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Gene expression pattern

Mouse submandibular gland mucin: embryo-specific mRNA and protein species

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Abstract

Mouse submandibular salivary gland (SMG) mucin is the primary histodifferentiation product of submandibular epithelia. We demonstrate marked differences between embryonic, neonatal, and adult SMG mucin mRNA and protein by Northern and Western blot analyses: E17 and 1-day-old neonates exhibit two unique mucin transcripts (1.20 and 0.85 kb) which are approximately 19% greater or smaller in size than the single (1.01 kb) adult transcript. Two embryonic protein isoforms (M_r~110 and 152 kDa) are immunodetected compared to a single adult protein (M_r~136 kDa), with the larger (~152 kDa) embryonic isoform persisting in neonatal glands. Mucin transcripts are localized to the branching epithelia in E14 and older SMGs, with increased hybridization signal being seen in terminal bud and proacinar epithelial cells with age; a significant 26% increase in transcript levels is detected by RNase protection assay between E14 and E19. By contrast, submandibular mucin protein is not immunodetected until E17, being primarily immunolocalized to terminal bud and proacinar epithelial cell membranes. Our data clearly shows that substantial qualitative differences exist between embryonic and adult SMG mucin mRNA and protein.

Keywords: Submandibular gland; Embryonic mucin; Embryo; Neonate; Morphogenesis; Mouse development; Northern blot; RNase protection; In situ hybridization; Western blot; Inmmunohistochemistry

Submandibular salivary gland (SMG) morphology and physiology has been extensively studied in adult rodents (for reviews see Pinkstaff, 1993; Tabak, 1995; Denny et al., 1997). Salivary mucin, the primary product of mature SMG epithelium, is exclusively synthesized and secreted into the oral cavity by seromucous acinar cells which histodifferentiate beginning 2–3 weeks postnatal (Srinivasan and Chang, 1979; Vreugdenhil et al., 1982; Denny et al., 1988). Little is known about embryonic SMG mucin expression. We investigated possible qualitative differences between embryonic and adult SMG mucin mRNA and protein. Surprisingly, the embryonic transcript notably differs from the adult (Fig. 1A): E17 SMGs exhibit two mucin transcripts (1.20 and 0.85 kb) which are approximately 19% greater or smaller in size than the single 1.01-kb transcript seen in the adult here and previously (Denny et al., 1996); mucin transcripts in newborn (1-day-old) mice which are nursing and exhibit milk in their stomachs are similar to the embryonic species (Fig. 1A).

Marked protein differences between embryonic and adult are also seen (Fig. 1B). A broad adult mucin band (M_r~136 kDa) is immunodetected as previously reported; the smear spanning from ~110 to 155 kDa is associated with the high degree of glycosylation and known SMG mucin protein heterogeneity (Albone et al., 1994; Tabak, 1995; Denny et al., 1996, 1997). By contrast, two embryonic protein isoforms (M_r~152 and 110 kDa) present as discrete, narrow bands. Since the embryonic protein isoforms are substantially larger in size than predicted by the transcript sizes (<36 kDa), we conclude that embryonic proteins are also glycosylated. However, the narrower appearing embryonic bands suggest decreased glycosylation and heterogeneity in embryonic mucin compared to adult. Only the larger (~152 kDa) isoform is observed in neonatal glands even though embryonic and neonatal transcripts are similar (Fig. 1B). Since the neonatal band is wider than embryonic, it suggests increased glycosylation and/or heterogeneity with birth.

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SMG mucin transcripts are first detected on E14 in the branching epithelia, being absent from the surrounding undifferentiated mesenchyme (Fig. 2A); a marked increase in transcript expression is seen in terminal bud and proacinar epithelia with a concomitant decrease in ductal cells with increasing age (Fig. 2C). With birth, a strong hybridization signal is detected throughout terminal bud and proacinar cell epithelia (Fig. 2D). Using RNase protection assay, we demonstrate a significant 16% increase in transcript level between E14 and E17 (29.98 ± 1.74 vs. 34.90 ± 1.04; $t_5 = 4.64$, $P < 0.01$) (Fig. 1C,D); by E19 there is a significant 26% increase in transcript level (29.98 ± 1.74 vs. 37.63 ± 3.30; $t_4 = 3.56$, $P < 0.05$) (Fig. 1C,D).

Even though mucin transcripts are expressed beginning on E14, mucin protein is not immunodetected until E17, being localized to the cell membrane of a few terminal sac and proacinar epithelia cells (Fig. 2E). By E18, there is a marked increase in immunodetectable mucin protein, with the protein primarily immunolocalized to the cell membrane, and to a lesser extent, in the cytoplasm (Fig. 2F). After birth, although mucin protein distribution becomes more restricted to proacinar cells (Fig. 2G), it is still primarily localized to cell membranes and not within proacinar cells. Our results clearly demonstrate for the first time substantial qualitative differences between embryonic and adult SMG mucin mRNA and protein.

1. Materials and methods

1.1. Animal mating and tissue collection

B10.A/SgSn (B10.A) mice were purchased from Jackson Laboratories (Bar Harbor, ME). The research conformed to the stipulations of USC Animal Review Committee. Animals were bred and plug day is designated day 0 of gestation. Embryos were collected, staged according to Theiler (1989), and SMGs dissected as previously described (Jaskoll et al., 1994).

1.2. Northern blot and RNase Protection Assays

A ribroprobe was generated from a plasmid containing a 160-bp mouse cDNA mucin fragment insert corresponding to mouse bp 209 and 369 of 5′ non-repeat domain of the full length 1.00-kb mucin (*Muc10*) (Denny et al., 1996); the

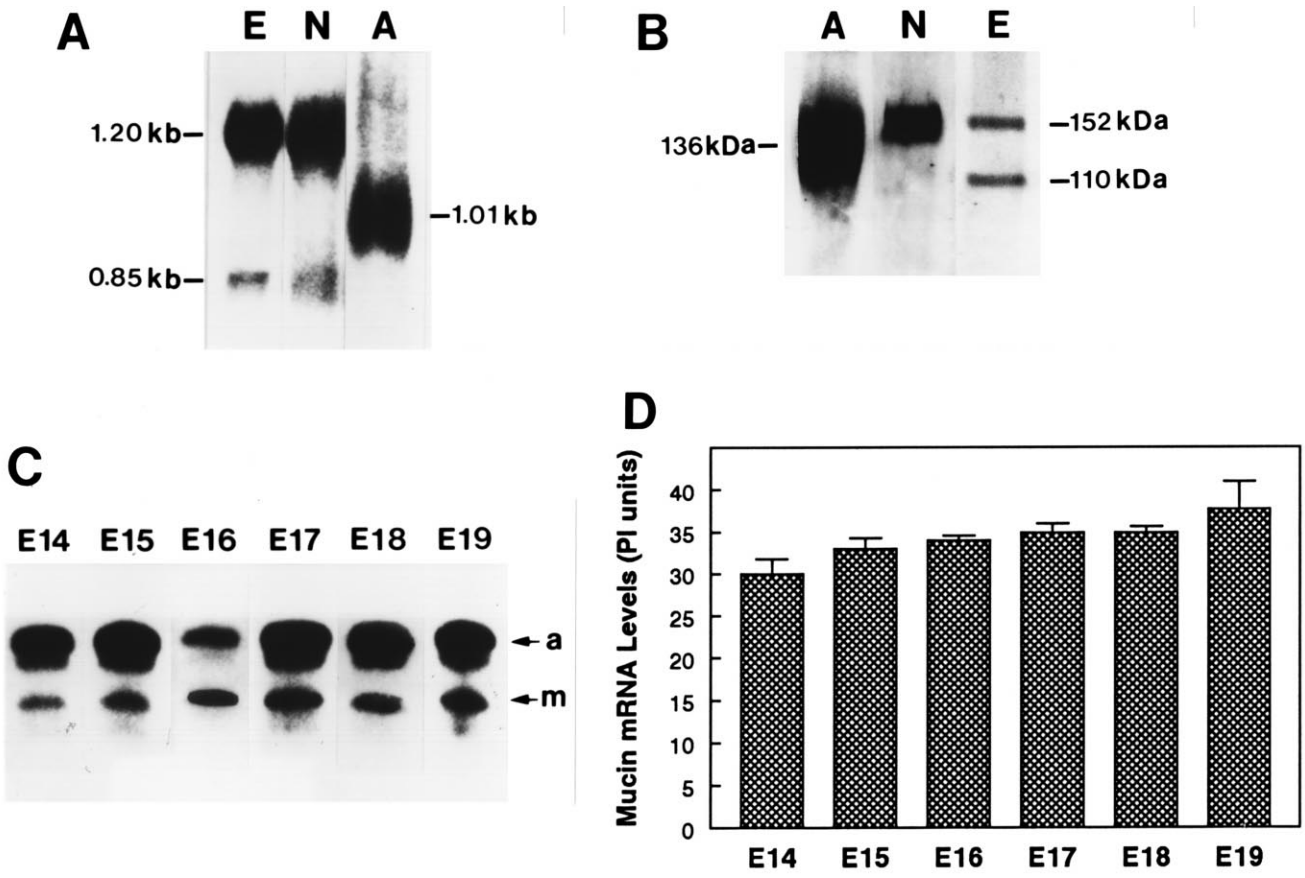

Fig. 1. Developmental expression of SMG mucin. (A) Northern blot analysis of E17 (E), neonatal (N) and adult (A) transcript. (B) Western blot analysis of E17 (E), neonatal (N), and adult (A) protein. (C) RNase protection assays were conducted to compare the steady-state levels of embryonic mucin mRNA in E14–El9 SMGs. A 150-nt protected mucin (m) fragment is detected in all SMGs. As an internal control, a protected 250-nt β-actin (a) fragment was used. (D) Embryonic SMG mucin transcript levels. Bars represent mean phosphor imaging (PI) units with their 95% confidence limits. E14: 29.98 ± 1.74; E15: 32.99 ± 1.24; E16: 33.83 ± 0.51: E17: 34.90 ± 1.04: E18: 34.86 ± 0.77; E19: 37.63 ± 3.30.

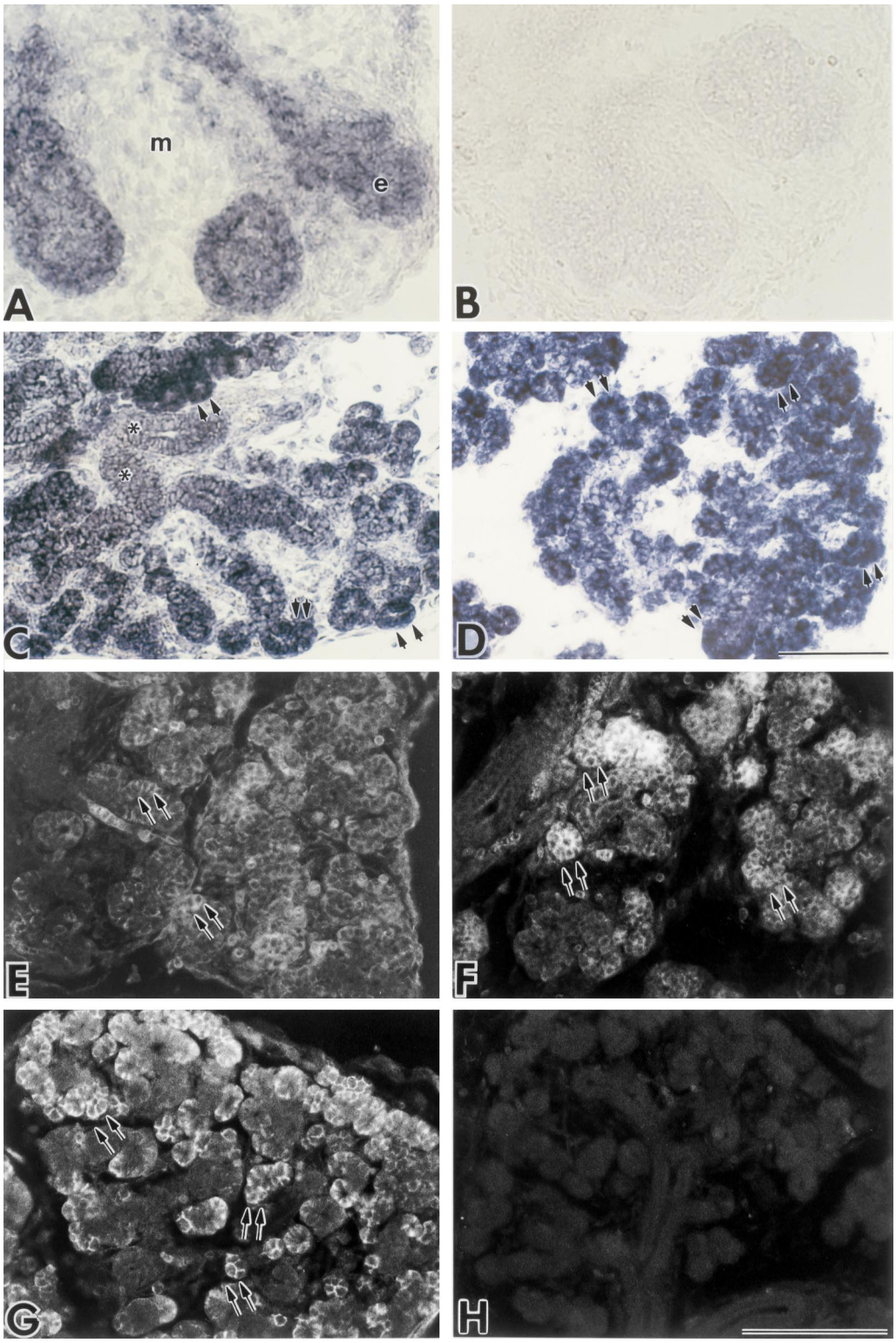

Fig. 2. Spatiotemporal distribution of SMG mucin. (A–D) Mucin transcript expression in E14 to 1-day-old neonatal SMGs by in situ hybridization. (A) E14 SMG hybridized with antisense probe demonstrates the presence of mucin transcripts in the epithelia (e) and absence from the mesenchyme (m). (B) E14 SMG hybridized with sense probe; no hybridization signal is seen. (C). E18 SMG hybridized with antisense probe. (D).One-day-old neonatal SMG hybridized with antisense probe. A marked increase in hybridization signal in the terminal bud and proacinar epithelial cells (arrow heads) and a decrease in ductal cells (*) is seen with development. (E–H) Distribution of mucin protein was determined by indirect immunofluorescence in E17 to 1-day-old neonatal SMGs. (E) E17 SMG; (F) E18 SMG; (G) 1-day-old neonatal SMG; (H) E18 control slide. SMG mucin protein is primarily immunodetected in the cell membranes (arrows) of terminal bud and proacinar epithelial cells. In the neonate (G), mucin protein expression is more restricted to proacinar cells. Bar, 100 μm.

cDNA adult mouse mucin sequence has been shown to be specific for mouse SMG mucin (Denny et al., 1996). Ribroprobe specificity was determined by Northern blot analysis of adult SMG total RNA; a ~1.01-kb transcript was detected which is identical to that reported using the full length cDNA probe (Denny et al., 1996). A linearized plasmid containing a 360-bp mouse β-actin cDNA insert was obtained from Ambion (Austin, TX). Total RNA was isolated from E17, 1-day-old neonatal, and adult SMGs and Northern blot analyses were conducted as previously described (Melnick et al., 1996a,b): six to ten independent samples were prepared (E17: 12–20 SMGs pooled by litter; N: six glands/three animals; A: two glands/animal). X-ray film of the same blot was exposed 18 h to visualize adult mucin and 72 h to visualize E17 and newborn mucin. Steady-state levels of mucin transcripts in E14–El9 SMGs were measured with an RNase protection assay essentially as described in Melnick et al. (1996b): E15–El9 samples were a pool of 12–20 glands from one litter; the E14 sample consisted of 20–40 glands pooled from two litters. Mucin mRNA probe protects a 150-nt fragment from digestion by RNases; the specificity of this probe for SMG mucin was demonstrated by analyzing total RNA from adult SMGs, E17 lungs, and E14 palatal shelves; a 150-nt protected fragment is seen in adult SMGs and is absent from embryonic pulmonary and palatal tissues (data not shown). β-Actin transcripts provided an internal standard for each assay; a 250-nt protected fragment is detected. Protected RNAs were quantified by phosphor imaging and normalized to the amount of protected β-actin present in each sample. Ratios were mathematically transformed to meet the analytical assumptions of normality and homoscedasticity, means were calculated, and differences between mean ratios were compared by the *t*-test (Sokal and Rohlf, 1981).

1.3. In situ hybridization

E14 to newborn SMGs were processed and in situ hybridization was performed essentially as described in Melnick et al. (1996a) using a digoxigenin (DIG)-labeled ribroprobe (Melnick et al., 1996a). As a negative control, sections were hybridized with sense RNA probes (Fig. 2B). As a positive control, sections were pretreated with RNase prior to hybridization with the antisense RNA probes. In both the positive and negative controls, no hybridization signal was detected.

1.4. Immunolocalization and western blot analysis

E15 to 1-day-old neonatal SMGs were collected, processed and indirect immunofluorescent experiments conducted as described in Jaskoll et al. (1994) using a monospecific anti-mouse mucin polyclonal antibodies specific for mouse acinar-cell-specific mucin (Denny et al., 1988, 1989). Controls consisted of sections incubated in the absence of primary antibodies; controls were routinely negative. Mucin protein was not detected in E15 or E16 glands (data not shown). For Western blot analyses, E17 (112 glands from 14 litters), 1-day old neonatal (20 glands/ten animals) and adult (eight glands/four animals) SMGs were lysed with 1% NP 40 lysate buffer containing 1 mM PMSF, 1 μM leupeptin, at room temperature for 1 h and transferred to 4°C for 5 h. The lysates were centrifuged at 14 000 rev./min for 15 min to remove cool temperature precipitate protein and then dialyzed overnight at 4°C to remove low molecular weight ($<$10 kDa) proteins. Equivalent amounts of total protein (60 μg) were fractionated by SDS-PAGE, immunoblotted and immunostained essentially as described previously (Jaskoll et al., 1996). Blots were incubated overnight at 4°C with anti-mucin antibody and then incubated in alkaline phosphatase conjugated anti-rabbit IgG (Pierce Scientific, Rockford, IL). The controls consisted of blots incubated in the absence of primary antibodies: controls were routinely negative (data not shown).

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