Antisense-targeted immuno-EM localization of the pre-mRNA path in the spliceosomal C complex

Elmar Wolf[1], Berthold Kastner[*], Reinhard Lührmann[*]

Department of Cellular Biochemistry

Max Planck Institute for Biophysical Chemistry

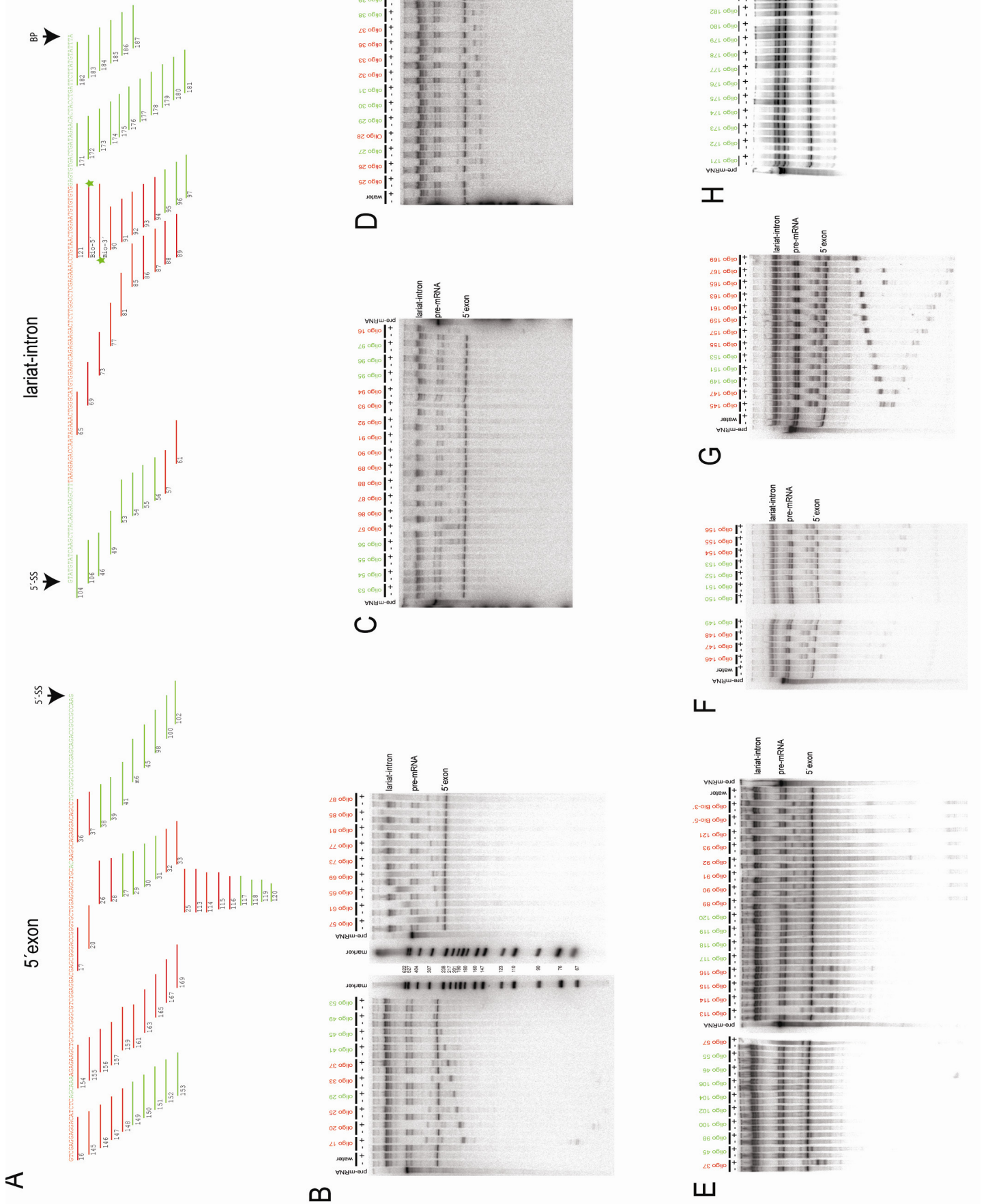
37077 Göttingen

Germany

[1]present address: Theodor-Boveri-Institut, Biocenter, Universität Würzburg, Am Hubland, 97074 Würzburg, Germany

Supplementary materials

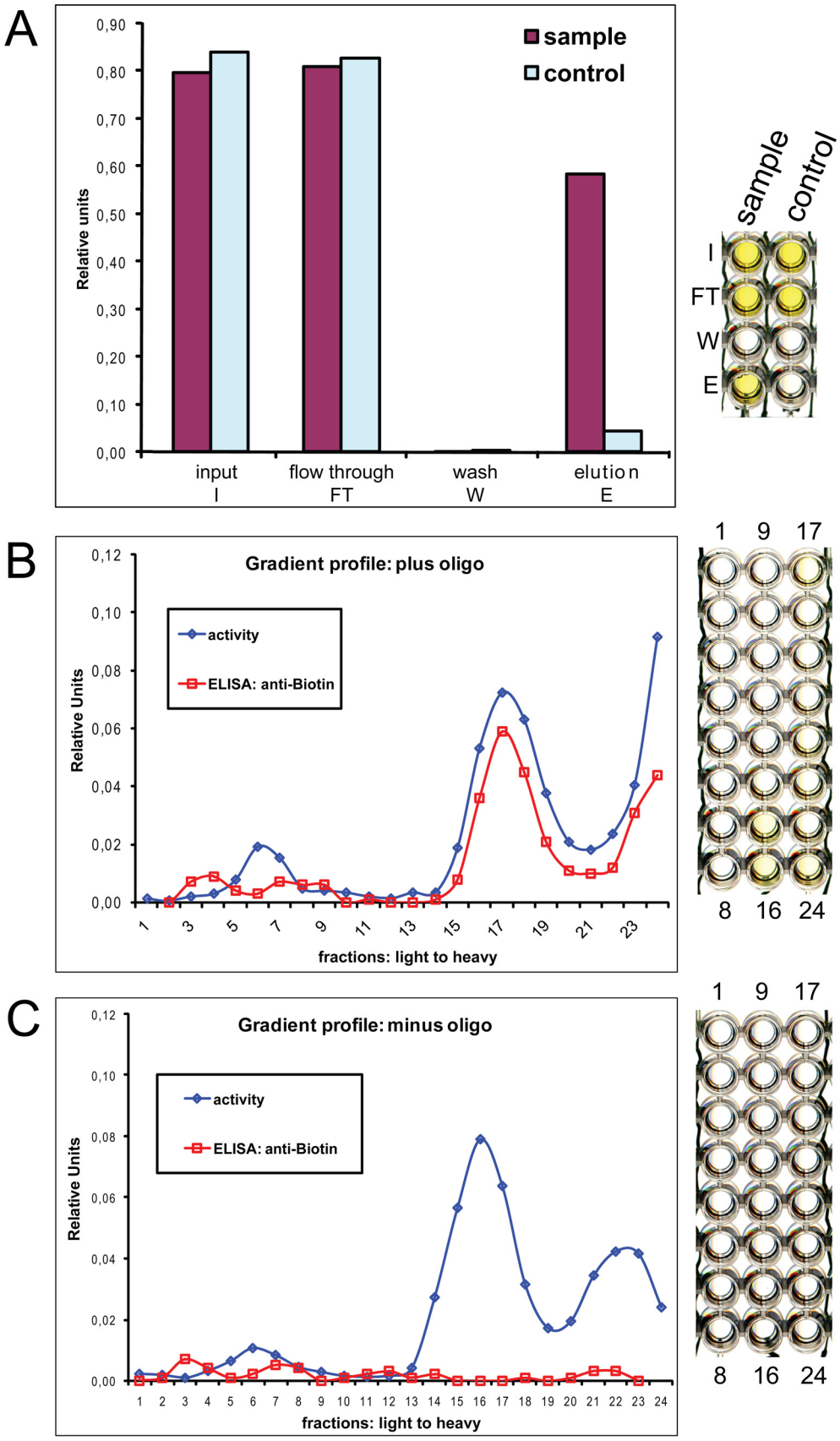
Figure S1


A
lariat-intron
5'-SS
BP
5'exon
5'-SS
B
lariat-intron
pre-mRNA
5'exon
marker
pre-mRNA
water
C
lariat-intron
pre-mRNA
5'exon
pre-mRNA
D
lariat-intron
pre-mRNA
5'exon
water
E
lariat-intron
pre-mRNA
5'exon
pre-mRNA
F
lariat-intron
pre-mRNA
5'exon
pre-mRNA
water
G
lariat-intron
pre-mRNA
5'exon
pre-mRNA
water
H
lariat-intron
pre-mRNA
5'exon
pre-mRNA

Legend Figure S1: Accessibility of pre-mRNA regions within the C complex to digestion by RNase H upon addition of complementary oligodeoxyribonucleotides to HeLa nuclear extract. C complexes assembled on ^{32}P-labeled PM5 pre-mRNA were incubated together with DNA oligonucleotides complementary to selected pre-mRNA regions. Pre-mRNA bound by DNA is then cleaved by the RNase H present in the extract. The cleavage products were separated by gel electrophoresis and visualized by autoradiography of the gels. An intact C complex is indicated by the presence of the splicing intermediates (cleaved 5' exon and the intron lariat), and their degradation implies accessibility of the sequence in the C complex. All of the cleavage products showed the expected gel-electrophoretic migration behavior after RNase H digestion.

(A) The oligonucleotide number shown defines the sequence of the oligonucleotide used. The complementary binding sites of these oligonucleotides on the pre-mRNA sequence are shown in. Oligonucleotides that led to cleavage of the target region of the pre-mRNA in complex C are shown in red. The oligonucleotides shown in green did not lead to cleavage. (B) Initial RNase H screen with DNA oligonucleotides complementary to various regions of the pre-mRNA. At the right of each gel the positions of the unspliced pre-mRNA and splicing intermediates are shown; length standards are also shown, with the respective numbers of nucleotides. (C–H) High-resolution RNase H screen with DNA oligonucleotides complementary to the regions where accessible pre-mRNA becomes inaccessible. The borders between accessible and inaccessible regions were mapped with oligonucleotides spaced 2 nucleotides apart. (E) RNase H screen of the 5' splice site and test of the oligonucleotide lengths and oligonucleotide modifications. The accessibility for cleavage by RNase H at the 5' splice site and the influence of oligonucleotide length (right-hand autoradiogram, left half) was checked. In addition to the 12 nucleotide-long oligonucleotides used in the screen (e.g., oligonucleotide 113), the use of shorter oligonucleotides also led to cleavage, but no cleavage was observed with an oligonucleotide length of 8 nucleotides (oligonucleotide 117). We also investigated whether terminal biotin-labeling affected binding of the oligonucleotide and therefore the activity of RNase H (right-hand autoradiogram, right-hand side). Efficient binding and digestion by RNase H was not prevented by biotinylation either at the 3' end (oligonucleotide Bio-3') or at the 5' ends (Bio-5'); compare with the non-biotinylated oligonucleotide 121.

Figure S2


A
0,90
0,80
0,70
0,60
0,50
0,40
0,30
0,20
0,10
0,00
Relative units
sample
control
input
I
flow through
FT
wash
W
elution
E
sample
control
I
FT
W
E
B
Gradient profile: plus oligo
activity
ELISA: anti-Biotin
Relative Units
0,12
0,10
0,08
0,06
0,04
0,02
0,00
1 3 5 7 9 11 13 15 17 19 21 23
fractions: light to heavy
1 9 17
8 16 24
C
Gradient profile: minus oligo
activity
ELISA: anti-Biotin
Relative Units
0,12
0,10
0,08
0,06
0,04
0,02
0,00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
fractions: light to heavy
1 9 17
8 16 24

Legend Figure S2: Confirmation of the specific binding of the anti-biotin antibody to oligonucleotide-labeled C complexes. (A) Detection by ELISA of anti-biotin antibodies in the purification of labeled and unlabeled C complexes. Anti-biotin antibodies were added to oligonucleotide-labeled C complexes (biotinylated oligonucleotide 'label intron anchoring site'; see Fig. 2 of the main text) and to unlabeled C complexes. Photographs of the original ELISA plates (right) and their quantitative photometric assessment (left) are shown. After the addition of antibodies a strong ELISA signal was seen (yellow coloration, see "I" and "input"). After incubation with the antibody, both C-complex samples were affinity-purified on an amylose matrix. While the C complexes bound to the matrix, most of the anti-biotin antibodies were found in the flow-through, and in the final washing step no antibodies were detected. When the oligonucleotide-labeled C complexes were eluted, they were found to contain anti-biotin antibodies. In contrast, hardly any antibody was co-eluted together with the unlabeled C-complex. This shows, that most of the anti-biotin antibodies bind specifically the biotinylated oligonucleotide with which the c complexes are labeled. (B,C) The (anti-biotin–C complex) immunocomplexes remain intact during the subsequent GraFix centrifugation. To investigate the association of the anti-biotin antibody with the C complex during the GraFix gradient centrifugation (which preceded the EM preparation), anti-Biotin antibody-labbeld and affinity-purified C complexes were, then subjected to GraFix centrifugation and finally fractionated. In the fractions, the quantities of C complex were measured by scintillation counting (blue curve) and the quantities of antibodies were measured by ELISA (red curve). The purified oligonucleotide-labeled and anti-biotin-treated C complexes (B) sedimented around fraction 17, where the anti-biotin antibodies also sedimented. Unlabeled C complexes (C) sedimented in comparable fractions (peak at 16; blue curve). However, unlike the oligonucleotide-labeled complexes, no antibody was detected in these fractions. The small quantity of co-eluted anti-biotin antibodies migrated in the light fractions of the gradient (3–8) and were not associated with complex C.