Detection and Localization of Actin mRNA Isoforms in Chicken Muscle Cells by In Situ Hybridization Using Biotinated Oligonucleotide Probes

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We have developed in situ hybridization methodology for nonisotopically labeled oligonucleotide probes to detect cellular mRNA with improved speed, convenience, and resolution over previous techniques. Previous work using isotopically labeled oligonucleotide probes characterized important parameters for in situ hybridization (Anal Biochem 166:389, 1987). Eleven oligonucleotide probes were made to coding and noncoding regions of chick β-actin mRNA and one oligonucleotide probe to chick α-cardiac actin mRNA. All the probes were 3' end-labeled with bio-11-dUTP using terminal transferase, and the labeled probes were hybridized to chicken myoblast and myotube cultures. The hybridized probe was detected using a streptavidin-alkaline phosphatase conjugate. Our assay for the success of probe hybridization and detection was the demonstration of β-actin mRNA highly localized in the lamellipodia of single cells (Lawrence and Singer, Cell 45:407, 1986) as well as the expression of α-cardiac actin mRNA and the repression of β-actin mRNA in differentiating myoblasts and in myotubes. With the α-cardiac probe, we found that this mRNA was distributed all over the cytoplasm of myotubes and differentiated (bipolar) single cells and negative in undifferentiated single cells and at the ends of myotubes. When β-actin probes were used, two of 11 probes were highly sensitive, and, in pooling them together, the localization of β-actin mRNA in fibroblastic single cells was evident at the leading edge of the motile cells, the lamellipodium. β-Actin mRNA was not detected in myotubes except at the ends where contact was made with substrate. This indicates that both β and cardiac actin mRNA can coexist in the same myotube cytoplasm but at different locations.

Key words: β-actin mRNA, α-cardiac actin mRNA, poly-(A)⁺ mRNA

In situ hybridization is a technique that demonstrates the morphological disposition of DNA or RNA within cells, tissue sections, or chromosome preparations. Intracellular mRNA is hybridized with the complementary probe, and the hybrids within their morphological context are detected by probe labeled in a variety of ways,

nonisotopic or isotopic. The technique was originally developed in 1969 [1,2] and nick-translated probes [3–11], single stranded cDNA probes [12], single stranded antisense RNA probes [13–15], random primed synthetic DNA probes [16], and oligonucleotide probes [17–22] have been applied to in situ hybridization. Single stranded nucleic acid probes have certain advantages; since they are single stranded, they do not reanneal, unlike the nick-translated probe, and they can also be used to detect strandedness.

With the use of radiolabeled DNA probes, our laboratory [5,17] has analyzed in situ hybridization with respect to a number of parameters, e.g., optimal concentrations of probe to saturate all the target sites, size of the probe, and hybridization conditions in order to obtain the highest signal-to-noise ratio. The use of radiolabeled probes ensures high sensitivity but carries several disadvantages. For autoradiographic analysis, the exposure time can be long, and the resolution obtained is relatively poor. As an alternative, biotin-labeled probes [3,23–25] or digoxigenin-labeled probes [26,27] or chemically modified, acetylaminofluorene (AAF)-labeled probes [28,29] have been used for nonisotopic in situ hybridization. Biotin-, digoxigenin-, or AAF-labeled probes can be detected by immunocytochemical methods using specific antibodies or, for biotin modified probes, by coupling with a streptavidin-enzyme conjugate [30].

Oligonucleotides have some advantages over cloned probes as has been detailed elsewhere [17,22]. These have proven very efficient probes for hybridization [17–22] and have an advantage in specificity in that they can be designed to discriminate closely homologous mRNA sequences [22]. In this study, we wished to combine the advantages of in situ hybridization using oligonucleotide probes with the speed, convenience, and resolution of nonisotopic label. Others have reported the nonisotopic detection of oligonucleotide probes in situ [see, e.g., 31,32]; however, our interest has been to develop these probes for the use of intracellular localization of mRNA in conjunction with distinction of the isoforms of actin expressed during muscle development. To optimize the use of these probes for this purpose, we analyzed a number of variables similar to the methodological approach used earlier for radiolabeled probes [17]. The oligonucleotide probes made to coding and noncoding regions of β-actin mRNA [33] and α-cardiac actin mRNA [34,35] (51–55 nucleotides), which were used in this previous work to optimize hybridization conditions were biotinylated enzymatically using terminal deoxynucleotidyl transferase. Detection of the probes was by alkaline phosphatase conjugated to streptavidin. Intracellular localization of β-actin mRNA within lamellipodia of motile cells [6] was used as an assay for the resolution of the probe. Using this method we have shown the presence of cardiac actin mRNA predominantly in myotubes [36] and β-actin mRNA in single cells served as an assay for distinction of the two isoforms. The work presented here details methodological aspects of using oligonucleotide probes and extends previous work [36] on actin mRNA localization. In addition, we describe the presence of both isoforms simultaneously in the same myotube cytoplasm associated with different cytological structures.

MATERIALS AND METHODS

Synthesis, Purification, and Labeling of Oligonucleotide Probes

DNA probes were synthesized by the phosphoramidite method [37] using an Applied Biosystems DNA synthesizer model 380A. The probes were purified by gel

electrophoresis on 15% acrylamide gels containing 7 M urea, transferred to plastic wrap, and placed on a fluorescent chromatography plate; DNA was visualized by ultraviolet (UV) shadowing, and the desired band was excised and eluted from the gel and dried in a speedvac.

For 3' end-labeling, 100 ng of oligonucleotide in a total volume of 50 μl was incubated with 40 μM bio-11-dUTP (BRL), 140 mM potassium cacodylate, 30 mM Tris HCl, pH 7.6, 1 mM $CoCl_2$, 0.1 mM DTT, and 20 U terminal deoxynucleotidyl transferase (BMB) at 37°C for 30 min. For 5' end-labeling, 100 ng of DNA probe was incubated at 37°C for 30 min with 100 μCi of γP^{32}-ATP (>3,000 Ci/mM; Amersham), 25 mM $MgCl_2$, 25 mM β-mercaptoethanol, 175 mM Tris HCl, pH 7.5, and 10 U T_4 polynucleotide kinase (Amersham) in 50 μl volume. The reaction products were purified by gel filtration on 1 × 20 cm G-50 Sephadex column. Oligonucleotides were tested for biotinylation by spotting on filters and detected using the BRL DNA detection kit. Radiolabeling was assessed by gel electrophoresis on 15% acrylamide followed by exposure to X-ray film.

Preparation of Oligonucleotide Probes

Oligonucleotide probes (51–55 nucleotides) were made to various regions of the β-actin [33] and α-cardiac actin [34,35] sequences (G + C = 43–50%) as listed in Table I. Three probes were made to the coding region of β-actin, having homologies, respectively, of 94%, 72%, and 88% to α-cardiac actin; 94%, 85%, and 74% to α-skeletal actin [38], and 88%, 80%, and 77% to α-smooth muscle actin [39]. One probe made to the coding region of α-cardiac actin has 36%, 88%, and 73% homology with β-actin, α-skeletal muscle actin, and α-smooth muscle actin, respectively. Nine additional probes were made to 5'-untranslated and 3'-untranslated regions of β-actin

TABLE I. List of Oligonucleotide Probes Made to α-Cardiac and β-Actin mRNA*

S.N.	Oligo	Corresponding nt position	Homology (%)			
			α-Cardiac	β-Actin	α-Skeletal	α-Smooth muscle
α-Cardiac coding region						
1	KLT-9	1630–1680	100	36	88	73
β-Actin coding region						
2	KLT-5	548–601	94	100	94	88
3	KLT-23	1442–1495	72	100	85	80
4	KLT-22	1967–2017	88	100	74	77
3'-Untranslated region of β-actin						
5	KLT-4	2644–2698	25	100	20	16
6	KLT-14	2750–2800	15	100	20	23
7	KLT-15	2801–2851	21	100	27	33
8	KLT-18	2954–3004	—	100	—	—
9	KLT-6	3006–3060	—	100	—	—
10	KLT-19	3061–3111	—	100	—	—
11	KLT-20	3112–3162	—	100	—	—
5'-Untranslated region of β-actin						
12	KLT-40	(−959)–(−909)	37	100	35	39

*Oligonucleotide probes (51–55 bases) were made to β-actin [33] and α-cardiac actin [35] from the coding and noncoding regions of mRNA. Percentage homology was compared with α-skeletal [38] and α-smooth [39] actin mRNA.

having very little or no homology with other actin genes. Oligodeoxyadenylic acid (55 nucleotides) and oligothymidilic acid (55 nucleotides) were made as negative and positive controls for in situ hybridization. DNA probes were biotinated or were end-labeled by use of γP^{32} ATP. On some preparations, both labels were used simultaneously.

Cell Culture

Skeletal myoblasts were isolated from the pectoral muscle of 12-day chicken embryos and plated at a density of 10^6 cells per 100 mm plate containing 0.5% gelatin coated, autoclaved glass coverslips (22 mm). Cells were grown in minimal essential medium (MEM), 10% heat-inactivated fetal calf serum, and 2% chicken serum. After about 48 hr of incubation, cells consisted of a mixture of undifferentiated and differentiated myoblasts and early myofibers. Coverslips containing these cells were rinsed twice with Hank's balanced salt solution (HBSS) and fixed for 15 min in 4% paraformaldehyde (Fisher) in phosphate-buffered saline containing 5 mM $MgCl_2$. After fixation, the coverslips were washed twice with 70% ethanol and stored in 70% ethanol at 4°C. To examine the cells with different probes, a large number of coverslips were prepared from one cell batch with uniform density. Just prior to hybridization, coverslips were cut in half with diamond pencil; one-half was directly hybridized to probe and the other half treated with RNase and then hybridized to the same probe to have a negative control for nonspecific hybridization.

RNase Treatment

Cells were rehydrated in phosphate-buffered saline (PBS) containing 5 mM $MgCl_2$ for 10 min at room temperature and rinsed twice with 2× SSC. Mixture of RNase A (0.2 mg/ml), RNase T1 (0.2 mg/ml), and RNase T2 (100 U/ml) was prepared in 2× SSC, 30 μl of the solution was put on the parafilm, and the half coverslip was placed cell side down on the solution. The coverslips were sealed with another layer of parafilm and incubated at 37°C for 1 hr in a humidified incubator, and the coverslips were washed three times with 2× SSC to remove the traces of RNases. These coverslips can also be stored at 4°C in 70% ethanol.

Hybridization and Washing

Cells stored in 70% ethanol at 4°C, were rehydrated with PBS plus 5 mM $MgCl_2$ for 10 min at room temperature, followed by 10 min in 0.1 M glycine, 0.2 M Tris, pH 7.4, at room temperature. Prior to hybridization, the coverslips containing cells were incubated in 50% formamide (Sigma), 2× SSC and 5 mM NaH_2PO_4, pH 7.0, at 65–70°C for 10 min. Two nanograms of the DNA probe, 5 μg of sonicated salmon sperm DNA and 10 μg of *Escherichia coli* tRNA were lyophilized, resuspended in 5 μl of 100% deionized formamide, and heated at 90°C for 2 min. The DNA solution was mixed with 5 μl of hybridization buffer so that the final concentration of probe was 200 ng/ml in 50% formamide, 2× SSC, 1% bovine serum albumin (BSA), 10 mM Vanadyl complex, and 10% dextran sulfate containing 0.5 mg/ml of salmon sperm DNA and 1 mg/ml of *E. coli* tRNA. Total hybridization solution (10 μl) was put on the parafilm, and the half coverslip (11 × 22 m) was placed cell side down on the hybridization mixture. The cells were incubated at 37°C for 2-3 h in a humidified incubator. After hybridization, coverslips were washed successively 30 min each with 50% formamide, 2× SSC at 37°C; twice in 50% formamide, 1× SSC at 37°C; and twice in

1× SSC at room temperature. For P[32]-labeled probe, the coverslips were counted in PBS in a scintillation counter. No-probe controls and RNase treated cells were also hybridized and washed in the same way.

Colorimetric Detection of Hybridized Biotinated Oligonucleotide Probe

Biotin-labeled oligonucleotide probe was detected by covalently linked streptavi-din-alkaline phosphatase (SA-AP provided by BRL [30] or purchased from Dako). The use of the conjugate was in 4× SSC as described previously for the streptavidin biotinated alkaline phosphatase procedure [7,8]. After hybridization and washing, the coverslips were rinsed in 4× SSC, 0.01% Triton for 5 min; then cells on the coverslip were incubated in 30 μl of SA-AP conjugate at a concentration of 0.6 μg/ml in 4× SSC, 1% BSA for 30 min at 37°C in a humidified incubator. The coverslips were washed at room temperature twice in the Triton buffer for 10 min each and once in 0.1 M Tris HCl, pH 9.5, 0.1 M NaCl, 50 mM $MgCl_2$ for 1 min. The color development was performed by placing the coverslips in the above solution containing NBT (330 μg/ml) and BCIP (167 μg/ml) in dark for appropriate times. During the development, the coverslips were viewed by microscopy, and, when signal was evident, the reaction was terminated by washing two times in cold H_2O. The coverslips were air-dried and mounted cell side down on the slides using an aqueous mounting medium (Crystal Mount, Biomeda). Alternatively, the coverslips were stored dried at room temperature.

RESULTS
In Situ Hybridization Using Biotinated Oligonucleotides

The experimental procedure used for this work is schematized in Figure 1. When the samples were exposed to the streptavidin-alkaline phosphatase (SA-AP) conju-gate, a time-dependent nonspecific background coloring of the cells occurred. For maximal signal-to-noise ratio, it was necessary to minimize the color development time. Half of a coverslip was hybridized with biotinylated probe (signal), and the other half coverslip (background) was exposed to the same protocol but without probe. The probe used for actin mRNA (KLT-5) gave a moderate to weak signal and hence served as a means to evaluate signal-to-noise ratio more critically. After exposure to SA-AP conjugate, samples were taken out at appropriate times, and results are shown in Table II. The result showed that the color was detectable in 40 min in the experimental cells but not in the control cells until 80 min of incubation. Signal was maximal at 60 min, with minimal background; longer incubation increased the background gradually. Different conjugate preparations of streptavidin-alkaline phos-phatase require standardization using this procedure. For a positive control, we used biotin-labeled oligo(dT), which showed that the color reaction was distributed through-out the cytoplasm of all cells in the culture (Fig. 2A). For a negative control, we used biotinylated oligo(dA), where cells in the culture did not show any signal (Fig. 2C). Additionally, the cells were also treated with RNase A, RNase T1, and RNase T2 to digest the mRNA completely, and these cells were then hybridized with biotin-labeled oligo(dT). Alkaline-phosphatase color was not detectable in any of these cells (Fig. 2B, Table III).

We have described in a previous paper [17] how the choice of oligonucleotide probe made to a particular sequence is very important for efficient in situ hybridiza-

METHODOLOGY FOR NON-ISOTOPIC
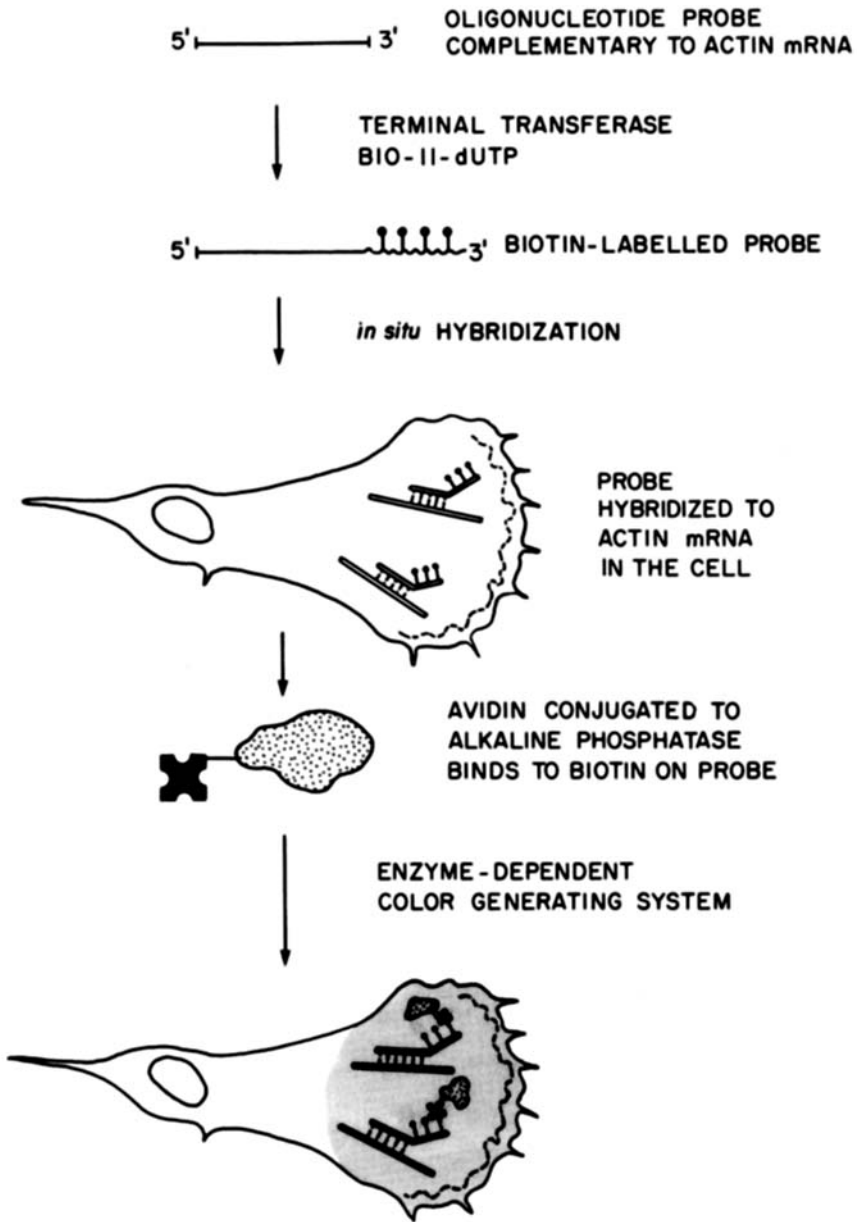## *in situ* HYBRIDIZATION USING OLIGONUCLEOTIDES

Fig. 1.   Schematic diagram of procedure.

TABLE II. Development of Alkaline-Phosphatase Color With Time*

	20 min	40 min	60 min	80 min	100 min	120 min
KLT-5	−	− +	+	+ +	+ +	+ + +
No probe	−	−	−	− +	+	+ +

*Samples from the same cell batch were hybridized to biotinylated probe (KLT-5) or no probe and detected with SA-AP. Samples were removed from the developing solution with time and checked under the microscope. Seventy minutes was chosen as the optimal time for signal-to-noise ratio.

tion. One probe can give high levels of hybridization, whereas another to the same message may have poor hybridization efficiency. This may result from intrinsic probe properties such as intramolecular hybridization or from properties of the mRNA such as protein binding sites or secondary structure. Prior to hybridization cells were heated at 65–70°C in 50% formamide, 2× SSC in order to reduce the secondary structure of RNA, but this did not change the hybridization signal. Various probes were made to coding and noncoding regions of β-actin and α-cardiac actin mRNAs to determine the optimal probe for quantitative detection of their gene expression. We analyzed the hybridization efficiency of these probes, after hybridization and detection with SA-AP conjugate. Table III illustrates the efficiency characteristic of each β-actin probe detected in single cells. Two probes, KLT-23 and KLT-19, had maximal hybridization efficiency, and the rest of the probes had adequate efficiency, with the exception of three, which were decidedly inferior. This observation was confirmed using scintillation counting to measure the probe (P^{32}-labeled), which was hybridized to cells on coverslips [17]. The saturation values of some of these probes were determined from scintillation counting as a comparison with the alkaline phosphatase signal. Good correlation between counting and microscopy exists with the best probe (KLT-19). The other probes showed less signal by either method. α-Cardiac actin probe (KLT-9) showed alkaline-phosphatase color in differentiated single cells and myotubes [36], but not in undifferentiated single cells, and so also served as a control for nonspecific background.

To increase the signal-to-noise ratio by pooling oligonucleotide probes, we selected two probes (KLT-23 and KLT-19) from Table III made to β-actin. Biotin-labeled probes were hybridized individually and combined (mixed in equal molar ratio) with the cells. As determined by decreased development time, the signal with mixture of oligonucleotide probes increased equal to the sum of the individual probes. This result was also confirmed by the saturation level obtained using P^{32}-labeled probes for KLT-14 and KLT-16, showing that signal of each probe can sum (Fig. 3).

Intracellular Localization of β-actin and α-cardiac Actin mRNA

We next looked at the intracellular distribution of total poly-(A) mRNA in the cell. As is shown in Figure 2A, the alkaline phosphatase color is distributed throughout the cytoplasm of differentiated and undifferentiated single cells as well as myotubes. Hybridization to poly-(A) mRNA generally resulted in strong signal in the nucleus and perinuclearly, more over the central region of the cell than over the peripheral region. Extended cell processes showed less signal with the oligo(dT) probe relative to the perinuclear region.

Using β-actin probes, the signal in the hybridized cell revealed that the distribution of β-actin mRNA was highly nonhomogeneous as was reported previously [6]. For
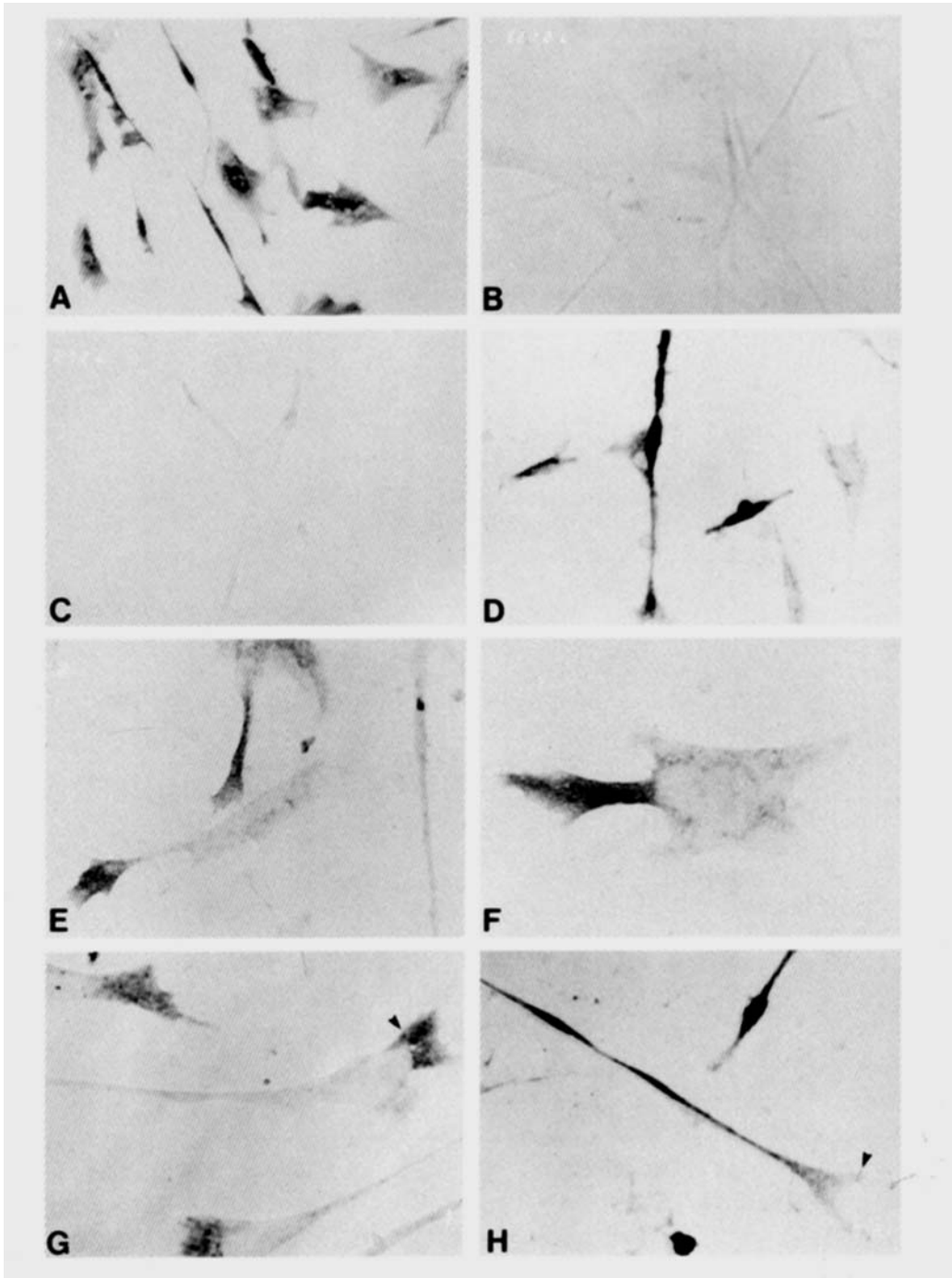
Figure 2.

TABLE III. Summary of Results After Hybridization Using Oligonucleotide Probes*

P^{32} hybridized (pg)	−RNase		+RNase		Oligo
	Myoblast	Myotubes	Myoblast	Myotubes	
	+ + + + +	+ + + + +	−	−	T55
	−	−	−	−	A55
	−	+ + + +	−	−	KLT-9
	+ +	−	−	−	KLT-5
	+ + + +	−	−	−	KLT-23
	+	−	−	−	KLT-22
	+ + +	−	−	−	KLT-4
8.0	+	−	−	−	KLT-14
5.0	+ +	−	−	−	KLT-15
4.2	+ +	−	−	−	KLT-18
	+	−	−	−	KLT-6
14.0	+ + + +	−	−	−	KLT-19
7.0	+ +	−	−	−	KLT-20
	+ +	−	−	−	KLT-40

*Probes listed in Table I were biotinated with bio-11-dUTP, then hybridized to 2-day chicken muscle cultures. The hybridized probe was detected with SA-AP conjugate. In the case of oligo(dT) and oligo(dA), the color development of alkaline-phosphatase time was 10 min, whereas in other cases the time was 70 min. Oligo(dT) shows all cells (differentiated as well as undifferentiated) to be positive. When oligo(dA) was used, the same cells were negative. The α-cardiac actin-specific probe shows that differentiated myotubes were positive and that undifferentiated cells were negative. β-Actin probes show undifferentiated cells were positive, and differentiated myotubes were much weaker. RNase eliminated all signal. The hybridization efficiency of some β-actin probes was evaluated by saturation hybridization using P^{32} probes and expressed as pg hybridized/10^5 cells (left column).

the localization of β-actin mRNA, the two best probes (KLT-23 and KLT-19) were chosen from Table III and hybridized to the cells, and the hybridized cells consistently showed the alkaline phosphatase color at the cell periphery, the lamella, or extended process of a motile cell [6], indicating that the β-actin mRNA is concentrated in that part of the cell. The color distribution and the morphology of cells are illustrated in Figure 2E and F.

When the α-cardiac actin probe (KLT-9) was used to detect the α-cardiac actin mRNA, it was found that this mRNA was distributed throughout the cytoplasm of

Fig. 2. Detection and localization of mRNAs for actin isoforms. Biotin-labeled oligonucleotide probes were hybridized to cultures of chicken myoblasts and myotubes, and the hybridized probe was detected with streptavidin-alkaline phosphatase (SA-AP). In A–C, the color development time was 10 min. In D–H, the time was 70 min. A: Cells were hybridized to the biotinylated oligo(dT) probe; all the cells in the field were positive and in the undifferentiated single cell, poly-(A) mRNA was more predominant near the nucleus. B: Cells were treated with an RNase mixture (RNase A, T$_1$, T$_2$) and then hybridized to oligo(dT). No signal was detected. C: Oligo(dA) was used as a negative control. D: α-Cardiac actin message-specific probe (KLT-9) was used for hybridization. Most fibroblastic single cells are negative, whereas myotubes and differentiated (bipolar) single cells are positive. α-Cardiac actin mRNA is distributed throughout the cytoplasm. E, F: Two β-actin probes (KLT-19 and KLT-23) were mixed together in equimolar ratio before hybridization. Most single cells contain β-actin mRNA located at the leading edge of the cell, the lamellipodium. Myotubes and differentiated (bipolar) single cells had decreased signal for β-actin mRNA. G: Same as E and F. β-Actin mRNA was present in the tip of the myotube (arrowhead), and the rest of the myotube has no signal for β-actin mRNA. H: Same as D. α-Cardiac mRNA was absent in the tip of the myotube (arrowhead) and present all over the myotube.
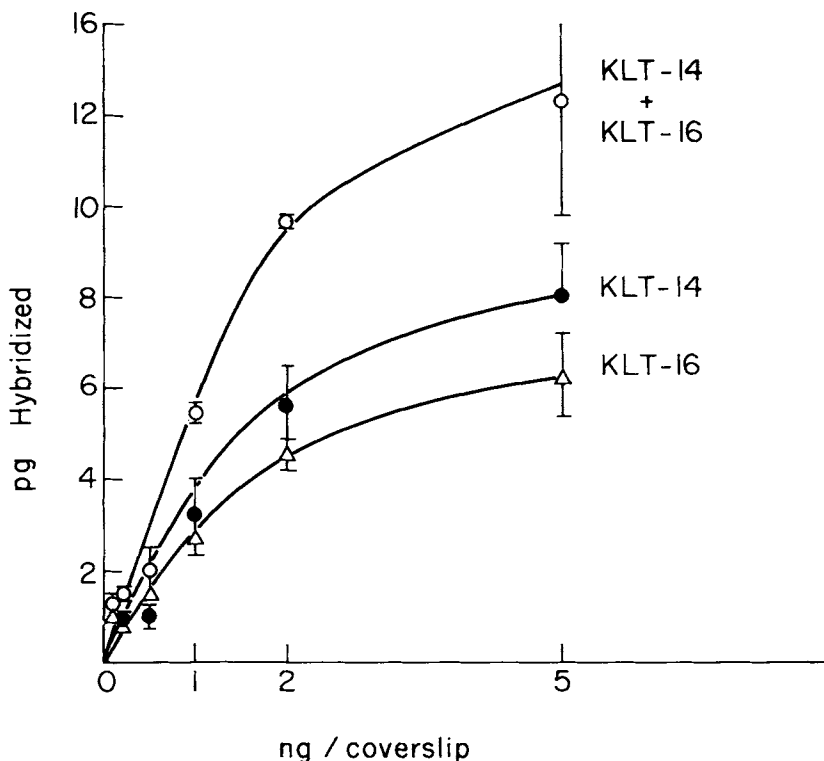
Fig. 3.   Increase in signal obtained after pooling of oligonucleotide probes. Two oligonucleotide probes were made to the 3'-untranslated region of β-actin mRNA (KLT-14, KLT-16, nucleotide 2851-2901) [34]. Saturating amounts of KLT-14, KLT-16, and KLT-14 + KLT-16 (combined in equimolar concentration) were added to the coverslip of chicken fibroblasts (10^5 cells); hybridization was for 3 hr.

differentiated single cells but was nearer the nucleus than the periphery and was homogeneously distributed in myotubes (Fig. 2D, H). It was substantially reduced in undifferentiated single cells (on the right in Fig. 2D). However, in many myotubes, where the ends splay out in attachment to the substrate, β-actin mRNA was substantially enriched specifically in this region (Fig. 2G) but was absent in the rest of the myotube. The myotube (vertical on the right) in Figure 2E has less β-actin mRNA than the single cells, even though it is a much thicker structure. Therefore, β-actin mRNA expression must decrease in differentiated myotubes, except at the ends where it is restricted. α-Actin mRNA was confined to the juxtanuclear areas and did not extend into the myotube tips (Fig. 2H). This indicates that both isoforms of actin message can coexist in the same cytoplasm.

DISCUSSION

In the present paper we demonstrate the use of nonisotopic labeled oligonucleotide probes for sensitive, in situ hybridization capable of subcellular resolution. These probes have advantages over radioactive probes, such as stability, safety, speed, and convenience in addition to superior resolution. Due to proportionately decreased target size, oligonucleotide probes have decreased sensitivity compared with a much

larger cDNA probe such as with the actin (1.8 kb) message. In nondifferentiated fibroblastic cells, there are approximately 2,000 β-actin mRNA molecules per cell [5,34]. Using one oligonucleotide probe to β-actin, we are detecting an accumulated target of 110 kb representing the activity of 2,000 alkaline phosphatase molecules per cell, assuming 100% hybridization efficiency, and one streptavidin-alkaline phosphatase per oligonucleotide. Both these assumptions are reasonable, since we have previously shown high hybridization efficiency using radioactive probes [17], and, since approximately 10 nucleotides are added by the terminal transferase (data not shown), steric hindrance would most likely prevent more than one conjugate from detecting the probe. We have noted differing efficiencies of hybridization depending on the specific probe, even though they are all targetted to the same message. This could be due to the secondary structure of the probe or RNA target or RNA-associated proteins at that site. Hence the choice of the probe for a message greatly affects the signal-to-noise ratio.

Because actin mRNA is highly localized, this sensitivity is possible. Nonisotopic detection, due to its higher resolution, compresses the signal into a smaller space, since the probe is detected at its site of hybridization. If actin mRNA were not localized, the sensitivity of nonisotopic detection would decrease significantly, since it would be difficult to distinguish it from background. Currently, a disadvantage of nonradioactive probes is the lack of a convenient method of quantitation. Other studies have also presented evidence showing in situ hybridization using biotinylated oligonucleotides on tissue sections, detected by alkaline phosphatase, for POMC RNA in the pituitary [31] and for vasopressin or calcitonin in nerve or carcinoma [32].

When myoblasts start to differentiate into postmitotic myoblasts or fuse into myotubes, the β-actin mRNA is decreased and α-cardiac actin mRNA is increased [see also 36]. Not only is the expression of α-cardiac and β-actin messages inversely related during differentiation but they exist in morphologically distinct regions of the cell. β-Actin mRNA is confined to the periphery of single myoblasts and fibroblasts and myotubes, particularly in motile or substrate adherent regions [6]. α-Actin mRNA is perinuclear in its location in postmitotic single cells and in myotubes [36]. The coexistence of β- and α-actin in the myotube (and possibly even in single, differentiated myoblasts) suggests that a mechanism exists for the differential localization of each message isoform. This localization may indicate functional differences in these regions; for instance, β-actin in substrate adherence and α-actin in sarcomere assembly. In contrast to the actin messages, poly-(A) RNA was detected throughout the cell and was most intense around or in the nucleus. Similar results were obtained with HeLa cells [40]. Current work directed toward methods of double labeling of oligonucleotides using analogs such as digoxigenin may resolve questions concerning the precise regional distribution of each message within the same cell. The characterization of nonisotopic labeling also allows these probes to be detected with the electron microscope using colloidal gold antibodies providing even higher resolution localization (Bassell, et al., in preparation).

ACKNOWLEDGMENTS

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