A Cell-specific Glycosylated Silk Protein from Chironomus thummi Salivary Glands

CLONING, CHROMOSOMAL LOCALIZATION, AND CHARACTERIZATION OF cDNA*

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Chironomid salivary glands contain 40 cells dedicated to the synthesis of a relatively small ensemble of silk proteins. Glands in some species contain a special lobe composed of 4 cells distinguishable from the others. We have cloned a lobe-specific cDNA from Chironomus thummi salivary glands. Northern blots of salivary gland RNA demonstrated that the cDNA hybridizes to a 2.5-kilobase transcript present only in the special lobe. In situ hybridization mapped the gene encoding this cDNA to region A2b on polytene chromosome IV, the locus of the special lobe-specific Balbiani ring a. The deduced amino acid sequence encodes a protein with a calculated molecular mass of 77 kDa and numerous potential glycosylation sites; it appears unrelated to other known chironomid silk proteins. Polyclonal antibody, raised against a cDNA-encoded fusion protein, reacted exclusively with a special lobe-specific 160-kDa silk protein. Lectin binding studies indicate that the immunoreactive 160-kDa protein contains both N- and O-linked glycan moieties. We conclude that glycosylation most likely contributes to the difference between calculated and apparent molecular masses and that this cDNA encodes the special lobe-specific silk protein previously described as ssp160 (Kolesnikov, N. N., Karakin, E. I., Sebeleva, T. E., Meyer, L., and Serfling, E. (1981) Chromosoma 83, 661–677).

Silks are produced by a wide variety of arthropods including spiders and larvae of hundreds of insect species. Few silks are well-characterized, but emerging evidence suggests that differences in biochemical and biophysical properties are attributable to constituents that vary considerably among species (1). In fact, silk proteins from silkworms (2–4), spiders (5), and larvae of hundreds of insect species. Few silks are well-characterized, but emerging evidence suggests that differences in biochemical and biophysical properties are attributable to constituents that vary considerably among species (1). In fact, silk proteins from silkworms (2–4), spiders (5), and

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The nucleotide sequences reported in this paper have been submitted to the GenBank™/EMBL Data Bank with accession number(s) U24265.

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The abbreviations used are: BR, Balbiani ring; spX, silk protein with an apparent molecular mass of X; ssp160, special silk protein 160; PCR, polymerase chain reaction; ConA, concanavalin A; GNA, Galanthus nivalis agglutinin; PNA, peanut agglutinin; kb, kilobase(s); bp, base pair(s).

EXPERIMENTAL PROCEDURES

Salivary Gland Dissection—C. thummi thummi (Novosibirsk strain) was raised (22) on powdered dolomitic limestone which appeared to
increase the amount of special lobe secretion stored in the gland. Sali-

vory glands, dissected from fourth instar larvae and prepupa, were

rinsed in 0.8% NaCl and fixed in 70% ethanol. The special lobe was

identified by its location and distinctive appearance and cut away

from the gland. For RNA extracts, the remainder of the gland was taken as

an average amount of special lobe secretion stored in the gland. Sali-

tography. The resulting probe had a specific activity of 3.3

M13BM20(Boehringer Mannheim) to yield M13BM20[S

cleotides were reduced to 20

M13BM20[32P]RNA was synthesized from special and main
cDNA from four clones from

M13BM20(−N) was cut with Alul, and the 288-bp fragment (cDNA

nucleotides 1086-1373) was blunt-end-ligated to the filled-in SalI site

in the expression vector pET12b (Novagen). This placed the cDNA

in-frame with the ompT leader. Transformants of Escherichia coli

strain BL21(DE3)pLysS (33) were identified by colony hybridization to a

PCR cDNA probe. The construct was confirmed by DNA sequencing.

To assay for production of fusion protein, antibody production,

leader. Transformants of E. coli strains containing pET12b. The antibody was then diluted and used for West-

ern blotting (38). For competition studies, lysate from BL21 (E)

DE3)pLysS-containing pET12b with the cDNA insert (i.e. with fusion protein)

was used for adsorption.

Western Blotting—For analysis of salivary gland proteins, samples

of either special and distal-main lobes (cells plus secretion) or secretion

alone were solubilized with guanidine HCI, reduced and carboxymeth-

yalbumin as a standard. Those containing fusion protein were pooled,
dialyzed against water, and lyophilized. Approximately 0.5 mg of fusion

protein was recovered from 5 mg of protein loaded on each preparative

gel. The identity of the fusion protein was confirmed by amino acid

Analysis.

Antibody Production and Purification—Rabbits were immunized

subcutaneously at multiple sites with a total of 170 μg of purified fusion

protein emulsified with Freund's adjuvant (36). Booster injections of

100 μg and 280 μg were given on days 14 and 31, respectively. Immune

serum was collected on day 49.

Affinity-purified antibodies were obtained by chromatography (37)

using 500 μg of gel-purified fusion protein coupled to Affi-Gel 10 (Bio-

Rad). To remove antibodies against the ompT leader and co-purified E.

coli proteins, affinity-purified antibody (14 μg of protein in 1 ml of 1% non-

fat dry milk, 20 ml Tris-HCl, pH 7.5, 0.5 μM NaCl, 0.02% NaN3) was

adsorbed for 4 h at 20°C with 275 μg of protein from a control lysate of

isopr1-thio-β-D-galactopyranoside-induced BL21(DE3)pLysS cells

containing pET12b. The antibody was then diluted and used for West-

ern blotting (38). For competition studies, lysate from BL21 (DE3)pLysS-containing pET12b with the cDNA insert (i.e. with fusion protein)

was used for adsorption.

Lectin Binding—Western blots were incubated with digoxigenin-

labeled lectins that were subsequently detected with an anti-digoxige-

nin antibody conjugated to alkaline phosphatase. GNA, PNA, and ConA

binding were done according to the manufacturer's protocols (Boeh-

ringer Mannheim), except that the PNA and ConA were diluted to 5

μg/ml and 3% bovine serum albumin (Fraction V) was used as a block-

ing agent for ConA.

To examine protein binding to lectins, special lobe proteins were

extracted as described above (16) and dialyzed against TBS (50 mM

Tris-HCl, pH 7.5, 150 mM NaCl) keeping the protein concentration

less than 25 μg/ml to avoid precipitation. Dialysate was equilibrated

with an equal volume of ethylene glycol in TBS. Those containing

(labeled glucose) in TBS plus 1 μM each MgCl2, CaCl2, and MnCl2, for

2 h at room temperature with constant mixing. Supernatants contain-

ing unbound protein were removed, and the resin was rinsed thor-

oughly. To release bound protein, resin was then incubated for 1 h in

2 volumes of binding buffer containing 1 μM mannan (GNA-Sepharose)

(45) or 0.2 M lactose (PNA-Sepharose) (46). The supernatant was re-

moved, dialyzed against water, and proteins were precipitated with

acetic. These fractions were subjected to SDS-polyacrylamide gel

electrophoresis and immunoblotted.

RESULTS

Cloning a Special Lobe-specific cDNA—Our search for a
cDNA clone encoding a special lobe-specific protein began by con-

paring double-stranded, 32P-labeled cDNA made from spe-

2 For simplicity, we refer to the nonspecial lobe portion of the gland as the “main lobe” because those cells all appear similar to each other. However, anatomical distinctions between different regions of the gland have been made (7).

3 S. T. Case, C. Cox, W. C. Bell, R. T. Hoffman, J. Martin, and R. Hamilton, manuscript in preparation.
cial and main lobe poly(A)⁺ RNA on an agarose gel (Fig. 1). Several discrete (1.5-, 1.8-, and 4.5-kb) cDNAs were present in both samples and 5.5- and ~20-kb cDNAs were enriched in the main lobe. However, the most striking result was an abundant 2.5-kb cDNA that was present only in the special lobe sample. Even with extensive exposure, no corresponding band was seen in main lobe material. This cDNA was used to make a size-fraction library in λgt22.

A radiolabeled special lobe-specific cDNA probe was made by subtractive hybridization against an excess of main lobe poly(A)⁺ RNA. To check probe specificity, we first hybridized the probe to a Northern blot containing special and main lobe RNA. Hybridization was seen only to a 2.5-kb special lobe RNA (Fig. 2A). This confirmed the lobe specificity of the probe and demonstrated that only one size class of special lobe-specific transcripts was detected.

To determine the proportion of special lobe-specific transcripts in the entire gland, we screened two C. thummi whole gland cDNA libraries with the special lobe-specific subtracted probe. 7.7% of 1.2 × 10⁶ clones in a primary λgt23 library and 1.8% of 1.8 × 10⁶ clones in an amplified λZAP library were hybridized. However, when this subtracted probe was used to screen the 2.5-kb size fraction λgt22 library, 92% of the insert-containing clones hybridized. The first four clones selected had identical restriction enzyme cleavage patterns, suggesting that they are representative of cDNA molecules taken from the agarose gel. One, λ160.1, was chosen for further characterization.

To determine the size of the RNA from which this cDNA was derived, primers complementary to flanking vector sequences were used to amplify and radiolabel the cloned 2.5-kb cDNA by PCR. Northern blots of special and main lobe-derived RNA showed specific hybridization only to a 2.5-kb RNA from special lobes (Fig. 2B). Subsequent rehybridization of this blot with a probe for 5.5-kb sp220 mRNA verified that lack of hybridization to main lobe transcripts was not due to RNA degradation (Fig. 2C). We conclude that the cloned cDNA is a near full-length copy of a 2.5-kb special lobe-specific RNA.

Localizing the Gene—The chromosomal location of the gene encoding this cDNA was determined by in situ hybridization to salivary gland polytene chromosomes. The hybridization signal of the PCR-amplified and digoxigenin-labeled cDNA probe was localized to region A2b of chromosome IV (Fig. 3). Samples from two subspecies, C. thummi thummi and C. thummi piger, both gave the same result. Region A2b on polytene chromosomes in main lobe cells contains a thick, condensed band that forms BRa in special lobe cells (41).
that region I is comprised of six almost-perfect tandem copies of the hexameric repeat TSSNST (Fig. 5). Region II has very similar hexamers; variations, including a 2-residue deletion, appear limited to the first three residues. Region III is separated from the others by the central basic core and is most divergent; however, seven 6–11-residue segments can be aligned by a conserved N\textsubscript{X}T motif. These patterns suggest that internal sequence duplication has occurred during evolution, preserving the N\textsubscript{X}T motifs.

While there is no definitive consensus for sites involved in O-linked glycosylation, residues that are modified tend to be located +1 or −3 residues from a proline (e.g. P(S/T) or (S/T)XXP) or located near other serines, threonines, or alanines (43). Based on these criteria, Thr\textsubscript{60}, Ser\textsubscript{185}, Ser\textsubscript{251}, Thr\textsubscript{335}, Ser\textsubscript{581}, Thr\textsubscript{683}, and Ser\textsubscript{727} are possible candidates for O-glycosylation along with the numerous serines and threonines which neighbor each other.

Identifying the Encoded Protein—A 288-bp \textsc{Alu}I fragment of \textit{cDNA} encoding the central, basic region was subcloned into pET12b to provide inducible expression of an \textit{ompT/cDNA}-encoded fusion protein. Anti-fusion protein antibody was reacted with Western blots made from total salivary gland proteins separated by electrophoresis on SDS-polyacrylamide gels. Immunoreactivity was limited to one special lobe protein with an apparent molecular mass of about 160 kDa (Fig. 6A). To demonstrate that immunoreactivity was due to epitopes encoded by the \textit{cDNA}, a parallel blot was reacted with antibody that had been preadsorbed with fusion protein (Fig. 6B). No immunoreactive band was seen, even when the color reaction was deliberately overdeveloped. To determine whether the immunoreactive protein was secreted into the lumen, we examined samples of purified secretion. An immunoreactive 160-kDa protein was present in special lobe secretion only (Fig. 6C). Based on lobe specificity, electrophoretic mobility, and secretion into the lumen, the immunoreactive protein fits the criteria defining ssp160 (20).

Examining Carbohydrate Moieties—Since the \textit{cDNA}-encoded protein has numerous glycosylation sites which, if used, could contribute to differences in calculated and apparent molecular masses, Western blotted proteins were reacted with lectins for detection of carbohydrate. Nearly all stainable proteins are common to special and main lobes, except the 160-kDa protein present only in the special lobe lane (Fig. 7A). N-linked oligosaccharides react with ConA, which binds to internal and nonreducing terminal \(\alpha\)-mannosyl residues (44). Over a dozen ConA-binding proteins were common to both special and main lobes (Fig. 7B); however, one additional ConA-binding protein, migrating at 160 kDa (arrow) was present only in the special lobe. GNAbindsonly those glycans having a terminal mannose linked to mannose (45). Consequently, while all GNA-binding proteins react with ConA, only a subset of the N-glycosylated proteins reacting with ConA are detected with GNA. At least two GNA-binding proteins were present in both special and main lobes (Fig. 7C); however, one additional ConA-binding protein, migrating at 160 kDa (arrow) was present only in the special lobe. GNA binds only those glycans having a terminal mannose linked to mannose (45). Consequently, while all GNA-binding proteins react with ConA, only a subset of the N-glycosylated proteins reacting with ConA are detected with GNA. At least two GNA-binding proteins were present in both special and main lobes (Fig. 7C); however, one additional ConA-binding protein, migrating at 160 kDa (arrow) was present only in the special lobe. GNA binds only those glycans having a terminal mannose linked to mannose (45). Consequently, while all GNA-binding proteins react with ConA, only a subset of the N-glycosylated proteins reacting with ConA are detected with GNA. 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capable of depleting immunoreactive ssp160 from extracts of special lobe proteins, and that the immunoreactive protein was specifically released from the lectins by incubation with cognate sugars (Fig. 8).

**DISCUSSION**

Chironomid silk proteins have been studied most extensively in *C. tentans*. The molecular biological data base for the major proteins is complete (6), and studies are underway to reveal how they fold and assemble into fibers (17, 48). However, *C. tentans* evidently lacks characteristics of special lobe-specific BR and protein (18, 49). The silk protein data base for species that have a special lobe (*C. pallidivittatus* (50) and *C. thummi* (51, 52)) is small but growing (40), but comparable data for special lobe proteins is hard to come by. Salivary glands in these species are smaller (~1 mm in length), and yields of special lobe poly(A)1 RNA (2 ng/lobe) and silk proteins (<100 ng/lobe) are limited. This project required the manual dissection of over 4000 salivary glands. Nonetheless, a special lobe-specific cDNA has been acquired, and the encoded protein has been identified. We conclude that s160.1 cDNA encodes the major, if not only,
special lobe-specific silk protein in C. thummi salivary glands. Its mRNA is lobe-specific (Fig. 2) and cDNA most abundant (Fig. 1). Its gene resides in lobe-specific B5A (Fig. 3). The encoded immunoreactive protein is absent from main lobes and found in special lobes and their secretion (Fig. 6). These characteristics fit criteria defining ssp160 (20); however, there is a discrepancy between the calculated (77 kDa) and apparent (160 kDa) molecular mass of these proteins (Figs. 4 and 6). The presence of 77-kDa dimers is possible but unlikely since, for Western blotting, protein extraction included denaturation in 6 M guanidine HCl, reduction of disulfide bonds, and covalent modification of cysteinyl sulfhydryl groups. An alternative explanation is that decreased electrophoretic mobility is due to protein glycosylation. For example, N- and O-linked sugars comprise 50–70% of the mass of some glycoproteins (53). ssp160 can incorporate label from [3H]glucosamine (20), and the amino acid sequence inferred from the cDNA (Fig. 4) contains numerous sites for potential glycosylation. Lectin binding coupled with Western blotting experiments showed that the 160-kDa special lobe-immunoreactive protein contains both O- and N-linked sugars (Figs. 7 and 8). These results indicate that the cDNA-encoded and immunoreactive proteins are the same. To estimate how much glycosylation contributes to the mobility of this protein, we attempted enzymatic removal of sugars followed by immunoblotting with anti-fusion protein antibody to detect the resulting protein core. Despite successful removal of sugars from purified control proteins and lack of detectable proteolysis, repeated attempts to deglycosylate silk proteins were inconclusive; lectin binding was abolished, but we failed to detect an immunoreactive band. Since the bacterially expressed fusion protein could not have contained carbohydrate epitopes, the evident loss of immunoreactivity could not be due to loss of sugars per se. A more plausible explanation is that detection was hampered by the heterogeneous distribution of partially deglycosylated products. Thus, we conclude that ssp160 cDNA does, in fact, encode ssp160.

The primary structure of ssp160 is novel. A survey (54) of data bases revealed no overall similarities but some related regions. For example, ssp160’s putative hydrophobic leader sequence duplication of regions preserving the N-linked sugars is unknown. ssp160 is unusual even among Chironomids salivary glands are dedicated to producing large amounts of a small ensemble of silk proteins. This is reflected in the cDNA banding pattern seen for both special and main lobes; several distinct cDNAs are common to both lobes, and their sizes coincide with mRNAs for small and midsize silk proteins. The amount of ssp160 cDNA (the 2.5-kb special lobe-specific band in Fig. 1) is remarkable, prompting expectations of proportionally high levels of ssp160; however, Coomassie-stained gels* and AuroDye-stained blots (Fig. 7) indicate that neither ssp160, nor any other special lobe-specific protein, accumulates to such levels. This contrasts with other silk proteins whose steady-state level of mRNA and protein coincide (59–61). These observations suggest that either translational control of ssp160 synthesis or its half-life in the gland differs dramatically from that of other silk proteins.

The role of the special lobe and ssp160 is uncertain. There are no reported differences in either tube-building behavior, structure of silken feeding/pupation tubes, or properties of silk in species that do and do not have special lobes. The molecular probes and antibodies acquired in this study will enable us to examine Beermann’s granules for the presence of ssp160 and begin a phylogenetic investigation of the evolution and expression of ssp160-encoding genes that may lead to elucidation of the function of the special lobe.

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REFERENCES

1. Kaplan, D., Adams, W. W., Farmer, B., and Viney, C. (1993) Silk Polymers: Materials Science and Biotechnology, American Chemical Society, Washington, D.C.
2. Oshima, Y., and Suzuki, Y. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 5363–5367
3. Gage, L. P., and Manning, R. F. (1980) J. Biol. Chem. 255, 9444–9450
4. Manning, R. F., and Gage, L. P. (1980) J. Biol. Chem. 255, 9451–9457
5. Xu, M., and Lewis, R. V. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 7120–7124
6. Wieslander, L. (1994) Proc. Nutidical Acids Res. 22, 275–313
7. Case, S. T., and Wieslander, L. (1992) Results Probl. Cell Differ. 13, 187–226
8. Lamb, M., and Daneshbakt, B. (1979) Cell 17, 835–848
9. Skoglund, U., Anderson, K., Strandberg, B., and Daneshbakt, B. (1986) Nature 319, 356–360
10. Olins, D. E., Olins, A. L., Levy, H. A., Durfee, R. C., Margile, S. M., Timlin, E. P., and Dover, S. D. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 598–600
11. Francene, C., Edström, J.-E., Dowedall, A. W., and Miller, O. L. J. (1982) EMBO J. 1, 59–62
12. Kiselova, E. V. (1988) FEBS Lett. 258, 251–253
13. Herting, T., Eppenberger, H. M., and Leski, M. (1983) Chromosoma 88, 194–200
14. Galli, J., and Wieslander, L. (1993) J. Biol. Chem. 268, 11888–11893
15. Galli, R., Rydland, L., Riedel, N., Kluding, H., and Eppenberger, H. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 1448–1452
16. Kao, W. Y., and Case, S. T. (1985) J. Cell Biol. 101, 1044–1051
17. Wildman, S. E., and Case, S. T. (1986) J. Biol. Chem. 261, 10879–10893
18. Beermann, W. (1961) Chromosoma 12, 1–25
19. Grossbach, U. (1977) Results Probl. Cell Differ. 8, 147–196
20. Kolesnikov, N. N., Karakin, E. I., Sebatellie, T. E., Meyer, L., and Selrings, E. (1981) Chromosoma 83, 661–677
21. Kloezel, J. A., and Laufer, E. H. (1969) J. Urol. 92, 15–36
22. Kiknadze, I. I., Lopatin, O. E., Kolesnikov, N. N., and Gunderina, L. I. (1990) In Animal Species for Developmental Studies (Dettlafl, T. A., and Vassetzky, S. G.) eds. Vol. 1, pp. 133–138, Consultants Bureau, New York
23. Case, S. T., and Daneshbakt, B. (1978) J. Biol. Chem. 253, 224–229
24. Bailey, J. M., and Davidson, N. (1976) Anal. Biochem. 70, 75–85
25. Han, J. H., and Rutter, W. J. (1987) Nature Acids Res. 15, 6304
26. Short, J. M., Fernandez, A. A., and Huse, W. D. (1988) Nature Acids Res. 16, 7583–7600
27. Benton, W. D., and Davis, R. W. (1977) Science 196, 180–182
28. Davis, M. M., Cohen, D. I., Nielsen, E. A., Steinmetz, M., Paul, W. E., and Hood, L. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 2194–2198
29. Doman, K. B., and Faldon, D. (1977) Methods Enzymol. 45, 335–350
30. Honoré, B., Madsen, P., and Jeffers, H. (1993) J. Biochem. Biophys. Methods 27, 39–48
31. Shedd, E. R., Key, H. G., and Hanks, J. E. (1988) Chromosoma 96, 353–359
32. Postell, J., and Kahafop, F. C. (1982) Nuclid Acids Res. 10, 51–59
33. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorf, J. W. (1990) Methods Enzymol. 185, 60–89
34. Laemmli, U. K. (1970) Nature 227, 680–685
35. Smith, P. K., Kohno, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Geoke, N. M., Olson, B. J., and Klenk, D. C. (1985) Anal. Biochem. 155, 76–85
36. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
37. Drennan, T. D., and Case, S. T. (1987) Gene (Amst.) 55, 55–65
38. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 4350–4354
39. Stolarovich, D., and Mantelaro, R. C. (1986) Anal. Biochem. 156, 341–347
40. Brunlle, L. L., Bogachev, S., Kolesnikov, N. N., Wilke, J. H., and Case, S. T. (1995) Comp. Biochem. Physiol. 104B, 731–738

* R. T. Hoffman and S. T. Case, unpublished data.
41. Kiknadze, I. I., Zainiev, G. A., Panova, T. M., Iostomina, A. G., Zacharenko, L. P., and Potapov, W. A. (1985) Biol. Zbl. 104, 113–123
42. Spiro, R. G. (1973) Adv. Prot. Chem. 27, 349–467
43. Wilson, I. B. H., Gavel, Y., and von Heijne, G. (1991) Biochem. J. 275, 529–534
44. Debray, H., Decout, D., Strecker, G., Spik, G., and Montreuil, J. (1981) Eur. J. Biochem. 117, 41–55
45. Shibuya, N., Goldstein, I. J., Van Damme, E. J., and Peumans, W. J. (1988) J. Biol. Chem. 263, 728–734
46. Lotan, R., Skutelsky, E., Danon, D., and Sharon, N. (1975) J. Biol. Chem. 250, 8518–8523
47. Kobata, A. (1992) Eur. J. Biochem. 209, 483–501
48. Smith, S. V., Correia, J. J., and Case, S. T. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 945–954
49. Grossbach, U. (1969) Chromosoma 28, 136–187
50. Grund, C., Saiga, H., and Edstrom, J.-E. (1987) Res. Probl. Cell Differ. 14, 69–80
51. Baumlein, H., Wobus, U., Gerbi, S. A., and Kafatos, F. C. (1982) Nucleic Acids Res. 10, 3893–3904
52. Galli, J., and Wieslander, L. (1994) J. Mol. Biol. 238, 482–488
53. Dwek, R. A. (1995) Science 269, 1234–1235
54. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
55. Snyder, M., Hunkapiller, M., Yuen, D., Silvert, D., Fristrom, J., and Davidson, N. (1982) Cell 29, 1027–1040
56. Garrison, K., Mackrell, A. J., and Fessler, J. H. (1991) J. Biol. Chem. 266, 22899–22904
57. Olson, P. F., Fessler, L. I., Nelson, R. E., Sterne, R. E., Campbell, A. G., and Fessler, J. H. (1990) EMBO J. 9, 1219–1227
58. Kordeli, E., Lambert, S., and Bennett, V. (1995) J. Biol. Chem. 270, 2352–2359
59. Dresn, T. D., Lohr, M., and Case, S. T. (1988) J. Biol. Chem. 263, 21–27
60. Dignam, S. S., Yang, L., Loezi, M., and Case, S. T. (1989) J. Biol. Chem. 264, 9444–9452
61. Dignam, S. S., and Case, S. T. (1990) Gene (Amst.) 88, 133–140
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