position based on a J1 hybrid map of 11p (*22*). DNA probes for known genes on chromosome 11p were provided as follows: cosmid K40 [F. S. Collins and S. M. Weissman, *Prog. Nucleic Acid Res. Mol. Biol.* **31**, 315 (1984)] containing the β-globin gene cluster (HBBC) by N. Guttmann-Bass (Jerusalem, Israel) plasmids phins321 and pPTHg108 [G. I. Bell, D. S. Gerhard, N. M. Fong, R. Sanchez-Pescador, L. B. Rall, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6450 (1985)] containing the insulin gene (INS) and the parathyroid hormone gene (PTH), respectively, by G. Bell (University of Chicago) and H. Kronenberg (Massachusetts General Hospital); plasmid pT24-Hras (Shih and Weinberg, above) containing the Harvey-*ras* proto-oncogene 1 (HRAS1) by H. E. Ruley (Cold Spring Harbor Laboratory); phage λ15B [P. C. Watkins *et al.*, *DNA* **6**, 205 (1987)] containing the follicle-stimulating hormone β polypeptide (FSHB) by P. Watkins (Bethesda Research Laboratories); and plasmid p14L (*22*) containing a centromeric nonalphoid repeat element by T. Glaser (Oregon Health Sciences University). Cosmid clones mapping to the long arm of chromosome 11 were isolated from a genomic library constructed in cosmid vectors sCos-1 (G. A. Evans, K. A. Lewis, B. E. Rothenberg, *Gene*, in press) by using DNA from a somatic cell hybrid TG5D1-1 carrying 11q13-11qter as the only human material in a mouse erythroleukemia (MEL) cell background [C. L. Maslen *et al.*, *Genomics* **2**, 66 (1988)]. Cosmid clones were isolated by cross-hybridization with total human DNA and archived in 96-well microtiter plates; many of the clones were organized into "contigs" of overlapping clones by using a pooled hybridization multiplex procedure (G. A. Evans and K. A. Lewis, *Proc. Natl. Acad. Sci. U.S.A.*, in press). Cosmid clones are designated by archive grid coordinates: 1.1, 1.16, 2.23 (XB1), 3.16, 3.17 (XB2), 4.13, 4.16 (XB4), 5.8, 6.6, 7.21 (XB10), 7.24 (SRPR), 8.5, 8.15 (XB11), 9.4, 9.27, 11.25 (PBGD), 11.34 (XH5), 13.27 (AP0A1), 17.26 (ZD7), 18.4 (ZA7), 18.29 (ZD8), 19.18 (ZC7), 19.21 (ZC9), 22.7 (ZB6), 23.2, 23.20, and 23.23 (ZD5). [The nomenclature of these clones according to *Nomenclature for Physical Mapping of Complex Genomes* (Document AO2, available from National Technical Information Service, Department of Commerce, Springfield, VA) is SALKc-3-1.1, SALKc-3-4.16, and so forth, but only the grid coordinate numbers are used here.] Cosmids containing known genetic loci were located by using cDNA or oligonucleotide probes (for example, PBGD), and additional anonymous loci (for example, XB1 or ZB6) containing as yet uncharacterized genes were identified by the presence of putative HTF islands (G. Hermanson and G. A. Evans, unpublished data). Some cosmid clones carrying genes known to be located on 11q, CL15 (PYGM), ETS1a (ETS1), HNCAM2.2 (NCAM), HTHY1 (THY1), and T3δ1.7 (CD3D) were isolated from a human genomic cosmid library constructed in cosmid vector pWE15 [G. M. Wahl *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2160 (1987)]. Where appropriate, the clone designation is followed by the genetic loci in parentheses.

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21. The J1 hybrid cell line, possessing a standard complement of hamster chromosomes, but retaining only human chromosome 11, was isolated originally by C. P. Jones, P. Wuthier, T. T. Puck [*Somatic Cell Genet.* **1**, 235 (1975)]. Cell surface antigen loci on the short arm of human chromosome 11 were used as selective markers to isolate a panel of deletion derivatives of the J1 cell line after mutagen treatment [F. T. Kao, C. Jones, T. T. Puck, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 193 (1976)]; these lines have been used extensively to map a variety of DNA clones on chromosome 11 [J. F. Gusella *et al.*, *ibid.* **77**, 2829 (1980); (*22*)]. The panel of hybrids used here possesses segments of 11p with varying internal deletions spanning the WAGR region.
22. T. Glaser, D. Housman, W. H. Lewis, D. Gerhard, C. Jones, *Somatic Cell Mol. Genet.*, in press.
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25. Metaphase spreads were prepared from human lymphocyte cultures with normal karyotypes (46, XX and 46, XY). In order to obtain elongated chromosomes, cultured cells were treated with Colcemid (0.1 μg/ml) for 10 to 30 min, and hypotonic treatment (0.075*M* KCl) was carried out for 12 to 18 min, followed by standard methanol-acetic acid fixation. An optional synchronization of the lymphocyte cultures was carried out as described [J. J. Yunis, *Science* **191**, 1268 (1976)]. Probe labeling was generally carried out by nick translation, substituting dTTP with (i) bio-11-dUTP [D. J. Brigati *et al.*, *Virology* **126**, 32 (1983); (ii) DNP-11-dUTP [dinitrophenol-*N*-hydroxysuccinimide ester was used to convert 5-(3-amino)allyl-dUTP to DNP-11-dUTP as described by P. R. Langer, A. A. Waldrop, D. C. Ward, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6633 (1981)], or (iii) with dTTP and digoxigenin-11-dUTP (Dig-11-dUTP) (Boehringer Mannheim) in a ratio of 3:1. Unincorporated nucleotides were separated from the probe DNA by centrifugation through 1 ml of Sephadex G-50 (Pharmacia) columns in the presence of 0.1% SDS.

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U1-Specific Protein C Needed for Efficient Complex Formation of U1 snRNP with a 5′ Splice Site

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One of the functions of U1 small nuclear ribonucleoprotein (snRNP) in the splicing reaction of pre-mRNA molecules is the recognition of the 5′ splice site. U1 snRNP proteins as well as base-pair interactions between U1 snRNA and the 5′ splice site are important for the formation of the snRNP–pre-mRNA complex. To determine which proteins are needed for complex formation, the ability of U1 snRNPs gradually depleted of the U1-specific proteins C, A, and 70k to bind to an RNA molecule containing a 5′ splice site sequence was studied in a nitrocellulose filter binding assay. The most significant effect was always observed when protein C was removed, either alone or together with other U1-specific proteins; the binding was reduced by 50 to 60%. Complementation of protein C–deficient U1 snRNPs with purified C protein restored their 5′ splice site binding activity. These data suggest that protein C may potentiate the base-pair interaction between U1 RNA and the 5′ splice site.

THE U1 SMALL NUCLEAR RIBONUcleoprotein (snRNP) particle is the most abundant member of the class of major snRNPs (U1, U2, U4+U6, and U5) that are essential cofactors in mRNA splicing (*1–3*). The 165-nucleotide (nt) U1 RNA is complexed with at least ten proteins, which can be divided into two classes, U1-specific proteins (70k, A, and C) and common U snRNP proteins (B′, B, D, D′, E, F, and G) (*4*). One of the functions of U1 snRNP in pre-mRNA splicing is the recognition of the 5′ splice site (*5, 6*). Although base-pairing between the 5′ end of U1 snRNP and the 5′ splice site is crucial for this recognition step (*7*), a role for U1 snRNP proteins has also been demonstrated (*5, 8*). The identity of the essential snRNP proteins and their exact functions are not known.

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We used U1 snRNPs that were gradually depleted of the U1-specific proteins (A, C, and 70k), to investigate whether one or more of these proteins are involved in 5′ splice site binding (Fig. 1A). U1 snRNP particles, deficient in protein C (ΔC), protein A (ΔA), or proteins A and C [Δ(A,C)] were obtained by chromatography of snRNPs U1 to U6 (which had been affinity purified by antibody to m_3G) on Mono Q ion-exchange resin at elevated temperatures (25° to 37°C) (*9*). A U1 snRNP particle lacking 70k, A, and C [Δ(70k,A,C)] was obtained by Mono Q chromatography at 37°C. Depletion of the respective U1-specific proteins by this procedure was, however, not always 100% effective. The ΔA and Δ(A,C) U1 snRNPs used for these studies, for example, contained residual amounts of A protein [less than 5% of the amount of A present in wild-type (wt) U1 snRNPs]. The fraction of U5 snRNPs contaminating the wt and ΔC U1 snRNPs was about 10 and 5%, respectively, as estimated by RNA gel

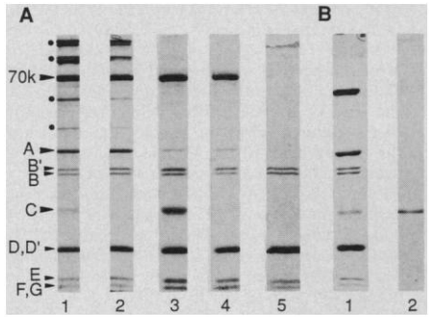


Fig. 1. Protein composition of the various types of U1 snRNPs used for the binding studies as determined by SDS–polyacrylamide gel electrophoresis. (**A**) A mixture of snRNPs U1 to U6 was isolated from HeLa nuclear splicing extracts by anti-m_3G affinity chromatography (*13*) and then subjected to Mono Q chromatography at room temperature as described (*9*). This gel shows intact U1 snRNPs (wt U1 snRNPs) (lane 1), U1 snRNPs deficient in protein C (lane 2), protein A (lane 3), or protein A and C (lane 4). For the isolation of U1 snRNPs lacking all U1-specific proteins 70k, A, and C (lane 5), wt U1 snRNPs were chromatographed again on a Mono Q column at 37°C. On Mono Q columns, 20*S* U5 snRNPs containing the U5-specific proteins characterized by molecular masses 40, 52, 100, 102, 116, and 200 (mostly a double band) kD elute at salt concentrations similar to those at which wt and ΔC U1 snRNP particles elute (*9, 10*). This explains the presence of the U5-specific proteins in addition to the U1 snRNP proteins in lanes 1 and 2 (marked by dots). (**B**) Essentially pure wt U1 snRNPs (lane 1) were obtained by Mono Q chromatography of snRNPs U1 to U6 isolated from HeLa nuclear extracts, which had been prepared at 50 m*M* Mg^{2+} concentrations (*13*). For the isolation of pure C protein (shown in lane 2) the flow through from the Mono Q column of snRNPs U1 to U6 at 25°C (see above) containing the dissociated proteins C and A together with U2-specific proteins A′ and B″ was chromatographed on a Mono S column. Protein C eluted at 200 m*M* KCl.

electrophoresis (*11*). The presence of U5 snRNPs in the respective U1 snRNP fractions does not at all influence complex formation of U1 snRNPs with the 5′ splice site (see below).

The ability of the various U1 snRNP species shown in Fig. 1 to bind to a 172-nt RNA molecule encompassing the 5′ splice site of the second intron of rabbit β-globin gene (5′ SS RNA) was compared by a nitrocellulose filter binding assay (*8*). Table 1 illustrates the specificity of the assay. Efficient complex formation of the wt U1 snRNP with an RNA molecule was critically dependent on the presence of a 5′ splice site sequence. Binding to a 143-nt RNA molecule encompassing only the 3′ splice site and the branch point of the second intron of rabbit β-globin gene (denoted 3′ SS RNA) was only marginal (about 6% of 3′ SS RNA is retained on a filter as compared to 38% of 5′ SS RNA). Furthermore, efficient binding of wt U1 snRNP to the 5′ splice site RNA required an intact 5′ end of U1 RNA. This is demonstrated by our findings that site-directed cleavage of the 5′ terminus of U1 RNA with ribonuclease (RNase) H reduced binding by about 75%, whereas treatment of U1 snRNPs with RNase H in the presence of an unspecific DNA oligonucleotide reduced binding by only about 34% (*12*). Collectively, the data indicate that the binding of wt U1 snRNP to the 5′ SS RNA is largely dependent on base-pairing between the 5′ splice site and the 5′ end of U1 RNA under our experimental conditions.

In addition, U1 snRNPs used for Table 1, experiment 4, were derived from HeLa nuclear extracts prepared at high Mg^{2+} concentrations (*13*) and were essentially free of U5 snRNPs (for the protein composition see Fig. 1B, lane 1). These nuclear extracts were prepared under conditions that caused the U5-specific proteins to dissociate from the 20*S* U5 snRNPs; the remaining core U5 snRNP particles elute from the Mono Q column at salt concentrations higher than those of the U1 snRNP particles (*10*). Thus, the presence or absence of U5 snRNPs does not affect the complex formation between U1 snRNPs and the 5′ splice site (compare the results of experiments 1 and 4).

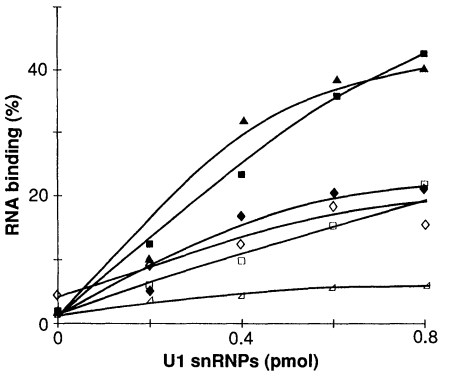
Depletion of protein C showed the most pronounced effect on RNA binding (Fig. 2). Binding of ΔC U1 snRNPs to the 5′ SS RNA was reduced by about 50% as compared to wt U1 snRNPs. In contrast, selective depletion of the A protein showed only a marginal effect on the binding ability of ΔA U1 snRNPs. The level of 5′ SS RNA binding activity of Δ(A,C) U1 snRNPs was similar to that for ΔC U1 snRNP particles. Loss of the 70k protein in addition to proteins C and A [Δ(70k,A,C) U1 snRNP] did not further decrease the binding of the U1 snRNP particles to the 5′ splice site. The residual binding activity of ΔC, Δ(A,C), and Δ(70k,A,C) U1 snRNPs to the 5′ SS RNA was significantly above the background values obtained when the 3′ SS RNA was used as a control substrate.

The importance of protein C in the recognition of the 5′ splice site by U1 snRNPs was further substantiated by the following experiment. When Δ(A,C) U1 snRNPs were preincubated with increasing amounts of purified C protein (see Fig. 1B, lane 2, for the purity of the C protein) binding activity of the particles was restored to a level similar to that observed for ΔA U1 snRNPs (Fig. 3). Since protein C by itself does not show any binding of the 5′ SS RNA (Fig. 3), we can assume that protein C restores the 5′ SS RNA binding activity of Δ(A,C) U1 snRNP

Table 1. Efficient complex formation between U1 snRNP and a 5′ splice site RNA requires an intact 5′ end of U1 RNA as determined by a filter binding technique. The nitrocellulose filter binding assay was performed essentially as described by Tatei *et al.* (*8*). U1 snRNPs (0.8 pmol) were incubated together with either 5′ SS RNA or 3′ SS RNA (10 fmol, 25,000 cpm), and *Escherichia coli* transfer RNA (100 μg/ml), and bovine serum albumin (50 μg/ml) in buffer A (10 m*M* tris-HCl, *p*H 7.5, 10 m*M* KCl, 2 m*M* $MgCl_2$, 0.1 m*M* EDTA, 0.5 m*M* dithioerythritol) in a final reaction volume of 100 μl. After incubating for 1 hour at 0°C, the incubation mixture was filtered through a nitrocellulose filter (0.45 μm) by the use of a slot blot microfiltration minifold (Schleicher and Schuell). Filters were washed with 200 μl of buffer A, dried, and subjected to autoradiography. For quantitative evaluation, individual slots were excised from the filter and ^{32}P labeling (counts/min) was measured. The table shows the percent of radioactivity retained on the filters. Experiments were done in triplicate samples from a single preparation. Standard errors of the values given in the table were less than ±8%. Intact wt U1 snRNPs, that is, U1 snRNPs with their full complement of proteins, were used in experiment 1. In experiment 2, wt U1 snRNPs were treated with RNase H in the presence of DNA oligonucleotide 1 (5′-TGCCAGGTAAGTAT-3′), which is complementary to the 5′ end of U1 RNA, for 2 hours at 30°C, before incubation with 5′ SS RNA or 3′ SS RNA (*20*). Cleavage of the 5′ end of U1 RNA under these conditions was >95% efficient as determined by RNA gel electrophoresis (*11*). In experiment 3, wt U1 snRNPs were treated with RNase H as in experiments 1 and 2, but in the presence of an unspecific DNA oligonucleotide (oligo 2, 5′-GGGGTAAATTCTTTGCCA-3′) so as to leave the 5′ end of U1 RNA intact. Whereas the U1 snRNPs used in experiments 1 to 3 contained in addition about 10% 20*S* U5 snRNPs (see Fig. 1A, lane 1), the U1 snRNPs used in experiment 4 were essentially free of U5 snRNPs (see Fig. 1B, lane 1). Comparison of the results obtained in experiments 1 and 4 demonstrated that the presence or absence of U5 snRNPs in the U1 snRNP preparation does not significantly affect binding of U1 snRNPs to the 5′ splice site under these conditions. The 5′ SS RNA and 3′ SS RNA were constructed as described (*21*). Transcription of the respective Bluescript plasmids with T7 RNA polymerase was carried out in the presence of [α-^{32}P]CTP (cytidine triphosphate) with standard protocols (*22*).

Experiment	RNase H	Oligo 1	Oligo 2	Radioactivity (percent retained)	
				5′ SS RNA	3′ SS RNA
1	−	−	−	38	6
2	+	+	−	9	5
3	+	−	+	25	5
4	−	−	−	42	5

Fig. 2. Comparison of the ability of U1 snRNPs gradually depleted of U1-specific proteins to bind to 5′ SS RNA. RNA binding activities were assayed by the nitrocellulose filter binding technique essentially as described in Table 1. Experiments were carried out with at least two independent snRNP preparations, and triplicate samples were taken from a single preparation for each point. Standard errors were less than ±8%; ▲, wt U1 snRNP; ■, ΔA U1 snRNP: ◆, ΔC U1 snRNP; □, Δ(A,C), U1 snRNP; and ◇, Δ(70k,A,C) U1 snRNP; in addition, Δ represents the level of binding of the various types of U1 snRNPs to the 3′ SS RNA.



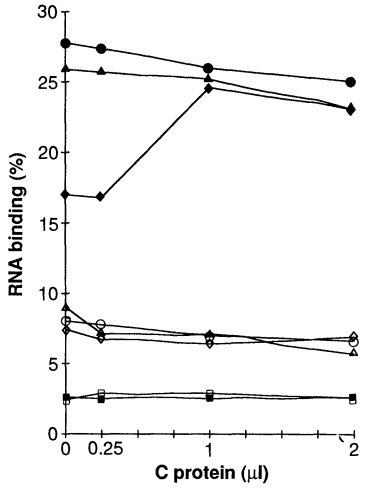


Fig. 3. Protein C restores 5′ SS RNA binding activity of Δ(A,C) U1 snRNPs. The ability of purified U1-specific protein C (see Fig. 1B, lane 2) to restore 5′ SS RNA binding activity of C-deficient U1 snRNPs was measured by the nitrocellulose filter binding techniques as described in Table 1. In order to allow assembly of protein C, Δ(A,C) U1 snRNPs or, as controls, ΔA U1 snRNPs and wt U1 snRNPs were incubated with the amounts of protein C indicated on the abscissa for 30 min at 0°C in buffer A before addition of 5′ SS RNA or 3′ SS RNA. The concentration of protein C was estimated to be 0.2 to 0.4 pmol/μl. Experiments were carried out with at least two independent snRNP preparations, and triplicate samples were taken from a single preparation for each point. Standard errors were less than ±8%; ◆, Δ(A,C) U1 snRNPs + C + 5′ SS RNA; ▲, ΔA U1 snRNP + C + 5′ SS RNA; ●, wt U1 snRNP + C + 5′ SS RNA; ◇, Δ(A,C) U1 snRNP + C + 3′ SS RNA; △, ΔA U1 snRNP + C + 3′ SS RNA; ○, wt U1 snRNP + C + 3′ SS RNA; and ■, C + 5′ SS RNA; and □, C + 3′ SS RNA.

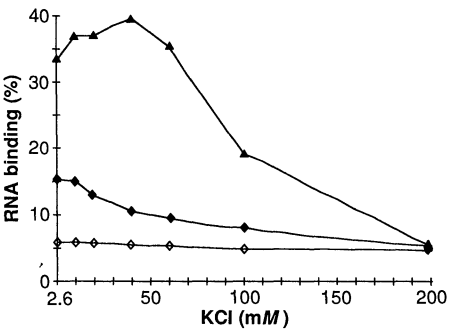


Fig. 4. Inhibition of 5′ SS RNA binding activity of U1 snRNPs by increasing KCl concentrations. The dependence on KCl concentration of the binding of U1 snRNPs to 5′ SS RNA was measured by the nitrocellulose filter assay as described in Table 1. Experiments were carried out with at least two independent snRNP preparations, and triplicate samples were taken from a single preparation for each point. Standard errors were less than ±8%; ▲, wt U1 snRNP + 5′ SS RNA; ◆, Δ(A,C) U1 snRNP + 5′ SS RNA; and ◇, wt U1 snRNP + 3′ SS RNA.

by successful assembly with the Δ(A,C) U1 snRNP particles. The stimulatory effect of protein C is specific for the interaction of U1 snRNP with the 5′ SS RNA, as no increase of binding of Δ(A,C) U1 snRNPs to the 3′ SS RNA was observed in the presence of purified C protein (Fig. 3). Qualitatively the same results were obtained when ΔC U1 snRNPs were complemented with isolated C protein.

The residual binding of U1 snRNPs to the 5′ SS RNA in the absence of protein C (Figs. 3 and 4) appears to involve base-pair interaction with U1 RNA. This is indicated by our finding that cleavage by RNase H of the 5′ end of U1 RNA in Δ(A,C) U1 snRNPs, for example, completely abolished complex formation with the 5′ SS RNA (*14*). Thus, one function of protein C could be to specifically potentiate the base-pair interaction between U1 RNA and the 5′ splice site.

Binding of the 5′ SS RNA by wt U1 snRNP was drastically inhibited at KCl concentrations greater than 50 m*M* and approached background levels at 200 m*M* KCl (Fig. 4). The reverse effect, that is, stabilization of U1 snRNP–5′ splice site complex formation, would have been expected if the interaction were only due to RNA-RNA base-pairing. The effect is specific since interaction of U1 snRNP with the 3′ SS RNA was only marginally affected by higher KCl concentrations (Fig. 4). The possibility that the inhibitory effect might simply be due to a KCl-dependent shielding of the 5′ end of U1 RNA or due to dissociation of protein C from U1 snRNP can be excluded for the following reasons: (i) The 5′ end of U1 RNA is accessible to RNase H–directed cleavage at these KCl concentrations (*15*). (ii) At 0°C U1 snRNPs do not lose protein C during Mono Q chromatography at KCl concentrations between 100 and 350 m*M* (*9*). We therefore interpret these data to indicate that ionic interactions between certain U1 snRNP proteins and the pre-mRNA (probably the phosphodiester backbone) contribute significantly to stable complex formation of U1 snRNP with a 5′ splice site and that increasing KCl concentrations interfere with these ionic protein–RNA interactions. The nature of the U1 snRNP proteins contributing to such a proteinaceous pre-mRNA binding site at the surface of U1 snRNP is not yet clear. Although protein C is a good candidate, additional U1 snRNP proteins also appear to be important in this respect. This is indicated by our finding that the complex formation of Δ(A,C) U1 snRNPs with 5′ SS RNA is still sensitive to varying salt concentrations though in a less drastic way as compared to wt U1 snRNPs (Fig. 4). More experiments are needed to clarify this point.

The primary structure of protein C has recently been determined by cDNA cloning (*16, 17*). Protein C is peculiar in its high proline content, and, furthermore, most of the proline residues are organized in clusters. It will be necessary to investigate whether these proline-rich domains may be involved in the specific augmentation by protein C of the U1 snRNP–5′ SS RNA complex formation demonstrated in this report. The recognition of a 5′ splice site region by U1 snRNPs may be even more complex if one considers the possibility that additional factors in the cell may first associate in a sequence-dependent manner with the 5′ splice site. Indirect evidence for the existence of such proteinaceous factors has recently been provided (*18*).

Although our report underscores the importance of protein C for the efficient complex formation of U1 snRNP with a 5′ splice site, the role of the other two U1-specific proteins remains to be explored. They may contribute to other functions of U1 snRNP during splicing, such as interaction with factors recognizing the 3′ splice site region (*19*) or communication with additional snRNPs in the spliceosome.

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21. The 5′ SS RNA represents a 172-nt T7 RNA polymerase transcribed RNA molecule including 37 nt of the polylinker region of Bluescript plasmid (Stratagene) and 135 nt from the rabbit β-globin gene between positions 476 and 611. This RNA molecule encompasses the 5′ splice site of the second intron from rabbit β-globin. The 3′ SS RNA comprises the 94-nt region from rabbit β-globin gene between positions 1022 and 1116. This RNA encompasses the branch point and the 3′ splice site of the second intron. In addition the 3′ SS RNA contains at its 5′ end 49 nt from the polylinker region of the Bluescript plasmid. The 5′ SS containing T7 transcription plasmid was constructed by insertion of the second intron of rabbit β-globin gene flanked by some exon sequences as Bam HI–Bgl II fragment (nt 476 to 1196) into the Bam HI linearized Bluescript plasmid. Before transcription the construct has been linearized with Nco I at nucleotide position 611. The 3′ SS containing T7 transcription plasmid was prepared by insertion of a fragment of rabbit β-globin gene between the restriction sites for Apa I (position 1022) and Bgl II (position 1196) into the Bluescript plasmid which was opened at the Pst I and Hinc II sites after creation of appropriate ends. Before transcription the construct was linearized with Eco RI at position 1116. All restriction site positions refer to the sequencing data of van Ooyen *et al.* [A. van Ooyen, J. van den Berg, N. Mantei, C. Weissmann, *Science* **206**, 337 (1979)].
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Mechanism of Insect Resistance to the Microbial Insecticide *Bacillus thuringiensis*

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Receptor binding studies show that resistance of a laboratory-selected *Plodia interpunctella* strain to a *Bacillus thuringiensis* insecticidal crystal protein (ICP) is correlated with a 50-fold reduction in affinity of the membrane receptor for this protein. The strain is sensitive to a second type of ICP that apparently recognizes a different receptor. Understanding the mechanism of resistance will provide strategies to prevent or delay resistance and hence prolong the usefulness of *B. thuringiensis* ICPs as environmentally safe insecticides.

PROSPECTS FOR THE FUTURE OF MICROBIAL insecticides are bright. In addition to conventional applications, recent advances in plant transformation with ICP genes from the entomopathogenic bacterium *Bacillus thuringiensis* (*1*) provide an exciting new approach to insect control in which transgenic plants produce their own protective proteins. Moreover, increasing limitations on the use of chemical pesticides stimulate interest in alternative strategies in pest control. Obviously, the extent to which insects are able to develop resistance to *B. thuringiensis* ICPs will be an essential determinant for the continued success of these insecticides. Significant resistance to *B. thuringiensis* has not been observed during its use over more than three decades. However, in laboratory selection experiments, high levels of resistance were obtained in the Indian meal moth (*Plodia interpunctella*) (*2*). In strains selected by rearing on a *B. thuringiensis*–treated diet, the median lethal dose (LD_{50}) increased to as much as 250 times those of the sensitive parent strains (*3*). Subsequently, lower levels of resistance have been selected in the almond moth (*Cadra cautella*) (*3*) and the tobacco budworm (*Heliothis virescens*) (*4*). Conventional applications of *B. thuringiensis* are currently increasing and commercialization of transgenic crops expressing ICPs is envisaged within a few years. Thus, the challenge is to develop deployment strategies that will minimize the potential for development of field resistance (*5*). Therefore, it is critical to understand the mechanism involved in pest resistance to these toxins.

We investigated the mechanism of resistance to *B. thuringiensis* ICPs in a *P. interpunctella* strain (strain 343) selected for a high level of resistance against Dipel, a commercial formulation of a crystal-spore mixture of *B. thuringiensis* var. *kurstaki* (*2*). It has been shown that this *P. interpunctella* strain did not exhibit similar levels of resistance to certain other *B. thuringiensis* strains (*6*). In this context, it should be noted that crystals of most *B. thuringiensis* strains contain more than one type of ICP. Different classes of ICPs exhibit striking differences in their insecticidal spectrum (*7, 8*) and may bind to distinct receptor sites (*9*), presumably of glycoprotein nature (*10, 11*). We have taken into account this substantial heterogeneity in *B. thuringiensis* ICPs by studying two distinct ICPs. Two essential factors in the pathway of toxic action of *B. thuringiensis* ICPs are proteolytic activation of the protoxin (around 130,000 daltons) to the active toxin (around 66,000 daltons) (*12*) and binding of the toxin to receptors on the brush border membrane of the midgut epithelium (*9–11*). The contribution of receptor binding and protease activity in the mechanism of resistance has been studied.

Crystals of Dipel preparations consist of a mixture of ICPs belonging to the CryIA and to the CryII family (*7*). We compared resistance levels to Dipel and cloned ICPs of the CryIA(b) type [Bt2, from *B. thuringiensis* var. *berliner* 1715 (*13*)] and the CryIC type [Bt15, from *B. thuringiensis* var. *entomocidus* HD110 (*14*)]. Resistance levels to both the protoxin and the activated toxin were determined. Resistance to Dipel was confirmed by the toxicity data for the sensitive strain (S strain) and for the strain selected for Dipel resistance (R strain) (Table 1). High levels of resistance are also observed for CryIA(b) protoxin and CryIA(b) toxin. Since similar resistance levels were obtained with CryIA(b) protoxin and in vitro–activated CryIA(b) toxin, resistance is apparently not due to lack of proteolytic activation of protoxin in the midgut of the R strain. In contrast to the ICP of the CryIA(b) type, there is no resistance to CryIC protoxin and CryIC toxin. This ICP is not present in Dipel crystals. Moreover, we observed a marked increase in sensitivity to CryIC pro-

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