Journal of Cellular Biochemistry 57:423–431 (1995)

Noncoordinate Changes in the Steady-State mRNA Expressed From Aldolase A and Aldolase C Genes During Differentiation of Chicken Myoblasts

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Abstract In chickens, as in all vertebrates, tissue-specific expression of aldolase isozymes A, B, and C is developmentally coordinated. These developmental transitions in aldolase expression have been studied most extensively by charting enzyme activity during normal and abnormal development of specific vertebrate tissues. Indeed, aldolase expression has been a key marker for normal differentiation and for retrodifferentiation during carcinogenesis. Aldolase expression during chicken myoblast differentiation offers a model for investigating the regulatory mechanisms of these developmental transitions at the level of gene expression. For these studies, cDNAs encoding the most isozyme-specific regions of both chicken aldolase A and C were cloned. The chicken aldolase A cDNA represents the first report of this sequence. Aldolase steady-state mRNA expression was measured during chicken myoblast differentiation in primary cultures using RNase protection assays with cRNA probes generated from these aldolase cDNA clones. Steady-state mRNA for aldolase C, the predominant embryonic aldolase isozyme in chickens, did not significantly change throughout myoblast differentiation. In contrast, expression of steady-state mRNA for aldolase A, the only aldolase isozyme found in adult skeletal muscle, was not detected until after myoblast fusion was approximately 50% completed. Aldolase A expression gradually increased throughout myoblast differentiation until approximately 48 h after fusion was completed when there was a dramatic increase. These results are contrasted with those of Turner et al. (1974) [Dev Biol 37:63–89] that showed a coordinated switch in isozyme activities between the embryonic aldolase C and the muscle-specific aldolase A. This discordant expression indicates that the aldolase A and C genes may employ different regulatory mechanisms during myoblast differentiation.

Key words: cDNA, RNase protection, DNA sequence

The fructose-1,6-bisphosphate aldolases (EC 4.1.2.13) exist as three isozymes, A, B, and C, which are encoded on unlinked genes in vertebrates [Tolan et al., 1987]. These isozymes are all responsible for the reversible aldol cleavage of fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate and dihyroxyacetone phosphate. They resemble each other in molecular weight (40,000 daltons), tertiary structure, and overall catalytic mechanism, but each isozyme has distinct immunochemical reactivity, catalytic properties and tissue-specific expression [Penhoet et al., 1966, 1967, 1969; Horecker et al., 1975]. In adult vertebrates, aldolase A is expressed in tissues, such as muscle, that have high rates of glycolysis. It is also the predominant isozyme in mammalian embryos. Aldolase B has a role in fructose metabolism in the liver, kidney, and small intestine due to its substantial activity toward fructose-1-phosphate. It is also the isozyme best suited for gluconeogenesis. Aldolase C, which has catalytic properties intermediate to those of aldolase A and B, is expressed primarily in brain and other neural tissues. Aldolase C is also the predominant aldolase isozyme in the avian embryo [Lebherz and Rutter, 1969].

This tissue-specific expression in adult vertebrates results from three developmental patterns of aldolase isozyme expression. In the first pattern, which has been observed in mammalian muscle development, a relatively low level of aldolase A is expressed in the embryo until close to birth, when a dramatic increase occurs [Weber, 1965]. This large increase in aldolase A expression is the result of a promoter switch in

Received June 26, 1994; accepted August 8, 1994.

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overlapping transcription units from a nonspecific, or housekeeping, promoter to a muscle-specific promoter [Joh et al., 1986; Maire et al., 1987; Colbert and Ciejek-Baez, 1988; Concordet et al., 1993]. The second pattern involves an apparently coordinated switch in aldolase isozyme expression. This pattern has been observed in several tissues: in developing mammalian liver, where aldolase B is expressed with a reciprocal disappearance of aldolase A [Weber, 1965]; in hepatocarcinoma, where this coordinated switch is reversed [Shapira et al., 1963; Matsushima et al., 1968; Numazaki et al., 1984]; and in developing chicken muscle, where aldolase A is expressed with a reciprocal disappearance of aldolase C [Turner et al., 1974]. The third pattern has been observed in the developing mammalian brain where the expression of the embryonic aldolase A is joined by the coexpression of aldolase C [Lebherz and Rutter, 1969].

The regulatory mechanisms that govern these patterns of developmental isozyme expression have been most extensively studied in mammalian muscle, where the first pattern arises from a transcriptional switch in *cis*. The apparently coordinated switches found in the second pattern are less well characterized. Chicken myoblast differentiation during early myogenesis in primary cultures offers a model for investigating the regulatory mechanisms of this second expression pattern. In studies of chicken myogenesis, Turner et al. [1974] reported a coordinated switch from aldolase C isozyme expression to aldolase A isozyme expression after the completion of myoblast fusion. This reciprocal transition during chicken myogenesis is similar to the pattern observed in liver development, where transitions from A to B and from C to B occur in mammals and chickens, respectively [Weber, 1965; Ono et al., 1990].

The patterns of aldolase gene expression were investigated by examining the pattern of aldolase steady-state mRNA in differentiating chicken myoblasts. Chicken aldolase A and C cDNAs were cloned from the most isozyme-specific regions and used to generate cRNA aldolase probes for RNase protection analysis. The steady-state mRNA levels were measured as a function of time in primary cultures, and the results suggested that the aldolase A and C genes are not coordinately expressed during chicken myoblast differentiation and may be differentially regulated.

MATERIALS AND METHODS

Culturing of Chicken Myoblasts

Rhode Island Red fertilized eggs were purchased from Hardy's Hatchery (Essex, MA). Chicken myoblasts were isolated from thigh tissue of 11-day stage 37 chick embryos [Hamburger and Hamilton, 1951] and were prepared as single-cell suspensions as described by Hausman and Velleman [1981]. These suspensions were resuspended in DMEM (Dulbecco's modified Eagle medium, Gibco), preplated on a 1% gelatin-coated plates, and incubated for 1 hr at 37°C to enrich for nonadherent skeletal myoblasts. Aliquots of 3–5 × 10^6 nonadherent cells were cultured on 1% gelatin-coated plates in 8–10 ml DMEM containing 10% heat-inactivated FBS (fetal bovine serum, Gibco) and an antibiotic/antimycotic mixture (100 U penicillin, 0.1 *mg streptomycin*, 0.25 *μg amphotericin* B) (Gibco).

Isolation of RNA

Total RNA was isolated from juvenile chicken brain or cultured myoblasts/myotubes using a modification of the method described by Chomczynski and Sacchi [1987] [Xie and Rothblum, 1991]. Total RNA of adult chicken muscle was isolated from polysomes [Lebherz and Doyle, 1976]. Poly $(A)^+$ RNA was purified using an oligo-dT cellulose column (Collaborative Research) [Aviv and Leder, 1972] or was isolated directly from cell or tissue lysates [Badley et al., 1988].

cDNA Amplification

First strand aldolase cDNAs were synthesized in reactions (20 μl) containing 2–3 μg poly $(A)^+$ RNA, 40 U RNAsin (Promega), 200 μM dNTPs, 3 mM $MgCl_2$, 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 50 pmol of 3′-primer and 200 U MMLV (Moloney murine leukemia virus) reverse transcriptase (Gibco). After 1 h at 37°C, the entire contents of the synthesis reaction were used in the amplification reaction (100 μl) with 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1–2 mM $MgCl_2$, 200 μM dNTPs, 100 pmol of 5′-primer, 50 pmol of 3′-primer and 2.5 U *Taq* DNA polymerase (Perkin-Elmer Cetus). The PCR reaction parameters were as follows: 94°C, 90 sec; 37°C, 90 sec; 72°C, 90 sec for 5 cycles, and 94°C, 90 sec; 42°C, 90 sec; 72°C, 180 sec for 30 cycles. The PCR primers for cloning the aldolase C cDNAs were (5′ to 3′): AS-C, GCCGGATCCTGCTGAAGC-

CCAACATG; d(X)T, GGCGAATTCTTTTTTT-TTT(C/A/G); UT-C, GGCGAATTCGGTCTC-TACCCGTTTATT. The PCR primers for cloning the aldolase A cDNAs were: GA322, GGCG-GATCCGCCGCCCAGGAGGAGTA; d(X)T; CA329, AAGAGGGCCTTGGCCAAC.

Southern Blot Analysis

A double-wick method [Southern, 1975] was used to transfer DNA that was separated on an agarose gel to a Zeta-probe (BioRad) nylon membrane [Sambrook et al., 1989]. Prehybridization was for 1 h at 42°C in either of the following hybridization solutions: (1) 6× SSC (1× = 150 mM NaCl, 15 mM sodium citrate, pH 7.0), 50% formamide, 0.5% SDS, 5× Denhardt's, 1 mM EDTA, 100 μg/ml denatured sonicated salmon sperm DNA; or (2) 0.5 M NaH_2PO_4, pH 7.2, 7% SDS, 1 mM EDTA, 100 μg/ml denatured sonicated salmon sperm DNA. Hybridization was for 4 h at 42°C in fresh hybridization solution that included a radiolabeled denatured DNA probe. The DNA probe was radiolabeled with α-[^{32}P]-dATP as described by Feinberg and Vogelstein [1983], using random octamers instead of random hexamers or by 5′-end-labeling with γ-[^{32}P]-ATP as described by Sambrook et al. [1989]. After hybridization, the blot was washed in 2× SSC, 0.1% SDS for 1 h at 50°C.

In Situ Plaque-Lift Hybridizations

Some modifications were made to the method described by Sambrook et al. [1989]. Duplicate filters (Colony/Plaque Screen #NEF-978, Dupont/NEN Research Systems) were laid on top-agarose plates for 5 min. The filters were dried for 5 min prior to denaturation in 0.5 N NaOH for 10 min and neutralization in 0.5 M Tris–HCl, pH 8.0, for 10 min. The filters were dried and were then washed in 0.05 M Tris–HCl, pH 8.0, 0.01 M EDTA, 0.2% SDS prior to hybridization. Hybridization occurred in 6× SSC, 0.5% SDS, 5× Denhardt's, 1 mM EDTA, 100 μg/ml denatured sonicated salmon sperm DNA at room temperature for 4 h. Filters were washed in 2× SSC, 0.1% SDS for 30 min at 50°C.

DNA Sequence Determination

Sequences were determined by the method of Sanger et al. [1977], which was modified by substituting c^7-deaza-dGTP for dGTP [Barr et al., 1986]. The sequence of single-stranded M13 clones was determined as previously described [Tolan and Penhoet, 1986]. The sequence of double-stranded DNA clones was determined using T7 DNA polymerase (USB). The manufacturer's protocol was modified by adding 5 pmol of the oligonucleotide primer to the denatured DNA (3–4 μg) immediately following neutralization and prior to precipitation. In addition, following resuspension, the enzyme was added directly to the labeling mixture without prior dilution.

In Vitro Transcription

The aldolase subclones, pRMCA and pRMCC, were linearized with *Hin*dIII and *Bam*HI, respectively. The purified linearized DNA template was treated with 0.2 mg/ml proteinase K, 0.5% SDS for 30 min at 37°C, the reaction was extracted, and the DNA was precipitated [Sambrook et al., 1989]. The in vitro transcription was performed as described by Green et al. [1983], using either SP6 or T7 RNA polymerase (Promega) in a volume of 4 μl. After 2-h incubation at room temperature, the DNA template was removed by digestion with 8 U DNase I (RNase free) for 15 min at 37°C. The radiolabeled cRNA probe was extracted, precipitated and resuspended in DEPC (diethylpyrocarbonate)-treated water. The specific activity of the radiolabeled RNA was 1–2 × 10^8 cpm/μg as determined by Cerenkov counting [Robyt and White, 1987].

RNase Protection Assays

RNase protection assays using RNase I (Promega) were performed using the method described by Brewer et al. [1992], with some modifications. Total RNA (2.5–15 μg) was vacuum dried from an ethanol suspension and dissolved in 30 μl hybridization buffer. The cRNA probe (5–10 × 10^4 cpm) was added, and the mixture was denatured for 10 min at 85°C before hybridization overnight at 55°C. The RNA hybrids were digested for 1 h at 37°C with 10–20 U RNase I in 300 μl digestion buffer. The digestion was stopped with 3.3 μl 10% SDS. The double-stranded RNA was precipitated using 1 μg yeast tRNA as carrier, washed with 70% ethanol, dried for 5 min, and resuspended in 2 μl DEPC-treated water. After adding 3 μl loading buffer (95% formamide, 12.5 mM EDTA, 0.1% xylene cyanol, 0.04% bromphenol blue), the samples were denatured for 5 min at 85°C, loaded onto an 8% polyacrylamide/7 M urea gel, and subjected to electrophoresis. A control cRNA probe

for chicken β-actin [Bergsma et al., 1986] was used to indicate that equivalent amounts of RNA were loaded.

Data Analysis

The intensity of autoradiographic bands from protected RNA fragments were measured with a LKB Bromma UltroScan XL Enhanced Laser Densitometer using the software program, GelScan XL, from Pharmacia LKB Biotechnology, Inc. (Piscataway, NJ). The intensity of each band was measured as a peak in the densitometric scan with an area given by absorbance units × *mm*. This area represented the steady-state level of mRNA expressed in the culture at a given time interval.

Determination of Myoblast Fusion

Fusion of skeletal myoblasts was measured by visualization of at least three nuclei aligned in groups with no apparent separating membranes [Knudsen and Horowitz, 1977], using a fluorescence and phase-contrast Zeiss (MC100) microscope. The myoblast nuclei from sequential time intervals in culture were stained with the nuclear fluorochrome, Hoechst's dye #33342, fixed in methanol and counterstained with malachite green before mounting under cover slips using 70% glycerol, 30% 0.1 M KPO_4, pH 7.4, 5% *n*-propyl gallate, 0.1 μg/ml diaminophenolindol. At each time interval, the percentage of nuclei within fused myoblasts was calculated as the mean number of these nuclei divided by the total number of nuclei in each of three camera frames. This determination was used as a direct morphological indicator of myoblast differentiation. To account for the differences in the number of nuclei from different culture experiments, the data from each experiment at a given time interval were divided by the number of nuclei before normalization to 96 h in culture.

RESULTS AND DISCUSSION

Cloning Chicken Aldolase C cDNA

A cDNA of aldolase C was cloned using a 5′-primer derived from a conserved region in the active site of class I aldolases [Rottmann et al., 1987] and a 3′-d(X)T primer that annealed to the beginning of the poly $(A)^+$ tail. Aldolase C cDNA was PCR-amplified from juvenile chicken brain poly $(A)^+$ RNA using the primers AS-C and d(X)T. The identity of the PCR product was confirmed by Southern blot analysis (data not shown), using a human aldolase B probe [Tolan and Penhoet, 1986]. A PCR product of the correct size was cloned into M13mp18. Southern blot and DNA sequence analyses showed that 18% of the clones were positive. Two positive clones, which were generated from independent PCR-amplifications, were characterized. One clone, B4A, showed a 145-bp (base pair) deletion when compared to the other clone, 20N9. This discrepancy was resolved in a third independent PCR-amplification that used the 3′-primer, UT-C, which was specific to the 3′-end of the untranslated region. The resulting clone, BGC8-1, was identical to 20N9 (Fig. 1). The complete sequences in both directions of these two independent and identical PCR-generated clones were determined in order to be confident of the sequence reported in Figure 1. The deletion in B4A, as well as other incomplete clones detected with Southern blot analysis, were considered PCR artifacts.

Discrepancies between the identical sequences determined for BGC8-1 & 20N9 and the chicken aldolase C cDNA sequence reported by Ono et al. [1990] were found in both the translated and the 3′-untranslated regions (Fig. 1). At amino acid positions 236, 297, 338, and 361, the discrepancies were in the wobble position. At amino acid position 254, the codon was reported by Ono et al. [1990] as Ser instead of Thr. Thr is conserved at this position in all vertebrate aldolases [D. Tolan, R. Meighan-Mantha, T. Reichert, unpublished observations for this site as well as others described below]. Likewise, the codon at position 255 was reported as Pro instead of Ala, which is conserved in almost all vertebrate aldolases. The codon at position 292 was reported as Phe instead of Val. Vertebrate aldolases encode either Val, Pro or Leu at this position. The codon at position 293 was reported as Ala instead of Arg. Other vertebrate aldolase encode either Lys or Arg at this position. Lastly, the codon at position 335 was reported as Trp instead of Gly. Other aldolase C's encodes Gly at this position; no other aldolases encode Trp at this position. There were also discrepancies in the 3′-untranslated region at base positions 40, 104–111, 190, 200, and 228. The differences at the wobble positions and in the untranslated region could be due to polymorphisms, as was found in the rabbit aldolase A mRNA [Tolan et al., 1984]. However, the differences that cause amino acid substitutions are more likely to be errors in the sequence reported by Ono et al.

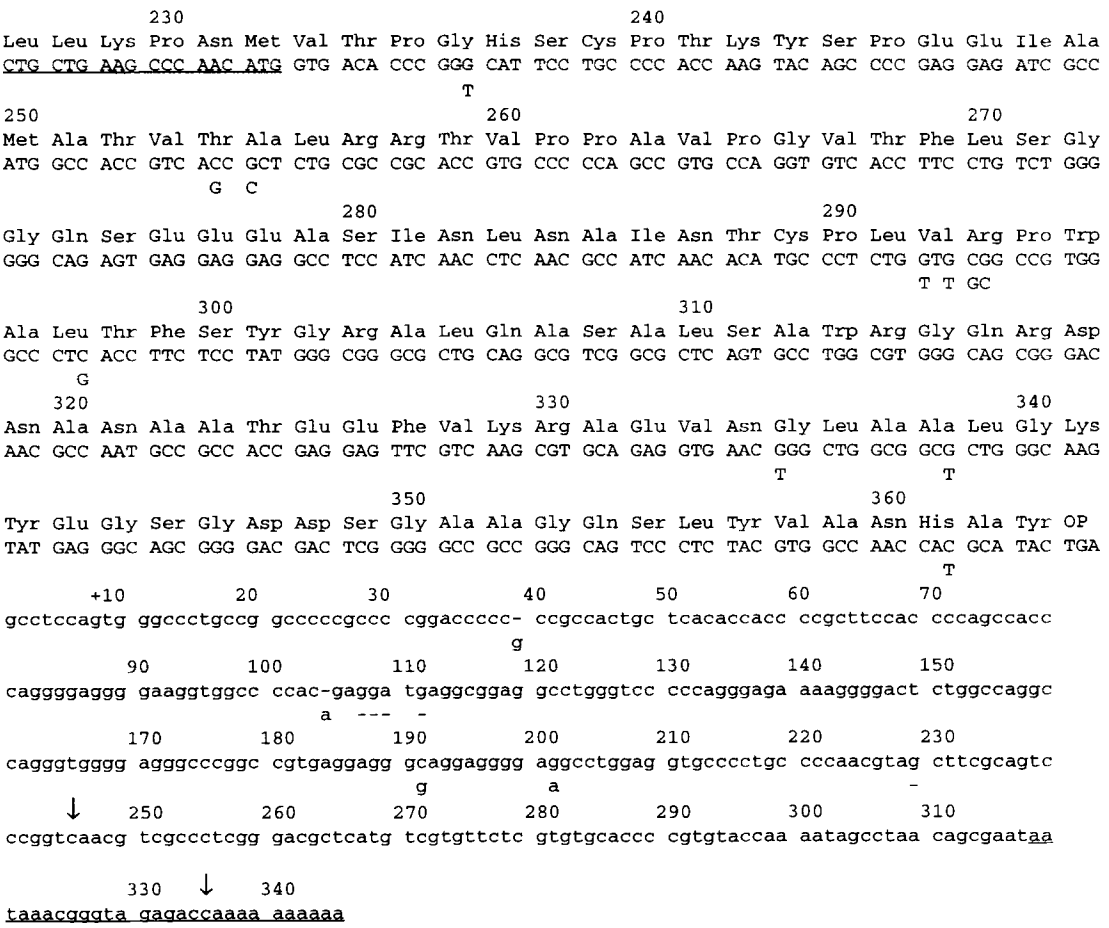

Fig. 1. Partial sequence of a chicken aldolase C cDNA. The nucleotide sequence of BGC8-1/20N9 is shown with the derived amino acid sequence shown above. Differences between this sequence and that reported by Ono et al. [1990] are depicted below. The translated region is in uppercase and the untranslated region is in lowercase. Underlined sequences indicate the positions of the PCR primers. Dashes represent the bases that are missing in either of the sequences. The region indicated between the downward arrows was subcloned into a *Sma*I and *Eco*RI-digested pGEM-3 vector, creating pRMCC, and was used as a DNA template for in vitro transcription.

[1990]. All the discrepancies that cause amino acid substitutions lack evolutionary conservation at these positions. The evolutionary conservation, and the repeated independent sequence determinations, support the validity of the DNA sequence reported here.

Cloning Chicken Aldolase A cDNA

The cDNAs for aldolase A were cloned from adult breast muscle poly(A)$^+$ RNA using a 5′-primer, GA322, derived from a conserved region of aldolase A, and the 3′-primer, d(X)T. The identity of the PCR product was confirmed by Southern blot analysis using a rabbit aldolase A probe [Tolan et al., 1984]. A PCR-product of the correct size was cloned into M13mp18 and screened by in situ plaque-lift hybridization. The DNA sequences of four positive clones were determined in both directions. Two of the four clones were identical, except for a nine base deletion. Therefore, PCR-amplification was repeated changing the 5′-primer to CA329, which was common to all three clones, and using different 3′-primers specific to the 3′-untranslated regions of each clone. Southern blot analysis of the PCR-amplified RNAs showed that both adult breast and thigh muscle contained only two of these three aldolase A mRNA sequences (data not shown). The complete sequence in both directions was determined for each of these sequences; 322A-1 and 322A-3 (Fig. 2). Each inde-

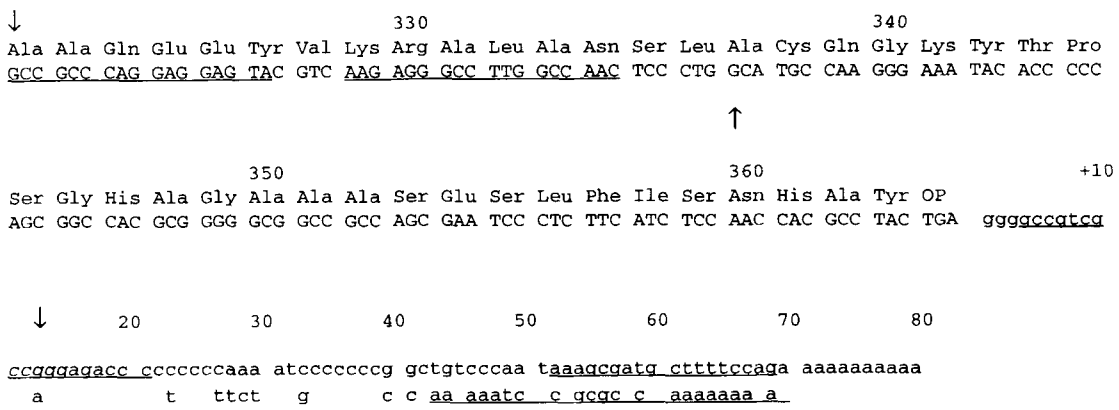

Fig. 2. Partial sequences of chicken aldolase A cDNAs. The nucleotide sequence of the aldolase A cDNA clone, 322A-1 is shown with the derived amino acid sequence shown above. Differences between this sequence and that of another clone, 322A-3, are depicted below. The translated region is in uppercase and the untranslated region is in lowercase. Underlined sequences indicate the positions of the PCR primers. The region between the downward arrows was subcloned into an *Acc*I and *Sph*I-digested pGEM-4Z vector, creating pRMCA. pRMCA had the region encoding from Ala-322 to the *Sph*I site (upward arrow) inserted in the antisense direction at that *Sph*I site, including a 30-bp fragment of the multicloning region of M13mp18 (*Bam*HI to *Sph*I), and the region between this internal *Sph*I site (upward arrow) and the *Nci*I site (second downward arrow) inserted in the sense direction. This latter part of the insert was used as a DNA template for in vitro transcription.

pendently generated clone had the identical sequence in the protein coding region, but distinct sequences in the 3′-untranslated region. This is the first report of any DNA clone from the chicken aldolase A gene.

The derived amino acid sequence of chicken aldolase A had an Ala at position-348 and Ile at position-358, which are similar to the residues found among other mammalian aldolase A sequences [Tolan et al., 1984, 1987; Joh et al., 1986; Mestek et al., 1987]. These two positions are the only variable sites in mammalian aldolase As. Position 347, normally a conserved Gln in mamalian aldolase As, has been substituted by a His in chickens. This His-347 in the chicken aldolase A sequence is unique among all known aldolases.

These two mRNA sequences for chicken aldolase A differ chiefly around the polyadenylation signal and could represent separate 3′-exons. The existence of multiple polyadenylation signals has been documented in mouse aldolase B [Maine et al., 1992], chicken aldolase B [Burgess and Penhoet, 1985] and human aldolase B [Tolan and Penhoet, 1986]. In addition, the utilization of alternative polyadenylation sites has been demonstrated in mouse aldolase B [Maine et al., 1992]. This is consistent with the possibility that more than one transcript may exist from both the chicken aldolase A and C genes. In the chicken aldolase C sequence, the polyadenylation signal appears as two overlapping signals (AATAATAAA; see Fig. 1). This would generate mRNAs that are slightly different in length. Consistent with this, two aldolase C-specific fragments that differed by six bases were detected in RNase protection assays (data not shown). In the aldolase A cDNA clones described above, the differences in size and sequence suggest that there are alternative or incomplete 3′-untranslated regions that have not been determined. From the size of other vertebrate aldolase A mRNAs it seems unusual that the complete 3′-untranslated region is only 80 bp, although the mouse brain aldolase A 3′-untranslated region is 185 bp [Mestek et al., 1987].

Aldolase Steady-State mRNA Expression During Differentiation of Chicken Myoblasts

Aldolase steady-state mRNA expression during the differentiation of chicken myoblasts in primary cultures was measured using RNase protection assays with cRNA aldolase probes. The cRNA aldolase probes of isozyme-specific regions were generated by in vitro transcription from subclones, pRMCA and pRMCC, that contained aldolase A and C cDNA inserted into pGEM vectors, respectively (see Figs. 1, 2). Skeletal thigh myoblasts were cultured over a period of 168 h, and the morphological changes during myoblast differentiation were measured by determining nuclei number and percent fusion. The fragments protected by the cRNA probes were

verified by their size, which was determined by co-migration of a DNA sequencing ladder, and by using RNA from appropriate embryonic and adult tissues known to express aldolase A or C, or both. At each time point, the amount of all the protected aldolase mRNA fragments was determined by densitometric analysis. The normalized intensities versus time in culture are averages of two or three experiments (Fig. 3).

During the differentiation of chicken myoblasts in primary cultures, the expression of aldolase C steady-state mRNA remained relatively constant (Fig. 3A). A typical RNase protection experiment for aldolase C is shown in Figure 4A. Aldolase C steady-state mRNA was maximally expressed at 40–48 h in culture. However, this initial increase in expression from 0 to 48 h appeared to be a consequence of the culture system. An abundant embryonic gene, β-actin, with a constant level of expression [Bergsma et al., 1986], showed a similar early recovery of expression in primary cultures followed by an expected constant level of expression (Fig. 4C). At 144 h and 168 h, the mean of the normalized intensity/nuclei for aldolase C mRNA was 50–75% of the maximal level; however, the deviation in these experiments suggested that these differences were not significant.

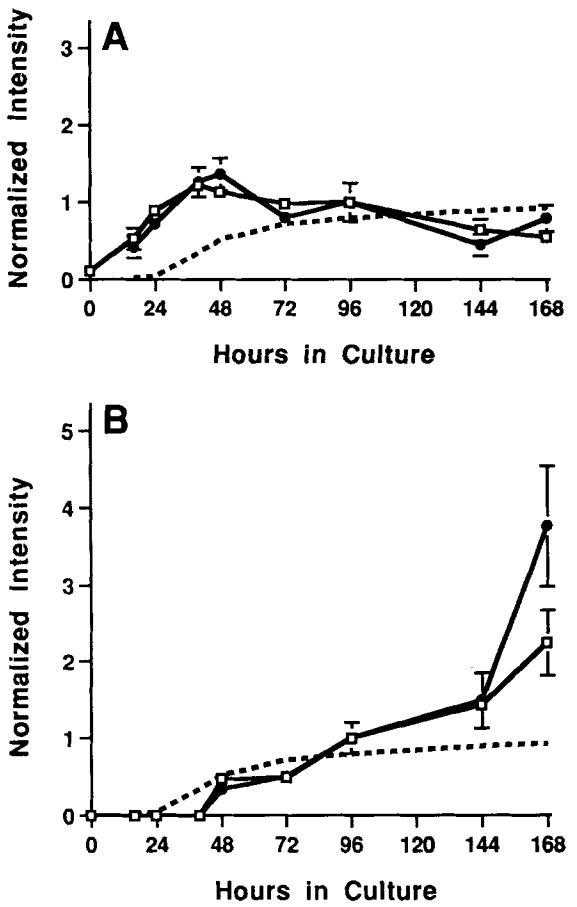

Fig. 3. Aldolase expression during differentiation of chicken myoblasts. Intensity was normalized to 96 h. Open squares represent normalized intensity, and the error bars indicate the standard deviation from the mean of three culture experiments. Closed circles represent normalized (intensity/nuclei), and the error bars indicate the standard deviation from the mean of two culture experiments. The dashed line represents the percentage of myoblasts fused where 1 = 100%. **A:** Aldolase C mRNA expression is plotted versus h in culture. **B:** Aldolase A mRNA expression is plotted versus h in culture.

In contrast to aldolase C expression, aldolase A steady-state mRNA was not expressed prior to fusion (Fig. 3B). Aldolase A expression was first detected at 48 h in culture, when fusion was approximately 50%. Expression gradually increased until around 144 h in culture when a dramatic increase was observed. By 168 h, aldolase A expression was four times greater than that at 96 h, which marked the completion of fusion. A typical RNase protection experiment for aldolase A is shown in Figure 4B. This biphasic change, an initial induction of mRNA at fusion then a subsequent large increase, is interesting and has not been described previously. The sensitivity of RNase protection assays, relative to Northern or dot-blot analysis, may be the main reason for this observation.

The findings of Turner et al. [1974] that measured the expression pattern of aldolase C and A at the protein level during early myogenesis showed an apparently coordinated switch in isozyme expression with the midpoint of the switch at approximately 7 days in primary culture. This previous work was performed using similar myoblast differentiation cultures as described here. In contrast to the activity levels, the steady-state mRNA levels does not support this apparently coordinated switch in expression. The aldolase A activity that was detected, starting at day 5 of myoblast differentiation [Turner et al., 1974] closely corresponded to the large increase in aldolase A steady-state mRNA starting at day 6 (144 h, Figs. 3B, 4B). By contrast, the aldolase C activity decreased starting at day 5 of myoblast differentiation [Turner et al., 1974], while the aldolase C steady-state mRNA level remained relatively unchanged through day 7 (168 h, Figs. 3A, 4A).

The results from the steady-state mRNA levels clearly indicate that the pattern of aldolase

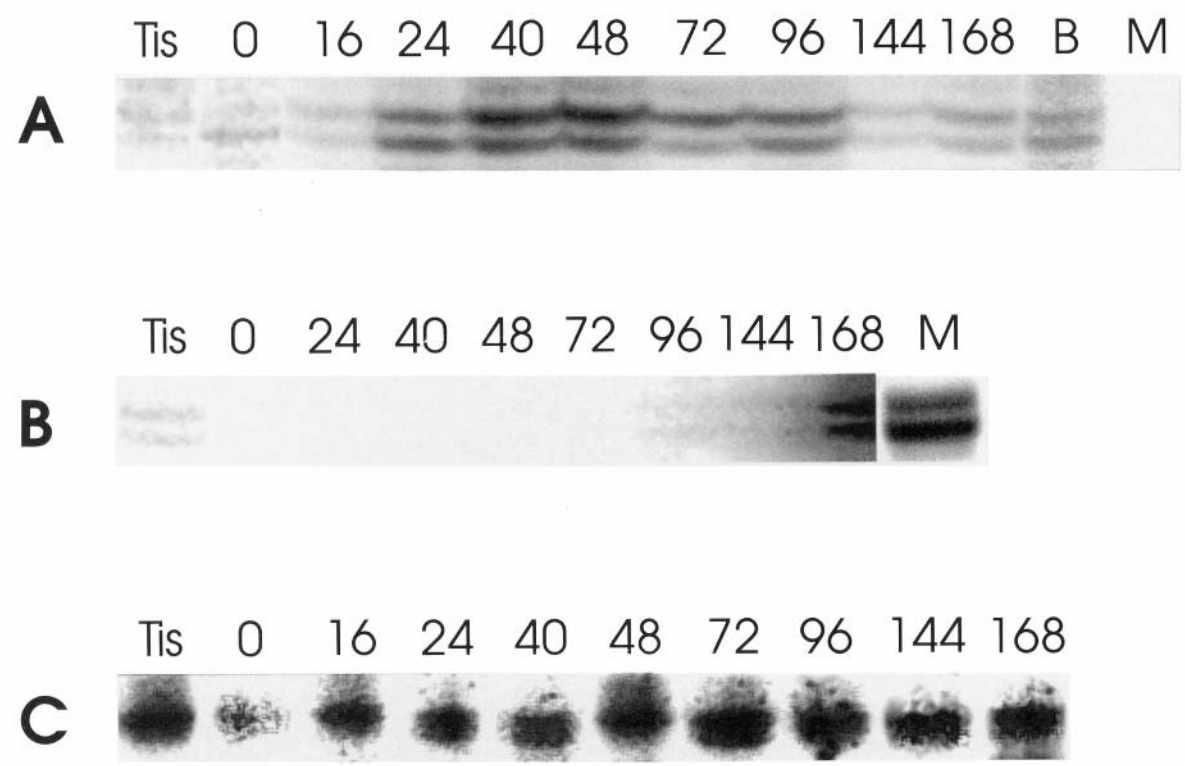

Fig. 4. RNase protection assays of steady-state mRNA during differentiation of chicken myoblasts. The numbers above denote the h in culture with tissue controls designated as: Tis, stage 37 embryonic skeletal muscle; B, adult brain; and M, adult skeletal muscle. **A:** Autoradiograph from a typical experiment with aldolase C cRNA. Five μg total RNA was used for all the time intervals, except for 0 h, which was 10 μg. Exposure was for 16 days without an intensifying screen, except for 0 h which 7 days. **B:** Autoradiograph from a typical experiment with aldolase A cRNA. Five μg total RNA was used for all the time intervals, except for M, where 3 μg was used. Exposure was for 16 days without an intensifying screen. The intensity of M was purposely lightened for clarity. **C:** Autoradiograph from a typical experiment with β-actin cRNA. Five μg total RNA was used for all the time intervals. Exposure was for 7 days without an intensifying screen. Autoradiographs were scanned with a PDI, Inc. (Huntington Station, NY) model DNA35 densitometer using the software program, Quantity One Dimensional Analysis, version 2.2, at the Vincent T. Lombardi Cancer Center, Georgetown University Medical Center. The backgrounds were normalized to correct for overexposure.

gene expression is not coordinately regulated during myoblast differentiation. It appears that the expression of steady-state mRNA from the aldolase A and C genes is regulated by different mechanisms. Perhaps aldolase A expression is primarily regulated at transcription, like other muscle-specific genes [Buckingham, 1989], while post-transcriptional control may contribute significantly to the regulation of aldolase C expression. Consistent with this possibility, Maine and Ciejek-Baez [1991] found that aldolase B expression in the mouse liver is regulated primarily by the initiation of transcription, while aldolase A expression (the embryonic form in mammals) was regulated by post-transcriptional processing. In addition, in mouse muscle cell lines, which can undergoe differentiation, there was a persistence of transcription from the embryonic specific promoter [Colbert and Ciejek-Baez, 1992]. In summary, the developmental switches that control aldolase gene expression appear to be complex.

ACKNOWLEDGMENTS

We are grateful to Drs. Robert Hausman and Nadia Rosenthal and for their advice and helpful discussions, to Dr. Chris Li and Johanna Phillips for their help with the microscopy, to Tanya Reichert for help with the protein sequence alignments and Dr. Elise Drouin for her help with the densitometric analysis. This work was aided by PHS grant DK-38821 from the National Institutes of Health.

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