lysate were immune-precipitated with antiferritin antibody and analyzed by SDS-PAGE and fluorography, all as described (*13, 26, 27*).
18. A slight inhibition of ferritin synthesis by Desferal was seen only at a low heme concentration (10 μM). However, this effect was prevented by preincubation with either Zn^{2+}–BGDP IX (Fig. 2) or Sn^{4+}–protoporphyrin IX (*15*), both potent inhibitors of heme oxygenase (which is normally responsible for rapid heme turnover) (*28*). This result suggests that at low heme concentrations, the effect of Desferal is a result of inhibition of the recycling of iron released by heme oxygenase action. By contrast, heme oxygenase inhibitors did not prevent the inhibition by Desferal of Tf-induced ferritin synthesis. Inhibition of heme oxygenase by Zn^{2+}–BGDP IX was so severe that addition of 50 μM heme to treated cells resulted in cell lysis (Fig. 2), which presumably resulted from an inability to detoxify such a high concentration of heme. Inhibition of heme oxygenase by metalloporphyrins was confirmed by direct measurement (N. Abraham).
19. The large amount of SA required to inhibit ferritin synthesis induced by FAC or Tf is consistent with other observations that suggest the existence of a large intracellular excess of ALA dehydratase (*29*). In our initial studies, 1.0 mM SA inhibited heme synthesis by greater than 98% when added 4 hours before the pulse labeling of heme with either ^{59}Fe- or ^{14}C-ALA. However, 1.0 mM SA had little effect on total protein synthesis, on ferritin synthesis in the presence or absence of inducers, or on cell growth rate. Cells grown for 5 days in 1.0 to 2.0 mM SA as described (*14*) produced cell numbers and protein mass indistinguishable from cells grown in control media. Cells grown in 2.5 to 5.0 mM SA grew 77% as fast as control cells, as indicated by the same criteria. These results suggest that only a small fraction of the cell's ALA-dehydratase activity is sufficient to supply all normal heme requirements. This result is also consistent with our finding that protein synthesis is inhibited only 4% and 24% by 7.5 mM and 15 mM SA, respectively (*15*).
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Occurrence of Sialic Acids in *Drosophila melanogaster*

Jürgen Roth,* Andreas Kempf, Gerd Reuter, Roland Schauer, Walter J. Gehring

Sialylated oligosaccharides, which are cell type–specific and developmentally regulated, have been implicated in a variety of complex biological events. Their broad functional importance is reflected by their presence in a wide variety of phyla extending from *Echinodermata* through higher vertebrates. Here, sialic acids are detected throughout development in an insect, *Drosophila*. Homopolymers of α 2,8-linked sialic acid, polysialic acid, are developmentally regulated and only expressed during early *Drosophila* development.

Sialic acids comprise a large family of closely related derivatives of *N*-acetylneuraminic acid and *N*-glycolylneuraminic acid (*1*). Terminal glycosylation sequences, in particular sialylated oligosaccharides that are cell type–specific and developmentally regulated, have been implicated in a variety of complex biological events (*2*). The expression of various specific sialyltransferases is the basis for the synthesis of such glycosylation sequences (*3*). Sialic acids can be found in a variety of chemical linkages, including linkage to different penultimate sugars of a glycosylation sequence. Specific sequences expressed in one context may be critical for biological recognition but may have no function in another context (*2*) as shown in studies of sialic acids during organogenesis (*4*), cell differentiation (*5*), endothelial cell leukocyte adhesion molecule (ELAM-1)–mediated cell adhesion (*6*), interaction of viruses and certain pathogenic bacteria with their host cells (*7*), and tumor cell invasiveness (*8*). Sialic acids are found in the *Echinodermata* and in most chordates

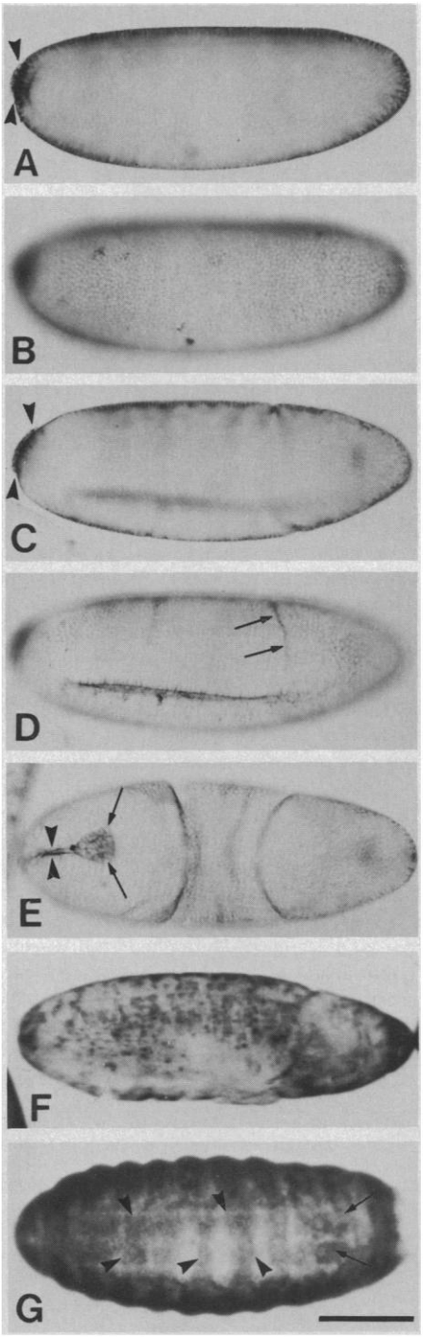


Fig. 1. Cytochemical detection of sialic acid in *Drosophila* embryos with use of the *Limax flavus* lectin (*11*). (**A**) Whole mount of a blastoderm stage embryo (*20*) showing intensely stained pole cells (arrowheads) and stained blastoderm cells. (**B**) Same embryo as in (A) at another plane of focus to demonstrate the honeycomb pattern of lectin staining on blastoderm cells. (**C** and **D**) Early gastrulation stage embryo. Staining of pole cells [arrowheads in (C)] and blastoderm cells is evident, along with additional labeling along the ventral [arrowheads in (D)] and cephalic [arrows in (D)] furrows. (**E**) Rapid phase of germ band elongation. Dorsal view of an embryo that exhibits staining of the ventral furrow (arrowheads) and the invaginating pole cells (arrows) as well as the honeycomb staining pattern. (**F**) Embryo with fully extended germ band. Small groups of cells at both sides of the ventral furrow are more intensely stained than neighboring cells. (**G**) Embryo after germ band shortening and during dorsal closure exhibits staining in the ventral nervous system (arrowheads) and the brain ganglia (arrows). In all photographs embryos are oriented with the anterior end to the right. Scale bar = 0.1 mm.

Fig. 2. Expression of polysialic acid during *Drosophila* development demonstrated by immunoblot analysis with MAb 735 (*21*). (**A**) In homogenates from 18-day, embryonic mouse brain and 12- to 18-hour *Drosophila* embryos a high molecular weight band was revealed. Time segments 0 to 6 hours, 6 to 12 hours, 12 to 18 hours, and 18 to 24 hours are followed by first, second, and third instars. (**B**) A polysialic acid–positive double band at ≈210 kD was detected during 14 to 18 hours of development. Endosialidase N pretreatment of nitrocellulose (**C**) almost completely abolishes staining. Time segments: 0 to 2 hours, cleavage divisions and syncytial blastoderm; 2 to 4 hours, cellular blastoderm, gastrulation, and amnioproctodeal invagination; 4 to 6 hours, stomodeal invagination; 6 to 8 hours, germ band shortening; 8 to 10 hours, germ band shortening; 10 to 12 hours, head involution and closure of midgut; 12 to 14 hours, dorsal closure; 14 to 16 hours, closure of embryo; 16 to 24 hours, fully developed embryo and hatching.

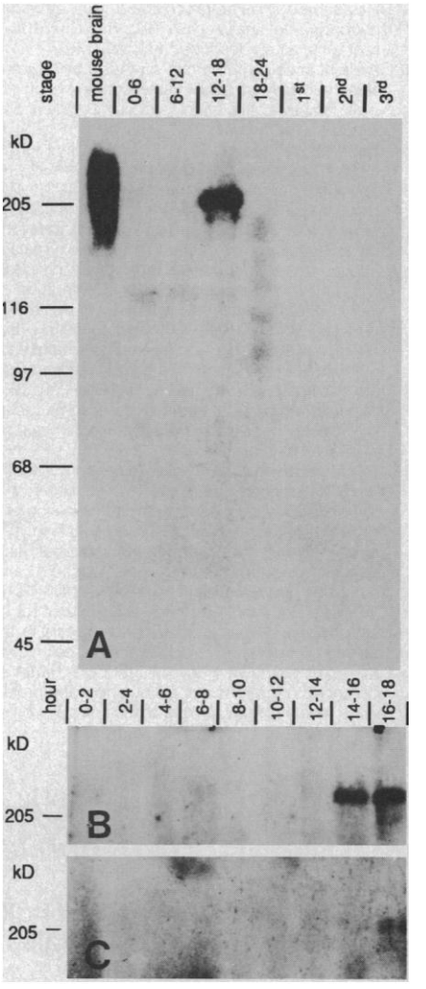
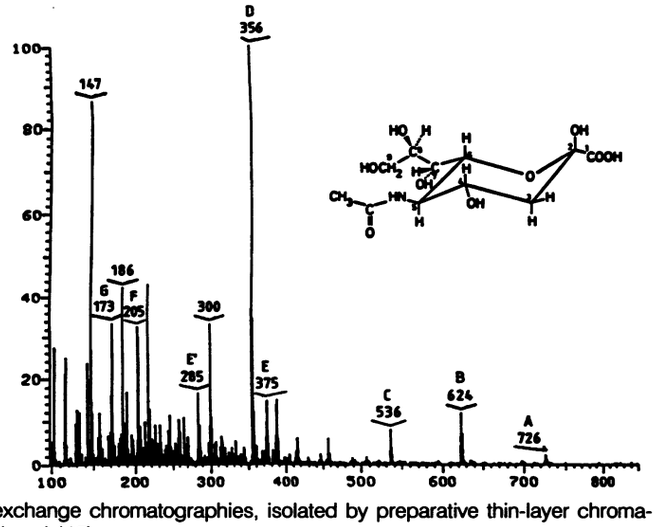


Fig. 3. Electron-impact mass spectrum (70 eV) of *N*-acetylneuraminic acid as per-*O*-trimethylsilyl derivative after gas-liquid chromatography–mass spectrometry isolated from 12- to 18-hour *Drosophila* embryos. The same mass spectrum was obtained from 0- to 6-hour embryos. The characteristic fragment ions A through G are indicated together with the structure of *N*-acetylneuraminic acid (*15*). The embryos were lyophylized (14.6 g), and the sialic acids were liberated by mild acid hydrolysis, purified by ion-exchange chromatographies, isolated by preparative thin-layer chromatography, and further analyzed (*15*).



but have been encountered only occasionally in prokaryotes (*9*).

Conclusive evidence for the presence of sialic acids in *Annelida*, *Arthropoda*, and *Mollusca* is missing, and the analysis of several insects including *Musca domestica*, *Calliphora erythrocephala*, and *Drosophila melanogaster* has yielded negative results. Although lepidopteran insect cell lines have recently been shown to possess the glycosyltransferase genes for the synthesis of N-linked, complex-type oligosaccharides, activation of the genes was observed only under certain conditions such as transfection with recombinant baculovirus/human plasminogen complementary DNA (*10*).

We found sialic acids in *D. melanogaster* by the use of lectin-gold histochemistry, Western blotting, and gas-liquid chromatography–mass spectroscopy (GLC-MS). Sialic acids were detected throughout the development of *Drosophila*, from blastoderm to third instar larvae stage. Polysialic acid (homopolymers of α 2,8-linked sialic acid), however, showed a developmentally regulated expression.

For cytochemistry the *Limax flavus* lectin, which exhibits high specificity for sialic acids, was applied (*11*). At the blastoderm stage, all cells exhibited staining, although the pole cells stained most intensely (Fig. 1, A and B). By electron microscopy, the plasma membrane, the Golgi apparatus, and cytoplasmic vesicles of blastodermal cells showed lectin labeling. The staining pattern remained during gastrulation and in early germ-band elongation, during which additional intense labeling along the ventral and cephalic furrow was seen (Fig. 1, C, D, and E). In embryos with a fully extended germ band, groups of cells on both sides of the ventral furrow were stained (Fig. 1F). Embryos after germ-band shortening showed staining in all structures, although the most intense staining was in the nervous system (Fig. 1G). No lectin staining was observed in various controls (*12*).

To test if homopolymers of α 2,8-linked sialic acid [polysialic acid (PSA)] were expressed during *Drosophila* development, we used the monoclonal antibody MAb 735, which specifically recognizes PSA (*13*). By Western blot analysis, PSA expression was restricted to 14- to 18-hour embryos (Fig. 2, A and B). Pretreatment with bacteriophage-associated endosialidase N (*14*) and preabsorption of MAb 735 with colominic acid (*Escherichia coli* K1 capsular polysaccharide composed of homopolymers of α 2,8-linked sialic acid) abolished the immunostaining (Fig. 2C).

The structural analysis of sialic acids in 0- to 6-hour and 12- to 18-hour embryos was performed by a combination of GLC-MS (*15*) (Fig. 3). Colorimetric quantification by the periodic acid–thiobarbituric acid test was used to determine the amount of sialic acid (Neu5Ac) in 0- to 6-hour-old and 12- to 18-hour-old *Drosophila* embryos and gave 5 μg and 4.9 μg per gram of dry weight, respectively. The media used for culture of *Drosophila* did not contain sialic acids (*15*).

Thus, sialic acid is endogenous in *Drosophila* embryos. In bacteria, the capability to synthesize sialic acids was thought to be acquired only late in evolution after symbiotic interactions with hosts. Genetic and structural analysis, however, suggests that this is an inherent synthetic function (*16*). Therefore, sialic acids seem to have arisen early in evolution. The PSA in *Drosophila* embryos is probably on the neural cell adhesion molecule (N-CAM) (*17*). The detec-

J. Roth, A. Kempf, W. J. Gehring, Department of Cell Biology, Biocenter, University of Basel, CH-4056 Basel, Switzerland.
G. Reuter and R. Schauer, Institute of Biochemistry, University of Kiel, D-2300 Kiel, Germany.

*Present address and to whom correspondence should be addressed: Division of Cell and Molecular Pathology, Department of Pathology, University of Zürich, Schmelzbergstrasse 12, CH-8091 Zürich, Switzerland.

tion of PSA is of particular interestbecause this polyglycan seems important in regulating the adhesive properties of N-CAM during neuronal development (*18*). In larvae and adult of the fly *Calliphora vicina*, a nonsulfated, glucuronic acid moiety similar to the L2/HNK-1 carbohydrate epitope of several cell adhesion molecules, among them N-CAM, was recently detected (*19*). These data suggest a high degree of phylogenetic conservation of functionally important glycans.

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Modality-Specific Retrograde Amnesia of Fear

Jeansok J. Kim and Michael S. Fanselow

Emotional responses such as fear are rapidly acquired through classical conditioning. This report examines the neural substrate underlying memory of acquired fear. Rats were classically conditioned to fear both tone and context through the use of aversive foot shocks. Lesions were made in the hippocampus either 1, 7, 14, or 28 days after training. Contextual fear was abolished in the rats that received lesions 1 day after fear conditioning. However, rats for which the interval between learning and hippocampal lesions was longer retained significant contextual fear memory. In the same animals, lesions did not affect fear response to the tone at any time. These results indicate that fear memory is not a single process and that the hippocampus may have a time-limited role in associative fear memories evoked by polymodal (contextual) but not unimodal (tone) sensory stimuli.

The hippocampus is thought to serve a temporary function in the storage of memory because, when the hippocampus is damaged, recent but not remote memories are impaired (*1*). This memory syndrome is known as "retrograde amnesia," and it suggests that with the passage of time memories are stabilized (or consolidated) elsewhere in the brain (for example, in the neocortex). Because the hippocampus is not thought to be essential for learning and memory in most cases of simple classical conditioning (*2*), retrograde amnesia as a result of hippocampal damage has heretofore not been directly assessed in basic associative paradigms. Therefore, we tested whether retrograde amnesia occurs in rats by using a simple classical fear-conditioning procedure.

Department of Psychology, University of California, Los Angeles, CA 90024.

Long-Evans female rats underwent 15 tone–foot shock pairings (tone: 2000 Hz, 90 dB, 30 s; foot shock: 1 mA, 2 s) in a distinctive chamber (*3*). A short (3-min), fixed intertrial interval was used to ensure reliable fear conditioning to both tone and chamber. After training, bilateral electrolytic lesions were made in the hippocampus either 1, 7, 14, or 28 days later ($n = 8$) (*4*). Figure 1 shows a transverse section from the brain of a typical rat in the hippocampus-lesioned group. For unlesioned controls the electrode was lowered to the hippocampus without passing current ($n = 6$). Additionally, control lesions were made 1 day after training in the area of neocortex overlying the hippocampus ($n = 8$). All animals were given 7 days to recover after surgery before testing.

To test fear conditioning associated with context (the chamber), each rat was placed back in the chamber for 8 min. The foot shock and tone were not given during this test. The amount of fear conditioned to the chamber was assessed by scoring freezing