There are few satellite DNA-binding proteins isolated, none of them directly from the NM. Our long-term aim is to understand the role of these proteins in heterochromatin formation and in heterochromatin association with NM.

Extracts from hand-isolated nuclear envelopes from frog oocytes were tested for the specific DNA-binding activity to (T2G4)116. A fragment of *Tetrahymena* telomere from a YAC plasmid was used as a labelled probe in a gel-shift assay. The DNA-protein complexes from the assay were cut out and injected into a guinea pig. The antibodies (AB) obtained stained one protein with an m.w. of about 70 kD in the nuclear envelope of the oocyte, nothing in the inner part of the oocyte, and 70 kD and 120 kD in the frog liver nuclei. The immunofluorescent AB stained fine patches on the oocyte nuclear envelope and a number of intranuclei spots in the frog blood cells.

The electron-microscope immuno-gold technique showed that the protein is localized in the outer surface of the oocyte nuclear envelope in cup-like structures. DNA-binding activity to the same sequence has been tested and found in the mouse nuclear matrix extracts. The activity could be eluted from the DEAE52 ion exchange column in 0.15 NaCl. The activity could be competed out with the fragment itself but not with *E. coli* DNA in the same amounts. AB stained a 70-kD protein in active fractions after ion exchange chromatography. In nuclear matrix preparations, the AB recognized a 120-kD protein as well. The AB caused hypershift of the complexes on the gel shift assay. The AB has some affinity to the keratins. In the mouse cell culture 3T3 line the staining is intranuclei, with fine dots forming chains surrounding dark areas, which do not correspond to the nucleoli.

Similar results were observed when a mouse cell line was transformed with head-and tail-less human keratin constructs (Bader et al., 1991, *J Cell Biol* **115**:1293). These results suggest that the nuclear proteins detected with the AB may be natural analogs of this artificial keratin construct. The pattern of staining did not resemble the picture of telomere-specific staining. Possibly the protein recognized intragenomic (T2G4)2 sequence, which is present in 25% of murine GenBank sequences rather than telomere. We are going to do immunocytochemical investigations of frog and mouse development in order to determine the point when transcription of the 120- kD protein is initiated and the staining becomes intranuclear.

As a continuation of the previous project the multiple alignment of all the *Alu* sequences from GenBank is going on. We are also trying to obtain antibodies to the main *Alu*-binding proteins to find out how many proteins could be bound to *Alu* sequence.

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*Protein-Binding DNA Sequences

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POU domain of Oct-2 transcription factor binds octamer sequence ATGCAAAT and a number of degenerated sequences. It has been shown that POUs and POUh domains recognize left and right parts of the oct-sequence, respectively. The recognized sequences are partly overlapped in the native octamer. In the degenerated recognition sites these core sequences may be separated with a spacer up to four nucleotides. The obtained data changed our view on the number and structure of potential targets recognized on DNA by POU proteins.

Protein-DNA binding is realized due to interaction of a conservative amino acid residues with a DNA target. In POU proteins amino acid residues in positions 47 (Val), 50 (Cys) and 51 (Asn) of POUh domain are absolutely conservative. In order to examine a possible role of Val47 we substituted this residue by each of the 19 other amino acid residues and the interaction of the mutant proteins was investigated with homeospecific site and its variants (ATAANNN) and with oct sequence. It was shown that Ile47 mutant retains the affinity and specificity. Val replacement for Ser, Thr or His partially reduce the affinity.

Asn47 mutant sharply relax the specificity of protein-DNA recognition. Mutants at 47 position have much stronger effects on binding to homeospecific sites than to octamer motifs. Our data indicate that there is not a simple mono-letter code of protein/DNA recognition. It has been shown that this recognition is determined not only by the nature of the radicals involved in the contact but also by the structure of DNA binding domain as a whole and probably by cooperative interaction of POUs and POUh domains.

Proposals for 1997. The role of Cys50 in POU domain/DNA recognition will be investigated. This residue is absolutely conservative in POU proteins but it is variable in relative homeo-proteins. Our preliminary data allow to suppose that residue at position 50 of POU homeodomain have a key role in discrimination between TAAT-like and octamer sequences. The role of the nuleotides flanking DNA target will be investigated.

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Relevant Publications

1. Stepchenko A.G. (1994) Noncanonical oct-sequences are targets for mouse Oct-2B transcription factor. FEBS Letters, V.337, P.175-178.
2. Stepchenko A.G., Polanovsky O.L. (1996) Interaction of Oct proteins with DNA. Molecular Biology, V.30, P.296-302.
3. Stepchenko A.G., Luchina N.N., Polanovsky O.L. The role of conservative Val47 for POU homeodomain/DNA recognition. FEBS Letters, in press.