A possible general mechanism of the signal transfer switching on the α/β-interferons expression. 1. The local cell membrane deformation as the first induction stage

Alexander V. Karpov

Danylo Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine
154 Zabolotny str., Kyiv, 252143

A hypothetic mechanism of the external effect of viruses, synthetic polyanions, double-stranded RNAs, and synthetic double-stranded polyribonucleotides upon a cell is explained. This effect is known to be followed by the α/β-IFN induction. Double-stranded RNAs and polynucleotides inducing such process are thought to change their conformation in an outer ionic perimembrane layer of cells and to induce the specific local deformation of cell membranes giving a signal for the α/β-IFN induction. In the case of viruses such a membrane deformation appears on the stage of virus penetration into the cell. The α/β-IFN inducers of intercalating nature are proposed to cause the interferonogenesis in vivo forming complexes with exogenic RNA molecules and stabilizing their double-stranded sites with a partial complementarity.

The synthesis of interferons (IFNs) as well as of many other cytokines is regulated by induction, i. e. by the extracellular IFN genes activation [1]. The data concerning the α/β-IFNs confirm their activation both by viruses and by a lot of different high and low molecular weight compounds. The IFN-inductive effect of natural double-stranded RNAs (dsRNAs) and of synthetic polynucleotides has been studied in detail [1—4]. However, the earliest IFN-inducing stage as well as some initial inducer-cell interactions and the role of such interactions for the signal transfer followed by the gene expression have not been yet completely investigated.

Almost all the hypotheses describing the IFN induction mechanism by dsRNAs and synthetic polynucleotides are based on the presumption the dsRNA-directed induction process to be completely due to some intracellular events [2, 4]. The main argument permitting to propose such a presumption is the fact that the polyribonucleotides having been interacted with the cell penetrate later into it, so these compounds are with no doubt to influence the regulation of intracellular processes. Besides, the dsRNAs are thought to participate in the IFN induction because they are known to be able to bind some specific proteins — IFN-induced protein kinase and 2′,5′-oligoadenylatesynthetase — and to change their functional properties [5]. The same presumption is thought to be right for the cases of virus-induced IFN synthesis, the stage of dsRNAs formation able to induce such synthesis having place during reproduction cycles of both DNA- and RNA-containing viruses [2, 6]. The IFN-inducing effect of low molecular weight compounds has been never postulated to be a result of the dsRNAs effect. The mechanism of the IFN induction by tilorone-HCl and its derivatives as well as by some other intercalating IFN inducers is believed to be due to their direct interaction with cell genome [3].

The theory postulating the polynucleotide penetration into the cell as a necessary step of the IFN induction has not been accepted by all the authors. An alternative point of view has been proposed concerning the importance of polynucleotide interactions with the outer cell membrane for the IFN induction. Such an opinion is based, first of all, on the experimental data with a poly(I)-poly(C)-complex

immobilized on an insoluble carrier. This complex unable to penetrate into the cell has been, however, detected to induce the IFN synthesis [7, 8]. Besides, a high quantity of the native and active poly(I)-poly(C)-complex has been isolated from cell membranes following cellular IFN synthesis [9].

In the cases of the virus-induced IFN synthesis there is also a pool of experimental data which cannot be interpreted from the point of view based on the crucial role of intracellular dsRNA structures as conductors of the induction signal. Some purified virus envelope proteins have been shown to induce the IFN synthesis [10, 11]. Simultaneously, the activity of the Newcastle disease virus inducing the IFN synthesis has been detected to be as sensitive to the guanidine, urea, 2-mercaptoethanol, and heat treatment as the virus infectivity. However, both the UV-irradiation and the nitrous acid treatment inactivating the viral RNA cause the exponential virus infectivity loss accompanied with the decrease of the INF-inducing activity according to the two-phase kinetics [12]. Finally, sometimes there is no correlation between the virus RNA replication and the IFN synthesis.

I am not going to reject the opinion concerning the important role of the intracellular dsRNA taking part in the developing of the signal for the IFN synthesis. However, I think that another process taking place during the primary inducer-cell interactions participates also in this signal development; this process is a specific local deformation of the cell outer membrane followed by a series of molecular events.

The primary nucleic acid-cell interaction is due to the nucleic acid binding by specific cell receptors. The existence of such presumably protein receptors on the cell surface was earlier postulated [13]. Later nucleic acids receptors were isolated and purified, they were also confirmed to be proteins [14—16]. According to the general receptors function, the next post-binding stage is the active transfer of the receptor-nucleic acid complex into the cell; it should be noted this process to be accompanied by the specific deformation of the membrane areas where the complex formation and transfer had earlier taken place.

So it should be expected the polynucleotides binding by the cell receptors to result the analogue or similar result. According to our up-to-date models, the cell membranes have on their surface an outer perimembrane layer as thick as 10—15 μm [14, 17] — glyocalyx functioning as a cellular cation exchanger. The glyocalyx structure presenting a specific giant «polyanion» is formed by the carboxylic groups of N-acetylneuraminic acid as well as by ionized phosphate groups residues and protein amino groups residues [18]. This polyanion participates in a lot of metabolic processes accompanied by the dynamic absorption of cations transferred from and into the cell through the cell pores determining the electric potential of the plasmatic membrane surface [18, 19]. The dsRNAs effect on the cell (as well as the effect of synthetic polyionic IFN inducers including polycarboxylates, polysulphates, and polyphosphates [20] taking place without any receptor-mediated binding) is thought to be accompanied by moderate cationic gradients changes followed by cooperative transitions in membranes. The next additional binding of cations leads to the shortening of outer membrane areas causing (due to mutual cations' repulsion) the formation of the external fluctuating mosaic structure. As a result of new factors action some fluctuating holes may also appear on the inner membrane surface, such a phenomenon having been well described in scientific literature [21].

At first sight such a process seems to be independent on the quantity of strands of the IFN-inducing polynucleotide as well as on the nature of its sugar residue. Such an opinion, however, is in contradiction to the well-known fact concerning the IFN-inducing polynucleotides structure; without any doubt they are found to contain ribose in their phosphate-sugar chains and to be double-stranded molecules [4]. I think the specificity of the IFN-induction by double-stranded polyribonucleotides may be due to their conformational properties and their molecular electrostatic potentials [22].

Finally, there is also another probable hypothetical mechanism causing the cell membrane deformation following its interaction with dsRNA molecules. The double-stranded polyribonucleotides are known to keep the so-called A-conformation having 11 base pairs per a helix step as well as the A'-conformation with 12 base pairs per a helix step. The first conformation is usually found in solutions of physiological pH and ionic strength values. The increased salt concentrations cause the polyribonucleotide transition to the A'-conformation [2]. Such an A-A'-transition having place in any double-stranded polyribonucleotide bound by more than one receptor, the following decrease of the contour length of double-stranded sequences should cause the specific cell membrane deformation in the binding area. My opinion is that the possibility of the mentioned conformational transition may be due to the glyocalyx ionic environment.

An indirect proof permitting to suppose the existence of this last mechanism is the fact that the IFN-inducing polyribonucleotides lose almost always

their induction properties after their ribose component modifications; this component is mostly responsible for their conformation. Such a conclusion is a result of data the analysis concerning a lot of double-stranded polyribonucleotides modified in different positions [4]. Taking this analysis into consideration we are now able to understand the inability of DNA molecules as well as of double-stranded deoxyribonucleotides and DNA-RNA-copolymers to cause the IFN induction [4]; due to conformational hindrances they are all unable to be IFN inducers as they do not possess any A-conformation at physiological ionic strength values [23].

All the nucleotides being IFN inducers — native dsRNAs as well as synthetic homo- and heteropolymers — meet the case concerning the inducers structure (they are all double-stranded and ribose-containing); however, different compounds belonging to this group of substances are of different IFN induction ability, the poly(I)-poly(C)-complex possessing the best one [4]. The differences between a lot of synthetic polynucleotides are explained as a result of different length of their monomeric chains in the reaction medium; the chains are able also to interact forming some partially double-stranded sequences simultaneously in several points. The complexes obtained are not strictly double-stranded along all their strands; they contain double-stranded regions of different lengths interrupted by branching regions, loops, and perhaps also by some one-stranded regions as well as by contacts with some neighbour polynucleotide chains [24]. All these interactions lead to the formation of the reticulate «tertiary structure» of polynucleotide complex molecules. Such a structure is a well-known one for natural dsRNAs. There is an opinion [25] the IFN-inducting ability of any polyribonucleotide complex is dependent on its complexity degree influencing the complex-cell interactions followed by the effective/non-effective α/β-IFN synthesis. The data mentioned above are also applicable to the poly(I)-poly(C)-complex whose components are different in their ability to form their own secondary structure. The poly(I), for example, is able to form double-stranded structures at physiological pH and ionic strength values, this process being dependent on temperature and salt concentration [4]; the poly(C) molecules are found to develop a single-stranded helix [26]. So the interactions of these polynucleotides with their different original configurations leads with the highest probability to the development of the «tertiary structure» mentioned above and responsible for the highest ability of the poly(I)-poly(C)-complex to induce the IFN synthesis.

From the point of view of this hypothesis, the most important is the fact that the similar tertiary structure interacting with the cell membrane is to assure the highest density of membrane components contacts with double-stranded polynucleotide regions situated on any local membrane area. It should be noted such a regularity to be accompanied by increased complexity of polynucleotide structures correlating with their IFN-inducting ability. Such considerations have already experimental support [25].

In the frame of my hypothesis it is also possible to interpret the IFN-inducing mechanism of some compounds named above and belonging to the classical intercalating substances (i. e. actinomycin D, antrachinone derivatives, trypaflavine, acrydine orange) [1] as well as of the best known low molecular weight IFN-inducing compound — tilorone-HCl and its derivatives able also to intercalate [27, 28]. We have recently demonstrated the one-stranded RNA molecule to interact with tilorone ones forming specific IFN-inducing complexes acting both *in vitro* and *in vivo* [29, 30]. To explain this phenomenon, we have postulated tilorone to stabilize any partially complemented areas spontaneously developed in solution of any one-stranded RNA preparation. Tilorone is described to induce the IFN synthesis *in vivo* only, but not in the *in vitro* cultivated cells. This tilorone property can be explained from the data mentioned previously in this review; this compound appears to interact with small RNA molecules present in the intercellular space *in vivo* but absent, however, in cell cultures [31]. In the process of extracellular interactions of such molecules with some tilorone's stable double-stranded regions may be also developed; they are able to act as the IFN-inducers.

So I think the IFN-inducing ability is the property of the tilorone-RNA complex but not of this low molecular compound itself. A similar situation, i. e. the stabilization of double-stranded regions of extracellular RNA molecules while their interaction with intercalating ones followed by IFN-inducing effect of these complexes, is to develop always in every IFN-inducing experiment with intercalating compounds.

It would also like to note another physicochemical property of tilorone which might have been correlated with its higher IFN-inducing ability comparing to other intercalating substances. The tilorone molecule contains lateral chains linked to a fluorene nucleus; so the stacking interaction between its chromophore and the DNA bases has been found to be rather lower comparing to the other «classical» intercalating compounds. So the lateral intercalating is here more profitable from the energetic point of view, the intercalated tilorone side chains having been located in DNA grooves [32]. If this situation takes

also place with the double-stranded RNA molecules the regional weakening of the negative charge in the sugar-phosphate dsRNA backbone is to cause the rapprochement of the nearest double-stranded regions and the formation of the tertiary reticulate structure discussed above. The similar consequences are to be caused due to tilorone binding to the neighbour single-stranded regions possessing already some double-stranded areas.

Finally, the proposed hypothesis concerning the crucial role of the local cell membrane deformation in the process of the signal transfer necessary for the IFN synthesis permits also to interpret the IFN-inducing mechanism of viral infections. In fact, the viruses are demonstrated to interact with the cell membranes in the loci of their contact, this primary interaction influencing significantly the cell metabolism and the cell membrane changes [33]. In particular, the process of the influenza virus penetration into the chicken embryo fibroblasts is shown to be accompanied by the local modifications of hydrophobic plasmatic membrane zones in the points of virus binding [34]. The interactions of the human immunodeficiency virus (HIV) and the Sendai virus with the cell followed by the syncytia formation cause also the deepest changes in cell membrane lipids, their destabilization and re-orientation [35]. The situation observed here possesses a lot of features inherent to the result of the local membrane deformation. The fact itself that the dsRNA-directed IFN synthesis takes place earlier comparing to the virus-inducted one [1, 2] may be due to the cell membrane deformation differences caused by these IFN-inducing agents.

It remains to mention that the IFN-inducing effect of a such non-specific physical factor as laser irradiation cannot be interpreted in the frame of current hypotheses [36].

To summarize all the data given in this review, I would like to note all the discussed cases of the cell primary interactions with the different types of IFN-inducing compounds are accompanied with the same process, i. e. with the local cell membrane deformation. I think this process to be a rather general cell reaction developing after the extracellular influence of all the IFN inducers; this process somewhat similar to the simple mechanical effect on the cell possesses, however, some specificity inhibiting the IFN system induction after any extracellular signal.

It is well known that the development of the extracellular specific signal due to the influence of many biologically active substances interacting with the cell membrane leads to the transfer of this signal mediated by so-called «secondary messengers» (cAMP, cGMP, Ca^{2+} ions) [37]. A similar situation can be also observed following the mechanical influence on the cell membrane [38—40]. It seems very probable the principal role in the process of the signal transfer after the membrane deformation to be played exactly by the secondary messengers both in a lot of described cases of gene regulations and in the process of the genes activation coding the α- and β-IFNs synthesis.

Unfortunately, we have not meanwhile obtained but the fragmentary data permitting to discuss the problem concerning the direct effect of the cell membrane state on the IFN induction. For example, the cell treatment by neuraminidase or by concanavalin A are described to inhibit the IFN synthesis without any effect on the level of poly(I)-poly(C) adsorption by cells [41]. It has been also shown the cell treatment by ouabaine, a glycoside substance belonging to the specific inhibitors of Mg^{2+}, Na^{+}, K^{+}, and ATP transport through the cell membrane, stops completely the influenza B virus induced IFN synthesis without changing the virus adsorption. Such an effect has been completely eliminated after the preparation's washing off from the cells [42]. All these data convince the hypothesis concerning the principal role of the glycocalyx and perimembrane ions state in the process of the signal transfer.

It think the next investigations concerning the cell membrane function in the IFN induction process are to verify the validity of this hypothesis and to determine the nature of the signal switching on the induction of the α- and β-IFN synthesis.

О. В. Карпов

Можливий універсальний механізм передачі сигналу до експресії генів α/β-інтерферонів. 1. Локальна деформація клітинної мембрани як початковый етап індукції

Резюме

На основі даних літератури і власних досліджень зроблено спробу встановити гіпотетичний механізм зовнішнього впливу на клітину вірусів, синтетичних поліаніонів, дволанцюгових РНК і синтетичних дволанцюгових полірибонуклеотидів, який призводить до індукції α/β-інтерферонів. Припускається, що ця індукція у випадку дволанцюгових РНК і полінуклеотидів обумовлена конформаційними змінами даних полімерів в примембранному іонному шарі клітин; далі ці зміни викликають специфічну локальну деформацію клітинної мембрани, що є одним з сигналів до індукції α/β-інтерферонів. У випадку вірусів подібна деформація мембрани здійснюється на стадії їхнього проникнення у клітину. Індуктори α/β-інтерферонів інтеркаляторної природи, скоріш за все, викликають інтерфероногенез in vivo за рахунок утворення комплексу з екзогенними молекулами РНК, стабілізуючи їхні дволанцюгові ділянки з частковою комплементарністю.

А. В. Карпов

Возможный универсальный механизм передачи сигнала к экспрессии генов α/β-интерферонов. 1. Локальная деформация клеточной мембраны как начальный этап индукции

Резюме

На основании данных литературы и собственных исследований предпринята попытка установить гипотетический механизм внешнего воздействия на клетку вирусов, синтетических полианионов, двуспиральных РНК и синтетических двуспиральных полирибонуклеотидов, приводящий к индукции α/β-интерферонов. Предполагается, что эта индукция в случае двуспиральных РНК и полинуклеотидов обусловлена конформационными изменениями данных полимеров в примембранном ионном слое клеток; далее эти изменения вызывают специфическую локальную деформацию клеточной мембраны, являющуюся одним из сигналов к индукции α/β-интерферонов. В случае вирусов подобная деформация мембраны происходит на стадии их проникновения в клетку. Индукторы α/β-интерферонов интеркаляторной природы, скорее всего, вызывают интерфероногенез in vivo вследствие образования комплекса с экзогенными молекулами РНК, стабилизируя их двуспиральные участки с частичной комплементарностью.

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