.

M. Dubois, P. Atten, P. Bergé
La Recherche n° 185, vol. 18, p. 191 (1987).

NONLINEAR ELECTRODYNAMICS IN CELL MEMBRANE TRANSDUCTIVE COUPLING OF ELECTROMAGNETIC FIELDS AND HUMORAL STIMULI

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A connected picture of the sequence and energetics of major events that couple humoral stimuli (hormones, neurotransmitters, antibodies) from surface receptor sites to the cell interior is now emerging from use of weak electromagnetic (EM) fields to manipulate inward and outward signal streams through cell membranes.

1. Intramembranous Protein Particles (IMPs) and the Fluid Mosaic Cell Membrane Model."

There has been a remarkable growth in knowledge of the structural and functional organization of cell membranes through use of other sophisticated research tools. On the one hand, these studies have revealed numerous strands of protein (intramembranous particles, IMPs) inserted into the thin double layer of fat molecules that forms the plasma membrane. On the other, studies of both electrical and chemical intercellular communication have focused on specialized regions of contact between adjacent cell membranes. These regions form gap-junctions that couple cells electrically and chemically.

IMPs span the membrane from inside to outside. They have external protrusions into the fluid surrounding the cell. Their outer tips are terminal glycoprotein strands that sense electric fields and form receptor sites for chemical stimulating molecules. They have functional contacts inside cells with key elements of the cell machinery, including enzymes and the numerous fine tubes of the cytoskeleton. They make functional contact inside the cell with key elements of the cell machinery, including enzymes and the numerous fine tubes of the cytoskeleton. IMPs "float" in the sea of lipid molecules of the plasma membrane, leading to the generally accepted "fluid mosaic model of the cell membrane (Singer and Nicolson, 1972). Thus, these intramembranous strands form signaling pathways by which external stimuli are sensed and conveyed to the cell interior.

2. Intercellular Communication Through Gap Junctions.

The regions of contact between membranes of adjacent cells form gap junctions that couple cells electrically and chemically (Robertson, 1963). These junctions are perforated by numerous tiny tubes (connexons) that span the entire membrane that allow ionic coupling and metabolic cooperation in transfer of essential metabolic substances between cells (Fletcher et al., 1987). Disruption of intercellular communication through gap junctions leads to serious disorders of growth control, including tissue repair and neoplastic transformation (Loewenstein, 1981). Our studies indicate a synergic action of chemical cancer promoters with weak EM fields in modification of gap junction functions. Disruption of gap junctions is now viewed as a prime factor in cancer promotion and tumor formation (Adey, 1988a and b; Trosko, 1987; Yamasaki, 1987).

3. Electrical Benchmarks in Physiological Organization of Cells and Tissues.

The membrane potential of approximately 0.1 V exists across the extremely thin lipid plasma membrane, typically about 40° thick; a membrane so thin that in consequence there is an enormous electric gradient of 10^5V/cm across the cell membrane. Physiological electric oscillations in fluid surrounding cells are many orders of magnitude weaker than this natural barrier of the membrane potential. These weak pericellular gradients have therefore been considered too weak to play a physiological role.

Nevertheless, many organisms including man are sensitive to tissue gradients in the range 0.1-100 mV/cm. These sensitivities have been observed in many tissues and cell cultures, including lymphocytes, and cells from liver, ovary, skin, bone, cartilage and nervous tissue (see Adey, 1981, 1984, 1988a and b for reviews).

These interactions require appropriate consideration of the role of cooperative processes and associated nonlinear electrodynamics at cell membranes revealed with imposed EM fields (Adey and Lawrence, 1984). These cooperative phenomena are in the realm of nonequilibrium thermodynamics, far removed from traditional equilibrium models of cellular excitation that have focused on depolarization of the membrane potential and on associated massive changes in ionic equilibria across the cell membrane.

3. Cooperative Processes Initiated by EM Fields at Cell Surfaces, with Amplification of Initial Signals.

Extracellular current flow in tissue associated with physiological activity and with imposed EM fields spreads longitudinally through narrow gutters separating adjacent cells. Initial events associated with these pericellular fields and with binding of stimulating molecules at their receptor sites elicit a highly cooperative modification of Ca^{2+} binding to glycoproteins along the membrane surface. This is an amplifying stage. From concurrent manipulation of initial molecular binding events with imposed EM fields, there is evidence of a far greater Ca^{2+} efflux than is accounted for in the events of receptor-ligand binding.

Furthermore, there is striking evidence for the nonequilibrium character of this modification in Ca^{2+} binding in its occurrence in quite narrow windows in stimulus frequency and amplitude (Adey, 1988b). "Tuning curves" of altered Ca^{2+} efflux from tissues as a function low frequencies in imposed fields were first described by Bawin et al. (1975, 1976) with maximum sensitivities around 16 Hz. Neither size nor geometry of the test biota are primary determinants of these interactions. Fields in the same frequency range (1-100 Hz) also show intensity windows for Ca^{2+} efflux with induced tissue electric gradients typically in the range 10^{-7}-10^{-1} V/cm. Windowing in frequency, amplitude and time also occurs in a wide range of Ca-dependent cell surface and intracellular mechanisms, including allogeneic cytotoxicity and enzymatic responses to EM field interactions at cell surfaces.

Cooperativity found in these functional linkages in biological systems may be defined as ways in which components of a macromolecule, or a system of macromolecules, act together to switch from one stable state of a molecule to another. These joint actions frequently involve phase transitions, hysteresis, and avalanche effects in input-output

relationships. Initial triggers to cooperative processes may be weak and the amplified responses orders of magnitude larger, raising questions about thresholds and the minimum size of an effective triggering stimulus. Most important is the thermal Boltzmann (kT) noise in the system. At room temperature, this is 0.02 eV and is the basis for molecular collisional interactions. The sensing of a gradient of 10^{-7} V/cm modeled on this threshold would require a cooperative molecular system extending over 300 m. The abundant evidence that extracellular gradients from 10^{-1} V/cm down to this level are biologically significant in systems of cellular dimensions is a strong reminder of the importance of better understanding molecular and morphological substrates of this transductive coupling.

4. Three Stages in Transmembrane Signaling from Cell Surface to Interior

There is a minimal sequence of three steps in transductive coupling between cell surfaces and intracellular enzymatic systems and organelles. Each step is Ca-dependent. a) The first weak electrochemical events at molecular receptor sites and in detection of EM fields are sensed by cell surface glycoproteins. b) These amplified surface events are signaled to the cell interior along transmembrane portions of IMPs. c) Internally there is signal coupling to intracellular enzymes and through the cytoskeleton to the nucleus and other organelles.

The long strands of membrane receptor proteins for the human epidermal growth factor (EGF), the nerve growth factor (NGF) and the insulin receptor have been studied as models of coupling proteins in transmembrane signaling. In all three, the molecule appears to cross the membrane only once. The intramembranous segment is extremely short, and is composed of 23 predominantly hydrophobic amino acids, probably incapable of supporting ionic or protonic conduction. Nevertheless, addition of EGF to human epidermal cell cultures causes a 2-4-fold increase in cytoplasmic free Ca within 30 sec, all derived from extracellular sources and without a change in membrane potential Moolenaar et al., 1986; Ullrich et al., 1985).

We have hypothesized that this transmembrane signaling may involve nonlinear vibration modes in helical proteins, leading to Davydov soliton waves (Davydov, 1979; Scott, 1985). Although evidence for soliton waves in DNA and helical proteins remains inconclusive, studies of millimeter microwave absorption in macromolecules, bacteria and simple cellular systems have revealed highly nonlinear absorption in the frequency range 10-100 GHz (see Adey, 1988b and c for reviews).

5. Molecular Markers of Transductive Coupling Through Cell Membranes: Cytotoxicity Studies and Intracellular Enzyme Activities.

Is cooperative modulation of cell surface Ca binding a first step that culminates in Ca-dependent physiological responses in key enzyme systems within cells?

We have shown that cell membrane mechanisms that mediate cytotoxic destruction of target cells by direct contact (allogeneic T lymphocytes targeted against lymphoma cells, for example) are modulated by weak pericellular EM fields. Three groups of intracellular enzymes respond to signals initiated at cell membranes as a response to EM field exposure: a) membrane-bound adenylate cyclase involved in activation of protein kinases through conversion of ATP to cAMP; b) cAMP-independent protein kinases that perform

messenger functions (Fig. 1); c) ornithine decarboxylase (ODC), essential for growth in all cells by its participation in synthesis of polyamines essential for DNA formation.

6. Models of Cooperative Organization in Physiological Systems.

Experiments cited above imply that interactions occurring at athermal levels between biological substrates and low frequency components of EM fields must take place in biomolecular systems exhibiting dynamic patterns of organization. Historically, these patterns have been studied in populations of elements in terms of complex flow patterns. They can undergo sudden transitions to new self-maintaining arrangements that are relatively stable over time. They are dissipative in nature, since they are sustained by continuous energy inputs and are thus far from equilibrium with respect to at least one important system parameter. As nonequilibrium processes, they may be exhibit resonant or windowed phenomena, an important aspect of their occurrence in tissue interactions with EM fields.

Self-sustained oscillations in biological systems have also been modeled on the requirement for interaction of regular external perturbations with internal oscillations, thus synchronizing the system to the external drives (entrainment). There is a sharp frequency response, exhibiting both frequency and intensity windows and rather irregular behavior near the entrainment region. A further increase in energy of external driving fields, both static and periodic, leads to sequences of period-doubling bifurcations, alternating with quasi-periodic and irregular regions (chaos). As a consequence, a regularly driven self-oscillating system may exhibit intrinsic chaotic behavior, even though the underlying dynamic is strongly deterministic (Kaiser, 1984). Finally, still higher levels of energy input destabilize the system (collapse), leading to the onset of propagating pulses (solitons). A nonlinear temporal structure is thus replaced by a nonlinear spatiotemporal structure in a dispersive process.

7. Summary

Use of weak EM fields to study the sequence and energetics of events that couple stimuli from surface receptor sites to the cell interior has identified the cell membrane as a primary site of interactions with these low frequency fields. Field modulation of cell surface chemical events indicates a major amplification of initial weak triggers associated with binding of hormones, antibodies and neurotransmitters to their specific binding sites. Calcium ions play a key role in this stimulus amplification.

It is at the atomic level, rather than the molecular, that physical, rather than chemical events now appear to shape the flow of signals and transmission of energy in biomolecular systems. These recent observations have opened doors to new concepts of communication between cells as they whisper together across barriers of cell membranes.

8. References

1. Adey, W.R. (1981) Physiol. Rev. 61:435.
2. Adey, W.R. (1984) In: Nonlinear Electrodynamics in Biological Systems, W.R. Adey and A.F. Lawrence, eds. Plenum, New York.

MARDI 5 JUILLET 1988

9 h - 12 h 30

ETUDES NOUVELLES SUR LES STRUCTURES ET INTERACTIONS MACROMOLECULAIRES

NEW STUDIES OF MACROMOLECULAR STRUCTURES AND INTERACTIONS

SELF-CATALYSIS AND ENZYMATIC ACTIVITY IN RNA. Thomas R. Cech, Howard Hughes Medical Institute, University of Colorado, Boulder, CO 80309-0215, U.S.A.

Chemical reactions in a living cell rarely take place by themselves, but are generally catalyzed by macromolecules called enzymes. It was long thought that all enzymes were proteins. The finding that RNA (ribonucleic acid), a form of the genetic material, can in some cases act as an enzyme overturns this principle, and may throw new light on the origin of life.

Self-catalyzed RNA Splicing.

Eukaryotic genes are often interrupted by stretches of non-coding DNA called intervening sequences (IVS) or introns. RNA polymerases transcribe both the exons (coding sequences) and the intervening sequences to give large precursor RNAs. The intervening sequences are subsequently removed by a cleavage-ligation process known as RNA splicing.

In the case of a pre-ribosomal RNA from *Tetrahymena*, a ciliated protozoan, we found that accurate RNA splicing occurs *in vitro* in the absence of protein (1,2). Splicing requires only a divalent cation (Mg^{2+} or Mn^{2+}), a monovalent cation, and some form of the nucleoside guanosine. The splicing activity is intrinsic to the IVS RNA. RNA self-splicing exemplifies intramolecular catalysis in that specific cleavage-ligation reactions are accelerated far beyond the basal uncatalyzed rate (> 10^{10}-fold).

Tetrahymena pre-rRNA splicing is a two-step mechanism (3). Both steps occur by transesterification, an exchange of phosphate esters that produces no net change in the number of ester linkages. This mechanism explains how RNA ligation can take place without an external energy source as is often provided by ATP or GTP hydrolysis. In the first transesterification, the 3'-hydroxyl of a free guanosine molecule (GTP, GDP, GMP, or guanosine) acts as the nucleophile, attacking the 5' splice site (Fig. 1, top). This reaction leaves a 3' hydroxyl group at the end of the 5' exon, which can then act as the nucleophile for the second transesterification reaction, exon ligation.

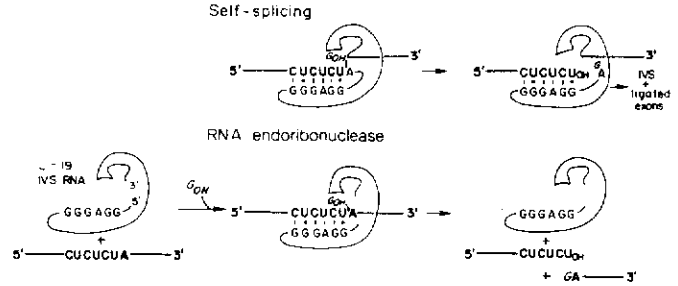

Fig. 1. A model for the endonuclease action of L - 19 IVS RNA. The mechanism is an intermolecular version of the first step of pre-rRNA self-splicing. Thin letters and lines, IVS sequences; bold letters and thick lines, exon sequences (top) or substrate RNA sequences (bottom); the G in italics is a free guanosine nucleotide or nucleoside.

Self-splicing has also been demonstrated for a number of other rRNA precursors and mRNA precursors from fungal mitochondria and bacteriophage T4 as well as nuclei (reviewed in ref. 3). In many cases these undergo self-splicing by the same guanosine-dependent transesterification mechanism described for *Tetrahymena* nuclear pre-rRNA splicing; in other cases self-splicing occurs by a lariat-formation mechanism analogous to that described for nuclear pre-mRNA splicing.

The *Tetrahymena* IVS RNA as an Enzyme

After its excision from the *Tetrahymena* pre-rRNA, the IVS RNA retains catalytic activity. For example, shortened forms of the IVS RNA, or "ribozyme", can perform a variety of cleavage-ligation reactions with oligoribonucleotide substrates (4,5). These include nucleotidyl transfer reactions that allow the ribozyme to function as a sequence-specific endoribonuclease (Fig. 1, bottom). The ribozyme works with multiple turnover, thereby satisfying the definition of an enzyme. The sequence specificity of these reactions can be altered in a predictable manner by site-specific mutagenesis of the active site of the ribozyme (5).

The ribozyme can also catalyze RNA polymerization reactions (4,6). For example, pentacytidylic acid (C_5) can be extended by the successive addition of mononucleotides derived from a guanylyl-(3'-5')-nucleotide (GpN). C's or U's are added to C_5 to generate chain lengths of 10 to 11 nucleotides, with longer products being generated at greatly reduced efficiency (6). The reaction is analogous to that catalyzed by a replicase with C_5 acting as the primer, GpN's as the nucleoside triphosphates, and a sequence in the IVS providing a template. The demonstration that an RNA enzyme can catalyze net elongation of an RNA primer supports theories of prebiotic RNA self-replication.

Structure of the Active Site for RNA Catalysis.

Some of the sequences and structures of the IVS that are involved in substrate sequence specificity and in catalysis have been identified. A tertiary-structure model of the active site of this RNA enzyme has been constructed (Fig. 2) based on comparative sequence analysis of related group I intervening sequences (7,8), data on the accessibility of each nucleotide to chemical and enzymatic probes, and principles of RNA folding derived from a consideration of the structure of tRNA determined by X-ray crystallography (9).

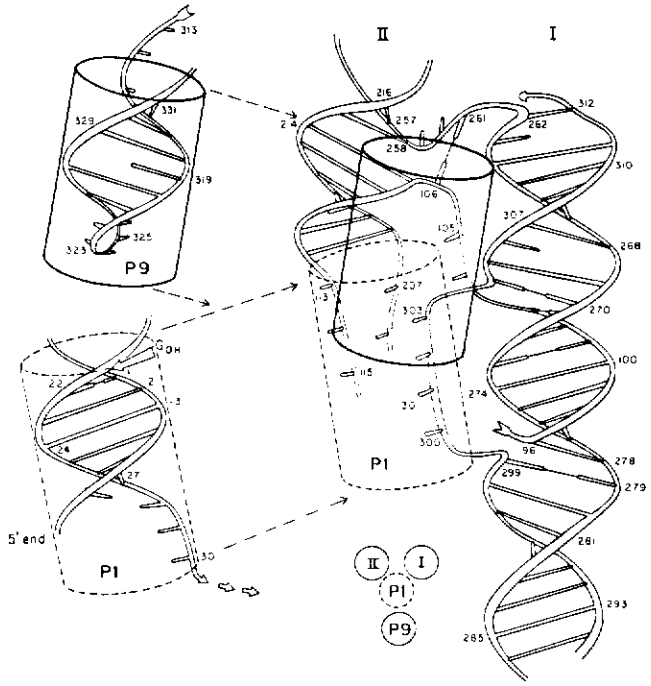

Fig. 2. Three-dimensional structure model of the active site of a ribozyme. Possible interaction of the 5' splice-site duplex (P1, dashed cylinder) with the catalytic center of the *Tetrahymena* IVS RNA is shown. Paired element P9 (solid cylinder) might interact with P1 from the side opposite that of domains I and II of the catalytic center as shown. Small diagram at bottom depicts top view of the model. See Kim and Cech (9) for details.

In the model, the catalytic center has a two-helix structural framework composed of the base-paired segments of the group I conserved sequence elements. The structural framework supports and orients the conserved nucleotides that are adjacent to the base-paired sequence elements; these conserved nucleotides are proposed to form the active site and to bind the 5' splice-site duplex and the guanine nucleotide substrate (Fig. 2). Tests of the model are in progress.

References

1. Cech, T.R., Zaug, A.J. and Grabowski, P.J., *In vitro* Splicing of the Ribosomal RNA genes of *Tetrahymena*: Involvement of a Guanosine Nucleotide in the Excision of the Intervening Sequence, *Cell* 27, 487-496 (1981).
2. Kruger, K., Grabowski, P.J., Zaug, A.J., Sands, J., Gottschling, D.E. and Cech, T.R., Self-splicing RNA: Autoexcision and Autocyclization of the Ribosomal RNA Intervening Sequence of *Tetrahymena*, *Cell* 31, 147-157 (1982).
3. Cech, T. R., The Chemistry of Self-Splicing RNA and RNA Enzymes, *Science* 236, 1532-1539 (1987).
4. Zaug, A. J. and Cech, T. R., The Intervening Sequence RNA of *Tetrahymena* is an Enzyme, *Science* 231, 470-475 (1986).
5. Zaug, A. J., Been, M. D. and Cech, T. R., The Tetrahymena Ribozyme Acts like an RNA Restriction Endonuclease, *Nature* 324, 429-433 (1986).
6. Been, M. D. and Cech, T. R., RNA as an RNA Polymerase: Net Elongation of an RNA Primer Catalyzed by the *Tetrahymena* Ribozyme, *Science* 239, 1412-1416 (1988).
7. Michel, F., Jacquier, A. and Dujon, B., Comparison of Fungal Mitochondrial Introns Reveals Extensive Homologies in RNA Secondary Structure, *Biochimie* 64, 867-881 (1982).
8. Davies, R. W., Waring, R. B., Ray, J. A., Brown, T. A. and Scazzocchio, C., Making Ends Meet: A Model for RNA Splicing in Fungal Mitochondria, *Nature* 300, 719-724 (1982).
9. Kim, S.-H. and Cech, T. R., Three-dimensional Model of the Active Site of the Self-Splicing rRNA Precursor of *Tetrahymena*, *Proc. Natl. Acad. Sci. USA* 84, 8788-8792 (1987).

GENETIC ANALYSIS OF SMALL NUCLEAR RIBONUCLEOPROTEIN PARTICLES (snRNPs) IN YEAST. Christine Guthrie, Department of Biochemistry and Biophysics
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The process by which introns are removed from precursors to messenger RNA (pre-mRNA) in eukaryotic nuclei shares certain fundamental properties with splicing reactions of the group II type (1). Since the latter can occur autocatalytically in vitro, catalysis must be achieved by the formation of active sites comprised solely of RNA. Highly conserved structural elements which reside primarily within the intron have been shown to underly the geometry required for function. In contrast, the only conserved features of nuclear pre-mRNA introns are restricted to short regions at or near the splice junctions, and splicing takes place in association with an extensive trans-acting machinery dubbed the spliceosome. Work during the last several years has revealed that prominent trans-acting factors are U1, U2, U4, U5 and U6, five small nuclear RNAs (snRNAs) which exist as ribonucleoprotein complexes termed snRNPs (2). Presumably the low information content in cis within this class of introns is compensated by the participation of the snRNPs, which act in trans to impart the appropriate architecture for catalysis.

In principle, both RNA- and protein-based interactions contribute to communications between the snRNPs and the intron and the snRNPs with one another. In cognizance of group II reactions, however, it seems evident that nuclear pre-mRNA splicing evolved from an RNA machine. As with the recent revolution in our understanding of the ribosome, the mechanism may be most directly appreciated by seeking to establish the RNA-mediated aspects of the reaction. We have thus chosen to focus our efforts on the snRNA components of the spliceosomal snRNPs. In particular, we have carried out our studies on the snRNAs from the budding yeast Saccharomyces cerevisiae because of the ability to exploit the facile genetic techniques available in this organism. Moreover, this has allowed us to obtain information on snRNA structure from a broad phylogenetic distance. The power of comparative sequence analysis to identify functionally important features of sequence and structure has been clearly demonstrated in the tour-de-force studies of rRNA pioneered by Woese, Noller and their colleagues (3).

Our knowledge of the complete set of spliceosomal snRNAs from yeast has provided several unexpected insights into the evolutionary constraints operating on the five snRNA species. Interestingly, the similarity between the yeast and mammalian homologues varies considerably. The structure of yeast U2 RNA is quite surprising: the S. cerevisiae RNA is more than six times as long as its human counterpart (1,175 vs. 189 ntes.) and almost all the sequence similarity is in the first 80 nucleotides, where the two RNAs are 75% identical (4). Likewise, yeast U5 (5) and U1 (6) RNAs are respectively 1.5 and 3.5 times the length of their mammalian counterparts, with which they share only limited sequence and structural similarity. In contrast to the other spliceosomal RNAs, however, U4 and U6 are very close in size to those in mammals. The primary structure of yeast and human U4 is significally different. However, yeast U6 is virtually identical in size,

sequence and structure to its mammalian homologue (7). These apparent constraints argue for a central role in the splicing process and suggest that U6 snRNA is in close contact with several components of the machinery.

Each of the yeast snRNAs is encoded by a single copy gene (in striking contrast to the large multi-gene familes characteristic of snRNAs in higher eukaryotes) and we have shown, using the technique of one-step gene replacement, that the genes are essential for growth (4, 5, 7). To prove that these snRNAs are required for splicing in vivo, we have engineered their conditional synthesis, by fusing the coding sequences to a promoter whose activity can be controlled in response to carbon source (GAL1); thus, by shifting cells from galactose to glucose, we can inhibit further expression from this essential gene and observe the accumulation of unspliced pre-mRNAs as the snRNA is diluted out during subsequent cell divisions (5). With these tools in hand, we are now attempting to understand how the RNAs mediate the splicing reaction.

The process of splicing can be broken down into several types of recognition events: 1) interactions between signals in the substrate and trans-acting factors, and 2) the coalescence of these factor-site assemblies into a higher order structure, the mature spliceosome. The archetype of snRNA-mediated recognition events is the interaction between U1 and the 5' splice site. Steitz and colleagues first proposed that this occurs by complementarity between the 5' splice junction and highly conserved sequences at the 5' terminus of U1 (8). We have recently subjected this model to direct genetic tests by the creation of base changes in U1 predicted to compensate for 5' splice site mutations which inhibit the reaction. These experiments utilize a chimeric splicing substrate which has an easily assayable phenotype: proper splicing of an actin-HIS4 fusion allows cells to grow on media containing the histidine precursor histidinol (Hol). Thus, we can test the hypothetical base-pairing by asking if cells containing a fusion with 5' splice site mutations (and are thus Hol-) regain the ability to grow on Hol after transformation with a plasmid carrying an appropriately engineered snRNA gene. This biological suppression assay is followed by biochemical analyses which directly test whether splicing of the mutant fusion is restored. In this way we have demonstrated base-pairing interactions between U1 and the 5' splice site and between U2 and sequences upstream of the 3' splice site (9). Interestingly, the latter interaction appears to mimic a structure known to be essential for autocatalytic group II splicing, an intermolecular helix containing a "bulged" adenosine residue.

Our understanding of the snRNP-snRNP interactions which mediate the formation of higher order structure is currently quite limited. By far the best understood interaction between snRNP components is the association between U4 and U6, which are ordinarily isolated together in a single snRNP particle. We have obtained recent phylogenetic data that allow us to describe an extensive base pairing interaction between the two molecules (7). This interaction, which confers a high temperature stability (Tm ~53 degrees C), is of particular interest in light of the unexpected finding that spliceosomal complexes about to undergo the

first nucleolytic cleavage event have lost U4, but retain U6. Thus the association between U4 and U6 is dynamic and requires a substantial structural rearrangement. We are now trying to understand the mechanism which mediates this long sought-after example of an RNA "conformational switch." Ongoing mutational analysis of the U4 and U6 RNAs should shed light on this fascinating process.

Finally, we are using genetics to establish which structural features of the snRNAs serve as recognition sites for binding of snRNP proteins. One approach to this problem is the isolation of extragenic (i.e., second site) suppressors of snRNA mutations. For example, a set of polypeptides which associate with each of the splicoeosomal snRNAs, termed the "Sm" proteins, are known to bind to a consensus sequence rich in pyrimidines. Mutations in this element confer a lethal phenotype; rare viable cells may arise by the selection of mutations in the Sm proteins which allow them to recognize the altered site (or, simply to bind with higher affinity to all sites). We have validated the efficacy of this general type of approach by isolating a suppressor of a mutant intron. The mutated locus encodes a dominant, trans-acting, allele-specific suppressor. These and other properties argue that we have identified a structural gene encoding a component of the splicing machinery that physically interacts with the intron (10). We have recently cloned the suppressor gene and shown that the wild-type locus is essential for viability. Sequence analysis and genetic mapping are underway; together with the generation of antibodies to the gene product, and of conditionally lethal point mutations within the gene, these experiments should uncover the structure and function of this essential splicing factor.

(1) Cech, T., and Bass, B. 1986. Ann. Rev. Biochem. 55:599. (2) Guthrie, C. and Patterson, B. 1988. Ann. Rev. Genetics, in press. (3) Noller, H. 1984. Ann. Rev. Biochem. 53:119. (4) Ares, M. 1986. Cell 47:49. (5) Patterson, B. and Guthrie, C. 1987. Cell 49:613. (6) Siliciano, P., Jones, M. H., and Guthrie, C. 1987. Science 237:1484. (7) Brow, D. and Guthrie, C. 1988. Nature, in press. (8) Lerner, M., Boyle, J., Mount, S., Wolin, S., and Steitz, J. 1980. Nature 283:220. (9) Parker, R., Siliciano, P., and Guthrie, C. 1987. Cell 49:220. (10) Couto, J., Tamm, J., Parker, R., and Guthrie, C. 1987. Genes and Develop. 1:445.

MARDI 5 JUILLET 1988

14 h 30 - 18 h

METHODES NOUVELLES D'ANALYSE DES STRUCTURES

NEW STRUCTURAL METHODS