Генноинженерная биотехнология

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THE INTERPHASE CHROMATIN SPECIAL STATE ZONES

The possibility is demonstrated of RecA protein of E. coli introduction into eukaryotic cell with preservation of its biologicall activity. The character of this protein affinity to chromatin of different degree of condensation is shown on cytological level (undirect immunofluorescent method combined with cytophotometry) for different stages of cellular cycle (protein is not tested in meta- and anaphase chromosomes).

The presence of bacterial RecA protein in the nuclei and cytoplasm of cells both in vitro and in vivo is confirmed with the help of immunoelectron microscopy.

Studies were carried out on cultures of HeLa and Ltk⁻ cells and also in vivo on hepatocytes after direct injection on RecA protein and plasmid pKCR2 enclosed in liposomes into the liver of adult mice line BALB/c.

Proceeding from the experimental data obtained and also considering the fact that RecA protein mainly connects with single-stranded DNA, the assumption is done about existence of special state chromatin zones (SSCZ). For these zones the active affinity to RecA protein serum is character with intensive fluorescence, thus they may correspond to some actively expressing genes, gathered in clusters.

Introduction. The product of the gene *recA* of *Escherichia coli* due to its great significance for normal functioning of cellular genome occupies particular place among all products of the bacterial genes, revealed during two last decades. Polyfunctioning of this protein with comparatively small weight (37 800 D), taking participation in the processes of recombination, replication, reparation, mutagenesis, cell division, synthesis of new proteins, induction of prophages [1, 2] is suprising.

There may exist some SOS-functions both in eukaryotic and prokaryotic cells. It was shown even in the early studies, that mammalian cells might response the inhibition of replication by induction of new proteins (during the inhibition of proliferation in the *E. coli* cells the sygnal causing induction of *RecA* protein is generated). Proliferation of the lymphoid cells, peripheral lymphocytes, primary fibroblasts of the skin has been inhibited by the delay of the replication. It has been found that under these conditions the synthesis of two new proteins is induced, one of them is localised in nucleus (nuclear localization of products supposes their fermentive nature and interaction with DNA) [3]. May the products of genes *recA* (or their analoges) with different origin substitute functionally one another? The presence of protein factors in eukaryots functional analogous to *recA* protein of *E. coli* is shoun in a number of studies.

Thus the protein *RecA*, isolated from *Ustilago maydis*, realises homological coupling of overspyralised DNA with ss-DNA, requiring ATP for the reaction [4]. Specifical to ss-DNA and ss-RNA endonuclease was isolated from mouse cells plasmocytome mitochondria [5]. Activity of endonuclease specifical to ss-DNA in a large extend is similar to the enzymes from fungus mitochondrias, whose absence results in disturbances in the processes of DNA reparation and recombination.

Partially purified factor specifical to ss-DNA dependent ATP activity and promoting DNA homologous pairing with heteroduplex formation and DNA strands transferation between corresponding DNA-substrates has been isolated from human skin fibroblasts nuclear extract by chromatography on biogel [6]. Activity of this factor was tested in experiments with phages circular ss-DNA in linear ss-DNA. Factor-catalized reactions were found similar to those, in which protein *recA* from *E. coli* participates.

So extensive spectrum of eukaryotic proteins which are able *in vitro* to fulfil *RecA* protein functions such as homologous pairing of superhelical DNA with ss-DNA, transfer of DNA strands between corresponding substrates has been found. For some of them, for example, for RRA1 [7] nuclear localization has been shown. The presence of the same elements in the system of general recombination of pro- and eukaryots lets us assume under certain conditions the possibility of the participation of *RecA* protein of *E. coli* in the processes of homologous recombination and reparation of the eukaryotic genome. However the possibility of penetration of *RecA* protein through the nuclear membrane and its ability to interact effectively with eukaryotic DNA being in part of chromatin has been studied to our day in a few works [8—10]. For solving of this problem at first one had to make sure of the possibility of *RecA* protein of *E. coli* penetration inside eukaryotic cell and, in part, into nucleus. Choice of method of test penetration of *RecA* protein in cytoplasm and nucleus was of principal importance. As it was known that *in vivo RecA* protein is related with DNA while carrying out this work for its test indirect immunofluorescent (IF) method was chosen to define visually penetration and localization of *RecA* protein in nuclei of eukaryotic cells. It was suspected that use on the one side of the method permiting to make visual the connection of *RecA* protein with chromatin and, on the other side, of suitable model system (rapidly deviding cells of HeLa culture) would allow to determine on the morphological level the character of the connection of *RecA* protein with chromatin of different degree of spiralization of the isolated stages of cellular cycle.

In addition to method described above the method of immunochemical electron microscopy was applied to clear up some questions concerning the localization of *RecA* protein in different cellular compartments.

Thus an attempt of the spatial distinguishing of the expressing sites of chromatin (in which there must be firm connection of *RecA* protein with single-stranded DNA) from the rest of unreplicating chromatin mass was made.

Beside this it was supposed that in differentiated cells, such as, for example, hepatocytes of adult mice, in which mitotic cell-divisions took place rarely, evidence of connection of *RecA* protein with expressing genes might be useful for determination of their morphological and structural localization of these expressing genes in nucleus. Initially the presence of the so called special state chromatin zones (SSCZ) in nuclei, in which there were actively expressing genes gathered in clusters was postulated.

Materials and methods. Objects of study. Studies *in vitro* were carried out on culture of HeLa cells from the collection of cells of Sankt-Petersburg Institute of cytology of AS of Russia and Ltk- cells from the collection of cells of IMB & G of Ukraine. Culture were cultivated on the Eagle medium containing 10 % bovine serum and antibiotics — penicillin (200 v/ml) and streptomycin (1 mg/ml). In system in vivo experiments were made on hepatocytes of mouses of BALB/c line (2-month age, mascular) for one varient the group of 2-3 animals was picked out.

Extraction of RecA protein. Purified *RecA* protein — polypeptide with molecular weight 37 800 D — was extracted from *E. coli* cells superproducing this protein (stamp DH 1, defective on the system of recombination) under the condition of SOS-function by nalidixic acid [11]. Cells of *E. coli* DH 1 transformated by plasmid *px13* had high content

of protein owing to the presence of *spr* mutation and persuation by nalidixic acid. Cultivation of *E. coli* for accumulation of its biomass was carried out on the medium LB (1 % of pepton, 0.5 % yeast extract, 1 % NaCl). The protein was purified on a Sepracryl S-200 column [12]. Purity of *RecA* protein on the SDS electroporation in 14 % polyacrylamid gel was 96-98 %. Functional activity of *RecA* protein was controlled by its ability to hydrolise ATP in the presence of single-stranded DNA. Controlled by such way activity was 1.8 ± 0.4 pM ATP/mg of protein in hour [13].

Plasmid. *BamHI-SalGI* fragment from plasmid *px13*, containing full-size *recA* gene of *E. coli*, was cloned on *BamHI*-site (with filling in by DNA-polymerase of uncomplementary ends) on plasmid *pKCR* [14]. After restriction analyse thf clones, in which *recA* gene was in the direct orientation to the promoter SV40, were selected. Resulting plasmid *pKCR2* was induced in mammalian cells *in vitro* and *in vivo*.

Liposomes. To transfer *RecA* protein and plasmid DNA negatively-charged unilamellar liposomes were used 10 to 100 nm in diameter with next compounds: lecitin : cholesterol : phosphatidic acid : phosphatidylethanolamin in proportion 7 : 2 : 1 : 0.2, received by ultrasonic method with phase conversation [15, 16].

RecA protein incorporation in liposomes was carried out by adding of protein in lipid mixture on the «gel» stage in the process of liposome preparation. DNA plasmid's *pKCR2* were included into liposomes by Ca-fusion method and amount of liposome's included DNA was 7-10 % from that was introduced. Sizes, lamellarity and safety of liposomes were controlled in electrone microscope on negatively stained grids [17].

Experiments in vitro. HeLa cells were sown on the cover glasses and were cultured under standard conditions in Eagle's medium with additions for 24 h.

Cells were transfected with plasmid *pKCR2* by the Ca-phosphate and DEAE-dextran technique [21]. Besides, introduction of plasmid *pKCR2* into cells in part of liposomes was applied.

RecA protein when it was introduced into mammalian cells in vitro was added directly to cultural medium in account of 0.5 mg/ml with next cells exposition during 30 min. Then medium was withdrown and cells were subjected to glycerol shock (15 % glycerol) for 3 s.

Besides, in other varients of experiments HeLa cells were incubated with pure *RecA* protein without liposomes and with unloaded liposomes. Fixation of preparations in several changes of aceton cooled up to 4 °C for 20 min was conducted after 24 h after treatment.

Experiments in vivo. Volume of material, which was introduced directly in the mouse liver was 60 μl/individual. In variations of injections of plasmid *pKCR2* being in part of liposomes mixture contained 30 μg of lipids and 10 μg of plasmid DNA. Animals were sacrified and liver was extracted 24 h and 48 h after injection of material. Hepatocytes was prepared as early described [15] from the same lobe of liver in which the material was injected.

Receiving and purification of serums. Shinshila race rabbits were immunized by electrophoratively purified *RecA* protein on the next scheme. During 3 weeks *RecA* protein was induced in acount 100 μg/individual in the region of two cervical and two popliteal lymphadens of rabbits with seven-day intervals. The first immunization was carried out with complete Freind adjuvant and the next two — with uncomplete one.

In a week after third injection it was done buster-injection —100 μg of *RecA* protein was injected through rabbit's ear vein and blood collection was made in 7-8 days after last injection. Antibody titer was determined by double gel-diffusion method (1 : 16-1 : 32). Gamma-globulin fraction was isolated by double precipitation of serums by ammonium sulphate with 33 % of saturation with following dialysis to 0.0175 M of sodium phosphated buffer with pH 6.3. In emergency gamma-globulins

were additionally purified on ion exchange column with cellulose (1.5×25 sm) using the same buffer. IgS fraction removed from ion exchange column was concentrated by PEG-3500-4000 and was kept under 4 °C with addition of NaN_3.

Before staining of cytological preparations serum to *RecA* protein was purified additionally 1-2 times by liver powder [19]. Labelled by fluoresceinisothiocyanit (FITC) donkey's antirabbit globulin was purified from unconnected fluorochrom on the 1 % agarose.

Working dilution of serum to *RecA* protein for preparations staining was 1 : 5-1 : 10; luminescent — 1 : 16.

Studying of cytological preparations. *RecA* protein was tested by undirect IF method [23]. Preparations were looked through on the luminescent microscope ML-2 with photometrical addition on the 520 nm and with the probe 0.5 μm. The objective X70 for water immersion was used. Stained *RecA* protein fluoresced in green area of spectrum. Measurement on the each varient of experiments were done on 50 cells with measuring by probe fluorescene of nucleus and cytoplasm separately.

Electron immunocytochemistry. 24 h after injections of material the cubes of liver tissue were fixed in 2.5 % glutaraldehyde prepared on the 0.1 M sodium-cacodylate buffer (pH 7.4) at 4 °C for 2.5 h. After rinsing in the same buffer the tissue was postfixed in 2 % osmium tetroxid. Then after dehydration in a graded series of alcohols the samples were embedded in Epon-Araldite.

Monolayers of HeLa cells were cultured on pieces of lavsan film and after 24 h of transfection processed according classic scheme with the addition of 2 % sucrose at the first steps. The film with cells was embedded in special metal rings by «sandwich» method. Under the control of light microscope separate groups of cells were separated from film, fastened to standard epoxy blocks and then sectioned as usually.

Ultrathin sections for immunochemical assay were cut on ultratome LKB III (Sweden) and mounted on parlodian metal grids without any film-coating.

To identify the molecules of *RecA* protein the method of indirect immunolabelling was used that is based on the formation of special linking: antigene — antibody — protein *A* — colloidal gold. Biospecific probe «protein A — colloidal gold» (PAG) was obtained from scientific-industrial centre «BIOFROMEKOLOGIA» (Saratov, Russia). The main characteristics of the probe were the following: the mean diameter of the gold particle 20.6 nm; concentration $3.5 \cdot 10^{12}$ pt/ml. The final concentration of gold probe was defined cytophotometrically upon the absorption OD_{520} and settled for electron microscopy in ranges 0.1-0.2 by diluting the initial reagent 20-40 times. To avoid the formation of gold depositions on thin sections the probe after diluting was centrifugated (up to 5 min with 10 000 rot/min) with the subsequent control in electron microscope. The labelling of thin sections was settled according to method [21] with special attention on blocking procedure, i. e. preliminary incubation of sections on the drops of bull serum albumin to diminish the unspecific labelling.

After incubation in drops of PAG the grids with sections were rinsed thoroughly in phosphate buffer saline and distilled water and then, avoiding them from final drying, stained with uranyl acetat followed by lead citrate. The preparations were examined in «TESLA» electrone microscope at 90 kV.

Experiments with HeLa culture. Controls: 1) cells persuated by unloaded «empty» liposomes during 30 min with subsequent glycerine shock of 30 s of duration; 2) cells without any treatment (intact cells) and stained with serum to *RecA* protein; 3) intact cells, stained with serum to bacterial β-galactosidase.

Fluorescence of cytoplasm under staining of control intact cells with serum to *RecA* protein was not considerable and on quantitative indices approached to values of autoluminescence (luminescence of unstained fi-

xed cells) (fig. 1, *a*). The chromatin net was slightly revealed in nuclei (fig. 1, *c*). After preparations staining with serum to bacterial β-galactosidase no fluorescence of cytoplasm was noted and no morphological structures were revealed (fig. 1, *d*) in nuclei; the luminescence of cells corresponded to autoluminescence level.

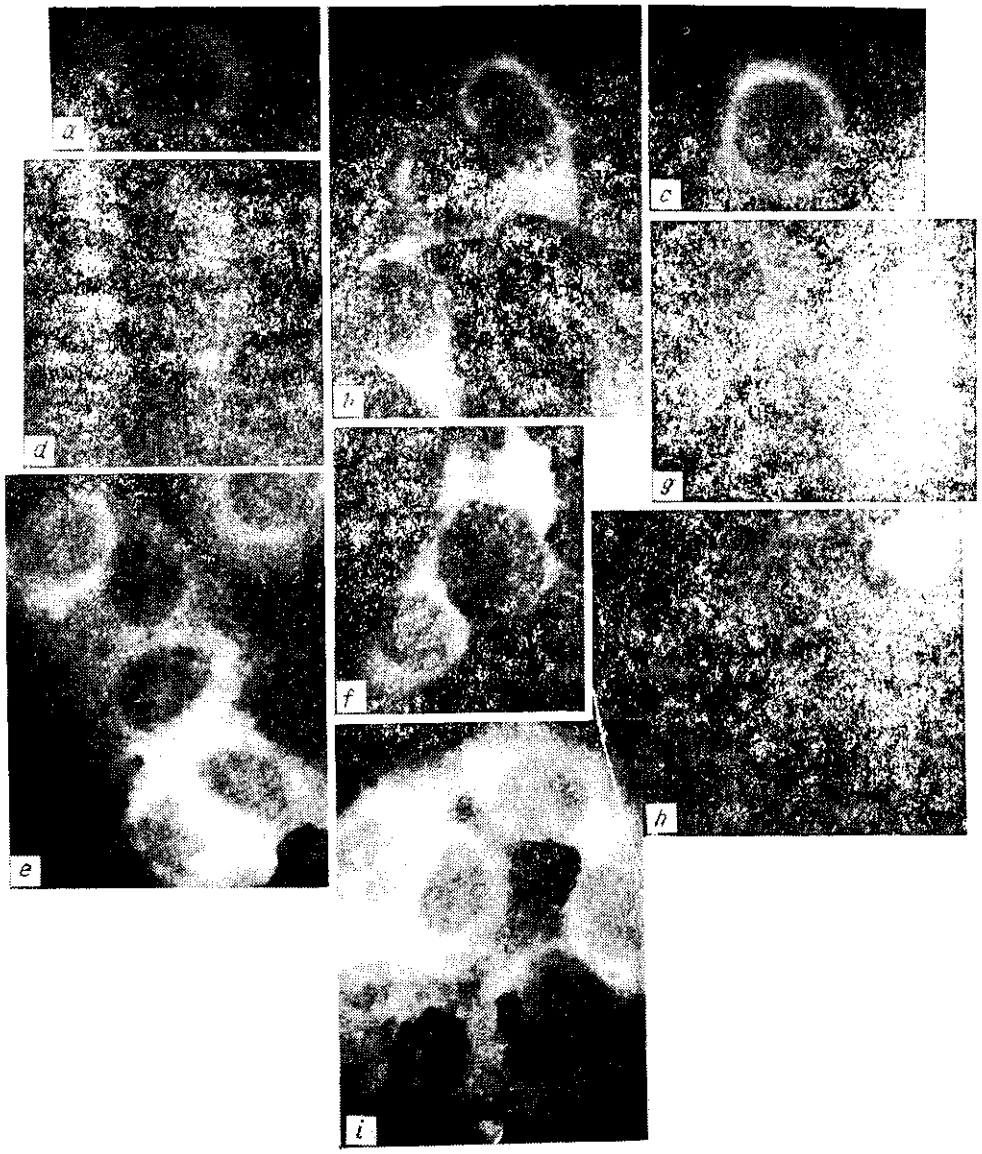

Fig. 1. Immunofluorescence of HeLa cells (controls: *a*—*d*): *a* — autoluminescence; *b* — treatment with unloaded «empty» liposomes, serum to *RecA* protein; *c* — cell without any treatment stained with antiserum to *RecA protein*; *d* — cell without any treatment stained with antiserum to β-galactosidase; *e* — after 30 min exposition with pure *RecA* protein; *f, g, h* — after 30 min exposition with *RecA* protein enclosed in liposomes; *i* — after contact with plasmid *pKCR2* enclosed in liposomes; *g, i* — groups of metaphase chromosomes that do not reveal any fluorescence

After staining of preparations previously treated by «empty» liposomes with antibodies to *RecA* protein of *E. coli* dim luminescence of cytoplasm was noted and slightly fluorescent chromatin and nucleolus could be identified (fig. 1, *b*).

Obviously that after staining of preparations with serum to bacterial *RecA* protein chromatin structures of nucleus revealed themselves, while after using of other serum — to bacterial β-galactosidase — they did not.

On these grounds it may be suppossed that there are specifical to antiserum to *RecA* protein determinants in the structure of eukaryotic chromatin, and it may testify that there are functional analogues of *RecA* protein of *E. coli* in eukaryotic nucleus. But low intensity of luminescence of stained chromatin structure shows that either the quality of such proteins is not considerable, or their affinity with antibodies received by immunization of animals by *RecA* protein of *E. coli* is not large.

Incubation of HeLa cells with pure RecA protein and RecA protein, enclosed in liposomes. At is was demonstrated in seria of experiments, described in detail in previous publication [8], the bright fluorescence was observed in cytoplasm and nuclei on cell preparations 24 h after their incubation for 0.5 h with pure *RecA* protein or *RecA* protein enclosed in liposomes. Increasing of luminescence intensity of this cellular structures (in comparison with control varients) after treatment of preparations with serum to bacterial *RecA* protein of *E. coli* was explained as a consequence of *E. coli* protein penetration in eukaryotic cell and its nucleus. In such cases the localization of fluorescence on nuclear chromatin structures was observed visualy. Karyoplasm of nuclei did not luminescence and went on to be dark, unfluorescent (fig. 1, *e*, *f*, *g*, *h*). Fluorescence distribution on length of chromatin thread is ununiform and next to intensive luminescent sites there are sections of chromosomes without luminescence.

Observation of chromatin luminescence peculiarities on the different stages of cellular cycle allowed to note reccurence in its fluorescence depending on degree of chromatin condensation. Nuclei with greatly decondensated chromatin, according to stages of G and S periods of cellular cycle by visual observations differed also by intensive fluorescence. Luminescence of nuclei decreased according to chromatin condensation in the late prophase and was completely absent in metaphasic chromosomes (fig. 1, *g*, *i*). For this photometrical measurements demonstrated increasing of quantity (in relative units) of tested *RecA* protein in cytoplasm, which exceeded one in next undivided cells, thus, bacterial *RecA* protein during metaphase—anaphase could be tested only in cytoplasm. Nuclei luminescence was renewed after the formation of nucleus covers around separated groups of chromosomes and decondensation of chromatin, formation of nucleolus in telophasic nuclei.

So from visual observations or contrasting of chromatin after treatment of HeLa cells with pure *RecA* protein or *RecA* protein in compound of liposomes the next conclusion may be done:

1) in described experiments it was shown that *RecA* protein of *E. coli* penetrated in eukaryotic cell and nucleus that is proved by appearance in them of specifical fluorescence;

2) in nucleus protein is associated with chromatin and thus its visualization on chromosomes after staining of preparations by antibodies to *RecA* protein becomes possible;

3) *RecA* protein independent on the way of introduction into eukaryotic cell (in compound of liposomes or without them) preserves its biological activity and, obviously, functions in eukaryotic cells with revealing of some (for example, DNA-connecting) biochemical activities;

4) *RecA* protein of *E. coli* association with chromatin unmasked by undirect IF method depends on the degree of chromosomes condensation and charges during cellular cycle. In compactly condensated metaanaphasic chromosomes protein is not revealed, diffusely dispersing in cytoplasm. Simultaneously with karyokinesis protein begins to be revealed associated with chromatin;

5) connection of *RecA* protein with chromatin is observed not along whole chromatin strand and brightly fluorescent sites alternate with sections of chromatin, uncontrasted by protein.

Gene recA introduction into HeLa cells. Fullsized gene *recA* of *E. coli* was cloned in plasmid *pKCR* [14] on *BamHI* site. Plasmid was introduced in HeLa culture by several ways (Ca-phosphatic and

DEAE-dextran and in compound of liposomes), in experiments we have chosen two last ones as giving stable results. Beside, this the possibility of introduction of plasmid DNA enclosed in liposomes into cultivated cells was studied. Presence of protein-product in nuclei and cytoplasm was determined 24 h after transfection.

RecA protein according to the presence of fluorescence was revealed both in nuclei and in cytoplasm of transformated cells. Cells fluorescence (fig. 1, *i*) did not differ from one under influence of pure *RecA* protein on HeLa preparations or of protein being contained in liposomes. The same regularities in character of luminescence of chromatin in dependence of degree of its spiralization were observed. In cells transformed by plasmid *pKCR2* enclosed in liposomes (approximatelly 36 %), different quantities (in relative units) of protein-product were revealed [8]. For this in nuclei of some cells the content of *RecA* protein greatly exceeded one in nuclei of cells from other variations of experiments — after influence by pure *RecA* protein or by protein enclosed in liposomes. Untransformated cells (about 64 %) showed fluorescence within the limits of luminescence of cells treated by unloaded «empty» liposomes, that is they were on the level of control.

So this study has shown:

1) the possibility of introduction of *recA* gene in part of plasmid *pKCR2* in HeLa cells by DEAE-dextran method or using liposomes with the effect of transfection;

2) product of *recA* gene expression in nucleus is associated with chromatin.

Experiments in vivo. Controls: 1) hepatocytes of animals without any treatment; 2) hepatocytes of animals after injection in liver of unloaded liposomes; preparation in both cases were stained with serum to RecA protein; 3) besides preparation from liver of intact animals for comparison were stained with serum to β-galactosidase.

Morphological revealings of chromatin in nuclei of intact animals (fig. 2, *a*) and after introduction of unloaded «empty» liposomes (fig. 2, *b*) under staining of preparations of liver by antibodies to bacterial *RecA* protein were the same with one in analogous experiments in HeLa culture. Chromatin was contrasted slightly though after injection of «empty» liposomes the structure of nucleus revealed more clear. Controlled staining of hepatocytes preparations with antiserum to bacterial β-galactosidase did not reveal any structures in nuclei (fig. 2, *c*).

In variants of experiments — after injection into liver of *RecA* protein (fig. 2, *d*) or plasmid *pKCR2* (fig. 2, *f*) enclosed contained in liposomes fluorescence in hepatocyte nuclei was observed after two days. Vizualized chromatin was seen as thin luminescent net (fig. 2, *d*), or in other nuclei on the more compactly condensated chromatin treads intensive luminescent regions was marked (fig. 2, *f*). Photometrical measurements fixed considerable amounts of *RecA* protein in nuclei and cytoplasm of hepatocytes.

So it was shown that:

1) after injection into liver of *RecA* protein being contained in liposomes or plasmid coding expression of bacterial *RecA* protein the penetration of injected material into separate hepatocytes takes place;

2) *RecA* protein in hepatocytes is tested in nuclei and cytoplasm after both variants of introduction;

3) in nuclei *RecA* protein is associated with chromatin and after staining by antibodies to bacterial *RecA* protein is vizualized on the nucleus structures;

4) staining of hepatocyte preparations of intact animals allows to observe slight luminescence of chromatin, that is the evidence of some determinants in eukaryotic chromatin connected with antibodies to bacterial *RecA* protein.

The immunoelectron revealing of RecA protein in vitro and in vivo. On fig. 3 (*a* — *i*) one can observe the frag-

ments of ultrathin sections of mouse liver and HeLa culture cells after incubation with antibodies for bacterial *RecA* protein followed by PAG complexes. Judging by distribution of colloidal gold particles *RecA* protein is revealed both in nuclei and in cytoplasm of those two test-systems.

In cytoplasm the greatest amount of gold particles is located over the channels of granular endoplasmic reticulum (GER) and clusters of free ribosomes and polysomes (fig. 3, *a*). In some cases the label is disposed directly in cytosol. The high level of PAG binding is shown for

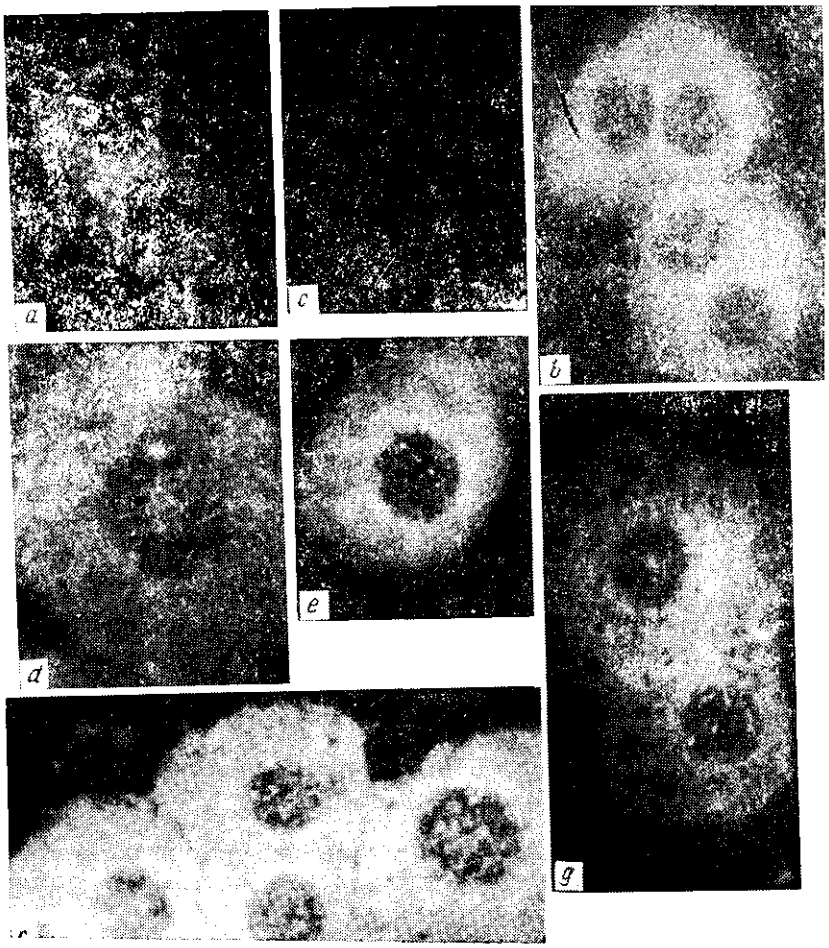

Fig. 2. Immunofluorescence of mice hepatocytes 2 days after injection of «empty» liposomes (*b*), *RecA* protein of *E. coli* (*d*, *g*) and plasmid *pKCR2* (*e*, *f*) enclosed in liposomes. Controls: hepatocytes of intact animals stained with antibodies to *RecA* protein of *E. coli* (*a*) and to bacterial β-galactosidase (*c*)

some autophagic vacuoles (fig. 3, *b*), which can be explained by proteolysis of deposited *RecA* protein in these structures. Less intensively the mitochondrion and microbodies are labelled. Very scanty labelling of the background level is observed over Golgi elements and in the vesicles of agranular endoplasmatic reticulum.

In the nuclei both of hepatocytes and of HeLa cells the label is disposed unevenly. The tendency is seen for its predominance over perimembrane chromatin, perinuclear space and nuclear membrane complexes (fig. 3, *a*, *c* — *g*). In some cases after the injection of liposomes containing plasmid *pKCR2* the peculiar chains of gold particles can be observed, located perpendicularly to the nuclear membrane and crossing it (fig. 3, *g*). On the control grids no deposition of colloidal gold is found, thus, such «chains» probably correspond to the true distribution of pro-

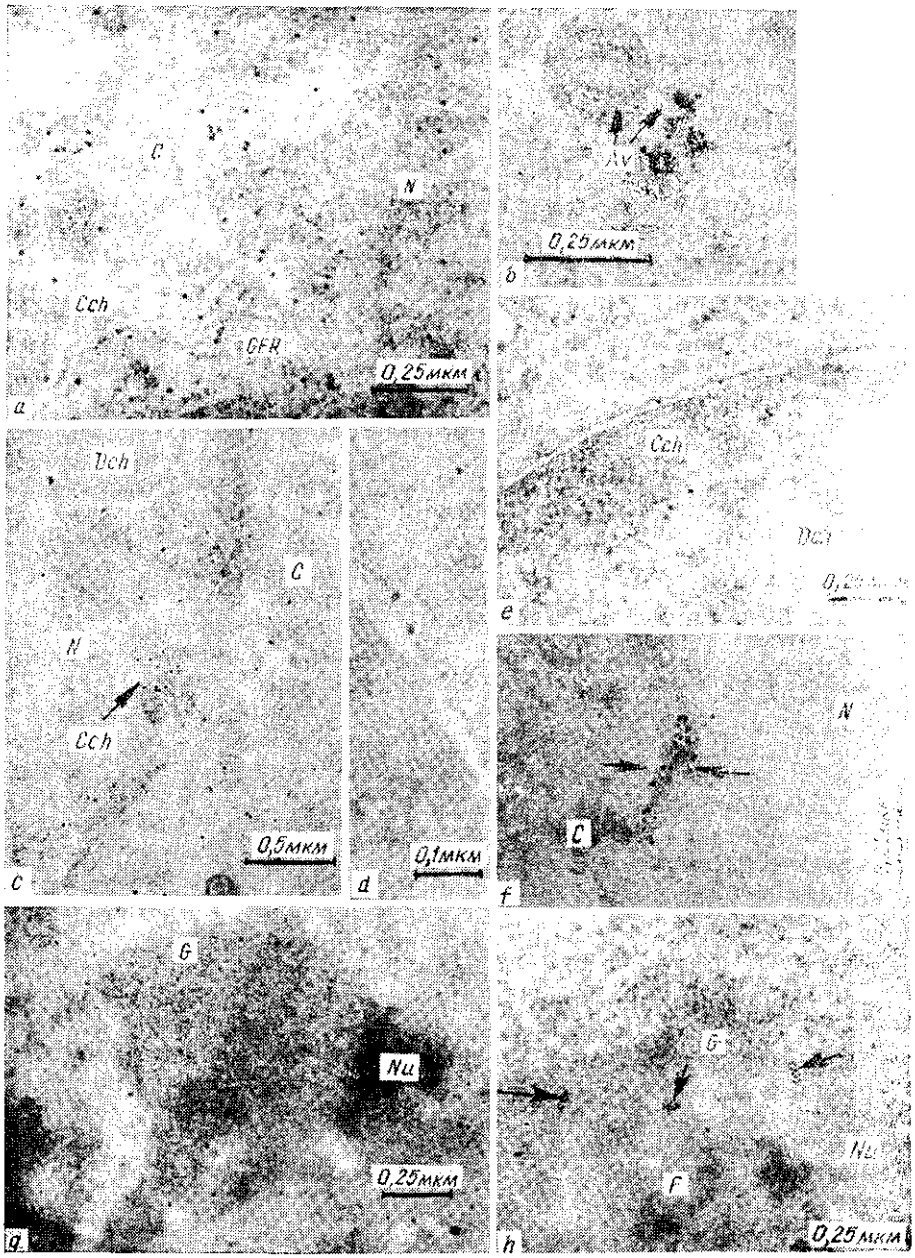

Fig. 3. Electron immunocytochemistry of hepatocytes (*a*, *b*, *d*, *e*, *g*) and HeLa culture cells (*c*, *f*, *h*) 24 h after injection/applying of liposomes containing *RecA* protein (*a*—*e*) or plasmid *pKCR2* (*f*, *g*, *h*). All sections are stained with rabbit antiserum to *RecA* protein and protein *A* — colloidal gold complexes according to [21]. Enlargements: *a* — 64 000; *b* — 96 000; *c* — 34 000; *d* — 12 000; *e* — 55 000; *f* — 67 000; *g* — 48 000; *h* — 42 000. Abbreviations: *N* — nucleus; *Nu* — nucleolus; *P* — nuclear pore complex; *Av* — autophagic vacuole; *CcH* — condense chromatin; *Dch* — disperse chromatin; *G* and *F* — granular and fibrillar components of nucleoli; *GER* — granular endoplasmic reticulum; *C* — cytoplasm. Arrows indicate the clusters of colloidal gold grains

tein molecules and depict to some extent their transport from cytoplasm to the nucleus.

The attempt to identify the localization of gold particles with certain structural type of chromatin had met some methodological difficulties. According to method [21], the preliminary etching of sections should be done (with 10 % H_2O_2) to facilitate the access of reagents to antigenic determinants. The binding of the latter with antibodies after these pro-

cedure raised to some extent but at the same time the contrast of ultrastructures beneath the label reduced. Nevertheless it can be affirmed that label is mainely localized not in the mass of perimembrane chromatin but on the periphery of its condensed clusters, in the border between the condense chromatin and the disperse one. It is here, in the bordering zone, that the perichromatin fibrils are situated — the sites of initial despiralization of expressing chromatin [22].

Both after injection of bacterial protein and of plasmid with its gene the nucleoli are labelled intensively in two test-systems. There is however certain difference between the distribution of probe over hepatocyte nucleoli in comparison with those of the HeLa cells.

The nucleoli of hepatocytes (often having nucleolonemal and sometimes compact structure) are labelled more intensively and gold particles are distributed more evenly (fig. 3, *h*). There is no preference of label to fibrillar or granular zones and only on the periphery of nucleolonemal loops there is sometimes the label of more than 2 particles.

In cells of HeLa culture, where nucleoli often have one large vacuole and well-defined borders between granular and fibrillar components, the label is distributed unevenly forming clusters of 5-10 particles (fig. 3, *i*). These latter are placed either between two zones or over granular component, mostly on the periphery of organelle. It is here, in granular component of nucleolus, that most mature sorts of RNA are placed, the ribosomes precursors [23].

Both in HeLa cells and in hepatocytes the intensity of labelling in different nucleoli even on the same section varies greatly — from abundant to practically zero.

In control immunochemical variants the ultrathin sections were incubated with PAG omitting the preliminary incubation with antibodies. On such sections there were practically no label at all, which is the evidence for comparatively high specificity of method. Nevertheless some methodological aspects of this investigation should be discussed. The authors of method [21], having applied it to the variety of tissues concluded that in principle one can obtain the high specific label of protein for tissue embedded in routine conditions, without usage of low temperature embedding media. However in all these papers the proper proteins of the cells were investigated whereas in our work the protein was foreign. May be in such case the antigene determinants are particularly tender and can be distroyed in the process of outine embedding. On the other hand, taking in mind that the serum used was polyclonal, it is possible that some portion of protein molecules with high specificity could be washed out from sections of the step of blocking. Both these reasons explain to some extent the fact that the label obtained, although specific enough, is less intensive in whole comparing with IF method, where the preparatory procedure is shorter and less harmful for antigene determinants.

Thus the presence of bacterial *RecA* protein in the nuclei and cytoplasm of cells both *in vitro* and *in vivo* is confirmed with the help of immunoelectron microscopy. In cytoplasm the protein is revealed in the channels of granular endoplasmatic reticulum, in clusters of free ribosomes and polysomes and also in some autophagic vacuoles. In the cell nuclei of both systems the protein is distributed unevenly — with the displacement to the periphery of organelles. In the nucleoplasm *RecA* protein is bound presumably with perichromatin fibrils the sites of initial despiralization of expressing chromatin. Nucleoli, that in both test-systems depose the protein quite intensively although very unequally, accumulate it mainly in the granular component. There is no substantial difference in the intercellular distribution of *RecA* protein after its own penetration to the cell or as the plasmid containing *recA* gene.

Conclusion. Proceeding from our investigations one can conclude that *RecA* protein is able to recognise not only «naked» DNA, but also DNA in part of chromatin of yeast [13] and chromatin of higher euka-

ryots. Available in literature data don't contradict to the possibility of prokaryotic proteins participation in substitution of eukaryotic proteins functions *in vivo* and *in vitro* [1, 2, 24]. Thus *RecA* protein of *E. coli in vitro* interacts equally effective both with pro- and eukaryotic DNA. There is a number of proteins with functional specialities of *RecA* protein of *E. coli* in eukaryotic cells [4-7], so functioning of *RecA* protein in eukaryotic cells *in vivo* in spite of unsufficient studying of this question seems to be interesting.

We have demonstrated possibility of *RecA* protein of *E. coli* introduction into eukaryotic cell with preservation of its fermentative activity. Both the product itself and *recA* gene expression products penetrated in nuclei of HeLa cells and hepatocytes *in vivo* with equal success and after cytological preparations treatment by rabbit's serum to *RecA* protein the protein was revealed in nuclei to be associated in whole with chromatin. It is suprising that chromatin and nucleoli in control preparations also became available to microscopic observation, one could see it very well in the intact HeLa cells treated by «empty» liposomes. Besides, it is obviously that *RecA* protein affinity with chromatin definitely depends on degree of chromatin condensation and, correspondingly, on cellular cycle phases. It can be cytologically proved by metaphasic and anaphasic chromosomes groups being revealed as dark structures on brightly fluorescent cytoplasm background while interphase nuclei chromatin fluorescence brightly.

Proceeding from of these data and also based on those facts that *RecA* protein mainly connects with single-stranded DNA and that genes are localized on chromosome ununiformly the assumption was done about existence of special state chromatin zones (SSCZ). We postulate ununiform distribution of interphasic chromosome. Structuraly they are localized in nucleus in such a way, that majority of expressing genes are collected in separate clusters. Length of each chromosome amounts to santimeters. That's why each of them in limit of nucleus with the diameter of thousands times smaller than interphase chromosome length may form pluralcurves-repetitions. And under unocasional distribution sections with intensive expressing genes (or at all expressing) are situated one next another. So functional compartments being special state chromatin zones are formed in nucleus. In result expressing sites spationaly standout for the rest mass of unexpressing chromatin.

One may suspect different amounts of SSCZ for different tissues, this number would be depend also on stage of cellular cycle. In its turn sizes of SSCZ must depend on degree of functional loading on the cell, and also on the age of the cell.

Photometrical measurements often demonstrated higher acount of *RecA* protein in interphasic nuclei, than in cytoplasm. Apparently the possibility of *RecA* protein to contrast chromatin that we have seen may be used in cytological practice for carrying out experiments with this protein, studying of expression of this gene *in vivo* and for studying of functional activity of chromatin.

Р е з ю м е. Показано можливість введення до еукаріотичної клітини *RecA*-білка *E. coli* із збереженням його біологічної активності. На цитологічному рівні (непрямий імунофлюоресцентний метод з цитофотометрією) визначено характер зв'язування *RecA*-білка з хроматином різного ступеню спіралізації на окремих стадіях клітинного циклу (у мета- і анафазних хромосомах білок не тестується). Присутність бактеріального білка у цитоплазмі і ядрах клітин *in vivo* та *in vitro* підтверджена також імуноелектронною мікроскопією. Виходячи з отриманих експериментальних даних та враховуючи переважне зв'язування *RecA*-білка з онДНК, зроблено припущення про існування зон особливого стану хроматина (ЗОСХ), які характеризуються активним зв'язуванням з сироваткою до бактеріального *RecA*-білка та інтенсивною флюоресценцією і можуть відповідати зібраним у кластери активно експресуючим генам. Експерименти виконано на культурах клітин HeLa і Ltk$^-$, а також *in vivo* на гепатоцитах після прямого введення білка та плазміди *pKCR2* у складі ліпосом до печінки дорослих мишей лінії BALB/c.

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